Molecular systematics of flyingfishes (Teleostei: Exocoetidae): evolution in the epipelagic zone

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The flyingfish family Exocoetidae is a diverse group of marine fishes that are widespread and abundant in tropical and subtropical seas. Flyingfishes are epipelagic specialists that are easily distinguished by their enlarged fins, which are used for gliding leaps over the surface of the water. Although phylogenetic hypotheses have been proposed for flyingfish genera based on morphology, no comprehensive molecular studies have been performed. In the present study, we describe a species-level molecular phylogeny for the family Exocoetidae, based on data from the mitochondrial cytochrome b gene (1137 bp) and the nuclear RAG2 gene (882 bp). We find strong support for previous morphology-based phylogenetic hypotheses, as well as the monophyly of most currently accepted flyingfish genera. However, the most diverse genus Cheilopogon is not monophyletic. Using our novel flyingfish topology, we examine previously proposed hypotheses for the origin and evolution of gliding. The results support the progressive transition from two-wing to four-wing gliding. We also use phylogenetic approaches to test the macroecological effects of two life history characters (e.g. egg buoyancy and habitat) on species range size in flyingfishes. © 2010 The Linnean Society of London, Biological Journal of the Linnean Society, 2011, 102, 161–174.


INTRODUCTION

The marine epipelagic zone is one of the largest and most productive habitats on Earth, although it exhibits remarkably low species diversity (Angel, 1993). Survival in epipelagic habitats presents a number of specific challenges for fishes, including the rarity of substrate for egg deposition and refuges, and a highly patchy distribution of resources (Parin, 1968; Hamner, 1995; Allen & Cross, 2006). Epipelagic fishes are also exposed to predators and powerful abiotic forces (e.g. ocean currents) during all phases of their lifecycles. As a result, epipelagic species exhibit an array of specialized adaptations. Reproductive characteristics that compensate for the absence of benthic substrate (e.g. buoyant eggs or egg filaments for attachment to floating debris and vegetation) and adaptations for predator avoidance (e.g. defensive spines, cryptic coloration, and protective schooling behaviour) are present in many epipelagic taxa (Hamner, 1995; Nelson, 2006). Among the most spectacular adaptations to epipelagic habitats is the aerial behaviour of flyingfishes (and certain species of squids), which make gliding leaps from the water, presumably to evade predators (Mohr, 1954; Evans & Sharma, 1963; Fish, 1990; Gillett & Ianelli, 1991; Davenport, 1992; Davenport, 1994; Kutschera, 2005).

The flyingfish family Exocoetidae includes approximately 50 species that are distributed across the tropical and subtropical regions of the Paciﬁc,
Atlantic, and Indian oceans. A key element of epipelagic food webs, flyingfishes feed on zooplankton and transfer energy from lower levels of the trophic system to top predators (Parin, 1968). As the predominant form of middle-sized nekton (i.e. actively swimming organisms < 1 m in length) in the open ocean, flyingfishes are a critical source of food for pelagic predators such as dolphinfishes, tunas, billfishes, cetaceans, and pelagic seabirds (Parin, 1968). The most distinct feature of flyingfishes is their greatly enlarged paired fins, which allow glides above the surface of the water (Davenport, 1994). Some species have greatly enlarged pectoral fins (two-wing gliders; also described as monoplane gliders by Breder, 1930), whereas others have greatly enlarged pectoral and pelvic fins (four-wing gliders; also described as biplane gliders by Breder, 1930). Four-wing flyingfishes can glide up to 400 m, and can accomplish turns and altitude changes, whereas two-wing gliders travel shorter distances, usually in a straight line (Davenport, 1994).

Flyingfishes show variation in life history and reproductive biology. Although all species are oviparous, some have specialized egg structures that allow attachment of eggs to floating debris and seaweed, whereas others lay buoyant eggs on the surface of the open ocean (Collette et al., 1984). However, some species return to, or continuously occupy, coastal habitats to complete their life cycle, whereas others spend their entire lives far offshore in pelagic habitats. Geographical range size varies considerably among species, from locally restricted to circumtropical. For example, Fodiator rostratus is endemic to the nearshore waters of the eastern tropical Pacific (Parin, 1995), whereas Exocoetus volitans has a largely pantropical distribution (Parin & Shakhovskoy, 2000). Life-history traits, such as dispersal ability of eggs and larvae, have been demonstrated to affect geographic ranges of other marine taxa, including invertebrates and fishes (Bowen & Avise, 1990; Palumbi, 1992; Knowlton, 1993; Burton, 1998; Palumbi, 2004; Lester et al., 2007; Galarza et al., 2008; Ehle, Toonen & Bowen, 2009; for a review, see Cowen & Sponaugle 2009). However, correlations between life history characters and species range size have not been investigated in flyingfishes.

The Exocoetidae has been proposed as a monophyletic group within the order Beloniformes based on both morphological (Bruun, 1935; Parin, 1961; Collette et al., 1984; Dasilao & Sasaki, 1998) and molecular studies (Lovejoy, 2000; Lovejoy, Iranpour & Collette, 2004). However, phylogenetic hypotheses within the family Exocoetidae have been entirely based on morphological characters and have focused on genus-level relationships. Parin (1961; see also Bruun, 1935) proposed an evolutionary scheme that grouped seven genera into four subfamilies: Fodiatorinae, Parexocoetinae, Exocoetinae, Cypselurinae (with the latter containing the genera Prognichthys, Cypselurus, Cheilopogon, and Hirundichthys) (Fig. 1A). Collette et al. (1984) produced a subfamily-level analysis, based on morphological characters, that matched Parin (1961) (Fig. 1A). More recently, Dasilao & Sasaki (1998; see also Dasilao, Sasaki & Okamura, 1997) produced a cladistic analysis based on 41 morphological characters (Fig. 1B), which provided further support for the trees proposed by Parin (1961) and Collette et al. (1984). The morphology-based trees suggest a stepwise evolution of gliding capability, progressing from two-wing gliding (Fodiator, Parexocoetus, and Exocoetus), to four-wing gliding (Cypselurinae). These authors also proposed that Oxyporhamphus, a taxon that shares features with both flyingfishes and halfbeaks, should be considered a basal member of Exocoetidae (Dasilao et al., 1997). Lovejoy et al. (2004) presented a molecular phylogenetic analysis for beloniform fishes that included eight flyingfish species in seven genera. This analysis closely agreed with the flyingfish relationships based on morphological studies, although Parexocoetus and Fodiator were grouped as sister taxa.

Flyingfishes are an excellent group for studying the evolution of epipelagic adaptations, biogeography, and marine diversification. However, a species-level phylogeny is a prerequisite for such investigations. In the present study, we describe the first molecular

phylogeny for the Exocoetidae based on mitochondrial and nuclear genes. Our objectives were: (1) to generate a species-level molecular phylogeny for flyingfishes and compare this with previous morphology-based hypotheses; (2) to test the monophyly of currently accepted flyingfish subfamilies and genera; (3) to reconstruct the evolution of flyingfish gliding strategies; and (4) to test whether species range size is correlated with variation in egg buoyancy and habitat preference.

MATERIAL AND METHODS

TAXON SAMPLING

Specimens were collected in the field or donated by collaborators, with tissues stored in 95% ethanol. Voucher specimens have been deposited in museum collections (Table 1). In total, 65 flyingfish individuals (representing 31 species and seven genera) and ten outgroup individuals (representing five species and four genera) were included.

MOLECULAR DATA COLLECTION

Genomic DNA was extracted using DNeasy kits (Qiagen). The mitochondrial cytochrome b (cytb) gene (1137 bp) was amplified by polymerase chain reaction (PCR) and primers ExoCBFwd (5′-GGACTTTATGAYTTGAAAAACCATCGTTG-3′) and ExoCBRev (5′-AACCTTCGACGCTTGCCCTACAAG GCCG-3′), which were designed using published data from actinopterygian (Sevilla et al., 2004) and beloniform fishes (Lovejoy & Collette, 2001; Lovejoy et al., 2004).

PCR for cytb amplifications were performed with 8 mg/L of bovine serum albumin, 1 × Taq Polymerase Buffer, 0.2 mM of each dNTP, 2.8 mM MgCl₂, 7.5 mol of each primer, 1.25 U of Taq DNA Polymerase (Fermentas Inc.) and approximately 125 ng of genomic DNA. RAG2 amplifications were performed with 8 mg/L of bovine serum albumin, 1 × Taq Polymerase Buffer, 0.2 mM of each dNTP, 2.0 mM MgCl₂, 5 mol of each primer, 1 U of Taq DNA Polymerase, and approximately 250 ng of genomic DNA.

PCR was performed using the following conditions for cytb: initial denaturation at 95 °C for 30 s; followed by 35 cycles of 95 °C for 30 s, 50 °C for 60 s, and 72 °C for 90 s; followed by an extension at 72 °C for 5 min. RAG2 amplifications used the conditions: initial denaturation at 94 °C for 120 s; followed by 40 cycles of 94 °C for 30 s, 50 °C for 60 s, and 72 °C for 120 s; followed by a final extension at 72 °C for 7 min. Sequencing was completed using internal sequencing primers ExoFwd1 (5′-GCYACCCCT CACCCGATTTTACC-3′) and ExoRev1 (5′-CTT TRTATGAAAGTGGGTG-3′) (cytb), and F16b-Ch (5′-CTATTGACCTGAGTTTG-3′) and R17-Ch (5′-GAGTCAGGTCAGTGAGTG-3′) (RAG2). Sequences were examined, edited, and aligned using Sequencer, version 4.6 (Gene Codes Corporation).

PHYLOGENETIC ANALYSIS

Maximum parsimony (MP) analyses were conducted using the combined evidence dataset (both genes), as well as for cytb and RAG2 separately. Saturation analyses indicated that inclusion of cytb third positions was appropriate. Heuristic searches were implemented using PAUP* (Swofford, 2000), with tree bisection–recombination (TBR) branch swapping and 10 000 random taxon addition replicates. MP bootstrap analyses were performed using an equally weighted heuristic search with 1000 replicates, 100 addition sequence replicates, and TBR branch swapping. For all analyses, the outgroup taxon Zenarchopterus buffonis was used to root phylogenetic trees.

For Bayesian analysis, MrModeltest, version 2.3 (Nylander, 2004) was used to select models of evolution based on Akaike information criteria (Posada & Buckley, 2004). MrModeltest was run on the combined dataset, as well as for cytb and RAG2 separately. Bayesian Inference (BI) analyses were performed using MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003) and were conducted on the combined evidence dataset (both genes) as well as for cytb and RAG2 separately. For all analyses, convergence between concurrent runs was assessed by PSRF values approaching 1.000 and an even distribution of posterior probabilities (Ronquist & Huelsenbeck, 2003). A general time reversible model with variable sites and a gamma shaped distribution (GTR+I+Γ) was applied and run for 20 million generations (sampling every 1000 trees and discarding 25% as burn-in). For the combined dataset, the ‘unlink’ command was used to estimate parameters independently for each gene.

RECONSTRUCTING THE EVOLUTION OF GLIDING STRATEGIES

To reconstruct the evolution of gliding in flyingfishes, gliding strategy was categorized as a multistate character and optimized on our trees using the ‘trace’ command in MacClade, version 4.07 (Maddison & Maddison, 2005). Breder’s (1930) proposed distinction
Table 1. Data for specimens used in the present study, voucher catalogue information, collection localities, and GenBank accession numbers

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between two-wing and four-wing gliding in flying-fishes has largely been followed in the literature (Fish, 1990; Davenport, 1994); thus, gliding strategy was coded as a multistate character with the states ‘absent’, ‘two-wing’, and ‘four-wing’. The elongated dorsal fin of *Parexocoetus* may serve as an additional gliding surface, resulting in three-wing gliding (R. L. Pitman, pers. observ.); however, this phenomenon has not been formally described and thus will not be specifically addressed in the present study (see below).

**RANGE SIZE AND LIFE-HISTORY CHARACTERS**

To test whether flyingfish species range size is correlated with certain life history characters, we used the concentrated-changes test (CCT) of Maddison (1990; see Maddison & Maddison, 1992) as implemented by MacClade, version 4.07 (Maddison & Maddison, 2005). The CCT determines whether changes in a particular character (the dependent character) are concentrated on branches that have a specified state for a second character (the independent character). Specifically, we tested the macroecological predictions that: (1) flyingfishes with buoyant eggs have larger geographic ranges than those with nonbuoyant eggs and (2) flyingfishes that complete their entire lifecycle far offshore have larger geographic ranges than those that include an inshore component to their lifecycle.

Data on egg buoyancy, habitat, and geographic range size were determined from the literature (see Supporting information, Table S1) and coded as binary characters. Each species in our phylogeny was coded as having eggs that are either nonbuoyant (0) or buoyant (1), habitat preference that is either meroepipelagic (0) or holoepipelagic (1), and a range size that is either limited to a single ocean (0) or spans multiple oceans (1). We defined the two habitat states based on Parin’s (1968) work, where meroepipelagic species are defined as using coastal (continental shelf) waters during some period of their lives, whereas holoepipelagic species are defined as taxa that complete all life stages in the open ocean (off of the continental shelf). We use the presence of a species in either one or multiple oceans as a coarse proxy for more precise measurements of species range size because other procedures, such as digitizing areas from maps, could not be completed for a
reasonable number of species (i.e. the majority of exocoetids). Pending improved biogeographic data for flyingfish species, we consider that this approximation allows reasonable, albeit conservative, tests of our hypotheses.

To implement the CCT, redundant operational taxonomic units (multiple representatives of the same species) were pruned from trees and polytomies were resolved manually (a necessity for the test). Characters were optimized with equivocal reconstructions resolved using both DELTRAN and ACCTRAN, however, the DELTRAN method was preferred because it does not force an increase in the number of observed character reversals (Maddison & Maddison, 2005).

RESULTS

PHYLOGENETIC RELATIONSHIPS

A total of 2019 bp were amplified and sequenced from the mitochondrial cytb gene (1137 bp) and the nuclear RAG2 gene (882 bp). Of these, 1289 characters were constant, 51 were variable but parsimony uninformative, and 679 were parsimony informative. MP analyses yielded 1059 equally parsimonious trees of 2464 steps each, and a strict consensus is shown in Figure 2. Most nodes are well-supported, with 45 of 60 nodes having BS > 80. The family Exocoetidae, excluding Oxyporhamphus, is found to be monophyletic. Also, recognized subfamily, genus, and species-level groupings were generally monophyletic. An exception is the genus Cheilopogon, which was divided into two well-supported clades, named here Cheilopogon Clade A and Cheilopogon Clade B. Separate analyses of cyt b and RAG2 (not shown) produced results that were largely congruent with the combined evidence trees. The BI combined evidence analysis produced phylogenetic reconstructions that were largely consistent with MP; however, less resolution was observed for some major clades (Fig. 3). There were some differences between the MP and BI trees. Both analyses strongly supported the monophyly of flyingfishes but positioned Fodiator as the sister group to all other flyingfishes, whereas BI showed Parexocoetus in that position. By contrast to MP, BI failed to provide evidence for a monophyletic Cypselurinae (Prognichthys, Cypselurus, Cheilopogon, and Hirundichthys), and also failed to support the monophyly of Hirundichthys. Finally, MP showed Cheilopogon clade B (see below) as the sister group of all other Cheilopogon + Cypselurus + Prognichthys, whereas BI placed Cheilopogon clade A in that position. In general, BI nodes that conflicted with the MP results showed relatively low posterior probabilities.

When compared with BI, our combined MP analysis was better resolved, and more congruent with previous morphology-based phylogenies (Parin, 1961; Collette et al., 1984; Dasilao & Sasaki, 1998). Our MP results agree with morphology in placing Fodiator as the basal flyingfish lineage rather than Parexocoetus (Figs 1, 2). Also, MP supports the monophyly of Cypselurinae, a node supported by several anatomical synapomorphies (Collette et al., 1984; Dasilao & Sasaki, 1998). Given the congruence between our combined MP analysis and previous morphological investigations, as well as the higher resolution of the MP analysis, we use the combined MP tree as our preferred hypothesis of flyingfish relationships.

FLYINGFISH GLIDING

Our finding of a monophyletic Exocoetidae supports the idea that true gliding evolved a single time in this group. The earliest condition within flyingfishes, based on character optimization, is the two-wing state (exhibited by Fodiator, Parexocoetus, and Exocoetus) (Fig. 4). Four-wing gliding had a single origin within Cypselurinae, and is relatively derived (Fig. 4).

EGG BUOYANCY, HABITAT PREFERENCE, AND RANGE SIZE

Figure 5 summarizes the optimization of egg buoyancy, habitat preference, and range size characters on our preferred flyingfish species phylogeny. For egg buoyancy, the plesiomorphic condition is nonbuoyant eggs, with buoyant eggs evolving multiple times: once in Exocoetus, once in Prognichthys, and one or more times in Cheilopogon clade A. For habitat preference, the plesiomorphic condition is meroepipelagic, and the holoepipelagic state has evolved in several clades, including Exocoetus, Hirundichthys, Cheilopogon furcatus, Prognichthys, and Cheilopogon clade A. Range size exhibits a complex pattern of evolution, with eight bidirectional changes between the restricted (single ocean) and widespread (two or more oceans) states.

Using the CCT, we were unable to reject the null hypothesis that large species ranges (occupying two or more oceans) have evolved randomly with respect to lineages that exhibit buoyant eggs (CCT P-value = 0.13). Thus, having buoyant eggs does not appear to affect the evolution of flyingfish species ranges. However, CCT did reject the null hypothesis that large species ranges have evolved randomly with respect to lineages that are holoepipelagic (CCT P-value = 0.032). This indicates that large range sizes are more likely to evolve in lineages with flyingfish species that are holoepipelagic.
As in previous studies (Parin, 1961; Collette et al., 1984; Dasilao & Sasaki, 1998), we found strong support for the monophyly of flyingfishes. We also found molecular support for the monophyly of each of the four subfamilies (Fodiatorinæ, Parexocoetinæ, Exocoetinæ, and Cypselurinæ) and the previously proposed pattern of phylogenetic relationships among these subfamilies (Bruun, 1935; Parin, 1961; Collette et al., 1984; Dasilao & Sasaki, 1998). By contrast to Dasilao et al. (1997), who proposed a sister group relationship between the traditionally recognized...
Figure 3. Phylogram from combined Bayesian analysis of cytochrome b (cytb) and recombination activating gene 2 (RAG2) sequence data. Numbers above nodes indicate Bayesian posterior probabilities (* = 1.00).
Exocoetidae and the genus *Oxyporhamphus*, the present study confirms the result of Lovejoy *et al.* (2004) in placing *Oxyporhamphus* with *Hemiramphus*. This finding has implications for reconstructions of the earliest evolution of gliding.

Our molecular results support the monophyly of most currently recognized flyingfish genera. In general, our analyses included high proportions of the recognized species within each genus; we included five of six recognized species of *Prognichthys*, five of eight *Hirundichthys*, four of five *Exocoetus*, three of three *Parexocoetus*, and one of two *Fodiator* (numbers of recognized species from Froese & Pauly, 2010). This level of sampling strengthens our case of the monophyly of these genera. Our taxon sampling was weaker for the more diverse genera *Cypselurus* (three of ten recognized species included) and *Cheilopogon* (ten of 33 recognized species included) (Parin, 2009; Froese & Pauly, 2010). Our results indicate that the latter genus, the most diverse and morphologically variable within flyingfishes, is not monophyletic. Support for this result was high, based on consistency across analyses and bootstrap and posterior probability values. Several *Cheilopogon* subgenera have been proposed (Parin, 1961), although our limited species sampling for this genus makes it difficult to determine how well our clade A and clade B correspond to these subgeneric designations.

Several studies have questioned whether *Cypselurus* and *Cheilopogon* are distinct genera (Bruun, 1935; Staiger, 1965; Gibbs & Staiger, 1970; Dasilao & Sasaki, 1998). However, our molecular results support the contention of Parin (1961) and Collette *et al.* (1984) that these are distinct taxa, with *Cypselurus* more closely related to *Prognichthys* than it is to either of the *Cheilopogon* clades.

**EVOLUTION OF FLYINGFISH GLIDING STRATEGIES**

The gliding behaviours of flyingfishes have long been of interest to evolutionary biologists (Darwin, 1872; Möbius, 1878; Dunford, 1906; Breder, 1930; Fish, 1990; Davenport, 1994; for a review, see Kutschera, 2005). Exocoetids exhibit a range of gliding capabilities, from weak gliders like *Fodiator*, to the two-winged *Exocoetus* that glide short distances (tens of metres), to the four-winged *Cypselurinae* that can glide hundreds of metres (Fish, 1990; Davenport, 1994). The evolutionary trajectory of gliding in flyingfishes has been discussed (Dasilao & Sasaki, 1998; Kutschera, 2005) and most recent studies have concluded that a progressive evolution of gliding took place, from two-wing to four-wing (Parin, 1961; Collette *et al.*, 1984; Dasilao *et al.*, 1997; Dasilao & Sasaki, 1998). The results of the present study support the hypothesis that two-winged (two-wing) gliding evolved first in flyingfishes and four-winged (four-wing) gliding evolved more recently.

Although gliding has frequently and traditionally been considered a two-state character (two-wing and four-wing), both anatomical and functional analyses suggest a more complex pattern of evolution. Dasilao & Sasaki (1998) presented a detailed reconstruction of the evolution of anatomical features associated with gliding. Their scenario describes a progression, with the following characters added sequentially: (1) enlarged pectoral fins and associated muscles at the exocoetid node; (2) more greatly enlarged pectoral fins at the *Exocoetus* + *Cypselurinae* node; and (3) enlarged pelvic fins at the *Cypselurinae* node. This scenario would thus define three groups characterized by different suites of morphological features related to gliding. Data collected by Fish (1990) lend support to this idea. Fish (1990) measured body mass, wing area, and tail area for several flyingfish genera, and calculated the aerodynamic parameters of wing loading and aspect ratio. He found differences between the three groups described above in a combination of characteristics, including % wing area composed of pectoral fin, deviations (or lack thereof) from geometric scaling for wing span and wing area, and wing aspect ratio (Fish, 1990). The molecular phylogeny presented here agrees with the three-step scenario of gliding evolution. However, we suggest that further phylogenetic optimizations of detailed functional characters, such as the very high wing aspect ratio in *Exocoetus* (Fish, 1990), and the use of the laterally inclined dorsal fin as a gliding surface in *Parexocoetus* (R. L. Pitman, pers. observ.), represent autapomorphies that deserve further investigation.

Reconstructing the earliest origin of gliding behaviour and anatomy in the Exocoetidae will depend on an accurate assessment of the family’s nearest relatives. Dasilao *et al.* (1997) placed *Oxyporhamphus* as the sister group to flyingfishes, based on morphology. *Oxyporhamphus* is a genus of two epipelagic species that exhibit limited jumping and gliding behaviour.

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**Figure 4.** Genus level phylogenetic hypothesis for flyingfishes, simplified from maximum parsimony analysis of the full dataset (see Figure 2), and showing the evolution of gliding strategies. Gliding illustrations *sensu* Davenport (2003).

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and have been grouped with either halfbeaks (Gill, 1864; Regan, 1911; Bruun, 1935; Parin, 1961; Norman, 1966; Collette et al., 1984; Collette, 2004) or flyingfishes (Nichols & Breder, 1928; Hubbs, 1935; Dasilao et al., 1997). In contrast with Dasilao et al. (1997), molecular studies place Oxyporhamphus with Hemiramphus (Lovejoy et al., 2004; present study). Swim bladder morphology provides additional evidence for the latter relationship (Tibbetts et al., 2007). The placement of Oxyporhamphus away from the flyingfishes suggests that some aspects of gliding behaviour and anatomy have evolved independently within beloniform fishes. However, resolution of this issue depends on an analysis with more extensive sampling of halfbeaks, particularly Hemiramphus.

The selective pressures responsible for the origin and elaboration of gliding in flyingfishes remain unresolved. The consensus is that predator avoidance is the most reasonable explanation (Mohr, 1954; Evans & Sharma, 1963; Fish, 1990; Gillett & Ianelli, 1991; Davenport, 1992; Davenport, 1994; Kutschera, 2005). Flyingfishes share epipelagic habitats with

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high-speed predators such as billfishes (Istiophoridae), dolphins (Coryphaenidae), tunas (Scombridae), and dolphins (e.g. Scomberella spp.), and diet analyses suggest that these taxa feed on exocoetids (Olson, 1982; Olson & Boggs, 1986; Richard & Barbeau, 1994; Oxenford & Hunte, 1999). Unlike reef or shore habitats, which offer natural cover for fishes, flyingfish habitat is largely refuge free. Thus, mechanisms of predator evasion are at a premium, and the predator avoidance hypothesis posits that gliding evolved as a means of escaping a suddenly hostile environment. This historical hypothesis is difficult to test; however, it might be possible to test for correlations between the presence of particular types of predators and types of gliding behaviour. For example, field observations (R. L. Pitman, pers. observ.), indicate that Exocoetus co-occurs with tunas, whereas Cypselurinae individuals are frequently the prey of dolphinfishes. Tunas (Scombridae) hunt in large, fast-swimming schools, and may be avoided best by two-wing flyingfishes that are able to exit the water quickly and without the need of a ‘taxiing’ phase (Hubbs, 1935; Fish, 1990). On the other hand, dolphins (Coryphaenidae) actively pursue prey (Davenport, 1994) and may be evaded best by the longer glides, faster speeds, and abrupt changes in direction achieved by four-wing flyingfish species. Habitat modeling of these predator/prey systems could provide tests of these hypotheses.

**Egg Buoyancy, Habitat, and Species Range Size**

Our analyses using CCT suggest that flyingfishes may provide tests of these hypotheses. However, it might be possible to test for correlations between the presence of particular types of predators and types of gliding behaviour. For example, field observations (R. L. Pitman, pers. observ.), indicate that Exocoetus co-occurs with tunas, whereas Cypselurinae individuals are frequently the prey of dolphinfishes. Tunas (Scombridae) hunt in large, fast-swimming schools, and may be avoided best by two-wing flyingfishes that are able to exit the water quickly and without the need of a ‘taxiing’ phase (Hubbs, 1935; Fish, 1990). On the other hand, dolphins (Coryphaenidae) actively pursue prey (Davenport, 1994) and may be evaded best by the longer glides, faster speeds, and abrupt changes in direction achieved by four-wing flyingfish species. Habitat modeling of these predator/prey systems could provide tests of these hypotheses.

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**SUPPORTING INFORMATION**

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

**Table S1.** Species, gliding strategies, species distributions, geographic range sizes, habitat, and egg buoyancy characteristics used for concentrated changes tests.

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