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 Novellia 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 Kermande 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 mm² 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 *trnLUA* group1 intron and the *trnLF* 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 MgCl₂, 0.9 mL of each primer at 10-mM concentration, 0.12 mL of 1% BSA, and 0.12 mL of Inmollase 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 MgCl₂, 0.9 mL of each primer at 10-mM concentration, 0.12 mL of 1% BSA and 0.08 mL of Inmollase 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 Akaiake Information Criterion as the measure of model fit. 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* monophyly 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 monophyly 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 monophyly 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  |
|                  | 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     | KF495343     | 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 | NSW866403     | 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  | HM992439  |
| *R. cuspidata*   | New Zealand |            | M.A.M. Renner     |        |                 |               |              | GOET      | HM991973  |
| *R. decora*      |          |            | I. Holz & Franzaring | 4060  |                 |               |              | DUKE      | HM992013  |
| *R. kojana*      | Japan     |            | M. Mizutani       | 14255  |                 |               |              | DUKE      | HM992013  |
| *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 | NSW896760     | 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    | NSW853020     | KF495327     | KF495266  | KF495366  |
| *R. novae-hollandiae* | Australia | Qld | M.A.M. Renner     | 6341   | 27 March 2012   | NSW869746     | 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    | KF495349    | 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. Pocs  |                   | 03281.C |            | EGR           | HM992035    | HM992385   | HM992464 |
| *R. retroflexa*  | Vanuatu    | E.A. Brown    |                   | 05517  |                   | NSW          | KF495317    | KF495257   | KF495376 |
| *R. sp.indet.*   | Fiji       | E.A. Brown    |                   | 05632  |                   | 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     | 6024   | 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*    | 9  | 5.778 | 17        | 10 (15.6)   |
| *R. novae-hollandiae* | 5  | 1.6   | 4         |             |
| *R. ocellata*    | 4  | 6     | 9         | 9 (15.3)    |
| *R. pulchella*   | 9  | 3.6   | 12        |             |
| *R. tasmanica*   | 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 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 shiny silver, 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 _Abrodicymum 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 uniaxial to bipinnately branched, 0.6–1.0 mm, at the female perichaetium up to 1.5 mm wide. Stems in cross section elliptoid, 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 ⅓ 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 peristomes, 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 2002.
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*.
Contribution to the bryoflora of Australia, V. Radula 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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