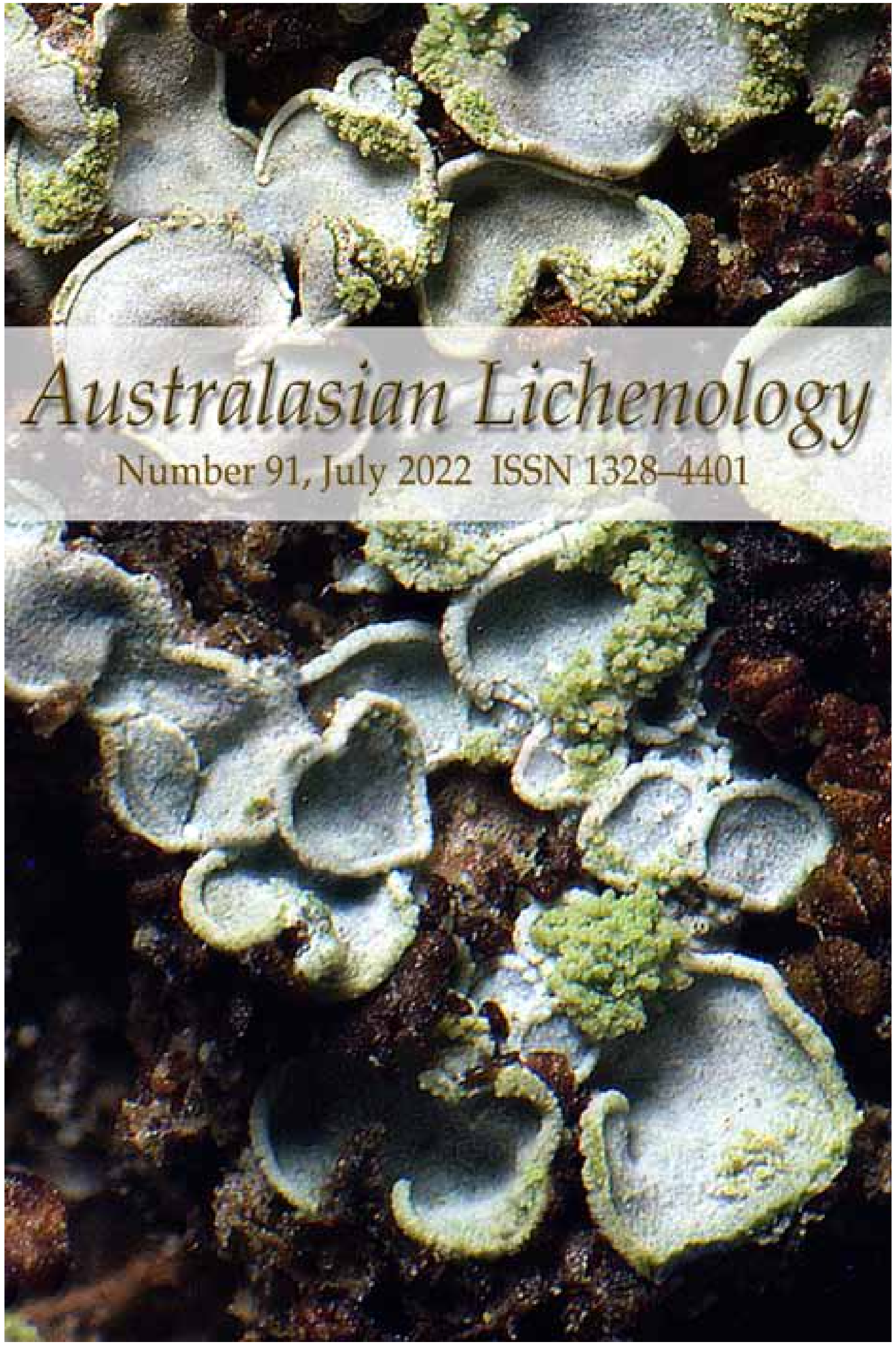

Australasian Lichenology

Number 91, July 2022 ISSN 1328–4401

Australasian Lichenology

Number 91, July 2022 ISSN 1328–4401

Normandina pulchella is readily identified by its distinctive blue-green colour and its ear-like squamules, which have raised and strongly inrolled margins. It colonizes a range of substrata, including rock, tree bark, moist humus, leaves, and even other lichens, and it's moderately tolerant of air pollution. It often produces dense patches of moss-green soredia on the surface and margins of its squamules, but ascomata and conidiomata are unknown. Occasional reports of perithecia have mostly been dismissed as fruiting bodies produced by *Lauderlindsaya borreri* or other lichenicolous Ascomycetes. Often overlooked because of its small size, it's nearly cosmopolitan in its distribution.

1 mm

CONTENTS

Synonymy in species of *Trapelia* (lichenized Ascomycota, Trapeliaceae) from Australia

Cécile Gueidan
Australian National Herbarium, National Research Collections Australia,
CSIRO, Clunies Ross Street, Canberra, A.C.T. 2601, Australia
e-mail: Cecile.Gueidan@csiro.au

John A. Elix
Research School of Chemistry, Building 137,
Australian National University, Canberra, A.C.T. 2601, Australia
e-mail: John.Elix@anu.edu.au

Abstract

Species of the genus *Trapelia* are notoriously variable and difficult to identify. Although previous taxonomic studies have taken advantage of molecular data to confirm the placement and delimitation of species, most Australian taxa, including the recently described *T. atrocarpa*, *T. terrestris* and *T. rosettiformis*, have not been the subject of molecular study. Here, ITS sequences were generated for 11 species of *Trapelia* occurring in Australia and integrated to a dataset of mostly European *Trapelia* taxa. As a result, due to poor phylogenetic and morphological differentiations, synonymies are proposed for the three species *T. atrocarpa* (syn. *T. terrestris*), *T. pruinosa* (syn. *T. rosettiformis*) and *T. placodioides* (syns *T. thieleana* and *T. occidentalis*). Together with a few Welsh *T. elacista* specimens, *T. atrocarpa* is found to be closely related to *T. elacista*. A key to the nine *Trapelia* species currently accepted from Australia is provided.

Introduction

Trapelia M.Choisy is a small but widely distributed genus of crustose to squamulose species occurring on various substrata (bark, rock and soil). Among other characters, it is distinguishable by its hemiangiocarpic apothecia that rupture the upper cortex during their development, aseptate ascospores, and crustose to squamulose or placodioid thalli (Kantvilas *et al.* 2014). *Trapelia* belongs to the family Trapeliaceae, together with several other genera, including *Lambiella* Hertel, *Placopsis* (Nyl.) Linds., *Placynthiella* Elenkin, *Rimularia* Nyl. and *Trapeliopsis* Hertel & Gott.Schneid. (Resl *et al.* 2015). *Trapelia* is currently not monophyletic, as the cephalodiate genus *Placopsis* has been shown to be nested within this genus (Kantvilas *et al*. 2014; Resl *et al.* 2015; Schneider *et al.* 2016). The first molecular revisions of *Trapelia* species focused on Australian taxa (Kantvilas *et al.* 2014) and those from Great Britain and the Falkland Islands (Orange 2018). Those two studies showed that several inferred lineages were unrecognized and that the diversity of *Trapelia* species had been underestimated. They also highlighted the difficulties in circumscribing taxa with pronounced morphological and anatomical variation.

Fourteen species of *Trapelia* have been described or reported from Australia (Kantvilas & Elix 2007; Kantvilas *et al.* 2014; Elix & McCarthy 2019, 2020a, 2020b; McCarthy 2020), including the widespread and probably cosmopolitan *T. coarctata* (Sm.) M.Choisy and *T. placodioides* Coppins & P.James, as well as the Australasian *T. macrospora* Fryday and eleven Australian endemics, *viz. T. atrocarpa* Elix & P.M.McCarthy (Fig. 2), *T. calvariana*, Kantvilas & Lumbsch (Fig. 3), *T. concentrica* Elix & P.M.McCarthy (Fig. 4), *T. crystallifera* Kantvilas & Elix (Fig. 5), *T. kosciuszkoensis* Elix (Fig. 6), *T. lilacea* Kantvilas & Elix (Fig. 7), *T. occidentalis* Elix, *T. pruinosa* Elix & P.M.McCarthy (Fig. 10), *T. rosettiformis* Elix & P.M.McCarthy (Fig. 11), *T. terrestris* Elix & P.M.McCarthy and *T. thieleana* Kantvilas, Lumbsch & Elix. In an effort to verify the integrity of the recently described species, we have undertaken a molecular study of the available Australian material of *Trapelia*.

Material and methods

Morphology and chemistry

For the most part this study is based on herbarium material in CANB. Observations and measurements of photobiont cells, thallus and apothecium anatomy, asci, ascospores and pycnidia were made on hand-cut sections mounted in water and 10% KOH (K). Asci were also observed in Lugol's Iodine (I), with and without pretreatment in K. Medullary sections were treated with 10% sulfuric acid (H_2SO_4) and apothecial sections with 50% nitric acid (N). Chemical constituents were identified by thin-layer chromatography (Elix 2022) and comparison with authentic samples.

Molecular: *taxon and gene sampling*

Herbarium specimens of Australian *Trapelia* were used for molecular analysis (Table 1). The fungal DNA barcode ITS (the internal transcribed spacer region) was chosen as a phylogenetic marker for this study. In total, sequences were generated from 44 Australian specimens, including five type specimens. Sequence data available in GenBank for *Trapelia* species and those from closely related genera (*Lambiella*, *Orceolina*, *Placopsis*, *Placynthiella*, *Rimularia* and *Trapeliopsis*; Kantvilas *et al.* 2014; Resl *et al.* 2015; Orange 2018) were also added to the dataset. The genera *Lambiella* and *Rimularia* were chosen as an outgroup based on previous phylogenetic studies (Kantvilas *et al.* 2014; Resl *et al.* 2015). In total, the dataset included 150 specimens, 112 of them from the genus *Trapelia*.

Molecular: *DNA extractions, amplification and sequencing*

Two methods were used to generate ITS sequences (Table 1). For 17 taxa, a long-read amplicon sequencing approach as described in Gueidan *et al.* (2019) and Gueidan & Li (2022) was used. In brief, specimens were extracted using the Invisorb Spin Plant Mini Kit (Stratec Molecular, Berlin, Germany), ITS amplified with ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) tagged with a universal primer sequence, then reamplified with indexed universal primers. The amplicon library was prepared and sequenced on a Sequel I platform at Macrogen (Seoul, Republic of South Korea). For the other 27 taxa, genomic DNA was obtained using a protocol modified from Zolan & Pukkila (1986), as described in Gueidan *et al.* (2007). The primers ITS1F and ITS4 were used to amplify ITS. One micro-litre of a 1, 1/10, or 1/100 dilution of genomic DNA was added to the following PCR mix: 5 µl MyFi buffer (Bioline, London, UK), 1 µl of each primer (10 µM), 1 µl MyFi DNA polymerase, and water to a total volume of 25 µl. The PCR reactions were performed on a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany). The PCR program for ITS was as follows: 3 min at 95°C, followed by 35 cycles of the three steps 30 sec at 95°C (denaturation), 30 sec at 53°C (annealing), 90 sec at 72°C (extension), and a final extension time of 10 min at 72°C. PCR product clean-up and Sanger sequencing were carried out by Macrogen (Seoul, Republic of South Korea).

Molecular: *alignments and phylogenetic analyses*

Sanger sequences were edited using Sequencher v. 5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan, USA). PacBio sequences were edited using SMRT Tools v. 7.0.1 (Pacific Biosciences, Menlo Park, CA, USA) and denoised with DADA2 (Callahan *et al.* 2016), as described in Gueidan & Li (2022). GenBank and newly obtained sequences were manually aligned using Mesquite v. 3.61 (Maddison & Maddison 2011), and ambiguously aligned regions were manually delimited and excluded as in Lutzoni *et al.* (2000). jModelTest2 v. 2.1.10 (Darriba *et al.* 2012) was used to infer the substitution model as implemented on the CIPRES Web Portal (http://www.phylo.org; Miller *et al.* 2010). The dataset was then analyzed using a Bayesian approach with MrBayes v. 3.2.6 (Ronquist *et al.* 2011), as implemented on the CIPRES Web Portal. Two analyses of four chains were run for 5 million generations and trees were sampled every 500 generations. For the two runs, parameter convergence and required burn-in sample were checked using Tracer v. 1.6 (Rambaut *et al.* 2014). The remaining trees were used to estimate the posterior probabilities with the "compute consensus" command in PAUP* (Swofford 2002). The consensus tree was computed with the sumt command in

MrBayes and visualized in PAUP*. Additional support values were obtained from a tree search and a RAxML fast bootstrap analysis of 1,000 pseudoreplicates using the CIPRES Web Portal (RAxML v. 8.2.12; Stamatakis *et al.* 2005, 2008). The tree figure was edited with Illustrator v. 26.0.3 (Adobe, San Jose, CA, USA).

Results and discussion

Although the overall thallus morphology of the Australian *Trapelia* species was highly variable, the distinctly squamulose species (*T. crystallifera* and *T. pruinosa*) could be reliably distinguished from the remaining crustose and areolate taxa. The most reliable traditional taxonomic character was thallus chemistry, namely gyrophoric acid (major) versus 5-*O*-methylhiascic acid (major). Average ascospore size was also a useful distinguishing character, but the presence or absence of calcium oxalate in the thallus and of surface pruina proved of no consequence.

For the molecular analysis, the ITS alignment had a total of 1,155 characters. After introns and ambiguous regions were excluded, 459 characters remained, including 273 distinct alignment patterns. The proportion of missing data in the alignment was 10.48%. For the Bayesian analysis, parameters and tree topology rapidly converged, and a burn-in of 10% of the trees was removed from the tree sample. The Bayesian consensus tree is shown in Figure 1, with posterior probabilities (PP). Bootstrap support (BS) obtained with RAxML are also reported on the tree. The resulting tree topology does not disagree with Resl *et al.* (2015), with the clade including *Placopsis*, *Placynthiella*, *Trapelia* and *Trapeliopsis* supported as monophyletic (98% PP, 88% BS). However, due to our limited data (ITS only), the relationships among the four genera are not supported (Fig. 1). *Placynthiella* is supported as monophyletic (100% PP and BS), and appears as sister to the three other genera, but with low support (100% PP, 73% BS). *Trapeliopsis* and *Placopsis* are both resolved as monophyletic (99% PP and 63% BS, and 100% PP and BS, respectively), but they are nested within *Trapelia*, although with no support.

Within *Trapelia*, the placement and monophyly of species treated in Orange (2018) were confirmed (Fig. 1). This included the non-Australian species *T. collaris*, *T. corticola*, *T. elacista*, *T. glebulosa*, *T. involuta*, *T. obtegens*, *T. sitiens*, *T. stipitata* and *T. tristis*. The two newly sampled Australian species *T. concentrica* and *T. crystallifera* are also supported as monophyletic (100% PP and 95% BS, and 100% PP and BS, respectively). The cosmopolitan and generitype species *T. coarctata* is also monophyletic (99% PP, 85% BS), but none of the Australian material sampled corresponded to that taxon. Moreover, no Australian material of *T. macrospora* could be sourced, but the sister relationship between a specimen from New Zealand and *T. sitiens* is confirmed (Orange 2018). As in Orange (2018), specimens of *T. placodioides* and *T. thieleana* clustered in a single clade, although with low support (100% PP, 68% BS). No clear delimitation can be drawn between those two species based on ITS. Similarly, based on ITS, no clear delimitation can be drawn between the Australian species *T. terrestris* and *T. atrocarpa* (including three European specimens of *T. elacista*), nor between *T. pruinosa* and *T. rosettiformis*. The Australian species *T. lilacea* is well supported as sister to the clade formed of *T. pruinosa* and *T. rosettiformis* (100% PP, 97% BS). Australian specimens of the species *T. calvariana* cluster together with unidentified specimens of *Trapelia* from the Falklands Islands (Orange 22381, 23379, 23172), although with no support.

Based on these phylogenetic results, morpho-anatomical observations were carried out on Australian material. Due to absence of unambiguous diagnostic characters for several species, four synonymies were proposed (see Taxonomy section). As mentioned above, the overall thallus morphology of the Australian *Trapelia* species is highly variable, both within the distinctly squamulose species (*T. crystallifera* [Fig. 5] and *T. pruinosa* [Figs 10 and 11]) as well as among the crustose and areolate taxa. The most reliable traditional taxonomic character was thallus chemistry, namely the occurrence of gyrophoric acid (major) or 5-*O*-methylhiascic acid (major), as well as the average ascospore size. More particularly, the squamulose *T. pruinosa* and *T. rosettiformis* were found to be synonymous even though the type of the former has a dense, coarsely crystalline surface pruina and relatively small, more or less flat squamules to 1.2 mm wide, whereas the latter has an epruinose upper surface and large, elevated, rosette-forming squamules up to 4 mm wide (compare Figs 10 and 11). The crustose or microareolate *T. atrocarpa* and *T. terrestris* were primarily differentiated by the presence of large quantities of calcium oxalate in the former. However, molecular studies confirmed that this was of no taxonomic significance. The morphological variation within *T. placodioides* was particularly variable, from subsquamulose-areolate and esorediate (as in *T. occidentalis*, Fig. 9), to continuous-crustose and esorediate or with sparse surface granules (as in *T. thieleana*) to continuous-crustose with definite soralia (Fig. 8).

A previous molecular taxonomic investigation of *Trapelia* (Orange 2018) had inferred, based on ITS, a highly supported clade for *Trapelia elacista* (100% maximum likelihood bootstrap). This clade was divided into two relatively well-supported groups, one of which contained, among others, the Welsh specimens Orange 23623, 22891 and 23456. Those two clades were considered as a single species, because no morphological and few phylogenetic divergences could be found (Orange 2018). In our phylogeny (Fig. 1), those three specimens cluster together with all specimens of *T. atrocarpa* and *T. terrestris* (now *T. atrocarpa*, because the two species are synonymized below), although with moderate support (72% BS). The close relationship between *T. elacista* and *T. atrocarpa* is supported by their chemistry (gyrophoric acid major) and their morphology (growth form, ascocarp development and ascospore size). Because those two species are phylogenetically distinct and have disjunct geographic distributions, they were not synonymized. Further work with broader taxon and gene sampling will be necessary to further test their delimitation.

Nine species of *Trapelia* are currently accepted from Australia, eight of which are represented in our tree (*T. atrocarpa*, *T. coarctata*, *T. concentrica*, *T. crystallifera*, *T. lilacea*, *T. macrospora*, *T. placodioides* and *T. pruinosa*). No sequences from *T. kosciuszkoensis* could be obtained as part of this study. Whether or not *T. coarctata* actually occurs in Australia remains to be determined; no recent collections of it were encountered in the present study. A key to the Australian *Trapelia* species is provided below.

Taxonomy

New synonyms

Trapelia atrocarpa Elix & P.M.McCarthy, *Australas. Lichenol.* **86**, 102 (2020)
Syn. nov. *Trapelia terrestris* Elix & P.M.McCarthy, *Australas. Lichenol.* **87**, 43 (2020)

Trapelia pruinosa Elix & P.M.McCarthy, *Australas. Lichenol.* **86**, 105 (2020)
Syn. nov. *Trapelia rosettiformis* Elix & P.M.McCarthy, *Australas. Lichenol.* **87**, 41 (2020)

Trapelia placodioides Coppins & P.James, *Lichenologist* **16**, 257 (1984)
Syn. nov. *Trapelia thieleana* Kantvilas, Lumbsch & Elix, *Austral. Syst. Bot.* **27**, 400 (2015)
Syn. nov. *Trapelia occidentalis* Elix, in J.A. Elix & P.M. McCarthy, *Australas. Lichenol.* **87**, 40 (2020)

Key to *Trapelia* in Australia

1 Soralia present 2
1: Soralia absent 3

2 Thallus squamulose or subsquamulose .... **T. pruinosa**[1]
2: Thallus crustose, coherent, continuous .... **T. placodioides**

3 Thallus squamulose 4
3: Thallus crustose; surface continuous, rimose or areolate 5

4 Thallus containing 5-*O*-methylhiascic acid (major) .... **T. pruinosa**
4: Thallus containing gyrophoric acid (major) .... **T. crystallifera**

5 Ascospores 17–34 × 12–20 µm; apothecia persistently immersed; alpine 6
5: Ascospores 9–30 × 4–15 µm; apothecia immersed at first, then adnate to sessile ... 7

6 Ascospores 25–34 µm long; hypothecium 150–180 µm thick; gyrophoric acid (major); Tasmania .... **T. macrospora**
6: Ascospores 17–30 µm long; hypothecium 70–100 µm thick; 5-*O*-methylhiascic acid (major); N.S.W. .... **T. kosciuszkoensis**

7: Thallus surface scabrid, mealy, coarsely crystalline; disc pruinose at least in part ... . **T. concentrica**
7: Thallus surface smooth to rugulose, not crystalline; disc epruinose 8

8 Thallus containing 5-*O*-methylhiascic acid (major).. 9
8: Thallus containing gyrophoric acid (major) 10

9 Ascospores 16–23 × 9–15 µm; conidia 10–17 µm long; thallus containing additional 5-methoxylecanoric acid .... **T. lilacea**
9: Ascospores 11–19 × 5–11 µm; conidia 13–30 µm long; thallus lacking 5-methoxylecanoric acid .... **T. calvariana**

10 Thallus micro-areolate; ascospores 15–30 µm long........................... **T. atrocarpa**
10: Thallus forming extensive patches; ascospores 14–21 µm long.... **T. placodioides**

[1] in older, markedly pruinose specimens, the pruina often erodes, and such specimens can appear sorediate.

Acknowledgements

We thank Dr Chris Cargill and Ms Judith Curnow of CANB for their kind cooperation in providing us with ready access to key collections. Ms Lan Li is also thanked for her valuable support with the molecular work. The molecular work was partly funded by a BushBlitz Taxonomy Research grant to CG.

References

Callahan, BJ; McMurdie, PJ; Rosen, MJ; Han, AW; Johnson, AJA; Holmes, SP (2016): DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**, 581–583.
Darriba, D; Taboada, GL; Doallo, R; Posada, D (2012): jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.
Elix, JA (2022): *A Catalogue of Standardized Chromatographic Data and Biosynthetic Relationships for Lichen Substances*, 6th edn. Published by the author, Canberra.
Elix, JA; McCarthy, PM (2019): *Trapelia concentrica* (lichenized Ascomycota, Trapeliaceae), a new species from south-eastern Australia, with a key to the genus in Australia. *Australasian Lichenology* **85**, 46–50.
Elix, JA; McCarthy, PM (2020a): Three new species of *Trapelia* (lichenized Ascomycota, Trapeliaceae) from eastern Australia. *Australasian Lichenology* **86**, 102–108.
Elix, JA; McCarthy PM (2020b): Three new species and a new record of *Trapelia* (lichenized Ascomycota, Trapeliaceae) from Australia. *Australasian Lichenology* **87**, 40–47.
Gardes, M; Bruns, TD (1993): ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**, 113–118.

Gueidan, C; Roux, C; Lutzoni, F (2007): Using a multigene phylogenetic analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). *Mycological Research* **111**, 1145–1168.
Gueidan, C; Elix, JA; McCarthy, PM; Roux, C; Mallen-Cooper, M; Kantvilas, G (2019): PacBio amplicon sequencing for metabarcoding of mixed DNA samples from lichen herbarium specimens. *MycoKeys* **53**, 73–91.
Gueidan, C; Li, L (2022): A long-read amplicon approach to scaling up the metabarcoding of lichen herbarium specimens. *MycoKeys* **86**, 195–212.
Kantvilas, G; Elix, JA (2007): Additions to the lichen family Agyriaceae Corda from Tasmania. *Bibliotheca Lichenologica* **95**, 317–333.
Kantvilas, G; Leavitt, SD; Elix, JA; Lumbsch, HT (2014): Additions to the genus *Trapelia* (Trapeliaceae: lichenised Ascomycetes). *Australian Systematic Botany* **27**, 395–402.
Lutzoni, F; Wagner, P; Reeb, V; Zoller, S (2000): Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49**, 628–651.
McCarthy, PM (2020): *Checklist of the Lichens of Australia and its Island Territories*. Australian Biological Resources Study, Canberra. Version 1 March 2020. http://www.anbg.gov.au/abrs/lichenlist/introduction.html
Maddison, WP; Maddison, DR (2011): *Mesquite*: *a modular system for evolutionary analysis*, version 2.75.
Miller, MA; Pfeiffer, W; Schwartz, T (2010): Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, pp. 1–8.
Orange, A (2018): A new species level taxonomy for *Trapelia* (Trapeliaceae, Ostropomycetidae) with special reference to Great Britain and the Falkland Islands. *Lichenologist* **50**, 3–42.
Rambaut, A; Suchard, MA; Xie, D; Drummond, AJ (2014): *Tracer v1.7.1*. Available from: http://beast.bio.ed.ac.uk/Tracer.
Resl, P; Schneider, K; Westberg, M; Printzen, C; Palice, Z; Thor, G; Mayrhofer, H; Spribille, T (2015): Diagnostics for a troubled backbone: testing topological hypotheses of trapelioid lichenized fungi in a large-scale phylogeny of Ostropomycetidae (Lecanoromycetes). *Fungal Diversity* **73**, 239–258.
Ronquist, F; Huelsenbeck, J; Teslenko, M (2011): MrBayes version 3.2: Tutorials and Model Summaries, 183 pp. Available from: http://mrbayes.sourceforge.net/mb3.2_manual.pdf
Schneider, K; Resl, P; Spribille, T (2016): Escape from the cryptic species trap: lichen evolution on both sides of a cyanobacterial acquisition event. *Molecular Ecology* **25**, 3453–3468.
Stamatakis, A; Ludwig, T; Meier, H (2005): RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* **21**, 456–463.
Stamatakis, A; Hoover, P; Rougemont, J (2008): A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* **75**, 758–771.
Swofford, DL (2002): *PAUP* version 4.0 b10. Phylogenetic analysis using parsimony* (**and other methods*). Sinauer, Sunderland, MA.
White, TJ; Bruns, T; Lee, S; Taylor, JW (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in Innis, MA; Gelfand, DH; Sninsky, JJ; White, TJ (eds), *PCR Protocols: A guide to methods and applications*, pp. 315–322. Academic Press, New York.
Zolan, ME; Pukkila, PJ (1986): Inheritance of DNA methylation in *Coprinus cinereus*. *Molecular and Cellular Biology* **6**, 195–200.

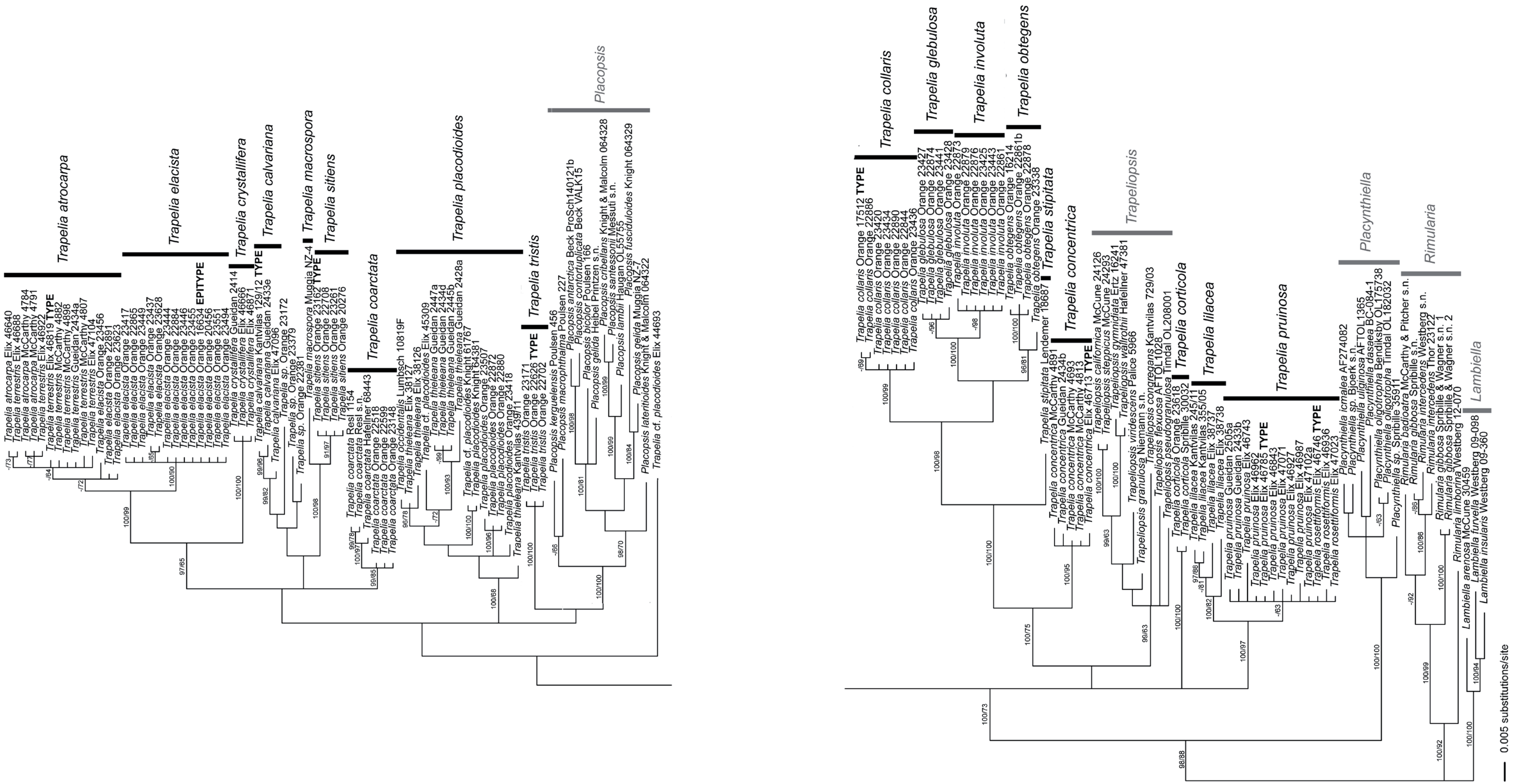

Figure 1. Phylogenetic relationships within the family Trapeliaceae, with a focus on the genus *Trapelia* and Australian *Trapelia* species. Bayesian consensus tree with posterior probabilities and bootstrap values (PP/BS) shown above or below the branches when greater than 95% and 60%, respectively. *Lambiella* and *Rimularia* were used as outgroups. Type specimens of *Trapelia* species are indicated in the tree.

	Species	Collection number, Herbarium	Collection site	ITS accession
1	*Trapelia atrocarpa*	Elix 46640 (CANB)	ACT, Australia	**OM955152***
2	*Trapelia atrocarpa*	McCarthy 4784 (CANB)	ACT, Australia	**OM955153**
3	*Trapelia atrocarpa*	McCarthy 4791 (CANB)	ACT, Australia	**OM955154**
4	*Trapelia calvariana*	Kantvilas 129/12 (HO) - TYPE	TAS, Australia	KU672613
5	*Trapelia* cf. *calvariana*	Gueidan 2433e (CANB)	ACT, Australia	**OM955156***
6	*Trapelia* cf. *calvariana*	Elix 47096 (CANB)	ACT, Australia	**OM955155**
7	*Trapelia coarctata*	Resl 1154 (GZU)	Austria	KR017098
8	*Trapelia coarctata*	Resl s.n. (cultured mycobiont: GZU)	Austria	KR017092
9	*Trapelia coarctata*	Hafellner 68443 (GZU)	Austria	KR017097
10	*Trapelia coarctata*	Orange 22518 (NMW)	Falkland Islands	KX961313
11	*Trapelia coarctata*	Orange 22599 (NMW)	Falkland Islands	KX961314
12	*Trapelia coarctata*	Orange 23148 (NMW)	Falkland Islands	KX961335
13	*Trapelia collaris*	Orange 17512 (NMW) - TYPE	Wales	KX961309
14	*Trapelia collaris*	Orange 23420 (NMW)	Wales	KX961344
15	*Trapelia collaris*	Orange 23434 (NMW)	Wales	KX961350
16	*Trapelia collaris*	Orange 22886 (NMW)	Wales	KX961331
17	*Trapelia collaris*	Orange 22890 (NMW)	Wales	KX961333
18	*Trapelia collaris*	Orange 22844 (NMW)	Wales	KX961316
19	*Trapelia collaris*	Orange 23436 (NMW)	Wales	KX961351
20	*Trapelia concentrica*	McCarthy 4891 (CANB)	NSW, Australia	**OM955157***
21	*Trapelia concentrica*	Gueidan 2434b (CANB)	ACT, Australia	**OM955158***
22	*Trapelia concentrica*	McCarthy 4693 (CANB)	NSW, Australia	**OM955159**
23	*Trapelia concentrica*	McCarthy 4813 (CANB)	ACT, Australia	**OM955160**
24	*Trapelia concentrica*	Elix 46713 (CANB) - TYPE	ACT, Australia	**OM955161**
25	*Trapelia corticola*	Orange 23618 (NMW)	Wales	KY797788
26	*Trapelia corticola*	Spribille 30032 (GZU)	Idaho, USA	KR017135
27	*Trapelia crystallifera*	Gueidan 2414 (CANB)	ACT, Australia	**OM955162***
28	*Trapelia crystallifera*	Elix 46666 (CANB)	ACT, Australia	**OM955163***
29	*Trapelia crystallifera*	Elix 46871 (CANB)	NSW, Australia	**OM955164**
30	*Trapelia elacista*	Orange 23417 (NMW)	Wales	KX961342
31	*Trapelia elacista*	Orange 22865 (NMW)	Wales	KX961319
32	*Trapelia elacista*	Orange 23449 (NMW)	Wales	KX961357
33	*Trapelia elacista*	Orange 23437 (NMW)	Wales	KX961352
34	*Trapelia elacista*	Orange 23444 (NMW)	Wales	KX961355
35	*Trapelia elacista*	Orange 22884 (NMW)	Wales	KX961330
36	*Trapelia elacista*	Orange 23446 (NMW)	Wales	KX961356
37	*Trapelia elacista*	Orange 23455 (NMW)	Wales	KX961360
38	*Trapelia elacista*	Orange 16634 (NMW) - EPITYPE	England	KX961308
39	*Trapelia elacista*	Orange 20456 (NMW)	Wales	KX961311
40	*Trapelia elacista*	Orange 23551 (NMW)	Germany	KX961382
41	*Trapelia elacista*	Orange 23628 (NMW)	Wales	KY797791
42	*Trapelia elacista*	Orange 23456 (NMW)	Wales	KX961361
43	*Trapelia elacista*	Orange 22891 (NMW)	Wales	KX961334
44	*Trapelia elacista*	Orange 23494 (NMW)	England	KX961372
45	*Trapelia elacista*	Orange 23623 (NMW)	Wales	KY797790
46	*Trapelia glebulosa*	Orange 23427 (NMW)	Wales	KX961348
47	*Trapelia glebulosa*	Orange 22874 (NMW)	Wales	KX961322
48	*Trapelia glebulosa*	Orange 23441 (NMW)	Wales	KX961353
49	*Trapelia glebulosa*	Orange 23428 (NMW)	Wales	KX961349
50	*Trapelia involuta*	Orange 22879 (NMW)	Wales	KX961326
51	*Trapelia involuta*	Orange 22876 (NMW)	Wales	KX961324
52	*Trapelia involuta*	Orange 23425 (NMW)	Wales	KX961347
53	*Trapelia involuta*	Orange 22873 (NMW)	Wales	KX961321
54	*Trapelia involuta*	Orange 23443 (NMW)	Wales	KX961354
55	*Trapelia involuta*	Orange 22861 (NMW)	Wales	KX961318
56	*Trapelia lilacea*	Kantvilas 245/11 (HO)	TAS, Australia	KU672611
57	*Trapelia lilacea*	Kantvilas 355/05 (HO)	TAS, Australia	KU672612
58	*Trapelia lilacea*	Elix 38737 (CANB)	WA, Australia	**OM955165***
59	*Trapelia lilacea*	Elix 38738 (CANB)	WA, Australia	**OM955166***
60	*Trapelia macrospora*	Muggia NZ-4 (GZU)	North Island, New Zealand	KR017102
61	*Trapelia obtegens*	Orange 16214 (NMW)	England	KX961306
62	*Trapelia obtegens*	Orange 22878 (NMW)	Wales	KX961325
63	*Trapelia obtegens*	Orange 22861b (NMW)	Wales	KX961317
64	*Trapelia obtegens*	Orange 23338 (NMW)	Wales	KX961339
65	*Trapelia occidentalis*	Lumbsch 10819F (CANB)	WA, Australia	**OM955167**
66	*Trapelia placodioides*	Orange 23507 (NMW)	England	KX961374
67	*Trapelia placodioides*	Orange 22872 (NMW)	Wales	KX961320
68	*Trapelia placodioides*	Orange 22880 (NMW)	Wales	KX961327
69	*Trapelia placodioides*	Orange 23418 (NMW)	Wales	KX961343
70	*Trapelia placodioides*	Knight 64381 (OTA)	South Island, New Zealand	KU844758
71	*Trapelia* cf. *placodioides*	Knight 61767 (OTA)	South Island, New Zealand	KU672615
72	*Trapelia* cf. *placodioides*	Elix 44693 (CANB)	QLD, Australia	**OM955168**
73	*Trapelia* cf. *placodioides*	Elix 45309 (CANB)	NSW, Australia	**OM955169**
74	*Trapelia pruinosa*	Gueidan 2505a (CANB)	ACT, Australia	**OM955170***
75	*Trapelia pruinosa*	Gueidan 2433b (CANB)	ACT, Australia	**OM955171***
76	*Trapelia pruinosa*	Elix 46743 (CANB)	NSW, Australia	**OM955172***
77	*Trapelia pruinosa*	Elix 46962 (CANB)	ACT, Australia	**OM955173**
78	*Trapelia pruinosa*	Elix 46785 (CANB) - TYPE	ACT, Australia	**OM955174**
79	*Trapelia pruinosa*	Elix 46843 (CANB)	NSW, Australia	**OM955175**
80	*Trapelia pruinosa*	Elix 47071 (CANB)	NSW, Australia	**OM955176**
81	*Trapelia pruinosa*	Elix 46927 (CANB)	ACT, Australia	**OM955177**
82	*Trapelia pruinosa*	Elix 46987 (CANB)	ACT, Australia	**OM955178**
83	*Trapelia pruinosa*	Elix 47102a (CANB)	ACT, Australia	**OM955179**
84	*Trapelia rosettiformis*	Elix 46746 (CANB) - TYPE	NSW, Australia	**OM955180**
85	*Trapelia rosettiformis*	Elix 46936 (CANB)	ACT, Australia	**OM955181**
86	*Trapelia rosettiformis*	Elix 47023 (CANB)	ACT, Australia	**OM955182**
87	*Trapelia sitiens*	Orange 23162 (NMW) - TYPE	Falkland Islands	KX961336
88	*Trapelia sitiens*	Orange 22708 (NMW)	Falkland Islands	KY800909
89	*Trapelia sitiens*	Orange 23261 (NMW)	Falkland Islands	KY800910
90	*Trapelia sitiens*	Orange 20276 (NMW)	Falkland Islands	KX961310
91	*Trapelia stipitata*	Lendemer 18687 (GZU)	Pennsylvania, USA	KR017096
92	*Trapelia terrestris*	Elix 46688 (CANB)	ACT, Australia	**OM955183**
93	*Trapelia terrestris*	Elix 46922 (CANB)	NSW, Australia	**OM955184**
94	*Trapelia terrestris*	Elix 46819 (CANB) - TYPE	ACT, Australia	**OM955185**
95	*Trapelia terrestris*	McCarthy 4882 (CANB)	ACT, Australia	**OM955186**
96	*Trapelia terrestris*	McCarthy 4898 (CANB)	NSW, Australia	**OM955187**
97	*Trapelia terrestris*	Gueidan 2434a (CANB)	ACT, Australia	**OM955188***
98	*Trapelia terrestris*	McCarthy 4807 (CANB)	ACT, Australia	**OM955189***
99	*Trapelia terrestris*	Elix 47104 (CANB)	ACT, Australia	**OM955190**
100	*Trapelia thieleana*	Elix 38127 (CANB)	WA, Australia	KU672616
101	*Trapelia thieleana*	Kantvilas 439/11 (HO) - TYPE	WA, Australia	KU672614
102	*Trapelia thieleana*	Gueidan 2447a (CANB)	ACT, Australia	**OM955191***
103	*Trapelia thieleana*	Gueidan 2434d (CANB)	ACT, Australia	**OM955192***
104	*Trapelia thieleana*	Gueidan 2428a (CANB)	ACT, Australia	**OM955193***
105	*Trapelia thieleana*	Gueidan 2445b (CANB)	ACT, Australia	**OM955194***
106	*Trapelia thieleana*	Elix 38126 (CANB)	WA, Australia	**OM955195**
107	*Trapelia tristis*	Orange 23171 (NMW)	Falkland Islands	KX961337

Table 1. List of taxa included in the molecular phylogeny, with collection numbers, herbaria, countries of collection and ITS accession numbers. Accession numbers in bold correspond to sequences generated as part of this study. Sequences generated with the long read amplicon sequencing protocol are indicated by a star. These sequences correspond to material collected and/or sequenced as part of a BushBlitz Taxonomy Research project.

108	*Trapelia tristis*	Orange 22626 (NMW) - TYPE	Falkland Islands	KX961315
109	*Trapelia tristis*	Orange 22702 (NMW)	Falkland Islands	KY800908
110	*Trapelia sp.*	Orange 23379 (NMW)	Falkland Islands	KX961341
111	*Trapelia sp.*	Orange 23172 (NMW)	Falkland Islands	KX961338
112	*Trapelia sp.*	Orange 22381 (NMW)	Falkland Islands	KX961312
113	*Placopsis kerguelensis*	Sochting 9398 (C)	Crozet Islands	AY212814
114	*Placopsis antarctica*	Beck ProSch140121b (M)	Antarctica	MH670330
115	*Placopsis bicolor*	Poulsen 166 (C)	Kerguelen Islands	AY212817
116	*Placopsis contortuplicata*	Beck VALK15/01aIII (M)	Antarctica	MH670337
117	*Placopsis cribellans*	Knight & Malcolm 064328 (OTA)	South Island, New Zealand	KU844762
118	*Placopsis fusciduloides*	Knight 064329 (OTA)	South Island, New Zealand	KU844756
119	*Placopsis gelida*	Heibel & Printzen s.n. (ESS)	Portugal	AF274091
120	*Placopsis gelida*	Muggia NZ-7 (GZU)	South Island, New Zealand	KR017055
121	*Placopsis lambii*	Haugan OL55755 (O)	Norway	AY212819
122	*Placopsis lateritioides*	Knight & Malcolm 064322 (OTA)	South Island, New Zealand	KU844740
123	*Placopsis macrophthalma*	Poulsen 227 (C)	Kerguelen Islands	AY212820
124	*Placopsis santessonii*	Messuti s.n. (hb. Lumbsch)	Chile	AY212826
125	*Placynthiella dasaea*	BC-084-1 (WSL)	Switzerland	KX132967
126	*Placynthiella icmalea*	Lumbsch 12059a (hb. Lumbsch)	Germany	AF274082
127	*Placynthiella oligotropha*	Timdal OL182032 (O)	Norway	MK811853
128	*Placynthiella oligotropha*	Bendiksby OL175738 (O)	Norway	MK812378
129	*Placynthiella uliginosa*	AFTOL-ID 1365 (DUKE)	*not available*	HQ650633
130	*Placynthiella sp.*	Bjoerk s.n. (GZU)	Canada	MH636005
131	*Placynthiella sp.*	Spribille 35911 (GZU)	Montana, USA	MH636004
132	*Trapeliopsis californica*	McCune 24126 (OSC)	Oregon, USA	AF353567
133	*Trapeliopsis congregans*	Kantvilas 729/03 (FR)	TAS, Australia	MH636006
134	*Trapeliopsis flexuosa*	AFTOL-ID 1028 (DUKE)	*not available*	HQ650634
135	*Trapeliopsis granulosa*	Niemann s.n. (ESS)	Sweden	AF274087
136	*Trapeliopsis gymnidiata*	Ertz 16241 (BR)	Canary Islands	MN483160
137	*Trapeliopsis pseudogranulosa*	Timdal OL208001 (O)	Norway	MK812568
138	*Trapeliopsis steppicus*	McCune 24293 (OSC)	Washington, USA	AF353574
139	*Trapeliopsis viridescens*	Palice 5966 (hb. Palice)	Czech Republic	KR017155
140	*Trapeliopsis wallrothii*	Hafellner 47381 (GZU)	Canary Islands	AF353575
141	*Rimularia badioatra*	McCarthy & Pitcher s.n. (MSC)	Newfoundland, Canada	KR017120
142	*Rimularia gibbosa*	Spribille s.n. (GZU)	Austria	KR017129
143	*Rimularia gibbosa*	Spribille & Wagner s.n. 1 (GZU)	Montana, USA	KR017111
144	*Rimularia gibbosa*	Spribille & Wagner s.n. 2 (GZU)	Montana, USA	KR017107
145	*Rimularia intercedens*	Thor 23122 (UPS)	Sweden	KR017134
146	*Rimularia intercedens*	Westberg s.n. (S)	Austria	KR017119
147	*Rimularia limborina*	Westberg 12-070 (S)	Norway	KR017108
148	*Lambiella arenosa*	McCune 30459 (OSC)	Oregon, USA	MF464549
149	*Lambiella furvella*	Westberg 09-098 (S)	Sweden	KR017118
150	*Lambiella insularis*	Westberg 09-360 (S)	Sweden	KR017101

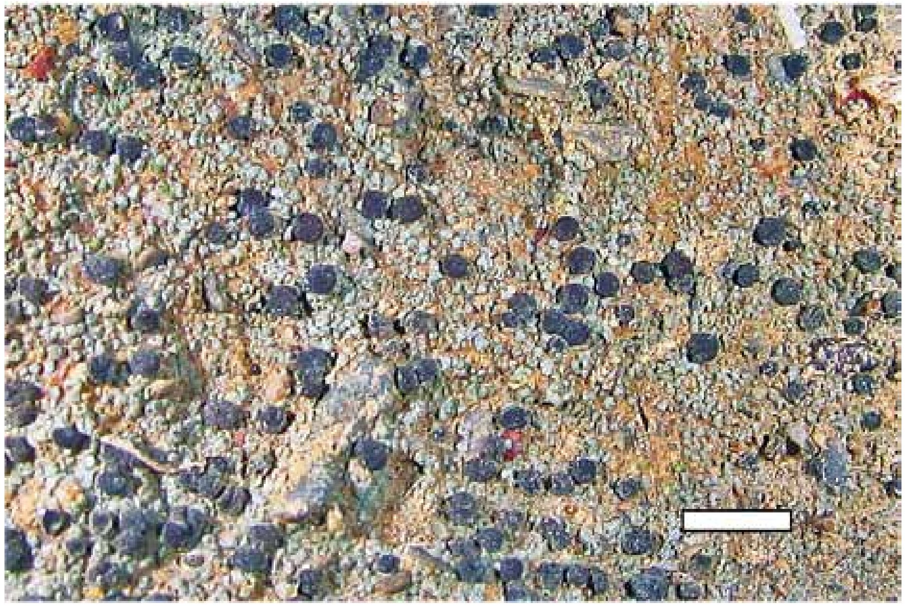

Figure 2. *Trapelia atrocarpa* (holotype of *T. terrestris* in CANB). Scale: 2 mm.

Figure 3. *Trapelia calvariana* (*Elix 47106* in CANB). Scale: 2 mm.

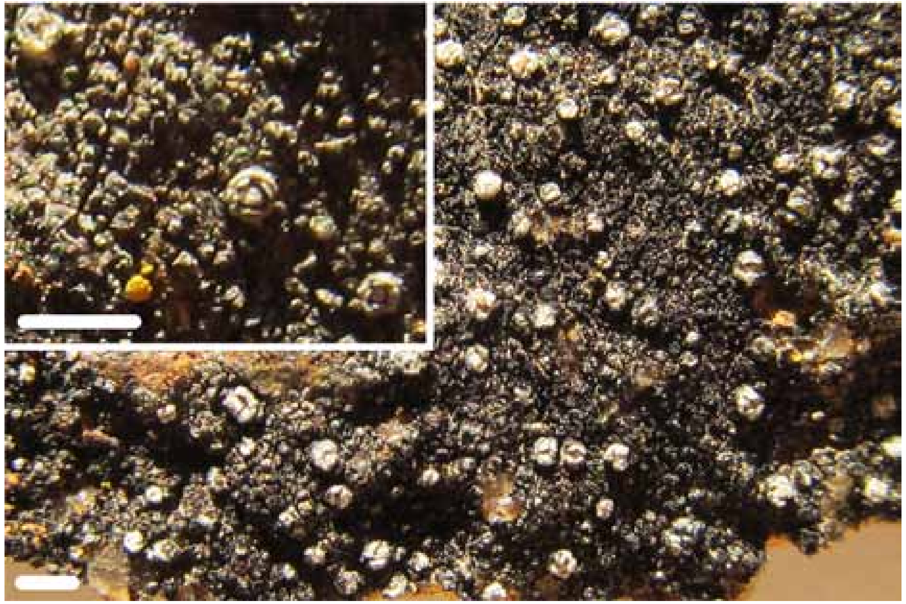

Figure 4. *Trapelia concentrica* (*McCarthy 4693* in CANB). Scale: 1 mm (2 mm in close-up).

Figure 5. *Trapelia crystallifera* (*Elix 47058* in CANB). Scale: 2 mm.

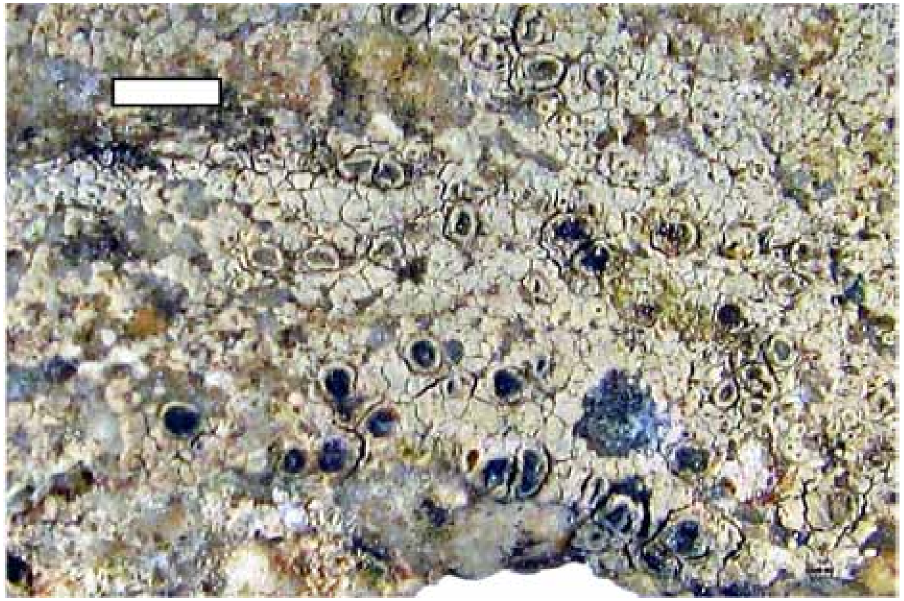

Figure 6. *Trapelia kosciuszkoensis* (holotype in CANB). Scale: 2 mm.

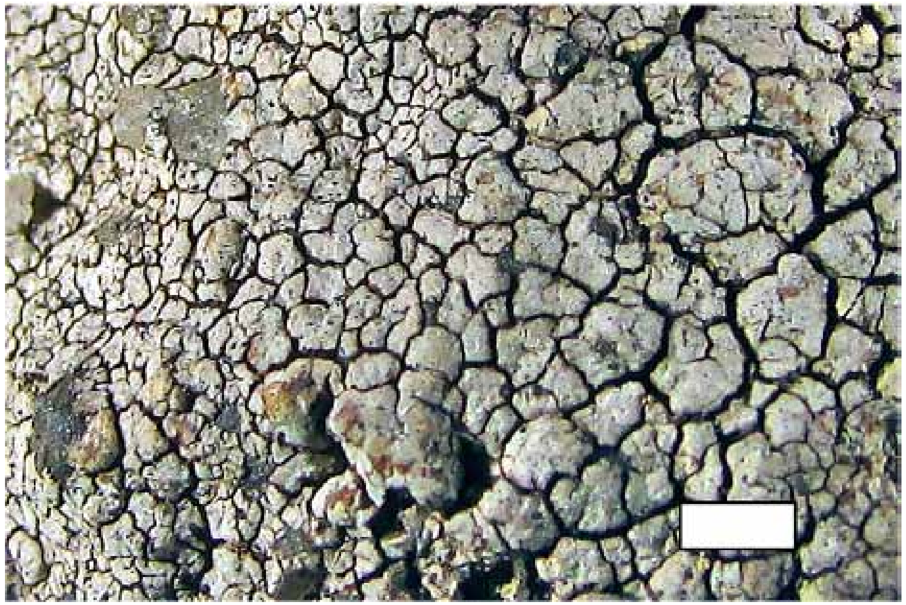

Figure 7. *Trapelia lilaceae* (*Elix 3221* in CANB). Scale: 2 mm.

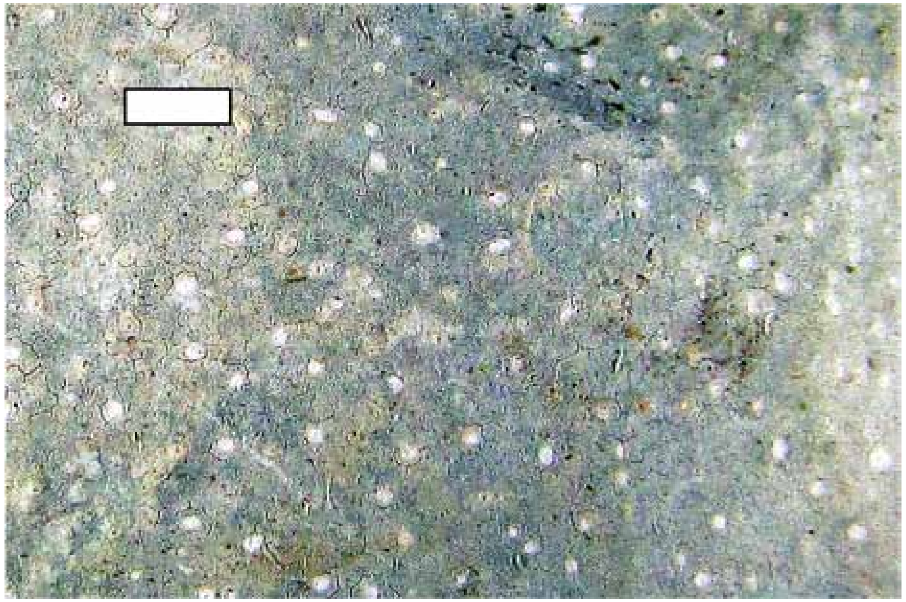

Figure 8. *Trapelia placodioides*, sorediate form (*Elix 45309* in CANB). Scale: 1 mm.

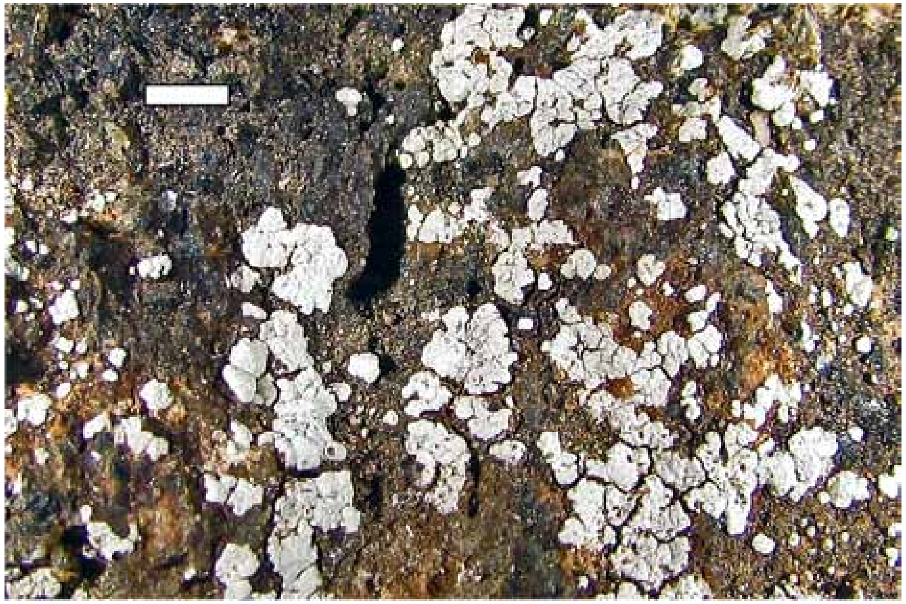

Figure 9. *Trapelia placodioides*, areolate form (*Lumbsch 10819F* in CANB). Scale: 2 mm.

Figure 10. *Trapelia pruinosa* (holotype of *T. rosettiformis* in CANB). Scale: 1 mm.

Figure 11. *Trapelia pruinosa* (holotype in CANB). Scale: 2 mm.