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# Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): A new perspective based on whole mitogenome sequences from 75 higher teleosts

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**Abstract**

The fishes currently recognized as members of the order Gasterosteiformes (sticklebacks, pipefishes, and their relatives) number 278 species, classified into two suborders (Gasterosteoidei and Syngnathoidei), 11 families and 71 genera. Members of this group exhibit unique appearances, many of which are derived from armored bodies with bony plates in various forms. Although recent molecular phylogenetic studies have repeatedly questioned the monophyly of this order, none of the studies examined all of the representative families and the phylogenetic reality of the group has remained unclear. In this study, we examined whole mitochondrial genome (mitogenome) sequences from 13 gasterosteiform species representing all 11 families in the order, and subjected them to partitioned maximum likelihood and Bayesian analyses, with additional data from other percomorphs and outgroups (75 mitogenome sequences considered overall, including 10 newly determined). The resultant phylogenies indicated explicitly that previously recognized members of Gasterosteiformes had diverged basally within the Percomorpha into three different clades with the following subgroups: Syngnathoidei, Gasterosteoidei (minus Indostomidae), and Indostomidae. Monophyly of the order Gasterosteiformes and any combinations of the three subgroups were confidently rejected by statistical tests. Syngnathoidei (together with Dactylopteroidei) formed a monophyletic group, a sister-group relationship between Gasterosteoidei (minus Indostomidae) and Zoarcoidei was reconfirmed and Indostomidae was nested within the Synbranchiformes, rendering the latter group paraphyletic. Our study demonstrates a new perspective of gasterosteiform phylogeny, which will provide fundamental information for future studies of phylogeny, systematics, and evolution.


*Keywords:* Mitogenomics; Gasterosteiformes; Teleostei; Long PCR

## 1. Introduction

Gasterosteiformes (sticklebacks, pipefishes, and their relatives) is one of 14 orders of the Percomorpha (sensu Miya et al., 2005), the most diversified (>15,000 spp.) crown clade of teleosts. Most members exhibit a unique appearance, such as armored bodies with bony plates in various forms (Fig. 1), with many also having tube-shaped mouths adapted for suction feeding (Svensson, 1988). They also exhibit diverse locomotory modes: some species swimming in a vertical position, with the snout downwards (Centriscoidea) or upwards (Syngnathidae), and some crawling slowly over the sea floor (Pegasidae; Herold and Clark, 1993). In addition to such remarkable diversity, they also exhibit various reproductive modes (Clutton-Brock and Vincent, 1991). For example, mature male gasterosteids and an aulorhynchid species construct nests with glue-

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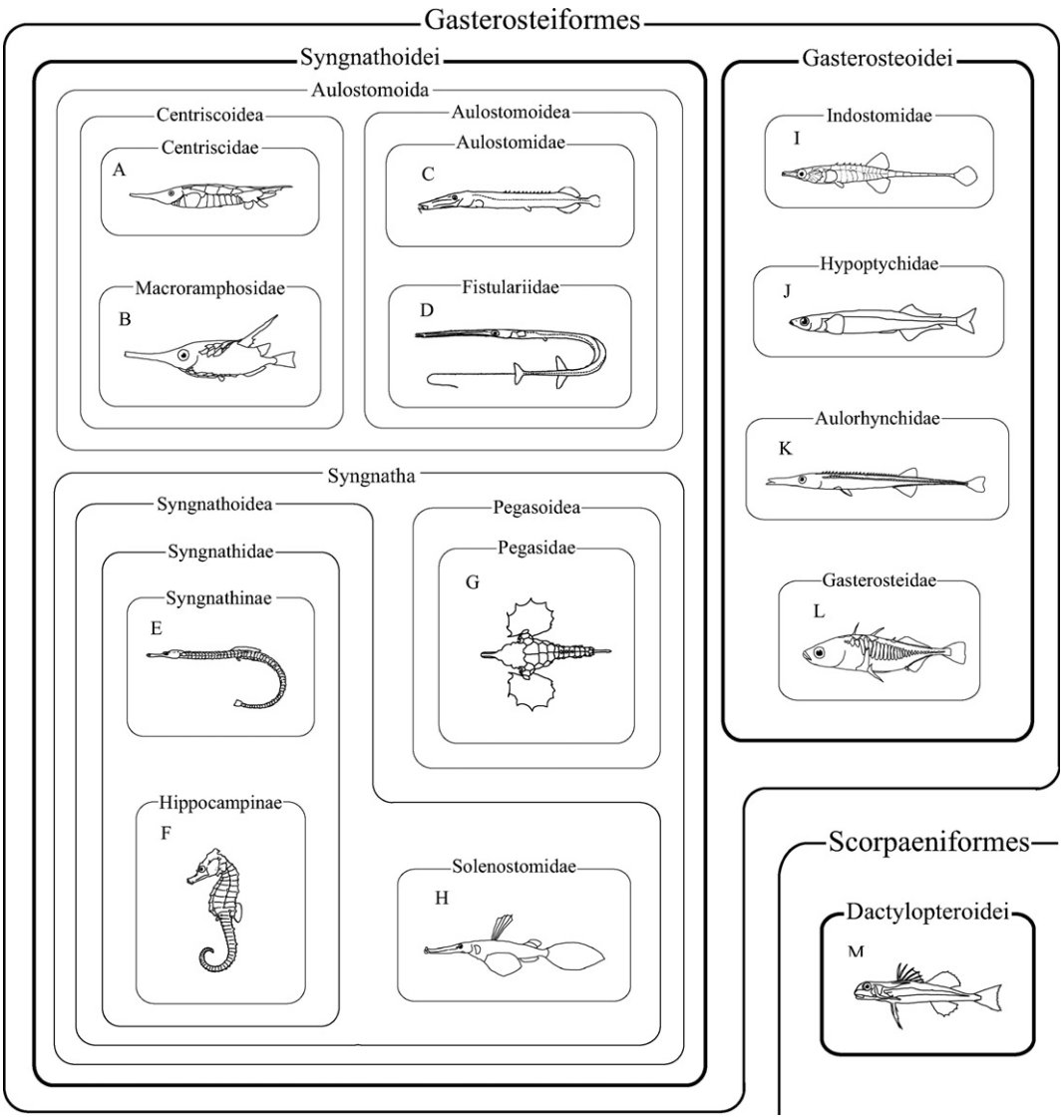


Fig. 1. Schematic representation of gasterosteiforms and dactylopteroids. (A) *Aeoliscus strigatus* (Centriscidae); (B) *Macroramphosus scolopax* (Macroramphosidae); (C) *Aulostomus chinensis* (Aulostomidae); (D) *Fistularia commersonii* (Fistulariidae); (E) *Microphis brachyurus* (Syngnathinae, Syngnathidae); (F) *Hippocampus kuda* (Hippocampinae, Syngnathidae); (G) *Eurypegasus draconis* (Pegasidae; dorsal view); (H) *Solenostomus cyanopterus* (Solenostomidae); (I) *Indostomus paradoxus* (Indostomidae); (J) *Hypoptychus dybowskii* (Hypoptychidae); (K) *Aulorhynchus flavidus* (Aulorhynchidae); (L) *Gasterosteus aculeatus* (Gasterosteidae); (M) *Dactyloptena tiltoni* (Dactylopteridae, Dactylopteroidei). Classifications follow Nelson (2006).

like substances (Limbaugh, 1962; Wootton, 1976), and syngnathid females attach eggs to males in a special area on the undersurface of the trunk or tail (Breder and Rosen, 1966; Wootton, 1976; Wilson et al., 2001, 2003).

Gasterosteiformes includes 278 species, currently classified into two suborders (Gasterosteoidei and Syngnathoidei), 11 families and 71 genera (Nelson, 2006). Most gasterosteoids are distributed in cold and temperate fresh or brackish waters of the northern hemisphere, whereas syngnathoids occur in temperate and tropical shallow, coastal marine habitats (Nelson, 2006). Their body sizes also differ greatly, adult *Indostomus paradoxus* (Indostomidae) attaining only 2.4–2.7 cm in standard length (SL), whereas *Fistularia tabacaria* (Fistulariidae) attains 1.8 m SL. The order also includes threespine stickleback (*Gasterosteus aculeatus*), the behavioral characteristics of which have attracted much attention (Tinbergen, 1951; Wootton, 1976). In addition, many populations of *Gasterosteus aculeatus* have rapidly adapted to diverse freshwater environments and exhibit unprecedented phenotypic diversity (Bell and Foster, 1994), the species being an excellent subject for studies of parallel evolution (Shapiro et al., 2004; Colosimo et al., 2005). The genome database of *Gasterosteus aculeatus* having been recently determined, its importance in biological research is expected to increase (Kingsley et al., 2004).

The Gasterosteoidei and Syngnathoidei were originally placed in different lineages based on morphological characters (Fig. 2), McAllister (1968) including the two groups in distantly related positions in his phylogeny of teleosts