Laterality is Universal Among Fishes but Increasingly Cryptic Among Derived Groups

Authors: Hori, Michio, Nakajima, Mifuyu, Hata, Hiroki, Yasugi, Masaki, Takahashi, Satoshi, et al.

Laterality is Universal Among Fishes but Increasingly Cryptic Among Derived Groups

Michio Hori[1], Mifuyu Nakajima[2], Hiroki Hata[3], Masaki Yasugi[4], Satoshi Takahashi[5], Masanori Nakae[6], Kosaku Yamaoka[7], Masanori Kohda[8], Jyun-ichi Kitamura[9], Masayoshi Maehata[10], Hirokazu Tanaka[8,11], Norihiro Okada[12,13], and Yuichi Takeuchi[14]*

[1]*Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan,* [2]*Department of Biology, Stanford University, Stanford, CA 94305, USA,* [3]*Graduate School of Science and Engineering, Ehime University, Bunkyocho 2-5, Matsuyama 790-8577, Japan,* [4]*National Institute for Basic Biology, Higashiyama 5-1, Myodaiji, Okazaki, Aichi 444-8787, Japan,* [5]*Research Group of Environmental Sciences, Nara Woman's University, Kitauoya Nishimachi, Nara 630-8506, Japan,* [6]*Department of Zoology, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan,* [7]*Kochi University, Monobe-B, Nankoku, Kochi 783-8502, Japan,* [8]*Department of Biological Science, Osaka City University, Sugimotocho, Sumiyoshi-ku, Osaka 558-8585, Japan,* [9]*Mie Prefectural Museum, Isshinden-kouzubeta 3060, Tsu, Mie 514-0061, Japan,* [10]*Kobe Gakuin University, Arise 518, Ikawadanicho, Nishi-ku, Kobe 651-2180, Japan,* [11]*Institute of Ecology and Evolution, University of Bern, Hinterkappelen 3032, Switzerland,* [12]*Department of Life Sciences, National Cheng Kung University, Tainan 701, Taiwan,* [13]*Foundation for Advancement of International Science, Tsukuba 305-0821, Japan,* [14]*Department of Anatomy, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama 930-0194, Japan*

Laterality has been studied in several vertebrates, mainly in terms of brain lateralization and behavioral laterality, but morphological asymmetry has not been extensively investigated. Asymmetry in fishes was first described in scale-eating cichlids from Lake Tanganyika, in the form of bilateral dimorphism in which some individuals, when opening their mouths, twist them to the right and others to the left. This asymmetry has a genetic basis, and is correlated with lateralized attack behaviors. This has subsequently been found in fishes from numerous taxa with various feeding habits. The generality of such morphological laterality should thus be investigated in as wide a range of fishes as possible. Using specific indicators of lateral differences in mandibles and head inclination, we find that representative species from all 60 orders of extant gnathostome fishes (both bony and cartilaginous) possess morphological laterality. Furthermore, we identify the same laterality in agnathans (hagfish and lamprey), suggesting that this trait appeared early in fish evolution and has been maintained across fish lineages. However, a comparison of asymmetry among groups of bony fishes reveals, unexpectedly, that phylogenetically more recent-groups possess less asymmetry in body structures. The universality of laterality in fishes indicates a monophyletic origin, and may have been present in the ancestors of vertebrates. Ecological factors, predator–prey interactions in particular, may be key drivers in the evolution and maintenance of dimorphism, and may also be responsible for the cryptic trend of asymmetry in derived groups. Because lungfish and coelacanths share this trait, it is likely that tetrapods also inherited it. We believe that study of this morphological laterality will provide insights into the behavioral and sensory lateralization of vertebrates.

Key words: morphological asymmetry, dimorphism, anti-symmetry, fish evolution, frequency-dependent selection

INTRODUCTION

Behavioral and sensory lateralization is widely recognized in various vertebrates (Roger and Andrew, 2002). Studies of lateralization have focused primarily on humans and higher vertebrates, mainly examining cerebral lateralization and related behavioral or sensory differentiation between right and left sides. However, morphological asymmetry, such as differences between the right and left sides of kinetic or sensory systems, has not been explored in depth, except for differences in the brain and nervous system. Recent studies of laterality in fishes have revealed that behavioral laterality is always associated with morphological asymmetry, which is characteristically represented as dimorphism in populations, i.e., every population is composed of

both lefty and righty individuals at different rates. The goals of this paper are (1) to demonstrate that all fishes have laterality and, therefore, that the origins of laterality are at least as old as those of fish, and (2) to discuss the significance of the maintenance of dimorphic laterality in most populations. Specifically, we direct our attention to predator–prey interactions in biotic communities as the primary factor responsible for the maintenance of dimorphism of laterality in fish.

The first and most notable example of laterally asymmetric bodies in fishes was found in scale-eating cichlids in Lake Tanganyika (Liem and Stewart, 1976; Hori, 1991, 1993; Hata et al., 2013), in which laterality was associated with hunting techniques. Each population consists of righty (the right side of the head faces slightly forward, and the mouth opens to the left) and lefty (*vice versa*) individuals. Righty (lefty) individuals attack the right (left) sides of their prey fish from behind to tear off scales (Hori, 1993; Takeuchi et al., 2012, 2016). The behavioral laterality associated with bilateral asymmetry has also been found in fishes from a wide range of trophic levels and taxa, including cichlids (Hori et al., 2007; Takeuchi and Hori, 2008), gobies (Seki et al., 2000; Yasugi and Hori, 2011), bettas (Takeuchi et al., 2010), largemouth bass (Nakajima et al., 2007; Yasugi and Hori, 2011), characins (Hata et al., 2011), poeciliids (Matsui et al., 2013), and anglerfish (Yasugi and Hori, 2016). Nearly all of the individuals in these studies could be classified as either righty or lefty individuals.

Laterality appears to have a genetic basis, as indicated by studies on parents and their offspring in wild species (a goby: Seki et al., 2000; two cichlids: Hori et al., 2007) and in breeding experiments (a cichlid: Hata et al., 2012; medaka: Hata et al., 2012; and zebrafish: Hata and Hori, 2012), in which righty and lefty offspring were born in a Mendelian ratio with left dominant over right. Additionally, genetic studies have identified genomic loci corresponding to mandibular asymmetry (Stewart and Albertson, 2010).

The degree of laterality in individuals has been quantified based primarily on two measures: (i) differences between the right and left mandibles (Hori, 1993; Seki et al., 2000; Takeuchi and Hori, 2008; Yasugi and Hori, 2011; Takeuchi et al., 2012; Hata et al., 2013), more specifically, the height of the posterior ends of the right and left mandibles (HMPE) (Hori et al., 2007; Hata et al., 2013) (Fig. 1A), and (ii) the angle θ formed by the head and vertebrae (Hata et al., Yasugi and Hori, 2011; Nakajima et al., 2007) (Fig. 1B), in which the head of each individual inclines laterally, such that one side of the head faces forward (Hori et al., 2007). Laterality identified in every individual using these two methods has been consistent (Yasugi and Hori, 2011; Hata et al., 2013).

In general, lateral asymmetry in species or populations can be divided into three categories based on the frequency distribution of measured laterality: fluctuating asymmetry (FA) with a unimodal and symmetrical distribution, directional asymmetry (DA) with a unimodal distribution shifted from symmetry, and anti-symmetry (AS) with a bimodal distribution (Palmer and Strobeck, 1992). Recent studies examining the asymmetry of fishes have shown bimodal distributions, suggesting AS (Hori et al., 2007; Nakajima et al., 2007; Takeuchi and Hori, 2008; Seki et al., 2000; Takeuchi et al., 2010, 2012, 2016; Hata et al., 2011, 2013; Yasugi and
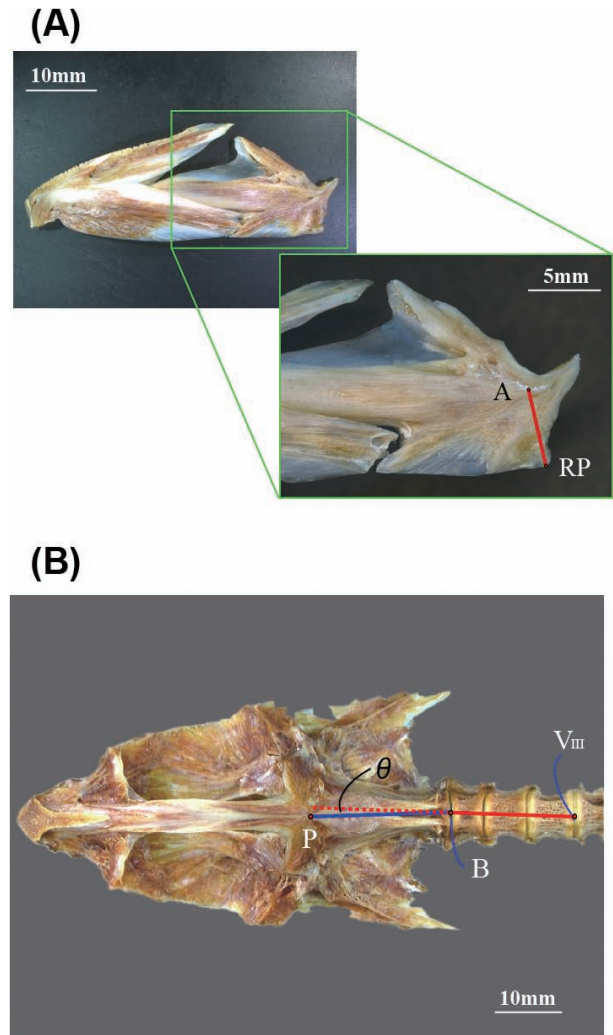

(A)

(B)

Fig. 1. Bone measurements used to identify fish laterality. **(A)** The height of the mandible at the posterior end (HMPE) is the distance from A (the deepest point on the suspensoriad articulation facet of the anguloarticular) to RP (the ventral corner of the retroarticular process). **(B)** Ventral view of a skeleton showing data points for head angle (θ), defined as the angle between the line extended from V_{III}–B (dashed red line) and P–B. B: the sagittal posterior end of the basioccipital; P: the center of the posterior portion of the parasphenoid; V_{III}: the sagittal posterior end of the third vertebra. Both panels show the Japanese amberjack (*Seriola quinqueradiata*).

Hori, 2011, 2012; Matsui et al., 2013). Here, to ascertain the type of asymmetry, we examined frequency distributions of two measures in two species of bony fishes—amberjack and catfish—as arbitrary representatives of dominant taxa in marine and freshwater environments, respectively, as well as in one cartilaginous fish, a stingray.

Next, to assess the prevalence of lateral asymmetry among fishes and to identify trends in the degree of laterality during evolution, we measured the laterality of individuals from at least one arbitrary representative species from all 62 extant orders of fishes (Nelson, 2006). The species examined and the measurements of the 62 orders are listed in the electronic supplementary material (Supplementary Table S1). We measured mandibular asymmetry and head angle

(as described above) in 10–12 individuals from one to several populations of each species, except for five rare species in which only a few individuals ($n < 6$) were available.

MATERIALS AND METHODS

Collection of fish specimens

To define the frequency distribution of laterality, we collected approximately 100 individuals each of Japanese amberjack (*Seriola quinqueradiata*, Perciformes), Amur catfish (*Silurus asotus*, Siluriformes), and Japanese stingray (*Dasyatis akajei*) as arbitrary representatives of dominant marine and freshwater bony fish taxa, and a marine cartilaginous fish, respectively. Next, to determine the presence and degree of asymmetry in each fish order, we chose at least one arbitrary representative species from all 62 orders of extant fishes based on their availability. We then collected 10–12 individuals from one to several populations of each species, except for five rare species in which only a few individuals ($n < 6$) were available (see Supplementary Table S1 for details). Animal care and operations were performed in accordance with the Regulation on Animal Experimentation at Kyoto University.

Measurements of morphological laterality in fish specimens

Laterality of gnathostome fishes was quantified by two measures: the difference in the height of the mandible at the posterior end (HMPE) of the right and left mandibles (Fig. 1A for bony fishes and electronic supplementary material, Supplementary Figure S2A for cartilaginous fish) and the angle from the vertebrae to the head (θ in Fig. 1B for bony fishes and Supplementary Figure S2B for cartilaginous fishes) following Hata et al. (2013). In cartilaginous fishes (except in chimaeras), the sustentaculum functions as the mandibular surface of the hyomandibular-mandibular joint, and the nodule on the ventral corner of the mandibular cartilage (VC) functions as the attachment point of the depressor mandibularis (Dean and Motta, 2004; Wilga, 2005), such that the HMPE provides a functional measure equivalent to that of bony fishes. Despite its taxonomic position, the sturgeon is grouped with cartilaginous rather than bony fishes in this paper, as its skeleton is cartilaginous and the mechanical structure of its mouthparts resembles that of cartilaginous fishes (Carroll and Wainwright, 2003).

To prepare skeletal material, samples of all specimens, except those of the coelacanth, were gently boiled, and tissues were removed carefully by hand. For agnathan fishes (hagfish and lamprey), the mouthparts (a pair of spinose cartilage and flat cartilaginous bones, respectively, which may be homologous to the mandible of gnathostome fishes; Kuratani, 2012) were measured (Supplementary Figures S5A and S6A). We also measured the angle from the vertebrae to the head (Supplementary Figures S5B and S6B). Specimens of agnathan fishes were moderately boiled to expose mouthparts for measurement and then transparentized to measure θ. On each skeletal specimen of both gnathostome and agnathan fishes, the data points were marked with a fine pen under a binocular microscope, and HMPE and θ were measured using a digital microscope (VHX-100, Keyence Co., Inc., Osaka, Japan) to within 0.005 mm and 0.05° of accuracy, respectively. Data were measured horizontally using a level scope attached to a monocular microscope of our own design. Specifically, specimens were secured with a small clip that had a universal joint connected to a base comprising a magnet, which was then placed on a two-dimensional gonio-stage. Manipulating the gonio-stage and monitoring via the level scope kept the specimens level. We used the median of three measurements from each specimen. Each measurement was obtained by repositioning the specimen on the microscope stage. For the coelacanth, computerized axial tomography (CAT) scan images of two frozen specimens stored at the Tokyo Institute of Technology were supplied, and the two measures were obtained using a DICOM viewer (INTAGE Realia, K.G.T., Inc.,

Tokyo, Japan). The index of asymmetry (IAS) for the difference between right and left mandible heights was given by $[2 \times (R - L) / (R + L)] \times 100$, where R and L are the right and left HMPE, respectively. Fish with positive IAS values and θ were defined as righty, and those with negative values were defined as lefty. Note that the definition of laterality used here and in recent studies differs from that used in earlier papers (Hori, 1991, 1993; Seki et al., 2000), which defined individuals with the mouth opening to the right as 'right-handed' or 'dextral'. The terminology used in the present study, 'lefty', reflects the fact that the left mandible of such 'right-handed' fish is larger than the right mandible (Hori et al., 2007; Takeuchi and Hori, 2008; Yasugi and Hori, 2011, 2012), and that the left eye is dominant (Takeuchi et al., 2010).

Classification and statistical examination of asymmetry

The asymmetries of morphological characteristics were categorized as FA, DA, or AS for each species using the frequency distributions of the laterality indices. To define the asymmetries of the IAS and θ in each species, we fitted three models to the frequency distributions of our measurements: (1) FA model, which assumes a normal distribution with a mean of 0 and standard deviation (s.d.) equal to the s.d. of the data; (2) DA model, which assumes a normal distribution of both the mean μ ($\neq 0$) and s.d. of the data; and (3) AS model, which assumes two normal distributions with the same s.d. and means at $\pm\mu$ (bimodal distribution). The two normal distributions with means $+\mu$ and $-\mu$ correspond to the frequency distributions of righty and lefty fish, respectively. The means, s.d., and ratios of righty to lefty individuals in each model were estimated by maximum likelihood. For each species, we calculated the Akaike information criterion (AIC) of the three models using the R package IASD 1.0.7. The models with the lowest AIC values were selected as the best explanations of the asymmetries in morphological characteristics.

Phylogenetic grouping of fishes

The classification of extant fish orders used here follows Nelson (2006). For evolutionary analysis, we categorized orders within Osteichthyes (bony fishes) as follows: (1) sarcopterygians (lobe-finned fishes), (2) primitive actinopterygians (ray-finned fishes), and (3) lower, (4) intermediate, and (5) higher teleosts (one of three infraclasses of ray-finned fishes). These categories were based on those proposed by Nelson (2006), but are slightly modified in light of recent phylogenetic hypotheses, as shown in Supplementary Figure S1 (Betancur-R et al., 2013). Although Nelson et al. (2016) changed their classification of extant orders from those in Nelson (2006), the fish categories mentioned above are consistent with those of the former (group memberships are shown in Fig. 3A and Supplementary Table S1). Within the teleosts, we also categorized fishes into an ecological group 'deep-sea fishes' (those living at depths below 200 m) due to their distinctive degrees of asymmetry (see Discussion). The degree of laterality of each category was calculated as the average distance for laterality intensity (DLI), which was measured from the origin to a point (x, y) representing each species, where x is the standardized absolute value of IAS and y is the standardized absolute value of θ.

Degree of lateral asymmetry during growth

To compare the degree of lateral asymmetry among fishes of various ages, we collected 60 largemouth bass (*Micropterus salmoides*) individuals of various lengths from a single population in Lake Biwa, Japan, in 2004–2008. We measured the standard length, fish age by counting circuli on the opercular bone (Menon, 1950; Campana, 2001), IAS, and θ of every individual. Then we used analysis of variance (ANOVA) to assess the effects of age on IAS and θ.
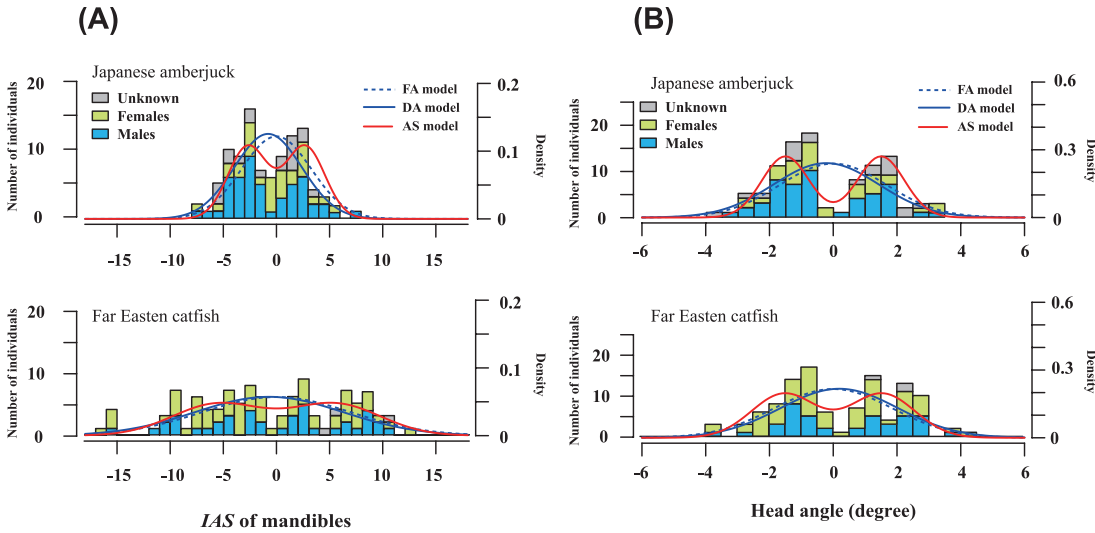
Fig. 2. Frequency distributions of **(A)** the index of asymmetry (*IAS*) of the mandibles and **(B)** head angle (*θ*) of the Japanese amberjack (*Seriola quinqueradiata*, *n* = 100; upper) and Amur catfish (*Silurus asotus, n* = 110; lower). Lines show the probability densities derived from the three models (see Methods). The model selected by the lowest Akaike information criterion (AIC) value is indicated by the thick line.
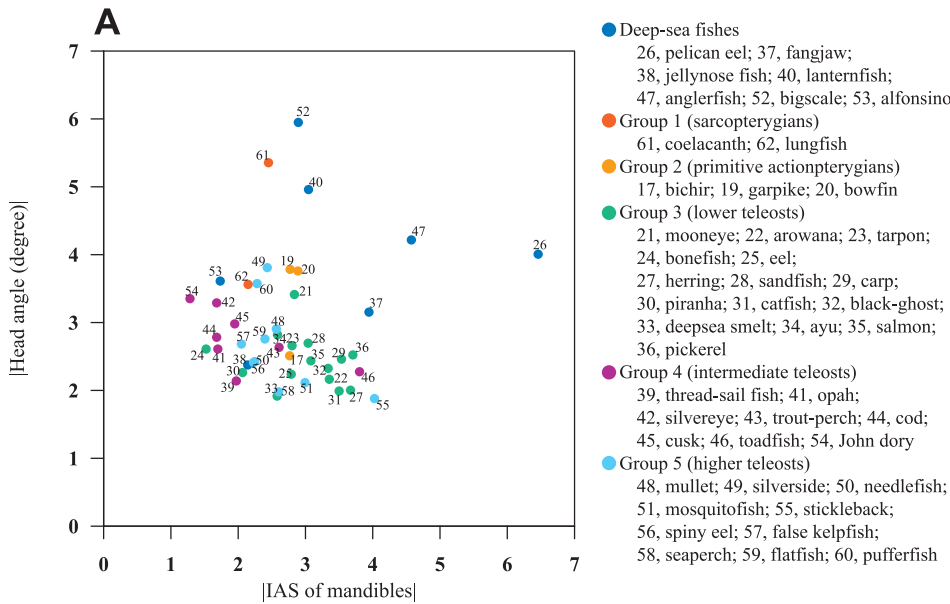
● Deep-sea fishes
    26, pelican eel; 37, fangjaw;
    38, jellynose fish; 40, lanternfish;
    47, anglerfish; 52, bigscale; 53, alfonsino
● Group 1 (sarcopterygians)
    61, coelacanth; 62, lungfish
● Group 2 (primitive actionpterygians)
    17, bichir; 19, garpike; 20, bowfin
● Group 3 (lower teleosts)
    21, mooneye; 22, arowana; 23, tarpon;
    24, bonefish; 25, eel;
    27, herring; 28, sandfish; 29, carp;
    30, piranha; 31, catfish; 32, black-ghost;
    33, deepsea smelt; 34, ayu; 35, salmon;
    36, pickerel
● Group 4 (intermediate teleosts)
    39, thread-sail fish; 41, opah;
    42, silvereye; 43, trout-perch; 44, cod;
    45, cusk; 46, toadfish; 54, John dory
● Group 5 (higher teleosts)
    48, mullet; 49, silverside; 50, needlefish;
    51, mosquitofish; 55, stickleback;
    56, spiny eel; 57, false kelpfish;
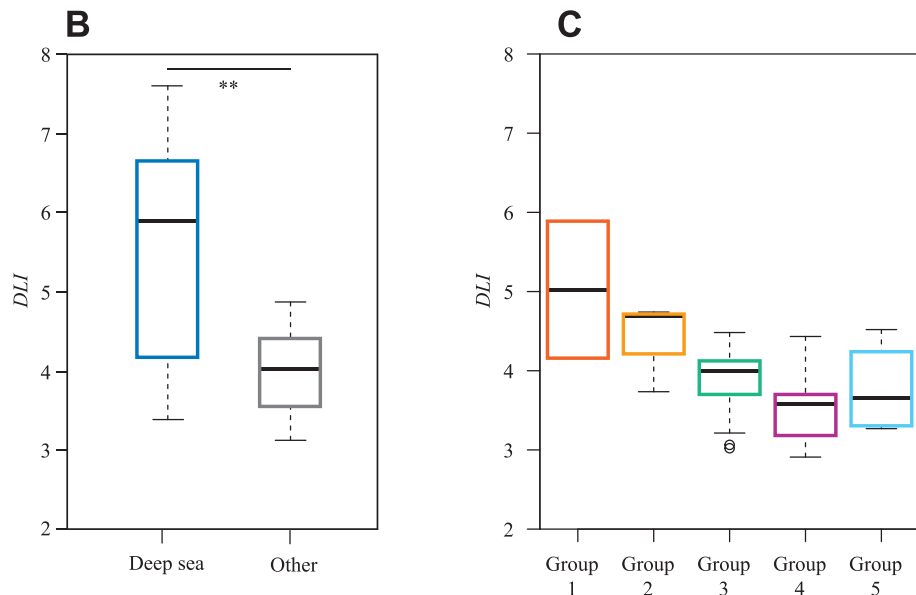   58, seaperch; 59, flatfish; 60, pufferfish

Fig. 3. Degrees of laterality among extant teleost fishes. **(A)** Laterality quantified by the absolute values of the index of asymmetry (*IAS*) of the mandibles and the head angle (*θ*) in each representative species from all extant orders of teleost fishes. Index numbers correspond to the extant orders of fishes in Nelson (2006). Species are grouped into six categories (deep-sea fishes and five phylogenetic groups) indicated by colors. Each axis was standardized by the mean and standard deviation (s.d.) of all original values. **(B)** Comparison of the distance of laterality intensity (*DLI*) between the seven species of deep-sea fishes and the other teleost fishes (range, outlier, lower and upper quartiles, and median). P-values are from the Wilcoxon rank sum test. **, $P <$ 0.01. **(C)** Comparison of *DLI* among five phylogenetic groups (range, outlier, lower and upper quartiles, and median).

RESULTS

Types of asymmetry

Our measurements of amberjack and catfish were best explained by the AS model, which indicates that the focal laterality in both species was AS. Histograms of mandibular asymmetry and head angle showed bimodal distributions in both sexes, with few individuals near 0 (Fig. 2). AS was the best-fitting model for these distributions among the three asymmetry types, per the AIC values. AS has been detected in the laterality of several fish orders (Takeuchi and Hori, 2008; Yasugi and Hori, 2011; Hata et al., 2011, 2013; Matsui et al., 2013) and was also found in a cartilaginous fish, the Japanese stingray, in the present study (Supplementary Figure S2).

Except for the six species with small sample sizes, every species examined was composed of both righty and lefty individuals, and the absolute degree of laterality was always greater than 0 for both measures (Supplementary Table S1). The degree of laterality measured in the present study did not appear to change during growth (Supplementary Figure S3). The distributions of both measures were mostly explained by the AS model, with only three exceptions in mandibular asymmetry (Supplementary Table S1). Among the exceptions, two showed DA, which is likely due to biased proportions of morphs among the samples (one or two individuals of minority morph). The third exception was a flatfish (Pleuronectiformes), which showed FA. However, our measurements of head angle in flatfishes demonstrated that each population was composed of both righty and lefty individuals. Flatfishes have DA bodies with both eyes on the same side of the face. The upper and lower jaws on the side with the eyes are usually shorter than those on the opposite side. The *IAS* of the mandibles in flatfishes may be biased by the restricted form of the mouthparts (Supplementary Figure S4). In the likelihood ratio test between FA and AS, AS was significantly better than FA for both measures among all species with ≥10 samples (Supplementary Table S1). Therefore, all fishes may exhibit laterality.

Although agnathans have no mandibles, their mouthparts and head angle showed AS distributions (Supplementary Figures S5 and S6). Therefore, the phylogenetic occurrence of focal laterality may date back to the origin of fishes.

Trends in fish phylogeny

The absolute values of our two laterality measures (*IAS* of the mandibles and θ) in all orders of extant bony fishes revealed functional and evolutionary trends (Fig. 3A). First, deep-sea fishes had high levels of asymmetry except for one species (jellynose fish). The distances from the origin of the two axes in Fig. 3A to the points representing each species (*DLI*) were significantly greater for the seven deep-sea fishes than for the other fishes (Wilcoxon rank sum test, n = 45, P = 0.010; Fig. 3B). Because the exploitation of extreme depths evolved independently among deep-sea fishes (Nelson, 2006), extreme asymmetry seems adaptive for life in the deep sea, as discussed below. Second, the absolute values of *IAS* and θ in other fishes had a significant negative correlation (Spearman's rank correlation test, n = 38, ρ = −0.402, P = 0.012; Fig. 3A), suggesting trade-offs between the two structures in developing laterality.

'Ancient fishes,' such as lungfish, coelacanths, and gars, had greater *DLI*s than 'modern fishes,' such as perciforms and scorpaeniforms. We categorized bony fishes (except the deep-sea fishes) into five phylogenetic groups (see Methods). The *DLI* values had a significant negative correlation with the phylogenetic order of the groups (Spearman's rank correlation test, n = 38, ρ = −0.352, P = 0.030; Fig. 3C), implying that the degree of morphological laterality was reduced progressively during the evolution of bony fishes. The phylogenetic trends in laterality among cartilaginous fishes are shown in Supplementary Figure S7; more derived species, such as rays and skates, had lower *DLI* values.

DISCUSSION

Our results indicate that AS laterality is universally shared among fishes, but that the degree of asymmetry has become increasingly weaker in more derived groups. However, functional (behavioral) laterality seems not to be reduced even in derived groups, since many fishes have prominent behavioral laterality, including cichlids (Hori, 1993; Takeuchi and Hori, 2008), gobies (Seki et al., 2000), largemouth bass (Nakajima et al., 2007; Yasugi and Hori, 2011), and bettas (Takeuchi et al., 2010), all of which belong to derived groups. This cryptic trend in morphological laterality should be considered in depth. Here, we discuss 1) why morphological and behavioral laterality has developed in fish and why fish have AS rather than DA laterality; 2) the relationship between the two measures we used to evaluate morphological laterality, its implications for body form, and how this relates to the progressive weakening of morphological laterality in fish evolution; 3) the origin and legacy of fish laterality; and 4) the relationship between laterality in fishes and that in aquatic invertebrates.

Behavioral advantage has been proposed as a driving force behind lateral asymmetry among fishes. The general fish body plan facilitates a bilateral motor pattern. The body is propelled by side-to-side undulations. When a fish makes a dashing movement, it first bends its body either to the right or left and subsequently straightens it (Wilga, 2005). The locomotive speed of this initial motion is crucial in predator–prey interactions (Cantalupo et al., 1995; Takeuchi et al., 2012; Yasugi and Hori, 2012). If one side of the body is mechanically dominant, i.e., has more muscle and effective support tissue than the opposite side, the initial use of this side can produce stronger propulsion and higher speed than that generated by non-specialists with completely symmetrical bodies (Takeuchi et al., 2012). Therefore, morphological asymmetry and laterally disproportionate usage (behavioral laterality) may be associated and mutually reinforcing.

Although asymmetrical bodies seem advantageous, why are fish dimorphic rather than monomorphic? Behavioral laterality inevitably leads to biased directions of attack (Nakajima et al., 2007; Yasugi and Hori, 2012) and evasion (Izvekov et al., 2009; Yasugi and Hori, 2012). These biases may provoke negative, frequency-dependent selection. For instance, of the two lateral morphs among scale-eaters, the numerically dominant morph was more likely to fail at feeding than the less common morph, probably because prey fishes are more vigilant on the side more frequently attacked by the major morph (Hori, 1993; Takahashi and Hori, 1994, 1998). Among pursuit-type piscivores, predation success

was higher if the laterality of the predator and its prey were opposite (so-called 'cross predation') than if they were the same ('parallel predation') (Yasugi and Hori, 2011, 2012). Conversely, when luring-type piscivores face prey fish during a predation event, parallel predation was more successful (Yasugi and Hori, 2016). This difference in success also seems to cause an alternating advantage between righties and lefties in both predator and prey fishes and results in the dynamic maintenance of laterality (Nakajima et al., 2004). This advantage is shared by predators that attack the same prey fishes and by prey fishes that share a common predator (Takahashi and Hori, 2005). Natural selection through predator-prey interactions may play a major role in maintaining anti-symmetric dimorphism in fish communities (Stearns and Hoekstra, 2000; Hedrick, 2005; Sinervo and Calsbeek, 2006). We suggest that this is the very reason why morphological laterality has progressively weakened during fish evolution, which we discuss below.

The two measures of fish laterality used here represent the most distinctly asymmetric features. The morphological implications of head angle are intuitive. If the body is asymmetric due to differential development of the right and left sides, the more developed side may be structurally dominant over the opposite side. The more developed side should, therefore, be convex, as suggested in Fig. 1. Zebrafish were found to have a trunk muscle volume that was laterally biased in relation to the laterality of the fish (Heuts, 1999), although there was a significant bias towards escaping in one direction at the population level. The morphological implication of the *IAS* of the HMPE can be also understood by applying the principle of leverage; i.e., the HMPE acts as a line between the effort point (i.e., where the ligament is attached) and the fulcrum point of the lever (i.e., the articulation of the mandible) (Westneat, 1990). Thus, the difference between the right and left HMPE may cause the force and speed of opening to differ between the right and left mandibles, causing the mouth to twist as it opens. Because of this morphological asymmetry, the fish can open its mouth toward one side to a greater extent than through head inclination alone. In any lateralized behavior, these factors may work together. For example, in the shrimp-eating cichlid (*Neolamprologus fasciatus*; Takeuchi and Hori, 2008) and the scale-eating cichlid (*Perissodus microlepis*; Takeuchi et al., 2016), each morph exhibits an advantage (greater hunting success) when the fish uses the dominant side of its body during foraging. The direction to which the body was bent, the eye used to aim at the target, and the dominant side of the mandible were all consistent.

The negative relationship between the two measures found among extant bony fishes (Fig. 3A) suggests a trade-off between asymmetry in the mandibles and head angle. Although this relationship may reflect the constraints imposed by morphogenesis, it may be ultimately affected by differences in lifestyles and modes of locomotion among fish groups. Fishes with a large head angle, such as silvereye, John Dory, and puffer fish (#42, 54, and 60, respectively, in Fig. 3A), are deep-bodied, whereas those with high *IAS* values, such as herring, salmon, and pickerel (#27, 35, and 36, respectively, in Fig. 3A), have slender bodies (defined as having a high body length to diameter ratio). Perciforms are highly variable in body shape. Among them, slender fishes,

such as amberjack, are adapted to prolonged high-speed swimming and a pelagic lifestyle, whereas deep-bodied fishes, such as sea bream, are adapted to quick turns and a demersal lifestyle (Azuma, 2006). This relationship between body shape and swimming style may apply to all bony fishes except those with extreme body shapes, such as deep-sea fishes and eels. Thus, we propose that deep-bodied fishes are suited to tilting the head to either side during asymmetric behaviors, whereas slender fishes are suited to having asymmetric mandibles due to their streamlined bodies and smaller head angles adapted to rapid and prolonged cruising. A more symmetrical bodyline is adaptive for high-speed swimming (Gosline, 1971). One of the fastest-moving fishes, the flying fish *Prognichthys brevipinnis*, has the smallest head angle (Supplementary Table S1).

The universality of morphological laterality in fishes suggests a monophyletic origin. We hypothesize that the evolution of fish laterality began with lancelets or agnathans, and that laterality has been maintained over the course of fish evolution. Ecological factors, particularly predator–prey interactions, are likely key drivers in the evolution of lateral dimorphism in every fish population, which should be maintained by frequency-dependent selection mediated through minority advantage (Hori, 1993; Stearns and Hoekstra, 2000; Hedrick, 2005; Sinervo and Calsbeek, 2006). Although laterality is inevitable for effective locomotion in every fish species, they must also conceal their own laterality from appearance, as such laterality would indicate a preferred attacking direction of predators or escape direction of prey. Laterality may therefore have become increasingly cryptic during fish evolution. In this context, the extreme asymmetry in deep-sea fishes (Fig. 3A) may be explained as follows; since they live in the dark and rely only slightly on vision, they have little need to conceal their morphological laterality.

In addition to the origin of laterality, the remarkable laterality of sarcopterygians (coelacanths and lungfish; #61 and 62, respectively, in Fig. 3A), which share a common ancestor with terrestrial tetrapods (amphibians, reptiles, birds, and mammals), provides another implication for the universality of laterality, particularly with respect to the traits inherited by tetrapods from fish. Lateralized behaviors have been evaluated in animals ranging from fish to mammals (Roger and Andrew, 2002) with emphasis on brain lateralization, which is thought to facilitate enhanced performance or faster responses (Vallortigara and Rogers, 2005; Dadda et al., 2010). By contrast, the laterality of behaviors in fishes, including hunting techniques (Hori, 1993; Nakajima et al., 2007; Takeuchi and Hori, 2008; Hata et al., 2011; Yasugi and Hori, 2012; Takeuchi et al., 2012, 2016), scouting behaviors (Matsui et al., 2013), and social behaviors (Takahashi and Hori, 2008; Takeuchi et al., 2010), are correlated with the morphological laterality that we have demonstrated here. This documentation of morphological laterality in fish may lead to a deeper understanding of laterality in other vertebrates.

Morphological AS has also been known in aquatic invertebrate groups, including Arthropoda and Mollusca (Palmer, 2005). Our recent studies on shrimp (Takeuchi et al., 2008), crayfish (Tobo et al., 2012), and cuttlefish (Lucky et al., 2012) demonstrated that their morphological AS is associated with behavioral laterality, which is involved with escaping from

predators or attacking prey. Like fish, these animals move actively in aquatic environments. They suffer minimal effects from gravity due to buoyancy, which may allow them to develop bilateral body asymmetry. By contrast, in terrestrial environments, the effect of gravity may force animals to have a laterally symmetric body supported by limbs in a balanced arrangement. Morphological laterality in terrestrial animals may thus have been weakened, although functional (behavioral) laterality seems to remain, as many studies indicate (e.g., Rogers and Andrew, 2002). Although it is difficult to speculate on the phylogenetic relationship of laterality between aquatic invertebrates and fishes, it is possible that all members of Bilateria share laterality as a homology, given the commonness of morphological laterality among invertebrate phyla (Palmer, 2005). In addition, Arthropoda and Mollusca are phylogenetically older than fishes (Moody and Zhuravlev, 2001), and behavioral laterality seems to have already existed among Cambrian trilobites and/or their predators (Babcock, 1993); fish did not appear until the end of this period. Therefore, it is possible that laterality developed first in Arthropoda, and then probably in Cephalopoda (e.g., Nautiloidea in the Ordovician: Babcock, 1993). The Cambrian also saw the explosive development of predator–prey interactions, which were driven by newly acquired eyesight in most animal phyla (Gould, 1989). We speculate that the development of the anti-symmetric laterality in Bilateria may be closely linked to such interspecific interactions where appearance is crucial to detect prey and/or escape from predators. Although it is unclear whether the laterality of fish is homologous with that of aquatic invertebrates, the anti-symmetric laterality of fish may have been developed through predator-prey interactions in ancient aquatic communities. Understanding the laterality of fish may also shed new light on the community structures of aquatic ecosystems.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

MH planned and headed the project, and wrote the manuscript. MNakajima collected the materials and refined the manuscript. MH, HH, MY, MNakae, KY, MK, JK, MM, HT, and YT collected materials and carried out the measurements, ST and HT cover the statistical examination, and NO obtained coelacanth specimens and their CT image data. All authors discussed the results and commented on the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online (URL: http://www.bioone.org/doi/suppl/10.2108/zs160196).

Supplementary Table S1. List of species analyzed and model-fitting results.

Supplementary Figure S1. Classification and phylogenetic tree of the extant orders of teleost fishes used in this study.

Supplementary Figure S2. Laterality of the Japanese stingray (*Dasyatis akajei*).

Supplementary Figure S3. Relationship between growth and laterality in largemouth bass (*Micropterus salmoides*).

Supplementary Figure S4. Laterality of the flatfishes.

Supplementary Figure S5. Laterality of the hagfish.

Supplementary Figure S6. Laterality of the lamprey.

Supplementary Figure S7. Degrees of laterality in extant cartilaginous fish orders and sturgeons.

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