

**MORPHOLOGICAL AND GENETIC DIFFERENTIATIONS OF THE STALKED
BARNACLE *HETERALEPAS JAPONICA* AURIVILLIUS, 1892,
WITH DESCRIPTION OF A NEW SPECIES OF
HETERALEPAS PILSBRY, 1907, FROM THE PHILIPPINES**

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ABSTRACT. – Species of *Heteralepas* are called “naked barnacles” because they have no external shell plates or valves on the capitulum. Taxonomy in *Heteralepas* is often confusing due to the lack of calcareous hard characters for comparison and the high degree of intra-specific morphological variations in the stalk, shape of capitulum and the cirral segments of the barnacles. In the present study, barnacles initially identified as *Heteralepas japonica* collected in Philippine, Japanese and Taiwanese waters exhibit diagnostic morphological and genetic differentiation. *Heteralepas japonica* from the Philippines had one to three crests on the carinal margin, whilst the Japan and Taiwan population had at most one crest on the carinal region. The wall of the capitulum of the Japanese and Taiwanese population is thicker than the Philippine populations. Phylogenetic analysis of COI and 12S sequences from individuals initially identified as *H. japonica* resulted in two distinct clades with high nodal support (> 85% for all analyses). The first clade consisted of all *H. japonica* from the Philippines, while the three *H. japonica* individuals from Taiwan and the single individual from Japan form the second clade. The sequence divergences among the Philippine and Taiwanese clades were ~ 14% and ~ 8.5% for uncorrected p-distance in the COI and 12S regions, respectively. These values were much greater than sequence divergences within each clade ($\leq 2.1\%$). Results suggest that *Heteralepas* from the Philippines and Taiwan are two different species. Type locality of *H. japonica* is at Hirado Strait, West Kyushu, Japan and the morphological features of the Japan and Taiwan populations matched with the previous descriptions of *H. japonica* collected from Japanese waters. We, therefore, recognise the Philippine form as a new species, described herein.

KEY WORDS. – *Heteralepas japonica*, Barnacles, new species, Cirripedia, *Heteralepas cantelli*.

INTRODUCTION

Stalked barnacles of the genus *Heteralepas* Pilsbry, 1907, are called “naked barnacles” because they have no external shell plates or valves on the capitulum. *Heteralepas* is also characterized by having the inner rami of the cirri V and VI much reduced in length in comparison to the corresponding outer rami. Taxonomy in *Heteralepas* is often confusing due to lack of the hard parts for comparisons and the high degree of intra-specific morphological variations in the stalk, shape of capitulum and the cirral segments of the barnacles (see

Zullo & Newman, 1964; Foster, 1978). *Heteralepas indicus* (Gruvel, 1902), for example, had been considered to be a distinct species because it had a longer stalk length when compared to *H. japonica* (Aurivillius, 1892). Nilsson-Cantell (1927) studied the morphological variation of *H. japonica* and concluded *H. indicus* was a variety of *H. japonica* due to the high degree of morphological variations in the stalk length of *H. japonica*. Nilsson-Cantell (1927) also pointed out that the length of caudal rami and the segment number, which were used to classify *Heteralepas*, did exhibit a high degree of variation and these may not be diagnostic

Table 1. Sampling station details from the Philippines (Stations 1–6), Taiwan (Station 7) and Japan (Station 8).

Station No.	Station code	Date	Latitude		Longitude		Min. depth (m)	Max. depth (m)
			Deg.(°)	Min. (°)	Deg. (°)	Min. (°)		
1	CP2362	26 May 2005	8	56.50	123	32.70	679.0	684.0
2	CP2381	28 May 2005	8	43.30	123	19.00	280.0	275.0
3	CP2335	22 May 2005	9	34.30	123	37.80	733.0	743.0
4	CP2332	22 May 2005	9	38.80	123	45.90	418.0	477.0
5	CP2331	22 May 2005	9	39.20	123	47.50	255.6	262.8
6	CP2372	27 May 2005	8	38.70	123	16.00	255.0	231.0
7	CD 380	25 Jul. 2007	24	37.12	122	9.88	329.0	329.0
8	JP	16 Mar. 2007	Collected at fish market in Sakai, Minabe, Wakayama					

CP, French beam trawl; CD, Oregon beam trawl.

characters for species identification. Nilsson-Cantell (1927) and Foster (1978) proposed that a detailed revision of *H. japonica* species complex is needed but no studies to date have been conducted.

Heteralepas japonica was described in the Hirado Strait, W. Kyushu, Japan (33°10'N 129°18'E, see Aurivillius, 1892) and was reported to be widely distributed in the Indo-Pacific and Australian waters and is commonly found at 100–400 m depth. Specimens initially referred to *H. japonica* collected in the Philippines from the PANGLAO 2005 expedition and in Taiwanese waters showed a high degree of morphological variations. In the present study, we showed that the Philippine form is different from the Taiwan and Japan form from a combined morphological and molecular analysis and regards the Philippine form as a new species and described herein.

MATERIALS AND METHODS

Morphological analysis. – Specimens referred to *Heteralepas japonica* in Philippine and Taiwanese waters were collected by French beam trawls and Oregon beam trawls respectively. A single specimen of *H. japonica* collected from the fish market at Sakai, Minabe, Wakayama was included in the present study as a representative specimen of *H. japonica* because it was collected relatively close to the type locality. Upon collection, all barnacles were preserved in 95% ethanol. Comparison of the external morphological features among barnacles collected from different stations and regions (Table 1) included the capitulum length (CL), width (CW), peduncle length, width, orifice height, number of crests on the carinal margin, and the thickness of the capitulum (digital vernier calliper, ± 0.1 mm; Fig. 1A). Variations in the external morphological characters between the specimens from the Philippines, Japan and Taiwan were analysed using multivariate analysis (PRIMER 6, Plymouth Routine in Multivariate Analysis; Clarke, 1993). The data was square root transformed prior to analysis. A similarity matrix was calculated between the *Heteralepas* samples using the Bray-Curtis index. Non-metric Multidimensional Scaling (nMDS) was conducted to generate the two dimensional plots of the external morphology among *Heterlepas* from the Philippines, Japan and Taiwan. Analysis of Similarity (ANOSIM) was

conducted to test the differences in morphological characters between the Philippine and Taiwanese populations and SIMPER (Similarity Percentage) analysis was used to detect any significant discriminate parameters. The Japanese specimen was not included in ANOSIM and SIMPER analysis as there was only a single specimen.

The mouth parts and cirri of *Heteralepas* from the Philippines, Japan and Taiwan were further analysed using light microscopy and scanning electron microscopy (SEM) to study the variation of setal types of cirri and mouth parts (Chan et al., 2008). Barnacles were dissected and prepared for SEM following Chan et al. (2007a, b).

Specimens examined are deposited in the National Museum of the Philippines, Manila, the Philippines (NMCR), Biodiversity Research Museum of the Biodiversity Research Center, Academia Sinica (ASIZCR), the research collections in the Coastal Ecology Laboratory, Biodiversity Research Center, Academia Sinica (CEL) and National Museum of Natural Science, Taiwan (NMNS).

Molecular analysis. – Total genomic DNA was extracted from whole soft tissue of individual barnacles using the commercial QIAamp Tissue Kit (QIAGEN). Primers for amplifying partial sequences of mitochondrial COI and 12S rDNA were obtained from Folmer et al. (1994) and Mokady et al. (1994). The amplifications were conducted in a reaction mix containing 1 μ L of template DNA, 1 \times PCR reaction buffer, 2 mM MgCl₂, 200 nM of each primer, 200 μ M dNTPs, 1.5 units of *Taq* polymerase (Amersham) and ddH₂O to a total volume of 50 μ L. The PCR profile was as follows: 3 min at 94°C for initial denaturation, then 33 cycles of 30 s at 94°C, 30 s at 47°C, 40 s at 72°C with the final extension for 3 min at 72°C. The PCR products were then purified using QIAquick gel purification kit (QIAGEN) according to manufacturer's instructions. Sequences were generated using the same sets of primers and determined using an Applied Biosystem (ABI) 3100 automated sequencer using the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol.

Sequence analysis. – Sequences were aligned using CLUSTAL W (Thompson et al., 1994) with default gap

weighting parameters and adjusted by eye. Alignment of COI sequences was confirmed by translating the aligned DNA sequences into amino acid sequences. Sequence data from the two genes were first analyzed separately to determine any nodes with high conflicts among different datasets. For a combined analysis, phylogenetic congruence among the two datasets was tested under parsimony criterion using incongruent length difference (ILD) test (Farris et al., 1994) implemented in PAUP* v4.0 b10 as the partition-homogeneity test (Swofford, 2000). Sequences from two deep sea barnacles species from the Philippines, *Octolasmis orthogonia* (Darwin, 1851) and an unidentified *Paralepas* sp. were included as outgroups.

Four methods of phylogenetic inference were applied to the datasets: neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) using PAUP*, and Bayesian inference (BI) using MrBayes v.3.12 (Huelsenbeck & Ronquist, 2001). Modeltest 3.7 (Posada & Crandall, 1998) was used to select the best-fit model of nucleotide substitution for each dataset in ML and BI analyses. Kumura 2 parameter distance was used for the NJ analyses with 1000 replicates used for bootstrapping. MP analyses were performed using heuristic search and tree-bisection-reconnection (TBR) with 1000 random sequence addition replicates. Character states were unordered and equally weighted. Gaps were treated as missing data. Bootstrap support for the most parsimonious tree was evaluated using 1000 replicates with 100 random sequence addition replicates. For ML analysis, the heuristic search was performed with 100 random sequence addition replicates and support for individual clades was obtained from 100 bootstrap (BP) replicates with 1 random sequence addition replicates. The Bayesian analysis was run with four Markov Chain Monte Carlo (MCMC) chains for 2 000 000 generations started from a random tree. The chain was sampled every 100 generations and the first 4000 trees were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PP). Three replicates of these Bayesian runs were conducted as described to ensure convergence was repeatable.

RESULTS

Morphological analysis. – Capitulum of *Heteralepas* specimens from the Philippines, Japan and Taiwan had no external valves and the colour of the capitulum and peduncle is pale yellow. In the Philippine samples, the carinal region had 1–3 distinct crests (Figs. 1B, 6A; indicated by arrows) whilst the Japanese and Taiwanese specimen had at most one crest (Figs. 2A, 5A). The shape of the capitulum varied among sites and between individuals within site (Fig. 1B). From nMDS ordination plots, the morphology of the Philippine, Japanese and Taiwanese populations were separated into two distinct clusters (Fig. 1C), the Philippine population in one cluster and the Japanese and Taiwanese populations in other cluster. The differences in morphological characters between the Taiwanese and Philippine populations were further supported from significant results in the ANOSIM

($r = 0.7$, $p < 0.05$). Both *Heteralepas* populations from the Philippines, Japan and Taiwan were similar in the size of capitulum and with variable length of peduncle. However, SIMPER analysis showed that number of crests in the carinal region and thickness of the capitulum account for a total of 70% differences between the two populations. *Heteralepas* population from the Philippines had 1–3 crests on the carinal region, whilst the Taiwanese specimens had at most 1 crest. Taiwanese specimens had significantly thicker capitulum wall ($0.83 \text{ mm} \pm 0.11$, $n = 6$) than the Philippine specimens ($0.41 \pm 0.13 \text{ mm}$, $n = 25$).

Cirrus I of the specimens from the Philippines, Japan and Taiwan have one filamentary appendage at the basal region (Fig. 2B, F). Inner rami of cirrus I of the Taiwan and Japan samples are shorter than the outer ramus. The inner ramus has 10–12 segments, while the outer ramus has 16–18 segments (Fig. 2B). For the Philippine specimen, the inner ramus (10 segments) is shorter than the outer rami (20 segments; Figs. 2F, 6I). Compared to the Taiwanese and Japanese specimens, the base of the inner ramus of the Philippine samples is slightly swollen and expanded (Fig. 2F). Setae on cirrus I of the Philippine sample are serrulate type (Fig. 6J). Morphology of the cirrus II–VI is similar among the Philippine, Taiwanese and Japanese specimens. The inner and outer rami of cirrus II–cirrus IV are similar in length for Taiwan, Japan and Philippine specimens. In cirrus V and VI, the outer rami are much longer than the inner rami for the Philippine, Taiwanese and Japanese specimens (Fig. 2C, G). Outer rami of cirrus V and VI ranged from 60–65 segments and the inner rami ranged from 15–20 segments. Each segment of the cirri II–VI of both populations had 1 or 2 strong spines on the lateral sides (Figs. 4D, E; 6K, L). Caudal rami were present in all specimens. The Japanese sample has 12 segments (Fig. 2D), Taiwanese samples has 9 segments (Fig. 4A, B) whilst the Philippine sample has 6 segments (Fig. 2H). The lengths of the caudal rami for both Japanese and Philippine samples are within the basipode of cirrus VI.

Mouth parts of the specimens from the three locations are similar (Fig. 3). For all samples, mandibles have four large teeth (Figs. 3A, E; 5H; 6D). Maxillule are strongly notched, with two–three spines on the upper notch (Figs. 3B, F; 5F; 6F). The mandibulatory palps are elongated with dense serrulate setae (Figs. 3C, G; 5B, C). Maxilla are globular with two patches of serrulate setae (Figs. 3D, H; 5J, K; 6G, H). Labrum concaved with numerous strong teeth (Figs. 5D, E; 6B, C).

Molecular analysis. – A total of 28 individuals of the specimens that are initially referred to *Heteralepas japonica* (24 samples collected from the Philippines three from Taiwan and one from Japan) were sequenced for the two genes. All sequences are deposited in GenBank (Accession nos. EU884136–EU884174 and FJ694788–FJ694789). The aligned COI fragments consisted of 639 bp of which 115 out of the 210 variable sites are parsimony informative. The aligned 12S rRNA sequences include 336 bp, of which 41 of the 121 variable sites are parsimony informative. The trees

constructed from individual genes do not show great conflict in topology (nodes different among trees with support > 70% in ML analysis) and there is no significant incongruence between data from the two genes as revealed by ILL test ($P = 1$). Therefore, we concatenated the sequences of the two genes, resulting in a dataset with 975 characters for analysis. The best-fit model selected for the combined dataset using Modeltest is TVM+I+G.

Parsimony analysis resulted in 1,623 most parsimonious trees of 497 steps, a consistency index of 0.845 and a retention index of 0.865. These trees differ from each other the arrangement of internodes which receive low support. The strict consensus tree reveals two major clades and most of the individuals within each of these clades form a polytomy. The overall topology recovered from the other three phylogenetic inference methods resulted in trees that are congruent with the MP. Therefore, results from all of the three analyses are shown together based on the BI tree with bootstrap values ≥ 50 and PP value ≥ 0.5 shown on the corresponding branches (Fig. 7).

In all analyses, individuals of *Heteralepas japonica* were consistently divided into two distinct clades with high nodal support (> 85% for all analyses). The first clade consisted of all specimens from the Philippines, while the three individuals from Taiwan and the single specimen from Japan form the second clade. The two clusters corresponded to the two forms identified by morphological analysis. However, the relationships between individuals within each clade are poorly resolved. The sequence divergences among the two clades were high. They are differentiated by ~ 14% and ~ 8.5% for uncorrected p-distance in the COI and 12S regions respectively. These values were much greater than sequence divergences within each clade ($\leq 2.1\%$). The data suggests *Heteralepas* from Philippine belongs to a different species from the Taiwan-Japan clade.

SYSTEMATICS

LEPADIFORMES Buckeridge & Newman, 2006

HETERALEPADOMORPHA Newman, 1987

HETETRALEPAIDAE Nilsson-Cantell, 1921

Heteralepas Pilsbry, 1907

Heteralepas cantelli new species

(Figs. 2E–H; 3E–H; 6)

Material examined. – Holotype. NMCR. CL 11.68 mm, CW 10.08 mm, Maribojoc Bay, Bohol Sea, the Philippines, Panglao 2005 Stn. CP2331 (9°39.2'N 123°47.5'E, muddy substratum, 22 May 2005). Paratypes. NMNS-6006-001 > 50 specimens, CL 7.97–14.5 mm, CW 4.78–11.78 mm, data same as holotype. ASIZCR000213, 1 specimen, CL 13.02 mm, CW 8.03 mm. Panglao 2005 Stn. CP2391 (9°30.3'N 123°43.0'E, sandy substratum; Table 1). ASIZCR000214, > 50 specimens, CL 7.97–14.5 mm, CW 4.78–11.78 mm, Panglao 2005 Stn. CP2331 (9°39.2'N 123°47.5'E, muddy substratum).

CEL-PANGLAO-08, > 100 specimens, CL 7.97–14.5 mm, CW 4.78–11.78 mm, Panglao 2005 Stn. CP2332 (9°38.8'N 123°45.9'E, muddy substratum; Table 1). CEL-PANGLAO-09, > 100 specimens, CL 5.3–18.1 mm, CW 3.6–9.80 mm, Panglao 2005 Stn. CP2362 (8°56.5'N 123°32.7'E, sandy substratum; Table 1). CEL-PANGLAO-10, > 10 specimens, CL 7.62–14.7 mm, CW 5.07–10.08 mm, Panglao 2005 Stn. CP2372 (8°38.7'N 123°16.0'E, sandy/muddy substratum; Table 1). CEL-PANGLAO-11, > 100 specimens, CL 10.29–17.90 mm, CW 5.88–12.5 mm. Panglao 2005 Stn. CP2381 (8°43.3'N 123°19.0'E, sandy substratum; Table 1).

Comparative material. – *Heteralepas japonica* (Aurivillius, 1892). Taiwan: Stn. CD 380 (24°37.1'N 122°9.8'E, depth 329 m, 25 Jul.2007). Japan: (collected at fish market in Sakai, Minabe, Wakayama, coll. Y. Yusa. 16 Mar.2007).

Diagnosis. – Capitulum rounded, wall thin (< 0.08 mm). Cirrus I has one filamentary appendage. Inner rami shorter than outer rami in cirri V–VI.

Descriptions. – Capitulum without any valves. Shape of capitulum and peduncle length variable (Fig. 1B). Orifice crenulated. Carinal margin of capitulum and pedicle with 1–3 crests (Figs. 1B, 2A). Maxilla bilobed, with dense serrulate setae. Maxillule strongly notched, two strong setae on upper notch, blade shaped cuspidate setae on the cutting margin (Figs. 3F, 6F). Mandible with four large teeth including inferior angle, denticles blade shaped (Figs. 3A, 6D, E). Labrum concave, with 28 small teeth (Fig. 6B, C). Maxilla triangular with setae on superior margin (Fig. 3G). Mandibulatory palp triangular with two patch of dense setae on the margin (Figs. 3H, 6G, H). Cirrus I maxillipeds, unequal rami, inner rami shorter, six segmented, outer rami longer, 12 segmented (Fig. 2F). Cirrus I with bidentate serrulate setae (Fig. 6, I, J). Base of cirrus I with one filamentous appendage (Fig. 2F). Cirri II–IV long and slender, with serrulate type setae. Lengths of rami of cirri V and VI unequal, inner rami obviously shorter than outer rami. Number of segments variable in rami of cirri, inner rami and outer rami of cirrus II 24 and 26, 47 and 46 in cirrus III, 47 and 47 in cirrus IV, 50 and 19 in cirrus V, 49 and 18 in cirrus VI (Fig. 2G). Each segment of cirrus II–VI with a pair of long spine (Fig. 6K, L). Caudal appendage present, 6 segmented, 1/4 length of cirrus exopodite of cirrus VI (Fig. 2H).

Etymology. – It is an honor to name the new species after C. A. Nilsson-Cantell who contributed to the barnacle taxonomy and pointed out the great morphological variations and identification problems in *Heteralepas japonica*.

Distribution. – Presently known only from Philippine waters.

Remarks. – *Heteralepas cantelli*, new species, is morphologically close to *H. japonica*, but the thickness of capitulum wall exhibit diagnostic differences. *Heteralepas japonica* has thicker capitulum wall (0.83 ± 0.11 , $n = 6$ from Taiwanese samples) than *H. cantelli*, new species, (0.41 ± 0.13 mm, $n = 25$ from Philippine samples). In the present study, although the number of crests on the dorsal side of the capitulum appears to be more in *H. cantelli*, new species.

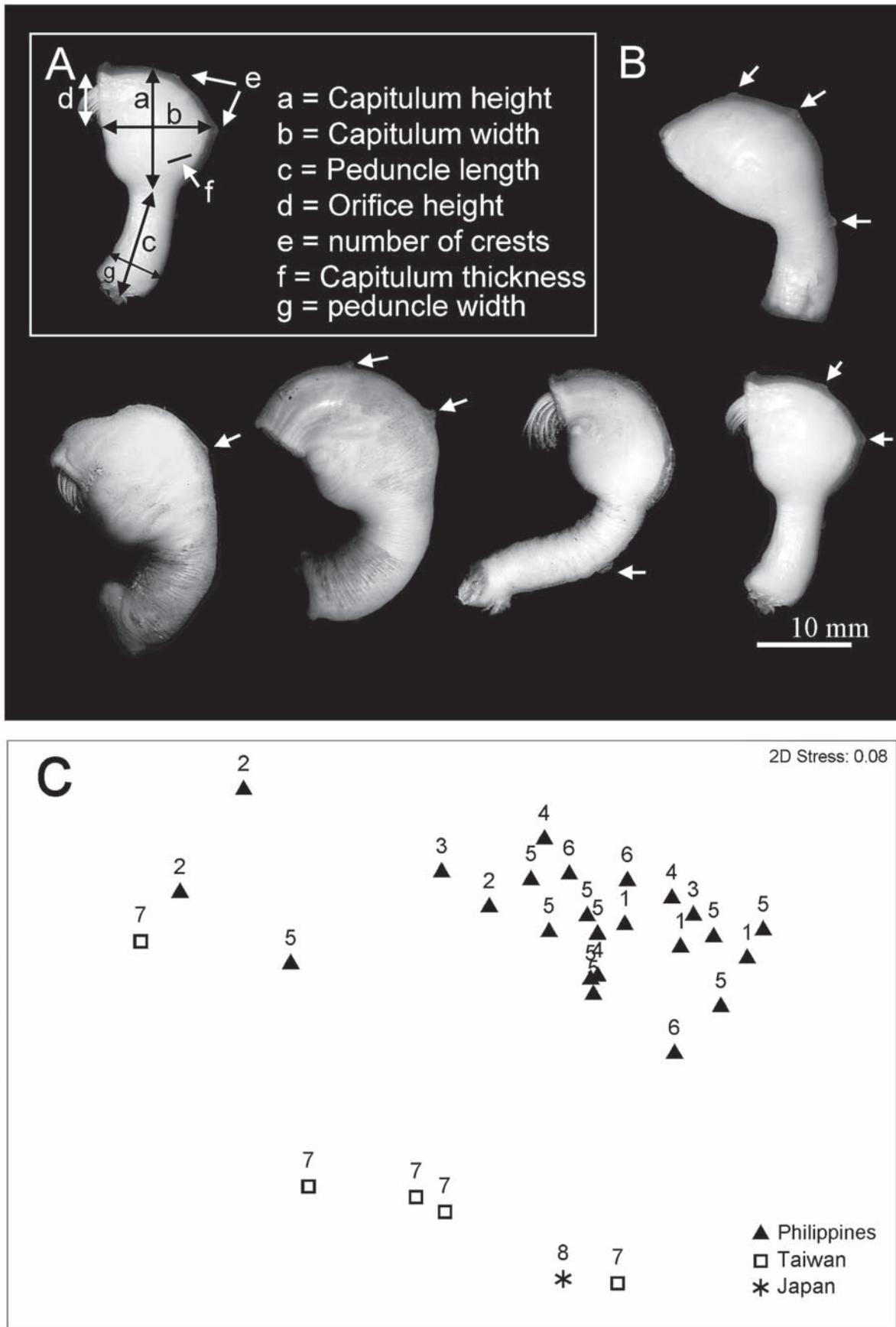


Fig. 1. A, morphological parameters measured in the external features of *Heteralepas* species for multivariate analysis. Capitulum thickness were measured by cutting a small hole on the capitulum and the thickness of the capitulum was measured using vernier caliper; B, morphological variation in the capitulum shape and number of crests (indicated by arrows) on the carinal region of *Heteralepas cantelli*, new species, from the Philippine population; C, nMDS ordination plots on the morphological divergence among the Philippines, Japan and Taiwan populations. Number denotes the collecting station (see Table 1 for details).

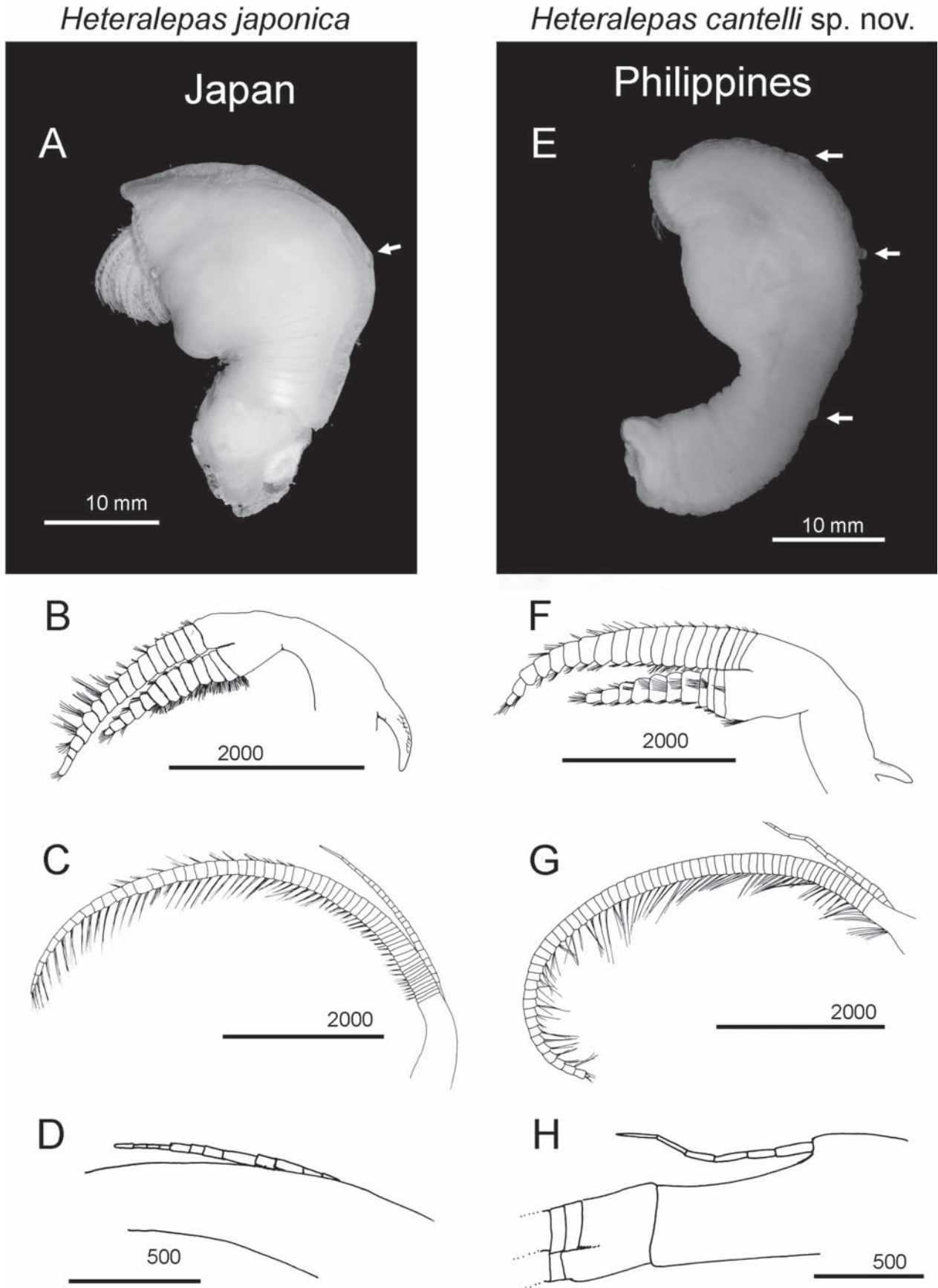


Fig. 2. *Heteralepas japonica* from Japan: A, external morphology, showing the capitulum and peduncle; B, Cirrus I, showing the filamentary appendage at the basal region; C, cirrus VI; D, caudal appendages. *Heteralepas cantelli*, new species, from the Philippines; E, external morphology, showing the capitulum and peduncle. Arrows indicate the crests on the dorsal region; F, cirrus I; G, cirrus VI. H. Caudal appendages. Scale bars: A, E in mm; B–D, F–H in μm .

H. japonica (Japan)

H. cantelli sp. nov. (the Philippines)

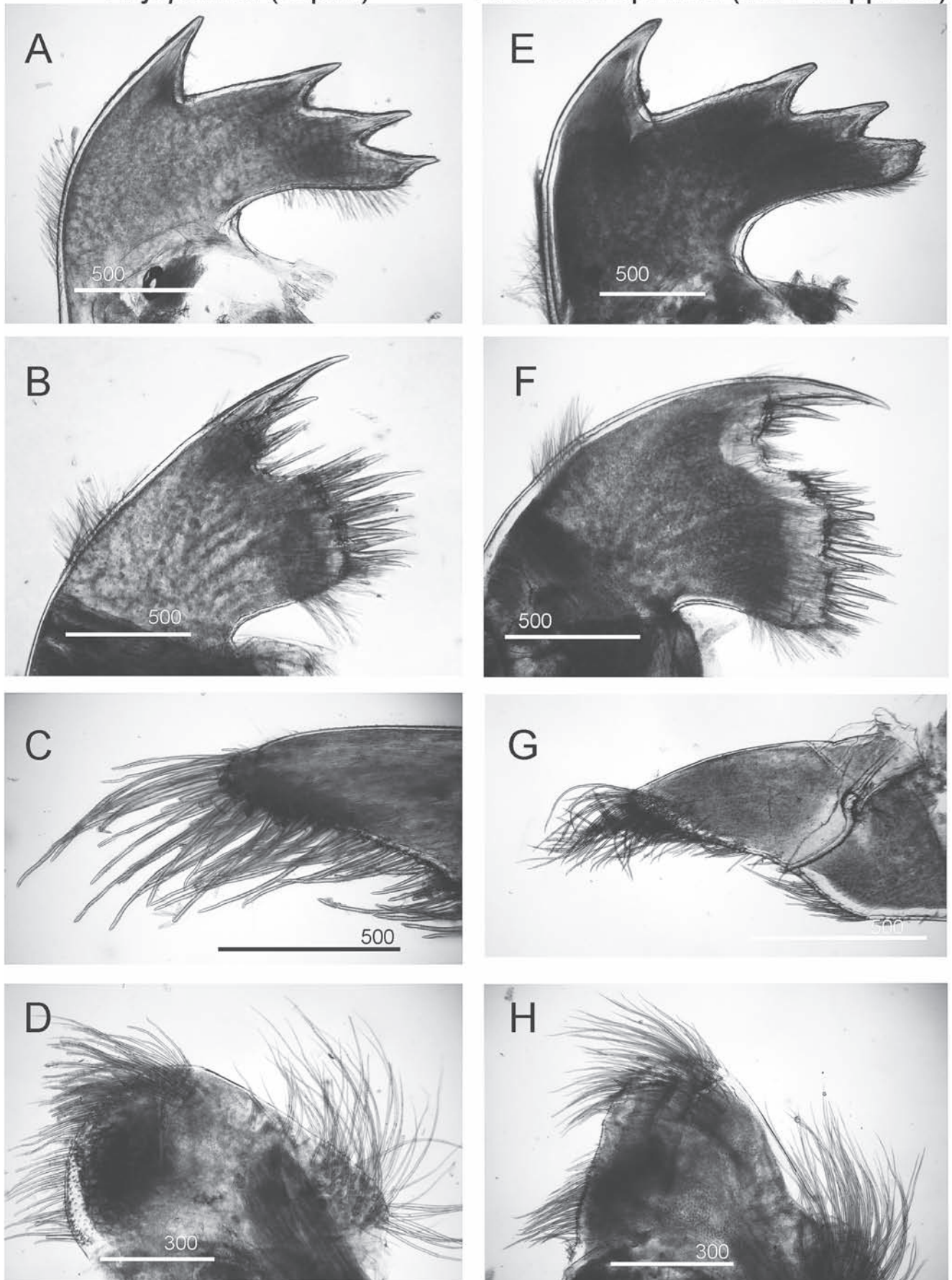


Fig. 3. Light micrographs. *Heteralepas japonica* from Japan: A, mandible; B, maxillule; C, mandibulatory palp; D, maxilla. *Heteralepas cantelli*, new species, from the Philippines: E, mandible; F, maxillule; G, mandibulatory palp; H, maxilla. Scale bars in µm.

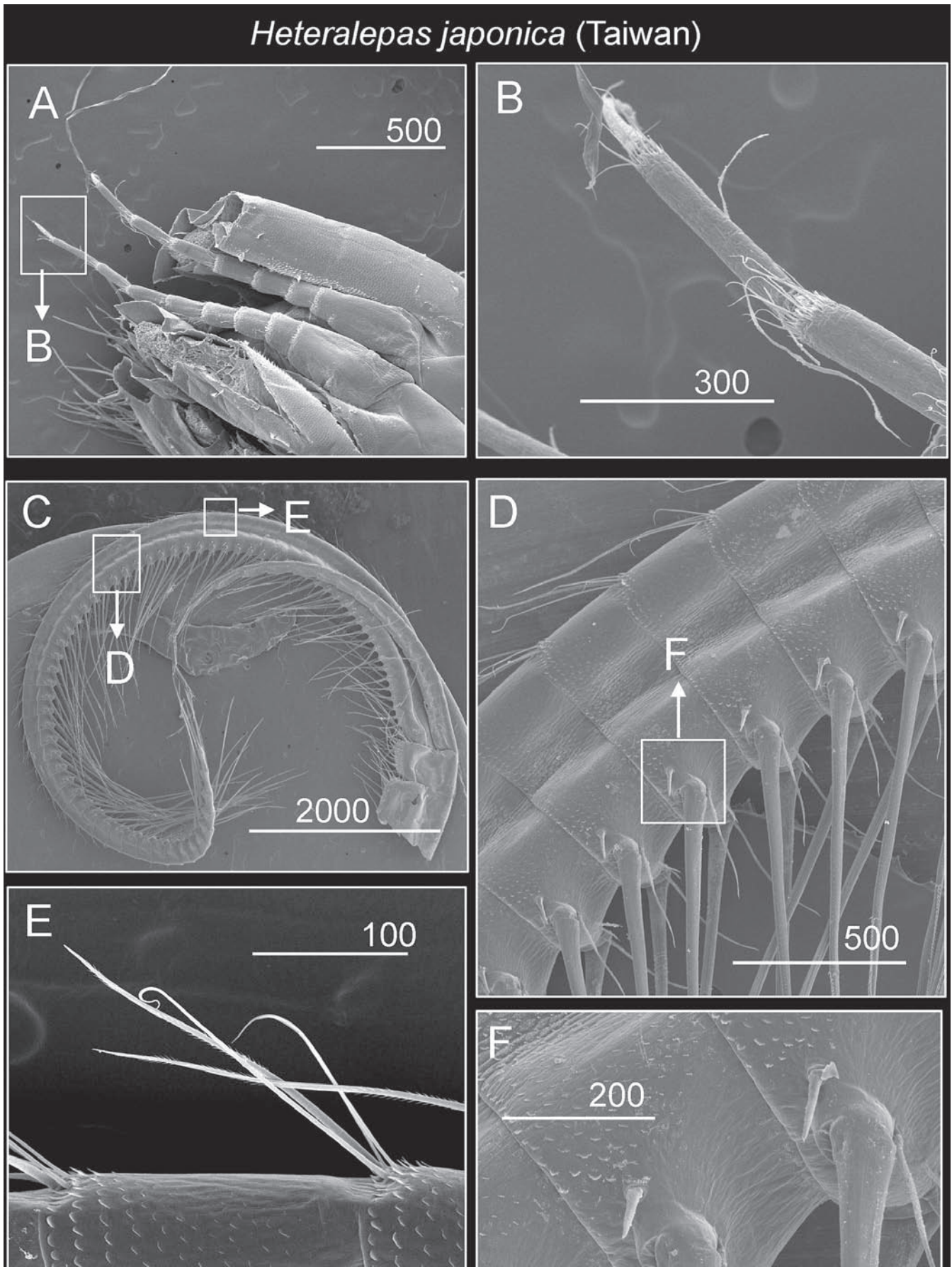


Fig. 4. *Heteralepas japonica* from Taiwan. Scanning electron micrographs images: A, caudal appendages; B, tip of caudal appendages; C, cirrus VI; D, intermediate segment of cirrus VI; E, simple setae on the dorsal side of intermediate segment of cirrus VI; F, strong spine on the lateral side of the intermediate segment of cirrus VI. Scale bars in µm.

Heteralepas japonica (Taiwan)

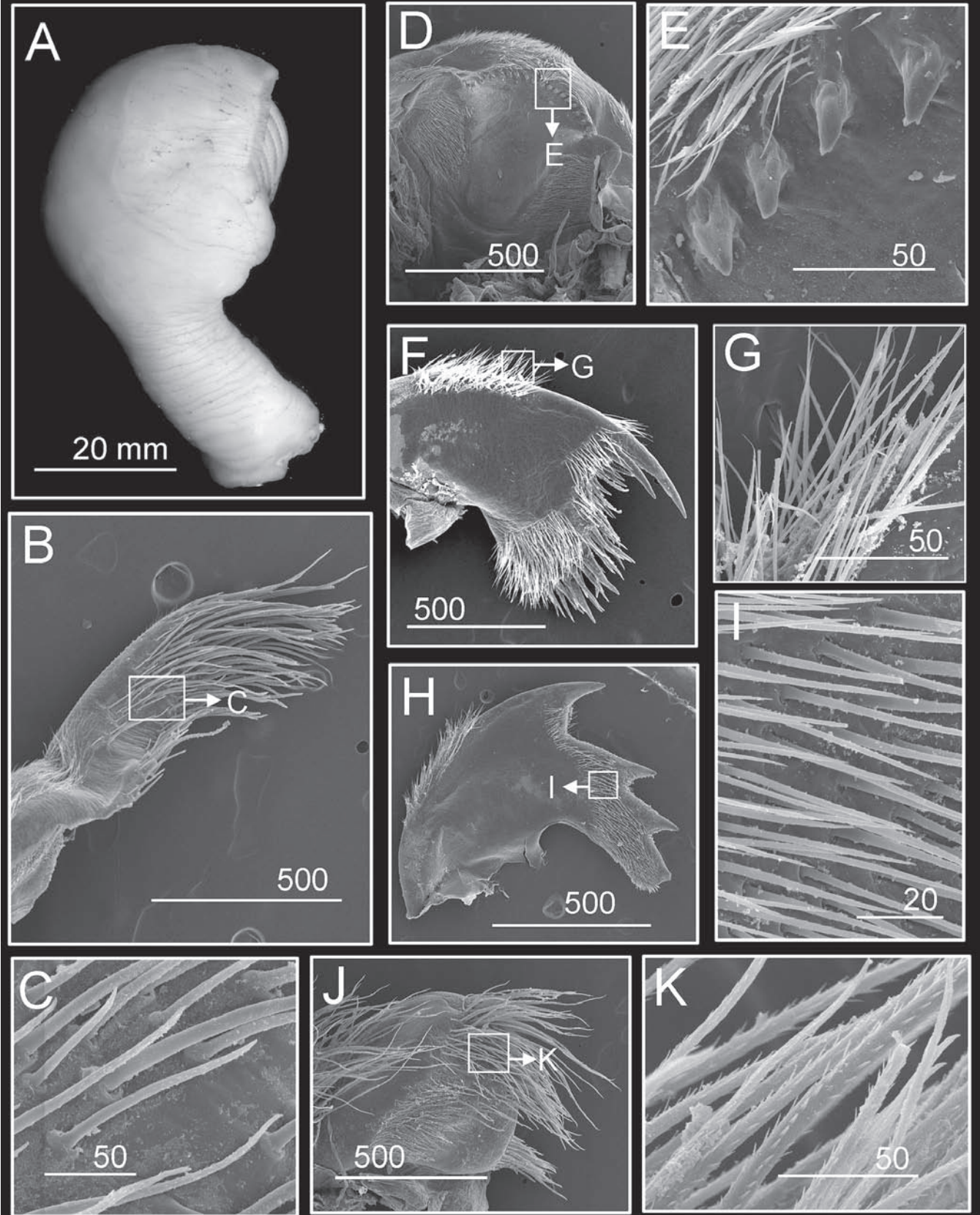


Fig. 5. *Heteralepas japonica* from Taiwan: A, external morphology, showing capitulum and peduncle. Scanning electron micrographs: B, mandibulatory palp; C, serrulate setae on mandibulatory palp; D, larvum; E, teeth on labrum; F, maxillule; G, dense setae on the lateral region of the maxillule; H, mandible; I, blade shaped setae on mandibles; J, maxilla; K, serrulate setae on maxilla. Scale bars: A in mm; B–K in μm.

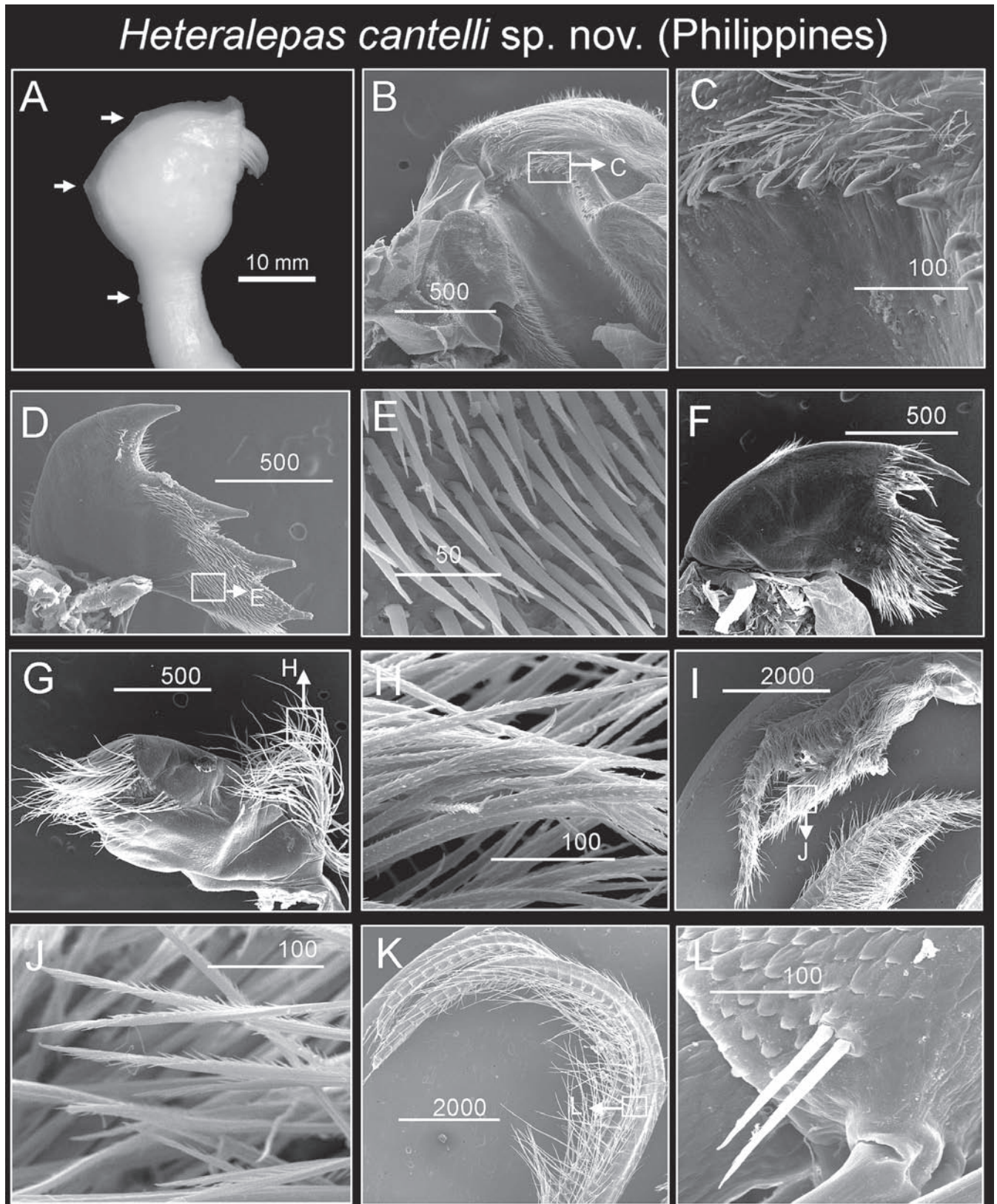


Fig. 6. *Heteralepas cantelli*, new species, from the Philippines: A, side view showing capitulum. Arrows indicate the crests on the carinal margin of the capitulum. Scanning electron micrographs: B, labrum; C, teeth on labrum; D, mandible; E, blade shaped setae on mandible; F, maxillule; G, maxilla; H, serrulate setae on maxilla; I, cirrus I; J, serrulate setae on cirrus I; K, cirrus IV; L, paired setae on segment of cirrus IV. Scale bars: A in mm; B–L in μm .

Number of crests on the dorsal side of capitulum has been shown to be a variable character. In the present study, this character was not suggested to be use for discriminate *H. japonica* and *H. cantelli*, new species.

DISCUSSION

The high degree of morphological variation in *Heteralepas* has led to taxonomic confusion in the genus (Nilsson-Cantell, 1927; Zullo & Newman, 1964; Foster, 1978). In the present study, we studied the *Heteralepas* from Japan, Taiwan and the Philippines and resulted into two different populations which exhibit diagnostic morphological and genetic differences. *Heteralepas japonica* from Japan and Taiwan did not have > 2 crests on the carinal region of the capitulum and the wall of capitulum is thicker than the Philippine specimens.

Individuals from Japan and Taiwan and the Philippines were also differentiated in the mitochondrial COI and 12S sequences, which Japan and Taiwan fall into one clade and the Philippine populations in the other clade. The sequence divergence between the two clades is comparable to the interspecific differences reported in other barnacle species (e.g. ~ 9% for COI in *Euraphia*, Wares, 2001; 7.5–13% for COI in *Chthamalus*, Tsang et al., 2008; ~ 14–17% for COI and 3–4% for 12S in *Tetraclita*, Chan et al., 2007a, b). Thus, the morphological and molecular evidence indicates that the Philippine and Taiwanese forms represent two different species. The type locality of *Heteralepas japonica* was Hirado Strait, southern Japan (33°10' N 129°18'E, see Aurivillius, 1892). Descriptions on *H. japonica* in Aurivillius 1892, however, was brief and it mentioned the capitulum without plates and made of chitin, the dorsal side forming a low crest with equal distance spines (Aurivillius, 1892). Caudal

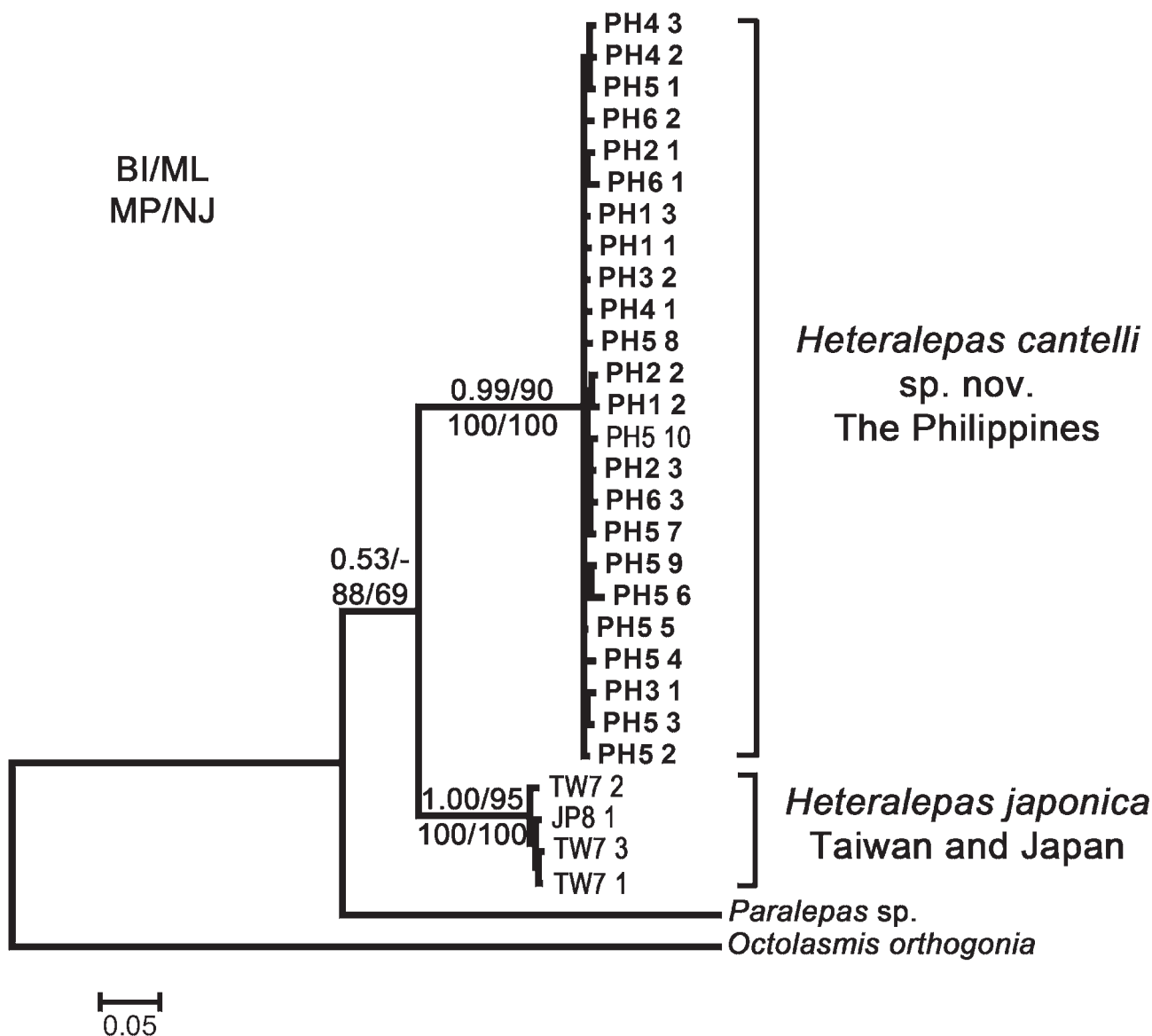


Fig. 7. Bayesian inference topology of the three *Heteralepas* populations with the outgroup taxa. The percentages of bootstrap replicates for NJ, MP and ML, and posterior probability of BI are shown above the corresponding branch for the node with values > 50% in at least two analytical methods. See Table 1 for the station number for the individual collected from. Region abbreviation: PH = Philippine, TW = Taiwan, JP = Japan. Station (see Table 1) and individual numbers follow the abbreviations for region.

appendages 9–10 segments (Aurivillius, 1892). Inner ramus of the cirrus VI had 15 segments and the outer ramus 48 segmented. Peduncle is two thirds the length of the capitulum (Aurivillius, 1892). The Taiwan, Japan and Philippine samples matched the brief description in Aurivillius, 1892. In the present study, we considered the Japan samples represent the type *H. japonica* as it was collected relatively close to the type locality and the external morphology of the Japan samples matched with the figure and description of *H. japonica* in Hiro (1933), in which the specimens were collected from Japanese waters. We, therefore, designate the Philippine samples as a new species.

Beside *Heteralepas indicus* which considered as synonym to *H. japonica* (Nilsson-Cantell, 1927), *H. dubia* was recorded in Disaster Bay in New Zealand by Broch (1922) was also considered as synonym to *H. japonica* (Foster, 1978; Liu & Ren, 2007). The morphological features of *H. dubia*, including the number of crests, segments number of cirri and caudal appendages were fall into the range of morphological variations described by Nilsson-Cantell (1927) (see Foster, 1978). Broch (1922) mentioned *H. dubia* has a single crest which appeared on the junction between the capitulum and peduncle and the cutting edge of the second and third tooth of mandibles are serrated. These characters did not appears in *Heteralepas cantelli*, new species. The present study, therefore, considers *H. cantelli*, new species, and *H. dubia* are separate species.

The deep sea is long acknowledged to contain high faunal diversity (Hessler & Sanders, 1967). However, our current understanding and estimations are based primarily on morphological identification of the species. The genetic structure of the deep sea animals remains poorly studied. This often results in overlooking the presences of cryptic species, especially in the case of *Heteralepas*. In the present study, the new species in identified in the Philippines, *Heteralepas cantelli*, new species, also had a number of morphological varieties that are genetically indistinguishable. These morphotypes possibly induced by different environmental conditions. Phenotypic plasticity had been well-documented in acorn barnacles in that the cirral length and shell shape can be affected by physical and biological factors (Barnes & Powell, 1950; Marchinko, 2003; Marchinko & Palmer, 2003; Chan & Hung, 2005; Jarrett, 2008). For pedunculate barnacles, *Lepas anatifera* Linnaeus, 1758 also reported to have several morphological varieties (Liu & Ren, 2007). *Heteralepas japonica* often occurs in large clumps and such crowded conditions can affect the morphological developments of barnacles (Barnes & Powell, 1950). High-level intraspecific morphological variation combined with the presence of cryptic species leads to difficulties in recognising the species, which complicates the study of deep sea biodiversity and ecology. Thus, integrated morphological and molecular investigations can help clarify the taxonomic status and diversity of morphologically variable deep sea species.

Heteralepas is characterized by having no shell plates in the capitulum and has been suggested to be plesiomorphic with

regards to barnacle phylogeny. However, recent molecular analysis showed that barnacles from Heteralepadidae (*Paralepas* and *Heteralepas*) are actually polyphyletic, suggesting that genera within this family are the result of separate evolutionary events (Pérez-Losada et al., 2008; Høeg et al. 2009). Further studies should include a wide sampling of species in Heteralepadidae to contribute to a revision of the phylogeny using both morphological and molecular analysis.

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