

Cryptic diversity of the acrothoracican barnacle *Armatoglyptes taiwanus* in the Indo-Pacific waters, with description of a new species from the Mozambique Channel collected from the MAINBAZA cruise

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ABSTRACT

Cirripedes of the superorder Acrothoracica are normally found as epizoic borings on marine calcareous substrates. *Armatoglyptes taiwanus* (Utinomi, 1950) is a lithoglyptid acrothoracican barnacle reported from different parts of the Indo-Pacific. Recent studies have demonstrated phylogenetic breaks between the Indian and Pacific Oceans populations in widespread Indo-Pacific marine organisms due to isolation events during the Pleistocene glaciations. It is possible that *A. taiwanus* represents a cryptic species complex in the Indo-Pacific, which the previous studies have failed to identify from morphology alone. In the present study, we analyzed the morphology and the sequence divergence of the 12S rDNA of *A. taiwanus* from the Indo-Pacific region, including Taiwan and the Philippines in the Pacific, and Phuket Island (Thailand) and the Mozambique Channel in the Indian Ocean, to test whether *A. taiwanus* is a cryptic species across its geographical range. The results showed that *A. taiwanus* has a homogeneous population structure in Taiwan, the Philippines, and Phuket Island (sequence divergence < 1%). Specimens from the Mozambique Channel, although morphologically similar to *A. taiwanus*, have a greater sequence divergence of 9.4% from *A. taiwanus* in the Pacific, and thus appeared to represent a new species, described herein as *Armatoglyptes flexuosus* n. sp. Although both species

KEY WORDS

Cirripedia,
Pleistocene vicariance,
phylogeny,
12S rDNA,
SEM,
morphology,
cryptic species,
new species.

are morphologically similar, *A. flexuosus* n. sp. has more strongly bent/recurved posterior processes of the opercular bars and feebler armament of the orificial knob than does *A. taiwanus* from Taiwan (type locality). Phylogenetic analysis showed that populations of *A. flexuosus* n. sp. from the Mozambique Channel and *A. taiwanus* from the Pacific region are indeed closely related. Populations of their common ancestor may have become isolated and underwent speciation during the Pleistocene glaciations.

RÉSUMÉ

Diversité cryptique chez les balanes acrothoraciques Armatoglyptes taiwanus de la zone Indo-Pacifique, avec la description d'une nouvelle espèce collectée lors de la campagne océanographique MAINBAZA, au large du Mozambique.

Les cirripèdes appartenant au super-ordre Acrothoracica sont généralement considérés comme des foreurs épizoïques des substrats durs calcaires en milieu marin. *Armatoglyptes taiwanus* (Utinomi, 1949) est une balane acrothoracique signalée dans différentes régions de la zone Indo-Pacifique. Des études récentes sur différents organismes marins de cette zone ont montré qu'il existait une séparation phylogénétique entre les populations de l'océan Indien et du Pacifique, induite par des épisodes d'isolement pendant les glaciations du Pléistocène. Dans ce contexte, *A. taiwanus* pourrait présenter un complexe d'espèces cryptiques dans l'Indo-Pacifique, hypothèse non confirmée par de précédentes études basées uniquement sur la morphologie. Le but de cette étude est d'analyser la morphologie et la diversité génétique (gène 12S rADN) de spécimens d'*A. taiwanus* provenant de la région Indo-Pacifique dont Taiwan, les Philippines et l'île de Pucket (Thaïlande) ainsi que du canal du Mozambique (océan Indien) afin de tester la présence d'espèces cryptiques à travers une large distribution géographique. Les résultats montrent que la structure de la population de *A. taiwanus* est homogène pour les zones de Taiwan, des Philippines et de Pucket (divergence entre les séquences < 1 %). Bien que morphologiquement similaires à *A. taiwanus*, les spécimens du canal du Mozambique présentent une importante divergence avec les spécimens d'*A. taiwanus* de Taiwan (divergence de 9,4 %) et peuvent donc être considérés comme une nouvelle espèce, décrite dans le présent article comme *Armatoglyptes flexuosus* n. sp. Bien que ces deux espèces soient morphologiquement similaires, *A. flexuosus* n. sp. présente un processus postérieur de la barre operculaire plus fortement incurvé et une plus faible ornementation de l'orifice que chez l'espèce *A. taiwanus* de Taiwan (localité type). Les analyses phylogénétiques ont démontré que les populations d'*A. flexuosus* n. sp. du Canal du Mozambique et d'*A. taiwanus* de la zone Pacifique sont étroitement reliées. Les populations de leur ancêtre commun pourraient avoir été isolées au cours des épisodes glaciaires du Pléistocène.

MOTS CLÉS

Cirripèdes,
vicariance du Pléistocène,
phylogénie,
12S rADN,
MEB,
morphologie,
espèces cryptiques,
espèce nouvelle.

INTRODUCTION

Cirripedes of the superorder Acrothoracica are normally found as epizoic borings on marine calcareous substrates (Tomlinson 1969, 1987). Acrothoracican

barnacles are dioecious, with large-sized females living in self-excavated borings and dwarf males attached externally to females (Kolbasov 2009). The main taxonomic reviews of the Acrothoracica are those of Tomlinson (1969), Kolbasov & Newman

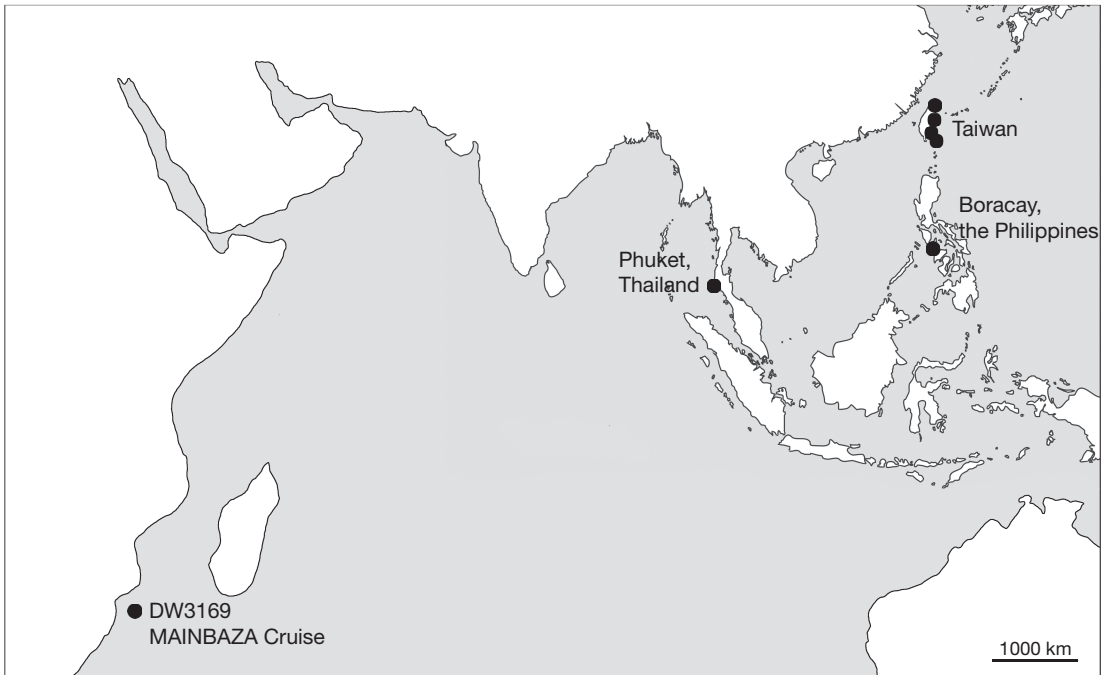


FIG. 1. — Sampling locations of acrothoracican barnacles in the present study. He-Ping-Dao and Shen-Ao-Keng in Taiwan are very close together and are jointly represented by one dot in northernmost Taiwan.

(2005) and Kolbasov (2009). In the latest taxonomic revision of the superorder Acrothoracica Gruvel, 1905, Kolbasov, Newman & Høeg (*in* Kolbasov 2009) rearranged the acrothoracican species into two new orders, the Lithoglyptida and Cryptophialida (Kolbasov 2009). The Lithoglyptida consist of the families Lithoglyptidae Aurivillius, 1892 and Trypetesidae Kruger, 1940, having a wide aperture, a large, saddle-shaped labrum, and well developed mouth cirri. Within the Lithoglyptidae, the new genus *Armatoglyptes* was proposed by Kolbasov & Newman (2005) to house the species with four pairs of terminal cirri and a pair of two-segmented caudal appendages with no basal pedestal.

Armatoglyptes taiwanus (Utinomi, 1950) (originally described as *Balanodytes taiwanus* but currently re-assigned to *Armatoglyptes* and considered to be a senior synonym of *Armatoglyptes habeii* (Tomlinson, 1963); see Chan *et al.* *in press*) is a lithoglyptid acrothoracican barnacle that inhabits a wide range of calcareous substrates, including mollusc

shells, dead coral skeletons, and barnacle shells in the Indo-Pacific (see Chan *et al.* *in press*; Kolbasov 2009). Tomlinson (1969) noted a high degree of intraspecific morphological variation in *Armatoglyptes taiwanus* (as *A. habeii* in Tomlinson 1969) but found it difficult to discriminate species by a morphological approach only. Phylogenetic breaks have been reported in species distributed between the Indian and Pacific Oceans (Lavery *et al.* 1996; Benzie *et al.* 2002; Teske *et al.* 2005; Tsang *et al.* 2008), probably as the result of speciation following the isolation of populations in the two major oceans by the emergence of the Sunda and Sahul Shelves during the Pleistocene glaciations (Voris 2000). It is thus possible that *A. taiwanus* as well consists of a cryptic species complex showing only slight morphological variation across the Indo-Pacific, but which might be identified by molecular approaches (see Chan *et al.* 2007, *in press*; Tsang *et al.* 2008). In the present study, we examined collections of the acrothoracicans of the genus *Armatoglyptes* from Taiwan, the Philippines,

TABLE 1. — Gene bank accession number and locality of all samples collected in present study.

Specimen number	Species	Locality	12S rDNA
Acro_sp_3	<i>Armatoglyptes taiwanus</i> (Utinomi, 1950)	He-Ping-Dao, Taiwan	HQ412387
Acro_sp_4	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412388
Acro_sp_5	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412389
Acro_sp_6	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412390
Acro_sp_8	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412391
Acro_sp_10	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412392
Acro_sp_11	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412393
Acro_sp_12	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412394
Acro_sp_13	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412395
Acro_sp_14	<i>Armatoglyptes taiwanus</i>	Shih-Ti-Ping, Taiwan	HQ412396
Acro_sp_15	<i>Armatoglyptes taiwanus</i>	Shih-Ti-Ping, Taiwan	HQ412397
Acro_sp_16	<i>Armatoglyptes taiwanus</i>	Shih-Ti-Ping, Taiwan	HQ412398
Acro_sp_22	<i>Armatoglyptes taiwanus</i>	Kata Noi Beach, Phuket Island, Thailand	HQ412399
Acro_sp_23	<i>Armatoglyptes taiwanus</i>	Kata Noi Beach, Phuket Island, Thailand	HQ412400
Acro_sp_24	<i>Kochlorine hamata</i> Noll, 1872	Kata Noi Beach, Phuket Island, Thailand	HQ412401
Acro_sp_26	<i>Kochlorine hamata</i>	Kata Noi Beach, Phuket Island, Thailand	HQ412402
Acro_sp_27	<i>Kochlorine hamata</i>	Kata Noi Beach, Phuket Island, Thailand	HQ412403
Acro_sp_28	<i>Kochlorine hamata</i>	Kata Noi Beach, Phuket Island, Thailand	HQ412404
Acro_sp_31	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412405
Acro_sp_46	<i>Armatoglyptes taiwanus</i>	Boraycay Island, the Philippines	HQ412406
Acro_sp_47	<i>Armatoglyptes taiwanus</i>	Boraycay Island, the Philippines	HQ412407
Acro_sp_52	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412408
Acro_sp_53	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412409
Acro_sp_54	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412410
Acro_sp_59	<i>Armatoglyptes mitis</i> (Tomlinson, 1969)	Da-Xhi deep-sea fish market, Taiwan	HQ412411
Acro_sp_66	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412412
Acro_sp_69	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao, Taiwan	HQ412413
Acro_sp_72	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412414
Acro_sp_73	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412415
Acro_sp_76	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412416
Acro_sp_77	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412417
Acro_sp_78	<i>Armatoglyptes taiwanus</i>	Aquarium shop, supplied from the Philippines	HQ412418
Acro_sp_82	<i>Armatoglyptes taiwanus</i>	Lanyu Island, Taiwan	HQ412419
Acro_sp_84	<i>Armatoglyptes taiwanus</i>	Lanyu Island, Taiwan	HQ412420
Acro_sp_85	<i>Armatoglyptes taiwanus</i>	Lanyu Island, Taiwan	HQ412421
Acro_sp_86	<i>Armatoglyptes taiwanus</i>	Lanyu Island, Taiwan	HQ412422
Acro_sp_97	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412423
Acro_sp_99	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412424
Acro_sp_107	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412425
Acro_sp_108	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412426
Acro_sp_109	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412427
Acro_sp_110	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412428
Acro_sp_113	<i>Armatoglyptes taiwanus</i>	San-Ao-Keng, Taiwan	HQ412429
Acro_sp_116	<i>Armatoglyptes mitis</i>	He-Ping-Dao fish market, Taiwan	HQ412430
Acro_sp_118	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412431
Acro_sp_121	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412432
Acro_sp_122	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412433
Acro_sp_123	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412434
Acro_sp_124	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412435
Acro_sp_125	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412436
Acro_sp_126	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412437
Acro_sp_127	<i>Armatoglyptes taiwanus</i>	He-Ping-Dao fish market, Taiwan	HQ412438
Acro_sp_213	<i>Armatoglyptes flexuosus</i> n. sp.	Mozambique Channel, Almirante Leite Bank, Indian Ocean	HQ412439
Acro_sp_215	<i>Armatoglyptes flexuosus</i> n. sp.	Mozambique Channel, Almirante Leite Bank, Indian Ocean	HQ412440

TABLE 1. — Continuation.

Specimen number	Species	Locality	12S rDNA
Acro_sp_242	<i>Armatoglyptes taiwanus</i>	Shi-Ti-Ping, Taiwan	HQ412441
Acro_sp_243	<i>Armatoglyptes taiwanus</i>	Shi-Ti-Ping, Taiwan	HQ412442
Acro_sp_256	<i>Armatoglyptes taiwanus</i>	Green Island, Taiwan	HQ412443
Acro_sp_261	<i>Trypetesa habeii</i> Utinomi, 1962	He-Ping-Dao, Taiwan	HQ412444

Phuket Island in Thailand, and trawl samples taken by the MAINBAZA expedition in the Mozambique Channel of the western Indian Ocean. Combined molecular and morphological study shows that the specimens from the Mozambique Channel represent a new species, *Armatoglyptes flexuosus* n. sp., which is described herein.

MATERIAL AND METHODS

Fresh and live specimens of *Armatoglyptes taiwanus* were collected and fixed in 100% ethanol from different gastropod shells occupied by hermit crabs (Table 1) in He-Ping-Dao (25°09'48"N, 121°45'44"E), Shen-Ao-Keng (25°07'25"N, 121°49'10"E), Shih-Ti-Ping (23°29'28"N, 121°30'46"E), Lanyu Island (22°03'31"N, 121°30'28"E), and Green Island (22°40'46"N, 121°29'24"E) in Taiwan; Kata Noi Beach on Phuket Island (2 samples in *Thais* sp. shells; 7°48'31"N, 98°17'54"E), Thailand; Boracay Island (11°58'53"N, 121°54'31"E) in the Philippines; and *Conus* shells occupied by hermit crabs purchased from an aquarium shop in Taiwan but imported from the Philippines and preserved in 100% ethanol. Specimens of the new species *Armatoglyptes flexuosus* n. sp. came from Warén dredge samples (DW3169; 26°11'00"S, 35°01'00"E) collected by the MAINBAZA expedition, in the Mozambique Channel (Fig. 1; for details about the MAINBAZA expedition, see the acknowledgement section) and were extracted from corals which were fixed in 100% ethanol upon collection. The margins of the burrow containing a barnacle were treated in 2% HCl, or the burrow was mechanically destroyed to remove the animal. This method preserves the calcareous plates characterizing some acrothoracican species. The isolated barnacles were boiled in potassium

hydroxide solution for 1 min and the cirri, caudal appendages and mouth parts were dissected and observed under compound microscopes (Zeiss Scope A1) and observed under magnification of 400× (Zeiss Plan APOchromat 40x/0.95) and 1000× (Zeiss Plan APOchromat 100x/1.4 oil), using glycerin slide mounts. Parts of the mantle sac and cirri of selected species were further observed using a scanning electron microscope (SEM). Adult female of *A. flexuosus* was post-fixed in 2% OsO₄ for 2 h, dehydrated in acetone, critical point dried in CO₂, sputter coated with gold, and examined in SEM (FEI Quanta 2000) with accelerating voltages of 25 kV, following the methods in Chan *et al.* (in press).

MOLECULAR STUDIES

Total genomic DNA was extracted and purified from the soft tissue of individual barnacles (Table 1), using the commercial QIAamp Tissue Kit (QIAGEN). Mitochondrial 12S rDNA (12S) was chosen as the molecular marker. Partial sequences were amplified by the primers of Mokady *et al.* (1994) and the *Taq* DNA Polymerase Master Mix (Ampliqon, Denmark), following the manufacturer's instructions. Polymerase chain reaction (PCR) was done under an initial 95°C denaturation for 2.5 min, a 30-cycle amplification of 30 sec at 96°C, 30 sec at 48°C, and 1 min at 72°C, and a final 72°C extension for 3 min. The amplicons with the same sets of primers were sent to a commercial company (Genomics BioSci & Tech, Taiwan) for purification and automated sequencing.

All the sequences obtained were visually edited. Default gap weighting parameters were used to align the sequences in CLUSTAL W (Thompson *et al.* 1994), while both missing data and gaps were treated as missing data and excluded from the analysis. The haplotype and nucleotide diversities as well as the

Kimura's two-parameter (K2P) distance (Kimura 1980) were calculated using DnaSP version 5 (Librado & Rozas 2009) and MEGA version 4.1 (Tamura *et al.* 2007), respectively. A phylogenetic neighbor-joining (NJ) tree was generated using MEGA with 1000 bootstrap replicates, based on K2P distance. A Bayesian inference (BI) tree was generated using Mr-Bayes version 3.12 (Ronquist & Huelsenbeck 2003). The *a priori* parameters were specified according to Hasegawa, Kishino and Yano's models (Hasegawa *et al.* 1985) with invariable sites (HKY+I), which were found to be the optimal substitution model based on the corrected Akaike information criterion implemented in jModelTest version 0.1.1 (Posada 2008). Two independent Markov-chain-Monte-Carlo (MCMC) searches with random starting points were conducted until the divergence between them became stationary. Trees were sampled every 10000 cycles in 10000000 generations with the burn-in value set to discard the first quarter of the sampling trees. *A posteriori* possibility was then calculated from the sampled trees to illustrate the statistical confidence of BI.

Mitochondrial 12S rDNA sequences of *Armatoglyptes mitis* (Tomlinson, 1969) and *Trypetesa habeii* Utinomi, 1962, collected in Taiwan, and *Kochlorine hamata* Noll, 1872, from Kata Noi Beach, Phuket, Thailand (Table 1) were included in the analysis. *Trypetesa habeii* and *K. hamata* were designated as the outgroups.

SYSTEMATICS

Superorder ACROTHORACICA Gruvel, 1905

Order LITHOGLYPTIDA

Kolbasov, Newman & Høeg, 2009

Family LITHOGLYPTIDAE Aurivillius, 1892

Subfamily LITHOGLYPTINAE Aurivillius, 1892

Genus *Armatoglyptes* Kolbasov & Newman, 2005

Armatoglyptes flexuosus n. sp.
(Figs 2-6)

TYPE MATERIAL. — **Mozambique Channel**. MAINBAZA, stn DW3169, 26°11'00"S, 35°01'00"E, 450 m, ♀ holotype boring in the coral *Pavona* sp. (MNHN-Ci3215).

OTHER MATERIAL EXAMINED. — **Mozambique Channel**. MAINBAZA, stn DW3169, 26°11'00"S, 35°01'00"E, 450 m, 5 ♀♀ (MNHN-Ci3215) and 4 dwarf ♂♂ attached to their exteriors.

DISTRIBUTION. — At present, only recorded from the Mozambique Channel, at 450 m deep.

ETYMOLOGY. — The Latin adjective *flexuosus* denotes the strongly bent (recurved) posterior processes of the opercular bars, a diagnostic character of this new species.

DIAGNOSIS. — *Armatoglyptes* having opercular bars with bifid teeth, posterior processes L-shaped, strongly bent/recurved at tip region.

DESCRIPTION

Female

Shape of burrow opening narrow oval or spindle-shaped (Fig. 4A, B). Opercular bars *c.* 400 µm long, armed with series of bifid teeth, simple setae, and small, swollen cuticular knobs (Figs 2A, B; 3A, B, F, I, J); posterior processes strongly anteriorly bent/recurved and becoming L-shaped, with bifid teeth and simple setae along their length (Figs 2A, B; 3B, G); comb collar with long, feather-like cuticular projections, fused at their bases (Fig. 3E, F). Orificial knob distinct, shallow and globular, surface with setae and rare simple or bifid teeth (Figs 2C; 3C). Lateral surfaces of opercular area covered with sparse simple setae and rows of massive multifid scales, lateral bars undeveloped (Fig. 3H).

Maxilla triangular (Fig. 5A), with setae on exterior and inferior margins. Maxillule with two long and sharp upper and one smaller lower cuspidate setae, notch with short sharp seta, lower 2/3 of cutting edge with numerous short, sharp setae (Figs 2E; 5B). Mandible with three large teeth and two smaller teeth close to the lower margin, tiny sharp denticle inserted in each gap between them (from 2nd to 5th), inferior angle with two sharp denticles and sparse setae (Figs 2D; 5C). Mandibular palp trapezoidal (ice-hockey stick shaped), with simple setae on oblique distal part along exterior margin (Fig. 5D).

Mouth cirri consisting of long, curved coxa, quadrangular basis, and three-segmented rami, anterior ramus slightly shorter than posterior, rami and basis with plumose setae (Fig. 2F). Four pairs of terminal cirri. Anterior surfaces of their annuli armed with two pairs of long distal and short

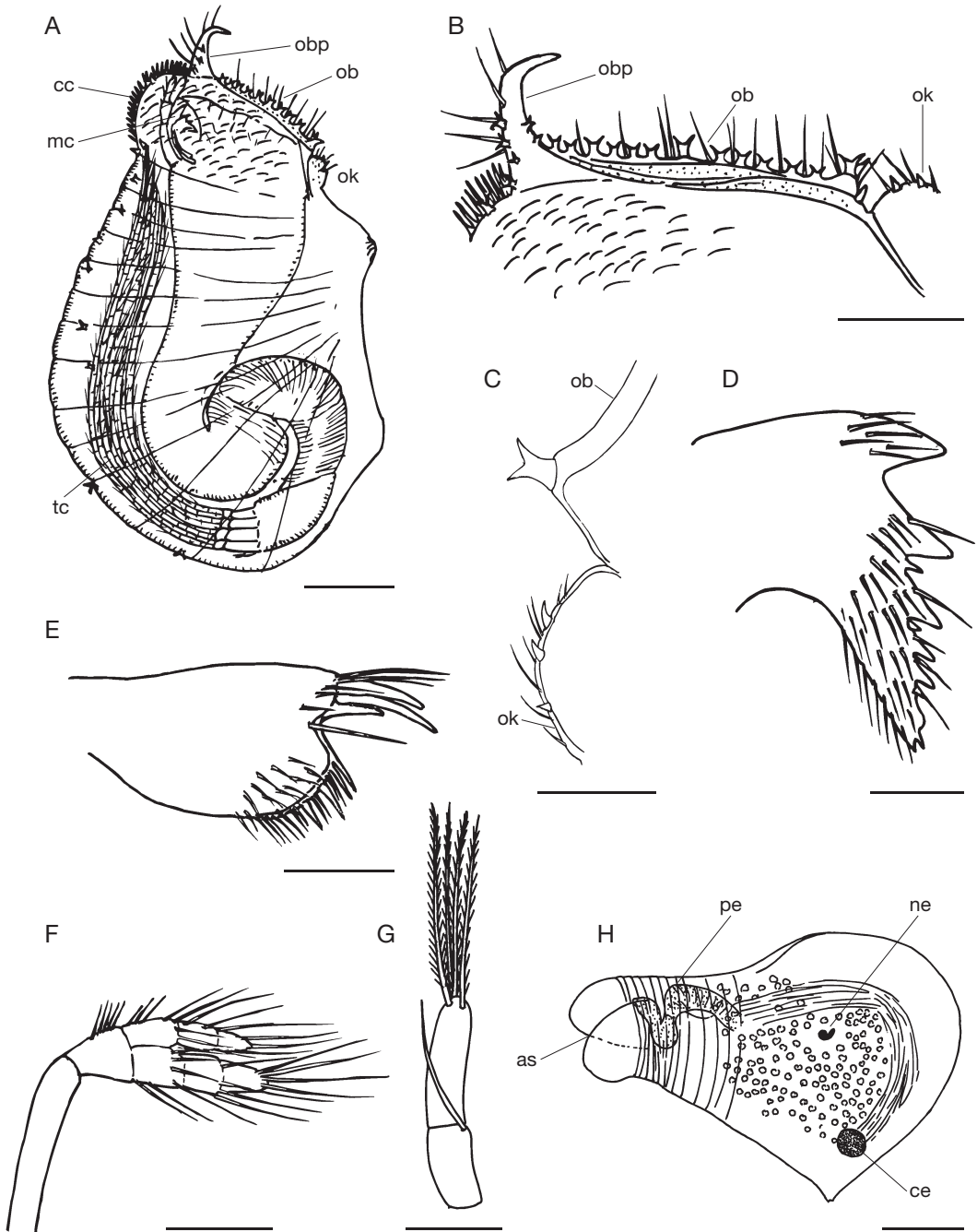


FIG. 2. — *Armatoglyptes flexuosus* n. sp., general morphology: **A-G**, female; **H**, dwarf male, general view, lateral; **A**, general view, lateral; **B**, magnified view of the opercular area; **C**, orifical knob; **D**, mandible; **E**, maxillule; **F**, mouth cirrus I; **G**, caudal appendage. Abbreviations: **as**, apertural slit; **cc**, comb collar; **ce**, cyprid compound eye; **mc**, mouth cirri; **ne**, naupliar eye; **ob**, opercular bar; **obp**, posterior process of opercular bar; **ok**, orifical knob; **pe**, penis; **tc**, terminal cirri. Scale bars: A, B, D, E, H, 100 μm; C, 25 μm; F, 20 μm; G, 50 μm; H, 10 μm.

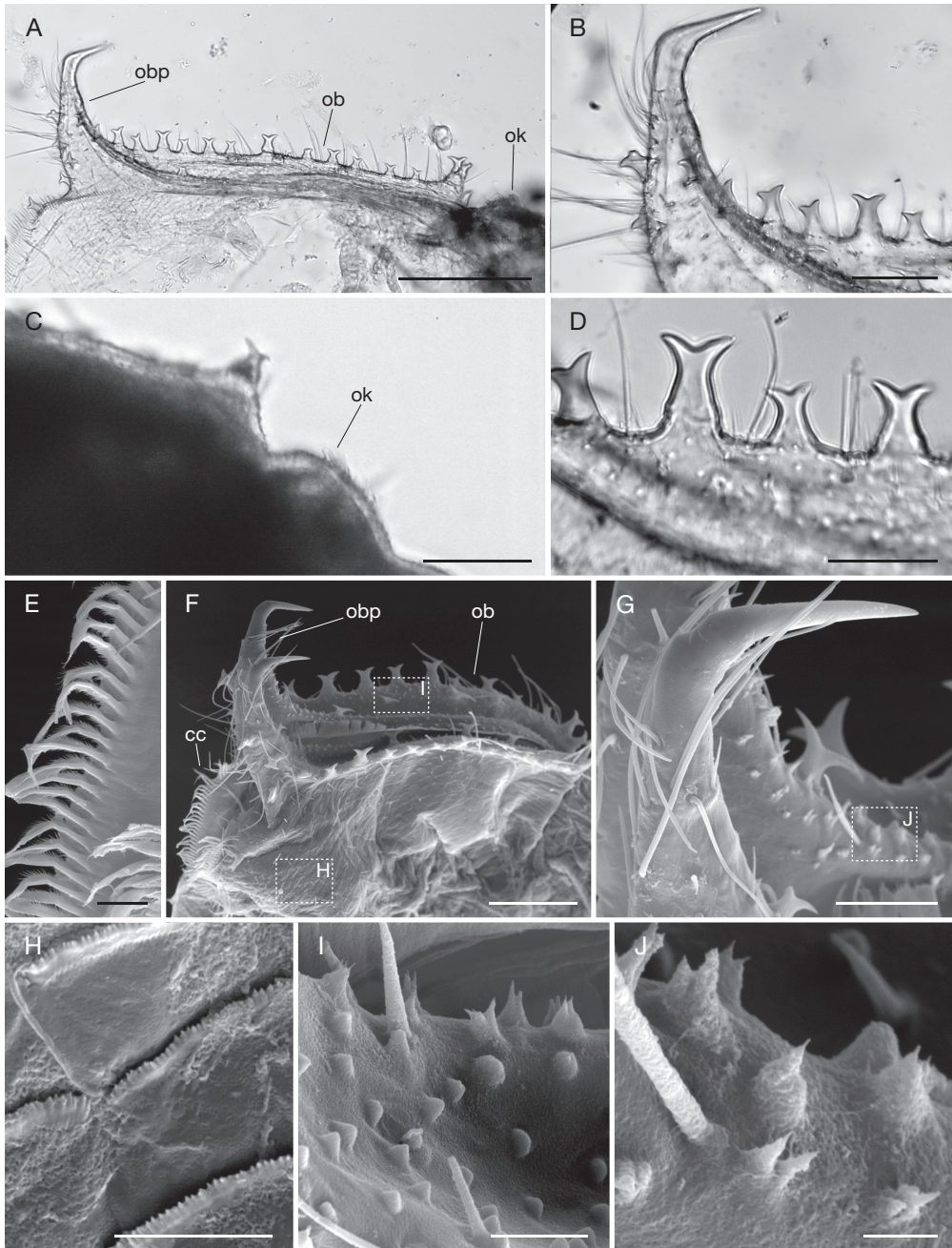


FIG. 3. — *Armatoglyptes flexuosus* n. sp., armament of opercular area: **A-D**, light microscopy; **E-J**, SEM microscopy; **A**, opercular bar showing the “L-curved” posterior process; **B**, posterior process of opercular bar; **C**, orificial knob; **D**, bifid teeth of opercular bar; **E**, projections of comb collar; **F**, opercular bars; **G**, posterior process of opercular bar; **H**, massive multifid scales on lateral surface of opercular area; **I**, button-like projections on opercular bar; **J**, sharp denticles on opercular bar. Abbreviations: **cc**, comb collar; **ob**, opercular bar; **obp**, opercular bar posterior process; **ok**, orificial knob. Scale bars: A, F, 100 µm; B, 50 µm; C, 25 µm; D, G, 20 µm; E, H, I, 10 µm; J, 5 µm.

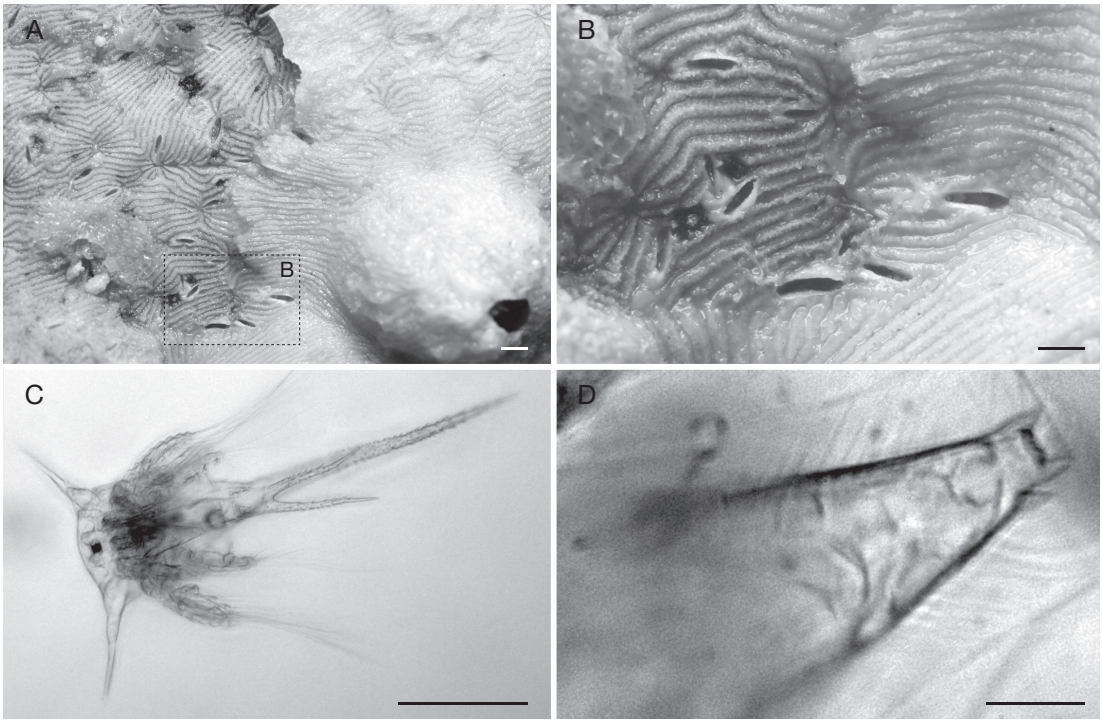


FIG. 4. — **A, B**, *Armatoglyptes flexuosus* n. sp.; **C, D**, *A. taiwanus* (Utinomi, 1950); **A, B**, burrow openings in the coral *Pavona* sp.; **C**, naupliar instar II, showing frontolateral horns and appendages; **D**, magnified view of well developed labrum of naupliar instar II. Scale bars: A, B, 1000 µm; C, 100 µm; D, 25 µm.

medial setae, posterior surfaces with pair of distal setae (Fig. 5F). Caudal appendages two-segmented, without pedestal, basal segment with simple distal seta, terminal segment with four plumose distal setae (Figs 2G; 5E).

Dwarf male

Cyprids of dwarf male with elongated carapace (Fig. 6A), posterior end truncate (Fig. 6B), with a pair of typical antennules, attachment segment III shoe-shaped (Fig. 6C). Posterior lattice organs 3-4 and unpaired large central pore distinctly observed on carapace surface (Fig. 6E, F). After settlement, dwarf male metamorphoses within carapace, shed cypris cuticle with thoracopods coming to lie in posterior part (Fig. 6D, E). Newly developed young dwarf male globular, with short posterior end terminating in distinct apertural slit (Fig. 6G, H), bearing antennules of typical cypris morphology,

and lacking long attachment stalk (Fig. 6G, I). Mature dwarf male pear-shaped, with elongated posterior end, apertural slit long (Figs 2H; 6J, K), cuticle of posterior end wrinkled with circular striations, without mantle teeth (Figs 2H; 6K, L). Terminal part of long, whip-shaped penis observed in posterior part of mature male (Fig. 2H). Dwarf males retaining compound eyes and naupliar eye from cypris (Figs 2H; 6G, J).

REMARKS

The mouthparts and caudal appendages of *A. flexuosus* n. sp. are similar to those of *A. taiwanus*, but the new species has strongly curved, “L-shaped” posterior processes of the opercular bars, which are different from the slightly, gradually curved posterior processes, normally having a sharp curved spine or spines beneath their tips, that are characteristic of *A. taiwanus*.

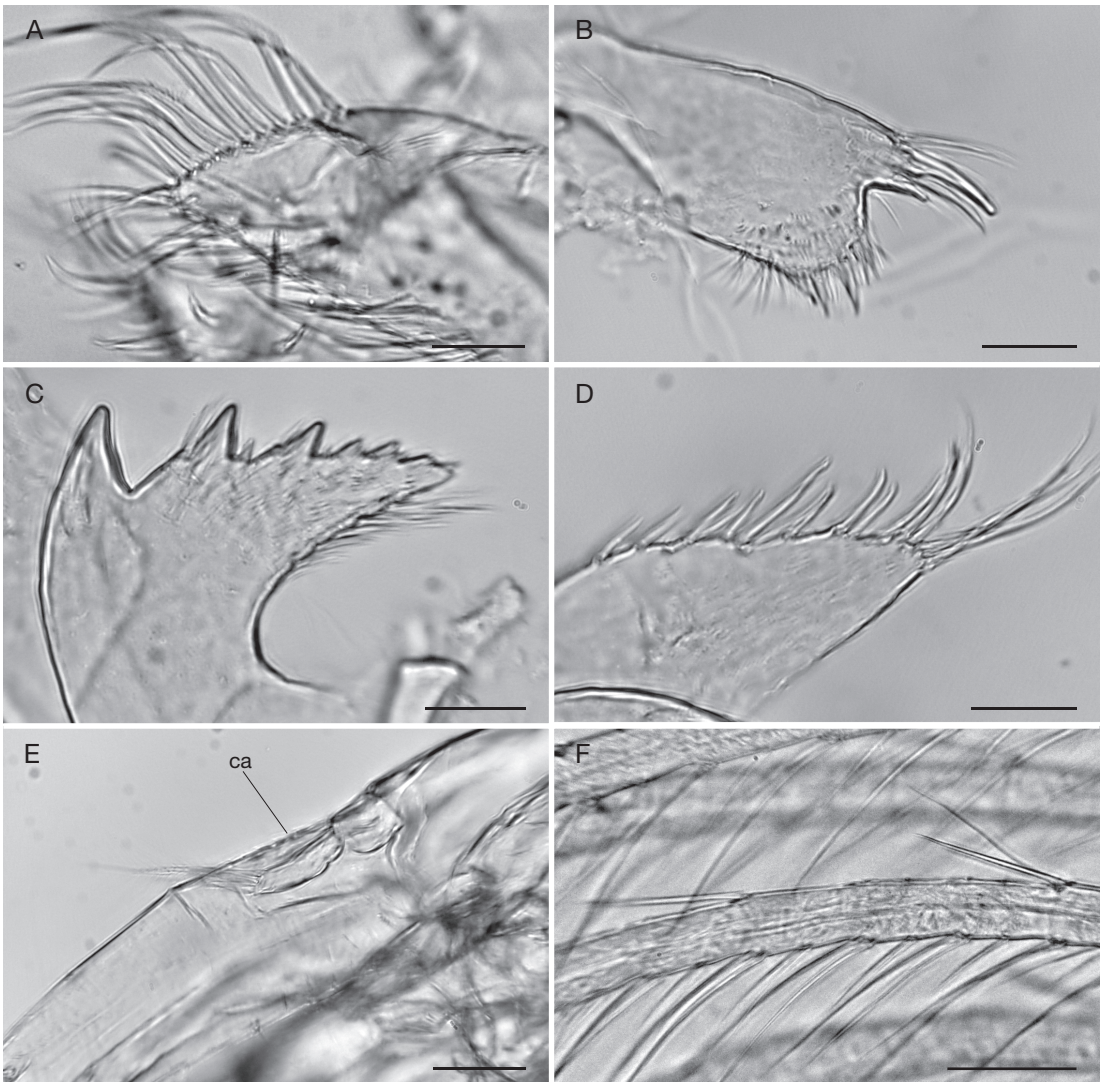


FIG. 5. — *Armatoglyptes flexuosus* n. sp., mouthparts: **A**, maxilla; **B**, maxillule; **C**, mandible; **D**, mandibular palp; **E**, caudal appendages; **F**, intermediate segments of cirrus VI. Abbreviation: **ca**, caudal appendages. Scale bars: A–D, F, 100 μ m; E, 50 μ m.

Armatoglyptes flexuosus n. sp. is also close to *A. mitis* in having an armed orificial knob and similar mouthparts, but differs by the L-shaped posterior processes of the opercular bars; *A. mitis* has conical posterior processes without a bent tip. *Armatoglyptes stirni* (Turquier, 1978), *A. thomasi* (Kolbasov & Newman, 2005) and *A. wilsoni* (Tomlinson, 1969) also have anteriorly bent posterior processes of the opercular

bars like those in *A. flexuosus* n. sp., but these species lack an armed orificial knob (Kolbasov 2009).

Armatoglyptes flexuosus n. sp. was found at 450 m and is thus a deep-water acrothoracican. This record is the deepest for the genus *Armatoglyptes* (Kolbasov 2009); *A. taiwanus* ranges from 0 to 220 m, *A. mitis* from 0 to 78 m, *A. stirni* from 90 to 390 m, *A. thomasi* from 0 to 3 m, and *A. wilsoni* from 4 to 18 m.

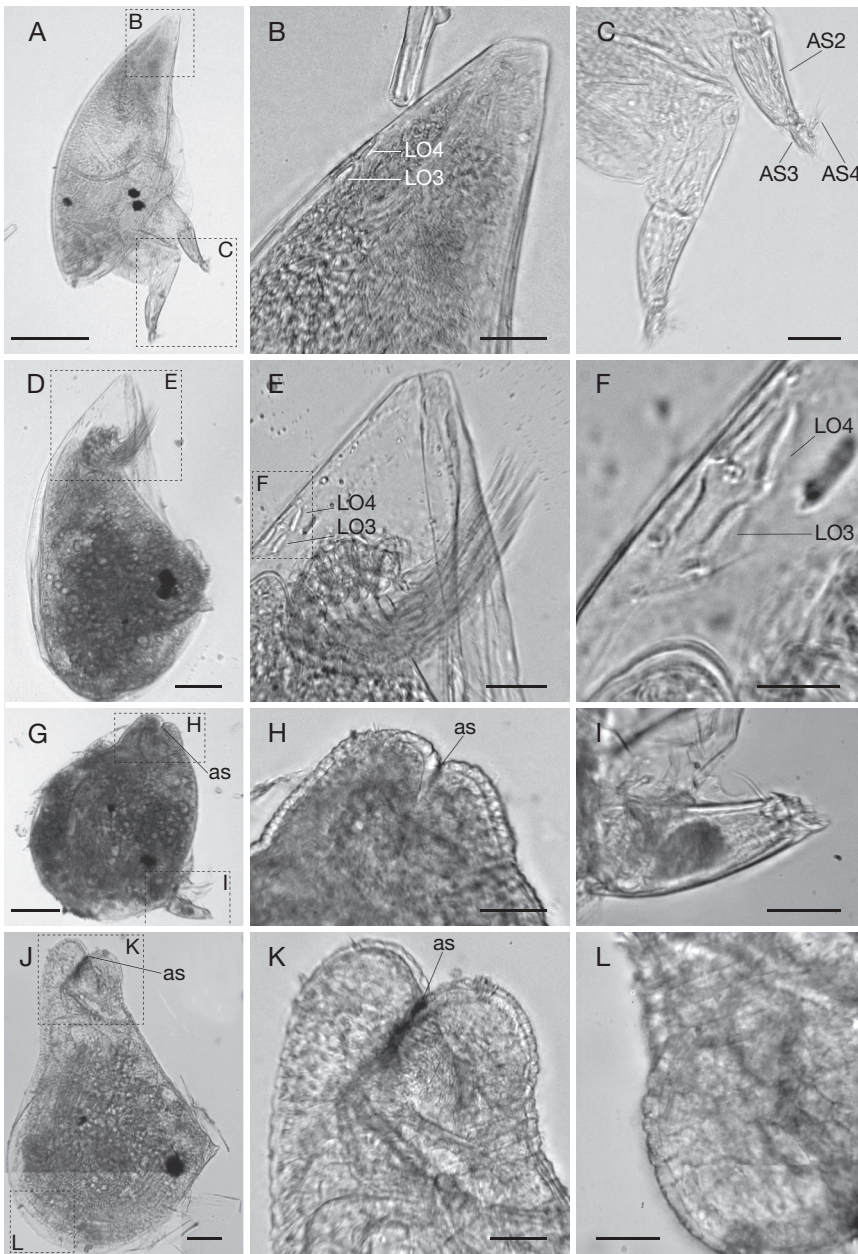


FIG. 6. — *Armatoglyptes flexuosus* n. sp., dwarf males at different stages: **A**, just settled cyprid of dwarf male; **B**, posterior part of carapace of settled cyprid; **C**, cypris antennules; **D**, newly developed dwarf male, in prior to shed off cypris carapace; **E**, posterior part of shed carapace; **F**, posterior lattice organs (3rd and 4th pairs) on carapace surface; **G**, young, immature male; **H**, posterior end of immature dwarf male with apertural slit; **I**, attachment antennule of dwarf male; **J**, mature dwarf male; **K**, posterior end of mature dwarf male with apertural slit; **L**, surface of cuticle at posterior end of dwarf male, showing circular striation. Abbreviations: **as**, apertural slit; **AS2**, **AS3**, **AS4**, 2nd, 3rd and 4th segments of cypris antennule; **LO 3-4**, posterior lattice organs 3 and 4. Scale bars: A, D, G, J, 10 µm; B, C, F, 3 µm; E, H, I, K, L, 5 µm.

TABLE 2. — Sample size and genetic diversity of five studied acrothoracican species. Diversity could not be calculated for *Trypetesa habeii* Utinomi, 1962 due to a single sample. Abbreviations: **N**, total number of specimens examined; **n**, number of sequences obtained; **Length**, the aligned length of the markers; **n_h**, number of haplotypes; **H**, haplotype diversity; **π**, nucleotide diversity; **N. A.**, not applicable. For collection site of acrothoracican species, refer to text. *Kochlorine hamata* Noll, 1872 was collected in Phuket, Thailand for comparative studies in the present study.

Species	N	n	Length	n _h	H	π
<i>Armatoglyptes taiwanus</i> (Utinomi, 1950)	49	49	306-307	12	0.84 ± 0.04	0.0108 ± 0.0023
<i>Armatoglyptes flexuosus</i> n. sp.	2	2	306	1	0.00 ± 0.00	0.0000 ± 0.0000
<i>Armatoglyptes mitis</i> (Tomlinson, 1969)	2	2	303	2	0.83 ± 0.22	0.0033 ± 0.0017
<i>Kochlorine hamata</i> Noll, 1872	4	4	305	2	0.50 ± 0.07	0.0016 ± 0.0009
<i>Trypetesa habeii</i> Utinomi, 1962	1	1	305	1	N. A.	N. A.

TABLE 3. — Kimura-2-parameter genetic (K2P) distances within and between each acrothoracican species studied here. No intraspecific K2P distance could be calculated for *Trypetesa habeii* Utinomi, 1962 due to a single sample. Abbreviations: **12S**, 12S rDNA; **At**, *Armatoglyptes taiwanus* (Utinomi, 1950); **Af**, *A. flexuosus* n. sp.; **Am**, *A. mitis* (Tomlinson, 1969); **Kh**: *Kochlorine hamata* Noll, 1872; **Th**, *Trypetesa habeii* Utinomi, 1962; **N. A.**, not applicable.

12S	At	Af	Am	Kh	Th
At	0.011				
Af	0.094	0.000			
Am	0.229	0.253	0.003		
Kh	0.369	0.368	0.360	0.002	
Th	0.375	0.386	0.350	0.425	N. A.

MOLECULAR ANALYSIS

A total of 58 partial sequences, the length of which ranged from 303 to 307 bp, were obtained for *Armatoglyptes taiwanus*, *A. flexuosus* n. sp., *A. mitis*, *Kochlorine hamata* and *Trypetesa habeii* (Tables 1; 2). There are 138 polymorphic sites in the whole dataset, of which 116 are parsimony informative, and which define 18 haplotypes. The haplotype diversity (*h*) of *A. taiwanus* is 0.84 ± 0.04 , and the nucleotide diversity is (π) 0.0108 ± 0.0023 . The two sequences of *A. flexuosus* n. sp. obtained are identical, so both *h* and π are zero. *Armatoglyptes taiwanus* demonstrated the highest haplotype and nucleotide diversities, as well as the greatest intraspecific genetic distance (K2P = 0.011) among all the acrothoracican species studied herein (Tables 2; 3). The interspecific K2P distance among the five studied species ranged from 0.094 to 0.425, with

Armatoglyptes spp. differing from each other by 0.253 at most. In the phylogenetic tree based on these data (Fig. 7), *A. taiwanus* and *A. flexuosus* n. sp. are the two most closely related species, with the smallest interspecific K2P distance (0.094; Table 3). The congeneric species *A. mitis* is relatively distantly related to these two species (Fig. 7), differing in K2P from *A. taiwanus* and *A. flexuosus* n. sp. by 0.229 and 0.253 respectively.

DISCUSSION

On the basis of molecular analysis, the widely distributed Indo-Pacific species, *A. taiwanus* is confirmed as distinct. Samples from the Philippines, Taiwan, and Phuket Island in the Andaman Sea showed a very little divergence in the 12S rDNA region (< 1%), which suggests that these populations are conspecific. Most species of acrothoracican barnacles have been thought to brood their naupliar larvae inside the female mantle cavity, with only cyprids being released into the open environment (Kolbasov *et al.* 1999). Therefore, how can some acrothoracican barnacles gain a wide geographical distribution if they lack planktonic naupliar stages in their life cycle? Kolbasov *et al.* (1999) and Kolbasov & Hoeg (2000) observed nauplii within the mantle cavities of *A. mitis* and *A. taiwanus* (= *A. habeii*) that lacked capsular membrane, had a well developed labrum and fronto-lateral horns, and which were obviously ready to swim. They supposed that these acrothoracicans may

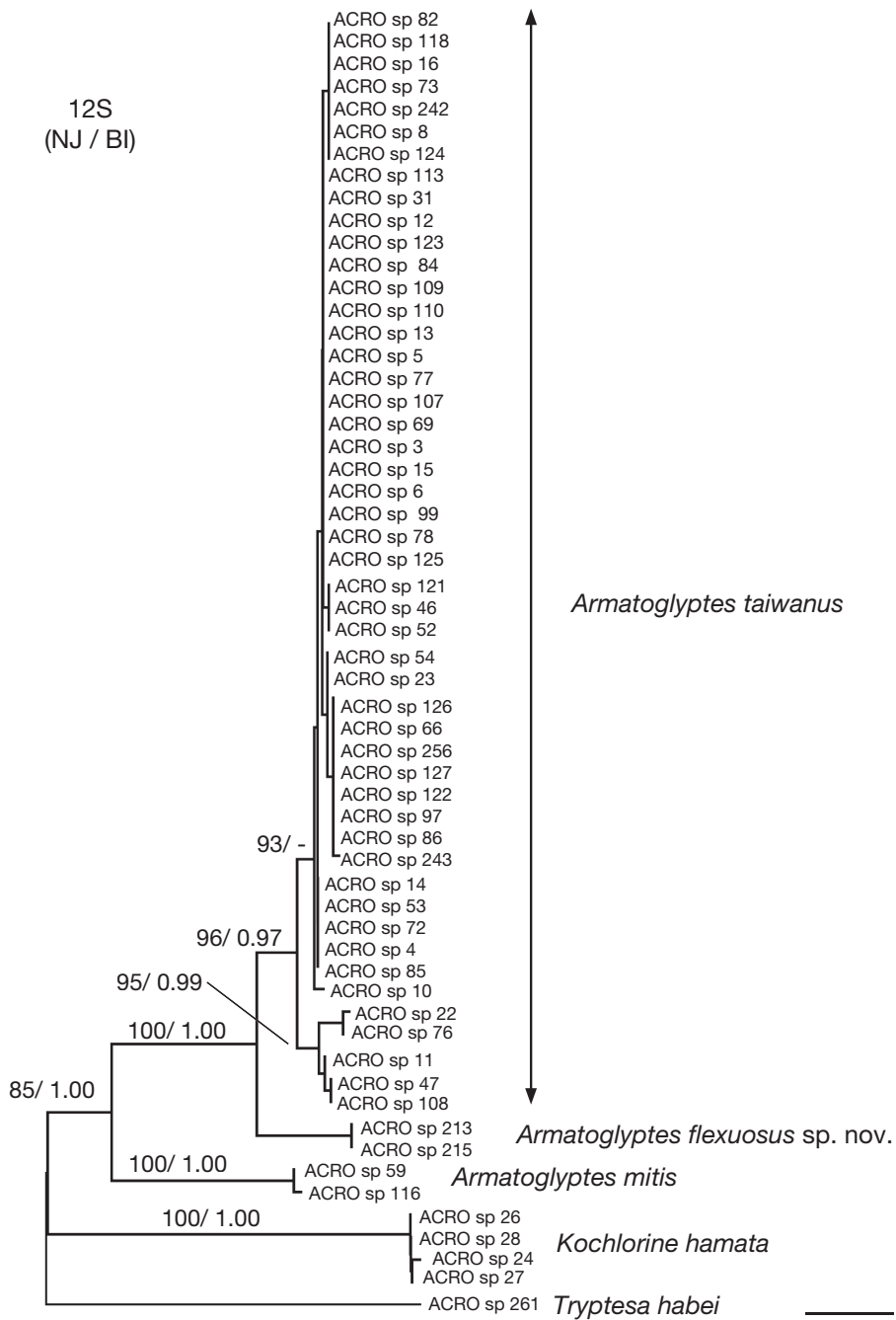


FIG. 7. — Phylogenetic neighbor-joining (NJ) tree of five studied acrothoracican species, based on 12S rDNA region. The topology of the Bayesian inference tree (BI) is identical with that of the NJ tree. Bootstrap statistical significance of NJ (left) and a posteriori possibility of BI (right) are shown on the branches, for values of 50 or more. Scale bar: 0.05.

possess free-swimming nauplii, as do species of the genera *Berndtia* Utinomi, 1950 and *Trypetesa* Norman, 1903 along with *Lithoglyptes indicus* Aurivillius, 1892. In fact, observation of live specimens of *Armatoglyptes taiwanus* in the present study revealed that this species releases eggs into the water column and that nauplius larvae hatch immediately after egg release. Nauplii of instar II have well developed labrum, long frontolateral horns, a bifurcate thoraco-abdominal process, and a very long caudal spine (Fig. 3C, D). These characters suggest these nauplii are active swimmers and planktotrophic. Dissected adult females of *A. taiwanus* rarely contained a single cypris larva inside (all naupliar larvae should be released for dispersal), which may represent a future dwarf male (Kolbasov *et al.* 1999). On the basis of this circumstantial evidence, we suggest that *Armatoglyptes* species have planktonic naupliar larval instars in their life cycle and are probably able to disperse widely. A description of the complete larval development of any species of *Armatoglyptes* is an urgent task for the future.

Armatoglyptes samples from the Mozambique Channel in the Indian Ocean displayed a sequence divergence greater than the one found among populations of *A. taiwanus* in Pacific and Andaman Sea waters, as well as a significant morphological difference from those populations. In the present study, the sequence divergence in the 12S gene of *A. taiwanus* between Mozambique and other locations reached 12%. Previous studies showed that interspecific sequence divergence in 12S is 3.4–4.2% in intertidal barnacles *Tetraclita* Schumacher, 1817 (Chan *et al.* 2007), 4.1–5.0% and 9.2–13.9% in coral-associated barnacle *Cantelliuss* Ross & Newman, 1973 and *Savignium* Leach, 1825 respectively (Mokady *et al.* 1999) and in the high shore barnacle *Chthamalus* Ranzani, 1817, it even reaches 15–18%. On this basis, we recognize the Mozambique Channel specimens as a distinct new species. The phylogenetic analysis of the 12S rDNA region showed that *A. taiwanus* and *A. flexuosus* n. sp. are more closely related to each other than either is to *A. mitis*. From the 12S phylogenetic tree, it appeared that *A. taiwanus* from the Pacific Ocean and Andaman Sea populations consists of

two clades with very high bootstrap values. The differences between these two clades were less than 2%, which suggests that these differences rather represent intraspecific variance. Both clades consisted of samples from Taiwan, the Philippines and Thailand, suggesting there are no distinct distribution of these clades.

Armatoglyptes flexuosus n. sp., *A. taiwanus* (= *A. habei*), and *A. mitis* are morphologically very close and may well actually be sub-species freely capable of hybridization. Specimens having morphological characters of both *A. taiwanus* and *A. flexuosus* n. sp. were formerly assigned to the single species *A. habei* (Tomlinson 1969; Kolbasov & Høeg 2000). Kolbasov & Høeg (2000) considered that the only principal difference between *A. mitis* and *A. taiwanus* from different regions is the armament of the posterior processes of the opercular bars, consisting in *A. taiwanus* of one or several strong, spur-like spines that are absent in *A. mitis*. However, both species may exhibit the full range of spine development, and very often individuals can be found in which one posterior process bears a clear spine while the other bears only a bifid tooth. Kolbasov & Høeg (2000) supposed that these forms represent hybrids, which can coexist in similar areas and depths, and may even inhabit the same host as their parent species.

In the present study, specimens of *A. taiwanus* with different sorts of armament of the posterior processes of the opercular bars were found, some of which may be hybrid forms. However, we could not identify hybrids solely based on the mitochondrial marker gene. To identify hybrids of this “complex species” in future studies, we recommend analyzing additional genetic markers, in particular nuclear genes. Nevertheless, both *A. mitis* and *A. flexuosus* n. sp. represent clear morphospecies, because each has a characteristic armament of the posterior processes of the opercular bars (conical with bifid teeth, and “L-shaped” with bifid teeth respectively). L-shaped posterior processes have not been reported in any previously described species of this genus. We have provided additional molecular evidence (mitochondrial 12S rDNA) confirming that the latter is a new species.

The acrothoracian fauna of southern African waters has been studied extensively. Berndt (1907a) and Tomlinson (1969) reported *Weltneria spinosa* Berndt, 1907 from South Africa. Barnard (1924) described a dubious species, *Kochlorine bihamata* Barnard, 1924, from the Cape of Good Hope. *Australophialus turbonis* (Barnard, 1925) and *A. utinomii* (Tomlinson, 1969) also were found in Qolora, Cape Province, South Africa (Tomlinson, 1969). *Kochlorine boqueti* Turquier, 1977, *K. floridana* Wells & Tomlinson, 1966, *Trypetesa spinulosa* Turquier, 1976, *Tomlinsonia asymetrica* (Turquier & Carton, 1976), and *Cryptophialus longicollatus* Berndt, 1907 have been reported from Madagascar and vicinity (Berndt 1907b; Tomlinson 1969; Turquier 1976, 1977; Turquier & Carton 1976; Kolbasov 2009). Three species of *Armatoglyptes*, viz. *A. cornutus*, *A. mitis*, and *A. taiwanus* (as *A. habeii*), have been reported from the Seychelles (Kolbasov 2009). The present study is the first report of the genus *Armatoglyptes* in waters off Mozambique.

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