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RAPID SPECIATION AND ECOLOGICAL DIVERGENCE IN THE AMERICAN SEVEN-SPINED GOBIES (GOBIIDAE, GOBIOSOMATINI) INFERRED FROM A MOLECULAR PHYLOGENY

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Abstract.—The American seven-spined gobies (Gobiidae, Gobiosomatini) are highly diverse both in morphology and ecology with many endemics in the Caribbean region. We have reconstructed a molecular phylogeny of 54 Gobiosomatini taxa (65 individuals) based on a 1646-bp region that includes the mitochondrial 12S rRNA, tRNA-Val, and 16S rRNA genes. Our results support the monophyly of the seven-spined gobies and are in agreement with the existence of two major groups within the tribe, the *Gobiosoma* group and the *Microgobius* group. However, they reject the monophyly of some of the Gobiosomatini genera. We use the molecular phylogeny to study the dynamics of speciation in the Gobiosomatini by testing for departures from the constant speciation rate model. We observe a burst of speciation in the early evolutionary history of the group and a subsequent slowdown. Our results show a split among clades into coastal-estuarian, deep ocean, and tropical reef habitats. Major habitat shifts account for the early significant acceleration in lineage splitting and speciation rate and the initial divergence of the main Gobiosomatini clades. We found that subsequent diversification is triggered by behavior and niche specializations at least in the reef-associated clades. Overall, our results confirm that the diversity of Gobiosomatini has arisen during episodes of adaptive radiation, and emphasize the importance of ecology in marine speciation.

Key words.—Adaptive radiation, Caribbean, diversification rates, marine fish, mitochondrial DNA.

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Adaptive radiation, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage has engaged biologists for over a century (Osborne 1902; Simpson 1953; Schluter 2000). Adaptive radiations are excellent systems in which to study the factors that influence speciation processes. The astonishing biological diversity of these systems may follow the evolution of key innovations in morphological, physiological, or behavioral traits (Stanley 1979; Sanderson and Donoghue 1994). Alternatively, the colonization of novel habitats is thought to provide new ecological opportunity or release of competition, and hence enable lineages to colonize new adaptive zones (Simpson 1953; Schluter 2000). Molecular phylogenies provide a robust framework to study the temporal pattern of speciation and diversification in lineages that have undergone adaptive radiation (Johns and Avise 1998; Lovette and Bermingham 1999). The branching pattern of a phylogenetic tree can be used to detect changes in speciation rate through time, and to detect asymmetries between contemporaneous clades in their number of descendant species (Nee et al. 1994a; Nee et al. 1996; Sanderson and Donoghue 1996; Pybus and Harvey 2000). Such information is needed to distinguish speciation bursts from stochastic background rates and to identify historical factors underlying the emergence of ecological and phenotypic divergence within a lineage.

With the accumulation of phylogenetic data it has become evident that a considerable amount of biological diversity has arisen during episodes of adaptive radiation (Schluter 2000). Approximately 13,000 marine teleosts are known, which account for about 61% of the total teleost diversity (Nelson 1994). Nevertheless, in contrast to the wealth of studies documenting fish adaptive radiations in lacustrine environments (e.g. Fryer and Iles 1972; Echelle and Kornfield 1984; Schluter 1996), only a few have focused on marine fishes (Johns and Avise 1998; Eastman and McCune 2000).

The American seven-spined gobies (Gobiidae: Gobiosomatini; proper spelling of the tribe from Gobiosomini to Gobiosomatini corrected by Smith and Baldwin 1999) represent a possible case of a marine adaptive radiation (Hoese and Larson 1985). The Gobiosomatini display a great diversity in color, ecology, and behavior; include over 130 species in 24 genera; and represent some 40% of the total New World gobioid genera and perhaps as many as 50% of the species (Birdsong and Robins 1995). The Gobiosomatini are endemic to the New World, and occur in the western Atlantic from Massachusetts to Argentina, and in the eastern Pacific from the northern Gulf of California to Chile, although most of the species are found in the Caribbean region. Some genera are either restricted to the Atlantic or to the Pacific, but most have representatives in both oceans. Members of the Gobiosomatini display a remarkable diversity in morphology and are highly selective with regard to habitat (Böhlke and Robins 1968). Some are found in shallow fresh waters, brackish waters, and estuaries with mud, shell, gravel, or algae-covered substrate. Others are found in coral or rocky reefs and shelf slopes at depths exceeding 500 meters (Appendix).

Differences in dentitions indicate specialized diets, although little information for most species (especially the benthic genera) is available (Böhlke and Robins 1968). Some members of the subgenus *Elacatinus* are known to remove ectoparasites from other fishes (cleaning behavior) whereas others are associated with sponges. One species (*Nes longus*)

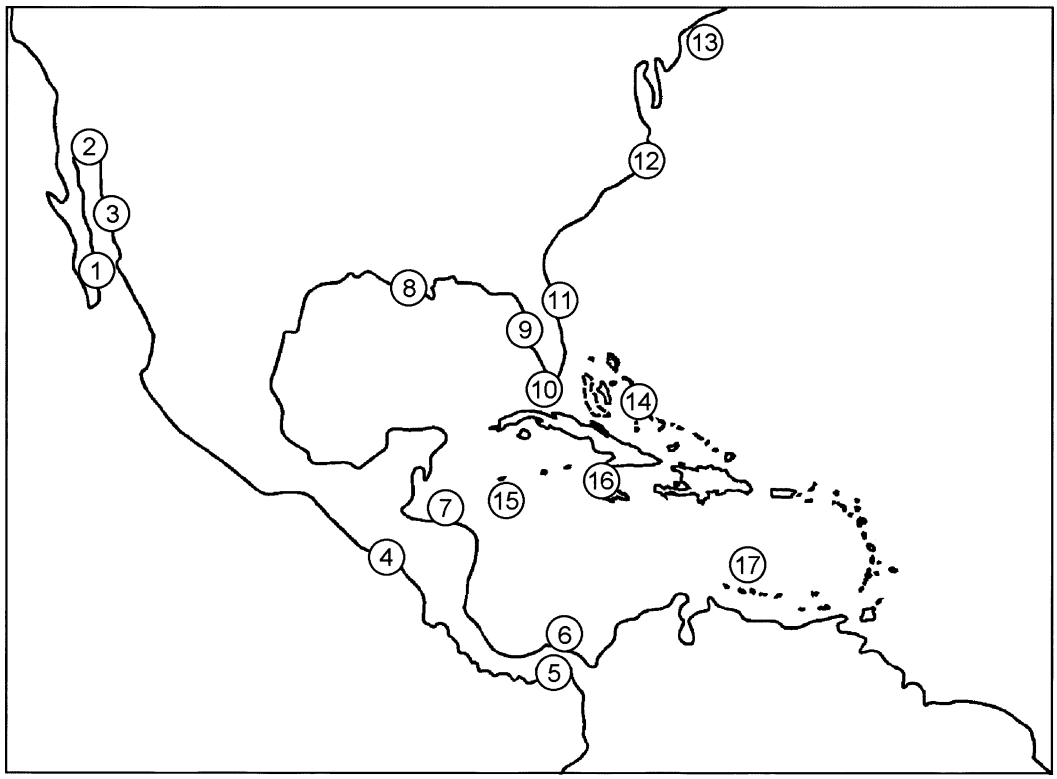


FIG. 1. Map of the Caribbean region with the sampling localities for the Gobiosomatini taxa. Proximate sampling locations have been gathered into a single number (see Appendix for more details).

lives commensally with snapping shrimp whereas a close association with sea urchins is found in *Ginsburgellus novemlineatus* and *Tigrigobius multifasciatum* (Böhlke and Robins 1968).

Phylogenetic hypotheses of the tribe based on morphological characters are rather inconclusive, perhaps because different authors have used distinct sets of characters and taxa, and due to the potential of high levels of homoplasy resulting from body size reduction and/or adaptations to extreme habitats (e.g. soft mud bottom, sponge-dwelling; Böhlke and Robins 1969; Hoese 1971; Van Tassell 1998). The aim of this study was to establish a mtDNA-based molecular phylogeny of the American seven-spined gobies to study the dynamics of speciation in this ecomorphologically and behaviorally highly diverse group of marine fishes. A robust phylogenetic framework will permit us (1) to test for departures from the constant speciation rate model and to identify peak periods of cladogenesis, (2) to detect asymmetries between contemporaneous clades in their number of extant species, and (3) to map habitat shifts onto the phylogeny and test their association with divergence or diversification events during the evolutionary history of the Gobiosomatini.

MATERIAL AND METHODS

Samples and Sequences

To assess the molecular phylogeny of the American seven-spined gobies, 65 individuals (plus four outgroups) were collected from 17 localities (Fig. 1 and Appendix). Whole fish were preserved in 70–100% ethanol, and voucher specimens were deposited in the American Museum of Natural History, New York (Appendix; for identification numbers, GenBank accession numbers, and museum voucher numbers, see the Electronic Appendix, available from the *Evolution* Editorial Office at evolution@asu.edu). Here, we follow Böhlke and Robins's (1968) classification of the genus *Gobiosoma* into the subgenera *Austrogobius*, *Elacatinus*, *Gobiosoma*, *Garmannia*, and *Tigrigobius* and use their criteria to assign subgeneric status to eastern Pacific species not included in their study.

Total genomic DNA was isolated from white muscle tissue or fin clips by proteinase K/SDS digestion, phenol-chloroform extraction, and ethanol precipitation (Kocher et al. 1989). Partial mitochondrial 12S and 16S rRNA genes were polymerase chain reaction (PCR) amplified using the universal primers L1091 and H1478 (Kocher et al. 1989), and 16Sar-L and 16Sbr-H (Palumbi et al. 1991), respectively. A PCR product of approximately 1300 bp that connects the above mentioned gene fragments (including the 3′ end of the 12S rRNA, the complete tRNA-Val, and the 5′ end of the 16rRNA genes) was amplified with the primers fish-12F1 (5′-TGA AGG AGG ATT TAG CAG TAA G-3′) and fish-16SR1 (5′-AAG TGA TTG CGC TAC CTT CGC AC-3′). These primers also work in a variety of other acanthomorph fish (L. Rüber, pers. obs.). The primer fish-12SF2 (5′-TCT CTG TGG CAA AAG AGT-3′) was used as an internal sequencing primer. Polymerase chain reaction amplifications were conducted in 25 μl reactions containing 75 mM tris-HCl (pH 9.0), 2 mM $MgCl_2$, 0.4 mM of each dNTP, 0.4 μM of each primer, template DNA (10-100 ng), and Taq DNA polymer-

ase (one unit, Biotools, Madrid, Spain), using the following program: one cycle of 2 min at 94°C, 35 cycles of 60 sec at 94°C, 60 sec at 48–54°C, 90 sec at 72°C, and finally, one cycle of 5 min at 72°C.

After PCR purification using an ethanol/sodium acetate precipitation, samples were cycle-sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (V3.0) in 10 μl reactions, and following manufacturer's instructions (Applied Biosystems, Foster City, CA), with 3.25 pmol of primer, 3 μl of Terminator Ready Reaction Mix and 5% dimethyl sulfoxide. The cycling profile for the sequencing reaction consisted of 25 cycles of 10 sec at 96°C, 5 sec at 50°C, and 4 min at 60°C. Cycle-sequencing products were purified using MultiScreen plates (Millipore, Billerica, MA), and were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Sequences have been deposited in GenBank (see Electronic Appendix).

Sequence Alignment and Phylogenetic Analysis

Orthologous DNA sequences were aligned with Clustal X (Thompson et al. 1997) using the default settings, and alignments were further optimized by eye. Equivocal alignment positions in gap-rich areas were excluded from the analyses because of uncertainty in homology assignment. Modeltest version 3.06 (Posada and Crandall 1998) was used to determine the substitution model that best fit the dataset. The hierarchical likelihood-ratio tests (LRT) implemented in Modeltest selected the GTR + I + Γ model (proportion of invariable sites = 0.33; $\alpha = 0.54$, empirical base frequencies: A = 0.33; C = 0.26; G = 0.21; T = 0.20; substitution rates: A-C = 3.22; A-G = 8.51; A-T = 2.98; C-G = 0.95; C-T = 14.63; G-T = 1.00). These settings were subsequently used for maximum-likelihood (ML) analyses and to estimate ML distances for minimum-evolution (ME) analyses. Maximum-parsimony (MP) analyses were conducted with heuristic searches (TBR branch swapping, MULTREES option effective, and 10 random stepwise additions of taxa). Transversions (Tv) were given two times the weight of transitions based on an empirical Ts:Tv ratio of 1.97. Robustness of the inferred trees was tested using nonparametric bootstrapping (Felsenstein 1985) with 1000 and 500 pseudoreplicates for the ME and MP analyses, respectively. The quartet puzzling method (Strimmer and von Haeseler 1996) was used to test the robustness of the ML analyses using 10,000 puzzling steps. All above-mentioned phylogenetic analyses were conducted with PAUP* version 4.0b8 (Swofford 2002).

A Bayesian inference (BI) of Gobiosomatini phylogeny was performed with MrBayes version 3.01 (Huelsenbeck and Ronquist 2001) by Markov chain Monte Carlo (MCMC) sampling for one million generations (four simultaneous MC chains; sample frequency 100; burn-in 100,000 generations; chain temperature 0.2) under the GTR + I + Γ model.

Alternative phylogenetic hypotheses were tested using the Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa 1999). A LRT (Huelsenbeck and Crandall 1997) was performed with ML trees with and without a molecular clock constraint. To date major cladogenetic events and to perform tests of diversification rates, ultrametric trees were constructed using the nonparametric rate smoothing (NPRS) method (Sanderson 1997) as implemented in TreeEdit version 1.0 (Rambaut and Charleston 2001). To roughly estimate divergence times between major clades, we applied a molecular clock using the Tv rate of the 12S and 16S rRNA genes of 0.14% per million years (Ritchie et al. 1996).

Tests of Diversification Rate

We investigated whether rates of cladogenesis have changed through time in the seven-spined gobies using the constant-rate (CR) test (Pybus and Harvey 2000; Pybus and Rambaut 2002). We also applied the Monte Carlo constant-rates (MCCR) test (Pybus and Harvey 2000) that takes incomplete taxon sampling into account using the program MCCRTest (Pybus 2000). The CR and MCCR tests are conducted under the assumption that diversification occurs equally among lineages. Therefore, we used three approaches to detect nonstochastic differences in species diversity among lineages. First, we performed three-taxon tests as implemented in LRDIVERSE version 0.8 (Sanderson and Donoghue 1996; Sanderson and Wojciechowski 1996). Second, the *B*1 index was used as a measure of tree imbalance (Kirkpatrik and Slatkin 1993). Third, the relative cladogenesis statistic (Nee et al. 1994b, 1995, 1996) as implemented in End-Epi version 1.0 (Rambaut et al. 1997) was used to identify lineages with higher than expected rates of cladogenesis. The relative cladogenesis statistic uses a simple birth-death model to calculate the probability that any ancestral lineage has more than *a* extant tips given *k* ancestral lineages and *n* extant tips in total. End-Epi version 1.0 was also used for generating semilogarithmic lineage through time (LTT) plots. All these analyses were based on a smaller phylogeny (excluding intraspecific data) with 54 ingroup species obtained using Modeltest, ML, and NPRS as described above.

RESULTS

Phylogeny of the American Seven-Spined Gobies

The alignment of the 12S rRNA, tRNA-Val, and 16S rRNA gene nucleotide sequences of 65 Gobiosomatini individuals with *Gobius* and *Vanneaugobius* as outgroups consisted of 2195 positions. A total of 1646 positions were analyzed, of which 782 were invariant and 638 were phylogenetically informative under the parsimony criterion. Pairwise sequence divergence between ingroup taxa varied from zero to 22.6% (17.8% excluding *T. multifasciatum* and *Psilotris batrachodes* that had the longest branches) and substitutions were not saturated (as indicated by a Tv:Ts vs. patristic distance plot; not shown).

The tree reconstructed by the Bayesian phylogenetic analysis is shown in Fig. 2. Two main lineages corresponding to the *Microgobius* group and the *Gobiosoma* group of Birdsong et al. (1988) were recovered within the Gobiosomatini (Fig. 2). The *Microgobius* group consists of two clades, whereas at least seven major clades were identified in the *Gobiosoma* group (Fig. 2). Maximum-parsimony phylogenetic analyses with a 2:1 Tv:Ts weighting scheme resulted in two shortest trees of 6665 steps. Different Tv:Ts weighting schemes (e.g. 1:1 and 4:1) resulted in similar and congruent MP trees (not shown). The ME phylogenetic analysis recovered two trees

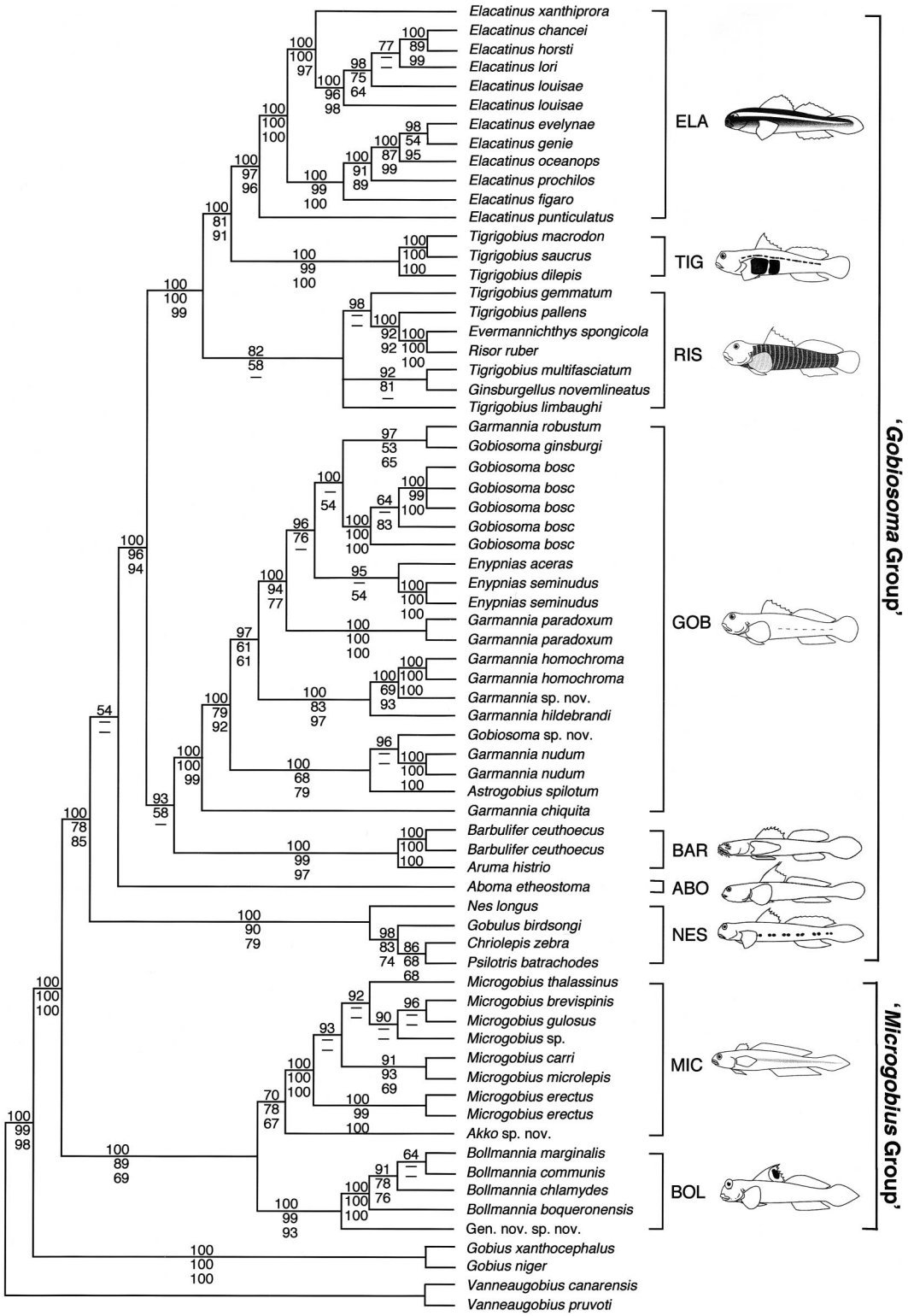


FIG. 2. Phylogenetic relationships of the American seven-spined gobies based on a Bayesian phylogenetic analysis of mitochondrial 12S rRNA, tRNA-Val, and 16S rRNA sequence data using the GTR + I + Γ evolutionary model. The number above each node refers to the Bayesian posterior probability (shown as percentage) derived from 9000 Markov chain Monte Carlo sampled trees. Bootstrap values (>50%) for minimum-evolution (GTR + I + Γ distances) and maximum-parsimony (2Tv:1Ts) phylogenetic analyses are shown below branches (upper and lower values, respectively). The two Gobiosomatini groups (*Gobiosoma* group and *Microgobius* group) and the nine Gobiosomatini clades used in the text are indicated. Clades are: ELA, *Elacatinus*; TIG, *Tigrigobius*; RIS, *Risor*; GOB, *Gobiosoma*; BAR, *Barbulifer*; NES, *Nes*; ABO, *Aboma*; MIC, *Microgobius*; BOL, *Bollmannia*. Outline drawings adapted from Hoese and Larson (1985) and Humann (1994) except *Aboma* (drawn directly from the specimen).

(score = 3.86). The topology of the ML tree (−ln likelihood = 23,763.45) is shown in Figure 3A. The MP, ME, and ML trees showed nearly identical topologies to the BI tree (Fig. 2 and 3A). In contrast to MP, ME, and ML phylogenetic analyses where *Aboma* was resolved as the most basal clade of the *Gobiosoma* group (although with no bootstrap or quartet puzzling support), the BI phylogenetic analysis resolved *Aboma* after the *Nes* clade (a topology which was not rejected under ML using the SH test: $P = 0.95$).

Our results reject the monophyly of the genus *Gobiosoma* including the subgenera *Austrogobius*, *Elacatinus*, *Gobiosoma*, *Garmannia*, and *Tigrigobius* (Figs. 2 and 3, SH test: $P = 0.00$) as defined by Böhlke and Robins (1968). The genus *Enypnias* is placed deep within a *Gobiosoma* clade that includes the subgenera *Austrogobius*, *Garmannia*, and *Gobiosoma* (Figs. 2 and 3A) although the SH test did not reject a topology where the genus *Enypnias* was placed basal to the latter three subgenera ($P = 0.06$). Furthermore, the subgenus *Tigrigobius* is not monophyletic (SH test: $P = 0.02$).

For several species, more than one individual was included in the phylogenetic analyses. Conspecifics of a given species were resolved monophyletically with the only exception of *Elacatinus louisae* sampled from the Bahamas and Jamaica. The paraphyly of *E. louisae* is supported by results from an extensive phylogeographic study of the genus *Elacatinus* (M. S. Taylor, pers. comm.). The highest intraspecific sequence divergence (uncorrected *p*-distance, 7.6%) was observed between two individuals of *Garmannia nudum* from El Salvador and Panama. Differences in scale count are found in north-south populations of *G. nudum* (Hoese 1976). Therefore, both molecular and morphological data suggest a potential case of cryptic species, a situation often encountered in marine species (Knowlton 1993). Within *Gobiosoma bosc*, identical sequences were found between two individuals from Long Island and Chesapeake Bay. These specimens clustered with an individual from eastern Florida to the exclusion of two individuals from western Florida and the Gulf of Mexico. This intraspecific pattern is concordant with the existence of zoogeographic boundaries in Florida that are also found in many coastal maritime animals in this area (reviewed in Avise et al. 1987; Avise 2000).

Rates of Evolution

A likelihood-ratio test with (−ln L = 23,988.51) and without (−ln L = 23,763.45) the molecular clock enforced rejected overall constancy of rates of evolution in the Gobiosomatini ($\delta = 450.12$, df = 67, $P \ll 0.001$). To further explore such rate heterogeneity, we plotted the mean path lengths (±SD) across the ME tree for each clade (Fig. 4). The *Bollmannia* and *Microgobius* clades showed the lowest rates of evolution, whereas the *Elacatinus*, *Tigrigobius*, *Risor*, and *Barbulifer* clades showed the highest (Fig. 4). The *Risor* and *Nes* clades had large standard deviations due to the extremely long branches of *T. multifasciatum* and *Psilotris*, respectively (Figs. 3A and 4). In the absence of rate constancy we used the NPRS method to construct an ultrametric tree (Fig. 3B) based on the ML tree, which was used for further analyses.

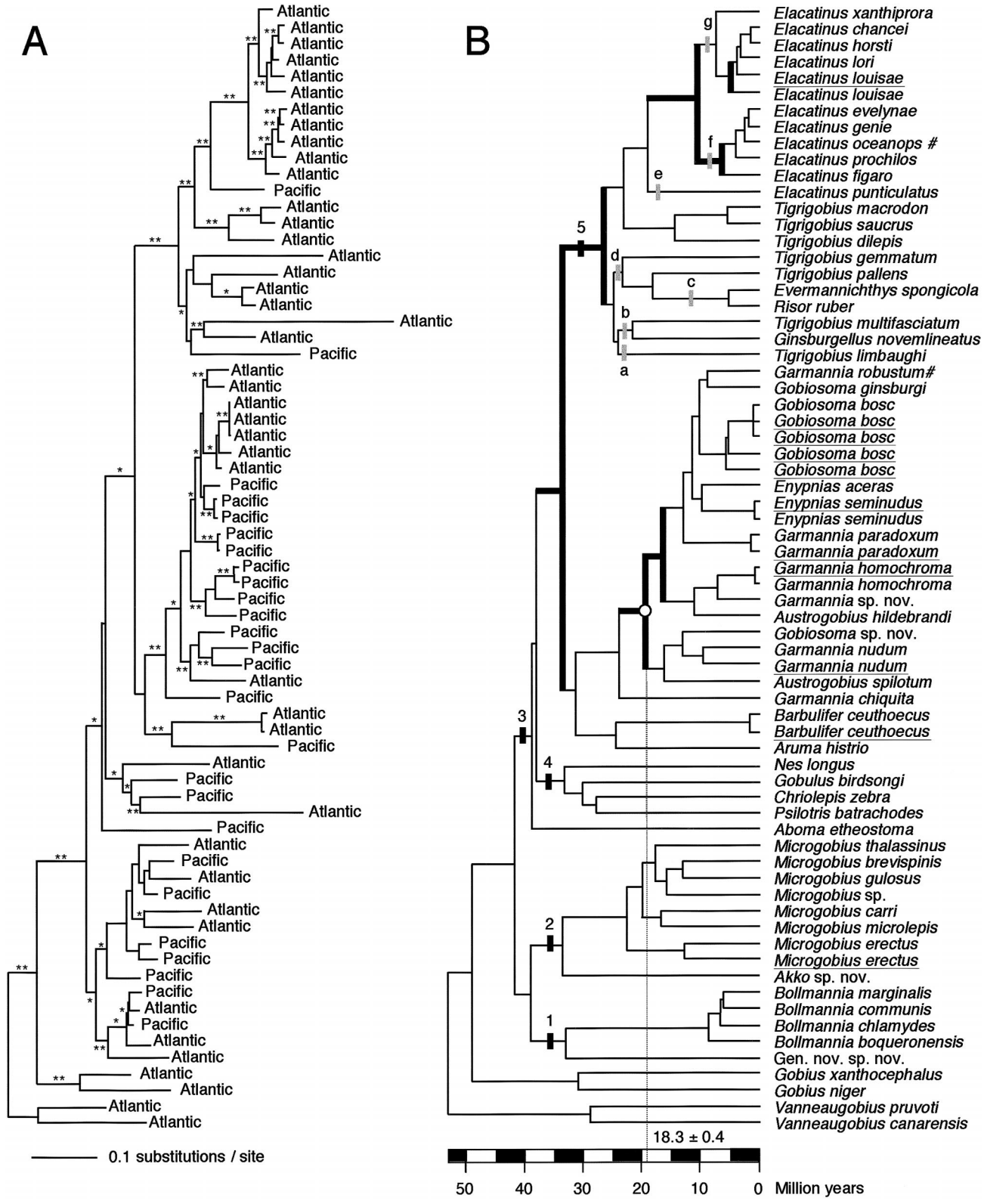
Calibration of a molecular clock with a Tv rate of 0.14% per million years allowed us to roughly date the main cladogenetic events that occurred in Gobiosomatini history (Fig. 3B). The origin of the American seven-spined gobies and the initial divergence into the *Gobiosoma* and *Microgobius* groups date back to the late Eocene (around 40 million years ago; Fig. 3B). Divergence into the nine major Gobiosomatini clades occurred during the Oligocene (around 35–28 million years ago; Fig. 3B). These nine clades have both Pacific and Atlantic representatives indicating that other factors besides biogeography may have been important in the evolution of the Gobiosomatini. Interestingly, Pacific lineages are mostly resolved in a basal position within each main clade, with the exception of *Bollmannia*. Subsequent diversification of the main clades occurred during the Miocene-Pliocene (22–3 million years ago). In the case of the *Elacatinus* + *Tigrigobius* + *Risor* clade, the timing of the main cladogenetic events correlates with the origin and diversification of the Caribbean coral reef fauna (Budd et al. 1995).

Diversification Rate through Time

A LTT plot of the Gobiosomatini was estimated based on the NPRS tree. The LTT plot is convex (Fig. 5) indicating a burst of speciation early in the history of the group. Convex

→

FIG. 3. (A) Maximum-likelihood tree based on the GTR + I + Γ evolutionary model. Robustness of the nodes was tested with the quartet puzzling method. Nodes with 50–69% (*) or 70–100% (**) support are indicated. Atlantic or Pacific origin of each species is indicated. (B) Ultrametric tree based on the nonparametric rate smoothing analysis. Two datasets were used for the constant-rates/Monte Carlo constant-rates tests. Taxa that were excluded for the 54 ingroup taxa dataset are underlined and the additional two taxa that were excluded for the 52 ingroup taxa dataset are marked with a number symbol (#) (see text for details). Thicker lines indicate lineages that have significantly higher than expected diversification rate using the relative cladogenesis statistic ($P < 0.05$; 54 ingroup taxa dataset). Black bars indicate major habitat transitions during the evolution of the Gobiosomatini: 1, generally deep ocean habitat over mud; 2, generally coastal habitats mostly over silt/mud substrate; 3, generally coastal-estuarine habitats over diverse substrates; 4, generally on outer edge of coral or rocky reefs over fine or coarse sand substrate with or without rocks; 5, rocky and coral reef habitats. Gray bars illustrate some behavioral adaptations in the *Elacatinus* + *Tigrigobius* + *Risor* clade: a, cleaning behavior mostly on moray eels, no apparent cleaning stations (M. S. Taylor, pers. comm.); b, in association with sea urchins; c, sponge-dwelling (mostly internal cavity of sponges), with morphological adaptations (Böhlke and Robins 1969); d, exclusively or occasionally found inside chiton (*Choneplax lata*) burrows (Taylor and Van Tassell 2002); e, facultative cleaning behavior mostly on moray eels, no apparent cleaning stations, in the Gulf of California mostly in association with sea urchins (M. S. Taylor, pers. comm.); f, obligate cleaning behavior (generally get food by cleaning but may feed from other sources, maintain cleaning stations), primarily coral-dwellers. Sponge-dwelling has occasionally been observed in *E. figaro* (Rocha et al. 2000), *E. prochilos* (Whiteman and Côté 2002), and *E. oceanops* (J. L. Van Tassell, pers. obs.), but cleaning stations have not been observed when species associate with sponges. Occasional sponge-dwelling behavior in these species may be explained by high inter- and intraspecific competition of newly settled juveniles for cleaning stations on coral heads. g, sponge-dwelling (tube sponges), mostly in sponge cavity (not inside sponge canals) sometimes on outside of sponges. The scale bar below the tree shows the time scale resulting from a calibration of the molecular clock (Tv rate of 0,14% per million years) based on the circled node.


A
Atlantic
Pacific
0.1 substitutions / site
B
Elacatinus xanthiprora
Elacatinus chancei
Elacatinus horsti
Elacatinus lori
Elacatinus louisae
Elacatinus louisae
Elacatinus evelynae
Elacatinus genie
Elacatinus oceanops #
Elacatinus prochilos
Elacatinus figaro
Elacatinus punticulatus
Tigrigobius macrodon
Tigrigobius saucrus
Tigrigobius dilepis
Tigrigobius gemmatum
Tigrigobius pallens
Evermannichthys spongicola
Risor ruber
Tigrigobius multifasciatum
Ginsburgellus novemlineatus
Tigrigobius limbaughi
Garmannia robustum#
Gobiosoma ginsburgi
Gobiosoma bosc
Gobiosoma bosc
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Gobiosoma bosc
Enypnias aceras
Enypnias seminudus
Enypnias seminudus
Garmannia paradoxum
Garmannia paradoxum
Garmannia homochroma
Garmannia homochroma
Garmannia sp. nov.
Austrogobius hildebrandi
Gobiosoma sp. nov.
Garmannia nudum
Garmannia nudum
Austrogobius spilotum
Garmannia chiquita
Barbulifer ceuthoecus
Barbulifer ceuthoecus
Aruma histrio
Nes longus
Gobulus birdsongi
Chriolepis zebra
Psilotris batrachodes
Aboma etheostoma
Microgobius thalassinus
Microgobius brevispinis
Microgobius gulosus
Microgobius sp.
Microgobius carri
Microgobius microlepis
Microgobius erectus
Microgobius erectus
Akko sp. nov.
Bollmannia marginalis
Bollmannia communis
Bollmannia chlamydes
Bollmannia boqueronensis
Gen. nov. sp. nov.
Gobius xanthocephalus
Gobius niger
Vanneaugobius pruvoti
Vanneaugobius canarensis
18.3 ± 0.4
50 40 30 20 10 0 Million years

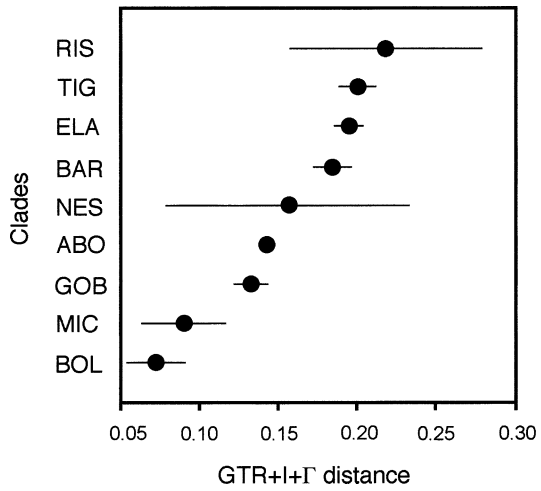

FIG. 4. Path lengths (average ± SD) based on GTR + I + Γ distances across the minimum-evolution tree from the most recent ancestor to the members of the nine Gobiosomatini clades are shown. Clade designations as in Figure 2.

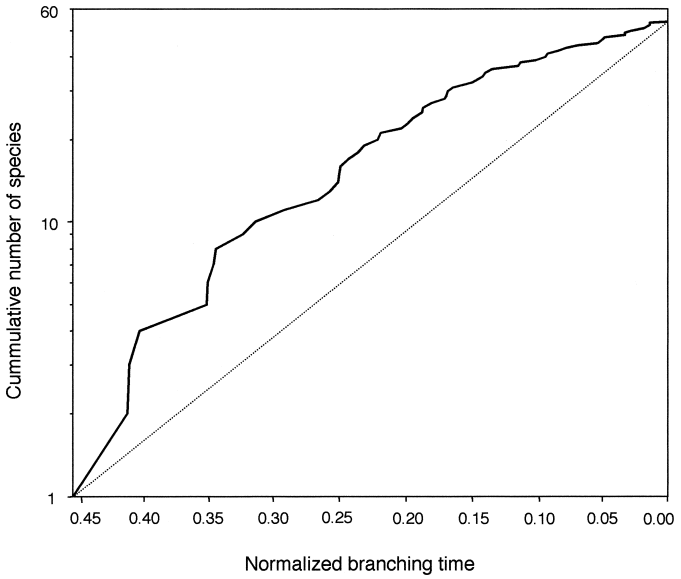

FIG. 5. Semilogarithmic plot of lineages through time (LTT) using the 54-taxa dataset. The constant-rate (CR) model is rejected under the CR/Monte Carlo constant-rate tests if the internal nodes are significantly closer to the root (convex LTT plot) than expected under the pure birth model (dotted line; Pybus and Harvey 2000).

LTT plots might also occur due to incomplete taxon sampling (Nee et al. 1994a). To rule out the effect of incomplete taxon sampling on changes in the rate of cladogenesis through time we used the CR/MCCR tests, which assume that diversification occurs equally among lineages. To test such an assumption we performed the three-taxon test, the *B*1 test, and the relative cladogenesis statistic.

Assuming a hypothetical outgroup consisting of one species, the three-taxon test indicated a significantly higher diversification rate at the origin of the Gobiosomatini, but when assuming two hypothetical outgroup species, the test results were nonsignificant (Table 1). With this test, *Aboma* shows a significantly slower diversification rate when it is placed basal to the *Gobiosoma* group as indicated by the MP, ME, and ML phylogenetic analyses (Table 1).

The *B*1 test rejected the hypothesis of equal diversification rates across lineages ($n = 54$, $B1 = 25.41$, $P < 0.05$). Based on the NPRS tree, the relative cladogenesis statistic identified several events of unusually rapid diversification rates (Fig. 3B). Significantly higher than expected diversification rates were found at the base of the *Elacatinus* + *Tigrigobius* + *Risor* + *Gobiosoma* clade, at the base of the *Gobiosoma* and the *Elacatinus* clades, and at the base of the two main clades within *Elacatinus* (Fig. 3B). We removed one species from both the *Elacatinus* (*E. oceanops*) and the *Gobiosoma* (*Garmannia robustum*) clades, resulting in a new phylogeny (52 ingroup dataset). This dataset was not rejected under the *B*1 test ($n = 52$, $B1 = 25.04$, $P > 0.05$).

Following Pybus and Harvey (2000), we applied the CR/MCCR tests to both the 54 and 52 ingroup datasets. In both

TABLE 1. Results of three-taxon tests of shifts in diversification rates (Sanderson and Donoghue 1994) obtained for selected Gobiosomatini clades. Calculations were performed with LRDIVERSE version 0.8 (written by M. Sanderson) using Monte-Carlo simulations (1000 replicates). Relative age of internal nodes was set to zero and the diversification rate of internal branches was set equal to the average of the three terminal taxa. Asterisk indicates significance at the 5% level.

Outgroup	Ingroup 1	Ingroup 2	*P*-value for null model
Unknown 1 sp	*Microgobius* group 37 spp[1]	*Gobiosoma* group 94 spp[2]	0.983*[7]
Unknown 2 spp	*Microgobius* group 37 spp[1]	*Gobiosoma* group 94 spp[2]	0.926
Microgobius group 37 spp[1]	*Aboma* clade 1 sp[3]	remaining *Gobiosoma* group 91 spp[4]	0.971*[8]
Microgobius group 37 spp[1]	*Nes* clade 28 spp[3]	remaining *Gobiosoma* group 64 spp[4]	0.132
Tigrigobius clade 4 spp[5]	*Elacatinus puncticulatus* (Pacific)	*Elacatinus* (Atlantic) 14 spp[6]	0.913
Elacatinus puncticulatus	*Elacatinus* (sponge-dweller) 6 spp	*Elacatinus* (cleaner) 7 spp	0.837

[1] Including *Palatogobius* and *Parella* according to Birdsong et al. 1988.
[2] Including *Eleotrica, Gymneleotris, Ophiogobius, Pariah, Pycnomma, Robinsichthys,* and *Varicus* according to Birdsong (1988), Birdsong et al. (1988) and Van Tassell (1998).
[3] Assuming that either the *Aboma* or the *Nes* clade is the most basal lineage within the *Gobiosoma* group, respectively.
[4] *Eleotrica* and *Ophiogobius* cannot be assigned to any clade within the *Gobiosoma* group, and therefore were left out of these calculations, whereas *Gymneleotris, Pariah, Pycnomma, Robinsichthys,* and *Varicus* were assigned to the *Nes* clade according to Birdsong (1988) and Van Tassell (1998).
[5] Including *T. zebrella* according to Van Tassell (1998).
[6] Including *E. astronasum,* which is a plankton feeder.
[7] Higher diversification rate in the two ingroups ($P = 0.352$).
[8] Slower diversification rate in ingroup 1 ($P = 0.650$).

TABLE 2. Test of the null hypothesis that per-lineage speciation and extinction rates have remained constant through time in the Gobiosomatini using the constant-rate and Monte Carlo constant-rate tests (Pybus and Harvey 2000).

Phylogeny	Number of sampled species (x)	True number of species (y)	γ[1]	Critical value of γ (at 5% level)[2]
54 taxa dataset	54	131	-3.45 ($P < 0.05$)	-3.28
52 taxa dataset	52	131	-3.58 ($P < 0.05$)	-3.30

[1] Calculated with Genie version 3.0 (Pybus and Rambaut 2002).
[2] Distribution obtained from 10,000 replicates using MCCRTest (Pybus 2000).

cases, the hypothesis of a constant-rate model is rejected (Table 2). Our data, therefore, provide strong phylogenetic evidence that after an initial burst, overall speciation rate has decreased through time in the American seven-spined gobies.

DISCUSSION

Systematics and Taxonomy

The phylogeny of the New World Gobiosomatini was recovered based on mitochondrial 12S and 16S rRNA and tRNA-Val gene sequence data using representatives of the Gobiinae genera *Gobius* and *Vanneaugobius* from the temperate eastern Atlantic-Mediterranean region as outgroups (Figs. 2 and 3). The monophyly of the Gobiosomatini was highly supported and is concordant with the results from Birdsong (1975), who defined the tribe based on the presence of seven spines in the first dorsal fin, a dorsal fin pterygiophore formula of 3–221110, and a vertebral count of 11 + 16 − 17. Changes in the above-mentioned character states have occurred in *Risor* and *Evermannichthys* (Birdsong 1975). Both genera display variability in the dorsal pterygiophore pattern and, in the latter, also in vertebral number and arrangement. The unusual morphology of these genera may be associated with their highly evolved, probably obligate, sponge-dwelling habits (Beebe 1928; Böhlke and Robins 1969; Birdsong 1975). The close sister-group relationship of these two taxa in the molecular phylogeny further suggests different levels of adaptation to sponge dwelling within a common evolutionary lineage (Böhlke and Robins 1969). Our results also support the division of the tribe into the *Gobiosoma* group and the *Microgobius* group, which was based on the presence of a hypural fusion in the former group (Birdsong et al. 1988; Figs. 2 and 3). Although a hypural fusion is missing in *Aboma*, this genus is placed within the *Gobiosoma* group with high bootstrap, quartet puzzling, and Bayesian support values (Figs. 2 and 3). This phylogenetic placement is concordant with cheek myology and papillae patterns (Van Tassell 1998). Uncertainty remains whether this genus is the most basal member of the *Gobiosoma* group or resolves after the *Nes* clade. Hence, with the current molecular data we cannot determine whether the absence of the hypural fusion in *Aboma* represents the plesiomorphic condition or a secondary loss.

Gobiosoma, the largest genus within the seven-spined gobies, has been divided into as few as four or as many as seven subgenera based on squamation, cephalic pore patterns, or sensory papillae patterns (Ginsburg 1933; Böhlke and Robins 1968; Hoese 1971; Van Tassell 1998). Furthermore, there is little agreement on the species composition of each subgenus. Our molecular phylogeny provides a robust framework to accomplish a taxonomic revision of the genus *Gobiosoma* (sensu Böhlke and Robins 1968). Given our molecular results, it seems reasonable to elevate some of the former subgenera to a generic rank (e.g. *Elacatinus*). Future taxonomic work will also have to take into account that *Tigrigobius* is not monophyletic, and that the genus *Enypnias* may be nested within a clade that includes the subgenera *Gobiosoma*, *Garmannia*, and *Austrogobius*.

Patterns of Diversification

With over 130 species, the Gobiosomatini is one of the most successful groups of tropical marine fishes in the New World, and has been suggested to be an example of an adaptive radiation (Hoese and Larson 1985). Using the molecular phylogeny as a robust framework, we performed several statistical tests to characterize the patterns of diversification of the group, and to test the hypothesis of constant speciation rate in the group. According to the three-taxon test, a higher diversification rate at the origin of the tribe is supported only by assuming a single hypothetical outgroup species (Table 1). Unfortunately, the outgroup of the Gobiosomatini is currently not known. The possibility that more than one species forms the outgroup of the Gobiosomatini needs to be tested. Several Gobiinae genera, namely *Odondebuenia*, *Corcyrogobius*, *Gorogobius*, *Egglestonichthys*, and *Callogobius* have been proposed to be closely related to the Gobiosomatini (Miller and Tortonese 1969; Miller 1972; 1978; Miller and Wongrat 1979; but see Birdsong 1988; Birdsong et al. 1988; Van Tassell 1998). Hence, it remains uncertain whether the origin of the Gobiosomatini was associated with an increase in diversification rate according to the three-taxon test. On the other hand, the relative cladogenesis statistic identified unusually rapid diversification rates associated with several cladogenetic events in the *Gobiosoma* group (Fig. 3B).

Both the LTT plot and the CR/MCCR tests indicate an early burst of speciation within the Gobiosomatini followed by a slowdown (Fig. 5 and Table 2). Such a pattern of change in speciation rate is typical of adaptive radiations (Johns and Avise 1998; Lovette and Bermingham 1999) and has been documented with similar tests for only two groups of marine fishes, icefishes and rockfishes (Johns and Avise 1998). The decrease in speciation rate in such adaptive radiations may be due to a decrease of speciation opportunities as ecological and geographical spaces are filled (Schluter 2000).

Factors Underlying Gobiosomatini Evolution

Our molecular phylogeny suggests that ecology rather than biogeography has played an important role in the evolution of the seven-spined gobies. We observed that shifts in ecology seem to be associated with specific cladogenetic events (shown in Figure 3B). Ecological shifts are either associated with recent speciation events, and thus closely related taxa exhibit pronounced ecological differences, or instead are associated with the long-term persistence and subsequent ra-

diation of lineages, and thus most shifts would be observed between distantly related lineages, that is, close to the root of the tree (Barraclough et al. 1999; Barraclough and Nee 2001). In the phylogeny of the seven-spined gobies, major habitat shifts observed conform to the second case. They are located at the origin of the main Gobiosomatini lineages (Fig. 3B). The majority of the *Microgobius* species studied are found in coastal habitats mostly over mud/silt substrate (Appendix). In contrast, the species from the *Bollmannia* clade, which was resolved as sister group of the *Microgobius* clade, are generally found in deeper ocean habitats below 20–30 meters depth, over mud or coral sand flats; as far as it is known species of both groups live in burrows (Appendix). The members of the *Gobiosoma*, *Barbulifer*, and *Aboma* clades are best characterized as shallow coastal or estuarine species that inhabit the continental coasts in both oceans (Appendix). Major ecological shifts from continental coastal-estuarine habitats into tropical reef or reef-edge areas are associated with the origin of the *Elacatinus*, *Tigrigobius*, and *Risor* clades and the *Nes* clade, respectively (Fig. 3B and Appendix). These major habitat shifts are concordant with the burst of speciation early in the evolutionary history of the Gobiosomatini detected in our analysis (Fig. 5 and Table 2).

Major habitat shifts may account for the initial divergence within the Gobiosomatini, but they cannot alone explain the subsequent diversification within each clade. We propose that microhabitat and behavioral specializations may have been crucial in the radiation of the clades. For example, members of the three clades *Elacatinus*, *Tigrigobius*, and *Risor*, which are found in rocky or coral reef habitats, exhibit astonishing specializations, that seem to have a single origin (Fig. 3B). *Tigrigobius multifasciatum* and *Ginsburgellus novemlineatus*, two species that are associated with sea urchins, were consistently grouped as sister species (although a long branch attraction effect cannot be discarded in this case). *Risor ruber* and *Evermannichthys spongicola*, two highly specialized sponge-dwelling taxa, were grouped together. The Atlantic *Elacatinus*, the neon gobies, are separated into two distinct clades, one comprising species that remove ectoparasites from fishes and maintain cleaning stations (obligate cleaning behavior), the other comprising species that live in association with sponges (Fig. 3B). This latter behavior seems to be more derived among neon gobies, since the most basal member of the group, the Pacific *E. puncticulatus* shows facultative cleaning behavior. Unfortunately, we cannot determine the origin of the plankton feeding behavior of *E. astronasus* (Colin 1975) a species not included in this analysis. It is interesting to note that in the Indo-Pacific some Labridae (mostly from the genus *Labroides*) show cleaning behavior (Grutter 1999), and that these species show striking convergence in body shape and coloration with *Elacatinus* from the Caribbean Sea. The behavioral dichotomy between cleaners and sponge-dwellers likely played an important role in the diversification of these fishes that are often found in sympatry. According to the relative cladogenesis statistic, a recent radiation event may be associated with the origin of cleaning and sponge-dwelling behaviors (Fig. 3B). Facultative cleaning behavior has evolved independently in two Pacific species, *Tigrigobius limbaughi* and *T. digueti* (species not included in this study). It is likely that similar behavioral and niche specialization may have been important in the diversification of the other Gobiosomatini clades. However, with the exception of striking examples such as *Nes longus*, that lives commensally with snapping shrimp (Appendix), almost no behavioral and ecological data are available from the other clades to further evaluate this hypothesis.

The association of an early burst of speciation with major habitat shifts, and the subsequent diversification linked to specializations in behavior and microhabitat, reinforces our interpretation of an adaptive radiation in the American seven-spined gobies. The observation of early lineage divergence due to major habitat shift in the Gobiosomatini is in agreement with increasing data that support the important role of invasions of novel habitats in adaptive divergence in speciation (Schluter 2000). To further test this interpretation, more ecomorphological data are required to establish phenotype-environment correlations (Schluter 2000).

Marine Speciation

It is challenging to reconcile speciation with the vast extension and apparent absence of physical barriers in marine environments. Under these conditions, organisms with a pelagic larval stage and the potential for long distance dispersal would be expected to form populations with very little or no spatial structure (Palumbi 1994; Shulman and Bermingham 1995; Mora and Sale 2002; Palumbi and Warner 2003), which reduces the opportunity for speciation mechanisms to operate. However, recent studies indicate that the dispersal of pelagic larvae may be restricted by factors such as settlement preferences and persistence ability of the larvae in new settlements (Öhman et al. 1998). Therefore, self-recruitment in marine populations may be a substantial factor counteracting gene flow and setting the conditions for local adaptation. The importance of self-recruitment is suggested by the persistence of endemic species with pelagic larvae on small isolated islands (Robertson 2001), the unexpected genetic subdivision of many marine populations (Shulman and Bermingham 1995; Miya and Nishida 1997; Taylor and Hellberg 2003), and new information on the behavior of pelagic larvae enhancing near-shore retention (Jones et al. 1999; Swearer et al. 1999; Cowen et al. 2000).

Mechanisms of speciation that are thought to be important in terrestrial and lacustrine systems such as ecological adaptation and sexual selection have been barely explored in marine systems. However, new data are accumulating that highlight the importance of these mechanisms on marine speciation (McMillan et al. 1999; Bernardi et al. 2002; Streelman et al. 2002; Taylor and Hellberg 2003).

We have reconstructed the phylogeny of the gobiid New World tribe Gobiosomatini to investigate the processes driving cladogenesis in this speciose group. We find that major habitat shifts seem to be directly related with the origin and divergence of the main clades and that their subsequent diversification seems to be related to behavior and niche specializations. Therefore, our results are in agreement with the two-phase scenario proposed by Streelman et al. (2002) for the evolution of parrotfishes. In these fishes an ecomorphological split into reef and seagrass types seems to have played

a significant role in the initial divergence of the major lineages within the group, whereas color and breeding behavior seem to have been involved in subsequent diversification (Streelman et al. 2002). An initial divergence followed by diversification has also been documented in lacustrine fishes such as cichlids from the East African Great Lakes. This successful group of fishes has undergone a rapid adaptive radiation in which first adaptation to major habitats (sand-dwellers and rock-dwellers) occurred, followed by diversifications through divergent selection on ecomorphological traits and mate recognition systems (Albertson et al. 1999; Danley and Kocher 2001).

Speciation in both terrestrial and marine systems may be driven by similar evolutionary mechanisms, although they may operate at different temporal and spatial scales (Bowen et al. 2001; Colborn et al. 2001). Understanding of speciation in the marine realm will benefit from the accumulation of more phylogenetic data combined with an improvement of our knowledge on the ecology and behavior of marine fishes.

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APPENDIX

Summary of specimens, sample locations, distribution, and ecological/behavioral data. Specimen identification numbers, GenBank accession numbers, and museum voucher numbers are given in the Electronic Appendix (currently available from the *Evolution* Editorial Office at evolution@asu.edu).

Genus	Subgenus	Species	Locality (locality number in Fig. 1)	Distribution	Ecology and behavior
Gobiosomatini					
Aboma		*etheostoma*	Panama, Panama Bay (5)	Gulf of California from Sonora to Colombia	Coastal estuaries in fine mud with some sand
Akko		species novum	El Salvador, Golfo de Fonseca (4)	Panama to Peru	Black mud bottom at 20–30 meter depths
Aruma		*histrio*	Mexico, La Paz (1)	Gulf of California	Shallow waters under cobble rocks
Barbulifer		*ceuthoecus*	Panama, Colon (6)	Bahamas, south Florida, to Venezuela and Central American coast	Shallow waters associated with macroalgae or grasses
Barbulifer		*ceuthoecus*	Florida Keys, Marathon (10)	Bahamas, south Florida, to Venezuela and Central American coast	Shallow waters associated with macroalgae or grasses
Bollmannia		*boqueronensis*	Florida Keys, Marathon (10)	South Florida, Greater Antilles, Venezuela	Coral sand flats in or near burrows, deeper than 30 meters
Bollmannia		*chlamydes*	El Salvador, Golfo de Fonseca (4)	Costa Rica to northern Peru	Mud bottoms, ocean deeper than 20 meters
Bollmannia		*communis*	Gulf of Mexico, Louisiana (8)	Gulf of Mexico	Mud bottoms, ocean
Bollmannia		*marginalis*	El Salvador, Departamento La Libertad (4)	southern Gulf of California; Costa Rica to Ecuador	Mud bottoms, ocean
Chriolepis		*zebra*	Mexico, San Carlos (3)	Gulf of California	Rock reef areas under small rocks
Enypnias		*aceras*	El Salvador, Golfo de Fonseca (4)	Panama to El Salvador	Rock reef habitat in coarse sand, shell habitat at bottom of rock ledges
Enypnias		*seminudus*	Panama, Panama Bay (5)	Gulf of California to Colombia	Coastal, in sand-rock habitat
Enypnias		*seminudus*	Panama, Panama Bay (5)	Gulf of California to Colombia	Coastal, in sand-rock habitat
Evermannichthys		*spongicola*	Florida Keys, Marathon (10)	North Carolina to Dry Tortugas	Sponge dweller generally inside logger head sponges
Genus novum		species novum	Curacao (17)	Curacao	Deep water rocky habitats—schools in water column near rock cliffs
Ginsburgellus		*novemlineatus*	Curacao (17)	Bahamas, Cayman Islands, Antilles to Venezuela	Under sea urchins in shallow waters
Gobiosoma	*Austrogobius*	*spilotum*	Panama, Colon (6)	Panama, Colon-Atlantic	Coastal waters in sandy and hard mud substrate
Gobiosoma	*Elacatinus*	*chancei*	aquarium specimen	Southern Bahamas, Puerto Rico, Virgin Islands, Lesser Antilles	Sponge dweller—in and around sponges, especially tube sponges
Gobiosoma	*Elacatinus*	*evelynae*	aquarium specimen	Bahamas, south to Lesser Antilles and islands off Venezuela	Coral heads and large sponges, cleaner fish, congregate in cleaning stations
Gobiosoma	*Elacatinus*	*figaro*	aquarium specimen	Brazil	Coral heads in shallow water and sponges in deeper waters, cleaner fish, congregate in cleaning stations
Gobiosoma	*Elacatinus*	*genie*	Grand Cayman (15)	Bahamas and Grand Cayman Islands	Coral heads and large sponges, cleaner fish, congregate in cleaning stations
Gobiosoma	*Elacatinus*	*horsti*	Curacao (17)	Curacao, Bonaire, Netherlands Antilles	Sponge dweller, medium-depth reefs, generally tube sponges
Gobiosoma	*Elacatinus*	*louisae*	Bahamas, Lee Stocking Island (14)	Bahamas to Belize	Sponge dweller, medium-depth reefs, generally tube sponges
Gobiosoma	*Elacatinus*	*louisae*	Jamaica, St. Ann's Bay (16)	Bahamas to Belize	Sponge dweller, generally tube sponges

APPENDIX. Continued.

Genus	Subgenus	Species	Locality (locality number in Fig. 1)	Distribution	Ecology and behavior
Gobiosoma	*Elacatinus*	*oceanops*	Florida Keys, Sugar Loaf Key (10)	Bahama Islands west to Honduras and Gulf of Mexico	Coral heads, cleaner fish, congregate in cleaning station
Gobiosoma	*Elacatinus*	*prochilos*	aquarium specimen	Lesser Antilles, Barbados, St. Croix, Jamaica, Belize, Yucatan, northern Gulf of Mexico	Coral heads and sponges, cleaner fish
Gobiosoma	*Elacatinus*	*puncticulatus*	Mexico, La Paz (1)	Gulf of California to Panama and Ecuador	Rocky reef areas in small caves and depressions, facultative cleaner
Gobiosoma	*Elacatinus*	*lori*	Grand Cayman (15)	Gulf of Honduras	Sponge dweller, in and around sponges, especially tube sponges
Gobiosoma	*Elacatinus*	*xanthiprora*	Caribbean, Honduras (7)	Florida Keys and Jamaica	Sponge dweller, medium-depth reefs, generally tube sponges
Gobiosoma	*Garmannia*	*chiquita*	Mexico, Puerto Penasco (2)	Gulf of California	Intertidal waters, rock with sand substrate
Gobiosoma	*Garmannia*	*hildebrandi*	Panama Canal (5)	Panama Canal	Rock and mud bottom in Panama Canal, fresh and brackish water
Gobiosoma	*Garmannia*	*homochroma*	Panama Canal (5)	Panama Canal	Fresh water mud-rock sediments
Gobiosoma	*Garmannia*	*homochroma*	Panama Canal (5)	Panama Canal	Fresh water mud-rock sediments
Gobiosoma	*Garmannia*	*nudum*	Panama Bay (5)	Peru to El Salvador and Atlantic coast of Panama Canal	Coastal waters in rocky habitats with mud/sand or shell substrate
Gobiosoma	*Garmannia*	*nudum*	El Salvador, Golfo de Fonseca (4)	Peru to El Salvador and Atlantic coast of Panama Canal	Coastal waters in rocky habitats with mud/sand or shell substrate
Gobiosoma	*Garmannia*	*robustum*	Florida, Indian River (11)	Gulf of Mexico, Texas to Florida, Atlantic coast of Florida	Estuarine and coastal, mud, sand, weed habitats
Gobiosoma	*Garmannia*	*paradoxum*	Panama Bay (5)	West coast of Mexico to Ecuador	Coastal waters in rocky habitats with sand, mud and/or shell substrate
Gobiosoma	*Garmannia*	*paradoxum*	Panama Bay (5)	West coast of Mexico to Ecuador	Coastal waters in rocky habitats with sand, mud and/or shell substrate
Gobiosoma	*Garmannia*	species novum	Panama Canal (5)	Panama Canal	Rock and mud bottom in Panama canal
Gobiosoma	*Gobiosoma*	*bosc*	Florida, Indian River (11)	Atlantic coast Massachusetts to southern Florida, Gulf of Mexico, southern Florida to Mexico	Estuarine environment in association with oyster shell, rocks, etc
Gobiosoma	*Gobiosoma*	*bosc*	Gulf of Mexico, Louisiana (8)	Atlantic coast Massachusetts to southern Florida, Gulf of Mexico, southern Florida to Mexico	Estuarine environment in association with oyster shell, rocks, etc.
Gobiosoma	*Gobiosoma*	*bosc*	New York, Long Island, south shore (13)	Atlantic coast Massachusetts to southern Florida, Gulf of Mexico, southern Florida to Mexico	Estuarine environment in association with oyster shell, rocks, etc.
Gobiosoma	*Gobiosoma*	*bosc*	Florida, St. Petersburg (9)	Atlantic coast Massachusetts to southern Florida, Gulf of Mexico, southern Florida to Mexico	Estuarine environment in association with oyster shell, rocks, etc.
Gobiosoma	*Gobiosoma*	*bosc*	Virginia, Chesapeake Bay (12)	Atlantic coast Massachusetts to southern Florida, Gulf of Mexico, southern Florida to Mexico	Estuarine environment in association with oyster shell, rocks, etc.
Gobiosoma	*Gobiosoma*	*ginsburgi*	Virginia, Chesapeake Bay (12)	New York to Georgia	Estuarine environment, oyster reef and mud substrate
Gobiosoma	*Gobiosoma*	species novum	Mexico, Puerto Penasco (2)	Gulf of California	Coastal waters rock with sand substrate
Gobiosoma	*Tigrigobius*	*dilepis*	Honduras, Caribbean (7)	British West Indies, Bahama Islands, Cayman Islands, British Honduras, Jamaica	Coral reef, on vertical walls, sponges, or coral heads
Gobiosoma	*Tigrigobius*	*gemmatum*	Grand Cayman (15)	Bahamas to Lesser Antilles and Venezuela	Coral flats found only in holes in coral cement

APPENDIX. Continued.

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Gobiosoma	*Tigrigobius*	*limbaughi*	Mexico, Gulf of California (1)	Gulf of California	Rocky reef areas in small caves and depressions, cleaning behavior
Gobiosoma	*Tigrigobius*	*macrodon*	Florida Keys, Marathon (10)	Southern Florida, Cuba to Haiti	Coral flats under rock ledges and interstices of the calcareous rubble
Gobiosoma	*Tigrigobius*	*multifasciatum*	Bahamas, Lee Stocking Island (14)	Bahamas, Cuba, Cayman Islands south to Barbados and Curacao	Under sea urchins or rocks in shallow waters
Gobiosoma	*Tigrigobius*	*pallens*	Jamaica, St. Ann's Bay (16)	Bahamas and throughout the Caribbean Sea	Coral reef on vertical walls and in holes in coral cement
Gobiosoma	*Tigrigobius*	*saucrus*	Bahamas, Lee Stocking Island (14)	Florida Keys, Bahamas, Jamaica, Virgin Islands, and Belize	Coral reef, on the surface of large, rounded boulder corals
Gobulus		*birdsongi*	Panama Canal (5)	Panama Canal	Fresh water rocky mangrove streams
Microgobius		*brevispinis*	Panama Canal (5)	Southern Baja and Gulf of California to Panama	Beach areas with silt/mud and some sand/broken shell substrate
Microgobius		*carri*	Florida Keys, Marathon (10)	Gulf of Mexico to Lesser Antilles	Coarse sand substrate near coral reefs, above burrows in the sand
Microgobius		species	El Salvador, Golfo de Fonseca (4)	Gulf of California to Peru	Mostly silt/mud, some broken shell substrate in coastal habitats
Microgobius		*erectus*	Panama, Bahia Honda (5)	Gulf of California to the Gulf of Panama	Ocean in silt/mud substrate, 15–30 meter depths
Microgobius		*erectus*	El Salvador, Departamento La Libertad (4)	Gulf of California to the Gulf of Panama	Ocean in silt/mud substrate, 15–30 meter depths
Microgobius		*gulosus*	Florida, St. Petersburg (9)	Virginia to Texas	Estuarine and fresh waters, fine silt/mud habitats
Microgobius		*microlepis*	Florida Keys, Marathon (10)	Bahamas to Yucatan	Inshore waters over calcareous silt shell bottoms
Microgobius		*thalassinus*	Florida, Sarasota (9)	Virginia to Texas	Silt/mud substrate with oyster, sponge habitats, coastal
Nes		*longus*	Florida Keys, Marathon (10)	Bahamas to Yucatan and Venezuela	Coral reef areas, inhabit burrows in fine coral sand, lives commensally with snapping shrimp
Psilotris		*batrachodes*	Jamaica, St. Ann's Bay (16)	Bahamas, Puerto Rico, Belize, Honduras, to Colombia	Coral reef areas in small cracks with sand bottom
Risor		*ruber*	Florida Keys, Marathon (10)	Bahamas and Florida, south through the Antilles	Sponge dweller, generally found on or in barrel sponges
Outgroups					
Gobius		*xanthocephalus*	Spain, Gran Canaria, Puerto Rico	eastern Atlantic and Mediterranean	Coastal waters in fine sand with rock substrate
Gobius		*niger*	Spain, Gran Canaria, Puerto Rico	eastern Atlantic and Mediterranean	Coastal waters on sand with grass
Vanneaugobius		*canarensis*	Spain, Lanzarote, Mala	Canary Islands, Madeira and Guinea	Coastal under small rocks on sand substrate
Vanneaugobius		*pruvoti*	Spain, Lanzarote, Mala	West Africa, Canary Islands	Deep water >45 meters under rocks on sand substrate