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

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Abstract

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

Keywords: *Rutilus rutilus*, resource polymorphism, postglacial range expansion, 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; Gavrilets, 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 & Skulason, 1996; Schluter, 2000; Lucek *et al.*, 2014). Plasticity can initially promote differentiation (Snorrason & Skulason, 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 macropyhtes 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

98 distribution across Europe (Kottelat & Freyhof, 2007), iii) its ability to inhabit an
99 array of different environments (including streams and the pelagic and littoral
100 zones of lakes (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015), iv) its large population
101 sizes, and v) its modest economic importance, resulting in little to no direct
102 management. Additionally, the roach in this study (Figure 1) often coexist with
103 adaptive radiations of whitefish and are ecologically similar to some of the
104 shallow water whitefish species (Hudson *et al.*, 2011; Vonlanthen *et al.*, 2012;
105 Doenz *et al.*, 2018), thus providing the potential for ecological niche shifts of
106 roach in response to interspecific interactions, as has been shown for other fish
107 species (Persson, 1983; Braband, 1985; Faulks *et al.*, 2015).
108 Previous allozyme studies implicate that genetic differentiation in roach
109 occurs predominantly between, but not within, drainage systems as a result of
110 different colonization events following the last glaciation period (Laroche *et al.*,
111 1999; Hänfling *et al.*, 2004). Roach from lakes with distinct colonization histories
112 often differ in body shape, potentially as a response to different predation
113 regimes (Scharnweber *et al.*, 2013) or varying levels of intra- (Svanbäck *et al.*,
114 2008) or interspecific competition (Faulks *et al.*, 2015). However, in cases where
115 genetic data were available, phenotypic differentiation showed only minor
116 association with the level of genetic differentiation, suggesting that plasticity
117 may often underlie phenotypic differences among roach populations
118 (Scharnweber *et al.*, 2013; Faulks *et al.*, 2015). The aforementioned studies were,
119 however, conducted in relatively shallow lakes, which might not provide the
120 same ecological opportunities for genetic and adaptive differentiation as large,
121 deep, and oligotrophic lakes do (Seehausen & Wagner, 2014). In addition, studies
122 of lacustrine populations compared different drainages that were likely
123 independently colonized, potentially resulting in different evolutionary
124 contingencies (Svanbäck *et al.*, 2008; Scharnweber *et al.*, 2013; Faulks *et al.*,
125 2015). By integrating phenotypic data of roach from seven large pre-alpine lakes
126 with genomic and ecological (i.e. stable isotopic) data of five of these, we assess
127 to which degree allopatric populations from lakes within the same drainage
128 system that are connected by rivers differ from each other. We further test for
129 intralacustrine differentiation of roach caught over different substrates within
130 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 *Sbf*I 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 *Sbf*I 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 PRINTREADS. 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 ≤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 $^{13}C/\ ^{12}C$ 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 $\delta^{13}C$ 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 (Brienz 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 Brienz 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: ρ = 0.958; $t_{1,3}$ = 5.78, p = 0.010). Pairwise F_{ST}s were neither correlated with differences in phosphate levels (Mantel test: $\boldsymbol{r}_M$ = 0.114, p = 0.600) nor with pairwise phenotypic (P_{ST}) differentiation among lake populations (PC2: $\boldsymbol{r}_M$ = 0.088, p = 0.466; PC3: $\boldsymbol{r}_M$ = -0.113, p = 0.690). P_{ST} was likewise not correlated with differences in phosphate amongst lakes (PC2: $\boldsymbol{r}_M$ = 0.367, p = 0.165; PC3: $\boldsymbol{r}_M$ = -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 Brienz (δ^{13}C of -22.63 ± 1.80) to a more omnivorous diet within Lake Hallwil (δ^{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 Brienz

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

362 diversification remains unassessed for most other fish species (Seehausen &
363 Wagner, 2014). Roach have a broad geographic distribution in Europe and occur
364 in a variety of habitats – including deep and ultraoligotrophic lakes that provide
365 a wide range of potential niches to diversify, making roach a good candidate to
366 look for diversification (Svanbäck *et al.*, 2008; Faulks *et al.*, 2015). We found
367 evidence for intraspecific differentiation between roach populations from
368 ultraoligotrophic lakes and lakes with a higher trophic level, as well as some
369 diversification within the ultraoligotrophic Lake Brienz.
370 Former studies suggested that roach often adapt to their local environment
371 and become phenotypically differentiated, e.g. along a littoral-pelagic axis as a
372 result of intra- and interspecific competition (Svanbäck *et al.*, 2008; Faulks *et al.*,
373 2015) or predation (Scharnweber et al., 2013), and this phenotypic
374 differentiation has often been attributed to phenotypic plasticity (Svanbäck et al.,
375 2008; Faulks et al., 2015). Studying roach from pre-alpine lakes in Switzerland,
376 we found subtle yet significant genetic differentiation amongst populations from
377 different pre-alpine lakes, where populations from ultraoligotrophic lakes are
378 genetically more distinct (Figure 3b). This is consistent with recent colonization,
379 potentially combined with ongoing gene flow within the Aare/Rhine drainage.
380 Alternatively, the effective population size may be too large for drift to become a
381 dominant factor (Gillespie, 2001). We also found roach from Lake Geneva to
382 cluster closely with individuals from the nearby Aare/Rhine system (Figure 3).
383 This suggests a common origin, potentially due to historical connectivity as
384 observed for other fish species (Vonlanthen *et al.*, 2007; Gouskov & Vorburger,
385 2016) or supplementary human translocations. Despite their low level of genetic
386 differentiation (Table 2), roach differed both phenotypically (Figure 2b, Table
387 S1) and ecologically (Figure 4) between lakes. Individuals differed
388 predominantly in their head shape, with the population from Lake Brienz being
389 most distinct, showing a slender head and more subterminal orientation of the
390 mouth (Figure 2). The observed phenotypic changes among roach from different
391 lakes hint towards a functional and potentially adaptive response related to
392 feeding regimes that differ between lakes (Wainwright & Barton, 1995).
393 Phenotypic changes in head and body shape similar to the ones observed here
394 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; Gavrilets, 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 δ13C 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

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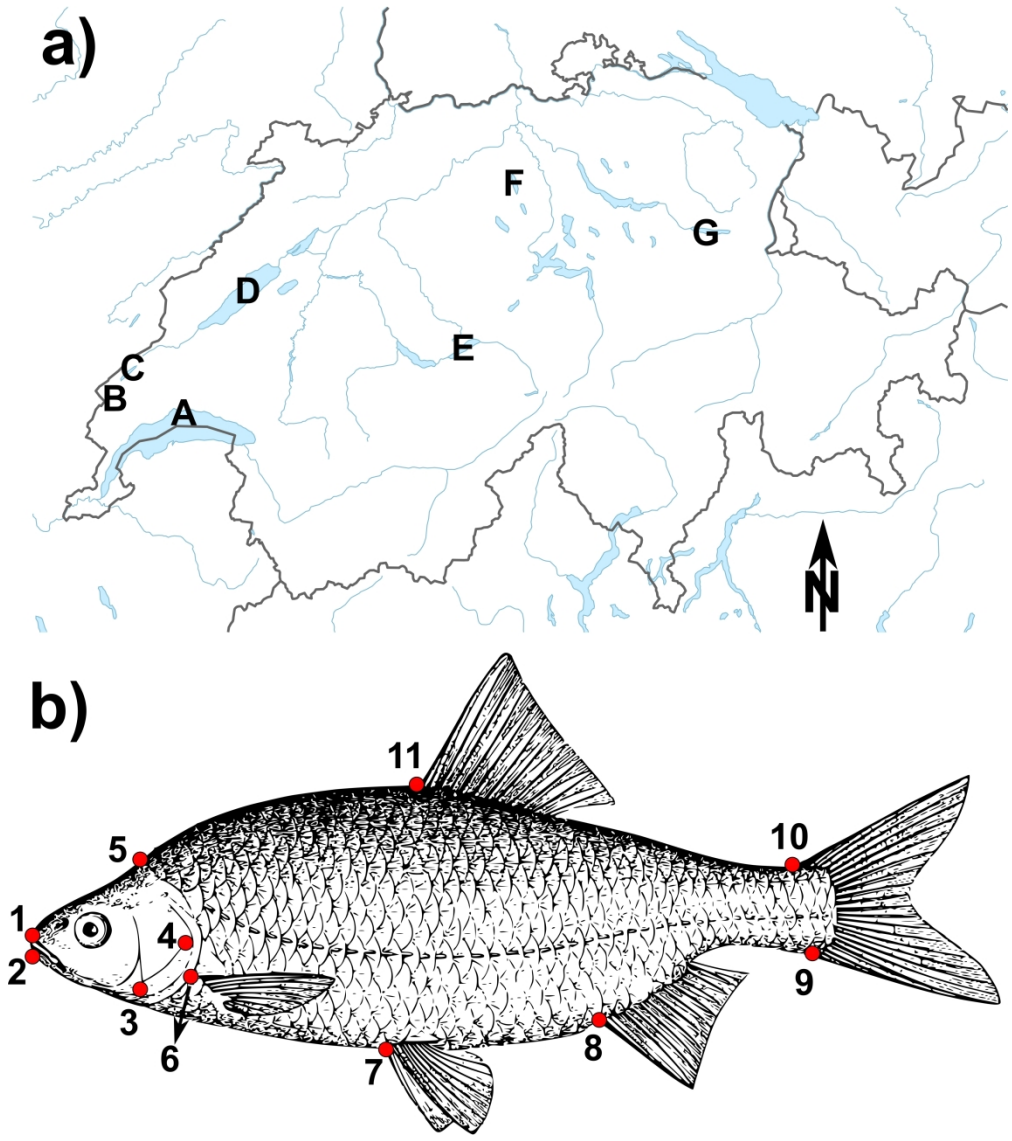
Table 1: Characteristics of each sampled lake and the sample size of each data set. For each lake, the coordinates, the trophic status based on dissolved phosphate (parts per million – ppm), its elevation, and maximal depth are given. In addition to the sampling date, depth range where individuals were 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
Brienz	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

	Ho	**Brienz**	**Hallwil**	**Geneva**	**Neuchatel**	**Walen**
Brienz	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	

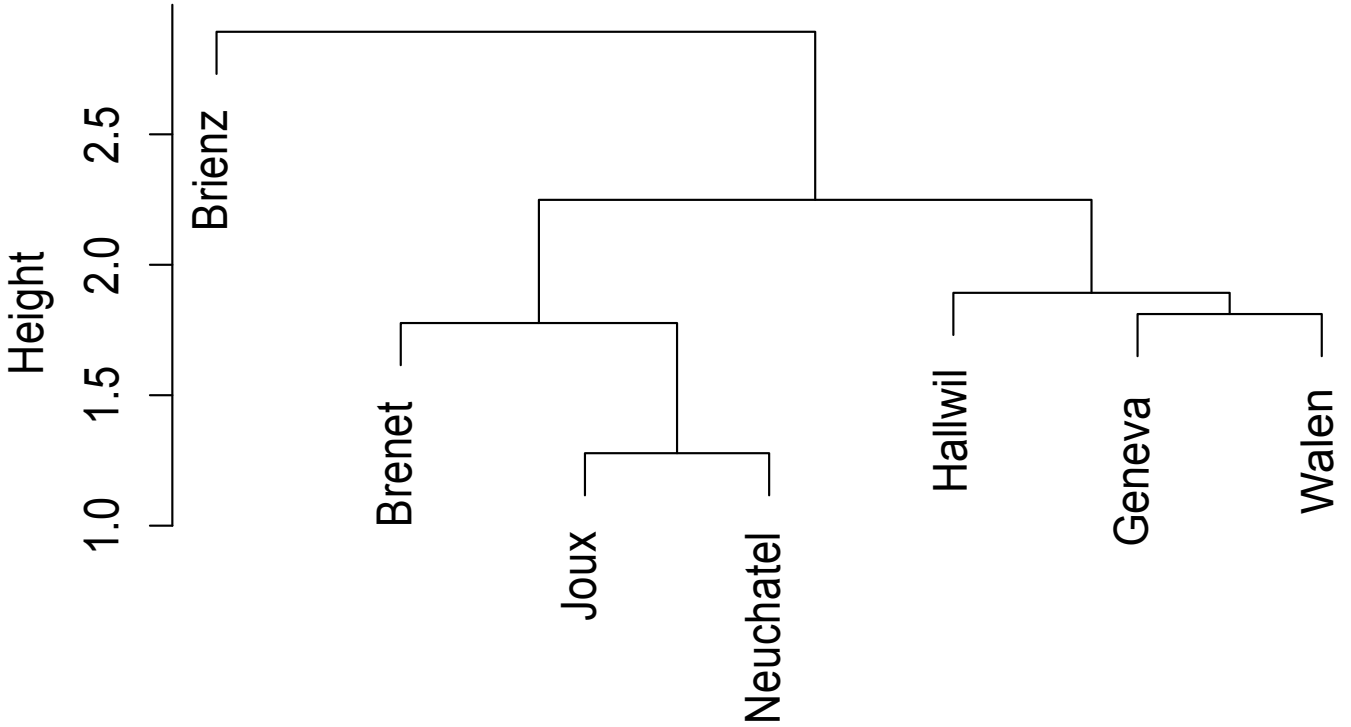
867



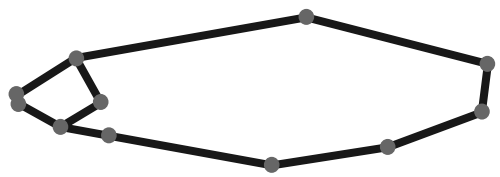
a) Map of Switzerland with the sampled lakes indicated: A) Geneva, B) Joux, C) Brenet, D) Neuchatel, E) Brienz, F) Hallwil, and G) Walen (see Table 1 for details). b) Roach (Rutilus rutilus) with the 11 morphological landmarks used: 1) anterior tip of snout, 2) anterior tip of lower lip, 3) anterior & 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.

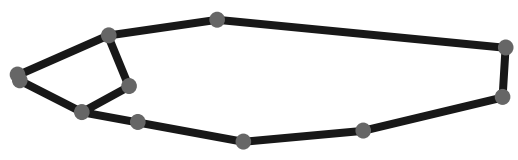
389x439mm (300 x 300 DPI)

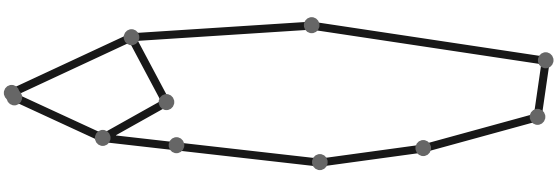
a)

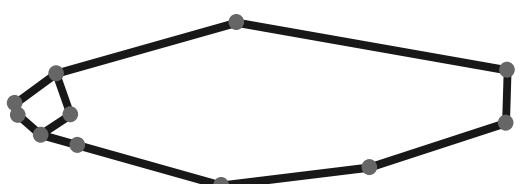

Height
1.0
1.5
2.0
2.5
Brienz
Brenet
Joux
Neuchatel
Hallwil
Geneva
Walen

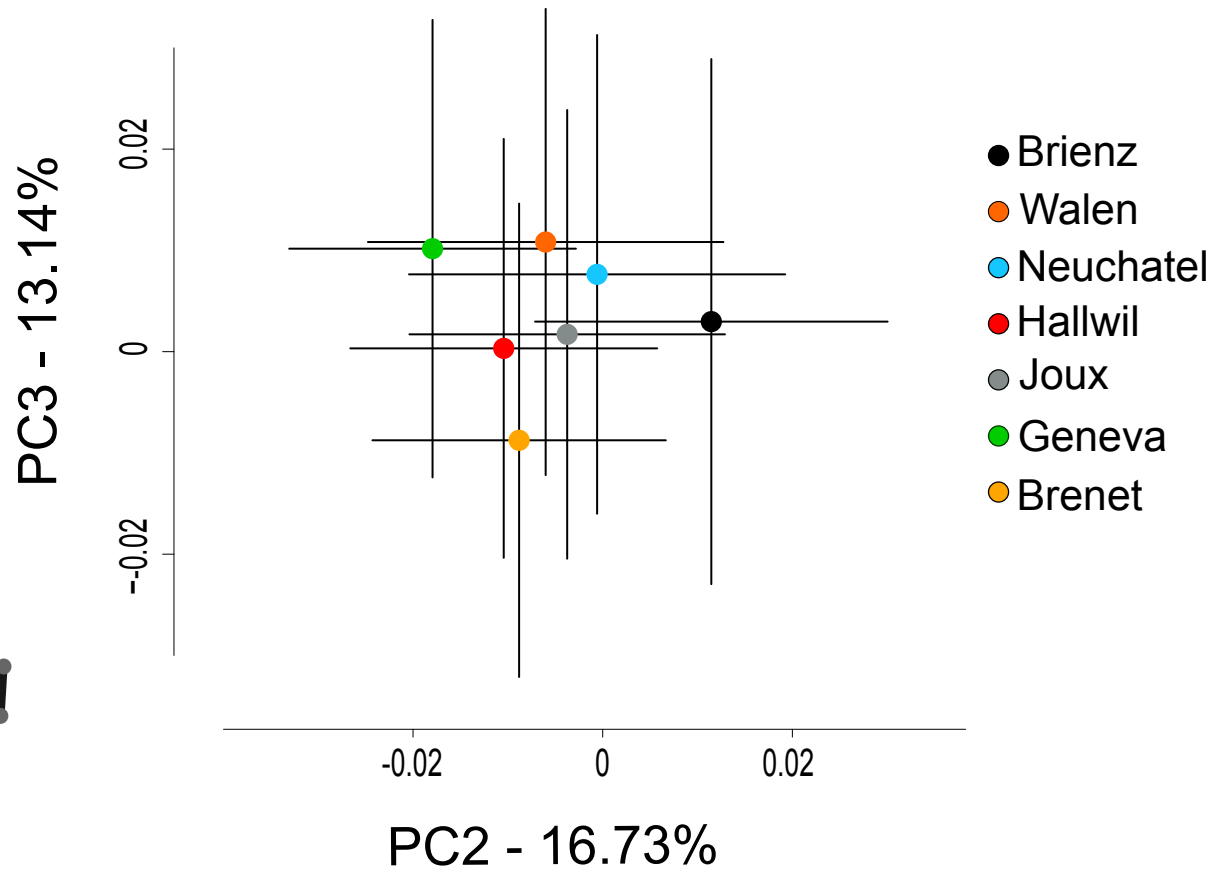
b)

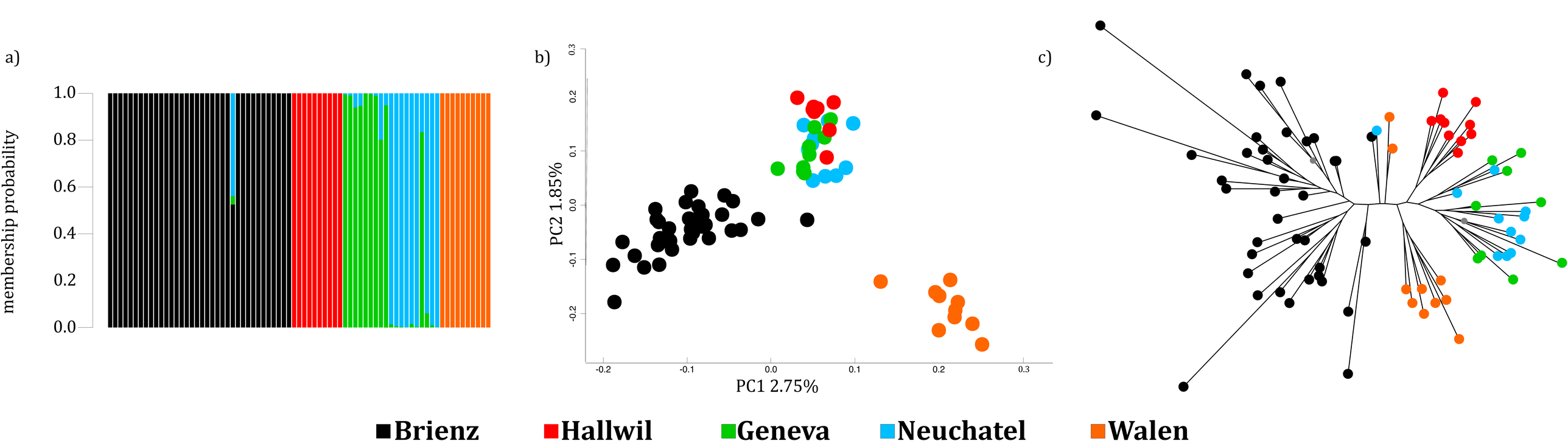


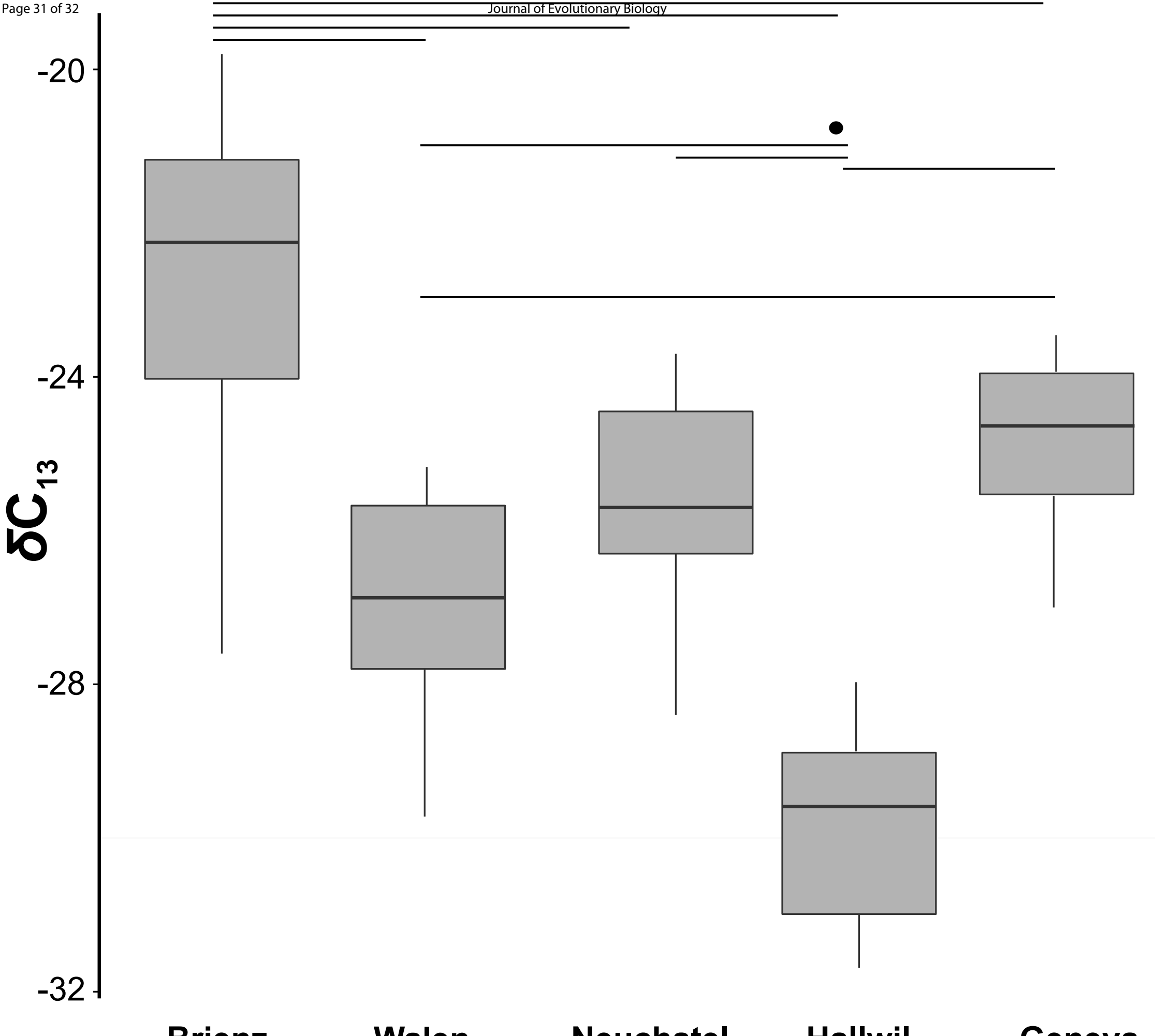


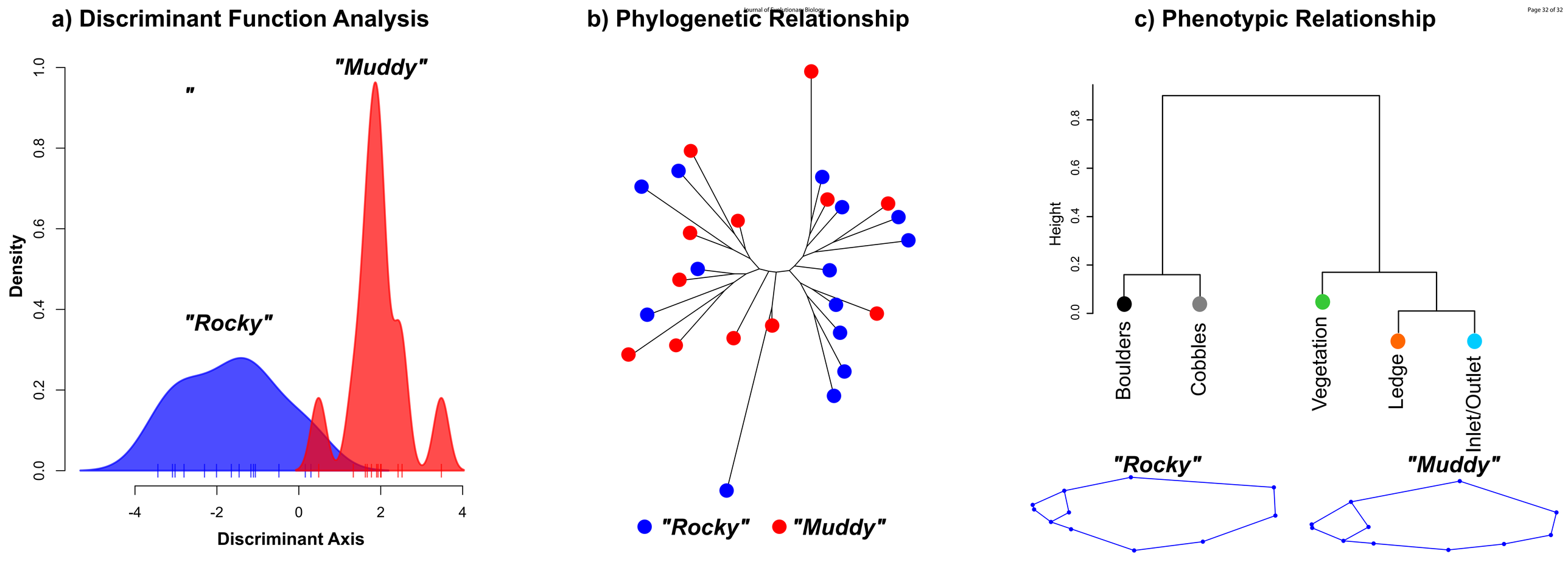





PC3 - 13.14%
0.02
0
-0.02
-0.02
0
0.02
PC2 - 16.73%
Brienz
Walen
Neuchatel
Hallwil
Joux
Geneva
Brenet


a)
membership probability
1.0
0.8
0.6
0.4
0.2
0.0
b)
PC2 1.85%
PC1 2.75%
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.2
-0.1
0.0
0.1
0.2
0.3
c)
Brienz
Hallwil
Geneva
Neuchatel
Walen


-20
-24
-28
-32
δC13
Brienz
Walen
Neuchatel
Hallwil
Geneva


a) Discriminant Function Analysis
"Muddy"
"
"Rocky"
Density
0.0
0.2
0.4
0.6
0.8
1.0
-4
-2
0
2
4
Discriminant Axis
b) Phylogenetic Relationship
"Rocky"
"Muddy"
c) Phenotypic Relationship
Height
0.0
0.2
0.4
0.6
0.8
Boulders
Cobbles
Vegetation
Ledge
Inlet/Outlet
"Rocky"
"Muddy"

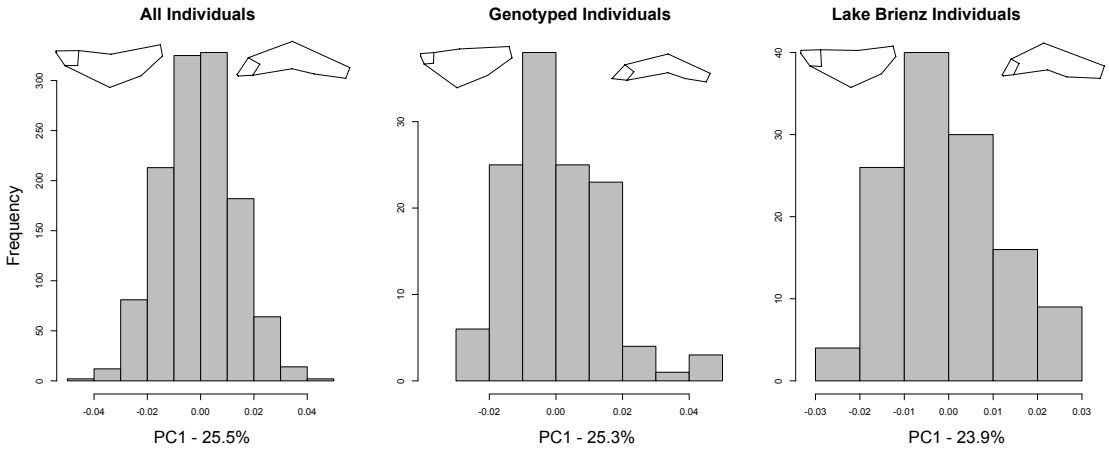


Figure S1: Histograms based on PC1 scores using size-corrected geometric morphometric data. Shown are the distributions using individuals from all lakes, using only genotyped individuals, and using only individuals from Lake Brienz. PC1 data was removed from subsequent analysis for all datasets because 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		***p-value***	
		PC2	**PC3**
Brenet	Brienz	<0.001	0.000
	Geneva	0.984	0.015
	Joux	0.006	0.000
	Geneva	<0.001	0.000
	Neuchatel	<0.001	0.000
	Walen	0.978	0.000
Brienz	Geneva	<0.001	0.973
	Joux	<0.001	0.998
	Geneva	<0.001	0.154
	Neuchatel	<0.001	0.419
	Walen	<0.001	0.606
Geneva	Joux	0.021	0.999
	Geneva	0.036	0.050
	Neuchatel	<0.001	0.152
	Walen	0.882	0.325
Joux	Geneva	<0.001	0.032
	Neuchatel	0.431	0.093
	Walen	0.993	0.398
Geneva	Neuchatel	<0.001	0.973
	Walen	0.014	1.000
Neuchatel	Walen	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**	***p-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	-