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Phylogeny and species boundaries in the gobiid genus *Gnatholepis* (Teleostei: Perciformes)

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Species of the goby genus *Gnatholepis* Bleeker, 1874, are common inhabitants of shallow tropical seas worldwide. In this study, mitochondrial DNA sequence (ND2 gene), from 349 *Gnatholepis* individuals sampled from across the South and Central Pacific and Caribbean, is used to infer phylogeny and determine species boundaries. Seven species of *Gnatholepis* are recognized: the Indo-Pacific *G. anjerensis* (Bleeker, 1851) [*G. cauerensis* (Bleeker, 1853) is a synonym]; *G. scapulostigma* Herre, 1953; *G. davaoensis* Seale, 1910; *G. knighti* Jordan & Evermann, 1903; *G. gymnocara* Randall & Greenfield, 2001; *G.* sp. Randall & Greenfield, 2001; and the Atlantic/Caribbean *G. thompsoni* Jordan, 1904. Results from the molecular phylogeny are compared with a previous morphology-based revision of the genus in order to establish which morphological characters diagnose species in correspondence with the molecular phylogeny.

ADDITIONAL KEYWORDS: Caribbean – Gobiidae – Hawaii – Indo-Pacific – phylogeography – species concepts.

INTRODUCTION

Species of the gobiid fish genus *Gnatholepis* are common benthic inhabitants of nearshore coral reef habitats throughout the tropical Pacific and western Atlantic. *Gnatholepis* individuals are usually small [adult size 25–40 mm standard length (SL)], pale with dark mottling and markings, and generally inconspicuous in their habitat. *Gnatholepis* is classified in the gobiid subfamily Gobionellinae and is unusual among gobionellines in that it inhabits marine reef environments; most gobionellines are found in fresh or brackish water. *Gnatholepis* is characterized by dorsal-fin counts of VI + I, 10–12, anal-fin counts of I, 11–12, pectoral fin ray counts of 14–19, a fused ventral pelvic disc, and a lower lip with ventral flaps on each side, giving them a distinctive, ventrally protuberant snout in anterior view. *Gnatholepis* shares with other gobionellines the presence of paired interorbital pores and a dorsal pterygiophore interdigitation formula of 3–12210 (Birdsong, Murdy & Pezold, 1988; Pezold, 1993; Randall & Greenfield, 2001). In a recent preliminary review of Indo-Pacific species, Randall & Greenfield (2001) recognized five species, one with four subspecies (Table 1), but indicated that due to the plasticity in individual coloration and lack of meristic variation in *Gnatholepis* morphology, molecular data would be required for a definitive assessment of species number and identity.

This study seeks to determine the relationships and species boundaries within *Gnatholepis* using DNA sequence data, with comparison to morphological characters. The species *G. thompsoni*, *G. scapulostigma*, *G. anjerensis*, *G. davaoensis* and *G.* sp. were sequenced; *G. gymnocara* was not sequenced but was examined for morphology. All of the above listed species are found in the Pacific and/or Indian Oceans with the exception of *G. thompsoni*, which is known from the Caribbean and Western Atlantic. Dense sampling of individuals was used within the most widespread Indo-Pacific species, *G. scapulostigma* and *G. anjerensis*, in order to quantify species boundaries and evaluate the correspondence of previously described morphological characters with the molecular data. Among Pacific species, different authorities recognize

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Table 1. Character distribution in species of Indo-Pacific *Gnatholepis* according to Randall & Greenfield (2001). Pectoral ray count is given as range (mode)

Species	Interorbital bar	Shoulder spot	Pectoral count	Head scales	Geographic range
G. anjerensis	Incomplete, at rear of pupil	Present	14–17 (16)	Present	Red Sea to Hawaiian & Society Is.
G. cauerensis					
ssp. *cauerensis*	Present, at pupil centre	Variably present	16–19 (17)	Present	East Africa to Society Is.
ssp. *australis*	Present, at pupil centre	Absent	17–19 (18)	Present	Rarotonga to Pitcairn
ssp. *hawaiiensis*	Present, at pupil centre	Present	16–19 (17)	Present	Hawaiian Is.
ssp. *pascuensis*	Present, at pupil centre	Present	18–19 (19)	Present	Easter Is.
G. davaoensis	Incomplete, at rear of pupil	Absent	15–17 (17)	Present	Ryukyus to Solomon Is.
G. gymnocara	Unspecified	Absent	15–18 (17)	Absent	Northern Australia
G. sp.	Unspecified	Absent	15–18 (16–17)	Absent	Northern Australia

various species and synonymies. Traditionally, the morphological character used to distinguish *G. scapulostigma* from other species is the presence of a spot on the shoulder, just dorsal to the pectoral fin base, either entirely black or in the form of a black ring with a pale centre. *Gnatholepis scapulostigma* possesses the spot, and *G. cauerensis* lacks it (Lieske & Myers, 1999; Akihito *et al*., 2002). However, Myers (1999) indicated that both *G. scapulostigma* and *G. cauerensis* possessed the shoulder spot, and only *G. anjerensis* lacked it. Allen (1997) included only *G. scapulostigma* and indicated that it possesses the shoulder spot. Randall (1983, 1995) regarded *G. anjerensis* and *G. cauerensis* (both without spot) as synonyms, and Randall, Allen & Steene (1997) indicated that *G. scapulostigma* possesses the spot, and that *G. inconsequens* is a junior synonym. Hoese (1986) treated the South African *Gnatholepis* species simply as *G.* sp. 1 and *G*. sp. 2, distinguished by the counts of pectoral fin rays, number of scales in longitudinal series, and the anterior extent of cheek scalation.

In the preliminary review of Randall & Greenfield (2001), five species were treated: *G. anjerensis*, *G. cauerensis* (with four subspecies), *G. davaoensis*, and the new species *G. gymnocara* and *G*. sp. Randall & Greenfield (2001) considered *G. anjerensis* and *G. cauerensis* to be distinct species, and *G. scapulostigma* and *G. inconsequens* Whitley, 1958, to be synonyms of *G. cauerensis*. Randall & Greenfield (2001) did not use the shoulder spot as a species-diagnostic character; instead, they used the modal pectoral fin ray count (modal count is 16 in their *G. anjerensis* vs. 17, 18 or 19 for their subspecies of *G. cauerensis*, but ranges of counts overlap) and the extent and position of a stripe of pigment dorsal to and between the eyes. Under their species categories, *G. anjerensis* has an incomplete interorbital bar positioned slightly posterior of the centre of the pupils. *Gnatholepis anjerensis* was described as having the shoulder spot, although the spot is not present in the illustration of the neotype, and is variably present or absent in other examples of *G. anjerensis* (Randall & Greenfield, 2001: plate 1). The spot is also variably present or absent in their four subspecies of *G. cauerensis*; their diagnostic character for this species is the presence of a complete interorbital pigment bar positioned directly dorsal to the pupils of the eyes. Randall & Greenfield (2001) additionally indicated that the Atlantic *G. thompsoni* could not be distinguished morphologically from Pacific *G. cauerensis*, but refrained from synonymizing the two pending a comparison of their DNA. Winterbottom & Emery (1986) also indicated that they could not separate *G. thompsoni* from their *Gnatholepis* material from the Chagos Archipelago. Most meristic and morphological characters are stable among *Gnatholepis* species; the aim of this study is to use DNA sequence data as another source of information regarding *Gnatholepis* relationships and species boundaries, and then re-evaluate some previously postulated species-diagnostic characters in light of the new phylogeny.

MATERIAL AND METHODS

DNA SEQUENCE

Gnatholepis for DNA analysis were collected using SCUBA, hand nets and quinaldine from 35 sites in the Society Islands, Tuamotu Archipelago, Cook Islands, Fiji and Hawaii, at depths ranging between 1 and 23 m. Figure 1 shows the localities for all the individuals examined for this study, and sites from which samples used in DNA analysis were taken are indicated with arrows. *Gnatholepis* were invariably found

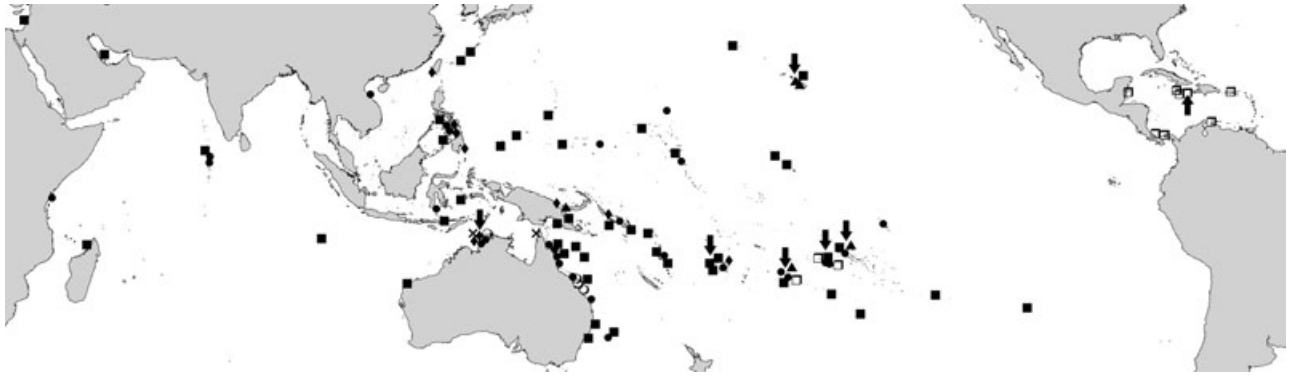

Figure 1. Map of localities for all *Gnatholepis* specimens examined for this study. Sites from which samples used in DNA analysis were taken are indicated with arrows. *Gnatholepis anjerensis* (●); *G. scapulostigma* (■); *G. davaoensis* (◆); *G. knighti* (▲); *G. thompsoni* (□); *G. gymnocara* (○); *G.* sp. (×). Many other locality records for *Gnatholepis* exist, but only specimens for whom identity has been confirmed by examination of morphology and/or DNA sequence are included in this figure.

on sandy or silty substrates, near rocks, coral heads or some other structure in which they shelter when pursued. Two specimens of *G. davaoensis* were provided by David Greenfield (University of Hawaii) from collections in Fiji, and three specimens of *G.* sp. were provided by Helen Larson (Museum and Art Gallery of the Northern Territory) from three sites in the Northern Territory of Australia. Sequence data were also collected from two specimens of *G. thompsoni* obtained from rotenone stations at the Caribbean island of Navassa (vouchers: LACM 54098.016 and LACM 54113.005), and sequences for an additional three specimens of *G. thompsoni* was obtained from GenBank (AF322985, AF391488 and AF391487). DNA sequence data were collected from 349 individuals in total. *Gnatholepis gymnocara* was not sequenced in this study, but that species was examined for morphology. Formalin-fixed vouchers were preserved for each species collected for sequencing (*Gnatholepis anjerensis*: Moorea, French Polynesia, LACM 54117.002; LACM 54118.005; LACM 54119.001; LACM 54124.005; LACM 54125.002; LACM 55960.004; LACM 55960.006; *G. scapulostigma*: Moorea, French Polynesia, LACM 54117.003; LACM 54120.004; LACM 54125.003; LACM 55960.005; LACM 55962.001; Oahu, Hawaii, LACM 55973.001, LACM 55973.002); other specimens were fixed in 90% ethanol. Muscle tissue was used for total genomic DNA extraction, performed with the QIAamp Tissue Kit (Qiagen). PCR was performed using primers GOBYL5464 (5′-GGTTGAGGRGGCCTMAACCARAC-3′) and GOBYH6064 (5′-CTCCTACTTAGAGCTTT GAAGGC-3′; both10 μM, Thacker, 2003) and Gibco Life Technologies Taq DNA polymerase, with a profile of 94 °C for 90 s, followed by 40 cycles of 94 °C/15 s denaturation, 45–50 °C/45 s annealing and 70 °C/60 s extension. These primers amplify a 498 base pair fragment comprising the 3′ half of the mitochondrial NADH dehydrogenase subunit two (ND2) gene. The same primers (1 μM) were used for cycle sequencing with the Big Dye terminator/Taq FS ready reaction kit and run on an ABI 377 automated sequencer (Applied Biosystems). The heavy and light strands were sequenced separately. The resultant chromatograms for the heavy and light strands were reconciled using SEQUENCHER (GeneCodes Corp.) to check basecalling, and aligned with respect to the translated to amino acid sequence using the mammalian mtDNA code. The alignment was trivial because most of the amino acids were conserved; no gaps were inserted in the sequences. Aligned nucleotide sequences were exported as NEXUS files for analysis using the parsimony criterion with PAUP* version 4.0b8 (Swofford, 2001). Due to the size of the data set and the high ratio of terminals to informative characters (246 of 498 characters were parsimony-informative), a heuristic search was performed, and 10 000 most parsimonious trees were saved. A strict consensus of these shortest trees was constructed. The related species, *Awaous guamensis* and *Stenogobius hawaiiensis* (GenBank accession numbers AF391482 and AF391493), were included and designated as outgroups. A previous study (Thacker, 2003) showed that these two genera are closely related to *Gnatholepis*. Decay indices (Bremer, 1988) were calculated with PAUP* and TREEROT v.2 (Sorenson, 1999). DNA sequences for all specimens were deposited in GenBank (accession numbers AF504305–7, AF537620–AF537863 and AY184836–AY184928). Comparisons of depth distributions were performed with the Mann–Whitney *U*-test, as implemented in STATISTICA for Macintosh, version 4.0 (Statsoft Corp.).

MORPHOLOGY

Gnatholepis specimens in the collections of the Australian Museum, the Bishop Museum, the California Academy of Sciences, the United States National Museum of Natural History and the Natural History Museum of Los Angeles County were examined (Fig. 1) for external morphology and coloration, specifically for the presence or absence and position of an interorbital pigment bar, a spot dorsal to the pectoral fin, and other pigmentation characters such as lateral blotches and lines, a horizontal slash on the cheek, and any spots or other pigmentation on the fins. Specimens of all *Gnatholepis* species treated by Randall & Greenfield (2001) were examined for morphology, including all of their holotypes (see Appendix for specimen list). Morphological characters were recorded from specimens of *Gnatholepis* collected from various Pacific and Caribbean localities; these specimens were also used for DNA sequencing.

RESULTS

DNA SEQUENCE

The strict consensus of most parsimonious trees inferred based on DNA sequence data was 1238 steps, with consistency index 0.356, retention index 0.946 and rescaled consistency index 0.337. The 349 *Gnatholepis* sampled from across the Pacific basin and Caribbean fall into several groups (Fig. 2), and Bremer supports for nodes subtending species and deeper nodes are strong. The most basal *Gnatholepis* species is *G.* sp., a shallow, small-bodied species known from Northern Australia. It shares with *G. gymnocara* the lack of scales on the cheek, opercle and the median predorsal region of the nape, as well as the lack of enlarged teeth in the anterior portion of the lower jaw.

The remainder of the individuals examined form three groups. The first consists of the two individuals of *G. davaoensis*, the second of individuals of *G. scapulostigma* and *G. thompsoni* (Clade I in Fig. 2) and the third of *G. anjerensis* individuals (Clade II of Fig. 2). Within the individuals classified as *G. anjerensis* (Clade II) are three groups: one consisting of most individuals and including samples from across the Pacific; a second including some of the individuals collected from Moorea, French Polynesia and Rarotonga, Cook Islands; and a third including all the individuals collected from Hawaii. The relationships of each of these two clades as distinct from the majority of *G. anjerensis* individuals are supported by high Bremer support values, and within each of the three groups, most of the individuals fall into a broad polytomy. The Hawaii individuals were collected in shallow water (8 m or less), in silty/sandy habitat

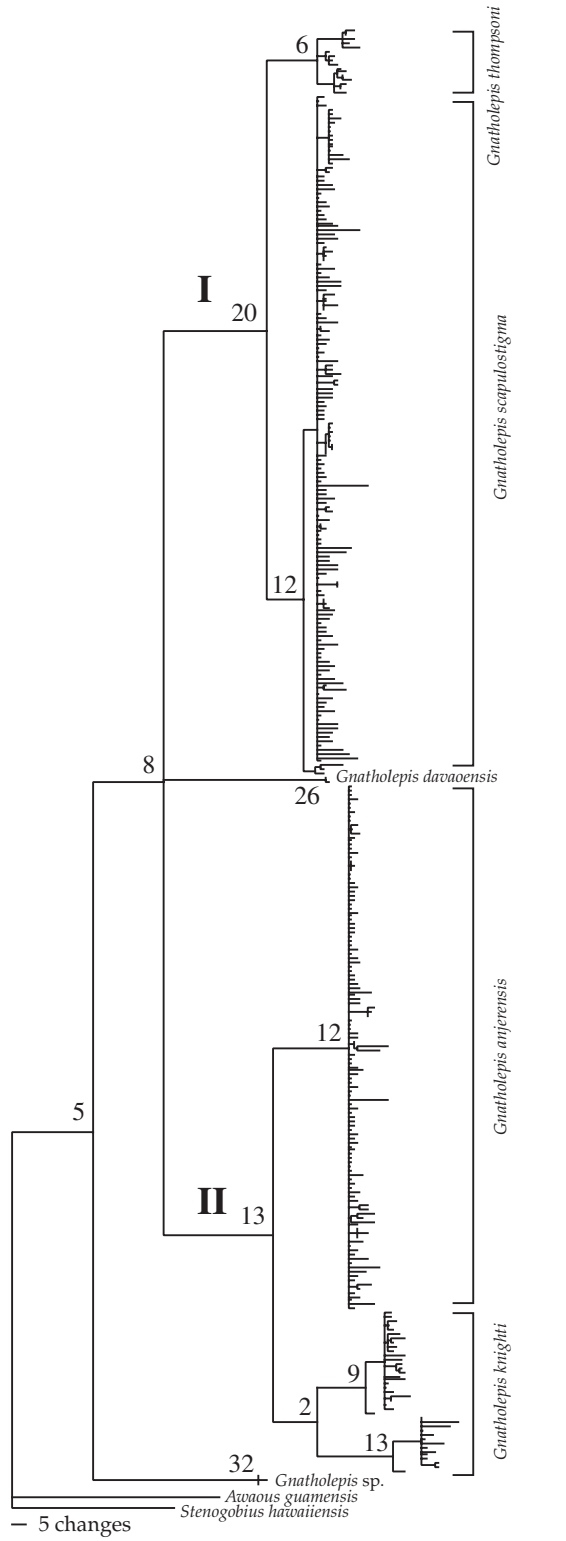

Figure 2. Strict consensus of most parsimonious trees obtained in cladistic analysis of DNA data; numbers at nodes are decay indices. Two large clades (I and II) are present, each of which contains smaller clades that are accorded specific status.

featuring stands of dead coral and algae. Those in the clade sister to the Hawaii individuals occupied a very shallow habitat (less than 2 m), with mixed sand/pavement substrate and also a great deal of rock, dead coral and algae. By the phylogenetic species concept, each of these three clades could be recognized as a distinct species (Mishler & Theriot, 2000; Wheeler & Platnick, 2000). Examination of sequenced specimens for morphological characters that could be used to distinguish the Hawaii individuals from those in their sister clade, however, did not yield any such characters. Therefore, the clade containing Hawaii individuals plus those from Rarotonga and Moorea are treated as a single species, separate from *G. anjerensis*.

The species *G. scapulostigma* and *G. thompsoni* (Clade I), although diagnosable based on DNA data, are incompletely geographically segregated and also lack distinguishing morphological characters. Five of the 16 individuals in the *G. thompsoni* clade were sampled from the Caribbean; the other samples were collected from Rarotonga, Cook Islands, and Bora Bora, Tahaa and Moorea in the Society Islands.

MORPHOLOGY

All specimens used for DNA sequencing, plus others from museum collections, were examined for external morphology. Because of the stability in meristic characters, pigmentation patterns were emphasized as possible species-diagnostic markers. The coloration among *Gnatholepis* individuals was observed to vary widely; variable characteristics included the overall density of mottling, presence or absence of two to six fine lateral stripes, a vertical stripe on the cheek that crosses the suborbital horizontal bar, and a series of five to six large, lateral blotches, decreasing in size from anterior to posterior. The fin pigmentation also varied and included fins that were transparent, lightly mottled with fine pigment spots, densely marked with spots in the form of vertical stripes, or (anal and second dorsal fins only) clear with black distal edges. All of these characters varied continuously among specimens, with no combination unambiguously diagnosing any group. However, some generalizations could be made: *G. anjerensis* may attain a larger maximum size (the largest specimen encountered in this study was 51.1 mm SL; most *G. scapulostigma* range from 20 to 35 mm SL), tend to be more uniformly and darkly mottled than *G. scapulostigma* or *G. thompsoni*, and are more likely to exhibit some degree of spotting or mottling on the fins. Conversely, large lateral blotches and the series of fine lateral pigment lines are more common in *G. scapulostigma* and *G. thompsoni*, and these species are also often paler overall, with clear fins or soft dorsal and anal fins with distal black edges. Pigmentation characters were useful, however, in distinguishing *G. davaoensis*; this species consistently featured prominent dark spots on the anal fin. In live specimens, these spots are edged in red and alternate with rows of yellow spots (Randall & Greenfield, 2001). *Gnatholepis gymnocara* and *G.* sp. (of Randall & Greenfield, 2001) were also confirmed to lack scales on the cheek, nape and opercle, and to lack enlarged teeth medially in the lower jaw.

The distribution of pigmentation characters among individuals was compared with the clades revealed by the molecular phylogenetic analysis. Individuals in clade I of the phylogeny (*G. thompsoni* and *G. scapulostigma*) both feature the presence of a pigment blotch at the shoulder; this blotch is lacking in clade II individuals. *Gnatholepis inconsequens* was considered a synonym of *G. scapulostigma* by Randall *et al.* (1997). The holotype of *G. inconsequens* (AMS IB 3916) is badly stained green and it is not possible to identify whether or not it possesses the shoulder spot; otherwise, it does appear to correspond with *G. scapulostigma* and the synonymy of these two taxa is supported. Within clade II, individuals in the largest clade (*G. anjerensis*) did not have a shoulder spot, although the dorsal area did feature sparse dark freckling as well as orange spots in live individuals. The individuals from the Hawaii clade and its sister clade did have pigmentation on the shoulder but in the form of a dark horizontal stripe or streak, not a round spot. The differences in shoulder pigmentation are illustrated in Figure 3; these differences may also be observed in the many illustrations of *Gnatholepis* species presented in Randall & Greenfield (2001).

The shoulder pigmentation character agrees with clades delineated in the molecular analysis, but these clades are not in agreement with the characters described in the preliminary review of Randall & Greenfield (2001). In their review, the characters used to distinguish between *G. anjerensis* and *G. cauerensis* were the modal pectoral fin ray count and interorbital pigmentation: *G. anjerensis* was reported as having an incomplete black bar dorsally between the eyes, positioned slightly posterior to the centre of the pupils, whereas in *G. cauerensis* the bar was described as extending completely from one eye to the other across the top of the head, directly dorsal to the pupils (Table 1). In examination of both *Gnatholepis* from across the central Pacific and preserved museum *Gnatholepis* specimens, almost none of the specimens exhibited a complete interorbital bar, but positional differences in the pigment at the dorsal rim of the orbit were observed to vary as described by Randall & Greenfield (2001). Individuals in the Clade I of Figure 2 have the shoulder spot and would be classified as *G. thompsoni* or *G. scapulostigma*. Of these 179 individuals, most (163/179 or 91%) had the pigment dorsal to the eyes present directly above the pupils,

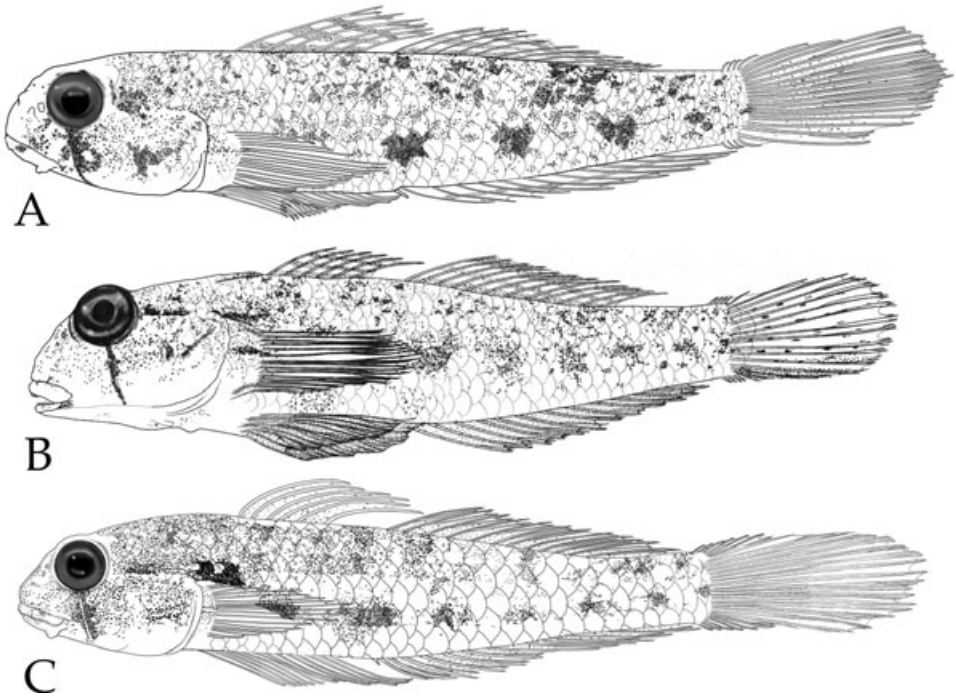

Figure 3. Left lateral views of A, *Gnatholepis anjerensis* (LACM 55960–4; 30.3 mm SL), B, *G. knighti* (LACM 55973.001; 26.7 mm SL), C, *G. scapulostigma* (LACM 55960–5; 34.4 mm SL). The meristic counts for these species overlap and there is a great deal of intra- and interspecific variation in colour pattern for most characters. The morphological character that distinguishes these species and accords with clades revealed in the DNA sequence analysis is the presence of a spot dorsal to the pectoral fin, with or without a pale centre (in *G. scapulostigma* and *G. thompsoni*), absence of such a spot (in *G. anjerensis*), or presence of a narrow dash-shaped mark dorsal to the pectoral fin (in *G. knighti*).

the character used by Randall & Greenfield (2001) to delineate *G. cauerensis*. The remaining 9% (16/179) of individuals possessed interorbital pigment present posterior to the centres of the pupils. Clade II of Figure 2 includes individuals without the shoulder spot (characteristic of *G. anjerensis*) or with a dash-shaped mark (Hawaiian samples and their sister clade). Among these individuals, 52% (85/165) had the interorbital pigment directly above the centre of the pupils [characteristic of Randall & Greenfield's (2001) *G. cauerensis*] and the remaining 48% (80/165) possessed the condition of pigment posterior to the centre of the pupils, the diagnostic character of *G. anjerensis* in Randall & Greenfield (2001).

The DNA phylogeny indicates that the presence or absence and configuration of shoulder pigmentation is the appropriate species-diagnostic character to use in delimiting Pacific individuals of *Gnatholepis* [excluding *Gnatholepis davoensis*, *G. gymnocara* and *G.* sp. of Randall & Greenfield (2001)]. The morphology among widespread Pacific *Gnatholepis* varies extensively, and no other morphological characters were identified that could serve to conclusively differentiate these species. One ecological factor, habitat depth, does exhibit significant variation between the groups delineated by the shoulder spot character; those without the spot (*G. anjerensis*) or with a dash (Hawaiian samples and their sister clade) are generally found at depths shallower than 4 m, and those with the spot (*G. scapulostigma*) are generally found deeper than 4 m. The nonparametric Mann–Whitney *U*-test was used to determine whether or not a significant difference in depth distribution existed between *G. anjerensis* and *G. scapulostigma*; recorded depths were compared for the samples collected in this study, the museum samples examined and the pooled samples. In each case, the depth distributions were highly significantly different ($P < 10^{-7}$).

Within the clade of individuals originally identified as *Gnatholepis anjerensis* is a clade including all of the individuals sampled from Hawaii and some of the individuals from Rarotonga, Cook Islands, and Moorea, Society Islands. These individuals are diagnosable morphologically by the presence of a dash-shaped mark on the shoulder. The morphology of these individuals corresponds well to the description of *G. knighti* Jordan and Evermann, 1903, also a shallow-water *Gnatholepis* species described from Hawaii (Jordan & Evermann, 1903). The molecular and morphological evidence presented here indicates that

G. knighti is a valid species. The holotype of *G. knighti* (USNM 50653) is lost, but paratypes are still extant. Below, *G. knighti* is rediagnosed and a review of the type material is given.

SYSTEMATICS

FAMILY GOBIIDAE
GENUS *GNATHOLEPIS* BLEEKER, 1874

Gnatholepis Bleeker, 1874: 318. Described as a subgenus of *Stenogobius*. Type species *Gobius anjerensis* Bleeker, 1851, by original designation.

GNATHOLEPIS KNIGHTI JORDAN & EVERMANN, 1903: 204 (FIG. 2)

G. anjerensis Randall & Greenfield, 2001 (part), Myers, 1999 (part).
G. cauerensis Randall, 1983, 1995 (part), Lieske and Myers, 1999 (part).

Holotype: USNM 50653, lost.

Paratypes: AMNH 2294 (1), ANSP 24222 (1), missing, BPBM 1699 (1), CAS 1497 (1), missing, CAS 14982 [ex IU 9812] (1), CAS 43092 [ex IU 10477] (1), CAS SU 7468 (7); FMNH 3969 (1); MCZ 28902 (1).

Other material: LACM 55973–1, a 26.7 mm SL male; LACM 55973–2, a 27.3 mm female and 21.1 mm individual of undetermined sex.

Etymology: This species was named in honour of Knight Starr Jordan, the son of David Starr Jordan.

Diagnosis: A *Gnatholepis* (as diagnosed by Randall & Greenfield, 2001) with dorsal fin elements VI + I, 11; anal fin elements I, 11; pectoral fin rays 15–16; scales covering body, nape and rear of opercle; teeth in outer row of upper jaw enlarged to form canines; tongue bilobed. Colour in alcohol: dorsal third of body with clusters of small pigment spots; a series of six to seven blotches midlaterally decreasing in size from anterior to posterior; dark dash-shaped stripe or streak just dorsal to pectoral fin; additional dark marks at centre of pectoral fin base, obliquely on opercle, and extending posteriad from eye; dark line extending ventrad from centre of eye. Dorsal fins with series of three lateral dusky stripes, caudal fin with numerous small black spots, and anal and pelvic fins overall dusky.

Remarks: Gnatholepis knighti are found in shallow water in Hawaii and South Pacific islands, and may be distinguished from other *Gnatholepis* by the presence of a dash-shaped mark on the shoulder, dorsal to the pectoral fin.

DISCUSSION

Dense sampling of DNA sequence among *Gnatholepis* individuals from the Pacific and Caribbean has allowed inference of phylogeny and identification of species boundaries that had previously been supported only by conflicting morphological characters. In this study, seven species are recognized: *G. thompsoni*, *G. scapulostigma*, *G. davaoensis*, *G. anjerensis*, *G. knighti*, *G. gymnocara* and *G.* sp. The morphology of all *Gnatholepis* species was examined, and DNA sequences were obtained for all species except *G. gymnocara*, for which suitable tissue was not available. The morphological character that distinguishes *G. thompsoni* and *G. scapulostigma* from the other species is the presence of a shoulder spot; *G. knighti* is distinguished by a dash-shaped shoulder mark, and characters diagnostic for the other species are listed in Table 2. No additional characters were identified that could distinguish *G. thompsoni* from *G. scapulostigma*, in agreement with other studies (Winterbottom & Emery, 1986; Randall & Greenfield, 2001). Currently, *G. thompsoni* populations in the Pacific and Caribbean are separated by the isthmus of Panama and have been so for at least three million years (Marshall *et al*., 1979; Knowlton & Weigt, 1998). However, it is not appropriate to assume that populations of *G. thompsoni* were sundered by the closure of the isthmus; *Gnatholepis* are not known from the Eastern Pacific (Fig. 1), and sampling of DNA sequences for *Gnatholepis* from the Western Pacific, Indo-Pacific and

Table 2. Character distribution in species of *Gnatholepis* according to this study

Species	Shoulder spot	Male anal spots	Head scales	Geographic range
G. anjerensis	Absent	Absent	Present	Red Sea to Hawaiian & Society Is.
G. scapulostigma	Present	Absent	Present	East Africa to Easter Is.
G. knighti	Dash	Absent	Present	Hawaiian Is., Cook Is., Society Is.
G. davaoensis	Absent	Black and red, yellow	Present	Ryukyus to Solomon Is.
G. thompsoni	Present	Absent	Present	Caribbean and Atlantic
G. gymnocara	Absent	Absent	Absent	Northern Australia
G. sp.	Absent	Blue plus broken black line	Absent	Northern Australia

Indian Ocean was not performed for this study. It is possible that the invasion of the Caribbean by *G. thompsoni* occurred from the east rather than the west. The eastern Pacific barrier, between Polynesia and the Americas, may have acted as a deterrent to the spreading of *Gnatholepis* individuals from the Pacific eastward into the Caribbean (Ekman, 1953; White, 1994).

Confirmation of the shoulder spot as the species diagnostic character for *G. scapulostigma* and *G. thompsoni*, to the exclusion of *G. anjerensis* and other *Gnatholepis* species, contradicts the conclusions of Randall & Greenfield (2001). They used the extent and position of the interorbital pigment to separate Pacific species, and identified four subspecies within one of their species based on modal differences in pectoral fin ray count. Randall & Greenfield's (2001) types, and other specimens for their nominal subspecies, would be classified differently if the presence or absence of the shoulder spot were used as the species-diagnostic character. Their *G. anjerensis* and *G. c. cauerensis* include individuals that would be classified as either *G. anjerensis* or *G. scapulostigma*, with different species boundaries. Randall & Greenfield's (2001) other subspecies of *G. cauerensis* (*G. c. australis*, *G. c. hawaiiensis* and *G. c. pascuensis*) would all be classified as *G. scapulostigma* based on the configuration of their shoulder pigment. Their *G. c. australis* is described as being very pale and lacking the shoulder spot, but examination of their holotype and other specimens reveals that the spot, although faint, is present. Two names have been used for widespread Pacific *Gnatholepis* species lacking the shoulder spot: *G. anjerensis* (Bleeker, 1851) and *G. cauerensis* (Bleeker, 1853). The older name, *G. anjerensis*, takes priority, and identity of this species is confirmed by comparison with Randall & Greenfield's (2001) neotype, which lacks the shoulder spot.

In the widespread species of *Gnatholepis*, intraspecific variability has hindered attempts to delineate species based on morphological data. Among these species, meristic values overlap and pigment can exhibit large amounts of variation depending on the particular substrate conditions that the fish inhabit. For this situation, molecular data provide another source of evidence to delimit species boundaries. Although DNA sequence patterns are not practical for use as species identification markers under most circumstances, molecular data can be used in tandem with morphology where morphology may be contradictory or extremely labile. Wiens & Penkrot (2002) compared morphology and mitochondrial DNA data with regard to their utility in delimiting species boundaries in recently diverged species and found that mtDNA was preferred because of its faster rate of change. In this study, DNA sequence revealed two large groups of individuals within *Gnatholepis*; the corresponding morphological marker distinguishing these groups is the configuration of the shoulder spot. The new phylogeny also confirmed the distinctness of a third group, with dash-shaped shoulder pigment and corresponding to a species named in 1903: *G. knighti*.

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APPENDIX

INSTITUTIONAL ABBREVIATIONS

AMS	Australian Museum, Sydney
BPBM	Bernice P. Bishop Museum, Honolulu, Hawaii, USA
CAS	California Academy of Sciences, San Francisco, California, USA
LACM	Natural History Museum of Los Angeles County, California, USA
USNM	United States National Museum of Natural History, Washington, D. C., USA

MATERIAL EXAMINED

Gnatholepis anjerensis

Abaiang Atoll, Gilbert Is., AMS I.18053–010 (7); Lapu Lapu Mkt, Philippines, AMS I.21931–005 (8); Marquesas, Nuku Hiva, AMS I.22176–033 (7); Yeppoon, Qld, AMS I.22877–016 (19); East Arm, Darwin, NT, AMS I.23948–013 (2); Gunn Point, NT, AMS I.24694–004 (78); Moreton Bay, Qld, AMS I.24711–002 (2); Moorea, French Polynesia, AMS I.30940–006 (7); Sabina Point, Qld, AMS I.34301–054 (20), AMS I.34309–004 (1); Townsend Island, Qld, AMS I.34318–053 (20); Sabina Point, Qld, AMS I.34405–001 (4); One Tree Island, Qld, AMS I.35852–003 (3); Vanuatu, Santa Maria Is., AMS I.37915–040 (1); Lord Howe Is., BPBM 14829 (1); Sulawesi, Bunaken Is., BPBM 26651 (1) neotype; Ras Iwefine, Kenya, BPBM 27230 (1); Maldive Is., Male atoll, BPBM 34404 (3); Solomon Is., CAS 200237 (6); Nukulau Is., Fiji, CAS 213212 (7); Negros Oriental, Philippines, CAS 51515 (15), CAS 51516 (12); Khanh Hoa, Nhatrang Bay, Vietnam, CAS 58304 (1); Raroia, French Polynesia, CAS 59407 (3); Maldives, CAS 66708 (2); Elangalap, Falarik Is., CAS 95416 (1); Pohnpei, Micronesia, CAS 95425 (2); Moorea, French Polynesia, LACM 54117 (2), LACM 54118.005 (2), LACM 54119.001 (6), LACM 54124.005 (2), LACM 54125.002 (1), LACM 55960.004 (8); Wake Island, LACM W56-255 (6).

Gnatholepis davaoensis

Lapu Lapu Mkt, Philippines, AMS I.21931–004 (5); Amilao, Philippines, AMS I.24136–001 (1); East Arm, Darwin, NT, AMS I.24676–014 (12); East Point, Darwin, NT, AMS I.24678–007 (10); Solomon Islands, New Georgia Is., AMS I.31088–002 (1); Madang Province, Papua New Guinea, AMS I.32492–004 (2), AMS I.32492–009 (1); Hou Pi Hu, Taiwan, BPBM 18670 (1) neotype; Negros Oriental, Philippines, CAS 200236 (4), CAS SU 26293(22), CAS 30806 (1), CAS 51508 (8), CAS 51511 (2), CAS 51513 (2), CAS 51514 (3), CAS51519 (1), CAS 51769 (1), CAS 75215 (3); Madang, Papua New Guinea, CAS 65724 (1).

Gnatholepis gymnocara

Townsend Is., Qld, AMS I.34318–051 (1) holotype; Wide Bay, Teebar Creek, Qld, AMS IB.1276 (1); Caloundra, Qld, AMS I. 22102–001 (1); Darwin, East Point, NT, AMS I.23930–011 (2); Townsend Is., Qld, AMS I.34318–032 (42).

Gnatholepis knighti

Oahu, Kaneohe Bay, LACM 55973.001 (1); LACM 55973.002 (2); Hawaii, Hilo, CAS SU 7468 (7), CAS

43092 (1); Umboi Is., Papua New Guinea, USNM 297217 (1).

Gnatholepis thompsoni

Panama, Caledonia Bay, LACM 20636 (2); Limon, Costa Rica, LACM 36220.17 (2); Navassa Is., LACM 54098.016 (1), LACM 54113.005 (6); Jamaica, Pedro Cays, LACM 5973.000 (3), LACM 5974.000 (26); Puerto Rico, Crashboat Basin, LACM 6522.13 (3); Curacao, LACM 6727.5 (3); Jamaica, Montego Bay, LACM 8937.008 (6).

Gnatholepis scapulostigma

New Hebrides, AMS IB 3607 (1); Heron Island, Qld, AMS IB 3916 (1), AMS IB 4004 (2); Lord Howe Is., NSW, AMS I.17410–002 (1); One Tree Island, Qld, AMS I.17445–049 (1); Suva, Fiji, AMS I.18354–030 (3); Beqa lagoon, Fiji, AMS I.18448–024 (2); Ceram Marsegoe Bay, Indonesia, AMS I.18469–122 (7); Seal Rocks, NSW, AMS I.18659–001 (1); Lizard Island, Qld, AMS I.18805–002 (4); Sydney harbour, NSW, AMS I.19500–003 (1); North-west cape, WA, AMS I.19641–022 (2); One Tree Island, Qld, AMS I.20561–007 (3); Cape Melville, Qld, AMS I.20755–062 (6); Raine Island, Qld, AMS I.20757–072 (14); Cape Melville reef, Qld, AMS I.20774–107 (10); Raine Is., Qld, AMS I.20775–142 (6); Lizard Is., Qld, AMS I.21540–006 (2); Israel, Shurat el Mankta, AMS I.21874–001 (2); Sombrero Is., Philippines, AMS I.21908–002 (8); Cabon Is., Philippines, AMS I.21918–018 (11); Anilao, Philippines, AMS I.21922–009 (4); Solomon Is., New Georgia Is., AMS I.22128–011 (1); Escape Reef, Qld, AMS I.22613–041 (3), AMS I.22633–072 (12); Japan, Miyake-Jima, AMS I.23492–015 (1); Middleton Reef, Qld, AMS I.27138–055 (6), AMS I.27142–030 (6); Elizabeth Reef, Qld, AMS I.27149–037 (3), AMS I.27155–019 (2), AMS I.27156–035 (7); Japan, Amitori Bay, AMS I.27366–002 (1); Japan, Iriomote-Jima, Amitori Bay, AMS I. 27367007 (1); Madagascar, Nosy Be, AMS I.28108–038 (4); French Polynesia, Moorea, Tareu pass, AMS I.28947–014 (14); Cocos-Keeling, Horsburgh Is., AMS I.28992–009 (1); Cocos Keeling, Keeling Is., AMS I.28993–009 (4), AMS I.28999–010 (2); North Solitary Is., NSW, AMS I.30310–034 (1); Holmes Reef, Qld, AMS I.30465–101 (14); Reef 11102, Qld, AMS I.33703–074 (1); Reef 10–403/10–400, Qld, AMS I.33710–051 (1); Far North GBR, Qld, AMS I.33711–064 (2); Coral Sea, Ashmore reef, AMS I.33715–096 (4), AMS I.33717–071 (1), AMS I.33719–047 (9), AMS I.33721–036 (1); Coral Sea, Boot reef, AMS I.33749–122 (33); Indonesia, Flores, AMS I.34501–018 (1); Vanuatu, Emae Is., Sulua Bay, AMS I.37323–013 (1); Vanuatu, AMS I.37340–021 (1), AMS I.37340–032 (1); Marshall Is., Enewetak, AMS I.37692–002 (1), AMS I.37704–003 (1), AMS I.37709–003 (1), AMS I.37719–006 (1); Solomon Islands, AMS I. 39002–058 (4), AMS I.39010–100 (1), AMS I.39011–056 (1); Solomon Is., Santa Cruz Is., AMS I.39040–061 (1); Guam, Tumon Bay, AMS I.40826–001 (1); Popote Bay, Tahiti, BPBM 8108 (2); Line Is., Palmyra Atoll, BPBM 9393 (1); Moorea, Papetoi pass, BPBM 12032 (1); Austral Is., Rurutu, BPBM 13715 (4); Cook Is., Rarotonga, BPBM 13980 (1); Fiji, Mbengga barrier reef, BPBM 14591 (2); Ryukyu Is., Ishigaki, BPBM 15021 (2); Maui, Lahaina, BPBM15131 (13); Papua New Guinea, Kranket Is., BPBM 15534 (1); Papua New Guinea, Port Moresby, BPBM 15924 (1); Pitcairn Is., Oeno Atoll, BPBM 16532 (7); Pitcairn Is., BPBM 16846 (2); Rapa Is., BPBM 17276 (1); Oahu, Pokai Bay, BPBM 17800 (1); Line Is., Fanning Is., BPBM 28073 (3); Enewetak Is., BPBM 31327 (2); Easter Is., BPBM 32850 (1), BPBM 32851 (2), BPBM 32852 (1), BPBM 32853 (1); Saudi Arabia, Jana Is., BPBM 33350 (2); Maldive Is., Male Atoll, BPBM 34390 (2); Midway Atoll, BPBM 34770 (2); Oahu, Pupukea, BPBM 37847 (1); Hawaii, Kona coast, BPBM 37861 (2); Rapa Is., BPBM 38375 (1); Fiji, Suva, CAS 213191 (3); Fiji, Makuluva Is., CAS 213217 (1); Kiribati, CAS 51303 (8); CAS 51304 (10); Tahiti, Society Is., CAS 51305 (6); Yap lagoon, CAS 51306 (4); Belau, CAS 51307 (3), CAS 51308 (1); Vanuatu, Palikulo Bay, CAS 51548 (1); Moorea, Society Is., CAS 51602 (8); Ashmore Is., Australia, CAS 57292 (5); Pohnpei Lagoon, Micronesia, CAS 95420 (5), CAS 95424 (2), CAS 95426 (1), CAS 95427 (2); Raroia, French Polynesia, CAS 95430 (2); Moorea, French Polynesia, LACM 54120.004 (1), LACM 55960.005 (6), LACM 55962.001 (1).

G. sp.

Darwin, NT, AMS I.24677–003 (12); Prince of Wales Is., Qld, AMS I.19356–016 (61).