



# Resource trait specialisation in an introduced fish population with reduced genetic diversity

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Received: 27 March 2019 / Accepted: 25 March 2020  
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**Abstract** Ecological specialisation is hypothesised to play a major role in the evolution of phenotypic diversity, especially following the colonisation of novel habitats. For example, cichlid fishes provide some of the most remarkable evidence for rapid ecological diversification. Here, we capitalised on a recently ( $\leq 40$  years ago) introduced population of red devils (*Amphilophus labiatus*) in Australia to investigate adaptive phenotypic responses to a novel environment. We used stomach content analyses,

morphometrics and laboratory experiments to test for covariation between diet and size of an important trophic trait, lip size. We found that, while maximum lip size in the study population was smaller than in the species' natural range, the proportions of algae, insects and fish remains in the diet covaried with lip size. However, contrary to predictions, we found no evidence for lip development to be plastic under laboratory conditions in relation to substrate complexity or food manipulation, nor did we find any relationship between lip morphology and feeding performance in adults. Single nucleotide polymorphism data, in turn, suggested that the introduced population has reduced standing genetic variation,

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**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10530-020-02264-y>) contains supplementary material, which is available to authorized users.

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which potentially influences both phenotypic plasticity and diversity, in comparison to native populations. Together, the results suggest that morphological variation in a key trophic trait can respond rapidly to diet in a novel environment, despite reduced genetic diversity in the population.

**Keywords** Plasticity · *Amphilophus* cichlid · Ecological specialisation · RAD tag sequencing · Introduced species · Phenotypic diversity

## Introduction

Ecological specialisation to local environments is considered to be a major driver of phenotypic diversity, both within and between populations (Schluter 2000; Sorenson et al. 2003; Savolainen et al. 2006; Muschick et al. 2011). For example, sympatric intraspecific morphs that show differential niche use (i.e. segregation in habitat and/or diet) may represent the intermediate stages of population divergence (Smith and Skúlason 1996; Barluenga and Meyer 2004). Well-known examples of this phenomenon include benthic and limnetic populations of sticklebacks (McKinnon and Rundle 2002) and trophic specialisation in whitefish (Lu and Bernatchez 1999). Such examples are often associated with the colonisation of novel, isolated habitats (e.g. lakes and islands) where ecological opportunities allow for rapid adaptation to empty niches (see Elmer et al. 2010a). However, due to the potentially rapid nature of these events, their beginnings are rarely witnessed, and the underlying processes and mechanisms that facilitate ecological diversification are therefore still not completely understood.

In new habitats, the ability to respond plastically to novel food sources can be critical in promoting the divergence of trophic morphology (Wente and Phillips 2003; West-Eberhard 2003; Ledon-Rettig et al. 2008; Wund 2012). In this respect, adaptive phenotypic plasticity (i.e. the ability of an organism's phenotype to vary in response to its environment) appears to be a major contributor to population diversification (Smith and Skúlason 1996; Ghalambor et al. 2007; Pfennig et al. 2010), particularly within novel environments (Kocher 2004; Doebeli et al. 2005; Pfennig and McGee 2010). Nevertheless, the role of phenotypic

plasticity in adaptation has been controversial (Pfennig et al. 2010) and has previously been viewed as an impediment to evolutionary change (see Schlichting 2004), despite being taxonomically widespread (Meyer 1987; West-Eberhard 2003; Yeh and Price 2004; Pfennig et al. 2010). However, morphological differences among populations may also be accompanied by ecological and genetic differences that are suggestive of various stages of reproductive isolation (Skúlason et al. 1999; Hendry et al. 2000; Adams and Huntingford 2004; Hendry 2009). For example, Pfennig and McGee (2010) found that clades which display resource polyphenism (alternative phenotypes in response to different environmental stimuli) are more species rich, suggesting that resource polyphenism may promote diversification.

Phenotypic plasticity is considered to play a major role in the explosive adaptive radiations of cichlid fishes (West-Eberhard 1989; Clabaut et al. 2007; Muschick et al. 2011). A common feature of adaptive radiations, including those seen in cichlids, is that species occupying similar niches often display convergent evolution (Schluter and Nagel 1995; Salzburger 2009; Losos 2011). A prominent example of convergent evolution in cichlids is the development of large hypertrophied lips, a key functional trophic trait that has evolved repeatedly in different cichlid lineages in Africa (Arnegard and Snoeks 2001; Salzburger et al. 2005; Oliver and Arnegard 2010; Colombo et al. 2013; Henning et al. 2017) and the Americas (Klingenberg et al. 2003; Elmer et al. 2010a, b; Colombo et al. 2013; Manousaki et al. 2013; Burrell 2015; Machado-Schiaffino et al. 2017). In this regard, researchers have proposed alternative hypotheses to explain the development of hypertrophied lips (Fryer 1959; Yamaoka 1997; Arnegard and Snoeks 2001; Ferry et al. 2012). For example, Greenwood (1974) suggested that large lips could be an adaptation to reduce the mechanical shock of striking against rocks during foraging. Currently, however, the consensus hypothesis is that hypertrophied lips most commonly facilitate a suction feeding mechanism, whereby invertebrates, particularly crustaceans, are sucked up, from crevices and gaps in between rocks (Barlow and Munsey 1976; Klingenberg et al. 2003; Colombo et al. 2013; Manousaki et al. 2013; Baumgarten et al. 2015; Machado-Schiaffino et al. 2017). Yet, few studies have tried to experimentally disentangle among these alternative hypotheses.

Independent of the primary function of large lips in cichlid fishes, phenotypic plasticity may play a role in their development (Machado-Schiaffino et al. 2014). In particular, in captive individuals, large hypertrophied lips reduce in size over time (Barlow and Munsey 1976), indicating that there may be a plastic component to this trait. Interestingly, within their native range, large-lipped cichlids often coexist with closely related smaller-lipped species, with the two types tending to otherwise only differ in regard to their ecological niches (Colombo et al. 2013; Baumgarten et al. 2015). Moreover, large-lipped forms appear to be able to rapidly evolve from small-lipped populations (Elmer et al. 2010a), with theory supporting the hypothesis that phenotypic plasticity may promote rapid evolutionary responses to novel conditions (Behera and Nanjundiah 2004; Fierst 2011).

The red devil (*Amphilophus labiatus*) is an omnivorous cichlid with large, hypertrophied lips. The red devil is a member of the Midas cichlid species complex, a group of Neotropical cichlids that has diversified rapidly, particularly after colonising new environments (Barlow and Munsey 1976; Elmer et al. 2010a, 2013). In the Nicaraguan native range, there is little neutral genetic differentiation between the red devil and its more common, sympatric and small-lipped congener, the Midas cichlid (*Amphilophus citrinellus*) (Barluenga and Meyer 2004, 2010). Nevertheless, hybrid or intermediate lip phenotypes are absent or very rare. In recent decades (within 40 years), a feral population of red devil cichlids (or possibly a hybrid) has become established in Hazelwood Pondage in south-eastern Australia (Corfield et al. 2008). Importantly, this introduction into an isolated lake is analogous to a founder population naturally colonising a novel environment (sensu Elmer et al. 2010a, 2013). For example, in both cases, individuals may need to adjust foraging behaviours and resource trait use in response to a change in both the available sources and type of food. Hence, the feral red devil population provides an excellent opportunity to both observe phenotypic responses to a novel environment and to test the predictions that plasticity and resource polymorphism play an important role in facilitating adaptive diversification after a recent colonisation event.

We set out to investigate whether morphology (i.e. lip size) and diet covary in the introduced red devil population. Our primary focus was lip size, because

this key trophic trait can change rapidly in *Amphilophus* populations, suggesting that phenotypic plasticity could potentially play an important role. Because hypertrophied lips are typically associated with an increased head elongation (Klingenberg et al. 2003), we also included head morphology in our analyses. To this end, we combined laboratory-based behavioural experiments with morphological assays and genomic analyses of samples collected both in the introduced and native lake environments, in Australia and Nicaragua, respectively. First, we experimentally investigated whether among individual morphological variation in the introduced population reflected differences in feeding performance in regard to substrate complexity and diet. Second, focusing on laboratory-reared juveniles from the same population, we tested the extent to which developmental plasticity shapes key trophic traits, in particular, lip size and head morphology in red devils. Third, we compared both the mean and variation in relative lip size between individuals from the native range and the introduced Australian population. Last, using genomic data, we examined genetic diversity of fish both in the native and introduced range to test whether there is evidence of a bottleneck in the introduced population, putatively limiting standing genetic variation, which, in turn, might influence phenotypic diversity and plastic responses.

## Materials and methods

### Link between morphology and diet in the introduced red devil population

To investigate whether morphology covaries with diet in the introduced population, we collected adult red devils from Hazelwood Pondage, Australia, in December 2011 and September–November 2012, using bait traps, hand-lines and nets. We euthanized the fish with a lethal dose of clove oil solution and then dissected and removed the entire stomach and gut of 81 individuals. We later classified food items into the following categories and determined their volume (in % of total gut content): insects (terrestrial and aquatic), fish (remains, scales and eggs), plant material and algae, and other (e.g. silt). Molluscs, crustaceans and zooplankton were also found in gut samples, but were only found in less than 2% of individuals and,

therefore, these food items were not considered in further analyses. Lip size and head morphology (standardised for body size), in turn, was assessed by analysing a digital photo (Nikon D5200 Digital SLR) taken of the right lateral side of each individual, with a 1-mm grid paper as a scale. Using IMAGEJ 1.50b (National Institutes of Health, Rasband 1997), we measured the area of the lip and the head, which—when compared to the area of the whole body (excluding fins)—allowed us to calculate each trait as a percentage of body area. We defined the head as the region between the snout and the most posterior edge of the operculum.

To investigate whether standardised lip size covaries with diet in the introduced population, we performed a multivariate analysis of variance (MANOVA) test (*sensu* Bolnick et al. 2014). To meet the assumption of normality of data, the proportional values of food item categories found in gut content samples (insects, fish remains, plant material, and algae) were all log transformed and then fitted as a multivariate response variable, with standardised lip size as the explanatory fixed variable, and standard body length as a covariate. Next, we carried out post-hoc significance tests, specifically by conducting four separate univariate analysis of variance (ANOVA) tests for each of the four food item categories, with lip size as the fixed variable, and standard body length as a covariate. We also assessed the strength of the correlation (Pearson's correlation) between lip size and head morphology. All statistical analyses were conducted using R 3.0.0 software (R Development Core Team).

### Feeding performance experiment

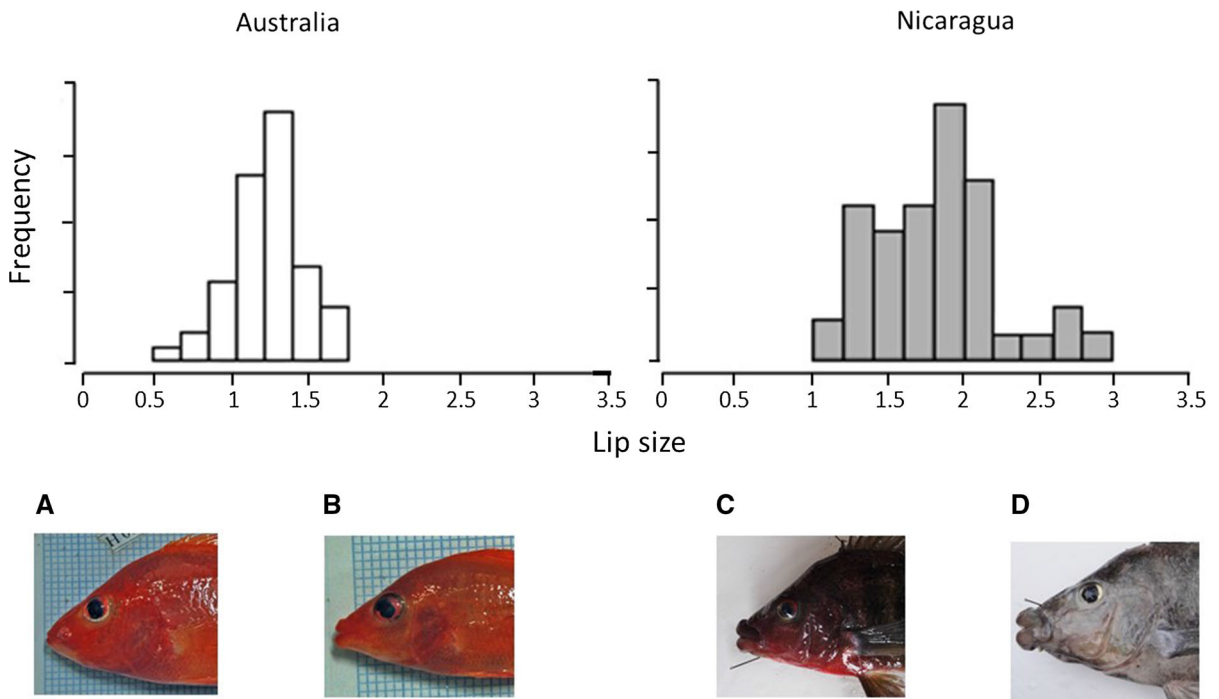
We carried out a controlled laboratory experiment to determine whether variation in morphology (standardised lip size and head morphology) is linked to differences in feeding performance in relation to substrate complexity and diet.

For this purpose, we collected sub-adults ( $n = 88$ , standard length 60–75 mm) from Hazelwood Pondage in early 2013 using the same methods as described above. Fish were transported back to the laboratory and were then sorted based on lip size. Standardised lip size and head morphology were assessed from photographs, as detailed above. Each individual was tagged in the caudal peduncle with a visible implant

elastomer for identification (Willis and Babcock 1998). Finally, all individuals were also weighed ( $\pm 0.01$  g), on an electronic balance.

Using the above data, we found that mean lip size, in the introduced population, was 1.2% of standard body area (Fig. 1). To ensure that we had a representative sample of lip sizes in all our experimental tanks, we categorized individuals with lip size above 1.2% as 'large' and below 1.2% as 'small' lipped. The two groups of fish (i.e. relatively large lips versus small lips) were then split equally between eight 250 l tanks maintained at 25 °C on a 12:12 day night cycle (stocking density = 11 fish of unknown sex per tank). Using a 2 × 2 factorial design, we tested the effects of substrate type (fine grain sand versus cobbles and coarse river rocks) and diet type (thawed frozen adult brine shrimp versus Otohime EP3 fish food pellets) on the feeding performance of the fish (2 tanks per treatment combination). We purposely selected two substrate types that differed in particle size, and consequently, in the size of the interstitial spaces formed between substrate particles. We did this to test whether large and small lip phenotypes differ in their success at extracting food from different substrate surfaces or from gaps between particles (see Baumgarten et al. 2015). The particle size of the fine-grained sand was < 1.5 mm in diameter, compared to cobbles, which ranged in diameter from 5 to 20 mm, and coarse river rocks, which ranged in diameter from 55 to 150 mm. In the native range, the diet of the red devil primarily consists of hard-shelled invertebrates and small arthropods, while they also eat plant material, seeds, fish and fish scales (Colombo et al. 2013; Sowersby et al. unpublished data). We therefore selected foods differing in their characteristics for collecting and handling to reflect the naturally varied diet of the species. Fish were kept under experimental conditions and fed 5 days a week for eight weeks. Food (limited to 2 g per tank) was placed into each aquarium in a container that was pulled along the bottom of the tank with the aid of fishing line and weights so that it was evenly distributed on the substrate before fish could start eating. Fish were weighed and photographed each week. All fish survived the eight-week experiment and weight gain during this time period was used as an indicator of feeding performance.

To assess the influence of substrate and diet type on the feeding performance (weight gain) of individuals



**Fig. 1** The distribution of lip size (relative to body size) in the introduced Australian population (left), and from the native population in Nicaragua (right). The photographs demonstrate,

**a, b**, the variation in lip size in the introduced Australian population, and **c, d**, the variation in lip size in native populations in Nicaragua

with differing lip size, we fitted a linear mixed-effects model, using the ‘lme4’ package in R. The full model was fitted with substrate treatment, diet treatment and the interaction between the two as explanatory fixed factors. Standardised lip area (as a continuous variable) and standardised head area were fitted as covariates. The response variable, weight gain, was square root transformed to meet the assumption of normality of data. To account for the potential interdependence between individuals in a treatment tank, tank ID was added as a random effect. The full model was simplified by stepwise removal of non-significant interaction terms (Crawley 2012), using log likelihood ratio tests (with  $\alpha = 0.05$ ).

#### Experimental assessment of developmental plasticity

In a separate, longitudinal experiment, we also investigated the extent to which lip size and head morphology develop plastically in response to substrate complexity and foods of differing hardness.

Sub-adults, collected at the same time and using the same methods as those described previously, were brought back into the laboratory where they were housed in large tanks (4000 l) maintained at 25 °C on a 12:12 day night cycle (stocking density = ~ 50 fish per tank). Approximately 5 months after collection, breeding pairs were allowed to form naturally in 1500 l tanks. During the study, we were successful in obtaining offspring from three pairs, resulting in three separate clutches. After fry were free-swimming for 14 days, 96 were collected and removed from the parents for use in the experiment.

To assess the role that substrate complexity and the hardness of consumed food items plays in influencing the development of lip and head morphology, while controlling for differences between families, we used a 2 × 2 factorial split-clutch experimental design. Specifically, for each clutch, the siblings were split across 4 same sibling tanks (200 l), representing the 4 possible treatment combinations of food hardness (soft versus hard pellets) and substrate complexity (sand versus coarse gravel and cobbles), i.e. in total 12 tanks were used. Here, we manipulated the hardness of the

food, so that in soft food replicates pellets were soaked in water for 10 min prior to feeding, while in the hard food replicates non-manipulated (hard) pellets were used (and immediately consumed by the fish). Otherwise, the method for delivering the food was the same as that described in the previous experiment. For the first 3 months, fish ( $n = 96$ ) subsisted on pellets that were 1.7 mm in diameter, before progressing onto 3.1 mm pellets for the rest of the experimental period. The entire experiment lasted 14 months, after which, the fish were photographed (left lateral side).

We compared the standardised lip size and head morphology (i.e. area) of each individual ( $n = 96$ ) in IMAGEJ 1.50b using the same methods as described above.

To assess the influence of substrate complexity and food hardness on developmental plasticity, we fitted two separate linear mixed-effects models, using the package 'lme4', in R. In particular, we had standardised lip size and standardised head area (measured at the end of the experimental period) as the response variables in the two models, respectively. In all cases, substrate treatment and diet treatment were fitted as explanatory fixed factors and, to account for the design of the experiment and the potential interdependence between siblings, clutch ID as a random effect. Each full model was then simplified by stepwise removal of non-significant interaction terms (Crawley 2012), using log likelihood ratio tests (with  $\alpha = 0.05$ ).

#### Morphological comparisons with fish from the native range

We quantified the morphology of wild-caught red devils from their native range in Nicaragua ( $n = 41$ ) and compared these with the fish of the feral, Australian population ( $n = 82$ ). Red devils were collected from six locations in Lake Nicaragua in late 2013 (supplementary information) and at multiple times in Australia, (supplementary information), between late 2011 and early 2014, using the same methods as outlined above. Fish were individually photographed (left lateral side) for morphological analyses (see Table S1 in supplementary information for sample size and locations).

Using the same methods as above, we measured variation in standardised lip size in a sub-set of native and introduced fish. We used a Shapiro–Wilk

normality test to assess the distribution of lip size in the introduced and native range.

#### Genetic diversity

Differences in the extent of plasticity and phenotypic variation in the introduced vs. natural populations may be due to different levels of genetic diversity, which could result from, for example, a population bottleneck. To test this possibility, we compared the level of genetic diversity in the introduced population with individuals from the native range using Restriction site associated DNA (RAD)-sequencing, a reduced representation population genomic method. We extracted DNA (DNeasy Blood and Tissue Kit, QIAGEN) from ethanol preserved samples of individuals from the introduced population in Australia and from six locations in Lake Nicaragua (see supplementary information). Samples were digested using the SbfI enzyme following a standard RAD-sequencing protocol (Developed by M. Roesti, Basel University 2011). We pooled 40 (barcoded) individuals into 5 libraries (total  $n = 200$ ). Each library was sequenced (single-end) on separate Illumina HiSeq2500 lanes (200 bp reads) at the ETH Zurich Department of Biosystems Science and Engineering in Basel, Switzerland.

Since no reference genome is currently available for *Amphilophus* species, we used a de novo assembly approach implemented in Stacks v 1.30 to identify RAD loci and estimate genome-wide diversity (Catchen et al. 2011, 2013). To ensure that only high quality reads were used in our analysis, we first trimmed sequenced reads to 175 bp and removed any with an average Phred quality score  $< 20$ . Quality screening and sample demultiplexing was performed using the Stacks *process\_radtags* module.

Restriction site associated DNA loci were first identified within individuals using the *ustacks* module, allowing a minimum of 5 reads per stack, a maximum of 2 stacks per locus and up to 4 nucleotide mismatches within and between stacks. SNPs were called at the individual level using the standard Stacks SNP model with  $\alpha = 0.01$ . Following this step, we then used a subset of 50 individuals with the highest read depths from across the native and introduced range to create a de novo RAD loci catalogue. In short, this catalogue acts as a database of identified loci amongst all individuals. Care was taken to ensure individuals of both sexes were included to prevent any

sex biases. Catalogue construction was performed using *cstacks* with a maximum of 4 mismatches permitted between loci amongst individuals.

Once our de novo reference catalogue was complete, the remaining 150 individuals were matched against it using *stacks* and we used the *populations* module to perform final dataset filtering and to estimate population genetic statistics. Polymorphic RAD loci were only included if they occurred in at least 50% of individuals originating from each of the six locations, had a minimum read depth of  $20 \times$  and had a minor allele frequency  $> 0.1$ . For our final dataset, we used linear mixed-effects models to test for differences in observed heterozygosity and nucleotide diversity amongst the populations. In particular, we fitted a separate model for the two response variables, with polymorphic RAD loci fitted as a random effect in both models.

## Results

### Link between morphology and diet in the introduced red devil population

The most abundant food item categories found in the gut of the introduced population were plant material (23%), algae (22%) and macroinvertebrates (14%) (supplementary information).

A MANOVA revealed a significant difference in the proportions of food item categories in gut/stomach with respect to standardised lip size ( $F_{1,63} = 6.904$ ,  $p = 0.001$ ), the covariate factor body size was not significant ( $F_{1,63} = 0.42$ ,  $p = 0.79$ ). To test what was driving this pattern, we ran separate univariate ANOVAs for the food item categories, which revealed that the proportion of insects ( $F_{1,63} = 13.19$ ,  $p = 0.0005$ ) and fish remains ( $F_{1,63} = 6.21$ ,  $p = 0.015$ ) in the gut content were significantly higher as lip size decreased (Fig. 2a). However, the significant relationship between fish remains and lip size should be treated with caution, due to an outlier in the data (MANOVA can be sensitive to outliers) (Fig. 2b). In contrast to insects and fish remains which were associated with a decrease in lip size, the proportion of algae in gut content samples significantly increased as lip size increased, in the introduced population ( $F_{1,63} = 11.23$ ,  $p = 0.0013$ , Fig. 2c). We did not find any significant relationship between plant material and

lip size ( $F_{1,63} = 1.06$ ,  $p = 0.31$ ). Lip and head area (both standardised for body size) correlated positively (Pearson's correlation,  $r = 0.36$ ,  $df = 80$ ,  $p = 0.0007$ ).

### Feeding performance experiment

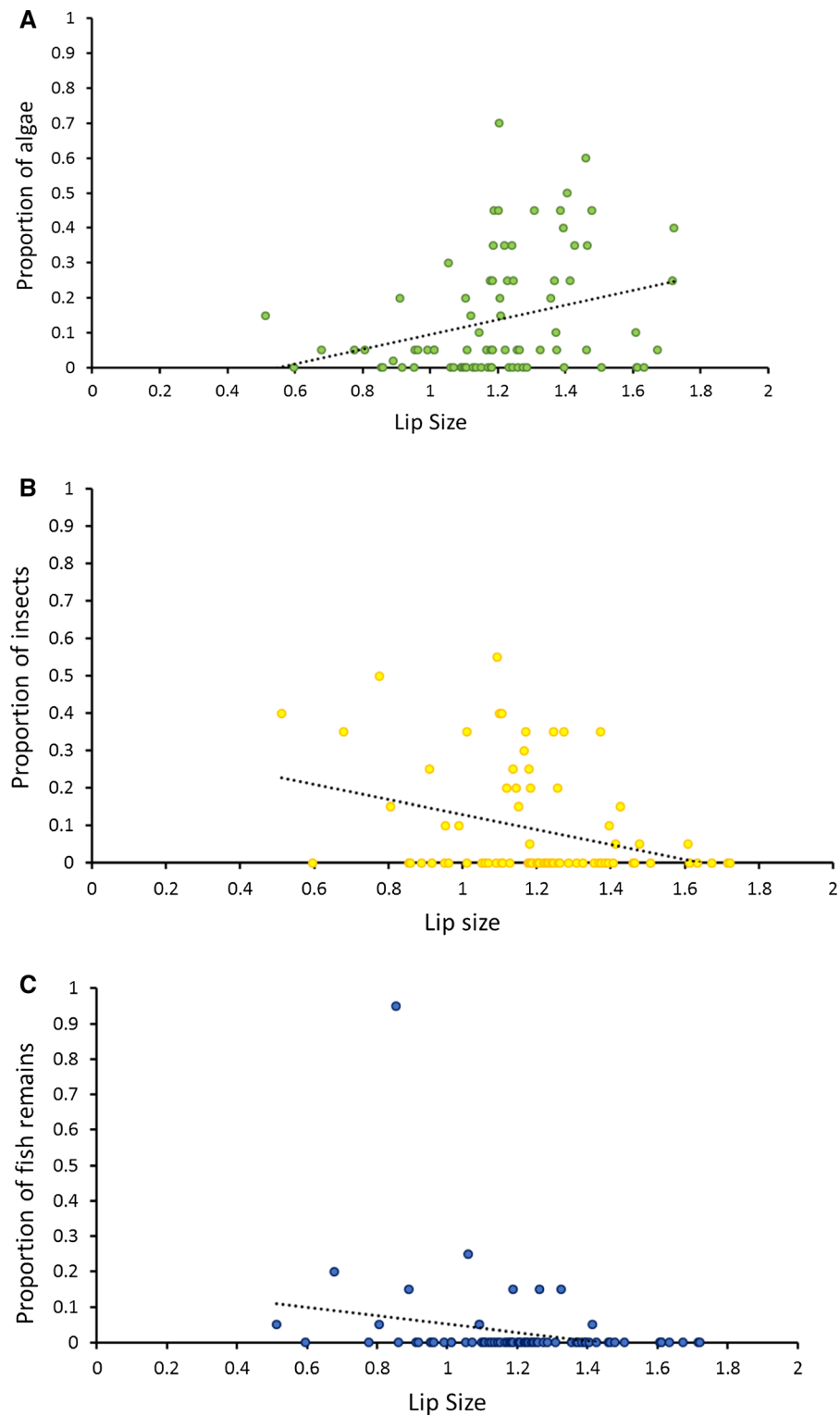
Regarding the feeding performance of wild-caught fish (from the introduced population) under laboratory conditions, the interaction between substrate and diet treatments was not significant ( $\chi^2 = 0.0022$ ,  $df = 1$ ,  $p = 0.96$ ), and it was subsequently removed from the model. The diet treatment had a significant effect ( $\chi^2 = 121.91$ ,  $df = 1$ ,  $p < 0.0001$ ), with individuals consuming pellets gaining more weight, compared to those that consumed shrimp. The substrate complexity treatment ( $\chi^2 = 0.69$ ,  $df = 1$ ,  $p = 0.41$ ), and the covariates, standardised lip size ( $\chi^2 = 2.51$ ,  $df = 1$ ,  $p = 0.11$ ) and standardised head area ( $\chi^2 = 0.13$ ,  $df = 1$ ,  $p = 0.72$ ) did not have a significant effect on weight gain.

### Experimental assessment of developmental plasticity

To assess whether the standardised resource use traits—i.e. standardised lip size and standardised head area—responded plastically to substrate treatment or food hardness in the common garden experiment, we ran a separate linear mixed-effects model for each of the response variables. In both cases, we found the interaction between substrate complexity and food hardness to be non-significant (standardised lip size,  $\chi^2 = 0.33$ ,  $df = 1$ ,  $p = 0.56$ ; standardised head area,  $\chi^2 = 0.14$ ,  $df = 1$ ,  $p = 0.71$ ). We then refitted both models without the interaction term. The simplified models showed that neither substrate complexity nor food hardness had a significant effect on standardised lip size (substrate,  $\chi^2 = 2.42$ ,  $df = 1$ ,  $p = 0.12$ ; food,  $\chi^2 = 0.99$ ,  $df = 1$ ,  $p = 0.32$ ) or standardised head area (substrate,  $\chi^2 = 0.07$ ,  $df = 1$ ,  $p = 0.79$ ; food,  $\chi^2 = 3.25$ ,  $df = 1$ ,  $p = 0.071$ ).

### Morphological comparison with fish from the native range

We found that lip size relative to body size was larger in the Nicaraguan population ( $n = 41$ , mean  $\pm$  SD:  $1.81\% \pm 0.17$ ) compared to the introduced Australian population ( $n = 82$ , mean  $\pm$  SD:  $1.21\% \pm 0.95$ ;



**Fig. 2** Proportion of food item categories found in the gut and stomach content of red devil cichlids in the introduced population, **a** proportion of algae (light green), **b** proportion of insects (yellow), and **c** fish remains (blue)



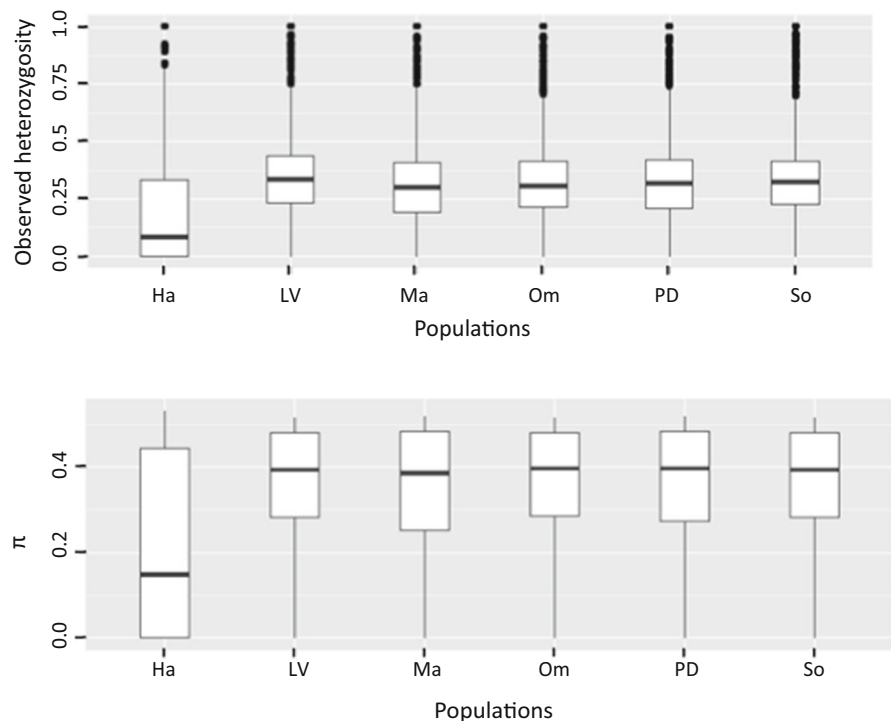
Fig. 1). The distribution of lip size was not significantly different from a normal distribution in either the native (Shapiro–Wilk Normality Test,  $W = 0.96$ ,  $p = 0.94$ ) or feral ( $W = 0.97$ ,  $p = 0.28$ ) populations. Mean standard length in Nicaragua was 16.9 cm (range 11.4–22.2 cm) and 11.7 cm (range 7.2–20.1 cm) in Australia.

### Genetic diversity

Our de novo assembly identified 341 226 unique RAD tags, reduced to 6226 RAD loci and 8038 SNPs following filtering for RAD loci which were polymorphic, occurring in all six populations (sampling locations) in > 50% individuals, had a MAF of > 0.1 and were sequenced to at least  $20 \times$  depth. In the final dataset, an average of 1.29 SNPs occurred on each RAD tag.

We found mean observed heterozygosity was 38% lower in the introduced population compared to populations from the native range ( $F_{5,40185} = 739.81$ ,  $p < 0.0001$ ; Fig. 3). Similarly, nucleotide diversity was 34% lower in the introduced population relative to the native range ( $F_{5,40185} = 1003.6$ ,  $p < 0.0001$ ; Fig. 3).

**Fig. 3** Boxplots showing the distribution of observed heterozygosity (upper panel) and nucleotide diversity (lower panel) estimated from 8038 SNPs occurring in all six populations. Note the considerable reduction of both diversity measures in the introduced Hazelwood population (Ha) compared to native Nicaraguan populations (Lv, Ma, Om, Pd and So)



### Discussion

According to theory, ecological specialisation is predicted to be a major driver of phenotypic diversity, especially in response to novel environmental stimuli. Here, we found a significant correlation between standardised lip size and diet in an introduced red devil cichlid fish population. Specifically, as lip size increased, so did the proportion of algae in the diet, while the proportion of insects and fish remains decreased. In the laboratory, fish exposed to different substrates and diet treatments did not show significant links between phenotypic variation and feeding performance. Similarly, there was no apparent plastic developmental response in lip size or head morphology to substrate treatments or food hardness. Finally, compared to individuals in the native range, the lip size of the introduced red devils is smaller, and the introduced population also has significantly lower genetic diversity.

We found a greater proportion of algae in the stomach and gut content of individuals with relatively larger lips, whereas individuals with smaller lips had consumed a greater proportion of insects and fish remains. This result is in accordance with the observed

variation in trophic morphology being linked to the exploitation of different resources (Elmer et al. 2010a). Similar patterns of resource trait variation have been hypothesised to eventually facilitate population divergence in respect to ecological niche. For example, the exploitation of vacant ecological niches is a primary hypothesis for the rapid diversification of finches on the Galapagos Islands (Grant and Grant 2008), with strong intraspecific competition potentially leading to the rapid evolution of different niche use within populations (Bolnick 2001). However, such patterns of apparent ecological specialisation do not necessarily lead to genetic differentiation between the ecomorphs. For instance, there is no observable genetic differentiation between individuals differing in lower pharyngeal jaw morphology in the polymorphic Minkley's cichlid (*Herichthys minckleyi*), with the trait, instead, being developmentally plastic (Kornfield and Koehn 1975; Hulsey et al. 2005).

In the current study, the wide range of food items exploited by the feral population was related to resource use morphology, with lip size being associated with types of commonly consumed food items. This finding suggests that the population may be undergoing an adaptive diversification process, which is resulting in a more efficient exploitation of available food sources (see Bolnick et al. 2002). This type of ecological specialisation has been offered as the main explanation for the rapid diversification within the Midas cichlid species complex (Barluenga et al. 2006). As an example, a large lipped *Amphilophus* phenotype has rapidly (within ~ 100 years) evolved from a small lipped form in Crater Lake Apoyeque (Elmer et al. 2010a), with lip size being linked to ecological niche use of the fish. In particular, in direct contrast to our findings in Australian red devils, the large lipped form of Lake Apoyeque, consumes more insects, whereas the small lipped form eats more algae (Elmer et al. 2010a).

In several fish species, large hypertrophied lips appear to be an adaptation for foraging on rocky surfaces, in particular for invertebrates (Colombo et al. 2013; Baumgarten et al. 2015). This is assumed to be the case also in red devils in their native range in Lake Nicaragua and Lake Managua, where crustaceans and hard-shelled invertebrates form the majority of their diet (Colombo et al. 2013). However, across different species (and populations), large lips seem to have evolved for different functions and to exploit a range

of resources (Agrawal and Mittal 1991). For example, in multiple African cichlid species, large lips have a high density of taste buds and may be acting as an accessory gustatory organ (Arnegard and Snoeks 2001; Oliver and Arnegard 2010). Large lips may also be an adaptation for reducing the mechanical shock of striking rocks during foraging (Greenwood 1974). Furthermore, large hypertrophied lips appear to have evolved from a large range of ancestral states in cichlids, such as the algivorous *Lobochilotes* (Wagner et al. 2009; Muschick et al. 2011), omnivorous *Amphilophus* (Barluenga et al. 2006; Colombo et al. 2013), and piscivorous *Crenicichla* (Burrell et al. 2013). In the introduced red devil population, large lips may reduce the mechanical shock of foraging on rocky surfaces, or, considering the gut content of large lipped individuals, may be especially useful in removing algae from benthic surfaces, as in the kissing gourami (*Helostoma temminckii*) (Ferry et al. 2012). The absence of crustaceans in the introduced habitat (personal observations), a key food source of red devils in their native range (Colombo et al. 2013), may also have contributed to both the overall smaller lip size and the use of lips for processing alternative food sources. We were not able to distinguish between aquatic and terrestrial insects in our gut content analysis. Therefore, whether individuals with smaller lips primarily feed on insects from the water surface or lake substrate remains unresolved.

Variation in lip size and head morphology did not influence feeding performance under different substrate complexity or diet treatments. We did, however, find a significant effect of diet type on weight gain, which was not associated with morphological variation and, instead, is most likely due to nutritional differences between the two food types. In contrast to our study, Baumgarten et al. (2015) found that *Haplochromis chilotes*, a large-lipped cichlid, is more efficient at extracting food from crevices, when compared to the small lipped congener, *Haplochromis nyererei*. Why did we not detect such a link between feeding performance and morphology in the laboratory, despite the covariation between lip size and diet in the population studied? It remains possible that our experiment tested environmental conditions that were not the same as those that have resulted in the observed relationship between phenotypic variation (lip size) and diet. For example, in the introduced population, larger lips may be employed for removing algae from

rocky surfaces rather than for drawing food items from gaps and crevices between rocks. In the laboratory, the growth of epiphytic algae on gravel and pebbles was very limited, especially when compared to the availability of the offered food sources.

One motivation for our laboratory experiments was that surprisingly little is known about the mechanisms of individual level diet specialisation, despite the phenomenon appearing to be relatively widespread in nature (Bolnick et al. 2002, 2003). However, our experiments did not provide any evidence for developmental plasticity in lip size or head morphology in response to the assessed environmental conditions. This is in contrast with some other studies on cichlids, which have found support for variation in trophic traits arising from differences in feeding regime (Witte et al. 1989; Stauffer and Snick 2004; Muschick et al. 2011). For example, Meyer (1987) found within sibling differences in the head/snout shape of jaguar cichlids, *Parachromis managuensis*, when they were raised under diet treatments that required different modes of feeding. In red devils, exposure to more than the two particular environmental conditions assessed in this study, while using larger sample sizes, might help unravel the potentially complex mechanisms underlying the development of large hypertrophied lips.

The introduced red devils had a much lower genetic diversity than populations in the native range, which is a common feature of introduced populations (Barrett 1991; Kinziger et al. 2011) and populations that have recently colonized new environments following range expansions (Bernatchez and Wilson 1998). Individuals of the introduced population presumably had a lower genetic diversity already before their establishment in Hazelwood Pondage because of their origin from the aquarium trade. The level of standing genetic variation is relevant for adaptive divergence and the rapid evolution of phenotypically similar traits among independent populations (Barrett and Schluter 2008; Schluter and Conte 2009). Population bottlenecks and founder events may reduce variation at adaptive loci segregating in a population, potentially reducing phenotypic diversity and the availability of phenotypic variance for selection to act upon (Barrett and Schluter 2008). Yet, increased genetic variation has been hypothesised to increase the potential for phenotypic plasticity, as plasticity itself is often a heritable trait (Scheiner 1993; Pigliucci 2005). Therefore, an interesting avenue for future research would be to test for

phenotypic plasticity of the trophic traits in a population of red devils from their native range, in which the current results show genetic variation to be much higher than in our introduced population.

In summary, we found that an introduced red devil population is phenotypically highly variable in regard to lip size. Furthermore, we found that lip size covaries with diet, suggestive of lip size being linked to ecological differences. This association may be an initial sign of adaptive diversification within the population, with variation in a key trophic trait, hypertrophied lips, being used to capitalise different resources. In the context of biological invasions, our findings highlight the potential for introduced species to exploit unoccupied niches and, in so doing, promote specialisation and diversification. Under experimental conditions, we did not find morphological differences to be associated with feeding performance in the ecological conditions we assessed, nor did we observe any plasticity in these traits during development. Hence, in this introduced population, ecological factors other than those commonly acknowledged (and tested by us) seem to be driving the diet specialisation in red devils with different morphologies. Notably, such morphology-dependent specialisation has taken place despite reduced standing genetic variation in the population.

**Acknowledgements** We thank Carlos Garita Alvarado, Fiona Kang, Rowan Jacques-Hamilton, Nicholas Deal, Rachel Fetherston, Eeling Ng, Ruby Albury, Andrej Hohmann, Stefanie Forster, Madeleine De Jong and Matthew Simpson for logistic support and animal collection, and members of the Wong and Chapple Labs, Monash University, who assisted with animal husbandry. We also thank Bernd Egger, Christian Beisel Melinda Hofmann, Mariana Leal Cardín and Gema Aguilera García for their assistance in the laboratory, with preparation of samples for genetic analysis. We thank the staff of the DNA Database of Japan (DDBJ) for their assistance with the use of the NIG supercomputer to conduct population genomic analyses. Lastly, we thank David Chapple, Walter Salzburger, Marius Röstl, Bernd Egger and Alysha Heimberg for their advice in regard to genetic analysis.

**Author contributions** WS, TKL and BMW formulated the idea, developed methodology, and collected the field data. MB organised, and participated in, data collection in Nicaragua. WS wrote the first draft of the manuscript, conducted laboratory experiments and prepared samples for sequencing. WS, TKL and MR performed statistical analyses. All authors contributed to writing of the manuscript.

**Funding** This study was funded by a Holsworth Wildlife Endowment, and a Linnean Society of New South Wales Small Research Grant, both awarded to WS. The research also received support from the SYNTHESYS Project (<https://www.synthesys.info/>), which is financed by European Community Research Infrastructure Action under the FP7 “Capacities” Program (to TKL and MB).

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Collection and experimental procedures were approved by the Animal Ethics Committee of Monash University, Australia (BSCI/2012/23), and complied with all relevant State and Federal laws. Collection in Nicaragua was approved by the Ministerio del Ambiente y los Recursos Naturales (MARENA), Permit Number: 013-102013.

### References

- Adams CE, Huntingford FA (2004) Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic Charr. *Biol J Linn Soc* 81:611–618. <https://doi.org/10.1111/j.1095-8312.2004.00314.x>
- Agrawal N, Mittal AK (1991) Epithelium of lips and associated structures of the Indian major carp, *Catla catla*. *Jpn J Ichthyol* 37:363–373. <https://doi.org/10.1007/BF02905362>
- Allendorf FW, Lundquist LL (2003) Introduction: Population biology, evolution, and control of invasive species. *Conserv Biol* 17:24–30. <https://doi.org/10.1046/j.1523-1739.2003.02365.x>
- Arnegard ME, Snoeks J (2001) New three-spotted cichlid species with hypertrophied lips (Teleostei: Cichlidae) from the deep waters of Lake Malaŵi/Nyasa, Africa. *Copeia* 2001:705–717
- Barlow GW, Munsey JW (1976) The red devil - Midas - arrow cichlid species complex in Nicaragua. In: Thorson TB (ed) *Investigations of the ichthyofauna of Nicaraguan Lakes*. School of Life Sciences, University of Nebraska-Lincoln, pp 359–369
- Barluenga M, Meyer A (2004) The Midas cichlid species complex: Incipient sympatric speciation in Nicaraguan cichlid fishes? *Mol Ecol* 13:2061–2076. <https://doi.org/10.1111/j.1365-294X.2004.02211.x>
- Barluenga M, Meyer A (2010) Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evol Biol* 10:326. <https://doi.org/10.1186/1471-2148-10-326>
- Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439:719–723. <https://doi.org/10.1038/nature04325>
- Barrett SCH (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D, Holsinger KE (eds) *Genetics and conservation of rare plants*. Oxford University Press, New York and Oxford, pp 3–30
- Barrett RD, Schluter D (2008) Adaptation from standing genetic variation. *Trends Ecol Evol* 23:38–44. <https://doi.org/10.1016/j.tree.2007.09.008>
- Baumgarten L, Machado-Schiaffino G, Henning F, Meyer A (2015) What big lips are good for: on the adaptive function of repeatedly evolved hypertrophied lips of cichlid fishes. *Biol J Linn Soc* 115:448–455. <https://doi.org/10.1111/bj.12502>
- Behera N, Nanjundiah V (2004) Phenotypic plasticity can potentiate rapid evolutionary change. *J Theor Biol* 226:177–184. <https://doi.org/10.1016/j.jtbi.2003.08.011>
- Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Mol Ecol* 7:431–452. <https://doi.org/10.1046/j.1365-294x.1998.00319.x>
- Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R (2002) Measuring individual-level resource specialization. *Ecology* 83:2936–2941. [https://doi.org/10.1890/00129658\(2002\)083\[2936:MILRS\]-2.0.CO;2](https://doi.org/10.1890/00129658(2002)083[2936:MILRS]-2.0.CO;2)
- Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. *Am Nat* 161:1–28. <https://doi.org/10.1086/343878>
- Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusia AJ, Knight R, Caporaso JG, Svanbäck R (2014) Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nat Commun* 5:1–13. <https://doi.org/10.1038/ncomms5500>
- Burruss ED, Duarte A, Serra WS, Gangloff MM, Siefferman L (2013) Species-specific ontogenetic diet shifts among Neotropical Crenicichla: Using stable isotopes and tissue stoichiometry. *J Fish Biol* 82:1904–1915. <https://doi.org/10.1111/jfb.12117>
- Burruss ED (2015) Cichlid fishes as models of ecological diversification: Patterns, mechanisms, and consequences. *Hydrobiologia* 748:7–27. <https://doi.org/10.1007/s10750-014-1960-z>
- Catchen JM, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An analysis tool set for population genomics. *Mol Ecol* 22:3124–3140. <https://doi.org/10.1111/mec.12354>
- Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2011) Stacks: Building and genotyping loci de novo from short-read sequences. *G3* 1:171–182. <https://doi.org/10.1534/g3.111.000240>
- Clabaut C, Bunje PME, Salzburger W, Meyer A (2007) Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. *Evolution* 61:560–578. <https://doi.org/10.1111/j.1558-5646.2007.00045.x>
- Colombo M, Diepeveen ET, Muschick M, Santos ME, Indermaur A, Boileau N, Barluenga M, Salzburger W (2013) The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. *Mol Ecol* 22:670–684. <https://doi.org/10.1111/mec.12029>
- Corfield J, Diggles B, Jubb C, McDowall RM, Moore A, Richards A, Rowe DK (2008) Review of the impacts of introduced ornamental fish species that have established

- wild populations in Australia. Prepared for the Department of the Environment, Water, Heritage and the Arts, Canberra, ACT. [www.environment.gov.au/biodiversity/publications/index.html](http://www.environment.gov.au/biodiversity/publications/index.html)
- Crawley MJ (2012) Mixed-effects models. The R book. Wiley, United Kingdom, pp 681–714
- Doebeli M, Dieckmann U, Metz JA, Tautz D (2005) What we have also learned: adaptive speciation is theoretically plausible. *Evolution* 59:691–695. <https://doi.org/10.1554/04-154>
- Elston DA, Moss R, Boulinier T, Arrowsmith C, Lambin X (2001) Analysis of aggregation, a worked example: number of ticks on red grouse. *Parasitology* 122:563–569. <https://doi.org/10.1017/S0031182001007740>
- Elmer KR, Lehtonen TK, Kautt A, Harrod C, Meyer A (2010a) Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. *BMC Biol* 8:60. <https://doi.org/10.1186/1741-7007-8-60>
- Elmer KR, Kusche H, Lehtonen TK, Meyer A (2010b) Local variation and parallel evolution: morphological and genetic diversity across a species complex of Neotropical crater lake cichlid fishes. *Philos T R Soc B* 365:1763–1782. <https://doi.org/10.1098/rstb.2009.0271>
- Elmer KR, Lehtonen TK, Fan S, Meyer A (2013) Crater lake colonization by Neotropical cichlid fishes. *Evolution* 67:281–288. <https://doi.org/10.1111/j.1558-5646.2012.01755.x>
- Ferry LA, Konow N, Gibb AC (2012) Are kissing gourami specialized for substrate-feeding? Prey capture kinematics of *Helostoma temminckii* and other Anabantoid fishes. *J Exp Zool* 317:571–579. <https://doi.org/10.1002/jez.1749>
- Fierst JL (2011) A history of phenotypic plasticity accelerates adaptation to a new environment. *J Evol Biol* 24:1992–2001. <https://doi.org/10.1111/j.1420-9101.2011.02333.x>
- Fryer G (1959) The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. *P Zool Soc Lond* 132:153–281. <https://doi.org/10.1111/j.1469-7998.1959.tb05521.x>
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407. <https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Grant PR, Grant B (2008) How and why species multiply: the radiation of Darwin's finches. Princeton University Press, Princeton
- Greenwood PH (1974) The cichlid fishes of Lake Victoria, East Africa: The biology and evolution of a species flock. British Natural History Museum, London, pp 134
- Hendry AP (2009) Ecological speciation! Or the lack thereof? *Can J Fish Aquat Sci* 66:1383–1398. <https://doi.org/10.1139/F09-074>
- Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* 290:516–518. <https://doi.org/10.1126/science.290.5491.516>
- Henning F, Machado-Schiaffino G, Baumgarten L, Meyer A (2017) Genetic dissection of adaptive form and function in rapidly speciating cichlid fishes. *Evolution* 71:1297–1312. <https://doi.org/10.1111/evo.13206>
- Hulseley CD, Hendrickson DA, García de León FJ (2005) Trophic morphology, feeding performance and prey use in the polymorphic fish *Herichthys minckleyi*. *Evol Ecol Res* 7:303–324
- Kinziger AP, Nakamoto RJ, Anderson EC, Harvey BC (2011) Small founding number and low genetic diversity in an introduced species exhibiting limited invasion success (speckled dace, *Rhinichthys osculus*). *Ecol Evol* 1:73–84. <https://doi.org/10.1002/ece3.8>
- Klingenberg CP, Barluenga M, Meyer A (2003) Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. *Biol J Linn Soc* 80:397–408. <https://doi.org/10.1046/j.1095-8312.2003.00246.x>
- Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid fish model. *Nat Rev Genet* 5:288–298. <https://doi.org/10.1038/nrg1316>
- Kornfield IL, Koehn RK (1975) Genetic variation and speciation in new world cichlids. *Evolution* 29:427–437. <https://doi.org/10.2307/2407255>
- Ledon-Rettig CC, Pfennig DW, Nascone-Yoder N (2008) Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evol Dev* 10:316–325. <https://doi.org/10.1111/j.1525-142X.2008.00240.x>
- Lehtonen TK, Kvarnemo C (2015) Infections may select for filial cannibalism by impacting egg survival in interactions with water salinity and egg density. *Oecologia* 178:673–683. <https://doi.org/10.1007/s00442-015-3246-1>
- Losos JB (2011) Convergence, adaptation, and constraint. *Evolution* 65:1827–1840. <https://doi.org/10.1111/j.1558-5646.2011.01289.x>
- Lu G, Bernatchez L (1999) A study of fluctuating asymmetry in hybrids of dwarf and normal lake whitefish ecotypes (*Coregonus clupeaformis*) from different glacial races. *Heredity* 83:742–747. <https://doi.org/10.1046/j.1365-2540.1999.00625.x>
- Machado-Schiaffino G, Henning F, Meyer A (2014) Species-specific differences in adaptive phenotypic plasticity in an ecologically relevant trophic trait: hypertrophic lips in Midas cichlid fishes. *Evolution* 68:2086–2091. <https://doi.org/10.1111/evo.12367>
- Machado-Schiaffino G, Henning GF, Meyer A (2017) Incipient speciation driven by hypertrophied lips in Midas cichlids fish? *Mol Ecol* 26:2348–2362. <https://doi.org/10.1111/mec.14029>
- Manousaki T, Hull PM, Kusche H, Machado-Schiaffino G, Franchini P, Harrod C, Elmer KR, Meyer A (2013) Parsing parallel evolution: ecological divergence and differential gene expression in the adaptive radiations of thick-lipped Midas cichlid fishes from Nicaragua. *Mol Ecol* 22:650–669. <https://doi.org/10.1111/mec.12034>
- McKinnon JS, Rundle HD (2002) Speciation in nature: the threespine stickleback model systems. *Trends Ecol Evol* 17:480–488. [https://doi.org/10.1016/S0169-5347\(02\)02579-X](https://doi.org/10.1016/S0169-5347(02)02579-X)
- Meyer A (1987) Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 41:1357–1369. <https://doi.org/10.2307/2409100>

- Muschick M, Barluenga M, Salzburger W, Meyer A (2011) Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol Biol* 11:116. <https://doi.org/10.1186/1471-2148-11-116>
- Oliver MK, Arnegard ME (2010) A new genus for *Melanochromis labrosus*, a problematic Lake Malawi cichlid with hypertrophied lips (Teleostei: Cichlidae). *Ichthyol Explor Fres* 21:209–232
- Pfennig DW, McGee M (2010) Resource polyphenism increases species richness: a test of the hypothesis. *Philos T R Soc B* 365:577–591. <https://doi.org/10.1098/rstb.2009.0244>
- Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol Evol* 25:459–467. <https://doi.org/10.1016/j.tree.2010.05.006>
- Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol* 20:481–486. <https://doi.org/10.1016/j.tree.2005.06.001>
- Rasband W (1997) ImageJ (version 1.50b). National Institutes of Health, Bethesda. <https://www.rsbl.info.nih.gov/ij>
- Salzburger W (2009) The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol Ecol* 18:169–185. <https://doi.org/10.1111/j.1365-294X.2008.03981.x>
- Salzburger W, Mack T, Verheyen E, Meyer A (2005) Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the Haplochromine cichlid fishes. *BMC Evol Biol* 5:17. <https://doi.org/10.1186/1471-2148-5-17>
- Savolainen V, Lexer C, Anstett M-C, Hutton I, Clarkson JJ, Norup MV, Powell MP, Springate D, Salamin N, Baker WJ (2006) Evolutionary biology: sympatric plant speciation in islands? *Nature* 441:210–213. <https://doi.org/10.1038/nature05217>
- Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol Evol S* 24:35–68. <https://doi.org/10.1146/annurev.es.24.110193.000343>
- Schlichting CD (2004) The role of phenotypic plasticity in diversification. Oxford University Press, New York and Oxford
- Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, New York and Oxford
- Schluter D, Conte GL (2009) Genetics and ecological speciation. *P Natl Acad Sci USA* 106:9955–9962. <https://doi.org/10.1073/pnas.0901264106>
- Schluter D, Nagel LM (1995) Parallel speciation by natural selection. *Am Nat* 146:292–301. <https://doi.org/10.1086/285799>
- Skúlason S, Snorrason SS, Jonsson B (1999) Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr. In: Magurran AE, May RM (eds) *Evolution of biological diversity*. Oxford University Press, New York and Oxford, pp 70–92
- Smith TB, Skúlason S (1996) Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu Rev Ecol Evol S* 27:111–133. <https://doi.org/10.1146/annurev.ecolsys.27.1.111>
- Sorenson MD, Sefc KM, Payne RB (2003) Speciation by host switch in brood parasitic indigobirds. *Nature* 424:928–931. <https://doi.org/10.1038/nature01863>
- Stauffer JR, van Snick GE (2004) Phenotypic plasticity: Its role in trophic radiation and explosive speciation in cichlids (Teleostei: Cichlidae). *Anim Biol* 54:137–158. <https://doi.org/10.1163/1570756041445191>
- Wagner CE, McIntyre PB, Buels KS, Gilbert DM, Michel E (2009) Diet predicts intestine length in Lake Tanganyika's cichlid fishes. *Funct Ecol* 23:1122–1131. <https://doi.org/10.1111/j.1365-2435.2009.01589.x>
- Wente WH, Phillips JB (2003) Fixed green and brown color morphs and a novel color-changing morph of the Pacific tree frog *Hyla regilla*. *Am Nat* 162:461–473. <https://doi.org/10.1086/378253>
- West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Evol S* 20:249–278. <https://doi.org/10.1146/annurev.es.20.110189.001341>
- West-Eberhard MJ (2003) *Developmental plasticity and evolution*. Oxford University Press, New York and Oxford
- Willis TJ, Babcock RC (1998) Retention and in situ detectability of visible implant fluorescent elastomer (VIFE) tags in *Pagrus auratus* (Sparidae). *New Zeal J Mar Fresh* 32:247–254. <https://doi.org/10.1080/00288330.1998.9516823>
- Witte F, Barel C, Hoogerhoud R (1989) Phenotypic plasticity of anatomical structures and its ecomorphological significance. *Neth J Zool* 40:278–298. <https://doi.org/10.1163/156854289X00309>
- Wund MA (2012) Assessing the impacts of phenotypic plasticity on evolution. *Integr Comp Biol* 52:5–15. <https://doi.org/10.1093/icb/ics050>
- Yamaoka K (1997) Trophic ecomorphology of Tanganyikan cichlids. In: Kawanabe H, Hori M, Nagoshi M (eds) *Fish communities in Lake Tanganyika*. Kyoto University Press, Kyoto, pp 25–26
- Yeh PJ, Price TD (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* 164:531–542. <https://doi.org/10.1086/423825>
- Zuur AF, Hilbe J, Ieno EN (2013) *A beginner's guide to GLM and GLMM with R: A frequentist and Bayesian perspective for ecologists*. Highland Statistics Ltd., United Kingdom

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