Zoological Studies **51**(3): 415-423 (2012)

Zoological Studies

The Blue Velvet Angelfish *Centropyge deborae* sp. nov., a New Pomacanthid from the Fiji Islands, Based on Genetic and Morphological Analyses

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(Accepted November 4, 2011)

Kang-Ning Shen, Hsuan-Ching Ho, and Chih-Wei Chang (2012) The blue velvet angelfish *Centropyge deborae* sp. nov., a new pomacanthid from the Fiji Islands, based on genetic and morphological analyses. *Zoological Studies* **51**(3): 415-423. A new species of angelfish, *Centropyge deborae* sp. nov., is described from 6 specimens collected in the Fiji Is. It is morphologically similar to the midnight angelfish *C. nox*, but differs in having a metallic-blue luster over the black ground color of the fish, black pectoral-fin rays with a transparent fin membrane when alive, and 13 or 14 dorsal-fin spines, and by lacking a yellowish patch at the pectoral-fin base. Genetic analyses of mitochondrial 16S and cytochrome oxidase subunit I and nuclear ETS2 markers confirmed a species-level separation of *C. deborae* sp. nov., and rejected the hypothesis that *C. deborae* sp. nov. is a suspected hybrid of the sympatric congeners *C. nox* and *C. tibicen* or *C. bispinosa*, each of which also bears a metallic-blue luster. http://zoolstud.sinica.edu.tw/Journals/51.3/415.pdf

Key words: *Centropyge nox*, New species, Hybridization.

Marine angelfishes are perciform fishes of the family Pomacanthidae. They are found on shallow reefs in the tropical Atlantic, Indian, and Pacific Oceans, but primarily in the western Pacific. The family contains 8 genera with about 82 species worldwide (Nelson 2006). Because of their striking color patterns either as juveniles or adults, as well as their variety of sizes, from the approximately 3-cm pygmy *Centropyge* to the 45-cm adult *Pomacanthus* species, the pomacanthids are popular ornamental fishes of considerable value and importance in the world aquarium trade (Wood 2001).

Numerous examples of natural hybridization in pomacanthids were reported since the publication of Allen's (1985) book on the family. The number of reported pomacanthid hybrids is the 2nd highest for marine fish species, behind that of butterflyfishes (Pyle and Randall 1994). Pyle and Randall (1994) reviewed and proposed at least 16 examples of pomacanthid hybrids that could be documented by morphometric and meristic data. Among them, the majority were in *Centropyge*, with 7 hybrids; there were also 5 hybrids in *Pomacanthus*, 2 in *Chaetodontoplus*, and one each in *Apolemichthys* and *Holacanthus*. The reality of hybridization and the relationship between the hybrid and either of the suspected parent species can be confirmed when genetic analyses are incorporated. In terms of *Centropyge* genetics, for instance, the 3 Atlantic pygmy *Centropyge* congeners were confirmed to be a single genetic clade by the genealogy of the mitochondrial (mt)DNA control region (Bowen et al. 2006, Rocha et al. 2007). The flame angelfish

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C. loriculus lacked significant genetic partitioning among 3 color morphs and among sampling locations in the central Pacific when the molecular diversity of the mitochondrial cytochrome *b* gene was compared (Schultz et al. 2007).

The midnight angelfish, also known as the black pygmy angelfish, *C. nox* (Bleeker 1853), is the only member in the family Pomacanthidae with a uniformly black body (Myers 1991). It was recorded in the western Pacific from the Ryukyu Is. to Indonesia, the Great Barrier Reef, New Caledonia, and Micronesia (Myers 1991 1999, Pyle 2001, Allen and Adrim 2003). Two photographs of an unidentified 95-mm *Centropyge* angelfish were reported in the Japanese aquarium magazine, *Marine Aquarist* (no. 56, Summer 2010, p. 94), along with the following text translated from the Japanese: "A mysteriously black pygmy angelfish imported from the Fiji Is. in the South Pacific might be a new finding of a small-sized angelfish. The fish would be regarded as the midnight angelfish *C. nox* by aquarium-fish importers. However, a metallic-blue luster on the black color of the fish was never seen in the adult *C. nox*. The color of the pectoral fin rays was black, and the fin fold was transparent, being distinctive from the solely black color of the pectoral fin of *C. nox*. The body shape, head outline, and metallic-blue luster of the fish resembled the yellow-fin angelfish *C. flavipectoralis* Randall and Klausewitz 1977 and the blue-fin dusky angelfish *C. multispinis* (Playfair 1867) from the Indian Ocean."

At almost the same time the unknown *Centropyge* was reported in the Japanese magazine, 6 identically colored individuals (Fig. 1A, B) were obtained through the marine aquarium trade by the 1st author in Aug. 2010 in Taiwan. Our speculation, without referring to the above-mentioned magazine report, also centered on the suspicious relationship between the possibly new fish and *C. nox* (Fig. 1C). However, instead of *C. flavipectoralis* and *C. multispinis* being the potential parents of a hybrid or sister-groups to the possible new fish, the sympatrically overlapping

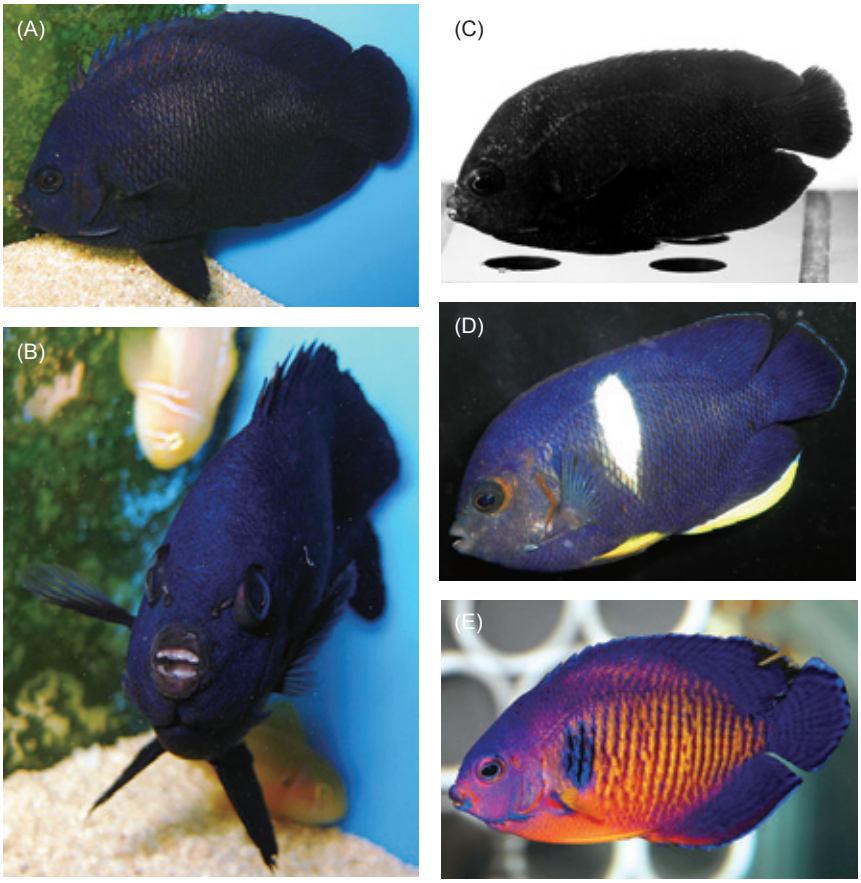

Fig. 1. Living individuals of 4 *Centropyge* species: (A, B) *Centropyge deborae* sp. nov.; (C) *C. nox*; (D) *C. tibicen*; and (E) *C. bispinosa*. Photographs were taken by W. Smith (A, B) and C.L. Lin (C-E).

Pacific congeners *C. tibicen* (Cuvier, 1831) and *C. bispinosa* (Günther, 1860), both of which bear a metallic-blue luster on the body, were thought to have a higher probability of being parent species with *C. nox* of the unknown *Centropyge* (Fig. 1D, E).

By substantial genetic analyses of mitochondrial and nuclear DNA markers, a species-level separation of the 4 angelfishes was confirmed, while a hypothesized hybridization relationship was rejected. Thus, this study presents genetic and morphological evidence to support these 4 distinct congeners, and to name and describe the new *Centropyge* species.

MATERIALS AND METHODS

Specimen collection

Six specimens of the new *Centropyge* examined were collected in the Bligh Water, east of Yasawa Is., western Fiji and purchased from a commercial dealer engaged in the legal aquarium trade in Taiwan. For both genetic and morphological comparisons, 13 additional individuals of *C. nox*, 4 *C. tibicen*, and 13 *C. bispinosa* were also obtained from the legal aquarium trade which had been imported from either the Philippines or Indonesia.

The type series of the new species and comparative materials are cataloged in the Pisces Collection of the National Museum of Marine Biology and Aquarium (NMMB-P), Checheng, Pingtung, Taiwan, and the Smithsonian National Museum of Natural History (USNM), Washington, DC, USA.

Morphological analysis

Standard length (SL) and head length (HL) in millimeters were used throughout. Morphometric measurements and methods for taking the meristic data followed Randall and Rocha (2009). SL, body depth, caudal-peduncle length, caudal-peduncle depth, predorsal length, preanal length, prepelvic length, lengths of the fin spines and rays, and caudal fin length were taken from x-rays of the fish. The remaining measurements were taken directly from the fish using electronic calipers and were rounded to the nearest 0.1 mm. Dorsal-, anal- and caudal-fin spines and rays were counted on x-rays. Pectoral-fin rays were counted on both sides, except for those that were damaged.

Genetic analysis

Epaxial muscles of the trunk were removed and preserved in 95% ethanol prior to DNA extraction. Genomic DNA was extracted from muscle tissue using a DNA Purification Kit (Bioman, Taipei, Taiwan), preserved in TE buffer, and then quantified and diluted to 1 ng/μl for a polymerase chain reaction (PCR). Three different categories of molecular markers, 1 nuclear marker (ETS2), and 2 mitochondrial markers (16S and cytochrome oxidase subunit I (COI)) were applied. ETS2 is an intron from a nuclear oncogene originally used as a conserved mammalian single-locus DNA marker (Lyons et al. 1997). The ETS2 primers, based on flanking exonic regions, also amplify fish DNA (van Herwerden et al. 2002, Klanten et al. 2004). Primers of ETS2 (ETS2F: 5'-AGCTGTGGCAGTTTCTTCTG-3' and ETS2R: 5'-CGGCTCAGCTTCTCGTAG-3' (Lyons et al. 1997)), 16S ribosomal (r)RNA (LR-J-12887: 5'-CCGGTCTGAACTCAGATCACGT-3' and LRN-13398: 5'-CGCCTGTTTACCAAAAACAT-3' (Simon et al. 1994)), and COI (FishF2: TCGACTAATCATAAAGATATCGGCAC and FishR2: ACTTCAGGGTGACCGAAGAATCAGAA (Ward et al. 2005)) were PCR-amplified in a Biometra TGradient Thermocycler with a 15-μL reaction volume that contained 0.2 μm dNTPs, 1.5 μL of 10× PCR buffer (Bioman), 0.5 μm each of the forward and reverse primers, 0.2 U *Taq* DNA polymerase (Bioman), and 10 ng of template DNA. DNA amplification of the 3 target genes was carried out using the following PCR conditions: 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for ETS2, 54°C for 16S, and 52°C for COI for 15 s, and extension at 72°C for 30 s. A final extension was at 72°C for 10 min. PCR products of the mitochondrial 16S and COI genes were directly sequenced using the same primer for the PCR. PCR products of ETS2; however, had 2 bands of around 500 and 135 bp in size; sequencing from both directions could exclude noise from sequencing the small fragments to obtain an entire 474-bp ETS sequence. Sequences were analyzed on an automated ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA, USA) at the Taiwan Normal University Sequencing Facility (Taipei, Taiwan).

All genes were automatically aligned using MAFFT vers. 6 (Katoh et al. 2002). Phylogenetic trees were reconstructed using Maximum-likelihood (ML) and Neighbor-joining (NJ) analyses with the Bayesian information criterion (BIC) and 1000

bootstraps in MEGA5 (Tamura et al. 2011). For the mitochondrial 16S gene, the transversion model with invariable sites of the Kimura 2-parameter (K2) + G (gamma = 0.05) was selected according to the best-fitting substitution model test. For the mitochondrial COI gene, the transversion model with invariable sites K2 + I (I = 0.67) was selected. For the nuclear ETS2 gene; however, the Jukes-Cantor (JC) model with a uniform substitution rate was selected. Pairwise the *p*-distances including both transitions and transversions were also calculated using MEGA5. Nodes with bootstrap values of ≥ 70% were considered to be well supported (Hillis and Bull 1993). All sequences obtained in this study were deposited in GenBank (JQ904543-JQ904590).

RESULTS

Centropyge deborae sp. nov.
(Figs. 1A-B, 2-4, Tables 1, 2)

Material examined: Holotype: NMMB-P 12641, 67.4 mm SL, western (17°9.208'S, 177°41.397'E) to eastern part (17°14.349'S, 178°41.397'E) of the Bligh Water, Fiji Is., South Pacific Ocean, 5-25 m in depth, Aug. 2010, coll. W. Smith (Fig. 2)

Paratypes: NMMB-P 12642, 57.9 mm SL; NMMB-P 12643, 56.9 mm SL; NMMB-P 12644, 69.5 mm SL; USNM 404486, 64.0 mm SL; USNM 404487, 63.1 mm SL; all collected from near the holotype locality.

Etymology: We took the recommendation of Walt Smith, who collected the type series and provided the underwater photograph and detailed collecting data of the new species for this study, to name the fish after his wife, Deborah Smith. Deborah is an Israelitic name that does not exist in Latin. The genitive form of the non latin feminine word is "deborahae". For reasons of euphony, however, the "deborae" was finally adapted. The new common English name of this species should be the blue velvet angelfish.

Fig. 2. *Centropyge deborae* sp. nov.: Holotype, NMMB-P12641, 67.4 mm standard length.

Diagnosis: Dorsal-fin spines XIII or XIV (usually XIV); dorsal-fin rays 16 or 17 (usually 16); anal-fin rays 17 or 18 (usually 17); pectoral-fin rays 15 or 16 (usually 16); scales large, about 43 or 44 in longitudinal series; gill rakers 5 or 6 + 12 or 13; supraneural bone 1; body depth 1.7-1.8 in SL; head length 3.2-3.5 in SL; last dorsal spine longest, 1.2-1.4 in HL; preopercular spine (10.0%-12.8% SL), slightly longer than eye diameter; relatively deep cheek depth (13.6%-15.5% SL); color when alive bluish-black with whitish posterior caudal-fin margin, pectoral fin rays black, and fin membrane transparent; color in alcohol uniformly black with a transparent posterior caudal-fin margin.

Description: The following data are given for the holotype. Those in parentheses are the range of the type series, if different from that of holotype. Dorsal fin XIV, 16 (XIII or XIV, XIII in 1 paratype; 16 or 17, 17 in 1 paratype); anal fin III, 17 (17 or 18, 18 in 1 paratype); all dorsal- and anal-fin rays branched, the last to base; pectoral-fin rays 16 (15 or 16, 15 on 1 side of 69.5-mm paratype), upper 2 and lowermost unbranched; pelvic fin I, 5; principal caudal-fin rays 17, upper and lower unbranched; upper procurrent caudal rays 4 (3 or 4); lower procurrent caudal-fin rays 3; longitudinal scale series 43 (43 or 44); pored lateral-line scales 31 + 7 = 38 (30-33 + 5-7 = 35-40); pseudobranchial filaments 13 (12 or 13); branchiostegal rays 6; gill rakers 6 + 13 = 19 (5 or 6 + 12 or 13 = 17-19); vertebrae 10 + 14; supraneural 1.

Body moderately deep, depth 1.7-1.8 in SL, and compressed, width 3.0-3.4 in depth; HL 3.2 (3.2-3.5) in SL; dorsal profile of head forming an angle of about 75°, with slight concavity above eye and slight convexity before dorsal fin; snout short, length 3.4 (3.2-3.6) in HL; interorbital width 4.0 (3.5-4.0) in HL; cheek depth 2.2 (1.9-2.3) in HL; caudal-peduncle depth 2.1 (1.9-2.1) in HL; caudal-peduncle length 4.1 (3.7-4.3) in HL.

Mouth small and terminal, maxilla reaching below anterior nostril, and strongly oblique when fully closed, forming an angle of about 60° to horizontal axis of head and body; lower jaw not projecting; jaws slightly protractible; lips broad, median depth of upper lip about 1/2 orbital

diameter; teeth in jaws in 4 rows, inner rows progressively shorter; teeth close-set, long and slender, about twice as wide as thick, tips slightly incurved, expanded, and tricuspid; central cusp of teeth largest and strongly pointed; no teeth on palate; tongue short and rounded, set far back in mouth; gill membranes narrowly attached to isthmus; gill rakers relatively long, about 1/3 length of gill filaments.

Anterior nostril a short fleshy tube with small opening, about 1/3 eye diameter before anterior margin of eye; posterior nostril a narrow elliptical aperture, close to anterior nostril. Strong spine at corner of preopercular, its length 4.1 (3.7-4.1) in SL; lower 1/2 of posterior margin of opercle with 9 or 11 (9-12) small serrae; posterior margin of preopercle with 18 or 20 (16-20), small and unevenly spaced serrae, some as tiny nodules; lower margin of preopercle with 1 or 2 (1-3) small serrae; margin of subopercle with 3 or 4 (3 or 4) small serrae; preorbital with 6 or 7 (6-8) small serrae.

Dorsal part of lateral line strongly arched to middle of body, curving downward to end near rear base of dorsal fin; extra separate lateral line on midlateral part of caudal peduncle; scales on body not in regular rows, coarsely ctenoid, with up to 21 cteni, continuing as ridges across exposed part of scales; many scales on body with auxiliary scales (also ctenoid); scales smaller on head, progressively smaller anteriorly; scales extending out on dorsal and anal fins as rows of narrow oblique ridges, scales progressively smaller distally; no scales on 1st 2 dorsal-fin spines and membranes or about outer 1/2 of next 2 spines and membranes; caudal fin densely covered with very small scales; rays of pectoral fins with a row of closely set, quadrangular scales, only those basally on rays with a few cteni; pelvic fin with small ctenoid scales on rays.

Origin of dorsal fin above 1st lateral-line scale, predorsal length 2.4 (2.4-2.7) in SL; 1st dorsal-fin spine 3.9 (2.7-3.9) in HL; 2nd dorsal-fin spine damaged in holotype, 2.0-2.4 in HL in paratypes; last dorsal-fin spine longest, 1.4 (1.2-1.4) in HL; 8th or 9th dorsal-fin ray longest, length 1.3 (1.2-1.3) in HL; origin of anal fin below base of 7th dorsal-fin spine, preanal length 1.6 (1.6-1.7) in SL; 1st anal-fin spine 2.1 (1.9-2.4) in HL; 2nd anal-fin spine 1.6 (1.4-1.7) in SL; 3rd anal-fin spine longest, its length 1.2 (1.1-1.4) in HL; 8th or 9th anal-fin ray longest, length 1.3 (1.2-1.4) in HL; origin of pelvic fins below midbase of pectoral fins, prepelvic length 2.6 (2.6-2.8) in SL; pelvic spine 1.7 (1.4-1.7) in HL; 1st pelvic-fin ray longest, reaching posterior to anus, 1.1 (1.1-1.4) in HL.

Coloration: Uniformly bluish-black with a narrow white posterior margin on caudal fin, black pectoral-fin rays, and transparent fin membrane when alive. In alcohol, uniformly black with a narrow posterior transparent margin on caudal fin.

Distribution: Known from the type series collected from the Bligh Water, east of Yasawa Is., Fiji.

Genetic analysis: In total, 596 bp of the 16S gene was amplified for *C. deborae* sp. nov. and *C. nox*. The *p*-distance between *C. deborae* sp. nov. and *C. nox* was 0.013-0.015, with transitions in all 8 variable sites. The 16S gene sequence of *C. tibicen* had 2 nucleotide deletions. The *p*-distance between *C. deborae* sp. nov. and *C. tibicen* ranged 0.027-0.03, with 1 nucleotide transversion among the 16 variable sites. The 16S sequence of *C. bispinosa* had 1 nucleotide deletion. The *p*-distance between *C. deborae* sp. nov. and *C. bispinosa* ranged 0.054-0.057, with 34 variable sites (9 transversions and 25 transitions).

In total, 648 bp of the COI gene was amplified for all *C. deborae* sp. nov. The *p*-distances between *C. deborae* sp. nov. and *C. nox* were 0.039-0.051, with 26 variable sites (3 transversions and 23 transitions). The *p*-distances between *C. deborae* sp. nov. and *C. tibicen* ranged 0.111-0.114, with 20 nucleotide transversions among the 74 variable transition sites. The *p*-distances between *C. deborae* sp. nov. and *C. bispinosa* ranged 0.153-0.156, with 99 variable sites (27 transversions and 72 transitions).

Phylogenetic reconstruction using both the ML and NJ phylogenetic trees for the mitochondrial 16S and COI genes suggested that *C. deborae* sp. nov. genetically differs from all other examined congeners, which was supported by very high bootstrap values (Fig. 3). *Centropyge nox* was the most-closely related species to *C. deborae* sp. nov., followed by *C. tibicen*. *Centropyge bispinosa* was the most divergent species from the others (Fig. 3).

In addition, all 4 species exhibited a homozygote in the nuclear ETS2 gene. The *p*-distance between *C. deborae* sp. nov. and *C. nox* was 0.002, with only 1 nucleotide transition, while between *C. deborae* sp. nov. and *C. tibicen*, the *p*-distance was 0.005 with 2 transition sites. The *p*-distances between *C. deborae* sp. nov. and *C. bispinosa* ranged 0.0323-0.0342, with 14 variable sites represented by 11 transitions, 2 transversions, and 1 nucleotide deletion.

The phylogenetic tree of the nuclear ETS2 gene constructed using both the ML and NJ methods also indicated that *C. deborae* sp. nov. was closer to, but differed from, *C. nox* (Fig. 4A). The lower bootstrap support value was due to the smaller mutation rate in this nuclear intron gene between species. One nucleotide difference at the 172nd nucleotide within 474 bp of the ETS2 gene was recognized between *C. deborae* sp. nov. and *C. nox* (Fig. 4B). This single-nucleotide polymorphism (SNP) in *C. deborae* sp. nov., compared to the other 3 *Centropyge* species examined, and a homozygote genotype in the ETS2 gene for all 4 species, suggested that *C. deborae* sp. nov. is unlikely to be a hybrid of the other 3 species.

DISCUSSION

Except for the metallic-blue luster, coloration patterns clearly differed among *C. deborae* sp. nov., *C. tibicen*, and *C. bispinosa*. The living *C. nox* exhibits a uniformly black body color with a yellow patch at the pectoral-fin base. The living *C. deborae* sp. nov. instead reveals a bluish-black body color with no yellow patch at the pectoral-fin base (Fig. 1).

Comparisons of the morphometric and meristic characters of the 4 *Centropyge* species

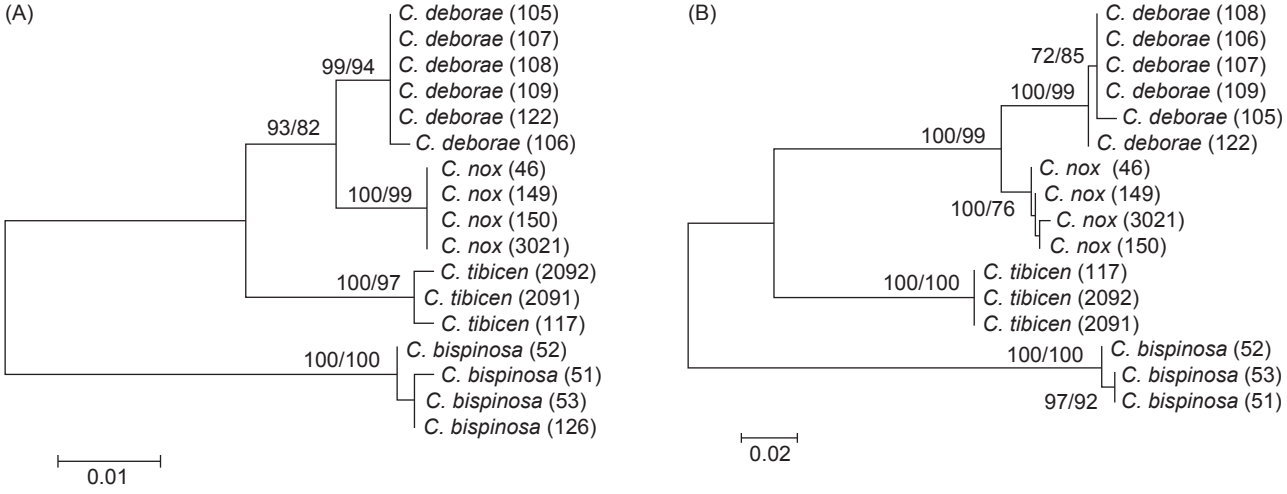

Fig. 3. Phylogenetic trees reconstructed using the (A) mt 16S and (B) cytochrome oxidase subunit I (COI) genes of 4 *Centropyge* species. Values above the branches are respective bootstrap values for the Neighbor-joining (NJ) and Maximum-likelihood (ML) analyses.

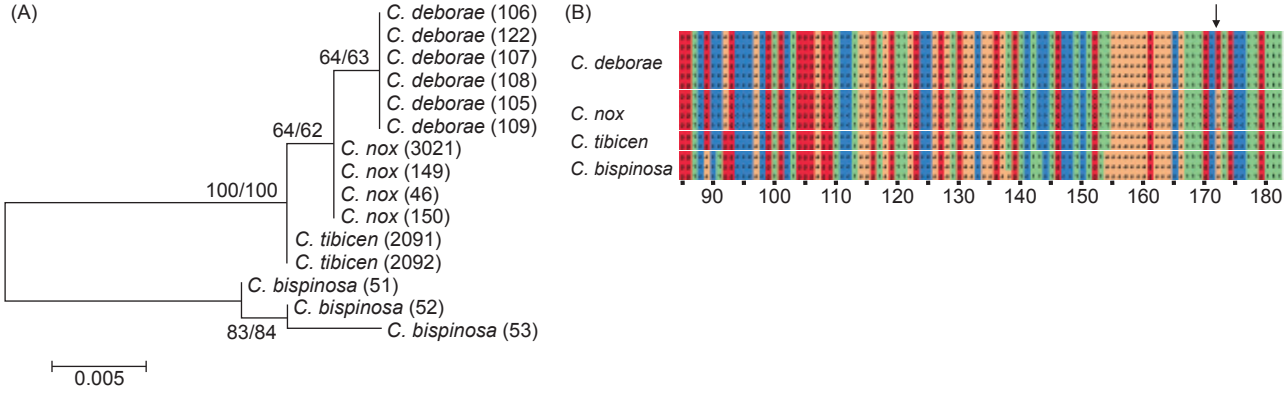

Fig. 4. (A) Phylogenetic trees reconstructed using the nuclear intron ETS2 gene of 4 *Centropyge* species. Values above the branches are respective bootstrap values for the Neighbor-joining (NJ) and Maximum-likelihood (ML) analyses. (B) One nucleotide difference (black rectangle) at the 172nd nucleotide in the homozygote nuclear ETS2 of *C. deborae* sp. nov. The number under the sequence alignment is the number of PCR-amplified ETS2 nucleotides.

Table 1. Morphometric data of the 4 *Centropyge* species

	C. deborae sp. nov.			*C. nox*		*C. tibicen*		*C. bispinosa*	
	Holotype	Type series (*n* = 6)		*n* = 13		*n* = 4		*n* = 13	
SL (mm)	67.4	56.9-69.5		38.0-53.8		43.2-71.7		43.3-66.1	
Proportion (% SL)		Mean (Range)	S.D.	Mean (Range)	S.D.	Mean (Range)	S.D.	Mean (Range)	S.D.
Body depth	55.2	56.7 (55.2-59.1)	1.4	52.9 (50.9-59.7)	2.2	53.4 (51.2-56.3)	2.3	52.6 (48.3-56.5)	2.2
Body width	16.9	17.9 (16.5-19.5)	1.2	17.9 (15.6-20.2)	1.4	19.5 (18.3-21.9)	1.6	17.8 (15.8-19.6)	1.0
Head length	31.5	30.1 (28.6-31.5)	1.2	30.2 (28.9-32.6)	1.0	28.8 (26.7-29.9)	1.4	29.2 (26.5-30.9)	1.3
Snout length	9.3	9.0 (8.1-9.4)	0.5	10.1 (8.8-12.1)	1.0	10.5 (8.3-13.5)	2.2	9.1 (7.3-10.4)	0.9
Cheek depth	14.4	14.5 (13.6-15.5)	0.6	13.3 (12.2-14.4)	0.6	13.9 (12.0-14.6)	1.2	12.0 (11.4-12.7)	0.4
Upper jaw length	6.7	7.1 (6.7-7.6)	0.3	7.7 (6.9-9.5)	0.7	7.8 (6.5-10.2)	1.6	7.8 (6.2-8.7)	0.8
Orbital diameter	10.5	10.4 (9.8-10.9)	0.4	11.1 (10.4-12.4)	0.5	10.6 (9.2-11.6)	1.1	11.3 (10.0-12.7)	0.9
Interorbital width	7.9	8.3 (7.9-8.6)	0.3	9.1 (8.1-10.4)	0.7	8.5 (6.7-10.0)	1.4	9.2 (8.2-10.3)	0.6
Preopercular spine	12.8	11.5 (9.5-12.8)	1.4	7.7 (6.1-9.5)	1.2	7.5 (5.8-9.7)	1.8	7.4 (6.4-8.2)	0.7
Caudal-peduncle depth	15.1	15.2 (14.6-15.9)	0.5	14.2 (13.7-14.7)	0.3	15.3 (14.2-16.3)	1.0	14.0 (13.2-15.5)	0.8
Caudal-peduncle length	7.7	7.4 (6.7-7.9)	0.5	7.0 (6.2-9.0)	0.9	6.2 (5.6-7.3)	0.7	6.7 (4.7-9.0)	1.4
Predorsal length	41.1	40.7 (37.1-41.8)	1.8	41.2 (38.7-43.8)	1.3	38.1 (35.0-40.4)	2.2	41.0 (39.0-47.3)	2.1
Prepelvic length	37.2	37.4 (35.8-38.7)	1.0	39.5 (36.6-42.2)	1.9	40.2 (37.3-41.7)	1.9	38.3 (36.3-43.0)	1.9
Preanal length	61.3	62.4 (59.7-63.9)	1.6	62.1 (59.3-64.3)	1.5	63.5 (61.6-66.5)	2.2	62.2 (57.2-65.2)	2.4
1st dorsal-fin spine	8.0	9.2 (7.8-10.7)	1.1	8.5 (7.6-9.4)	0.7	9.4 (8.1-10.2)	0.9	8.1 (6.6-9.2)	0.8
2nd dorsal-fin spine	-	13.0 (12.3-14.2)	0.9	13.7 (10.9-15.4)	1.1	13.3 (11.3-14.2)	1.3	12.4 (10.3-13.4)	0.8
Longest dorsal-fin spine	22.7	22.6 (21.0-24.8)	1.5	22.0 (19.8-23.8)	1.3	22.4 (20.4-24.4)	1.7	21.4 (18.0-23.6)	1.5
Longest dorsal-fin ray	24.5	24.1 (22.3-24.6)	1.0	22.1 (20.0-25.0)	1.5	21.9 (19.3-23.3)	1.8	22.3 (18.7-26.8)	2.4
1st anal-fin spine	14.7	13.7 (12.4-15.6)	1.2	14.6 (12.3-17.0)	1.4	14.1 (12.5-15.2)	1.2	13.5 (11.7-21.1)	2.4
2nd anal-fin spine	19.4	18.5 (17.1-20.4)	1.2	20.1 (18.8-22.1)	1.0	18.8 (18.1-19.4)	0.6	18.0 (14.8-20.4)	1.4
3rd anal-fin spine	25.5	24.2 (22.2-26.2)	1.5	24.3 (21.8-26.2)	1.4	24.1 (23.1-25.8)	1.1	21.4 (19.2-23.6)	1.4
Longest anal-fin ray	26.3	23.8 (21.2-26.3)	1.9	24.0 (22.4-28.2)	1.7	22.9 (20.6-25.4)	2.0	23.4 (20.6-25.9)	1.4
Caudal-fin length	22.7	23.6 (22.7-24.6)	0.9	22.9 (20.9-25.0)	1.1	25.5 (23.3-27.3)	1.6	26.2 (22.2-29.8)	2.6
Pectoral-fin length	24.8	25.0 (22.4-26.9)	1.8	27.0 (23.4-32.4)	2.4	27.0 (26.6-27.7)	0.5	28.2 (22.9-32.1)	2.7
Pelvic-fin spine length	18.2	19.1 (17.8-20.6)	1.1	20.4 (15.9-25.8)	2.6	18.2 (15.6-20.0)	1.9	16.4 (11.5-21.9)	3.1
Pelvic-fin length	27.9	24.8 (22.6-27.9)	2.2	20.7 (13.7-30.1)	5.7	29.5 (28.9-30.2)	0.6	22.6 (15.9-33.2)	4.7

Table 2. Distributions of selected meristic data of the 4 *Centropyge* species. Those of pectoral-fin rays were counted on both sides. Some specimens had damaged characters and were thus not included in the table

		Dorsal-fin spines			Dorsal-fin rays				Anal-fin rays				Pectoral-fin rays		
	n	13	14	15	15	16	17	18	16	17	18	19	15	16	17
C. deborae sp. nov.	6	1	5			5	1			5	1		1	11	
C. nox	13			13	10	3			1	1	11			21	4
C. tibicen	4		4			4				3	1			3	5
C. bispinosa	13		11	2		4	8	1		6	6	1	4	22	

		Body scales				Upper pored scales										Lower pored scales						
	n	42	43	44	45	27	28	29	30	31	32	33	34	35	36	1	2	3	4	5	6	7
C. deborae sp. nov.	6		2	4			1		1	2		2				1				1	1	3
C. nox	13		5	4	4	1		1		4	4	3							4	3	3	3
C. tibicen	4		1	1	2					1	1	1	1									4
C. bispinosa	13	1	7	4	1					1	1	6	1	1	2			5	3	3	1	1

		Pseudobranchial filaments						Upper gill rakers					Lower gill rakers						
	n	11	12	13	14	15	16	3	4	5	6	7	11	12	13	14	15	16	17
C. deborae sp. nov.	6		1	5						3	3			2	4				
C. nox	13	2	7	3		1		2	3	7	1			10	2	1			
C. tibicen	4					2	1			3	1		1	2	1				
C. bispinosa	13	2	3	6	1	1		1	2	3	4	3					3	6	4

are respectively provided in tables 1 and 2. *Centropyge deborae* sp. nov. differed from *C. nox* in usually having 14 dorsal-fin spines (vs. 15 in *C. nox*); usually 16 dorsal-fin rays (vs. 15); and mainly 17 anal-fin rays (vs. 18). *Centropyge deborae* sp. nov. can be distinguished from *C. tibicen* by having 12 or 13 pseudobranchial filaments (vs. 15 or 16). It can be further distinguished from *C. bispinosa* by having mainly 16 dorsal-fin rays (vs. 17); and 12 or 13 lower-limb rakers on the 1st gill arch (vs. 15-17). Some intraspecific variation was observed in the type series of *Centropyge deborae* sp. nov. The 56.9-mm paratype had only 28+1 pored lateral-line scales compared to 30-33 + 5-7 in the other type specimens. The fewer pored lateral-line scales may be attributed to individual variation.

Acknowledgments: We thank W.S. Yee, C.K. Chong, J.Y. Choo, and P.Y. Teng (University of Malaysia, Terengganu, Malaysia) for taking x-rays and data; T. Iwamoto (California Academy of Sciences, San Francisco, CA, USA) for reviewing an early draft; D. Nolf (Royal Belgian Institute of Natural Sciences, Brussels, Belgium) for etymological advice; C.J. Yeh (National Dong Hwa University, Checheng, Pingtung, Taiwan) and S.Y. Wang (National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan) for curatorial assistance; and the anonymous referees for critical comments. This study was financially supported by the research grants NMMBA100200352, 100200354, and NSC99-2631-H-291-001 from various agencies.

REFERENCES

Allen GR. 1985. Butterfly and angelfishes of the world. Vol. 2. Atlantic Ocean, Caribbean Sea, Red Sea and Indo-Pacific. Hong Kang: MERGUS Publishers.

Allen GR, M Adrim. 2003. Coral reef fishes of Indonesia. Zool. Stud. **42:** 1-72.

Bleeker P. 1853. Vierde bijdrage tot de kennis der ichthyologische fauna van Amboina. Natuurkundig Tijdschrift voor Nederlandsch Indië **5:** 317-352.

Bowen BW, A Muss, LA Rocha, WS Grant. 2006. Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus *Centropyge*) indicates a recent invasion from the Indian Ocean. J. Hered. **97:** 1-12.

Cuvier G, A Valenciennes. 1831. Histoire naturelle des poissons. Tome septième. Livre septième. Des Squamipennes. Livre huitième. Des poissons à pharyngiens labyrinthiformes. Histoire naturelle des poissons. **7:** i-xxix+1-531, pls. 170-208.

Günther A. 1860. Catalogue of the acanthopterygian fishes in the collection of the British Museum. 2. Squamipinnes, Cirrhitidae, Triglidae, Trachinidae, Sciaenidae, Polynemidae, Sphyraenidae, Trichiuridae, Scombridae, Carangidae, Xiphiidae. London: British Museum. Catalogue of the fishes in the British Museum **2:** i-xxi+1-548.

Hillis DM, JJ Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42:** 182-192.

Katoh K, K Misazawa, KI Kuma, M Takashi. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. **30:** 3059-3066.

Klanten S, L van Herwerden, JH Choat, D Blair. 2004. Patterns of lineage diversification in the genus *Naso* (Acanthuridae). Mol. Phylogen. Evol. **32:** 221-235.

Lyons LA, TF Laughlin, NG Copeland, NA Jenkins, JE Womack, SJ O'Brien. 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet. **15:** 47-56.

Myers RF. 1991. Micronesian reef fishes. A practical guide to the identification of the coral reef fishes of the tropical central and western Pacific. 2nd revised ed. Barrigada, Guam: Coral Graphics.

Myers RF. 1999. Micronesian reef fishes. A comprehensive guide to the coral reef fishes of Micronesia. 3rd revised ed. Barrigada, Guam: Coral Graphics.

Nelson JS. 2006. Fishes of the world. New York: J. Wiley.

Playfair RL, A Günther. 1867. The fishes of Zanzibar, with a list of the fishes of the whole east coast of Africa. London: Voorst.

Pyle RL. 2001. Family Pomacanthidae. *In* KE Carpenter, VH Niem, eds. Species identification guide for fishery purposes. The living marine resources of the western central Pacific. Bony fishes part 3 (Menidae to Pomacentridae). Rome: Food and Agricultural Organization, pp. 3266-3286.

Pyle RL, JE Randall. 1994. A review of hybridization in marine angelfishes (Perciformes: Pomacanthidae). Environ. Biol. Fish. **41:** 127-145.

Randall JE, W Klausewitz. 1977. *Centropyge flavipectoralis*, a new angelfish from Sri Lanka (Ceylon) (Pisces: Teleostei: Pomacanthidae). Senckenberg. Biol. **57:** 235-240.

Randall JE, LA Rocha. 2009. *Chaetodontoplus poliourus*, a new angelfish (Perciformes: Pomacanthidae) from the tropical western Pacific. Raffles Bull. Zool. **57:** 511-520.

Rocha LA, MT Craig, BW Bowen. 2007. Phylogeography and the conservation of coral reef fishes. Coral Reefs **26:** 501-512.

Schultz JK, RL Pyle, E DeMartini, BW Bowen. 2007. Genetic connectivity among color morphs and Pacific archipelagos for the flame angelfish, *Centropyge loriculus*. Mar. Biol. **151:** 167-175.

Simon C, F Frati, A Beckenbach, B Crespi, H Liu, P Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. **87:** 651-701.

Tamura K, D Peterson, N Peterson, G Stecher, M Nei, S Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28:** 2731-2739.

van Herwerden L, CR Davies, JH Choat. 2002. Phylogenetic and evolutionary perspectives of the Indo-Pacific grouper *Plectropomus* species on the Great Barrier Reef,

Australia. J. Fish Biol. **60:** 1591-1596.
Ward RD, TS Zemlak, BH Innes, PR Last, PD Hebert. 2005. DNA barcoding Australia's fish species. Phil. Trans. R. Soc. Lond. B Biol. Sci. **360:** 1847-1857.
Wood E. 2001. Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Herefordshire, UK: Marine Conservation Society.