

The evolutionary origin of flatfish asymmetry

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All adult flatfishes (Pleuronectiformes), including the gastronomically familiar plaice, sole, turbot and halibut, have highly asymmetrical skulls, with both eyes placed on one side of the head. This arrangement, one of the most extraordinary anatomical specializations among vertebrates, arises through migration of one eye during late larval development. Although the transformation of symmetrical larvae into asymmetrical juveniles is well documented^{1–7}, the evolutionary origins of flatfish asymmetry are uncertain^{1,2} because there are no transitional forms linking flatfishes with their symmetrical relatives^{8,9}. The supposed inviability of such intermediates gave pleuronectiforms a prominent role in evolutionary debates^{10–16}, leading to attacks on natural selection¹¹ and arguments for saltatory change^{14,15}. Here I show that *Amphistium* and the new genus *Heteronectes*, both extinct spiny-finned fishes from the Eocene epoch of Europe, are the most primitive pleuronectiforms known. The orbital region of the skull in both taxa is strongly asymmetrical, as in living flatfishes, but these genera retain many primitive characters unknown in extant forms. Most remarkably, orbital migration was incomplete in *Amphistium* and *Heteronectes*, with eyes remaining on opposite sides of the head in post-metamorphic individuals. This condition is intermediate between that in living pleuronectiforms and the arrangement found in other fishes. *Amphistium* and *Heteronectes* indicate that the evolution of the profound cranial asymmetry of extant flatfishes was gradual in nature.

Teleostei Müller, 1846

Acanthomorpha Rosen, 1973

Percomorpha Rosen, 1973

Pleuronectiformes Bleeker, 1859

Heteronectes chaneti gen. et sp. nov.

Etymology. The generic name references a disused collective name for flatfishes (Heterosomata), as well as the incomplete orbital migration characterizing this taxon (Greek *heteros*, different; *nectri*, swimmer). The specific name honours B. Chanet and his contributions to the study of fossil pleuronectiforms^{8,9,17}.

Holotype. NHMW 1974.1639.24 + 1974.1639.25 (part and counterpart), Naturhistorisches Museum, Vienna (NHMW). Total length is 142 mm; standard length is 111 mm.

Horizon and locality. Bolca, possibly Monte Postale locality, northern Italy. Lower Eocene (Ypresian; SBZ11)¹⁸.

Diagnosis. Stem pleuronectiform differing from *Amphistium* in the following characters: the dorsal- and anal-fin spines are robust, being much broader anteroposteriorly than rays in each fin (rather than a similar thickness); the ventral margin of the anal fin is concave (rather than convex; possible autapomorphy); the anal fin comprises three spines and no fewer than 32 soft, bifurcating rays (rather than 22–21).

The most notable feature of *Heteronectes* is its high degree of cranial asymmetry, manifested primarily in the orbital region of the braincase. This pattern does not arise from taphonomic distortion.

There is neither torsion-induced damage between the orbital region and the posterior of the braincase nor deformation of other components of the skull (Fig. 1a, b).

As in living flatfishes^{2,19}, the frontal bones of *Heteronectes* are unequal in size and shape. The right-side frontal bone is broad, rectangular and ventrally extensive, whereas the left-side frontal bone is reduced to a narrow, curved splint that defines the upper margin of the migrated orbit. The orbital margin fails to extend beyond the dorsal midline. This differs from the arrangement in all living adult flatfishes, in which the migrating eye passes completely over the top of the head to a position on the opposite side of the skull (Fig. 2c). Thus, whereas *Heteronectes* shares a displaced orbit with pleuronectiforms, this genus shows a remarkable intermediate condition in which the eyes remain on opposite sides of the head.

Heteronectes closely resembles *Amphistium*, another percomorph also known from Bolca (*A. paradoxum*)^{9,20} and younger (Lutetian) deposits of France (Paris Basin; *A. altum*)²¹. *Amphistium* has been allied with numerous acanthomorph groups⁹, although many authors noted—and dismissed—a resemblance to pleuronectiforms. The most recent examination of *Amphistium* rejected similarities shared by this genus and flatfishes as primitive or homoplastic, but could not determine its relationships within Percomorpha⁹. Although their systematic interpretations diverged, all previous accounts of *Amphistium* agreed that this genus has a symmetrical skull. Renewed study, using conventional techniques and computed tomography, unequivocally shows that *Amphistium* is characterized by conspicuous cranial asymmetry centred in the orbital region (Figs 1c–f and 2b). Just as in *Heteronectes*, the migrated orbit of *Amphistium* does not extend beyond the dorsal midline. The mode and degree of asymmetry is identical between multiple specimens of *Amphistium*, clearly demonstrating that it reflects a genuine anatomical pattern rather than post-mortem deformation.

Many lines of evidence indicate that the observed cranial morphology in these Eocene fossils represents the adult condition, not an ontogenetic stage preceding complete orbital migration characteristic of all other flatfishes. First, all individuals of *Amphistium* and *Heteronectes* are an order of magnitude longer than the size at which eye migration begins in most living flatfishes (between 10 mm and 15 mm)³. There is no indication that primitive flatfishes might have metamorphosed at larger body sizes, because the eye passes over the dorsal midline at 13 mm in the anatomically conservative *Psettodes*²². Second, unlike living flatfishes in even the late stages of eye migration^{4,5,7}, *Amphistium* and *Heteronectes* have completely mineralized skulls, with all component ossifications present. Third, the consistent morphology of all *Amphistium* specimens—which show identical degrees of asymmetry despite ranging in size between 103 mm (MNHN 10878b/Bol87) and 200 mm (BMNH P.16138) in standard length—indicates this is the adult arrangement.

Amphistium and *Heteronectes* share with flatfishes the specialization of displaced orbits, an unambiguous synapomorphy of that clade¹⁹. Several other derived features, unrelated to asymmetry, link

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Amphistium to flatfishes. These characters, which cannot be checked in the incomplete material of *Heteronectes*, include an anteriorly extensive first dorsal fin, a procumbent first pterygiophore of the dorsal fin, and anteriorly curved neural spines in the abdominal region. However, apart from these flatfish synapomorphies, *Amphistium* and *Heteronectes* display a series of generalized percomorph characters lost or transformed in other pleuronectiforms. These genera retain a single supraneural (uncertain in *Heteronectes*; absent in crown-group pleuronectiforms), lack a pseudomesial bar (a neomorph that frames the migrated orbit in crown-group pleuronectiforms⁶), and possess a generalized 'perciform' caudal endoskeleton²³ comprising a full complement of uroneurals, epurals and independent hypurals. *Amphistium* and *Heteronectes* also bear fin spines in both the anal and dorsal fins. This diagnostic acanthomorph

feature^{24,25} is only found in the plesiomorphic *Psettodes* among living flatfishes^{2,19}. In the context of a phylogenetic analysis, this distribution of characters indicates that *Amphistium* and *Heteronectes* branch from pleuronectiform stem, outside the crown clade that contains living forms plus all other known fossil examples (Fig. 2a). Placement of *Heteronectes* and *Amphistium* as successive taxa along the flatfish stem is notable because it suggests that the observed asymmetry in these genera represents an evolutionary intermediate between generalized conditions and the arrangement found in crown-group pleuronectiforms, rather than a secondary development of incomplete orbital migration from an ancestor showing complete transit.

Specimens of *Amphistium* include both dextral and sinistral morphs (Figs 1c–f and 2b). This is consistent with the pattern seen

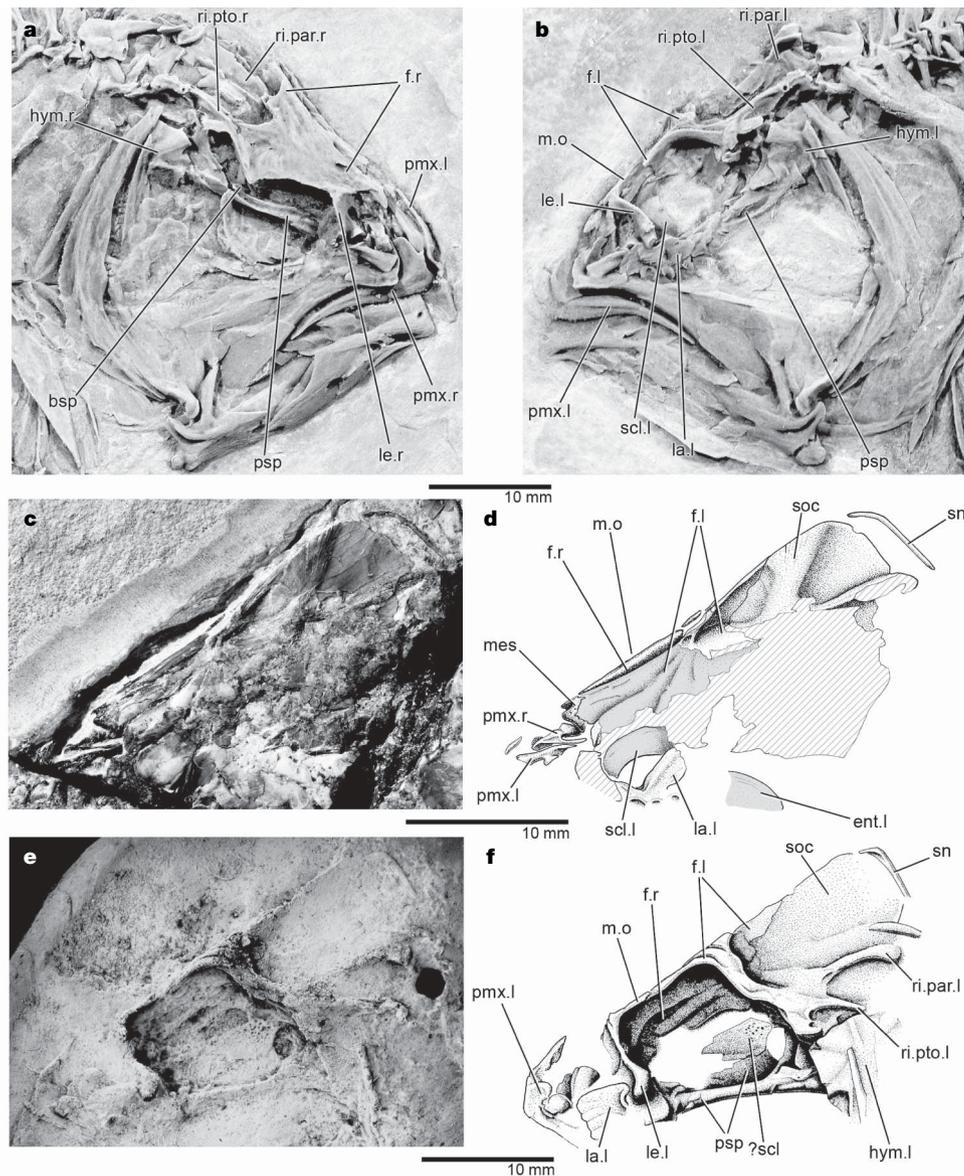


Figure 1 | Skulls of primitive pleuronectiforms showing incomplete orbital migration intermediate between generalized fishes and living flatfishes.

a, *Heteronectes chaneti* gen. et sp. nov., holotype, NHMW 1974.1639.25 (dextral morph); transfer preparation dusted with ammonium chloride and presented in right-lateral view. **b**, Counterpart, NHMW 1974.1639.24; transfer preparation dusted with ammonium chloride and presented in left-lateral view, showing migrated orbit. **c**, *Amphistium paradoxum*, Muséum national d'Histoire naturelle, Paris (MNHN), MNHN 10878b/Bol87 (sinistral morph); specimen presented in left-lateral view (photo credit: C. Lemzaouda, MNHN). **d**, Interpretive drawing. Solid grey shading indicates

impression; diagonal hatching indicates damaged bone. **e**, *Amphistium altum*, Natural History Museum, London (BMNH), BMNH P. 3940 (dextral morph); silicone peel dusted with ammonium chloride and presented in left-lateral view, showing migrated orbit. **f**, Interpretive drawing. bsp, basisphenoid; ent, entopterygoid; f, frontal; hym, hyomandibular; la, lacrimal; le, lateral ethmoid; m.o, dorsal margin of migrated orbit; mes, mesethmoid; pmx, premaxilla; psp, parasphenoid; ri.par, parietal/epioccipital ridge; ri.pto, pterotic ridge; scl, sclerotic ring; sn, supraneural; soc, supraoccipital; names followed by 'r' or 'l' indicate right or left feature, respectively; '?' indicates uncertain identification. Scale bars, 10 mm.

in the morphologically primitive living flatfish *Psettodes*, where the two morphs occur in near equal frequency, but differs from the condition in more derived forms, which typically occur almost exclusively as one morph or the other²⁶. *Amphistium* indicates that indiscriminate orbital migration is primitive for flatfishes, with dominant left- or right-eyed migration representing a derived arrangement. This conforms to a general pattern in the evolution of directional asymmetries that arise late in development, in which groups often first pass through an antisymmetric stage where individuals occur as both dextral and sinistral forms at near-equal frequencies²⁷.

Amphistium and *Heteronectes* deliver the first clear picture of flatfish origins, a hotly contested issue in debates on the mode and tempo of evolution owing to the unclear adaptive value of incomplete eye migration. Flatfishes formed a cornerstone of early arguments against natural selection¹¹, forcing Darwin¹² to respond with a scenario that invoked the inheritance of acquired traits, similar to Lamarck's theory of flatfish origins espoused 60 years earlier¹⁰. Goldschmidt asserted that the first flatfish must have arisen suddenly as a "hopeful monster", bypassing any intermediate forms, and gave this group a

key position in his arguments for saltatory evolution^{14,15}. The discovery of stem flatfishes with incomplete orbital migration refutes these claims and demonstrates that the assembly of the flatfish bodyplan occurred in a gradual, stepwise fashion. Thus, the evolutionary origin of flatfish asymmetry resembles its developmental origin, with increasing degrees of orbital migration transforming a symmetrical precursor into a fully asymmetrical form (Fig. 2c).

Questions about the possible selective advantage of incomplete orbital transit arise from the discovery of stem flatfishes. Clues are given by living taxa, which often prop their bodies above the substrate by depressing their dorsal- and anal-fin rays²⁸. Similar behaviour might have permitted *Amphistium* and *Heteronectes*—both of which have long median-fin rays—the use of both eyes while on the sea floor. The unusual morphology and resting orientation of pleuronectiforms have been interpreted as adaptations for prey ambush¹⁶, and it is clear that stem flatfishes, like morphologically primitive living forms, were piscivorous; one specimen of *Amphistium* (MCSNV V.D.91+92) contains the remains of a fish nearly half its own length. The fossil record rejects the intuitive notion that

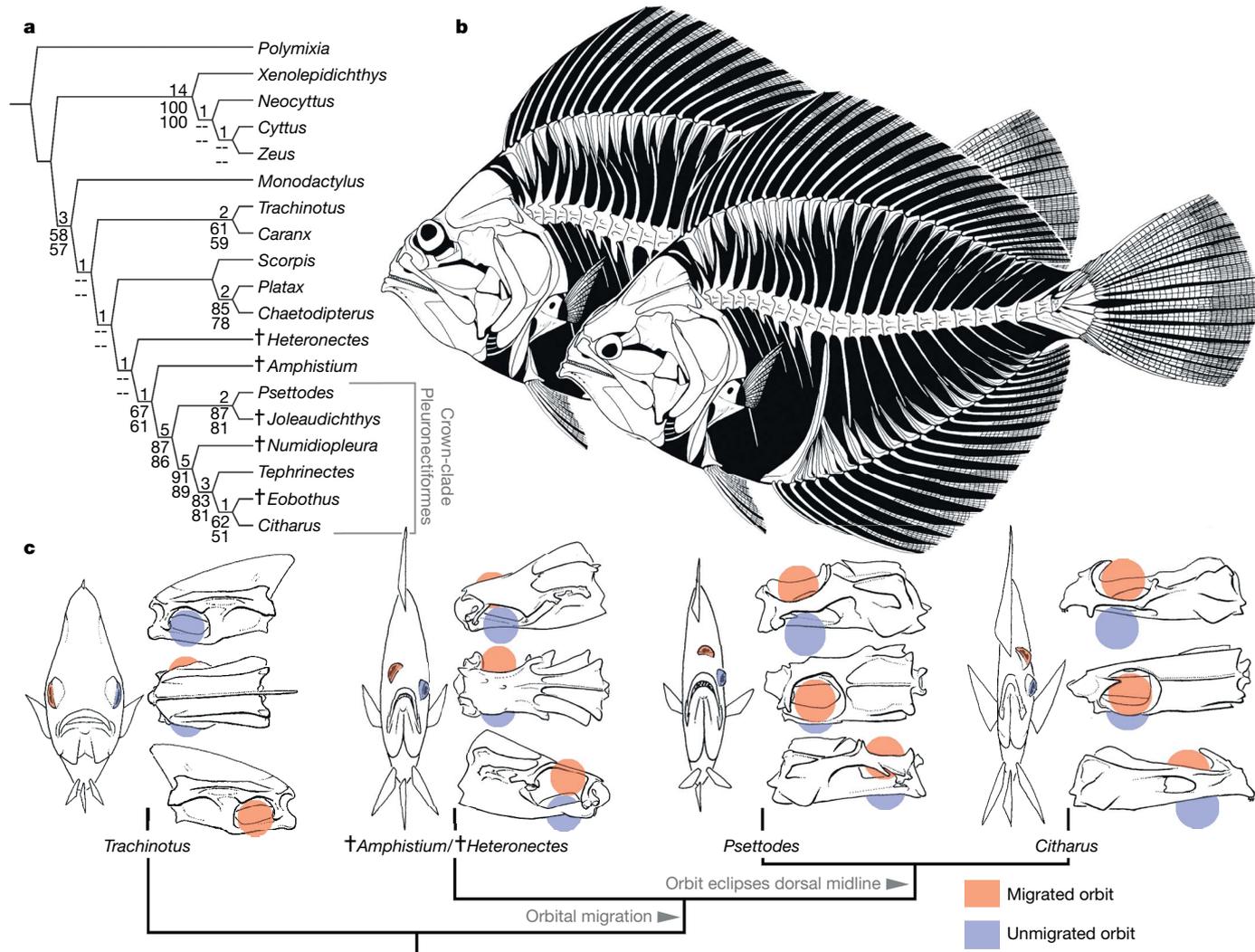


Figure 2 | Phylogenetic placement of *Heteronectes* and *Amphistium* and implications for the origin of cranial asymmetry in flatfishes. **a**, Topology arising from the analysis of a matrix comprising 19 taxa coded for 58 morphological characters (8 ordered) (number of cladograms = 1; cladogram length = 135; consistency index = 0.50; retention index = 0.74; rescaled consistency index = 0.37). *Heteronectes* and *Amphistium* are placed as successively more crownward plesions on the flatfish stem. Unordered analyses reconstruct these taxa in the same position. Numbers at nodes indicate Bremer decay index, bootstrap support and jackknife support, from

top to bottom, respectively. Extinct taxa are marked (†) and ‘-’ indicates that bipartition occurs in fewer than half of cladograms arising from bootstrap or jackknife analysis. Previous placements of *Amphistium* outside Pleuronectiformes are rejected. See Supplementary Information for full details of the analysis. **b**, Reconstruction of *Amphistium*, showing sinistral (front) and dextral (back) individuals in the left lateral view (modified from ref. 20). **c**, Simplified cladogram adapted from **a** showing the progression of orbital migration across flatfish phylogeny. Neurocrania are depicted in left lateral (top), dorsal (middle) and right lateral (bottom) views.

incomplete orbital migration might be maladaptive. Stem flatfishes with this condition range over two geological stages and derive from localities that also yield crown pleuronectiforms with full cranial asymmetry^{8,9}.

Amphistium and *Heteronectes* are contemporaries of the earliest members of many derived pleuronectiform lineages^{8,29}, including the oldest known sole¹⁷. The sudden appearance of anatomically modern pleuronectiform groups in the Palaeogene period matches the pattern repeated by many acanthomorph clades^{24,29}. Inferring interrelationships between higher groups in this explosive radiation has proved difficult, and an unresolved bush persists³⁰. Documenting the origin of these clades is vital to understanding the roots of modern biodiversity, because acanthomorph fishes comprise nearly one-third of living vertebrate species³⁰. Stem representatives—such as *Amphistium* and *Heteronectes* in the case of pleuronectiforms—have yet to be identified for many acanthomorph clades^{24,29}, but their recognition might prove invaluable in delivering a stable hypothesis of interrelationships for this exceptional vertebrate radiation.

METHODS SUMMARY

Computed tomography scans were carried out the University of Texas at Austin. The positions of *Amphistium* and *Heteronectes* were inferred by parsimony analysis of a morphological data set. Details of these analyses and further anatomical data are given in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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