

Supplementary information

Laterality is universal among fishes but increasingly cryptic among derived groups

Supplementary Table 1. List of the species analyzed and the model-fitting results. Fish species analyzed as representatives of each extant fish orders are listed with both Latin and common names, sample sizes, Akaike information criterion (AIC) values of the model fitted to the two measurements (bold: AIC of the selected model), sampling locations, and sources. *P*-values in LRT are from likelihood ratio test between FA model and AS model. Common names follow those used by FishBase (<http://www.fishbase.org/>). The index numbers of fish orders follow the classification used in Nelson (2006). Phylogenetic groupings for teleosts in this study, however, are slightly modified from Nelson's system based on Betancur-R et al. (2013).

Supplementary Figure 1. Classification and phylogenetic tree of the extant orders of teleost fishes used in this study. Phylogenetic trees are modified following Betancur-R et al. (2013). The classification is based on Nelson (2006), with a slight modification following Betancur-R et al. (2013) as shown in Supplementary Table 1 (colors are the same as those in Fig. 3A). The blue plot shows deep-sea fishes.

Supplementary Figure 2. Laterality of the Japanese stingray (*Dasyatis akajei*). (A, B) Cartilaginous bones showing the data points used for measurements. (A) Ventral view of the jaw arch and a close-up of the left mandibular cartilage showing the data points for the index of asymmetry (*IAS*) of the mandibles. The arrow indicates the height of the mandible at the posterior end (HMPE), which is the distance from the postero-dorsal edge of the sustentaculum in the mandibular cartilage (PE) to the ventral corner of the mandibular cartilage (VC). In cartilaginous fishes (except in chimaeras), the sustentaculum functions as the mandibular surface of the hyomandibular-mandibular joint, and the nodule on the 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 teleost fishes. (B) Ventral view of head and vertebrae showing data points for the head angle (θ), which is the angle between the head and vertebrae on the ventral side of the anterior part of the skeleton. CC: the small foramen near the center of the cranial cartilage; PC: the midline of the postero-ventral margin of the cranium; PV: the first large process on the ventral sagittal line of the vertebra. (C, D) Frequency distributions of the (C) *IAS* of the mandibles and (D) θ in the Japanese stingray. 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.

Supplementary Figure 3. Relationship between growth and laterality in largemouth bass (*Micropterus salmoides*). The absolute values of the index of asymmetry (*IAS*) of mandibles and the head angles (θ) in three size classes did not differ significantly (ANOVA, $P = 0.2379$ for *IAS* and $P = 0.7791$ for θ). Individuals were sampled from the same population in Lake Biwa, Japan, in 2004–2008. Vertical bars indicate standard deviations (s.d.). Small fish were primarily 0⁺ ($n = 20$, standard length [SL; mean \pm s.d.] = 76.1 \pm 4.1

mm), medium fish were primarily 1⁺ ($n = 20$, SL = 125.1 \pm 13.6 mm), and large fish were primarily $\geq 2^+$ ($n = 20$, SL = 173.4 \pm 16.8 mm).

Supplementary Figure 4. Laterality of the flatfishes. Frequency distributions of the (A) index of symmetry (*IAS*) of the mandibles and (B) head angles in flathead flounder (*Hippoglossoides dubius*; upper) and olive flounder (*Paralichthys olivaceus*; lower). The lines are the same as those used in Figure 2. In both species, the model with the best fit to the distributions of *IAS* was fluctuating asymmetry, whereas the best model for head angle was anti-symmetry (see Supplementary Table 1).

Supplementary Figure 5. Laterality of the hagfish. (A, B) Data for measurements from inshore hagfish (*Eptatretus burgeri*). (A) Ventral view of the mouthparts of a moderately boiled fish sample. A pair of flat cartilaginous structures behind the tooth plates is exposed. FC: the length of the flat cartilaginous structure used for the index of asymmetry (*IAS*). (B) Dorsal view of the anterior part of the body in a transparent preparation showing data for the head angle. CN: the center of the cartilaginous rings of the nasal tube; MC: the posterior end of the membranous cranium; DP: the dorsal datum point equidistant from MC to CN–MC on the sagittal line. Head angle (θ) is defined as the angle of the line extending anteriorly from MC–DP to CN–MC. A fish with a clockwise positive angle is defined as left type, whereas a fish with a negative angle is defined as right type. The definitions of right and left types are consistent with those shown in the ventral view of Figure 1. (C, D) Frequency distributions of (C) *IAS* of the flat cartilaginous structure and (D) θ in inshore hagfish. The lines are the same as those used in Figure 2. Both distributions showed anti-symmetry (see Supplementary Table 1).

Supplementary Figure 6. Laterality of the lamprey. (A, B) Data points used for measurements in arctic lamprey (*Lampetra japonica*). (A) Ventral view of a mouthpart in which a pair of spinose cartilaginous structures behind the suction cup are exposed in a moderately boiled fish sample. SC: the length of the spinose cartilage used for the index of asymmetry (*IAS*). (B) Dorsal view of the anterior part of the body in a transparent specimen showing the data points used for head angle (θ) on the ventral sagittal line. PD: the anterior end of the posterior dorsal cartilage; PO: the posterior end of the postero-occipital cartilaginous ring; DP: the dorsal datum point equidistant to PD–PO. θ is defined as the angle from the line extending anteriorly from PO–DP to PD–PO. A fish with a clockwise positive angle is defined as left type, whereas a fish with a negative angle is defined as right type. (C, D) Frequency distributions of the (C) *IAS* of spinose cartilaginous structures and (D) θ in arctic lamprey. The lines are the same as those in Figure 2. Both distributions showed anti-symmetry (see Supplementary Table 1).

Supplementary Figure 7. The degree of laterality in extant cartilaginous fish orders and sturgeons. Two measures of the laterality, the index of asymmetry (*IAS*) of the mandibles and the head angle, are displayed as in Figure 3A. Fish species are grouped into four color-coded categories.

Supplementary References

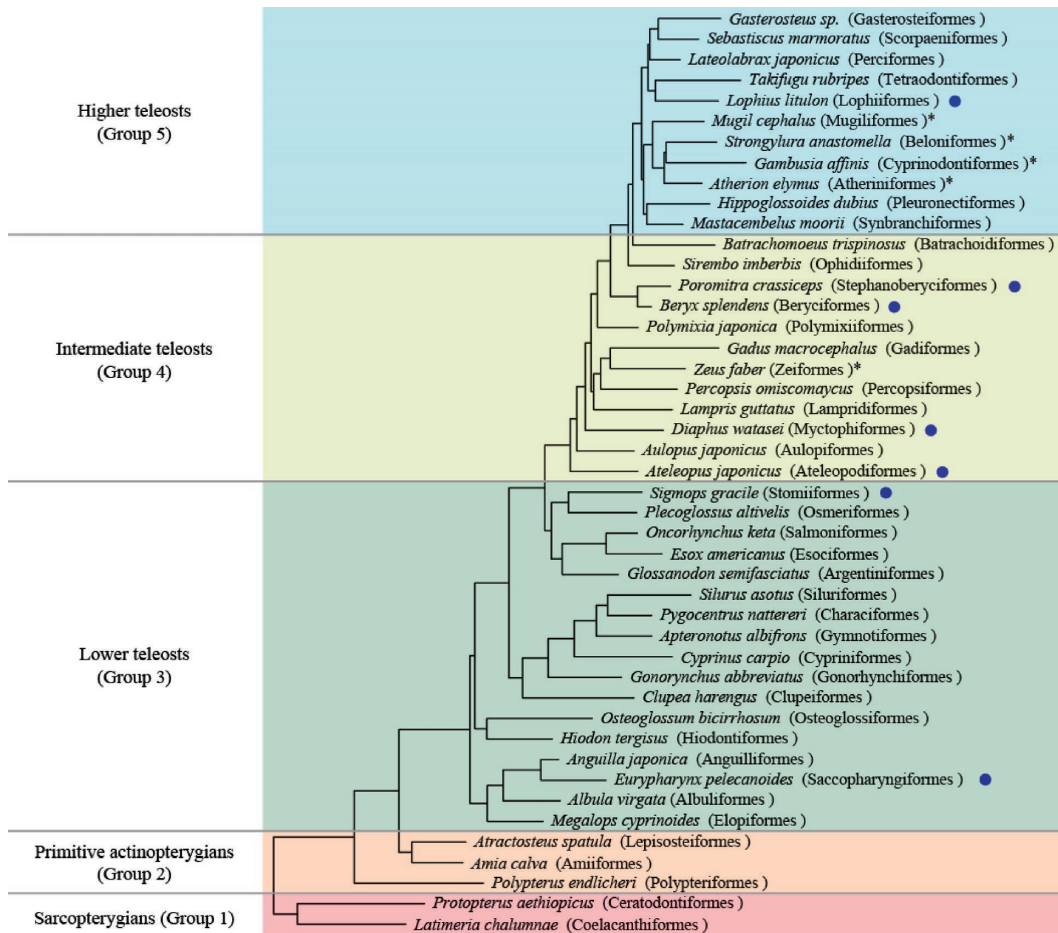
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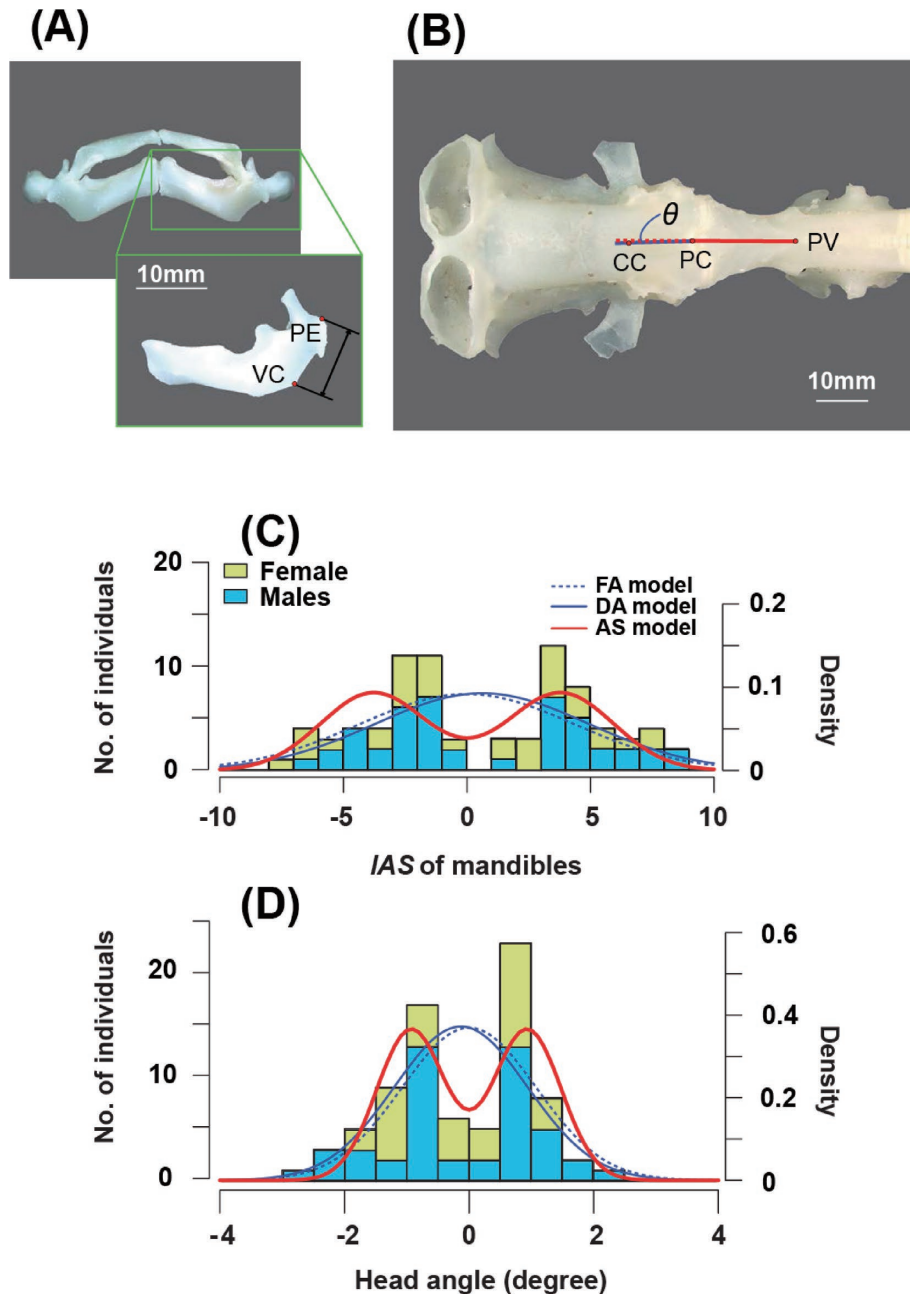
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Supplementary Table S1. List of species analyzed and model-fitting results. Fish species analyzed as representatives of each extant fish orders are listed with both Latin and common names, sample sizes, Akaike information criterion (AIC) values of the model fitted to the two measurements (bold: AIC of the selected model), sampling locations, and sources. P-values in LRT are from likelihood ratio test between FA model and AS model. Common names follow those used by FishBase (<http://www.fishbase.org>). The index numbers of fish orders follow the classification used in Nelson (2006). Phylogenetic groupings for teleosts in this study, however, are slightly modified from Nelson's system based on Betancur-R et al. (2013).

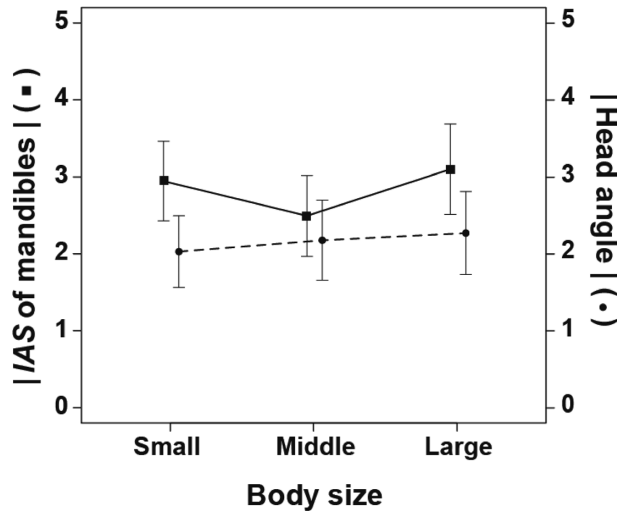
No. of order #	Group in Fig. 3	Order Name	Species Name †	English Name	Sample size * n	IASI mean	S.D.	length mean	S.D.	D/L	AIC in IAS of mandibles Fmodel	AIC in IAS of mandibles Dmodel	LRT p [‡] or AS to FA	AIC in angle between the head and vertebrae Fmodel	AIC in angle between the head and vertebrae Dmodel	LRT p [‡] or AS to FA	Locality of materials	Source of materials	
1	-	Mxyciformes	<i>Engraulis mordax</i>	Inshore bayfish	11(5)	3.65	1.09	1.82	0.76	0.76	62.63	64.21	52.36	<0.001	44.42	50.17	48.18	0.016 The Pacific of southern	Collected by ourselves
2	-	Percyiformes	<i>Chimarra phantasma</i>	Arctic lamprey	11(5)	4.11	1.94	4.06	1.28	1.28	65.52	67.76	51.90	<0.001	55.91	67.07	<0.001 Northern Japan	Purchased from fisherman	
3	-	Channichthyiformes	<i>Chimaera phantasma</i>	Silver chimaera	10(7)	4.82	1.33	2.63	0.85	0.85	62.58	63.85	51.93	<0.001	55.91	67.07	<0.001 Northern Japan	Collected by ourselves	
4	-	Hexanchiformes	<i>Heterodontus japonicus</i>	bullhead shark	10(4)	3.56	2.42	2.10	0.98	0.98	57.43	59.36	44.55	0.027	46.22	48.27	0.053 The Pacific of southern	Purchased from fisherman	
5	-	Oreoloobiformes	<i>Chiloscyllium punctatum</i>	Brownbanded bamboo shark	10(6)	4.08	2.74	1.77	0.83	0.83	53.66	55.30	43.77	<0.001	47.95	49.75	0.065 East China Sea	Courtesy of Dr. K. Sato (Oknawa Churaumi Aquarium)	
6	-	Lamniformes	<i>Lamna ditropis</i>	Salmon shark	11(3)	2.86	2.03	2.74	0.86	0.86	66.58	62.02	65.89	0.101	56.42	57.43	<0.001 The Pacific of eastern	Purchased from fisherman	
7	-	Carcharhiniformes	<i>Megachasma pelagios</i>	Spotted smooth-bound shark	5(5)	4.08	0.86	1.69	0.57	0.57	57.30	55.39	47.08	<0.001	45.98	45.39	0.002 The Pacific of southern	Purchased from fisherman	
8	-	Hexanchiformes	<i>Hoplostethus medius</i>	Sharnose seven gill shark	1(1)	3.46	1.31	2.42	0.35	0.35	-	-	-	-	-	-	-	East China Sea	Preserved in Hokkaido University Museum
9	-	Echinorhiniformes	<i>Acipenser baeri</i>	Bramble shark	1(1)	3.46	1.31	2.42	0.35	0.35	-	-	-	-	-	-	-	East China Sea	Preserved in Hokkaido University Museum
10	-	Squaliformes	<i>Squalus acanthias</i>	Piked dogfish	10(3)	3.61	1.30	1.20	0.53	0.53	57.25	57.36	51.54	0.005	33.67	37.29	0.059 The Pacific of eastern	Purchased from fisherman	
11	-	Squaliformes	<i>Squalus paucispinis</i>	Japanese angelfish	10(5)	3.43	1.44	2.65	1.10	1.10	56.64	58.27	53.52	0.024	48.03	51.34	0.021 The Pacific of southern	Purchased from fisherman	
12	-	Pristigasteriformes	<i>Pristigaster japonicus</i>	Japanese starhake	3(1)	4.62	1.43	3.19	0.85	0.85	-	-	-	-	-	-	-	East China Sea	Preserved in Hokkaido University Museum
13	-	Pseudocricetiformes	<i>Paralichthys oblongus</i>	Japanese steper ray	1(1)	4.62	1.43	3.19	0.85	0.85	-	-	-	-	-	-	-	East China Sea	Preserved in Hokkaido University Museum
14	-	Pseudocricetiformes	<i>Pseudocricetodon japonicus</i>	Japanese skate	1(1)	3.69	1.37	1.26	0.66	0.66	62.27	62.91	57.33	0.008	42.54	42.49	0.072 The Pacific of southern	Preserved in KAYUKAN Aquarium	
15	-	Rajiformes	<i>Oxymonacodon kuroki</i>	Ocellate spot skate	10(4)	3.69	1.37	1.26	0.66	0.66	62.27	62.91	57.33	0.008	42.54	42.49	0.072 The Pacific of southern	Preserved in KAYUKAN Aquarium	
16	-	Mylobatiformes	<i>Dasyatis akabei</i>	Japanese spiny shark	80(29)	3.86	2.00	1.33	0.47	0.47	56.08	58.08	51.90	0.013	34.43	35.08	0.024 The Pacific of southern	Purchased from fisherman	
17	2	Polypteriformes	<i>Polypterus endlicheri</i>	Saddled biotid	10(7)	4.74	1.11	1.74	0.76	0.76	62.04	61.84	49.51	<0.001	24.12	24.07	0.010 The Pacific of southern	Collected by ourselves	
18	4	Acipenseriformes	<i>Acipenser sp.</i>	Sturgeon	12(7)	4.42	1.74	2.23	0.95	0.95	62.04	61.84	49.51	<0.001	24.12	24.07	0.010 The Pacific of southern	Collected by ourselves & Purchased from pet shop	
19	2	Lepistosteiformes	<i>Aracostatus spania</i>	Alligator gar	10(6)	4.75	1.83	3.06	1.07	1.07	73.36	74.18	67.02	0.004	45.87	47.15	0.008 Lake Tanganyika & other	Collected by ourselves & Purchased from pet shop	
20	2	Aminiformes	<i>Aminia calva</i>	Bowfin	10(5)	4.95	1.12	3.04	0.91	0.91	62.80	64.85	58.30	0.010	53.92	55.08	0.010 Western Japan	Purchased from farmer	
21	3	Hiodontiformes	<i>Hiodon tergisus</i>	Mooneye	10(4)	4.86	1.36	2.76	0.58	0.44	62.87	64.85	58.30	0.010	53.92	55.08	0.010 Western Japan	Purchased from pet shop	
22	3	Osteoglossiformes	<i>Osteoglossum</i>	Arwana	10(3)	5.75	1.25	1.75	0.74	0.74	62.77	64.43	52.41	<0.001	51.12	52.91	<0.001 Georgia, USA	Courtesy of Dr. M. Kibbey & Purchased from pet shop	
23	3	Elopiiformes	<i>Megalops cyprinoides</i>	Indo-Pacific tarpon	10(3)	4.80	1.97	2.15	0.78	0.78	65.82	64.75	50.65	<0.001	43.16	42.69	0.024 Brasil	Courtesy of Dr. M. Kibbey & Purchased from pet shop	
24	3	Albirostriformes	<i>Albia virgata</i>	Bonfish	10(6)	2.61	1.08	2.11	0.36	0.36	63.31	62.63	49.75	0.018	46.72	46.31	0.002 Culture in Southeast Asia	Courtesy of Dr. A. Kosier (Waikiki Aquarium)	
25	3	Anguilliformes	<i>Anguilla japonica</i>	Japanese eel	10(7)	4.78	1.18	1.81	0.72	0.72	55.10	53.10	39.13	<0.001	46.04	47.48	<0.001 Hawaii, USA	Courtesy of Dr. A. Kosier (Waikiki Aquarium)	
26	3	Alepocephaloformes	<i>Parasaurichthys argenteus</i>	Blain eel	10(3)	6.29	1.62	3.14	1.24	1.24	62.26	62.81	49.54	<0.001	43.67	44.29	0.015 Western of Japan	Collected by ourselves & Purchase from fishery	
27	3	Capniiformes	<i>Caponichthys akabei</i>	Yellowfin shiner	10(3)	6.29	1.62	3.14	1.24	1.24	62.26	62.81	49.54	<0.001	43.67	44.29	0.015 Western of Japan	Collected by ourselves & Purchase from fishery	
28	3	Goanichthyoformes	<i>Goanichthys akabei</i>	Bighead black sandfish	11(7)	5.21	1.47	2.18	1.01	1.01	60.37	71.92	50.47	<0.001	52.52	53.97	0.047 The Pacific of southern	Collected by ourselves & Purchase from fishery	
29	3	Cypriniformes	<i>Cyprinus carpio</i>	Common carp	10(5)	6.06	2.76	1.99	0.84	0.84	68.29	70.28	66.42	0.049	45.81	47.33	0.024 Western Japan	Collected by ourselves & Purchase from fishery	
30	3	Characiformes	<i>Pycnogonius nattereri</i>	Red piranha	3(54)	3.54	1.30	1.83	0.57	0.57	56.90	57.54	51.44	0.006	43.36	44.11	0.001 South America	Purchased from pet shop	
31	3	Shariiformes	<i>Shirahia aonae</i>	For Eastern catfish	110(53)	6.00	3.65	1.61	0.91	0.91	74.25	743.06	44.12	0.007	44.92	45.54	<0.001 Western of Japan	Collected by ourselves	
32	3	Argentiniformes	<i>Apteronotus albifrons</i>	Black ghost	10(4)	5.72	1.26	1.88	0.64	0.64	65.74	67.73	50.88	<0.001	43.55	45.54	<0.001 Cultured in Southeast Asia	Purchased from pet shop	
33	3	Argentiniformes	<i>Glossogobius aureus</i>	Deepsea smelt	10(5)	4.42	1.76	1.55	0.65	0.65	63.35	67.48	57.48	0.014	40.78	42.53	0.024 Sea of Japan	Purchased from fisherman	
34	3	Osmoeriformes	<i>Pleurostomus xiphioides</i>	Ayu	12(5)	4.43	1.21	2.28	0.67	0.67	63.25	64.57	59.29	<0.001	46.77	48.45	<0.001 Western Japan	Collected by ourselves	
35	3	Salmoiformes	<i>Oncorhynchus keta</i>	Chum salmon	10(2)	5.28	1.69	1.97	0.60	0.60	64.62	63.83	56.78	0.002	44.82	44.45	<0.001 Northern Japan	Purchased from fisherman	
36	3	Esociformes	<i>Esox americanus</i>	Stellar pike	10(4)	6.35	1.39	2.04	0.68	0.68	67.80	68.84	52.84	<0.001	45.71	47.21	0.003 Ohio, USA	Courtesy of Dr. M. Kibbey (Ohio State University)	
37	3	Stomiiformes	<i>Siniperca kneri</i>	Redfin pangasius	11(5)	6.76	1.26	2.55	1.21	1.05	75.64	77.39	55.46	<0.001	56.06	56.17	0.003 The Pacific of southern	Collected by ourselves	
38	Deep	Alepocephaloformes	<i>Alepocephalus japonicus</i>	Pacific jellynose fish	12(10)	3.67	1.25	3.17	0.61	0.61	68.56	66.36	52.84	<0.001	42.50	42.77	<0.001 The Pacific of southern	Collected by ourselves	
39	Deep	Muraeniformes	<i>Muraena japonica</i>	Japanese threadfin eel	10(6)	4.58	1.52	1.92	0.80	0.80	62.30	64.05	51.26	0.039	44.28	45.21	0.022 The Pacific of southern	Collected by ourselves	
40	Deep	Muraeniformes	<i>Muraena helvetica</i>	Whiting	10(6)	5.22	1.62	4.01	1.68	1.68	64.34	66.05	55.90	0.001	59.76	61.31	0.022 The Pacific of southern	Collected by ourselves	
41	4	Lampridiformes	<i>Lampris guttatus</i>	Opah	10(3)	2.91	1.72	2.11	0.80	0.80	54.75	54.75	48.49	0.413	46.67	46.45	0.008 The Pacific of southern	Purchased from fisherman	
42	4	Polymniiformes	<i>Polymnia japonica</i>	Silver eye	10(5)	2.88	1.12	2.66	1.24	1.24	52.95	54.35	50.47	0.011	51.91	53.75	0.064 The Pacific of southern	Collected by ourselves	
43	4	Perciformes	<i>Percopsis omiscomaycus</i>	Trout-perch	10(6)	4.47	1.08	2.13	0.82	0.82	60.90	62.44	47.86	<0.001	46.93	48.12	0.019 Ohio, USA	Courtesy of Dr. M. Kibbey (Ohio State University)	
44	4	Gobiiformes	<i>Gadus macrocephalus</i>	Pacific cod	10(7)	2.88	1.00	2.25	0.92	0.92	52.58	53.57	45.39	0.002	48.11	48.07	0.019 The Pacific of northern	Purchased from fisherman	
45	4	Ophidiiformes	<i>Stenobrama harti</i>	Golden eel	10(6)	3.34	1.00	2.41	1.02	1.02	55.35	55.33	46.24	<0.001	49.59	51.56	0.026 The Pacific of southern	Collected by ourselves & Purchase from fishery	
46	4	Batrachoidiformes	<i>Batrachomus</i>	Three-spined padfish	10(3)	6.52	1.46	1.84	0.60	0.60	68.35	67.52	53.81	<0.001	43.58	44.47	0.002 Southeast Asia	Purchased from pet shop	
47	Deep	Lophiiformes	<i>Lophius litulon</i>	Angler fish	12(8)	7.84	2.53	3.41	1.15	1.15	62.22	68.58	85.94	0.001	66.72	64.99	<0.001 The Pacific of southern	Collected by ourselves & Purchase from fishery	
48	5	Mugiliformes	<i>Mugil cephalus</i>	Flathead mullet	11(4)	4.40	1.51	2.35	0.87	0.87	67.02	68.70	59.45	0.002	53.44	55.28	0.005 Western Japan	Collected by ourselves	
49	5	Blennioformes	<i>Atheron japonicus</i>	Bearded silverside	10(6)	4.17	0.88	3.08	0.86	0.86	59.35	61.09	43.77	<0.001	43.77	43.77	<0.003 Western Japan	Collected by ourselves	
50	5	Blennioformes	<i>Strongylotus omiscomella</i>	Needlefish	10(8)	3.85	1.14	1.96	0.66	0.66	58.15	56.40	48.82	<0.001	44.88	44.44	0.003 Western Japan	Collected by ourselves	
51	5	Blennioformes	<i>Pringichthys brevipinnis</i>	Shortfin flyingfish	10(6)	5.28	0.92	1.30	0.27	0.27	63.25	61.63	49.05	<0.001	41.05	41.05	<0.001 The Pacific of southern	Collected by ourselves	
52	5	Channichthyiformes	<i>Chimaera microps</i>	Chimaera	10(6)	4.58	1.52	1.92	0.80	0.80	62.30	64.05	51.26	0.039	44.28	45.21	0.022 The Pacific of southern	Collected by ourselves	
53	Deep	Stenobrambiformes	<i>Stenobrama harti</i>	Crested bream	10(6)	4.96	1.13	4.81	2.25	2.25	62.91	64.77	62.23	<0.001	63.75	65.56	0.061 The Pacific of southern	Collected by ourselves	
54	4	Zoetiformes	<i>Zelus faber</i>	Skipjack	12(6)	2.97	1.25	2.92	1.42	1.42	54.05	53.43	50.56	0.023	53.90	55.16	0.073 The Pacific of southern	Purchased from fisherman	
55	5	Gasterosteiformes	<i>Gasterosteus sp.</i>	Three-spined stickleback	10(5)	6.90	2.02	0.63	2.71	1.23	55.89	57.89	52.49	<0.001	62.19	64.09	0.031 Northern Japan (the Sea of	Purchased from fisherman	
56	5	Synbranchiiformes	<i>Mastacembela moorii</i>	Spiny eel	12(3)	3.36	1.92	1.50	0.41	0.41	69.66	71.81	60.34	<0.001	39.38	41.35	0.001 Lake Tanganyika	Collected by ourselves	
57	5	Scopelogasteriformes	<i>Schizocottus narmoratus</i>	False killifish	10(5)	3.41	0.81	2.17	0.85										



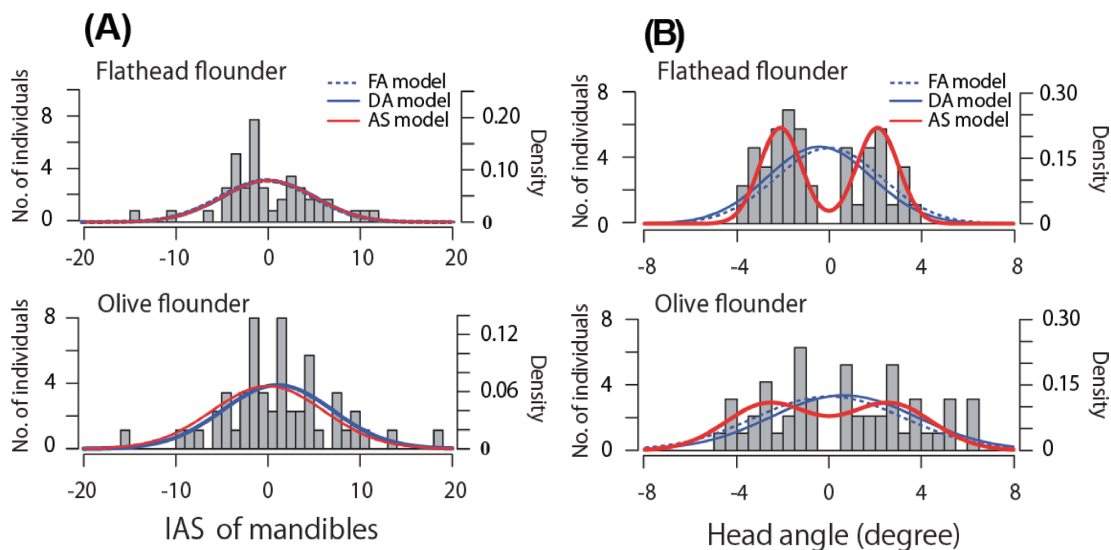
Supplementary Figure S1. Classification and phylogenetic tree of the extant orders of teleost fishes used in this study. Phylogenetic trees are modified following Betancur-R et al. (2013). The classification is based on Nelson (2006), with a slight modification following Betancur-R et al. (2013) as shown in Supplementary Table 1 (colors are the same as those in Fig. 3A). The blue plot shows deep-sea fishes.



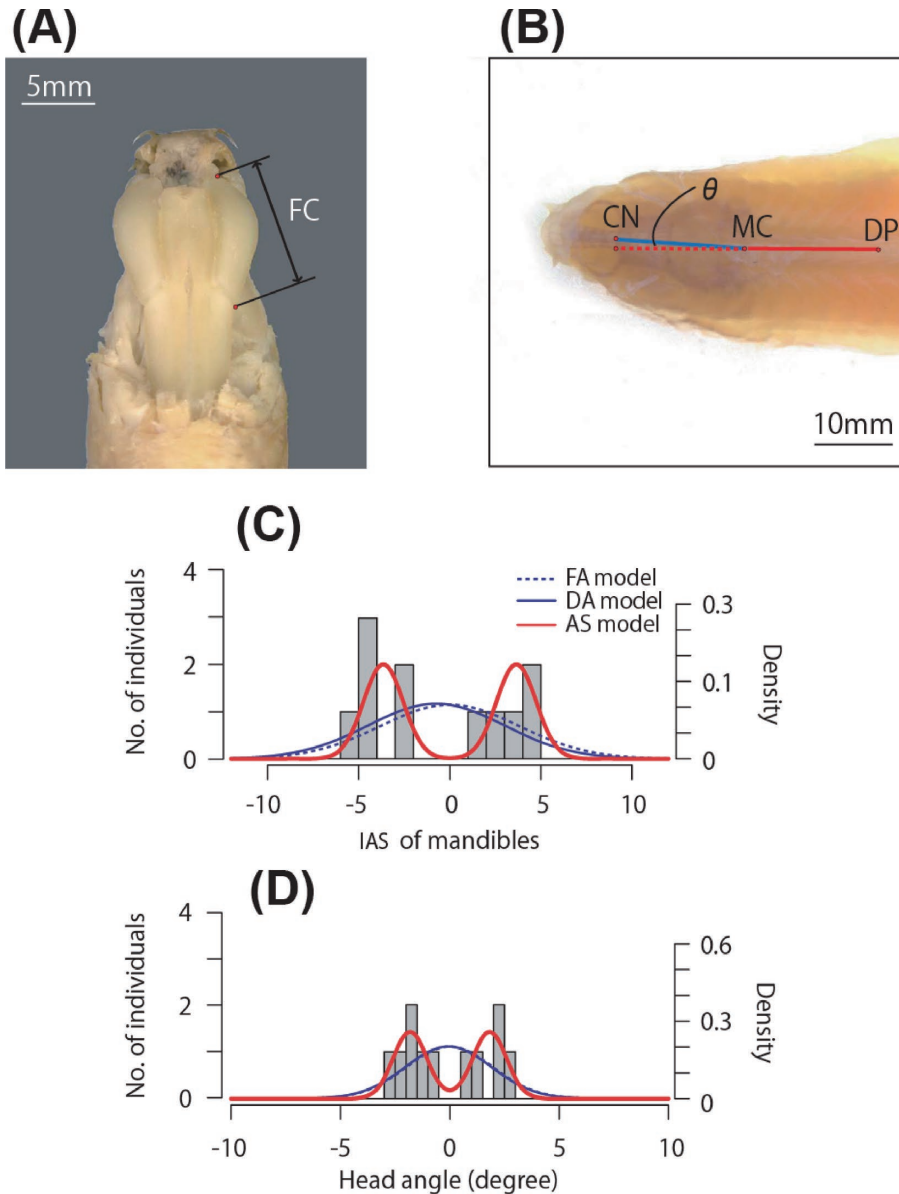
Supplementary Figure S2. Laterality of the Japanese stingray (*Dasyatis akajei*). **(A, B)** Cartilaginous bones showing the data points used for measurements. **(A)** Ventral view of the jaw arch and a close-up of the left mandibular cartilage showing the data points for the index of asymmetry (*IAS*) of the mandibles. The arrow indicates the height of the mandible at the posterior end (HMPE), which is the distance from the postero-dorsal edge of the sustentaculum in the mandibular cartilage (PE) to the ventral corner of the mandibular cartilage (VC). In cartilaginous fishes (except in chimaeras), the sustentaculum functions as the mandibular surface of the hyomandibular-mandibular joint, and the nodule on the 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 teleost fishes. **(B)** Ventral view of head and vertebrae showing data points for the head angle (θ), which is the angle between the head and vertebrae on the ventral side of the anterior part of the skeleton. CC: the small foramen near the center of the cranial cartilage; PC: the midline of the postero-ventral margin of the cranium; PV: the first large process on the ventral sagittal line of the vertebra. **(C, D)** Frequency distributions of the **(C)** *IAS* of the mandibles and **(D)** θ in the Japanese stingray. 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.



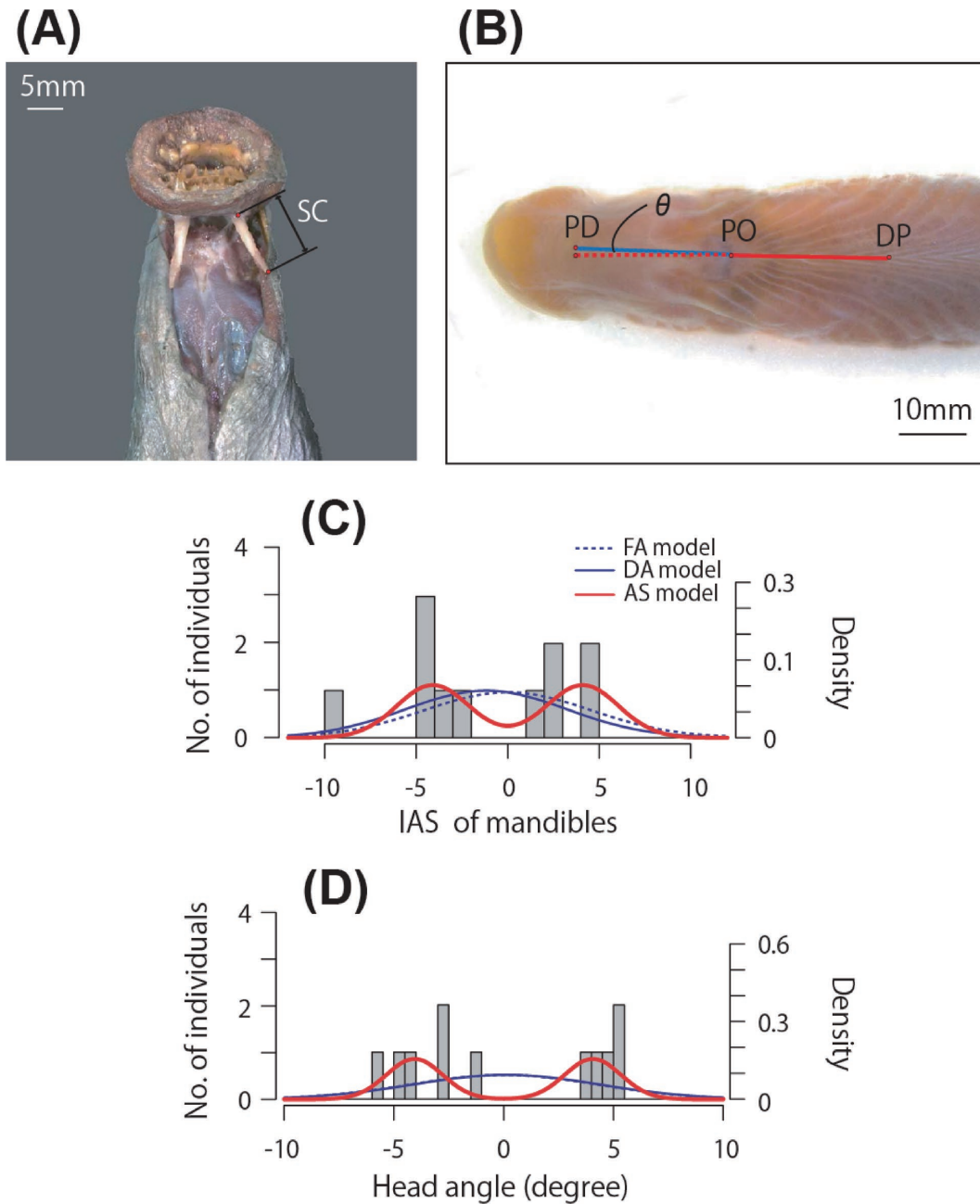
Supplementary Figure S3. Relationship between growth and laterality in largemouth bass (*Micropterus salmoides*). The absolute values of the index of asymmetry (*IAS*) of mandibles and the head angles (θ) in three size classes did not differ significantly (ANOVA, $P = 0.2379$ for *IAS* and $P = 0.7791$ for θ). Individuals were sampled from the same population in Lake Biwa, Japan, in 2004–2008. Vertical bars indicate standard deviations (s.d.). Small fish were primarily 0⁺ ($n = 20$, standard length [SL; mean \pm s.d.] = 76.1 \pm 4.1 mm), medium fish were primarily 1⁺ ($n = 20$, SL = 125.1 \pm 13.6 mm), and large fish were primarily $\geq 2^+$ ($n = 20$, SL = 173.4 \pm 16.8 mm).



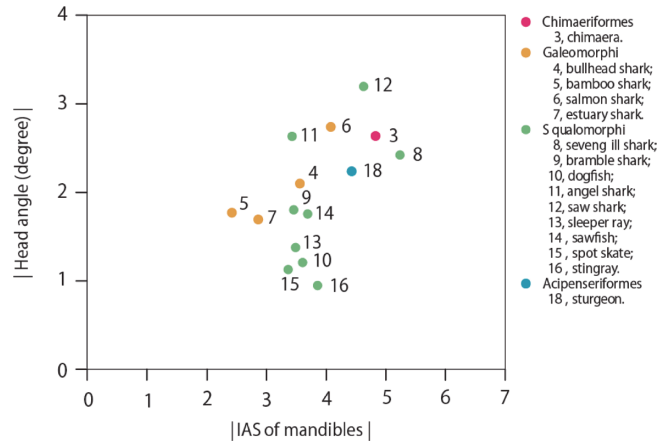
Supplementary Figure S4. Laterality of the flatfishes. Frequency distributions of the (A) index of symmetry (*IAS*) of the mandibles and (B) head angles in flathead flounder (*Hippoglossoides dubius*; upper) and olive flounder (*Paralichthys olivaceus*; lower). The lines are the same as those used in Fig. 2. In both species, the model with the best fit to the distributions of *IAS* was fluctuating asymmetry, whereas the best model for head angle was anti-symmetry (see Supplementary Table 1).



Supplementary Figure S5. Lateralization of the hagfish. **(A, B)** Data for measurements from inshore hagfish (*Eptatretus burgeri*). **(A)** Ventral view of the mouthparts of a moderately boiled fish sample. A pair of flat cartilaginous structures behind the tooth plates is exposed. FC: the length of the flat cartilaginous structure used for the index of asymmetry (IAS). **(B)** Dorsal view of the anterior part of the body in a transparent preparation showing data for the head angle. CN: the center of the cartilaginous rings of the nasal tube; MC: the posterior end of the membranous cranium; DP: the dorsal datum point equidistant from MC to CN–MC on the sagittal line. Head angle (θ) is defined as the angle of the line extending anteriorly from MC–DP to CN–MC. A fish with a clockwise positive angle is defined as left type, whereas a fish with a negative angle is defined as right type. The definitions of right and left types are consistent with those shown in the ventral view of Fig. 1. **(C, D)** Frequency distributions of **(C)** IAS of the flat cartilaginous structure and **(D)** θ in inshore hagfish. The lines are the same as those used in Fig. 2. Both distributions showed anti-symmetry (see Supplementary Table 1).



Supplementary Figure S6. Laterality of the lamprey. **(A, B)** Data points used for measurements in arctic lamprey (*Lampetra japonica*). **(A)** Ventral view of a mouthpart in which a pair of spinose cartilaginous structures behind the suction cup are exposed in a moderately boiled fish sample. SC: the length of the spinose cartilage used for the index of asymmetry (IAS). **(B)** Dorsal view of the anterior part of the body in a transparent specimen showing the data points used for head angle (θ) on the ventral sagittal line. PD: the anterior end of the posterior dorsal cartilage; PO: the posterior end of the postero-occipital cartilaginous ring; DP: the dorsal datum point equidistant to PD–PO. θ is defined as the angle from the line extending anteriorly from PO–DP to PD–PO. A fish with a clockwise positive angle is defined as left type, whereas a fish with a negative angle is defined as right type. **(C, D)** Frequency distributions of the **(C)** IAS of spinose cartilaginous structures and **(D)** θ in arctic lamprey. The lines are the same as those in Fig. 2. Both distributions showed anti-symmetry (see Supplementary Table 1).



Supplementary Figure S7. Degrees of laterality in extant cartilaginous fish orders and sturgeons. Two measures of the laterality, the index of asymmetry (*IAS*) of the mandibles and the head angle, are displayed as in Fig. 3A. Fish species are grouped into four color-coded categories.