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PHYLOGENETIC RELATIONSHIPS OF INDO-PACIFIC CORAL GOBIES OF THE GENUS *GOBIODON* (TELEOSTEI: GOBIIDAE), BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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ABSTRACT

Gobiodon species are coral-commensal gobiid fishes which occur throughout much of the Indo-Pacific Region. Species-level phylogenetic relationships were analyzed using mitochondrial DNA sequences. Portions of the 12S and 16S rRNA mitochondrial genes were selected for analysis. A search was made for the most parsimonious trees (maximum parsimony), the result of which was two trees with a consistency index of 0.620 and length of 753 steps. Bootstrap support and decay values were calculated for each resolved node. Many parts of the trees were well-supported, but with lower support at intermediate levels. Monophyly of *Gobiodon* is strongly supported (bootstrap support 100%, decay value 33). Inclusion of a set of morphological characters in a total evidence analysis provided additional support at some nodes resolved by the molecules-only analysis and also allowed a number of new resolutions. In the total evidence tree the specialized deep-bodied, compressed species, such as *Gobiodon histrio* (Valenciennes, 1837), *Gobiodon unicolor* (Castelnau, 1873), and *Gobiodon brochus* (Harold and Winterbottom, 1999), form a monophyletic group, whereas the molecular analysis has them paraphyletic. In both analyses, the morphologically generalized species, *Gobiodon quinquestrigatus* (Valenciennes, 1837), and related species form a clade which is sister group to all other *Gobiodon* species.

Gobiodon species live in obligate commensal association with reef-building corals through much of the tropical Indo-Pacific Region. There is considerable diversity in body shape and pigmentation among species. Some species, such as *Gobiodon quinquestrigatus* (Valenciennes, 1837), has a relatively generalized body form with only slight compression, whereas some species are quite deep-bodied and very compressed (e.g., *Gobiodon brochus* Harold and Winterbottom, 1999). With regard to pigmentation, some species, such as *Gobiodon ceramensis* (Bleeker, 1852), are uniformly pigmented and lack markings, whereas others, such as *Gobiodon erythrospilus* Bleeker, 1875 and *Gobiodon histrio* (Valenciennes, 1837), have variable patterns of stripes, bands and dots of orange against a green background (Munday et al., 1999). In addition to this morphological diversity, some species have been demonstrated to be protogynously hermaphroditic (Cole, 1990), and some (*G. histrio*) have been shown to undergo bi-directional sex change, with some males observed to change back to females (Munday et al., 1998). All studied species of *Gobiodon* exhibit a specialized pattern of habitat use and tend to occupy only a select group of coral species hosts (Patton, 1994; Munday et al., 2004). These and other species of coral associates represent an important component of reef diversity, yet to be studied in adequate detail. Our immediate goal was reconstruction of interspecific phylogenetic relationships. Resultant phylogenies will be used later in studies of biogeography, and evolution of ecological and behavioral attributes.

Gobiodon contains more than 30 nominal species, but of these named species we currently recognize up to 19 as valid: *Gobiodon acicularis* Harold and Winterbottom, 1995, *Gobiodon albofasciatus* Sawada and Arai, 1972 (questionable), *Gobiodon axillaris* De Viz, 1884, *G. brochus*, *G. ceramensis*, *Gobiodon citrinus* (Rüppell, 1838), *G. erythrospilus*, *Gobiodon fulvus* Herre, 1927, *Gobiodon heterospilos* Bleeker, 1856, *G. histrio*, *Gobiodon micropus* Günther, 1861, *Gobiodon oculolineatus* Wu, 1979, *Gobiodon okinawae* Sawada, Arai, and Abe, 1972, *Gobiodon prolixus* Winterbottom and Harold, 2005, *G. quinquestrigatus*, *Gobiodon reticulatus* Playfair, 1867, *Gobiodon rivulatus* (Rüppell, 1830), *Gobiodon spilophthalmus* Fowler, 1944, and *Gobiodon unicolor* (Castelnau, 1873). As a result of our surveys of museum collections and specimens obtained in the field we recognize as many as six additional undescribed species.

Research on goby phylogenetics has been fragmentary and deals mainly with higher level relationships within the suborder Gobioidei based on a variety of morphological characters (e.g., Harrison, 1989; Hoese and Gill, 1993), but recently has been shifting toward analysis at various taxonomic levels using molecular data (e.g., Dawson et al., 2001; Thacker and Cole, 2002; Thacker, 2003; Munday et al., 2004). Harold and Winterbottom (1999) documented some morphological characters that bear information on the phylogenetic relationships among *Gobiodon* species. In particular, they suggested that a paired set of grooves under the head (interopercular-isthmus groove, first noted by D. F. Hoese) represents a complex set of underlying osteological and soft tissue structures, the presence of which indicates likely monophyly of a subset of the species. This putative synapomorphy is present in *G. brochus*, *G. erythrospilus*, *G. fulvus*, *G. histrio*, *G. micropus*, *G. unicolor*, and at least three undescribed species: *Gobiodon* sp. A, *Gobiodon* sp. B, and *Gobiodon* sp. C (see Munday et al., 1999 for information on these designations for undescribed species). Those species also share a relatively compressed body form, a feature which is also thought to be synapomorphic. Although our efforts to date have mostly contributed to the knowledge of species diversity of these fishes (Winterbottom and Emery, 1986; Harold and Winterbottom, 1995, 1999; Munday et al., 1999; Winterbottom and Harold, 2005), we have also been able to accumulate enough whole specimens and tissue samples to allow for the current phylogenetic analysis.

Here we present a phylogenetic analysis based on partial sequences of 12S and 16S rRNA mitochondrial DNA of 15 *Gobiodon* species (listed in Table 1), four of which are undescribed, to address the following two questions: (1) What are the interrelationships among *Gobiodon* species using DNA sequences alone, and (2) what is the impact on the tree(s) of including a set of morphological characters in a total evidence analysis? (see Nixon and Carpenter, 1996).

Materials and Methods

Molecular Analysis.—Most voucher specimens and tissue samples of *Gobiodon* species and outgroups *Paragobiodon echinocephalus* (Rüppell, 1830) and *Paragobiodon xanthosomus* (Bleeker, 1852) were collected by co-author PLM at: Lizard and One Tree Islands, Queensland, Australia; Motupore Island, Bootless Bay, Papua New Guinea; and New Britain (Table 1). Other outgroup gobioids, *Dormitator maculatus* (Bloch, 1792), *Eleotris amblyopsis* (Cope, 1871), and *Ctenogobius shufeldti* (Jordan and Eigenmann, 1887) were collected in estuaries of South Carolina. Tissue samples and vouchers are deposited at the Grice Marine Laboratory (GMBL).

Table 1. List of taxa for which portions of 12S and 16S rRNA mitochondrial genes were sequenced in this study, with total number of specimens for each taxon, collection localities, voucher specimen catalog numbers, and GenBank Accession numbers. Additional information on localities available on request. GBR = Great Barrier Reef, PNG = Papua New Guinea.

Taxon	Locality	Voucher Catalog no.	GenBank Accession numbers	
			16S	12S
Outgroup taxa				
Eleotridae				
Dormitator maculatus	Charleston Harbor, South Carolina	GMBL 5355	EF443265	EF540555
Eleotris amblyopsis	Charleston Harbor, South Carolina	GMBL 5356	EF443266	EF540556
Gobiidae				
Ctenogobius shufeldti	South Santee River, South Carolina	GMBL 5361	DQ648194	DQ650658
Paragobiodon echinocephalus	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4444	EF443261	EF540557
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4445	EF443262	–
Paragobiodon xanthosomus	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4436	EF443263	EF540558
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4438	EF443264	EF540559
Ingroup taxa				
Gobiodon sp. A	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4481	EF443267	–
	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4482	EF443268	EF540560
Gobiodon sp. B	Motupore Island, Bootless Bay, PNG	GMBL 5357	EF463067	EF540561
	Motupore Island, Bootless Bay, PNG	GMBL 5358	EF463068	EF540562
Gobiodon sp. C	Schumann Island, Kimbe Bay, New Britain, PNG	GMBL 4491	–	EF540563
	Schumann Island, Kimbe Bay, New Britain, PNG	GMBL 4492	EF463069	–
Gobiodon sp. D	Schumann Island, Kimbe Bay, New Britain, PNG	GMBL 4495	EF463070	EF540564
Gobiodon acicularis	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4461	EF463071	EF540565
	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4462	EF463072	EF540566
Gobiodon axillaris	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4446	EF463073	–
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4447	EF463074	EF540567

Table 1. Continued.

Taxon	Locality	Voucher Catalog no.	GenBank Accession numbers	
			16S	12S
Gobiodon brochus	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4471	EF463075	EF540568
	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4472	EF463076	EF540569
Gobiodon ceramensis	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4466	EF527238	EF540570
	Lagoon, Lizard Island, GBR, Qld., Australia	GMBL 4467	EF527239	EF540571
Gobiodon citrinus	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4506	EF527240	EF540572
	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4505	EF527241	EF540573
Gobiodon erythrospilus	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4430	EF527242	EF540574
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4431	EF527243	EF540575
Gobiodon histrio	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4425	EF527244	EF540576
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4426	EF527245	EF540577
Gobiodon okinawae	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4497	EF527246	EF540578
	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4498	EF527247	EF540579
Gobiodon quinquestrigatus	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4450	EF527248	–
	Horseshoe Reef, Lizard Island, GBR, Qld., Australia	GMBL 4451	EF527249	EF540580
Gobiodon rivulatus	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4503	EF527250	EF540581
	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4504	EF527251	EF540582
	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4509	EF527252	EF540583
Gobiodon unicolor	Lagoon, One Tree Island, GBR, Qld., Australia	GMBL 4477	EF527254	EF540584

Methods for tissue processing and analysis of DNA generally follow those described by Hillis et al. (1996). Pectoral fin clips were dissected from fresh specimens and preserved in sarcosyl-urea solution. After about 2 mo of storage and digestion, a 250 µl aliquot was removed for glass bead DNA isolation. Portions of the 12S and16S rRNA genes were amplified by Polymerase Chain Reaction (PCR) with the following conditions (with some variation, particularly of template, for optimization of the reaction), with a total volume of 50 µl per tube: template 2 µl, L20 primer 2 µl (10 µM), H22 primer 2 µl (10 µM), MgSO4 2.5 µl (134 mM), dNTP 4 µl (2.5 mM), Taq. DNA polymerase 0.5 µl (50×), 10× Kocher buffer 5 µl, sterile distilled water 32 µl. The PCR profile was 94 °C for 3 min, followed by 35 cycles consisting of 94 °C for 45 s denaturation, 50 °C for 45 s annealing, and 72 °C for 2 min 30 s extension.

Primer sequences are as follows:

12S rRNA
L1091short: AAACTGGGATTAGATACCCCACTAT
H1478: TGACTGCAGAGGGTGACGGGCGGTGTGT
(Kocher et al., 1989)

16S rRNA
16Sar (L20): CGCCTGTTTATCAAAAACAT
16Sbr (H22): CCGGTCTGAACTCAGATGACGT
(Palumbi, 1996)

Amplification of a 50 µl total sample volume was done using a PTC-100 Programmable Thermal Controller (MJ Research, Inc.). Isolation of DNA, amplification, and purification were done at the Marine Resources Research Laboratory (SC DNR) or the Hollings Marine Laboratory, Charleston, SC. The PCR product was sequenced at the Biotechnology Resource Laboratory, Medical University of South Carolina and the Molecular Core Facility at the Grice Marine Laboratory. Sequence editing and manual alignment was done with the aid of DNASIS software (Hitachi Software Engineering Co. Ltd.) and BioEdit (Ibis Therapeutics, Carlsbad, CA). Sequences were deposited on GenBank, with their respective Accession numbers shown in Table 1.

Phylogenetic analysis (maximum parsimony) was performed using PAUP* version 4.0b10 (Swofford, 1998) with the branch-and-bound (BandB) tree-searching algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data and therefore uninformative. Multiple equally parsimonious trees were summarized with a strict consensus tree. Levels of nodal support were determined by (1) a bootstrap analysis using PAUP*4 with the heuristic tree building option and 1000 replicates, and (2) a decay analysis (Bremer, 1988) using TreeRot.v2 (Sorenson, 1999). Nodal support values reported in the text are given in parentheses, with the bootstrap support value first as a percent, followed by the decay value. The trees are labeled with these values, with the bootstrap value above the branch and the decay value below.

The issue of whether or not to use weighted or unweighted characters is unresolved in the systematic literature, often leading to publications containing numerous rival topologies for the same data set and with no clear indication of preference. In this paper we avoid a priori weighting schemes (e.g., transitions vs transversions), considering only unweighted characters representative of a pattern requiring an evolutionary process explanation. Our use of unweighted characters follows, for example, Thacker's (2003) phylogenetic analysis of gobioid fishes based on sequences of the three mitochondrial genes ND1, ND2, and COI.

Another contentious issue is that of total evidence vs comparisons of trees based upon partitions of the data (e.g., morphology vs molecules, separate consideration of various regions of the genome). In the present study we have chosen to analyze the sequences of the 12S and 16S rRNA genes as one dataset, and the molecular plus morphological characters as another

dataset, rather than as separate partitions. The resultant tree or consensus of multiple trees in each of the two analyzes will represent globally parsimonious solutions (Eernisse and Kluge, 1993; Nixon and Carpenter, 1996).

Total Evidence Analysis.—For the purpose of examining as much evidence of relationships as is currently available some aspects of *Gobiodon* morphology are combined with the molecular data in a total evidence analysis. Some of the morphological characters were described and discussed by Harold and Winterbottom (1999), who described the much of the morphology needed in order to understand the majority of the utilized characters. The character states were obtained by microscopic examination of whole alcohol-preserved specimens and those that had been histologically prepared following the standard clearing and staining procedures as presented by Potthoff (1984) and Taylor and Van Dyke (1985). Terminology for anatomical features described below and referred to in Figures 1 and 2 follows Hoese and Gill (1993). Other terminology, relating to morphology of *Gobiodon* species, follows Harold and Winterbottom (1999). We surveyed these characters for the same series of five outgroup taxa used in the molecular analysis. Assumptions and program settings used in PAUP*4 for the molecular parsimony analysis were also used in the combined analysis. Institutional abbreviations are as listed in Leviton et al. (1985).

Results

Phylogenetic Analysis of Molecular Data.—Sequencing of the small subunit rRNA (12S) yielded a fragment of about 400 base pairs in length, and the large subunit (16S) a fragment of up to about 550 base pairs. The phylogenetic analysis resulted in two equally parsimonious trees, each with a length of 753 steps, consistency index (CI), excluding uninformative characters = 0.620, and retention index (RI) = 0.694. Of the 999 total characters 625 were constant, 128 were parsimony-uninformative, and 246 were parsimony-informative. The strict consensus tree (Fig. 3) is fully resolved, except at node K, which is a trichotomy. In terms of nodal support, bootstrap support values range from < 50% to 100%, decay values from 1 to 28.

Strong support is indicated for the outgroup nodes B, *Gobiodon* + *Paragobiodon* (bootstrap support 100%: decay value 21), and A, *Ctenogobius* + *Gobiodon* + *Paragobiodon* (99%: 16). The most highly supported node is that of the ingroup (*Gobiodon*) at node C (100%: 28). Within *Gobiodon* there is considerable variation in the level of support. Two subgroups of morphologically similar, somewhat generalized, species have strong support: node Q, *G. quinquestrigatus* + *G.* sp. D + *G. rivulatus* (100%: 20), and node O, *G. acicularis* + *G. ceramensis* + *G. citrinus* + *G. okinawae* (99%: 7). Within the node O group there are two pairs of phylogenetically related species: node M, *G. acicularis* + *G. ceramensis* (63%: 2), and node I, *G. okinawae* + *G. citrinus* (50%: 1). We recognize two "forms" of *G. quinquestrigatus*, *G. quinquestrigatus* in the strict sense (GBR, Great Barrier Reef), and *Gobiodon* sp. D (*Gobiodon* cf. *G. quinquestrigatus*), a morphologically similar form that was found inhabiting different coral hosts from other *G. quinquestrigatus* (Munday et al., 1999). The two forms are strongly supported on the tree as sister-taxa (100%: 10).

Another group of species, at node E, but in this case morphologically specialized, all with deep and compressed body form, is well supported as a monophyletic group (92%: 4). Within the node E quartet of species the two extremely similar species *G. histrio* and *G. erythrospilus* are weakly supported at node F as sister taxa (61%: 1), while the two other, rather dissimilar, species at node G, *G. unicolor* and *G. axillaris*, are well-supported (92%: 5). Another cluster of somewhat similar species occurs at node K, comprising *G. brochus*, and *Gobiodon* sp. A, *Gobiodon* sp. B, and *Gobiodon*

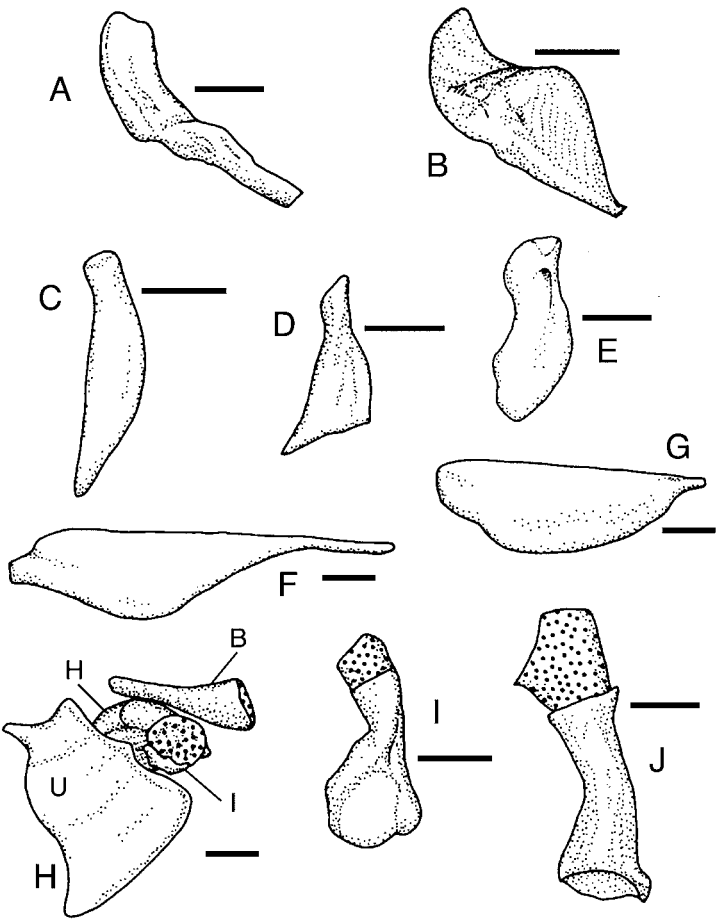

Figure 1. Osteological features of the head skeleton in selected *Gobiodon* species. (A) *Gobiodon acicularis* metapterygoid, (B) *Gobiodon brochus* metapterygoid, (C) *Gobiodon quinquestrigatus* lacrimal, (D) *G. acicularis* lacrimal, (E) *Gobiodon histrio* lacrimal, (F) *G. quinquestrigatus* interoperculum, (G) *G. brochus* interoperculum, (H) *G. quinquestrigatus* interhyoid ossification and associated bones, (I) *G. quinquestrigatus* interhyal, (J) *G. acicularis* interhyal. Abbreviations: B, basihyal; H, left hypohyal; I, "interhyoid" ossification; U, urohyal. A–H right lateral view, I–J right medial view. Scale bars A–I = 0.5 mm, C = 0.25 mm.

sp. C, with *Gobiodon* sp. A and *Gobiodon* sp. B clearly indicated as sister-taxa at node H (100%: 18). *Gobiodon brochus* and *Gobiodon* sp. C are placed in an unresolved trichotomy with clade H, although neither of the two rival trees have these two species as sister-taxa.

Genetic distance among taxa is reported as ranges of uncorrected "*p*" distances (pairwise distances) at various taxonomic levels in Table 2. For sister group relationships that are strongly supported by our analysis "*p*" ranges from the lowest values for morphologically similar but likely distinct species with different habitat-use attributes (Munday et al., 1999) ($p = 0.012$ for *G. quinquestrigatus* vs *Gobiodon* sp. D) to morphologically distinct sister-species pairs (e.g., 0.029 for *G. axillaris* + *G. unicolor,* and 0.044 for *G. quinquestrigatus* + *G. rivulatus*). One pair of distinct, undescribed

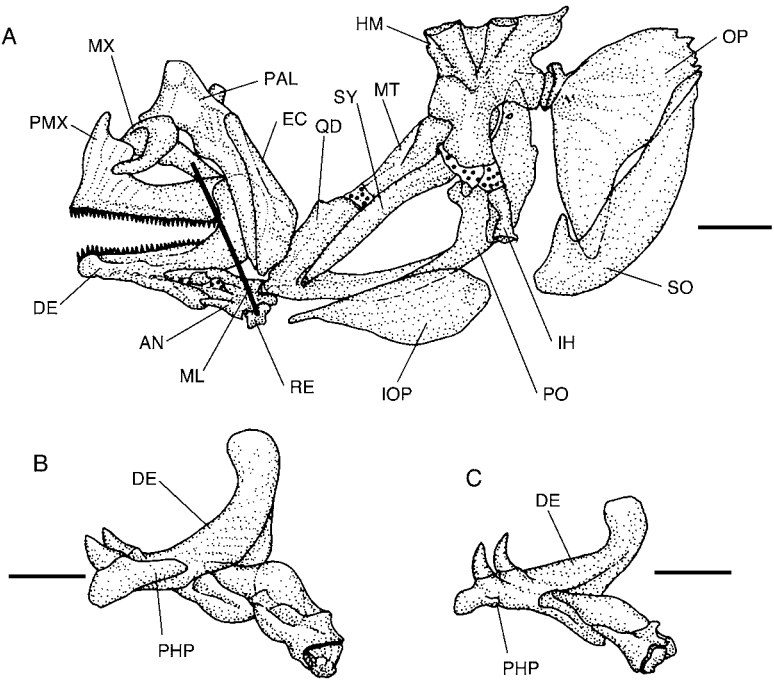

Figure 2. Jaws, suspensorium, and opercular bones of representative *Gobiodon* species. (A) *Gobiodon acicularis* jaws, suspensorium, and opercular bones, (B) *Gobiodon histrio* lower jaw, (C) *Gobiodon quinquestrigatus* lower jaw. Abbreviations: AN, anguloarticular; DE, dentary; EC, ectopterygoid; HM, hyomandibula; IH, interhyal; IOP, interoperculum; LA, lacrimal; ML, maxillomandibular ligament; MT, metapterygoid; MX, maxilla; OP, operculum; PAL, palatine; PMX, premaxilla; PHP, protractor hyoidei process; PO, preoperculum; QD, quadrate; RE, retroarticular; SO, suboperculum; SY, symplectic. A–C right medial views. Scale bars A–C = 1 mm.

species, *Gobiodon* sp. A (western Pacific) and *Gobiodon* sp. B (Papua New Guinea), have a low *p* value (0.014), approximating the value for the two morphologically similar forms *G. quinquestrigatus* and *Gobiodon* sp. D.

Phylogenetic Analysis With Morphological and Molecular Data Combined.—The following morphological characters and associated states (state number in parentheses) were recorded for the set of *Gobiodon* species and designated outgroup gobioids (character-state matrix in Table 3) for use in the total evidence analysis. Three of these characters (1, 2, and 6) and their states were described by Harold and Winterbottom (1999), who suggested that some states of these characters are probably derived and may be of use in the analysis of interspecific relationships among *Gobiodon* species.

Character 1. Deep groove between isthmus and interopercle: (0) absent, (1) present (e.g., *G. brochus*, indicated by arrow in fig. 3, Harold and Winterbottom, 1999).

Character 2. Configuration of anterior margin of interopercle and associated ligament attaching to retroarticular: (0) anterior margin not prolonged and attaching to retroarticular by elongate ligament (e.g., *G. quinquestrigatus*, fig. 2B, in Harold and Winterbottom, 1999), (1) anterior margin of interopercle prolonged and attaching to retroarticular by a short ligament (e.g., *G. brochus*, fig. 2A, in Harold and Winterbottom, 1999).

Table 2. Summary of pairwise uncorrected "*p*" genetic distance values for sister-taxa and other putatively closely-related species pairs depicted in phylogenetic tree based on molecular data (Fig. 3).

Taxon pair	*p*
Gobiodon	
G. quinquestrigatus–Gobiodon sp. D (cf. *G. quinquestrigatus*)	0.012
Gobiodon sp. A–*Gobiodon* sp. B	0.014
G. ceramensis–G. acicularis	0.019
G. histrio–G. erythrospilus	0.020
G. histrio–Gobiodon sp. A	0.073
G. erythrospilus–Gobiodon sp. A	0.020
G. unicolor– G. axillaris	0.029
G. okinawae–G. citrinus	0.043
G. quinquestrigatus–G. rivulatus	0.044
G. rivulatus–Gobiodon sp. D (cf. *G. quinquestrigatus*)	0.045
G. brochus–Gobiodon sp. A	0.072
G. brochus–Gobiodon sp. B	0.073
G. brochus–Gobiodon sp. C	0.066
Paragobiodon	
P. echinocephalus–P. xanthosomus	0.020

Character 3. Metapterygoid form: (0) shallow, with little dorsal extension (Fig. 1A), (1) relatively deep, with dorsomedial blade-like process (Fig. 1B).

Character 4. Squamation: (0) scales present covering most of body, (1) scales absent, (2) scales present on caudal peduncle and nearly completely absent on remainder of body (present in *G. erythrospilus* in association with lateral line).

Character 5. Dentary shape: (0) triangular (e.g., *G. quinquestrigatus*, fig. 2B, in Harold and Winterbottom, 1999), (1) elongate, recurved (e.g., *G. brochus*, fig. 2A, in Harold and Winterbottom, 1999).

Character 6. Post-symphysial canine teeth: (0) present, (1) absent.

Character 7. Jaw dentition: (0) jaw teeth of two or three sizes (i.e., lengths), with several rows of small inner teeth and an outer row of much larger teeth (some taxa have an additional row of innermost teeth of intermediate size, (1) jaw teeth relatively villiform (of approximately uniform size).

Character 8. Form of protractor hyoidei muscle attachment process on ventral surface of lower jaw: (0) process absent (Fig. 2A), (1) large, well-developed process near dentary symphysis (Fig. 2B), (2) small, poorly-developed process about half-way along ventral surface of dentary (Fig. 2C).

Character 9. Lacrimal shape: (0) elongate and narrow (Fig. 1C), (1) ventrally expanded, triangular to quadrilateral shape (Fig. 1D), (2) broad and spatulate (Fig. 1E).

Character 10. Interopercle shape: (0) anteriorly elongate, expanded and deep posteriorly (Fig. 1F), (1) shallow and lance-like overall, (2) deep, subelliptical (Fig. 1G).

Character 11. Form of maxillomandibular ligamentous tissue: (0) diffuse connective tissue, (1) thickened, cord-like highly organized connective tissue (Fig. 2A).

Character 12. Ossification of "interhyoid cartilage"; a disc of cartilage located between the paired hypohyals has a posterior ossification of varying forms: (0) ossification absent or only a minute ossification present (if present, its length much less than the diameter of the cartilage disc with which it associates, (1) large ossification present, its length nearly equal to the diameter of the cartilage disc (Fig. 1H).

Table 3. Distribution of states of 24 morphological characters, as described in text, among species of *Gobiodon* and five gobioid outgroup taxa.

	Characters																							
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Outgroup taxa																								
Dormitator maculatus	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Eleotris amblyopsis	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctenogobius shufeldti	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Paragobiodon echinocephalus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1
Paragobiodon xanthosomus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1
Ingroup taxa																								
Gobiodon sp. A	1	1	1	2	1	0	0	1	2	0	1	1	0	0	0	1	0	0	1	0	0	0	1	1
Gobiodon sp. B	1	1	1	2	1	0	0	1	0	1	1	1	0	0	0	1	0	1	1	0	0	0	1	1
Gobiodon sp. C	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1
Gobiodon sp. D	0	0	1	1	0	0	0	2	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1
Gobiodon acicularis	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1
Gobiodon axillaris	0	1	1	1	1	0	0	1	1	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1
Gobiodon brochus	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	0	1	1	0	1	1	1	1
Gobiodon ceramensis	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1
Gobiodon citrinus	0	0	0	2	0	0	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1
Gobiodon erythrospilus	1	1	1	2	1	0	0	1	2	0	1	1	0	0	0	1	0	0	1	0	0	0	1	1
Gobiodon histrio	1	1	1	1	1	0	0	1	2	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1
Gobiodon okinawae	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1
Gobiodon quinquestrigatus	0	0	1	1	0	0	0	2	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1
Gobiodon rivulatus	0	0	1	1	0	0	0	2	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1
Gobiodon unicolor	1	1	1	1	1	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1

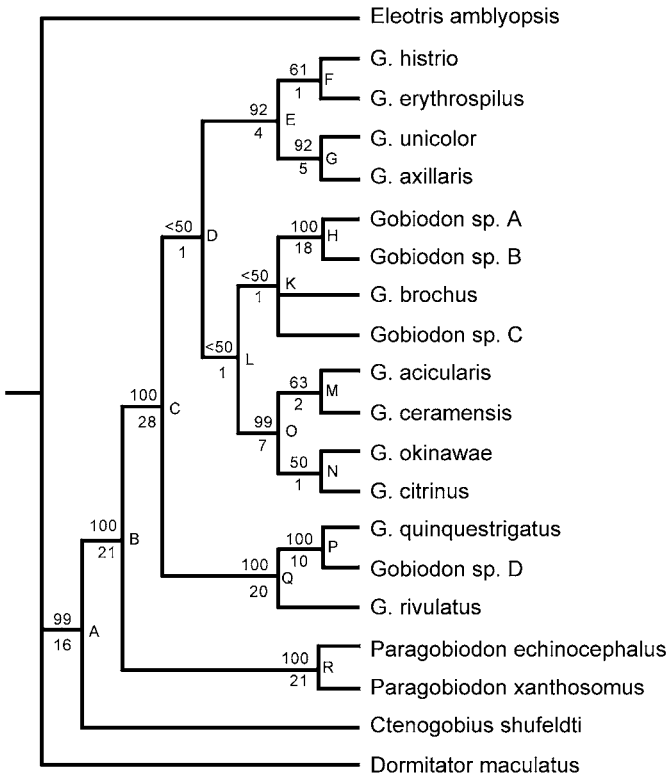

Figure 3. Phylogenetic analysis of molecular data: Strict consensus tree of two equally parsimonious trees based on portions of 12S and 16S rRNA mitochondrial genes (999 base pairs total, including gaps). Bootstrap support values, based on 1000 replicates and heuristic branch swapping, are given above the branch at each node; decay values are below the branch. See text for descriptive statistics and other data.

Character 13. Narrow, facial vertical pigment bars consisting of light pigment bordered by dark lines: (0) absent, (1) present (e.g., *G. citrinus* and *G. quinquestrigatus*, figs. 4 and 9, respectively, in Munday et al., 1999).

Character 14. Number of narrow, facial vertical pigment bars (light pigment bordered by dark): (0) fewer than five facial pigment bars (e.g., *G. citrinus*, fig. 4 in Munday et al., 1999), (1) five or more facial pigment bars (e.g., *G. quinquestrigatus*, fig. 9 in Munday et al., 1999).

Character 15. Interorbital reticulate pigmentation pattern on dorsal surface of head, between eyes: (0) absent (pigmentation uniform between eyes), (1) reticulate pattern present.

Character 16. Broad, diffuse vertical brown to orange pigment bars on head: (0) absent, (1) present on lateral surfaces of head; bars occasionally present as series of broad spots in a linear arrangement (e.g., *Gobiodon* sp. A *and Gobiodon* sp. B, figs. 15 and 16, respectively, in Munday et al., 1999). or as diffuse, oblique bars behind the eye (e.g., *G. brochus*, fig. 14 in Munday et al., 1999).

Character 17. Dark pigment spot on dorsal portion of opercular membrane: (0) absent, (1) present (e.g., *G. histrio*, fig. 5, in Munday et al., 1999).

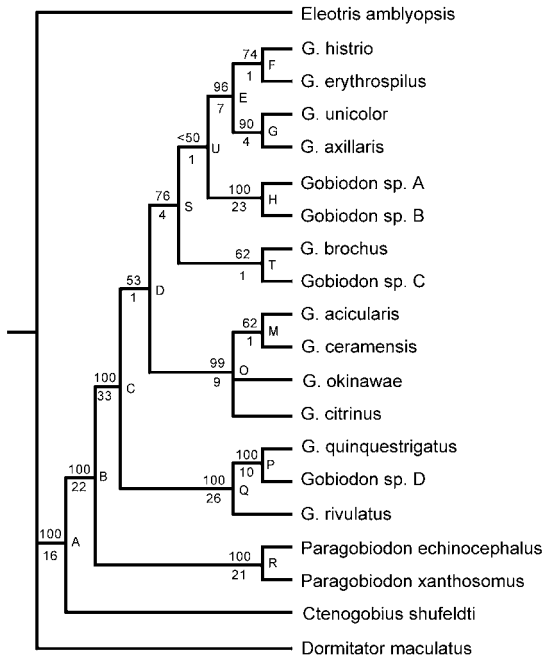

Figure 4. Phylogenetic analysis of morphological and molecular data in a total evidence analysis: strict consensus tree of two equally parsimonious trees based on analysis of 999 molecular and 26 morphological characters. Bootstrap support values, based on 1000 replicates and heuristic branch swapping, are given above the branch at each node; decay values are below the branch. See text for descriptive statistics and other data.

Character 18. Length (height) of cheek papillae: (0) short, maximally as long as wide, (1) elongate, longer than wide.

Character 19. Body shape: (0) fusiform to slightly compressed (e.g., *G. acicularis* and *G. ceramensis*, figs. 1 and 3, respectively, in Munday et al., 1999), (1) deep and highly compressed (e.g., *G. histrio*, fig. 5, in Munday et al., 1999).

Character 20. Interhyal shape: (0) basally expanded, triangular (Fig. 1I), (1) dorsal and ventral extremities of approximately equal width (Fig. 1J).

Character 21. Dentigerous pad anterior to dentary symphysis: (0) absent, (1) present (e.g., *G. brochus*, figs. 4 and 5, in Harold and Winterbottom, 1999).

Character 22. Diffuse pigment bars posterodorsally of eye: (0) absent, (1) present (e.g., *G. brochus*, fig. 14 in Munday et al., 1999).

Character 23. Configuration of pelvic fins: (0) pelvic fins separate, not united by membrane, (1) pelvic fins united by membrane.

Character 24. Coral-commensal life habit, with thickened epidermal mucus layer: (0) absent, (1) present.

The phylogenetic analysis of the combined morphological and molecular matrix resulted in two equally parsimonious trees with a length of 807 steps, CI, excluding uninformative characters, = 0.612, and RI = 0.703. Of the 1024 total characters 626 were constant, 128 were parsimony-uninformative, and 270 were parsimony-informative. The strict consensus tree (Fig. 4) is fully resolved, except the trichotomy at

node O. Bootstrap support values range from < 50% (node F) to 100%, decay values range from 1 to 33.

Inclusion of the morphological characters in this combined analysis has various effects in comparison to the trees derived exclusively from molecular data (Fig. 4 compared with Fig. 3). Most nodes remain topologically unchanged. The principal differences relate to new resolutions for several of the nodes that were resolved, although weakly-supported, in the molecular analysis. Those nodes in the molecular analysis (Fig. 3) had bootstrap values < 50% and decay values of 1 (in particular, nodes D, K, and L). In the total evidence tree (Fig. 4) the node H species (*Gobiodon* sp. A and sp. B) are the sister group of the node E quartet (*G. histrio*, *G. erythrospilus*, *G. unicolor*, and *G. axillaris*), albeit with weak support at node U (< 50%: 1). Importantly, node T (Fig. 4) comprising *G. brochus* plus *Gobiodon* sp. C resolves (62%: 1) and forms the sister group (node S) of all of the other deep-bodied, compressed species (76%: 4). In this topology the quartet of morphologically generalized species at node O in both trees (99%: 9 in total evidence analysis) resolve as the sister group (node D) of the entire clade of deep-bodied, compressed species. The weakly supported (50%: 1), resolved node N (*G. citrinus* plus *G. okinawae*) in the molecular analysis (Fig. 3) has collapsed at node O in the combined analysis (Fig. 4).

Discussion

Of the 999 total characters in the molecular analysis, 246 were parsimony informative, 625 were constant, and 128 parsimony uninformative, which means a substantial proportion of sites (753 out of 999 sites) are not available for resolving phylogenetic branching. Extensive areas of the 12S and 16S fragments that were sequenced are apparently conserved across these taxa. Rates of change in 12S and 16S may be relatively low compared with other mitochondrial regions (e.g., cyt b), and therefore frequently avoided for interspecific analysis. Nevertheless, we have found significant interspecific variation and applied it to this phylogenetic problem. Similarly, a number of studies (e.g., Miya and Nishida, 1996; Banford et al., 2002) have also used sequences of the 12S and 16S regions either on their own or in combination with other regions in effectively resolving genealogy at the interspecific level.

The overall pattern of support within the ingroup *Gobiodon* shows robustness towards the tips and weakness towards the base, although the total evidence analysis is affected by the problem to a lesser degree than the molecular analysis on its own. Incorporation of sequence data from other mitochondrial genes or from nuclear genes, and inclusion of more morphological characters, may help to rectify the problem in later analyses. The trees presented here are proposed as working hypotheses of genealogical relationships among the *Gobiodon* species and will be tested in the future.

In comparing the resultant trees from the two analyses it looks like the total evidence trees pose two important advantages over the molecular trees: (1) number of nodes with weak support (measured according to those ≤ 50% bootstrap values) is four for the molecular analysis but two for the total evidence analysis (taking into account the collapse of node N), and (2) the compressed, deep-bodied species form a paraphyletic group in the molecular analysis with the morphologically generalized, species of node O (e.g., *G. acicularis*) nested within them, but are monophyletic in the total evidence trees (node S). For these reasons, we prefer the total evidence trees as representative of the available data.

Monophyly of *Gobiodon* is strongly supported by our analyses, as reported for the combined analysis of 100% bootstrap and decay value 33 (node C, Fig. 4). This is an expected result based on the unusual morphology of these species, most of them sharing a relatively deep body in comparison to other gobies, and with minor exception (Suzuki et al., 1995), a complete lack of scales. Support values from the total evidence analysis are cited throughout this section, except where stated otherwise. There is some evidence that *Paragobiodon* may also be monophyletic (100%: 21), although we have included only two of the contained species, *P. echinocephalus* and *P. xanthosomus*, in this analysis. According to node B of our analysis, and mainly molecular character evidence, *Paragobiodon* is the likely sister group of *Gobiodon* (100%: 22). The two genera are unusual among gobiids, with their thickened epidermal mucus layers, and associated coral-commensal life habits. Our morphological analysis is too cursory in terms of numbers of characters and taxonomic coverage to discover robust synapomorphies among the genera.

Among other well-supported results from our analysis is the resolution of a clade at node E (96%: 7) comprising *G. histrio*, *G. erythrospilus*, *G. unicolor*, and *G. axillaris*, and another at node O (99%: 9) comprising *G. acicularis*, *G. ceramensis*, *G. okinawae*, and *G. citrinus*. The latter clade makes morphological sense, with these somewhat similarly-shaped species all having nearly uniform villiform jaw teeth (character 7, state 1). On the other hand, the node E clade is a little more complicated. *Gobiodon histrio* and *G. erythrospilus* have long been considered closely related, and in some cases, *G. erythrospilus* has been placed as a junior synonym of *G. histrio* (e.g., Munday et al., 1999; Randall, 2005). Others have considered them morphologically similar but distinct species (Suzuki et al., 1995; Harold and Winterbottom, 1999) on the basis, for example, of the presence of an opercular spot only in *G. histrio*, and presence of nearly continuous orange stripes on the flank of *G. histrio* as contrasted by rows of orange dots in *G. erythrospilus* (Munday et al., 1999: figs. 5 and 6; Munday et al., 2004: figs. 1D,E). In addition to these similarities, the two species have about 2% genetic distance ($p = 0.020$), which is probably more consistent with species-level recognition as opposed to conspecific populations, although such interpretation is admittedly controversial. Our recognition of these two species as sister taxa conflicts with Munday et al. (2004), who used D-loop sequences in maximum likelihood and Bayesian analyses to show these two species to be sequential, basal taxa among a total of five *Gobiodon* species analyzed: their topology in tree notation is (*histrio* (*erythrospilus* (*brochus* (sp. A, sp. B)))). Other *Gobiodon* species pairs clearly distinguishable on morphological grounds have genetic distances of about 2% (e.g., *G. ceramensis* vs *G. acicularis*: $p = 0.019$). *Gobiodon* sp. A and *Gobiodon* sp. B are also well-supported as sister taxa (100%: 23), a result consistent with Munday et al. (2004), although their analysis included only five representatives of the genus and may be affected by low taxon sampling. Our results also agree with Munday et al. (2004) in recognizing the form "*Gobiodon* sp." (Suzuki et al., 1995), here referred to *Gobiodon* sp. A, and topologically separable from *G. histrio* and *G. erythrospilus* based on our molecular and total evidence analyses. Our analysis also reveals that *Gobiodon* sp. A has a much greater genetic distance from *G. histrio* ($p = 0.073$) than *G. histrio* and *G. erythrospilus* do from each other ($p = 0.020$). *Gobiodon* sp. A is, however, genetically similar to *G. erythrospilus* ($p = 0.020$), although with a p value similar to that of other species considered distinct here.

Both *G. histrio* and *G. erythrospilus* have an interopercular-isthmus groove (character 1, state 1) as does *G. unicolor*, but the fourth species in the node E clade, *G. axillaris*, does not. The lack of the feature is probably not plesiomorphic, but a derived and reductive feature. However, *G. axillaris* has a deep and rather compressed body form (character 20, state 1), a likely major derived feature shared with the other clade members, especially *G. histrio* and *G. erythrospilus*, although the feature is probably derived at a phylogenetic level slightly below that of node E, most likely at node S (Fig. 4). Elsewhere on the tree the groove and compressed body morphology is found in *Gobiodon* sp. A, *Gobiodon* sp. B, *Gobiodon* sp. C, and *G. brochus*. The relationships of those species to each other and to the two well supported clades at nodes E and O are partially resolved according the molecular analysis, with a trichotomy at node K (< 50%: 1), but fully resolved in the total evidence trees (sequential nodes S and U). We find it surprising that two species, *G. brochus* and *Gobiodon* sp. C, which resolve as sister species in total evidence analysis (Fig. 4, node T) and have a shared and very unusual morphological specialization (Character 22, state 1: presence of a dentigerous pad anterior to dentary symphysis), are not resolved as sister species in the molecular-based analysis.

To conclude the phylogenetic interpretations, we find that the molecular and morphological data were in some cases in conflict, but largely complementary, leading to a well-resolved consensus tree for *Gobiodon* species. In both analyses, there are weakly supported nodes but the topologies of the total evidence trees had, overall, more support than the molecular analysis. Low support values for some of the nodes in the molecular-based tree, particularly at node K at which the relationships of *Gobiodon* sp. A, *Gobiodon* sp. B, *Gobiodon* sp. C, and *G. brochus* are partially unresolved, may be due to the following factors: insufficient numbers of character state transformations at that level, saturation of sites giving high levels of character conflict (homoplasy), and/or an ancestral hybridization event.

Based on our analyses, some initial biogeographical interpretations can be made. Both analyses support *Gobiodon* sp. A and sp. B as sister species. They are morphologically distinct (Munday et al., 1999, 2004), yet have a rather low genetic distance (p = 0.014). Munday et al. (2004) proposed that *Gobiodon* sp. B, endemic on the southern coast of Papua-New Guinea, may be derived from sp. A, a broadly distributed western Pacific species (Japanese archipelago to the Australian Great Barrier Reef), through sympatric speciation. Another, but less likely explanation, is the possibility that these two species could have split from a common ancestor in allopatry through vicariance during times of cooler climate and modified ancestral distribution patterns, although distinguishing between these two hypotheses may prove to be difficult.

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Appendix

Comparative material examined for morphological characters. One specimen cleared and stained from each lot unless otherwise indicated in parentheses.

Ctenogobius shufeldti GMBL 93-004 uncat.; *Dormitator maculatus* GMBL 01-001 uncat.; *Eleotris amblyopsis* GMBL 75-199 uncat.; *Gobiodon* species (sp. A) ROM 68986, GMBL 4481CS; *Gobiodon* sp. B GMBL PM090398 uncat.; *Gobiodon* sp. C GMBL 4515; *Gobiodon* sp. D GMBL 4495 (2); *Gobiodon* DFH sp. 11 ROM 1485CS (4); *Gobiodon* RW sp. 1 ROM 1483CS (2); *Gobiodon* RW sp. 2 1484CS (2); *Gobiodon* RW sp. 4 ROM 1516CS (2); *G. acicularis* CAS 81515, CAS 81516 (5), ROM 1603CS (2); *G. albofasciatus* ROM 1604CS; *G. axillaris* ROM 1522CS; *G. brochus* GMBL ROM 1482CS, 1609CS (2); *G. ceramensis* ROM 1608CS (2); *G. citrinus* CAS 58552 (3), ROM 1479CS, USNM 324028; *G. erythrospilus* ROM 68119; *G. fulvus* ROM 509CS, 1613CS, 37079; *G. histrio* ROM 1477CS (2); *G. micropus* ROM 1480CS; *G. okinawae* ROM 1521CS; *G. prolixus* USNM 223236; *G. quinquestrigatus* ROM 1478CS (2), 1492CS (2), 1493CS, 1494CS (2), 1515CS, 1600CS; *G. reticulatus* USNM 334750 (5); *G. rivulatus* ROM 577CS (10), 1476CS (2); *G.* nr *unicolor* ROM 1615CS, 1616CS; *G. unicolor* USNM 260493; *Paragobiodon echinocephalus* GMBL 4444; *P. melanosomus* ROM 1495CS, USNM 245254 (5); *P. modestus* ROM 665CS, 1496CS; *P. xanthosomus* ROM 1481CS, 45458.