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(*Rutilus rutilus*) of large prealpine lakes**

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Allopatric and sympatric diversification within roach (*Rutilus rutilus*) of large prealpine lakes

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- 1 **Allopatric and sympatric diversification within roach (*Rutilus rutilus*) of**
- 2 **large prealpine lakes**
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5 **Abstract**

6 Intraspecific differentiation in response to divergent natural selection between
7 environments is a common phenomenon in some lineages of northern
8 freshwater fishes, especially salmonids and stickleback. Understanding why
9 these taxa diversify and undergo adaptive radiations while most other fish
10 species in the same environments do not, remains an open question. The
11 possibility for intraspecific diversification has rarely been evaluated for most
12 northern freshwater fish species. Here, we assess the potential for intraspecific
13 differentiation between and within lake populations of roach (*Rutilus rutilus*) – a
14 widespread and abundant cyprinid species - in lakes in which salmonids have
15 evolved endemic adaptive radiations. Based on more than 3,000 polymorphic
16 RADseq markers, we detected low but significant genetic differentiation between
17 roach populations of two ultraoligotrophic lakes and between these and
18 populations from other lakes. This, together with differentiation in head
19 morphology and stable isotope signatures, suggests evolutionary and ecological
20 differentiation among some of our studied populations. Next, we tested for
21 intralacustrine diversification of roach within Lake Brienz, the most pristine lake
22 surveyed in this study. We found significant phenotypic evidence for ecological
23 intralacustrine differentiation between roach caught over a muddy substrate and
24 those caught over a rocky substrate. However, evidence for intralacustrine
25 genetic differentiation is at best subtle and phenotypic changes may therefore be
26 mostly plastic. Overall, our findings suggest roach can differ between ecologically
27 distinct lakes, but the extent of intralacustrine ecological differentiation is weak,
28 which contrasts with the strong differentiation among endemic species of
29 whitefish in the same lakes.

30
31 **Keywords:** *Rutilus rutilus*, resource polymorphism, postglacial range expansion,
32 stable isotopes, RADseq

Introduction

Much of the existing species diversity of freshwater fish in northern climate zones is of recent origin, having evolved since the end of the last glaciation period ~12kyrs ago (Hewitt, 1999; Seehausen & Wagner, 2014). During the invasion of newly available freshwater bodies and associated range expansion, many fish species colonized a variety of different environments, and as a result may have experienced competitive release that may have triggered adaptive diversification (Bolnick *et al.*, 2007; Stroud & Losos, 2016). Divergent selection between habitats frequently led to the emergence of ecologically distinct populations, ecotypes, and species. Divergence of populations may for example occur as a response to different predation regimes (Walsh & Reznick, 2009; Scharnweber *et al.*, 2013), different parasites (Karvonen & Seehausen, 2012), different feeding regimes (Schluter, 1996; Jonsson & Jonsson, 2001; Svanbäck & Eklöv, 2004), or as a response to interactions among several of these and other variables (Seehausen & Wagner, 2014). The same factors when combined with intraspecific competition may also drive intraspecific diversification in sympatry (Rosenzweig, 1978; Dieckmann & Doebeli, 1999; Gavrillets, 2004; Svanbäck & Bolnick, 2007), e.g., within a lake where ecologically distinct individuals may occupy different niches. Such intralacustrine diversification of fish has received an ample amount of interest to study adaptive radiation (Schluter, 1996; Bolnick & Fitzpatrick, 2007; Seehausen & Wagner, 2014). Evidence for intraspecific sympatric diversification and adaptive radiation among temperate freshwater fishes is, however, restricted to relatively few taxonomic groups, particularly salmonids and a few cases of threespined stickleback (*Gasterosteus aculeatus*) (Seehausen & Wagner, 2014). These are classic examples of adaptive radiations, i.e., the diversification of a single taxon into phenotypically, ecologically, and genetically differentiated populations or ultimately species (Schluter, 2000). Comparatively, few studies have explored taxa beyond these classical cases to better understand why some fish taxa form adaptive radiations while others do not, and therefore, a study bias cannot be ruled out (reviewed in Seehausen & Wagner, 2014). Comparative investigations of other common taxa are consequently needed.

Cases of intralacustrine diversification in temperate freshwater fish often involve differentiation along a pelagic-benthic axis, leading to the evolution of sympatric planktivorous pelagic and benthivorous benthic species (Seehausen & Wagner, 2014). A second axis of diversification includes segregation along depth gradients such as in Arctic charr (*Salvelinus alpinus*; Jonsson & Jonsson, 2001) or whitefish (*Coregonus* sp.; Vonlanthen *et al.*, 2009). The range and discreteness of vacant niches and available food resources in an ecosystem may determine the number of resource-specific ecotypes that can evolve (Nosil & Sandoval, 2008; Wagner *et al.*, 2014; Lucek *et al.*, 2016). In the case of intraspecific diversification, adaptive phenotypic differentiation may initially emerge through divergent selection on standing genetic variation (Barrett & Schluter, 2008), phenotypic plasticity, or a combination of both (Smith & Skúlason, 1996; Schluter, 2000; Lucek *et al.*, 2014). Plasticity can initially promote differentiation (Snorrason & Skúlason, 2004; Pfennig *et al.*, 2010), and depending on the stability of the selective regime, divergent phenotypes may become genetically fixed through phenotypic canalization, genetic assimilation, or genetic accommodation (Crispo, 2008; Thibert-Plante & Hendry, 2011). On the other hand, plasticity may shield the genome from the effects of selection and prevent genetic fixation (Price *et al.*, 2003; Ghalambor *et al.*, 2007). If reproductive isolation cannot evolve, adaptive variation may sometimes be maintained by intraspecific resource polymorphisms either through adaptive phenotypic plasticity (Pfennig *et al.*, 2010) or frequency dependent selection (Svanbäck & Bolnick, 2007).

Here, we test for the presence of intraspecific differentiation and diversification in a widespread and abundant fish species of postglacial lakes – the roach (*Rutilus rutilus*). Roach are often considered to be generalist feeders (Persson, 1983), but may specialize on part of the food spectrum, such as zooplankton, to avoid predation and/or interspecific competition (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). Roach have also been shown to, in some cases, undergo ontogenetic dietary shifts, e.g. from zooplankton to macrophytes or mussels (Prejs *et al.*, 1990; Vejříková *et al.*, 2017). Roach represent an ideal candidate to test for intraspecific diversification, given i) its broad dietary niche providing the ecological opportunity to explore a wide range of the available niche space and thus to potentially adapt to one or more niches, ii) its wide

distribution across Europe (Kottelat & Freyhof, 2007), iii) its ability to inhabit an array of different environments (including streams and the pelagic and littoral zones of lakes (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015), iv) its large population sizes, and v) its modest economic importance, resulting in little to no direct management. Additionally, the roach in this study (Figure 1) often coexist with adaptive radiations of whitefish and are ecologically similar to some of the shallow water whitefish species (Hudson *et al.*, 2011; Vonlanthen *et al.*, 2012; Doenz *et al.*, 2018), thus providing the potential for ecological niche shifts of roach in response to interspecific interactions, as has been shown for other fish species (Persson, 1983; Braband, 1985; Faulks *et al.*, 2015).

Previous allozyme studies implicate that genetic differentiation in roach occurs predominantly between, but not within, drainage systems as a result of different colonization events following the last glaciation period (Laroche *et al.*, 1999; Hänfling *et al.*, 2004). Roach from lakes with distinct colonization histories often differ in body shape, potentially as a response to different predation regimes (Scharnweber *et al.*, 2013) or varying levels of intra- (Svanbäck *et al.*, 2008) or interspecific competition (Faulks *et al.*, 2015). However, in cases where genetic data were available, phenotypic differentiation showed only minor association with the level of genetic differentiation, suggesting that plasticity may often underlie phenotypic differences among roach populations (Scharnweber *et al.*, 2013; Faulks *et al.*, 2015). The aforementioned studies were, however, conducted in relatively shallow lakes, which might not provide the same ecological opportunities for genetic and adaptive differentiation as large, deep, and oligotrophic lakes do (Seehausen & Wagner, 2014). In addition, studies of lacustrine populations compared different drainages that were likely independently colonized, potentially resulting in different evolutionary contingencies (Svanbäck *et al.*, 2008; Scharnweber *et al.*, 2013; Faulks *et al.*, 2015). By integrating phenotypic data of roach from seven large pre-alpine lakes with genomic and ecological (i.e. stable isotopic) data of five of these, we assess to which degree allopatric populations from lakes within the same drainage system that are connected by rivers differ from each other. We further test for intralacustrine differentiation of roach caught over different substrates within Lake Brienz. As Brienz is the most pristine lake that we studied (Figure 1, Table

1), it is also the most likely lake to reveal if intralacustrine diversification evolved in roach as a response to local ecological opportunities. This is because the fish fauna of this lake experienced relatively little human impact, i.e. did not undergo a phase of eutrophication and re-oligotrophication during the second part of the 20th century like many other Swiss lakes (Vonlanthen *et al.*, 2012), and is one of the few pre-alpine lakes that still hosts its whole adaptive radiation of whitefish (Hudson *et al.*, 2011; Vonlanthen *et al.*, 2012; Doenz *et al.*, 2018).

Materials and Methods:

Study area and sampling

We sampled 1,223 roach from seven pre-alpine Swiss lakes between September 2011 and October 2012 (Figure 1a; Table 1). All lakes belong to the Aare/Rhine drainage except for Lake Geneva, which belongs to the Rhone drainage. However, former biogeographic work found that *R. rutilus* from the Rhone drainage formed a genetic cluster with specimens from the Rhine drainage (Larmuseau *et al.*, 2009), potentially reflecting human translocations and/or natural drainage crossings, which has been observed for other fish species between the Aare/Rhine drainage and Lake Geneva (Vonlanthen *et al.*, 2007; Gouskov & Vorburger, 2016). All specimens were collected during *Project Lac*, a large fish diversity assessment of pre-alpine lakes that aimed to probe all available littoral substrates and depth-related habitats for each lake using a standardized gillnet approach (reviewed in Alexander *et al.* (2015a; b)). Briefly, for each lake the littoral habitats (<5 m deep) were classified based on substrate composition and particle size, macrophyte morphology and density, and proximity to an inwardly or outwardly flowing watercourse. Fishing was subsequently performed using a combination of two different gillnet protocols that combined different mesh sizes to reduce size-selective catch biases (described in detail in Alexander *et al.* 2015a). Nets were set in a randomized way within the available area of both benthic and littoral habitats. Netting effort reflected the relative abundance of each habitat with a minimum number of three nets per habitat (Alexander *et al.*, 2015a; b). Following capture, the total length of each specimen was measured and each sample was photographed on the left side for further morphological analyses. From a subset of specimens,

muscle tissue samples were collected for further genetic and stable isotopic analyses (Table 1).

Assessing phenotypic differentiation

We quantified individual shape phenotypes based on 11 landmarks (Figure 1b) in TPSDIG2 (Rohlf, 2015) and subsequently conducted a Procrustes fit on the obtained shape data in MORPHOJ 1.05e (Klingenberg, 2011) for (i) all lake populations (ii) Lake Brienz specimens, (iii) roach used in the genetic and stable isotopic analyses (see Table 1). We corrected Procrustes coordinates for size by performing a regression against standard length, retaining the residuals. To identify the major axes of phenotypic variation, we performed a principal component (PC) analysis on each size-corrected dataset. The scores along the second and third PC axes in the overall data set were tested for differentiation among lake populations using ANOVAs with *post hoc* Tukey-Kramer tests. PC1 of size-corrected landmarks (accounting for 25.5%, 23.9%, and 25.3% of the total variance for the overall data set, the Lake Brienz data set, and the genetic/isotopic data set, respectively) was not analyzed because it was driven by the bending of the fish and therefore, represented a non-biological artifact (Figure S1). We calculated pairwise Mahalanobis distances among lake populations as well as substrate types within Lake Brienz, and estimated their significances with 10,000 bootstrap replicates. To further assess the degree of phenotypic differentiation among lake populations, we calculated P_{ST} following Kaeuffer *et al.* (2012). P_{ST} is a unit-less and scale-free proportional measurement of pairwise difference, here using the scores of PC2 and PC3. For each P_{ST} , we established the 95% confidence interval using a resampling approach with 1,000 replicates following the procedure by Lucek *et al.* (2013). Finally, we tested for an association between pairwise P_{ST} and F_{ST} , and between P_{ST} and pairwise differences in phosphate levels of lakes (Table 1), using Mantel tests in R 3.1.1 with 10,000 bootstrap replicates to determine significance.

To assess phenotypic differentiation within Lake Brienz, we calculated Mahalanobis distances between individuals from different substrates using PC2 scores. Based on the observed clustering of phenotypes (see Results), we combined individuals from different substrates into broader substrate categories,

i.e. rocky (boulders, cobble) and muddy (ledge, inlet/outlet, vegetation). We subsequently performed an ANOVA on individual PC2 and PC3 scores to test for a difference between individuals from these broader substrate categories.

Genomics

We prepared two restriction site-associated (RAD) genomic libraries using *SbfI* restriction sites following Lucek *et al.* (2018). Libraries contained DNA from 42 and 50 individually barcoded specimens, respectively. Each library was single-end sequenced on one lane of an Illumina HiSeq 2000 platform together with ~10% bacteriophage PhiX genomic DNA (Illumina Inc., San Diego, CA, USA) to increase complexity at the first 10 sequenced base pairs. Reads without the complete *SbfI* recognition sequence were subsequently discarded. Using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), we removed any reads with at least one base with a Phred quality score <10 or more than 5% of base pairs with quality <30. This approach yielded 102.6 million high quality reads for analysis.

Given the lack of a reference genome for roach, we generated a *de novo* assembly using all filtered reads for all individuals having more than 250k reads with USTACKS (Catchen *et al.*, 2011). The following settings were used: minimum stack size of 75 reads, allowing a maximum of two base pairs of difference for stacks to be merged and excluding loci with unusually high coverage to avoid repetitive regions. The *de novo* assembly consisted of 49,772 contigs and was used to map reads for each individual with BWA MEM 0.7.17 (Li, 2013). We also aligned raw sequencing reads against the PhiX 174 reference genome (accession: NC_001422; Sanger *et al.*, 1977) masking known variants. We then used the PhiX-alignments to create a base quality score recalibration table for each library using BASERECALIBRATOR from GATK v. 3.7-0 (McKenna *et al.*, 2010). We subsequently recalibrated the base quality scores of each roach alignment to remove potential library effects with the GATK tool PRINTRADS. We called genotypes with UNIFIEDGENOTYPER implemented in GATK v. 3.7-0, considering only bases with a mapping quality >20. Using VCFTOOLS v. 0.1.14 (Danecek *et al.*, 2011), we filtered the resulting VCF file, where we set genotypes with quality < 28 or depth < 6 to missing. We further applied a minor-allele frequency cut-off of

0.03 considering only biallelic SNP positions with $\leq 20\%$ missing data. Following all filtering steps, a total of 3,865 polymorphic SNPs were available for the subsequent analyses comprising all lakes and 4,721 polymorphic SNPs for the Lake Brienz dataset.

We estimated the level of pairwise genetic differentiation between roach populations from different lakes using pairwise locus-by-locus F_{ST} s in GENODIVE v. 2.0b27 (Meirmans & Van Tienderen, 2004). Significances were assessed with 10,000 permutations, applying a Bonferroni correction for the pairwise comparisons. To calculate the probability of each individual to be assigned to its sample population, we employed a discriminant function analysis on principal components (DAPC) with ADEGENET (Jombart *et al.*, 2010) in R based on the first ten PC axes and the four leading discriminant axes. We further used ADEGENET to calculate the observed heterozygosity (H_0) for each roach population. SNPRELATE (Zheng *et al.*, 2012) was used to perform a PC analysis based on the genomic data.

We used RAxML 8.2.11 (Stamatakis, 2014) to test for genetic differentiation among individuals from different lakes as well as among individuals caught over different substrates within Lake Brienz. In both cases we implemented a generalized time-reversible (GTR) model with optimized substitution rates and a gamma model of rate heterogeneity. We then applied an ascertainment bias correction for each dataset to account for the fact that we only used polymorphic SNPs. Significances were assessed using 1000 bootstrap replicates. We also tested for intralacustrine genetic differentiation between individuals caught over a muddy or rocky substrate within Lake Brienz using DAPC based on the 20 leading PC axes accounting for 80% of the total variation and also calculated the average locus-by-locus genetic differentiation (F_{ST} s) between individuals from the two substrates in GENODIVE.

Stable Isotope Analysis

We obtained the stable isotopes signature of individuals using muscle tissue for 12 to 28 individuals per lake (Table 1). In fish, differences in $^{13}\text{C}/^{12}\text{C}$ ratios fall along a gradient where low values indicate a diet dominated by plant and algae matter, while increased values reflect a shift towards higher trophic levels (Post, 2002). To further obtain isotopic baseline values, we collected snails

(*Lymnaeidae* sp. and *Planorbidae* sp.) at the time each lake was sampled (Table 1), except for lakes Neuchatel and Geneva, where we collected baseline material in September 2013. All samples were dried at 55°C for 48 hours. Dry mass (0.5-1.0 mg) was subsequently analyzed with internal reference standards (18 Sucrose [IAEA-CH-6], 18 IAEA-N2, and 18 caffeine [IAEA-600]). The remaining uncertainty as estimated by the standards was 0.08‰ (VPDB). The stable isotopic signature was used to i) test for differences in the stable isotopic signature among populations from different lakes with an ANOVA followed by a TukeyHSD *post hoc* decomposition, ii) to test for an association between morphology and diet across all lake populations by regressing the baseline corrected $\delta^{13}\text{C}$ values against the scores of the second and third phenotypic PC axes, respectively, iii) to determine if the trophic status as measured by the phosphate level of a lake (Table 1) affected the diet of the respective roach population by using an ANOVA, and lastly iv) to test for ecotypic differentiation based on stable isotopes between individuals caught over a rocky or muddy substrate within Lake Brienz using a Mann-Whitney test.

Results

Differentiation among roach from different lakes

Roach differed phenotypically between lakes along both the second ($F_{6,1216} = 45.19$, $p < 0.001$) and third PC axes ($F_{6,1216} = 16.55$, $p < 0.001$), accounting for 16.7% and 13.1% of the overall shape variance, respectively (Figure 2, Table S1). The *post hoc* decomposition suggests that individuals from lakes Brienz and Brenet account for most of the variation captured by the two PC axes (Table S2). Variation along PC2 was driven by differences in the position of the mouth (landmarks 1 & 2) and the position of the pelvic and pectoral fins (landmarks 6 & 7). In contrast, PC3 was mainly driven by differences in the position of the dorsal fin (Table S1). This resulted in a group of specimens from lakes Walen, Neuchatel, Hallwil, Joux, Geneva, and Brenet with a terminal mouth and a more anterior dorsal fin and a second group consisting of roach from Lake Brienz, which had a compact head, a subterminal mouth and a posteriorly placed dorsal fin (Figure 2).

Consistent with a single colonizing lineage, the degree of pairwise genetic differentiation among lake populations was generally low ($F_{ST} \leq 0.040$) but

significant (Table 2). The low level of genetic differentiation between the roach populations from lake Neuchatel and Geneva is consistent with a recent drainage crossing (Larmuseau *et al.*, 2009) and/or human translocations. Despite the low level of genetic differentiation, 99% of all individuals were correctly assigned to their lake of origin by DAPC (Figure 3a). The genetic PC analysis showed a clustering of ultraoligotrophic (Brienzen and Walen) and mesotrophic lake populations (Hallwil, Geneva, and Neuchatel) along PC1, accounting for 2.75% of the total genetic variation (Figure 3b). Our phylogenomic reconstruction showed a clustering similar to the DAPC assignment (Figure 3c), where individuals from Brienzen seemed most distinct, whereas individuals from Geneva and Neuchatel clustered together. However, bootstrapping yielded no significant node support, suggesting substantial levels of gene flow. Levels of heterozygosity (H_o) differed marginally among lake populations (Table 2), and this variation was negatively correlated with the phosphate levels (see Table 1) observed in each lake (Pearson correlation: $\rho = 0.958$; $t_{1,3} = 5.78$, $p = 0.010$). Pairwise F_{ST} s were neither correlated with differences in phosphate levels (Mantel test: $r_M = 0.114$, $p = 0.600$) nor with pairwise phenotypic (P_{ST}) differentiation among lake populations (PC2: $r_M = 0.088$, $p = 0.466$; PC3: $r_M = -0.113$, $p = 0.690$). P_{ST} was likewise not correlated with differences in phosphate amongst lakes (PC2: $r_M = 0.367$, $p = 0.165$; PC3: $r_M = -0.151$, $p = 0.613$).

Stable isotopes indicate significant trophic differentiation of roach amongst lakes ($F_{4,77} = 47.49$, $p < 0.001$), where all but two *post hoc* comparisons (Neuchatel-Geneva and Neuchatel-Walen) were significant. Stable isotopes range from a more herbivorous diet in Lake Brienzen ($\delta^{13}C$ of -22.63 ± 1.80) to a more omnivorous diet within Lake Hallwil ($\delta^{13}C$ of -29.72 ± 1.16 ; Figure 4). However, the stable isotopic values were neither correlated with individual scores along the second ($F_{1,80} = 0.01$, $p = 0.990$) or third ($F_{1,80} = 0.19$, $p = 0.665$) phenotypic PC axes, nor were they correlated with differing phosphate levels ($F_{1,3} = 1.14$, $p = 0.365$).

Diversification within Lake Brienzen

Pairwise Mahalanobis distances suggested phenotypic clustering of individuals caught over “rocky” (boulder, cobbles) vs. “muddy” (ledge,

vegetation) substrates (Figure 5c; Table S3). Individuals caught close to the inlet or outlet clustered with the muddy substrate group and were subsequently included in this substrate category (Figure 5). Consistent with this clustering, we found significant phenotypic differentiation between individuals caught over muddy and rocky substrates along the second ($F_{1,81}=12.77$, $p<0.001$) but not third ($F_{1,81}=0.01$, $p=0.902$) PC axes. Variation along PC2 was driven by morphological differences in the position of the dorsal, caudal, and pelvic fin (landmarks 11, 9 and 7), while PC3 was driven by the placement of the dorsal (landmark 11) and pectoral fin (landmark 6) and the position of the mouth (landmarks 1 & 2). The two phenotypic clusters did not differ in their diet assessed by stable isotopes ($W = 61.5$, $p = 0.540$).

Our phylogenomic reconstruction did not yield any significant clustering by substrate (Figure 5b). Concordantly, there was no genome-wide differentiation between individuals caught over muddy or rocky substrate ($F_{ST} = -0.001$, $p = 0.759$). When using a discriminant function analysis that maximizes the differentiation among substrates, a bimodal distribution occurred along the discriminant axis, supporting some genetic differentiation (Figure 5a). Indeed, we found five SNPs among the total of 4,721 polymorphic SNPs within Lake Brienz that showed a $F_{ST} > 0.3$, each belonging to a different contig (Table S4). To identify potential genes involved in substrate-related differentiation, we further matched each contig against the NCBI nucleotide collection on the 26th of October 2018 using megablast (Boratyn *et al.*, 2013). Of the five contigs, two overlapped with known genes: i) *FSTL5: Follistatin-related protein 5* and ii) *PCSK5: Proprotein convertase subtilisin/kexin type 5* – a gene involved in neuromast deposition within the lateral line system in zebrafish, where a deficiency resulted in reduced spatial awareness and sensing of the environment (Chitramuthu *et al.*, 2010).

Discussion

Postglacial diversification of roach

Understanding why some species undergo diversification, while others do not, remains a conundrum. Evidence for species diversification among temperant freshwater fish comes from a small range of taxa, while intraspecific

diversification remains unassessed for most other fish species (Seehausen & Wagner, 2014). Roach have a broad geographic distribution in Europe and occur in a variety of habitats – including deep and ultraoligotrophic lakes that provide a wide range of potential niches to diversify, making roach a good candidate to look for diversification (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). We found evidence for intraspecific differentiation between roach populations from ultraoligotrophic lakes and lakes with a higher trophic level, as well as some diversification within the ultraoligotrophic Lake Brienz.

Former studies suggested that roach often adapt to their local environment and become phenotypically differentiated, e.g. along a littoral-pelagic axis as a result of intra- and interspecific competition (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015) or predation (Scharnweber *et al.*, 2013), and this phenotypic differentiation has often been attributed to phenotypic plasticity (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). Studying roach from pre-alpine lakes in Switzerland, we found subtle yet significant genetic differentiation amongst populations from different pre-alpine lakes, where populations from ultraoligotrophic lakes are genetically more distinct (Figure 3b). This is consistent with recent colonization, potentially combined with ongoing gene flow within the Aare/Rhine drainage. Alternatively, the effective population size may be too large for drift to become a dominant factor (Gillespie, 2001). We also found roach from Lake Geneva to cluster closely with individuals from the nearby Aare/Rhine system (Figure 3). This suggests a common origin, potentially due to historical connectivity as observed for other fish species (Vonlanthen *et al.*, 2007; Gouskov & Vorburger, 2016) or supplementary human translocations. Despite their low level of genetic differentiation (Table 2), roach differed both phenotypically (Figure 2b, Table S1) and ecologically (Figure 4) between lakes. Individuals differed predominantly in their head shape, with the population from Lake Brienz being most distinct, showing a slender head and more subterminal orientation of the mouth (Figure 2). The observed phenotypic changes among roach from different lakes hint towards a functional and potentially adaptive response related to feeding regimes that differ between lakes (Wainwright & Barton, 1995). Phenotypic changes in head and body shape similar to the ones observed here were indeed found to occur in response to differences in resource use in other

fish (Anker, 1974; Barel, 1983; Pfaender *et al.*, 2009). Given the lack of an association between the degree of phenotypic and genetic differentiation, the observed phenotypic changes likely represent a plastic response to varying environmental pressures, as has been proposed for other roach populations (Scharnweber *et al.*, 2013; Faulks *et al.*, 2015).

Intralacustrine diversification in Lake Brienz

Habitat-dependent divergent selection can lead to the evolution of distinctly adapted ecotypes within a system (Schluter, 2000; Nosil, 2012). When combined with intra- and interspecific competition, divergent selection can lead to differences in prey utilization between individuals from structurally contrasting environments. These factors are common drivers of diversification among postglacial freshwater fishes (Rosenzweig, 1978; Dieckmann & Doebeli, 1999; Gavrillets, 2004; Svanbäck & Bolnick, 2007). Both intra- and interspecific competition, such as with perch (*Perca fluviatilis*), have been shown to drive resource polymorphism in roach from Swedish lakes (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). This may similarly apply for roach in Lake Brienz where perch are the most abundant fish species caught, followed by roach and whitefish. Roach were moreover restricted to depths <3m, overlapping with perch and part of the whitefish species, thus providing the potential for interspecific competition (Alexander *et al.*, 2015a; Doenz *et al.*, 2018).

Substrate-related phenotypic differentiation is common among freshwater fishes, where adaptive phenotypic changes often occur in head shape, as a response to different feeding regimes (Caldecutt & Adams, 1998; McGee *et al.*, 2013), and in fin position or body shape in response to different swimming regimes (Walker, 1997; Hendry *et al.*, 2011). Within Lake Brienz, we found roach to show evidence for such substrate-related intralacustrine phenotypic diversification, as individuals fell into two phenotypic clusters (Figure 5). Individuals caught over muddy substrates showed a more caudal position of the dorsal fin, consistent with adaptation to more active swimming in cyprinid fish (Felley 1984). This, together with an elongated snout and a more terminal mouth (Figure 5), could reflect feeding on more pelagic prey as has been found for other lake-dwelling roach populations (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). In

contrast, individuals caught over a rocky substrate had a more anterior dorsal fin, consistent with increased manoeuvrability in structured environments such as between rocks. The compact head and sub-terminal mouth of fish from a rocky substrate is also often associated with a predominantly benthic feeding strategy (Wainwright & Barton, 1995). To which degree these phenotypic differences are associated with selective feeding strategies, e.g., if and to which extent fish caught over a muddy vegetated substrate feed on macrophytes, remains unknown as we relied solely on stable isotope data. With the latter we found no association between phenotypes and resource use. This, however contrasts with the increased range of stable isotope values found for roach in Lake Brienz (Figure 4) and could reflect limited power to distinguish differences in microhabitats given our restricted sample sizes. However, stable isotopes represent a long-term average diet, and the observed phenotypic segregation shown here may be seasonal (Post, 2002).

Average genome-wide differentiation between the two substrate-related phenotypic groups was absent (i.e. $F_{ST} = -0.001$), and no apparent clustering occurred in our phylogenetic reconstruction (Figure 5). This is also consistent with plasticity acting as the main driver for the observed phenotypic differentiation. However, a discriminate function analysis that captured the differences between the two groups suggests a bimodal distribution of individuals (Figure 5a). Among the five markers that showed the highest degree of genetic differentiation between substrates (Table S4), one occurred within the gene *PCSK5* that is involved in lateral line development (Chitramuthu *et al.*, 2010). The lateral line organ is important for spatial awareness and sensing of the environment, and the observed genetic differences could suggest divergent selection between the two substrates that differ in their complexity, being consistent with the detected differences in body shape (Figure 4). Genomic differentiation at only few target loci is consistent with a very early stage of divergence-with-gene flow, where further differentiation depends on the evolution of barriers to gene flow (Nosil, 2012). The absence of significant genomic differentiation could also reflect a limited resolution given the restricted number of polymorphic SNPs available for our analyses (Wagner *et al.*, 2013).

The slight differentiation of roach of different habitats contrasts with the co-occurring adaptive radiation of whitefish, which had a similar timespan as roach to evolve in Lake Brienz, i.e. since the retreat of the glaciers ~12kyrs ago. Within Lake Brienz, there are a total of four genetically differentiated whitefish species, segregated along the water depth and pelagic-benthic axes, which are distinct in their morphology, including the gill rakers (Doenz *et al.*, 2018), thus suggesting adaptation to different trophic niches (Roesch *et al.*, 2013). Given the abundances of perch and whitefish in Lake Brienz (Alexander *et al.*, 2015a; Doenz *et al.*, 2018), the limited degree of diversification in roach could be a result of different factors. i) Interspecific competition may have constrained roach from diversifying. ii) If the observed phenotypic differentiation (Figure 5) is primarily due to phenotypic plasticity, the latter could have constrained diversification by shielding the genome from selection, thus decreasing the potential for genetic divergence (Price *et al.*, 2003; Ghalambor *et al.*, 2007). iii) The fundamental niche of roach may be narrower than that of whitefish, preventing roach to explore otherwise available niche space. For example, roach prefer warmer water and are therefore restricted to the shallow zones of lakes, while whitefish can tolerate colder water, allowing them to explore the deeper sections of lakes (Coutant 1977, Kottelat & Freyhof, 2007). iv) Recent genomic work suggests that adaptive diversification in stickleback and whitefish often occurs from standing genetic variation in genomic regions that show structural changes, including inversions (Jones *et al.*, 2012; Marques *et al.*, 2016) or chromosomal rearrangements (Dion-Côté *et al.*, 2016). Such structural genomic rearrangements may then facilitate diversification through coupling of co-adapted alleles (Butlin & Smadja, 2018). Given the limited evidence for genetic differentiation in roach (Figure 5, Table S4), such genomic features may be lacking, which may constitute a genetic constraint that impedes diversification and the build-up of genetic barriers to gene flow (Seehausen *et al.*, 2014).

Conclusions

Intraspecific differentiation in response to habitat-dependent divergent selection is thought to be a major driver of diversification and adaptive radiation in freshwater fish, yet evidence comes from only a few taxonomic groups (Seehausen & Wagner, 2014). Combining phenotypic, ecological, and genomic data, we show differentiation between lake populations of roach from ultraoligotrophic lakes and lakes with a higher trophic level within the same drainage system, potentially in response to different abiotic and biotic factors. In one ultraoligotrophic lake, we also found evidence for intralacustrine diversification with different phenotypes being associated with distinct substrates. However, given the lack of genetic differentiation, phenotypic changes are likely to be mostly plastic, where the lack of diversification may also reflect genomic constraints. This needs to be investigated in the future. Taken together, our study reveals striking differences in the degree of phenotypic and genetic differentiation between this lineage of roach and the lineage of whitefish that has undergone impressive adaptive radiations in the same lakes. However, our study also indicates the potential for more subtle intraspecific differentiation and diversification in a widespread and abundant freshwater fish species, especially in ultraoligotrophic lakes. This may similarly apply to other fish species and highlights the importance to study both an ecologically and a geographically broad range of populations within a species to assess cryptic biodiversity (Bickford *et al.*, 2007).

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Data accessibility

BAM files with aligned de-multiplexed and base quality score recalibrated reads are available through the short read archive (www.ncbi.nlm.nih.gov/sra). BioProject ID: PRJNA533015. Phenotypic and stable isotopic data are available through DRYAD: XXXXX.

525

526 **Figure legends**

527 Figure 1: a) Map of Switzerland with the sampled lakes indicated: A) Geneva, B)
528 Joux, C) Brenet, D) Neuchatel, E) Brienz, F) Hallwil, and G) Walen (see Table 1 for
529 details). b) Roach (*Rutilus rutilus*) with the 11 morphological landmarks used: 1)
530 anterior tip of snout, 2) anterior tip of lower lip, 3) anterior & 4) posterior point
531 of operculum, 5) junction where the dorsolateral part of the head and body fuse,
532 anterior insertion points of the 6) pectoral, 7) pelvic and, 8) anal fin, 9) ventral
533 and 10) dorsal junction of the caudal peduncle and tail, 11) anterior insertion of
534 the dorsal fin.

535

536 Figure 2: Phenotypic relationships across lake populations. a) Mahalanobis
537 distance dendrogram. b) Principal component (PC) analysis of body shape for all
538 seven-lake populations. Shown are the mean values across the second and third
539 PC axes with the 95% confidence interval for each population. Changes in body
540 shape are further indicated.

541

542 Figure 3: Population genomic structure across different lake populations. a)
543 Individual-based assignment probabilities based on a discriminant function
544 analysis of PC components (DAPC). b) Principal component analysis based on
545 3,865 polymorphic SNPs. c) RAxML phylogeny tree depicting the genetic
546 relationship of all roach (no significant bootstrap support except two nodes with
547 >50% support highlighted by a grey dot).

548

549 Figure 4: Boxplot summarizing the variance in $\delta^{13}\text{C}$ among roach from different
550 lakes. Horizontal bars indicate significant comparisons ($p < 0.05$) after a *post hoc*
551 Tukey-Kramer ANOVA decomposition (see main text for details).

552

553 Figure 5: Differentiation of roach within Lake Brienz based on: a) discriminant
554 function analysis of genetic data comparing individuals assigned to different
555 substrate groups (rocky vs. muddy). b) RAxML phylogeny tree depicting the
556 genetic relationship of Brienz roach (no significant bootstrap support). c)
557 Morphological relationship based on Mahalanobis distances between different

558 substrates. Morphological differences between individuals caught over rocky
559 (boulders and cobble) and muddy (ledge, inlet/outlet, and vegetation) substrates
560 are indicated.

561

562

References

- Alexander, T.J., Vonlanthen, P., Periat, G., Degiorgi, F., Raymond, J.-C. & Seehausen, O. 2015a. Evaluating gillnetting protocols to characterize lacustrine fish communities. *Fish Res* **161**: 320–329.
- Alexander, T.J., Vonlanthen, P., Periat, G., Degiorgi, F., Raymond, J.C. & Seehausen, O. 2015b. Estimating whole-lake fish catch per unit effort. *Fish Res* **172**: 287–302.
- Anker, G.C. 1974. Morphology and kinetics of the head of the stickleback, *Gasterosteus aculeatus*. *Trans Zool Soc London* **32**: 311–416.
- Barel, C.D.N. 1983. Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). *Neth J Zool* **33**: 357–424.
- Barrett, R.D.H. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol Evol* **23**: 38–44.
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., *et al.* 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol* **22**: 148–155.
- Bolnick, D.I. & Fitzpatrick, B.M. 2007. Sympatric speciation: models and empirical evidence. *Annu Rev Ecol Evol S* **38**: 459–487.
- Bolnick, D.I., Svanbäck, R., Araujo, M.S. & Persson, L. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *P Natl Acad Sci Usa* **104**: 10075–10079.
- Boratyn, G.M., Camacho, C., Cooper, P.S., Coulouris, G., Fong, A., Ma, N., *et al.* 2013. BLAST: a more efficient report with usability improvements. *Nucleic Acids Res* **41**: W29–W33.
- Braband, A. 1985. Food of roach (*Rutilus rutilus*) and ide (*Leuciscus idus*):

596 significance of diet shifts for interspecific competition in omnivorous fishes.
597 *Oecologia* **66**: 461–467.
598

599 Butlin, R.K. & Smadja, C.M. 2018. Coupling, reinforcement, and speciation. *Am Nat*
600 **191**: 155–172.
601

602 Caldecutt, W. & Adams, D. 1998. Morphometrics of trophic osteology in the
603 threespine stickleback, *Gasterosteus aculeatus*. *Copeia* 827–838.
604

605 Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H. 2011.
606 Stacks: building and genotyping Loci de novo from short-read sequences. *G3* **1**:
607 171–182.
608

609 Chitramuthu, B.P., Baranowski, D.C., Cadieux, B., Rousselet, E., Seidah, N.G. &
610 Bennett, H.P.J. 2010. Molecular cloning and embryonic expression of zebrafish
611 PCSK5 co-orthologues: Functional assessment during lateral line development.
612 *Dev Dyn* **239**: 2933–2946.
613

614 Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among
615 natural selection, adaptation and gene flow. *J Evol Biol* **21**: 1460–1469.
616

617 Coutant, C.C. 1977. Compilation of temperature preference data. *J Fish Res Board*
618 *Can* **34**: 739–745.
619

620 Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., *et al.*
621 2011. The variant call format and VCFtools. *Bioinformatics* **27**: 2156–2158.
622

623 Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric
624 speciation. *Nature* **400**: 354–357.
625

626 Dion-Côté, A.-M., Symonová, R., Lamaze, F.C., Pelikánová, Š., Ráb, P. & Bernatchez,
627 L. 2016. Standing chromosomal variation in Lake Whitefish species pairs: the
628 role of historical contingency and relevance for speciation. *Mol Ecol* **26**: 178–192.

629

630 Doenz, C.J., Bittner, D., Vonlanthen, P., Wagner, C.E. & Seehausen, O. 2018. Rapid
631 buildup of sympatric species diversity in Alpine whitefish. *Ecol Evol* **8**: 9398–
632 9412.

633

634 Faulks, L., Svanbäck, R., Eklöv, P. & Östman, Ö. 2015. Genetic and morphological
635 divergence along the littoral–pelagic axis in two common and sympatric fishes:
636 perch, *Perca fluviatilis* (Percidae) and roach, *Rutilus rutilus* (Cyprinidae). *Biol J*
637 *Linn Soc* **114**: 929–940.

638

639 Gavrillets, S. 2004. *Fitness landscapes and the origin of species*. Princeton
640 University Press, Princeton, MA, USA.

641

642 Ghalambor, C.K., McKay, J., Carroll, S.P. & Reznick, D.N. 2007. Adaptive versus
643 non-adaptive phenotypic plasticity and the potential for contemporary
644 adaptation in new environments. *Funct Ecol* **21**: 394–407.

645

646 Gillespie, J.H. 2001. Is the population size of a species relevant to its evolution?
647 *Evolution* **55**: 2161–2169.

648

649 Gouskov, A. & Vorburger, C. 2016. Postglacial recolonizations, watershed
650 crossings and human translocations shape the distribution of chub lineages
651 around the Swiss Alps. *BMC Evol Biol* **16**: 185.

652

653 Hänfling, B., Durka, W. & Brandl, R. 2004. Impact of habitat fragmentation on
654 genetic population structure of roach, *Rutilus rutilus*, in a riparian ecosystem.
655 *Conserv Genet.* **5**:247.

656

657 Hendry, A.P., Hudson, K., Walker, J.A., Räsänen, K. & Chapman, L.J. 2011. Genetic
658 divergence in morphology-performance mapping between Misty Lake and inlet
659 stickleback. *J Evol Biol* **24**: 23–35.

660

661 Hewitt, G.M. 1999. Post-glacial re-colonization of European biota. *Biol J Linn Soc*

662 **68**: 87–112.

663
664 Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive
665 radiations from a single hybridogenic ancestral population. *P R Soc B* **278**: 58–66.

666
667 Jombart, T., Devillard, S. & Balloux, F. 2010. Discriminant analysis of principal
668 components: a new method for the analysis of genetically structured populations.
669 *BMC Genet.* **11**: 94.

670
671 Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J., *et al.*
672 2012. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*
673 **484**: 55–61.

674
675 Jonsson, B. & Jonsson, N. 2001. Polymorphism and speciation in Arctic charr. *J*
676 *Fish Bio* **58**: 605–638.

677
678 Kaeuffer, R., Peichel, C.L., Bolnick, D.I. & Hendry, A.P. 2012. Parallel and
679 nonparallel aspects of ecological, phenotypic, and genetic divergence across
680 replicate population pairs of lake and stream stickleback. *Evolution* **66**: 402–418.

681
682 Karvonen, A. & Seehausen, O. 2012. The role of parasitism in adaptive
683 radiations—when might parasites promote and when might they constrain
684 ecological speciation? *Inter J Ecol* **2012**: 1–20.

685
686 Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric
687 morphometrics. *Mol Ecol Resour* **11**: 353–357.

688
689 Kottelat, M. & Freyhof, J. 2007. *Handbook of European freshwater fishes*. Kottelat,
690 Cornol, Switzerland.

691
692 Larmuseau, M.H.D., Freyhof, J., Volckaert, F.A.M. & Van Houdt, J.K.J. 2009.
693 Matrilinear phylogeography and demographical patterns of *Rutilus rutilus*:
694 implications for taxonomy and conservation. *J Fish Biol* **75**: 332–353.

695

696 Laroche, J., Durand, J.D. & Bouvet, Y. 1999. Genetic structure and differentiation
697 among populations of two cyprinids, *Leuciscus cephalus* and *Rutilus rutilus*, in a
698 large European river. *Can J Fish Aquat Sci.* **56**:1659-1667.

699

700 Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with
701 BWA-MEM. *arXiv.org* 1303.3997v2.

702

703 Lucek, K., Keller, I., Nolte, A.W. & Seehausen, O. 2018. Distinct colonization waves
704 underlie the diversification of the freshwater sculpin (*Cottus gobio*) in the
705 Central European Alpine region. *J Evol Biol* **31**:1254-1267.

706

707 Lucek, K., Kristjánsson, B.K., Skulason, S. & Seehausen, O. 2016. Ecosystem size
708 matters: the dimensionality of intralacustrine diversification in Icelandic
709 stickleback is predicted by lake size. *Ecol Evol* **6**:5256-5272.

710

711 Lucek, K., Sivasundar, A. & Seehausen, O. 2014. Disentangling the role of
712 phenotypic plasticity and genetic divergence in contemporary ecotype formation
713 during a biological invasion. *Evolution* **68**: 2619–2632.

714

715 Lucek, K., Sivasundar, A., Roy, D. & Seehausen, O. 2013. Repeated and predictable
716 patterns of ecotypic differentiation during a biological invasion: lake-stream
717 divergence in parapatric Swiss stickleback. *J Evol Biol* **26**: 2691–2709.

718

719 Marques, D.A., Lucek, K., Meier, J.I., Mwaiko, S., Wagner, C.E., Excoffier, L.,
720 Seehausen, O. 2016. Genomics of rapid incipient speciation in sympatric
721 threespine stickleback. *Plos Genet* **12**:e1005887.

722

723 McGee, M.D., Schluter, D. & Wainwright, P.C. 2013. Functional basis of ecological
724 divergence in sympatric stickleback. *BMC Evol Biol* **13**: 277.

725

726 McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., *et al.*
727 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-

728 generation DNA sequencing data. *Genome Res* **20**: 1297–1303.

729

730 Meirmans, P.G. & Van Tienderen, P. 2004. GENOTYPE and GENODIVE: two

731 programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol*

732 *Notes* **4**: 792–794.

733

734 Nosil, P. 2012. *Ecological Speciation*. Oxford University Press, Oxford, UK.

735

736 Nosil, P. & Sandoval, C.P. 2008. Ecological niche dimensionality and the

737 evolutionary diversification of stick insects. *PLoS ONE* **3**: e1907.

738

739 Persson, L. 1983. Effects of intra- and interspecific competition on dynamics and

740 size structure of a perch *Perca fluviatilis* and a roach *Rutilus rutilus* population.

741 *Oikos* **41**: 126–132.

742

743 Pfaender, J., Schliewen, U.K. & Herder, F. 2009. Phenotypic traits meet patterns of

744 resource use in the radiation of “sharpfin” sailfin silverside fish in Lake Matano.

745 *Evol Ecol* **24**: 957–974.

746

747 Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. &

748 Moczek, A.P. 2010. Phenotypic plasticity's impacts on diversification and

749 speciation. *Trends Ecol Evol* **25**: 459–467.

750

751 Post, D.M. 2002. Using stable isotopes to estimate trophic position models

752 methods and assumptions. *Ecology* **83**: 703–718.

753

754 Prejs, A., Lewandowski, K. & Stańczykowska-Piotrowska, A. 1990. Size-selective

755 predation by roach (*Rutilus rutilus*) on zebra mussel (*Dreissena polymorpha*):

756 field studies. *Oecologia* **83**: 378–384.

757

758 Price, T.D., Qvarnström, A. & Irwin, D.E. 2003. The role of phenotypic plasticity in

759 driving genetic evolution. *P R Soc B* **270**: 1433–1440.

760

761 Roesch, C., Lundsgaard-Hansen, B., Vonlanthen, P., Taverna, A., Seehausen, O.
 762 2013. Experimental evidence for trait utility of gill raker number in adaptive
 763 radiation of a north temperate fish. *J Evol Biol* 26: 1578–1587.
 764
 765 Rohlf, F. J. 2006. Version 2.10. Department of Ecology and Evolution, State
 766 University, Stony Brook, New York.
 767
 768 Rosenzweig, M.L. 1978. Competitive speciation. *Biol J Linn Soc* **10**:275–289.
 769
 770 Sanger, F., Air, G.M., Barrell, B.G., Brown, N.L., Coulson, A.R., Fiddes, C.A., *et al.*
 771 1977. Nucleotide sequence of bacteriophage phi X174 DNA. *Nature* **265**: 687–
 772 695.
 773
 774 Scharnweber, K., Watanabe, K., Syväranta, J., Wanke, T., Monaghan, M.T. &
 775 Mehner, T. 2013. Effects of predation pressure and resource use on
 776 morphological divergence in omnivorous prey fish. *BMC Evol Biol* **13**: 132.
 777
 778 Schluter, D. 1996. Ecological speciation in postglacial fishes. *Phi. Trans R Soc B*
 779 **351**: 807–814.
 780
 781 Schluter, D. 2001. The ecology of adaptive radiation. *Heredity* **86**:749-750.
 782
 783 Seehausen, O. & Wagner, C.E. 2014. Speciation in freshwater fishes. *Annu Rev Ecol*
 784 *Evol S* **45**: 621–651.
 785
 786 Seehausen, O., Butlin, R.K., Keller, I., Wagner, C.E., Boughman, J.W., Hohenlohe,
 787 P.A., *et al.* 2014. Genomics and the origin of species. *Nat Rev Genet* **15**: 176–192.
 788
 789 Smith, T.B. & Skúlason, S. 1996. Evolutionary significance of resource
 790 polymorphisms in fishes, amphibians, and birds. *Annu Rev Ecol Evol Syst* **27**:111–
 791 133.
 792
 793 Snorrason, S.S. & Skúlason, S. 2004. Adaptive speciation in northern freshwater

794 fishes In: Dieckmann, U., & Doebeli, M.: *Adaptive speciation*, p. 210–228.
795 Cambridge University Press, Cambridge, UK.
796
797 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-
798 analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
799
800 Stroud, J.T. & Losos, J.B. 2016. Ecological opportunity and adaptive radiation.
801 *Annu Rev Ecol Evol Syst* **47**: 507–532.
802
803 Svanbäck, R. & Bolnick, D.I. 2007. Intraspecific competition drives increased
804 resource use diversity within a natural population. *P R Soc B* **274**: 839–844.
805
806 Svanbäck, R. & Eklöv, P. 2004. Morphology in perch affects habitat specific
807 feeding efficiency. *Funct Ecol* **18**: 503–510.
808
809 Svanbäck, R., Eklöv, P., Fransson, R. & Holmgren, K. 2008. Intraspecific
810 competition drives multiple species resource polymorphism in fish communities.
811 *Oikos* **117**: 114–124.
812
813 Thibert-Plante, X. & Hendry, A.P. 2011. The consequences of phenotypic
814 plasticity for ecological speciation. *J Evol Biol* **24**: 326–342.
815
816 Vejříková, I., Eloranta, A.P., Vejřík, L., Šmejkal, M., Čech, M., Sajdlová, Z., *et al.* 2017.
817 Macrophytes shape trophic niche variation among generalist fishes. *PLoS ONE*
818 **12**: e0177114.
819
820 Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Müller, R., Lundsgaard-
821 Hansen, B., *et al.* 2012. Eutrophication causes speciation reversal in whitefish
822 adaptive radiations. *Nature* **482**: 357–362.
823
824 Vonlanthen, P., Excoffier, L., Bittner, D., Persat, H., Neuenschwander, S. &
825 Largiadèr, C.R. 2007. Genetic analysis of potential postglacial watershed
826 crossings in Central Europe by the bullhead (*Cottus gobio* L.). *Mol Ecol* **16**: 4572–

4584.

Vonlanthen, P., Roy, D., Hudson, A.G., Largiadèr, C.R., Bittner, D. & Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus* sp.). *J Evol Biol* **22**: 498–514.

Wagner, C.E., Harmon, L.J. & Seehausen, O. 2014. Cichlid species-area relationships are shaped by adaptive radiations that scale with area. *Ecol Lett* **17**: 583–592.

Wagner, C.E., Keller, I., Wittwer, S., Selz, O.M., Mwaiko, S., Greuter, L., *et al.* 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Mol Ecol* **22**: 787–798.

Wainwright, P.C. & Barton, A.R. 1995. Predicting patterns of prey use from morphology of fishes. *Environ Biol Fishes* **44**: 97–113.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. *Biol J Linn Soc* **61**: 3–50.

Walsh, M.R. & Reznick, D.N. 2009. Phenotypic diversification across an environmental gradient: a role for predators and resource availability on the evolution of life histories. *Evolution* **63**: 3201–3213.

Zheng, X.W., Levine, D., Shen, J., Gogarten, S.M., Laurie, C. & S, W.B. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* **28**: 3326–3328.

Table 1: Characteristics of each sampled lake and the sample size of each data set. For each lake, the coordinates, the trophic status based on dissolved phosphate (parts per million – ppm), its elevation, and maximal depth are given. In addition to the sampling date, depth range where individuals were sampled are indicated. Samples sizes for morphology, genomics and stable isotopes are provided. Phosphate levels are based on measurements taken in 2002.

Lakes	Geographic coordinates		Trophic status		Elevation (m)	Maximum Depth (m)	Sampled	Depth Range (m)	Numbers of Samples		
	Latitude	Longitude	Phosphate (ppm)	Trophic Level					Morphology	Genomics	Stable Isotopes
Brien	47°48'E	45°49'N	3	Oligotrophic	564	260	Sept 2011	1.0 – 12.0	190	41	28
Brenet	6°19'E	46°40'N	29	Eutrophic	100	18	Sept 2011	1.9 – 20.0	342	-	-
Hallwil	8°12'E	47°17'N	16	Mesotrophic	449	28	Oct 2012	1.9 – 20.0	94	10	13
Joux	6°17'E	46°38'N	16	Mesotrophic	100	32	Sept 2011	1.1 – 15.0	257	-	-
Geneva	6°33'E	46°26'N	23	Mesotrophic	372	310	Sept 2012	0.5 – 42.0	102	9	12
Neuchatel	6°55'E	46°59'N	6	Oligotrophic	429	152	Sept 2011	1.2 – 37.0	208	10	15
Walen	9°12'E	47°07'N	4	Oligotrophic	419	151	Oct 2012	1.1 – 27.0	30	10	14

862 Table 2: Observed heterozygosity (H_o) of each lake population as well as the
863 pairwise genetic differentiation (F_{ST}) among populations. F_{ST} values are
864 presented in the lower triangle and Bonferroni corrected significance levels in
865 the upper triangle.

866

	<i>H_o</i>	Brien	Hallwil	Geneva	Neuchatel	Walen
Brien	0.265		<0.001	<0.001	<0.001	<0.001
Hallwil	0.257	0.032		<0.001	<0.001	<0.001
Geneva	0.257	0.026	0.032		<0.001	<0.001
Neuchatel	0.264	0.025	0.025	0.005		<0.001
Walen	0.265	0.038	0.036	0.030	0.026	

867

a) Map of Switzerland with the sampled lakes indicated: A) Geneva, B) Joux, C) Brenet, D) Neuchatel, E) Brienzi, F) Hallwil, and G) Walen (see Table 1 for details). b) Roach (*Rutilus rutilus*) with the 11 morphological landmarks used: 1) anterior tip of snout, 2) anterior tip of lower lip, 3) anterior & 4) posterior point of operculum, 5) junction where the dorsolateral part of the head and body fuse, anterior insertion points of the 6) pectoral, 7) pelvic and, 8) anal fin, 9) ventral and 10) dorsal junction of the caudal peduncle and tail, 11) anterior insertion of the dorsal fin.

389x439mm (300 x 300 DPI)

a)

membership probability

b)

c)

■ Brienzen

■ Hallwil

■ Geneva

■ Neuchatel

■ Walen

a) Discriminant Function Analysis

b) Phylogenetic Relationship

c) Phenotypic Relationship

Figure S1: Histograms based on PC1 scores using size-corrected geometric morphometric data. Shown are the distributions using individuals from all lakes, using only genotyped individuals, and using only individuals from Lake Brienz. PC1 data was removed from subsequent analysis for all datasets because PC1 was driven by the bending of the fish, shown by the deformation grids for each dataset.

Table S1: Principal component (PC) scores for each landmark (see Figure 1 for details) along PC axes 1-3. For each axis, the percentage of variance explained is indicated. Also indicated are the standardized PC scores for axes 2 and 3 showing the relative importance of each trait (see main text for details).

Landmark	PC1 (25.5%)	PC2 (16.7%)	PC2 Standardized	PC3 (13.1%)	PC3 Standardized
x1	-0.014	0.474	0.992	0.066	0.087
y1	-0.331	0.012	0.026	-0.034	0.045
x2	0.008	0.478	1	0.064	0.084
y2	-0.326	-0.045	0.095	-0.06	0.079
x3	0.115	-0.057	0.118	-0.131	0.173
y3	-0.062	0.12	0.25	0.014	0.018
x4	0.007	-0.323	0.676	-0.204	0.268
y4	0.147	-0.001	0.003	0	0
x5	-0.234	-0.16	0.336	-0.231	0.303
y5	-0.015	-0.187	0.392	-0.056	0.073
x6	0.109	-0.348	0.728	-0.215	0.283
y6	0.207	0.051	0.108	0.029	0.038
x7	0.012	-0.338	0.708	0.239	0.314
y7	0.479	-0.083	0.174	-0.06	0.079
x8	-0.171	0.034	0.072	0.166	0.219
y8	0.157	-0.052	0.11	0.001	0.001
x9	0.029	0.22	0.46	-0.27	0.356
y9	-0.306	0.049	0.102	0.012	0.016
x10	0.147	0.167	0.349	-0.245	0.322
y10	-0.317	0.021	0.043	-0.001	0.002
x11	-0.008	-0.146	0.306	0.76	1
y11	0.367	0.117	0.245	0.157	0.206

Table S2: Tukey-Kramer *post hoc* results, with a Bonferroni correction applied, for PC2 and PC3 scores detailing significant morphological differences amongst lake populations.

Lake Comparisons		<i>p-value</i>	
		PC2	PC3
Brenet	Brienzen	<0.001	0.000
	Geneva	0.984	0.015
	Joux	0.006	0.000
	Geneva	<0.001	0.000
	Neuchatel	<0.001	0.000
	Walén	0.978	0.000
Brienzen	Geneva	<0.001	0.973
	Joux	<0.001	0.998
	Geneva	<0.001	0.154
	Neuchatel	<0.001	0.419
	Walén	<0.001	0.606
Geneva	Joux	0.021	0.999
	Geneva	0.036	0.050
	Neuchatel	<0.001	0.152
	Walén	0.882	0.325
Joux	Geneva	<0.001	0.032
	Neuchatel	0.431	0.093
	Walén	0.993	0.398
Geneva	Neuchatel	<0.001	0.973
	Walén	0.014	1.000
Neuchatel	Walén	0.669	0.993

Table S3: Mahalanobis distances calculated between habitat groups within Lake Brienz, with their associated *p*-values based on 10,000 bootstraps.

Comparison	Mahalanobis distance	<i>p</i>-value
Boulders – Cobble	1.58	0.110
Boulders – Ledge	2.39	<0.001
Boulders – Affluent/Effluent	1.69	<0.001
Boulders - Vegetation	1.84	<0.001
Cobble – Ledge	1.81	0.056
Cobble – Affluent/Effluent	1.72	0.001
Cobble – Vegetation	1.52	0.130
Ledge – Affluent/Effluent	1.52	0.080
Ledge – Vegetation	1.50	0.272
Affluent/Effluent – Vegetation	0.93	0.755

Table S4: List of SNPs showing an $F_{ST} > 0.3$ between individuals caught over rocky or muddy substrates in Lake Brienz. Also, presented are the SNPs, contig IDs, and the locus specific F_{ST} . Each contig was compared to the NCBI nucleotide collection. Gene annotations for contigs that aligned with a known gene in other fish species are given.

SNP ID	Contig ID	F_{ST}	Gene annotation
SNP_1198	consensus_3971	0.333	FSTL5: Follistatin-related protein 5
SNP_2379	consensus_10858	0.374	-
SNP_2439	consensus_11289	0.307	PCSK5: Proprotein convertase subtilisin/kexin type 5
SNP_3301	consensus_18419	0.323	-
SNP_4437	consensus_38170	0.319	-