Zoosyst. Evol. 93 (1) 2017, 95–104 | DOI 10.3897/zse.93.10794

PENSOFT

museum für naturkunde

Diversity and taxonomy of Vietnamese *Pollicaria* (Gastropoda, Pupinidae)

Russell L. Minton[1], Phillip M. Harris[2], Ernest North[1], Do Van Tu[3]

1 *Division of Natural Sciences, University of Houston Clear Lake, 2700 Bay Area Boulevard MC 39, Houston, Texas 77058 USA*
2 *Department of Biological Sciences, University of Alabama, Box 870345, Tuscaloosa, Alabama 35487 USA*
3 *Vietnam Academy of Science and Technology, 18 Hoàng Quốc Việt, Nghĩa Đô, Cầu Giấy, Hà Nội, Vietnam*

http://zoobank.org/07186867-987F-4661-894D-359FE9B5B4CF

Corresponding author: *Russell L. Minton* (minton@uhcl.edu)

Received 4 November 2016
Accepted 6 January 2017
Published 3 February 2017

Academic editor:
Matthias Glaubrecht

Key Words

Pollicaria rochebruni
Pollicaria crossei
Pollicaria mouhoti
Vietnam
pupinid

Abstract

Species in the genus *Pollicaria* (Gastropoda: Pupinidae) are conspicuous members of the southeast Asian land snail fauna. Originally erected in 1856, both the genus and its constituent species have been reorganized multiple times with the most recent treatment published in 2013. Collections of Vietnamese *Pollicaria* during 2014 and 2015 raised questions of the utility of currently used diagnostic shell characters and identification keys in species identification. An examination of the authors' collections, combined with museum specimens, suggested that at least three species of *Pollicaria* occur or have historically occurred in Vietnam. It is suggested that *P. rochebruni* is a senior synonym of *P. crossei* and treat both taxa as conspecific. A second species, *P. mouhoti*, was believed to only occur in Cambodia, Laos, and Thailand. A possible third species, based only on previous karyotypic work, is discussed. Our data further suggest that shell features such as color and size lack consistent utility in species-level identifications in *Pollicaria*.

Introduction

The genus *Pollicaria* was erected by Gould (1856) to include large pupinid snails of southeastern Asia possessing the following characters: a thick ovate shell, distorted and flattened in the adult; a circular aperture; and an internal shoulder or groove inside the palatal edge of the peristome (Pain 1974). The taxonomy and systematics of *Pollicaria* have remained in flux for many years. At the same time he erected the genus, Gould (1856) described *Cyclostoma pollex* Gould, 1856 and made it the type species of *Pollicaria*. Benson (1859) erected the genus *Hybocystis* with *Megalomastoma gravidum* Benson, 1856 from Burma as the type species, recognizing that *C. pollex* was synonymous with the previously described *M. gravidum*. Haines described *C. myersii* Haines, 1858 from Siam, and three more species were described in *Hybocystis*: *H. mouhoti* Pfeiffer, 1862 from the Lao Mountains of Cambodia; and *H. elephas* de Morgan, 1885 and *H. jousseaumi* de Morgan, 1885 from the Perak region of Malaysia. Benson (1860) recognized that *Hybocystis* was a junior synonym of *Pollicaria*, but the former name remained in use until Pain's (1974) revision. Crosse (1885) revised *Hybocystis* into two sections, one with *H. elephas* and its junior synonym *H. jousseaumi* and the other with *H. gravida*, *H. mouhoti*, and *H. myersii*. In 1887, two species of *Hybocystis* were described from Tonkin (northern Vietnam), *H. rochebruni* Mabille, 1887 and *H. crossei* Dautzenberg & d'Hammonville, 1887. Dautzenberg and Fischer (1905) equated *H. rochebruni* and *H. crossei* with *H. gravida*, indicating that any shell differences between species were not consistent but rather existed in a continuum between extremes.

Pain (1974) revised the then six nominal *Pollicaria* species into three based on shell morphology: *P. gravida*, including *P. rochebruni* and *P. crossei*; *P. elephas*; and *P. myersii*, including its junior synonym *P. mouhoti*. Kongim et al. (2010) provided karyotype analyses for *P. gravida*, *P. elephas*, and *P. myersii*, along with

P. mouhoti as a separate species. They found karyotype differences between all four species and found two distinct karyotypes in Vietnamese *P. gravida*; their "big brown shell" and "small orange shell" samples differed in the centromere position of one chromosome pair (Kongim et al. 2010:127). However, they did not karyotype any topotypic *P. gravida* from Burma. Most recently, Kongim et al. (2013) reassessed *Pollicaria* using a combination of shell, radula, and reproductive characters and recognized six separate species: *P. gravida*, *P. myersii*, *P. mouhoti*, *P. elephas*, *P. rochebruni*, and *P. crossei*. They correctly recognized that not all *Pollicaria* possess the shouldering inside the peristome, and assigned the different karyotypes of Vietnamese *P. gravida* (Kongim et al. 2010) to *P. rochebruni* ("big brown shell") and *P. crossei* ("small orange shell"). The two species were diagnosed in their key based on shell size and color; *P. rochebruni* is large (>40 mm) with a brownish shell, while *P. crossei* is small (<35 mm) with a bright orange shell (Kongim et al. 2013). Both species are thus separate from the small yellow-shelled *P. gravida* from Burma. Kongim et al. (2013) also provided revised distributions for all species and figured type and additional material.

While identifying Vietnamese land snails collected in 2015 by PMH and DVT, we found Kongim et al. (2013) to be problematic in identifying specimens of *P. rochebruni* and *P. crossei*. We noted specifically that smaller (30-35 mm) reddish-brown shells and larger (35-40 mm) orange shells were left unidentifiable by the key, and that species-specific characters of the key conflicted with the comparative characters listed by Kongim et al. (2013:28). Of additional interest were records of putative *P. mouhoti* from Lào Cai, Vietnam (Solem 1966), a species that Kongim et al. (2013) had restricted to Cambodia, Laos, and Thailand, and internet references to additional species in northeast Vietnam and southern China. In an effort to resolve the taxonomic and distribution issues presented by Kongim et al. (2013), we undertook a morphological and molecular examination of Vietnamese *Pollicaria* specimens, along with original descriptions and other literature relevant to the genus, to address two research questions. First, are *P. rochebruni* and *P. crossei* separate valid species? Second, beyond those specimens listed in Solem (1966), is there evidence of additional *Pollicaria* species in Vietnam?

Materials and methods

Specimens collected by the authors were vouchered at the Carnegie Museum of Natural History (CM), Philadelphia, Pennsylvania. Collection data for Vietnamese *Pollicaria* came from the following museums: Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (CUMZ); Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZ); Florida Museum of Natural History, University of Florida, Gainesville, Florida (FLMNH); Museum National d'Histoire Naturelle, Paris, France (MNHN), Royal Belgian Institute of Natural Sciences (RBINS), and National Museum of Natural History, Smithsonian Institute, Washington D.C. (USNM). Specimens from MNHN, CUMZ, and RBINS were not made available, so we treated the identifications and locality data given in Kongim et al. (2013) as correct. All Vietnamese *Pollicaria* holdings from FLMNH, MCZ, and USNM were borrowed, identified by the authors based on Kongim et al. (2013), latitude and longitude coordinates estimated, and mapped with DIVA-GIS (Hijmans et al. 2008).

We also searched the Internet for shell dealers selling *Pollicaria* specimens from Vietnam and surrounding areas. While we lacked the funding to purchase specimens outright, we examined shell images and locality information from the dealer websites. Requests to formally include images and additional information in this manuscript went unanswered. We accepted that this information was not equivalent to that of vouchered specimens, but could be important in determining the diversity and distribution (Turney et al. 2015) of *Pollicaria* species.

Live *Pollicaria* specimens were collected in Vietnam by PMH, DVT, and local farmers in 2015, preserved in ethanol, and DNA was successfully sequenced from two of them. One individual collected south of Hoàng Liên National Park had a reddish-brown shell we identified as *P. rochebruni*; the other was collected from Cúc Phương National Park and had an orange shell that was consistent with *P. crossei sensu* Kongim et al. (2013). Using foot tissue we followed a standard CTAB and phenol/chloroform DNA extraction method (Saghai-Maroof et al. 1984), then amplified one mitochondrial (16S) and two nuclear (18S and 28S) gene fragments by PCR. Amplification conditions were the same for all fragments and included an initial denaturing step (94ºC for 120 s), 30 cycles (94ºC for 60 s, 50ºC for 60 s, 72ºC for 120 s), and a final extension step (72ºC for 10 minutes). We employed the following primer pairs: 16Sar and 16Sbr (Palumbi et al. 1991), 18S1F and 18S4R (Giribet et al. 1996), and 28SD1F and 28SD6R (Park and Ó Foighil 2000). Sequences were generated with the Sanger method on an ABI 3730xl by Genewiz (South Plainsfield, New Jersey, USA). Contigs were assembled in Geneious (BioMatters Ltd.) and combined with Cyclophoroidea data taken from GenBank (Table 1). Each gene fragment was aligned separately using MUSCLE (Edgar 2004) and the appropriate substitution model selected by Bayesian information criterion in IQTREE (Nguyen et al. 2015). The models chosen were HKY+G4 (Hasegawa et al. 1985) for 16S, JC+I+G4 (Jukes and Cantor 1969) for 18S, and TN+I+G4 (Tamura and Nei 1993) for 28S. The combined three gene dataset was analyzed under maximum likelihood in IQTREE allowing each gene partition to be optimized under its own model. Branch support was estimated using 10,000 ultra-fast bootstrap replicates (Minh et al. 2013) in IQTREE.

Results

A review of the original descriptions of both *P. rochebruni* and *P. crossei* suggested that Mabille (1887a) and

Table 1. GenBank accession numbers for all taxa included in the phylogenetic analysis.

Species	16S	18S	28S
Acroptychia bathiei	HM753491	HM753437	HM753380
Acroptychia milloti	HM753492	HM753438	HM753381
Adelopoma tucma	HM753534	HM753450	HM753393
Aperostoma palmeri	DQ093479	DQ093435	DQ093458
Arinia paricostata	HM753500	HM753441	HM753384
Bellamya bengalensis	FJ405724	FJ405678	FJ405623
Cochlostoma elegans	HM753489	HM753435	HM753378
Cochlostoma roseoli	HM753490	HM753436	HM753379
Cochlostoma septemspirale	HM753497	HM753423	HM753367
Cyclophorus aurantiacus	JX474723	KJ407088	KF319206
Cyclophorus bensoni	JX474670	KJ407082	KF319138
Cyclophorus cantori	JX474718	KJ407089	KF319193
Cyclophorus hirasei	AY010505	AF055644	HM003647
Cyclophorus latus	HM753484	HM753430	HM753374
Cyclotus taivanus	HM753485	HM753431	HM753375
Diplommatina canaliculata	HM753504	HM753445	HM753388
Diplommatina gomantongensis	HM753509	HM753451	HM753394
Diplommatina rubicunda	HM753520	HM753477	HM753417
Diplommatina rubra	HM753514	HM753456	HM753399
Japonia sp.	HM753486	HM753432	HM753376
Leptopoma vitreum	JX474741	KJ407147	KF319214
Opisthostoma austeni	KC250904	KC250930	KC250955
Opisthostoma hailei	KC250897	KC250923	KC250948
Opisthostoma mirabile	HM753529	HM753466	HM753406
Opisthostoma obliquedentatum	HM753530	HM753467	HM753407
Opisthostoma platycephalum	KC250905	KC250931	KC250956
Opisthostoma tenerum	KC250902	KC250928	KC250953
Palaina albata	HM753531	HM753469	HM753409
Palaina striolata	HM753533	HM753470	HM753410
Pollicaria cf. crossei	KY359073	KY359075	KY359077
Pollicaria rochebruni	KY359072	KY359074	KY359076
Pseudopomatias eos	KP271244	n/a	n/a
Pseudopomatias maasseni	KM103252	n/a	n/a
Pupina hosei	HM753493	HM753439	HM753382
Pupinella swinhoei	HM753494	HM753440	HM753383

Dautzenberg and d'Hamonville (1887) described shells of the same species from Tonkin (Figure 1). Authors of both species provided nearly identical data for the size and shape of the shell, aperture, apex, and sutures, and the number of whorls (Table 2). Mabille (1887a) did not provide information on the color of *P. rochebruni*, but did note the interior shoulder of the peristome. Dautzenberg and d'Hamonville (1887a) gave the color of *P. crossei* as reddish-brown, did not mention the shouldered peristome, but did note the triangular breathing device. The illustration of *P. rochebruni* in Mabille (1887b) clearly shows the breathing triangle, though the shouldered peristome is not immediately apparent in the illustrated *P. crossei* shell (Dautzenberg and d'Hamonville 1887). No mention is made in either description of any small orange shells.

A total of 87 museum specimens (Figure 2) was examined and an additional 44 specimens were collected by PMH and DVT. Many shells were weathered and slightly pitted, and most were light tan to medium brown in color. Of the 131 total specimens, 124 possessed a shoulder inside the peristome and seven did not. Using the key to *Pollicaria* in Kongim et al. (2013:25) we were able to identify 12 specimens to species level: three *P. rochebruni*, two *P. crossei*, and seven *P. mouhoti*. Seventeen specimens had a peristome with the interior shoulder, but were between 35 and 40 mm in height and excluded by the key. The remaining 119 specimens also had an interiorly shouldered peristome, but had shell size and coloration combinations different than those given in the key. Using the table of comparative characters given later in Kongim et al. (2013:28), we identified 43 *P. rochebruni*, two *P. crossei*, and seven *P. mouhoti* from the same set of 131 specimens. The remaining shells did not fit any combination of characters given in the table. The seven putative *P. mouhoti* specimens more closely resembled the specimens of *P. myersii* figured in Kongim et al. (2013:27) than the lectotype of *P. mouhoti* figured therein.

Table 2. Morphological character comparison taken from the original descriptions of *Pollicaria rochebruni* and *P. crossei*.

	P. rochebruni	***P. crossei***
Height	39 mm	39 mm
Diameter	21 mm	20 mm
Color	n/a	Reddish-brown
Peristome	Thick, upturned, with a channel in the upper portion of the lip	White, circular, thickened, with a triangular air space at the lip.
Whorls	6.5 to 7 convex, flattened	7 convex, flattened on the upper part
Aperture	Circular	Circular
Shell	Ovate-pupiform, striate, and distorted	Pupiform, striate, and distorted
Sutures	Impressed	Linear and impressed
Apex	Obtuse	Obtuse

Pollicaria specimens are advertised for sale at five websites: eBay.com, yhshells.com, femorale.com, conchology.be, and topseashells.com. Taken together, these specimens suggest that the known distribution of *Pollicaria* species should be expanded to include Guangxi autonomous region and Yunnan province in southern China. These two regions are adjacent to the northern borders of Vietnam, Laos, and Myanmar. Shells from these two regions were identified by their sellers as *P. crossei*, *P. gravida*, and *P. rochebruni*. All but two Chinese specimens possessed the interiorly shouldered perisotome; the other two possessed the flared lip and no shoulder seen in *P. mouhoti* and *P. myersii*. Shell sizes varied up to 45 mm in length, and shell colors varied from light tan through orange, reddish brown, dark brown, and dark purple. Vietnamese *Pollicaria* specimens varied similarly in color and size to the Chinese samples, and all possessed the interior shoulder in the peristome. These specimens were labelled as *P. crossei* and *P. rochebruni*.

DNA sequences from our putative *P. rochebruni* and *P. crossei* specimens (Figure 3) were identical for all three genes. Alignment lengths for each gene were 566 positions for 16S, 430 for 18S, and 838 for 28S. Our phylogenetic analysis produced a single tree (log-likelihood -17304.7519)

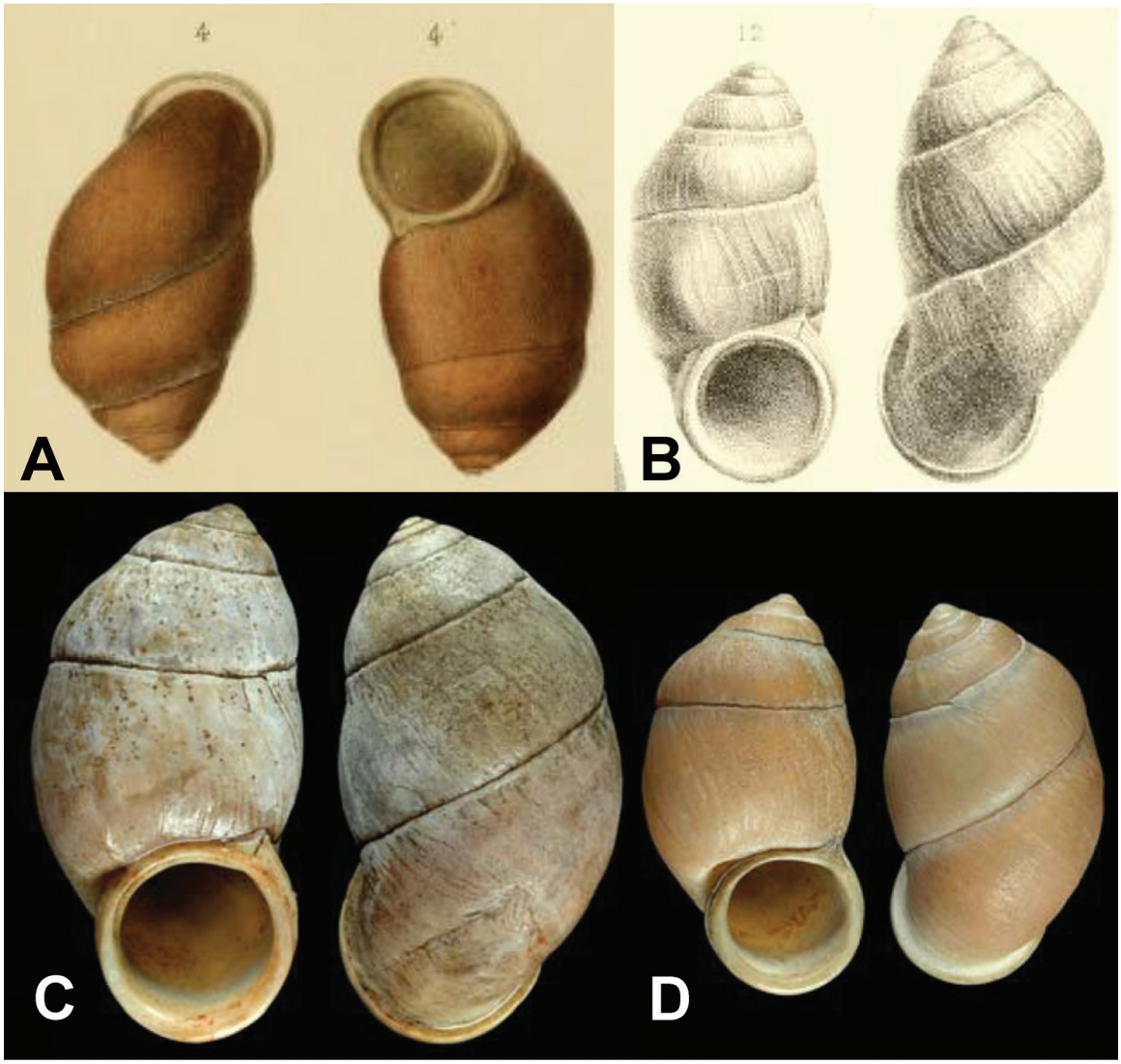

Figure 1. Original illustrations and type material of Vietnamese *Pollicaria*. **A**. *Pollicaria crossei* from Dautzenberg and d'Hamonville (1887). **B**. *P. rochebruni* from Mabille (1887b). No figure was provided in Mabille (1887a). **C**. *P. rochebruni*, lectotype MNHN 21305. **D**. *P. crossei*, lectotype MNHN 21304. Lectotype images from Kongim et al. (2013).

that recovered a monophyletic, well-supported Pupinidae sister to Cochlostomatidae. *Pollicaria* was resolved as sister to a clade of all other pupinids (Figure 4).

Discussion

Based on museum, online, and our own data, we believe that at least two, and possibly three, species of *Pollicaria* occur or have historically occurred in Vietnam. We consider the first, *P. rochebruni*, to comprise both *P. rochebruni sensu stricto* and *P. crossei* pre-Kongin et al. (2013). We treat *P. rochebruni* and *P. crossei* as synonyms based on the similarity of their original descriptions, the natural variation seen among shells of both species, and preliminary molecular evidence. The name *P. rochebruni* (publication date 14 May 1887) has priority over *P. crossei* (1 July 1887) and should be applied from hereon. We follow Kongim et al. (2013) in keeping *P. rochebruni* separate from *P. gravida*, given the lack of specimens of *Pollicaria* with an internally shouldered peristome from between Burma and Vietnam. This implies that the two species are geographically separate; based on the underlying geology, those regions separated approximately 40 million years ago (Yin 2010). However, since museum records only document presence not absence, future collections may suggest a continuous distribution. Additional karyotype and sequence analysis is needed to confirm or refute the separation between topotypic *P. gravida* and *P. rochebruni*. The revised distribution of *P. rochebruni* now includes Vietnam south to Đà Nẵng, with a possibility of stretching northeast into southern China (Figure 5).

We identified the second Vietnamese *Pollicaria* species as *P. mouhoti*, based on Solem's (1966) identifying collections of shells with peristomes lacking an internal shoulder and possessing a flared and partially reflexed lip to that species. We identified seven museum specimens which keyed out to *P. mouhoti* based on Kongim et al.

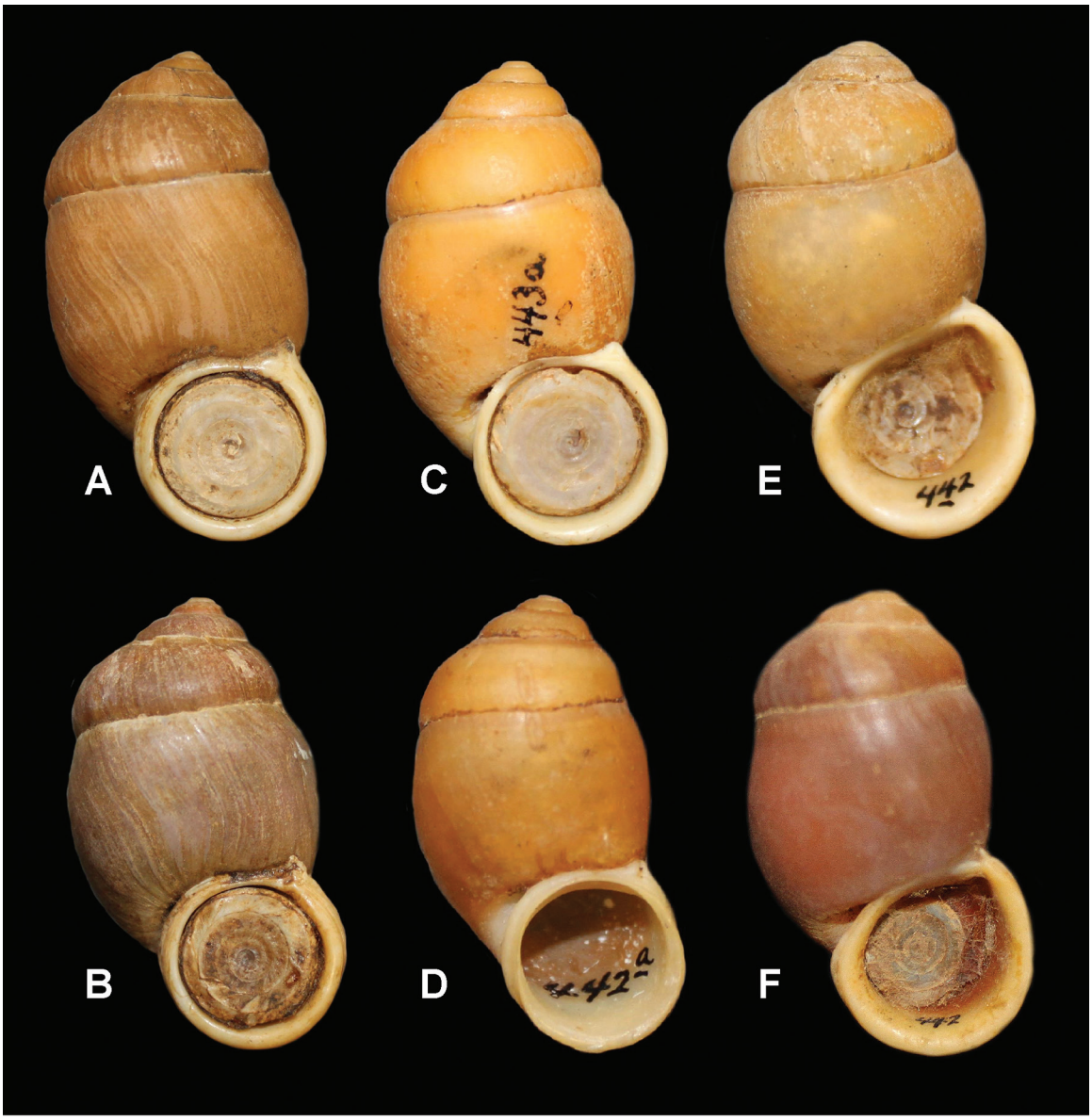

Figure 2. Vietnamese *Pollicaria* from museum collections. **A** and **B**. FLMNH 130267, *Pollicaria rochebruni*, Tonkin Province, 36.9 mm and 34.4 mm respectively. **C**. FLMNH 164622 (in part), *P.* cf. *crossei sensu* Kongim et al. (2013), Thất Khê, Tonkin Province. 28.5 mm. Labeled as *P. rochebruni*. **D**. FLMNH 164621 (in part), *P.* cf. *crossei sensu* Kongim et al. (2013), Pak-Kha, Tonkin Province, 24.6 mm. Labeled as *P. mouhoti*. **E** and **F**. FLMNH 164620, *P. mouhoti*, Gia Phu, Tonkin Province, 32.5 mm and 30.9 mm respectively.

(2013). Shells of *P. mouhoti* were collected by Solem (1966) in Vat Son, and we found additional museum specimens from Thái Nguyên, Gia Phu, Muong, and Bắc Kạn. Internet records suggest similar shells can be found in southern China, but it is unknown if they are the same species. The distribution of *P. mouhoti* in Vietnam appears restricted to isolated areas in the north.

A possible third Vietnamese *Pollicaria* species is based on karyotype differences that Kongim et al. (2010) uncovered in nominal *P. gravida* from Vietnam. Kongim et al. (2013) assigned their two *P. gravida* karyoptyes to *P. rochebruni* and *P. crossei*, the former having a large brown shell and the latter a small orange shell. We did not generate chromosome spreads for any individuals, but based on the figures in Kongim et al. (2010) we find the karyotypes between the brown and orange individuals to be equivocal. We also feel the radula differences listed in Kongim et al. (2013) are non-diagnostic characters. Kongim et al. (2010) believed that their small, orange-shelled snail was the result of sympatric speciation due to chromosomal evolution as outlined in King (1993). This hypothesis is consistent with studies of similar patterns of intra- and interspecific karyotype variation seen elsewhere (e.g. Ayala and Coluzzi 2005). Whereas authors have provided possible selective pressures that may accompany the karyotypic divergence (e.g. elevation and temperature), there is insufficient data available to pose any similar hypothesis for this *Pollicaria* species. Given

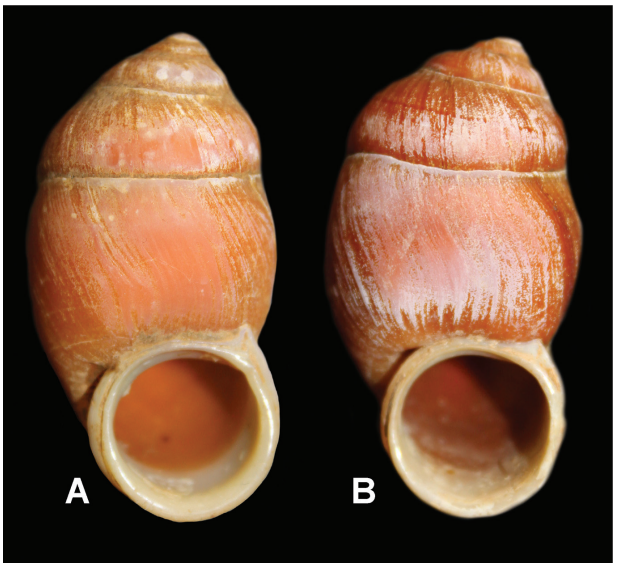

Figure 3. *Pollicaria* specimens sequenced in this study. **A**. CM 155302, orange phenotype (cf. *P. crossei sensu* Kongim et al. [2013]), 38.6 mm. **B**. CM 155307, red-brown phenotype (cf. *P. rochebruni*), 24.7 mm.

our synonymizing of *P. crossei* with *P. rochebruni*, if the individuals with the unique karyotype represent a new species it will require a new name. The known distribution of this possible taxon is limited to Cúc Phương National Park and Hữu Liên nature preserve (Kongim et al. 2013).

Our preliminary molecular evidence suggests that shell color and size may not be reliable for consistent species identification in Vietnamese *Pollicaria*. Sequences from one small, reddish-brown shelled snail (cf. *P. rochebruni*) and one large, orange-shelled snail (cf. *P. crossei*) were identical at three genetic loci. Our orange-shelled snail was larger than the nominal *P. crossei* in Kongim et al. (2013), and was collected alongside similarly-sized individuals with brownish and reddish shells. We feel both of our sequenced specimens represent *P. rochebruni*, supporting our belief that shell color in *Pollicaria* species can vary greatly. This contrasts with Kongim et al. (2013), who felt that shell color was diagnostic for many species.

Despite our best efforts and those of Kongim et al. (2013) to untangle the taxonomy of *Pollicaria*, we recognize the need for more work on the genus. Karyotypes of topotypic *P. gravida* remain unknown so no comparisons to the Vietnamese and other taxa can be made. Additional field work in southeastern Asia and southern China is needed to determine the true ranges of each species.

Systematic accounts

Family Pupinidae

Genus *Pollicaria* Gould, 1856

Type species. *Megalostoma gravidum* Benson, 1856 (=*Cyclostoma pollex* Gould, 1856), designated by Pain (1974).

Pollicaria rochebruni (Mabille, 1887)

Hybocystis rochebruni Mabille 1887a:12; Mabille 1887b: 138, pl. 2, figs 12 and 13; Dautzenberg and Fischer 1905:171
Pollicaria rochebruni – Kobelt 1902: 290; Kongim et al. 2013: 35, figs 5D and 5E
Hybocystis crossei Dautzenberg and d'Hamonville 1887: 220, pl. 8, fig. 4; Kobelt and Möllendorf 1899: 137; Kobelt 1902: 290; Dautzenberg and Fischer 1905:171
Pollicaria crossei – Kongim et al. 2013: 37, fig. 5F
Hybocystis gravida – Pain 1974: 174.
Pollicaria gravida – Kongim et al. 2010: 127 ("big brown shell")

Type Designation and locality. Lectotype MNHN 21305, Tonkin. Paralectotypes MNHN 25855, Tonkin (Kongim et al. 2013).

Material examined. All material from Vietnam / Tonkin Province. The number in parentheses is the number of shells from each lot assigned to the species. Locality names have been corrected wherever possible. – CM 155301, south of QL279, Thẩm Dương, Văn Bàn District, Lao Cai (7); CM 155302, Vườn quốc gia Cúc Phương, Lạc Sơn District, Hòa Bình (3); CM 155303, south of QL279, Thẩm Dương, Văn Bàn District, Lao Cai (7); CM 155304, limestone mountain at bridge into Tan Moui, near intersection of QL279 and DT234B, southwest of Chi Lăng, Lạng Sơn (6); CM 155305, ca. 60 m north of the intersection of QL1A and DT234B, Chi Lăng, Lạng Sơn (6); CM 155306, limestone mountain off QL279 at H9/155 road marker, ca. 1.1 km southwest of Dong Mo and 0.9 km southwest of Chi Lăng, Lạng Sơn (1); CM 155307, Công An huyện Cao Phong at QL6, Cao Phong, Hòa Bình (15); FLMNH 130259, Thất Khê (4); FLMNH 130265, Phu Ly (3); FLMNH 130623, Thái Nguyên (1); FLMNH 130258, Bắc Kạn (3); FLMNH 130261, Thái Nguyên (3); FLMNH 130262, Thái Nguyên (3); FLMNH 130624, Phi Mi (1); FLMNH 130260, Thái Nguyên (2); FLMNH 130268 (1); FLMNH 243794, Thái Nguyên (2); FLMNH 271764, Ninh Bình Province, Cúc Phương National Park, path to fairy cave, primary forest (1); FLMNH 164619, Thái Nguyên (2); FLMNH 164620, Gia Phu (2); FLMNH 164621, Pak-Kha (2); FLMNH 164622, Thất Khê (3); FLMNH 164623, Chợ Rã (4); FLMNH 130267 (2); FLMNH 242525 (2); FLMNH 192609, Thất Khê (6); FLMNH 130266 (1); MCZ 180001, vicinity of Lake Ba Bể (2); MCZ 180002 (2); MCZ 180003, Thất Khê (2); MCZ 180004, vicinity of Lake Ba Bể (3); MCZ 180005, Thái Nguyên (1); MCZ 180006, Thái Nguyên (4); MCZ 180007, Pak-Kha (4); MCZ 180008, Bảo Lạc (2); MCZ 180009, Pak-Kha (1); MCZ 197272, Pak-Kha (4); MCZ 380546 (1); USNM 207972, Lào Cai (1); USNM 207973, Lào Cai (1); USNM 207974, Chợ Rã (1); USNM 207975, Bắc Kạn (1); USNM 207976, Chợ Rã (1); USNM 207977, Chợ Rã (1); USNM 207978, Muong (1); USNM 207979, Muong (1); USNM 207980, Vân Sơn (1); USNM 207981, Vân Sơn (1); USNM 220045, Ba Bể (3); USNM 335963, Bắc Kạn (2).

Literature records (not examined). All from Vietnam, taken from Kongim et al. (2013). CUMZ 1523, Phong Na

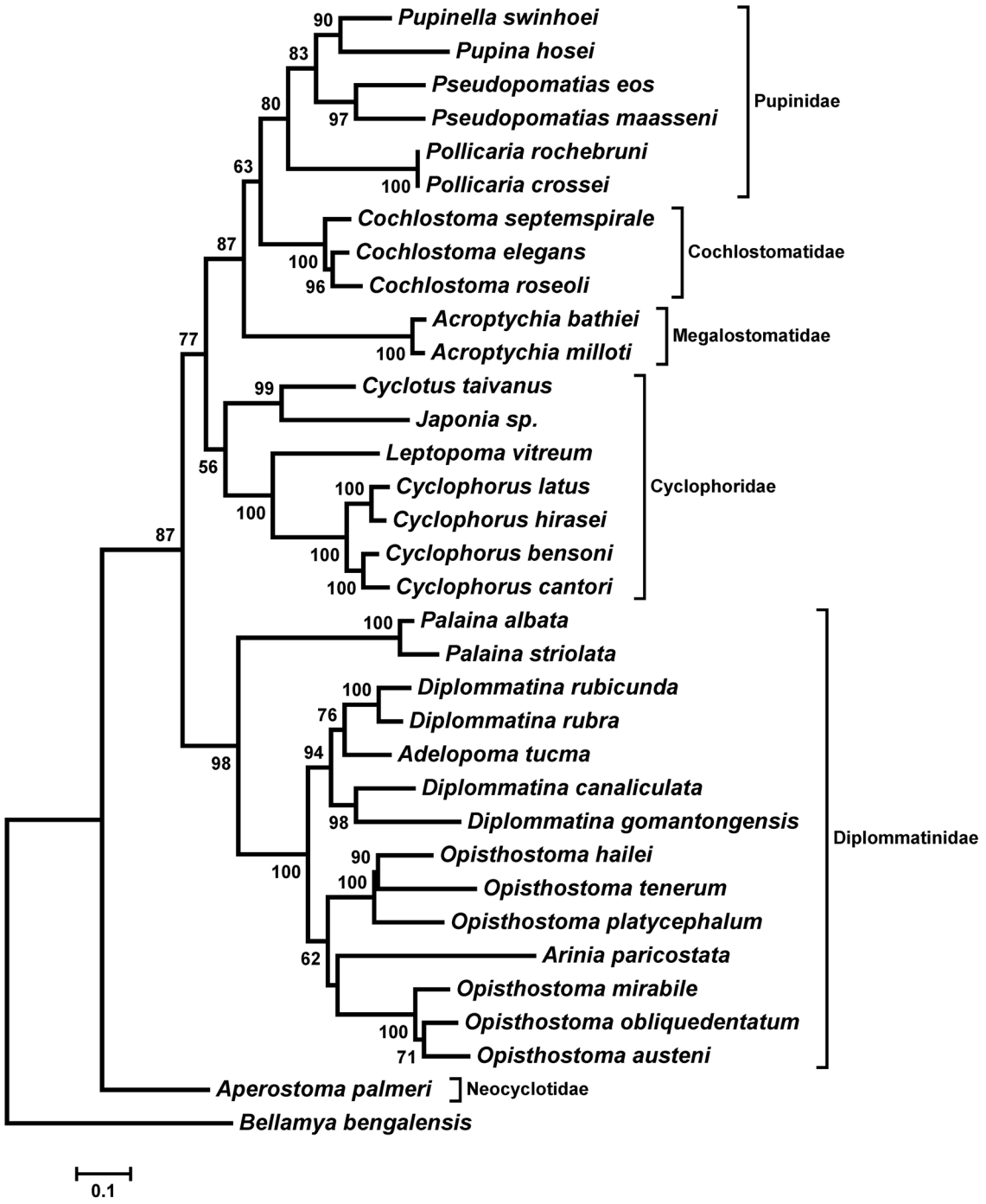

Figure 4. Phylogenetic tree (log likelihood= -17304.7519) based on three ribosomal genes (mt 16S, nuclear 18S and 28S) showing *Pollicaria* as sister to all other pupinid taxa. Values at branches are bootstrap values based on 10,000 ultrafast replicates.

National Park, Quảng Bình; CUMZ 1532, Cúc Phương National Park, Ninh Bình; CUMZ 1539, Phong Na National Park, Quảng Bình; CUMZ 1552, Phong Na National Park, Quảng Bình; CUMZ 1556, Bạch Mã National Park; CUMZ 1568, Phong Na National Park, Quảng Bình; CUMZ 1568, Cúc Phương National Park, Ninh Bình; CUMZ 1573, Cúc Phương National Park, Ninh Bình; CUMZ 1587, Cúc Phương National Park, Ninh Bình; CUMZ 1589, Khe Sen, Đà Nẵng; CUMZ 1594, Hữu Liên Nature preserve; MNHN 21304, Than Moi, Tonkin; RBINS 525390, Than Moi, Tonkin.

Description. Pupoid shell, to 45 mm length and 20 mm diameter, color varying from tan to orange, reddish-brown, and dark brown, possibly dark purple. Old specimens are often finely pitted. Whorls 5-7, transverse growth lines present, sutures linear and slightly impressed, apex obtuse and occasionally distorted to the left (assuming standard

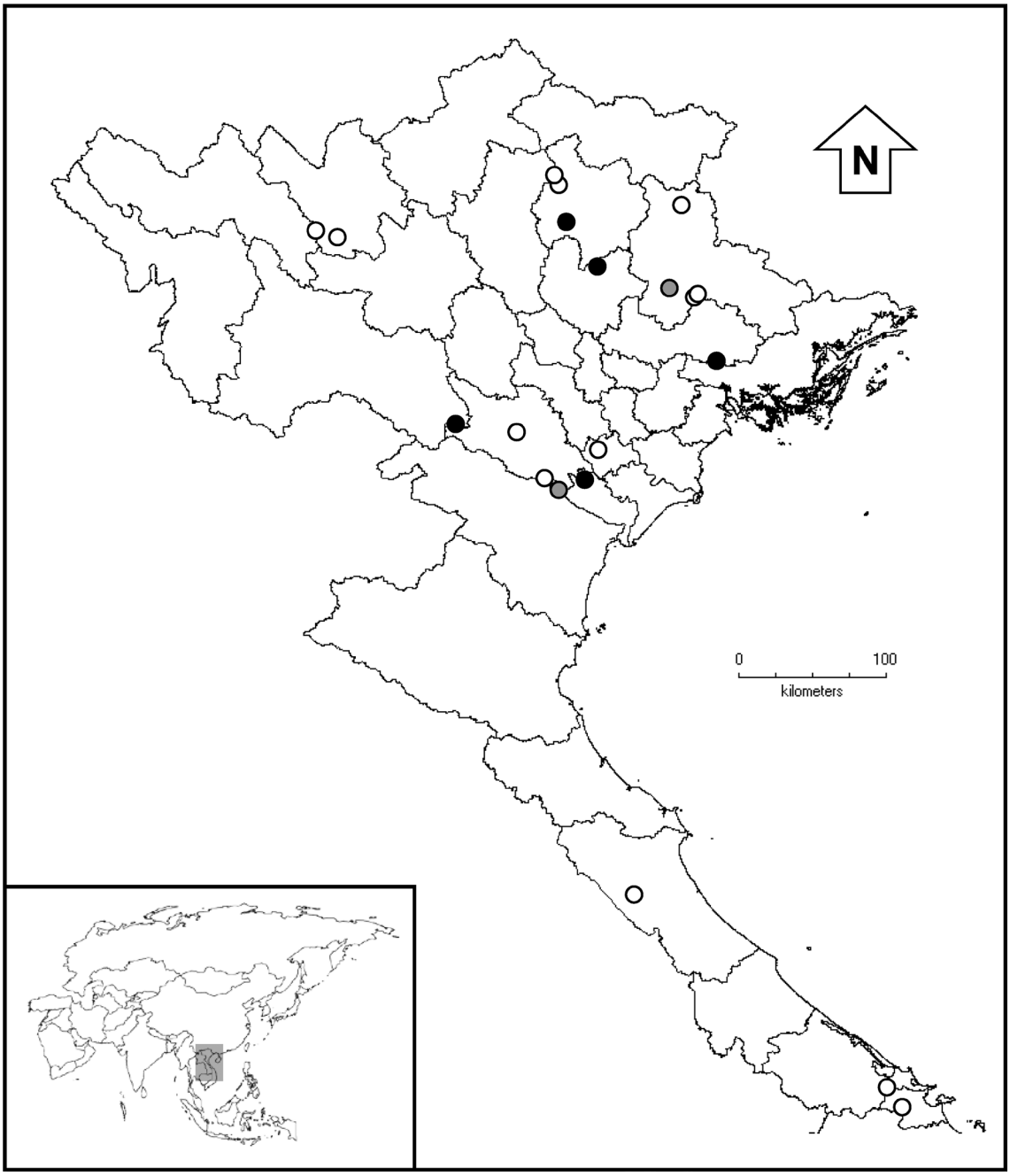

Figure 5. Revised distribution map for North Vietnamese *Pollicaria* species. *Pollicaria rochebruni* is present at all sites shown. Black dots indicate the presence of *P. mouhoti*, while gray dots indicate the presence of the putative new species of Kongim et al. (2013). Coordinates for localities taken from museum data were estimated from historical sources.

anatomical position), spire short. Penultimate body whorl may appear inflated relative to whorls on either side. Aperture circular with a right posterior triangular breathing structure. Peristome thickened, white to shell colored, with a thin parietal declining shoulder inside; umbilicus narrow. Operculum concentric, thick, and calcareous.

Radula with teeth in a 2-1-1-1-2 arrangement, based on the figure in Kongim et al. (2013: Figure 6E). Central tooth with three cusps, the middle one longer that the others. Lateral teeth with one large cusp and one smaller cusp on the central side. Marginals with one large central cusp flanked by one smaller cusp on each time. Kongim et al. (2013) described the radula as having more cusps per tooth than illustrated.

Distribution. Northern Vietnam south to Đà Nẵng (Figure 5). Possibly in Guangxi autonomous region and Yunnan Province, China.

Discussion. By synonymizing *P. rochebruni* and *P. crossei*, we suggest that all *Pollicaria* specimens from Vietnam that possess an interiorly shouldered peristome are a single species, excluding some smaller (< 35 mm) individuals with bright orange shells. We found live specimens of *P. rochebruni* at the bases of and in the valleys between karst outcrops in northern Vietnam. Reports from local farmers indicated that snails are more abundant during the spring rains, when native residents eat them as a delicacy. Some Chinese shells found for sale on the Internet are consistent morphologically with *P. rochebruni* but may represent a separate species.

Acknowledgements

RLM was supported by Faculty Research Support Funds through UHCL. PMH was supported in part by the UA Research Grants Committee, and a UA College Academy of Research, Scholarship, and Creativity award. Images of original figures (*P. rochebruni* and *P. crossei*) were downloaded from Biodiversity Heritage Library and are in the public domain. Images in figure 1 are reproduced from Kongim et al. (2013) under the terms of the Creative Commons Attribution License 3.0.

References

Ayala FJ, Coluzzi M (2005) Chromosome speciation: humans, drosophila, and mosquitos. Proceedings of the National Academy of Sciences USA 102(S1): 6535–6542. https://doi.org/10.1073/pnas.0501847102

Benson WH (1856) Characters of seventeen new forms of the Cyclostomacea from the British Provinces of Burmah, collected by W. Theobald, jun., Esq. Annals and Magazine of Natural History Series 2 17(99): 225–233. https://doi.org/10.1080/00222935608697501

Benson WH (1859) Observations on the shell and animal of *Hybocystis*, a new genus of Cyclostomatidae, based on *Megalomastoma gravidum* and *Otopoma blennus* B.; with notes on other living shells from India and Burmah. Annals and Magazine of Natural History Series 3 4(20): 90–93. http://www.tandfonline.com/doi/abs/10.1080/00222935908697091

Benson WH (1860) Notes on the subgenus *Corilla*, H. and A. Adams; and on the group *Plectopylis*, Benson; also on *Pollicaria*, Gould, and *Hybocystis*, Benson. Annals and Magazine of Natural History Series 3 6(32): 98–100. http://www.tandfonline.com/doi/abs/10.1080/00222936008697288

Crosse H (1885) Etude monographique sur les espèces du genre *Hybocystis* de Benson. Journal de Conchyliologie 33: 180–193. http://biodiversitylibrary.org/page/15979546

Dautzenberg P, d'Hamonville L (1887) Description d'Espèces nouvelles du Tonkin et observations sur quelques autres Mollusques de la même région. Journal de Conchyliologie 35: 213–225. http://biodiversitylibrary.org/page/15921460

Dautzenberg P, Fischer P (1905) Liste de Mollusques récoltés par M. le Capitaine de Frégate Blaise au Tonkin, et descriptions d'espèces nouvelles. Journal de Conchyliologie 53 (2nd quarter): 85–234. http://biodiversitylibrary.org/page/16292495

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Giribet G, Carranza S, Baguña J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada+Arthropoda clade. Molecular Biology and Evolution 13(1): 76–84. https://doi.org/10.1093/oxfordjournals.molbev.a025573

Gould AA (1856) [Descriptions of fourteen shells]. Proceedings of the Boston Society of Natural History 6: 11–16. http://biodiversitylibrary.org/page/9493210

Haines WA (1858) Descriptions of four new species of terrestrial shells from Siam. Annals of the Lyceum of Natural History of New York 6(1): 157–158. https://doi.org/10.1111/j.1749-6632.1858.tb00358.x

Hasegawa M, Kishino H, Yano T-A (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22(2): 160–174. https://doi.org/10.1007/BF02101694

Hijmans R, Guarino L, Mathur P, Jarvis A (2008) DIVA-GIS. http://www.diva-gis.org

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (Ed.) Mammalian Protein Metabolism (vol. 3). Academic Press, 21–132. https://doi.org/10.1016/B978-1-4832-3211-9.50009-7

King M (1993) Species Evolution: the Role of Chromosome Change. Cambridge University Press, Cambridge, 360 pp.

Kongim B, Sutcharit C, Tongkerd P, Tan S-HA, Quynh NX, Naggs F, Panha S (2010) Karyotype variations in the genus *Pollicaria* (Caenogastropoda: Pupinidae). Zoological Studies 49(1): 125–131. http://zoolstud.sinica.edu.tw/Journals/49.1/125.pdf

Kongim B, Sutcharit C, Naggs F, Panha S (2013) Taxonomic revision of the Elephant Pupinind snail genus *Pollicaria* Gould, 1856 (Prosobranchia, Pupinidae). ZooKeys 287: 19–40. https://doi.org/10.3897/zookeys.287.4617

Mabille MJ (1887a) Molluscorum Tonkinorum diagnoses. Imprimé par A. Masson, Meulan, 18 pp.

Mabille MJ (1887b) Sur quelques mollusques du Tonkin. Bulletin de la Société Malacologique de France 4: 73–164. http://biodiversitylibrary.org/page/14883892

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

de Morgan J (1885) Mollusques terrestres et fluviatiles du Royaume de Pérak et des pays voisins (Presqu'ile Malaise). Bulletin de la Société Zoologique de France 10: 353–428. https://doi.org/10.5962/bhl.part.14300

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) Iq-tree: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Pain T (1974) The land operculate genus *Pollicaria* Gould (Gastropoda), a systematic revision. Journal of Conchology 28(3): 173–178.

Park J-K, Ó Foighil D (2000) Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. Molecular Phylogenetics and Evolution 14(1): 75–88. https://doi.org/10.1006/mpev.1999.0691

Palumbi S, Martin A, Romano S, McMillan W, Stice L, Grabowski G (1991) The Simple Fool's Guide to PCR, Version 2.0. University of Hawaii, Honolulu.

Pfeiffer L (1862) Descriptions of thirty-six new land shells, from the collection of H. Cuming, Esq. Proceedings of the Zoological Society of London 3: 268–278. https://doi.org/10.1111/j.1469-7998.1862.tb06529.x

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard R (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences USA 81(24): 8014–8018. https://doi.org/10.1073/pnas.81.24.8014

Solem A (1966) Some non-marine molluscs from Thailand, with notes on classification of the Helicarionidae. Spolia Zoologica Musei Hauniensis 24: 1–110. http://www.vliz.be/imisdocs/publications/261969.pdf

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3): 512–526. http://mbe.oxfordjournals.org/content/10/3/512.long

Turney S, Cameron ER, Cloutier CA, Buddle CM (2015) Non-repeatable science: assessing the frequency of voucher specimen deposition reveals that most arthropod research cannot be verified. PeerJ 3: e1168. https://doi.org/10.7717/peerj.1168

Yin A (2010) Cenozoic tectonic evolution of Asia: a preliminary synthesis. Tectonophysics 488(1–4): 293–325. https://doi.org/10.1016/j.tecto.2009.06.002