

# A comprehensive overview of the developmental basis and adaptive significance of a textbook polymorphism: head asymmetry in the cichlid fish *Perissodus microlepis*

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**Abstract** Identifying the evolutionary and developmental bases of adaptive phenotypes is of central interest in evolutionary biology. Cichlid fishes have been a useful research model due to their extraordinary phenotypic diversity reflecting adaptations to often very narrow niches. Among them, the scale-eating *Perissodus microlepis* is considered to be a textbook example for balanced polymorphism: its asymmetric head and handed behavior is thought to be maintained by negative frequency-dependent selection *via* prey–

predator interactions. However, several contradictory findings and open questions have emerged in recent years, challenging our understanding of this model. Here, we review existing evidence for both genetic and non-genetic effects influencing head asymmetry, the association between morphological asymmetry and behavioral laterality, and the identification of signatures of balancing selection. Recent technological and theoretical developments have opened new exciting research avenues that can help identifying the drivers of adaptive traits in *P. microlepis* and other nonmodel organisms, and we discuss promising directions worth exploring. We highlight the importance of using integrative approaches that analyze genetic, environmental, and epigenetic variation in natural populations to aid a comprehensive understanding of why cichlids are so diverse and how

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evolution has produced and continues to generate such a vibrant and often complex phenotypic diversity.

**Keywords** Diversity · Asymmetry · Plasticity · Balancing selection · Negative frequency-dependent selection · Genome scans

## Introduction

Evolutionary biologists aim to uncover the processes facilitating the striking diversity of life forms on our planet and their adaptations to the environment they inhabit. Many mechanisms drive phenotypic divergence and adaptation of species or populations (e.g., Meyer, 1990; Seehausen et al., 1999; Barluenga & Meyer, 2004; Reimchen & Nosil, 2008; van der Sluijs et al., 2013; Fraser et al., 2014; Morgans et al., 2014; Sandkam et al., 2015). However, relatively few studies have addressed the mechanisms that lead to stable divergent phenotypes within populations apparently independent of factors such as sex or life stage. Examples of this kind of polymorphism are seen in some occurrences of bilateral asymmetries (e.g., Palmer, 2009; Schilthuisen, 2013). Here, left and right individuals differ from a typically bilateral symmetric organism, a pattern that has repeatedly emerged in both vertebrates and invertebrates and is thought to be adaptive (Ludwig, 1932; Palmer, 2004, 2009; Compagnucci et al., 2014; Palmer, 2016, e.g., Lucky et al., 2012; Tobo et al., 2012; Matsui et al., 2013; Kurvers et al., 2017). The evolutionary and developmental processes underlying such polymorphisms and adaptations often remain unclear or explored only theoretically. One natural model for this research is *Perissodus microlepis* (Boulenger, 1898, Fig. 1), a cichlid fish notably renowned for the peculiar intraspecific polymorphism in its asymmetric cranio-facial anatomy, particularly at the mouth level (Fig. 2). It is considered a textbook example of balancing selection and of extreme ecological specialization (Hori, 1993; Lee et al., 2015).

Here we review the current state of knowledge on the puzzling diversity of *P. microlepis*. While the evolutionary mechanisms and genetic basis underlying such a remarkable adaptation were initially thought to be quite simple (Hori, 1993), this trait is more complex than previously proposed, particularly

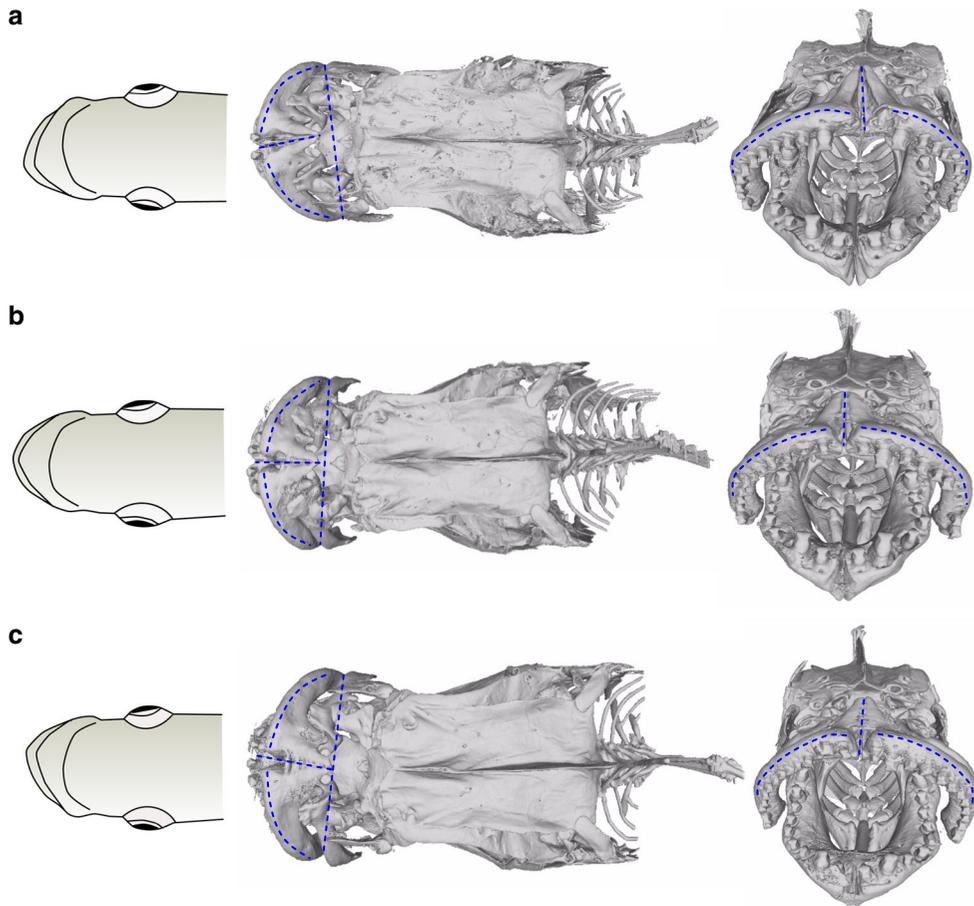


**Fig. 1** The scale-eating cichlid fish *Perissodus microlepis* endemic to Lake Tanganyika surrounded by juveniles (picture courtesy of Heinz H. Büscher)

in the light of recent findings (Table 1). We highlight the most important questions that remain still unanswered and provide an overview of potentially promising approaches to address the architecture of head asymmetry. We also outline some pitfalls that limit their application to the study of *P. microlepis* and other nonmodel study systems. As some of these frameworks have already been extensively described elsewhere (e.g., Lehner, 2013; Henning & Meyer, 2014; Kratochwil & Meyer, 2015; Pardo-Diaz et al., 2015; Bernatchez, 2016; Ashton et al., 2017; Kumar & Kocour, 2017; Pasaniuc & Price, 2017), here we specifically aim to survey research strategies that are potentially useful to answer open issues regarding the evolutionary and developmental bases of adaptive diversity in *P. microlepis*, focusing particularly on those that investigate signatures of balancing selection. Finally, we conclude with an outlook on the approaches that can be beneficial for studies aiming to bridge the gap between phenotype, genotype, and environment of adaptive traits such as head asymmetry.

## Background: balancing selection and its importance in adaptation

Adaptations are the outcome of natural selection, i.e., the differential survival and reproduction (fitness) of individuals due to differences in phenotype (Darwin, 1859). If the trait that affects individuals' fitness is (at least partly) heritable, its underlying genomic architecture can be modified by selection. Depending on the effect that selection has on allele/genotype frequencies, it can be classified as positive (favors



**Fig. 2** Three alternative states of head asymmetry: left (a), symmetric (b), and right (c). From left to right: top view of external head morphology, dorsal, and frontal view of skull (CT

scans with blue, dashed guidelines; CT scans courtesy of Dominique Adriaens and Barbara De Kegel)

advantageous mutations), negative (removes disadvantageous variants), or balancing selection (fitness is context dependent; Ewens & Thomson, 1970; Kaplan et al., 1989; Fournier-Level et al., 2013; Vitti et al., 2013). Balancing selection includes heterozygote advantage, temporally and/or spatially heterogeneous selection, and positive and negative frequency-dependent selection (Levene, 1953; Ewens & Thomson, 1970; Gillespie, 1973; Hedrick et al., 1976; Via & Lande, 1985). While positive frequency-dependent selection can lead to convergent or parallel evolution and thus decrease diversity, selection that favors rarer variants (negative frequency-dependent selection) is one of the most powerful forces maintaining polymorphism (Wright, 1969; Ayala & Campbell, 1974; Gromko, 1977; Charlesworth, 2006).

In the recent decades, evolutionary biologists have mainly focused on positive and negative selection due to their intuitive importance in adaptation (Nielsen, 2005; e.g., Gerrard & Meyer, 2007; Salzburger et al., 2007; Salzburger, 2009; Diepeveen & Salzburger, 2011; Fan et al., 2011; Diepeveen et al., 2013; Keller et al., 2013; Brawand et al., 2014; Henning & Meyer, 2014; Xia et al., 2015; Pavlova et al., 2017). In contrast, balancing selection has not been widely studied, especially in fish (reviewed in López et al., 2015; Bernatchez, 2016; Kumar & Kocour, 2017; Yue & Wang, 2017). Nonetheless, balancing selection can have major influences on evolutionary processes, particularly in adaptation. In fact, it: (i) provides an exceptional source of functional variants that can mediate later adaptations (“standing variation”; Barrett & Schluter, 2008; Andrés et al., 2009; Messer &

**Table 1** Summary of hypotheses aiming to explain head morphological asymmetry and/or handed behavior in *Perissodus microlepis*

Focus	Proposed hypothesis	Supporting evidences	References
Distribution of head shapes	Bimodal	The presence of conspicuous morphs by visual inspection bimodal distribution in the height of the mandible posterior end, and height of the mandible posterior process	Hori (1993), Nakajima et al. (2004), Hori et al. (2007), Takahashi & Hori (2008), Stewart & Albertson (2010), Takeuchi et al. (2012), Hata et al. (2013), Takeuchi et al. (2016)
	Unimodal	The presence of some nearly symmetric individuals; continuous distribution in the degree of head bending angle centered on zero	Hori (1991), Lee et al. (2010), Van Dooren et al. (2010), Kusche et al. (2012), Hata et al. (2013), Lee et al. (2015)
Genetic basis	Stochastic, not inherited trait	Stochastic or partially random direction of asymmetry in most occurrences of antisymmetry	Palmer (2004), Palmer (2010)
	One Mendelian locus	Nonactively feeding juveniles with skewed jaws; field observations of parent–offspring frequencies; identification of UNH2101, a conserved locus for jaw asymmetry in East African cichlids	Hori (1993), Hori et al. (2007), Stewart & Albertson (2010)
	Complex polygenetic basis	Unimodal distribution of head shapes; additive genetic component; UNH2101 unlinked to external head asymmetry; estimates of narrow-sense heritability; identification of numerous SNPs associated to external craniofacial anatomy	Stewart & Albertson (2010), Van Dooren et al. (2010), Kusche et al. (2012), Lee et al. (2015), Raffini et al. (2017), Raffini et al. (2018a)
Environmental effects	Simple exclusive genetic basis	Nonactively feeding juveniles with skewed jaws; field observations of parent–offspring frequencies	Hori (1993), Hori et al. (2007)
	Quantitative trait	Unimodal distribution of head shapes; effect of phenotypic plasticity and feeding experience; parent–offspring frequencies; narrow-sense heritability estimates; influence of both additive genetic and environmental component	Stewart & Albertson (2010), Van Dooren et al. (2010), Kusche et al. (2012), Lee et al. (2012), Lee et al. (2015), Takeuchi et al. (2016), Raffini et al. (2018a, b)
Relationship between morphological and behavioral asymmetry	Handed behavior as follower	Nonactively feeding juveniles with skewed jaws; gradual establishment of lateralized behavior during development positively correlated with head asymmetry; feeding behavior linked to head asymmetry	Hori (1993), Takeuchi et al. (2016), Raffini et al. (2018a)
	Handed behavior as driver	Influence of environmental factors; laterality possibly expressed earlier and more conspicuous than asymmetry in morphology; potential involvement of <i>habenula</i>	Palmer (2010), Stewart & Albertson (2010), Van Dooren et al. (2010), Kusche et al. (2012), Lee et al. (2012), Lee et al. (2017)
	Developmental independence	Weak correlation between morphological and behavioral asymmetry in lab-reared fish potential involvement of brain and eye size asymmetry	Ichijo et al. (2017), Lee et al. (2017), Raffini et al. (2018b)

Petrov, 2013; Whitlock, 2015; De Filippo et al., 2016), (ii) actively “protects” polymorphisms from forces such as gene flow or drift (Yeaman & Otto, 2011;

Bernatchez, 2016), and (iii) helps explain the long-term maintenance of variation, as proposed in *Perissodus microlepis* (Hori, 1993).

### *Perissodus microlepis*: a useful model for evolutionary biology

Adaptive radiations are ideal to investigate the mechanisms underlying biodiversity and adaptation (Simpson, 1953). An exceptional system to study these processes is exemplified by the family Cichlidae, a group of tropical freshwater fishes that are famous for their spectacular rapid and hyper diverse adaptive radiation, especially those inhabiting the three large East African Great Lakes (Victoria, Tanganyika, and Malawi; Meyer, 1993; Kocher, 2004; Salzburger & Meyer, 2004; Seehausen, 2006). This burst of diversity involves the expansion of an ancestral lineage into a variety of related forms specialized to fit different environments or ways of life, and includes speciation and adaptation to divergent ecological niches (Schluter, 2000; Gavrillets & Losos, 2009; Larsen, 2011). Within sometimes extremely short timeframes (e.g., more than 500 species of Lake Victoria cichlids arose in less than 100,000 years, Meyer et al., 1990; Stiassny & Meyer, 1999; Verheyen et al., 2003; Elmer et al., 2009), cichlids have evolved an extraordinary number of species (~2000) and a wide array of diversity in morphology, coloration, and behavior. They represent one of the most diverse and species-rich radiations in vertebrates (reviewed in Salzburger & Meyer, 2004; Seehausen, 2006; Salzburger, 2009; Henning & Meyer, 2014; Kratochwil & Meyer, 2015; Meyer, 2015). Thus, this group of fish provides an excellent model to investigate the evolution of adaptations and to understand the underlying mechanisms of divergence within and between species (reviewed in Salzburger & Meyer, 2004; Salzburger, 2009; Takahashi & Koblmüller, 2011; Henning & Meyer, 2014; Kratochwil & Meyer, 2015; Meyer, 2015).

*Perissodus microlepis* (Fig. 1) is a cichlid fish endemic to Lake Tanganyika (East Africa). It is a relatively recent species (approximately 3,600,000 years bp; Koblmüller et al., 2007) of the tribe Perissodini, one of the last to emerge in the course of the primary Tanganyika radiation (Koblmüller et al., 2007). This group of fishes went through an ecological expansion from deep-water generalist carnivorous predators to shallow-water, highly specialized lepidophagous (fish scale eater) hunters; *P. microlepis* is one of the most specialized Perissodini's lineages (Takahashi et al., 2007b; Stewart & Albertson, 2010). A recent phylogenomic study reveals

hybridization between Perissodini and the deep-water Benthochromini, a mechanism which may have provided new genetic variation for adaptation and ecological specialization (Irissari et al., in press). Eating scales removed from the flanks of other fishes without killing the prey is a highly specialized, yet relatively widespread feeding behavior among fishes. It has independently evolved at least 19 times and is known in 50 teleost species up to date, including cichlids (Martin & Wainwright, 2013). In fact, scales are highly nutritious and one of the most abundant and renewable, thus stable, food sources (Sazima, 1983; Nshombo et al., 1985; Martin & Wainwright, 2013). In Perissodini, the transition between generalist to scale-eating behavior is reflected in their morphological variation, particularly in discrete shifts and sided differences in craniofacial skeletal anatomy mirroring species' divergence in habitat preferences and predation strategies (Stewart & Albertson, 2010).

*Perissodus microlepis* is famously known for its peculiar intraspecific phenotypic diversity. Both left-bending ("left" morph, L hereafter) and right-bending ("right" morph, R) heads are commonly observed within natural populations (Fig. 2a, c; Hori, 1993; Hori et al., 2007; Kusche et al., 2012; Hata et al., 2013; Lee et al., 2015). This kind of polymorphism makes this fish a striking example of bilateral asymmetry, where left or right individuals differ from typically bilaterally symmetric specimens having two mirror image halves (Hori, 1993; Palmer, 2004; Koblmüller et al., 2007; Takahashi et al., 2007a, b). This fish is mainly a shallow-water scale eater that attacks a broad spectrum of prey species (Nshombo et al., 1985; Takahashi et al., 2007b; Takeuchi et al., 2016; Kovac et al., 2018). However, prey are vigilant against predators and readily escape in open waters where attacks are more difficult; consequently, *P. microlepis* predation success is quite low (about 20%; Hori, 1987). During hunting attempts, *P. microlepis* very quickly approaches its victim from behind, taking advantage of the fish's blind angle, and finally tears off scales from flank with quick body rotations and twists (Nshombo et al., 1985; Takahashi et al., 2007a, b; Takeuchi et al., 2012). Most individuals exhibit preference for one of the two sides of prey (Hori, 1993; Lee et al., 2010; Van Dooren et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016; Indermaur et al., in press). For most other fish species, sneaking-up from behind and attacking a fish

in a pointed angle would not work well as their mouths would only have a minimal contact area with the prey's flank. In contrast, *P. microlepis* is supported by its bending head that maximizes the contact area between mouth and prey's flank even in sharp assault angle, enabling it to attack from a more posterior, thus less visible, orientation (Hori, 1993; Takahashi et al., 2007a, b; Palmer, 2010; Van Dooren et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016).

Head morphological asymmetry and lateralized hunting behavior are linked: right morphs preferentially remove scales from the prey left side, and *vice versa* for the left morph individuals (Hori, 1993; Palmer, 2010; Van Dooren et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016; Lee et al., 2017; Indermaur et al., in press). Functional morphology and kinematics analyses further suggest that head bending facilitates increased feeding success. In fact, they indicated a lateralization in the speed and force between the opposite sides of the lower jaw while rotating to remove scales, and kinetic differences in body flexion during attacks made from the preferred or nonpreferred direction (Stewart & Albertson, 2010; Takeuchi et al., 2012; Takeuchi & Oda, 2017). Other features, such as aggressive mimicry (Nshombo, 1994; Hori & Watanabe, 2000; Koblmüller et al., 2007), teeth, and body morphology (Takahashi et al., 2007; Takahashi et al., 2007b), may contribute to increase the success rate of this specialized feeding even more.

#### A putative textbook model of balanced polymorphism

In a key study, Hori (1993) investigated the frequencies of L and R morph individuals in *P. microlepis* across a period of 11 years. Morph frequencies fluctuated cyclically every 4–5 years around a 50:50 ratio and were stable over time within a given population (Fig. 2A in Hori, 1993). The morphs relative abundance has been proposed to be regulated by the selective advantage of the rare phenotype over the abundant one (negative frequency-dependent selection). According to this hypothesis, prey survive the attacks and might learn to protect the flank that is more often attacked from the more abundant head form thereby selecting against the more abundant morph. A recent field-based cage experiment showed

that *P. microlepis* from experimental populations with both the L and R morphs have higher attack success compared to monomorphic groups (Indermaur et al., in press). Balancing selection thus likely maintains the polymorphism in head shapes over time (Hori, 1993; Nakajima et al., 2004; Indermaur et al., in press). Disassortative mating (i.e., preference for a mating partner with a dissimilar phenotype) might also contribute to stabilize this polymorphism (Takahashi & Hori, 2008) but more recent studies did not support this hypothesis (Lee et al., 2010; Kusche et al., 2012, but see Raffini et al., 2017).

*Perissodus microlepis* soon became widely cited as a spectacular textbook example of negative frequency-dependent selection through prey–predator interactions and a fascinating case of extreme adaptive ecological specialization, an iconic occurrence of antisymmetry (i.e., bilateral asymmetry in which the abundance of left and right morph individuals is equal, a bimodal distribution of phenotypes; Palmer, 2004). It is also a promising model to investigate neuronal circuits (Hori, 1993; Palmer, 1996; Stewart & Albertson, 2010; Takeuchi et al., 2012; Ichijo et al., 2017; Lee et al., 2017). However, several incongruous findings and unaddressed issues emerged in recent years, particularly regarding the trait distribution of head asymmetry, its genetic basis, the influence of non-genetic (environmental) cues, as well as the causal association between morphological asymmetry and behavioral laterality (Table 1). Although these topics are interconnected, for sake of clarity each of them is reviewed individually below.

#### The distribution of head asymmetry

Despite its head morphological asymmetry being one of the main features of *P. microlepis*, the distribution of this phenotypic trait is still questioned, particularly whether it is a continuous or a discrete trait and how to best measure it. The accuracy of phenotyping is also crucial for understanding its genetic bases and underlying evolutionary processes. In particular, a discrete bimodal distribution of head shapes could suggest that diversifying selection mainly acts on a relatively simple genetic architecture (as originally proposed by Hori, 1993), while a continuous unimodal phenotypic pattern would indicate a more complex, quantitative trait where the phenotype depends on the cumulative

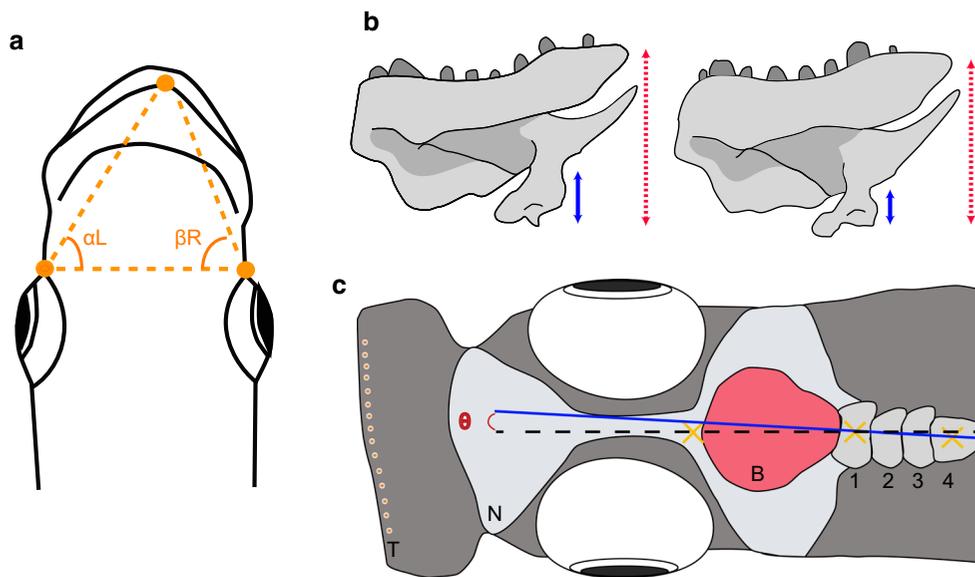
effect of multiple genetic and non-genetic factors (Lynch & Walsh, 1998).

Initially, two clear external head phenotypes were described in natural populations: left and right, without an intermediate morph (i.e., a bimodal distribution; Liem & Stewart, 1976; Hori, 1991, 1993; Nakajima et al., 2004; Hori et al., 2007; Takahashi & Hori, 2008). The right morph is easily recognized by the right orientation of the premaxilla ascending process, the left side of its head exposed to the front, and a right lower jaw that is longer than the left one; the opposite features characterize the left morph (Fig. 2a, c; Nakajima et al., 2004; Kusche et al., 2012; Takeuchi et al., 2012). A comparative morphometric analysis of skeletal morphology and asymmetry from dissected skulls of seven species of the Perissodini tribe showed sided differences in the length of retroarticular processes, mouth orientation, maxilla and premaxilla thickness, nasal bone curvature, and in the functional mechanics of the jaw opening and closing lever systems (Stewart & Albertson, 2010). In particular, prominent asymmetries were evident in stained heads of *P. microlepis* larvae still having yolk, specifically in the jaw direction, hyoid length, curvature, and pharyngeal jaw dentition (Stewart & Albertson, 2010). A clear bimodal distribution of head shapes was described in adults by eye examinations of external craniofacial shape and by image analysis software measurements of the mandible posterior end height (Fig. 3b; Takeuchi et al., 2012). Recently, this method was adjusted to include the length of the entire posterior process (Fig. 3b) to make it easily applicable to small fish, and a sharp dimorphism was described throughout all the developmental stages, as expected according to the antisymmetry model (Takeuchi et al., 2016).

However, other reports revealed that this morphological asymmetry might be less clear than previously described. Adults with less pronounced head bending and difficulties in determining asymmetry by visual inspection alone were repeatedly reported since the first investigations focusing on *Perissodus* (Hori, 1991; Lee et al., 2010; Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche et al., 2012). A quantitative assessment of *P. microlepis* external head asymmetry using the difference in the angle from the pre-orbital processes to the premaxillary joint derived from a dorsal view pictures of stained larvae (head bending angle, Fig. 3a; Hori et al., 2007; Kusche et al.,

2012; Raffini et al., 2017) indicate that head shapes are continuously and unimodally (and not bimodally as previously described) distributed early in development, presenting also near-symmetric samples (Fig. 2c; Stewart & Albertson, 2010). Procrustes shape analysis of external head landmarks in live wild-caught adult fish identified only a weak asymmetry (i.e., a trait distribution that is not bimodal but presents more data in the tails and less around the peak compared to a normal distribution) with peaks at low values of head bending angles, and no significant deviations from unimodality (Van Dooren et al., 2010). Analysis of the head bending angle in both laboratory-bred and wild-caught juveniles showed unimodal distributions of head shapes, and, interestingly, a markedly lower amount of asymmetry in fish raised in laboratory compared to those captured in Lake Tanganyika (Lee et al., 2012). These findings were confirmed in a detailed analysis of wild-caught adult and lab-reared juvenile fish, where external head asymmetry was quantified from photographs using the head bending angle and landmark-based geometric morphometric analyses (Kusche et al., 2012). A continuous distribution of head bending angle centered onto zero was observed also in adults collected in the wild and their broods, and in *P. microlepis* juveniles compared to a brood of the symmetric fish *Astatotilapia burtoni* having the same size (Lee et al., 2015).

The reason for the different distributions of head shapes in previous studies might have arisen by differences in measuring methodology or the use of different populations. A single sample of 50 wild-caught adults from a single sampling site was used to compare three approaches (Hata et al., 2013): (a) the head bending angle from dorsal view photos (Fig. 3a; Hori et al., 2007; Kusche et al., 2012; Raffini et al., 2017), (b) the difference in the mandible posterior end height between the left and right lower jaw (Fig. 3b; Albertson et al., 2005; Stewart & Albertson, 2010) and (c) the angle from the vertebrae to the neurocranium tip (Fig. 3c; Hata et al., 2013). In the first case (method a), the trait better fit a unimodal distribution, but a more pronounced trait dimorphism was observed using the last two procedures (b and c). Since measurement error was lower using the length of the retroarticular processes (b), this method was proposed to be more reliable. It would also avoid preservation artifacts in soft tissues that might affect the



**Fig. 3** Methods to quantify head asymmetry in *P. microlepis*. **a** Based on external head morphology: the degree of head bending angle. Top view of the head. Three points corresponding to the most anterior part of the eye sockets and the tip of the snout were recorded. From these, the angles at each of the eye sockets were drawn. The degree of asymmetry was measured as the difference between the angle at the left ( $\alpha_L$ ) and right ( $\beta_R$ ) eye. Positive values indicate left-bending individuals, whereas negative results are indicative of right-bending fish. **b–c** Based on skeletal measurements: **b** the differences in the height of the

mandible posterior end (blue continuous line) or the entire process (red dashed line) between the left and right lower jaws. **c** The neurocranium–vertebrae angle. Ventral view of the head. The degree of asymmetry is calculated as the angle  $\theta$  between the line passing from the vertebrae (indicated with numbers; blue continuous line) and the line connecting the 1st vertebra to the neurocranium (N; red dashed line). Right-bending individuals are defined by positive values of  $\theta$ , while left-bending individuals show negative  $\theta$  values. *T* tooth, *B* brain

measurement of external head shape (Hata et al., 2013). While the use of skeletal elements (b and c) could be more precise, the biological significance of head asymmetry and its function may be better captured using external head morphology (a). In fact, the prey–predator interaction occurs mainly at the mouth level, where *P. microlepis* contacts the prey’s flank to remove scales, and might results from the interactions of different tissues and not bones alone. From a methodological point of view, the above-mentioned analysis of skeletal material (methods b and c) implied the dissection of bone(s) from surrounding tissues, thus destroying the integrity of specimens and potentially introducing alterations, with important drawbacks that should be considered in morphometric analyses. In addition, traditional linear morphometric measurements, widely used to estimate head asymmetry in *P. microlepis* with relatively few exceptions (Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche et al., 2012; Raffini et al., 2017, 2018a, b), are known to be less

effective in detecting differences in shape compared to geometric morphometrics approaches (Rohlf & Marcus, 1993; Kassam et al., 2003). Another potential source of measurement bias could arise due to the handedness of the observer(s) leading to artificial differences between sides (Helm & Albrecht, 2000). Future studies should consider non-destructive (e.g., imaging techniques such as CT-scanning) and landmark-based methodologies together with a rigorous application of procedures to minimize measurement bias and error, a source of inaccuracy that has been too often overlooked (reviewed in Fruciano, 2016).

#### The genetic architecture of head asymmetry

Head asymmetry in *P. microlepis* is thought to have a genetic component. The heritability of this trait was initially deduced from the presence of juveniles with skewed jaws before the ontogenetic dietary shift toward eating scales (Hori, 1993; Stewart & Albertson, 2010). However, the direction of asymmetry

could be purely stochastic and not inherited, as observed in most occurrences of antisymmetry (Palmer, 2004, 2010). In addition, head asymmetry could be in part genetically determined and in part random (Palmer, 2010). Yet, these two random determinations of asymmetry did not reconcile with several findings that emerged in the last decade. These included parents–offspring frequencies (Hori, 1993; Hori et al., 2007; Palmer, 2010; Stewart & Albertson, 2010; Lee et al., 2015), a significant heritable component of this phenotype estimated using quantitative measurements of the head bending angle and parent–offspring regressions (Lee et al., 2015), or the presence of several single nucleotide polymorphisms (SNPs) associated with external head morphs (Raffini et al., 2017; Raffini et al., 2018a). These lines of evidence seem to suggest that gene(s) may contribute to head polymorphism in *P. microlepis*.

The inheritance mode and genomic architecture potentially underlying head asymmetry is also unclear. Field observations and visual assessments of external head phenotypes initially suggested a simple Mendelian trait, a locus with two alleles, “dextral” (“R”) and “sinistral” (“L”), with R dominant over L (Hori, 1993). Conversely, a precise measurement of offspring morph frequencies is difficult in the field due to the presence of brood farming out behavior (i.e., care for unrelated brood; Yanagisawa, 1985; Hori, 1993; Sefc, 2011; Lee et al., 2016). To potentially avoid the effects of brood mixing, fry from wild-caught pairs were carefully selected for stage, size, and vicinity to the conspecific brood and then raised in laboratory. Following this, the previous genetic model was modified to include dominance but homozygous lethality of the allele R (Hori et al., 2007). This same genetic determination was also suggested by a study that identified a conserved locus for jaw asymmetry in East African cichlids, which showed a size polymorphism at a microsatellite locus (UNH2101) segregating with head morphs in *P. microlepis* (two alleles: A linked to the R morph, and B associated to the L morph, with AA homozygous lethal; Stewart & Albertson, 2010). However, this marker was later shown to be unlinked to head asymmetry in studies employing multiple populations and families (Lee et al., 2010; Lee et al., 2015). More importantly, the single locus model would not be able to account for the presence of nearly symmetric morphs (Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche

et al., 2012), and does not fit the reported and expected parents–offspring frequencies (Palmer, 2010). These contradictory findings were most likely related to the low reliability of the previous brood estimates (phenotyping based on visual inspection alone and the absence of genetic parentage analyses to avoid brood mixing; Palmer, 2010; Lee et al., 2015; Lee et al., 2016). A more recent study (Lee et al., 2015) controlling for these confounders showed that this phenotype is unlikely to have a simple Mendelian genetic basis, but it is rather a complex, multilayer trait with a weak additive genetic component (18–22%; Stewart & Albertson, 2010; Lee et al., 2015). Later, the identification of numerous SNPs linked to head asymmetry further support the idea that a polygenic basis might contribute to this polymorphism (Raffini et al., 2017; Raffini et al., 2018a), which appears to be the architecture characterizing many traits (Orr, 1998; Pritchard & Di Rienzo, 2010; Rockman, 2012; Bernatchez, 2016). Interestingly, the presence of phylogeographic structuring in this fish (Koblmüller et al., 2009; Raffini et al., 2017; Raffini et al., 2018b) has recently opened up the possibility that the putative genetic basis of head asymmetry might also have a relevant geographic component, i.e., being nonparallel across the distribution range of this species (Raffini et al., 2017; Raffini et al., 2018b). Additional data are required to shed light on the heritable variation of head asymmetry in this iconic cichlid fish.

#### Environmental influences on head asymmetry

The role of non-genetic factors in shaping *P. microlepis*' polymorphism was first suggested by the observation of a unimodal distribution of head shapes in wild-caught fish (Hori, 1991; Palmer, 2010; Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche et al., 2012; Lee et al., 2015). The first study to explicitly test whether head asymmetry is influenced by environmental factors controlled the feeding environment (Van Dooren et al., 2010). Ten wild-caught adult fish showed preference in attacking the prey's left flank. They were randomly subdivided into two experimental groups and forced to experience a different feeding treatment for 6 months: one was allowed to only attack the side of its preference (left flank), while the other was forced to attack the right side. At the end of the experiment, the preferred-side group exhibited an increased magnitude of the head

bending angle toward the right, while the change in asymmetry was not significant in samples forced to feed on the nonpreferred flank. These results suggested that head asymmetry is at least to some extent phenotypically plastic and can be modified in adult fish (Van Dooren et al., 2010). In addition, the observed lower degree of head asymmetry in laboratory-reared samples predominantly fed with flake food compared to those collected in the wild (Kusche et al., 2012; Lee et al., 2012), and the positive correlation between the number of ingested scales and the amount of head asymmetry (Takeuchi et al., 2016) provided indirect lines of evidence for the contribution of non-genetic factors such as predation mode and feeding experience to the head bending angle. Narrow-sense heritability estimates of head asymmetry *via* parent–offspring regression further indicated that this trait is strongly influenced by non-genetic effects (80%, Lee et al., 2015). A recent investigation using stable isotopes analysis suggested that the degree of head bending angle is influenced by random and nonrandom interindividual variation in feeding experience, foraging behavior (individual specialization) and intraspecific competition (Raffini et al., 2018a).

Thus, head asymmetry is likely the results of processes acting at multiple levels, from genes to environment, particularly the feeding environment. Specifically, in the light of most recent findings, the direction of external craniofacial asymmetry (left or right) might be at least partially genetically determined, and its polymorphism could still be maintained by negative frequency-dependent selection (as suggested by Hori, 1993, Indermaur et al., in press), while the degree of head bending angle might be influenced by both a putative genetic basis and a plastic response due to feeding experience and intraspecific competition (Raffini et al., 2018a). Therefore, the influence of balancing selection on this trait might not be so straightforward as initially described (and expected from a bimodal trait, Hori, 1993), but it is coupled with additional mechanisms such as plasticity likely generating a unimodal distribution of head shapes (Raffini et al., 2018a).

Morphological asymmetry and handed behavior: which is the “driver” and which is the “follower”?

Morphological head asymmetry in *P. microlepis* has been linked to behavioral laterality (handed feeding

behavior) since the first publication of a field experiment and stomach content analysis showing that the right morph attacks prey on its left flank, while the opposite applies to the left morph (Hori, 1993). Later, a laboratory experiment based on a larger number of wild-caught fish indicated that attack preference is not rigorously bimodal but multimodal, with the presence of few discrete behaviorally different groups (Van Dooren et al., 2010). In addition, observed behavioral responses in fish forced to remove scales from their nonpreferred side suggested that the direction of handed behavior is not plastic, i.e., cannot be strongly influenced by environmental factors such as feeding experience. Then, behavioral laterality appears to be a more conspicuous trait closer to a bimodal distribution of phenotypes (as expected in the antisymmetry model) than morphological asymmetry (Van Dooren et al., 2010). These findings were supported by further analyses of wild-caught adults and laboratory-bred juveniles. The preferred prey’s flank could be predicted from head morph in adults (Lee et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016), while juveniles exhibited a clearly lateralized attacking behavior even when the degree of head asymmetry was still slight (Lee et al., 2012). Handedness mainly followed a bimodal distribution with considerable variation in preference strength between individuals (Lee et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016). Under laboratory conditions, younger fish (2 and 3 months of age) showed a more marked handedness compared to 7-month-old individuals (Lee et al., 2012). However, a recent study on stomach content analyses of wild-caught samples and a predation experiment suggested that lateralized behavior emerges gradually during development, with early juveniles attacking both prey’s flanks, and then becoming increasingly biased toward one side (Takeuchi et al., 2016; Takeuchi & Oda, 2017). Finally, behavioral laterality has been shown to be related to kinetic, neuroanatomical, and brain transcriptional asymmetry (Lee et al., 2017; Takeuchi & Oda, 2017), particularly in the *tectum opticum* and *habenula*, an integration center that regulates motor behavior after sensory information (reviewed in Matsumoto & Hikosaka, 2007; Bianco & Wilson, 2009; Chen et al., 2009; Gutiérrez-Ibáñez et al., 2011; Ichijo et al., 2017; Mizumori & Baker, 2017). Asymmetry in external craniofacial anatomy (and most likely, lateralized behavior) is also

significantly associated with asymmetry in eye size, possibly suggesting that a cerebral asymmetric information flow might contribute to the establishment of lateralized neuronal circuits, including the ones responsible for motor response to visual stimuli (Raffini et al., 2018b). Then, natural selection due to social interactions may act on these neuronal circuits that process information from the outer world, and the subsequent response, and are likely involved in laterality and/or asymmetry (Ichijo et al., 2017; Lee et al., 2017; Raffini et al., 2018b).

The central question thus remaining is which comes first in ontogeny and phylogeny: morphological asymmetry or behavioral laterality? In other words, is morphological asymmetry the “driver” or the “follower” in *P. microlepis*? On the one hand, it has been initially proposed that morphological polymorphism has a strict genetic basis and its peculiar shape is useful only if fish attack the prey at the side correlated with the mouth opening direction, suggesting that handedness is likely the follower in ontogeny and possibly phylogeny directed through natural selection (Hori, 1993). This hypothesis is supported by the presence of juveniles still feeding on the yolk sac (i.e., not yet eating scales) already exhibiting skewed heads (Hori, 1993; Stewart & Albertson, 2010), as well as the gradual establishment of lateralized behavior during development, that is increasingly positively correlated with head asymmetry likely through learning (Van Dooren et al., 2010; Takeuchi et al., 2016; Takeuchi & Oda, 2017). In addition, individuals less successful at removing scales, possibly due to their nearly symmetric heads, seem to compensate this nutrients source through alternative food (Raffini et al., 2018a). Interspecific (prey–predator) and intraspecific (competition between L, R, symmetric morph) antagonistic interactions (Hori, 1993; Ichijo et al., 2017; Raffini et al., 2018a, b) and tradeoffs between disruptive selection *via* scale-eating efficiency favoring more asymmetric heads and its negative effect on other kind of performances (such as swimming ability; Takeuchi et al., 2016) may jointly play a central role in *P. microlepis*. In this fish, individuals seem to modify (“adapt”) their own predation behavior and prey items (ecological niche) to fit their morphology (individual specialization and niche construction, Raffini et al., 2018a). This learning-based process might be guided by the *habenula* (Lee et al., 2017; Takeuchi & Oda, 2017; Raffini et al., 2018a), known to dynamically

switch behavioral responses according to inputs from several parts of the brain including sensory systems, existing memories on trial-by-trial outcomes, and learned associations between context/cues and behavior (Mizumori & Baker, 2017).

On the other hand, a substantial contribution of external factors on head polymorphism, and feeding experiments, and the observation that laterality might be expressed earlier in development and is more conspicuous than morphology (Van Dooren et al., 2010; Lee et al., 2012) support the idea that handedness amplifies or even induces morphological asymmetry, i.e., behavioral laterality is the driver in *P. microlepis* (Palmer, 2010; Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche et al., 2012; Lee et al., 2012). It has been recently suggested that cerebral asymmetry stimulates lateralized behavior, which in turn leads to plastic changes in head morphology (Lee et al., 2017). In addition, a simple genetic basis might underlie handedness (Van Dooren et al., 2010; Lee et al., 2012; Lee et al., 2017) or brain asymmetry (Ichijo et al., 2017; Lee et al., 2017), and thus have an indirect rather than direct effect on morphological asymmetry. The unimodal distribution of head shapes might, then, result from correlated effects (Van Dooren et al., 2010) or reflect among-individual variation in the degree of handed behavior (Lee et al., 2012).

Finally, a third view that is currently emerging suggests that handed feeding behavior and head morphological asymmetry in *P. microlepis* are not necessarily linked to each other by a direct causative relationship, but they may develop independently or be induced or amplified, and directed by a common upstream mechanism, such as cerebral asymmetry in the habenular complex or eyes (Ichijo et al., 2017; Lee et al., 2017; Raffini et al., 2018b). Then, the entire head including brain might be implicated in this polymorphism, which is complex and involves the interaction of a putative genetic basis of multiple potentially independent traits and the environment (Raffini et al., 2018b).

Clarifying the ontogenetic relationship between head morphological asymmetry and behavioral laterality in *P. microlepis*, complemented with knowledge from other species, can also inform the evolution and adaptive significance of these two traits and their association in Perissodini as well as other cichlids and a variety of vertebrate and invertebrate lineages (e.g.,

Takeuchi & Hori, 2008; Davison et al., 2009; Yasugi & Hori, 2011; Lucky et al., 2012; Tobo et al., 2012). Stable morphological and behavioral polymorphisms in bilateral symmetry (i.e., excluding fluctuating asymmetries) have been mainly explored within the single species (as seen in *P. microlepis*, reviewed above; other examples: Takeuchi & Hori, 2008; Davison et al., 2009; Yasugi & Hori, 2011; Lucky et al., 2012; Tobo et al., 2012) or individually across phylogeny (e.g., asymmetry in craniofacial anatomy but not behavioral laterality in Perissodini, Stewart & Albertson, 2010; other examples: Palmer, 1996, 2009; Miyashita & Palmer, 2014; Harrington et al., 2016; Blum & Ott, 2018). So far, they have been jointly analyzed within an inclusive phylogenetic framework only in a few studies predominantly involving the link between cerebral asymmetry and handed behavior, none of them including cichlid fishes (examples reviewed in Bisazza et al., 1998; Palmer, 2016; Rogers, 2017). Clearly, a more detailed, and comprehensive ontogenetic and evolutionary studies are needed of the origin and the persistence of asymmetric polymorphisms in *P. microlepis* and other organisms. In the following paragraphs, we outline how the current biological research can help to this aim, and the issues that limit the application of the available approaches in nonmodel organisms such as *P. microlepis*.

### A roadmap to understand the mechanisms underlying asymmetry in *P. microlepis*

#### Toward coupling phenotype and genotype

The gap between phenotypic and genotypic variation has classically been bridged using “forward genetics” approaches (reviewed e.g., in Nielsen, 2005; Vitti et al., 2013; Kratochwil & Meyer, 2015, examples in cichlids: Salzburger et al., 2007; Navon et al., 2017). Unfortunately, husbandry difficulties and the paucity of *a priori* knowledge about genome and candidate genes/pathways have limited the application of this framework to *P. microlepis* (Lee et al., 2010; Raffini et al., 2017). This fish and other nonmodel organisms can better benefit from “reverse genetics” methods (reviewed e.g., in Nielsen, 2005; Nosil et al., 2009; Butlin, 2010; Martin & Jiggins, 2013; Vitti et al., 2013; Kratochwil & Meyer, 2015; Wellenreuther &

Hansson, 2016, examples in cichlids: Kautt et al., 2012; Keller et al., 2013).

Comparative population genomics and brain transcriptome approaches have already successfully provided a set of candidate loci for morphological asymmetry and behavioral laterality in *P. microlepis* (Raffini et al., 2017; Raffini et al., 2018a; Lee et al., 2017). In addition, head polymorphism in this fish is thought to be maintained by negative frequency-dependent selection (Hori, 1993). Thus, the regions of the genome underlying this trait should exhibit signatures of balancing selection, facilitating the identification of such loci (methods, challenges, and references in Supplementary Information S1). However, detecting footprints of selection using genome scans could be challenging, particularly for *P. microlepis*. In fact, these approaches are currently based on statistics that perform poorly in natural populations, require extensive and detailed knowledge of the species, its population history and genomic architecture, as well as the availability of a precise type of data (details in Supplementary Information S1). Additional data are clearly needed to verify and expand the original results (Hori, 1993) in the light of more recent findings.

Further insights into the nature of this potentially adaptive trait may be offered by the inclusion of species exhibiting (e.g., in Perissodini: *P. straeleni*) or not (e.g., in Perissodini: *P. paradoxus*, *P. elaviae*, *P. multidentatus*, *P. hecqui*, *Haplotaxodon microlepis*, Stewart & Albertson, 2010) asymmetry and/or handedness as well as different developmental stages. Up to date, interspecific comparative approaches in *P. microlepis* have been limited to one molecular phylogenetic (Takahashi et al., 2007b) and one functional morphology (Stewart & Albertson, 2010) study of adult Perissodini fishes. However, these two studies mainly focused on the evolution of feeding ecology in this cichlid tribe and the associated functional skeletal craniofacial anatomy. Comparative analyses of adult/larval morphological and behavioral phenotypes or heritability patterns across these Perissodini species have not been presented so far, yet they could elucidate the genetic, ontogenetic and phylogenetic basis of asymmetry. In particular, the developmental processes underpinning head polymorphism in *P. microlepis* remain obscure up to date, mainly due to difficulties in obtaining embryos in laboratory. Current and future technological advancements (e.g., in

humans: Li et al., 2018; Rivron et al., 2018) might facilitate developmental and comparative “omics” studies (reviewed in e.g., Liu et al., 2015).

Some studies observed phylogeographic structure among *P. microlepis* populations (Koblmüller et al., 2009; Lee et al., 2010; Raffini et al., 2017); however, the effects of this population subdivision and demographic history on the phenotypic and genetic bases of head asymmetry have not been fully investigated. Neutral population processes such as mutation, gene flow and genetic drift can play an important role in phenotypic/genetic diversity and hence adaptation, especially for polygenic traits (reviewed in Hedrick, 2011; Yeaman, 2015; Bernatchez, 2016; Casillas & Barbadilla, 2017, examples in cichlids: Koblmüller et al., 2011; Husemann, 2013; Sefc et al., 2017). Particularly, hybridization have a relevant impact on intra- and interspecific divergence (reviewed in Schwenk et al., 2008; Abbott et al., 2013), as seen in cichlids (e.g., Nichols et al., 2015; Kautt et al., 2016; Meier et al., 2017; Irissari et al., in press). A deeper phylogenomic study could clarify whether gene flow between Perissodini and Benthochromini (Irissari et al., in press) has provided a source of adaptive variation. Therefore, exploring neutral dynamics in *P. microlepis* and their potential effects on head polymorphism could be useful to gain a more comprehensive understanding of this interesting study model and limit the incidence of false discoveries (as discussed in Raffini et al., 2017).

On the way to a more inclusive approach:  
environmental effects and interactions

Non-genetic factors can strongly influence phenotypic variation, adaptation, and evolution (e.g., Meyer, 1987; West-Eberhard, 2003; Bonduriansky et al., 2012; Grenier et al., 2016; Schneider & Meyer, 2016), as seen in *P. microlepis*. The effect of environmental cues is clearly evident in phenotypically plastic traits, in which a single genotype is able to produce diverse phenotypes according to external influences (Bradshaw, 1965), also known as developmental plasticity when induced phenotypes are irreversible (reviewed in Forsman, 2014; Schneider & Meyer, 2016). Head asymmetry in *P. microlepis* appears to be plastically influenced by feeding experience and to involve learning and memory (neuronal plasticity, Stewart & Albertson, 2010; Van Dooren

et al., 2010; Lee et al., 2012; Takeuchi et al., 2016; Raffini et al., 2018a). Additional studies are needed in this cichlid fish to better understand the environmental background of its polymorphism. However, some powerful experimental approaches typically used in plasticity studies (reviewed in Aubin-Horth & Renn, 2009; Bonduriansky et al., 2012; de Villemereuil et al., 2016) could be limited by husbandry difficulties in *P. microlepis*.

The presence of plasticity in *P. microlepis* has already been described in Van Dooren et al. (2010). A potential limit of this study is that it did not consider the presence of different head morphs, as only individuals with right-bending head were tested. Considering that the environmental responsiveness of a trait depends on its heritable component (e.g., Parsons et al., 2016), and head morphs seems to be at least in part genetically differentiated (Hori, 1993; Hori et al., 2007; Koblmüller et al., 2009; Stewart & Albertson, 2010; Van Dooren et al., 2010; Kusche et al., 2012; Lee et al., 2015; Raffini et al., 2017, 2018a, b), the levels of plastic response could be different in different phenotypes. Investigating plasticity in both morphs (or considering the unimodal distribution of this trait) possibly using larger sample sizes could shed light on the environmental component of asymmetry. If this is coupled with time-series transcriptome analyses, in which the temporal expression dynamics of genes that respond to external *stimuli* (diet in our case) that mediate the plastic response is analyzed before and after the inductive cue (e.g., in cichlids: Schneider et al., 2014), the onset and regulatory network shaping plasticity in *P. microlepis* could be better understood. Furthermore, behavioral handedness might be less plastic than morphological asymmetry (Van Dooren et al., 2010; Lee et al., 2012; Takeuchi et al., 2012; Takeuchi et al., 2016). An appropriate study design comparing both the heritable and environmental components of these two traits (morphological asymmetry and behavioral laterality) could help clarifying their relationship and addressing which one comes first in ontogeny and also in phylogeny if other key species (e.g., other Perissodini cichlids) are included. Comparative phylogenetic approaches may also elucidate whether plasticity, genetic assimilation, or similar processes contributed to the Perissodini radiation guided by ecological specialization and the associated modifications in the craniofacial structures including asymmetry

(Takahashi et al., 2007b; Stewart & Albertson, 2010), similarly to that observed in the pharyngeal jaw of East African cichlid fish species (Gunter et al., 2017).

#### Linking phenotype, genotype, and environment: epigenetic variation

Lying at the interface between genotype and environment, epigenetics (“non-genetic inheritance” or “trans-generational plasticity”) considerably contributes to complex phenotypes (reviewed, and examples in Wong et al., 2005; Richards, 2006; Aubin-Horth & Renn, 2009; Johannes et al., 2009; Esteller, 2011; Bonduriansky et al., 2012; Kilvitis et al., 2014; Kratochwil & Meyer, 2015; Bernatchez, 2016; Chen et al., 2016; Miska & Ferguson-Smith, 2016; Triantaphyllopoulos et al., 2016; Ashton et al., 2017; Ocklenburg et al., 2017, examples in cichlids: Lenkov et al., 2016; Chen et al., 2017), and this might be the case of *P. microlepis*. For example, epigenetic processes explain part of the “missing heritability” of human handedness (Yang et al., 2010; Ocklenburg et al., 2017). Similarly, examining epigenetic variation in *P. microlepis* may offer interesting insights into mechanisms shaping this polymorphism, particularly the link between genetic and non-genetic effects (e.g., Johannes et al., 2009) as well as the ontogenetic and molecular background of asymmetry (e.g., Ocklenburg et al., 2017). In addition, variations in diet have been shown to be translated into epigenetic modifications with transgenerational effects (reviewed in Triantaphyllopoulos et al., 2016). Considering that feeding behavior seems to play a prominent role in *P. microlepis* (Van Dooren et al., 2010; Kusche et al., 2012; Lee et al., 2012; Takeuchi et al., 2016; Raffini et al., 2018a), investigating how epigenetic processes—if at all—influence head asymmetry in this cichlid fish at the intraindividual level (e.g., changes associated to learning and plasticity) as well as between generations (e.g., effect of parental feeding behavior on broods laterality) could further shed light on the eco-evolutionary and developmental dynamics underlying this interesting head polymorphism.

#### Conclusion

Our understanding of the processes underpinning head asymmetry in *P. microlepis*, and polymorphism and

adaptation in general, has considerably advanced in the recent decades. Nevertheless, we are far from resolving this evolutionary puzzle. Former studies have highlighted that head asymmetry in this cichlid fish might be more complex than initially described. In particular, many aspects of head asymmetry remain to be elucidated: (i) its phenotypic and ontogenetic patterning, (ii) the underlying genetic and non-genetic factors, (iii) the influence of negative frequency-dependent selection, and (iv) the association between morphological asymmetry and behavioral laterality in ontogeny and phylogeny. We emphasized the importance of considering integrative approaches that can clarify (i) its phenotypic (morphological and behavioral), genetic, environmental, and epigenetic variation and interactions, (ii) the developmental and evolutionary basis of this polymorphism, and (iii) the occurrence of (balancing) selection. *P. microlepis* provides a model with exciting opportunities to inform the evolution of asymmetry and handedness as well as of adaptive phenotypic diversity in this fish and other organisms. This interesting study system contributes to our understanding of why cichlids are so diverse and how evolution has produced and continues generating such a spectacular and often complex diversity.

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