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The oceanic bathypelagic realm (1000–4000 m) is a nutrient-poor habitat. Most fishes living there have pelagic larvae using the rich waters of the upper 200 m. Morphological and behavioural specializations necessary to occupy such contrasting environments have resulted in remarkable developmental changes and life-history strategies. We resolve a long-standing biological and taxonomic conundrum by documenting the most extreme example of ontogenetic metamorphoses and sexual dimorphism in vertebrates. Based on morphology and mitogenomic sequence data, we show that fishes currently assigned to three families with greatly differing morphologies, Mirapinnidae (tapetails), Megalomycteridae (bignose fishes) and Cetomimidae (whalefishes), are larvae, males and females, respectively, of a single family Cetomimidae. Morphological transformations involve dramatic changes in the skeleton, most spectacularly in the head, and are correlated with distinctly different feeding mechanisms. Larvae have small, upturned mouths and gill arches on copepods. Males have huge gapes with long, horizontal jaws and specialized gill arches allowing them to capture larger prey. Males cease feeding, lose their stomach and oesophagus, and apparently convert the energy from the bolus of copepods found in all transforming males to a massive liver that supports them throughout adult life.

Keywords: Cetomimidae; Megalomycteridae; Mirapinnidae; ontogenetic transformation; sexual dimorphism; whalefishes


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Figure 1. Life stages and selected skeletal elements of cetomimid whalefishes. (a) Eutaeniophorus festivus postlarva, BSKU 51970, 56 mm SL, approximately 816 mm TL, photo courtesy of Masanori Nakamachi, ‘Sea Fishes of Japan’ © YAMAKEI Publishers Co., Ltd. P. brevis: (b) postlarva, Cozumel, Mexico, photo courtesy of Donald Hughes; (c) postlarva, KPM NI13654: (i) photo courtesy of Yasuhiro Morita, (ii) photo courtesy of Sandra Raredon, USNM; (d) larva, MCZ 59910, 13 mm SL, photo courtesy of Chris Kenaley, © President and Fellows of Harvard College; (e) Ataxolepis apus adult male, USNM 391648: (i) dorsal view of nasal organs, (ii) lateral view of viscera, enlarged liver on left, enlarged testes dorsal and ventral right, intestine middle right. (f) Gyrinomimus sp., juvenile female, NE Pacific, photo courtesy of Bruce Robison, MBARI. (g(i), h(i), i(i)) Cranium and anterior vertebrae, and (g(ii), h(ii), i(ii)) left jaws, palatine arch, suspensorium and opercular bones of (g) E. festivus postlarva, USNM 391655, 60 mm SL, (h) A. apus adult male, USNM 391649, 58 mm SL and (i) C. regani female, USNM 391657, 93 mm SL, respectively. Blue ‘ovals’ enclose maxillae, premaxillae and rostral cartilage, which, in (h(ii)) are fused to each other and to broken nasals. (g–i) Photo courtesy of G.D.J.
Three deep-sea fish families are one

Figure 2. (a) ML tree derived from analyses of whole mitogenome sequences from 15 specimens using RAxML v. 7.0.4. Numerals beside internal branches indicate bootstrap values (only 50% and above are shown) based on 1000 replicates. Scale indicates expected number of substitutions per site; red asterisks, larvae; blue asterisk, male. Long-finned whalefish C. regani Zugmayer, 1914: (b) USNM 391563; (c) MCZ 60609 (inset, enlarged nasal organ); (d) BMNH 1957.7.20.1.00, holotype of P. gulosus (inset, elongate nasal rachis); (e) USNM 392646; (f) USNM 391656. Photo courtesy of S. Raredon and G.D.J.
larval megalomycterids was thus established. Fortuitously, a transforming specimen of the cetomimid long-finned whalefish *Cetostoma regani* (figure 2e) was captured shortly thereafter.

2. MATERIAL AND METHODS


DNA from 34 individuals of all five whalefish ‘families’ representing 10 genera and 16 presumed species plus two melanoids as outgroups was analysed (see table S1 in the electronic supplementary material, including GenBank numbers). Whole mitochondrial genome (mitogenome) sequences for nine species were newly determined and used with an additional six such sequences available from GenBank (total 15 species). The mitogenomes (approx. 16 500 bp) were determined using a combination of long and short polymerase chain reactions and direct cycle sequencing techniques following the methods of Miya & Nishida (1999).

For the remaining 21 individuals, we determined partial sequences of the 16S ribosomal RNA (rRNA) gene (approx. 575 bp). Unambiguously aligned mitogenome sequences from 15 specimens were divided into five partitions (first, second and third codon positions of the 13 protein-coding, rRNA and tRNA genes; total = 15 886 positions) and subjected to the partitioned maximum-likelihood (ML) analysis using RAxML v. 7.0.4 (Stamatakis 2006). We estimated the best-scoring ML tree using a general time reversible (GTR) + gamma model of sequence evolution with 1000 bootstrap replicates. The resulting ML tree was then used as a backbone constraint (–r option in RAxML) for subsequent ML analysis using unambiguously aligned, partial sequences of the 16S rRNA gene from all 36 specimens. We similarly estimated the best-scoring ML tree using a GTR + gamma model of sequence evolution with 1000 bootstrap replicates. More details of the DNA methods are in the electronic supplementary material.

3. RESULTS AND DISCUSSION

We identified three specimens in transition from larval/juvenile stage to adult. The 41.7 mm *C. regani* taken in an open net fished to a depth of 5110 m in the southeastern Atlantic is a late transforming female that retains only 3–4 of the 8–10 pelvic-fin rays found in the larvae; pelvic-fin rays are lacking in the other 184 female specimens of this most common whalefish (figure 2c). This species has uniquely high dorsal- and anal-fin ray counts of 26–37 compared with 11–22 rays for all other taxa in the family, allowing links with *P. gulosus* larvae/postlarvae and *Cetomimoides parri* males (figure 2c). The 35 mm holotype of *P. gulosus* (figure 2d) collected in a closing net between 700–1400 m is an early transforming specimen with a full complement of 10 pelvic-fin rays, moderately long jaws and a gut full of copepods. Although the nasal organ is incompletely developed, the elongate, thickened median rachis (figure 2d inset) indicates that the individual would have developed into a male. The 34 mm holotype of *M. teevani* described above was caught in an open net fished to a depth of 1650 m. Histology of the gonad reveals good spermatogenic tissue with pre-spermatids (H. G. Moser 2006, personal communication).

A detailed osteological description of the three life stages is beyond the scope of this paper, but images of the various stages shown in figures 1 and 2 illustrate the amazing ontogenetic transformations that result in extraordinary sexual dimorphism. These transformations include major changes in jaw length, depth and angle, and concomitant radical modifications of the suspensorium and angle of attachment of the skull to the vertebral column (figure 1g–i). Females develop taxon-specific gift arch structure and males exhibit hyperossification of most bones. Of the latter, most remarkable are fusion of the first vertebra to the occiput and of the hypertrophied nasal, lacrimal and upper jawbones (figure 1h), our first clue that males do not feed.

Transformed males lack an oesophagus and stomach, but retain a vestigial, thin-walled intestine containing copepod tests; a massive liver and paired gonads fill the peritoneal cavity (figure 1e(ii)). Most of the largest juveniles have a gut swollen with copepods (40–200 +), visible externally in life as a swollen orange bulge. This bulus of copepods must provide the nutrition required to generate the large liver that sustains the male through the rest of its life. This is unnecessary in females that continue to feed and may reach more than 40 cm. The transforming female *Cetostoma* has neither a gut full of copepods nor a massive liver.

The most striking feature of the larvae is the streamer that grows from the caudal-fin rays, just visible in the smallest 4–5 mm larvae, but extending an estimated 75 cm in the largest postlarva photographed (figure 1a). The two largest photographed specimens (figure 1ac), both with copepod-gorged guts, lost their streamers during capture. The most striking streamer is that of *Parataeniophorus brevis*, with ornamentation reminiscent of a siphonophore (figure 1b,c). One can only speculate regarding the possible advantages and disadvantages of this remarkable appendage in feeding versus predator avoidance. Videos of live female whalefish show that their locomotion involves both rapid swimming with sinuous body waves and slow swimming with undulations of dorsal and anal fins (see video A in the electronic supplementary material).

In recent years, additional tissues have become available, with the total mitogenomic analyses that provided the ML tree (figure 2a) from one male specimen, three larvae representing two species and six species of females in five genera. The linking of larval *P. gulosus* with *C. regani* is confirmed, with an ML tree based on 16S rRNA analyses (see figure S1 in the ESM) including two larvae and nine females of this species. Larval *Eutaeniophorus* and male *Ataxolepis* are embedded within the genera *Cetominus* and *Gyrinominus*. With outgroups of the stephanocytiiform *Rondeletiidae* and *Barbourisidiidae*, the generic relationships of the cetomimids largely follow those proposed by Paxton (1989). The basal position of *Procetichthys* is confirmed, while notable differences include the more basal position of *Cetostoma* and the paraphyly of *Gyrinominus*. Further analyses combining morphologic and genetic data are planned, while tissues from additional genera and larvae are needed. With the synonymy of the three families confirmed, the next challenge is to link the three life stages of each species. Meristic data establish *Mirapinna esau* as the postlarva of *Procetichthys kreffti* and suggest that *Parataeniophorus bertelseni* is the larva of *Ditropichthys storeri*.

Although remarkable ontogenetic transformations occur in a few other deep-sea fish families (e.g. Giganturidae), and prominent sexual dimorphism is widespread among vertebrates, the extraordinary combination of both that we have documented here for the whalefishes is unparalleled within Vertebrata.

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