

Eastern Australian Land Snail Species Closely Related to *Austrochloritis porteri* (Cox, 1868), with Description of a New Species (Mollusca, Eupulmonata, Camaenidae)

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ABSTRACT. The systematic taxonomy of three currently accepted species of *Austrochloritis* Pilsbry, 1891 from central eastern New South Wales (*A. nundinalis* Iredale, 1943, *A. niangala* Shea & Griffiths, 2010, and *A. kaputarensis* Stanisic, 2010) is revised based on comparative morpho-anatomy and mitochondrial phylogenetics. In addition, the status of two undescribed candidate taxa identified as *Austrochloritis* spp. NE3 and SN39, respectively, is assessed. These species and candidate species are closely related to the type species of the genus, *Austrochloritis porteri* (Cox, 1866) from southern Queensland based on a recently published mitochondrial phylogeny.

Comparative analyses of shell and reproductive anatomy revealed that the members of the *A. porteri* clade exhibit a rather similar morphology overall. Based on subtle, yet consistent differences in shell and reproductive features, we consider *A. nundinalis* as an accepted species. The species *A. niangala*, *A. kaputarensis*, and NE3 are considered as synonyms of each other and preference is given to the name *A. niangala* by First Reviewers Choice. The candidate taxon SN39 represents a new species, which is herein described as *Austrochloritis copelandensis* sp. nov.

Introduction

Austrochloritis Pilsbry, 1891 is taxonomically a comparatively diverse, yet morphologically rather homogeneous land snail genus endemic to eastern Australia (e.g., Stanisic *et al.* 2010). *Austrochloritis* species, with a few exceptions, are overall similar externally, having rather small, depressed and ‘hairy’ shells of dull brown colour. Because of their similar nondescript appearance, most species are currently difficult to identify based on the classification system introduced by Stanisic *et al.* (2010), which relies exclusively on shell characters in combination with the documented or presumed distribution of species. Nearly all species, both currently accepted and unaccepted, are known only from their shells while taxonomically critical information

on their comparative anatomy is almost entirely lacking. Exceptionally, we recently described the reproductive anatomy of two species: *Austrochloritis porteri* (Cox, 1866), the type species of the genus, and *A. specularis* Shea & Griffiths, 2010, a species from the Northern Tablelands of New South Wales (Shea and Köhler 2019).

In addition, we scrutinized the current systematic classification by analysing the differentiation in mitochondrial DNA sequences (Köhler, Criscione, and Shea 2020). This study has revealed widespread incongruence between the current species-level classification and the branching patterns of the mitochondrial trees. Most significantly, we found that many species as currently delineated were non-monophyletic in the phylogenetic trees. This incongruence may be attributed to a wide range

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of potential problems relating to the systematic significance of available morphological and molecular evidence. For example, the sole reliance of the current classification on shell characters is problematic because shell features may be homoplastic due to their potentially adaptive nature. It is well-documented that shell characteristics may be conserved in species pursuing identical life styles while living in similar environments as the result of balancing selection. Such morphological conservatism may lead to an underestimation of the true taxonomic diversity in morphologically conserved groups as was demonstrated for the northern Australian camaenid *Mesodontrachia* by Criscione & Köhler (2013). On the other hand, shell characters may also be polymorphic within species that inhabit heterogeneous environments, which may lead to an overestimation of taxonomic diversity (e.g., Davison & Clarke, 2000; Criscione & Köhler, 2016a).

However, the mitochondrial markers underpinning our previous phylogenetic hypothesis are not without potential caveats either as several mechanisms may cause the non-monophyly of species in mtDNA trees, such as paralogy, retention of ancestral polymorphisms, or introgressive hybridization, to name just a few (e.g., Funk & Omland, 2003; Ballard & Whitlock, 2004). Indeed, there are several well-documented cases in which mtDNA markers proved to be unreliable for the delineation of species, especially in cerithioidean freshwater snails (e.g., Köhler & Deen, 2010; Köhler, 2016, 2017; Whelan & Strong, 2016). However, mtDNA has so far appeared as a reliable source of systematic information in a wide range of Australian camaenids (e.g., O'Neill *et al.*, 2014; Criscione & Köhler, 2014a,b, 2016b; Taylor *et al.*, 2015; Johnson *et al.*, 2016; Köhler & Burghardt, 2016).

Putting the difficulties with species delineation aside, our molecular phylogenetic study has highlighted the importance of the Hunter Valley as an effective biogeographic barrier separating temperate and subtropical species of *Austrochloritis*. Species on either side of this divide revealed remarkably different biogeographic patterns: One of widespread parapatry of species south of the Hunter Valley and of potentially widespread sympatry north of it. Usually, species occurring in sympatry with each other were found not to belong to closely related main clades in the phylogenetic tree.

Based on the observation of widespread sympatry of possibly morphologically cryptic species north of the Hunter Valley, we also consider the existence of undescribed, morphologically rather cryptic species, as another possible explanation for the observed incongruence between molecular phylogeny and morphological species delineation. Hence, to resolve the prevailing ambiguity in the delineation of *Austrochloritis* species, it is critical to base any systematic appraisal on more comprehensive evidence, such as previously ignored morpho-anatomical and genetic data. In addition, increasing the number of studied specimens and populations should be useful to improve our understanding of the amounts of variation within and between species especially in cases where sympatry of morphologically cryptic species has been postulated (Köhler *et al.*, 2020).

Our mitochondrial phylogenies (Köhler *et al.*, 2020) contained several well-differentiated and well-supported main clades (clades A–E), which are postulated to represent well-differentiated species or species groups within *Austrochloritis*. One of these clades, Clade B, contained

sequences of the type species, *A. porteri* (Cox, 1868), three additional nominal species (*A. nundinalis* Iredale, 1943, *A. niangala* Shea & Griffiths, 2010, *A. kaputarensis* Stanisic, 2010), and two supposedly undescribed species (*Austrochloritis* sp. NE3 and SN39). Each of these taxa or candidate taxa was represented by just two sequences and one of them, *A. niangala*, was found not to be monophyletic.

In the present study, we assess the variation in shell and reproductive anatomy among representatives of this clade in combination with a slightly increased sampling of mitochondrial DNA sequences in order to more objectively delimit distinct species.

Materials and methods

This study is based on examination of all relevant samples housed in the collections of the Australian Museum, Sydney (AM), including historic and newly collected material, both wet and dry. A Leica MZ8 stereo microscope with a drawing apparatus was used to examine the reproductive anatomy of ethanol preserved samples by means of anatomical dissection. Bodies were removed from shells prior to dissection and shells were photographed. Shell height (H) and width (W) were measured with callipers accurate to 0.5 mm. We also counted the number of whorls (N) as shown by Köhler (2011). Selected shells were cleaned by gently brushing in warm soapy water, dried, mounted on carbon specimen tabs, and coated with gold for scanning electron microscopy.

DNA was extracted from small pieces of foot muscle by use of 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 (Chiba 1999) and 16Sbd1 (Sutcharit, Asami, and Panha 2007) and L1490 and H2198 (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. Both strands of PCR fragments were purified with ExoSAP (Affymetrix) and 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 contig using CodonCode Aligner v. 3.6.1 (CodonCode Corporation, Dedham, MA). New sequences have been deposited in GenBank. 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. We used the online version of Gblocks (Version 0.91b) (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 data set. Four partitions were designated: The entire 16S fragment plus each of the three codon positions of the COI fragment. The best-fit model of nucleotide substitution was identified for each sequence partition separately using ModelFinder included in IQ-Tree (Kalyaanamoorthy *et al.*, 2017). Phylogenetic relationships were estimated by employing a Maximum Likelihood-based method of tree reconstruction using the program IQ-Tree (Nguyen *et al.*, 2015). Nodal

support of the best ML tree was estimated by performing 10,000 ultrafast bootstraps in IQ-Tree (Minh *et al.*, 2013).

Our operational criterion for the delimitation of species was to test whether candidate species were phenotypically distinct from each other (Sites & Marshall, 2004). Candidate taxa were initially delimited by grouping specimens in accordance with their shell morphology with reference to the current taxonomy. In a second step we assessed if these groups can be confirmed as consistently distinct groups with respect to their comparative reproductive anatomy and if they are recovered as monophyletic clusters in the mitochondrial phylogeny. We also employed basic statistics of morphometric characters to assess the morphometric similarity or distinctiveness of the candidate taxa. Candidate taxa that could be distinguished consistently by comparative morpho-anatomy and are also well-differentiated from each other in the mtDNA tree, were accepted as distinct species. Candidate species that could not be consistently distinguished from each other were lumped together.

Abbreviations. *Morpho-anatomy*: *at*, atrium; *ag*, albumen gland; *bc*, bursa copulatrix; *bh*, head of bursa copulatrix; *d*, number of dry shells per lot; *ep*, epiphallus; *fl*, flagellum; *H*, height of shell; *N*, number of whorls; *p*, penis; *pv*, penial verge; *pw*, penial wall; *rm*, penial retractor muscle; *so*, spermoviduct; *va*, vagina; *vd*, vas deferens; *w*, number of preserved specimens per lot; *W*, width of shell. *Geographic*: *HS*, homestead; *NP*, National Park; *NR*, Nature Reserve; *NSW*, New South Wales.

Results

Mitochondrial phylogenetics

We constructed a DNA sequence dataset by concatenating partial sequences of the mitochondrial genes cytochrome *x* oxidase subunit 1 (COI) and 16S rRNA (16S). The final dataset contained sequences of 24 individuals (four of *A. nundinalis*, three of *A. kaputarensis*, one of *A. niangala*, three of *Austrochloritis* sp. NE3, four of *Austrochloritis* sp. SN39, as well as seven sequences of other *Austrochloritis* species that were used as outgroup to root the tree). This sampling included sequences of topotypic specimens of *A. kaputarensis* and *A. niangala*, the holotype and paratype of the newly described species, and material from close to the type locality of *A. nundinalis* (i.e., from Sheba Dam).

In this dataset three COI sequences of in-group taxa were missing whereas the 16S sequences were completely sampled. All COI sequences had a length of 655 bp after pruning of the primer sites while the multiple 16S sequence alignment had a length of 782 bp after trimming of ends and removing ambiguously aligned sections by using Gblocks. For the phylogenetic analysis a data partition was applied, which allowed parameters for each codon position of the COI fragment and the 16S fragment to be modelled independently. The model test implemented in IQ-Tree identified the following models of sequence evolution as the best-fit models for the different partitions by means of the Bayesian Information Criterion: TVM+F+I+G for 16S,

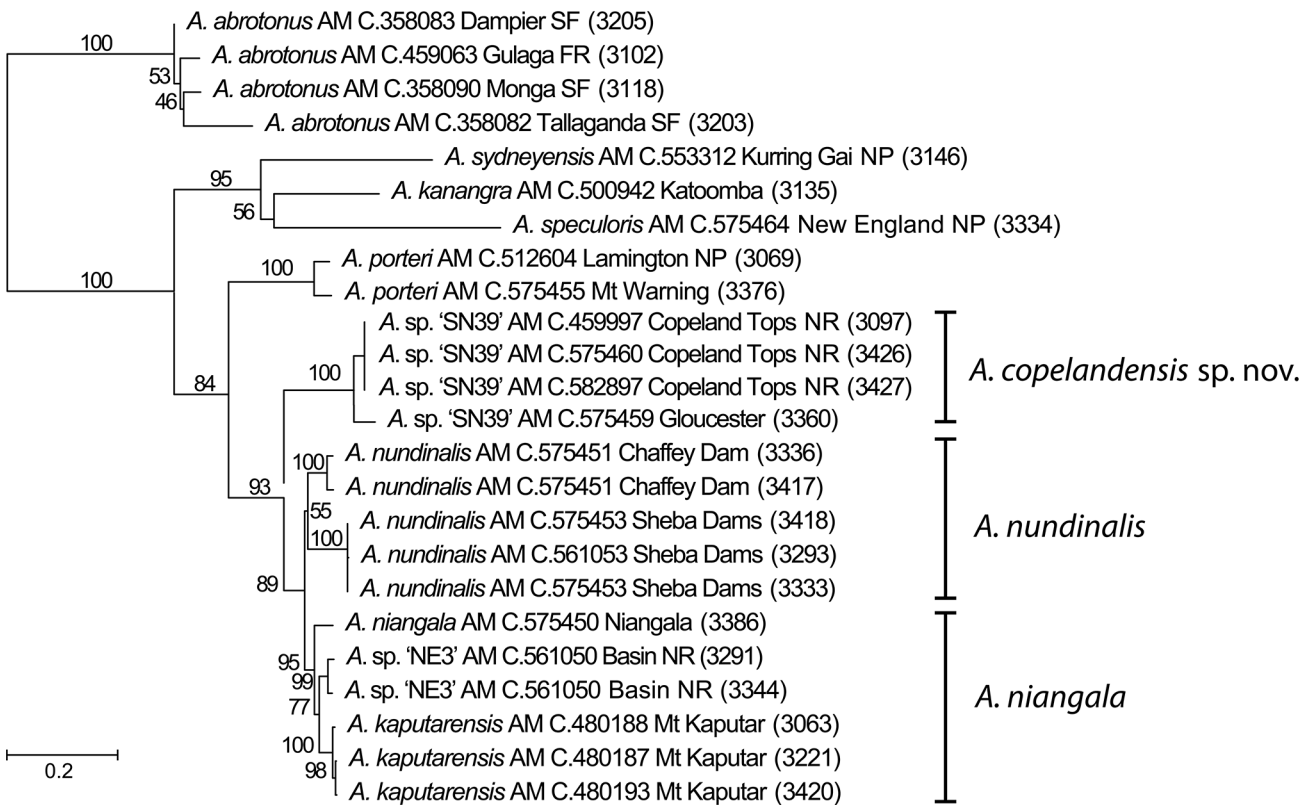


Figure 1. Bootstrap consensus tree for 10,000 ultrafast Maximum Likelihood bootstraps as based on Maximum Likelihood Analysis of the concatenated 16S and COI sequence dataset. Sequences of *Austrochloritis abrotonus* Shea & Griffiths, 2010 were used to root the tree.

F81+F+I for 1st, TIM+F+G for 2nd, and TN+F+G for 3rd codon positions in COI. These models were applied in the partitioned Maximum Likelihood analysis.

The bootstrap consensus tree of the Maximum Likelihood Analysis confirmed *A. porteri* as the sister group to the in-group consisting of *A. nundinalis*, *A. niangala*, *A. kaputarensis* as well as the candidate species *A. sp. NE3* and *A. sp. SN39* with good statistical support (Fig. 1). All nominal and candidate species formed monophyletic clusters. However, the bootstrap support for the monophyly of *A. nundinalis* was low as individuals from two distinct locations formed two well-differentiated sub-clades. The taxa *A. kaputarensis*, *A. niangala* and *Austrochloritis sp. NE3* together formed a clade with comparatively short internal branches. The maximum uncorrected pairwise distances in 16S were low within this clade: 1.1 % among three sequences of *A. kaputarensis* and 1.5 % between two sequences of *Austrochloritis sp. NE3*. However, the maximum pairwise distance between all sequences of this clade was 4.3 % (for six sequences), which was comparable with the maximal p-distance of 6.7 % among the five sequences of *A. nundinalis* and the distance between the two sequences of *Austrochloritis sp. SN39*: 3.2 %.

Comparative morpho-anatomy

All examined nominal and candidate species are characterized by exhibiting a largely similar shell morphology and reproductive anatomy. However, some minor, yet consistent differences exist.

We found that *A. niangala*, *A. kaputarensis*, and *A. sp. NE3* were effectively indistinguishable in both shell morphology (shell size, shape, periostracal projections, sculpture, coloration) and reproductive anatomy (anatomical detail, relative lengths of penis, epiphallus, penial verge, and bursa copulatrix, development of flagellum, bursa head, penial pilasters).

Austrochloritis nundinalis exhibited a very similar reproductive anatomy compared to the above-mentioned taxa. However, it could be distinguished from them by its significantly larger shell and by a different shape of the periostracal projections covering its shell (curved instead of straight).

Austrochloritis sp. SN39 had a very similar shell compared to *A. nundinalis* in terms of shape and size. However, it differed from all other taxa by having a well-reflected aperture and a much longer and pointed penial verge.

Systematics

Family Camaenidae Pilsbry, 1895

Genus *Austrochloritis* Pilsbry, 1891

Type species: *Helix porteri* Cox, 1866, by original designation. For diagnosis and taxonomic details refer to Stanistic *et al.* (2010) and Shea & Köhler (2019).

Austrochloritis nundinalis Iredale, 1943

Austrochloritis nundinalis Iredale, 1943: 64 (probable syntypes AM C.112312, 2d, from Nundle, NSW); Stanistic *et al.*, 2010: 384, fig. 570.

Type material examined. Probable syntypes, AM C.112312 (2 d; Nundle) (Fig. 2A).

Non-type material examined: AM C.171350 (3 d; Ponderosa Forest Park, Nundle SF, E of Nundle, -31.47° 151.26°; alt. 1,250 m; eucalypt forest), AM C.171513 (9 d; Sheba Dams, 14 km SE of Nundle, -31.498° 151.195°; tall, moist eucalypt forest), AM C.335318 (1 d; 5 km ESE of Nundle, -31.48° 151.18°), AM C.339647 (4 d; Chaffey Dam, NW end of wall, S of Woolomin, -31.345° 151.135°; open eucalypt woodland), AM C.459889 (1 d; Nundle SF, -31.48° 151.37°; sclerophyll forest), AM C.459901 (4 d; same as AM C.459889), AM C.575259 (15 d; E side of Chaffey Dam, 5 km N of Bowling Alley Point, -31.37° 151.14°; alt. 550 m; dry sclerophyll woodland), AM C.575451 (3 w; same as AM C.575259); AM C.575260 (2 d; Sheba Dams camping area, east of Nundle, -31.50° 151.20°; alt. 1,173 m; moist sclerophyll forest), AM C.575453 (3 w; same as AM C.575260).

Taxonomic remarks

The original description is rather uninformative, stating that this species is similar to *A. porteri*, but differs by its higher spire and denser hair (Iredale, 1943: 64). Iredale's description does not contain an explicit type designation. Subsequently, Stanistic *et al.* (2010: 384) stated that *A. nundinalis* was "distinguished [from other congeners] by combination of relatively large size, flattened spire, fine moderately long setae and weakly reflected lip".

Table 1. Shell dimensions (*H* = height, *W* = width, *N* = number of whorls) of mature shells of *Austrochloritis nundinalis*, *A. niangala* and *A. copelandensis sp. nov.*

		H	W	N
<i>A. nundinalis</i>		9.3	15.7	3.5
	(n = 19)	(7.7–11.3)	(13.8–17.3)	(3.3–3.6)
<i>A. niangala</i>		7.4	12.7	3.5
	(n = 20)	(6.2–8.8)	(11.1–15.0)	(3.2–3.9)
<i>A. copelandensis sp. nov.</i>		9.0	16.1	4.1
	(n = 2)	(8.9–9.0)	(15.8–16.3)	(4.1–4.2)

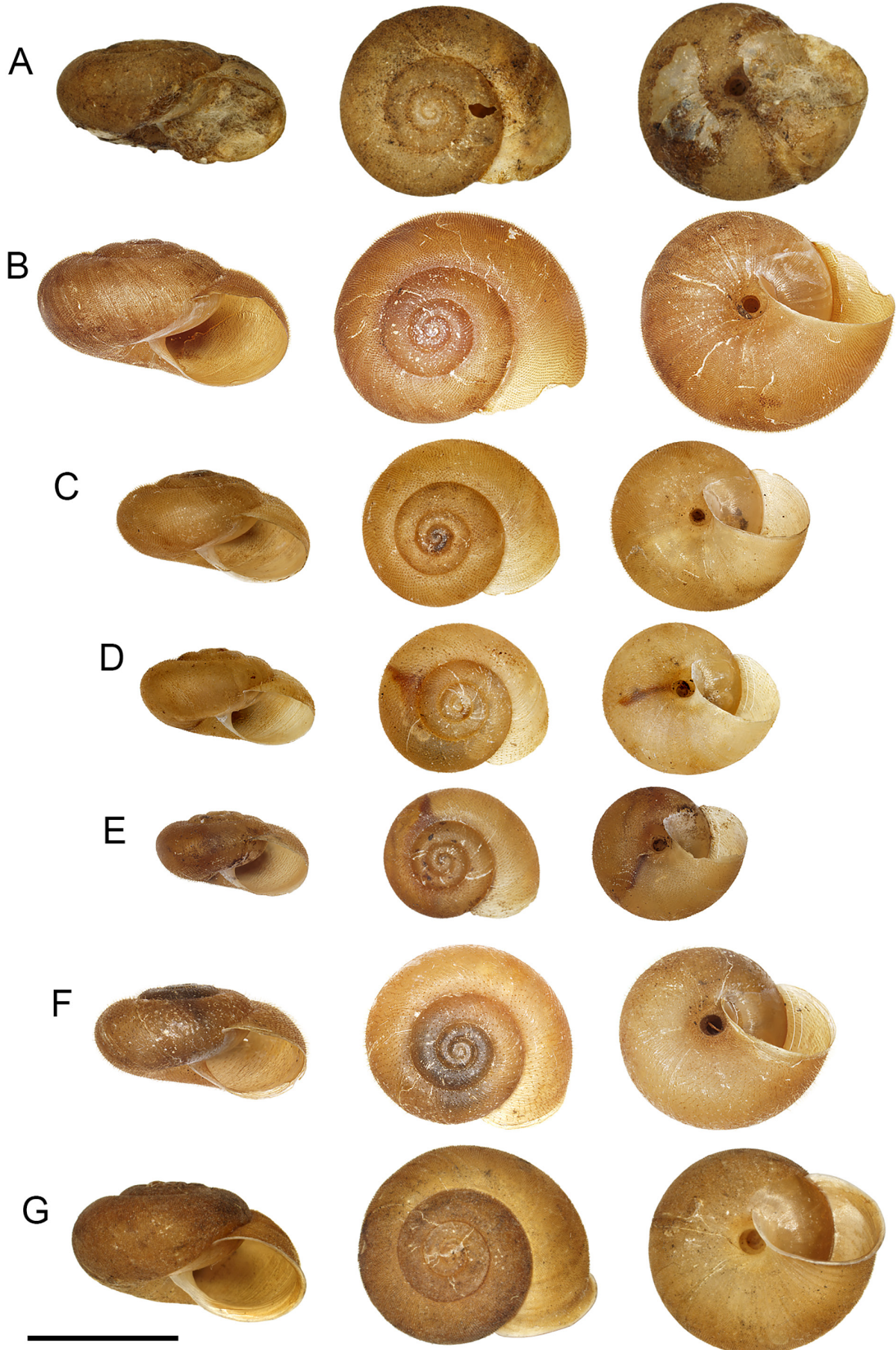


Figure 2. Shells (front, top, and umbilical view). (A, B) *Austrochloritis nudinalis*: A, probable syntype AM C.112312 (Nundle, NSW); B, AM C.575260 (Sheba Dams, E of Nundle); (C–F) *Austrochloritis niangala*: C, holotype, AM C.339934 (ESE of Tamworth); D, paratype of *A. kaputarensis*, AM C.452038 (NE of Manilla); E, AM C.478658 (Mt Kaputar); F, AM C.561050 (Georges Mountain, Basin NR); G, *Austrochloritis copelandensis* sp. nov., holotype AM C.582897 (Copeland Tops). Scale bar = 10 mm.

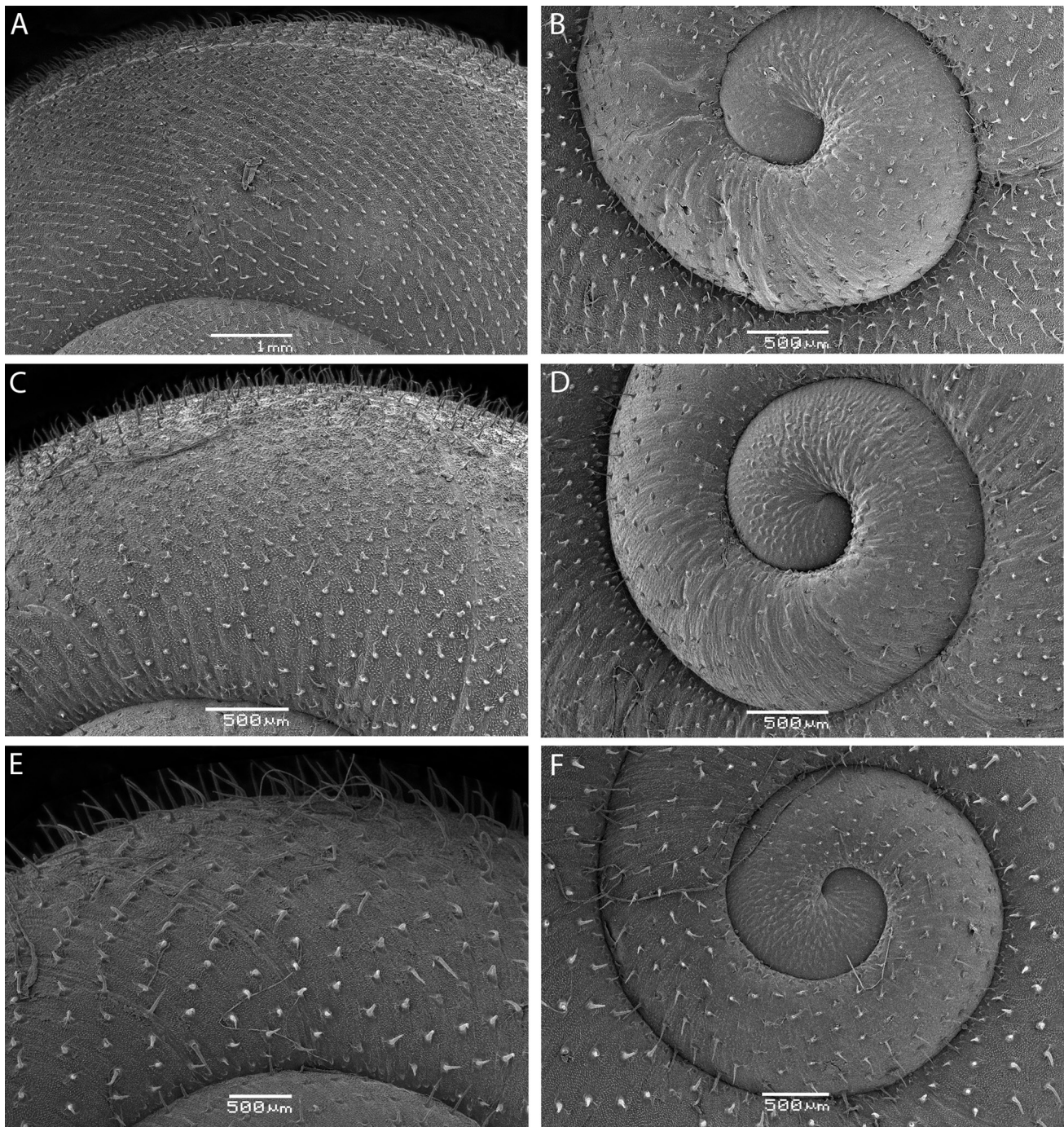


Figure 3. Scanning electron micrographs of shells of *Austrochloritis* (teleoconch viewed from above, protoconch viewed from above). (A, B) *Austrochloritis mundinalis*, AM C.171513, Nundle; (C, D) *A. niangala*, AM C.339933, Niangala; (E, F) *Austrochloritis* sp. NE3, AM C.561050). Scale bars: A = 1 mm, B–F = 0.5 mm.

Description

Shell (Figs 2A,B, 3A,B). Medium sized (W=13.8–17.3 mm, H=7.7–11.3 mm, N = 3.3–3.5; for n = 19; Table 1), depressedly subglobose, whorls rounded to slightly shouldered in cross-section, gradually increasing in diameter, suture moderately incised; protoconch sculpture of rugose radials with coarse pustules at apex, periostracal setae extending well onto protoconch; teleoconch sculpture of low growth lines and corrugations with periostracal sculpture of curved, crowded, short setae; interstitial microsculpture of very fine wavy periostracal ridgelets and scales; last whorl

strongly descending behind aperture in mature individuals; aperture moderately tilted forward from axis of coiling, without thickened or reflected outer lip and without sulcus behind lip; umbilicus narrowly open with U-shaped profile; shell colour pale yellowish to dark reddish brown.

Reproductive anatomy (Fig. 4). Penis cylindrical, without penial sheath, inner penial wall sculpture of many well-developed longitudinal pilasters, epiphallus about two to three times longer than penis, with well-developed finger-like epiphallic flagellum at distal end, about as long as penis; epiphallus communicates with penis through broadly conical,

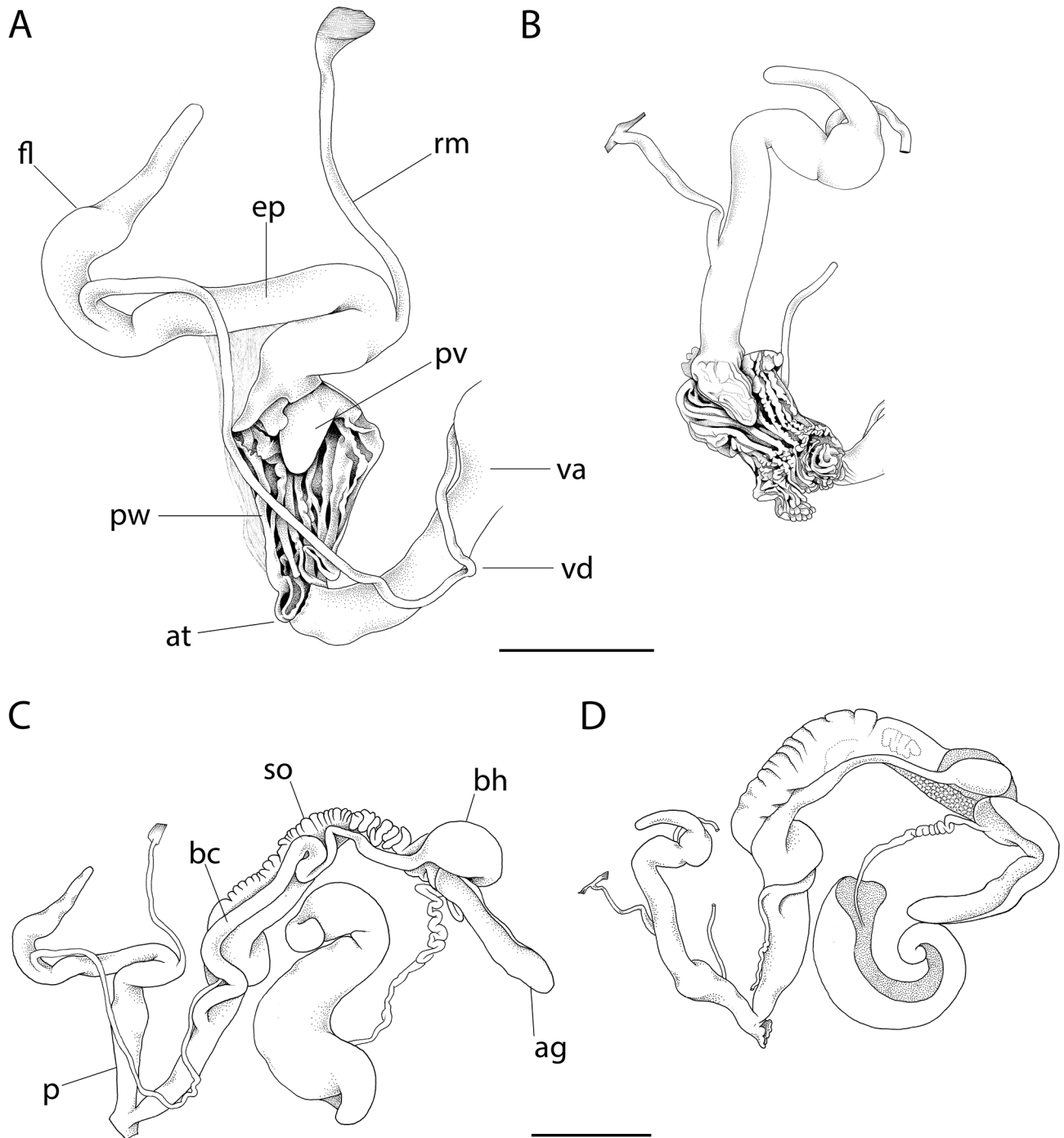


Figure 4. Reproductive anatomy of *Austrochloritis nundinalis*. (A) Penial anatomy, AM C.575453, Sheba Dams, E of Nundle; (B) penial anatomy, AM C.575451, Chaffey Dam; scale bar = 3 mm. (C) Reproductive system, AM C.575453, Sheba Dams, E of Nundle; (D) reproductive system, AM C.575451, Chaffey Dam; scale bar = 5 mm.

free penial verge, penial verge comprising about half to one third of penis length; penial retractor muscle attached to distal third of epiphallus, vas deferens entering head of epiphallus through single pore just below base of epiphallallic flagellum; vagina cylindrical, about as long as penis, inner wall with prominent longitudinal pilasters; bursa copulatrix long, tubular with inflated bulb-like head, about as long as oviduct to one quarter longer, head reaching base of albumen gland; hermaphroditic duct inserting into head of talon (based on three dissected specimens).

Comparative remarks

Reproductive morphology was variable among dissected specimens even within a single population: Penial length varied from about as long as vagina to half of vaginal length and the bursa copulatrix varied in length from about equivalent to the oviduct to substantially longer. The lack of a reflected outer lip and sulcus and setae extending well onto the protoconch distinguish this species from most other congeners. *Austrochloritis nundinalis* differs from *A. porteri*

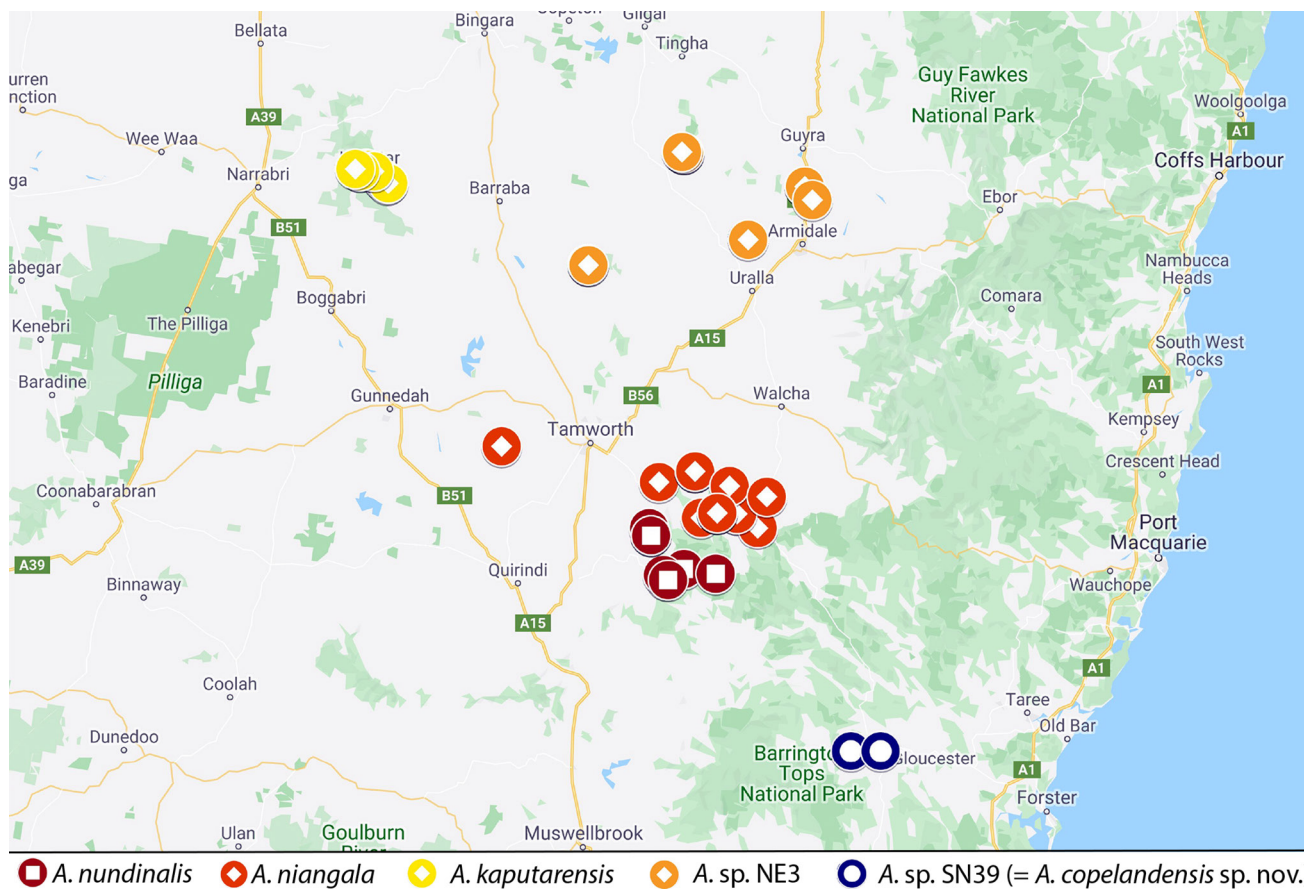


Figure 5. Occurrence records of *Austrochloritis nundinalis*, *A. niangala* (= *A. niangala*, *A. kaputarensis*, *Austrochloritis* sp. NE3), and *A. copelandensis* sp. nov. (= *Austrochloritis* sp. SN39) from the malacological collection of the Australian Museum. Source of base map: Google Maps.

most notably in having a proportionally longer epiphallus, a different penial verge morphology (conical, smooth vs. elongated, sculptured), and a wider flagellum (Shea & Köhler, 2019). In addition, *A. nundinalis* has a significantly smaller shell. For a comparison with *A. niangala* refer below.

Distribution and ecology

Austrochloritis nundinalis is so far only known from near Nundle, where it is mainly found at altitudes between 550–1,418 m (Fig. 5). It is found in sclerophyll forests, on granitic and basaltic bedrock; under logs, rocks and shed bark around base of trees. It seals to the underside of substrates with a tough parchment-like epiphragm in dry conditions.

Austrochloritis niangala Shea & Griffiths, 2010

Austrochloritis niangala Shea & Griffiths, 2010 in Stanistic, Shea, Potter & Griffiths, 2010: 384–385, 536 (holotype AM C.339934 from ESE of Tamworth, NE New South Wales, -31.177° 151.295°).

Austrochloritis kaputarensis Stanistic, 2010 in Stanistic, Shea, Potter & Griffiths, 2010: 384–385, 536 (holotype QM MO32626 from Dawson Spring, Mt Kaputar, -30.267° 150.167°); **new synonym**.

Type material examined. Holotype of *Austrochloritis niangala*, AM C.339934 (1d, ESE of Tamworth, -31.177° 151.295°) (Fig. 2C), Paratypes, AM C.339935 (1 d, same as holotype), AM C.339933 (3 d, SE of Tamworth, near Three Corner Swamp, 300 m E of “Carnegie” HS). Paratypes of *Austrochloritis kaputarensis*, AM C.450448 (8 d, NE of Manilla, Warrabah NP, -30.267° 150.167°), AM C.452038 (1 d, same as AM C.450448).

Non-type material examined. AM C.339566 (1 w, ESE of Tamworth, 3.4 km E of Niangala, -31.30° 151.44°), AM C.339936 (1 d, 40 km S of Walcha, -31.34° 151.51°), AM C.446747 (1 d, Oakey Creek Nature Reserve, -31.10° 150.62°), AM C.339817 (1 w, 32 km SE of Kootingal, -31.21° 151.17°), AM C.108379 (1 w, 3.4 km E of Niangala, -31.30° 151.44°), AM C.339816 (2 w, same as holotype), AM C.478245 (7 d, same as holotype), AM C.339928 (2 w, S of Walcha, Ingleba Ck, N of Branga Plains HS,

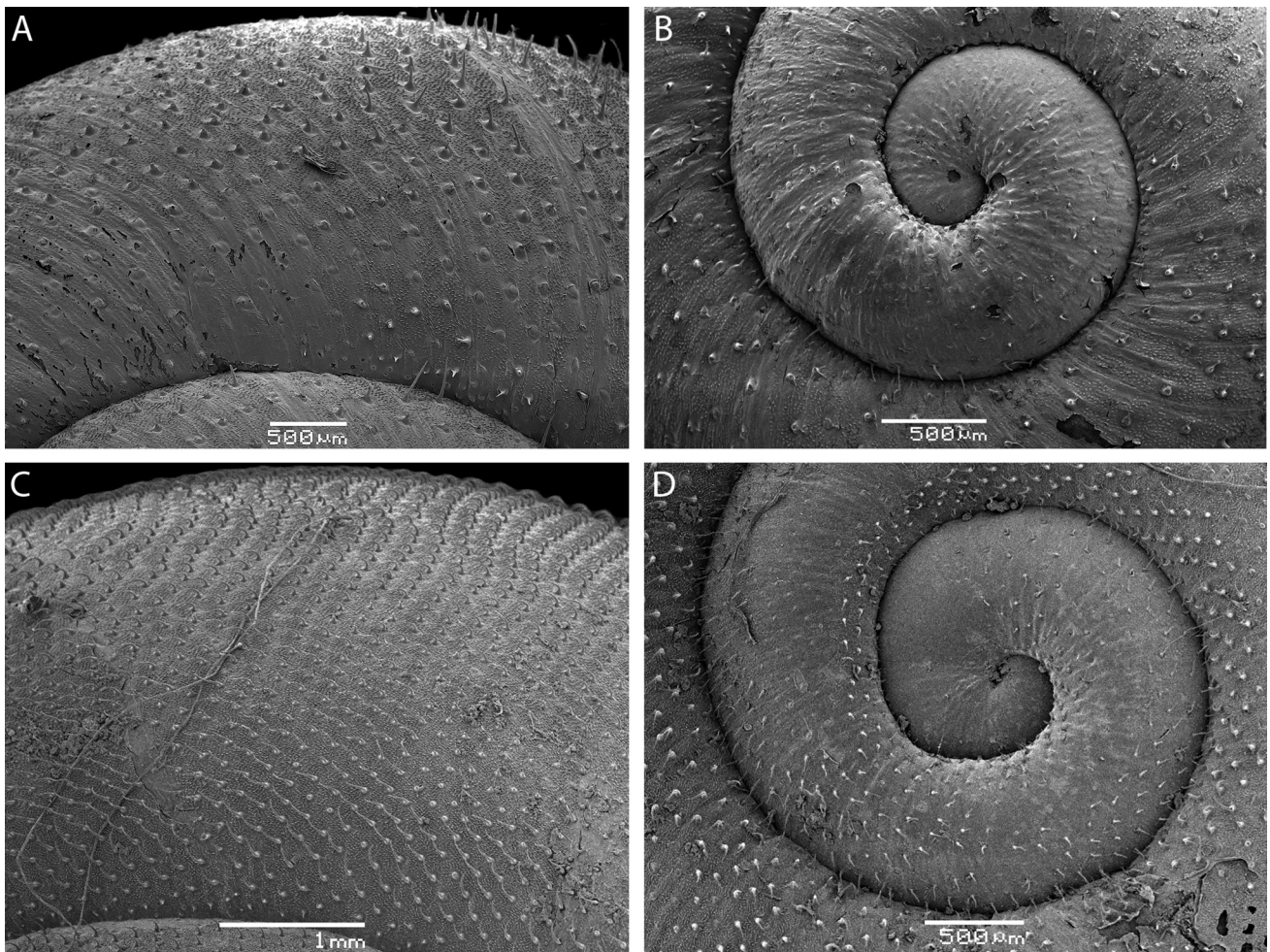


Figure 6. Scanning electron micrographs of shells of *Austrochloritis* (teleoconch viewed from above, protoconch viewed from above). (A, B) *Austrochloritis kaputarensis* AM C.478659, Mt Kaputar; (C, D) *Austrochloritis copelandensis* sp. nov., paratype, AM C.459997, Copeland Tops.

-31.25° 151.54°), AM C.575258 (3 d, Niangala-Weabonga road, -31.30° 151.37°), AM C.575450 (3 w, 1d, Niangala-Weabonga road, -31.30° 151.37°), AM C.557123 (2 d, 30 km N of Armidale, Thunderbolts Cave, -30.33° 151.67°), AM C.557124 (2 d, same as AM C.557123), AM C.519090 (1 d, Mt Kaputar NP, Coryah Gap, -30.28° 150.13°), AM C.480187 (3 w, same as AM C.519090), AM C.519095 (1 d, Mt Kaputar NP, West Kaputar Rock lookout, -30.28° 150.15°), AM C.519099 (10 d, Mt Kaputar NP, Barraba Track, -30.29° 150.18°), AM C.480188 (4 w, same as AM C.519099), AM C.478644 (4 d, Mt Kaputar NP, Barraba Track, -30.31° 150.21°), AM C.478832 (4 d, same as AM C.478644), AM C.478653 (5 d, Mt Kaputar NP, South East Fire trail, -30.32° 150.23°), AM C.478658 (8 d, Mt Kaputar NP, eastern park entrance, -30.32° 150.23°), AM C.478674 (3 d, Mt Kaputar NP, below Dawson's Spring, -30.28° 150.16°), AM C.477015 (1 w, Mt Kaputar NP, Dawson's Spring, -30.28° 150.16°), AM C.480193 (1 w, Mt Kaputar NP, 4.4 km up from Green Camp, -30.28° 150.12°), AM C.164945 (4 w, NW of Armidale, W of Guyra, Georges Mountain, -30.23° 151.25°), AM C.339673 (1 d, same as AM C.164945), AM C.339674 (1 d, same as AM C.164945), AM C.375155 (1 w, 1 d, 21.4 km W of Armidale on Bundarra Rd, -30.49° 151.48°), AM C.210134 (4 d, N of Armidale, Tilbuster Ponds, -30.36° 151.68°), AM C.561050 (3 w, 10 d, Georges Mountain, Basin NR, -30.23° 151.25°).

Nomenclatural and taxonomic remarks

Both species, *A. niangala* and *A. kaputarensis*, were described in the same publication based on shell characters only. Their original descriptions are indicative of their rather close similarity. The purported differences are mainly in the length and density of periostracal setae. However, the reproductive anatomy was previously not documented. We are unable to identify any consistent morphological or anatomical difference between these two taxa. Shells are virtually identical in shape and size and no significant and consistent difference in the density and length of periostracal was observed. 16S sequences assigned to both taxa, respectively, differed from each other by on average 4.3 % p-distance, which may well be in the range of intraspecific genetic variation given the comparatively large geographic distances between the sequenced populations (Fig. 5). Based on the lack of consistent morpho-anatomical differentiation in concert with the rather low amount of mitochondrial differentiation, we consider both taxa as synonyms. Preference is here given to the name *A. niangala* by First Reviewers Choice. The candidate species *Austrochloritis* sp. NE3, which has been identified during curatorial work in the collections of the AM, is also considered as conspecific with *A. niangala* for exhibiting a consistent morpho-anatomy and also because it falls within the sub-clade formed by sequences of *A. niangala* (Fig. 1).

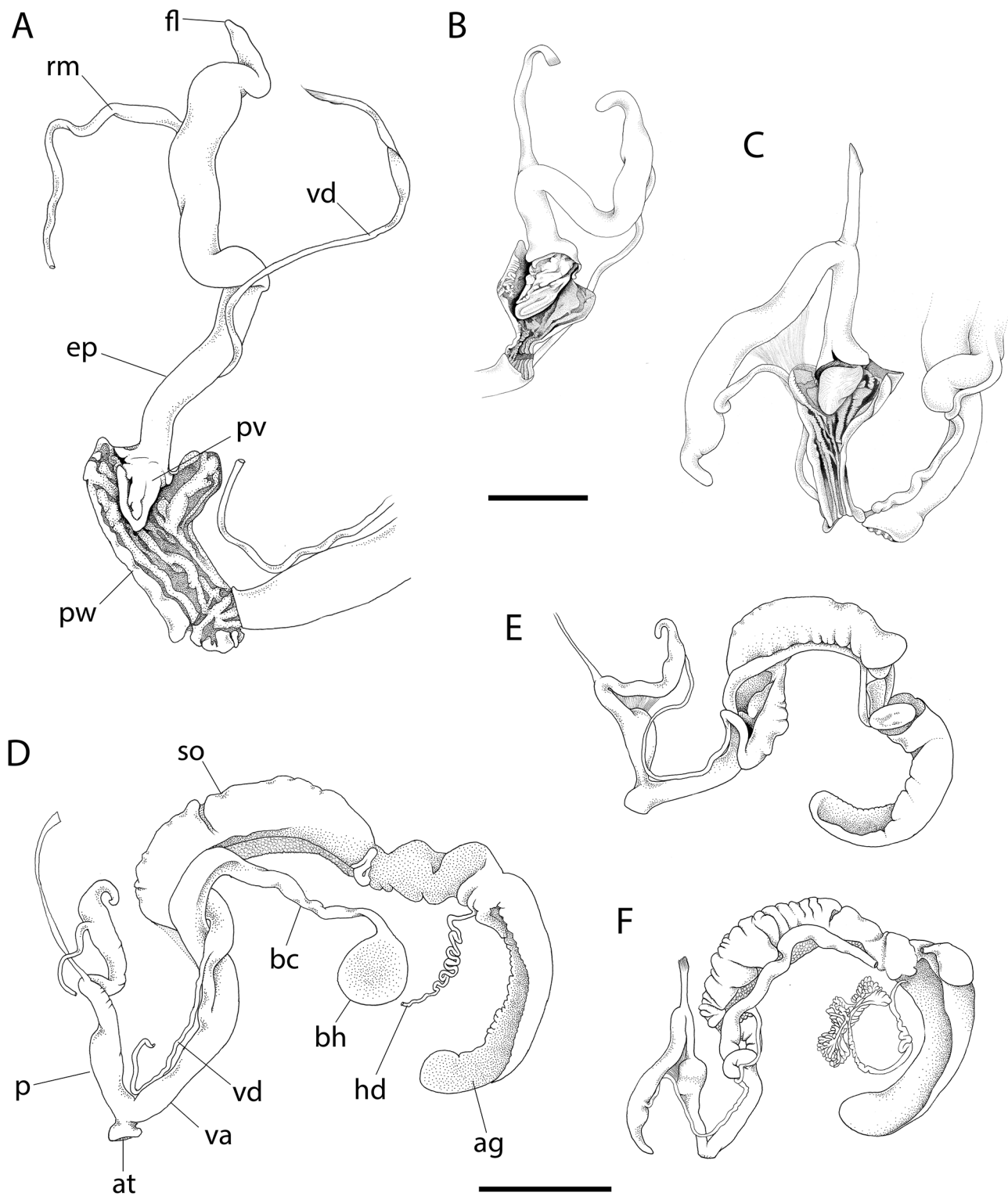


Figure 7. Reproductive anatomy of *Austrochloritis niangala*. (A–C) Penial anatomy: A, AM C.575450, *A. niangala*, Niangala; B, AM C.480188, *A. kaputarensis*, Mt Kaputar; C, AM C.375155, *Austrochloritis* sp. NE3, Bundarra; scale bar = 2 mm. (D–F) Reproductive system: D, AM C.575450, *A. niangala*, Niangala; E, AM C.480188, *A. kaputarensis*, Mt Kaputar; F, AM C.375155, *Austrochloritis* sp. NE3, Bundarra; scale bar = 5 mm.

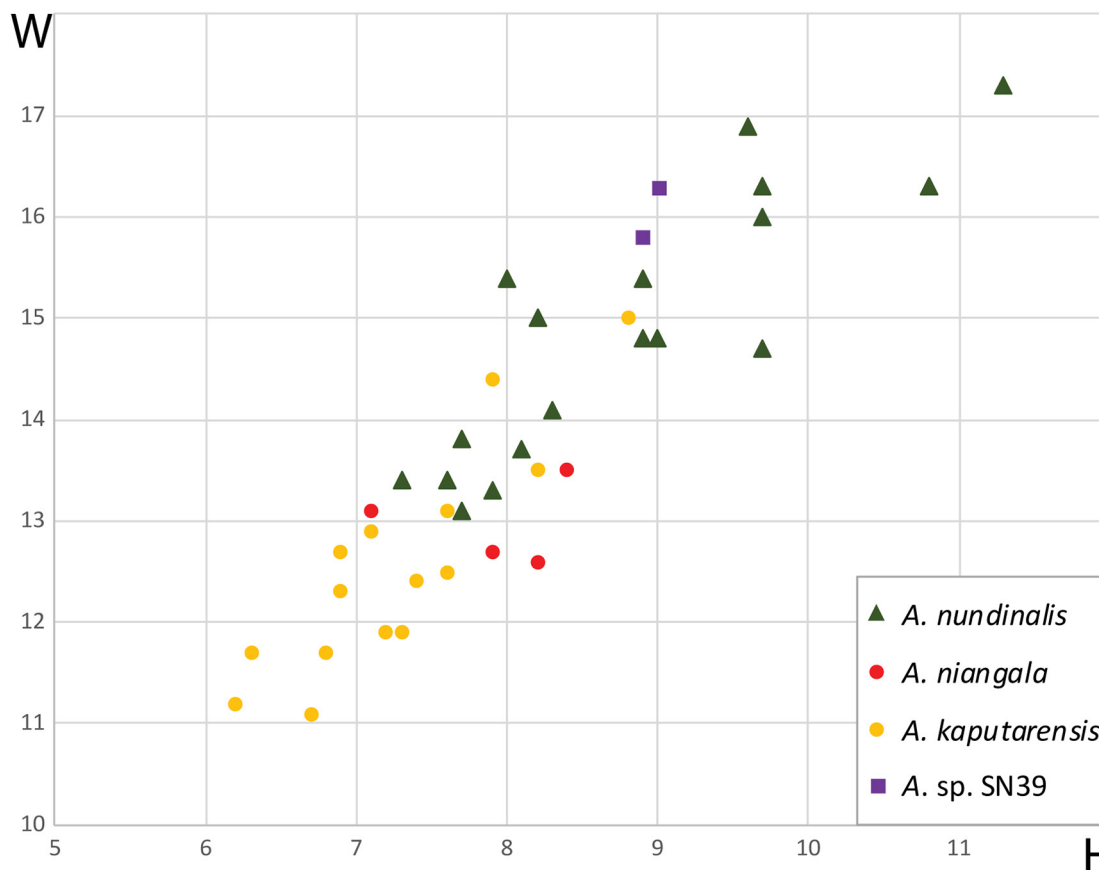


Figure 8. Comparison of shell dimensions of *Austrochloritis nundinalis*, *A. niangala*, *A. kaputarensis*, and *A. copelandensis* sp. nov. by ordination of shell height against width.

Description

Shell (Figs 2C–F, 3C–F, 6A,B). Medium sized ($W=11.1$ – 15.0 mm, $H=6.2$ – 8.8 mm, $N = 3.2$ – 3.9 ; for $n = 20$; Table 1), turbinate to depressedly globose, with rounded to slightly shouldered whorls that regularly increase in diameter, sutures rather deeply incised; protoconch sculpture of rugose radials with coarse pustules at apex and with periostracal setae extending well on protoconch; teleoconch sculpture of low growth lines and corrugations with periostracal sculpture of rather straight, moderately widely spaced and occasionally long setae, interstitial microsculpture of very fine wavy periostracal ridgelets and scales; end of last whorl descending strongly below whorl plane on reaching sexual maturity; aperture moderately tilted forward from axis of coiling, without thickened or reflected outer lip and without sulcus behind lip; umbilicus moderately open with U-shaped profile; shell colour pale yellowish brown.

Reproductive anatomy (Fig. 7). Penis cylindrical, narrowing toward genital opening, no penial sheath, penial sculpture of corrugated interlocking longitudinal filaments, distally giving rise to longitudinal rows of strap-like filaments, epiphallus twice as long as penis, with well-developed finger-like epiphallic flagellum at distal end, penial verge broadly conical, with wide longitudinal groove, length equivalent to between about half and one third of length of penis, free; penial retractor attached to proximal third of epiphallus, vas deferens entering head of epiphallus through single pore just below base of epiphallic

flagellum; vas deferens narrow to broad at its junction with apex of epiphallus, later tapering to a narrow tubule; vagina cylindrical, as long as penis, inner wall with prominent longitudinal anastomosing pilasters and filaments; bursa copulatrix long and broad, particularly at its base, folded or kinked several times and about as long as oviduct, with inflated bulb-like head, aligning with base of albumen gland; hermaphroditic duct inserting into head of talon.

Comparative remarks

Austrochloritis niangala can be distinguished from *A. nundinalis* by its smaller shell (Fig. 8) and by having straight instead of curved periostracal setae. Both species exhibit a very similar reproductive anatomy, but differ somewhat in the relative length of the bursa copulatrix (longer in *A. nundinalis*) and the penial verge (occasionally partly attached to penial wall in *A. nundinalis*, free in *A. niangala*). Like *A. nundinalis*, the lack of a reflected outer lip and the setae extending well onto the protoconch are shell features that distinguish *A. niangala* from most other congeners. Despite occurring in relative close proximity near Nundle, both species are separated by genetic p-distances of on average 7% (minimum 5.9%).

Material from the Mt Kaputar NP previously described as *Australochloritis kaputarensis* does not consistently or significantly differ in shell or reproductive characters from material from near the type locality of *A. niangala* and is therefore considered as conspecific.

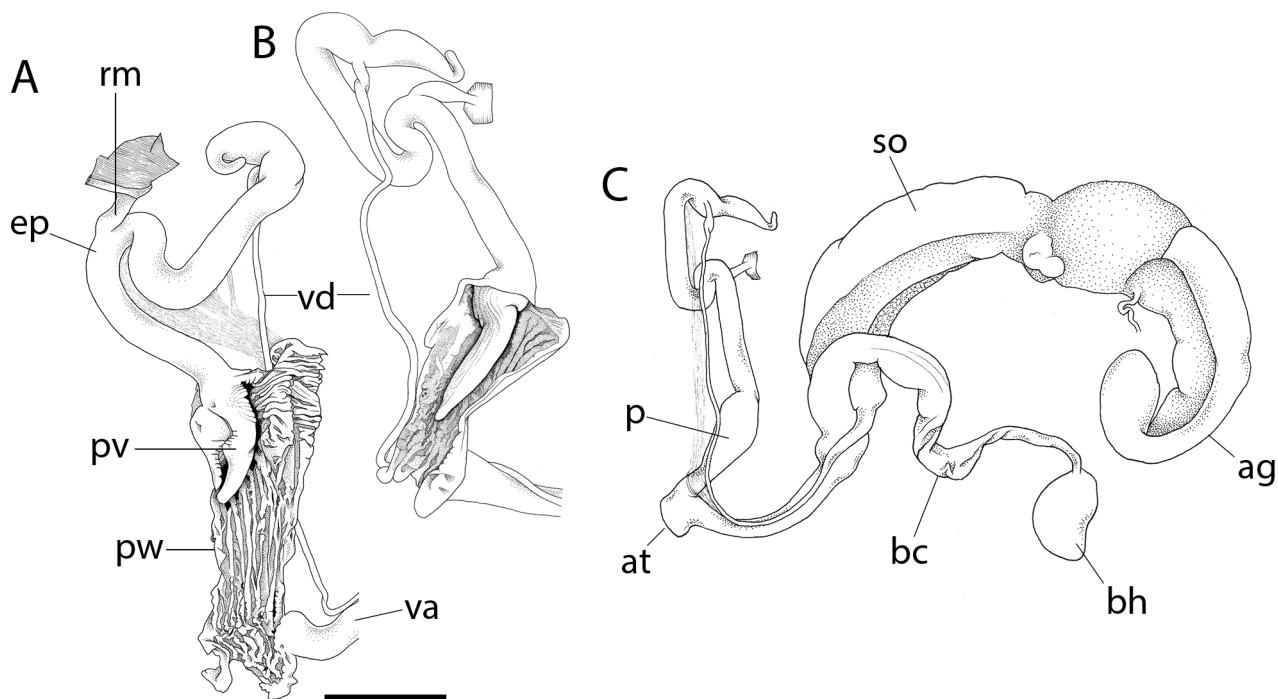


Figure 9. Reproductive anatomy of *Austrochloritis copelandensis* sp. nov. (A) Penial anatomy, paratype, AM C.575460, Copeland Tops; (B) penial anatomy, holotype, AM C.582897, Copeland Tops; (C) reproductive system, paratype, AM C.575460, Copeland Tops; scale bar = 3 mm.

Distribution and ecology

Austrochloritis niangala lives in dry to moist sclerophyll forests on the New England Plateau from Mt Kaputar in the west to near Guyra in east and E of Tamworth in the south (Fig. 5). Most of its range is heavily fragmented into very small remnant patches of forests as large parts of the species' natural range has been cleared for agriculture.

Austrochloritis copelandensis sp. nov.

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Figs 2G, 6C,D, 9A,B

Holotype AM C.582897 (1 w, dissected, sequenced; Fig. 2G) from NSW, Copeland Tops SCA, off Scone road, Hidden Treasure Walking Track, -32.00° 151.83°; leg. Köhler, Xie, Shea, 3/6/2018. **Paratypes** AM C.575460 (2 w, one dissected, sequenced), as for the holotype; AM C.459997 (1w, Copeland Tops SCA, Hidden Treasure Walking Track).

Non-type material examined. AM C.575265 (1 w, Bucketts Mountains off Bucketts Road nr Gloucester, Bucketts Scenic Walk, -32.00° 151.94°).

Description

Shell (Figs 2G, 6C–D). Medium sized to large (W = 13–17 mm), H = (8.5–11 mm); for n = 4); discoidal in shape with a flat to low domed spire, with on average 4.5 rounded to slightly shouldered whorls that increase gradually in diameter; sutures deeply incised; protoconch sculpture of low radials with rugose pustules at apex and with periostracal setae extending well onto protoconch; teleoconch sculpture of low growth lines and corrugations

with periostracal sculpture of strongly curved, crowded and short setae; interstitial microsculpture of very fine wavy periostracal ridgelets and scales; end of last whorl descending strongly below whorl plane on reaching sexual maturity; aperture moderately tilted forward from axis of coiling, with thickened and strongly reflected white outer lip and with shallow sulcus behind lip; umbilicus widely open with U-shaped profile; shell colour from pale yellowish brown to reddish brown, sometimes with a darker mid peripheral colour band.

Reproductive anatomy (Fig. 9). Penis cylindrical, narrowing toward genital opening, no penial sheath; penial sculpture of corrugated interlocking transverse to longitudinal filaments, distally giving rise to longitudinal rows of strap-like filaments; epiphallus more than twice as long as penis, with well-developed finger-like epiphallic flagellum at distal end, moderately long, broad at base, tapering to blunt apex and kinked; penial verge elongately conical and curved with tapering apex, free to partially attached to penial wall, vergic slit longitudinal along length of verge. Verge length equivalent to between about half to two thirds of length of penis. Penial retractor half the length of epiphallus from its base; vas deferens entering head of epiphallus through single pore just below base of epiphallic flagellum; vas deferens narrow to broad at its junction with base of apex of epiphallus but later tapering to a narrow tubule; vagina cylindrical, as long as or one and one quarter longer than penis, inner wall with prominent longitudinal anastomosing pilasters and filaments; bursa copulatrix long and broad, particularly at its base, folded or kinked several times and as long as or slightly longer than oviduct with inflated bulb-like head, aligning with base of albumen gland; hermaphroditic duct inserting into head of talon.

Comparative remarks

Austrochloritis copelandensis is conchologically rather similar to *A. nundinalis*, especially in regard to its comparatively large size (both still being smaller than *A. porteri*) (Fig. 8). However, both species differ from each other in that shells of similar size have about 0.5 more whorls in *A. copelandensis* than *A. nundinalis*. Shells of *A. copelandensis* differ from both *A. nundinalis* and *A. niangala* by having a well-reflected apertural lip and sulcus and a somewhat wider umbilicus. The reproductive anatomy of all three species is rather similar, but *A. copelandensis* differs from *A. nundinalis* and *A. niangala* by having a much longer and more slender penial verge. *Austrochloritis copelandensis* co-occurs with other *Austrochloritis* species: At the type locality, Copeland Tops, the other species resembles *A. nambucca* Iredale, 1943 while at the second known site, Bucketts Range, the second species is *A. disjuncta* (Gude, 1906). Both species are not closely related with *A. copelandensis* based on the mitochondrial phylogeny presented by Köhler *et al.* (2019).

Distribution and ecology

This species lives in scree, dry vine thickets and dry rainforest in the, Manning Valley (Fig. 5), where it has been found under logs and in rock piles. Only two occurrences are currently known; both located in a distance of about 25 km from each other at altitudes of 236 and 348 m on sedimentary laminated siltstones and sandstones (Copeland Tops) or rhyolite (Bucketts Range) bedrock, respectively.

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