

The physiology of rapid ecological specialization: A look at the Midas cichlids

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Understanding the process of speciation is a primary goal of evolutionary biology, yet the question of whether speciation can reach completion in the presence of gene flow remains controversial. For more than 50 years, the cichlids of Africa, and more recently those in South and Central America, have served as model systems for the study of speciation in nature. Cichlids are distinguished by their enormous species richness, their diversity of behavioural and trophic adaptations, and their rapid rate of divergence. In both Africa and South and Central America, the repeated interaction of geology, new founder events and adaptive evolution has created a series of natural experiments with speciation occurring both within and between waterbodies of differing ages. In the “From the Cover” paper in this issue of the *Journal of Molecular Ecology*, Raffini, Schneider, Franchini, Kautt and Meyer move beyond the question of which mechanisms drive speciation, and instead show that divergent morphologies and physiologies translate into adaptive traits. They investigate differences in physiology and gene expression profiles in a benthic/limnetic species pair of Midas cichlids in a 24,000-year-old Nicaraguan crater lake. While recently diverged, these two species demonstrate significant ecological, but limited genetic differentiation. The authors find that the distinct morphotypes translate into relevant differences in swimming performance and metabolic rates that correspond to differential gene expression profiles. Hence, the authors take an integrative approach examining the impacts of morphological differences on performance and niche partitioning: an approach that can advance our understanding of the drivers of morphological and physiological divergence during speciation.

KEYWORDS

benthic, ecological speciation, gene expression, limnetic, macroevolution, swimming

Midas cichlids are a Central American species flock of the genus *Amphiophus* that inhabit a series of freshwater lakes and rivers in western Nicaragua (Figure 1). This system centres around two large lakes of tectonic origin (Lake Nicaragua and Lake Managua) that formed a single lake ~500,000 years ago, but since have been only intermittently connected by the Tipitapa River. More recent volcanic activity along the Central American Volcanic Arc has created several young and hydrologically isolated crater lakes in the region (Kutterolf, Freundt, Perez, Wehrmann, & Schmincke, 2007). The oldest of these crater lakes is the 24,000-year-old Lake Apoyo, which

serves as the study site in Raffini, Schneider, Franchini, Kautt, and Meyer (2020). It is estimated that ancestral cichlids arrived in the Nicaragua Lake system around 100,000 years ago, with the two larger and older lakes subsequently serving as the source of founding populations for the crater lakes as they formed (Barlengua & Meyer, 2010). Mitochondrial data derived from individuals from Lake Apoyo point to a single colonization event of a small number (~260; Kautt, Machado-Schiaffino, & Meyer, 2016) of benthic individuals (Barlengua & Meyer, 2004). Similar to the African cichlids, cichlids in the Nicaragua Lake system rapidly speciated in parallel

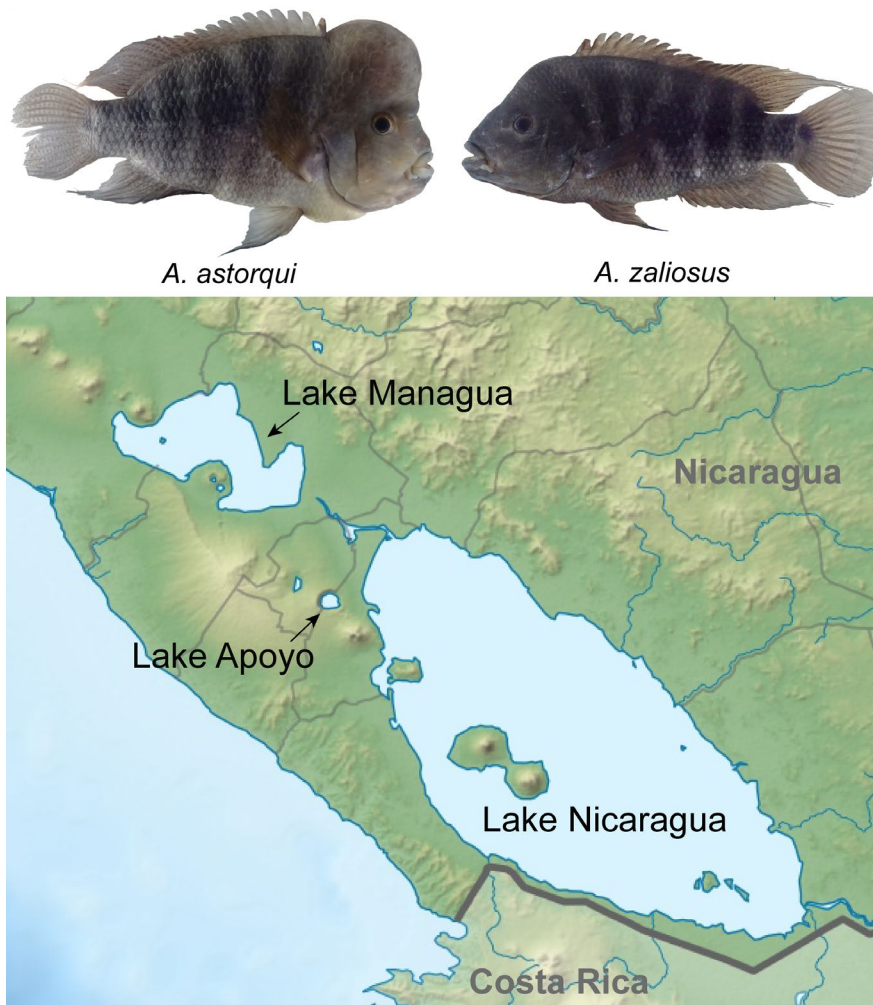


FIGURE 1 Species and area of study in Raffini et al. (2020)

within each crater lake after colonization. With an average depth of only 15 m, Lake Nicaragua does not host a limnetic morphotype. On the other hand, Lake Apoyo reaches at least 125 m and hosts six described endemic species of Midas cichlid, including five benthic and a single limnetic species. Similar divergences along benthic/limnetic morphotypes have been observed in several freshwater taxa, including three-spined sticklebacks (Schluter & McPhail, 1992), African cichlids (Hulseley, Roberts, Loh, Rupp, & Streelman, 2013), salmonids (Langeland, L'Abée-Lund, Jonsson, & Jonsson, 1991) and whitefish (Bernatchez, Chouinard, & Lu, 1999). Research shows that niche divergence along the benthic/limnetic axis alleviates competition for food resources and that a more streamlined (elongated) morphology typifies the limnetic morphotype, including *Amphiophus zaliosus* in Lake Apoyo.

Comparing individuals of a limnetic/benthic species pair from Lake Apoyo (*A. zaliosus*/*A. astorquii*; Figure 1) Raffini et al. (2020) investigate differences in physiology and gene expression profiles. Recently diverged (<2,000 generations; Kautt et al., 2016), these two species demonstrate significant ecological (Table 1; Kautt, Machado-Schiaffino, & Meyer, 2018) but very limited genetic differentiation with low but significant pairwise F_{ST} values recorded ($F_{ST} = 0.089$ based on mitochondrial DNA, Barlunga

TABLE 1 Interspecific differences previously documented for the two species (see references in Raffini et al., 2020)

Interspecific differences	
Body shape	Deep-bodied vs. streamlined
Habitat	Benthic vs. limnetic
Diet	Algae/molluscs vs. insects/fish; distinct stable isotope signatures
Microbiome	Distinct gut microbiomes
Mating	Assortative mating
Genetics	Shallow divergences but form distinct genetic clusters

& Meyer, 2010). A more recent data set based on single nucleotide polymorphisms indicates five genetic clusters among the six recognized species in Lake Apoyo, with both *A. zaliosus* and *A. astorquii* unambiguously assigned to distinct clusters (Kautt et al., 2016). Using body shape analysis, the authors confirm the distinct morphological traits of each species. They then examined how swimming ability and O_2 consumption differed between the species during swimming trials (critical/sustained swimming speed and sprinting [burst swimming]), and generated transcriptomic

profiles for individuals after sustained swimming speed and at rest. They determined that while the two species did not differ in sprinting ability or O₂ consumption at rest, the limnetic *A. zalius*, with its more streamlined shape, demonstrated greater ability for sustained swimming with higher O₂ demand compared to the benthic *A. astorquii*. Swimming ability and O₂ intake were correlated with differences in gene expression patterns in gills, and to a lesser extent in white muscle tissue. Together, the data indicate that the streamlined morphology of *A. zalius* was probably the result of selection for increased cruising performance in the open water habitat. Importantly, the streamlining greatly diminished its nuchal hump, a secondary sexual trait in male Midas cichlid fishes that is strongly preferred by females, indicating a trade-off between physiological efficiency and reproductive success.

The use of gene expression profiles to study ecological speciation has increased over the past decade and can be used to reveal the link between morphology, physiology and adaptation. In some cases, differences between conspecific populations or between recognized species correlate with processes that underlie adaptive divergence. For instance, populations of the North American lake whitefish *Coregonus clupeaformis* display two morphotypes: normal and dwarf. Using microarrays to study sympatric populations in two separate lakes in parallel, Derome, Duchesne, and Bernatchez (2006) revealed similar patterns of differential gene expression in white muscle tissue of the two morphotypes. They found significant over-expression of genes involved in metabolism and regulation of muscle contraction in dwarf whitefish in both lakes. Raffini et al. (2020) take this a step further by examining gene expression in individuals immediately after exercise. They showed significant differences in gene expression profiles of limnetic and benthic species during both rest and exercise phases, with the greatest number of affected genes in gills during exercise. Some of the differentially expressed genes they identified have been conclusively linked with vital functions in fish. In particular, during exercise, three genes (*fh11b*, *tph1a* and *cart2*) were down-regulated in the gills of limnetic *A. zalius* when compared to its benthic counterpart. Calcium uptake has been shown to be negatively correlated with the expression of *fh11b* in gills, where calcium regulation occurs in fish (Pillar et al., 2017). During hypoxic conditions, such as in the benthic environment of Lake Apoyo, *tph1a* is down-regulated. This gene mediates oxidative stress responses, including the expression of serotonin, which in turn activates branchial vasoconstriction in their gills to conserve oxygen (Rahman & Thomas, 2014). Finally, the *cart2* gene is an appetite and metabolic regulatory gene which has been reported to be up-regulated after feeding and down-regulated during fasting in many fish species (Rønnestad et al., 2017). The limnetic *A. zalius* of Lake Apoyo feed on fish and insects, as well as their larvae, which are evasive and patchily distributed. The variation in gene expression and the associated differences in O₂ demand and optimal cruising speeds documented by Raffini et al. (2020) may serve as adaptations to the specific diets and microhabitats of these two morphotypes. By demonstrating transcriptional differences, their study provides new evidence that the demonstrated physiological changes may be

heritable traits with adaptive significance toward increased feeding efficiency for each species' respective environmental and ecological role.

The integrative approach used by Raffini et al. (2020) suggests that the benthic/limnetic specializations resulting from morphological and physiological trade-offs have probably contributed to the establishment of reproductive isolation in less than 2,000 generations (Kautt et al., 2016). However, the level of morphological divergence and degree of ecological adaptation probably reflect a long list of processes driven by gene flow, environmental gradients and selection. Translating these microevolutionary patterns and processes to a macroevolutionary scale is not a trivial task. Yet, the authors make a good start, and there is an opportunity to use similar approaches in other systems to test hypotheses concerning the correlation between morphological and physiological divergence. By characterizing the physiological drivers that have been hypothesized to play a role in the divergence of these species, they have begun to uncover a potential answer as to why adaptation was able to occur in such a young and small lake.


AUTHOR CONTRIBUTIONS

All authors contributed to the writing of this manuscript.

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REFERENCES

- Barlengua, M., & Meyer, A. (2004). The Midas cichlid species complex: Incipient sympatric speciation in Nicaraguan cichlid fishes? *Molecular Ecology*, *13*, 2061–2076. <https://doi.org/10.1111/j.1365-294X.2004.02211.x>
- Barlengua, M., & Meyer, A. (2010). Phylogeography, colonization, and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evolutionary Biology*, *10*, 326. <https://doi.org/10.1186/1471-2148-10-326>
- Bernatchez, L., Chouinard, A., & Lu, G. (1999). Integrating molecular genetics and ecology in studies of adaptive radiation: Whitefish, *Coregonus* sp., as a case study. *Biological Journal of the Linnean Society*, *68*(1–2), 173–194. <https://doi.org/10.1006/bjil.1999.0337>
- Derome, N., Duchesne, P., & Bernatchez, L. (2006). Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Molecular Ecology*, *15*, 1239–1249. <https://doi.org/10.1111/j.1365-294X.2005.02968.x>
- Hulsey, C. D., Roberts, R. J., Loh, Y. H. E., Rupp, M. F., & Streelman, J. T. (2013). Lake Malawi cichlid evolution along a benthic/limnetic axis. *Ecology and Evolution*, *3*(7), 2262–2272. <https://doi.org/10.1002/ece3.633>
- Kautt, A. F., Machado-Schiaffino, G., & Meyer, A. (2016). Multispecies outcomes of sympatric speciation after admixture with the source population in two radiations of Nicaraguan crater lake cichlids. *PLoS Genetics*, *12*(2), e1006157. <https://doi.org/10.1371/journal.pgen.1006157>
- Kautt, A. F., Machado-Schiaffino, G., & Meyer, A. (2018). Lessons from a natural experiment: Allopatric morphological divergence and sympatric diversification in the Midas cichlid species complex are largely

- influenced by ecology in a deterministic way. *Evolution Letters*, 2(4), 323–340. <https://doi.org/10.1002/evl3.64>
- Kutterolf, S., Freundt, A., Perez, W., Wehrmann, H., & Schmincke, H. U. (2007). Late Pleistocene to Holocene temporal succession and magnitudes of highly-explosive volcanic eruptions in west-central Nicaragua. *Journal of Volcanology and Geothermal Research*, 163(1), 55–82. <https://doi.org/10.1016/j.jvolgeores.2007.02.006>
- Langeland, A., L'Abée-Lund, J. H., Jonsson, B., & Jonsson, N. (1991). Resource partitioning and niche shift in Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta*. *The Journal of Animal Ecology*, 60(3), 895–912. <https://doi.org/10.2307/5420>
- Pillar, N., Pleniceanu, O., Fang, M., Ziv, L., Lahav, E., Botchan, S., ... Shomron, N. (2017). A rare variant in the FHL1 gene associated with X-linked recessive hypoparathyroidism. *Human Genetics*, 136(7), 835–845. <https://doi.org/10.1007/s00439-017-1804-9>
- Raffini, F., Schneider, R. F., Franchini, P., Kautt, A. F., & Meyer, A. (2020). Diving into divergence: Differentiation in swimming performances, physiology and gene expression between locally adapted sympatric cichlid fishes. *Molecular Ecology*. <https://doi.org/10.1111/mec.15304>
- Rahman, M. S., & Thomas, P. (2014). Restoration of tryptophan hydroxylase functions and serotonin content in the Atlantic croaker hypothalamus by antioxidant treatment during hypoxic stress. *Frontiers in Neuroscience*, 8, 130. <https://doi.org/10.3389/fnins.2014.00130>
- Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jonsson, E., & Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Frontiers in Endocrinology*, 8, 73. <https://doi.org/10.3389/fendo.2017.00073>
- Schluter, D., & McPhail, J. D. (1992). Ecological character displacement and speciation in sticklebacks. *The American Naturalist*, 140(1), 85–108. <https://doi.org/10.1086/285404>

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