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Despite their importance as evolutionary and ecological model systems, the phylogenetic relationships among gasterosteiforms remain poorly understood, complicating efforts to understand the evolutionary origins of the exceptional morphological and behavioural diversity of this group. The present review summarizes current knowledge on the origin and evolution of syngnathids, a gasterosteiform family with a highly developed form of male parental care, combining inferences based on morphological and molecular data with paleontological evidence documenting the evolutionary history of the group. Molecular methods have provided new tools for the study of syngnathid relationships and have played an important role in recent conservation efforts. Despite recent insights into syngnathid evolution, however, a survey of the literature reveals a strong taxonomic bias towards studies on the species-rich genera *Hippocampus* and *Syngnathus*, with a lack of data for many morphologically unique members of the family. The study of the evolutionary pressures responsible for generating the high diversity of syngnathids would benefit from a wider perspective, providing a comparative framework in which to investigate the evolution of the genetic, morphological and behavioural traits of the group as a whole. © 2011 The Authors

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INTRODUCTION

The Syngnathidae (seahorses and pipefishes) are a large family of close to 300 marine, brackish and freshwater species (Froese & Pauly, 2010), all of which share an exceptional form of reproduction, male pregnancy (Wilson *et al*., 2001; Stölting & Wilson, 2007). This key feature of the group has made them important model organisms for the study of sexual selection (Clutton-Brock & Vincent, 1991; Kvarnemo & Ahnesjö, 2002; Berglund & Rosenqvist, 2003; Wilson *et al*., 2003; Jones *et al*., 2005).

In addition to their unique reproductive behaviour, syngnathids are well known for their highly specialized morphology, and the diversity of morphological forms found in this group has complicated efforts to understand their evolutionary origins and pattern of diversification. This exceptional morphological variation is reflected in the current taxonomy of the group: 14 of the 54 currently recognized syngnathid genera are monotypic (Froese & Pauly, 2010), and the majority of genera are composed of fewer than three species.

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The family Syngnathidae has traditionally been included as a member of the order Gasterosteiformes, which includes 11 families in two suborders: the Gasterosteoidei, with the Hypoptychidae, Gasterosteidae and Aulorhynchidae; and the Syngnathoidei, with the Indostomidae, Aulostomidae, Fistulariidae, Macroramphosidae, Centriscidae, Pegasidae, Solenostomidae and Syngnathidae. The evolutionary history of the Gasterosteiformes itself has been the subject of controversy since its inception because of the derived and highly reductive morphology of its constituent families. Major monophyletic groups of families within the Gasterosteiformes have long been recognized, including Gasterosteidae and Aulorhynchidae (as superfamily Gasterosteoidea), Aulostomidae and Fistulariidae (Aulostomoidea), Centriscidae and Macroramphosidae (Centriscoidea), and Syngnathidae and Solenostomidae (Syngnathoidea) (Fig. 1; Orr, 1995). Studies using morphology have proposed monophyly of the order and suggested sister groups based on weak evidence, and while the close relationships of superfamilies is well supported (Fig. 1), the relationships among these family pairs remains unclear (Pietsch, 1978; Johnson & Patterson, 1993; Orr, 1995; Keivany & Nelson, 2006). In contrast, molecular evidence clearly refutes the monophyly of the Gasterosteiformes, placing gasterosteoids close to the cottoid–zoarcoid lineage (Imamura & Yabe, 2002), excluding the Indostomidae, and placing both groups distant from syngnathoids. While the sister group of syngnathoids remains unknown and the relationships among syngnathoid lineages are poorly resolved, the family Solenostomidae (ghost pipefishes) has been faithfully recovered as the sister group of the Syngnathidae in both morphological and molecular analyses, an evolutionary relationship key to understanding the evolution of male parental care in the family Syngnathidae.
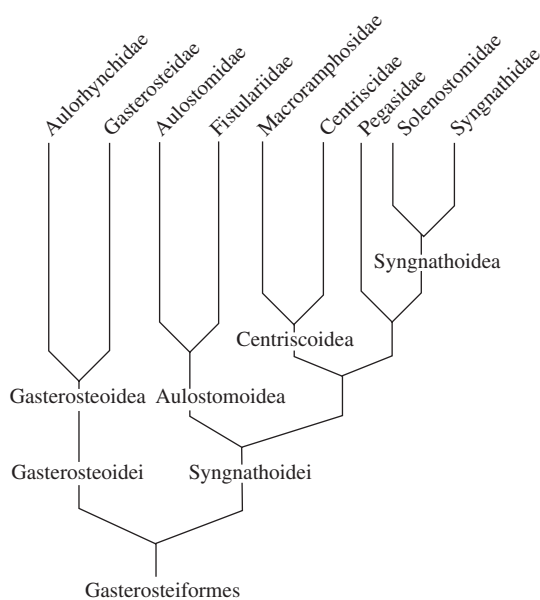

FIG. 1. Phylogenetic relationships and superfamily classification of the Gasterosteiformes based on morphological analysis (after Orr, 1995). Note: the Hypoptychidae and Indostomidae are excluded from this classification scheme (see Fig. 2 for other hypotheses on higher level relationships in this group).

HIGHER LEVEL PHYLOGENETICS: MORPHOLOGY VS. MOLECULES

MORPHOLOGICAL ANALYSES

Linnaeus (1758) codified the prevailing concept of a wide separation between the currently recognized gasterosteoids and syngnathoids in the 10th edition of *Systema Naturae*. Gasterosteids were placed in the Artedian Thoracici, while syngnathoids were divided into two groups, the Abdominales and the Branchiostegi. *Pegasus* was classified separately from the syngnathoids as a chondropterygian (Gill, 1884). In a major revision as part of the final edition of *Systema Naturae*, Linnaeus (1768) reclassified the syngnathoids *Centriscus*, *Syngnathus* and *Pegasus* together in the Amphibia Nantes, reflecting his erroneous belief that all these fishes possessed both lungs and gills, a contention which perplexed many of his contemporaries (Pietsch, 1995). The position of *Fistularia* and the gasterosteids remained unchanged in this edition of *Systema Naturae*. Linnaeus' systematic groupings of gasterosteoids and syngnathoids were retained for almost half a century, with only minor changes in taxonomic nomenclature during this time.

As part of his effort to classify fishes into natural groups on the basis of unique characters, Cuvier (1816) placed gasterosteiforms in three widely separated lineages and established two key new groups: the Lophobranches and 'Les Bouches en Flute'. Cuvier's Lophobranches united the pegasids, solenostomids and syngnathids, while 'Les Bouches en Flute' comprised the aulostomoids and centriscoids, marking the first time that these two lineages were closely associated. Cuvier & Valenciennes (1829) tentatively placed the Gasterosteidae in a primarily scorpaeniform group, foreshadowing current ideas of scorpaeniform affinities based on molecular data.

The ideas of two American workers, Cope and Gill, had a broad influence on the thoughts of later researchers in gasterosteiform systematics. In establishing a filing system for use at the National Museum of Natural History, Washington DC, U.S.A. Gill (1872) was influenced in his ideas of the relationships of gasterosteiforms by Cope (1871). Gill's Lophobranchii contained only the Solenostomidae and the Syngnathidae [although *Hippocampus* was placed within its own family, the Hippocampidae, for reasons detailed by Gill (1896)], while his Hemibranchii was composed of the Gasterosteiformes, with presently recognized gasterosteoids and aulostomoids, and the Centrisciformes, containing only centriscoids [Fig. 2(a)]. Noting that a linear series of names is inadequate to express genetic relationships, he outlined the evolutionary lineage of fishes and depicted the Lophobranchii arising from the Hemibranchii. These ideas of gasterosteiform evolution followed Cope (1871), except for the placement of the Pegasidae, which was not included. Gill (1884) published a detailed analysis of the Hemibranchii, which contained all gasterosteiforms except syngnathids and solenostomids and argued that the Aulorhynchidae and Gasterosteidae were only slightly differentiated, even going as far as suggesting that they might appropriately be united into one family.

The landmark studies of Jungersen (1908, 1910) provided the first detailed accounts of the morphology of the Centriscidae and Macroramphosidae in 1908 and the Aulostomidae, Fistulariidae, Syngnathidae and Solenostomidae in 1910. In the first of these papers, he concluded that the Solenichthys (the Syngnathoidei less Pegasidae) was a natural group and that the Hemibranchii (Gasterosteoidei) and Pegasidae were not closely related to this group. He argued that several morphological characters
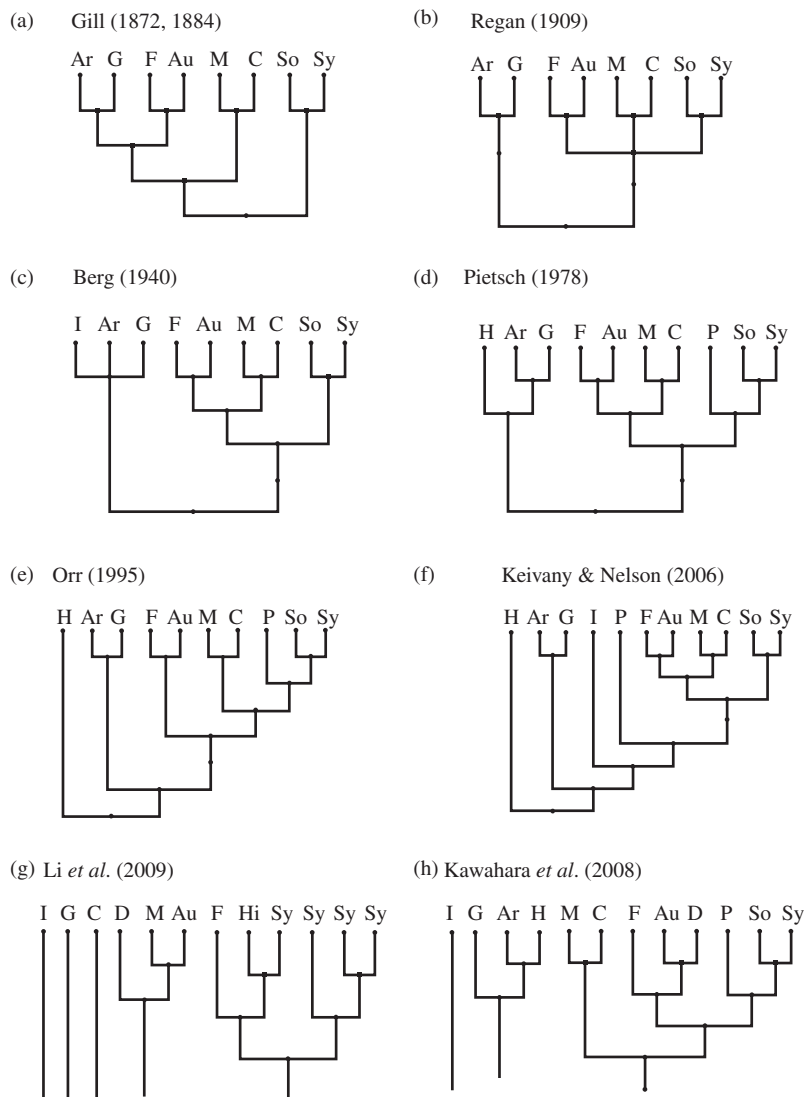
(a)　　Gill (1872, 1884)

(b)　　Regan (1909)

(c)　　Berg (1940)

(d)　　Pietsch (1978)

(e)　　Orr (1995)

(f)　　Keivany & Nelson (2006)

(g) Li *et al.* (2009)

(h) Kawahara *et al.* (2008)

FIG. 2. Eight (a–h) morphological and molecular-based hypotheses on the interrelationships of families of gas-
terosteiforms. Ar, Aulorhynchidae; Au, Aulostomidae; C, Centriscidae; D, Dactylopteridae; F, Fistulari-
idae; G, Gasterosteidae; H, Hypoptychidae; Hi, *Hippocampus;* I, Indostomidae; M, Macroramphosidae;
P, Pegasidae; So, Solenostomidae; Sy, Syngnathidae.

were 'found united in the whole community of forms' (his Solenichthys), plac-
ing centriscoids and aulostomoids together as a natural group and solenostomids and
syngnathids as a separate but closely related lineage. He also concluded that gasteros-
teoids and pegasids belonged within scorpaeniforms, and were not closely related to
syngnathoids, based on investigations that were never published (Jungersen, 1908,
1915). Regan (1909) was heavily influenced by the work of Jungersen (1908) and
recognized monophyletic groups comparable to the Aulostomoidea, Centriscoidea

and Syngnathoidea, though he did not specify their relationships to one another [Fig. 2(b)]. In later work, Regan (1913) suggested that gasterosteids were closely related to scorpaeniform fishes, reiterating the earlier conclusions of Cuvier & Valenciennes (1829; see above).

In his influential classification, Berg (1940) listed the Gasterosteiformes in three separate orders [Fig. 2(c)]: the Gasterosteiformes, Syngnathiformes and Pegasiformes. The Gasterosteiformes contained the gasterosteoids as well as the Indostomidae, although as *incertae sedis* [the phylogenetic affinities of this family have remained controversial since its initial description (Prashad & Mukerji, 1929)]. The Syngnathiformes included all syngnathoids, except the Pegasidae (which was placed in a separate order at the end of the classification), divided into two suborders following Jungersen (1910): the Aulostomoidei, with aulostomoids and centriscoids, and the Syngnathoidei, with solenostomids and syngnathids. Perhaps the most important higher level work in pre-cladistic systematic ichthyology was the classification of Greenwood *et al.* (1966), which summarized the state of knowledge of the evolutionary relationships of the main phyletic trends of teleosts. Following Berg's ideas of gasterosteiform relationships, Greenwood *et al.* (1966) tentatively brought all families together into a united Gasterosteiformes, indicating that the relationships of this group were 'still to be assessed'.

Although a description of the cladistic method, which emphasizes the pre-eminence of shared evolutionary novelties (*i.e.* synapomorphy) in revealing evolutionary relationships, was first published by Hennig (1950), the influence of this methodology was not felt in ichthyology until the English translation of his work became available in the U.S.A. (Hennig, 1966). The change in systematic rigour with the introduction of cladistic techniques was immense, as the methodology was applied to systematic studies of many taxa of fishes in the early 1970s. In the first cladistic treatment of the Gasterosteiformes, Pietsch (1978) firmly supported an expanded Gasterosteiformes, including the Pegasidae and adding the Hypoptychidae (the Indostomidae was not formally included in this analysis) [Fig. 2(d)]. Johnson & Patterson (1993) added new characters to corroborate Pietsch's (1978) hypotheses of a monophyletic Gasterosteiformes, presented evidence to support monophyly of the Syngnatha (Pegasidae, Solenostomidae and Syngnathidae) and suggested that the Indostomidae should be placed within this group. The Gasterosteiformes were placed into a large group (Smegmamorpha), which included the Synbranchoidei, Mastacembeloidei, *Elassoma*, Mugilomorpha and Atherinomorpha, a relationship which has been called into question in subsequent studies (Springer & Orrell, 2004).

Orr (1995) conducted a phylogenetic analysis of the Gasterosteiformes, primarily based on osteology, and examined the limits, monophyly and relationships of the 11 families of the order. His results supported the monophyly of the Gasterosteiformes and reinforced the monophyly of the traditional major family pairs within the order: the Gasterosteoidea, Aulostomoidea, Macroramphosoidea [=Centriscoidea of Keivany & Nelson (2006)] and Syngnathoidea [Figs 1 and 2(e)]. The Pegasidae was found to be the sister group of the terminal clade containing the Solenostomidae and Syngnathidae. Orr (1995) excluded *Indostomus* from the Gasterosteiformes and suggested a sister group relationship between the Hypoptychidae and the order Gasterosteiformes [Figs 1 and 2(e)].

Understanding the limits and constituents of the taxonomic groups under investigation is critical to a phylogenetic analysis. The influence of taxonomic sampling can

be seen in the morphological analysis of Keivany & Nelson (2006), who compiled data of their own with the analyses of several previous morphological analyses in an analysis of all families recently considered to be members of the Gasterosteiformes. They assumed monophyly of the group (Johnson & Patterson, 1993; Orr, 1995; Britz & Johnson, 2002) and used the smegmamorphs of Johnson & Patterson (1993) as outgroups. Their resulting tree [Fig. 2(f)] placed *Indostomus* as a basal syngnathoid followed by the Pegasidae, which is sister of the Syngnathoidea, the sister of the Aulostomoidea and Centriscoidea [=Macroramphosoidea of Orr (1995)].

Differences among recent morphological analyses of the group demonstrate the instability of the current understanding of gasterosteiform evolution. Orr's (1995) and Keivany & Nelson's (2006) most parsimonious phylogenetic trees were weakest at the nodes of most interest in syngnathoid evolution, the position of the Pegasidae and relationship of the Aulostomoidea and Centriscidae. Outgroup choice and the representation of in-group taxa may have strong effects on the resolution of relationships within a group of interest. With few exceptions, differences in taxonomic representation among morphological and molecular analyses have produced incongruous results in studies of gasterosteiform families.

MOLECULAR ANALYSES

Two recurring results have been found in all higher level molecular studies published to date: (1) the paraphyly of the Gasterosteiformes and (2) the placement of the Gasterosteoidei together with the cottoid–zoarcoid lineage of Imamura & Yabe (2002). As most higher level molecular studies have had a broad taxonomic focus, taxon sampling of traditional gasterosteiform members has been incomplete, making it difficult to evaluate the relationships of the group as a whole. Early analyses based on complete mitochondrial DNA genome sequences of gasterosteids, *Hypoptychus* and *Indostomus* consistently placed gasterosteids and *Hypoptychus* basal to the cottoid–zoarcoid lineage and positioned *Indostomus* within the Synbranchiformes (Miya *et al.*, 2001, 2003, 2005).

As nuclear sequences have come to be integrated into molecular analyses, incongruencies between molecular and morphological analyses of gasterosteiform relationships have become more pronounced. Within an extensive acanthomorph data set, Chen *et al.* (2003) sampled the gasterosteoid *Spinachia* and the syngnathoids *Aulostomus* and *Macroramphosus* and analysed sequence data from two nuclear (28*S* rDNA and rhodopsin) and two mitochondrial genes (12*S* and 16*S* rDNA). *Spinachia* was placed basally among a miscellaneous assortment of fishes, while *Aulostomus* and *Macroramphosus* were found to be closely related and situated in a well-supported lineage together with *Dactylopterus*. The analysis of combined mtDNA (12*S* and 16*S* rDNA) and nDNA (28*S* rDNA, histone H3 and TMO-4c4) data sets by Smith & Wheeler (2004) and Smith & Craig (2007) consistently recovered a relationship of gasterosteids with the cottoid–zoarcoid lineage of Imamura & Yabe (2002). Although *Aulostomus* was the only syngnathoid sampled in these two studies, it was found to be distantly related to gasterosteoids, consistent with the earlier results of Chen *et al.* (2003). Dettai & Lecointre (2005) added taxa and two portions of a novel nuclear locus [mixed lineage leukaemia-like gene (MLL)] to the data set of Chen *et al.* (2003). In all their analyses, the Gasterosteiformes was paraphyletic,

and gasterosteoids were placed again at the base of a cottoid–zoarcoid clade distant from syngnathoids. Oddly, while their analysis of the MLL gene recovered a monophyletic Syngnathidae, their total-evidence phylogenetic analysis (including previously published rhodopsin, 28*S* rDNA and mtDNA 12*S* and 16*S* rDNA data) placed *Syngnathus* in a clade distant from all other syngnathoids, including *Hippocampus* (Dettai & Lecointre, 2005), a result that has not been found in any other study of syngnathoid phylogenetics.

Further emphasizing the contrast between molecular and morphological studies of gasterosteiforms, the supertree analysis of Li *et al*. (2009), which sampled four nuclear genes across a broad array of acanthomorphs, placed gasterosteiforms in five different clades [Fig. 2(g)]: indostomids among synbranchiforms, fistularids together with syngnathids, aulostomids and macroramphosids with dactylopterids, and centriscids in a polytomy among miscellaneous perciforms. In a separate tree, gasterosteoids were placed with the cottoid–zoarcoid lineage. With the exception of gasterosteoid and indostomid relationships, none of these ideas, particularly the paraphyly of syngnathoid superfamilies, are supported by morphological data.

In the most comprehensive molecular study of gasterosteiform relationships to date, Kawahara *et al*. (2008) used complete mitochondrial genome sequences of representatives of all gasterosteiform families (including the Solenostomidae for the first time). Kawahara *et al*. (2008) found the Gasterosteiformes to be paraphyletic, again recovering the Gasterosteoidei together with cottoids and zoarcoids. *Indostomus* was placed with the Synbranchiformes, and members of the Syngnathoidei were placed together with dactylopterids in a polytomy with percomorphs [Fig. 2(h)]. Kawahara *et al*. (2008) supported a sister group relationship between the Pegasidae and the Syngnathidae and Solenostomidae, as in Orr (1995) [but in contrast with Keivany & Nelson (2006)], while the basal placement of the Centriscoidea by Kawahara *et al*. (2008), and the grouping of the Aulostomoidea with *Dactylopterus* (as the sister of *Aulostomus*) contrasted with both morphological analyses {Orr, 1995; Keivany & Nelson, 2006; though the relative position of these two superfamilies was admittedly only weakly supported in these earlier studies [Fig. 2(e), (f)]}. In contrast to all previous morphological analyses, Kawahara *et al*. (2008) found that the Syngnathoidei was closely related to the Gobioidei.

If the Gasterosteiformes are paraphyletic and *Indostomus* is allied with the Synbranchiformes, what is the sister group of the Syngnathoidei and where is its systematic position? While the Gasterosteoidei appears to be related to the cottoid–zoarcoid lineage, the placement of the Syngnathoidei within the acanthomorph bush remains uncertain. Morphological analyses have provided no alternative to the hypothesis of a sister group relationship between the Gasterosteoidei and the Syngnathoidei, and the results of molecular analyses have been incongruent. Among molecular analyses, the Syngnathoidei (plus Dactylopteridae) was found to be monophyletic only by Kawahara *et al*. (2008). Although morphological characters may ultimately be discovered which support a sister group relationship among other acanthomorphs, it is probable that questions related to the origins of the group will require a broadened search for appropriate outgroups (Smith & Craig, 2007). Molecular analyses of deep-level phylogenetic relationships may be susceptible to positively misleading evidence, but the ability to rapidly screen a broad diversity of distantly related taxa means that future studies on gasterosteiform relationships will heavily emphasize this form of data (Smith & Craig, 2007).

FAMILY-LEVEL PHYLOGENETICS

While higher level phylogenetic analyses have proven problematic for syngnathoids, the evolutionary relationships among members of the family Syngnathidae have been resolved with greater confidence. As outlined above, Linnaeus recognized syngnathids in his earliest classification of animals and described both seahorses and pipefishes in the first edition of *Systema Naturae* (Linnaeus, 1735). Following the descriptions of many new syngnathid species during the 18th and early 19th centuries, the first systematic description of the group was published by Kaup (1856), who considered the location and degree of development of the male brood pouch in dividing the family Syngnathidae into four subfamilies (Doryrhampinae, Nerophinae, Hippocampinae and Syngnathinae). Subsequent authors adopted this classification system (Duméril, 1870), and although several authors have used a different system of syngnathid classification (Kuiter, 2000; Nelson, 2006), these four subfamilies are still in common use today. Wilson & Rouse (2010) have proposed a revision of syngnathid subfamilies based on the results of a mtDNA-based phylogeny.

Duncker (1912, 1915) was the first to recognize that syngnathids could be naturally partitioned into two major groups based on the location of the male brood pouch. His Gastrophori (literally, abdominal-bearing) and Urophori (tail-bearing) were further divided into six subfamilies [Kaup's (1856) four plus the Gastrotokeinae = Syngnathoidinae and Solenognathinae, with three in both the Gastrophori and Urophori] according to the complexity of male brooding structures (Duncker, 1912). Unfortunately, Duncker's planned 'Synopsis of the Syngnathidae' was never published, and his rich collection of syngnathid fishes housed in the Natural History Museum, Hamburg, Germany was lost during the bombing of the city in 1939–45 (Thiel *et al.*, 2009).

In the first explicitly phylogenetic analysis of syngnathid evolution, Herald (1959) used Duncker's (1912, 1915) system of classification to develop an evolutionary hypothesis on the origin and diversification of the group. According to Herald (1959), the Syngnathidae diverged early in its evolution into tail and trunk-brooding species. Following the development of a rudimentary form of male brooding in both these lineages, brood-pouch complexity evolved in parallel in the Gastrophori and Urophori, resulting in the fully enclosed pouch of the seahorse and the highly developed brooding structures found in some gastrophorine species. Herald (1959) suggested that the brooding structures of urophorine pipefish with partially enclosed brood pouches could be further subdivided into monophyletic lineages according to their method of closure (inverted, semi-inverted, overlapping and everted) and proposed a multistage model by which the fully enclosed pouch of the seahorse was derived from pipefish ancestors with an everted brooding structure.

C. Dawson was the most recent researcher to tackle family-level syngnathid relationships from a global perspective (Overstreet & Poss, 1993). In revisions of North Atlantic (Dawson & Vari, 1982), Indo-Pacific (Dawson, 1985) and Mediterranean syngnathids (Dawson, 1986), Dawson made major strides towards clarifying the taxonomy of the group, synonymizing many species that had been previously described on the basis of regional collections. Only 294 of the 558 nominal species of syngnathids are presently considered to be valid (Froese & Pauly, 2010), and Dawson was responsible for the reclassification of 185 (>30%) of these nominal taxa. Over

a career spanning more than five decades, Dawson also described 37 new species of syngnathids, 36 of which are still recognized as valid (Froese & Pauly, 2010).

In the first family-level molecular phylogenetic study of the Syngnathidae, Wilson *et al*. (2001) used a suite of three mtDNA markers to reconstruct the evolutionary history of the group. This study included 34 species distributed across 16 of the presently recognized 54 syngnathid genera, with a strong emphasis on the two most species-rich genera in the family, *Hippocampus* and *Syngnathus*. While the taxonomic sampling of this study was far from complete, these first molecular phylogenetic results provided several key insights into the evolution of the family. Wilson *et al*.'s (2001) study supported Duncker's (1912) morphologically based grouping of the Urophori and Gastrophori, indicating that trunk- and tail-brooding lineages diverged early in the evolution of the family, but suggested that several major pouch types within each of these lineages had independent evolutionary origins, challenging the phylogenetic model proposed by Herald (1959). This study also supported a close evolutionary relationship between *Syngnathus* pipefish and *Hippocampus*, contradicting Herald's (1959) theory on the origin of the seahorse brood pouch [though the limited taxonomic sampling of Wilson *et al*. (2001) did not permit a robust test of this hypothesis]. A recently published phylogenetic analysis of syngnathids has provided compelling evidence that trunk brooding may have been secondarily acquired in *Syngnathoides biaculeatus* (Bloch 1785) (Fig. 3; Wilson & Rouse, 2010), making this species of particular interest in studies aimed at understanding the functional and morphological changes associated with the evolution of male pregnancy in this group.

Unfortunately, while the mtDNA-based molecular phylogeny of Wilson *et al*. (2001) provides strong support for the early divergence of the Urophori and the Gastrophori and supports the monophyly of several major in-group taxa, much of the backbone of the syngnathid phylogenetic tree remains poorly resolved, complicating efforts to fully understand the pattern of speciation and diversification in the group. Several follow-up studies have broadened the species sampling of this original phylogeny for the three mtDNA genes used in the original Wilson *et al*. (2001) analysis (Wilson *et al*., 2003; Wilson & Rouse, 2010) and have addressed important questions related to the evolution of the family, but the timing of key evolutionary events during the early evolution of the Urophori remain unresolved. Further resolution of the syngnathid family tree will probably only be achieved with the use of nDNA loci, whose slower rate of evolution may be more appropriate for resolving deeper level divergences in this group. A major phylogenetic analysis of the Syngnathidae using both nDNA and mtDNA markers and including a much wider taxonomic sample of species is currently underway (H. Hamilton, pers. comm.) and is expected to provide higher resolution at the deeper nodes in the syngnathid phylogeny. The most up-to-date phylogenetic tree of the family Syngnathidae, based on mtDNA sequence data, is provided in Fig. 3.

FOSSIL RECORDS

All members of the Syngnathidae have a dermal skeleton composed of body plates, and this group is consequently relatively well represented in the marine fossil record (Fig. 4). The availability of a rich fossil record is an essential prerequisite for
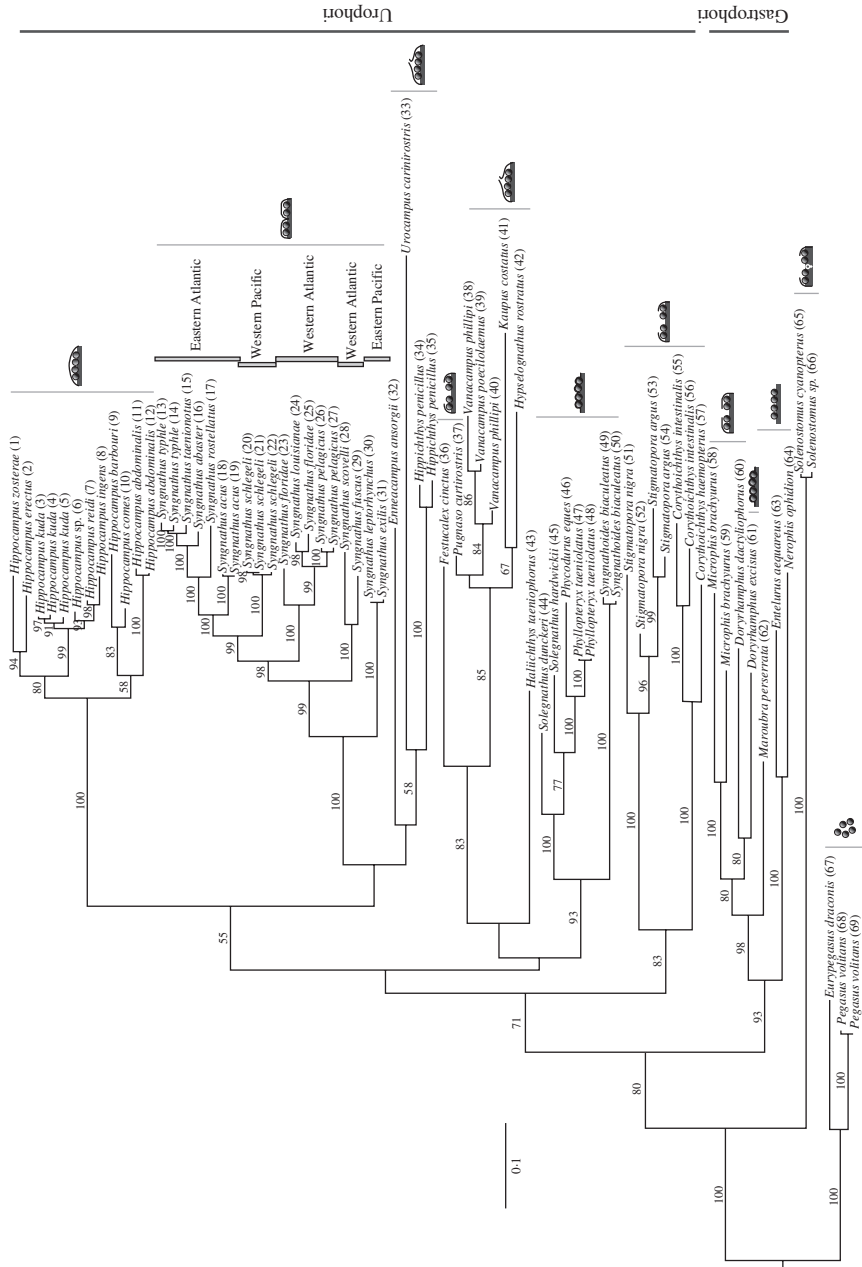
Fig. 3. Legend on next page.

studies aimed at the evolutionary origins of a group and, together with molecular data, can provide a temporal framework in which to study both its origin and pattern of diversification.

Syngnathoid fossil finds are richest in the Mediterranean Sea and Black Sea regions, but large numbers of Oligocene and Miocene fossils have also been collected from Paratethys sites in central Europe (Fig. 4). In addition to this rich European fossil record, fossils of three syngnathid species have been collected from the Modelo and Puente formations of southern California (Fritzsche, 1980). Mirroring the confusion surrounding the higher level relationships among contemporary taxa, considerable debate has surrounded the taxonomic placement of many syngnathoid fossils. Many fossil species remain undescribed, and while the fossil species discussed here are believed to represent valid taxa, a comprehensive consideration of the more problematic gasterosteiform fossils awaits a detailed review.

The oldest syngnathoid fossils date from the early Eocene [48–50 million years before present (B.P.)], and the majority of these fossil taxa have been collected from the rich fossil beds of the Monte Bolca formation in northern Italy (Blot, 1980). Six fossil syngnathoids have been collected from Eocene deposits in this region, representing five distinct taxonomic lineages (Fig. 4). Species of the now extinct Ramphosidae, a family which has been suggested to be a sister group to the extant Pegasidae (Pietsch, 1978), have been collected from Eocene sites in Monte Bolca (Blot, 1980) and from a second locality in Jutland, Denmark (Nielsen, 1960). Three additional Monte Bolca fossils are believed to represent morphologically-distinct syngnathoid lineages, and *Solenorhynchus elegans* (Heckel 1853) and *Calamostoma breviculum* Agassiz 1833 are thought to be intermediate forms between the Solenostomidae and the Syngnathidae (Orr, 1995). The oldest syngnathid fossil is believed to be *Prosolenostomus lessenii* Blot 1980, a species that has been placed in the stem lineage of the Syngnathidae on the basis of its absence of dorsal and pelvic fins (Orr, 1995). While the age of *Prosolenostomus* indicates that the family Syngnathidae is at least 50 million years old, the high diversity of syngnathoid fossils found in Eocene deposits suggests that the family probably predates this period.

While the fossils collected from the Eocene deposits of Monte Bolca cannot be confidently assigned to an extant genus, more recent fossils have been partitioned to either the Urophori or the Gastrophori on the basis of their brooding structures. The genus *Hipposyngnathus* is the oldest gastrophorine lineage yet discovered and

FIG. 3. Maximum-likelihood molecular phylogenetic tree of the Syngnathidae, based on complete cytochrome *b* and partial 12*S* rDNA and 16*S* rDNA sequence data, with individual partitions for each of the mtDNA data sets and a common GTR + I + G model of evolution. Specimen numbers indicated in parentheses (Table SII for a full list of sequence accessions). Tree reliability was estimated using bootstrap resampling (200 replicates; bootstrap values >50% indicated). Brood-pouch cross-sections illustrate pouch variation [🐛, fully enclosed pouch; 🐛, enclosed pouch (everted); 🐛, enclosed pouch (inverted); 🐛, enclosed pouch (semi); 🐛, rudimentary pouch with plates and skin-folds; 🐛, individual egg compartments; 🐛, unprotected eggs; 🐛, female brooding; 🐚, free spawning] among the major lineages of trunk- (Gastrophori) and tail-brooding (Urophori) species. The trunk-brooding *Syngnathoides biaculeatus* has recently been shown to cluster together with urophorine species, suggesting that trunk brooding has evolved independently in this lineage (Wilson & Rouse, 2010). Note the low level of support for the backbone of the syngnathid tree. Methodological details for this analysis are provided in Appendix SI.

(a)

(b)

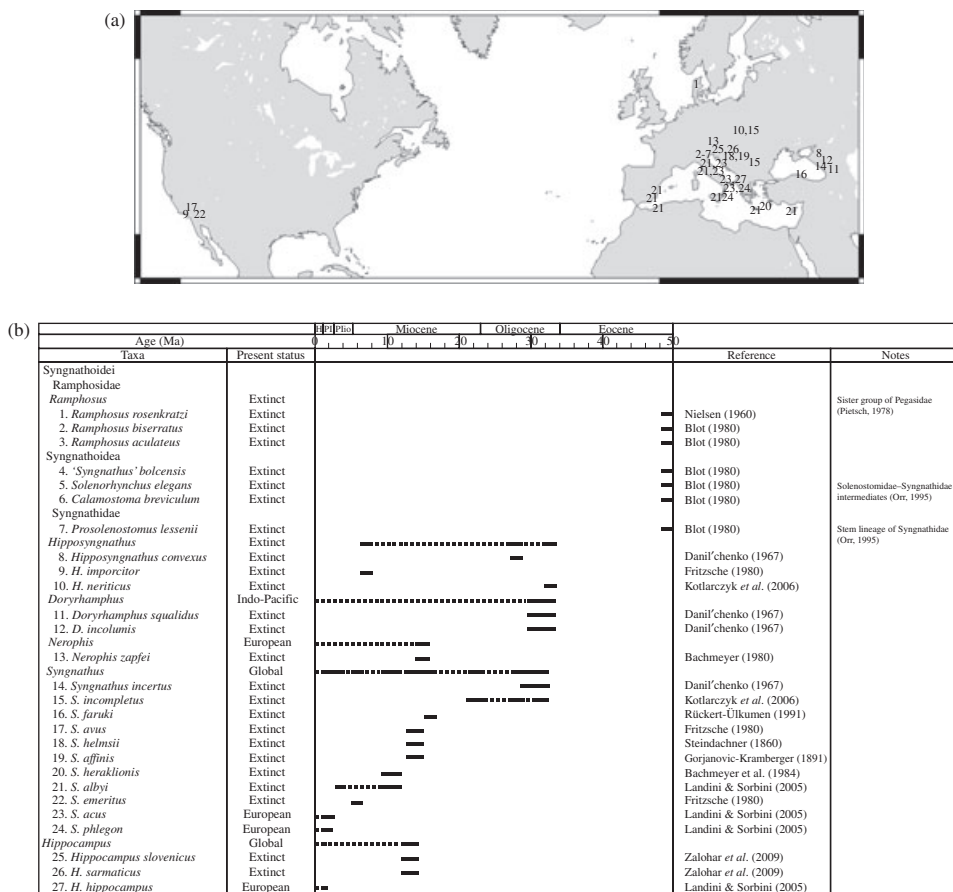| Taxa	Present status	Age (Ma)	Reference	Notes
Syngnathoidei				
Ramphosidae				
Ramphosus	Extinct			Sister group of Pegasidae (Pietsch, 1978)
1. *Ramphosus rosenkratzi*	Extinct		Nielsen (1960)	
2. *Ramphosus biserratus*	Extinct		Blot (1980)	
3. *Ramphosus aculeatus*	Extinct		Blot (1980)	
Syngnathoidea				
4. *'Syngnathus' bolcensis*	Extinct		Blot (1980)	
5. *Solenorhynchus elegans*	Extinct		Blot (1980)	Solenostomidae–Syngnathidae intermediates (Orr, 1995)
6. *Calamostoma breviculum*	Extinct		Blot (1980)	
Syngnathidae				
7. *Prosolenostomus lessenii*	Extinct		Blot (1980)	Stem lineage of Syngnathidae (Orr, 1995)
Hipposyngnathus	Extinct			
8. *Hipposyngnathus convexus*	Extinct		Danil'chenko (1967)	
9. *H. imporcitor*	Extinct		Fritzsche (1980)	
10. *H. neriticus*	Extinct		Kotlarczyk *et al.* (2006)	
Doryrhamphus	Indo-Pacific			
11. *Doryrhamphus squalidus*	Extinct		Danil'chenko (1967)	
12. *D. incolumis*	Extinct		Danil'chenko (1967)	
Nerophis	European			
13. *Nerophis zapfei*	Extinct		Bachmeyer (1980)	
Syngnathus	Global			
14. *Syngnathus incertus*	Extinct		Danil'chenko (1967)	
15. *S. incompletus*	Extinct		Kotlarczyk *et al.* (2006)	
16. *S. faruki*	Extinct		Rückert-Ülkumen (1991)	
17. *S. avus*	Extinct		Fritzsche (1980)	
18. *S. helmsii*	Extinct		Steindachner (1860)	
19. *S. affinis*	Extinct		Gorjanovic-Kramberger (1891)	
20. *S. heraklionis*	Extinct		Bachmeyer et al. (1984)	
21. *S. albyi*	Extinct		Landini & Sorbini (2005)	
22. *S. emeritus*	Extinct		Fritzsche (1980)	
23. *S. acus*	European		Landini & Sorbini (2005)	
24. *S. phlegon*	European		Landini & Sorbini (2005)	
Hippocampus	Global			
25. *Hippocampus slovenicus*	Extinct		Zalohar *et al.* (2009)	
26. *H. sarmaticus*	Extinct		Zalohar *et al.* (2009)	
27. *H. hippocampus*	European		Landini & Sorbini (2005)	

FIG. 4. The syngnathoid fossil record. (a) A summary of published reports of syngnathoid fossils, indicating (a) the locality of the fossil description (above) and (b) the approximate age of the fossil deposits based on data reported in the original publications. ——: described fossils; ·······: presumed presence based on more recent fossil finds and the existence of contemporary taxa (Landini & Sorbini, 2005).

represents an extinct lineage of pipefishes that persisted from the early Oligocene until the Miocene (Fig. 4). Three fossil species have been described from this genus, two from early Oligocene deposits in the Caucasus and Carpathian basins [*Hipposyngnathus convexus* Danil'chenko 1960 and *Hipposyngnathus neriticus* Jerzmañska 1968 (Danil'chenko, 1967; Kotlarczyk *et al*., 2006)] and one from Miocene deposits of southern California (*Hipposyngnathus imporcitor* Fritzsche 1980), indicating that this genus achieved a wide distribution during its existence.

The earliest gastrophorine fossils with clear affinities for extant genera are the early Oligocene *Dunckerocampus incolumis* Danil'chenko 1967 and *Dunckerocampus squalidus* Danil'chenko 1967 from the Caucasus (Danil'chenko, 1967) and the Miocene *Nerophis zapfei* Bachmeyer 1980 (Bachmayer, 1980) of eastern Austria, indicating the extended evolutionary history of these two lineages (Fig. 4). The modern *Nerophis* is found in the temperate coastal habitats along the European coastline; *Dunckerocampus* spp. are currently restricted to the Indo-West Pacific Ocean (Froese & Pauly, 2010).

Both *Hippocampus* and *Syngnathus* are well represented in the urophorine fossil record. *Syngnathus* has the richest fossil record of any extant syngnathid genus, and 11 fossil *Syngnathus* species are currently recognized. The oldest *Syngnathus* fossils date from the early Oligocene (Danil'chenko, 1967; Kotlarczyk *et al.*, 2006), and the genus has an almost uninterrupted fossil record from this period until the present day (Fig. 4). The highest diversity of *Syngnathus* fossils has been found in the pre-Messinian deposits of the Miocene Mediterranean Sea, though the existence of *Syngnathus avus* Jordan & Gilbert 1919 in southern Californian formations indicates that the genus has been present on the Pacific Coast of North America for at least 15 million years. While at least four *Syngnathus* species were present in the Miocene Mediterranean Sea (15 M B.P.), subsequent deposits are dominated by a single widespread species (*Syngnathus albyi* Sauvage 1870), which has been collected from many sites around the modern Mediterranean Sea basin (Landini & Sorbini, 2005). *Syngnathus albyi* appears to have persisted through the Messinian salinity crisis in the Mediterranean Sea (*c.* 6 M B.P.), but is replaced in more recent Pliocene and Pleistocene deposits by the modern *Syngnathus acus* L. 1758 (Fig. 4).

While a Pleistocene fossil of the extant *Hippocampus hippocampus* (L. 1758) was long the only known representative of this genus in the fossil record (Landini & Sorbini, 2005), two Miocene fossils have been described from Sarmatian deposits in Slovenia [*Hippocampus sarmaticus* Zalohar, Hitij & Kriznar 2009 and *Syngnathus slovenicus* Zalohar, Hitij & Kriznar (Zalohar *et al.*, 2009)], considerably extending the age of this lineage. The two Slovenian seahorse fossils are clearly distinct, with *H. sarmaticus* showing affinities for the modern *Hippocampus trimaculatus* Leach 1814 and *H. slovenicus* bearing a resemblance to contemporary pygmy seahorses (Zalohar *et al.*, 2009). Again, major morphological differences between these two fossils suggest that the seahorse lineage predates the Miocene, consistent with recent molecular clock reconstructions of the group (Teske & Beheregaray, 2009).

INTRAGENERIC RELATIONSHIPS

Both phylogenetic and phylogeographic studies have heavily emphasized the evolution of seahorses (genus *Hippocampus*) and *Syngnathus* pipefishes, providing insights into the biogeography of these two genera. A 2004 issue of *Molecular Phylogenetics and Evolution* saw the publication of back-to-back articles on the evolutionary relationships of the seahorse. The first, by Casey *et al.* (2004), analysed full-length cytochrome *b* sequences in a sample of 22 seahorse species, including multiple representatives of each species sampled. A second study by Teske *et al.* (2004) supplemented the Casey *et al.* (2004) cytochrome *b* data set with 16*S* rDNA sequence data and two nuclear genes (*RP*1 and *aldolase*) and included a total of 30 seahorse species. The analysis of Teske *et al.* (2004) produced a highly resolved phylogeny of the seahorse genus and provided important insights into the evolutionary history of the group.

On the basis of the placement of the Australian *Hippocampus breviceps* Peters 1869 and Australian and New Zealand *Hippocampus abdominalis* Lesson 1827 at the base of the seahorse phylogeny and the south-west Pacific Ocean distribution of several outgroup species, Teske *et al.* (2004) hypothesized an Australian origin for the genus *Hippocampus*. The main group of seahorses falls into three major clades, two

with Indo-Pacific affinities and one with a circumglobal distribution, a lineage which is thought to have had its origin following the colonization of the Atlantic Ocean (Teske *et al.*, 2004). This lineage subsequently recolonized the Indo-West Pacific Ocean, giving rise to a species-rich group including the widespread *Hippocampus kuda* Bleeker 1852. The alternative hypothesis, that Atlantic Ocean seahorse species are the descendents of repeated colonizations of this region, however, could not be statistically rejected. Subsequent biogeographic analyses indicate that long-distance dispersal events have placed an important role in the origin of new seahorse lineages in the Indo-West Pacific (Teske *et al.*, 2005) and Atlantic Oceans (Teske *et al.*, 2007).

The original family-level phylogeny of the Syngnathidae included 11 species of *Syngnathus* pipefishes from Europe, Asia and North America (Wilson *et al.*, 2001). While the relationships among *Syngnathus* pipefishes were poorly resolved using the pruned data set published in the original paper (third codons of cytochrome *b* were removed due to mutational saturation at high genetic distances), the full data set used for the updated family-level phylogeny published here provides a high degree of resolution within *Syngnathus*, with strong support for all major nodes in the *Syngnathus* phylogeny (Fig. 3).

Similar to the results observed for *Hippocampus*, there is clear geographical structure in *Syngnathus* biogeography. The most basal lineages in the *Syngnathus* phylogeny are restricted to the Pacific coast of North America [*Syngnathus exilis* (Osburn & Nichols 1916) and *Syngnathus leptorhynchus* Girard 1854], and the remainder of the phylogeny is divided into three major clades, a widespread Atlantic coast group [*Syngnathus fuscus* Storer 1839 and *Syngnathus scovelli* (Everman & Kendall 1896)], a second clade containing Atlantic coast and Caribbean species, and a third clade containing the western Pacific *Syngnathus schlegeli* Kaup 1856 and a monophyletic clade of European pipefishes (Fig. 3). The close phylogenetic relationships between clades on east and west coasts of North America and Eurasia suggest that interoceanic dispersal may be relatively common in *Syngnathus*, a pattern which mirrors that detected in *Hippocampus*. Interestingly, given that the oldest *Syngnathus* fossils are from Oligocene sites in eastern Europe, the present-day phylogeny of the genus appears to reflect the consequences of extensive speciation and extinction during the evolution of the group.

PHYLOGEOGRAPHY

Recent publications concerning the evolution and origin of syngnathids have been dominated by phylogeographic investigations of individual species. Here again, studies have focused almost exclusively on *Hippocampus* and *Syngnathus* (Table SI), providing an excellent data set in which to identify general patterns in the contemporary phylogeography of these two genera.

Despite differences in the spatial scales (from 10^1 to 10^4 km), the number of individuals screened (from <10 to >300) and the markers used (mtDNA and nDNA sequence data, microsatellites and allozymes), the majority of studies on both seahorses and pipefishes have found moderate-to-high genetic diversity and strong phylogeographic structure, consistent with large effective population sizes and low dispersal potential of syngnathid species. Studies conducted on northern-hemisphere

temperate water species have typically found evidence of recent population expansions, consistent with the colonization of northern habitats following the end of the last glacial maximum (*c.* 20 k B.P.). The large number of phylogeographic studies published over the past decade cannot be reviewed in detail here (see Table SI for a list of published studies), but a number of case studies serve to illustrate how phylogeographic analyses have contributed to a greater understanding of syngnathid evolution.

PANMIXIA IN POPULATIONS OF *H. ABDOMINALIS*

In contrast to the majority of phylogeographic studies on syngnathid species, which have found evidence of strong population structuring in both seahorses and pipefishes, recent investigations by Armstrong (2001) and Nickel (2009) found a surprising lack of phylogeographic structure in New Zealand and Australian populations of *H. abdominalis*. Armstrong (2001) used a combination of morphological characters, mtDNA sequence data and allozymes to investigate the hypothesis that Australian and New Zealand populations of *H. abdominalis* represent distinct species (Kuiter, 2001). While morphological variation in *H. abdominalis* was high, the majority of this variation was found within populations, with no diagnostic differences between the putative species. Allozyme markers could not distinguish between New Zealand and Australian populations, though some evidence of geographic structure was detected in mtDNA data, where the majority of New Zealand individuals formed a monophyletic clade. Armstrong (2001) rejected the hypothesis that *H. abdominalis* represented more than a single species, though molecular data do appear to indicate that populations in these two regions may be in the early stages of divergence.

In a study aimed at investigating the conservation status of New Zealand populations of *H. abdominalis*, Nickel (2009) used multiple mtDNA markers and nuclear microsatellites to investigate the phylogeographic structure of a large number of populations on both the north and south islands of the country. Although Nickel (2009) observed a remarkable range of morphological and colour variation among individuals [consistent with the results of Armstrong (2001)], and high genetic diversity at both mtDNA and microsatellite loci, no evidence of phylogeographic structure was found. The distribution of mtDNA haplotypes was found to be consistent with a recent population expansion, leading Nickel (2009) to suggest that New Zealand may have been colonized relatively recently by Australian seahorses, a situation which could help to explain the lack of population structure observed in the region. The application of molecular dating to these data should permit a robust test of timing of the colonization of New Zealand by *H. abdominalis*.

IDENTIFYING PHYLOGEOGRAPHIC BREAKS: THE IMPORTANCE OF SCALE

The broad distributions of many syngnathids make this group particularly well suited for studying the population genetic consequences of environmental variation across time and space. The Pacific Coast pipefish *S. leptorhynchus* is one of the most widespread pipefish species and is distributed across almost 4000 km of coastline between the Alexander Archipelago, Alaska, U.S.A. and Baja California,

Mexico. Morphological variation in *S. leptorhynchus* is high, a finding which has led some researchers to suggest that it may actually comprise more than a single species (Herald, 1941). On the basis of meristic variation, Herald (1941) suggested that *S. leptorhynchus* could be divided into a northern and southern species, with a biogeographic boundary near Point Conception in southern California.

Two recent studies have used molecular markers to study the Pacific Coast phylogeography of *S. leptorhynchus* (Louie, 2003; Wilson, 2006). While both studies covered the same geographic range, Louie (2003) used a high-density sampling strategy, surveying almost 400 individuals sampled from 29 coastal sites, while Wilson (2006) sampled larger numbers of individuals from four collection localities. Both studies found evidence of the post-glacial colonization of northern sites following the end of the last glacial cycle and detected evidence of a phylogeographic break between sites in the north-west Pacific Ocean and those farther south. Interestingly, while the phylogeographic break identified by Wilson (2006) was found between central Oregon and California, U.S.A., the mtDNA data of Louie (2003) suggested that this break was located nearly 500 km farther north, in northern Washington. While the statistical approaches used in these two studies were not identical, differences in their conclusions can be largely attributed to differences in their sampling strategies, illustrating the potential challenges inherent in interpreting the results of molecular analyses.

COMPARATIVE PHYLOGEOGRAPHY OF SEAHORSES: NEW INSIGHTS *VIA* SPECIES-LEVEL COMPARISONS

In what is undoubtedly the most impressive analysis of comparative phylogeographic structure in a group of syngnathids, Lourie *et al.* (2005) investigated the phylogeographic structure of four south-east Asian seahorse species inhabiting a region dominated by the presence of the Sunda Shelf, a large shallow sea which has been repeatedly exposed as land during the sea-level reductions associated with Pleistocene glacial cycles. Lourie *et al.* (2005) sampled widely (11–29 populations of each species) and, most importantly, used the same fragment of the mtDNA cytochrome *b* gene for all species, facilitating the direct comparison of phylogeographic structure. Differences in the contemporary population genetic structure of the four *Hippocampus* species could be directly attributed to species-specific habitat and ecological differences. While genetic diversity was high in all species, population genetic structuring was especially pronounced in *Hippocampus barbouri* Jordan & Richardson 1905 and *H. kuda*, two shallow-water species, while phylogeographic structure was more modest in the deep-water *Hippocampus spinosissimus* Weber 1913 and *H. trimaculatus* (Lourie *et al.*, 2005).

Differences in phylogeographic structure among these species appear to reflect differences in their relative dispersal abilities. While both deep-water species appear to have colonized the Sunda Shelf since its most recent flooding (*c*. 15 k B.P.), *H. barbouri* has not yet spread into the region, and the large number of private haplotypes found in shelf populations of *H. kuda* suggest that these lineages are derived from a refugial population. In an innovative use of haplotype frequency data, Lourie *et al.* (2005) inferred dispersal potential of the four study species by examining the geographic distribution of individual haplotypes, an approach which serves as an indirect estimate of relative dispersal. The scale of the Lourie *et al.* (2005) investigation exceeds that of most previously published phylogeographic studies on the

group [Woodall (2009) provides a comparably impressive data set for European sea-horses], and such comparative studies offer one of the best means by which the factors contributing to population and species-level divergence can be identified.

CONCLUSIONS

Researchers have long been interested in the evolutionary relationships among syngnathids, and the past several decades have provided new insights into the evolution of the group. The application of molecular methods has proven particularly effective in illuminating more recent evolutionary events, while both molecular and morphological analyses have failed to provide a well-resolved model of deeper level relationships, complicating efforts to understand the evolutionary origins of the family Syngnathidae. Although the sister group relationship of the Solenostomidae and the Syngnathidae appears to be robust, the relationships among other major groups within the order Gasterosteiformes remain poorly understood. Comparative ontogenetic studies may help to complement morphological analyses based on adult specimens, and a broadened analysis using molecular methods may provide an evolutionary hypothesis that could be corroborated with further morphological investigations.

While the large number of recent studies on syngnathid phylogeography and phylogenetics (and on many other aspects of syngnathid biology) have clarified several aspects of syngnathid evolution, most of these studies have been restricted to the two most species-rich genera of the family, seahorses (genus *Hippocampus*), and pipefishes of the genus *Syngnathus*. Although these publications have been prompted in part by considerable conservation interest in seahorse species (Foster & Vincent, 2004), they tend to overlook the majority of morphological and ecological variation found in the rest of the family. As many syngnathid species inhabit the same sensitive environments inhabited by seahorses, they offer potentially useful comparative systems in which to investigate how ecological variation influences species susceptibility to habitat degradation. At the same time, a wider focus on the diversity of forms found in this family promises to provide a deeper understanding of the evolutionary pressures which have influenced its diversification.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

APPENDIX SI. Methodological details for phylogenetic reconstruction.

TABLE SI. Syngnathid phylogeography, recent studies. Genetic markers: Microsats (Microsatellites), mtDNA (mitochondrial DNA), cyt*b* (cytochrome *b*), COI (cytochrome oxidase subunit I), CR (control region).

TABLE SII. GenBank accession numbers for specimens included in phylogenetic analysis (Fig. 3).

APPENDIX SII. Supplementary References.

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