Graeteriella (Graeteriella) longifurcata, new species, a stygobitic cyclopoid species (Copepoda: Cyclopoida: Cyclopidae) from Central Vietnam

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Abstract.—A new cyclopoid species belonging to the genus *Graeteriella* is described from a limestone cave in central Vietnam. This is the first record of the genus from the Oriental region. It is assigned as the fifth member of the subgenus *Graeteriella* Rylov, 1948 based on its unique 1-segmented leg 5 armed with two spines/setae, lacking protopodal seta, and 2-segmented rami of legs 2–4 in female. The new species is distinguished from its congeneric species of the subgenus from Europe by having longer caudal rami with spiniform outer caudal seta, by the absence of coxal seta on leg 4, and by the seta/spine armature of leg 5, that is, an inner stout spine and a shorter outer pinnate seta on the free exopodal segment of leg 5.

Keywords: karst, limestone cave, Oriental region, SEM

The genus *Graeteriella* Brehm, 1926 is characteristic in having the character combination as follows: P5 is fully incorporated into the fifth pedigerous somite without protopodal seta; genital double-somite is broad or normally developed, with broad seminal receptacle; antennule is very short, 11-segmented; P5 is 1-segmented, bearing a free exopodal segment with two setae/spines (Dussart & Defaye 1995, Pesce 1996).

Rylov (1948) divided the genus *Graeteriella* into two subgenera *Graeteriella* and *Paragraeteriella*, which are differentiated by two key characters: segmentation of exopods of P1–P4 (2-segmented in *Graeteriella* while 3-segmented in *Paragraeteriella*) and the setal armature of caudal ramus (with 3 apical caudal setae in *Graeteriella*, whereas 4 in *Paragraeteriella*). However, three later described species, *G. brehmi* Lescher-Moutoué, 1968, *G. rouchi* Lescher-Moutoué, 1968, and *G. boui* Lescher-Moutoué, 1974, are regarded as belonging to the subgenus *Graeteriella*, although they have normally developed apical caudal setae. Therefore, the only criterion differentiating the two subgenera is the segmentation of exopods of P2–P4 in female. Currently, the subgenus *Graeteriella* comprises four valid species, and their distributions are still confined to the Palaearctic (Rylov 1948, Lescher-Moutoué 1968a, 1968b, 1974; Pesce 2012).

According to Walter (2013), the genus *Graeteriella* Brehm, 1926 comprises 10 species, including *G. operculata* (Chappuis, 1917) and *G. surinamensis* Brehm, 1926. However, *G. operculata* was redescribed by Rocha & Bjornberg (1987) and transferred to *Muscocyclops* by the presence an outer basal seta on the fifth pedigerous somite, which is absent from the genus *Graeteriella*. Unfortunately, no information is available on the exact character state of P5 of *G. surinamensis*.

In Vietnam, studies on groundwater copepods have been few and most focused on the orders Harpacticoida and Calanoida.

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Borutzky (1967) and Apostolov (2007) recorded nine harpacticoid species from groundwater in North Vietnam. Almost all of them could be considered as stygophilic species, but only a few species are genuine stygobites. Dang & Ho (2001) described two new calanoid species, Nannodiaptomus phongnhaensis and Neodiaptomus curvispinosus, from Phong Nha cave in Phong Nha-Ke Bang National Park, Central Vietnam. Brancelj (2005) established a new genus for a stygobitic calanoid species, Hadodiaptomus dumonti, from a cave in Cuc Phuong National Park, North Vietnam. Recently, Tran & Chang (2012) described two new harpacticoid species, Microarthridion thanhi and Nitokra vietnamensis from anchialine caves in the karst area of Ninh Binh province, North Vietnam. Studies on groundwater cyclopoids are lacking in Vietnam. So far, only a rare stygobitic species, Tropocyclops chinei Dang, 1967, is known from caves in Hoa Binh province, North Vietnam (Dang 1967).

In 2011, the first author (DLT) participated in the project of "Nature Conservation and Sustainable Management of Natural Resources in the Phong Nha-Ke Bang National Park Region," which has a well-developed karst system comprising about 80 caves and has been surveyed by Vietnamese and British scientists since 2005 (Limbert 2012). In this study, we describe a new stygobitic cyclopoid species of the genus *Graeteriella*, which was found during the expedition. This is the first record of the genus *Graeteriella* from the Oriental region as well as outside of Europe.

Materials and Methods

Samples were taken from a siphon lake inside Thien Duong cave (17°31'09.1"N, 106°13'23.0"E) in Phong Nha-Ke Bang National Park, Quang Binh province, Central Vietnam. The siphon lake is a permanently dark section; the distance from the cave exit is about 1200 m. Copepods were collected on surface water with a conical plankton net of 80 μ m mesh and fixed and stored in 80% ethanol.

Specimens were dissected and mounted in glycerol or lactophenol. The mounted specimens were observed with a differential interference contrast microscope (Olympus BX51 and CH40). All drawings were made with the aid of a camera lucida.

Material used for SEM observation was fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2–7.4) for 2 hours, followed by fixation in 1% cold osmium tetroxide (at about 5°C) in the same buffer for 12 hours. After dehydration through a graded series of ethanol (70, 75, 80, 90 and 100%) for 30 minutes each, the material was critical point dried, coated with goldpalladium, and examined with a scanning electron microscope Jeol JEM 5410 LV operated at 15 KV.

Type specimens are deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam and in the Department of Biological Science, Daegu University (DB).

Abbreviations used in the text and figure legends follow the conventional ones frequently used for the taxonomy of copepods: enp-1 to enp-2 or exp-1 to exp-2, the first to second endopodal or exopodal segments of each leg, respectively; P1–P6, first to sixth legs. Sewell's system is adopted for seta/spine armature of P1–P4, where setae are denoted by Arabic numerals, and spines by Roman numerals (cf. Huys & Boxshall 1991, figs. 1, 5, 7).

Systematics Family Cyclopidae Rafinesque, 1815 Subfamily Cyclopinae Rafinesque, 1815 Genus Graeteriella Brehm, 1926 Subgenus Graeteriella Rylov, 1948 Graeteriella (Graeteriella) longifurcata, new species Figs. 1–6

Type locality.—A small siphon lake in Thien Duong cave (17°31′09.1″N,



Fig. 1. *Graeteriella* (*Graeteriella*) *longifurcata* (\mathcal{Q}). A, habitus, dorsal; B, habitus, lateral; C, pediger 5 and genital double somite, ventral; D, pediger 5 and genital double somite, lateral; E, anal somite and caudal rami, dorsal; F, anal somite and caudal rami, ventral. Scale bars = 100 µm.



Fig. 2. *Graeteriella* (*Graeteriella*) *longifurcata* (\mathcal{Q}). A, antennule; B, antenna; C, D, mandible; E, maxillule; F, maxilla; G, maxilliped. Scale bars = 50 μ m (A), 10 μ m (B–G).



Fig. 3. Graeteriella (Graeteriella) longifurcata (). A–D, P1–P4. Scale bar = 100 μ m.



Fig. 4. Graeteriella (Graeteriella) longifurcata (δ). A, habitus, dorsal; B, habitus, lateral; C, antennule; D, labrum. Scale bars = 100 μ m.



Fig. 5. Graeteriella (Graeteriella) longifurcata (3). A, P5, P6, and urosome, ventral; B–E, P1–P4. Scale bar = 100 μ m.



Fig. 6. *Graeteriella* (*Graeteriella*) *longifurcata*. SEM micrographs. A–G, female. A, habitus, dorsal; B, anal somite and caudal rami, dorsal; C, anal somite with anal operculum; D, anal somite and caudal rami, ventral; E, pediger 5 and genital double somite, ventral; F, P5 and P6; G, mouthparts; H, male habitus, dorsal.

106°13′23.0″E), distance from the exit about 1200 m, Phong Nha-Ke Bang National Park, Quang Binh province, Central Vietnam.

Material examined.—Holotype ♀ (IEBR-COP3390), 9 Aug 2011, leg. D. L. Tran, dissected and mounted on slide in glycerol. Allotype & (IEBR-COP3391), type locality, 9 Aug 2011, leg. D. L. Tran, dissected and mounted on slide in glycerol. Paratypes: 3 ♀♀ (IEBR-COP3392-3394) and 2 ඊඊ (IEBR-COP3395, 3396), dissected and mounted on slide in glycerol, collection details same as in holotype and allotype. Other materials: 15 99, 4 33, and 2 copepodites (IEBR-COP-AED02.23.068), undissected and preserved in 80% ethanol, collection details same as in holotype; 7 \Im \Im , 1 \eth (DB20053), stored in 80% ethanol, deposited in the Department of Biological Science, Daegu University.

Description of female.—Body (Figs. 1A, B, 6A) colorless; 526 µm long (ranging 480– 552 µm, n = 8), length/width ratio 2.99 (ranging 2.78–3.14, n = 8). Prosomeurosome boundary distinctly defined between fourth pedigerous somite and fifth pedigerous somite; prosome/urosome length ratio 1.86; cephalothorax 2.08 times as wide as genital double-somite ratio (ranging 1.97–2.22, n = 8). Dorsal surfaces of prosomites and urosomites ornamented with innumerable pits.

Prosoma comprising cephalothorax, incorporating first pedigerous somite and 3 free pedigerous somites; elongate, oval, slightly slender; greatest width at posterior end of cephalothorax. Cephalothorax slightly wider than long, or nearly as long as wide (length/width ratio 0.99, ranging 0.97-1.01, n = 8), representing about 33% of total body length; posterior lateral angle round; sensilla scattered on dorsal surface (8 sensilla in dorsal view). Nauplius eye absent. Pedigerous somites 2 to 4 each with 2–4 sensilla dorsally and laterally, with narrow and elongate posterior lateral angle. Posterior margins of prosomites with narrow and smooth hyaline fringe.

Urosomites ornamented with irregularly crenate hyaline fringe along posterior margin both dorsally and ventrally. Genital somite and first abdominal somite (Figs. 1C, D, 6E) fused to form genital double somite, with lateral chitinous suture marking line of fusion; anterior part expanded with dorsolateral recesses at level of sixth legs; slightly wider than long (about 1.01 times as wide as long); with serrated hyaline fringe along posterior margin both dorsally and ventrally, serration finer ventrally. Seminal receptacle, anterior part wide, rounded medially; posterior part developed, much larger than anterior part, sac-like. Copulatory pore small, ovoid, situated at anterior one-third of double-somite; copulatory duct narrow, siphon-shaped and slightly curved. Third and fourth urosomites cylindrical, much narrower than genital double-somite, without sensilla dorsally; third urosomite about 1.4 times as long as fourth. Anal somite (Figs. 1E, F, 6B–D) much wider than long, length/width ratio 0.61 (ranging 0.56–0.67, n = 8), ornamented with 2 sensilla on dorsal surface and transverse row of minute spinules along posterior margin. Anal operculum semicircular with round and smooth posterior margin, extending posteriorly beyond posterior margin of anal somite in lateral view (Fig. 1B); anal operculum 0.57 times as wide as total anal somite (ranging 0.56–0.61, n = 8).

Caudal rami (Figs. 1E, F, 6B, D) subcylindrical, nearly parallel, narrow and elongate, 4.2 times as long as wide (ranging 3.8–4.5, n = 8); greatest width at base of caudal ramus, slightly narrowing posteriorly, then again slightly wider near distal end, inner and outer margins smooth. Lateral caudal seta short, spiniform, inserted slightly dorsally at about middle of caudal ramus, armed with transverse row of 6–8 spinules ahead of its base. Outer caudal seta strong, spiniform, about 0.5 times as long as caudal ramus, flanking 3–4 spinules at its base. Outer terminal caudal seta stout, spiniform, slightly shorter than outer caudal seta. Inner terminal caudal seta well developed, about 3.5 times as long as caudal ramus, heterogeneously ornamented, proximal third nearly bare, next third pinnate, distal third plumose. Inner caudal seta minute, naked, about 0.3 times as long as outer caudal seta. Dorsal caudal seta slender, bare, about two thirds times as long as caudal ramus, issuing from inner distal corner of caudal ramus.

Antennule (Fig. 2A) short, 11-segmented, nearly reaching to posterior margin of cephalothorax. Segments 1, 3, 7, and 8 stout and long; segments 2, 4, and 5 relatively short; relative length ratio of antennular segments 6.0 : 1.8 : 2.9 : 1.4 : 1.0: 2.5: 4.3: 4.5: 2.7: 3.7: 3.9. Segment 5 with short, robust, spiniform seta anterodistally; all setae of segments 1-4 and segments 6-10 slender and bare. Segment 8 with 1 long, slender aesthetasc, slightly extending over posterior end of segment 10. Segment 11 with 3 long pinnate setae, 3 bare setae apically, and 1 bare seta on inner margin. Setal formula: 6,3,5,2,1,2, 3, 2 + aesthetasc, 2, 2, 7.

Antenna (Fig. 2B) 4-segmented, comprising coxobasis and 3-segmented endopod. Coxobasis cylindrical, twice as long as wide, with 1 inner distal plumose seta. Exopod absent. Enp-1 with 1 inner naked seta; enp-2 with 4 inner naked and 1 stout subapical seta, with spinule row outer distally; enp-3 cylindrical, about twice as long as wide, bearing 4 geniculate and 3 slender setae apically, armed with 2 spinule rows at outer margin and outer distal corner.

Labrum small, representing trapezoidal plate, without spinules or setules on surface; cutting edge concave, with about 12 minute teeth.

Mandible (Fig. 2C, D) with well-developed coxal gnathobase bearing about 11 strong teeth along distal margin, with 1 naked seta at dorsal corner. Mandibular palp 1-segmented, with 2 long and 1 small naked seta.

Maxillule (Fig. 2E) with praecoxal arthrite with 3 strong apical spines fused to arthrite base and 6 elements along inner margin, proximal plumose seta longest, others naked. Palp consisting of coxobasis and endopod; coxobasis armed with 3 setae distally on basal endite (2 naked slender setae and 1 pinnate seta), with minute spinules distomedially. Exopod represented by 1 naked seta on outer margin of coxobasis; endopod 1-segmented with 2 naked and 1 pinnate setae.

Maxilla (Figs. 2F, 6G) 5-segmented. Praecoxa fused to coxa; praecoxal endite with 2 plumose setae. Coxa with 2 coxal endites; proximal endite represented by 1 naked seta; distal endite subcylindrical, elongate, armed with 2 setae apically. Basis forming bifurcate claws with 1 naked seta inserted to base of distal claw. Enp-1 with 1 smooth seta on inner margin. Enp-2 relatively small, with 1 pinnate and 3 naked setae.

Maxilliped (Figs. 2G, 6G) 4-segmented, composed of syncoxa, basis, and 2-segmented endopod. Syncoxal endite with 1 pinnate and 1 naked seta inner distally. Basis bearing 1 pinnate and 1 naked seta, ornamented with curved setule row at inner proximal margin and 3–4 minute spinules at outer distal margin. Enp-1 armed with 1 stout pinnate seta. Enp-2 small, with 3 naked setae.

P1–P4 (Fig. 3A–D), both endopods and exopods 2-segmented. P1–P3, exopod shorter than endopod, while slightly longer than endopod in P4. Seta/spine armature of P1–P4 as follows:

	Coxa	Basis	Exopod	Endopod
P1	0–1	1-1	I-0; III,1,4	0–1; 1,I,3
P2	0-1	1-0	I-0; III,1,4	0-1; 1,I,4
P3	0-1	1-0	I-0; III,,1,4	0-1; 1,I,4
P4	0-0	1-0	I-0; III,1,3	0–1; 1,I,3

P1–P4 (Fig. 3A–D), with pits scattered irregularly on surfaces. Intercoxal scler-

ites with paired lateral lobes produced distally, with rounded and smooth posterior margin; both anterior and posterior surfaces smooth without transverse spinule or setule row. Coxa with spinule row at outer distal corner; coxal seta present in P1-P3, while absent in P4. Bases of P1–P3 produced inner distally, with setule row along inner distal margin, but lacking in P4; medial basal seta of P1 stout and spiniform, nearly reaching middle of enp-2. Exp-2 of P1-P4 each with 3 outer spine (spine formula 3,3,3,3), similar in length to each other; setal formula 5,5,5,4. Enp-1 of P1-P4 each with 1 inner distal seta; enp-2 with setal formula 4,5,5,4. P4 enp-2 elongate, about 2.6 times as long as wide; armed with single spine apically, about 0.75 times as long as enp-2; outer distal seta short, not exceeding beyond apical spine.

P5 (Figs. 1C, D, 6E, F) with protopodal segment incorporated into fifth pedigerous somite, lacking outer basal seta, situating rather laterally; free exopodal segment very small, quadrate, about 1.7 times as wide as long, bearing 1 spine apically and 1 outer plumose seta subapically; spine about 1.5 times longer than seta.

P6 (Figs. 1D, 6F) small, forming semicircular plate, inserted dorsolaterally on genital double-somite, armed with 2 short spines.

Description of male.—Body (Figs. 4A, B, 6H) colorless, 486 μ m in length (ranging 467–505 μ m, n = 5), relatively more slender than females (length/width ratio 3.48, ranging 3.30–3.60, n = 4), greatest width at posterior end of cephalothorax; cephalothorax 2.02 times as wide as genital double-somite ratio (ranging 1.96–2.11, n = 4); length ratio of prosome to urosome 1.20 (ranging 1.16– 1.24, n = 4). Prosome elongate-oval; ornamentation on dorsal surfaces nearly same as in female. Cephalothorax 0.32 times as long as body (ranging 0.31–0.33, n = 4). Genital somite (Fig. 5A) swollen laterally, about 1.2 times wider than long, posterior margin serrated dorsally; 2 ovoid spermatophores visible inside in dorsal view; paired genital opercula pyriform, each armed with inner spine and minute spiniform seta on outer distal corner, representing P6.

Antennule (Fig. 4C) 15-segmented, relatively longer than that of female; geniculate between segments 8 and 9, and between segments 13 and 14; segments 4, 5, 6, and 9 relatively short; segments 13, 14, and 15 elongate; segment 10 large and swollen, forming 2 obtuse processes in middle of anterior margin; segments 1, 4, and 15 each bearing 1 slender aesthetasc; segment 11 with 1 spiniform seta; all setae slender and naked, except for pinnate apical seta on segment 15. Setal formula: 7+aesthetasc, 3, 1, 1+aesthetasc, 0, 1, 1, 1, 1, 2, 1, 0, 0, 1, 8+aesthetasc.

Other characters of mouthparts, caudal rami and P1–P4 (Fig. 5B–E) similar to those of female.

Etymology.—The specific name *longifurcata* is taken from the Greek *longus* (meaning long) and *furcata* (meaning 'with caudal rami'). The name refers to the narrow and elongate caudal rami of the new species.

Ecology.—Specimens were found in the surface water of a small siphon lake in the dark zone of Thien Duong cave. The small cavern lake is situated at about 1200 m from cave exit and has the size dimensions of 5.5 m (W) x 8.0 m (L), with a muddy bottom containing sand. When sampling completed on 9 Aug 2011, water quality of the lake showed the following: water temperature 22.4°C; pH 6.87; DO 5.3 mg/l; electrical conductivity 116 μ S/cm; salinity 0.00%.

This species co-occurred with Nannodiaptomus phongnhaensis Dang & Ho, 2001 (Calanoida), Tropocyclops sp. (Cyclopoida), and several specimens of

Character	G. unisetigera	G. brehmi	G. rouchi	G. boui	G. longifurcata
Habitus	vermiform	cyclopiform	cyclopiform	cyclopiform	cyclopiform
Body length	?	465 μm	500 μm	500 μm	532 μm
Caudal ramus, length/width ratio	-	1.7	2.2	2.5–2.7	3.8-4.5
Outer terminal caudal setae	absent	normal	normal	normal	spiniform
Anal operculum	triangular	semicircular	semicircular	semicircular	semicircular
P1–P4 exp-2, spine formula	3,4,4,4	3,3,3,3	3,3,3,3	3,4,4,4	3,3,3,3
P1–P4 exp-2, setal formula	5,?,4,4	5,5,5,5	5,(5),(5),5	5,5,5,5	5,5,5,4
P2–P4 enp-2, setal formula	3,?,2,2	4,5,6,4	3,4,5,3	4,5,5,4	4,5,5,4
P4 enp-2, number of apical spine	1	2	2	2	1
P4, coxal seta	present	present	present	present	absent
♂ P4, segmentation of exp/enp	3/2	3/3	3/3	2/2	2/2
ở P6, number of apical setae/spines	2	3	3	2	2
Locality (habitat)	Europe & Russia (caves, wells)	France (ground waters)	France (ground waters)	France (ground waters)	Vietnam (cave)

Table 1.—Character comparison of *Graeteriella* (*Graeteriella*) longifurcata with congeneric species in the subgenus *Graeteriella*.

unidentified harpacticoids and parabathynellids.

Remarks.—As shown in Table 1, reduction of segmentation of legs in both sexes and in the setal and/or spine armature of legs occurs widely in members of the genus Graeteriella, supposedly due to the subterranean habit. Sometimes, reduction also appears in the number of caudal setae, accompanied with malformation of them. Graeteriella longifurcata is characterized in having elongate caudal rami, as suggested in the specific name, with spiniform outer and outer terminal caudal setae, which discriminate it from all known congeners. Above all, this new species is most remarkable in showing loss of coxal seta of P4 in both sexes. Furthermore, a single apical spine on P4 enp-2 is also a decisive characteristic distinguishing it from congeners in the subgenus *Graeteriella*, except for *G*. *unisetigera*.

Of the two subgenera of the genus *Graeteriella*, this new species can be assigned to the subgenus *Graeteriella* Rylov, 1948 in having 2-segmented rami of P2–P4 in both sexes.

Among four recognized species of the subgenus *Graeteriella* (see Table 1), *G. unisetigera* (Graeter, 1908), described from Europe and Russia, shares a single apical spine on P4 enp-2 with *G. longi-furcata*; however, it is easily distinguished from the new species, as well as other congeneric species, by a vermiform body shape, the absence of outer caudal seta, and the triangular anal operculum (instead of semicircular in others).

Graeteriella longifurcata is similar to G. brehmi Lescher-Moutoué, 1968 and G.

rouchi Lescher-Moutoué, 1968, both described from France, in showing the same spine formula 3,3,3,3, but it clearly differs from the two congeneric species by 2-segmented exopods and endopods of P4 in male (versus 3-segmented in *G. brehmi* and *G. rouchi*); number of setae on P2–P4 exp-2 (4 setae in *G. longifurcata* versus 5 setae in *G. brehmi* and *G. rouchi*); setal formula of P2–P4 enp-2 (4,5,5,4 in *G. longifurcata* versus 4,5,6,4 in *G. brehmi* and 3,4,5,3 in *G. rouchi*); and a single apical spine on P4 enp-2 (versus 2 spines in *G. brehmi* and *G. rouchi*).

Graeteriella longifurcata much resembles *G. boui* Lescher-Moutoué, 1974 from France in sharing 2-segmented rami of P1–P4 in male; 2 seta/spine elements on P6 in both sexes; and setal formula of P2–P4 enp-2 (4,5,5,4). However, the new species is distinguished from *G. boui* by the armature of exopodal segment of P5 (armed with a spine and a seta versus 2 pinnate setae in *G. boui*); spine formula of 3:3:3:3 (3:4:4:4 in *G. boui*); setal formula of 5:5:5:4 (5:5:5:5 in *G. boui*); and single apical spine on P4 enp-2 (2 spines in *G. boui*).

To date, members of this genus have been reported only from the ground waters in Europe. This is the first record of the genus *Graeteriella* outside Europe and reported for the first time from the Oriental region.

Key to the species of subgenus Graeteriella

- 1. Spine formula 3,3,3,3 for P1–P4 exopods 2
- Spine formula 3,4,4,4 for P1-P4 exopods 4
- 2. Coxal seta of P4 absent in both sexes; outer and outer terminal caudal setae spiniform *G. longifurcata*
- 3. Setal arrangement of P1–P4 enp-2 4,5,6,4..... *G. brehmi*
- Setal arrangement of P1-P4 enp-2 3,4,5,3..... G. rouchi

- 4. With single apical spine on P4 enp-2; anal operculum triangular; P4 exopod in male 3-segmented *G. unisetigera*
- With 2 apical spines on P4 enp-2; anal operculum semi-circular; P4 exopod in male 2-segmented .. G. boui

Acknowledgments

First author (DLT) thanks Dr. Phi Vuong Tuong (The 69 Institute, Ho Chi Minh Mausoleum Protection High Command, Vietnam) for his assistance in operating a scanning electron microscope. Second author (CYC) is deeply grateful to Dr. Janet W. Reid (Virginia Museum of Natural History, U.S.A.) and Dr. Chad Walter for providing him with many references. Authors also appreciate two anonymous reviewers for their critical comments that greatly improved the manuscript. This work was supported in part by a research grant of Daegu University, Korea in 2009.

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Associate Editor: Janet W. Reid.