

Table 2
Species sequenced for this study

Species	Source	GenBank Accession Nos.
Odontobutidae		
<i>Odontobutis obscura</i>	Akihisa Iwata, Japan	AF391330, AF391402, AF391474
Eleotridae: Eleotrinae		
<i>Eleotris sandwicensis</i>	Small bottom trap, stream, North Oahu, Hawaii	AF391333-4, AF391405-6, AF391477-8
<i>Erotelis smaragdus</i>	Bottom tow, Twin Cays, Belize	AF391355, AF391427, AF391499
<i>Hypseleotris aurea</i>	Peter Unmack, Gascoyne River, WA, Australia	AF391392, AF391464, AF391536
<i>Hypseleotris compressa</i>	Peter Unmack, Ross River, Qld., Australia	AF391366, AF391438, AF391510
<i>Hypseleotris klunzingeri</i>	Peter Unmack, Barcoo River, Qld., Australia	AF391393, AF391465, AF391537
<i>Mogurnda adspersa</i>	Peter Unmack, Ross River, Qld., Australia	AF391367, AF391439, AF391511
<i>Ophieleotris aporos</i>	Peter Unmack, Ross River, Qld., Australia	AF391368, AF391440, AF391512
<i>Philypnodon grandiceps</i>	Peter Unmack, Glenelg River, Vic., Australia	AF391386, AF391458, AF391530
Xenisthmidae		
<i>Xenisthmus</i> sp.	Mark Westneat, Santa Cruz Island, Solomon Islands	AF391372, AF391444, AF391516
Gobiidae: Gobionellinae		
<i>Acanthogobius flavimanus</i>	Scott Matern, Sacramento River Delta	AF391381, AF391453, AF391525
<i>Awaous guamensis</i>	Brent Tibbats, Guam	AF391338, AF391410, AF391482
<i>Chaenogobius annularis</i>	Ho Young Suk, Korea	AF391365, AF391437, AF391509
<i>Ctenogobius saepepallens</i>	Plankton tow, Carrie Bow Cay, Belize	AY077595-6, AY077602-3, AY077609-10
<i>Eucyclogobius newberryi</i>	CAS 86280; San Gregorio Creek, California	AF391361, AF391433, AF391505
<i>Evorthodus minutus</i>	Jim Van Tassell, Mazatlan, Mexico	AY077593, AY077600, AY077607
<i>Gillichthys mirabilis</i>	Nancy Aguilar, California	AF391340, AF391412, AF391484
<i>Gnatholepis cauerensis</i>	Quinaldine, Moorea, Society Islands	AF391364 & 75, AF391436 & 47, AF391508 & 19
<i>Gnatholepis scapulostigma</i>	Quinaldine, Moorea, Society Islands	AF391376, AF391448, AF391520
<i>Gnatholepis thompsoni</i>	Quinaldine, Carrie Bow Cay, Belize	AF391343-4, AF391415-6, AF391487-8, AY077594, AY077601, AY077608
<i>Gobiopterus semivestita</i>	Peter Unmack, Milingandi Creek, NSW, Australia	AF391387, AF391459, AF391531
<i>Mugilogobius</i> sp.	Brent Tibbats, Guam	AF391356, AF391428, AF391500
<i>Mugilogobius rivulus</i>	Peter Unmack, Leaders Creek, NT, Australia	AY077592, AY077599, AY077606
<i>Pandaka lidwilli</i>	Tony Gill, Innes Park Creek, Qld., Australia	AY077590-1, AY077597-8, AY077604-5
<i>Stenogobius hawaiiensis</i>	Brent Tibbats, Guam	AF391349, AF391421, AF391493
<i>Typhlogobius californiensis</i>	Nancy Aguilar, California	AF391345, AF391417, AF391489
Gobiidae: Gobiinae		
<i>Amblyeleotris wheeleri</i>	Quinaldine, Moorea, Society Islands	AF391383, AF391455, AF391527
<i>Amblygobius nocturnus</i>	Quinaldine, Moorea, Society Islands	AF391379, AF391451, AF391523
<i>Amblygobius phalaena</i>	Quinaldine, Moorea, Society Islands	AF391369 & 78, AF391441 & 50, AF391513 & 22
<i>Asterropteryx semipunctatus</i>	Quinaldine, Moorea, Society Islands	AF391377, AF391449, AF391521
<i>Barbulifer ceuthoecus</i>	Quinaldine, Carrie Bow Cay, Belize	AF391353, AF391425, AF391497
<i>Bathygobius cocosensis</i>	Quinaldine, Rangiroa, Tuamotu Atolls	AF391388, AF391460, AF391532
<i>Bathygobius curacao</i>	Quinaldine, Pelican Cays, Belize	AF391354, AF391426, AF391498
<i>Cabillus tongarevae</i>	Quinaldine, Moorea, Society Islands	AF391382, AF391454, AF391526
<i>Callogobius sclateri</i>	Quinaldine, Moorea, Society Islands	AF391390, AF391462, AF391534
<i>Coryphopterus dicrus</i>	Kathleen Cole, Carrie Bow Cay, Belize	AF391395, AF391467, AF391539
<i>Coryphopterus hyalinus</i>	Kathleen Cole, Carrie Bow Cay, Belize	AF391326, AF391398, AF391470
<i>Coryphopterus personatus</i>	Kathleen Cole, Carrie Bow Cay, Belize	AF391325, AF391397, AF391469
<i>Coryphopterus punctipectophorus</i>	Kathleen Cole, Carrie Bow Cay, Belize	AF391396, AF391468, AF391540
<i>Ctenogobius feroculus</i>	Quinaldine, Moorea, Society Islands	AF391363, AF391435, AF391507
<i>Eviota afelei</i>	Quinaldine, Moorea, Society Islands	AF391391, AF391463, AF391535
<i>Fusigobius neophytus</i>	Quinaldine, Moorea, Society Islands	AF391374, AF391446, AF391518
<i>Fusigobius signipinnis</i>	Mark Westneat, Santa Cruz Island, Solomon Islands	AF391370, AF391442, AF391514
<i>Gobiodon histrio</i>	Rob Reavis, Captive stock	AF391360, AF391432, AF391504
<i>Gobiosoma macrodon</i>	Colette St. Mary, Florida	AF391348, AF391420, AF391492
<i>Lophogobius cyprinoides</i>	Kathleen Cole, Florida	AF391362, AF391434, AF391506
<i>Priolepis cincta</i>	Quinaldine, Moorea, Society Islands	AF391385, AF391457, AF391529
<i>Priolepis eugenius</i>	David Greenfield, Hawaii	AF391329, AF391401, AF391473
<i>Risor ruber</i>	Colette St. Mary, Florida	AF391351-2, AF391423-4, AF391495-6
<i>Valenciennea strigata</i>	Quinaldine, Moorea, Society Islands	AF391384, AF391456, AF391528

Table 2 (continued)

Species	Source	GenBank Accession Nos.
Gobiidae: Oxudercinae		
<i>Periophthalmus barbarus</i>	Nancy Aguilar, Nigeria	AF391339, AF391411, AF391483
<i>Pseudapocryptes elongatus</i>	CAS 90433, Yangon Fish Market, Myanmar	AF391394, AF391466, AF391538
<i>Scartelaos histophorus</i>	Nancy Aguilar, Australia	AF391346, AF391418, AF391490
Gobiidae: Amblyopinae		
<i>Odontamblyopus rubicundus</i>	CAS 90432, Yangon Fish Market, Myanmar	AF391371, AF391443, AF391515
Gobiidae: Sicydiinae		
<i>Sicyopterus lagocephalus</i>	Quinaldine, stream, Moorea, Society Islands	AF391389, AF391461, AF391533
<i>Stiphodon elegans</i>	Brent Tibbats, Guam	AF391350, AF391422, AF391494
Microdesmidae		
<i>Cerdale floridana</i>	Plankton tow, Carrie Bow Cay, Belize	AF391337, AF391409, AF391481
<i>Gunnellichthys monostigma</i>	Yuji Ikeda, Japan	AF391373, AF391445, AF391517
<i>Microdesmus bahianus</i>	Plankton tow, Carrie Bow Cay, Belize	AF391347, AF391419, AF391491
<i>Microdesmus longipinnis</i>	Richard Heard, Gulf Coast of Mississippi	AF391341-2, AF391413-4, AF391485-6
Ptereleotridae		
<i>Nemateleotris magnifica</i>	Aquarium supplier	AF391327-8, AF391399-1400, AF391471-2
<i>Ptereleotris microlepis</i>	Quinaldine, Moorea, Society Islands	AF391380, AF391452, AF391524
<i>Ptereleotris monoptera</i>	Aquarium supplier	AF391357, AF391429, AF391501
<i>Ptereleotris zebra</i>	Aquarium supplier	AF391358-9, AF391430-1, AF391502-3
Kraemeriidae		
<i>Kraemeria cucicularia</i>	Akihisa Iwata, Japan	AF391331-2, AF391403-4, AF391475-6
Schindleriidae		
<i>Schindleria pietschmanni</i>	Plankton tow, Kaneohe Bay, Oahu	AF391335, AF391407, AF391479
<i>Schindleria praematura</i>	Plankton tow, Palmyra Atoll, Line Islands	AF391336, AF391408, AF391480

Unless otherwise indicated, tissues were collected by the author and where known the collection method is indicated. CAS indicates the specimen was from the tissue collection of the California Academy of Sciences, San Francisco; other species are uncataloged holdings of the Natural History Museum of Los Angeles County. Species are grouped by family and subfamily, and separate GenBank accession numbers are given for each gene.

seconds of extension added at each step. These long (~2500 bp) fragments were quantified on a 1.5% low melting point agarose gel stained with ethidium bromide, bands were visualized and photographed under UV light, cut from the gel and DNA purified from the bands using the QIAquick gel extraction kit (Qiagen, Chatsworth, CA). The long PCR fragments were used as template for four shorter PCR reactions using the primer pairs: L3827/H4644; L4500/H5191; L5219/H5766; and L5758/H6313 (Sorenson et al., 1999). These amplifications were performed with AmpliTaq or AmpliTaq Gold DNA polymerase (Perkin–Elmer, Foster City, CA). PCR was performed with a profile of 94 °C for 3 min, followed by 35 cycles of 94 °C/15 s denaturation, 50–55 °C/20 s annealing and 70 °C/1 min extension.

In other cases, particularly amplifications of the COI gene, PCR reactions were performed directly from genomic DNA with the goby-specific primers listed in Table 3, using the enzymes and PCR profile given above. PCR products were run out on a low melting point agarose gel, visualized and photographed, then cut out and purified with the QIAquick kit. Using the same primers (1 μM rather than 10 μM solution) the short PCR fragments were cycle sequenced using rhodamine dye terminator/Taq FS or Big Dye terminator ready reaction kits (Perkin–Elmer, Foster City, CA) and run on an ABI 377XL automated sequencer. Both the heavy

and light strands were sequenced separately for each short PCR fragment. The resultant chromatograms for the heavy and light strands were reconciled in Sequence Navigator (Perkin–Elmer, Foster City, CA), or Sequencher (Gene Codes, Ann Arbor, MI) to check basecalling, translated to amino acid sequence using the universal mtDNA code, and aligned by eye. There were no ambiguities or gaps in the alignment; all the gaps present in the final matrix were due to missing data and

Table 3
Goby-specific primers used for amplification of ND1, ND2, and COI genes

Primer	Sequence
GOBYL3543	GCAATCCAGGTCAGTTTCTATC
GOBYH4389	AAGGGGGCYCGGTTTGTTTC
GOBYL4201	GTTGCMCAAACMATTTTCHTATGAAG
GOBYH4937	GGGGTATGGGCCCGAAAGC
GOBYL4919	CCCATACCCGAAAATGATG
GOBYH5513	GAGTAGGCTAGGATTTTWCGAAGYTG
GOBYL5464	GGTTGAGGRGGCTMAACCARAC
GOBYH6064	CTCCTACTAGAGCTTTGAAGGC
GOBYL6468	GCTCAGCCATTTTACCTGTG
GOBYH7127	ACYTCTGGGTGACCAAAGAATC
GOBYL7059	CCCTGCMGGTGGAGGAGACCC
GOBYH7696	AGGCCTAGGAAGTGTGAGGGGAAG
GOBYL7558	TTTGCWATTATGGCWGGATTGT
GOBYH8197	ATTATTAGGGCGTGGTCTGTG

All primers are given in the 5'–3' direction.