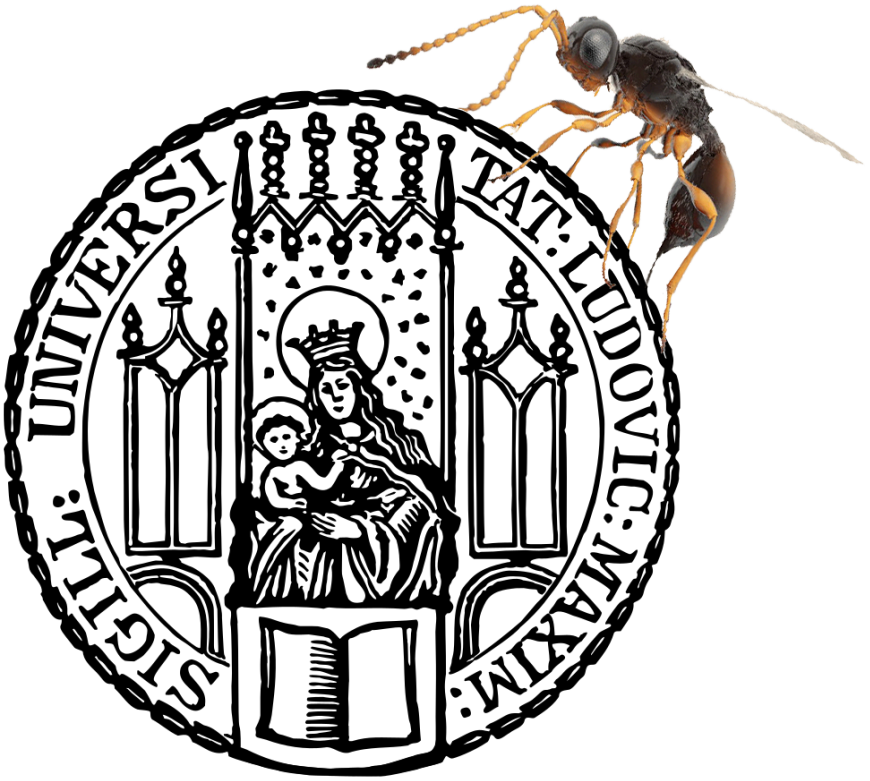

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)
an der Fakultät für Biologie der
Ludwig-Maximilians-Universität München

Illuminating the Dark Taxon Diapriidae (Hymenoptera) by Integrative Taxonomy

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vorgelegt von:
Jeremy Hübner
München, Juni 2024

COVER: *Xenomorphia resurrecta* Krogmann, van de Kamp & Schwermann, 2018 3D reconstruction in fossilized parasitized fly puparium, modified from Van De Kamp et al. 2018.

Erstgutachter:	Prof. Dr. Gerhard Haszprunar
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Tag der mündlichen Prüfung:	21.01.2025

EIDESSTATTLICHE ERKLÄRUNG

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt ist.

München, den .....28.05.2024..... .....

Jeremy Hübner

ERKLÄRUNG

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

München, den .....28.05.2024..... .....

Jeremy Hübner

Diese Dissertation wurde angefertigt
unter der Leitung des Promotionskomitee bestehend aus

Prof. Dr. Gerhard Haszprunar,
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Dr. Stefan Schmidt

an der Zoologischen Staatssammlung München (SNSB-ZSM) und
der Fakultät für Biologie der Ludwig-Maximilians-Universität München.

“Without taxonomy, phylogeny is impoverished, ecology is deprived of one of its fundamental units of currency, and conservation biology loses focus and aim. Taxonomy can have a profound and instant impact on conservation planning and decisions and may even be so potent as to force some taxonomists to consider concealing locality data to prevent the exploitation of newly described, commercially marketable species.”

(Agnarsson & Kuntner, 2007)

This thesis is dedicated to my beloved family and all
my friends for supporting me.

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ABBREVIATIONS

AI	Artificial Intelligence
ASAP	Assemble Species by Automatic Partitioning
BIN	Barcoding Index Number
BOLD	Barcode of Life Database
CF.	Confer (Latin, meaning ‘compare with’)
CNN	Convolutional neural network
COMB. N.	New combination
COI	Cytochrome Oxidase subunit 1
DNA	Desoxyribonucleic acid
DT	Dark Taxa
ETOH	Ethanol
FR	First record
GBOL	German Barcode Of Life - Project
IUCN	International Union for the Conservation of Nature
ML	Maximum-Likelihood
NOM. NUD.	Nomen nudum
OTU	Operational Taxonomic Unit
UCE	Ultra Conserved Elements
SNSB-ZSM	Bavarian State Collection of Zoology
SP. N./SP. NOV.	New species

LIST OF PUBLICATIONS

Andersen, T., Höcherl, A., Hübner, J., Chimeno, C., Lin, X.-L., & Baranov, V. (2023). New species and records of Pseudochironomini Sæther, 1977 (Diptera, Chironomidae) from the Dominican Republic. *Biodiversity Data Journal*, *11*(e111925). https://doi.org/10.3897/BDJ.11.e111925

Baranov, V., Lin, X.-L., Hübner, J., & Chimeno, C. (2024). Uncovering the hidden diversity of non-biting midges (Diptera, Chironomidae) from central Namibia, using morphology and DNA barcodes. *African Invertebrates*, *65*(1), 13–36. https://doi.org/10.3897/afrinvertebr.65.111920

Chimeno, C., Hausmann, A., Schmidt, S., Raupach, M. J., Doczkal, D., Baranov, V., Hübner, J., Höcherl, A., Albrecht, R., Jaschhof, M., Haszprunar, G., & Hebert, P. D. N. (2022). Peering into the Darkness: DNA Barcoding Reveals Surprisingly High Diversity of Unknown Species of Diptera (Insecta) in Germany. *Insects*, *13*(1), 82. https://doi.org/10.3390/insects13010082

Chimeno, C., Hübner, J., Seifert, L., Morinière, J., Bozicevic, V., Hausmann, A., Schmidt, S., & Müller, J. (2023). Depicting environmental gradients from Malaise trap samples: Is ethanol-based DNA metabarcoding enough? *Insect Conservation and Diversity*, *16*(1), 47–64. https://doi.org/10.1111/icad.12609

Hübner, J., Chemyreva, V. G., Macek, J., & Kolyada, V. A. (2024). A review of the genus *Zygota* (Hymenoptera, Diapriidae) in Germany with taxonomic notes on this genus and its distinction from *Pantoclis*. *ZooKeys*, *1207,* 325-353. https://zookeys.pensoft.net/article/121725/

Hübner, J., Chemyreva, V. G., & Notton, D. (2023). Taxonomic and nomenclatural notes on *Geodiapria longiceps* Kieffer, 1911 (Hymenoptera, Diapriidae) and synonymy of the genus *Geodiapria* Kieffer, 1910. *ZooKeys*, *1183*, 1–11. https://doi.org/10.3897/zookeys.1183.110952

Hübner, J., Deines, V., & Anton, J. L. (in review). A new record of gynandromorphism in *Trichopria nigra* (Hymenoptera, Diapriidae, Diapriinae). *Spixiana*, *47* (1), 79-82.

Hübner, J., Gabel, H., Deines, V., Kreiling, A. K. & Notton, D. G. (2024). Review of Diapriidae (Hymenoptera) of the Faroe Islands. *Spixiana*, *47* (1), 83-92.

Hübner, J. J., & Chemyreva, V. (2024). Review of German *Spilomicrus* Westwood (Hymenoptera, Diapriidae, Spilomicrini). *Biodiversity Data Journal*, *12*, e114515. https://doi.org/10.3897/BDJ.12.e114515

Krueger, T., Cross, A. T., Hübner, J., Morinière, J., Hausmann, A., & Fleischmann, A. (2022). A novel approach for reliable qualitative and quantitative prey spectra identification of carnivorous plants combining DNA metabarcoding and macro photography. *Scientific Reports*, *12*(1), 4778.

Shirali, H., Hübner, J., Both, R., Raupach, M. J., Schmidt, S., & Pylatiuk, C. (2024). Speed it up! Recognition of parasitoid wasps using a neuronal network. *Invertebrate Systematics, 38*, IS24011. https://doi.org/10.1071/IS24011

Wolz, M., Rabl, D., Höcherl, A., Hübner, J., Tschorsnig, H.-P., Whitmore, D., Leroy, B., Weisser, W., Mitesser, O., Zakharov, E. V., Hebert, P. D. N., Liebhold, A. M., & Müller, J. (in review). Response of parasitoid communities to insecticide application during a *Lymantria dispar* outbreak in mixed oak forests. *Journal of Applied Ecology, 61*(11), 2774-2785.

The following publications were written within the scope of GBOL III but do not meet the scope of this dissertation on Diapriidae and will therefore not be discussed in detail: Andersen et al., 2023; Baranov et al., 2024; Krueger et al., 2022 and Wolz et al., 2024. The publication by Hübner et al. on a record of gynandromorphism is not discussed further in this paper due to its review status.

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FIGURE 1. Sexual dimorphism. **A** *Diapria cava* Notton, 1993 male specimen **B** the yet undescribed female **C** *Spilomicrus antennatus* (Jurine, 1807) wingless female **D** *S. simplex syn. n.*, established as the unknown male of *S. antennatus* in Hübner & Chemyreva 2024. Scale bar: 0.5mm.

FIGURE 2. Comparison of **A** the host *Solenopsis fugax* and **B** its parasitoid *Lepidopria pedestris*. Body features like the shape of the female antenna, the small eye diameter, the modified petiolus and the body hair are highlighted with red arrows. The image **A** is modified from https://anthouse.es/

FIGURE 3. Diaprioidea diversity profiles based on Chao1. **A** Belytinae **B** Diapriinae **C** Ismaridae. The empirical (BIN counts; dotted blue) and estimated (Chao1; red) diversity profiles are quantified by Hill numbers for values of the diversity order (q) from 0–3 with 95% confidence intervals (shaded areas based on bootstrap analysis of 100 permutations). Species richness is depicted by q = 0; Shannon diversity by q = 1; and Simpson diversity by q = 2.

FIGURE 4. Different diversity estimation plots based on iNext (Hsieh et al., 2016). **A**, **B** Accumulation curve of OTU diversity **C**, **D** OTU diversity based on sample coverage **E**, **F** Sample coverage based on abundance. Yellow: Belytinae, blue: Diapriinae, red: Ismaridae.

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TABLE 1. Summary of the species estimates and diversity findings.

TABLE 2. Excerpt of the most important results of the new German Checklist: COMB. N. = combinatio nova, SP. N.= species nova, FR= first record.

DECLARATION OF CONTRIBUTIONS AS CO-AUTHOR

1. CHAPTER: Taxonomy

1.1 Hübner, J., Chemyreva, V. G. and David Notton. 2023. “Taxonomic and Nomenclatural Notes on *Geodiapria longiceps* Kieffer, 1911 (Hymenoptera, Diapriidae) and Synonymy of the Genus *Geodiapria* Kieffer, 1910” *ZooKeys* 1183: 1–11: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing the manuscript, critically revision of the manuscript and approval of the final version.

1.2 Hübner, J., and Vasilisa Chemyreva. 2024. “Review of German *Spilomicrus* Westwood (Hymenoptera, Diapriidae, Spilomicrini)” *Biodiversity Data Journal* 12: e114515: Data curation, formal analysis, investigation, methodology, visualization, writing the manuscript, critically revision of the manuscript and approval of the final version.

1.3 Hübner, J., Chemyreva, V., Macek, J., and Viktor Kolyada. 2024. “A review of the genus *Zygota* (Hymenoptera: Diapriidae) in Germany" *ZooKeys* 1207: 325-353: Data curation, formal analysis, investigation, methodology, visualization, writing the manuscript, critical revision of the manuscript and approval of the final version.

2. CHAPTER: Innovative approaches

2.1 Chimeno, C., Hübner, J., Seifert, L., Moriniere, J., Bozicevic, V., Hausmann, A., Schmidt, S., and Jörg Müller. 2023. “Depicting Environmental Gradients from Malaise Trap Samples: Is Ethanol-based DNA Metabarcoding Enough?” *Insect Conservation and Diversity* 16(1): 47–64. https://resjournals.onlinelibrary.wiley.com/doi/10.1111/icad.12609 (October 25, 2023): Formal analysis, methodology (supporting), writing the manuscript; writing, critical revision of the manuscript.

2.2 Shirali, H., Hübner, J., Both, R., Raupach, M., Schmidt, S., and Christian Pylatiuk. 2024. “Speed it up! Recognition of parasitoid wasps using a neuronal network” *Invertebrate Systematics* 38, IS24011. https://doi.org/10.1071/IS24011: Preprint available: https://doi.org/10.1101/2024.01.01.573817: Investigation, writing the methods section of the manuscript, critical revision of the manuscript and approval of the final version.

3. CHAPTER: Biodiversity assessment and species records

3.1 Chimeno, C., Hausmann, A., Schmidt, S., Raupach, M., Doczkal, D., Baranov, V., Hübner, J., Höcherl, A., Albrecht, R., Jaschof, M., Haszprunar, G., and Paul D. N. Hebert. 2022. "Peering into the Darkness: DNA Barcoding Reveals Surprisingly High Diversity of Unknown Species of Diptera (Insecta) in Germany." *Insects* 13(1): 82. https://www.mdpi.com/2075-4450/13/1/82 (October 25, 2023). Drafting the work or revising it critically for important intellectual content, critical revision of the manuscript and approval of the final version.

3.2 Hübner, J., Gabel, H., Deines, V., Kreiling, A. K. and David Notton. 2024. "Review of Diapriidae (Hymenoptera) of the Faroe Islands" *Spixiana*. *47* (1): 83-92: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization, writing the manuscript, critical revision of the manuscript and approval of the final version.

3.3 Hübner J., and Caroline Chimeno. 2024. "Species Estimates for Germany" (unpublished material, not in manuscript form, not planned for publication outside this thesis): Conceptualization, data curation, investigation, project administration, supervision, visualization.

3.4 Hübner J. 2024. "A new German Diaprioidea Checklist" (unpublished material, not in manuscript form, not planned for publication outside this thesis): Conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization, writing the manuscript.

SUMMARY

ABSTRACT (English)

Background

Among insects, Hymenoptera (primarily bees, wasps and ants) is probably the most speciose and thus successful animal taxon worldwide. Their biodiversity and specimen numbers are unsurpassed, which is one of the reasons why insects play a prominent role (mainly, but not only) in terrestrial ecosystems. Pollination, pest control and food source are just a few examples of the ecosystem services these arthropods provide to the planet. However, most animal species on earth are insects, it is estimated that up to 80 % of hymenopteran species diversity is still unknown to science. At the same time, extinction rates of all taxonomic kingdoms are at their highest due to human impact on the planet. The increasing disappearance of species makes it all the more urgent for taxonomists to describe unknown species more quickly. This endeavor is particularly difficult in remote areas and with very diverse, often small, cryptic taxa, the so-called "Dark Taxa" (DT). With these groups, even identifying the genus is often a significant hurdle for a “non-specialist”. And while the diversity of (not only hymenopteran) insect species is greatest in the tropics, thousands of species are probably still undescribed even in a supposedly well-researched western country like Germany.

The German Barcode of Life (GBOL) project aims to catalog as many animal species as possible in Germany by obtaining the sequence of the mitochondrial CO1 gene, which is used as a barcode (the so-called Barcode Index Number (BIN)) or proxy to genetically distinguish between different taxa. The third phase of the GBOL project, the GBOL III: Dark Taxa project, was launched to tackle multiple Dark Taxa, assess their diversity, and test and apply new integrative taxonomic approaches to achieve an efficient increase in knowledge. The parasitoid wasp families Diapriidae and Ismaridae are the subject of this work. This species-rich but highly understudied group is treated with an integrative taxonomic approach.

Results

Because the phenotype rather than the genotype interacts with other species and the environment, the first chapter of this dissertation deals with the “traditional” work of a taxonomist: revisions and species descriptions. On the base of roughly 10 000 DNA-barcoded

specimens, a few genera were picked for further evaluation. Section 1.1 evaluated a rare and questionable diapriid species. The genus *Geodiapria* Kieffer, 1911 was described by monotypy as *Geodiapria longiceps* Kieffer, 1911. What was already suspected in the past could be proven through DNA barcoding, phylogenetics and morphology: *Geodiapria* is a junior synonym of *Basalys* Westwood, 1833 and the species is now valid as *Basalys rufocinctus* (Kieffer, 1909). In addition, *Loxotropa longiceps* Wasmann, 1909, syn. nov., and *L. rufosignata* Kieffer, 1911, syn. nov. could also be established as synonyms. The latter species was recorded for the first time for Corsica, Germany, Norway and Sweden (Hübner et al., 2023).

Section 1.2 aimed at a much more speciose diapriid genus, *Spilomicrus* Westwood, 1832. Prior to this review, twelve valid species were recorded for Germany in the latest diapriid checklist. Applying the sample procedure mentioned above, major contributions to the *Spilomicrus* systematics were archived: *Spilomicrus simplex* Tomsik, 1947 (which was only described as a macropterous male) was placed in synonymy with *S. antennatus* Jurine, 1807 (which was only known from the brachypterous female). On the other hand, *S. thomsoni* Kieffer, 1911 was removed from synonymy with *S. hemipterus* Marshall, 1868 and confirmed as a valid species. *S. thomsoni* could be recorded for Germany together with *S. crassiclavis* Marshall, 1868, *S. lusitanicus* Kieffer, 1910 and *S. diversus* Chemyreva, 2021 for the very first time. In addition, three new species were described: *S. brevimalaris* sp. nov., *S. flavecorpus* sp. nov. and *S. politus* sp. nov.. 23 barcodes and an updated taxonomic key were provided to improve the capability of easily identifying *Spilomicrus* species genetically and morphologically (Hübner & Chemyreva, 2024).

The last section of this chapter, section 1.3 was dedicated to the genus *Zygota* Förster, 1856. While the two genera *Zygota* and *Pantoclis* Förster, 1856 previously were hard to distinguish, new morphological characters could be established to interpret each genus confidently. As a consequence the following new combinations were introduced for no less than 13 species: *Pantoclis brevinervis* (Kieffer, 1909) comb. n., *P. brevipennis* (Kieffer, 1908) comb. nov., *P. caecutiens* (Kieffer, 1908) comb. n., *P. cursor* (Kieffer, 1908) comb. nov., *P. fossulata* (Thomson, 1858) comb. nov., *P. fuscata* (Thomson, 1858) comb. nov., *P. hemiptera* (Thomson, 1858) comb. nov., *P. microtoma* (Kieffer, 1909) comb. nov., *P. soluta* (Kieffer, 1907) comb. nov., *P. striata* (Kieffer, 1909) comb. nov., *P. subaptera* (Thomson, 1858) comb. nov., *P. sulciventris* (Kieffer, 1909) comb. nov. and *P. unicolor* (Kieffer, 1908) comb. nov.. *Zygota walli* sp. nov. was described as new to science and *Zygota balteata* Macek, 1997, *Z.*

comitans Macek, 1997, *Z. spinosipes* (Kieffer, 1908), *Z. sordida* Macek, 1997, *Z. angularis* Macek, 1997 and *Z. vigil* Nixon, 1957 were recorded for the first time in Germany. *Zygota caligula* Buhl, 1997 is placed in synonymy with *Z. congener* (Zetterstedt, 1840) (Hübner, Chemyreva, et al., 2024).

The second chapter provides an insight into broader approaches and the implementation of innovative technologies. Section 2.1 discusses the usage of preserving ethanol as DNA sources instead of the actual insect bulk material. Various studies (e.g. Erdozain et al. 2019; Marquina et al. 2019) have shown that the preservative fluid can be used as a non-destructive alternative to classic destructive metabarcoding of the insect bulk material itself. Unfortunately, both DNA sources, the bulk material and the ethanol, produce significantly different sequencing results: the fluid tends to contain proportionally more DNA of small, soft-bodied insects while the bigger, more robust insects are significantly better represented in the bulk material. It is therefore advisable to use both methods at the same time. It could be shown through our study that ecological information, the seasonality of flying insects could be conserved to a certain degree, but also, that this data has to be interpreted carefully (Chimeno, Hübner, et al., 2023).

Another non-destructive, straight forward, but less fine scaled method to identify insects is presented in section 2.2. The GBOL dataset for diapriids was used for an innovative artificial intelligence (AI) approach. Recently, Wührl et al. (2022) presented a machine that was equipped with a pipetting robot and an imaging unit powered by an AI. This device was able to identify, photograph and sort small (up to 5 mm) insects down to family level. As it is true for many Dark Taxa, there might be taxa with high individual counts, or different taxa that look very much alike. A convolutional neural network (CNN) was trained on images of 11 genera of Diapriinae (male and female), to enable it to distinguish specimens down to genus level. The dataset was separated into images for training, validation and testing and also took control groups (“other hymenopterans”, “non-hymenoptera”) into account. The AI ended up identifying a specimen’s genus with a 96% success rate, depending on the amount of material available for the training of the CNN (Shirali et al., 2024).

The last chapter contains several manuscripts (one unpublished), dealing with biodiversity assessment. Section 3.1 is a manuscript on the diapriid fauna of the Faroe Islands. The diapriids of the islands were historically evaluated twice by Kryger & Schmiedeknecht (1938). Kryger’s material and some freshly caught specimens were reidentified. It turned out that most of the prior identifications were either not valid anymore or fell simply short. Only

two species were correctly determined by those authors highlighting the importance of accessibility and reevaluating of historic collections. The new checklist for Faroe Islands records: *Basalys abruptus* (Thomson, 1859) (first record), *Basalys longipennis* (Kieffer, 1911) (first record), *Trichopria aptera* (Ruthe, 1859), *Zygota parallela* (Thomson, 1858) (first genus record), *Pantoclis similis* (Thomson, 1858) (first record), *Pantoclis trisulcata* Kieffer, 1907, *Synacra atracta* Macek, 1995 (first genus record), *Miota exsecta* Wall, 1998 (first record), *Aclista alticollis* (Thomson, 1858) (first genus record) and *Aclista* cf. *insolita* Nixon, 1957 (first record) (Hübner, Gabel, et al., 2024).

When facing not only a small insect community with few species but hyper-diverse Dark Taxa, it is important to have at least some extrapolated species estimates to assess the potential number of unknown diversity. Section 3.2 extrapolates species numbers of dipteran Dark Taxa based on DNA barcodes for Bavaria and Germany. Those were obtained using Chao1 species ratios. Here, Dark Taxa (Cecidomyiidae, Phoridae, Sciaridae, Chironomidae) proved themselves to contain in average way higher rates of hidden diversity than "common", less diverse and better investigated diptera families (Asilidae, Calliphoridae, Drosophilidae, Ephydridae, Muscidae, Sarcophagidae, Stratiomyidae, Syrphidae, Tabanidae, Tachinidae and Tipulidae). In total, 1800-2200 dipteran species are still unknown to science for Germany alone, according to our data (Chimeno et al., 2022).

Section 3.3 used the same approach, Chao1, to estimate the diapriid diversity of Germany. This unpublished material appraises the diversity for Ismaridae and the two subfamilies Diapriinae and Belytinae separately. The basis for the analyses are Operational Taxonomic Units (OTUs) obtained from the diapriid dataset. Ismaridae are estimated to be represented by nine species in Germany, which is accurate. For Diapriinae, 233 OTUs were observed in Bavaria alone, making it an estimated German Diapriinae number of 391. The more diverse, but less sampled Belytinae were represented in the dataset by 262 OTUs (Bavaria) and their actual species number is calculated to be up to 561 for Germany.

Lastly, section 3.4 represents the yet unpublished updated checklist of Diaprioidea of Germany. The latest checklist up to now was published by Blank (2001) and listed 289 species, of which 20 species have turned out to be invalid in the meantime. However, Blank's study was based mainly on literature and the collection from Hubert Hilpert, a diapriid taxonomic specialist. The new checklist for Germany consists of Blank's records, new records and newly described species for the country since 2001, and history records that had been overlooked. In addition, German records from the online source Fauna Europaea were

used as well. In total, 363 species were recorded nationwide. In addition to that, 189 BINs were recorded which were only identified down to genus level.

Conclusion

This dissertation shows how integrative taxonomy can be used to tackle highly diverse, cryptic insects using the worldwide distributed hymenopteran families Diapriidae and Ismaridae as an example. It demonstrates how innovative approaches can help to accelerate the species handling and identification process. Within this work five new synonyms, fourteen new combinations, thirtyfour new national records (twentyseven only for Germany, seven for Faroe Islands) and four new species were described. Three insect genera, *Geodiapria*, *Spilomicrus* and *Zygota* got a revision for Germany.

In addition to the published material, new species estimates for Germany were calculated via Chao1: based on roughly 8800 successfully barcoded specimens (mostly from Bavaria and the subfamily Diapriinae) there might be up to 966 diapriid species nationwide what is roughly three times as much as the previous record showed.

Lastly, a new diapriid checklist is provided for Germany, including all available DNA-barcodes. Based on the obtained data within the project, current and historic literature and online sources, 363 species could be recorded. In addition, 189 BINs are provided, whose voucher material could only be identified down to species level. There is still a lot of work to record and describe the several hundreds of other German species of Diapriidae.

In summary, the integrative taxonomic approach has made it possible to make significant contributions to the taxonomic study of diapriids within a limited period of time. Nevertheless, most diapriid species have not yet been discovered worldwide, and this taxon, like many other insect families, is still in need of further in-depth research.

ABSTRACT (German)

Hintergrund

Unter den Insekten gehören die Hymenoptera (unter anderem Bienen, Wespen und Ameisen) zu den evolutionär erfolgreichsten Tierordnungen der Welt. Ihre Arten- und Individuenzahlen sind unübertroffen, was einer der Gründe ist, warum Insekten allgemein (hauptsächlich, aber nicht nur) in terrestrischen Ökosystemen eine herausragende Rolle

spielen. Bestäubung, Schädlingsbekämpfung und Nahrungsquelle sind nur einige Beispiele für die Ökosystemleistungen, die diese Arthropoden für unseren Planeten erbringen. Obwohl die meisten Tierarten auf der Erde Insekten sind, ist schätzungsweise bis zu 80 % der Hymenoptera-Artenvielfalt der Wissenschaft noch unbekannt. Gleichzeitig waren jedoch die Aussterberaten in allen Reichen der Lebewesen dieses Planeten nie höher aufgrund des menschlichen Einflusses. Das immer schnellere Verschwinden von Arten macht es für Taxonomen umso dringlicher, unbekannte Arten schneller zu beschreiben. Besonders schwierig ist dieses Unterfangen in abgelegenen Gebieten und für sehr diverse, oft winzig kleine und kryptische Taxa, die sogenannten "Dark Taxa" (DT). Bei diesen Gruppen stellt oft bereits die Identifizierung der Gattung eine signifikante Hürde dar für einen “Nicht-Spezialisten”. Doch obwohl die Vielfalt der (nicht nur hymenopteren) Insektenarten in den Tropen am größten ist, sind selbst in einem vermeintlich gut erforschten westlichen Land wie Deutschland wahrscheinlich noch Tausende von Arten unbeschrieben.

Das Projekt German Barcode of Life (GBOL) zielte darauf ab, so viele Tierarten wie möglich in Deutschland zu katalogisieren, indem das mitochondriale CO1-Gen sequenziert wird. Die Sequenz, die sogenannte Barcode Index Number (BIN), wird einer Art zugeordnet oder kann alternativ selbst als Proxy zur genetischen Unterscheidung verschiedener Taxa verwendet werden. Die dritte Phase von GBOL, das Projekt GBOL III: Dark Taxa, wurde eingeleitet, um mehrere Dark Taxa zu untersuchen, ihre Vielfalt zu evaluieren und neue integrative taxonomische Ansätze zu testen und anzuwenden für einen effizienten Wissenszuwachs. Zu diesem Zweck sind die beiden Familien parasitoider Wespen, Diapriidae und Ismaridae (zusammen Teil der Diaprioidea), Gegenstand dieser Arbeit. Diese artenreiche, aber sehr wenig untersuchte Gruppe wird mit einem integrativen, taxonomischen Ansatz untersucht.

Ergebnisse

Da der Phänotyp und nicht der Genotyp mit anderen Arten und der Umwelt interagiert, befasst sich das erste Kapitel dieser Dissertation mit der "traditionellen" Arbeit eines Taxonomen: Revisionen und Artbeschreibungen. Aus etwa 10.000 genetisch gebarcodeten Individuen wurden einige Gattungen für eine weitere Bewertung ausgewählt. In Abschnitt 1.1 wurde eine sehr seltene und taxonomisch fragliche Diapriidengattung bearbeitet. Die Gattung *Geodiapria* Kieffer, 1911 wurde monotypisch als *Geodiapria longiceps* Kieffer, 1911 beschrieben. Was bereits in der Vergangenheit vermutet wurde,

konnte durch DNA-Barcoding, Phylogenetik und Morphologie bestätigt werden: *Geodiapria* ist ein Junior-Synonym von *Basalys* und die Art ist nun als *Basalys rufocinctus* (Kieffer, 1909) valide. Darüber hinaus konnten auch *Loxotropa longiceps* Wasmann, 1909, syn. nov. und *L. rufosignata* Kieffer, 1911, syn. nov. synonymisiert werden unter dem neuen Namen. Außerdem wurde die Art zum ersten Mal für Korsika, Deutschland, Norwegen und Schweden nachgewiesen (Hübner et al., 2023).

Abschnitt 1.2 befasst sich mit einer viel artenreicheren Diapriidengattung, *Spilomicrus* Westwood, 1832. Vor dieser Revision wurden in der letzten Diapriiden-Checkliste zwölf valide Arten für Deutschland aufgeführt. Der integrative Taxonomie-Ansatz möglichte es, wichtige Beiträge zur Systematik von *Spilomicrus* zu leisten: *Spilomicrus simplex* Tomsik, 1947 (die nur als makropteres Männchen beschrieben wurde) wurde in Synonymie mit *S. antennatus* Jurine, 1807 (von der nur vom brachypteren Weibchen bekannt war) gestellt. Andererseits wurde *S. thomsoni* Kieffer, 1911 aus der Synonymie mit *S. hemipterus* Marshall, 1868 entfernt und konnte zusammen mit *S. crassiclavis* Marshall, 1868, *S. lusitanicus* Kieffer, 1910 und *S. diversus* Chemyreva, 2021 erstmals für Deutschland nachgewiesen werden. Darüber hinaus wurden drei neue Arten beschrieben: *S. brevimalaris* sp. nov., *S. flavecorpu*s sp. nov. und *S. politus* sp. nov. Insgesamt 23 DNA-Barcodes und ein aktualisierter dichotomer Schlüssel wurden publiziert, um die genetische und morphologische Bestimmung von *Spilomicrus*-Arten zu erleichtern (Hübner & Chemyreva, 2024).

Der letzte Abschnitt dieses Kapitels, Abschnitt 1.3, war der Gattung *Zygota* Förster, 1856 gewidmet. Während die beiden Gattungen *Zygota* und *Pantoclis* Förster, 1856 früher schwer zu unterscheiden waren, konnten neue morphologische Merkmale ermittelt werden, die eine sichere Interpretation der beiden Gattungen ermöglichen. Infolgedessen wurden nicht weniger als 13 neue Kombinationen etabliert: *Pantoclis brevinervi*s (Kieffer, 1909) comb. n.; *P. brevipennis* (Kieffer, 1908) comb. nov.; *P. caecutiens* (Kieffer, 1908) comb. n.; *P. cursor* (Kieffer, 1908) comb. nov.; *P. fossulata* (Thomson, 1858) comb. nov.; *P. fuscata* (Thomson, 1858) comb. nov.; *P. hemiptera* (Thomson, 1858) comb. nov.; *P. microtoma* (Kieffer, 1909) comb. nov.; *P. soluta* (Kieffer, 1907) comb. nov.; *P. striata* (Kieffer, 1909) comb. nov.; *P. subaptera* (Thomson, 1858) comb. nov.; *P. sulciventris* (Kieffer, 1909) comb. nov. und *P. unicolor* (Kieffer, 1908) comb. nov.. *Zygota walli* sp. nov. wurde als neu für die Wissenschaft beschrieben. *Zygota balteata* Macek, 1997, *Z. comitans* Macek, 1997, *Z. spinosipes* (Kieffer, 1908), *Z. sordida* Macek, 1997, *Z. angularis* Macek, 1997 und *Z. vigil* Nixon, 1957 wurden

zum ersten Mal in Deutschland nachgewiesen. *Zygota caligula* Buhl, 1997 wird in Synonymie mit *Z. congener* (Zetterstedt, 1840) gestellt (Hübner, Chemyreva, et al., 2024).

Das zweite Kapitel soll einen Einblick in umfassendere Ansätze und die Umsetzung innovativer Technologien geben. Abschnitt 2.1 befasst sich mit der Verwendung von Ethanol als DNA-Quelle, anstatt die Biomasse der Insekten-Sammelprobe selbst zu verwenden. Verschiedene Studien (z. B. Erdozain et al. 2019; Marquina et al. 2019) haben gezeigt, dass Ethanol als nicht-destruktive Alternative zum klassischen (destruktiven) Metabarcoding des Insektenmaterials selbst prinzipiell verwendet werden kann. Leider führen beide DNA-Quellen, das Gewebe und der Ethanol, zu signifikant unterschiedlichen Sequenzierungsergebnissen: die Flüssigkeit enthält tendenziell proportional mehr DNA von kleinen, weichen Insekten, während die größeren, robusteren Insekten im Bulk-Material weitaus besser vertreten sind. Daher ist es empfehlenswert, beide Methoden gleichzeitig zu nutzen. Nichts desto trotz konnte gezeigt werden, dass ökologische Informationen und die Saisonabhängigkeit von Fluginsekten bis zu einem gewissen Grad erhalten bleiben, aber eben auch, dass diese Daten vorsichtig interpretiert werden müssen (Chimeno, Hübner, et al., 2023).

Eine weitere zerstörungsfreie, weniger aufwendige, aber auch weniger fein abgestufte Methode zur Identifizierung von Insekten wird in Abschnitt 2.2 vorgestellt. Der GBOL-Datensatz für Diapriidae wurde für einen innovativen Einsatz künstlicher Intelligenz (KI) verwendet. Wührl et al. (2022) stellten kürzlich ein Gerät vor, das mit einem Pipettierroboter und einer von einer KI betriebenen bildgebenden Einheit ausgestattet war. Dieses Gerät war in der Lage, kleine (bis zu 5 mm) Insekten bis auf Familien-Niveau zu identifizieren, zu fotografieren und zu sortieren. Dark Taxa zeichnen sich oft durch hohe Individuenzahl oder hohe kryptische Diversität aus. Um Individuen zumindest bis auf Gattungsebene zu unterscheiden, wurde ein neuronales Netzwerk (CNN) mit Bildern von elf Gattungen der Diapriinae (Männchen und Weibchen) trainiert. Der Datensatz an Bildern wurde dreigeteilt für das Training, die Validierung und den Test. Zusätzlich wurde gegen eine Kontrollgruppe (“andere Hymenoptera”, “Nicht-Hymenoptera”) getestet. Die KI identifizierte die Gattung der Tiere mit einer Erfolgsquote von bis zu 96 %, abhängig von der Menge des für das Training des CNN verfügbaren Materials (Shirali et al., 2024).

Das letzte Kapitel enthält mehrere Manuskripte (eines davon unveröffentlicht), die sich mit der Bewertung der biologischen Vielfalt befassen. Abschnitt 3.1 ist ein Manuskript über die Diapriidenfauna der Färöer Inseln. Die Diapriidae der Inseln wurden in der Vergangenheit

zweimal von Kryger und Schmiedeknecht (1938) und Petersen (1956) untersucht. Das Material von Kryger und einige frisch gefangene Exemplare wurden neu identifiziert. Es stellte sich heraus, dass die meisten der historischen Bestimmungen entweder nicht mehr gültig oder einfach unzureichend waren. Nur zwei Arten wurden von den Autoren korrekt bestimmt, was die Bedeutung der Zugänglichkeit und Aufarbeitung historischer Aufsammlungen unterstreicht. Die neue Checkliste für die Färöer-Inseln enthält: *Basalys abruptus* (Thomson, 1859) (Erstnachweis), *Basalys longipennis* (Kieffer, 1911) (Erstnachweis), *Trichopria aptera* (Ruthe, 1859), *Zygota parallela* (Thomson, 1858) (Erstnachweis), *Pantoclis similis* (Thomson, 1858) (Erstnachweis), *Pantoclis trisulcata* Kieffer, 1907, *Synacra atracta* Macek, 1995 (Erstnachweis der Gattung), *Miota exsecta* Wall, 1998 (Erstnachweis), *Aclista alticollis* (Thomson, 1858) (Erstnachweis der Gattung) und *Aclista* cf. *insolita* Nixon, 1957 (Erstnachweis) (Hübner, Gabel, et al., 2024).

Wenn man es nicht nur mit einer kleinen Insekten Gemeinschaft mit wenigen Arten zu tun hat, sondern mit hyperdiversen Dark Taxa, ist es wichtig, zumindest einige extrapolierte Artenschätzungen zu haben, um die potenzielle Anzahl der unbekannten Vielfalt zu beurteilen. In Abschnitt 3.2 werden die Artenzahlen der Dipteren Dark Taxa auf der Grundlage von DNA-Barcodes für Bayern und Deutschland extrapoliert. Diese wurden anhand von Chao1 ermittelt. Dabei zeigte sich, dass die bearbeiteten Dark Taxa (Cecidomyiidae, Phoridae, Sciaridae, Chironomidae) im Durchschnitt weitaus höhere Raten an kryptischer oder unbekannter Diversität aufweisen als "gewöhnliche", weniger vielfältige und besser untersuchte Dipterenfamilien (Asilidae, Calliphoridae, Drosophilidae, Ephydridae, Muscidae, Sarcophagidae, Stratiomyidae, Syrphidae, Tabanidae, Tachinidae und Tipulidae). Unseren Schätzungen zufolge sind insgesamt allein in Deutschland noch 1800-2200 Dipterenarten der Wissenschaft unbekannt (Chimeno et al., 2022).

In Abschnitt 3.3 wurde derselbe Ansatz, Chao1, verwendet, um die Diapriidenvielfalt in Deutschland abzuschätzen. In diesem unveröffentlichten Material wird die Diversität für Ismaridae und die beiden Unterfamilien Diapriinae und Belytinae separat voneinander evaluiert. Die Grundlage für die Analysen bilden Operational Taxonomic Units (OTUs) aus dem Diapriidendatensatz. Es wird geschätzt, dass die Ismaridae in Deutschland mit neun Arten vertreten sind, was den empirischen Daten entspricht. Für Diapriinae wurden in Bayern 233 OTUs gefunden, Schätzungen zufolge könnten es bis zu 391 Arten in Deutschland sein. Die deutlich diverseren, aber weniger beprobten Belytinae waren im Datensatz mit 262 OTUs (Bayern) vertreten. Ihre tatsächliche Artenzahl wird auf bis zu 561 (Deutschland) geschätzt.

Der Abschnitt 3.4 schließlich stellt die noch unveröffentlichte aktualisierte Checkliste der Diaprioidea Deutschlands dar. Die bisher letzte Checkliste wurde von Blank (2001) veröffentlicht und führte 289 Arten auf, von denen sich 20 Arten inzwischen als ungültig erwiesen haben. Blanks Studie basierte jedoch hauptsächlich auf Literatur und der Sammlung von Hubert Hilpert, einem Taxonomen und Diapriiden-Spezialisten. Die neue Checkliste für Deutschland besteht aus Blanks Nachweisen, neuen Nachweisen und neu beschriebenen Arten für das Land seit 2001, sowie historischen Nachweisen, die übersehen worden waren. Zusätzlich wurden die Nachweise von der Fauna Europaea integriert. Insgesamt wurden bundesweit 363 Arten erfasst. Hinzu kommen 189 BINs, die nur bis auf Gattungsebene bestimmt wurden.

Schlussfolgerungen

In dieser Dissertation wird am Beispiel der weltweit verbreiteten Hautflügler-Familien Diapriidae und Ismaridae gezeigt, wie die integrative Taxonomie zur Erforschung hyper-diverser, kryptischer Insekten eingesetzt werden kann. Es wird aufgezeigt, wie innovative Ansätze dazu beitragen können, den Umgang mit dem Material und den Identifizierungsprozess zu beschleunigen. Im Rahmen dieser Arbeit wurden fünf neue Synonyme, dreizehn neue Kombinationen, vierunddreißig neue nationale Nachweise (siebenundzwanzig nur für Deutschland, sieben für die Färöer Inseln) und vier neue Arten beschrieben. Drei Insektengattungen, *Geodiapria*, *Spilomicrus* und *Zygota*, wurden für Deutschland überarbeitet.

Zusätzlich zum publizierten Material wurden mit Hilfe von Chao1 neue Artabschätzungen für Deutschland berechnet: Auf der Grundlage von etwa 8800 erfolgreich gebarcodeten Specimens (hauptsächlich aus Bayern und der Unterfamilie Diapriinae) könnte es bundesweit bis zu 966 Diapriidenarten geben, was etwa dreimal so viel ist, wie die bisherige Erfassung ergab.

Abschließend wird eine neue Diapriiden-Checkliste für Deutschland vorgelegt, die auch alle verfügbaren DNA-Barcodes enthält. Basierend auf den im Rahmen des Projekts gewonnenen Daten, aktueller und historischer Literatur sowie Online-Quellen konnten 363 Arten erfasst werden. Zusätzlich werden 189 BINs angegeben, deren Belegmaterial nur bis auf Genusniveau bestimmt werden konnte. Es gibt noch viel Arbeit, um die mehreren hundert weiteren Diapriidae-Arten in Deutschland zu erfassen und zu beschreiben.

Zusammenfassend konnte gezeigt werden, dass es der integrative taxonomische Ansatz ermöglicht hat, innerhalb eines begrenzten Zeitraums bedeutende Beiträge zur taxonomischen Untersuchung der Diapriidae zu leisten. Dennoch sind die meisten Diapriidenarten weltweit noch unbeschrieben und daher bedarf dieses Taxon, wie viele andere Insektenfamilien auch, noch weiterer eingehender Forschung.

INTRODUCTION

Insects in general

Insects make up 75% of all animal species on the planet (Leandro & Jay-Robert, 2019), and are therefore one of the most important taxa worldwide. In addition to their enormous diversity and high numbers of individuals - which adds up to 10 quintillion at any point in time according to May (1988), equaling to about 1.4 billion specimens per human - insects play key roles in various ecosystems, fields of research, and in the food industry. They fulfill various ecosystem services that humanity depends on such as pollination, food source or pest control. In addition, they provide many animal-based products such as beeswax and silk.
Insects have inhabited the planet since the Ordovician, dating back 485–444 million years (Misof et al., 2014) when terrestrial plants appeared on Earth, and have conquered all types of habitats, such as land, the air, and even limnic and marine bodies of water. Even Antarctica is occupied by one dipteran species, *Belgica antarctica* Jacobs, 1900 (Chironomidae).

Stork (2018) estimates that about 80 % of all insect species are yet to be discovered. In general, most of this hidden diversity on the planet can be found in the tropics. Yet, to find undescribed species, one does not have to travel to the tropics to be successful. Several studies (e.g. Morinière et al., 2019, Hausmann et al., 2013, Chimeno et al. 2022, 2023) show that there might be several thousand species of insects in Germany alone, even though this country's entomofauna is supposed to be well investigated and has a long history of entomological research.
But while the majority of insect species are still unknown to science, global diversity is rapidly shrinking. Extinction rates have never been higher in the recent past (Cafaro, 2015; De Vos et al., 2015; Pimm et al., 2014): the current pace at which species die out today surpasses the extinction rate at the end of the Cretaceous Period, 65 million years ago (Raven et al., 2011). And since this historical event is referred to as the 5th mass extinction, it is more than justified to coin the recent development in the planet's diversity as the 6th mass extinction. Human induced climate change, environmental pollution, usage of pesticides and exploitation of resources lead to enormous reduction in insect biomass (Hallmann et al., 2017) and drive the species' diversity into a major crisis. The work of Hallmann et al. (2017) raised wide attention and awareness among the general public and has even led to at least

some conservational efforts and the funding of insect related projects such as the GBOL project (Hausmann et al., 2020).

But since there are still considerably high rates of hidden diversity, even the best conservation measures are limited in their effect. The International Union for the Conservation of Nature (IUCN) lists e.g. only 679 Hymenoptera worldwide, of which 498 species live in the Palearctic. From the planet's "biodiversity hotspot" on the other hand, the Neotropics, there are only 69 records listed. No conservation status is known for 334 species (so half of all Hymenoptera on the Red List) due to deficient data (*IUCN*, 2023). The majority of Hymenoptera, hundreds of thousands of species, are not even considered here. And consequential, unknown species, their biology and potentially key interaction with the environment can of course not be considered at all, if not properly investigated.

Taxonomic impediment

Although it might be common sense that there is a significant need to further investigate unknown diversity, the implementation of this much needed research is even nowadays difficult. The so-called "taxonomic impediment" is a term that describes the situation in which taxonomists lack the resources and mainly specialized manpower to advance in their field (Engel et al., 2021; Rodman & Cody, 2003), although there have never been more taxonomists than today (Costello et al., 2013; Joppa et al., 2011). There is a lack in the amount of specialized taxonomists, and the researchers who work on systematics are unevenly spread among different taxa and locations. Less students getting into taxonomy is another aggravating trend (Coleman, 2015). Reasons for that are low job prospects and a general conception that descriptive taxonomy does not require special skills and can be replaced by new approaches such as DNA barcoding (Agnarsson & Kuntner, 2007). It is also problematic that species groups with larger, more colorful and less diverse species (Coleoptera, Lepidoptera, larger Hymenoptera) are apparently studied by far more taxonomists. Unfortunately, the highest diversity of insects (and highest individual counts) can be found among the smallest species. Those groups, such as the dipteran family Cecidomyiidae, are inconspicuous in their appearance, often tiny and highly diverse (Chimeno, Schmidt, et al., 2023). Yet, they get very little taxonomic attention. The other counterproductive relation is the location taxonomists are working at: there are way more scientists with often better resources in well-studied western countries than in the regions of

the world, the “diversity hotspots”, where much more hidden diversity is expected (Agnarsson & Kuntner, 2007).

Dark Taxa: Diapriidae

The above referred to, tiny, inconspicuous and hyper-diverse insect taxa are called “Dark Taxa” (DT) (Hartop et al., 2022). Prior to analysis and species identification, their fragile but characteristic body features and their small size can make even handling and preparation for morphological and genetic analyses difficult. They have an exceptionally high number of undiscovered species (~90 %) and are often found in large numbers in their habitat, such as Cecidomyiidae and Chironomidae (Diptera). Many Dark Taxa are also found among the Hymenoptera, such as some (sub)families of the Braconidae, Chalcidoidea and Proctotrupoidea.

One of those Dark Taxa is the hymenopteran family Diapriidae. Diapriidae Haliday, 1833 is a mircohymenoptera family of insects that consists of tiny (1.5–4.5 mm) parasitoid wasps. Most species show (in part extreme) sexual dimorphism (Fig. 1). Taxonomists differentiate between three subfamilies within the Diapriidae: Diapriinae Haliday, 1833, Belytinae Förster, 1856 and Ambositrinae Masner, 1961. Diapriids and their sister taxon, the Ismaridae (both, together with the non-palaearctic families Monomachidae, Maamingidae, Austroniidae are referred to as Diaprioidae), are a cosmopolitan group with its highest suggested diversity in the tropics (Johnson, 1992). The diapriid subfamily Ambositrinae is limited in its occurence to what is known as the Gondwanan distribution (Australia, New Guinea, New Zealand and South America) (Naumann, 1982). A single diapriid species can be distributed over several continents and biogeographic realms e.g. *Spilomicrus formosus* Jansson, 1942 that is known from Belgium, Czech Republic, Denmark, Finland, Germany, Great Britain, Ireland, Japan, Norway, Russia, Slovakia, Sweden (all Palaearctic), Canada, United States (both Nearctic). About 2000 species are described worldwide so far but species estimates range between 4500 (Johnson, 1992) and up to 50 000 (pers. comm. P. Hebert) potential taxa. And even in a supposingly well-investigated western country like Germany with a long tradition of entomological research, diapriid diversity is vastly understudied.

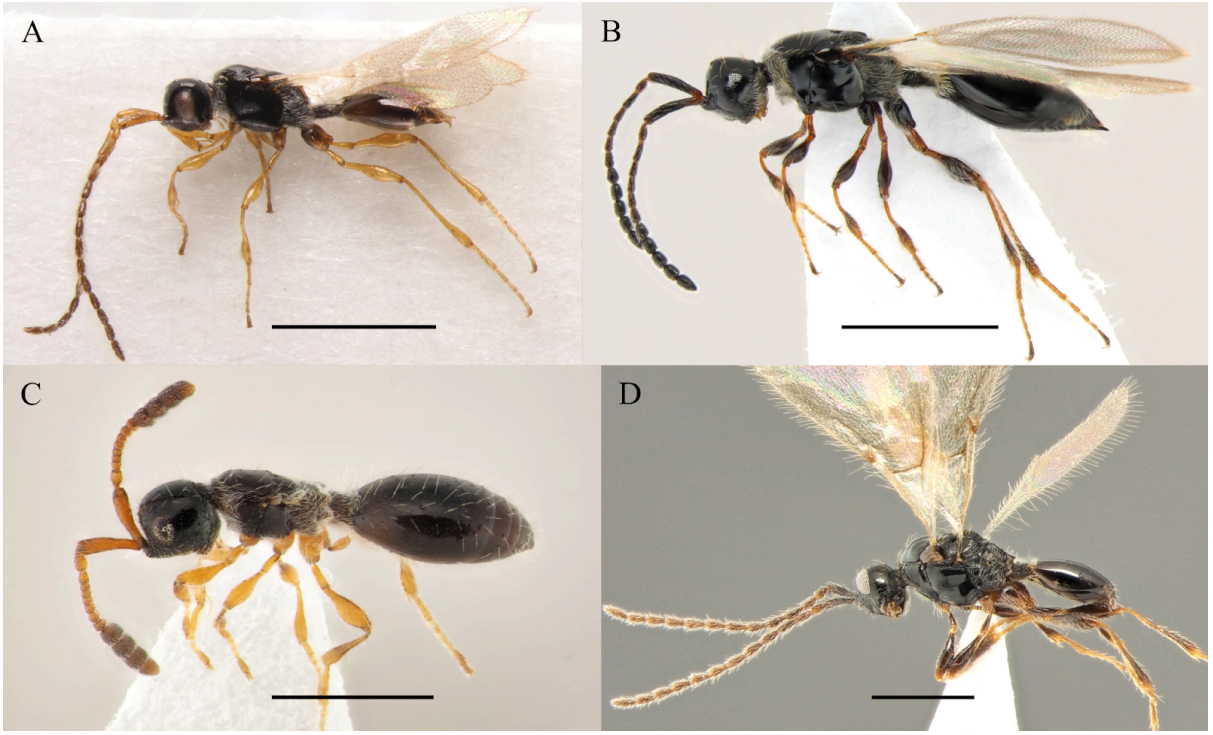

FIGURE 1. Sexual dimorphism. **A** *Diapria cava* Notton, 1993 male specimen **B** the yet undescribed female **C** *Spilomicrus antennatus* (Jurine, 1807) wingless female **D** *S. simplex syn. n.*, established as the unknown male of *S. antennatus* in Hübner and Chemyreva (2024). Scale bar: 0.5mm.

The biology of individual diapriid species is barely known. While Ismaridae are hyperparasitoids of Dryinidae (Hymenoptera), most other Diaprioidea taxa live as solitary or gregarious endoparasitoids of dipteran larvae and pupae (Goulet & Huber, 1993; Hoffmeister, 1989; Yoder, 2007). Other parasitoid lifestyles are also common: *Spilomicrus formosus,* for instance, is known to be a pseudohypoparasitoid of Pipunculidae (Diptera) (Masner, 1991). In general, host records are sparse and often only observable through breeding experiments. Hoffmeister (1989) found that the most profound knowledge of host records were among the subfamily Diapriinae in North America with 22% known relations, while the worst ratio was also recorded in the United States: the hosts of only 0,4% of the Belytinae are established, which was represented by a single species. A few more host records are scattered over the literature: Huggert (1979) for instance contributed several relations, as well as e.g. Notton (1991). Yoder (2007) recorded a summary of several host records. In general, Diapriidae prefer damp, shady and moist habitats which is also reflected by their known hosts: a lot of taxa are known to parasitize on Mycetophilidae (Nematocera) which thrive in those conditions due to the diverse funga. Additional other hosts have been recorded such as Staphylinidae (Coleoptera), other Nematocera, Acalyptratae, Calyptratae, Syrphidae,

Phoridae (all Diptera), and even Formicidae (Hymenoptera) (Hoffmeister, 1989; Notton, 1991). Well adapted to their host's life conditions are several species out of different genera of the Diapriinae subfamily: myrmecophile species such as e.g. *Solenopsia imitatrix* Wasmann, 1899 and *Lepidopria pedestris* Kieffer, 1916 have been investigated by scientists all over the world (Borowiec, 2013; Huggert & Masner, 1983; Lachaud & Passera, 1982; Notton, 1994b; Staverløkk & Ødegaard, 2021; Wasmann, 1909). Most of these species have developed modified body features to mimic the ant's anatomy (Fig. 2). The general body color, reduced eye diameter, body hair, shape of the antenna and shortened wings are common adaptations. Even the proactive shortening of the wings has been observed by Gösswald (1929) to be less likely to get their cover blown while inhabiting the ant nest. *Lepidopria pedestris* even developed a "scale" on its petiolus that resembles a similar structure in its host, *Solenopsis fugax* (Latreille, 1798).

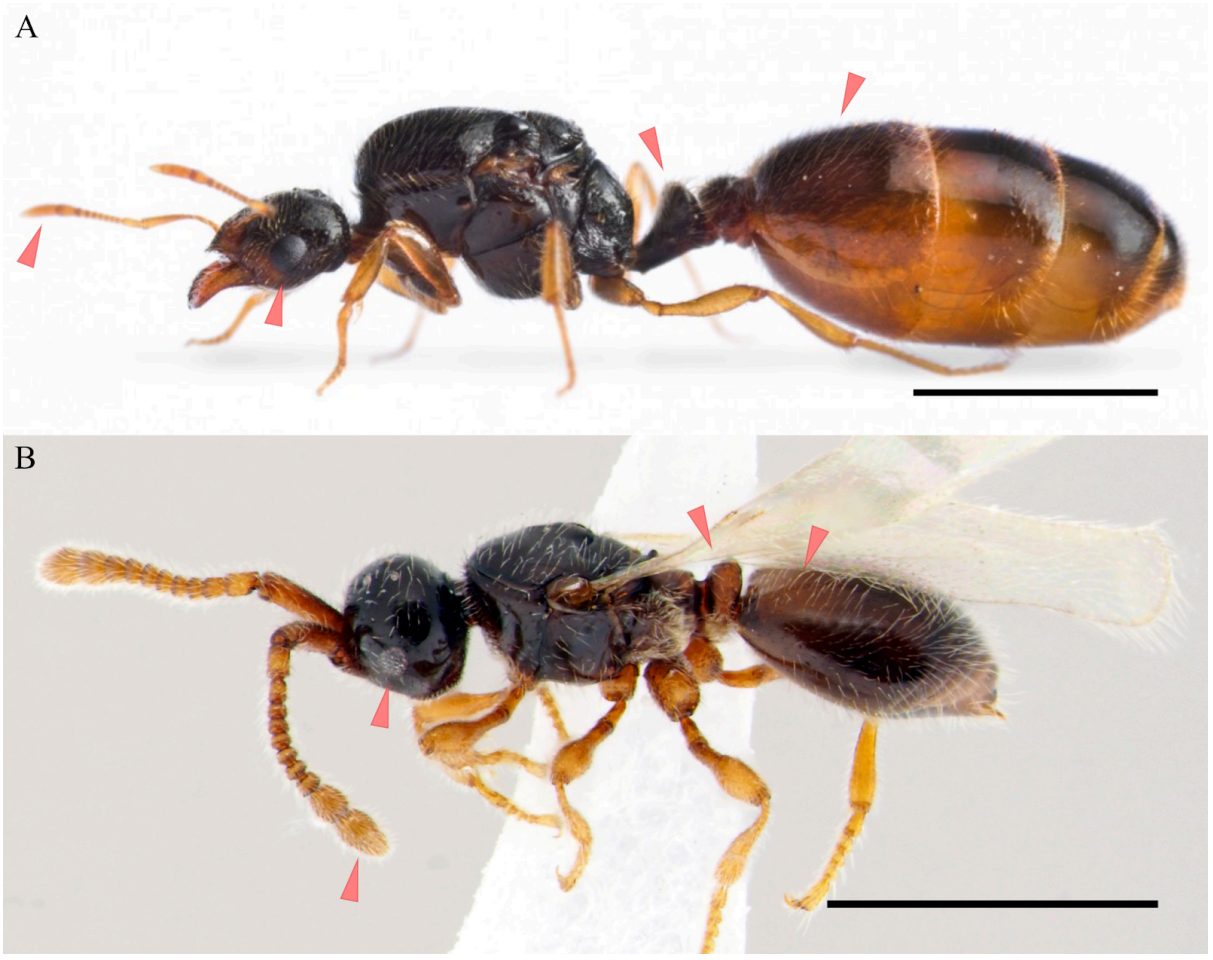

FIGURE 2. Comparison of **A** the host *Solenopsis fugax* and **B** its parasitoid *Lepidopria pedestris*. Body features like the shape of the female antenna, the small eye diameter, the modified petiolus and the body hair are highlighted with red arrows. The image **A** is modified from https://anthouse.es/. Scale bar: 0.5mm.

But even in the rather rare cases where the host-parasitoid relationship is known, most immature stages after hatching from the host pupa or larva are completely unknown to science, with the exception of a few species (Coon, 2000; Hoffmeister, 1989; Kazimírová & Vallo, 1999).

But not only the biology of those parasitoids is challenging, but also their taxonomy and associated literature: diapriid taxonomy of the early days was shaped by authors like Kieffer (e.g. 1911, 1916), Ashmead (1893) and Förster (1856). Due to many circumstances, Kieffer's literature is especially unsatisfactory to work with today: types were not designated and even if so, the information about the remainings are lost. Most of Kieffer's material is supposed to be in the National History Museum in Paris, so the access is quite limited to those specimens. Another challenge taxonomists face when working with Kieffer's literature is the choice of characters he used to establish species or genus boundaries. Those characters have often been proven to be variable and since the author barely took intraspecific variation into account, many species descriptions are too vague. Many homonyms is one consequence taxonomists struggle with today (Macek, 1989b).
Therefore, various authors such as Chemyreva (Chemyreva, 2014, 2015a, 2015b, 2018, 2020, 2021a, 2021b), Chemyreva & Kolyada (Chemyreva & Kolyada, 2013, 2018, 2019a, 2019b, 2021b, 2021a), Kolyada & Chemyreva (2016), Macek (1989b, 1989a, 1990, 1990, 1993, 1995c, 1995b, 1995d, 1995a, 1996, 1997a, 1997b, 1997c, 1998, 2000, 2001, 2005, 2006, 2007), Masner (1959, 1964, 1965, 1974, 1976, 1991), Chemyreva et al. (2021), Masner & Garcia (2002), Nixon (1957, 1980), Notton (1991, 1992, 1993, 1994a, 1994b, 1995, 1999), Szabo (1960, 1961, 1977) and Wall (1963, 1967, 1971, 1980, 1993, 1998, 2000, 2023) took the challenge after all these years and revised many diapriid taxa. Despite those major contributions towards the palaearctic diapriid taxonomy in recent years, a huge chunk of the diversity is still hidden and many taxonomic relations remain questionable. Not only are there many demanding genera such as *Aclista* or *Basalys* that seem to present such a difficulty that it has been avoided by taxonomists for the most part. Even higher level identification is not always intuitive, even for specialists.

Due to huge distribution patterns, ambiguous literature and simply due to their body features (miniscule size, monochromatic body coloration, sexual dimorphism, intraspecific variation), diapriid species are presented in various homonyms and synonyms causing confusion and

make it nearly impossible to non-specialized researchers to come up with a correct identification. Diverse taxa like *Belyta depressa* Thomson, 1858 with 15 or more synonyms are no exception among Diapriidae. On the other hand, there are many, often questionable genera, that were only described by monotypy, such as *Geodiapria* Kieffer, *Solenopsiella* Dodd, *Gymnopria* Loiácono, *Heteropria* Kieffer, *Hexapria* Kieffer, *Viennopria* Jansson or *Labolips* Förster just to name a few. The status of these often rare species is often questionable and are in demand to be looked into. *Viennopria* for instance is probably a junior synonym of *Trichopria* Ashmead.

Diapriid taxonomy can even be unclear on family level. The family Ismaridae Thomson, 1858 was originally described as a tribe, Ismarini, that consists of two genera, *Ismarus* Haliday, 1835 and *Entomius* Herrich-Schäffer, 1840 (the latter was first changed to subgeneric status and then synonymized with *Ismarus*). The tribe gained a century later subfamily status (Ismarinae Hellen, 1964) before Sharkey et al. (2012) elevated the taxon to family status. The latest investigations by Blaimer et al. (2023) using of around 1100 loci of UCEs suggest that Ismaridae (in their analysis represented by one *Ismarus* specimen) should have the the same taxonomic status as the three diapriid subfamilies Ambositrinae, Belytinae and Diapriinae. Blaimer et al. (2023) do not specifically call for an taxonomic adjustment of the aforementioned taxon but it would seem logical since their dataset is the most complete molecular evaluation yet and it would resemble Hellen's (1963) morphological interpretation.

Diapriidae, like many parasitoid wasps, fulfill an important ecological role by controlling pest species. But since only little is known about host relations and the biology of most species, research is still in heavy demand. A few species are of commercial interest due to their capabilities to significantly reduce pest species. *Trichopria drosophilae* (Perkins, 1910) is a species of agricultural interest since it is able to parasitize on the invasive japanese pest *Drosophila suzukii* (Matsumura, 1931). Artificially bred specimens get set free in cherry or plum plantations and are now established in Germany. Other species from the genus of *Synacra* are currently under investigation in experiments to evaluate their potential worth as pest control within the ParaDrosu project at the Insect Technology Center in Berlin, Germany.

Integrative Taxonomy

When facing all challenges simultaneously like the taxonomic impediment, insect biomass loss and the ongoing biodiversity crisis, solely morphology based approaches have proven to be just too ineffective and time consuming for a diversity evaluation.

Taxonomy itself has to be innovated and has to take advantage of the latest advancements in various methodologies.

Instead of spending extensive periods of time trying to sort through similar looking insect material based on morphology only, reversing the process bares several advantages. This reverse taxonomy approach was used within the GBOL project and relied heavily on the DNA-barcoding of the CO1 locus ahead of the morphological evaluation (Hartop et al., 2022). This process made it possible for an inexperienced newcomer in the field of entomology to tackle the diversity of a complex and cryptic taxon of micro-hymenoptera. Since the material is sequenced and barcoded first, the material can be sorted into monophyletic groups effortlessly prior to the identification (Hebert et al., 2003). Even without further taxonomic knowledge, it is possible to distinguish between different genera and identify some species by the comparison with the available online references. Apart from the simple comparison of sequences, platforms like BOLD (https://www.boldsystems.org/) provide further opportunities e.g. retracing the distribution of a target taxon. Another useful application of DNA barcoding is the effortless alignment of sexual dimorphic specimens. Since the differences between male and female can be so striking in diapriids that it is often hard to properly align both sexes of one species (Fig. 1). Sometimes, DNA-barcoding can help rectify species or even genus concepts that might have been challenged or wrongfully interpreted in the past (Hübner et al., 2023).

Relying only on one approach, morphology or genetics, has been proven to be insufficient or just less effective. Limiting the research to morphology is time consuming and relies heavily on the experience and knowledge of the taxonomist. Morphological concepts are often interpreted subjectively and are limited to the physical specimens one has at his disposal. The solely usage of genetics has its own challenges. Hybridisation, endosymbionts, unresolved lineages, contaminations, DNA quality, pseudogenes, intra- and interspecific variation, etc. can all have a severe influence on the significance and validity of sequence data and their interpretation. Different cluster algorithms (e.g. BOLD, ASAP (Puillandre et al., 2021), SpeciesIdentifier (Meier et al., 2006)) group the same records differently and not all pivotal parameters are always comprehensible. Many studies have discussed the validity of BINs or

OTUs as proxy or as decisive character to describe new species (Blagoev et al., 2009; Collins & Cruickshank, 2013; Goldstein & DeSalle, 2011; Klopfstein et al., 2016; Morinière et al., 2019; Packer et al., 2009; Pires & Marinoni, 2010; Sharkey et al., 2012; H. T. Taylor & Harris, 2012) with varying results and opinions.

So therefore, the best method has proven to be an integrative approach taking the advantage of both complementary methodologies. At the same time, taxonomy is not limited to those two exclusively. 3D-imaging (as e.g. used in Van De Kamp et al. (2018)), different (meta-)barcoding variants (section 2.1 and 3.2) or artificial intelligence (AI) (section 2.2), are just a few additional, rapidly evolving approaches that can be put to good use in modern taxonomy. Machine learning is among the most promising of today's research and new applications of AI get established almost on a daily basis.

RESULTS

1. CHAPTER: Taxonomy

In the framework of three taxonomic research papers, significant contributions could be accomplished. Through the usage of integrative taxonomy, new combinations, new species descriptions and new records could be established. Those findings are represented in the following three publications.

Spilomicrus hemipterus
Marshall, 1868

SECTION 1.1: *Geodiapria* review

One of many questionable taxa, the genus *Geodiapria* Kieffer, 1910 (Diapriinae) described by subsequent monotypy represented by *Geodiapria longiceps* Kieffer, 1911, was found and DNA barcoded. The barcoding in combination with the the analysis of a taxonomic tree revealed what has been already suspected by Pschorn-Walcher (1957): *Geodiapria* is a junior synonym of the genus *Basalys* Westwood, 1833. Furthermore, new synonyms could be established: *Loxotropa longiceps* Wasmann, 1909 (nec. *Basalys longiceps* Ashmead, 1893) and *Loxotropa rufosignata* Kieffer, 1911. Since *Basalys longiceps* is preoccupied, the new valid name, designated by first revisor action, is *Basalys rufocintus* (Kieffer, 1911).

Hübner, J., Chemyreva, V. G., & Notton, D. (2023). Taxonomic and nomenclatural notes on *Geodiapria longiceps* Kieffer, 1911 (Hymenoptera, Diapriidae) and synonymy of the genus *Geodiapria* Kieffer, 1910. *ZooKeys*, *1183*, 1–11. https://doi.org/10.3897/zookeys.1183.110952

Basalys rufocinctus
(Kieffer, 1911)

ZooKeys

ZooKeys 1183: 1–11 (2023)
DOI: 10.3897/zookeys.1183.110952

Research Article

Taxonomic and nomenclatural notes on *Geodiapria longiceps* Kieffer, 1911 (Hymenoptera, Diapriidae) and synonymy of the genus *Geodiapria* Kieffer, 1910

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Abstract

This paper reviews the status of *Geodiapria* and its nominotypical and only included species *G. longiceps*. *Geodiapria* was previously understood to be very similar to, and doubtfully separated from the genus *Basalys*. We use integrative taxonomy (morphology, DNA-barcoding, phylogenetic tree building) to show that the valid name for what was *G. longiceps* Kieffer, 1911 is now *Basalys rufocinctus* (Kiefer, 1911) and that *Geodiapria* is consequently a junior synonym of *Basalys* **syn. nov.** The following taxa are new synonyms of *B. rufocinctus*: *Loxotropa longiceps* Wasmann, 1909, **syn. nov.**, *G. longiceps* Kieffer, 1911, **syn. nov.**, *L. rufosignata* Kieffer, 1911, **syn. nov**. *Basalys rufocinctus* is newly reported from Corsica, Germany, Norway and Spain.

Key words: *Basalys rufocinctus*, DNA-barcoding, first record, integrative taxonomy, parasitoid wasp, species concepts

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Introduction

Parasitoid wasps of the family Diapriidae are speciose and distributed worldwide, and while about 50% of its diversity is estimated to be unknown to science, there are few experts working on this family. Small size (c. 1–4 mm), wide distribution, cryptic diversity, sexual dimorphism, and previous poor taxonomy and lack of critical study of types are some of the problems researchers face when dealing with Diapriidae. The taxonomy of this group still therefore presents many interesting challenges. The status of the genus *Geodiapria* and its single included species *G. longiceps* Kieffer, 1911 has been a taxonomic problem for some time because of its close relation to *Basalys*, in particular species such as *B. rufocinctus* (Kiefer, 1911) with similar distinctive reddish flattened petiolar hairs. The question this paper seeks to resolve is whether or not *Geodiapria* is valid. *Geodiapria* was first described in a key by Kieffer (1910) who separated it from *Loxotropa* auctt. (now *Basalys* in part) and *Basalys* sensu stricto simply by the lack of a basal vein, adding later (Kieffer 1911a)

that the form of the head, longer than wide and a little wider in front than behind, was also distinctive. It was clearly similar to *Basalys* because Kieffer had previously considered the same material to be a *Loxotropa* auctt. (Wasmann, 1909). Kieffer (1911b) then described two species of *Loxotropa* auctt. with the same distinctive reddish flattened petiolar hairs: *L. rufosignata* said to have a head slightly longer than wide and reduced wings without distinct veins; and *L. rufocincta* with an almost square head and with an almost hyaline basal vein. Pschorn-Walcher (1957) examined the type of *G. longiceps* and considered *Geodiapria* to be very close to *Loxotropa* auctt., noting that the absence of the basal vein could be a consequence of wing reduction, but did not make a decision on the validity of *Geodiapria* because of lack of material. Since more material is now available, it is timely to reexamine the question of the validity of *Geodiapria* using an integrative approach combining morphotaxonomy and DNA barcoding (Ratnasingham and Hebert 2007). We examined 18 examples including types of four relevant nominal species, including *L. rufosignata* and *L. rufocincta*, and provide an up to date nomenclatural summary, presenting the first genetic results, including the DNA-barcode placing *Geodiapria* in its proper context.

Material and methods

The specimens of *B. rufocinctus* used for the CO1 DNA barcoding were collected in July 2021 in the Dammbach Valley (Spessart Nature Park) on an orchard meadow, using a Malaise trap. The sequencing was conducted at Canadian Centre for DNA Barcoding (Guelph, Canada) using a voucher recovery protocol. Tree building was undertaken using IQ TREE (server version 1.6.12, Trifinopoulos et al. 2016) using the default settings with 1000 generations. MODELFINDER determined GTR+F+I+G4 to be the best fitting substitution model. The resulting tree was edited using FIGTREE v. 1.4.4 (Rambaut 2010) and INKSCAPE v. 1.1 (https://inkscape.org/de/).

Repository acronyms:

DNPC	David Notton personal collection, United Kingdom
MCSN	Museo Civico di Storia Naturale "Giacomo Doria", Genoa, Italy
MNHN	Muséum national d'Histoire naturelle, Paris, France
NHME	Natural History Museum, Maastricht, Netherlands
NHMUK	Natural History Museum, London, United Kingdom
SNSB-ZSM	Bavarian State Collection, Munich, Germany

Taxonomy

***Basalys* Westwood, 1833**

Basalys Westwood, 1833: 343. Type species *Basalys fumipennis* Westwood, 1833 by monotypy.
Loxotropa auctt. nec Förster, 1856.
Geodiapria Kieffer, 1910: 707, syn. nov. Type species *G. longiceps* Kieffer, 1911 by subsequent monotypy.

Notes. Other generic synonyms are omitted from the above list for simplicity. A diagnosis and detailed description of *Basalys* was given by Masner and García (2002), hence, only a brief diagnosis is given here. Further information on synonyms can be obtained from Johnson (1992).

Diagnosis. Small, smooth and shining wasps; head and mesosoma with long scattered hairs; antennal shelf usually distinctly prominent; female antenna 12-segmented, with strongly abrupt 3- or 4-segmented clava; male antenna 14-segmented with A4 distinctly modified; fore wing with submarginal vein slightly remote from fore margin of wing, costal vein absent, stigmal vein often moderately developed, basal vein always present in macropterous forms, straight, usually strongly pigmented, perpendicular to but never contiguous with submarginal vein.

Remarks. We discovered that the type species of *Geodiapria*, that is *G. longiceps*, is a *Basalys*, a synonym of *B. rufocinctus* (see below) and so *Geodiapria* becomes a junior synonym of *Basalys* syn. nov.

Basalys rufocinctus (Kieffer, 1911)

Loxotropa longiceps Wasmann, 1909: 68, 172, syn. nov., preoccupied nec *B. longiceps* (Ashmead, 1893).
Geodiapria longiceps Kieffer, 1911a: 897, syn. nov., preoccupied nec *B. longiceps* (Ashmead, 1893).
Loxotropa rufocincta Kieffer, 1911b: 916, 939 takes precedence over *L. rufosignata* by first revisor action.
Loxotropa rufosignata Kieffer, 1911b: 914, syn. nov.

BIN number. BOLD_BIN: AEW6196 (Ratnasingham and Hebert 2007).

Type material. ***Holotype*** ♀ of *Loxotropa longiceps* labelled: "Allotype ♂ (!)/ Solenopsia imitatrix/ Wasmann, err. det.!; Holotype ♀/ Geodiapria longiceps/ Kieffer, 1911; Loxotropa/ longiceps n. sp./ ♀ Kieff.; 5.98. Exaet./ b. Solenopsis; Solenopsis m/ Kol. 293. sang [=colony #293 of *Formica sanguinea*]." (NHME) (Fig. 2). ***Holotype*** ♀ of *Geodiapria longiceps* - the same specimen as the holotype of *Loxotropa longiceps* q.v. ***Holotype*** ♀ of *Loxotropa rufosignata* labelled: "Is. Giglio/ IV.1902/ G. Doria; Loxotropa/ rufosignata; ♀" (MCSN) (Fig. 3). ***Syntypes*** 2♀ 3♂ of *Loxotropa rufocincta*: 2♀ labelled: "Holotype [sic – there is no original designation]; Bitche; Loxotropa/ rufocincta; Muséum Paris/ 1957/ coll. Kieffer. 2♂ labelled: Loxotropa/ rufocincta; Bitche; ♂; Allotype; Muséum Paris/ 1957/ coll. Kieffer. ♂ labelled: Paratype; Muséum Paris/ 1957/ coll. Kieffer; Bitche" (MNHN).

Other material. **Denmark** • ♀; N. E. Zealand, Tisvilde Hegn; 56°02'N, 12°04'E; 4 May 1994; P.N. Buhl leg. (DNPC). **France** • ♂; Corsica, Corse du Sud, Basteli-caccia nr. Ajaccio; 41°55'N, 08°30'E; 14–21 Jun. 1996; C. Villemant leg.; Malaise trap, *Quercus suber* stand (DNPC) • ♀; Gard, Mont Ventoux, Malaucène; 44°13'N, 05°08'E; 1–8 Jul. 1997; C. Villemant leg.; maquis, *Quercus ilex* (DNPC) • ♂; same locality; 5–12 Aug. 1997; C. Villemant leg.; maquis, *Quercus ilex* (DNPC). **Germany** • ♀; Bavaria, Dammbach, Dammbachtal; 49°51'58"N, 09°19'30"E; 338 m a.s.l.; 16 Jul. 2021; J. Hübner leg.; nutrient poor grassland; ZSM-HYM-42434-GO2 (BOLDSYSTEMS Process ID: DTIII5299-22; GenBank

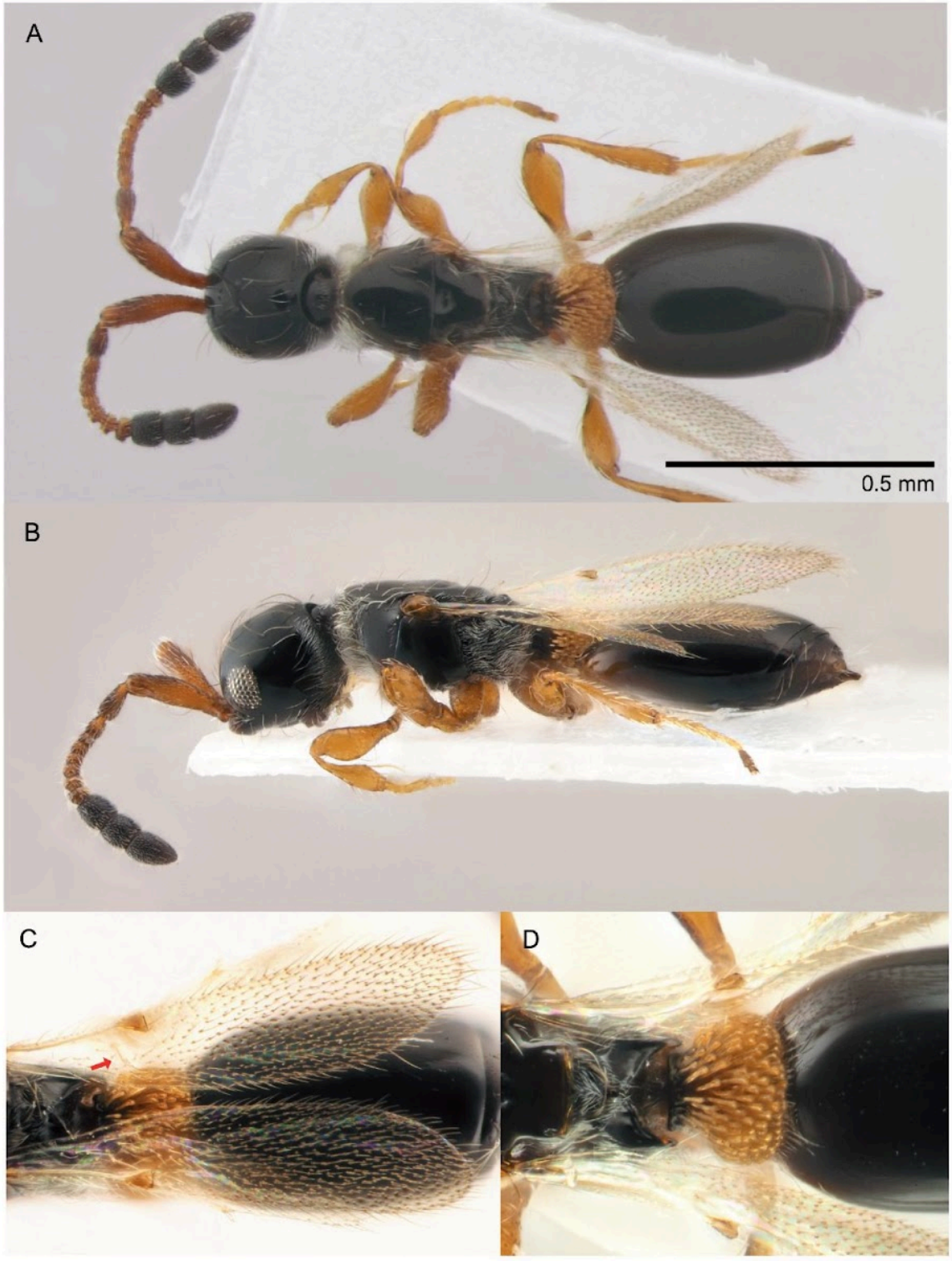

Figure 1. *Basalys rufocinctus* (Kieffer, 1911) ♀: **A** habitus, dorsal view **B** habitus, lateral view **C** wing with reduced venation (arrow) **D** close-up of petiole.

Figure 2. Holotype ♀ of *Loxotropa longiceps* (Wasmann, 1909), the same specimen is also the holotype ♀ of *Geodiapria longiceps* Kieffer, 1911: **A** habitus, lateral view **B** labels.

accession ID: OR450821) (SNSB-ZSM) • ♀ same locality; 16 Jul. 2021; J. Hübner leg.; nutrient poor grassland; ZSM-HYM-42433-H11 (BOLDSYSTEMS Process ID: DTIII5225-22; GenBank accession ID: OR450822) (DNPC). **Norway** • ♀; Onsøy, Hankø Bloksberg, EIS 20, Ø; 3–29 Jun. 1995; O. Hanssen & J.I.I. Båtvik leg.;pitfall trap (DNPC). **Spain** • ♀; Granada, Calahonda; Jul. 1987; L. Lockey leg.; Malaise trap, (DNPC) • ♀; Granada, Sierra Nevada; 1600 m a.s.l.; 10 Apr. 1959; C. Besuchet leg. (NHMUK). **United Kingdom** • ♀; Cheshire, Abbotts Moss; 53°12'27"N, 02°36'23"W; 12 Oct. 1990; D.G. Notton leg.; swept, stream (DNPC) • 3♀; Norfolk, Santon Downham; 52°27'45"N, 00°40'29"E; 15 Aug. 1984; J. Field leg.; Malaise trap, heath with *Betula* and *Pinus* (DNPC) • 1♂; same locality; 18–25 Aug. 1983; J. Field leg. (DNPC).

Diagnosis. ***Female*** Head elongate, rounded, about 1.2 times as long as wide; frons without angles or teeth; antenna 12-segmented with abrupt 3-segmented clava; A11 transverse in lateral view, as long as wide in dorsal view; A6–A9 transverse in lateral view (Fig. 1A); mesonotum and scutellum slightly convex in longer winged individuals, almost flat in shorter winged individuals (Fig. 1B), anterior pronotum with a ruff of whitish setae; anterior scutellar pit small and transverse, less than one third the width of the scutellum; propodeum with medial keel slightly raised anteriorly, less so in short winged individuals; fore wing variable in length, at most extending well beyond apex of gaster, at least reaching anterior margin of petiole; basal vein present in longer winged individuals although hard to see as it is fine and barely pigmented, absent in shorter winged individuals; femora of all legs broadened medially, fore femora 2.2–2.3 times as long as wide in lateral view, with sharp keel ventrally; petiole densely covered dorsally and laterally with long orange flattened setae (Fig. 1D); basal margin of large tergite with two whitish hair tufts more or less concealed under petiolar setae; disc of large tergite normally bare, although the shortest winged individuals, e.g. the type of *L. rufosignata*, may have some long setae. ***Male*** As for female except antenna 14-segmented with A4 expanded posteriorly subtriangular with a fine flange; A5 elongate, flagellar segments becoming shorter towards apex, A13 more or less quadrate; fore wing variable in length at least reaching apex of gaster, at most extending well beyond it; basal vein present, fine, barely pigmented; femora slightly less broadened than female. Body length 1.3–2.2 mm (♀); 1.5–2.4 mm (♂).

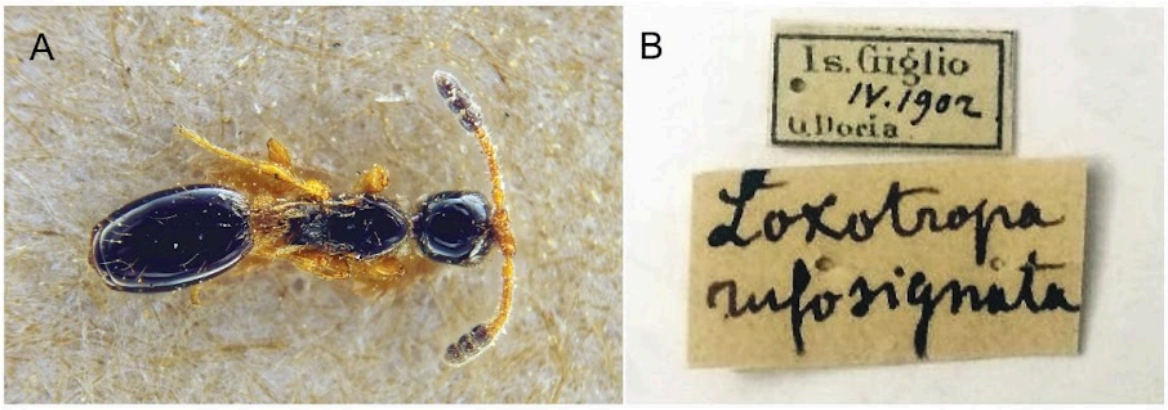

Figure 3. Holotype ♀ of *Loxotropa rufosignata* Kieffer, 1911: **A** habitus, dorsal view **B** labels.

Distribution. Czechia (Macek 1989 as *B. rufocincta* [sic]); Denmark (Buhl 1998 as *B. rufocincta* [sic]) confirmed here; France - mainland France (Kieffer 1911b as *L. rufocincta*) confirmed here; France - Corsica (new record); Germany (new record); Italy (Kieffer 1911b as *L. rufosignata*); Netherlands (Wasmann 1909 as *L. longiceps*); Norway (new record); Spain (new record); Sweden (Hedqvist 2007 as *B. ruficincta* [sic]); United Kingdom (Nixon 1980 as *B. rufocincta* [sic]) confirmed here.

Biology. Host unknown. *Basalys rufocinctus* has previously been considered to be a myrmecophile but the evidence is weak. Of all the specimens we have seen only one, Wasmann's, was found in an ant nest, in a mixed colony of *Solenopsis fugax* and *Formica sanguinea*, and may have entered the nest by accident. Wasmann provided no ethological observations to demonstrate myrmecophily and the species has no obvious morphological adaptation for myrmecophily when compared to other *Basalys*.

Remarks. From the extensive material examined we recognised only one taxon, diagnosed above, and with more variation than previously understood. Most importantly we found that the head was always elongate when seen from above, also significant variation in fore wing length, and expression of the basal vein which was present and weakly pigmented in longer winged individuals, becoming hyaline and then altogether absent in shorter winged individuals. This taxon is therefore a *Basalys* since there is no significant morphological difference: some other species of *Basalys* are known to have elongate heads, also some other *Basalys* have the basal vein absent in short-winged individuals. Based on our examination of the type specimens we consider all four nominal species above, including *Geodiapria longiceps*, belong to this taxon.

Further support for the generic placement of *B. rufocinctus* is based on genetic analyses. A representative ML tree (Appendix 1; *Idiotypa maritima* (Haliday, 1833) as outgroup, 1000 generations) with 76 Diapriini specimens shows *B. rufocinctus* nested within a *Basalys* clade (Appendix 1). The obtained sequences are publicly available on the BOLDSYSTEMS platform (Ratnasingham and Hebert 2007).

Some nomenclatural notes are necessary:

1. We differ from some authors in recognising *Loxotropa longiceps* as a nominal species separate from, and not just a combination of, *Geodiapria longiceps*. *Loxotropa longiceps* is available from Wasmann's (1909) paper

where the name is first used. The name is made available by indication (ICZN 1999: Code art. 12.2.1) since Wasmann refers to his description (Wasmann, 1899) of a specimen previously misidentified as a male of *Solenopsia imitatrix* Wasmann, 1899. Although Wasmann attributes the name to Kieffer, the author of the name is actually Wasmann because he was responsible for publishing the name and writing the prior description (ICZN 1999: Code art. 50.1). The oldest available name for the taxon is thus *L. longiceps* Wasmann, 1909.

2. As *L. longiceps* is transferred to *Basalys* it becomes a secondary junior homonym of *B. longiceps* (Ashmead, 1893) so is invalid.
3. The next oldest available name is *G. longiceps* described as new by Kieffer (1911a). The date of publication is early 1911: evidence comes from the NHMUK copy which has a library stamp 25 Feb. 1911, and the page bound into the end of vol. 10 of *Species des Hyménoptères d'Europe et d'Algérie* which says 1 Mar. 1911.
4. As *G. longiceps* is transferred to *Basalys* it becomes a secondary junior homonym of *B. longiceps* (Ashmead, 1893) so is invalid.
5. The next oldest available names are *L. rufosignata* Kieffer, 1911b and *L. rufocincta* Kieffer, 1911b which were published simultaneously in mid-1911: the page bound into the end of vol. 10 of *Species des Hyménoptères d'Europe et d'Algérie* says 1 Jun. 1911.
6. Since the only two remaining potentially valid names are published simultaneously, we here make a first revisor action to determine precedence thus: *L. rufocincta* has precedence over *L. rufosignata*. We have chosen *L. rufocincta* because this is the more widely used name.
7. *L. longiceps*, *G. longiceps* and *L. rufosignata* are all new synonyms of *L. rufocincta*.
8. The valid name is thus *Basalys rufocinctus*, a combination first recognised by Nixon (1980).
9. Despite previous misspellings, when in combination with *Basalys*, the correct spelling of the species epithet is *rufocinctus*; the gender of *Basalys* is masculine (Notton (2014).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization and methodology, all authors; resources, all authors; writing – original draft, all authors; writing – review and editing, all authors; supervision, David Notton; funding acquisition, Jeremy Hübner and Vasilisa Chemyreva. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Appendix 1

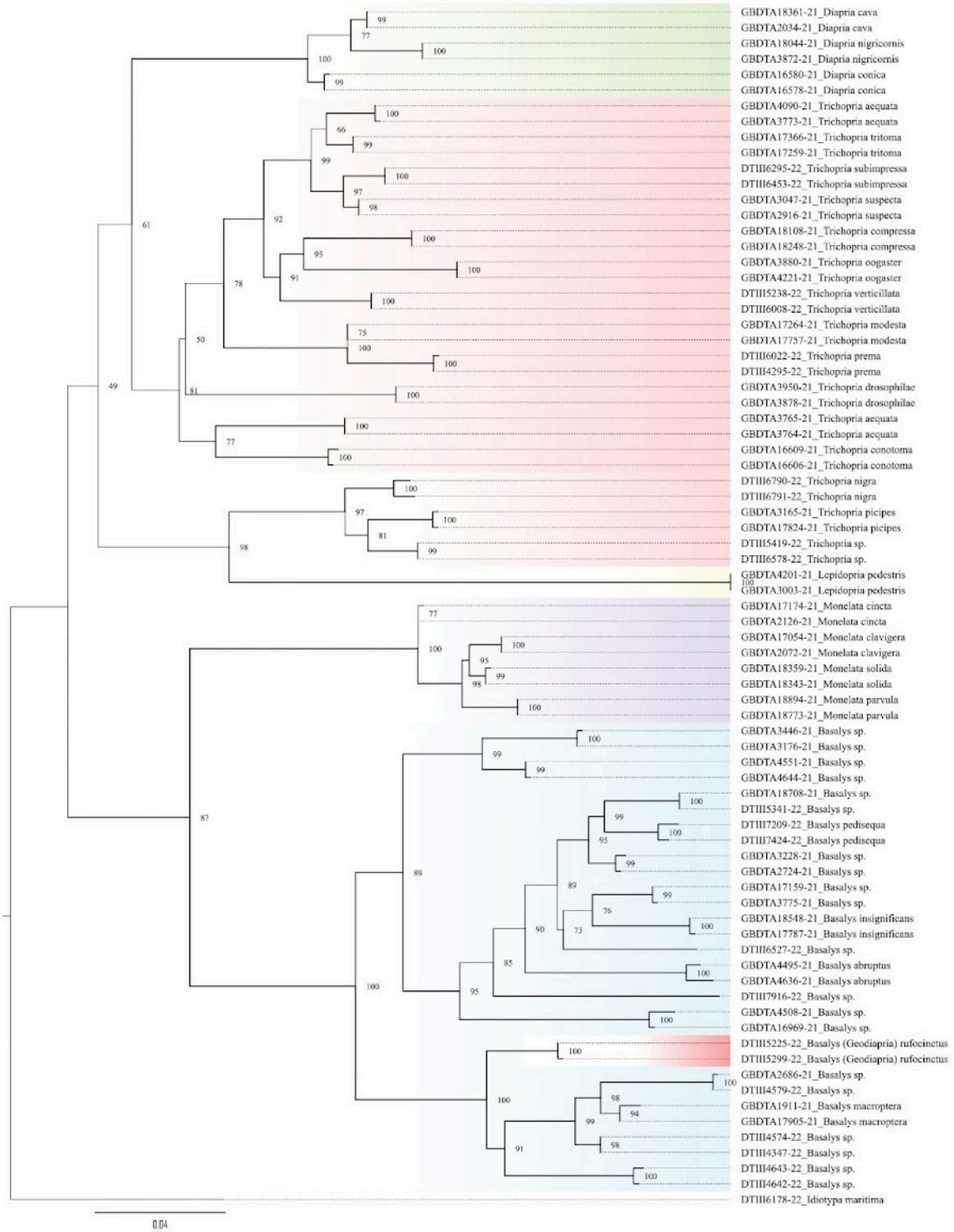

Figure A1. Maximum-likelihood tree of 76 Diapriini specimens, with *Idiotypa maritima* as outgroup. The different genera are color-coded, the numbers on the nodes represent the bootstrap values. Files are openly accessible online at TREEBASE (Piel et al. 2009; http://purl.org/phylo/treebase/phylows/study/TB2:S30685).

Supplementary material 1

Supplementary data

Authors: Jeremy Hübner, Vasilisa G. Chemyreva, David G. Notton

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1183.110952.suppl1

SECTION 1.2: *Spilomicrus* review

A detailed revision of the genus *Spilomicrus* Westwood, 1832 was conducted. Twenty species were recorded for Germany which surpassed the former number by five. Three new species were described: *Spilomicrus brevimalaris* sp. nov., *S. flavecorpus* sp. nov. and *S. politus* sp. nov.. In addition, 23 barcodes were recorded. Species newly recorded for the country are *S. thomsoni* Kieffer, 1911, *S. crassiclavis* Marshall, 1868, *S. lusitanicus* Kieffer, 1910 and *S. diversus* Chemyreva, 2021. *S. thomsoni* was removed from synonymy while *S. simplex* was placed in synonymy with *S. antennatus* Jurine, 1807.

Hübner, J. J., & Chemyreva, V. (2024). Review of German *Spilomicrus* Westwood (Hymenoptera, Diapriidae, Spilomicrini). *Biodiversity Data Journal*, *12*, e114515. https://doi.org/10.3897/BDJ.12.e114515

Spilomicrus antennatus
Jurine, 1807

Biodiversity Data Journal 12: e114515
doi: 10.3897/BDJ.12.e114515

Taxonomy & Inventories

Review of German *Spilomicrus* Westwood (Hymenoptera, Diapriidae, Spilomicrini)

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ZooBank: urn:lsid:zoobank.org:pub:F1FCE190-8E38-47E1-8285-23D0CC9010FE

Abstract

Background

This study provides an integrative taxonomy-based review for the genus *Spilomicrus* Westwood in Germany using DNA barcoding and classic morphology.

New information

Spilomicrus simplex Tomsik, 1947 is placed in synonymy with *S. antennatus* Jurine, 1807; *Spilomicrus thomsoni* Kieffer, 1911 is removed from synonymy with *S. hemipterus* Marshall, 1868. A lectotype is designated for *Spilomicrus nigripes* Thomson, 1858. Newly recorded for Germany are the following species: *S. thomsoni* Kieffer, 1911, *S. crassiclavis* Marshall, 1868, *S. lusitanicus* Kieffer, 1910 and *S. diversus* Chemyreva, 2021. Three species, *Spilomicrus brevimalaris* **sp. nov**., *S. flavecorpus* **sp. nov**. and *S. politus* **sp. nov**. are described as new to science. The 23 DNA-barcodes with species identification present a substantial addition over the previous German checklist. This study aims to update the

number of nationwide known *Spilomicrus* species from fifteen to twenty. Furthermore, a new key to identify all European *Spilomicrus* species is provided.

Keywords

checklist, DNA-barcoding, integrative taxonomy, key, new records, new species, new synonymy, parasitoid wasps

Introduction

This study provides a review of the diaprid genus *Spilomicrus* (Diapriinae, Spilomicrini) in Germany. Diapriidae are parasitoid wasps that are referred to as a "dark taxon" because they are hyper-diverse and it is assumed that a large proportion of the species diversity remains hidden (Hartop et al. 2022). The genus *Spilomicrus* contains more than 100 described species that are worldwide distributed. As is the case for many dark taxa, it is difficult to identify species of *Spilomicrus* because they are miniscule and depict high levels of sexual dimorphism, as well as intraspecific variation. Additionally, many species have distribution areas that can span over several continents and biogeographic areas. *S. formosus* and *S. stigmaticalis*, for example, are found in Europe, Asia and North America (Masner 1991, Notton 1999, Chemyreva 2021). As a consequence, there are many synonyms that refer to the same species although, for example, Masner (1964), Masner and Muesebeck (1968), Notton 2014 and Chemyreva (2021) have made *major* contributions to rectify some. Although much taxonomic effort has been recently aimed at the description of new species in the Palaearctic (Chemyreva 2015, Chemyreva 2018), it is believed that most representatives of this genus are still unknown to science (Masner 1991). Moreover, it is assumed that a large proportion of the diaprid fauna is found in the tropics, where not much diaprid research has been conducted up to date.

The most recent diversity evaluation that was conducted for Germany was done so over twenty years ago by Blank (2001). Here, fourteen *Spilomicrus* species were recovered, of which two (*S. basalyformis* and *S. nigripes*) have been synonymised since. Additionally, although *S. nigripes* had already been synonymised 21 years earlier by Nixon (1980), this species was still treated as a valid taxon in Blank's checklist. Two species, *S. bipunctatus* Kieffer, 1911 and *S. nigriclavis* Marshall, 1868 which have been documented (Tomsik 1947) and even originally described (Kieffer 1911) from Germany, are missing from the checklist. Further single records of species in Germany were established by Notton (1999), such as *S. formosus*, which was not included in the aforementioned checklist either. Ultimately, this means that fifteen species are currently acknowledged as being present in Germany.

Overall, the German diaprid fauna is expected to resemble the European species communities which have been recently examined in detail by Chemyreva (2021). To reduce redundancies, we refer to illustrations in Chemyreva's aforementioned and Notton's (1999) work when reporting our findings. Species of *Spilomicrus* can be identified using the

generic keys by Nixon (1980) and Masner (1991). Generic and species synonymy is documented in Chemyreva (2021).

In order to tackle this megadiverse "dark taxon", we take advantage of DNA barcoding (Hebert et al. 2003) which is a method that uses the DNA of specimens for species identification. Every animal species on the planet has highly conserved elements in their (mito-)genome that can be used to identify specimens using their DNA only by comparing those sequences with a reference library. The CO1 barcode is a widely used and reliable proxy to distinguish insect species. Every taxon obtains one (or more) species-specific identifiers, the Barcode Index Number (BINs) that are stored online and are publicly available (Ratnasingham and Hebert 2007). One of the many advantages of DNA barcoding is the possibility of associating different specimens, which may have been erroneously described as separate species, based on high levels of sexual dimorphism, to the same species. Overall, DNA barcoding has been proven to be a reliable, fast and cost-efficient method for species identification. Still, it should not be applied exclusively, as the DNA barcode does not always provide the resolution to display the true taxonomic relationships of diverse and complex species (Raupach et al. 2016). Therefore, classic morphology is crucial for the interpretation of a species hypothesis (Schlick-Steiner et al. 2010). In this study, we apply an integrative and complementary approach to review the genus *Spilomicrus*. In this manner, we are increasing the rigour of the taxonomic study because we are combining the advantages that each method provides on its own.

Materials and methods

Most of the material was collected in Germany in various collecting events, mainly in Bavaria in the framework of GBOL III: Dark Taxa project. Part of the investigated specimens were taken from the Hilpert collection. All specimens are stored at the Bavarian State Collection of Zoology in Munich. In addition, type material from various museums was examined. For species identification, we applied an integrative taxonomy approach, using all resources possible: barcoded and non-barcoded material, as well as genetic and morphological identification methods. Based on the CO1 barcodes which we obtained from the Canadian Centre for DNA Barcoding (CCDB, https://ccdb.ca/resources/), a Maximum-Likelihood tree was calculated using IQ TREE (online tool, Trifinopoulos et al. (2016)) with a subset of 45 *Spilomicrus* sequences and *Labolips innupta* as an outgroup to display (Suppl. material 1). The tree was edited using FigTree version 1.4.4 (Rambaut 2010) and Inskape version 1.1.1 (2021, available from: https://inkscape.org/de). All barcoding data (628 records) are stored and accessible in the dataset DS-SPILO ([will be published when accepted]) online on the BOLD platform (www.barcodinglife.org). A table of localities with detailed information on each specimen is attached and also online available on the GBIF platform (https://www.gbif.org/dataset/62c523f3-f065-4677-8124-8cf9b56dd8fb and Suppl. material 2). All the examined type material is listed in the Taxon treatments. We conducted BIN distance analyses (the so-called "Barcode Gap") to examine how molecularly close questionable MOTUs are with MEGA11 (Tamura et al. 2021) and Assemble Species by

Automatic Partitioning (ASAP; Puillandre et al. (2021)). DNA barcodes were obtained from BOLD on the 20 Sept 2023.

The morphological terminology and abbreviations follow Hymenoptera Anatomy ontology (Yoder 2010); the measurements follow Yoder (2004), Chemyreva (2015) and Chemyreva (2018). The general distribution of species was obtained and updated from Notton (1999), Blank (2001) and Chemyreva (2021). The new records are marked with an asterisk (*). Series of images were taken using an Olympus OM-D camera mounted on a Leica M125 C binocular and stacked using Helicon Focus (Version 8).

The following abbreviations for locations in Germany are used: BW = Baden-Wuerttemberg, BY = Bavaria, HE = Hesse, NRW = North Rhine-Westphalia. Museum acronyms: HNHM – Hungarian Natural History Museum, Budapest, Hungary; MNHN – National Museum of Natural History, Paris, France; MZLU – Lund Museum of Zoology, Sweden; NHRS – Swedish Museum of Natural History, Stockholm, Sweden; MMBC – Moravian Museum, Brno, Czech Republic; ZISP – Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Taxon treatments

Spilomicrus Westwood, 1832

Nomenclature

Type species *Spilomicrus stigmaticalis* Westwood, 1832, by original monotypy.

Diagnosis

A detailed diagnosis of the genus was given by Masner (1991) and by Masner and García (2002) and, therefore, we only provide a short diagnosis including the most important features.

Medium-sized (1.5–4.5 mm long) melanic wasps. Head subglobose, with mouthparts in lateral view hypognathous; antenna 13-segmented, in females with clava more- or less abrupt, in males antenna thread-like, A4 modified in almost all species. Mesosoma moderately to distinctly wider than high; scutellum with 2 anterior pits and, in most species, with 2 lateral pits and row of smaller posterior pits along posterior margin; forewing with costal vein tubular to nebulous, submarginal vein tubular, marginal vein relatively short, postmarginal and stigmal veins rudimentary or absent; basal vein rarely tubular, in most species nebulous or absent; other veins, at most, nebulous or absent; legs slender to stout, with or without trochanters. Petiole cylindrical in most species; anterior margin of T2 straight, without median cleft or emargination (rarely with 2 lateral folds filled with pilosity); base of S2 arcuate, with moderate to strong cushion of pilosity.

The following part lists all the *Spilomicrus* species found within the framework of the GBOL III project. In comparison to the whole European *Spilomicrus* fauna, three

species could not be recorded for Germany and are, therefore, not documented here: *S. sanbornei* Masner, 1991, *S. cursor* Kieffer, 1911 and *S. latus* Chemyreva, 2021. In addition to the morphology, we provide the barcoding information in the form of the BINs and, if necessary, genetic distances for closely-associated taxa. Illustrations are given for the newly-described taxa and the closest sister taxa for a better understanding of the morphological characters and differences. All other species have already been well described and illustrated in Chemyreva (2021).

Spilomicrus abnormis Marshall, 1868

- Barcode of Life AEP5852

Nomenclature

Spilomicrus abnormis Marshall, 1868 : 202.

Spilomicrus minimus Kieffer, 1911. Synonymised by Nixon (1980).

Description

Illustrated in Chemyreva (2021): fig. 1.

Distribution

Czech Republic, Germany*, Hungary, Ireland, Korea, Moldova, Netherlands, Poland, Russia.

Spilomicrus annulicornis Kieffer, 1911

- Barcode of Life ADF4870

Nomenclature

Spilomicrus annulicornis Kieffer, 1911 : 788.

Description

Illustrated in Chemyreva (2021): fig. 2.

Distribution

Austria, Czech Republic, Finland, France, Germany, Netherlands, Russia (European part), United Kingdom.

Spilomicrus antennatus (Jurine, 1807)

- Barcode of Life AEE0914

Nomenclature

Psilus antennatus Jurine, 1807 : 319.

Basalys californica Ashmead, 1893. Synonymised by Masner (1991).

Eriopria nigra Kieffer, 1910. Synonymised by Notton (2004).

Eriopria rufithorax Kieffer, 1910. Synonymised by Notton (2004).

Scutellipria quinquepunctata Szabo, 1961. Synonymised by Chemyreva (2021).

Spilomicrus simplex Tomsik, 1947 : 33, 34, 40. **Syn. nov.** Fig. 1B, D and E

Figure 1. doi

Spilomicrus antennatus. **A** male lateral; **B** lectotype *S. simplex* lateral; **C** *S. antennatus* female lateral; **D** lectotype *S. simplex* dorsal **E** corresponding labels.

Material

Lectotype:

a. scientificName: *Spilomicus simplex* Tomsik, 1947; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *antennatus*; scientificNameAuthorship: (Jurine, 1807); continent: Europe; eventDate: 1946; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: 1061/Ent; recordedBy: P. Laurer; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; identificationRemarks: designated by Chemyreva (2021), Fig. 1B, D, E; ownerInstitutionCode: MMBC; occurrenceID: 698B71B1-AA08-5BC3-BF96-06C746452B4A

Distribution

Austria, Bulgaria, Czech Republic, Germany, Hungary, Romania, Slovakia, Switzerland, United Kingdom, United States.

Notes

What was already suspected by some researchers could be established using DNA barcoding of specimens of each sex (only one female was available, but numerous males). Our obtained sequences were too short to be included in the attached tree (Suppl. material 1), but a female specimen, collected at the Institute's garden in Munich was sequenced and stored in the framework of the Global Malaise trap project (project code GMGMW in BOLD) (Sones et al. 2023). The average genetic distance in between all examined specimens was 0.27%. The common *Spilomicus simplex* Tomsik, that was only described as a male (Fig. 1B, D and E) is a junior synonym of *S. antennatus* (Jurine), which was only described as a female (Fig. 1C).

Spilomicrus bipunctatus Kieffer, 1911

- Barcode of Life AEC7259

Nomenclature

Spilomicrus bipunctatus Kieffer, 1911 : 284, 289.

Description

Illustrated in Chemyreva (2021): fig. 4.

Distribution

Azerbaijan, Czech Republic, Estonia, France, Germany, Hungary, Italy, Moldova, Netherlands, Poland, Russia (European part), Slovakia, Ukraine, United Kingdom.

Spilomicrus brevimalaris Huebner & Chemyreva sp. nov.

- Barcode of Life AEC2138
- ZooBank F47D7379-D468-424F-A72E-97CE9D66C116

Materials

Holotype:

a. scientificName: *Spilomicrus brevimalaris*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *brevimalaris*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Ammergau Alps; verbatimElevation: 901; decimalLatitude: 47.606; decimalLongitude: 10.841; eventID: dv.hale1.05; samplingProtocol: malaise trap; eventDate: 18-Jul-2016; individualCount: 1; sex: male;

lifeStage: adult; catalogNumber: ZSM-HYM-33100-G04; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AEC2138; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: CBACBA07-062E-5C74-B9EE-8C7847C5FED0

Paratypes:

a. scientificName: *Spilomicrus brevimalaris*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *brevimalaris*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Ammergau Alps; verbatimElevation: 1430; decimalLatitude: 47.5718; decimalLongitude: 10.8807; eventID: dd.amg9.02; samplingProtocol: malaise trap; eventDate: 22-Jul-2015; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: BC-ZSM-HYM-25934-G09; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AEC2138; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: E02F385D-99B0-5BFB-B947-7FE75E5C9CD1

b. scientificName: *Spilomicrus brevimalaris*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *brevimalaris*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Baden-Wuerttemberg; locality: Malsch; verbatimElevation: 120; decimalLatitude: 48.884; decimalLongitude: 8.32; eventID: dd.mgart2.13; samplingProtocol: malaise trap; eventDate: 16-Aug-2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-33108-G09; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AEC2138; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: 9BF2145E-DC54-5FE1-AF53-FCC9ADB054C3

Description

Male. Body length 1.4 mm; forewings reaching far beyond apex of metasoma; antenna 0.9 times as long as body.

Head: black; in dorsal view 1.35 times as wide as long, as wide as mesosoma. Temples behind eyes gradually receding posteriorly. Tentorial pit tiny. Malar sulcus absent. Clypeus weakly convex, oval, 1.7 times as wide as high. Mandible reddish-brown, elongate, with upper tooth slightly shorter than lower tooth. Palpi yellow. Eye oval, with scattered long setae; 0.6 times as high as head and 3.8 times as high as malar space. Frons above base of toruli smooth. Postgenal cushion scanty (Fig. 2). **Antennae**: A1 slightly curved, smooth; its apical rim with small lamellae. A2 not compressed. A2–A13 brown, A13 1,3 times as long as A12. Antennomeres length to width ratios in lateral view as in Fig. 2C. **Mesosoma**: dark brown, as wide as high. Neck bare, with shallow longitudinal grooves. Pronotum with median area and pronotal corner pubescent, pronotal cushion dense; pronotal corner weakly prominent, rounded; lateral area of pronotum smooth, bare medially. Tegula brown, large. Mesoscutum convex, 1.2 times as wide as long. Humeral sulcus distinct and narrow. Scutellum slightly convex. Anterior scutellar pits large, circular, smooth inside, with narrow septum. Axillar depression finely pubescent and smooth. Lateral scutellar pit broad. Posterior scutellar pits distinct. Mesopleuron shining bare and smooth, with subalar

ridge, longitudinal wrinkles postero-ventrally above middle coxa and sculpture around epicnemial pit. Epicnemial pit tiny, without pubescence inside. Sternaulus absent. Ventral side of mesopleuron scarcely pubescent. Metanotum pubescent, finely sculptured, with three weakly-projecting keels on metascutellum. Propodeum entirely pubescent and coarsely rugose. Median propodeal keel in lateral view projecting into high spine anteriorly (Fig. 2A). All legs slender, pale brown with separated trochantelli. **Wings**: Stigmal vein as long as width of marginal vein. Costa, basal and cubital veins sclerotised and weakly pigmented. **Metasoma**: Petiole 1.9 times as long as wide, cylindrical, entirely longitudinally grooved. Petiole pubescent ventrally and dorsally in anterior part. T2 2.8 times as long as petiole, mainly bare and smooth, with small bunch of setae laterally at anterior margin. T3–T5 sparsely pubescent with semi-erect long setae, smooth. T6 small, setose and bare. T7 tapered, setose. S3–S7 with scattered setae, smooth.

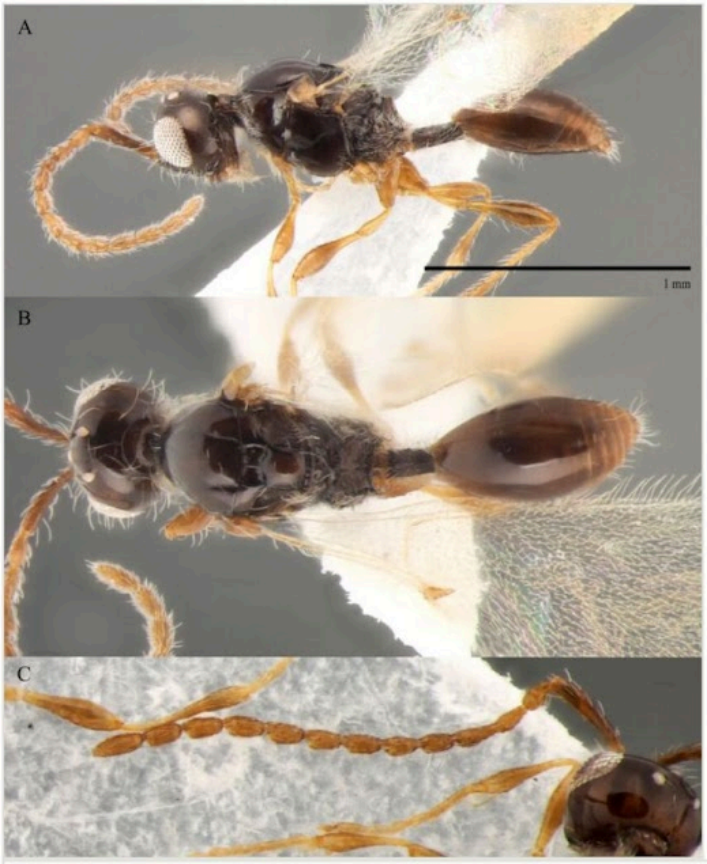

Figure 2. doi

Male holotype *Spilomicrus brevimalaris* sp. nov. (ZSM-HYM-33100-G04; BOLD:AEC2138). **A** lateral; **B** dorsal; **C** antenna.

Female. Body length 1.6–1.7 mm. Wings 0.9–1.0 times as long as the body. Pleurostomal distance 0.8 times as long as shortest distance between eyes (Fig. 3C). Malar distance 0.7 times as long as largest diameter of eye. Antennae brown, clavate with abrupt 5-segmented clava, A13 without ventral pit, A4–A8 moniliform and slightly elongate, A10–A13 with distinct MGS brush ventrally. Scutellum transverse, 0.8 times

as long as wide (measured without anterior scutellar pits) (Fig. 3B). Petiole elongate, 1.3–1.4 as long as wide. T2–T8 smooth. S3–S5 smooth. S6 smooth and densely setose. A more detailed description of the female is given by Chemyreva (2021). The females of *S. brevimalaris* sp. nov. were mistakenly described in Chemyreva (2021) as *S. lusitanicus*.

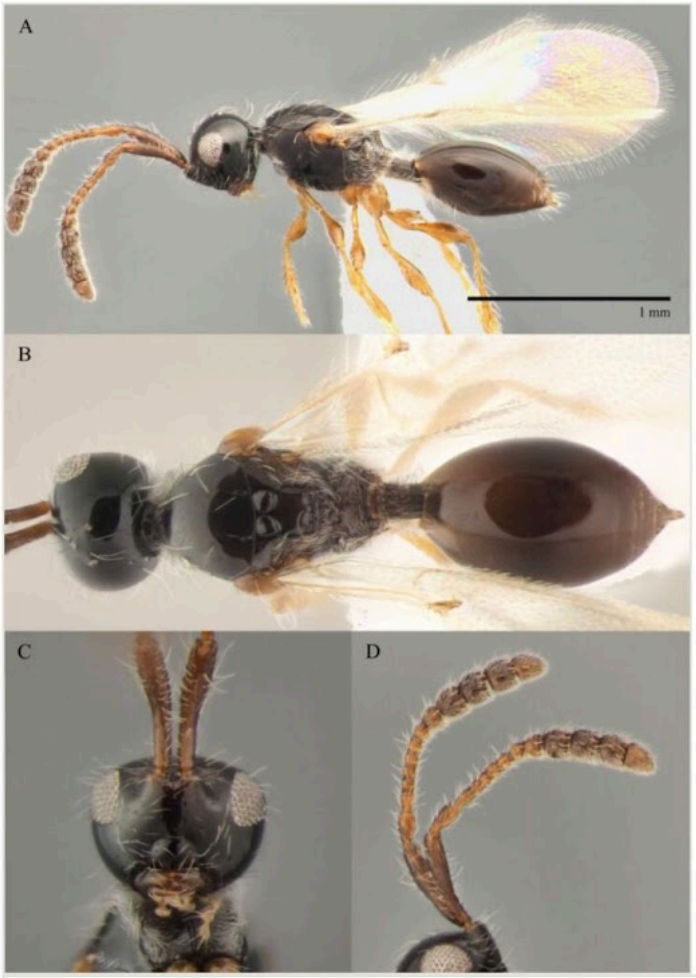

Figure 3. doi

Female paratype *Spilomicrus brevimalaris* sp. nov. (ZSM-HYM-33108-G09; BOLD:AEC2138). **A** lateral; **B** dorsal; **C** face frontal; **D** antenna.

Diagnosis

Male. Body length 1.3–2.1 mm. Face without malar sulcus, pleurostomal distance slightly wider than shortest distance between eyes (Fig. 4B). Malar distance 0.20-0.25 times as long as largest diameter of eye. Front smooth. Antennae brown, slender and long, with A5–A12 2.0–2.7 times as long as wide in dorsal view. A4 1.1–1.4 times as long as A3 and with keel and emargination reaching 0.55–0.60 of the segment length. Notauli extending to the half of mesoscutum length. Scutellum convex, as long as wide (measured without anterior scutellar pits) (Fig. 2B). Propodeum with weakly-arcuate emargination in dorsal view between plicae. Basal vein and distal part of CU dark and

sclerotised. Marginal vein short, less than 1.5 times as long as wide. Petiole elongate, about 1.5–2.0 times as long as wide. T2 pubescent at the base. S8 setose and densely micropunctate.

Figure 4. doi

Faces of the males. **A** *Spilomicrus lusitanicus*; **B** *Spilomicrus brevimalaris* **sp. nov.**; **C** *Spilomicrus flavecorpus* **sp. nov.**

Etymology

The name of this species is a composite Latin masculine adjective derived from "brevis" and "malar" and refers to the short malar distance typical for the males of the new species.

Distribution

Germany, Russia (European part). Further BIN records are online available for Italy and Norway. Probably further distributed around western Europe.

Notes

The male specimen was used in this case as a holotype, since there is no possibility to use females for the *S. lusitanicus*-species group (both species, *S. brevimalaris* sp. nov. and *S. flavecorpus* sp. nov., are very close to *S. lusitanicus* (Kieffer)). There are two reasons for that: 1) the female for the *S. lusitanicus* is unknown; 2) The most reliable feature to determine this species is the length of the malar space, but this feature does not work for the female determination.

The Russian material that was recorded by Chemyreva (2021) as (the closely related) *S. lusitanicus* actually belongs to *S. brevimalaris* sp. nov.

Spilomicrus compressus Thomson, 1859

- Barcode of Life ACH2501

Nomenclature

Spilomicrus compressus Thomson, 1859 : 369.

Spilomicrus carinatus Kieffer, 1911. Synonymised by Notton (2004).

Spilomicrus crassipes Kieffer, 1911. Synonymised by Notton (2004).

Description

Illustrated in Chemyreva (2021): fig. 5.

Distribution

Belarus, Czech Republic, Estonia, France, Germany, Hungary, Poland, Russia (European part), Sweden, Ukraine, United Kingdom.

Spilomicrus crassiclavis Kieffer, 1911

- Barcode of Life AEP5849

Nomenclature

Spilomicrus crassiclavis Kieffer, 1911 : 788, 797.

Spilomicrus pelion Nixon, 1980. Synonymised by Notton (1999).

Description

Illustrated in Notton (1999): figs. 2, 7–9, 17 and 19.

Distribution

Czech Republic, Denmark, Finland, Germany*, Japan, Norway, Sweden, United Kingdom.

Spilomicrus diversus Chemyreva, 2021

- Barcode of Life ADF4749

Nomenclature

Spilomicrus diversus Chemyreva, 2021 : 19.

Materials

Holotype:

a. scientificName: ***Spilomicrus diversus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet:

diversus; scientificNameAuthorship: Chemyreva, 2021; continent: Europe; country: Georgia; stateProvince: Abkhazia; locality: Bzipi River; decimalLatitude: 43.363916; decimalLongitude: 40.495772; samplingProtocol: yellow pan trap; eventDate: 11–14-Aug-2015; individualCount: 1; sex: female; lifeStage: adult; recordedBy: Chemyreva; identifiedBy: V. Chemyreva; dateIdentified: 2021; ownerInstitutionCode: ZISP; occurrenceID: 3C510A1D-F303-5A3C-88AA-E130F0615F94

Paratypes:

a. scientificName: *Spilomicrus diversus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *diversus*; scientificNameAuthorship: Chemyreva, 2021; continent: Europe; country: Russia; stateProvince: Samara Prov.; locality: Zhigulevskii Nature Reserve; eventDate: Jul-28-2009; individualCount: 1; sex: female; lifeStage: adult; recordedBy: Chemyreva; identifiedBy: V. Chemyreva; dateIdentified: 2021; ownerInstitutionCode: ZISP; occurrenceID: BF6D2FE7-2D0B-50DC-9AD9-8E49765AF5DF

b. scientificName: *Spilomicrus diversus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *diversus*; scientificNameAuthorship: Chemyreva, 2021; continent: Europe; country: Russia; stateProvince: Samara Prov.; locality: Zhigulevskii Nature Reserve; eventDate: Jul-28-2009; individualCount: 1; sex: male; lifeStage: adult; recordedBy: Chemyreva; identifiedBy: V. Chemyreva; dateIdentified: 2021; ownerInstitutionCode: ZISP; occurrenceID: D95FF450-21D9-5D16-BD48-C1D871E63B28

c. scientificName: *Spilomicrus diversus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *diversus*; scientificNameAuthorship: Chemyreva, 2021; continent: Europe; country: Russia; stateProvince: Samara Prov.; locality: Adygea, Belaya River; eventDate: 19–24-Aug-2009; individualCount: 1; sex: female; lifeStage: adult; recordedBy: K. Tomkovich; identifiedBy: V. Chemyreva; dateIdentified: 2021; ownerInstitutionCode: ZISP; occurrenceID: 41886139-9703-5131-AFE2-DE3224575F4F

d. scientificName: *Spilomicrus diversus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *diversus*; scientificNameAuthorship: Chemyreva, 2021; continent: Europe; country: Russia; stateProvince: Krasnoyarsk Terr.; locality: 70 km of Kryuchkovo Station; eventDate: 4–23-Jul-2009; individualCount: 1; sex: female; lifeStage: adult; recordedBy: K. Tomkovich; identifiedBy: V. Chemyreva; dateIdentified: 2021; ownerInstitutionCode: ZISP; occurrenceID: 24DE85E8-29C9-5635-8E4B-E2551DC573E8

Diagnosis

Female. Face with malar sulcus visible in the form of shallow furrow. Malar distance 0.47 times as long as largest diameter of eye. Front behind scapus with two small holes (as in the male, Fig. 5 C). Head in dorsal view with temples receding behind eyes, as wide as mesosoma. Antennae (Fig. 6 D) with dark abrupt 5-segmented clava, A2–A8 pale brown, A13 narrower than A12, with pit ventrally; A11–A12 about 2.3 times as wide as A5. Notauli absent. Scutellum transverse. Sternaulus smoothed medially and weakly visible anteriorly and posteriorly. Posterior margin of propodeum without arcuate emargination in dorsal view between plicae. Petiole slightly to distinctly elongate. Base of T2 bare.

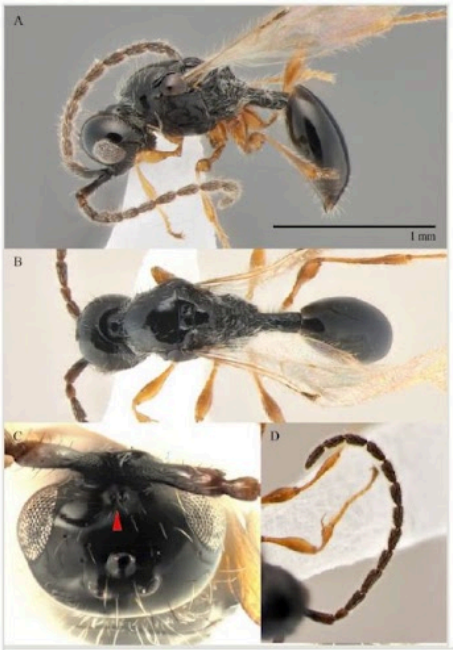

Figure 5. doi

Male *Spilomicrus diversus* (ZSM-HYM-42367-C03; BOLD:ADF4749). **A** lateral; **B** dorsal; **C** head dorsofrontal, small oval holes marked with red arrow; **D** antenna.

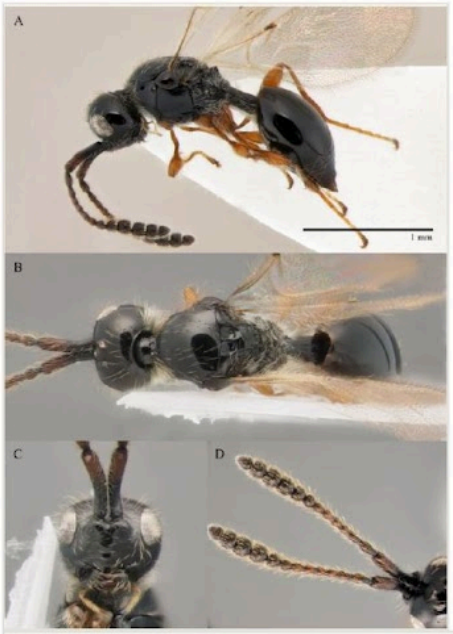

Figure 6. doi

Female *Spilomicrus diversus* (ZSM-HYM-42318-D01; BOLD:ADF4749). **A** lateral; **B** dorsal; **C** face; **D** antenna dorsal.

Male. Antennae filiform (Fig. 5D), in dorsal view A5–A12 more than twice as long as wide; A4 slightly longer than A3, with shallow excavation and keel running from base to 0.6 of the segment length. Petiole elongate, at least 1.5 times as long as wide.

Distribution

Abkhazia, Czech Republic, Georgia, Germany*, Poland, Russia (European part: Samara Prov., Republic Adygea; Siberia: Krasnoyarskiy Terr.).

Notes

Based on new data on intraspecific variability of the *Spilomicrus diversus* and re-investigation of the type series, we conclude that some specimens should be excluded from the type series. The front sculpture of the specimens from the Far East of Russia (Primorskiy Terr. and Sakhalin Area) is significantly different from both *S. diversus* and *S. politus* sp. nov. and these specimens (paratypes) must be excluded from the type series.

Spilomicrus flavecorpus Huebner & Chemyreva sp. nov.

- Barcode of Life AAU9373
- ZooBank 3E306FCD-F79B-4E1A-968A-B1B1C1E2FF44

Materials

Holotype:

a. scientificName: ***Spilomicrus flavecorpus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***flavecorpus***; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Rhön Mountains; verbatimElevation: 780; decimalLatitude: 50.512; decimalLongitude: 10.069; eventID: dd.kerm1.06; samplingProtocol: malaise trap; eventDate: 26-Jun-2017; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: ZSM-HYM-33097-H02; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AAU9373; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: D5DD109A-59EF-5A24-8473-55D2E1F44C9E

Paratypes:

a. scientificName: ***Spilomicrus flavecorpus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***flavecorpus***; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Bavarian Forest National Park; decimalLatitude: 49.04; decimalLongitude: 13.377; samplingProtocol: malaise trap; eventDate: 15-Jul-2013; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: BC-ZSM-HYM-21586-H02; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AAU9373; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: 8B6FA11B-3B86-5434-998A-EFEEAF2E1CCF

b. scientificName: ***Spilomicrus flavecorpus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***flavecorpus***; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Marktredwitz; verbatimElevation: 625; decimalLatitude: 50.011; decimalLongitude: 12.044; eventID: 5938_3_For; samplingProtocol: malaise trap; eventDate: 16-Jul-2019; individualCount: 1; sex: female;

lifeStage: adult; catalogNumber: ZSM-HYM-42359-C01; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AAU9373; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: A47590DD-1EC2-5409-AA89-46B91902D85E

c. scientificName: *Spilomicrus flavecorpus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *flavecorpus*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Atzmannsberg; verbatimElevation: 550; decimalLatitude: 49.825; decimalLongitude: 11.963; eventID: 6137_4_For; samplingProtocol: malaise trap; eventDate: 11-Jul-2019; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-42363-E04; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:AAU9373; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: 150B177D-86F3-56D7-95CE-F9C02E399311

Description

Male. Body length 1.9 mm; forewings reaching far beyond apex of metasoma; antenna 0.8 times as long as body.

Head: brown; in dorsal view 1.05 times as wide as long, as wide as mesosoma. Temples behind eyes gradually receding posteriorly. Tentorial pit tiny. Malar sulcus absent. Clypeus weakly convex, oval, 1.85 times as wide as high. Mandible brown, elongate, with upper tooth shorter than lower tooth. Palpi yellow. Eye oval, with few scattered long setae; 0.4 times as high as head and 1.7 times as high as malar space. Frons above base of toruli smooth. Postgenal cushion scanty (Fig. 7A, B). **Antennae**: brown. A1 slightly curved, smooth; its apical rim with small lamellae. A2 not compressed. A13 1.4 times as long as A12. Antennomeres length to width ratios in lateral view as in Fig. 7A, C. **Mesosoma**: brown, 1.1 times as wide as high. Neck with few scattered setae and shallow longitudinal grooves. Pronotum with median area scarcely setose and pronotal corner densely pubescent, pronotal cushion dense; pronotal corner weakly prominent, rounded; lateral area of pronotum smooth, bare medially. Tegula brown, large. Mesoscutum convex, 1.25 times as wide as long. Humeral sulcus distinct and narrow. Scutellum slightly convex. Anterior scutellar pits large, circular, smooth inside, with narrow septum. Axillar depression finely pubescent and smooth. Lateral scutellar pit broad. Posterior scutellar pits small. Mesopleuron shining bare and smooth, with small depression next to epicnemial pit, subalar ridge below tegula and longitudinal wrinkles postero-ventrally above middle coxa. Epicnemial pit tiny, without pubescence inside. Sternaulus absent. Ventral side of mesopleuron scarcely pubescent. Metanotum pubescent, finely sculptured, with three weakly-projecting keels on metascutellum. Propodeum entirely pubescent and finally sculptured. Median propodeal keel in lateral view high raised anteriorly (Fig. 7A). All legs slender, pale brown with separated trochantelli. **Wings**: Stigmal vein as long as width of marginal vein. Costal, basal and cubital veins sclerotised and weakly pigmented. **Metasoma**: Petiole entirely pubescent. T2 4.5 times as long as petiole, mainly bare and smooth, with small bunch of setae laterally at anterior margin. T3–T5

sparsely pubescent with semi-erect long setae, smooth. T6 small, setose and bare. T7 tapered, setose. S3–S7 with scattered setae, smooth.

Figure 7. doi

Male holotype *Spilomicrus flavecorpus* sp. nov. (ZSM-HYM-33097-H02; BOLD: AAU9373). **A** lateral; **B** dorsal, head width and mesosoma width marked with arrows; **C** antenna.

Female. Body length 1.7–1.8 mm. Pleurostomal distance 0.74 times as long as shortest distance between eyes (Fig. 8C). Malar distance 0.73 times as long as largest diameter of eye (Fig. 8C). Antennae brown, clavate with abrupt 5-segmented clava, A13 without ventral pit, A4–A8 moniliform and transverse, A10–A13 with distinct MGS brush ventrally (Fig. 8D). Petiole as long as wide. T2–T6 smooth. T7–T8 weakly punctured. S3–S5 smooth. S6 smooth and densely setose.

Figure 8. doi

Female paratype *Spilomicrus flavecorpus* sp. nov. (ZSM-HYM-42363-E04; BOLD: AAU9373). **A** lateral; **B** dorsal; **C** face frontal; **D** antenna.

Diagnosis

Male. Face without malar sulcus, pleurostomal distance 0.9 times as wide as shortest distance between eyes (Fig. 4C). Malar distance 0.42 times as long as largest diameter of eye. Front smooth. Antennae brown, filiform, with A5 1.3 and A12 1.6 times as long as wide in dorsal view. A4 1.1 times as long as A3 and with keel and emargination reaching to half of the segment (Fig. 7C). Notauli marked as short grooves posteriorly (Fig. 7B). Scutellum convex, as long as wide (measured without anterior scutellar pits) (Fig. 7B). Propodeum with weak emargination between plicae. Marginal vein 1.25 times as long as wide. Petiole slightly elongate, about 1.1 times as long as wide. T2 pubescent at the base. S8 setose and densely micropunctate.

Etymology

The name of this species is a composite Latin masculine adjective derived from the adverb “flave” (yellowly) and “corpus” and refers to the colouration of the body.

Distribution

Germany. Further BIN records are online available for Canada. Probably further distributed around the Palaearctic and Nearctic.

Notes

The reason for the selection of the holotype is analogous to that of *S. brevimalaris* sp. nov. (check Notes).

Spilomicrus flavipes Thomson, 1858

- Barcode of Life ACL2543

Nomenclature

Spilomicrus flavipes Thomson, 1858: 369.

Spilomicrus szelenyii Szabo, 1977. Synonymised by Chemyreva (2021).

Description

Illustrated in Chemyreva (2021): fig. 8.

Distribution

Czech Republic, France, Germany, Hungary, Ireland, Moldova, Mongolia, Poland, Russia, Sweden, United Kingdom.

Spilomicrus formosus Jansson, 1942

- Barcode of Life AAU9811

Nomenclature

Spilomicrus formosus Jansson, 1942 : 215.

Description

Illustrated in Notton (1999): figs. 3, 4, 10–12, 18 and 20.

Distribution

Belgium, Canada, Czech Republic, Denmark, Finland, Germany, Great Britain, Ireland, Japan, Norway, Russia, Slovakia, Sweden, United States.

Spilomicrus hemipterus Marshall, 1868

- Barcode of Life ADM6694

Nomenclature

Spilomicrus hemipterus Marshall, 1868 : 202.

Spilomicrus inaequalis Tomsik, 1941: 34, 38, 42. Synonymised by Chemyreva (2021). Fig. 9A and B.

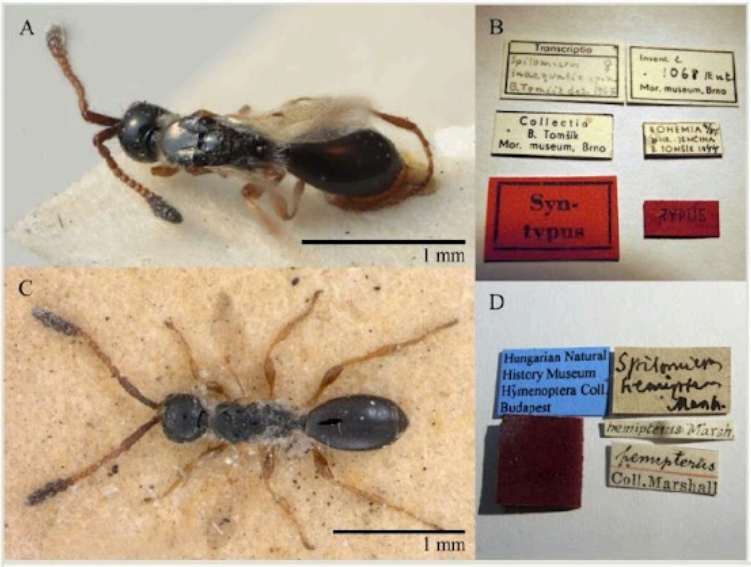

Figure 9. doi

Female lectotypes. **A** *Spilomicrus inaequalis* dorsal; **B** corresponding labels; **C** *Spilomicrus hemipterus* dorsal; **D** corresponding labels.

Spilomicrus pedisequus Kieffer, 1916: 784, 787. Synonymised by Nixon (1980).

Materials

Lectotypes:

a. scientificName: ***Spilomicrus hemipterus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***hemipterus***; scientificNameAuthorship: Marshall, 1868; continent: Europe; eventDate: 1944; individualCount: 1; sex: female; lifeStage: adult; recordedBy: B. Tomsik; otherCatalogNumbers: BOLD:ADM6694; identificationRemarks: designated in Chemyreva (2021), Fig. 17C, D; ownerInstitutionCode: MNHN; occurrenceID: 5CFDD1B7-4855-5101-B094-8EE5657DAC38

b. scientificName: ***Spilomicrus inaequalis***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***hemipterus***; scientificNameAuthorship: Marshall, 1868; continent: Europe; individualCount: 1; sex: female; lifeStage: adult; recordedBy: Marshall; otherCatalogNumbers: BOLD:ADM6694; identificationRemarks: designated in Chemyreva (2021), Fig. 17A, B; ownerInstitutionCode: MMBC; occurrenceID: 3E890838-0263-587C-86F8-418105C938B7

Diagnosis

Malar sulcus partly developed, shallow; neck of prothorax bare anteriorly; pleurostomal distance distinctly shorter than distance between eyes in front view; temples behind eyes gradually receding posteriorly; male A4 with keel reaching 0.7 of the segment length, A4 0.65 times as long as A3, widened apically; antenna distinctly bicolorous with more abrupt clava; female A9 without or with weakly indicated MGS brush; A13 with distinct small pit ventrally; A13 in dorsal and lateral views narrower than A12; A9 distinctly narrower and shorter than A10; notauli present in the form of broad grooves posteriorly; sternaulus absent; wings reaching to one-fourth of metasoma length to distinctly beyond the apex of metasoma; female petiole elongate, about 1.2 times as long as wide.

Distribution

Austria, Croatia, Czech Republic, France, Germany, Hungary, Moldova, Netherlands, Poland, Russia (European part), Switzerland, Ukraine, United Kingdom.

Spilomicrus integer Thomson, 1859

- Barcode of Life ADF4750

Nomenclature

Spilomicrus integer Thomson, 1859 : 369.

Spilomicrus major Vollenhoven, 1879. Synonymised by Chemyreva (2021).

Description

Illustrated in Chemyreva (2021): fig. 10.

Distribution

Czech Republic, France, Germany, Hungary, Netherlands, Poland, Romania, Russia (European part), Slovakia, Sweden, Ukraine, United Kingdom.

Spilomicrus lusitanicus (Kieffer, 1910)

- Barcode of Life AEK2205

Nomenclature

Tritopria lusitanica Kieffer, 1910 : 749, male. Fig. 10E, G and H.

Figure 10. doi

Male types. **A** holotype of *Spilomicrus noctiger* Szabó; **B** corresponding labels; **D**, **F** lectotype of *Spilomicrus gracilicornis* Kieffer (designated by Notton (2004)); **C** corresponding labels; **E**, **H** lectotype of *Trichopria lusitanica* Kieffer (designated by Chemyreva (2021)); **G** corresponding labels.

Spilomicrus gracilicornis Kieffer, 1911. Synonymised by Chemyreva (2021). Fig. 10C, D and F.

Spilomicrus noctiger Szabo, 1977. Synonymised by Chemyreva (2021). Fig. 10A and B.

Materials

Holotype:

a. scientificName: *Tritopria lusitanicus* Kieffer, 1910; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *lusitanicus*; scientificNameAuthorship: (Kieffer, 1910); continent: Europe; eventDate: 1956; individualCount: 1; sex: male; lifeStage: adult; recordedBy: Kieffer; identifiedBy: V. Chemyreva; dateIdentified: 2021; identificationRemarks: designated by Chemyreva (2021), Fig. 11E, G, H; ownerInstitutionCode: MNHN]; occurrenceID: 5274E46C-F7BF-506B-A39B-7027769199D7

b. scientificName: *Spilomicrus noctiger* Szabo, 1997; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *lusitanicus*; scientificNameAuthorship: (Kieffer, 1910); continent: Europe; eventDate: Jul-13-1970; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: 2775; recordedBy: P. L. G. Benoit; identifiedBy: V. Chemyreva; dateIdentified: 2021; identificationRemarks: designated by Chemyreva (2021), Fig. 11A, B; ownerInstitutionCode: HNHM; occurrenceID: 64EB8BCA-C3A7-599F-94FC-B9DBD87BA7EB

Lectotype:

a. scientificName: *Spilomicrus gracilicornis* Kieffer, 1911; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *lusitanicus*; scientificNameAuthorship: (Kieffer, 1910); continent: Europe; eventDate: 1956; individualCount: 1; sex: male; lifeStage: adult; recordedBy: Kieffer; otherCatalogNumbers: BOLD:AEK2205; identifiedBy: V. Chemyreva; dateIdentified: 2021; identificationRemarks: designated by Chemyreva (2021), Fig. 11C, D, F; ownerInstitutionCode: MNHN; occurrenceID: 6E75EA16-1397-560F-8A1D-6B47EF00B5D2

Diagnosis

Male. Body length 1.9–2.5 mm. Face without malar sulcus, pleurostomal distance slightly wider than shortest distance between eyes. Malar distance 0.45–0.55 times as long as largest diameter of eye. Front smooth. Antennae dark brown, slender and long, with A5–A12 2.3–3.3 times as long as wide. A4 1.2–1.25 times as long as A3 and with keel and emargination reaching to 0.5–0.55 of the segment length (Fig. 11D). Notauli almost complete, but shallow anteriorly (Fig. 11B). Scutellum convex, 1–1.2 times as long as wide (measured without anterior scutellar pits). Propodeum with not deep emargination between plicae in dorsal view. Basal vein and distal part of CU dark and sclerotised. Marginal vein short, less than 1.5 times as long as wide. Petiole elongate, 1.7–1.8 times as long as wide. T2 pubescent at the base. S8 almost smooth, with few setae and very weak elongated wrinkles.

Distribution

Algeria, Austria, Czech Republic, France, Germany*, Hungary, Italy*, Portugal, Russia (European part).

Figure 11. doi

Male *Spilomicrus lusitanicus* (ZSM-HYM-42423-H01; BOLD:AEK2205). **A** lateral; **B** dorsal; **C** antenna lateral; **D** antenna dorsal.

Notes

The most important features of this species, such as malar and pleurostomal distances cannot be examined in the lectotype of *Tritopria lusitanica* because the face of the type specimen is hidden in glue. However, secondary diagnostic characters (proportions of the remaining antennomeres, width of the head and proportions of the scutellum) lead us to believe that all type specimens belong to a single species and correspond with the examined material mentioned above under the name *Spilomicrus lusitanicus*. The females are unknown. The females described by Chemyreva (2021) belong to the *S. brevimalaris* sp. nov.

Spilomicrus modestus Tomsik, 1947

- Barcode of Life AEJ2099

Nomenclature

Spilomicrus modestus Tomsik, 1947 : 33, 39, 42.

Description

Illustrated in Chemyreva (2021): fig. 13.

Distribution

Austria, Czech Republic, Finland, Germany, Hungary, Moldova, Russia (European part and East Siberia), Ukraine.

Spilomicrus nigriclavis Marshall, 1868

- Barcode of Life AEK0961

Nomenclature

Spilomicrus nigriclavis Marshall, 1868 : 228.

Spilomicrus punctatus Kozlov, 1978 : 591, nom. praeocc., non *Spilomicrus punctatus* (Cameron, 1889).

Spilomicrus kozlovi Notton, 2014. Synonymised by Chemyreva (2021).

Spilomicrus nigriclavis var. *armatus* Kieffer, 1911 : 781, nom. praeocc., non *Spilomicrus armatus* (Ashmead, 1893).

Spilomicrus nigriclavis var. *subarmatus* Kieffer, 1912. Synonymised by Chemyreva (2021).

Description

Illustrated in Chemyreva (2021): fig. 14.

Distribution

France, Germany, Netherlands, Russia (European part), Sweden, United Kingdom.

Spilomicrus politus Huebner & Chemyreva sp. nov.

- Barcode of Life AER1505
- Barcode of Life ACZ2358
- ZooBank E9C61643-B816-4379-97E5-A71D2603E8B1

Materials

Holotype:

a. scientificName: ***Spilomicrus politus***; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***politus***; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Munich; verbatimElevation: 516; decimalLatitude: 48.164; decimalLongitude: 11.497; eventID: gb.botgar1.10; samplingProtocol: malaise trap; eventDate: 01-Sep-2021; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-42456-C12; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:ACZ2358; identifiedBy: V. Chemyreva I J.

Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: 2C22789C-3D9D-5594-9D9E-71E8AC0ABD87

Paratypes:

a. scientificName: *Spilomicrus politus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *politus*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Baden-Wuerttemberg; locality: Gaggenau; verbatimElevation: 340; decimalLatitude: 48.821; decimalLongitude: 8.388; eventID: dd.mbach.05; samplingProtocol: malaise trap; eventDate: 21-Aug-2011; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-42369-G02; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:ACZ2358; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: EF90EABD-F85F-52CF-8EE2-796379EB829F

b. scientificName: *Spilomicrus politus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *politus*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Munich; verbatimElevation: 516; decimalLatitude: 48.164; decimalLongitude: 11.497; eventID: gb.botgar1.09; samplingProtocol: malaise trap; eventDate: 11-Aug-2021; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-42373-F02; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:ACZ2358; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: E1875FF2-D75C-554D-B113-04D0EE157E8C

c. scientificName: *Spilomicrus politus*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *politus*; scientificNameAuthorship: Huebner & Chemyreva, 2023; continent: Europe; country: Germany; stateProvince: Bavaria; locality: Paehl; verbatimElevation: 720; decimalLatitude: 47.941; decimalLongitude: 11.183; eventID: dd.pmor5.06; samplingProtocol: malaise trap; eventDate: 27-Aug-2020; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: ZSM-HYM-42466-G05; recordedBy: Huebner & Chemyreva; otherCatalogNumbers: BOLD:ACZ2358; identifiedBy: V. Chemyreva I J. Huebner; dateIdentified: 2023; ownerInstitutionCode: SNSB-ZSM; occurrenceID: C7CE6D4E-9617-55DE-A7AD-03ADCFFD1528

Description

Female (holotype). Body length 1.8 mm; forewing extending far beyond apex of metasoma; antenna 0.68 times as long as body. **Head**: black, in dorsal 0.95 times as wide as metasoma. Tentorial pits absent. Clypeus weakly convex, 0.6 times as high as wide. Mandible dark brown, elongate, its upper tooth slightly shorter than lower tooth. Palpi yellow. Eye oval, with scattered long setae, 0.42 times as high as head and 1.9 times as high as malar space. Postgenal cushion dense. **Antennae**: A1 slightly curved, broadened apically, finely coriaceous; its apical rim simple. A2 not compressed. Apical half of A1 and A2–A8 dark brown, A9–A13 dark brown. Antenna A10–A13 with MGS brush, flattened on ventral side. A10–A12 as long as wide. A13 distinctly narrower than A12 and 1.1 times as long as A12. Antennomers length to width ratios in dorsal view as in Fig. 12A and D; A13 with small shallow ventral tip. **Mesosoma**: black, as wide as high. Neck bare, with longitudinal grooves. Pronotum with median area and pronotal

corner pubescent, pronotal cushion dense; pronotal corner weakly prominent, rounded; lateral area of pronotum smooth and bare. Tegula dark brown, large. Mesopleuron smooth, shiny and bare, with subalar ridge. Sternaulus absent. Epicnemial pit tiny and bare inside. Ventral side of mesopleuron pubescent. Mesoscutum 1.25 times as wide as long, without notauli. Humeral sulcus distinct and narrow. Anterior scutellar pitscircular with short and low elongate keels posteriorly (Fig. 12B). Lateral scutellar pit broad. Posterior scutellar pits distinct. Metanotum sparse pubescent, coarsely sculptured, metascutellum with three low longitudinal keels. Propodeum pubescent and coarsely rugose, its posterior margin without arcuate emargination in dorsal view between plicae. Median propodeal keel projecting into high spine anteriorly. All legs slender, pale brown, with separated trochantelli. **Wings**: Marginal vein elongate, twice as long as its median width. Stigmal vein as wide as width of marginal vein. Costa and basal veins sclerotised, weakly pigmented. **Metasoma**: Petiole cylindrical, 1.3 times as long as wide, striate, weakly setose dorsally (with hirsute belt medially) and densely pubescent ventrally. T2 about 3.9–4.5 times as long as petiole, smooth and bare. T3–T6 and S3–S6 with few erect long setae, almost smooth (with small area of micropunctures medially). T5 weakly expanded laterally. T7 subtriangle, with long setae around spiracles. S6 pointed, more densely pubescent on the top.

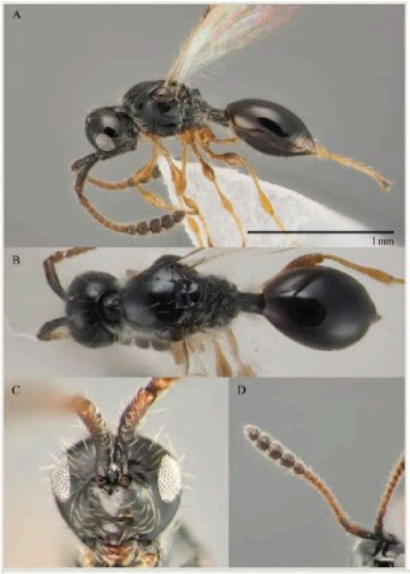

Figure 12. doi

Female holotype *Spilomicrus politus* sp. nov. (ZSM-HYM-42456-C12; BOLD: ACZ2358). **A** lateral; **B** dorsal; **C** face; **D** antenna dorsal.

Male (BOLD: AER1505). Body length 1.6 mm. Similar to female, but differs by the following features: antenna filiform, A2–A13 brown, A1 dark brown (Fig. 13A, B and D); A4 with keel running from base to 0.7 of the segment; A4 as long as A3 and 1.2 times as long as A5; A5-A10 about twice as long as wide in dorsal view; malar space 0.47 times as long as pleurostomal distance and 0.54 times as long as largest diameter of eye; petiole twice as long as wide; T2 2.8 times as long as petiole. S8 densely micropunctate.

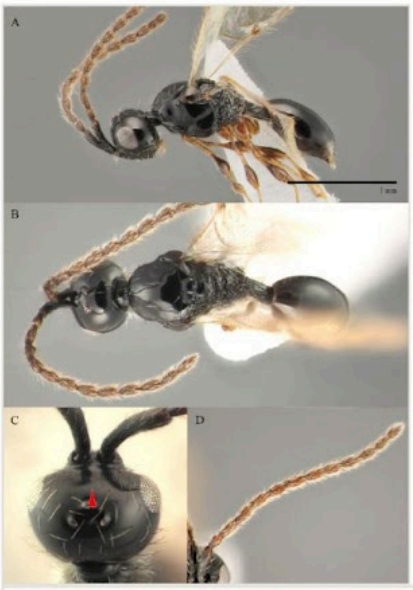

Figure 13. doi

Male *Spilomicrus politus* sp. nov. (ZSM-HYM-42318-B01; BOLD:AER1505). **A** lateral; **B** dorsal; **C** head dorsal without holes, bare area marked with red arrow; **D** antenna.

Diagnosis

The species closely resembles *S. diversus* Chemyreva, 2021 from which it can be distinguished by the combination of the following features: A11 and A12 2.7 times as wide as A5 (A11–A12 about 2.3 times as wide as A5 in *S. diversus*); the malar sulcus is totally absent (visible in the form of shallow furrow in *S. diversus*); frons above base of toruli smooth (Fig. 13C) (with two small round and shallow depressions in *S. diversus*).

Etymology

The name of the new species is a Latin masculine adjective "*politus*" (smooth).

Distribution

Estonia, Georgia (Republic of Abkhazia and Autonomous Republic of Adjara), Germany, Romania, Russia (European part).

Notes

The new species *Spilomicrus politus* sp. nov. was assigned two BINs, BOLD:ACZ2358 and BOLD:AER1505. It was not reliably possible to separate those two BINs into two morphologically sound species. The distance between those two BINs is 1.74%, whereas the distances to *Spilomicrus diversus* (BOLD:ADF4749) are 2.59% (BOLD:ACZ2358) and 3.12 % (BOLD:AER1505), the distance to *S. modestus* is 13.6%. The fact that both BINs of the *S. politus* sp. nov. differ in under 2% of the bases in their sequences leads to the suspicion that the specimens might just be one species.

Spilomicrus rufitarsis Kieffer, 1911

- Barcode of Life AEK1604

Nomenclature

Spilomicrus rufitarsis Kieffer, 1911 : 786.

Spilomicrus pseudocursor Szabo, 1974 : 497. Synonymised by Chemyreva (2021).

Description

Illustrated in Chemyreva (2021): fig. 15.

Distribution

Algeria, Austria, Czech Republic, France, Germany, Hungary, Ireland, Italy, Netherlands, United Kingdom.

Spilomicrus stigmaticalis Westwood, 1832

- Barcode of Life ADS1706
- Barcode of Life ACU1243

Nomenclature

Spilomicrus stigmaticalis Westwood, 1832 : 129, female.

Spilomicrus nigripes Thomson, 1859. Synonymised by Nixon (1980). Fig. 14.

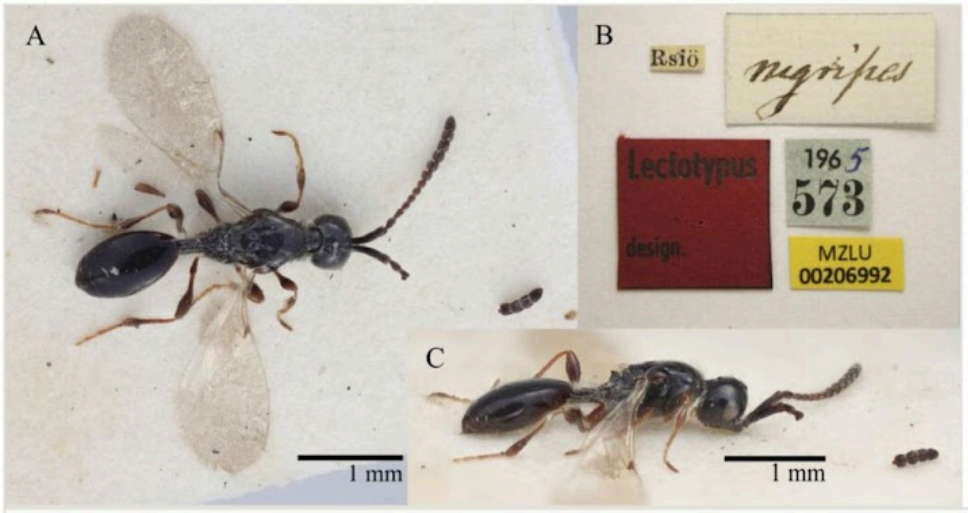

Figure 14. doi

Female lectotype of the *Spilomicrus nigripes* Thomson, 1858. **A** dorsal; **B** corresponding labels; **C** lateral.

Spilomicrus basalyformis Marshall, 1868. Synonymised by Chemyreva (2021).

Spilomicrus armatus Ashmead, 1893. Synonymised by Masner (1991).

Spilomicrus tripartitus Kieffer, 1911. Synonymised by Nixon (1980).

Spilomicrus pilicornis Szabo, 1977b. Synonymised by Chemyreva (2021).

Spilomicrus barbatus Szabo, 1983. Synonymised by Chemyreva (2021).

Spilomicrus mediofurcatus Szabo, 1983. Synonymised by Chemyreva (2021).

Material

Lectotype:

a. scientificName: ***Spilomicrus nigripes***, Thomson, 1859; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: ***Spilomicrus***; specificEpithet: ***stigmaticalis***; scientificNameAuthorship: Westwood, 1832; continent: Europe; country: Sweden; locality: Ringsjon in Skíne; eventDate: 1965; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: MZLU 00206992; recordedBy: Thomson; otherCatalogNumbers: BOLD:ACU1243; identifiedBy: V. Chemyreva; dateIdentified: 2023; identificationRemarks: designated here, Fig. 14; ownerInstitutionCode: MZLU; occurrenceID: 1BA744EC-3DAD-5F67-BA17-27BF144BA292

Distribution

Algeria, Azerbaijan, Austria, Canada, Czech Republic, Finland, France, Georgia, Germany, Hungary, Ireland, Italy, Kazakhstan, Moldova, Netherlands, Poland, Russia (European part and Siberia), Slovakia, Sweden, Switzerland, Ukranie, United Kingdom, United States.

Notes

Spilomicrus stigmaticalis is a fairly common, widely distributed species. The species contains two BINs, BOLD:ADS1706 and BOLD:ACU1243. Still, all sequences are clustered as one single taxon using the BOLD cluster analysis and the ASAP algorithm. Not only is the genetic distance between those BINs small (1.9%), they also show medium to high intraspecific variation of up to 2.2% (mean distance 0.6%). In addition to that, we were not able to distinguish both genetic clades morphologically in both sexes, not even using the genitalia. It was only possible to find identifying morphological characters to distinguish between the females. Due to the genetic and morphological proximity of both clades, we will keep them together as one species. A lectotype is designated for *Spilomicrus nigripes* Thomson, 1858 (Fig. 14).

Spilomicrus thomsoni Kieffer, 1911

- Barcode of Life ADF4747
- Barcode of Life ADX1651

Nomenclature

Spilomicrus thomsoni Kieffer, 1911 : 787, 798.

Material

Lectotype:

a. scientificName: *Spilomicrus thomsoni*; kingdom: Animalia; phylum: Arthropoda; class: Insecta; order: Hymenoptera; family: Diapriidae; genus: *Spilomicrus*; specificEpithet: *thomsoni*; scientificNameAuthorship: Kieffer, 1911; continent: Europe; country: Sweden; stateProvince: SmÍland; individualCount: 1; sex: female; lifeStage: adult; recordedBy: C. H. Boheman; institutionCode: NHRS-HEVA; collectionCode: 000016369; ownerInstitutionCode: NHRS; source: designated by Chemyreva 2021; occurrenceID: 051550F1-0B2D-5654-9E40-7E0ED1AF0AA2

Diagnosis

Malar sulcus partly developed, shallow; neck of prothorax bare anteriorly; pleurostomal distance distinctly shorter than distance between eyes in front view; temples behind eyes gradually receding posteriorly; male A4 cylindrical, with keel reaching 0.55 of the segment length, A4 0.73–0.80 times as long as A3; antenna gradually darkened towards the top, with non-abrupt clava; female A9 with distinct MGS brush; A13 with small pit ventrally; A13 in dorsal and lateral views narrower than A12; A9 distinctly narrower and shorter than A10; notauli present in the form of broad grooves posteriorly; sternaulus absent; wings reaching to apex of metasoma to distinctly beyond it; female petiole elongate, about 1.2 times as long as wide. Lectotype illustrated in Fig. 15.

Figure 15. doi

Female lectotype of *Spilomicrus thomsoni*. **A** dorsal; **B** corresponding labels; **C** broken off body parts.

Distribution

Czech Republic (Tomsik 1947), Finland, Germany*, Moldova, Russia (European part), Sweden, Ukraine.

Notes

There are two BINs within *Spilomicrus thomsoni*, BOLD:ADF4747 and BOLD:ADX1651 which differ in only 0.1% from each other. Although the cluster methods of ASAP and BOLD separate the two clades and show very low intraspecific genetic variation, we could not tell them morphologically apart. Therefore, we will refer to them as being one species until further analyses might change that interpretation.

On the other hand, we can separate the *Spilomicrus thomsoni* taxon from *S. hemipterus* genetically and morphologically. This is why we removed *S. thomsoni* from synonymy with *S. hemipterus*.

Identification keys

Key to the European *Spilomicrus* species (modified and updated after Chemyreva (2021))

Females
(female of *S. lusitanicus* unknown)

1	Ventral margin of clypeus with pointed or rounded deflexed median projection; mandibles short, with upper tooth much shorter than lower tooth	2
–	Ventral margin of clypeus with rounded reflexed median projection (fig. 17, *2* [arrow], *4* in Chemyreva (2021)); mandibles elongate, with upper tooth only slightly shorter than lower tooth (fig. 17, *4* in Chemyreva (2021))	4
2	Antennae with clava 5- or 6-segmented; in front view, ventral margin of clypeus rounded, blunt; mesosoma depressed, no more than 0.8 times as high as wide, mesoscutum weakly convex; median propodeal keel low, hardly raised anteriorly	***S. sanbornei***
–	Antennae with clava 7- or 8-segmented; in front view, ventral margin of clypeus triangular, pointed; mesosoma less depressed, at least 0.9 times as high as wide, mesoscutum strongly convex; median propodeal keel distinctly raised anteriorly to form a high projection	3
3	Antennal clava 7-segmented, A8–A12 strongly transverse; notauli weakly convergent anteriorly or subparallel, developed in posterior fourth or absent	***S. crassiclavis***
–	Antennal clava 8-segmented, A8–A12 subquadrate or elongate; notauli distinctly divergent anteriorly and always developed at least in posterior third	***S. formosus***
4	(1) All femora broad, with very short stalks (fig. 5, *9*; fig. 11, *9*; fig. 14, *2* in Chemyreva (2021)); clypeus more than twice as wide as high (fig. 5, *2*; fig. 11, *1*; fig. 14, *5*; fig. 15, *2* in Chemyreva (2021))	5

–	All femora slender, with long stalks (fig. 8, *1*, *8* in Chemyreva (2021)); clypeus less than twice as wide as high (fig. 1, *2*; fig. 2, *1*; fig. 4, *3* in Chemyreva (2021)) [except *S. stigmaticalis*]	8
5	Antenna with abrupt 6-segmented clava, A3–A7 yellowish, A8–A13 dark brown (fig. 5, *4*, *5* in Chemyreva (2021)); hind femur longitudinally deeply grooved (with distinct sharp margins) on ventral side for reception of tibia (fig. 5, *7* in Chemyreva (2021))	***S. compressus***
–	Antenna with non-abrupt clava, uniformly reddish-brown to black (fig. 11, *5*; fig. 14, *4* and fig. 15, *3* in Chemyreva (2021)); hind femur with smooth bare area or shallow depression on ventral side or not modified	6
6	Clava slender, A11 about 1.5 times as wide as A4 in dorsal view and about 1.25 times, in lateral view (fig. 14, *4* in Chemyreva (2021)); notauli developed in posterior half and narrow throughout (fig. 14, *3* in Chemyreva (2021))	***S. nigriclavis***
–	Clava wider, A11 about twice as wide as A4 in dorsal view and about 1.75 times, in lateral view (fig. 11, *5* and fig. 15, *3*, *4* in Chemyreva (2021)); notauli developed only in the form of oval or round posterior point or (if they are longer) distinctly broadened posteriorly (fig. 11, *4* and fig. 15, *6* in Chemyreva (2021)), sometimes completely absent	7
7	Neck of prothorax with short longitudinal grooves posteriorly; notauli developed at least in posterior third of mesoscutum (fig. 15, *6* in Chemyreva (2021)); propodeum with median keel strongly raised anteriorly (fig. 15, *6* in Chemyreva (2021)); A13 as long as A12	***S. rufitarsis***
–	Neck of prothorax entirely smooth (fig. 11, *3* in Chemyreva (2021)); notauli developed on mesoscutum only in the form of small posterior pits to completely absent (fig. 11, *4* in Chemyreva (2021)); propodeum with median keel slightly raised anteriorly (fig. 11, *4* in Chemyreva (2021)); A13 about 1.3–1.4 times as longer A12	***S. latus***
8	(4). Base of T2 pubescent (fig. 3, *3* and fig. 12, *4* in Chemyreva (2021))	9
–	Base of T2 bare (fig. 1, *4* in Chemyreva (2021))	11
9	Micropterous (fig. 1 C. fig. 3 in Chemyreva (2021)); T2 with scattered long setae (fig. 3, *1*, *3* in Chemyreva (2021)); scutellum strongly transverse, without posterior scutellar pits (fig. 3, *3* in Chemyreva (2021)); head subquadrate in dorsal view (fig. 3, *2* in Chemyreva (2021)); ocelli absent	***S. antennatus***
–	Macropterous (Fig. 3A); T2 bare (fig. 12, *2* in Chemyreva (2021)); scutellum slightly transverse to elongate, with posterior scutellar pits (fig. 12, *4* in Chemyreva (2021)); head transverse in dorsal view; ocelli present	10

10	Propodeum with deep emargination between plicae, plicae slightly convergent posteriorly (Fig. 3B)	***S. brevimalaris* sp. nov.**
–	Propodeum with not deep emargination between plicae, plicae not convergent posteriorly (Fig. 8B)	***S. flavecorpus* sp. nov.**
11	(8). T2 with numerous scattered long setae (fig. 6, *1* in Chemyreva (2021)); two posterior ocelli absent (fig. 6, *4* in Chemyreva (2021))	***S. cursor***
–	T2 bare; all ocelli present	12
12	Propodeum with deep arcuate emargination of posterior margin between plicae in dorsal view (fig. 2, *2*; fig. 9, *6*; fig. 10, *8* and fig. 17, *3* in Chemyreva (2021)); body mainly larger than 2.0 mm	13
–	Propodeum with weak arcuate emargination of posterior margin between plicae in dorsal view (fig. 1, *4*; fig. 7, *1* and fig. 13, *4* in Chemyreva (2021)); body mainly smaller than 2.0 mm	19
13	Sternaulus complete (fig. 17, *1* in Chemyreva (2021)); A13 without pit ventrally; A13 in dorsal and lateral views not narrower than A12; clava elongate, A9 as wide and as long as A10 [not always in *S. flavipes*] (fig. 2, *3* and fig. 17, *5*, *6* in Chemyreva 2021)	14
–	Sternaulus absent at least medially (fig. 9, *7* in Chemyreva (2021)); A13 with distinct small pit ventrally; A13 in dorsal and lateral views narrower than A12; clava fusiform [not always in *S. hemipterus*], A9 distinctly narrower and shorter than A10 (fig. 4, *5*, *6*; fig. 9, *3*, *4* and fig. 10, *3*, *5* in Chemyreva (2021))	17
14	Head in front view with transverse wrinkles on antennal shelf (fig. 8, *2* in Chemyreva (2021)); temples distinctly, but gradually receding behind eyes in dorsal view (fig. 8, *5* in Chemyreva (2021))	***S. flavipes***
–	Head in front view without wrinkles on antennal shelf (fig. 2, *1* and fig. 17, *4* in Chemyreva (2021)); temples parallel behind eyes in dorsal view (fig. 2, *4* and fig. 17, *7* in Chemyreva (2021))	15
15	A3–A6 pale brown and clava black; tentorial pit absent to very tiny (punctiform) (fig. 2, *1* in Chemyreva (2021)); scutellum parallel-sided to narrowed posteriorly (fig. 2, *2* in Chemyreva 2021); A3 1.5 times as long as A2 (fig. 2, *3* in Chemyreva (2021))	***S. annulicornis***
–	A1–A13 black; tentorial pit distinct (Fig. 16C); scutellum slightly broadened posteriorly (Fig. 16D); A3 equal to 1.2 times as long as A2 (Fig. 16E)	***S. stigmaticalis***

16	(13). Notauli usually absent, when rarely present, then expressed only in the form of two narrow incisions; malar sulcus totally absent	***S. integer***
–	Notauli present in the form of broad grooves posteriorly; malar sulcus present, partly developed or fully visible in the form of a shallow groove	17
17	Neck of prothorax pubescent anteriorly (fig. 4, *7* in Chemyreva (2021)); pleurostomal distance distinctly longer than distance between eyes in front view (fig. 4, *3* in Chemyreva (2021)); temples behind eyes parallel or even weakly divergent posteriorly in dorsal view (fig. 4, *7* in Chemyreva (2021))	***S. bipunctatus***
–	Neck of prothorax bare anteriorly (fig. 9, *6* in Chemyreva (2021)); pleurostomal distance distinctly shorter than distance between eyes in front view (Fig. 17, Fig. 18C); temples behind eyes gradually receding posteriorly	18
18	Female antennae distinctly bicolor with abrupt 5-segmented clava (Fig. 17 D); A10–A13 with MGS brush (all multiporous gustatory sensillae on the antenna) on its ventral side	***S. hemipterus***
–	Female antennae more or less monochrome with non-abrupt clava (Fig. 18 D); A9–A13 with MGS brush on its ventral side	***S. thomsoni***
19	(12). Notauli in the form of short grooves on mesoscutum posteriorly (fig. 1, *4* in Chemyreva (2021)); malar sulcus not deep, but completely developed throughout (fig. 1, *2* in Chemyreva (2021))	***S. abnormis***
–	Notauli totally absent; malar sulcus absent or incompletely developed (fig. 7, *2*, *5* and fig. 13, *2* in Chemyreva (2021))	20
20	Head in dorsal view with temples parallel behind eyes (fig. 13, *5* in Chemyreva (2021)); petiole subquadrate to transverse (fig. 13, *4* in Chemyreva (2021)); antennae entirely brown, moniliform, without clava (fig. 13, *3* in Chemyreva (2021))	***S. modestus***
–	Head in dorsal view with temples receding behind eyes (fig. 7, *8* in Chemyreva (2021)); petiole slightly elongate to 1.8 times as long as wide (Fig. 12B, Fig. 6B); antennae with dark abrupt 5-segmented clava, A2–A8 pale brown (Figs 6, 12D)	21
21	Front behind scapus with two small oval and not deep holes (as in Fig. 5C)	***S. diversus***
–	Front behind scapus smooth (as in Fig. 13C)	***S. politus* sp. nov.**

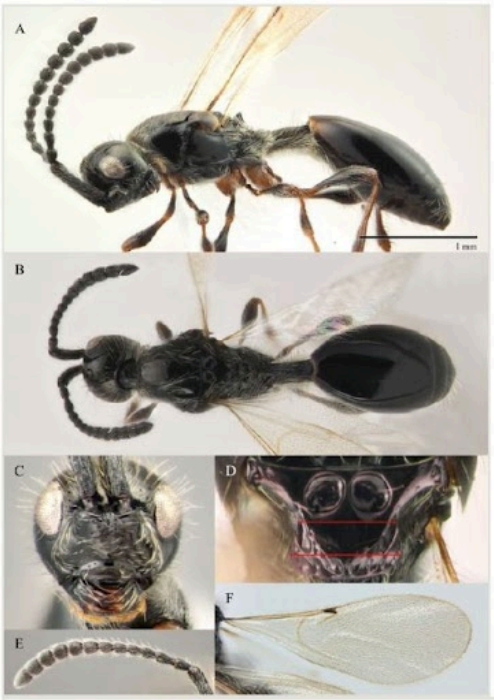

Figure 16. doi

Female *Spilomicrus stigmaticalis* (ZSM-HYM-42423-H02, BOLD:ADS1706). **A** lateral; **B** dorsal; **C** face; **D** scutellum highlighted red, arrows mark the basal broadening; **E** antenna; **F** wing.

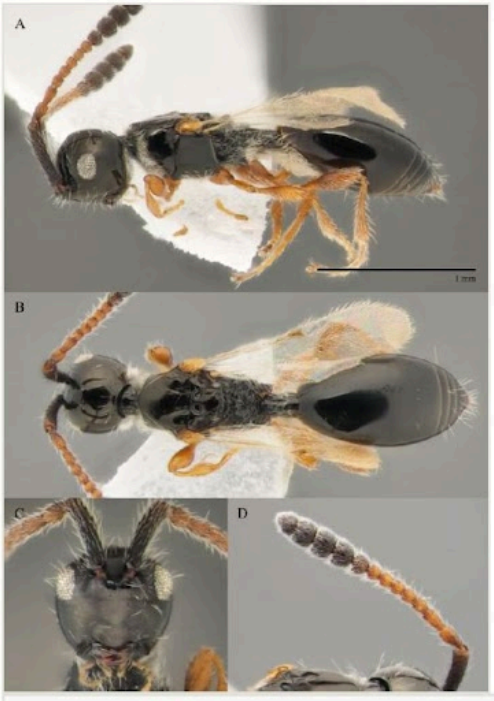

Figure 17. doi

Female *Spilomicrus hemipterus* (ZSM-HYM-42322-F02; BOLD:ADM6694). **A** lateral; **B** dorsal; **C** face; **D** antenna.

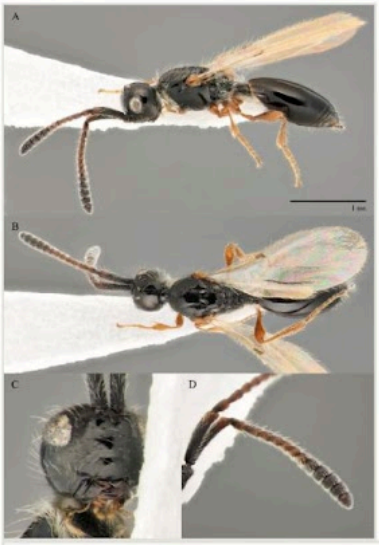

Figure 18. doi

Female *Spilomicrus thomsoni* (ZSM-HYM-42321-H08; BOLD:ADF4747). **A** lateral; **B** dorsal; **C** face; **D** antenna.

Males (males of *S. cursor* and *S. nigriclavis* unknown)		
1	Ventral margin of clypeus with pointed or rounded deflexed median projection; mandibles short, with upper tooth much shorter than lower tooth	2
–	Ventral margin of clypeus with small rounded reflexed median projection (fig. 17, *2* [arrow], *4* in Chemyreva (2021)); mandibles elongate, with upper tooth slightly shorter than lower tooth	4
2	In front view, ventral margin of clypeus rounded, blunt; A4 with moderately deep, curved emargination; mesosoma distinctly depressed, no more than 0.8 times as high as wide, mesoscutum weakly convex; median propodeal keel low, hardly raised anteriorly	***S. sanbornei***
–	In front view, ventral margin of clypeus triangular, acuminate; A4 with at most a shallow emargination; mesosoma less depressed, at least 0.9 times as high as wide, mesoscutum strongly convex; median propodeal keel raised anteriorly to form a high projection	3
3	Eye sparsely hairy; A4 with carina over-reaching 0.7 of the segment	***S. crassiclavis***
–	Eye bare; A4 with carina not over-reaching basal half of the segment	***S. formosus***
4	(1). Clypeus transverse, more than twice as wide as high (fig. 5, *2*; fig. 11, *1* and fig. 15, *2* in Chemyreva (2021))	5

–	Clypeus rounded, less than twice as wide as high (fig. 1, *2*; fig. 2, *1* and fig. 4, *3* in Chemyreva (2021))	8
5	A4 distinctly longer than A3	***S. stigmaticalis***
–	A4 distinctly shorter than A3	6
6	A5–A12 at least twice as long as wide (fig. 5, *3* in Chemyreva (2021)); legs yellowish-brown	***S. compressus***
–	A5–A12 at most 1.5 times as long as wide (fig. 11, *6* and fig. 15, *5* in Chemyreva (2021)); legs dark brown	7
7	Neck with short longitudinal grooves posteriorly; notauli developed at least in posterior half of mesoscutum (fig. 15, *6* in Chemyreva (2021)); propodeum with median keel strongly raised anteriorly; A3 1.1–1.3 times as long as A4 (fig. 15, *5* in Chemyreva (2021))	***S. rufitarsis***
–	Neck entirely smooth (fig. 11, *3* in Chemyreva (2021)); notauli developed on mesoscutum in the form of small pits posteriorly to absent (fig. 11, *4* in Chemyreva (2021)); propodeum with median keel slightly raised anteriorly; A3 1.5–1.6 times as long as A4 (fig. 11, *8* Chemyreva (2021))	***S. latus***
8	(4). Base of T2 pubescent (fig. 3, *3*; fig. 12, *4* in Chemyreva (2021))	9
–	Base of T2 bare (fig. 1, *4* in Chemyreva (2021))	12
9	A4 without emargination and keel (fig. 16, *6* in Chemyreva (2021))	***S. antennatus***
–	A4 with emargination and keel (fig. 12, *8*, *9* in Chemyreva (2021))	10
10	Malar space 0.2–0.22 times as long as largest diameter of eye (Fig. 4B) and 0.24–0.27 times as long as distance between pleurostoma	***S. brevimalaris* sp. nov.**
–	Malar space more than 0.42 times as long as largest diameter of eye (Fig. 4 A and C) and 0.40–0.45 times as long as distance between pleurostoma	11
11	Head narrower than mesosoma (Fig. 11B); A5–A12 more than 2.3 times as long as wide (Fig. 11C and D); scutellum as long as wide or distinctly elongated	***S. lusitanicus***
–	Head as wide as to wider than mesosoma in dorsal view (Fig. 7B); A5–A12 about 1.3 times as long as wide (Fig. 7A and C); scutellum distinctly transverse	***S. flavecorpus* sp. nov.**
12	(8). A3 distinctly longer than A4 (fig. 4, *2*; fig. 9, *8* and fig. 10, *6*, *7* in Chemyreva (2021))	13

–	A3 as long as or shorter than A4 (fig. 1, *7*; fig. 2, *7*; fig. 7, *4*; fig. 12, *9* and fig. 17, *10* in Chemyreva (2021))	16
13	Notauli present (fig. 4, *1* and fig. 9, *6* in Chemyreva (2021)); keel on A4 not reaching apex of the segment (fig. 4, *2* and fig. 9, *8* in Chemyreva (2021)); malar sulcus present (partly developed or fully visible in the form of a shallow groove)	14
–	Notauli absent (fig. 10, *8* in Chemyreva (2021)); keel on A4 reaching apex of the segment (fig. 10, *6* in Chemyreva (2021)); malar sulcus totally absent (fig. 10, *2* in Chemyreva (2021))	***S. integer***
14	Neck of prothorax pubescent anteriorly (fig. 4, *7* in Chemyreva (2021)); pleurostomal distance distinctly longer than distance between eyes in front view (fig. 4, *3* in Chemyreva (2021)); temples behind eyes parallel or even divergent posteriorly in dorsal view (fig. 4, *7* in Chemyreva (2021))	***S. bipunctatus***
–	Neck of prothorax bare anteriorly (fig. 9, *5*, *6* in Chemyreva (2021)); pleurostomal distance distinctly shorter than distance between eyes in front view (fig. 9, *2* in Chemyreva (2021)); temples behind eyes usually convergent posteriorly (fig. 9, *5* in Chemyreva (2021))	15
15	A4 0.65 times as long as A3, widened apically with keel reaching 0.7 of the segment length (Fig. 19a)	***S. hemipterus***
–	A4 0.73 times as long as A3, cylindrical with keel reaching 0.55 of the segment length (Fig. 19b)	***S. thomsoni***
16	(12). A4 with projection at base of keel and with bare smooth area along this keel (fig. 2, *7*; fig. 8, *6*, *7* and fig.17, *10* in Chemyreva (2021)); sternaulus complete; body usually longer than 2.0 mm	17
–	A4 without projection at base of keel and without bare smooth area along this keel (fig. 1, *7*; fig. 7, *4* and fig. 13, *6* in Chemyreva (2021)); sternaulus absent medially; body usually shorter than 2.0 mm	19
17	Propodeum with deep arcuate emargination of posterior margin between plicae in dorsal view (fig. 8, *8* and fig. 17, *3* in Chemyreva (2021)); A3–A5 in lateral view equal to each other in width; pubescence of A3–A13 less dense, semi-erect (fig. 8, *6* and fig. 17, *9* in Chemyreva (2021))	12
–	Propodeum with weak arcuate emargination of posterior margin between plicae in dorsal view; A4 in lateral view wider than A3 and A5; pubescence of A3–A13 more dense, recumbent (fig. 2, *7* in Chemyreva (2021))	***S. annulicornis***

18	Head in front view with transverse wrinkles on the top of antennal shelf (fig. 8, *2*); antenna pale brown to brown, emargination on A4 shallow (fig. 8, *6* in Chemyreva (2021)); mesoscutum smooth anteriorly and with notauli developed in posterior half	***S. flavipes***
–	Head in front view without transverse wrinkles on the top of antennal shelf (fig. 17, *4* in Chemyreva (2021)); antennae dark brown to black, emargination on A4 deep (Fig. 20A, B. fig. 17, *10* in Chemyreva (2021)); mesoscutum with notauli completely developed throughout, shallow anteriorly (Fig. 20B. fig. 17, *7* in Chemyreva (2021))	***S. stigmaticalis***
19	(16). Notauli developed in the form of short posterior grooves (fig. 1, *4* in Chemyreva (2021)); malar sulcus complete, shallow (fig. 1, *2* in Chemyreva (2021))	***S. abnormis***
–	Notauli totally absent (fig. 7, *1* and fig. 13, *4* in Chemyreva (2021)); malar sulcus absent (fig. 7, *2*, *5* and fig. 13, *2* in Chemyreva (2021))	20
20	Head in dorsal view subrectangular, with temples parallel behind eyes (fig. 13, *5* in Chemyreva (2021)); A5–A12 1.1–1.3 times as long as wide (fig. 13, *6* in Chemyreva (2021)); petiole subquadrate to weakly elongate	***S. modestus***
–	Head in dorsal view with temples receding behind eyes (fig. 7, *8* in Chemyreva (2021)); A5–A12 about twice as long as wide (fig. 7, *4* in Chemyreva (2021)); petiole elongate, at least 1.5 times as long as wide	21
21	Front behind scapus with two small oval and not deep holes (Fig. 5C)	***S. diversus***
–	Front behind scapus smooth (Fig. 13C)	***S. politus* sp. nov.**

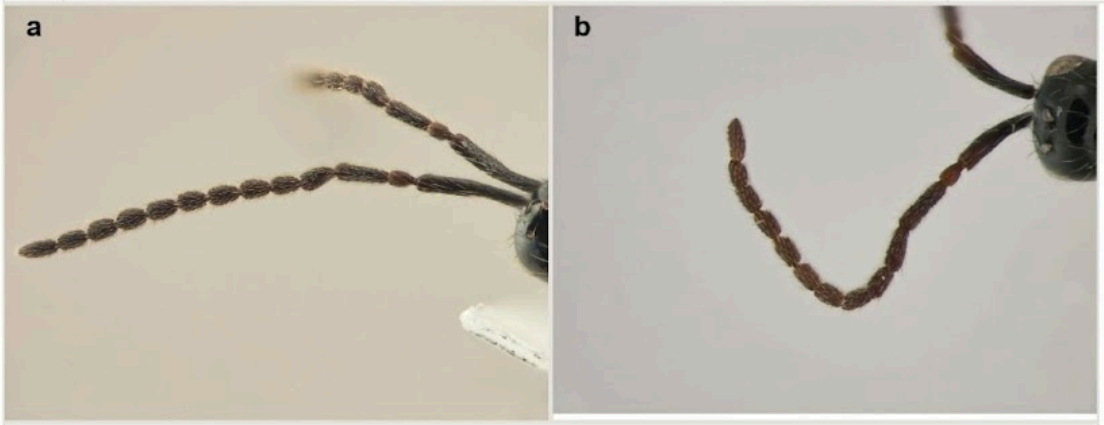

Figure 19.

Male antennae.

a: *Spilomicrus hemipterus* (ZSM-HYM-42425-B06; BOLD:ADM6694); doi
b: *Spilomicrus thomsoni* (ZSM-HYM-33122-A05; BOLD:ADF4747). doi

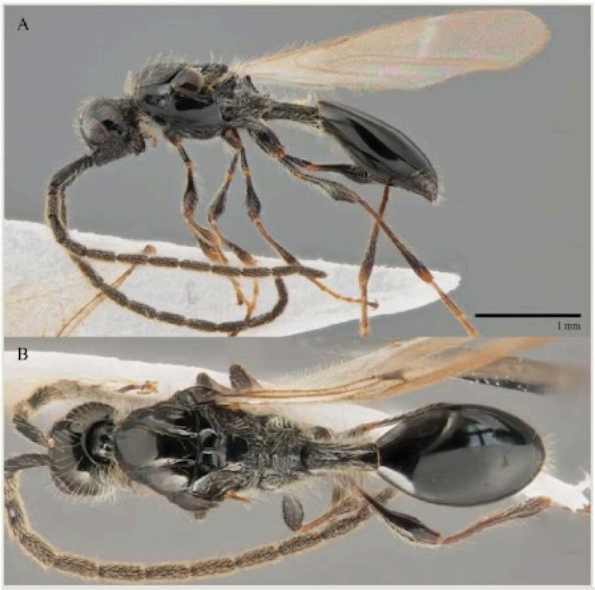

Figure 20. doi

Male *Spilomicrus stigmaticalis* (ZSM-HYM-42320-F08, BOLD:ADS1706). **A** lateral; **B** dorsal.

Discussion

DNA barcoding is revolutionising taxonomy research, especially when researchers are dealing with hyper- and cryptic-diverse insect taxa of small body size and variable morphological characters (Fernandez-Triana 2022). Although DNA barcoding is a great tool at hand, it has its own limitations. Various researchers (see Meier et al. (2006), Raupach et al. (2016), Ferrer-Suay et al. (2018) and Pollmann et al. (2023)) have attempted to examine the accuracy of DNA barcodes for species identification and have found discrepancies in the results depending on the targeted taxon. Heteroplasmy, NUMTs, hybridisation, recent speciation, phylogeographic effects, introgression and/or incomplete lineage sorting, endosymbionts and their combinations can all have an effect on sorting of genetic material, as well as simply high variation in the (mostly used) mitochondrial genes. Analysing different (nucleic) loci can equalise some challenges like multiple gene copies (NUMTs) and can help to interpret the actual taxonomic reality more reliably. Still, one of the *major* difficulties to assign a new BIN is the threshold value of difference between two sequences (usually 2% variance in the CO1 sequence). While some species can have intraspecific variation of up to 9.6% (Huemer et al. 2014) and are still considered to be one valid species, other taxa show the opposite: for example, the geometrid taxa *Boudinotiana notha* and *B. touranginii* are known to be two clearly separated species, though both share the same Barcode (Hausmann et al. 2013). It is also worth mentioning that the BIN system is dynamic and that BINs can change over time, depending on the amount of data available. Using an integrative approach, traditional morphology in combination with genetic analyses provides the opportunity to obtain a more accurate hypothesis on the taxonomic status of a taxon. Our study found evidence that the just recently described *Spilomicrus diversus* Chemyreva, 2021 is, indeed, composed of at least two species.

Although we were able to assign a BIN to the described species, *S. diversus*, *S. politus* sp. nov., on the other hand, received two BINs which only differ in 1.74% of the sequences within our dataset. The slim molecular variation in combination with a lack of morphological characters led us to the hypothesis that the two BINs align both with the same species. Therefore, we described only one new taxon, *S. politus*, with the corresponding BINs (BOLD:AER1505 and BOLD:ACZ2358). When first described in 2021, the species *S. diversus* was known to show a highly diverse morphology, as the name suggests. As a consequence of our barcoding results, one paratype had to be excluded from the series.

Another questionable case we faced was *Spilomicrus stigmaticalis* Westwood. While only an insufficient difference could be examined between the two haplotypes of the female, the males could not be distinguished morphologically at all. Interspecific variation was detected to be relatively low, while the intraspecific variation was rather high. Incomplete lineage sorting might be a reason for that, since allopatric/geographic factors, as well as seasonality could be ruled out. Taking both factors, genetics and morphology, into account, we decided to keep those two BINs in one species, *S. stigmaticalis*.

On the other hand, *S. thomsoni* was a relatively clear case. The two BINs (BOLD:ADF4747 BOLD:ADX1651), corresponding with the morphological determination, were genetically close (0.1%), while the taxon could be separated from *S. hemipterus* both genetically and morphologically.

There are still many taxonomic questions remaining regarding the Palearctic species of *Spilomicrus*. The high level of the sexual dimorphism in *S. crassiclavis* (Notton 1999) (only males were included in the current research) and the genetic relatedness of the species reported from Europe and North America (*S. antennatus*) or from the Western Palaearctic and the Eastern Palaearctic (*S. formosus*, *S. crassiclavis*, *S. abnormis*, *S. diversus* and *S. flavipes*) have not been verified yet. It has to be noted that a tree, based on a CO1-barcode alone, cannot be expected to resolve "deep" nodes correctly. Therefore, it is not suprising that, for example, the *formosus* species-group does not appear monophyletic (and if it is, indeed, monophyletic Notton (1999)). Additionally, as is true for many diaprid species, there are not too many host records.

Acknowledgements

We are grateful to Dr. Rune Bygebjerg (MZLU) and Dr. Hege Vårdal (NHRS) for the photos of the type specimens and to Dr. Zoltan Vas (HNHM) and Dr. Claire Villemant (MNHN) for their help and providing the opportunity to examine the type material.

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Supplementary materials

Suppl. material 1: Phylogenetic ML-tree doi

Authors: Huebner J. and Chemyreva V.
Data type: taxonomic tree based on CO1 data
Brief description: Phylogenetic ML-tree of 45 *Spilomicrus* sequences with the outgroup *Labolips innupta*.
Download file (92.93 kb)

Suppl. material 2: Table of localities doi

Authors: Huebner J.
Data type: occurrences
Brief description: This table lists all the location data for each specimen that was caught within the project. Not listed are the lectotypes stored at other museums that were investigated. All the available information is printed on the labels in the image tables.
Download file (106.66 kb)

SECTION 1.3: *Zygota* and *Pantoclis* review

In the past, the genera *Zygota* Förster, 1856 and *Pantoclis* Förster, 1856 have historically been hard to distinguish. We provide a set of characters to clearly tell them apart. As a consequence the following 13 new combinations were established: *Pantoclis brevinervis* (Kieffer, 1909) comb. n., *P. brevipennis* (Kieffer, 1908) comb. n., *P. caecutiens* (Kieffer, 1908) comb. n., *P. cursor* (Kieffer, 1908) comb. n., *P. fossulata* (Thomson, 1858) comb. n., *P. fuscata* (Thomson, 1858) comb. n., *P. hemiptera* (Thomson, 1858) comb. n., *P. microtoma* (Kieffer, 1909) comb. n., *P. soluta* (Kieffer, 1907) comb. n., *P. striata* (Kieffer, 1909) comb. n., *P. subaptera* (Thomson, 1858) comb. n., *P. sulciventris* (Kieffer, 1909) comb. n. and *P. unicolor* (Kieffer, 1908) comb. n. In total, 18 species of the genus *Zygota* were recorded nationwide. One of those, *Z. walli* sp. nov. was described as new to science. Recorded for the first time in Germany are *Zygota balteata* Macek, 1997, *Z. comitans* Macek, 1997, *Z. spinosipes* (Kieffer, 1908), *Z. sordida* Macek, 1997, *Z. angularis* Macek, 1997 and *Z. vigil* Nixon, 1957. *Zygota caligula* Buhl, 1997 is placed in synonymy with *Z. congener* (Zetterstedt, 1840).

Hübner, J., Chemyreva, V. G., Macek, J., & Kolyada, V. A. (2024). A review of the genus *Zygota* (Hymenoptera, Diapriidae) in Germany with taxonomic notes on this genus and its distinction from *Pantoclis*. *ZooKeys*, *1207,* 325-353. https://zookeys.pensoft.net/article/121725/

Pantoclis sp.

ZooKeys

ZooKeys 1207: 325–353 (2024)
DOI: 10.3897/zookeys.1207.121725

Research Article

A review of the genus *Zygota* (Hymenoptera, Diapriidae) in Germany with taxonomic notes on this genus and its distinction from *Pantoclis*

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Abstract

This study provides a comprehensive overview of the genus *Zygota* Förster combining DNA barcoding and current morphology. Nineteen species of *Zygota* were found throughout Germany, including the newly described species *Zygota walli* **sp. nov.** First species records for Germany are: *Zygota balteata* Macek, 1997; *Z. comitans* Macek, 1997; *Z. spinosipes* (Kieffer, 1908); *Z. sordida* Macek, 1997; *Z. angularis* Macek, 1997 and *Z. vigil* Nixon, 1957. We also clarify diagnoses for the two related genera, *Pantoclis* Förster and *Zygota* to designate the boundaries of the *Zygota* genus and propose new synonymies: *Zygota caligula* Buhl, 1997 is a junior synonym of *Z. congener* (Zetterstedt, 1840); *Z. reticulata* Kozlov, 1978 is a junior synonym of *Z. ruficornis* (Curtis, 1831). Thirteen species of *Zygota* sensu Nixon (1957) are transferred to the genus *Pantoclis* with the following new combinations proposed: *Zygota brevinervis* (Kieffer, 1908) (= *Pantoclis brevinervis* (Kieffer, 1909), **comb. nov.**); *Z. brevipennis* (Kieffer, 1908) (= *P. brevipennis* (Kieffer, 1908), **comb. nov.**); *Z. caecutiens* (Kieffer, 1908) (= *P. caecutiens* (Kieffer, 1908), **comb. nov.**); *Z. cursor* (Kieffer, 1908) (= *P. cursor* (Kieffer, 1908), **comb. nov.**); *Z. fossulata* (Thomson, 1858) (=*P. fossulata* (Thomson, 1858), **comb. nov.**); *Z. fuscata* (Thomson, 1858) (= *P. fuscata* (Thomson, 1858), **comb. nov.**); *Z. hemiptera* (Thomson, 1858) (= *P. hemiptera* (Thomson, 1858), **comb. nov.**); *Z. microtoma* (Kieffer, 1909) (= *P. microtoma* (Kieffer, 1909), **comb. nov.**); *Z. soluta* (Kieffer, 1907) (= *P. soluta* (Kieffer, 1907), **comb. nov.**); *Z. striata* (Kieffer, 1909) (= *P. striata* (Kieffer, 1909), **comb. nov.**); *Z. subaptera* (Thomson, 1858) (= *P. subaptera* (Thomson, 1858), **comb. nov.**); *Z. sulciventris* (Kieffer, 1909) (= *P. sulciventris* (Kieffer, 1909), **comb. nov.**), and *Z. unicolor* (Kieffer, 1908) (= *P. unicolor* (Kieffer, 1908), **comb. nov.**).

Key words: Checklist, DNA-barcoding, integrative taxonomy, new records, new species, new synonymy, parasitoid wasps

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Introduction

This article deals with the parasitoid wasps of the genus *Zygota* Förster (Diapriidae, Belytinae, Belytini), comprising mostly medium-sized (2.5–4.0 mm long) melanic and pubescent specimens with brightly colored appendages.

The genus has 75 described species worldwide, of which most are described from the Palearctic and Nearctic (Johnson 1992; Buhl 1995, 1997, 1998; Macek 1997). Although common, little is known about their biology and their hosts. In the past, morphology-based taxonomy of *Zygota* led to confusion and many reinterpretations of the genus. The generic diagnosis, key to the species of Central Europe, and diagnostic remarks based on available types were given by Macek (1997). According to the original description of the genus given by Förster (1856) *Zygota* can be easily distinguished from other Belytinae genera by the strengthened marginalis, open radial cell, and emarginated fore tibiae in males (Förster 1856). Förster's vague diagnosis was misinterpreted by the later authors Ashmead (1893, 1902) and Kieffer (1909), which Macek (1997, 2007) has pointed out in his revisionary works. He clarified the identity based on the designation of the neotype of *Zygota abdominalis* (Nees, 1834), and completed a revision of available types. However, the boundary between *Zygota* and its sister genus *Pantoclis* Förster is still unclear, as some species remained falsely placed inside *Zygota*. Nixon (1957) and later Kozlov (1978) placed all Belytini species with an open radial cell and unpunctured scutellum [except some few *Belyta* species (Macek 1995)] in the genus *Zygota*. The same genus concept was applied in Johnson's (1992) world catalog. Although the diagnosis of the genus *Zygota* was given by Macek (1997), the generic affiliation of many species was not discussed. For example, the taxonomy of the 14 species from 39 Palearctic species of *Zygota* listed by Johnson (1992) is still questionable. The genus *Pantoclis* has never been defined conclusively to exclude it from other Belytinae, because the diversity and lack of knowledge of *Pantoclis* species makes it extremely difficult to define. To understand the genus concept of *Zygota*, it must be distinguished from *Pantoclis*. We will, therefore, present a diagnosis for each.

Currently, there are 38 known species of *Zygota* in the Palearctic Region (Johnson 1992, Buhl 1995, 1997, Macek 1997). Full taxonomic treatments of the genera are given by Macek (1997) (only *Zygota*) and cataloged by Johnson (1992) (both, *Zygota* and *Pantoclis*). Macek (1997) has given a taxonomic interpretation only for 18 of these species. The present study thus aims to clarify the diagnosis of *Zygota* and the taxonomic position of the remaining 20 species, which are not discussed in Macek (1997). This revision is mostly based on material collected in Bavaria, Germany, in the framework of the German Barcode of Life (GBOL) III: Dark Taxa project (Hausmann et al. 2020). The most recent diversity evaluation that has been conducted for Germany was done over twenty years ago by Blank (2001). In his work, twenty *Zygota* taxa were recovered, of which two, *Z. excisipes* (Kieffer, 1916) and *Z. norvegica* (Kieffer, 1912), have been synonymized with *Z. excisor* (Zetterstedt, 1840) and *Z. ruficornis* (Curtis, 1831), respectively. For *Zygota subclausa* (Kieffer, 1907), Macek (1995, 1997) proposed the new combination *Belyta subclausa* (Kieffer, 1907). In total, 19 species of *Zygota* were reliably identified for the German fauna.

Material and methods

Most of the examined material was collected within the GBOL III project as well as from earlier collecting events in Bavaria and Baden-Wuerttemberg (Germany) led by the Bavarian State Collection of Zoology in Munich (SNSB-ZSM). Further

material originates from the collection of the National Museum in Prague (NMPC) and the Russian collections in St. Petersburg (ZISP). In addition, type material from the Zoological Museum in Copenhagen (ZMUC) and the Natural History Museum (NHM) in London was examined. All specimens were morphologically identified as far as possible, including the closely related genus *Pantoclis*. Afterwards, individuals were Sanger sequenced under the usage of a voucher recovery approach. The genetic information was obtained at the Canadian Centre for DNA Barcoding (CCDB) in Guelph by the application of a voucher recovery protocol (https://ccdb.ca/). All mitochondrial CO1 sequences were aligned in MEGA11 (Tamura et al. 2021), and the alignment was then used to construct maximum likelihood trees with the online program IQ TREE version 2.0 (Trifinopoulos et al. 2016) using the default settings (1000 bootstrap alignments, substitution model: TIM+F+I+G4, 1000 iterations). Editing was done using FIGTREE version 1.4.4 (Rambaut 2010) and INKSCAPE version 1.1.1 (2021, available from: https://inkscape.org/de). Clustering and BIN-distance-analyses were conducted to infer species barriers among the CO1 barcodes using MEGA11 as well as ASAP (Puillandre et al. 2021). Suppl. material 3 gives an overview of the genetically examined material and the clustering results. All molecular data and collection metadata are publicly available on the Barcode of Life Data System (BOLD) platform (http://www.barcodinglife.org, Ratnasingham and Hebert 2007) in the dataset [DS-ZYGPAN dx.doi.org/10.5883/DS-ZYGPAN]. It is important to note that analysis was conducted on data that was downloaded from BOLD on 27 February 2024. Therefore, the results are based on the BIN-statuses of that time.

The morphological terminology and abbreviations follow those proposed by Yoder (2004) and as used in Hymenoptera Anatomy Ontology (Yoder et al. 2010); the measurements follow Yoder (2004) and Chemyreva (2015, 2018). Terms of relative position follow Goulet and Huber (1993). The terms of sculpture description follow Eady (1968). The accurate taxonomic treatments of the genera and species *Zygota* and *Pantoclis* are given in Macek (1997) and Johnson (1992). Taxa that have received an updated taxonomic treatment, such as new species or synonyms, are newly diagnosed here. Sufficiently detailed diagnoses for all other species were given by Macek (1997). The general distribution of species was obtained and updated from Blank (2001), Wall (1963), Buhl (1995, 1997), Macek (1997), and Chemyreva et al. (2023). New records are marked with an asterisk (*). The following abbreviations for locations in Germany are used: BW= Baden-Württemberg, BY= Bavaria. Museum acronyms: SNSB-ZSM – Bavarian State Collection of Zoology, Munich; ZISP – Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia; ZMUC – Zoological Museum, University of Copenhagen. A series of images were taken using an Olympus OM-D camera mounted on a Leica M125 C binocular and stacked using HELICON FOCUS (Version 8).

Taxonomy

Genus *Pantoclis* Förster, 1856

Type species. *Pantoclis barycera* Förster, 1861 (Figs 1A, B, 5E).

Diagnosis. Body black to yellowish brown; males macropterous, females alate to brachypterous or wingless; occipital carina always with occipital pit

(Fig. 1B, red arrow); fore tibiae of males always unmodified with homogeneous pubescence (Fig. 3H); submetapleural carina usually present, complete (Fig. 2A, green arrow) [if submetapleural carina missing, then venation as described below]; radial cell open to closed, variable in shape (Fig. 14); radialis not parallel to parastigma [if parallel (Fig. 3G, J) then angle between stigmal and marginal veins as described below]; angle between stigmal and marginal veins 130 degrees (Fig. 3G, J) or more; S2 always smooth, without punctured area on it in anterior half (Fig. 4F); male genitalia usually slender, apex of aedeagus distinctly convex (Fig. 5I–L), lanceolate (Fig. 5F–L), rather truncate (Fig. 5E) [if genitalia short and stout with rounded aedeagus then fore wing with a closed radial cell], digitus usually diminished (Fig. 5E–L) [if not then fore wing with closed radial cell]; ovipositor usually long, at least as long as length of T2 [if ovipositor short then fore wing with closed radial cell].

Genus *Zygota* Förster, 1856

Zygota Förster, 1856: 128, 131, 133, 135. Type species: *Belyta abdominalis* Nees van Esenbeck, designated by Ashmead (1893).

Carinia Kieffer, 1905: 140. Type: *Carinia nitida* Kieffer, by monotypy and original designation. Synonymized with *Aclista* Förster by Kieffer (1910), with *Zygota* Förster by Muesebeck (1951).

Diagnosis. Body always black (only metasoma very rarely brown); males and females alate; occipital carina with or without occipital pit (Fig. 1C–F, red arrows); fore tibiae modified in some males or bear several stiff setae (Fig. 3B, E, F, I); submetapleural carina missing (Fig. 2B), or reduced; radial cell long, open at apex (except *Z. croton* Fig. 3C); radialis long and almost parallel to parastigma (Fig. 3D); angle between stigmal and marginal veins at most 120 degrees; some species with small depression (Fig. 4B) or micro-puncture sculpture on S2 in anterior half (Fig. 4A, C–E, green arrows); male genitalia short and stout, apex of aedeagus truncate or rounded, digitus large (Fig. 5A–D); complete ovipositor always short, at most as long as pygidium (8^{th} + 9^{th} tergite above, 7^{th} sternite below).

Remarks. Based on the diagnoses and original descriptions of the species *Zygota caecutiens* (Kieffer, 1908), Z. *hemiptera* (Thomson, 1858), Z. *microtoma* (Kieffer, 1909), Z. *soluta* (Kieffer, 1907) and the generic diagnoses of *Zygota* and *Pantoclis*, these four species should be excluded from *Zygota* and considered as part of *Pantoclis*; *Pantoclis caecutiens* (Kieffer, 1908), comb. nov., *P. hemiptera* (Thomson, 1858), comb. nov., *P. microtoma* (Kieffer, 1909), comb. nov. and *P. soluta* (Kieffer, 1907), comb. nov. Moreover, based on the study of the type specimens the following species are transferred from *Zygota* to *Pantoclis*: *Pantoclis brevinervis* (Kieffer, 1909), comb. nov., *P. brevipennis* (Kieffer, 1908), comb. nov., *P. cursor* (Kieffer, 1908), comb. nov., *P. fossulata* (Thomson, 1858), comb. nov., *P. fuscata* (Thomson, 1858), comb. nov., *P. striata* (Kieffer, 1909), comb. nov., *P. subaptera* (Thomson, 1858), comb. nov., *P. sulciventris* (Kieffer, 1909), comb. nov. and *P. unicolor* (Kieffer, 1908), comb. nov. (see also Suppl. material 2 for an overview of type locations and the museums where the specimens are stored).

***Zygota abdominalis* (Nees, 1834)**
Figs 1D, E, 4B, 5B–D

Belyta abdominalis Nees, 1834: 344, male.
Zygota abdominalis: Macek 1997: 37, male, female, neotype designation.

BOLD BIN. BOLD:AEJ6743.

Material examined. GERMANY: BY: NGS Schwarzes Moor, 09-Aug-2017, 1 ♂; Paehl, 21-Mar-2020, 24-Apr-2020, 4 ♂; Ammer mountains, 27-Aug-2016, 1 ♂; Kehlheim, 10-Apr-2017, 1 ♂; Balderschwang, 21-Sept–12-Oct-2017, 1 ♀, 4 ♂; Kehlheim, 23-Aug–08-Sept-2017, 1 ♂; NSG Romberg, 18-May–09-Jun-2018, 2 ♂; Paehl, 24-Apr–08-May-2020, 7 ♂; Rhoen mountains, 27-Jun–11-Jul-2018, 2 ♂; Ketterschwang, 01–16-Jul-2019, 1 ♂; Grafenreuth, 01–15-Jul-19, 4 ♂. BW: Malsch, 27-Jun–09-Jul-2011, 2 ♂; Gaggenau-Sulzbach, 02–21-Aug-2011, 1 ♀.

Distribution. Europe: Czech Republic, Germany, Poland, Russia (European part).

***Zygota angularis* Macek, 1997**

Zygota angularis Macek, 1997: 54, male, female.

BOLD BIN. BOLD:ACQ5437.

Material examined. GERMANY: BY: Mittenwald, 30-Jul-2021, 1 ♂; Rhoen mountains, 11-Jul-2018, 3 ♂.

Distribution. Europe: Czech Republic, Germany*, Slovenia.

***Zygota balteata* Macek, 1997**

Zygota balteata Macek, 1997: 40, male, female.

BOLD BIN. No BIN.

Material examined. GERMANY: BY: NSG Fellinger Mountain, 08-Jun-2013, 1 ♀, Grafenaschau, 2013, 1 ♀.

Distribution. Europe: Czech Republic, Germany*, Slovenia.

***Zygota breviuscula* (Thomson, 1858)**
Figs 2B, 3E, 4A

Belyta breviuscula Thomson, 1858: 176, female.
Aclista sulcata Kieffer, 1909. Synonymized by Macek (1997).
Zygota larides Nixon, 1957. Synonymized by Macek (1997).

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Ammer mountains, 05-Oct-2016, 1 ♀; Oberstdorf, 10–24-Jul-2016, 24-Jul-2016 and 28-Jun-2016, 15 ♂.

Distribution. Europe: Austria, Czech Republic, Germany, Hungary, Italy, Russia (European part), Slovenia, Sweden.

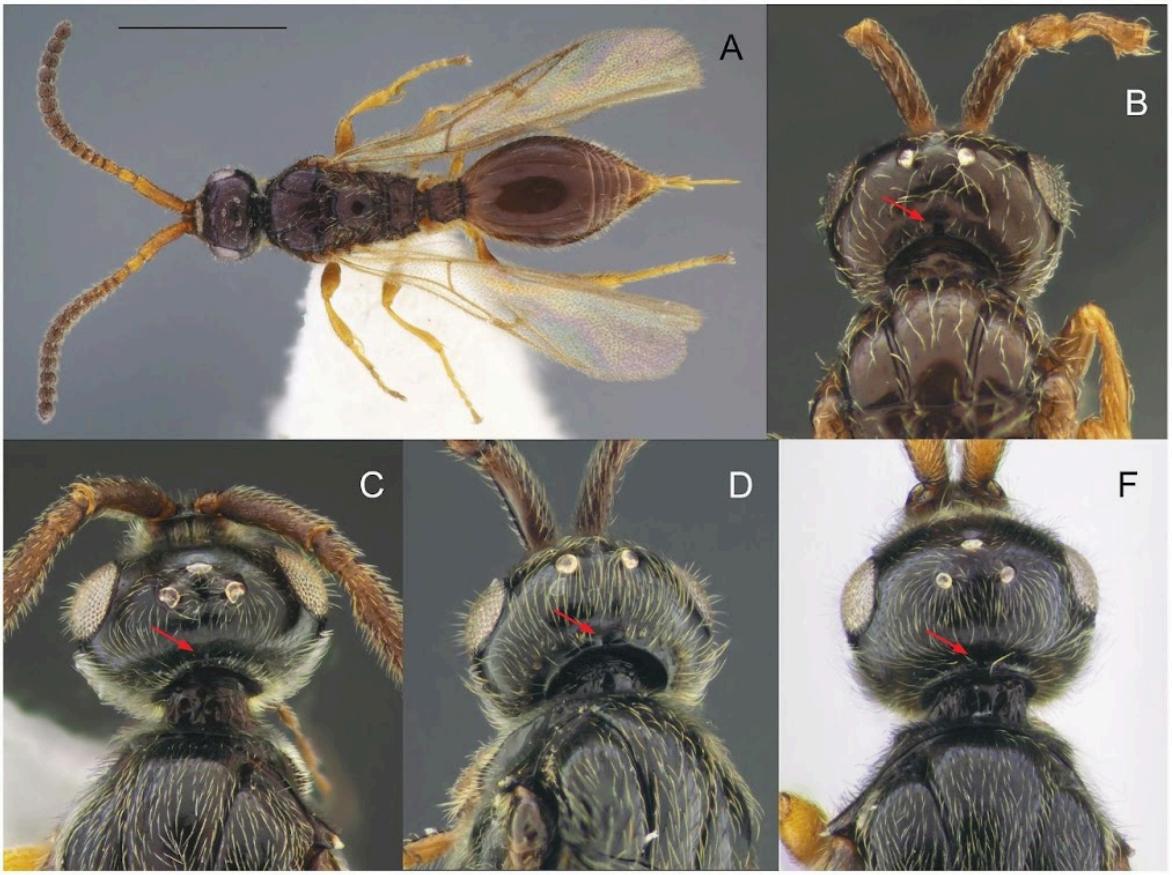

Figure 1. Morphological characters to identify the closely related genera *Zygota* and *Pantoclis* **A, E** female **B, C, D** males **A, B** *P. barycera* **C** *Z. walli* sp. nov. **D, E** *Z. abdominalis*. Scale bars: 1 mm (**A**); 0.5 mm (**B–F**).

Zygota claviscapa (Thomson, 1858)

Belyta claviscapa Thomson, 1858: 175, female, male.
Aclista brevicornis Kieffer, 1909. Synonymized by Macek (1997).

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Garmisch-Partenkirchen, 2–13-Aug-2018, 3 ♂; Oberstdorf, 28-Jun-2016, 2 ♂; Grafenreuth, 1–15-Jul-19, 1 ♂.

Distribution. Europe: Austria, Czech Republic, England, Germany, Hungary, Ireland, Poland, Russia (European part), Scotland, Slovenia, Sweden.

Zygota comitans Macek, 1997

Zygota comitans Macek, 1997: 47, female, male.

BOLD BINs. BOLD:AEL3896, BOLD:AEJ0891.

Material examined. GERMANY: BY (BOLD:AEL3896): Moos, Isarmuendung, Hartholzauwald, 16-Jun-2021, 1 ♂; Chiemgauer Alpen, Ruhpolding, Fischbach, 02-Aug-2016, 1 ♂; Paehl, 24-Apr-2020, 1 ♂. BY (BOLD:AEJ0891): Berchtesgaden,

Figure 2. Morphological characters to identify the closely related genera *Pantoclis* (**A**) and *Zygota* (**B**) **A** *Pantoclis* spp., male **B** *Z. breviuscula*, male. Green arrow – submetapleural carina. Scale bars: 0.3 mm.

Bartholomae, NP Berchtesgarden, Wald, 13-Sep-2017, 1 ♀; Gaggenau, Michelbach, 21-Aug-2011, 1 ♀; Paehl, Niedermoor w Goasl, 19-Sep-2020, 1 ♀. BY (unsequenced material): Rhoen mountains, 27-Jun–11-Jul-2018, 3 ♂; Grafenaschau, 2013, 1 ♂; Oberstdorf, 28-Jun-2016, 1 ♂.

Distribution. Finland, Germany*, Poland, Slovenia, Sweden.

***Zygota congener* (Zetterstedt, 1840)**
Figs 6A–F, 7A–F

Psilus (*Belyta*) *congener* Zetterstedt, 1840: 415, female, male.
Zygota caligula Buhl, 1997: 53, female. Syn. nov.

BOLD BIN. BOLD:AAI8609.

Material examined. ***Holotype*** of *Zygota caligula*: Norway: Mosvik, 14-Aug-1994, "MT. JT:19", "Smafa", P.N. Buhl det. 1996, Holotype, ZMUC 00021242, Zygota caligula, 1 ♀. Germany: BY: Garmisch-Partenkirchen, 02-Aug-2018, 13-Aug-2018, 09-Oct-2018, 4 ♂; Grafenaschau, 2013, 1 ♂ (Fig. 6E)

Diagnosis. Both sexes: postmarginal vein distinctly shorter than radial cell length (Fig. 7F); occipital pit present; mesopleuron with only small bare area medially or entirely pubescent (Fig. 6D); axillar depression with scattered setae and only 2 verriculate tubercles; propodeal spiracle distinctly enlarged (Fig. 6A); base of T2 with lateral corners (Fig. 6A); S2 without micro-puncture sculpture anteriorly. **Female:** female antenna with A6–A14 about 1.25 times as long as wide (Fig. 7B, C); T2 punctuated (Fig. 7B, C); T8 (apical) with median keel between cerci (Fig. 6E). **Male:** A3 strongly emarginate (Fig. 6B); fore tibia slightly modified, weakly humped interiorly, entirely pubescent and with a row of enlarged setae along its inner side (Fig. 6C); genitalia as in *Z. walli* sp. nov. and *Z. abdominalis* (Fig. 5A–D), digitus armed with 3 or 4 teeth.

Remarks. The female of *Zygota congener* is best recognized by the large propodeal spiracles (Fig. 6A) and the sharp median keel between the cerci on the apical tergite of the female (Fig. 6E). These two characters, together with other peculiarities of the morphology of *Z. congener*, correspond to the

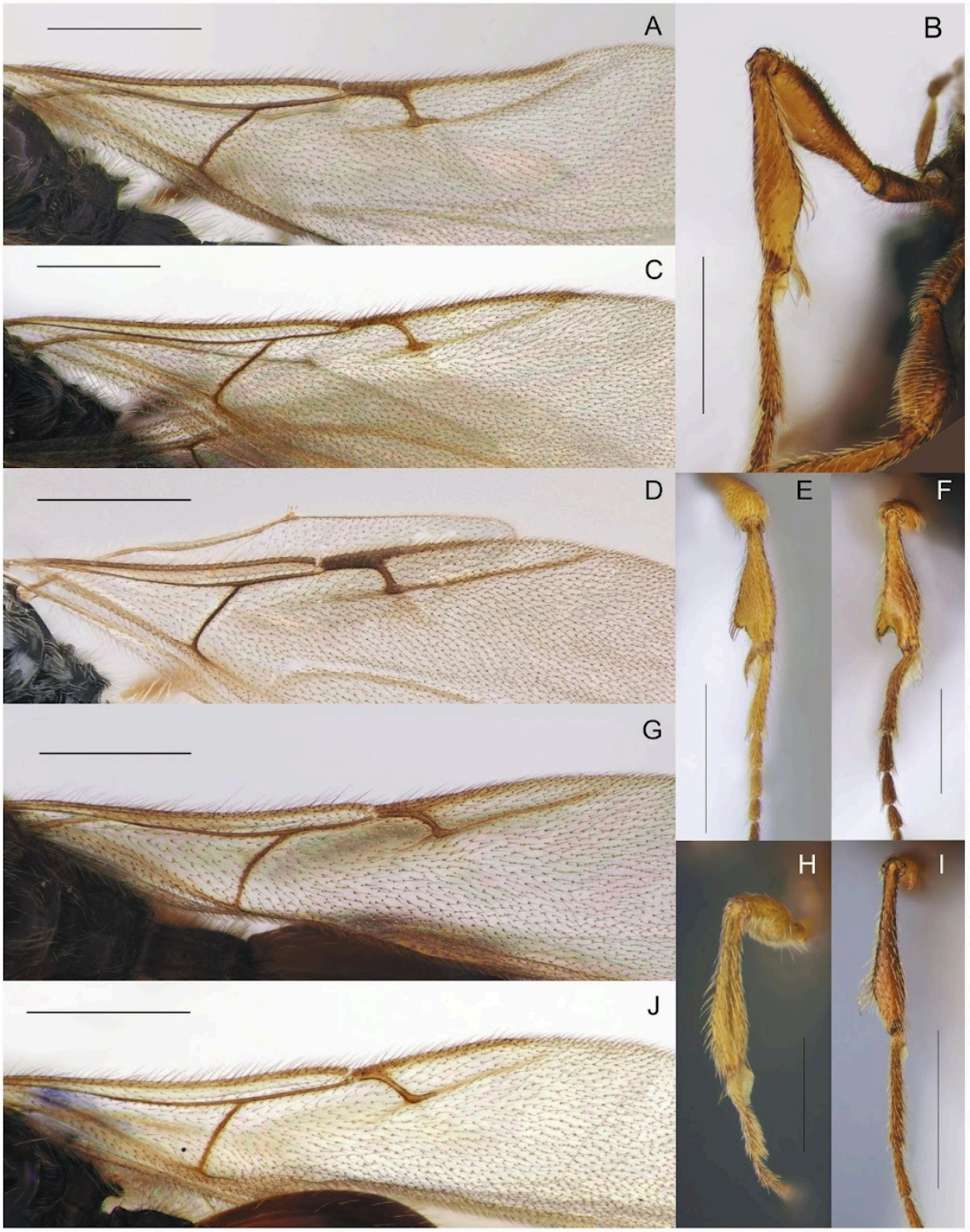

Figure 3. Venation (**A, C, D, G, J**) and fore tibia (**B, E, F, H, I**) morphology of males **A** *Zygota bensoni* **B** *Z. sordida* **C** *Z. croton* **D** *Z. walli* sp. nov. **E** *Z. breviuscula* **F** *Z. walli* sp. nov. **H** *Pantoclis* sp. **I** *Z. croton* **G, J** *Pantoclis* spp. Scale bars: 0.5 mm (**A–E**, **G, I, J**); 0.3 mm (**F, H**).

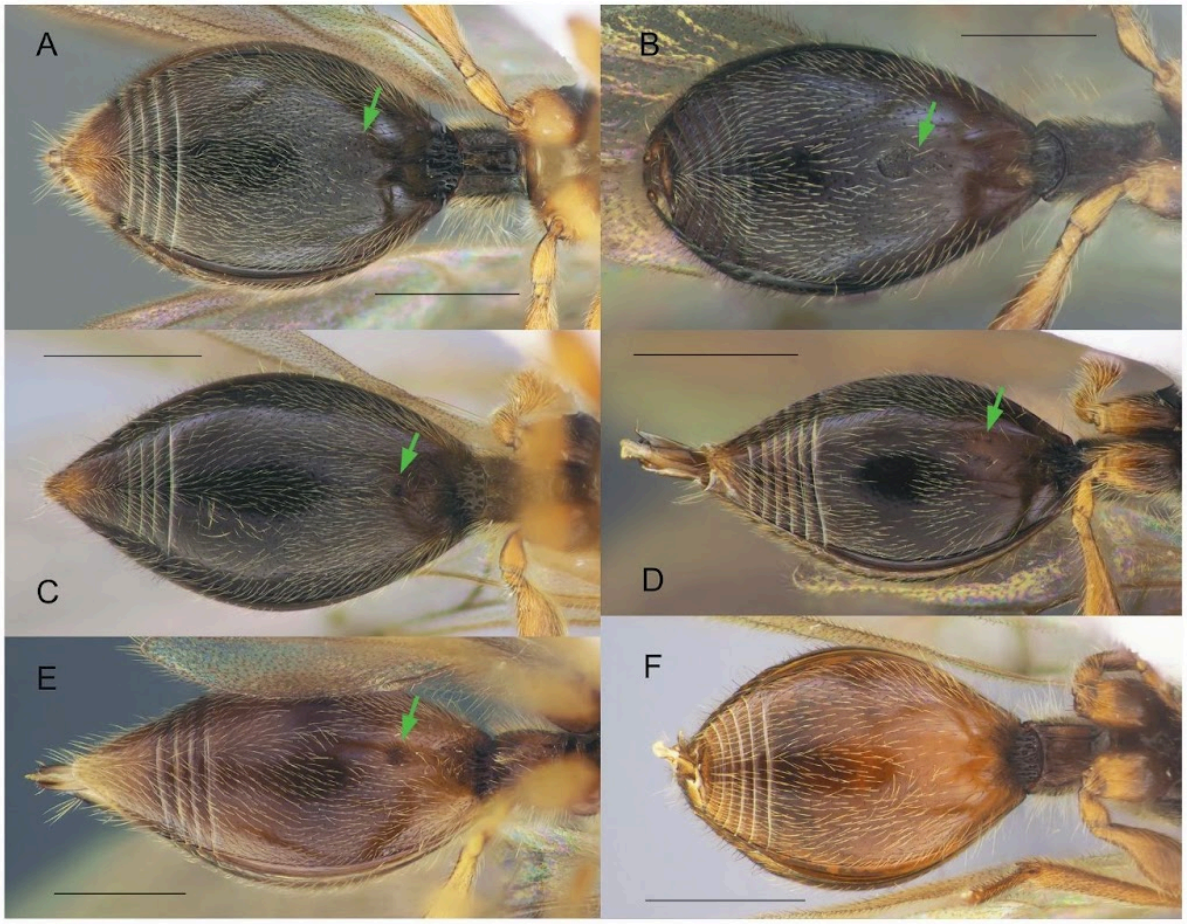

Figure 4. Ventral side of metasoma of females (**A, C, E**) and males (**B, D, F**) **A** *Zygota breviuscula* **B** *Z. abdominalis* **C, D** *Z. pubescence* **E** *Z. walli* sp. nov. **F** *Pantoclis* sp. Scale bar: 0.5 mm.

characters of the holotype of *Z. caligula* Buhl. For this reason, *Z. caligula* is considered here to be a junior synonym of *Z. congener*.

Distribution. Austria, Czech Republic, Denmark, Finland, Germany, Russia (European part), Slovenia, Sweden.

Zygota croton Nixon, 1957

Fig. 3C, I

Zygota croton Nixon, 1957: 29, 62, male, female.

BOLD BIN. BOLD:AEK1965.

Material examined. GERMANY: BY: Mittenwald, 30-Jul-2021, 1 ♂; Garmisch-Partenkirchen, 05-Jul-2018, 18-Jul-2018, 02-Aug-2018, 13-Aug-2018, 1 ♀, 16 ♂; Oberstdorf, 10-24-Jul-2016, 1 ♂.

Distribution. Europe: Austria, Czech Republic, France, Germany, Russia (European part), Scotland, Slovenia, Sweden.

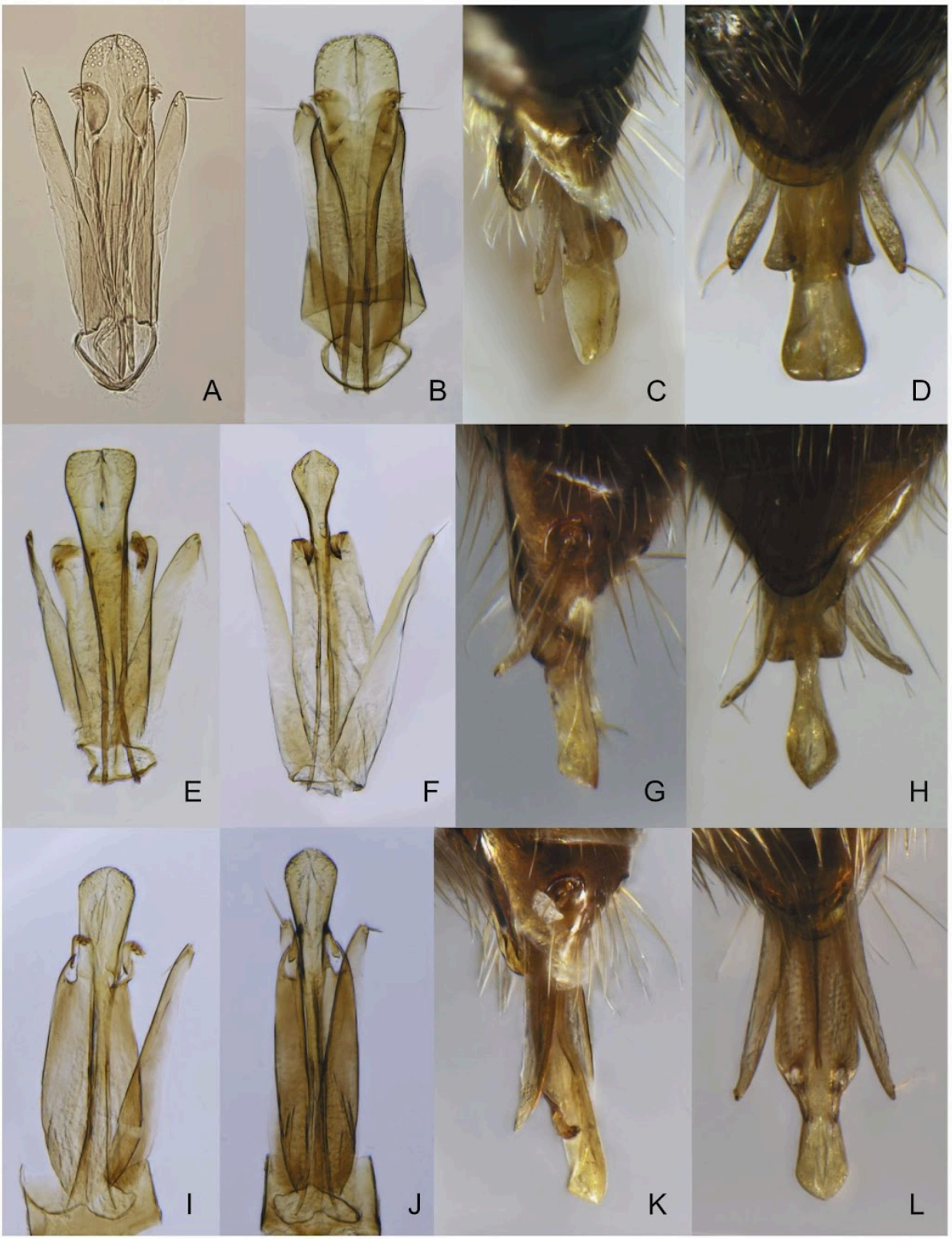

Figure 5. Male genitalia of *Zygota* and *Pantoclis* **A** *Z. walli* sp. nov. **B–D** *Z. abdominalis* **E** *P. barycera* **F–H** *Pantoclis* sp. 1 **I–L** *Pantoclis* sp. 2 **C, G, I, K** lateral view **A, B, D, E, F, H, J, L** ventral view.

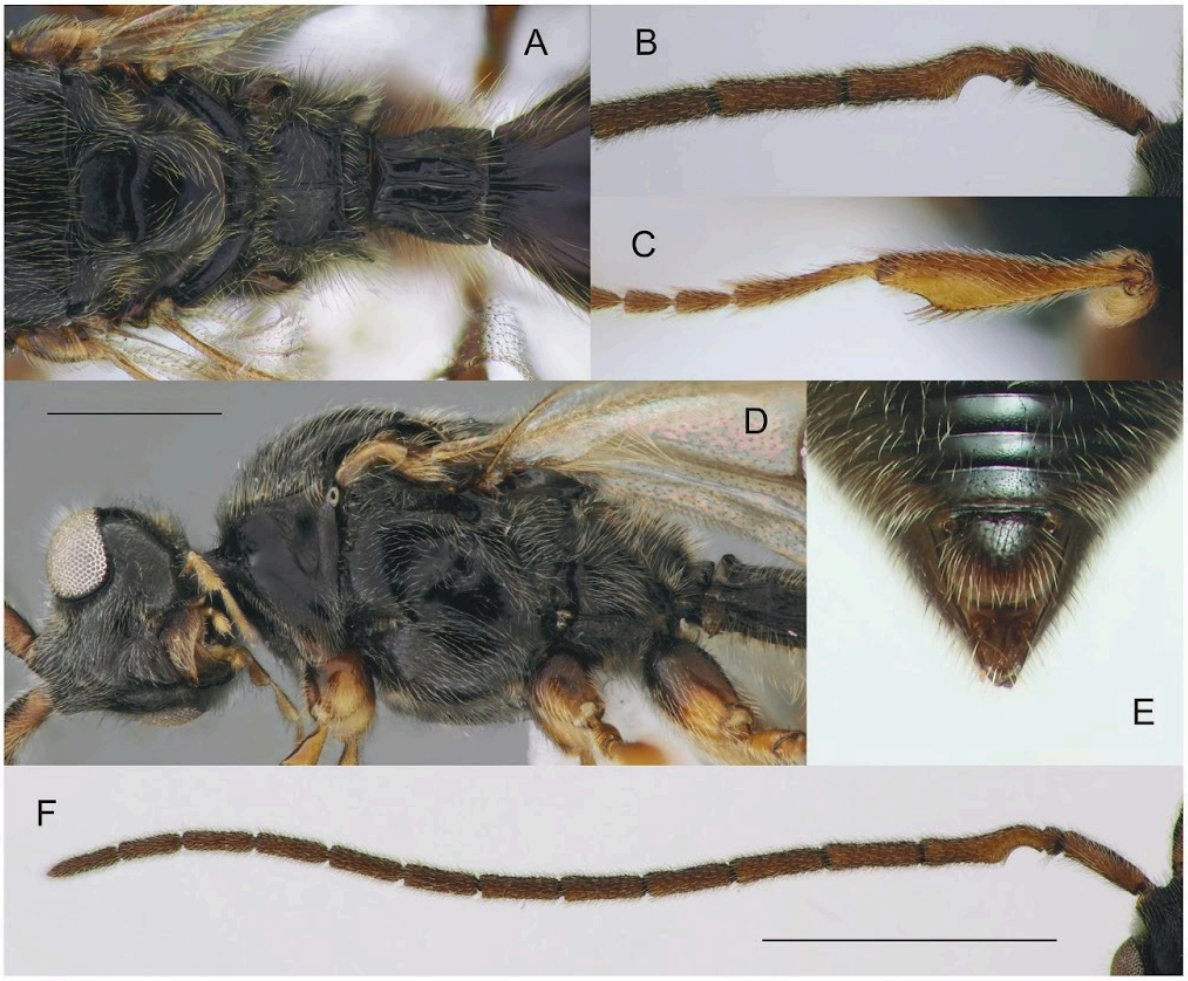

Figure 6. *Zygota congener*, male (**B–D, F**) and female (**A, E**) **A** mesosoma and petiole in dorsal view **B** A1–A5 in ventral view **C** fore tibia **D** head and mesosoma in lateral view **E** apex of metasoma in dorsal view (*Z. caligula* Buhl, holotype) **F** antennae in ventral view. Scale bars: 0.5 mm (**D**); 1 mm (**F**).

***Zygota excisor* (Zetterstedt, 1840)**

Psilus (*Belyta*) *excisor* Zetterstedt, 1840: 415, male.
Aclista lanceolata Kieffer, 1909. Synonymized by Macek (1997).
Aclista lanceolata var. *fuscicornis* Kieffer, 1909. Synonymized by Macek (1997).
Aclista semirufa Kieffer, 1909. Synonymized by Macek (1997).
Aclista (*Zygota*) *excisipes* Kieffer, 1908. Synonymized by Macek (1997).

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Lohr am Main, 06-Sep-2016, 1 ♂; Rhoen mountains, 11-Jul-2018, 1 ♂; Oberstdorf, 28-Jun-2016, 1 ♀; Ruhpolding, 19-Jul-2016, 1♂; Garmisch-Partenkirchen, 13-Aug-2018, 1 ♂.

Distribution. Europe: Austria, Czech Republic, Germany, Hungary, Italy, Poland, Russia (European part), Slovenia, Sweden.

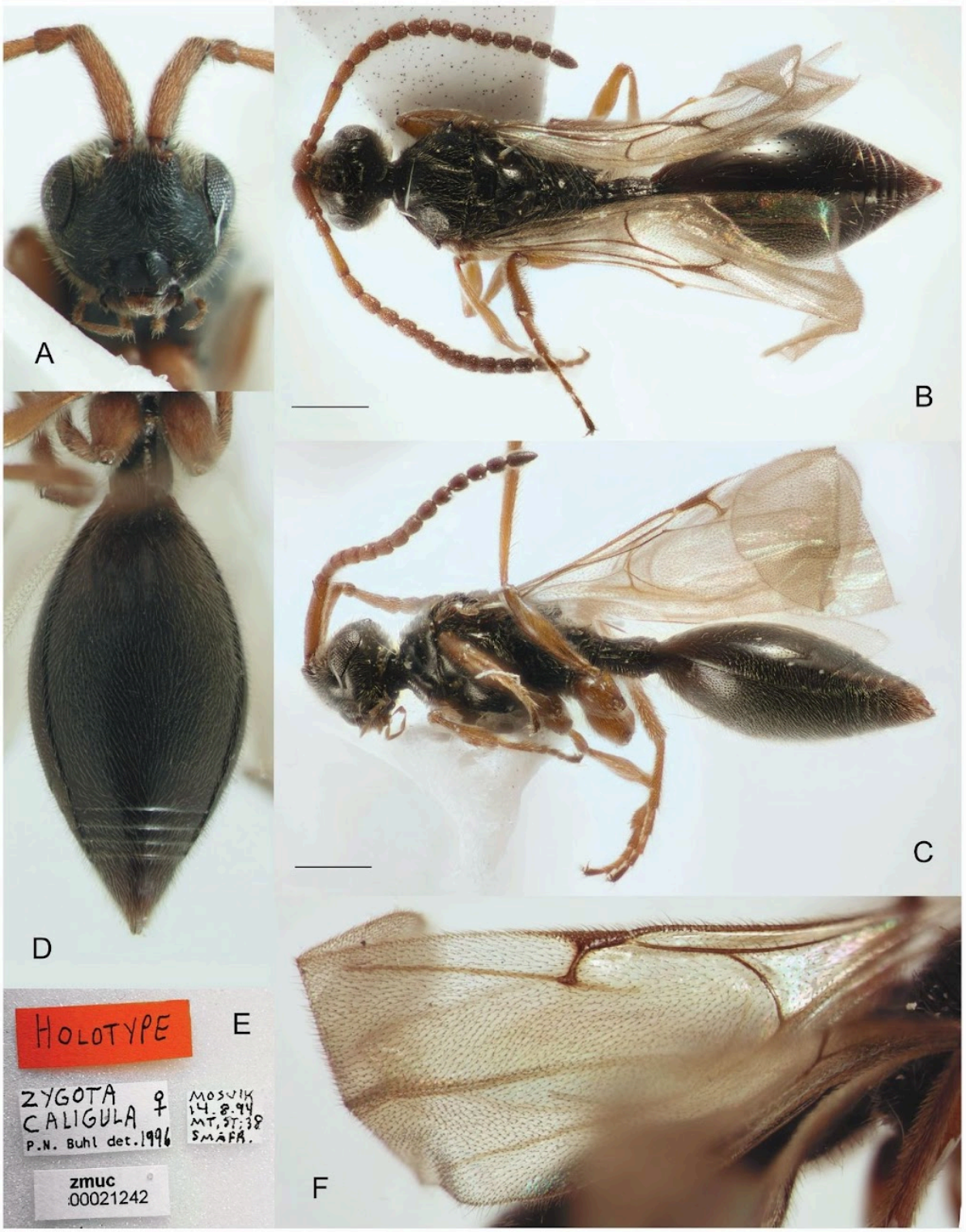

Figure 7. Holotype of the *Zygota caligula* Buhl **A** face **B** body in dorsal view **C** body in lateral view **D** metasoma, ventral view **E** type material labels **F** fore wing venation. Scale bar: 0.5 mm.

***Zygota nigra* (Thomson, 1859)**

Belyta nigra Thomson, 1859: 175, female.
Aclista lanceolata Kieffer, 1909. Synonymized by Macek (1997).

BOLD BIN. BOLD:AEJ4945.

Material examined. GERMANY: BY: Mittenwald, 30-Jul-2021, 3 ♂, 1 ♂; Garmisch-Partenkirchen, 05-Jul-2018, 13-Aug-2018, 11-Sep-2018, 3 ♂.

Distribution. Europe: Algeria, Czech Republic, Germany, Russia (European part), Slovenia, Sweden.

***Zygota parallela* (Thomson, 1859)**

Belyta parallela Thomson, 1859: 175, male.
Aclista macroneura Kieffer, 1909. Synonymized by Macek (1997).

BOLD BINs. BOLD:ACU1498, BOLD:AEJ0893.

Material examined. (BOLD:ACU1498) GERMANY: BY: Berchtesgaden, 11-Jun-2017, 3 ♂; Rhoen mountains, 27-Jun–11-Jul-2018, 2 ♀, 1 ♂; NSG Metzgergraben, 25-Jun-2016, 1 ♂; NSG Metzgergraben, 10–25-Jun-2016, 10 ♀, 37 ♂; Oberstdorf, 24-Jul-2016, 1 ♀, 17 ♂; Oberstdorf, 28-Jun-2016, 12 ♂; Siegenburg, 08–26-May-2017, 4 ♂; Grafenreuth, 01–15-Jul-2019, 1 ♀, 1 ♂; Paehl, 24-Apr-08-May-2020, 6 ♂; Rhoen mountains, 27-Jun-18-Jul-2018, 10 ♂; NSG "Schwarzes Moor", 26-Jun–18-Jul-2017, 4 ♂. Material examined (BOLD:AEJ0893). GERMANY: BY: Sugenheim, 24-May-2021, 1? (ZSM-HYM-42355-A04); Garmisch-Partenkirchen, 13-Aug-2018, 1 ♀; Markt Nordheim, 02-May-2019, 1 ♂.

Distribution. Europe: Austria, Czech Republic, Germany, Hungary, Poland, Scotland, Slovenia, Sweden.

***Zygota praetor* Nixon, 1957**

Zygota praetor Nixon, 1957: 58, 62, male, female.

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Oberstdorf, 24-Jul-2016, 1 ♂.

Distribution. Europe: Czech Republic, Denmark, Germany, Ireland, Slovenia, Sweden.

***Zygota pubescens* (Kieffer, 1909)**
Fig 4C, D

Aclista lanceolata var. *pubescens* Kieffer, 1909: 473. Female.
Pantoclis cameroni: Kieffer 1907. Synonymized by Macek (1997).

BOLD BIN. BOLD:ACC4346.

Material examined. GERMANY: BY: Mittenwald, 13-Jul-2021, 1 ♂; Paehl, 21-Mar-2020, 24-Apr–08-May-2020, 2 ♀, 1 ♂; Ketterschwang, 01–16-Jul-2019, 1 ♂; Balderschwang, 21-Sep–12-Oct-2017, 3 ♂; Rhoen mountains, 27-Jun–11-Jul-2018, 5 ♂; Garmisch- Partenkirchen, 02-Aug-2018, 1 ♀; NSG Allacher Lohe, 01-Sep-2021, 1 ♂; NSG Allacher Lohe, Munich, 08-Jun–23-Jun-2021, 3 ♂; NSG Metzgergraben, 10–25-Jun-2016, 2 ♂; Siegenburg 08–26-May-2017, 2 ♂; Oberstdorf, 10–24-Jul-2016, 2 ♂.

Distribution. Europe: Austria, Czech Republic, Germany, Italy, Russia (European part), Scotland, Slovenia, Sweden.

Zygota ruficornis (Curtis, 1831)

Fig. 8A–I

Cinetus ruficornis Curtis, 1831: 380, female.
Aclista dentatipes Kieffer, 1908: 447. Synonymized by Macek (1997).
Aclista norvegica Kieffer, 1912: 20. Synonymized by Macek (1997).
Zygota reticulata Kozlov, 1978: 575, female. Syn. nov.

BOLD BINs. BOLD:AEX2887, BOLD:AEK5610, BOLD:AEY0233.

Material examined. ***Holotype*** of *Zygota reticulata*: RUSSIA: Kola Peninsula, Lake Vud'yavr basin, Khibiny Mountains, Kol'sk Mt., 18-Jun-1931, Fridolin leg., 1 ♀ (Fig. 8I). GERMANY: BY (BOLD:AEX2887): Mittenwald, 30-Jul-2021, 1 ♂. BY (BOLD:AEY0233): Paehl, 08-May-2020, 1 ♂; Mittenwald, 13-Jul-2021, 1 ♂. BY (BOLD:AEK5610): Mittenwald, 30-Jul-2021, 3 ♂; Garmisch-Partenkirchen, 18-Jul-2018, 02-Aug-2018, 4 ♂. BY (unsequenced material): Garmisch-Partenkirchen, 05-Jul-2018, 18-Jul-2018, 02-Aug-2018, 09-Oct-2018, 4 ♂; Garmisch-Partenkirchen, 13-Aug-2018, 1 ♀, 9 ♂; Bad Windsheim, 12-Jul-2020, 1 ♂; Aub, 21-May-2020, 1 ♂; Grettstadt, 20-May-2020, 1 ♀; Oberstdorf, 28-Jun-2016, 1 ♀, 6 ♂; Rhoen mountains, 27-Jun-11–Jul-2018, 21 ♂; Grafenreuth, 01–15-Jul-2019, 7 ♂; NSG Metzgergraben, 10–25-Jun-2016, 15 ♂; NSG Romberg, 18-May–09-Jun-2018, 3 ♂; Ketterschwang, 01–16-Jul-2019, 3 ♂; Siegenburg, 08–26-May-2017, 2 ♂; Garmisch-Partenkirchen, 02–13-Aug-2018, 2 ♂; NSG "Schwarzes Moor", 26-Jun–18-Jul-2017, 2 ♂; Paehl, 24-Apr–08-May-2020, 2 ♂; Kehlheim, 29-Jun–13-Jul-2017, 1 ♂; Lohr a. M., 03–14-Jun-2018, 1 ♂; NSG Allacher Lohe, Munich, 08–23-Jun-2021, 1 ♂. BW (unsequenced material): Malsch, 27-Jun–09-Jul-2011, 1 ♀, 4 ♂.

Diagnosis. Both sexes: postmarginal vein distinctly shorter than radial cell length (Fig. 8F); occipital pit present; mesopleuron with only small bare area on it medially or entirely pubescent (Fig. 8D); axillar depression with scattered setae and only 2 verriculate tubercles; base of T2 with small lateral corners (Fig. 8A). **Female:** T2 finely granulate (Fig. 8A); T8 without transverse or elongate carinae on it (Fig. 8B); S2 with a small pit in anteriorly half (as in Fig. 4C, green arrow). **Male:** A3 weakly emarginate (Fig. 8H); fore tibia broadened, with sharp projection and a row of strong setae on the top of it, bare at the apex on its anterior surface (Fig. 8G); S2 with a small area of micropuncture in anteriorly half (as in Fig. 4E, green arrow); digitus armed

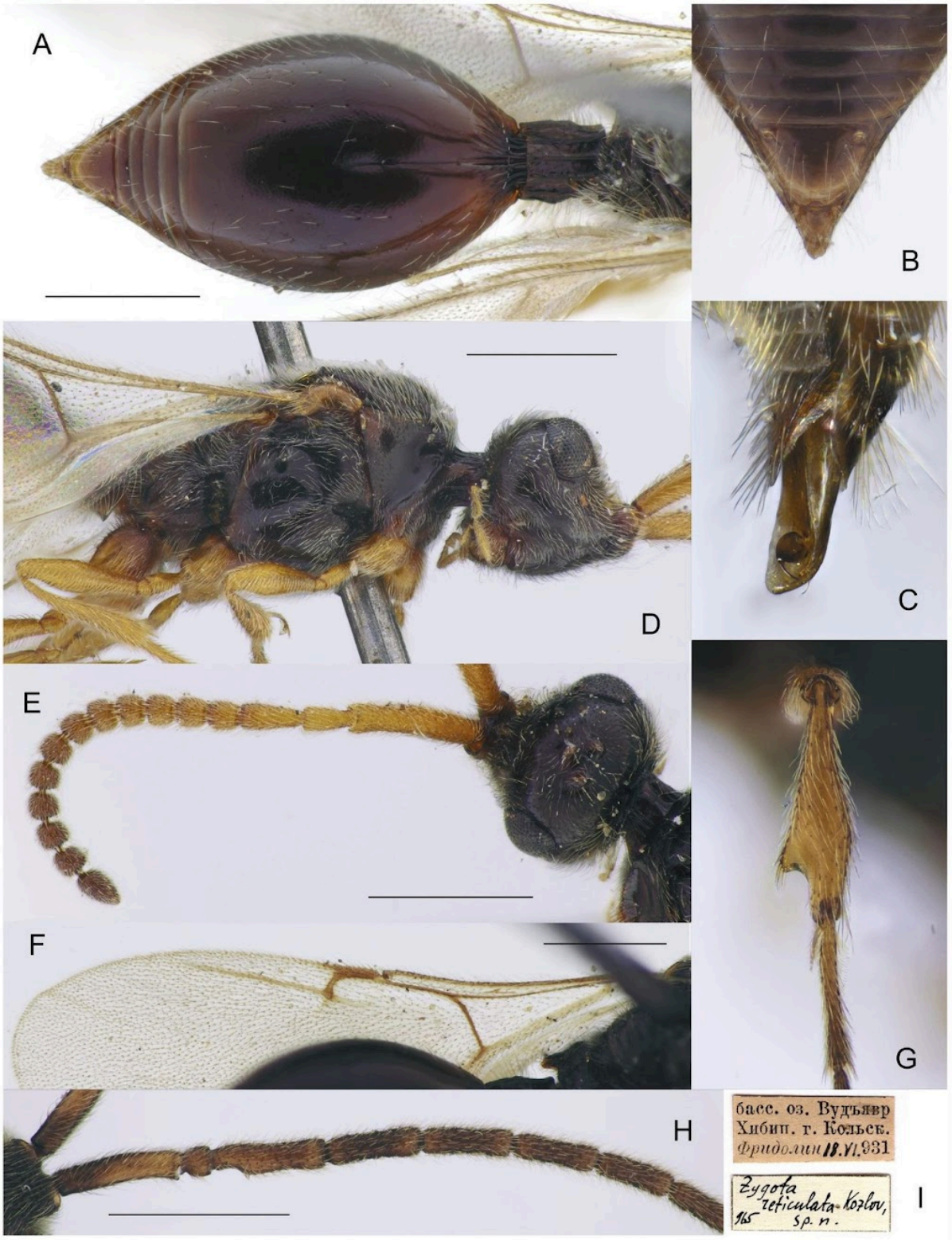

Figure 8. *Zygota ruficornis* male (**C, G, H**) and female (Z. *reticulata* Kozlov, holotype) (**A, B, D, E, F**) **A** metasoma, dorsal view **B** apex of metasoma, dorsal view **C** genitalia, lateral view **D** head and mesosoma, lateral view **E** antennae, dorsal view **F** fore wing **G** fore tibia **H** antenna, proximal part **I** label of the holotype. Scale bar: 0.5 mm.

with 1 long curved spine; spine extending from digitus at significant angle and not pushed towards it (Fig. 8C).

This species is very similar to *Z. pubescens* except as follows: female antenna stout, with A6–A14 distinctly transverse (A6–A14 subquadrate in Z. *pubescens*); male genitalia armed with a spine, which extends from digitus at significant angle (this spine pushed towards digitus in Z. *pubescence*). Both species are very common in Germany.

Distribution. Europe: Austria, Czech Republic, France, Germany, Hungary, Norway, Poland, Russia (European part), Scotland, Slovenia.

Zygota sordida Macek, 1997

Fig. 3B

Zygota sordida Macek, 1997: 11, female, male.

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Paehl, 24-Apr-2020, 1 ♂; Oberstdorf, 10–24-Jul-2016, 1 ♂.

Distribution. Europe: Austria, Czech Republic, Germany*, Slovenia.

Zygota spinosa (Kieffer, 1908)

Aclista (*Zygota*) *spinosa* Kieffer, 1908: 448, male.
Zygota comes Nixon, 1957: 63, male. Synonymized by Macek (1997).
Zygota loris Nixon, 1957: 59, female. Synonymized by Macek (1997).

BOLD BINs. BOLD:AEL5584, BOLD:AER0775.

Material examined. GERMANY: BY (BOLD:AEL5584): Mittenwald, 13-Jul-2021, 30-Jul-2021, 2 ♂; Garmisch-Partenkirchen, 02-Aug-2018, 13-Aug-2018, 11-Sep-2018, 5 ♀, 6 ♂. BY (BOLD:AER0775): Garmisch-Partenkirchen, 02-Aug-2018, 1 ♂; Garmisch-Partenkirchen, 11-Sept-2018, 1 ♂.

Distribution. Austria, Czech Republic, Germany, Slovenia, Switzerland.

Zygota spinosipes (Kieffer, 1908)

Aclista (*Zygota*) *spinosipes* Kieffer, 1908: 446, male.

BOLD BIN. BOLD:ACK3325, BOLD:AEY9457.

Material examined. Germany: BY (BOLD:ACK3325): Mittenwald, 30-Jul-2021, 1 ♀, 1 ♂; Garmisch-Partenkirchen, 11-Sep-2018, 2 ♀; NP Berchtesgaden, 09-Aug-2017, 1 ♀. BY (BOLD:AEY9457): Garmisch-Partenkirchen, 13-Aug-2018, 1 ♀; Mittenwald, 30-Jul-2021, 1 ♂, 1 ♀. BY (unsequenced material): Oberstdorf, 28-Jun-2016, 1 ♀.

Distribution. Europe: Czech Republic, Germany*, Italy, Russia (European part), Sweden.

***Zygota vigil* Nixon, 1957**
Figs 9A–C, 10A–G

Zygota vigil Nixon, 1957: 65, male.

BOLD BIN. No BIN.

Material examined. GERMANY: BY: Garmisch-Partenkirchen, 18-Jul-2018, 1 ♂.

Diagnosis. Slender specimens with postmarginal vein clearly shorter than radial cell length (Fig. 9); marginal vein slightly longer than parastigma (Fig. 9C); occipital pit absent; mesopleuron with only small bare area medially (Fig. 10B); axillar depression with scattered setae and only 2 verriculate tubercles; petiole in dorsal view pubescent anteriorly; S2 without micro-puncture sculpture on its anterior half (Fig. 10C); emargination on A3 distinct but not deep, extending to 0.35 of the segment length; fore tibia not modified, entirely pubescent and with several enlarged setae along its inner side (Fig. 10D); petiole with inarticulated elongate carinae (Fig. 10E); base of T2 without lateral corners (Fig. 10E); digitus with two narrow and long spines (Fig. 9B).

Distribution. Europe: Austria, Germany*.

Remark. This species was described by Nixon based on a single male from Austria, but the type of the species was not found (J. Monks pers. com.). Unfortunately, it was not possible to create a BIN from the obtained sequence of the *Zygota vigil* male due to its length (461bp).

***Zygota walli* sp. nov.**
https://zoobank.org/DC1B6471-36AC-4653-9044-4D277DFF9DF3
Figs 1C, 3D, F, 4E, 5A, 11A–F, 12A–E

BOLD BIN. BOLD:ACF9113, BOLD:AER4128.

Material examined. ***Holotype*** GERMANY. BY: Platt, Garmisch-Partenkirchen, 09-Oct-2028, lat. 47.406, long. 11.009, dv.zugsp6.6, ZSMHYM42437-A07, GBOL III leg., BOLD:ACF9113, SNSB-ZSM, 1 ♀.

Paratypes. BY (BOLD:ACF9113): Mittenwald, 13-Jul-2021, 30-Jul-2021, 1 ♀, 2 ♂; Garmisch-Partenkirchen, 05-Jul-2018, 09-Oct-2018, 2 ♀, 1 ♂.

Other material. GERMANY: BY (BOLD:AER4128): Garmisch-Partenkirchen, 2-Aug-2018 1 ♂; Mittenwald, 30-Jul-2021, 1 ♂; Garmisch-Partenkirchen, 09-Oct-2018, 1 ♂. BY (unsequenced material): Rhoen mountains, 11-Jul-2018, 1 ♂; Oberstdorf, 28-Jun-2016, 1 ♀; Garmisch-Partenkirchen, 13-Aug-2018, 1 ♂.

Diagnosis. Both sexes: postmarginal vein distinctly shorter than radial cell length (Figs 3D, 11B); occipital pit absent (Figs 1C, 11C); mesopleuron with only small bare area medially or entirely pubescent (Fig. 11D); axillar depression with scattered setae and only 2 verriculate tubercles; base of T2 with lateral corners (Fig. 12B); S2 with small sculptured area anteriorly (Fig. 4E, green arrow). **Female:** T2 mainly smooth with few scattered micropunctures (Fig. 12B); T8 with distinct transverse carinae (Fig. 11E, 12A). **Male:** A3 distinctly emarginated (Fig. 12C); fore tibia distinctly modified, broadened with sharp projection and a row of strong setae on the top of it, bare at the apex on its anterior sur-

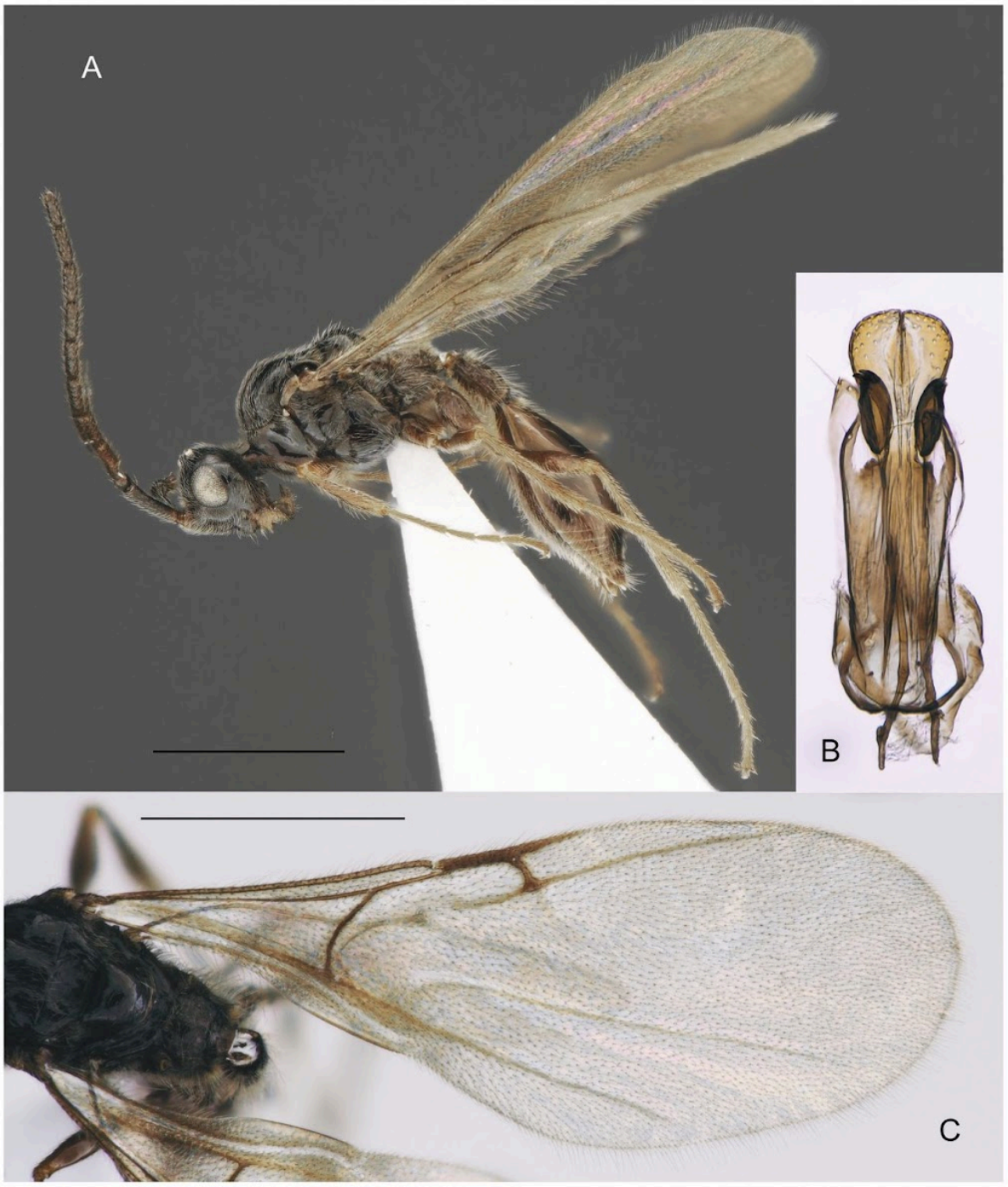

Figure 9. *Zygota vigil* Nixon, male **A** whole insect in lateral view **B** male genitalia **C** fore wing venation. Scale bar: 1 mm.

face (Fig. 3F); digitus armed with 3 teeth (Fig. 5A). *Zygota walli* sp. nov. differs from all other species mentioned by Macek (1997) in the absence of the occipital pit (Fig. 1C, red arrow).

Description. Female (*holotype*). Body length 3.2 mm, antenna length 2 mm, wing length 2.6 mm. Body mainly black with metasoma dark brown; antennae, palpi, mandibles, tegula, legs and venation brown (Fig. 11B).

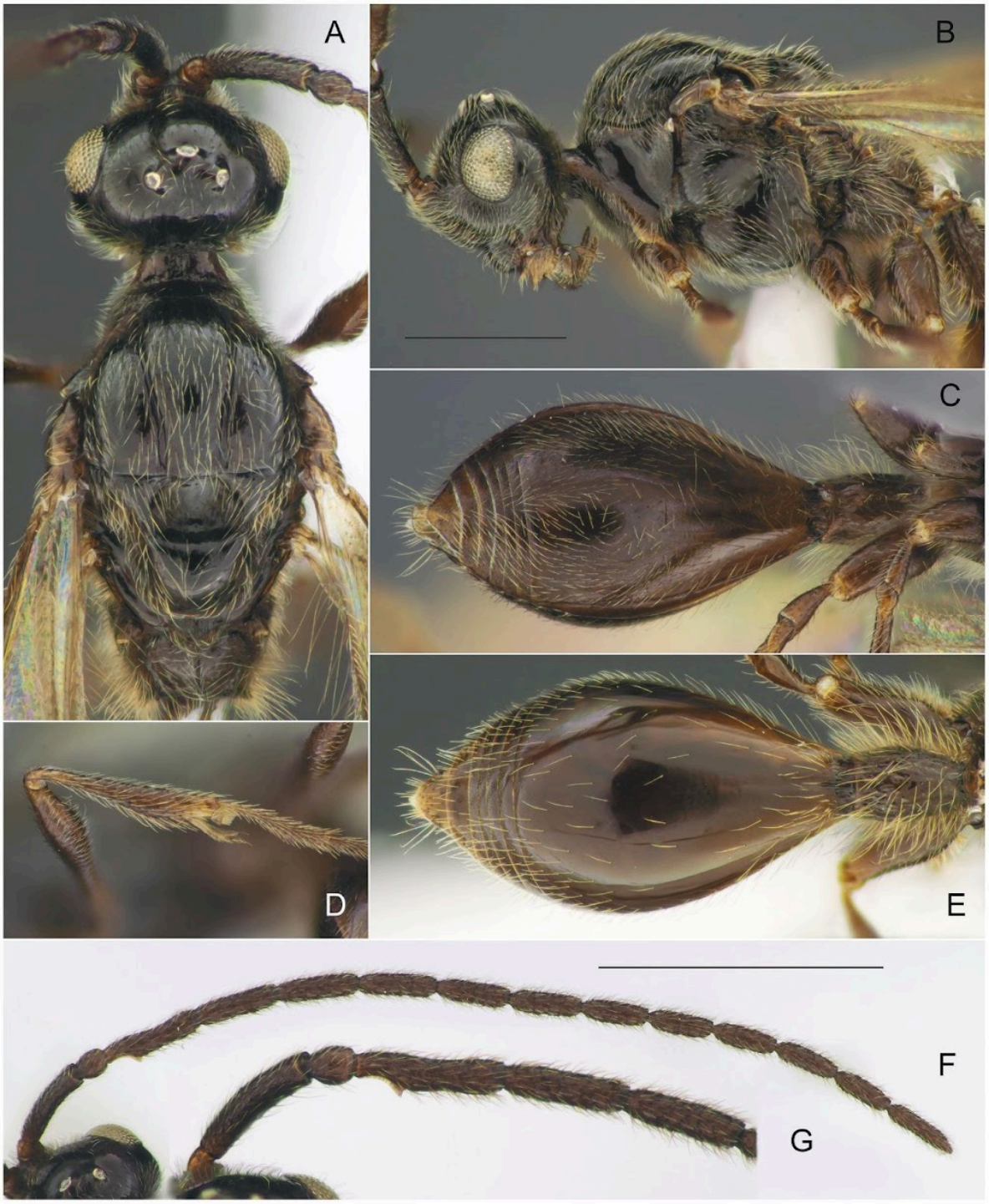

Figure 10. *Zygota vigil* Nixon, details of morphology, male **A, B** head and mesosoma in dorsal (**A**) and lateral (**B**) views **C, E** metasoma, in ventral (**C**) and dorsal (**E**) views **D** fore tibia **F, G** antennae in dorsal view. Scale bars: 0.5 mm (**B**); 1 mm (**F**).

Head in dorsal view as long (measured with antennal shelf) as wide. Toruli separated from each other by narrow and shallow furrow and from front posteriorly with deep pubescent depression. Ocelli small, OOL twice as long as POL. Eye densely pubescent. Eye diameter 1.2 as long as malar space. Pleurostomal distance as

Figure 11. *Zygota walli* sp. nov. female holotype (ZSMHYM42437-A07) **A** face **B** whole body in dorsal view **C** head, dorsal view **D** head and mesosoma in lateral view **E** apex of metasoma, dorso-lateral view **F** head and mesosoma in lateral view. Scale bar: 1 mm.

long as malar space. Occipital carina narrow, almost smooth, without occipital pit (Fig. 11C). Head in lateral view as high as long, in frontal view subtriangular, with face smooth and shining. Antennal shelf rugose below toruli in frontal view. Subantennal furrows very short (Fig. 11A). Epistomal sulcus distinct, clypeus convex and smooth. Tentorial pits situated in small hollows. Mandibles not prominent.

Antennae 15-segmented (Figs 11B, 12E). A1 cylindrical, as long as A2–A5 combined, slightly curved, with simple apical rim. A3–A14 as long as wide

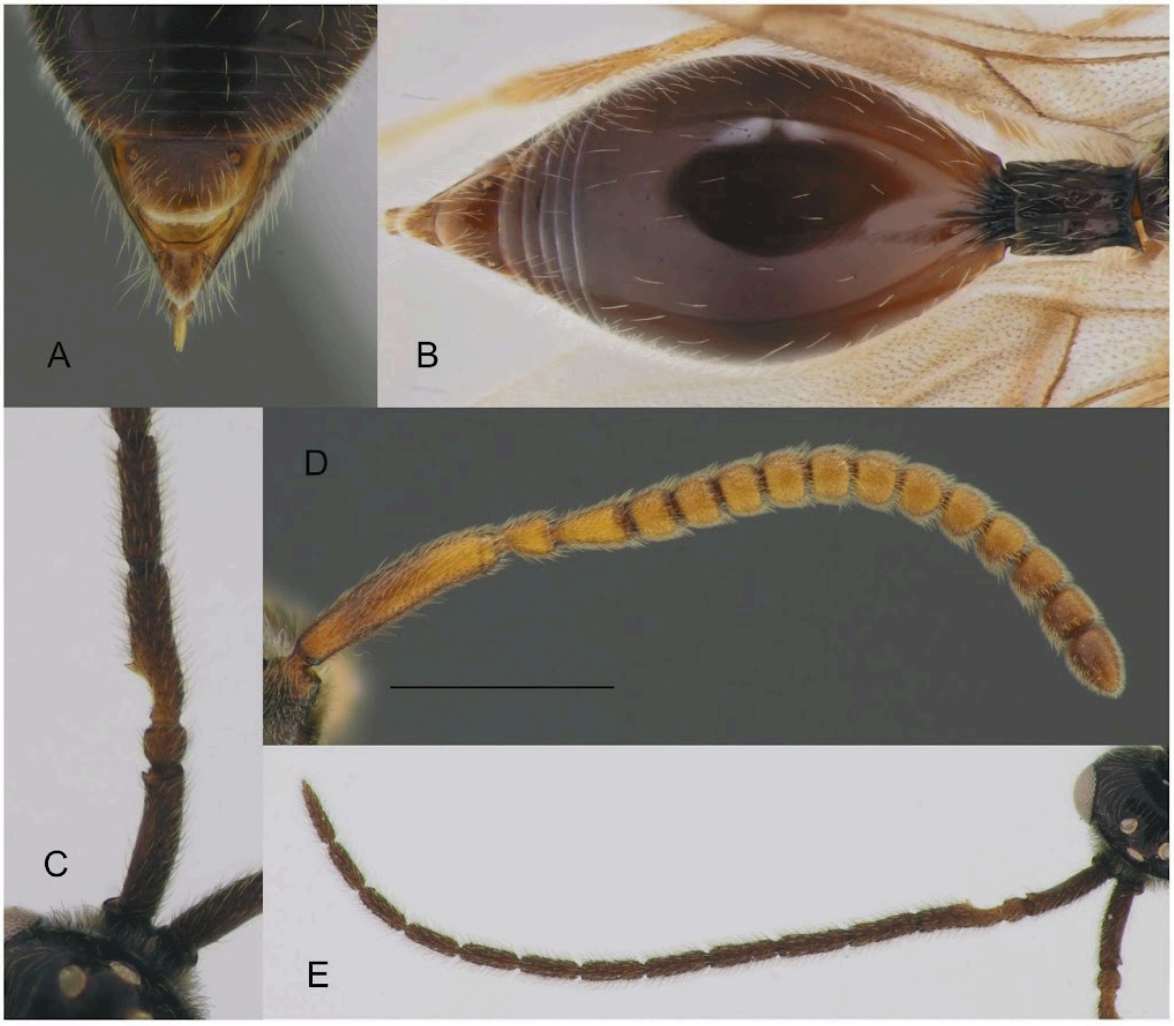

Figure 12. Details of *Zygota walli* sp. nov. morphology, female (**A, B, D**) and male (**C, D**) **A** apex of metasoma **B** metasoma in dorsal view **C** A1–A4 in dorsal view **D** antenna in lateral view **E** antenna in dorsal view. Scale bar: 0.5 mm.

to slightly transverse: A7–A9 weakly wider than A13–A14. A15 1.7 times as long as wide.

Mesosoma convex, 1.2 times as wide as the head. Pronotal shoulders weakly convex, with transverse carina between them. Epomia with long lower branch and short lateral branch. Lateral part of pronotum strongly impressed, smooth and shining. Mesonotum convex, with percurrent notauli, converging posteriorly. Scutellum convex, smooth, with oval anterior scutellar pit. Axillar depressions smooth, densely pubescence, with a pair of vericulate tubercles. Mesopleuron smooth with deep mesopleural pit, with epicnemial and acetabular bridges (Fig. 11D). Metascutellum with strong median carina and lateral carinas. Metanotal trough smooth and bare. Propodeum slightly transverse, with round posterior rim. Median keel of propodeum simple. Both plicae parallel to each other, slightly projecting posteriorly. Lateral side of propodeum below plicae with lateral longitudinal carina, slightly projecting posteriorly. Fore tibia simple with homogeneous strengthened bristles on the inner side.

***Wings*.** Marginal vein strongly developed, 3.9 times as long as wide (measured medially) and 1.45 times as long as distance from it to basal vein. Radial cell open, radialis long and nebulous (Fig. 11B). Postmarginal vein slightly shorter than stigmal vein; stigmal and postmarginal veins form 65° angle, stigmal vein 0.5 times as long as marginal vein.

Petiole cylindrical, entirely covered with semi-erect pubescence and elongate keels, ventrally with a row of verriculate tubercles. Base of T2 with slightly indicated lateral corners, short medial furrow and straight striation flanked at each side (Fig. 12B). S2 entirely pubescent, base of S2 with group of verriculate tubercles. Apical tergite (T8) with transverse sharp keel (Figs 11E, 12A), smooth and bare anteriorly and smooth and setose posteriorly from the transverse keel.

Male. Head distinctly transverse, as wide as mesosoma. Antennae 14-segmented with A4–A14 cylindrical, A3 with keel and emargination extending to 0.35–0.40 of the segment length (Fig. 12C, E). Fore tibia modified, acutely angled on the inner side and covered at the top with several minute bristles (Fig. 3F). Excavation on the fore tibia bare and shining in frontal view. Postmarginal vein 0.5–1.5 times as long as marginal vein (Fig. 3D). Marginal vein 1.3 times as long as distance from it to basal vein or slightly shorter. Petiole 1.5–2.1 times as long as its median width.

Etymology. This newly described species is named after the diapriid taxonomist Ingmar Wall who made himself a name in the Diapriidae research for years.

Distribution. Europe: Germany (Bavaria).

Discussion

As a result of our study, new combinations were proposed for 13 of 20 species which have a yet questionable taxonomic position, and two names (*Zygota caligula* Buhl and *Z. reticulata* Kozlov) were considered synonyms. One species of the genus *Zygota*, *Z. maura* (Kieffer, 1910) remains unstudied and inexplicable. Based on the emarginated fore tibia in males, mentioned in the original description, this species should be without doubt classified in the genus *Zygota* (Kieffer 1910). However, the type specimen of this species has not been found, and the description is not detailed enough to allow further conclusions at the species level or potential synonymies. The types of the two species *Z. strigata* Kozlov, 1978 and *Z. groenlandica* Buhl, 1995 were examined, and both are valid taxa of *Zygota*. *Zygota cilla* Nixon, 1957 and *Z. vigil* Nixon, 1957 were not included in Maceḱ s (1997) revision because of the lack of relevant material. Nixon (1957) based both species on a single female (*Z. cilla*) and a single male specimen (*Z. vigil*), yet neither type has been found. The first discovery of a male *Z. vigil* since the description of the species is given here. A female of *Z. cilla*, which is unique in its morphology (Nixon 1957), was not found during this research. Thus, the taxonomic position of all Palearctic species (Johnson 1992, Buhl 1995, 1997, Macek 1997) listed in *Zygota* but not mentioned in Maceḱ s (1997) revision, are discussed in this article.

Molecular-based analysis, which was conducted in the framework of this and previous works of GBOL III, has recovered rather poor results for the genus *Zygota* (and others of the Belytinae tribes Cinetini and Belytini; ~68% sequencing success rate) when compared to other diapriid taxa (~90%). Therefore, we recommend future studies invest their efforts into the development of a specific primer set to improve sequencing success. Nevertheless, we significantly

improved the amount of genetic information that is available online. Prior to this study, BOLD listed a total of 391 public records that were assigned to 26 BINs globally. Our dataset DS-ZYGPAN presents 178 *Zygota* records and 19 BINs from Germany alone (see also Suppl. material 3).

In this study, some *Zygota* morphospecies were assigned to more than one BIN. This can happen for a variety of reasons: incomplete lineage sorting, heteroplasmy, NUMTs, hybridisation, recent speciation, cryptic species, phylogeographic effects, introgression or endosymbionts or their combinations can influence the outcome of genetically sorting of different OTUs (Raupach et al. 2016). Another factor that plays a key role in the construction of a BIN is the DNA barcoding gap difference between the highest intra- and smallest interspecific variation of a certain taxon. A typical threshold in the genetic distance between two species ranges from 10–15%, but this can vary immensely (Meier et al. 2006, Hebert et al. 2016, Raupach et al. 2016). In our case, 10–15% was indeed a fitting value to delimit species with CO1. A MEGA mean group distance analysis (Suppl. material 3) confirmed our morphological findings, namely, that specimens assigned to the same morphological species all displayed smaller genetic distances between one another than between other morpho-species: *Z. comitans* (mean group distance within all sequences of the BIN: 7%), *Z. spinosa* (5.4%), *Z. parallela* (5.8%), *Z. spinosipes* (6.3%), *Z. ruficornis* (three BINs; 5.3%, 3.5%, 4.3%) and *Z. walli* sp. nov. (2.6%). The corresponding specimens of each BIN cluster together in the taxonomic ML-tree (see Suppl. material 1). An ASAP analysis of the genetic material confirmed the BIN clusters for the genus *Zygota*. The highly variable genus *Pantoclis*, on the other hand, displayed less resemblance when comparing the BINs with ASAP clusters. All of those questionable records were only represented by one or two sequences in our dataset which might explain their uncertain placement.

A subset of the available CO1 sequence data of species of the tribe Belytini was used to construct a phylogenetic ML-tree (Fig. 13). Here, the genera *Zygota* and *Pantoclis* were displayed as well-supported sister groups within the Belytini. Fig. 14 shows a more detailed tree with records from all *Pantoclis* BINs we investigated. The data show that some species with an open radial cell are grouped and demonstrate close genetic relationships with species that clearly belong to *Pantoclis* and have a closed radial cell. These findings suggest that the character state of the radial cell reduction cannot be used as an appropriate feature for genus designation. Nixon (1957) also noticed these differences between *Zygota* species and the group of *Pantoclis* species with an open radial cell. He proposed to aggregate them into the *Z. fuscata* – species group "... because of the form of the radial cell and better development of the radialis, this group is transitional between *Pantoclis* and *Zygota* and has perhaps more relationships to the former genus [*Pantoclis*] than to *Zygota* s. str." (Nixon 1957). Nixon placed six species (*Z. fuscata*, *Z. microtoma*, *Z. striata*, *Z. brevinervis*, *Z. soluta*, *Z. fossulata*) in the *Z. fuscata* – species group which have been transferred to *Pantoclis* here.

In addition, the species transferred to the genus *Pantoclis* in this research are not similar to *Zygota* species in other key characteristics. Unlike *Zygota* species, males of *Pantoclis* never display a modified fore tibia and most of them have slender genitalia with lanceolate apex of aedeagus and a diminished digitus. On the contrary, some *Zygota* males have the digitus with a single strong curved spine, while similar structures are not known for the *Pantoclis* species. All females of *Zygota* show a very short ovipositor, while many *Pantoclis* females (with closed or

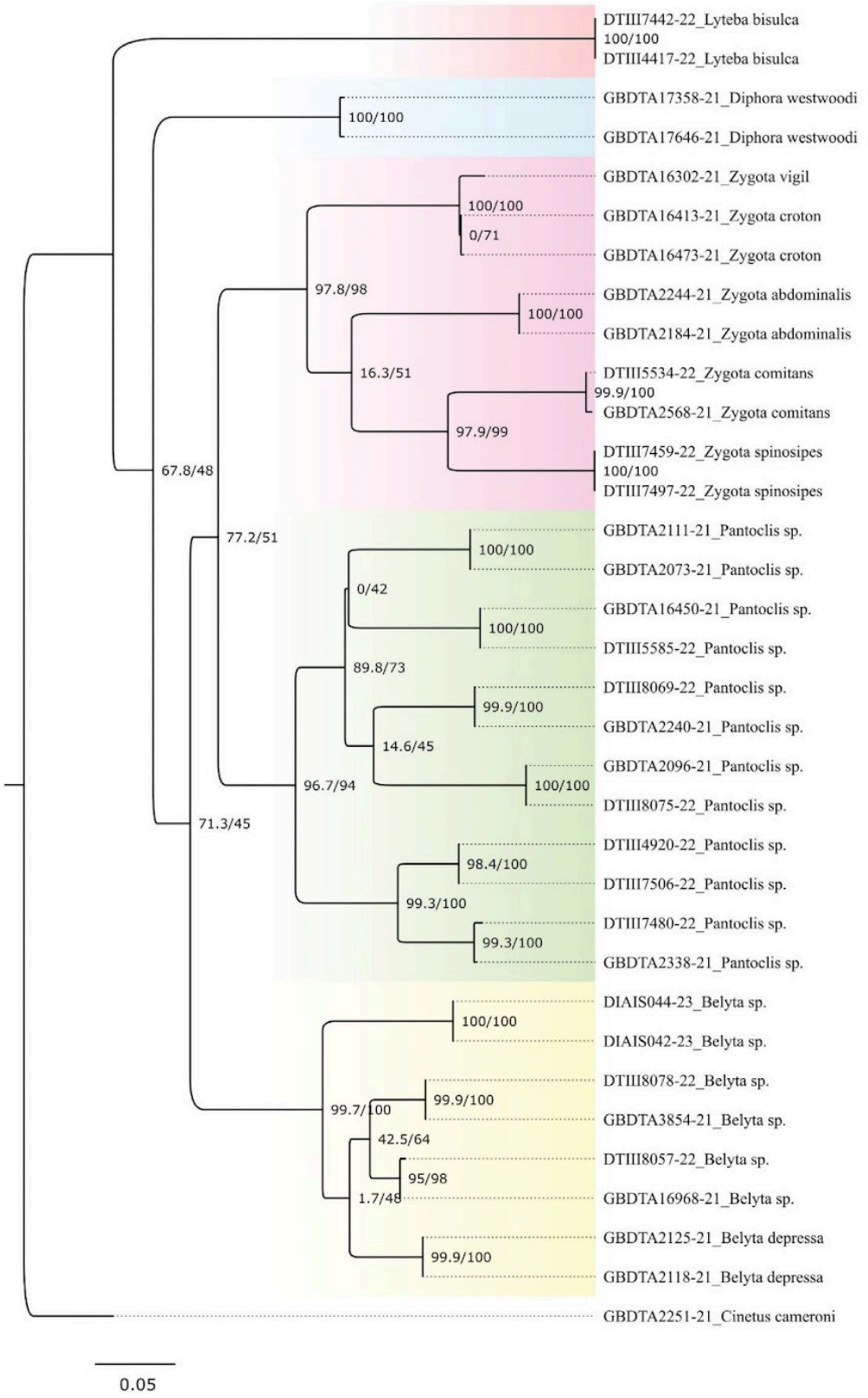

Figure 13. Phylogenetic ML consensus tree of barcoded Belytini specimens with bootstrap/jackknife values and *Cinetus cameroni* as an outgroup.

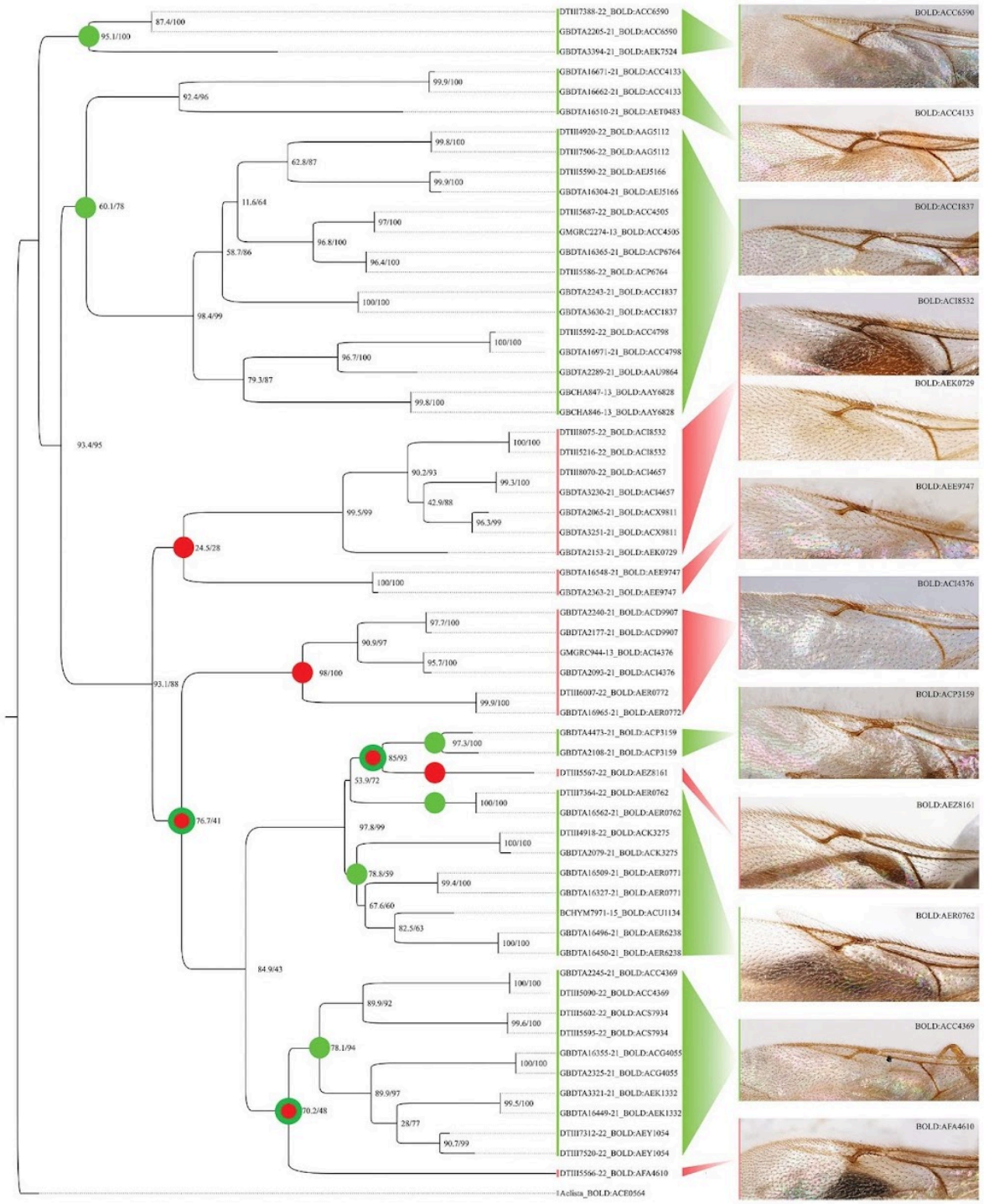

Figure 14. Phylogenetic ML tree of barcoded *Pantoclis* material and the polyphyletic appearance of their wing venation. Green represents the taxa with a closed radial cell while species with an open cell are color-coded red. Each node's support is displayed by the bootstrap and the jackknife values. *Aclista* was used as an outgroup.

open radial cell) show a long ovipositor (Fig. 1A). Thus, combining this morphological information with our understanding of the genus *Pantoclis* (see the diagnosis of the genus proposed above), and taking data on the venation variability based

on the molecular data into consideration, we propose in this study, new combinations for 13 species previously listed in the genus *Zygota* (Suppl. material 2).

Because a detailed revision of *Pantoclis* is still lacking, it is important to note that the diagnosis presented here is preliminary. The high amount of variation in the morphology and the large species richness of the genus suggest that *Pantoclis* is paraphyletic. On the other hand, as a consequence of the taxonomic changes proposed here, the monophyly of the *Zygota* is now less controversial based on species morphology.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: VC. Data curation: JH, VC. Formal analysis: VK, JM, JH, VC. Funding acquisition: JH. Investigation: VK, JH, JM, VC. Project administration: VC. Resources: JH. Validation: VK, VC. Visualization: JH, VC. Writing – original draft: JH, JM, VC. Writing – review and editing: VK.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

ML-tree with a subset of all *Pantoclis* and *Zygota* BINs available from our data with one *Aclista* sequence as outgroup

Authors: Jeremy Hübner, Vasilisa Chemyreva, Jan Macek, Victor Kolyada

Data type: png

Link: https://doi.org/10.3897/zookeys.1207.121725.suppl1

Supplementary material 2

Type information for the taxonomically treated and transferred *Zygota* species

Authors: Jeremy Hübner, Vasilisa Chemyreva, Jan Macek, Victor Kolyada

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1207.121725.suppl2

Supplementary material 3

Cluster analyses of the genetic results

Authors: Jeremy Hübner, Vasilisa Chemyreva, Jan Macek, Victor Kolyada

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1207.121725.suppl3

2. CHAPTER: Innovative approaches

This chapter is dedicated to the usage of innovative approaches for monitoring insects. The first manuscript evaluates the suitability of preserving fluids as a DNA source for metabarcoding and its utilization in ecological frameworks. The second manuscript displays an artificial intelligence based approach to fastly and reliably sort insect specimens.

Ismarus flavicornis
(Thomson, 1858)

SECTION 2.1: Ecological gradients

Metabarcoding of arthropod bulk material has proven itself to be a helpful tool in species community assessment. A less destructive source of DNA, the preservative ethanol has already been shown to detect significantly different species compositions for the same samples, making it hardly credible for diversity analyses. Ecology on the other hand uses subsets of data due to the countless factors shaping it. To test, if ecological information gets conserved, both DNA from ethanol and from the tissue got sequenced for various habitat types. Our results show that only seasonality and for only some taxa could be preserved in the DNA obtained from the preserving ethanol. It should therefore be used cautiously.

Chimeno, C., Hübner, J., Seifert, L., Morinière, J., Bozicevic, V., Hausmann, A., Schmidt, S., & Müller, J. (2023). Depicting environmental gradients from Malaise trap samples: Is ethanol-based DNA metabarcoding enough? *Insect Conservation and Diversity*, *16*(1), 47–64. https://doi.org/10.1111/icad.12609

Scorpioteleia longepetiolata (Thomson, 1858)

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ORIGINAL ARTICLE

Insect Conservation and Diversity

Depicting environmental gradients from Malaise trap samples: Is ethanol-based DNA metabarcoding enough?

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Abstract

1. DNA metabarcoding is revolutionising biodiversity research, as it offers researchers a holistic taxonomic approach across lineages. Many studies are dedicated to testing its application and optimising workflows. One topic of discussion is the nature of samples used for sequencing and comparing taxonomic results.
2. However, in ecological and environmental studies, where scientists always work with subsets of species, it may be less important whether different methods provide different subsets but more important if ecological and environmental information is conserved equally.
3. Numerous studies have successfully applied destructive and non-destructive metabarcoding approaches to evaluate patterns in biodiversity and in this respect, we aim to determine for the very first time whether environmental information is also conserved in the preservative ethanol of terrestrial arthropod bulk samples.
4. To test this, we applied DNA metabarcoding on tissue DNA and on ethanol-based DNA of the same Malaise trap samples. The arthropod material was collected with eight traps located in three different habitats: forest, meadow, and riparian.
5. We identified more than 3000 operational taxonomic units and demonstrate that ethanol-based DNA sequencing did not provide information on ecological gradients, except for the case of seasonal patterns, which was well conserved for some taxa.
6. The conserved seasonality is an interesting starting point for further investigations. Until future research has provided more successful results, we recommend researchers dealing with terrestrial ecosystems to be careful when using ethanol DNA.

KEYWORDS
arthropod communities, arthropod tissue, DNA barcoding, ecological gradients, ethanol-based DNA, Malaise traps, metabarcoding, preservative ethanol, tissue-based DNA

INTRODUCTION

Evaluating the state of an ecosystem requires adequate monitoring of biodiversity (Liu et al., 2021). This includes having knowledge on the inhabiting communities at one or more ecological levels and assessing changes over time and space (Coissac et al., 2012; Niemelä, 2000). Arthropods are especially suitable as ecological indicators, because they are abundant, species rich, and sensitive to slight environmental changes due to their functionality in an ecosystem (Medhi et al., 2021; Schowalter, 2017). However, identifying arthropod species using conventional morphological approaches is challenging, often dependent on specialised taxonomists (the availability of which is in decline), and time-consuming (Chimeno et al., 2022; Ji et al., 2013; Morinière et al., 2016; Yu et al., 2012).

Following the advent of DNA barcoding (Hebert et al., 2003), molecular approaches have become more frequent in biomonitoring surveys (Cristescu, 2014; Hardulak et al., 2020; Shokralla et al., 2012). One approach that is expediting biodiversity monitoring is DNA metabarcoding (Liu et al., 2020; Makiola et al., 2020). This method extends single species delimitation to the identification of entire communities holistically by extracting genetic material from entire bulk samples and sequencing a standard DNA marker via high-throughput sequencing (HTS) (Aylagas et al., 2018; Cristescu, 2014; Hardulak et al., 2020; Ji et al., 2013; Keck et al., 2017; Meusnier et al., 2008; Taberlet et al., 2012; Yu et al., 2012). Not only does DNA metabarcoding enable highly standardised, reliable and cost-efficient community analysis, it also enables biodiversity assessments of larger community subsets across a broad range of ecosystems (Liu et al., 2021; Morinière et al., 2016). Analysis of biodiversity patterns driven by ecological gradients is therefore much more comprehensive than in conventional biomonitoring where scientists are often limited to the evaluation of few key taxa (Bohan et al., 2017; Keck et al., 2017; Mandelik et al., 2010; Porter et al., 2014).

Although DNA metabarcoding has become a well-established method (Shum & Palumbi, 2021), a consensus workflow is still lacking in some fields of research (e.g. studies on terrestrial arthropods; see Elbrecht & Leese, 2015). Numerous studies are therefore dedicated to testing its robustness across protocols (Deagle et al., 2014; Hardulak et al., 2020; Ji et al., 2013; Marquina et al., 2019). One subject of debate, for example, is the nature of samples used for sequencing. Homogenisation of arthropod tissue has quickly become a favoured approach, because most DNA is released upon tissue destruction. More DNA, however, comes at a cost of losing the specimen's structural integrity, which erases any possibility for subsequent morphological analysis (Aylagas et al., 2016, 2018).

Due to its non-destructive nature and easy application, the interest for ethanol-based DNA sequencing has greatly increased in recent years. Instead of regarding ethanol as a mere preservative that is discarded upon specimen analysis, it could be poured out, filtered and its contents subjected to molecular analysis. Thus, ethanol-based DNA metabarcoding can provide an extensive community analysis all while keeping the specimens intact (Erdozain et al., 2019; Marquina et al., 2019). Studies testing the consistency of taxonomic results between the use of specimen tissue and preservative ethanol are still sparse, and those that have provide divergent results. Studies conducted on freshwater benthic macroinvertebrates were overall more successful (Hajibabaei et al., 2012; Zizka et al., 2019) than those conducted on terrestrial arthropods (see Kirse et al., 2022; Linard et al., 2016; Marquina et al., 2019), and when examining real-life Malaise trap samples of terrestrial arthropods, Marquina et al. (2019) recovered significantly different arthropod communities with each approach, displaying little to almost no overlap between OTUs of the same samples. The authors therefore concluded that when dealing with Malaise trap samples, the ethanol-based DNA should not be used a sole substitute to tissue DNA, but at most be regarded as a complementary source of information (Marquina et al., 2019).

In this study, we also aim at comparing detected arthropod communities across methods but in a different context. In ecology, where researchers always work with subsets of communities, identical taxonomic recovery may not always be as crucial as the conservation of ecological and environmental information. Ji et al. (2013) were the first to examine the reliability of metabarcoding for depicting ecological trends among the homogenised tissue of arthropod communities. Since then, numerous studies have successfully applied destructive metabarcoding approaches to evaluate patterns in biodiversity (see Barsoum et al., 2019; Liu et al., 2021; Watts et al., 2019). In this respect, we aim to determine for the very first time whether environmental information is also conserved in the preservative ethanol of terrestrial arthropod bulk samples. We compare results of tissue homogenate metabarcoding with that of the preservative ethanol of the same samples to see whether we obtain similar ecological patterns among our communities. If this were the case, the preservative ethanol can in fact be regarded as a valuable non-destructive source of DNA for metabarcoding applications in environmental research. To answer our question, we set up Malaise traps to capture arthropod communities from different localities and habitats. For direct comparison we performed, for each bulk sample, metabarcoding on (1) the homogenised arthropod tissue and (2) the ethanol-based DNA.

MATERIALS AND METHODS

Arthropod sampling

In 2019, we installed eight Malaise traps in the Bavarian Forest National Park, which is located in southeast Germany along the border with the Czech Republic (Figure 1). Six traps, ranging from 650 to 800 m.a.s.l., were set in the catchment areas of the streams Kolbersbach, Grosse Ohe, and Kleine Ohe: one was installed directly above each stream using wooden beams, and one in the surrounding forest. Two further traps were installed in open meadows located in Kolbersbach and Bergerau. All traps were in operation from the end of April to September. The collection bottles were replaced every 2 weeks with new ones that were distinctive to the specific trap. All collection bottles had been bleached prior to the start of the experiment, and between collection events, the bottles were cleaned with distilled

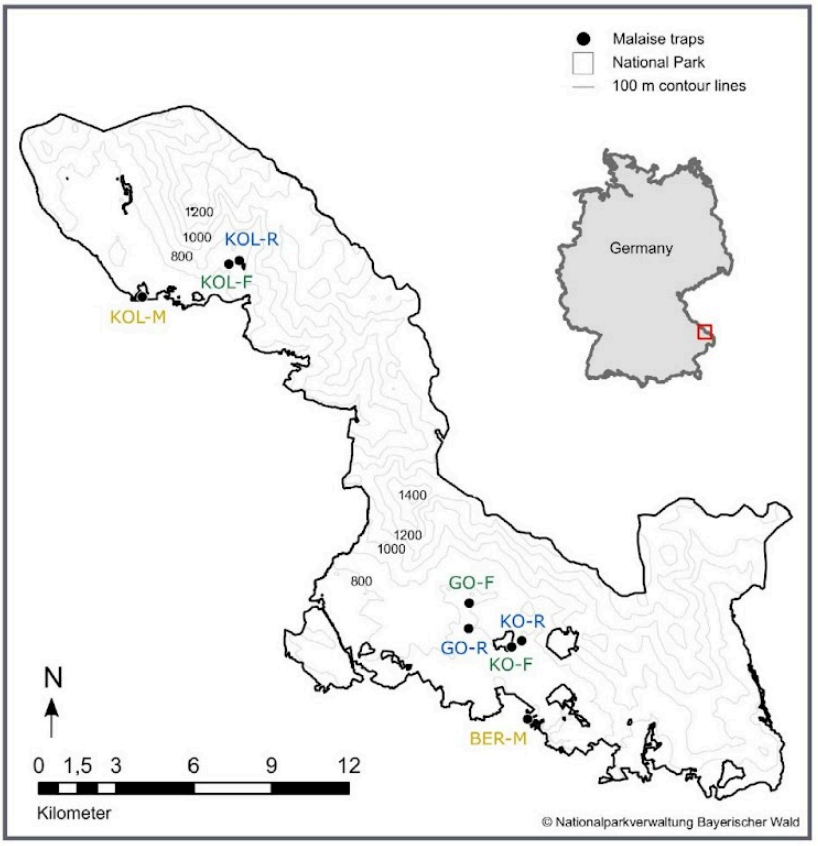

FIGURE 1 Location of the eight Malaise traps that were set up in the Bavarian Forest National Park and in operation from April to September 2019. KOL (Kolbersbach); GO (Große Ohe); KO (Kleine Ohe); BER (Bergerau); R (riparian); F (forest); M (meadow)

water and ethanol. The 80% ethanol (1 vol% MEK) was used for arthropod sampling.

Laboratory procedures

In the laboratory, we processed each sample individually to avoid cross-contaminations. We used cellulose tea bags to separate the arthropod tissue from its preservative ethanol (first phase ethanol used for sampling). A fresh bag was used for each sample. We weighed the tissue and transferred it to fresh 96% ethanol. We subsampled 50 ml of the ethanol (after thorough mixing) which we filtered (using sterile cellulose nitrate filters for vacuum filtration, 0.45 µm) and stored individually in 96% ethanol at −30°C until analysis (Advanced Identification Methods GmbH, Leipzig, Germany). The arthropod tissue and the ethanol filters were dried separately overnight in an oven at 60–70°C to remove all residual ethanol. We homogenised the arthropod tissue and the ethanol filters separately with stainless steel beads in a FastPrep 96 (MP Biomedicals) and used a 90:10 solution of animal lysis buffer (buffer ATL, Qiagen DNeasy Tissue Kit, Qiagen, Hilden, Germany) and Proteinase K for lysis, which was performed overnight in a 56°C oven. All samples were cooled to room temperature for subsequent DNA extraction. We took 200 µl aliquots of each lysate from which DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. PCR was performed using 5 µl of the extracted genomic DNA, 12.5 µl Plant MyTAQ (Bioline, Luckenwalde, Germany), and 1 µl HTS adapted mini-barcode primers mlCOIntF 5′–GGW ACW GGW TGA ACW GTW TAY CCY CC–3′ and dgHCO 5′–TAA ACT TCA GGG TGA CCA AAR AAY CA–3′ (see Morinière et al., 2016, 2019). We used the following PCR profile of 95°C for 5 min; 3 cycles of 96°C for 15 s; 48°C for 30 s; 65°C for 90 s; then 30 cycles of 96°C for 15 s; 55°C for 30 s; 65°C for 90 s and a final extension of 76°C for 10 min.

We examined amplification success and fragment lengths via gel electrophoresis, cleaned up the amplified DNA using ExoSap (Thermo Fisher), and resuspended it in 50 µl molecular grade water for each sample. Illumina Nextera XT (Illumina Inc., San Diego, USA) indices were indexed to the samples using a second PCR reaction. We used standard Illumina i5/i7 indices. Here, the same annealing temperature (55°C) was used as in the first PCR reaction, but with fewer cycles (7). Ligation success was confirmed by gel electrophoresis and DNA

concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad, USA), which resulted in ~52 ng/μl for the tissue samples and ~24 ng/μl for the ethanol samples. We measured the DNA concentrations for each tagged sample, then pooled samples together (taking each PCR product into account) in order to obtain 40 μl pools that comprised concentrations of 100 ng/μl DNA each. The pools were purified using MagSi-NGSprep Plus (Steinbrenner Laborsysteme GmbH) beads. A final elution volume of 20 μl was used for HTS, which was performed on an Illumina MiSeq using v3 chemistry (2 × 300 bp, 600 cycles, maximum of 25 million paired-end reads). We aimed at obtaining 250 k RAW reads (125 k paired-end after merging) per sample. Overall, we used six negative controls per 96-well plate: two negative controls of DNA extractions, two amplicon PCR negative controls, and two indexing PCR negative controls.

Bioinformatic analysis

Briefly, we merged the paired-end reads using USEARCH v11.0.667_i86linux32 (Edgar, 2010). We trimmed adapters using CUTADAPT (Martin, 2011) and all reads that did not contain them were filtered out. Quality filtering, de-replication, chimera filtering, and clustering were carried out using the VSEARCH suite v2.9.1 (Rognes et al., 2016). We quality-filtered all reads containing more than one expected error per read, and then de-replicated them, first at the sample level, and then again at the combined dataset level after concatenating all sample files into one large FASTA file. This file was also filtered for singletons (reads that only occur once in the entire dataset). To save processing power, we pre-clustered the reads at 98% identity before chimera filtering using the VSEARCH centroids algorithm. As recommended by Rognes et al. (2016), we then carried out de novo chimera filtering, followed by the final round of clustering into OTUs at 97% identity.

In order to create the OTU table, the reads had to be mapped back to the created OTUs. To do this, we used a Perl script obtained from Rognes et al. (2016) to recover all quality- and chimera-filtered reads from the individual samples, including singletons, as well as reads that were previously removed by the two rounds of de-replication (https://github.com/torognes/vsearch/wiki/VSEARCH-pipeline). To reduce likely false positives, we excluded read counts in the OTU table that constituted less than 0.01% of the total number of reads in the sample. We then blasted the OTUs in Geneious (v.10.2.5; Biomatters, Auckland, New Zealand) and following methods described in the study by Morinière et al. (2016). We first blasted against a local copy of the NCBI nucleotide database (downloaded from ftp://ftp.ncbi.nlm.nih.gov/blast/db/) and then also against a custom database built from data downloaded from BOLD (www.boldsystems.org; Ratnasingham & Hebert, 2007, 2013), including taxonomy and BIN information. We exported the resulting CSV files from Geneious, including the OTU ID and NCBI/BOLD annotations for each detected OTU, and then combined them with the OTU table generated by the bioinformatic pre-processing pipeline. To provide another measure of control other than BLAST, we then classified OTUs into taxa using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang et al., 2007) trained on a cleaned COI dataset of Arthropods and Chordates (plus outgroups; Porter & Hajibabaei, 2018). We filtered out all OTUs where the combined number of reads in the negative control samples constituted more than 20% of the total number of reads. Finally, we annotated the OTUs using NCBI taxonomic information (downloaded from https://ftp.ncbi.nlm.nih.gov/pub/taxonomy/).

Statistical analysis

All analyses were performed using R version 3.6.3 (R Core Team, 2012), and the packages *vegan* version 2.5-7 (Oksanen et al., 2020), *iNEXT* version 2.0.20 (Hsieh et al., 2020), *rtk* version 0.2.6.1 (Saary et al., 2017), *stats* version 3.4.3 (included in the standard R). An example R script and input data sets are deposited on Figshare (doi: https://doi.org/10.6084/m9.figshare.c.5666860.v2). We evaluated metabarcoding results of tissue- and ethanol-based DNA for all arthropods, then individually for each of the top five biodiverse arthropod orders in our dataset. For all arthropods and each individual order, we created an OTU × sample table with associated environmental variables (sites, habitats, seasonality) and sample type (tissue and ethanol). All reads were converted to presence/absence (Yu et al., 2012).

For statistical testing, the OTU dataset was rarefied to the lowest number of reads to equalise the sampling effort (via *rtk*; *rtk* package). To test whether community compositions differ based on associated environmental variables, we performed permutation multivariate analysis of variance (PERMANOVA) (via *adonis2*; *vegan* package; Jaccard dissimilarity method; 999 permutations). This method is best for testing compositional differences among multiple factors (Anderson, 2017). To differentiate between location and dispersion effects, we applied a beta dispersion test analogous to Levene's test (via *betadisper*; *vegan* package) and an *F*-test (via *permutest*; *vegan* package). In cases of unequal dispersion, we used a Tukey test (via *TukeyHSD*; *stats* package) to locate the variables responsible for inner group variation.

To visualise and compare environmental trends between the tissue- and the ethanol-based DNA communities, we used non-metric dimensional scaling ordinations (NMDS; via *metaMDS*; *vegan* package) or multidimensional scaling (PCoA; via *cmdscale*; *stats* package) of Jaccard dissimilarity matrices. We used the functions *vegdist* (to calculate a dissimilarity matrix), *ordiplot* (plotting function), *ordiellipse* (to add ellipses to ordination plot), and *ordispider* (to add spider graphs to the plot) from the *vegan* package. We created an ordination of each sample type (tissue and ethanol) for all arthropods, then for each of the top five most abundant arthropod orders.

We performed an alpha-diversity analysis (via *iNEXT*) of tissue- and ethanol-based DNA for the entire arthropod dataset. *iNEXT* uses observed sample incidence data (presence–absence data) to compute diversity estimates for sample-size and coverage-based rarefaction and extrapolation (R/E) curves using Hill numbers (Chao & Chiu, 2016): Indices such as the Shannon index and Simpson diversity have always been used by biologists to portray biological diversity in a

TABLE 1 Malaise trap sample information

Location	Habitat	Malaise traps	Malaise trap samples
Kleine Ohe	Forest	1	11
	Riparian	1	11
Große Ohe	Forest	1	11
	Riparian	1	11
Kolbersbach	Forest	1	11
	Riparian	1	11
	Meadow	1	10
Bergerau	Meadow	1	11

Note: Number of bulk samples obtained for each location and habitat type.
Abbreviation: PERMANOVA, permutation multivariate analysis of variance.

given system; however, researchers have demonstrated that the non-linearity of these metrics can mislead researchers when evaluating their results. Thus, diversity values were converted into equivalents, also known as Hill numbers, to overcome these shortcomings (Chao & Chiu, 2016; Cox et al., 2017; Jost, 2006). Hill numbers differ among themselves only by an exponent q, providing results for species richness ($q = 0$), Shannon diversity ($q = 1$) and Simpson diversity ($q = 2$). Chao and Jost (2012) established coverage-based R/E methods, which standardise samples by completeness rather than by size in sample-based approaches (see Colwell et al., 2012), which is highly dependent on the sampling effort. Integrating both approaches offers the best of both worlds: a consolidated framework for (1) estimating species richness and (2) statistical conclusions. For each sample type (tissue and ethanol), we constructed a list of presence–absence data for each habitat (samples × OTUs) to obtain the correct input format. All three measures of Hill numbers (q) were used in our analysis, but we only look at the species richness ($q = 0$) in this study. We created three plots for each sample type: a sample-size-based R/E curve plot, a sample completeness curve plot, and a coverage-based R/E plot.

RESULTS

Overall, we collected 87 Malaise trap samples throughout the season (Table 1). In total, 174 samples were sequenced: 87 tissue samples and their corresponding ethanol. From all samples, we detected 3636 OTUs belonging to six phyla, namely Arthropoda (3620 OTUs), Annelida (5 OTUs), Chordata (3 OTUs), Platyhelminthes (4 OTUs), Mollusca (3 OTUs), and Tardigrada (1 OTU). Limiting our analyses to arthropods, we recovered 2725 OTUs from tissue-based DNA, 1823 OTUs from ethanol-based DNA, and 934 (25.8%) from both (Figure 2a). These belong to 31 orders, of which the top five most diverse are (from most to least diverse): Diptera (1554 OTUs), Lepidoptera (610 OTUs), Hymenoptera (555 OTUs), Coleoptera (392 OTUs) and Hemiptera (132 OTUs) (Figure 2b). Together, these orders represent 89.6% of all arthropod OTUs. In total, 49.5% more arthropod OTUs were recovered from analysis of tissue-based DNA than ethanol-based DNA.

Tissue-based DNA sequencing results of all arthropods

PERMANOVA analysis found a significant difference in community compositions based on trap site, habitat type, and seasonality (all adonis2 $p = 0.001$) (Table 2). The measured significance among sites, however, also includes dispersion effects that are caused by uneven sample distribution among the trap site Große Ohe. Interaction effects were significant between habitats and sites (adonis2 $p = 0.002$), habitats and seasonality (adonis2 $p = 0.001$), and sites and seasonality (adonis2 $p = 0.032$) but not for all three together. Consistent with the statistical results, the NMDS plot (Figure 3a; Figure S1a) reveals clear distinctions in communities based on habitat type and along a chronological seasonal gradient. Differences based on trap sites are not as prominent.

Sample-size-based rarefaction curves show that the forests (1743 OTUs) are the richest habitats that we sampled, followed by riparian (1413 OTUs) and lastly the meadow habitats (1401 OTUs) (Figure 4a; left). Extrapolation to double the sampling units reveals that both terrestrial habitats (forest, meadow) display a higher species richness than the riparian habitats and that at least 25% more OTUs could have been obtained for each habitat type (forest +25.9% OTUs; riparian +26.9%; meadow +27.9%). Sample coverage was highest for samples collected in the forest (90.6%) and riparian (90.5%) habitats (Figure 4a; right). Doubling the sampling effort would not have provided a much higher coverage for these habitats. Sample coverage was lowest for the meadow landscapes (85.8%), which is due to the lower number of sampling units for this habitat type; our extrapolation curve shows that a very similar coverage would have been obtained with more sampling effort. Coverage-based rarefaction and extrapolation curves show similar results when comparing to sample-size-based R/E: the highest species richness was found among the forest habitats. Furthermore, species diversity within the terrestrial habitats was higher than that of the riparian habitats at equivalent coverage levels.

Ethanol-based DNA sequencing results of all arthropods

Hypothesis testing of the ethanol-based DNA results found no significant difference in community compositions based on trap site (adonis2 $p = 0.463$) nor habitat type (adonis2 $p = 0.073$; with dispersion effects) (Table 1). Testing for seasonality revealed a significant difference in community composition (adonis2 $p = 0.001$) with the inclusion of dispersion effects. Tukey testing revealed that samples collected from Week 18 to 24 are dispersed highly differently than those from Week 26 to 38. Interaction effects were significant between trap sites and seasonality (adonis2 $p = 0.005$). In the NMDS plot, samples are plotted into two distinct groups, with those on the left side being more dispersed than those on the right (Figure 3b; Figure S1b). There is no clear distinction between the different habitats nor between trap sites, and samples are not plotted along a chronological seasonal gradient. Furthermore, gradient lines for collection Weeks 32–38 are missing.

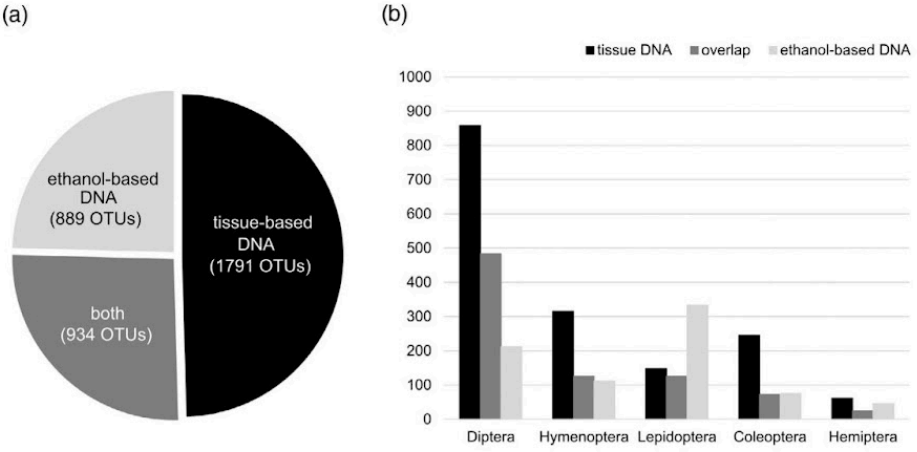

FIGURE 2 (a) Source of detected OTUs. Pie chart displaying the number of OTUs found with each metabarcoding approach. (b) OTU abundances per order. The bar chart shows results for the top five most abundant orders; together, these represent 89.6% of all arthropod OTUs detected throughout all samples.

TABLE 2 Statistical analysis of all arthropod OTUs (rarefied)

		PERMANOVA					Permutest
DNA source	Variables	Df	SS	R^2	F	Pr (>F)	P
Tissue-based DNA	Site	3	24.271	0.07801	3.0618	0.001***	0.001***
	Habitat	2	36.995	0.11890	7.0004	0.001***	0.891
	Week	1	28.128	0.09040	10.6448	0.001***	0.996
	Site:Habitat	2	0.9315	0.02994	1.7626	0.002**	
	Site:Week	3	10.294	0.03309	1.2986	0.039*	
	Habitat:Week	2	10.358	0.03329	1.9600	0.001***	
	Site:Habitat:Week	2	0.4163	0.01338	0.7877	0.913	
	Residuals	71	187.608	0.60299			
	Total	86	311.133	100.000			
Ethanol-based DNA	Site	3	0.6735	0.02768	0.9504	0.483	0.394
	Habitat	2	0.7430	0.03054	1.5726	0.054	0.023*
	Week	1	32.637	0.13413	13.8157	0.001***	0.002**
	Site:Habitat	2	0.3951	0.01624	0.8362	0.588	
	Site:Week	3	14.996	0.06163	2.1160	0.006**	
	Habitat:Week	2	0.5635	0.02316	1.1927	0.216	
	Site:Habitat:Week	2	0.4215	0.01732	0.8921	0.521	
	Residuals	71	167.725	0.68931			
	Total	86	243.325	100.000			

Note: Results of PERMANOVA (testing for differences in OTU community compositions) and permutation tests (P. test) via permutest (checking for homogeneity of multivariate dispersion) based on 999 permutations. Significance codes: 0 "***"; 0.001 "**"; 0.01 "*"; 0.05 "."; 1 " ".
Abbreviation: PERMANOVA, permutation multivariate analysis of variance.

Sample-size-based rarefaction curves show that the forests are the richest habitats that we sampled (1349 OTUs), followed by the riparian (1207 OTUs) and the meadow habitats (747 OTUs) (Figure 4b; left). Extrapolation to double the sampling units shows similar curves for the forest and riparian habitat, displaying a much higher species richness than the meadow habitats. At least 29% more species could have been obtained for each habitat (forest +30.3% OTUs; riparian + 29.8% OTUs; meadow +>36.8% OTUs) when doubling the sampling effort. Sample coverage was highest among the riparian habitats (91.7%), followed by the forest (91.6%) and lastly the

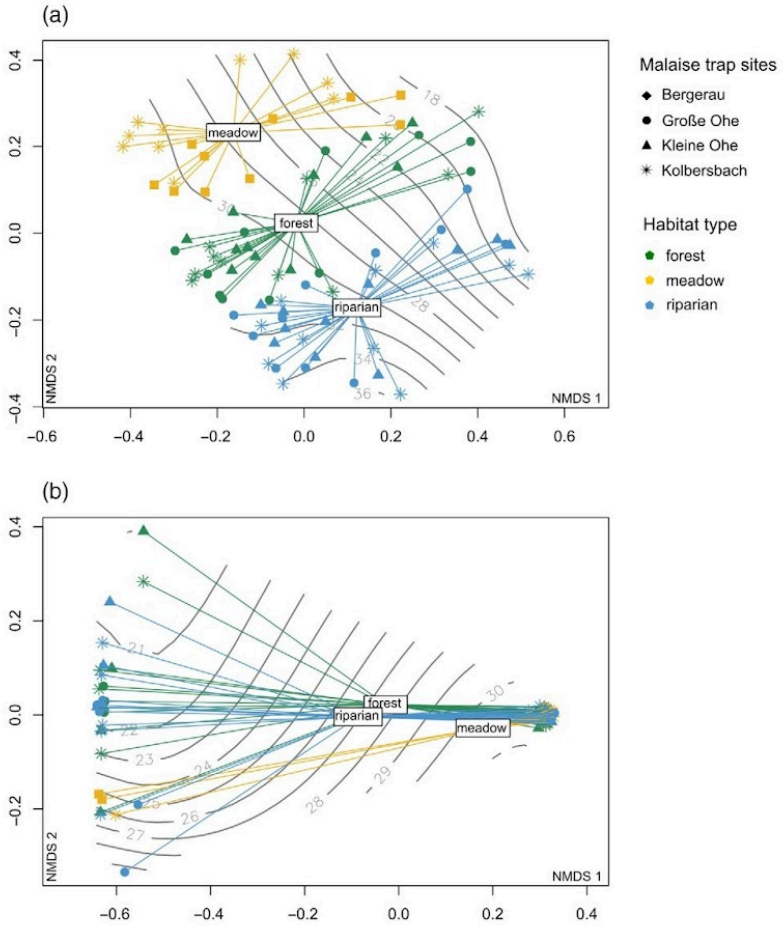

FIGURE 3 Non-metric dimensional scaling (NMDS) plots of arthropod community compositions of samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the NMDS plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39. (a) Arthopod communities of tissue-based DNA; NMDS of tissue-based DNA sequencing (3D analysis; stress = 0.1492). (b) Arthopod communities of ethanol-based DNA; NMDS of ethanol-based DNA sequencing (3D analysis; stress = 0.039). Ellipses are 95% CI of centroids for each sample type.

meadow (88.9%) habitats (Figure 4b; right). Doubling the sampling effort would not have provided a much higher coverage for these habitats.

Analysis of the most abundant orders

We performed individual statistical analyses for each of the top five most abundant arthropod orders in our dataset. For each of the five orders, analysis of the tissue-based OTUs depicted highly significant differences in community compositions based on each of the three environmental variables (Table 3). The majority of the significant results are driven by location effects only: For almost all orders, sample dispersion was homogenous among habitat types (exception: Hymenoptera) and among collection events. Sample dispersion, was not homogenous among site types for Diptera, Hymenoptera, and Coleoptera. Consistent with these statistical results, the NMDS/PCoA plots reveal clear distinctions in communities based on habitat type and along a chronological seasonal gradient (Figures 5a,c,e and 6c; Figures S2a,c,e and S3a,c). Sample clustering based on trap site is not clearly visible.

For every order, analysis of the ethanol-based OTUs displayed no significant differences in community compositions based on sites nor based on habitats (Table S1). Accordingly, in the NMDS/PCoA plots, there is no clear clustering as samples originating from different habitats and sites overlap one another (Figures 5b,d,f and 6b,d; Figures S2b,d,f and S3b,d). Testing for community differences based

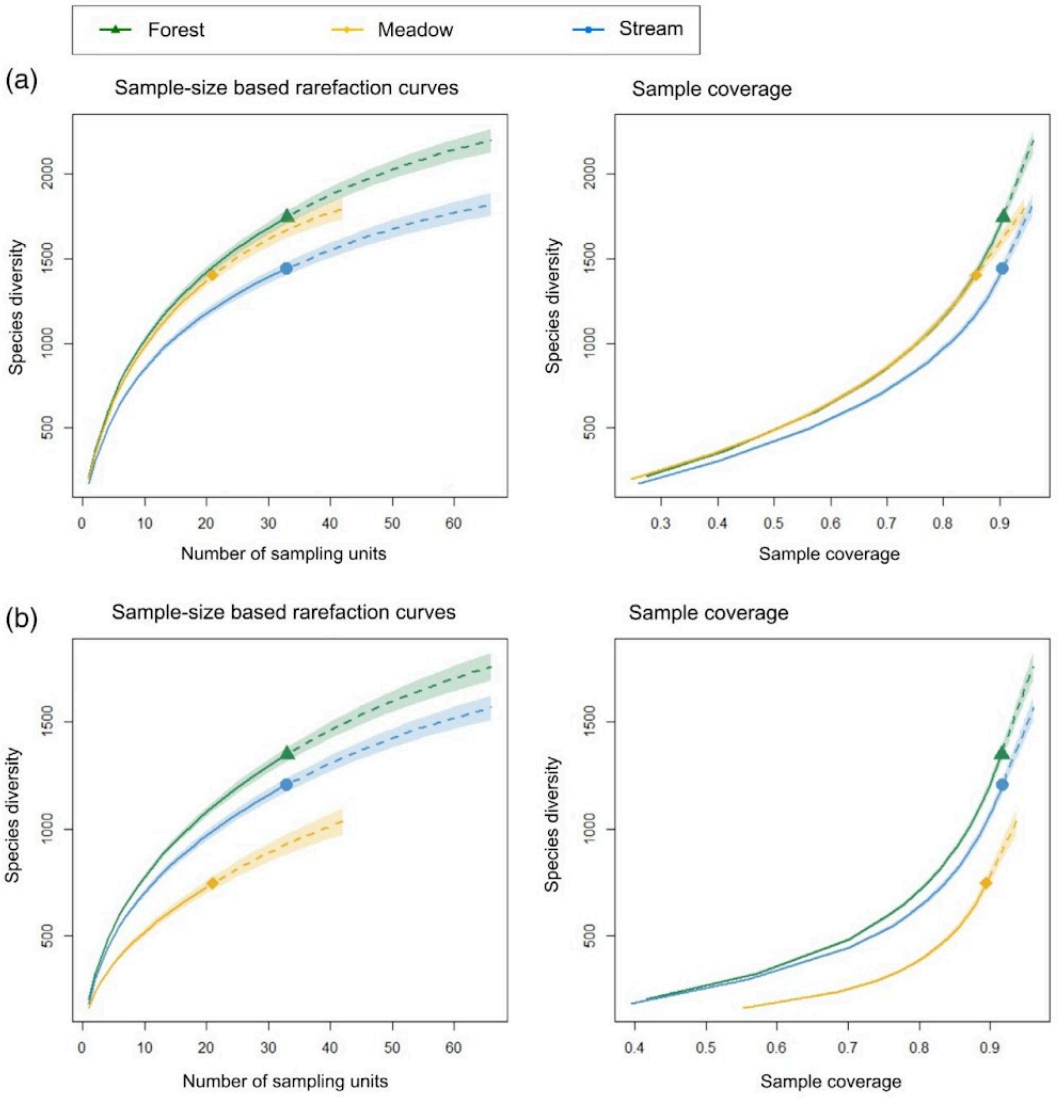

FIGURE 4 Rarefaction and extrapolation curves for $q = 0$ (species richness): (a) Arthopod communities of tissue-based DNA; sample-size-based rarefaction and extrapolation; (b) Arthopod communities of ethanol-based DNA; coverage levels for each habitat (meadow, forest, riparian). Solid lines represent rarefaction, while dashed lines represent extrapolation up to the double of sampling units. Shaded areas represent the 95% confidence interval using the bootstrap method on the basis of 100 repetitions.

on seasonality revealed highly significant results (all adonis2 $p = 0.001$) for all orders except for Hemiptera (adonis2 $p = 0.102$). Accordingly, samples of Hymenoptera and Coleoptera are plotted along a clear chronological seasonal gradient, however, less so for Diptera, and not at all for Lepidoptera. Samples of Lepidoptera were not homogenously dispersed throughout collection events. Although statistical analysis depicted no significant difference in community compositions of Hemiptera based on seasonality, samples are plotted along a chronological gradient in the Principal Coordinate Analysis (PCoA) ordination (Figure 6d; Figure S3d).

DISCUSSION

Discrepant arthropod communities

We detected completely different arthropod communities based on the DNA source used for sequencing (Figure 3c), which is congruent with findings of previous studies (see Elbrecht et al., 2017; Kirse et al., 2022; Marquina et al., 2019). For example, the preservative ethanol of an arthropod sample is more likely to contain the DNA of soft-bodied individuals because they release their DNA more freely into

TABLE 3 Statistical analysis of individual arthropod orders (rarefied)

		PERMANOVA					Permutest
Diptera	**Variables**	**Df**	**SS**	R^2	*F*	Pr (>*F*)	*P*
Tissue-based DNA	Site	3	22.200	0.07504	2.8960	0.001***	0.001***
	Habitat	2	34.308	0.11597	6.7132	0.001***	0.709
	Week	1	26.279	0.08883	10.2844	0.001***	0.911
	Site:Habitat	2	0.9900	0.03347	1.9373	0.001***	
	Site:Week	3	0.9553	0.03229	1.2461	0.064	
	Habitat:Week	2	0.8364	0.02827	1.6365	0.006**	
	Site:Habitat:Week	2	0.3811	0.01288	0.7457	0.935	
	Residuals	71	181.423	0.61325			
	Total	86	295.838	100.000			
Ethanol-based DNA	Site	3	0.9106	0.02918	0.8816	0.771	0.353
	Habitat	2	0.7696	0.02466	1.1176	0.222	0.531
	Week	1	17.614	0.05643	5.1159	0.001***	0.905
	Site:Habitat	2	0.5214	0.01671	0.7572	0.937	
	Site:Week	3	13.953	0.04470	1.3509	0.029*	
	Habitat:Week	2	0.6922	0.02218	1.0052	0.435	
	Site:Habitat:Week	2	0.7158	0.02293	1.0395	0.346	
	Residuals	71	244.450	0.78321			
	Total	86	312.113	100.000			
Hymenoptera	**Variables**	**Df**	**SS**	R^2	*F*	Pr (>*F*)	*P*
Tissue-based DNA	Site	3	1.979	0.05536	1.7995	0.001***	0.005***
	Habitat	2	2.262	0.06327	3.0852	0.001***	0.01**
	Week	1	1.918	0.05366	5.2331	0.001***	0.84
	Site:Habitat	2	0.985	0.02755	1.3435	0.021*	
	Site:Week	3	1.084	0.03032	0.9857	0.511	
	Habitat:Week	2	0.705	0.01972	0.9618	0.567	
	Site:Habitat:Week	2	0.790	0.02210	1.0775	0.274	
	Residuals	71	26.029	0.72802			
	Total	86	35.753	100.000			
Ethanol-based DNA	Site	3	0.9530	0.03167	0.9032	0.632	0.534
	Habitat	2	0.7099	0.02359	1.0093	0.416	0.488
	Week	1	20.410	0.06783	5.8030	0.001***	0.649
	Site:Habitat	2	10.128	0.03366	1.4398	0.066	
	Site:Week	3	12.143	0.04036	1.1508	0.218	
	Habitat:Week	2	0.8514	0.02830	1.2104	0.176	
	Site:Habitat:Week	2	0.7971	0.02649	1.1331	0.251	
	Residuals	64	225.099	0.74810			
	Total	79	300.895	100.000			
Coleoptera	**Variables**	**Df**	**SS**	R^2	*F*	Pr (>*F*)	*P*
Tissue-based DNA	Site	3	1.918	0.05018	1.5507	0.002***	0.002***
	Habitat	2	1.938	0.05073	2.3513	0.001***	0.056*
	Week	1	2.107	0.05513	5.1109	0.001***	0.383
	Site:Habitat	2	0.867	0.02269	1.0517	0.321	
	Site:Week	3	1.320	0.03455	1.0677	0.227	

(Continues)

TABLE 3 (Continued)

Coleoptera	Variables	Df	SS	R^2	*F*	Pr (>*F*)	*P*
	Habitat:Week	2	1.212	0.03171	1.4699	0.004***	
	Site:Habitat:Week	2	0.822	0.02151	0.9970	0.442	
	Residuals	68	28.031	0.73350			
	Total	83	38.215	100.000			
Ethanol-based DNA	Site	3	10.180	0.03768	1.0945	0.292	0.061
	Habitat	2	0.4528	0.01676	0.7303	0.870	0.219
	Week	1	15.747	0.05828	5.0793	0.001***	0.559
	Site:Habitat	2	0.4536	0.01679	0.7316	0.865	
	Site:Week	3	11.681	0.04323	1.2559	0.136	
	Habitat:Week	2	0.9149	0.03386	1.4754	0.065	
	Site:Habitat:Week	2	0.6636	0.02456	1.0702	0.357	
	Residuals	67	207.721	0.76883			
	Total	82	270.179	100.000			
Lepidoptera	**Variables**	**Df**	**SS**	R^2	*F*	Pr (>*F*)	*P*
Tissue-based DNA	Site	3	1.879	0.05602	1.4913	0.001***	0.108
	Habitat	2	1.734	0.05169	2.0642	0.001***	0.147
	Week	1	1.280	0.03817	3.0485	0.001***	0.884
	Site:Habitat	2	0.804	0.02398	0.9577	0.597	
	Site:Week	3	1.563	0.04660	1.2406	0.010**	
	Habitat:Week	2	1.362	0.04062	1.6222	0.001***	
	Site:Habitat:Week	2	0.981	0.02926	1.1684	0.093	
	Residuals	57	23.936	0.71367			
	Total	72	33.540	100.000			
Ethanol-based DNA	Site	3	0.5955	0.02709	0.9858	0.430	0.452
	Habitat	2	0.6804	0.03095	1.6893	0.075	0.028*
	Week	1	38.188	0.17369	18.9634	0.001***	0.013*
	Site:Habitat	2	0.3038	0.01382	0.7542	0.634	
	Site:Week	3	15.042	0.06841	2.4898	0.006**	
	Habitat:Week	2	0.4878	0.02219	1.2112	0.226	
	Site:Habitat:Week	2	0.2980	0.01355	0.7399	0.666	
	Residuals	71	142.978	0.65031			
	Total	86	219.863	100.000			
Hemiptera	**Variables**	**Df**	**SS**	R^2	*F*	Pr (>*F*)	*P*
Tissue-based DNA	Site	3	27.244	0.10038	2.5727	0.001***	0.313
	Habitat	2	20.499	0.07553	2.9036	0.001***	0.19
	Week	1	0.7811	0.02878	2.2128	0.009**	0.959
	Site:Habitat	2	0.8555	0.03152	1.2118	0.190	
	Site:Week	3	13.171	0.04853	1.2437	0.121	
	Habitat:Week	2	0.8404	0.03096	1.1904	0.207	
	Site:Habitat:Week	2	0.9230	0.03401	1.3074	0.113	
	Residuals	50	176.493	0.65029			
	Total	65	271.405	100.000			
Ethanol-based DNA	Site	3	11.624	0.06741	0.8683	0.784	0.712
	Habitat	2	0.8268	0.04794	0.9264	0.623	0.889

(Continues)

TABLE 3 (Continued)

Hemiptera	Variables	Df	SS	R^2	F	Pr (>F)	P
	Week	1	0.5979	0.03467	1.3398	0.118	0.004**
	Site:Habitat	2	0.7798	0.04522	0.8737	0.721	
	Site:Week	3	16.973	0.09843	1.2679	0.054	
	Habitat:Week	2	0.7129	0.04134	0.7988	0.882	
	Site:Habitat:Week	2	0.7580	0.04395	0.8493	0.774	
	Residuals	24	107.098	0.62104			
	Total	39	172.449	100.000			

Note: Results of PERMANOVA (testing for differences in OTU community compositions) and permutation tests (P. test) via permutest (checking for homogeneity of multivariate dispersion) based on 999 permutations. Significance codes: 0 "***"; 0.001 "**"; 0.01 "*"; 0.05 ".": 1 " ".
Abbreviation: PERMANOVA, permutation multivariate analysis of variance.

the preservative fluid than higher sclerotised individuals (Elbrecht et al., 2017; Morinière et al., 2016). In general, small-bodied or fragile individuals are also more apt to be detected in the preservative fluid because their bodies (or detached parts) may pass through the mesh of the filter during sample processing (Marquina et al., 2019). Comparing each community, we find that metabarcoding results of tissue DNA resulted in sample compositions that are coherent with typical catchings of Malaise traps: predominantly Diptera and Hymenoptera, followed by other orders in much lower abundances (Geiger et al., 2016; Gressitt & Gressitt, 1962; Karlsson et al., 2020; Matthews & Matthews, 2017; Moeed & Meads, 1987; Schmidt et al., 2019; Skvarla, 2015). In contrast, we recovered a strikingly high proportion of Lepidoptera when metabarcoding the ethanol-based DNA. Of all lepidopteran OTUs that we recovered in total, more than half of these were detected exclusively in the ethanol-based DNA, making Lepidoptera the most abundant order after Diptera. Interestingly, Lepidoptera is also the only (abundant) order for which we recovered more OTUs from the ethanol-based DNA than from the tissue DNA. We believe that this may be explained by several interacting factors: First, Lepidoptera possess soft-bodied abdomens, meaning that the DNA of these individuals is easily released into the preservative ethanol (Elbrecht et al., 2017; Morinière et al., 2016). Second, a large proportion of Germany's lepidopteran fauna are small-bodied microlepidoptera meaning that the DNA of these individuals is more likely to be concealed by that of larger ones in the tissue (Herrich-Schäffer & Hübner, 1843; Marquina et al., 2019). Thus, these individuals are likely underrepresented in the tissue and overrepresented in the ethanol. Third, both macro- and microlepidoptera serve as important food sources for other arthropods (Strazanac & Butler, 2005). Because some species are known to regurgitate their stomach contents when coming in contact with ethanol (Marquina et al., 2019), we believe that a substantial proportion of lepidopteran OTUs recovered in the ethanol may in fact be gut-based DNA.

Depicting ecological gradients

As expected, we found that communities recovered from the tissue DNA depicted clear biodiversity patterns based on environmental factors (see Barsoum et al., 2019; Liu et al., 2021; Watts et al., 2019). All statistical tests that we performed on tissue DNA revealed highly significant differences in communities for all three variables (sites, habitats, seasonality) individually, but also as a result of interaction effects. We created ordinations to obtain visual overviews of the sample data and in all cases, the environmental trends depicted in the plots were coherent with the statistical results. Environmental trends were strongest for Diptera, which was expected because Malaise traps are very efficient at catching flies; hence, sample size and sample representativeness are much higher for this order than for others (e.g. Coleoptera, Lepidoptera) (Matthews & Matthews, 2017).

Metabarcoding the ethanol-based DNA of the same Malaise trap samples demonstrated that ecological trends were only partly conserved in the preservative fluid. Habitats and sites had no effect on community compositions, but seasonality did. For all orders (except Hemiptera), statistical analysis depicted highly significant differences in communities driven by seasonality (adonis2 $p = 0.001$). Seasonal gradients were strongest among Hymenoptera and Coleoptera, and permutation testing validated that these differences were only driven by location effects. Gradients were not as prominent for Diptera and not at all visible for Lepidoptera. Although statistical testing found that seasonality had a significant effect on lepidopteran communities, we believe that this result is strongly driven by dispersion effects and that we may be dealing with a type II statistical error. Permutation testing revealed that communities collected in the first half of the season were more dispersed than those collected in the second half (permu $p = 0.009$), and the box plot of Tukey's results displayed absolutely no overlap between these groups. Interestingly, although we measured no significant difference in hemipteran community compositions based on seasonality, samples are clearly plotted along a chronological gradient in the ordination. In this case, we suspect that we may be possibly dealing with a type I statistical error, but further analyses are needed.

We are not certain as to why seasonal trends in the ethanol are better conserved among some groups and lesser so among others. However, we speculate that a group's trophic level may have a meaningful impact, as arthropod specimens that fall prey to other arthropods

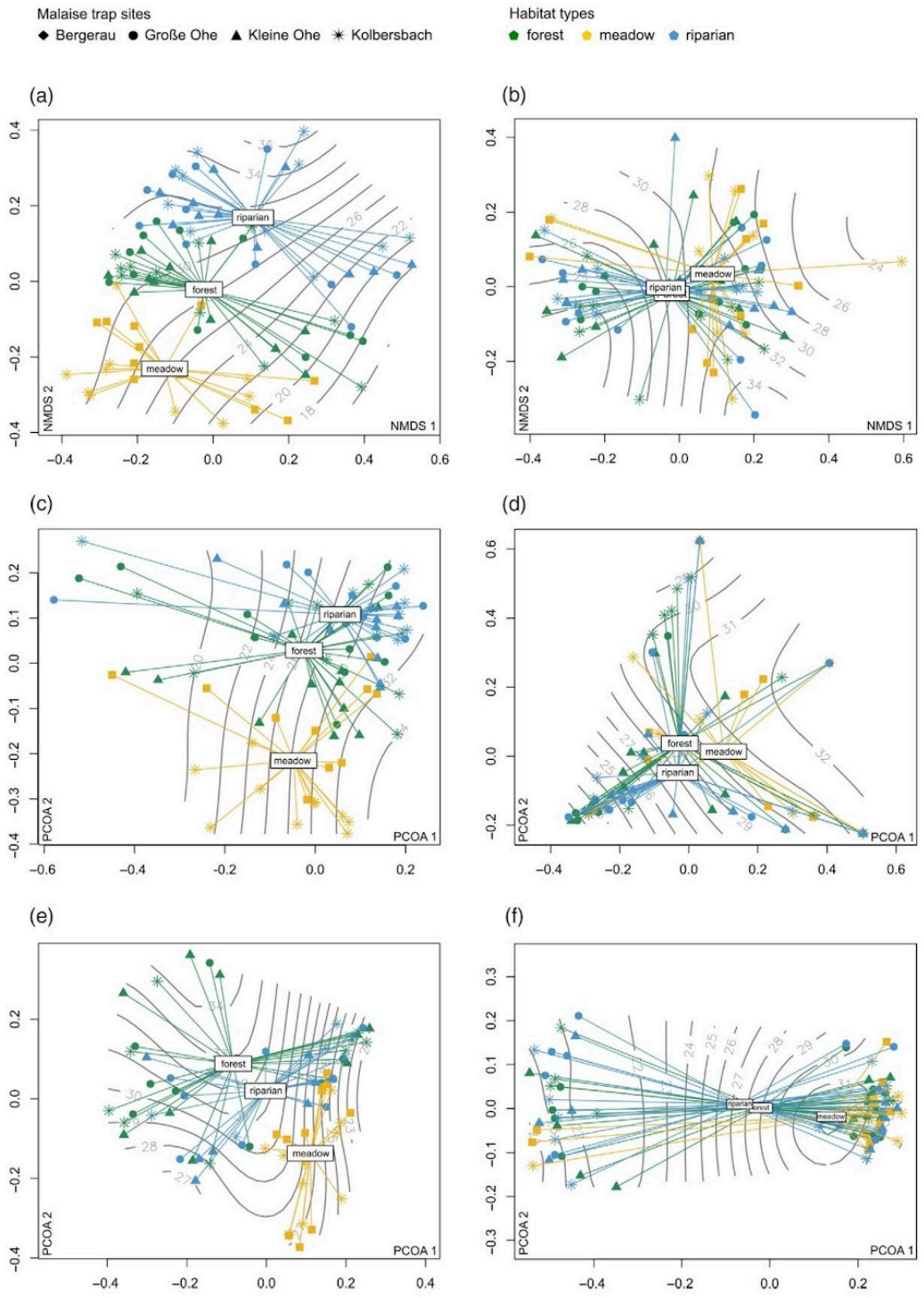

FIGURE 5 Legend on next page.

are introduced into the ethanol as gut content (Marquina et al., 2019). Differing temporal-based factors (e.g. predator–prey interactions, predator metabolic rates, time elapsed since prey consumption) would especially skew natural patterns of abundances because gut-based DNA of the same species is introduced into the ethanol at odd points of time. In addition, there are numerous methodological, environmental

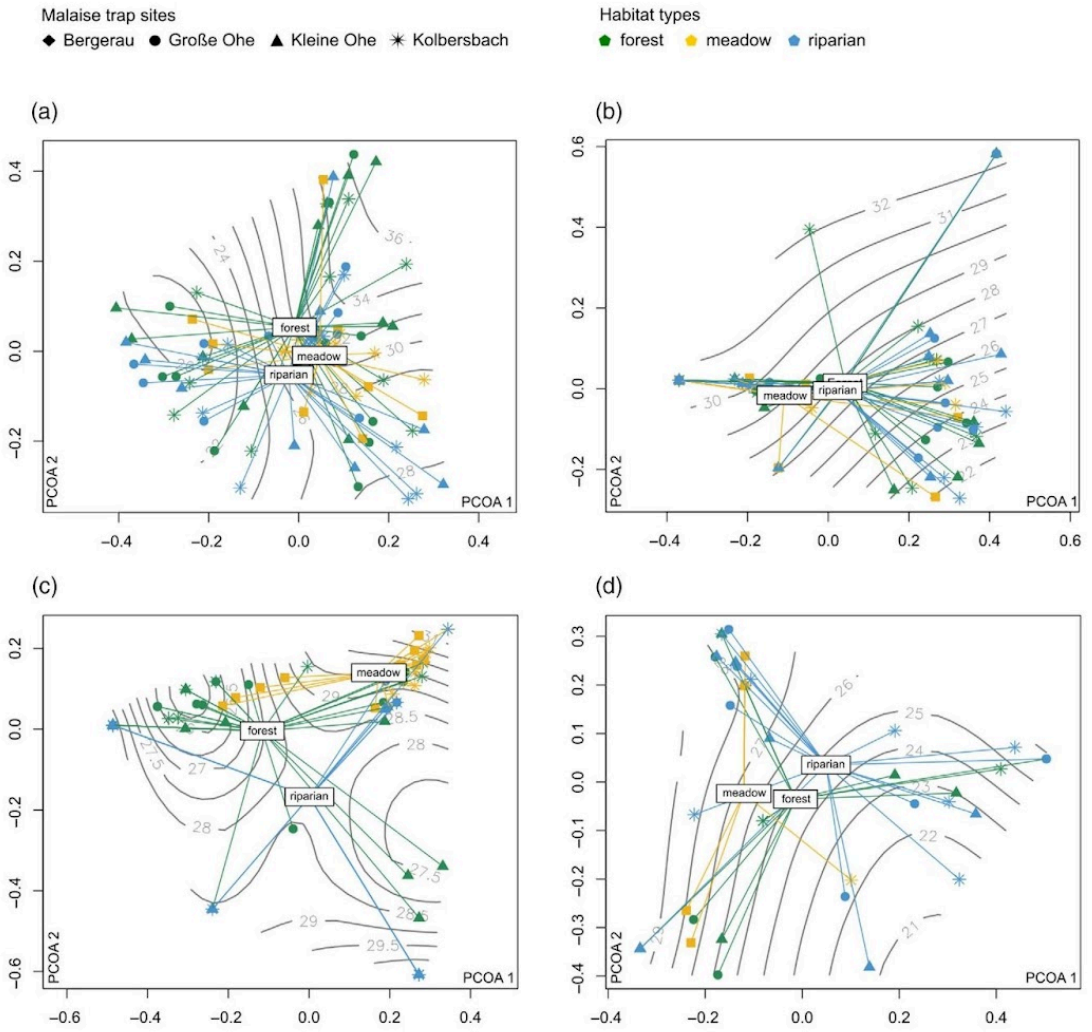

FIGURE 6 Non-metric dimensional scaling (NMDS)/PCoA plots of individual orders. Coleoptera: (a) tissue-based DNA sequencing (PCoA); (b) ethanol-based DNA sequencing (PCoA). Hemiptera: (c) tissue-based DNA sequencing PCoA; (d) ethanol-based DNA sequencing PCoA. Samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the NMDS plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39.

and biological/physiological factors that have a direct influence on success rates of gut content sequencing (Eitzinger et al., 2013; Greenstone et al., 2010; von Berg et al., 2008). With too many sources of bias that are introduced into the analysis of ethanol-based DNA, and no possibility of discriminating between ingested and captured arthropods, seasonal patterns are especially prone to distortion among groups that include many prey species. In our study, seasonal gradients were best depicted among Hymenoptera, Coleoptera, and Hemiptera, but lesser

FIGURE 5 NMD/PCoA plots of individual orders. Diptera: (a) tissue-based DNA sequencing (3D analysis; stress = 0.1649); (b) ethanol-based DNA sequencing (2D analysis; stress = 0.1533). Hymenoptera: (c) tissue-based DNA sequencing (PCoA); (d) ethanol-based DNA sequencing (PCoA) and Lepidoptera: (e) tissue-based DNA sequencing (PCoA); (f) ethanol-based DNA sequencing (PCoA). Samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the non-metric dimensional scaling (NMDS) plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39.

so (or not at all) for Diptera and Lepidoptera. We believe that because the former orders encompass species that are less susceptible to falling prey to other arthropods, they are also less likely to be introduced into the ethanol of our samples as gut content. Typical predators of Coleoptera, Hymenoptera, and Hemiptera are, for example, birds, bats, and frogs (Britannica, 2022). Other arthropods that predate on these taxa include Odonata and Araneae, both of which are lesser represented in our dataset. In contrast, predators of Diptera and Lepidoptera are very well represented in our Malaise trap samples, as these include many taxa of Hymenoptera, Coleoptera, Diptera, and Araneae (Flint & Dreistadt, 1998).

Sequencing ethanol-based DNA failed at depicting spacial patterns. We detected no significant differences among trap sites nor among habitats for all orders. Consistent with previous findings, alpha-diversity assessment demonstrated that the ethanol-based DNA (1) failed at discriminating between the terrestrial and riparian habitats and (2) underrepresented the magnitude of arthropod diversity within every single habitat (see Erdozain et al., 2019; Linard et al., 2016). Recently, Zenker et al. (2020) conducted DNA metabarcoding exclusively on the preservative ethanol of automatic light trap samples to compare the alpha and beta diversity of arthropod communities in Brazil. Unfortunately, they did not examine or process the tissue of these samples at all, so no reference was available as a guideline to their interpretations. Observing our alpha-diversity curves, we strongly believe that the sole use of preservative ethanol can clearly lead to false conclusions, and we therefore discourage its sole use until further research has been conducted.

Overall, we find that ethanol-based DNA sequencing did not provide information on ecological gradients, except for the case of seasonal patterns. The conserved seasonality among some taxa is an interesting starting point for further investigations but until more research has provided more successful results, we recommend researchers dealing with terrestrial ecosystems to be careful when using ethanol-based DNA. It is important to note that in this study, we used 80% ethanol (1 vol% MEK) for arthropod sampling. We conducted DNA extractions in spring 2020 following the collection season (April–October 2019). According to Marquina et al. (2021), this concentration of ethanol is too low for ideal DNA preservation over time. We therefore highly encourage others to use 95% ethanol for sampling to guarantee optimal DNA preservation.

Non-destructive DNA extractions as a promising alternative

A striking subject of today's (and the future's) research concerns the advancing methodology of non-destructive DNA extractions. Numerous studies dedicated to the development of non-destructive methodologies for sequencing are emerging, showing that it is possible to extract DNA (although in smaller quantities) from specimens while keeping their structural integrity intact (Batovska et al., 2021; Carew et al., 2018; Kirse et al., 2022; Marquina et al., 2022; Martins et al., 2019; Martoni et al., 2022; Nielsen et al., 2019). Such protocols roughly consist of leaching DNA from whole individuals by temporarily submerging them in a digestive buffer (Castalanelli et al., 2010; Krosch & Cranston, 2012; Nielsen et al., 2019; Porco et al., 2010; Wong et al., 2014). While various studies have tested non-destructive DNA extractions on single arthropod specimens or samples of mock communities (see Castalanelli et al., 2010; Marquina et al., 2022; Nielsen et al., 2019), we only found one study that did so on real-life bulk samples of terrestrial arthropods from Malaise traps (see Kirse et al., 2022). Malaise traps are especially challenging to process as they can contain hundreds to thousands of individuals (Geiger et al., 2016), each displaying various degrees of sclerotisation, which require different incubation times for adequate non-destructive DNA extraction (Elbrecht et al., 2017). Moreover, there are many options in which non-destructive DNA extractions can be performed, ranging from an optional step of sample sorting, to the choice of lysis buffer, to incubation times of specimen in the fluid, to the protocol used for extraction (Kirse et al., 2022; Marquina et al., 2022; Martoni et al., 2022). With so many factors, numerous researchers are in the process of testing these different options in determining which combination is most effective. One very recent study is especially interesting as the authors conducted comparative analysis on real-life (however sorted) Malaise trap samples (see Kirse et al., 2022). The authors were able to demonstrate that when choosing the right protocol, non-destructive analysis can provide comparable results in terms of species richness and community composition.

On the basis of these results, we believe that in time, non-destructive DNA extractions will become the preferred technique for obtaining DNA from terrestrial arthropod bulk samples. Not only is the sample integrity conserved for further studies, this technique is also quick and provides a lower contamination risk in comparison to traditional tissue-based approaches (Kirse et al., 2022). On this note, we highly encourage future work to test whether ecological trends are also conserved in the OTUs recovered from such analyses. We strongly believe that this is the case as Kirse and authors have shown that they recovered comparable OTU communities in their study using both methods.

CONCLUSION

Returning to the topic of ethanol-based DNA, we recommend researchers dealing with terrestrial ecosystems to be careful when using this approach. These results are not comparable to those obtained using the traditional destructive approaches. However, we do invite researchers in the field of aquatic ecology to look into our research question. Overall, preservative ethanol sequencing on aquatic macroinvertebrates has provided better results as these communities are dominated by soft-bodied specimens—thus, it would be expected that environmental trends are better conserved in the ethanol of such samples.

AUTHOR CONTRIBUTIONS

Caroline Chimeno: Formal analysis (lead); software (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead).

Jeremy Hübner: Formal analysis (equal); methodology (supporting); writing – original draft (equal); writing – review and editing (supporting). **Linda Seifert:** Investigation (lead); methodology (lead); writing – review and editing (supporting). **Jérôme Morinière:** Methodology (lead); software (equal); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting). **Vedran Bozicevic:** Data curation (equal); methodology (equal); software (equal); writing – original draft (supporting); writing – review and editing (supporting). **Axel Hausmann:** Supervision (equal); writing – original draft (supporting); writing – review and editing (supporting). **Stefan Schmidt:** Supervision (equal); writing – original draft (supporting); writing – review and editing (supporting). **Jörg Müller:** Conceptualization (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available on Figshare under the following dois:Chimeno et al. (2022): R Script accompanying Manuscript. figshare. Software (https://doi.org/10.6084/m9.figshare.20222322.v2); Chimeno et al. (2022): Entire Arthropod Dataset (Input data for R script).xlsx. figshare. Dataset (https://doi.org/10.6084/m9.figshare.19397132.v1); Chimeno et al. (2022): Sample Metadata. figshare. Dataset (https://doi.org/10.6084/m9.figshare.19377122.v1); Chimeno et al. (2022): OTU Table. figshare. Dataset (https://doi.org/10.6084/m9.figshare.15029157.v2); Chimeno et al. (2022): Fastq Files. figshare. Dataset (https://doi.org/10.6084/m9.figshare.19376666.v1).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. NMDS plots (without lines) of arthropod community compositions of samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the NMDS plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39. (a) NMDS of tissue-based DNA sequencing (3D analysis; stress = 0.1492). (b) NMDS of ethanol-based DNA sequencing (3D analysis; stress = 0.039). Ellipses are 95% CI of centroids for each sample type.

Figure S2. NMD/PCoA plots of individual orders (without lines). Diptera: (a) tissue-based DNA sequencing (3D analysis; stress = 0.1649); (b) ethanol-based DNA sequencing (2D analysis; stress = 0.1533). Hymenoptera: (c) tissue-based DNA sequencing (PCoA); (d) ethanol-based DNA sequencing (PCoA) and Lepidoptera: (e) tissue-based DNA

sequencing (PCoA); (f) ethanol-based DNA sequencing (PCoA). Samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the NMDS plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39.

Figure S3. NMDS/PCoA plots of individual orders (without lines). Coleoptera: (a) tissue-based DNA sequencing (PCoA); (b) ethanol-based DNA sequencing (PCoA). Hemiptera: (c) tissue-based DNA sequencing PCoA; (d) ethanol-based DNA sequencing PCoA. Samples collected from four sites (Bergerau, Große Ohe, Kleine Ohe, Kolbersbach) covering three habitat types (riparian, forest and meadow). Sites are different symbols and habitats are different colours. Points nearest in plot space have similar species assemblages. In the NMDS plots, seasonality is displayed with ordisurf and ranges from calendar Week 18 to 39.

Table S1. Statistical analysis of the individual arthropod orders (conducted on rarefied dataset). Results of PERMANOVA (testing for differences in OTU community compositions) and permutation tests (P. test) via permutest (checking for homogeneity of multivariate dispersion) based on 999 permutations.

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SECTION 2.2: Artificial Intelligence

Diapriidae are, like many Dark Taxa, hyperdiverse and quite abundant. Not only does it take taxonomic specialists to distinguish them, the sorting process is also really time-consuming due to their high abundance. In order to get around those obstacles, three artificial learning models (ConvNeXt, BEiTv2, and YOLOv8) were trained on over 2200 images to identify eleven different Diapriinae genera and to distinguish both sexes. That proof-of-concept achieved up to 96% accuracy in genus identification and even higher success in determining the sex of specimens.

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Image-based recognition of parasitoid wasps using advanced neural networks

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ABSTRACT

Hymenoptera has some of the highest diversity and number of individuals among insects. Many of these species potentially play key roles as food sources, pest controllers and pollinators. However, little is known about the diversity and biology and ~80% of the species have not yet been described. Classical taxonomy based on morphology is a rather slow process but DNA barcoding has already brought considerable progress in identification. Innovative methods such as image-based identification and automation can further speed up the process. We present a proof of concept for image data recognition of a parasitic wasp family, the Diapriidae (Hymenoptera), obtained as part of the GBOL III project. These tiny (1.2–4.5 mm) wasps were photographed and identified using DNA barcoding to provide a solid ground truth for training a neural network. Taxonomic identification was used down to the genus level. Subsequently, three different neural network architectures were trained, evaluated and optimised. As a result, 11 different genera of diaprids and one mixed group of 'other Hymenoptera' can be classified with an average accuracy of 96%. Additionally, the sex of the specimen can be classified automatically with an accuracy of >97%.

Keywords: AI, artificial intelligence, biodiversity, Diapriidae, DNA barcoding, genus classification, Hymenoptera, image-based identification, integrative taxonomy, machine learning, neural network architectures, taxonomic identification.

Introduction

Although the highest (insect) diversity is known to occur in the tropics (Godfray *et al.* 1999; Dunn and Fitzpatrick 2012), several recent studies (e.g. Chimeno *et al.* 2022, 2023) suggest that there is a very high number of unknown arthropod species in Germany. Most of these taxa are among the insect domains Diptera and Hymenoptera, and referred to as 'dark taxa' (Hartop *et al.* 2022). The highest diversity and individual numbers among insects also occur in the small-bodied groups (but not because of the size; Rainford *et al.* 2016), making even basic tasks such as specimen handling and mounting a challenge (Morinière *et al.* 2019). Although many of these species play potentially key roles in all types of habitats as food sources, pest controllers, pollinators, etc. little is known about the diversity and biology (Dunn and Fitzpatrick 2012). Hallmann *et al.* (2017) recorded a devastating 75% decline in insect biomass within 27 years. That number is especially concerning due to the fact that 30% of all predicted species (Eukaryotes and Prokaryotes) worldwide are insects (Mora *et al.* 2011) and also because up to 80% of insects are as yet undescribed (Stork 2018). Consequently, politicians have become increasingly aware of the ongoing biodiversity crisis and projects such as GBOL III: Dark Taxa were funded to learn more about hidden insect diversity (Hausmann *et al.* 2020). Although the extinction rate of numerous taxa is higher than ever (De Vos *et al.* 2015), descriptive taxonomy and morphological identification of such complex insect groups remains a rather slow process. One of the advancements in species identification and delineation, the DNA barcoding approach (Hebert *et al.* 2003), has helped increase the rate of the process of species

identification, the detection of new species, the evaluation of species complexes and the interpretation of unclear systematics (Blagoev *et al.* 2009; Goldstein and DeSalle 2011; Hübner *et al.* 2023). Combining innovative methods with classic morphology is a cost- and time-efficient means of tackling hidden diversity (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010).

Another promising new technology that is growing in prominence is advanced artificial intelligence (AI). There are many examples of how to advance biological research with these new technologies. Toscano-Miranda *et al.* (2022) listed and compared, for example, the applications of AI in pest control. Folliot *et al.* (2022) used machine learning applications in combination with acoustics to monitor pollinating insects, wood use and ecological interactions in a forest. Wührl *et al.* (2022) presented a promising state-of-the-art insect sorting device, the 'DiversityScanner', powered by a convolutional neural network (CNN). This device identified specimens to family level with a success rate of up to 100% (on average 91.4%), depending on the family they belonged to. Similarly, Borowiec *et al.* (2022) discussed the application of deep learning across various ecological and evolutionary studies, highlighting the potential in predictive modelling and pattern recognition in complex biological data.

The better and more finely scaled these automated identifications become, however, the more opportunities arise for advances in insect research. One potential application could be to only highlight specimens that are not possible to align with a certain group that the algorithm is able to recognise. Targeted evaluation without the expensive and time-consuming hand-picking would be possible (Wührl *et al.* 2022).

As is true for the DNA barcoding system, neural networks can only be as good as the reference on which these are based or with which trained. As barcodes change over time (Hebert *et al.* 2003), depending on the data available for the clustering algorithms, neural networks can distinguish categories based on the quantity and quality of the images used for training.

Our study is based on data from a parasitoid wasp family, the Diapriidae (Hymenoptera) that was obtained in the framework of the GBOL III project (Hausmann *et al.* 2020). These parasitoids play important roles in the ecosystem, e.g. for pest control and are used commercially in agriculture (e.g. *Trichopria drosophilae* to fight the invasive pest *Drosophila suzukii*; Rossi Stacconi *et al.* 2019). Although these tiny (1.2–4.5 mm) wasps occur worldwide, the biology is barely known (Johnson 1992). The known diversity of Diapriidae is limited to ~2000 described species and this is likely only the tip of the iceberg (P. Hebert, pers. comm.). In the framework of GBOL III: Dark Taxa project, one of the two local subfamilies was further examined as a proof of concept of how to approach highly diverse groups with disproportionately high rates of unknown diversity. The GBOL dataset is highly suitable for classification with AI because thousands of specimens were photographed, barcoded and (therefore reliably and fine-scale) identified, allowing a robust foundation for network training. Genetic results were morphologically confirmed and new findings were examined further. Our work should be interpreted as proof of concept that AI can be a valuable, rapid means of evaluating extremely species-rich taxa with high levels of cryptic diversity or bulk samples.

Materials and methods

Dataset

The dataset used for automated classification includes 11 genera of parasitoid wasps, of which 10 belong to the family Diapriidae and subfamily Diapriniiae. Only one taxon, the genus *Ismarus,* is from the family Ismaridae. Both the Diapriinae and Ismaridae were selected for the proof of concept because the diversity, while still challenging, is significantly less incomprehensible and the identification less demanding than for the more diverse and abundant subfamily Belytinae. The specimens were mostly collected in southern Germany, mainly in Bavaria. Since 2011, Malaise traps have been set regularly to cover various (even the most specialised) habitats, ranging from private gardens to the high alpine region. A complete list of evaluated specimens and associated location data are available in Hübner and Shirali (2024). A standardised integrative taxonomic approach consisting of DNA barcoding and morphology was used to identify the specimens: specimens were preliminary identified (to genus if possible and sex) and sequenced (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010; Chimeno *et al.* 2023). The Sanger sequencing of the preliminarily identified material was conducted at the CCBD in Guelph, Canada (see https://ccdb.ca/) using a voucher recovery approach. Genetic results were uploaded to the *BOLD* platform (see https://www.boldsystems.org/) for cross-referencing. After the molecular analysis, all questionable specimens were re-evaluated morphologically. Images of other hymenopteran species were pooled into another group, 'other Hymenoptera', comprising 121 images of other Hymenoptera such as Braconidae, Ichneumonidae, Chalcidoidea and also some Diapriidae that did not belong to the 10 previously mentioned genera because these belonged to the subfamily Belytinae. The word 'class' hereinafter will refer to target groups that belong together and are to be sorted. This does not refer to the taxonomic hierarchical term.

We employed two systems for image capturing: an Olympus camera E-M10 with a Novoflex Mitutoyo Plan Apo 5× microscope lens, controlled by *OM Capture* software (ver. 3.0, see https://www.om-digitalsolutions.com/en/) was used to take deep-focused images by stacking 70–130 individual images; and we took images with a

prototype of the Entomoscope (Wührl *et al.* 2024). All specimens were photographed in ethanol, mimicking the light and sample conditions used for the DiversityScanner. All images were subsequently stacked using *Helicon Focus* (ver. 8, see https://www.heliconsoft.com/heliconsoft-products/helicon-focus/). We used 2257 colour images in our study, as summarised in Table 1. One additional test dataset, including non-Hymenoptera specimens, has been curated to evaluate our pipeline's performance to exclude non-target species using an outlier detection model. This step is vital to avoid misclassifications in practical applications, such as mistakenly identifying a honey bee (Apis) as a target Hymenoptera species. Detailed taxonomy and the number of images in these test datasets are presented in Table 2. DNA barcoding and morphological (expert knowledge) methods were applied to identify the species. All images are available in Hübner and Shirali (2024).

Data preprocessing

In the computer vision field, the efficiency of model training and classification accuracy is significantly influenced by the quality and preparation of input images. This section delineates the preprocessing steps to prepare the insect image dataset for effective machine-learning model training.

Table 1. Taxa and the number of images used for training, validation and testing the neural network split by sex.

Genus	Training	Validation	Testing
Aneurhynchus	104	11	20
Basalys	306	35	60
Coptera	85	9	17
Entomacis	71	8	14
Idiotypa	42	5	19
Ismarus (Ismaridae)	61	7	12
Monelata	115	13	23
Paramesius	110	13	22
Psilus	56	6	10
Spilomicrus	114	12	22
Trichopria	564	63	111
Other Hymenoptera	93	10	18
Female	713	79	140
Male	915	103	180
Unknown	93	10	18
Total	1721	192	338

Crop and resize using *Grounding DINO*

To enhance the model's focus on the insect and to minimise background noise, images are first cropped to the Region of Interest (ROI) using the *Grounding DINO* model (Liu *et al.* 2023), as depicted in Fig. 1. This model employs a zero-shot object detection approach, leveraging image and text features to predict bounding boxes around the insect based on the text prompt 'Insect. Wasp. Wings.' with a box threshold of 0.29 and text threshold of 0.25. These cropped images are resized to a uniform size of 224 × 224 pixels. This standardisation step preserves critical insect features for further processing.

Data augmentation

To enrich the dataset and prevent overfitting, data augmentation techniques such as horizontal and vertical flip, rotation (−30° to +30°), horizontal shift (1–8% of the image width), vertical shift (1–8% of the image height) and zooming in or out (up to 8%) are applied. These techniques help the model learn from a more diverse representation of insect features.

Final dataset compilation

The preprocessed images are compiled into the final dataset and randomly split into a training dataset (~69%), a validation dataset (~11%) and a testing dataset (~20%), considering class imbalance to effectively assess the model's performance and generalisability. These steps ensure that the dataset is thoroughly prepared for the subsequent model training and evaluation phases, establishing a solid foundation for precise, robust insect classification.

Deep learning model architectures

Three different deep learning models were selected and evaluated in this study: *ConvNeXt* (Li *et al.* 2022), *BEiTv2* (Peng *et al.* 2022) and *YOLOv8* (G. Jocher, A. Chaurasia and J. Qui, see https://github.com/ultralytics/ultralytics). These models were selected for proficiency in handling complex computer vision tasks, particularly in identification and classification. Our approach is grounded in transfer learning and fine-tuning methodologies, ensuring that the models are adapted to our specific requirements.

ConvNeXt XLarge (Li *et al.* 2022) is an advanced convolutional neural networks (CNNs) variant known for the

Table 2. Test dataset for outlier detection.

Label	Descriptor	Image count
Diapriidae, Belytinae	Parasitoid wasp	52
Other insects	e.g. Aleothripidae: *Aleothrips*, Coleoptera: *Anisandrus*, Phoridae: *Megaselia*	149

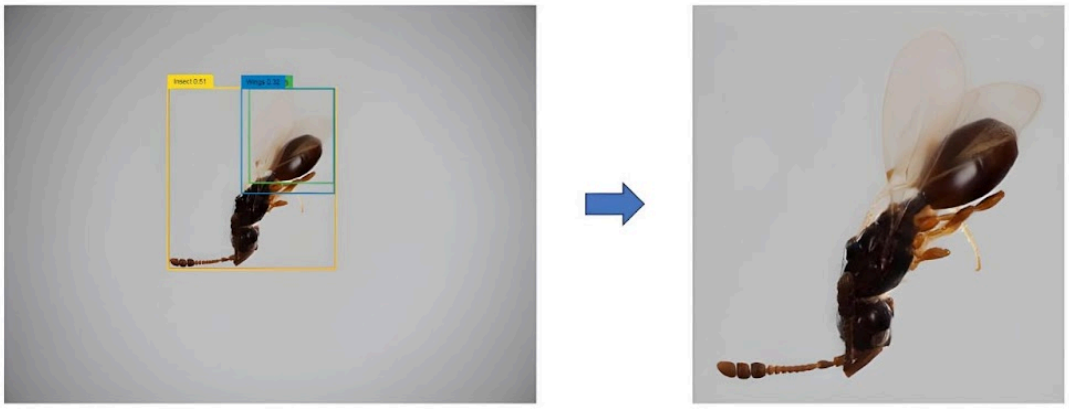

Fig. 1. Object detection using *Grounding DINO* with subsequent cropping is visualised.

exceptional feature extraction capabilities. This incorporates multiple layers designed to process and interpret intricate image details, leveraging advanced activation functions and optimisers to ensure efficient learning and high classification accuracy. The model supports multi-label classification with a sigmoid activation function and handles an image size of 224 × 224 pixels with a batch size of 32 and stochastic depth regularisation with a rate of 0.3. The class weights are similarly adjusted for genera classes. The second model is *BEiTv2* (Peng *et al.* 2022), a Transformer-based model adapted to understanding and interpreting complex image patterns. The unique attention mechanism is instrumental in identifying subtle variations within images, making this a crucial tool for ensuring model stability and robustness under diverse imaging conditions. The model processes images of 224 × 224 pixels with a batch size of 32 and employs a dropout regularisation of 0.3 applied to Attention-MLP (multilayer perceptron) blocks. Class weights for genera classes are weighted by a factor of three. The third model is *YOLOv8*, the latest iteration in the *YOLO* (You Only Look Once) series, selected for the rapid object detection capabilities that are also suitable for classification tasks. The architecture, balanced for speed and accuracy, makes this ideal for real-time applications for which immediate, precise classification is essential. Owing to the framework's limitation in not supporting multi-label classification, we train two separate models, one for genus classification and one for sex determination. Both models leverage ImageNet pre-training weights (Russakovsky *et al.* 2015) and all layers are made trainable, an approach that maximises learning from our dataset. These models are designed for multi-output classification, utilising a softmax activation function. The models are capable of processing larger images of 640 × 640 pixels, operating with a batch size of 64 and incorporating a dropout regularisation of 0.3. The class weights for both models are set to default. This configuration ensures optimal performance and accuracy in our classification tasks. In conclusion, the architecture of each model has been tailored to meet the specific requirements of this project. *ConvNeXt*'s advanced convolutional approach, *BEiT*'s attention-based mechanism, and *YOLO*'s speed and precision collectively contribute to the successful implementation of the classification tasks in this study.

Training setup and process

A standard personal computer with a powerful NVIDIA RTX 4080 GPU was used with *Python* (ver. 3.10), *TensorFlow* (ver. 2.10.1), *PyTorch* (ver. 2.0.1), *Keras*, *CUDA* (ver. 11.7) and *Anaconda* software was used for classification. This integrated environment provides the efficiency and flexibility to train deep learning models. During the training process, all three models were trained for a maximum of 150 machine-learning epochs using the AdamW optimiser with a consistent learning rate of 0.001. We employed a four-fold cross-validation approach to optimise model performance. This allowed us to assess the models' performance on different subsets of the data, mitigating the risk of overfitting and providing a more robust evaluation of the generalisation capabilities. In addition to cross-validation, we also applied early stopping, model check pointing, and learning rate reduction techniques, with training progress monitored. Notably, model weights were saved whenever improvements were observed during validation. *BEiTv2* and *YOLOv8* utilised categorical cross entropy for loss functions, whereas *ConvNeXt* employed binary cross entropy.

Outlier detection

An algorithm for automatic classification is expected to reliably differentiate between insects that belong to the predefined classes for classification and specimens that do not belong to these classes. To enhance this capability, we implemented a preliminary filtering stage using an outlier

detection model prior to our main image classifier. This allowed the automatic filtering of collections that had not previously been presorted for the predefined classes. This outlier detection model classified a specimen into one of the two groups, 'Hymenoptera for classification' and 'Non-Hymenoptera'. 'Hymenoptera for classification' includes specimens within the Hymenoptera genera we targeted for detailed analysis. The second group, 'Non-Hymenoptera,' consisted of all other insect specimens that do not belong to the order Hymenoptera. This broad category includes a variety of insects, some examples of which are provided in Table 2 as other insects. This prefiltering is carried out by a one-class support vector machine (OCSVM) based on the *BEiTv2* – a pretrained deep learning model with ImageNet weights. Da Silva Puls *et al.* (2023) have demonstrated that ViTs perform best for this task. During this process, the classification layer is removed, leaving the model to serve as an effective feature extractor. This model transforms the input images into a lower-dimensional feature space, capturing low-level and high-level image features. Subsequently, a OCSVM on these feature representations extracted from the training dataset is trained. Any new testing data that falls within the boundary of the OCSVM is assigned to the trained class and data points outside the boundary are declared as outliers or Non-Hymenoptera.

Principal Component Analysis (PCA) is subsequently employed to reduce the dimensionality of the data from 1024 to 128 features per image to maintain data quality while reducing computational complexity. In this next step, the data are normalised using the mean and variance of the training dataset. Subsequently, the OCSVM is trained on the reduced, normalised feature representations. This approach does not involve training a neural network, therefore there is no need for a separate validation dataset. Instead, the validation dataset is combined with the training dataset for training the OCSVM on the positive class, making this suitable for detecting outliers that, in this context, are the other insects.

The entire approach is implemented using the open-source machine learning library Scikit-learn (Pedregosa *et al.* 2011). Parameter tuning is performed through a grid search to optimise the OCSVM's performance.

Results

Classification performance metrics

The performance metrics for genus and sex classification of the three different deep learning (DL) models are provided in Table 3. The performance metrics include the test classification accuracy and the *F*1-score for the best model selected across four training runs using fourfold cross-validation.

The performance metrics show that *BEiTv2* consistently outperforms the other models in genus and sex classification tasks. *ConvNeXt XLarge* also exhibits strong performance, while *YOLOv8* performs competitively, albeit with lower accuracy and *F*1-score than in the two other models. For this reason, only the classification results of the best-performing model, *BEiTv2* are presented below.

Table 3. Performance metrics of three different deep learning architectures for genus and sex classification.

Architectures	Genus accuracy	Genus *F*1-score	Sex accuracy	Sex *F*1-score
BEiTv2	0.96	0.95	0.97	0.98
ConvNeXt XLarge	0.94	0.94	0.95	0.96
YOLOv8	0.89	0.90	0.94	0.94

A value of one corresponds to 100%.

The classification results for the 11 predefined classes of Hymenoptera and one 'Other_Hymenoptera' class are depicted in a confusion matrix in Fig. 2 and 3 for the tasks of genus and sex classification.

In addition, the graphs of the classification results for training and validation accuracy, and loss for the *BEiTv2* model are given in Fig. 4, 5 and 6. These figures represent the best fold of the cross-validation training process. These provide a comprehensive view of the model's learning progress throughout training, illuminating the overall performance and convergence behaviour.

The figures show a steady increase in accuracy and corresponding decrease in loss, suggesting the model is learning effectively. Notably, the close alignment of the training and validation curves indicates that the model is not overfitting, performing similarly on both seen and unseen data. Moreover, the absence of a plateau in improvement or a significant gap between training and validation performance suggests that underfitting is not occurring. Hence, the model exhibits a balanced learning trajectory, suggesting robustness and reliability when applied to similar unseen data.

Class activation maps

Class Activation Mapping (CAM) (Zhou *et al.* 2016) is a technique used for generating heat maps to highlight class-specific regions of images that impact the classification result. In Fig. 7, heat maps for two different insect specimens are provided as examples: the genus *Paramesius* (top) and *Spilomicrus* (bottom). The left side represents heat maps associated with the predicted genus. The antennae, head and thorax are consistently significant in predicting the genus. On the right side, the heat maps related to sex prediction are displayed, in which the antennae are crucial for sex prediction. These results show that the classification algorithm considers features similarly to how a taxonomic expert would.

Identification of non-target Hymenoptera

The outlier detection method was assessed using two different test datasets: one is described in Table 2 and the other is

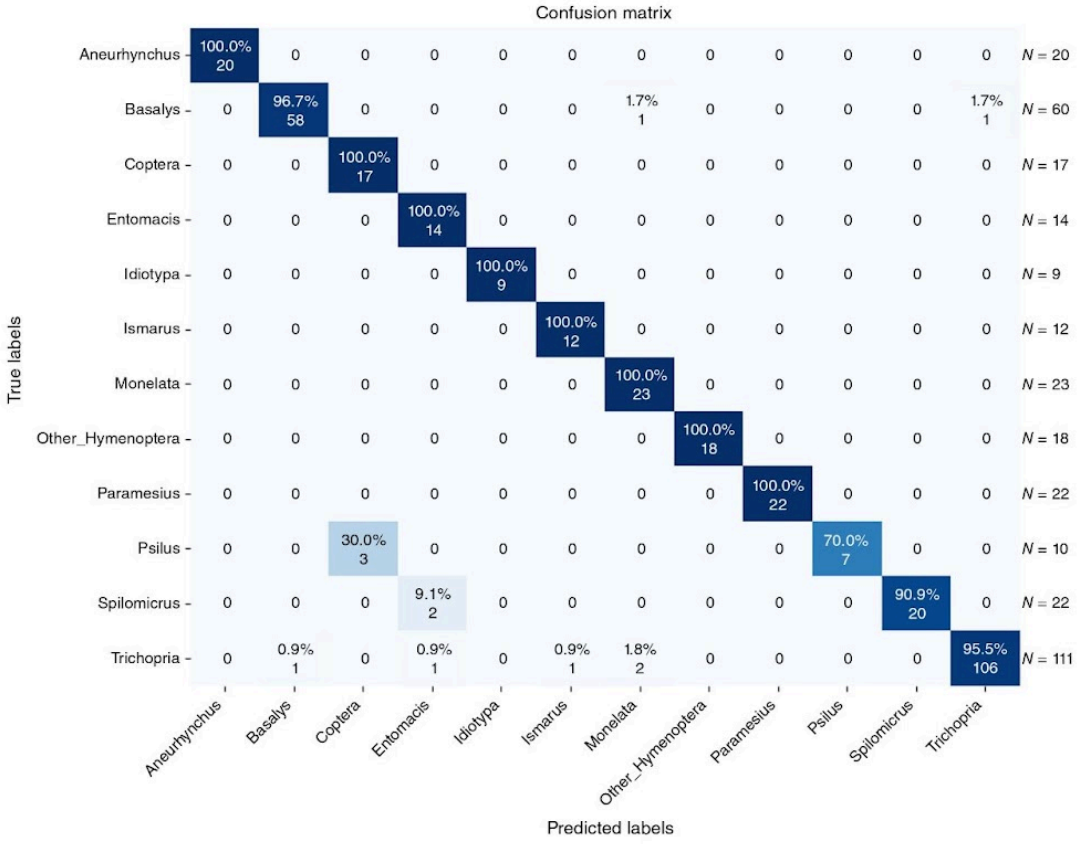

Fig. 2. Confusion matrix with genus classification results of the *BEiTv2* model.

a split of our main dataset. The results are visualised in Fig. 8. The method misclassified 23 of the total of 652 resulting images.

In the context of our study, 'inliers' are images that the outlier model correctly identifies as belonging to the category of Hymenoptera but not necessarily to the specific target genera of Diapriidae that are our classification focus. Conversely, 'outliers' are images that do not belong to the category of Hymenoptera and are therefore beyond the focus of our model's training criteria.

Notably, this approach achieved 100% accuracy on the test split of our dataset as expected because our outlier model was trained specifically on this dataset. Regarding the 'Other Insects' images, this model demonstrated prowess by identifying 90.6% of the images as outliers. This indicates the model's ability to distinguish these insects from Hymenoptera effectively. The Diapriidae and Belytinae images, as part of 'Other Hymenoptera,' presented a unique challenge. There were variations in image quality, background and differences in camera sources. Despite these challenges, our model detected 82.7% of the images as inliers, underscoring the potential for accurate classification even under adverse conditions. Overall, these results demonstrate the model's robustness and accuracy in classifying closely related but non-target Hymenoptera species, even under non-ideal conditions.

Discussion

The network approach demonstrated is restricted to the European Diapriidae fauna, particularly the subfamily Diapriinae because within the framework of the GBOL III project, specimens and species of this subfamily were investigated and barcoded as proof of concept. The Diapriidae (even if the dataset is limited to German material only) is simply too diverse and complex to be investigated in such a short period of time. Nevertheless, most of the genera that were subject to our approach are distributed worldwide. Also, there are many species, e.g. *Spilomicrus formosus* that even inhabit several continents (in this case, Europe, Asia and North America), making our DL model a powerful

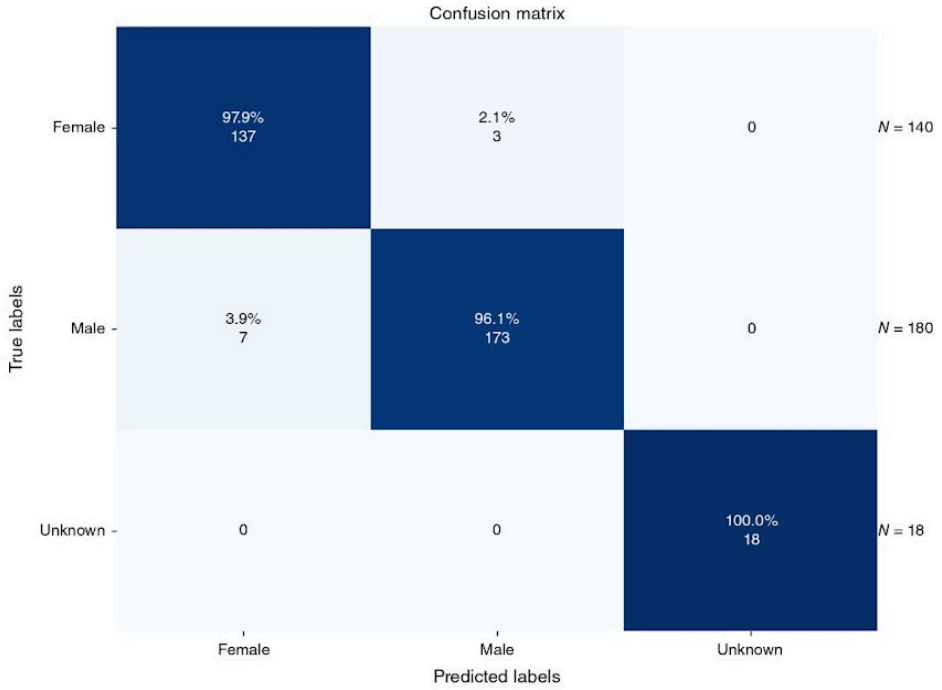

Fig. 3. Confusion matrix with sex classification results of the *BEiTv2* model.

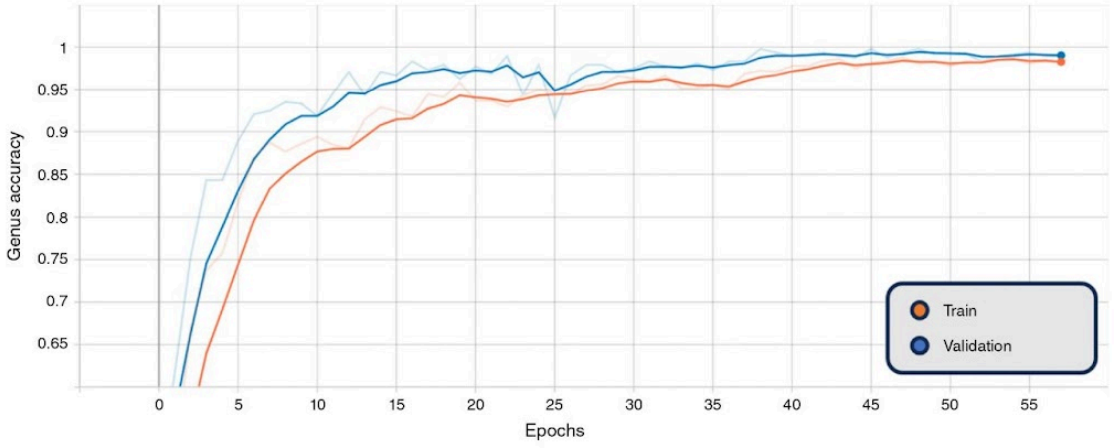

Fig. 4. Smoothed training (orange) and validation (blue) genus accuracy, *BEiTv2*, with the original graph transparent. Note: 'Epoch' refers to a machine-learning epoch.

tool regarding the fact that over 90% of the sampling area of the barcoded material was limited to Bavaria, Germany.

The success rate at which the DL model was able to distinguish between different genera was high (up to 100%). Exceptions could be detected distinguishing between the genera *Psilus* and *Coptera*. A closer examination of these was not surprising as genera are closely related and appear highly similar. Although *Psilus* was described by Panzer (1801) and *Coptera* by Say (1836) 35 years later, confusion remained regarding distinguishing between these over a century after description (Nixon 1980). The most reliable morphological feature is the wing that is folded lengthwise

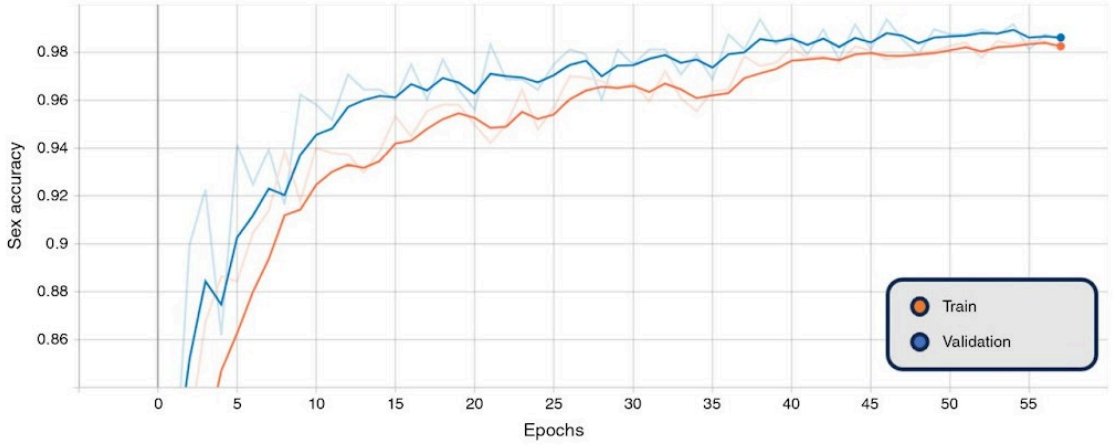

Fig. 5. Smoothed training (orange) and validation (blue) sex accuracy, *BEiTv2*, with the original graph transparent. Note: 'Epoch' refers to a machine-learning epoch.

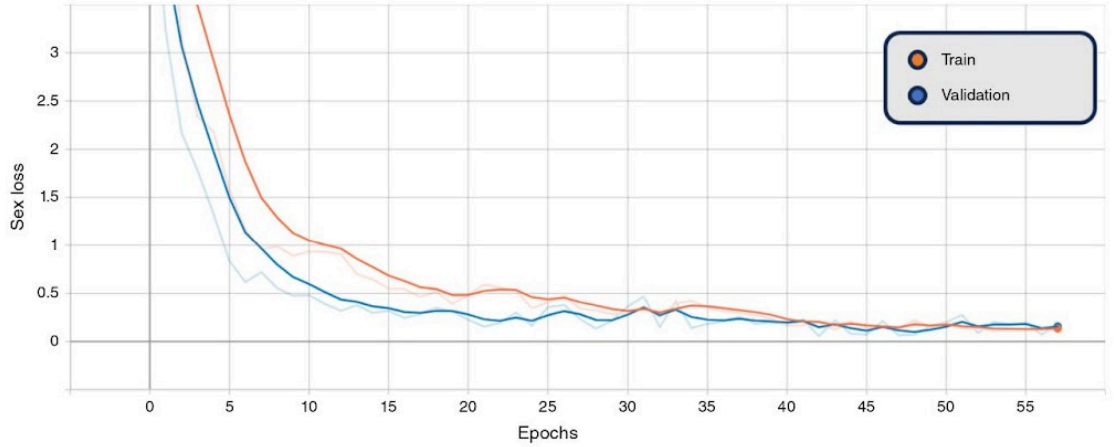

Fig. 6. Smoothed training (orange) and validation (blue) combined genus and sex loss, *BEiTv2*, with the original graph transparent. Note: 'Epoch' refers to a machine-learning epoch.

in *Coptera* and without a fold in *Psilus*. However, both genera usually lie on the sides with applied wings and therefore distinguishing between these without changing the position to a dorsal view is almost impossible. Another obstacle we faced was that there was not enough material to train the models on rare taxa. *Idiotypa, Diapria* or *Tetramopria* are genera with low species and individual counts.

The class activation heatmaps highlight, as expected, the antennae of the insects that taxonomists also use to distinguish between sexes. What was less expected was that the CAMs highlighted the head region. Although the head shape could be used to identify genera, other body features would be used by a specialist. Wing venation (that is often not visible in the images) and the shape of the abdomen (that is not always helpful and dependent on orientation) would be more intuitive for distinguishing *Paramesius* and *Spilomicrus* (example provided in Fig. 7). Therefore, CAMs may have the potential to find descriptive characters for species descriptions in future.

Although the algorithm cannot identify these to genus level, the family can be determined and therefore used to specifically sort for rare, unidentifiable specimens that would save even a specialist vast amounts of time due to the generally high specimen numbers of most diaprids.

In furthering this research, we developed a web application, DiapriidaeClassificationApp, to make the identification process more accessible and user-friendly (see https://gitlab.kit.edu/kit/iai/ber/diapriidaeclassificationapp). However, noting that the application's accuracy is highly dependent

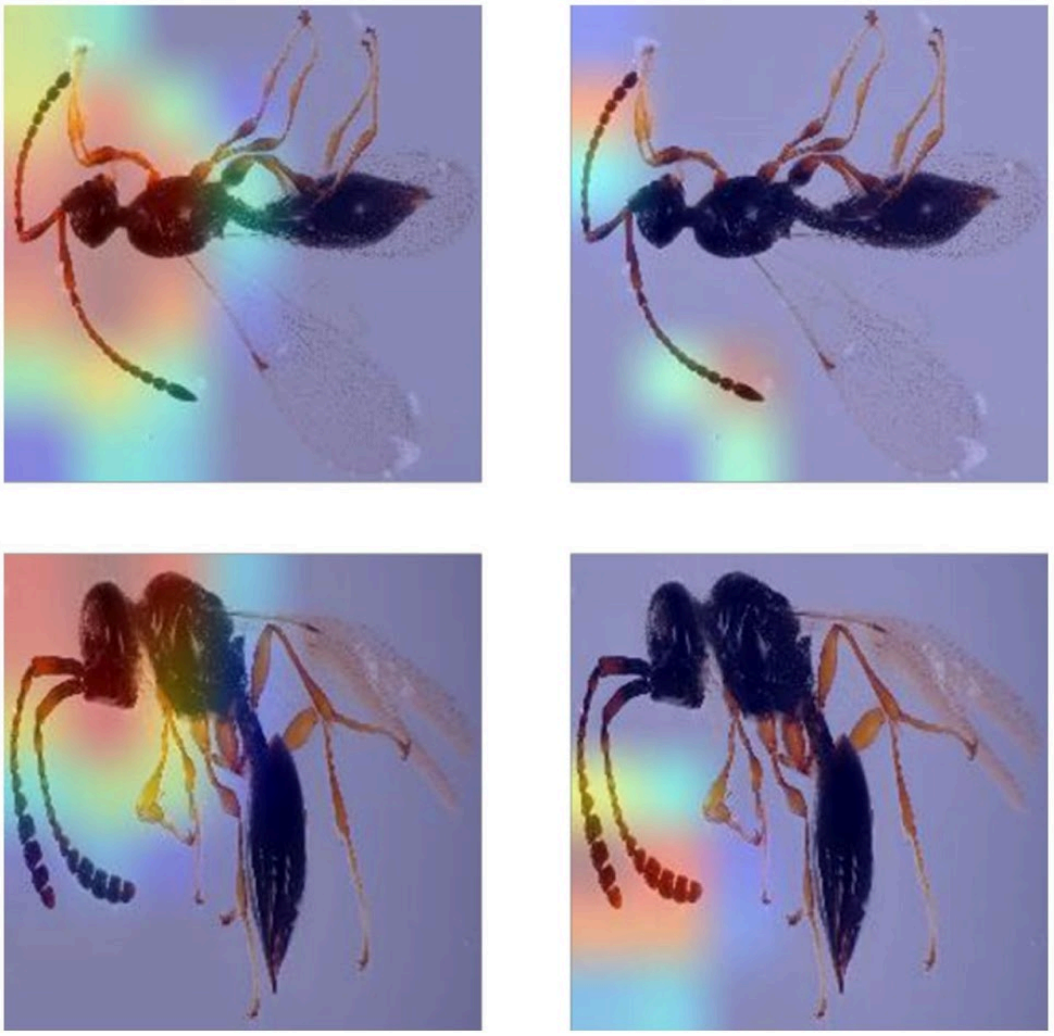

Fig. 7. Class activation heatmaps for genus classification (left) and sex classification (right). Red areas indicate regions with higher weighting in the classification.

on the quality of the images used is crucial. Only high-quality lab images with consistent, comparable illumination are suitable for the app's analysis. Images taken with a smartphone, that often vary in quality and lighting conditions, are unlikely to yield reliable results. This limitation emphasises the need for standardised image-capturing methods to ensure the app's effectiveness in species identification.

Conclusion

AI has been proven to be a reliable and efficient tool for identifying the highly diverse taxon Diapriinae to genus level in Europe. One of the greatest advantages lies in the fact that a user does not need a profound knowledge of morphology or other taxonomic experience to achieve identification results. Making these groups available for completely different research fields, such as ecology or pest control, is a significant advancement and an affordable, non-invasive alternative to (*meta*-) barcoding-based species identification. This technology should be further developed and can be applied to a wide variety of species groups, e.g. other parasitoid wasps. Another potential application could be to power the DiversityScanner with the new DL models to allow more accurate delimitations and targeted specimen selection.

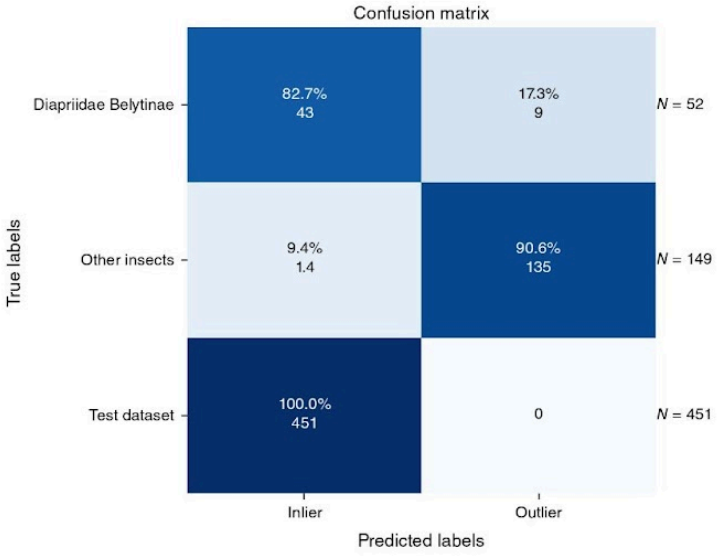

Fig. 8. Confusion matrix for outlier detection.

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Data availability. All images are available in Hübner and Shirali (2024). Additionally, a preprint version of this article is available in Shirali *et al.* (2024).

Conflicts of interest. The authors declare that they have no conflicts of interest.

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Dedication. We dedicate this paper to the memory of Stefan Schmidt, who sadly passed away. Stefan's significant contributions to the research were invaluable, and his expertise and dedication greatly aided the completion of this work. He will be deeply missed.

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3. CHAPTER: Biodiversity assessments and Species records

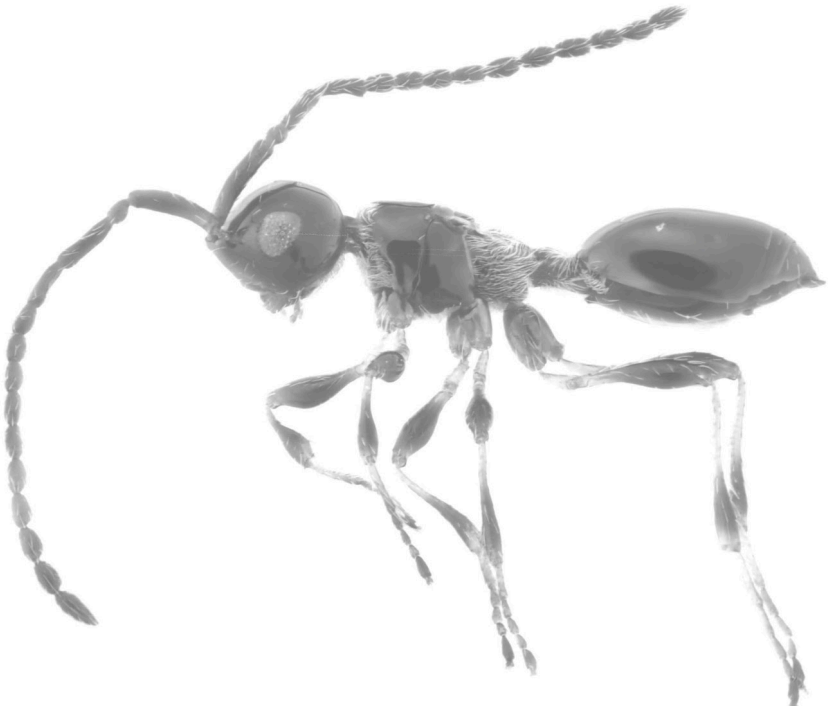
This last chapter deals with biodiversity and hidden entomofauna assessment in general. A taxonomist has to be able to apply different methods and approaches, morphologically and genetically. Its tasks may be very specific, requiring a meticulous review of historical material, or may extend quite differently to the assessment of diversity estimates of entire communities or habitats. Both cases will be presented in this last chapter.

Diphora westwoodii
Förster, 1856

SECTION 3.1: Diapriidae of the island Koltur (Faroe Islands, Denmark)

The diapriid fauna of the Faroe Islands were evaluated the last time in 1956 (Petersen). The reevaluation of the historic material and a new collection turned out to be overdue: most species misidentified. As a result of our study, the following species could be recorded: *Basalys abruptus* (Thomson, 1859) (first record), *Basalys longipennis* (Kieffer, 1911) (first record), *Trichopria aptera* (Ruthe, 1859), *Zygota parallela* (Thomson, 1858) (first genus record), *Pantoclis similis* (Thomson, 1858) (first record), *Pantoclis trisulcata* Kieffer, 1907, *Synacra atracta* Macek, 1995 (first genus record), *Miota exsecta* Wall, 1998 (first record), *Aclista alticollis* (Thomson, 1858) (first genus record) and *Aclista* cf. *insolita* Nixon, 1957 (first record).
In addition to the morphological identifications we provide some sequence information.

Hübner, J., Gabel, H., Deines, V., Kreiling, A. K. & Notton, D. G. (2024). Review of Diapriidae (Hymenoptera) of the Faroe Islands. *Spixiana*, *47* (1), 82-93.



Trichopria aptera
(Ruthe, 1859)

Review of Diapriidae of the Faroe Islands

(Hymenoptera, Diapriidae)

Jeremy Hübner, Hannes Gabel, Viktor Deines, Agnes-Katharina Kreiling & David G. Notton

Hübner, J., Gabel, H., Deines, V., Kreiling, A.-K. & Notton, D. G. 2024. Review of Diapriidae of the Faroe Islands (Hymenoptera, Diapriidae). Spixiana 47 (1): 83–92.

The Faroe Islands are isolated in the North Sea but, despite sparse vegetation, are home to numerous insects. The diapriid fauna was previously studied by Kryger & Schmiedeknecht (1938) and Petersen (1956), but taxonomic knowledge has advanced significantly so a reevaluation is necessary. This study aims to update the diapriid checklist for the Faroes by reviewing historic material and some recent collections from the island Koltur. We identified ten species: *Aclista alticollis* (Thomson, 1858), *Aclista* cf. *insolita* Nixon, 1957, *Basalys abruptus* (Thomson, 1858), *B. longipennis* (Kieffer, 1911), *Miota exsecta* Wall, 1998, *Pantoclis similis* (Thomson, 1858), *P. trisulcata Kieffer*, 1907, *Synacra atracta* Macek, 1995, *Trichopria* ? *aptera* (Ruthe, 1859), and *Zygota parallela* (Thomson, 1858) and found nine taxa new to the Faroes: *Aclista*, *Synacra*, *Zygota*, *A. alticollis*, *B. abruptus*, *B. longipennis*, *M. exsecta*, *P. similis* and *S. atracta*. In addition, we provide CO1 barcode sequences for *A. alticollis*, and an identification key for all Faroese diapriids.

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Introduction

The Faroe Islands are an archipelago in the North Atlantic Ocean north of Scotland, south east of Iceland and west of Norway. The subpolar oceanic climate is characterized by cold (12°C) summers, and stormy, wet but mild (~5°C) winters demanding high rates of adaptation of flora and fauna (Cappelen & Laursen 1998). While some diapriids can have huge distribution areas that span several continents, we expect the diapriid fauna of the Faroes to be similar to those of the nearby islands and coastal areas of Iceland, Greenland, Norway and Scotland which have comparable terrain and climate.

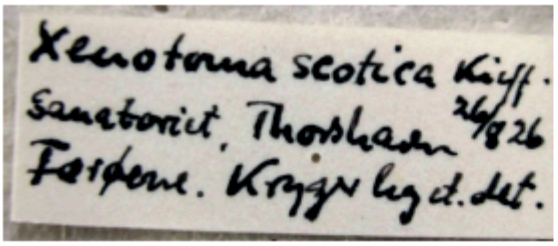

Fig. 1. Example of a handwritten label of the female *Pantoclis trisulcata* specimen by J. P. Kryger: *Xenotoma scotica* Kieffer, Streymoy Is., Sanatoriet near Tórshavn, ♀, 26.8.1926, leg. and det. J. P. Kryger.

The diapriid fauna was previously evaluated only by Kryger & Schmiedeknecht (1938) who specifically studied Faroese Hymenoptera and by Petersen (1956) in the context of his study of Icelandic Hymenoptera. They recorded: *Aclista macroneura* Kieffer, 1909, now *Zygota parallela* (Thomson, 1858); *Cinetus fuscipes* (Kieffer, 1907); *Loxotropa aptera* (Ruthe, 1859), now *Trichopria aptera*; *L. suecica* Kieffer, 1911, now *Basalys suecicus*; *L. thomsoni* Kieffer, 1911, now *T. nigricornis* (Marshall, 1868); *Pantoclis trisulcata* Kieffer, 1907; *Xenotoma gracilicornis* Kieffer, 1910, now *Pantolyta flaviventris* (Thomson, 1858); and *X. scotica* Kieffer, 1910, now *Belyta sanguinolenta* (Nees, 1834). Other authors studying the Faroese fauna (Landt 1800, Hansen 1881) did not mention any diapriids from the Faroe Islands.

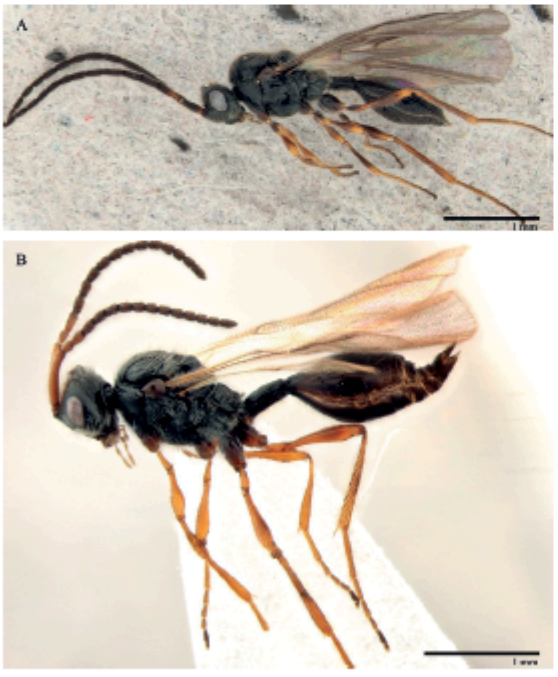

Fig. 2. *Aclista alticollis*: **A.** male habitus, lateral; **B.** female habitus, lateral.

Almost seventy years since the last evaluation of Faroese diapriid taxonomy has advanced greatly and it is time to review the fauna again. This study is the first integrative taxonomic study of Faroese diapriids combining traditional morphology of the historic material identified by Kryger & Schmiedeknecht (1938) together with recently collected material which we have barcoded.

Material and methods

Fifty-six freshly caught specimens were examined for this study. All specimens were collected by Agnes Kreiling in 2021 and 2022 on the island of Koltur (Faroe Islands) using pitfall traps and Malaise traps, dry mounted on card points and deposited at the SNSB-ZSM and FOMNH collections. Of these DNA sequencing was attempted for sixteen specimens: the CO1 barcodes of two species (ZSM-IRT-Koltur-1 and ZSM-IRT-Koltur-3) were successfully obtained and uploaded on the Barcode of Life database (www.boldsystems.org). All sequences are publicly available in the BOLD project KODIA. Historical material from the collection of Jens Peter Kryger (NHMD) was also examined for this study. Twenty-five specimens from one collection event in 1826 comprising card mounted and ethanol preserved specimens of which the latter were dried and mounted. We have referred to Kryger & Schmiedeknecht (1938) to interpret the locality labels of J. P. Kryger (Fig. 1).

Repository acronyms

FOMNH – Faroe Islands National Museum, Koltur island
NHMD – Zoological Museum of the Natural History Museum of Denmark
SNSB-ZSM – Bavarian State Collection of Zoology in Munich, Germany

Taxonomic part

Aclista alticollis (Thomson, 1858)

Fig. 2A–B

Nomenclature: *Acoretus alticollis* Thomson, 1858: 157, ♀. *Xenotoma nigra* Kieffer, 1907: 23,25, ♀. Synonymized by Nixon (1957). *Pantoclis cilipes* Kieffer, 1907: 31, 37, ♂. Synonymized by Nixon (1957). *Anectata* (*Acoretus*) *fallax* Kieffer, 1909: 544, ♀. Synonymized by Nixon (1957). *Anectata* (*Acoretus*) *alticollis* var. *aestivalis* Kieffer, 1909: 547, ♀. *Anectata* (*Acoretus*) *alticollis* var. *isotoma* Kieffer, 1909: 547, ♀.

Examined material: FAROE ISLANDS: Koltur Is., N61.98487, W6.96508, 3♂, 1♀, 17.6.2022, Malaise trap, leg. A. Kreiling, det. J. Hübner/J. Macek (SNSB-ZSM), one male sequenced (BOLD:ACR7790). Koltur Is., N61.98473, W6.96653, 2♀, 26.7.2021, pitfall trap, KolturM1, leg. A. Kreiling, det. J. Hübner/J. Macek (SNSB-ZSM), one male sequenced (BOLD:ADU5289).

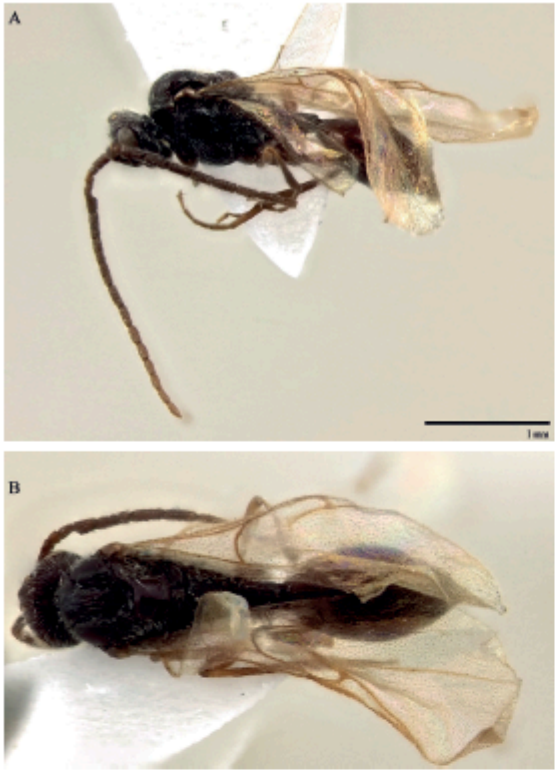

Fig. 3. *Aclista* cf. *insolita* male: **A.** habitus, lateral; **B.** habitus, dorsal.

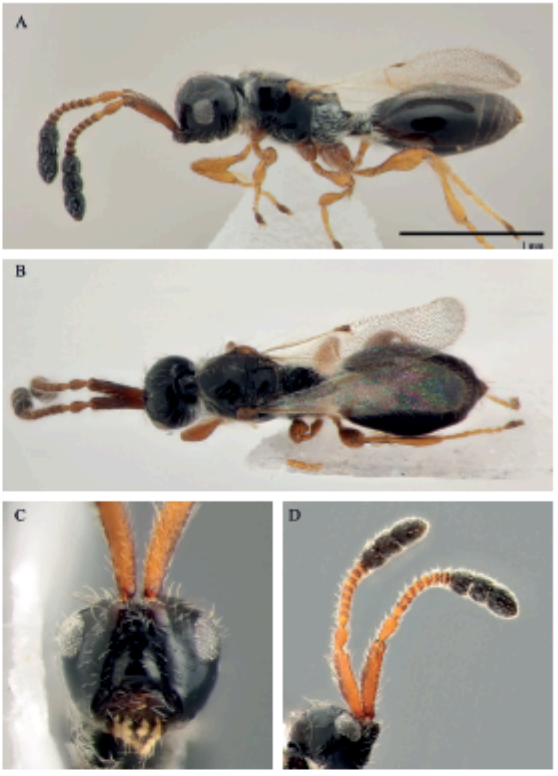

Fig. 4. *Basalys abruptus* female: **A.** habitus, lateral; **B.** habitus, dorsal; **C.** face; **D.** antennae, lateral.

Distribution. A common species widely distributed in North-West Europe (e.g. Nixon 1957). This is the first record of the species for the Faroe Islands. In addition the Barcode of Life database (www.boldsystems.org) has sequences from Denmark, Finland and Canada.

Notes. Sequence information was obtained for four of the Faroese specimens above and the species was assigned two BINs: ADU5289 and ACR7790. Both BINs were already recorded for Canada, Denmark and Finland, but only identified down to genus level. This is the first record of the genus *Aclista* from the Faroe Islands. *A. alticollis* was previously represented on BOLD by five other BINS, representing 106 specimens from Belarus, Canada, Denmark, Finland, Germany, Norway. It may be a species complex, although possibly some of these BINs are misidentified.

Aclista cf. *insolita* Nixon, 1957
Fig. 3A–B

Examined material: FAROE ISLANDS: Streymoy Is., Tórshavn, 2♂, 4.6.1926, leg. J. P. Kryger, *Xenotoma gracilicornis* det. Kryger (FOMNH).

Notes. Kryger & Schmiedeknecht (1938) identified the material as *Xenotoma gracilicornis*, now *Pantolyta flaviventris* according to Chemyreva & Kolyada (2021), however it does not belong to *Pantolyta* and no other Faroese *Pantolyta* was seen during the current study. *P. flaviventris* should be removed from the checklist of the Faroe Islands.

Basalys abruptus Thomson, 1858
Figs 4A–D, 5A–D

Nomenclature: *Basalys abrupta* Thomson, 1858: 368, ♀. Incorrect termination. *Loxotropa convexa* Kieffer 1911: 932. Synonymized by Nixon (1980).

Examined material: FAROE ISLANDS: Koltur Is., N61.98487, W6.96508, 3♂, 2♀, 17.6.2022, Malaise trap, Koltur-M3, leg. A. Kreiling, det. J. Hübner/D. Notton (SNSB-ZSM). Koltur Is., N61.98473, W6.96653, 2♂, 2♀,

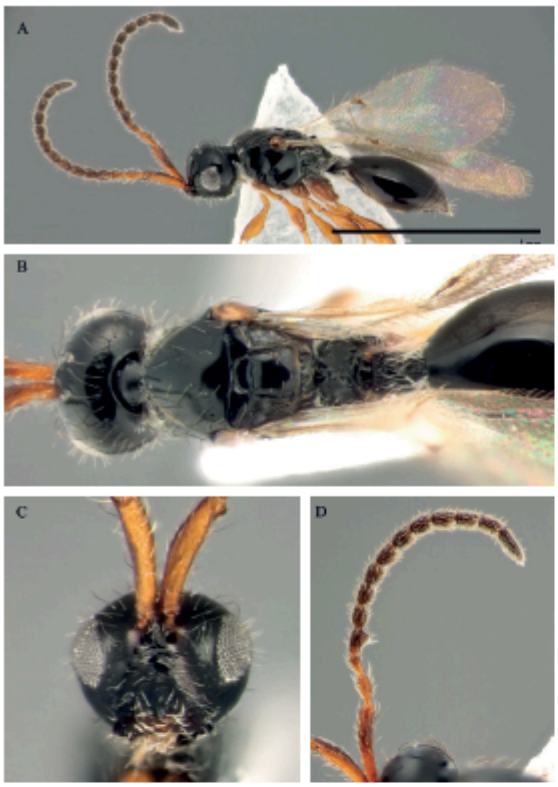

Fig. 5. *Basalys abruptus* male: **A.** habitus, lateral; **B.** habitus, dorsal; **C.** face; **D.** antenna.

26.7.2021, pitfall trap, Koltur-OCR, leg. A. Kreiling, det. J. Hübner/D. Notton (SNSB-ZSM). Koltur Is., N61.99041, W6.97188, 12♂, 27♀, 3.8.2022, pitfall trap, Koltur-KMM, leg. A. Kreiling, det. J. Hübner/D. Notton (SNSB-ZSM). Streymoy Is., Sanatoriet near Tórshavn, ♀, 28.7.1926, leg. J. P. Kryger, *Loxotropa suecica* det. Kryger (FOMNH); Sanatoriet near Tórshavn, ♂, 14.7.1926, leg. J.P. Kryger, *L. suecica* det. Kryger (FOMNH); Tórshavn, ♀,14.7.1926, leg. J. P. Kryger, *L. suecica* det. Kryger (FOMNH).

Distribution. A common and widespread species in Europe (e. g. Hellén 1963, Kozlov 1978, Nixon 1980). This is the first record for the Faroe Islands. In addition the Barcode of Life database (www.boldsystems.org) has sequences from Bulgaria, Germany, Norway and Canada.

Notes. We have followed the interpretation of *Basalys abruptus* given by Nixon (1980) who saw type material. Kryger & Schmiedeknecht (1938) identified the Faroese material above as being *Loxotropa suecica* (Kieffer, 1911) now *B. suecicus* according to Johnson (1992), however we are not certain of the correct interpretation of that name and *B. suecicus* should be removed from the checklist for the Faroe Islands.

Fig. 6. *Basalys longipennis* female: **A.** habitus, lateral; **B.** habitus, dorsal.

Basalys longipennis (Kieffer, 1911)
Fig. 6A–B

Nomenclature: *Loxotropa longipennis* Kieffer, 1911: 932, ♀.

Examined material: FAROE ISLANDS: Streymoy Is., Sanatoriet near Tórshavn, 2♀, 28.7.1926, leg. J.P. Kryger, *Loxotropa suecica* det. Kryger (FOMNH).

Distribution. A widespread species in Europe (e. g. Kozlov 1978, Nixon 1980). This is the first record for the Faroe Islands. In addition the Barcode of Life database (www.boldsystems.org) has a sequence from Norway.

Notes. We have followed the interpretation of *Basalys longipennis* given by Nixon (1980) who saw type material. Kryger & Schmiedeknecht (1938) identified the Faroese material above as *Loxotropa suecica* (Kieffer, 1911) now *B. suecicus*, however we are not certain of the correct interpretation of that name as we have not seen the type and *B. suecicus* should be removed from the checklist for the Faroe Islands.

Miota exsecta Wall, 1998
Fig. 7A–D

Nomenclature: *Miota exsecta* Wall, 1998: 62, 65, fig. 7, ♂.

Examined material: FAROE ISLANDS: Streymoy Is., Sanatoriet near Tórshavn, ♂, 28.7.1926, leg. J. P. Kryger, *Cinetus fuscipes* det. Kryger (FOMNH); Tórshavn, 1♂, 1♀, 24.7.1926, leg. J. P. Kryger, *C. fuscipes* det. Kryger (FOMNH); Sanatoriet near Tórshavn, 2♂, 15.7.1926, leg. J.P. Kryger, *C. fuscipes* det. Kryger (FOMNH); Sanatoriet near Tórshavn, ♂, 28.7.1926, leg. J. P. Kryger, *C. fuscipes* det. Kryger (FOMNH).

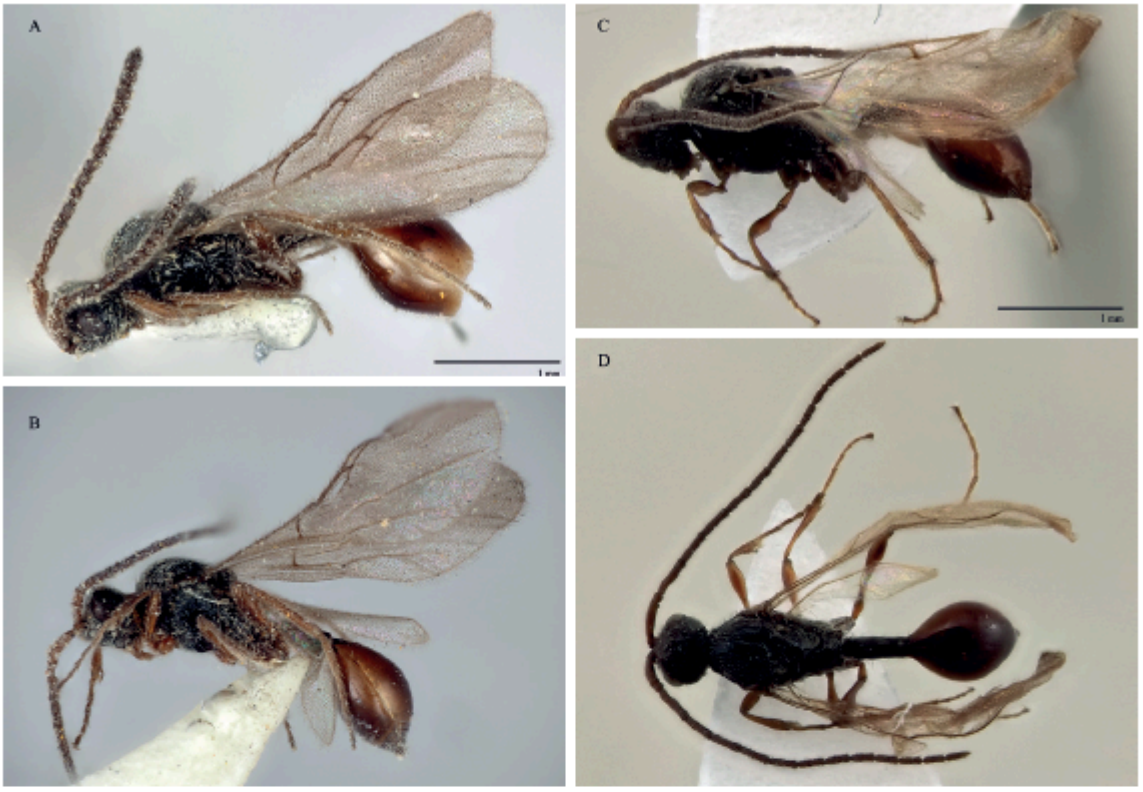

Fig. 7. *Miota exsecta*: **A.** female habitus, lateral; **B–C.** male habitus, lateral; **D.** male habitus, dorsal.

Distribution. This species is poorly known in Europe having only recently been described from material collected in Germany and Switzerland (Wall 1998). This is the first record for the Faroe Islands.

Notes. Kryger & Schmiedeknecht (1938) identified the material as *Cinetus fuscipes* however it is not a *Cinetus* and no other Faroese *Cinetus* was seen during this study. *C. fuscipes* should be removed from the checklist for the Faroe Islands. The female of *M. exsecta* was previously unknown but we have not described it here as the only available specimen is damaged.

Pantoclis similis (Thomson, 1858)
Fig. 8A–D

Nomenclature: *Belyta similis* Thomson, 1858: 172, ♀. *Pantoclis rufiventris* Kieffer, 1907: 32, 39, ♀. Synonymized by Nixon (1957).

Examined material: FAROE ISLANDS: Streymoy Is., Sanatoriet near Tórshavn, ♂, 28.7.1926, leg. J. P. Kryger, *Xenotoma scotica* det. Kryger (FOMNH); Sanatoriet near Tórshavn, ♂, 26.8.1926, leg. J. P. Kryger, *X. scotica* det. Kryger (FOMNH); Sanatoriet near Tórshavn, ♂, 22.7.1926, leg. J. P. Kryger, *X. scotica* det. Kryger (FOMNH).

Distribution. A common and widespread species in Europe (e. g. Nixon 1957, Hellén 1964, Kozlov 1978). This is the first record for the Faroe Islands. In addition the Barcode of Life database (www.boldsystems.org) has sequences from Norway.

Notes. Kryger & Schmiedeknecht (1938) identified the material as *Xenotoma scotica*, now *Belyta sanguinolenta* according to Macek (1996), however it does not belong to *Belyta* and no other Faroese *Belyta* was seen during the current study. *B. sanguinolenta* should be removed from the checklist of the Faroe Islands. The examined material was identified using Nixon's (1957) key.

Pantoclis trisulcata Kieffer, 1907
Fig. 9A–C

Nomenclature: *Pantoclis trisulcata* Kieffer, 1907: 32, 40, ♀.

Examined material: FAROE ISLANDS: Streymoy Is., Sanatoriet near Tórshavn, ♀, 26.8.1926, leg. J. P. Kryger, *Xenotoma scotica* det. Kryger (FOMNH); Sanatoriet near

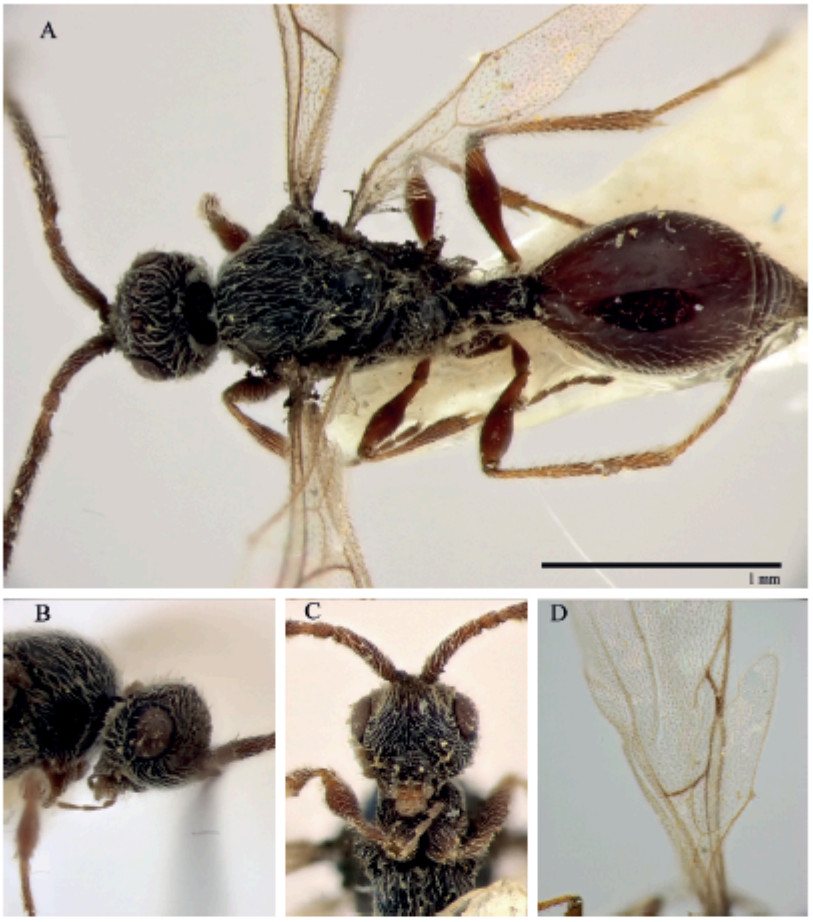

Fig. 8. *Pantoclis similis* male: **A.** habitus, dorsal; **B.** head, lateral; **C.** face, frontal; **D.** wing venation.

Tórshavn, ♀, 26.8.1926, leg. J. P. Kryger, *X. scotica* det. Kryger (FOMNH); Tórshavn, old garden at Landavegur (as gamle havn), ♀, 2.6.1926, leg. J. P. Kryger, *X. scotica* det. Kryger, *Pantoclis trisulcata* det. B. Petersen (FOMNH).

Distribution. A common and widespread species in Europe (e.g. Nixon 1957, Hellén 1964, Kozlov 1978). Previously recorded from the Faroe Islands by Petersen (1956). In addition the Barcode of Life database (www.boldsystems.org) has sequences from Finland and Norway.

Notes. Kryger & Schmiedeknecht (1938) identified the material as *Xenotoma scotica*, now *Belyta sanguinolenta* according to Macek (1996), however it does not belong to *Belyta* and no other Faroese *Belyta* was seen during the current study. *B. sanguinolenta* should be removed from the checklist of the Faroe Islands. Petersen (1956) realized the error and correctly redetermined it as *Pantoclis trisulcata*.

Synacra atracta Macek, 1995
Fig. 10A–B

Nomenclature: *Synacra* (*Paratelopsilus*) *atracta* Macek, 1995: 477, figs 3, 10, 16, ♀♂.

Examined material: FAROE ISLANDS: Streymoy Is., Ljósávatn Lake south of Tórshavn, ♂, 15.6.1926, leg. J. P. Kryger, *Xenotoma scotica* det. Kryger (FOMNH).

Distribution. A widespread species in Europe (e.g. Macek 1995). This is the first record of the species for the Faroe Islands. In addition the Barcode of Life database (www.boldsystems.org) has sequences from Norway.

Notes. Kryger & Schmiedeknecht(1938) identified the material as *Xenotoma scotica*, now *Belyta sanguinolenta* according to Macek (1996), however it does not belong to *Belyta* and no other Faroese *Belyta* was seen during the current study. *B. sanguinolenta* should be removed from the checklist of the Faroe Islands. This is the first record of the genus *Synacra* from the Faroe Islands.

Fig. 9. *Pantoclis trisulcata* female: **A.** habitus, lateral; **B.** habitus, dorsal.

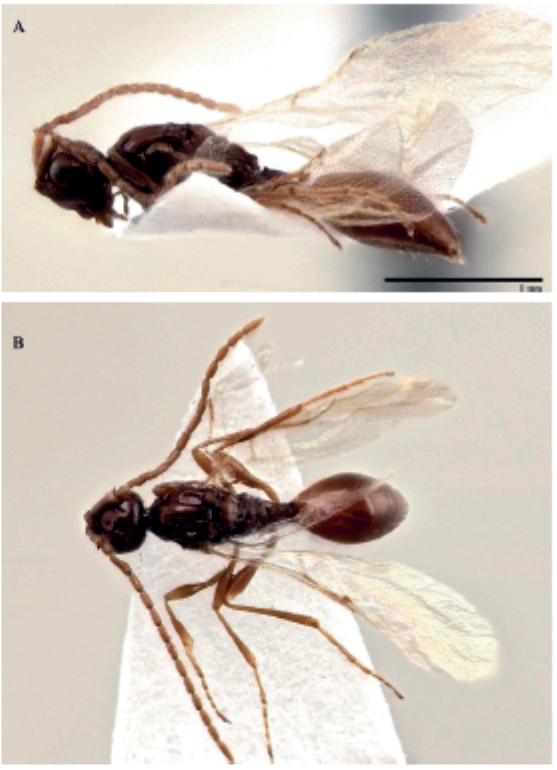

Fig. 10. *Synacra atracta* male: **A.** habitus, lateral; **B.** habitus, dorsal.

Trichopria ? *aptera* (Ruthe, 1859)
Fig. 11A–B

Nomenclature: *Diapria aptera* Ruthe, 1859: 313, ♀.

Examined material: FAROE ISLANDS: Koltur Is., N61.99041, W6.97188, altitude 87 m, ♂, 3.8.2022, pitfall trap, sampling event 2022-08-03-KMM-P, leg. A. Kreiling, det. J. Hübner/H. Gabel (SNSB-ZSM). Streymoy Is., Sanatoriet near Tórshavn, ♀, 15.7.1926, leg. J. P. Kryger, *Loxotropa thomsoni* det. Kryger (FOMNH); Tórshavn, ♂, 22.8.1926, leg. J. P. Kryger, *L. aptera* det. Kryger (FOMNH); Tórshavn, ♂, 24.7.1926, leg. J. P. Kryger, *L. thomsoni* det. Kryger (FOMNH); Tórshavn, ♂, 26.8.1926, leg. J. P. Kryger, *L. thomsoni* det. Kryger (FOMNH).

Distribution. The distribution of this species is poorly known owing to taxonomic problems explained below. Previously recorded from the Faroe Islands by Kryger & Schmiedeknecht (1938) and Petersen (1956).

Notes. Kryger & Schmiedeknecht (1938) found one male specimen, which they identified as *L. aptera* Ruthe, 1859, now *Trichopria aptera*, and four male specimens they identified as *L. thomsoni* Kieffer, 1911 now *T. nigricornis* (Marshall, 1868). From the same material however Petersen (1956) did not recognise two different species and made *L. thomsoni* a junior synonym of *L. aptera*. Unfortunately there is uncertainty over the correct interpretation of *T. aptera* because the type from Iceland has been missing since 1859 (Ruthe 1859, Petersen 1956, Notton 1995), this species belongs to a particularly difficult species group and Icelandic material has not been critically revised. Problems with Petersen's synonymy were outlined by Notton (1995) and since then Notton has seen material suggesting there are two closely related brachypterous *Trichopria* in Iceland, either of which could be *T. aptera*. For the time being we are following the morphological concept of Petersen (1956) bearing in mind that this may include more than one species, pending a detailed revision of Icelandic material which is unfortunately outside the scope of the current paper. However since *L. thomsoni* is not certainly synonymized with *T. aptera*, *T. nigricornis* should be removed from the checklist for the Faroe Islands.

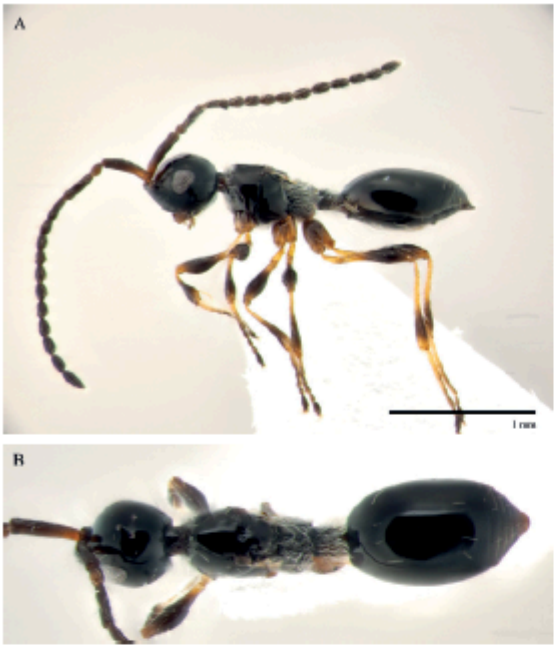

Fig. 11. *Trichopria ? aptera* male: **A.** habitus, lateral; **B.** habitus, dorsal.

Zygota parallela (Thomson, 1858)
Fig. 12A–B

Nomenclature: *Belyta parallela* Thomson, 1858: 175, ♂. *Aclista macroneura* Kieffer, 1909: 469, ♂. Synonymized by Macek (1997).

Examined material: FAROE ISLANDS: Esturoy Is., Eiði (as Ejde), ♂, 8.8.1926, leg. J. P. Kryger, *A. macroneura* det. Kryger (FOMNH). Streymoy Is., Sanatoriet near Tórshavn, ♂, 22.7.1926, leg. J. P. Kryger, *A. macroneura* det. Kryger (FOMNH); Tórshavn, ♂, 14.6.1926, leg. J. P. Kryger, *A. macroneura* det. Kryger (FOMNH); Tórshavn, north of Viðarlundin park, also known as Plantajan (as n. for Plantagen), ♂, 27.6.1926, leg. J. P. Kryger, *A. macroneura* det. Kryger (FOMNH).

Distribution. A widespread species in Europe (e.g. Macek 1997). Previously recorded from the Faroe Islands by Kryger & Schmiedeknecht (1938) as *Aclista macroneura*.

Notes. This is the first record of the genus *Zygota* from the Faroe Islands.

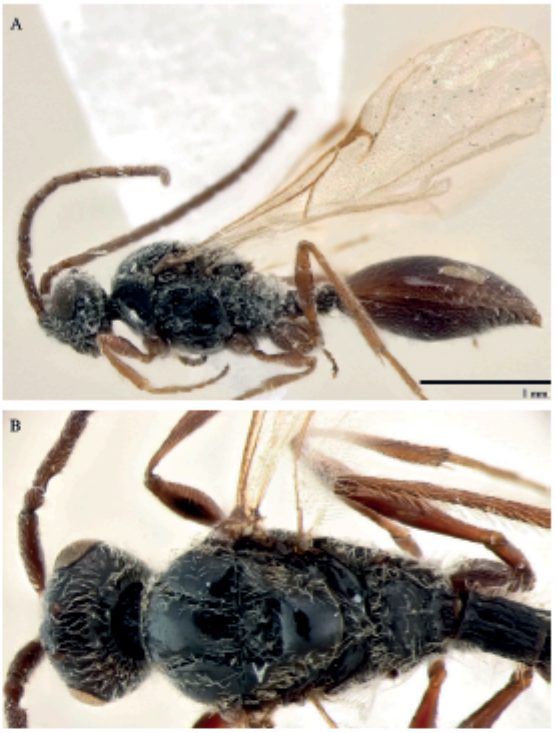

Fig. 12. *Zygota parallela* male: **A.** habitus, lateral; **B.** head, mesosoma and petiole, dorsal.

Provisional key to Diapriidae of the Faroe Islands

The male of *Basalys longipennis* is unknown.

1 Notauli absent 2
– Notauli present 6
2 Antenna 12-segmented (females) 3
– Antenna 14-segmented (males) 5
3 Antennal club gradually expanded; base of large tergite without hair tufts ..... *Trichopria ? aptera*
– Antennal club with abrupt 3-segmented club; base of large tergite with hair tufts 4
4 Antennal segment 11 slightly but distinctly transverse ..... *Basalys abruptus*
– Antennal segment 11 slightly elongate ..... *Basalys longipennis*
5 Wings vestigial; base of large tergite without hair tufts ..... *Trichopria ? aptera*
– Wings extending beyond apex of metasoma; base of large tergite with hair tufts ..... *Basalys abruptus*

6 Radial cell open 7

– Radial cell closed 8

7 Mandibles long, without eredge straight, barely overlapping, together forming a backwards directed beak; apex of scape with sharp flanges; female with 12 antennal segments .... *Synacra atracta*

– Mandibles without eredge curved, overlapping, together not beak-like; apex of scape without sharp flanges; female with 15 antennal segments ..... *Zygota parallela*

8 Marginal vein about as long as its distance from basal vein ..... *Miota exsecta*

– Marginal vein much shorter than its distance from basal vein 9

9 Mandibles with lower tooth long, sickle-shaped, more or less widely crossing; apex of poststigmal vein posteriorly directed; petiole more than about twice as long as wide 10

– Mandibles with lower tooth not long, sickle-shaped, not widely crossing; apex of poststigmal vein basally directed; petioleless less than 1.5 times as long as wide 11

10 Mandibles shorter, not so widely crossing ....*Aclista alticollis*

– Mandibles longer, conspicuously sickle-shaped and widely crossing at tips .. *Aclista* cf. *insolita*

11 The two lateral keels of the propodeum closer together; radial cell shorter; smaller darker overall ..... *Pantoclis trisulcata*

– The two lateral keels of the propodeum not so close; radial cell longer; larger overall, and usually with parts of body and legs reddish/yellowish ..... *Pantoclis similis*

Discussion

Even today with advanced methodologies and literature Diapriidae is a difficult taxon; their taxonomy is subject to constant reevaluation, even at genus level, so it is understandable that earlier works (Kryger & Schmiedeknecht 1938, Petersen 1956) now need updating. Our study adds and updates significantly the previously limited understanding of the diapriid fauna of the Faroe Islands and corrects some taxonomic mistakes. We show the value of reevaluating historic material, complemented with specimens collected using pitfall and Malaise traps, to cover a wider geographical, temporal and ecological envelope, sampling as many species as possible. Using integrative methods, as we have initiated with *Aclista alticollis*, will allow further species to be identified using comparison of CO1 barcodes complementing morphological data and placing them in their wider genetic context. The specific biology of many diapriids is unknown and there are no biological observations for the Faroe Islands, yet the Faroe Islands offer a unique opportunity in diapriid research, the constantly humid climate favours their dipteran hosts, e.g. at lat least 30 species of Mycetophilidae fungus gnats have been recorded (Kjærandsen & Jorgensen 1992; pers. comm. J. Kjærandsen) an important host group for belytine diapriids, and even though there are no native trees there is a diverse range of fungi, over 600 species (Vesterholt 1998) which provide food for these fly hosts. Consequently we believe there will be further opportunities to discover more diapriid species and uncover their biology.

Acknowledgements

We thank Dr. Lars Vilhelmsen for the loan of historic material from FOMNH, which made accurate interpretationof the species of previous authors possible. We are also grateful to Dr Vasilisa Chemyreva and Jan Macek, who helped to identify some challenging specimens and Inger Lund for help interpreting the handwritten specimen labels of J. P. Kryger. Our work is part of the GBOL III: Dark Taxa project and was funded by the German Federal Ministry of Education and Research (FKZ 16LI1901B). Fieldwork was conducted as part of a general species inventory on Koltur funded by Aage V. Jensens Fonde.

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SECTION 3.2: Peering into darkness

Dark Taxa are even in Germany so cryptic and diverse that it is not even known how many species there might be. This study estimates the diversity of four Diptera families (Cecidomyiidae, Chironomidae, Phoridae, and Sciaridae) based on more than 48,000 DNA barcodes. Those estimates were compared to those of less diverse and better studied Diptera families. It was demonstrated that there are at least 1800–2200 species unknown to science in the country.

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Article

Peering into the Darkness: DNA Barcoding Reveals Surprisingly High Diversity of Unknown Species of Diptera (Insecta) in Germany

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Simple Summary: Roughly two-thirds of the insect species described from Germany belong to the orders Diptera (flies) or Hymenoptera (wasps, bees, ants and sawflies). However, both orders contain several species-rich families that have received little taxonomic attention until now. This study takes the first step in assessing these "dark taxa" families and provides species estimates for four challenging groups of Diptera (Cecidomyiidae, Chironomidae, Phoridae and Sciaridae). The estimates given in this paper are based on the sequencing results of over 48,000 fly specimens that have been collected in southern Germany via Malaise traps that were operated for one season each. We evaluated the fraction of species in our samples belonging to well-known fly families in order to estimate the species richness of the challenging "dark taxa" (DT families hereafter). Our results suggest a surprisingly high proportion of undetected biodiversity in a supposedly well-investigated country: at least 1800–2200 species await discovery and description in Germany in these four families.

Abstract: Determining the size of the German insect fauna requires better knowledge of several megadiverse families of Diptera and Hymenoptera that are taxonomically challenging. This study takes the first step in assessing these "dark taxa" families and provides species estimates for four challenging groups of Diptera (Cecidomyiidae, Chironomidae, Phoridae, and Sciaridae). These estimates are based on more than 48,000 DNA barcodes (COI) from Diptera collected by Malaise traps that were deployed in southern Germany. We assessed the fraction of German species belonging to 11 fly families with well-studied taxonomy in these samples. The resultant ratios were then used to estimate the species richness of the four "dark taxa" families (DT families hereafter). Our results suggest a surprisingly high proportion of undetected biodiversity in a supposedly well-investigated country: at least 1800–2200 species await discovery in Germany in these four families. As this estimate is based on collections from one region of Germany, the species count will likely increase with expanded geographic sampling.

Keywords: Diptera; insects; dark taxa; taxonomic impediment; species estimates; DNA barcoding; biodiversity; German insect fauna

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1. Introduction

Although the Central European insect fauna is considered to be well studied, gaps in knowledge of its taxonomy and biodiversity remain [1]. About 33,300 species of insects

are documented from Germany, of which roughly two-thirds of these taxa belong to one of the two orders: Diptera (flies) and Hymenoptera (wasps, bees, ants, and sawflies) [1–8]. However, both orders contain several species-rich families which have received less attention than others in Germany's long history of taxonomic research [1]. This reflects the confluence of several factors, such as extreme species richness combined with a high rate of cryptic diversity and, most importantly, the limited taxonomic attention directed to small specimens (<2 mm) whose morphological characteristics are difficult to evaluate. Successful identification of species in these groups using morphology is time-consuming and requires taxonomic expertise, the availability of which is decreasing [9–14]. This imbalance of few researchers but high species numbers still awaiting documentation is commonly referred to as the taxonomic impediment [9,15,16]. Against the backdrop of a worldwide decline in insect abundance, the taxonomic impediment is an alarming constraint to biodiversity surveys [17–21]. One such constraint is noticeable in the framework of DNA barcoding applications, where species proxies (Barcode Index Numbers, BINs) often lack a linkage to a known species [22]. Page [22] coined the term "dark taxa" for these nameless BINs, and in 2020, Hausmann et al. [1] used it to address species-rich, taxonomically challenging groups of insect families whose diversity remains mostly undescribed. These include certain families of non-brachyceran Diptera (mosquitoes, gnats, midges), some families of Brachycera (flies), and nearly all families of parasitoid Hymenoptera (wasps) which often make up the majority of the insect biodiversity present in environmental and bulk samples [23]. With the shortage of taxonomic specialists, the functional role of "dark taxa" in ecosystems is far too understudied, meaning that they cannot be included in biomonitoring or conservation surveys.

The most recent project in the German Barcode of Life initiative, GBOL III: Dark Taxa, was launched in mid-2020 to tackle these challenging groups. Its two main goals are: (1) to study various DT families using an integrative taxonomic approach which combines morphological and sequence data [1,24], and (2) to expand the DNA barcode reference library established by three earlier initiatives (Barcoding Fauna Bavarica, GBOL I, GBOL II) [24–26]. Work conducted by GBOL II generated a reference library for the order Diptera based on 50,963 COI sequences, data that provided barcodes for 5200 BINs [13]. A recent commentary on this study presented a classical dipterist's perspective on the situation for the better-known families of Diptera [27]. It explored ways to extend the involvement of expert taxonomists in assigning Linnean names to BINs. However, the challenge in implementing similar work on DT families was not addressed, highlighting the need to seek new approaches so these taxa can finally become more accessible to research.

This study begins this effort by considering the German fauna of four DT families of Diptera which lack estimates of their species numbers: Cecidomyiidae (gall midges), Chironomidae (non-biting midges), Phoridae (scuttle flies), and Sciaridae (dark-winged fungus gnats) (Figure 1). To address this goal, we examine the diversity of these DT families in our Malaise trap collections. We employ BIN data resulting from the sequence analysis of samples from southern Germany and use these results to estimate the extent of undocumented biodiversity in these families in Bavaria and Germany. An important backbone to our calculations is species numbers inferred from essential contributions of Germany's over 200-year-long history of taxonomy [5–8,28–38].

Figure 1. Selected representatives of the DT families analyzed in our study: Cecidomyiidae (**top left**); Phoridae (**top right**); Sciaridae (**bottom left**) and Chironomidae (**bottom right**). Scale bars represent 1 mm.

2. Materials and Methods

2.1. Malaise Tap Sites

In 2012, the Global Malaise Trap Program was launched by the Centre for Biodiversity Genomics (CBG) at the University of Guelph to provide a global overview of arthropod diversity [39]. As part of this project, 14 Malaise traps were deployed at various sites in Germany (Figure 2 and Table 1). In 2012, one trap was operated from May to September in the Bavarian Forest National Park (BFNP), a conifer-dominated montane forest. In 2014, 12 Malaise traps were placed along an altitudinal transect (1036–2160 m) in the Allgäu Alps, ranging from the Oytal to the Schochen and Nebelhorn Mountains. Traps in lower altitudes (Oytal) were deployed in May, whereas those in higher altitudes (Schochen and Koblat) were deployed in June. All traps in the Allgäu Alps were operated until October. Finally, in 2017, one trap was deployed at the Bavarian State Collection of Zoology (ZSM) in Munich, which is situated in a residential neighborhood rich in backyard gardens. This trap was operated from April to December. Altogether, the sampled sites represent a heterogeneous array of habitats typical of southern Germany. The specifics of trap deployment (habitat type, site, orientation, height) strongly influence its catch [40]. Collection dates varied among sites but are detailed in Table A1. Denatured ethanol (80%) was used to preserve specimens.

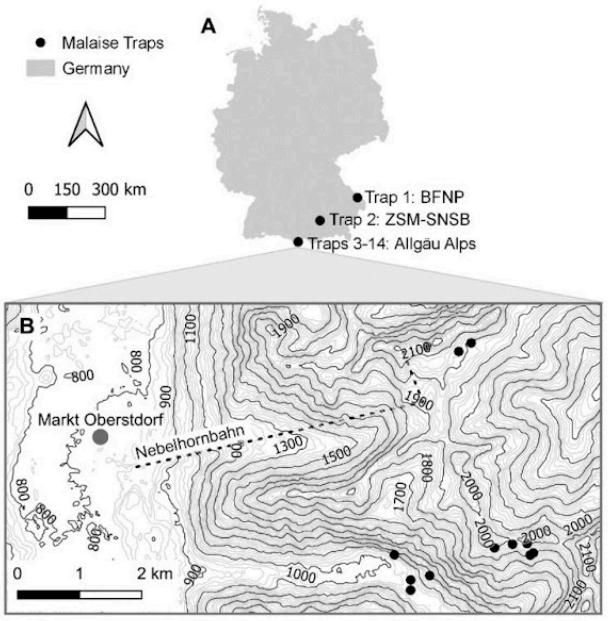

Figure 2. Malaise trap sites. Locations where the 14 Malaise traps were deployed in 2012, 2014, and 2017 ((**A**,**B**) shows enlarged map of Allgäu Alps) as Germany's contribution to the Global Malaise Trap Program.

Table 1. Malaise trap information. Trap site, exact location, elevation, and habitat type.

Site	Trap	Coordinates	Elevation	Habitat
BFNP	Trap 1	48.9509° N 13.422° E	842 m	Natural forest
ZSM	Trap 2	48.1648° N 11.4849° E	519 m	Urban, pre-alpine meadow
Allgäu Alps: Oytal	Trap 3	47.39205° N 10.34093° E	1122 m	Lake rock face
Allgäu Alps: Oytal	Trap 4	47.38903° N 10.34846° E	1200 m	Cone of scree
Allgäu Alps: Oytal	Trap 5	47.38842° N 10.34440° E	1056 m	Rough pasture
Allgäu Alps: Oytal	Trap 6	47.38695° N 10.34438° E	1036 m	River
Allgäu Alps: Schochen	Trap 7	47.39202° N 10.36991° E	1930 m	Alpine grassland
Allgäu Alps: Schochen	Trap 8	47.39232° N 10.37057° E	1908 m	Spring
Allgäu Alps: Schochen	Trap 9	47.39368° N 10.36926° E	2032 m	South-exposed ridge with Blaugras-Horstseggenrasen
Allgäu Alps: Schochen	Trap 10	47.39307° N 10.36229° E	2010 m	South-exposed rock
Allgäu Alps: Schochen	Trap 11	47.39360° N 10.36615° E	1980 m	Snow bed
Allgäu Alps: Koblat	Trap 12	47.42223° N 10.34783° E	2160 m	South-exposed rock face
Allgäu Alps: Koblat	Trap 13	47.42147° N 10.35465° E	2033 m	Snow bed
Allgäu Alps: Koblat	Trap 14	47.42272° N 10.35730° E	2005 m	Mountain pine bush

2.2. Processing of Specimens

Samples from two sites (BFNP, ZSM) were sent directly to the CBG for analysis. Due to funding constraints, roughly every second weekly sample from the BFNP and every fourth weekly sample from the ZSM were selected for DNA barcode analysis. Based on the number of specimens in the samples that were processed, the full year of collecting at these sites yielded about 52,000 and 130,000 specimens, respectively. Using morphology, specimens from these locales were sorted to an order prior to sequence analysis and to a family after analysis. In total, tissue samples or whole individuals of 62,073 specimens (29,481 from BFNP; 32,592 from ZSM) were transferred to 96-well microplates for DNA

extraction. Samples from the Allgäu Alps were sorted by a dipterist at the ZSM before being dispatched in 96-well microplates to the CBG for sequence analysis. Rough estimates suggest the Allgäu samples included well over a million specimens, but funding was only available to process about 2% of them (20,250 specimens).

At the CBG, specimens were processed using standard protocols for DNA extraction, PCR amplification of the barcode region of COI, and sequencing. Specimens from the BFNP and the Allgäu Alps were Sanger sequenced on an ABI 3730XL [41], while specimens from the ZSM were sequenced on Sequel [42].

2.3. Data Analysis

All specimen metadata and sequence data were uploaded to the Barcode of Life Data System (BOLD), an online workbench and database [32]. These data are publicly available in three datasets: DS-BFNP, DS-ZSMTRAP and DS-ALGALPS. Each sequence $\geq$ 300 base pairs (bp) was automatically assigned to a Barcode Index Number (BIN) already in BOLD if sequence similarity based on the (RESL-) BIN algorithm was fulfilled [43]. Sequences $\geq$ 500 bp which did not find a match served as founders of new BINs. All data were downloaded on 8 February 2021 for further analysis. Therefore, the present results correspond to BINs assigned at that time (BIN assignments can change as new sequences are added to BOLD). Employing BINs as a proxy for species, we employed Chao1 [44] to estimate species counts for the dipteran families selected for analysis. We then calculated the ratio between the observed number of BINs in our samples to the estimate of species richness generated by Chao1 to ascertain the proportion of species at the sampling sites that have not been captured by our Malaise traps and that await analysis. We also generated continuous diversity profiles that illustrated variation in three standard metrics of biodiversity, which are quantified by Hill numbers (q): species richness (q = 0), Shannon diversity (q = 1), and Simpson diversity (q = 2) [34]. Hill numbers are a mathematically consolidated group of diversity indices which include relative species abundances in order to quantify biodiversity [45]. All calculations were performed in R version 3.3.6 with the Chao1 estimates calculated using the *SpadeR* package [46].

2.4. Extrapolating Species Numbers

We selected, more or less randomly, 11 dipteran families whose taxonomy and fauna have been intensively studied to date in order to assess the fractions of the Bavarian and German faunas represented in our samples (Table 2). By comparing the known species counts for these 11 families with the species recovered from our Malaise traps, we could estimate the percentage of these taxa that were recovered, providing a basis for estimating the completeness of our sampling. These values could then be used to estimate species diversity for our four DT families: Cecidomyiidae—gall midges; Chironomidae—non-biting midges; Phoridae—scuttle flies, and Sciaridae—dark-winged fungus gnats.

Species numbers for Germany and for Bavaria were obtained from extensive literature (Table 2). For each family where a species count for Bavaria was unavailable, we adopted a count equal to 0.80 of the species number for Germany. This value was conservative because where species lists were available for both Bavaria and Germany, the ratio often exceeded 0.80 (Table 2). Moreover, this proportion corresponds to past evidence that Bavaria hosts 80–85% of the German fauna in well-studied invertebrate groups, both terrestrial and limnic [2,47].

Table 2. Species numbers for 15 families of Diptera. Species numbers for the Bavarian and German faunas are shown for 11 families of Diptera with well-established taxonomy and for four families with limited knowledge (Cecidomyiidae, Chironomidae, Phoridae, Sciaridae). *—estimated at 80% of German fauna.

Taxon	Bavarian Species Count	German Species Count	Species Count Bavaria/Germany
Asilidae	68 [28]	85 [29]	0.80
Calliphoridae	50 *	62 [35]	0.80 *
Drosophilidae	64 [28]	81 [37]	0.79
Ephydridae	140 *	174 [38]	0.80 *
Muscidae	267 *	334 [48]	0.80 *
Sarcophagidae	107 *	134 [35]	0.80 *
Stratiomyidae	59 [28]	71 [30,48]	0.83
Syrphidae	389 [28]	458 [31]	0.85
Tabanidae	47 [28]	58 [8,48]	0.81
Tachinidae	361 [28]	501 [48]	0.72
Tipulidae	120 [33]	142 [32]	0.85
Cecidomyiidae	328 [38]	859 [5–8]	0.38
Chironomidae	576 [28]	781 [5–8]	0.74
Phoridae	302 *	378 [5–8]	0.80 *
Sciaridae	231 [28]	343 [43]	0.67
All Diptera	7635 *	9544 [8]	0.80 *

We estimated species numbers for the DT families through the following steps:

1. We calculated a Recovery Ratio by dividing the number of BINs detected through sequencing by the species count for each of the 15 families and for all Diptera (BIN/species ratio). This approach generated a ratio for each well-known family, for each DT family, and for all Diptera.
2. We estimated the maximum number of species for each "dark taxon" for both Germany and Bavaria by dividing its BIN count by the average BIN/species ratio of all 11 well-known families.
3. We estimated the minimum species number for each "dark taxon" by dividing all Diptera BINs by all Diptera species (i.e., 9544). Because this calculation includes numerous families with cryptic diversity, the resultant values underestimate the diversity of the DT families.

In the same fashion, we extrapolated species numbers employing the Chao1 values for the four DT families.

3. Results

3.1. Sequencing Results

COI sequences were recovered from 85.4% of the insects (70,293/82,323) that were analyzed (Table 3) and success was even higher for Diptera (91%). Diptera comprised nearly two thirds of the specimens that were analyzed and more than half of the resultant BINs. When results for Diptera from the three collection sites were pooled, the resulting 48,230 COI sequences were assigned to 4863 BINs and included species from 85 families. Across all sites, roughly 20% of the BINs were new to BOLD and almost 70% of them were Diptera with representatives from 56 families. Almost half of all dipteran BINs (2146; 44.1%) and 55% of the new dipteran BINs belonged to the four DT families.

Table 3. Sequence results for the three sampling sites. Total sample size, number of processed specimens, sequences recovered, BINs, BINs new to BOLD, Diptera specimens, and Diptera BINs.

	BFNP	ZSM	Allgäu Alps	Total
Samples (trap × collection events)	1 × 9 = 9	1 × 10 = 10	8 × 7 + 4 × 10 = 96	100
All				
Specimens	29,481	32,592	20,250	82,323
COI sequences (% success)	25,217 (85.6%)	28,923 (88.7%)	16,152 (79.8%)	70,293 (85.4%)
BINs (% new to BOLD)	2565 (19.4%)	3870 (15.8%)	4043 (23.0%)	8790 (23.8%)
Diptera				
Specimens (% of all specimens)	23,114 (78%)	15,448 (47%)	14,238 (70%)	52,800 (64%)
COI sequences (% success)	20,909 (91%)	14,983 (97%)	12,338 (87%)	48,230 (91%)
BINs (in % of all BINs)	1571 (61%)	1676 (43%)	2632 (65%)	4863 (55%)
Diptera BINs new to BOLD	375	260	736	1413
DT BINs new to BOLD (% of all new Diptera BINs)	337 (90%)	215 (83%)	215 (29%)	780 (55%)

3.2. Estimation of Taxon Diversity Using BIN/Species Ratios

The 11 well-known families of Diptera displayed BIN/species ratios that ranged from 0.19–0.60 (ø 0.33 ± 0.9) for Bavaria and from 0.15–0.48 (ø 0.27 ± 0. 7) for Germany (Table 4, Figure A1a). Dividing all Diptera BINs by all known Diptera species produced a ratio of 0.64 for Bavaria and 0.51 for Germany. While one DT family (Chironomidae) possessed a ratio (0.38, Germany) that overlapped the upper end of the values for the 11 well-known families, the other three had far higher ratios. In fact, the BIN count for Phoridae and Sciaridae nearly matched the known species count for Germany, while the count for Cecidomyiidae exceeded it.

Table 4. Fifteen families of Diptera, 11 with well-developed taxonomy and four that are less well known. The number of BINs recovered in this study is followed by the known species count for Bavaria and Germany, the ratio of species counts for Bavaria and Germany, and BIN/Species ratios for Bavaria and Germany.

Taxa	BINs	Bavarian Species	German Species	Bavarian/German Species	BINs/Bavarian Species	BINs/German Species
Asilidae	13	68	85	0.80	0.19	0.15
Calliphoridae	22	50	62	0.80	0.44	0.35
Drosophilidae	27	64	81	0.79	0.42	0.34
Ephydridae	32	140	174	0.80	0.23	0.18
Muscidae	160	267	334	0.80	0.60	0.48
Sarcophagidae	35	107	134	0.80	0.33	0.26
Stratiomyidae	14	59	71	0.83	0.24	0.20
Syrphidae	131	389	458	0.85	0.34	0.29
Tabanidae	9	47	58	0.81	0.19	0.16
Tachinidae	126	361	501	0.72	0.35	0.25
Tipulidae	43	120	142	0.85	0.36	0.30
Average values					0.33 ± 0.9	0.27 ± 0.7
Cecidomyiidae	1163	328	859	0.38	3.55	1.35
Chironomidae	296	576	781	0.74	0.51	0.38
Phoridae	348	302	378	0.80	1.15	0.92
Sciaridae	339	231	343	0.72	1.47	0.99
Average values					1.67 ± 0.9	0.91 ± 0.3
All Diptera	4863	7635	9544	0.80	0.64	0.51

3.3. Estimation of Taxon Diversity Using Chao1/Species Ratios

Chao1 estimates of species richness were obtained for the 15 families of Diptera (Table 5). BIN/Chao1 ratios averaged 0.76 for the 11 well-known families. The diversity profiles for 10 of these families showed overlap between the species richness in our samples

and that estimated to occur at the sites sampled by our Malaise traps (Hill number q = 0, Figure 3). Muscidae was the sole exception as its predicted diversity was considerably higher than currently recognized. Chao1/species ratios ranged from 0.21–0.82 (0.46 ± 0.2) for Bavaria and from 0.16–0.66 (0.37 ± 0.2) for Germany (Table 5).

Table 5. Proportion of undocumented Diptera biodiversity for Bavaria and Germany based on Chao1 estimates for 15 families.

Taxon	BINs	Chao1	BIN/Chao1	Bavarian Species	German Species	Chao1/ Bavarian Species	Chao1/ German Species
Asilidae	13	16	0.81	68	85	0.24	0.16
Calliphoridae	22	28	0.79	50	62	0.56	0.45
Drosophilidae	27	38	0.71	64	81	0.59	0.47
Ephydridae	32	88	0.36	140	174	0.63	0.51
Muscidae	160	220	0.73	267	334	0.82	0.66
Sarcophagidae	35	41	0.85	107	134	0.38	0.31
Stratiomyidae	14	16	0.88	59	71	0.27	0.23
Syrphidae	131	158	0.83	389	458	0.41	0.34
Tabanidae	9	10	0.90	47	58	0.21	0.17
Tachinidae	126	153	0.82	361	501	0.42	0.31
Tipulidae	43	59	0.73	120	142	0.49	0.42
Average values						0.46 ± 0.2	0.37 ± 0.2
Cecidomyiidae	1163	1937	0.60	328	859	5.91	2.25
Chironomidae	296	479	0.62	576	781	0.83	0.61
Phoridae	348	432	0.81	302	378	1.43	1.14
Sciaridae	339	468	0.72	231	343	2.03	1.36
Average values						2.55 ± 1.7	1.34 ± 0.5
All Diptera	4863	6927	0.70	7635	9544	0.91	0.73

The BIN/Chao1 ratios for the DT families were similar to those for the well-known families, ranging from 0.60–0.81 (ø 0.69 ± 0.8). The diversity profiles for all four families (Figure 4) showed no overlap between observed and estimated species richness (i.e., Hill number q = 0). Chao1/species ratios indicated coverages of 0.83–5.91 for Bavaria and 0.61–2.25 for Germany (Table 5). Excluding Chironomidae, all DT families possessed ratios well above 1. Considering all Diptera, our samples recovered about 70% of the species estimated to occur at the study sites, meaning that as many as 6927 BINs of Diptera could have been collected during sampling. Chao1/species ratios were 0.91 for Bavaria and 0.73 for Germany.

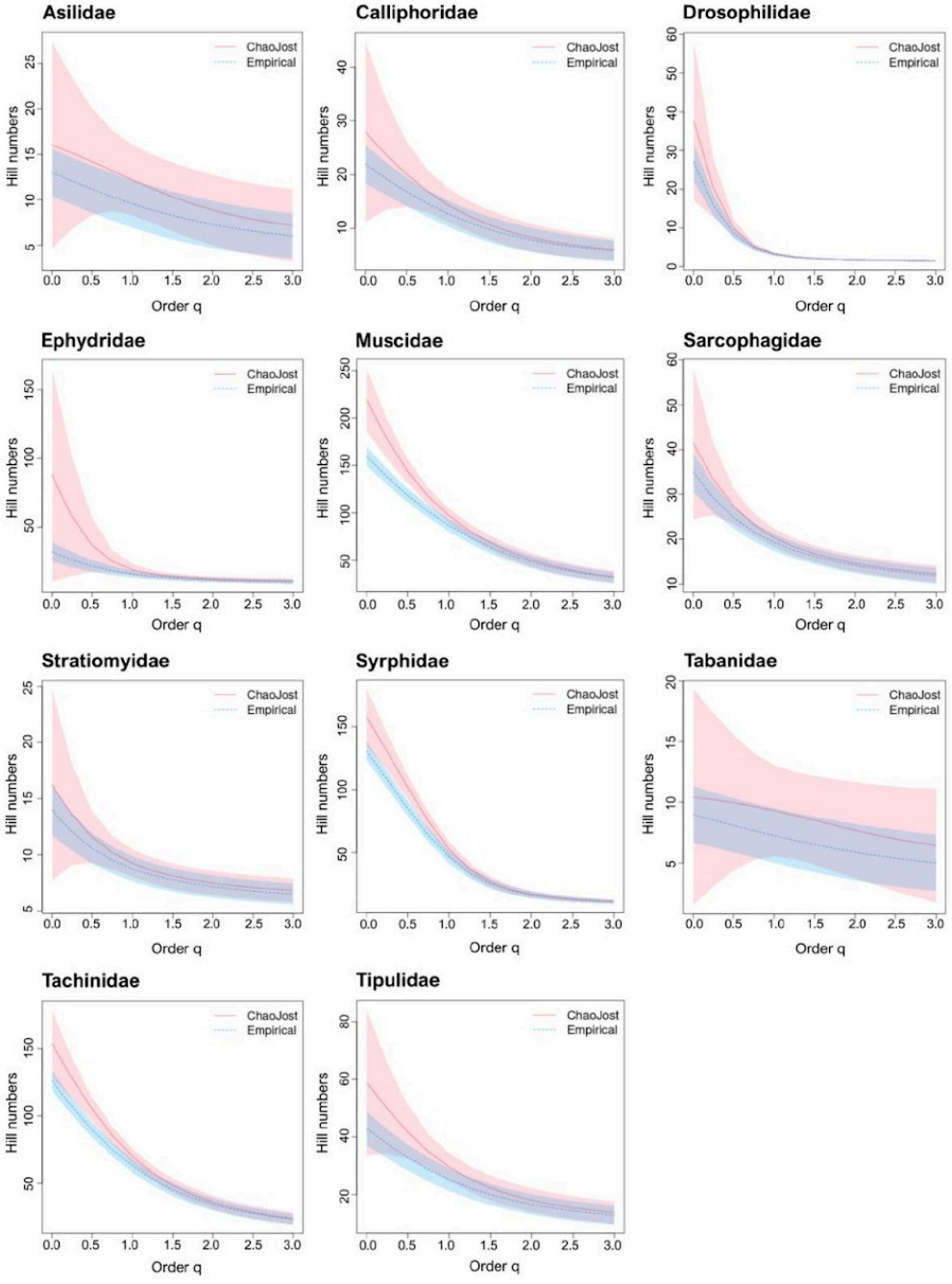

Figure 3. Diversity profiles for 11 well-known taxa. The empirical (BIN counts; dotted blue) and estimated (Chao1; red) diversity profiles for communities where Malaise traps were deployed, as quantified by Hill numbers for each of the 11 well-known families for values of the diversity order (q) from 0–3 with 95% confidence intervals (shaded areas based on bootstrap analysis of 100 permutations). Species richness is depicted by q = 0; Shannon diversity by q = 1; and Simpson diversity by q = 2.

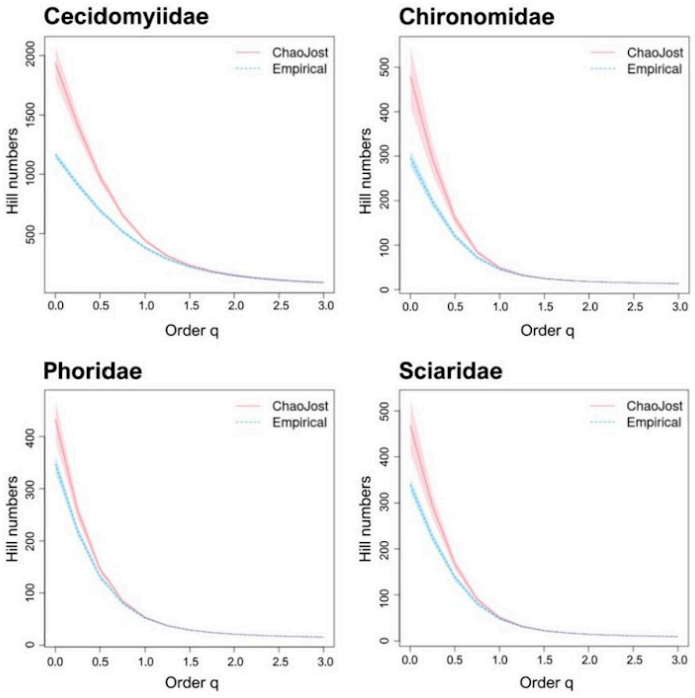

Figure 4. Diversity profiles for the four DT families. The empirical (BIN counts; dotted blue) and estimated (Chao1; red) diversity profiles for communities where Malaise traps were deployed, as quantified by Hill numbers for each of the four "dark taxa" families for values of the diversity order (q) from 0–3 with 95% confidence intervals (shaded areas based on bootstrap analysis of 100 permutations). Species richness is depicted by q = 0; Shannon diversity by q = 1; and Simpson diversity by q = 2.

3.4. Extrapolating Species Numbers

We employed the two ratios to estimate the number of species in the DT families. First, we used BIN/species ratios to extrapolate species numbers based on the number of observed BINs. Second, we used the Chao1/species ratios to estimate species numbers based on the estimated BIN diversity. The first approach generates more conservative values than the second. We divided the number of observed BINs by the (BIN or Chao1)/species ratio for all Diptera to calculate minimum species numbers. To obtain an upper limit, we divided the number of observed BINs for each family by the average (BIN or Chao1)/species ratio for all well-known families. The following calculation is presented below (e.g., Sciaridae).

As 339 Sciaridae BINs were recovered, the minimum species estimate for Bavaria was 530 (339/0.64), while the upper estimate was 1027 (339/0.33). Similarly, the number of species in Germany could be estimated as ranging from 665 (339/0.51) to 1255 (339/0.27) species. By making similar calculations for each DT family, an overall estimate for total species numbers in Bavaria and Germany was obtained (Table 6). The number of species that await discovery in each region can then be obtained by subtracting the number of known species from these estimates.

Table 6. BINs and calculated estimates. Total number of BINs recovered for each family from all traps, our calculated estimates, number of recorded species, and potential amplitude of new records for Bavaria and Germany.

Dark Taxa	BINs	Estimates Bavaria	Bavarian Species	New Records Bavaria	Estimates Germany	German Species	New Records Germany
BIN/species ratio							
Cecidomyiidae	1163	1817–3524	328	1489–3196	2280–4307	859	1421–3448
Chironomidae	296	463–897	576	0–321	580–1096	781	0–315
Phoridae	348	544–1055	302	242–753	682–1289	378	304–911
Sciaridae	339	530–1027	231	299–796	665–1256	343	322–913
Chao1/species ratio							
Cecidomyiidae	1937	2129–4211	328	1801–3883	2653–5235	859	1794–4376
Chironomidae	479	526–1041	576	0–465	656–1295	781	0–514
Phoridae	432	475–939	302	173–637	592–1168	378	214–790
Sciaridae	468	514–1017	231	283–786	641–1265	343	298–922

In total, we recovered 2146 BINs for the DT families which is 22% of the total count of dipteran species known from Germany. Our conservative estimate suggested that just the DT families comprise about 3300–6500 species in Bavaria versus 4200–7900 in Germany. Based on the current species count for Diptera in Bavaria (7635) and Germany (9544), and our estimate of new record, this implies an increase of 25–66% and by 19–59% respectively.

By comparison, the Chao1 analysis suggested that 3316 BINs of the DT families occurred at our sampling sites, a 54% increase from current estimates. Based on this approach, there about 2200–5800 species in Bavaria and 2200–6600 in Germany that may still await documentation. Hence, this approach raises the species count for Diptera by 29–75% for Bavaria and by 22–69% for Germany.

4. Discussion

Although members of the order Diptera comprise almost a third of Germany's insect fauna, the true diversity of the four highly diverse families [1] examined in this study is likely much higher than previously assumed [13,38]. By assessing the number of BINs sequenced from our collections and extrapolating species numbers, we obtained an initial estimate of their species numbers. Our results suggest that at least 1900–2200 dipteran species await discovery in Bavaria versus 1800–2200 in Germany. Although our species estimates were only based on sequencing Bavarian specimens, they are likely a good approximation of diversity in Germany as 80–85% of the invertebrate species found in Germany occur in Bavaria [2,36]. While Bavaria does have some habitats (e.g., alpine) that are not found in other regions of Germany, other habitats (e.g., coastal marshes) are absent [2], meaning that species specialized in the latter habitats will not occur in the state.

4.1. DNA Barcoding: Using BIN Numbers as Proxies for Species Numbers

Prior studies [49] have demonstrated that DNA barcoding is not only effective for specimen identification, but is also valuable for estimating species numbers [50–53]. Although there is strong correspondence between BIN counts and species numbers [49,54], several factors can lead to differences [54]. For example, COI numts can lead to the overestimation of species numbers if they are preferentially amplified in some specimens [55–58]. Conversely, the introgression of mitochondrial DNA (mtDNA), incomplete lineage sorting, and recent speciation can lead to underestimation of species numbers [59–61]. Other factors that challenge COI-based species identifications include heteroplasmy [62] and the homogenization of mtDNA haplotypes due to the maternally inherited endosymbiont *Wolbachia* [63,64]. These underlying molecular factors can lead the BIN algorithm on BOLD to assign members of a single species to several BINs or to assign several species to a single BIN. In groups

with well-developed taxonomic systems, the BIN algorithm typically underestimates the true species count by about 10% as it was designed to deliver a conservative value for species diversity [65]. In addition to this internal constraint, two operational factors may have led our study to substantially underestimate actual species numbers:

1. Limited geographic sampling as our data originates from few sites in Bavaria only, covering a tiny fraction of habitat types otherwise present.
2. Limited funding constrained analysis to just 5% of the 1.2 million specimens that were collected.

4.2. BIN & Chao1/Species Ratios: Well-Known Families versus DT Families

We assessed the completeness of the species coverage provided by our Malaise trap samples in two ways. First, we calculated the ratio of the BINs recovered for each family and its known species count for Bavaria and Germany. We then made the same calculation employing Chao1 estimates, which, in contrast to the first approach, includes species that were present at our sampling sites but not caught nor sequenced. Thus, it is important to note that our first approach generates more conservative values than the second. By calculating the BIN/Chao1 ratios for each taxon, we were able to make the proportion of diversity that was not captured tangible.

Overall, the resulting (BIN or Chao1)/species ratios were much higher for the DT families than for the well-known ones (Tables 4 and 5). Average ratios among the well-known families were well under 1 (ranging from 0.33–0.46 for Bavaria and 0.27–0.37 for Germany), indicating that our collections only included a fraction of the known diversity from Bavaria and Germany. This was expected because we only sampled few sites and only processed a fraction of our dipteran specimens. The much higher ratios for the DT families (average ranging from 1.67–2.55 for Bavaria and 0.91–1.34 for Germany) strongly suggest the presence of undescribed, unknown species. The Cecidomyiidae were the most dramatic case as we detected 1163 BINs, a value 35% higher than the species count for this family in Germany [8]. In fact, a quarter of all Diptera BINs belonged to this family, reinforcing conclusions from earlier studies indicating that this is the most diverse family of flies [13,49]. For example, extensive sampling at sites across Canada [49] revealed more than 10,000 BINs, a result which suggested that the Cecidomyiidae may include two million species worldwide. The Bavarian fauna has received little taxonomic attention as only 328 species are recorded versus a likely count of 687 species based on the presumption that 80% of the German fauna occurs there. By contrast, our analysis of 7148 specimens revealed 1163 BINs, a count for Bavaria which is threefold higher than the number of recorded species. Chironomidae was an exception among our DT families, as we obtained ratios that were consistent with those of the well-known families (Table 5). Although Chironomidae is a dark taxon, extensive research concerning the systematics, taxonomy, and nomenclature of European and Neotropical species has and is being conducted at the Bavarian State Collection of Zoology (ZSM) by the late Ernst Fittkau (former director of the ZSM) and his students including Martin Spies, the current editor of the Chironomid Home Page [66]. We therefore expect that the chironomid fauna of Bavaria and Germany is well documented and that, in contrast to the other DT families, a much lower amplitude of new species will be discovered in the following years of GBOL III. Among the well-known families, the Muscidae displayed the highest BIN/species indicating that the current species count considerably underrepresents its actual diversity. As a result, the Muscidae should also be recognized as a DT family.

4.3. Discrepancies in Taxa Coverage in Our Malaise Traps

Our estimated species counts for the DT families are based on the presumption that recovery success for the 11 families with strong taxonomy is a useful predictor of recovery success for the DT families. Our results did reveal threefold differences in recovery success among the well-known families, being lowest for Asilidae and Tabanidae and highest for the Muscidae. In our study, we used Malaise traps as a source of insect material, because

they enable sampling of high numbers of flying insects, especially Diptera [67–69]. However, a bias favoring the sampling of some taxa over others is always present, meaning that the community captured with such traps does not depict the true insect community of a sampled site [67]. Furthermore, the setup of a Malaise trap in terms of site choice, orientation, and above-ground-level is another source of bias, and these factors strongly influence sampling results [40]. To incorporate such variations, we used different approaches for extrapolating species numbers including Chao1 estimate calculations, which consider the unsampled taxa present at the sampling sites. The resulting Chao1 values indicated that we only recovered about 70% of the dipteran species present at the sites. In this manner, we obtained BIN estimates for each family that consider recovery success and unsampled taxa. Our results indicate that more than 3316 more BINs await detection, a total that would raise the number of Dipteran species in Germany by a third.

5. Conclusions

In this study, we aimed at estimating the number of species in the Bavarian and German faunas for four families of Diptera that are prime examples of "dark taxa". Our estimates were inferred from the analysis of sequence data, reproducible genetic patterns, rather than on speculations. The confidence intervals on these estimates are broad (Table 5), reflecting the various factors that influence any effort to gauge species diversity. Despite our limited geographic sampling effort, our results strongly suggest that a surprisingly high proportion of Germany's biodiversity is yet to be discovered.

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Institutional Review Board Statement: Not applicable.

Data Availability Statement: The datasets containing all sequence data are publicly available in three datasets on the Barcode of Life Data System: DS-BFNP, DS-ZSMTRAP and DS-ALGALPS.

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Appendix A

Table A1. Collection events for each Malaise trap.

Site	Trap	Processed Collection Events
BFNP 2012	1	8 May; 22 May; 8 June; 20 June; 4 July; 25 July; 12 August; 3 September; 22 September 2012.
ZSM-SNSB	2	10 April; 8 May; 5 June; 3 July; 31 July; 28 August; 25 September; 23 October; 20 November; 29 December 2017.
Allgäu Alps: Oytal	3	4 May; 17 May; 1 June; 16 June; 5 July: 20 July; 7 August; 29 August; 2 October; 27 October 2014.
Allgäu Alps: Oytal	4	4 May; 17 May; 1 June; 16 June; 5 July: 20 July; 7 August; 29 August; 2 October; 27 October 2014.
Allgäu Alps: Oytal	5	4 May; 17 May; 1 June; 16 June; 5 July: 20 July; 7 August; 29 August; 2 October; 27 October 2014.
Allgäu Alps: Oytal	6	4 May; 17 May; 1 June; 16 June; 5 July: 20 July; 7 August; 29 August; 2 October; 27 October 2014.
Allgäu Alps: Schochen	7	21 June; 4 July; 17 July; 6 August; 4 September; 29 September; 19 October 2014.
Allgäu Alps: Schochen	8	21 June; 4 July; 17 July; 6 August; 4 September; 29 September; 19 October 2014.
Allgäu Alps: Schochen	9	21 June; 4 July; 17 July; 6 August; 4 September; 29 September; 19 October 2014.
Allgäu Alps: Schochen	10	21 June; 4 July; 17 July; 6 August; 4 September; 29 September; 19 October 2014.
Allgäu Alps: Schochen	11	21 June; 4 July; 17 July; 6 August; 4 September; 29 September; 19 October 2014.
Allgäu Alps: Koblat	12	23 June, 4 July, 17 July; 8 August; 8 September; 5 September, 27 September; 20 October 2014.
Allgäu Alps: Koblat	13	23 June, 4 July, 17 July; 8 August; 8 September; 5 September, 27 September; 20 October 2014.
Allgäu Alps: Koblat	14	23 June, 4 July, 17 July; 8 August; 8 September; 5 September, 27 September; 20 October 2014.

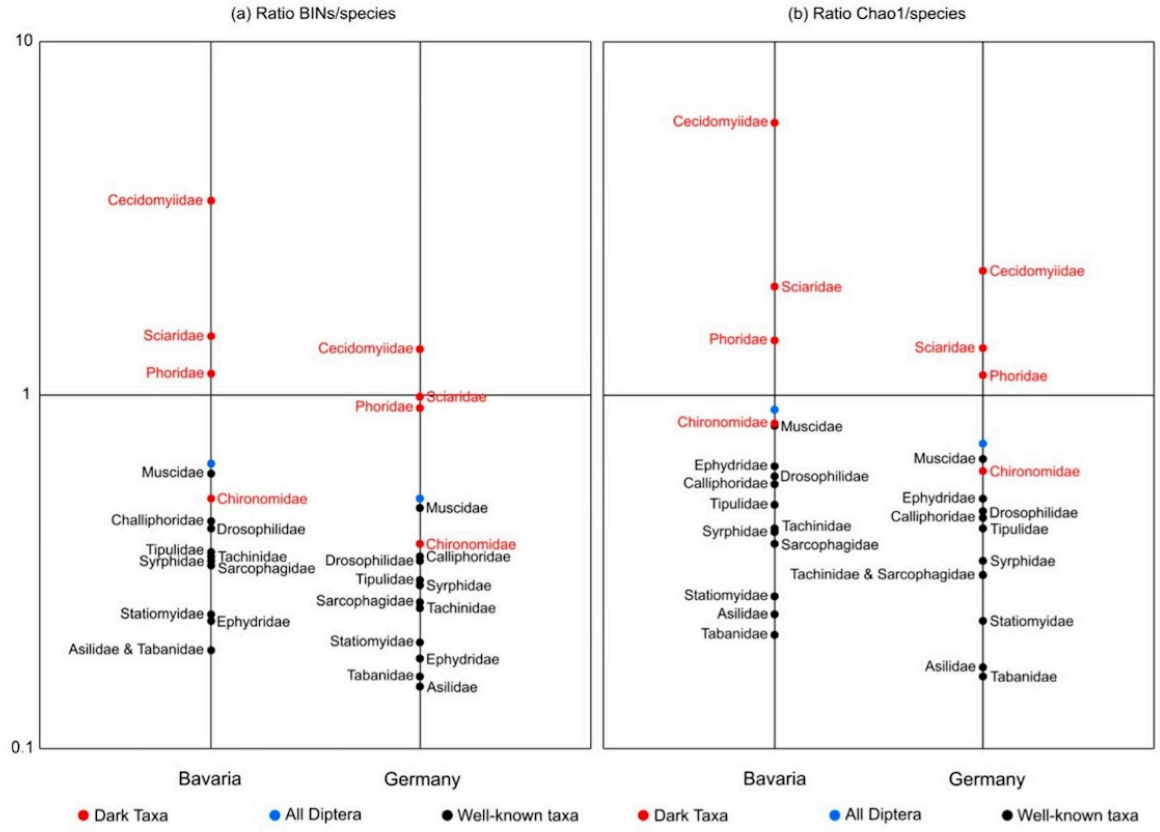

Figure A1. Ratio of BIN or Chao1 counts versus recorded species counts ratios for each family on a logarithmic scale. (**a**) BINs/species and (**b**) Chao1/species for well-known families, problematic families, and for all Diptera for Bavaria and Germany.

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SECTION 3.3: Species Estimates for Germany (unpublished material)

INTRO AND DATASET

The following part contains unpublished species estimates based on the barcoding information obtained within the framework of GBOL III. The last species checklist and diversity estimates for Diaprioidae were published over 20 years ago by Stefan Blank in the framework of "Fauna Germanica" (Blank, 2001). At that time, 289 species were recorded while estimated species diversity was expected to be "significantly higher".

Both, the known diversity and the estimates (Table 1), do not resemble proportionally the amount of barcoded and investigated specimens within the two subfamilies of Diapriidae. The reason to mainly focus on the less diverse and less abundant Diapriinae was simply of a practical nature: this thesis and the usage of integrative taxonomy is a proof of concept. When facing a hyper-divers Dark Taxa like Diapriidae, there is no claim to completeness in the limited time of a PhD project. Another limitation of the dataset lies in the fact that most of the sample locations are in Bavaria. Bavaria is expected to inhabit around 80% of the German entomological biodiversity (Blank, 2001; Chimeno et al., 2022; Haszprunar, 2009), so the listed estimates are not corrected for the whole nation and can be seen as a very conservative approximation.

Section 3.4 further discusses the status quo of the known-species list and why the numbers of Blank (2001) have to be used with caution.

RESULTS

Table 1 summarizes and compares the known species numbers (Blank, 2001), the obtained genetic information and the estimates that are based upon the OTUs. Fig. 3 shows the plots of the diversity profiles/species numbers based on Chao1 and compares it to the obtained empirical data. Fig. 4 plots the accumulation curve of OTU diversity (A, B), the OTU diversity based on sample coverage (C, D) and the sample coverage based on abundance (E, F). The plots of both figures have been created with R using the iNext package (Hsieh et al., 2016).

Since Ismaride are only represented by one species-poor genus, *Ismarus*, the dataset covered the diversity well (also see Figs 3C, 4B, D, F). The estimates (9.5) barely surpass the

obtained genetic data (9). Since the genus was recently revised twice (Kim et al., 2018; Kolyada & Chemyreva, 2016) and two new species were described for Western Palaearctic (United Kingdom) there is a good chance for them to be found in Northern Germany. *I. similis* Kim, Notton & Lee, 2018 and *I. distinctus* Kim, Notton & Ødegaard, 2018.

The estimates for the diapriid subfamily Diapriinae differ significantly from the Ismaridae in many aspects. 229 BINs / 233 OTUs were obtained from 7489 barcoded specimens. This number is over twice as many as the recorded 98 species from the latest checklist. Based on the data obtained, there might be up to 313 (Chao1 estimate + standard error) in Bavaria or even 20% more species in Germany (391).

TABLE 1. Summary of the species estimations and diversity findings.

Taxon	German species [1]	Specimens	BINs	OTUs	Chao1 estimates	s. e.	iNext estimates	max. German estimates
Ismaridae	4	147	9	9	9.5	± 1.32	9.5	14
Diapriinae	98	7489	229	233	289.9	± 23.29	263.6	391
Belytinae	161	1173	234	262	409.2	± 39.89	336.1	561
Summe	263[1] (360)	8809	472	504	708.6	± 64.5	609.2	**966**

[1] Species numbers according to Blank (2001) corrected for synonyms (and species counts included records that study left out).

Only 161 Belytinae were known in Germany so far. 234 BINs or 262 OTU could be obtained from barcoding 1173 specimens. Chao1 estimates the species richness to be up to 449 (Chao1 + s.e.) for Bavaria or 561 accordingly for Germany. The iNext diversity estimates are significantly lower since the Belytinae subfamily, due to its high diversity and high specimen counts severely undersampled, as expected. The graphs of Fig. 4 A, C, E clearly show that a higher sampling effort would lead to higher estimates. The slopes of Belytinae (yellow color coded) are steeper for every category mentioned above than for the Ismaridae (red) and Diapriinae (blue) with the sample sampling effort. Fig. 3 clearly shows that the empirical and estimated Hill numbers (based on Chao1) are the furthest apart for Belytinae in comparison to the other two taxa.

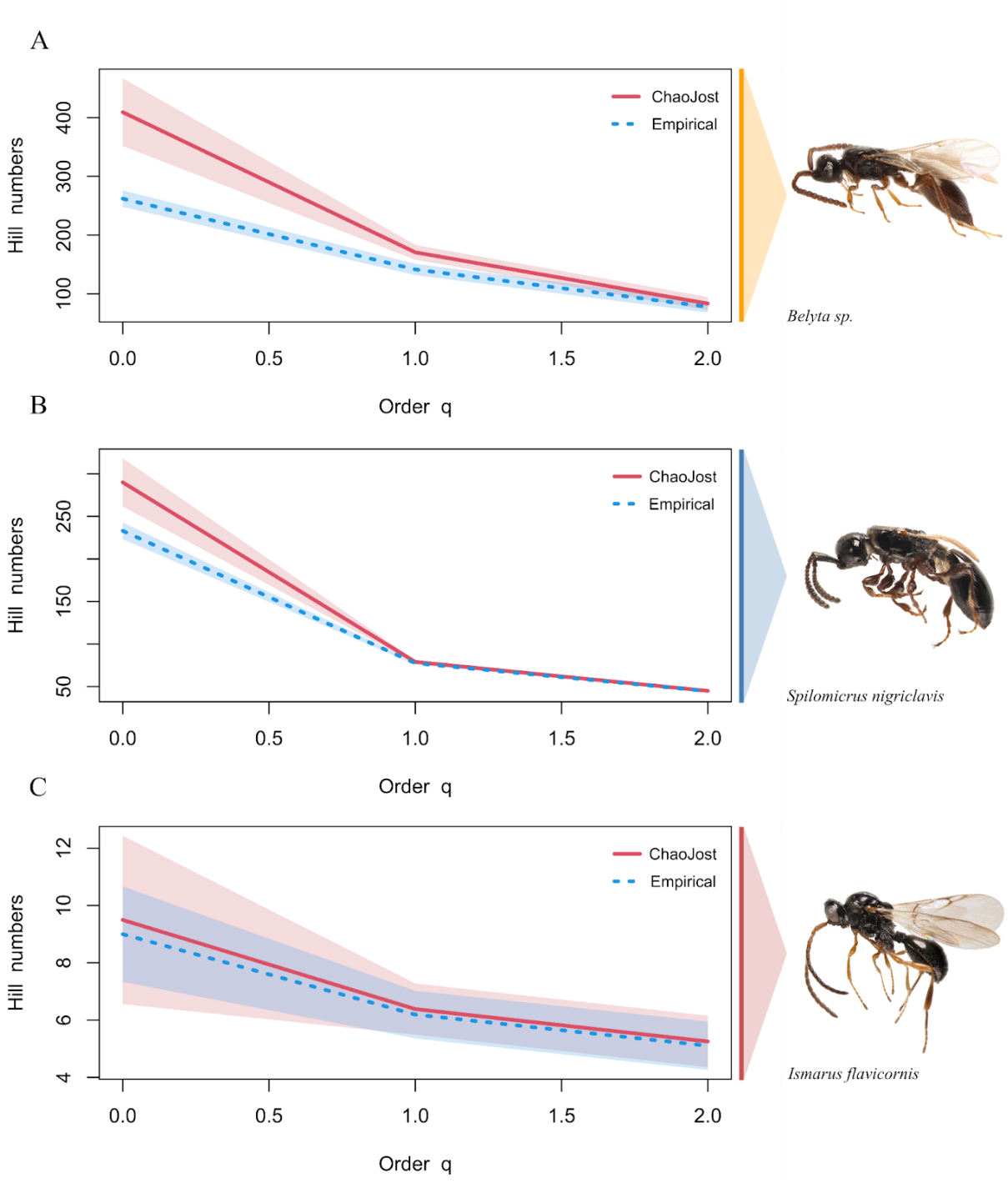

FIGURE 3. Diaprioidea diversity profiles based on Chao1. **A** Belytinae **B** Diapriinae **C** Ismaridae. The empirical (BIN counts; dotted blue) and estimated (Chao1; red) diversity profiles are quantified by Hill numbers for values of the diversity order (q) from 0–3 with 95% confidence intervals (shaded areas based on bootstrap analysis of 100 permutations). Species richness is depicted by q = 0; Shannon diversity by q = 1; and Simpson diversity by q = 2.

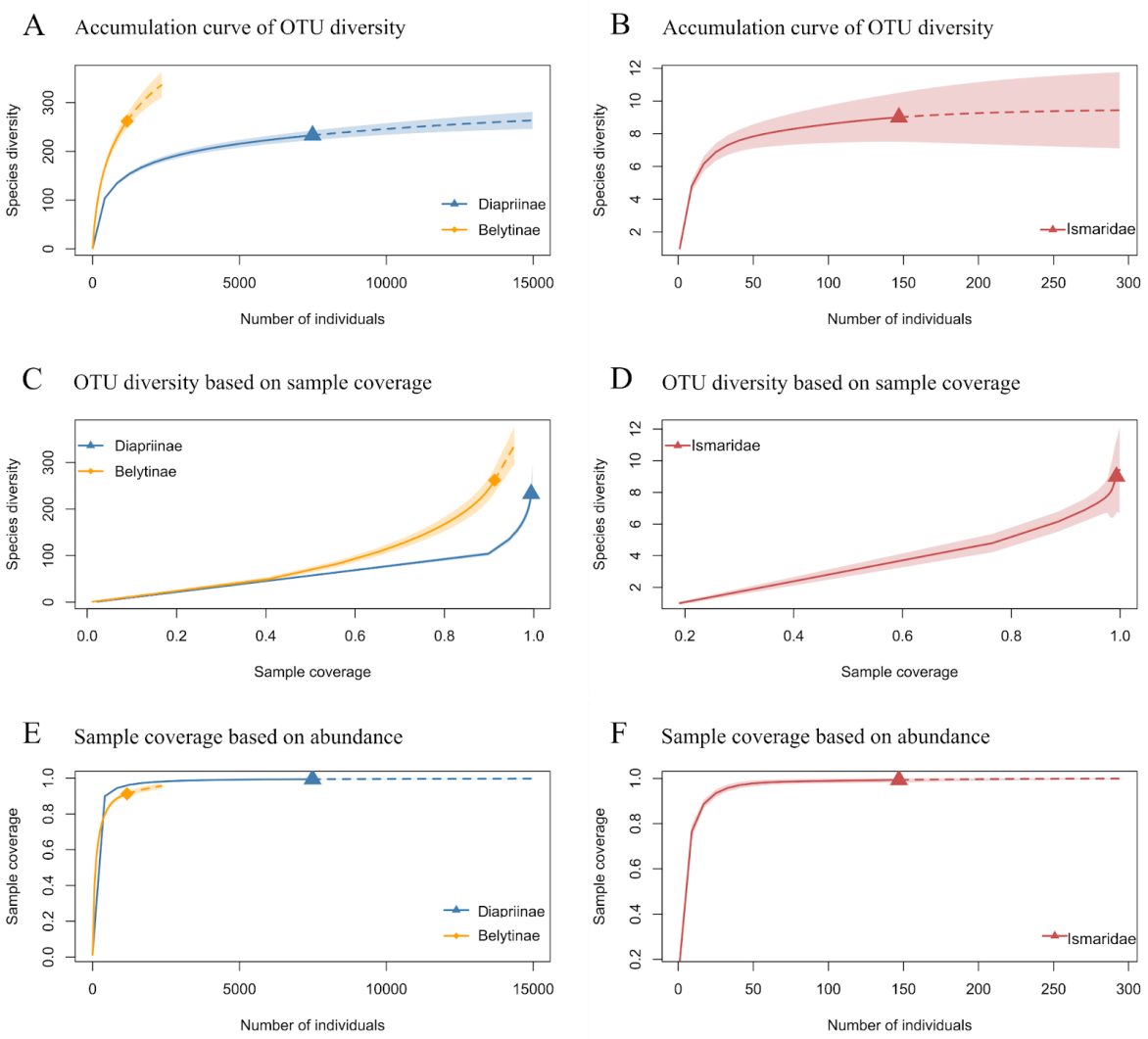

FIGURE 4. Different diversity estimation plots based on iNext (Hsieh et al., 2016). **A**, **B** Accumulation curve of OTU diversity **C**, **D** OTU diversity based on sample coverage **E**, **F** Sample coverage based on abundance. Yellow: Belytinae, blue: Diapriinae, red: Isamridae.

In total, 8809 specimens were successfully barcoded. That resulted in 472 BINs and 504 OTUs. Chao1 estimates the diapriid diversity at up to 773 (with s.e) for Bavaria or accordingly to up to 966 species nationwide.

If those estimates prove themself to be correct, the diversity of Diaprioidea in Germany has to be two and a half times as high as evaluated in 2001 (corrections included, check section 3.4.)

SECTION 3.4: A new German Diaprioidea Checklist (unpublished material)

The following table lists all taxonomic contributions that enhanced the German Checklist of Diaprioidea. It is limited to the new combinations, first records and new species that were established. In the appendix is an updated, but unpublished checklist attached that records all known species in the country: it combines the latest checklist by Blank (2001), the records from historic and current literature and all the species that have been found within the framework of this project. In addition, all BINs that could only be identified down to genus level are listed since there are potential unknown species among them.

Blank (2001) recorded in total 289 different diaprioid species sorted by federal state based on the species concepts as Hubert Hilpert interpreted them (Hilpert himself published several papers (e.g. 1989a, 1989b) on Diapriidae). Parts of his notable collection are stored at the SNSB-ZSM and were used to compare identified material. 21 of those recorded 289 species have been established as synonyms in the meantime. 23 species got a generic transfer, so Blank recorded essentially 268 taxa for Germany. By closer evaluation of the historic literature, another 92 could have been recorded, which elevates the total number of known species to 360 in the year 2001. Within the GBOL project, 184 species could be identified and have a BIN at the same time. The genetic data alone is composed of 474 BINs. Because 475 specimens were barcoded late in the project and did not get assigned a BIN, a BOLD-wide cluster analysis was conducted, which is implemented in the BOLD workbench. The algorithm sorted all available 9817 records (sequences with and without BIN) into 504 OTUs.

In summary, 364 species could be recorded for Germany in the framework of this thesis. This number is composed of records by Blank, the addition of the overlooked historically recorded species in the literature, species found since 2001, all first records and species descriptions within the project and a few records from the online platform Fauna Europaea (https://fauna-eu.org/) [1]. Another 189 BINs were only identified down to genus level for various reasons. So that number bears the potential of the record of another 189 species

[1] The records for Diaprioidea were downloaded at the end of 2020. Those records could not be reevaluated while writing this thesis since the homepage is hosted at the Natural History Museum in Berlin which recently fell victim to a hacker assault. Their IT infrastructure took severe damage, and the webpage as well as many other server functions are down. Reinstating the old status quo is an ongoing process and has not been reached by the completion of this thesis. Nevertheless, the platform has not been updated since 2013, which makes it less likely that important records were missed hereby.

nationwide. The fact that the DNA barcoding all research relied on was heavily focused on the less diverse and less spread subfamily Diapiinae has to be highlighted at this point once again.

All 9817 specimen records (9274 sequences, 474 BINs) obtained within this dissertation are uploaded online at the BOLD platform in the project DIAIS. It is not yet publicly available due to ongoing taxonomic investigation e.g. of the genus *Lyteba* and e.g. the description of a new species, *Lyteba maceki* sp. nov.. Access can be granted upon request.

TABLE 2. Excerpt of the most important results of the new German Checklist: COMB. N. = combinatio nova, FR= first record, NOM. NUD. = nomen nudum, SP. N.= species nova. The name in gray font is a nomen nudum at the moment, the descriptions will be published in 2024.

Family	Subfamily	Tribe	Genus	Species	Author	Status
Diapriidae	Belytinae	Belytini	*Lyteba*	*maceki*	Chemyreva, Hübner, Kolyada, Ødegaard, 2024	SP. N./NOM. NUD.
Diapriidae	Belytinae	Belytini	*Pantoclis*	*caecutiens*	(Kieffer, 1908)	COMB. N.
Diapriidae	Belytinae	Belytini	*Pantoclis*	*fuscata*	(Thomson, 1858)	COMB. N.
Diapriidae	Belytinae	Belytini	*Pantoclis*	*hemiptera*	(Thomson, 1858)	COMB. N.
Diapriidae	Belytinae	Belytini	*Pantoclis*	*mese*	Nixon, 1957	FR
Diapriidae	Belytinae	Belytini	*Pantoclis*	*microtoma*	(Kieffer, 1909)	COMB. N.
Diapriidae	Belytinae	Belytini	*Pantoclis*	*soluta*	(Kieffer, 1907)	COMB. N.
Diapriidae	Belytinae	Belytini	*Zygota*	*angularis*	Macek, 1997	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*balteata*	Macek, 1997	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*comitans*	Macek, 1997	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*sordida*	Macek, 1997	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*spinosipes*	(Kieffer, 1908)	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*vigil*	Nixon, 1957	FR
Diapriidae	Belytinae	Belytini	*Zygota*	*walli*	Hübner, Chemyreva, Kolyada, Macek 2024	SP. N.
Diapriidae	Belytinae	Cinetini	*Scorpioteleia*	*cebes*	(Nixon, 1957)	FR
Diapriidae	Belytinae	Pantolytini	*Opazon*	*frigidum*	Macek, 1995	FR
Diapriidae	Belytinae	Pantolytini	*Pantolyta*	*flaviventris*	(Thomson, 1858)	FR

Diapriidae	Belytinae	Pantolytini	*Pantolyta*	*rufiventris*	(Kieffer, 1909)	FR
Diapriidae	Belytinae	Pantolytini	*Pantolyta*	*sciarivora*	(Kieffer, 1907)	FR
Diapriidae	Belytinae	Pantolytini	*Pantolyta*	*seticornis*	(Kieffer, 1910)	FR
Diapriidae	Belytinae	Pantolytini	*Synacra*	*azepylopria*	Chemyreva & Kolyada, 2019	FR
Diapriidae	Belytinae	Pantolytini	*Synacra*	*paupera*	Macek, 1995	FR
Diapriidae	Diapriinae	Diapriini	*Basalys*	*rufocinctus*	(Kieffer, 1911)	FR; COMB. N.
Diapriidae	Diapriinae	Diapriini	*Diapria*	*cava*	Nixon, 1993	FR
Diapriidae	Diapriinae	Diapriini	*Diapria*	*luteipes*	Nixon, 1993	FR
Diapriidae	Diapriinae	Diapriini	*Lepidopria*	*pedestris*	Kieffer 1916	FR
Diapriidae	Diapriinae	Diapriini	*Monelata*	*aphrodite*	(Nixon) 1980	FR
Diapriidae	Diapriinae	Diapriini	*Monelata*	*clavigera*	Priesner, 1953	FR
Diapriidae	Diapriinae	Diapriini	*Tetramopria*	*cincticollis*	Wasmann, 1899	FR
Diapriidae	Diapriinae	Diapriini	*Trichopria*	*drosophilae*	(Perkins, 1910)	FR
Diapriidae	Diapriinae	Diapriini	*Viennopria*	*lacustris*	(Schulz, 1911)	FR
Diapriidae	Diapriinae	Psilini	*Coptera*	*punctiventris*	(Kozlov, 1978)	FR
Diapriidae	Diapriinae	Psilini	*Psilus*	*frontalis*	(Thomson, 1859)	FR
Diapriidae	Diapriinae	Psilini	*Psilus*	*rufipes*	(Thomson, 1859)	FR
Diapriidae	Diapriinae	Spilomicrini	*Entomacis*	*hajeki*	Macek, 2000	FR
Diapriidae	Diapriinae	Spilomicrini	*Paramesius*	*belytoides*	Marshall, 1867	FR
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*brevimalaris*	Hübner & Chemyreva, 2024	SP .N.
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*crassiclavis*	Kieffer, 1911	FR
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*diversus*	Chemyreva, 2021	FR
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*flavecorpus*	Hübner & Chemyreva, 2024	SP. N.
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*lusitanicus*	(Kieffer, 1910)	FR
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*politus*	Hübner & Chemyreva, 2024	SP. N.
Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus*	*thomsoni*	Kieffer, 1911	FR
Ismaridae			*Ismarus*	*apicalis*	Kolyada & Chemyreva, 2016	FR

GLOBAL DISCUSSION

Taxonomy over time

When Swedish naturalist Carl Linnaeus started a structured framework that taxonomists still work with over 250 years later, he estimated that there are less than 30 000 species on the planet (Linnaeus, 1735). Up to now, about 1.2 million species have been described so far. That leaves approximately about 86% of the taxa on land, and 91% in the sea unknown to science (including unicellular organisms) (Mora et al., 2011). Considering the last 20 years, about 6200 eukaryote species have been described annually, at a cost per new species of around US $48,500 per species (Carbayo & Marques, 2011). Keeping this pace, it would take researchers another 1200 years and 303000 taxonomists to record every single undescribed species (Mora et al., 2011). In addition, those numbers do not factor in the ongoing biodiversity crisis with extinction rates that are between 100 and 1000 times higher than the pre-human levels (Pimm et al., 1995).

Taxonomy's challenges

Taxonomy as it is conducted for the most part today needs changes in order to keep a mere chance countering the effects of accelerating diversity loss. Modern taxonomists need to learn way more methods to work in the field and people need to dare to go new, sometimes seemingly radical ways to innovate the field (e.g. Blagoev et al. 2009; Fernandez-Triana 2022; Goldstein and DeSalle 2011; Meier et al. 2006; Sharkey et al. 2012; Wührl et al. 2022). Obviously not all approaches are right out of the gate applicable for any taxon and are often in need of improvements and standards. DNA barcode based species description, turbo-taxonomy, metabarcoding of eDNA or preserving fluids etc. have been (in part) rightly so criticized (Baker et al., 2009; Ebach & Carvalho, 2010; Meier et al., 2022; Packer et al., 2009; Pires & Marinoni, 2010; H. R. Taylor & Harris, 2012), but as a result, approaches could be improved. At the end of the day, one of the most fundamental questions is still the same Linneaus asked himself: what is a species? This almost philosophical question has been dealt with by many researchers and philosophers (e.g. Mayr 1988, 1996, 1999; Ruse 1969; Wilkins 2007). Can we tell two species apart by their morphological characters without knowing their behavior or biology (which is often the case for Dark Taxa)? Where does intraspecific variation end and interspecific variation start? When does an isolated population

become a new species? Is there a threshold value in genetic distances between BINs or OTUs that determines whether or not two specimens belong to the same species or not? All those questions are legitimate and the answer can't always be the same for each species on the planet, not even only for animal species. Despite all the (partly justified) criticism and the challenges posed by some innovative methods, we must not forget that even the classical morphological species is also only a species hypothesis.

Taxonomy's future

So another approach to deal with those challenges would be to rethink the role of a taxonomic specialist. Instead of focusing on a highly sophisticated narrow group, future researchers have to widen their scope. Being highly specialized is still in demand for highly complex and diverse taxa, but modern taxonomists need to broaden their horizon. There are simply not enough jobs in taxonomy, enough time etc. With all the new technologies on hand, it is even for less specialized experts possible to make significant contributions to a certain taxon's taxonomy.

Technological advances in (non-invasive) metabarcoding, AI-powered automated specimen sorting using machines are all opportunities that grant the chance to save a taxonomist's greatest resource: time at hand.

A further focus should also be placed on the perspective of a taxonomist. A specialist's scope should also be on diversity itself and less only on a single taxon. The methods used within the scope of this thesis are widely applicable to all kinds of (insect) groups. The diapriid research was, although limited in time, considerably successful and produced a notable gain in knowledge. It has to be highlighted once again: highly specialized taxonomists are not obsolete by those new approaches, they are still needed and have to be integrated in innovative projects, such as GBOL. But in times of global biodiversity crisis, research has to up the pace to examine hidden diversity before it gets extinct. Cooperation across borders has always been important and is more so today than ever. Bringing together all kinds of resources (specialized taxonomic knowledge, experience in innovative digital approaches, acquiring funding, research communication, ect.) is the way forward.

One point gets easily overlooked: employment options need to be created for young researchers that are so desperately needed in taxonomy. We have shortcomings in educating them, way too less man-power and still, whenever we educate young early-career

scientists/taxonomists it is a struggle to find employment. That drives them away in (better) paid positions e.g. in the private sector.

Not only in order to raise money for future jobs in taxonomy it is imperative to start getting ordinary people involved. Insects don't have a lobby and are not perceived as animals worth protecting as it is the case for many mammals such as the polar bear, orangutan, wild cats etc.. While the general public is aware that bees are important for pollination and honey production, most of them are not aware that there are around 600 wild bee species and many more pollinators that keep our ecosystem and agriculture alive. Insects are expected to play a more and more important role in food production as a cheap protein source, as it is already the case in some cultural circles. But while in Western countries the perception of what insects can do for humans (apart from causing an annoying itch on summer vacation) is only slowly changing, the fact that diversity itself is one of the most important divers for a functioning ecosystem is still not being taken into account. The more people understand that humanity and the planet depend on a strong and diverse (insect) fauna, the more opportunities there will be in the future to study, protect and utilize the ecological services of insects. There are countless ways to gain attention in today's digital world and those have to be taken advantage of: social media, citizen science, public reach out, exhibitions etc.

CONCLUSION

Diapriidae or Dark Taxa in general are just the tip of the iceberg when it comes to hidden, undiscovered species diversity on the planet. And while diversity is decreasing at an alarming and accelerating pace, it is impeccable to get a hold on all the biodiversity being present and its biology in order to find a suitable way of protecting it. Descriptive taxonomy today has merely gone faster and more efficient than when it was established 250 years ago. Innovative methods that combine the advantages of complementary branches of taxonomy are needed to get the most out of the data at hand.

This thesis presents different integrative approaches to streamline the whole process of taxonomy work. From mechanically sorting out the insect bulk material for target taxa (and supported by AI), through plating, species identification, new species description to public availability of the data, this work streamlined all processes. Although over 90% of all evaluated specimens were only caught in Bavaria and although the focus of barcoding layed primarily on the way less diverse and less abundant subfamily Diapriinae, substantial taxonomic contribution could be accomplished.

There are still many diapriid genera left that badly need a revision, such as *Alista, Belyta, Cinetus, Pantoclis* (all Belytinae) or *Basalys* (Diapriinae). Due to their high intraspecific variation it is challenging for taxonomists to distinguish between traits that are informative and characters that are variable. Barcoding these species will give researchers a great chance to easily sort obtained material according to their BIN or OTU and even align the opposing sexes before a detailed morphological analysis.

It has been shown within the framework of this dissertation that DNA barcoding is just one out of many new approaches that might change how we conduct or how we maybe even have to conduct taxonomy in the future. Generally speaking, descriptive taxonomy is in an urgent need to get faster and more efficiently. The tools or approaches such as AI, metabarcoding, turbo taxonomy, DNA-barcoding, UCEs etc. are openly available at any researcher's disposal and have to be taken advantage of. Not everybody has to have every single competence or knowledge in every aspect but by combining forces and resources, great scientific contributions can be accomplished.

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APPENDIX

iNext estimates and metrics

```
List of 1
 $ Ismarus: num [1:503] 0 0 0 0 0 0 0 0 0 0 ...
> outputiNEXT <- iNEXT(all, q=0, datatype="abundance",nboot=100,se=T,conf=0.95)
> outputiNEXT
Compare 1 assemblages with Hill number order q = 0.
$class: iNEXT

$DataInfo: basic data information
  Assemblage   n S.obs     SC f1 f2 f3 f4 f5 f6 f7 f8 f9 f10
1    Ismarus 147     9 0.9933  1  1  0  0  0  0  1  0  0   1

$iNextEst: diversity estimates with rarefied and extrapolated samples.
$size_based (LCL and UCL are obtained for fixed size.)

   Assemblage   m        Method Order.q       qD   qD.LCL   qD.UCL        SC
SC.LCL    SC.UCL
1     Ismarus   1   Rarefaction       0 1.000000 1.000000  1.00000 0.1903830
0.1570471 0.2237189
10    Ismarus  73   Rarefaction       0 8.236685 7.286299  9.18707 0.9855646
0.9742462 0.9968830
20    Ismarus 147      Observed       0 9.000000 7.523804 10.47620 0.9932892
0.9810626 1.0000000
30    Ismarus 217 Extrapolation       0 9.305003 7.362515 11.24749 0.9974109
0.9909996 1.0000000
40    Ismarus 294 Extrapolation       0 9.429393 7.190018 11.66877 0.9990918
0.9955526 1.0000000

NOTE: The above output only shows five estimates for each assemblage; call
iNEXT.object$iNextEst$size_based to view complete output.

$coverage_based (LCL and UCL are obtained for fixed coverage; interval length is
wider due to varying size in bootstraps.)

   Assemblage        SC   m        Method Order.q       qD    qD.LCL    qD.UCL
1     Ismarus 0.1903830   1   Rarefaction       0 1.000000 0.9039924  1.096008
10    Ismarus 0.9855646  73   Rarefaction       0 8.236685 6.4380577 10.035311
20    Ismarus 0.9932892 147      Observed       0 9.000000 6.7977004 11.202300
30    Ismarus 0.9974109 217 Extrapolation       0 9.305003 6.8483658 11.761640
40    Ismarus 0.9990918 294 Extrapolation       0 9.429393 6.8528825 12.005904

NOTE: The above output only shows five estimates for each assemblage; call
iNEXT.object$iNextEst$coverage_based to view complete output.

$AsyEst: asymptotic diversity estimates along with related statistics.
  Assemblage         Diversity Observed Estimator      s.e.      LCL       UCL
1    Ismarus  Species richness  9.00000  9.496599 1.2236692 9.000000 11.894946
2    Ismarus Shannon diversity  6.19099  6.384267 0.3886445 5.622538  7.145997
3    Ismarus Simpson diversity  5.10489  5.252570 0.4264517 4.416740  6.088400
> plot(outputiNEXT, type=1, se=T, show.legend=T, col=
c("indianred3"),title("Accumulation curve of BIN diversity",adj=0,line=1.5))
Error in if (show.main == TRUE) title(main = paste("Order q =", ORDER[j])) :
  argument is of length zero
> plot(outputiNEXT, type=2, se=T, show.legend=T, col=
c("indianred3"),title("Sample coverage based on abundance",adj=0,line=1.5))
```

```
Error in if (show.main == TRUE) title(main = paste("Order q =", ORDER[j])) :
  argument is of length zero
> plot(outputiNEXT, type=3, se=T, show.legend=T, col= c("indianred3"),title("BIN
diversity based on sample coverage",adj=0,line=1.5))
Error in if (show.main == TRUE) title(main = paste("Order q =", ORDER[j])) :
  argument is of length zero
> all_d_b<-list(diapriinae_all,belytinae_all)
> names(all_d_b)<-c("Diapriinae", "Belytinae")
> str(all_d_b)
List of 2
 $ Diapriinae: num [1:503] 0 0 0 0 0 0 0 0 0 0 ...
 $ Belytinae : num [1:503] 1 11 5 72 3 3 1 6 1 4 ...
> outputiNEXT <- iNEXT(all_d_b, q=0,
datatype="abundance",nboot=100,se=T,conf=0.95)
> outputiNEXT
Compare 2 assemblages with Hill number order q = 0.
$class: iNEXT

$DataInfo: basic data information
  Assemblage    n S.obs     SC  f1 f2 f3 f4 f5 f6 f7 f8 f9 f10
1 Diapriinae 7489   233 0.9941  44 17 17 11 13  6  9  6  7   2
2  Belytinae 1173   262 0.9122 103 36 27 21 16 18  2  7  4   1

$iNextEst: diversity estimates with rarefied and extrapolated samples.
$size_based (LCL and UCL are obtained for fixed size.)

   Assemblage     m        Method Order.q       qD   qD.LCL   qD.UCL         SC
   SC.LCL     SC.UCL
1  Diapriinae     1   Rarefaction       0   1.0000   1.0000   1.0000 0.02225240
0.02132923 0.02317558
10 Diapriinae  3744   Rarefaction       0 203.3208 196.6048 210.0367 0.98849247
0.98702539 0.98995955
20 Diapriinae  7489      Observed       0 233.0000 222.6134 243.3866 0.99412532
0.99267345 0.99557720
30 Diapriinae 11036 Extrapolation       0 250.4506 236.2603 264.6408 0.99592596
0.99432458 0.99752733
40 Diapriinae 14978 Extrapolation       0 263.6460 244.8351 282.4570 0.99728753
0.99581335 0.99876170
41  Belytinae     1   Rarefaction       0   1.0000   1.0000   1.0000 0.01199777
0.01030734 0.01368820
50  Belytinae   586   Rarefaction       0 195.9317 187.6653 204.1981 0.84747373
0.83310683 0.86184064
60  Belytinae  1173      Observed       0 262.0000 247.3788 276.6212 0.91224331
0.89793762 0.92654899
70  Belytinae  1729 Extrapolation       0 303.5415 282.5113 324.5718 0.93700561
0.92061264 0.95339858
80  Belytinae  2346 Extrapolation       0 336.0709 307.0039 365.1379 0.95639590
0.94012537 0.97266643

NOTE: The above output only shows five estimates for each assemblage; call
iNEXT.object$iNextEst$size_based to view complete output.

$coverage_based (LCL and UCL are obtained for fixed coverage; interval length is
wider due to varying size in bootstraps.)
```

```
   Assemblage         SC     m        Method Order.q         qD      qD.LCL
qD.UCL
1  Diapriinae 0.02225240     1   Rarefaction       0   1.000000   0.9764067
1.023593
10 Diapriinae 0.98849247  3744   Rarefaction       0 203.320769 192.5324892
214.109049
20 Diapriinae 0.99412532  7489      Observed       0 233.000000 214.4988347
251.501165
30 Diapriinae 0.99592596 11036 Extrapolation       0 250.450583 227.1726768
273.728489
40 Diapriinae 0.99728753 14978 Extrapolation       0 263.646045 236.0604317
291.231658
41  Belytinae 0.01199791     1   Rarefaction       0   1.000012   0.9469222
1.053103
50  Belytinae 0.84747373   586   Rarefaction       0 195.931672 179.3596756
212.503668
60  Belytinae 0.91224331  1173      Observed       0 262.000000 231.9730589
292.026941
70  Belytinae 0.93700561  1729 Extrapolation       0 303.541517 264.1935242
342.889509
80  Belytinae 0.95639590  2346 Extrapolation       0 336.070886 288.0390313
384.102742

NOTE: The above output only shows five estimates for each assemblage; call
iNEXT.object$iNextEst$coverage_based to view complete output.

$AsyEst: asymptotic diversity estimates along with related statistics.
  Assemblage         Diversity  Observed Estimator       s.e.       LCL
UCL
1  Belytinae  Species richness 262.00000 409.22161 34.1931378 342.20429
476.23893
2  Belytinae Shannon diversity 141.46911 170.57495  6.8904246 157.06997
184.07993
3  Belytinae Simpson diversity  77.88130  83.34885  5.2735416  73.01290
93.68481
4 Diapriinae  Species richness 233.00000 289.93357 16.8807584 256.84789
323.01925
5 Diapriinae Shannon diversity  77.42590  79.02127  1.0984632  76.86832
81.17422
6 Diapriinae Simpson diversity  44.67684  44.93897  0.8476946  43.27752
46.60042
> plot(outputiNEXT, type=1, se=T, show.legend=T, col=
c("steelblue","orange"),title("Accumulation curve of BIN
diversity",adj=0,line=1.5))
```

Chao1 estimates and metrics

```
ISMARIDEA
(1) BASIC DATA INFORMATION:

                                      Variable Value
    Sample size                              n   147
    Number of observed species               D     9
    Coverage estimate for entire dataset     C 0.993
    CV for entire dataset                   CV 0.852
    Cut-off point                            k     2

                                                 Variable Value
    Number of observed individuals for rare group       n_rare     3
    Number of observed species for rare group           D_rare     2
    Estimate of the sample coverage for rare group      C_rare 0.667
    Estimate of CV for rare group in ACE                CV_rare     0
    Estimate of CV1 for rare group in ACE-1            CV1_rare     0
    Number of observed individuals for abundant group   n_abun   144
    Number of observed species for abundant group       D_abun     7

NULL


(2) SPECIES RICHNESS ESTIMATORS TABLE:

                            Estimate  s.e. 95%Lower 95%Upper
    Homogeneous Model         10.000 1.871    9.090   20.064
    Homogeneous (MLE)          9.000 0.590    9.000   10.758
    Chao1 (Chao, 1984)         9.497 1.315    9.029   17.394
    Chao1-bc                   9.000 0.590    9.000   10.758
    iChao1 (Chiu et al. 2014)  9.497 1.315    9.029   17.394
    ACE (Chao & Lee, 1992)    10.000 1.871    9.090   20.064
    ACE-1 (Chao & Lee, 1992)  10.000 1.871    9.090   20.064
    1st order jackknife        9.993 1.407    9.127   16.765
    2nd order jackknife       10.000 2.425    9.066   24.207


(3) DESCRIPTION OF ESTIMATORS/MODELS:

Homogeneous Model: This model assumes that all species have the same incidence
or detection probabilities. See Eq. (3.2) of Lee and Chao (1994) or Eq. (12a) in
Chao and Chiu (2016b).

Chao2 (Chao, 1987): This approach uses the frequencies of uniques and duplicates
to estimate the number of undetected species; see Chao (1987) or Eq. (11a) in
Chao and Chiu (2016b).

Chao2-bc: A bias-corrected form for the Chao2 estimator; see Chao (2005).

iChao2: An improved Chao2 estimator; see Chiu et al. (2014).

ICE (Incidence-based Coverage Estimator): A non-parametric estimator originally
proposed by Lee and Chao (1994) in the context of capture-recapture data
analysis. The observed species are separated as frequent and infrequent species
groups; only data in the infrequent group are used to estimate the number of
```

```
undetected species. The estimated CV for species in the infrequent group
characterizes the degree of heterogeneity among species incidence probabilities.
See Eq. (12b) of Chao and Chiu (2016b), which is an improved version of Eq.
(3.18) in Lee and Chao (1994). This model is also called Model(h) in
capture-recapture literature where h denotes "heterogeneity".

ICE-1: A modified ICE for highly-heterogeneous cases.

1st order jackknife: It uses the frequency of uniques to estimate the number of
undetected species; see Burnham and Overton (1978).

2nd order jackknife: It uses the frequencies of uniques and duplicates to
estimate the number of undetected species; see Burnham and Overton (1978).

95% Confidence interval: A log-transformation is used for all estimators so that
the lower bound of the resulting interval is at least the number of observed
species. See Chao (1987).
> Diversity(ismarus,"abundance",q=c(0,1,2))

(1) BASIC DATA INFORMATION:
                              Variable Value
    Sample size                      n   147
    Number of observed species       D     9
    Estimated sample coverage        C 0.993
    Estimated CV                    CV 0.852

(2) ESTIMATION OF SPECIES RICHNESS (DIVERSITY OF ORDER 0):

                            Estimate s.e. 95%Lower 95%Upper
    Chao1 (Chao, 1984)           9.5  1.3      9.0     17.4
    Chao1-bc                     9.0  0.6      9.0     10.8
    iChao1                       9.5  1.3      9.0     17.4
    ACE (Chao & Lee, 1992)       9.7  1.5      9.1     17.8
    ACE-1 (Chao & Lee, 1992)     9.9  2.0      9.1     21.1

        Descriptions of richness estimators (See Species Part)

(3a) SHANNON ENTROPY:

                       Estimate  s.e. 95%Lower 95%Upper
     MLE                  1.823 0.068    1.691    1.956
     Jackknife            1.855 0.068    1.722    1.988
     Chao & Shen          1.846 0.066    1.718    1.975
     Chao et al. (2013)   1.854 0.068    1.721    1.986

        MLE: empirical or observed entropy.
        Jackknife: see Zahl (1977).
        Chao & Shen: based on the Horvitz-Thompson estimator and sample coverage
method; see Chao and Shen (2003).
        see Chao and Shen (2003).
            Chao et al. (2013): A nearly optimal estimator of Shannon entropy;
see Chao et al. (2013).
            Estimated standard error is computed based on a bootstrap method.
```

```
(3b) SHANNON DIVERSITY (EXPONENTIAL OF SHANNON ENTROPY):

                      Estimate  s.e. 95%Lower 95%Upper
     MLE                 6.191 0.409    5.390    6.992
     Jackknife           6.392 0.424    5.562    7.222
     Chao & Shen         6.337 0.405    5.544    7.131
     Chao et al. (2013)  6.384 0.421    5.558    7.210

(4a) SIMPSON CONCENTRATION INDEX:

          Estimate    s.e. 95%Lower 95%Upper
     MVUE  0.19038 0.01999  0.15120  0.22957
     MLE   0.19589 0.01986  0.15697  0.23481

        MVUE: minimum variance unbiased estimator; see Eq. (2.27) of Magurran
(1988).
        MLE: maximum likelihood estimator or empirical index; see Eq. (2.26) of
Magurran (1988).

(4b) SIMPSON DIVERSITY (INVERSE OF SIMPSON CONCENTRATION):

          Estimate    s.e. 95%Lower 95%Upper
     MVUE  5.25257 0.43179  4.40627  6.09887
     MLE   5.10489 0.40516  4.31078  5.89900

(5) CHAO AND JOST (2015) ESTIMATES OF HILL NUMBERS

       q ChaoJost 95%Lower 95%Upper Empirical 95%Lower 95%Upper
     1 0    9.497    6.561   12.433     9.000    7.336   10.664
     2 1    6.384    5.492    7.276     6.191    5.362    7.020
     3 2    5.253    4.355    6.151     5.105    4.260    5.950

        ChaoJost: diversity profile estimator derived by Chao and Jost (2015).
            Empirical: maximum likelihood estimator (observed index).


DIAPIINAE
> ChaoSpecies(diapriinae_all ,"abundance", k=2,conf=0.95)

(1) BASIC DATA INFORMATION:

                                        Variable Value
    Sample size                                n  7489
    Number of observed species                 D   233
    Coverage estimate for entire dataset       C 0.994
    CV for entire dataset                     CV 2.053
    Cut-off point                              k     2

                                                 Variable Value
    Number of observed individuals for rare group    n_rare    78
    Number of observed species for rare group        D_rare    61
    Estimate of the sample coverage for rare group   C_rare 0.436
    Estimate of CV for rare group in ACE            CV_rare     0
    Estimate of CV1 for rare group in ACE-1        CV1_rare     0
```

```
    Number of observed individuals for abundant group   n_abun  7411
    Number of observed species for abundant group       D_abun   172

NULL


(2) SPECIES RICHNESS ESTIMATORS TABLE:

                             Estimate   s.e. 95%Lower 95%Upper
    Homogeneous Model         311.941 26.255  274.841  381.939
    Homogeneous (MLE)         233.000  3.641  246.666  262.251
    Chao1 (Chao, 1984)        289.934 23.287  259.338  356.070
    Chao1-bc                  285.549 21.315  257.454  345.920
    iChao1 (Chiu et al. 2014) 301.858 17.581  275.077  345.684
    ACE (Chao & Lee, 1992)    311.941 26.255  274.841  381.939
    ACE-1 (Chao & Lee, 1992)  311.941 26.255  274.841  381.939
    1st order jackknife       276.994  9.380  262.102  299.507
    2nd order jackknife       303.989 16.245  278.591  343.535


(3) DESCRIPTION OF ESTIMATORS/MODELS:

Homogeneous Model: This model assumes that all species have the same incidence
or detection probabilities. See Eq. (3.2) of Lee and Chao (1994) or Eq. (12a) in
Chao and Chiu (2016b).

Chao2 (Chao, 1987): This approach uses the frequencies of uniques and duplicates
to estimate the number of undetected species; see Chao (1987) or Eq. (11a) in
Chao and Chiu (2016b).

Chao2-bc: A bias-corrected form for the Chao2 estimator; see Chao (2005).

iChao2: An improved Chao2 estimator; see Chiu et al. (2014).

ICE (Incidence-based Coverage Estimator): A non-parametric estimator originally
proposed by Lee and Chao (1994) in the context of capture-recapture data
analysis. The observed species are separated as frequent and infrequent species
groups; only data in the infrequent group are used to estimate the number of
undetected species. The estimated CV for species in the infrequent group
characterizes the degree of heterogeneity among species incidence probabilities.
See Eq. (12b) of Chao and Chiu (2016b), which is an improved version of Eq.
(3.18) in Lee and Chao (1994). This model is also called Model(h) in
capture-recapture literature where h denotes "heterogeneity".

ICE-1: A modified ICE for highly-heterogeneous cases.

1st order jackknife: It uses the frequency of uniques to estimate the number of
undetected species; see Burnham and Overton (1978).

2nd order jackknife: It uses the frequencies of uniques and duplicates to
estimate the number of undetected species; see Burnham and Overton (1978).

95% Confidence interval: A log-transformation is used for all estimators so that
the lower bound of the resulting interval is at least the number of observed
```

```
species. See Chao (1987).
> Diversity(diapriinae_all,"abundance",q=c(0,1,2))

(1) BASIC DATA INFORMATION:
                              Variable Value
    Sample size                      n  7489
    Number of observed species       D   233
    Estimated sample coverage        C 0.994
    Estimated CV                    CV 2.053

(2) ESTIMATION OF SPECIES RICHNESS (DIVERSITY OF ORDER 0):

                            Estimate s.e. 95%Lower 95%Upper
    Chao1 (Chao, 1984)         289.9 23.3    259.3    356.1
    Chao1-bc                   285.5 21.3    257.5    345.9
    iChao1                     301.9 17.6    275.1    345.7
    ACE (Chao & Lee, 1992)     266.3 10.6    251.1    294.4
    ACE-1 (Chao & Lee, 1992)   275.5 14.8    254.9    315.5

        Descriptions of richness estimators (See Species Part)

(3a) SHANNON ENTROPY:

                       Estimate  s.e. 95%Lower 95%Upper
     MLE                  4.349 0.014    4.322    4.377
     Jackknife            4.368 0.014    4.341    4.396
     Chao & Shen          4.370 0.014    4.342    4.399
     Chao et al. (2013)   4.370 0.014    4.342    4.397

        MLE: empirical or observed entropy.
        Jackknife: see Zahl (1977).
        Chao & Shen: based on the Horvitz-Thompson estimator and sample coverage
method; see Chao and Shen (2003).
        see Chao and Shen (2003).
            Chao et al. (2013): A nearly optimal estimator of Shannon entropy;
see Chao et al. (2013).
            Estimated standard error is computed based on a bootstrap method.

(3b) SHANNON DIVERSITY (EXPONENTIAL OF SHANNON ENTROPY):

                       Estimate  s.e. 95%Lower 95%Upper
     MLE                 77.426 1.083   75.304   79.548
     Jackknife           78.923 1.113   76.741   81.105
     Chao & Shen         79.077 1.128   76.867   81.287
     Chao et al. (2013)  79.021 1.107   76.852   81.191

(4a) SIMPSON CONCENTRATION INDEX:

          Estimate    s.e. 95%Lower 95%Upper
     MVUE  0.02225 0.00049  0.02130  0.02321
     MLE   0.02238 0.00049  0.02143  0.02334

        MVUE: minimum variance unbiased estimator; see Eq. (2.27) of Magurran
(1988).
```

```
       MLE: maximum likelihood estimator or empirical index; see Eq. (2.26) of
Magurran (1988).

(4b) SIMPSON DIVERSITY (INVERSE OF SIMPSON CONCENTRATION):

          Estimate    s.e. 95%Lower 95%Upper
     MVUE 44.93897 1.03785 42.90479 46.97315
     MLE  44.67684 1.02568 42.66651 46.68718

(5) CHAO AND JOST (2015) ESTIMATES OF HILL NUMBERS

       q ChaoJost 95%Lower 95%Upper Empirical 95%Lower 95%Upper
     1 0  289.934  261.753  318.115   233.000  223.273  242.727
     2 1   79.021   77.320   80.722    77.426   75.764   79.088
     3 2   44.939   43.361   46.517    44.677   43.117   46.237

        ChaoJost: diversity profile estimator derived by Chao and Jost (2015).
            Empirical: maximum likelihood estimator (observed index).

BELYTINAE
> ChaoSpecies(belytinae_all ,"abundance", k=2,conf=0.95)

(1) BASIC DATA INFORMATION:

                                        Variable Value
    Sample size                                n  1173
    Number of observed species                 D   262
    Coverage estimate for entire dataset       C 0.912
    CV for entire dataset                     CV 1.564
    Cut-off point                              k     2

                                                 Variable Value
    Number of observed individuals for rare group       n_rare   175
    Number of observed species for rare group           D_rare   139
    Estimate of the sample coverage for rare group      C_rare 0.411
    Estimate of CV for rare group in ACE               CV_rare     0
    Estimate of CV1 for rare group in ACE-1           CV1_rare     0
    Number of observed individuals for abundant group   n_abun   998
    Number of observed species for abundant group       D_abun   123

NULL


(2) SPECIES RICHNESS ESTIMATORS TABLE:

                             Estimate   s.e. 95%Lower 95%Upper
    Homogeneous Model         460.847 44.334  391.132  568.199
    Homogeneous (MLE)         265.180  1.844  263.107  271.137
    Chao1 (Chao, 1984)        409.222 39.887  349.382  510.039
    Chao1-bc                  403.852 38.211  346.440  500.300
    iChao1 (Chiu et al. 2014) 434.890 29.399  386.181  502.704
    ACE (Chao & Lee, 1992)    460.847 44.334  391.132  568.199
    ACE-1 (Chao & Lee, 1992)  460.847 44.334  391.132  568.199
    1st order jackknife       364.912 14.344  340.415  397.062
```

```
    2nd order jackknife        431.829 24.831  389.706  487.845


(3) DESCRIPTION OF ESTIMATORS/MODELS:

Homogeneous Model: This model assumes that all species have the same incidence
or detection probabilities. See Eq. (3.2) of Lee and Chao (1994) or Eq. (12a) in
Chao and Chiu (2016b).

Chao2 (Chao, 1987): This approach uses the frequencies of uniques and duplicates
to estimate the number of undetected species; see Chao (1987) or Eq. (11a) in
Chao and Chiu (2016b).

Chao2-bc: A bias-corrected form for the Chao2 estimator; see Chao (2005).

iChao2: An improved Chao2 estimator; see Chiu et al. (2014).

ICE (Incidence-based Coverage Estimator): A non-parametric estimator originally
proposed by Lee and Chao (1994) in the context of capture-recapture data
analysis. The observed species are separated as frequent and infrequent species
groups; only data in the infrequent group are used to estimate the number of
undetected species. The estimated CV for species in the infrequent group
characterizes the degree of heterogeneity among species incidence probabilities.
See Eq. (12b) of Chao and Chiu (2016b), which is an improved version of Eq.
(3.18) in Lee and Chao (1994). This model is also called Model(h) in
capture-recapture literature where h denotes "heterogeneity".

ICE-1: A modified ICE for highly-heterogeneous cases.

1st order jackknife: It uses the frequency of uniques to estimate the number of
undetected species; see Burnham and Overton (1978).

2nd order jackknife: It uses the frequencies of uniques and duplicates to
estimate the number of undetected species; see Burnham and Overton (1978).

95% Confidence interval: A log-transformation is used for all estimators so that
the lower bound of the resulting interval is at least the number of observed
species. See Chao (1987).
> Diversity(belytinae_all,"abundance",q=c(0,1,2))

(1) BASIC DATA INFORMATION:
                              Variable Value
    Sample size                      n  1173
    Number of observed species       D   262
    Estimated sample coverage        C 0.912
    Estimated CV                    CV 1.564

(2) ESTIMATION OF SPECIES RICHNESS (DIVERSITY OF ORDER 0):

                           Estimate s.e. 95%Lower 95%Upper
    Chao1 (Chao, 1984)        409.2 39.9    349.4    510.0
    Chao1-bc                  403.9 38.2    346.4    500.3
    iChao1                    434.9 29.4    386.2    502.7
    ACE (Chao & Lee, 1992)    366.4 22.4    330.9    420.1
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    ACE-1 (Chao & Lee, 1992)    405.2 35.1    351.2    491.9

        Descriptions of richness estimators (See Species Part)

(3a) SHANNON ENTROPY:

                        Estimate  s.e. 95%Lower 95%Upper
     MLE                   4.952 0.038    4.877    5.027
     Jackknife             5.115 0.042    5.032    5.198
     Chao & Shen           5.097 0.040    5.019    5.175
     Chao et al. (2013)    5.139 0.044    5.053    5.226

        MLE: empirical or observed entropy.
        Jackknife: see Zahl (1977).
        Chao & Shen: based on the Horvitz-Thompson estimator and sample coverage
method; see Chao and Shen (2003).
        see Chao and Shen (2003).
            Chao et al. (2013): A nearly optimal estimator of Shannon entropy;
see Chao et al. (2013).
            Estimated standard error is computed based on a bootstrap method.

(3b) SHANNON DIVERSITY (EXPONENTIAL OF SHANNON ENTROPY):

                        Estimate  s.e. 95%Lower 95%Upper
     MLE                 141.469 5.063  131.545  151.393
     Jackknife           166.437 6.519  153.659  179.215
     Chao & Shen         163.604 6.115  151.618  175.591
     Chao et al. (2013)  170.575 6.901  157.050  184.100

(4a) SIMPSON CONCENTRATION INDEX:

          Estimate    s.e. 95%Lower 95%Upper
     MVUE  0.01200 0.00089  0.01025  0.01374
     MLE   0.01284 0.00089  0.01110  0.01458

        MVUE: minimum variance unbiased estimator; see Eq. (2.27) of Magurran
(1988).
        MLE: maximum likelihood estimator or empirical index; see Eq. (2.26) of
Magurran (1988).

(4b) SIMPSON DIVERSITY (INVERSE OF SIMPSON CONCENTRATION):

          Estimate    s.e. 95%Lower 95%Upper
     MVUE 83.34885 5.17341 73.20898 93.48873
     MLE  77.88130 4.54682 68.96954 86.79307

(5) CHAO AND JOST (2015) ESTIMATES OF HILL NUMBERS

       q ChaoJost 95%Lower 95%Upper Empirical 95%Lower 95%Upper
     1 0  409.222  351.888  466.556   262.000  248.111  275.889
     2 1  170.575  158.090  183.060   141.469  131.916  151.022
     3 2   83.349   72.257   94.441    77.881   68.136   87.626

        ChaoJost: diversity profile estimator derived by Chao and Jost (2015).
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Updated German Checklist

This is the latest Diaprioidea checklist for Germany. 364 species have been recorded in total. Those records are put together from the last, modified checklist (Blank, 2001), Fauna Europaea, (historic) literature, and the records obtained within the framework of GBOL. In addition, 189 BINs are listed that were, for various reasons, only identified down to genus level. The two species marked with an asterisk (*) are two species that are about to be described in the framework of a revision of the genus *Lyteba* and the tribe Pantolytini. They are therefore marked as nomina nuda. The description of *Acanosema sana* is still pending.

The sources for each record as indicated in the list:

Blank 2001	included in Blank 2001
Fauna Europaea	only recorded in the online platform Fauna Europaea
BOLD DIAIS	only recorded within GBOL, records online available in DIAIS project
BOLD DIAIS BIN	record identified only down to genus level, online available in DIAIS project

#	Family	Subfamily	Tribe	Species	Author	Record	BIN	Source
1	Diapriidae	Belytinae	Belytini	*Belyta abrupta*	Thomson, 1858		BOLD:ADF7999	Blank 2001
2	Diapriidae	Belytinae	Belytini	*Belyta acuta*	Kieffer, 1909		no BIN	Blank 2001
3	Diapriidae	Belytinae	Belytini	*Belyta bicolor*	Jurine, 1807		BOLD:AEK1964	Blank 2001
4	Diapriidae	Belytinae	Belytini	*Belyta borealis*	Whittacker, 1931		no BIN	Blank 2001
5	Diapriidae	Belytinae	Belytini	*Belyta depressa*	Thomson, 1858		BOLD:AEJ6744, BOLD:AEK7556, BOLD:AEL4523, BOLD:AEY0870, BOLD:ACH3380, BOLD:ACH3381, BOLD:AEE9464, BOLD:AEJ6092	Blank 2001
6	Diapriidae	Belytinae	Belytini	*Belyta elegans*	Kieffer, 1909		no BIN	Blank 2001
7	Diapriidae	Belytinae	Belytini	*Belyta elongata*	Thomson, 1858		no BIN	Blank 2001
8	Diapriidae	Belytinae	Belytini	*Belyta insignis*	(Kieffer, 1909)		BOLD:AEJ0892, BOLD:AER0764, BOLD:AER0767	Blank 2001
9	Diapriidae	Belytinae	Belytini	*Belyta moniliata*	Cameron, 1887		BOLD:ADF7999, BOLD:AEJ0892	Blank 2001
10	Diapriidae	Belytinae	Belytini	*Belyta nixoni*	Macek, 1996		no BIN	Blank 2001
11	Diapriidae	Belytinae	Belytini	*Belyta pelias*	Nixon, 1957		BOLD:AER0765	Blank 2001
12	Diapriidae	Belytinae	Belytini	*Belyta petiolaris*	Nees, 1834		no BIN	Blank 2001
13	Diapriidae	Belytinae	Belytini	*Belyta rugosicollis*	Kieffer, 1909		BOLD:AFA3243	Blank 2001
14	Diapriidae	Belytinae	Belytini	*Belyta sanguinolenta*	Nees, 1834		BOLD:ACH2189, BOLD:AEJ2535, BOLD:ACC1835, BOLD:ACG8044, BOLD:ADU7694	Blank 2001
15	Diapriidae	Belytinae	Belytini	*Belyta seron*	Nixon, 1957		BOLD:ACI4762	Blank 2001
	Diapriidae	Belytinae	Belytini	*Belyta sp*			BOLD:ABA1018	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Belyta sp*			BOLD:ACI5003	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Belyta sp*			BOLD:ACP7578	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Belyta sp*			BOLD:ADM4620	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Belyta sp*			BOLD:AFA3107	BOLD DIAIS BIN
16	Diapriidae	Belytinae	Belytini	*Belyta subclausa*	(Kieffer, 1907)		BOLD:ACT9617, BOLD:AEY0012	Blank 2001
17	Diapriidae	Belytinae	Belytini	*Belyta validicornis*	Thomson, 1858		BOLD:AEJ3994, BOLD:AEK0177, BOLD:AAU8736, BOLD:AEX0923	Blank 2001
18	Diapriidae	Belytinae	Belytini	*Cinetus cursor*	(Curtis, 1831)		no BIN	Blank 2001
19	Diapriidae	Belytinae	Belytini	*Diphora westwoodi*	Förster, 1856		BOLD:AER0776	Blank 2001
20	Diapriidae	Belytinae	Belytini	*Lyteba bisulca*	(Nees, 1834)		BOLD:ACE0017	Blank 2001
21	Diapriidae	Belytinae	Belytini	*Lyteba carinifrons*	(Kieffer, 1909)		BOLD:AEJ3996	Blank 2001
22	Diapriidae	Belytinae	Belytini	*Lyteba maceki*	Chemyreva, Hübner, Kolyada, Ødegaard*	NOM. NUD.	BOLD:ADZ9395	BOLD DIAIS BIN
23	Diapriidae	Belytinae	Belytini	*Macrohynnis lepidus*	Mayr, 1904		BOLD:AEJ5165	Blank 2001
24	Diapriidae	Belytinae	Belytini	*Miota acuminata*	(Zetterstedt, 1840)		no BIN	Blank 2001
25	Diapriidae	Belytinae	Belytini	*Pamis ione*	Nixon, 1957		no BIN	Blank 2001
26	Diapriidae	Belytinae	Belytini	*Pantoclis barycera*	Förster, 1861		no BIN	Blank 2001
27	Diapriidae	Belytinae	Belytini	*Pantoclis brevicornis*	Kieffer, 1909		no BIN	Blank 2001
28	Diapriidae	Belytinae	Belytini	*Pantoclis caecutiens*	(Kieffer, 1908)	COMB. N.	no BIN	Blank 2001
29	Diapriidae	Belytinae	Belytini	*Pantoclis carinata*	(Thomson, 1858)		no BIN	Blank 2001
30	Diapriidae	Belytinae	Belytini	*Pantoclis eulimine*	Nixon, 1957		no BIN	Blank 2001
31	Diapriidae	Belytinae	Belytini	*Pantoclis evanescens*	Kieffer, 1909		no BIN	Blank 2001
32	Diapriidae	Belytinae	Belytini	*Pantoclis fuscata*	(Thomson, 1858)	COMB. N.	BOLD:AER0772	BOLD DIAIS
33	Diapriidae	Belytinae	Belytini	*Pantoclis fuscicoxa*	Kieffer, 1909		no BIN	Blank 2001
34	Diapriidae	Belytinae	Belytini	*Pantoclis gaudens*	Nixon, 1957		no BIN	Blank 2001
35	Diapriidae	Belytinae	Belytini	*Pantoclis hemiptera*	(Thomson, 1858)	COMB. N.	no BIN	Blank 2001
36	Diapriidae	Belytinae	Belytini	*Pantoclis hirtistilus*	Kieffer, 1909		BOLD:AEK1332, BOLD:AAY6828	Blank 2001
37	Diapriidae	Belytinae	Belytini	*Pantoclis leviventris*	(Kieffer, 1907)		no BIN	Blank 2001
38	Diapriidae	Belytinae	Belytini	*Pantoclis longipennis*	(Thomson, 1858)		no BIN	Blank 2001
39	Diapriidae	Belytinae	Belytini	*Pantoclis mese*	Nixon, 1957	FR	BOLD:ACC4505	BOLD DIAIS
40	Diapriidae	Belytinae	Belytini	*Pantoclis microcera*	Kieffer, 1909		no BIN	Blank 2001
41	Diapriidae	Belytinae	Belytini	*Pantoclis microtoma*	(Kieffer, 1909)	COMB. N.	no BIN	Blank 2001
42	Diapriidae	Belytinae	Belytini	*Pantoclis numen*	Nixon, 1957		no BIN	Blank 2001
43	Diapriidae	Belytinae	Belytini	*Pantoclis obscuripes*	Kieffer, 1907		no BIN	Blank 2001
44	Diapriidae	Belytinae	Belytini	*Pantoclis opaca*	(Thomson, 1858)		no BIN	Blank 2001
45	Diapriidae	Belytinae	Belytini	*Pantoclis ruralis*	Nixon, 1957		no BIN	Blank 2001
46	Diapriidae	Belytinae	Belytini	*Pantoclis soluta*	(Kieffer, 1907)	COMB. N.	BOLD:AEK0729	BOLD DIAIS
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AAG5112	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AAU9135	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AAU9864	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AAU9879	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACB2486	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACC1837	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACC4369	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACC4798	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACC4799	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACC6590	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACD9907	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACG4055	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACI4376	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACI4657	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACI8532	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACP3159	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACP6764	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACR5407	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACS7934	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACT9556	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:ACX9811	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEE9747	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEJ5166	BOLD DIAIS BIN

	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEJ5888	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEJ8197	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEK7524	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AER0762	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AER0771	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AER6238	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AET0483	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEX0184	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEX8394	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEY1054	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEY4811	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AEZ8161	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AFA4610	BOLD DIAIS BIN
	Diapriidae	Belytinae	Belytini	*Pantoclis sp*			BOLD:AFN1601	BOLD DIAIS BIN
47	Diapriidae	Belytinae	Belytini	*Pantoclis striola*	(Thomson, 1858)		no BIN	Blank 2001
48	Diapriidae	Belytinae	Belytini	*Pantoclis subatricornis*	Kieffer, 1916		no BIN	Blank 2001
49	Diapriidae	Belytinae	Belytini	*Pantoclis sulcata*	(Thomson, 1858)		BOLD:ACC4133	Blank 2001
50	Diapriidae	Belytinae	Belytini	*Pantoclis trisulcata*	Kieffer, 1907		BOLD:ACK3275	Blank 2001
51	Diapriidae	Belytinae	Belytini	*Paroxylabis semirufa*	Kieffer, 1907		no BIN	Blank 2001
52	Diapriidae	Belytinae	Belytini	*Paroxylabis spinifer*	Nixon, 1957		no BIN	Blank 2001
53	Diapriidae	Belytinae	Belytini	*Synbelyta fuscipennis*	(Thomson, 1858)		BOLD:ACC0797	Blank 2001
	Diapriidae	Belytinae	Belytini	*Synbelyta sp*			BOLD:AEI1669	BOLD DIAIS BIN
54	Diapriidae	Belytinae	Belytini	*Zygota abdominalis*	(Nees, 1834)		BOLD:AEJ6743	Blank 2001
55	Diapriidae	Belytinae	Belytini	*Zygota angularis*	Macek, 1997	FR	BOLD:ACQ5437	BOLD DIAIS
56	Diapriidae	Belytinae	Belytini	*Zygota balteata*	Macek, 1997	FR	no BIN	BOLD DIAIS
57	Diapriidae	Belytinae	Belytini	*Zygota breviuscula*	(Thomson, 1858)		no BIN	Blank 2001
58	Diapriidae	Belytinae	Belytini	*Zygota claviscapa*	(Thomson, 1858)		no BIN	Blank 2001
59	Diapriidae	Belytinae	Belytini	*Zygota comitans*	Macek, 1997	FR	BOLD:AEJ0891, BOLD:AEL3896	BOLD DIAIS
60	Diapriidae	Belytinae	Belytini	*Zygota congener*	(Zetterstedt, 1840)		BOLD:AAI8609	Blank 2001
61	Diapriidae	Belytinae	Belytini	*Zygota croton*	Nixon, 1957		BOLD:AEK1965	Blank 2001
62	Diapriidae	Belytinae	Belytini	*Zygota excisor*	(Zetterstedt, 1840)		no BIN	Blank 2001
63	Diapriidae	Belytinae	Belytini	*Zygota nigra*	(Thomson, 1858)		BOLD:AEJ4945	Blank 2001
64	Diapriidae	Belytinae	Belytini	*Zygota parallela*	(Thomson, 1858)		BOLD:AEJ0893, BOLD:ACU1498	Blank 2001
65	Diapriidae	Belytinae	Belytini	*Zygota praetor*	Nixon, 1957		no BIN	Blank 2001
66	Diapriidae	Belytinae	Belytini	*Zygota pubescens*	(Kieffer, 1909)		BOLD:ACC4346	Blank 2001
67	Diapriidae	Belytinae	Belytini	*Zygota ruficornis*	(Curtis, 1831)		BOLD:AEK5610, BOLD:AEX2887, BOLD:AEY0233	Blank 2001
68	Diapriidae	Belytinae	Belytini	*Zygota sordida*	Macek, 1997	FR	no BIN	BOLD DIAIS
69	Diapriidae	Belytinae	Belytini	*Zygota spinosa*	(Kieffer, 1908)		BOLD:AER0775, BOLD:AEL5584	Blank 2001
70	Diapriidae	Belytinae	Belytini	*Zygota spinosipes*	(Kieffer, 1908)	FR	BOLD:ACK3325, BOLD:AEY9457	BOLD DIAIS
71	Diapriidae	Belytinae	Belytini	*Zygota vigil*	Nixon, 1957	FR	no BIN	BOLD DIAIS
72	Diapriidae	Belytinae	Belytini	*Zygota villosa*	Macek, 1997		no BIN	Blank 2001
73	Diapriidae	Belytinae	Belytini	*Zygota walli*	Hübner, Chemyreva, Kolyada, Macek 2023	SP. NOV.	BOLD:AER4128, BOLD:ACF9113	BOLD DIAIS
74	Diapriidae	Belytinae	Cinetini	*Aclista acuta*	(Kieffer, 1909)		BOLD:AEK1333, BOLD:ADZ8519	Blank 2001
75	Diapriidae	Belytinae	Cinetini	*Aclista alticollis*	(Thomson, 1858)		BOLD:ACE0564, BOLD:ADE1776	Blank 2001
76	Diapriidae	Belytinae	Cinetini	*Aclista analis*	(Kieffer, 1909)		BOLD:ACG3883	Blank 2001
77	Diapriidae	Belytinae	Cinetini	*Aclista angusta*	(Kieffer, 1909)		BOLD:AEL6574, BOLD:AER0778	Blank 2001
78	Diapriidae	Belytinae	Cinetini	*Aclista boops*	(Thomson, 1858)		no BIN	Blank 2001
79	Diapriidae	Belytinae	Cinetini	*Aclista brachycera*	(Kieffer, 1910)		no BIN	Blank 2001
80	Diapriidae	Belytinae	Cinetini	*Aclista cantiana*	(Curtis, 1831		no BIN	Blank 2001
81	Diapriidae	Belytinae	Cinetini	*Aclista clito*	Nixon, 1957		no BIN	Blank 2001
82	Diapriidae	Belytinae	Cinetini	*Aclista dubia*	(Kieffer, 1909)		BOLD:AEH7830	Blank 2001
83	Diapriidae	Belytinae	Cinetini	*Aclista elevata*	(Thomson, 1858)		BOLD:AAG8164	Blank 2001
84	Diapriidae	Belytinae	Cinetini	*Aclista filiformis*	(Kieffer, 1907)		BOLD:AEJ9239	Blank 2001
85	Diapriidae	Belytinae	Cinetini	*Aclista folia*	Nixon, 1957		BOLD:AEZ5826, BOLD:AEJ5162	Blank 2001
86	Diapriidae	Belytinae	Cinetini	*Aclista fractinervis*	(Kieffer, 1910)		no BIN	Blank 2001
87	Diapriidae	Belytinae	Cinetini	*Aclista fuscicornis*	(Kieffer, 1909)		no BIN	Blank 2001
88	Diapriidae	Belytinae	Cinetini	*Aclista haemorrhoidalis*	(Kieffer, 1910)		no BIN	Blank 2001
89	Diapriidae	Belytinae	Cinetini	*Aclista insolita*	Nixon, 1957		no BIN	Blank 2001
90	Diapriidae	Belytinae	Cinetini	*Aclista janssoni*	Nixon, 1957		BOLD:ACG5663	Blank 2001
91	Diapriidae	Belytinae	Cinetini	*Aclista marshalli*	(Kieffer, 1910)		no BIN	Blank 2001
92	Diapriidae	Belytinae	Cinetini	*Aclista mycale*	Nixon, 1957		BOLD:ACR5432	Blank 2001
93	Diapriidae	Belytinae	Cinetini	*Aclista neglecta*	(Kieffer, 1907)		no BIN	Blank 2001
94	Diapriidae	Belytinae	Cinetini	*Aclista parvula*	(Kieffer, 1910)		no BIN	Blank 2001
95	Diapriidae	Belytinae	Cinetini	*Aclista praeclara*	Nixon, 1957		no BIN	Blank 2001
96	Diapriidae	Belytinae	Cinetini	*Aclista prolongata*	(Kieffer, 1907)		BOLD:AEJ7778	Blank 2001
97	Diapriidae	Belytinae	Cinetini	*Aclista rufopetiolata*	(Nees, 1834)		BOLD:ACC1986	Blank 2001
98	Diapriidae	Belytinae	Cinetini	*Aclista soror*	(Kieffer, 1909)		no BIN	Blank 2001
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACC2806	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACC4158	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACI4375	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACI4524	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACK4946	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACL6636	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ACR2222	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ADF8227	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ADM5959	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ADU2593	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ADU5289	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*			BOLD:ADV2939	BOLD DIAIS BIN

	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEG4377	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEH6916	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEJ3995	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEJ5746	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEJ6742	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEJ7597	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEJ9241	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEK1331	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEK5608	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEK5609	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEL0172	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEL3224	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEL5201	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEN6427	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AER8715	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AES7433	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AES8380	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEU4740	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEW9726	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEZ1954	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEZ1957	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEZ1961	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEZ6730	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Aclista sp*		BOLD:AEZ7807	BOLD DIAIS BIN
99	Diapriidae	Belytinae	Cinetini	*Aclista stigma*	Kieffer, 1909	no BIN	Blank 2001
100	Diapriidae	Belytinae	Cinetini	*Aclista striolata*	(Thomson, 1858)	BOLD:ACE0564	Blank 2001
101	Diapriidae	Belytinae	Cinetini	*Aclista subaequalis*	(Kieffer, 1910)	no BIN	Blank 2001
102	Diapriidae	Belytinae	Cinetini	*Cinetus abdominalis*	Wall, 1998	no BIN	Blank 2001
103	Diapriidae	Belytinae	Cinetini	*Cinetus angustatus*	Kieffer, 1910	no BIN	Blank 2001
104	Diapriidae	Belytinae	Cinetini	*Cinetus brevipeliolalus*	Thomson, 1858	no BIN	Blank 2001
105	Diapriidae	Belytinae	Cinetini	*Cinetus cameroni*	Kieffer, 1916	BOLD:ACH2466	BOLD DIAIS
106	Diapriidae	Belytinae	Cinetini	*Cinetus carpentieri*	Kieffer, 1910	no BIN	Blank 2001
107	Diapriidae	Belytinae	Cinetini	*Cinetus decipiens*	Kieffer, 1910	no BIN	Blank 2001
108	Diapriidae	Belytinae	Cinetini	*Cinetus ditomus*	(Kieffer, 1910)	no BIN	Blank 2001
109	Diapriidae	Belytinae	Cinetini	*Cinetus elatior*	Nixon, 1957	BOLD:ADS2676	Blank 2001
110	Diapriidae	Belytinae	Cinetini	*Cinetus ennius*	Nixon, 1957	no BIN	Blank 2001
111	Diapriidae	Belytinae	Cinetini	*Cinetus eximius*	Wall, 1998	no BIN	Blank 2001
112	Diapriidae	Belytinae	Cinetini	*Cinetus fuscipes*	(Kieffer, 1907)	no BIN	Blank 2001
113	Diapriidae	Belytinae	Cinetini	*Cinetus iridipennis*	Lepeletier & Serville 1825	BOLD:ACH3984, BOLD:ACH3287, BOLD:ACH3983	Blank 2001
114	Diapriidae	Belytinae	Cinetini	*Cinetus lusitanicus*	(Kieffer, 1907)	no BIN	Blank 2001
115	Diapriidae	Belytinae	Cinetini	*Cinetus piceus*	Thomson, 1858	BOLD:ACU1661	Blank 2001
116	Diapriidae	Belytinae	Cinetini	*Cinetus princeps*	Nixon, 1957	no BIN	Blank 2001
117	Diapriidae	Belytinae	Cinetini	*Cinetus problematicus*	Wall, 1998	no BIN	Blank 2001
118	Diapriidae	Belytinae	Cinetini	*Cinetus procleus*	Nixon, 1957	no BIN	Blank 2001
119	Diapriidae	Belytinae	Cinetini	*Cinetus procris*	Nixon, 1957	no BIN	Blank 2001
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:ACC1834	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:ACC4347	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEE6403	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEI7598	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEJ0438	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEJ4944	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEJ4946	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEJ6090	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEK0175	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEK1963	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AER0761	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AER0773	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AER0774	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AER0777	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AET0482	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEX1134	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEY8321	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AEZ5802	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Cinetus sp*		BOLD:AFL8709	BOLD DIAIS BIN
120	Diapriidae	Belytinae	Cinetini	*Miota antennata*	Wall, 1998	no BIN	Blank 2001
121	Diapriidae	Belytinae	Cinetini	*Miota atriceps*	(Kieffer, 1910)	BOLD:AFM2025	BOLD DIAIS
122	Diapriidae	Belytinae	Cinetini	*Miota badensis*	Wall, 1998	no BIN	Blank 2001
123	Diapriidae	Belytinae	Cinetini	*Miota brevicornis*	(Kieffer, 1910)	no BIN	Blank 2001
124	Diapriidae	Belytinae	Cinetini	*Miota breviscapa*	Wall, 1998	no BIN	Blank 2001
125	Diapriidae	Belytinae	Cinetini	*Miota confusa*	Wall, 1998	no BIN	Blank 2001
126	Diapriidae	Belytinae	Cinetini	*Miota curta*	Wall, 1998	no BIN	Blank 2001
127	Diapriidae	Belytinae	Cinetini	*Miota distinguenda*	Wall, 1998	no BIN	Blank 2001
128	Diapriidae	Belytinae	Cinetini	*Miota exsecta*	Wall, 1998	no BIN	Blank 2001
129	Diapriidae	Belytinae	Cinetini	*Miota flavidicornis*	(Kieffer, 1910)	no BIN	Blank 2001
130	Diapriidae	Belytinae	Cinetini	*Miota gigantea*	Wall, 1998	no BIN	Blank 2001
131	Diapriidae	Belytinae	Cinetini	*Miota incisa*	(Kieffer, 1910)	no BIN	Blank 2001
132	Diapriidae	Belytinae	Cinetini	*Miota kiefferi*	Buhl, 1997	no BIN	Blank 2001
133	Diapriidae	Belytinae	Cinetini	*Miota macrocera*	(Kieffer, 1910)	no BIN	Blank 2001
134	Diapriidae	Belytinae	Cinetini	*Miota microgaster*	(Kieffer, 1910)	no BIN	Blank 2001
135	Diapriidae	Belytinae	Cinetini	*Miota monilicornis*	(Kieffer, 1910)	no BIN	Blank 2001
136	Diapriidae	Belytinae	Cinetini	*Miota perplexa*	(Kieffer, 1910)	no BIN	Blank 2001
137	Diapriidae	Belytinae	Cinetini	*Miota polita*	(Thomson, 1858)	no BIN	Blank 2001

138	Diapriidae	Belytinae	Cinetini	*Miota prolongata*	(Kieffer, 1910)		no BIN	Blank 2001
139	Diapriidae	Belytinae	Cinetini	*Miota recta*	Wall, 1998		no BIN	Blank 2001
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AAJ8191	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AAU9534	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AAU9762	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:ACJ8038	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AEG0602	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AEI9974	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AEJ5164	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AEK0728	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AEK2636	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AER0766	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AER1449	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AFB4654	BOLD DIAIS BIN
	Diapriidae	Belytinae	Cinetini	*Miota sp*			BOLD:AFK2111	BOLD DIAIS BIN
140	Diapriidae	Belytinae	Cinetini	*Miota thomsoni*	Wall, 1998		no BIN	Blank 2001
141	Diapriidae	Belytinae	Cinetini	*Miota transiens*	(Nixon, 1957)		no BIN	Blank 2001
142	Diapriidae	Belytinae	Cinetini	*Miota wuerttembergensis*	Wall, 1998		no BIN	Blank 2001
143	Diapriidae	Belytinae	Cinetini	*Pantoclis armatus*	(Haliday, 1831)		no BIN	Blank 2001
144	Diapriidae	Belytinae	Cinetini	*Scorpioteleia cebes*	(Nixon, 1957)	FR	BOLD:AEJ0439	BOLD DIAIS
145	Diapriidae	Belytinae	Cinetini	*Scorpioteleia longepetiolata*	(Thomson, 1858)		BOLD:AEJ1273	Blank 2001
146	Diapriidae	Belytinae	Pantolytini	*Acanopsilus heterocerus*	(Haliday, 1857)		BOLD:AEC2586	Blank 2001
147	Diapriidae	Belytinae	Pantolytini	*Acanosema nervosum*	(Thomson, 1858)		no BIN	Blank 2001
148	Diapriidae	Belytinae	Pantolytini	*Acanosema productum*	(Kieffer, 1908)		BOLD:AFA3833	Blank 2001
149	Diapriidae	Belytinae	Pantolytini	*Acanosema rufum*	Kieffer, 1908		BOLD:AEJ4541	Blank 2001
150	Diapriidae	Belytinae	Pantolytini	*Acanosema sana**	Chemyreva & Hübner	NOM. NUD.	BOLD:ADU8555	BOLD DIAIS
	Diapriidae	Belytinae	Pantolytini	*Acanosema sp*			BOLD:ACR2617	BOLD DIAIS BIN
	Diapriidae	Belytinae	Pantolytini	*Acanosema sp*			BOLD:AEJ5745	BOLD DIAIS BIN
	Diapriidae	Belytinae	Pantolytini	*Acanosema sp*			BOLD:AEJ7596	BOLD DIAIS BIN
	Diapriidae	Belytinae	Pantolytini	*Acanosema sp*			BOLD:AER0763	BOLD DIAIS BIN
	Diapriidae	Belytinae	Pantolytini	*Acanosema sp*			BOLD:AER0770	BOLD DIAIS BIN
151	Diapriidae	Belytinae	Pantolytini	*Acanosema tenuicornis*	(Kieffer, 1908)		BOLD:AFB4850, BOLD:AEL6576	Blank 2001
152	Diapriidae	Belytinae	Pantolytini	*Anommatium ashmeadi*	Mayr, 1904		BOLD:ACI4064	Blank 2001
153	Diapriidae	Belytinae	Pantolytini	*Aprestes variicornis*	(Kieffer, 1909)		no BIN	Blank 2001
154	Diapriidae	Belytinae	Pantolytini	*Opazon apertum*	(Kieffer, 1908)		BOLD:AEL8415	Blank 2001
155	Diapriidae	Belytinae	Pantolytini	*Opazon frigidum*	Macek, 1995	FR	no BIN	BOLD DIAIS
156	Diapriidae	Belytinae	Pantolytini	*Opazon parvulum*	(Haliday, 1857)		BOLD:ACM1547	Blank 2001
157	Diapriidae	Belytinae	Pantolytini	*Pantolyta atrata*	Förster, 1861		no BIN	Blank 2001
158	Diapriidae	Belytinae	Pantolytini	*Pantolyta flaviventris*	(Thomson, 1858)	FR	BOLD:AEH3144, BOLD:AFB0678	BOLD DIAIS
159	Diapriidae	Belytinae	Pantolytini	*Pantolyta hadrosoma*	1858) 1993		BOLD:AEL5583	Blank 2001
160	Diapriidae	Belytinae	Pantolytini	*Pantolyta macrocera*	(Thomson, 1858)		BOLD:AFA3984	BOLD DIAIS
161	Diapriidae	Belytinae	Pantolytini	*Pantolyta marginalis*	(Kieffer, 1909)		BOLD:AEK0176	Blank 2001
162	Diapriidae	Belytinae	Pantolytini	*Pantolyta micans*	(Macek, 1998)		no BIN	Blank 2001
163	Diapriidae	Belytinae	Pantolytini	*Pantolyta nigrocincta*	(Kieffer, 1909)		no BIN	Blank 2001
164	Diapriidae	Belytinae	Pantolytini	*Pantolyta nitida*	(Thomson, 1858)		no BIN	Blank 2001
165	Diapriidae	Belytinae	Pantolytini	*Pantolyta nixoni*	Macek, 1993		BOLD:AEJ4543	Blank 2001
166	Diapriidae	Belytinae	Pantolytini	*Pantolyta pallida*	Kieffer, 1908		no BIN	Blank 2001
167	Diapriidae	Belytinae	Pantolytini	*Pantolyta pseudosciarivora*	(Macek, 1998)		BOLD:AEE7712, BOLD:AEL6575, BOLD:AFA7499	BOLD DIAIS
168	Diapriidae	Belytinae	Pantolytini	*Pantolyta radialis*	(Hellen, 1964)		no BIN	Blank 2001
169	Diapriidae	Belytinae	Pantolytini	*Pantolyta rufiventris*	(Kieffer, 1909)	FR	BOLD:ACC0804, BOLD:AFA3986	BOLD DIAIS
170	Diapriidae	Belytinae	Pantolytini	*Pantolyta sciarivora*	(Kieffer, 1907)	FR	BOLD:ACR3837	BOLD DIAIS
171	Diapriidae	Belytinae	Pantolytini	*Pantolyta semirufa*	Kieffer, 1908		no BIN	Blank 2001
172	Diapriidae	Belytinae	Pantolytini	*Pantolyta seticornis*	(Kieffer, 1910)	FR	BOLD:AFA3985	BOLD DIAIS
	Diapriidae	Belytinae	Pantolytini	*Pantolyta sp*			BOLD:ADF5830	BOLD DIAIS BIN
	Diapriidae	Belytinae	Pantolytini	*Pantolyta sp*			BOLD:AEK7555	BOLD DIAIS BIN
173	Diapriidae	Belytinae	Pantolytini	*Pantolyta stylata*	Kieffer, 1908		no BIN	Blank 2001
174	Diapriidae	Belytinae	Pantolytini	*Polypeza ciliata*	(Thomson, 1858)		no BIN	Blank 2001
175	Diapriidae	Belytinae	Pantolytini	*Psilomma dubium*	Kieffer, 1908		BOLD:AEK1962	Blank 2001
176	Diapriidae	Belytinae	Pantolytini	*Psilomma fuscicornis*	Kieffer, 1908		BOLD:AEZ2007	Blank 2001
177	Diapriidae	Belytinae	Pantolytini	*Psilomma fusciscapis*	Förster, 1861		no BIN	Blank 2001
178	Diapriidae	Belytinae	Pantolytini	*Psilommacra olygomera*	Macek, 1990		no BIN	Blank 2001
179	Diapriidae	Belytinae	Pantolytini	*Synacra atracta*	Macek, 1995		no BIN	Blank 2001
180	Diapriidae	Belytinae	Pantolytini	*Synacra azepylopria*	Chemyreva & Kolyada 2019	FR	BOLD:ADW2839	BOLD DIAIS
181	Diapriidae	Belytinae	Pantolytini	*Synacra brachialis*	(Nees, 1834)		BOLD:AEB7128	Blank 2001
182	Diapriidae	Belytinae	Pantolytini	*Synacra giraudi*	(Kieffer, 1910		BOLD:AEP5857	Blank 2001
183	Diapriidae	Belytinae	Pantolytini	*Synacra holconota*	Kieffer, 1910		BOLD:AEH2733	Blank 2001
184	Diapriidae	Belytinae	Pantolytini	*Synacra paupera*	Macek, 1995	FR	BOLD:ABA1079	BOLD DIAIS
185	Diapriidae	Belytinae	Pantolytini	*Synacra sociabilis*	(Kieffer, 1904)		BOLD:ADU9017	Blank 2001
186	Diapriidae	Diapriinae	Diapriini	*Basalys abruptus*	Thomson, 1859		BOLD:ADI3723	Blank 2001
187	Diapriidae	Diapriinae	Diapriini	*Basalys amphoralis*	(Tomsik, 1949)		no BIN	Blank 2001
188	Diapriidae	Diapriinae	Diapriini	*Basalys angelikae*	(Hilpert, 1989)		no BIN	Blank 2001
189	Diapriidae	Diapriinae	Diapriini	*Basalys badeniensis*	(Hilpert 1989)		no BIN	Blank 2001
190	Diapriidae	Diapriinae	Diapriini	*Basalys bechtalensis*	(Hilpert, 1989)		no BIN	Blank 2001
191	Diapriidae	Diapriinae	Diapriini	*Basalys breisgauensis*	(Hilpert, 1989)		no BIN	Blank 2001
192	Diapriidae	Diapriinae	Diapriini	*Basalys brunnipes*	(Nees, 1834)		no BIN	Fauna Europaea
193	Diapriidae	Diapriinae	Diapriini	*Basalys ciliatus*	(Kieffer, 1911)		no BIN	Blank 2001
194	Diapriidae	Diapriinae	Diapriini	*Basalys claudiae*	(Hilpert, 1989)		BOLD:AEC4025	Blank 2001
195	Diapriidae	Diapriinae	Diapriini	*Basalys collaris*	Kieffer, 1911		BOLD:ABW3139	Blank 2001
196	Diapriidae	Diapriinae	Diapriini	*Basalys crassiceps*	(Kieffer, 1911)		no BIN	Blank 2001
197	Diapriidae	Diapriinae	Diapriini	*Basalys cuneiformis*	(Tomsik, 1949)		no BIN	Fauna Europaea

198	Diapriidae	Diapriinae	Diapriini	*Basalys cymocles*	Nixon, 1980		no BIN	Blank 2001
199	Diapriidae	Diapriinae	Diapriini	*Basalys depressus*	(Hilpert, 1989)		no BIN	Blank 2001
200	Diapriidae	Diapriinae	Diapriini	*Basalys dispar*	(Nees, 1834)		no BIN	Blank 2001
201	Diapriidae	Diapriinae	Diapriini	*Basalys erythropus*	Kieffer, 1911		no BIN	Blank 2001
202	Diapriidae	Diapriinae	Diapriini	*Basalys formicarum*	(Kieffer, 1911)		no BIN	Fauna Europaea
203	Diapriidae	Diapriinae	Diapriini	*Basalys fumipennis*	Westwood, 1833		no BIN	Blank 2001
204	Diapriidae	Diapriinae	Diapriini	*Basalys helicicola*	(Kieffer, 1911)		no BIN	Blank 2001
205	Diapriidae	Diapriinae	Diapriini	*Basalys hilleri*	(Hilpert, 1989)		no BIN	Blank 2001
206	Diapriidae	Diapriinae	Diapriini	*Basalys insignificans*	Nixon, 1980		BOLD:AEK2203	Blank 2001
207	Diapriidae	Diapriinae	Diapriini	*Basalys koenigi*	(Hilpert, 1989)		no BIN	Blank 2001
208	Diapriidae	Diapriinae	Diapriini	*Basalys longipennis*	(Kieffer, 1911)		BOLD:AFA6659, BOLD:ADV9157	Blank 2001
209	Diapriidae	Diapriinae	Diapriini	*Basalys luctuosus*	Kieffer, 1911		no BIN	Fauna Europaea
210	Diapriidae	Diapriinae	Diapriini	*Basalys luteipes*	Kieffer, 1911		no BIN	Fauna Europaea
211	Diapriidae	Diapriinae	Diapriini	*Basalys macroptera*	(Kieffer, 1911)		BOLD:AEJ8647	BOLD DIAIS
212	Diapriidae	Diapriinae	Diapriini	*Basalys minutissimus*	(Hilpert, 1989)		no BIN	Blank 2001
213	Diapriidae	Diapriinae	Diapriini	*Basalys neglectus*	(Herrich-Schaeffer, 1838)		no BIN	Fauna Europaea
214	Diapriidae	Diapriinae	Diapriini	*Basalys oberbergensis*	(Hilpert, 1989)		no BIN	Blank 2001
215	Diapriidae	Diapriinae	Diapriini	*Basalys orion*	Nixon, 1980		BOLD:AAU9056	Blank 2001
216	Diapriidae	Diapriinae	Diapriini	*Basalys parvus*	Thomson, 1859		no BIN	Blank 2001
217	Diapriidae	Diapriinae	Diapriini	*Basalys pedisequa*	(Kieffer, 1911)		BOLD:AAU9130, BOLD:AFA5382	Blank 2001
218	Diapriidae	Diapriinae	Diapriini	*Basalys picipes*	(Herrich-Schaeffer, 1838)		no BIN	Fauna Europaea
219	Diapriidae	Diapriinae	Diapriini	*Basalys rheni*	(Hilpert, 1989)		no BIN	Blank 2001
220	Diapriidae	Diapriinae	Diapriini	*Basalys rufiscapus*	(Nees, 1834)		no BIN	Fauna Europaea
221	Diapriidae	Diapriinae	Diapriini	*Basalys rufocinctus*	(Kieffer, 1911)	FR, COMB. NOV	BOLD:AEW6196	BOLD DIAIS
222	Diapriidae	Diapriinae	Diapriini	*Basalys sagittarii*	(Hilpert, 1989)		no BIN	Blank 2001
223	Diapriidae	Diapriinae	Diapriini	*Basalys scoticus*	(Kieffer, 1911)		no BIN	Fauna Europaea
224	Diapriidae	Diapriinae	Diapriini	*Basalys silvaticus*	(Hilpert, 1989)		no BIN	Blank 2001
225	Diapriidae	Diapriinae	Diapriini	*Basalys singularis*	Nixon, 1980		BOLD:ABW3139	Blank 2001
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AAN7572	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ACJ0974	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ACU2017	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADI1836	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADS7744	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADU0708	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADU6366	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADV0554	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADW1303	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADW4615	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:ADX0568	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEE3053	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEG0486	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEG1855	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEG8505	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEG9203	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEJ0013	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEJ6027	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEK2595	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEL3212	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEL5299	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEP5853	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AER1422	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AER1498	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AES7028	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AET3760	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEZ4136	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Basalys sp*			BOLD:AEZ4326	BOLD DIAIS BIN
226	Diapriidae	Diapriinae	Diapriini	*Basalys tenuis*	(Herrich-Schaeffer, 1838)		no BIN	Fauna Europaea
227	Diapriidae	Diapriinae	Diapriini	*Basalys tripartitus*	(Marshall, 1868)		no BIN	Blank 2001
228	Diapriidae	Diapriinae	Diapriini	*Basalys tritomus*	Thomson, 1859		BOLD:ACB1614	Blank 2001
229	Diapriidae	Diapriinae	Diapriini	*Basalys tuberculatus*	Kieffer, 1911		BOLD:ABW3139	Blank 2001
230	Diapriidae	Diapriinae	Diapriini	*Basalys weisweilensis*	(Hilpert, 1989)		no BIN	Blank 2001
231	Diapriidae	Diapriinae	Diapriini	*Diapria cava*	Nixon, 1993	FR	BOLD:AEJ8830	BOLD DIAIS
232	Diapriidae	Diapriinae	Diapriini	*Diapria clavata*	Herrich-Schaeffer 1838		no BIN	Fauna Europaea
233	Diapriidae	Diapriinae	Diapriini	*Diapria conica*	(Fabricius, 1775)		BOLD:ACH2737	Blank 2001
234	Diapriidae	Diapriinae	Diapriini	*Diapria filicornis*	Herrich-Schaeffer 1838		no BIN	Fauna Europaea
235	Diapriidae	Diapriinae	Diapriini	*Diapria luteipes*	Nixon, 1993	FR	BOLD:AEZ0207	BOLD DIAIS
236	Diapriidae	Diapriinae	Diapriini	*Diapria melanocorypha*	Ratzeburg 1848		no BIN	Fauna Europaea
237	Diapriidae	Diapriinae	Diapriini	*Diapria nigricornis*	Thomson, 1859		BOLD:AAU8803	Blank 2001
238	Diapriidae	Diapriinae	Diapriini	*Diapria solitaria*	(Hartig, 1834)		no BIN	Fauna Europaea
239	Diapriidae	Diapriinae	Diapriini	*Lepidopria pedestris*	Kieffer 1916	FR	BOLD:ACP4428	BOLD DIAIS
240	Diapriidae	Diapriinae	Diapriini	*Monelata aphrodite*	(Nixon) 1980	FR	BOLD:AEK1602	BOLD DIAIS
241	Diapriidae	Diapriinae	Diapriini	*Monelata cincta*	(Haliday, 1857)		BOLD:AEB7246	Blank 2001
242	Diapriidae	Diapriinae	Diapriini	*Monelata clavigera*	Priesner, 1953	FR	BOLD:AEJ4869	BOLD DIAIS
243	Diapriidae	Diapriinae	Diapriini	*Monelata parvula*	(Nees, 1834)		BOLD:AEJ0896	BOLD DIAIS
244	Diapriidae	Diapriinae	Diapriini	*Monelata petiolaris*	(Nees, 1834)		no BIN	Fauna Europaea
245	Diapriidae	Diapriinae	Diapriini	*Monelata solida*	(Thomson, 1859)		BOLD:AAG7877	Blank 2001
	Diapriidae	Diapriinae	Diapriini	*Monelata sp*			BOLD:AEJ6238	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Monelata sp*			BOLD:AEZ8636	BOLD DIAIS BIN

246	Diapriidae	Diapriinae	Diapriini	*Solenopsia imitatrix*	Wasmann, 1899		no BIN	Blank 2001
247	Diapriidae	Diapriinae	Diapriini	*Tetramopria aurocincta*	Wasmann, 1899		BOLD:AED2241	Blank 2001
248	Diapriidae	Diapriinae	Diapriini	*Tetramopria cincticollis*	Wasmann, 1899	FR	BOLD:AEK1339	BOLD DIAIS
249	Diapriidae	Diapriinae	Diapriini	*Trichopria aequata*	(Thomson, 1859)		BOLD:AEK3065, BOLD:AEK7669, BOLD:AEL0018, BOLD:AEJ8979, BOLD:AEL5289, BOLD:AEP5859, BOLD:ACG4391, BOLD:ACP4337, BOLD:ACR4507, BOLD:AEJ3049	Blank 2001
250	Diapriidae	Diapriinae	Diapriini	*Trichopria atrata*	Notton, 1994		BOLD:AEJ9123, BOLD:AEJ6239	Blank 2001
251	Diapriidae	Diapriinae	Diapriini	*Trichopria basalis*	(Thomson, 1859)		BOLD:AAN8179	Blank 2001
252	Diapriidae	Diapriinae	Diapriini	*Trichopria biarticulata*	Hilpert, 1989		BOLD:AEC1618	Blank 2001
253	Diapriidae	Diapriinae	Diapriini	*Trichopria bifoveata*	Kieffer, 1912		no BIN	Blank 2001
254	Diapriidae	Diapriinae	Diapriini	*Trichopria bipunctata*	Kieffer, 1911		BOLD:ACJ0448, BOLD:AEJ7405	Blank 2001
255	Diapriidae	Diapriinae	Diapriini	*Trichopria breisgauensis*	Hilpert, 1989		no BIN	Blank 2001
256	Diapriidae	Diapriinae	Diapriini	*Trichopria cameroni*	(Kieffer, 1909)		BOLD:ADF4751	Blank 2001
257	Diapriidae	Diapriinae	Diapriini	*Trichopria cilipes*	(Kieffer, 1904)		no BIN	Fauna Europaea
258	Diapriidae	Diapriinae	Diapriini	*Trichopria compressa*	(Thomson, 1859)		BOLD:AEP5858	Blank 2001
259	Diapriidae	Diapriinae	Diapriini	*Trichopria conotoma*	(Kieffer, 1911)		BOLD:AEK1603	BOLD DIAIS
260	Diapriidae	Diapriinae	Diapriini	*Trichopria crassifemur*	Nixon, 1980		no BIN	Blank 2001
261	Diapriidae	Diapriinae	Diapriini	*Trichopria credne*	Nixon, 1980		BOLD:AEX5225	Blank 2001
262	Diapriidae	Diapriinae	Diapriini	*Trichopria drosophilae*	(Perkins, 1910)	FR	BOLD:ABX6634	BOLD DIAIS
263	Diapriidae	Diapriinae	Diapriini	*Trichopria evanescens*	Kieffer, 1911		no BIN	Blank 2001
264	Diapriidae	Diapriinae	Diapriini	*Trichopria fucicola*	Walker 1834		BOLD:AAG7876, BOLD:ACK0109, BOLD:ADF4872, BOLD:ADH3269	Fauna Europaea
265	Diapriidae	Diapriinae	Diapriini	*Trichopria halterata*	(Kieffer, 1909)		BOLD:AEP5850, BOLD:ACJ7867	Fauna Europaea
266	Diapriidae	Diapriinae	Diapriini	*Trichopria hyalinipennis*	(Thomson, 1859)		no BIN	Blank 2001
267	Diapriidae	Diapriinae	Diapriini	*Trichopria incrassata*	(Jansson, 1955)		no BIN	Fauna Europaea
268	Diapriidae	Diapriinae	Diapriini	*Trichopria modesta*	(Ratzeburg, 1848)		BOLD:AAG8165	Blank 2001
269	Diapriidae	Diapriinae	Diapriini	*Trichopria moria*	(Thomson, 1858)		no BIN	Fauna Europaea
270	Diapriidae	Diapriinae	Diapriini	*Trichopria nigra*	(Nees, 1834)		BOLD:ABA5937, BOLD:ADU7317, BOLD:AEE0622	Blank 2001
271	Diapriidae	Diapriinae	Diapriini	*Trichopria nigricornis*	(Marshall, 1868)		no BIN	Blank 2001
272	Diapriidae	Diapriinae	Diapriini	*Trichopria nixoni*	Notton, 1995		BOLD:AAM7487, BOLD:ADS7869, BOLD:ADY7851, BOLD:ADF5094	Blank 2001
273	Diapriidae	Diapriinae	Diapriini	*Trichopria oogaster*	(Thomson, 1859)		BOLD:AEK1997, BOLD:ADF4869	Blank 2001
274	Diapriidae	Diapriinae	Diapriini	*Trichopria picipes*	(Nees, 1834)		BOLD:ABA5937, BOLD:ADU4810	Blank 2001
275	Diapriidae	Diapriinae	Diapriini	*Trichopria polita*	Notton, 1993		BOLD:AEJ9589	BOLD DIAIS
276	Diapriidae	Diapriinae	Diapriini	*Trichopria prema*	Nixon, 1980		BOLD:AEP5860, BOLD:ADF4871	Blank 2001
277	Diapriidae	Diapriinae	Diapriini	*Trichopria quadrifida*	Notton, 1994		BOLD:AEK2030	BOLD DIAIS
278	Diapriidae	Diapriinae	Diapriini	*Trichopria sociabilis*	Masner, 1965		BOLD:AEJ7405, BOLD:ACJ5673, BOLD:AEK0576, BOLD:ADF5094	Blank 2001
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ABX8318	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ACC6687	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ACM7725	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ACP2906	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ADC1554	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ADF4415	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ADF4868	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ADX3813	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:ADY9865	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEJ5091	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEK0960	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEK2204	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEP5861	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AER1495	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEW8662	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEY8109	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEZ1469	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEZ2624	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEZ3981	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Diapriini	*Trichopria sp*			BOLD:AEZ5366	BOLD DIAIS BIN
279	Diapriidae	Diapriinae	Diapriini	*Trichopria subimpressa*	(Kieffer, 1911)		BOLD:AAP6718, BOLD:ACR1931, BOLD:AEP5848	Fauna Europaea
280	Diapriidae	Diapriinae	Diapriini	*Trichopria suspecta*	(Nees, 1834)		BOLD:ABY3110, BOLD:AEJ1088, BOLD:ACH3222, BOLD:AEJ2214	Blank 2001
281	Diapriidae	Diapriinae	Diapriini	*Trichopria tenuicornis*	(Thomson, 1859)		BOLD:ACH3222, BOLD:AER1500, BOLD:AAP6714, BOLD:ABA5906, BOLD:AEE1299, BOLD:AEJ3368	Blank 2001
282	Diapriidae	Diapriinae	Diapriini	*Trichopria tritoma*	(Thomson, 1859)		BOLD:ADF4414, BOLD:AEJ6793	BOLD DIAIS
283	Diapriidae	Diapriinae	Diapriini	*Trichopria verticiliata*	(Latreille, 1805)		BOLD:AEJ5798, BOLD:ACH3554, BOLD:AEJ7337	Blank 2001
284	Diapriidae	Diapriinae	Diapriini	*Trichopria wasmanni*	(Kieffer, 1911)		no BIN	Blank 2001
285	Diapriidae	Diapriinae	Diapriini	*Viennopria lacustris*	(Schulz, 1911)	FR	BOLD:AEY0567	BOLD DIAIS
286	Diapriidae	Diapriinae	Psilini	*Aneurhynchus ariadne*	Nixon, 1980		BOLD:AEC9571	Blank 2001
287	Diapriidae	Diapriinae	Psilini	*Aneurhynchus crinicornis*	Wall 1971		no BIN	Fauna Europaea
288	Diapriidae	Diapriinae	Psilini	*Aneurhynchus depressus*	Wall 1971		no BIN	Fauna Europaea
289	Diapriidae	Diapriinae	Psilini	*Aneurhynchus flavicornis*	Wall 1971		no BIN	Fauna Europaea
290	Diapriidae	Diapriinae	Psilini	*Aneurhynchus galesiformis*	Westwood, 1832		BOLD:AEC4348, BOLD:AEE6654	Blank 2001
291	Diapriidae	Diapriinae	Psilini	*Aneurhynchus gracilicornis*	Wall 1971		BOLD:ACW1208	Fauna Europaea
292	Diapriidae	Diapriinae	Psilini	*Aneurhynchus gracilis*	Wall 1971		no BIN	Fauna Europaea
293	Diapriidae	Diapriinae	Psilini	*Aneurhynchus longicornis*	Thomson, 1859		BOLD:ACU2378	Blank 2001
294	Diapriidae	Diapriinae	Psilini	*Aneurhynchus macrotomus*	Vollenhoven 1874		no BIN	Fauna Europaea
295	Diapriidae	Diapriinae	Psilini	*Aneurhynchus mese*	Nixon, 1980		no BIN	Blank 2001
296	Diapriidae	Diapriinae	Psilini	*Aneurhynchus nodicornis*	Marshall, 1867		BOLD:AEJ7595	BOLD DIAIS

297	Diapriidae	Diapriinae	Psilini	*Aneurhynchus obliquus*	Kieffer, 1911		no BIN	Blank 2001
298	Diapriidae	Diapriinae	Psilini	*Aneurhynchus oviventris*	Thomson, 1859		BOLD:ACP6875	Blank 2001
299	Diapriidae	Diapriinae	Psilini	*Aneurhynchus pentatomus*	Thomson, 1859		no BIN	Blank 2001
300	Diapriidae	Diapriinae	Psilini	*Aneurhynchus ruficornis*	Thomson, 1859		BOLD:ACP6875, BOLD:ACT8995, BOLD:ADF4732, BOLD:ADF4748	Blank 2001
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:ACI4528	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:ACZ1559	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:ADS9987	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEE3015	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEI1053	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEJ0435	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEK1334	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEK1966	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEP5851	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AER6750	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Aneurhynchus sp*			BOLD:AEZ0846	BOLD DIAIS BIN
301	Diapriidae	Diapriinae	Psilini	*Aneurhynchus tritomus*	Wall 1971		no BIN	Fauna Europaea
302	Diapriidae	Diapriinae	Psilini	*Aneurhynchus trivialis*	Kieffer 1911		no BIN	Fauna Europaea
303	Diapriidae	Diapriinae	Psilini	*Aneurhynchus uniformis*	Wall 1971		no BIN	Fauna Europaea
304	Diapriidae	Diapriinae	Psilini	*Aneuropria foersteri*	(Kieffer, 1910)		no BIN	Blank 2001
305	Diapriidae	Diapriinae	Psilini	*Coptera inaequalifrons*	(Jansson, 1942)		BOLD:ACU1446	Blank 2001
306	Diapriidae	Diapriinae	Psilini	*Coptera maura*	(Kieffer, 1911)		no BIN	Fauna Europaea
307	Diapriidae	Diapriinae	Psilini	*Coptera punctiventris*	(Kozlov, 1978)	FR	BOLD:ADV7592, BOLD:ADW9663	BOLD DIAIS
308	Diapriidae	Diapriinae	Psilini	*Labolips innupta*	Haliday, 1857		BOLD:ABA6052	Blank 2001
309	Diapriidae	Diapriinae	Psilini	*Psilus acutangulus*	(Jansson, 1942)		BOLD:AEY5223	BOLD DIAIS
310	Diapriidae	Diapriinae	Psilini	*Psilus caecutiens*	(Marshall, 1867)		BOLD:ACZ2777	Blank 2001
311	Diapriidae	Diapriinae	Psilini	*Psilus cornutus*	Panzer, 1801		BOLD:AEC6405, BOLD:AEW9234, BOLD:AEC3476	Blank 2001
312	Diapriidae	Diapriinae	Psilini	*Psilus egregius*	(Herrich-Schaeffer, 1840)		no BIN	Fauna Europaea
313	Diapriidae	Diapriinae	Psilini	*Psilus foersteri*	(Kieffer, 1911)		no BIN	Fauna Europaea
314	Diapriidae	Diapriinae	Psilini	*Psilus frontalis*	(Thomson, 1859)	FR	BOLD:ADB9395, BOLD:AEX7805	BOLD DIAIS
315	Diapriidae	Diapriinae	Psilini	*Psilus fuscipennis*	(Curtis, 1831)		BOLD:AEJ9045, BOLD:ACZ1426	Blank 2001
316	Diapriidae	Diapriinae	Psilini	*Psilus puncticeps*	(Kieffer, 1911)		no BIN	Blank 2001
317	Diapriidae	Diapriinae	Psilini	*Psilus rufipes*	(Thomson, 1859)	FR	BOLD:ADB9394, BOLD:AEO7017	BOLD DIAIS
	Diapriidae	Diapriinae	Psilini	*Psilus sp*			BOLD:ADL2013	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Psilus sp*			BOLD:ADY2123	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Psilus sp*			BOLD:AEY1509	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Psilus sp*			BOLD:AFA2227	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Psilini	*Psilus sp*			BOLD:AFA3164	BOLD DIAIS BIN
318	Diapriidae	Diapriinae	Psilini	*Psilus submonilis*	(Kieffer, 1911)		BOLD:AEH1087, BOLD:AEJ9044	BOLD DIAIS
319	Diapriidae	Diapriinae	Spilomicrini	*Entomacis bipunctata*	(Kieffer, 1911)		BOLD:AED0714	Blank 2001
320	Diapriidae	Diapriinae	Spilomicrini	*Entomacis graeffei*	Kieffer, 1916		BOLD:AEJ2832, BOLD:AEK5540	BOLD DIAIS
321	Diapriidae	Diapriinae	Spilomicrini	*Entomacis hajeki*	Macek, 2000	FR	BOLD:AEC2590, BOLD:AEP5855	BOLD DIAIS
322	Diapriidae	Diapriinae	Spilomicrini	*Entomacis muscorum*	(Dahl, 1912)		no BIN	Fauna Europaea
323	Diapriidae	Diapriinae	Spilomicrini	*Entomacis penelope*	Nixon, 1980		BOLD:AEP5854	Blank 2001
324	Diapriidae	Diapriinae	Spilomicrini	*Entomacis perplexa*	(Haliday, 1857)		BOLD:ACH3280, BOLD:ADV1474, BOLD:AEC0428	Blank 2001
325	Diapriidae	Diapriinae	Spilomicrini	*Entomacis platyptera*	(Haliday, 1857)		BOLD:AEP5803, BOLD:AEP5856, BOLD:ACJ0885	Blank 2001
	Diapriidae	Diapriinae	Spilomicrini	*Entomacis sp*			BOLD:ABA8019	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Spilomicrini	*Entomacis sp*			BOLD:ACJ0375	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Spilomicrini	*Entomacis sp*			BOLD:ACJ2782	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Spilomicrini	*Entomacis sp*			BOLD:AEZ6218	BOLD DIAIS BIN
	Diapriidae	Diapriinae	Spilomicrini	*Entomacis sp*			BOLD:AEZ6250	BOLD DIAIS BIN
326	Diapriidae	Diapriinae	Spilomicrini	*Idiotypa mariae*	Gregor, 1939		BOLD:AEP5847	BOLD DIAIS
327	Diapriidae	Diapriinae	Spilomicrini	*Idiotypa maritima*	(Haliday, 1833)		BOLD:ADF4867, BOLD:AEZ1854	BOLD DIAIS
328	Diapriidae	Diapriinae	Spilomicrini	*Paramesius belytoides*	Marshall, 1867	FR	BOLD:AEC6551	BOLD DIAIS
329	Diapriidae	Diapriinae	Spilomicrini	*Paramesius bifoveatus*	Kieffer 1911		no BIN	Fauna Europaea
330	Diapriidae	Diapriinae	Spilomicrini	*Paramesius brachypterus*	Thomson, 1859		BOLD:ACZ3261, BOLD:AEJ1825	Blank 2001
331	Diapriidae	Diapriinae	Spilomicrini	*Paramesius crassicornis*	Thomson, 1859		BOLD:AEC5675, BOLD:AER6527	Blank 2001
332	Diapriidae	Diapriinae	Spilomicrini	*Paramesius dolichocerus*	Kieffer 1911		no BIN	Fauna Europaea
333	Diapriidae	Diapriinae	Spilomicrini	*Paramesius nervosus*	(Nees, 1834)		no BIN	Fauna Europaea
334	Diapriidae	Diapriinae	Spilomicrini	*Paramesius rufipes*	(Fonscolombe, 1832)		BOLD:ADX3587	Blank 2001
335	Diapriidae	Diapriinae	Spilomicrini	*Paramesius westwoodi*	Fergusson, 1977		no BIN	Blank 2001
336	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus abnormis*	Marshall, 1868		BOLD:AEP5852	Blank 2001
337	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus acuminatus*	(Herrich-Schaeffer, 1838)		no BIN	Fauna Europaea
338	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus annulicornis*	Kieffer, 1911		BOLD:ADF4870	Blank 2001
339	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus antennatus*	(Jurine, 1807)		BOLD:AEE0914	Blank 2001
340	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus bipunctatus*	Kieffer 1911		BOLD:AEC7259	Fauna Europaea
341	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus brevimalaris*	Hübner & Chemyreva, 2023	SP. NOV.	BOLD:AEC2138	BOLD DIAIS
342	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus clavatus*	(Herrich-Schaeffer, 1838)		no BIN	Fauna Europaea
343	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus compressus*	Thomson, 1859		BOLD:ACH2501	Blank 2001
344	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus crassiclavis*	Kieffer, 1911	FR	BOLD:AEP5849	BOLD DIAIS
345	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus diversus*	Chemyreva, 2021	FR	BOLD:ADF4749	BOLD DIAIS
346	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus flavecorpus*	Hübner & Chemyreva, 2023	SP. NOV.	BOLD:AAU9373	BOLD DIAIS
347	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus flavipes*	Thomson, 1859		BOLD:ACL2543	Blank 2001
348	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus formosus*	Jansson, 1942		BOLD:AAU9811	Blank 2001
349	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus hemipterus*	Marshall, 1868		BOLD:ADM6694	Blank 2001

350	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus integer*	Thomson, 1859		BOLD:ADF4750	Blank 2001
351	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus lusitanicus*	(Kieffer, 1910)	FR	BOLD:AEC2138, BOLD:AEK2205	BOLD DIAIS
352	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus modestus*	Tomsik, 1947		BOLD:AEJ2099	Blank 2001
353	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus nigriclavis*	Marshall, 1868		BOLD:AEK0961	BOLD DIAIS
354	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus politus*	Hübner & Chemyreva, 2023	SP. NOV.	BOLD:ACZ2358, BOLD:AER1505	BOLD DIAIS
355	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus rufitarsis*	Kieffer, 1911		BOLD:AEK1604	Blank 2001
356	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus stigmaticalis*	Westwood, 1832		BOLD:ACU1243, BOLD:ADS1706	Blank 2001
357	Diapriidae	Diapriinae	Spilomicrini	*Spilomicrus thomsoni*	Kieffer, 1911	FR	BOLD:ADF4747, BOLD:ADX1651	BOLD DIAIS
358	Ismaridae			*Ismarus apicalis*	Kolyada & Chemyreva 2016	FR	BOLD:ADA6314	BOLD DIAIS
359	Ismaridae			*Ismarus campanulatus*	(Herrich-Schaeffer, 1840)		no BIN	Fauna Europaea
360	Ismaridae			*Ismarus dorsiger*	(Haliday, 1831		BOLD:AEJ7515, BOLD:ADA6020	Blank 2001
361	Ismaridae			*Ismarus flavicornis*	(Thomson, 1859)		BOLD:ADA7493, BOLD:AEJ0302	Blank 2001
362	Ismaridae			*Ismarus halidayi*	(Förster, 1850)		BOLD:AEJ7514, BOLD:ADA6019	Blank 2001
363	Ismaridae			*Ismarus longicornis*	(Thomson, 1859)		no BIN	Fauna Europaea
364	Ismaridae			*Ismarus rugulosus*	Förster, 1850		BOLD:AFB5847, BOLD:ACM9864	Blank 2001