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Adaptive radiation of Arctic charr (*Salvelinus alpinus*) in three Norwegian lakes

- niche segregation, phenotypic and genetic variation

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Adaptive radiation of Arctic charr (*Salvelinus alpinus*) in three Norwegian lakes - niche segregation, phenotypic and genetic variation

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Abstract

Understanding the ecological and physical factors driving the origin of species, and which ones are shaping new intraspecific diversity, are the "holy grail" of evolutionary biology. Adaptive radiation is the evolutionary process that can generate diversification of phenotypes and genotypes across different environments, differentiating a single ancestor into different forms and species. Under ecological speciation, local adaptation through natural selection drives the divergence of populations, evolving reproductive isolation and leading to the formation of new eco-morphs, populations, and ultimately, species. A good example of polymorphic species is Arctic charr (*Salvelinus alpinus*), which has the flexibility to occupy different niches (i.e. a specific range of abiotic and biotic factors that a species has specialised) in a lake. For example, in fish, the specialisation into a specific niche can favour divergence among morphs, showing differences in morphology, growth, maturity, spawning time and site, developing reproductive barriers among the morphs. This thesis focuses on phenotypic and genetic divergence of Arctic charr morphs. The main objectives are to investigate trophic niche segregation (i.e. diet choice and habitat use), morphological and genetic differences among sympatric Arctic charr morphs from three different lakes in Norway (Tinnsjøen, Tårnvatn and Skøvatn). Two Arctic charr morphs were found coexisting in Lake Skøvatn, three morphs in Lake Tårnvatn and four morphs in Lake Tinnsjøen. Two novel morphs were found in Lake Tinnsjøen and Skøvatn. All morphs showed divergence in life history, genetics, phenotype, diet and habitat use. The piscivore morphs fed mainly on fish and were found in the profundal habitat of Lake Tinnsjøen and Tårnvatn. The planktivore morphs were feeding mainly on zooplankton, and were found across different habitats from these three lakes. Life-history traits and habitat use was similar among the small-sized profundal morphs, but the morph in Skøvatn presented differences in diet choice compared to the morph from Lake Tårnvatn. Finally, the Abyssal morph was found in the deep-profundal habitat in Lake Tinnsjøen, presenting similarities with cave fish such as white coloration, reduced eyes and small brain regions. Parallel evolution could be responsible for the similarities found among some of the Arctic charr morphs across these three lakes. These findings show how selection pressures can sometimes lead to similar outcomes in similar environments. However, phenotypic plasticity may also be an important component during the early stages of niche specialization. These morphs are likely under ecological speciation, where natural selection could play an important role in the adaptive divergence of morphs, contributing to reproductive isolation. Arctic charr polymorphism could be a case of adaptive radiation, explaining their diversity across different freshwater systems.

Sammendrag

Å forstå hvordan økologiske og fysiske faktorer fører til opprinnelsen av nye arter, og hvilke faktorer som former ny intraspesifikk diversitet, er den "hellige gral" i evolusjonær biologi. Adaptiv radiasjon er den evolusjonære prosessen som kan føre til diversifisering av fenotyper og genotyper i ulike miljøer, og som kan splitte en forfaders form eller linje i nye former og arter. I den økologiske artsdannelsesprosessen vil lokal tilpasning gjennom naturlig seleksjon drive oppsplittingen av populasjoner, noe som vil føre til evolusjon av reproduktiv isolasjon og dermed dannelsen av nye økotyper, morfer, populasjoner og til slutt arter. Et godt eksempel på en polymorf art er røya (*Salvelinus alpinus*) som kan ha ulike nisjer (det vil si tilpasning til spesifikke abiotiske og biotiske forhold) i en innsjø. Spesialiseringen til en bestemt nisje kan favorisere divergensen mellom morfene, noe som kan lede til forskjeller i utseende, vekst, kjønnsmodning, gytetid og sted, og over tid utvikle reproduktive barrierer mellom morfene. Denne doktorgradsavhandlingen fokuserer på fenotypisk og genetisk divergens av røyemorfer. Hovedmålet er å undersøke nisjesegregering på trofisk nivå (det vil si diettvalg og habitatbruk), ved å studere morfologiske og genetiske forskjeller mellom røyemorfer fra tre forskjellige ferskvannsystemer i Norge (Tinnsjøen, Tårnvatn og Skøvatn). To røyemorfer ble funnet eksisterende sammen i Skøvatn, tre morfer i Tårnvatn, og fire i Tinnsjøen. To nye morfer ble funnet i Tinnsjøen og Skøvatn. Alle morfene viste forskjeller i livshistorie, genetikk, fenotype, diettvalg og habitatbruk. De fiskespisende morfene ernærte seg hovedsakelig av fisk, og ble funnet i den profundale delen av Tinnsjøen og Tårnvatn. Planktivore morfer spiste hovedsakelig dyreplankton, og ble funnet i flere ulike habitater i disse tre innsjøene. Livshistoriekarakterer og habitatbruk var lignende hos de småvokste dypvannsmorfene, men morfen i Skøvatn viste forskjeller i diettvalg sammenlignet med morfen fra Tårnvatn. Den nye dypvannsmorfen som ble funnet i den dypere delen av Tinnsjøen har likhetstrekk med hulefisk som hvitt skinn, underutviklede øyne og små hjerneregioner. Parallell evolusjon kan være en forklaring for likhetene som er funnet blant noen av morfene i disse tre ferskvannssystemene. Funnene viser hvordan seleksjonspress kan føre til lignende resultater i samme miljøer, der fenotypisk plastisitet også kan være en viktig mekanisme i tidlige stadier av nisjespesialisering. Disse morfene er sannsynligvis i en økologisk artsdannelsesprosess, der naturlig seleksjon spiller en viktig rolle i den adaptive divergensen av morfer, og bidrar til reproduktiv isolasjon. Adaptiv radiasjon kan forklare tilpasninger og diversitet hos den polymorfe røya i ulike vann.

Preface

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Contents

List of Papers

This PhD thesis includes the following papers:

Paper I   Østbye, K., Hagen, M.H., **Tamayo, A. M. P.**, Hagenlund, M., Vogler, T., & Præbel, K. (2020). *"And if you gaze long into an abyss, the abyss gazes also into thee"*: four morphs of Arctic charr adapting to a depth gradient in Lake Tinnsjøen. *Evolutionary applications*, *13*(6), 1240-1261. https://doi.org/10.1111/eva.12983

Paper II   **Tamayo, A. M. P**., Devineau, O., Præbel, K., Kahilainen, K. K., & Østbye, K. (2020). A brain and a head for a different habitat: Size variation in four morphs of Arctic charr (*Salvelinus alpinus* (L.)) in a deep oligotrophic lake. *Ecology and Evolution*, *10*(20), 11335-11351. https://doi.org/10.1002/ece3.6771

Paper III   **Tamayo, A. M. P**., Bhat, S., Østbye, K., Bitz-Thorsen, J., & Præbel, K. Chromosomal inversions, polymorphism and local adaptation in four sympatric Arctic charr morphs. *Manuscript*.

Paper IV   Moccetti, P., Siwertsson, A., Kjær, R., Amundsen, P. A., Præbel, K., **Tamayo, A. M. P.**, Power, M., & Knudsen, R. (2019). Contrasting patterns in trophic niche evolution of polymorphic Arctic charr populations in two subarctic Norwegian lakes. *Hydrobiologia*, *840*(1), 281-299. https://doi.org/10.1007/s10750-019-3969-9

Introduction

Species concepts: a systematic debate

Species are a basic unit that are compared in different fields such as biology, ecology, physiology, evolution, genetics and systematics. The definition of species has been a controversial topic for a long time and different species concepts have been proposed to classify the diversity of species. One of the systematists to define the species concept based on plants was John Ray, who defined a species as a set of plants which breed within their range of variation (Ray, 1686). Linnaeus was the next person to describe a wider species concept based on a sexual system, mainly describing sexual traits and floral structure (Linnaeus, 1753). Ray and Linnaeus used the species concept based on typology. De Candolle defined species as a group of individuals with similar characteristics, which can produce fertile offspring (De Candolle, 1813). Some decades later, Darwin considered species as fundamental units of evolution, which could originate rapidly depending on the environmental conditions (Darwin, 1859). During centuries, different species concepts have been proposed to clarify the definition of species. For instance, one of these concepts is the "Biological Species Concept", defining a species as a group of populations that interbreed, and which are reproductively isolated from other populations, occupying a specific niche (Mayr, 1942, 1982). Another concept is the "Typological or Morphological species concept", stating that a species is a community or group of communities with specific morphological traits (Regan, 1925). The "Ecological Species Concept" is mainly based on ecological competition, where similar individuals have similar needs and thus, are expected to compete (Colinvaux, 1986). These are some of the proposed species concepts among others (e.g. evolutionary, cohesion, phylogenetic), suggesting different criteria for defining a species (see review e.g. Coyne & Orr, 2004). Thus, defining a species is not a simple decision to make since it is sometimes possible to combine more than one concept, such as the ecological and morphological species concepts. Furthermore, we must also take into consideration that the speciation process, regardless of species concepts applied, is an ongoing process with units at different stages of divergence. This further implies that defining the specific limits of what is a species is a complex task.

In nature, we can also find intraspecific variation (e.g. polymorphic species), which can lead to difficulties in their classification depending on the species concept used. The classification of polymorphic species (i.e. different forms within a species that can have phenotypic variation depending on the environment where they live) together with cryptic species (i.e. different species classified under one species name) have challenged the species concept. Thus, the work presented in this thesis contributes to this needed knowledge as it reveals phenotypic and genetic variation within a polymorphic species, and potential drivers.

Adaptive radiation and reproductive isolating barriers

Knowledge about adaptive genetic and ecological diversity is important to understand the mechanisms behind speciation. There are different mechanisms of speciation such as natural selection, sexual selection, hybridization, drift and polyploidy (Schluter, 1996; Coyne & Orr, 2004). Adaptive radiation is the diversification of genotypes and phenotypes in heterogeneous environments as a result of divergent natural selection (Schluter, 2000a; b), where populations adapting to different niches diverge. Thus, these populations can develop reproductive isolation, leading to new species through ecological speciation (Schluter, 2000a; Rundle & Nosil, 2005; Schluter & Conte, 2009; Skúlason *et al.*, 2019), which is the development of reproductive isolation between populations or within a population as a result of their adaptation to different environments driven by divergent natural selection (Schluter, 2000b, 2001; Rundle & Nosil, 2005; Nosil, 2012). Adaptive radiation can arise when populations accumulate different and incompatible mutations, evolving reproductive isolation (Mani & Clarke, 1990; Nosil & Flaxman, 2011). Phenotypic plasticity (i.e. ability of a genotype to produce several phenotypes in different environments) can also contribute to population divergence, where natural selection can act (Smith & Skúlason, 1996; Pigliucci, 2001; Pigliucci *et al.*, 2006; Skúlason *et al.*, 2019).

Divergent selection can also act in specific regions of the genome, creating regions with large differentiation known as genomic islands of divergence (Nosil *et al.*, 2009; Feder *et al.*, 2012). Genomic islands can grow in size under divergent hitchhiking (i.e. the fixation of a neutral loci, which is closely linked to another loci that is under selection), showing a decrease in the recombination rate and gene flow in nearby regions (Via & West, 2008; Nosil *et al.*, 2009;

Feder *et al.*, 2012; Via, 2012; Kulmuni *et al.*, 2020). Chromosomal rearrangements (i.e. reorganisations of chromosome structure that can alter the function of one or several genes) can also generate genomic islands (Kirkpatrick & Barton, 2006; Feder & Nosil, 2009; Via, 2012). An example of chromosome rearrangements are inversions, which can suppress recombination and can cause large linkage disequilibrium, increasing differentiation in specific locations of the genome and developing determinate phenotypes, which can be associated with local adaptation (Kirkpatrick & Barton, 2006; Hoffmann & Rieseberg, 2008; Slatkin, 2008; Kirkpatrick, 2010; Berg *et al.*, 2017).

Divergent selection might also arise from interspecific or intraspecific interactions such as predation and competition, or from environmental heterogeneity (Schluter, 2000b; Nosil, 2012). Hybridisation (i.e. interbreeding of different populations or species) and introgression (i.e. inclusion of alleles from one gene pool of one entity to another gene pool of a divergent entity via hybridisation and backcrossing) can happen after secondary contact (i.e. different populations that have been isolated during a period of time prior to contact, which in some cases could re-establish gene flow; Anderson & Hubricht, 1938; Anderson, 1949; Schluter, 2001, 2009; Johannesson *et al.*, 2020). In this scenario, selection could decrease fitness of intermediate hybrids and drive local adaptations in divergent populations (Schluter, 2001, 2009), causing reproductive isolation and speciation. Reproductive isolation could include premating (i.e. prevent individuals to mate) and postmating (i.e. prevent fertilisation or formation of fertile or viable offspring) isolating barriers (Coyne & Orr, 2004; Schluter, 2009; Schluter & Conte, 2009). Premating isolating barriers includes, for instance, mechanical, behavioural, spatial and temporal isolation. Postmating isolating barriers can be prezygotic, which includes copulatory behavioural isolation and gametic isolation, and postzygotic isolating barriers, which can have extrinsic mechanisms involving ecological inviability and behavioural sterility, or intrinsic mechanisms including inviability and sterility of hybrid.

Ecological speciation and speciation reversal

Reproductive isolation mechanisms can depend on environmental and ecological conditions (Vines & Schluter, 2006), as has been observed in three-spined stickleback (*Gasterosteus aculeatus*), cichlids and whitefish (Seehausen *et al.*, 1997; Candolin *et al.*, 2007; Vonlanthen *et al.*, 2012; Feulner & Seehausen, 2019). These species have changed in some cases their

mating strategies as a result of human-induced eutrophication, which affects zooplankton and benthic invertebrate diversity and biomass (Straile & Geller, 1998; Jeppesen *et al.*, 2000; Blumenshine *et al.*, 2015). The introduction of new species to freshwater systems can also alter morph (i.e. distinct forms of a species) diversity. For instance, Bhat *et al.*, (2014) suggested a breakdown of reproductive isolation between two morphs of European whitefish (*Coregonus lavaretus*) caused by the invasion of a new competitor, vendace (*Coregonus albula*). In the case of three-spined sticklebacks, their reproductive behaviour changed due to the presence of the exotic American signal crayfish (*Pascifasticus leniusculus*), collapsing benthic and limnetic sticklebacks into a hybrid swarm (i.e. population of hybrids originated by interbreeding and hybridisation, reducing reproductive isolation between populations or species; Taylor *et al.*, 2006; Velema *et al.*, 2012). Another study by Gow *et al.*, (2006) suggested historical introgression of three-spined sticklebacks, which was associated with historical human-induced habitat disturbance and the introduction of a predator, the coho salmon (*Oncorhynchus kisutch*). In all these cases, the genetic homogenisation could lead to speciation reversal (i.e. increase of gene flow between populations or species due to the loss of environmental heterogeneity, reducing their genetic and ecological differentiation, resulting in a decrease of reproductive isolation; see also Seehausen *et al.*, (2008)). The ecological speciation process seems to be common in species from postglacial lakes (Skúlason *et al.*, 1999; Schluter, 2000a; Klemetsen, 2010), where there are heterogeneous environments and low competition, offering ecological opportunities for divergent natural selection to produce phenotypic and genetic differentiation among morphs (Schluter, 2000a; Rundle & Nosil, 2005; Nosil, 2012). Therefore, changes in the environment could affect the direction and strength of natural selection and gene flow over time, where the speciation process is started but never completed (McKay & Zink, 2015). For instance, populations could diverge, leading to the formation of new species, or could shift to the opposite direction (Seehausen *et al.*, 1997; Gow *et al.*, 2006; Taylor *et al.*, 2006; Candolin *et al.*, 2007; Velema *et al.*, 2012; Vonlanthen *et al.*, 2012; Bhat *et al.*, 2014), where gene flow may act, preventing complete isolation and thus, the completion of speciation. This process is defined as "Sisyphean evolution" (McKay & Zink, 2015). Thus, the identification of loci that shows signatures of divergent selection within sympatric species will help to understand the underlying mechanisms in the process of reproductive isolation.

Diversification within species and speciation pump

Fish species likely recolonised new environments every time the ice retreated during the Pleistocene ice age, creating lakes in North America and Eurasia (Pielou, 1992; Schluter, 1996; Hewitt, 1999). The repeated glacial cycles during the Pleistocene has isolated geographically different populations, known as the "speciation pump", being relevant for species diversification (Haffer, 1969; Avise *et al.*, 1998; Bernatchez & Wilson, 1998; Wilson *et al.*, 2009; Schoville *et al.*, 2012). Morphs can show different stages of divergence within freshwater systems, which offer opportunities for secondary contact and for allopatric/sympatric divergence across and within species (Schluter, 1996; Smith & Skúlason, 1996; Taberlet *et al.*, 1998; Hewitt, 2004; Swenson & Howard, 2005). Thus, these lakes can behave like islands, having heterogeneous fauna and limited contact between different areas (Schluter, 1996), where populations act as separate evolutionary units.

Brain diversity across different environments

Fish species occupy different habitats with specific environmental conditions, developing determinate brain designs depending on ecological and behavioural demands, where fish could develop similar solutions in similar environments (Kotrschal *et al.*, 1998). Brain morphology can vary among and within species, which has been associated with evolutionary history, recent adaptation, and differences in mating, habitat use, feeding ecology and social interactions (Huber *et al.*, 1997; Kotrschal *et al.*, 1998; Day *et al.*, 2005; Shumway, 2008; Kolm *et al.*, 2009; Crispo & Chapman, 2010). Environmental conditions can affect the brain morphology. For instance, pressure increases with lake depth, whereas light decreases, producing morphological changes such as specialised or reduced eye size, and the importance of the lateral line can increase (Kotrschal *et al.*, 1998). The brain size can also decrease with increasing depth as a result of energy constraints (Kotrschal *et al.*, 1998; Isler & van Schaik, 2006), which is caused by a limitation of food resources in deepwater habitats. Thus, understanding differences in brain morphology provides valuable insights into the mechanisms contributing to adaptation.

Parallel evolution and sympatric speciation

As discussed above, ecological speciation could occur through resource competition and niche differentiation. However, parallel speciation can happen between populations in different

postglacial lakes, developing similar traits (e.g. behaviour, morphology and life-history) in habitats with similar environmental conditions and selection pressure (Schluter, 1996; Wood *et al.*, 2005). Similar traits in different freshwater systems has been observed in Arctic charr (*Salvelinus alpinus*; Knudsen *et al.*, 2016a), sticklebacks (Boughman *et al.*, 2005; Kaeuffer *et al.*, 2012) and whitefish (Østbye *et al.*, 2006), which could show parallelism among similar morphs in independent lake systems (Colosimo *et al.*, 2005; Siwertsson *et al.*, 2013; Jacobs *et al.*, 2020; Salisbury *et al.*, 2020; Wang *et al.*, 2020; Magalhaes *et al.*, 2021).

In a lake, sympatric speciation can arise when there is colonisation of available niches by different genetic lineages, under secondary contact, or when a single genetic lineage enters to the lake and diverges into several populations as a result of competition, or alternatively results from combining both processes (Endler, 1977, 1982; Schluter, 1996; Smadja & Butlin, 2011; Moan *et al.*, 2016). Ideally, we should consider all putative causes and processes jointly in our inference of the driving factors such as mutation coupled with genetic drift, convergent or divergent natural selection, and genomic rearrangements that could fix different alleles in different groups, generating most likely reproductive isolation and leading to speciation (Schluter, 1996, 2000b, 2009; Gavrilets, 2004; Coyne, 2007; Fitzpatrick, 2012; Nosil, 2012). Several of these procersses likely play an important role in the polymorphism of these species and in the ecological speciation of these complex systems. However, there is still a gap of knowledge regarding mechanisms behind reproductive isolation, and thus, the speciation process. My thesis addresses this knowledge gap by looking into ecological and genetic differentiation in three deep lakes with different number of Arctic charr morphs, contributing to increase the understanding of these processes.

Resource polymorphism in fish species

Resource polymorphism has been recorded in different species originated by phenotypic plasticity, genetic basis, or both, defined as the occurrence of different morphs or forms within a species originated by the opportunity of different available niches, presenting differences, for instance, in diet, behaviour, morphology, and life history traits (Skúlason & Smith, 1995; Smith & Skúlason, 1996; Skúlason *et al.*, 2019). This divergence in the phenotype and in the genotype are likely driven by natural selection, which can be an important factor for local adaptation (Schluter, 2000a). Polymorphism exists within some freshwater fish species that

live in postglacial lakes, showing phenotypic and life-history diversity among different morphs (Schluter, 2000a). There are several examples of families with species showing polymorphism such as rainbow smelt (*Osmerus mordax*; Osmeridae; Taylor & Bentzen, 1993), pumpkinseed sunfish (*Lepomis gibbosus*; Centrarchidae; Robinson *et al.*, 1993) and brown trout (*Salmo trutta*; Salmonidae; Ferguson & Mason, 1981). There is also examples of three-spined stickleback and Eurasian perch (*Perca fluviatilis;* Schluter, 1993; Svanbäck & Eklöv, 2002; Figure 1), which show polymorphism as a result of disruptive selection (Bolnick & Lau, 2008; Svanbäck & Persson, 2009). The salmonid genera of *Salvelinus* and *Coregonus* are good examples of resource polymorphism, holding between two to eight morphs in the different postglacial lakes (Guiguer *et al.*, 2002; Power, 2002; Kahilainen *et al.*, 2004; Kahilainen & Østbye, 2006; Smalås *et al.*, 2013; Skoglund *et al.*, 2015; Muir *et al.*, 2016; Markevich *et al.*, 2018; Arostegui & Quinn, 2019; Doenz *et al.*, 2019; Doenz & Seehausen, 2020), showing divergence in growth rates, age, morphology and colour, among others (Walker *et al.*, 1988; Sandlund *et al.*, 1992; Kahilainen & Østbye, 2006; Præbel *et al.*, 2013). Within the *Salvelinus* genus (Figure 1-2), there are several examples of polymorphic species such as Arctic charr, Lake charr (*Salvelinus namaycush*), brook charr (*Salvelinus fontinalis*) and Dolly Varden charr (*Salvelinus malma;* Bourke *et al.*, 1997; Klemetsen, 2010; Muir *et al.*, 2016; Markevich *et al.*, 2018). However, we need a better understanding of resource polymorphism across species and the different processes that can contribute to the divergence within sympatric species.
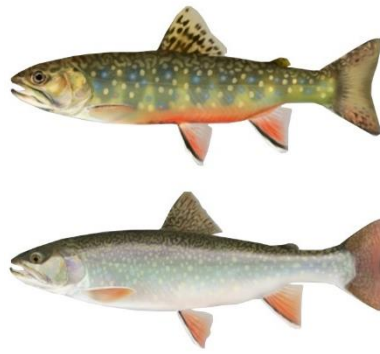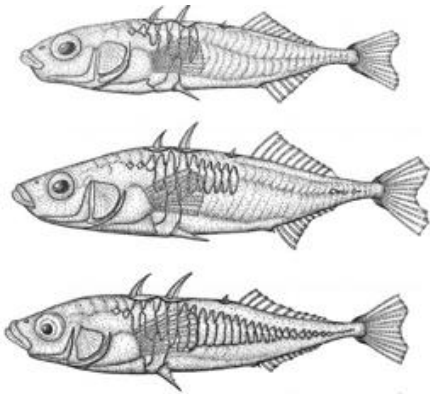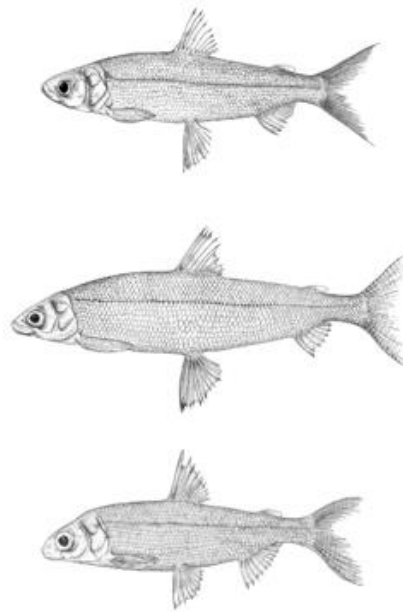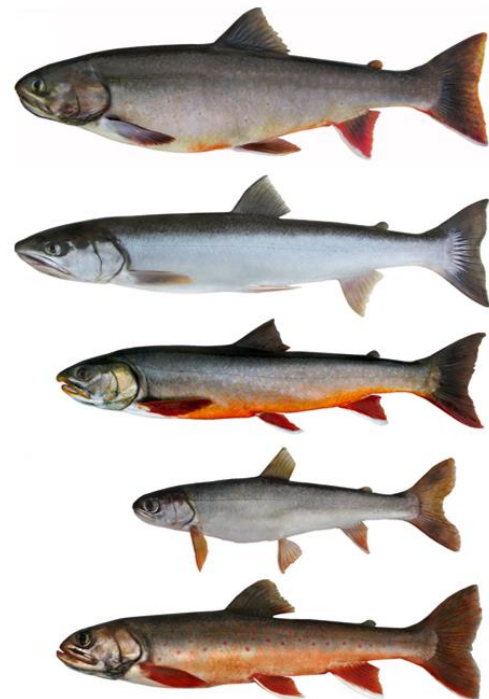
Figure 1. Polymorphic species. (a) Perch with two morphs (Skúlason *et al.*, 2019). (b) Brook charr with fluvial and lake resident (Arostegui & Quinn, 2019). (c) Three-spined stickleback with low-, partial- and complete-plated morphs (Bell & Foster, 1994). (d) Densely rakered, large sparsely rakered and small sparsely rakered whitefish (Harrod *et al.*, 2010). (e) Lake charr with lean, humper, siscowet and redfin (Muir *et al.*, 2014). (f) Dolly Varden charr with white, longhead, nosed, smallmouth and bigmouth morphs (Markevich *et al.*, 2018).

The Arctic charr species complex

Postglacial lakes are excellent systems for studying the mechanisms driving ecological speciation and adaptive phenotypic diversity. In these lakes, various fish species can be found under different divergent stages, showing different levels of reproductive isolation (Smith & Skúlason, 1996; Skúlason *et al.*, 2019). These species reveal phenotypic variation among morphs, showing local adaptation (Schluter, 2000a). A good example of phenotypic variation is found in Arctic charr, which is polymorphic in several freshwater systems in the Northern hemisphere such as in Lake Skogsfjordvatn and Lake Fjellfrøsvatn in Norway, Loch Rannoch and Loch Tay in Scotland, and Lake Thingvallavatn in Iceland (Jonsson *et al.*, 1988; Walker *et al.*, 1988; Adams *et al.*, 1998; Simonsen *et al.*, 2017). Shallow freshwater systems are relatively well described, whereas deep-water morphs from deep lakes have remained far less studied. Arctic charr morphs differ in characteristics such as body shape and size, life-history traits, diet and habitat use (Johnson, 1980; Riget *et al.*, 1986; Jonsson *et al.*, 1988; Walker *et al.*, 1988; Hindar & Jonsson, 1993; Snorrason *et al.*, 1994; Skúlason & Smith, 1995; Smith & Skúlason, 1996; Jonsson & Jonsson, 2001; Klemetsen, 2010; Parsons *et al.*, 2010, 2011; Salisbury *et al.*, 2018, 2020; Doenz *et al.*, 2019; Skúlason *et al.*, 2019; Jacobs *et al.*, 2020).

In Norway, Lake Fjellfrøsvatn and Skogsfjordvatn have two replicated morphs (Figure 2; Klemetsen *et al.*, 1997; Smalås *et al.*, 2013; Knudsen *et al.*, 2016a). Both lakes harbour the littoral spawning omnivorous morph (LO-morph), which feeds on zooplankton and macrobenthos, and the profundal benthivorous morph (PB-morph), which feeds on bottom benthic invertebrates (Klemetsen et al., 1997; Smalås et al., 2013). Lake Skogsfjordvatn also presents an additional third morph, the piscivorous morph (PP-morph), which feeds on other individuals of Arctic charr and three-spined stickleback (Smalås *et al.*, 2013; Knudsen *et al.*, 2016b). In the case of lakes Skøvatn and Tårnvatn, management surveys suggested that there

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are two and three putative morphs, respectively. These morphs have similarities with the ones found in Fjellfrøsvatn and Skogsfjordvatn, with a LO-morph and a PB-morph. Additionally, Lake Tårnvatn harbours a PP-morph similar to the one in Lake Skogsfjordvatn. In the deep oligotrophic Lake Tinnsjøen, four morphs have been identified: dwarf and planktivore morphs (Hindar *et al.*, 1986), a profundal morph described by the local fishermen as "Gautefisk" (Brabrand, 1994), and a small white morph discovered by a ROV submarine (Søreide *et al.*, 2006). There are no previous studies describing the four morphs together, and little is known regarding their life history and resource use.
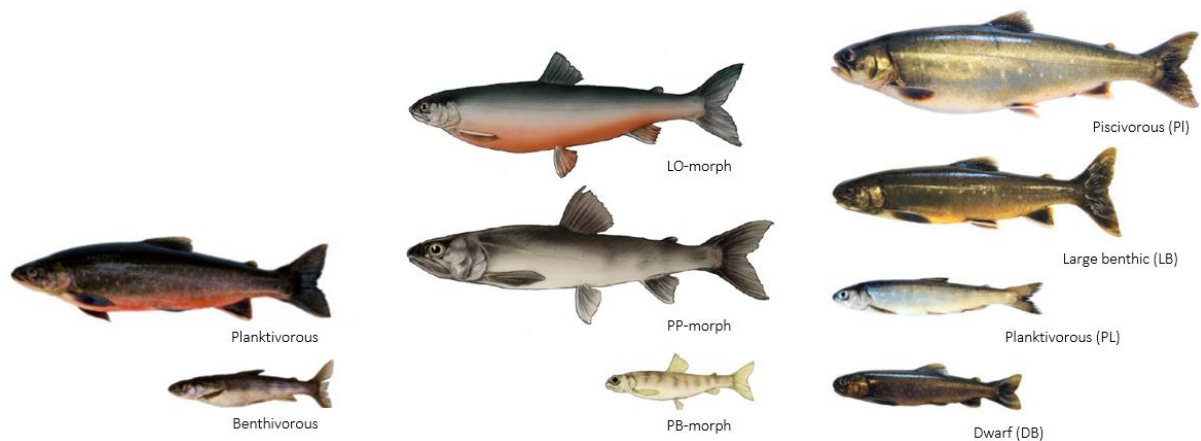
Figure 2. Examples of Arctic charr systems harbouring two to four morphs in Loch Tay (Scotland; Jacobs *et al.*, 2020), Skogsfjordvatn (Norway; Simonsen *et al.*, 2017) and Thingvallavatn (Iceland; Johnston *et al.*, 2004).

Objectives

The aim of this study was to understand ecological and genetic patterns, and processes underlying phenotypic and genotypic variation within the polymorphic Arctic charr. This study was performed in three Norwegian lakes (Lake Skøvatn, Tårnvatn and Tinnsjøen), which harbour two, three and four Arctic charr morphs, respectively. The Arctic charr in these three lakes can be seen as being on different temporal stages along the speciation continuum, or reflecting the filling of niche availability in these lakes, which differ in their ecological opportunity for adaptive radiation. Here, we investigated trophic niche segregation (i.e. diet and habitat use), morphological and genetic differences among Arctic charr morphs.

The main objectives of this thesis were to:

1. Investigate the genetic and phenotypic divergence of the four morphs of Arctic charr in Lake Tinnsjøen and examine the Holarctic phylogeography to infer putative lineages that colonised Lake Tinnsjøen (Paper I).

2. Investigate the variation of olfactory organs, head, eye, and brain regions of Arctic charr along a depth gradient to reveal putative association of sensory capacities, which could be related to environmental constraints, foraging and mating habitats in Lake Tinnsjøen (Paper II).

3. Investigate the genetic basis of four sympatric morphs of Arctic charr in Lake Tinnsjøen to identify putative local adaptation, which could suggest an early stage of ecological speciation (Paper III).

4. Investigate parallelism of trophic divergence and genetic differentiation among Arctic charr morphs from two lakes in Norway, which could occur under similar environmental conditions developing similar phenotypes (Paper IV).

Material and methods

In Paper I, Lake Tinnsjøen and four outgroup populations of Arctic charr from north, south, east and west of Lake Tinnsjøen (River Leirfossvassdraget, Lake Tyrivatn, Lake Femund and Lake Vatnevatnet) were sampled in 2013-2015 (Figure 3). Fish were collected from four habitats (littoral, pelagial, shallow-moderate profundal and deep-profundal) in Lake Tinnsjøen. Phenotypic (i.e. based on external morphology) and genetic (i.e. microsatellite and mtDNA) data were collected from four morphs of Arctic charr, two profundal morphs (Dwarf and Piscivore), the Planktivore morph and the deep-profundal benthivore morph (Abyssal). Sex, body length, weight, maturity and age (i.e. counting the year rings from otoliths) were recorded from Arctic charr morphs. We included a set of 30 landmarks to capture the body shape of the fish. A total of 10 microsatellite loci were included for genetic analysis to estimate the genetic differentiation (F_{st}) and the genetic structure of the four morphs. A total of 13 Norwegian CytB-mtDNA sequences and 75 haplotypes were used to show the Holarctic phylogeography and identify the lineages that colonised Lake Tinnsjøen.
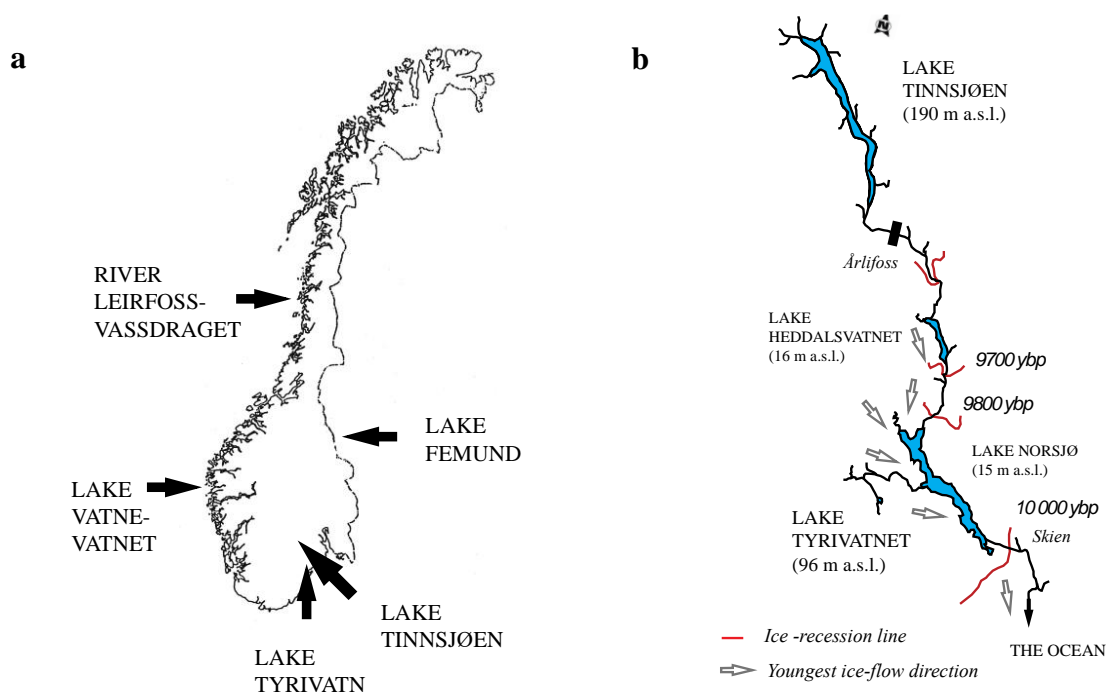
Figure 3. (a) Map of the study area from Lake Tinnsjøen and the four outgroups sampled in Norway. (b) River Skiensvassdraget wherein Lake Tinnsjøen is situated. Red lines denote dated ice-recession lines in years before present (ybp) from Bergstrøm (1999). Gray arrows denote

the youngest ice-flow direction in the end of the Pleistocene glaciation from Bergstrøm (1999). The black bar indicates the upper deposits of marine sediments (Bergstrøm, 1999). The figures are from Østbye *et al.*, (2020).

In Paper II, 72 individual Arctic charr were included from Lake Tinnsjøen, which were collected previously in Paper I (Planktivore (n = 25), Piscivore (n = 13), Dwarf (n = 22), and Abyssal (n = 12)). Geometric morphometrics were performed to study variation of the head. In addition, five brain regions (i.e. olfactory bulb, telencephalon, optic tectum, cerebellum, and hypothalamus), olfactory rosettes and age (i.e. based on otoliths) were measured. Recursive partitioning methods (i.e. random forest) were performed to identify the most important variables to predict the four morphs. ANOVAs and post hoc Tukey's HSD analyses were also included to test whether there were differences among the morphs.

In Paper III, a total of 125 individuals of four Arctic charr morphs, which were collected previously in Paper I, were included to study the genetic variation by using Next-Generation-Sequencing (NGS), specifically the RAD-sequencing method. Neutral loci and loci putatively under divergent selection were identified to estimate the genetic structure of the four morphs. Chromosome inversions, candidate genes under putative divergent selection and their biological function were also detected. The linkage disequilibrium between a set of loci putatively under divergent selection were estimated.

Finally, in Paper IV, two subarctic lakes were sampled from Norway, Tårnvatn and Skøvatn in October 2016. Three habitats (littoral, pelagic and profundal) were included in Tårnvatn and two habitats (littoral and profundal) in Skøvatn. All individuals were classified based on their external morphology. A total of 10 microsatellite loci were used to estimate the genetic differentiation (F_{st}) and the genetic structure. Parasites (i.e. richness, prevalence and abundance) and prey items (i.e. prey types divided into five categories and estimation of the proportion of each prey type) from stomach contents were identified to compare trophic ecology among the morphs and between the lakes. A sample of muscle tissue was cut from each fish for stable isotope analyses based on $\delta^{13}C$ and $\delta^{15}N$. A non-metric multidimensional scaling analysis (NMDS) based on the Bray-Curtis index of similarity from both lakes and

Schoener's similarity index were included to visualise the diet of Arctic charr individuals and to study dietary niche overlap, respectively.

Results and discussion

Adaptive radiation in sympatric morphs of Arctic charr (Paper I)

We identified four morphs in Lake Tinnsjøen living in four habitats (pelagial, littoral, shallow-moderate profundal and deep-profundal; Figure 4b). We identified a new morph that has not been found in other Arctic charr systems, the Abyssal morph. This morph lives in the deep-profundal habitat of Lake Tinnsjøen and presents similarities with cave species, such as white coloration and reduced eyes (Jones *et al.*, 1992; Moran *et al.*, 2015; Krishnan & Rohner, 2017). We found genetic differentiation in field assigned morphs based on microsatellites (F_{st} range of 0.119-0.199) and in "genetically pure" (based on cut-off values of q) morphs (F_{st} range of 0.088-0.212), suggesting a certain degree of reproductive isolation among the morphs. We further observed differences in the life-history traits, where the Piscivore morph had the largest age span ($\bar{x}$= 9.2 years) and weight ($\bar{x}$= 267 g), whereas the Planktivore morph showed the lowest age span ($\bar{x}$= 2.9 years), followed by the Dwarf morph ($\bar{x}$= 4.8 years). The Abyssal morph had the lowest weight ($\bar{x}$= 2.2 g) and a larger age span ($\bar{x}$= 5.0 years) than the Planktivore and the Dwarf morphs. These differences in life history are most likely an indication of local adaptations, where adaptive divergence could be benefited by low or intermediate levels of gene flow (Garant *et al.*, 2007), which could be the case in these four Arctic charr morphs. When comparing Lake Tinnsjøen and the four outgroups with the Holarctic distribution of Arctic charr, we identified 10 endemic CytB-mtDNA haplotypes in Lake Tinnsjøen (Figure 4a, c-d). This could support intralacustrine diversification, where a single common ancestor from one genetic lineage could have colonised Lake Tinnsjøen. Since these endemic haplotypes were only found in Lake Tinnsjøen, it suggests that the morphs likely originated in sympatry. Arctic charr could have colonised Lake Tinnsjøen approximately in < 9700 ybp (Bergstrøm, 1999), most likely from the south following the Norwegian coastline and upward. Considering an average generation time of 5 years, this represents a maximum of 2000 generations since deglaciation, which is a rapid divergence of the morphs across the different habitats.

The phenotypic variation within species can be related to differences in environmental conditions, known as ecosystem size (Jacobs *et al.*, 2020). Ecosystem size can be an indicator of niche opportunities in a freshwater system (Recknagel *et al.*, 2014). Lakes with large depths and surface areas can present more ecological niches, as has been seen in Arctic charr, showing more diverse populations in larger lakes (Recknagel *et al.*, 2014, 2017; Doenz *et al.*, 2019; Jacobs *et al.*, 2020). This could be the case in Lake Tinnsjøen (surface area of 51.4 km^2 and maximum depth of 460 m), presenting larger ecological opportunities than shallow lakes, where the morphs could develop different adaptive responses. Thus, the combination of different environmental conditions and ecological opportunity in deep lakes could lead to adaptive divergence, where selective forces could be stronger than in other lakes, developing new traits such as in the case of the Abyssal morph in Lake Tinnsjøen. The Arctic charr diversity could also be due to factors such as gene flow, genomic architecture, selection and demographic history, developing parallel phenotypes in similar environments (Schluter, 2000b; Conte *et al.*, 2012; Kowalko *et al.*, 2013; Elmer *et al.*, 2014; Jacobs *et al.*, 2020). Thus, it is important to consider the combination of these different factors, which could be involved in the Arctic charr diversity, to understand the underlying processes behind ecological speciation and adaptive radiation.

Several studies of Arctic charr showed morphs with similar phenotypes across different lakes with similar conditions (Klemetsen et al., 1997; Knudsen et al., 2016; Moccetti et al., 2019; Sandlund et al., 1992; Smalås et al., 2013). These morphs can be under parallel evolution, where repeated natural selection likely produces phenotypic parallelism. For example, Gander Lake shares some characteristics with Lake Tinnsjøen (i.e. deep and oligotrophic lake). Gander Lake contains a pale form of Arctic charr that is mainly found in deeper parts of the lake than the dark form, revealing differences in morphology, habitat, life-history traits and diet most likely caused by divergent selection (O'Connell & Dempson, 2002; Power *et al.*, 2005; Gomez-Uchida *et al.*, 2008). This pale-coloured pattern has also been observed in other systems less deep such as Lock Rannoch and Loch Ericht in Scotland (Gardner *et al.*, 1988; Fraser *et al.*, 1998). Traits observed in the Abyssal morph such as pigmentation loss and eye reduction, could be an adaptation to dark habitats, as has been observed, for instance, in fish and arthropods living in caves (Jones *et al.*, 1992; Protas *et al.*, 2006; Krishnan & Rohner, 2017).

These features are likely a result of parallel evolution, where similar selection pressures can lead to similar structures or phenotypes, as well as similar life-histories.

a

b

PLANKTIVORE MORPH

PISCIVORE MORPH

DWARF MORPH

ABYSSAL MORPH

c

0.002

Russia

USA

Russia

Ru/Ca/Us/No

Russia

Russia/USA

Russia/Canada/USA

Canada

Russia

Russia

Russia

Root

Canada/ Russia Fennoscandia

Noraway - Lake Tinnsjøen

Russia USA

Russia

1

2

5

d

Transbaikalia - Kamchatka - Bering Sea

Quebec - Taimyr - Chucotka - Fennoscandia

Norway - Lake Tinnsjøen only

h33
h32
h17
h18
h21
h2
h15
h1
h14
h16
h13
h10
h12
h6
h5
h4
h3
h11
h7
h8
h9

31

Figure 4. (a) Distribution of 88 mtDNA-cytochrome B mtDNA haplotypes compared with major clades in different colours according to figure c. White circles denote haplotypes not well supported in figure c. (b) Four morphs found of Arctic charr in Lake Tinnsjøen. (c) Circular phylogenetic tree of sequences mapped in figure a. Here, a total of 13 Norwegian sequences and 75 haplotypes retrieved from GenBank (using a cut-off of 200 highly similar BLAST sequences) are compared. Here, haplotype 31 was found to be the most ancestral when rooted with three distant salmonid taxa (*Salmo trutta*, *Oncorhynchus kisutch*, and *Coregonus lavaretus*) (tree not shown). Major supported clades have different colors. Main geographical regions are named on the outer circle. (d) A minimum spanning network of haplotypes (not frequencies) in the major light purple clade (#1) comprising Lake Tinnsjøen with geographical areas described. Haplotypes in red were found in Lake Tinnsjøen. The figures are from Østbye *et al.*, (2020).

Sensory structure divergence among Arctic charr morphs (Paper II)

We found that brain size varied among the four Arctic charr morphs in Lake Tinnsjøen. Both random forest analysis (Figure 5a) and ANOVAs revealed eye area, habitat and number of lamellae as the most important variables in discriminating the four morphs. These results suggest variation in habitat use and most likely also in foraging and mating behaviours, where vision and smell could play an important role. We also quantified head shape among the morphs, where the six first principal component axes explained 78.1% of the variation, where three of the four morphs were more distinct than the generalist Planktivore morph (Figure 5b).

The Piscivore and the Planktivore morphs presented the largest absolute optic tectum size, which is likely associated with living in a well-illuminated habitat. The Abyssal morph presented the largest olfactory bulb relative to the optic tectum. This enlargement of the olfactory bulb could be explained by a larger importance of olfactory perception. The small eyes and small optic tectum found in the Abyssal morph could be associated with the dark habitat where they lived. This reduction in size is likely explained by a decrease in the vision importance related to an increase in depth (Kotrschal *et al.*, 1998). Living in a deepwater habitat, where there is limited food and low or absent light, a decrease of vision can be a way to save energy (Moran *et al.*, 2015). These differences in eye, optic tectum and olfactory

bulb are likely related to mating behaviour, where coloration might play an important role in well-illuminated habitats. In the case of the Abyssal morph, which is pale compared to the other three morphs, the coloration could be less important to potential mates than in the other morphs that live in a well-illuminated habitat. A previous study in Arctic charr also found differences in the number and size of lamellae relative to size (Olsén, 1993). This pattern was also identified in our study, where the number and size of lamellae decreased in the smallest fish, the Abyssal morph, and increased in the largest fish, the Piscivore morph. The Abyssal morph showed the smallest absolute brain regions compared with the other three morphs. There are different factors such as food availability and light that could constrain the brain size, as has been seen in cave-dwelling fish species, which developed a small brain and showed a reduction of the optic tectum (Jeffery, 2008; Moran *et al.*, 2015).

The mosaic evolution hypothesis postulates that selection pressures act on determinate brain regions, which develop independently from each other, reducing energy cost to maintain unnecessary neural tissue (Liem, 1978; Barton & Harvey, 2000; Hager *et al.*, 2012). There are studies that have found this pattern among and within species, which can be attributed to differences in diet, life history traits, presence of conspecifics and environmental conditions (Sherry *et al.*, 1989; Garamszegi & Eens, 2004; Kihslinger *et al.*, 2006; Kihslinger & Nevitt, 2006; Gonda *et al.*, 2009; Kolm *et al.*, 2009). This pattern has been also observed in Lake Tinnsjøen, which is most likely explained by the divergences in diet and habitat use. These differences found among the morphs in Lake Tinnsjøen most likely evolved from local adaptation driven by natural selection or adaptive phenotypic plasticity. Thus, further experimental studies are needed to understand what drives mosaic brain evolution and how this led to a large diversity in brain morphology.

a

Distribution of minimal depth and its mean
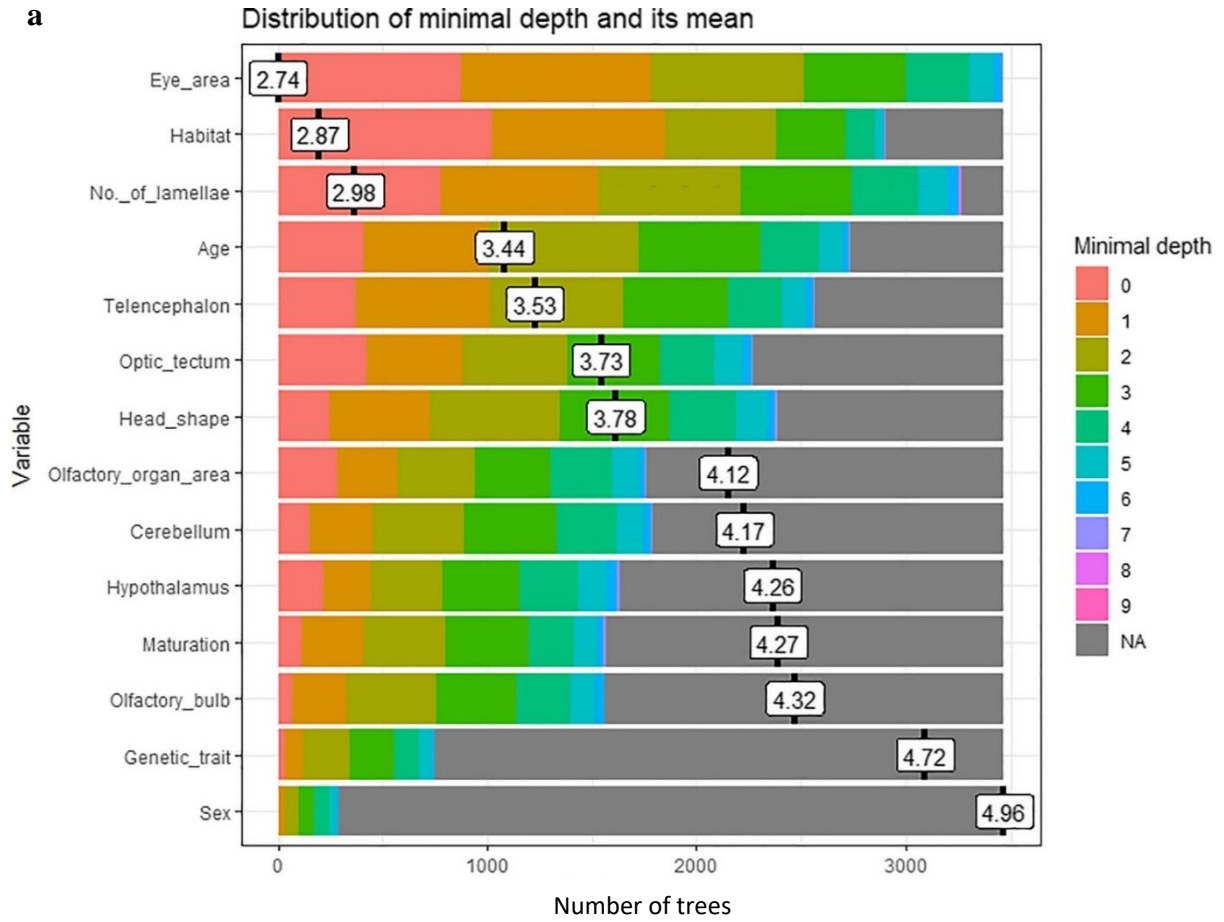
b
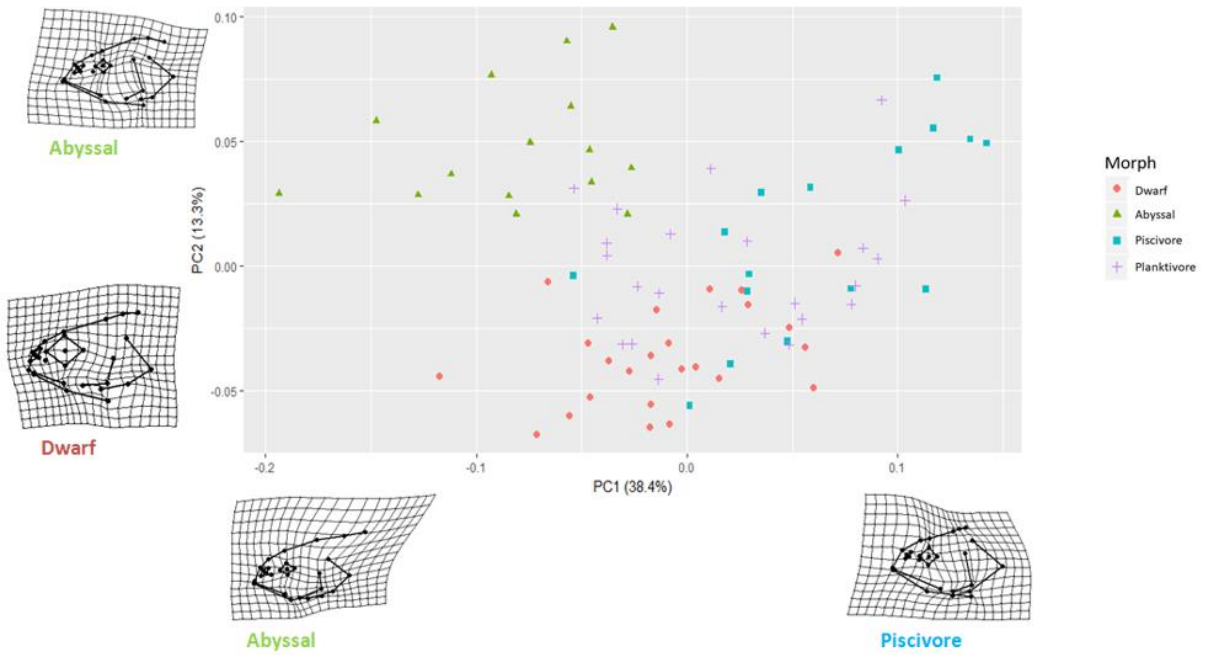
Figure 5. (a) Variable importance based on minimum depth from the random forest analysis, which represents the consensus across trees (i.e., the higher the variables and the lower the depth on this figure, the more frequently and early the variable was selected to make the split, i.e., the more important the variable is). Results from the random forest analysis for the response variable morph. Note that we used the residuals of the measured variables obtained from the log-log regressions to correct for size. Number of trees grown were set to 5,000. The importance of the variables is measured with the minimal depth (indicated with different colours inside the horizontal bar for each variable) and its mean (indicated in the white box). Minimal depth is the average distance between the root of a tree and the node/split where a given variable was used. Smaller values of the minimal depth indicate early contribution of the variable, that is, more discriminating power. NAs represent all variables not picked for a given split. (b) Principal component analysis of head shape illustrating extremes of head shape morphology in Arctic charr (red: Dwarf, green: Abyssal, blue: Piscivore, purple: Planktivore). The first two principal components are shown for the four morphs. Wireframe images illustrate head shape differences along the two first PC axes. The figures are from Tamayo *et al.*, (2020).

Chromosomal inversions and local adaptation in the polymorphic Arctic charr (Paper III)

We identified genetic differences among the four morphs from Lake Tinnsjøen, presenting loci putatively under divergent selection across the genome (1132 SNPs; Figure 6). Regarding population structure, the DAPC based on the subset with loci putatively under divergent selection separated the morphs in four genetic clusters. These results are also supported by the findings based on microsatellite data from Paper I. We identified candidate genes involved in 119 significant biological functions (GOs) and found ten chromosomal inversions along the Arctic charr genome, which could play a role in morph divergence by reducing or suppressing recombination in these regions (Kirkpatrick & Barton, 2006; Hoffmann & Rieseberg, 2008). There were also differences in the allele frequency among the morphs, especially in the subset with the loci putatively under divergent selection. These results showed that the two profundal morphs were grouped together, while the Abyssal and Planktivore morphs were clustered together, showing some similarities in the allele frequency in specific loci. The

morph diversity could be a sign of local adaptation, occurring in habitats where environmental conditions vary (Kawecki & Ebert, 2004; Garcia de Leaniz *et al.*, 2007; Fraser *et al.*, 2011), and where different selection pressures can contribute to genetic differences (Lamichhaney *et al.*, 2012). Thus, the four morphs living in Lake Tinnsjøen are likely under divergent selection pressures, evolving specific phenotypes and genotypes.

We identified genes within chromosomal inversions associated with different biological functions. Polymorphism in Arctic charr could be related to a set of genes involved in specific phenotypes, which could be found within these chromosomal inversions. Thus, these chromosomal inversions could be relevant for the morph divergence, playing an important role in adaptation and speciation as has been seen in other polymorphic species (Ayala & Coluzzi, 2005; Kirkpatrick & Barton, 2006; Hoffmann & Rieseberg, 2008; Ayala *et al.*, 2013; Kirubakaran *et al.*, 2016; Hooper & Price, 2017; Wellenreuther & Bernatchez, 2018). These regions with clustered genes can have a low degree of recombination or suppression, accumulating genetic variation (Kirkpatrick & Barton, 2006; Feder & Nosil, 2009), which could favour reproductive barriers among Arctic charr morphs. A study in three-spined sticklebacks also found a set of SNPs clustered in specific regions across the genome, specifically to one chromosome, which showed low to intermediate recombination (Marques *et al.*, 2016). However, they also identified other regions as genomic islands, which were not exclusively of low recombination. This suggests that there are other factors such as life history-driven and habitat-driven divergent selection which act together with gene flow and recombination, creating patterns of genomic divergence (Marques *et al.*, 2016).

We also identified other genes involved in biological functions such as neurogenesis, brain, eye and inner ear development that were not within inversions. These genes could be also involved in multiple phenotypic traits, most likely varying among the morphs depending on the habitat use and trophic niche preferences. For instance, vision could be less important in dark habitats (Huber *et al.*, 1997; Kotrschal *et al.*, 1998), leading to a decrease in size of the brain, the eye, and the optic tectum (Jeffery, 2008; Moran *et al.*, 2015). In Lake Tinnsjøen, the decrease in size of the brain and eye has also been observed in the Abyssal morph, which lives in the deep-profundal habitat, where likely there is low food quality/quantity, absence of light and large pressure (Tamayo *et al.*, 2020). However, the Piscivore, Planktivore and Dwarf

morphs showed a larger brain and eye (Tamayo *et al.*, 2020), most likely associated with living in habitats with more light.

The specific genes identified are most likely involved in Arctic charr polymorphism, which could be driven by natural selection. The genetic architecture (i.e. genetic basis of a specific phenotypic trait and its variational properties) could play an important role in morph diversification. Thus, the divergence at the phenotypic and genomic level could increase the differences among the morphs, contributing to their adaptive responses and reproductive isolation.
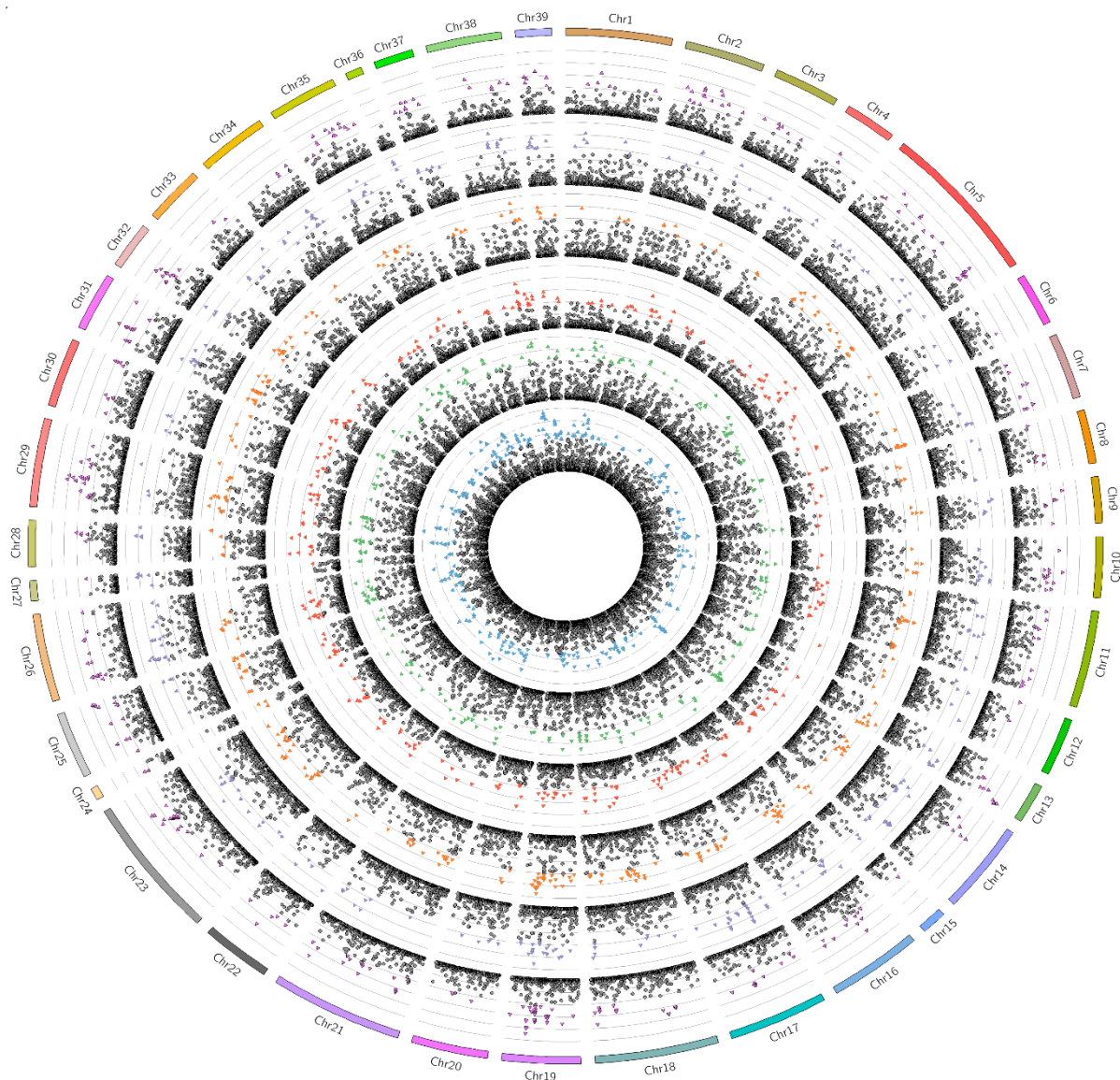
Figure 6. Manhattan plot of genome-wide association study showing the distribution of pairwise differentiation (F_{st}, ranging from 0 to 1) across the genome for all 6 morph comparisons of Arctic charr. Each grey dot shows a single SNP pairwise F_{st} estimates and the

colored triangles are the SNPs under putative selection, with non-overlapping 10-kb sliding windows across the genome. From internal to external circles, it shows F_{st} between: Abyssal-Dwarf (highlight in blue the SNPs under putative selection), Piscivore-Abyssal (green), Piscivore-Dwarf (red), Piscivore-Planktivore (orange), Planktivore-Abyssal (purple), Planktivore-Dwarf (pink). The figure is from Tamayo *et al*. (manuscript).

Parallel evolution in Arctic charr morphs from two subarctic lakes (Paper IV)

We investigated parallelism in trophic niches in Arctic charr morphs from two Norwegian lakes (Tårnvatn and Skøvatn). We found genetic differences based on microsatellites between the Arctic charr morphs from Skøvatn and Tårnvatn. In Tårnvatn, the genetic divergence was larger between the LO- and PB-morphs, and between the LO- and the PP-morphs (F_{st}= 0.134 and 0.121, respectively) than between the PB- and PP-morphs (F_{st}= 0.042). This has also been observed in another two Arctic charr systems, Fjellfrøsvatn and Skogsfjordvatn (Præbel *et al.*, 2016; Simonsen *et al.*, 2017). These genetic differences together with differences in the trophic niche and life-history (Kjær, 2018) revealed three different deep-water morphs in Tårnvatn and Skøvatn (Figure 7c-d).

In Tårnvatn, we observed a dietary shift in the PP-morph, where small young individuals fed mainly on profundal benthic prey and large older individuals fed mainly on *Gammarus lacustris* and fish (Figure 7a). The dietary shift started with a change in body length at 20 cm, approximately, which concurs with previous studies (Amundsen, 1994; Knudsen *et al.*, 2016b). The PP-morph showed a boost in its growth at an age between 7 to 9 years (Kjær, 2018), likely explained by the shift in feeding to larger-energetic rich preys (Claessen *et al.*, 2002; Hammar, 2014; Borgstrøm *et al.*, 2015).

The LO-morph from both lakes had a wide diet based on zooplankton and littoral benthos (Figure 7a, 7c-d). In both lakes, the LO-morphs showed a similar pattern of parasite prevalence, mainly infected by *Dibothriocephalus* spp. and *Crepidostomum* spp., similar life-history traits (Kjær, 2018), and a relatively wide isotopic range. In Tårnvatn, the LO-morph had lower $\delta^{13}C$ and $\delta^{15}N$ mean values than PB- and PP-morphs, which showed similar mean values (Figure 7b). However, the LO-morph showed larger $\delta^{13}C$ and lower $\delta^{15}N$ than the PB-morph in Skøvatn (Figure 7b). The LO-morphs from both lakes showed the largest growth rates, and reached maturity at intermediate size and age in comparison with the profundal-dwelling

morphs (Kjær, 2018). Thus, parallelism in habitat use, diet, morphology and life history of the LO-morphs was found in both lakes, which has been also detected in the LO-morphs from other lakes in this region (Knudsen *et al.*, 2016a; Siwertsson *et al.*, 2016). Parallel patterns observed in different lakes can be due to similar natural selection pressures leading to similar traits (Schluter, 2000a; Schluter *et al.*, 2004; Østbye *et al.*, 2006; Kaeuffer *et al.*, 2012; Siwertsson *et al.*, 2016; Jacobs *et al.*, 2020).

However, there were differences in the PB-morph from both lakes, which showed divergence in feeding preferences, where the PB-morph fed mainly on zooplankton in Skøvatn, whereas it fed mainly on profundal benthic prey in Tårnvatn (Figure 7a). The diet choice of the PB-morph in Tårnvant has been also observed in other freshwater systems in this region (Klemetsen, 2010; Knudsen *et al.*, 2016a). The PB-morph also showed a larger parasite prevalence in Tårnvatn than the PB-morph in Skøvatn with exception of *Dibothriocephalus* spp., which was more prevalent in Skøvatn. Both PB-morphs from Tårnvatn and Skøvatn showed similar life-histories differing from the PP-morph, which had the lowest growth, the largest size and a high longevity (Kjær, 2018). Although, PB-morphs from both lakes also showed similar habitat use and morphologies (Klemetsen et al., 1997; Smalås et al., 2013; Kjær, 2018), these morphs did not use the same trophic niche. The PB-morph in Skøvatn differed from the other PB-morphs found (Klemetsen, 2010; Knudsen *et al.*, 2016a). Thus, it was denominated as small-sized deep-water planktivorous (PZ) morph to be distinguished from the other PB-morphs. There are other systems in Norway (Telnes & Sægrov, 2004), central Europe (Brenner, 1980), and Russia (Alekseyev *et al.*, 2002) with a morph that shares similarities with the PZ-morph. Thus, population divergence can also happen among populations living in similar environmental conditions, caused by biotic drivers such as predation, parasitism, and competition (Meyer & Kassen, 2007; Karvonen & Seehausen, 2012; Ingley *et al.*, 2014), or genetic architecture variation as a product of genetic drift or gene flow (Barton & Charlesworth, 1984; Hendry & Taylor, 2004; de Brito *et al.*, 2005; Bolnick & Nosil, 2007).

a

| | Tårnvatn | | | Skøvatn | |

Tårnvatn

LO-morph
n = 48

PB-morph
n = 24

PP-morph
n = 21

Skøvatn

LO-morph
n = 54

PB-morph
n = 31

Zooplankton

Profundal benthos

Gammarus lacustris

Littoral benthos

Fish

Prey abundance (%)

b

Tårnvatn

- ○ LO-morph
- △ PB-morph
- ◆ PP-morph

δ¹⁵N (‰)

Pelagic prey

Littoral prey

δ¹³C (‰)

Skøvatn

- ○ LO-morph
- △ PB-morph

δ¹⁵N (‰)

Pelagic prey

δ¹³C (‰)

c

d
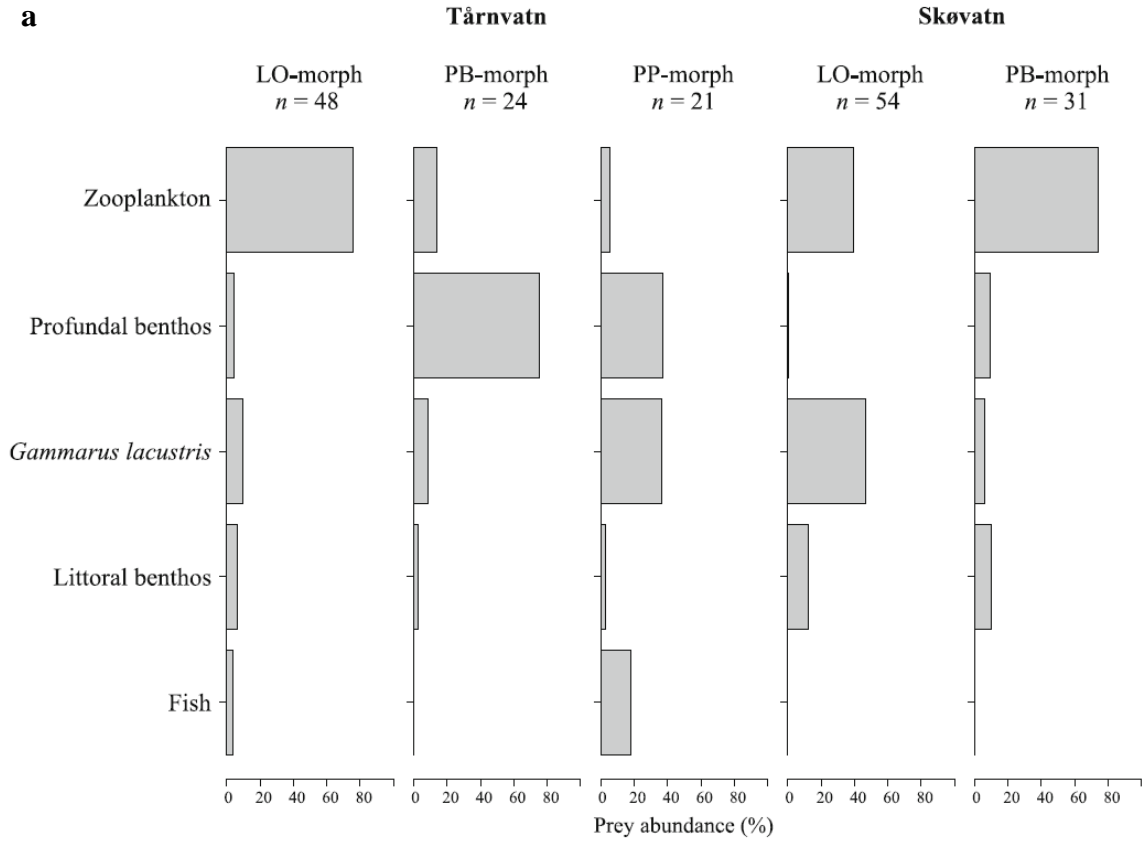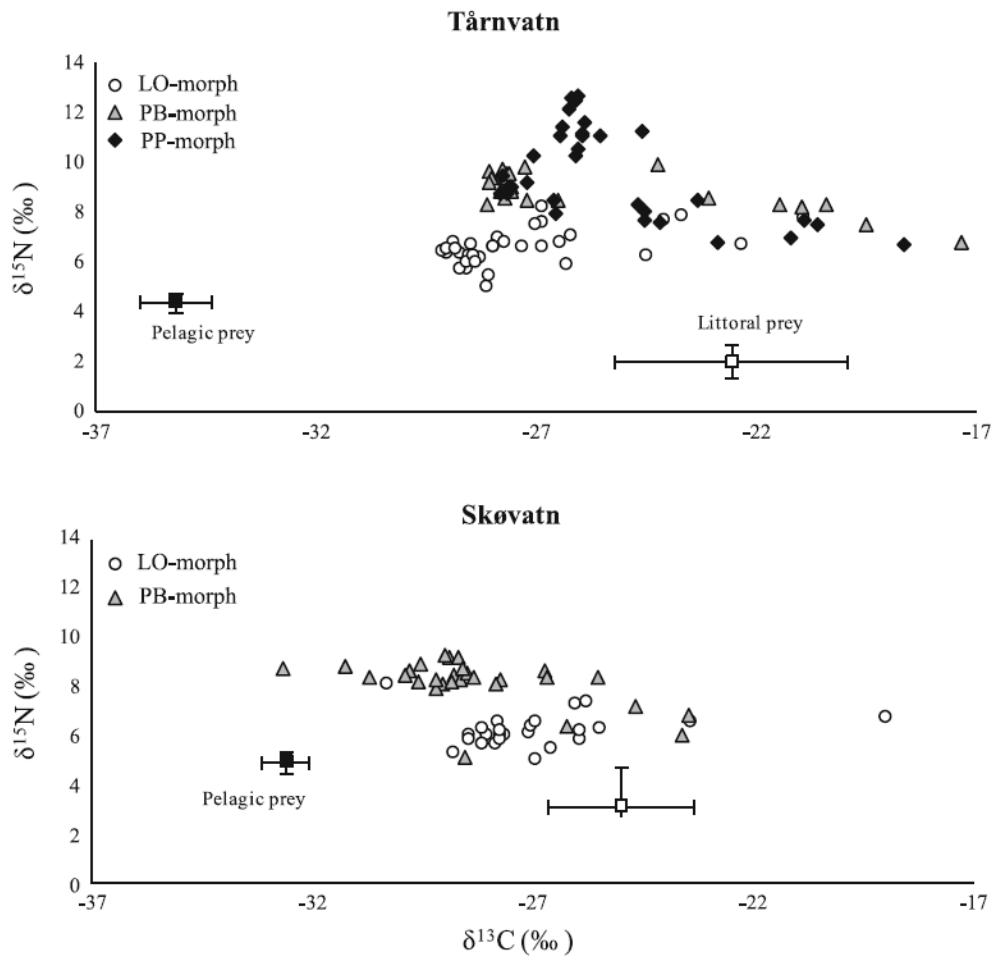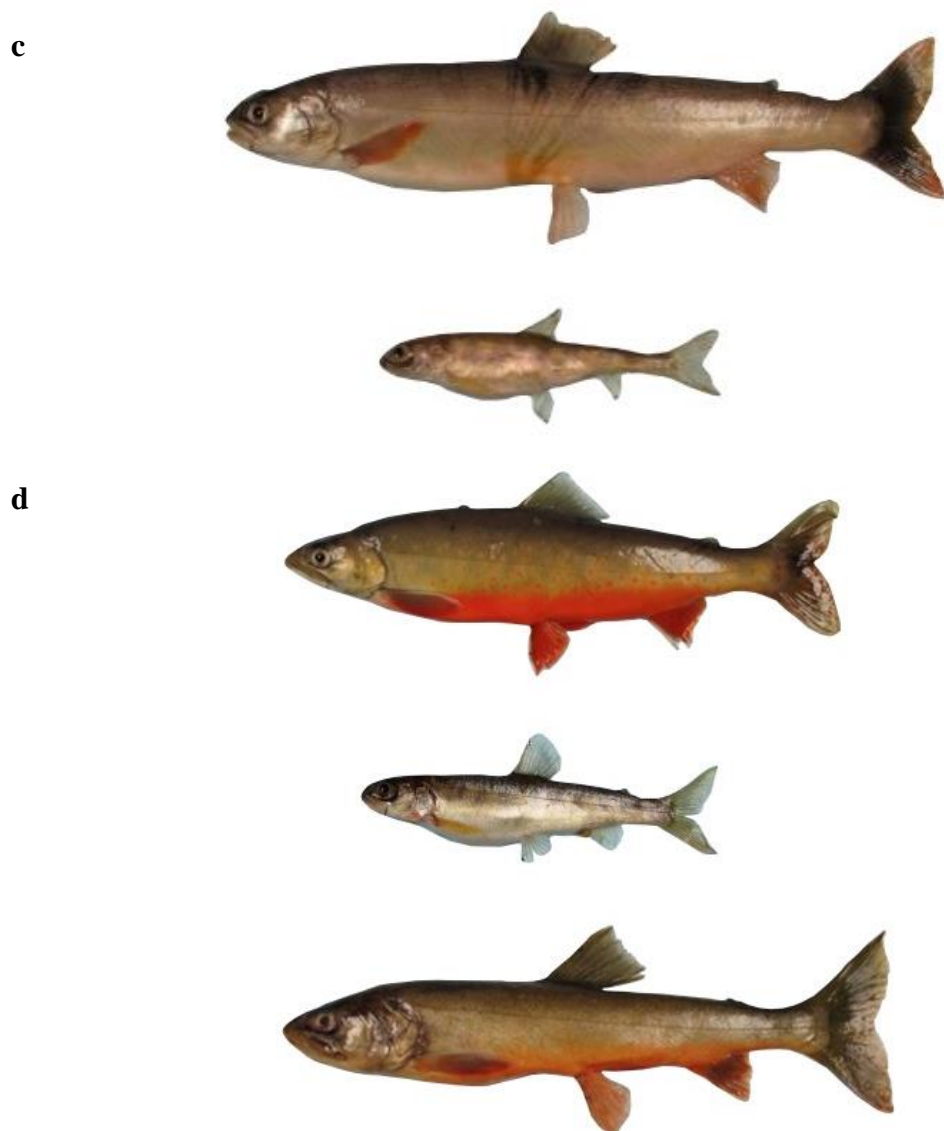
Figure 7. (a) Percent abundances of the major prey groups found in the stomach content of the different Arctic charr morphs from Tårnvatn and Skøvatn (October 2016). (b) Stable isotope biplots displaying the δ^{13}C and δ^{15}N values of dorsal muscle tissue samples of Arctic charr caught in Tårnvatn and Skøvatn in October 2016. The LO-morphs are represented by white dots (n = 34, 29, respectively), the PB-morphs (n = 25, 25, respectively) by grey triangles, and the PP-morphs in Tårnvatn (n = 32) by black diamonds. Mean values (± SD) of pelagic (black squares) and littoral (white squares) prey sampled in June 2017 are also given. (c) Two Arctic charr morphs from Skøvatn: the LO-morph (upper) and the PZ-morph (bottom). (d) Three Arctic charr morphs from Tårnvatn: the LO-morph (upper), the PB-morph (middle) and the PP-morph (bottom). The figures (a) and (b) are from Moccetti *et al.*, (2019), and (c) and (d) are from Kjær (2018).

General discussion

In this thesis, I studied ecological and genetic patterns by investigating trophic niche segregation, morphological and genetic differences among Arctic charr morphs from three Norwegian lakes, which harbour two to four morphs. The results show phenotypic and genetic divergence across Arctic charr morphs from three freshwater systems (Paper I-IV). Lake Skøvatn is inhabited by a littoral omnivorous morph (LO-morph), and a new profundal morph (PZ-morph; Paper IV). Lake Tårnvatn harbours a littoral omnivorous morph (LO-morph), a profundal piscivore morph (PP-morph) and a profundal benthivorous morph (PB-morph; Paper IV). Finally, Lake Tinnsjøen harbours the Planktivore morph, the Piscivore morph, the Dwarf morph, and a new Abyssal morph (Paper I).

The genetic and phenotypic diversity among the lakes could be caused by parallel phenotypic evolution, where different genetic factors could lead to the same phenotypic outcome in similar environmental conditions. Morphs were specialised in different habitats, where conditions such as temperature, light, pressure and quantity and quality of prey vary. These differences were reflected in traits such as head and body morphology, coloration, eye and mouth size (Paper I, II and IV), and in the brain (Paper II).

Origin and divergence of polymorphic species

The colonisation of postglacial lakes occurred during the deglaciation in the Pleistocene, which offered the opportunity to occupy new environments. These lakes are characterised by habitats with different foraging resources and low productivity (Klemetsen, 2010). Polymorphism in Arctic charr and other species has been documented in postglacial lakes (Schluter, 1993; Guiguer *et al.*, 2002; Kahilainen *et al.*, 2004; Muir *et al.*, 2014; Markevich *et al.*, 2018; Guðbrandsson *et al.*, 2019; Doenz & Seehausen, 2020), where morphs can differ in characteristics such as foraging and habitat use (Schluter, 1993, 2000b; Jonsson & Jonsson, 2001; Kahilainen & Østbye, 2006).

The origin of divergent morphs can be determined by historical events, where morphs from a lake can have common or different origins depending on the colonisation events. This polymorphism can be explained by abiotic (e.g. ecosystem size) and biotic factors (e.g. interspecific competition; Skúlason & Smith, 1995; Schluter *et al.*, 1996; Vamosi, 2005;

Siwertsson *et al.*, 2010; Recknagel *et al.*, 2017; Jacobs *et al.*, 2020; Thibert-Plante *et al.*, 2020; Öhlund *et al.*, 2020). Species will most likely diverge into different morphs in lakes with absent or reduced interspecific competition and with large depths and surface areas due to a larger niche availability (i.e. ecological opportunity). Environments with larger complexity and diversity of available resources could favour local adaptations (Recknagel *et al.*, 2017). In the freshwater systems from this study, Tårnvatn, Skøvatn and Tinnsjøen are deep and oligotrophic lakes, which have surface areas of 3.2, 6.2 and 51.4 km^2 and maximum depth of 53, 119 and 460 m, respectively. Regarding the fish community, Lake Tårnvatn consists of Arctic charr and perch. Lake Skøvatn has Arctic charr, brown trout and Atlantic salmon (*Salmo salar*). Lake Tinnsjøen harbours Arctic charr, brown trout, perch and Eurasian minnow (*Phoxinus phoxinus*). Thus, the larger morph diversity in Lake Tinnsjøen in comparison with Tårnvatn and Skøvatn, is most likely explained by the lake's depth and the surface area. A larger diversity in Arctic charr, sticklebacks, cichlids and whitefish has also been observed in lakes with large surface area and depth (McPhail, 1993; Alekseyev *et al.*, 2002; Vonlanthen *et al.*, 2009; Siwertsson *et al.*, 2010; Wagner *et al.*, 2014; Recknagel *et al.*, 2014; Hooker *et al.*, 2016; Recknagel *et al.*, 2017; Doenz *et al.*, 2019).

Does parallelism always occur under similar environmental conditions?

Parallel evolution can happen when environmental conditions are similar, where morphs can develop similar characteristics in different freshwater systems (i.e. phenotypic parallelism; (Elmer & Meyer, 2011; Elmer *et al.*, 2014; Laporte *et al.*, 2015; Hooper & Price, 2017; Oke *et al.*, 2017; Thompson *et al.*, 2019; Jacobs *et al.*, 2020; Salisbury *et al.*, 2020). Phenotypic parallelism could be caused by variation in selective pressures and genetic factors such as mutation, drift and gene flow (Hendry *et al.*, 2001; Schluter *et al.*, 2004; Bolnick & Nosil, 2007; Maan & Seehausen, 2011; Kaeuffer *et al.*, 2012; Moore *et al.*, 2016). In postglacial lakes, morphs can show parallel phenotypic and genetic divergence, where divergent natural selection plays an important role in the morph diversification (Taylor & Bentzen, 1993; Robinson & Wilson, 1994; Schluter *et al.*, 1996; Pigeon *et al.*, 1997; Gíslason *et al.*, 1999; Taylor, 1999; Rundle *et al.*, 2000; Schluter, 2000a; Rogers *et al.*, 2013).

Our results revealed parallelism in diet and habitat use among some Arctic charr morphs in different freshwater systems (Paper I and IV). For instance, the piscivore morph was mainly

found in the shallow-moderate profundal habitat in Tinnsjøen and it was only found in the profundal habitat in Tårnvatn, where older individuals fed on fish. Parallelims in diet and habitat use was found in the piscivore morphs from Tinnsjøen, Tårnvatn and Skogsfjordvatn (Smalås *et al.*, 2013; Knudsen *et al.*, 2016b; Paper I, II, IV). The planktivore morph fed mainly on zooplankton and on littoral benthos. This morph was found in the littoral habitat in Skøvatn, in littoral, pelagic and profundal habitats in Tårnvatn, and in the pelagial, littoral and shallow-moderate profundal habitats in Tinnsjøen. Parallelism in habitat and diet choice was also found among the LO-morphs from Skøvant, Tårnvatn, Tinnsjøen, Fjellfrøsvatn and Skogsfjordvatn (Knudsen *et al.*, 2016a; Siwertsson *et al.*, 2016; Paper I, IV). However, there was non-parallelism in diet preference between the PZ-morph in Skøvatn and the PB-morph in Tårnvatn (Paper IV).

Regarding life-history traits, there were also similar patterns among Arctic charr morphs. For instance, the LO-morph in Skøvatn and Tårnvatn showed the fastest growth rate and reached maturity at a medium size and age in comparison with PB- and PZ-morphs (Kjær, 2018). The PB- and PZ-morphs had low growth-rates and reached maturity at early ages and at small body sizes (Kjær, 2018), showing a similar life span than the Dwarf morph in Tinnsjøen (Paper I). This has also been observed in the small-sized profundal morphs from Fjellfrøsvatn and Skogsfjordvatn (Klemetsen *et al.*, 1997; Smalås *et al.*, 2013). Parallel patterns regarding life history, morphology, diet and habitat use have been recorded in the PB-morph from Tårnvatn, Fjellfrøsvatn and Skogsfjordvatn (Klemetsen *et al.*, 1997; Knudsen *et al.*, 2016a; Siwertsson *et al.*, 2016; Saltykova *et al.*, 2017; Paper IV). The PP-morph in Tårnvatn showed a delay in maturity, had the lowest growth and the largest length (Kjær, 2018). The PP-morph also showed the largest life span in Tinnsjøen and Tårnvatn (Kjær, 2018; Paper I). Thus, these similarities found among Arctic charr morphs across different lakes could be explained by adaptation to environments with similar selection pressures developing similar traits, which could be related to the interaction of environmental conditions, stochasticity and evolutionary contingencies (Jacobs *et al.*, 2020).

These differences among the morphs could also be detected at the genomic level, where the genomic architecture variation could play an important role in the morph divergence. A relevant feature of genomic architecture is chromosomal inversions, which can reduce or suppress recombination in specific areas along the genome (Kirkpatrick & Barton, 2006;

Hoffmann & Rieseberg, 2008; Feder & Nosil, 2009; Kirkpatrick, 2010). Chromosomal inversions could contribute to parallel adaptive divergence (Barrett & Schluter, 2008; Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010; Faria *et al.*, 2019; Morales *et al.*, 2019), acting as a reserve of adaptive standing variation that could favour rapid adaptation (Colosimo *et al.*, 2005; Steiner *et al.*, 2007; Tishkoff *et al.*, 2007; Morales *et al.*, 2019). In this thesis, it was identified chromosomal inversions along the Arctic charr genome (Paper III). These chromosomal inversions might increase genome divergence among Arctic charr morphs, leading to the development of specific phenotypes associated with local adaptation (Kirkpatrick & Barton, 2006; Berg *et al.*, 2017). Such variations among morphs could lead to a rapid adaptive radiation.

Adaptive ecological speciation in Arctic charr morphs?

As discussed above, these morphs showed phenotypic and genetic differences (Paper I-IV). For instance, their divergence in life-history, morphology, diet and habitat use, are most likely driven by different selection pressures, which can vary depending on the environment. For ecological speciation to happen, a source of divergent selection is needed (Rundle & Nosil, 2005). Divergent selection can arise from environmental differences, sexual selection and ecological interactions, evolving reproductive isolation between populations and leading to ecological speciation (Rundle & Nosil, 2005). Divergent selection can arise when there are populations occupying different environments that have specific abiotic and biotic characteristics such as habitat structure, composition of species (predators and/or competitors) or resources (Schluter, 2000b; Rundle & Nosil, 2005). Environmental conditions can change the direction and strength of selection and gene flow (McKay & Zink, 2015). Gene flow can reduce morph divergence, decreasing reproductive barriers and thus, speciation reversal could happen (Seehausen *et al.*, 2008). For the formation of species, different evolutionary forces have to be considered such as drift, selection and mutations, together with the combination of ecological opportunity, creating genetic differences within species.

Adaptation favours speciation when populations develop reproductive barriers (i.e. premating and postmating isolation), reducing gene flow and arising genetic differences among populations (Coyne & Orr, 2004; Thompson *et al.*, 2019). Under postmating isolation, there is a reduction of hybrid fitness due to their intermediate phenotype, having a mismatch

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between their environment and their phenotype (Coyne & Orr, 2004). In this thesis, Arctic charr morphs showed genetic divergence (Paper I-IV), even though there were hybrid individuals, which could have a lower fitness compared with pure individuals. The presence of hybrids in Arctic charr morphs shows an incomplete reproductive isolation (Paper I-IV), since hybridisation is still happening among the morphs, as reported in other studies (e.g. Gíslason *et al.*, 1999; Samusenok *et al.*, 2006; Gordeeva *et al.*, 2015; May-McNally *et al.*, 2015; Guðbrandsson *et al.*, 2019; Salisbury *et al.*, 2020). Thus, Arctic charr morphs from these three lakes studied could be undergoing an adaptive radiation, which is in an early phase.

Arctic charr morphs also showed phenotypic divergence such as variation in head and body size, coloration, weight, and age at maturation (Paper I, II and IV). These different traits among the morphs could also be a reproductive barrier (e.g. premating isolation). Reproductive isolation might have arisen in parallel in similar environments across these different freshwater systems (Paper I and IV), showing parallel evolution. Parallel evolution could play an important role in the phenotypic parallelism of the morphs, since not all genetic differences are always shown in the phenotype and different genetic routes, genetic parallelism, or both, might lead to same phenotypes (Reed & Frankham, 2001, 2003; Moss *et al.*, 2003; Mitchell-Olds *et al.*, 2007; Laporte *et al.*, 2015; Oke *et al.*, 2017). Thus, similar morphs could arise across different lakes with similar environments. Arctic charr morphs from the three Norwegian lakes studied likely are under ecological speciation, where ecologically-based divergent selection could lead to morph divergence, causing reproductive isolation that might evolve as a result of adaptation to different environments.

Conservation and management issues

Our capacity to delimit among and within species level is difficult due to the multiplicity of species concepts (Zachos, 2018) and the intraspecific diversity observed, which is frequently underestimated. Thus, the lack of resolution leads to uncertainty to decide what should be conserved and managed. At the intraspecific level, different categories have been used for conservation units (CUs; i.e. population units found within species used for management and conservation purposes) such as evolutionary significant units (ESUs; Moritz, 1994; Funk *et al.*, 2012), management units (MUs; Moritz, 1994) and subspecies or ecological races (Braby *et al.*, 2012). Recent advances in molecular techniques and analytical methods such as next-

generation sequencing (NGS) techniques and machine learning, could also help to delineate conservation units. Modern genomic techniques could also reinforce gaps that have been previously detected in phenotypic and environmental data (Crandall *et al.*, 2000; Funk *et al.*, 2012), providing information regarding adaptive variation within species (Funk *et al.*, 2012). In these three Norwegian lakes studied, we will have two MUs in Lake Skøvatn, three MUs in Lake Tårnvatn and four MUs in Lake Tinnsjøen following Moritz (1994), corresponding with the morphs found in each lake. It is relevant to conserve the local adaptive variation within each lake to preserve the genetic and phenotypic diversity of Arctic charr morphs.

In this thesis, Arctic charr morphs displayed phenotypic and genetic diversity across three Norwegian lakes, offering the opportunity to study ongoing speciation processes. The conservation of the morphs' ecological niches is necessary to preserve habitat characteristics, in turn influencing, spawning sites and feeding sources. Anthropogenic impacts such as fishing, pollution or habitat loss could affect the morph composition, potentially reducing their genetic diversity through genetic drift or selection (Schaffer & Elson, 1975; Ricker, 1981). For instance, the harvest of one specific morph through fishing will reduce its abundance, potentially favouring and giving new or different opportunities (e.g. food source and habitat) to other morphs. Once the fishing is terminated, it is difficult to predict whether the morphs proportion will be recovered. Thus, for conservation and management purposes, Arctic charr morphs could be considered together within each lake, reducing the disturbance into these lakes to preserve their diversity and ongoing evolutionary processes.

Conclusions

In this thesis, I investigated phenotypic, genetic and genome-wide diversity of Artic charr morphs and highlighted putative processes behind polymorphism and genetic diversity. These results showed phenotypic and genetic divergence among the morphs, which could be due to phenotypic plasticity, genetic basis, or both.

Comparing the three Norwegian freshwater systems, a lower diversity in Arctic charr morphs was observed in smaller lakes (Paper IV), which could vary depending on ecological opportunity and colonisation history. Parallelism in morphology and habitat use was found among some of the Arctic charr morphs from Tinnsjøen, Tårnvatn and Skøvatn (Paper I, IV).

Natural selection is likely the cause of parallel evolution, where Arctic charr morphs develop similar traits in different lakes that have similar environmental conditions (Paper I, IV). Divergence was also detected in brain regions of the four morphs from Lake Tinnsjøen (Paper II). Natural selection might act on specific regions of the brain associated with divergence in sensory capacities given by different preferences and constraints, for instance, in habitat and diet.

Chromosomal rearrangements can be an important factor in the divergence of sympatric morphs, reducing gene flow in these regions and favouring reproductive isolation of morphs at early stages of the speciation process (Paper III).

In conclusion, the studied Arctic charr morphs are likely at early stages along the ecological speciation trajectory, showing phenotypic and genetic divergence across different environments. Arctic charr morphs are likely undergoing an adaptive radiation, explaining the Arctic charr polymorphism. Adaptation to specific environments could cause the development of reproductive barriers among morphs. These polymorphic Arctic charr systems should be managed as whole-lake units until we fully understand the evolutionary dynamics and persistence of sympatric morphs, and as they represent an important legacy of the Arctic charr. These Arctic charr systems could be examples of speciation within species level.

Future research and perspectives

Arctic char is a complex species for studying phenotypic and genetic variation, and many questions are yet to be resolved. To investigate further phenotypic variation and trophic niche, a seasonal schedule for sampling could be done to observe seasonal differences in feeding and in parasite communities among Arctic charr morphs. Regarding relationship between brain and behaviour, it would be interesting to study, for instance, feeding behaviour and food intake, by looking at differences in the neuropeptides and doing a detailed mapping of these neuropeptides to investigate pleiotropic functions. In addition, examination of neuron density and gene expression levels in response to selection pressures for brain region sizes would help to understand mosaic evolution and morphological divergence in the brain sections. Furthermore, measurements at protein and gene expression levels of genes correlated with

craniofacial morphogenesis and eye development could be done to investigate possible niche specialisation among Arctic charr morphs from Lake Tinnsjøen.

Comparison of replicate monomorphic and polymorphic systems of Arctic charr from different habitats using genome-wide approach will give us a better insight and understanding of the variation of these morphs across the genome.

Conducting experimental studies on different morphs would be interesting to identify changes in the phenotype and in the genotype simulating different conditions. These studies will help to identify whether these variations are due to phenotypic plasticity, genetic basis or both. These studies could be also implemented to identify changes in the morphology of the whole brain and in specific brain region and to measure gene expression levels.

Finally, CRISP/Cas technology is another interesting tool to implement, which can knockout genes in non-model organisms. The application of this method could provide more knowledge about different aspects of Arctic charr such as foraging behaviour, helping to highlight possible mechanisms behind feeding behaviour divergence.

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"And if you gaze long into an abyss, the abyss gazes also into thee": four morphs of Arctic charr adapting to a depth gradient in Lake Tinnsjøen

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Abstract

The origin of species is a central topic in biology. Ecological speciation might be a driver in adaptive radiation, providing a framework for understanding mechanisms, level, and rate of diversification. The Arctic charr *Salvelinus alpinus* L. is a polymorphic species with huge morphological and life-history diversity in Holarctic water systems. We studied adaptive radiation of Arctic charr in the 460-m-deep Lake Tinnsjøen to (a) document eco-morphology and life-history traits of morphs, (b) estimate reproductive isolation of morphs, and (c) illuminate Holarctic phylogeography and lineages colonizing Lake Tinnsjøen. We compared Lake Tinnsjøen with four Norwegian outgroup populations. Four field-assigned morphs were identified in Lake Tinnsjøen: the planktivore morph in all habitats except deep profundal, the dwarf morph in shallow-moderate profundal, the piscivore morph mainly in shallow-moderate profundal, and a new undescribed abyssal morph in the deep profundal. Morphs displayed extensive life-history variation in age and size. A moderate-to-high concordance was observed among morphs and four genetic clusters from microsatellites. mtDNA suggested two minor endemic clades in Lake Tinnsjøen originating from one widespread colonizing clade in the Holarctic. All morphs were genetically differentiated at microsatellites (F_{ST}: 0.12–0.20), associated with different mtDNA clade frequencies. Analyses of outgroup lakes implied colonization from a river below Lake Tinnsjøen. Our findings suggest postglacial adaptive radiation of one colonizing mtDNA lineage with niche specialization along a depth–temperature–productivity–pressure gradient. Concordance between reproductive isolation and habitats of morphs implies ecological speciation as a mechanism. Particularly novel is the extensive morph diversification with depth into the often unexplored deepwater profundal habitat, suggesting we may have systematically underestimated biodiversity in lakes.

In a biological conservation framework, it is imperative to protect endemic below-species-level biodiversity, particularly so since within-species variation comprises an extremely important component of the generally low total biodiversity observed in the northern freshwater systems.

1 | INTRODUCTION

Revealing processes behind adaptive diversity, and formation of species, are central themes in evolutionary biology. Although studied for a long time, the mechanisms for adaptive radiation and speciation appear enigmatic. Our consensus understanding is that adaptive radiation by natural selection has been important in the origin of populations and species (Darwin, 1859;Mayr, 1942;Schluter, 2000). In a biological conservation framework, we should center less on species moving toward protecting biological diversity below the species level, which reflects ongoing natural (non)adaptive speciation processes. The low aquatic species diversity in the north means that the within-species variation is an extremely important component of the total biodiversity (Chavarie, Howland, Harris, & Tonn, 2015;Fraser, Weir, Bernatchez, Hansen, & Taylor, 2011;Moore et al., 2014;Reist, Power, & Dempson, 2013). Thus, the speciation process as a fundamental question in evolutionary biology has also important and practical relevance in applied biological conservation (Coates, Byrne, & Moritz, 2018).

Scientists continuously search for ideal study systems and species groups, to illuminate how speciation processes are acting under evolutionary scenarios and timescales. Here, highly recognized model species used as rewarding looking-glasses into the species-formation process comprise, for example, Darwin's finches on the Galapagos Islands, European-Mediterranean sparrows, the *Anolis* lizards, cichlid fishes, the threespined stickleback, and sunflowers (Grant & Grant, 2008;Hermansen et al., 2011;Miller, Rosti, & Schluter, 2019;Moyers & Rieseberg, 2016;Salazar, Castañeda, Londoño, Bodensteiner, & Muñoz, 2019;Salzburger, 2018). The polymorphic northern freshwater fishes of *Coregonus* and *Salvelinus* species complexes are becoming increasingly recognized as good model systems in this regard (Bernatchez, 2004;Jonsson & Jonsson, 2001;Klemetsen, 2010). Speciation is a complex issue (e.g., Wilkins, 2018), where the theoretical–empirical framework presents avenues for adaptive diversification in speciation (Gavrilets, 2004;Seehausen & Wagner, 2014;Suzuki & Chiba, 2016). Across examples of adaptive radiation, similarities exist for patterns and processes, where one could tailor models specifically to each species system to derive an understanding of mechanisms by empirically parameterizing theoretical models (Gavrilets & Vose, 2007;Thibert-Plante et al., 2020). The insight from theoretical–empirical analyses can point toward important areas where we

need to fill knowledge gaps that surface through predictive theoretical models when attempting to add empirical values.

In the ice-covered northern Eurasian hemisphere, the late Pleistocene ice sheet set the frame for colonization and postglacial adaptation to lakes as the maximum extent of the ice sheet occurred at ca. 21, 000 years before present (ybp) and deglaciation at ca. 10–20, 000 ybp (Hughes, Gyllencreutz, Lohne, Mangerud, & Svendsen, 2016;Mangerud et al., 2004;Patton et al., 2017). The Pleistocene ice age started ca. 2.58 million years before present, with alternating phases of glaciation (of roughly 70, 000–100, 000 years' duration) and interglacials (10, 000–30, 000 years' duration) (Andersen & Borns, 1994;Lorens, Hilgen, Shackleton, Laskar, & Wilson, 2004;Rapp, 2015). The Pleistocene ice age dynamics represents a long time series where flora and fauna likely repeatedly colonized new land and retracted to glacial refugia. Such conditions created opportunities for allopatric differentiation, secondary contact, and sympatric diversification among and within species (Hewitt, 2004;Swenson & Howard, 2005;Taberlet, Fumagalli, Wust-Saucy, & Cosson, 1998). Thus, Holarctic lakes comprise a unique window into the adaptive diversification process of colonizing Arctic charr (*Salvelinus alpinus*, L) where the degree and rate of novel, or parallel adaptations, can be studied by contrasting old versus young glacial geological systems represented by genetic lineages and carbon-isotope-dated lakes. Ecological opportunity for diversification via intraspecific competition and niche radiation in species-poor postglacial lakes may be an important mechanism in morph and species formation in several fish taxa (Robinson & Wilson, 1994;Seehausen & Wagner, 2014;Siwertson et al., 2010). One mechanism that could build up reproductive isolation as a secondary product is termed ecological speciation (Hendry, 2009;Rice, 1987) and could have been central in adaptive proliferation of morphs into all lake niches. With regard to sympatric Arctic charr morphs, several evolutionary scenarios are hypothesized (see also Seehausen & Wagner, 2014). First, the lake could have been colonized by divergent genetic lineages (associated with different morphs) coming into secondary contact after separation for thousands of years in glacial refugia. Secondly, sympatric morphs may represent a real intralake sympatric adaptive diversification after colonization of one genetic lineage (comprising one initial ancestral morph). Thirdly, a combination of such scenarios could have occurred, generating temporal dynamics in gene pool sharing via expansion–contraction, adaptive divergence,

speciation reversal, introgression and hybrid swarm dynamics, and subsequent divergence based on novel combinations of genetic variants to be selected upon. Under such adaptive diversification mechanisms, also genetic drift and phenotypic plasticity may be important processes (Häkli, Østbye, Kahilainen, Amundsen, & Præbel, 2018;Seehausen & Wagner, 2014;West-Eberhard, 1989). The highly polymorphic Arctic charr species complex has a Holarctic distribution and is the most cold adapted northern freshwater fish species, where some populations are anadromous, while most populations are stationary in freshwater (Klemetsen, 2010;Taylor, 2016). Arctic charr occupy species-poor Holarctic lakes, suggesting ecological opportunity for adaptive radiation into available niches (Klemetsen, 2010;Knudsen, Klemetsen, Amundsen, & Hermansen, 2006). Many Arctic charr lakes apparently only harbor a generalist morph, supported by the relative few studies revealing polymorphism. Some of these monomorphic populations, with a generalist morph, utilize both littoral and pelagial habitats through ontogenetic habitat shifts (Klemetsen, 2010). In a much fewer set of lakes, two more or less distinct morphs, for example, a littoral and a pelagic morph, may co-occur (Hooker et al., 2016;Westgaard, Klemetsen, & Knudsen, 2004), suggesting lake-specific temporal persistence of niches for the evolution and coexistence of two different morphs. In a very few lakes, a third morph is found in the profundal, termed the profundal morph, coexisting with, for example, the littoral and pelagic morph (Moccetti et al., 2019;Skoglund, Siwertsson, Amundsen, & Knudsen, 2015). Only in one single lake worldwide, namely Lake Thingvallavatn in Iceland, four sympatric morphs are reported having radiated into all lake niches: a small and large benthic morph, a pelagic morph, and a piscivore morph (Jonsson et al., 1998). Arctic charr morphs that adapt to divergent niches may show parallelism among lakes

with independent origin of morph pairs (Gordeeva, Alekseyev, Matveev, & Samusenok, 2015). Here, similar morphs can evolve through parallel or nonparallel evolutionary routes revealing similar gene expression as seen in independently derived morph replicates of two genetic lineages (Atlantic and Siberian lineage) in Arctic charr (Jacobs et al., 2020). This suggests the presence of a highly robust adaptive system in the Arctic charr complex for deriving the same evolutionary outcome from different genetic starting points (historical contingency: adaptive standing genetic diversity, genomic architecture) as response to similar selection pressures. However, there are often lake-specific differences in morph variance in, for example, niche occupation, phenotype, and life history (Knudsen, Amundsen, Primicerio, Klemetsen, & Sørensen, 2007;Moccetti et al., 2019). This large-scale parallel evolution in Holarctic lakes, with similar morphs appearing, is a unique feature when studying natural selection and early stages in the speciation continuum, making the Arctic charr species complex an excellent model system in evolutionary biology and eco-evo-devo studies.

Here, we report on a new Arctic charr system harboring a striking diversity in phenotypes and life histories, apparently associated with a depth–temperature–productivity–pressure gradient in the 460-meter-deep oligotrophic Lake Tinnsjøen in Norway (Box 1). The history before our study is as follows. In 1944, in the occupied Norway during the Second World War, the Norwegian partisans sunk the railway ferry *D/F Hydro* carrying an estimated 20 barrels with 500 kilo of heavy water (D_2O) in Lake Tinnsjøen. The German occupation government had the purpose to construct an atomic bomb back home in Germany using D_2O (Dagbladet, 2018;National Geographic, 2018). It has been debated whether this Second World War famous sabotage action hampered or stopped Hitler's attempt

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BOX 1 We got involved with this nice man named Louis many years back during our own PhD (Kjartan) and PostDoc work (Kim), being kindly invited to his lab in Quebec for collaboration. We were not there at the same time, but Louis and we shared the same love to studies of adaptive radiation and ecological speciation in Coregonus (of course!). We worked on understanding evolutionary and genetic patterns and processes underlying the vast phenotypic and genotypic variation found in the European whitefish complex. A daunting and life-long task, that we never would have been able to advance if not for the tremendous contribution and insights from Louis, especially from the Lake whitefish crossings, and his pioneering work on enabling and using genomic tools in non-model species. We also still remember our discussions a late evening in 2012 in Mondsee, Austria, where you encouraged us to undertake this study in Arctic charr! Based on our long-term friendship it is evident that Louis is a strong scientific person, but he has not traded off important ordinary down-to-earth traits such as good mood, being able to party, going fishing and hunting. Particularly, his strong social side is an essential positive trait to mention, as Louis has run his lab as an integrated social unit where the atmosphere is relaxing, and competitive, and based on a curiosity-driven mindset. In such a rewarding environment, filled with top-notch personnel and state of the art technologies, even untrained naive hillbilly-rascals from Norway and Denmark were able to learn fast and efficiently. Louis has the brilliant ability to really listen to his students and colleagues, and indeed a special nose for cutting-edge studies that needs to be conducted for the common good for the scientific society. Louis has been very influential for both of us with regard to our mind-sets in our scientific careers, and as a friend, colleague and collaborator in our scientific projects. We are indeed very fond of this Basque-Quebecois-Canadian guy and look forward to the years to come.

to produce the atomic bomb. Almost 50–60 years later, in 1993 and 2004, a Norwegian team on their search for the sunken ferry, making a Second World War news report regarding the presence of heavy water on the ferry, was able to locate it at 430 meters depth using a ROV submarine. At the same time, they also observed small fish residing at the bottom. The team successfully retrieved two fish specimens that were later classified as Arctic charr (Søreide, Dolmen, & Hindar, 2006). The knowledge about the Arctic charr diversity within Lake Tinnsjøen up to that date comprised a study by Hindar, Ryman, and Ståhl (1986) showing that a dwarf and planktivore morph grouped together (being statistically different from each other) compared to yet other Norwegian lakes when analyzing allozymes. From old age, local fishermen in Lake Tinnsjøen have recognized a rare deepwater morph of Arctic charr locally named "Gautefisk" ("Gaute" is a Norwegian male name, and "fisk" is fish in Norwegian). This morph has different coloration from other morphs in the lake, and different body proportion, weighing up to 4–6 kg (Brabrand, 1994). Thus, when summarizing available information, a set of four morphs were suggested in Lake Tinnsjøen.

As no progress occurred considering scientific studies on the small white fish from the bottom of the lake from the ROV team, we conducted a fish survey in the lake in 2013 to document the occurrence of morphs. We set up three main research topics with regard to the Lake Tinnsjøen Arctic charr diversity: (a) to document eco-morphology and life-history traits (body shape, proportional catch in habitat, age, weight) of field-assigned morphs, (b) to estimate reproductive isolation of field-assigned morphs or fish assessed using unbiased methods (microsatellites), and (c) to illuminate the phylogeography and ancestral lineages colonizing Lake Tinnsjøen (mtDNA-CytB sequences). To accomplish these tasks, we collected fish in different habitats in the pelagial, littoral, shallow-moderate profundal and in the deep profundal. In the field, we classified fish to morphs from exterior phenotype, while in the laboratory, we assessed morphological (body shape) and genetic divergence using mtDNA and nDNA markers. We further performed a Holarctic phylogeography retrieving online genetic sequences to evaluate lineages colonizing Lake Tinnsjøen. The strength of association of field-assigned morphs and genetically identified morphs using microsatellites (i.e., genetic clusters or populations) was tested. We compared mtDNA and nDNA in Lake Tinnsjøen with four Norwegian outgroup lakes. Using a putative ancestor below in the same drainage, we compared body shape to the Lake Tinnsjøen morphs.

2 | METHODS

2.1 | Material used for different analyses

The material used for the different analyses is summarized in Appendix S1: Table S1.

2.2 | Study area, fish sampling, and field-assigned morphs

Lake Tinnsjøen (60 38 15.6 N, 11 07 15.2 E) is a long (35 km), large (51.38 km²), and deep (max depth of 460 m, 190 m mean depth) oligotrophic lake in southeastern Norway (Figure 1a,b) (NVE, 1984). High mountain sides surround the lake descending steeply into the lake resulting in a relatively small littoral area compared to an extensive pelagic volume and a large profundal area. In the southern and northern ends of the lake, larger littoral areas exist. The littoral zone is exposed to the elements such as wind and waves. The shoreline is monotonous with few bays and only two small islands. The littoral zone is composed mostly of bedrock, large boulders, smaller rocks, and sand in less exposed areas and in the deeper layers. The pelagic zone is extensive. The profundal appears to differ structurally in shallow and deep areas—composed of bedrock, boulders, sand, and larger-sized organic matter in shallow areas, while more fine particulate organic detritus dominates in the deep profundal areas (based on organic matter on catch equipment and from videos by the Norwegian Broadcasting Company (www-link; no longer valid)). A survey in Lake Tinnsjøen in June 2006 by Boehrer, Golmen, Løvik,

FIGURE 1 (a) Norway with Lake Tinnsjøen and the four outgroups sampled. (b) River Skiensvassdraget wherein Lake Tinnsjøen is situated. Red lines denote dated ice-recession lines in years before present (ybp) from Bergstrøm (1999). Gray arrows denote the youngest ice-flow direction in the end of the Pleistocene glaciation from Bergstrøm (1999). The black bar indicates the upper deposits of marine sediments. (c) The four nominal field-assigned Arctic charr morphs (FA-morphs) observed within Lake Tinnsjøen (note: fish scaled to the same length)
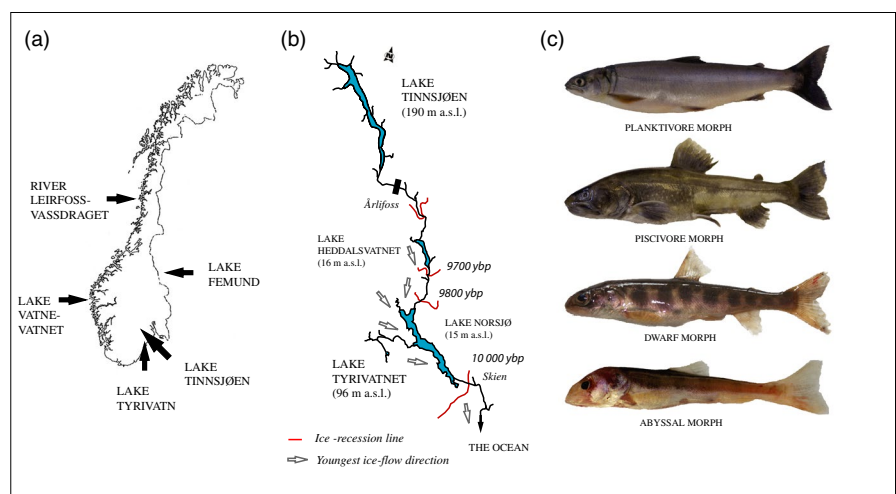
Rahn, and Klavness (2013) gave an oxygen concentration of 11.5–12.0 mg/L from surface down to 460 m depth, a temperature profile from 4.0 to 3.3°C from 50 to 460 meters depth, conductivity of 10.0–8.0 μS/cm from 0 to 460 m depth, and dissolved oxygen ranging from 90% to 85% from 0 to 460 m depth. Lund (1948) sampled Lake Tinnsjøen once a month from December 1946 to December 1947 and found that below ca 80 m depth, the temperature was at a constant 4°C (depth stratified), while warming up to ca. 18–20°C in top layer in summer. Thus, Lake Tinnsjøen offers a divergent temperature profile (and light, pressure, and productivity in habitats, depths, and niches) in pelagic and littoral–benthic depth gradients from surface to 460 m.

We collected Arctic charr from Lake Tinnsjøen during 2013 and from four additional Norwegian outgroup populations (see below) north, west, east, and south of Lake Tinnsjøen in 2013–2015 (Figure 1a). Fish were caught in four lake habitats (can be viewed as crude nominal niches for individuals and morphs) in Lake Tinnsjøen using equipment described below. At this stage, we do not reveal the exact sampling sites until the taxonomic status of the new abyssal morph has been described and conservation biology authorities in Norway have considered the situation with regard to its conservation value. Particularly relevant here are the population size and uniqueness of the new discovered morph, and what conservation status it merits. As the lake has steep mountain sides entering the

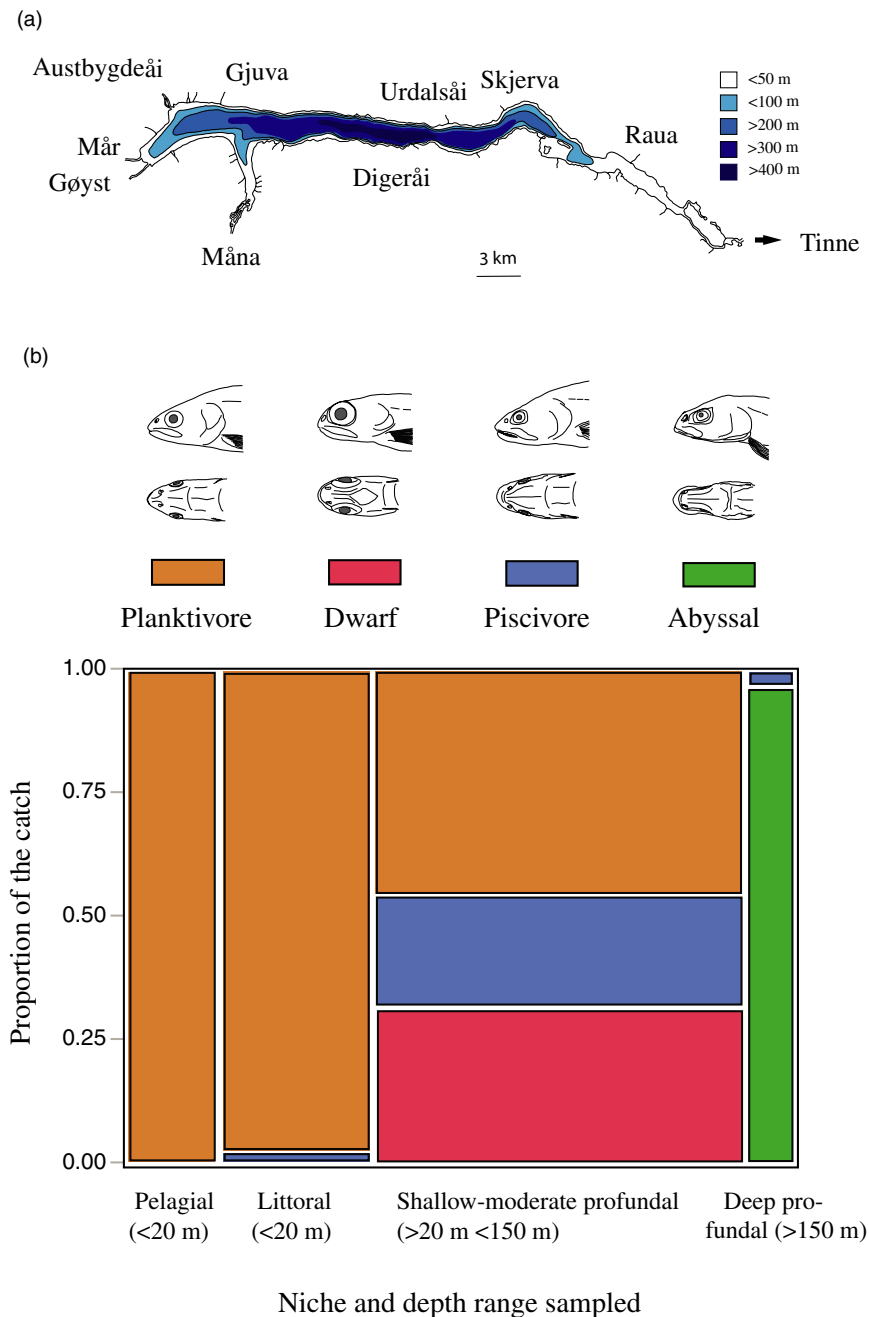**FIGURE 2** (a) A crude bathymetric map of Lake Tinnsjøen (modified from The Norwegian Water resources and Energy Directorate; http//gis3.nve.no/metadata/tema/DKBok1984/Dybdekart_1984.htm) (NVE, 1984). (b) Association between the catch of the four FA-morphs in the four lake habitats in Lake Tinnsjøen. A drawing of representative heads (lateral and ventral views) of each of the four FA-morphs is given in the top panel

lake, it is hard to place equipment precisely at predetermined positions. Thus, habitat and depth ranges fished were grouped to be able to compare catch among four nominal lake habitats. The four lake habitats (nominal niches) sampled (and defined by us) in Lake Tinnsjøen in 2013 were as follows: (a) the pelagial (gillnets at < 20 m depth, in areas with depths of > 30 m, and > 50 meters from the shore), (b) the littoral (gillnets from shore < 20 m depth), (c) the shallow-moderate profundal (gillnets, traps, and hook and line from shore at > 20 m and < 150 m depth), and (d) the deep profundal (traps at > 150 m depth, >100 m from the shore).

Sampling was conducted with gillnets, baited anchored longlines, and traps. Initially, we aimed at fishing with a standardized effort **x** equipment in all niches, but due to the experimental nature of fishing Arctic charr at depths > 150 m, and the low fish density, it was difficult to obtain sufficient sample sizes. Thus, we intensified the effort in the different habitats with the catch methods that worked best. As such, the material obtained may not be fully representative of fish populations at all depths and habitats, but represents an opportunistic sampling strategy under quite challenging fishing conditions. We used different monofilament series coupled in gangs when fishing with gillnets. In the pelagial, we used a 12-panel multimesh Nordic series (each net: 6 × 60 m) with mesh sizes (in the following order) of 43, 19.5, 10.0, 55.0, 12.5, 24, 15.5, 35.0, 29.0, 6.3, 5.0, and 10.0 mm (knot to knot) and extended Jensen floating series (each net: 6 × 25 m) with mesh sizes of 13.5, 16.5, 19.5, 22.5, 26.0, 29.0, 35.0, 39.0, 45.0, and 52.0 mm. In the littoral, we used extended Nordic and Jensen littoral net series (each net: 1.5 × 60 m or with the same mesh size as in the pelagic zone) including extra nets of some of the largest meshes. We used traps at 20–60 m depth, and Jensen littoral net series (see above for specifications) and hook and line down to 150 m depth in the shallow-moderate profundal. In the deep profundal, we used traps baited with cheese at 150–350 m depth. The baited anchored longlines (ca 220 m long; 3–4 mm line; 180 hooks; size 1, 1/0, and 2), aimed at catching piscivorous Arctic charr, were placed vertically close to the shoreline (<100 m) and in a few cases horizontally at the bottom. As these attempts resulted in a low catch, the hook and line approach was not used extensively. Nets and baited lines were
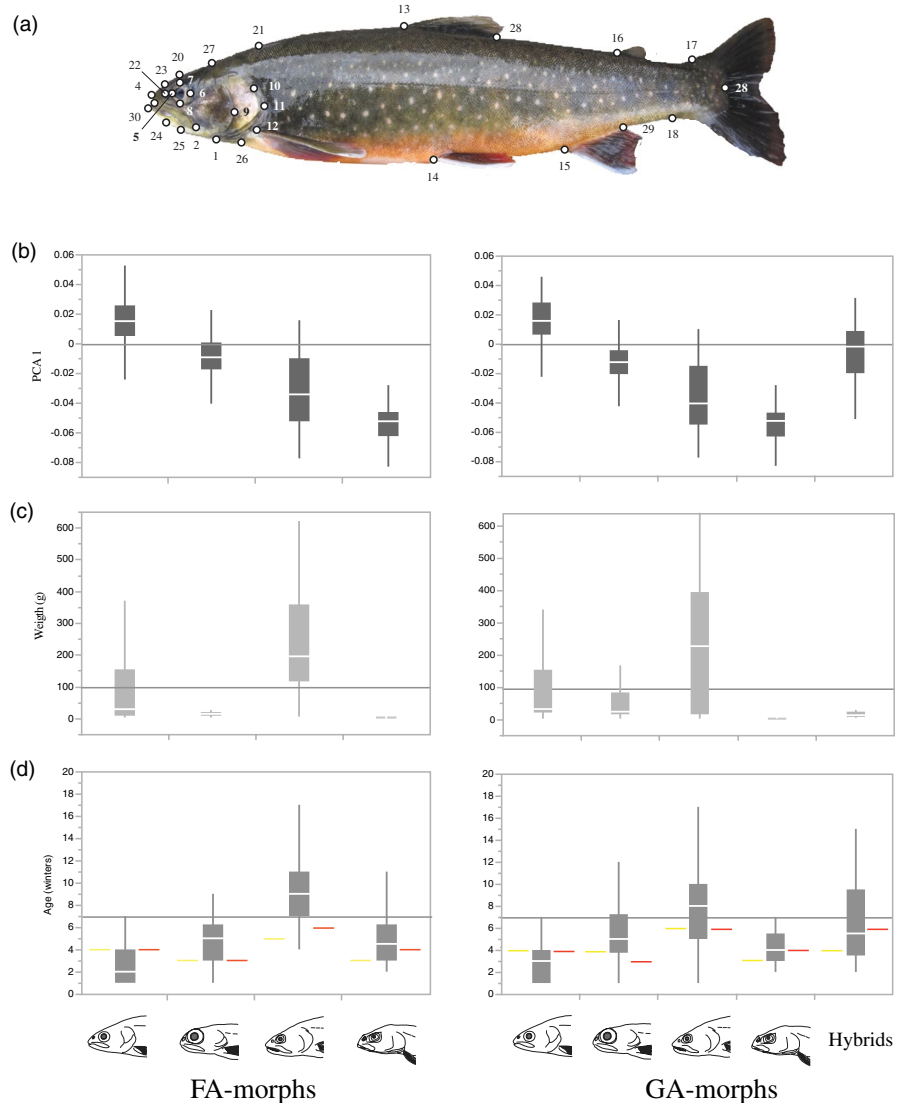
FIGURE 3 (a) The 30 landmarks used for body shape analyses in Lake Tinnsjøen: 1. lower edge of preoperculum, 2. edge of maxillary bone, 3. mouth opening, 4. tip of snout, 5.–8. eye positions, 9. mid-edge of preoperculum, 10. posterior edge of preoperculum, 11. posterior edge of operculum, 12. pectoral fin, 13. and 28. dorsal fin, 14. pelvic fin, 15. and 29. anal fin, 16. adipose fin, 17. upper tail root, 18. lower tail root, 19. end of the side line organ, 20. top of head, 21. back above pectoral fin, 22. nostril, 23. over nostril, 24, under-jaw, 25. edge of mouth, 26. lower edge of operculum, 27. transition zone from head to body, and 30. edge of lower lip. (b) Principal component axis 1 versus respectively FA-morphs (left panel) and GA-morphs (right panel) based on the 30 landmarks. (c) Weight versus FA-morphs and GA-morphs. (d) Age versus FA-morphs and GA-morphs. The youngest sexually mature male (yellow line) and female (red line) are given. The graphs denote median values (white horizontal line), the 25% to 75% percentiles (solid blocks), and the 10% to 90% percentiles (gray vertical line). In figure a–c, arbitrarily selected horizontal lines have been imposed for helping out visual comparisons among the four FA-morphs and the four GA-morphs, and in two panels compared

checked after 12 hr, and traps could be out for 48 hr. A motorized winch was used for hauling equipment. All catch was grouped in lake habitats (nominal niches) despite different types of gear used. A total effort of 42 Nordic multimesh and 225 Jensen-net nights, 1,001 trap nights, and 27 line nights were implemented in fishing. Besides Arctic charr, we caught brown trout, perch (*Perca fluviatilis*), and Eurasian minnow (*Phoxinus phoxinus*) (catch statistics not reported as being minute, <10 fish in few locations). The lake only holds the four fish species. The Eurasian minnow was introduced in Lake Tinnsjøen recently (1960–1970s).

Fish were killed using an overdose of benzocaine and transported dead on ice to the field laboratory at Lake Tinnsjøen. In the field, all the fish were subjectively assigned to four nominal morphs based on exterior morphology: (a) planktivore, (b) dwarf, (c) piscivore, and (d) abyssal (see representative individuals in Figure 1c). Each fish was classified as one of the four morphs despite variation within morphs and uncertainties. This field assignment of morphs was labeled as field-assigned morphs (hereafter FA-morphs). Length and weight were recorded, with sex and maturity stage, and age from otoliths in the laboratory. A DNA sample was taken in the field and stored on 96% EtOH for use in analyses (description below).

The four additional Norwegian outgroup populations of Arctic charr were situated to the north (River Leirfossvassdraget; anadromous sea-running), west (Lake Vatnevatnet), east (Lake Femund), and south (Lake Tyrivatn) of Lake Tinnsjøen (Figure 1a). The three latter Arctic charr populations were stationary in freshwater. The sampling equipment, effort, and placement varied among lakes comprising gillnets with at least 16.5, 19.5, 22.5, and 29.0 mm (knot to knot) and/or modified Jensen series or Nordic multimesh panels set in littoral, pelagic, and profundal areas. In the laboratory, these four outgroup populations were analyzed as described above for Lake Tinnsjøen. A DNA sample was stored in 96% EtOH for genetic analyses. These four populations were used as selected outgroups in microsatellite analyses, in mtDNA-based phylogenetic analyses, and partly in the morphological analyses. Arctic charr in Lake Tyrivatn was inferred as a putative "ancestral state" founder that could have colonized Lake Tinnsjøen, and was thus used for comparative purposes in microsatellite, mtDNA, and morphometric analyses (Figure 1a,b). This was anticipated as the lake is situated far below Lake Tinnsjøen in the same water system (see argumentation of likely colonization route in discussion). The real founding population into Lake Tinnsjøen is currently unknown.

2.3 | Eco-morphological and life-history trait divergence in the charr morphs

In Lake Tinnsjøen, the association between habitat occurrence and FA-morphs was tested using χ^2 statistic in JMP 11.2 (SAS institute Inc, 2013). See bathymetric map in Figure 2a. The main purpose here was to reveal the association between FA-morphs and habitat at catch; however, we are aware of the putative bias in having used different fishing gear in different habitats.

Geometric morphometric analysis using landmarks to reveal body shape was conducted using Lake Tinnsjøen only, and secondly Lake Tinnsjøen and Lake Tyrivatn in the river drainage to the south of Lake Tinnsjøen. In the latter analysis, the idea was to evaluate the phenotype of the putative ancestral founder that could have colonized Lake Tinnsjøen, and how the Arctic charr in Lake Tyrivatn was morphologically assigned to the FA-morphs in Lake Tinnsjøen. A Canon EOS 550d camera (Canon lens EFS 18–55 mm and macro-lens EFS 60 mm; F20 ISO1600 AV, blitz) was used to photograph (JPEG) fish. Photographs were taken in a Styrofoam box with a permanent standardized light. Fish were placed in natural position with their left side fronting the camera. All fish which had inflated swim bladders were carefully punctuated so that inflation did not affect body shape. After digitalization in TpsUtil 1.53 (Rohlf, 2004a), transforming JPEG to tps-files, landmarks were scored in TpsDig2 2.16 (Rohlf, 2004b). A set of 30 landmarks (real and semi-landmarks) were used to capture the body shape of fish, with main focus on the head region (Figure 3a). Similar landmarks have been used in other studies, but there is no consensus regarding the position or number of landmarks to be used. A transparent film with imposed lines helped setting semi-landmarks. To minimize interindividual scoring bias, all landmarks were set by one person. In MorphoJ 1.06 (Klingenberg, 2011), using the TpsDig2 file, extreme outliers were removed from both datasets after an outlier analysis, followed by a Procrustes fit analysis. A principal component analysis with eigenvalues was conducted for each dataset. As there were still body length effects on shape after PC analyses in MorphoJ (likely due to allometric growth), we corrected for body length using a regression of log centroid size on body shape (PC axes 1–5) in MorphoJ (Klingenberg, 2011) in both datasets, then saving the residuals for further analyses.

To evaluate how concordant body shape was to FA-morphs in Lake Tinnsjøen, we used a discriminant analysis in JMP 11.2 (SAS institute Inc, 2013) with linear, common covariance using residuals from the five PC axes in MorphoJ. Similarly, we tested morphological resemblance in body shape of the FA-morphs with their putative ancestral founder from Lake Tyrivatn combining shape data from Lake Tyrivatn in one analysis. Assignment percentages to the categories were recorded for both analyses.

A subset of the catch (see Section 3.2) was used for determining age from otoliths, immersed in 95% EtOH, and read using a microscope (Kristoffersen & Klemetsen, 1991). An unfortunate challenge was encountered as the Arctic charr heads had been stored in unbuffered formalin, which partly prevented age reading in some fish due to unbuffered formalin eating up parts of the otoliths. However, for the age-determined fish used, we were confident in their age. Further, it was difficult to determine maturity stage in some fish. This situation prevented a thorough life-history analysis at this stage. Thus, we present age and body weight distributions revealing the youngest sexually mature male and female (also for body weight distributions).

2.4 | Estimating the degree of reproductive isolation of field-assigned morphs

A set of 11 microsatellites were amplified and analyzed after procedures in Moccetti et al. (2019) (Appendix S1: Table S2a,b). 3%–6% negative controls per plate and 4% replicate samples were included in the analysis to control cross-contamination and consistency of genotypes. All negative samples were blank in the fragment analysis, and all replicate samples had matching genotypes. The genotypes were scored in GeneMapper 3.7 (Applied Biosystems) using automatic binning in predefined allelic bins. All genotypes were subsequently verified by visual inspection independently by two persons.

Deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD (Guo & Thompson, 1992) was estimated using GENEPOP 4.6 (Raymond & Rousset, 1995;Rousset, 2008) implementing an exact test. The presence of LD may lead to erroneous conclusions if loci do not have independent evolutionary histories. Loci exhibiting significant LD should be excluded from analyses. False discovery rate (FDR) corrections (Pike, 2011) were used to test for significant HWE and LD adjusting p-values for multiple tests. The results showed that out of 40 tests of departures from HWE, significant deviations were not found in any loci or populations after FDR correction. Significant LD was discovered between loci SCO204 and SCO218. Thus, locus SCO204 was removed, and a total of 10 loci were used in the subsequent analyses.

GENEPOP 4.6 (Raymond & Rousset, 1995;Rousset, 2008) was used to calculate the number of alleles, expected and observed heterozygosity, and genetic divergence between populations (F_{ST}) using log-likelihood-based exact tests. The software HP-RARE 1.0 (Kalinowski, 2005) was used to calculate standardized private allelic richness (A_p) and standardized allelic richness (A_r) accounting for differences in sample size. A_p and A_r were calculated with rarefaction using the minimum number of genes in the samples, that is, 28 genes.

The software MICRO-CHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) was used to check for null alleles, stutter errors, large allele dropout, and size-independent allelic dropout. Of the ten loci, MICRO-CHECKER found one locus to exhibit homozygote excess, potentially due to null alleles, being SalF56SFU. Due to the presence of null alleles, the program FREENA (Chapuis & Estoup, 2007;Chapuis et al., 2008) was run to correct for this using the ENA method (Excluding Null Alleles). The FREENA software was run with 5,000 replicates, and corrected F_{ST} values were used.

Genetic differentiation (F_{ST}) was estimated in GENEPOP 4.6 (Raymond & Rousset, 1995;Rousset, 2008) comparing Lake Tinnsjøen and the four outgroup lakes, the four FA-morphs, and the four outgroup lakes, and among revealed genetically defined morphs (termed GA-morphs, with a definition of genetic morphs being $q > 0.7$ based on STRUCTURE results; see details below) in Lake Tinnsjøen. F_{ST} values are presented with and without the ENA method.

To determine the most likely number of genetic clusters (K), the software STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) was run using 500,000 burn-in steps and 500,000 Markov chain Monte Carlo (MCMC) repetitions with 10 iterations, considered as a high enough number to reach convergence. STRUCTURE was run a first time with the individuals from Lake Tinnsjøen and the four Norwegian outgroups: Lake Femund, Lake Tyrivatn, Lake Vatnevatnet, and River Leirfossvassdraget. Secondly, a hierarchical approach was performed where the population that deviated the most from the remainder of the populations was removed, and all remaining populations were run a second time. This was repeated until no more clustering was found. The number of genetic clusters was estimated by calculating the logarithmic probability (LnP(K)) and ΔK which is based on changes in K (Evanno, Regnaut, & Goudet, 2005). The most likely number of clusters was determined using STRUCTURE HARVESTER (Earl & Vonholdt, 2012). According to recommendations by Hubisz, Falush, Stephens, and Pritchard (2009), STRUCTURE was also run with the LOCPRIOR function which incorporates geographical sampling locations using default values. Based on K-clusters results from the STRUCTURE analysis, we assigned different genetic populations or morphs in Lake Tinnsjøen (GA-morphs). Here, assignment analyses were based on K-clusters of individuals with q-values of > 0.7 to its own cluster, evaluated as belonging to this population. Individuals with q-values < 0.7 were interpreted as being hybrids of unsure population origin. We further contrasted Lake Tinnsjøen with the four outgroup lakes.

In Lake Tinnsjøen, as for FA-morphs, association between habitat occurrence and GA-morphs was tested using χ^2 statistic in JMP 11.2 (SAS institute Inc, 2013). Further, a discriminant analysis in JMP 11.2 (SAS institute Inc, 2013) was used to test for association between GA-morphs and FA-morphs to reveal how concordant these two different morph-assignment methods were.

As an alternative way to test genetic differentiation, we first conducted a principal component analysis in Genetix 4.05.2 (Belkhir, Borsa, Chikh, Raufaste, & Bonhomme, 2004) based on microsatellite alleles. Then, we tested for differentiation among the lakes for PC1 and PC2 using a nonparametric multiple comparison test (Steel–Dwass all pairs) in JMP 11.2 (SAS institute Inc, 2013). Further, we used the same approach for testing differentiation, now along PC1–3, for four FA-morphs in Lake Tinnsjøen as described above, by only subsetting Lake Tinnsjøen from the five-lake dataset.

2.5 | Phylogeography and the ancestral lineages colonizing Lake Tinnsjøen

DNA was isolated from pectoral fins using the E-Z96 Tissue DNA Kit (Omega Bio-tek) following the manufacturer's instructions. Quality and quantity of isolated DNA were assessed using a NanoDrop spectrophotometer and agarose gel electrophoresis. An 851-base pair fragment of the mitochondrial DNA (mtDNA) cytochrome B (CytB) gene was amplified using a standard primer pair, FishCytB_F (5' ACCACCGTTGTTATTCAACTACAAGAAC 3') and TrucCytB_R (5' CCGACTTCCGGATTACAAGACCG 3') (Sevilla et al., 2007) in 10 μl polymerase chain reactions (PCRs). The reactions consisted of 1 μl 10 x PCR buffer, 0.3 μl 10 μM dNTP, 0.5 μl of each of the 10 μM

F and R primers, 5.5 µl ddH$_2$O, 0.2 µl Finnzymes DyNAzyme EXT Polymerase, and 2 µl DNA template (0.4–0.8 µg). The cycling profile consisted of an initial 5-min denaturation step at 94°C, and 32 cycles of 94°C for 30 s, 57°C for 35 s, and 70°C for 1 min, followed by a final 10-min elongation step at 70°C. The products were treated with ExoZAP™ to remove leftover primers and dNTPs, before running the standard BigDye reaction, using the above primer set in 3.5 µM concentrations. The products were cleaned by precipitation, before sequencing them on an ABI 3130XL Automated Genetic Analyzer (Applied Biosystems), using 80-cm capillaries. All sequences were manually trimmed and verified in Geneious 10 (Biomatters).

For phylogeographical analyses using cytochrome B, the 851-base pair-long sequences were aligned in Mega 7.0.26 using default settings (Kumar, Stecher, & Tamura, 2016). Sequences were interpreted mostly based on both forward and reverse readings (but in a few cases, only one sequence direction was readable). A set of 115 Norwegian sequences were obtained where the sample size was 21–22 for the four Lake Tinnsjøen FA-morphs and 5–9 for the four Norwegian outgroup lakes (Table 5).

For larger scale comparison of phylogeny, highly similar sequences were retrieved using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Appendix S1: Table S2c,d). A cutoff of 200 highly similar sequences were downloaded from BLAST (including various *Salvelinus* taxa), aligned as described above and analyzed with sequences from Lake Tinnsjøen and the four Norwegian outgroup lakes.

The best substitution model for the combined dataset (115 Norwegian and 200 BLAST sequences) was interpreted using online server IQ-Tree (http://www.iqtree.org/) with 10,000 ultrafast bootstrap iterations (Nguyen, Schmidt, von Haeseler, & Minh, 2015). Here, the best substitution model revealed was TN + F + I (Tamura & Nei, 1993) (Appendix S1: Table S3).

A circular phylogenetic tree using the TN + F + I model was visualized in Treview 1.6.6 (Page, 1996) using all the 88 observed haplotypes from the joint dataset from the 115 Norwegian sequences and 200 BLAST sequences. Earlier, in another tree, we initially used three outgroup taxa to reveal the most ancient haplotypes in the charr sequences: *Salmo trutta* (GenBank accession;

LT617532.1), *Oncorhynchus kisutch* (KJ740755.1), and *Coregonus lavaretus* (AJ617501.1). This tree is not shown, but the most ancestral *Salvelinus* sp. sequence revealed from this analysis is presented in the results as the root in the tree.

A map was made (ESRI, 2017) for the joint dataset of the 88 sequences and plotted geographically with regard to a set of selected major clade configurations. Subjective clade definition and selection was done to basically visualize the large-geographical-scale patterns of sequences (although alternative clade definitions do exist).

A major large-scale phylogenetic branch including the Lake Tinnsjøen haplotypes was used for drawing a minimum spanning network in PopART (http://popart.otago.ac.nz) (Bendelt, Forster, & Röhl, 1999), when not considering frequencies of haplotypes. This major clade, which harbored 21 haplotypes, had good statistical support (89%) from the remaining haplotypes and was selected for further resolution, covering a large geographical range. The purpose with this branch selection was to have an in-depth look at the putative radiation and geographical distribution of the closest genetic relatives to the Lake Tinnsjøen morphs.

For five lakes and FA-morphs (arranged by mtDNA clades in Lake Tinnsjøen), the number of haplotypes was listed along with genetic diversity estimators in DnaSP v6.11.01 (Rozas et al., 2017). For Lake Tinnsjøen, the association of FA-morphs or GA-morphs with the three mtDNA clade frequencies was tested using χ^2 statistic in JMP 11.2 (SAS institute Inc, 2013).

3 | RESULTS

3.1 | Fish catch and field-assigned morphs

A total of 754 fish were caught in Lake Tinnsjøen, being 457 Arctic charr, 294 brown trout, and 3 perch, and a small number of European minnow (not quantified). For Arctic charr, 63 fish (13.8% of the total catch of Arctic charr) were caught in the pelagial, 105 fish (23.0%) in the littoral, 256 fish (56.0%) in the shallow-moderate profundal, and 33 fish (7.2%) in the deep profundal (Table 1). For brown trout, 101 fish were caught in the pelagial, 131 in the littoral,

TABLE 1 The Arctic charr ($N = 457$) collected in Lake Tinnsjøen in 2013 using different sampling equipment. – denotes equipment not used in that habitat (niche), while a value of 0 denotes equipment used, but no catch in that habitat. The sampling effort was not standardized precluding catch per unit effort

Lake habitat sampled	Habitat code	Depth (m) range	N fish total	Benthic nets	Floating nets	Lines	Traps
Pelagial[a]	PEL	0–20	63	–	63	0	–
Littoral[b]	LIT	0–20	105	105	–	0	0
Shallow-moderate profundal[c]	SDP	20–150	256	173	–	9	74
Deep profundal[d]	ABY	150–350	33	–	–	1	32

[a]Deposited at < 20 m depth, over depths of > 30 m, and > 50 meters from shore.

[b]From shore at < 20 m depth.

[c]From shore at > 20 m and < 150 m depth.

[d]Deposited at > 150 m depth > 100 m from shore.

TABLE 2 Number and catch percentage of the total catch (*N* = 457) partitioned into field-assigned morphs (FA-morphs) in the four lake habitats. The bottom row summarizes the number and catch percentage in the four habitats across the morphs, and the last two columns similarly summarize the catch of the morphs. The abbreviations for the four habitat codes (PEL, LIT, SDP, and ABY) are defined in the footnote of Table 1

FA-morphs	PEL *N*	%	LIT *N*	%	SDP *N*	%	ABY *N*	%	In morph	%
Planktivore	63	13.8	102	22.3	117	25.6	–	–	282	61.7
Dwarf	–	-	1	0.2	80	17.5	–	–	81	17.7
Piscivore	–	–	2	0.4	59	12.9	1	0.2	62	13.6
Abyssal	–	–	–	–	–	–	32	7.0	32	7.0
Across morphs	63	13.8	105	23.0	256	56.0	33	7.2	457	

TABLE 3 Assignment percentage based on discriminant analysis of PC axes 1–5 for body shape comparing the four FA-morphs in Lake Tinnsjøen. The diagonal values denote "correct" back assignment to original population or morph categories

Comparison	Individuals	Planktivore	Dwarf	Piscivore	Abyssal
Planktivore	266	(88.0)	10.5	1.1	0.4
Dwarf	77	9.1	(74.0)	15.6	1.3
Piscivore	55	–	29.1	(61.8)	9.1
Abyssal	26	–	–	11.5	(88.5)

and 62 in the profundal. European minnow and perch were only caught in the littoral.

In Lake Tinnsjøen, the field-assigned morphs based on visual appearance (FA-morphs, *N* = 457) revealed 282 fish (61.7%) of the planktivore morph, 81 fish (17.7%) of the dwarf morph, 62 fish (13.6%) of the piscivore morph, and 32 fish (7.0%) of the abyssal morph (Table 2).

3.2 | Eco-morphological and life-history trait divergence in the charr morphs

In the contingency analysis of FA-morphs by habitat, the association was significant (*N* = 457, *df* = 9, R^2 (U) = 0.400, likelihood ratio test; χ^2 = 387.92, *p* < .0001) (Figure 2b). The planktivore morph was caught in the pelagial (22.3% of the catch within morph), littoral (36.2%), and shallow-moderate profundal (41.5%), but not in the deep profundal (0%). The dwarf morph was primarily caught in the shallow-moderate profundal (98.8%) appearing at 20–70 m depths, and only rarely in the littoral (1.2%). The piscivore morph was primarily caught in the shallow-moderate profundal (95.2%), and rarely in the littoral (3.2%) and deep profundal (1.6%). The abyssal morph was only caught in the deep profundal habitat (100.0%).

With regard to body shape, the first five PC axes were used for analyses capturing a large part of the variation. For Lake Tinnsjøen, PC axes 1–5 explained 45%–4% of the variation in body shape, with a summed variation of 81.5% (PC1 45%, PC2 14%, PC3 13%, PC4 6%, and PC5 4%, respectively). When testing for concordance of body shape and FA-morphs (Wilks' lambda 0.20, *F* = 61.25, *df* = 15, *p* < .0001), it was a moderate-strong concordant assignment ranging from 61.8% (piscivore morph) to 88.5% (abyssal morph) (Table 3, Figure 3b).

For life-history analyses, a subset of 182 out of 457 Arctic charr were successfully used for age analyses (FA-morphs: planktivore = 85, dwarf = 34, piscivore = 37, abyssal = 26, GA-morphs; planktivore = 55, dwarf = 30, piscivore = 35 abyssal = 25, hybrids = 10). FA-morphs and GA-morphs were visually contrasted regarding weight and age distribution, suggesting large difference among morphs (Figure 3c,d). It seems that the planktivore morph has the lowest age span (1–7 years; mean of 2.9), followed by a roughly equal life span in the dwarf (1–9; 4.8) and abyssal morph (2–11; 5.0). The piscivore morph has the longest life span (4–19; 9.2). There were large differences in weight, where the piscivore morph had the largest size (min–max range of 6–1,816 g; mean of 267 g) followed by the planktivore morph (1–370; 82). The dwarf morph was smaller (2–105; 23), with the abyssal morphs being minute (1–4; 2.2). There was some variation in the youngest sexually mature males (3–6 years) and females (3–6 years) in FA- and GA-morphs. The comparison of FA-morphs and GA-morphs broadly gave the same picture with regard to age and weight patterns (Figure 3c,d).

When comparing body shape in Lake Tinnsjøen and Lake Tyrivatn, back assignment (Wilks' lambda 0.30, *F* = 31.96, *df* = 20, *p* < .0001) showed that Lake Tyrivatn had highest assignment to itself (71.8%), then planktivore morph (18.8%), and lower to dwarf (6.3%) and piscivore (3.1%), and no fish were assigned to abyssal morph (Appendix S1: Table S4).

3.3 | Estimating the degree of reproductive isolation of field-assigned morphs

The combined hierarchical STRUCTURE analysis of Lake Tinnsjøen and the four outgroup lakes first showed that there were four separate genetic clusters in Lake Tinnsjøen (Figure 4a,d, Appendix S1: Table S5, hierarchical STRUCTURE plot in Appendix S1: Figure
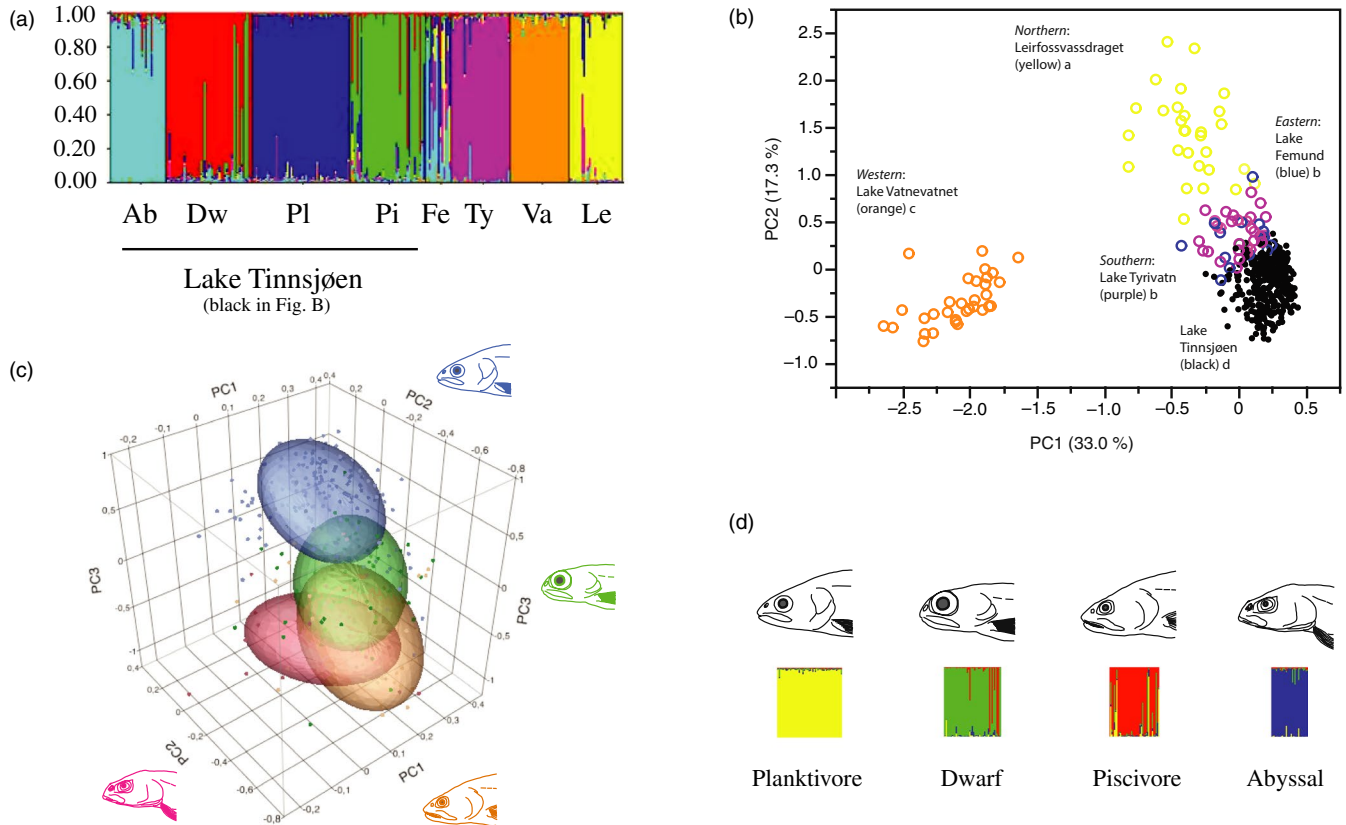
FIGURE 4 (a) STRUCTURE plot for K = 8 genetic clusters based on the 10 microsatellites for the four Lake Tinnsjøen FA-morphs and for the four Norwegian outgroup lakes. Abbreviations: Lake Tinnsjøen (Ab = abyssal morph; Dw = dwarf morph; Pl = planktivore morph; Pi = piscivore morph); Fe = Lake Femund; Ty = Lake Tyrivatn; Va = Lake Vatnevatnet; and Le = River Leirfossvassdraget River. (b) PCA plot of microsatellite alleles partitioned into the five lakes studied (different letters denote significant differences on PC1; colors match figure a). (c) Three-dimensional PCA plot of microsatellite alleles for the four FA-morphs in Lake Tinnsjøen only (a subset of the four lakes visualized in figure b). The colors in graphs represent heads of the four FA-morphs on the sides of the graph. (d) STRUCTURE plot for K = 4 based on microsatellites in the FA-morphs in Lake Tinnsjøen. Note that colors in figure c and d are different and do not correspond to the same morphs across figures

Comparison	Individuals	FA-planktivore	FA-dwarf	FA-piscivore	FA-abyssal
GA-planktivore	166	(94.6)	3.0	2.4	–
GA-dwarf	74	28.4	(55.4)	16.2	–
GA-piscivore	41	4.9	17.9	(78.0)	–
GA-abyssal	29	–	–	–	(100.0)
GA-hybrids	34	14.7	55.9	26.5	2.9

TABLE 4 Association between genetically assigned morphs (GA-morphs) based on microsatellite-based STRUCTURE analysis (q > 0.70) and the subjectively field-assigned morphs (FA-morphs). The group GA–hybrids is fish with a q-value < 0.70 and as such could not be assigned to any specific GA-morph. Values are percentages within morphs using genetic assignment in GA-morphs compared to FA-morphs. The diagonal values denote "correct" back assignment to original population or morph categories

S1). The contingency analysis of FA-morphs and GA-morphs was significant (N = 344, Df = 12, R^2 (U) = 0.563, likelihood ratio test; χ^2 = 453.75 and p < .0001) (Table 4). Association ranged from 55.4% (dwarf morph) to 100% (abyssal morph). This implies four genetic populations in Lake Tinnsjøen, concordant with the FA-morphs. In the combined analysis of Lake Tinnsjøen and outgroup lakes, using principal component on microsatellites, the variation explained

along the first two axes was: PC1 (33.0%) and PC2 (17.3%). When contrasting teh FA-morphs within Lake Tinnsjøen, it was evident that four out of the six comparisons were significantly different for PC1 (q = 2.57, alpha = 0.05), and five of six were significantly different for PC2 (Figure 4c). For PC1, the piscivore morph was not different from the abyssal morph, and the planktivore morph was not different from the dwarf. Along PC2, the dwarf morph was not

TABLE 5 The observed mtDNA haplotypes in Lake Tinnsjøen and in the four Norwegian outgroup lakes. Colors represent three clades where haplotypes group together in the phylogenetic tree (Figure 5b). Summary statistics for genetic variation in the morphs and lakes are also given

| Units Haplotype	N fish	Planktivore	Dwarf	Piscivore	Abyssal	Tinnsjøen	Leirfoss	Vatnevatnet	Femund	Tyrivatn
Clade I h1	45	1	9	5	2	17	5	8	9	6
h2	1	–	–	–	–	–	–	1	–	–
h10	1	–	–	1	–	1	–	–	–	–
h13	1	–	1	–	–	1	–	–	–	–
Clade II h5	1	–	–	–	1	1	–	–	–	–
h6	23	2	6	1	14	23	–	–	–	–
h7	1	–	1	–	–	1	–	–	–	–
h8	1	1	–	–	–	1	–	–	–	–
h9	1	–	1	–	–	1	–	–	–	–
h11	1	–	–	–	1	1	–	–	–	–
h12	1	1	–	–	–	1	–	–	–	–
Clade III h3	37	16	3	14	4	37	–	–	–	–
h4	1	1	–	–	–	1	–	–	–	–
N base pairs	851	851	851	851	851	851	851	851	851	851
N sequences	115	22	21	21	22	86	5	9	9	6
N haplotypes	13	6	6	4	5	12	1	2	1	1
Variable/ singletons	11/8	5/4	5/3	3/1	4/2	10/7	0/0	1/1	0/0	0/0
Parsim. inf. sites	3	1	2	2	2	3	0	0	0	0
Hapl. diversity	0.709	0.476	0.743	0.519	0.576	0.711	0	0.222	0	0
Nucleot. Div. (Pi)	0.00131	0.00086	0.00125	0.00116	0.00078	0.00124	0	0.00026	0	0

different from the abyssal morph, while for PC3, piscivore and abyssal morph did not differ significantly. In the contingency analysis of habitat-specific catch by the four revealed GA-morphs, the association was significant ($N = 344$, $df = 12$, R^2 (U) = 0.4283, likelihood ratio test; $\chi^2 = 302.55$ and $p < .0001$), although less than 20% of cells in the tests had expected count < 5 (suggesting x^2 to be suspect) (Appendix S1: Table S6). The same general pattern emerged as for the FA-morphs by habitat-specific catch contingency analysis, implying that the GA-morphs have different habitat use when compared among themselves.

Genetic differentiation was significant among all the four FA-morphs (also when using ENA correction) showing a range in F_{ST} of 0.119–0.199 (F_{ST} with ENA correction: 0.119–0.195) (Appendix S1: Table S7). When only considering the "genetically pure" GA-morphs

($q > 0.7$), F_{ST} ranged from 0.088 to 0.212 (F_{ST} with ENA correction: 0.087–0.212) (Appendix S1: Table S8).

The combined hierarchical STRUCTURE analysis of Lake Tinnsjøen and the four Norwegian outgroup lakes secondly revealed eight distinct genetic clusters comprising each of the four morphs in Lake Tinnsjøen and each of the four Norwegian outgroup lakes (Figure 4a,d, Appendix S1: Table S5, Appendix S1: Figure S1). The combined analysis of Lake Tinnsjøen and the four Norwegian outgroup lakes using principal components on microsatellites secondly showed that all the lakes were significantly different along PC1 and PC2 (Steel–Dwass method; $q = 2.72$, alpha = 0.05) except for Lake Tyrivatn and Lake Femund that were not significantly differentiated (Figure 4b). Here, Lake Tinnsjøen was most similar to Lake Tyrivatn and Lake Femund. The number of alleles in

FIGURE 5 (a) Distribution of 88 mtDNA-cytochrome B mtDNA haplotypes compared with major clades in different colors according to figure b. White circles denote haplotypes not well supported in figure b. (b) Circular phylogenetic tree of sequences mapped in figure a. Here, a total of 13 Norwegian sequences and 75 haplotypes retrieved from GenBank (using a cutoff of 200 highly similar BLAST sequences) are compared. Here, haplotype 31 was found to be the most ancestral when rooted with three distant salmonid taxa (*Salmo trutta*, *Oncorhynchus kisutch*, and *Coregonus lavaretus*) (tree not shown). Major supported clades have different colors. Main geographical regions are named on the outer circle. (c) A minimum spanning network of haplotypes (not frequencies) in the major light purple clade (#1) comprising Lake Tinnsjøen with geographical areas described. Haplotypes in red were found in Lake Tinnsjøen

(a)

(b)

(c)
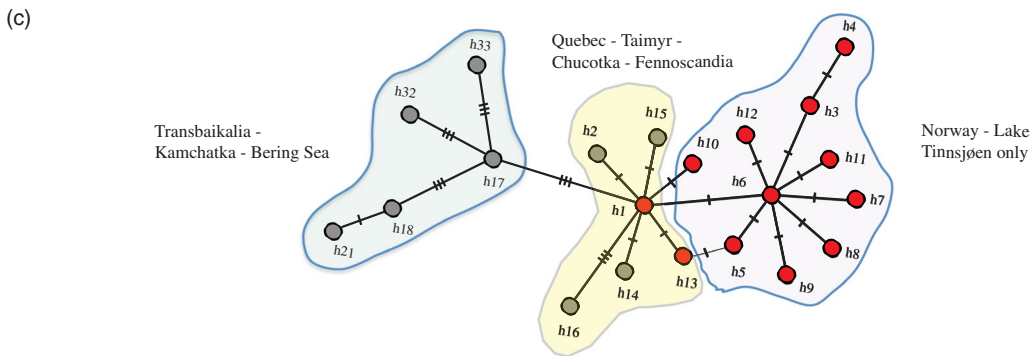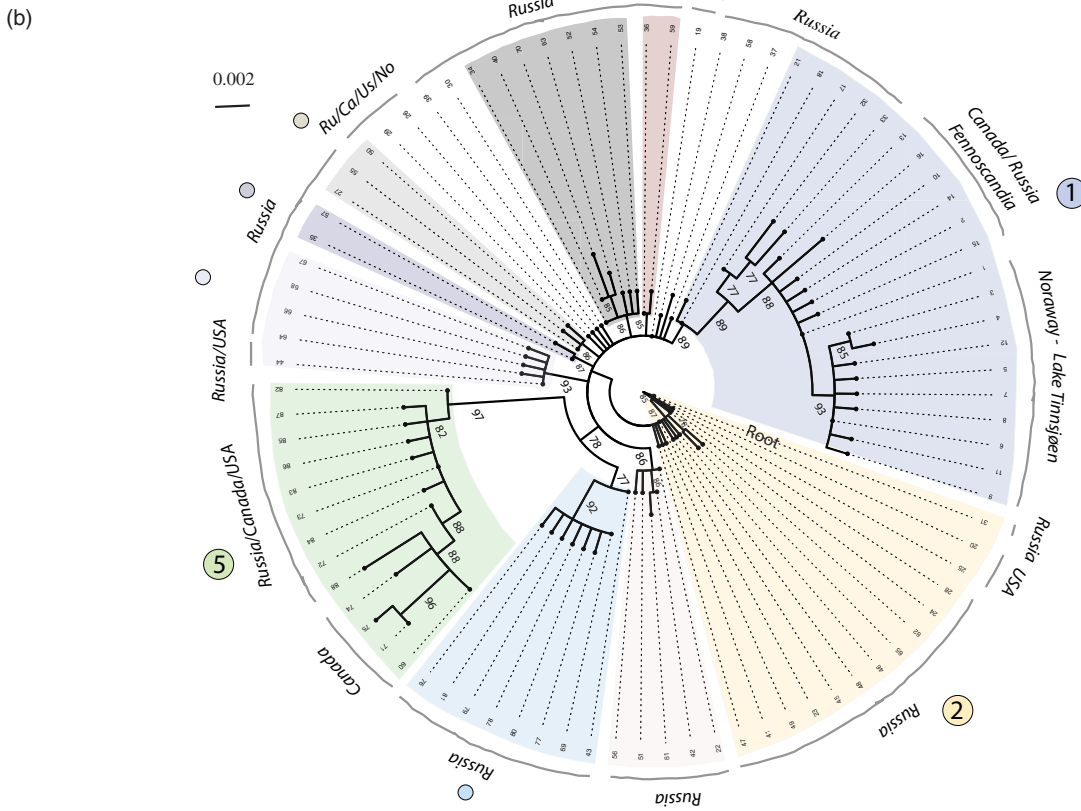
FA-morphs and outgroup lakes ranged from 76 (Lake Vatnevatnet) to 143 (planktivore morph), standardized private allele richness from 0.13 (piscivore) to 0.69 (River Leirfossvassdraget), standardized allelic richness from 6.02 (Lake Vatnevatnet) to 8.63 (planktivore morph), F_{is} from −0.012 (Lake Tyrivatn and Femund) to 0.118 (River Leirfossvassdraget), heterozygosity from 0.128 (piscivore morph) to 0.820 (Lake Tyrivatn and Femund), and gene diversity from 0.567 (Lake Vatnevatnet) to 0.761 (River Leirfossvassdraget) (Appendix S1: Table S9). Genetic differentiation among the four FA-morphs and the four outgroup lakes was all significant showing a range in F_{ST} of 0.080–0.291 (F_{ST} ENA correction: 0.085–0.286) (Appendix S1: Table S7). Here, the planktivore, piscivore, and abyssal morphs were most similar to Lake Femund, while the dwarf morph was most similar to Lake Tyrivatn. When using Lake Tinnsjøen as one group compared with the four outgroup lakes, all were significant, with F_{ST} ranging from 0.057 to 0.272 (F_{ST} with ENA correction: 0.057–0.269) (Appendix S1: Table S10). Here, Lake Tinnsjøen was most similar to Lake Femund.

3.4 | Phylogeography and the ancestral lineages colonizing lake Tinnsjøen

A set of 13 haplotypes (h1–13) were found in the combined dataset of Lake Tinnsjøen and the four Norwegian outgroup lakes (Table 5). The 13 haplotype sequences obtained in our study are deposited on GenBank (accession numbers: MT276144–MT2761569). Here, 12 of the 13 haplotypes were only found in Lake Tinnsjøen (which lacked h2). The four outgroup lakes all had haplotype h1, which also occurred in all of the four FA-morphs, while only one outgroup lake, Lake Vatnevatnet, had an additional haplotype h2.

From the samples in the larger scale phylogeography (Figure 5a,b), a total of 75 new haplotypes were retrieved from BLAST, comprising 88 haplotypes including the 13 Norwegian haplotypes (Appendix S1: Table S2c,d). Comparing these 75 haplotypes to the ones found in Norway revealed that only h1 (in five lakes) and h13 (in one lake) were found outside Lake Tinnsjøen and the four Norwegian outgroups. Lake Tinnsjøen harbored a set of 10 endemic haplotypes (h3–h12).

The major branch in Figure 5b (light purple; #1) including Lake Tinnsjøen haplotypes was used for drawing a minimum spanning network, not considering frequencies of haplotypes. This major clade with 21 haplotypes had good statistical support (89%), covering a large geographical range (Figure 5b). Within the light purple clade, a total of 6 haplotypes or subclades were supported with good statistical bootstrap values between 77% and 93%.

In Figure 5b, the phylogeny of the 13 haplotypes in Lake Tinnsjøen reveals moderate-to-high bootstrap support for clustering of three "clades": clade I (h1, h2, h10, h13) with bootstrap support of 88%, clade II (h5–h9, h11, h12) with bootstrap support of 93%, and clade III (h3, h4) with bootstrap support of 85%. Here, clade I consisted of more haplotypes (i.e., h13–18, h21, h32, h33) found outside Lake Tinnsjøen and the four Norwegian outgroup

lakes. One haplotype link, h5–h13, had unresolved cluster groupings, where it was interpreted that h5, being one mutational step away from h1, belonged to clade II rather than to clade I and that h13 belonged to clade I. The tree topology in Figure 5b and network in Figure 5c support the evaluation. When using FA-morphs in Lake Tinnsjøen as units, the number of haplotypes ranged from 4 in the piscivore morph to 6 in the dwarf and planktivore morph (Table 5).

In Lake Tinnsjøen, the percentage (Table 5) of the three clades in FA-morphs showed that the planktivore morph consisted of mostly clade III (77.3%), and less of clade II (18.2%) and clade I (5%). The dwarf had most of clade I (47.6%) and clade II (38.1%) and less of clade III (14.3%). The piscivore morph had most of clade III (66.7%) and less of clade I (28.6%) and clade II (4.8%). Finally, the abyssal morph had most of clade II (72.7%) and less of clade III (18.2%) and clade I (9.1%). The contingency analysis of FA-morphs and mtDNA clades was significant (N = 86, Df = 6, R^2 (U) = 0.2524, likelihood ratio test; χ^2 = 46.062 and p < .0001) although less than 20% of cells in the tests had expected count < 5 (suggesting x^2 to be suspect). Here, the planktivore and piscivore morphs had more of clade III, and the abyssal and dwarf morphs had more of clade II than other morphs. The association between GA-morphs and mtDNA clades was also significant (N = 79, df = 8, R^2 (U) = 0.3585, likelihood ratio test; χ^2 = 60.245 and p < .0001) although less than 20% of cells in the tests had expected count < 5 (suggesting x^2 to be suspect). The same pattern as described above for FA-morphs appeared.

The genetic diversity (Table 5) of FA-morphs ranged from a low haplotype diversity of 0.476 (planktivore morph) to a high 0.743 (dwarf morph) with the abyssal morph having a value of 0.576 in Lake Tinnsjøen, and from 0 to 0.222 (highest in Lake Vatnevatnet) in outgroup lakes. In Lake Tinnsjøen combined, the haplotype diversity was found to be 0.711. Similarly for nucleotide diversity, a low value was seen for the abyssal morph (0.00078) and a higher value for the dwarf morph (0.00128), while the four outgroup lakes varied from 0 to 0.00026 (highest in Lake Vatnevatnet). In Lake Tinnsjøen combined, the nucleotide diversity was 0.00124.

4 | DISCUSSION

We revealed four Arctic charr morphs associated with four habitats in the pelagial (<20 m), littoral (<20 m), shallow-moderate profundal (20–150 m), and deep profundal (150–350 m) in Lake Tinnsjøen. A novel finding was the abyssal morph in the deep profundal which has not yet been described before in the worldwide Arctic charr species complex. Field assignment from exterior appearance, and laboratory geometric landmark analyses, supported the distinction into four morphs. Life-history parameters also supported morph separation based on size, age, and maturity patterns. We evaluated that the four morphs were differentiated with regard to habitat use based on catch, and in their life history, suggesting association between phenotypic divergence and catch habitat. This implies adaptive niche proliferation with morphological specialization (due to phenotypic

plasticity and/or genomic hardwiring) toward different environmental conditions along the depth–temperature–productivity–pressure gradient in the lake. We found that the four field-assigned morphs were genetically divergent at microsatellite loci (F_{ST}: 0.12–0.20), indicating some form of reproductive isolation among morphs. Further, there was a close association between field-assigned morphs and unbiased genetic analyses (microsatellites) revealing four distinct genetic clusters in the lake, supporting morph differentiation. The genetic differentiation was, partly, also supported by the mtDNA analysis revealing differential clade associations of morphs. We also find it reasonable to postulate that members of one widespread Holarctic mtDNA lineage colonized Lake Tinnsjøen, likely suggesting one single common ancestor that later diversified into the observed four sympatric morphs. Further, the 10 endemic haplotypes found in Lake Tinnsjøen support a mechanism of intralacustrine diversification. Given that this adaptive radiation occurred after the lake became ice-free (<10,000 years), it represents a rapid diversification in lake niches with associated phenotypic modifications. When considering a 5-year mean generation time, it corresponds to a maximum of 2,000 generations of evolution. Thus, we found empirical support for evaluating the three main research questions addressed. However, the degree of morphological differentiation, and niche radiation, in Lake Tinnsjøen reveals an extension of specialization into the deep profundal niche. Thus, this highlights an intriguing general question in speciation research of polymorphic fish in lakes: Have we systematically underestimated the degree and rate of adaptive radiation into profundal niches?

4.1 | What are the main drivers in adaptive radiation of sympatric morphs?

Is there a repeatable pattern in niche use in sympatric morph? Imagine the colonization of a barren lake after the ice age with all lake niches available for utilization. Here, founders will likely utilize the most energetically profitable niche first, depending upon the lake-specific morphometry with regard to the highest fitness gain in the littoral or pelagial niche. Thus, the starting point for adaptive proliferation may be highly contingent on what niche(s) is actually holding the highest fitness reward among the available lake niches. This will also apply in a situation with presence of another species being a resource competitor or predator. Based on the number of sequence of morphs from monomorphic to four morph systems, it seems that there is a predictable temporal pattern in evolutionary branching associated with niche radiation. Here, the littoral (or pelagial) may be the first niche to be filled, then the pelagial (or littoral), and then the profundal, with a piscivore morph originating putatively due to growth threshold dynamics from one of the units, or evolving independently. Adding upon this complexity, moving away from an assumption of only three discrete niches in a given lake, one can imagine that there could be gradients of predictable fitness along environmental variation such as the depth–temperature–productivity–pressure gradient in Lake Tinnsjøen. Indeed, a study

on polymorphic European whitefish (*Coregonus lavaretus*) in the Swizz Alpine Lake Neuchâtel suggested adaptive diversification and buildup of reproductive isolation along ecological gradients when assessing morphs spawning at different time and place (Vonlanthen et al., 2009). Morphological diversification in the north American cisco (*Coregonus* ssp.) species complex has also been related to adaptation by depth in the Canadian Lake Nipigon (Turgeon, Estoup, & Bernatchez, 1999). Ohlberger, Brännström, and Dieckmann (2013) who used an adaptive-dynamics model, calibrated with empirical data, found support for an evolutionary diversification of the two German Lake Stechlin *Coregonus* sp. morphs likely being driven by selection for physiologically depth-related optimal temperatures. In the 1.6-km-deep Lake Baikal, Russia, one of the oldest freshwater lakes on earth, adaptive radiations have occurred in several taxa such as reflected by the depth gradient and the environmental niche radiation of the freshwater sculpins (*Cottidae*, *Abyssocottidae*, and *Comephoridae*) (Goto, Yokoyama, & Sidelva, 2014). Also, speciation along depth gradients in the ocean is strongly suggested (Ingram, 2011). A study by Chavarie et al. (2018) tested a multitrait depth gradient diversification of morphs in lake trout (*Salvelinus namaycush*) in Bear Lake in Canada, but did not find a strong association in differentiation with depth (but, partly association with genetic structure). In comparison with these studies, it seems reasonable to infer that there is a depth–temperature–productivity–pressure gradient with different fitness rewards reflecting an adaptive landscape whereupon the four Arctic charr morphs within Lake Tinnsjøen can adapt. Such a gradient may not necessarily be discrete with regard to environmental sustainable conditions, but could reflect a continuum, or a holey adaptive landscape (see Gavrilets, 2004). A recent study by Jacobs et al. (2020) revealed the complexity in inferring mechanisms behind origin of replicate Arctic charr morphs. These authors suggested that similar Arctic charr morphs could originate through parallel or nonparallel evolutionary routes as revealed in gene expression being highly similar between independently derived replicates of the same morph. They highlighted that variability in the Arctic charr with regard to predicting phenotypes was contingent on a set of factors such as demographic history, selection response, environmental variation, genomic architecture, and genetic association with specific morphs. Thus, revealing mechanisms in speciation trajectories in the Arctic charr complex is indeed a challenging task.

A novel finding in our study was the appearance of the deep profundal abyssal morph with its distinctive phenotypic features, apparently being adaptations to the cold, dark, and low-productive high-pressure environment in deeper parts of the oligotrophic Lake Tinnsjøen. Our finding of the four morphs could reflect a continuum of divergence from surface to the deep profundal environments. This implies large differences in yearly cumulative temperature sum at different depths and productivity, likely strongly affecting life-history evolution. In shallow Fennoscandian lakes, the littorals seem to have the highest biotic production, followed by the pelagial and profundal (Kahilainen, Lehtonen, & Könönen, 2003). In the 1.6-km-deep Lake Baikal, oligochaetes was found from the surface down to maximum depth, comprising

up to 70%–90% of biomass and numbers in the bottom fauna (Snimschikova & Akinshina, 1994). In the same lake, biomass of benthos decreased with depth, with an increasing proportion of oligochaetes. In comparison with the Baikal studies, we assume that the biotic prey production for Arctic charr is highest in the pelagial in the deep Lake Tinnsjøen (with small littoral areas) and lower in the benthic–littoral, and the least in the deep profundal. As such, a temperature and food production gradient likely exists in Lake Tinnsjøen from more productive pelagic and littoral areas down to the shallow profundal and deep profundal. Also, as pressure increases by one atmosphere every 10 meters of depth, it should further have marked impacts on adaptations evolved in various traits, being particularly evident in the small abyssal morph with its curved head, upturned mouth, and small eye size. Thus, both abiotic factors and ecological opportunity likely determine the potential of adaptive divergence in deepwater lakes as already implied in studies on Arctic charr in the profundal habitat (Klemetsen, 2010;Knudsen et al., 2006), and in European whitefish (*Coregonus lavaretus*) (Siwertson et al., 2010). In deep lakes such as Tinnsjøen (460 m) and Gander Lake in Canada (288 m; O´Connell, Dempson, & Power, 2005), selective forces for habitat and niche occupation could be even stronger than previously anticipated, selecting for traits that have not been seen in other morphs from other lakes. In Lake Tinnsjøen, the small eyes in the abyssal morph bear apparent similarities with eye reduction seen in cave fishes (e.g., Krishnan & Rohner, 2017). This seems somehow logical given that cave environments often can be described as nutrient-poor, cold, and harboring few co-occurring species.

It is pertinent to pose the question whether the Lake Tinnsjøen morphs have originated due to ecological speciation mechanisms. According to the ecological theory of adaptive radiation and ecological speciation (Bernatchez, 2004;Hendry, 2009;Schluter, 2000, 2009), our four morphs do seem to fit well to an ongoing diversification process according to several of the expectations from this theory (see also Hendry, Nosil, & Rieseberg, 2007;Thibert-Plante & Hendry, 2010a, b, 2011). However, the process of ecological speciation is complex and remains to be tested awaiting ecological niche studies and using higher resolution genetic markers under an evolutionary scenario framework comparing simulated and empirical data. As a crucial and fundamental basis in ecological theory, we would also here, in our newly discovered Lake Tinnsjøen system, expect a niche-specific fitness trade-off in adaptations to evolve so that no one phenotype will be optimal in all the available lake niches. Thus, the saying "*Jack of all trades, master of none, but oftentimes better than master of one*" might nicely reflect the early postglacial stages of the ongoing evolutionary dynamics in adaptive radiation of Arctic charr.

4.2 | Genetic divergence of sympatric morphs in the radiation of Arctic charr

In the Holarctic, the pattern of adaptive diversification in Arctic charr into lake niches seems to be that most lakes hold only one morph

(e.g., littoral), fewer lakes have two morphs (e.g., littoral and pelagic), and even fewer lakes have three morphs (e.g., littoral–pelagic and profundal), while only Lake Thingvallavatn, Island, so far has been reported to harbor four morphs (small and large benthic, planktivore, and piscivore). Several studies have compared Arctic charr among lakes with regard to their genetic differentiation (where there may be lakes holding more than one morph of Arctic charr) revealing a microsatellite F_{ST} range of 0.003–0.657 when contrasted in Holarctic lakes (Appendix S1: Table S11; including references). The presence of two morphs associated (or not) with genetic clusters has been found in a number of Arctic charr lakes revealing a F_{ST} range of 0.006–0.381 (Appendix S1: Table S11, including references). Few lakes harbor three morphs revealing an F_{ST} range of 0.017–0.497 (Appendix S1: Table S11; including references). A set of four morphs (small and large dark and small and large pale morphs) have been described from Gander Lake in Canada (O'Connell & Dempson, 2002;Power, O'Connell, & Dempson, 2005). Gomez-Uchida, Dunphy, and O´Connell, & Ruzzante (2008) tested the dark and pale morphs and found an F_{ST} (θ) of 0.136, suggesting two genetic clusters. Currently, it is unknown whether the four morphs in Gander Lake constitute four genetic clusters. The classic textbook example of adaptive radiation in Arctic charr comes from a continental plate rift lava lake, Lake Thingvallavatn, in Iceland. Here, a set of four morphs of Arctic charr has been described: large benthic, small benthic, planktivorous, and piscivorous morphs (Sandlund et al., 1992). Kapralova et al. (2011) studied three of these morphs (small benthic, large benthic, and planktivorous) and found F_{ST} (theta) varying between 0 and 0.07. As such, the genetic status of the four Lake Thingvallavatn morphs remains partly unresolved to date with regard to microsatellite differentiation. In our study of the Arctic charr in Lake Tinnsjøen, we estimated F_{ST} values between 0.119 and 0.199 among the four morphs, being much more differentiated than the morphs compared in Lake Thingvallavatn. However, the range in genetic differentiation among morphs in Lake Tinnsjøen lies within the range among lakes (F_{ST}: 0.003–0.657), among two-morph sympatric systems (F_{ST}: 0.006–0.381), and within the three-morph sympatric systems (F_{ST}: 0.017–0.497). Genetic divergence in mtDNA was also implied among the four sympatric morphs in Lake Tinnsjøen as the morphs were associated with different clade frequencies. With regard to mtDNA divergence of sympatric Arctic charr morphs, much fewer studies exist, mostly at regional or lake-specific scales to reveal the pattern of divergence (Alekseyev et al., 2009;Salisbury, McCracken, Keefe, Perry, & Ruzzante, 2019;Verspoor, Know, Greer, & Hammar, 2010). The Arctic charr morphs in Lake Thingvallavatn display low mtDNA differentiation (Danzmann, Ferguson, Skúlason, Snorrason, & Noakes, 1991;Escudero, 2011;Volpe & Ferguson, 1996), and not all morphs are compared, barring a full contrast of the four morphs in Lake Tinnsjøen. Thus, it appears that no direct comparison can be made to relevant studies on Arctic charr considering mtDNA results from Lake Tinnsjøen. However, using the same line of argument as in Alekseyev et al. (2009) and Gordeeva, Alekseyev, Kirillov, Vokin, and Samusenok (2018), one could imply a case of sympatric origin of the four Lake Tinnsjøen morphs as they have endemic haplotypes not

yet seen outside the lake. However, that could also reflect limited geographical coverage nearby, or far from, Lake Tinnsjøen. Thus, one should be cautious when interpreting these results.

Genetic divergence (using different markers) among sympatric Arctic charr morphs in lakes throughout the Holarctic varies widely, and we expect them to do so given their different evolutionary histories, genetic load and evolvability, biotic and abiotic environmental conditions, and ecological opportunities to radiate. Indeed, there are systems with one to four morphs in different lakes, but only few studies have addressed nuclear and mtDNA markers at the same time. In Lake Tinnsjøen, we have described four morphs that are different with regard to microsatellites and with regard to frequencies of mtDNA haplotypes. The evolutionary branching in their phylogeny and the high number of endemic haplotypes in Lake Tinnsjøen could support an intralacustrine origin of these morphs. However, the evolutionary scenarios remain to be tested in detail using a set of higher resolution markers. Although the Arctic charr species complex has been studied for a long time, researchers still need to address the important mechanisms underlying origin, presence, and temporal persistence of sympatric morphs. Thus, a multimethod-based eco-evo-devo approach with ecological, morphological, and life-history studies (Skúlason et al., 2019) and state-of-the-art genomics as performed in Lake Thingvallavatn (Gudbrandsson et al., 2018, 2019) seem to be a good avenue, as well as the methods applied in Jacobs et al. (2020) contrasting two independent replicate lineage radiations of the Arctic charr. Whether or not Lake Tinnsjøen represents a true sympatric speciation process remains to be tested using a combined set of genetic markers to contrast evolutionary scenarios.

4.3 | Origin and timing of colonization into Lake Tinnsjøen

Identifying whether an ongoing adaptive radiation has a monophyletic origin or results from parallel colonization of several morphs or secondary contact is a daunting task. To provide some initial evidence, we sequenced a mtDNA-cytochrome B fragment in the four morphs from Lake Tinnsjøen and Arctic charr from four comparative Norwegian populations to the south, west, east, and north of Lake Tinnsjøen. Additionally, we contrasted these results in a Holarctic context, to identify the likely linage(s) colonizing Lake Tinnsjøen. These analyses suggested that the founders of Lake Tinnsjøen carried the *h1* haplotype, widespread in the Holarctic (clade I), subsequently giving rise to clade II (*h5, h7, h8, h9, h11, h12*) and clade III (*h3, h4*), as novel haplotypes within the lake (see also Appendix S2: Information S1 for a phylogenetic discussion). The Norwegian outgroup lakes were all dominated by the haplotype *h1*, and only Lake Vatnevatnet had an additional haplotype *h2*, providing little information about possible routes of colonization into Lake Tinnsjøen. It is therefore relevant to address the glacial geological conditions surrounding the area of Lake Tinnsjøen for evaluating the potential of colonization direction and timing of founder

events. The maximum extension of the Eurasian Late Weichselian ice sheet occurred ca 21–23,000 years before present (ybp) (Hughes et al., 2016;Patton et al., 2017). Around 15,000 ybp, the retreating ice margin was close to the Norwegian coast, and the ice stream in the Skagerrak Sea broke up in the Norwegian channel (Longva & Thorsnes, 1997). In southern Telemark county, wherein Lake Tinnsjøen is situated, the ice sheet extended all the way to the coast ca 13,000 ybp (Bergstrøm, 1999). Around 12,000 ybp, the coast was ice-free (Longva & Thorsnes, 1997). The ice sheet retreated in a northwestern direction. An ice-recession line southeast of Lake Heddalsvatnet, situated below Lake Tinnsjøen in the same drainage (River Tinne), was dated to 9,700 ybp by Bergstrøm (1999). Further, marine sediment deposits were recorded (www.ngu.no) close to the village of Årlifoss 11 km southeast of Lake Tinnsjøen in River Tinne (see Figure 1b for the position of the upper limit of marine deposits). A sediment core study from Lake Skogstjern in the lower part of the Skiensvassdraget River by Wieckowska-Lüth, Kirleis, and Doerfler (2017) revealed a lake formation dating at ca 10,500 ybp. The outlet of Lake Tinnsjøen is situated 50 km (estimated current waterway distance) northwest of Lake Heddalsvatnet. Lake Tinnsjøen was glaciated, and we thus assume that it could not have been accessible for fish immigration prior to that period—setting a crude frame for colonization to < 9,700 ybp. We further infer that the fish colonization has proceeded from the southeast through the River Skienselva, or alternatively through any existing nonidentified proglacial lakes situated southeast of Lake Tinnsjøen. This is also logic given the elevation level of the landscape surrounding Lake Tinnsjøen, where colonization along the suggested direction is most likely as the alternative routes imply crossing mountains and elevated slopes. The estimated ice-flow directions (Figure 1b; Bergstrøm, 1999) support that the Arctic charr colonized Lake Tinnsjøen along the River Skienselva from the coastline and upward. As the Arctic charr can be anadromous and live short periods in the sea (Klemetsen, 2010), and as the Skagerrak area at certain times during deglaciation was carrying a brackish water upper layer (Gyllencreutz, Backman, Jakobsen, Kissel, & Arnold, 2006;Jiang, Björck, & Svensson, 1998), it seems reasonable to infer that the Arctic charr came from the south and colonized Lake Tinnsjøen from the coast.

4.4 | Conservation biology and management of biodiversity below the species level

Lake Tinnsjøen harbors four significantly genetically differentiated Arctic charr morphs, representing breeding populations with restricted gene flow. These morphs have likely been formed in sympatry within the lake postglacially and may stem from ongoing adaptation to available habitats and resources (niches), as previously implied in yet other Arctic charr systems (Skúlason, Snorrason, & Jónsson, 1999;Snorrason & Skúlason, 2004). If the evolution of these morphs is the result of response to past or prevailing selection pressures, that is, that phenotypic and life-history differentiation reflects specific adaptation to local conditions, then

this may have important management implications. Previously, species have often been considered as the overriding unit in conservation approaches; however, in the last century, also smaller conservation units with the purpose of preserving intraspecific diversity and evolutionary legacies have been developed (Crandall, Bininda-Emonds, Mace, & Wayne, 2000;Fraser & Bernatchez, 2001;Waples, 1991). Here, one such attempt is evolutionarily significant units (ESUs), defined by Waples (1991), who stated that ESUs are *populations that exhibit substantial reproductive isolation and constitute an important component of the evolutionary legacy of the species.* Moritz (1994) further suggested a more restrictive use of the term ESU and added the criterion that populations should exhibit *reciprocal monophyly for mtDNA haplotypes and significant genetic differentiation at nuclear loci.* Moritz (1994) also suggested the use of a second term, management units (MUs), which excluded the need for reciprocal monophyly, but with the criteria of populations exhibiting significant differentiation at nuclear loci. Crandall et al. (2000) suggested an inclusive ESU concept, as it is mainly focused on historical legacy than on preservation of functional diversity, suggesting the ESU concept should be more holistic including ecology. Fraser and Bernatchez (2001) put forward an adaptive evolutionary conservation approach, considering that a context-based framework should be applied, being more dynamic than strict single criteria or definitions. Mable (2018) discuss the importance of fitness and adaptive potential, species definitions in conservation, type and level of genetic variation, and importance of understanding adaptive processes in the wild for management approaches.

So how do the four Arctic charr morphs in Lake Tinnsjøen fit to concepts issued above? First, the four morphs in Lake Tinnsjøen are not reciprocally monophyletic from each other, but seem to share same haplotypes, although in different frequencies (and with some endemic haplotypes in each morph). The large-scale phylogeography comparison in our study implies radiation in one of the main branches (clade 1 in Figure 5) where the haplotypes (*h3–h13*) in Lake Tinnsjøen are not found elsewhere with the exception of haplotype *h1* which is seen in other populations (i.e., Norway, Finland, Sweden, Russia, Canada). The four morphs are significantly differentiated in their nuclear markers (microsatellites). As such, Lake Tinnsjøen as a whole could be evaluated as comprising one ESU according to Waples (1991), but not according to Moritz (1994). According to Moritz (1994), we would have four MUs in Lake Tinnsjøen, corresponding to the four morphs. In line with Fraser and Bernatchez (2001) and Mable (2018), we support the idea that criteria for evaluation should be more holistic and dynamic considering adaptive diversity and the need to conserve the processes that generate it. The complex Arctic charr system in Lake Tinnsjøen contains extensive genetic variation, but also extensive life-history variants and phenotypic diversity spanning from the miniscule white abyssal charr to the large piscivore charr. To preserve this degree of local adaptive variation, it is vital to maintain the genetic integrity of the local populations and thereby to conserve the evolutionary potential of the whole lake ecosystem that generated this

diversity. Overfishing and other harvest-related threats are generally not an issue in Lake Tinnsjøen as there is limited use of three of the four morphs. However, other anthropogenic effects such as pollution and in-lake fish farming could influence water chemistry and enrich the ecosystem with nutrients. Thus, it is imperative to conserve the four morphs together in an undisturbed lake ecosystem. One should prevent negative impacts from, for example, introduction of new species of deepwater-dwelling piscivore fishes that potentially could decimate the worldwide rare abyssal morph. Hence, the degree of phenotypic and life-history diversity in Lake Tinnsjøen suggests that the four morphs comprise an important evolutionary legacy of the Arctic charr species complex and offers a rare research window into an ongoing speciation process. As the goal in conservation biology should be to conserve ecological viability and evolutionary processes, capturing the adaptive landscape for evolutionary changes (Fraser & Bernatchez, 2001), the Lake Tinnsjøen ecosystem should merit international biological conservation. In biological conservation, we should not disturb ongoing processes of natural selection, and aim to protect the active units below the species level not only focusing on species conservation. We suggest that the Norwegian management authorities should merit Lake Tinnsjøen special biodiversity protection as it is one of the most divergent Arctic charr systems seen worldwide.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

K.P. and K.Ø. conceived/designed the study (equal project leaders). All authors contributed to the field sampling (with exception of A-M.P.T.). Basic genetic work in the laboratory was conducted by M.H.H, M.H., and K.P. Analyses of fish morphology were done by M.H.H. and M.H. Genetic analyses were done by K.P., M.H., M.H.H., A.-M.P.T., and K.Ø. The main body of the manuscript was written by K.Ø., K.P., and M.H.H. with significant contributions from all co-authors. All authors read and approved the final manuscript.

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Fish were sampled after initial consent from local authorities at Tinn County Administration giving us permission to fish in Lake Tinnsjøen after consent was approved also by the local landowners. The local landowners gave us the oral permission to fish on their land. No other permit or ethics approval is needed in Lake Tinnsjøen in order to sample Arctic charr when the main purpose is to use Arctic charr for scientific studies.

DATA AVAILABILITY STATEMENT

Parts of the data used in this study are available as online Appendix in the electronic version of the article. The reason why not all data have been freely distributed is the current unknown status of the abyssal morph described in our survey. As such, conservation authorities should evaluate the taxonomic status and conservation need of the morph before information on specific sampling locations and catches may be released. This is a logic precautionary conservation biological approach as the observed new abyssal morph in the deep profundal habitat (150–350 m) may have a small/vulnerable population size. Further, as this morph is not found elsewhere in the world, it merits the highest conservation status possible. Lake Tinnsjøen represents a unique window into speciation for scientists.

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SUPPORTING INFORMATION

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2

ORIGINAL RESEARCH

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A brain and a head for a different habitat: Size variation in four morphs of Arctic charr (*Salvelinus alpinus* (L.)) in a deep oligotrophic lake

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Abstract

Adaptive radiation is the diversification of species to different ecological niches and has repeatedly occurred in different salmonid fish of postglacial lakes. In Lake Tinnsjøen, one of the largest and deepest lakes in Norway, the salmonid fish, Arctic charr (*Salvelinus alpinus* (L.)), has likely radiated within 9,700 years after deglaciation into ecologically and genetically segregated Piscivore, Planktivore, Dwarf, and Abyssal morphs in the pelagial, littoral, shallow-moderate profundal, and deep-profundal habitats. We compared trait variation in the size of the head, the eye and olfactory organs, as well as the volumes of five brain regions of these four Arctic charr morphs. We hypothesised that specific habitat characteristics have promoted divergent body, head, and brain sizes related to utilized depth differing in environmental constraints (e.g., light, oxygen, pressure, temperature, and food quality). The most important ecomorphological variables differentiating morphs were eye area, habitat, and number of lamellae. The Abyssal morph living in the deepest areas of the lake had the smallest brain region volumes, head, and eye size. Comparing the olfactory bulb with the optic tectum in size, it was larger in the Abyssal morph than in the Piscivore morph. The Piscivore and Planktivore morphs that use more illuminated habitats have the largest optic tectum volume, followed by the Dwarf. The observed differences in body size and sensory capacities in terms of vision and olfaction in shallow and deepwater morphs likely relates to foraging and mating habitats in Lake Tinnsjøen. Further seasonal and experimental studies of brain volume in polymorphic species are needed to test the role of plasticity and adaptive evolution behind the observed differences.

KEYWORDS
adaptive radiation, ecological speciation, evolution, phenotypic plasticity, polymorphism, species complex

1 | INTRODUCTION

Resource polymorphism occurs in a range of different species with intraspecific morphs, originating from phenotypic plasticity, adaptive evolution, or both of these processes, thorough use of different habitat and diet as a response to ecological opportunity within available niches (Skúlason et al., 2019). Resource polymorphism is specially common in the salmonid genera of *Salvelinus* and *Coregonus*, which often show phenotypical divergence in pelagic and benthic niches in postglacial lakes (e.g., Guiguer, Reist, Power, & Babaluk, 2002; Kahilainen, Malinen, Tuomaala, & Lehtonen, 2004; Muir, Hansen, Bronte, & Krueger, 2016; Smalås, Amundsen, & Knudsen, 2013). The most common occurrence of polymorphism consists of two sympatric morphs inhabiting well-lit littoral and pelagic habitats, whereas some large and deep lakes can have more pronounced resource polymorphism with 3–8 morphs, including deepwater profundal morphs (Doenz, Krähenbühl, Walker, Seehausen, & Brodersen, 2019; Kahilainen & Østbye, 2006; Markevich, Esin, & Anisimova, 2018; Power, O'Connell, & Dempson, 2005; Skoglund, Siwertsson, Amundsen, & Knudsen, 2015). Moreover, growth rates, spawning habitat and time, age, size, and colour patterns at sexual maturity can also differ amongst sympatric morphs in these genera (Kahilainen & Østbye, 2006; Sandlund et al., 1992; Walker, Greer, & Gardner, 1988). While such morphological and life-history differences of *Salvelinus* and *Coregonus* are increasingly well documented throughout their distribution range, there are no previous studies on putative divergence in sensory capacities in terms of brain structure.

Brain morphology varies across vertebrate taxa, with the development of different structures depending on factors such as environmental conditions (e.g., oxygen and pressure), predation, habitat, diet, and social interactions (e.g., Crispo & Chapman, 2010; Day, Westcott, & Olster, 2005; Edmunds, Laberge, & McCann, 2016; Harvey, Clutton-Brock, & Mace, 1980; Yopak, Lisney, Collin, & Montgomery, 2007). Occupying different environments requires different traits, which can varies with depth (Caves, Sutton, & Johnsen, 2017). For instance, adaptations to a deepwater habitat in freshwater and marine systems can involve changes in morphology (e.g., eye size), lowered rates of metabolism, variation in the oxygen transport system, and fatty acid composition (e.g., Evans, Præbel, Peruzzi, Amundsen, & Bernatchez, 2014; Kahilainen & Østbye, 2006; Radnaeva et al., 2017; Seibel & Drazen, 2007). Brain morphology can also be affected by depth, turbidity, and feeding type, such as the development of a larger optic tectum and larger eyes in fish feeding on active prey in well-illuminated habitats and low turbidity (Huber, van Staaden, Kaufman, & Liem, 1997). Natural selection may act on the brain, targeting morphology, and adaptive function of different regions under divergent selection, also being active below the species level such as morphs using different niches (Gonda, Herczeg, & Merilä, 2013; Merilä & Crnokrak, 2001).

Regarding metabolism, energetic costs can constrain the development of the brain size as it is one of the most energetically expensive organs (Kotrschal et al., 2013; Laughlin, van Steveninck, & Anderson, 1998). The increase in size and complexity of the brain can be a trade-off between selection for cognitive benefits and the cost of production and maintenance of the brain (Gonda et al., 2013; Kotrschal et al., 2014). The brain, as the controller of behaviour and eco-physiological functions, can be under developmental canalization (i.e., the ability of a genotype to produce one or a few targeted phenotypes in different environments, presenting a lack of plasticity) or under phenotypic plasticity (Ghalambor, McKay, Carroll, & Reznick, 2007; Gottlieb, 1991). Since phenotypic plasticity may be either adaptive, or nonadaptive, not all plasticity will necessarily provide a fitness advantage (Ghalambor et al., 2007).

Brain structure of fish is similar to other vertebrates (Kotrschal, Van Staaden, & Huber, 1998). In fish, olfactory organs are composed by lamellae and are attached to the olfactory nerves. These nerves are connected to the olfactory bulb, which processes information about odours, and it is thus involved in social communication, feeding and mating behaviour, and predator recognition (Chivers & Smith, 1993; Dulka, 1993; Hara, Sveinsson, Evans, & Klaprat, 1993; Landry, Garant, Duchesne, & Bernatchez, 2001; Milinski et al., 2005). An enlargement of the olfactory bulb can be found in fish that live in environments with high predation risk (Gonda, Valimaki, Herczeg, & Merila, 2012). The telencephalon and hypothalamus are related to more complex activities such as learning, memory and social tasks (Demski, 1983; Kotrschal et al., 1998). For instance, fishes living in structured environments show a larger telencephalon (Huber et al., 1997). The hypothalamus is also involved in regulating reproductive and feeding behaviour (Kulczykowska & Vázquez, 2010; White & Fernald, 1993). Gonda et al., (2012) found a reduction of the hypothalamus in the presence of predation in nine-spined sticklebacks that were less aggressive and took less risks to feed than in absence of predators (Herczeg & Välimäki, 2011).

Eyes and the optic tectum are involved in vision, and both of these structures are used as an indicator of visual capabilities and importance (Huber et al., 1997; Lisney, Bennett, & Collin, 2007). The cerebellum is in charge of several tasks such as motor coordination, proprioception (i.e., movement and balance), and eye movement (Demski, 1983). In addition, habitat complexity can also influence the brain regions, increasing the cerebellum and telencephalon size, and decreasing the olfactory bulb (Pollen et al., 2007). Social environment seems to affect the brain as well, increasing the optic tectum size and decreasing the olfactory bulb when fish live in groups (Gonda, Herczeg, & Merilä, 2009). Many of the above brain volume

FIGURE 1 (a) Study area of Lake Tinnsjøen, Norway. Exact sampling positions are not reported until more information is available about the population status of the new Abyssal morph. 1:125,000 using ArcGIS (ESRI 2015). (b) Sexually mature fish from each of the four Arctic charr morphs in Lake Tinnsjøen. From top to bottom: Piscivore (greyish colour and inflated swim bladder; Body length: 217.38 ± 92.96, mean ± *SD*), Planktivore (male in spawning dress with orange belly; 177.27 ± 63.35), Dwarf (brown with inflated swim bladder; 113.82 ± 21.50), and Abyssal (sunken eyes; 78.58 ± 10.66). (Photo © K. Østbye)

(a)

(b)
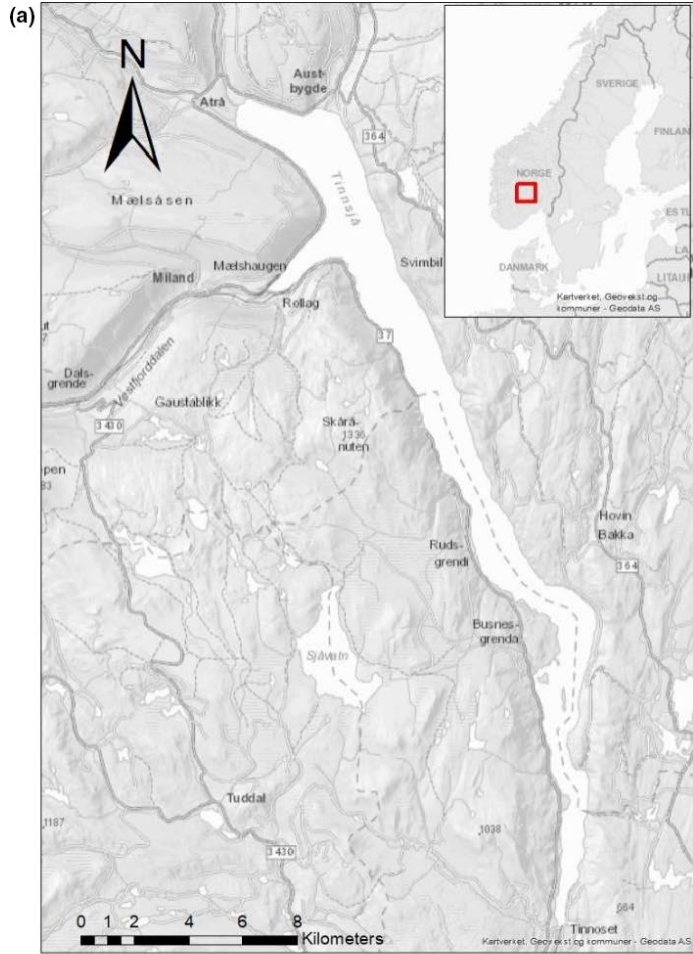
studies have been conducted with shallow water species in lakes with well-illuminated habitats lacking strong vertical gradients of light, temperature, pressure, and prey availability. Such conditions prevail in many deep and oligotrophic lakes inhabited by polymorphic fish, but we do not know the potential effects of such depth gradients and habitat selection on corresponding brain morphology.

In the deep oligotrophic Lake Tinnsjøen, in southern Norway, four Arctic charr morphs coexist along steep depth gradients (Figure 1). This lake contains two profundal morphs, the Dwarf and Piscivore morphs, one Planktivore, a habitat generalist morph, and one deep-profundal benthivore morph, the Abyssal morph (Østbye et al., 2020). All these morphs presented differences in body size and coloration (Østbye et al., 2020). The Piscivore is the largest morph having a large, robust head and elongated black/grey body, showing a piscivorous behaviour, feeding on other fish, while the Dwarf is a small-bodied morph with a pale brown coloration often with parr marks, feeding on macrobenthos and zooplankton. The Planktivore is a moderately sized morph with a darkish coloration on the upper part of the body with silvery sides, and feeds on zooplankton. Finally, the minute Abyssal morph is a tiny fish with a pale bluish-whitish body colour, light purple coloration on parts of its head, and it feeds on the soft-profundal-bottom benthic invertebrates. These striking phenotypic differences coupled with largely contrasting environmental conditions in their habitats, strongly imply putative sensory divergence in different lake habitats.

In this study, we tested how different habitat use along a depth gradient may correspond to head morphology and brain volume in the four Arctic charr morphs in Lake Tinnsjøen. First, we aimed to detect clustering of four morphs based on combined differences in ecomorphology, population genetics, life-history traits, and brain volume, using recursive partitioning methods. Secondly, we aimed to compare brain variation and sensory traits among the four morphs. We hypothesised that the morphs (i.e., Planktivore, Piscivore, and Dwarf) living in habitats with more light radiation will have a larger optic tectum than the Abyssal morph. We hypothesised that the Abyssal morph will have developed a better smell perception due to lack of light in the deep-profundal habitat (Yopak et al., 2019), showing abundant lamellae, larger surface of the olfactory rosette, and a developed olfactory bulb. Finally, we hypothesised that the Abyssal morph will have the smallest brain regions due to prey resource limitation in their habitat.

2 | METHODS

2.1 | Study area

Lake Tinnsjøen (60°38′15.6″N, 11°07′15.2″E, elevation 191 m.a.s.l.) in Telemark county, southern Norway (Figure 1) is one of the largest lakes in Norway (51.4 km^2), and one of the deepest in Europe (max. depth 460 m). It is an oligotrophic lake harbouring Arctic charr, brown trout (*Salmo trutta*), a small population of perch (*Perca fluviatilis*) and the recently introduced minnow (*Phoxinus phoxinus*).

According to Boehrer, Golmen, Løvik, Rahn, and Klaveness (2013), oxygen concentration in June 2006 ranged from 11.5 to 12.0 mg/L, dissolved oxygen from 90% to 85% at 0–460 m depth, and temperature ranged from 4.0 to 3.3°C at 50–460 m. A river connected the lake to the sea during the most recent postglacial period (9,700 years to present; Bergstrøm, 1999), which suggests that the fish fauna colonized the lake naturally after deglaciation via this river.

2.2 | Fish collection

We sampled fish using gillnets, traps and baited anchored longlines in August–October 2013 (Østbye et al., 2020). We sampled in four habitats: (i) the pelagial (setting gillnets positioned more than 50 m from shore and 20–30 m depth in midwater using a 12-panel multimesh Nordic series with mesh sizes in this order of 43, 19.5, 10.0, 55.0, 12.5, 24, 15.5, 35.0, 29.0, 6.3, 5.0 and 10.0 mm and Jensen floating series with mesh size of 13.5, 16.5, 19.5, 22.5, 26.0, 29.0, 35.0, 39.0, 45.0 and 52.0 mm), (ii) the littoral (gillnets within 20 m from the shore using Nordic and Jensen littoral net series), (iii) the shallow-moderate profundal (Jensen littoral net series, traps, and hook-line between 20 and 150 m depth), and (iv) the deep profundal (setting traps >150 m depth and >100 m from the shoreline using longlines of 220 m long and 3 to 4 mm line with 180 hooks; see more detailed information in Østbye et al., 2020). In the field, we assigned each individual to one of the four morphs (called field-assigned morphs: FA morphs) based on differences in body and head appearance and coloration. We also measured body length and determined the sex and maturation stage visually (i.e., mature if the gonads covered more than half of the body cavity length; immature otherwise). We euthanized the fish with an overdose of benzocaine, and we preserved the heads in formalin (10% unbuffered).

2.3 | Genetic analyses

We had 72 individuals with both genetic and morphological data for each individual (field assigned morphs: Planktivore (*n* = 25), Piscivore (*n* = 13), Dwarf (*n* = 22), and Abyssal (*n* = 12); Figure 1). In the deep-profundal habitat, only the Abyssal morph was caught. The Dwarf and Piscivore morphs were caught in the shallow-moderate profundal habitat. The Planktivore morph was caught in the pelagial (*n* = 8), littoral (*n* = 6), and shallow-moderate profundal habitat (*n* = 11). We used 10 microsatellite markers to classify the fish into genetic clusters (*K*; see Østbye et al., 2020). Herein, we used allele frequencies to identify the genetic clusters of Arctic charr (genetic assigned morphs, GA-morphs) with the software STRUCTURE (Pritchard, Stephens, & Donnelly, 2000). We included a predictor variable to test whether pure and hybrid individuals differed based on *q*-value (i.e., using admixture proportions of individuals; Bhat et al., 2014), considering a threshold value of *q* > 0.7 for genetically pure individuals (i.e., belonging to a unique cluster), and *q* < 0.7 for hybrids (Anderson & Thompson, 2002;

Harrison, 1993). In Lake Tinnsjøen, 48 fish were genetically pure (genetic cluster 1 was the Planktivore morph ($n = 12$), genetic cluster 2 was the Piscivore morph ($n = 15$), genetic cluster 3 was the Dwarf morph ($n = 13$), and genetic cluster 4 was the Abyssal morph ($n = 8$)) and 24 hybrids were also identified (Østbye et al., 2020). All the analyses below are based on the genetic classification (GA-morphs).

2.4 | Head morphometrics

We photographed the left side of each fish using a digital camera (Canon EOS 350D), and we preprocessed the photographs with tpsUtil v.1.26 (Rohlf, 2004). We digitized a set of 30 common anatomical landmarks in tpsDIG2 v.2.22 (Rohlf, 2015) to capture head variation (Figure 2a), which we included for landmark-based geometric morphometrics and statistical analyses. In addition, we measured the width (W) and height (H) of the eye in tpsDIG2 to calculate the eye area.

2.5 | Age determination

We determined the age based on otoliths, which are more reliable than scales especially in Arctic charr (Christensen, 1964). We opened the skull dorsally under a microscope and removed the olfactory rosette and the brain from the olfactory bulb to the spinal cord to collect the otoliths. We used a microscope to count the rings of the dorsal part of otoliths for determining age. We then burned one otolith with a gas flame for ca. 5 s and broke it in half to count the rings from the lateral side under a microscope, as a further confirmation of age.

2.6 | Neuroanatomy

Following Pollen et al. (2007), we measured five brain regions (Figure 2b–d): olfactory bulb, telencephalon, optic tectum, cerebellum, and hypothalamus. We measured the width (W) of each brain structure from the dorsal and ventral image of the brain, as well as the length (L) and height (H) from lateral views of the left hemisphere (Figure 2b–d). We used an ellipsoid model to estimate the volume ($V = 1/6 \ \pi(LWH)$) of each brain region (Huber et al., 1997).

2.7 | Olfactory rosettes

We dissected the olfactory rosettes and the nasal organ and stored them in 70% ethanol (Figure 2e). We measured the width (W) and length (L) using a micrometer under the microscope in order to calculate the surface area of each olfactory rosette ($A = 1/4\pi(WL)$). We also counted the number of olfactory lamellae in each rosette.

2.8 | Statistical analysis

2.8.1 | Quantification of diversity in the morphs

We conducted a principal component analyses (PCA) to evaluate the variation in head shape among morphs. We standardized for size with a Generalized Procrustes Analysis (Adams, Rohlf, & Slice, 2004; Zelditch, Swiderski, Sheets, & Fink, 2004). We then conducted a PCA using the package geomorph (Adams & Otárola-Castillo, 2013). We calculated the centroid size (i.e., as a measure of size) for each individual to use in further analyses (i.e., analysis of variance (ANOVA), post hoc Tukey's HSD and random forest).

To account for allometric relationships, we used log-log regression approach for each morphological measurement, using body length as predictor. We performed ANOVAs and post hoc Tukey's HSD for all the variables to know whether there were differences among the morphs. We used the residuals from the regressions for the ANOVAs, post hoc Tukey's HSD, and random forest analyses to account for size.

2.9 | Morph prediction using random forest

Recursive partitioning methods are used in fields such as genetics, psychology, medicine, and epidemiology (Qi, Bar-Joseph, & Klein-Seetharaman, 2006; Segal, Barbour, & Grant, 2004; Shen, Ong, Li, Hui, & Wilder-Smith, 2007; Ward, Pajevic, Dreyfuss, & Malley, 2006), but less in ecology (Cui et al., 2019; Cutler et al., 2007; Desantis, 2019; Kargar, Akhzari, & Saadatfar, 2019). To differentiate between morphs, including all the variables measured above, we used a random forest approach with 10-fold cross-validation and 5 repeats per fold. To predict the four GA-morphs, we opted for a random forest approach rather than a regression because there was a higher number of parameters than observations (Strobl, Malley, & Tutz, 2009). We thus discuss the variable importance rather than parameters estimates below (Rossi, Amaddeo, Sandri, & Tansella, 2005). Compared to principal components or discriminant analyses, random forests are more flexible (Kuhn & Johnson, 2013; Zhang & Aires-de-Sousa, 2007; Zumel & Mount, 2014), are robust to overfitting, have internal cross-validation, and often outperforms more classical approaches in terms of prediction accuracy (Johnston, Johnston, Kennedy, & Florence, 2008; Kuhn & Johnson, 2013; Palmer, O'Boyle, Glen, & Mitchell, 2007; Svetnik et al., 2003; Zhang & Aires-de-Sousa, 2007).

Random forest is an ensemble method, which builds many decision trees to obtain more accurate classifications (Cutler et al., 2007; Strobl et al., 2009). We generated 5,000 trees, with 3 variables considered for each split (Bischl et al., 2016), which was calculated as the square root of the number of predictors. To train the random forest model, we included the variables habitat (littoral, pelagial, shallow-moderate profundal and deep-profundal), genetic trait (pure/hybrid), sex (male/female), maturation (mature/immature), number of olfactory lamellae, area olfactory rosette, eye area, all volumes of the different brain regions (olfactory bulb, telencephalon, optic tectum, cerebellum, hypothalamus), head size (i.e., as the centroid size for each individual calculated as the average of x and y coordinates of all landmarks), and the
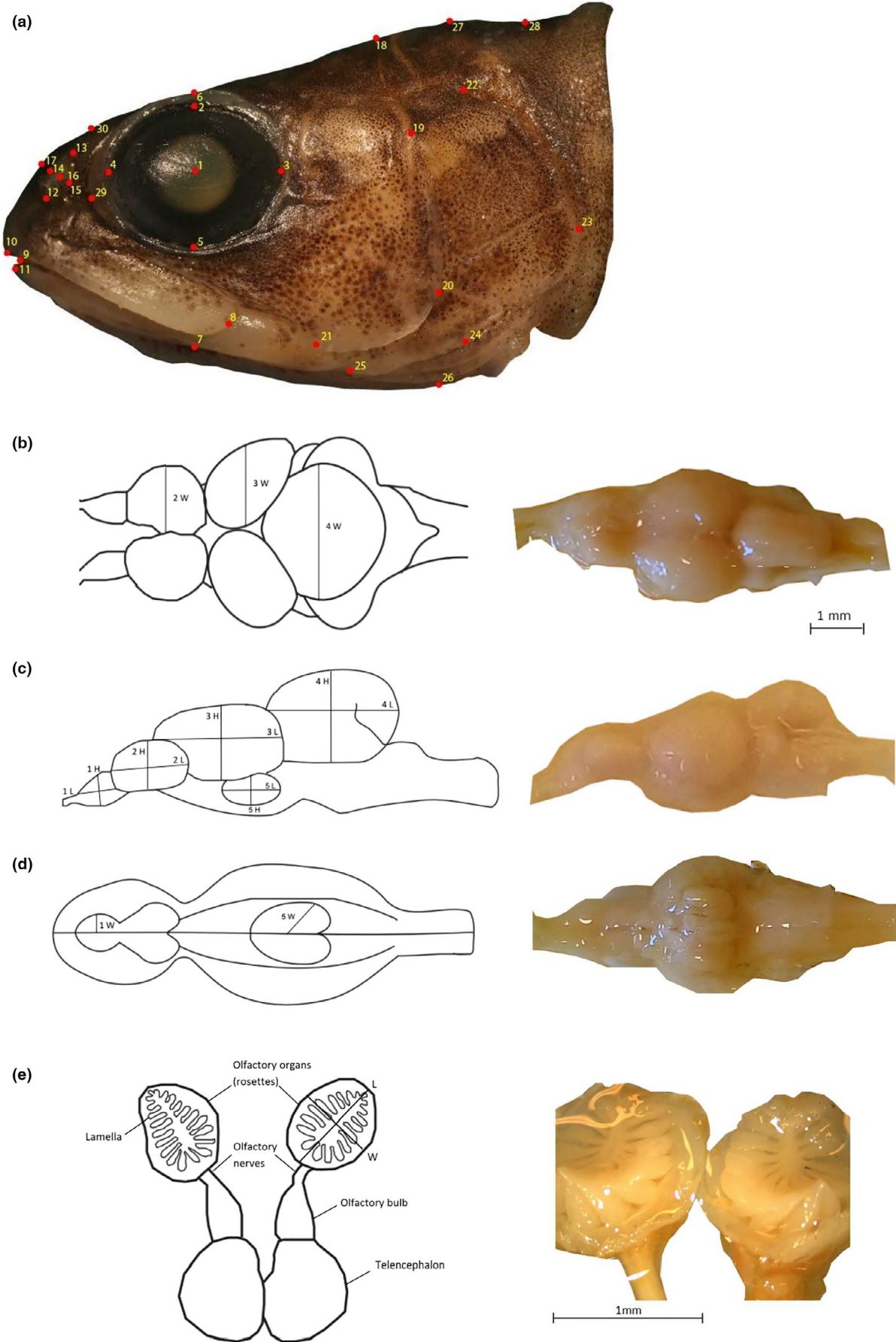
(a)

(b)

(c)

(d)

(e)

FIGURE 2 (a) Anatomical landmarks (30 points) used to measure the head shape of Arctic charr. Photo from male Dwarf morph, which was a genetically pure individual ($q = 0.85$). Landmarks used for head shape analysis: 1. Central point of the eye, 2. Dorsal extreme of bony orbit of the eye, 3. Posterior extreme of bony orbit of the eye, 4. Anterior extreme of bony orbit of the eye, 5. Ventral extreme of bony orbit of the eye, 6–7. Perpendicular line following landmarks 1, 2 and 5, 8. Posterior point of the upper jaw, 9. Central point of the closed mouth, 10. Anterior point of the upper jaw, 11. Anterior point of the lower jaw, 12. Ventral extreme of nostril, 13. Dorsal extreme of nostril, 14. Anterior point of nostril, 15. Posterior point of nostril, 16. Central point of nostril, 17. Perpendicular line following landmarks 14, 15 and 16, 18. Starting point of the line of preoperculum, 19. Upper point of the preoperculum, 20. Point of maximum curvature of the preoperculum, 21 Lower point of preoperculum, 22. Upper point of the operculum, 23. Posterior point of the bony operculum, 24. Point of curvature of the operculum, 25. Lower point of operculum, 26. Perpendicular line following landmark 20 to the bottom of the fish, 27. Middle point between landmarks 18 and 28, 28. Starting point of the line of operculum, 29. Socket of the eye, 30. Perpendicular line from landmark 29. (b) Dorsal, (c) lateral and (d) ventral view of the brain, illustrating the five brain regions studied (1: olfactory bulb, 2: telencephalon, 3: optic tectum, 4: cerebellum, 5: hypothalamus). For each brain region, the length (L), height (H), and width (W) were measured. (e) Olfactory lamellae of Arctic charr and illustration of olfactory organs with lamellae and olfactory nerves attached with the olfactory bulb, which is connected with the telencephalon
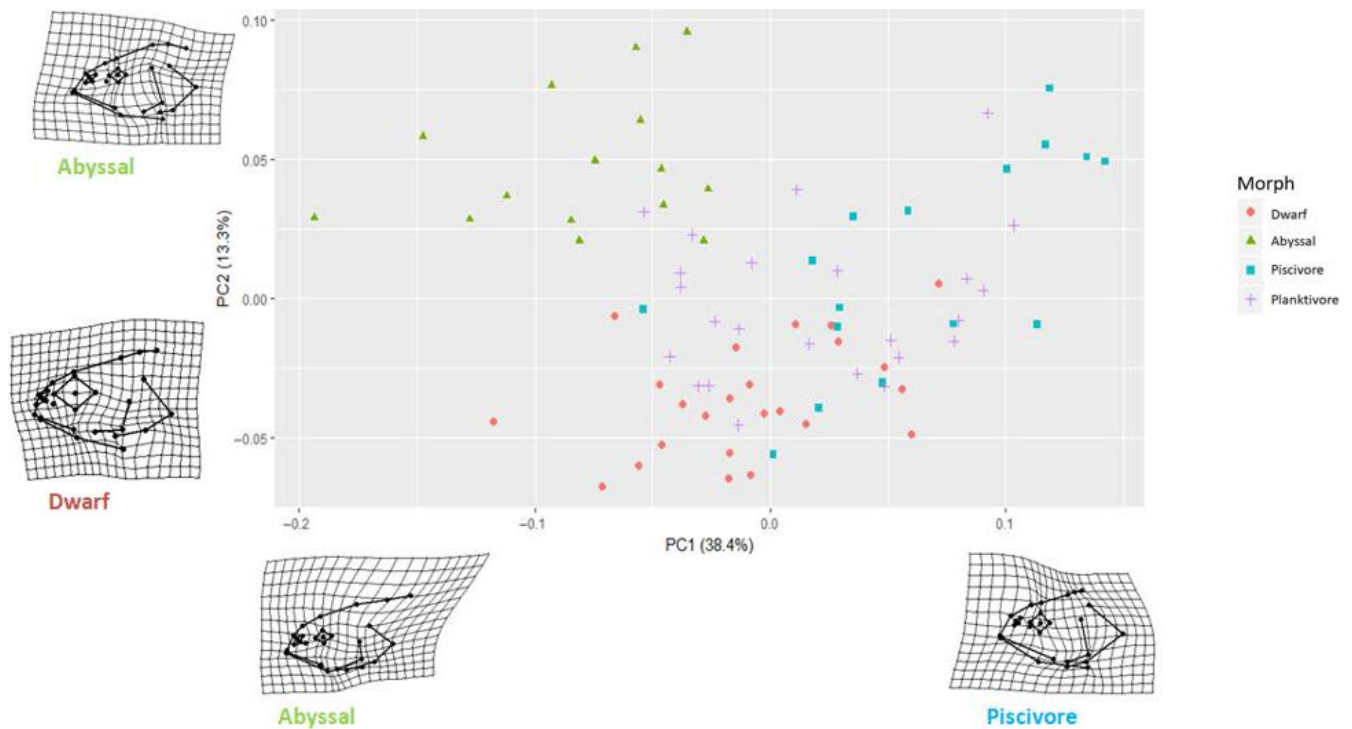

FIGURE 3 Principal component analysis of head shape illustrating extremes of head shape morphology in Arctic charr (red: Dwarf, green: Abyssal, blue: Piscivore, purple: Planktivore). The first two principal components are shown for the four morphs. Wireframe images illustrate head shape differences along the two first PC axes

age of fish. We then identified the most important variables to predict the four morphs. Note that we used the residuals of the variables obtained from the log-log regressions to correct for size. We estimated the accuracy of the random forest. We assessed the relative contribution of variables to the classification with variable importance, ranking predictors by the mean minimal depth (Ishwaran, Kogalur, Chen, & Minn, 2011; Paluszynska, Biecek, & Jiang, 2019). We used accumulated local effects (ALE) plots to visualise the variables influence in the prediction of the model (Friedman, 2001). When the ALE values are positive, there is a higher probability to belong to a specific class. We only report the ALE plots for the most important variables. We used R packages ranger (Wright, Wager, & Probst, 2019) for the analysis, iml (Molnar, 2018) for the ALE plots, and randomForestExplainer

(Paluszynska et al., 2019) for the variable importance plot. All statistical analyses were performed in R version 3.6.1 (R Core Team, 2019).

3 | RESULTS

3.1 | Quantification of diversity in the morphs—head shape

To quantify differences in head shape among morphs, we retained the first six principal component axes (PC) explaining 78.1% of the variation in head shape across morphs. The first two PC separated three of the four morphs by head morphology, except the Planktivore
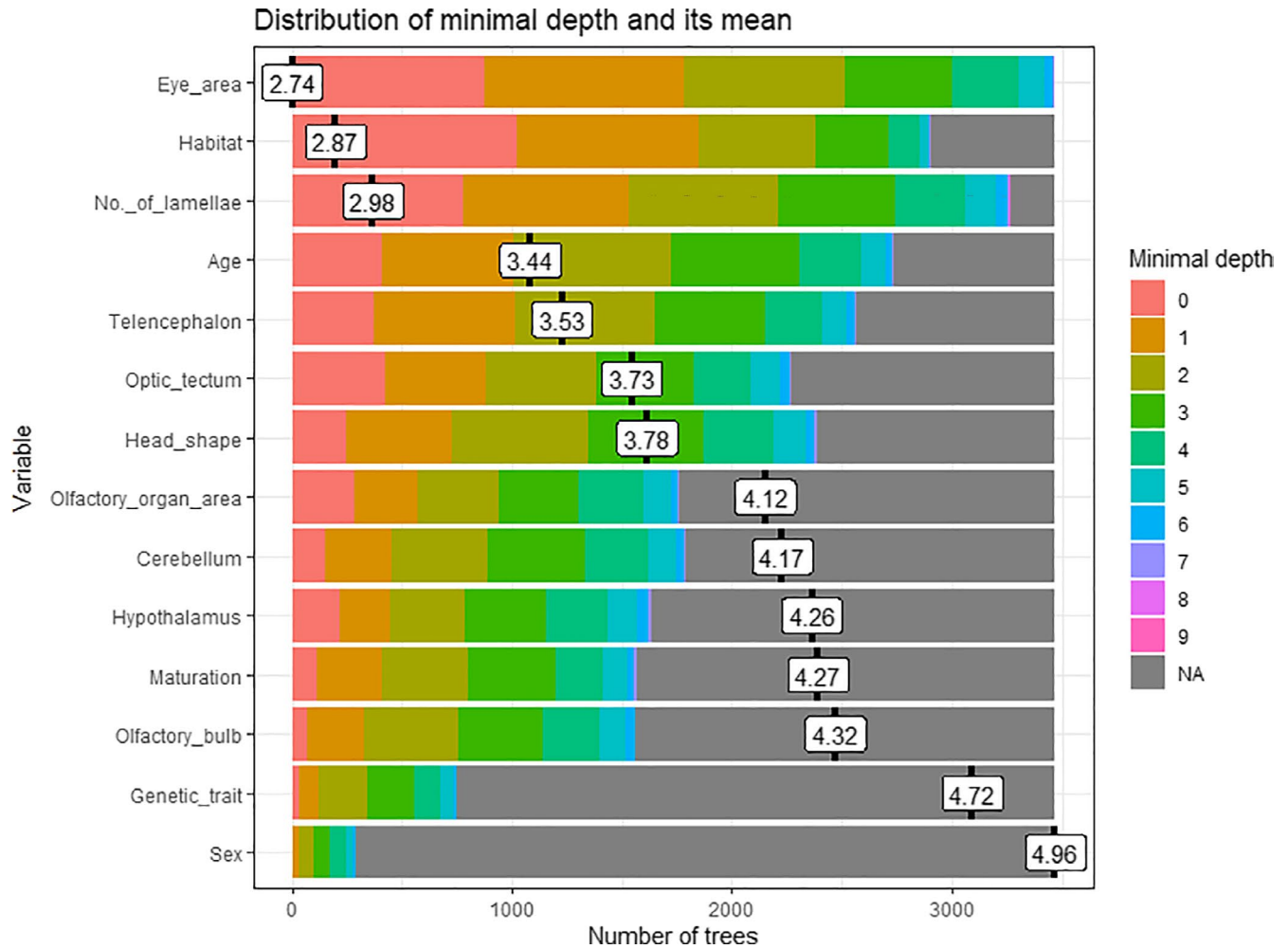
Distribution of minimal depth and its mean

FIGURE 4 Variable importance based on minimum depth from the random forest analysis, which represents the consensus across trees (i.e., the higher the variables and the lower the depth on this figure, the more frequently and early the variable was selected to make the split, i.e., the more important the variable is). Results from the random forest analysis for the response variable morph. Note that we used the residuals of the measured variables obtained from the log-log regressions to correct for size. Number of trees grown were set to 5,000. The importance of the variables is measured with the minimal depth (indicated with different colours inside the horizontal bar for each variable) and its mean (indicated in the white box). Minimal depth is the average distance between the root of a tree and the node/split where a given variable was used. Smaller values of the minimal depth indicate early contribution of the variable, that is, more discriminating power. NAs represent all variables not picked for a given split

morph, which overlapped with the Piscivore and Dwarf morphs (Figure 3). The first PC (38.4% of total variance) revealed two different head shapes corresponding to Abyssal and Piscivore morphs. The Piscivore had a larger head depth and a larger eye than the Abyssal. The second PC (13.3% variance) separated Abyssal from Dwarf, where the Abyssal morph had the smallest eyes and the Dwarf morph had relatively larger eye size than the other three morphs.

3.2 | Morph prediction using random forest

For the morph classification, the prediction accuracy was 80%. The most important variables to predict the morph class were eye area, habitat, and number of lamellae (Figure 4). These variables were selected for early in the trees, which indicates that they have a great role in partitioning the data.

FIGURE 5 (a) Accumulated local effect (ALE) plots for habitat. Bars indicate the contribution of a given predictor, relative to the overall prediction of the model (at ALE of y = 0). Here, positive values of bars indicate higher prediction for a specific morph and negative values indicate lower effect on predicting morph (i.e., lower probability to be a determinate morph in that specific habitat). For instance, the probability of being Abyssal morph is higher in the deep-profundal habitat. (b) Accumulated local effect plots for number of lamellae and eye area residuals,. ALE plots show the marginal effect of a variable on the predictions from the model. For instance, deep-profundal habitat (a) and an eye area residuals smaller than −0.8 (b) have a high contribution on predicting the Abyssal morph. Lines indicate the contribution of these predictors, relative to the overall model prediction. The maximum values of line indicate highest prediction of given morph, for example, prediction of Dwarf morph is highest with eye area residuals larger than 0.5 and number of lamellae ranging from 9 to 11, whereas for Abyssal morph are <−0.8 and <6, respectively
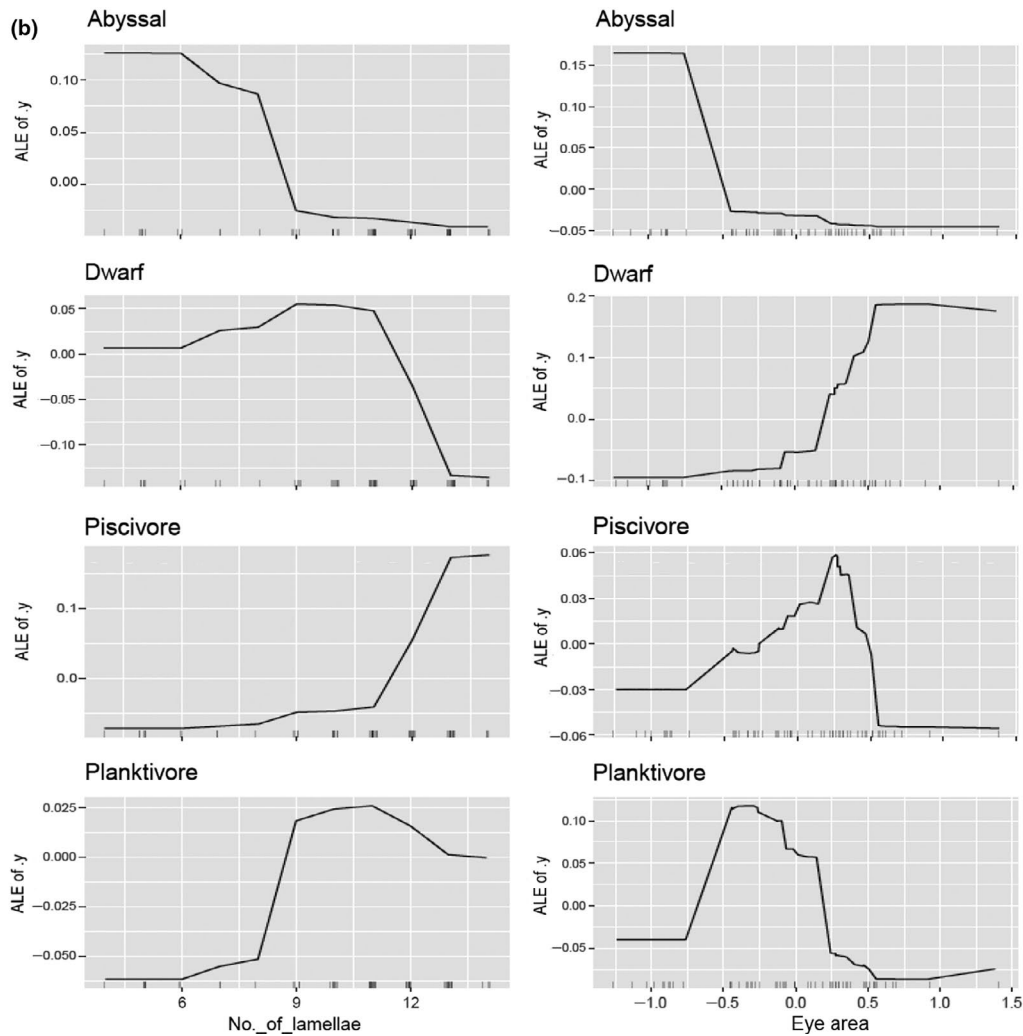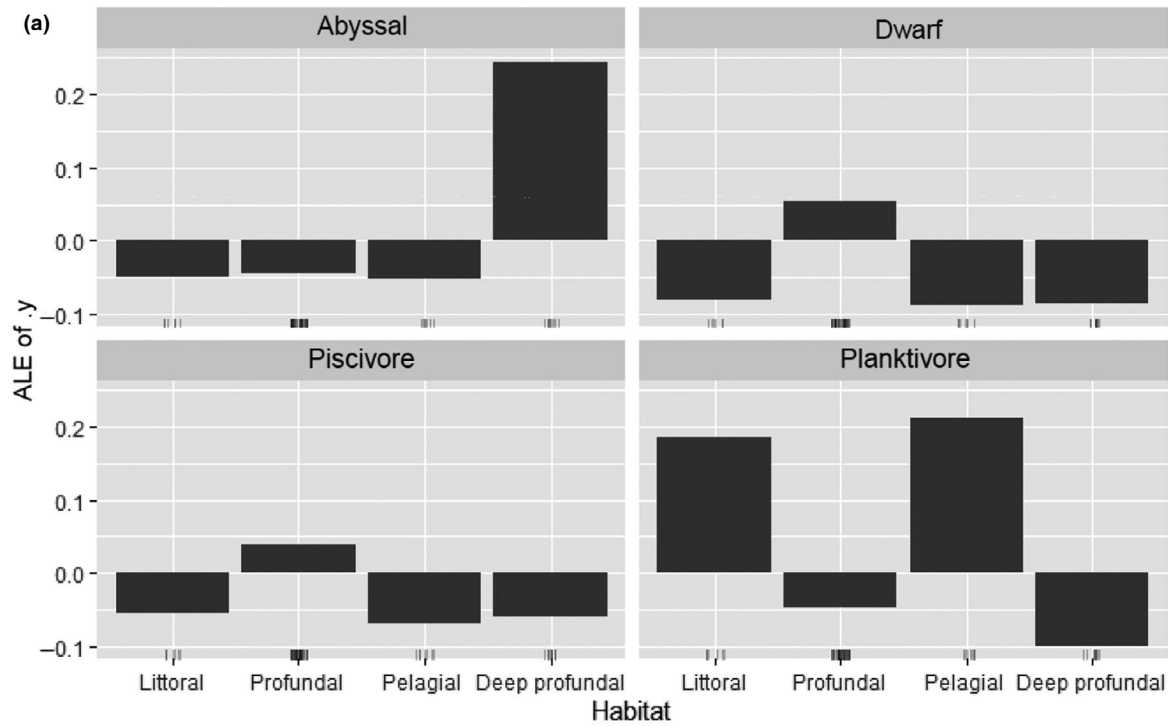
(a)

(b)

TABLE 1 Summary of the Arctic charr measurements: number of individuals (*n*), sex (number of males/females), mean body length (±*SD*), mean of number of lamellae (±*SD*), mean of the absolute size of olfactory organ area (±*SD*), and mean of the absolute brain region volume (±*SD*) measured from four Arctic charr morph in Lake Tinnsjøen

Morph	*n*	Sex	Body length (mm)	Number of lamellae	Olfactory organ area (mm²)	Olfactory bulb (mm³)	Telencephalon (mm³)	Optic tectum (mm³)	Cerebellum (mm³)	Hypothalamus (mm³)
Abyssal	12	6/6	78.58 ± 10.66	5.67 ± 1.07	0.31 ± 0.12	0.10 ± 0.04	0.37 ± 0.16	0.78 ± 0.23	0.72 ± 0.30	0.17 ± 0.07
Dwarf	22	12/10	113.82 ± 21.50	10.45 ± 1.37	0.89 ± 0.27	0.24 ± 0.06	0.91 ± 0.37	3.32 ± 1.24	2.33 ± 1.00	0.48 ± 0.16
Piscivore	16	10/6	217.38 ± 92.96	12.75 ± 0.93	2.11 ± 1.30	0.74 ± 0.70	3.20 ± 3.07	11.31 ± 9.35	7.77 ± 6.96	1.28 ± 1.13
Planktivore	22	10/12	180.36 ± 67.42	11.41 ± 1.53	1.49 ± 0.64	0.54 ± 0.62	1.52 ± 1.10	10.14 ± 7.56	6.40 ± 4.90	0.86 ± 0.43

The variables from the ALE plots showed the effects and how they changed across the different classes (e.g., morphs).

For habitat, the fish caught in the deep-profundal habitat were predicted to most likely be the Abyssal morph (Figure 5a). For the shallow-moderate profundal habitat, the Dwarf or the Piscivore morphs were predicted. For the littoral and pelagial habitat, the Planktivore morph was predicted.

For the number of lamellae, the Abyssal morph was predicted to have less than 6 lamellae (Figure 5b, first panel). The Dwarf morph was predicted to have between 9 and 11 lamellae. The Piscivore morph was predicted to have more than 11 lamellae, and the Planktivore morph between 9 and 11.

For the eye area residuals, the Abyssal morph was predicted to have a value smaller than −0.8 (Figure 5b, second panel). The Dwarf morph was predicted to have the eye area residuals larger than 0.5. If the eye area residuals ranges from −0.3 to 0.3, the model predicted belonging to the Piscivore class. There is a higher prediction of being Planktivore morph when the eye area residuals were between −0.8 and −0.3.

3.3 | Brain region and olfactory organ variation

The four morphs varied in brain region volumes. The largest absolute brain volumes were found in the Piscivore morph, followed by the Planktivore morph, whereas the Abyssal morph had the smallest (Table 1). The largest absolute brain region in all the morphs was the optic tectum, whereas the smallest was the olfactory bulb (Table 1). The optic tectum and the cerebellum were both larger in the Piscivore and the Planktivore morphs in comparison with the other two morphs (Table 1). The Abyssal morph had the smallest absolute optic tectum size compared with the other morphs. Within the Abyssal morph, the largest region was the optic tectum, followed by the cerebellum (Table 1). Comparing the Abyssal and the Piscivore morphs, the olfactory bulb represented a 12.8% and a 6.5% of the optic tectum in size, respectively (Table 1). In the case of the Dwarf and the Planktivore, the olfactory bulb represented a 7.2% and a 5.3% of the optic tectum in size, respectively. Therefore, there is an increase of the olfactory bulb in size in the Abyssal morph compared with the other three morphs.

Results from the ANOVA revealed all traits were significantly different ($p < .05$), except genetic trait, sex, and olfactory bulb (Table 2). Eye area, habitat, and number of lamellae were the only variables that had significant differences across all morph comparisons, except in one comparison (Piscivore–Planktivore, Dwarf–Piscivore, and Dwarf–Planktivore, respectively), being the same variables selected as most important in the random forest. The Piscivore morph had the highest number of lamellae followed by the Planktivore morph, whereas the Abyssal morph had the lowest number of lamellae (Table 1). The Abyssal morph presented significant differences in the olfactory organ area, habitat, number of lamellae, optic tectum, hypothalamus, and eye area when it was compared with the other three morphs (Table 2). The most different morph was the Abyssal when compared with the Piscivore and Planktivore morphs (Table 2).

4 | DISCUSSION

We found differences in the brain sizes among the four morphs of Arctic charr corresponding to their niche utilization. The optic tectum was the largest absolute brain region in the Piscivore and Planktivore morphs, which could be related to using a habitat with more light than the other two morphs. Comparing the olfactory bulb with the optic tectum in size, the olfactory bulb was larger in the Abyssal morph than in the other three morphs, suggesting that smell likely has a more relevant role than in the other morphs. The Piscivore morph presented the largest brain region volumes, whereas the Abyssal had the smallest, followed by the Dwarf morph. In the random forest analysis, eye area, habitat, and number of lamellae were the most important variables to classify the morphs suggesting differences in foraging and mating behaviour as well.

Based on the head morphology of Arctic charr, three of the four morphs were more distinguishable than the Planktivore morph (i.e., the most generalist morph).

4.1 | Random forest analyses verify four morphs of Arctic charr

The deep-profundal Abyssal morph presented the largest morphological differences compared with the other morphs, presenting a very distinct head shape and the smallest eyes and body length. The Dwarf and Piscivore morphs have evolved common head and body shapes, likely through parallel adaptation for occupying the

shallow-moderate profundal habitat, and both differ from the Planktivore morph, which has small eyes and head compared with the body size. Although both profundal morphs differ in head and body size (e.g., the Dwarf morph has smaller head, mouth, and body than the Piscivore morph), this is probably associated with diet preferences. From our results, it appears that there is certain selection pressure on vision or smell depending on the habitat and foraging behaviour. For instance, the morphs living in low light conditions could rely more on their vision developing larger eyes to detect their prey. Normally, the Piscivore morph of Arctic charr lives in the pelagic or littoral habitats (Adams et al., 1998; Power et al., 2005), but in Fennoscandia it seems that the piscivore morph mainly occupies the profundal habitat such as in Lake Tinnsjøen and Lake Skogsfjordvatn, Norway (Skoglund et al., 2015). It is likely that the Piscivore morph in Lake Tinnsjøen utilises several habitats in the lake, such as littoral, shallow-moderate profundal, and deep-profundal to seek for prey due to low density of fish (Østbye et al., 2020).

Both profundal morphs had larger eyes than the other morphs, but the Dwarf morph had the largest relative eye size. The larger eye size in the Dwarf morph may be favoured for feeding on small prey in habitats of low light conditions, whereas the eye size in the Piscivore could be due to feeding on more active prey, which can facilitate the detection of prey (Huber et al., 1997; Schliewen et al., 2001). The Piscivore also had a larger mouth, more robust head and larger body size than the other three morphs, which could be adaptations to enhance predation on other fish. Adams et al. (1998) also reported larger eye size in other piscivore morphs

TABLE 2 Results from ANOVAs and post hoc Tukey's HSD tests indicating the difference of trait means and significant level tests between Arctic charr morphs

	ANOVA		Tukey's HSD tests					
	$F_{3,68}$	p	Abyssal–Dwarf	Abyssal–Piscivore	Abyssal–Planktivore	Dwarf–Piscivore	Dwarf–Planktivore	Piscivore–Planktivore
Genetic trait	2.56	.06	−0.08	0.27	−0.12	0.35	−0.05	−0.39
Habitat	135.50	.00***	3.00***	3.00***	2.09***	−8.88e−16	−0.91***	−0.91***
Sex	0.37	.78	0.05	0.13	−0.05	0.08	−0.09	−0.17
No. of lamellae	75.15	.00***	4.79***	7.08***	5.74***	2.30***	0.95	−1.34*
Olfactory organ area	10.88	.00***	0.52***	0.43***	0.39***	−0.09	−0.13	−0.04
Olfactory bulb	1.65	.19	0.29	0.23	0.18	−0.06	−0.12	−0.05
Telencephalon	4.51	.01**	0.28	0.29	−0.06	0.00	−0.34*	−0.34*
Optic tectum	11.96	.00***	0.66***	0.49**	0.76***	−0.18	0.10	0.28
Cerebellum	4.63	.01**	0.43*	0.26	0.48**	−0.16	0.06	0.22
Hypothalamus	4.59	.01**	0.48**	0.37*	0.39*	−0.11	−0.09	0.02
Eye area	59.66	.00***	1.43***	1.01***	0.93***	−0.42***	−0.50***	−0.09
Age	9.56	.00***	0.88	2.21*	−1.03	1.33	−1.91**	−3.24***
Head size	4.64	.01**	301.10	811.66**	596.38*	510.56	295.28	−215.28
Maturation	4.39	.01**	0.27	0.13	−0.23	−0.15	−0.50**	−0.35

Note: We used the residuals of the measured variables obtained from the log-log regressions to correct for size. Level of significance (p):
*.01 < p ≤ .05; **.001 < p ≤ .01; ***p ≤ .001.

of Arctic charr likely related to predation behaviour, reaching a larger size and living longer than the other morphs. Morphological differences suggest different evolutionary pressures across and within habitats.

Our random forest analysis indicates that eye area, habitat, and number of lamellae seem to be good indicators for classifying morphs. These variables also showed differences among the morph comparisons in the ANOVA analyses, suggesting these predictors could have an important role in the morph diversity. The accuracy of the random forest was 80%. Here, having a larger dataset would most likely give a higher accuracy.

4.2 | Habitat specialization and optic tectum volume

Living in a deepwater habitat means adaptation to the darkness, high pressure, low temperature, monotony, and a limitation in food resources (e.g., low prey densities). The limits of food abundance likely varies temporally and seasonally, affecting, for example, the fatty acid composition in the brain (Menzies, 1965; Patton, 1975; Roots, 1968). A reduction of vision can be a strategy to save energy in habitats with limited food and where vision can be not needed for feeding or predation avoidance (Moran, Softley, & Warrant, 2015). The Abyssal morph had the smallest eyes and optic tectum. The reduction in eye size across depth can indicate a decrease in the importance of vision due to a decrease in light irradiance (Huber et al., 1997). In addition, studies on cave and surface forms of the Mexican blind cavefish (*Astyanax mexicanus*) and medaka (*Ozyzias latipes*) showed that an increase in eye size, promoted by light irradiance, can affect the growth of the optic tectum (Ishikawa, Yoshimoto, Yamamoto, & Ito, 1999; Ishikawa et al., 2001; Soares, Yamamoto, Strickler, & Jeffery, 2004). Therefore, an increase of light would drive an increase in the size of the eye and optic tectum. In Lake Tinnsjøen, there is no light at 460 m, explaining the small eyes and small optic tectum size found in the Abyssal morph compared with the other three morphs, where vision can be more important. The presence of visual stimuli, such as bioluminescence, may determine the eye and optic tectum sizes in these kind of environments such as observed in the deep sea (Wagner, 2001).

The differences found in eye size and optic tectum, and even in the olfactory bulb, can be related to mating behaviour. Arctic charr has characteristically bright breeding coloration with a red belly and secondary sexual traits such as lower jaw type, which shows pronounced individual variation and potentially contribute to mate selection (Janhunen, Peuhkuri, Primmer, Kolari, & Piironen, 2011; Kekäläinen, Vallunen, Primmer, Rättyä, & Taskinen, 2009). A distinguished coloration may be important for female mate preferences in well-illuminated habitats, where vision will be of higher importance than in dark habitats. In Lake Tinnsjøen, the pale coloration presented in the Abyssal morph most likely indicates a lesser importance of coloration in mating than in the other three morphs living in habitats with more light. Sensory-driven divergence in visual capacities during speciation has been documented for cichlids

as well, with a clear link to mate selection (Seehausen et al., 2008). However, we still have a very limited amount of studies of colour vision in Arctic charr morphs (Kahilainen et al., 2016). Major histocompatibility complex (MHC) genes can influence mating choice and kin recognition through olfaction, where females can reject mates with high differentiation in the MHC genotypes (Landry et al., 2001; Milinski et al., 2005; Olsén, Grahn, Lohm, & Langefors, 1998). Wild Arctic charr populations show differences in MHC genotypes within and among morphs, and diversity of polymorphisms in MHC can be linked to a lower amount of parasites (Conejeros et al., 2014; Eizaguirre & Lenz, 2010; Kekäläinen et al., 2009). Large variation in ecological niches and colouration of different Arctic charr morphs in Lake Tinnsjøen would provide a nice setting for parasite and MHC genotype studies as well as experimental tests for sexual selection potentially acting on phenotypes.

4.2.1 | Smell perception capacities

All morphs presented differences in the number of lamellae and the Abyssal morph showed differences in the olfactory organ area when compared with the other morphs. The Piscivore was the morph with the largest absolute size of the olfactory bulb, olfactory organ, and largest number of lamellae, followed by the Planktivore and the Dwarf morphs, whereas the Abyssal had the smallest. Wagner (2001) found that species relying more on visual foraging have larger optic tectum than species relying on the smell, which have a larger olfactory bulb. However, the combination of different stimuli and the occupation of different habitats may have determined the sensory preferences, developing specific brain regions independently.

Regarding olfactory lamellae, previous studies have found that the size of the olfactory organ and the number of lamellae increases with fish size (Atta, 2013; Halama, 1982; Kasumyan, 2004; Kudo, Shinto, Sakurai, & Kaeriyama, 2009). These findings were also corroborated by Olsén (1993), who founded that the size and number of lamellae increased with the body size of Arctic charr reared in the laboratory. Our study also supports these studies, presenting the largest number of lamellae and larger olfactory organ area in the largest morph (i.e., Piscivore morph) and the smallest in the minute morph (i.e., Abyssal morph).

4.2.2 | Brain region volumes differ among the morphs

In this study, the brain regions had different volumes among the morphs. The five small brain regions found in the Abyssal morph could be a response to low availability of energy through food (e.g., food quality/quantity), as has been found in a study of *Poecilia mexicana* that live in cave habitats and has a reduction of the optic tectum size and the total brain size (Eifert et al., 2015). A small brain can be a strategy to reduce energy expenditure in cave habitats

(Tobler, 2008; Tobler et al., 2006); this can also apply to deep-profundal habitat in Lake Tinnsjøen, where environmental parameters, such as light, temperature, and low resources, in many ways resemble a similar habitat to caves. Thus, is it the lack of light or food, or both that caused brain reduction in cave fish and in the deep-profundal Abyssal morph? As the brain is an energetically expensive organ, a reduction of the relative brain size likely reflects a decrease in their metabolic rate, as seen in other species (Poulson, 1963, 2001; Shi et al., 2018). These small brain region sizes are probably due to a reduction in the physical space of the skull, constraining the brain size. Head morphology of the Abyssal morph and its cranial space may force some modifications on the structure of the brain regions due to spatial constrains (Striedter & Northcutt, 2006). Hypoxia can also be another factor that can reduce the brain size, as observed in other species (Chapman & Hulen, 2001). However, Lake Tinnsjøen is an oxygen-rich deep-water lake across the different habitats. Therefore, oxygen is not likely to be a factor constraining the brain size. Pressure might also have an effect on the brain size, especially in the deep habitat where the Abyssal morph lives. Thus, we have to consider different factors when it comes to brain morphology depending on the habitat where the morphs live.

According to the _mosaic evolution hypothesis_, each brain region is able to develop independently from the others (Hager, Lu, Rosen, & Williams, 2012; Liem, 1978). Our study supports this hypothesis, where the foraging behaviour and habitat specialisation of the different morphs most likely explain the variation in the brain regions we observed. Previous studies have found that, depending on environmental conditions, presence of conspecifics and ecological and behavioural conditions, there are certain brain regions that can be more important, and more developed than others (Gonda et al., 2009; Kihslinger, Lema, & Nevitt, 2006; Kihslinger & Nevitt, 2006; Kotrschal et al., 1998; Lisney et al., 2007). Thus, the pattern observed in brain region differentiation in the four Arctic charr morphs could be due to a rather complex set of putative explanations.

5 | CONCLUSIONS

In summary, we found differences among morphs in body size, eye area, and number of lamellae, which were associated with habitats and diet used by morphs. For instance, large body size is attained from energy rich prey, that is, fish, in the case of the Piscivore morph or productive habitats in the Planktivore morph. It seems that living in different habitat conditions, such as lack of light and food limitation, affects brain morphology as showed in the small brain regions of the Abyssal morph. The optic tectum was the largest in the Piscivore and Planktivore morphs living in more illuminated habitats compared to the Abyssal, which had the smallest, suggesting a less developed vision. These clear relationships between brain traits and habitats suggest long-term niche specialization, which may originate from phenotypic plasticity or adaptive evolution. These relationships warrant further empirical and experimental studies. As our

study present the first brain region study from _Salvelinus_, there is need for studies in other polymorphic species, such as _Coregonus_ and _Cottus_, to test the generality of our findings.

AUTHOR CONTRIBUTION

Ana-Maria Peris Tamayo: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Writing-original draft (lead); Writing-review & editing (lead). **Olivier Devineau:** Formal analysis (equal); Supervision (equal); Writing-review & editing (equal). **Kim Præbel:** Conceptualization (lead); Data curation (equal); Funding acquisition (lead); Project administration (lead); Resources (lead); Supervision (equal); Writing-review & editing (equal). **Kimmo K. Kahilainen:** Conceptualization (supporting); Formal analysis (supporting); Supervision (supporting); Writing-review & editing (equal). **Kjartan Østbye:** Conceptualization (lead); Data curation (equal); Funding acquisition (lead); Project administration (lead); Resources (lead); Supervision (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

Data are available in the repository Dryad at: https://doi.org/10.5061/dryad.15dv41nvt.

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Chromosomal inversions, polymorphism and local adaptation in four sympatric Arctic charr morphs

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Abstract

Understanding patterns of divergence within species is important to predict their phenotypic and genetic diversity across environmental gradients. Structural changes such as chromosomal inversions likely contribute to local adaptation in the presence of gene flow. The salmonid Arctic charr (*Salvelinus alpinus*) has a circumpolar distribution and can show different morphs within the same lake. In this study, we used single-nucleotide polymorphisms (SNPs) to investigate the genetic basis of four Arctic charr morphs (i.e. Piscivore, Planktivore, Dwarf and Abyssal morphs) from Lake Tinnsjøen, Norway. We identified ten chromosomal inversions in the Arctic charr genome and a set of candidate genes involved in 119 biological functions such as generation of neurons, neuron differentiation and regulation of neurogenesis. Outlier loci putatively under divergent selection discriminated better the genetic structure of the four morphs than neutral loci. The genomic differentiation among the four Arctic charr morphs suggests habitat-specific selection pressures, which likely lead to local adaptation when there are differences in environmental conditions. Arctic charr morphs could be at the early stages of the ecological speciation process. This freshwater

system offers a unique opportunity for further studies to investigate the early stages of ecological segregation and reproductive isolation.

Introduction

Revealing population genetic basis can help to determine how populations respond to different environments and can contribute to understand the adaptive phenotypic variation (Stinchcombe & Hoekstra, 2008). Changes in the genetic basis and in the genomic architecture might introduce new adaptive variants that can lead to adaptive divergence and reproductive isolation, giving us the chance to study the earlier stages of speciation (Renaut et al., 2011). Ecological speciation occurs when populations develop reproductive barriers leading to population divergence and the formation of new species adapted to specific environments. Preferences towards different environments might lead to adaptation and diversification of their phenotypes and genotypes, where divergent selection can act and contribute to their genomic divergence (Nosil et al., 2009; Schluter, 2000).

Phenotypic diversification can originate from similar or different genomic bases depending on selected mutations (Rougeux et al., 2019). Genomic architecture of ecological speciation can vary depending on population divergence and the outcome of reproductive isolation and adaptation, influenced by drift, selection and migration (Rogers et al., 2013; Rougeux et al., 2019). Natural selection can originate different species by favouring specific alleles depending on the environment, which can reduce gene flow between populations and lead to reproductive isolation (Schluter, 2009). At the genome level, natural selection modifies phenotypic diversity, changing the genomic architecture of ecological speciation by increasing genetic differentiation in specific regions (Nosil et al., 2009; Renaut et al., 2011). Migration can be important for the evolution of local adaptations at the population level, depending on the strength and timing of gene flow, where adaptive divergence can decrease with high levels of migration (Garant et al., 2007; Kirkpatrick & Ravigné, 2002; Kisel & Barraclough, 2010; Moore & Hendry, 2005; Smadja & Butlin, 2011; Yeaman & Whitlock, 2011).

Fusion and fission of chromosomes produce variations in the genomic architecture and in the number of chromosomes (Kasahara et al., 2007; Nakatani et al., 2007). Genomic architecture can also be altered by duplications in the whole genome (Nugent et al., 2017), which can play

an important role in evolutionary history (Smith et al., 2013). Duplications can increase the number of genes in genomes, which can favour different gene functions in larger genomes, allowing complex interactions (Meyer & Peer, 2005). These duplications can affect different traits in the phenotype such as pigmentation diversity (Braasch et al., 2009). For instance, two whole genome duplications occurred in the common ancestor of vertebrates, one in the ancestor of teleost fish and one in the ancestor of salmonid fish (Allendorf & Danzmann, 1997; Berthelot et al., 2014; Lien et al., 2016; Macqueen & Johnston, 2014). Thus, salmonids have experienced four whole-genome duplication events (Waples et al., 2015) and their genes present disomic and tetrasomic patterns, even though tetrasomic inheritance seems to occur only in males (Allendorf & Danzmann, 1997).

Patterns of divergence between sympatric species are reflected in specific genomic regions (Llopart et al., 2005; Mallet et al., 2007; Yatabe et al., 2007), defined as genomic islands (Harr, 2006; Nosil et al., 2009; Turner et al., 2005; Wu, 2001). These genomic islands originate from divergent selection, which can be observed within and between populations and species (Berg et al., 2015; Nosil et al., 2009). Genomic islands can originate by hitchhiking (i.e. fixation of a neutral locus due to linkage with another locus; Kulmuni et al., 2020; Via, 2012) or by processes that can decrease recombination (e.g. chromosomal rearrangements such as inversions, translocations or transposable elements) across the genome (Feder & Nosil, 2009; Kirkpatrick & Barton, 2006). Chromosomal rearrangements seem to play an important role in conserving polymorphism in specific traits (Conrad & Hurles, 2007). For instance, chromosomal inversions can be drivers of adaptation, which affect recombination patterns causing recombination suppression and elevated linkage disequilibrium (Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010; Kirkpatrick & Barton, 2006; Slatkin, 2008). There are several examples including mosquitoes (Ayala & Coluzzi, 2005), butterflies (Kandul et al., 2007) and fish (Berg et al., 2016; Sodeland et al., 2016), where inversion polymorphism was associated with adaptation with gene flow. Low recombination in chromosomal rearrangements could increase the divergence in different parts of the genome, expressing specific phenotypes related to local adaptation (Berg et al., 2017; Kirkpatrick & Barton, 2006). Such genomic islands can be more effective if they include genes involved in adaptation or in reproductive isolation with low degree of recombination, such as genes within an inversion (Gavrilets, 2004; Kirkpatrick & Barton, 2006; Nachman & Payseur, 2012; Yeaman & Whitlock,

2011). These groups of close genes in a chromosome, which are inherited together, can affect both different and related traits, favouring specific combinations of genotypes (Nosil et al., 2009; Sinervo & Svensson, 2002). Other studies suggest that genomic islands can also grow in size due to the spill over effect of strong selection, decreasing gene flow in the regions nearby (Via, 2012; Via & West, 2008). The growth variability can depend on the number of islands that are involved in affecting a specific trait under selection (Nosil et al., 2009). For instance, more islands involved in capturing new mutations could promote the increase of the island size (Nosil et al., 2009).

Genetic diversity of postglacial freshwater fishes provides relevant insights in several processes such as local adaptation and speciation (Abbott et al., 2013; Schluter, 2009). Within freshwater systems, phenotypic divergence can evolve due to the availability of different niches, leading to a rapid adaptive radiation (Schluter, 2000; Smith & Skúlason, 1996). Phenotypic and genetic diversity of fish among and within postglacial lakes could be related to local adaptation (Bernatchez et al., 2016; Gagnaire et al., 2013). Some of the species from postglacial lakes are Arctic charr (*Salvelinus alpinus*; e.g. Jonsson & Jonsson, 2001), Atlantic salmon (*Salmo salar*; e.g. Klemetsen et al., 2003), stickleback (*Gasterosteus aculeatus*; e.g. Bell & Foster, 1994) and lake whitefish (*Coregonus clupeaformis*; e.g. Pigeon et al., 1997).

Arctic charr is a polymorphic species, showing variations in morphology, growth rate, spawning time, sexual maturity, feeding behaviour, habitat utilization, body size, and coloration (Alexander & Adams, 2000; Johnson, 1980; Jonsson et al., 1988; Jonsson & Jonsson, 2001; Klemetsen, 2010). The reproductive isolation between different freshwater systems of Arctic charr can vary, for instance, due to differences in the exploitation of resources and resource competition (Bernatchez et al., 1996; Schluter, 1996). Arctic charr can hold different morphs as has been observed in systems such as Lake Fjellfrosvatn (Klemetsen et al., 1997, 2002; Westgaard et al., 2004) and Lake Skogsfjordvatn (Knudsen et al., 2016; Skoglund et al., 2015; Smalås et al., 2013) in Norway, Lake Thingvallavatn in Iceland (Johnston et al., 2004; Jonsson et al., 1988; Sandlund et al., 1992), Gander Lake in Canada (Power et al., 2005), Transbaikalian lakes in Russia (Alekseyev et al., 2019; Gordeeva et al., 2015), and Loch Dughaill in Scotland (Hooker et al., 2016).

In Norway, Lake Tinnsjøen presents four distinct morphs of Arctic charr that differ, for instance, in body, brain and head sizes, coloration and habitat use (Østbye et al., 2020; Tamayo et al., 2020). The Piscivore morph feeds on fish and is mainly found in the shallow-moderate profundal habitat. The Planktivore morph feeds on zooplankton and is found in the littoral, pelagic and shallow-moderate profundal habitats. The Dwarf morph feeds on macrobenthos and zooplankton and lives in the shallow-moderate profundal habitat. Finally, the Abyssal morph feeds on soft-profundal-bottom benthic invertebrates from the deep-profundal habitat. These morphs also show differences associated with their habitat use. For instance, the Abyssal morph has the smallest eye, head and absolute brain regions, which seems related to the habitat conditions where they live (i.e. light and food constrains; Tamayo et al., 2020). However, the Piscivore, Planktivore and Dwarf morphs have larger eye, head and absolute brain regions than the Abyssal morph, and live in habitats that are more illuminated and with larger prey resources (Tamayo et al., 2020). Ecological speciation most likely is the mechanism behind the morph divergence in Lake Tinnsjøen.

The aim of this study is to investigate the genetic basis and putative processes underlying the phenotypic and genetic variation of four Arctic charr morphs from Lake Tinnsjøen, Norway. First, we identified putatively neutral loci and outlier loci (i.e. loci putatively under divergent selection) to determine the population genetic structure. Second, we detected chromosomal inversions and assessed the pattern of linkage disequilibrium (LD) between a set of loci putatively under divergent selection to investigate the genetic divergence among Arctic charr morphs. Third, we identified candidate genes under putative divergent selection and their biological functions.

Materials and Methods

Study area and sample collection

Lake Tinnsjøen is an oligotrophic and subartic lake situated at 191 m.a.s.l (60°38'15.6" N, 11°07'15.2" E) with a surface area of 51.4 km² and a maximum depth of 460 m. Arctic charr, brown trout (*Salmo trutta*), and small populations of perch (*Perca fluviatilis*) inhabit Lake Tinnsjøen. Minnow (*Phoxinus phoxinus*) has likely been introduced to the lake in the 1960-1970s, and comprises a small population residing close to the shoreline.

We conducted the fish collection in 2013 (see details in Østbye et al., (2020)). We sampled in four habitats: littoral (gillnets up to 20 m from shore), pelagic (gillnets > 50 m from shore, 20-30 m depth), shallow-moderate profundal (gillnets, traps and hook-line from shore, 20-150 m depth), and (iv) deep-profundal (setting traps >100 m from shore, > 150 m depth). In the field, we classified all Arctic charr into four morphs based on morphological characteristics: Dwarf (n= 34), Piscivore (n= 33), Planktivore (n= 36) and Abyssal (n= 22).

DNA extraction, quality assessment and quantification

We extracted the genomic DNA from muscle tissue using Dneasy 96 blood and tissue kit (QIAGEN). We used 500 nanograms of DNA per individual to digest with the restriction enzyme SbfI-HF (New England Biolabs). We prepared all RAD-libraries following the protocol of Benestan et al., (2015). Every individual had a unique barcode of 6bp. We selected DNA fragment sizes ranging from 300 to 800bp for downstream library preparation. We sequenced libraries on an Illumina HiSeq4000 platform (Novogene (HK) Company Limited, Hong Kong, China), using 2x150 paired-end chemistry.

Data quality filtering and genotyping parameters

We demultiplexed the reads using STACKS v.2.1 (Rochette et al., 2019) with process_radtags. We mapped the reads to *Salvelinus* sp. reference genome (Christensen et al., 2018; GCA_002910315.2) using BWA version 0.7, (Li, 2013) allowing a maximum of four mismatches. We used STACKS v.2.1 to call SNPs from the mapping results. The RAD locus had to be present in all the four populations (-p) and in at least 60% of the individuals in each population containing the locus (-r). We set the minimum minor allele frequency (-min_maf) to 0.01 and the maximum observed heterozygosity (-max-obs-het) to 0.5. We only retained a single SNP per each RAD-tag to avoid possible linkage disequilibrium. We observed high rates of PCR duplication in the dataset. However, we suspected most of the duplicates were false positives and were caused by the narrow insert size distribution of the sequencing libraries. It is shown by the negative correlation between the width of the insert size distribution and the duplication rate. Therefore, we decided to keep all the data for the subsequent analyses.

We sequenced a total of 125 samples, of which 90 samples passed our filtering criteria. We obtained a total of 21429 SNPs from placed and unplaced contigs after the filtering in STACKS.

The final dataset used for further analysis contained 16895 SNPs based on placed contigs. The obtained data set was exported to VCFTOOLS 0.1.15 (Danecek et al., 2011) and PGDSpider v. 2.1.1.5 (Lischer & Excoffier, 2012) for downstream analyses.

Population differentiation

We calculated Weir and Cockerham's F_{st} (1984) across non-overlapping 10-kb sliding windows using VCFTOOLS 0.1.15. We plotted all SNPs for each genomic region. We produced Manhattan plots using CIRCOS software v 0.69-8 (Krzywinski et al., 2009). We calculated the F_{st} threshold with the F_{st} distribution from the upper 95% percentiles for all the 39 observed chromosomes. We inferred loci outside the neutral envelope (i.e. outside the upper 95% percentiles of F_{st} distribution) as loci putatively under divergent selection (i.e. candidate outliers; Cayuela et al., 2020). There were 1132 outlier SNPs above this threshold identified as loci putatively under divergent selection.

Rearrangement patterns

We identified candidate inversions by using Liu et al., (2018) script in R (R Core Team, 2020), where the F_{st} is calculated between SNPs across different populations. We set up a threshold of 0.1 for the allele frequency, a sliding window size of 10^7, a sliding step of 5000000, a F_{st} threshold of 0.01, a minimum size of 200 bases for an inversion, a minimum distance of 200 bases between neighbouring SNPs and a minimum of 3 SNPs for an inversion. We also plotted frequencies of haplotypes inferred for the different morphs.

Gene Ontology (GO) enrichment analysis

We used BEDTOOLS v2.25.0 (Quinlan & Hall, 2010) with the intersect function to find the overlap between genomic features by comparing the genome annotation and a set of loci putatively under divergent selection (-wb), to know which SNPs were falling within a gene in the genome. We also used BEDTOOLS v. 2.25.0 with the window function to find which of these SNPs were close to a gene within 1Kb. After the identification of the genes, we used WebGestalt (Liao et al., 2019) for functional enrichment analysis to find the biological function of these genes by using human gene set for the analysis. We used WebGestalt based on Overrepresentation Enrichment Analysis (ORA) method (Khatri et al., 2012). We used *P* value

< 0.05 and a minimum of 5 genes per category as cut-off criteria to perform the analysis. In addition, we identified the top 10 most significant categories from these biological functions. We identified the genes and biological function for 283 SNPs identified in the loading plot of the discriminant analysis of principal components (DAPC; see section below).

Linkage disequilibrium and population structure analyses

We calculated the linkage disequilibrium (LD) for the 1132 SNPs putatively under divergent selection. We used the R package 'vcfR' (Knaus & Grünwald, 2017) for extracting genetic distances and the 'Ldheatmap' package (Shin et al., 2006) to calculate Lewontin's D'. We estimated the average LD (i.e. mean of all pairwise LD values from the matrix) for each morph.

We used STRAUTO V1.0 (Chhatre & Emerson, 2017) to execute STRUCTURE V2.3.4 (Pritchard et al., 2000). This was used to reveal the population structure of three sets of SNPs: whole set of SNPs based on placed contigs (16895 SNPs), putatively neutral loci (15763 SNPs) and loci putatively under divergent selection (1132 SNPs). We assumed admixture model (NOADMIX = 0), use of sampling location as prior (LOCPRIOR = 1), and correlated allele frequency (FREQSCORR = 1). We set the genetic clusters (K) from one to seven performing five replications. The burn-in period was 50000 and Markov chain Monte Carlo iterations were 75000. We used STRUCTURE HARVESTER (Earl & vonHoldt, 2012) and CLUMPAK (Kopelman et al., 2015) to process and plot the STRUCTURE results. We identified delta K and the best K-value for each data set in STRUCTURE with CLUMPAK (Kopelman et al., 2015).

We analysed the genetic structure using DAPC for the three sets of SNPs (whole set of SNPs, putatively neutral loci and loci putatively under divergent selection). We performed these analyses using 'adegenet' package in R (Jombart & Ahmed, 2011).

We identified a set of 283 SNPs from the loci putatively under divergent selection, which showed a larger contribution in the DAPC, using 'adegenet' package in R. We plotted this set of SNPs using DAPC analysis. Then, we identified the two chromosomes that had the larger number of SNPs, and a DAPC was executed for each of these two chromosomes.

We calculated the minor allele frequency (MAF) for the three sets of SNPs (whole set of SNPs, putatively neutral loci and loci putatively under divergent selection) using --freq in PLINK

(Purcell et al., 2007). We depicted the results in a heatmap and in a hierarchical dendrogram using the 'heatmap.2' function in 'gplots' package (Warnes et al., 2020) in R.

Results

Genome-wide association analysis: Manhattan plots

The six comparisons among the four morphs showed outlier SNPs (i.e. loci putatively under divergent selection) across the whole genome (Figure 1). For the comparison between the Abyssal-Dwarf morphs, there were 306 outlier SNPs. For the Piscivore-Abyssal morphs, there were 286 outlier SNPs. For the Piscivore-Dwarf morphs and the Piscivore-Planktivore morphs, there were 309 and 348 outlier SNPs, respectively. For the Planktivore-Abyssal morphs and the Planktivore-Dwarf morphs, there were 343 and 365 outlier SNPs, respectively. There was a total of 1132 loci putatively under divergent selection across all comparisons, without counting repetitive loci.

There were five chromosomes across the different pairwise comparisons that had the largest number of outlier SNPs. For the comparison between the Abyssal-Dwarf morphs, it was chromosome Chr21 with 20 outlier SNPs. For the Piscivore-Abyssal morphs and the Piscivore-Dwarf morphs, it was chromosome Chr5 with 19 and 20 outlier SNPs, respectively. For the Piscivore-Planktivore morphs, it was chromosome Chr19 and Chr23, each of them with 26 outlier SNPs. For the Planktivore-Abyssal morphs and the Planktivore Dwarf morphs, it was chromosome Chr17 with 22 SNPs and Chr19 with 29 outlier SNPs, respectively.

There were only four out of 39 chromosomes without outlier SNPs in some comparisons: in chromosome Chr15 for the Dwarf-Abyssal morphs, in Chr24 for the Planktivore-Abyssal morphs, in Chr27 for the Piscivore-Planktivore morphs, and in Chr36 for the Piscivore-Planktivore morphs and the Planktivore-Dwarf morphs.

Rearrangement patterns: Chromosomal inversions

We identified inverted regions across different chromosomes: Chr12 (28.54-29.25 Mb), Chr15 (11.30-11.34 Mb), Chr17 (14.89-16.81 Mb), Chr19 (26.67-28.75 Mb), Chr23 (43.59-45.98 Mb), Chr30 (14.63-15.72 Mb), Chr32 (11.31-13.24 Mb), Chr34 (28.19-28.62 Mb), Chr35 (12.35-12.38 Mb) and Chr38 (22.95-23.08 Mb). For Chr12 and Chr23, the Abyssal and the Planktivore

morphs were almost fixed for one haplotype, respectively, whereas the Piscivore morph was almost fixed for the alternative haplotype. For Chr30, the Dwarf and the Piscivore morphs were almost fixed for the same haplotypes, whereas the Abyssal and Planktivore morphs showed intermediate frequencies (Figure 2). The Piscivore morph showed large haplotype frequencies in Chr12, Ch17, Ch19, Ch23 and Ch38, whereas the Planktivore morph had low to intermediate frequencies (Figure 2).

Biological function of genes

From the 1132 SNPs putatively under divergent selection, there were 695 SNPs within a gene, 38 SNPs close to a gene, and 33 SNPs within inversions.

From 645 genes identified, we found 531 genes related to biological processes such as biological regulations, metabolic processes and response to stimulus (Figure 3). We found 119 significant biological functions (GOs) that were involved in functions such as regulation of cell development, circadian rhythm, and brain, eye and inner ear development (Table 1). We also identified top 10 GOs such as generation of neurons, neuron differentiation and regulation of neurogenesis (Table 1).

From 33 SNPs found within inversions, there were 18 genes involved in 54 GOs such as brain and inner ear development. Three of these GOs were within the top 10 GOs, which were involved in generation of neurons, neuron differentiation and neurogenesis.

Linkage disequilibrium and population structure

We analysed the linkage disequilibrium (LD) of the SNPs putatively under divergent selection identified in the Manhattan plots (1132 SNPs; Figure 1). By using pairwise LD correlations, we found variation in D' among morphs (Figure S1). The mean D' value decreased from 0.80 in Piscivore, 0.72 in Abyssal, 0.67 in Dwarf, and 0.61 in Planktivore morphs.

The STRUCTURE analysis, including all 16895 SNPs, suggested as the most likely scenarios of clustering K = 4-7. At K = 4, there was a separation between the Planktivore and the Abyssal morphs, without distinguishing the Piscivore and the Dwarf morphs (Figure S2). At K = 2, the Piscivore, Dwarf and Abyssal morphs were clustered together, whereas the Planktivore morph was separated in another genetic cluster. For the subset with the putatively neutral

loci (15763 SNPs), the most likely configuration was K = 3 (Figure S3), showing a similar pattern to K = 2. At K = 3, the Piscivore, Dwarf and Abyssal morphs were clustered together, whereas the Planktivore morph has its own genetic cluster. Regarding the loci putatively under divergent selection (1132 SNPs), the most likely configurations were also K = 4-7 (Figure S4). The dataset with the loci putatively under divergent selection showed a better separation of all four morphs (Figure S4) compare to the whole set of SNPs and the set with putatively neutral loci (Figure S2, S3).

The DAPC was conducted to show the population structure of 90 individuals and encompassed the whole set of SNPs (16895 SNPs). In this DAPC, the Dwarf and the Planktivore morphs showed overlap with the Abyssal morph (Figure 4a). In the DAPC with the putatively neutral loci (15763 SNPs), Dwarf morph showed overlap with the other three morphs (Figure 4b). The DAPC with the loci putatively under divergent selection (1132 SNPs) could discriminate the four morphs (Figure 4c).

For the SNPs with a larger contribution in the DAPC, we found a set of 283 SNPs (Figure 5a). The DAPC for these 283 SNPs showed four separate clusters (Figure 5b). The largest number of SNPs was identified in chromosomes Chr5 (17 SNPs) and Chr26 (23 SNPs). The DAPC performed for Chr5 and Chr26 separately had similar clustering patterns (Figure 5c-d).

From these 283 SNPs, we found 171 genes involved in the 119 significant GOs, such as generation of neurons, neuron differentiation, regulation of neurogenesis, brain and eye development. Chr5 had 10 genes involved in 76 GOs (e.g. brain, inner ear and eye development) and Chr26 had 14 genes involved in 73 GOs (e.g. neuron development, regulation of growth and cell morphogenesis). The genes found in both chromosomes were involved in all top 10 GOs except from one (synapse organisation), where the genes in Chr5 were not involved.

The minor allele frequency (MAF) of the whole set of loci (16895 SNPs), putatively neutral loci (15763 SNPs) and loci putatively under divergent selection (1132 SNPs) are shown in Figure 6. The colour pattern ranges from red to yellow, showing lowest to highest allele frequencies. Dendrograms represent highly correlated SNPs or genetically similar morphs with shorter branches, whereas less correlated SNPs or more distant morphs are represented with longer

branches. The morphs were similarly grouped by the heatmaps (Figure 6a-c). However, the groupings were clearly differentiated when using the subset with the loci putatively under divergent selection (Figure 6c). The heatmap showed a similar pattern between the two profundal morphs, the Piscivore and the Dwarf morphs, clustering them together (Figure 6c). There were also some similarities among the Planktivore, the Piscivore and the Dwarf morphs in some of the loci with low MAF, where the Abyssal morph showed larger values (Figure 6c). The dendrogram clustered together the Abyssal and the Planktivore morphs, showing some similarities in specific loci (Figure 6c).

Discussion

We studied genome-wide patterns of divergence in four sympatric morphs of Arctic charr from Lake Tinnsjøen. We identified 1132 SNPs putatively under divergent selection. From these 1132 SNPs, we found 283 SNPs that had a larger contribution in the DAPC. Regarding population structure, both of these subsets (i.e. with 1132 and 283 SNPs, respectively) were successful in identifying the four genetic clusters in the DAPC. Arctic charr morphs still show gene flow between them, which suggests that the morphs could be at the early stages of reproductive isolation. These results are also supported by a genetic analysis based on microsatellite data from Lake Tinnsjøen, where the four morphs were separated into four different genetic clusters (Østbye et al., 2020). We also identified 10 chromosomal inversions along the Arctic charr genome. The Dwarf and Piscivore morphs were almost fixed for the same haplotypes in Chr30. The Abyssal and Planktivore morphs showed almost fixation for one haplotype in Chr12 and Chr23, respectively, whereas the Piscivore morph showed almost fixation for the alternative haplotype. Variations in the allele frequency of chromosomal inversions might be related to differences in behaviour and environmental conditions, as has been reported in other studies (Arostegui et al., 2019; Ayala et al., 2013, 2014; Berg et al., 2016; Kapun et al., 2016; Rane et al., 2015; Wellenreuther & Bernatchez, 2018). Thus, chromosomal inversions found in the Arctic charr genome could favour the morph divergence. We also identified a set of candidate genes involved in 119 biological functions, most likely highlighting distinct signals of adaptation among the four morphs living in different environments. We found a larger LD in the Piscivore and Abyssal morphs compared with the Dwarf and Plantivore morphs. The increase of LD could be caused by chromosomal inversions,

or it could be due to small population size most likely caused by a bottleneck (Slatkin, 2008), which could be the case in the Abyssal morph. Finally, we found differences in the allele frequency using different sets of loci, especially observed in the subset containing the loci putatively under divergent selection. Changes in the allele frequency from different populations most likely happen when there are differences in selection pressures leading to genetic divergence of markers, which originated by selection (Lamichhaney et al., 2012).

Genomic differentiation among the morphs in Lake Tinnsjøen suggests habitat-specific selection pressures leading to local adaptation. Local adaptation has been recorded in several salmonid species and it can occur in populations that live in habitats with different environmental conditions (Fraser et al., 2011; Garcia de Leaniz et al., 2007; Kawecki & Ebert, 2004; Taylor, 1991). At the population level, local adaptations can happen in the presence of gene flow (Garant et al., 2007), which might also be the case in the Arctic charr morphs from Lake Tinnsjøen. These morphs are likely exposed to different selection pressures, developing differences not only in the phenotype but also in the genotype. Thus, the loci putatively under divergent selection might be associated with local adaptation, which likely show functional divergence among Arctic charr morphs.

Local adaptation mechanism can favour chromosomal inversions, leading to the divergence of populations (Kirkpatrick & Barton, 2006). Chromosomal inversions can cause genomic islands (Barth et al., 2017; Berg et al., 2016; Kirubakaran et al., 2016; Sodeland et al., 2016). In addition, chromosomal inversions have been found between species and within polymorphic species, where inversions can contribute to the speciation process (Ayala & Coluzzi, 2005; Kandul et al., 2007; Kirubakaran et al., 2016; Noor et al., 2001). Inversions can reduce or suppress recombination (Kirkpatrick & Barton, 2006; Slatkin, 2008), and can be associated with adaptation with gene flow (Ayala & Coluzzi, 2005; Berg et al., 2017; Kandul et al., 2007; Noor et al., 2001), increasing the linkage disequilibrium. Furthermore, other studies in sticklebacks (Jones et al., 2012), herring (*Clupea harengus*; Lamichhaney et al., 2017) and rainbow trout (*Oncorhynchus mykiss;* Pearse et al., 2014) have also found differences in behaviour and habitat use related to chromosomal rearrangements. The set of genes found in chromosomal inversions could be involved in specific phenotypes responsible of the polymorphism within Arctic charr, which could favour adaptation and reproduction isolation. Thus, chromosomal inversions found in Arctic charr could play an important role in the

adaptation and speciation process, as has been previously reported (Berg et al., 2017; Kirkpatrick, 2010; Sodeland et al., 2016).

A set of loci were associated to functional genes in this study (e.g. generation of neurons, neuron differentiation, regulation of neurogenesis, neurogenesis, and brain, eye and inner ear development) that could play a role in the divergence of Arctic charr morphs. Organs such as brain, eye, and ear most likely vary among morphs associated with habitat and diet preferences. A small brain size can be a strategy to save energy, especially in habitats with low resources, whereas a reduction in the eye size can be related to the lack of light (Eifert et al., 2015), where the decrease of the importance of vision might be associated with an increase of depth (Kotrschal et al., 1998). Light and food constraints seem to affect the brain and the eye morphology of these Arctic charr morphs, developing small brain regions and small eyes in the Abyssal morph, whereas the other three morphs living in habitats with more light and food resources presented larger eyes and brain regions (Tamayo et al., 2020). For instance, the Piscivore and the Planktivore morphs showed the largest optic tectum, which could be associated with well-illuminated habitats, whereas the Abyssal morph showed a larger olfactory bulb in comparison with the optic tectum size, which could be associated with a larger importance of the smell perception (Tamayo et al., 2020). These four morphs also differed in traits such as body size, age, coloration, which could be related to living in specific environmental conditions (Østbye et al., 2020). This phenotypic diversity among the morphs most likely implies adaptive sensory divergence driven by natural selection related to the occupation of different habitats.

Polymorphic species such as Arctic charr are particularly interesting because they offer us the opportunity to study how the early stages of adaptive radiation looks like (Yoder et al., 2010). Regarding resource polymorphism, it is not clear whether is due to phenotypic plasticity, genetic basis or a combination of both, which can occur when there is intraspecific morphs that can differ in resource use (Skúlason et al., 2019). The divergence in the phenotype of Arctic charr morphs most likely is driven by selection where specific genes might be involved, contributing to their differentiation. Additionally, phenotypic plasticity might be responsible for differences in certain traits among the morphs, decreasing the detection of other differences between phenotypes and genotypes (Lucek et al., 2014). Phenotypic plasticity likely plays an important role in the early stage of reproductive isolation in the Arctic charr

morphs from Lake Tinnsjøen. Thus, phenotypic plasticity may contribute to differences such as growth, maturity, and time and place of spawning. In addition, the interaction between factors such as recombination and selection can contribute to maintaining the genetic differentiation among populations (Feder & Nosil, 2009), which might vary with chromosomal rearrangements.

Conclusion

This study provided in-depth results on genetic divergence, genome-wide differentiation, and putative biological functions of loci under divergent selection in four morphs of Arctic charr in Lake Tinnsjøen. There were chromosomal inversions along the genome, which might reduce gene flow in these regions, suppressing or reducing recombination. We also identified a set of genes that were associated with biological functions, which could influence specific phenotypes depending on the environment. Thus, these genes and chromosomal inversions likely contribute to the divergence of these four sympatric morphs caused by local adaptation. Lake Tinnsjøen offers a unique system to understand the mechanisms involved during early stages of reproductive isolation and ecological speciation. Further research is needed to improve our understanding of factors influencing the evolutionary processes in different populations and the origin of these differences.

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Author contribution

AMPT: Concept and idea. AMPT; SB; KØ; JBT; KP: Study design, methods, data gathering and interpretation. AMPT conducted the bioinformatics and statistical analyses with inputs from SB, KØ and KP. AMPT wrote the manuscript with contribution from all the authors.

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Figure legends

Figure 1. Manhattan plot of genome-wide association study showing the distribution of pairwise differentiation (F_{st}, ranging from 0 to 1) across the genome for all 6 morph comparisons of Arctic charr. Each grey dot shows a single SNP pairwise F_{st} estimate and the coloured triangles are the loci putatively under divergent selection, with non-overlapping 10-kb sliding windows across the genome. From internal to external circles, it shows F_{st} between: Abyssal-Dwarf (highlighted in blue are SNPs putatively under divergent selection), Piscivore-Abyssal (green), Piscivore-Dwarf (red), Piscivore-Planktivore (orange), Planktivore-Abyssal (purple), Planktivore-Dwarf (pink). Chromosome names correspond to the following NCBI accession numbers: Chr1= NC_036838.1; Chr2= NC_036839.1; Chr3= NC_036840.1; Chr4= NC_036841.1; Chr5= NC_036842.1; Chr6= NC_036843.1; Chr7= NC_036844.1; Chr8= NC_036845.1; Chr9= NC_036846.1; Chr10= NC_036847.1; Chr11= NC_036848.1; Chr12= NC_036849.1; Chr13= NC_036850.1; Chr14= NC_036851.1; Chr15= NC_036852.1; Chr16= NC_036853.1; Chr17= NC_036854.1; Chr18= NC_036855.1; Chr19= NC_036856.1; Chr20= NC_036857.1; Chr21= NC_036858.1; Chr22= NC_036859.1; Chr23= NC_036860.1; Chr24= NC_036861.1; Chr25= NC_036862.1; Chr26= NC_036863.1; Chr27= NC_036864.1; Chr28= NC_036865.1; Chr29= NC_036866.1; Chr30= NC_036867.1; Chr31= NC_036868.1; Chr32= NC_036869.1; Chr33= NC_036870.1; Chr34= NC_036871.1; Chr35= NC_036872.1; Chr36= NC_036873.1; Chr37= NC_036874.1; Chr38= NC_036875.1; Chr39= NC_036876.1. There was a total of 1132 SNPs, note that some loci were shared among comparisons.

Figure 2. Frequency of haplotypes within chromosomal inversions for the four Arctic charr morphs. From top left to right bottom: Chr12 (28.54-29.25 Mb), Chr15 (11.30-11.34 Mb), Chr17 (14.89-16.81 Mb), Chr19 (26.67-28.75 Mb), Chr23 (43.59-45.98 Mb), Chr30 (14.63-15.72 Mb), Chr32 (11.31-13.24 Mb), Chr34 (28.19-28.62 Mb), Chr35 (12.35-12.38 Mb) and Chr38 (22.95-23.08 Mb).

Figure 3. Bar plots of the biological process, cellular component and molecular function categories obtained from the gene set using WebGestalt software.

Figure 4. Genetic relationships among individuals from all populations assessed using discriminant analysis of principal components (DAPC). (a) Based on the whole set of SNPs

(16895 SNPs). (b) Based on the subset of putatively neutral loci (15763 SNPs). (c) Based on the subset of loci putatively under divergent selection (1132 SNPs). Abbreviation of Arctic charr morphs: Abyssal morph (Ab); Dwarf morph (Dw); Piscivore morph (Pi) and Planktivore morph (Pl).

Figure 5. (a) Loading plot based on the DAPC analyses shows the contribution of each SNP. From a total of 1132 SNPs, there were 283 SNPs that showed the largest contribution (i.e. SNPs above the threshold are delimited with a grey dashed line). (b) DAPC for the 283 SNPs found in the loading plot. There were 2 chromosomes with the largest number of SNPs, (c) Chr5 and (d) Chr26. Abbreviation morphs: Abyssal morph (Ab); Dwarf morph (Dw); Piscivore morph (Pi) and Planktivore morph (Pl).

Figure 6. Heatmap of the allele frequencies for each SNP in the four morphs of Arctic charr. (a) Based on the whole set of SNPs (16895 SNPs). (b) Based on the subset of putatively neutral loci (15763 SNPs). (c) Based on the subset of loci putatively under divergent selection (1132 SNPs).
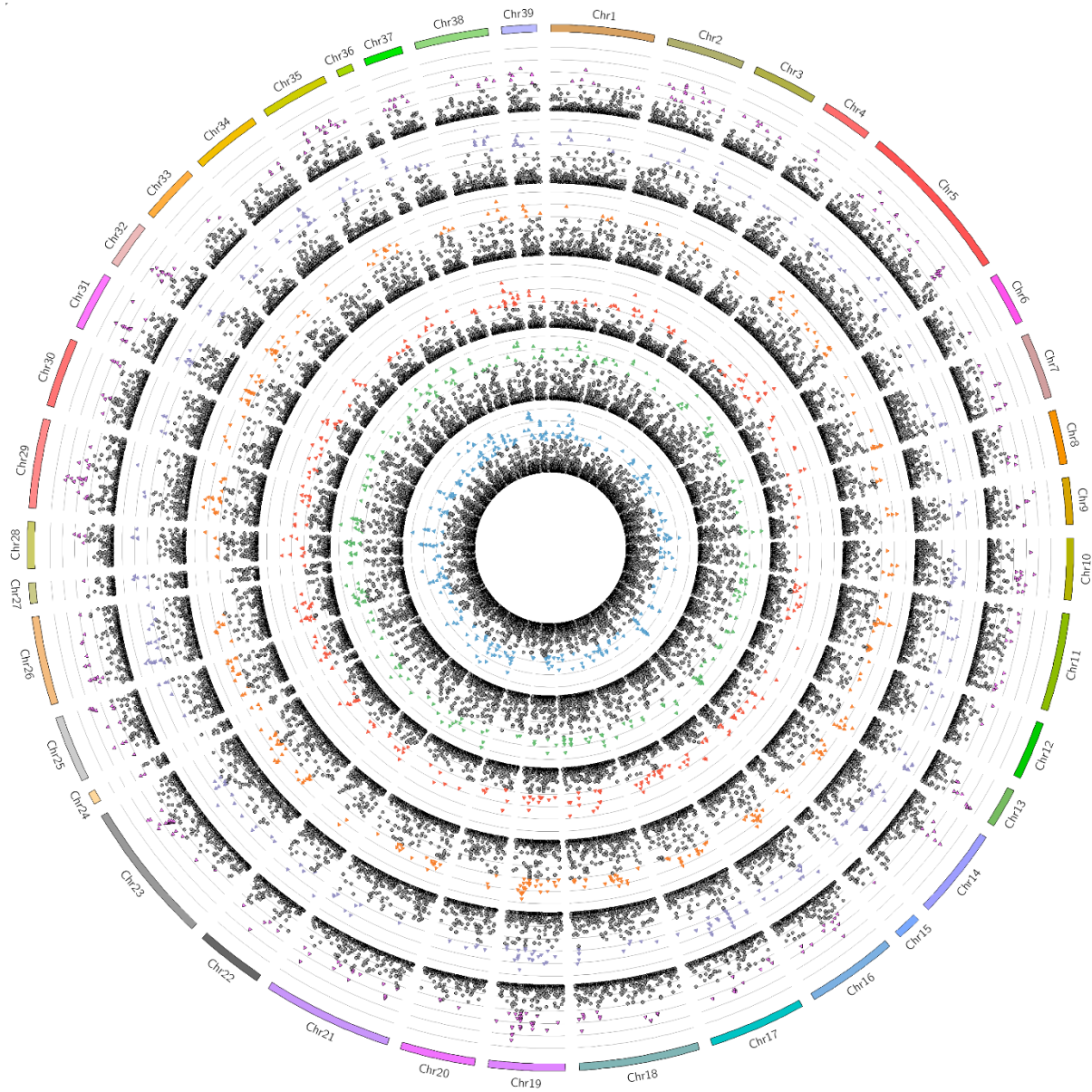
Figures

Figure 1.
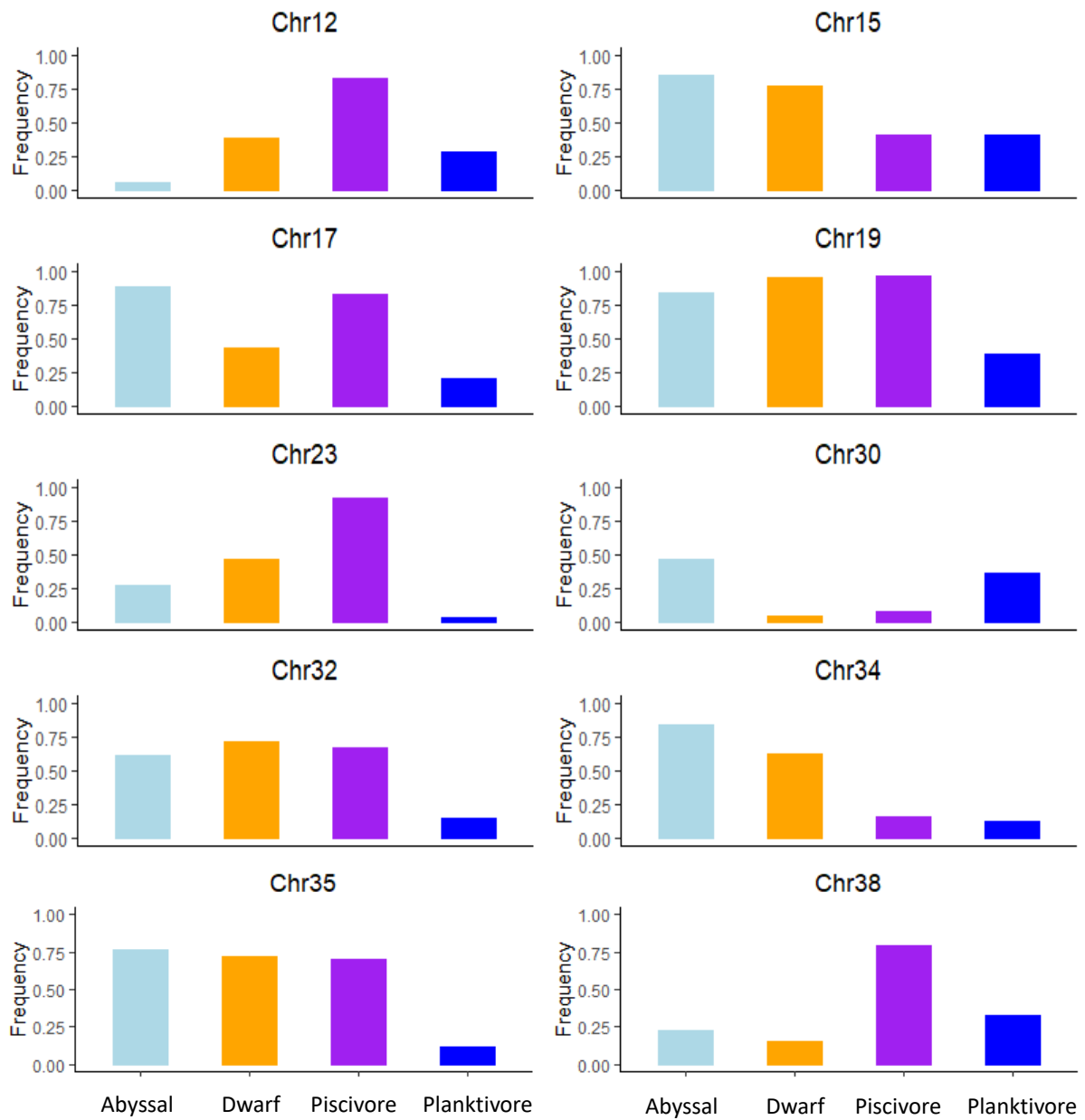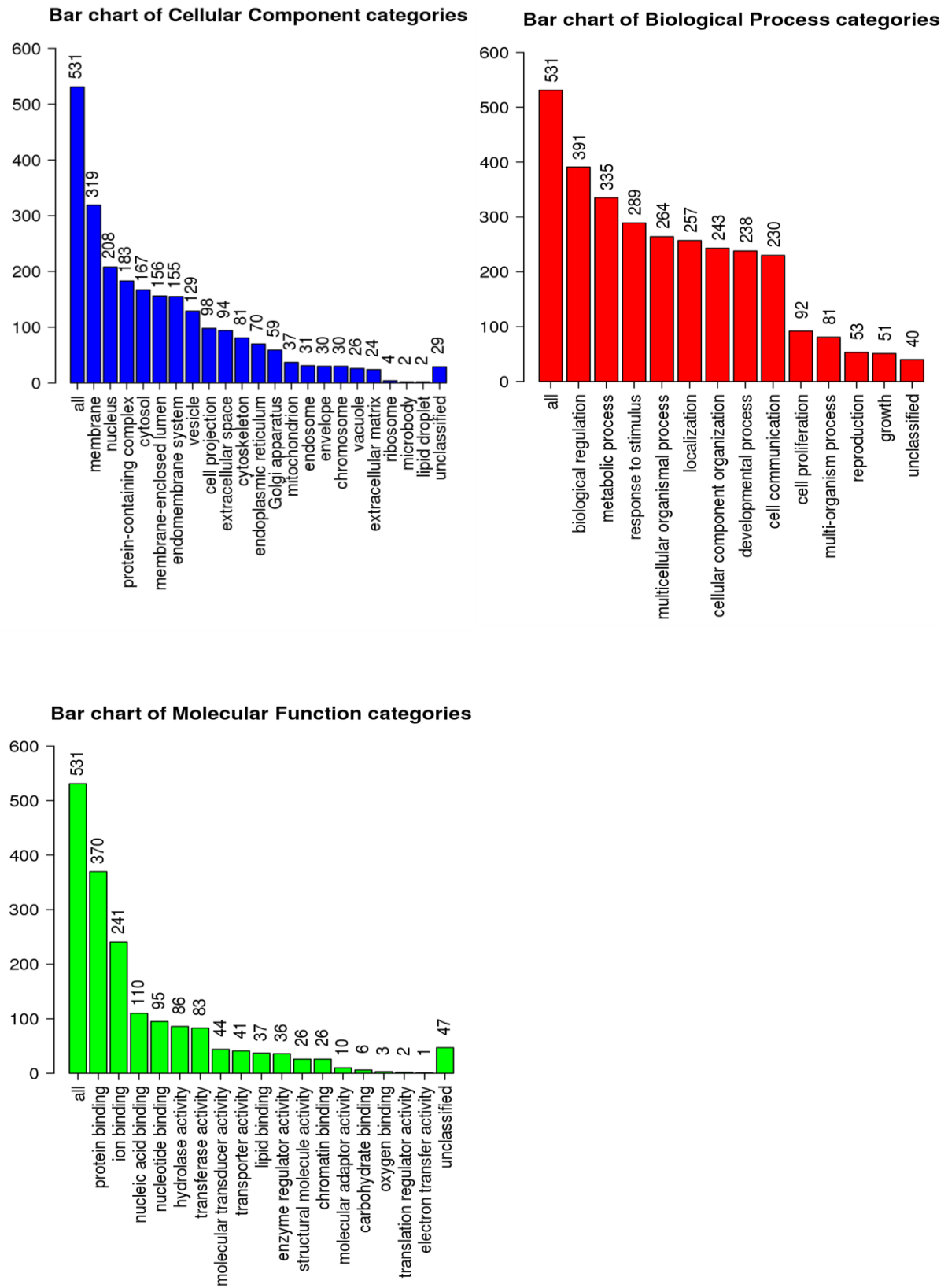
Figure 2.

Figure 3.

Bar chart of Cellular Component categories

Bar chart of Biological Process categories

Bar chart of Molecular Function categories
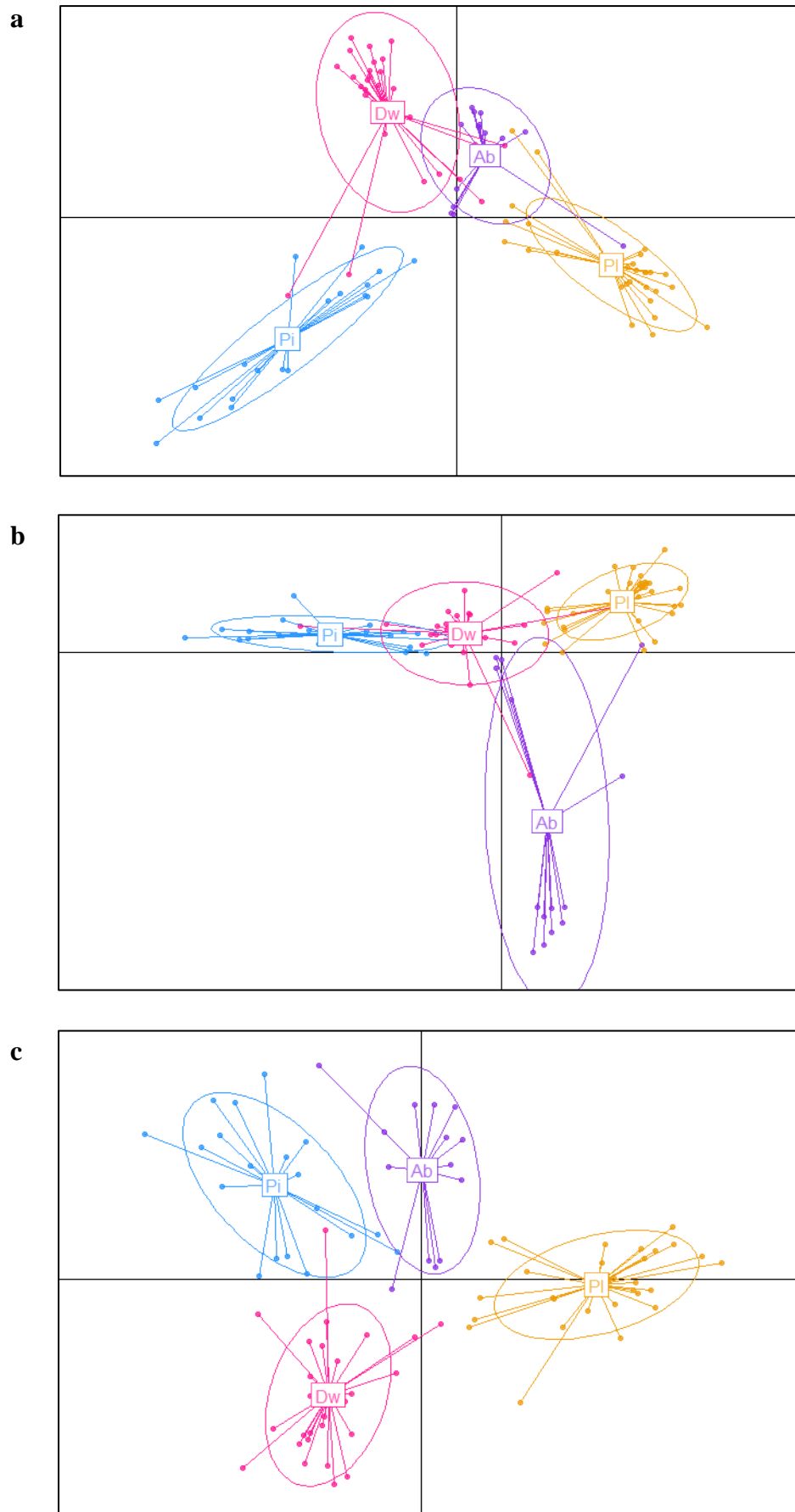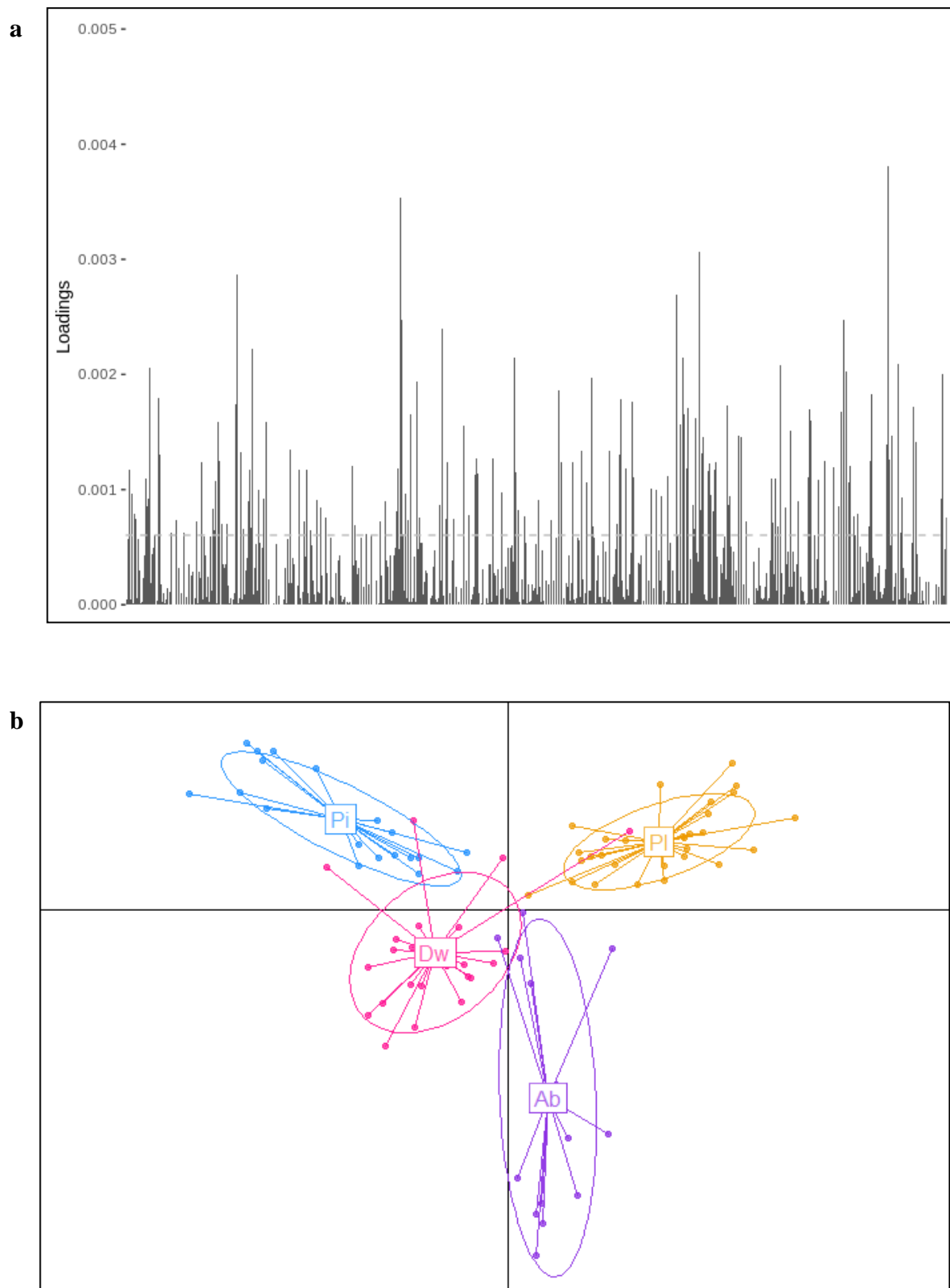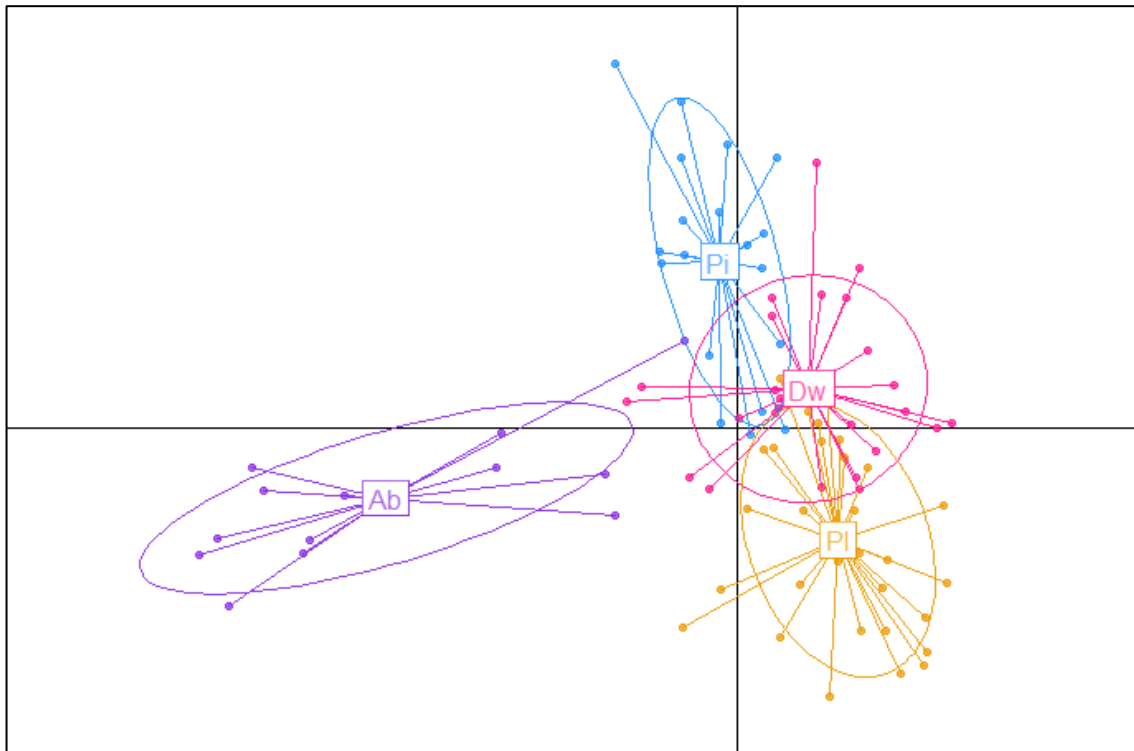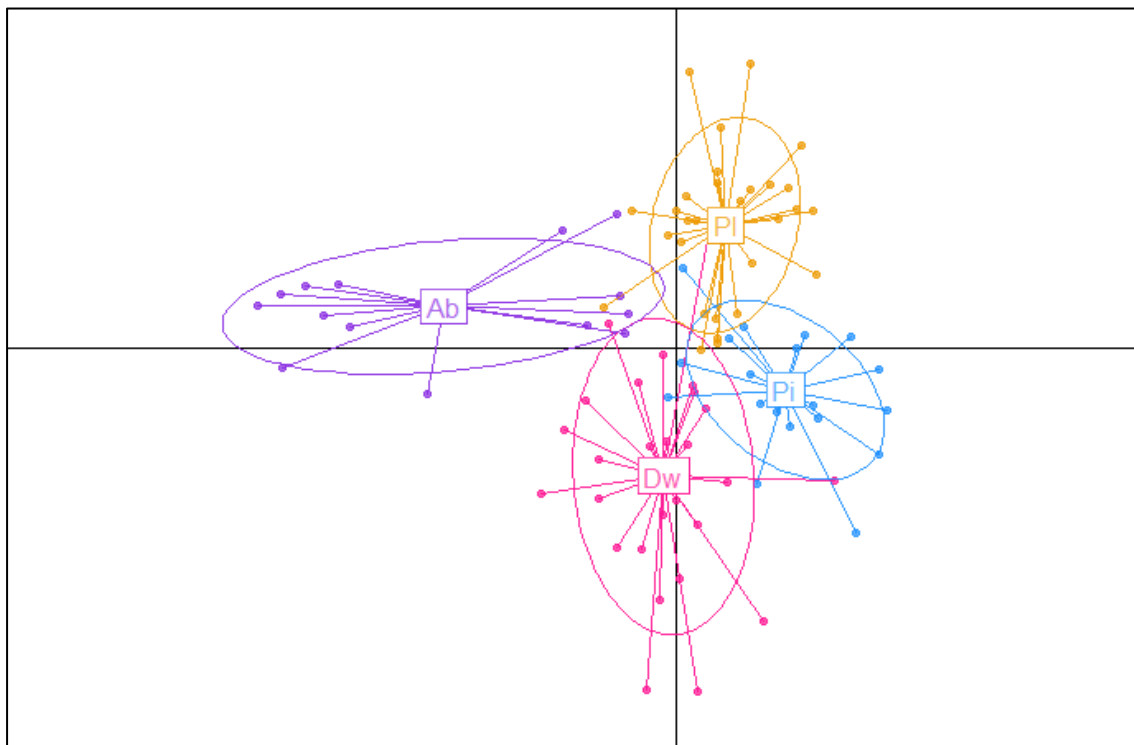
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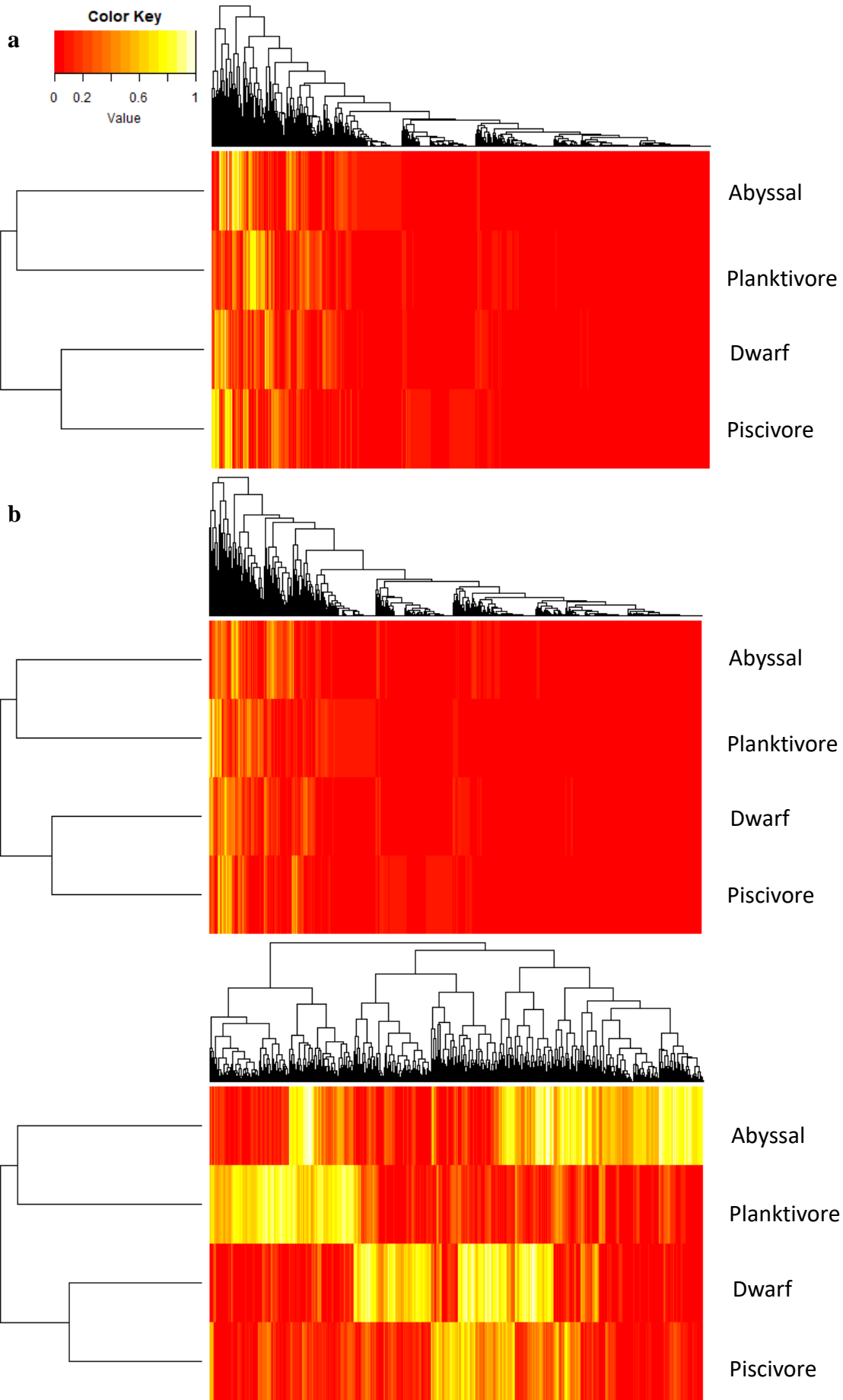
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Figure 6.

Table 1. Summary table of Gene Ontology (GO) enrichment results showing gene set obtained from Overrepresentation Enrichment Analysis (ORA) method. Significance level of *P*< 0.05 and false discovery rate (FDR) < 0.05*.* Top 10 GOs are the ten first gene sets.

Gene set	Description	*P*-value	FDR
GO:0048699	generation of neurons	8.5176E-13	9.3421E-09
GO:0030182	neuron differentiation	6.2623E-12	2.3873E-08
GO:0050767	regulation of neurogenesis	8.1821E-12	2.3873E-08
GO:0050808	synapse organization	8.7066E-12	2.3873E-08
GO:0022008	neurogenesis	1.2706E-11	2.7872E-08
GO:0048667	cell morphogenesis involved in neuron differentiation	4.7261E-11	8.4331E-08
GO:0051960	regulation of nervous system development	5.3822E-11	8.4331E-08
GO:0045664	regulation of neuron differentiation	7.2593E-11	9.9526E-08
GO:0010769	regulation of cell morphogenesis involved in differentiation	9.9139E-11	1.2082E-07
GO:0060284	regulation of cell development	1.1269E-10	1.236E-07
GO:0099173	postsynapse organization	1.5719E-10	1.5673E-07
GO:0048666	neuron development	1.9381E-10	1.7714E-07
GO:0016477	cell migration	2.9643E-10	2.1623E-07
GO:0031175	neuron projection development	3.0342E-10	2.1623E-07
GO:0007155	cell adhesion	3.0701E-10	2.1623E-07
GO:0022604	regulation of cell morphogenesis	3.2972E-10	2.1623E-07
GO:0120039	plasma membrane bounded cell projection morphogenesis	3.5312E-10	2.1623E-07
GO:0048858	cell projection morphogenesis	3.7255E-10	2.1623E-07
GO:0010975	regulation of neuron projection development	3.7457E-10	2.1623E-07
GO:0022610	biological adhesion	4.0569E-10	2.2248E-07
GO:0048812	neuron projection morphogenesis	5.1215E-10	2.6681E-07
GO:0120035	regulation of plasma membrane bounded cell projection organization	5.3518E-10	2.6681E-07

GO:2000026	regulation of multicellular organismal development	7.1117E-10	3.3214E-07
GO:0000904	cell morphogenesis involved in differentiation	7.2678E-10	3.3214E-07
GO:0031344	regulation of cell projection organization	8.475E-10	3.7182E-07
GO:0032990	cell part morphogenesis	1.0057E-09	4.2425E-07
GO:0040011	locomotion	1.0688E-09	4.3419E-07
GO:0006928	movement of cell or subcellular component	1.7706E-09	6.9358E-07
GO:0050770	regulation of axonogenesis	2.1729E-09	8.2179E-07
GO:0022603	regulation of anatomical structure morphogenesis	2.8983E-09	1.0596E-06
GO:0050807	regulation of synapse organization	3.1649E-09	1.1198E-06
GO:0048870	cell motility	3.4481E-09	1.146E-06
GO:0051674	localization of cell	3.4481E-09	1.146E-06
GO:0050803	regulation of synapse structure or activity	5.5903E-09	1.8034E-06
GO:0030334	regulation of cell migration	5.9107E-09	1.8522E-06
GO:0000902	cell morphogenesis	1.0847E-08	3.3048E-06
GO:2000145	regulation of cell motility	2.5921E-08	7.6839E-06
GO:0051130	positive regulation of cellular component organization	2.6843E-08	7.7477E-06
GO:0032989	cellular component morphogenesis	2.9329E-08	8.2481E-06
GO:0120036	plasma membrane bounded cell projection organization	3.1039E-08	8.3779E-06
GO:0043113	receptor clustering	3.2082E-08	8.3779E-06
GO:0051049	regulation of transport	4.4361E-08	1.0812E-05
GO:0007268	chemical synaptic transmission	8.5869E-08	1.9621E-05
GO:0051336	regulation of hydrolase activity	4.0356E-07	0.00007904
GO:0007010	cytoskeleton organization	1.0527E-06	0.0001804
GO:0007411	axon guidance	1.8087E-06	0.00027175
GO:0010721	negative regulation of cell development	1.9595E-06	0.00028656
GO:0035639	purine ribonucleoside triphosphate binding	2.0562E-06	0.00029674
GO:0044087	regulation of cellular component biogenesis	2.1472E-06	0.0002981

GO:0008283	cell proliferation	2.2624E-06	0.00030634
GO:0032555	purine ribonucleotide binding	2.4767E-06	0.00033128
GO:0023057	negative regulation of signaling	2.6998E-06	0.00034836
GO:0070723	response to cholesterol	4.7797E-06	0.00054268
GO:0010648	negative regulation of cell communication	4.8212E-06	0.00054268
GO:0051048	negative regulation of secretion	4.8489E-06	0.00054268
GO:0030036	actin cytoskeleton organization	5.2843E-06	0.00057385
GO:0060443	mammary gland morphogenesis	5.3461E-06	0.00057486
GO:0050769	positive regulation of neurogenesis	6.1553E-06	0.00064805
GO:0035305	negative regulation of dephosphorylation	9.8499E-06	0.00094766
GO:0001654	eye development	9.9908E-06	0.00095286
GO:0007045	cell-substrate adherens junction assembly	1.5402E-05	0.0013515
GO:0050772	positive regulation of axonogenesis	1.9623E-05	0.0016145
GO:0019199	transmembrane receptor protein kinase activity	1.9623E-05	0.0016145
GO:0060997	dendritic spine morphogenesis	2.1041E-05	0.0016969
GO:0051271	negative regulation of cellular component movement	2.1653E-05	0.0017335
GO:0048598	embryonic morphogenesis	2.2775E-05	0.0018009
GO:0042127	regulation of cell proliferation	2.3478E-05	0.0018393
GO:0002009	morphogenesis of an epithelium	2.4995E-05	0.0019171
GO:0001764	neuron migration	3.0805E-05	0.0022984
GO:0098742	cell-cell adhesion via plasma-membrane adhesion molecules	3.7832E-05	0.0027663
GO:0043547	positive regulation of GTPase activity	3.9133E-05	0.0028053
GO:0014065	phosphatidylinositol 3-kinase signaling	4.1146E-05	0.0029116
GO:0051963	regulation of synapse assembly	4.3957E-05	0.0030514
GO:0032940	secretion by cell	6.3497E-05	0.0041262
GO:0071801	regulation of podosome assembly	6.4734E-05	0.0041262
GO:0005543	phospholipid binding	6.4836E-05	0.0041262
GO:0030038	contractile actin filament bundle assembly	6.5488E-05	0.0041262

GO:0010977	negative regulation of neuron projection development	0.00009593	0.0056873
GO:0034613	cellular protein localization	0.00011368	0.0064606
GO:0040008	regulation of growth	0.0001197	0.0066982
GO:0045197	establishment or maintenance of epithelial cell apical/basal polarity	0.00012761	0.0069981
GO:2000177	regulation of neural precursor cell proliferation	0.00013111	0.0071541
GO:0031267	small GTPase binding	0.00013963	0.0075072
GO:0008584	male gonad development	0.00014125	0.0075573
GO:0003013	circulatory system process	0.00014653	0.0077641
GO:0015833	peptide transport	0.00015159	0.0079038
GO:0042981	regulation of apoptotic process	0.00015277	0.0079038
GO:0007265	Ras protein signal transduction	0.00017194	0.0086111
GO:0007623	circadian rhythm	0.00017936	0.008782
GO:0019900	kinase binding	0.00018861	0.0091132
GO:0031683	G-protein beta/gamma-subunit complex binding	0.00020889	0.0097081
GO:0016301	kinase activity	0.00021097	0.0097634
GO:0061387	regulation of extent of cell growth	0.00021717	0.0099778
GO:0051223	regulation of protein transport	0.00021742	0.0099778
GO:0097106	postsynaptic density organization	0.00021907	0.010011
GO:0035239	tube morphogenesis	0.00029832	0.012933
GO:0001932	regulation of protein phosphorylation	0.00034144	0.014566
GO:0051272	positive regulation of cellular component movement	0.00034264	0.014566
GO:0018108	peptidyl-tyrosine phosphorylation	0.0003557	0.014722
GO:0045936	negative regulation of phosphate metabolic process	0.00036912	0.015075
GO:0007420	brain development	0.00041395	0.016332
GO:0001736	establishment of planar polarity	0.00043159	0.016552
GO:1990778	protein localization to cell periphery	0.00047168	0.017901

GO:0071559	response to transforming growth factor beta	0.00054874	0.020265
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	0.00059954	0.021212
GO:0043549	regulation of kinase activity	0.00062141	0.021569
GO:0051496	positive regulation of stress fiber assembly	0.00068039	0.023321
GO:0016570	histone modification	0.00073651	0.024703
GO:0030097	hemopoiesis	0.0007606	0.025356
GO:0010558	negative regulation of macromolecule biosynthetic process	0.00078896	0.026064
GO:0051254	positive regulation of RNA metabolic process	0.00080778	0.026447
GO:0022038	corpus callosum development	0.0009337	0.029512
GO:0048839	inner ear development	0.00094775	0.02987
GO:0097688	glutamate receptor clustering	0.0011882	0.035608
GO:0032269	negative regulation of cellular protein metabolic process	0.0013794	0.040561
GO:0010647	positive regulation of cell communication	0.0013857	0.040638
GO:0048066	developmental pigmentation	0.001532	0.043419
GO:0045580	regulation of T cell differentiation	0.0016042	0.045
GO:0048771	tissue remodeling	0.0016732	0.046342

Figure S1. Heatmap of pairwise linkage disequilibrium measured by D' for each morph between all possible pairs of loci putatively under divergent selection (1132 SNPs). The scale colour ranges from low (white) to high (dark blue) values of D'.
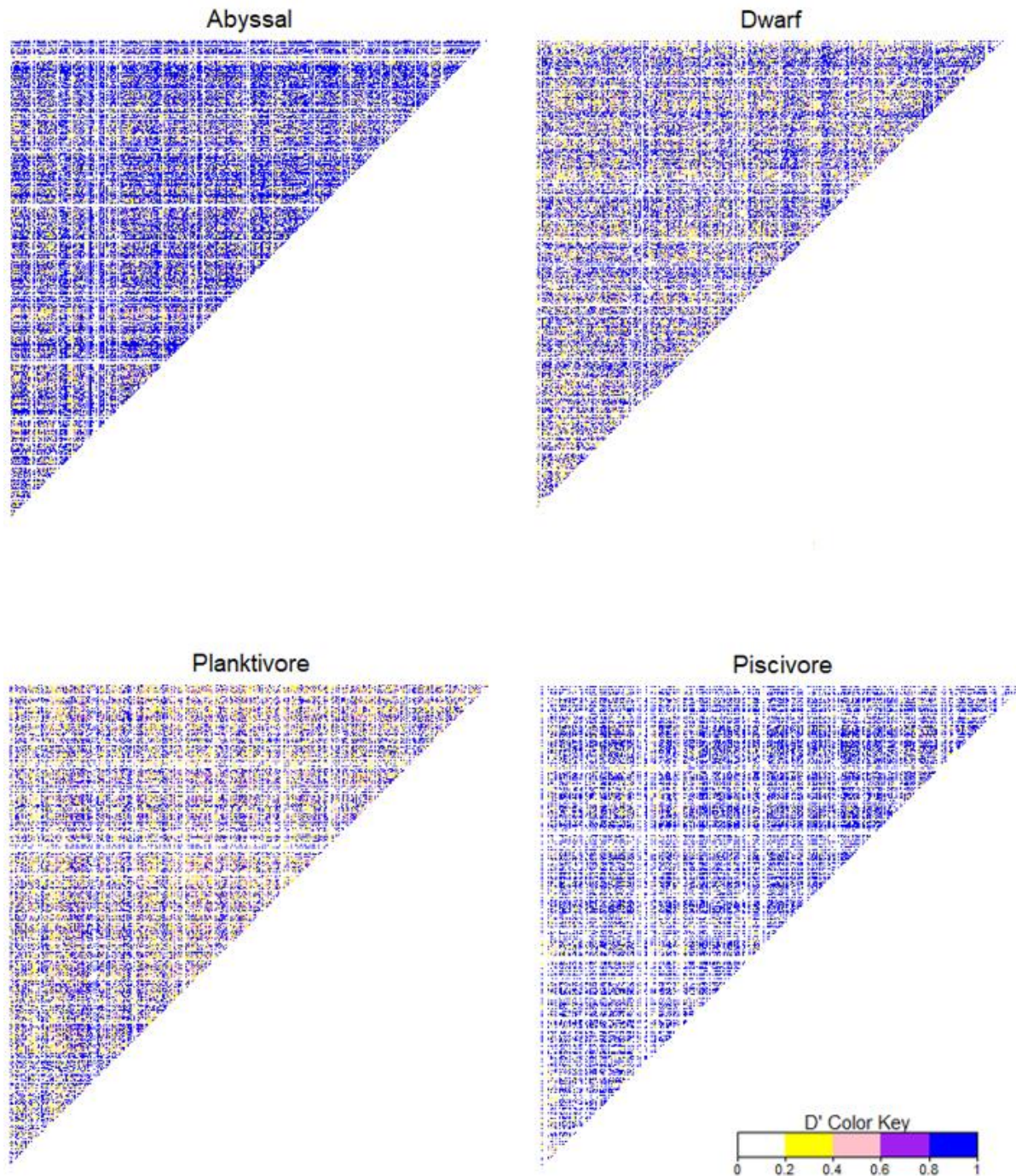
Figure S2. Population structure analysis based on the whole set of SNPs (16895 SNPs). Individual admixture proportions are shown assuming numbers of genetic clusters (K) ranging from 2 to 7 using CLUMPAK software.
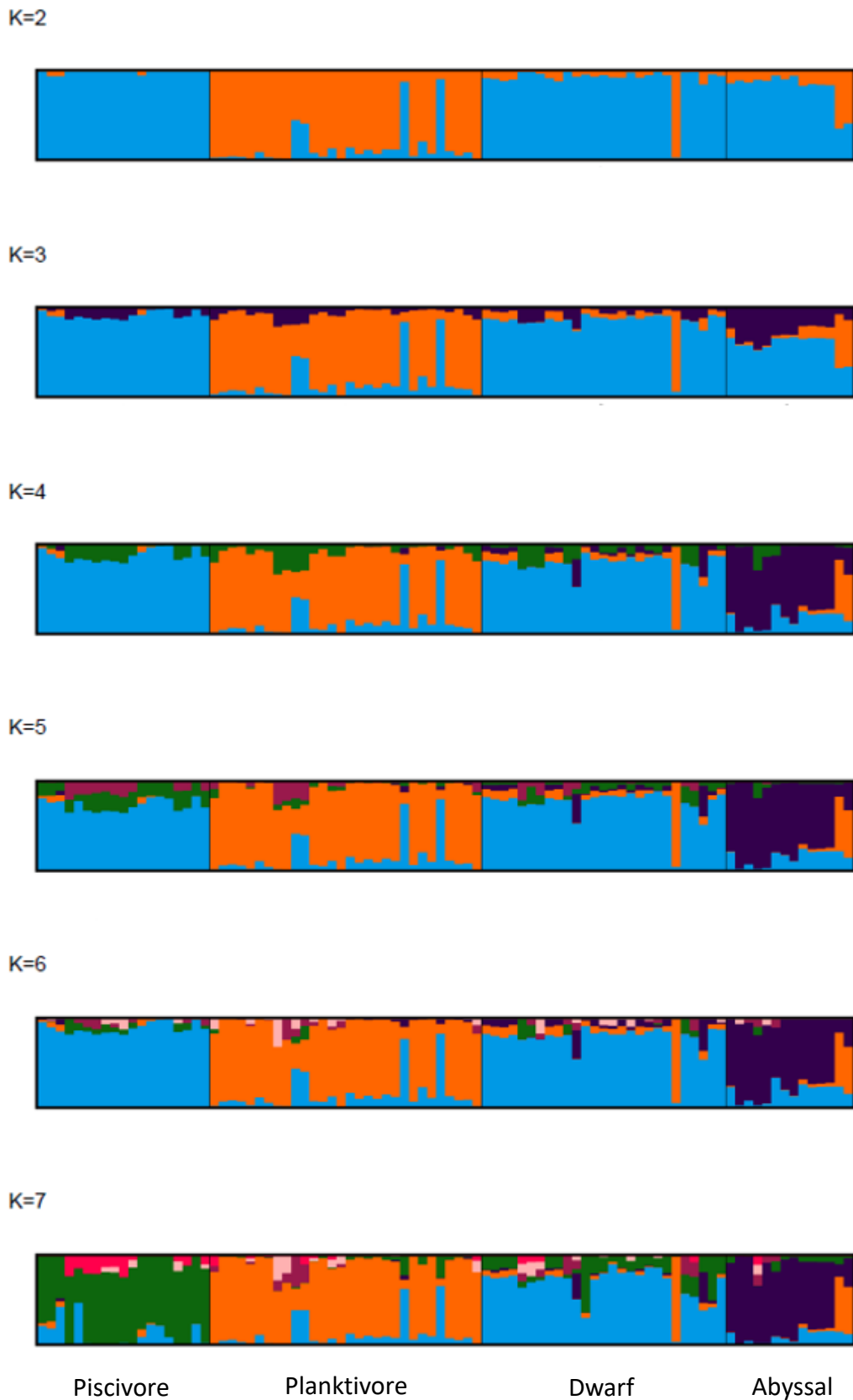
K=2

K=3

K=4

K=5

K=6

K=7

| Piscivore | Planktivore | Dwarf | Abyssal |

Figure S3. Population structure analysis based on the subset of putatively neutral loci (15763 SNPs). Individual admixture proportions are shown assuming numbers of genetic clusters (K) ranging from 2 to 7 using CLUMPAK software.
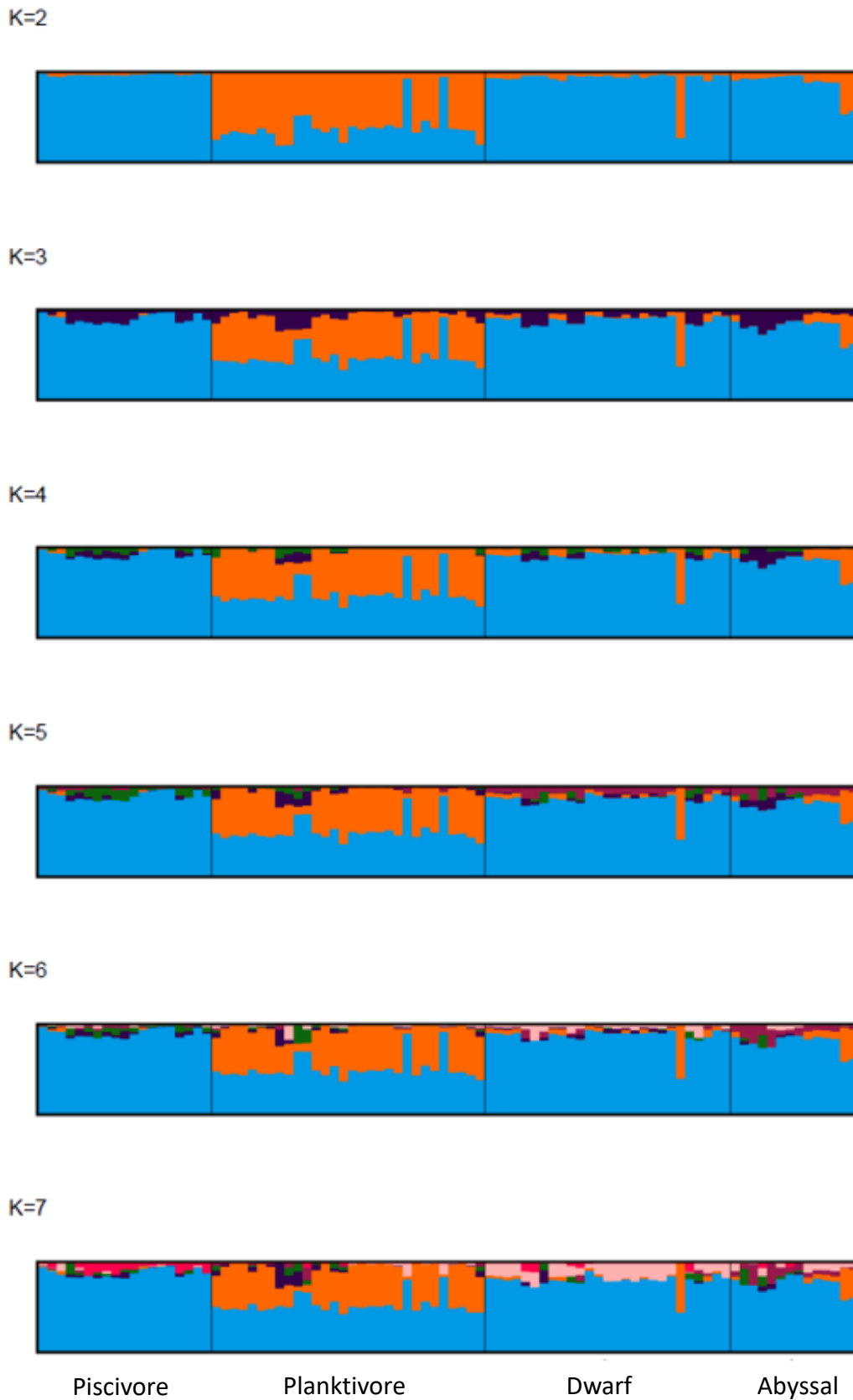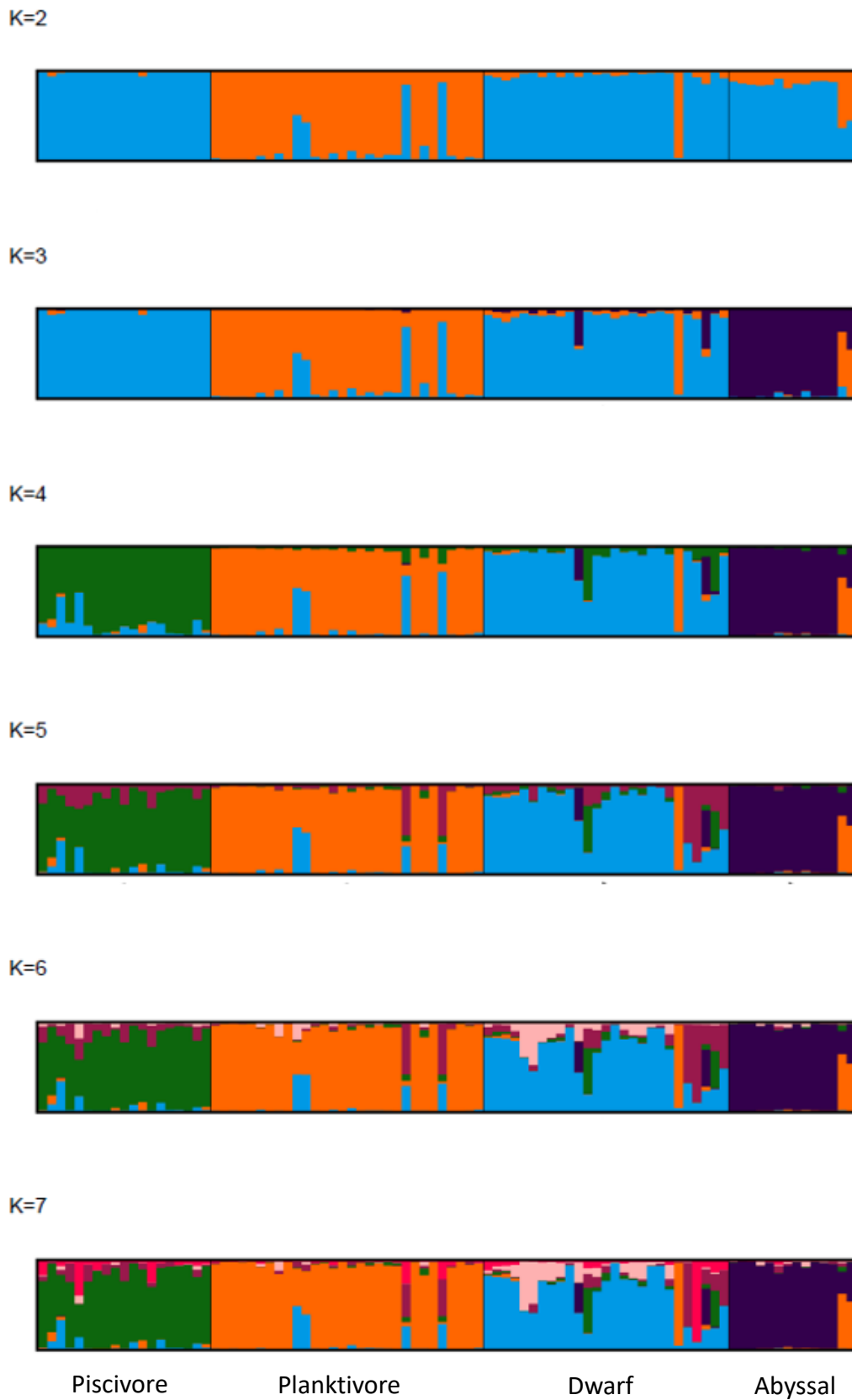
Figure S4. Population structure analysis based on the subset of loci putatively under divergent selection (1132 SNPs). Individual admixture proportions are shown assuming numbers of genetic clusters (K) ranging from 2 to 7 using CLUMPAK software.

4

CHARR III

Contrasting patterns in trophic niche evolution of polymorphic Arctic charr populations in two subarctic Norwegian lakes

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Abstract Parallelism in trophic niches of polymorphic populations of Arctic charr was investigated in two similar subarctic lakes, Tårnvatn and Skøvatn, in northern Norway. Analysis of eleven microsatellite loci confirmed, respectively, the existence of three and two genetically differentiated morphs. Three methods were used to describe their trophic niches: habitat choice and stomach contents for the recent feeding behaviour, and trophically transmitted parasites and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) as proxies for the longer term trophic niche differences. The results showed a distinct segregation in trophic resource utilization of the different morphs. Tårnvatn has three morphs: a littoral omnivorous (LO), a small-sized profundal benthivorous (PB), and a large-sized profundal piscivorous (PP). In contrast, a novel Arctic charr morph was discovered in Skøvatn: a small-sized profundal zooplanktivorous-morph (PZ), which when compared to the sympatric LO-morph, had distinct stable isotope values and a contrasting parasite community. A parallelism in habitat choice and external morphology was found among the small-sized, deep-water morphs and between the upper-water, omnivorous LO-morphs in both lakes. There was a no parallel pattern in diet choice between the PB- and the PZ-morphs. These findings show how evolution can produce diverse outcomes, even among

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systems with apparently similar environmental and ecological conditions.

Introduction

A resource polymorphism is defined as the occurrence of distinct morphs specialized in different resource use within a single species (Skúlason & Smith, 1995). Polymorphic populations of several fish species have repeatedly been found in postglacial lakes, especially within the genera *Salvelinus*, *Gasterosteus*, and *Coregonu*s (Skúlason & Smith, 1995; Skúlason et al., 1999; Amundsen et al., 2008; Klemetsen, 2013). Since polymorphisms are considered to be an important step in an ecologically induced speciation process (Wimberger, 1994; Gíslason et al., 1999; Snorrason & Skúlason, 2004; Amundsen et al., 2008; Siwertsson et al., 2013a), freshwater systems of recent origin are viewed as hotspots for investigating the function and role of ecological components in divergent evolution (Schluter, 1996; Snorrason & Skúlason, 2004; Klemetsen, 2010). Similar ecological niches and environments in many isolated postglacial lakes have resulted in parallel adaptations in the morphology, behaviour, physiology, and life-history traits of several fish species (Endler, 1986; Schluter, 2000), including Arctic charr, *Salvelinus alpinus* (L.) (Skúlason & Smith, 1995; Klemetsen, 2010), which is the target species of the present study.

The initial step in the evolutionary divergence of northern fishes has been suggested to be competition for discrete habitats and food resources, which allow fish to specialize and segregate in distinctive niches (Wimberger, 1994; Skúlason & Smith, 1995; Jonsson & Jonsson, 2001; Adams et al., 2003; Garduño-Paz & Adams, 2010). A repeatedly found pattern of trophic niche segregation in postglacial lakes occurs along the benthic-pelagic resource axis, with benthivorous morphs exploiting the littoral area, and planktivorous and/or piscivorous morphs residing in the pelagic zone (Wimberger, 1994; Skúlason & Smith, 1995; Schluter, 1996; Sigursteinsdóttir & Kristjánsson, 2005). The

degree of divergence within lakes varies considerably, with containing completely reproductively isolated morphs (populations) and other showing variable levels of reproductive isolation within a common species (Gíslason et al., 1999; Skúlason et al., 1999; Hendry et al., 2009; Klemetsen, 2010). The frequent incidents of parallel evolution observed in several fish taxa, such as e.g. Arctic charr and three-spined stickleback (*Gasterosteus aculeatus* L.), are considered strong evidence of ecologically induced divergence, as they are unlikely to arise solely by genetic drift or other nonecological mechanisms (Schluter & Nagel, 1995; Schluter, 1996, 2001; Nosil & Rundle, 2009; Kaeuffer et al., 2012; Saltykova et al., 2017).

Arctic charr is considered to be a highly variable and plastic species, showing a myriad of differences in coloration, morphology, ecology, and life history traits (Johnson, 1980; Skúlason et al., 1999; Alexander & Adams, 2000; Jonsson & Jonsson, 2001; Klemetsen, 2010). Polymorphic Arctic charr may represent two (e.g. Fjellfrøsvatn; Klemetsen et al., 1997), three (e.g. Loch Rannoch; Adams et al., 1998), and even four (e.g. Thingvallavatn; Sandlund et al., 1992) distinct morphs within a single lake (Smith & Skúlason, 1996; Jonsson & Jonsson, 2001; Klemetsen, 2010; Jacobs et al., 2018). The evolution of phenotypic and ecological divergence in Arctic charr has mediated the accumulation of genetic differences among the morphs both when occurring as allopatric and polymorphic sympatric morphs (e.g. Gomez-Uchida et al., 2008; Power et al., 2009; Præbel et al., 2016; Jacobs et al., 2018; O'Malley et al., 2019). Most Arctic charr morphs are segregated along the littoral-pelagic axis, but deep-water living Arctic charr morphs adapted to the profundal habitat have also been described in a few lakes (Klemetsen, 2010; Markevich & Esin, 2018). The present study addresses the trophic niche utilization of polymorphic Arctic charr populations in two subarctic lakes, with special emphasis on the trophic ecology of profundal-dwelling morphs.

Two well-studied examples of profundal Arctic charr morphs are those in lakes Fjellfrøsvatn and Skogsfjordvatn, northern Norway (Klemetsen et al., 1997; Knudsen et al., 2006, 2016a, b; Amundsen et al., 2008; Smalås et al., 2013). In both lakes, there are two distinct, replicated morphs: a littoral spawning omnivorous 'LO-morph' feeding on littoral macrobenthos and zooplankton, and a small-sized profundal spawning benthivorous 'PB-morph' that forages on soft-

bottom benthic invertebrates (Klemetsen et al., 1997; Smalås et al., 2013). Additionally, Skogsfjordvatn hosts a rare profundal spawning piscivorous 'PP-morph' that feeds mostly on conspecific Arctic charr and, to a lesser extent, on three-spined stickleback (Smalås et al., 2013; Knudsen et al., 2016b). Within each lake the different morphs are clearly segregated in habitat and diet, as reflected by their stable isotope values and parasite loads (e.g. Knudsen et al., 2016a, Siwertsson et al., 2016), and in life history strategies and morphology (e.g. Smalås et al., 2013; Skoglund et al., 2015). The different morphs were first classified on the basis of external morphological functional traits including: body and head shape, eye and mouth size, and coloration (Knudsen et al., 2007; Skoglund et al., 2015; Saltykova et al., 2017; Simonsen et al., 2017), and have subsequently been shown to be reproductively isolated (Klemetsen et al., 1997; Smalås et al., 2017) and genetically distinct based on microsatellite loci (Præbel et al., 2016; Simonsen et al., 2017).

Recent fish management surveys of additional northern Norwegian lakes have suggested that lakes Tårnvatn and Skøvatn, similarly harbour polymorphic Arctic charr (three and two putative morphs, respectively), with the varieties morphologically resembling those described from Skogsfjordvatn and Fjellfrøsvatn. These preliminary observations suggest that both Tårnvatn and Skøvatn harbour a normal growing LO-morph and potentially a small-sized PB-morph. In addition, Tårnvatn appears to host a large-growing profundal piscivorous morph similar to the PP-morph found in Skogsfjordvatn. The two lakes have similar fish communities, are deep, dimictic, oligotrophic, and experience analogous subarctic climates similar to Fjellfrøsvatn and Skogsfjordvatn. Although little was known about the ecology and life history of the putative morphs in the two lakes, the same nomenclatures (i.e. LO, PB, PP) were initially used to label the morphs in Tårnvatn and Skøvatn.

The primary goal of the present study was to explore any parallelism in the evolution of sympatric Arctic charr morphs in Tårnvatn and Skøvatn. To establish whether the putative morphs were genetically separated and the extent of divergence, the genetic differentiation was examined using microsatellites and Bayesian clustering. The trophic ecology of the Arctic charr morphs was then contrasted within and between the two lakes using stomach contents to describe short-term resource use

and trophically transmitted parasites and stable isotopes analysis (SIA) to evaluate at longer, ecologically relevant time scales (Post, 2002; Knudsen et al., 2011, 2014; Hayden et al., 2014). Further, any concordance with the sympatric morph classifications reported from Fjellfrøsvatn and Skogsfjordvatn was assessed (Klemetsen et al., 1997; Knudsen et al., 2006, 2016a, b; Amundsen et al., 2008; Smalås et al., 2013; Præbel et al., 2016; Simonsen et al., 2017). Four hypotheses were addressed. Firstly, we hypothesised that the sympatric Arctic charr morphs in both lakes were genetically differentiated. Secondly, we hypothesised that the sympatric Arctic charr morphs would show trophic niche divergence in habitat and diet within each of the two study lakes, with the divergence being stable over time (i.e. similar based on gut contents, parasite community and SIA). Thirdly, it was hypothesised that the Arctic charr morphs display evolutionary parallelism when compared to morphs known to exist in Fjellfrøsvatn and Skogsfjordvatn (Knudsen et al., 2016a; Siwertsson et al., 2016), with the LO-morphs showing a generalist foraging behaviour and feeding on pelagic zooplankton and littoral benthos, and the small-sized deep-water morphs specializing in feeding on profundal soft-bottom macroinvertebrates. Finally, it was hypothesised that the putative PP-morph in Tårnvatn would exhibit a distinctive piscivorous feeding strategy, preying upon small-sized charr (i.e. cannibalism) in the profundal habitat.

Materials and methods

Study area description and field sampling

Tårnvatn and Skøvatn are subarctic lakes situated at 107 and 180 m, respectively, above sea level at 69°N in northern Norway. They have surface areas of 3.2 and 6.2 km² and maximum depths of 53 and 119 m, respectively. Both lakes are dimictic, oligotrophic, and are usually icebound from December to May. The linear distance between the two water bodies is about 33 km. Tårnvatn has a very simple fish community, consisting entirely of land-locked Arctic charr and brown trout (*Salmo trutta* L.). Skøvatn is an open system directly connected to sea with a 14-km-long unobstructed river and hosts mostly resident Arctic charr and brown trout, but also small stocks of

anadromous Arctic charr, brown trout, and Atlantic salmon (*Salmo salar*). The Secchi disk transparency was measured to be approximately 8 and 10 m in Tårnvatn and Skøvatn, respectively. The euphotic depth (< 1% of surface light) was estimated as two times the Secchi disk-depths and was standardized to 15 m in both lakes.

Fishing was conducted during the lake turnover period in late October 2016 in the littoral (1.5 m high benthic nets, 0–10 m depth), profundal (1.5 m high benthic nets, 15–35 m depth), and pelagic habitats (6 m high floating nets set offshore above 35 m depth) using multi-mesh gillnets 40 m long with mesh sizes from 10 to 45 mm (knot to knot) set overnight (see details in Smalås et al., 2013). The number of multi-mesh benthic nets used to survey the littoral and profundal habitats was, respectively, four and six in Tårnvatn, and six and four in Skøvatn. Two multi-mesh floating nets were set out in the pelagic zone in Tårnvatn, whereas, in Skøvatn, the pelagic zone was omitted from the sampling due to unfavourable weather conditions. Additionally, standard sized nets having only a single mesh size (6, 8, 10, 12, 20, 25, 30, 40 mm) were used to increase sample sizes of all morphs in both lakes. The habitat use of the different Arctic charr morphs was assessed based on catch per unit effort (CPUE expressed as number of fish caught per 100 m^2 multi-mesh gill-net per night) in the different habitats.

All Arctic charr were classified into different morph groups according to their external morphology (e.g. head and body shape and colour). The morphological characterization of the different morphs was based on criteria developed from previous studies of polymorphic charr in northern Norway (Klemetsen et al., 1997; Skoglund et al., 2015; Saltykova et al., 2017). In Tårnvatn, individuals were sorted into three distinct morphs (LO, PB, and PP), and in Skøvatn, into two morphs (LO and PB) (see Appendix Figs. 1, 2). The LO-morph adult fish had typical Arctic charr breeding coloration with a red–orange abdomen, a generally silvered dorsal area, and paired fins edged in white. The head, mouth, and eyes were relatively small compared to the body size. Juvenile fish generally displayed parr marks along the lateral sides of the body. The PB-morph had a small and deep body, with a relatively large head and a blunt snout, and round, big eyes. The colouration of the PB-morphs differed between the two lakes. In Tårnvatn, the mature PB-

morph charr had a pale yellow–brown coloration with a brass hue, usually with very pale parr marks. In contrast, the PB-morph in Skøvatn had clear parr marks and a more silvery body colour. The PP-morph in Tårnvatn had a slender elongated body shape, a robust, large, pointed head with sharp teeth on the palate and the tongue. The head, caudal fin, and back were very dark, with shades of grey and black. The abdomen and the flanks were generally opaque orange in colour, with white bordered paired fins similar to the LO-morph.

Genetic analyses

To establish the extent of genetic divergence among the morphs in Tårnvatn and Skøvatn, a small sample of gill-lamella was cut out from each fish and stored in 96% ethanol. DNA was extracted using an E-Z96 Tissue DNA Kit (OMEGA Bio-tek®) following manufacturer instructions. A total of 133 individuals were included in the genetic analysis (Table 1). Eleven microsatellite loci were amplified in two multiplex polymerase chain reactions (PCR) using forward labelled primers (Appendix Table 1). The PCRs consisted of 1.25 μL QIAGEN® Multiplex PCR Master Mix, 0.25 μL primer mix (multiplex panel Sal_Mp1 or 2), 0.5 μL water, and 5–10 ng template DNA. The general PCR profile for all multiplex reactions was: 95°C for 15 min followed by 25 cycles of 94°C for 30 s, Ta for 3 min, and 72°C for 1 min, with a final 60°C extension for 30 min, where Ta was 60°C and 55°C for Sal_Mp1 and 2, respectively. The analysis included 3% blank and 3% replicate samples,

Table 1 Samples of Arctic charr included in the genetic analysis

Lake	Morph	Code	n	H_e	F_{IS}
Tårnvatn (Tv)	LO	TvLO	21	0.638	− 0.058
	PB	TvPB	23	0.693	− 0.030
	PP	TvPP	30	0.593	− 0.012
Skøvatn (Sv)	LO	SvLO	29	0.737	0.052
	PB	SvPB	30	0.729	0.021

Number of morphs and individuals (*n*) and their code (Morph) in the genetic analysis are given. Expected heterozygosity (H_e) and F_{IS} is also given per morph. None of the F_{IS} values displayed significant deviations from Hardy–Weinberg expectations

which were blank or matched to the original samples, respectively. The PCR products were separated on an ABI 3130XL Automated Genetic Analyzer (Applied Biosystems) using LIZ500 as an internal standard, and the alleles were scored using the GeneMapper 3.7 software (Applied Biosystems). Each genotype was automatically binned in predefined allelic bins by the GeneMapper software and verified by visual inspection.

Departures from Hardy–Weinberg equilibrium (HWE) among loci within populations and among populations, and linkage disequilibrium (LD) among loci within populations were estimated using GENE-POP 4.0 (Rousset, 2007). All pair-wise estimates were corrected using Bonferroni corrections (Rice, 1989). The locus Sco204 was removed from the subsequent analysis as it was consistently linked with Sco218 across all populations, and with Sco220 in TvPP and SvLO, and with SMM22 in SvLO. Hence, all subsequent estimates were based on variation at 10 microsatellite loci. Summary statistics for each locus per population were estimated in GenAlEx 6.5 (Peakall & Smouse, 2006) (Appendix Table 2).

The genetic divergence between morphs within lakes was estimated by F_{ST} (Weir & Cockerham, 1984) and tested for statistical significance using 10,000 bootstraps in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010). Divergence among morphs within lakes was inferred using Bayesian clustering as implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000; Hubisz et al., 2009). The most likely number of populations (K) and their admixture (q) within each lake was estimated using a model assuming admixture and correlated allele frequencies. The LOCPRIOR option was used to assist the clustering as recommended by the software documentation in situations with weak genetic divergence among populations in the dataset. The model was tested with 50,000–150,000 burn-ins and Markov chain Monte Carlo (MCMC) replicates from 100,000 to 300,000. The optimal condition considering computational time versus model convergence was found to be 100,000 burn-ins and 200,000 MCMCs. The analysis was repeated 10 times for each K, and the most likely K per lake was estimated by assessing the mean LnP(K) and ΔK as implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012).

Diet composition

Stomachs were removed and stored in 70% ethanol. The number of empty stomachs was low in both lakes (19.1% and 6.6% in Tårnvatn and Skøvatn, respectively). Prey items were identified and sorted to the lowest practical taxonomic groups, and their contribution to the total stomach fullness was evaluated (0–100%) following Amundsen (1995). A total of 12 different prey types were identified in the 180 stomachs analysed for both lakes (see Appendix Table 4 for details). Rarefaction curves indicated that sample sizes in this study produced a good approximation of the diet diversity for the different morphs (Appendix Fig. 3). The different prey types were divided into five categories: zooplankton (limnetic cladocerans and copepods), littoral benthos (gastropods, larvae of stoneflies, caddisflies, and fish eggs), *Gammarus lacustris* (littoral amphipod), profundal benthos (chironomid larvae, *Pisidium* sp. mussels and *Acanthocyclops* sp. benthic copepods), and fish (Arctic charr). The proportion of each prey type in the diet was estimated as percent prey abundance following Amundsen et al. (1996). Dietary niche overlap between the different Arctic charr morphs was quantified using Schoener's (1970) similarity index. To visualize the diet of individual Arctic charr in the two lakes, a non-metric multidimensional scaling analysis (NMDS) based on the Bray–Curtis index of similarity was computed using relative prey abundance. The analysis was executed using the vegan package (Oksanen et al., 2013) in R version 3.3.1. (R Core Team, 2016). For the NMDS analysis, the LO- and PP-morph individuals were divided in two size groups to explore possible ontogenetic diet shifts. In the LO-morph, the division of small (< 16 cm) and large (> 16 cm) individuals was based on the onset of maturation sizes for the LO-morph observed, 17 cm and 16 cm, respectively, for Tårnvatn and Skøvatn (Kjær, 2018). The size-group division was also compared with that reported for earlier studies of polymorphic Arctic charr populations in the same region (Amundsen et al., 2008; Knudsen et al., 2016a) that contrasted the trophic niche of adult small-sized profundal morphs with juveniles of the upper-water (LO) morph. The threshold size for the PP-morph in Tårnvatn was set at 20 cm based on the piscivorous diet shift size reported for the PP-morph in Skogsfjordvatn (Knudsen et al., 2016b).

Parasite communities

Past diet was inferred from trophically transmitted parasites in a subset of Arctic charr from each morph. Trophically transmitted parasites reside in specific prey types and are ingested together with the prey. These parasites can live in the Arctic charr host for months or years (depending of the parasite life expectancy, Table 4) and act as tracers of long-term feeding patterns (Knudsen et al., 1996, 2008). For the purposes of this study, particularly relevant parasites were transmitted to Arctic charr by the amphipod *G. lacustris* (the cestode *Cyathocephalus truncatus*), insect larvae (the trematodes *Crepidostomum* spp. and *Phyllodistomum umblae*), and different species of pelagic copepods (the cestodes *Eubothrium salvelini*, *Proteocephalus* sp., and *Dibothriocephalus* spp.) (Knudsen, 1995; Knudsen et al., 1997, 2007, 2014; Jonsson & Jonsson, 2001; Siwertsson et al., 2016). All parasite species are in the adult stage in the Arctic charr except for larval *Dibothriocephalus* spp. (former *Diphyllobothrium* spp., see Waeschenbach et al., 2017). Prevalence (i.e. proportion of individuals infected in a host morph) and abundance (i.e. average number of parasites in host fish from a given morph) were calculated for each parasite species following Bush et al. (1997). Rarefaction curves indicated that sample sizes in this study produced a good approximation of the parasite diversity for the different morphs (Appendix Fig. 4). Individual species richness of trophically transmitted parasites is related to the diet niche width, since utilization of a larger range of different prey types is associated with higher infection risks from a multitude of food transmitted parasites. Thus, differences in individual parasite species richness between morphs were tested using non-parametric Mann–Whitney U tests to account for non-normality. Differences between morphs in the abundance of single parasite species were tested using generalized linear models (GLMs), specifying Poisson distributions typically used for count data. Whenever pairwise tests were performed, a Bonferroni correction was applied (Rice, 1989) such that for all tests when comparing morphs within the two lakes (four pairwise comparisons) a P value < 0.0125 was considered statistically significant.

Stable isotope analysis

For stable isotope analyses, a muscle tissue sample from each fish was cut from the dorsal area posterior to the dorsal fin and above the lateral line and immediately frozen. Littoral zoobenthos (*G. lacustris*, insect larvae, and snails) and pelagic zooplankton samples from both lakes were collected and used to explore baseline differences in stable isotope values between the major lake habitats (Fig. 6). Zooplankton sampling from the whole water column was carried out using a plankton net (diameter 26 cm, mesh size 90 μm) hauled three times vertically from a depth of 15 m to the surface. Benthic littoral macroinvertebrates were sampled using a benthos hand square net. Both zooplankton and littoral benthos samples were immediately frozen. Littoral benthos samples were sorted into *G. lacustris*, Megaloptera, Ephemeroptera, Trichoptera, Plecoptera, Chironomidae, and molluscs. Only the soft body tissue of molluscs was prepared for analyses. Samples were dried at 60°C for 24 h, homogenised using mortar and pestle, and weighed (0.3 ± 0.05 mg) into tin capsules. The analyses were performed at the University of Waterloo, Canada, on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyser (CHNS-O EA1108, Carlo Erba, Milan, Italy) with an analytical precision of $\pm 0.2‰$ ($\delta^{13}C$) and $\pm 0.3‰$ ($\delta^{15}N$). Analytical accuracy was established through the repeat analysis of internal laboratory standards calibrated against International Atomic Energy Agency standards CH6 for carbon and N1 and N2 for nitrogen. Analytical precision was established by the repeat analysis of one in ten samples. All results were reported in conventional delta notation (δ) relative to international standard Vienna Peedee Belemnite, VPBD, for $\delta^{13}C$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}N$ (Mariotti, 1983). As tissue samples had C:N values < 4, lipids were neither extracted nor corrected for using mathematical models (Jardine et al., 2013). Due to the non-normality of stable isotope values, Kruskal–Wallis and pair-wise Mann–Whitney U tests were used to statistically test for differences in $\delta^{13}C$ and $\delta^{15}N$ values among the morphs within the two lakes. Whenever pairwise tests were performed, a Bonferroni correction was applied (Rice, 1989).

Results

Genetic analyses

The five samples of morphs were all in HWE (Table 1), and none of the loci displayed deviation from HWE after Bonferroni corrections. Ten of 225 pairwise comparisons showed significant LD, but only one (OMM1105 vs SMM22 in SvLO) of 225 remained significant after Bonferroni corrections. The number of alleles per morph varied from one (Sco215 in SvLO, TvLO, TvPP, and TvPB) to 19 in SvLO (Sco218) (Appendix Table 2). The genetic variation (expected heterozygosity, H_e) of the Arctic charr morphs was higher in Skøvatn (H_e = 0.729–0.739) than in Tårn-vatn (H_e = 0.593–0.693), and none of the morph samples displayed significant inbreeding signatures (Table 1).

In Tårnvatn, the LO-morph displayed F_{ST}s of 0.134 ($P < 0.001$) and 0.121 ($P < 0.001$) compared to the PB and PP-morphs, respectively (Table 2). The genetic divergence between the PP- and PB- morphs was lower (F_{ST} = 0.042), but significant ($P < 0.001$). The STRUCTURE analysis identified $K = 2$ or $K = 3$ clusters in Tårnvatn (Fig. 1A, B). In both cases, the LO-morph formed its own cluster, where PB- and PP-morphs grouped together for $K = 2$ (Fig. 1A). The groupings revealed by STRUCTURE followed the visual phenotypic classification of individuals completed in the field. The two morphs in Skøvatn showed a significant genetic divergence with an F_{ST} value of 0.041 (Table 2). The result was supported by the STRUCTURE analysis, which clustered the morphs in

two separate clusters according to their phenotype (Fig. 1C).

Habitat and diet

In Tårnvatn, the LO-morph (mean length ± S.D.: 20.6 ± 5.6 cm) was caught in all three habitats, but at highest densities in littoral and pelagic areas (CPUE: 16.7 and 31.7, respectively; Table 3). The diet of the LO-morph in Tårnvatn included chiefly zooplankton (exclusively cladocerans) and some littoral benthos, with *G. lacustris* as the main benthic prey (Fig. 2, Appendix Table 4). All individuals of the PB- and PP-morphs were caught at depths > 15 m in Tårnvatn (Table 3). The PB-morph (mean length ± S.D.: 14.0 ± 5.6 cm) largely exploited profundal benthic prey groups, mostly chironomid larvae (Fig. 2, Appendix Table 4). The PP-morph (mean length ± S.D.: 26.0 ± 11.8 cm) in Tårnvatn exhibited a broad diet including profundal benthos, *G. lacustris*, and a notable (18%) proportion of small-sized Arctic charr (Fig. 2, Appendix Table 4). The PP-morph had a high dietary similarity when compared with the PB-morph in the same lake (54%). In contrast, the diet of the LO- and PP-, and the LO- and PB-morphs in Tårnvatn were distinct (26% and 29% diet overlap, respectively).

In Skøvatn, all the LO-morph individuals were caught in littoral habitat (Table 3). The LO-morph (mean length ± S.D.: 18.5 ± 6.5 cm) had a wide diet comprised of zooplankton (cladocerans) and littoral benthos, with *G. lacustris* constituting the largest single benthic prey item (47%) (Fig. 2, Appendix Table 4). In contrast, the PB-morph in Skøvatn (mean length ± S.D.: 9.5 ± 1.7 cm) had highest CPUE in the profundal habitat (Table 3) and fed mainly on zooplankton, particularly on the cladocerans *Bosmina* and *Daphnia* spp. (Fig. 2, Appendix Table 4). The dietary overlap between the two morphs in Skøvatn was 49% (Schoener's similarity index).

When comparing the analogous morphs from the two lakes, the LO-morphs had the highest diet niche similarity of 53% (Fig. 2). Nevertheless, the two LO-morphs had different ontogenetic dietary patterns in the two lakes (Fig. 3). In Tårnvatn, there was little difference in diet between small (< 16 cm) and large (> 16 cm) individuals. In contrast, in Skøvatn there was a clear shift from a zooplanktivorous feeding behaviour in the small fish towards a mixed diet composed of benthic prey and zooplankton in the large

Table 2 Genetic divergence among morphs within and across lakes as inferred by F_{ST} (below diagonal) and the associated P values (above diagonal)

	SvLO	SvPB	TvLO	TvPB	TvPP
SvLO	–	***	***	***	***
SvPB	0.041	–	***	***	***
TvLO	0.129	0.120	–	***	***
TvPB	0.097	0.088	0.134	–	***
TvPP	0.159	0.133	0.121	0.042	–

Sv Skøvatn, *Tv* Tårnvatn

***P < 0.001

Fig. 1 Genetic structuring of Arctic charr morphs from Tårnvatn (**A**, **B**) and Skøvatn (**C**) as inferred by STRUCTURE. In the STRUCTURE analysis, black lines separate individuals from different morphs (as determined in the field) and each individual is represented by a thin vertical line, which is partitioned into K-coloured segments representing the individual's estimated membership fractions in K clusters. For each lake, the mean values of lnP(K) and ΔK are given in Appendix Table 3
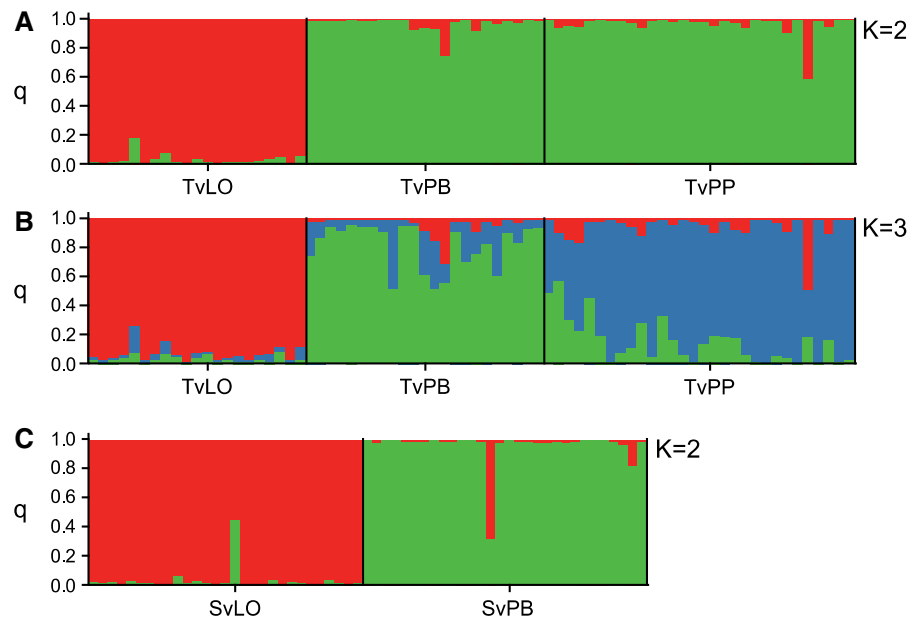
Table 3 Catch per unit effort (CPUE = number of fish caught per 100 m^2 multi-mesh gill-nets per night) of the Arctic charr morphs in the different habitats in Tårnvatn and Skøvatn

Habitat	Tårnvatn			Skøvatn	
	LO	PB	PP	LO	PB
Littoral	16.7 ($n = 40$)	0.0	0.0	24.0 ($n = 43$)	1.0 ($n = 2$)
Profundal	9.4 ($n = 18$)	10.0 ($n = 13$)	7.8 ($n = 18$)	0.0	3.0 ($n = 8$)
Pelagic	31.7 ($n = 39$)	0.0	0.0	–	–

The number (n) of fish caught in the different habitat is also provided

individuals (Fig. 3). The two small-sized deep-water PB-morphs, on the other hand, revealed contrasting feeding strategies in the two lakes with low dietary similarity (29%) (Figs. 2, 3, Appendix Table 4). The PB-morph in Skøvatn had the highest dietary similarity with the small LO-morphs from both lakes, feeding mainly on cladocerans (Fig. 3). The two PB-morphs showed no signs of ontogenetic dietary changes. The Tårnvatn PP-morph diet was distinctly different between small and large size-classes (Fig. 3). The small PP-morph (< 20 cm) almost exclusively consumed profundal benthos and had diet similar to the PB-morph in Tårnvatn (Fig. 3). The larger individuals (> 20 cm) relied predominantly on *G. lacustris* and fish, having the most distinctive diet of all the studied morphs (Fig. 3).

Parasite communities

In total, six different food-borne parasite genera were recorded in Arctic charr in both lakes, including four cestodes (pelagically transmitted *Dibothriocephalus* spp., *E. salvelini*, and *Proteocephalus* sp., and littoral *C. truncatus*) and two littoral benthic-transmitted trematodes (*Crepidostomum* spp. and *P. umblae*). No nematodes were found in any fish. All morphs in the two lakes harboured all six trophically transmitted parasites taxa.

In Tårnvatn, the PB-morph had the lowest parasite richness (mean number ± S.E.: 2.0 ± 0.2; Mann–Whitney *U* test: $P < 0.001$) (Fig. 4), whereas there was no significant difference in the number of parasite species between the LO- (3.7 ± 0.2) and PP- (3.4 ± 0.2) morphs (Mann–Whitney *U* test:
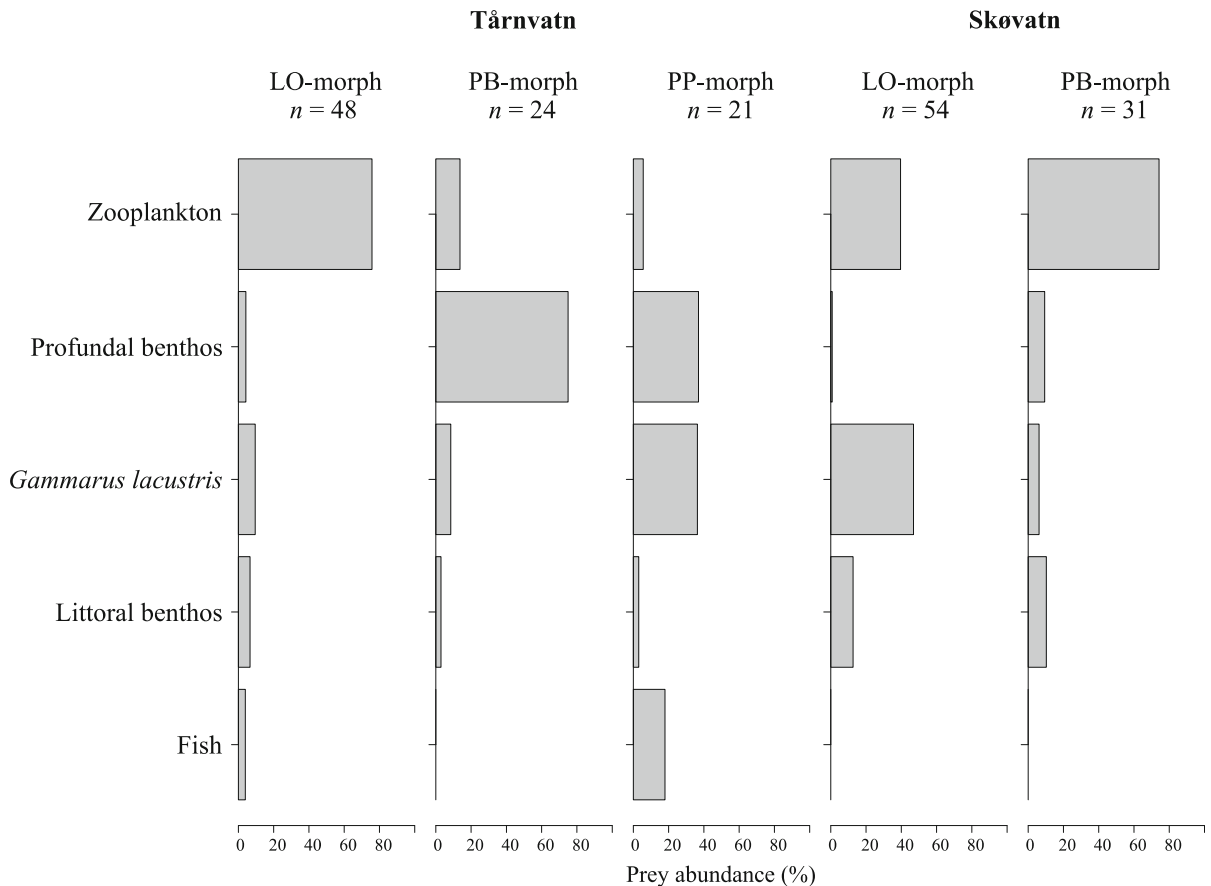
Fig. 2 Percent abundances of the major prey groups found in the stomach contents of the different Arctic charr morphs from Tårnvatn and Skøvatn (October 2016). For a more detailed diet description, see Appendix Table 4

P = 0.378) (Fig. 4). The prevalence in the LO- and PP- morphs was high for most of the parasite species, especially for *Dibothriocephalus* spp., *Crepidostomum* spp. and *E. salvelini* (Table 4). In contrast, the PB-morph had a low prevalence for all parasites, except for *Crepidostomum* spp., which infected a high proportion of individuals (Table 4). In Tårnvatn, the PP-morph had the highest total parasite abundance (mean number ± S.E.: 129.1 ± 37.7), followed by the LO-morph (67.9 ± 16.2), whereas the PB-morph had the lowest (35.3 ± 16.2). The LO-morph had the highest abundance of *P. umblae* and *Proteocephalus* sp. (GLMs: $P < 0.001$), whereas the PP-morph had the highest infection of *C. truncatus*, *Crepidostomum* spp., *E. salvelini*, and *Dibothriocephalus* spp. (GLMs: $P < 0.001$) (Fig. 5). In contrast, the PB-morph had low abundance for most of the parasites, with the lowest infections of *Proteocephalus* sp., *E. salvelini*,

and *Dibothriocephalus* spp. (GLMs: $P < 0.001$) (Fig. 5).

The LO-morph in Skøvatn had the highest parasite richness, harbouring up to six different parasites genera in one individual (mean number ± S.E.: 3.3 ± 0.3 S.E.) (Mann–Whitney U test: $P < 0.001$) (Fig. 4). In contrast, a lower parasite richness (1.5 ± 0.2) with a maximum of four parasite taxa was recorded in the PB-morph (Fig. 4). In Skøvatn, the LO-morph in general had a high parasite prevalence, with the greatest occurrence of *Dibothriocephalus* spp. and *Crepidostomum* spp. (Table 4). In contrast, the PB-morph showed a lower prevalence than the LO-morph for all parasites except for *Dibothriocephalus* spp., which was more frequently present in the PB-morph (Table 4). The LO-morph had a higher parasite abundance (mean number ± S.E.: 118.7 ± 33.6) compared to the PB-morph (67.9 ± 17.4). In Skøvatn, the highest mean
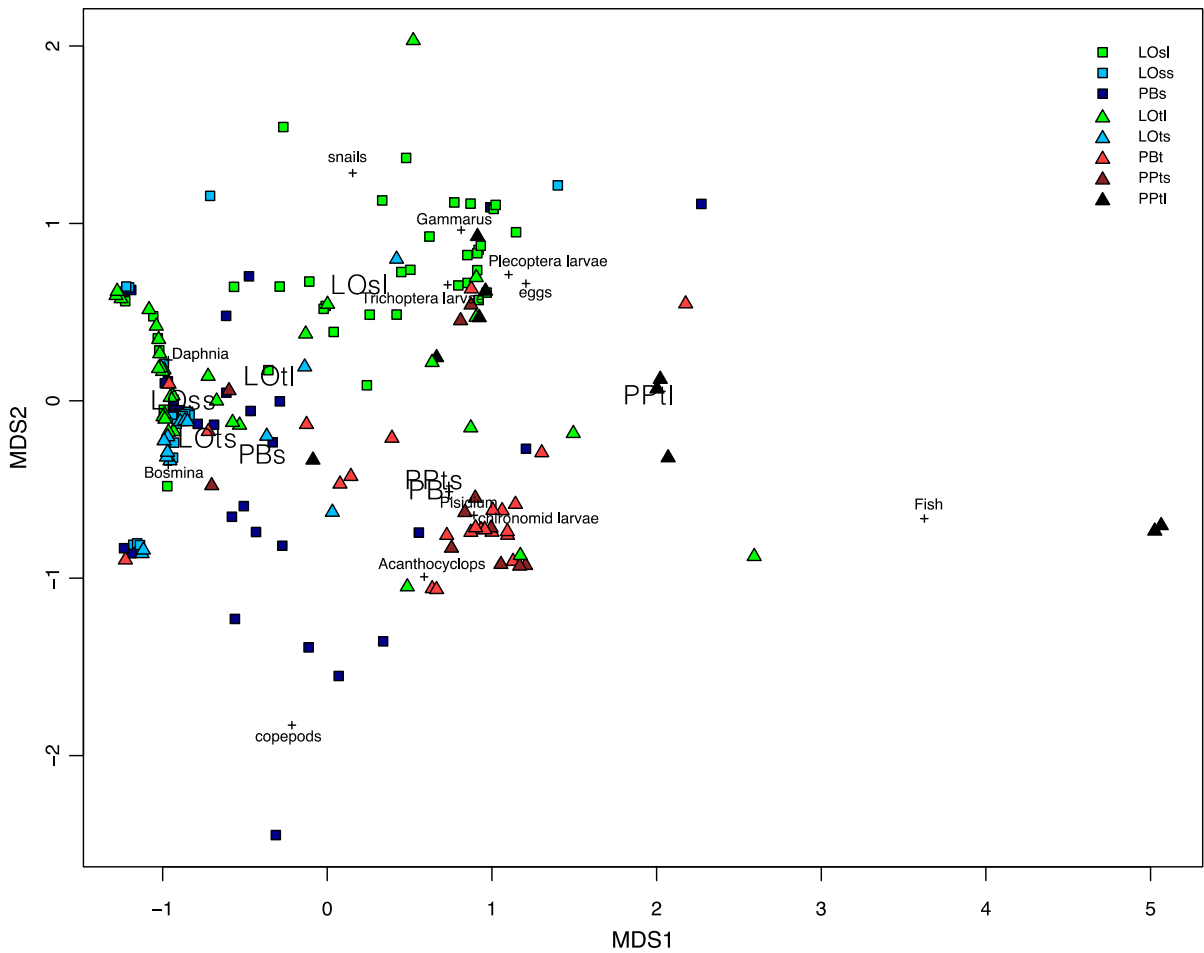
Fig. 3 Diet composition of individual Arctic charr of the various morphs in Tårnvatn and Skøvatn depicted by non-metrical multidimensional scaling (NMDS; stress = 0.12). LOts = small LO-morph (< 16 cm) in Tårnvatn (*n* = 15), LOtl = large LO-morph (> 16 cm) in Tårnvatn (*n* = 33), PBt = PB-morph in Tårnvatn (*n* = 24), PPts = small PP-morph (< 20 cm) in Tårnvatn (*n* = 10), PPtl = large PP-morph (> 20 cm) in Tårnvatn (*n* = 11), LOss = small LO-morph (< 16 cm) in Skøvatn (*n* = 19), LOsl = large LO-morph in Skøvatn (> 16 cm) (*n* = 37), PBs = PB-morph in Skøvatn (*n* = 35). The acronyms indicate average values for each morph in the two lakes
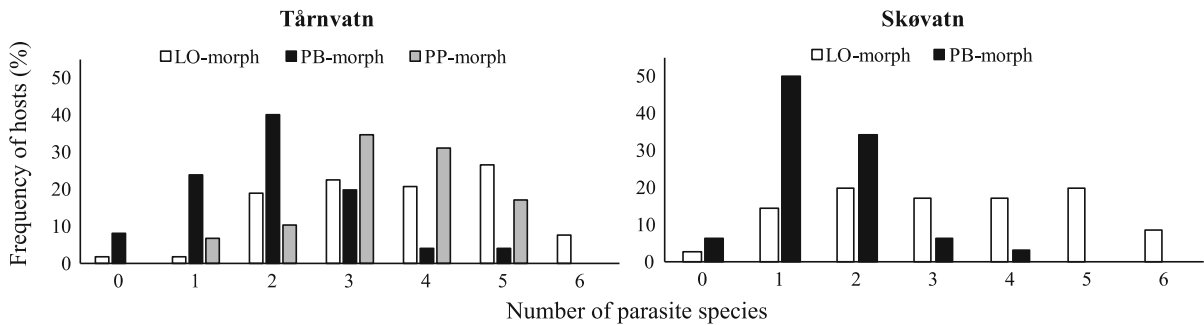

Fig. 4 Distribution (%) of the number of parasite species per host in the different morphs of Arctic charr in Tårnvatn (left) and Skøvatn (right)

Table 4 Prevalence (%) of the different parasite taxa found in the Arctic charr morphs in Tårnvatn and Skøvatn

Parasite species	Life expectancy in the host	Intermediate-host's habitat	Tårnvatn			Skøvatn	
			LO $n = 53$	PB $n = 25$	PP $n = 29$	LO $n = 35$	PB $n = 32$
C. truncatus	Months	L	22.6	16.0	37.9	54.3	12.5
P. umblae	1–2 years	L	54.7	8.0	17.2	42.9	3.1
Crepidostomum spp.	1–2 years	L	73.6	76.0	89.7	77.1	31.3
Proteocephalus sp.	1–2 years	P	69.8	40.0	41.4	25.7	9.4
E. salvelini	1–2 years	P	67.9	20.0	75.9	48.6	9.4
Dibothriocephalus spp.	many years	P	79.2	40.0	79.3	77.1	84.4

The life expectancy in the host and the intermediate host's habitat (L = Littoral and P = Pelagic) are also signed
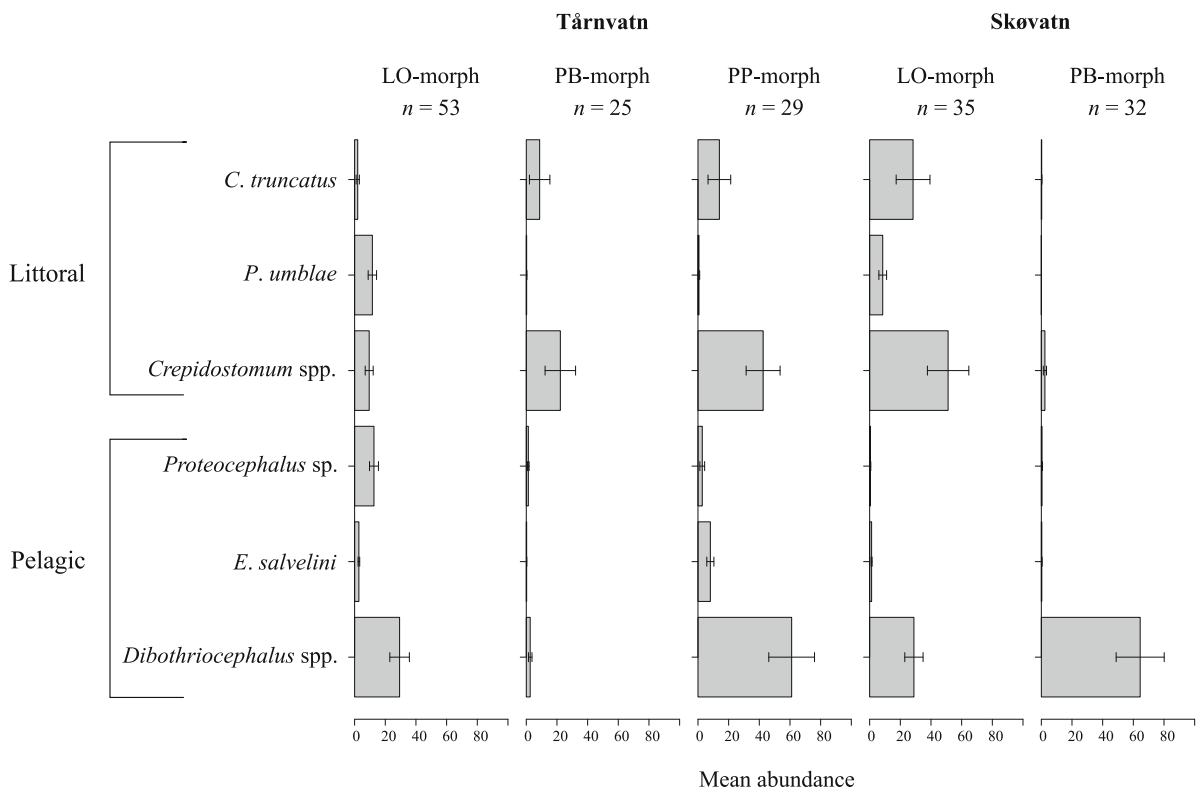

Fig. 5 Mean abundance (± S.E) of the six parasites genera found in the different Arctic charr morphs from Tårnvatn and Skøvatn (October 2016). The first three parasite species are associated with littoral feeding, the last three with pelagic

abundance in the LO-morph was found for *Crepidostomum* spp., followed by *Dibothriocephalus* spp., *C. truncatus*, and *P. umblae*, whereas the infection rate was very low for *E. salvelini* and *Proteocephalus* sp. (Fig. 5). On the other hand, the PB-morph generally had low infection levels, with significantly lower abundance for all parasites species (GLM: $P < 0.001$), except *Proteocephalus* sp. (GLM: $P = 0.791$) and *Dibothriocephalus* spp. (Fig. 5). The abundance of *Dibothriocephalus* spp. was higher in the PB-morph than in the LO-morph (GLM: $P < 0.001$) (Fig. 5).

Parasite species richness was similar across lakes between the two LO- morphs (Mann–Whitney U test: $P = 0.231$) and PB-morphs (Mann–Whitney U test: $P = 0.061$) (Fig. 4). Nevertheless, the LO- and PB-morphs in Tårnvatn had a lower total parasite abundance than the corresponding morphs in Skøvatn. A similar pattern of prevalence for the LO-morphs was observed in the two lakes, with the majority of fish infected by *Dibothriocephalus* spp. and *Crepidostomum* spp. However, the LO-morph in Tårnvatn showed a greater occurrence of pelagically transmitted parasites, but a lower prevalence of the *G. lacustris*-transmitted *C. truncatus* (Table 4). The PB-morph in Tårnvatn had a higher prevalence than the PB-morph in Skøvatn for all parasites, except for *Dibothriocephalus* spp., which was more prevalent in Skøvatn (Table 4). The two LO-morphs had significant differences in the abundance of all parasites species (GLM: $P < 0.001$) except for *Dibothriocephalus* spp. (GLM: $P = 0.700$) (Fig. 5). The PB-morph in Skøvatn had a higher abundance of *Dibothriocephalus* spp. than the PB-morph in Tårnvatn (GLM: $P < 0.001$), but lower abundances of *C. truncatus*, *Crepidostomum* spp. and *Proteocephalus* sp. (GLM: $P < 0.001$) (Fig. 5). The abundances of the other parasite species were not significantly different (GLMs: $P > 0.060$) (Fig. 5).

Stable isotope analysis

There were significant differences in the stable isotope values among the morphs in Tårnvatn (Kruskal–Wallis tests: $P < 0.001$). The PB- and PP-morphs had similar $\delta^{13}C$ mean values (Mann–Whitney U test: $P = 0.015$), but higher compared to the sympatric LO-morph (Mann–Whitney U tests: $P \leq 0.0125$) (Fig. 6A; Appendix Table 4). The LO-morph had the lowest $\delta^{15}N$ values (Mann–Whitney U test: $P < 0.001$) in comparison with the other morphs, which were similar (Mann–Whitney U test: $P = 0.339$) (Fig. 6A; Appendix Table 4).

In Skøvatn, the LO-morph had significantly higher $\delta^{13}C$ values than the sympatric PB-morph (Mann–Whitney U test: $P < 0.005$), but lower $\delta^{15}N$ (Mann–Whitney U test: $P < 0.001$) (Fig. 6B; Appendix Table 4).

Discussion

As predicted, all the sympatric Arctic charr morphs in the two lakes were genetically differentiated. In both lakes, genetic differences were evident between the LO- and the co-occurring profundal morphs as has been noted in earlier studies of analogous morph-pairs in Fjellfrøsvatn and Skogsfjordvatn (Præbel et al., 2016; Simonsen et al., 2017). The genetic differentiation was weaker, but still highly significant between the PB- and PP-morphs in Tårnvatn. Collectively, the results show that an intra-lacustrine divergence of the Arctic charr morphs is ongoing in both lakes and that all morphs can be genetically discriminated. There was also a clear separation in the trophic niches (habitat and diet) between the upper-water column (LO-morph) and profundal morphs within each lake. Niche segregation among the Arctic charr morphs in both Tårnvatn and Skøvatn was also supported by the differences between the temporally integrated trophic tracers (stable isotopes and parasites) that pointed to the persistence of trophic niche segregation over the ecologically relevant time scales of months (stable isotopes) or years (parasites). The resulting weight of evidence provided by the genetic differences, the clear trophic segregation, and life-history patterns (Kjær, 2018) strongly suggests the existence of two distinct deep-water morphs in Tårnvatn and one in Skøvatn. However, while the LO-morphs appeared to have similar trophic niches in both lakes, the PB-morphs were strikingly different. Although similar in appearance, life histories (Kjær, 2018), and habitat preference, the PB-morph in Skøvatn was feeding mainly on zooplankton while in Tårnvatn they were feeding on profundal benthos like in other lakes in the region (Klemetsen, 2010; Knudsen et al., 2016a).

The parallelisms in habitat choice and trophic tracers between the LO-morphs from the two study lakes were similar to patterns observed in earlier studies of morphs from the same geographic region (Knudsen et al., 2016a; Siwertsson et al., 2016). The LO-morphs in Tårnvatn and Skøvatn had a generalist trophic niche, with a mixed diet obtained from the littoral-pelagic habitat, a rich parasite community, and a relative broad isotopic range, similar to the niches described earlier for polymorphic populations in Fjellfrøsvatn and Skogsfjordvatn (Amundsen et al., 2008; Knudsen et al., 2011, 2016a; Siwertsson et al., 2016). Such a broad dietary niche typically occurs also

Fig. 6 Stable isotope biplots displaying the $\delta^{13}C$ and $\delta^{15}N$ values of dorsal muscle tissue samples of Arctic charr caught in Tårnvatn and Skøvatn in October 2016. The LO-morphs are represented by white dots ($n = 34$, 29, respectively), the PB-morphs ($n = 25$, 25, respectively) by grey triangles, and the PP-morphs in Tårnvatn ($n = 32$) by black diamonds. Mean values ($\pm$ SD) of pelagic (black squares) and littoral (white squares) prey sampled in June 2017 are also given
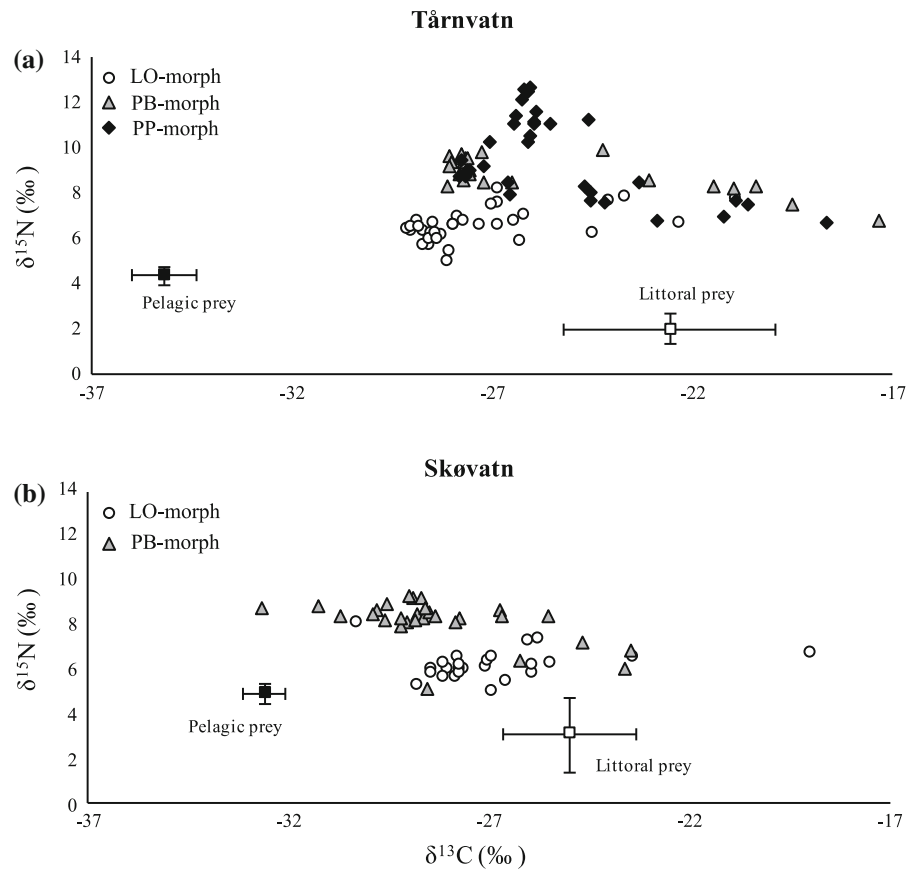
in monomorphic Arctic charr populations in the sub-Arctic region (Johnson, 1980; Amundsen, 1995; Klemetsen et al., 2003). In addition, the LO-morphs in Tårnvatn and Skøvatn share similar life history traits, particularly fast growth, similar maximal lengths (29–34 cm) and maturation at between 19 and 22 cm (Kjær, 2018). Thus, it seems reasonable to consider the LO-morph in both lakes to be analogous.

The adult PP-morph, in Tårnvatn only, displayed partly piscivorous foraging behaviour as hypothesised. A noticeable proportion (32.3%, Appendix Table 5) of individuals with empty stomachs was observed in the PP-morph as is commonly reported for piscivorous fish (Arrington et al., 2002; Vinson & Angradi, 2011; Amundsen, 2016). The PP-morph had a clear ontogenetic shift in foraging habits moving from a dominance of profundal benthic prey in the small young individuals to a diet composed by fish and *G. lacustris* in the large older fish, with $\delta^{15}N$ values in the 12–14‰ (Fig. 6A) consistent with heavy reliance on fish as prey (Guiguer et al., 2002). Similar to the PP-morph in

Skogsfjordvatn, the piscivorous diet shift occurred at an approximate length of 20 cm coincident with when individuals reached a size sufficient to prey on other fish (Knudsen et al., 2016b). As with other piscivorous Arctic charr morphs and in contrast to the sympatric LO- and PB-morph, the PP-morph had high accumulation of *Dibothriocephalus* spp. and *E. salvelini* (Frandsen et al., 1989; Siwertsson et al., 2016). These parasite species have the capacity to re-establish in piscivorous hosts (Curtis, 1984; Frandsen et al., 1989; Henriksen et al., 2016) and typically accumulate with age in the infected fish (Svenning, 1993; Knudsen & Klemetsen, 1994; Hammar, 2000; Knudsen et al., 2004). The PP-morph also had high infections of littoral-prey-transmitted *Crepidostomum* spp., reflective of the feeding on *G. lacustris* (Knudsen et al., 2008, 2014). Stable isotope values of the PP-morph further supported the contention of a mixed piscivorous-littoral benthivorous niche. Individuals with high $\delta^{15}N$ and low $\delta^{13}C$ values likely fed on conspecifics in the profundal zone (Jardine et al. 2003; Knudsen et al.

2016a, b), whereas individuals with low $\delta^{15}N$ and high $\delta^{13}C$ had values typical of littoral dwelling fish (Vander Zanden & Rasmussen, 1999; Jardine et al. 2003). Analogous to Skogsfjordvatn (Smalås et al., 2013), Kjær (2018) has shown that the PB- and PP-morphs have contrasting life history strategies, with the PB-morph having a significantly slower growth rate and earlier sexual maturation (approximately 5 years) than the PP-morph (approximately 7 years). Arctic charr is the only suitable fish prey that is available for the PP-morph in Tårnvatn, as only Arctic charr and brown trout are present. Juvenile brown trout do not commonly reside in the profundal zone, preferring to occupy streams or lacustrine littoral areas (L'Abée-Lund et al., 1992; Amundsen & Knudsen, 2009; Eloranta et al., 2013). Thus, the piscivorous PP-morph can only feed on small conspecifics. In contrast the PP-morph in Skogsfjordvatn is able to feed on both Arctic charr and three-spined sticklebacks (Knudsen et al., 2016b). Cannibalism in Arctic charr has been widely reported both as an outcome of ontogenetic niche shifts in large fish and as an occurrence of specialized piscivorous morphs (Amundsen, 1994, 2016; Svenning & Borgstrøm, 1995; Klemetsen et al., 2003; Knudsen et al., 2016b). Nevertheless, piscivorous charr morphs generally reside in shallow-water habitats (Sandlund et al., 1992; Adams et al., 1998). Skogsfjordvatn is one of the few described cases with a piscivorous morph residing entirely in the profundal zone (Smalås et al., 2013; Skoglund et al., 2015; Knudsen et al., 2016b) (but see Power et al., 2009). The presence of abundant and suitable prey fishes, i.e. the PB-morph and juvenile LO-morph in deep-waters, is probably a key factor in the local evolution of the PP-morph in Tårnvatn, as in Skogsfjordvatn, where a process of niche expansion in response to ecological opportunity has been suggested (Skoglund et al., 2015; Knudsen et al., 2016b).

In contrast to the LO-morphs, the PB-morphs from the two lakes showed both parallel and non-parallel patterns in trophic niche utilisation. As predicted, the PB-morph in Tårnvatn evidenced dietary specialization based on its stomach contents, preying profundal soft-bottom benthic invertebrates as has been reported for the PB-morphs in Fjellfrøsvatn and Skogsfjordvatn (Knudsen et al., 2006, 2016a). Specialization was also supported by the low species richness and infection rates (prevalence and abundance) of all examined parasites typical of the small-sized profundal morphs

(Knudsen et al., 1997; Siwertsson et al., 2016). Stable isotope values, on the other hand, suggested utilisation of a wide spectrum of prey resources along the littoral-pelagic-profundal habitat axis (Vander Zanden & Rasmussen, 1999). Thus, while dietary specialization as reflected in stomach contents and parasites is occurring, prey sourcing appears to occur from both littoral and profundal habitats. Deep-water morphs with a similar benthic feeding strategy have also been reported from Siberia (Alekseev & Pichugin, 1998), Canada (O'Connell et al., 2005), central Europe (Brenner, 1980), and Scandinavia (Hindar & Jonsson, 1982) (reviewed by Klemetsen, 2010), and with similar dichotomous use of deeper and shallower littoral habitats having been observed in the generally deep-water morph found in Gander Lake, Newfoundland (O'Connell et al., 2005; Power et al., 2012).

When compared to the benthivorous PB-morph in Tårnvatn and other lakes, the deep-water morph in Skøvatn used a different trophic niche despite identical life-history patterns, e.g. reduced growth and early maturation (Klemetsen et al., 1997; Smalås et al., 2013; Kjær, 2018). The zooplankton dominated diet of the Skøvatn deep-water morph was reflected by high infections of copepods-transmitted *Dibothriocephalus* spp. However, the lower $\delta^{13}C$ and higher $\delta^{15}N$ values when compared to the sympatric LO-morph, also suggested a greater reliance on profundal benthic resources (Hayden et al., 2014; Knudsen et al., 2016a, b). Since stable isotopes reflect diet over an approximate 3–4 month period before capture (Post, 2002; Buchheister & Latour, 2010; Knudsen et al., 2014), the Skøvatn deep-water morph likely consumed profundal prey during the early ice-free season when a high density of chironomid pupae emerge from the bottom substrate and zooplankton biomass is low (Klemetsen et al., 1992; Dahl-Hansen et al., 1994; Primicerio & Klemetsen, 1999; Amundsen et al., 2008; Mousavi & Amundsen, 2012; Kahilainen et al., 2016). The parasite community composition supported these findings as the small-sized profundal morph had low species richness and very low abundance for most parasites (except for *Dibothriocephalus* spp.), as typical of other deep-water morphs (Siwertsson et al., 2016). Since the Skøvatn profundal morph deviates clearly in its diet (zooplanktivory) from the benthivore PB-morph in Tårnvatn and elsewhere (Klemetsen, 2010; Knudsen et al., 2016a), and potentially spawns in deep-waters (Kjær,

2018), it is probably best denoted as a distinct small-sized deep-water planktivorous morph and is hereinafter referred using the acronym PZ ("Profundal spawning Zooplanktivore").

The PZ-morph in Skøvatn is the first documented case of a potential profundal planktivorous Arctic charr morph in northern Norway. Similar partly zooplanktivorous small-sized deep-water morphs have been described, e.g. in southern Norway (Telnes & Sægrov, 2004), in central Europe (Brenner, 1980), and in Transbaikalia (Alekseyev et al., 2002; Samusenok et al., 2006). Compared to zooplanktivory, one of the main advantages of a deep-water benthic diet may be lower parasite infections (Siwertsson et al., 2016) and associated higher fitness. A second advantage may be the year-round availability of prey items. The observed deviation from the more common deep-water benthivorous diet may be related to low productivity in the profundal zone, with the scarcity of deep-water benthic biomass inducing a shift to zooplanktivory. Overall, zooplankton is a generally more abundant resource in the late summer and autumn than profundal benthic invertebrates in many northern lakes (Primicerio & Klemetsen, 1999; Mousavi, 2002; Hayden et al., 2014; Kahilainen et al., 2016). As described for some monomorphic Arctic charr populations (e.g. Eloranta et al., 2010; Hayden et al., 2014; Kahilainen et al., 2016), the PZ-morph may alternate between benthivorous behaviour in winter and spring and zooplanktivory in autumn when zooplankton preys are abundant.

While parallelism in trophic ecology was evident in the LO-morphs from the two study lakes, the two small-sized profundal morphs differed substantially in their diets. The PB-morph in Tårnvatn along with the PB-morphs in Fjellfrøsvatn and Skogsfjordvatn are well-documented cases of parallel evolution in Arctic charr, given their similarity in habitat preferences, diet, parasite fauna, morphology, and life history (Knudsen et al., 2016a; Siwertsson et al., 2016; Saltykova et al., 2017). Parallel patterns are usually considered as evidence of similar selection pressures favouring the development of similar adaptive traits among fishes in postglacial lakes (Schluter, 2001; Sigursteinsdóttir & Kristjánsson, 2005; Kaeuffer et al., 2012; Præbel et al., 2013; Siwertsson et al., 2016; Saltykova et al., 2017; Häkli et al., 2018). Thus, the discrepancy in the dietary niche of the PB- and PZ-morphs in Tårnvatn and Skøvatn, respectively, is of great interest to improve the knowledge of evolutionary mechanisms driving adaptations.

The observed divergent patterns in local trophic adaptations (i.e. non-parallelism) of the PB- and the PZ-morphs of Arctic charr might have been promoted by differences in ecological and environmental factors occurring between the two lake systems (Kaeuffer et al., 2012; Kristjánsson et al., 2012; Siwertsson et al., 2013b; Saltykova et al., 2017). Such dissimilarities could be, e.g. in bathymetric conditions, productivity, and fish community, as Skøvatn (unlike Tårnvatn) hosts anadromous fish including Arctic charr, brown trout, and Atlantic salmon (Smalås & Henriksen, 2016). Alternatively, different adaptive responses may have been induced by the standing genetic variation of the colonizing ancestral populations (West-Eberhard, 1989) or as an outcome of genetic drift (Sigursteinsdóttir & Kristjánsson, 2005; Kaeuffer et al., 2012; Saltykova et al., 2017).

To conclude, the combined data describing habitat use, stomach contents, parasites, and tissue stable isotopes indicated clear trophic resource segregation between the genetically differentiated polymorphic Arctic charr morphs in Tårnvatn and Skøvatn. Results as described here are consistent with the occurrence of an ongoing process of trophic divergence, the consequences of which are reflected in a concomitant separation among the morphs in life history traits such as growth and maturation (Kjær, 2018). Furthermore, there were clear patterns of genetic divergence among the morph-pairs within these two lakes. Within the study lakes, a clear parallelism in habitat choice, external morphology, and life history was found for the upper-water omnivore LO-morphs and the small-sized deep-water morphs, suggesting the effect of parallel evolutionary processes along the depth gradient across lakes. Contrary to our hypotheses, there was an evident difference in dietary niches between the small-sized profundal benthivorous PB-morph and the zooplanktivorous PZ-morph indicating partially different evolutionary histories. Finally, the data describe for the first time in northern Norway the occurrence of the PZ-morph and the exclusively cannibalistic PP-morph from the deep-water environment. This study demonstrates how evolution can produce diverse outcomes, even among systems with apparently similar environmental and ecological conditions.
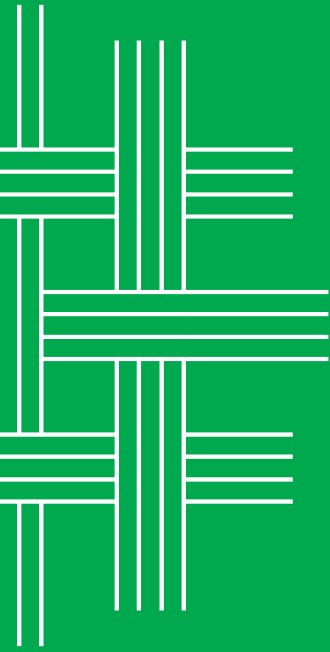
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Adaptive radiation is the evolutionary process that can generate diversification of phenotypes and genotypes across different environments, differentiating a single ancestor into different forms and species. Under ecological speciation, local adaptation through natural selection drives the divergence of populations, evolving reproductive isolation and leading to the formation of new eco-morphs, populations, and ultimately, species. A good example of polymorphic species is Arctic charr (*Salvelinus alpinus*), which has the flexibility to occupy different niches (i.e. a specific range of abiotic and biotic factors that a species has specialised) in a lake. The main objectives of this thesis are to investigate trophic niche segregation (i.e. diet choice and habitat use), morphological and genetic differences among sympatric Arctic charr morphs from three different lakes in Norway (Tinnsjøen, Tårnvatn and Skøvatn).

Two Arctic charr morphs were found coexisting in Lake Skøvatn, three morphs in Lake Tårnvatn and four morphs in Lake Tinnsjøen. Two novel morphs were found in Lake Tinnsjøen and Skøvatn. Life-history traits and habitat use was similar among the small-sized profundal morphs, but the morph in Skøvatn presented differences in diet choice compared to the morph from Lake Tårn-vatn. Parallel evolution could be responsible for the similarities found among some of the Arctic charr morphs across these three lakes. These morphs are likely under ecological speciation, where natural selection could play an important role in the adaptive divergence of morphs, contributing to reproductive isolation. Arctic charr polymorphism could be a case of adaptive radiation, explaining their diversity across different freshwater systems.