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Contribution to the bryoflora of Australia, V. *Radula tonitrua* sp. nov. from Queensland

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Abstract

Study of two recognised geographic lineages within *Radula novae-hollandiae sens. lat.* have resulted in the detection of morphological differences between individuals from the Queensland Wet Tropics, and those from New South Wales. Individuals from the Wet Tropics have perianths that are shorter at maturity (1.6–2.0 *v.* 3.8–4.4 mm), leaf lobes that usually bear numerous marginal gemmae, and leaf-lobules that are smaller and more quadrate. The morphological differences, particularly in perianth length, were not fully appreciated previously and provide evidence supporting the recognition of the Queensland Wet Tropics lineage as a distinct and new species, *Radula tonitrua*, which is here described. The degree of phylogenetic divergence and fixed molecular difference between *R. tonitrua* and *R. novae-hollandiae*, are comparable with the separation observed between *R. ocellata* and *R. pulchella*, another species pair exhibiting the same geographic disjunction.

Introduction

Paluma Range in Queensland is an isolated, large, rainforest island, surrounded by much drier areas. It is most notable for the northerly occurrence of several otherwise southern rainforest species, and for being a southern limit for many otherwise northern species. At the same time the Paluma Range is the type locality for several recently-described bryophyte taxa, including *Jubula hutchinsiae* ssp. *australiensis* Pócs and Cairns (2008) and *Cololejeunea cairnsiana* Pócs. There are other rare species too, originally reported for Australia based on collections from the Paluma Range, including *Cheilolejeunea ventricosa* (Schiffn.) X.L.He (Pócs and Streimann 2006) and *Nowellia langii* Pears. (Pócs *et al.* 2012). It is likely there are more novelties there to be discovered. During fieldwork collecting material for a revision of the genus *Frullania* in Australia, the first author collected, among other epiphyllous material, a tiny *Radula* species on the leaves of the filmy fern *Abrodictyum obscurum* (Blume) Ebihara & K.Iwats. in the notophyll vine forest of Birthday Creek in Paluma Range of Queensland, near Paluma settlement, at 850 m elevation. The plants bore acute leaves, and two pairs of female bracts, so belonged to subgenus *Odontoradula* K.Yamada. They were similar both to *Radula kojana* Steph. in their copious marginal gemmae and to *Radula novae-hollandiae* Hampe in their leaf and perianth shape. After careful examination it became clear that the specimen does not belong to *R. novae-hollandiae* and proved to be new to science, as both of the above species have much longer perianth (c. 4 mm long), while in the new species the mature perianth hardly exceeds 2 mm length. *Radula novae-hollandiae* infrequently produces gemmae, which are very abundant in the new species. Both known species have narrower stem medulla cells 8–12 μm diameter (in 5–12 or 19–27 rows respectively) while the stem medulla cells of the new

species are 10–20 µm diameter (in 6 rows). Its stem cortical cells are even larger, some to 30 µm diameter. Otherwise, the shape of sterile and perichaetial leaves (apart from the gemmae) are similar in all three species.

The relationships of 9 Australian species of *Radula* subgenus *Odontoradula* (Yamada 1979) were resolved using chloroplast DNA markers (Renner *et al.* 2013a, 2013b). In their revision of Australian *Radula* (Renner *et al.* 2013a), *Radula novae-hollandiae* was broadly circumscribed, and considered widespread in Australia, including Norfolk Islands, and occurring in New Zealand only in the Kermadec Islands. Two geographic lineages were resolved within *R. novae-hollandiae*, one in south-east Australia, the other in the Wet Tropics Bioregion of north-east Queensland. In this paper we reanalyse the molecular data from Renner *et al.* (2013a), which included *Radula novae-hollandiae s. lat.*, as part of our re-assessment of the plants from the Wet Tropics Bioregion of north-east Queensland, and separate these populations from *R. novae-hollandiae* as a new species.

Materials and Methods

Taxon sampling and molecular protocols

Sampling for DNA was based on material collected haphazardly throughout the Australasian geographical ranges reported for species of *Radula* subg. *Odontoradula*. This includes the Wet Tropics bioregion in north-eastern Queensland, along the coast and Great Dividing Range through New South Wales, Victoria, Tasmania and in New Zealand, for the purposes of the revision of *Radula* subg. *Odontoradula* published by Renner *et al.* (2013a). The objective of collecting was to sample individuals of each species from many sites across their range. Clean shoot tips comprising the meristem, embryonic leaves, and one or two nearly mature leaves were excised from each specimen until ~25–50 mm^2 of cleaned material was obtained, depending on plant size. DNA samples were either stored on silica gel or rapidly air-dried from wild-collected material to ensure plant material remained green and fungus-free.

Total genomic DNA was extracted using the DNeasy Plant Minikit (QIAGEN, Sydney, Australia), and three chloroplast markers were sequenced: (1) the *atpB–rbcL* spacer; (2) the plastid *trnL–F* region including the trnLUAA group1 intron and the *trnL–F* intergenic spacer, hereafter *trnL–F*; and (3) the *trnG* G2 intron. These regions were chosen because universal primers are available for all; they are known to exhibit sufficient variation to be informative at a species level (Stech and Quandt 2010) and two were used by Devos *et al.* (2011a, 2011b) to reconstruct the phylogeny of *Radula,* meaning a broader phylogenetic sampling context for the investigation of *R. novae-hollandiae* is available. Primer details were provided in Renner *et al.* (2013a). Polymerase chain reaction (PCR) was carried out as follows, for *trnL–F*, each 15-mL reaction contained 1.5 mL of 10´ PCR Buffer, 1.5 mL of 20 mM $MgCl_2$, 0.9 mL of each primer at 10-mM concentration, 0.12 mL of 1% BSA, and 0.12 mL of Immolase Taq (Bioline, Sydney, NSW). For the *atpB–rbcL* and *trnG*, each 15-mL reaction contained 1.5 mL of 10´ PCR buffer, 0.75 mL of 20 mM $MgCl_2$, 0.9 mL of each primer at 10-mM concentration, 0.12 mL of 1% BSA and 0.08 mL of Immolase Taq. Temperature profiles used for sequencing of *trnL–F* and *trnG* were 95°C for 10 min, then 35 cycles of 95°C for 1 min, 1 min at annealing temperature of 53°C, then 72°C for 1 min, followed by a final extension step of 72°C for 10 mins. The same profile, but with an annealing temperature of 50°C was used for atpB–rbcL. Cleaned PCR products were sequenced by Macrogen, South Korea (www.macrogen.com, accessed 24 February 2014) using the same primers as in PCR reactions.

Sequences were assembled using Geneious v.6 (Drummond *et al.* 2012), and consensus sequences were aligned by MUSCLE (Edgar 2004) on the CIPRES portal (Miller *et al.* 2010) and manually edited in BioEdit 5.0.9 (Hall 1999). jModelTest 2.1.10 (Darriba *et al.* 2012) to select optimal substitution models for each partition, from among 56 candidate models, with the corrected Akaike Information Criterion as the measure of model fit on the ML optimized tree. For the *atpB-rbcL* spacer an unequal frequency TVM+G model was selected as optimal, we used the next best model, which was GTR+G. For *trnG* an unequal frequency TPM1+I+G was selected as optimal, we used the next best fitting model, which was HKY+I+G; and for *trnL–F* an unequal frequency TPM1+G was selected as optimal, we used the next best fitting model but one, which was HKY+G, as this was easily implemented in BEAST.

We reconstructed relationships under maximum likelihood with iQTree (Nguyen *et al.* 2015), with a partitioned model (Cernomor *et al.* 2016) with a separate substitution model for each molecular marker, with the substitution models identified following model selection. We used the ultrafast bootstrapping approximation (Mihn *et al.* 2013; Hoang *et al.* 2018) as a measure of support for each branch. We estimated ultrametric trees summarising relationships using BEAST v.1.4.8 (Drummond and Rambaut 2007). Base frequencies were estimated, and six gamma categories were assigned for each substitution model, with all substitution models and clock models unlinked, but trees for the three partitions were linked. Substitution model priors followed default settings in BEAUTi v.1.7.2 (Drummond and Rambaut 2007). A separate uncorrelated log-normal relaxed clock modelled

substitution rates for each partition, with rates estimated relative to *atpB–rbcL*. A uniform prior with a range of 0–100 was applied to each clock, a speciation birth–death model (Gernhard 2008) with a uniform distribution was applied to node heights, and an unweighted pair-group mean aggregate (UPGMA) dendrogram was used as the starting tree. The phylogeny was not time-calibrated, however the branches in resulting ultrametric trees are proportional to time. The analysis was replicated three times, each was run for 20 million generations and sampled every 1000. Burn-in length and convergence were confirmed by comparing trace files in Tracer v.1.5 (Rambaut and Drummond 2009). After excluding the first 10% of samples as burn-in, the maximum clade credibility tree summarised the sample of trees from the posterior probability distribution.

Automatic barcode gap discovery (ABGD)

Automatic barcode gap discovery (ABGD) distinguishes distances resulting from coalescence from those resulting from divergence, by identifying the first significant peak in a plot of ranked pairwise genetic distances, which is interpreted as the gap separating intra- from inter-specific difference (Puillandre *et al.* 2012; Fontaneto *et al.* 2015). ABGD was applied to the concatenated chloroplast markers through the ABGD web portal (http://www.abi.snv.jussieu.fr/public/abgd/, accessed 16 November 2020), with default settings and distance matrices calculated using K80.

Generalised mixed Yule coalescent (GMYC) analysis

Generalised mixed Yule coalescent (GMYC) analysis (Pons *et al.* 2006) uses the expectation that coalescent branching within species occurs more rapidly than do speciation events between species. Therefore, species in gene trees form clusters of individuals on short branches separated from other such clusters by longer internal branches. Because they share the same gene tree by virtue of inheritance in plastid DNA, the concatenated chloroplast markers were used for estimation of an ultrametric gene tree. Single and multiple threshold model GMYC analyses were performed in R, ver. 3.5.2 (R Foundation for Statistical Computing) with the splits (see http://R-Forge.R-project.org/projects/splits/, accessed 16 November 2020) and ape (Paradis *et al.* 2004) packages, based on the ingroup, comprising subg. *Odontoradula*, only.

Fixed differences

Groups based on molecular data and morphological characters were compared in DnaSP v.5 (Librado and Rozas 2009). For each group, only individuals for which all three markers were sequenced were included.

Results

Voucher data and associated GenBank numbers for the molecular markers analysed for the ingroup samples belonging to *Radula* subg. *Odontoradula* are given in Table 1. *Radula novae-hollandiae* was resolved monophyletic with full support, in a sister relationship with *R. acuta*, a relationship that received support as measured by ultrafast bootstrap, but had low posterior probability. The *R. novae-hollandiae* plus *R. acuta* monophylum was in turn sister to a strongly supported clade containing *R. kojana*, and *R. apiculata* (Fig. 1). This clade was sister to *R. cuspidata*, and in turn in a fully supported sister relationship to *R. pulchella* plus *R. ocellata*. Parameter traces confirmed stationarity and convergence of all three runs, with effective sample sizes for tree likelihood and substitution model parameters greater than 200 in all three. Relationships resolved in maximum likelihood and Bayesian trees differed in the relationships close to the base of the tree, with *R. decora* plus *R. tasmanica* forming an unsupported monophylum sister to the remainder of the subgenus in the maximum likelihood tree, whereas in the Bayesian MCC tree *R. decora* plus *R. tasmanica* formed a monophylum with *R. plicata*, which was sister to the lineage containing *R. pulchella* and others, but without support. *Radula novae-hollandiae* was subdivided into two fully supported clades, one comprising individuals from north-east Queensland the other individuals from south-eastern Australia.

Automatic barcode gap discovery (ABGD)

ABGD results were in broad agreement, but exhibited some variation in grouping on the minimal prior inter-specific distance and the distance metric. *Radula ocellata* and *R. pulchella* were both oversplit under JC and K80 distances. While the north-east Queensland and south-east Australian lineages of *R. novae-hollandiae* were delimited as different clusters under JC and K80 distances metrics, under simple distance they were grouped together in a single cluster. All *Radula tasmanica* were grouped in a single cluster on JC and K80 distances, but were divided on geography into two clusters on the simple distance metric.

Table 1: Voucher specimens and associated GenBank numbers for sequences from three molecular markers analysed in this study, for ingroup accessions belonging to *Radula* subg. *Odontoradula*.

Species	Country	Region	Collector	number	Collection date	Voucher	atpB-rbcL	trnG	trnLF
R. acuta	Fiji	Viti Levu	M.A.M. Renner	5330	29 August 2011	NSW889318	KF495328	KF495267	KF495387
R. acuta	Fiji	Viti Levu	M.A.M. Renner	5349	29 August 2011	NSW889366	KF495330	KF495268	KF495389
R. acuta	Fiji	Viti Levu	M.A.M. Renner	5416	31 August 2011	NSW889521	KF495331	KF495269	KF495390
R. acuta	Fiji	Viti Levu	M.A.M. Renner	5503	1 September 2011	NSW890194	KF495334	KF495272	KF495393
R. acuta	Vanuatu	Sanma	E.A. Brown	s.n.	November 2006	NSW971056	KF495361	KF495302	KF495418
R. acuta	Fiji	Viti Levu	M.A.M. Renner	5346	29 August 2011	NSW889362	KF495329	–	KF495388
R. allisonii	New Zealand	North Island	M.A.M. Renner	6264	24 February 2012	NSW896403	KF495342	KF495280	KF495397
R. allisonii	New Zealand	North Island	M.A.M. Renner	6269	24 February 2012	NSW896414	KF495343	KF495281	KF495398
R. allisonii	New Zealand	North Island	P.J. de Lange	10144	22 September 2011	NSW973432	KF495367	KF495307	KF495423
R. allisonii	New Zealand	North Island	P.J. de Lange	s.n.	22 September 2011	NSW973436	KF495368	KF495308	KF495424
R. allisonii	New Zealand	South Island	M.A.M. Renner	6072	12 February 2012	NSW895347	KF495337	KF495275	KF495395
R. apiculata			T. Yamaguchi	1731		BR	HM992050	-	HM992478
R. cuspidata	New Zealand		M.A.M. Renner			AK280588	HM992002	HM992353	HM992439
R. decora			I. Holz & Franzaring	CH0060		GOET	HM991973	HM992327	HM992413
R. kojana	Japan		M. Mizutani	14255		DUKE	HM992013	HM992364	HM992447
R. novae-hollandiae	Australia	NSW	M.A.M. Renner	5261	14 April 2011	NSW875807	KF495322	KF495261	KF495381
R. novae-hollandiae	Australia	NSW	M.A.M. Renner	5274	15 April 2011	NSW875820	KF495323	KF495262	KF495382
R. novae-hollandiae	Australia	NSW	M.A.M. Renner	5883	16 December 2011	NSW898670	KF495354	KF495293	KF495410
R. novae-hollandiae	Australia	NSW	M.A.M. Renner	5024	22 September 2010	NSW973484	KF495369	KF495309	KF495425
R. novae-hollandiae	Australia	NSW	M.A.M. Renner	5036	22 September 2010	NSW973485	KF495370	KF495310	KF495426
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	2110	13 July 2005	NSW885020	KF495327	KF495266	KF495386
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6341	27 March 2012	NSW896746	KF495347	KF495285	KF495402
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6362	28 March 2012	NSW896816	KF495348	KF495286	KF495403
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6366	27 March 2012	NSW896820	KF495349	KF495287	KF495404
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6401	30 March 2012	NSW896902	KF495350	KF495288	KF495405
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6436	30 March 2012	NSW896975	KF495351	KF495289	KF495406
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6443	30 March 2012	NSW896982	KF495352	KF495290	KF495407
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6514	5 April 2012	NSW898725	KF495356	KF495295	KF495412
R. novae-hollandiae	Australia	Qld	M.A.M. Renner	6547	5 April 2012	NSW973523	KF495371	KF495311	KF495427
R. ocellata	Australia	Qld	M.A.M. Renner	5093	26 January 2011	NSW970362	–	KF495299	KF495415
R. ocellata	Australia	Qld	M.A.M. Renner	5090	26 January 2011	NSW970874	KF495360	KF495301	KF495417
R. ocellata	Australia	Qld	M.A.M. Renner	5091	26 January 2011	NSW973669	KF495372	KF495312	KF495428
R. ocellata	Australia	Qld	M.A.M. Renner	5108	28 January 2011	NSW973771	KF495373	KF495313	KF495429
R. ocellata	Australia	Qld	M.A.M. Renner	5053	24 July 2011	NSW973946	KF495374	KF495314	KF495430
R. ocellata	Australia	Qld	M.A.M. Renner	5054	24 July 2011	NSW973994	–	KF495315	KF495431
R. ocellata	Australia	Qld	J.A. Curnow	3664		CBG	HM992003	HM992354	HM992440

Species	Country	Region	Collector	number	Collection date	Voucher	atpB-rbcL	trnG	trnLF
R. plicata	New Zealand	North Island	M.A.M. Renner		4 January 2002	AK280391	HM992000	HM992351	HM992437
R. pulchella	Australia	NSW	M.A.M. Renner	5230	11 April 2011	NSW875776	KF495319	KF495259	KF495378
R. pulchella	Australia	NSW	M.A.M. Renner	5231	11 April 2011	NSW875777	KF495320	–	KF495379
R. pulchella	Australia	NSW	M.A.M. Renner	5276	15 April 2011	NSW875822	KF495324	KF495263	KF495383
R. pulchella	Australia	NSW	M.A.M. Renner	5304	6 June 2011	NSW877196	KF495325	KF495264	KF495411
R. pulchella	Australia	NSW	M.A.M. Renner	5030	22 September 2010	NSW877631	KF495326	KF495265	KF495385
R. pulchella	Australia	NSW	M.A.M. Renner	5885	16 December 2011	NSW898672	KF495355	KF495294	KF495411
R. pulchella	Australia	NSW	H. Streimann	63817		EGR	HM992030	HM992380	HM992459
R. retroflexa	Australia	Qld	M.A.M. Renner	2049	10 July 2005	NSW872738	KF495318	KF495258	KF495377
R. retroflexa	Australia	Qld	M.A.M. Renner	6279	24 March 2012	NSW896660	KF495344	KF495282	KF495399
R. retroflexa	Australia	Qld	M.A.M. Renner	6311	24 March 2012	NSW896700	KF495345	KF495283	KF495400
R. retroflexa	Australia	Qld	M.A.M. Renner	6313	24 March 2012	NSW896703	KF495346	KF495284	KF495401
R. retroflexa	Australia	Qld	M.A.M. Renner	6582	8 April 2012	NSW898461	KF495353	KF495292	KF495409
R. retroflexa	Australia	Qld	M.A.M. Renner	6523	5 April 2012	NSW898764	–	KF495296	KF495413
R. retroflexa	Australia	Qld	M.A.M. Renner	6524a	5 April 2012	NSW898872	KF495357	KF495297	KF495414
R. retroflexa	Australia	Qld	M.A.M. Renner	6565	6 April 2013	NSW970436	KF495359	KF495300	KF495416
R. retroflexa	Cook Islands	Rarotonga	P.J. de Lange	CK183	11 July 2010	NSW973421	KF495365	KF495305	KF495421
R. retroflexa	Cook Islands	Rarotonga	P.J. de Lange	CK180	8 July 2010	NSW973422	KF495366	KF495306	KF495422
R. retroflexa	Fiji	Kadavu	M.A.M. Renner	5719	September 2011	NSW973391	KF495364	KF495304	KF495420
R. retroflexa	Fiji	Viti Levu	M.A.M. Renner	5428	31 August 2011	NSW889533	KF495332	KF495270	KF495391
R. retroflexa	Fiji	Viti Levu	M.A.M. Renner	5431	31 August 2011	NSW889536	KF495333	KF495271	KF495392
R. retroflexa	Fiji	Viti Levu	M.A.M. Renner	5606	4 September 2011	NSW973390	KF495363	–	KF495419
R. retroflexa	Fiji		S. & T. Pócs	03281/C		EGR	HM992035	HM992385	HM992464
R. retroflexa	Vanuatu		E.A. Brown	05/517		NSW	KF495317	KF495257	KF495376
R. sp.indet.	Fiji		E.A. Brown	05/362		NSW	KF495316	KF495256	KF495375
R. tasmanica	Australia	Tas.	M.A.M. Renner	5935	23 January 2012	NSW895266	KF495335	KF495273	KF440509
R. tasmanica	Australia	Tas.	M.A.M. Renner	5985	26 January 2012	NSW909280	–	KF495298	KF440511
R. tasmanica	Australia	Tas.	M.A.M. Renner	6013	27 January 2012	NSW970369	KF495358	–	KF440513
R. tasmanica	Australia	Tas.	M.A.M. Renner	5956	24 January 2012	NSW972574	KF495362	KF495303	KF440510
R. tasmanica	New Zealand	South Island	M.A.M. Renner	6188	17 February 2012	NSW895514	KF495338	KF495276	KF440548
R. tasmanica	New Zealand	South Island	M.A.M. Renner	6198	17 February 2012	NSW895583	KF495339	KF495277	KF440484
R. tasmanica	New Zealand	South Island	M.A.M. Renner	6224	18 February 2012	NSW895669	KF495341	KF495279	KF440549
R. tasmanica	New Zealand	North Island	M.A.M. Renner		15 October 2001	AK280184	HM991998	HM992349	HM992435
R. weymouthiana	Australia	Tas.	M.A.M. Renner	6052	31 January 2012	NSW898459	–	KF495291	KF495408
R. weymouthiana	New Zealand	South Island	M.A.M. Renner	6064	12 February 2012	NSW895339	KF495336	KF495274	KF495394
R. weymouthiana	New Zealand	South Island	M.A.M. Renner	6201	17 February 2012	NSW895586	KF495340	KF495278	KF495396

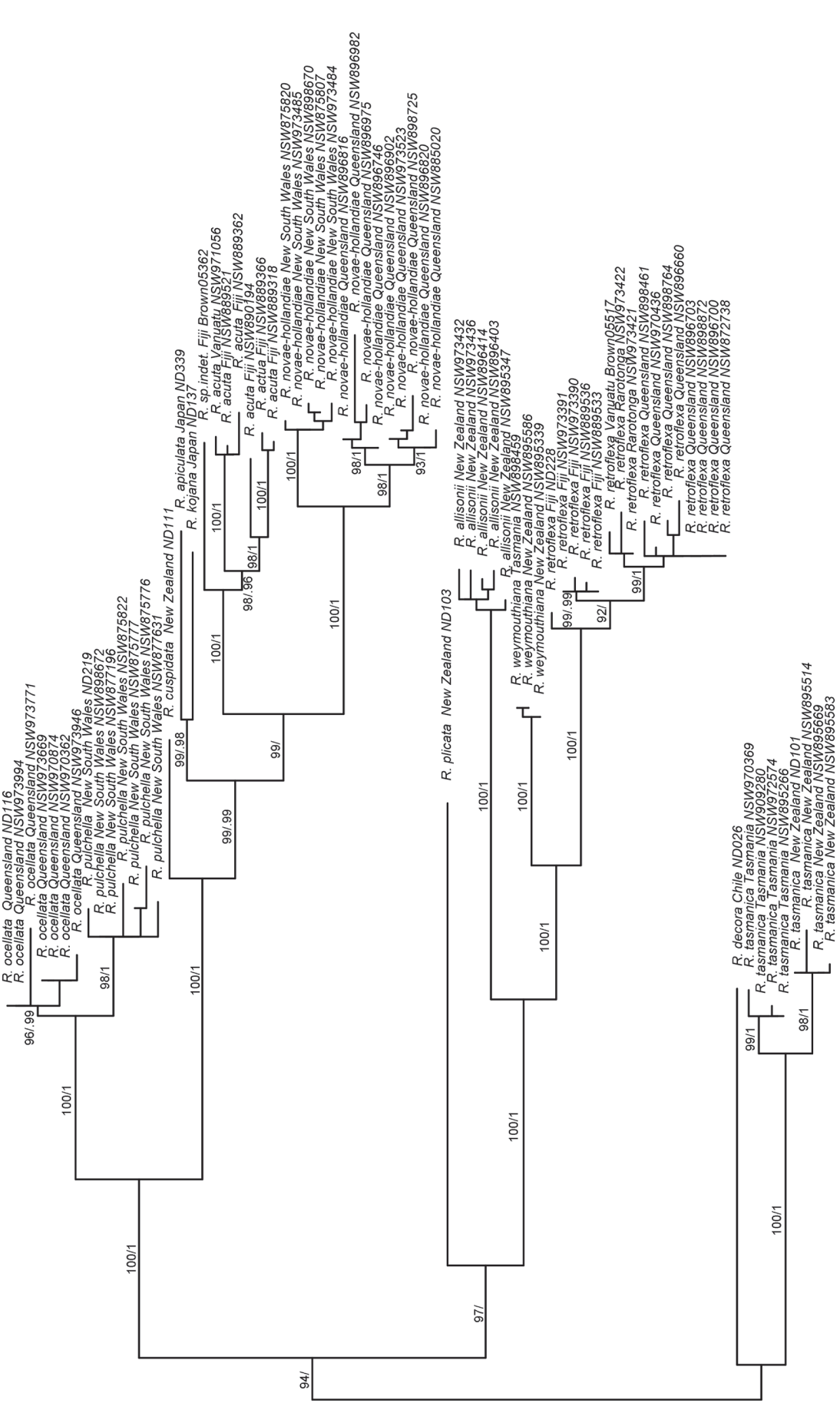

Fig. 1. Most likely tree reflecting relationships among individuals of *Radula* subg. *Odontoradula* included in this study. Numbers above branches are ultrafast bootstrap values from iQTree and posterior probability from BEAST.

Generalised mixed Yule coalescent (GMYC) analysis

Both the single and multiple threshold models were more likely than the null model of uniform (coalescence) branching rate (single: LGMYC = 552.9 vs. L0 = 546.3, 2DL = 26.1, P = 0.0014; multiple: LGMYC = 554.4 vs. L0 = 546.3, 2DL = 32.2, P = 0.0003). The multiple-threshold model provided a slightly better fit 2DL = 3.0. The single model fitted a switch resulting in 14 maximum likelihood (ML) clusters (confidence interval (CI: 7–14) and 21 ML entities (CI: 11–25). The multiple threshold model fitted three switches, resulting in 13 ML clusters (CI: 10–13) and 21 ML entities (CI: 14–25). The interpretation of geographic clades within *R. tasmanica* resulting in the only grouping difference between the two models, in the single threshold model interpreted these were interpreted as a phylogenetic divergence, but as coalescent branching in the multiple model. Both single and multiple threshold models identified three phylogenetic lineages within *R. novae-hollandiae*, one from south-east Australia, and two from the Wet Tropics of north east Queensland.

Fixed differences.

The number of fixed differences between individuals of *R. novae-hollandiae* from Queensland (=*R. tonitrua*) and New South Wales was 10, one more than separates *R. ocellata* and *R. pulchella*. The average number of differences between all pairs of individuals was slightly more than 15 in both species (Table 2).

Table 2. Number of fixed differences among sister lineages: n, the number of individuals included; k, the average number of nucleotide differences among individuals within populations; Mutations, the number of variable sites within species or populations. Differences are the number of fixed differences between individuals of each species or population pair, while the values in parentheses show the average number of differences between all pairs of individuals from each population.

		n	k	Mutations	Differences
R. tonitrua	Queensland	9	5.778	17	10 (15.6)
R. novae-hollandiae	New South Wales	5	1.6	4	
R. ocellata	Queensland	4	6	9	9 (15.3)
R. pulchella	New South Wales	9	3.6	12	
R. tasmanica	New Zealand	3	3.3	5	13 (15.2)
	Tasmania	2	1	1	

Discussion

Individuals of *Radula novae-hollandiae* from north-east Queensland and south-east Australia form two reciprocally monophyletic lineages which, in most analyses, are objectively diagnosable as two (or more) separate species. Improved modelling of sequence evolution, compared to Renner *et al*. (2013a), did not alter tree topology or branch lengths significantly, with the exception of the relationships between *R. decora* and *R. tasmanica* and the remainder of the subgenus in the ML tree. Despite these differences in topology within the MCC tree (see Fig. 2), the same groups were returned by objective species delimitation methods. The GMYC method is known to over-split entities (Luo *et al*. 2018), and this seems to have occurred in our analysis for *R. retroflexa*, *R. tasmanica*, *R. ocellata*, and also the Queensland lineage of *R. novae-hollandiae*. The depth of phylogenetic divergence, and number of fixed differences separating northern and southern lineages of *Radula novae-hollandiae* are both comparable with those separating *Radula pulchella* and *R. ocellata*, two sister species the latter of which was recognized on the basis of subtle, fixed, morphological differences from the first. These differences, associated with the presence of small accessory teeth around the lobule apex, were subsequently confirmed as reflecting a phylogenetic divergence by molecular data (Renner *et al.* 2013a). In contrast, the divergences between the northern and southern lineages of *R. novae-hollandiae* are shallower than those in *R. tasmanica*, which was interpreted, we believe correctly given the shared morphology of plants on both sides of the Tasman Sea, as within-species branching in the multiple threshold model. Northern and southern lineages of *R. novae-hollandiae* were not recognized as distinct by Renner *et al.* (2013a) because no morphological differences between northern and southern populations were detected by that study, hence *R. novae-hollandiae* appeared, like *R. tasmanica*, to present a case where geographically correlated divergences were possessed by single morphological entities. Subsequent investigation has demonstrated that this interpretation of morphological variation was incomplete, and that northern and southern lineages are morphologically diagnosable, on the basis of characters associated with reproductive structures generally considered critical for species circumscription within subg. *Odontoradula*, and *Radula* more broadly, in addition to lobule shape and propensity to asexual reproduction. A consistent interpretation of the molecular and morphological evidence, also compatible with overarching theory, is that the northern lineage of *R. novae-hollandiae* in the Wet Tropics

Bioregion comprises a new, undescribed species. *Radula novae-hollandiae,* in turn corresponds to the south-eastern lineage, from which the type specimen was derived. A single plant from the south-eastern lineage was the basis for the description of *Radula novae-hollandiae* in Renner *et al.* (2013a), and we refer readers there for a reasonably comprehensive description of it.

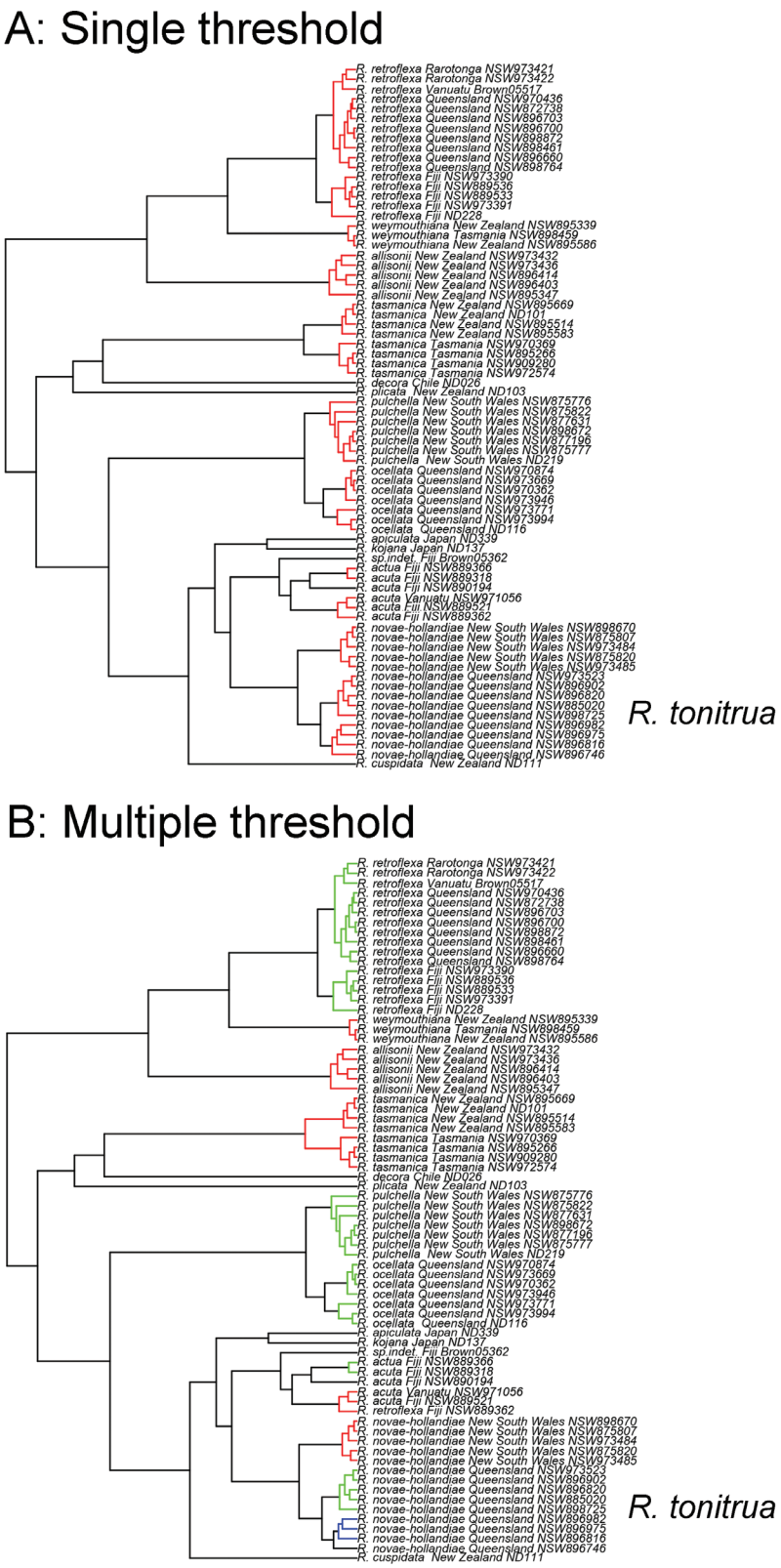

Fig. 2. GMYC analyses on MCC tree from BEAST, with phylogenetic branches in black, and coalescent branching in colour, as inferred using a single threshold between the two in A, and multiple, thresholds between the two in B, in which different colours are derived from different thresholds.

Taxonomic treatment

Radula tonitrua Pócs & M.A.M.Renner, *sp. nov*.

Diagnosis: *Radula tonitrua* is similar to *R. kojana,* but differs from by its much shorter perianth (1.6–2 mm long) and by the irregular shape and often much larger size (up to 120 µm long) of marginal gemmae, which in *R. kojana* are discoid or globular, usually not larger than 20 µm in diameter. The leaves of *R. kojana* are more concave and have a silky shine, which is not the case in *R. tonitrua.* It is similar in leaf and perianth shape to *R. novae-hollandiae* but separated by the presence of abundant marginal gemmae, which are not common in that species. *Radula tonitrua* differs from both by its stem medulla only 5 cells high and 6 cells wide, consisting of larger cells (10–20 µm in diameter).

Type: Australia: Queensland, Paluma Range State Forest 1.5 km NW of Paluma settlement. Birthday Creek, in the experimental area of James Cook University Tropical Biology Department. On *Abrodictyum obscurum* (Blume) Ebihara & K.Iwats. filmy fern leaves in wet, notophyll vine forest among boulders near the streamlet. At 840–870 m elevation, 18°59,\'9'S, 146°10'7–8'E *S. & T. Pócs 01121/AW,* accompanied by *A. Cairns, E.A. Brown & Ch. Cargill*, 20 June 2001. (holo: EGR, including 2 portions mounted on microslides; iso: BRI, CANB).

Description: (from the holotype). Epiphyllous (other specimens also lithophytic), forming loose mats, appressed to filmy fern leaves. Live plants dark green, pale green or brownish in herbarium. Shoots uni- to bipinnately branched, 0.6–1.0 mm, at the female perichaetium up to 1.5 mm wide. Stems in cross section ellipsoid, 50–100 µm (in average 5 cells) high and 60–120 µm (in average 6 cells) wide, constituted by 15–19 pale brown to rusty pigmented cortical cells, 20–40 µm long and 12–25 µm in diameter and 22–25 medulla cells, 10–20 µm in diameter, with pale yellow or colourless walls. All stem cells have evenly thickened (2 µm) walls except for the outermost wall of cortical cells (up to 3 µm thickness). Leaf insertions reach the midline of dorsal stem side but leave free 1–2 ventral cortical cell rows.

Leaf lobes asymmetrically ovate, somewhat falcate or deflexed, with obtuse to acute apex, 400–560 µm long and 250–300 µm wide on the main stem and 350–450 µm long and 200–320 µm wide on branches. Leaf lobes dorsally cover partially the stem, leaving free a zig-zag shaped zone in the lower part of stem, more imbricate upwards. Median lobe cells isodiametric polygonal, 19–25 µm in diameter, apical and marginal cells 12–16 µm. Cell walls evenly thin, with small triangular trigones. Leaf cell surface smooth. Lobe margins smooth to crenulate by bulging cells, often indicating origin of developing gemmae. Irregular shaped discoid or ribbon-like (sometimes bifurcate) gemmae copiously develop on the leaf margin (including perichaetial leaves and rarely the perianth mouth). In exceptional case the half surface of lobe is converted to gemmae or in other cases no gemmae develop at all. The gemmae are varied in size, consisting of a few to very many cells, in cases of ribbon-like ones up to 120 µm (12–15 cells) length and 50 µm (5–8 cells) width, uni- or rarely multilayered. The lobules rectangular, rhombic or triangular ovate, 1/3 to ½ length of the lobe, flat to slightly inflated in their postical half with obtuse to rounded apex. Angle between postical lobe margin and keel 130–140°. Interior lobule margin free from 1/10 to 1/4 of its length, the fused part straight, parallel to stem. Lobule cells similar to those of the lobe. Rhizoids of 8–10 µm of width, rare, develop from the slightly emergent lobule centre, pale brown, arranged in straight parallel bundles.

Perichaetium consists of 1–2 pairs of female bracts, which are much larger than ordinary leaves, up to 1000 µm length and 480 µm width, with acute apex, smooth or sometimes irregularly lobulate margin and triangular lobules of half lobe length. The angle of their postical margin to the stem is 155–170°. Cells and gemmae are similar to those of normal leaves. Mature perianth 1600–2500 µm long, at the mouth 500–600 µm wide, labia plane with gently undulate margin, unistratose in the upper third, conical in the upper two third tapering into the tubular stem perigynium of 250 µm width. In the perianth a young sporophyte was observed with cylindric capsule. Figs 1, 2.

Etymology: We understand that the name of type locality: Paluma State Forest, Paluma Range and Paluma National Park came from an aboriginal language in which "paluma" means thunder. Its Latin equivalent is "tonitrua", from which the species' binomial name is derived.

Additional specimens examined: Australia: Queensland, Paluma Range State Forest 1.5 km NW of Paluma settlement. Birthday Creek, 18°59'9'S, 146°10'7–8' E. 20 June 2001, *S. & T. Pócs 01121/BC,* accompanied by *A. Cairns, E.A. Brown & Ch. Cargill* (BRI); Daintree National Park, Mount Lewis, headwaters of Leichhardt Creek flowing down SW flanks of summit, 16°35'02"S, 145°16'33"E, 1150 m, 27 Mar. 2012, *M.A.M. Renner 6366, V.C. Linis & E.A. Brown*, NSW896820; Daintree National Park, Coast Range, Little Falls Creek catchment, immediately above the coral fern patch on the Manjal Jimalji track from Karnak to rock pinnacle, ENE of spot height 1198 m, 16°23'43"S, 145°17' 57"E, 1030 m, 28 Mar. 2012, *M.A.M. Renner 6377, V.C. Linis & E.A. Brown*, NSW896827; Main Coast Range, 19 km NNW of Mount Molloy, 16°31'S, 145°16'E, 1200 m, 30 June

1984, *H. Streimann 30249*, CANB8408604; Daintree National Park, Mount Lewis, upper Leichhardt Creek catchment, 16°34'59"S, 145°16'31"E, 1180 m, 27 Mar. 2012, *M.A.M. Renner 6341, V. C. Linis & E.A. Brown*, NSW896746; Kauri Creek, Mount Haig Road, Lamb Range, 22 km NE of Atherton, 17°08'S, 145°36'E, 800m, 27 June 1984, *H. Streimann 29887*, CANB8408242; Wooroonooran National Park, Bellenden Ker Range, Mulgrave River catchment, track to Choorichillum from end of Gourka Road, 17°22'48"S, 145°47'14"E, 1020 m, 30 Mar. 2012, *M.A.M. Renner 6401, V.C. Linis & E.A. Brown*, NSW896902; Palmerston Highway, Massey Creek, 8 km E of Ravenshoe, 17°37'S, 145°33'E, 1070 m, 8 Dec. 1990, *J.A. Curnow 3904*, CANB9409858; Wooroonooran National Park, South Johnston River catchment, Maple Creek, 17°40'59"S, 145°42'10"E, 590 m, 5 Apr. 2012, *M.A.M. Renner 6514, E.A. Brown & V.C. Linis,* NSW898725.

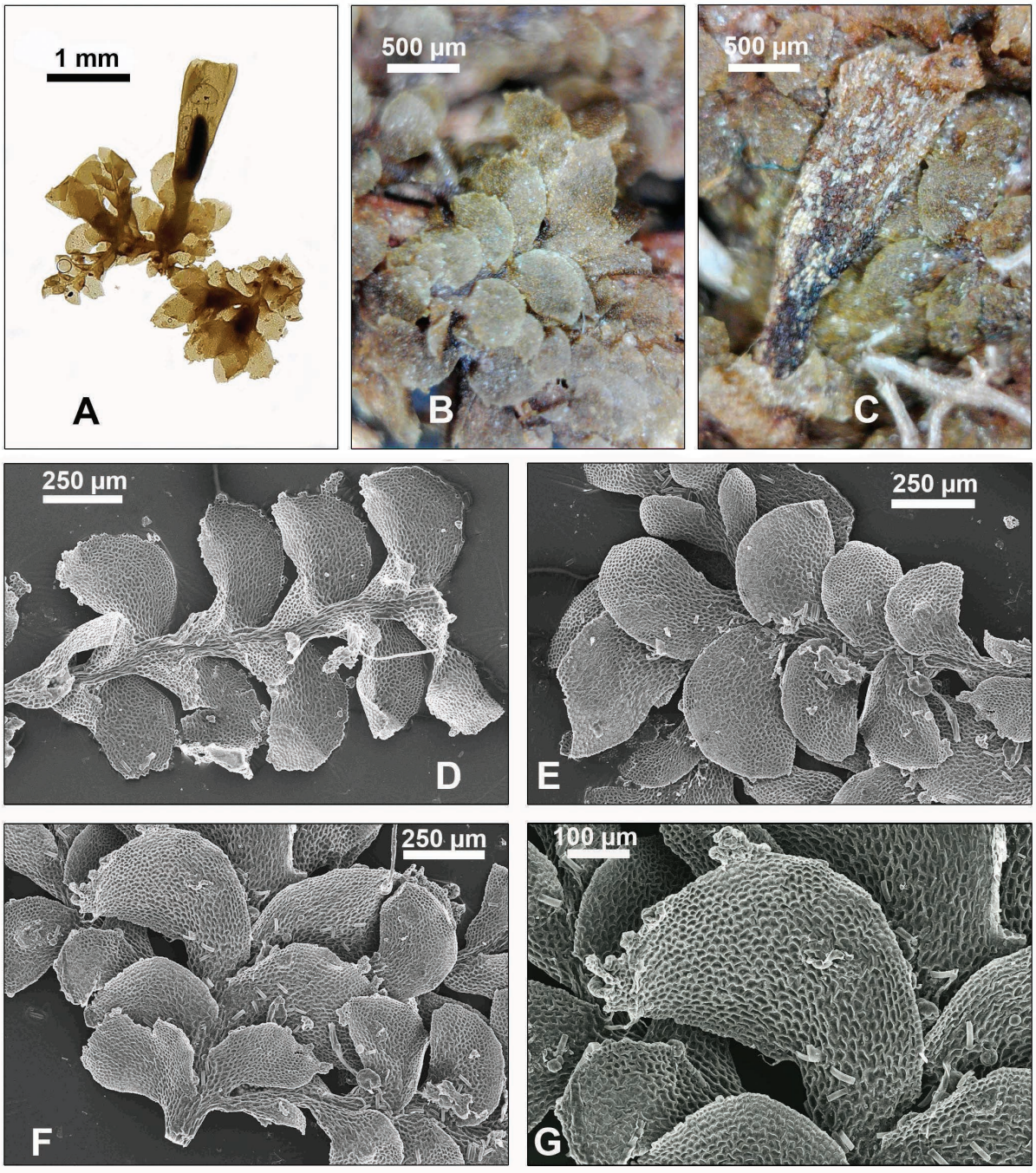

Fig. 3. *Radula tonitrua* A: Habit, ventral view. B, D, E and F: Habit, dorsal view. C: Mature perianth in dried herbarium material. G: Leaf, dorsal view. Images by T. Pócs from the holotype of *R. tonitrua*.

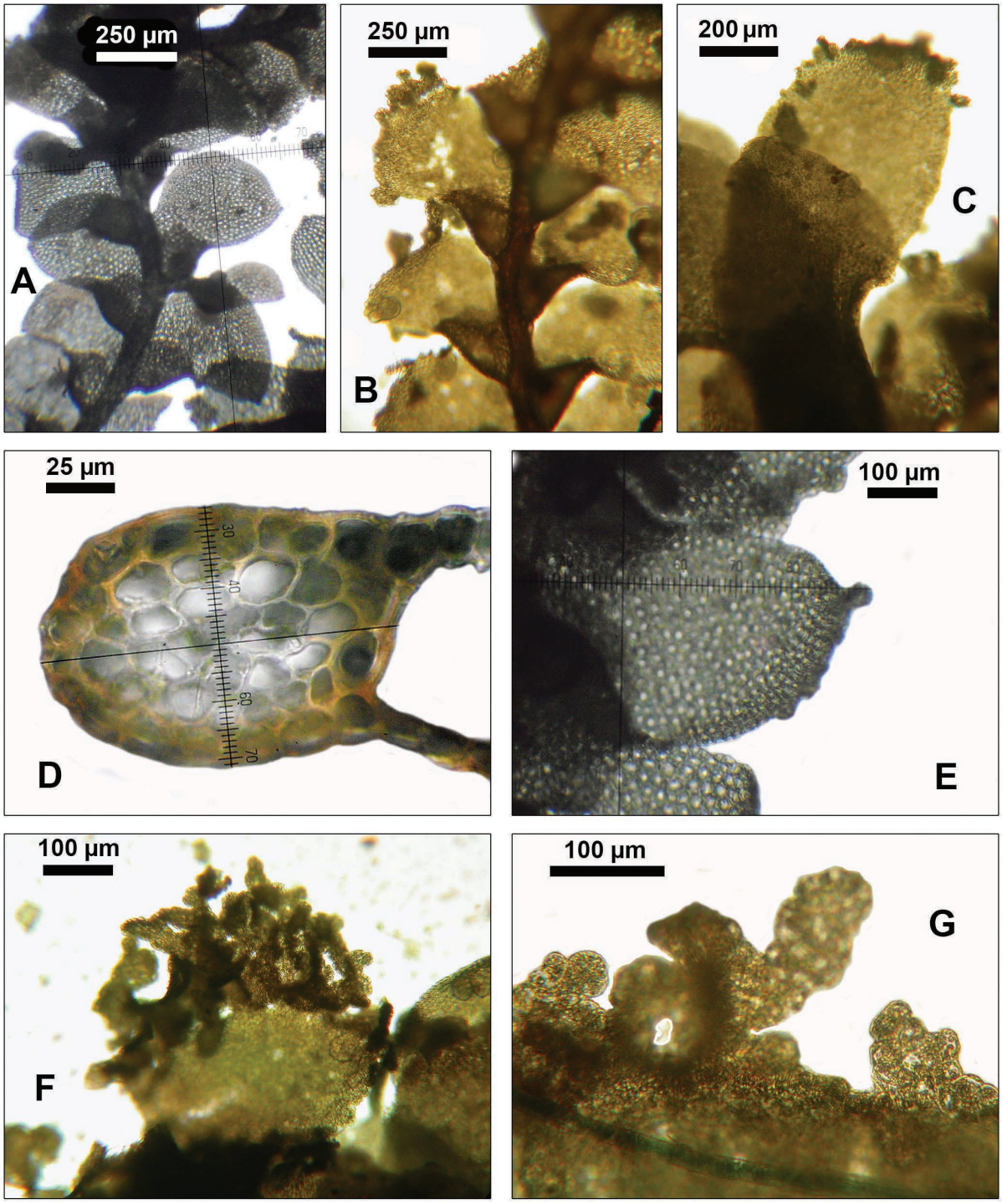

Fig. 4. *Radula tonitrua* A and B: Habit, ventral view. D: Leaf, ventral view. D: Cross section of main stem. E: Lobe margin with gemmae initials. E and G: Details of lobe margin with gemmae. Images by T. Pócs from the holotype of *R. tonitrua*.

Ecology. The specimens collected at the type locality were epiphyllous, growing on filmy fern leaves. The surrounding habitat is very wet with continuous high level of air moisture, near the cataracts of Birthday Creek. In other parts of the Wet Tropics Bioregion in north-eastern Queensland, *R. tonitrua* commonly grows in turfs on the sides of granite boulders, whether in association with permanent waterways or not. *Radula tonitrua* grows in association with a wide range of other lithophytic bryophytes, in particular *R. jovetiana* K.Yamada, *R. loriana* Castle, *R. myriopoda* M.A.M.Renner and *R. patens* K.Yamada, Lophocoleaceae spp., and Plagiochilaceae spp., and a variety of other mosses and liverworts, including Lejeuneaceae such as *Lopholejeunea muelleriana* var. *australis* (Steph.) B.Thiers & Gradst. (Renner *et al.* 2013a). Throughout its range, *R. tonitrua* inhabits wet tropical rainforests and montane rainforests from ~600 m to the tops of the highest peaks in the Wet Tropics, which range from 1200 to around 1600 m.

Revised couplets in key to species of *Radula* subg. *Odontoradula* (modified from Renner *et al.* 2013a)

4. Lobule margin irregularly crenulated because of bulging cells, lobules longitudinally rectangular. Apex of leaf lobules rounded to obtuse, apex of female bract lobes obtuse to acute..... 4a

   Lobule margin entire, lobules trullate. Apex of leaf lobes and female bract lobes rounded ..... *R. pugioniformis* M.A.M. Renner

4a. Perianths 1.6–2.0 mm long at maturity. Often with marginal gemmae on lobes of leaves and female bracts ..... *R. tonitrua* Pócs & M.A.M.Renner

   Perianths 3.8–4.5 mm long at maturity. Usually lacking gemmae..... *R. novae-hollandiae* Hampe

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