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A new cryptic species of land snail from the Northern Territory, Australia (Stylommatophora, Camaenidae, *Parglogenia*)

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https://zoobank.org/08CD0830-C657-4D7A-A8C4-8049BBA3E7A5

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Academic editor: Thomas von Rintelen + Received 23 August 2022 + Accepted 23 September 2022 + Published 31 October 2022

Abstract

Parglogenia cobourgensis **sp. nov.**, a new species of camaenid land snail is described from Cobourg Peninsula, Top End of the Northern Territory in Australia. This new species has a shell that is identical to the type species of the genus, *Parglogenia pelodes*, which also occurs in the Top End. However, both species clearly differ in their reproductive anatomy and are also well-differentiated in terms of mitochondrial phylogenetics. A single specimen of a *Parglogenia* species from Croker Island, West Arnhem Land, is hypothesized to represent a third species based on details of its reproductive anatomy. However, only a single historical specimen was available for study. We therefore refrain from formally naming this species because of the incomplete information at hand. *Helix subgranosa* Le Guillou, 1842, a nominal species previously placed in *Parglogenia*, is synonymized with *Xanthomelon durvillii* (Hombron & Jacquinot, 1841).

Key Words

Eupulmonata, Helicoidea, land snail, Stylommatophora, taxonomy

Introduction

The camaenid Parglogenia Iredale, 1938 is a monotypic genus endemic to the Top End of the Northern Territory in the Australian Monsoon Tropics. Its only known member is Parglogenia pelodes (Pfeiffer, 1846), which is known to occur in the western part of the Top End, including the surroundings of Darwin, West Arnhem Land, the Cobourg Peninsula, and the Tiwi Islands. Another camaenid that exhibits a rather similar shell in terms of overall size and shape is Arnemelassa creedi (Cox, 1868). Arnemelassa creedi replaces Parglogenia in the eastern part of the Top End and the species are not known to occur in sympatry. Despite their conchological similarity, both Parglogenia and Arnemelassa exhibit vastly different reproductive anatomies indicating that despite their similar shell these species are not closely related (Köhler 2012).

Historically, the identity and delimitation of *Parglogenia pelodes* has been somewhat controversial mainly because of the imprecise type locality and the fact that no type material was known to exist. To remove nomenclatural uncertainty, Köhler (2012) designated a neotype using a specimen that was previously illustrated by Solem (1979), notably including the taxonomically significant reproductive anatomy. As a consequence of this type designation, the type locality of *Parglogenia pelodes* was restricted to Darwin, Dudley Point. Köhler (2012) also figured the reproductive anatomy of another specimen of *P. pelodes* from Melville Island, which was found to correspond closely with the anatomy of the neotype as published by Solem (1979).

Subsequently, mitochondrial DNA sequences of *Parglogenia* and *Arnemelassa* have been included in a phylogenetic study of the Camaenidae from north-western Australia. This study confirmed that the taxa are

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indeed not immediately related although belonging to the same principal clade (Köhler and Criscione 2015). The sequences of *Parglogenia pelodes* used in this study were from specimens collected on the Cobourg Peninsula, about 200 km east of the type locality in Darwin. In the present study, we sequenced additional specimens of *P. pelodes*, including topotypic specimens from Darwin. In addition, we have examined the reproductive anatomy of museum specimens from Cobourg Peninsula and Croker Island from the north-eastern part of the known range of *Parglogenia*. These examinations have unearthed a previously unrecognised species, which is indistinguishable from *P. pelodes* in terms of its shell morphology but exhibits a sufficiently distinct reproductive anatomy to warrant its description as a new species.

Our report of a new, morphologically cryptic species in *Parglogenia* adds to earlier discoveries of cryptic camaenid land snail species in the Northern Territory. Notably, we showed earlier that the Top End harbours three cryptic species of *Xanthomelon* that cannot be distinguished from each other by their shell (Köhler and Burghardt 2016). The existence of such cryptic species has been mainly attributed to conservativism in shell shape in snails that are well adapted to living in a relatively homogenous, yet harsh environment (Criscione and Köhler 2013).

Material and methods

This study is based on analyses of ethanol preserved specimens and dry shell material, including types, deposited in the collections of the Australian Museum (AM) and the Museum and Art Gallery of the Northern Territory (MAGNT).

Dimensions of fully mature shells (as recognised by a finished apertural lip) were measured with callipers precise to 0.1 mm: Height of shell (**H**), diameter of shell (**D**). Whorls were counted as described by Köhler (2011). Genital anatomy was studied using a binocular microscope with drawing mirror.

Genomic DNA was extracted from small pieces of foot muscle using a QIAGEN DNA extraction kit for animal tissue following the standard procedure of the manual. Fragments of two mitochondrial genes, 16S rRNA (16S) and cytochrome c oxidase subunit 1 (COI), were amplified by PCR using the primer pairs 16Scs1 (5'-AAACAT-ACCTTTTGCATAATGG-3') (Chiba 1999) and 16Sbd1 (5'-CTGAACTCAGATCATGTAGG-3') (Sutcharit et al. 2007) and L1490 (5'-GGTCAACAAATCATAAAGA-TATTGG-3') and H2198 (5'-TAAACTTCAGGGTGAC-CAAAAAATCA-3') (Folmer et al. 1994), respectively. Reactions were performed with an annealing step of 60 s at 55 °C for 16S and at 50 °C for COI with elongation times of 60-90 s, respectively. PCR fragments were purified with ExoSAP (Affymetrix) and both strands were cycle sequenced by use of the PCR primers. Chromatograms were manually corrected for misreads, if necessary,

and forward and reverse strands were merged into one sequence contig using CodonCode Aligner v. 3.6.1 (Codon-Code Corporation). New sequences have been deposited in GenBank under the accession numbers ON534065-ON534074 and ON532885-ON532893, respectively. The 16S sequences were aligned using the online version of MAFFT (version 7.4) (Katoh et al. 2002) available at www. http://mafft.cbrc.jp/alignment/server/ by employing the iterative refinement method E-INS-i. We used the online version of Gblocks (Castresana 2000) to identify and remove unreliable alignment regions in the 16S alignment by employing options for a less stringent selection. The final sequence alignments of 16S and COI were concatenated into one partitioned dataset. Four partitions were designated: the entire 16S fragment plus each of the three codon positions of the COI fragment. Phylogenetic relationships were estimated by employing a maximum likelihood-based method of tree reconstruction (ML) by using IQ-TREE vs 1.6 (Nguyen et al. 2015). We used ModelFinder (Kalyaanamoorthy et al. 2017) integrated into IQ-Tree to identify the best-fit model of sequence evolution for each sequence partition. We employed Ultrafast Bootstrap Approximation (Minh et al. 2013) to estimate the statistical branch support of the best Maximum Likelihood tree.

Molecular results

Our sequence data set contained concatenated sequences of COI and 16S from 17 camaenids, of which nine sequences were of *Parglogenia*. The 16S alignment consisted of 732 base pairs and the COI of 655 base pairs. We selected several species that are more closely related to *Parglogenia* as outgroup representatives based on phylogenetic tree of north-western Australian Camaenidae presented by Köhler and Criscione (2015). Sequences of eight camaenids were retrieved from GenBank while sequences of nine samples have been produced herein. These new sequences are of seven individuals of *Parglogenia*, one individual of *Arnemelassa*, and one individual of *Chloritis eustoma* from the Solomon Islands (Table 1).

The phylogenetic tree (Fig. 1) shows all *Parglogenia* sequences to form a monophylum. The sequences of *Par-glogenia pelodes* from Darwin (Buffalo Creek and East Point Reserve) form the tight sister clade of *Parglogenia cobourgensis* sp. nov. from the Cobourg Peninsula.

Among the included species, *Parachloritis argilacea* from Timor-Leste is the sister taxon of *Parglogenia*. *Youwanjela wilsoni* from the Kimberley and *Chloritis eustoma* from Malaita, Solomon Islands are also more closely related.

Uncorrected genetic p-distances in COI were 0.14%– 0.4% (on average 0.3%) among sequences of *Parglogenia cobourgensis* (n = 3) and between 0 and 1.8% (on average 0.9%) among sequences of *Parglogenia pelodes* (n = 6). Interspecific genetic distances between both species ranged from 13.2% to 13.9% (on average 13.4%).



0.3

Figure 1. Maximum Likelihood bootstrap consensus tree for a concatenated alignment of mitochondrial 16S and COI sequences for selected Camaenidae from Northern Australia, Timor-Leste, and the Solomon Islands. Numbers next to branches indicate nodal support by means of ultrafast bootstrapping. Scale bar: 30% modelled sequence divergence.

Table	1. Sequences	used for p	hylogenetic	reconstruction [•]	with GenBank	and museum	registration r	numbers
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Species	Location	Museum Voucher	GenBank: 16S	GenBank: COI
Arnemelassa creedi	Australia, West Arnhem Land	AM C462990	HQ245442	KP085373
Arnemelassa creedi	Australia, West Arnhem Land	AM C459298	ON532885	ON534065
Chloritis eustoma	Solomon Islands, Malaita	AM C557142	ON532893	ON534074
Parachloritis argilacea	Timor-Leste	AM C471161	HG515231	HG515082
Parachloritis argilacea	Timor-Leste	AM C468971	HG515229	HG515084
Parglogenia cobourgensis	Australia, Cobourg Peninsula	AM C594396	KP085079	KP085395
Parglogenia cobourgensis	Australia, Cobourg Peninsula	AM C460965	HQ245508	ON534066
Parglogenia cobourgensis	Australia, Cobourg Peninsula	AM C460961	ON532886	ON534067
Parglogenia pelodes	Australia, Darwin, Buffalo Creek	NTM P63374	ON532887	ON534068
Parglogenia pelodes	Australia, Darwin, Buffalo Creek	NTM P63374	ON532888	ON534069
Parglogenia pelodes	Australia, Darwin, East Point Reserve	NTM P63373	ON532889	ON534070
Parglogenia pelodes	Australia, Darwin, East Point Reserve	NTM P63373	ON532890	ON534071
Parglogenia pelodes	Australia, Darwin, Buffalo Creek	AM C478455	ON532891	ON534072
Parglogenia pelodes	Australia, Darwin, Buffalo Creek	AM C478455	ON532892	ON534073
Torresitrachia bathurstensis	Australia, Kimberley	WAM S49549	KP085090	KP085409
Torresitrachia eclipsis	Australia, Kimberley	WAM \$49356	KP085093	KP085412
Youwanjela wilsoni	Australia, Kimberley	WAM \$49583	HE588141	KP085427

Taxonomy

Parglogenia Iredale, 1938

Type species. *Helix pelodes* Pfeiffer, 1846, by original designation.

Parglogenia pelodes (Pfeiffer, 1846)

- *Helix pelodes* Pfeiffer, 1846: 126 ('north coast of Australia'). Neotype FMNH 182450, Darwin, Dudley Point (12°24'S, 130°49'E), designated by Köhler (2012).
- *Helix prunum* Pfeiffer 1851 [in 1849–1853]: 54, pl. 74, figs 11–13; Reeve 1852 [in 1851–1854]: pl. 68, fig. 353; Cox 1868: 43, pl. 4, fig. 6 (not *Helix prunum* Férussac, 1821).
- Chloritis pseudoprunum Pilsbry, 1893: 271 ('Port Darwin'; Holotype ANSP 62411).
- Parglogenia pelodes Iredale 1938: 99; Solem 1979: 128–133, fig. 32, pls 11a, b.

Taxonomic remarks. Since its original description, the name *Helix pelodes* has been shrouded in uncertainty until relatively recently. Pfeiffer (1851 [in 1849–1853]), subsequently treated *H. pelodes* as a junior synonym of *Helix prunum* Férussac, 1821. Subsequently, Pilsbry



Figure 2. Shells. A. Parglogenia cobourgensis holotype AM C.594396; B. Parglogenia sp. 'Croker Island' AM; C. 121141. Scale bar: 10 mm.

(1890: 135) agreed with this synonymy, but accepted H. prunum as an eastern Australian species with uncertain systematic relationships. Based on this presumption, he considered that all previous authors had misapplied the name H. prunum for an unnamed species from Arnhem Land, for which Pilsbry (1893) introduced the new name Chloritis pseudoprunum. By contrast, Iredale (1938) and Solem (1979) considered that the supposed synonymy of H. pelodes with H. prunum was in error. While H. prunum continues to be a taxonomic enigma to this date (Köhler 2012), Helix pelodes was removed from its synonymy and treated as an accepted species, for which Iredale (1938) described the genus Parglogenia. This treatment rendered C. pseudoprunum a junior synonym of P. pelodes. Iredale (1938) included a second species in this genus, P. subgranosa (Le Guillou, 1842). Köhler (2012) rejected this treatment, arguing that the type of Helix subgranosa represented a juvenile shell of Xanthomelon durvillii (Hombron & Jacquinot, 1841). However, Köhler (2012) maintained H. subgranosa as a nomen inquirendum. To remove the remaining ambiguity, Helix subgranosa is herewith placed in the synonymy of X. durvillii.

Diagnosis. Shell relatively large (D = 14.3-31.5 mm, H = 10.8-20.3 mm; n = 92), with strongly and almost evenly elevated spire, comprising between 4.5 and 5.8 whorls. Apical sculpture of anastomosing ridges initially, becoming pustulated after first half whorl. Postapical whorls with microsculpture of very small, rather widely spaced setae and extremely fine ridgelets with weak radial ribs appearing on spire and body whorl. Umbilicus very narrow, partly covered by reflected lip, internally with crowded pustules. Body whorl globose, rounded, only slightly descending behind strongly reflected, thin, white lip. Shell light yellow brown, uniform. Vagina and

penis very long, atrium short, bursa copulatrix very short, without enlarged head. Free oviduct with glandular, convoluted walls. Vas deferens with bifurcated caecum on ascending arm and entering the epiphallus near insertion of penial retractor muscle without differentiation. A narrow, raised ridge separates the epiphallus from the penis; inner penial walls with longitudinal corrugated ridges; no penial sheath, verge, or epiphallic appendages present (Solem 1979; Köhler 2012).

Comparative remarks. Solem (1979) provided a detailed description of the shell and reproductive anatomy of this species based on examination of specimens from near Darwin. He remarked that the periostracal setae in *P. pelodes* were similar to those in *Semotrachia*, *Austrochloritis* and "*Chloritis argilacea*" (presently *Parachloritis argilacea*; see Köhler and Kessner 2014), but that the genital anatomy of *Parglogenia* was highly distinctive. Köhler (2012) illustrated the reproductive anatomy of a specimen of *P. pelodes* from Melville Island, which closely resembled that of specimens from Darwin. Shells of *P. pelodes*, including the holotype of *Chloritis pseudoprunum*, have been figured by Köhler (2012).

Parglogenia cobourgensis sp. nov.

https://zoobank.org/16BEC236-647A-49DF-954F-D346F576411C

Holotype. AUSTRALIA · 1 preserved specimen; Northern Territory, Cobourg Peninsula, 3.4 km NE of Black Point Ranger Station; 11°08'27.6"S, 132°10'12.0"E; vine thicket; leg. Vince Kessner, 4 Feb 2007; AM C.594396 (Fig. 2A).

Paratypes. AUSTRALIA · 1 preserved specimen; same data as holotype; AM C460965. 2 preserved specimens; Northern Territory, Cobourg Peninsula, Black Point nr barge landing; 11°9'18"S, 132°8'44"E; leg. Vince Kessner, 2 Feb 2007; AM C.460961.

Type locality. Australia, Northern Territory, Cobourg Peninsula, 3.4 km NE of Black Point Ranger Station (11°08'27.6"S, 132°10'12.0"E).

Etymology. For Cobourg Peninsula, where this species occurs.

Additional, non-type material. AUSTRALIA \cdot 1 dry shell; Northern Territory, Port Essington; 11°16'S, 132°9'E; leg. 1900; AM C.64926.

Description. Shell (Fig. 2A). Moderately large (D = 24.4-29.9 mm, H = 19.2-23.1 mm; n = 7), globose-conical with low domed spire, comprising 5 to 5.5 rounded whorls, moderately to rapidly increasing in diameter, separated by moderately to strongly incised suture. Teleoconch with microsculpture of widely to moderately spaced rounded pustules, in juveniles with short periostracal setae that are only retained along suture lines in adult specimens. Protoconch with microsculpture of elongate pustules arranged in oblique spirals. Umbilicus narrowly open. Outer lip moderately developed, outwardly reflected, without sulcus behind outer lip. Shell colour light brown, outer lip pale pinkish to white (Fig. 2A; based on 2 specimens).

Reproductive anatomy (Fig. 3C-F). Bursa copulatrix about a quarter to half of length of oviduct, with slightly enlarged to broad head, base broad above uterus junction becoming inflated and then narrowing before spermathecal head. Epiphallus broad and bulb-like or narrowing at apex, length equivalent to about one fifth to one tenth of length of penis; retractor muscle attached to apex of epiphallus; vas deferens attached at apex of epiphallus, next to penial retractor; with small bi-lobed caecum at about one quarter of its length. Penis straight to slightly kinked to bent above terminal end, cylindrical, about as long to twice as long as vagina. Penial walls very thick and muscular. Inner penial wall sculpture comprising lattice work of filaments below epiphallus, giving rise to longitudinal rows of interconnected thread-like filaments to corrugated pilasters toward genital pore; with one or two narrow, wrinkled longitudinal pilasters forming at around mid penis. Penial sheath absent (Fig. 3C-F; based on 2 specimens).

Head wart oval to trunk-shaped, about 2×3 to 3×5 mm in size (Fig. 3B); mantle roof typically camaenid (Fig. 3A).

Comparative remarks. Shell effectively indistinguishable from *Parglogenia pelodes*. Fewer shells of *P. cobourgensis* were available for examination. These completely overlapped in size range with *P. pelodes* occupying a smaller size range overall. This species differs from *P. pelodes* most conspicuously in having a completely different penial wall sculpture (*P. pelodes* has longitudinal corrugated ridges) and by having much shorter penis, epiphallus, vagina, and bursa copulatrix.

Distribution. The distribution of *Parglogenia cobourgensis* is difficult to delineate due to the paucity of suitable material. Here, the known distribution of *P. cobourgensis* is restricted to the Cobourg Peninsula and coastal parts of west Arnhem Land excluding Croker Island (see below). Denser sampling is required to delimitate the

distributions of *P. pelodes* and *P. cobourgensis* more accurately and to clarify the taxonomic status of the Croker Island population.

Parglogenia sp. nov. 'Croker Island'

Material examined. AUSTRALIA · 1 preserved specimen, 4 dried shells; Northern Territory, Croker Island, near airstrip; 11°10'0"S, 132°29'6"E; leg. 28 Mar 1980; AM C.121141 (Fig. 2B). 1 dried shell; Croker Island; 11°7'S, 132°33'E; leg. 28 Mar 1980; AM C.582514.

Description. Shell (Fig. 2B). Moderately large (D = 27.6–34.2 mm, H = 21.5–24.8 mm; n = 5), globose-conical with low domed spire, comprising 5 to 5.5 rounded whorls, moderately to rapidly increasing in diameter, separated by moderately to strongly incised suture. Teleoconch with microsculpture of widely to moderately spaced rounded pustules, in juveniles with short periostracal setae that are only retained along suture lines in adult specimens. Protoconch with microsculpture of elongate pustules arranged in oblique spirals. Umbilicus narrowly open. Outer lip moderately developed, outwardly reflected, without sulcus behind outer lip. Shell colour light brown, out lip pale pinkish to white (Fig. 2B).

Reproductive anatomy (Fig. 3G, H). Bursa copulatrix about a quarter to half of length of oviduct, with broad head, base broad above uterus junction, narrowing before spermathecal head. Epiphallus broad, narrowing at apex, length equivalent to about one fifth to one tenth of length of penis; retractor muscle attached to apex of epiphallus; vas deferens attached at apex of epiphallus, next to penial retractor; with small bi-lobed caecum at about one quarter of its length. Penis slightly kinked to bent above terminal end, cylindrical, about as long to twice as long as vagina. Penial walls very thick and muscular. Inner penial wall sculpture comprising lattice work of filaments below epiphallus, giving rise to longitudinal rows of interconnected thread-like filaments to corrugated pilasters toward genital pore; with one or two narrow, wrinkled longitudinal pilasters forming at around mid penis. Penial sheath absent (Fig. 3G, H).

Comparative remarks. Shell effectively indistinguishable from *P. pelodes* and *P. cobourgensis*. The few available specimens are at the larger end of the size distribution in this genus, but this might be a sampling artifact. Differs from *P. pelodes* most conspicuously in having a completely different penial wall sculpture that resembles *P. cobourgensis*. From *P. cobourgensis*, the Croker Island specimen differs in having a shorter bursa copulatrix, a broad spermathecal head, and a slightly longer epiphallus (one fifth of penis vs. one sixth to one tenth of penis length), inner penial wall has several longitudinal pilasters instead of only two.

Just a single historical specimen was available for dissection and not suitable for DNA extraction. For the paucity of suitable material, we refrain from a formal description of the Croker Island species.

Distribution. Presumably endemic to Croker Island.



Figure 3. Anatomy of *Parglogenia* species. **A–D.** *Parglogenia cobourgensis* holotype AM C.594396; **A.** Mantle roof; **B.** Head wart; **C.** Reproductive anatomy; **D.** Penial anatomy; **E. F.** *Parglogenia cobourgensis* paratype AM C.460961; **E.** Reproductive anatomy; **F.** Penial anatomy; **G. H.** *Parglogenia* sp. 'Croker Island' AM C. 121141; **G.** Reproductive anatomy; **H.** Penial anatomy. Abbreviations: ag, albumen gland; at, atrium; bc, bursa copulatrix; ca, caecum; ep, epiphallus; epv, efferent pulmonary vessel; hd, hermaphroditic duct; ipw, inner penial wall; me, mantle edge; mr, mantle roof; ne, nephridium; p, penis; re, rectum; so, spermoviduct; ue, ureter; va, vagina; vd, vas deferens; vt, ventricle. Scale bars: 10 mm (**A**, **D**, **F**, **G**); 2 mm (**B**).

Acknowledgements

Thanks are due to Vince Kessner for collecting much of the material used in the present study. We thank Isabel Hyman and Junn Foon for carefully reviewing an earlier version of this manuscript. Their comments have helped to improve the quality of this work.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Zoosystematics and Evolution

Jahr/Year: 2022

Band/Volume: 98

Autor(en)/Author(s): Köhler Frank, Shea Michael

Artikel/Article: <u>A new cryptic species of land snail from the Northern Territory</u>, <u>Australia (Stylommatophora, Camaenidae, Parglogenia) 427-433</u>