Genetic population structure and environmental impact on *Craspedacusta* at the medusa and polyp stages

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Summary

Craspedacusta, commonly known as the freshwater jellyfish, has spread worldwide rapidly and has been invading Europe for more than a century. It is thought to originate from the Yangtze River area in China. It features a metagenetic lifestyle, including the free swimming, planula larvae and dioecious medusae stages, as well as the benthic stages, comprising sessile polyps, cylindrical frustules and spherical podocysts. Benthic stages are easily dispersed and are so-called "good hitchhikers". Hence, *Craspedacusta* sp. shows important traits that are advantageous for effective dispersal and successful invasion into new habitats. Increasing anthropogenic activities profoundly accelerates the expansion. For example, intense international trade is a major source for intercontinental dispersal of non-native species. The presence of freshwater jellyfish can lead to a significant decrease and mortality of zooplankton, thus potentially influencing freshwater ecosystem dynamics. Therefore, there are growing concerns about the potential future impact of *Craspedacusta* on aquatic food webs.

The debate about the taxonomic classification within *Craspedacusta*, has lasted for over a century since the first discovery of the medusae stage. *Craspedacusta* has been given numerous names over time, according to ambiguous morphologic traits which are unreliable and confusing. In the present study, I investigated the spatio-temporal genetic diversity and structure among *Craspedacusta* population samples from central Europe, including medusa and polyp stages. This study is a first insight into the genetic diversity and the differentiation of *Craspedacusta* populations on large scales that allows a broader and deeper integrative understanding of the dispersal and invasion of this species. My results support recent findings that at least two mitochondrial lineages (Type 1, Type 2) with four main haplotypes (Type 1.1, 1.2, 2.1, 2.2), invaded Europe. Except for one lake, all lakes in this study were inhabited by only a single haplotype and gender of medusae, even when comparing different years. However, in contrast to medusae, polyps with distinct haplotypes co-existed in the same lake, even on very small scales, such as on a small stone. The haplotype identity of medusae and polyps that were found in a lake was sometimes inconsistent, for example, only one medusa haplotype appeared, while more haplotypes co-existed among polyps. At the medusa stage, gender and mitochondrial haplotype corresponded in large samples from numerous populations analysed.

A known environmental parameter that is thought to trigger life cycle transitions in *Craspedacusta* is temperature. Hence, in order to explore the temperature effect on the growth dynamics of regional *Craspedacusta* at the polyp stage, I studied their asexual reproduction

and population growth pattern in controlled laboratory experiments. *Craspedacusta* polyp populations were showing distinct growth rates at 18°C and 26°C, and developed significantly faster at a higher temperature. The number of frustules released by a single polyp, and the increase in polyp population size, were temperature dependent. Colonies with two connected polyps were the most common type at both temperatures. I conclude that increasing temperatures will further promote the rapid expansion of polyp populations and may thereby induce cascading effects in freshwater ecosystems. Additionally, I also investigated the effects of freshwater acidification on *Craspedacusta* at the polyp stage. I cultured polyps of *Craspedacuta* sp. from different clonal assemblages and clones at different pH levels, manipulated by weak acidification. My results indicated that *Craspedacusta* sp. polyps were quite insensitive to weak acidification with slight pH variations, at both low and high water temperatures. My findings further support the opinion that the polyps of *Craspedacusta* have a very broad tolerance to a large variety of environmental parameters, making them successful invaders into diverse freshwater ecosystems.

List of abbreviations and definitions

AMOVA	Hierarchical analysis of molecular variance
ANOVA	Analysis of variance
AU	Austria
bp	Base pair
Chl-a	Chlorophyll-a
COI	Cytochrome c oxidase subunit I
Colony	As defined in McClary (1959), all connected polyps sharing a same gastric cavity (single polyps were also considered as a colony)
Cl^-	Chloride
CR	Czech-Republic
DM	*Daphnia* Medium
DUI	Doubly uniparental inheritance
eDNA	Environmental DNA
ESD	Environmental sex determination
F_{ST}	Fixation index
GSD	Genotypic sex determination
hd	Haplotype diversity
HM	*Hydra* Medium
IAS	Invasive alien species
IBD	Isolation by distance
IBE	Isolation by environment
IG	Ingolstadt Germany
ITS	Internal transcribed spacer
K2P	Kimura 2-parameter model
LT	*Limnocnida tanganicae*
ML	Maximum Likelihood method
mtDNA	Mitochondrial DNA
Ne	Effective population size
NO_2^-	Nitrite
NO_3^-	Nitrate
N:P	The ratio of nitrogen to phosphorus

OG	Other areas in Germany
ORF	Open reading frame
PA1	Assemblage of Type 1.1 polyps from several lakes
PA2	Assemblage of Type 1.2 polyps from several lakes
PA3	Assemblage of Type 2.1 polyps from several lakes
PA4	Assemblage of Type 2.2 polyps from several lakes
PCA	Principal component analysis
pCO_2	Partial pressures of CO_2
rRNA	Ribosomal RNA
SE	Standard error
SMI	Strict maternal mitochondrial DNA inheritance
SO_4^{2-}	Sulfate
TP	Total phosphorus
tRNA	Transfer RNA
Type	mtDNA haplotype
π	Nucleotide diversity

1 General Induction

1.1 Invasive species

The study of species invasion was first put forward by Elton in the late 1950s in his influential book "*The ecology of invasions by animals and plants*" (Elton, 2020). Since then, more and more attention has been paid to the dynamics of invasive species. Invasive "alien species" (IAS) are defined as species that are non-native to an ecosystem under consideration and whose introduction causes, or is likely to cause, economic or environmental harm, or harm to human, animal, or plant health (Beck et al., 2008). IAS may migrate naturally to new areas, via wind, water flow or by attaching to other animals and plants. However, with increasing anthropogenic activities acting as further transportation vectors, biological invasion events are globally increasing year by year, intentionally or accidentally (Vitousek et al., 1997; Hulme et al., 2009). IAS are a considerable threat to native biodiversity and ecosystem dynamics (Lee, 2002; McGeoch et al., 2010). For example, in the 1950s, the introduction of a large predatory fish, the Nile perch (*Lates niloticus*), to Lake Victoria, to boost fisheries almost caused native haplochromine cichlid fish species to go extinct (Witte et al., 1992). The introduction simultaneously restructured ecosystem composition in this lake, resulting in a large biodiversity loss (Pringle, 2005).

Of particular interest is what makes a species invasive. As far as it is known, IAS typically possess some common life history traits, such as, rapid growth rate, short generation time, multiple reproductive strategies (e.g., reproduce sexually and asexually), strong dispersal capacity, high tolerance to a broad range of environmental conditions, good adaptability, and competitiveness (Sakai et al., 2001; Whitney and Gabler, 2008). The admixture of above characteristics supports the invasion success.

The process of invasion is profoundly important to understand invasion mechanisms. In general, it includes three key stages: introduction, establishment and spread (Fig. 1) (Kolar and Lodge, 2001; Sakai et al., 2001; Puth and Post, 2005). The introduction always starts from the dispersal of non-native species. Active dispersal is linked to the mobility of organisms themselves, like migration. Alternatively, they can also be distributed passively, such as by wind, water flow or animals (Bullock et al., 2018). Long distance dispersal allows invasions on large scales, and most are mediated by accidental or deliberate anthropogenic activities. For example, ballast

water from ships often results in the transfer of non-native species to other locations (Sieracki et al., 2014; Bailey, 2015). Multiple introductions are also a common occurrence (Wilson et al., 2009), increasing the genetic diversity and promoting the gene flow among populations. Such anthropogenically enhanced multiple invasions can help non-native species to overcome initial genetic bottlenecks, thus facilitating the success of invasions (Sakai et al., 2001).

However, the first step of initial introduction doesn't mean that non-native species are truly invasive. After initial arrival, species need to adapt to the novel surroundings and face two barriers throughout, survival and reproduction (Lockwood et al., 2013). Approximately 10% of introduced species successfully establish themselves and develop a self-sustaining population (Kolar and Lodge, 2001). Before their further spread, they often experience a lag period after initial colonization (Allendorf and Lundquist, 2003). This lag time is therefore seen as one of the characteristics to distinguish invasive species from non-invasive species (Allendorf and Lundquist, 2003). When a species keeps their distribution relatively localized, they are considered as non-invasive (Kolar and Lodge, 2001). However, invasive species usually spread rapidly and widely, by short-distance dispersal (diffusion dispersal), long-distance dispersal (saltation dispersal) and by other related dispersal mechanisms (Sakai et al., 2001, Lockwood et al., 2013). Rapid range expansion is always correlated with high dispersal rates (Barrett and Husband, 1990). Meanwhile, invaders are also likely to be reintroduced to their native ranges, termed cryptic back-introduction (Guo, 2005). Reports and research nevertheless on this back introduction are limited, but this process is nonetheless worth consideration. (Kolbe et al., 2017).

Concern over freshwater invasions has been increasing from the late 20th century onwards (Ricciardi and MacIsaac, 2011). Though freshwater resources account for only 2.5% of the world's water (Petersen et al., 2016), approximately 6% of all known species live in fresh water (Dudgeon et al., 2006). Even more, freshwater biodiversity is declining faster than observed in most terrestrial ecosystems (Ricciardi and Rasmussen, 1999; Sala et al., 2000). Currently, species which have been invading freshwater environments account for 26% of the 486 assessed alien species invading all types of environments (terrestrial, freshwater, and marine) (Nentwig et al., 2018).

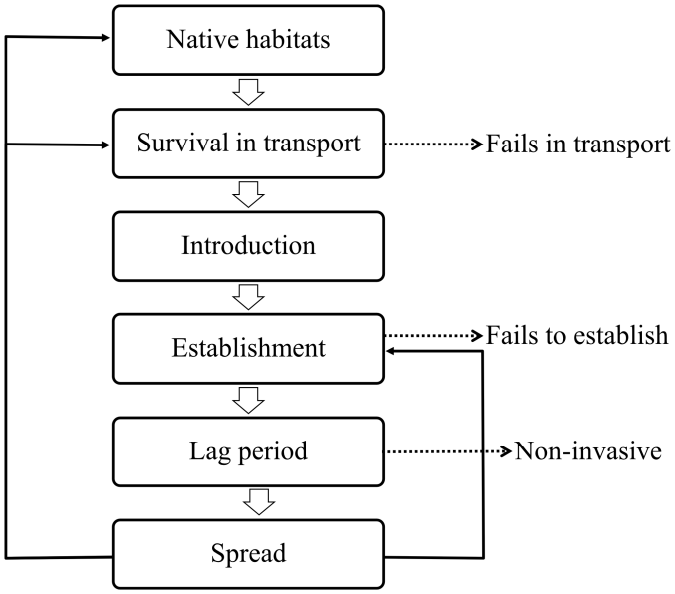

Fig. 1 Invasion process. (Modified from Kolar and Lodge, 2001 and Sakai et al., 2001)

1.2 Population genetics

Population generally refers to a group of interbreeding individuals of the same species living in the same geographical area at the same time (Rockwood, 2015; Hartl, 2020). However, population definition may differ between investigators due to their specific research aims (Rockwood, 2015). Theoretical population genetics started in 1908 when the Hardy-Weinberg principle was independently discovered by Godfrey Hardy and Wilhelm Weinberg (Dobzhansky, 1955), but the subsequent lack of analytical methods to detect the allelic differences between organisms limited the process of population genetics development (Hartl, 2020). Thanks to the technical advance of DNA sequencing coupled with its recent inexpensive and high-throughput possibilities, population genetics has been growing profoundly fast during the last decade (Hartl, 2020). Molecular markers are based on neutral theory (Kimura, 1979) and a great variety of markers have been widely used in population genetics (Khlestkina, 2014; Al-Samarai and Al-Kazaz, 2015). The appropriate selection of genetic markers is crucial to answer the questions in focus, rather than just using techniques that are available (Sunnucks, 2000).

Population genetics integrates ecological and evolutionary processes, called eco-evo interactions, and thereby contributes to an understanding of eco-evo dynamics (Fig. 2, Lowe et al., 2017). The fundamental mechanisms of evolution consist of natural selection, genetic drift, gene flow and mutation (Fig. 2, Lowe et al., 2017; Rowe et al., 2017). As the Hardy-Weinberg

law is a null model based on an idealized situation without evolutionary biological processes in operation, the departure from this law thus demonstrates the changes in allele frequency, and this must be induced by the evolutionary forces mentioned above (Hamilton, 2021).

Mutations, such as transitions, transversions and indels, are the ultimate source of all genetic variation (Rowe et al., 2017; Hamilton, 2021). The mutation rate illustrates how fast novel genetic variation is added to populations (Hamilton, 2021). Beneficial mutations increase survival and reproduction above the average fitness of populations while deleterious mutations reduce it and ultimately individuals with detrimental mutations fail to survive and reproduce (Hamilton, 2021). Notably, good or advantageous mutations, rather than bad or deleterious mutations, drive evolutionary adaptation (Keightley et al., 1998; Loewe and Hill, 2010). In ccontrast, indifferent, or neutral mutations have little effect on fitness (Loewe and Hill, 2010). In fact, mutations are often more harmful than beneficial or neutral, but such mutations are also often eliminated (Keightley et al., 1998). As deleterious mutations accumulate, small populations can decline due to mutational meltdown, while large populations are more resistant against this effect, because advantageous mutations are more likely to happen in large rather than small populations (Lowe et al., 2017).

Natural selection determines an individual's fitness, which is defined by that individual's survivorship and reproduction (Saccheri and Hanski, 2006). Individuals with beneficial adaptive traits are more likely to survive and reproduce and thus these adaptive traits are transmitted to their descendants, leading to adaptation to local environments (Kulpa and Leger, 2013). Underlying mechanisms relate to two types of natural selection: hard selection and soft selection. The mortality resulting from hard selection is often independent of population density or genotype frequency (Saccheri and Hanski, 2006; Lowe et al., 2017), which means different genotypes keep constant differences in fitness in a given environment (Reznick, 2016). Hard selection can result in additional mortality and subsequently has direct effects on population size (Saccheri and Hanski, 2006; Lowe et al., 2017). Soft selection depends on competition among genotypes, resulting in adaptive evolution and local adaptation (Lowe et al., 2017). This process is often population density and genotype frequency dependent, having little effect on population size (Saccheri and Hanski, 2006; Lowe et al., 2017). In practice, both selection types simultaneously work together (Saccheri and Hanski, 2006).

However, most of the variation within and between species is selectively neutral, having little or no fitness advantage or disadvantage, and is mainly resulting from genetic drift (Kimura,

1968; Hamilton, 2021). Genetic drift is the process by which allele frequencies in populations change from one generation to the next due to random sampling among gametes (Nielsen, 2005; Hartl, 2020; Hamilton, 2021). Genetic drift changes allele frequencies purely by chance and leads to the loss of a beneficial allele or fixation of a harmful allele in a population, ultimately resulting in the decline of genetic variation (Hamilton, 2021), and thus could determine the fate of a new mutation (Masel, 2011). Population size is associated with the strength of drift and the likelihood of inbreeding in populations (Franklin, 1980; Hamilton, 2021). The effect of genetic drift on small populations is particularly important, as changes in allele frequencies induced by sampling error are relatively small in large populations and inbreeding depression is more likely to happen in small populations due to either the fixation of deleterious recessive alleles, or the loss of heterozygosity (Franklin, 1980; Honnay, 2013; Lowe et al., 2017; Hartl, 2020). Moreover, the effects of natural selection are often small in small populations, leading to genetic drift being the dominant factor in such small populations after bottleneck and founder events (Franklin, 1980; Whitlock, 2000; Hamilton, 2021). Alternatively, genetic drift can also be important in times of rapid growth rates in large populations by acting on newly emerged or neutral mutations (Templeton, 2021). Therefore, the concept of effective population size (Ne) was introduced by Wright (1931), which is defined as the size of an ideal population that would experience as much genetic drift as observed in an actual population under investigation. Ne thereby allows the impact of genetic drift to be predicted. (Lowe et al., 2017; Hamilton, 2021). The values of Ne are often lower than the census number in a species (Charlesworth, 2009; Rowe et al., 2017). The rate of neutral genetic variation within a population lost by genetic drift is ½Ne per generation (Rowe et al., 2017). Moreover, populations with low Ne are more vulnerable to extinction (Newman and Pilson, 1997).

Gene flow refers to the successful movement of alleles, mediated by the movement of individuals (migration) or their gametes, between populations (Rowe et al., 2017; Hamilton, 2021). Hence, metapopulations of a species are connected by gene flow (Petit and Excoffier, 2009) and gene flow in turn accelerates the populations in becoming genetically similar (Rowe et al., 2017). Gene flow may either play a constraining role in evolution by preventing local adaptation or play a creative role by spreading new genes and combinations of genes towards a species. Hence, the role of gene flow for a particular species depends not only on the geographic distribution pattern, such as IBD - isolation by distance and IBE - isolation by environment, but also on the interactions between other evolutionary forces (Slatkin, 1987; Sexton et al., 2014). F-statistics, especially the fixation index F_{ST}, are commonly employed to

measure the genetic differentiation among subpopulations (Lowe et al., 2017). The value of F_{ST} ranges from 0 (no population subdivision) to 1 (complete fixation of different alleles in each population) (Bohonak, 1999).

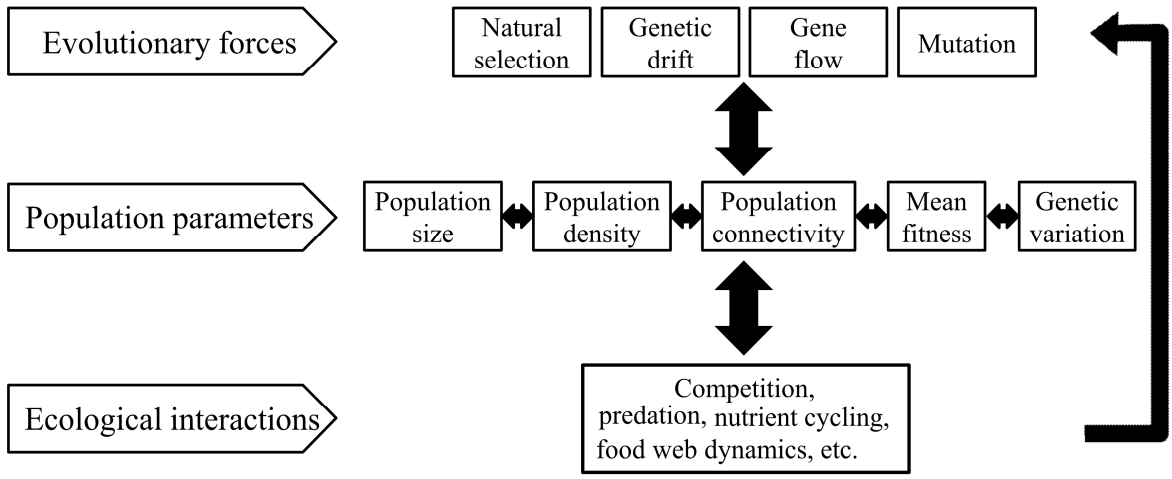

Fig. 2 Interactions between evolutionary and ecological processes. (Modified from Lowe *et al*., 2017)

1.3 Freshwater jellyfish

Most jellyfish live in marine waters, while some species distribute in inland waters as well, including fresh, brackish, and saline waters (Jankowski, 2001). Jellyfish living in inland waters mainly originate from the class Hydrozoa, and belong to the order Limnomedusae (Jankowski, 2001). To date, freshwater jellyfish are distinguished into two genera, *Limnocnida* and *Craspedacusta*. *Limnocnida* is a tropical genus, restricted to African and Indian regions (Dumont, 1994), while *Craspedacusta* is a cosmopolitan freshwater jellyfish, invading all continents except Antarctica due to its high invasiveness (Dumont, 1994; Jankowski et al., 2008). In addition, *Limnocnida* and *Craspedacusta* can co-exist in the same lake, however this was only described in Africa (Rayner and Appleton, 1989). Species identification within two genera is still controversial, thus the exact number of species of freshwater jellyfish is not fully known up to now.

1.4 *Craspedacusta*

1.4.1 Taxonomy

The genus *Craspedacusta* is positioned in the Phylum Cnidaria, Class Hydrozoa, Order Limnomedusae. A detailed phylogenetic relationship among members of the Phylum Cnidaria as derived from genomic and transcriptomic data (Kayal *et al.*, 2018) is shown in Fig. 3.

Kingdom **Animalia** Linnaeus, 1758

Subkingdom **Eumetazoa** Buetschli, 1910

Phylum **Cnidaria** Hatschek, 1888

Class **Hydrozoa** Owen, 1843

Subclass **Trachylinae** Haeckel, 1879

Order **Limnomedusae** Kramp, 1938

Family **Olindiidae** Haeckel, 1879

Genus **Craspedacusta** Lankester, 1880

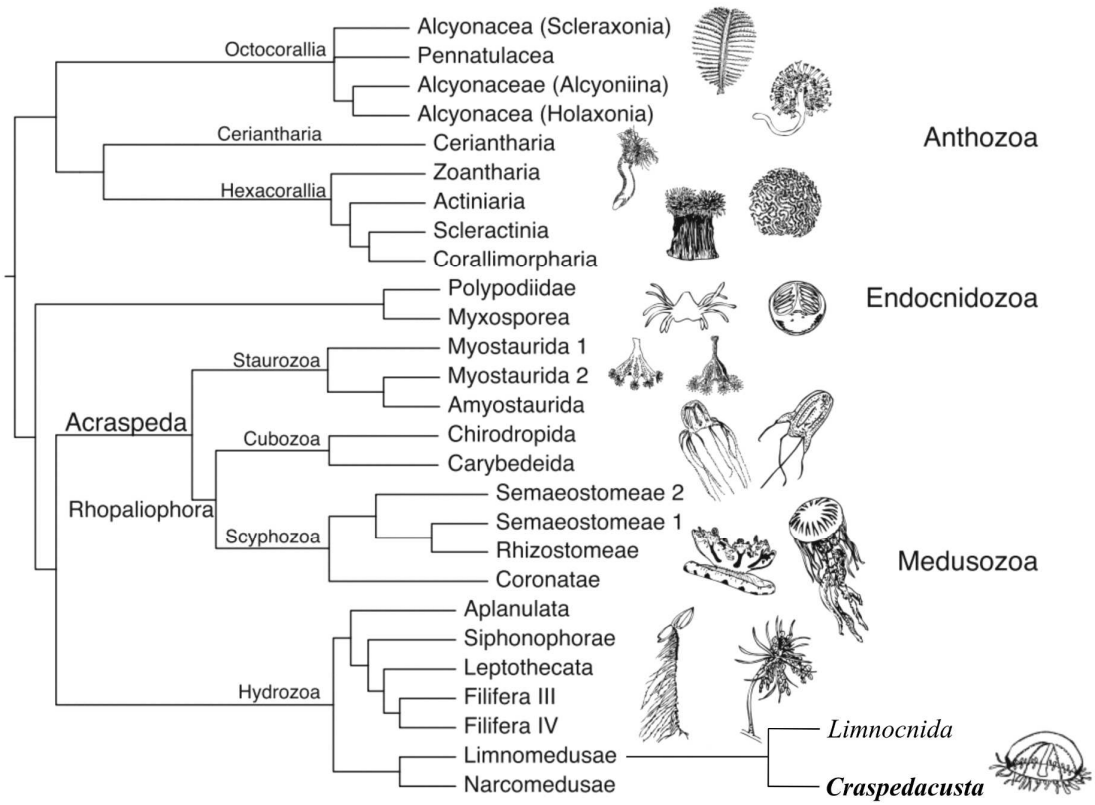

Fig. 3 Cnidarian phylogeny resolved from genomic and transcriptomic data. (Modified from Kayal *et al.*, 2018).

1.4.2 Life history

The life history of *Craspedacusta* is characterized by various forms of propagules and complex transformation among different life stages. In total, it has five distinct life stages, which are called polyp, frustule, podocyst, medusa and planula larvae respectively. The first three are benthic life stages but the latter two are pelagic. Additionally, two reproduction modes co-exist in the whole life cycle, frequent asexual reproduction, and sporadic sexual reproduction (Fig. 4).

Medusa, commonly known as jellyfish, is the best-known life stage of *Craspedacusta* for most people due to its easily visible size. The diameter of adult medusae can be around two centimetres (Acker and Muscat, 1976). *Craspedacusta* medusae usually appear at summer and autumn because high water temperature is assumed to trigger the formation of medusae (Lytle, 1961; McClary, 1959), which are budded from the body column of polyps as shown in Fig. 4. However, it is believed that high temperature is not the only factor to stimulate medusa budding and subsequent jellyfish blooms (Lytle, 1961; Folino-Rorem et al., 2016). The mechanism underlying medusa budding is ambiguous at present. Many parameters such as food abundance, light, oxygen, and CO_2 are considered to affect medusae occurrence as well (Dumont, 1994; Folino-Rorem et al., 2016; Minchin et al., 2016). Medusae are dioecious, and thus eggs or sperm are released from sexual mature females or males respectively. Planula larvae, subsequently, are formed from fertilized eggs and then differentiate to polyps.

Polyps are considered as the dominant stage during the whole life cycle of *Craspedacusta* (Payne, 1924; Acker and Muscat, 1976; Kato and Hirabayashi, 1991; Angradi, 1998), and they are the essential part for both sexual and asexual reproduction. They live in solitary or in colonies. As an example, Fig. 4 shows a two-polyp colony. Up to ten polyps forming a single colony sharing a common digestive tract have been already reported (McClary, 1959). In fact, the solitary polyp is able to grow into a several-polyp colony (Folino-Rorem et al., 2016; Marchessaux and Bejean, 2020). Within a colony, the same piece of food could be shared by different polyps through their mouth (Payne, 1924; Bushnell and Porter, 1967), located in the center of the capitulum, the head, surrounded by nematocysts (sting cells), which can discharge toxins to either defend themselves or catch prey (McClary, 1964; Folino-Rorem, 2015). Normally, polyps can attach to many kinds of substrates, such as stones, plastics, wood, plant

material, and even animals such as molluscs (Acker and Muscat, 1976; Duggan and Eastwood, 2012).

Frustules are asexually produced by polyps, especially under excess food conditions, and food is thought to be the main factor influencing frustule production (Lytle, 1961; Acker and Muscat, 1976). Similar as polyps, frustules attach to various substrates as well, but different from sessile polyps, frustules are able to crawl short distances, promoting the dispersal of *Craspedacusta* over short scales. Conversely, polyps can also transform themselves to frustules under adverse conditions, like starvation or low temperature (Fig. 4, Folino-Rorem, 2015).

Furthermore, both polyps and frustules could differentiate into podocysts, resting stages, which are produced under severe environmental conditions, such as drought, starvation periods or extreme temperatures (Payne, 1924; Acker and Muscat, 1976; Folino-Rorem, 2015). Podocysts are seen as a gene pool for the next growing season as they may be the only life form able to persist during cold winter periods (Acker, 1976). With improved environmental conditions podocysts can transform to polyps (Dunham, 1941). Similar as polyps and frustules, podocysts can be easily distributed by their ability to attach to other organisms, thereby potentially also allowing long-distance migration when hitchhiking, for example with migratory birds.

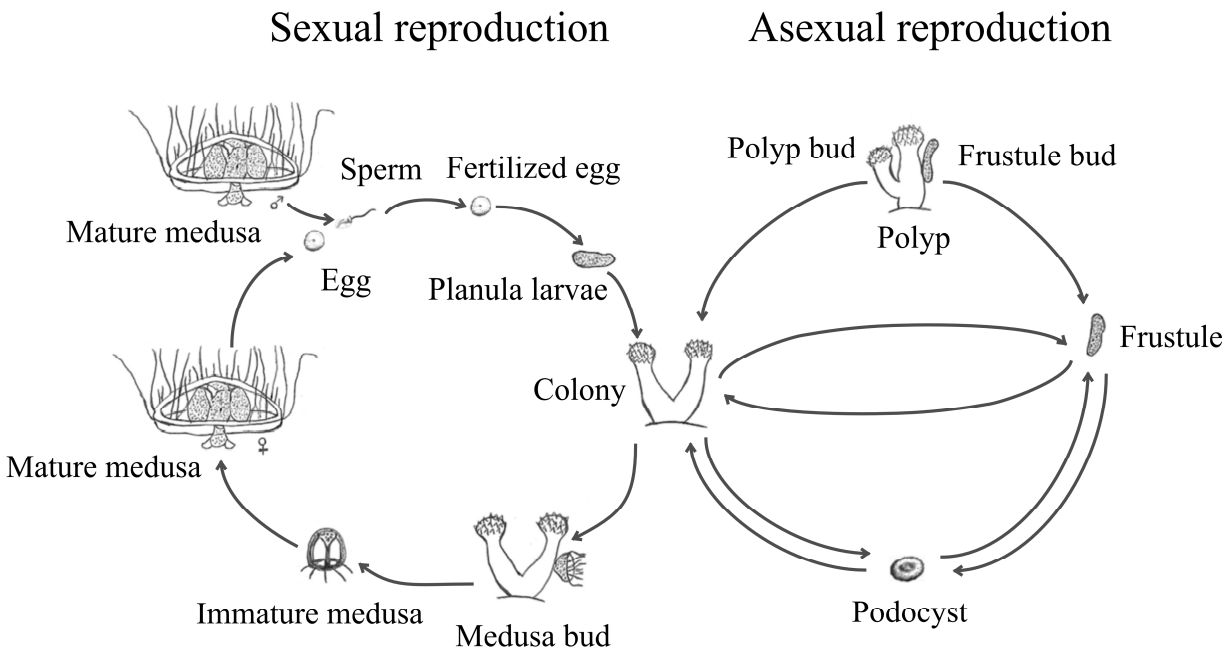

Fig. 4 Life cycle of *Craspedacusta*. (Modified after Folino-Rorem, 2015)

1.4.3 Distribution

Initially, Lankester assumed *Craspedacusta* to be tropical because the species was first detected within water lily tanks with warm water (Lankester, 1880). Additionally, as water lilies are tropical plants, he thus suggested that *Craspedacusta* might be introduced from tropical regions (Lankester, 1880). However, with more and more observations from diverse places it became evident that *Craspedacusta* is not a tropical genus but temperate (Kramp, 1950). Recently, the common agreement is that *Craspedacusta* originates from the Yangtze River area in China (Kramp, 1950). It prefers to live in lentic freshwater habitats, such as lakes, reservoirs, quarries, ponds, and slow flowing rivers (Dejdar, 1934; Lytle, 1960; Beckett and Turanchik, 1980; Fritz et al., 2007). It has been reported that it frequently occurs in man-made water bodies (Lytle, 1960; DeVries, 1992; Lewis et al., 2012). Currently the distribution of *Craspedacusta* is worldwide, except for Antarctica (Dumont, 1994; Jankowski et al., 2008).

However, whether the increasing observations of medusae occurrence is due to new introductions or local dispersal is indistinguishable (Fritz et al., 2009). Generally, almost all reports are based on the observations of medusae due to their visible size. From the life history described above, it becomes clear that medusae are budded from polyps, and polyps, frustules and podocysts all have high dispersal potential. Therefore, the difficulty of distinguishing the origin of polyps, frustules and podocysts is a big challenge owing to their minute size and the limited research to date. Simultaneously, this also reflects that the number of described *Craspedacusta* populations is seriously underestimated, and medusae observations are not enough to show a complete picture of the actual distribution (Duggan and Eastwood, 2012). In addition, the peculiar and puzzling occurrence of medusae makes it an extremely unpredictable indicator for the presence of this species (Thomas, 1950; Lytle, 1960; Brussock et al., 1985). In contrast, sessile polyps are more long-lived and have a broader range of tolerable environmental conditions (Folino-Rorem, 2015).

The underlying mechanisms of the worldwide distribution of *Craspedacusta* largely depend on its life history traits as mentioned in section 1.4.2. All benthic stages are good at attaching to transportation vectors and can travel around the world via hitchhiking, and the fast switch between producing podocysts and frustules can be seen as a strategy to overcome unpredictable and variable environments. For example, polyps have been found to hitchhike on zebra mussels, *Dreissena polymorpha* (Stanković and Ternjej, 2010), which is a highly invasive species as

well, and a recent study indicated that boat ramps facilitated this dispersal (Rodríguez-Rey et al., 2021). Therefore, it is quite likely that either zebra mussels' invasion indirectly contributes to the spread of polyps, or that polyps directly attach to boats to get propagated. In summary, the versatile vegetative propagules support their rapid dispersal and even rare sexual reproduction between dioecious medusae provides the chance for genetic recombination. The integration of asexual and sexual reproduction substantially helps this species to overcome multiple challenges during expansion, such as bottleneck and founder effects, thus successfully invading global lakes and rivers. Moreover, recent anthropogenic activities accelerate their long-distance dispersal across continents.

1.4.4 Species determination

The first observation of *Craspedacusta* in Europe was made by Mr. Sowerby in a water lily tank, located in the gardens of the Botanical Society, Regen's Park, London, in June 1880 (Lankester, 1880). Considered to be a new genus, Lankester proposed the name *Craspedacusta* (Lankester, 1880). Afterwards, the species was named *Craspedacusta sowerbii* in honour of the discovery by Mr. Sowerby (Lankester, 1880). Meanwhile, Allman (1880) discovered the same jellyfish as well and named it *Limnocodium Victoria*. Because of the priority rule, "*Craspedacusta"* got established. In the same year, Parsons (1885) found the hydroid stage in the same tank where the medusae had been detected. Initially, polyps were recognized as another species, named *Microhydra ryderi* (Potts, 1885) but whether they belonged to *Craspedacusta* remained ambiguous. In 1924, Payne (1924) cultured *Microhydra ryderi* from polyps to sexually mature medusa, showing that adult medusae of *Microhydra* were identical with *Craspedacusta* medusae. Hereafter, the distinction of the two main life stages, medusa and polyp, of *Craspedacusta* was recognized.

Craspedacusta is generally thought to originate from the Yangtze basin in China (Kramp, 1950). Since the Ming Dynasty, local Chinese people already recorded this organism and called it peach blossom fish, because it appeared during the peach blossom season, looked like peach blossom leaves, and swam like fish. The scientific documentation of this organism in China occured later than in Europe, but ancient Chinese reports and fairy tales mentioned the name peach blossom fish long before (He, 2005). The first scientific documentation in China was by Dr. Oka in 1907. Kawai, a Japanese captain, brought freshwater medusae found in the Yangtze River section near Yichang, to Tokyo. Oka then considered it was a new species and named it

Limnocodium Kawaii (Oka, 1907). Later, nevertheless, it was identified as a variety of *Craspedacusta sowerbii* (Oka and Hara, 1922). Moreover, Oka (Oka and Hara, 1922) described a new species found in Japan in 1922, *C. iseanum*, and only five individuals have been found up to now (Jankowski, 2001). Thereafter, local Chinese researchers started to pay attention to this species. Amongst such research, the most representative and profound local Chinese study is from Gaw and Kung in 1939. They found two different species in Kiating, Sichuan, China, *C. kiatingi* and *C. sinensis* (Gaw and Kung, 1939). More and more species were thereafter found in different regions in China, such as *C. xinyangensis* in Henan (He, 1980), *C. hangzhouensis* in Zhejiang (He, 1980), *C. sichuanensis* in Sichuan (He and Kou, 1984), *C. ziguiensis* and *C. brevinema* in Hubei (He and Xu, 1985, 2001) and *C. chuxiongensis* in Yunan (He et al., 2000). All such "new" species were identified by morphological characters only, despite morphological identification often being confused and controversial (Jankowski, 2001; He, 2003).

Due to the limited research on *Craspedacusta* and its plastic morphology, many questions on phylogenetic relationships within *Craspedacusta* remain unanswered. The lack of molecular evidence at that time resulted in this confusion. Species names are confusing and vary according to the species assignments in previous, mostly morphologically based studies (Jankowski, 2001; He, 2003; Fritz et al., 2009). Some individuals were named differently but molecular evidence later showed they are taxonomic synonyms (Zhang et al., 2009). Morphological identification alone cannot be a reliable approach of species determination. Proof from a molecular level is essential to identify species and even subspecies. Recently, the prevalence and extensive application of molecular markers has largely helped to uncover such taxonomic puzzles.

So far, four major mtDNA haplotypes seem to have invaded Europe based on mitochondrial markers. The first was reported by Fritz et al. 2009, based on specimens sampled at the medusa stage from Germany and Austria in 2006 and 2007. Subsequently the same haplotype was detected in Morocco (Africa) in 2015 (medusa stage, Oualid et al., 2019) and again in Germany between 2010 and 2011 (medusa stage, Grange et al., 2017). A second haplotype was found in Greece in 2014 (medusa stage, Karaouzas et al., 2015), the same as also found in China (medusa stage, Zou et al., 2012). Then a third haplotype was detected in southern Italy in 2017 (medusa stage, Shifani et al., 2019), which also occurred in Chile (South America) between 2014 and 2016 (medusa stage, Fuentes et al., 2019), in northern Italy in 2018 (medusa stage,

Morpurgo et al., 2021), in Canada in 2020 (medusa stage, Lüskow et al., 2021) and in USA (medusa stage, Collins et al., 2008). The fourth haplotype appeared in northern Italy in 2018 (medusa stage, Morpurgo et al., 2021), while a similar haplotype was already found in Switzerland in 2013 based on a singleton polyp sequence (unpublished). Overall, these four haplotypes were all part of a dataset described in Schachtl (2019), including 53 lakes from Africa, Austria, Czech Republic, Germany, and Greece between 2014 and 2017 based on both medusa and polyp stages; thereby providing a relatively comprehensive understanding of the distribution of medusae and polyps and their genetic diversity.

1.4.5 Ecological impact

Biotic impacts

As *Craspedacusta* are carnivorous and feed on zooplankton and fish eggs or larvae (DeVries, 1992; Spadinger and Maier, 1999), they have the potential to induce cascading food web effects, thereby affecting dynamics of freshwater ecosystems. Currently, most research on the ecological impacts of *Craspedacusta* is carried out at the medusa stage; this is due to its visible size and often dense bloom formation. However, it is still under debate whether the occurrence of medusae could profoundly influence aquatic food webs. For example, early work by Dodson and Cooper (1983) found evidence that medusae are not a threat to zooplankton stocks. However, recent evidence demonstrates that the presence of medusae can result in a significant decrease and mortality of zooplankton (Jankowski and Ratte, 2001; Boothroyd et al., 2002; Jankowski et al., 2005; Smith and Alexander, 2008, Himchik et al., 2021). It is well known that zooplankton plays a central role in aquatic food webs, transferring energy from primary producers to consumers, like invertebrates and fish (Lampert, 1997). Therefore, the loss of zooplankton by predation of freshwater jellyfish is a potential threat to fisheries and aquaculture. A recent report has shown that the estimated loss of fish products due to zooplankton reduction caused by the freshwater jellyfish *Craspedacusta* can reach up to 5740 t/year in a 400 km^2 reservoir in Ukraine (Himchik et al., 2021). Alternatively, *Craspedacusta* can also feed on fish eggs or larvae (DeVries, 1992; Spadinger and Maier, 1999), directly affecting the recruitment of fish. Furthermore, as already known from the short-lived but dense marine jellyfish blooms characterized by typical “boom and bust” cycles (Schnedler et al., 2018), the transient presence of enormous jellyfish biomass, and their subsequent decomposition can alter biogeochemical fluxes, microbial dynamics, and oxygen dynamics in

marine ecosystems both in the water column and on the seabed (Pitt et al., 2009; Sweetman et al., 2016; Guy-Haim et al., 2020; Tinta et al., 2020). Likewise, freshwater jellyfish blooms also appear in short-term boom and bust cycles and their degradation may cause similar effects in freshwater ecosystems. Given the potential adverse effects induced by *Craspedacusta* medusae on freshwater ecosystems, the increasing appearance of jellyfish blooms should be closely monitored and further studied.

In addition, as mentioned above (section 1.4.2), the nematocysts on medusa tentacles and polyp heads can eject toxins. For *Craspedacusta* such toxic effects are normally considered to be harmless for humans, as their nematocysts are not likely to penetrate their skin. So far, there is only one report on cutaneous envenomation by *Craspedacusta* medusae (Loeuillet et al., 2017). We therefore cannot fully rule out the possibility of potential detrimental effect of *Craspedacusta* to human health. Certainly, it can be helpful to analyse all venom components and amounts in *Craspedacusta* medusae and polyps for better risk assessments.

Abiotic effects on Craspedacusta growth patterns

Many studies have shown that temperature, osmotic pressure, light, pH, oxygen, water transparency, water current and CO_2 probably affect the growth and development of *Craspedacusta* individuals and populations (Brussock and Brown, 1985; Dumont, 1994; Folino-Rorem et al., 2016; Minchin et al., 2016; Zhang et al., 2016; Caputo et al., 2018). In brief, a detailed understanding of not only the dynamics of medusa populations but also the population growth dynamics of polyp populations is necessary to understand and potentially manage this invasive species. A mechanistic understanding of how environmental change may affect the population development of benthic polyps and consequently pelagic medusa is necessary to predict food web consequences of this “new” member of lake ecosystems and quantify potential harmful risks related to this species.

Global warming is leading to the temperature increase of surface waters in lakes worldwide (Schneider and Hook, 2010; O'Reilly et al., 2015), with projected increases in surface temperatures of about 2.5°C to 5.5°C by 2080-2100 (Woolway and Merchant, 2019). In addition, Pareeth et al. (2016) provided evidence that the increase rate of lake surface water temperatures during summer (0.36°C decade^{-1}) was higher than the mean annual increase rate of lake surface water temperatures (0.20°C decade^{-1}) based on a thirty-year dataset. The above cited long-term data all imply that higher lake temperatures at surface will become more

frequent, especially in summer. Currently, it is well known that increasing water temperatures induced by global warming promote marine jellyfish blooms (Purcell, 2007; Licandro et al., 2010; Boero et al., 2016) resulting in severe effects on the function of marine food webs and fisheries (Purcell, 2005; Richardson et al., 2009). The question arises whether similar effects from global warming on the structure and function of freshwater ecosystems will happen as well (Kundzewicz et al., 2008; Heino et al., 2009). Warming related enhancements of jellyfish abundances in lakes would even potentially introduce "new" food web structures and dynamics as jellyfish are not known from most temperate lakes since the introduction of *Craspedacusta* to Europe in the early 20th century.

Life cycle transitions in *Craspedacusta* are considered to be stimulated by temperature (McClary, 1959; Lytle, 1961; Acker and Muscat, 1976; DeVries, 1992; Folino-Rorem et al., 2016). The production of medusae is expected to strongly depend on temperature (McClary, 1959; Lytle, 1961). Warmer water temperature could initiate and enhance *Craspedacusta* medusa reproduction (Acker and Muscat, 1976; Lundberg et al., 2005; Minchin et al., 2016). However, far too little attention has been paid to whether increasing water temperature is also beneficial for the asexual propagation in the polyp stage. The asexual reproduction of *Craspedacusta* does not only include the polyp budding *per se* but also the production of frustules by polyps. The frustule buds from the side of the polyp, and it can move a short distance after falling from the polyp and subsequently differentiates to a polyp again, which provides a great pool for the flourishment of polyp populations (McClary, 1959; Dendy, 1978). If rapid polyp population expansion occurs at high temperatures, this would greatly accelerate medusae reproduction at warm seasons, which in turn may show considerable effects on freshwater ecosystems (Jankowski et al., 2005; Schachtl, 2019).

In addition to temperature, pH is also critical for maintaining the optimal growth and survival of aquatic organisms. Environmental parameters such as bedrock conditions, acid rain, wastewater discharge and carbon dioxide can affect the pH in water (Addy et al., 2004). Currently, elevated partial pressures of CO_2 (pCO_2) induced by burning fossil fuels has caused the decline of pH in marine ecosystems, as well as in freshwater ecosystems (Leduc et al., 2013). There has been extensive research on freshwater acidification, obviously leading to the loss of fish populations and subsequent adverse biological consequences (Henriksen, 1979; Raddum and Fjellheim, 1984; Sayer et al., 1993; Clair et al., 2007; Phillips et al., 2015; Hasler et al., 2018). A recent study describes that the growth rate of macrophytes and phytoplankton

increased in response to acidification, while conversely, growth was reduced in animals, such as fish, amphibians, and macroinvertebrates (Hasler et al., 2018). Research on the effects of acidification on cnidarians in general is an increasing research focus to date (Richardson and Gibbons, 2008; Klein et al., 2014; Nagelkerken et al., 2016; Tills et al., 2016; Chuard et al., 2019; Boco et al, 2020). Calcifying cnidarians (corals) are more strongly affected by ocean acidification than non-calcifying cnidarians such as jellyfish (Hoegh-Guldberg et al., 2007). Moreover, marine jellyfish are even expected to benefit from ocean acidification (Attrill et al., 2007).

A similar question arises for the effects of acidification on freshwater jellyfish, for example whether they are capable of tolerating acidification, and whether they could even gain advantages from these conditions. In general, the pH of most freshwater lakes ranges from 6 to 8 (Hasler et al., 2018) and seasonal pH fluctuations within a temperate lake is typically $\leqslant 1$ pH unit (Weisse and Stadler, 2007). The addition of carbonic acid to freshwater ecosystems accounts for only weak acidification, resulting in comparable small changes in environmental pH (1-2 pH units) (Hasler et al., 2018). It is hence interesting to know if and how *Craspedacusta* reacts to weak acidification in its environment, and how acidification potentially affects the development and growth dynamics of *Craspedacusta* polyps.

2 Research questions

In my thesis I tried to answer the following questions:

A. What is the genetic diversity of *Craspedacusta* at both medusa and polyp stages at the population level? To answer this question, I investigated the following aspects:

-) The genetic diversity of the *Craspedacusta* medusa stage in Germany &Europe
-) The genetic diversity of the *Craspedacusta* polyp stage in Germany &Europe
-) Is the genetic diversity similar/comparable at the polyp and medusa stages?
-) Is there a dominant genetic type existing at both stages?
-) Is there evidence for the presence of cryptic species?

B. What is the genetic structure of *Craspedacusta* at the medusae and polyp stages at the population level? To answer this question, I investigated following aspects:

-) What is the genetic structure of *Craspedacusta* medusae within a lake?
-) Is the genetic structure of medusa populations the same when comparing different populations?
-) What is the genetic structure of *Craspedacusta* polyps within a lake?
-) Is the genetic structure of polyp populations the same when comparing different polyp populations?
-) How large are differences in the genetic structure of medusa and polyp populations among lakes?
-) How large are differences in the genetic structure of polyp and medusa populations over time?
-) Is there a correlation between genetic distance and geographic distance when comparing *Craspedacusta* populations?

C. What are effects of basic abiotic variables on the development and growth patterns of *Craspedacusta* at the polyp stage? To answer this question, I investigated the following aspects:

-) How does increasing temperature affect the population growth dynamics of polyps and other benthic life stages of *Craspedacusta*?
-) How does weak acidification (pH decrease) affect the population growth dynamics of polyps and the production of frustules?
-) Are there differences in the growth dynamics of polyps of different genetic types?

3 Material and methods

3.1 Genetic population structure of medusae

3.1.1 Sampling

The majority of medusae used in my study originated from large available population samples of which only small subsets had been genotyped in a previous study (Schachtl, 2019). Jellyfish from eleven lakes in Germany, four lakes in the Czech-Republic and two lakes in Austria were sampled between June and October from 2015 to 2018. Medusae were caught by snorkelling and transported to the laboratory alive, whereupon they were preserved in 95% ethanol for later genetic analysis and stored at room temperature. In total, 870 individuals from 17 lakes were screened and included in my study (Fig. 5, Suppl. Tab. 1). Sample sets collected from single field sites during a single year were considered a population. Some lakes were re-sampled over time (Suppl. Tab. 1). The sampling design aimed to explore the genetic structure at different geographic scales and from year to year, to account for temporal changes. Therefore, samples were collected at local (from different lakes close to each other in a specific region separated by 0.3 to 5.5 km) and regional scales (from different geographic regions separated by 10 to 200 km) (Fig. 5, Suppl. Tab. 1). The focus of specimen collection at the local scale was a cluster of seven lakes distributed across approximately 5 km in the Ingolstadt Germany region (IG) in southern Germany (Fig. 5B, Suppl. Tab. 1). Other samples at the regional scale were from southern Germany (other areas in Germany (OG), four lakes), Austria (AU, two lakes) and Czech-Republic (CR, four lakes) (Fig. 5A, 5C and 5D, Suppl. Tab. 1). For the purpose of statistical analysis, individuals were assigned to four groups according to pairwise geographic distances between lakes. Groups included one local (IG) and three more distant regional groups (OG, AU and CR) (Fig. 5, Suppl. Tab. 1). In addition, all samples were pooled together in the "overall group" to analyse overall population patterns.

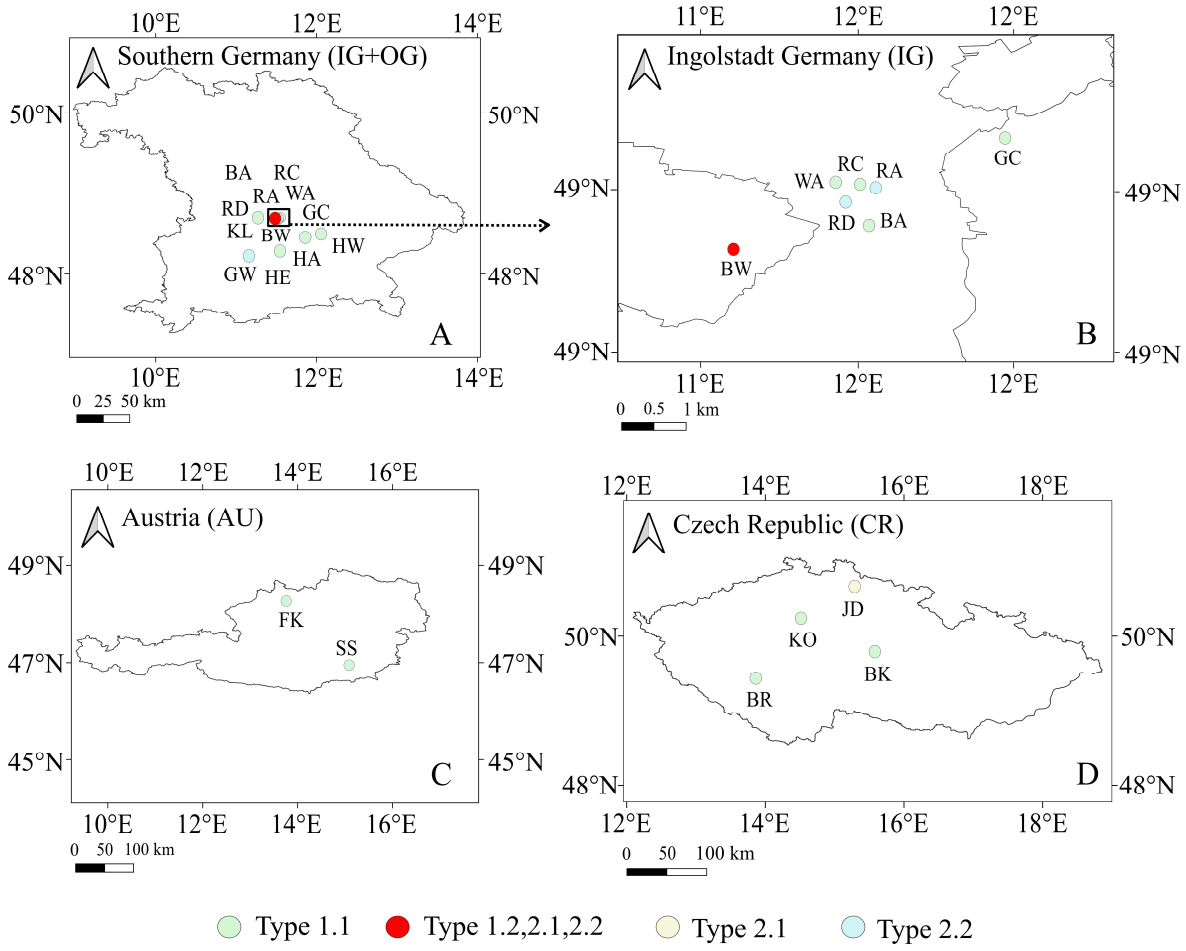

Fig. 5 Sampling locations for *Craspedacusta* medusae in Central Europe. Abbreviation of lakes as indicated in Suppl. Tab. 1. A: Southern Germany sampling (IG and OG (Other areas in Germany)); B: Ingolstadt Germany sampling (IG); C: Austria sampling (AU); D: Czech Republic sampling (CR). In the legend, green represents the lakes with only Type 1.1 medusae (including Type 1.1.2), yellow represents the lakes with only Type 2.1 medusae, blue represents the lakes with only Type 2.2 medusae, and red represents the lakes with mixed Type 1.2, 2.1, 2.2 medusae. The colour of haplotypes corresponds to Fig. 8 (section 4.1.1).

3.1.2 Jellyfish gender identification

All specimens were inspected for gender identity. With the aid of a binocular microscope, male and female individuals were identified by inspecting gonad tissues of mature medusae (see Schachtl, 2019). Eggs or sperm were identified in fresh and ethanol-preserved medusae, and the gender of all genotyped individuals was documented.

3.1.3 Sequencing

In total, 870 individuals were processed for mtDNA sequencing at the 16S rRNA locus. A subset of 337 individuals was additionally screened at the COI locus. DNA was extracted taking a piece from one gonad of a mature jellyfish using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufactures' protocol. DNA was eluted from silica columns in 200 µl AE buffer. This product was used as template. Polymerase chain reaction (PCR) was carried out in a total volume of 25 µl, containing 2 µl template DNA, 14.125 µl sterile H2O, 5 µl 5x reaction buffer, 0.5 µl dNTPs, 2.25 µl MgCl2, 0.5 µl of each primer (For 16S rRNA, 5'-TCGACTGTTTACCAAAAACATAGC-3'; 5'-ACGGAATGAACTCAAATCATGTAAG-3', Cunningham and Buss, 1993; for COI, 5'-CGCTGAGTATTTTCAACAAATCAC-3'; 5'-AACATGTGATGRGCCCAAAC-3', Sabine Gießler, pers. comm.) and 0.125 µl DNA polymerase (bioline MangoTaqTM). PCR amplification of 16S rRNA involved 5 min at 94°C, followed by 5 cycles of 50s at 94°C, 50s at 45°C and 1 min at 72°C and 30 cycles of 50s at 94°C, 50s at 50°C and 1 min at 72°C, ending by 5 min at 72°C. PCR amplification of COI started with 5 min at 94°C, followed by 30 cycles of 50s at 94°C, 50s at 58°C and 1 min at 72°C and a final step at 72°C for 5 min. The amplified products were verified on 1.2% agarose gels to ensure that they are the right target fragments without any contamination. Finally, PCR products were purified (see Schachtl, 2019) and sequenced in both forward and reverse directions using the same PCR primers and the BigDye v.3.1 sequencing kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer by the Genomics Service Unit at the LMU Biozentrum, Martinsried, Germany.

Due to the known high resolution and broader effectiveness of 16S rRNA barcoding in Cnidaria, especially in Hydrozoa (Collins at al., 2005; Moura et al., 2011; Zheng et al., 2014), all specimens were sequenced at the 16S locus. In addition, a subset of samples was sequenced at a second mtDNA locus, COI, to confirm the identification of two haplotypes (Type 1.1 and 1.2) which were based on 1 bp differences only when defined by 16S (Schachtl, 2019, Suppl. Tab. 2).

3.1.4 Environmental data

Water samples were collected in twenty lakes from southern Germany in July and August 2017 (Fig. 6, Suppl. Tab. 3). At each sampling site, temperature, pH, and dissolved oxygen were measured *in situ* in 1m intervals of the water column, with a multi-parameter probe (Exo 1©

YSI Inc., WTW GmbH, Xylem Inc.). The maximum depth was also recorded. Subsequently, 2L water samples from each lake were collected by an integrated tubular water sampler (KC Denmark A/S Research Equipment, Silkeborg). The water samples were then transported to the laboratory to analyze chemical parameters after a 250µm filtration to exclude mesozooplankton. Parameters such as total phosphorus (TP), chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), nitrite (NO_2^-), N:P, chlorophyll-a (Chl-a) were determined (Schachtl, 2019). In order to compare mean parameter values from the lakes which were of different depths, only values from the epilimnion were considered and averaged. To ascertain the presence of a thermocline occurrence within lakes, the shape of temperature-depth curves was used in respective plots. If a thermocline was observed, then the mean temperature, pH and dissolved oxygen values were calculated from the epilimnion layer. Otherwise, data from the whole water column were used to calculate respective means.

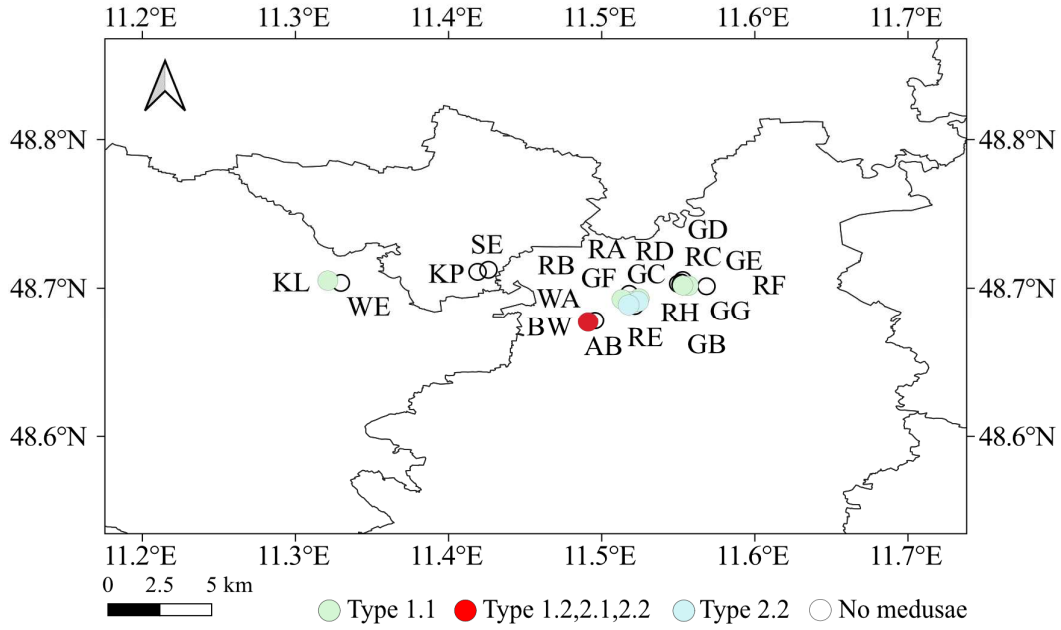

Fig. 6 Location of lakes to test for associations between physico-chemical parameters and medusa occurrence and haplotypes. Ten lakes without medusa and ten lakes with medusa occurrence were included in the 2017 lake survey in Southern Germany. Abbreviation of lakes as indicated in Suppl. Tab. 3. In the legend, green represents the lakes with only Type 1.1 medusae, blue represents the lakes with only Type 2.2 medusae, red represents the lakes with mixed Type 1.2, 2.1, 2.2 medusae and white represents the lakes without medusae occurrence. The colour of haplotypes corresponds to Fig. 8 (section 4.1.1).

3.1.5 Data analyses

MtDNA Phylogenies

For all individuals, the obtained PCR-products from the 16S rRNA and COI regions were sequenced in both directions and respective consensus sequences were generated in BioEdit 7.0.5.3 (Hall, 1999). Resulting consensus sequences were initially aligned using ClustalW (Thompson et al., 1994), with the default settings in BioEdit. The aligned datasets were corrected by eyes and gaps were introduced when necessary to minimize mismatches. Sequence datasets of 16S rRNA and COI were trimmed to a length of 593 bp and 600 bp, respectively. For later calculations, all sites containing gaps and missing data were eliminated from the aligned datasets.

The phylogenetic trees based on 16S rRNA and COI regions were both constructed by applying the Maximum Likelihood (ML) method based on the Kimura 2-parameter (K2P) model in MEGA 7, considering the estimated best-fit substitution models (Kumar et al., 2016). Bootstrap analyses (100 replicates) were performed to assess the relative robustness of internal nodes. To visualize mtDNA haplotype frequencies and their relatedness, the trees were displayed using appropriate settings at the iTOL online platform (Letunic and Bork, 2019).

The sequence of *Limnocnida tanganicae* (LT) from Lake Tanganyica in Africa sampled by my colleague Schachtl in 2015, was used as an outgroup in molecular phylogenies. *L. tanganicae* is also a freshwater jellyfish from the family Olindiidae and is only known to occur in Africa and India (Dumont, 1994; Jankowski, 2001).

Population diversity and differentiation

All subsequent analyses shown here were based on 16S mtDNA. Haplotype diversity (hd) and nucleotide diversity (π) were calculated in DnaSP 5.0 (Librado and Rozas, 2009). The levels of genetic diversity within and among different regions and years were compared using hierarchical analysis of molecular variance in Arlequin 3.5 (AMOVA; Excoffier and Lischer, 2010).

To test for a pattern of isolation by distance (IBD), I performed Mantel tests as implemented in Arlequin 3.5. Pairwise geographic distances were determined as shortest distances between sampling locations using Google Maps. One thousand permutations were conducted to assess

significance of correlations. Mantel tests were performed to test for local patterns (Ingolstadt Germany (IG), seven lakes), regional patterns (within OG (Other areas in Germany) (four lakes) and within CR (Czech-Republic) (four lakes)) and an overall pattern among all 17 lakes. Given that only two populations from Austria were available, a mantel test failed for the Austria region (AU).

Association between genetic structure and ecology

A principal component analysis (PCA, PRIMER v6, Clarke and Gorley, 2006) was performed to investigate the association between haplotype occurrence and environmental parameters (eleven physical and chemical variables) in all twenty lakes with special focus in six lakes in the Ingolstadt Germany region (IG) (Suppl. Tab. 3). Variables were normalized before analysis.

3.2 Mitochondrial genomes of medusae

3.2.1 Materials

The four specimens used for genomic analyses had been already genotyped at the COI and 16S loci (Schachtl, 2019) and were selected to represent each of the four major mtDNA haplotypes. Individuals were collected in three lakes in Germany (Type 1.1, lake Waldsee, 48°41'33.0"N, 11°30'47.9"E; Type 1.2, lake Neuer Baarer Weiher, 48°40'37.2"N, 11°29'28.0"E, and Type 2.2, lake near Reichertshofen, 48°41'28.0"N, 11°31'25.3"E) and one lake in Czech-Republic (Type 2.1, lake near Jílové/Držkova, 50°40'08.8"N, 15°17'21.5"E), preserved in 95% ethanol and kept at room temperature for genetic analysis (Tab. 7 (section 4.2.1)).

3.2.2 Sequencing

Genomic DNA was extracted from one gonad of a mature jellyfish using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufactures' protocol. Whole genome shotgun sequencing (WGS) was performed on an Illumina MiSeq (using v2.0 chemistry 500 cycles, 2x250 bp) to obtain genomic data from high abundance DNA. Using the Nextera XT DNA Library Prep kit (Illumina), libraries were obtained from total gDNA of one random representative sample from each of four mtDNA haplotypes using 2-5 ng DNA as template. Paired-end sequences were demultiplexed and subsequently merged using the VSEARCH suite v2.9.1 (Rognes et al. 2016). Similarly, merged reads from the four representative individuals

were mapped to a reference mitogenome from *C. sowerbii* (LN901194, Kayal et al. 2015, https://www.ncbi.nlm.nih.gov/) and assembled to obtain respective mitogenomes from the four mtDNA haplotypes. Available reference annotations were used to delimitate coding regions using the Geneious Prime 2021 software (Biomatters Ltd., Auckland, New Zealand). Respective individuals from each of the four mtDNA haplotypes were deposited at the SNSB - Zoologische Staatssammlung München (https://www.zsm.mwn.de/, contact: Eva Lodde-Bensch, lodde-bensch@snsb.de) under the voucher numbers ZSM 20211808-ZSM 20211811. The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. MZ508273 - MZ508276.

3.2.3 Data analyses

The phylogenetic tree was reconstructed by the concatenated protein coding regions using the Maximum Likelihood (ML) method, the Kimura 2-parameter (K2P) model in MEGA 7 (Kumar et al., 2016). The relative robustness of internal nodes was assessed by bootstrap analyses (1000 replicates).

Cnidarian mitogenomes published in Genbank, comprising two individuals from *Craspedcusta sowerbii* (JN593332, LN901194), five more Hydrozoan individuals from distantly related species (*Blackfordia virginica*: MW376866, *Cubaia Aphrodite*: JN700942, *Haliclystus antarcticus*: KU947038, *Haliclystus sanjuanensis*: JN700944, *Hydra oligactis*: NC_010214) and from the scyphozoan moon jellyfish *Aurelia aurita* (NC_008446), were used as references (downloaded from https://www.ncbi.nlm.nih.gov/).

3.3 Genetic population structure of polyps

3.3.1 Sampling

Polyp samples were randomly collected from lakes or rivers in Germany between July and November from 2015 to 2019 by the help of students and collegues (Fig. 7, Suppl. Tab. 5). Polyps collected from a single lake/river during a single year were considered a population. Colonies as defined in the literature (McClary, 1959) means single polyps and connected polyps sharing a same gastric cavity, starting with frustules differentiating into polyps at one or both ends. In the river Amper and in Langwieder See, whereupon no medusae have ever

been detected, polyps were found. All other lakes were characterized by their distribution of medusae belonging to different mtDNA haplotypes (Suppl. Tab. 5). Substrates, such as stones, twigs, wood, leaves from trees and lotus were randomly collected along the shoreline at a depth of 10-50 cm and were transported to the laboratory with the original lake/river water, and kept within small buckets (1L) in a climate chamber at 18°C. Substrates were then inspected for the presence of polyps by a binocular microscope with a maximum magnification of 25x, illuminated with a cold light source. Once polyps were found, they were gently removed from the substrate with a needle and transferred with a pipette to six-well plates (polystyrene; Greiner Bio-One, Kremsmünster, Austria) filled with *Hydra*/*Daphnia* medium (Folino-Rorem et al., 2016; Jeschke and Tollrian, 2000). After removing as much detritus as possible, polyps were transferred alive with forceps to the reaction tubes for DNA extraction. In total, 346 colonies from seven lakes and one river were genotyped (Suppl. Tab. 5).

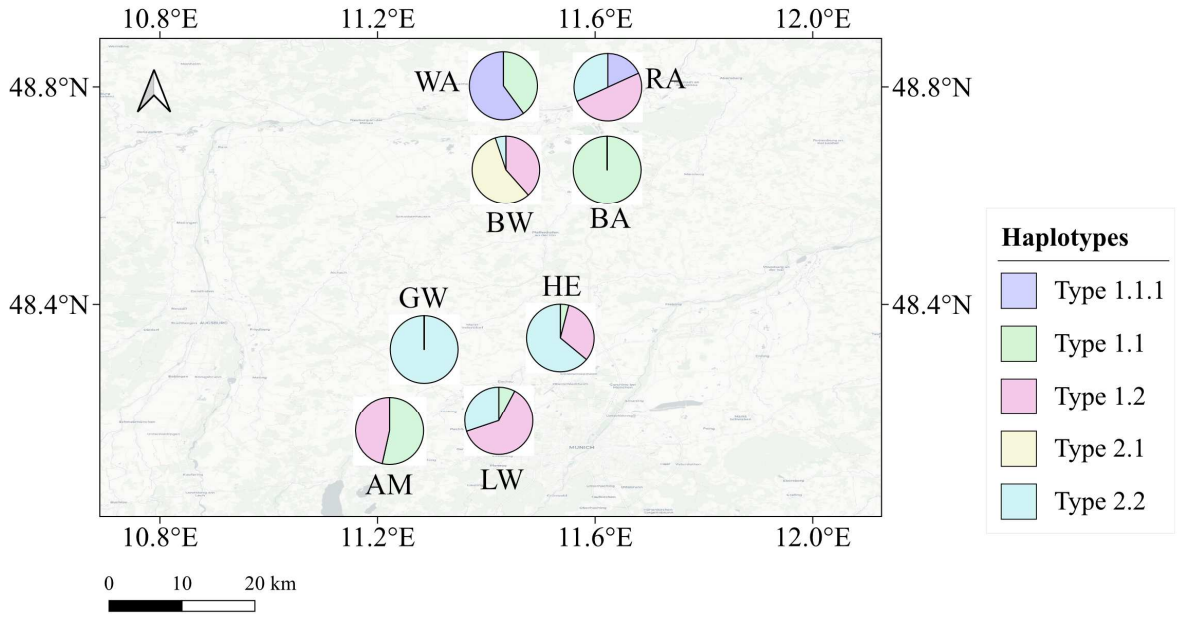

Fig. 7 Distribution and genetic diversity of polyp populations of *Craspedacusta*. Lake/river abbreviations follow those in Suppl. Tab. 5. Pie charts represent haplotype frequency in each lake/river; the colour of each haplotype is consistent with Fig. 14 (section 4.3.2).

3.3.2 Sequencing

In total, 346 colonies were processed for mtDNA sequencing. DNA was extracted taking a complete colony using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufactures' protocol. Amplification and sequencing followed procedures in section 3.1.3.

Initially, every colony was sequenced at both 16S rRNA and COI marker loci. It turned out that the electrophoretic received bands for COI PCR-products were much sharper than from 16S in polyps, and I thus decided to use COI sequencing for all following samples. In addition, more base pair differences were found in the COI than in 16S region among polyp mtDNA haplotypes. Hence, 16S sequencing data were only used to show consistency in haplotype identification (Suppl. Tab. 6). In short, both markers were applied on samples from Amper, Neuer Baarer Weiher and Langwieder See, while COI markers were exclusively used for all other lakes (Brandsee, Gernlindner Waldsee, Heiglweiher, Reichertshofen* Lake A and Waldsee).

3.3.3 Data analyses

I used ClustalW to align the sequences of 16S and COI, which were sequenced in both directions, according to the default settings in BioEdit 7.0.5.3 (Hall, 1999). The alignments were then corrected by eye and trimmed to a length of 600 bp, eliminating the positions with gaps and missing data for later analyses. Maximum Likelihood (ML) method combined with Kimura 2-parameter (K2P) distances in MEGA 7 were applied to generate the phylogenetic tree based on COI sequence alignments. The relative robustness of internal nodes was assessed by bootstrap analyses (100 replicates). I chose *Limnocnida tanganicae* (LT) (sampled and sequenced by Schachtl, 2019) as the outgroup as it belongs to freshwater jellyfish as well but is positioned within a different genus (Dumont, 1994; Jankowski, 2001). The trees were visualized using appropriate settings at the iTOL online platform (Letunic and Bork, 2019).

Here, all subsequent analyses were based on COI mtDNA sequencing. Haplotype diversity (h) and nucleotide diversity (π) were calculated in DnaSP 5.0 (Librado and Rozas, 2009). Hierarchical analysis of molecular variance (AMOVA), evaluated with 1000 permutations (Arlequin 3.5, Excoffier and Lischer, 2010), was applied to partition genetic variation to both within population components and among population comoponents, while also accounting for a temporal component between years (Φ_{ST}). In order to compare the genetic differences between lakes/rivers, we also calculated pairwise F_{ST}, where three temporal populations from Langwieder See (LW) were pooled together representing a whole large population, and simultaneously we calculated pairwise F_{ST} among those three temporal populations as well to reveal the variations between the three years. 100 permutations were used to evaluate the significance of F_{ST} and $p < 0.05$ was considered as significant. Additionally, a Mantel test was

performed to test the correlation between geographic and genetic distances (F_{ST}). I used shortest straight-line distances between sampling locations to estimate the pairwise geographic distance between sites.

3.4 Temperature effects on polyp population development

3.4.1 Materials

Polyps were taken from laboratory stock cultures raised at 18°C in a climate chamber under dark/dim light conditions. They were initially collected from field samples, and screened from stones, wood, and twigs, which were sampled from lakes (Baarer Weiher, Bodensee, Fridolfinger See, Gernlindener See, Gernlindner Waldsee and Langwieder See) or rivers (Amper and Maisach) along the shoreline during summer and autumn between 2018 and 2019 in southern Germany. Polyps from different lakes or rivers were separately cultured in six-well plastic culture plates (polystyrene; Greiner Bio-One, Kremsmünster, Austria) and fed with rotifers.

3.4.2 Food

Brachionus calyciflorus (Rotifera) were cultured in 800 ml *Daphnia* medium (Jeschke and Tollrian, 2000), refreshed three times a week, and fed with green algae (*Scenedesmus obliquus*) *ad libitum*. During refreshing, a 50 µm mesh was used to remove mature *B. calyciflorus* individuals as polyp food. Refreshed *B. calyciflorus* cultures were obtained by inoculating fresh medium with individuals smaller than 30 µm.

3.4.3 Standardization

The use of the term "colony" is described in section 3.3.1. Two colonies formed by two connected mature big polyps were used as set-ups for experimental units. They were randomly selected from stock polyp cultures, softly separated from the bottom using a needle, picked out by a pipette, seeded into a new well plate and cultured in the dark in an 18°C climate chamber. It should be noted that polyps were immediately fed with *B. calyciflorus* after they were transferred to the new habitat to prevent the transformation from polyps to frustules. Culture medium was refreshed three times a week and cultures were fed after medium exchange. After one week, polyps were starved for two days before the start of the experiment to standardize

the polyp conditions. The number of colony and polyp per well was finally checked to be sure that two colonies, namely four polyps, were in each well. Budded frustules and polyps were removed if they occurred. Standardized polyps were then exposed to the corresponding experimental temperatures.

3.4.4 Experimental design

Six replicates were set up at two different temperature treatments, 18°C and 26°C. 48 polyps, namely 24 colonies, were kept in six-well plates with two colonies per well in a volume of 10ml *Hydra* Medium (HM) (Folino-Rorem et al., 2016). When polyps reached the water surface, an additional 2ml of HM were added in each well. During experiments, medium was refreshed three times a week, and polyps were fed afterwards. Incubation water was replaced by fresh HM after counting the number of colonies, polyps, and frustules within wells. Polyps were *ad libitum* fed with *B. calyciflorus* after refreshing. Plates were placed in the dark at two different temperatures, 18°C and 26°C, respectively. The experiment lasted for 73 days. In order to measure the wet weight of polyps, ten two-polyp colonies were randomly selected from each well at the end of the experiment. Three replicates with ten frustules, length between 0.3 and 0.5mm, were randomly picked out from plates at each temperature. Tin cups (HEKAtech GmbH, Wegberg, Germany) were pre-weighed. Wet samples were placed in tin cups and weighed. A Sartorius microbalance (sensitivity 0.1µg) was used to determine weight.

3.4.5 Data analyses

Here, the total number of polyps is defined as the size of the polyp population. The growth rate of polyp populations and the production rate of frustules released by polyps was calculated by $g = (\ln N_t - \ln N_1)/t$, where N_1 and N_t are the total number of polyps or frustules initially and after t days. Since the initial number of frustules was 0, the number of frustules at day one and t days were transformed as N_1+1 and N_t+1, respectively. The growth rate of polyp populations and production rate of frustules at the last day of the experiment were used to test for statistically significant differences ($p < 0.05$) between treatments.

ANOVA analysis was used when normality (Shapiro Wilk) and homogeneity of variance (Levene's test) assumptions were fulfilled, otherwise an equivalent non-parametric Mann-Whitney test was used. A Welch ANOVA test was performed when normality (Shapiro Wilk)

was met but variances were not homogenous. All statistical tests were done as implemented in SPSS 19.0.

3.5 pH effects on polyp population development

3.5.1 Materials

From the available polyps in our laboratory, four assemblages were established by polyps with known mitochondrial haplotypes, randomly taken from different sampling sites to provide pooled polyp stocks for later experiments (Tab. 1). PA1 assemblage was made up of polyps from Amper and Bodensee, where we found Type 1.1 polyps; PA2 assemblage was built by polyps from Amper, Baarer Weiher, Fridolfinger See, Gernlindener See, Langwieder See and Maisach, where Type 1.2 polyps had been found; PA3 assemblage consisted of polyps from Neuer Baarer Weiher and Gernlindener See, where we discovered Type 2.1 polyps; PA4 assemblage included polyps from Neuer Baarer Weiher, Gernlindener See, Gernlindner Waldsee and Langwieder See, where Type 2.2 polyps were detected (Tab. 1). These four polyp stocks were kept in six-well plastic culture plates (polystyrene; Greiner Bio-One, Kremsmünster, Austria) with a volume of 10ml *Hydra* Medium per well (Folino-Rorem et al., 2016) at 18°C, refreshed once a week, and fed with *Brachionus calicyflorus*. Because the unintended transfer of frustules or resting stages can happen during the establishment of polyp stocks from the four haplotypes, the genotypic purity of assemblages was tested at the end of the experiment by resequencing subsamples from all experimental units. Feeding and medium exchange followed the described protocol above (section 3.4.2).

Tab. 1 Summary of individual assemblage composition and mtDNA haplotypes identification

Assemblages	Origin	Lake / river	Haplotype_beginning (One colony used for identification)	Haplotype_end (Three colonies used for identification)
PA1	Amper	river	1.1	1.2
	Bodensee	Lake	1.1	
PA2	Amper	river	1.2	1.2
	Baarer Weiher	Lake	1.2	
	Fridolfinger See	Lake	1.2	
	Gernlindener See	Lake	1.2	
	Langwieder See	Lake	1.2	
	Maisach	river	1.2	
PA3	Baarer Weiher	Lake	2.1	1.2
	Gernlindener See	Lake	2.1	
PA4	Baarer Weiher	Lake	2.2	2.2
	Gernlindener See	Lake	2.2	
	Gernlindner Waldsee	Lake	2.2	
	Langwieder See	Lake	2.2	

3.5.2 Standardization

Four frustules were transferred to each well of six-well plastic culture plates (polystyrene; Greiner Bio-One, Kremsmünster, Austria) using a pipette, kept in 10ml *Hydra* Medium (HM), and acclimatized at 18℃ in the dark without food to differentiate into polyps. Before the start of the experiment, the number of colonies in each well was standardized at two. During this period, if less than two colonies occurred in one well, more frustules were added until at least two colonies settled down. This process lasted for about three weeks. The use of the term "colony" is described in section 3.3.1.

3.5.3 Experimental approach

The pH was tested with a portable pH meter (Maozua), which was calibrated to an accuracy of 0.01 units by using 4.00, 6.86 and 9.18 buffer solutions. *Hydra* Medium (HM) treatment was always used as control as it is normally used to culture polyps in the laboratory. The pH was adjusted to the desired level with 0.2M $NaH_2PO_4 \cdot H_2O$ (pH = 4.5). Four levels, 8.5, 8, 7.8 and 7.6 were established to test the response of polyps. Every 2-3 days, the incubation water was

replaced by 10ml fresh solution after counting the number of colonies. The pH was measured before and after refreshing. Three replicates were set up for every treatment.

Two experiments were performed. Experiment 1 was conducted to explore the effect of a gradual change in pH, (within a range of one pH unit), on the asexual reproduction of polyps. Experiments were performed at two different temperatures to further examine whether potential pH effects on growth depend on temperature. The polyps were from four different assemblages (PA1, PA2, PA3, PA4), each of which included mixed polyps with same haplotypes from different lakes/rivers (Tab. 1, section 3.5.1). In total, 192 colonies were assigned to the eight combinations of two temperatures (18°C and 26°C) and four pH levels (8.5, 8, 7.8, 7.6). Each well contained two colonies in the beginning. Polyps were refreshed three times a week and were given *ad libitum* access to *Brachionus calyciflorus* after refreshing. During the experiments, all plates were covered with blue plastic lids (LEE-183-Moonlight Blue, ZILZ direct, Germany) to simulate moonlight which has been reported to favour medusa budding (Peukert, 2009). The experiment ran for 36 days. Experiment 2 aimed to examine clonal effects on asexual reproduction and their response to different pH at 26°C. Polyps originated from four different lakes. Three lakes are located in southern Germany, Langwieder See (LS), Fridolfinger See (FS) and Bodensee (BS) and one lake in Austria, Schwarzlsee (SS). The stock polyps from Bodensee (BS) were assigned as Type 1.1 and stock polyps from the other three lakes were all sequenced as Type 1.2. The factors tested in Experiment 2 were clones at four levels (LS, FS, BS, SS) and pH at two levels (8.5, 7.8). Altogether, 48 colonies were established. Polyps were also refreshed three times a week but were fed with exactly 20 *B. calyciflorus*/well. The experiment lasted for 39 days.

3.5.4 Molecular analyses

At the end of each experiment, three colonies were randomly picked from each treatment of the two experiments to confirm haplotype identify. DNeasy Blood & Tissue Kit (QIAGEN, Germany) was used for the DNA extraction following the manufactures' protocol. Amplification and sequencing followed procedures in section 3.1.3.

3.5.5 Data analyses

The pH variations in each treatment were measured and the mean pH was calculated according to the initial and final pH. Here, the total number of colonies was defined as the size of the

polyp population. The calculation of the growth rate of polyp populations and the production rate of frustules by polyps was the same as described in section 3.4.5. To find statistically significant differences ($p < 0.05$) on the number of colonies and frustules among different treatments, the growth rate of polyp populations and production rate of frustules by polyps at the last experimental day was compared.

The same ANOVA analyses as described in section 3.4.5 were used when appropriate. When not appropraite, equivalent non-parametric tests were applied, either Mann-Whitney tests (2 samples) or Kruskal-Wallis tests (more than 2 samples). SPSS 19.0 was employed for all statistical analyses.

4 Results

4.1 Medusa populations' genetic structure

4.1.1 MtDNA haplotypes and phylogenies

Four clusters were resolved based on the 16S mtDNA phylogeny (Fig. 8). Two main clusters 1 and 2 were clearly separated by 100% bootstrap support and both main clusters contained two sub-clusters each. These four mtDNA haplotypes were labelled with Type 1.1, Type 1.2, Type 2.1, and Type 2.2 (Fig. 8, Suppl. Tab. 1) according to haplotype IDs in Schachtl (2019). Individuals sequenced by both markers, 16S and COI, accounted for 38.7% of all samples. Haplotype consistency between 16S and COI sequence information was 100% regarding the fixed sites identifying the four different main haplotypes (Fig. 9, Suppl. Tab. 2). Only few of the Type 1.1 individuals shared an additional mutation defining Type 1.1.1 for COI (from Waldsee, Fig. 9b, Suppl. Tab. 2) and Type 1.1.2 for 16S was a singleton mutation in a single individual (from Geisenfeld * Lake C, Fig. 8, Suppl. Tab. 1). The mutations Type 1.1.1 and 1.1.2 were both nested in Type 1.1.

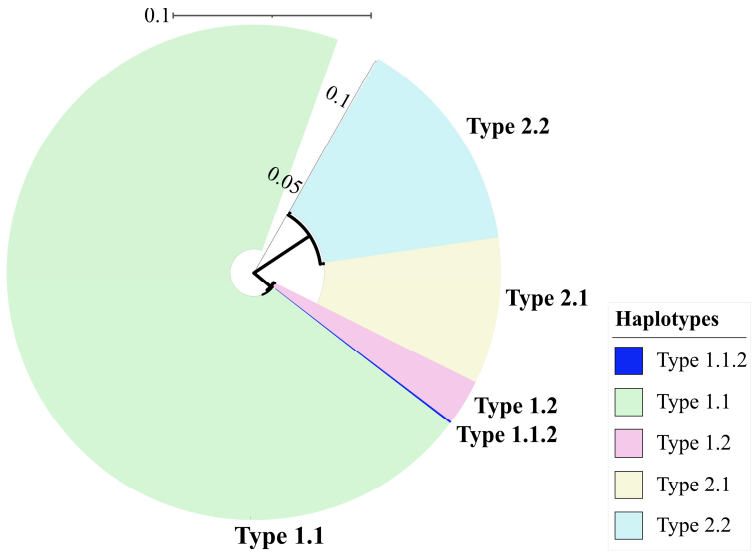

Fig. 8 Maximum Likelihood tree (ML-tree) based on the sequence variation of the mitochondrial 16S rRNA gene in *Craspedacusta* medusae (593 bp, 870 individuals). Shown is the frequency of mtDNA haplotypes and their genetic divergence (scale bar represents the K2P (Kimura 2-parameter) genetic distance). The branch widths represent bootstrap values between 0.6 and 1.

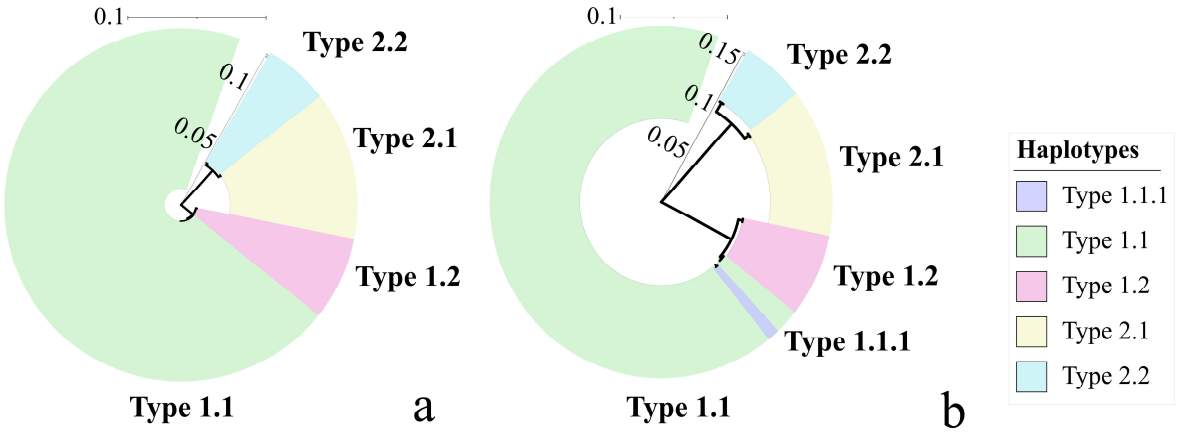

Fig. 9 Maximum Likelihood trees (ML-trees) of *Craspedacusta* medusae based on sequence variation of a subset of 337 individuals which were all genotyped at two mitochondrial loci: 16S (593bp, Fig. 9a) and COI (600bp, Fig. 9b). Shown is the consistency in haplotype-identification, the genetic divergence between haplotypes and their frequencies. The branch widths represent bootstrap values between 0.6 and 1. Scale bars indicating K2P (Kimura 2-parameter) genetic distance are given for each tree. For more details see Supp. Tab. 2.

4.1.2 Association between medusae gender and mtDNA haplotypes

Haplotype identity was associated with gender in both major lineages 1 (Type 1) and 2 (Type 2). In specific, all Type 1.1 (n=630) and Type 2.2 (n=128) individuals were females also the subtypes 1.1.1 and 1.1.2. On the contrary, all Type 1.2 (n= 26) and Type 2.1 (n=85) medusae were males. This indicated that the male and female medusae possess different mtDNA haplotypes also seen at a large population level including almost one thousand adult individuals. Notably, female medusae were most frequent in the population samples in both lineages (Fig. 8, Suppl. Tab. 1). About 72% of all screened individuals were 1.1 females and about 15% 2.2 females, meaning that about 90% of the individuals analysed were females and only about 10% were males. Regarding males, only 3% of all individuals belonged to the male Type 1.2 and almost 10% to the male Type 2.1. Notably, 16 lakes harboured a single gender: 12 lakes with Type 1.1-females, three lakes with Type 2.2-females and one lake with Type 2.1-males. Only in one lake, Neuer Baarer Weiher, mixed gender and haplotypes co-occurred. Interestingly, in the Ingolstadt Germany region (IG) in nearly all lake populations only females were found, as only in Neuer Baarer Weiher were males also found (Suppl. Tab. 1).

4.1.3 Haplotype frequencies

Overall, four different major mtDNA haplotypes were detected, additionally two rare subtypes (Type 1.1.1 and 1.1.2) were found among the most frequent Type 1.1. Individuals from the Type 1 lineage were the most frequent ones. At the overall scale, 630 individuals from eleven lakes belong to the Type 1.1 cluster (Fig. 8, Suppl. Tab. 1). Type 1.2 was the rarest haplotype and only found in Neuer Baarer Weiher (Fig. 8, Suppl. Tab. 1). Interestingly, there was no Type 1.1 individual found during three consecutive years from 2016 to 2018 in that lake, while Type 2.1 was the most frequent haplotype over time (Fig. 10, Suppl. Tab. 1). Overall, individuals from the Type 2 lineage were rare (Fig. 8, Suppl. Tab. 1). Type 2.1 was found in two lakes: Neuer Baarer Weiher and "Jílové / Držkova", while Type 2.2 was found in four lakes: Neuer Baarer Weiher, Reichertshofen* Lake D, Reichertshofen* Lake A and Gernlindner Waldsee. In the Ingolstadt Germany region (IG), other areas in Germany (OG) and in Czech-Republic (CR)), individuals from both lineages, Type 1 and 2, were found (Tab. 2, Suppl. Tab. 1). In Austria (AU), individuals from the two lakes sampled were only from Type 1.1. To summarize, Type 1.1 was consistently the most abundant haplotype. The overall frequencies of respective haplotypes and respective 1.1 subtypes were Type 1.1 (72.4%) > 2.2 (14.7%) > 2.1 (9.8%) > 1.2 (3.0%) > 1.1.1 (0.5%) > 1.1.2 (0.1%) (Fig. 8, Fig. 9, Suppl. Tab. 1).

4.1.4 Genetic diversity

Within lakes, genetic diversity was usually zero except in Geisenfeld* Lake C, where additionally to Type 1.1 also Type 1.1.2 (only 1 out of 40 individuals), and in Neuer Baarer Weiher, where three haplotypes co-occurred (Suppl. Tab. 1). Temporal variations in genetic diversity were only found in Neuer Baarer Weiher, where the haplotype diversity in different years was 0.154 (2016, two haplotypes), 0.655 (2017, 3 haplotypes) and 0.050 (2018, two haplotypes), respectively (Fig. 10, Tab. 3).

Measures of local, regional, and overall population diversity were generally moderate, with haplotype diversity ranging from 0.322 to 0.550 (Tab. 2). Nucleotide diversity was low, ranging from 0.015 to 0.020 (Tab. 2). At the local scale, genetic diversity was highest in the Ingolstadt Germany region (IG, 0.550) (Tab. 2).

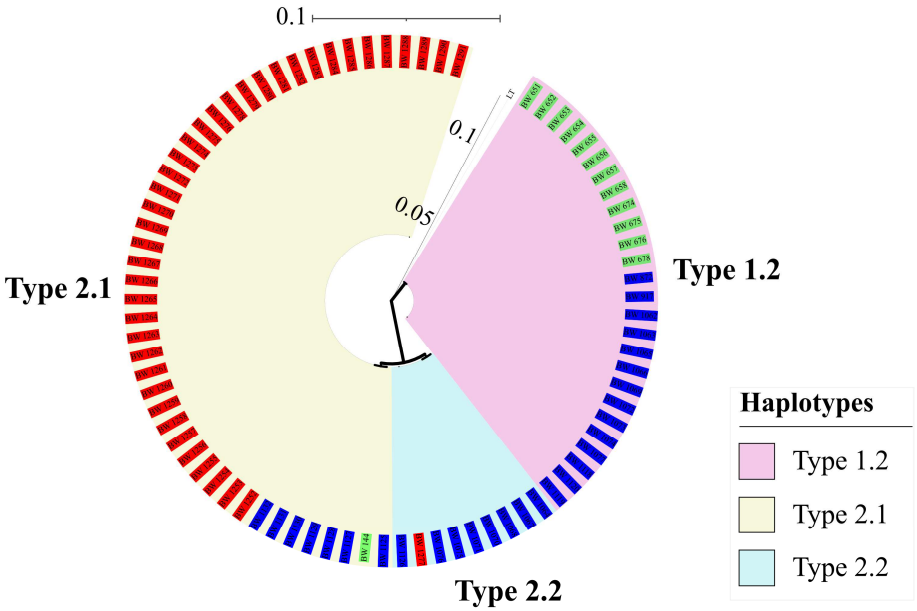

Fig. 10 Co-occurrence and frequency of mtDNA haplotypes among *Craspedacusta* medusa individuals from Neuer Baarer Weiher in 2016 (green bars), 2017 (blue bars) and 2018 (red bars) based on the variation of the mitochondrial 16S rRNA gene (593 bp, 82 individuals). Shown are phylogenetic relationships and genetic divergence between individuals in a Maximum Likelihood tree (ML-tree). Scale bar represents the K2P (Kimura 2-parameter) genetic distance. The branch widths represent bootstrap values between 0.6 and 1.

Tab. 2. Genetic diversity of *Craspedacusta* medusae in each region based on 16S rRNA. N_lakes: number of lakes within regions, Nseq: number of sequenced individuals, Nhap: number of haplotypes within regions, hd: haplotype diversity, π: nucleotide diversity, F: female, M: male. IG: Ingolstadt Germany, OG: Other areas in Germany.

Region	**N_lakes**	**Nseq**	**Haplotype frequency**					**Nhap**	**hd**	**π**
			1.1 F	**1.1.2 F**	**1.2 M**	**2.1 M**	**2.2 F**			
IG	*7*	*437*	*0.629*	*0.002*	*0.059*	*0.108*	*0.201*	*5*	*0.550*	*0.020*
OG	*4*	*200*	*0.800*	*0*	*0*	*0*	*0.200*	*2*	*0.322*	*0.015*
Germany (IG+OG)	11	637	0.683	0.002	0.041	0.074	0.200	5	0.487	0.018
Austria	2	80	1.000	0	0	0	0	1	0.000	0.000
Czech Republic	4	153	0.752	0	0	0.248	0	2	0.376	0.017
All	17	870	0.724	0.001	0.030	0.098	0.147	5	0.444	0.017

Tab. 3 Temporal genetic diversity for *Craspedacusta* medusae samples from three lakes in Germany based on 16S rRNA (*indicates closest town to lake). ∑: sum of sequenced individuals, Nhap: number of haplotypes, F: female, M: male.

Lake (Abb.)	***Latitude, Longitude***	**Country**	**Year**	**Individuals with Type**				∑	**Nhap**
				1.1 F	**1.2 M**	**2.1 M**	**2.2 F**		
Neuer Baarer Weiher (BW)	*48.6770, 11.4911*	Germany	2016	0	12	1	0	13	2
			2017	0	14	7	8	29	3
			2018	0	0	39	1	40	2
			BW total	0	26	47	9	82	3
Haselfurther Weiher (HW)	*48.4820, 12.0130*	Germany	2015	40	0	0	0	40	1
			2016	40	0	0	0	40	1
			HW total	80	0	0	0	80	1
Waldsee (WA)	*48.6925, 11.5133*	Germany	2015	37	0	0	0	37	1
			2016	40	0	0	0	40	1
			2017	40	0	0	0	40	1
			2018	40	0	0	0	40	1
			WA total	157	0	0	0	157	1

4.1.5 Spatial population structure

Hierarchical analysis of molecular variance (AMOVA) showed that independent of the spatial scale, the largest level of genetic differentiation was found among populations (Tab. 4). Regarding the overall pattern, there was no other significant variance component due to the low variation within populations (5%) and among regions (1%). Locally, 11% of the genetic variance was observed within populations, while no additional variance was found at the regional scale (Tab. 4).

The Mantel Test showed no significant support for isolation by distance (IBD) in *Craspedacusta* medusa populations on local (IG: r = -0.19, p = 0.78), regional (RG: r = 0.47, p = 0.25; CR: r = 0.11, p = 0.50) or overall scales (r = 0.02, p = 0.36).

Tab. 4 Spatial analysis of molecular variance (AMOVA) among 16S mtDNA sequences from *Craspedacusta* medusa individuals. Grouping of populations into regions as indicated in Suppl. Tab. 1. IG: Ingolstadt Germany, OG: Other areas in Germany, CR: Czech-Republic.

Pattern	Region	Source of variation	d.f.	Variance components	Percentage variation	Fixation indices	Significance
Local pattern	IG	among populations	6	0.443 Va	88.85	F_{ST} = 0.889	$p < 0.001$
		within populations	430	0.056 Vb	11.15		
		total	436	0.499			
Regional pattern	OG	among populations	3	0.500 Va	100.00	F_{ST} =1	$p < 0.001$
		within populations	196	0.000 Vb	0.00		
		total	199	0.500			
	CR	among populations	3	0.500 Va	100.00	F_{ST} = 1	$p < 0.001$
		within populations	149	0.000 Vb	0.00		
		total	152	0.500			
Overall pattern	all popula-tions	among regions	3	0.004 Va	0.88	F_{CT} = 0.009	$p = 0.07$
		among populations within regions	13	0.469 Vb	93.54	F_{SC} = 0.944	$p < 0.001$
		within populations	853	0.028 Vc	5.58	F_{ST} = 0.944	$p < 0.001$
		total	869	0.501			

4.1.6 Temporal population structure

Three lakes were sampled in different years, Neuer Baarer Weiher (2016, 2017, 2018), Haselfurther Weiher (2015, 2016) and Waldsee (2015, 2016, 2017, 2018) (Tab. 3). Interestingly, temporal variability in haplotype composition of populations was found only in Neuer Baarer Weiher while in the other two lakes the composition was stable up to four years (Tab. 3). More specifically, at least two haplotypes were found within the different years in Neuer Baarer Weiher despite low jellyfish densities and sample sizes in two years (Tab. 3). Notably, three haplotypes occurred in 2017 (Fig. 10, Tab. 3). Type 2.1 medusae were rare in 2016 and 2017 while they were the most abundant in 2018 (Fig. 10, Tab. 3). This was the reason why in Neuer Baarer Weiher, most of the variation was explained by the among-year component (71%), which was more than twice as high as the within-year component (29%) (Tab. 5).

Tab. 5 Temporal variation of haplotype composition in Neuer Baarer Weiher. Analysis of molecular variance (AMOVA) based on 16S mtDNA sequences of *Craspedacusta* medusae.

Source of variation	d.f.	Variance components	Percentage variation	Fixation indices	Significance
among years	2	0.351 Va	71.46		
within years	79	0.140 Vb	28.54	F_{ST}= 0.715	$p < 0.001$
total	81	0.491			

4.1.7 Association between haplotypes and environmental parameters

In total, twenty lakes were investigated (Fig. 6, Suppl. Tab. 3), ten lakes with medusae and ten lakes without medusae, to explore the relationship between medusa occurrence and environmental factors in lakes. In order to characterize lakes by ecological parameters, two main principal components were extracted based on eigenvalues > 1 in the twenty lakes PCA analysis (Tab. 6). Comparing the lakes with and without medusae (Fig. 11A), the first principal component, PC1, explained 30.1 % of the variance in the data which was mainly related to total phosphorus positively, and chloride, sulfate, and oxygen values negatively (Tab. 6, Suppl. Tab. 4). The second principal component, PC2, explained 26.4% of the variance (Tab. 6). Depth, temperature, and pH were negatively related to PC2 but nitrate, nitrite, nitrogen to phosphorus ratios and chlorophyll-a positively (Suppl. Tabl. 4). The ecological parameters of some lakes without medusae and with Type 1.1 medusae were similar (Fig. 11A). Type 2.2 medusae occurrence was related to high temperature, pH, and oxygen but low chlorophyll-a, nitrate, nitrite, and sulfate values. Notably, the lake condition for the occurrence of medusae with mixed haplotypes (Neuer Baarer Weiher) were different as detailed below.

Additionally, to explore the chemical and physical characteristics of closely located lakes and their association to medusae haplotype occurrences, a subset of six lakes located in the Ingolstadt Germany (IG) region, as defined in section 3.1.1, were selected because of their close geographical neighbourhood to each other but a simultaneous high diversity of haplotype composition of medusae (Suppl. Tab. 3). Based on eigenvalues > 1, PC1 and PC2 explained 54.3 % and 24.2 % of the variance respectively (Tab. 6). A similar pattern as seen by the PCA analysis of twenty lakes appeared (Fig. 11, Suppl. Tab. 4). The environmental parameters from lakes located only hundreds of meters apart were very diverse, and the most pronounced difference was found for Neuer Baarer Weiher.

Notably, in both PCA analyses, component loadings of environmental data from Neuer Baarer Weiher, the only lake which harboured three different haplotypes, placed this lake apart from all others. Neuer Baarer Weiher was special in showing relatively low pH, temperature and depth values and high chlorophyll-a, nitrate, nitrite, and total phosphorus values in contrast to other lakes (Fig. 11, Suppl. Tab. 4).

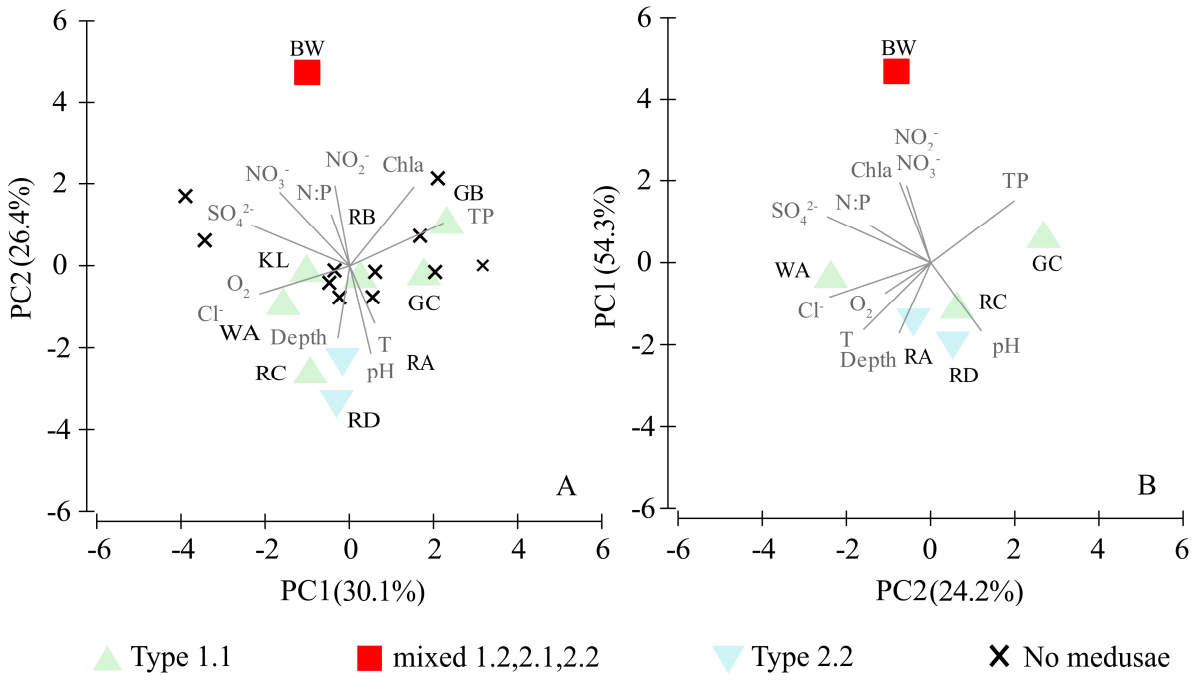

Fig. 11 Association between the occurrence of *Craspedacusta* medusa haplotypes and environmental factors. (A) PCA based on all twenty lakes, (B) PCA based on a subset of six closely located lakes in Ingolstadt Germany region. The axes were rotated in B with PC1 in the Y-axis and PC2 in the X-axis to allow for comparison. For selection of lakes and lake names see Suppl. Tab. 3.

Tab. 6 Eigenvalues of each PC in principal component analyses

Lakes	PC	Eigenvalues	Variation/%	Cum.Variation/%
All twenty lakes	1	3.31	30.10	30.10
	2	2.90	26.40	56.50
	3	1.14	10.30	66.80
	4	0.95	8.70	75.50
	5	0.87	7.90	83.40
Six closely located lakes in Ingolstadt Germany region	1	5.97	54.30	54.30
	2	2.66	24.20	78.40
	3	1.27	11.50	90.00
	4	0.58	5.30	95.20
	5	0.53	4.80	100.00

4.2 Medusa mitochondrial genomes

4.2.1 Mitogenome structure

The total lengths of complete mitochondrial genomes of each haplotype were ranging from 17861 to 19194 bp (Tab. 7). Consistent with already published mitochondrial genomes of *Craspedaceusta* (Zou et al. 2012; Kayal et al. 2015), 13 protein coding genes, 2 tRNAs, 2 rRNAs, and 2 putative ORFs were identified in the four individuals. The average GC content ranged from 39.2% to 43.2% (Tab. 7). All protein coding genes encoded with the typical initiation codon ATG and stop codon TAA and TAG but COI, which started with CTA and terminated with CAT. Two tRNAs were 71bp in size.

Notably, pairwise differences between Type 2.1 and 2.2 spanning the whole mitogenomes were about 8x greater between 2.1 and 2.2 Types than between 1.1 and 1.2 Types regarding bp differences and p-distances (Tab. 8). While several genes, such as ND5, ND4, ND2, CYTB, ND1 and ND6, showed base pair differences greater or equal to 20 between Types in cluster 2. Within cluster 1, the locus showing largest differences between Types was COI at a much lower level (8 bp differences) (Tab. 8).

Tab. 7 The length and GC content of four respective complete mitochondrial genomes of *Craspedacusta* medusae

Individual	Sample name	Lake	Country	Haplotype	Length	G+C
KS762	WA762	Waldsee	Germany	1.1	19194 bp	39.4%
KS872	BW872	Neuer Baarer Weiher	Germany	1.2	17861 bp	39.2%
KS411	JD411	Jílové/Držkova	Czech-Republic	2.1	18971 bp	43.2%
KS802	RA802	Reichertshofen* A	Germany	2.2	18774 bp	42.8%

Tab. 8 Characterization of mitochondrial genomes from the four common invasive *Craspedacusta* haplotypes. Shown are number of base pair differences and p-distances between haplotypes within cluster 1 (1.1/1.2) and cluster 2 (2.1/2.2) as defined in Fig. 12.

Name	Type	Length	bp differences		p-distances	
			1.1 / 1.2	2.1 / 2.2	1.1 / 1.2	2.1 /2.2
orf1611	ORF	1668	2	50	0.001	0.030
orf354	ORF	354	2	8	0.006	0.023
16S	rRNA	1780	2	11	0.001	0.006
CO1	Gene	1566	8	19	0.005	0.012
CO2	Gene	738	2	13	0.003	0.018
trnW	tRNA	71	0	2	0.000	0.028
ATP8	Gene	207	1	4	0.005	0.019
ATP6	Gene	705	1	12	0.001	0.017
CO3	Gene	786	3	19	0.004	0.024
trnM	tRNA	71	0	0	0.000	0.000
ND2	Gene	1350	4	44	0.003	0.033
ND5	Gene	1833	4	61	0.002	0.033
12S	rRNA	971	0	5	0.000	0.005
ND6	Gene	564	2	20	0.004	0.035
ND3	Gene	357	1	7	0.003	0.020
ND4L	Gene	300	2	9	0.007	0.030
ND1	Gene	999	4	32	0.004	0.032
ND4	Gene	1461	7	45	0.005	0.031
CYTB	Gene	1185	3	33	0.003	0.028
	overall		48	394	0.055	0.424

4.2.2 Mitogenome phylogeny

Fig. 12 shows phylogenetic relationships among mitochondrial genomes from my four representative *Craspedacusta* medusa samples and from some cnidarian mitogenomes that were previously published in Genbank (https://www.ncbi.nlm.nih.gov/). Regarding *C. sowerbii* mitogenomes, two divergent lineages (1 and 2) emerged supported by bootstrap values of 100% (Fig. 12). Two of the newly obtained *Craspedacusta* mitogenomes were grouped together in lineage 1. The other two new mitogenomes clustered with *C. sowerbii* from Genbank in a distinct lineage 2. While there was a clear subdivision among mitogenomes within lineage 2 (100% bootstrap support), mitogenomes within lineage 1 were highly similar. In lineage 2, Type 2.1 was highly similar to JN593332 from China (Zou et al. 2012) and clearly distinct from Type 2.2 which was almost identical to LN901194 (Kayal et al. 2015). Notably, differences within lineages spanning the whole coding region were much greater between 2.1 and 2.2 Types than between 1.1 and 1.2 Types which are consistent with single-locus-based differentiation described in Schachtl (2019).

The mitogenome data further support that at least two lineages and four haplotypes invaded Europe (Schachtl, 2019). In addition, the reference LN901194 was grouped with Type 2.2 while another one JN593332 was similar with Type 2.1. According to data deposited in the Genbank (Kayal et al. 2015, https://www.ncbi.nlm.nih.gov/), the origin of LN901194 was ambiguous but JN593332 was from China (Zou et al. 2012, https://www.ncbi.nlm.nih.gov/), which demonstrates that Type 2.1 has spread from China and undoubtedly invaded Europe.

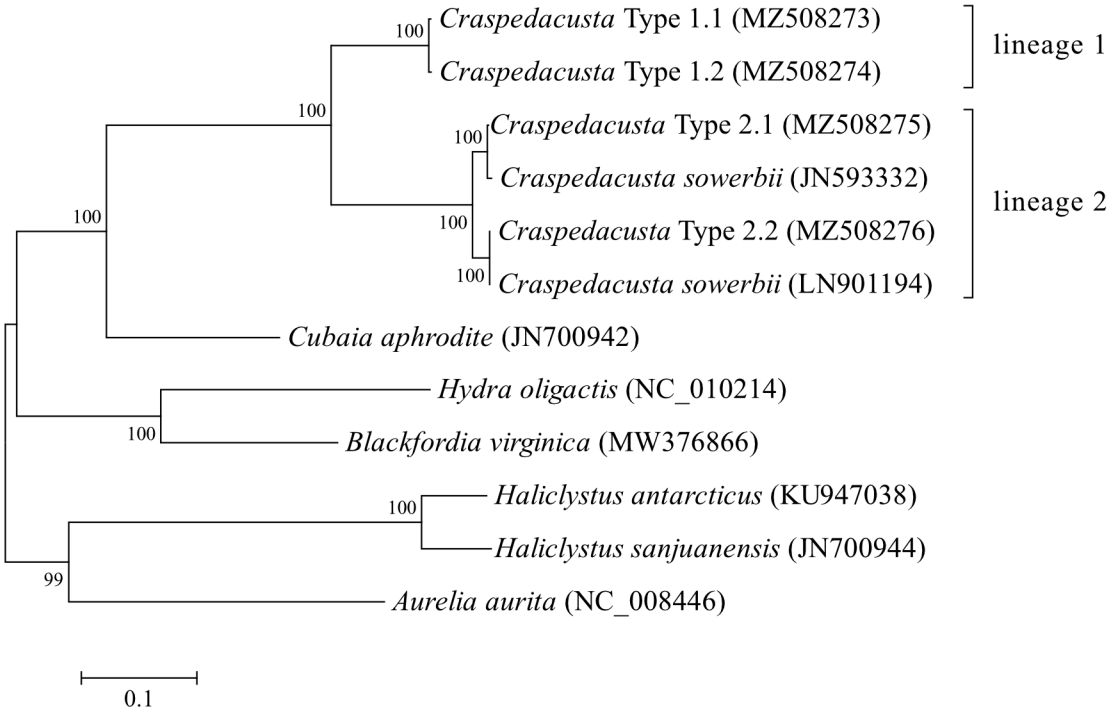

Fig. 12 Molecular phylogeny based on 13 concatenated protein coding regions from four invasive mtDNA haplotypes (Type 1.1, 1.2, 2.1, 2.2) of *Craspedacusta* in Europe and Cnidarian mitogenomes published in Genbank (two individuals from *C. sowerbii*, five more hydrozoan individuals from distantly related species and from the scyphozoan moon jellyfish *Aurelia aurita*. Genbank accession numbers for the sequences are indicated next to the species names). The reconstruction of the phylogenetic tree is based on the Maximum Likelihood (ML) method, bootstrap values are shown next to the nodes.

4.3 Polyp populations' genetic structure

4.3.1 Polyp occurrence frequency

Much effort was required to obtain enough polyp individuals from the field for population analyses. To get an impression, I counted the number of rocks and branches sampled in the Heiglweiher (HE) and in the Gernlindner Waldsee (GW). Four full buckets (each 1L) mostly with small stones were checked, specifically, there were 73, 60, 108 and 49 stones respectively in each bucket from HE (Tab. 9). From these samples, fifteen colonies were detected at appropriate magnification, found in two buckets and four stones only (Tab. 9). My colleagues and I sampled 612 stones from nine buckets in GW (Tab. 9). 20 colonies were detected in two buckets and three stones (Tab. 9). In terms of all samples, my colleagues and I screened about a thousand stones (and twigs) to achieve a population dataset with overall 346 colonies. In

addition, I documented the number of polyps in each colony in a subset 240 colonies, indicating that the colonies consisting of two polyps were the most frequent, followed by single polyps. Three or four polyps in a field colony was rare (Fig. 13, Tab. 10). The mean number of polyps in a colony from my samples was 1.80 ± 0.04 (Tab. 10).

Tab. 9 Stones sampled in Heiglweiher (HE), and the number of colonies and polyps found on stones.

Lake	Bucket	Number of sampled stones	Number of stones with polyps	Number of colonies	Number of polyps	Number of polyps per colony
Heiglweiher (HE)	1	73	1	5	10	2
	2	60	0	0	0	0
	3	108	0	0	0	0
	4	49	3	10	23	2.3
	total	290	4	15	33	2.2
Gernlindner Waldsee (GW)	1	84	0	0	0	0
	2	82	1	3	6	2
	3	167	0	0	0	0
	4	48	2	17	37	2.2
	5	50	0	0	0	0
	6	32	0	0	0	0
	7	37	0	0	0	0
	8	30	0	0	0	0
	9	82	0	0	0	0
	total	612	3	20	43	2.2

Tab. 10 Distribution of different types of polyp numbers in each colony based on 240 colonies data

Type	1 polyp per colony	2 polyps per colony	3 polyps per colony	4 polyps per colony	sum
Number of colonies	67	156	16	1	240
Number of polyps	67	312	48	4	431
Average number of polyps per colony (Mean ± SE)					1.80 ± 0.04

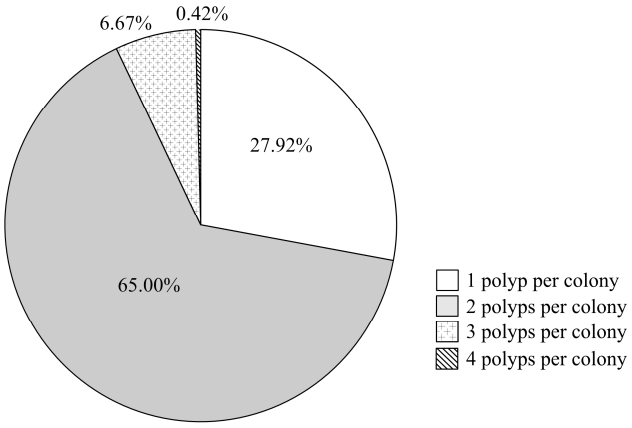

Fig. 13 Frequency of different colony sizes in field polyp populations

4.3.2 Genetic diversity

Analyses based on a 600 bp COI gene region for *Craspedacusta* polyps resulted in two main clades and five mtDNA haplotypes overall (Fig. 14, Suppl. Tab. 5). To be specific, haplotype diversity was the highest in the Reichertshofen* Lake A (RA) and the lowest nucleotide diversity was found in Waldsee (WA), due to the only one base pair difference between Type 1.1 and 1.1.1 (Suppl. Tab. 5). Notably, Type 1.1.1 was concurrently detected in Reichertshofen* Lake A (RA) and Waldsee (WA) (Suppl. Tab. 5). Colonies sequenced by both markers accounted for 57.2% of all samples, and their haplotype assignments were congruent (Suppl. Tab. 6).

Overall, the haplotype frequency was in the order: Type 1.2 (42.9%) > 2.2 (26.2%) > 1.1 (20.8%) > 1.1.1 (5.4%) > 2.1 (4.8%). Also, the most common haplotype in polyp populations was Type 1.2, consistent with a previous study (Schachtl, 2019). What should be noted was that the frequency of Type 1.1.1 exceeded Type 2.1 and the latter occurred only in Neuer Baarer Weiher (BW). Moreover, Type 1.1.1, one base pair variant from Type 1.1, was much more common than Type 1.1 in Waldsee (WA) (Suppl. Tab. 5). In the samples of the river Amper only Type 1 appeared represented by Type 1.1 and Type 1.2 individuals.

In terms of the genetic diversity among polyps, sometimes three haplotypes co-existed on the same stone/twig, for example, in Langwieder See (LW) and Reichertshofen* Lake A (RA), which showed the highest diversity indices (Fig. 15, Tab. 11). On the other hand, on some stones/twigs only a single haplotype was found, such as on all stones from Brandsee (BA) and Gernlindner Waldsee (GW) (Fig. 15, Tab. 11).

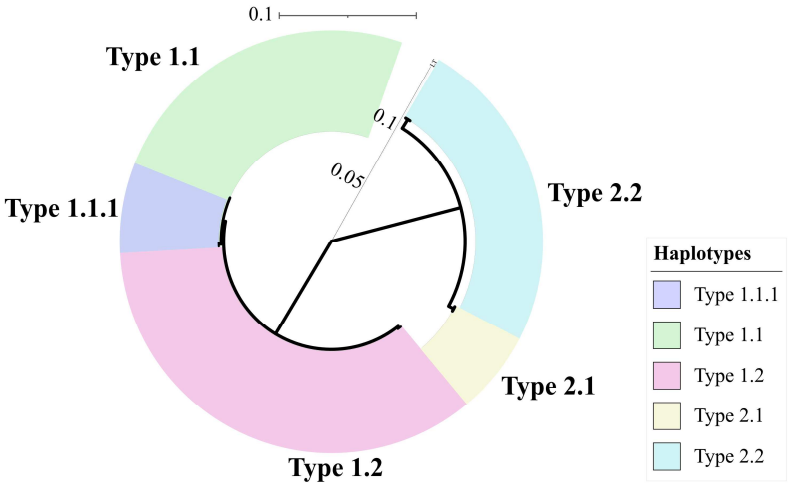

Fig. 14 Maximum likelihood tree (ML-tree) based on the sequence variation of the mitochondrial COI gene in polyps (600 bp, 346 individuals). Shown is the frequency of mtDNA haplotypes and their genetic divergence (scale bar represents the K2P (Kimura 2-parameter) genetic distance). The branch widths represent bootstrap values between 0.6 and 1, the colour of each haplotype is consistent with Fig. 8 (section 4.1.1).

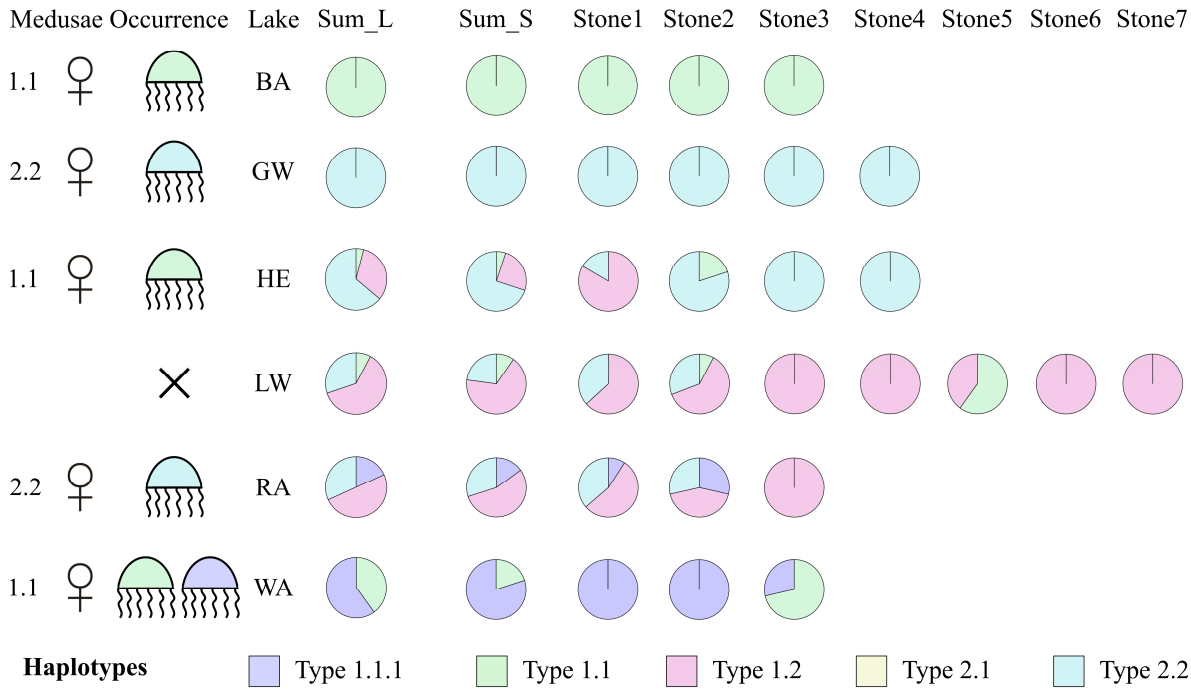

Fig. 15 Detection of polyp and medusa haplotypes within lakes and information on genetic diversity among polyps located on the same substrate. Sum_L: haplotype frequency in a lake including all polyps; Sum_S: haplotype frequency on all stones (with colony number $\geqslant$ 2); Stone 1-7: haplotype frequency on individual stones (Number of colonies $\geqslant$ 2); the colour of each haplotype is consistent with Fig. 8 (section 4.1.1).

Tab. 11 Genetic diversity of polyp populations on the same substrate based on mtDNA COI. ∑: sum of sequenced individuals; Nhap: number of haplotypes per stone/twig; hd: haplotype diversity; π: nucleotide diversity.

Lake (Abb.)	Substrate	Type 1.1	Type 1.1.1	Type 1.2	Type 2.1	Type 2.2	∑	N_hap	hd	π
Brandsee (BA) Medusae_1.1	stone1	10	0	0	0	0	10	1	0	0
	stone2	5	0	0	0	0	5	1	0	0
	stone3	23	0	0	0	0	23	1	0	0
Gernlindner Waldsee (GW) Medusae_2.2	stone1	0	0	0	0	5	5	1	0	0
	stone2	0	0	0	0	3	3	1	0	0
	stone3	0	0	0	0	15	15	1	0	0
	stone4	0	0	0	0	2	2	1	0	0
Heiglweiher (HE) Medusae_1.1	stone1	0	0	5	0	1	6	2	0.333	0.0522
	stone2	1	0	0	0	4	5	2	0.400	0.0627
	stone3	0	0	0	0	5	5	1	0	0
	stone4	0	0	0	0	4	4	1	0	0
Langwieder See (LW) No Medusae detected	stone1	0	0	19	0	11	30	2	0.480	0.0746
	stone2	2	0	16	0	8	26	3	0.542	0.0699
	stone3	0	0	7	0	0	7	1	0	0
	stone4	0	0	5	0	0	5	1	0	0
	stone5	6	0	4	0	0	10	2	0.533	0.0027
	stone6	0	0	3	0	0	3	1	0	0
	stone7	0	0	2	0	0	2	1	0	0
Reichertshofen* Lake A (RA) Medusae_2.2	stone1	0	1	6	0	4	11	3	0.618	0.0806
	stone2	0	2	3	0	2	7	3	0.762	0.0768
	stone3	0	0	2	0	0	3	1	0	0
Waldsee (WA) Medusae_1.1	twig1	0	16	0	0	0	16	1	0	0
	twig2	0	2	0	0	0	2	1	0	0
	twig3	5	2	0	0	0	7	2	0.476	0.0008

4.3.3 Population genetics

In general, there was high genetic differentiation among polyp populations (Φ_{ST}=0.452, p < 0.001) (Tab. 12). Genetic differentiation among populations (45.17%) and within populations (54.83%) was similar (Tab. 12). Pairwise comparisons revealed pronounced differences in

haplotype frequencies among polyp populations from different lakes/river in a range of F_{ST} between -0.03 and 1.00, the majority being statistically significant (Tab. 13). To be specific, polyp populations included only one haplotype in two lakes (Brandsee (BA) and Gernlindner Waldsee (GW)), while the population structures in others were relatively highly diverse (Fig. 7 (section 3.3.1), Suppl. Tab. 5). The result of the Mantel test showed that there was no significant correlation between genetic distance F_{ST} and geographical distance (r = -0.05, p = 0.57). The relationship between F_{ST} and geographical distance further indicated that genetic variation and geographical distance were not correlated (R^2 = 0.003, p = 0.796) (Fig. 16). The temporal population structure in Langwieder See (LW) indicated fluctuations over three years (Φ_{ST}=0.336, $p < 0.001$), which mainly resulted from variations within years (66.43%) (Tab. 14). A pairwise F_{ST} indicated clear differences between populations from 2018 and from the other two years (Tab. 15).

Tab. 12 Analysis of molecular variance (AMOVA) of *Craspedacusta* polyp populations

Source of variation	d.f.	Variance components	Percentage variation	Fixation indices	Significance
among populations	7	10.201	45.17	Φ_{ST}=0.452	$p < 0.001$
within populations	340	12.384	54.83		
total	347	22.585			

Tab. 13 Pairwise estimates of genetic differentiation (F_{ST}) of polyp populations between lakes/river. * $P < 0.05$, ** $P < 0.01$. Abbreviations of lake/river see Suppl. Tab. 5.

Lake/River	AM	BA	BW	GW	HE	LW	RA
BA	0.44**						
BW	0.60**	0.61**					
GW	0.99**	1.00**	0.40**				
HE	0.68**	0.69**	0.02	0.35**			
LW	0.22**	0.24**	0.17**	0.58**	0.19**		
RA	0.37**	0.40**	0.14**	0.69**	0.15*	-0.03	
WA	0.48**	0.61**	0.60**	1.00**	0.67**	0.24**	0.38**

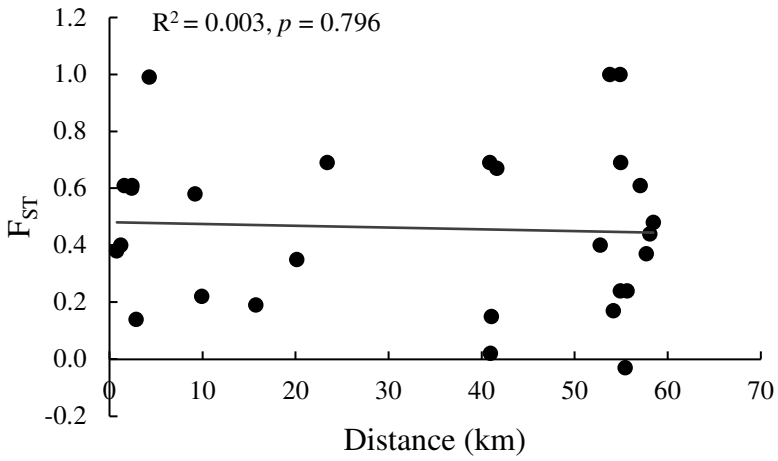

Fig. 16 Relationship between pairwise geographical distance (kilometer) and pairwise F_{ST} genetic distance of polyp populations.

Tab. 14 Temporal analysis of molecular variance (AMOVA) of polyp populations in Langwieder See (LW).

Source of variation	d.f.	Variance components	Percentage variation	Fixation indices	Significance
among years	2	8.300	33.57	Φ_{ST}=0.336	$p < 0.001$
within years	113	16.426	66.43		
total	115	24.726			

Tab. 15 Pairwise estimates of genetic differentiation (F_{ST}) of polyp populations between different years in Langwieder See (LW). * $P < 0.05$, ** $P < 0.01$.

Year	2015	2016
2016	-0.01	
2018	0.55^{**}	0.44^{**}

4.3.4 Correlation between the genetic structure of medusa and polyp populations

I compared the haplotype compositions of medusa and polyp populations inhabiting the same lakes. The haplotype of medusae coincided with that of polyp in Brandsee (BA), Neuer Baarer Weiher (BW), Gernlindner Waldsee (GW) and Waldsee (WA) (Fig. 15, Tab. 11). In spite of the consistency between the haplotype of medusae and polyps in Waldsee (WA), Type 1.1.1

outnumbered Type 1.1 at the polyp stage, contrary to the medusa stage, where I found only four individuals of Type 1.1.1 out of 160 individuals between 2015 and 2018. Type 1.1.1 appeared only in 2015. Surprisingly, only one haplotype of medusae was detected but three haplotypes of polyp co-occurred in the other two lakes, Heiglweiher (HE) and Reichertshofen* Lake A (RA). Moreover, although no medusae were detected in Amper (AM) and Langwieder See (LW), polyps with various haplotypes were found.

4.4 Mitochondrial genetic diversity

In a previous study by Schachtl (2019), the sampling size of medusae and polyps within most lakes were small, five medusae and three colonies per lake/river respectively. I compared the results from this small dataset with the outcome of my analysis based on much larger population sizes (20-40 individuals per sampling site). I found that, at the medusa stage, the genetic diversity in the dataset of Schachtl (2019) including more lakes was higher than the diversity in my dataset with a larger number of individuals analysed per population from a smaller number of lakes (Tab. 16). On the contrary, polyps' mitochondrial genetic diversity at the larger population level was near twice as large as the diversity found with the dataset of Schachtl (2019) (Tab. 16).

Tab. 16 Mitochondrial genetic diversity at different analysed population sizes. Nhap: number of haplotypes in each dataset, hd: haplotype diversity.

Stage	**small population size dataset (Schachtl, 2019)**			**large population size dataset (this study)**		
	sampling size	**Nhap**	**hd**	**sampling size**	**Nhap**	**hd**
medusa	5 ind/lake from 28 lakes	6	0.517	~40 ind/lake from 17 lakes	5	0.444
polyp	3 ind/lake or river from 39 lakes/rivers	5	0.446	15-83 ind/lake or river from 8 lakes/rivers	5	0.735

4.5 Polyp population growth responses to temperature

4.5.1 Polyp asexual reproduction

The asexual reproduction of polyps encompasses not only polyp population growth but also frustules production by polyps. Firstly, polyp growth followed an exponential growth pattern

at both temperatures (Fig. 17a, 17b) and polyp population development was at a significantly higher growth rate at 26℃ compared to 18℃ after 73 days ($p < 0.01$, Fig. 17g). Polyps experienced an extremely high growth at the beginning but then decreased to a roughly steady rate at both temperatures (Fig. 17e). While polyps grew very differently up to day 45 days at 26℃, their growth rate gently slowed down and the variations among wells became smaller. On the contrary, polyps cultured at 18℃ reached fast an almost constant growth rate, even though they showed a slight downward trend in last few days (Fig. 17e). The production of frustules also exhibited an exponential growth pattern (Fig. 17c, 17d), while the production rate pattern of frustules produced by polyps was totally different from that of the growth patterns of polyps themselves. Polyp growth patterns were mostly characterized by a first increase in production rate and then a decrease over time (Fig. 17f). Using data from the last day, a clear temperature effect on the production rate of frustules emerged as well ($p < 0.01$, Fig. 17h). Overall, the high temperature supported not only polyp population growth but also the production of frustules.

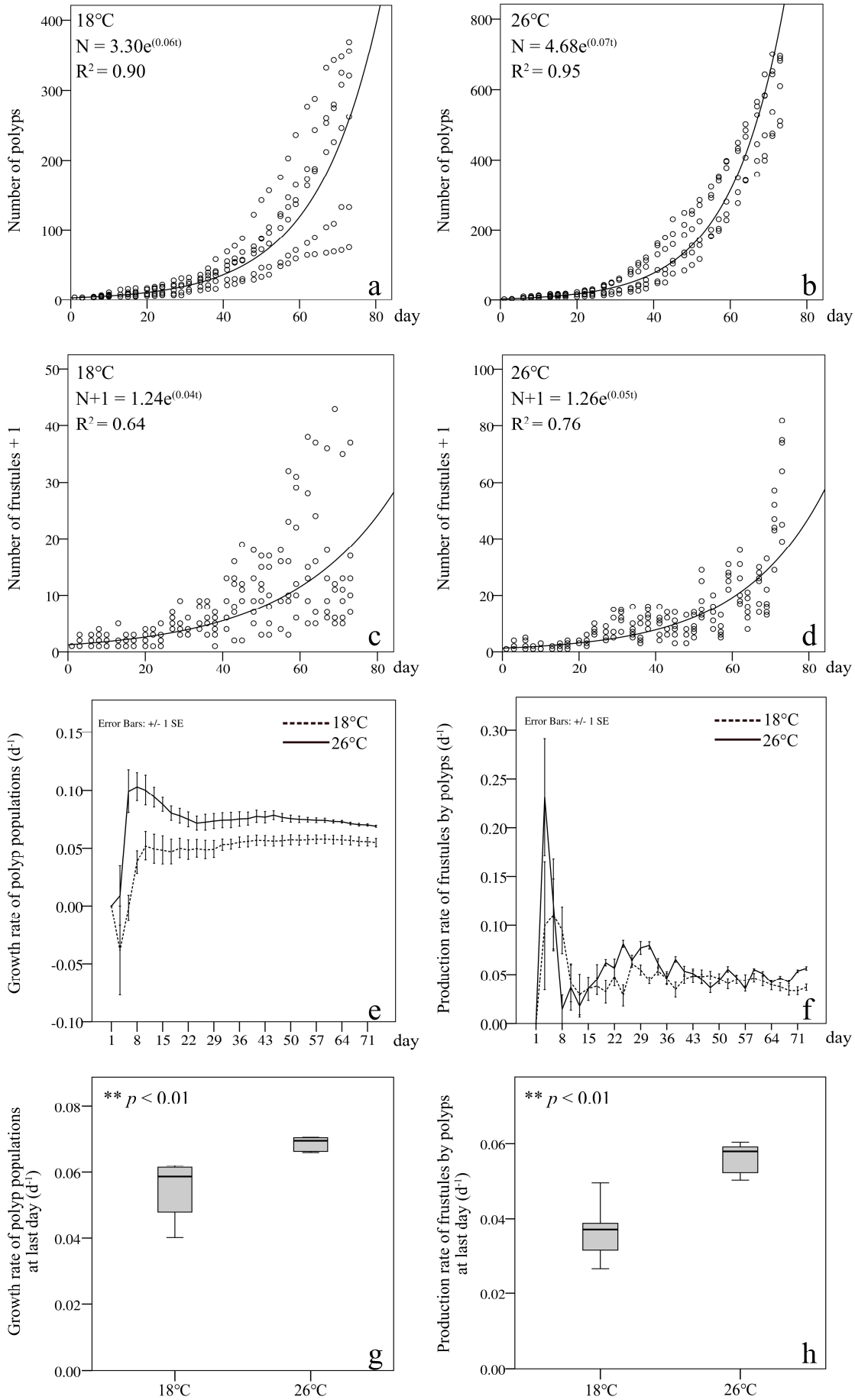
18°C
N = 3.30e(0.06t)
R2 = 0.90
Number of polyps
day
a
26°C
N = 4.68e(0.07t)
R2 = 0.95
Number of polyps
day
b
18°C
N+1 = 1.24e(0.04t)
R2 = 0.64
Number of frustules + 1
day
c
26°C
N+1 = 1.26e(0.05t)
R2 = 0.76
Number of frustules + 1
day
d
Error Bars: +/- 1 SE
18°C
26°C
Growth rate of polyp populations (d-1)
day
e
Error Bars: +/- 1 SE
18°C
26°C
Production rate of frustules by polyps (d-1)
day
f
** p < 0.01
Growth rate of polyp populations at last day (d-1)
18°C
26°C
g
** p < 0.01
Production rate of frustules by polyps at last day (d-1)
18°C
26°C
h

Fig. 17 Population dynamics of polyps and frustules at different temperatures. Polyp population growth pattern during 73 days at 18°C (a) and 26°C (b). Number of produced frustules during 73 days at 18°C (c) and 26°C (d). Growth rate of polyp populations during 73 days at both temperatures(e). Error bars represent standard errors. Production rate of frustules released by polyps during 73 days at both temperatures(f). Error bars represent standard errors. Growth rate of polyp populations at the last experimental day at 18°C and 26°C (g). * $p < 0.05$, ** $p < 0.01$. Production rate of frustules by polyps at the last experimental day at 18°C and 26° (h). * $p < 0.05$, ** $p < 0.01$.

4.5.2 Colony size

The average colony size fluctuated with polyp *per se* growth and the newly formed polyps from frustules (Fig. 18a). In general, colonies with two connected polyps were most common and accounted for 68% and 75% at 18°C and 26°C, respectively. The next frequent colony type consisted of one polyp with a percentage of only 23% and 14% at 18°C and 26°C, respectively. Colonies with three, four or more polyps were rare (Fig. 19). Additionally, the mean polyp number in a colony at day 73 was 1.88 ± 0.02 (mean ± SE) at 18°C and 2.06 ± 0.01 (mean ± SE) at 26°C, respectively, showing a notable temperature difference ($p < 0.05$, Fig. 18b).

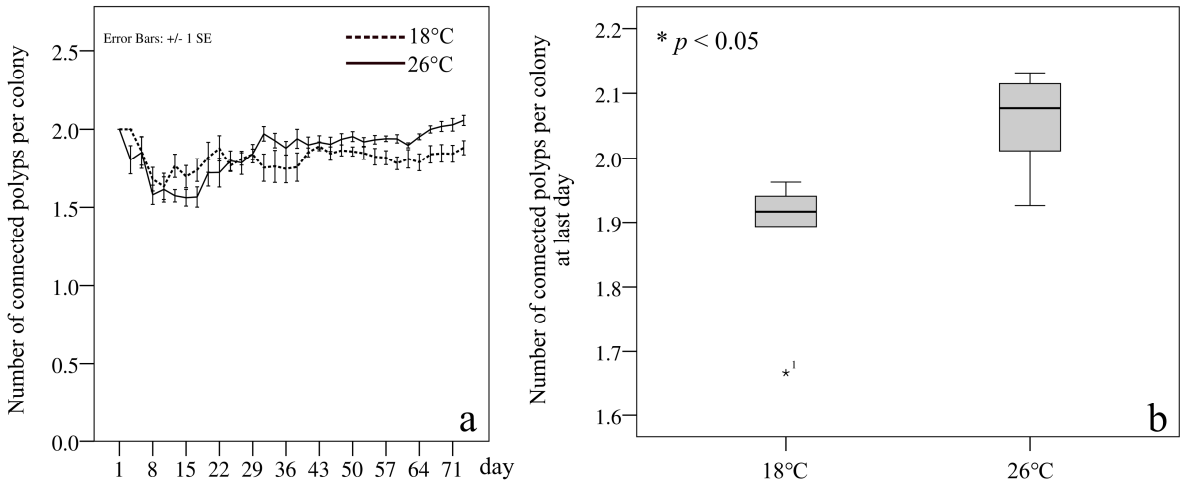

Fig. 18 The variation of polyp number per colony over 73 days (a) (Error bars represent standard errors.) and at the last experimental day (b) at 18°C and 26°C. * $p < 0.05$, ** $p < 0.01$.

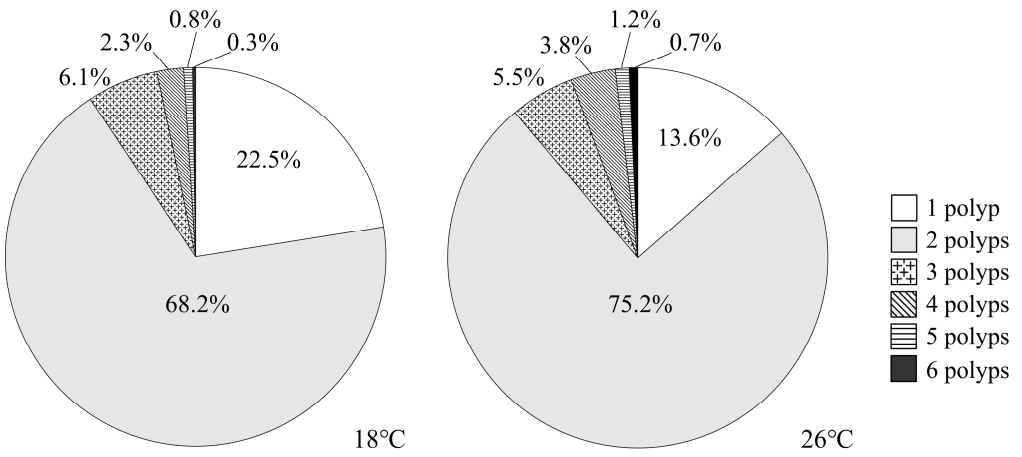

Fig. 19 The composition of colonies at the last experimental day at 18°C and 26°C.

4.5.3 Wet weight of polyps and frustules

Direct wet weight of polyps and frustules was measured to compare the weight of polyps and frustules at the respective treatments. The average wet weight of a single polyp was 2.25 ± 0.17µg (mean ± SE) and 2.47 ± 0.16µg (mean ± SE) at 18°C and 26°C, correspondingly, the difference was not significant ($p > 0.05$) (Fig. 20a). Similarly, mean frustule wet weight (0.70 ± 0.17µg at 18°C; 0.51 ± 0.13µg at 26°C; (mean ± SE)) was not statistically significant different between the two temperatures ($p > 0.05$) (Fig. 20b).

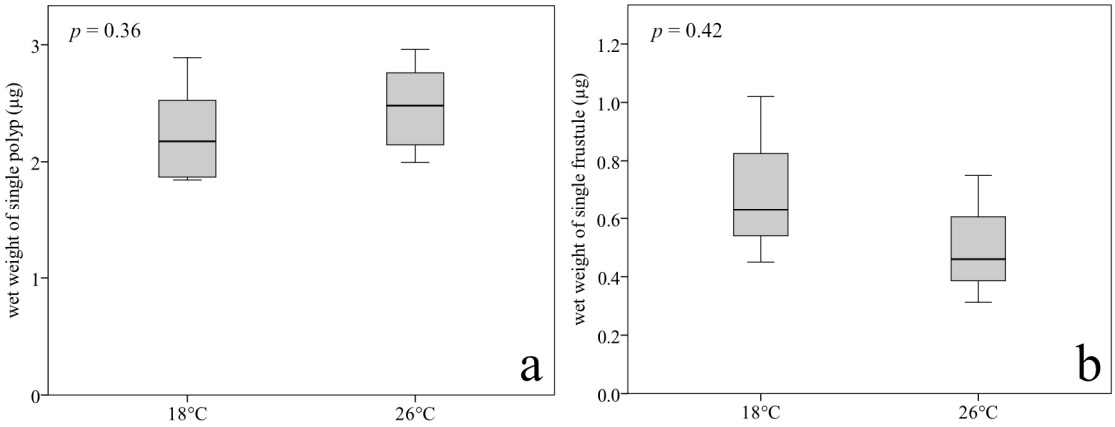

Fig. 20 Wet weight of single polyps (a) and frustules (b) at 18°C and 26°C.

4.5.4 Frustule production by polyps

Beside estimating the total frustule production rate by polyp populations, which is dependent on the number of polyps, I also calculated the number of frustules released per polyp. Frustule

production per polyp reached a peak at the initial experimental days and later on during days 25-35 at both temperatures (Fig. 21a). The mean number of frustules produced by a polyp during 73 days was respectively 0.15 ± 0.01 (mean ± SE) and 0.11 ± 0.01 (mean ± SE) at 18°C and 26°C, the difference was significant ($p < 0.01$, Fig. 21b).

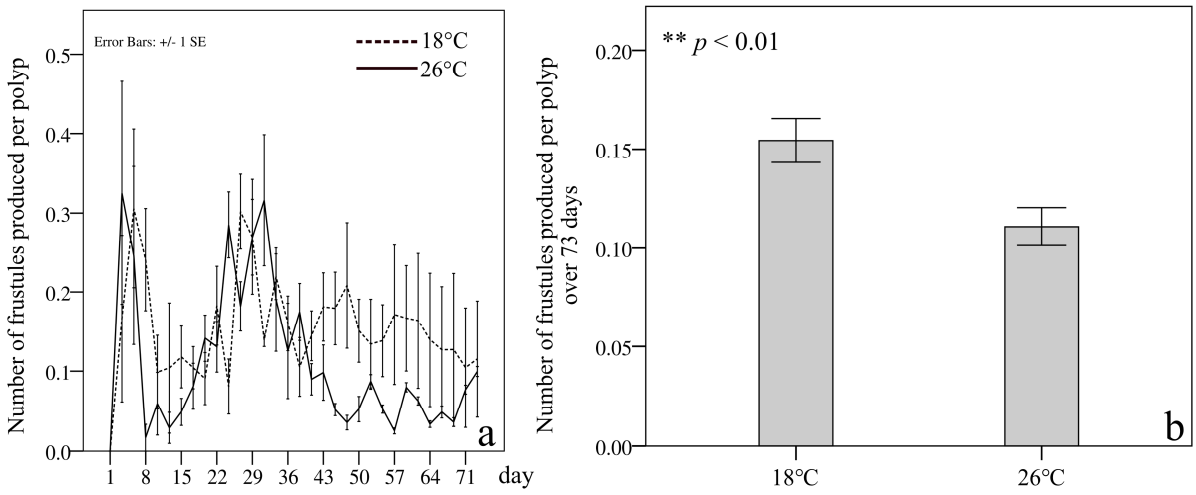

Fig. 21 The temporal variation of frustules produced by a polyp during 73 days (a) and the mean number of frustules released by a polyp per day over 73 days at different temperatures(b). Error bars represent standrd errors. * $p < 0.05$, ** $p < 0.01$.

4.6 Polyp population growth responses to weak pH variations

4.6.1 Initial water chemistry

In order to reach the desired experimental pH values, 0.2M $NaH_2PO_4 \cdot H_2O$ (pH = 4.5) was added to the growth medium (HM). The lower the experimental pH level was, the more fluctuations in pH occurred, especially for pH 7.6 (Fig. 22). Finally, the average pH levels of the different experimental treatments reached 8.5 ± 0.02, 8.0 ± 0.03, 7.8 ± 0.04 and 7.6 ± 0.04 (Mean ± SE) at both temperatures.

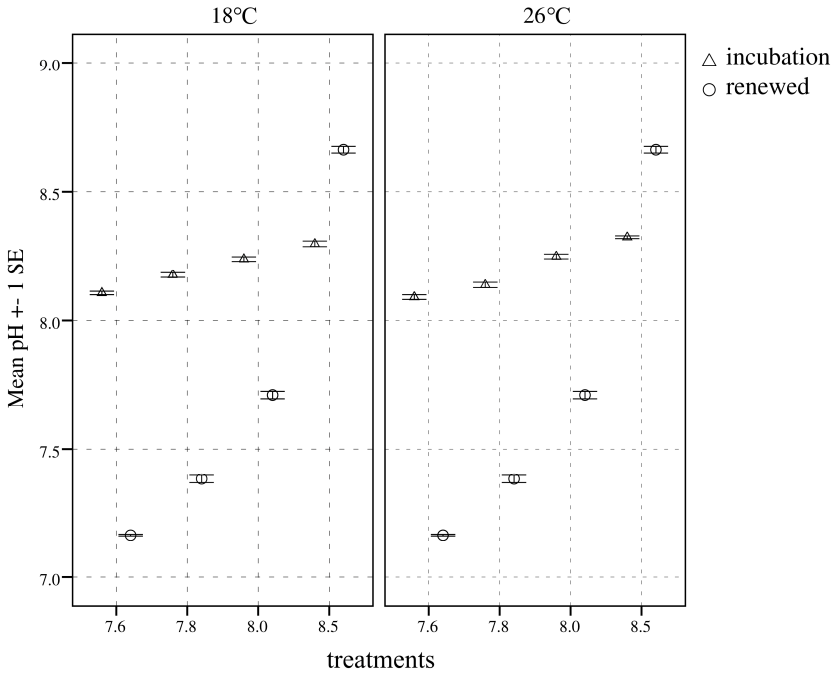

Fig. 22 pH fluctuations before and after refreshing the growth medium. Error bars represent standard errors.

4.6.2 Asexual reproduction of polyps

Over my first pH experiment, the growth rate of colonies and the production rate of frustules were lowest at pH 7.6, both at either 18°C or 26°C (Fig. 23). No significant difference in the growth rate of colonies and production of frustules was seen between the three pH treatments (8.5, 8.0, 7.8) at both temperatures (Fig. 23, 24). The significance of pH effects on the growth rate of polyps resulted from differences between the pH 7.6 treatments and the other three pH (8.5, 8.0, 7.8) treatments. Although no profound pH effects appeared among pH 8.5, 8.0 and 7.8 treatments, the growth patterns of polyps from different assemblages were inconsistent. Overall, assemblage type 1 and 3 (PA1 and PA3) always exhibited the highest growth rate. The growth rate was much lower for assemblage types 2 and 4 (PA2 and PA4) (Fig. 23, left). This was supported by ANOVA analysis showing significant differences between the growth rate of polyps at 18°C and 26°C (18°C: $F = 5.450$, $p < 0.05$; 26°C: $p < 0.05$). In terms of ability to produce frustules, polyps from four assemblages (PA1, PA2, PA3 and PA4) performed similarly at 18°C ($p > 0.05$) while PA1 and PA3 assemblages produced more frustules than PA2 and PA4 at 26°C ($p < 0.05$, Fig. 23). Within each assemblage, the effects of pH on the growth rate of populations and the production of frustules appeared from the difference between pH 7.6 treatments and all other treatments (Suppl. Tab. 7).

In my second pH experiment, the growth rate of colonies and the production rate of frustules by polyps were much lower compared to my first experiment (Fig. 24). Furthermore, no profound effect was found between the two experimental pH treatments (Suppl. Tab. 8), which was similar as seen in my first experiment. No significant differences in growth patterns between clones could be found between both pH treatments, neither for colony growth rate (pH 8.5: $p > 0.05$; pH 7.8: $p > 0.05$) nor for frustule production rate by polyps (pH 8.5: F=2.105, $p > 0.05$; pH 7.8: $p > 0.05$).

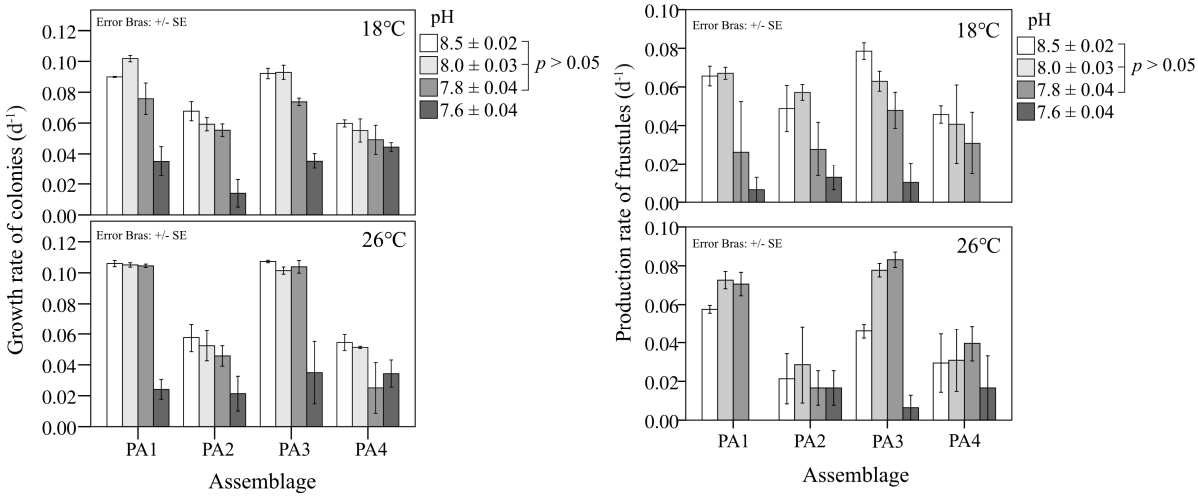

Fig. 23 The growth rate of colonies (left) and production rate of frustules (right) at different experimental treatments 1. Error bars represent standard errors.

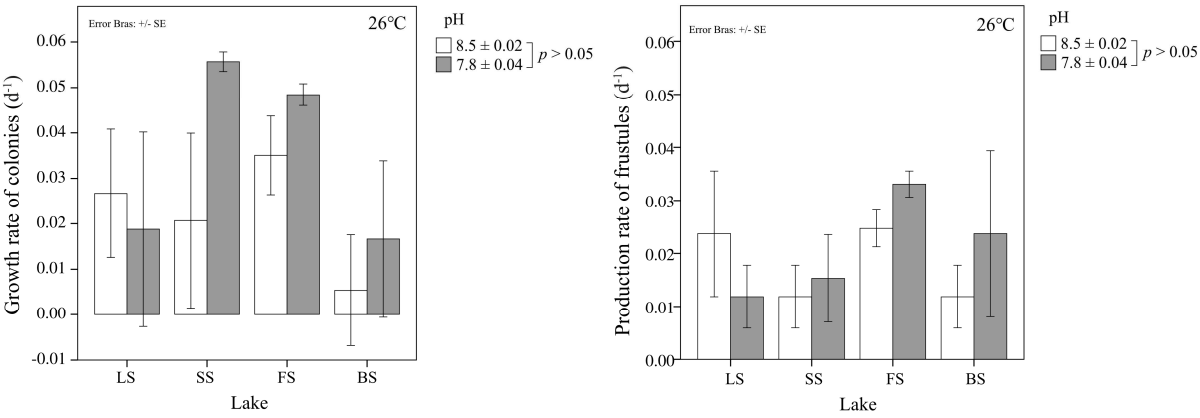

Fig. 24 The growth rate of colonies (left) and the production rate of frustules by polyps (right) from different lakes exposed at different pH treatments 2. Error bars represent standard errors.

4.6.3 Genetic differences among assemblages at the end of the experiment

Unexpectedly, at the end of the pH experiment 1, the sequencing results showed that the tested individuals from PA1and PA3 assemblages were not anymore from Type 1.1 and 2.1 but all from Type 1.2, while the haplotype of individuals from PA2 and PA4 assemblages was consistent with the initial incubation haplotype, the Type 1.2 and 2.2 respectively. Details about the initial composition of the experimental populations can be found in Tab. 1 (section 3.5.1). In my second pH experiment, the haplotype of polyps from each clone was consistent with the initial haplotypes (section 3.5.3). Here, the final haplotype of polyps from Langwieder See (LS), Fridolfinger See (FS) Schwarzlsee (SS) was Type 1.2 and Bodensee (BS) polyps belonged to Type 1.1.

5 Discussion

5.1 *Craspedacusta* genetic diversity on the population level

5.1.1 Genetic diversity of medusa populations

For more than a hundred years, it has been assumed that only one species, *C. sowerbii*, invaded Europe (Dumont, 1994). However, mitochondrial sequence divergence implies that more than one species might have successfully spread to Europe (Karaouzas et al., 2015; Oualid et al., 2019; Schifani et al., 2019). Nuclear ITS-barcoding already suggested that another species *C. kiatingii* is hidden among individuals identified as *C. sowerbii* (Fritz et al., 2009). Recently, it has been reported that two major mtDNA lineages (four haplotypes) invaded Europe, presumably representing different species (Schachtl, 2019). Theoretically, more species could be detected among more samples from broader geographic regions (Verberk, 2011). In my study, however, no more than the four previously reported main mitochondrial haplotypes were found, despite of large sample sizes at the population level, including previously unsampled lakes. In short, my study confirmed that no more than two lineages (Type 1 and 2) emerged among approximately nine hundred samples. Only subtypes of the already known haplotypes were additionally identified: one and four individuals with 1bp mutation at the 16S and COI locus, respectively, accounting for a very low proportion of all samples (no more than 1%). In summary, this reflects that multiple haplotypes have invaded a majority of the lakes in Europe and it is possible that every haplotype was successfully established in each of the investigated lake by repeated introductions.

Type 1.1 was the most frequent haploype among all sampled medusa individuals, followed by Type 2.2, 2.1 and 1.2. This was the same result as reported by Schachtl (2019), who sampled five medusa individuals within each lake but in more lakes. Type 1.2 was a rare haplotype and was only found in Neuer Baarer Weiher in 2016 and 2017 and this lake was the only lake with mixed lineages, haplotypes, and genders. Nevertheless, Schachtl (2019) found Type 1.2 medusae in another lake, Waldsee Schechen in Germany. Recently, Type 1.2 medusae were also detected in northern Italy (Morpurgo et al., 2021) and there was a single 16S sequence similar as Type 1.2 deposited in Genbank (accession number: MF000530, https://www.ncbi.nlm.nih.gov/), which was extracted from a polyp rather than a medusa.

Notably, female medusae were outnumbering males by far in both lineages, especially in the Type 1 lineage.

5.1.2 Genetic diversity of polyp populations

Compared with medusa populations, the overall genetic diversity of polyp populations (Suppl. Tab. 5, haplotype diversity = 0.735) was much higher, almost twice as much as for medusa (Tab. 2 (section 4.1.4), haplotype diversity = 0.444). The overall number of mtDNA haplotypes among polyp populations was the same as seen within medusa populations. In total five haplotypes were detected, which was one haplotype (Type 1.1.1) more than shown in a previous report (Schachtl, 2019). Compared with polyps from marine jellyfish (van Walraven et al., 2016; Seo et al., 2021), the reported genetic diversity of polyps from freshwater jellyfish is much lower. For example, twenty COI mtDNA haplotypes were found in polyp populations of moon jellyfish *Aurelia coerulea* in Jaran Bay, Korea (Seo et al., 2021). Regarding the abundance of the one base pair mutated Type 1.1.1 in polyps, a subtype of Type 1.1, it was only seen in very low frequency in medusa populations whereas it was very frequently observed in polyp populations, especially in Waldsee (WA), where it even outnumbered the main haplotype, Type 1.1. On the population level, Type 1.2 was also the most abundant haplotype, this is in accordance with a previous study (Schachtl, 2019). Interestingly, only the Type 1 lineage was found in the river Amper (AM), which may reflect that Type 1 is more broadly distributed and polyps from that lineage are able to live in river ecosystems. Certainly, more evidence is needed to clarify this. Besides, Type 1.1.1 was also found in two closeby lakes, Reichertshofen* Lake A (RA) and Waldsee (WA), which showed that gene flow occurred between these two lakes, demonstrating the effective dispersal of polyps. The distribution of the one base pair mutated Type 1.1.1 profoundly provides a valuable approach to analyse gene flow between lakes *in situ*. Additionally, the frequency of Type 2.1 was even lower than the mutated Type 1.1.1 and it was only detected in one lake, pointing towards either a limited dispersal capacity of this haplotype, or the special environmental conditions required for its survival and reproduction.

Due to the asexual reproduction of polyps, polyps located close to each other are likely to be clones. It hence could be hypothesised that polyps sitting on the same substrate were genetically identical. In the present study, even though the above hypothesis is supported by data from many stones and twigs, different polyp haplotypes co-existed on some stones as well, which

manifests a high mobility and genetic diversity of polyps in aquatic habitats. At least some haplotypes, such as Type 1.1, 1.2 and 2.2, can co-exist, although long-term coexistence among them is unknown.

5.1.3 Relationship between mitochondrial diversity and population size

In general, it is expected that the larger the effective population size is, the more neutral mutations will accumulate leading to higher genetic diversity (Frankham, 1996; Ellegren and Galtier, 2016). A general association between animal mtDNA variation and population size is however debated; for example, Bazin et al. (2006) demonstrated that population size had no effect on mitochondrial genetic diversity. In contrast, Mulligan et al. (2006) disagreed and claimed that mtDNA diversity and population size were positively related in animal groups with known or expected smaller populations, for example, human mtDNA diversity was successfully used to estimate related population sizes (Atkinson et al., 2008). Other determinants may be also important for the genetic diversity of a population, such as historical parameters, life histories of the species and its ecological strategies (Romiguier et al., 2014). In terms of *Craspedacusta*, information about haplotype diversity, genetic structure and life history strategies at large population levels is missing. Moreover, because of the interesting finding that in some lakes two or more haplotypes co-occurred at both medusa and polyp stages, one would expect to find higher diversity within *Craspedacusta* populations when the sampling effort is increased.

Comparing my data with the already mentioned population datasets including much fewer data (section 4.4), I found that it is not always the case that mitochondrial genetic diversity necessarily increases with population size. Additionally, opposite trends were found for different life stages of *Craspedacusta*. At one side, the mitochondrial genetic diversity among medusae from different lakes was already high in the datasets based on few analysed individuals because medusa populations within lakes were always fixed for a single but different haplotype. This means that only samples from more lakes would increase the medusa mitochondrial genetic diversity but not more samples within a lake, as shown in Tab. 16 (section 4.4). Conversely, polyps showed higher mitochondrial genetic diversity within a lake or river within my data, even on a small stone, suggesting that with increasing number of polyps sampled within a lake or river population, genetic diversity may increase. In summary, the detected mitochondrial genetic diversity of *Craspedacusta* could be affected by population size, depending on the investigated life stages. Additionally, the sampling strategy used may have

also resulted in some bias in diversity estimates, as for example, only shallow areas of some lakes were sampled. Hence, the sampling methods should be carefully evaluated and potentially modified depending on research aims. Moreover, my data can also be used in amalgamation with other datasets in order to generate large-scale databanks for studies that seek to have an increased sample size.

5.2 Genetic population structure

5.2.1 Genetic population structure at the medusa stage

Spatial genetic population structure

Dispersal potentially leads to gene flow and gene flow in turn promotes the spread of genotypes. It therefore has large consequences on local adaptation, population dynamics, range expansions, speciation, and the evolution of life-history traits. (Ouborg et al., 1999; Dieckmann et al., 1999; Sakai et al., 2001; Bowler and Benton, 2005; Hudina et al., 2014). In a species with limited natural dispersal ability by its own locomotion (short-distance dispersal, Liew and Curtis, 2004), long-distance dispersal by transportation vectors allows recolonization and facilitates genetic connectivity between discrete patches, thus promoting population differentiation and speciation in novel variable habitats (Trakhtenbrot et al., 2005; Medley et al., 2015; Jordano, 2017). Moreover, the long-distance dispersal, especially mediated by human activities, contributes greatly to the genetic admixture over large spatial scales (Suarez et al., 2001; Bullock et al., 2018). Hence, the association between genetic differentiation and geographic scale can reveal spatial patterns on species distribution (Wiens, 1989; Bowler and Benton, 2005; Pauchard and Shea, 2006). Furthermore, the successful invasion of invasive alien species is always based on a combination of successful short and long-distance dispersals (Sakai et al., 2001; Hastings et al., 2005).

Mantel tests indicated no significant relationships between genetic differentiation and geographical distance, reflecting no pronounced IBD (isolation by distance) pattern on the distribution of *Craspedacusta*, which demonstrates that gene flow occurs among regions. Contrary to the regional scale, the correlation coefficient in the Mantel tests at the local scale was negative, implying that closeby located populations were genetically dissimilar. Normally, a higher genetic differentiation is expected at a large scale than at a smaller spatial scale (Rundle and Nosil, 2005). However, in my study, the regional genetic diversity was lower than

the genetic diversity at the local scale. However, this was mainly caused by the only lake inhabiting mixed haplotypes of medusae, Neuer Baarer Weiher. This lake contributed to the largest genetic diversity on a local scale. Michels et al. (2003) also found a similar pattern of regional vs local diversity for *Daphnia pulex* and *Daphnia obtusa* populations in Flanders. They argued that studies on the polymorphism at few loci which are easy to score might conceil true local patterns which might appear with a larger subset of marker loci. This could also be the case in my study which is based on mtDNA-markers only. The application of nuclear markers or whole genome analyses would provide a higher genetic resolution between local populations and potentially reveal a geographic pattern. At present, however, high-resolving nuclear markers such as microsatellites are unpublished for *Craspedacusta* and whole genome genotyping at the population level is still too expensive.

Nervertheless, I found significant among-population variance at the medusa stage regardless of scale. This might largely result from the asexual reproduction mode of polyps, which is regarded to be the dominant life stage (DeVries, 1992). When only single-haplotype polyps existed in a single lake and different lakes potentially harbour completely distinct haplotypes, it would result in the single-haplotype medusae inhabiting lakes and obvious differentiations among populations and lakes. The consistency between haplotypes of budded medusae and their corresponding parental polyps has been confirmed in our laboratory. However, more data on polyp population structure is needed to support this assumption. Conversely, Elderkin et al (2007) reported more genetic variation within than between populations for the freshwater mussel *Amblema plicata*. It is therefore likely that the spatial genetic structure varies from species to species. Certainly, we cannot fully exclude some bias from sampling. It is possible that different haplotypes coexist, while their density was too low to find them within our monitoring program. For example, in the medusa population sampled in Neuer Baarer Weiher in 2018, I found that the frequency of the second medusa haplotype was only one in fourty analysed individuals. *Craspedacusta* is a vertically migrating species (Spadinger and Maier, 1999) and different haplotypes may differ in their migration behaviour. It is possible that some haplotypes of medusae prefer to stay in the deeper layers and could therefore be overlooked during sampling. This is a likely explanation and would explain that in several lakes multiple haplotypes were detected among polyps, while medusae populations seemed to be fixed for one haplotype.

Temporal genetic population structure

Due to the mostly irregular and sporadic occurrence of *Craspedacusta*, few data on the temporal distribution were reported so far. Along with increasing surface water temperatures, especially during summer and autumn, more and more medusae appeared and reached high abundances or even formed blooms (Acker, 1976; Lewis et al., 2012; Minchin et al., 2016). Therefore, in my study, all investigated sites were sampled between summer and autumn (from June to October). My results showed, except in lake Neuer Baarer Weiher, that all lakes were inhabited by the same mitochondrial haplotype of medusae over years. It seems that one lake is always inhabited by a single mitochondrial haplotype. Whether only one of the resident polyp haplotypes would be able to bud medusae in large numbers is unknown and needs to be proven. However, I found pronounced year-to-year changes in the composition of *Craspedacusta* medusa assemblages in the lake Neuer Baarer Weiher. A likely explanation for this is that shifts in critical environmental parameters occurred between years. Those shifts may have resulted in a shift in competitive advantage of different mitochondrial haplotype of polyps producing medusa. Hence, little is known about the underlying mechanism because the environmental data of Neuer Baarer Weiher in 2016 and 2018 are unavailable. To date, many studies have reported changes in the genetic composition within species over time, such as those observed in fish (Jaureguizar et al., 2006; Sirot et al., 2015), mussels (Atkinson and Vaughn, 2015), and zooplankton (Eloire et al., 2010; Nogueira et al., 2018; Usov et al., 2018) to name just a few examples. In the future, more attention needs to be paid to the temporal variation in haplotype frequencies of *Craspedacusta* polyps and medusae and their relationship to relevant environmental parameters.

5.2.2 Genetic population structure at the polyp stage

In the present study, I found high genetic diversity and differentiation among polyp populations, however, the genetic distance showed no correlation with geographical distance, suggesting the dispersal of polyps is not constrained by distance. Additionally, among-population and within-population structures were relatively the same for polyps. This is different from medusa populations, where most variation was explained by the among-population component due to the fixed haplotypes within most lakes. In contrast to the medusa population structures within lakes, polyp populations were always more diverse within a lake/river. Only in two lakes, a single mitochondrial haplotype was found among polyps.

I further detected significant spatial and temporal variation in the genetic structure of polyp populations. This could result from local adaptation since polyps of different haplotypes may exhibit distinct habitat preference, such as physical and chemical properties of water bodies, food abundance, biological competition, and so on. The polyps of various haplotypes should, in principle, be able to successfully disperse between lakes via passive transport (wind, birds, or human activities). For example, the pairwise geographic distances between Brandsee (BA), Neuer Baarer Weiher (BW), Reichertshofen* Lake A (RA) and Waldsee (WA) are ranging from 0.8 and 2.9 km (Fig. 7 (section 3.3.1)). This implies that the genetic structures of polyp populations in these lakes might be similar, although in fact they were significantly different (Tab. 17 (section 4.3.3)). The clear differences in genetic structure among polyp population from neighbouring lakes may point towards strong local adaptation.

5.2.3 Differences in the genetic structure of polyp and medusa stages

In my thesis, the genetic structures of medusae and polyps were analysed at the population level. In terms of the medusa stage, almost all populations did not show genetic variation, as a single haplotype was always predominant within lakes, except the population from Neuer Baarer Weiher, where more than one mtDNA haplotypes and mixed genders were found at each sampling from one year. Temporal changes occurred only in the Neuer Baarer Weiher with obvious variations in haplotype frequencies and genders, whereas in other lakes the same haplotype always appeared within a lake, even in different years. That a single gender of medusa is dominating the population of individual lakes is a common phenomenon which has been frequently reported worldwide (Rice, 1858; Payne, 1926; Kramp, 1950; Pennak, 1956; Acker and Muscat, 1976; DeVries, 1992; Zhang et al., 2016). Yet the reason behind is unrevealed. In my study, the particularity of Neuer Baarer Weiher which did not follow this pattern attracts my attention. I compared lakes with and without medusa appearance with Neuer Baarer Weiher. It became evident that the physicochemical water parameters of Neuer Baarer Weiher differed from all other lakes, possibly shaping the unique diverse genetic structures of this lake. However, the differences between lakes with and without medusae occurrence were not pronounced. What should be noted is that the physicochemical data are only from one year, data from several years are necessarily needed to get more insight into the mechanism underlying the sporadic and single-haplotype appearance of medusae.

In contrast, the genetic structure of polyps within a lake or river is more diverse than medusae even though a single fixed haplotype characterized some lakes as well. Besides, the higher diversity of polyps can even be seen by polyps living on the same substrate, where more than two haplotypes were able to co-exist. Interestingly, the differences of polyp population genetic structure between two adjacent lakes were generally significant. Giving the short distance, the transport of polyps is assumed to be easy and frequent, thus the closer two lakes are, the more similar their polyp populations should be. However, my result was contrary to this assumption. Hence, local adaptation is likely to structure polyp populations as different haplotypes may have distinct preferences for certain environmental parameters, such as temperature, pH, oxygen, and food.

5.3 Medusa sex determination

Another interesting phenomenon is that the gender of medusa was specified by mtDNA haplotype, and the number of observed females was far greater than of males. The gender of polyps cannot be identified by microscopic analyses, thus the sex ratio in polyp populations is totally unknown and therefore cannot be compared to the more easily identifiable gender structure of medusa populations. A fascinating phenomenon is that the sex ratio of medusae in a lake is extremely unbalanced. For example, in the medusa population of Neuer Baarer Weiher, the ratio of females to males was 12:1 in 2016, while the ratio was 1:39 in 2018. Besides the population in Neuer Baarer Weiher described in my data, most other populations were consisting of only one gender as also found in different other studies (Payne, 1924; Pennak, 1956; Fish,1971; Acker & Muscat, 1976; Boothroyd et al., 2002; Lundberg et al., 2005; Pérez-Bote et al., 2006; Minchin et al., 2016; Moreno-Leon & Ortega-Rubio, 2009; Oualid et al., 2019; Morpurgo et al., 2021). The general mechanisms of sex determination in animals are diverse and can include genotypic sex determination (GSD) and environmental sex determination (ESD) (Bachtrog et al., 2014). Sexual conflict or sexual antagonism occurs when traits express different optima in both sexes or conflict outcomes happen in male-female interactions (Chapman et al., 2003) and sex-biased inheritance patterns create this conflict and drive the evolution of male- or female- biased sex ratios (Bachtrog et al., 2014). Strict maternal mitochondrial DNA inheritance (SMI) applies to most animal species, nevertheless, an exception to SMI which is termed doubly uniparental inheritance (DUI) occurs in some bivalves, which may also be the only reports of mtDNA-associated sex determination (Breton et al., 2007; Breton et al., 2011). In brief, in most cases, females inherit the female genome

from their mother and transmit the female genome to both daughters and sons, while males inherit female and male genomes from both parents, but only male genomes are present in their gonads and gametes (Breton et al., 2007). This reflects that two divergent mtDNA-haplotypes can co-exist in somatic cells of male individuals. In terms of our freshwater jellyfish, sex is also mtDNA-associated, while most likely the DUI mechanism does not apply. I sequenced the manubrium (body tissue) and gonads (sex organ tissue), respectively, from one medusa individual of each of the four main haplotypes and I found always consistent haplotypes within individuals. Somatic and reproductive tissues in *Craspedacusta* medusae did not show different mitochondrial haplotypes in both genders and lineages (Type 1 and 2).

5.4 Temperature effects on polyp populations

5.4.1 Colony growth

The colony size, namely the number of polyps per colony, varied under different incubation temperatures in my study ($p < 0.05$), which reflects colony *per se* somatic growth. Previous studies also showed temperature dependent patterns, for example a colony had 3.8 polyps at 28°C, about 3.4 polyps at 25°C, and 5.9 polyps at 12°C after 90 days cultivation in filtered lake water (McClary, 1959). In addition, Folino-Rorem et al. (2016) reported that a colony had 2.2 polyps on average when polyps were cultured in *Hydra* Medium at 24°C over 34 days. A recent study indicated that the number of polyps per colony was up to 3.1 at 19°C, but 2.2 at 29°C cultured in reverse-osmosis filtered water for 80 days (Marchessaux and Bejean, 2020). In my study, a colony consisted of on average 1.9 polyps at 18°C and 2.1 polyps at 26°C when grown for 73 days in *Hydra* Medium. My results at 26°C are almost in accordance with the results described in Folino-Rorem et al. (2016) and Marchessaux and Bejean (2020), whereas the results at 18°C are showing much lower values compared to the results described in Marchessaux and Bejean (2020). In my study, the average polyp number per colony is significantly lower at 18°C than at 26°C, which is in contrast to the results from the other studies mentioned above. One reason for differences could be the different handling of frustules in the compared studies. Whether newly budded frustules are removed or kept in the culture vessel could make a large difference for the population development, as well as for the colony *per se* growth.

My results and the studies mentioned above are all based on laboratory experiments. In a field study collecting polyps from shells of the zebra mussel (*Dreissena polymorpha*) (Stanković

and Ternjej, 2010), it was observed that *Craspedacusta* colonies with two polyps (50.7%) were the most abundant followed by three-polyp colonies (21.1%). My field research on polyps also showed that two-polyp colonies were the most common (Fig. 13 (section 4.3.1)). In my experimental study, at either 18°C or 26°C treatment, two-polyp colonies accounted for over 65%, followed by single-polyp colonies (14-23%). It seems that generally two-polyp colonies might be the most abundant. Subsequently, the question arises, why two-polyp colonies are the most frequent? It is known that all connected polyps share one gastric cavity if a colony has more than one polyp (Bushnell and Porter, 1967). I observed further that connected polyps within one colony are able to cooperate in digesting the same prey. This behaviour is also described by Bushnell and Porter (1967) and Payne (1924). It is likely that the efficiency of two-polyp colonies to catch and digest prey is much higher than those of single polyp colonies. However, with increasing number of connected polyps in a colony, resource use efficiency might decrease, probably depending on food concentration. I assume that independent of temperature, food availability and food concentration determine the number of polyps per colony, yet this is poorly understood to date.

5.4.2 Polyp population dynamics

The understanding of population dynamics of invasive alien species is crucial for predicting invasion processes, as well as for management policies when the species may impose a risk of becoming harmful. Temperature is an essential parameter affecting population growth of aquatic animals, mediated by seasonal temperature fluctuations (Gillooly and Dodson, 2000; Winder et al., 2009). Previous studies (McClary, 1959; Folino-Rorem et al., 2016; Marchessaux and Bejean, 2020) illustrated polyp's growth only through colony size, which focused on the colony *per se* somatic growth alone, while population growth dynamics were not analyzed. Instead, we can have a deeper understanding on the development of polyp populations by surveying their population growth pattern and growth rate, depending not only on environmental factors such as temperature but also on resources such as food. It has been frequently reported in *Daphnia* that temperature effects were reduced when food conditions were limiting growth (Orcutt and Porter, 1984; Giebelhausen and Lampert, 2001). Hence, to avoid food limitation in our study, excessive food was given to the polyps to make sure they did not run into food limitation. Initially, the growth of polyp populations was highly fluctuating at both treatments and stabilizing after some time. This may reflect adaption to the experimental conditions. However, our results clearly show that polyps grow better at high

temperature and 26°C water temperature is not disadvantageous for this species. Increasing summer surface temperatures due to global warming would then promote not only the production of medusa and their growth dynamics (Acker and Muscat, 1976; Lundberg et al., 2005; Minchin et al., 2016) but also the growth of polyp populations. Hence, faster polyp population growth would subsequently further promote medusa populations, thereby enhancing potential trophic cascades within pelagic food webs.

5.4.3 Frustule production

The ability of polyps to produce frustules reveals the full asexual reproductive potential of polyps, as frustules are able to crawl after liberated from polyps, thereby largely accelerating the short-distance dispersal of polyps. It is thought that polyps start to produce frustules when polyps are in good nutritional condition and can use surplus assimilation for frustules production. On the other side, polyps could transform to frustules when environmental conditions get worse, and when polyps are in bad conditions, thereby producing a mobile life stage that can escape the potential poor environmental conditions (Folino-Rorem, 2015). In my study, the total production rate of frustules released by polyp populations at the last day is notably lower at 18°C than at 26°C. On the contrary, the mean number of frustules produced by a polyp throughout the entire experiment shows an opposite pattern, where a polyp produces more frustules at lower temperature, coinciding with the results of Marchessaux and Bejean (2020). At higher water temperature, increasing polyp populations produced in total more frustules, however, the mean number of frustules released by a polyp declined. The larger number of frustules at higher temperature was therefore the result of higher polyp growth and thereby larger polyp numbers, not of a higher production of frustules per polyp. Previous studies demonstrated that frustule production is more likely dependent on food quality and condition (Lytle, 1961; Acker and Muscat, 1976). However, my study shows that also temperature plays an essential role in the production of frustules. Investigating effects of the abiotic environment on the benthic life stages of *Craspedacusta* must therefore also include frustules dynamics and not only the polyp stage as mostly done in studies until yet.

5.5 pH effect on polyp populations

The worldwide spread of the invasive *Craspedacusta* is favoured by its clonally reproducing polyp stage and the dispersal of resting stages which need no food on their colonization routes

(Acker and Muscat, 1976; Duggan and Eastwood, 2012). pH is believed to influence the distribution of medusae and polyps (Folino-Rorem et al., 2016). There are very few experiments on the effects of the environment on polyp dynamics, hence I conducted experiments on the polyp stage to explore weak pH effects on the asexual reproduction of *Craspedacusta*.

I found that polyps from different assemblages and haplotypes grew similarly at different pH levels (8.5, 8.0, 7.8) in both of two independent experiments, suggesting polyps are insensitive to slight pH variations. Weak acidification seems to have no substantial effect on polyp population dynamics. Also, other aquatic organisms seem to be insensitive to weak acidification, for example, some species of diatoms (*Navicula corymbosa*, *Amphora coffeaeformis*, *N. mollis*, and *Cocconeis scutellum var. parva*) are also insensitive to minor pH changes within 1 pH unit (Hinga, 2002), and pH decrease within 1 pH unit had no effect on asexual reproduction of *Aurelia labiate* polyps (Winans and Purcell, 2010). One unanticipated finding nevertheless was that a significant pH effect was found between treatments with a pH of 7.6 and the other three pH treatments in my first experiment. These differences may be explained by the lower initial pH (about 7.1) in my cultures, in order to establish the average pH 7.6, which could harm the polyps. However, both medusae and polyps can be found in lakes ranging in pH values from pH 6.50 to pH 8.14, indicating that polyps are in general able to survive and reproduce in a broad range of pH conditions in the field.

Observed differences in growth dynamics showed some association to the genotypic composition of assemblages as determined by the different mtDNA haplotypes. Recent work has shown that four distinct mtDNA haplotypes of *Craspedacusta* polyps have been invading Europe and that the Type 1.2 is the most frequent one among polyps (Schachtl, 2019, section 4.3.2). In my first pH experiment, similarities among the two assemblages PA1 and PA3 might result from the fact that Type 1.2 obviously managed to reach dominance in both assemblages. The second pH experiment further presented that the growth rate of polyps and the production rate of frustules did not differ among clones and haplotypes. Clonal differences in response to pH changes are well known from a number of aquatic species, such as *Emiliania huxleyi* (Hinga, 2002), *Meseres corlissi* (Weisse et al., 2007), *Montipora digitata* and *Porites cylindrica* (Sekizawa et al., 2017). However, in both experiments, individuals from Type 1.2 (but originating from different lakes) were insensitive to minor pH changes, and there was no pH effect on population growth. But within all pH treatments differences between assemblages of

Type 1.2 emerged in the first experiment. The PA2 populations developed more slowly than PA1 and PA3 independent of pH. In contrast, in the second experiment there was no difference between population growth rate among 1.2 clones in any treatments. The different growth rate of 1.2 Typea could be explained by the fact that experimental clones differ at the nuclear level. This is because that first multi-locus microsatellite data from our group showed that individuals from different lakes who shared the same Type 1.2 haplotype differed at the nuclear level (Giessler et al. in preparation).

Another explanation for the uniform reponse could be that one specific clone outcompeted all others during the run of the experiment. Independent of the initial composition of the assemblages, it seems that Type 1.2 is the most competitive one, because at the end of the experiments, most diversely composed polyp assemblages at the setup of experiments turned out to be composed nearly exclusively of Type 1.2, which is also the most abundant haplotype in polyp populations found in the field. However, it cannot be ruled out that the original haplotypes were still present in low frequencies in respective assemblages because at the end of the experiments only three colonies per treatment and per assemblage were randomly picked for the genetic analysis. In summary, what becomes clear is that genetic differences among and between the four main haplotypes of polyps are important for responses to key environmental drivers despite the limited genetic diversity among *Craspedacusta* polyps.

The insensitivity of polyps to pH variations may be beneficial under potential future acidification by increasing CO_2 levels. However, whether this robustness allows for a future increase in the distribution and growth of *Craspedacusta* under global change scenarios remains open.

6 Outlook

In the following I highlight some of the research questions that in my opinion, still have to be resolved to gain further understanding of *Craspedacusta* dynamics, thereby allowing better predictions about the further impact of this highly invasive species.

6.1 Genetic structure of *Craspedacusta* in the native habitat

Craspedacusta is thought to originate from China, however genetic studies on *Craspedacusta* in China are relatively rare (Hu and Jiang, 2006; Zhang et al., 2009; Xu et al., 2010; Zou et al., 2012; Chi et al., 2016; Tao et al., 2019). Regarding invasive species, the investigation of their genetic structure in the native habitat is extremely critical. Comparing introduced and native populations can help to clarify whether there is reduced genetic variation or genetic loss in the invaded habitat associated with bottleneck and founder effects (Novak and Mack, 1993; Olden et al., 2004; Dlugosch and Parker, 2008; Templeton, 2021). In addition, as shown by my and previous work (Schachtl, 2019), medusa populations are mostly composed of one mtDNA haplotype and one gender. Hence, it would be interesting to know if this is also the case in the native ranges or whether their populations are composed of mixed mtDNA haplotypes and both sexes are more common. According to our latest microsatellite analysis, mtDNA Type 1.1 (common at medusa stage) is a hybrid. It would be very interesting to find and characterize the potential parental species in the native habitat, potentially helping to understand why Type 1.1 is the most successful invasive haplotype at the medusa stage and distributed worldwide.

6.2 Mechanism underlying sex determination

While only known thus far in some bivalves (Breton et al., 2007; Breton et al., 2011), our group found that the sex determination of *Craspedacusta* at the medusa stage is also mtDNA-associated, however the mechanism seems to differ from doubly uniparental inheritance (DUI) (see section 5.3). It is thus necessary to clarify whether their mode of sex determination is genotypic sex determination (GSD), environmental sex determination (ESD) or a mixture of both. Apart from the medusa stage, questions regarding sex determination arise in the other stages as well, especially the benthic stages involving polyps, frustules and even podocysts. It is interesting to know the potential sex determination in these benthic stages and how it works. Thus, it is important to find and develop potential specific gender markers to elucidate gender

composition and sex determination mechanisms in *Craspedacusta*. Furthermore, a reliable method to induce medusa budding in the laboratory would be of great help to shed light on the influence of environmental factors, such as temperature, light, and food on reproductive dynamics. For example, the anti-inflammatory drug indomethacin is reported to enhance the conversion of polyps to the sexual medusa stage in the moon jellyfish, *Aurelia aurita* (Kuniyoshi et al., 2012). Likewise, other known metamorphic inducers, such as indomethacin, H_2O_2, thyroxine and iodine and retinol (Cabrales-Arellano et al., 2017) can be tested to stimulate medusa budding. Finally, the molecular mechanism underlying medusa budding by polyps is needed to resolve these questions.

6.3 Sampling bias on the unbalanced sex ratio of medusae

According to previous published literature and our own studies, it seems extremely common that medusa populations consist only of a single gender, with few exceptions. The question arises whether an intensified monitoring protocol with larger sampling efforts will show further exceptions. What we should take care of is whether the development of female- and male-medusae is synchronous or not. This question could be examined under controlled conditions in the laboratory. If they are clear signs of asynchronously developments, such as described for the moon jellyfish *Aurelia* (Liu et al., 2018), single gender populations would most likely be a result of discrete sampling times, which may in turn result in missing the appearance of both sexes of medusae. It is therefore worth monitoring medusae occurrence in higher temporal resolutions over a continuous period to explore whether transition between both sexes and/or mtDNA haplotypes occurs within a year. Such dynamics can result in misleading information about the sex ratio and genetic structure within medusa populations if the sampling strategy does not take this into account. Furthermore, as we know that the gender in medusae is mtDNA haplotypes specific, the question arises whether reproductive isolation between these different lineages is existing or not.

6.4 Taxonomy within the genus *Craspedacusta*

Molecular evidence (genetic data) is critical to understand the diversification in *Craspedacusta*, however, more evidence from other sources is also needed to resolve the phylogenetic relationships and evolutionary history. These would be morphological data (data of organs, anatomy, and the structure of the body), physiological data (functioning of the parts of the

organism), ethological data (differences in behavior) and ecological data (differences in the response to the environment, preference and tolerance of environmental conditions, seasonal dynamics, geographical distribution, etc.) (Blackwelder, 1967; Wiley and Lieberman, 2011). For example, the metabarcoding of the gut content in polyps and medusae from field samples will allow to have a much better insight into the food preference of different haplotypes of polyps or medusae. In addition, resolving the 3D structure of, for example, cnidae can provide additional helpful information as cnidae are a critical feature for taxonomy in the phylum Cnidaria. Moreover, with the popularity of second and third generation sequencing, whole genome sequencing could help to delineate described species of *Craspedacusta* more precisely.

6.5 Food web effects induced by *Craspedacusta*

To date, experimental studies on effects of *Craspedacusta* on freshwater ecosystems are scarce. It is known that blooms of *Craspedacusta* may cause the significant reduction of zooplankton (Himchik et al., 2021). However, whether this further affects higher trophic levels, such as economically important fish stocks, is unknown. It has been already reported that *Craspedacusta* can feed on fish eggs or larvae (DeVries, 1992; Spadinger and Maier, 1999), while the ecological relationship between *Craspedacusta* and fish is unrevealed to date. It is therefore essential to survey the interactions between *Craspedacusta* and potential interacting trophic levels to provide appropriate management measurements if needed. At present, monitoring *Craspedacusta* populations is troublesome. Due to the sporadic and unpredictable occurrence of medusae and the difficulty to screen polyp populations, a concerted sampling strategy to monitor this invasive species does not yet exist. Applications of environmental DNA (eDNA) methods in Aquatic Ecology could be a future helpful method, as *Craspedacusta* has already been successfully detected in lakes using this method (Mychek-Londer et al., 2020). Therefore, monitoring the distribution and further dispersal of this invasive species can be facilitated, which subsequently helps to also gain a much better understanding of their recent and future effects on freshwater ecosystems.

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Supplementary Tables

Suppl. Tab. 1 Genetic diversity for *Craspedacusta* medusa populations from 17 lakes in Central Europe based on 16S rRNA. ∑: sum of genotyped individuals sampled between 2015 and 2018. Nhap: number of haplotypes per sampling, F: female, M: male.

Lake (Abb.)	***Latitude, Longitude***	**Sampling Time**	**Individuals with Type**					**∑**	**Nhap**
			1.1 F	**1.1.2 F**	**1.2 M**	**2.1 M**	**2.2 F**		
Brandsee (BA)	*48.6802, 11.5235*	Sep 17	39	0	0	0	0	39	1
Neuer Baarer Weiher (BW)	*48.6770, 11.491*	Aug 16	0	0	12	0	0	12	1
		Sep 16	0	0	0	1	0	1	1
		Aug 17	0	0	2	0	0	2	1
		Sep 17	0	0	12	0	7	19	2
		Oct 17	0	0	0	7	1	8	2
		Sep 18	0	0	0	39	1	40	2
		BW total	*0*	*0*	*26*	*47*	*9*	*82*	*3*
Geisenfeld* Lake C (GC)	*48.7019, 11.5530*	Aug 17	39	1	0	0	0	40	2
Reichertshofen* Lake A (RA)	*48.6911, 11.5237*	Sep 16	0	0	0	0	39	39	1
Reichertshofen* Lake C (RC)	*48.6911, 11.5193*	Aug 17	40	0	0	0	0	40	1
Reichertshofen* Lake D (RD)	*48.6887, 11.5176*	Aug 17	0	0	0	0	40	40	1
Waldsee (WA)	*48.6925, 11.5133*	Aug 15	37	0	0	0	0	37	1
		Jul 16	5	0	0	0	0	5	1
		Aug 16	35	0	0	0	0	35	1
		Jul 17	40	0	0	0	0	40	1
		Jul 18	40	0	0	0	0	40	1
		WA total	*157*	*0*	*0*	*0*	*0*	*157*	*1*
Germany Ingolstadt Region (IG)			***275***	***1***	***26***	***47***	***88***	***437***	***5***
Gernlindner Waldsee (GW)	*48.2202, 11.298*	Sep 18	0	0	0	0	40	40	1
Haager Weiher (HA)	*48.4503, 11.8283*	Aug 17	40	0	0	0	0	40	1
Haselfurther Weiher (HW)	*48.4820, 12.0130*	Aug 15	40	0	0	0	0	40	1
		Jul 16	40	0	0	0	0	40	1
		HW total	*80*	*0*	*0*	*0*	*0*	*80*	*1*
Kleiner Leitner Weiher (KL)	*48.7051, 11.3211*	Jul 17	40	0	0	0	0	40	1
Other areas in Germany (OG)			***160***	***0***	***0***	***0***	***40***	***200***	***2***
Feldkirchner Badesee IV (FK)	*48.3260, 14.0689*	Aug 15	40	0	0	0	0	40	1
Schwarzlsee (SS)	*46.9830, 15.4254*	Aug 15	40	0	0	0	0	40	1
Austria (AU)			***80***	***0***	***0***	***0***	***0***	***80***	***1***
Boreckáskalka (BK)	*49.7922, 15.580*	Sep 15	37	0	0	0	0	37	1
Blatna-Recicequarry (BR)	*49.4349, 13.8619*	Aug 16	40	0	0	0	0	40	1
Kojetice (KO)	*50.2407, 14.5157*	Sep 15	38	0	0	0	0	38	1
Jílové / Držkova (JD)	*50.6691, 15.2893*	Oct 15	0	0	0	38	0	38	1
Czech Republic (CR)			***115***	***0***	***0***	***38***	***0***	***153***	***2***
Overall (17 lakes)			***630***	***1***	***26***	***85***	***128***	***870***	***5***

Suppl. Tab. 2 Consistency of haplotype assignments of 337 medusa individuals from *Craspedacusta* populations in subsamples from 17 lakes which were genotyped at both mitochondrial loci (16S and COI) (*indicate closest town to lake). Nseq: number of sequenced individuals; Nhap: number of haplotypes in each lake; F: female; M: male.

Lake (Abb.)	Locus	individuals with Type					Nseq	Nhap
		1.1 F	1.1.1 F	1.2 M	2.1 M	2.2 F		
Brandsee (BA)	16S	5	0	0	0	0	5	1
	CO1	5	0	0	0	0	5	1
Borecká skalka (BK)	16S	3	0	0	0	0	3	1
	CO1	3	0	0	0	0	3	1
Blatná - Řečice * (BR)	16S	40	0	0	0	0	40	1
	CO1	40	0	0	0	0	40	1
Neuer Baarer Weiher (BW)	16S	0	0	26	9	9	44	3
	CO1	0	0	26	9	9	44	3
Feldkirchner Badesee IV (FK)	16S	40	0	0	0	0	40	1
	CO1	40	0	0	0	0	40	1
Geisenfeld* Lake C (GC)	16S	5	0	0	0	0	5	1
	CO1	5	0	0	0	0	5	1
Gernlindner Waldsee (GW)	16S	0	0	0	0	2	2	1
	CO1	0	0	0	0	2	2	1
Haager Weiher (HA)	16S	5	0	0	0	0	5	1
	CO1	5	0	0	0	0	5	1
Haselfurther Weiher (HW)	16S	45	0	0	0	0	45	1
	CO1	45	0	0	0	0	45	1
Jílové/Držkova (JD)	16S	0	0	0	38	0	38	1
	CO1	0	0	0	38	0	38	1
Kleiner Leitner Weiher (KL)	16S	5	0	0	0	0	5	1
	CO1	5	0	0	0	0	5	1
Kojetice (KO)	16S	4	0	0	0	0	4	1
	CO1	4	0	0	0	0	4	1
Reichertshofen* Lake A (RA)	16S	0	0	0	0	4	4	1
	CO1	0	0	0	0	4	4	1
Reichertshofen* Lake C (RC)	16S	5	0	0	0	0	5	1
	CO1	5	0	0	0	0	5	1
Reichertshofen* Lake D (RD)	16S	0	0	0	0	5	5	1
	CO1	0	0	0	0	5	5	1
Schwarzlsee (SS)	16S	40	0	0	0	0	40	1
	CO1	40	0	0	0	0	40	1
Waldsee (WA)	16S	47	0	0	0	0	47	2
	CO1	43	4	0	0	0	47	2
Overall (17 lakes)	16S	244	0	26	47	20	337	4
	CO1	240	4	26	47	20	337	5
	concatenate	240	4	26	47	20	337	5

Suppl. Tab. 3 Environmental characteristics of twenty lakes in Southern Germany. Abb.: abbreviations of lake names, –: concentration below detection level was scored as zero, N: no medusae occurred, 1.1/1.2/2.1/2.2: medusa haplotypes, highlighted in grey: lakes in Ingolstadt Germany region.

Lake	Abb.	Sampling date	Medusae occurrence	Depth	T	pH	O_2	Chla	TP	N:P	Cl^-	SO_4^{2-}	NO_2^-	NO_3^-
Alter Baarer Weiher	AB	14/08/2017	N	5.39	21.59	8.22	6.61	54.92	21.32	32	25.08	46.43	–	0.08
Geisenfeld* Lake B	GB	01/08/2017	1.1	3.73	22.99	8.14	6.14	28.03	17.86	27	12.90	32.62	–	–
Geisenfeld* Lake C	GC	22/08/2017	1.1	7.66	22.23	8.27	6.31	17.15	15.61	26	13.13	21.73	–	–
Geisenfeld* Lake D	GD	03/08/2017	N	5.72	24.96	8.20	6.70	21.75	17.27	33	13.52	15.65	–	–
Geisenfeld* Lake E	GE	03/08/2017	N	5.93	25.54	8.39	7.53	39.50	26.27	30	13.40	14.72	–	–
Geisenfeld* Lake F	GF	01/08/2017	N	4.60	22.07	8.34	6.84	28.91	16.88	41	13.77	13.38	–	–
Geisenfeld* Lake G	GG	23/08/2017	N	9.88	22.45	8.43	7.27	21.03	13.24	41	24.23	27.12	–	–
Kempesee	KP	14/08/2017	N	4.38	22.11	8.21	9.45	14.61	9.95	36	49.26	107.62	–	0.93
Kleiner Leitner Weiher	KL	18/07/2017	1.1	3.86	23.84	8.36	8.92	12.78	14.33	43	28.80	57.22	–	–
Neuer Baarer Weiher	BW	14/08/2017	1.2/2.1/2.2	6.24	22.08	7.84	7.58	37.38	15.88	41	25.30	58.35	0.08	2.09
Reichertshofen* Lake A	RA	12/07/2017	2.2	12.27	24.51	8.31	7.73	10.57	9.59	34	29.77	32.81	–	–
Reichertshofen* Lake B	RB	25/07/2017	1.1	6.36	23.47	8.30	7.39	20.32	13.09	39	34.33	33.18	–	–
Reichertshofen* Lake C	RC	23/08/2017	1.1	8.77	23.25	8.66	8.39	6.90	10.67	30	40.97	30.01	–	–
Reichertshofen* Lake D	RD	28/08/2017	2.2	10.60	23.89	8.44	8.12	8.59	11.38	–	45.35	36.28	–	–
Reichertshofen* Lake E	RE	01/08/2017	N	5.05	22.27	8.32	7.02	18.08	11.40	33	23.79	22.55	–	–
Reichertshofen* Lake F	RF	25/07/2017	N	8.22	23.03	8.13	7.55	20.00	13.49	32	51.74	36.77	–	–
Reichertshofen* Lake H	RH	22/08/2017	N	7.93	22.26	8.41	7.46	12.92	11.00	36	23.24	44.17	–	–
Seehof*	SE	22/08/2017	N	4.84	22.30	8.17	8.00	12.23	8.34	38	48.21	110.84	–	3.06
Waldsee	WA	12/07/2017	1.1	9.67	24.12	8.09	7.26	19.33	7.67	44	70.82	52.79	–	–
Weicheringer See	WE	18/07/2017	N	4.65	23.48	8.07	7.82	10.57	11.88	23	24.97	50.88	–	–

Suppl. Tab. 4 Eigenvectors (Coefficients in the linear combinations of variables making up PC's) in principal component analyses

Lakes	Variable	PC1	PC2	PC3	PC4	PC5
All twenty lakes	Depth	-0.057	-0.354	0.628	-0.223	-0.054
	T	0.119	-0.280	0.254	0.704	-0.340
	pH	0.100	-0.428	-0.354	-0.098	-0.028
	O_2	-0.395	-0.126	-0.212	0.447	-0.085
	Chl-a	0.306	0.386	0.070	0.058	-0.125
	TP	0.446	0.209	-0.097	0.362	-0.010
	N:P	-0.088	0.251	-0.111	-0.267	-0.887
	Cl^-	-0.425	-0.136	0.206	0.011	-0.165
	SO_4^{2-}	-0.468	0.199	-0.223	0.142	0.077
	NO_2^-	-0.071	0.392	0.501	0.097	0.096
	NO_3^-	-0.334	0.359	0.033	0.104	0.169
Six closely located lakes in Ingolstadt Germany region	Depth	0.344	-0.151	-0.036	-0.627	0.099
	T	0.327	-0.321	-0.042	-0.386	0.040
	pH	0.332	0.240	0.258	0.357	0.234
	O_2	0.149	-0.218	0.736	0.095	0.313
	Chl-a	-0.394	-0.149	-0.078	-0.084	-0.091
	TP	-0.304	0.397	0.118	-0.102	-0.108
	N:P	-0.185	-0.291	-0.439	0.225	0.753
	Cl^-	0.167	-0.483	-0.028	0.457	-0.414
	SO_4^{2-}	-0.227	-0.494	0.112	0.008	-0.229
	NO_2^-	-0.376	-0.115	0.286	-0.149	0.111
	NO_3^-	-0.376	-0.115	0.286	-0.149	0.111

Suppl. Tab. 5 Genetic diversity of polyp populations within each lake based on COI (*indicate closest town to lake). Nseq: number of sequenced colonies; Nhap: number of haplotypes in each lake/river; hd: haplotype diversity; π: nucleotide diversity.

Lake/River (Abb.)	Latitude	Longitude	Time	Individuals with Type					Nseq	Nhap	hd	π	Medusae occurrence
				1.1	1.1.1	1.2	2.1	2.2					
Amper (AM)	48.18	11.28	July-Sep 2018	23	0	20	0	0	43	2	0.509	0.0026	N
Neuer Baarer Weiher (BW)	48.68	11.49	July-Sep 2018	0	0	15	22	2	39	3	0.545	0.0746	1.2 & 2.1 & 2.2
Brandsee (BA)	48.68	11.52	July-Sep 2019	40	0	0	0	0	40	1	0.000	0.000	1.1
Gernlindner Waldsee (GW)	48.22	11.30	Aug-Oct 2019	0	0	0	0	26	26	1	0.000	0.000	2.2
Heiglweiher (HE)	48.31	11.53	Aug-Nov 2019	1	0	8	0	16	25	3	0.507	0.0753	1.1
Langwieder See (LW)	48.19	11.42	Nov 2015	1	0	12	0	2	15	3	0.362	0.0396	N
			Dec 2016	8	0	56	0	19	83	3	0.489	0.0560	
			July 2018	0	0	4	0	14	18	2	0.366	0.0573	
			total	9	0	72	0	35	116	3	0.522	0.0667	
Reichertshofen* Lake A (RA)	48.69	11.52	Sep 2019	0	4	11	0	7	22	3	0.645	0.0727	2.2
Waldsee (WA)	48.69	11.51	July-Aug 2019	14	21	0	0	0	35	2	0.494	0.0008	1.1 & 1.1.1
Overall				87	25	126	22	86	346	5	0.735	0.0690	-

Suppl. Tab. 6 Consistency of haplotype assignments of 198 colonies from polyp populations in subsamples from two lakes and one river which were genotyped at both mitochondrial loci (16S and COI) (*indicate closest town to lake). Nseq: number of sequenced colonies by 16SrRNA and COI; Nhap: number of haplotypes in each lake.

Lake/River (Abb.)	Locus	Individuals with Type					Nseq	Nhap
		1.1	1.1.1	1.2	2.1	2.2		
Amper (AM)	16S	23	0	20	0	0	43	2
	COI	23	0	20	0	0	43	2
Neuer Baarer Weiher (BW)	16S	0	0	15	22	2	39	3
	COI	0	0	15	22	2	39	3
Langwieder See (LW)	16S	9	0	72	0	35	116	3
	COI	9	0	72	0	35	116	3
Total	16S	32	0	107	22	37	198	4
	COI	32	0	107	22	37	198	4

Suppl. Tab. 7 Growth rates of colonies and production rates of frustules by polyps (Mean ± SE) from various assemblages at different treatments at the last experimental day in the first experiment.

Assemblages	T	pH	Growth rate of colonies			Production rate of frustules		
			value	method	significance	value	method	significance
PA1	18°C	8.5	0.09 ± 0.00	Welch ANOVA	F=14.734 $p < 0.05$	0.07 ± 0.01	Kruskal-Wallis test	$p > 0.05$
		8.0	0.10 ± 0.00			0.07 ± 0.00		
		7.8	0.08 ± 0.01			0.03 ± 0.03		
		7.6	0.04 ± 0.01			0.01 ± 0.01		
	26°C	8.5	0.11 ± 0.00	Welch ANOVA	F=39.638 $p < 0.05$	0.06 ± 0.00	Kruskal-Wallis test	$p < 0.05$
		8.0	0.11 ± 0.00			0.07 ± 0.00		
		7.8	0.10 ± 0.01			0.07 ± 0.01		
		7.6	0.02 ± 0.01			0.00 ± 0.00		
PA2	18°C	8.5	0.07 ± 0.01	one-way ANOVA	F=15.011 $p < 0.05$	0.05 ± 0.01	one-way ANOVA	F=4.092 $p < 0.05$
		8.0	0.06 ± 0.00			0.06 ± 0.00		
		7.8	0.06 ± 0.00			0.03 ± 0.01		
		7.6	0.01 ± 0.01			0.01 ± 0.01		
	26°C	8.5	0.06 ± 0.01	one-way ANOVA	F=2.939 $p > 0.05$	0.02 ± 0.01	one-way ANOVA	F=0.180 $p > 0.05$
		8.0	0.05 ± 0.01			0.03 ± 0.02		
		7.8	0.05 ± 0.01			0.02 ± 0.01		
		7.6	0.02 ± 0.01			0.02 ± 0.01		
PA3	18°C	8.5	0.09 ± 0.00	one-way ANOVA	F=45.766 $p < 0.05$	0.08 ± 0.00	Kruskal-Wallis test	$p < 0.05$
		8.0	0.09 ± 0.00			0.06 ± 0.01		
		7.8	0.07 ± 0.00			0.05 ± 0.01		
		7.6	0.04 ± 0.00			0.01 ± 0.01		
	26°C	8.5	0.11 ± 0.00	one-way ANOVA	F=11.126 $p < 0.05$	0.05 ± 0.00	Kruskal-Wallis test	$p < 0.05$
		8.0	0.10 ± 0.00			0.08 ± 0.00		
		7.8	0.10 ± 0.00			0.08 ± 0.00		
		7.6	0.03 ± 0.02			0.01 ± 0.01		
PA4	18°C	8.5	0.06 ± 0.00	one-way ANOVA	F=1.138 $p > 0.05$	0.05 ± 0.00	Kruskal-Wallis test	$p > 0.05$
		8.0	0.06 ± 0.01			0.04 ± 0.02		
		7.8	0.05 ± 0.01			0.03 ± 0.02		
		7.6	0.04 ± 0.00			0.00 ± 0.00		
	26°C	8.5	0.05 ± 0.01	Kruskal-Wallis test	$p > 0.05$	0.03 ± 0.02	Kruskal-Wallis test	$p > 0.05$
		8.0	0.05 ± 0.00			0.03 ± 0.02		
		7.8	0.03 ± 0.02			0.04 ± 0.01		
		7.6	0.03 ± 0.01			0.02 ± 0.02		

Suppl. Tab. 8 The differences of growth rate of colonies and production rate of frustules (Mean ± SE) from various lakes at different pH at the last experimental day in the second experiment.

Lakes	pH	Growth rate of colonies			Production rate of frustules		
		value	method	significance	value	method	significance
LS	8.5	0.03 ± 0.01	one-way ANOVA	F=0.096 $p > 0.05$	0.02 ± 0.01	Mann-Whitney U	$p > 0.05$
	7.8	0.02 ± 0.02			0.01 ± 0.01		
SS	8.5	0.02 ± 0.02	Welch ANOVA	F=3.265 $p > 0.05$	0.01 ± 0.01	Mann-Whitney U	$p > 0.05$
	7.8	0.06 ± 0.00			0.02 ± 0.01		
FS	8.5	0.04 ± 0.01	Mann-Whitney U	$p > 0.05$	0.02 ± 0.00	Mann-Whitney U	$p > 0.05$
	7.8	0.05 ± 0.00			0.03 ± 0.00		
BS	8.5	0.01 ± 0.01	one-way ANOVA	F=0.285 $p > 0.05$	0.01 ± 0.01	Mann-Whitney U	$p > 0.05$
	7.8	0.02 ± 0.02			0.02 ± 0.02		

Declarations

Eidesstattliche Erklärung

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

München, den ....02.08.2022..... .....

Yuanyuan Wang

Erklärung

Hiermit erkläre ich, *

[X] dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

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Yuanyuan Wang

*) Nichtzutreffendes streichen