Zoological Journal of the Linnean Society, 2023, **197**, 76–103. With 13 figures.

A small slug from a tropical greenhouse reveals a new rathouisiid lineage with triaulic tritrematic genitalia (Gastropoda: Systellommatophora)

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Received 5 April 2022; revised 2 June 2022; accepted for publication 9 June 2022

A small slug found in the tropical greenhouse of the Science Museum (MUSE) of Trento (Italy) turned out to be a species of the little-known systellommatophoran family Rathouisiidae. We undertook detailed comparative anatomical and molecular studies using specimens of the MUSE slug, *Rathouisia sinensis*, and sequences of other systellommatophoran species deposited in GenBank to conduct a systematic and phylogenetic assessment. Analysis of the genitalia of the MUSE slug and *R. sinensis* revealed an unusual triaulic tritrematic structure: two separate female ducts – one for egg release (oviduct), the other for intake of allosperm (vagina) – and a separate male duct for autosperm release. Analysis of the nucleotide sequences of several mitochondrial (*COI*, 16S rDNA) and nuclear (18S rDNA, ITS2 flanked by 5.8S and 28S rDNA fragments, *H3*) gene fragments supported assignation of the MUSE slug to Rathouisiidae, but also its distinction from the other rathouisiid genera *Atopos*, *Granulilimax*, *Rathouisia* and an undescribed genus from the Ryukyu Islands (Japan). Therefore, we decided to describe the MUSE slug as a new species in a new genus: ***Barkeriella museensis* gen. et sp. nov.** The species is certainly an alien introduced into the tropical greenhouse of MUSE, but its origin is unknown and calls for further investigation.

ADDITIONAL KEYWORDS: alien species – anatomy – *COI* mtDNA – Gastropoda – genitalia – 16S rDNA gene – 18S rDNA gene – internal transcribed spacer – new genera – new species.

INTRODUCTION

Plant nurseries, greenhouses, open-air garden centres and other similar facilities host a rich and well-known xenodiversity (e.g. Komala & Przybos, 2001: zooplankton; Horsák *et al.*, 2004 and Krumpálová & Holienková, 2018: molluscs; Szczepkowski *et al.*, 2014: fungi; Šestáková *et al.*, 2017: spiders). Indeed, in greenhouses, a diverse set of alien or cryptogenic species may establish communities consisting of common synanthropic species and occasional outdoor entities introduced with plants and other substrates (Kielhorn, 2008; Kolicka *et al.*, 2015; Richling & von Proschwitz, 2021). Horticulture is one of the pathways implied in species dispersal and introduction (Pergl *et al.*, 2017), allowing organisms that are normally relatively restricted to spread outside their natural range (Bergey *et al.*, 2014). Botanical gardens also play a role in the accidental introduction of foreign species, because they are final consumers in the worldwide

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[Version of record, published online 25 July 2022; http://zoobank.org/urn:lsid:zoobank.org:pub:20C04023-1BB3-4AE6-787-64E36AC5C0F0]

circuit of plant commerce involved in the cultivation and exchange of plants for recreation and research purposes (Heywood, 2011; Wang *et al.*, 2015).

These travelling organisms also provide readily accessible fresh material for taxonomists (Sherlock & Carpenter, 2009), offering the chance to discover novelties to science or to contribute to the knowledge of little-known taxa. For example, a new marasmioid fungus was discovered in the Botanical Garden of Teplice (Czech Republic) (Antonín, 1992). Two new species of the genus *Leptanilla* (Hymenoptera, Formicidae) were found in the Bogor Botanic Garden, West Java, Indonesia (Noerdjito & Kahono, 2001). More recently three new species of freshwater gastrotrichs, assigned to a new subgenus, were described from the Poznań Palm House (Kolicka *et al.*, 2013), while another new species of gastrotrich was discovered in the Jubilee Greenhouse of the Botanical Garden in Krakow (Kolicka, 2019). A new mite species of the genus *Phyllocoptes* (Acarida: Eriophyidae) was described from commercial greenhouses for rose production in Poland (Druciarek & Lewandowski, 2016), and a new species of *Megastylus* (Hymenoptera: Ichneumonidae) reared from larvae of keroplatid fungus gnats (Diptera) was discovered in 2016 in a Dutch orchid greenhouse (Humala *et al.*, 2017).

In this paper we report on a small slug found in the tropical Afromontane greenhouse of the Science Museum (MUSE) of Trento, northern Italy, which turned out to be an interesting discovery. A first superficial examination revealed that its foot had soleoles (transverse ridges), indicating that this small slug did not belong to the most diverse and successful land pulmonate gastropods, the stylommatophorans (order Stylommatophora Schmidt, 1855), but to the marginal marine intertidal and land systellommatophorans (order Systellommatophora Pilsbry, 1948, known also by an earlier name: Soleolifera Colosi, 1921).

The systellommatophorans are slug-like eupulmonates without external or internal shell. They have a dorsal mantle forming the notum, which covers the head and sides of the body, and a reduced or absent mantle cavity. They are hermaphrodite with genitalia presumed to be diaulic and ditrematous (the male and female genital ducts are distinct and open independently of each other) (Climo, 1980; Stanisic, 1998; Barker, 2001). They have a complex systematic nomenclatural history (e.g. Climo, 1980; Bouchet *et al.*, 2017), but are now recognized as a monophyletic group that has been regarded as the sister-group of the ellobioideans (Holznagel *et al.*, 2010; Jörger *et al.*, 2010; Dayrat *et al.*, 2011; Romero *et al.*, 2016). Teasdale (2017) suggested that they have sister-group relationships with stylommatophorans and constitute a clade – Geophila Férussac, 1819 – which in turn has a sister-group relation with ellobioideans. Consequently, Geophila was accepted by Bouchet *et al.* (2017) and Ponder *et al.* (2020) as an unranked taxon in molluscan classification, including Stylommatophora and Systellommatophora.

The systellommatophorans comprise three groups ranked as families: Onchidiidae Rafinesque, 1815, Rathouisiidae Heude, 1885 and Veronicellidae Gray, 1840. Based on their phylogenetic relationships, the onchidiids are assigned to the superfamily Onchidioidea, and the rathouisiids and the veronicellids to the superfamily Veronicelloidea (Bouchet *et al.*, 2017).

The onchidiids are a group of mainly marine slugs with an almost cosmopolitan distribution. According to MolluscaBase (2021) they include at least 84 species in 14 genera [or in 13, according to recent revision of the family by Goulding *et al.* (2022)] and are found in all oceans except polar waters; maximum diversity is reached in the tropical and subtropical Indo-Pacific, especially between the eastern Indian Ocean (Andaman Sea) and the tropical western Pacific (New Caledonia). Most onchidiids are amphibious and linked to marine coastal habitats, where they are found in the intertidal zone of rocky, sandy or muddy shores, coral rubble deposits and estuaries with mangrove forests. Two species of onchidiids are linked to brackish environments [*Onchidium typhae* Buchannan, 1800 and *O. stuxbergi* (Westerlund, 1883)] and tolerate fresh water, while three are considered fully terrestrial molluscs: *Onchidina australis* (Semper, 1880) always lives above the tidal zone, while *Semperoncis montanus* (Plate, 1893) and *S. ponsonbyi* (Collinge, 1901) have been described from high-elevation rainforests in the Philippines and Borneo, respectively. Onchidiid members are small to large slugs (maximum length usually not exceeding 70 mm, exceptionally reach 250 mm). Their body is usually oval, sometimes rounded, the foot is large, without soleoles, they have a single pair of tentacles bearing terminal eyes but the notum of some species bears accessory photoreceptors known as dorsal eyes. Major references to the onchidiids include Stanisic (1998), Barker (2001), Dayrat (2009) and Goulding *et al.* (2022).

The veronicellids are a group of terrestrial slugs with a pantropical distribution, commonly known as leatherleaf or leather-back slugs. According to MolluscaBase (2021) they include at least 123 species in 24 genera and are widespread in tropical and subtropical regions of the world, with some species having a pantropical distribution. The veronicellids generally occur in tropical and subtropical areas with a moist climate, especially in primary and secondary

forests, but can also live in drier habitats like savannas or in cooler environments such as mountain forests. Introduced species are usually synanthropic, frequently being found in gardens and suburban areas, occasionally in rural habitats with disturbed vegetation. Some are considered agricultural pests. They are medium-sized to large slugs (50–120 mm long). Their body is flattened dorsoventrally, elongated but not slender, and the notum is leathery, more or less smooth and relatively dry to the touch in comparison to most slugs; the perinotum carries repugnatory glands. The foot is large with many soleoles. They have two pairs of short tentacles: the upper one is contractile and bears terminal eyes; the lower has tactile function. Major references to veronicellids include Stanisic (1998), Barker (2001), Herbert & Kilburn (2004), Robinson & Fields (2010) and South (2012).

The rathouisiids are a relatively small group of terrestrial slugs with East Asian and Australasian distribution. According to Kimura *et al.* (2020a) and MolluscaBase (2021) they include at least 24 species in three genera and are found in China (*Rathouisia* Heude, 1884), Japan (*Granulilimax* Minato, 1989) and from South-East Asia to New Guinea and Australia (*Atopos* Simroth, 1891); an unassigned rathouisiid (of an undescribed genus) has been found in Ryukyu Islands, Japan (Kimura, 2020; Kimura *et al.*, 2020b). The rathouisiids are small to medium-sized slugs (maximum length rarely exceeding 50 mm, but up to 85 mm). Their body is slender, tapering to both ends; the notum is dry, leathery, weakly pustulous and usually has a central keel. The foot is narrow with many soleoles. They have two pairs of short tentacles: the upper one, contractile but not invaginable, bears terminal eyes; the lower is bifid and has a tactile function. The anterior part of the notum covers the head and only the upper tentacles emerge from it. They are oviparous and lay spherical to ellipsoidal eggs. Rathouisiids generally occur in tropical and subtropical rainforests from sea level to high elevations, deep in leaf litter, under rotting wood or in crevices of limestone rocks; *Atopos* species may climb trees. Rathouisiid's carnivorous diet is mainly composed of snails (they reportedly avoid other slugs), but they also consume fungi and decomposing plant matter. The prey is eaten either via the shell aperture or by boring holes into the shell surface by means of the radula. Major references to the rathouisiids include Stanisic (1998), Barker (2001), Barker & Efford (2004), Wu *et al.* (2006) and South (2012).

The aim of the present paper is to define the systematic setting of this systellommatophoran slug found in the tropical greenhouse of the Science Museum of Trento (Fig. 1) and to investigate its morphology and phylogenetic relationships based on nucleotide sequences of selected gene fragments.

Figure 1. Live specimen of *Barkeriella museensis* gen. et sp. nov. from the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy, Debora Barbato leg. 4.5.2019 (FGC 51191).

MATERIAL AND METHODS

Taxonomic sampling

The MUSE slug underwent morphological and molecular examination to establish its taxonomic setting and relationships. Morphological features were compared with those of the rathouisiids known in the literature and with the results of our re-examination of *Rathouisia sinensis* (Heude, 1882). Nucleotide sequences of selected genes from the slug were compared with the corresponding sequences of systellommatophorans deposited in GenBank. The stylommatophoran agriolimacid *Deroceras reticulatum* (Müller, 1774) or the hygromiid *Monacha pantanellii* (De Stefani, 1879) were used as outgroup.

Material examined

The material of the MUSE slug and *Rathouisia sinensis* is listed in Table 1. All specimens examined are kept in the F. Giusti collection (Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente, Università di Siena, Italy) (FGC) and in the Natural History Collections of Adam Mickiewicz University (Poznań, Poland) (NHC). The gene sequences from GenBank used for molecular research are listed in Supporting Information, Tables S1-S5, S9.

Morphological study

Live specimens were prepared by immersion in water to which drops of 70% spirit were slowly added until death, then fixed in 70% spirit buffered with sodium carbonate (slow drowning in pure water caused bodies to explode). The bodies were dissected by opening the upper surface of the notum under a light microscope (Wild M5A) using fine-pointed watchmaker's forceps. Anatomical organs were drawn using a Wild camera lucida. Some parts of the genitalia were measured using callipers (0.01 mm).

Table 1. Material used in research

No.	Species	Coordinates (lat., long.)	Country and site	Collector/ date/ no. of specimens (collection)	Used in research of anatomy (ANA)/ nucleotide sequences (GEN)
1	MUSE slug	46°03'45.03'N, 11°06'50.26'E	Italy, Trento, Museo delle Scienze	Debora Barbato, Gregorio Bolzonella/ 04.01.2019/ 2/ FGC 51205; Debora Barbato, Gregorio Bolzonella/ 10.02.2019/7/ FGC 51206; Debora Barbato, Gregorio Bolzonella/ 10.02.2019/ 2/ NHC/MOLL-2022-001 – 002; Danio Miserocchi/ 02.03.2019/ 6/ FGC 51168; Debora Barbato/ 04.05.2019/ 15/ FGC 51191	ANA/ GEN
2	*Rathouisia sinensis*	32°07'30.0'N, 118°47'12.2'E	China, Nanjing City	Ye Bin, Takahiro Hirano, Kazuki Kimura/ 20.04.2018/ 1/ KKC4481 (Kim1)	GEN
3	*Rathouisia sinensis*	32°06'27.7'N, 118°57'37.7'E	China, Jiangsu Province, Nanjing, Nandaheyuan, 100 m a.s.l.	Min Wu/ 21.09.2021/ 8/ Nan1-Nan8	ANA/ GEN

Specimens at different maturity stages were examined to collect reliable data on their external characters and on the position and structure of their internal organs. The study of most organs was relatively easy, that of the genitalia was particularly difficult due to the small dimensions and to the fact that the structures of the medial part of the genitalia were embedded in a large glandular structure, possibly consisting of a small, whitish albumen gland proximally and a large, subtransparent gelatinous uterine oviduct medium-distally. Interpretation of the structure of the genitalia was based only on gross morphology. Unfortunately, the scarcity of specimens prevented histological study, which would have been useful for interpretation of the proximal and medial sections of the genitalia.

Anatomical nomenclature usually followed the standard references for eupulmonates (e.g. Barker, 2001; Gómez, 2001; Ponder *et al.*, 2020). The proximal genitalia include the hermaphrodite gonad, the hermaphrodite duct, the albumen gland and the spermoviduct (uterine oviduct and prostate). The genitalia then divide into two separate female ducts and a male distal duct. In the monoaulic and diaulic reproductive systems, the female distal genitalia include the free oviduct (the duct between the distal end of the spermoviduct and the base of the bursa copulatrix duct) and the vagina (the duct between the base of the bursa copulatrix duct and the genital atrium or the female opening). In the triaulic type, the female distal genitalia include the free oviduct (the duct between the distal end of the spermoviduct and the oviductal opening) and the vagina (the duct between the oviduct and the vaginal opening). The male distal genitalia include the vas deferens (the thin duct between the distal end of the prostate and the proximal end of the penis) and the penis (the duct between the distal end of the vas deferens and the genital atrium or the male opening, often containing a penial papilla). In the systellommatophorans, with regard to the male distal genitalia, some authors use this terminology (e.g. Baker, 1925; Barker, 2001; Wiktor, 2003), but others prefer to name the penis as penial sheath and the penial papilla as penis (e.g. Odhner, 1917; Laidlaw, 1940; Dayrat *et al.*, 2016).

The descriptive terms (e.g. short vs. long, slender vs. wide, etc.) refer to comparisons of the same section of the genitalia in different taxa. The directional terms proximal, basal and initial denote the part closer to the gonad, whereas distal, apical, final and terminal denote the part closer to the gonopore in the case of the

ducts of the main axis of the genitalia (e.g. free oviduct, vagina, vas deferens, penis, etc.). The same terms denote the part closer to (proximal, basal or initial) or further from (distal, apical, final or terminal) the main axis of the genitalia in the case of blind structures radiating from the main axis of the genitalia (bursa copulatrix, penial/vaginal/atrial appendix, etc.).

Abbreviations: AG, albumen gland; BB, buccal bulb; BC, bursa copulatrix (gametolytic gland); BS, body side; BW, body wall; CF, carrefour; CG, carbonate granule; CM, cephalic retractor muscle; DSG, distal portion of Simroth's gland; F, foot; FO, free oviduct; H, head; HD, hermaphrodite duct; HG, hermaphrodite gonad; HP, hepatopancreas; In, intestine; ISG, intermediate portion of Simroth's gland; LG, lateral groove; LLB, large lobed body; LSG, left salivary gland; LT, lower tentacle; MGA, male genital atrium; N, notum; NR, nervous ring; OD, oviduct; Oe, oesophagus; OO, oviductal opening; Pb, proboscis; PC, pericardium; Pe, penis; PM, penial retractor muscle; PO, penial opening; PP, penial papilla; Pr, prostate; PSG, proximal portion of Simroth's gland; PV, prostatic vescicle; Re, rectum; RPC, renal-pulmonary complex; RSG, right salivary gland; RUT, right upper tentacle; SD, sperm duct/seminal duct; SG, Simroth's gland; SGD, salivary gland duct; SR, seminal receptacle; St, stomach; UOD, uterine oviduct; UT, upper tentacle; V, vagina; VD, vas deferens; VO, vaginal opening.

Molecular analysis

Small fragments of foot tissue from four MUSE slugs and seven *Rathouisia sinensis* alcohol-preserved specimens were used for total DNA extraction by Tissue Genomic DNA extraction mini kit (Genoplast) following the manufacturer's instructions. The purified DNA was used for analysis of the gene fragments listed in Table 2. Sequences of these gene fragments were obtained by polymerase chain reaction (PCR) with the use of the primers also listed in Table 2.

All amplifications were performed in a volume of 10 µL with the thermal regimes described in the Supporting Information, Table S6. The purity of the PCR products was verified by 1% agarose gel electrophoresis. Prior to sequencing, samples were purified with thermosensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Scientific). The amplified products were sequenced in both directions using the BigDye Terminator v.3.1 sequencing kit on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocols.

Sequences were edited by eye and alignments were performed using the programs BioEdit, v.7.0.6 (Hall, 1999; BioEdit, 2017) and ClustalW, implemented in BioEdit (Thompson *et al.*, 1994), respectively. The *COI* and *H3* sequences were aligned

Table 2. List of gene fragments (with their acronyms) and sequences of their primers used in molecular analysis

Primer	Sequence 5′–3′	References
***COI* – mitochondrial gene of cytochrome *c* oxidase subunit I, 5′-end**		
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* (1994)
HC02198	TAAACTTCAGGGTGACCAAAAAATCA	
16S rDNA – mitochondrial gene for RNA of large ribosome subunit		
16Scs1	AAACATACCTTTTGCATAATGG	Chiba (1999)
16Scs2	AGAAACTGACCTGGCTTACG	
18S rDNA – nuclear gene for rRNA of small ribosome subunit		
PCR amplification and sequencing		
18S-A1	CTGGTTGATCCTGCCAGTCATATGC	Vonnemann *et al.* (2005)
18S-1800	GATCCTTCCGACGGTTCACCTACG	
18S rDNA – nuclear gene for rRNA of small ribosome subunit		
Additional sequencing with the use of internal primers		
18S-400F	ACGGGTAACGGGGAATCAGGG	Vonnemann *et al.* (2005)
18S-400R	CCCTGATTCCCCGTTACCCGT	
18S-700F	GTCTGGTGCCAGCAGCCGCG	
18S-700R	CGCGGCTGCTGGCACCAGAC	
ITS2 – internal transcribed spacer 2 in nuclear gene for rRNA of large ribosome subunit with its flanking fragments (5.8S and 28S rDNA)		
LSU1	CTAGCTGCGAGAATTAATGTGA	Wade & Mordan (2000)
LSU3	ACTTTCCCTCACGGTACTTG	
***H3* – nuclear gene of histone 3**		
H3F	ATGGCTCGTACCAAGCAGAC(ACG)GC	Colgan *et al.* (1998)
H3R	ATATCCTT(AG)GGCAT(AG)AT(AG)GTGAC	

according to translated amino acid sequences. The ends of all sequences were trimmed. After trimming, the lengths of alignments of the *COI* were 566, 563, 551 or 549 bp, as needed in different analyses. Uncertain regions were removed from 16S rDNA alignments with GBLOCKS 0.91b program (Castresana, 2000; Talavera & Castresana, 2007) before phylogenetic analysis of this gene fragment. The lengths of alignments of 16S rDNA, 18S rDNA and *H3* sequences were 306, 1618, 267 positions, respectively. Sequences consisting of the 3′-end of 5.8S rDNA, ITS2 and the 5′-end of 28S rDNA of the MUSE slug were compared with other sequences obtained from GenBank and a resulting alignment was 774 positions long (54 5.8S rDNA + 680 ITS2 + 40 28S rDNA). The sequences were collapsed to haplotypes (*COI*, 16S rDNA, 18S rDNA, ITS2 flanked with 5.8S and 28S rDNA, *H3*) using the program ALTER (Alignment Transformation Environment) (Glez-Peña *et al.*, 2010). *COI* and 16S rDNA haplotypes were joined into concatenated sequences *COI* + 16S rDNA, which were 1028 positions long (573 *COI* + 455 16S rDNA). *COI* and 18S rDNA haplotypes were joined into concatenated sequences *COI* + 18S rDNA 2184 positions long (566 *COI* + 1618 18S rDNA). *COI*, 16S rDNA, ITS2 and *H3* haplotypes were eventually joined into concatenated sequences of *COI* + 16S rDNA + ITS2 + *H3*, 2122 positions long (614 *COI* + 458 16S rDNA + 54 5.8S rDNA + 662 ITS2 + 40 28S rDNA + 294 *H3*).

For each alignment file, best nucleotide-substitution models were specified according to the Bayesian information criterion (BIC): for *COI* – HKY+G+I (Hasegawa *et al.*, 1985; Kumar *et al.*, 2016); alternatively, for *COI* and for 16S rDNA, for concatenated *COI* + 18S rDNA and for concatenated *COI* + 16S rDNA + ITS2 + *H3* sequences – GTR+G+I (Nei & Kumar, 2000); for 18S rDNA sequences – K2+G+I (Kimura, 1980; Kumar *et al.*, 2016); for ITS2 – GTR+G (Nei & Kumar, 2000; Kumar *et al.*, 2016); for *COI* + 16S rDNA and for *H3* – T92+G+I (Tamura, 1992; Kumar *et al.*, 2016). The evolutionary distances between *COI* sequences were estimated using the Kimura 2-parameter method (Kimura, 1980) in MEGA7 (Kumar *et al.*, 2016). Neighbour-joining (NJ) analysis (Saitou & Nei, 1987) and maximum likelihood (ML) analysis were performed with MEGA7 (Kumar *et al.*, 2016). Calculated bootstrap values were mapped on the ML trees. In addition, Bayesian inference (BI) was conducted for concatenated *COI* + 16S rDNA, *COI* + 18S rDNA and *COI* + 16S rDNA + ITS2 + *H3* sequences with the use of program MRBAYES 3.2.6 (Ronquist & Huelsenbeck, 2003; Ronquist *et al.*, 2012). Four Monte Carlo Markov chains were run for one million generations, sampling every 100 generations (the first 25% of trees were discarded as 'burn-in'). Posterior probability (PP) values obtained on 50% majority rule consensus Bayesian tree were mapped on ML trees of concatenated sequences. Genetic species delimitation based on *COI* sequences was estimated by the assemble species by automatic partitioning (ASAP) clustering method (Puillandre *et al.*, 2021). ASAP analyses were performed on the online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) using three distance metrics (*p*-distances, JC69 and K80).

RESULTS

MORPHOLOGICAL ANALYSIS

The MUSE slug displays many distinctive anatomical features (Figs 2–4): a head with two couples of tentacles, of which the lower ones are short and bilobed; a finely granulated mantle forming a covering that wraps head and body up to the pedal groove with no sharp delimitation between the dorsal (perinotum) and the lateroventral sector (hyponotum) of the mantle covering (notum); a foot with soleoles (transverse ridges); an anterodorsal renal-pulmonary complex; a jaw is absent; the radula consists of many rows of unicuspidate hook-like teeth, with a central tooth present (Fig. 5); a small stomach; a unilobed digestive gland (hepatopancreas); an anus opening is anteriorly inside the right pedal groove, close to the oviductal opening; genitalia are complex and of triaulic (three ducts for gametes: one vaginal for receiving allosperm from partner, one oviductal for depositing the eggs, one penial for carrying autosperm to the penis) and tritrematic type (three genital openings: vaginal, oviductal and penial); a large and conspicuous glandular structure possibly consisting of a small, whitish albumen gland proximally and a large, subtransparent uterine oviduct medium-distally; and a Simroth's penial accessory gland.

Most of these features match those of the rathouisiid systellommatophorans (cf. Climo, 1980; Stanisic, 1998; Barker, 2001). Others are absolute novelties and have never hitherto been reported in systellommatophorans: (1) genitalia of triaulic tritrematic type; and (2) large conspicuous glandular structure embedding the organs of the medial genitalia (Fig. 3B).

Triaulic tritrematic genitalia are not known in any other group of Pulmonata. Triaulic tritrematic genitalia are known in some groups of 'opistobranchs', such as the dorid nudibranchs (Ponder *et al.*, 2020).

MOLECULAR ANALYSIS

Sixteen new nucleotide sequences from the MUSE slug were deposited in GenBank: three for each – *COI*, 16S rDNA, 18S rDNA and ITS2, as well as four

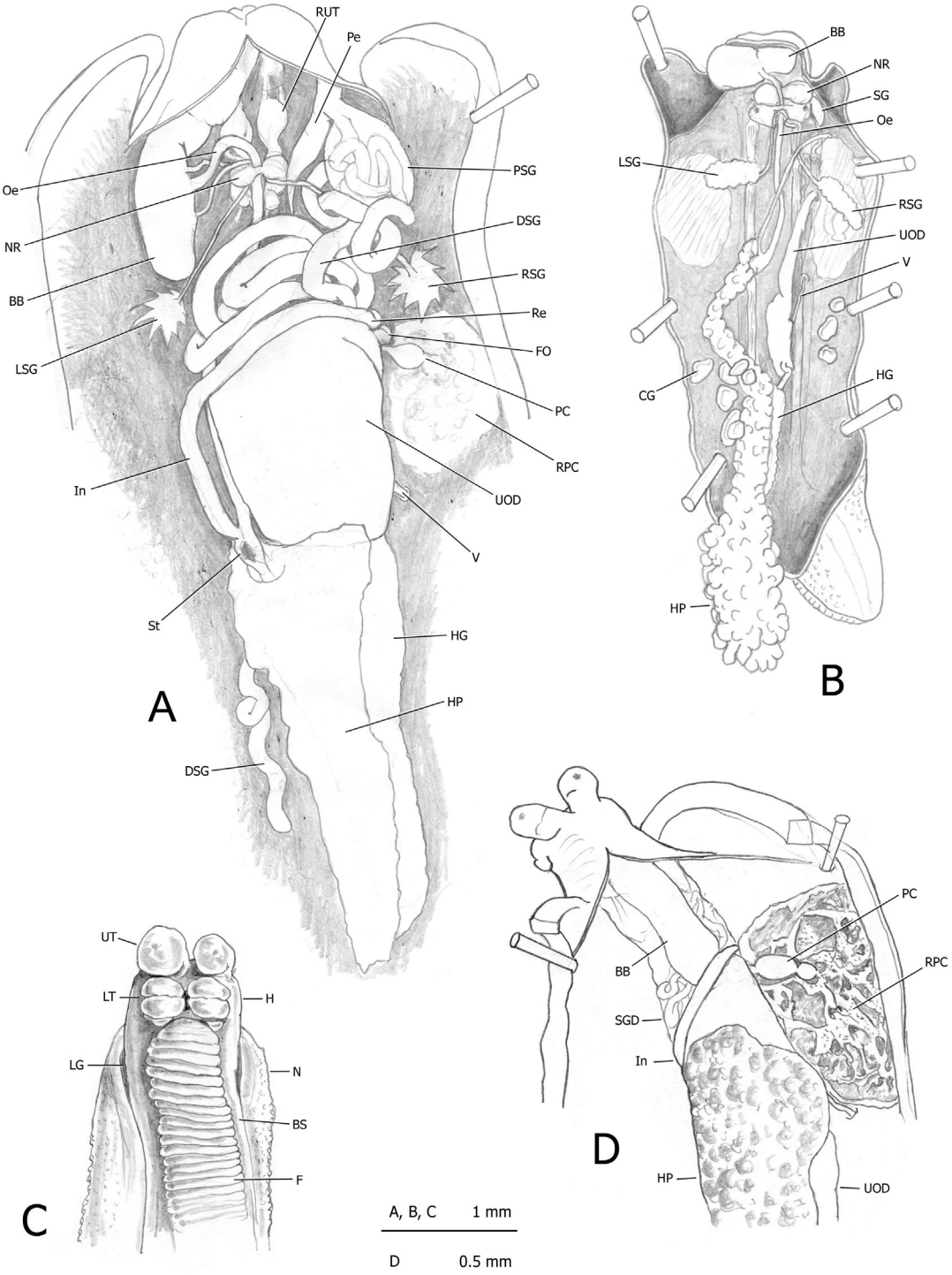


Figure 2. Body and internal anatomy of *Barkeriella museensis* gen. et sp. nov. from the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy, Debora Barbato leg. 4.5.2019 (FGC 51191): A, B, D, general view of internal anatomy of adult (A, D) and juvenile (B) specimens; C, head and anterior portion of body in ventral view. In A, salivary glands were partly cut away. See Morphological study for explanation of abbreviations.

for *H3* gene fragments (Table 3). GenBank resources are few for representatives of Rathouisiidae; only a few *COI* (Supporting Information, Table S1), 18S rDNA (Supporting Information, Table S2) and ITS2 (Supporting Information, Table S3) sequences have been deposited for species of the genera *Atopos*,

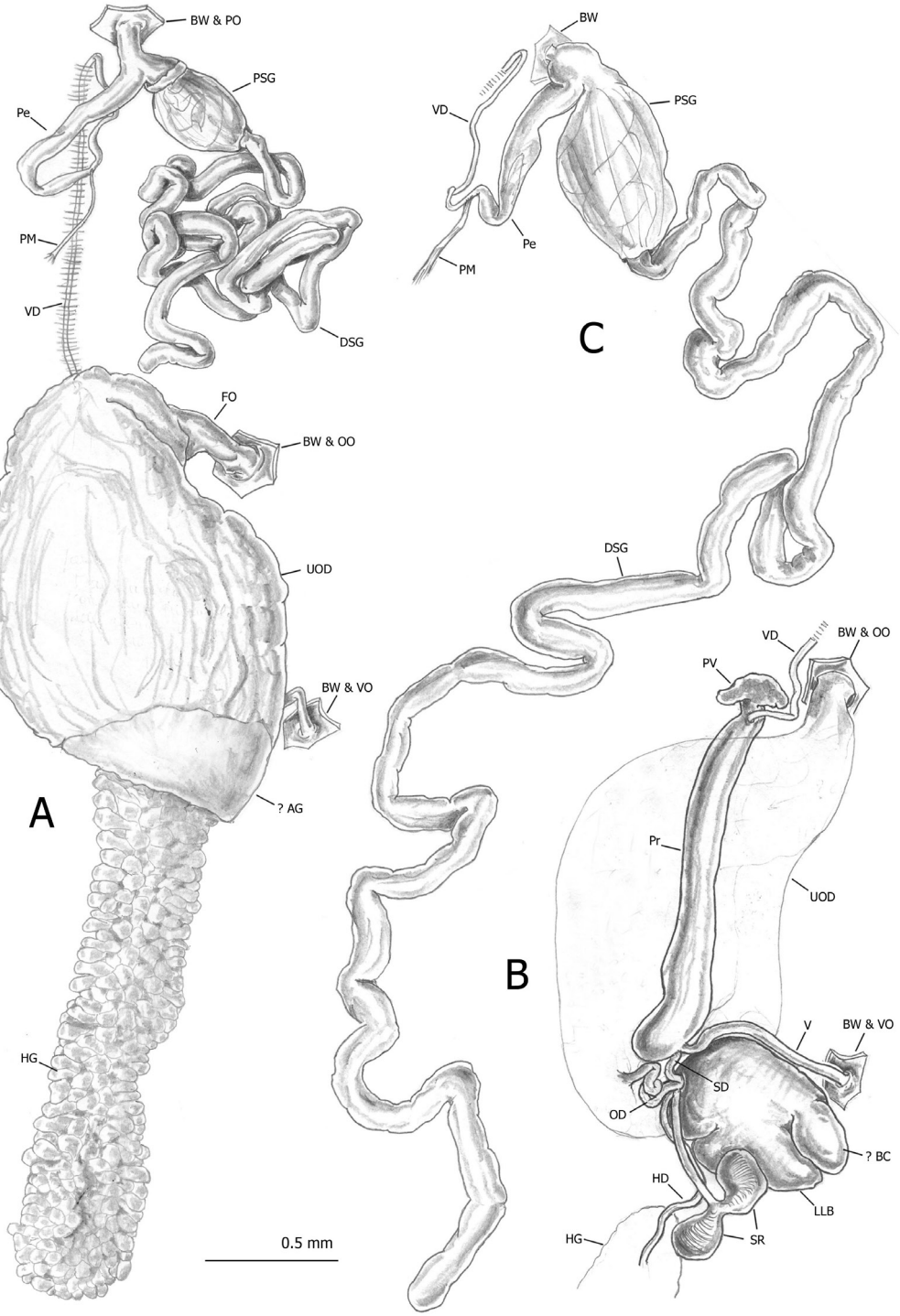


Figure 3. Genitalia of *Barkeriella museensis* gen. et sp. nov. from the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy, Debora Barbato leg. 4.5.2019 (FGC 51191): A, general view; B, distal female and proximal male genitalia; C, distal male genitalia. See Morphological study for explanation of abbreviations.

Granulilimax and unidentified one(s). No GenBank sequences for 16S rDNA (Supporting Information, Table S4) and *H3* (Supporting Information, Table S5) of Rathouisiidae were available. Moreover, there were no sequences for any species of the genus *Rathouisia* in GenBank. Therefore, 33 nucleotide sequences obtained as new records for *R. sinensis* were deposited in GenBank: seven each for *COI*, 16S rDNA, ITS2

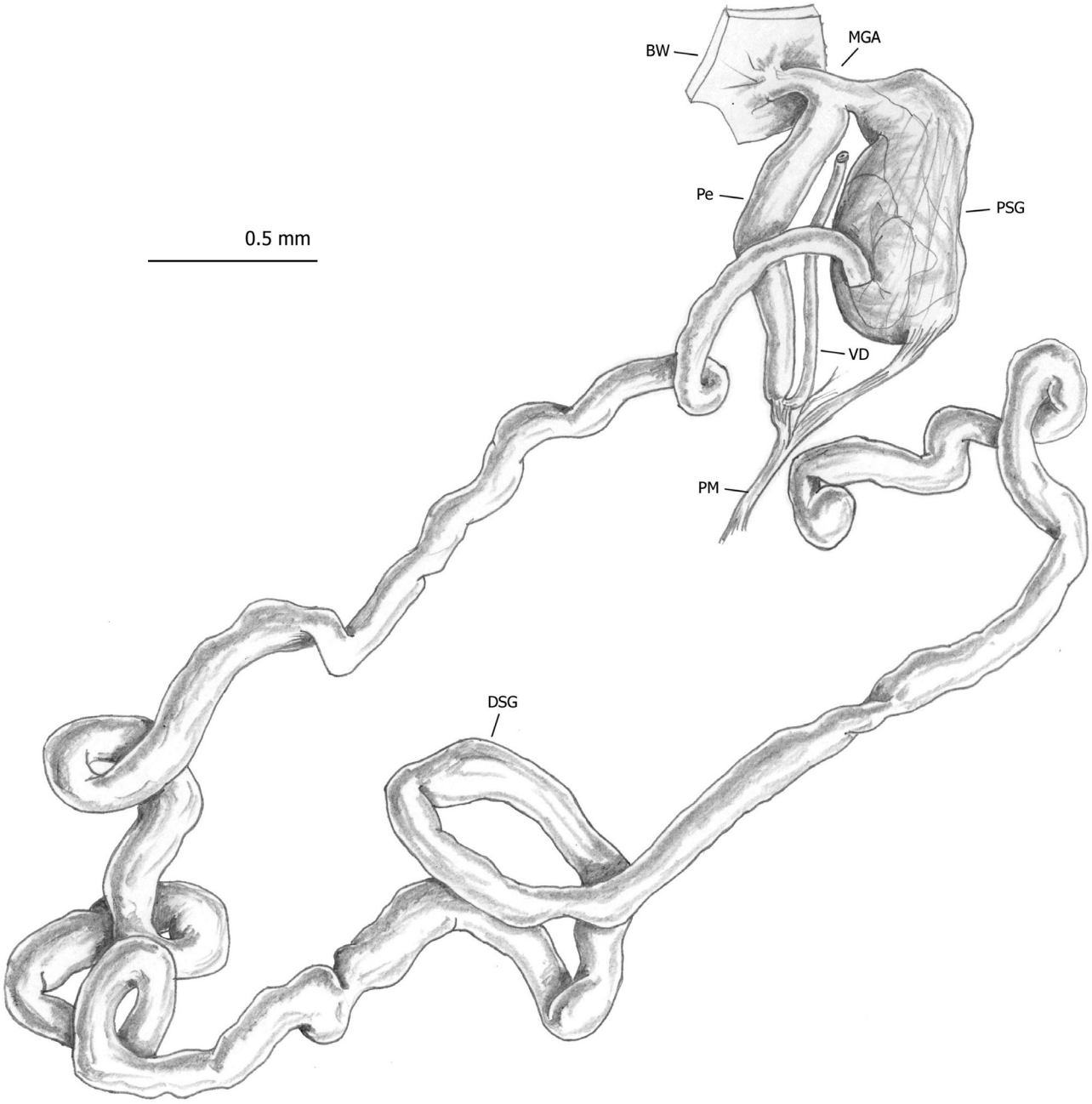


Figure 4. Distal male genitalia of *Barkeriella museensis* gen. et sp. nov. from the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy, Debora Barbato leg. 4.5.2019 (FGC 51191). See Morphological study for explanation of abbreviations.

and *H3*, as well as five for 18S rDNA gene fragments (Table 3). Two new sequences (one each for 16S rDNA and ITS2) of *Monacha pantanellii* were also deposited in GenBank (Table 3).

Among these new sequences from the MUSE slug, three *COI* (*COI* 1–*COI* 3), one 16S rDNA (16S 1), two 18S rDNA (18S 1 and 18S 2), one ITS2 (ITS2 1) and one *H3* (*H3* 1) haplotypes were identified (Table 3). Among new sequences of *R. sinensis*, six *COI* (*COI* 4–*COI* 9), three 16S rDNA (16S 2–16S 4), five 18S rDNA (18S 3–18S 7), three ITS2 (ITS2 2–ITS2 4) and one *H3* (*H3* 2) haplotypes were identified (Table 3).

For the remaining systellomatophoran families (Onchidiidae and Veronicellidae), more numerous sequences, especially of the *COI* gene, have been deposited in GenBank, which allowed us to compare the *COI* sequences from the MUSE slug and *R. sinensis* with other representatives of rathouisiids

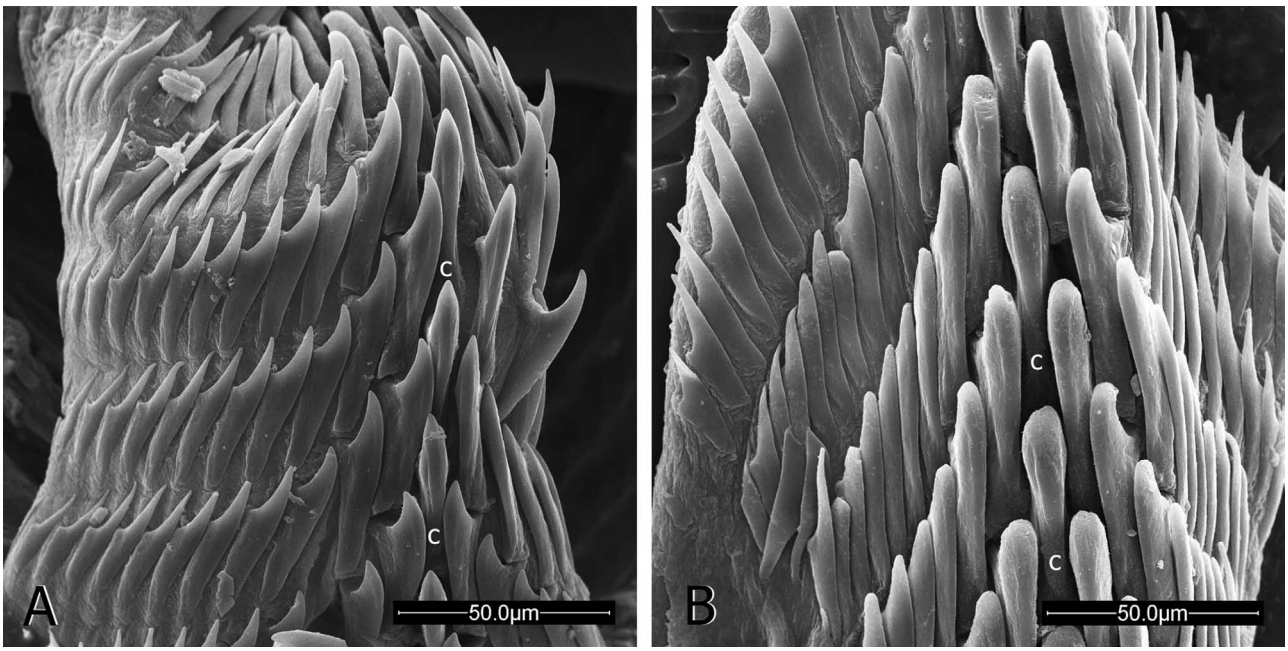


Figure 5. Radula of *Barkeriella museensis* gen. et sp. nov. from the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy, Debora Barbato leg. 4.5.2019 (FGC 51191): A, general view; B, detail of central sector [c, central teeth (A, B)].

and with *COI* sequences of onchidiids and veronicellids (Supporting Information, Figs S1–S4), as well as of the stylommatophoran slug family Philomycidae (Supporting Information, Figs S4, S5).

Sequences of the MUSE slug form a separate clade from clades of other rathouisiid genera, i.e. *Rathouisia*, *Granulilimax*, *Atopos*, as well as the unrecognized genus from the Ryukyu Islands. Sequences of rathouisiid genera clustered separately from sequences of veronicellid and onchidiid species in these analyses of single locus (*COI*) datasets (Supporting Information, Figs S1–S5). Moreover, in analyses of concatenated *COI* + 16S rDNA sequences, sequences of the MUSE slug and *R. sinensis* clustered together in the ML tree (Fig. 6), separately from sequences of veronicellid and onchidiid species.

Analysis of K2P distances (Supporting Information, Table S7) and ASAP species delimitation (Supporting Information, Fig. S6) of *COI* sequences confirm that the MUSE slug and *Rathouisia sinensis* belong to separate genera, different from other rathouisiid genera. However, no further conclusions are possible due to the small number of *COI* sequences of rathouisiid genera available.

Analyses of the single gene loci datasets gave similar results for the other genes (16S rDNA, 18S rDNA, *H3*; Supporting Information, Figs S7–S9, respectively) and show that the sequences from the MUSE slug and *R. sinensis* are different; in the case of 18S rDNA (Supporting Information, Fig. S8), they are also different from sequences of *Granulilimax* representatives. In each analysis, the sequences of the rathouisiid representatives were distinct from the sequences of two other systellommatophoran families (onchidiids and veronicellids). However, only in the case of ITS2, could the sequences from the MUSE slug and *R. sinensis* be compared with that of another rathouisiid species, i.e. *Atopos australis* (Heynemann, 1876) (Fig. 7).

Analysis of multilocus datasets of concatenated sequences *COI* + 16S rDNA + ITS2 + *H3* gene fragments (Fig. 8; Supporting Information, Table S8) and of concatenated sequences *COI* + 18S rDNA (Fig. 9) again showed that sequences of the MUSE slug and those of *R. sinensis* cluster separately. Moreover, in the analysis of concatenated *COI* + 18S rDNA, sequences of the MUSE slug cluster separately from those of *R. sinensis*, *G. fuscicornis* and the unidentified genus from Ryukyu (Fig. 9). Rathouisiid sequences cluster in a clade separate from clades of sequences from onchidiids and veronicellids (Figs 8, 9).

DISCUSSION

Rathouisiids are the least known group of systellommatophorans and this makes it difficult to establish a well-grounded systematic setting for the small slug from the MUSE tropical greenhouse. Currently, the rathouisiids are thought to include at least three genera: *Rathouisia* Heude, 1884 (type species *Vaginulus sinensis* Heude, 1882), *Atopos*

Table 3. New nucleotide sequences of mitochondrial and nuclear gene fragments obtained as results of this paper; ## GenBank accession number

Species	Voucher designation	COI		16S rDNA		18S rDNA		(5.8S rDNA) + ITS2 + (28S rDNA)		*H3*	
		Haplotype (no. specimens)	GenBank ##	Haplotype (no. specimens)	GenBank ##	Haplotype (no. specimens)	GenBank ##	Haplotype (no. specimens)	GenBank ##	Haplotype (no. specimens)	GenBank ##
MUSE slug	DCBC0050101IT01/ Rat1	-	-	16S 1 (3)	OM827013	-	-	-	-	*H3* 1 (4)	OM827043
	DCBC0050101IT01/ Rat2	*COI* 1 (1)	OM827003	-	-	18S 1 (2)	OM827024	ITS2 1 (3)	OM827032		OM827044
	DCBC0050101IT01/ Rat3	*COI* 2 (1)	OM827004		OM827014		OM827025		OM827033		OM827045
	DCBC0050101IT01/ Rat4	*COI* 3 (1)	OM827005		OM827015	18S 2 (1)	OM827026		OM827034		OM827046
Rathouisia sinensis	DCBC0050301CN01/ Kim1	*COI* 4 (1)	OM827006	16S 2 (1)	OM827016	-	-	ITS2 2 (1)	OM827035	*H3* 2 (7)	OM827047
	DCBC0050301CN02/ Nan1	*COI* 5 (1)	OM827007	16S 3 (1)	OM827017	18S 3 (1)	OM827027	ITS2 3 (1)	OM827036		OM827048
	DCBC0050301CN02/ Nan2	*COI* 6 (1)	OM827008	16S 4 (1)	OM827018	18S 4 (1)	OM827028	ITS2 4 (2)	OM827037		OM827049
	DCBC0050301CN02/ Nan3	*COI* 7 (1)	OM827009	16S 3 (1)	OM827019	18S 5 (1)	OM827029		OM827038		OM827050
	DCBC0050301CN02/ Nan4	*COI* 8 (1)	OM827010	16S 5 (1)	OM827020	18S 6 (1)	OM827030	ITS2 2 (2)	OM827039		OM827051
	DCBC0050301CN02/ Nan6	*COI* 9 (1)	OM827011	16S 4 (2)	OM827021	-	-		OM827040		OM827052
	DCBC0050301CN02/ Nan7	*COI* 6 (1)	OM827012		OM827022	18S 7 (1)	OM827031	ITS2 3 (1)	OM827041		OM827053
Monacha pantanellii	DCBC0020119IT01/ Fio8			16S 6 (1)	OM827023			ITS2 5 (1)	OM827042		

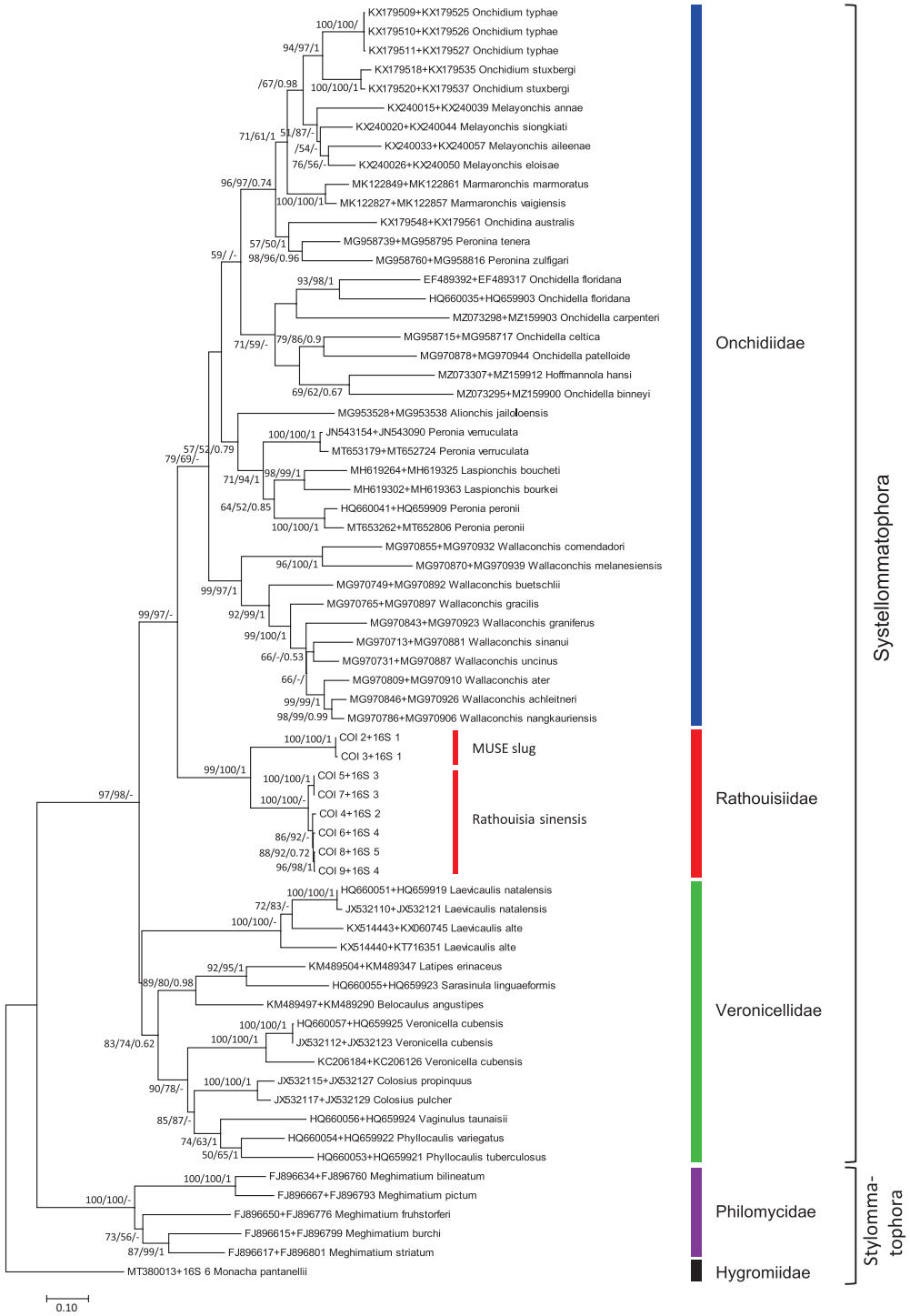


Figure 6. Maximum likelihood (ML) tree of concatenated *COI* + 16S rDNA haplotypes obtained from *Barkeriella museensis* gen. et sp. nov. and *Rathouisia sinensis* compared with sequences deposited in GenBank for representatives of the families Onchidiidae, Veronicellidae and Philomycidae (see Supporting Information, Tables S1, S4 for sequences obtained from GenBank for analysis). Concatenated sequences are listed with species names. They were 1028 positions in length (573 *COI* + 455 16S rDNA). Bootstrap support above 50% from ML (left) and NJ (middle) analysis, as well as posterior probabilities PP above 0.5 from Bayesian Inference analysis (right) are indicated next to the branches. Bootstrap analysis was run with 1000 replicates (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* sequences deposited in GenBank by us and by Pieńkowska *et al.* (2020) according to Table 3 and Supporting Information, Table S1, respectively.

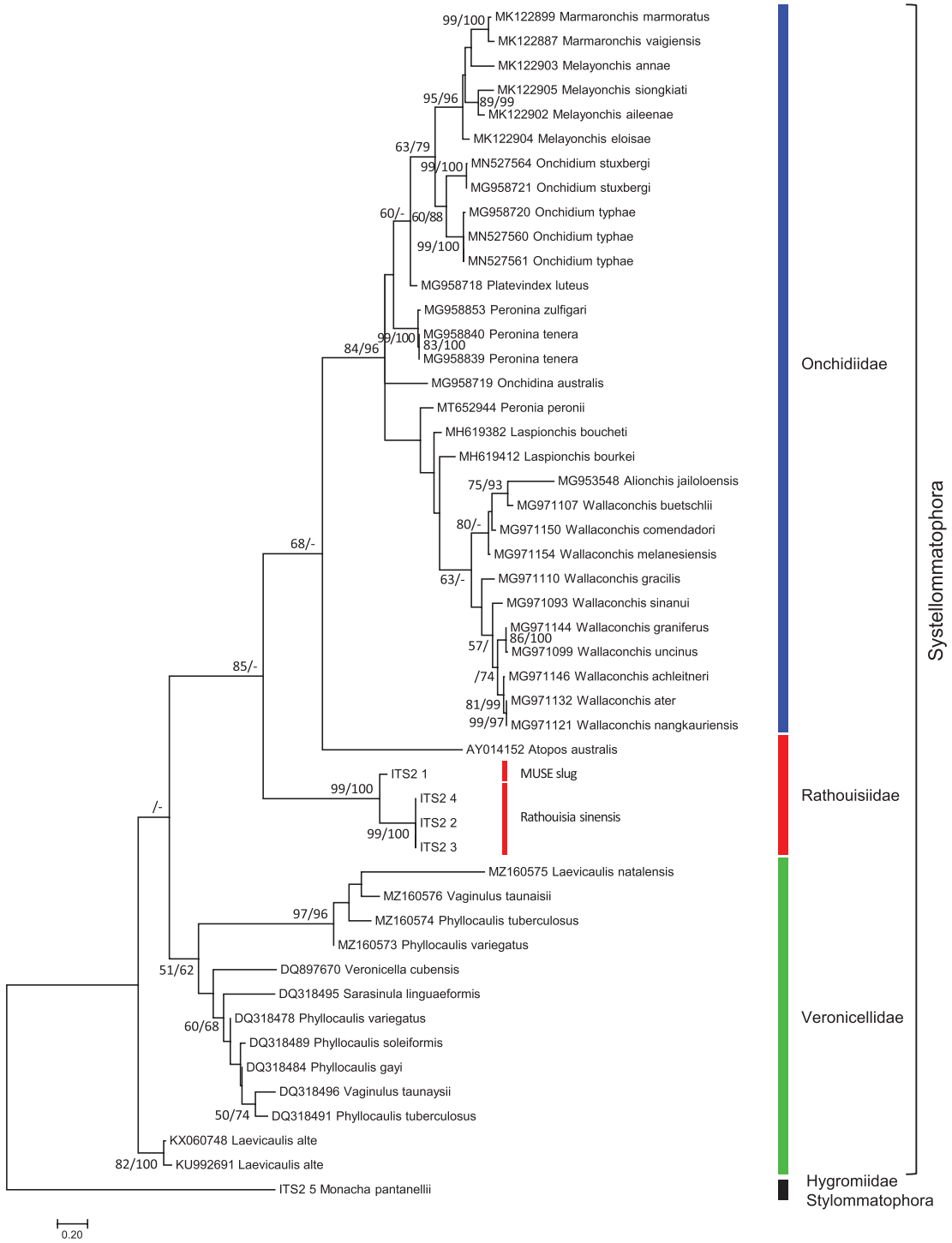


Figure 7. Maximum likelihood (ML) tree of ITS2 (flanked with 5.8S and 28S rDNA) sequences obtained from *Barkeriella museensis* gen. et sp. nov. (ITS2 1) and *Rathouisia sinensis* (ITS2 2–ITS2 4) compared with sequences deposited in GenBank for representatives of the other systellommatophoran families Onchidiidae and Veronicellidae (see Supporting Information, Table S3). ITS2 (flanked with 5.8S and 28S rDNA) sequences were 774 positions (54 5.8S rDNA, 680 ITS2, 40 28SrDNA) in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates by ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* sequence deposited in GenBank by us (see Table 3).

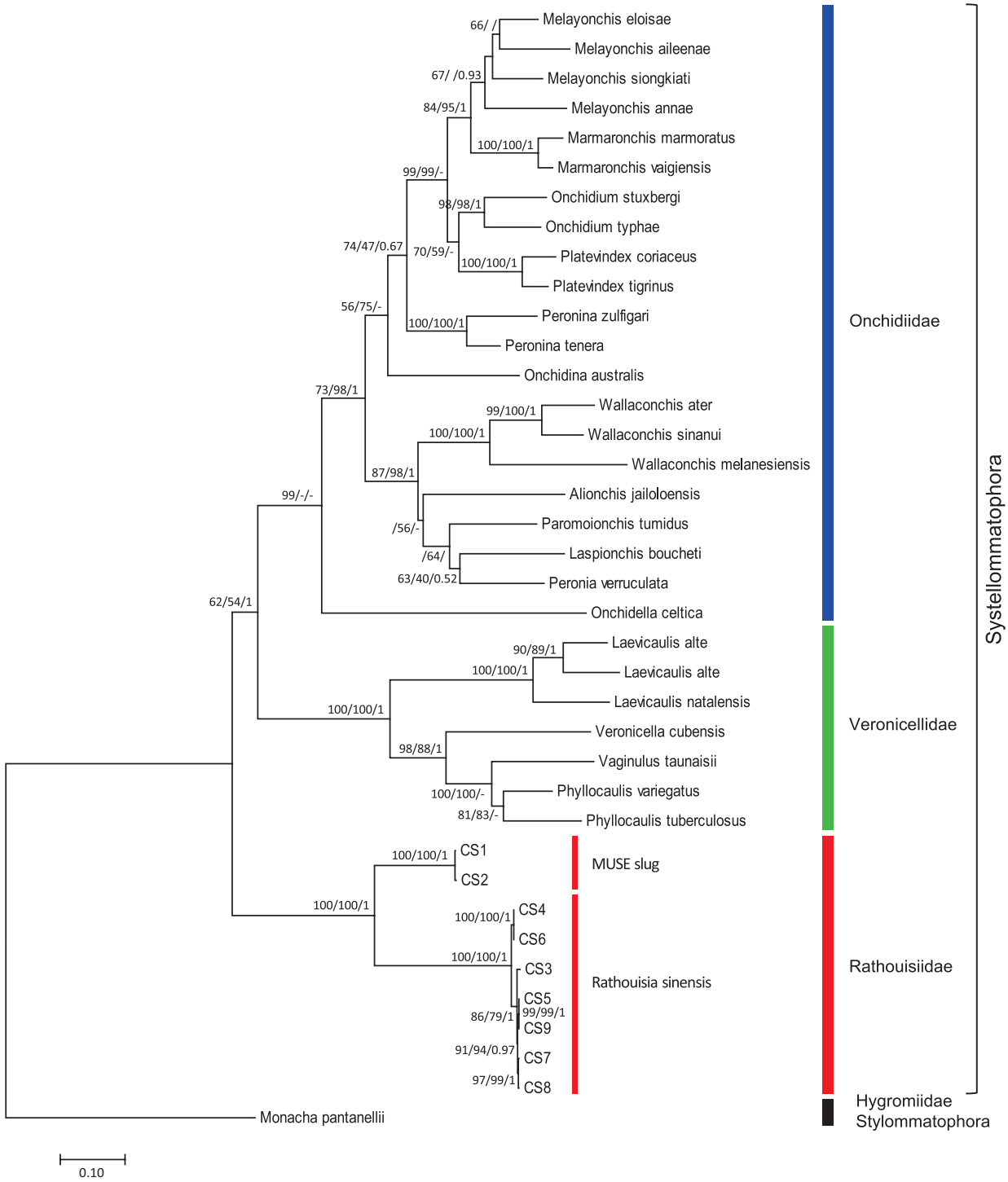


Figure 8. Maximum likelihood (ML) tree of concatenated *COI* + 16S rDNA + ITS2 (flanked with 5.8S and 28S rDNA) + *H3* sequences obtained from *Barkeriella museensis* gen. et sp. nov. and *Rathouisia sinensis* compared with sequences deposited in GenBank for representatives of the other systellommatophoran families Onchidiidae and Veronicellidae (see Supporting Information, Table S8 for concatenated sequence sets used in analysis). Concatenated sequences were 2122 positions in length (614 *COI* + 458 16S rDNA + 54 5.8S rDNA + 662 ITS2 + 40 28S rDNA + 294 *H3*). Bootstrap support above 50% from ML (left) and NJ (middle) analysis, as well as posterior probabilities above 0.5 PP, from Bayesian inference analysis (right) are indicated next to the branches. Bootstrap analysis was run with 1000 replicates (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* sequences (see Supporting Information, Table S8).

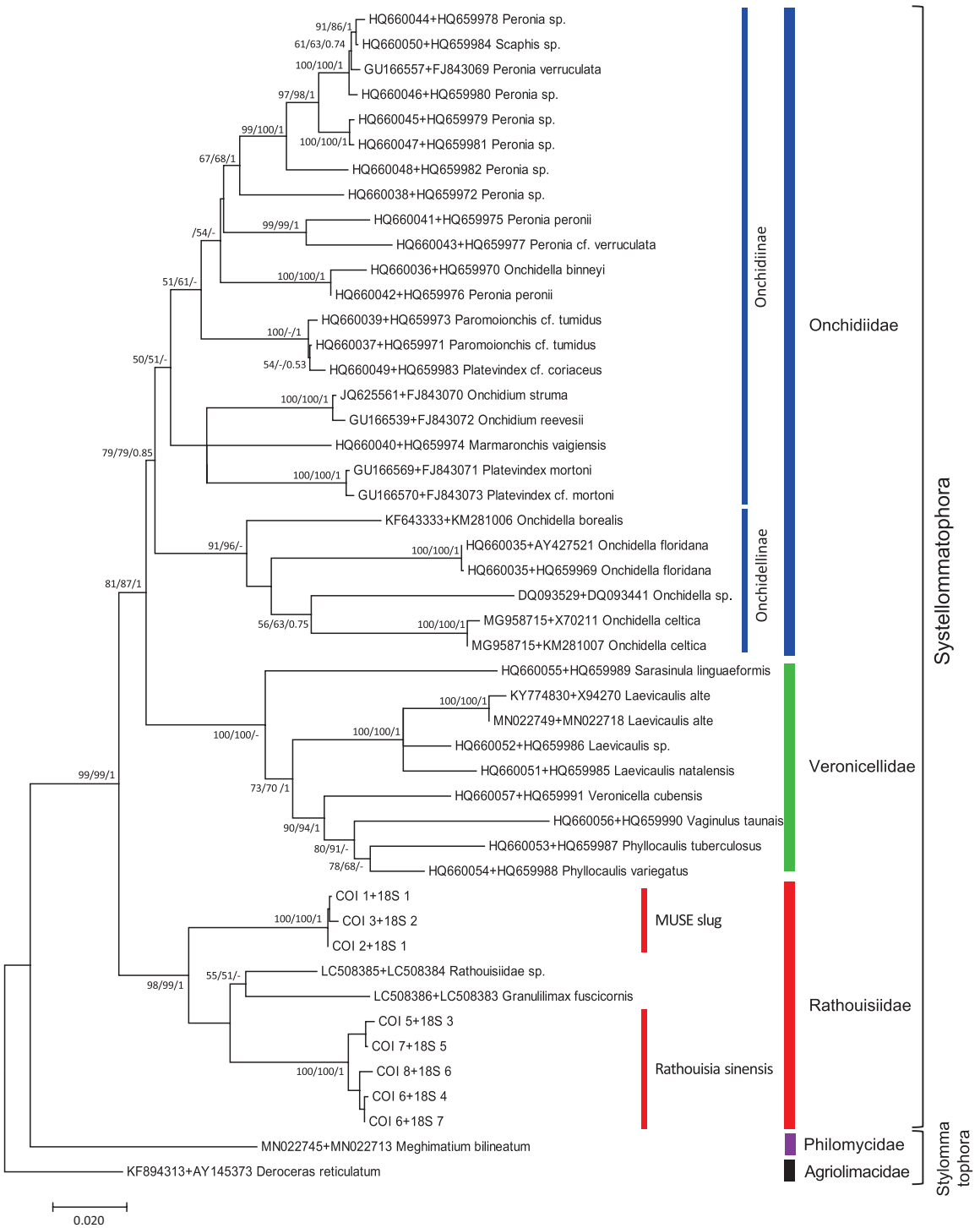
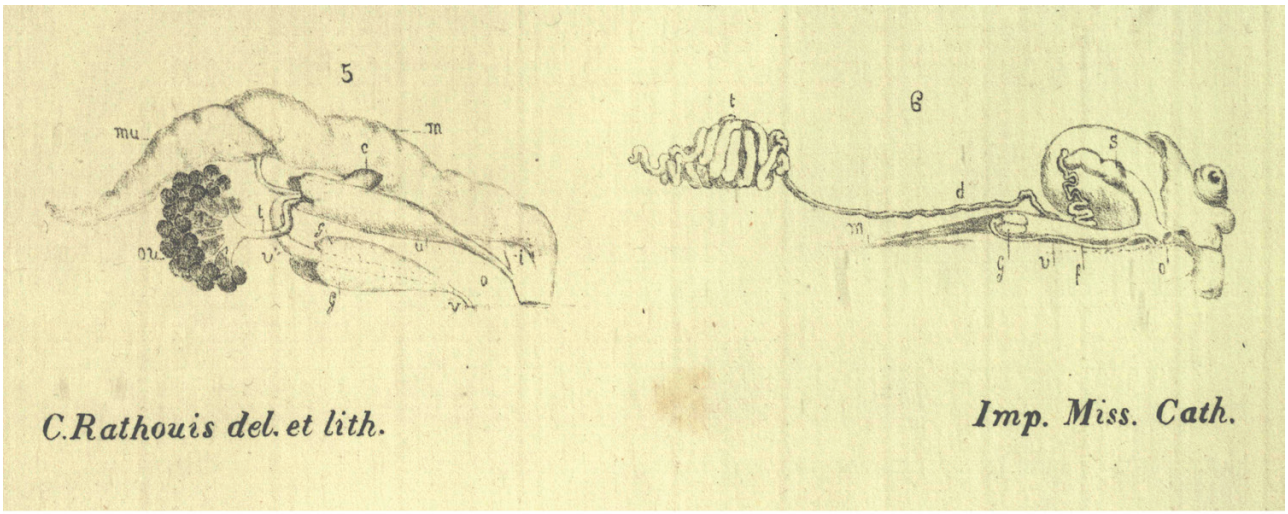


Figure 9. Maximum likelihood (ML) tree of concatenated *COI* + 18S rDNA sequences obtained from *Barkeriella museensis* gen. et sp. nov. and *Rathouisia sinensis* compared with sequences deposited in GenBank for representatives of the families Rathouisiidae, Onchidiidae and Veronicellidae (see Supporting Information, Tables S1, S2 for sequences obtained from GenBank for analysis). Concatenated sequences are listed with species names. They were 2184 positions in length (566 *COI* + 1618 16S rDNA). Bootstrap support above 50% from ML (left) and NJ (middle) analysis, as well as posterior probabilities PP above 0.5 (right) from Bayesian inference analysis are indicated next to the branches. Bootstrap analysis was run with 1000 replicates (Felsenstein, 1985). The tree was rooted with stylommatophoran *Deroceras reticulatum* KF894313 (*COI*) and AY145373 (18S rDNA) sequences deposited in GenBank by Rowson *et al.* (2014) and Passamaneck *et al.* (2004), respectively.

Simroth, 1891 (type species: *Atopos semperi* Simroth, 1891) and *Granulilimax* Minato, 1989 (type species: *Granulilimax fuscicornis* Minato, 1989) (Stanisic, 1998; Barker, 2001; Barker & Efford, 2004; Minato, 2015). Other taxa of the genus-groups, e.g. *Prisma* Simroth, 1891 (type species: ?*Veronicella prismatica* Tapparone Canefri, 1883), *Padangia* Babor, 1900 (type species: *Atopos schildii* Babor, 1900) and *Parapodangia* Ghosh, 1915 (type species: *Atopos gravelyi* Ghosh, 1915), are currently regarded as junior synonyms or subgenera of *Atopos* (Hoffmann, 1926; Laidlaw, 1940; De Wilde, 1984; Stanisic *et al.*, 2010). The genus *Atopos* was assigned to its own family Atopidae by Ihering (1892). Incidentally, this family name is not included in the Nomenclator of the gastropod family taxa by Bouchet *et al.* (2017).

Species from south-eastern China are assigned to *Rathouisia* (Collinge, 1902a; Heynemann, 1906; Hoffmann, 1926). MolluscaBase (2021) mentions four species: *R. sinensis* (Heude, 1882), *R. leonina* Heude, 1885, *R. pantherina* Heude, 1890 and *R. tigrina* Heude, 1885, regarding the latter three as taxa inquirenda. However, *R. leonina* is an unnecessary replacement name for *Vaginulus sinensis*, which Heude (1885) considered a homonym of *Vaginulus chinensis* Möllendorff, 1881 (Collinge, 1902a; Hoffmann, 1926). According to Heude (1890: 133), the two species (*R. pantherina* and *R. tigrina*) might correspond to only one taxon. The anatomy of *R. sinensis* has only been studied by Rathouis (1885) but, as already noticed by Sarasin & Sarasin (1899) and Collinge (1902a), the lithographic impression of his plate is so faint that many of the details are unclear (Fig. 10), and as stressed by Laidlaw (1940), his study also includes some misinterpretations of genital structures. Our re-examination of the gross anatomy of *R. sinensis* (Figs 11, 12) confirmed that the study by Rathouis (1885) illustrated the male distal genitalia correctly, much less the proximal genitalia and the female distal genitalia (see, for example, the confluence of prostate, oviduct and rectum) (Fig. 13). However, the most critical issue of his report is misinterpretation of most of the structures. Our re-examination shows that *R. sinensis* has triaulic tritrematic genitalia with complicated relationships between the proximal parts. Kimura *et al.* (2020a, b) suggested the existence of another undescribed species (a member of *Rathouisia* or an undescribed genus) from South Korea. Our molecular data on *COI* sequences (Supporting Information, Figs S1–S6)



F. 5. Appareil génital femelle.
v. La vulve cachée dans un sil'on du pied à un millimètre environ en arrière et en dedans de l'anus.
v'. Le vagin, gg. les glandes lubréfiantes (?) du vagin, rudimentaires avant l'état adulte.
ou. Les ovules, t. la trompe, u. l'utérus, ou mieux le réservoir des ovules ; o. l'oviducte.
c. Le réservoir, plus on moins pédicellé, des spermatozoïdes, ou poche copulatrice.
m. La glande muquense, mu. la glande muqueuse utérine; les cellules mucipares y sont de moitié moins grandes que celles de la glande m. dont elle est séparée par un septum ; avant l'état adulte elle est rudimentaire et elle s'atrophie après la ponte, son rôle serait principalement de fournir aux œufs leur albumine.
i. Le gros intestin.
n. b. Le vagin, l'utérus et la trompe sont tapissés d'un épithélium cylindrique à cellules longues et à cils vibratiles également très longs.

F. 6. Appareil génital mâle.
o. L'orifice, f. le flagellum fixé près de l'orifice.
G. Le gland avec sa gouttière pour le passage du sperme,
v. La verge, s. le réservoir séminal.
d. Le canal déférent, t. le testicule, m. le faisceau rétracteur émané du muscle latéral rétracteur des organes céphaliques etc.

Figure 10. Illustrations of the genitalia of *Rathouisia sinensis* and their legend published by Rathouis (1885).

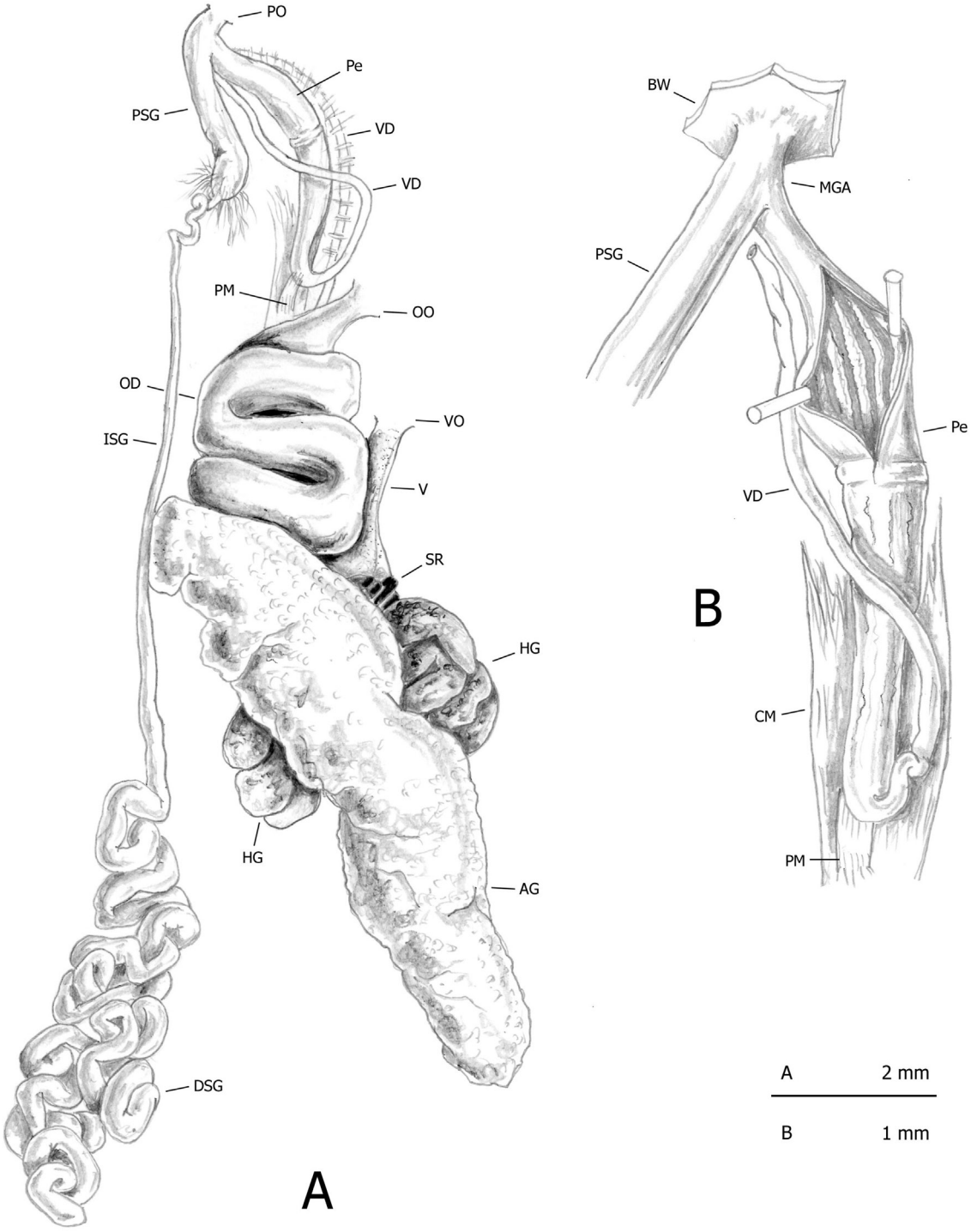


Figure 11. Genitalia of *Rathouisia sinensis* from Nandaheyuan, Nanjing, Jiangsu Province, China, M. Wu leg. 21.9.2021 (FGC 51209): A, general view; B, distal male genitalia. See Morphological study for explanation of abbreviations.

shows that the sequence LC598438 obtained by Kimura *et al.* (2020b) from a rathouisiid specimen from South Korea clusters with sequences obtained by us from *R. sinensis*, which strongly confirms the suggestion that they are conspecific.

About 30 named species from South-East Asia to New Guinea and northern Australia are attributed to *Atopos* (lists of *Atopos* species are given by: Heynemann, 1906: 37–38; Collinge, 1908: 119; Hoffmann, 1926: 26–27; another species, overlooked

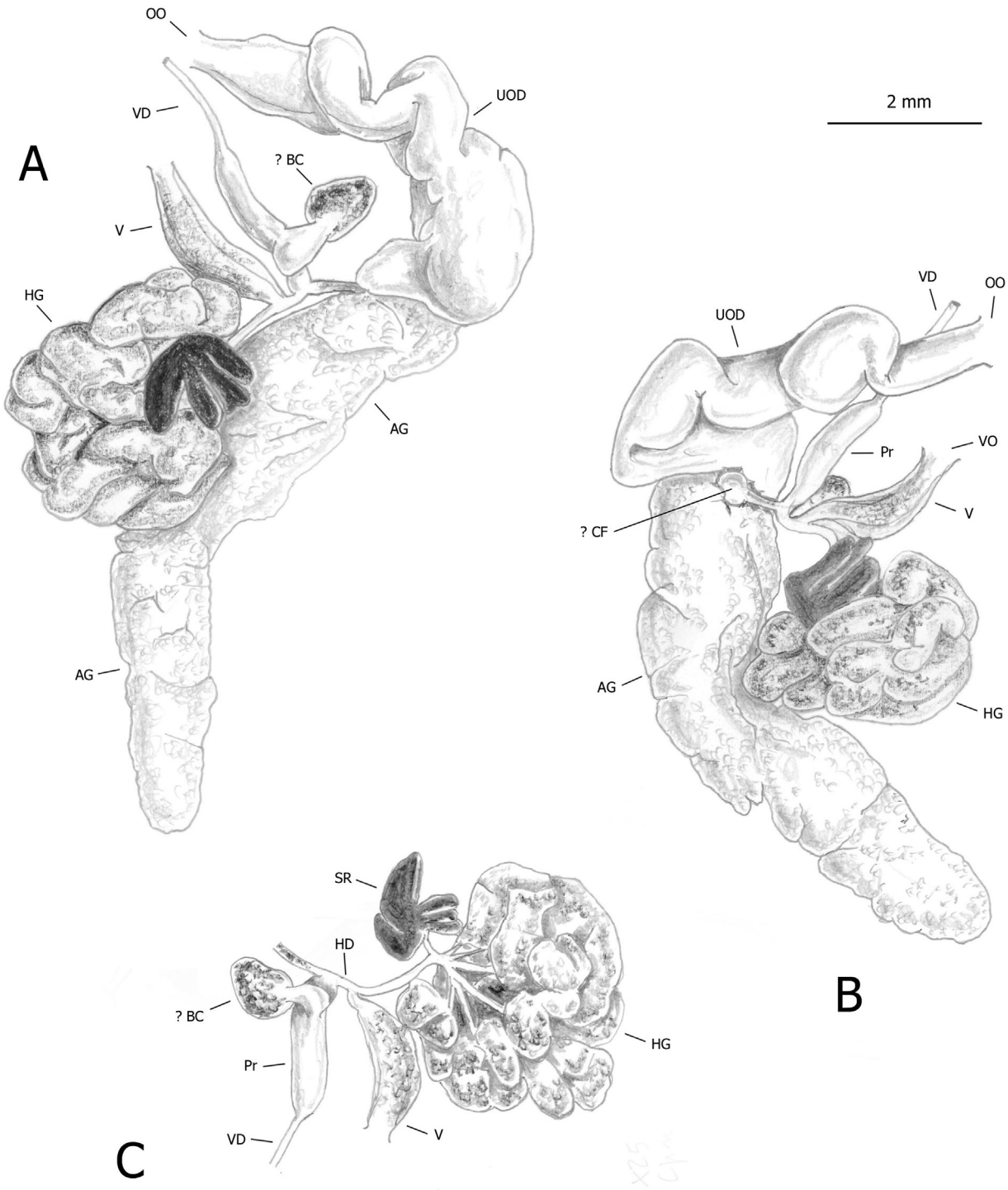


Figure 12. Genitalia of *Rathouisia sinensis* from Nandaheyuan, Nanjing, Jiangsu Province, China, M. Wu leg. 21.9.2021 (FGC 51209): A, gonad, male proximal genitalia and female distal genitalia; B, another view of gonad, male proximal genitalia and female distal genitalia; C, gonad, vagina and male proximal genitalia. See Morphological study for explanation of abbreviations.

in these lists, needs to be added: *Atopos gravelyi* Ghosh, 1915). MolluscaBase (2021) reports 23 species (although one, *A. heynemanni* (Simroth, 1891), as taxon inquirendum), but as stressed by De Wilde (1984), Wiktor (2003), Schilthuizen & Liew (2008) and Tan & Chan (2009), a modern revision of *Atopos* speciography

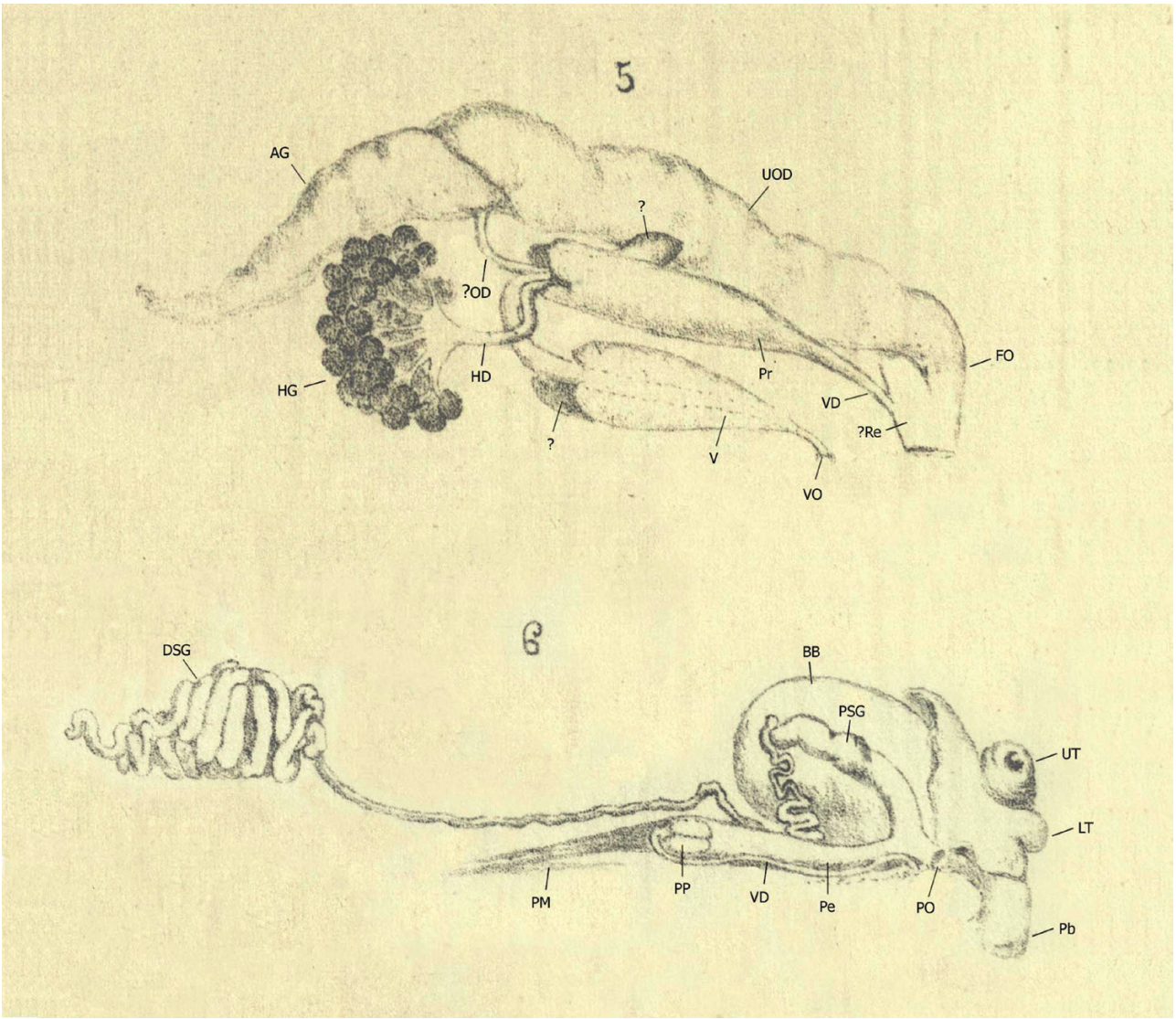


Figure 13. Illustrations of the genitalia of *Rathouisia sinensis* enlarged and re-interpreted according to our re-examination. See Morphological study for explanation of abbreviations.

is still lacking. Despite many detailed studies on their anatomy, most earlier contributions did not consider gross genital morphology, often dealing more with the histological structure of the organs (Simroth, 1891; Sarasin & Sarasin, 1899; Collinge, 1902a, 1903; Ghosh, 1912, 1913, 1914, 1915; Odhner, 1917; Laidlaw, 1940; De Wilde, 1984; Wiktor, 2003). Moreover, as pointed out by Laidlaw (1940), the separation of *Atopos* from *Rathouisia* is not at all clear and the paper by De Wilde (1984) remained the best contribution on the gross anatomy of an *Atopos* species.

The genus *Granulilimax* was originally described by Minato (1989) in the family Philomycidae, originally with only one species included from Japan. Subsequently, this slug was moved to the rathouisiids on the basis of anatomical re-examination by Tada & Yano (2010). Indeed, the authors demonstrated that *Granulilimax* showed rathouisiid features, such as the position of the anus, Simroth's gland and the absence of a jaw. It was also shown that Minato's (1989) anatomical description is incomplete and at least partly inaccurate (Kimura *et al.*, 2020a). This attribution was further confirmed by a new anatomical study by Hayase *et al.* (2018: fig. 3 1e–1i) and by a phylogenetic analysis based on mitochondrial and nuclear genes by Kimura *et al.* (2020a, b). Our molecular results also confirm attribution of *Granulilimax* to the family Rathouisiidae.

Kimura (2020) and Kimura *et al.* (2020a, b) suggested that other undescribed rathouisiid species, unassigned to genus, occur (or occurs) on Okinawa and other Ryukyu Islands. Our analysis of *COI* sequences (Supporting Information, Figs S1–S6) suggests that

they may represent a new rathouisiid genus. However, the lack of GenBank data on the sequences of nuclear genes does not permit this suggestion to be verified.

The MUSE slug and *R. sinensis* share the same triaulic tritrematic genital structure. Nevertheless, the MUSE slug differs in many other features. The multiacinous gonad is elongated and unpigmented (gonad consisting of a compact mass of black pigmented acini fused to one another in *R. sinensis*). The hermaphrodite duct ends inside a large lobed body embedded in the posterior side of the glandular structure, possibly consisting of the albumen gland and the uterine oviduct (the hermaphrodite duct first joins the duct ending in the seminal receptacles, then the vagina and then the prostate, ending in a small body – a sort of carrefour? – between the albumen gland and the uterine oviduct in *R. sinensis*). Two globular seminal receptacles with black pigmentation are positioned side by side, fused at the base (a group of black, variably long, sac-like receptacles are fused at base in *R. sinensis*). Three different ducts arise inside the large lobed body: one ends in the two seminal receptacles; one enters the posterior side of the glandular structure; and one enters the prostate (absent or possibly represented by the small body situated between the albumen gland and the oviduct in *R. sinensis*). The albumen gland is apparently fused to the uterine oviduct, forming a thick structure of translucent, glandular tissue (albumen gland long, easily distinguished from the twisted oviduct in *R. sinensis*). The vagina is long and slender and starts from the base of the prostate side by side with the end of the sperm duct (vagina short and wide, arising from hermaphrodite duct in *R. sinensis*). The bursa copulatrix is possibly represented by a lateral lobe of the large lobed body (bursa copulatrix possibly represented by a small globular structure joined to the proximal portion of the prostate in *R. sinensis*). The prostate is long, its distal end ending in a sort of vesicle and giving rise laterally to the vas deferens (prostate rather short, with a pedunculated globular vesicle, possibly representing the bursa copulatrix, arising at its base, and with the vas deferens arising at its distal end in *R. sinensis*). The penis is long and slender with an internal penial papilla at its base (penis rather long, divided into proximal and distal parts by a sort of ring, and without a penial papilla in *R. sinensis*). Simroth's gland is tubular, with its twisted proximal portion wrapped in a sort of oval sheath, thence forming a twisted bunch, and extending back along the left side of the glandular structure to end level with the gonad and the digestive gland (tubular, straight at its beginning, then extending back along the left side of the oviduct and ending as a twisted bunch on the left side of the albumen gland in *R. sinensis*).

Although it is not at all clear whether *Atopos* rathouisiids correspond to one or more taxa of the genus group, the MUSE slug differs (Figs 2–5) from the best-studied species of this group [i.e. *A. australis* and *A. smithi* (Collinge, 1902b)] in many characters, e.g. central tooth of radula present (central tooth of radula absent or vestigial in *Atopos*); genitalia of triaulic tritrematic type (genitalia of diaulic ditrematic type in *Atopos*); large conspicuous glandular structure possibly consisting of the albumen gland and the uterine oviduct embedding the organs of the medial section of the genitalia (albumen gland and uterine oviduct distinct in *Atopos*); and prostate not diverticle-like in the MUSE slug (prostate diverticle-like in *Atopos*) (see: Odhner, 1917; De Wilde, 1984; Wiktor, 2003).

It is more difficult to differentiate the MUSE slug from *Granulilimax*. The latter is only known from the description by Minato (1989) and the re-examination by Hayase *et al.* (2018: fig. 3 1e–1i). As stated by Tada & Yano (2010), Minato's (1989) description of the genitalia is not realistic. The new anatomical study by Hayase *et al.* (2018) only shows photos of the genitalia with many unclear details and so it cannot be a basis for a well-grounded morphological differentiation.

The MUSE slug differs (Figs 2–5) from *Granulilimax* by virtue of genitalia of the triaulic tritrematic type (*Granulilimax* has genitalia of the diaulic ditrematic type); large, conspicuous glandular structure, possibly consisting of the albumen gland and the uterine oviduct embedding the organs of the medial genitalia (albumen gland and uterine oviduct distinct in *Granulilimax*); and Simroth's gland with its twisted proximal portion wrapped by a sort of oval sheath, then digit-like for its whole length (Simroth's gland with its proximal portion enlarged, apparently bilobed, then with thin intermediate portion and a final digit-like portion in *Granulilimax*) (see: Hayase *et al.*, 2018: fig. 3 1e–1i).

Molecular identification of the MUSE slug was not possible, because there was no sequence deposited in GenBank identical or similar to those obtained for this species by our research (Table 3). However further molecular analyses of five different gene fragments, based on single locus (Supporting Information, Figs S1–S9) and multilocus datasets (Figs 6–9), clearly suggest that the MUSE slug belongs to Rathouisiidae, but cannot be assigned to any genus of this family. The sequences obtained from the MUSE slug cluster together in these analyses, but are always separate from sequences of the genera *Rathouisia*, *Granulilimax* and *Atopos*, as well as from representatives of the unnamed genus found in the Ryukyu Islands.

In conclusion, morphological and molecular analyses showed that the MUSE slug is a hitherto undescribed lineage in the rathouisiid systellommatophorans for which the name *Barkeriella museensis* is proposed. The origin of this species and its natural range remain obscure and require further research. Above all, a better

understanding of the family Rathouisiidae is needed, along with a revision of species assigned to the genera *Atopos*, *Barkeriella*, *Granulilimax* and *Rathouisia*, and possibly others, by means of integrative taxonomy (Dayrat, 2005), combining classic studies based on analysis of anatomical structures, ecology and phylogeography with molecular methods.

SYSTEMATICS

ORDER SYSTELLOMMATOPHORA PILSBRY, 1948

SUPERFAMILY VERONICELLOIDEA GRAY, 1840

FAMILY RATHOUISIIDAE HEUDE, 1885

BARKERIELLA GEN. NOV.

Zoobank registration: urn:lsid:zoobank:org:act:AC660310-BCA7-4EA4-B2C2-2194AFA7CE9A.

Type species: Barkeriella museensis sp. nov.

Etymology: The new genus is named after Gary Barker from Hamilton, New Zealand, a leading land gastropod biologist and author of *The biology of terrestrial molluscs* and *Natural enemies of terrestrial molluscs* and also the author of a major review on systellommatophoran gastropods (Barker, 2001).

Diagnosis: A genus of the rathouisiids characterized by mantle finely granulated with no sharp delimitation between the dorsal and lateroventral sector; radula consisting of many rows of unicuspidate hook-like teeth; central tooth present; genitalia of triaulic (three ducts for gametes: one vaginal for receiving allosperm from partner, one oviductal for conveying eggs to the outside, one penial for carrying autosperm to penis) and tritrematic type (three genital openings: vaginal, oviductal and penial); large, conspicuous glandular structure, possibly consisting of a small, whitish albumen gland proximally and a large subtransparent uterine oviduct medium-distally; single Simroth's penial accessory gland.

BARKERIELLA MUSEENSIS SP. NOV.

Zoobank registration: urn:lsid:zoobank:org:act:C8C98093-52BB-4E3B-AA82-7268F7FFAD97.

Type locality: Tropical greenhouse of the Science Museum (MUSE) of Trento, northern Italy.

Type material: Holotype: D. Barbato leg. 4.5.2019 (one ad. dissected, FCG 51208) and 31 paratypes: D. Barbato & G. Bolzonella leg. 4.1.2019 (two subad. sps. dissected, FGC 51205), D. Barbato & G. Bolzonella leg. 10.2.2019 (three subad. sps. dissected + four juv. sps. dissected, FGC 51206; one ad. + one juv. sps. NHC/MOLL-2022-001 – 002), D. Miserocchi leg. 2.3.2019 (six sps., FGC 51168), D. Barbato leg. 4.5.2019 (five juv. sps. dissected + seven juv. sps. 'exploded' + two ad. sps. dissected, FGC 51191).

Etymology: The new species was first discovered in the tropical greenhouse of the Science Museum (MUSE) of Trento, Italy. The species is named for the type locality.

Diagnosis: See genus diagnosis.

Description: Body (Figs 1, 2C): Crawling specimens, small and slender (length no more than 20 mm; width no more than 3 mm). Notum roundish, whitish in colour with a wide, greyish-violet band on each side, separated dorsally by a whitish line coinciding with a trace of keel; notum covered in coarse granulations with a thick mucus layer in irritated specimens; notum extending laterally to completely cover body sides as far as the pedal groove (no sharp delimitation between dorsal and lateroventral sectors of mantle covering); pedal groove separating notum and foot along length of body. In crawling specimens, head protrudes from notum, but in resting or irritated specimens is completely covered by the notum, and bears two white or pinkish upper tentacles and two apically bilobed lower tentacles. Foot long and rather slender with fine transverse ridges (soleolae).

Nervous system (Fig. 2A, B): not studied in detail; central system forms a concentrated ring, consisting dorsally of many ganglia (cerebral, buccal and visceropleural), more or less fused to one another and ventrally of a pair of pedal ganglia.

Renal–pulmonary complex (Fig. 2A, B, D): Located anterodorsally with atrophied pulmonary chamber; pericardium disposed obliquely and heart consisting of a single auricle and ventricle.

Digestive tract (Fig. 2A, B, D): consisting of buccal bulb with radula, oesophagus, two salivary glands, stomach and intestine. Jaw absent. Buccal bulb long and wide, occupying the internal part of head and neck and consisting anteriorly of the sac of the protrusible proboscis and posteriorly of the radular sac. Oesophagus long and slender, originating about halfway along length of buccal bulb, passing inside the nervous ring, reversing along the spermoviduct and entering the stomach anteriorly. Two large salivary glands discharge via two fine salivary ducts that pass inside the nervous ring into the buccal bulb. The stomach is small, receives a short, thin tube from the digestive gland posteriorly and continues anteriorly

with the intestine. Intestine long and slender, reversing and running first along the left, then along the anterior side of the uterine oviduct, and opening in the right pedal groove in the anterior one-fifth to one-quarter of the body, just anterior to the oviductal opening. Long, single-lobed digestive gland (hepatopancreas) in close contact with upper side of gonad and occupying dorsal part of body cavity as far as tip of tail.

Radula (Fig. 5A, B): Consisting of a sequence of many V-shaped rows of hook-like unicuspidate teeth, each with about 27 teeth, according to the formula 13L + 1C + 13L. The large central tooth and the first lateral teeth show a worn tip in most cases. The lateromarginal teeth, progressively reduced in dimension, have pointed tips.

Genitalia (Figs 2A, B, 3A–C, 4): Of triaulic tritrematic type (three ducts for gametes: one vaginal for receiving allosperm from partner, one oviductal for conveying eggs outside, one penial for carrying autosperm to penis; three genital apertures: vaginal, oviductal and penial).

Hermaphrodite gonad long, ventral with respect to the similarly long digestive gland; anterior side of gonad adheres to the posterior side of a large conspicuous glandular structure. This organ possibly consists of a small, whitish albumen gland proximally and a large, subtrasparent, gelatinous uterine oviduct distally. The glandular structure embraces a large, lobed body proximally and the prostate ventrally and continues with the short wide free oviduct that opens into the pedal groove on the right side of the body, about one-fifth to one-quarter of the way along the body, just posterior to the anus.

The large lobed body is a roundish non-glandular organ, in which a long, fine hermaphrodite duct ends and three other ducts arise: (1) a fine, long, straight duct ending into two seminal receptacles (i.e. where the allosperm received from a partner is stored, until it can fertilize eggs); (2) a fine, short, twisted duct ending on the posterior side of the glandular structure interpreted as an oviduct (to bring fertilized eggs into the uterine oviduct); and (3) a fine, short duct that enters the prostate and is interpreted as a sperm duct/seminal duct (to convey autosperm to the prostate and allosperm to the duct of the seminal receptacles?). An oval or roundish, variably pedunculate lobe, possibly a bursa copulatrix, is disposed on the other side of the large lobed body (i.e. the side opposite that on which the three ducts arise). The large lobed body possibly includes a fertilization chamber.

The prostate is a long tubular structure, adhering ventrally to the glandular structure and elongated anteriorly; proximally, near the point where the sperm duct enters, it receives another duct, interpreted as the vagina; distally it is connected to a sort of variably large vesicle, near the base of which the vas deferens arises.

The vagina is a long tubular duct that starts from the proximal portion of the prostate, extends along the anterior side of the large lobed body towards the right side of the body and ends with the vaginal opening in the pedal groove about one-third of the way along the body from the head.

The distal male genitalia include the vas deferens, the penis and Simroth's gland. The vas deferens consists of two portions: the initial (proximal) longer portion running inside the body wall, coinciding with the anterior part of the foot; the final (distal) shorter portion that re-enters the body cavity and finishes at the proximal end of the penis where the penial retractor also joins. The penis is a short tubular organ that ends in the male genital atrium side by side with Simroth's gland. Its proximal portion contains a pointed penial papilla. The genital atrium opens on the anterior right side of the head. Simroth's gland is a long tubular structure, pinkish in colour in adult specimens and consisting of two parts: the shorter initial portion (facing the distal penis) is twisted and enveloped in a transparent sheath, oval in shape; the longer final portion, which is free of the sheath, forms a bunch by twisting irregularly on itself, then extends backwards along the glandular structure to end level with the digestive gland and the gonad.

Shell: Absent. A variable number of vitreous, disc-like, ovoidal or roundish granules of variable size are scattered on the dorsal surface of the body cavity, up to the glandular structure and digestive gland.

Gene sequences: Partial sequences of the following genes characteristic of *Barkeriella museensis* were deposited in GenBank: *COI* – OM827003–OM827005; 16S rDNA – OM827013–OM827015; 18S rDNA – OM827024–OM827026; (5.8S rDNA) + ITS2 + (28S rDNA) – OM827032–OM827034; *H3* – OM827043–OM827046.

Remarks: It is not easy to trace the geographical origin of this slug: the family to which it belongs – the rathouisiids – has a natural range that encompasses the eastern Palaearctic, the Indomalayan and the northern Australasian regions. Its presence in the Afromontane greenhouse of MUSE in Trento (northern Italy) is surely linked to introduced exotic plants. While species of African vertebrates (fish, amphibians and birds) were introduced for display purposes, many soil invertebrates were unintentionally transported with root balls, despite the museum's strict control measures to prevent new invasions. Slugs can easily travel in this way as eggs or hatched individuals, and can spread into their surroundings (Bergey *et al.*, 2014). The greenhouse of MUSE (600 m^2; 130 species of plants; temperature

30 °C, humidity 70–80%) was completed in 2013 to recreate different montane forest environments of eastern Africa (namely those of the Udzungwa Mountains in eastern Tanzania), but not all the plant species used so far come from that area. Some trees, shrubs, herbs and vines native to South-East Asia or tropical America were chosen as substitutes for closely related or apparently similar eastern African plants, difficult or impossible to obtain through regular channels. Even the plants native to eastern Africa were not imported directly from there, but in most cases were purchased from plant nurseries in Italy or Europe or obtained by exchange with other institutions. In such facilities, plants from different continents are often raised in the same areas, allowing different pools of soil invertebrates to spread from one to the other. Thus, the rich soil fauna which inhabits the MUSE tropical greenhouse is composed of a mixture of species (flatworms, earthworms, woodlice, mites, centipedes, insects and molluscs) from several biogeographic regions.

All slug specimens were found in a restricted part of the greenhouse between January and May 2019. A subsequent survey in February 2022 was unsuccessful. The sector of the greenhouse where *B. museensis* was collected was radically restructured in the period 2020–22. Some plants were replaced and certain soil features were affected as a result. In that period, a population of the burrowing Suriname cockroach *Pycnoscelus surinamensis* (Linnaeus, 1758) spread throughout the greenhouse, reaching high densities everywhere and probably causing further changes to soil properties. The reorganization may have led to disappearance of the slug.

ACKNOWLEDGEMENTS

We thank the staff of MUSE (Trento, Italy) and, in particular, Francesca Rossi for permission to collect and active assistance during our samplings and Andrea Bianchi for information on the flora and fauna of the Afromontane greenhouse. Special thanks also to Gregorio Bolzonella (Siena, Italy) for his help during collecting, to Min Wu (Nanjing, China) for providing us with fresh specimens of *R. sinensis*, to Kazuki Kimura (Daegu, South Korea) for a preserved specimen of *R. sinensis*, Japanese references and his rathouisiid sequences and to Helen Ampt for the revision of the English language. The study was supported by the Adam Mickiewicz University, Poznań (Poland), project 526000/REZ_PROJEKT "Powrót do badań" of Andrzej Lesicki). The authors declare that they have no conflict of interest.

DATA AVAILABILITY

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov, and can be accessed with accession numbers OM827003-OM827053.

REFERENCES

Antonín V. 1992. Studies in marasmioid fungi – VIII. *Marasmius teplicensis*, a new species of the sect. *Sicci* growing in a greenhouse. *Česká Mykologie* **46:** 209–212.

Babor JF. 1900. Mittheilungen über Nacktschnecken in der Sammlung des k. k. naturhistorischen Hofmuseums. II. Erste vorläufige Mittheilung zu einer Monographie der Gattung *Atopos* Simroth. *Annalen des k.k. Naturhistorischen Hofmuseums Wien* **15:** 100–102.

Baker HB. 1925. North American Veronicellidae. *Proceedings of the Academy of Natural Sciences of Philadelphia* **77:** 157–184.

Barker GM. 2001. Gastropods on land: phylogeny, diversity and adaptive morphology. In: Barker GM, ed. *The biology of terrestrial molluscs*. Wallingford: CABI Publishing, 1–146.

Barker GM, **Efford M. 2004.** Predatory gastropods as natural enemies of terrestrial gastropods and other invertebrates. In: Barker GM, ed. *Natural enemies of terrestrial molluscs*. Wallingford: CABI Publishing, 279–403.

Bergey EA, **Figueroa LL**, **Mather CM**, **Martin RJ**, **Ray EJ**, **Kurien JT**, **Westrop DR**, **Suriyawong P. 2014.** Trading in snails: plant nurseries as transport hubs for non-native species. *Biological Invasions* **16:** 1441–1451.

BioEdit. 2017. *BioEdit 7.2*. Available online at https://bioedit.software.informer.com/7.2 (accessed 4 March 2021).

Bouchet P, **Rocroi J-P**, **Hausdorf B**, **Kaim A**, **Kano Y**, **Nützel A**, **Parkhaev P**, **Schrödl M**, **Strong EE. 2017.** Revised classification, nomenclator and typification of gastropod and monoplacophoran families. *Malacologia* **61:** 1–526.

Buchannan F. 1800. An account of the *Onchidium*, a new genus of the class of vermes, found in Bengal. *Transactions of the Linnean Society of London* **5:** 132–134.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Chiba S. 1999. Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: evidence from mitochondrial DNA sequences. *Evolution* **53:** 460–471.

Climo FM. 1980. Smeagolida, a new order of gymnomorph mollusc from New Zealand based on a new genus and species. *New Zealand Journal of Zoology* **7:** 513–522.

Colgan DJ, **McLauchlan A**, **Wilson GDF**, **Livingston S**, **Edgecombe GD**, **Macaranas J**, **Cassis G**, **Gray MR. 1998.** Histone H3 and U2 snRNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46:** 419–437.

Collinge WE. 1901. On the anatomy of a collection of slugs from N. W. Borneo; with a list of the species recorded from

that region. *Transactions of the Royal Society of Edinburgh* **40:** 295–312.

Collinge WE. 1902a. On the non-operculated land- and freshwater molluscs collected by the members of the 'Skeat Expedition' in the Malay Peninsula, 1899–1900. *Journal of Malacology* **9:** 71–95, pls 4–6.

Collinge WE. 1902b. Some notes on the genus *Prisma*, Simroth. *Journal of Malacology* **9:** 129–132, pl. 9.

Collinge WE. 1903. Contributions to a knowledge of Mollusca of Borneo (Part I). *Journal of Malacology* **10:** 79–82, pls 6–7.

Collinge WE. 1908. Description of a new species of slug of the genus *Atopos* from Java. *Journal of Conchology* **12:** 118–119.

Colosi G. 1921. Sul sistema dei gasteropodi. *Bollettino dei Musei di Zoologia ed Anatomia Comparata della Reale Università di Torino* **36:** 1–7, pl. 1.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85:** 407–415.

Dayrat B. 2009. Review of the current knowledge of the systematics of Onchidiidae (Mollusca: Gastropoda: Pulmonata) with a checklist of nominal species. *Zootaxa* **2068:** 1–26.

Dayrat B, **Conrad M**, **Balayan S**, **White TR**, **Albrecht C**, **Golding R**, **Gomes SR**, **Harasewych MG**, **de Frias Martins AM. 2011.** Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. *Molecular Phylogenetics and Evolution* **59:** 425–437.

Dayrat B, **Goulding TC**, **Apte D**, **Bhave V**, **Comendador J**, **Quáng NX**, **Tan SK**, **Tan SH. 2016.** Integrative taxonomy of the genus *Onchidium* Buchannan, 1800 (Mollusca, Gastropoda, Pulmonata, Onchidiidae). *ZooKeys* **636:** 1–40.

De Stefani C. 1879. Nuove specie di molluschi viventi nell'Italia centrale. *Bullettino della Società Malacologica Italiana* **5:** 38–48.

De Wilde JJA. 1984. The anatomy of an *Atopos*-species from Papua New Guinea with a discussion on its systematic position (Mollusca, Gastropoda, Rathouisiidae). *Indo-Malayan Zoology* **1:** 113–126.

Druciarek T, **Lewandowski M. 2016.** A new species in the genus *Phyllocoptes* Nalepa (Eriophyidae) from greenhouse roses in Poland. *Acarologia* **56:** 225–235.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Férussac d'Audebard de AEJ. 1819. *Histoire naturelle générale et particulière des mollusques terrestres et fluviatiles. Livraison 3*. Paris: Arthus-Bertrand, 17–56.

Folmer O, **Black M**, **Hoeh W**, **Lutz RA**, **Vrijenhoek RC. 1994.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Ghosh E. 1912. On the anatomy of *Atopos* (*Padangia*) *sanguinolenta* (Stoliczka, M.S.). *Records of the Indian Museum* **7:** 181–196, pls 25–27.

Ghosh E. 1913. Mollusca, I: Rathouisiidae. *Records of the Indian Museum* **8:** 209–227, pls 10–13.

Ghosh E. 1914. On the reproductive system of *Atopos* Simroth. *Journal and Proceedings of the Asiatic Society of Bengal New Series* **10:** 111–113.

Ghosh E. 1915. On the anatomy of a Burmese slug of the genus *Atopos*. *Records of the Indian Museum* **11:** 153–161, pls 16–19.

Glez-Peña D, **Gómez-Blanco D**, **Reboiro-Jato M**, **Fdez-Riverola F**, **Posada D. 2010.** ALTER: program-oriented format conversion of DNA and protein alignments. *Nucleic Acids Research* **38**(Suppl. 2): W14–W18.

Gómez BJ. 2001. Structure and functioning of the reproductive system. In: Barker GM, ed. *The biology of terrestrial molluscs*. Wallingford: CABI Publishing, 307–330.

Goulding TC, **Khalil M**, **Tan SH**, **Cumming RA**, **Dayrat B. 2022.** Global diversification and evolutionary history of onchidiid slugs (Gastropoda, Pulmonata). *Molecular Phylogenetics and Evolution* **168:** 107360.

Gray JE. 1840. Shells of molluscous animals. In: *Synopsis of the contents of the British Museum, 42nd edn*. London: Woodfall and son, 105–152.

Hall TA. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hasegawa M, **Kishino H**, **Yano T. 1985.** Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22:** 160–174.

Hayase Y, **Kimura S**, **Kawabe K**, **Yahashi M**, **Nishi H**, **Moriya S**, **Ywata A. 2018.** Land snail fauna on the crest of Mt Kanmuriyama, Gifu, Japan. *Kakitsubata* **43:** 1–27 [in Japanese].

Herbert D, **Kilburn D. 2004.** *Field guide to the land snails and slugs of eastern South Africa*. Pietermaritzburg: Natal Museum.

Heude PM. 1882. Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. *Mémoires concernant l'histoire naturelle de l'empire chinois par des pères de la Compagnie de Jésus* **1 (2):** 1–2, 1–87, pls 12–21.

Heude PM. 1884. Note sur un limacien nouveau de Chine. *Journal de Conchyliologie* **31:** 394–395.

Heude PM. 1885. Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. *Mémoires concernant l'histoire naturelle de l'empire chinois par des pères de la Compagnie de Jésus* **1 (2):** 89–132, pls 22–32.

Heude PM. 1890. Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. *Mémoires concernant l'histoire naturelle de l'empire chinois par des pères de la Compagnie de Jésus* **1 (4):** 125–188, pls 33–43.

Heynemann DF. 1876. Beschreibung eines neuen *Vaginulus*. *Journal des Museum Godeffroy* **12:** 159.

Heynemann DF. 1906. Die geographische Verbreitung der Nacktschnecken. Eine zusammenfassende kritische Darstellung unserer Kenntnisse derselben zu Anfang des 20. Jahrhunderts. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* **30:** 1–92.

Heywood VH. 2011. The role of botanic gardens as resource and introduction centres in the face of global change. *Biodiversity and Conservation* **20:** 221–239.

Hoffmann H. 1926. Die Nacktschnecken der Dänischen Expedition nach den Kei-Inseln. *Videnskabelige Meddelelser Dansk Naturhistorisk Forening* **81:** 15–36.

Holznagel WE, **Colgan DJ**, **Lydeard C. 2010.** Pulmonate phylogeny based on 28SrRNA gene sequences: a framework for discussing habitat transitions and character transformation. *Molecular Phylogenetics and Evolution* **57:** 1017–1025.

Horsák M, **Dvořák L**, **Juřičková L. 2004.** Greenhouse gastropods of the Czech Republic: current stage of research. *Malakológiai Tájékoztató* **22:** 141–147.

Humala AE, **Kruidhof HM**, **Woelke JB. 2017.** New species of *Megastylus* (Hymenoptera: Ichneumonidae: Orthocentrinae) reared from larvae of Keroplatidae fungus gnats (Diptera) in a Dutch orchid greenhouse. *Journal of Natural History* **51:** 83–95.

Ihering von H. 1892. Ueber *Atopos* Simroth. *Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft* **24:** 140–144.

Jörger KM, **Stöger I**, **Kano Y**, **Fukuda H**, **Knebelsberger T**, **Schrödl M. 2010.** On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. *BMC Evolutionary Biology* **10:** 323.

Kielhorn KH. 2008. A glimpse of the tropics – spiders (Araneae) in the greenhouses of the Botanic Garden Berlin-Dahlem. *Arachnologische Mitteilungen* **36:** 26–34.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

Kimura K. 2020. DNA barcoding of rathouisiid slugs on the Ryukyu Islands, Japan. *Molluscan Diversity Journal* **1:** 16140120.

Kimura K, **Sano I**, **Kameda Y**, **Saito T**, **Chiba S. 2020a.** Phylogenetic position of the Japanese land slug genus *Granulilimax* Minato, 1989 based on preliminary analyses of mitochondrial and nuclear genes. *American Malacological Bulletin* **37:** 53–61.

Kimura K, **Hirano T**, **Chiba S**, **Pak J-H. 2020b.** Note on occurrence of the land slug family Rathouisiidae Heude, 1885 from South Korea and its DNA barcode. *Biodiversity Journal* **11:** 1015–1019.

Kolicka M. 2019. Gastrotricha – not only in sediments: new epiphytic species of Chaetonotida from the Jubilee Greenhouse of the Botanical Garden in Kraków. *European Journal of Taxonomy* **511:** 1–100.

Kolicka M, **Kisielewski J**, **Nesteruk T**, **Zawierucha K. 2013.** Gastrotricha from the Poznań Palm House – one new sub-genus and three new species of freshwater Chaetonotida (Gastrotricha). *Zootaxa* **3717:** 231–279.

Kolicka M, **Dziuba MK**, **Zawierucha K**, **Kuczyńska-Kippen N**, **Kotwicki L. 2015.** Palm house – biodiversity hotspot or risk of invasion? Aquatic invertebrates: the special case of Monogononta (Rotifera) under greenhouse conditions. *Biologia* **70:** 94–103.

Komala Z, **Przybos E. 2001.** Zooplankton in the ponds with tropical plants in the greenhouses of the Botanical Garden of the Jagiellonian University in Kraków. *Folia Biologica (Krakow)* **49:** 225–228.

Krumpálová Z, **Holienková B. 2018.** Land snails in the Slovak open-air garden centres. *Ekológia (Bratislava)* **37:** 369–379.

Kumar S, **Stecher G**, **Tamura K. 2016.** MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33:** 1870–1874.

Laidlaw FF. 1940. Notes on some specimens of the genus *Atopos* (Mollusca: Pulmonata) with microphotographs illustrating points in the anatomy of the genus. *Bulletin of the Raffles Museum* **16:** 121–132.

Linnaeus C. 1758. *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Tomus I*. Stockholm: L. Salvius.

Minato H. 1989. A remarkable slug, *Granulilimax fuscicornis* n. gen. et sp. from Japan. *Venus (Japanese Journal of Malacology)* **48:** 255–258.

Minato H. 2015. A memorandum of the studies of Japanese land snails (14). Distribution of *Granulilimax fuscicornis* species complex (Systellommatophora: Rathouisiidae) and its bibliographic review. *Kainakama* **49:** 1–12.

Möllendorff von OF. 1881. Beiträge zur Molluskenfauna von Südchina. *Jahrbücher der Deutschen Malakozoologischen Gesellschaft* **8:** 302–312.

MolluscaBase eds. **2021.** *MolluscaBase*. Available at: http://www.molluscabase.org (accessed 14 July 2021).

Müller OF. 1774. *Vermium terrestrium et fluviatilium, seu animalium infusiorium, helminthicorum, et testaceorum, non marinorum, succincta historia, Vol.* **II**. Copenhagen & Leipzig: Heineck & Faber.

Nei M, **Kumar S. 2000.** *Molecular evolution and phylogenetics*. Oxford: Oxford University Press.

Noerdjito WA, **Kahono S. 2001.** Ant species diversity in the Bogor Botanic Garden, West Java, Indonesia, with descriptions of two new species of the genus *Leptanilla* (Hymenoptera, Formicidae). *Tropics* **10:** 379–404.

Odhner NH. 1917. Mollusca. In: Results of Dr. E. Mjöbergs Swedish scientific expeditions to Australia 1910–1913 XVIII. *Kungliga Svenska Vetenskapsacademiens Handlingar* **52:** 1–115, pls 1–3.

Passamaneck YJ, **Schander C**, **Halanych KM. 2004.** Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. *Molecular Phylogenetics and Evolution* **32:** 25–38.

Pergl J, **Pyšek P**, **Bacher S**, **Essl F**, **Genovesi P**, **Harrower CA**, **Hulme PE**, **Jeschke JM**, **Kenis M**, **Kühn I**, **Perglová I**, **Rabitsch W**, **Roques A**, **Roy DB**, **Roy HE**, **Vilà M**, **Winter M**, **Nentwig W. 2017.** Troubling travellers: are ecologically harmful alien species associated with particular introduction pathways? *NeoBiota* **32:** 1–20.

Pieńkowska JR, **Manganelli G**, **Giusti F**, **Barbato D**, **Kosicka E**, **Hallgass A**, **Lesicki A. 2020.** Redescription of *Monacha pantanellii* (De Stefani, 1879), a species endemic to the central Apennines, Italy (Gastropoda, Eupulmonata, Hygromiidae) by an integrative molecular and morphological approach. *ZooKeys* **988:** 17–61.

Pilsbry HA. 1948. *Land Mollusca of North America (north of Mexico), Vol.* **II,** *Part 2.* Philadelphia: Academy of Natural Sciences of Philadelphia, 521–1113.

Plate von L. 1893. Studien über opisthopneumone Lungeschnecken, II, Die Oncidiidien. *Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Thiere* **7:** 93–234, pls 7–12.

Ponder WF, **Lindberg DR**, **Ponder JM. 2020.** *Biology and evolution of the Mollusca, Vol.* **2**. Boca Raton, London, New York: CRC Press Taylor & Francis Group.

Puillandre N, **Brouillet S**, **Achaz G. 2021.** ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* **21:** 609–620.

Rafinesque CS. 1815. *Analyse de la nature ou tableau de l'univers et des corps organisés*. Palermo: published by the author.

Rathouis C. 1885. Quelques remarques sur les moeurs et habitudes du Rathouisia leonina. In: Heude PM. *Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. Mémoires Concernant l'Histoire Naturelle de l'Empire Chinois par des Pères de la Compagnie de Jésus* **1:** 131–132, pl. 32. https://doi.org/10.5962/bhl.title.50365.

Richling I, **von Proschwitz T. 2021.** Identification problems of travelling snail species – new exotic introductions to tropical greenhouses in Gothenburg, Sweden (Gastropoda: Achatinellidae, Strobilopsidae, Helicarionidae). *PeerJ* **9:** e11185.

Robinson DG, **Fields A. 2010.** *The leatherleaf slugs (family Veronicellidae)*. Conference Paper. Regional Workshop Mollusk Pests of Economic Importance, Centro Kellog, Escuela Agricola Panamericana, Zamorano, Honduras. Available at https://doi.org/10.13140/2.1.2936.4805. (accessed 28 July 2020).

Romero PE, **Weigand AM**, **Pfenninger M. 2016.** Positive selection on panpulmonate mitogenomes provide new clues on adaptations to terrestrial life. *BMC Evolutionary Biology* **16:** 164.

Ronquist F, **Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Ronquist F, **Teslenko M**, **Van der Mark P**, **Ayres DL**, **Darling A**, **Höhna S**, **Larget B**, **Liu L**, **Suchard MA**, **Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* **61:** 539–542.

Rowson B, **Anderson R**, **Turner JA**, **Symondson WO. 2014.** The slugs of Britain and Ireland: undetected and undescribed species increase a well-studied, economically important fauna by more than 20%. *PLoS One* **9:** e91907 (1–21).

Saitou N, **Nei M. 1987.** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4:** 406–425.

Sarasin P, **Sarasin F. 1899.** Die Land-Mollusken von Celebes. *Materialien zur Naturgeschichte der Insel Celebes* **2:** 1–248, pls 1–31.

Schilthuizen M, **Liew TS. 2008.** The slugs and semislugs of Sabah, Malaysian Borneo (Gastropoda, Pulmonata: Veronicellidae, Rathouisiidae, Ariophantidae, Limacidae, Philomycidae). *Basteria* **72:** 287–306.

Schmidt A. 1855. Der Geschlechtsapparat der Stylommatophoren in taxonomischer Hinsicht. *Abhandlungen des Naturwissenschaftlichen Vereins für Sachsen und Thüringen in Halle* **1:** 1–52, pls 1–14.

Semper C. 1880. Dritte Familie, Onchididae. In: Semper C, ed. *Reisen im Archipel der Philippinen. Zweiter Theil. Wissenschaftliche Resultate. Dritter Band. Landmollusken*. Wiesbaden: Kreidel, **3:** 225–264, pls 19–20, 22–23.

Šestáková A, **Suvák M**, **Krajčovičová K**, **Kaňuchová A**, **Christophoryová J. 2017.** Arachnids from the greenhouses of the Botanical Garden of the PJ Šafárik University in Košice, Slovakia (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpiones). *Arachnologische Mitteilungen* **53:** 19–28.

Sherlock E, **Carpenter D. 2009.** An updated earthworm list for the British Isles and two new 'exotic'species to Britain from Kew Gardens. *European Journal of Soil Biology* **45:** 431–435.

Simroth H. 1891. Über das Vagulinidengenus *Atopos* n. g. *Zeitschrift für Wissenschaftliche Zoologie* **52:** 593–615, pl. 37.

South A. 2012. *Terrestrial slugs: biology, ecology and control*. Berlin: Springer Science + Business Media.

Stanisic J. 1998. Order Systellommatophora. In: Beesley PL, Ross GJB, Wells A, eds. *Mollusca: the southern synthesis. Fauna of Australia*, *Vol.* **5,** *Part B*. Melbourne: CSIRO Publishing, 1060–1067.

Stanisic J, **Shea M**, **Potter D**, **Griffiths O. 2010.** *Australian land snails, Vol. 1. A field guide to eastern Australian species*. Mauritius: Bioculture Press for the Australian Museum.

Szczepkowski A, **Gierczyk B**, **Kujawa A. 2014.** Greenhouses of botanical gardens as a habitat of alien and native macrofungi: a case study in Poland. *Central European Journal of Biology* **9:** 777–795.

Tada A, **Yano S. 2010.** Some new descriptions on taxonomy, morphology and ecology in *Granulilimax fuscicornis* Minato, 1989. *Kagawa Seibutsu* **37:** 37–42.

Talavera G, **Castresana J. 2007.** Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56:** 564–577.

Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9:** 678–687.

Tan SK, **Chan S-Y. 2009.** New records of predatory slugs from Singapore with notes on their feeding behaviour. *Nature in Singapore* **2:** 1–7.

Tapparone Canefri C. 1883. Fauna malacologica della Nuova Guinea e delle isole adiacenti. Parte I. Molluschi estramarini. *Annali del Museo Civico di Storia Naturale di Genova* **19:** 5–313, pls 1–9.

Teasdale LC. 2017. *Phylogenomics of the pulmonate land snails*. Unpublished D. Phil. Thesis, University of Melbourne.

Thompson JD, **Higgins DG**, **Gibson TJ. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Vonnemann V, **Schrödl M**, **Klussmann-Kolb A**, **Wägele H. 2005.** Reconstruction of the phylogeny of the Opisthobranchia (Mollusca: Gastropoda) by means of 18S and 28S rRNA gene sequences. *Journal of Molluscan Studies* **71:** 113–125.

Wade CM, **Mordan PB. 2000.** Evolution within the gastropod molluscs; using the ribosomal RNA gene-cluster as an indicator of phylogenetic relationships. *Journal of Molluscan Studies* **66:** 565–570.

Wang C, **Zhang X**, **Pan X**, **Li Z**, **Zhu S. 2015.** Greenhouses: hotspots in the invasive network for alien species. *Biodiversity and Conservation* **24:** 1825–1829.

Westerlund CA. 1883. Noch einige von der Vega-Expedition gesammelte Mollusken. *Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft* **15:** 164–166.

Wiktor A. 2003. Terrestrial gastropods (Mollusca) of Province Madang in Papua-New Guinea. Part III. Pulmonata: Rathouisiidae, Ellobiidae, Succineidae, Agriolimacidae, Endodontidae (partim), Ariophantidae, Euconulidae, Subulinidae, Streptaxidae. *Folia Malacologica* **11:** 1–21.

Wu M, **Guo J-Y**, **Wan F-H**, **Qin Q-L**, **Wu Q**, **Wiktor A. 2006.** A preliminary study of the predatory terrestrial mollusk *Rathouisia leonina*. *The Veliger* **48:** 61–74.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. GenBank sequences of *COI* gene fragments for representatives of the families Rathouisiidae, Philomycidae, Onchidiidae and Veronicellidae used for comparison in molecular analysis, haplotypes are in bold (used for comparison of Rathouisiidae and Onchidiidae underlined). For additional references see Table S9.

Table S2. GenBank sequences of 18S rDNA gene fragments used for comparison in molecular analysis. For additional references see Table S9.

Table S3. GenBank sequences of ITS2 (flanked by 5.8S and 28S rDNA) fragments used for comparison in molecular analysis. For additional references see Table S9.

Table S4. GenBank sequences of 16S rDNA gene fragments used for comparison in molecular analysis. For additional references see Table S9.

Table S5. GenBank sequences of *H3* gene fragments used for comparison in molecular analysis. For additional references see Table S9.

Table S6. Thermal regimes for amplification of selected genes (see Table 2) and lengths of the amplification products.

Table S7. K2P genetic distances (%) between *COI* sequences of rathouisiid species used for analysis (see: Table 3; Supporting Information, Table S1).

Table S8. Concatenated sequences of *COI*, 16S rDNA, ITS2 (flanked by 5.8S and 28S rDNA) and *H3* gene fragments (2122 positions long: 614 *COI* + 458 16S rDNA + 54 5.8S rDNA + 662 ITS2 + 40 28S rDNA + 294 *H3*) used for ML, NJ and BI analysis (see Fig. 8).

Table S9. Additional references for GenBank gene fragments used for comparison in molecular analysis (listed in Supporting Information, Tables S1–S5).

Figure S1. Maximum likelihood (ML) tree of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences for representatives of the systellommatophoran families Rathouisiidae and Onchidiidae (see Supporting Information, Table S1). *COI* sequences were cut to 563 bp in length, onchidiid sequences were grouped for genera due to their large number. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT380013 sequence deposited in GenBank by Pieńkowska *et al.* (2020).

Figure S2. Maximum likelihood (ML) tree of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences for other rathouisiid species, as well as selected species representing particular genera of Onchidiidae (see Supporting Information, Table S1). *COI* sequences were cut to 549 bp. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 ML (left) and NJ (right) replicates (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT380013 sequence deposited in GenBank by Pieńkowska *et al.* (2020).

Figure S3. Maximum likelihood (ML) tree of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences for representatives of the families Rathouisiidae and Veronicellidae (see Supporting Information, Table S1). *COI* sequences were cut to 566 bp in length. Numbers next to the branches indicate bootstrap support above 50% for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT380013 sequence deposited in GenBank by Pieńkowska *et al.* (2020).

Figure S4. Maximum likelihood (ML) tree of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences for representatives of the families Rathouisiidae, Onchidiidae, Veronicellidae and Philomycidae (see Supporting Information, Table S1). *COI* sequences were cut to 549 bp. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT380013 sequence deposited in GenBank by Pieńkowska *et al.* (2020).

Figure S5. Maximum likelihood (ML) tree of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences for representatives of the families Rathouisiidae and Philomycidae (see Supporting Information, Table S1). *COI* sequences were cut to 551 bp in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT380013 sequence deposited in GenBank by Pieńkowska *et al.* (2020).

Figure S6. Graphical output of ASAP analysis (K80 substitution model) of *COI* haplotypes obtained from *Barkeriella museensis* (*COI* 1–*COI* 3), *Rathouisia sinensis* (*COI* 4–*COI* 9) and GenBank sequences from other Rathouisiidae genera (see Supporting Information, Table S1). *COI* sequences were 566 bp in length. A cladogram shows hierarchical clustering of sequences. Bar colours indicate unique partitions.

Figure S7. Maximum likelihood (ML) tree of 16S rDNA sequences obtained from *Barkeriella museensis* (16S 1), *Rathouisia leonina* (16S 2–16S 4) and GenBank sequences for representatives of the families Onchidiidae, Veronicellidae and Philomycidae (see Supporting Information, Table S4). 16S rDNA sequences were 306 positions in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* OM827023 (16S 6) sequence obtained in the present study (see Table 3).

Figure S8. Maximum likelihood (ML) tree of 18S rDNA sequences obtained from *Barkeriella museensis* (18S 1, 18S 2), *Rathouisia sinensis* (18S 4–18S 7) and GenBank sequences for representatives of the families Rathouisiidae, Onchidiidae, Veronicellidae and Philomycidae (see Supporting Information, Table S2). 18S rDNA sequences were 1618 positions in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Deroceras reticulatum* AY145373 sequence deposited in GenBank by Passamaneck *et al.* (2004).

Figure S9. Maximum likelihood (ML) tree of *H3* sequences obtained from *Barkeriella museensis* (*H3* 1), *Rathouisia sinensis* (*H3* 2) and GenBank sequences for representatives of the families Onchidiidae, Veronicellidae and Philomycidae (see Supporting Information, Table S5). *H3* sequences were 267 bp in length. Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates of ML (left) and NJ (right) analysis (Felsenstein, 1985). The tree was rooted with stylommatophoran *Monacha pantanellii* MT385778 sequence deposited in GenBank by Pieńkowska *et al.* (2020).