

# Sympatric and Allopatric Diversification in the Adaptive Radiations of Midas Cichlids in Nicaraguan Lakes



Julián Torres-Dowdall and Axel Meyer

**Abstract** The Nicaraguan Midas cichlid species complex is a natural experiment where fish from a large source population from turbid and shallow great lakes very recently (<20,000 years ago) colonized eight small crater lakes. The colonizers experienced completely novel environments in the clear and deep calderas. So far, 13 Midas cichlid species have been described, but more genetic clusters were identified. Although some of these species arose in allopatry, many more evolved in the absence of barriers to gene flow within two crater lakes. They contain small radiations of four and six endemics, respectively. These radiations constitute one of the few generally accepted empirical examples for sympatric speciation making them an ideal system for studying repeated evolution of adaptations and speciation at different levels of biological organization, including the genome level. Diversification occurred repeatedly in parallel including body morphology, coloration, color perception, and trophic structures such as pharyngeal jaws and hypertrophied lips. Additionally, parallel speciation happened in the two small crater lake radiations, where ecomorphologically similar species evolved repeatedly. Genomic differentiation associated with oligogenic traits (e.g., hypertrophic lips and coloration) is shallow, remaining polymorphisms, but much higher for polygenic traits (e.g., body shape and pharyngeal jaw morphology) that distinguish new species.

**Keywords** *Amphilophus citrinellus* · Speciation · Parallel evolution · Genetic polymorphism · Sympatric speciation · Natural experiment

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## 1 Introduction

One of the most debated topics in evolutionary biology is the role of geography in the origin of biodiversity. There is no doubt about the importance of geography for speciation, given its direct impact on gene flow and selection (Nosil 2012). However, disagreements continue about the necessity of physical barriers (e.g., geographic isolation) that impede gene flow for the evolution of new species and about how common speciation in the absence of such barriers is (i.e., speciation with gene flow; Mayr 1963; White 1978; Coyne and Orr 2004). As with many debates in evolutionary biology, the issue of geography was already foreshadowed in Darwin's writings. His views appeared to be ambiguous about the topic, but it is evident that he thought that it is feasible that speciation can occur under different biogeographic scenarios. However, emphasis on the role of natural selection during speciation and Darwin's "principle of divergence" facilitated the acceptance of the idea that speciation most commonly occurs independently of the geographic setting and is due to disruptive selection during competition for resources (Mayr 1963; Coyne and Orr 2004).

That speciation without geographic barriers, or in other words speciation with gene flow (the term "sympatric" was first introduced by Edward B. Poulton in 1904, see Mallet 2004), is possible prevailed until the emergence of a population-genetic thinking after the rediscovery of Mendel's laws (for a detailed revision of speciation during the first 100 years after "The Origin," see Mayr 1963). With an increasing understanding of genetics, the debate about the role of geography became focused on the impact of restriction of gene flow during speciation (Futuyma and Mayer 1980). This also affected the view of the involvement of natural selection in the process of speciation (Mayr 1963; Mallet 2004). In allopatric speciation, extrinsic factors (e.g., geographical barriers) cause a reduction of gene flow between diverging gene pools with natural selection acting only later, possibly contributing to further divergence. However, during sympatric speciation, natural selection plays a fundamental role in initially splitting apart the gene pools (Mayr 1963). The relevant question then became: Can natural selection effectively reduce gene flow among individuals without geographic isolation?

One of the strongest critics of the idea of sympatric speciation was Ernst Mayr who strongly influenced the view on that topic for several decades. In his book *Animal Species and Evolution* (1963), Mayr devoted a chapter to debunking the idea that speciation could proceed without geographic isolation. He critically analyzed the topic, finding no theoretical or empirical evidence in support of sympatric speciation, and concluded that "*All these considerations help to make clear why geographic isolation is a prerequisite for successful speciation*" (1963, p. 480). As part of this criticism, Mayr also attacked the idea of ecological speciation, arguing that there was no evidence that this is "*a process distinct from geographical isolation*" (Mayr 1963). Thus, the view that natural selection played either no role or only a minor role and that speciation was a by-product of geographic separation but not due to local adaptations to particular ecological conditions did prevail in

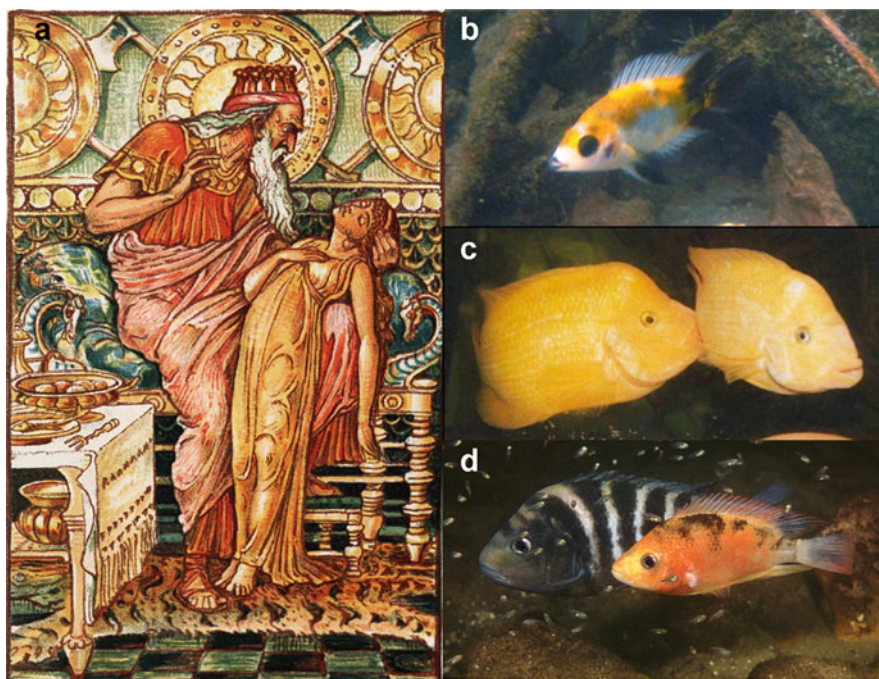
evolutionary biology for several decades. Natural selection became increasingly seen only as the key driving force on the path toward better adaptations within a population, but not as the mechanism for speciation (Meyer 2011).

As predicted by Mayr (1963, p. 451), the idea of sympatric speciation was raised again not long after he criticized it, mostly through genetic models by John Maynard Smith (Maynard Smith 1966; White 1978). In particular in the last decade or so, several models have shown that speciation with gene flow is theoretically possible, although it requires particular conditions to occur (Gavrilets 2004; Bolnick 2006; Bolnick and Fitzpatrick 2007; Gavrilets et al. 2007). This resurgence of interest in sympatric speciation was accompanied by a renewed emphasis on the role of natural selection as a driver of biological diversification, not only by making it possible for speciation to occur in sympatry, but because of its potential to accelerate the process in allopatry (Gavrilets 2004; Nosil 2012; Hendry 2016). Thus, it is now clear that sympatric speciation is not impossible as first postulated by Mayr (but see Mayr (2001) for his opinion on the examples of sympatric speciation in cichlids), but it does not appear to be particularly common. Only few empirical cases have emerged that appear to be best explained by sympatric speciation (reviewed in Coyne and Orr 2004; Bolnick and Fitzpatrick 2007; Bird et al. 2012; Meyer and Kautt 2014; Foote 2018). This paucity of convincing cases of sympatric speciation is mainly because ruling out other speciation mechanisms is extremely challenging. In particular, it requires rejecting the possibilities that (1) the observed phylogenetic pattern is the consequence of gene flow between nonreciprocal sister species occurring in sympatry and (2) speciation actually occurred in allopatry and current sympatry is due to secondary contact (Coyne and Orr 2004).

The Nicaraguan Midas cichlid species complex (*Amphilophus citrinellus* spp.) constitutes one of these few generally accepted empirical examples for sympatric speciation (Barluenga et al. 2006; see in the following). Midas cichlids very rapidly formed small radiations in an interesting geographic setting including two great lakes and several isolated crater lakes (Elmer et al. 2010a; Meyer 2011; see in the following). Thus, this species complex provides a valuable model for the study of the roles of biogeography and natural selection during the formation of adaptive radiations and speciation more generally. In the following, we discuss, with examples, how natural selection has facilitated divergence in allopatry and has repeatedly driven speciation in sympatry in this lineage. We then briefly discuss why, of all the species that inhabit the Nicaraguan lakes, only Midas cichlids radiated, whereas other fish lineages, including other cichlids, did not.

## 1.1 *Discovery of the Midas Cichlid Species Complex*

The name of the Midas cichlid species complex comes from the fact that a few individuals in the population would lose their melanophores during ontogeny and become “gold,” as if the King Midas from Greek mythology had touched them—King Midas was gifted, or cursed, with the ability to transform anything he touched



**Fig. 1** (a) The common name of the *Amphilophus citrinellus* species complex, Midas cichlids, is in reference to the King Midas of the Greek mythology, who transformed everything he touched into gold, including his own daughter (illustration by Walter Crane, 1893). During development, genetically determined gold individuals “transform into gold” by losing their melanophores and exposing the underlying xanthophores. (b) This process includes melanophore death and migration and sometimes results in beautiful mosaic fish with white, orange, and black body parts. (c) Gold Midas cichlids most often breed assortative; but (d) mixed couples are also seen in nature, probably affecting the pattern of genetic divergence between morphs (Photos **b** by Andreas Härer, **c** and **d** by Ad Konings)

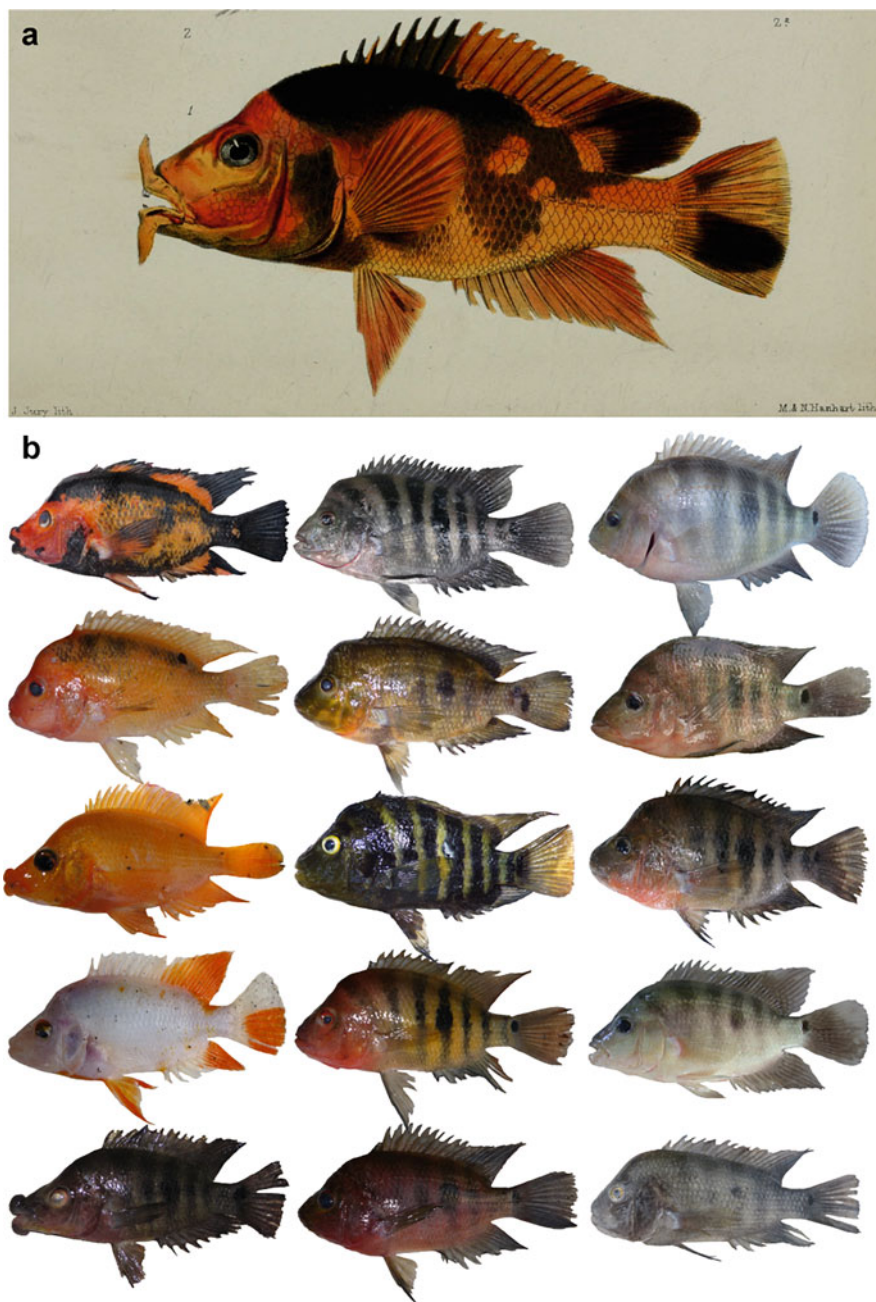
into gold (Fig. 1a). Louis Agassiz was the first to describe a Midas cichlid species from a specimen with hypertrophied lips, naming it *Amphilophus froebelii*. This species was presented as new to science during the meeting of the Boston Society of Natural History on October 6, 1858 (Agassiz 1859), and although the published article was written by a scribe, Agassiz is credited with the establishment of both the genus *Amphilophus* and the type species *A. froebelii*. The description given was quite vague, and no type specimen was designated. Therefore, although the genus name was maintained, the species name was considered a *nomen oblitum* because it went unused for more than half a century (Barlow and Munsey 1976; Stauffer and McKaye 2002). A few years later, in 1864, Albert Günther formally described the first two species of the Midas cichlid complex. First, he described *A. labiatus* (as *Heros labiatus*) from Lake Managua (Günther 1864a). Shortly after that, he described *A. citrinellus* (as *Heros citrinellus*) from Lake Nicaragua (Günther 1864b). Curiously, but maybe not too surprisingly, the original descriptions of both species

were done based on gold individuals (Fig. 2a). Later, it was clear that these species were polymorphic in terms of coloration (Figs. 1, 2, and 3, see Sect. 5). Within a few years, many additional species were described that subsequently were synonymized either to *A. citrinellus* (*H. basilaris* Gill and Bransford 1877, *Cichlasoma granadense* Meek 1907) or *A. labiatus* (*H. erythraeus* Günther 1867; *H. lobochilus* Günther 1867; *C. dorsatum* Meek 1907). This attests to one of the most distinctive, yet vexing, characteristics of the Midas cichlid species complex: its impressive amount of phenotypic variation, both within and across species (Fig. 2b). Seth E. Meek, in his *Synopsis of the Fishes of the Great Lakes of Nicaragua* wrote: “Of all the species [of] fishes in these lakes, this one is by far the most variable. I made many repeated efforts to divide this material listed below in from two to a half-dozen or more species. . .” (Meek 1907, p. 122). Later, he concluded: “it is possible that more than one species should be recognized here, and no doubt such will some day be the case. . .” Noticeably, he nonetheless split the two species currently accepted from the great lakes of Nicaragua into six species, two of which were described there as new species (Meek 1907).

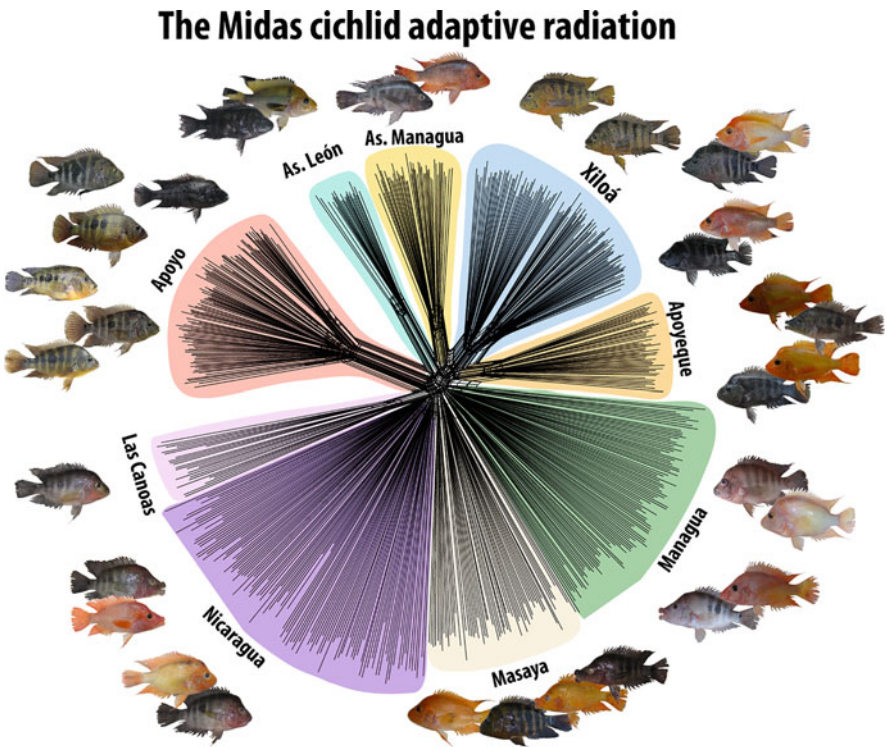
More than a hundred years passed until a new Midas cichlid species was described. George Barlow, who contributed significantly to our understanding of the ecology and behavior of Midas cichlids, together with John W. Munsey described *A. zaliosus*, the Arrow Midas cichlid, an endemic species from crater Lake Apoyo (Barlow and Munsey 1976). The description of this species brought renewed attention to the Midas cichlid lineage because it raised the possibility that *A. zaliosus* evolved in sympatry to its sister species. Five more endemic species were more recently described from the same crater lake, *A. chanco*, *A. flaveolus*, and *A. astorquii* by Stauffer et al. (2008); and *A. superciliosus* and *A. globosus* by Geiger et al. (2010b). Also four species endemic to crater Lake Xiloá (*A. amarillo*, *A. xiloensis*, and *A. sagittae* (Stauffer and McKaye 2002); and *A. viridis* (Recknagel et al. 2013)) were described during that time. These descriptions mattered not only because they led to the recognition of a second potential case of a small adaptive radiation within a crater lake, but also because *A. sagittae* has a striking morphological resemblance to *A. zaliosus* from Apoyo with a similarly elongated body shape associated with an open-water, limnetic life style. Thus, Midas cichlids not only constitute an example of multiple radiations, but the parallelism in these species also emphasizes the potentially deterministic role of natural selection in speciation and the formation of adaptive radiations (Elmer et al. 2010a, 2014).

Demographic models suggest that most Midas cichlid species arose within historical times (less than 2000 years ago) from a common ancestor inhabiting the great lakes (Kautt et al. 2016a, b, 2018, 2020; Machado-Schiaffino et al. 2017). To date, 13 species of Midas cichlids have been described; yet, more genetic clusters are being identified (Kautt et al. 2018). One critical point is that it is easier to determine species limits when they co-occur rather than when they are geographically isolated, and this problem is particularly tricky in the Midas cichlid system that is distributed in several completely isolated crater lakes and two large source lakes (Kautt et al. 2018). Thus, many times after a long collection day in Nicaragua, we found ourselves looking into a bucket full of fish, as Seth Meek





**Fig. 2** (a) Illustration of a gold, thick-lippy fish accompanying the description of *Amphilophus labiatus* by Albert Günther in 1864. (b) Midas cichlids are extremely variable along different phenotypic axes as depicted by these specimens collected from two lakes, Nicaragua and Masaya, with only two species described: *Amphilophus citrinellus* and *A. labiatus*. Most prominent is the variation in the abundance and distribution of melanophores, xanthophores, and leucophores (fish in the first column) and lip size (drawing in **a** and top and lowest three fish in the first column).



**Fig. 3** Neighbor-net split graph based on genetic distance (modified from Kautt et al. 2018), and pictures of the Midas cichlids within each lake. Representatives of the different species are depicted as isolated fish, whereas polymorphisms within species are depicted as overlapping fish (pictures by Andreas Kautt)

did more than a hundred years ago, and wondering if only one species is in our bucket or many of them.

2 The Ecological Setting

The country of Nicaragua is located in the Central American Isthmus (Fig. 4), and from west to east, it can be divided into the Pacific Coastal Plain, the Nicaraguan depression, the Interior Highlands, and the Atlantic Coastal Plain (Weyl 1980; Kutterolf et al. 2007). The Nicaraguan depression is considered as a tectonic graben

←  
**Fig. 2** (continued) Moreover, impressive variation in coloration is also seen in fish showing the “normal” pattern of melanophores’ distribution (fish in the second column). A third axis of phenotypic variation is seen in body shape (third column)



**Fig. 4** Geographic location and physical characteristics of the Nicaraguan great lakes and crater lakes



with a Late Miocene origin. This 45-km-wide depression contains two large lakes that formed during the late Pleistocene (Kutterolf et al. 2007). Lake Nicaragua (Cocibolca) is the largest lake in Central America and the 19th largest in the world, with an estimated surface area of 8300 km<sup>2</sup>. Lake Managua (Xolotlan) is smaller, but still large with a surface area of 1042 km<sup>2</sup> (estimates for both lakes vary, see Cole 1976; Meyer 2011). Both lakes are part of the San Juan River drainage that discharges into the Caribbean. Detailed analysis of the sediments of both lakes showed that there has never been a connection to the Pacific, as previously considered, and that these have always been freshwater lakes (Swain 1966). Both lakes are relatively shallow, with an average depth of only 9 and 12 meters for Managua and Nicaragua, respectively (Cole 1976). Lake Managua is located upstream of Lake Nicaragua, with its surface 9 m higher than the latter (Incer 1976). Both lakes are connected by the Tipitapa River, which flows through the Tisma Pond, but the connection is intermittent due to variation in the water levels of the two lakes (Montenegro Guillén 1992; Meyer 2011).

The Central American Volcanic Arc, which formed during the late Cretaceous due to the subduction of the Cocos plate beneath the Caribbean plate, crosses the Nicaraguan depression and both great lakes from north to south. This resulted in a series of 12 volcanic complexes in Nicaragua, some of which are still active today (Kutterolf et al. 2007). The calderas of inactive volcanoes eventually filled up with groundwater or rainwater and became crater lakes. In Nicaragua, there are eight crater lakes known to have populations of Midas cichlids (Figs. 2 and 3; Table 1). How these crater lakes were colonized is unclear, and several hypotheses have been proposed including “fish rains” produced by hurricanes, drops by piscivorous birds, and human activity (Villa 1976; Meyer 2011). Demographic models estimate that the colonization of the crater lakes occurred quite recently, with crater Lake Apoyo being the oldest colonization (1680 generations ago; Kautt et al. 2016a) and crater Lake Apoyeque the youngest (580 generations ago, one to one and a half generations per year is estimated for Midas cichlids; Machado-Schiaffino et al. 2017). The source of Midas cichlids of all crater lakes is the great Lake Managua, except Lake Apoyo whose source is the great Lake Nicaragua (Kautt et al. 2018).

The crater lakes inhabited by Midas cichlids differ to a certain degree in their physicochemical characteristics, including size, depth, water chemistry, and load of suspended particles (Fig. 4; Barlow et al. 1976; Recknagel et al. 2014; Kautt et al. 2018). Nonetheless, these crater lakes share several characteristics that depart from those in the great lakes in similar ways. For example, all crater lakes are significantly deeper and smaller, and their littoral zone is minuscule compared to that in the great lakes (Recknagel et al. 2014; Kautt et al. 2018). Additionally, the crater lakes are characteristically oligotrophic, having low levels of nutrients for primary production, hence contrasting with the eutrophic characteristics of the great lakes (Swain 1966). Thus, the crater lakes share a series of characteristics that provide a drastically different fish habitat than those of the great lakes. For these reasons, the Midas cichlid species complex provides a replicated system to the study of adaptive evolution and speciation under different geographical settings.

**Table 1** Fish diversity of the Nicaraguan lakes

a. Fish species reported from the Nicaraguan lakes											
		Crater lakes					Great lakes				
Family <sup>a</sup>	Species <sup>b</sup>	Apoyo	Masaya	Asosca León	Apoyeque	Xilola	Tiscapa	Asosca Managua	Lake Managua	Lake Nicaragua	
Atherinopsidae (3)	<i>Atherinella sardina</i>	x	x			x		x	x	x	
Cichlidae (18)	<i>Anatitlania nigrofasciata</i>		x			x			x	x	
	<i>Amphilophus longimanus</i>		x			x			x	x	
	<i>Amphilophus rostratus</i>					x			x	x	
	<i>Archocentrus centrarchus</i>					x			x	x	
	<i>Hypsophrys nematopus</i>		x			x			x	x	
	<i>Hypsophrys nicaraguensis</i>					x			x	x	
	<i>Parachromis dovii</i>		x			x			x	x	
	<i>Parachromis managuensis</i>	x	x	x	x	x	x	x	x	x	
Clupeidae (1)	<i>Dorosoma chavesi</i>		x			x			x	x	
Eleotridae (1)	<i>Gobiomorus dormitor</i>	x	x			x		x	x	x	
Heptapteridae (4)	<i>Rhamdia nicaraguensis</i>					x			x	x	
Poeciliidae (11)	<i>Poecilia gillii</i> <sup>c</sup>					x			x	x	
	<i>Poecilia mexicana</i> <sup>d</sup>	x	x	x	x	x			x	x	
Synbranchidae (1)	<i>Synbranchus marmoratus</i>					x			x	x	

b. Fish species only reported from the great lakes and the San Juan River	
Atherinopsidae (3)	<i>Atherinella hubbsi</i> , <i>A. milleri</i>
Bryconidae (1)	<i>Brycon guatemalensis</i>
Carcharhinidae (1)	<i>Carcharhinus leucas</i>
Centropomidae (2)	<i>Centropomus parallelus</i> , <i>C. pectinatus</i>
Characidae (10)	<i>Astyanax aeneus</i> , <i>A. cocinholca</i> , <i>A. fasciatus</i> , <i>Bramocharax bransfordii</i> , <i>Bryconamericus scleroparius</i> , <i>Carlana eigenmanni</i> , <i>Hemibrycon</i> sp., <i>Hyphessobrycon tortuguerae</i> , <i>Roeboides bouchellei</i> , <i>R. guatemalensis</i>
Cichlidae (18)	<i>Anatitlania septemfasciata</i> , <i>Amphilophus alfari</i> , <i>Cryptoheros spilurus</i> , <i>Herotilapia multispinosa</i> , <i>Oreochromis aureus</i> , <i>O. niloticus</i> , <i>Parachromis friedrichsthalii</i> , <i>P. loisellei</i> , <i>Tomocichla tuba</i> , <i>Vieja maculicauda</i>
Gerreidae (1)	<i>Eugerres plumieri</i>
Gymnotidae (3)	<i>Gymnotus carapo</i> , <i>G. cylindricus</i> , <i>G. maculosus</i>
Haemulidae (1)	<i>Pomadasys croco</i>
Heptapteridae (4)	<i>Rhamdia guatemalensis</i> , <i>R. laticauda</i> , <i>R. quelen</i>
Lepisosteidae (1)	<i>Atractosteus tropicus</i>
Loricariidae (1)	<i>Hemiancistrus aspidolepis</i>
Megalopidae (1)	<i>Megalops atlanticus</i>
Mugilidae (1)	<i>Agonostomus monticola</i>
Poeciliidae (11)	<i>Alfaro culturatus</i> , <i>Belonesox belizanus</i> , <i>Brachyraphis</i> sp., <i>Phallichthys amates</i> , <i>P. tico</i> , <i>Poecilia sphenops</i> , <i>Poeciliopsis gracilis</i> , <i>Priapichthys annexens</i> , <i>Xenophallus umbratilis</i>
Pristidae (3)	<i>Pristis pectinata</i> , <i>P. perotteti</i> , <i>P. pristis</i>
Rivulidae (1)	<i>Cynodonichthys isthmensis</i>

<sup>a</sup>Number in parenthesis indicates the number of species per family described for the great lakes, crater lakes, and San Juan River

<sup>b</sup>The list of species was extracted from ERM (2015), Härer et al. (2017b), and references within

<sup>c</sup>The phylogenetic position of the Nicaraguan taxon related to *P. gillii* is controversial (Bagley et al. 2015; Palacios et al. 2016; Härer et al. 2017b)

<sup>d</sup>*Poecilia mexicana* is most likely polyphyletic, including an undescribed taxon controversial (*P. sp. 'tipitapa'*; Bagley et al. 2015; Härer et al. 2017b)

## 2.1 Other Fish Species in Nicaraguan Lakes

Midas cichlids are one of the most common fish species in the great and crater lakes of Nicaragua, and they dominate most of these lakes in terms of fish biomass (Dittmann et al. 2012). Yet, they are not the only fish inhabiting the great and crater lakes. Surveys on the fish fauna of the great lakes and associated rivers have been scarce (Meek 1907; Astorqui 1971; ERM 2015; Härer et al. 2017a), but more than 50 species have been identified (Table 1). The great lakes count includes three species of elasmobranchs, the bull shark (*Carcharhinus leucas*) and two sawfish (*P. pristis* and *P. perotteti*), and the tropical gar (*Atractosteus tropicus*) among the most curious parts of their fish fauna. It is unfortunately likely that the sharks and the sawfish have been driven to extinction due to overfishing in the last decades. The most diverse family of bony fishes in the great lakes are the cichlids, with 17 species documented for the lakes, including two introduced species of tilapia (*Oreochromis niloticus* and *O. aureus*). Of these 17 cichlid species, only a subset colonized the crater lakes (Table 1, the composition varies across crater lakes) and only one cichlid species, besides Midas cichlids, is present in all the crater lakes (the top predator *Parachromis managuensis*). Curiously, this species is now very abundant in crater Lake Tiscapa, but was not found there by Meek one hundred years ago (Meek 1907), suggesting that humans might have introduced it into the lake in recent years. Studies on these species have been conducted in an effort to understand why Midas cichlids are the only lineage that radiated in the crater lakes (see Sect. 6) and not, for example, any other cichlid species or the livebearing fishes of the Family Poeciliidae, of which only two species are found in Xiloá, but seven more co-occur in the great lakes and in the San Juan River.

Much of this intra- and interspecific diversity is threatened. In 2013, the Hong Kong Nicaragua Canal Development Group (HKND) of China obtained a 50-year concession from the Nicaraguan government to build and use an interoceanic shipping canal. The construction of the canal is highly contested because of its potential social and ecological impacts (Meyer and Huete-Perez 2014; Huete-Perez et al. 2015, 2016). Currently, the project appears to be stalled, and for the last 3–4 years, there has been no official information from the Nicaraguan Government about the future of the canal. However, due to the major impacts to the environment and the imperilment of the fauna that its construction might cause, it is important to remain alert to the future decisions of the Nicaraguan government about the plans of the canal.

## 3 Parallel Phenotypic Divergence and Allopatric Speciation: Interlacustrine Radiation of Midas Cichlids

The allopatric origin of biodiversity is well-documented and, accordingly, not controversial. However, what role natural selection plays during allopatric speciation is still debated among evolutionary biologists. Genetic divergence between isolated



populations could be the result of nonselective processes (i.e., genetic drift); however, the process of speciation can be extremely slow under these conditions (Coyne and Orr 2004; Gavrillets 2004). Natural selection is thought to accelerate this process, and thus, it is considered fundamental in radiations occurring in allopatry (also referred to as geographic radiations, see Simões et al. 2016).

In Midas cichlids, variable levels of genetic differentiation were found in pairwise comparisons of populations inhabiting different lakes, from  $F_{st} \approx 0.05$ , when comparing fish from the two great lakes, to  $F_{st} \approx 0.5$ , when comparing populations from different crater lakes (Bunje et al. 2007; Kautt et al. 2018, 2020). This might be due to the fact that only a low number of individuals might have colonized each of the crater lakes from the great lakes, resulting in genetic bottlenecks that might have increased genetic divergence among geographically isolated populations (Bunje et al. 2007; Kautt et al. 2018). Demographic models estimated that only a few hundred individuals colonized each of the crater lakes (Kautt et al. 2016a, b, 2018, 2020; Machado-Schiaffino et al. 2017). However, these models also support the prediction that a second colonization event followed by admixture resulted in increased standing genetic diversity in most crater lake populations (Kautt et al. 2018, 2020). Natural selection is thought to have played a strong role driving phenotypic diversification by sorting this genetic diversity. This effect is most evident in the cases of parallel evolution that can be observed in the comparisons of the two small radiations of crater Lakes Xiloá and Apoyo (see Sect. 4.1; Elmer et al. 2010a, 2014; Kautt et al. 2016a), but also in consistent phenotypic changes seen across multiple crater lakes (e.g., Torres-Dowdall et al. 2017b; Kautt et al. 2018). In the following, we present and discuss two such examples, one considering morphological adaptations to the geomorphological characteristics of crater lakes, and a second one about the physiological adaptation of the visual system to the new photic conditions experienced in those lakes.

### ***3.1 Parallel Evolution in Body Shape After Crater Lake Colonization***

One of the most dramatic phenotypic changes observed in Midas cichlids after the colonization of crater lakes is in body shape. Midas cichlids from the great lakes Managua and Nicaragua have a deep body, where the ratio of body depth to standard length is high. This morphology is thought to be adaptive to the shallow, benthic environment they occupy in these great lakes. In the crater lakes, Midas cichlids evolved an elongated body shape compared to their ancestral populations (Klingenberg et al. 2003; Recknagel et al. 2014; Kautt et al. 2018). In some of these crater lakes, there was also an increase in variation in body shape (across and within species, see Sect. 4.1), but on average, there was a parallel change in body shape in all the crater lakes, where the ratio of body depth to length was reduced compared to great lake Midas cichlids (Kautt et al. 2018).

This divergence in body shape between the ancestral form and the derived crater lake Midas cichlids has been shown to be maintained under common garden conditions (Kautt et al. 2016b), suggesting that the trait is to some degree heritable. Fish body shape can plastically respond to environmental conditions (Wimberger 1992), such as water flow (Langerhans 2008; Franssen et al. 2013) or chemical cues (Abate et al. 2010; Torres-Dowdall et al. 2012). However, a split brood experiment exposing juveniles of four species of Midas cichlids to low versus high water flow for 6 months found no effect of treatment in the ratio of body depth to length (Kautt et al. 2016b). Instead, the three species from crater lakes maintained their more elongated body compared to great lake Midas cichlids. This is further supported by studies of F2 hybrid crosses finding significant QTL associated with body elongation (Franchini et al. 2014a). Interestingly, this study found that few genomic regions of large effect contributed to most of the divergence in Midas cichlids' body elongation. The study was conducted crossing two Midas cichlid species from crater Lake Apoyo, so this might not be representative of the genetic architecture of this trait across the whole radiation. However, demographic modeling indicated that morphological change occurred quite rapidly after crater lake colonization, but it slowed down later (i.e., the amount of change exponentially decreased with time since colonization; Kautt et al. 2018). This pattern is consistent with few alleles of large phenotypic effect contributing to change in body shape (e.g., Orr 2005; Kopp and Hermisson 2007).

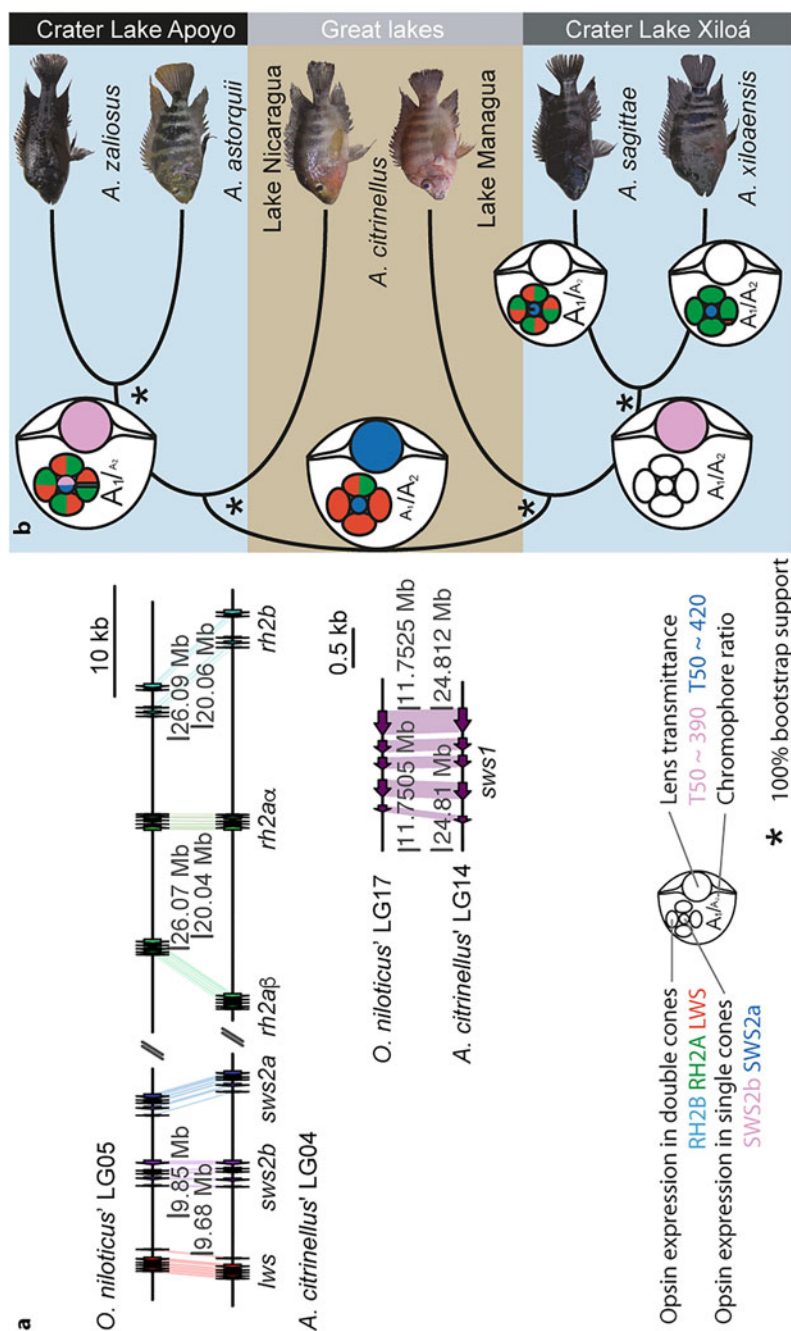
A similar pattern is observed across different fish species, like sticklebacks (*Gasterosteus* spp.; Schluter 1993) and whitefish (*Coregonus* spp.; Landry et al. 2007), where limnetic (elongated) and benthic (deep-bodied) morphs evolved repeatedly. This is also well-documented across cichlid lineages, for example, after the colonization of Ugandan crater lakes by haplochromine cichlids. Cichlids in 13 Ugandan crater lakes have evolved a more elongated, slender body shape than their source population from Lake Edward (Machado-Schiaffino et al. 2015). Why would crater lake cichlids evolve elongated body shapes consistently and repeatedly in distantly related species? The parallelism seen within the Midas cichlid radiation, and when comparing crater lake cichlids from Uganda and Nicaragua, strongly suggests that the evolution of body shape is driven by natural selection. The geomorphological characteristics of crater lakes might provide the ecological scenario where elongated body shapes might be advantageous. The steep banks and shores of crater lakes result in a reduced littoral zone that might render the limnetic zone ecologically more relevant for fish. In fact, the divergence in body shape between ancestral and derived Midas cichlid species is negatively correlated with the total littoral zone of the crater lake (Recknagel et al. 2014; Kautt et al. 2018). Models considering the biomechanics of swimming predict the evolution of body shape associated with habitat complexity (Webb 1994; Langerhans and Reznick 2010); and the colonization of more open-water habitats has been repeatedly shown to be related to the evolution of more elongated, streamlined body shapes (Schluter 2000; Gow et al. 2008; Landry and Bernatchez 2010). Midas cichlids with more elongated body shapes have higher swimming endurance than deep bodied fish

(Raffini et al. 2020), providing a functional link between the ecological setting of the crater lakes and the evolution of morphology.

In summary, all crater lake Midas cichlids have independently evolved elongated body shapes compared to that found in source population from the shallow great lakes, apparently as an adaptation for increasing swimming performance in the more open-water environment. This parallelism is suggesting an important role for natural selection acting on standing genetic variation originally introduced from the ancestral great lakes in driving morphological evolution in crater lake Midas cichlids toward a predictable adaptive outcome. However, other factors might also have contributed to the evolution of body shape. In particular, there is a negative correlation between amount of phenotypic change and size of the founder population (Kautt et al. 2018), suggesting that drift might be an important factor in shaping evolution (e.g., Kolbe et al. 2012). An alternative explanation for this pattern is that later admixture events in some crater lakes constrained the amount of phenotypic change. Unfortunately, it might be difficult to contrast these potential explanations with the current data and the small number of crater lakes that have been colonized by Midas cichlids.

### 3.2 *Parallel Evolution of the Visual System*

Visual sensitivity is another trait that evolved divergently and in parallel after colonization of at least two crater lakes (Apoyo and Xiloá) (Torres-Dowdall et al. 2017b). The visual sensitivity of an organism is the integration of several phenotypic characteristics of its eyes. For example, the part of the light spectrum that reaches animals' retinas is limited by their ocular media (e.g., cornea and lenses), which selectively filters out light of particular wavelengths—most often highly energetic short wavelengths that might damage the retina. Additionally, the visual pigments in the photoreceptors of the retina could have peak sensitivities at different parts of the light spectrum (Yokoyama and Yokoyama 1996; Bowmaker 2008). These peaks of sensitivity are the particular wavelength at which light absorption is at its maximum, which depends on the interaction of the two components of visual pigments (Cronin et al. 2014; Carleton et al. 2016). One component is the opsin protein, a transmembrane G-protein-coupled receptor. Vertebrates have different opsin gene paralogs coding for proteins with peak sensitivities at different parts of the spectrum (i.e., different spectral classes: short-, medium-, and long-wavelength sensitivity). Midas cichlids, like other cichlids (Carleton et al. 2016; Escobar-Camacho et al. 2017), have eight opsin paralogs; one sensitive under dim light conditions (rhodopsin) and seven sensitive under bright light and responsible for color discrimination (Fig. 5a). The second component of visual pigments is the chromophore, a light-absorbing molecule covalently bound to the opsin protein. When a photon of the proper wavelength hits a chromophore, it changes its configuration and initiates a transduction cascade by affecting the tertiary structure of the opsin protein (Fain et al. 2010). Aquatic vertebrates have two types of chromophores, one derived from



**Fig. 5** (a) Opsin gene synteny between the African Nile Tilapia (*Oreochromis niloticus*) and the Nicaraguan Midas cichlid (*Amphiphilopus citrinellus*). (b) Schematic representation of the phenotypic changes in the visual system of Midas cichlids associated with the colonization of the clear water crater lakes from the turbid water great lakes. In fish from two crater lakes, lenses became more transmissive and expression of *rh2a* increased and that of *lws* decreased. In all crater lake species, except for *A. xiloensis*, chromophore usage changed from mainly A2 to mainly A1, depicted in the figure as differences in font size (modified from Torres-Dowdall et al. 2017b)



vitamin A1 and a second one from vitamin A2. Switching from A1 to A2 chromophore usage shifts the sensitivity of a visual pigment toward longer wavelengths within the same spectral class (Wald 1961; Hárosi 1994). Midas cichlids have taken advantage of all these molecular mechanisms to adapt to new light conditions they encountered after the colonization of the crater lakes to shift their visual sensitivity in an integrated manner according to the prevailing light conditions (Torres-Dowdall et al. 2017b).

The great lakes Managua and Nicaragua are very shallow, and the wind keeps stirring the sediments of the bottom maintaining them suspended in the water column (Fig. 4). Hence, the great lakes are extremely murky and very little light penetrates into the water column. Contrary to this, the crater lakes are smaller, very deep, and surrounded by crater rims that protect them from the action of the wind. The result is that sediments rapidly precipitate and the water is very clear, allowing a lot of light to penetrate to greater depths. This has two consequences for the light environment in crater lakes; first, there is proportionally more light than in the great lakes at any given depth. Second, the light spectrum is broader than in the great lakes, particularly due to more available light at short wavelengths (Torres-Dowdall et al. 2017b). Given the importance of the visual system for all daily activities of these fish, strong selection pressure to adapt to these new light conditions is expected. Indeed, the visual system of crater lake Midas cichlids has evolved an increased sensitivity to light in the shorter part of the light spectrum (Torres-Dowdall et al. 2017b); and these modifications appear to have occurred through ontogenetic changes and to have been facilitated by adaptive developmental plasticity of the ancestral population (Härer et al. 2017b).

Given the broader light spectrum experienced by fish in the clear water crater lakes, it would be expected that the ocular media diverges from that found in fish from the turbid great lakes by becoming more transmissive. Indeed, crater lake Midas cichlids' crystalline lenses are clearer, allowing more light in the short-wavelength part of the spectrum to go through and to reach the retina. The increased breadth of light reaching the retina is expected to impose selection on the visual pigments in the photoreceptors of the retina favoring a shift in their sensitivities toward shorter wavelengths. This resulted in three changes in the visual pigments of crater lake Midas cichlids (Fig. 5b). First, opsins that were only expressed during early development in Midas cichlids from the great lakes, but not in adults, are retained in the adult phenotype of crater lake Midas cichlids (Härer et al. 2017b; Torres-Dowdall et al. 2017b). Midas cichlids, like other cichlid species (O'Quin et al. 2011; Carleton et al. 2016), undergo an ontogenetic shift in visual sensitivity due to differential patterns of opsin expression across ages. Larvae express a set of opsins that constitutes visual pigments sensitive at shorter wavelengths, including UV light. As development progresses, this set of expressed opsins changes, and this process ends with adults expressing opsins that constitute visual pigments with sensitivities at longer wavelengths (Härer et al. 2017b). However, crater lake Midas cichlids appear to have a paedomorphic visual system compared to fish from the ancestral great lakes. Adult Midas cichlids in the crater lakes express a set of opsin genes that resembles the phenotype seen in juveniles (~6-month-old fish)

from the great lakes and have overall short-wavelength shifted sensitivities (Härer et al. 2017b). Second, the pattern of expression of those opsins that are commonly expressed in adult fish from the great lakes was also modified. Crater lake Midas cichlids upregulated mid- and short-wavelength-sensitive opsin genes and downregulated long-wavelength sensitive ones, resulting in retinas with proportionally more photoreceptors sensitive at shorter wavelengths (Torres-Dowdall et al. 2017b). Finally, Midas cichlids from the crater lakes were also found to have short-wavelength shifted sensitivities compared to fish from the great lakes due to changes in chromophore usage (Torres-Dowdall et al. 2017b). Midas cichlids from the great lakes use predominantly A2 derived, but in the crater lakes most fish change chromophore usage to mainly A1 derived, effectively shifting visual pigment sensitivity toward shorter wavelengths. Thus, all these mechanisms contributed to tuning visual sensitivity toward shorter wavelengths, which are considered adaptive to the light environment of the crater lakes (Torres-Dowdall et al. 2017b). These changes are likely to be heritable to some extent, as the differences seen in nature between the fish from the crater lakes and their source population from the great lakes were maintained when fish were reared under common garden conditions in the laboratory. Interestingly, six other cichlid species that also colonized crater lakes have a short-wavelength shifted visual sensitivity compared to the ancestral populations from the great lakes, but the underlying molecular mechanisms are not exactly the same as those seen in Midas cichlids (Härer et al. 2018). These differences were mainly due to differences in the pattern of opsin expression in the crater lakes. While all species shifted sensitivities toward short wavelengths, only *Amatitlania siquia* retained a juvenile pattern of opsin expression in adults after colonizing the crater lakes, as Midas cichlids from crater Lake Apoyo did. Unlike Midas cichlids, four species changed the expression of green sensitive opsins. Nonetheless, in all cases, it resulted in a short-wavelength shifted visual sensitivity (Härer et al. 2018).

One question that remains is, how are the observed differences during ontogeny produced? The ontogenetic shift seen in visual sensitivities when comparing the derived Midas cichlid species from the crater lakes to their source populations in the great lakes may indicate differences in the thyroid metabolism of the retina of these fish. Thyroid hormone (TH) has been shown to play an important role in the developmental progression from short- to long-wavelength shifted visual sensitivities (Ng et al. 2001; Cheng et al. 2009; Novales Flamarique 2013). Comparisons of circulating levels of TH during development, indirectly measured using deiodinases (genes that are involved in TH metabolism) as proxies, between *A. citrinellus* from Lake Nicaragua and *A. astorquii* from crater Lake Apoyo have shown striking differences with *A. citrinellus* having higher levels of TH (Härer et al. 2017b). Moreover, this effect was validated by rearing Midas cichlids in water supplemented with TH, finding that the progression in opsin gene expression got accelerated (Härer et al. 2017b), as it occurs in other vertebrates (Roberts et al. 2006; Cheng et al. 2009). Disruption of the TH metabolism, by rearing Midas cichlids in complete darkness, also resulted in elevated levels of TH and acceleration of developmental progression from short- to long-wavelength shifted sensitivities (Karagic et al. 2018). Ongoing

experiments are aiming to determine if the retina regulates TH metabolism independently of the rest of the body, which will decouple the effect of TH on eye development from overall development.

## 4 Sympatric Speciation: Midas Cichlids' Intralacustrine Radiations

The likelihood of the evolution of reproductive isolation without barriers to gene flow (i.e., sympatric speciation) and how commonly it occurs in nature remains one of the most debated topics in evolutionary biology (Mayr 1963; Coyne and Orr 2004; Bolnick and Fitzpatrick 2007; Bird et al. 2012; Meyer and Kautt 2014). The idea of species evolving in sympatry through adaptation to different ecological niches is already present in Darwin's "On the Origin of Species" (1859), and it was for some time considered to being the most common mode of speciation (Mayr 1963; Coyne and Orr 2004). The idea that natural selection could have played a role in the process of speciation without geographic isolation was criticized by the proponents of the Modern Synthesis, especially by Ernst Mayr (reviewed in Bird et al. 2012). Following the publication of Mayr's influential book *Animal species and evolution* (Mayr 1963), most biologists considered allopatric speciation the most common mode of cladogenesis, and sympatric speciation was disregarded as unlikely or as an impossible process. However, the interest in sympatric speciation revitalized during the last 30 years, as theory showing that it is possible was produced and more credible empirical examples accumulated (Coyne and Orr 2004; Bolnick and Fitzpatrick 2007; Bird et al. 2012; Meyer and Kautt 2014).

However, verifying putative cases of sympatric speciation remains challenging because the process of speciation is traditionally thought to occur over long time scales. Thus, inferences about the probability of this event occurring in sympatry have to be drawn from indirect evidence, and most often alternative explanations cannot be completely discarded (Meyer and Kautt 2014). With the goal of guiding research, Coyne and Orr (2004) proposed four criteria that need to be satisfied for validating sympatric speciation as the most likely mode of speciation: (a) the considered species have a sympatric distribution; (b) there should be genetically based reproductive isolation; (c) there is a phylogenetic sister relationship between the species; and (d) there was no historic phase of geographic isolation. Very few cases satisfied these four criteria, and none of them does so without criticism (Bolnick and Fitzpatrick 2007; Meyer and Kautt 2014). One of the few accepted cases are the small adaptive radiations of Midas cichlids in Nicaraguan crater lakes (Barluenga et al. 2006). In the following, we examine the available evidence supporting this claim for the radiations in crater Lakes Apoyo and Xiloá (associated with body shape changes); then, we discuss the case of fish with thin and hypertrophied lips from two crater lakes and the great lakes; and finally, we argue for a role of pharyngeal jaws in this process.

#### **4.1 Rapid and Parallel Intralacustrine Speciation Along the Benthic-Limnetic Axis**

Midas cichlids are perhaps most well-known for sympatric speciation and parallel evolution of benthic and limnetic species within crater Lakes Apoyo and Xiloá (Barluenga et al. 2006; Elmer et al. 2010a, 2014; Kautt et al. 2012, 2016a). The limnetic species from crater Lake Apoyo, *A. zalius*, was the first species to be described from the crater lakes within the Midas cichlids species complex (Barlow and Munsey 1976). Since then, there have been many attempts to reconstruct the evolutionary history of this species and, in particular, to find out if it evolved in the absence of geographic barriers to gene flow. A case for sympatric speciation was first presented by Wilson et al. (2000) and later by Barluenga and Meyer (2004) and Barluenga et al. (2006). These studies used mitochondrial, microsatellite, and *AFLP* markers and concluded that crater Lake Apoyo Midas cichlids constitute a monophyletic clade; and therefore, *A. zalius* diverged in sympatry from *A. cf. citrinellus* from the same crater lake. At the time the other species from the lake had not been formally described yet. This interpretation was rapidly challenged, arguing that the results were also compatible with the alternative explanation of divergence in allopatry followed by a second colonization event of the lake by a second species (Schliewen et al. 2006). Subsequent work using different molecular markers have found support for the monophyly of Apoyo Midas cichlids (Geiger et al. 2010a; Kautt et al. 2012, 2016a, 2018, 2020; Elmer et al. 2014). The species from Lake Xiloá have received less attention, partly because the barrier isolating it from Lake Managua is small as the crater rim has eroded and recent oscillations in the water level of both lakes might have connected them. Thus, it has been assumed that several of these events must have occurred in the past contributing to the diversity of Midas cichlid of Xiloá. However, it was also concluded that the species within this lake constitute a monophyletic group (Kautt et al. 2012, 2016a, 2018, 2020; Elmer et al. 2014). Recently, Kautt et al. (2016a) pointed out that validating the fourth criteria proposed by Coyne and Orr (2004; no historic phase of geographic isolation) is more challenging than demonstrating monophyly, as secondary contact and introgressive hybridization might result in similar patterns (e.g., Martin et al. 2015). Using demographic models based on a comprehensive genome-wide dataset, they concluded that speciation within crater Lakes Apoyo and Xiloá occurred in sympatry and found only little evidence that secondary contact of partially divergent genetic pools was involved in the process (Kautt et al. 2016a). Thus, taking all evidence into account and considering the different methodologies used, there is strong support for the evolution of multiple Midas cichlid species in the absence of barriers to gene flow within the crater Lakes Apoyo and Xiloá, providing a very valuable, replicated system to study speciation with gene flow.

Three additional factors make the intralacustrine radiation in crater Lakes Apoyo and Xiloá truly remarkable. One is that the evolution of a limnetic species occurred in parallel in Apoyo and Xiloá (Elmer et al. 2010a, 2014). The level of parallelism is notable, with multiple traits varying in the same direction, including pharyngeal jaw,



body shape, and trophic niche (inferred from stable isotopes; Elmer et al. 2014). The most impressive parallelism is in body shape, where the angles of morphological divergence of *A. zaliosus* and *A. sagittae* from their respective source populations are almost identical (Kautt et al. 2018). The second factor is the speed at which these radiations occurred. Demographic models using genome-wide data suggest that the crater Lakes Apoyo and Xiloá were colonized less than 2000 generations ago and that the split among species occurred less than 900 generations ago (Kautt et al. 2016a). Finally, even though most of the attention has been paid to the divergence between benthic and limnetic species, at least four and three species that could be characterized as benthic have evolved within Apoyo and Xiloá, respectively (Stauffer and McKaye 2002; Stauffer et al. 2008; Geiger et al. 2010b; Kautt et al. 2016a). All these species appear to have originated within a very short period of time (on the order of a few hundred generations), thus, being better represented by a polytomy than dichotomous branching of species (Kautt et al. 2016a). Very little is known about the axes of variation within the benthic species in each of the lakes. However, comparative analyses of trophic niches (considering stable isotope signatures and gut microbiomes) are starting to clarify this issue (Franchini et al. 2014a; Härer et al. 2020). Thus, the extremely young Midas cichlid radiations present the opportunity to ask what are the conditions that promote and facilitate sympatric speciation.

Gavrilets et al. (2007) pointed out that speciation in crater lakes is theoretically possible if four conditions are met: (i) the strength of selection for local adaptation is intermediate; (ii) the traits controlling local adaptation are determined by few loci of major effect; (iii) carrying capacity of the crater lakes is intermediate; and (iv) the loci controlling for nonrandom mating have a strong effect. Unfortunately, measuring the strength of selection in nature has been challenging in the Midas cichlid lineage, so there is no direct support for the first condition. There have been a series of studies that allowed us to determine the plausibility of the other conditions. QTL analyses of a hybrid cross between the limnetic species (*A. zaliosus*), and one of the benthic species (*A. astorquii*) from Lake Apoyo found a few genomic regions associated with body shape (Franchini et al. 2014b, 2016a). Nonetheless, a large percentage of the body shape variation in the F2s remained unexplained with 12%–20% of the variation being explained by all the significant QTLs. Moreover, machine learning-based analyses on re-sequenced genomes of all species from crater Lakes Apoyo and Xiloá indicate the presence of numerous selection sweeps across the genome, suggesting a polygenic basis for trait divergence (Kautt et al. 2020). Thus, there is not much support for *condition ii*. Demographic models estimate much smaller population sizes for crater lake populations than those estimated for the great lakes, suggesting lower carrying capacity (Kautt et al. 2016a, b). Moreover, these models suggest that the process of disrupting selection did not start immediately after colonization, but that population size increased first, probably reaching carrying capacity for fish resembling the ancestral morph, and then Midas cichlids diversified within crater lakes (Kautt et al. 2016a, b, 2018). Thus, there is at least some support for *condition iii*. Finally, there is conflicting evidence for *condition iv*. Support for strong assortative mating comes from field surveys of Midas cichlid breeding

couples (Stauffer et al. 2008; Elmer et al. 2009) and mate choice experiments conducted under laboratory conditions (Kautt et al. 2020), where in both cases, fish mate strongly assortatively based on species identity. However, no assortative mating based on morphology was observed when females from one of the two crater lakes where the limnetic morph evolved were offered males of both morphs from the other lake. For example, Xiloá females of a benthic and a limnetic species were tested in community tanks with Apoyo males of a benthic and a limnetic species. Thus, the effect of the loci controlling nonrandom mating might be strong, but the mating cue is not necessarily associated with morphology.

A recent study using genomic markers identified a few wild-caught specimens from crater Lake Xiloá that appear to be hybrids between the limnetic species *A. sagittae* and its most closely related species, the benthic *A. xiloaensis* (Kautt et al. 2016a). This might suggest that reproductive isolation between the two young species is incomplete. Notably,  $F_{st}$  values between them are relatively high ( $F_{st} = 0.228$ ;  $P < 1 \times 10^{-14}$ ; Kautt et al. 2016a), suggesting that gene flow albeit being low does occur between these species. This finding is puzzling. An exciting potential explanation is that the putative hybrids are in the process of becoming a new, independent population occupying a distinct ecological niche and speciation is ongoing. This is supported by the finding of divergent morphology and significant  $F_{st}$  between the hybrids and the parental species (hybrids, *A. sagittae*:  $F_{st} = 0.109$ ; hybrids, *A. xiloaensis*:  $F_{st} = 0.129$ ; both  $P < 1 \times 10^{-14}$ ; Kautt et al. 2016a). Further ecological and genomic work will be required to validate this hypothesis.

In any case, the conditions proposed by Gavrillets et al. (2007) are barely met or not met at all in the small radiation of crater Lakes Apoyo and Xiloá. Yet, sympatric speciation did occur in these two crater lakes. Moreover, even when all the conditions were assumed to be true, in many cases the simulations did not result in sympatric speciation, and when it occurred, the time to divergence was relatively long (e.g., around 20,000 years). These results are not very compatible with our current findings on the system, where radiation occurred two independent times (out of six crater lakes) and relatively rapid (around 2000 generations) (Kautt et al. 2016a). Interestingly, a different model predicts a scenario that is highly similar to what is observed in the Midas cichlids from the crater lakes (Bolnick 2006). In particular, this model predicts the evolution of multiple species almost simultaneously and in a very short time span, resulting in hard polytomies. These predictions are strikingly similar to the results of demographic models in Midas cichlids (Kautt et al. 2016a). However, the model has a series of assumptions that were criticized on the base of their biological realism (e.g., Gavrillets 2004, 2005). One such assumption is that the trait of ecological relevance also plays a role in mate choice (e.g., magic trait). This could hold true for hypertrophied lips (e.g., Machado-Schiaffino et al. 2017; see Sect. 4.2), but it does not appear to be the case for body shape (see earlier). Another criticized assumption is that the ecological trait distribution is smaller than the resource distribution. This is possibly the case in the Midas cichlids, as the crater lakes differ significantly from the great lakes that served as source populations. New resources, not previously encountered in the great lakes, could have been available for fish after the colonization of the crater lakes. Still

another assumption, not yet studied in the Midas cichlid complex, is that females can sample at low cost a large number of males before mating. Violating this assumption still can result in sympatric speciation, unless the cost is really high (Bolnick 2006). Midas cichlids are currently very dense in the crater lakes they inhabit (Dittmann et al. 2012), so females could potentially be very choosy without paying a very high cost. However, current densities not necessary reflect those at the period when reproductive isolation evolved (Kautt et al. 2018). Thus, further exploring if the conditions of the model are satisfied in the Midas cichlid system is of major importance.

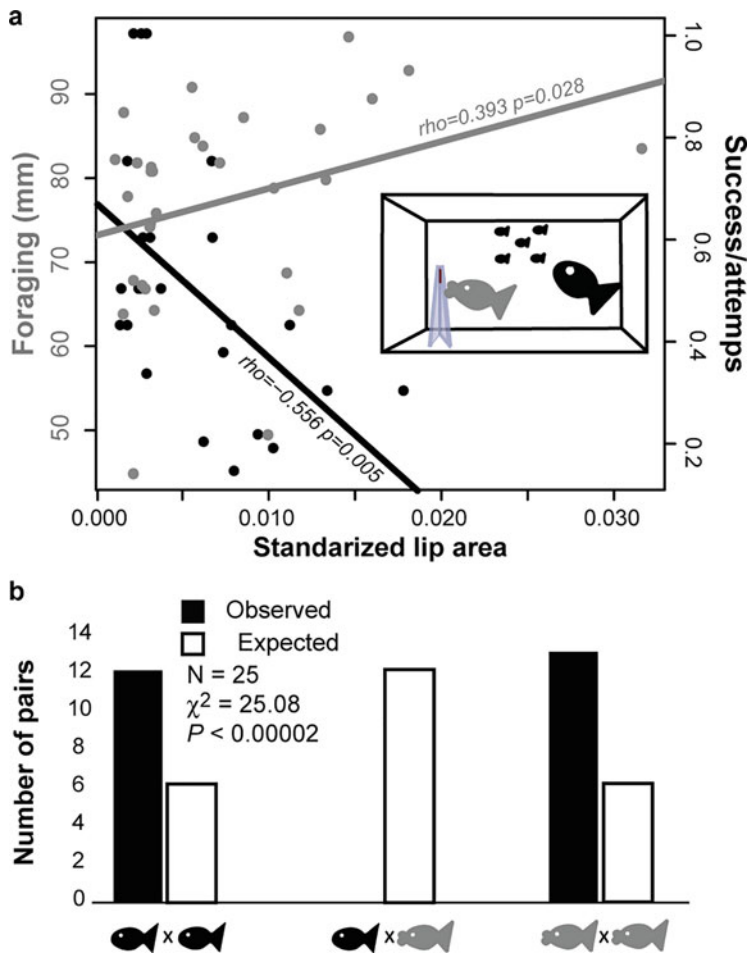
Incipient speciation along the limnetic–benthic axis might also be occurring in a third crater lake, Asososca Managua (Kusche et al. 2014; Kautt et al. 2016b). The Midas cichlid species in this crater lake (*A. tolteca*) satisfies some of the conditions necessary, although not sufficient, for sympatric speciation to occur (Nosil 2012). This species is highly variable in terms of ecomorphological traits, with some individuals having a limnetic-like and others a benthic-like body shape (Kusche et al. 2014; Kautt et al. 2016b). Importantly, this variation was associated with habitat use—limnetic-shaped fish were more common in the middle of the lake—and with trophic level—limnetic-shaped fish have a higher trophic level (Kautt et al. 2016b). Phenotypic plasticity does not seem to be the cause of this variation, as a split brood experiment rearing fish under different levels of water flow had no effect on body elongation (Kautt et al. 2016b). However, intermediate phenotypes are common in the lake, rendering a continuous, rather than a bimodal trait distribution. Moreover, the ecomorphological divergence was not associated with genetic divergence. This lack of genetic structuring was found in different studies using different molecular markers, all of them not finding any evidence for more than one genetic cluster in crater Lake Asososca Managua (Barluenga and Meyer 2010; Kusche et al. 2014; Kautt et al. 2016b, 2020). Hence, it seems possible that the processes of disruptive selection and assortative mating have been at work in *A. tolteca*, but that genetic differentiation has not built up at neutral markers across the genome yet. This is possible; neutral genetic differentiation may not be expected in the earliest stages of divergence (Elmer et al. 2010a; Colborne et al. 2016). Alternatively, the strengths of assortative mating and disruptive selection are not strong enough, and therefore, the population is stalled in its divergence (e.g., Matessi et al. 2001; Bolnick 2006). In any case, the Midas cichlid species complex presents three replicated cases of divergence along a benthic–limnetic axis, which are at different evolutionary stages due to the different history of the three crater lakes.

## 4.2 *Midas Cichlids with Hypertrophied Lips*

Hypertrophied lips have repeatedly evolved in the cichlid lineage, providing a striking case of convergent evolution (Kocher et al. 1993; Oliver and Arnegard 2010; Colombo et al. 2013; Manousaki et al. 2013; Burress 2014; Henning and Meyer 2014; Henning et al. 2017; Machado-Schiaffino et al. 2017). Species with

hypertrophied lips (also referred as thick-lipped species) can be found in the three African great lakes, among South American riverine cichlids of the genera *Crenicichla* and *Gymnogeophagus*, and in the Midas cichlid species complex. Only one species with hypertrophied lips has been formally described within the Midas cichlid complex, *Amphilophus labiatus*, which is found in the two great lakes, Managua and Nicaragua. But Midas cichlids with thick lips are also present in two crater lakes, Apoyeque and Masaya, and a few specimens were observed in crater Lake Xiloá (personal observation). Unlike *A. citrinellus*, *A. labiatus* is not found in the rivers connected to the lake nor in any other drainage where Midas cichlids are found. Thus, the current hypothesis is that lips evolved within the great lakes, although it is not clear if the trait is associated with population structure or if it evolved in the absence of extrinsic barriers to gene flow. There is evidence that in the great lakes, the genome of the two lineages became divergent enough to be called different species quite recently (Kautt et al. 2020). Whole-genome comparisons between *A. citrinellus* and *A. labiatus* suggest that there were high levels of gene flow between the two lineages until around ten thousand years ago (assuming 1.5 years generation time and mutation rates previously published for cichlids). Thus, these two species appear to have evolved in sympatry within the great lakes. There is compelling evidence that ecological divergence might have played a role, which combined with assortative mating, raises the interesting possibility that hypertrophied lips worked as a magic trait, affecting both, ecological performance and mate choice (Machado-Schiaffino et al. 2017).

Hypertrophied- and thin-lipped fish differ in their trophic niches (Elmer et al. 2010b; Colombo et al. 2013; Manousaki et al. 2013; Machado-Schiaffino et al. 2017). Hypertrophied lips are thought to help fish suck mollusks and arthropods from crevices among rocks, which is supported by the finding that fish with thick lips are more effective than thin-lipped fish in removing food items from structures resembling crevices (Baumgarten et al. 2015; Machado-Schiaffino et al. 2017). Moreover, *A. labiatus* fish that are forced to feed from crevice-like structures show adaptive plasticity, developing larger lips than individuals that feed from soft surfaces (Machado-Schiaffino et al. 2014). However, this trades off with the ability to capture evasive prey from the water column, a task in which thin-lipped fish are significantly more efficient (Fig. 6a; Machado-Schiaffino et al. 2017). Furthermore, gut content analyses (Elmer et al. 2010b; Colombo et al. 2013) show that the diet of thick-lipped Midas cichlids consists mainly of arthropods and hard-shelled invertebrates, whereas remains of fish and other evasive prey are the main items found in the guts of thin-lipped Midas cichlids from the same lake. This ecological divergence is also evidenced in stable isotope analyses finding that thick-lipped fish have a lower trophic level than thin-lipped Midas cichlids (Manousaki et al. 2013). The ecological divergence has been tested under natural conditions, where thick- and thin-lipped fish were kept in field enclosures associated with rocky or sandy habitats. As expected, thick-lipped Midas cichlids outperformed thin-lipped fish in the rocky environment, although no difference was found in the sandy habitat (Machado-Schiaffino et al. 2017). All these studies have provided strong evidence that there is ecological divergence between Midas cichlids with and without hypertrophied



**Fig. 6** (a) Thick- and thin-lipped Midas cichlids trade off in their ability in removing food items attached to crevices (e.g., invertebrate larvae, depicted as gray) and their success catching evasive prey (e.g., smaller fish, depicted in black). (b) Thick- and thin-lipped fish mate assortative, with no disassortative couples seen in laboratory crosses (modified from Machado-Schiaffino et al. 2017)

lips. Also, fish with intermediate phenotypes were shown to perform worse than the two extreme ones; strongly suggesting that hybrids would have lower fitness than either parental species, potentially resulting in extrinsic postzygotic isolation. Hence, natural selection is expected to have favored individuals that mate assortatively, facilitating the evolution of reproductive isolation.

There is strong evidence for premating reproductive barriers associated with lip morphology. A field survey in crater Lake Apoyeque found strong departure from random mating between thick- and thin-lipped Midas cichlids (Fig. 6b; Machado-Schiaffino et al. 2017). The number of disassortative couples found was significantly lower than the number expected given the relative abundance of both morphs. When



this experiment was repeated under laboratory conditions and using Midas cichlids from the great lakes, not a single disassortative couple was formed (Machado-Schiaffino et al. 2017). Thus, it is clear that mating is not random; however, it also suggests a cost for choosiness given that disassortative couples were seen in the field but not in the lab, although this could be due to the lake of origin of the species used in each experiment. It is also likely that intrinsic postzygotic isolation is evolving among these species, as a large number of malformed specimens were recovered from forced crosses between them (A.M. *unpublished data*).

The presence of hypertrophied lips gives the impression of similarity among *A. cf. labiatus* fish from different lakes. In fact, overall head shape shows parallel changes when comparing thick- and thin-lipped fish from the different lakes (Manousaki et al. 2013; A.M. *unpublished data*). However, overall body morphology is more similar within lakes than within morphs. This means that in general there is one axis of morphological divergence that is better explained by lake of origin, and a second axis that is better explained by lip morph. Thus, it remains unclear if hypertrophied lips evolved only once or multiple times in the Midas cichlid species complex. The most parsimonious would be to assume that it evolved once and then it spread into the two great lakes and crater lakes Apoyeque and Masaya, and several lines of data support this. Analyses of *AFLP* data found no differentiation between *A. labiatus* and *A. citrinellus* in Lake Nicaragua, suggesting that there are only islands of differentiation or simply that these two described species represent a single genetic pool (Kautt et al. 2012). RADseq data and data from re-sequenced genomes show that thick- and thin-lipped Midas cichlids genetically cluster by lake rather than by morph, but both datasets identify the two species as independent genetic clusters within the two great lakes (i.e.,  $F_{st}$  values are lower between morphs within lakes than across lakes within morphs; Machado-Schiaffino et al. 2017; Kautt et al. 2020). Nonetheless, genome-wide differentiation between morphs is the highest in the older great lakes but the lowest in the youngest crater Lake Apoyeque (Machado-Schiaffino et al. 2017; Kautt et al. 2020). This pattern of genomic differentiation across lakes agrees with a single origin of hypertrophied lips if gene flow is assumed to have occurred between the thick- and thin-lipped species of Midas cichlids, and this trait has a simple genetic architecture.

As mate choice experiments showed (Machado-Schiaffino et al. 2017), thick- and thin-lipped fish mate mostly assortatively. However, a very low number of disassortative couples are still observed in nature (at least in crater Lake Apoyeque) allowing for gene flow between species. Moreover, an increased cost of being choosy during mating could be expected when population densities are low, as might have occurred immediately after the colonization of the crater lakes (Machado-Schiaffino et al. 2017). These conditions would promote higher levels of introgression, which are expected to result in patterns of genetic differentiation similar to the ones observed (e.g., more divergence among lakes than between morphs and lower divergence in crater lakes).

Studies focusing on the genetic architecture of the trait also point to a single origin of hypertrophied lips. Crosses between thick- and thin-lipped species of Midas cichlids strongly suggest that the hypertrophied lip phenotype is controlled mainly

by one locus of major effect (although other loci of lower effect might be involved), different from what was found in African cichlid thick-lipped species (Henning et al. 2017). New analyses based on whole-genome resequencing on a large number of wild-caught specimens provide additional support to this conclusion (Kautt et al. 2020). In the otherwise flat landscape of genomic differentiation between Midas cichlids with different lip morphology within each of the lakes they inhabit, there are a few islands of high genomic differentiation, with one shared peak across all lakes. This peak coincides with a region of genomic association with lip morphology identified by a genome-wide association analysis. The fish with hypertrophied lips from the different lakes share a putatively causal haplotype in this region of differentiation that shows signatures of a selective sweep (Kautt et al. 2020).

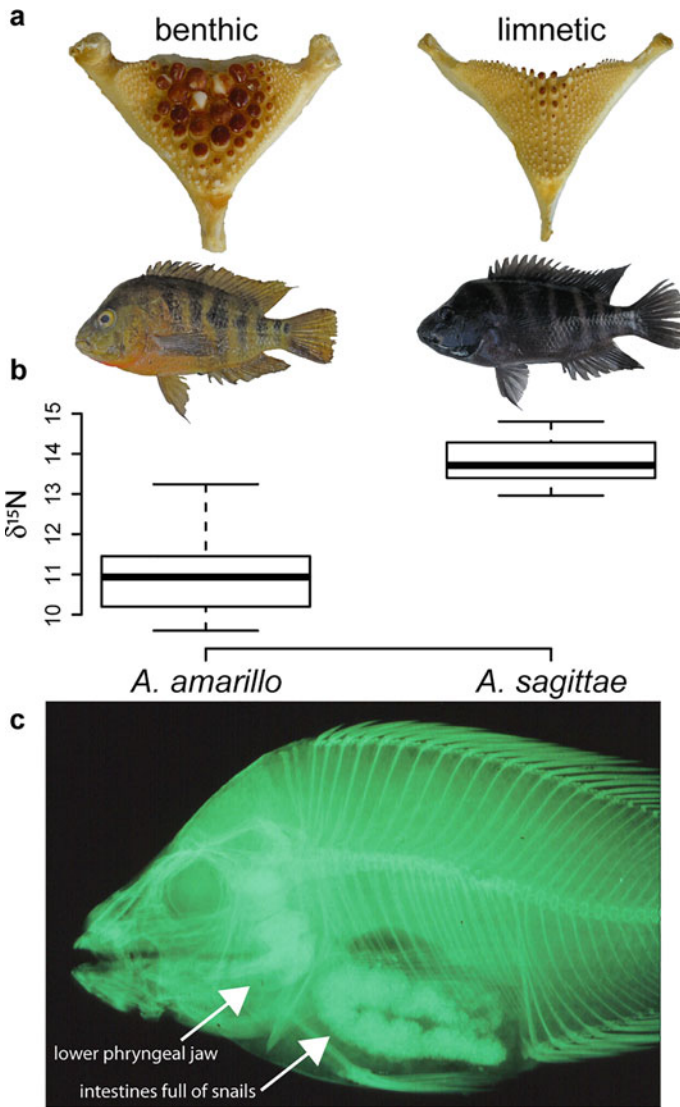
Current work is focused on reducing the genomic interval associated with hypertrophied lips, and most likely, the functional locus responsible for this phenotype will be found soon. Two inward rectifier potassium channel genes are located in the proximity of the region of genomic differentiation between morphs, and these might play a role in tissue swelling in fish with hypertrophied lips (Kautt et al. 2020), but further studies are required to determine if they play a role at all and what is their specific function. Identifying the causal locus will allow us to determine if the exact same mutation is responsible for hypertrophied lips in Midas cichlids across all the lakes; or if different mutations or even different loci are the underlying molecular cause (Elmer and Meyer 2011). Finding that all thick-lipped Midas cichlids share the same mutation would provide support to the hypothesis of a single speciation event, most likely in sympatry within one of the great lakes, followed by (1) colonization of other lakes by *A. cf. labiatus*, most likely at the same time as *A. cf. citrinellus*, and (2) some introgression occurring with *A. cf. citrinellus*. If this were the case, then the most plausible scenario is that few individuals of each species colonized the crater lakes after they became habitable. There, due to low population density and increased cost of finding mates, assortative mating broke down and gene flow increased between these species homogenizing their genomes. Divergent selection favored the maintenance of both morphotypes evidenced in the signatures of selection seen in the small region of genomic differentiation between morphs (Kautt et al. 2020). Whether the crater lake populations are currently diverging again or stalled in their current state as a stable polymorphism is a very interesting question that certainly would be a topic of future research in the system (Machado-Schiaffino et al. 2017). Nonetheless, it is notable that even though most of the conditions proposed by Gavrillets et al. (2007; see detail earlier) as favorable for sympatric speciation to occur are met in this system, particularly the oligogenic basis of the trait and the strong assortative mating, genome-wide differentiation associated with lip morphology is very low, contrary to what is seen in the small adaptive radiations of crater Lakes Apoyo and Xiloá where divergent traits are highly polygenic (Kautt et al. 2020).

#### 4.3 *The Pharyngeal Jaws and Their Role in Midas Cichlid Sympatric Speciation*

Pharyngeal jaws have long been suggested to play a pivotal role in the spectacular evolutionary success of cichlids (Liem 1973). This is because this second set of jaws allowed the decoupling of prey capturing and processing during feeding. In cichlid fish, the oral jaw is highly specialized for prey capturing potentially due to the effectiveness of the pharyngeal jaw in prey processing (Liem 1973; Hulsey et al. 2006). This functional modularity allows cichlids to exploit different niches and potentially adapt to new niches more rapidly than other fish can. The high diversity in the structure and mechanics of the pharyngeal jaws reflects this. For example, Central American cichlids show several transitions between robust pharyngeal jaws having few, large teeth (i.e., molariform) and more slender pharyngeal jaws having many small pointy teeth (i.e., papilliform) (Fig. 7; Hulsey 2006). The Midas cichlid adaptive radiation is not an exception with regard to the importance of the pharyngeal jaws. Many of the main axes of divergence seen across Midas cichlid species are associated with divergence in the pharyngeal jaws (Meyer 1990a, b; Barluenga et al. 2006; Manousaki et al. 2013; Elmer et al. 2014).

There is a clear relationship between diet and the lower pharyngeal jaw (LPJ) morphology. Both major types of LPJ, molariform and papilliform, are commonly found in Midas cichlid species. Molariform LPJs are more efficient in crushing hard-shelled prey, such as snails (Hulsey 2006). However, this advantage trades off with the ability to process soft prey for which papilliform LPJs are most efficient (Meyer 1989). For example, in crater Lake Xiloá, more than 90% of Midas cichlids with molariform LPJ are found to have eaten snails; whereas fewer than 20% of fish with papilliform LPJ were found to have snails in their stomach (Meyer 1990a). Moreover, the proportion of Midas cichlids with molariform LPJ is strongly associated with the abundance of snails in a lake. In crater Lake Masaya, snails are uncommon or absent and almost all Midas cichlids have papilliform LPJs, whereas in crater Lake Xiloá, where snails are abundant, up to 50% of the fish have a molariform LPJs (Fig. 7; Meyer 1990a). Although some of the variation in this trait is heritable, LPJ morphology is highly plastic in response to food hardness (Muschick et al. 2011).

The LPJ appears to have played a very important role in the process of sympatric divergence of Midas cichlids, both between thin- and thick-lipped fish as well as between benthic and limnetic fish. In the lakes where they coexist, thin- and thick-lipped fish differ significantly in LPJ morphology (Elmer et al. 2010b; Manousaki et al. 2013). It would be expected that thick-lipped fish commonly forage in rocky habitats and consume a large proportion of hard-shelled invertebrates (e.g., snails and arthropods; see Sect. 4.2). However, the opposite pattern is found in most lakes. The strongest differences are seen in fish from Lake Nicaragua, where *A. labiatus* (thick-lipped) have more papilliform LPJ than *A. citrinellus* (thin-lipped) (Manousaki et al. 2013). In other lakes, the data are inconclusive. In crater Lake Apoyeque, Elmer et al. (2010b) found thick-lipped fish to have more papilliform LPJs than thin-lipped fish, but the opposite pattern was found by Manousaki et al.



**Fig. 7** (a) Benthic (*A. amarillo*) and limnetic (*A. sagittae*) species from crater Lake Xiloá differ significantly in the morphology of their lower pharyngeal jaw (LPJ), with the former having molariform LPJ and the later a papilliform LPJ. (b) This is associated with differences in their diet as indicated by significant differences in the ratios of stable isotopes of nitrogen. (c) The lower trophic level seen in the benthic species is associated with a higher consumption of snails. X-ray photo of an *A. amarillo* specimen with its intestines full of fragmented pieces of snail shells

(2013). In crater Lake Masaya, no fish was found to have a clear molariform LPJ, but thick-lipped fish tend to have more robust LPJs than thin-lipped ones (Manousaki et al. 2013). Thus, difference in LPJ morphology has apparently contributed to

divergence between thick- and thin-lipped Midas cichlids, but not necessarily in a parallel manner across lakes (Manousaki et al. 2013).

LPJ morphology has evolved in parallel along the benthic–limnetic axis in crater Lakes Apoyo and Xiloá. The limnetic species of these two lakes (*A. zaliosus* and *A. sagittae* from Apoyo and Xiloá, respectively) have more gracile LPJs carrying many pointy teeth than the coexisting benthic species (Fig. 7; Barluenga et al. 2006; Elmer et al. 2014). The papilliform LPJs are expected to increase handling efficiency of live prey (Hulsey et al. 2005), in agreement with the higher proportion of evasive prey found in the limnetic species diet (Elmer et al. 2014). Analyses of a hybrid cross between the limnetic and a benthic species from crater Lake Apoyo revealed that the major *QTLs* for LPJ shape and for body shape co-localize. Thus, the integrated genetic architecture of these two traits might have played a fundamental role promoting rapid adaptive divergence in sympatry (Fruciano et al. 2016a).

Besides the work by Fruciano et al. (2016a), little has been done to determine the genetic architecture of LPJ morphology. The main reason for this is that the LPJ is a complex structure, and several different components have functional importance and need to be considered (e.g., pharyngeal jaw shape and weight, tooth size and number, and suture structure; Meyer 1990b). Even though Fruciano et al. (2016a) found a *QTL* for LPJ shape, no *QTL* was found for LPJ size. Other traits were not analyzed for this particular cross. Analyses of a hybrid cross between *A. labiatus* and *A. citrinellus* suggest that there are five small-effect *QTLs* for tooth size, but no significant ones for tooth number (Kautt et al. 2020). Analyses of hybrid crosses in African cichlids, on the other hand, did find some *QTLs* for tooth number, both in the pharyngeal and oral jaws (Hulsey et al. 2017). Although these two jaws are functionally and mechanically independent (Hulsey et al. 2006), they might not be genetically independent. The number of teeth in the pharyngeal and oral jaws are correlated; and the major *QTL* for these two traits are co-localized (Hulsey et al. 2017). It is still unclear if the same pattern is seen in Midas cichlid fish, but currently there is work being conducted in this area and soon these questions will be clarified. Thus, the Midas system could provide an ideal group of species to link genotype to phenotype in the pharyngeal jaws of cichlids.

## 5 The Intriguing Case of Color Polymorphism: Stable Polymorphism or In Vivo Speciation?

One of the most intriguing and interesting phenotypic variations in the Midas cichlid species complex is its polymorphism in coloration, with melanic and amelanistic, “gold” morphs. The gold morph can be found in almost all Midas cichlid populations, except for the species from crater lakes Tiscapa and Apoyo, where only a few gold specimens have been spotted, and two of the four species of Lake Xiloá (*A. amarillo* and *A. viridis*). When present, the gold morph constitutes a small proportion of the population, commonly between 5% and 10% of the fish, and this

value is roughly similar across all lakes and has been stable for at least the last 50 years (Barlow 1976; Torres-Dowdall et al. 2014; Kautt et al. 2020). Gold fish are born with melanophores, but at some point during development, these are lost due to apoptosis. The rest of the fish do not undergo such a significant change in coloration during ontogeny, and therefore have been commonly referred to as “normal,” in reference to its much greater frequency in nature, but “dark” or “melanic” are also commonly used. On average, fish that bear melanophores (i.e., dark pigment) are perceived as darker. However, since melanophores present motility in response to many factors, these fish can also be quite pale-gray. Whether pale grey can be consistently considered darker than orange is unclear, although it is possible when considered in terms of contrast with the environment. Of all the terms used to refer to this morph, melanic fish is technically more sound, as the difference with the gold morph is that they retain their melanophores. The gold morph of Midas cichlids has evoked the attention of researchers for a long time, and extensive work has been done to understand its evolution and ecology and its potential role in the adaptive radiation of this lineage; yet, the factors that influence the frequency of this polymorphism and its relationship to speciation are still unclear.

There is strong evidence that the gold phenotype is genetically determined. Crosses of gold and melanic fish have consistently resulted in a 3:1 gold to melanic ratio, strongly suggesting that the phenotype has a Mendelian genetic architecture, and it is controlled by a single locus with the gold allele being dominant to the melanic one (Barlow 1983; Henning et al. 2010, 2013). However, the causal locus has been evasive even though several attempts have been made to identify it. Ongoing work suggests that the same genomic region differentiates gold and melanic fish in all the populations where both morphs coexist. This region is quite small, comprising only 230 kilobases (Kautt et al. 2020). There is some evidence that this region might be a recombination hotspot, opening the possibility that different mutations within that region could underlie the gold phenotype. However, in all crosses conducted with pairs from different lakes, the same pattern of inheritance was found (e.g., 3:1 gold to melanic ratio), suggesting that it might have a common genetic basis across all populations. The gold locus is soon to be identified, which will allow us to test for the role of natural and sexual selection in the evolution and maintenance of phenotypic diversity in nature.

Irrespective of which is the causal locus for the gold phenotype, its effects are not evident in the early life of Midas cichlid fish. In fact, during the first few months of life, genetically determined gold and melanic fish are indistinguishable from each other. Changes in coloration rarely start before fish carrying the gold allele reach 60 mm of length. The process involves the death of most of the melanophores and the aggregation of some of them in particular regions of the body, in particular the fins and around the mouth area (Fig. 1b; Dickman et al. 1988). This pattern is strikingly similar to cases of vitiligo in humans. Concurrently with this process, xanthophores (yellow pigments) become dominant in the skin giving fish their characteristic orange coloration. Sometimes xanthophores only proliferate in a few parts of the body and leucophores (white pigments) become also abundant resulting in fish showing beautiful mosaic patterns (Fig. 1b). It is yet unclear what triggers the



onset of transformation, but the timing is variable among individuals. It has been even seen, on some rare occasions, that gold fish only transform after reaching sexual maturity. Some of this variation appears to be due to a dosage effect, where heterozygotes transform later than homozygous gold fish do (A.M. *unpublished data*). This delayed transformation of heterozygotes could play a role in the maintenance of this polymorphism across populations.

Several studies have shown that this polymorphism is not selectively neutral. Gold fish differ from melanic conspecifics in their diet, competitive ability, and risk of predation. There is compelling evidence suggesting that gold individuals have a competitive advantage over dark individuals (Barlow and Ballin 1976; Barlow and Wallach 1976; McKaye and Barlow 1976; Barlow 1983; Lehtonen 2014). Laboratory experiments demonstrated that when limiting foraging territories are available, juvenile gold Midas cichlids outcompete normal, melanic fish. Yet, this is not because juvenile gold fish are more aggressive, but because they get challenged less frequently by the more abundant melanic morph (Barlow and Ballin 1976). This is also apparent in natural populations, where gold fish were less likely to be attacked by another Midas cichlid than melanic fish (McKaye and Barlow 1976; Lehtonen 2014), but also intimidate normal fish that are up to 1.2 times their own size (Barlow 1983). There is evidence that between morph competition may have resulted in differences in their diet and associated trophic structures; yet, the evidence is inconclusive. Early studies suggested that pharyngeal jaws associated with consumption of evasive prey (e.g., papilliform) were more commonly found in gold individuals than melanic ones (Meyer 1990a). However, a more recent and larger study showed that gold individuals commonly have more robust pharyngeal jaws (e.g., molariform) than melanic fish in most lakes (except for fish in lakes Managua, Masaya, and Apoyeque; Kusche et al. 2015). This difference was also reflected in the trophic level estimated based on stable isotopes analysis, although variation across lakes was evident suggesting that there is no consistent pattern (Kusche et al. 2015). Curiously, there is evidence that gold fish fail to attain territories in shallow water, where breeding is most likely to be successful due to higher access to food (McKaye and Barlow 1976; McKaye 1980; but see Elmer et al. 2009). This was explained not by competitive ability, but as a mechanism to avoid predation by piscivorous birds (McKaye 1980; Barlow 1983).

When originally postulated, the competition–predation trade-off hypothesis suggested that visually oriented piscivorous birds were the main predators of Midas cichlids and that they would preferentially attack individuals of the conspicuous gold morph in shallow waters (McKaye 1980). This hypothesis could explain both, why gold Midas cichlids are more rare than individuals of the dark morph (Barlow 1983) and why gold individuals tend to breed deeper than melanic individuals, as by doing so they can escape predation by birds (McKaye and Barlow 1976). Different studies agree that color is not selectively neutral with regard to predation risk (Annett 1989; Kusche and Meyer 2014; Torres-Dowdall et al. 2014, 2017a); yet, it is not clear which of the morph is under higher risk of predation, or whether a more complex regime involving frequency dependency or interaction with environmental parameters (e.g., photic conditions) is at play. Field studies have shown that

piscivorous fish (e.g., *Parachromis managuensis* and *Gobiomorus dormitor*) are the main predators of Midas cichlids, but birds and crocodiles also prey upon them (Torres-Dowdall et al. 2014, 2017a). These studies also suggest that gold fish do not suffer higher predation risk than melanic fish do and that predation pressure does not decrease with depth. Thus, predation does not fulfill the expectations of the competition-predation trade-off hypothesis proposed to explain the maintenance of the polymorphism (e.g., McKaye 1980; Barlow 1983). Yet, the most common predator of Midas cichlids, *P. managuensis*, does indeed have a red shifted visual sensitivity (Härer et al. 2018) and, at least under laboratory conditions, it does attack gold fish more readily than melanic fish (Kusche and Meyer 2014). Thus, there is a disagreement between the tendency of Midas cichlids' main predator to attack gold fish and the pattern of predation observed in nature. A potential explanation for this is that predatory fish form search images of Midas cichlids, which will decrease the predation risk for gold fish given that they represent less than 10% of the population (Barlow 1983). Support for this hypothesis comes from the finding that small gold individuals, which are extremely rare in nature, have lower probability of a predator attack than melanic fish of the same size, but this difference disappears in large fish where the gold morph is more common (Torres-Dowdall et al. 2017a). There are still many open questions with regard to the role of predation in the maintenance of the Midas cichlid color polymorphism that demand answers. Some of these will surely be answered soon, once we identify the locus responsible for this phenotype allowing us to determine natural selection in nature.

An interesting aspect of the color polymorphism in Midas cichlids is that mating appears to be assortative (Fig. 1c, d; Barlow 1976; McKaye and Barlow 1976; McKaye 1980; Elmer et al. 2009). For example, in crater Lake Xiloá, pairs where both individuals are gold are more common (4% and 8% for *A. sagittae* and *A. xiloaensis*, respectively) than predicted given the small proportion of the population represented by gold fish (0.5% and 4%, respectively; Elmer et al. 2009). This result appears to be consistent in nature and in the lab, and across different species. Thus, given that mating is not random with regard to coloration and that color morphs are ecologically different in many regards (see earlier), this polymorphism is a prime candidate for divergence and speciation. Studies aimed to test for genetic differentiation between color morphs gave conflicting results. Microsatellite analyses concluded that there was some degree of differentiation between gold and normal fish from the great Lake Nicaragua (Wilson et al. 2000; Barluenga and Meyer 2004) and in crater Lake Xiloá between color morphs of *A. xiloaensis* (Elmer et al. 2009). However, recent analyses on a broader sampling scale (e.g., higher sample size per population and more populations) and with a much higher resolution (hundreds of markers obtained from restriction site associated DNA sequencing) did not find strong differentiation between morphs. Whole-genome resequencing data showed that there is an association between color and a very narrow region of the genome (Kautt et al. 2020). Thus, given our current knowledge, this trait is best understood as a polymorphism and probably not as a stage in incipient speciation. The fact that gold and melanic morphs are not selectively neutral and there is assortative mating suggests that there is potential for the evolution of reproductive

isolation. Yet, there is still a large number of disassortative couples that increase gene flow between the two morphs, therefore decreasing overall differentiation. For example, in Xiloá, disassortative couples represent over 5% and 23% of the total couples of *A. sagittae* and *A. xiloensis*, respectively (Elmer et al. 2009). A better understanding of selection in nature and the cost of assortative mating would be valuable to determine if this polymorphism is stable or if we are observing an onset of divergence.

## 6 Why Midas Cichlids?

The examples discussed earlier provide a general overview of the large degree of phenotypic diversity seen among Midas cichlid species complex in Nicaragua. This diversity is evident at different scales, from within population variation (i.e., polymorphisms), to morphological, physiological coupled with varying levels of genetic divergence within and among lakes. One of the most interesting open questions of the biodiversity of the Nicaraguan crater lake system is why Midas cichlids diversified and other lineages of cichlids, or any other species of fish for that matter, did not. This includes both, inter- and intralacustrine phenotypic differentiation, as other cichlid species do not differ particularly notably in ecomorphological traits among populations as Midas cichlids do (Fruciano et al. 2016b). There seems to be no easy answer to this question at this point, but some recently conducted studies can help to reject some hypotheses. One possible explanation is that Midas cichlids colonized the crater lakes prior to the other species. This priority advantage might have allowed them to occupy all empty niches, limiting the probability that other lineages also diversify (e.g., priority effects; Fukami 2015). However, Midas cichlids do not appear to have colonized the crater lakes before other species of cichlids did (Elmer et al. 2013; Franchini et al. 2017; Xiong et al. 2020). This seems to rule out a priority effect as an explanation. A second alternative is that the source populations of Midas cichlids might harbor higher levels of genetic diversity than other cichlid species providing a more “fertile” substrate for selection to act upon after the colonization of crater lakes. Recent analyses based on genome re-sequencing of around 500 individuals suggest that indeed most of the genetic diversity in the crater lakes was introduced with the original colonizations from the great lakes (Kautt et al. 2020). However, comparative demographic analysis between Midas cichlids and *Archocentrus centrarchus* from lakes where they co-occur does not support this hypothesis of greater levels of ancestral diversity (Franchini et al. 2017; Xiong et al. 2020). In fact, *A. centrarchus* from the great lakes harbor higher levels of genetic diversity than the coexisting Midas cichlids.

There are several potential explanations for the difference in diversification and speciation rates between Midas cichlids and other cichlid lineages that need to be explored. Firstly, Midas cichlids, in particular in the great lakes, are generalists consuming a high diversity of food items (e.g., Elmer et al. 2010b; Colombo et al. 2013; Manousaki et al. 2013). Other cichlid species appear to be more specialized in

their diets. For example, *P. managuensis* feed mainly on other fish, whereas *C. rostratus* feed on detritus (personal observation). Thus, Midas cichlids might be able to take advantage of new resources more easily and for disruptive selection to act upon this variation. Secondly, it is possible that mate choice is stronger or its cost lower in Midas cichlids than in other species (e.g., Bolnick 2006; Gavrillets et al. 2007). This is plausible as Midas cichlids have very large population sizes in most lakes (personal observations; Dittmann et al. 2012), which might allow choosy females to sample a large number of males at low cost. Thirdly, there could be peculiarities associated with the Midas cichlids' genome structure and transcription, such as novel microRNAs (Franchini et al. 2016; Xiong et al. 2018), that might facilitate rapid divergence in Midas cichlids (Brawand et al. 2014). This is something that we are currently exploring, but current evidence does not favor this hypothesis (Xiong et al. 2020). Finally, different levels of phenotypic plasticity across different Nicaraguan cichlid lineages might contribute to differences in their diversification rates. The role of phenotypic plasticity in adaptive evolution and speciation is highly contested. A classical view is that plasticity shields the genotype from selection (e.g., Meyer 1987; Schlichting 2004). But it has been also proposed that phenotypic plasticity might, instead, facilitate and even promote adaptive evolution and diversification, especially in the context of flexible stems (West-Eberhard 2003; Ghalambor et al. 2007; Schwander and Leimar 2011; Schneider and Meyer 2017). There are few, but interesting, cases providing evidence that phenotypic plasticity indeed might have contributed or accelerated adaptive evolution and diversification in nature (reviewed in Levis and Pfennig 2016). Midas cichlids show pronounced developmental plasticity in several traits (e.g., lower pharyngeal jaw (Muschick et al. 2011), lip size (Machado-Schiaffino et al. 2014), color vision (Härer et al. 2017b), and onset of color change (A.M. unpublished data)). But, also a lack of plasticity was found in body shape (Kautt et al. 2016b), and more experiments are needed to compare the reaction norms among ancestral and derived populations of Midas cichlids. Knowledge on phenotypic plasticity is, so far, limited in other Nicaraguan cichlids (but see Meyer 1987; Abate et al. 2010), especially with regard to comparisons between ancestral and derived populations. Variation in the ecology of Midas cichlids (e.g., generalists) and other cichlid species in Nicaraguan lakes (e.g., most are specialists) might favor different levels of phenotypic plasticity. Formal frameworks to test the role of plasticity in adaptation and diversification have been recently proposed (Levis and Pfennig 2016; Schneider and Meyer 2017). Following these guidelines might be fruitful in improving our understanding of why some cichlid lineages radiate while others do not. The Midas cichlid fishes of Nicaragua also provide one of the most interesting systems to address this important question in evolutionary biology. Although decades of research on various aspects of the biology of these fishes by several researchers already yielded many insights into fundamental problems in evolutionary biology, they still conceal numerous mysteries that are worth uncovering.

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