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Molecular phylogeny and systematics of Australian and East Timorese Stenothyridae (Caenogastropoda: Truncatelloidea)

Rosemary E. Golding*

Australian Museum, 6 College Street, Sydney, NSW 2010, Australia

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The Australian and East Timorese species belonging to the truncatelloid family Stenothyridae are revised using molecular data and morphological characters from the shell, operculum, radula and external and reproductive anatomy. The Australian species *Stenothyra australis* is redescribed and two previously recognised subspecies are shown to be synonyms. The New Guinean species *Stenothyra paludicola* van Benthem Jutting, 1963 is redescribed and recorded from the Torres Strait region of northern Australia, and two new subspecies of *S. paludicola* are described from the Northern Territory and East Timor; *S. paludicola topendensis* n. subsp. and *S. paludicola timorensis* n. subsp. respectively. *Stenothyra gelasinosa* n. sp. is described from Australia, comprising three allopatric subspecies; *S. gelasinosa gelasinosa* n. sp. and n. subsp. from the eastern seaboard, *S. gelasinosa phrixa* n. subsp. from northern Australia and *S. gelasinosa apiosa* n. subsp. from the Pilbara region of Western Australia. *Stenothyra frustillum* is considered a *nomen dubium*. Molecular phylogenetic analysis of these taxa and other Asian stenothyrids supports these systematic decisions and provides a preliminary interpretation of relationships within Stenothyridae.

Keywords: Rissooidea; microgastropod; mangrove; estuary; freshwater; biogeography; evolution; taxonomy; anatomy

Introduction

The Stenothyridae are a family of small-sized gastropods found in intertidal and shallow-water aquatic habitats in Asia and Australia. While many species are found in brackish estuaries, a large portion of stenothyrid diversity occurs in freshwater environments such as lakes or rivers, and a few taxa are found in unusual habitats such as hot springs (Kosuge 1969). Several major Asian river systems are sites of particularly high stenothyrid diversity (see Brandt 1968, 1970, 1974; Davis *et al.* 1986, 1988), and a few of those taxa have been described in detail. Less in known about estuarine stenothyrids, and many taxa named in the early literature are poorly defined and effectively ignored or overlooked in recent studies of estuarine gastropods. Uncertainty over the identity of many stenothyrid species results in speculative identifications in faunal checklists (e.g. Tan and Woo 2010). Attempts to catalogue estuarine gastropod diversity often result in the listing of undescribed stenothyrid taxa (e.g. Walthew 1995; Robba *et al.* 2003).

A preliminary survey of the literature recovered approximately 100 extant species-level names, belonging to approximately six genus-level groups. Many of these names may be synonyms and, given the lack of assessment of most of these taxa, it is not possible to produce a list of valid taxa. A previous estimate of stenothyrid diversity recognised approximately 60 species globally (Strong *et al*. 2008). Many fossil taxa have been named in several of the Recent genera and an additional six extinct genera have been proposed (Youlou 1978; Esu and Girotti 2010). Almost all Recent species are placed in the genus *Stenothyra* Benson, 1856, in which the monotypic subgenus *Incolaestuarium* Kuroda, 1962 is sometimes recognised. A small number of taxa belong to the Asian genus *Gangetia* Ancey, 1891, and the recently described genus *Farsithyra* Glöer & Pešić, 2009 contains a single Iranian species. The lack of generic structure and high proportion of taxa assigned to *Stenothyra* are probably a consequence of highly conserved shell morphology in the group. *Stenothyra* is easily recognised by its small, circular aperture and dorsoventrally compressed shell, but the characters used to distinguish species such as whorl proportions, size and shell sculpture recur in many species and are also known to be variable within single taxa (Davis *et al*. 1986).

Australia's stenothyrid fauna was initially sparsely recorded by C. Hedley and T. Iredale. A single taxon, *Stenothyra australis* Hedley, 1901 was described from Bowen, central Queensland, and subsequently placed in the genus *Obesitena* Iredale, 1943 with two additional subspecies, *Obesitena australis perdives* Iredale, 1943

*Email: rosemary.e.golding@gmail.com
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Table 1. Primer sequences, PCR annealing conditions and approximate product sizes for each fragment used in molecular analyses.

Gene	Approx. length (bp)	Annealing conditions	Primer sequence	Reference
COI	670	60 s, 45 °C	LCO1490: GGTCAACAAATCATAAAGATATTGG HCO2198: TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994
16S	470–540	60 s, 50–55 °C	16SARis: TGCCTGTTTAGCAAAAACAT 16SBRis: CCGGTCTGAACTCAGATCATGT	Criscione and Ponder 2012
28S	1340–1500	90 s, 56–58 °C	28SDK: GATCGGACGAGATTACCCGCTGAA LSU1600R: AGCGCCATCCATTTTCAGG	Strong *et al*. 2011 Williams *et al*. 2003

from Darwin, Northern Territory and *Obesitena australis wildiana* Iredale, 1943 from Nudgee, near Brisbane, southern Queensland. Since those original descriptions, no new information on the systematics of Australian Stenothyridae has been published. All three available names appear to belong to estuarine species, based on their coastal localities. Nothing is known of the anatomy, phylogenetic relationships, behaviour, ecology, geographic distributions or higher systematics of any Australian stenothyrid. Three species of *Stenothyra* were described by van Benthem Jutting (1963) from the southern coastline of New Guinea, but those species have not been subsequently reported.

This study aims to document the diversity of Australian stenothyrids using specimens presently held in museum collections as well as material collected from various regions of tropical Australia during this project. Species discrimination will be informed by shell morphology, observations of the living animal, microscopic anatomical details and molecular data. The detailed morphology-based treatment of a Chinese stenothyrid species complex by Davis *et al*. (1986, 1988) provides a good guide for the morphological treatment of Australian taxa. This study also uses molecular data to reconstruct the phylogenetic relationships of the examined Australian species of Stenothyridae and a small selection of Asian stenothyrids. A comprehensive global molecular phylogeny and systematic revision of Stenothyridae is long overdue, but unfortunately beyond the scope of this work.

Materials and methods

Material examined in this study was mainly sourced from the collections of the Australian Museum, supplemented by additional collections in tropical Queensland (September 2011) and northern Western Australia (June 2012) as part of a larger study of Australian mangrove microgastropods (see Golding in press). Representative specimens of most species were collected alive and photographed crawling in a petri dish of salt water, using a microscope-mounted camera. Records of pigmentation and foot/tentacle morphology are included in descriptions where known. The animals were preserved for either molecular (95–100% EtOH) or morphological (10% saltwater formalin or 70% EtOH) examination.

Dissections were performed under a stereomicroscope and illustrated using a *camera lucida*. Radulae were removed from dissected buccal masses by dissolution overnight in a warmed solution of sodium hydroxide. Shells, radulae and opercula were cleaned in a sonic water bath, mounted on stubs and thinly coated with gold for examination by scanning electron microscopy (performed by Sue Lindsay, Microscopy and Microanalysis Laboratory, Australian Museum) using a Zeiss Evo LS-15 machine. Shell measurements were made using a calibrated *camera lucida*, with whorl counts rounded to the nearest 1/4 whorl.

For molecular analysis, either entire animals or samples of foot tissue were processed using a DNeasy kit (Qiagen, Inc., Hilden, Germany) and QiaCube® robot to extract genomic DNA. Three gene fragments were amplified by polymerase chain reaction (PCR); COI and 16S from the mitochondrial genome and 28S from the nuclear genome. PCRs were performed in 25 µl volumes containing 1 × PCR buffer, 200 mM each dNTP, 2.0 mM $MgCl_2$, 0.5 mM forward and reverse primers, 1.25 units Taq polymerase, and approximately 50 ng DNA. Amplification followed a standard protocol with 35 cycles of 94 °C for 1 min, primer-specific annealing conditions (Table 1) and 72 °C for 1 min. Post-PCR products were purified using ExoSAP-IT proteinase solution (GE Healthcare, USA) and sequenced in both directions by Macrogen Inc. (Seoul, Korea).

Raw sequences were checked for misreads against their electropherogram and compiled using BioEdit v7.0.9 (Hall 1999). All sequences were deposited in GenBank with accession numbers provided in Table 2 (KC439688-KC439930). Ribosomal sequences were aligned using the online MAFFT v.6 server (Katoh *et al.* 2002) with the E-INS-i option (Katoh *et al.* 2005) implement for the 16S dataset and Q-INS-i (Katoh and Toh 2008) for the 28S dataset. COI sequences were translated using the invertebrate mitochondrial code and unambiguously aligned by amino acid sequence. Datasets were compiled using Mesquite v2.75 (Maddison and Maddison 2011) and uncorrected pairwise distances computed within and between taxa using MEGA5 (Tamura *et al.* 2011).

Australian species of Stenothyridae are represented in the molecular phylogenetic analyses by 4–16 specimens

Table 2. Identity, location/source, registration and GenBank accession numbers for specimens included in the molecular analyses. All sampling locations are in Australia unless otherwise stated. Sequences generated during this project are in bold and all other sequences were produced by Criscione and Ponder (2012). Taxa described in this manuscript and the accompanying manuscript (Iravadiidae) are named accordingly.

Family	Species	Locality/Source	AM reg.	COI	16S	28S
Stenothyridae	*Stenothyra australis* Hedley, 1901	Nudgee, Brisbane, QLD	C.470968	**KC439688**	**KC439810**	—
Stenothyridae	*S. australis*	Tin Can Bay, QLD	C.470957	**KC439689**	**KC439811**	—
Stenothyridae	*S. australis*	Turkey Beach, QLD	C.470943	**KC439690-1**	**KC439812-3**	—
Stenothyridae	*S. australis*	Magazine Cr., Bowen, QLD	C.470905	**KC439692-3**	**KC439814-5**	**KC439915**
Stenothyridae	*S. australis*	Magnetic Isl., QLD	C.470862	**KC439694-5**	**KC439816-7**	—
Stenothyridae	*S. australis*	Karumba, QLD	C.470895	**KC439696-8**	**KC439818-20**	—
Stenothyridae	*S. australis*	Rapid Cr., Darwin, NT	C.475889	**KC439699-700**	**KC439821-2**	—
Stenothyridae	*S. australis*	Dampier Cr., Broome, WA	C.475995	**KC439701-2**	**KC439823-4**	**KC439916**
Stenothyridae	*S. australis*	Cowrie Cove, Dampier Pen., WA	C.476021	**KC439703**	**KC439825**	—
Stenothyridae	*Stenothyra gelasinosa gelasinosa* n. sp. & n. subsp.	Woy Woy, NSW	C.475869	**KC439704-5**	**KC439826-7**	**KC439917**
Stenothyridae	*S. gelasinosa gelasinosa*	Nudgee, Brisbane, QLD	C.470969	**KC439706-7**	**KC439828-9**	—
Stenothyridae	*S. gelasinosa gelasinosa*	Magazine Cr., Bowen, QLD	C.470906	**KC439708-9**	**KC439830-1**	**KC439918**
Stenothyridae	*S. gelasinosa gelasinosa*	Magnetic Isl., QLD	C.470865	**KC439710-1**	**KC439832-3**	—
Stenothyridae	*S. gelasinosa gelasinosa*	Endeavour R., Cooktown, QLD	C.470869	**KC439712-3**	**KC439834-5**	**KC439919**
Stenothyridae	*Stenothyra gelasinosa phrixa* n. subsp.	Wallaby Isl., Weipa, QLD	C.470883	**KC439714**	**KC439836**	—
Stenothyridae	*S. gelasinosa phrixa*	East Woody Beach, Arnhem Land, NT	C.175248	**KC439715-6**	**KC439837-8**	—
Stenothyridae	*S. gelasinosa phrixa*	Rapid Cr., Darwin, NT	C.475888	**KC439717**	**KC439839**	**KC439920**
Stenothyridae	*Stenothyra gelasinosa apiosa* n. subsp.	Four Mile Cr., Port Hedland, WA	C.476011	**KC439718-9**	**KC439840-1**	—
Stenothyridae	*S. gelasinosa apiosa*	Nickol Bay, Karratha, WA	C.476017	**KC439720-1**	**KC439842-3**	**KC439921**
Stenothyridae	*Stenothyra paludicola topendensis* n. subsp.	Black Point Ranger Stat., Cobourg Pen., NT	C.458234	**KC439722-3**	**KC439844-5**	—
Stenothyridae	*S. paludicola topendensis*	Gumeragi Outstation, Cobourg Pen., NT	C.175257	**KC439724-5**	**KC439846-7**	—
Stenothyridae	*S. paludicola topendensis*	Coast track, Cobourg Pen., NT	C.175251	**KC439726**	**KC439848**	—
Stenothyridae	*S. paludicola topendensis*	NE of Towns River, Arnhem Land, NT	C.220253	**KC439727-8**	**KC439849-50**	—
Stenothyridae	*S. paludicola topendensis*	Numbulwar, Arnhem Land, NT	C.175259	**KC439729-30**	**KC439851-2**	—
Stenothyridae	*S. paludicola topendensis*	South Alligator R. floodplain, Kakadu, NT	C.175250	**KC439731-2**	**KC439853-4**	**KC439922**
Stenothyridae	*Stenothyra paludicola timorensis* n. subsp.	Manufahi, East Timor	C.475872	**KC439733-4**	**KC439855-6**	**KC439923**
Stenothyridae	*Stenothyra monilifera* Benson, 1856	Mahatchai, Bangkok, Thailand	C.474161	**KC439735-6**	**KC439857-8**	**KC439924**

(*Continued*)

Table 2. Continued

Family	Species	Locality/Source	AM reg.	COI	16S	28S
Stenothyridae	*S. monilifera*	Bang Poo, Bangkok, Thailand	C.474160	**KC439737**	**KC439859**	**KC439925**
Stenothyridae	*Stenothyra* cf. *polita* (A. Adams, 1851)	Bang Poo, Bangkok, Thailand	C.474159	**KC439738-9**	**KC439860-1**	**KC439926**
Stenothyridae	*Stenothyra* sp. 'Johor'	Tanjung Labuh, Johor, Malaysia	C.474158	**KC439740**	**KC439862**	**KC439927**
Stenothyridae	*Stenothyra* cf. *glabra* A. Adams, 1861	Mai Po, Hong Kong, China	C.474163	**KC439741-2**	**KC439863-4**	**KC439928**
Stenothyridae	*Stenothyra* cf. *hardouini* Morgan, 1885	Qi'ao Isl., Zhuhai, China	C.472947	**KC439743**	**KC439865**	—
Stenothyridae	*Stenothyra* cf. *divalis* (Gould, 1859)	Qi'ao Isl., Zhuhai, China	C.476089	**KC439744**	**KC439866**	**KC439929**
Stenothyridae	*S*. cf. *divalis*	Mai Po, Hong Kong, China	C.474162	**KC439745-6**	**KC439867-8**	**KC439930**
Stenothyridae	*Stenothyra* sp. 'Philippines'	Sampaloc Lake, Luzon, Philippines	C.416776	**KC439747-9**	**KC439869-71**	—
Iravadiidae	*Fluviocingula resima* (Laseron, 1956)	Karumba, QLD	C.470897	**KC439778**	**KC439900**	**KC439947**
Iravadiidae	*Pellamora australis* (Hedley, 1900)	Turkey Beach, QLD	C.470945	**KC439774**	**KC439896**	**KC439944**
Iravadiidae	*Pseudomerelina mahimensis* (Melvill, 1893)	Campwin Beach, QLD	C.470926	**KC439767**	**KC439889**	**KC439941**
Iravadiidae	*Nozeba topaziaca* (Hedley, 1908)	Tin Can Bay, QLD	C.470999	**KC439784**	**KC439906**	**KC439952**
Calopiidae	*Calopia imitata* Ponder, 1999	Woy Woy, NSW	C.475871	**KC439790**	**KC439912**	**KC439957**
Anabathridae	*Anabathron contabulatum* (Frauenfeld, 1867)	GenBank/AM tissue collections	C.466922	**KC439793**	KC109937	KC109989
Anabathridae	*Pisinna punctulum* (Philippi, 1836)	GenBank/AM tissue collections	C.463767	**KC439794**	KC109968	KC110020
Anabathridae	*Badepigrus pupoideus* (H. Adams, 1865)	GenBank/AM tissue collections	C.475761	**KC439795**	KC109942	KC109994
Anabathridae	*Nodulus contortus* (Jeffreys, 1856)	GenBank/AM tissue collections	C. 463766	**KC439796**	KC109966	KC110018
Assimineidae	*'Assiminea' capensis* (Sowerby, 1892)	GenBank/AM tissue collections	C.463732	**KC439797**	KC109939	KC109991
Falsicingulidae	*Falsicingula mundana* Yokoyama, 1926	GenBank/AM tissue collections	C.466899	**KC439798**	KC109957	KC110009
Truncatellidae	*Truncatella subcylindrica* (Linnaeus, 1767)	GenBank/AM tissue collections	C.463886	**KC439799**	KC109982	KC110035
Pomatiopsidae	*Coxiella striata* (Reeve, 1842)	GenBank/AM tissue collections	C.421184	**KC439800**	KC109948	KC110000
Hydrobiidae	*Hydrobia acuta* (Draparnaud, 1805)	GenBank/AM tissue collections	C.463786	**KC439801**	KC109959	KC110011
Tateidae	*Tatea rufilabris* (A. Adams, 1862)	GenBank/AM tissue collections	C.466927	**KC439802**	KC109980	KC110033
Clenchiellidae	*Clenchiella minutissima* (Wattlebled, 1884)	GenBank/AM tissue collections	C.475762	**KC439803**	KC109947	KC109999
Caecidae	*Caecum trachea* (Montagu, 1803)	GenBank/AM tissue collections	C.463777	**KC439805**	KC109945	KC109997
Tornidae	*Elachorbis subtatei* (Suter, 1907)	GenBank/AM tissue collections	C.466923	**KC439807**	KC109953	KC110005
Tornidae	*Scrupus* sp.	GenBank/AM tissue collections	C.466910	**KC439808**	KC109974	KC110027
Rissoidae	*Rissoina fasciata* A. Adams, 1851	GenBank/AM tissue collections	C.466913	**KC439809**	KC109972	KC110025

from many populations across a broad geographic range (Table 2). Non-Australian taxa are sparsely sampled and mostly represented by specimens from a single locality. Species from East Timor, Hong Kong, Thailand, Malaysia and the Philippines were included in this study but represent only a small fraction of global stenothyrid diversity. All of the foreign material was collected in mangrove or brackish-water habitats, except for material from the Philippines that was collected from a freshwater lake. Non-Australian taxa were provisionally identified where possible using the available literature (Brandt 1974, Davis *et al.* 1986). Some non-Australian specimens included in the molecular analysis could not be identified and may belong to undescribed taxa, but are not further treated here.

Sequences from additional, non-stenothyrid, taxa were also examined. Sequences of 16S and 28S gene fragments were recently obtained by Criscione and Ponder (2012) for a phylogenetic analysis of Rissooidea and Truncatelloidea, and some sequences from their study are included here. Truncatelloidea currently comprises approximately 23 families, including Stenothyridae (Criscione and Ponder 2012). During the present study additional COI sequences were generated from the original DNA extracts deposited in the Australian Museum collections (Criscione and Ponder 2012). Further sequences from two species of Calopiidae and several Iravadiidae generated as part of a larger project exploring Australian truncatelloid diversity were also included in the datasets.

Two datasets were constructed in order to test the position and monophyly of Stenothyridae in Truncatelloidea and also to examine the internal relationships and molecular diversity of the sampled representatives of Stenothyridae.

A Concatenated COI (excluding 3rd codon positions), 16S and 28S sequences with 100% coverage. Composed of 30 taxa, each represented by a single individual: 10 Stenothyridae, 19 other truncatelloids and outgroup *Rissoina fasciata* (Rissoidae). Total of 2607 base pairs (bp), consisting of: 446 bp COI, 564 bp 16S, 1597 bp 28S.

B Concatenated COI (including 3rd codon positions), 16S and 28S sequences with 100% coverage of the mitochondrial genes but only partial coverage of 28S (18 of 64 individuals). Composed of 15 taxa: 13 Stenothyridae (most represented by multiple individuals) and outgroups '*Assiminea*' *capensis* (Assimineidae) and *Truncatella subcylindrica* (Truncatellidae). Total of 2763 bp, consisting of: 669 bp COI, 517 bp 16S, 1577 bp 28S.

Phylogenetic hypotheses were explored using maximum likelihood (ML) and Bayesian Inference (BI) methods. ML analyses were performed using RAxML v7.3.2 (Stamatakis 2006) implemented in raxmlGUI v1.2 (Silvestro and Michalak 2011) with 1000 'thorough' bootstrap repetitions. Datasets were partitioned by gene and the GTRGAMMA model was implemented. BI analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Bayesian posterior probability support was estimated by running four Markov chains (10 million generations each, with trees sampled each thousand generations). The first 25% of trees were conservatively rejected as burn-in, and stationarity was confirmed by examination of the log likelihood plot using Tracer (Rambaut and Drummond 2007). A summary consensus tree with support indices was generated by MrBayes. Datasets were partitioned by gene, with the GTR + G + I model of sequence evolution selected for both datasets by MEGA5. Trees were visualised using FigTree v1.3.1 and rooted using the outgroup. Nodal support was considered high for Bayesian probabilities >95% and bootstraps >80%, and moderate for Bayesian probabilities 90–95% and bootstraps 70–80%. Nodes with lower support values were not considered significant.

Systematic descriptions are provided for all Australian taxa identified during this study. In addition, a new subspecies from East Timor is also described. A comprehensive description has been provided for *Stenothyra australis*; for the remaining taxa, only characters useful for discriminating species and subspecies are included in the descriptions. Unless otherwise stated, locations given for type materials examined are in Australia. Materials examined have been summarised here, but full details are provided in a supplementary file.

Abbreviations

States of Australia: NSW—New South Wales; NT—Northern Territory; QLD—Queensland; VIC—Victoria; WA—Western Australia.
Institutions: AMS—Australian Museum, Sydney; NTM—Northern Territory Museum; QM—Queensland Museum; RMNH—Rijksmuseum van Natuurlijke Historie; WAM—Western Australian Museum; ZMA—Zoological Museum Amsterdam.
Shell dimensions: SL—shell length; SD_1—lateral diameter of last whorl; SD_2—dorsoventral diameter of last whorl; AL—aperture length; AW—aperture width; PWC—protoconch whorl count; TWC—teleoconch whorl count; TWS—sculptured teleoconch whorl count.

Results

Sequence divergence

Mean uncorrected pairwise distances of COI within each lowest level taxonomic unit (species or subspecies) were <1.17% ($n = 2$–16) (Table 3). Mean p-distance between Australian species or non-conspecific subspecies ranged from 10.77% (*S. australis* vs. *S. gelasinosa gelasinosa*

Table 3. COI sequence divergence (including third codon base positions) between taxa (sample size *n*) represented by mean uncorrected pairwise distances (%). Values in bold are mean p-distance within each taxon. Taxa described or revised in this manuscript are named accordingly.

	n	*S. australis*	*S. g. gelasinosa*	*S. g. phrixa*	*S. g. apiosa*	*S.* cf. *divalis*	*S. monilifera*	*S.* sp. 'Johor'	*S. p. topendensis*	*S. p. timorensis*	*S.* cf. *glabra*	*S.* cf. *polita*	*S.* cf. *hardouini*	*S.* sp. 'Philippines'
Stenothyra australis	16	**0.86**												
Stenothyra gelasinosa gelasinosa n. sp. & n. subsp.	10	10.77	**0.17**											
Stenothyra gelasinosa phrixa n. subsp.	4	12.20	5.01	**1.17**										
Stenothyra gelasinosa apiosa n. subsp.	4	12.59	5.41	4.80	**0.67**									
Stenothyra cf. *divalis*	3	11.11	11.05	12.10	12.97	**0.06**								
Stenothyra monilifera	3	12.15	13.63	14.45	51.93	12.29	**0.40**							
Stenothyra sp. 'Johor'	1	11.95	11.58	12.52	13.49	11.91	11.11	—						
Stenothyra paludicola topendensis n. subsp.	11	13.41	12.60	13.63	13.46	11.55	13.04	11.33	**0.43**					
Stenothyra paludicola timorensis n. subsp.	2	12.91	12.03	13.27	13.30	10.81	12.16	11.96	3.00	**0.00**				
Stenothyra cf. *glabra*	2	13.24	12.47	13.79	13.75	13.05	12.31	11.81	10.30	10.76	**0.15**			
Stenothyra cf. *polita*	2	13.73	14.81	15.55	15.36	13.85	15.15	12.86	13.86	14.05	15.17	**0.60**		
Stenothyra cf. *hardouini*	1	14.24	12.12	13.34	14.35	11.81	13.80	11.36	11.37	11.06	12.93	14.50	—	
Stenothyra sp. 'Philippines'	3	14.68	14.08	14.42	15.87	13.15	14.55	12.95	12.23	11.56	13.00	16.39	13.55	**0.30**

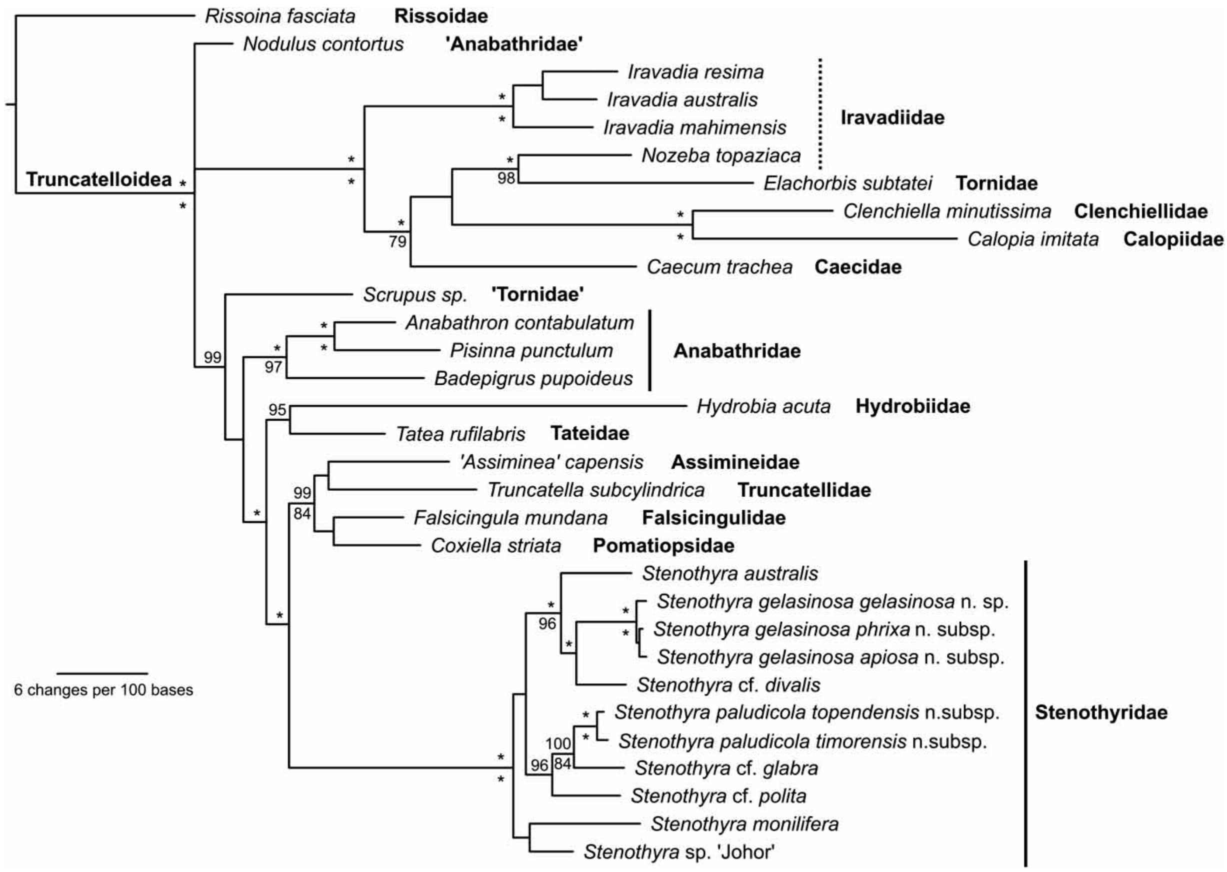

Figure 1. Summary tree from Bayesian analysis of concatenated COI (excluding 3rd codon position bases), 16S and 28S sequences (10 million generations, trees sampled every 1000 generations). Support indices are BI posterior probability (above nodes, >90%) and ML bootstraps (below nodes, >70%); asterisks indicate a support value of 100%. Family and higher level names are in bold. Taxa described in this manuscript are named accordingly.

n. sp. and n. subsp.) to 13.63% (*S. paludicola topendensis* n. subsp. vs. *S. gelasinosa phrixa* n. subsp.). COI sequence divergence between conspecific subspecies (see Systematics section) varied from 3.00% (*S. paludicola topendensis* n. subsp. vs. *S. paludicola timorensis* n. subsp.) to 5.41% (*S. gelasinosa gelasinosa* n. sp. and subsp. vs. *S. gelasinosa apiosa* n. subsp.).

Molecular phylogenies

Phylogenetic reconstruction by BI and ML methods produced consensus trees with identical topologies, with the exception of several weakly supported clades composed of closely-related populations from each subspecies. Only the BI summary tree is shown here, labelled with both Bayesian posterior probabilities (BPP) and bootstrap support values generated by ML analysis (Figs 1, 2).

The phylogenetic analysis of Truncatelloidea, including single representatives of most *Stenothyra* species examined here, found strong support for the monophyly of Stenothyridae (BPP and ML bootstrap values of 100%) and conflicting support (BPP = 100, ML bootstrap value not significant) for a sister relationship with a clade composed of Assimineidae, Truncatellidae, Falsicingulidae and Pomatioposidae (Fig. 1). Together, this group of families corresponds to clade F in the phylogeny produced by Criscione and Ponder (2012). Relationships between Stenothyridae and other truncatelloids were partially resolved by the phylogenetic analysis (Fig. 1), with strong support for a clade composed of *S. australis, S. gelasinosa* n. sp. and *S.* cf. *divalis*, and for a sister relationship between *S. paludicola* and *S.* cf. *glabra*.

The phylogenetic analysis of Stenothyridae with only two truncatelloid outgroups also provided strong support for the integrity of all species and subspecies of *Stenothyra* that were represented by more than one individual (Fig. 2). *Stenothyra australis, S. gelasinosa* n. sp. and *S.* cf. *divalis* formed a well-supported clade, with *S. australis* basal (Fig. 2). A relationship between *S. monilifera* and an unidentified species from the Malaysian Peninsula (*S.* sp. 'Johor') was supported by BPP (98%) but not by ML bootstrap values (<80%). Likewise, a clade comprising *S. paludicola* and *S.* cf. *glabra* was moderately supported by BPP values (92%), but not by ML bootstrap values

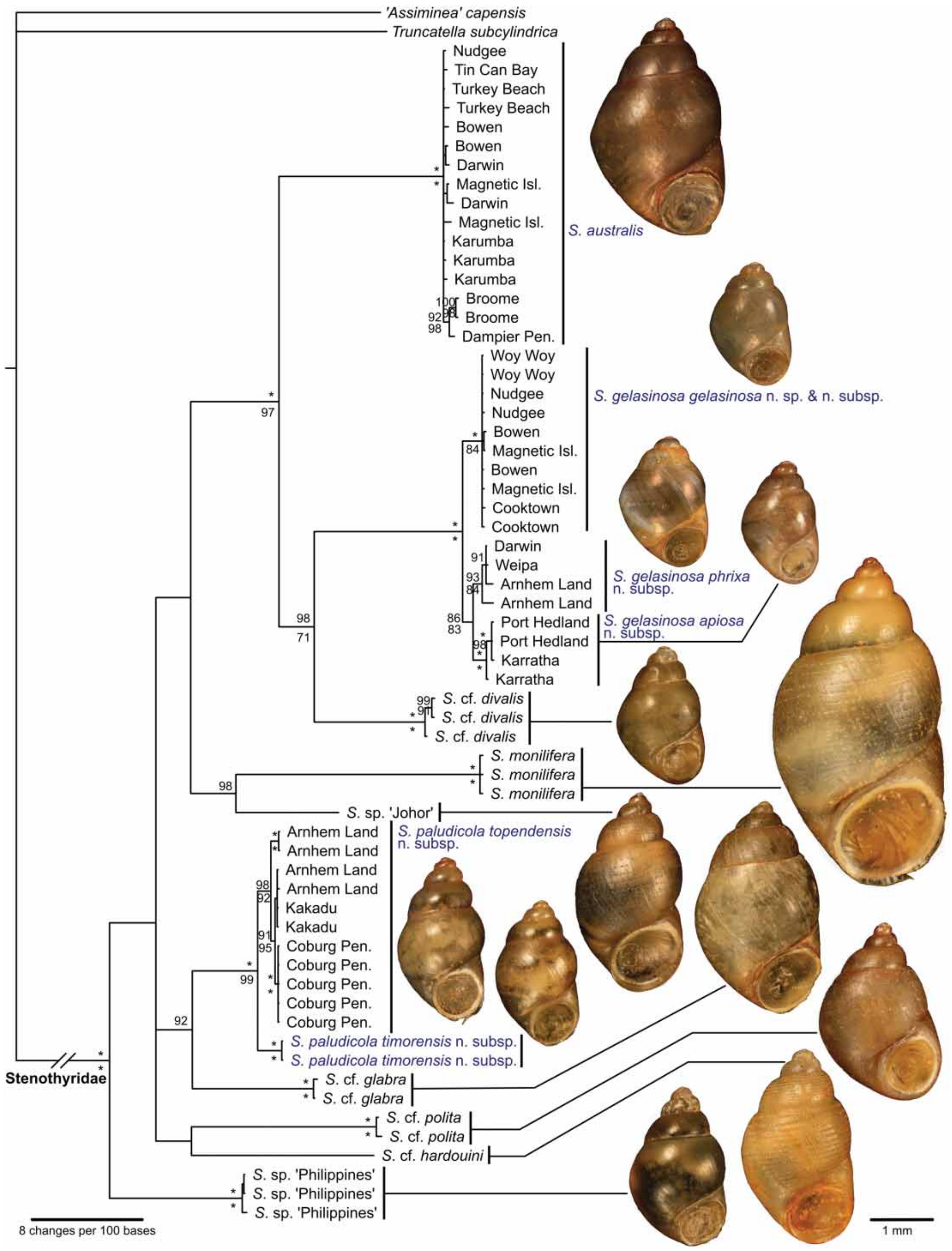

Figure 2. Summary tree from Bayesian analysis of concatenated COI (including 3rd codon position bases), 16S and 28S sequences (10 million generations, trees sampled every 1000 generations). Support indices are BI posterior probability (above nodes, >90%) and ML bootstraps (below nodes, >70%); asterisks indicate a support value of 100%. Taxa described in this manuscript are named accordingly and labelled in blue, with locality data given for each sample. Shell images are all to the same scale.

(<80%). Relationships between these clades and other branches of the tree were not well-supported in either BI or ML analyses (Fig. 2).

Systematics

Gastropoda
Caenogastropoda
Truncatelloidea Gray, 1840
Stenothyridae Tryon, 1866
Stenothyra Benson, 1856

Stenothyra australis Hedley, 1901

Stenothyra australis Hedley 1901: 724–725, pl. 48, fig. 10.
Obesitena australis Iredale 1943: 205.
Obesitena australis wildiana Iredale 1943: 205–206.
Obesitena australis perdives Iredale 1943: 205–206.

Table 4. Shell measurements of type and other specimens of *Stenothyra australis* and species synonymised with *S. australis*. Averages exclude measurements of the holotype of *S. nebularum* which were taken from a photograph.

Registration no.	Status	SL (mm)	SD_1 (mm)	SD_2 (mm)	AL (mm)	AW (mm)	PWC	TWC	TWS
O. australis perdives									
AMS C.100583	Ht	4.19	2.65	2.16	1.11	1.19	2	3 1/2	1 1/2
O. australis wildiana									
AMS C.030164	Ht	4.68	2.81	2.35	1.22	1.32	2	3 3/4	3/4
S. nebularum									
ZMA.MOLL.136312	Ht	3.00	1.88	?	0.82	0.93	?	?	?
S. australis									
AMS C.008975	Ht	3.72	2.36	1.89	0.96	1.02	2	3 1/4	1 1/4
AMS C.100778	Pt	3.70	2.28	1.81	1.00	1.00	1 3/3	3 1/4	1 1/2
AMS C.100778	Pt	3.19	1.91	1.60	0.87	0.85	2	2 3/3	1/2
AMS C.470968	—	3.66	2.26	1.79	1.00	1.04	1 3/4	3 1/2	1
AMS C.470968	—	3.65	2.30	1.84	1.00	1.11	2	3 1/4	1
Average or range ($n = 7$)		3.83	2.37	1.92	1.02	1.08	1 3/3–2	2 3/4–3 3/4	1/2–1/2

Notes: Ht, holotype; Pt, paratype; TWS, teleoconch whorls with spiral sculpture. See ‘Materials and methods’ for other abbreviations.

? *Stenothyra nebularum* van Benthem Jutting 1963: 447–448, fig. 12 [possible synonym].

Material examined

Holotype of Stenothyra australis. Bowen, Queensland, 20°01’S 148°15’E, coll. J. Brazier, c.1900, on mangrove leaves on beach (AMS C.008975). *Paratypes* of *Stenothyra australis*. Same data (AMS C.100778, 5). *Syntypes* of *Obesitena australis wildiana*. Nudgee, Queensland, 27°21’S 153°6’E, coll. C. J. Wild, c.1909 (AMS C.030164, 84). *Holotype* of *Obesitena australis perdives*. Darwin, Northern Territory, 12°28’S 130°50’E, coll. J. Laseron, c.1942-1945, on beaches (AMS C.100583). *Holotype* of *Stenothyra nebularum*, Robinson River Plantations, near Cloudy Bay, E of Port Moresby, Papua New Guinea, 10°09′*S* 148°47\′′E, coll. J. P. van Niel, 1958-59 (ZMA.MOLL.136312), examined from photograph only.

Other material. 1 dry lot from northern NSW; 12 wet lots and 18 dry lots from Queensland between Moreton Bay and Karumba; 2 wet lots and 10 dry lots from Northern Territory between Arnhem Land and the Tiwi Islands; 12 wet lots and 1 dry lot from Western Australia between Broome and Burrup Peninsula. See supplementary data for full list of material examined.

Redescription

Shell (Figs 2, 3). Ovate-conic, with rounded to angled inflation of last whorl, dorsoventrally compressed; spire moderately tall, conical; sutures shallow, upper whorls almost straight-sided; nonumbilicate; up to 5 3/4 whorls including protoconch, SL = 3.19–4.68 mm, SD_1 = 1.91–2.81 mm, SD_2 = 1.60–2.35 mm (ratio SD_1:SD_2 ~1.23) (Table 4). Slightly lustrous, golden to olive brown; periostracum thin. Sculpture on first 3/4 – 11/2 teleoconch whorls of 7–11 thin spiral ridges, terminating irregularly. Aperture smaller than preceding whorl, near circular, posterior margin simple; outer lip strongly prosocline.

Protoconch (Figs 3F, G). Dome-shaped; 13/4 – 2 whorls. Smooth, transitional varix well developed.

Operculum (Figs 4A, B). Near circular, with very weak angulation aligning with posterior apex of aperture; exterior surface with subcentral, paucispiral nucleus; interior surface with semicircular groove running inside umbilical margin, terminating at each end with equal-sized, perpendicular, semicircular ‘wings’.

Radula (n = 4) (Fig. 4C). Central tooth $2 - 3 + 1 + 2 - 3/3 - 4 + 3 - 4$; central cusp large, secondary cusps and basal denticles diminishing outwardly. Lateral teeth $4 - 5 + 1 + 8 - 9$. Marginal teeth with subequal cusps; inner marginal teeth with ~28 cusps on tip and distal half of outer edge; outer marginal teeth with ~10 cusps on distal third of inner edge.

External morphology and colouration in life (Fig. 5). Head-foot mottled grey and cream/yellow; darkest around eyes, on ventral surface of snout, sides of neck and dorsal surface of foot anterior to operculum. Dorsal surface of snout patterned with saddle-shaped outline of black pigment (sometimes appearing as two parallel transverse bands); lips unpigmented. Cephalic tentacles patterned with black bands interspaced with 7–12 narrow rings of cream/yellow pigment, closely spaced proximally but increasingly separated distally. Anterior foot with elongate lateral lobes, posterior margin rounded. Metapodial tentacle present, narrow, contractile, unpigmented; <25% of extended foot length, held straight and parallel to substrate in life. Mantle visible through transparent shell, mottled black and yellow with prominent broken ‘T-shaped’ pattern of black pigment; interior surface of anterior mantle margin black with scattered flecks of yellow pigment, especially around pallial tentacle. Pallial tentacle

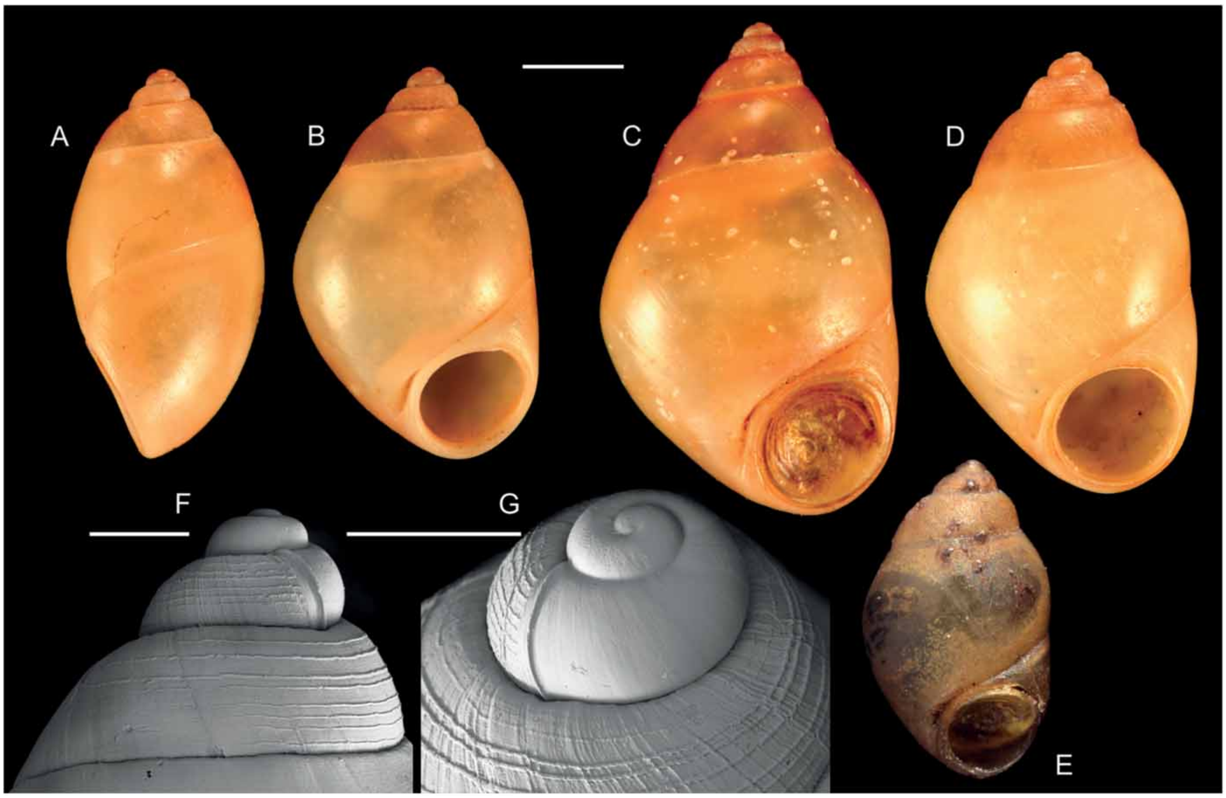

Figure 3. *Stenothyra australis* Hedley, 1901. **A,B,** Holotype of *S. australis*, Bowen, QLD, AMS C.008975; **C,** syntype of *S. australis wildiana*, Nudgee, QLD, AMS C.30164; **D,** holotype of *S. australis perdives*, Darwin, AMS C.100583; **E,** holotype of *S. nebularum*, Robinson River Plantations, E of Port Moresby, Papua New Guinea, photographed by Jeroen Goud; **F,** apical shell with periostracum, Turkey Beach, QLD, AMS C.470943; **G,** protoconch, Magnetic Island, QLD, AMS C.470862. Scale bars: A–E = 1 mm; F, G = 300 µm.

present on right side of anterior mantle margin; small, semi-circular lobe demarcated by ciliated ridge across base of tentacle; held against exterior surface of lip in life.

Male reproductive system (Figs 4D, E; 6A, C). Testis (te) multilobed, leading to broad, tubular vas deferens (vd) coiled in three loops (vd L1, L2, L3) with 3rd elongate loop positioned below testis. Seminal vesicle (sv) large, peanut-shaped, forming proximal vas deferens. Pallial prostate divided into digitiform lobes. Penis coiled, cylindrical; duct more or less straight, opening at distal end via penial stylet; black on inner margin, covered with white to yellow flecks; evenly ciliated. Penial stylet curved, spatulate 'shoehorn-shaped', with blunt, rounded tip; covered with flesh on dorsal surface, except for oval aperture at tip (presumed opening for sperm duct).

Female reproductive system (Figs 6B, D). Ovary ramifying; oviduct forming hairpin loop. Pallial oviduct divided into three glandular segments, opening posterior to anus near mantle margin. Seminal receptacle bulb connected by short duct to loop of sperm duct; sperm duct opening in posterior mantle cavity near accessory sperm pouch. Bursa copulatrix long, curved or bent double, muscular; opening to sperm duct near aperture.

Distribution (Fig. 7A)

Common in mangrove forests along the coast of tropical and subtropical Australia (possibly extending to southern New Guinea). The most southerly records in the collections of the Australian Museum are from the Clarence River in northern New South Wales and Karratha in central Western Australia. Recent field collections on the east coast of Australia found that the range of *S. australis* terminated around Brisbane. Sampling was not conducted south of Karratha, so it is possible that the species may occur further south on the west coast of Australia.

Remarks

Iredale's (1943) subspecies of *Stenothyra australis* are synonymous with the typical species as comparison of the type specimens shows only small differences that are within the variation of the species, and sequence data from specimens collected at all three type localities supports the existence of a single species-group taxon. The holotype of the Papua New Guinean species *S. nebularum* has similar spiral sculpture on the upper whorls and is only slightly smaller than some specimens (including one of the

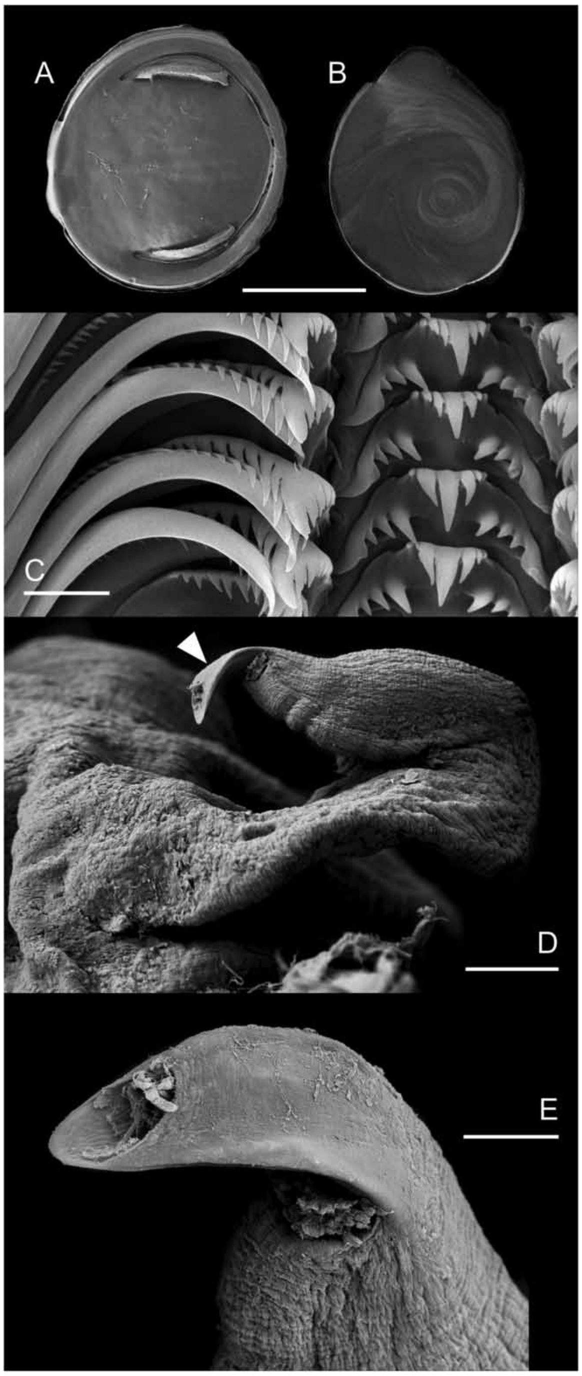

Figure 4. *Stenothyra australis* Hedley, 1901. **A,** Interior surface of operculum, Nudgee, QLD, AMS C.470968; **B,** exterior surface of operculum, Pallarenda, QLD, AMS C.331874; **C,** radula, AMS C.470968; **D, E,** critical point dried penis and penial stylet (white arrowhead), AMS C.470968. Scale bars: A, B = 500 μm; C = 10 μm; D = 100 μm; *E* = 30 μm.

paratypes) of *S. australis*. It is possible that *S. nebularum* is a junior synonym of *S. australis*. However, only photographs of the type specimens have been examined and the size difference may prove to be significant when further New Guinean material is examined. *Stenothyra australis* is generally larger than the other Australian species, and has a distinctively tall, conical spire with linear spiral ridges.

Stenothyra gelasinosa n. sp.

Remarks

Representative specimens of *Stenothyra gelasinosa* n. sp. form a monophyletic clade in the molecular analysis, but are arranged in three geographically discrete groups that are also differentiated by several morphological characters. These taxa are here recognised as subspecies of the *S. gelasinosa* n. sp. The shells of *S. gelasinosa* n. sp. are similar in shape to *Stenothyra saccata* van Benthem Jutting, 1963, from Papua New Guinea. However, the holotype of *S. saccata* is 30% taller than any specimen of *S. gelasinosa* n. sp. collected in Australia. The holotype of *S. saccata* was examined during this study from photographs only, and the presence or absence of the spiral pitted sculpture that characterises *S. gelasinosa* n. sp. could not be confirmed and were not mentioned in the original description. These Australian and New Guinean species are probably closely related, but at this stage there is sufficient evidence from the size of the animals to erect a new species for the Australian taxa.

Sequence data strongly supports the monophyly of *S. gelasinosa* n. sp. and each subspecies reciprocally. The subspecies have low sequence divergence within each taxon, but 4.80–5.41% divergence of the COI gene between subspecies. *Stenothyra gelasinosa* n. sp. can be distinguished from other Australian taxa by its small size, dome-shaped spire and spiral rows of oblong pits or dimples on the upper whorls. A comprehensive description is provided below for *S. gelasinosa gelasinosa* n. sp. & n. subsp., with comparative descriptions provided for the other two subspecies.

Etymology

Named for the sculpture of dimples (Greek, dimple = *gelasinos*) on the upper whorls that distinguishes this species from other Australian stenothyrids.

Stenothyra gelasinosa gelasinosa n. subsp.

Material examined

Holotype. Nudgee Beach, Queensland, 27°20′39″S 153°05′58″E, coll. R. Golding and S. A. Clark, 12 Oct. 2011, on leaf litter in pool in upper mangroves near road, below stormwater outlet (QM MO.80763). *Paratypes.* Same data (QM MO.80764, >20; AMS C.470969, >20). *Other material*. 3 wet lots from Broken Bay, New South Wales; 25 wet lots and 12 dry lots from Queensland between Moreton Bay and Cooktown. See supplementary data for full list of material examined.

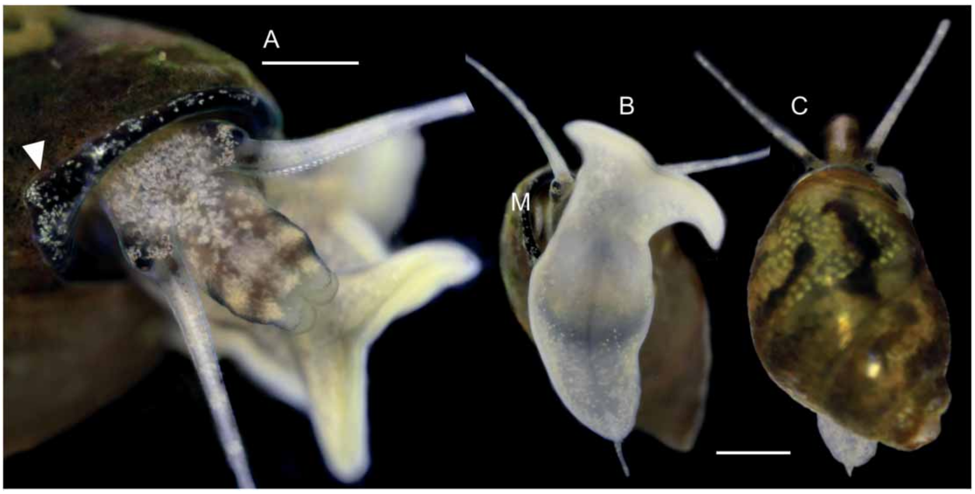

Figure 5. *Stenothyra australis* Hedley, 1901. **A,** Head and pallial tentacle (white arrowhead), Turkey Beach, QLD, AMS C.470943; **B,** ventral view of animal crawling on meniscus, AMS C.470943; **C,** dorsal view of live animal, River Head, AMS C.470843. Scale bars: A = 500 μm; B, C = 1 mm.

Description

Shell (Figs 2; 8B, C). Ovate, with rounded to angled inflation of last whorl, dorsoventrally compressed; spire short, dome-shaped; sutures moderately deep, upper whorls slightly convex; nonumbilicate; up to 4 1/4 whorls including protoconch, SL = 2.07–2.26 mm, SD_1 = 1.34–1.53 mm, SD_2 = 1.10–1.17 mm (ratio SD_1:SD_2 ~1.24) (Table 5). Slightly lustrous, pale golden brown; periostracum thin. Sculpture on first 3/4-1 teleoconch whorl of 9–12 spiral rows of oblong pits, terminating irregularly. Aperture smaller than preceding whorl, near circular, posterior margin slightly angulated; outer lip strongly prosocline.

Protoconch (Figs 8D, E). Dome-shaped; 1 3/4-2 whorls. Smooth; transitional varix well developed.

Operculum (Figs 9A, B). As for *S. australis*.

Radula (n = 3) (Fig. 9C). Central tooth 2 + 1 + 2/3 − 4 + 3 − 4; central cusp large, secondary cusps and basal denticles diminishing outwardly. Lateral teeth 3 − 4 + 1 + 8 − 9. Marginal teeth with subequal cusps; inner marginal teeth with ~30 cusps on tip and distal half of outer edge; outer marginal teeth with ~10 cusps on distal third of inner edge.

External morphology and colouration in life (Figs 10A, B). Head-foot transparent grey with mottled white pigment; darkest around eyes, on ventral surface of snout, dorsal midline of anterior foot and dorsal surface of foot anterior to operculum; eyes encircled by granules of canary-yellow pigment. Snout with bright patch of canary-yellow pigment on each lateral surface, surrounded by saddle-shaped outline of black pigment (often only faintly visible, usually appearing as two parallel transverse black lines) and overlaid with scattered cream to orange 'freckles' either side of snout midline; lips unpigmented. Cephalic tentacles transparent, patterned with 7–10 narrow rings of white pigment, black pigment restricted to diffuse band at base of each tentacle. Anterior foot with enlarged lateral lobes, posterior margin rounded. Metapodial tentacle present, narrow, contractile, with sparse granules of white pigment; size variable, 30–50% of extended foot length; mobile, usually held parallel to substrate or slightly upturned in life. Mantle visible through transparent shell, mottled black, white and yellow with prominent broken 'T-shaped' pattern of black pigment; interior surface of anterior mantle margin transparent with alternating bands of black and white to yellow pigment, one black band coinciding with pallial tentacle. Pallial tentacle present on right side of anterior mantle margin; small, semi-circular lobe demarcated by ciliary ridge across base of tentacle, with short, cylindrical tentacle at tip of lobe; held against exterior surface of shell in life.

Male reproductive system (Figs 9D, E, H; 11A). Testis, seminal vesicle and vas deferens as for *S. australis*. Penis cream or white, with sparse yellow flecks; cilia restricted to narrow strip along outer margin. Penial stylet rolled to form a hollow, curved, 'syringe' shape with open slit at tip; embedded in flesh, with presumed sperm duct opening via oval aperture near base.

Female reproductive system (Fig. 11B). As for *S. australis*.

Distribution (Fig. 7B)

Common in mangrove forests along the eastern coast of Australia. The most southern record is at Woy Woy, Broken Bay, just north of Sydney, although specimens were not detected at this site during the winter months. There are no records of *S. gelasinosa gelasinosa* between Woy Woy and Brisbane, but they are common around Brisbane and further north at least as far as Cooktown in northern

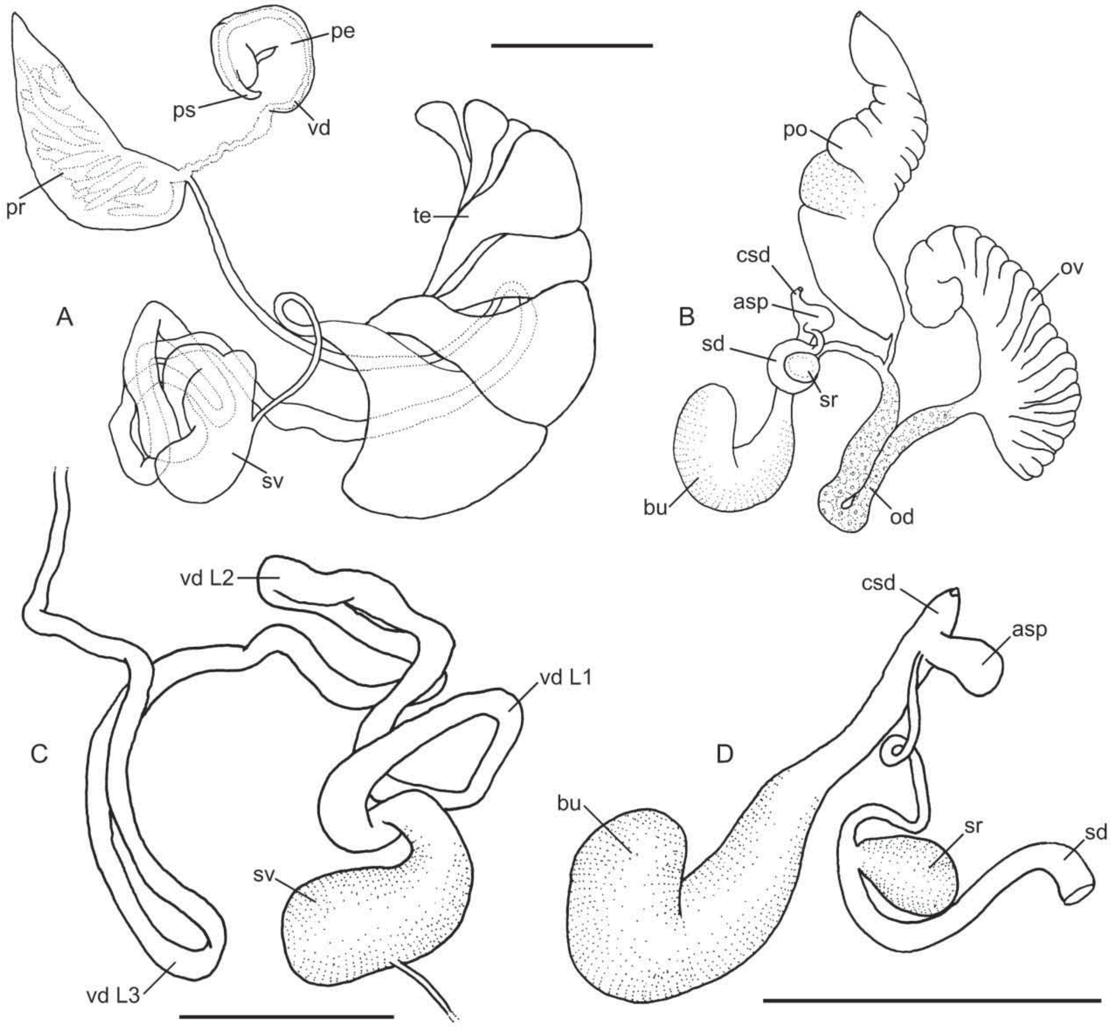

Figure 6. Anatomy of *Stenothyra australis* Hedley, 1901, from Nudgee, QLD, AMS C.470968. **A,** Male reproductive system, in natural position; **B,** proximal male reproductive system unravelled; **C,** female reproductive system, with pallial oviduct displaced to right; **D,** distal female reproductive system unravelled. Scale bars = 1 mm. Anatomical labels: asp, accessory sperm pouch; bu, bursa; csd, common sperm duct; od, oviduct; ov, ovary; pe, penis; po, pallial oviduct; pr, prostate; sd, sperm duct; sr, seminal receptacle; sv, seminal vesicle; te, testis; vd, vas deferens; vd L1, L2 and L3, 1st, 2nd and 3rd loops of vas deferens.

Queensland. Specimens collected at Weipa on the west side of Cape York belong to another subspecies of *S. gelasinosa* n. sp. It appears that *S. gelasinosa* s.s. does not extend around the tip of the Cape York Peninsula. *Stenothyra gelasinosa gelasinosa* lives in submerged mangrove leaf litter and can also be found living in estuaries on shallow subtidal *Zostera* seagrass (for example, at Woy Woy and Morton Bay).

Remarks

Stenothyra gelasinosa gelasinosa is intermediate in size between *S. gelasinosa phrixa* n. subsp. and *S. gelasinosa apiosa* n. subsp. Like *S. gelasinosa apiosa*, its protoconch is smooth and lacks the spiral sculpture of *S. gelasinosa phrixa*. Sequence divergence of the COI gene within *S. gelasinosa gelasinosa* is 0.17%, but compared to *S. gelasinosa phrixa* and *S. gelasinosa apiosa* the divergence is 5.01% and 5.41% respectively. Each subspecies has a distinct geographic distribution, and thus specimen locality is a good indicator of subspecies identity.

Stenothyra gelasinosa phrixa n. subsp.

Material examined

*Holotype.*W side of Wallaby Island, Weipa, Queensland, 12°38′16″S 141°50′26″E, coll. R. Golding and S. A. Clark, 28 Sep. 2011, in small pools with leaf litter and algae (QM MO.80765). *Paratypes.* Same data (QM MO.80766, 6; AMS C.470883, >20).

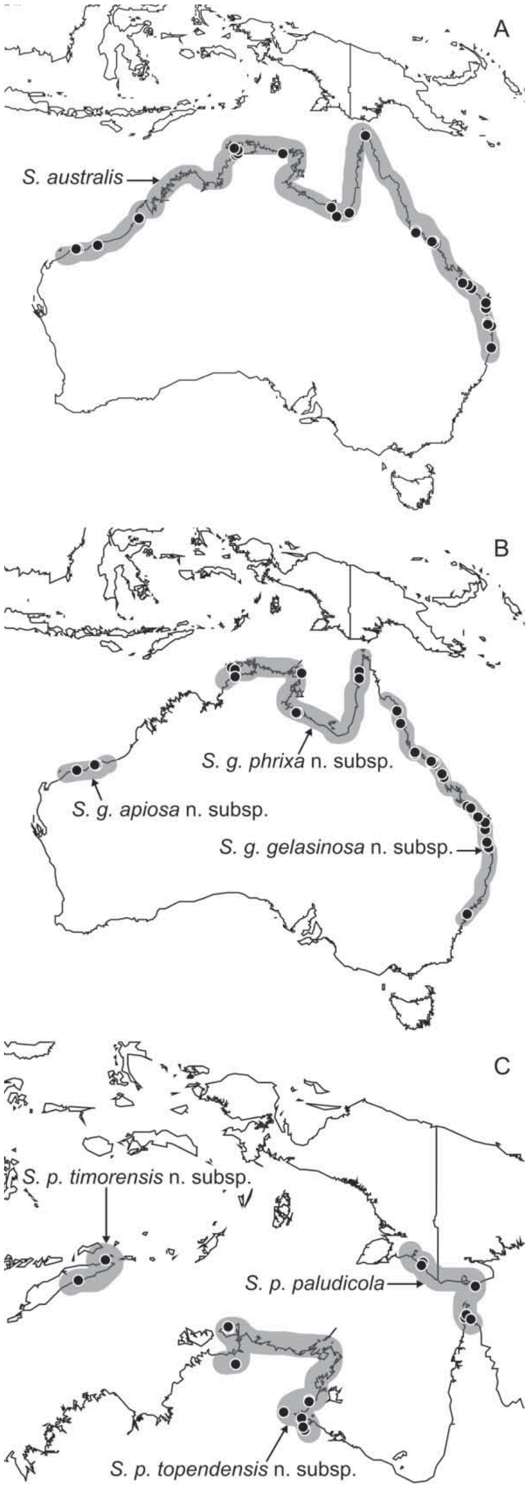

Figure 7. Distribution maps of Australian and Timorese Stenothyridae. Dots indicate samples examined during this study and labelled grey areas probable distributions inferred between known localities. **A,** *Stenothyra australis*; **B,** *Stenothyra gelasinosa* n. sp.; **C,** *Stenothyra paludicola*.

Other material. 3 wet lots from Queensland around Weipa; 7 wet lots and 11 dry lots from Northern Territory between Gulf of Carpentaria coast and the Tiwi Islands. See supplementary data for full list of material examined.

Description

Shell (Figs 2; 8F–I). As for *S. gelasinosa gelasinosa* n. sp. and n. subsp., except angled inflation of last whorl more pronounced; up to 4 1/2 whorls including protoconch, length 2.10–2.66 mm, diameter of last whorl 1.47–1.72 mm laterally, 1.22–1.53 mm dorsoventrally (ratio SD_1:SD_2 ~1.18) (Table 5). Slightly lustrous, pale golden, sometimes with faint white growth striae.

Protoconch (Figs 8H, I). As for *S. gelasinosa gelasinosa*, except for sculpture of ~20 delicate, undulating, spiral ridges.

Operculum and radula (n = 2). As for *S. gelasinosa gelasinosa*.

External morphology and colouration in life (Fig. 10C), *male* (Fig. 9F) *and female reproductive systems*. As for *S. gelasinosa gelasinosa*.

Distribution (Fig. 7B)

Found in the mangrove forests of Queensland and the Northern Territory between the western coast of Cape York Peninsula and Darwin. The precise western boundary of the distribution of *S. gelasinosa phrixa* is not known, but during recent fieldwork the species was not detected around Broome. Specimens from further south, at Port Hedland, belong to a different subspecies of *S. gelasinosa*(see below).

Remarks

Stenothyra gelasinosa phrixa is the largest of the three subspecies of *S. gelasinosa*. It has a slightly more pronounced inflation of the last whorl, and occasionally has faint white growth striae. The protoconch has a unique sculpture of spiral wavy lines, unlike the smooth protoconchs of the other subspecies of *S. gelasinosa*. Sequence divergence within the subspecies is 1.17%, but divergences from *S. gelasinosa gelasinosa* and *S. gelasinosa apiosa* n. subsp. are 5.01% and 4.80% respectively (Table 3).

Etymology

This subspecies is named for the characteristic sculpture on its protoconch, which resembles ripples on the surface of water (Greek, *phrix* = ripple).

Stenothyra gelasinosa apiosa n. subsp.

Material examined

Holotype. Nickol Bay, Karratha, Western Australia, 20°43′24″S 116°52′16″E, coll. R. Golding and M. Hill, 4 Jul. 2012, on leaf litter and filamentous roots in pools

Table 5. Shell measurements of type and other specimens of *Stenothyra gelasinosa* n. sp.

Registration no.	Status	SL (mm)	SD_1 (mm)	SD_2 (mm)	AL (mm)	AW (mm)	PWC	TWC	TWS
S. gelasinosa gelasinosa n. sp. & n. subsp.									
QM ex-C.470969	Ht	2.14	1.47	1.17	0.66	0.69	1 3/4	2 1/4	1
AMS C.470969	Pt	2.07	1.34	1.10	0.62	0.71	2	2 1/4	1
AMS C.470969	Pt	2.26	1.53	1.17	0.72	0.69	1 3/4	2 1/2	3/4
AMS C.470969	Pt	2.16	1.40	1.17	0.66	0.71	1 3/4	2 1/2	1 1/4
Average or range (n = 4)		2.16	1.44	1.16	0.66	0.70	1 3/4–2	2 1/4–2 1/2	3/4–1 1/4
S. gelasinosa phrixa n. subsp.									
QM ex-C.470883	Ht	2.24	1.55	1.28	0.69	0.69	1 3/4	2 1/2	3/4
QM ex-C.470883	Pt	2.34	1.60	1.34	0.72	0.81	1 3/4	2 1/2	3/4
QM ex-C.470883	Pt	2.10	1.47	1.22	0.67	0.72	1 1/2	2 1/2	3/4
AMS C.470887		2.66	1.72	1.53	0.78	0.79	1 3/4	2 3/4	1 1/4
Average or range (n = 4)		2.34	1.59	1.34	0.72	0.75	1 1/2–1 3/4	2 1/2–2 3/4	3/4–1 1/4
S. gelasinosa apiosa n. subsp.									
WAM S.82655	Ht	2.10	1.29	1.10	0.62	0.60	1 3/4	2 1/4	1/2
WAM S.82656	Pt	1.69	1.12	0.91	0.52	0.53	1 3/4	2	1/2
AMS C.476017	Pt	1.69	1.17	0.93	0.52	0.52	1 1/2	2	3/4
AMS C.476011		2.33	1.52	1.21	0.66	0.78	1 1/2	2 1/2	3/4
Average or range (n = 4)		1.95	1.28	1.04	0.58	0.61	1 1/2–1 3/4	2–2 1/2	1/2–3/4

Notes: Ht, holotype; Pt, paratype; TWS, teleoconch whorls with spiral sculpture. See 'Materials and methods' for other abbreviations.

between mangroves (WAM S.82655). *Paratypes.* Same data (WAM S.82656, 1; AMS C.476017, 3).
Other material. 3 wet lots and 1 dry lot from Karratha and Port Hedland, Western Australia. See supplementary data for full list of material examined.

Description

Shell (Figs 2; 8J–M). As for *S. gelasinosa gelasinosa*, except for moderately tall spire; up to 4 whorls, SL = 1.69–2.33 mm, SD_1 = 1.17–1.52 mm, SD_2 = 0.91–1.21 mm (ratio SD_1:SD_2 ~1.23) (Table 5). Slightly lustrous, golden to chestnut brown.
Protoconch (Figs 8L, M). As for *S. gelasinosa gelasinosa*, except 1 1/2 − −1 3/4 whorls.
Operculum and radula (n = 2). As for *S. gelasinosa gelasinosa*
External morphology and colouration in life (Figs 10D, E), *male* (Figs 9G) *and female reproductive systems*. As for *S. gelasinosa gelasinosa*.

Distribution (Fig. 7B)

In mangrove forests along the coast of Western Australia between Port Hedland and Karratha. The extent of the distribution beyond these two locations is unknown, but *S. gelasinosa apiosa* was not found during recent fieldwork in Broome or Derby.

Remarks

Stenothyra gelasinosa apiosa is the smallest subspecies of *S. gelasinosa*. It is often a slightly darker shade of brown, and has a slightly taller spire than *S. gelasinosa gelasinosa* or *S. gelasinosa phrixa*. Within-species COI sequence divergence is 0.67%, and the divergence from *S. gelasinosa gelasinosa* and *S. gelasinosa phrixa* is 5.41% and 4.80% respectively (Table 3).

Etymology

This subspecies is named for its remote distribution on the western coast of Australia (Greek, *apiosa* = far away).

Stenothyra paludicola van Benthem Jutting, 1963

Stenothyra paludicola van Benthem Jutting 1963: 446, fig. 11.

Remarks

Unlike other species of *Stenothyra* described here, *S. paludicola* lives in billabongs, swamps and coastal streams in brackish or mainly freshwater environments rather than coastal mangrove forests. It has a distinct shell shape, with the whorl profile more rounded and inflated (with correspondingly deeper sutures) than other Australasian stenothyrids. Molecular sequence data strongly supports the monophyly of *S. paludicola*, and the reciprocal monophyly of two subspecies within the taxon. Other distinguishing features of the species are the asymmetrical 'wings' on the interior surface of the operculum, less dorsoventrally compressed shell and small protoconch with punctate sculpture.

Stenothyra paludicola paludicola van Benthem Jutting, 1963

Stenothyra paludicola van Benthem Jutting 1963: 446, fig. 11.

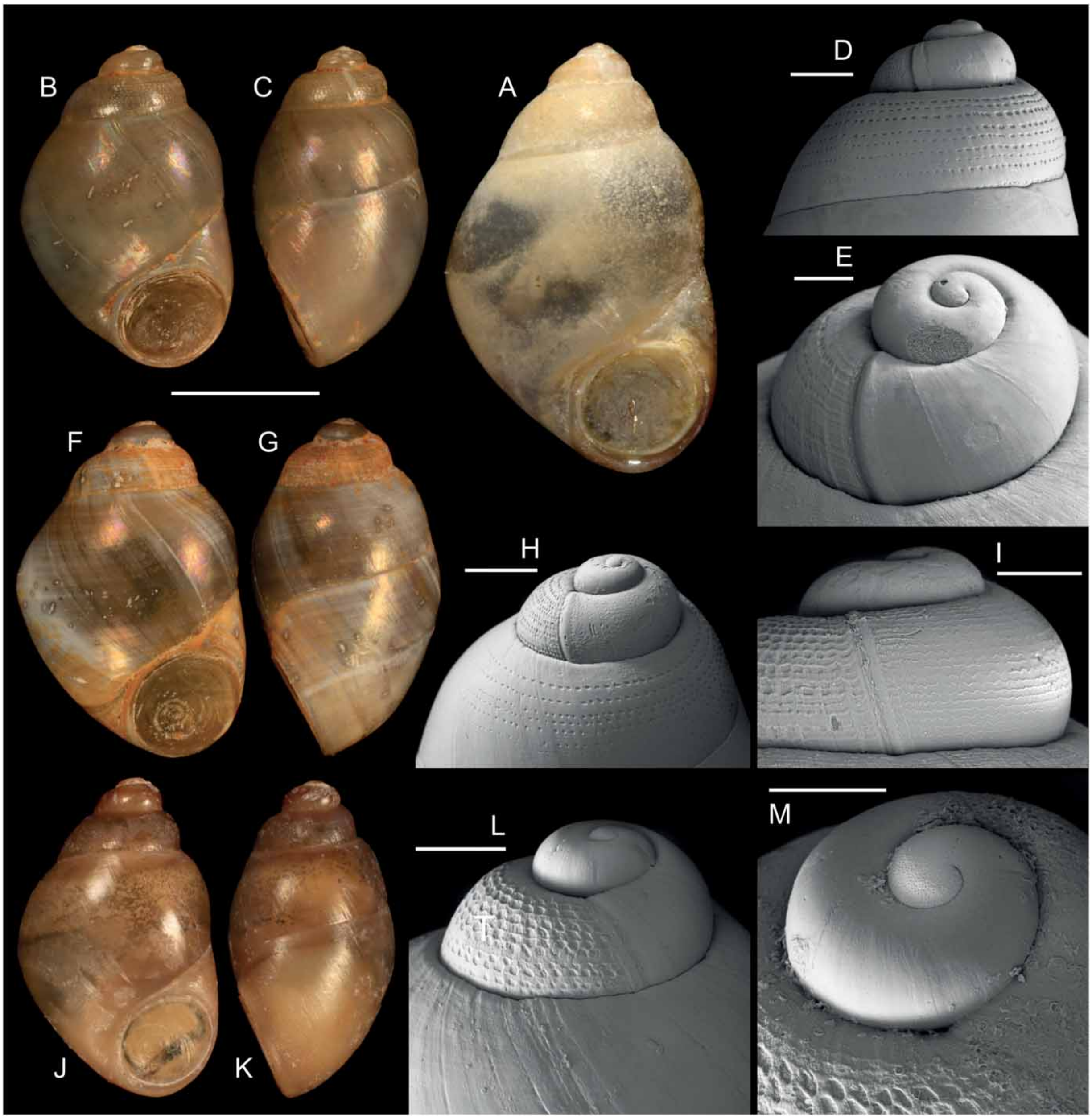

Figure 8. **A,** Holotype of *Stenothyra saccata* van Benthem Jutting, 1963, beach at Lampu Satu, near Merauke, Papua, Indonesia, RMNH.MOL.309666; photographed by Jeroen Goud. **B–E,** *Stenothyra gelasinosa gelasinosa* n. sp. **B,C,** Holotype, Nudgee, QLD, QM MO.80763;**D,E,** apical shell including protoconch, paratypes from Nudgee, QLD, AMS C.470969. **F–I,** *Stenothyra gelasinosa phrixa* n. subsp. **F,G,** Holotype, Weipa, QLD, QM MO.80765;**H,** apical shell, East Woody Beach, NT, AMS C.175248; **I,** protoconch, AMS C.175248. **J–M,** *Stenothyra gelasinosa apiosa* n. subsp. **J,K,** Holotype, Karratha, WA, WAM S.82655; **L,** apical shell from paratype, Karratha, WA, AMS C.476017; **M,** protoconch, Karratha, AMS C.476024. Scale bars: A–C, F, G, J, K = 1 mm; D, H, L = 200 µm; E, I, M = 100 µm.

Type material

Paratype. Beach at Lampu Satu, near Merauke, Papua, Indonesia, 8°29′00″S 140°22′00″E, coll. L. B. Holthuis, 4 Apr. 1955 (registration number unknown, 2).

Material examined

Holotype. Small pond in swampy area N of Merauke, Papua, Indonesia, 8°22′00″S 140°26′00″E, coll. L. B. Holthuis, 28 Mar. 1955 (RMNH.MOL.309872); examined from photograph only. *Paratype*. Same data (ZMA.MOLL.136314, 28); one specimen examined from photograph only.

Other material. 5 wet lots and 2 dry lots from Torres Strait and northern Cape York Peninsula, Queensland. See supplementary data for full list of material examined.

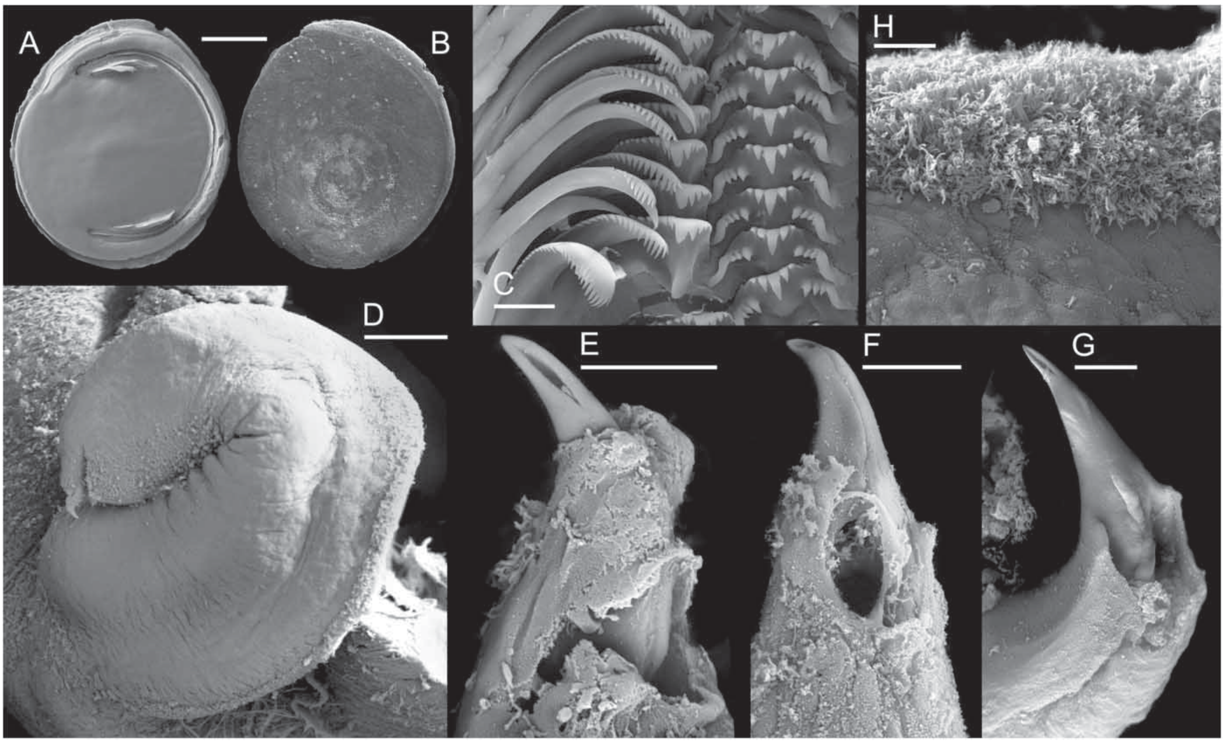

Figure 9. *Stenothyra gelasinosa* n. sp. **A–D,** *Stenothyra gelasinosa gelasinosa* n. subsp. **A,B,** Interior and exterior surfaces of operculum; **C,** radula; **D,** critical point dried penis. **E–G,** Penial stylets. **E,** *S. gelasinosa gelasinosa* n. subsp. **F,** *Stenothyra gelasinosa phrixa* n. subsp. paratype, Weipa, QLD, AMS C.470883; **G,** *Stenothyra gelasinosa apiosa* n. subsp., paratype, Karratha, WA, AMS C.476017. **H,** *S. gelasinosa gelasionsa* n. subsp. Detail of ciliated strip on penis. **A–E,** H paratypes from Nudgee, QLD, AMS C.470969. Scale bars: A, B = 200 μm; C, E–H = 10 μm; D = 100 μm.

Redescription

Shell (Figs 2; 12A–E). Ovate-conic, with evenly rounded last whorl, slightly dorsoventrally compressed; spire tall, convex to conical; sutures deep, upper whorls gently convex; nonumbilicate; up to 4 3/4 whorls including protoconch, SL = 3.02–3.38 mm, SD_1 = 1.79–2.02 mm, SD_2 = 1.53–1.74 mm (ratio SD_1:SD_2 ~1.11) (Table 6). Dull, pale to golden brown; periostracum thin. Sculpture on all teleoconch whorls of 10–15 very faint, irregular spiral bands of tiny pits. Aperture smaller than preceding whorls, near circular, posterior margin with weak angulation at apex; outer lip strongly prosocline.

Protoconch (Fig. 12E). Flat, $1/2 - -3/4$ whorls. Covered with minute punctate sculpture, transitional varix weakly developed.

Operculum (Figs 13A, B). As for *S. australis*, except for prominent angulation at posterior apex; semicircular 'wings' on interior surface asymmetrical, with anterior projection ~30% larger than posterior projection.

Radula (n = 2) (Fig. 13C). Central tooth $2 + 1 + 2/3 - 4 + 3 - 4$, central cusp large, secondary cusps and basal denticles diminishing outwardly. Lateral teeth $5 - 7 + 1 + 8 - 9$. Marginal teeth with subequal cusps; inner marginal teeth with ~28 cusps on tip and distal half of outer edge; outer marginal teeth with ~15 cusps on distal third of inner edge.

External morphology and colouration in life. The animal has not been observed alive in this study. Specimens preserved in 70% EtOH have two parallel black bands crossing the snout and scattered black flecks on the cephalic tentacles.

Male reproductive system (Figs 13D, E; 14A). Testis, seminal vesicle and vas deferens as for *S. australis*. Colour and ciliation of penis not observed in life. Penial stylet rolled to form a hollow, curved, 'syringe' shape with minute open slit at tip; embedded in flesh, but without any other obvious aperture for sperm duct.

Female reproductive system (Fig. 14B). As for *S. australis*.

Distribution (Fig. 7C)

Known from New Guinea in Papua, Indonesia from the type localities around Merauke and from Saibai, Horn and Prince of Wales Islands in the Torres Strait region of Australia (Fig. 5C). One locality at Utingu on the Cape York Peninsula is known from mainland Australia, but temporary, wet-season brackish-water lagoons are not

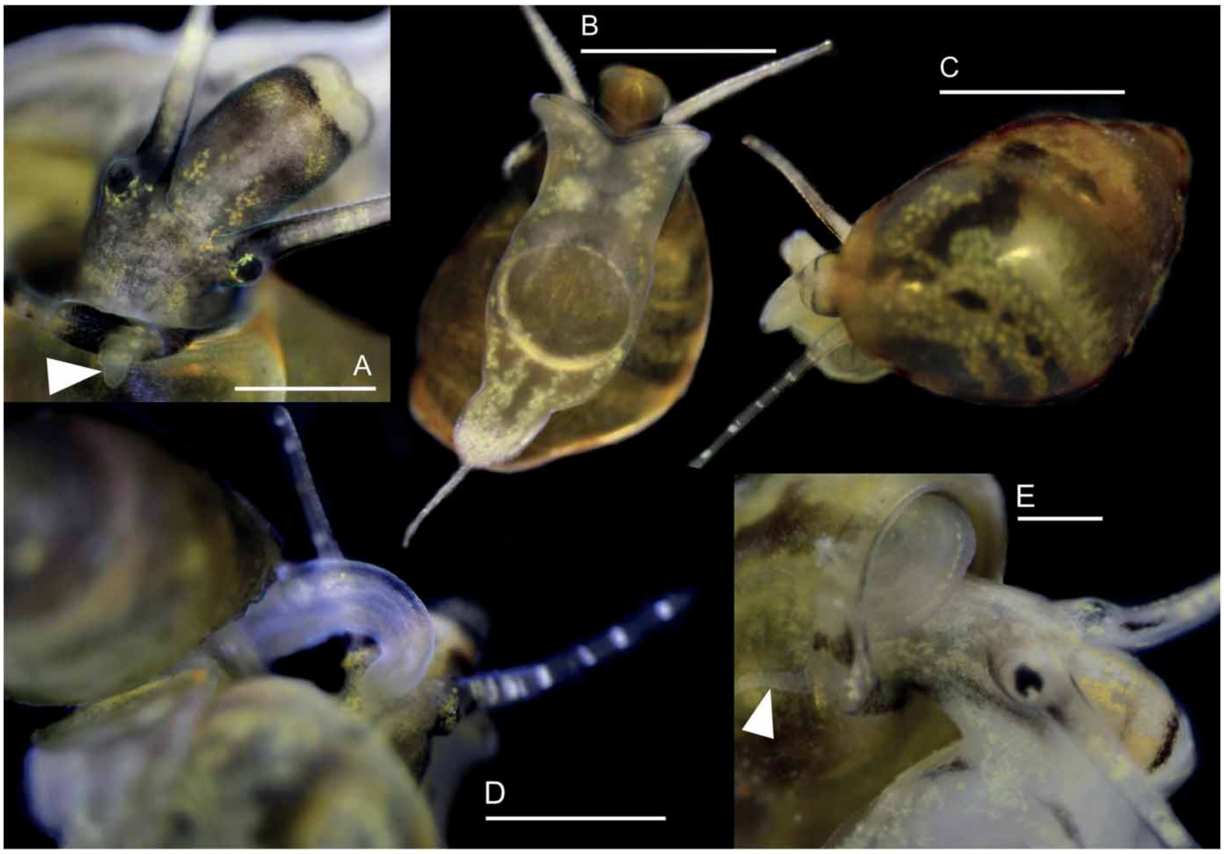

Figure 10. *Stenothyra gelasinosa* n. sp. **A, B,** *Stenothyra gelasinosa gelasinosa* n. subsp., Tin Can Bay, QLD, AMS C.470950: **A,** head and pallial tentacle (white arrowhead); **B,** ventral view of animal crawling; **C,** *Stenothyra gelasinosa phrixa* n. subsp., dorsal view of animal crawling, paratype, Weipa, QLD, AMS C.470883; **D, E,** *Stenothyra gelasinosa apiosa* n. subsp. **D,** copulating pair, paratypes, Karratha, WA, AMS C.476017; **E,** head and pallial tentacle (white arrowhead), Port Hedland, WA, AMS C.476003. Scale bars: A, D = 500 μm; B, C = 1 mm; E = 200 μm.

well-sampled in this area and the species may be more widespread. Found in permanent or seasonal freshwater to brackish pools (billabongs) behind mangroves and in brackish swamps.

Remarks

Specimens in the Australian Museum collections from Torres Strait and Cape York appear to be conspecific with the type material of *Stenothyra paludicola* from Merauke in New Guinea. The shells are very similar in size and shape, although sculptural details were not visible on the available photograph of the holotype. Unfortunately, the lack of freshly collected material prevents observation of the colouration and behaviour of the animal, and molecular data could not be obtained. There are sufficient morphological grounds for separating the other subspecies of *S. paludicola* from *S. paludicola paludicola*, with the shell of the latter subspecies having characteristic spiral bands of minute pitted sculpture.

Stenothyra paludicola topendensis n. subsp.

Material examined

Holotype. Swamp 2.7 km ENE from Black Point Range Station, S of the airstrip, Cobourg Peninsula, Northern Territory, 11°08′58″S 132°09′50″E, coll. V. Kessner, 3 Feb. 2007, in shallow water of large seasonal lake, abundant at edge of lake on sandy mud (NTM P.50628). *Paratypes*. Same data (NTM P.50629, >20; AMS C.458234, >20). *Other material*. 19 wet lots from Northern Territory between Gulf of Carpentaria coast and Coburg Peninsula. See supplementary data for full list of material examined.

Description

Shell (Figs 2; 12F–I). As for *S. paludicola paludicola*, except slightly narrower and taller proportionally; upper whorls strongly convex; up to 4 3/4 whorls including protoconch, SL = 2.81–3.26 mm, SD_1 = 1.66–1.77 mm, SD_2 = 1.47–1.60 mm (ratio SD_1:SD_2 ~1.10) (Table 6). Glossy golden brown; periostracum thin. Teleoconch entirely smooth with weak growth striae (Figs 8J–L).

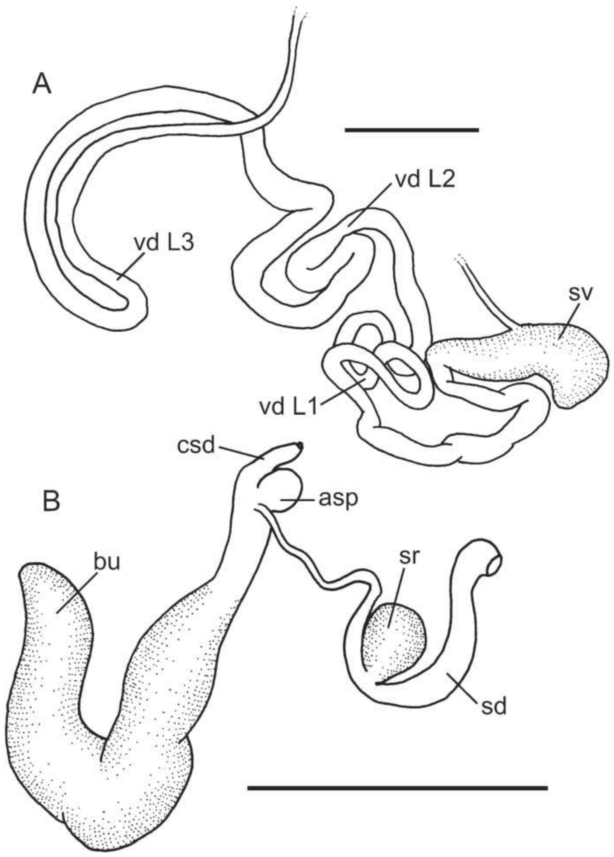

Figure 11. Anatomy of *Stenothyra gelasinosa gelasinosa* n. sp. from Nudgee, QLD, AMS C.470969. **A,** proximal male reproductive system unravelled; **B,** distal female reproductive system unravelled. Scale bars = 500 µm.

Protoconch (Figs 12H, I), *operculum and radula* (n = 3) (Fig. 13F). As for *S. paludicola paludicola*.
External morphology and colouration in life. The animal has not been observed alive in this study. Specimens preserved in 70% EtOH have an unpigmented snout and scattered black flecks on the base of the cephalic tentacles.
Male and female reproductive systems. As for *S. paludicola paludicola*.

Distribution (Fig. 7C)

Found in brackish to freshwater billabongs, rivers and streams in coastal to inland (but still tidally influenced) areas of the Northern Territory, between the western coast of the Gulf of Carpentaria, through Arnhem Land and Kakadu to the Cobourg Peninsula.

Remarks

Stenothyra paludicola topendensis is distinguished from *S. paludicola paludicola* by its unsculptured, glossy shell with more convex upper whorls. It is more similar to *S. paludicola timorensis* n. subsp., but has a slightly taller spire. It is also geographically isolated from the other subspecies of *S. paludicola*. Within-species sequence divergence of the COI gene is 0.43% and divergence from *S. paludicola timorensis* n. subsp. is 3.00% (Table 3).

Etymology

Named for the distribution of this subspecies along the tropical coastline of the Northern Territory, a region known to Australians as the 'Top End'.

Stenothyra paludicola timorensis n. subsp.

Material examined

Holotype. On Natarbora-Betano Road, Alas, south coast of Manufahi District, East Timor, 9°08′21″S 125°49′32″E, coll. V. Kessner and F. Köhler, 14 Nov. 2011, extremely common in 10 cm of water in tidal stream, on algae and sandy bottom (AMS C.476088). *Paratypes*. Same data (AMS C.475872, >20).
Other material. 2.3 km SW of Lautem, Lautem District, East Timor, 8°22′17″S 126°52′57″E, coll. V. Kessner, 18 May 2012, on sand and mud in tidal stream and shallow lagoon near beach, brackish water (AMS C.477095, >20).

Description

Shell (Figs 2; 12J–L). As for *S. paludicola paludicola*, except with shorter spire; up to 4 3/4 whorls including protoconch, SL = 2.60–2.96 mm, SD_1 = 1.66–1.83 mm, SD_2 = 1.45–1.79 mm (ratio SD_1:SD_2 ~1.09) (Table 6). Glossy, golden brown; periostracum thin. Teleoconch entirely smooth with weak growth striae.
Protoconch (Fig. 12L), *operculum and radula* (n = 2) (Fig. 13G). As for *S. paludicola paludicola*.
External morphology and colouration in life. The animal was not observed alive. Specimens preserved in 70% EtOH appear white with sparse black flecks on dorsal surface of head.
Male (Fig. 13H) *and female reproductive systems*. As for *S. paludicola paludicola*.

Distribution (Fig. 7C)

Known only from two shallow, tidal streams in East Timor, in the Manufahi and Lautem districts.

Remarks

This subspecies of *S. paludicola* is geographically isolated on the island of Timor. It has a similar shell to *S. paludicola topendensis* but is shorter spired and has less strongly convex upper whorls. COI sequence divergence within

Table 6. Shell measurements of type and other specimens of *Stenothyra paludicola*. Averages exclude measurements of the holotype of *S. paludicola paludicola* which were taken from a photograph.

Registration no.	Status	SL (mm)	SD_1 (mm)	SD_2 (mm)	AL (mm)	AW (mm)	PWC	TWC
S. paludicola paludicola								
RMNH.MOL.309872	Ht	3.1	1.8	?	1.0	1.0	?	?
AMS C.434332		3.34	1.91	1.70	0.94	0.96	1/2	4
AMS C.434332		3.38	2.02	1.74	0.98	1.00	1/2	4
AMS C.434332		3.17	1.89	1.68	0.98	1.06	3/4	4
AMS C.434332		3.02	1.79	1.53	0.87	0.96	3/4	4
Average or range (n = 4)		3.23	1.90	1.66	0.94	0.99	1/2–3/4	4
S. paludicola topendensis n. subsp.								
NTM P.50628	Ht	3.09	1.68	1.53	0.98	0.96	3/4	4
AMS C.458234	Pt	3.26	1.77	1.60	1.06	1.00	3/4	4
AMS C.458234	Pt	2.87	1.60	1.49	0.89	0.89	1/2	4
AMS C.458234	Pt	2.81	1.66	1.47	0.85	0.91	3/4	4
Average or range (n = 4)		3.01	1.68	1.52	0.95	0.94	1/2–3/4	4
S. paludicola timorensis n. subsp.								
AMS C.476088	Ht	2.77	1.60	1.53	0.87	0.91	1/2	4
AMS C.475872	Pt	2.96	1.83	1.79	0.91	0.98	1/2	4 1/4
AMS C.475872	Pt	2.60	1.66	1.45	0.89	0.96	1/2	4
AMS C.475872	Pt	2.83	1.79	1.57	0.85	0.98	3/4	3 1/2
Average or range (n = 4)		2.79	1.72	1.59	0.88	0.96	1/2–3/4	31/2–41/4

Notes: Ht, holotype; Pt, paratype. See 'Materials and methods' for other abbreviations.

the subspecies is 0%, and divergence from *S. paludicola topendensis* n. subsp. is 3.00% (Table 3).

Etymology

Named for the distribution of this species in East Timor.

Stenothyra frustillum* Benson, 1856 *nom. dub.

Stenothyra frustillum Benson 1856: 498.

Remarks

Stenothyra frustillum was described by Benson (1856) from a specimen in the Cuming collection, with the type locality as 'Australia'. Benson (1856) provided a brief description of the species but did not figure the type specimen, and it has not been subsequently figured or described by any author. The shell is small (1.5 mm long by 1 mm wide) and smooth, but otherwise typically stenothyrid. A recent request for the holotype revealed that it is lost from the collections of the Museum of Natural History in London (J. Ablett, pers. comm.). It is possible that this small species was based on a specimen of what is here described as *S. gelasinosa*. A specimen 1.5 mm long could belong to this taxon, but without type material, precise locality or detailed original description of *S. frustillum* it is impossible to resolve its identity. Consequently I consider *Stenothyra frustillum* a *nomen dubium*.

Discussion

The systematic results of this study have significantly increased the known stenothyrid diversity in the Australasian region. Prior to this study, one Australian species, *S. australis* (with two subspecies) and three New Guinean species were recognised, with no stenothyrids known to occur in East Timor. Here the two subspecies of *S. australis* have been synonymised with *S. australis* and *S. nebularum* is a probable synonym of that species. Three subspecies of the newly-named species *S. gelasinosa* are thought to be endemic to Australia and there are three subspecies of *S. paludicola* (one endemic to northern Australia, one occurring in both Australia and New Guinea, and one subspecies endemic to East Timor). *Stenothyra saccata* from New Guinea is still recognised as a valid species, but its relationship to the Australian fauna is unresolved. Clarification of the diversity of stenothyrids in this region will greatly improve our capacity to identify these species in future studies of estuarine gastropods. These findings should provide a basis for further species discovery in the region, as this revision is certainly not a complete account of their diversity. In particular, temporary habitats such as seasonal brackish to freshwater pools may be under-represented in surveys of tropical areas due to the difficulties associated with field work during the wet season. These pools and billabongs have been identified as key habitat for *S. paludicola*, and they may contain further undescribed stenothyrid species in other parts of Australia, New Guinea and South East Asia.

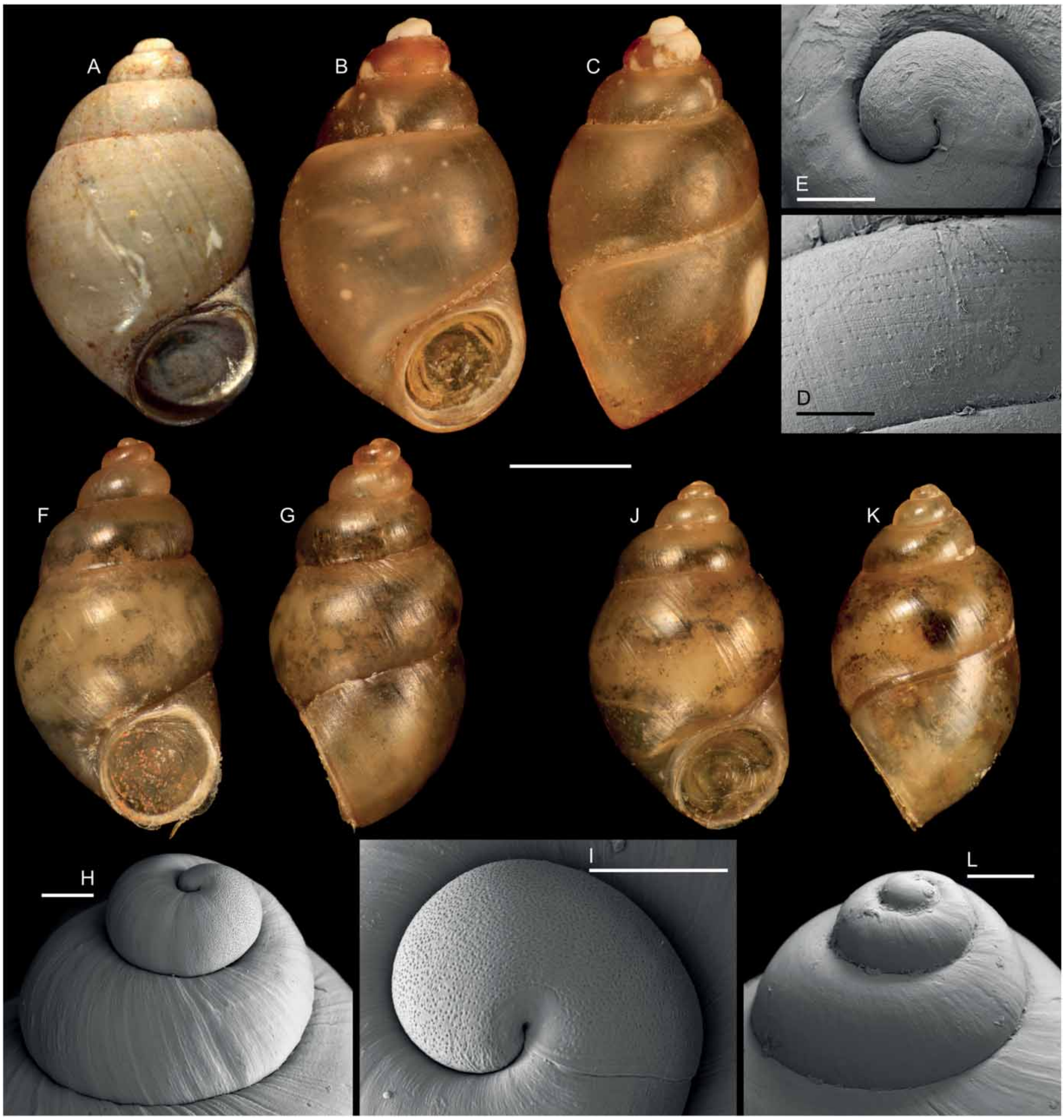

Figure 12. *Stenothyra paludicola* van Benthem Jutting, 1963. **A–E,** *Stenothyra paludicola paludicola* van Benthem Jutting, 1963. **A,** Holotype, N of Merauke, Papua, Indonesia, RMNH.MOL.309872, photographed by Jeroen Goud; **B,C,** representative specimen, Prince of Wales Island, Torres Strait, QLD, AMS C.434332; **D,** sculpture on last whorl, AMS C.434332; **E,** corroded protoconch, AMS C.434332. **F–I,** *Stenothyra paludicola topendensis* n. subsp. **F, G,** Holotype, Black Point Ranger Station, Cobourg Peninsula, NT, NTM P.50628; **H,** apical shell, paratypes from Black Point Ranger Station, Cobourg Peninsula, NT, AMS C.458234; **I,** protoconch, AMS C.458234. **J–L,** *Stenothyra paludicola timorensis* n. subsp. **J,K,** holotype, Manufahi District, East Timor, AMS C.476088; **L,** apical shell, paratype, Manufahi District, East Timor, AMS C.475872. Scale bars: A–C, F, G, J, K = 1 mm; D, E, H, I, L = 100 μm.

This study is the first use of molecular data to examine stenothyrid systematics and phylogeny. Analysis of sequence divergence and phylogenetic relationships demonstrates the utility of this data for species discrimination in Stenothyridae. Comparison of within-taxon and between-taxon uncorrected sequence divergence provided a sound basis for determining specific, subspecific and population-level differences. Less than 1.5% divergence of the COI gene was considered to meet intraspecific variation, while 3–5.5% was evidence of subspecific diversity and >10% was found between species. In this study, values of sequence divergence fell consistently into these three categories, without any intermediate values. Observation of mitochondrial sequence divergence in marine

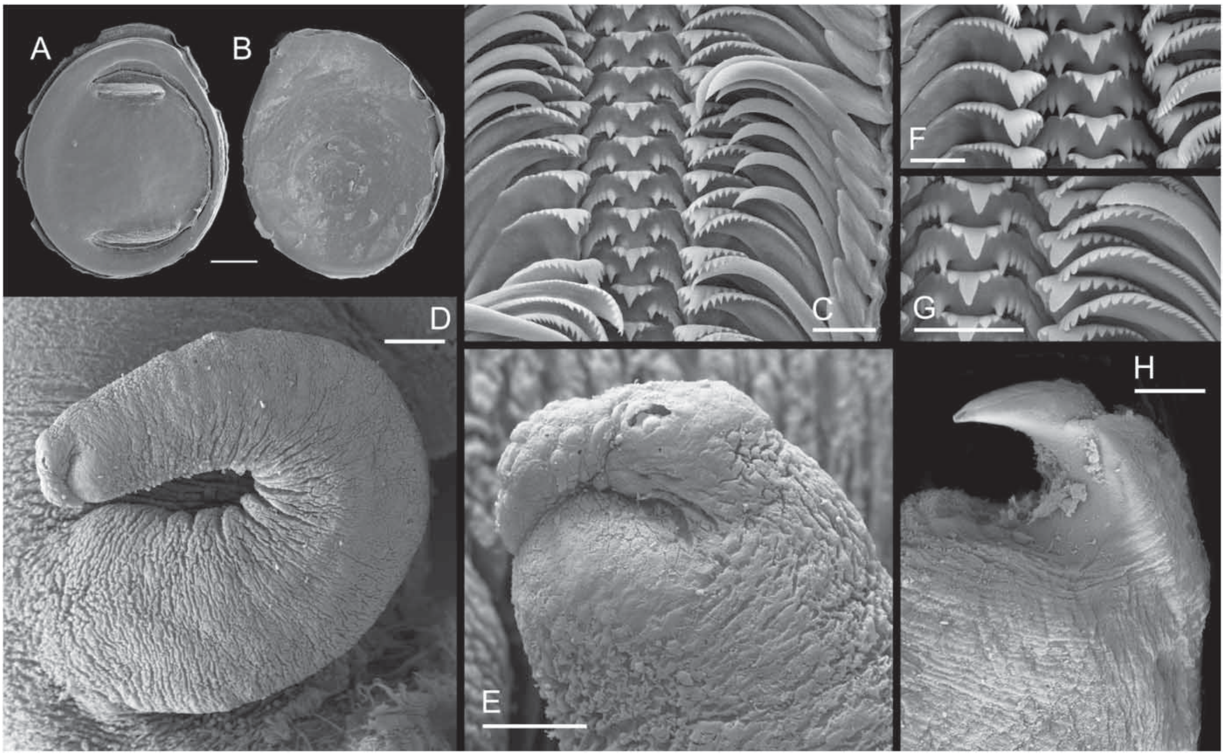

Figure 13. *Stenothyra paludicola* van Benthem Jutting, 1963. **A–E,** *Stenothyra paludicola paludicola* van Benthem Jutting, 1963. **A, B,** interior and exterior surfaces of operculum, Saibai, Torres Strait, QLD, AMS C.434319. **C,** Radula, AMS C.434319; **D,E,** critical point dried penis and detail of penial stylet, AMS C.434319. **F,** *Stenothyra paludicola topendensis* n. subsp., radula, paratype, AMS C.458234. **G,H,** *Stenothyra paludicola timorensis* n. subsp., paratypes, Manufahi District, East Timor, AMS C.475872. **G,** Radula; **H,** penial stylet. Scale bars: A, B = 200 μm; C, G, H = 20 μm; D, *E* = 50 μm; F = 10 μm.

invertebrates has previously shown that widely distributed species have higher levels of within-taxon sequence divergence (genetic structure), relative to species occupying a narrower geographic range (for example, Waters *et al.* 2005). Contrary to this expectation, *S. gelasinosa gelasinosa* has the lowest within-taxon COI sequence divergence (0.17%) among the Australian and East Timorese taxa, but is widely distributed along the coastline of eastern Australia. The extent of the distribution is largely due to the discovery of an isolated population in southern New South Wales (north of Sydney), detectable during the summer months but seemingly not in winter. It is possible that gene flow is maintained by the southerly distribution of larvae by the Eastern Australian Current, which extends to Sydney, allowing for population of suitable habitat during the warmer months but resulting in local extinctions in cooler seasons.

The collection of molecular data from multiple specimens sampled from a wide range of locations provided some insights into the phylogeographic patterns within and between species. While some taxa were widespread, others were found to be endemic to a single region. This contrast is most evident in *S. australis* and *S. gelasinosa*, both of which are found (often sympatrically) in the same mangrove forest microhabitat. *Stenothyra australis* is widespread across the northern coastline of Australia, from at least Brisbane to Karratha, with low genetic diversity across its broad range. *Stenothyra gelasinosa* occupies an almost identical range (extending further south on the east coast), but is separated into three molecularly and morphologically distinct subspecies. The distributions of these subspecies are abutting in one instance (across the tip of Cape York Peninsula) and separated by an unknown distance in another (the Kimberly region) (Fig. 7B), but molecular data confirms that, at least as far as the present material is concerned, they are not sympatric. These contrasting phylogeographic patterns in closely related species cannot be explained with the present data, but may reflect different life history or dispersal strategies.

Molecular phylogenetic analysis of Stenothyridae using multiple representatives of each taxon was a useful tool for resolving taxa, but was less informative about the deeper relationships in the group. A few sister-species relationships were well supported (e.g. *S. gelasinosa* and *S.* cf. *divalis* from Hong Kong), but many nodes lacked strong support from the BI and ML analyses. Based on this information, it is difficult to reconstruct the evolutionary history of Stenothyridae. This situation may be improved by the addition of other species, in particular from freshwater habitats. Only one truly freshwater

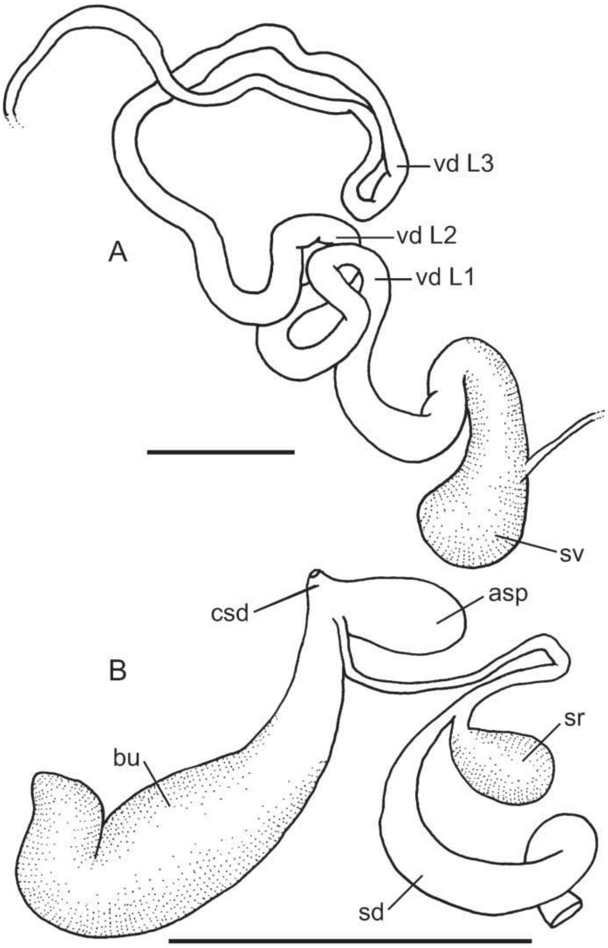

Figure 14. Anatomy of *Stenothyra paludicola paludicola* van Benthem Jutting, 1963, from Saibai, QLD, AMS C.434319. **A,** proximal male reproductive system unravelled; **B,** distal female reproductive system unravelled. Scale bars = 500 µm.

species was included in this study, an unidentified species from Sampaloc Lake on Luzon in the Philippines, which was positioned at the base of the tree. It is premature to speculate on the relationships between freshwater and estuarine species, but future studies may explore this interesting dimension of stenothyrid evolution.

Low sampling density and a lack of support for deeper nodes in the tree also prevent an assessment of genus-level nomenclature in Stenothyridae. Almost all named stenothyrids have been assigned to *Stenothyra*, a situation which poorly reflects their global diversity and subtle but distinct variety of shell and anatomical morphologies. Some clades recovered in the molecular analysis are morphologically distinct, such as *S. monilifera* and an unidentified species from Johor in peninsular Malaysia. These taxa both have larger shells etched all over with distinctive spiral rows of pits. Too little is known of these species to speculate on the need for generic recognition, but it is likely that further genus-level taxa will be required when a comprehensive systematic revision of Stenothyridae is undertaken.

The more broadly sampled phylogenetic analysis exploring the monophyly of Stenothyridae and its relationship with other truncatelloids produced a similar tree to that of Criscione and Ponder (2012). This result is unsurprising since the majority of the non-stenothyrid sequences were first used in that study, but increased sampling in Stenothyridae did not greatly alter the family-level relationships.

Anatomical examination of these species was restricted to external morphology and the details of the reproductive system and radula. The configuration of the reproductive anatomy was of particular significance because of inconsistent reports in the literature. Kosuge (1969) described the anatomy of three Japanese species, *S. edogawaensis* (Yokoyama, 1927), *S. thermaecole* Kuroda, 1962 and *S. japonica* Kuroda, 1962. Subsequently, Davis *et al*. (1986; 1988) produced a detailed anatomical description of the Chinese species *S. divalis* (Gould, 1859), S. jinghongensis Davis, Guo and Hoagland, 1986 and *S. hunanensis* Möllendorff, 1888. Davis *et al*. (1986) questioned the many discrepancies between the two anatomical accounts, in particular the lack of pallial prostate and configuration of the sperm storage organs and ducts in the female reproductive system of the Japanese species, as reported by Kosuge (1969).

The configuration of the reproductive system of three Australian taxa is presented here, and was found to be consistent among those taxa. When compared to the previous descriptions, the proximal male reproductive system is almost identical to the arrangement described by Kosuge (1969), with a bulbous, peanut-shaped seminal vesicle unlike the simple coiled portion of the vas deferens described for *S. divalis* (Table 7). However, a pallial prostate is definitely present, as reported by Davis (1986), but which was found to be absent by Kosuge (1969). The female reproductive system is structurally very similar to that of *S. divalis, S. jinghongensis* and *S. hunanensis*, with openings at both the posterior and anterior mantle cavity, single bursa duct connecting the bursa bulb to the common sperm duct, spherical seminal receptacle and an accessory sperm pouch. Kosuge's (1969) description of the female reproductive tract does not have much in common with the species examined here, and may describe the anatomy of a pomatiopsid rather than a stenothyrid due to the absence of an accessory sperm pouch and connection of the sperm duct to the bursa. The anatomy of the Japanese stenothyrids should be revisited to confirm or refute the reproductive ground plan of Stenothyridae proposed by Davis *et al*. (1986) and confirmed here for the Australian species.

Comparison between the Australian, Timorese and Chinese stenothyrid species for which detailed anatomical descriptions are available reveals several useful characters

Table 7. Anatomical characters differentiating six species of *Stenothyra*.

	S. divalis	*S. jinhongensis*	*S. hunanensis*	*S. australis*	*S. gelasinosa*	*S. paludicola*
Penial stylet presence/type	absent	narrow, cylindrical	narrow, cylindrical	wide, spatulate	narrow, cylindrical	narrow, cylindrical
Ejaculatory duct	simple	massively enlarged	simple	simple	simple	simple
Ciliation on penis	absent	anterior patch	central patch	absent	longitudinal strip	absent
Seminal vesicle	slender coiled tube	slender coiled tube	slender coiled tube	large peanut-shaped	large peanut-shaped	large peanut-shaped
Position of accessory sperm pouch relative to insertion of sperm duct	distal	distal	at same point	at same point	at same point	at same point
Bursa duct anterior to bursa sac	narrow tube	narrow tube	narrow tube	thick tube	thick tube	thick tube
Posterior pedal tentacle	short	short	short	elongate	elongate	?
Primary cusps on central tooth	2 + 1 + 2	3 + 1 + 3	2 − 3 + 1 + 2 − 3	2 − 3 + 1 + 2 − 3	2 + 1 + 2	2 + 1 + 2
Basal cusps on central tooth	4 + 4	5 − 6 + 5 − 6	3 − 4 + 3 − 4	3 − 4 + 3 − 4	3 − 4 + 3 − 4	3 − 4 + 3 − 4

for separating species and inferring relationships. The seminal vesicle is either an elongate convolution of the vas deferens (Chinese species), or a discrete bulbous structure (Australian/Timorese species). Anterior male and female reproductive structures are also informative, including the shape of the penial stylet (narrow or spatulate), ciliation patterns on the penis, enlargement of the ejaculatory duct (unique to *S. jinghongensis*), position of the accessory sperm pouch and thickness of the bursa duct (Table 7). With this very limited dataset, it is already possible to identify discrete anatomical characters, or combinations of characters, that distinguish species and groups of species. The addition of further taxa and anatomical characters will permit an examination of evolutionary patterns in Stenothyridae.

Supplementary data

Supplementary file 1. Full list of material examined.

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