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***Citharichthys darwini* n. sp., a new endemic flatfish from the Galápagos Archipelago (Teleostei: Pleuronectiformes: Paralichthyidae)**

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Abstract

A new flatfish, *Citharichthys darwini* n. sp., is described from the shores of Isla Isabela on the western side of the Galápagos Archipelago. Our recent collection from Tagus Cove in 1998 is the first record of the species on Isla Isabela since a series of specimens were collected at Tagus Cove and nearby by the Allan Hancock Expedition in 1934. *C. darwini* is a dwarf species with adults maturing at around 30 mm SL and the largest collected less than 60 mm SL. The new species is distinguished from other eastern Pacific members of the *Citharichthys*/*Etropus* group by a narrow body (maximum body width 39–45% SL), medium-sized mouth (upper jaw 31–35% HL), low dorsal and anal fin-ray counts (D 70–75, A 51–58), relatively few slender gill rakers (4–7 upper, 8–10 lower), and non-deciduous scales. The barcode mtDNA COI sequence (used by the Barcode of Life project) for the new species falls within the broad *Citharichthys*/*Etropus* clade, but is more than 16% divergent from other *Citharichthys* in the BOLD barcode database (including most of the known species). The nearest-neighbor sequence in the phenetic tree for paralichthyid flatfishes is an Atlantic species, *Citharichthys* sp., from the U.S. Virgin Islands. The species list of flounders and sanddabs (Paralichthyidae) for the Galápagos Islands is revised and expanded to six, including *Syacium maculiferum*, previously considered a Cocos Island endemic. *C. darwini* is apparently the only endemic flatfish (Paralichthyidae or Bothidae) in the Galápagos Archipelago. The new species is associated with the cooler water and coarse black volcanic sands of the recently emerged western islands in the chain.

Key words: Galápagos, fishes, flatfish, sanddab, endemic, new species, *Citharichthys darwini*, Paralichthyidae, barcode, DNA sequence, biogeography, species list, biodiversity.

Introduction

The taxonomy of tropical eastern Pacific flounders and sanddabs (Paralichthyidae) was stable for some time after a series of expeditions in the late 1800s, most notably the *Albatross* surveys, which resulted in the description of many of the flatfishes in the region (Gilbert 1890, Garman 1899). Those expeditions were followed by several more collections early in the last century that mostly confirmed prior species accounts. In the past two decades, however, a renewed interest in biodiversity has yielded four new species for the region (Murakami & Amaoka 1992, Hoshino & Amaoka 1999, van der Heiden & Mussot-Perez 1995, van der Heiden & Plascencia Gonzalez 2005), as well as the rediscovery of an old species (van der Heiden & Mitchell 1998).

Of the 32 species of paralichthyid flatfishes in the eastern Pacific Ocean, about a third are temperate northern species that extend south only to the Baja California peninsula and the adjacent Sea of Cortez and several more are from cool subtropical waters south of Colombia or Ecuador (Hensley 1995, Robertson & Allen 2008). In general, flounders and sanddabs are continental in distribution, paralleling the distribution of soft sediments. The offshore islands of the eastern Pacific have mostly hard substrate and are thus particularly depauperate in this family of flatfishes, with records of only a few species, mostly wide-ranging and some collected only once or twice on the offshore islands. Six species are now known for the Galápagos Islands (McCosker & Rosenblatt 2010 and this study); three species occur on Cocos Island, including one originally described as an endemic (Bussing & Lopez 2005, Garrison 2005); one or two reach the Islas Revillagigedos and Malpelo Island; and none have been collected on Clipperton Atoll, the farthest offshore island in the region (Robertson & Allen 2008).

The largest genus in the family Paralichthyidae is *Citharichthys*, the sanddabs, with nine tropical eastern Pacific species (now ten), five of which are found only around the northern subtropics, in Baja California and the adjacent Mexican coastline (Robertson & Allen 2008, van der Heiden *et al.* 2009). Only *C. gilberti* and *C. platophrys* are wide-ranging throughout the region, while *Citharichthys mariajorisae* van der Heiden & Mussot-Perez 1995 occurs from Southern Mexico to Panama and *Citharichthys gnathus* Hoshino & Amaoka 1999, described recently as a Galápagos endemic, is also found in Colombia (van der Heiden *et al.* 2009).

On a National Geographic Society/National Public Radio-sponsored expedition in May 1998 to the Galápagos Islands, we collected several small *Citharichthys* in 10–30 m depth while SCUBA diving at Tagus Cove on the island of Isabela (Albemarle). The sequence of the mtDNA barcode gene, COI, was obtained for a specimen as part of the campaign to barcode all fishes by the Barcode of Life project, Fish-BOL (Ward *et al.* 2009). The sequence proved to be in the *Citharichthys* clade, but not close to any of the several Pacific *Citharichthys* species in the BOLD database (including the two widespread species *C. gilberti* and *C. platophrys*). The nearest-neighbor sequence in the phenetic tree, still more than 17% divergent, was *Citharichthys* sp. from the Atlantic Ocean. The new specimens did not fit the description for the only known *Citharichthys* in the Galápagos Islands, *C. gnathus*, found only on rare patches of deep mud substrate in the archipelago. A subsequent review of museum collection records revealed that a series of small *Citharichthys* had been collected at Tagus Cove and nearby on Isla Isabela during the Allan Hancock expedition on the *Velero III* in 1934. These old specimens and the new ones we recently collected matched in morphology and meristics and represent a new species of *Citharichthys*, apparently endemic to the Galápagos Archipelago.

Materials and Methods

Type specimens of the new species are deposited in the Marine Vertebrate Collection of the Scripps Institution of Oceanography (SIO), the Los Angeles County Museum of Natural History (LACM), the California Academy of Sciences Ichthyology Collection (CAS), and the Smithsonian Institution, National Museum of Natural History (USNM). The fish from 1998 were collected by hand and immediately preserved in 90% ethanol. Ethanol-preserved specimens of other species in the *Cyclopsetta* group of the Paralichthyidae were collected for DNA sequencing from the Pacific Ocean in Baja California, Guanacaste in Costa Rica, and from larval samples collected over the Galápagos hydrothermal vents south of Cocos Island in 1985 (Victor 1987). Comparison species from the Atlantic were collected for this study in Panama, Belize, Quintana Roo (Mexico), and the U.S. Virgin Islands (Appendix 1). Comparison sequences from the Barcode of Life Database (BOLD) were graciously provided by Eduardo Balart of Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico; Andrew Bentley of the Ichthyology Division, Biodiversity Institute, University of Kansas; Ralph Imondi of Coastal Marine Biolabs; Jacob Lowenstein of the American Museum of Natural History; and Lourdes Vásquez Yeomans and Martha Valdez of ECOSUR, Unidad Chetumal, in Quintana Roo, Mexico (Appendix 1).

DNA extractions were performed with the NucleoSpin96 (Machery-Nagel) kit according to manufacturer specifications under automation with a Biomek NX liquid-handling station (Beckman-Coulter) equipped with a filtration manifold. A 652-bp segment was amplified from the 5' region of the mitochondrial COI gene using a variety of primers (Ivanova *et al.* 2007). PCR amplifications were performed in 12.5 µl volume including 6.25 µl of 10% trehalose, 2 µl of ultra pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM (NH₄)₂SO₄, 20mM Tris-

HCl (pH8.8), 2mM MgSO₄, 0.1% Triton X-100), 0.625 µl of MgCl₂ (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM), 0.0625 µl of *Taq* DNA polymerase (New England Biolabs), and 2 µl of template DNA. The PCR conditions consisted of 94°C for 2 min., 35 cycles of 94°C for 30 sec., 52°C for 40 sec., and 72°C for 1 min., with a final extension at 72°C for 10 min. Specimen information and barcode sequence data from this study were compiled using the Barcode of Life Data Systems (BOLD, www.barcodinglife.org; Ratnasingham & Hebert 2007). The sequence data is publicly accessible on BOLD and GenBank. Sequence divergence was calculated using BOLD with the Kimura 2-parameter (K2P) model generating a mid-point rooted neighbor-joining (NJ) phenogram to provide a graphic representation of the species divergence.

Measurements follow van der Heiden *et al.* (2009), with the following clarifications: length measurements are taken from the tip of the upper jaw; all lengths are horizontal spans except the oblique measurements of the upper jaw and fin lengths; upper jaw length is the maximum from the uppermost tip of the fleshy lip of the premaxilla to the farthest point on the rear end of the maxilla; orbit lengths are measured from the bony rims since the preservation of the eye is variable; snout length is the horizontal span before the anterior margin of the bony orbit of the upper eye; interorbital width is the bony width; pored lateral line scale counts are from the scale just above the end of the opercular opening to the last over the hypural plate, not counting additional pored scales on the caudal fin; dorsal and anal fin-ray counts include each of the last rays counted individually; vertebral counts include the urostylar centrum.

Citharichthys darwini, n. sp.

Figures 1–4.

Holotype. SIO 12-3075 (1) 33.3 mm SL, Ecuador, Galápagos Islands, Isla Isabela, Tagus Cove (-0.26°, -91.37°), B. Victor, G. Wellington, & C. Caldow, May 28, 1998.

Paratypes. SIO 12-3076 (2) 33.4–39.1 mm SL, same as holotype; LACM 57426-1 (1) 32.7, same as holotype; LACM 23671 (8) 33.4–42.4 mm SL, Ecuador, Galápagos Islands, Isla Isabela, Tagus Cove (-0.26°, -91.37°), Allan Hancock Expedition, prob. Jan. 13–15, 1934 (in LACM catalog as Jan. 10, but the Expedition was in transit and only arrived in Galápagos on Jan. 11 according to the Fraser (1943) itinerary); CAS 6895 (5) 20.8–49.3 mm SL, Ecuador, Galápagos Islands, Isla Isabela, Tagus Cove (-0.26°, -91.37°), Allan Hancock Expedition, Jan. 15, 1934; CAS 6862 (8) 19.3–50.2 mm SL, Ecuador, Galápagos Islands, prob. Isla Isabela, Albemarle Point (0.15°, -91.38°), Jan. 12, 1934 (in CAS catalog as “Charles Island, Jan. 12 1934”, but the expedition did not reach Charles Island (Floreana) until Jan. 16, 1934 (Fraser 1943), on Jan. 12 the expedition was at Albemarle Pt. and the Field Number in the catalog is, appropriately, HE 12-I-1934); USNM 101970 (4) 48.8–59.6 mm SL, Ecuador, Galápa-



Figure 1. *Citharichthys darwini*, holotype, 33.3 mm SL, male, SIO 12-3075.

gos Islands, Isla Isabela, Tagus Cove (-0.26°, -91.37°), Allan Hancock Expedition, Jan. 15, 1934; USNM 101896 (2) 43.6–45.5 mm SL, Ecuador, Galápagos Islands, Isla Isabela, Tagus Cove (-0.26°, -91.37°), Allan Hancock Expedition, Dec. 10, 1934.

Diagnosis. A small narrow-bodied species of *Citharichthys*, sharing the characters for the genus, i.e. a mostly straight lateral line, apparently uniserial and even-sized teeth, relatively long thin gill rakers without serrations, urogenital papilla on the blind side, asymmetric pelvic fin placement with ocular-side fin-base along ventral midline (i.e. the *Cyclopsetta* group *sensu* Hensley and Ahlstrom 1984), and a medium-sized mouth with upper-jaw length greater than 30% HL (vs. less in *Etropus*). *Citharichthys darwini* is distinguished from congeners by low fin-ray counts, D 70–75 A 51–58; low numbers of slender gill rakers, 4–7+8–10 (usually 6+9 or 6+10); a narrow body, maximum body width in adults 39–45% SL; low numbers of pored lateral line scales, 41–42; 10+25 vertebrae; and firmly attached (non-deciduous) scales.

Description. (range listed for variously included paratypes, depending on condition, with holotype in parentheses) Body flat and left-sided, elongate and oval, upper-body profile low, i.e. outline mostly symmetrical around lateral midline, maximum body width slightly forward of midbody, more than twice into SL, 37–45 (40)% SL (45% only in largest paratype, 42% maximum in types from 30 to 42 mm SL and 37% in 21.7 mm SL); lateral line mostly straight along the lateral midline but with a gradual slight curve above pectoral fin; caudal peduncle width 11–12 (11)% SL, caudal peduncle length (dorsal) 3–4 (4)% SL.

Head relatively small 24–29 (27)% SL; dorsal head profile mostly smooth with only slight notch in front of upper eye; dorsal head profile low, sloping gradually, maximum head depth (measured at the rear end of the operculum) 31–37 (37)% SL; eyes large with prominent rounded corneal tab over pupil (pupillary operculum), lower eye slightly forward of upper eye; upper orbit length 31–35 (34)% HL; interorbital space very narrow 2–3 (3)% HL; interorbital ridge moderately developed; snout short 13–18 (18)% HL; lower free preopercular margin straight, almost parallel to body axis, corner of preopercle smoothly curved, forming about right angle and corner well behind vertical at rear margin of eyes; no fleshy tabs or tentacles along preopercular or opercular margins on either ocular or blind side. Mouth medium; jaws mostly straight and parallel to head outline, no angle formed in maxillary or mandibular outline, only a slight indentation in the anterior cranial margin overlying upper jaw; rear end of maxilla extending to vertical through anterior quarter of orbit and below horizontal through lower edge of eye, length of upper jaw 31–35 (34)% HL; small bump below mandibular symphysis. Teeth caniniform to triangular, apparently uniserial (without dissection), small but stout, uniformly sized, movable (bend inward), extend full length of jaw on blind side, sometimes less than half length along ocular-side jaws.

Dorsal-fin rays 70–75, mode 72 (72); anal-fin rays 51–58, mode 56 (56); pectoral-fin rays on ocular side 10–11 (11), on blind side 8–9 (8); ocular-side pectoral fin 15–19 (15)% SL; blind-side pectoral fin 12–14 (14)% SL; pelvic-fin insertion asymmetrical with blind-side fin attached along ventral midline in advance of insertion of ocular-side fin, pelvic fins with 6 rays each (6), ocular-side pelvic fin 10–11 (11)% SL; blind-side fin 12–14 (12)% SL; caudal fin truncate, caudal-fin length 10–11% SL; 17 caudal-fin rays, rare 16. Vertebrae 10+25 (6 specimens).

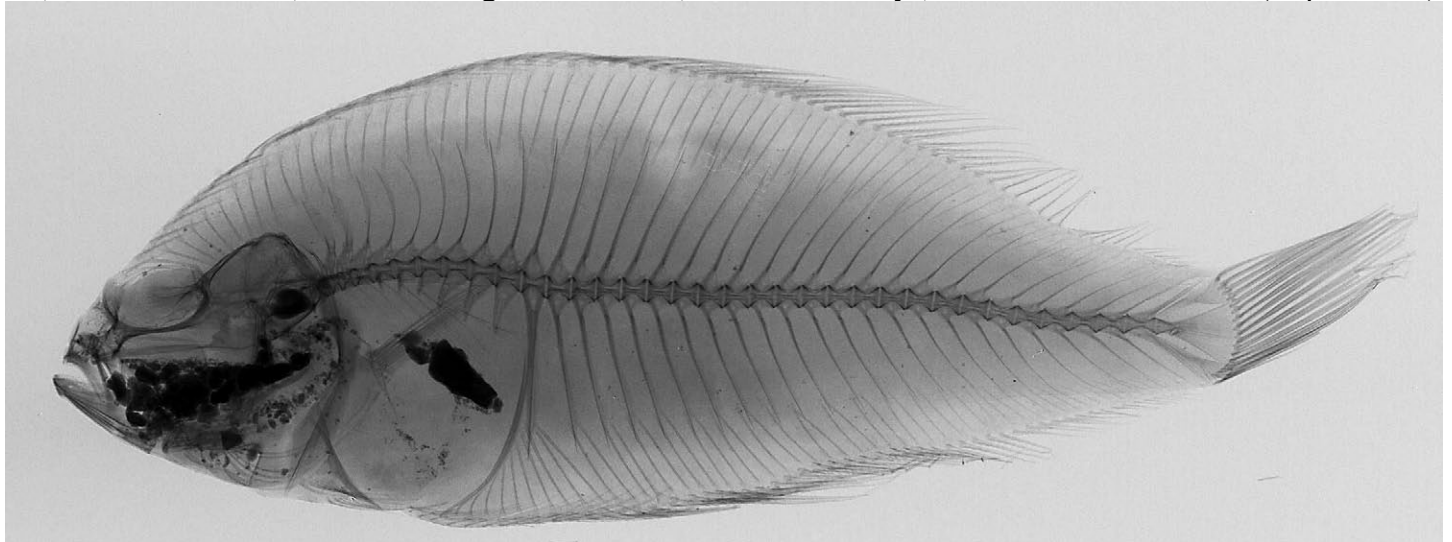


Figure 2. *Citharichthys darwini*, paratype, USNM 101896, radiograph courtesy Sandra Raredon, USNM.

Scales medium-sized, extending over entire body and head except snout, becoming distinctly smaller over pterygiophore zone near body edges (Fig. 3), moderately ctenoid to a few cycloid on ocular side, no secondary scales (but largest paratype has occasional small secondary scale apparent around lateral line on posterior body), cycloid on blind side; lines of small strongly ctenoid scales along lower portions of dorsal, anal, and caudal-fin rays, all scales firmly attached (no loss of scales on any types from 1998 collection); pored lateral-line scales on body 41–42 (39), plus up to 6 pored scales along the middle caudal-fin ray (scales intact for accurate counts primarily from 1998 specimens; pored series begins above end of opercular opening, with scale after pore in black spot, up to end of hypural plate, followed by several pored scales along caudal-fin rays not included in lateral-line counts). Urogenital papilla on blind side near anal fin origin, simple papilla on females, cone with accessory flaps in a *fleur-de-lis* form in males.

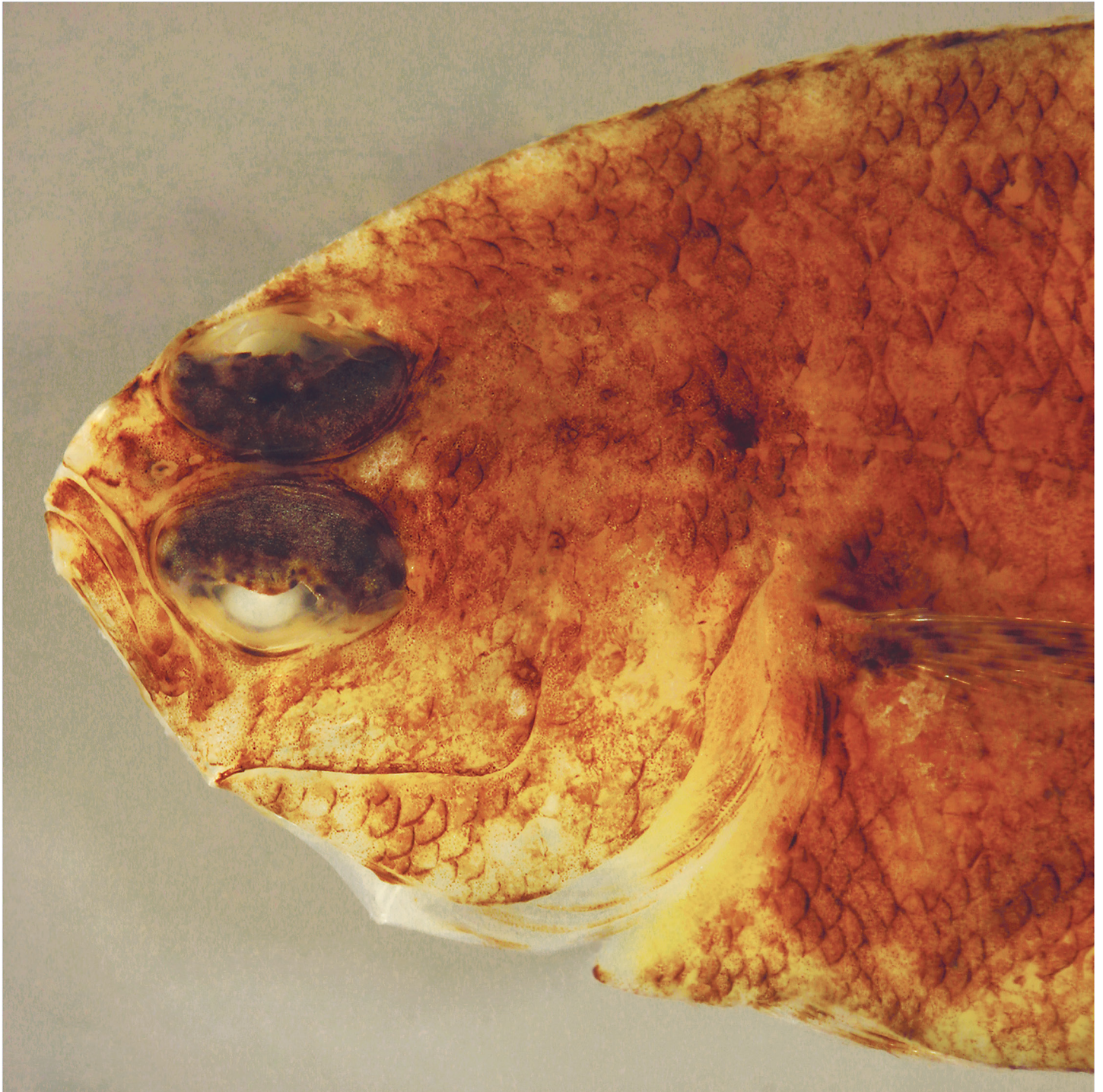


Figure 3. *Citharichthys darwini*, holotype, 33.3 mm SL, male, SIO 12-3075.



Figure 4. *Citharichthys darwini*, slender gill rakers on first arch.

Gill rakers on first arch medium-length and slender, without serrations, longest about half pupil diameter; upper limb 4–7, mode 6; lower limb (including rudiments and corner) 8–10, mode 10 (rare 8); total 12–17, mode 15 (Fig. 4).

Color in alcohol. Live colors not documented. Specimens placed directly into ethanol retain their marking pattern; the specimens from 1934 are variably marked, some similar to newer specimens (CAS 6895), some retaining mostly white markings (CAS 6862), and the remainder uniformly museum-brown with few remaining markings. For the newer specimens, the pattern on the ocular side of the body is mottled brown and pale, the outer edges of the scales within the brown patches are clearly outlined with dark pigment. There are a series of about 8 white or light spots about the size of the pupil arrayed along the body adjacent to the dorsal midline and about 6 near the ventral midline, as well as iridescent white patches on the rear interorbital and on the body facing and below the pectoral fin. The darkest patches amongst the mottling are along the lateral line, usually a patch just behind the mid-body followed by a light area and then one or two more dark patches before a dark patch on the base of the caudal fin (the fish in the 1934 collection from Albermarle Point (CAS 6862) have more intense white markings and reduced dark markings and likely reflect the patterning of individuals found on white or mixed sands, although preservation artifact cannot be

excluded). The ocular-side pectoral fin has dark patterning (not bands) including dark patches at the distal end of the upper (longest) fin rays and at the base of the three lower fin rays. The blind side is mostly uniformly light, except for some brown patterning on the underside of the jaws, the edge of the snout, a few patches along the body near the edges, and sometimes the urogenital papilla. Several small round deep internal melanophores are sometimes visible from the blind side in the body musculature near the base of the pterygiophores along the dorsal and anal fins and in a row or streak along the posterior midline of the underside.

Barcode DNA sequence. A 652-nucleotide sequence of the section of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype (Genbank accession number JX516097). Following the database management recommendation of the BOLD the sequence of the holotype is presented here as well:

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CCTGTATCTAGTATTCGGTGCCTGAGCCGGTATAGTTGGAACGGCATTAAAGCCTTCTCATCCGAGCC-
GAACTTAGTCAACCTGGAGCCCTTCTAGGAGACGACCAAATTTACAATGTAATCGTTACCGCA-
CATGCATTTCGTAATGATTTTCTTTATAGTAATGCCATTATGATTGGGGGCTTTGGAAACTGATTA-
ATTCCCCTTATGATCGGAGCCCCTGATATAGCTTTTCCACGAATAAATAATATGAGTTTCTGAC-
TACTGCCTCCTTCTTTCTTCTCCTTTTAGCATCTTCGGGTGTGCGAAGCAGGGGCAGGAACAG-
GCTGAACAGTTTATCCCCACTAGCAGGAAATCTTGCCACGCAGGAGCATCCGTAGACCTAAC-
GATCTTCTCCCTTCATCTTGCAAGGATTTTCTCTATTTTAGGAGCAATCAACTTTATTACAACCATTAT-
TAATATGAAGCCAACTCCGTCACAATGTACCAAATCCCTCTTTTCGTATGAGCTGTCCTTATTACT-
GCAGTACTACTCCTCCTCTCTCTACCAGTACTTGCTGCAGGTATCACCATGCTACTAACAGACC-
GAAACCTAAATAACAACCTTTCTTTGACCCGGCAGGGGGAGGAGATCCTATTCTATATCAACACCTG
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Etymology. The new species is named for Charles Darwin, who spent an “overpoweringly hot” October 1, 1835 exploring Tagus Cove and hiking into the Beagle Crater just south of the bay (quote from *The Voyage of the*

Beagle). Furthermore, the description serves as a somewhat belated recognition of the 150th anniversary of the publication of *The Origin of Species* in London in October 1860, mitigated to some small degree by the knowledge that the dilatory nature of the endeavor would not be particularly foreign to Darwin's sensibilities. The common name of Darwin's Sanddab is proposed.

Comparisons. The combination of low dorsal and anal fin-ray counts and low numbers of gill rakers distinguishes *C. darwini* from virtually all other *Citharichthys* species. The only eastern Pacific congener with fin-ray counts almost as low is *C. gordae*, which broadly overlaps the count for *C. darwini* (i.e. *C. gordae* mode of D 75 (72–79) and A 58 (53–62)), however the dorsal-fin count is generally higher and it has many more gill rakers (6–10 + 16–19) (van der Heiden *et al.* 2009). Most eastern Pacific *Citharichthys* have more gill rakers than *C. darwini*, only *C. stigmaeus* and *C. platophrys* share the 10 or fewer on the lower limb of the first arch. According to van der Heiden *et al.* (2009), some congeners, including *C. fragilis*, *C. gordae*, and *C. mariajorisae*, have deciduous or partially deciduous scales vs. firmly attached in *C. darwini*. In addition, *C. mariajorisae* has a notably wider body, with the maximum body width greater than 50% SL (van der Heiden & Mussot-Perez 1995).

Among Pacific congeners, *C. darwini* is closest in appearance and morphology to *C. platophrys*, which shares the low gill-raker count. In the key for eastern Pacific *Citharichthys* by van der Heiden (2009), *C. darwini* would key out to the line describing *C. platophrys*. There is a small overlap in dorsal and anal fin-ray counts; the lowest end of the range for *C. platophrys* (D 73–84, A 57–65; van der Heiden *et al.* 2009) coincides with the upper limit for *C. darwini*. Nevertheless, the DNA barcode sequences for the two species are 16.1% divergent and the lineages are about as distant from each other as they are from other congeners from both sides of the isthmus of Panama. Meristics clearly distinguish *C. darwini* from the known species of *Citharichthys* from the Atlantic Ocean. Hoshino *et al.* (2004) collated the counts for the genus from the Western Atlantic and, although several species share the low fin-ray counts of *C. darwini*, only *C. minutus* also has as few gill rakers; it can be distinguished from *C. darwini* by having many fewer lateral-line scales (34–36), as well as differing by 18.2% in the barcode DNA sequence.

The genus *Etropus* is very close to *Citharichthys* and the separation has been questioned for some time (Parr 1931, Leslie & Stewart 1986). Indeed, the barcode DNA sequences of some *Etropus* species fall within the *Citharichthys* clade (see below), although a phylogenetic study with more species and multiple genes is needed. *C. darwini*, like most *Citharichthys*, is separated from *Etropus* by a larger mouth; species of *Etropus* are characterized by a small mouth with the upper jaw length less than 30% HL and often the anterior cranial margin overlying the short upper jaw notably indented. Nevertheless, some species assigned to *Citharichthys* have small mouths, such as *C. arctifrons* (Parr 1931) and *C. minutus* (Cervigon 1996). *Etropus* species generally share low gill-raker counts with *C. darwini* although the gill rakers are described as stout or broad-based.

Among the eastern Pacific *Etropus*, both *E. ciadi*, with D 66–79 (mode 73) and A 52–62 (mode 57), and *E. delsmanni pacificus*, with D 71–77 (mode 75) and A 53–62 (mode 58), overlap the fin-ray counts for *C. darwini* (van der Heiden & Plascencia Gonzalez 2005 and Nielsen 1963, respectively). *E. delsmanni pacificus* differs in the generic character of a smaller mouth (24–30% HL; Nielsen 1963), along with a pronounced indentation in the anterior cranial margin overlying the short upper jaw (Fig. 5). The dorsal profile rises more sharply and the operculum is expanded to cover more of the cheek than



Figure 5 (right). *Etropus delsmanni pacificus*, USNM 383307, Coiba, Panama. Photograph courtesy Sandra Raredon, USNM



Figure 6. *Etropus delsmanni pacificus* holotype, ZMUC P-853137, Islas Perlas, Panama. Photograph courtesy Marcus Krag, Natural History Museum of Denmark.

the preoperculum, resulting in a distinctly wider head. These morphological differences can be quantified as follows (*E. delsmanni pacificus* vs. *C. darwini* respectively): first, the upper jaw length is less than the orbit diameter and much less than the horizontal span from the corner of the preoperculum to the edge of the operculum vs. equal or greater; and second, the body width at the end of the head is 39–43% SL (vs. 31–37%) (Fig. 6). In addition, *E. delsmanni pacificus* is slightly wider (on my measure from photographs of 4 types and a new specimen, USNM 383307) with a maximum body width from 43–46% SL (vs. 39–42% SL in the same size range), has fewer gill rakers (6–8 lower limb vs. 8–10), and does not have the sharp change in scale size near the body edges. They also appear to have deciduous scales and are found in deeper (45m) mud sediments along the coast of South America (Nielsen 1963). *E. ciadi* can be quickly distinguished by a wider body (>50% SL) and obviously deciduous scales (van der Heiden & Plascencia Gonzalez 2005).

DNA analysis. Most of the New World species comprising the *Cyclopsetta* group of paralichthyids have now been sequenced by various contributors to the Barcode of Life project. Almost all of the *Citharichthys* species are included, although a few lineages have only tentative specific identifications. In the neighbor-joining phenetic tree (generated by BOLD) of *Citharichthys* and *Etropus* (with *Syacium* and *Cyclopsetta* as outgroups), the nearest neighbor to *C. darwini* (i.e. sharing the same clade) is a juvenile *Citharichthys* sp. collected in the U.S. Virgin Islands, in the Atlantic (Fig. 7). There is, nevertheless, a large 17.8% sequence difference between the two species. Neighbor-joining trees as generated in BOLD are based on a clustering model (Ratnasingham & Hebert 2007) in which the nearest neighbor is not always the closest sequence in a simple similarity measure (as in the BLAST similarity model used by GenBank) or a probability-based model (such as the Hidden Markov model used in BOLD in the ID engine for fishes). Two sequences can have the same percent similarity in the sequence to a test sequence but may or may not cluster on the same branch of the tree assembled with a neighbor-joining model. In this case, *C. platophrys* is marginally more similar based on the simple similarity percentage, but is not as close as the USVI *Citharichthys* sp. in the phenetic tree.

Discussion. There are notably few museum records of paralichthyids from the Galápagos Islands and almost all are small specimens with tentative identifications. Upon review, it appears that many are *C. darwini* collected during the Allan Hancock Expedition of 1934 and subsequently divided up among the USNM, SIO, and CAS collections. The several additional museum specimens are small and often in poor condition; given the difficulty in distinguishing even genera in juvenile paralichthyid fishes, no firm identification can be made for them in the absence of DNA sequencing (not feasible in formalin-preserved specimens).

Previous surveys and listings of the fishes of the Galápagos Islands have not reported the Allan Hancock Expedition's collections of *Citharichthys* on Isla Isabela. Grove and Lavenberg (1997) list only three paralichthyid

Figure 7. The neighbor-joining tree based on the COI mtDNA sequences of New World *Citharichthys*, *Etropus*, *Cyclopsetta*, and *Syacium* (the *Cyclopsetta* group of paralichthyid flatfishes), following the Kimura two-parameter model (K2P) generated by BOLD (Barcode of Life Database). The scale bar at left represents a 2% sequence difference. Collection locations for specimens are indicated and *C. darwini* is highlighted with an asterisk. GenBank accession numbers and collection data corresponding to the sequences in the tree are listed in Appendix 1.



species (before the recognition of *C. gnathus* in 1999), including *Paralichthys woolmani*, *Syacium ovale*, and *C. gilberti*. The latter two are misidentified. The *S. ovale* record cited (LACM 44012) is *Syacium latifrons* by examination (see illustration by Richard Feeney, p. 860 in Grove and Lavenberg (1997)); i.e. the large male specimen has widely-separated eyes, D-87, A-70, and 8 lower-limb gill rakers, diagnostic for *S. latifrons* according to Murakami and Amaoka (1992) and van der Heiden and Mitchell (1998). The *C. gilberti* listing was decided by Grove and Lavenberg (1997) by process of elimination from the only two known tropical species at the time, based solely on a set of *Citharichthys* larvae collected off the Galápagos Islands by the Eastropac surveys in 1966 (Ahlstrom 1972). Those larvae were early stage and could not be identified to species (William Watson and Andrew Thompson, pers. comm.), but were noted in Grove and Lavenberg (1997) to have two extended dorsal fin spines compared to the three diagnostic for *C. platophrys*, and were assigned to *C. gilberti* based on that exclusion. They likely represent *C. darwini* or *C. gnathus*, the two species now known from the Galápagos Archipelago.

More recently, McCosker and Rosenblatt (2010) list four paralichthyid species for the Galápagos Islands, retaining *P. woolmani* and *S. ovale* and adding a widespread eastern Pacific flounder, *Hippoglossina bollmani*, and the newly recognized *C. gnathus*. Interestingly, all 19 of the *H. bollmani* records from the Archipelago (USNM 362263) were collected on the same day at the same trawl station (*Anton Bruun* Survey 791C, Sep. 21, 1966) as the type specimens (and only known collection) of *C. gnathus* in the Galápagos Islands (USNM 337310–337311; Hoshino & Amaoka 1999). That particular location has been recorded in Fraser (1943, p. 290) as one of the rare deep mud bottoms present in the Galápagos Islands: i.e. “mud and shell, between Daphne and Seymour at 55 fathoms”. The location of that trawl is listed as 95 m depth and from 0° 36' S to 0° 27' S in Hoshino and Amaoka (1999); however, with those coordinates the trawl starts on land on Isla Santa Cruz- the corrected coordinates should be 0° 26' S to 0° 27' S. Clearly, mud-associated continental species can be found when suitable habitat is available on the offshore islands. Another specimen of possible *H. bollmani* was collected by John McCosker on the Johnson Sea Link in 1995 at 300m off Isla Floreana (the coordinates in the CAS catalog for CAS 86410 indicate Devil's Crown on Floreana; not “off Isla Espanola” as listed), but its taxonomic status remains uncertain (McCosker & Rosenblatt 2010). In the most recent online checklist for Galápagos fishes, Tirado *et al.* (2013) list only *C. gnathus*, *H. bollmani*, and *P. woolmani* (and *S. ovale* under Bothidae).

Another small flatfish specimen (CAS 2984) not reviewed for prior species lists was collected in the Galápagos Islands by the Templeton Crocker Expedition (TCE 3723= CAS 2984–3085) on June 4, 1932, and it possesses serrated wide-based gill rakers consistent with *Syacium*. The 80.2 mm SL specimen has large eyes with the lower eye in advance of the upper, narrowing the identification to the widespread *S. latifrons* vs. *S. maculiferum*, the latter described as a Cocos Island endemic (Murakami & Amaoka 1992, van der Heiden & Mitchell 1998). The fin-ray count of D-86 and A-68 falls in the overlapping range between *S. latifrons* and *S. maculiferum*. The front-half body outline is distinctly more curved ventrally than dorsally (i.e. a low dorsal profile), a characteristic of *S. maculiferum*. In addition, the specimen has 9 lower-limb gill rakers on the first arch on both sides, a diagnostic feature for separating the two species (van der Heiden & Mitchell 1998). It is not surprising that *S. maculiferum* can reach the Galápagos Islands, since Cocos Island is the nearest offshore island in the region and I have collected numerous early-stage *S. maculiferum* larvae, identified by fin-ray counts and DNA barcoding, from the ocean over the Galápagos hydrothermal vents, located about 500 km south of Cocos Island and 350 km east of the Galápagos Archipelago (unpublished data; Victor 1987).

A review of museum records also revealed a set of late-stage *Citharichthys/Etropus* larvae collected off the southern Galápagos Islands by A. Ebeling on Nov. 28, 1955 (ET-H-60) about 80 km SSW of the island of Floreana (SIO-55-258). The larvae are in relatively good condition and are clearly paralichthyid larvae (Moser & Sumida 1996, Lyczkowski-Shultz & Bond 2006). They do not belong to the *Syacium/Cyclopsetta* group since they are missing the diagnostic horn-like cranial spines and ornate spikes at the corner of the preoperculum. They have the characters of the *Citharichthys/Etropus* group, i.e. limited body pigmentation with melanophores over the hindgut and cleithral symphysis, a melanophore streak along the posterior notochord, widely spaced melanophore streaks along the base of the dorsal and anal fin pterygiophores (spreading distally), a streak along a posterior myoseptum, small spines in irregular rows on the preoperculum, as well as some elongation of the first few dorsal-fin rays.

The 1955 larvae do not match the known larvae of any of the northern species described in Moser and Sumida (1996) or the descriptions of the tropical species *C. platophrys* or *C. gilberti* (Moser & Sumida 1996, Beltrán-

Leon & Herrera 2000), in particular not sharing the obvious tail bar of those species. Larvae of some Atlantic species of *Citharichthys* also have a single streak at that location, as do larvae of *Etropus crossotus*, the only eastern Pacific member of *Etropus* with described larvae (Moser & Sumida 1996, Beltrán-Leon & Herrera 2000, Lyczkowski-Shultz & Bond 2006). However, the Galápagos larvae differ in several ways from the described larvae of *E. crossotus*: they are more deep-bodied, are missing the melanophore at the pectoral axil, have only two widely separated pigment patches, about 20 rays apart, along the base of the anal-fin pterygiophores (vs. rows of melanophores along the bases of the anal-fin pterygiophores as well as along the insertion line of the rays), and have melanophores in a vertical collection over the hindgut (vs. horizontal along the ventral abdominal margin). The larvae from 1955 have a fin-ray count of D-87 and A-67, higher than that of most of the species in the group and excluding many, especially *C. darwini*, nevertheless the count does overlap the uppermost range for several species: i.e. *E. crossotus*, *C. gnathus*, *C. mariajorisae*, the southernmost-populations of *C. gilberti*, and slightly exceeds the range reported for *Etropus peruvianus* (van der Heiden & Mussot-Perez 1995, Hoshino & Amaoka 1999, van der Heiden & Plascencia Gonzalez 2005). Since *C. gilberti* larvae are reported to have a prominent tail bar, these larvae are not likely to be *C. gilberti*. Without fully matching fin-ray counts and given that several regional species are without larval descriptions, their species identity remains unknown and *C. gnathus* cannot be excluded.

There are now six species of paralicthyid flatfishes confirmed from the Galápagos Islands, yet only one, *P. woolmani*, is routinely encountered in surveys. *C. gnathus* and *H. bollmani* are found only on the scarce deep mud substrate in the Archipelago, *S. latifrons* and *S. maculiferum* are apparently rare with a single record each, and the new species, *C. darwini*, is documented only on Isla Isabela. This curious distribution for the new species may be related to the distinctive oceanographic conditions that develop on the western edge of the Galápagos Archipelago. The islands of Fernandina and Isabela are directly over the Galápagos hotspot and both have recently active volcanoes and are exceptionally dry. In addition, extensive local upwelling of colder deep water occurs seasonally in the area. This unusual combination of colder water and particularly light erosion sedimentation due to low precipitation and rocky shores (Edgar *et al.* 2004) creates a distinctive habitat. Tagus Cove itself is a breached tuff cone and is characterized by coarse black sands (Glynn & Wellington 1983). Areas of upwelling and cool water elsewhere in the eastern Pacific are typically near continental shores with a heavy sedimentation load and, concomitantly, a much finer substrate.

Other Material Examined. USNM 43424, Paralicthyidae, (1) Ecuador, Galápagos Islands, off Gardner (-1.35°, -89.65°), Albatross, trawl, 37m, Apr. 7, 1888 (catalogued as *C. gilberti*); USNM 362518/9, Paralicthyidae, (5) Ecuador, Galápagos Islands, Isla Santiago, west side (-0.20°, -90.85°), trawl 794E, 34m, Dec. 10, 1934 (catalogued as *Syacium* sp., but fin-ray counts are below the range for *Syacium* spp.); LACM 44012, *Syacium latifrons* (1) 202 mm SL, Ecuador, Galápagos Islands, Isla Isabela, Tagus Cove; CAS 2984, *Syacium maculiferum* (1) 80.2 mm SL, Ecuador, Galápagos Islands, Isla Santiago, James Bay; SIO 55-258, Paralicthyidae (4 larvae) 9.5–10.5 mm SL, Ecuador, Galápagos Islands, 80 km. SSW of Floreana.

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Genus	species	Collection site	Voucher	GenBank #	Collector
<i>Citharichthys</i>	<i>arctifrons</i>	USA, off Virginia	KU 27142	JX516098	K. Moots, KUI Tissue
<i>Citharichthys</i>	<i>arenaceus</i>	Panama, Portobelo	n7529af1244	HQ987852	B. Victor
<i>Citharichthys</i>	<i>arenaceus</i>	Panama, Portobelo	n762bc130	JX516095	B. Victor
<i>Citharichthys</i>	<i>cornutus</i>	USA, off Texas	KU 30202	KC170026	K. McNyset, KUI Tissue
<i>Citharichthys</i>	<i>darwini</i>	Isabela, Galápagos	SIO 12-3075	JX516097	B. Victor/G. Wellington
<i>Citharichthys</i>	<i>dinoceros?</i>	Quintana Roo, Mexico	ECO-CHLP3862	HM389452	L. Vásquez Yeomans
<i>Citharichthys</i>	<i>fragilis</i>	Baja California, Mexico	CBN 5221	JN312654	E. Balart, CIBNOR
<i>Citharichthys</i>	<i>fragilis</i>	Baja California, Mexico	CBN 5221	JN312655	E. Balart, CIBNOR
<i>Citharichthys</i>	<i>gilberti</i>	El Salvador	KU 40295	KC170027	A. Bentley, KUI Tissue
<i>Citharichthys</i>	<i>gilberti</i>	El Salvador	KU 40286	KC170028	A. Bentley, KUI Tissue
<i>Citharichthys</i>	<i>gymnorhinus?</i>	Belize	ECO-CHLP4278	HM390025	L. Vásquez Yeomans
<i>Citharichthys</i>	<i>macrops</i>	Quintana Roo, Mexico	ECO-CHLP5266	JN313699	L. Vásquez Yeomans
<i>Citharichthys</i>	<i>minutus</i>	Panama, Portobelo	n7528ac140	JX516092	B. Victor
<i>Citharichthys</i>	<i>minutus</i>	Panama, Portobelo	n7528ac200	JX516100	B. Victor
<i>Citharichthys</i>	<i>platophrys</i>	Galápagos Rift vents	gv85310cp122	JX516093	B. Victor
<i>Citharichthys</i>	<i>platophrys</i>	Galápagos Rift vents	gv85310cp79	JX516099	B. Victor
<i>Citharichthys</i>	<i>platophrys</i>	Galápagos Rift vents	gv85310cp90	JX516089	B. Victor
<i>Citharichthys</i>	<i>sordidus</i>	San Diego, CA	SIO 02-19	GU440282	P. Hastings, H.J. Walker
<i>Citharichthys</i>	<i>sordidus</i>	Ventura, CA	CMB VH-11	JQ672526	R. Imondi
<i>Citharichthys</i>	<i>sordidus</i>	Ventura, CA	CMB VH-11	JQ694029	R. Imondi
<i>Citharichthys</i>	<i>sp. a</i>	St. Thomas, USVI	st80628cu122	HQ987850	B. Victor
<i>Citharichthys</i>	<i>sp. a</i>	St. Thomas, USVI	st954p115	HQ987846	B. Victor
<i>Citharichthys</i>	<i>spilopterus</i>	Panama, Portobelo	n7529af150	HQ987853	B. Victor
<i>Citharichthys</i>	<i>spilopterus</i>	Panama, Portobelo	n762cc110	JX887474	B. Victor
<i>Citharichthys</i>	<i>spilopterus</i>	Panama, Portobelo	n7528bc137	JX887473	B. Victor
<i>Citharichthys</i>	<i>stigmaeus</i>	Puget Sound, WA	UW 48811	JQ354050	W. Pallson
<i>Citharichthys</i>	<i>stigmaeus</i>	Puget Sound, WA	UW 48784	JQ354051	W. Pallson
<i>Citharichthys</i>	<i>xanthostigma</i>	San Diego, CA	UW 119902	JQ354052	NMFS
<i>Citharichthys</i>	<i>xanthostigma</i>	San Diego, CA	SIO 02-19	GU440283	P. Hastings, H.J. Walker
<i>Cyclosetta</i>	<i>chittendeni</i>	USA, off Texas	KU 30228	JX516094	K. McNyset, KUI Tissue
<i>Cyclosetta</i>	<i>panamensis</i>	Galápagos Rift vents	gv85310cp90	JX887475	B. Victor
<i>Cyclosetta</i>	<i>querna</i>	Costa Rica	JHLOW00205	JX887476	J. Lowenstein
<i>Etropus</i>	<i>microstomus</i>	USA, off Virginia	KU 27165	JX516090	K. Moots, KUI Tissue
<i>Syacium</i>	<i>gunteri</i>	Panama, Portobelo	n761f190	JX887477	B. Victor
<i>Syacium</i>	<i>maculiferum</i>	Galápagos Rift vents	gv85310sm60	JX887478	B. Victor
<i>Syacium</i>	<i>micrurum</i>	Belize	cn10f133	HQ987876	B. Victor
<i>Syacium</i>	<i>papillosum</i>	Belize	cn11sy64	JX887479	B. Victor
<i>Syacium</i>	<i>papillosum</i>	Belize	ECO-CHLP3755	GU224775	L. Vásquez Yeomans
<i>Syacium</i>	<i>papillosum</i>	Quintana Roo, Mexico	ECO-CHLP4304	HM390055	L. Vásquez Yeomans

Appendix 1. Specimen data and GenBank accession numbers for the mtDNA COI sequences used in the phenogram in Fig. 7 (alphabetical order).