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A Molecular Phylogenetic Investigation of *Cyathopoma* (Prosobranchia: Cyclophoridae) in East Asia

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Yen-Chen Lee, Kuang-Yang Lue, and Wen-Lung Wu (2008) A molecular phylogenetics investigation of *Cyathopoma* (Prosobranchia: Cyclophoridae) in East Asia. *Zoological Studies* **47**(5): 591-604. The Cyclophoridae is the dominant group of operculated terrestrial snails in East Asia. The group consists of 4 subfamilies and about 300 species currently arranged in 34 genera. They occupy a range of habitats and exhibit considerable morphological diversity. Members of *Cyathopoma* are tiny white cyclophorid snails occurring in East Asia, Madagascar, and the Seychelles. The phylogenetic relationships of *Cyathopoma* are uncertain. In order to investigate the relationships among cyclophorids and within East Asian *Cyathopoma* species, we sequenced part of the mitochondrial cytochrome oxidase subunit I (COI) gene from 31 species of 9 genera of cyclophorids. We constructed phylogenetic trees using Neighbor-joining, Bayesian, and maximum-likelihood analyses. Phylogenetic relationships based on mitochondrial (mt)DNA sequences suggested that *Cyclophorus*, *Cyclotus*, *Leptopoma*, and *Platyrhaphe* are monophyletic. Combined with molecular and radular data, we concluded that *Cyathopoma* and *Cyclotus* are only distantly related. *Cyathopoma iota* has been considered to be a controversial member of this group. Through molecular and radular data, we found *Cya. iota* to be closer to *Cya. taiwanicum* than to *Cya. micron*, and concluded that *Cya. micron*, *Cya. ogaitoi*, *Cya. iota*, and *Cya. taiwanicum* belong to *Cyathopoma*. In addition we provide the first report of *Cya. ogaitoi* from Guizhou Province, China. http://zoolstud.sinica.edu.tw/Journals/47.5/591.pdf

Key word: Phylogeny, *Cyathopoma*, Cyclophoridae, East Asia, New record.

Recent studies on phylogenetic relationships within the molluscan class Gastropoda have involved morphological (Kay et al. 1998), ultrastructural (Healy 1996), anatomical (Kantor 1996), and molecular (e.g., McArthur and Koop 1999, Lydeard et al. 2002, Remigio and Hebert 2003) approaches. Those investigations provided new insights into gastropod affinities and classification and have enabled a rigorous testing of taxonomic relationships within the group. While gastropod phylogeny has received much recent attention (Tillier et al. 1992, Ponder and Lindberg 1997, Rosenberg et al. 1997, Thollesson 1999, Remigio and Hebert 2003), relationships within some land snail clades are still poorly understood. One of these is the Cyclophoridae which consists of 4 subfamilies and about 300 species currently arranged into 34 genera. The most generally accepted system of classification today partitions the diverse Cyclophoridae into 4 subfamilies (Vaught 1989, Millard 1996). The Cyclophorinae is extremely diverse; while the Spirotomatinae, Alycaeinae, and Pterocyclinae are less diverse. Although some investigators treat the Spirotomatinae and Alycaeinae as independent families (Azuma 1982, Higo and Goto 1993), their subordinate taxa are uncontentious. However, the traditional classification is based on shell morphology and anatomy, it may course by convergent. Phylogenetic knowledge of the Cyclophoridae is limited and thus there is great interest in resolving their phylogenetic issues.

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Furthermore, *Cyathopoma* was known as an Indian endemic before 1900 (Pilsbry 1900). It was proposed by Blanford (1861) based on *Cyathopoma filocinctum* Blanford, 1961. *Cyathopoma* were sculptured with spiral striate on shell surface and umbilicus, with multispiral calcific operculum. Ancey (1904) proposed *Nakadaella* for those with a smooth shell surface. However, *Nakadaella* was considered a subspecies of *Cyathopoma* or a synonym of *Jerdonica* Blanford, 1961 (subspecies of *Cyathopoma* without sculpture within umbilicus) by some authors (Pilsbry and Hirase 1905, Kuroda 1941, Azuma 1982). *Cyathopoma micron* (Pilsbry, 1900) was the first record of this tiny cyclophorid from East Asia. *Cyathopoma iota* (Pilsbry and Hirase 1904), *C. taiwanicum* Pilsbry and Hirase, 1905, *Cya. taiwanicum degeneratum* Pilsbry and Hirase, 1905, *Cya. nishinoi* Minato, 1980, and *Cya. ogaitoi* (Minato 1988) were subsequently reported from East Asia but their generic placement was doubtful. For example, *C. micron* was initially placed in *Cyclotus* (Pilsbry 1900) before being placed in *Nakadaella* by Ancey (1904) and *Cyathopoma* (Pilsbry and Hirase, 1905), but the generic status of *Cya. micron* remains controversial.

Cyathopoma ogaitoi was original described from Awaji Is., Japan. Prior to our discovery of several *Cya. ogaitoi* specimens at Leigong Mt., Guizhou Province, China in July 2006, there were no reports indicating that *Cyathopoma* occurs in the area between East Asia and India. This is the first report of *Cyathopoma* from this area.

Dentition of the radula has long been established as providing informative characters for the taxonomy of gastropods (Trochel 1856-1863, Habe 1942, Ponder and Lindberg 1996). Radulae fundamentally differ among different gastropod groups. However, radular morphology must be used with care when inferring relationships because there is marked convergence in the radulae of taxa that belong to different clades but occupy similar adaptive zones (Lindberg and McLean 1981). Cuspidal features, the number of teeth, and teeth shape play important roles in classification and have proven to be of particular value at the generic level (Kilburn 1988). Traditional classification of these tiny cyclophorids was based on shell and operculum morphology, while little attention has been paid to the radular morphology of cyclophorids, and most *Cyathopoma* radulae have not previously been described.

Over the past decade molecular approaches have proven their value not only in resolving phylogenetic issues, but also in providing an indication of the time scales of evolutionary divergence. Among the 13 protein-coding genes within the mitochondrial genome, the cytochrome oxidase subunit I (COI) has gained particular popularity for effectively resolving species (Hebert et al. 2004a b, Hogg and Hebert 2004, Barrett and Hebert 2005, Smith et al. 2006). In the case of possible cryptic species identification and identifying morphologically difficult species (Paquin and Hedin 2004), the COI gene provides a high degree of taxonomic resolution. Despite its broad use in resolving affinities at lower taxonomic levels, it has been little exploited to address deeper phylogenetic issues (Remigio and Hebert 2003). COI datasets may be suitable to infer the affinities at the generic level of cyclophorids.

The objectives of this study were (1) to test whether the genera of the *Cyclophoridae* are monophyletic, (2) to resolve the relationships among several species of *Cyathopoma*, (3) to understand the affinities between *Cyathopoma* and other cyclophorid genera, and (4) to investigate the microstructure of the radulae of *Cyathopoma*.

MATERIALS AND METHODS

DNA preparation and sequencing

Samples of 31 species representing 9 genera and including members from the 3 major subfamilies (the Cyclophorinae, Alycaeinae, and Pterocyclinae) were collected from 47 sites (Fig. 1, Table 1). We separated their shells and soft parts at the laboratory. Shells were thoroughly cleaned for identification, and the soft parts were stored at -80°C, except for very tiny species (e.g., *C. micron*) which were placed in pure ethanol until DNA extraction. DNA was extracted from the columellar muscle; the entire animal was used in the case of tiny species. We extracted DNA from separate individuals using a TEK-based protocol (Jiang et al. 1997) with minor modifications. Tissue was placed in TEK buffer (12.5 mM Tris-Cl (pH 7.3), 2.5 mM ethylenediaminetetraacetic acid, and 0.4% KCl), then ground with a glass pestle, and incubated at 57°C with 20 μl of proteinase K (20 mg/ml) for more than 2 h. The tissue extract was extracted at least twice with phenol and chloroform. A DNA extract (400 μl) was precipitated by adding 1000 μl of pure ice-cold

ethanol, and then placed at -20°C for 20 min. The DNA was pelleted by centrifugation. After a 70% ethanol rinse, the DNA was resuspended in distilled water and stored at -80°C for DNA amplification. Exactly 531 bp of the COI gene using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Williams et al. 2003) was used. Polymerase chain reactions (PCRs) contained 10-50 ng/μl template DNA, 10 pmol of each primer, 5 μl 10 × reaction buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% gelatin, and 1% Triton X-100), 0.4 μl 25 mM/μl dNTP, 0.2 μl 50 mM Mg^{2+}, and 0.4 μl *Taq* polymerase (5 units/μl) in a total volume of 50 μl. Thermal cycling was performed with initial denaturation for 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 47°C, and 50 s at 72°C, with an ultimate extension at 72°C for 10 min, and final holding at 4°C. PCR products were purified using a purification kit (AMP PCR purification, Beckman, Fullerton, California, USA) and then sequenced using an ABI 3700 autosequencer (PerkinElmer, Waltham, Massachusetts, USA).

Phylogenetic analyses

Sequences were combined with data of the outgroup (*Littoraria* scabra Linnaeus and *L. undulata* (Gray) with respective accession nos. of AJ488637 and AJ488635) from GenBank. Sequences were assembled and edited using Bioedit 5.0.9 (Hall 1999). All alignments employed Clustal X (Thompson et al. 1994) and were manually proofread. Codon positions within the COI gene were tested using the incongruence length difference (ILD) test of Farris et al. (1995), as implemented by the partition homogeneity test in PAUP 4.0b10 (Swofford 1998) (with 100 replicates). Sequence data were divided into 2 partitions, with the 1st and 2nd codon positions in one and the 3rd codon positions in the other. Two parts of the COI gene were congruent, and all codon positions were combined and used in the following analysis.

All data sets were subjected to Neighbor-joining (NJ) and maximum-likelihood (ML) analyses using PAUP 4.0b10 and a Bayesian analysis using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The substitution model used for the COI dataset corresponded to the transversional model, which ignores rate variations or includes invariable sites and rate variations among sites (TVM+I+G). The best models were found using Modeltest 3.06 (Posada and Crandall 1998). Before model fitting, full-length sequences were tested to confirm that there was no significant heterogeneity in base frequencies across taxa (χ^2 = 191.70, *d.f.* = 177, p = 0.2131) using DAMBE 4.5.50 (Xia and Xie 2001). NJ bootstrapping consisted of 1000 iterations. The reliability of the ML trees was estimated by the approximate likelihood ratio test (aLRT) (using custom-defined model base

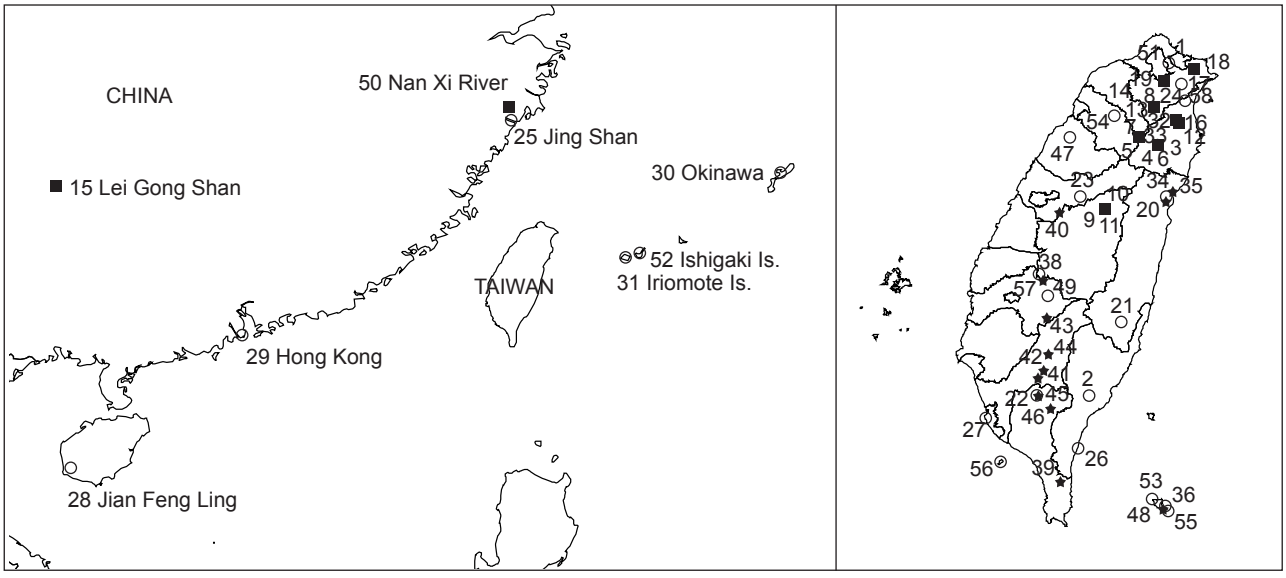

Fig. 1. East Asian map, with sampling sites indicated. Solid squares and asterisks respectively indicate sampling sites of the genera *Cyathopoma* and *Cyclotus*. Open circles indicate sampling sites of other genera.

frequencies: A = 0.3359, C = 0.1190, G = 0.1308, and T = 0.4144) using PHYML 3.0 (Guindon and Gascuel 2003). The Bayesian analysis was run for 2 x 10^6 generations, with a sample frequency of 100. The first 2000 trees were discarded, so that the final consensus was based on 18,000 trees. Support for nodes was expressed as posterior probabilities (calculated by MrBayes). For the constraint analysis, we conducted parsimony heuristic searches to find the best trees, and using the Kishino-Hasegawa test (Kishino and Hasegawa 1989), evaluated the resulting trees, which were consistent with traditional taxonomy.

Environmental scanning electron microscopy (ESEM) microstructural observations

The empty shell was glued to a SEM specimen stub for further observation. Radulae were removed from snails and soaked in a 0.5% NaOH solution to remove organic tissue adhering to the radula, then it was fixed in 90% ethanol. The radula was glued to the SEM specimen stub and 1 side of the marginal teeth was unfolded for further observation. Finally, the specimens were coated, in a vacuum, with gold-palladium. Specimens were then observed and photographed using an ESEM (FEI Quanta 200, Hillsboro, Oregon, USA).

RESULTS

Phylogenetic analysis

The aligned 531 bp of the COI gene data matrix included 263 variable sites, of which 246 (93.54%) were parsimony informative. The average *p*-distance was 0.200. No length difference from the outgroup was detected among members of the 3 subfamilies, the Cyclophorinae, Alycaeinae, and Pterocyclinae. Sequence divergences among the haplotypes ranged 0.002-0.275, the intra-generic range was 0.066-0.156, and inter-generic range was 0.197-0.269.

The inferred phylogenetic trees among haplotypes of the COI gene are shown in figures 2-4. Three monophyletic groups, *Cyclophorus*, *Cyclotus*, and *Leptopoma*, were present, with support values higher than 92% using the NJ, Bayesian, and ML methods. Although with lower support, members of the genus *Platyrhaphe* also had a monophyletic relationship. Relationships among the subfamilies of the Cyclophorinae,

Table 1. List of species included in the analysis, catalog numbers, and GenBank accession numbers (specimens are deposited in the Malacological Laboratory of the Biodiversity Research Center, Academia Sinica, Taipei, Taiwan)

Item	Subfamily, genus, and species	Catalog no.	Accession no.
	Alycaeinae		
	Chamalycaeus		
1	*Chamalycaeus varius* (Pilsbry and Hirase, 1905)	G050403-1	EU219770
	Dioryx		
2	*Dioryx swinhoei* (H. Adams, 1866)	G050110-1	EU219758
3	*Dioryx swinhoei* (H. Adams, 1866)	G050520-1	EU249291
	Cyclophorinae		
	Cyathopoma		
4	*Cyathopoma iota 1* (Pilsbry and Hirase, 1904)	G040916-21	EU219764
5	*Cyathopoma iota 1* (Pilsbry and Hirase, 1904)	G040916-22	EU249288
6	*Cyathopoma iota 1* (Pilsbry and Hirase, 1904)	G040916-23	EU249289
7	*Cyathopoma iota 2* (Pilsbry and Hirase, 1904)	G060504-5	EU219766
8	*Cyathopoma iota 3* (Pilsbry and Hirase, 1904)	G040608-2	EU249284
9	*Cyathopoma micron 1* (Pilsbry, 1900)	G021103-1	EU219768
10	*Cyathopoma micron 1* (Pilsbry, 1900)	G021103-2	EU249275
11	*Cyathopoma micron 1* (Pilsbry, 1900)	G021103-3	EU249276
12	*Cyathopoma micron 2* (Pilsbry, 1900)	G050222-3	EU219769
13	*Cyathopoma micron 3* (Pilsbry, 1900)	G040608-1	EU249283
14	*Cyathopoma micron 3* (Pilsbry, 1900)	G040608-3	EU249285

Table 1. (Cont.)

Item	Subfamily, genus, and species	Catalog no.	Accession no.
15	*Cyathopoma ogaitoi* (Minato, 1988)	G060722-1	EU249292
16	*Cyathopoma taiwanicum 1* Pilsbry and Hirase, 1907	G050615-3	EU219765
17	*Cyathopoma taiwanicum 2* Pilsbry and Hirase, 1905	G040224-10	EU249282
18	*Cyathopoma taiwanicum 2* Pilsbry and Hirase, 1905	G040224-2	EU219767
19	*Cyathopoma taiwanicum 3* Pilsbry and Hirase, 1905	G070124-4	EU249295
	Cyclophorus		
20	*Cyclophorus formosensis* Nevill, 1882	G011028-9	EU249274
21	*Cyclophorus formosensis* Nevill, 1882	G030204-B	EU249278
22	*Cyclophorus friesianus* Moellendorff, 1883	G060816-6	EU219746
23	*Cyclophorus latus* (Kuroda, 1941)	G031108-1	EU249281
24	*Cyclophorus latus* (Kuroda, 1941)	G040728-1	EU249287
25	*Cyclophorus martensianus* Moellendorff, 1874	G060724-2	EU219756
26	*Cyclophorus moellendorffi* Schmacker and Boettger, 1891	G030724-5	EU249280
27	*Cyclophorus moellendorffi* Schmacker and Boettger, 1891	G040725-1	EU249286
28	*Cyclophorus pyrostoma* Moellendorff, 1882	G060714-1	EU219755
29	*Cyclophorus subcarinatus* Meollendorff, 1882	G040727-1	EU219757
30	*Cyclophorus turgidus* Pilsbry, 1902	G021128-2	EU219754
31	*Cyclophorus turgidus* Kuroda, 1960	G021201-5	EU219753
	Cyclotus		
32	*Cyclotus taivanus* Pilsbry and Hirase, 1905	G031010-1	EU219786
33	*Cyclotus taivanus* Pilsbry and Hirase, 1905	G040916-7	EU249290
34	*Cyclotus taivanus dilatus* Lee and Wu, 2001	G011028-13	EU249273
35	*Cyclotus taivanus dilatus* Lee and Wu, 2001	G030302-C	EU249279
36	*Cyclotus taivanus diminutus* Kuroda and Kano in Lee and Wu, 2001	G030707-6	EU219790
37	*Cyclotus taivanus* Pilsbry and Hirase, 1905	G021201C	EU219789
38	*Cyclotus taivanus* H. Adams, 1870	G010225-3	EU249269
39	*Cyclotus taivanus* H. Adams, 1870	G010429-30	EU249270
40	*Cyclotus taivanus* H. Adams, 1870	G010527A	EU249271
41	*Cyclotus taivanus* H. Adams, 1870	G010701-12	EU249272
42	*Cyclotus taivanus* H. Adams, 1870	G041003-11	EU219788
43	*Cyclotus taivanus* H. Adams, 1870	G060805-16	EU249293
44	*Cyclotus taivanus* H. Adams, 1870	G060810-22	EU249294
45	*Cyclotus taivanus* H. Adams, 1870	G070121-4	EU219787
46	*Cyclotus taivanus* H. Adams, 1870	G070622-1	EU249296
	Japonia		
47	*Japonia formosana* Pilsbry and Hirase, 1905	G040321-1	EU219763
48	*Japonia lanyuensis* Lee and Wu, 2001	G040707-38	EU219761
49	*Japonia boonkioensis* Lee, Lue and Wu, 2008	G060805-3	EU219762
	Pilosphaera		
50	*Pilosphaera yentoensis* Lee, Lue and Wu, 2008	G060725-1	EU219772
51	*Pilosphaera zebra* (Pilsbry and Hirase, 1905)	G050429-3	EU219773
	Leptopoma		
52	*Leptopoma nitidum* Sowerby, 1843	G021128-1	EU249277
53	*Leptopoma tigris* Kuroda and Kano in Lee and Wu, 2001	G040707-1	EU219784
	Ptychopoma		
54	*Ptychopoma wilsoni* (Pfeiffer, 1865)	G040321-3	EU219785
	Pterocyclinae		
	Platyrhaphe		
55	*Platyrhaphe lanyuensis* Lee and Wu, 2001	G010825-35	EU219781
56	*Platyrhaphe minutus* (H. Adams, 1866)	G070120-2	EU219779
57	*Platyrhaphe swinhoei* (H. Adams, 1866)	G030615A-1	EU219774
58	*Platyrhaphe swinhoei* (H. Adams, 1866)	G070619-1	EU219776

Alycaeinae, and Pterocyclinae were uncertain because of the low bootstrap support.

Whether *Cya. micron*, *Cya. ogaitoi*, and *Cya. iota* belong to *Cyathopoma*, *Cyclotus*, or *Nakadaella* was controversial in the previous literature. However, all cladograms in this study indicated that these species were all in the same clade as *Cya. taiwanicum* instead of the genus *Cyclotus*. Particularly, *Cya. iota* was clustered with *Cya. taiwanicum* in a subclade, without resolution. In order to test the positions of these species in the classification, we performed 2 likelihood analyses between topologies constrained to match given mutually exclusive hypotheses. The constraints were designed to test these cyclophorid snails according to different contentions described in

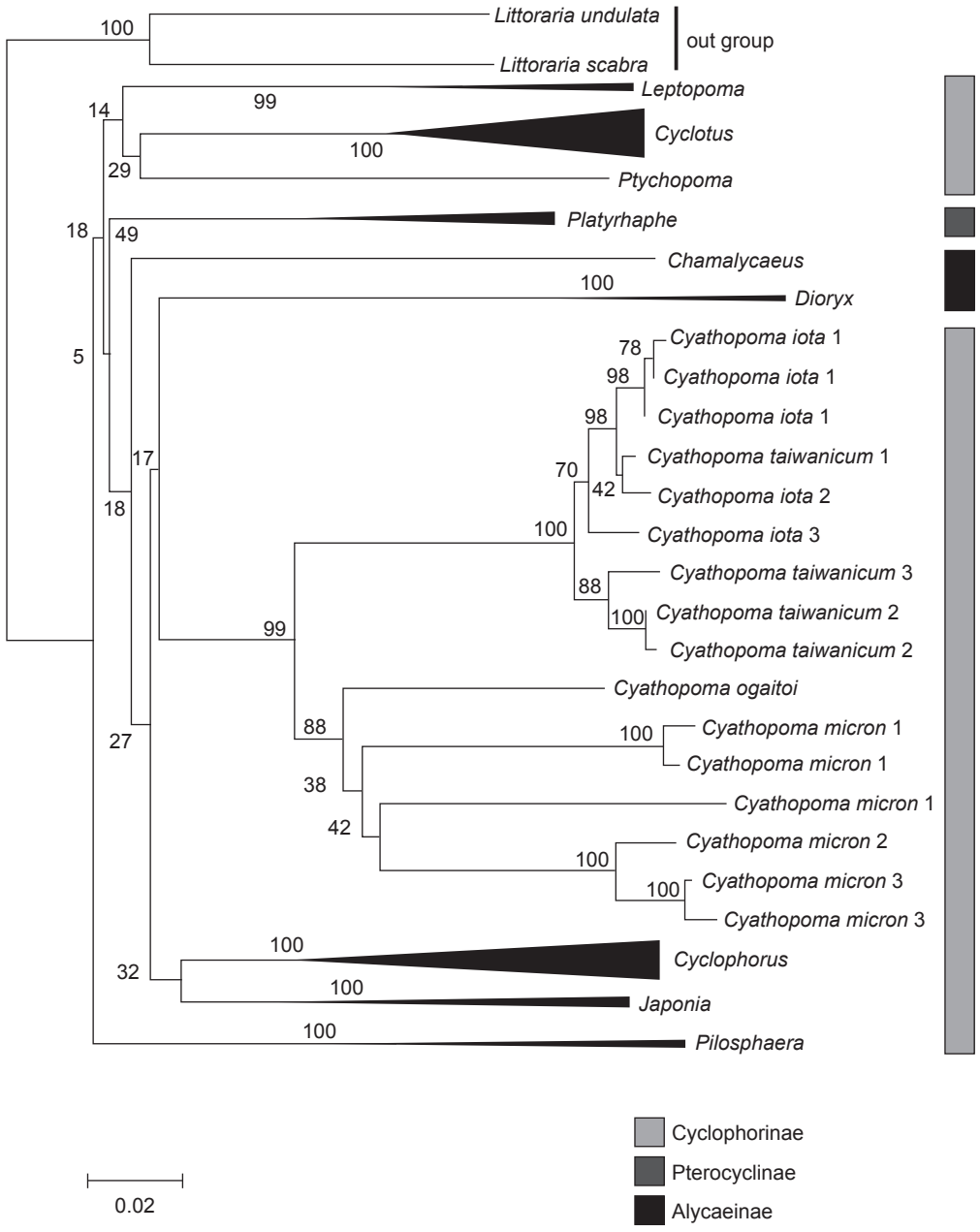

Fig 2. Molecular phylogenies of the Cycloporidae produced by Neighbor-joining (NJ) analysis of individual gene sequence data from the cytochrome oxidase subunit I gene. Relative branch support indices are given as bootstrap tests consisting of 1000 iterations. Different numbers after the scientific name indicate different sampling sites.

the previous literature. We performed a Kishino-Hasegawa likelihood evaluation to test these contentions. Using the constraints option in PAUP, we conducted parsimony heuristic searches (with specifics described below) to find the best trees that were consistent and inconsistent with the monophyly of these clades. The sets of trees consistent and inconsistent with the constraint were then compared using the Kishino-Hasegawa test (Kishino and Hasegawa 1989). For the (*micron, ogaitoi, iota*, and genus *Cyclotus*) clade issue, the COI data significantly rejected the monophyly of this clade, 1 "best" tree (with a tree length of 2034 and -InL of 10,308.285) supporting a monophyly had significantly lower support than the 37 best-supported trees (with a tree length of 1993 and

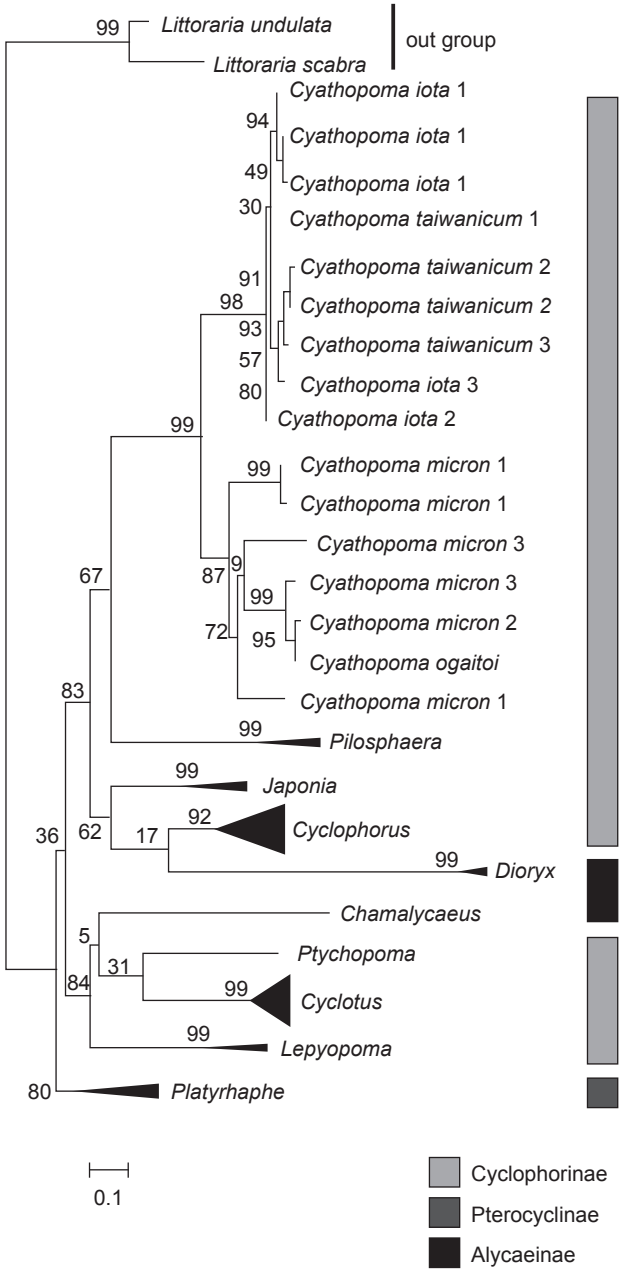

Fig. 3. Molecular phylogenies of the Cycloporidae produced by maximum-likelihood (ML) analysis of individual gene sequence data from the cytochrome oxidase subunit I gene. Relative branch support indices are given as the approximate likelihood ratio test (aLRT). Different numbers after the scientific name indicate different sampling sites.

-lnL values of 10,171.656-10,180.462; $p = 0.0001$). To resolve the *Cya. micron*, *Cya. ogaitoi*, and *Cyclotus* clade, all 182 best-supported trees had significantly lower support ($p \leq 0.0491$) than the 37 trees which did not match the constraint.

Some investigators considered *Cya. iota* to be a subspecies of *Cya. micron* (Higo and Goto 1993, Lee and Wu 2001), and we performed the Kishino-Hasegawa likelihood evaluation to test this. The 783/792 best-supported trees had significantly lower support ($p < 0.05$) than the 37 trees which did not match the constraint. Trees that were not significantly worse had a nearly significant p value of < 0.06. Furthermore, if (*Cya. micron* and *Cya. ogaitoi*) and (*Cya. iota* and *Cya. taiwanicum*) were separate groups, the divergence among these 2

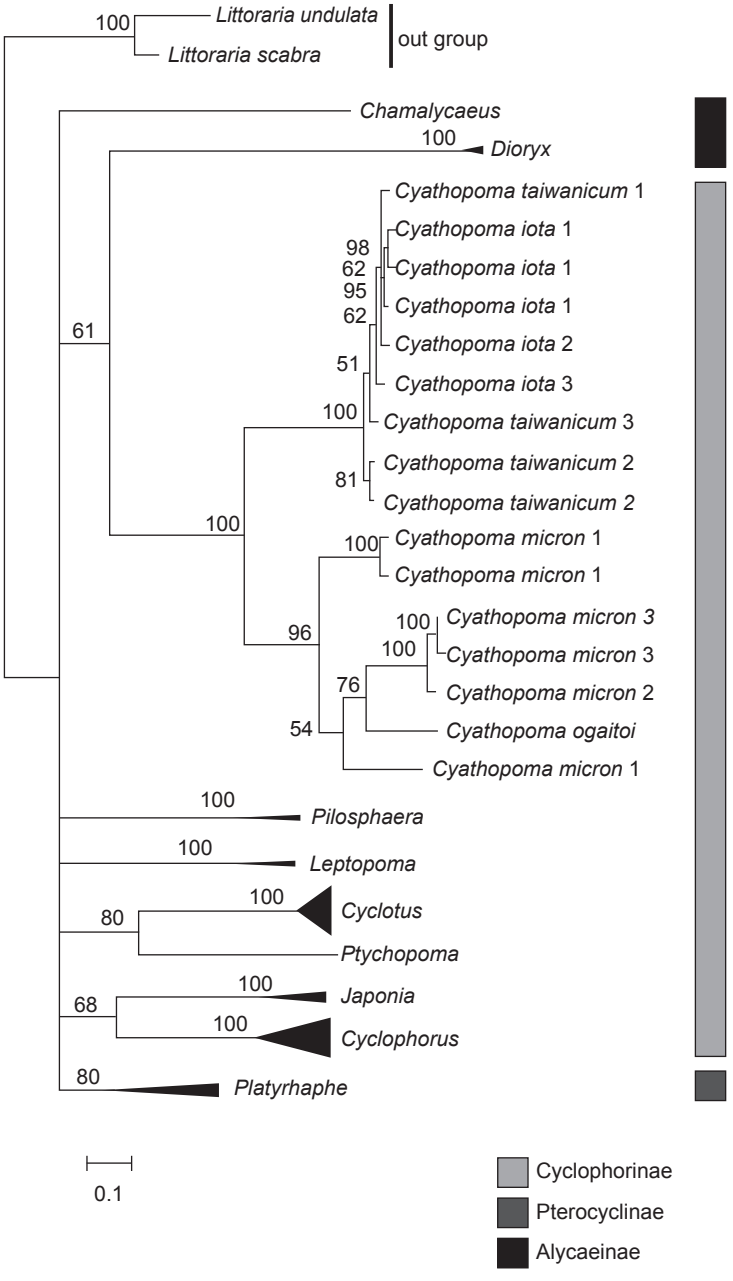

Fig. 4. Molecular phylogenies of the Cycloporidae produced by a Bayesian analysis of individual gene sequence data from the cytochrome oxidase subunit I gene. Relative branch support indices are given as Bayesian probabilities. Different numbers after the scientific name indicate different sampling sites.

groups was 0.151 which was moderately lower than that among other genera (Table 2). We concluded that *Cya. micron*, *Cya. ogaitoi*, and *Cya. iota* had closer relationships with *Cya. taiwanicum* than with members of the genus *Cyclotus*, while *Cya. micron*, *Cya. ogaitoi*, *Cya. iota*, and *Cya. taiwanicum* belong to the genus *Cyathopoma*.

ESEM microstructure

Radular morphology was very similar between *Cya. iota* and *Cya. taiwanicum*. Both possessed the same number of teeth cusps and general shape. They had a scoop-shaped central tooth, 5 cusps on the convex side, and 7-9 irregular tiny cusps on the convex side; the inner lateral teeth possessed 5 cusps and the outer lateral teeth 6 or 7 cusps within the same individual; and marginal teeth had 6 small cusps on the inner side and 3 large cusps on the outer side (Figs. 5D, 6D). The radulae of *Cya. micron* and *Cya. ogaitoi* were similar to that described above, but the cusps on the teeth were shorter and broader (Figs. 7D, 8D). Furthermore, the outer lateral teeth had fewer cusps (5 or 6 in number). The protocochs of *Cya. iota* and *Cya. taiwanicum* exhibited a granular surface (Figs. 5B, 6B), but those of *Cya. micron* and *Cya. ogaitoi* were relatively smooth (Figs. 7B, 8B). The operculae of *Cya. iota*, *Cya. micron*, *Cya. ogaitoi*, and *Cya. taiwanicum* were almost the same shape (Figs. 5C, 6C, 7C, 8C).

In addition, we examined 4 subspecies of *Cyclotus taivanus* H. Adams, 1870. The radulae within the *Cyclotus* we examined were similar to each other: namely 5 cusps on the central tooth, 4 cusps on the inner lateral teeth, 4 cusps on the outer lateral teeth, and 3 cusps on the marginal teeth (Fig. 9). The only distinct difference was the shape of the marginal teeth. The marginal teeth of *Cyc. taivanus adams* Pilsbry et Hirase, 1905 were sickle-shaped, while the other 3 species exhibited hook-shaped marginal teeth.

DISCUSSION

Although molecular relationships among the 3 major subfamilies of cyclophorids are uncertain, our results show that many taxa in traditional classifications of cyclophorids are non-monophyletic. Because the Cyclophorinae, Spirotomatinae (not included in this study), Alycaeinae, and Pterocyclinae in a Linnean system are considered to be equal entities, problems associated with ranks and synonymy arise.

Cyathopoma micron was initially placed in the genus *Cyclotus* (Pilsbry 1900), then placed in the genus *Nakadaella* by Ancey in 1904. Pilsbry and Hirase (1905) placed *micron* in the genus *Cyathopoma*. Subsequently, Kuroda (1941) and Chang (1984) recognized this species as belonging to the genus *Cyathopoma*, while Lai (1990) and Lee and Wu (2001) placed it in the genus *Cyclotus*. *Cyathopoma iota* was considered a closer relative to *Cya. micron* than to *Cya. taiwanicum* (Pilsbry and Hirase 1904, Higo and Goto 1993, Lee and Chen 2003). However, based on our Kishino-Hasegawa test results, molecular phylogenetics, and radular data, *Cya. iota* is closer to *Cya. taiwanicum* than to *Cya. micron*. Ancey (1904) proposed the genus *Nakadaella* for *micron*, but this species does not differ from typical forms

Table 2. Average pairwise differences among genera of the Cyclophoridae using cytochrome oxidase subunit I dataset

	1	2	3	4	5	6	7	8	9	10
1. *Chamalycaeus*										
2. *Dioryx*	0.242									
3. (*micron, ogaitoi*)	0.212	0.228								
4. (*iota, taiwanicum*)	0.226	0.246	0.151							
5. *Cyclophorus*	0.223	0.223	0.217	0.219						
6. *Cyclotus*	0.217	0.266	0.221	0.222	0.223					
7. *Japonia*	0.232	0.256	0.216	0.221	0.213	0.231				
8. *Leptopoma*	0.234	0.257	0.222	0.229	0.234	0.212	0.218			
9. *Platyrhaphe*	0.207	0.230	0.190	0.207	0.202	0.197	0.202	0.203		
10. *Pterocyclus*	0.215	0.245	0.206	0.235	0.231	0.206	0.231	0.213	0.194	

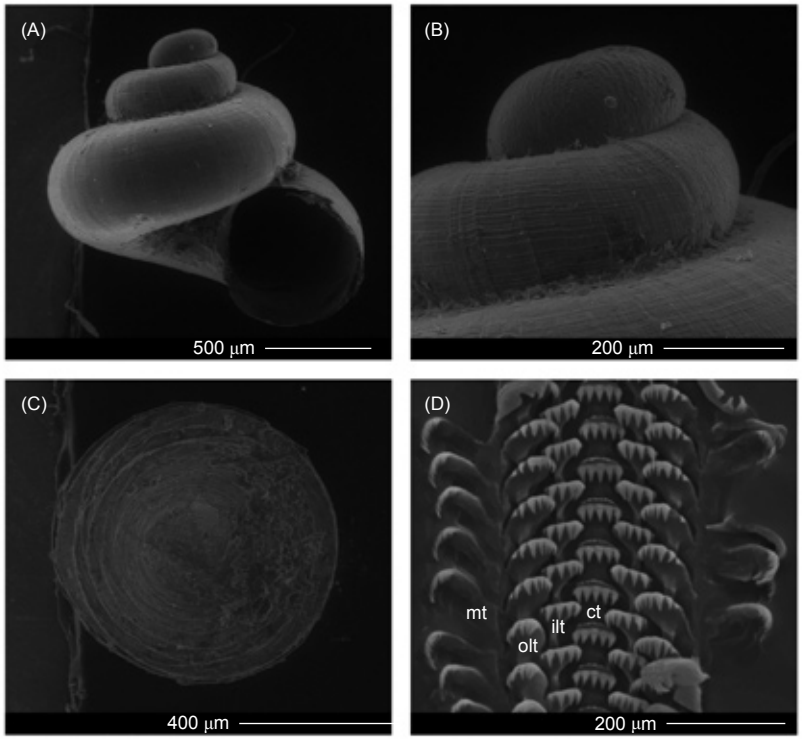

Fig. 5. Environmental scanning electron microscopic photograph of *Cyathopoma iota* (Pilsbry and Hirase, 1904). (A) Shell, lateral view; (B) protoconch; (C) operculum (outer view); (D) radula. ct, central tooth; ilt, inner lateral teeth; olt, outer lateral teeth; mt, marginal teeth.

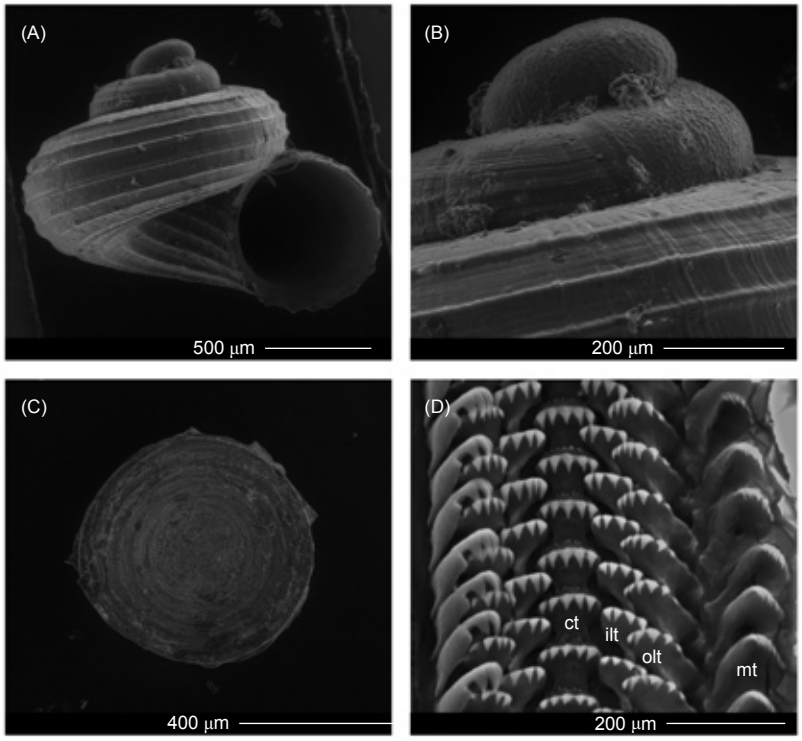

Fig. 6. Environmental scanning electron microscopic photograph of *Cyathopoma taiwanicum* Pilsbry and Hirase, 1905. (A) Shell, lateral view; (B) protoconch; (C) operculum (outer view); (D) radula. ct, central tooth; ilt, inner lateral teeth; olt, outer lateral teeth; mt, marginal teeth.

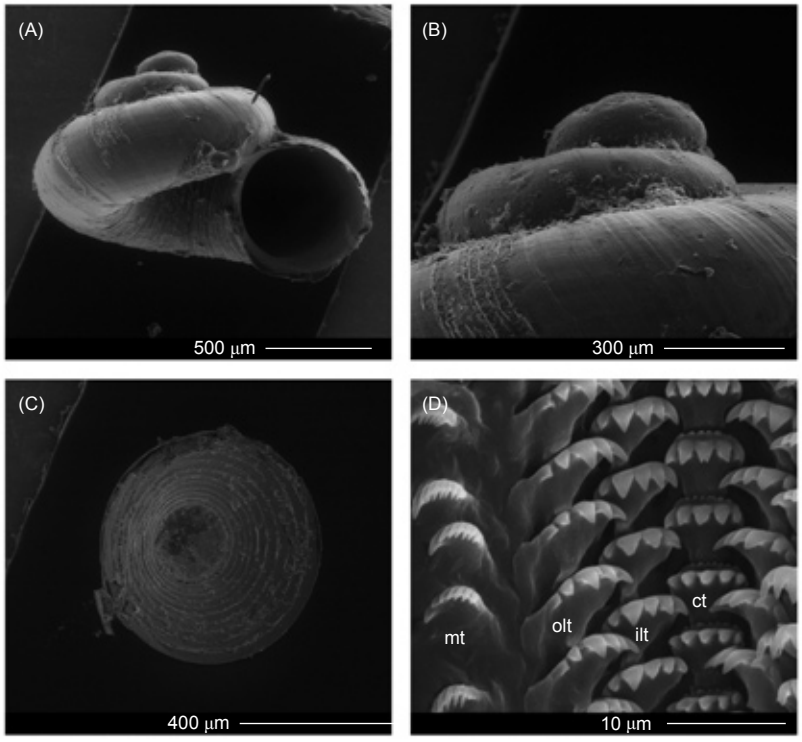

Fig. 7. Environmental electron scanning microscopic photograph of *Cyathopoma micron* (Pilsbry, 1900). (A) Shell, lateral view; (B) protoconch; (C) operculum (outer view); (D) radula. ct, central tooth; ilt, inner lateral teeth; olt, outer lateral teeth; mt, marginal teeth.

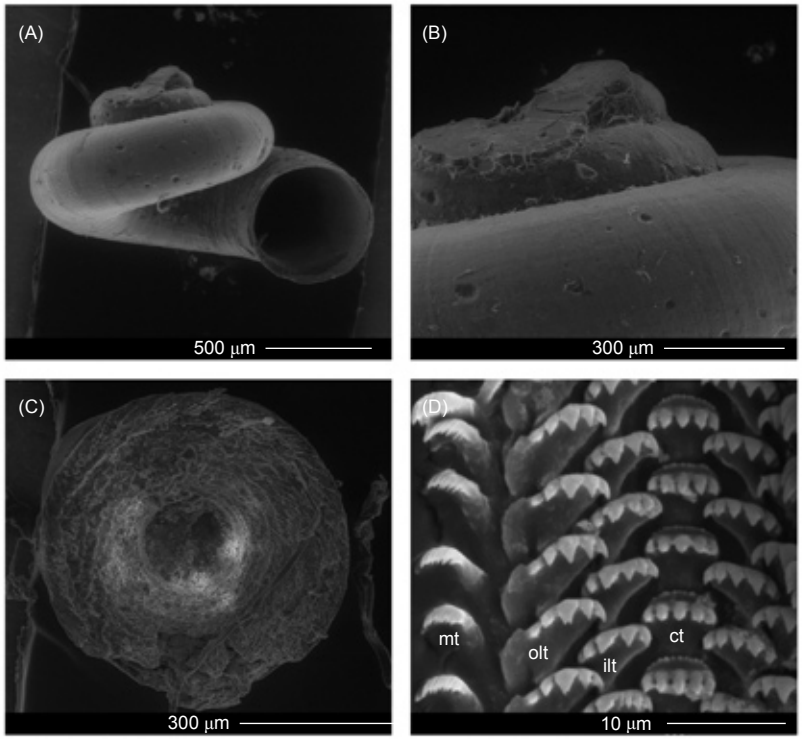

Fig. 8. Environmental electron scanning microscopic photograph of *Cyathopoma ogaitoi* (Minato, 1988). (A) Shell, lateral view; (B) protoconch; (C) operculum (outer view); (D) radula. ct, central tooth; ilt, inner lateral teeth; olt, outer lateral teeth; mt, marginal teeth.

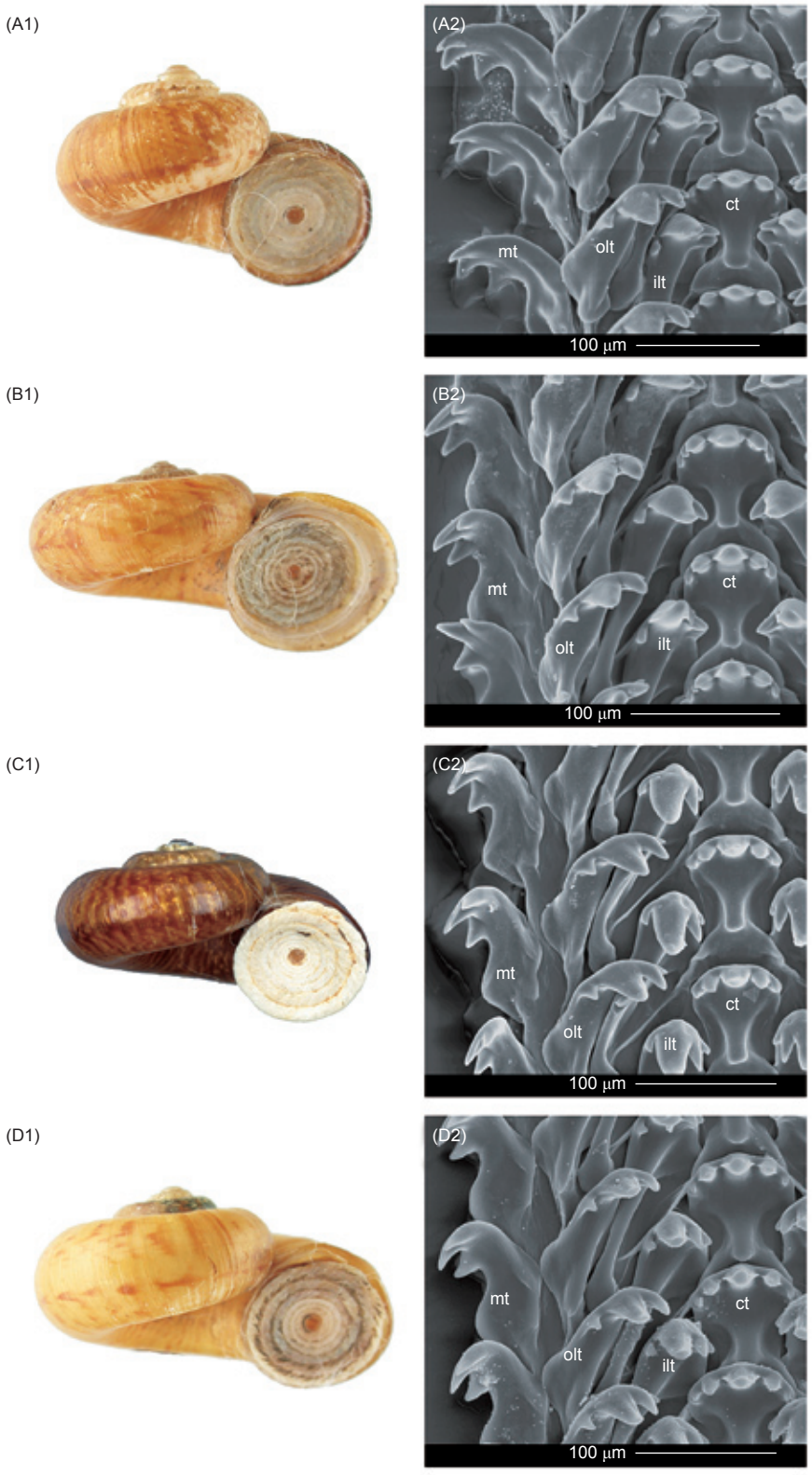

Fig. 9. Environmental scanning electron microscopic photograph of *Cyclotus* species. (A1) Shell of *Cyc. taivanus adams* Pilsbry et Hirase, 1905, lateral view; (A2) radula of *Cyc. t. adams*; (B1) shell of *Cyc. t. dilatus* Lee et Wu, 2001, lateral view; (B2) radula of *Cyc. t. dilatus*; (C1) shell of *Cyc. t. peraffinis* Pilsbry et Hirase, 1905, lateral view; (C2) radula of *Cyc. t. peraffinis*; (D1) shell of *Cyc. t. taivanus* H. Adams, 1870, lateral view; (D2) radula of *Cyc. t. taivanus*.

of *Jerdonia* W. and H. Blanford, 1861 except for the absence of spiral striations (Pilsbry and Hirase 1905), and the genus *Nakadaella* was retained as a subgenus for smooth species (Pilsbry and Hirase 1905). *Jerdonia* is similar to *Cyathopoma* but without sculpture within the umbilicus. However, our results indicate that the smooth shell of *Cya. micron* may be a plesiomorphic character, and *Nakadaella* is a synonym for *Cyathopoma*.

In all cladograms *Cya. iota* and *Cya. taiwanicum* were clustered without resolution. All *Cya. iota* specimens were reported from mountain regions exceeding 1000 m in elevation (Lee and Chen 2003), except the types, and we suspect that *Cya. iota* may be an ecotype of *Cya. taiwanicum*. In a comparison of the radulae between these species and *Cyclotus*, the serrate cusps on the convex side of the central teeth in *Cyathopoma* are not present in *Cyclotus*. In addition, the fine serrate cusps on the inner side of the marginal teeth are not present in *Cyclotus*. Thus the radulae of *Cyathopoma* are clearly distinct from those of *Cyclotus*. Based on molecular and radula data, we concluded that *Cyathopoma* and *Cyclotus* are distant relatives. *Cyathopoma* micron cannot be placed in *Cyclotus*. However, Indian species were not included in this study. Obviously, further studies are needed which would include the Indian species in order to understand the phylogenetic relationships of these interesting snails.

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