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A new species of land snail, *Xanthomelon amurndamilumila*, from the North East Isles off Groote Eylandt (= Ayangkidarrba), Gulf of Carpentaria, Australia (Stylommatophora, Camaenidae)

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Abstract

This paper introduces *Xanthomelon amurndamilumila* **sp. nov.**, a newly-discovered land snail species inhabiting the North East Isles, offshore from Groote Eylandt (= Ayangkidarrba), north-western Gulf of Carpentaria, in the Northern Territory, Australia. Specimens of this species were first collected during the 2021 Bush Blitz expedition to Groote Eylandt, a large offshore archipelago previously unexplored for land snails. The taxonomic status of the new species was established through a comprehensive analysis of comparative morphology and mitochondrial genetics: *X. amurndamilumila* forms a maximally supported clade closely related to *X. arnhemense* and is characterised by a unique combination of morphological characteristics, including smaller shell size, distinctive sculpture of collabral ridges and specific features of its reproductive anatomy. The genetic divergence and phylogenetic relationships suggest historical isolation. While the discovery of *X. amurndamilumila* **sp. nov.** enriches our understanding of land snail diversity in the Northern Territory, its conservation status is of concern on North East Island because of habitat degradation caused by feral deer.

Key Words

distribution, Gastropoda, Helicoidea, Pulmonata, taxonomy

Introduction

The genus *Xanthomelon* E. von Martens, 1860 comprises several species of Australian camaenid land snail, typically characterised by their large, globose shells of mostly uniform yellowish-brown to ochre colour. The distribution of this genus encompasses the Australian Monsoon Tropics as well as eastern Queensland. Of the 11 currently accepted species of *Xanthomelon*, five occur in the 'Top End' of the Northern Territory, according to the latest revision of the genus (Köhler and Burghardt 2016). These species exhibit varying degrees of morphological distinctive characteristics, including some that are highly similar with one another and have distributional ranges that vary from narrow to wide. It is a testament to our rather poor knowledge of land snails in the 'Top End' generally that two of these five species have been described only a few years ago. *Xanthomelon darwinense* was only described in the 21st century (Köhler and Burghardt 2016), even though it occurs in the Territories' capital, Darwin and is readily distinguished from most other congeners by its much smaller size. This species has a narrow distribution in and around Darwin and is currently known only from two separate locations.

In contrast, *X. arnhemense* Köhler & Burghardt, 2016 has a wide distribution, encompassing most of

Arnhem Land. However, this species had also remained unrecognised for at least a century due to its highly cryptic nature, even though specimens are well represented in museum collections. However, shells of *X. arnhemense* are indistinguishable from those of its close ally, *X. interpositum* Iredale, 1938. Indeed, these two species can be distinguished only through comparative reproductive anatomy or genetics. The previously-poor documentation of *Xanthomelon* is but one example of how the scarcity of modern systematic studies impairs the documentation of the Territories' land snail fauna to this day. The recent discovery of another undescribed species of land snail in the Northern Territory, *Parglogenia cobourgensis* Köhler & Shea, 2022, which had been overlooked for centuries due to its externally cryptic shell, is another example illustrating our slow progress towards a more complete documentation of the land snail fauna of the Northern Territory (Köhler and Shea 2022).

The presumably still incomplete knowledge of Australian land snails also hampers our ability to manage and conserve the fauna that has already been described. Indeed, Willan et al. (2009) showed that four species of *Torresitrachia* Iredale, 1939 described in 2009, which are endemic to the Daly Basin Bioregion and have very limited distribution ranges, are under threat of extinction due to the impact of changed fire regimes and the transformation of native savannah by introduced grasses.

To remedy the lack of documentation of biotic patterns throughout the Northern Territory, more systematic studies are urgently needed that employ contemporary methods and analyse evidence from multiple sources of information to reliably identify and delimit species. Oftentimes, new species may be discovered when understudied museum samples are examined, especially by using novel scientific techniques. *Parglogenia cobourgensis* and *Xanthomelon arnhemense* are two such species that were discovered in museum collections. Secondly, new discoveries may be made in hitherto under-surveyed areas.

In the present study, we examine newly-collected samples of a presumed new species that were collected on small islets offshore from Groote Eylandt, which were never surveyed for land snails previously. The specimens examined here were first collected in 2021 during the Bush Blitz expedition to Groote Eylandt that aimed to close a pre-existing survey gap for multiple groups of organisms through a targeted survey (Willan and Bourke 2022).

Preliminarily identified in the field by one of us (RCW) as a putative new species of *Xanthomelon*, we here employ comparative morphology and mitochondrial genetics to resolve its taxonomic status. To ensure an accurate taxonomic assessment, we compare all new data collected on this putative new species with the current knowledge of the *Xanthomelon* species in the 'Top End' as summarised in the latest systematic revision by Köhler and Burghardt (2016).

Materials and methods

The present study is based on samples collected on North East Island (= Amburrkba) and Hawk Island (= Ayangkijirumanja) in the Gulf of Carpentaria during the Bush Blitz on Groote Eylandt conducted in 2021. Additional specimens were collected in 2022 on Lane Island (= Milyekaluwakba) and also on Hawk Island (Fig. 1).

Most of the specimens have been deposited in the malacological collection of the Museum and Art Gallery of the Northern Territory in Darwin (**NTM**). Supplementary specimens have been deposited in the malacological collection of the Australian Museum in Sydney (**AM**).

Material was collected by hand while conducting visual searches of habitats on North East Island, the largest of the North East Isles. Living specimens were fixed and preserved in 95% ethanol. Shells were photographed and reproductive anatomy was studied using a binocular microscope with drawing mirror. Height of shell (**H**) and diameter (**D**) of fully mature shells as recognised by a finished apertural lip were measured with callipers precise to 0.1 mm and shell whorls were counted as described by Köhler (2011).

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′-AAACATACCTTTTGCATAATGG-3′) (Chiba 1999) and 16Sbd1 (5′-CTGAACTCAGATCATGTAGG-3′) (Sutcharit et al. 2007) and L1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and H2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-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 90 and 60 s, respectively. PCR fragments were purified with ExoSAP (Affymetrix) and both strands were cycle sequenced by use of the PCR primers. Chromatograms were merged into one sequence contig using Sequencher (Gene Codes Corporation. Ann Abor) and misreads were manually corrected where necessary. New sequences have been deposited in GenBank under the accession numbers OR610390-OR610392 and OR612298-OR612302. For the phylogenetic analyses, we built a sequence dataset by incorporating all available GenBank sequences of *Xanthomelon* (16S and COI). The 16S sequences were aligned using the online version of MAFFT (version 7.4) (Katoh et al. 2002) available at http://mafft.cbrc.jp/alignment/server/ by employing the iterative refinement method E-INS-i. The final sequence alignments of 16S and COI were concatenated into one partitioned dataset. Two partitions were designated, one for each gene fragment. Phylogenetic relationships were estimated by employing a Maximum Likelihood-based method of tree reconstruction (ML) using IQ-TREE v. 2.3 (Nguyen et al. 2015). We used the integrated ModelFinder (Kalyaanamoorthy et al. 2017) to identify the

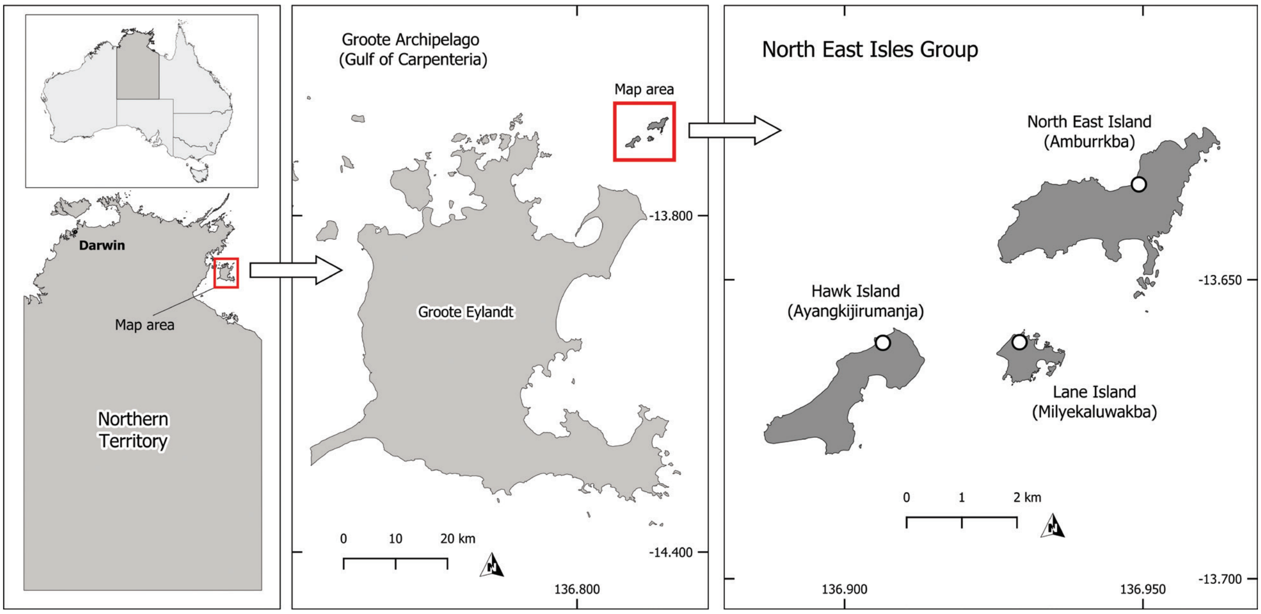

Figure 1. Map of the North East Isles off Groote Eylandt, Arnhem Land, Northern Territory. Specimens of *Xanthomelon amurndamilumila* sp. nov. have been found on North East Island as well as Hawk and Lane Islands. Small circles indicate sampling sites.

best-fit models 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.

Abbreviations used: **16S** = 16S rRNA; **COI** = cytochrome c oxidase subunit 1; **H** = shell height; **D** = shell diameter; **SD** = standard deviation.

Results

Mitochondrial phylogenetics

The concatenated sequence dataset of COI and 16S contained sequences from 79 individuals representing all the presently known *Xanthomelon* species (i.e. *X. arnhemense*, *X. darwinense* Köhler & Burghardt, 2016, *X. distractum* Iredale, 1938, *X. durvillii* (Hombron & Jacquinot, 1840), *X. interpositum*, *X. jannellei* (Le Guillou, 1842), *X. magnidicum* Iredale, 1938, *X. obliquirugosum* (E. A. Smith, 1894), *X. pachystylum* (L. Pfeiffer, 1845), *X. saginatum* Iredale, 1938) and five individuals of the presumed new species of *Xanthomelon* from the North East Isles (i.e. two from North East Island and three from Lane Island). In addition, we included *Quistrachia leptogramma* (L. Pfeiffer, 1846) as the outgroup to root the tree. This taxon was selected because *Qusitrachia* is the sister group of *Xanthomelon* in the phylogeny of north-western Australian Camaenidae (Köhler and Criscione 2015).

The COI sequences had a length of 655 bp and the 16S alignment consisted of 797 base pairs. Several samples missed either a COI or a 16S sequence, but all species were represented by individuals with complete sampling of both markers. ModelTest identified the General Time Reversible model with a gamma distributed rates (GTR+G+I) as the best-fit model of sequence evolution for both the COI and 16S sequences.

The Maximum Likelihood phylogram revealed all sequences of the putatively new species formed a maximally supported clade in a maximally supported sister group relationship with *X. arnhemense* (Fig. 2).

Uncorrected p-distances in COI ranged from 0.9% to 1.5% (average: 1.2%) amongst sequences of the putative new species (i.e. intraspecific genetic differentiation) and from 7.9% to 10.4% (average: 8.8%) between sequences of the putative new species and *X. arnhemense* (i.e. interspecific genetic differentiation). For comparison, the intraspecific p-distances in *X. arnhemense* ranged from 2.3 to 7.5% (on average 4.8%).

Comparative morphology

The putative new species from the North East Isles differed from most congeners in having relatively strong collabral ribs on the shell. Collabral ribs are also present in *X. durvillii* and *X. arnhemense*, but are considerably weaker in development. We measured 75 specimens of the putative new species (54 from North East Island, 12 from Hawk Island, 9 from Lane Island) and found that the shells on average were significantly smaller than *Xanthomelon* shells from the closest land mass, which is mainland Groote Eylandt (= *X. arnhemense*; see Köhler and Burghardt (2016)) (Fig. 3). Shells of the putative new species measured between 13.6 and 21.5 mm in height (mean = 17.7 mm, SD = 2.1) and between 15.5 and 24.2 mm in diameter (mean = 19.6, SD = 1.9). By contrast, 25 shells of *X. arnhemense* from mainland Groote Eylandt were between 19.1 and 37.1 mm (mean = 28.8 mm, SD = 4.7) high and between 21.6 and 39.5 mm (mean = 30.2 mm, SD = 4.8) wide (Fig. 3).

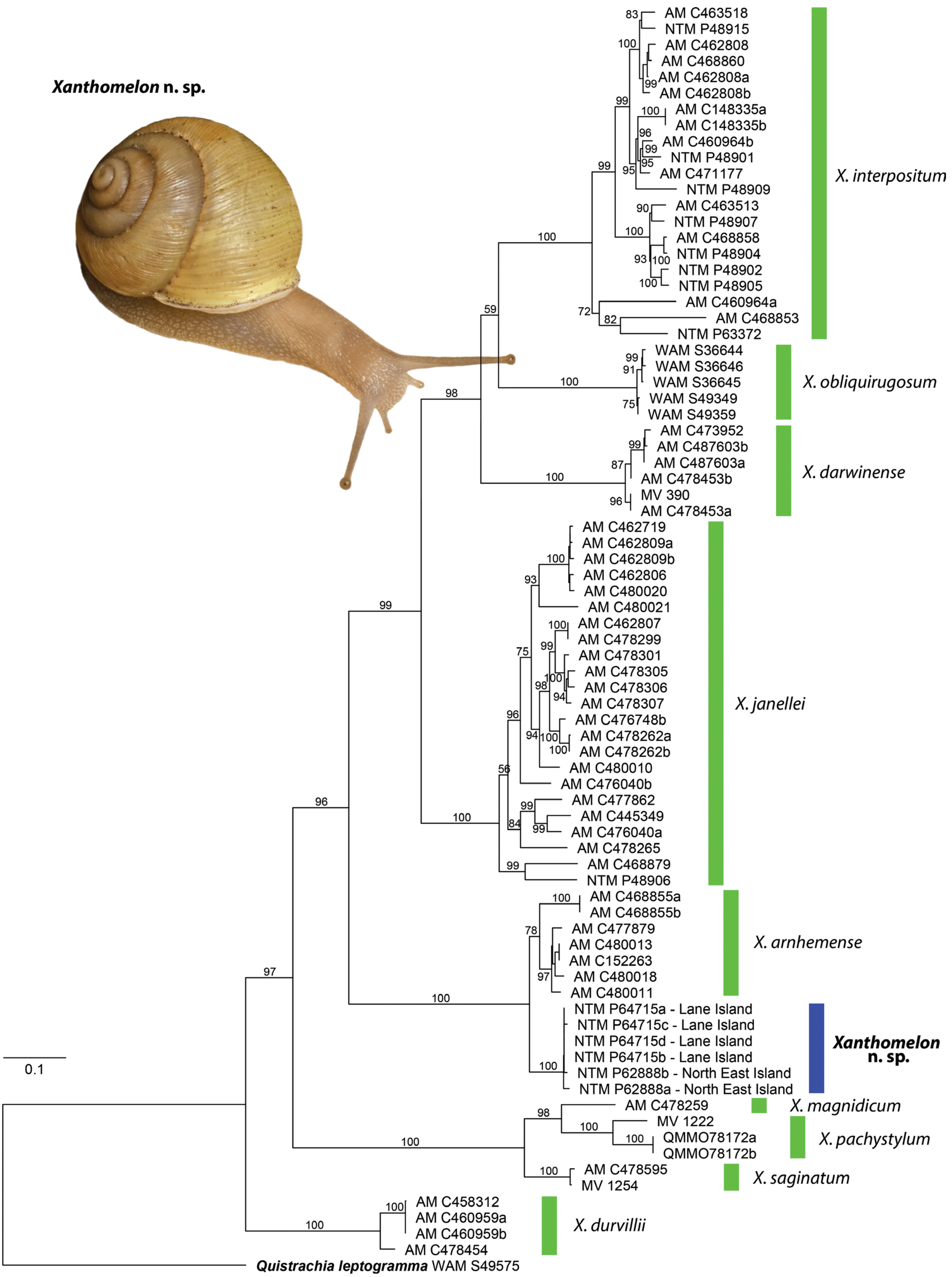

Figure 2. Maximum Likelihood phylogram based on analysis of a concatenated alignment of partial 16S and COI sequences using IQ-Tree. Numbers on branches indicate nodal support by 10,000 ultrafast bootstrap replicates. Sequences of *Quistrachia leptogramma* were used to root this tree. Scale bar indicating modelled evolutionary distance of 10%.

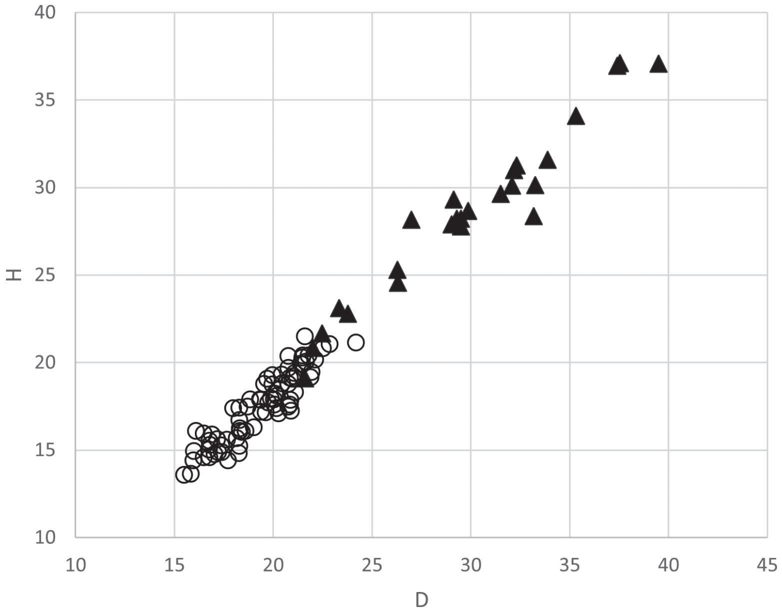

Figure 3. Shell size comparison between the putative new species (circles) and *X. arnhemense* from adjacent Groote Eylandt (full triangles) by plotting shell height (H) against shell diameter (D).

The reproductive anatomy of two specimens from North East Island was examined and found to differ significantly from the reproductive anatomy of *X. durvillii*, *X. interpositum* and *X. darwinense* as depicted by Köhler & Burghardt (2016: fig. 6A-C). Amongst other features, all these species differed most noticeably in having a long epiphallus (1.5 to 3 times longer than penis). By contrast, the epiphallus in specimens from the North East Isles is much shorter relative to the length of the penis (approx. 0.75 of penis length). Both *X. jannellei* and *X. arnhemense* have a similar configuration of reproductive organs, particularly lacking a well-developed epiphallus (i.e. epiphallus shorter than penis; see Köhler & Burghardt (2016: fig. 6D-E). Of these two species, *X. jannellei* is distinguished from the other two species in having a densely pustulated apical portion of the inner penial wall, a feature which is not exhibited by *X. arnhemense* or the putative new species. Indeed, the putative new species and *X. arnhemense* do not differ notably in their reproductive anatomy.

Taxonomic description

Xanthomelon amurndamilumila sp. nov.

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Type locality. Australia • Northern Territory, Groote Eylandt (= Ayangkidarrba), North East Isles, North East Island (= Amburrkba), 13°38'13.2"S, 136°56'34.5"E.

Holotype NTM P.65134 (1 preserved specimen, dissected, shell broken; Fig. 4), from type locality, coll. A.J. Bourke, 18 June 2021.

Paratypes NTM P.62888 (14 preserved specimens; Fig. 5B), AM C.548628 (4 preserved specimens), from type locality, coll. A.J. Bourke, 18 June 2021.

Additional (non-type) specimens examined. North East Isles, North East Island (= Amburrkba), NTM P.62774 (59 dried shells), coll. R.C. Willan & A.J. Bourke, 18 June 2021; NTM P.62904 (23 dried shells), coll. R.C. Willan & A.J. Bourke, 18 June 2021; North East Isles, Lane Island (= Milyekaluwakba), NTM P.64715 (4 dried shells), coll. P. Barden, 15 November 2022, NTM P.64717 (7 dried shells), coll. P. Barden, 15 November 2022; North East Isles, Hawk Island (= Ayangkijirumanja), NTM P.62775 (14 dried shells), coll. R.C. Willan & A.J. Bourke, 22 June 2021, P.64716 (1 dried shell), coll. P. Barden, 15 November 2022.

Description. *Shell* (Fig. 5). Comparatively small for genus (H = 13.6–21.5 mm, D = 15.5–24.2 mm), broadly conical to almost globose in shape, with moderately elevated spire (H/D = 0.81–0.99). Teleoconch entirely covered with well-developed collabral ridges. Whorls with well-rounded periphery, weakly shouldered below well-incised suture. Last whorl rapidly descending just behind aperture; apertural lip thick, slightly reflected, white, parietal wall calloused. Umbilicus narrow, open, partly concealed by reflected columellar lip; collabral ribs ornamented with very small pustules inside umbilical cavity. Shell colour uniform, light brown.

Reproductive anatomy (Fig. 4). Penis rather thick, uncoiled, slightly bent, with short epiphallus (shorter than penis), embedded in thin semi-transparent penial sheath; vagina short, thick; bursa copulatrix thick, comparatively short, with bulbous head, extending up to half of spermoviduct; vas deferens thick; proximal part of penial wall thickened, inner penial wall proximally with two smooth pilasters and several strongly developed oblique accessory pilasters, distally with dense pustulation (based on dissections of two specimens).

Etymology. The specific name for this new species of land snail, *amurndamilumila*, comes directly from the

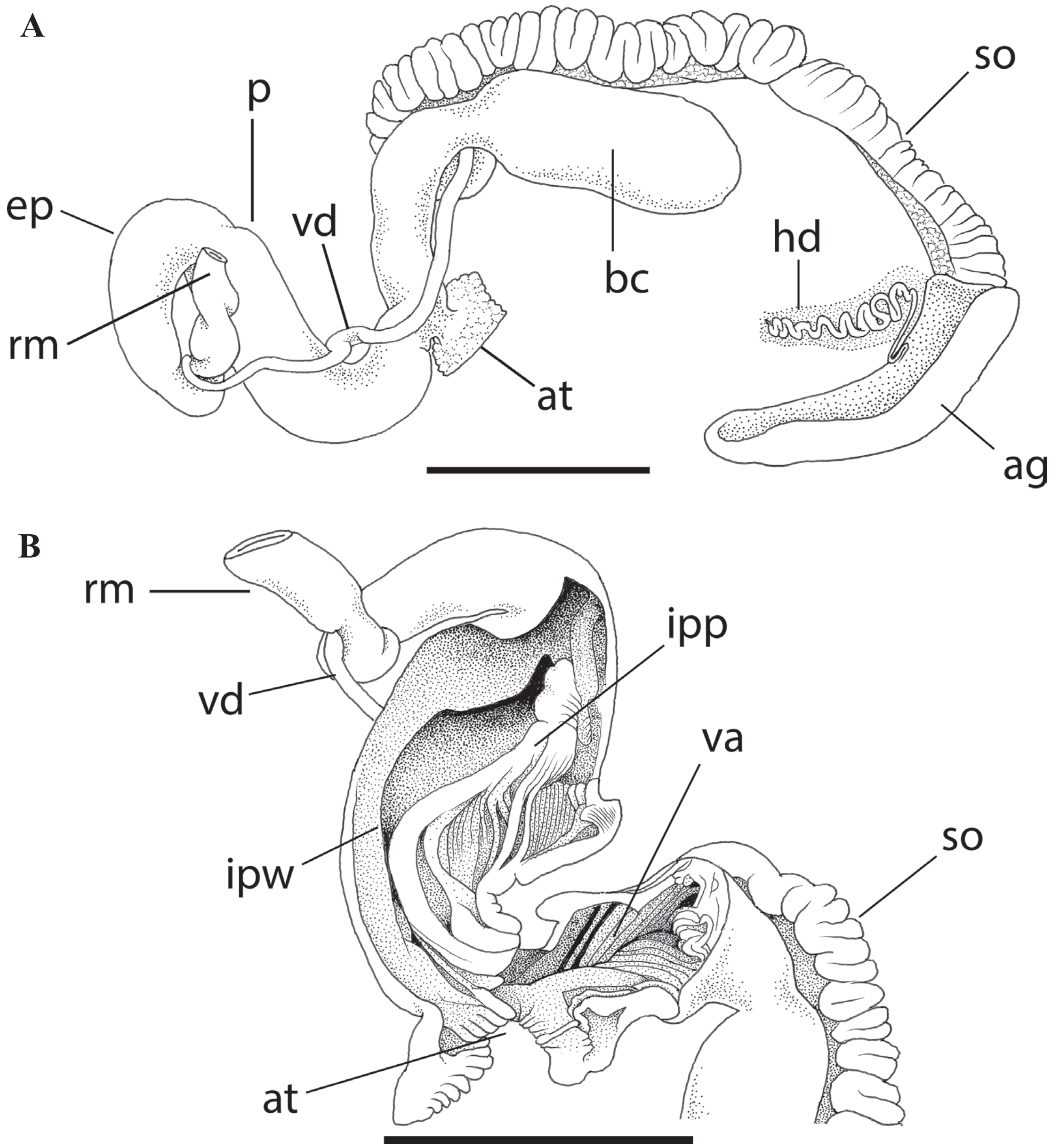

Figure 4. Reproductive anatomy of *Xanthomelon amurndamilumila* sp. nov., based on dissection of the holotype NTM P.65134. **A** Genital system; **B** Penial interior. Abbreviations used: ag, albumen gland; at, atrium; bc, bursa copulatrix; ep, epiphallus; hd, hermaphroditic duct; ipp, penial wall pilasters, ipw, inner penial wall; p, penis; rm, retractor muscle, so, spermoviduct; va, vagina; vd, vas deferens. Scale bars: 5 mm.

Amamalya Ayakwa language spoken by the Anindilyakwa Aboriginal people living on Groote Eylandt and Bickerton Island in the Gulf of Carpentaria. The word, which was provided by the Groote Eylandt Language Centre in consultation with the authors of this paper, means bumpy or corrugated (as in a sheet of corrugated iron) and it is a reference to the regular collabral ribs that ornament the shell. According to the Groote Eylandt Language Centre, the official pronunciation of the word is a-murn-DA-muhluh-muhla. The name is intended as a noun in apposition. Incidentally, *Xanthomelon arnhemense*, which is the cognate species living on mainland Groote Eylandt, has the Anindilyakwa name of yimurnderrma (pers. comm. P. Barden).

Comparative remarks. *Xanthomelon amurndamilumila* differs from other congeners in the Northern Territory by having well-developed collabral ridges (absent in *X. interpositum*, *X. darwinense* and *X. jannellei*), smaller shell size (larger shells in *X. arnhemense*, *X. durvillii* and *X. interpositum*) and in relative length of the epiphallus (1.5–5 times longer than penis in *X. durvillii*, *X. arnhemense* and *X. interpositum*). Furthermore, *X. jannellei* differs by having an extensive field of pustules on the apical half of the inner penial wall (refer to comparative morphology above for details and to Köhler and Burghardt (2016) for descriptions of other species).

Distribution. Known to occur only on the three islands comprising the North East Isles – North East Island

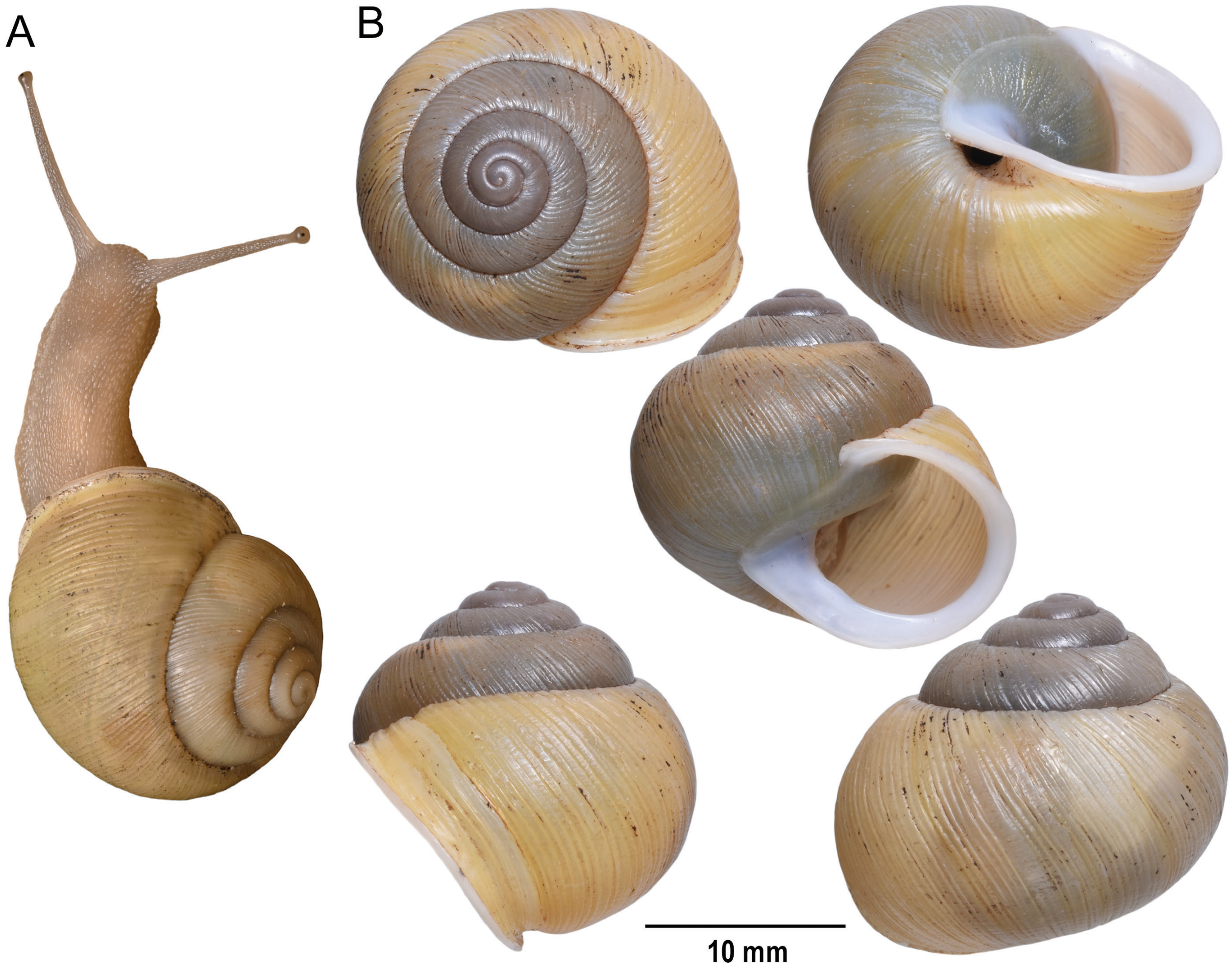

Figure 5. Shell morphology of *Xanthomelon amurndamilumila* sp. nov. **A** Living specimen from North East Island, not to scale; **B** Paratype NTM P.62888. Scale bar: 10 mm.

itself (= Amburrkba), Hawk Island (= Ayangkijirumanja) and Lane Island (= Milyekaluwakba) (Fig. 1).

Threatening processes. The presence of thousands of long dead *Xanthomelon* shells scattered on the surface across North East Island suggests deleterious changes in vegetation have occurred sometime during the last 100 years. Current inspection of North East Island shows that it is heavily damaged through over-browsing by feral Javan Rusa Deer (*Rusa timorensis*) (Willan and Bourke 2022). This over-browsing and habitat destruction puts *Xanthomelon amurndamilumila* potentially at risk of extinction on that island.

Discussion

The mitochondrial phylogeny revealed that the putative new *Xanthomelon* species on the North East Isles is most closely related to *X. arnhemense*, which occurs on Groote Eylandt, the large land mass closest to the North East Isles (Fig. 1). In fact, both species are sister species forming a single clade in the mitochondrial phylogeny of *Xanthomelon* (Fig. 2). The genetic divergence between both species (p-distances of on average 8.8% in COI) is consistent with the amount of interspecific genetic divergence found in this genus (between 14.5% and 19.1%; Köhler and Burghardt (2016)), although perhaps at the lower end of the range. This genetic distance supports the conclusion that there has been a considerable duration of evolutionary isolation of the populations on the North East Isles and that there is no evidence for contemporary genetic exchange between them and *X. arnhemense* on Groote Eylandt.

The largely identical reproductive anatomy is testimony to the close phylogenetic relationship between both species. We hypothesise that the putative new species likely diverged from *X. arnhemense* because of peripatric speciation after an historical event of long-distance dispersal from mainland Groote Eylandt, but that there has been little if any genetic exchange between the two of them subsequently. That both species now form a sister pair of clades in the mtDNA phylogeny shows that there has been a sufficiently long period of isolation to allow any pre-existing ancestral polymorphisms to sort out.

However, comparative morphology provides the strongest arguments for the recognition of the *Xanthomelon* species presently living on the North East Isles as an independent species. The North East Isles species is characterised by several unique features, which distinguish it from any other species of *Xanthomelon*, including *X. arnhemense* (refer to taxonomic description for details).

The close relationship of both species is also evident from their closely similar reproductive anatomy. However, the significant differences in shell size and sculpture in combination with substantial amounts of mitochondrial variation between both taxa are indicative of the existence of largely distinct gene pools in both forms, hence indicative of the distinct species status of the North East Isles species.

We conclude that molecular and morphological data support the conclusion that the *Xanthomelon* snails from the North East Isles indeed represent a distinct species or an evolutionarily distinct entity, that has a distinct gene pool (as expressed in a consistently distinct morphology) and with a high probability of lacking genetic admixture with *X. arnhemense* (as expressed in substantial mitochondrial differentiation).

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