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A New *Meghimatium* Slug (Pulmonata: Philomycidae) from Taiwan

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Chi-Li Tsai and Shi-Kuei Wu (2008) A new *Meghimatium* slug (Pulmonata: Philomycidae) from Taiwan. *Zoological Studies* 47(6): 759-766. *Meghimatium burchi* sp. nov. is described based on its small size (12-16.5 mm in body length), and its genitalia with a uniquely expanded proximal end of the vas deferens. DNA sequence analysis within the family Philomycidae indicated that *M. burchi* belongs to the Taiwanese clade. Specimens of this new species were collected from the watershed of Koan-Tau Mt. of Hwei-Sun Agriculture Farm in Taiwan's Central Mountain Range. The holotype is deposited at the National Museum of Natural Science, Taichung, Taiwan (catalog no. NMNS-4609-001). <http://zoolstud.sinica.edu.tw/Journals/47.6/759.pdf>

Key words: Philomycidae, *Meghimatium*, New species, Morphology, Molecular analyses.

The philomycids are aulacopod slugs in which the mantle covers the entire back. There is a large shell sac but no shell, and the foot sole is undivided. The genital orifice is on the right side of the head. The cephalic retractor muscles are completely separate and are inserted towards the ventral side of the lateral body walls (Runham and Hunter 1970).

Philomycus was used to name all Asian philomycid slugs (Cockerell 1890, Collinge 1901, Collinge 1903, Hoffmann 1924, Kuroda 1941, Simroth 1902). Benson (1842) first used the generic name *Incilaria*, and reported *I. bilineata* as its type species. Keferstein (1866) dissected both *I. bilineata* from Yokohama, Japan, and *Meghimatium striatum* from Java, Indonesia. He concluded that both *Incilaria* and *Meghimatium* should be grouped in the family Philomycidae. Runham and Hunter (1970) mentioned that the Oriental philomycids are separated into the genera *Meghimatium* and *Incilaria*, and the American species are separated into the genera *Philomycus* and *Pallifera*. In the past *I. bilineata* was used quite extensively for

species which occurred in Japan, Korea, Taiwan, and China (Keferstein 1866, Yen 1937, Azuma 1995, Chang et al. 1995). However, the generic name *Incilaria* has disappeared from the literature because the definition of the genus *Incilaria* (Benson, 1842) can be applied to almost all genera of philomycids. Wiktor et al. (2000) placed the generic name *Incilaria* as a junior synonym of the genus *Meghimatium* van Hasselt (1823).

Tsai et al. (2005) compared 4 species of the genus *Meghimatium* from Taiwan. According to their sizes, there were the large-sized *M. fruhstorferi* and *M. rugosum*, and the medium-sized *M. bilineatum* and *M. pictum*. Other than those mentioned above, 4 small-sized *Meghimatium* specimens were not included in that report. The small-sized specimens were suspected of being young of other *Meghimatium* species. However, after studying the genitalia, they were actually determined to be mature specimens, as they had fully developed genitalia and a morphology which differed from the above-mentioned 4 species. Thus, they were possibly a new species

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(Wu and Tsai 2003). These specimens were also suspected of being a species of the genus *Pallifera*, introduced from America. Therefore, more studies were carried out on some members of the American genera *Philomycus*, *Pallifera*, and *Megapallifera*. The preliminary results of DNA sequences and phylogenetic analyses indicated that this small-sized species is an endemic Taiwan *Meghimatium*. The authors herein report it as a new *Meghimatium* species.

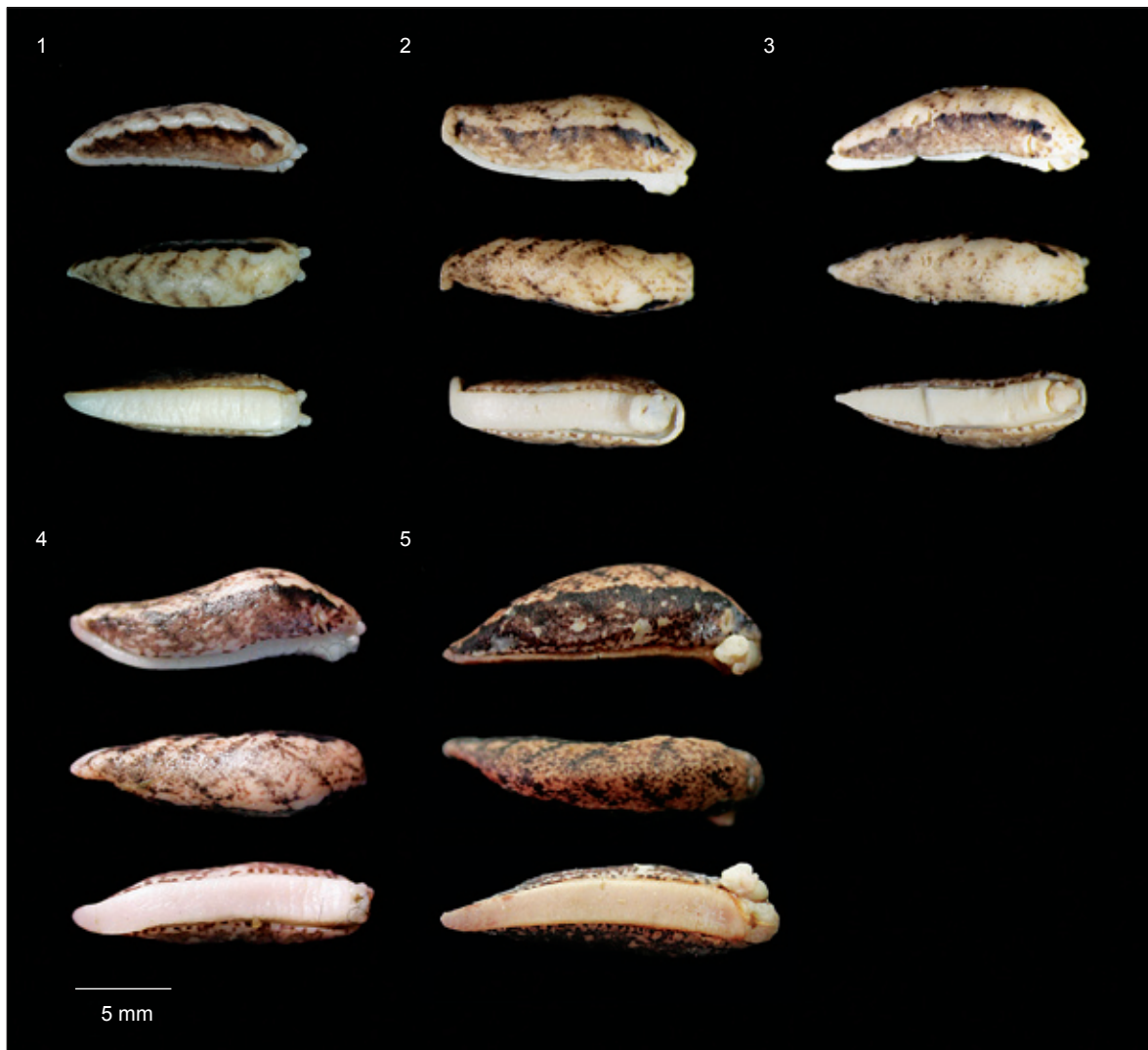
MATERIALS AND METHODS

Meghimatium burchi was collected from the

watershed of Koan-Tau Mt., Huei-Sun Agricultural Farm in Taiwan's Central Mountain Range. The collecting site is at 24°04.54'N, 121°02.10'E.

Originally 4 specimens were catalogued into the Taiwan Endemic Species Research Institute, Mollusks Collection (ESRI-Moll.) no. 61. The holotype was designated and deposited at the National Museum of Natural Science, Taichung, Taiwan (catalog no. NMNS-4609-001). The remaining 3 specimens were designated as paratypes, ESRI-Moll. no. 61.

For dissection, slugs were drowned in water overnight, fixed in 70% alcohol, which was changed twice, and finally preserved in 70% alcohol. Dissection was done under a Leica Wild



Figs. 1-5. Right side, dorsal and ventral views of *Meghimatium burchi* sp. nov. 1. Holotype; 2. paratype 1; 3. paratype 2; 4. paratype 3; and 5. topotype.

MZ12.5 binocular dissecting microscope (Leica Microscopy and Scientific Instruments Group, Heerburg, Switzerland). Drawings were made with the aid of a camera lucida attached to the microscope. Both jaw and radula were separated, cleaned, observed and photographed under a Hitachi scanning microscope (model 3000N, Tokyo, Japan).

DNA sequence comparison

DNA samples were isolated from 8 philomycid species (5 Taiwanese *Meghimatium* and 3 American philomycids), and 1 newly introduced *Arion distinctus* and 1 Taiwanese *Deroceras laeve* as the outgroup (Table 1). Cytochrome oxygenase subunit I (COI) data of *A. subfuscus* were obtained from GenBank (AY987914). The Arionidae and Philomycidae both belong to the Arionoidea. *Deroceras laeve* belongs to the Limacoidea. Muscle tissue from the foot was used for all DNA extractions. Genomic DNAs were extracted using a Puregene DNA purification kit according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN, USA). Universal COI primers (Folmer et al. 1994) were used to amplify the target fragment of mitochondrial (mt)DNA. Optimal PCR conditions were as follows: 3 min at 92°C, 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s, and a final 7 min extension at 72°C in a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA, USA). Sequences were aligned with the software BIOEDIT (vers. 7.0.1) (Hall, 1999).

Phylogenetic analyses based on distance, maximum-parsimony (MP), and maximum-likelihood (ML) methods were conducted using PAUP 4.0 b10 (Swofford 2002). The optimal model of nucleotide substitutions was evaluated by the likelihood ratio test in MODELTEST 3.7 (Posada and Crandall 1998), by selecting a TVM+I+G model with a proportion of invariable sites (I) of 0.4805 and a gamma distribution shape parameter of 1.1363. Based on this model, a distance matrix and Neighbor-joining (NJ) tree were constructed. Using the above model parameters, a ML tree was obtained by a heuristic search with the tree bisection reconnection (TBR) branch-swapping algorithm. An MP tree was created with equal weighting and heuristic parsimony with the closest additional sequence. The confidence nodes were evaluated by the bootstrap method (Felsenstein 1985) with 1000 replications in the NJ, MP, and ML trees.

Family Philomycidae Gray, 1847
Genus *Meghimatium* van Hasselt, 1823
***Meghimatium burchi* sp. nov.**

Specimens examined: ESRI-Moll. no. 61; 4 specimens; watershed of Koan-Tau Mt. in Huei-Sun Agriculture Farm, Nantou Co., 12 Feb. 2003; Collectors Wu, Lin, and Tsai. (holotype and paratypes). ESRI-Moll. no. 566; 1 specimen; data same as above; 16 Apr. 2005; Collector Tsai (topotype).

Measurements: (after preservation in 70%

Table 1. Philomycid, arionid, and agriolimacid species analyzed, collecting locality and date, and GenBank accession number

Taxon	Location	Date	Accession no.
Philomycidae			
<i>Meghimatium bilineatum</i>	Jiji Township, Nantou Co., Taiwan	2002 Dec. 24	EF128225
<i>Meghimatium pictum</i>	Taman, Wulai Township, Taipei Co., Taiwan	2001 Apr. 7	EF128224
<i>Meghimatium frustorferi</i>	Dongyan Mt., Fusing Township, Taoyuan Co., Taiwan	2004 Sept. 2	EF128223
<i>Meghimatium rugosum</i>	Neilinger Mt., Zhuoxi Township, Hualien Co., Taiwan	1999 Apr. 24	EF128222
<i>Meghimatium burchi</i>	watershed of Koan-Tau Mt., Nantou Co., Taiwan	2005 Apr. 16	EF105117
<i>Philomycus carolinianus</i>	Carter Co., TN, USA (Florida Museum of Natural History)	2001 May 30	EF128221
<i>Megapallifera ragsdalei</i>	Seatey Co., AK, USA (Florida Museum of Natural History)	1999 May 8	EF128220
<i>Pallifera marmorea</i>	Barry Co., MO, USA (Field Museum of Natural History)	1982 Apr. 28	EF128219
Arionidae			
<i>Arion distinctus</i>	Wuling Farm, Taichung Co., Taiwan	2006 Oct. 12	EF128218
Agriolimacidae			
<i>Deroceras laeve</i>	Guansi, Hsinchu Co., Taiwan	2002 Dec. 17	EF128217

alcohol)

Holotype (Fig. 1): 12 mm L, 3.4 mm W, 3.4 mm H.

Paratype 1 (Fig. 2): 13 mm L, 3.7 mm W, 4.4 mm H.

Paratype 2 (Fig. 3): 13.1 mm L, 3.6 mm W, 3.7 mm H (specimen dissected for genitalia).

Paratype 3 (Fig. 4): 15 mm L, 4.4 mm W, 4.7 mm H (specimen dissected for preparing radula, Figs. 7-9; drawing of the genitalia, Fig. 8).

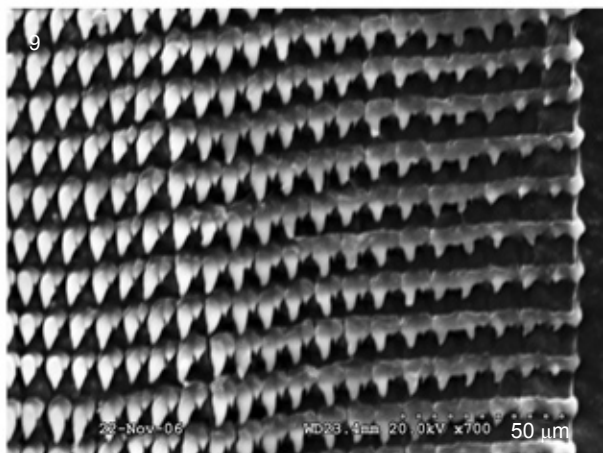
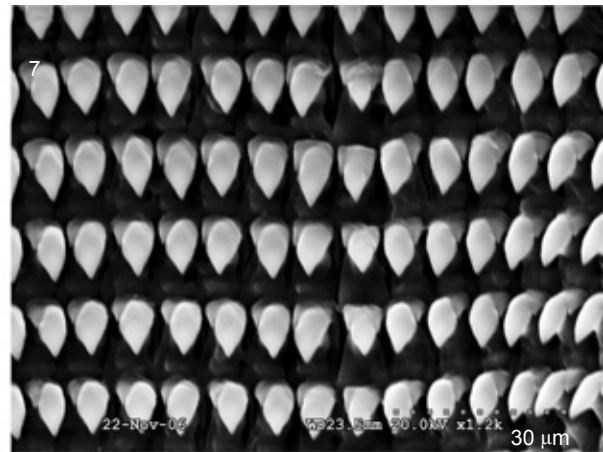
Topotype (Fig. 5): 16.5 mm L, 4.7 mm W, 5 mm H (specimen used for molecular work, table 1; jaw, Fig. 6).

Description: Body coloration (after preservation in alcohol): creamy-brown, dorsal with 3-6 diagonal streaks projecting to rear from both lateral margins between which are scattered reticulated

streaks (Figs. 1-5). Lateral sides generally darker than dorsal side; genital pore located at 1/7 body length from anterior end. Sole white.

Jaw (Fig. 6): Arcuate and an aulacognathic condition (Barker 1999) with about 11-16 fused plates in topotype jaw.

Radula (Figs. 7-9): 18 mm long and 4 mm wide. Radula with about 140 transverse rows; each transverse row with a central tooth and about 34 lateral and marginal teeth. Central tooth with a pointed cusp and a slit on both sides of it; lateral teeth with an elongated triangular cusp, and a slit on outer side of cusp (Figs. 7, 8) which becomes more distinct and appears as a separate denticle (Fig. 9); outwardly marginal teeth with cusps and denticles becoming smaller and eventually disappearing (Fig. 9).



Figs. 6-9. 6. *Meghimatium burchi* sp. nov. Jaw (from topotype). 7. *Meghimatium burchi* sp. nov. Radula, showing central tooth (7th tooth from the right side) and lateral teeth (from paratype 3). 8. *Meghimatium burchi* sp. nov. Radula, showing central tooth (4th tooth from the right side) and lateral teeth (from paratype 3). 9. *Meghimatium burchi* sp. nov. Radula showing right side of lateral and marginal teeth (from paratype 3).

Genitalia (Fig. 10): Ovotestis (Ot) of moderate size, about 2 mm in diameter and 1 mm in height; hermaphroditic duct (Hd) of moderate length (about 3 mm in length) and convoluted; spermoviduct (Spov) long (about 6 mm in length) and thick; free oviduct (Od) about 3 mm long; spermatheca (Sp) oval-shaped and its duct (Sd) about 1.5 mm

with a moderate diameter, slightly expanded at its base (Bsd); vagina (V) short; vas deferens (Vd) separated from spermoviduct (Spov). Vas deferens (Vd) about 7 mm long: its 3/7 proximal end (Pvd) wide and rest of 4/7 of distal end thin and opening into penis (P) where a fairly stout penial retractor muscle (Prm) is attached. Penis

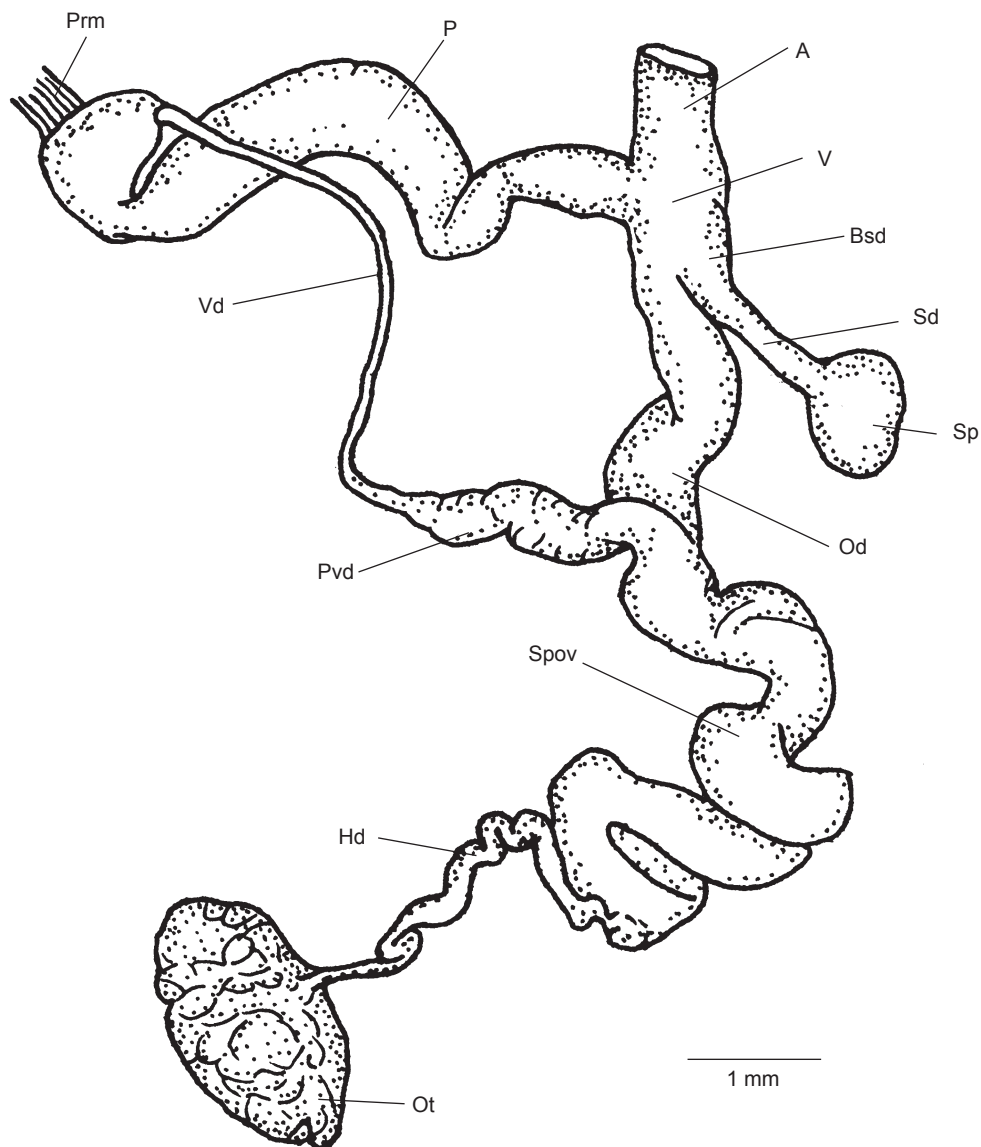


Fig. 10. Genitalia of *Meghimatium burchi* sp. nov. (from paratype 3). A, Atrium; Bsd, base of spermathecal duct; Hd, hermaphroditic duct; Od, oviduct; Ot, ovotestis; Prm, penial retractor muscle; Pvd, proximal end of the vas deferens; P, penis; Sd, spermathecal duct; Sp, spermatheca; Spov, spermoviduct; V, vagina; Vd, vas deferens.

vermiform and long, folded, diameter of its proximal portion about 3 times thicker than its distal portion. Vagina, penis, and duct of spermatheca opening into a short (1 mm long), bellow-shaped atrium (A).

Habitat: Humid, deciduous forests.

Distribution: Taiwan (type locality only).

Molecular data: Ten 658-bp sequences were obtained from this study and deposited with other species in GenBank (Table 1). This sequence is a partial COI gene, and its translation resulted in a 219-amino acid fragment. From those COI data, similar results were obtained from the phylogenetic trees produced by the NJ, MP, and ML methods. *Meghimatium* species from Taiwan formed a well-supported monophyletic clade. American philomycid species formed a well-supported clade. These 2 clades were not sister to each other. They exhibited strong to moderate statistical support for the 2 main clades (clade Taiwan bootstrap values NJ/MP/ML: 87%/64%/97%; clade America bootstrap values: 90%/96%/78%) (Fig. 11). *Meghimatium burchi* belongs to the monophyletic Taiwanese ingroup, although its size and external characters were similar to those of the American *Pallifera*. In addition, resulting topologies obtained from analyses of the amino acid dataset from different methodologies were highly congruent with those obtained from the nucleotide analyses.

Remarks: The jaw morphology of *M. burchi* sp. nov. is similar to those of the medium-sized Taiwanese *Meghimatium* (*M. bilineatum* and *M. pictum*) and American *Pallifera* and *Megapallifera*,

but differs from those of large-sized *Meghimatium* (*M. frustorferi* and *M. rugosum*), which are similar to those of American *Philomycus*. The radular morphology of *M. burchi* sp. nov. is similar to those of the other 4 Taiwanese species.

Genitalia of *M. burchi* sp. nov. differ from those of the other 4 Taiwanese *Meghimatium* in the proximal end of sperm duct expanding then narrowing before entering the penis, and the penial length being fairly long (see also Tsai et al. 2005).

The COI data exhibited 2 main clades (Fig. 11). *Meghimatium burchi* sp. nov. belongs to the monophyletic Taiwanese ingroup, although its size and external characters are similar to those of the American *Pallifera*. The hypothesis that *M. burchi* sp. nov. may possible be an exotic American *Pallifera* species is, therefore, eliminated.

Etymology: This small-sized species is named in honor of Dr. John B. Burch, Prof. Emeritus, Univ. of Michigan, Ann Arbor, MI, who has devoted his entire life to researching freshwater and land mollusks, and who is also a teacher and mentor of the junior author.

DISCUSSION

Benson (1842) described a new species *I. bilineata*. His definition of the genus *Inciliaria* as "Corpus elongatum, postice attenuatum, repens, undique velo marginatum. Tentacula quatuor, superioribus oculiferis, inferioribus integris.

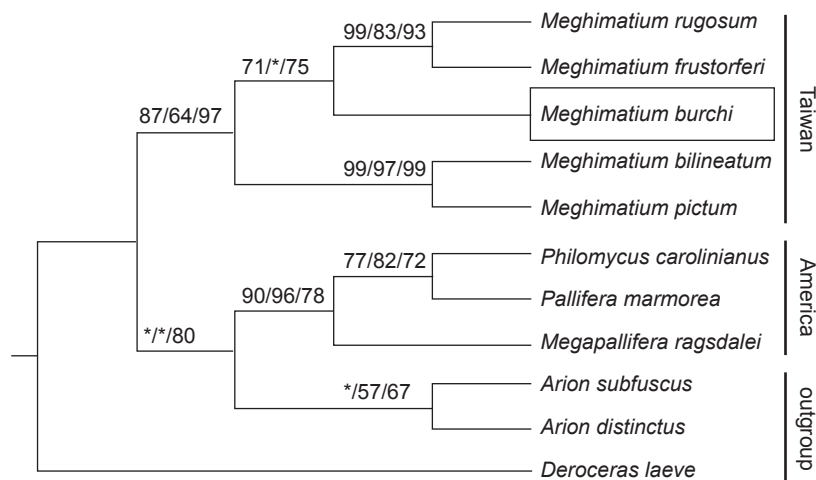


Fig. 11. Bootstrap consensus tree of 11 slug taxa from 658 base pairs of the cytochrome oxidase subunit I (COI) gene, obtained by Neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) methods. Numbers above the nodes indicate bootstrap values (1000 replicates) for the NJ/MP/ML methods. COI data of *Arion subfuscus* were obtained from GenBank AY987914. *Arion subfuscus*, *A. distinctus*, and *Deroceras laeve* were used as the outgroup. * Indicates that the node is not present.

Foramen commune in latere dextro, non procul ab extremitate antica veli situm” can be applied to almost all genera of philomycids.

Wiktor et al. (2000) mentioned that “Asian *Meghimatium* differs from the 2 American genera, i.e. *Philomycus* and *Pallifera* in the lack of external glandular layer around atrium. In addition, *Meghimatium* differs from the externally similar *Philomycus*, in the absence of a special organ comprising a calcified dart-like stimulator”.

From the point of view of body size, *M. burchi* sp. nov. is the smallest size of all the other 4 Taiwanese *Meghimatium* (Tsai et al. 2005) and all known Southeast Asian species (Hasselt 1824, Laidlaw 1937). There are 2 small species reported from Southeast Asia, namely *M. striatum* Hasselt, 1824 and *M. uniforme* Laidlaw 1937. *Meghimatium striatum* was originally reported from Java, Indonesia with an average mature body length of 45 mm, and its yellowish body is marked by 5 longitudinal purplish-brown bands. The type locality of *M. uniforme* is Mount Kinabalu, North Borneo. This species has an average mature body length of 20 mm and has a uniform grayish-brown ground color with no markings on its dorsal body. The body lengths of these 2 species are larger than that of *M. burchi*.

A comparison of these 3 small-sized *Meghimatium* species showed that the proximal ends of the vas deferens (pvd) are all expanded, a unique character which is not shown by the other 4 Taiwanese *Meghimatium* species. *Meghimatium striatum* possesses a distinctly long vagina, and the penis of *M. uniforme* had almost disappeared and was not notable. The above characteristics of *M. striatum* and *M. uniforme* distinctly differ from those of *M. burchi*. In the future, DNA analytical results will be necessary to discuss the phylogenetic relationships of these 3 small-sized Asian *Meghimatium*.

The American philomycid species are classified into 3 genera: *Philomycus*, *Megapallifera*, and *Pallifera* based on body sizes and the presence or absence of a dart sac. Asian philomycid species can be classified by the apparent body size and reproductive morphology, such as the type of spermathecal duct, size of the atrium, and having either a normal or expanded proximal portion of the vas deferens. Whether the Asian philomycid species should be classified into additional genera should rely heavily on molecular data from future studies.

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