



Standard Paper

Phylogenetic evidence for an expanded circumscription of *Gabura* (*Arctomiaceae*)

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Abstract

Since the advent of molecular taxonomy, numerous lichen-forming fungi with homoiomerous thalli initially classified in the family *Collemataceae* Zenker have been transferred to other families, highlighting the extent of morphological convergence within Lecanoromycetes O. E. Erikss. & Winka. While the higher level classification of these fungi might be clarified by such transfers, numerous specific and generic classifications remain to be addressed. We examined the relationships within the broadly circumscribed genus *Arctomia* Th. Fr., which has been the recipient of several transfers from *Collemataceae*. We demonstrated that *Arctomia insignis* (P. M. Jørg. & Tønberg) Ertz does not belong to *Arctomia* s. str. but forms a strong monophyletic group with *Gabura fascicularis* (L.) P. M. Jørg. We also confirmed that *Arctomia borbonica* Magain & Sérusiaux and the closely related *Arctomia insignis* represent two species. We formally transferred *A. insignis* and *A. borbonica* to the genus *Gabura* Adans. and introduced two new combinations: *Gabura insignis* and *Gabura borbonica*. We reported *Gabura insignis* from Europe (Scotland and Ireland) for the first time. While material from Europe and North America is genetically almost identical, specimens from Madagascar, South Africa and Reunion Island belong to three distinct phylogenetic lineages, all of which are present in the latter area and may represent distinct species. In its current circumscription, the genus *Gabura* may contain up to six species, whereas *Arctomia* s. str. includes only two species (*A. delicatula* Th. Fr. and *A. teretiuscula* P. M. Jørg.). The *Gabura insignis* group is shown to have an unexpectedly large, subcosmopolitan distribution. With the extended sampling from *Arctomiaceae* Th. Fr., the placement of *Steinera sorediata* P. James & Henssen in the genus *Steinera* Zahlbr. is confirmed and the presence of a new *Steinera* species from Chile is highlighted.

Key words: biogeography, Ireland, lichenized fungi, Scotland, species delimitation

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Introduction

Within the Lecanoromycetes O. E. Erikss. & Winka, the subclass Ostropomycetidae Reeb *et al.* has a high diversity of ascomata, thallus forms, symbiont preferences and ecological habits. Relationships among orders inside Ostropomycetidae have been difficult to resolve (e.g. Baloch *et al.* 2010; Resl *et al.* 2015). Phylogenetic revision of the Lecanoromycetes (Miadlikowska *et al.* 2014) placed the *Arctomiales* S. Stenroos, Miadl. & Lutzoni as a sister group to the *Ostropales* Nannf. with weak support, as part of a relatively well-supported clade which also contains *Trapeliales* B. P. Hodk. & Lendemer. Resl *et al.* (2015) performed a series of topological hypothesis testing and rejected the sister group relationship of *Arctomiales* and *Ostropales*. Instead, the authors recognized *Arctomiaceae* as part of the

order *Baeomycetales* Lumbsch, Huhndorf & Lutzoni based on its sister relationship to *Xylographaceae* Tuck. and *Trapeliaceae* M. Choisy ex Hertel. within a large clade that includes *Baeomycetaceae* Dumort. and *Hymeneliaceae* Körb. A recent revision of the orders and families within the Lecanoromycetes, based on a temporal banding approach (Kraichak *et al.* 2018), confirmed this topology and reduced the *Arctomiales* into synonymy with *Baeomycetales*.

Arctomiaceae is the only family of the Ostropomycetidae to associate with cyanobacteria of the order *Nostocales* T. Cavalier-Smith as their main photobionts (Miadlikowska *et al.* 2014). Morphology-based revisions classified most lichens with jelly-like, homoiomerous thalli in the family *Collemataceae* Zenker, until Wedin *et al.* (2009) showed that fungi forming such thalli represent a much broader phylogenetic diversity. Furthermore, it has been shown that the identity of the cyanobiont is likely to influence the thallus structure. For example, Magain & Sérusiaux (2014) demonstrated that in the genus *Fuscopannaria* P. M. Jørg., thalli of the *Kroswia* morphotype involve cyanobionts from a unique clade of *Nostoc* Vaucher ex Bornet & Flahault,

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which is phylogenetically distant from other *Fuscopannaria* photobionts. Recently, several species classified elsewhere have been transferred to *Arctomiaceae* based on molecular evidence, for example *Moelleropsis humida* (Kullh.) Coppins & P. M. Jørg. (currently *Gregorella humida* (Kullh.) Lumbsch; Lumbsch *et al.* 2005), *Collema fasciculare* (L.) F. H. Wigg (currently *Arctomia fascicularis* (L.) Otálora & Wedin; Otálora & Wedin 2013) and *Massalongia intricata* Øvstedal (currently *Steinera intricata* (Øvstedal) Ertz; Ertz *et al.* 2017). Spribille & Muggia (2013) also discussed the possibility of two other *Steinera* species, *S. polymorpha* P. James & Henssen and *S. sore-diata* P. James & Henssen, belonging in *Arctomiaceae*.

Otálora & Wedin (2013) recognized *Collema fasciculare* as a member of *Arctomiaceae* under the name of *Arctomia fascicularis*, despite the anatomical differences in comparison to other species of the genus but in the absence of convincing phylogenetic evidence for the recognition of a new genus. Later on, Jørgensen (2014) proposed to resurrect the name *Gabura* Adans. to accommodate *Collema fasciculare* as *Gabura fascicularis* (L.) P. M. Jørg. (spelt as ‘*fasciculare*'). Currently, the family *Arctomiaceae* contains five accepted genera: *Arctomia* Th. Fr., *Gabura*, *Gregorella* Lumbsch, *Steinera* Zahlbr. and *Wawea* Henssen & Kantvilas. Jørgensen & Palice (2016) suggested that the morphology of *Leptogium insigne* P. M. Jørg. & Tønsberg resembled that of *Gabura fascicularis*, and that this species might also be a member of the genus *Gabura*. However, the formal transfer was never completed due to lack of molecular evidence. *Leptogium insigne* was later transferred to the genus *Arctomia* as *A. insignis* P. M. Jørg. & Tønsberg (Ertz *et al.* 2017). The geographical distribution of *A. insignis* remains unclear because the species is often confused with the morphologically similar but unrelated *Leptogium brebissonii* Mont., which has been reported from Australia, Brazil, France, Montenegro, Portugal and Tenerife. In contrast, *A. insignis* is found on the west coast of North America, including Alaska, British Columbia, California, Oregon (the type locality) and Washington, where *L. brebissonii* is thought not to occur (Carlberg 2012). Another new species, *A. borbonica*, morphologically resembling *A. insignis*, was described from Reunion Island by Magain & Sérusiaux (2012). Later, despite the absence of genetic evidence, this species was synonymized with *A. insignis* (as *Leptogium insigne*) by Jørgensen & Palice (2016).

Since many recent studies revealed numerous cases of similar morphotypes representing assemblages of distinct, genetically isolated lineages (e.g. Lücking *et al.* 2014, 2017; Magain *et al.* 2017) and because cosmopolitan species seem to be the exception rather than the rule for lichen-forming fungi (e.g. Moncada *et al.* 2014; Lücking *et al.* 2017; Magain *et al.* 2017), the aims of this project were to 1) reconstruct a multilocus phylogeny for the family *Arctomiaceae*; 2) re-evaluate the circumscriptions of genera using monophyly as the grouping criterion; 3) assess the taxonomic validity of *A. borbonica* and *A. insignis*. We present a four-locus phylogeny of the family *Arctomiaceae*, with a focus on the genus *Gabura*. We assessed the generic affinity of *Arctomia insignis* based on expanded taxon sampling. We also tested whether *A. borbonica* represents a distinct lineage sister to *A. insignis*. In addition, we assessed the phylogenetic placement of recently acquired putative specimens of *A. insignis*, and other members of *Arctomiaceae*, in the context of the most comprehensive taxon sampling of the family to date.

Material and Methods

Taxon sampling and DNA sequence acquisition

We collected eight specimens morphologically identified as *Arctomia insignis* or *A. borbonica* from the USA (Oregon), Ireland, Scotland, Madagascar, Reunion Island and South Africa, as well as one specimen identified as *G. fascicularis* from Chile. For each of the nine specimens, we sequenced four loci of the mycobiont DNA: the entire ITS regions (internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2; ITS), the nuclear large subunit ribosomal RNA gene (nuLSU), the mitochondrial small subunit ribosomal RNA gene (mtSSU), and the protein-coding RNA polymerase II largest subunit (*RPB1*), following protocols described in Magain & Sérusiaux (2012, 2015). We also sequenced ITS of the type material of *A. borbonica* (N952), mtSSU and nuLSU for a specimen of *Steinera sore-diata* from the type locality, and ITS and mtSSU for two unidentified collections of *Steinera* from Chile. We generated 10 new ITS sequences, nine nuLSU, 12 mtSSU and six *RPB1*, giving a total of 37 new sequences (Table 1). We downloaded sequences from GenBank representing the diversity of *Baeomycetales* and selected orders from Ostropomycetidae (Kraichak *et al.* 2018) and added sequences of *Lecanora intumescens* (*Lecanorales* Nannf.) to root the tree.

We assembled single-locus datasets using Mesquite v. 3.1.1 (Maddison & Maddison 2016). Sequences were aligned using MAFFT v. 7.305b with default settings (Katoh *et al.* 2002). Substitutions and indels between sequences of the *A. insignis/A. borbonica* group were counted after alignment. After manual exclusion of ambiguously aligned characters, we combined them into two datasets: a three-locus dataset of 63 specimens containing nuLSU (903 characters), mtSSU (605 characters) and *RPB1* (654 characters); a four-locus dataset containing, in addition to the three loci, the ITS sequences (653 characters) of eight representatives of the *A. insignis/A. borbonica* group (Table 1). We also assembled a two-locus dataset (ITS and mtSSU with 486 and 790 characters, respectively) for the sequences from the three *Steinera* specimens along with all the sequences for ITS and mtSSU available in GenBank for this genus.

Phylogenetic and species discovery analyses

We delimited six subsets for the three-locus dataset (nuLSU, mtSSU, *RPB1* 1st, 2nd and 3rd codon positions, and the intron of *RPB1*) and seven subsets for the four-locus dataset (addition of ITS to the six subsets) and ran PartitionFinder2 v. 2.1.1 (Lanfear *et al.* 2016), searching all models with the greedy algorithm and BIC as the model selection criterion to determine the best partitioning of the data. For the four-locus dataset, four subsets were delimited: the first was composed of ITS and the intron of *RPB1* (GTR + G), the second of mtSSU (GTR + I+G), the third of nuLSU and *RPB1* 1st and 2nd codon positions (GTR + I+G), and the fourth of *RPB1* 3rd codon positions (GTR + I+G). The same four subsets were delimited in the three-locus dataset, except that ITS was not included.

We ran RAXML v. 8.2.9 (Stamatakis 2006) on the two datasets using the best partition schemes with the GTRGAMMA model, performing 1000 bootstrap pseudoreplicates and searching for the best maximum likelihood (ML) tree simultaneously. RAXML analysis with similar settings was completed on the two-locus *Steinera* dataset, which was partitioned according to the loci. In addition, we performed a MrBayes v. 3.2.6 analysis (Ronquist &

Table 1. Specimens of Ostropomycetidae used in the 3- and 4-locus datasets for phylogenetic and species discovery analyses. Voucher information (for *Arctomiaceae*) is provided and/or details of the source of the previously published sequences and GenBank reference numbers for each locus. Newly generated sequences are in bold. Details of the outgroup *Lecanora intumescens* (*Lecanorales*) are also provided.

Species	Voucher/source	nuITS	nuLSU	mtSSU	RPB1
1. Arctomiaceae					
<i>Arctomia delicatula</i> 1	Sweden, <i>Palice</i> s.n. 2002 (F), <i>Wedin et al. 2005</i> , <i>Lumbsch et al. 2007a</i>	–	AY853355	AY853307	DQ870929
<i>A. delicatula</i> 2	USA: Alaska, <i>Spribille</i> 37968 (GZU), <i>Resl et al. 2015</i>	–	KR017191	KR017335	KR017488
<i>A. interfixa</i>	USA: Washington, <i>Thomson</i> 15456 (WIS), <i>Lumbsch et al. 2005</i>	–	DQ007345	DQ007348	–
<i>A. teretiuscula</i>	China: Sichuan, <i>Lumbsch et al. 2005</i>	–	DQ007346	DQ007349	DQ870930
<i>Gabura borbonica</i>	Reunion Island, <i>Magain & Sérusiaux</i> N952 (LG)	MK571781	JX030030	JX030032	JX030034
<i>G. fascicularis</i>	Spain, <i>Aragon & Martinez</i> 3417 (MA), <i>Otålor & Wedin 2013</i>	–	–	KC118988	KC118991
<i>G. fascicularis</i>	Sweden, <i>Karström</i> 562 (UPS), <i>Otålor & Wedin 2013</i>	–	–	KC118987	KC118993
<i>G. fascicularis</i>	Spain, <i>Aragon</i> 1041/95 (MA), <i>Otålor & Wedin 2013</i>	–	–	KC118989	KC118992
<i>G. cf. fascicularis</i>	New Zealand, <i>Wedin</i> 8753 (S), <i>Otålor & Wedin 2013</i>	–	–	KC118990	KC118994
<i>G. cf. fascicularis</i>	Chile, <i>Magain</i> P6184 (DUKE)	–	MK571797	MK571786	MK570286
<i>G. insignis</i>	USA: Oregon, <i>McCune</i> 23460 (OSC)	–	EU166329	–	–
<i>G. insignis</i>	Ireland, <i>Sérusiaux</i> N3786 (LG)	MK571780	MK571798	MK571787	MK570287
<i>G. insignis</i>	UK: Scotland, <i>Sérusiaux</i> N4892 (LG)	–	MK571799	MK571788	–
<i>G. insignis</i>	USA: Oregon, <i>DiMeglio</i> 322 P6281 (OSC)	MK571777	MK571800	MK571789	MK570288
<i>G. insignis</i>	USA: Oregon, <i>DiMeglio</i> 321 P6282 (OSC)	MK571778	MK571801	MK571790	–
<i>G. insignis</i>	USA: Oregon, <i>DiMeglio</i> 320 P6283 (OSC)	MK571779	MK571802	MK571791	MK570289
<i>G. cf. insignis</i> 1	Reunion Island, <i>Magain & Sérusiaux</i> N1025 (LG)	–	JX030031	JX030033	JX030035
<i>G. cf. insignis</i> 1	Madagascar, <i>Sérusiaux</i> L6223 (LG)	MK571782	MK571803	MK571792	MK570290
<i>G. cf. insignis</i> 2	Reunion Island, <i>Sérusiaux</i> N1868 (LG)	MK571783	MK571804	MK571793	MK570291
<i>G. cf. insignis</i> 2	South Africa: Cape Town, <i>R. Vargas</i> s. n. (DNA N8003) (BOL)	MN560140	–	MN559070	–
<i>Gregorella humida</i>	Germany, <i>Lumbsch & Zimmermann</i> s. n. (F), <i>Wedin et al. 2005</i>	–	AY853378	AY853329	–
<i>Steinera intricata</i>	Subantarctic Is., <i>Smith</i> 10530, <i>Ertz et al. 2017</i>	–	MF893015	MF893055	MF893122
<i>S. isidiata</i>	Subantarctic Is., <i>Ertz</i> 20869 (BR), <i>Ertz et al. 2017</i>	–	MF893018	MF893018	MF893125
<i>S. latispora</i>	Subantarctic Is., <i>Ertz</i> 20591 (BR), <i>Ertz et al. 2017</i>	–	MF893021	MF893061	MF893127
<i>S. lebouvieri</i>	Subantarctic Is., <i>Ertz</i> 20867 (BR), <i>Ertz et al. 2017</i>	–	MF893024	MF893064	MF893130
<i>S. membranacea</i>	Subantarctic Is., <i>Ertz</i> 19126 (BR), <i>Ertz et al. 2017</i>	–	MF893026	MF893066	MF893132
<i>S. molybdoplaca</i>	Subantarctic Is., <i>Ertz</i> 18651 (BR), <i>Ertz et al. 2017</i>	–	MF893032	MF893072	MF893138
<i>S. pannarioides</i>	Subantarctic Is., <i>Ertz</i> 19096 (BR), <i>Ertz et al. 2017</i>	–	MF893038	MF893078	MF893142
<i>Steinera</i> sp. PCC9	Chile, <i>Nelson & Wheeler</i> 2483 (Nelson Pers. Hb.)	MK571784	–	MK571795	–
<i>Steinera</i> sp. PCC10	Chile, <i>Nelson & Wheeler</i> 2479 (Nelson Pers. Hb.)	MK571785	–	MK571796	–
<i>S. soreliata</i>	New Zealand, <i>Galloway</i> 5984a (GZU)	–	MK571805	MK571794	–
<i>S. subantarctica</i>	Subantarctic Is., <i>Gremmen</i> 94-245, <i>Ertz et al. 2017</i>	–	MF893041	MF893081	–
<i>Wawea fruticulosa</i>	Australia: Tasmania, <i>Kantvilas</i> (F, HO), <i>Lumbsch et al. 2005, 2007a</i>	–	DQ007347	DQ871023	DQ871005
2. Other taxa					
<i>Absconditella</i> sp.	<i>Lumbsch et al. 2004</i>	–	AY300825	AY300873	–
<i>Agyrium rufum</i>	<i>Lumbsch et al. 2007b</i>	–	EF581826	EF581823	EF581822
<i>Aspicilia contorta</i>	<i>Miadlikowska et al. 2006</i>	–	DQ986782	DQ986876	DQ986852

(Continued)

Table 1. (Continued.)

Species	Voucher/source	nuITS	nuLSU	mtSSU	RPB1
<i>Coccotrema maritimum</i>	Schmitt <i>et al.</i> 2001	–	AF329164	AF329163	–
<i>Gyalectaria diluta</i>	Schmitt <i>et al.</i> 2010	–	GU980982	GU980974	–
<i>Icmadophila ericetorum</i>	Miadlikowska <i>et al.</i> 2006	–	DQ883694	DQ986897	DQ883723
<i>Lecanora intumescens</i>	Lumbsch <i>et al.</i> 2004, Ekman <i>et al.</i> 2008	–	AY300841	AY300892	AY756386
<i>Lepra amara</i>	Lumbsch <i>et al.</i> 2001, 2004, 2007a	–	AF274101	AY300900	DQ870965
<i>L. subventosa</i>	Lumbsch <i>et al.</i> 2004, 2007a, Schmitt <i>et al.</i> 2006	–	AY300854	DQ780302	DQ870981
<i>Neobelonia</i> sp.	Lumbsch <i>et al.</i> 2004	–	AY300830	AY300879	–
<i>Ochrolechia parella</i>	Lumbsch <i>et al.</i> 2001, Schmitt <i>et al.</i> 2001, Lumbsch <i>et al.</i> 2007a	–	AF274097	AF329173	DQ870959
<i>O. tartarea</i>	Lumbsch <i>et al.</i> 2004	–	AY300848	AY300899	–
<i>Orceolina kerguelensis</i>	Schmitt <i>et al.</i> 2003a, Lumbsch <i>et al.</i> 2007a	–	AY212830	AY212853	DQ870963
<i>Pertusaria leioplaca</i>	Lumbsch <i>et al.</i> 2004, 2007a	–	AY300852	AY300903	DQ870973
<i>P. paramerae</i>	Schmitt <i>et al.</i> 2006, 2010	–	DQ780326	DQ780293	GU981012
<i>P. pertusa</i>	Lumbsch <i>et al.</i> 2001, Schmitt <i>et al.</i> 2003b	–	AF279300	AF381565	DQ870978
<i>P. pustulata</i>	Schmitt <i>et al.</i> 2006, 2010	–	DQ780332	DQ780297	GU981013
<i>Placopsis bicolor</i>	Schmitt <i>et al.</i> 2003b	–	AY212834	AY212857	–
<i>Protothelenella corrosa</i>	Schmitt <i>et al.</i> 2005, Lumbsch <i>et al.</i> 2007a	–	AY607734	AY607746	DQ870988
<i>Stictis populorum</i>	Lumbsch <i>et al.</i> 2004	–	AY300833	AY300882	–
<i>S. radiata</i>	Wiklund & Wedin 2003	–	AY340575	AY340532	–
<i>Thamnolia vermicularis</i>	Wedin <i>et al.</i> 2005, Lumbsch <i>et al.</i> 2007a	–	AY853395	AY853345	DQ915599
<i>Thelenella antarctica</i>	Schmitt <i>et al.</i> 2005	–	AY607739	AY607749	–
<i>Thelenella muscorum</i>	Schmitt <i>et al.</i> 2005, 2009	–	AY607731	AY607743	FJ941910
<i>Thelotrema lepadinum</i>	Slovenia, Oct. 1997, U. Arup, Staiger <i>et al.</i> 2006	–	DQ431924	DQ431957	–
<i>Trapelia chiodectonoides</i>	Schmitt <i>et al.</i> 2003b, Lumbsch <i>et al.</i> 2007a	–	AY212847	AY212873	DQ870999
<i>Trapeliopsis granulosa</i>	Lumbsch <i>et al.</i> 2005, 2007a	–	AF274119	AF381567	DQ871001
<i>Thrombium epigaeum</i>	Schmitt <i>et al.</i> 2005	–	AY607741	AY607751	–
<i>Varicellaria lactea</i>	Schmitt <i>et al.</i> 2003b, Lumbsch <i>et al.</i> 2007a	–	AF381557	AF381564	DQ870971
<i>Xalocoa ocellata</i>	Fernandez-Brime <i>et al.</i> 2011	–	HQ659183	HQ659172	DQ366252

Huelsenbeck 2003) as implemented on the CIPRES portal (Miller *et al.* 2010), using the same partition scheme and the best models of nucleotide substitutions as determined by PartitionFinder2. We conducted two runs of four chains for 50 million generations, sampling every 1000th generation, and discarding the first 10 000 trees (20%) as burn-in.

We further generated single-locus chronograms for the *Gabura* clade for the mtSSU (16 taxa, 763 characters) and RPB1 (12 taxa, 653 characters) because they were the two loci with the lowest quantity of missing data. We determined the best substitution models using MrModeltest2 v. 2.3 (Nylander 2008) for the mtSSU (substitution model GTR), the RPB1 1st and 2nd codon positions (GTR), RPB1 3rd codon position (K80), and for the non-coding regions (JC). We ran BEAST v. 1.8.4 (Drummond & Rambaut 2007) for 50 million generations, sampling every 1000th generation, with default priors. The RPB1 dataset was partitioned into three subsets (1st and 2nd codon positions together, 3rd codon positions, and non-coding regions)

and the analysis was run with unlinked substitution models. We removed 10% of the trees as burn-in and generated a maximum clade credibility tree. These trees were used for GMYC (General Mixed Yule Coalescent model, Pons *et al.* 2006) species discovery analyses using R (R Core Team 2016) package splits v. 1.0 (Ezard *et al.* 2009). We also ran the PTP (Poisson Tree Process, Zhang *et al.* 2013) species discovery method as implemented on the portal <https://species.h-its.org> (last accessed 27 September 2019) on the four-locus phylogram of the *Gabura* group, using default parameters. Additional GMYC and PTP tests were performed on phylogenetic trees after pruning selected taxa, to test the sensitivity of the analyses.

Results and Discussion

The topologies of the ML trees resulting from the three- and four-locus datasets match, including branch lengths and bootstrap support (four-locus tree is shown; Fig. 1). Overall, the majority of

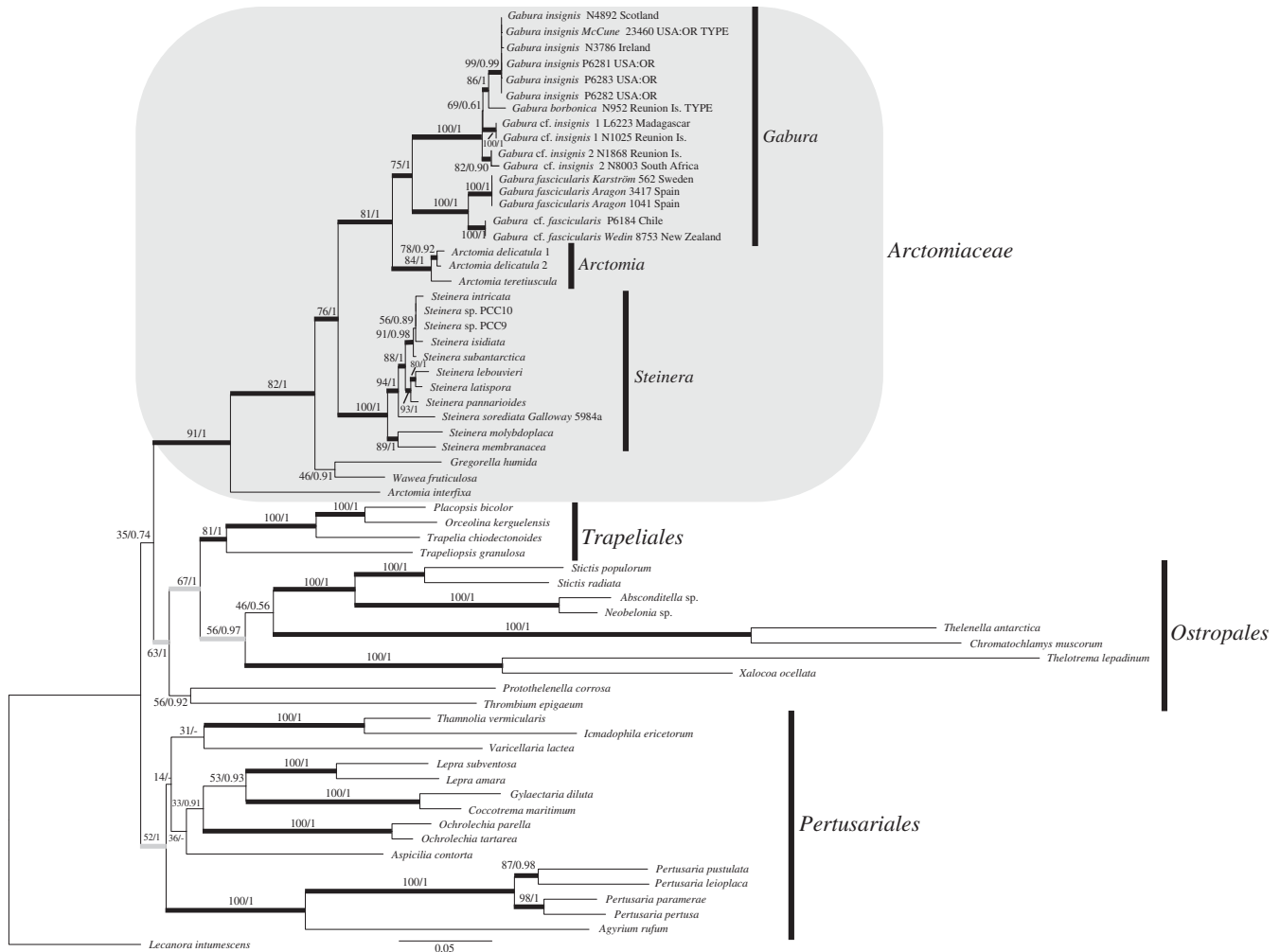


Fig. 1. Phylogenetic tree (best ML tree) of taxa in the Ostropomycetidae resulting from the RAxML analysis on a four-locus dataset (ITS, nuLSU, mtSSU, *RPB1*) for a total of 2815 characters and 63 specimens. ML bootstrap values and Bayesian posterior probabilities are shown above or below branches, before and after the slash, respectively. Thick black branches received ML bootstrap support value ≥ 70 , while thick grey branches received Bayesian posterior probability ≥ 0.95 but MS bootstrap support value < 70 . The grey box highlights the family Arcctomiaceae. *Lecanora intumescens* is used to root the tree.

branches in the phylogenetic tree of the Ostropomycetidae are highly supported, except for deep relationships including the placement of the family Arcctomiaceae in relation to the remaining orders and the relationships among them (although PP support is usually high, bootstrap values are below 70%) (Fig. 1). Contrary to Kraichak *et al.* (2018), the order Trapeliales is nested in Ostropales, with *Protothelenella corrosa* (Körb.) H. Mayrhofer & Poelt and *Thrombium epigaeum* (Pers.) Wallr. (*Protothelenellaceae*) placed outside of the clade containing Ostropales and Trapeliales. Our topology does not support the inclusion of Arcctomiales and Trapeliales in Baeomycetales. However, our sampling for the latter order does not include all families (Kraichak *et al.* 2018). Our topology is not in conflict with the phylogeny presented in Otálora & Wedin (2013), where phylogenetic relationships inside the family Arcctomiaceae were mostly unresolved due to low bootstrap support.

We recovered a monophyletic family Arcctomiaceae with similar internal relationships as reported in Magain & Sérusiaux (2012) and Ertz *et al.* (2017). *Arctomia interfixa* (Nyl.) Vain. appears as the first-diverging lineage in the family, and clearly does not belong to *Arctomia* s. str., as already reported in other studies (Magain & Sérusiaux 2012; Otálora & Wedin 2013). The

two monotypic genera *Gregorella* and *Wawea* are resolved outside of the clade comprising the remaining genera in the family (*Arctomia*, *Gabura* and *Steinera*).

Within the genus *Steinera*, *S. membranacea* Ertz & R. S. Poulsen and *S. molybdoplaca* (Nyl.) Zahlbr. form a sister clade to the remaining species of the genus followed by *S. soreidiata*. Two unidentified specimens from Chile (PCC9 and PCC10) cluster with *S. intricata* (Øvstedal) Ertz and *S. isidiata* Ertz & R. S. Poulsen. The two-locus analysis focusing on *Steinera* suggests that the newly added collections represent a new species, sister to a clade composed of *S. isidiata* and *S. subantarctica* (Øvstedal) Ertz (Fig. 2).

The genus *Steinera* is sister to a clade composed of *Arctomia* s. str. (*A. delicatula* Th. Fr., the type species of the genus, and *A. teretiuscula* P. M. Jørg.) and representatives of the genus *Gabura* (Fig. 1), including a sister relationship of *Gabura fascicularis* with representatives of the *G. insignis*/*G. borbonica* group (formal combinations which have been introduced in this study to accommodate *Arctomia insignis*/*A. borbonica*). As currently delimited, and with the exclusion of the isolated species *A. interfixa*, the genus *Arctomia* is characterized by crustose-squamulose, semi-gelatinous thalli and biatorine apothecia

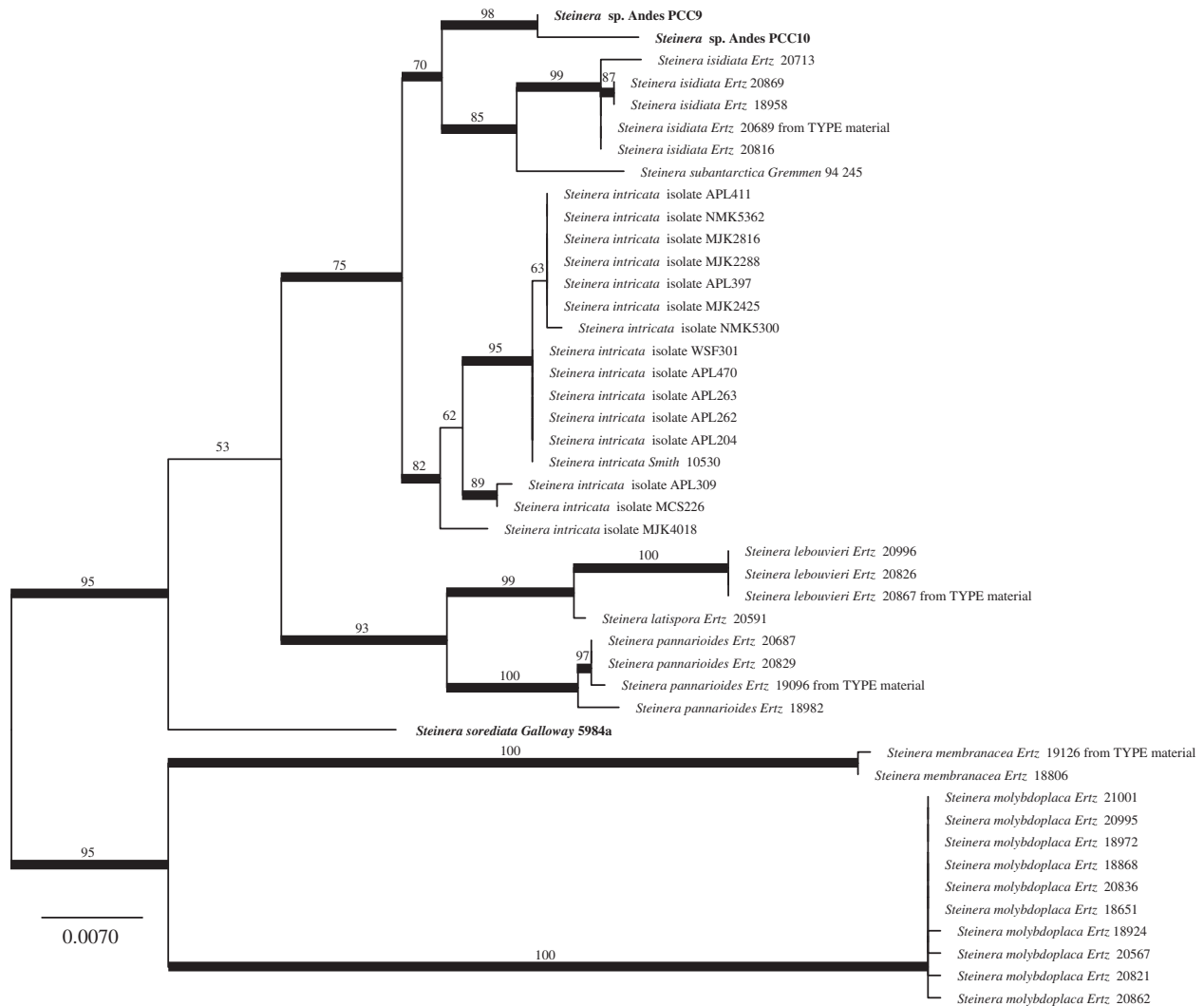


Fig. 2. Phylogenetic tree of *Steinera* species resulting from the RAxML analysis on a two-locus dataset (ITS, mtSSU) for a total of 45 taxa and 1276 characters. ML bootstrap values are shown above branches. Thick black branches received ML bootstrap support value ≥ 70 . Newly sequenced specimens are shown in bold. The tree was rooted according to Fig. 1.

(Henssen 1969; Jørgensen 2003; Otalóla & Wedin 2013). Other characters referred to by Jørgensen (2007), such as the absence of amyloid structures in the ascus apex and fusiform, multiseptate ascospores, are no longer autapomorphic within the family because they are also present in the *Collema fascicularis* group (Otalóla & Wedin 2013).

Gabura fascicularis is split into two well-supported and geographically distant groups: one with European specimens (from Spain and Sweden) and the second with specimens from Chile and New Zealand (Fig. 1). The *G. insignis* group is composed of four well-supported clades including *G. insignis* s. str., containing a sequence from the type material (McCune 23460) from Oregon, together with other specimens from Oregon, Scotland and Ireland (Fig. 1). The origin of the type material was erroneously reported as 'Washington, Lane Co., near mouth of Gwynn Creek on Pacific Ocean' (Jørgensen & Tønsberg 2010) although it comes from Lane County in Oregon. The second clade is represented by the type specimen of *G. borbonica* (N952) and is sister to *G. insignis* s. str. (Fig. 1). A specimen from Reunion Island previously identified as *Arctomia borbonica* (N1025, Magain & Sérusiaux 2012) and one specimen from

Madagascar (L6223) were placed in a separate lineage, whereas another specimen from Reunion Island (N1868) together with a single collection from South Africa (N8003) is part of another separate clade, representing early divergence events within the *G. borbonica*/*G. insignis* species complex (but with relatively low support; BS = 69, PP = 0.61) (Fig. 1). These four distinct lineages revealed within the *G. insignis* group probably represent separate species. GMYC species discovery analyses of *RPB1*, as well as PTP analysis of the four-locus dataset (15 specimens), suggest the presence of four species in this species complex, whereas GMYC analysis of mtSSU and PTP analysis of the 16-specimen dataset (but with PP = 0.11, Fig. 3C) suggest that they all belong to the same single species (Fig. 3). These analyses are sensitive to sampling, as removal of a single taxon from the mtSSU (data not shown) or from the combined PTP analyses (Fig. 3C & D) resulted in the recognition of several species in the group. In this case, species delimitation results are inconclusive and need to be interpreted with caution.

The four lineages of the *G. insignis*/*G. borbonica* complex form a monophyletic clade and could represent a single species corresponding to a broadly defined and widely distributed *G. insignis*,

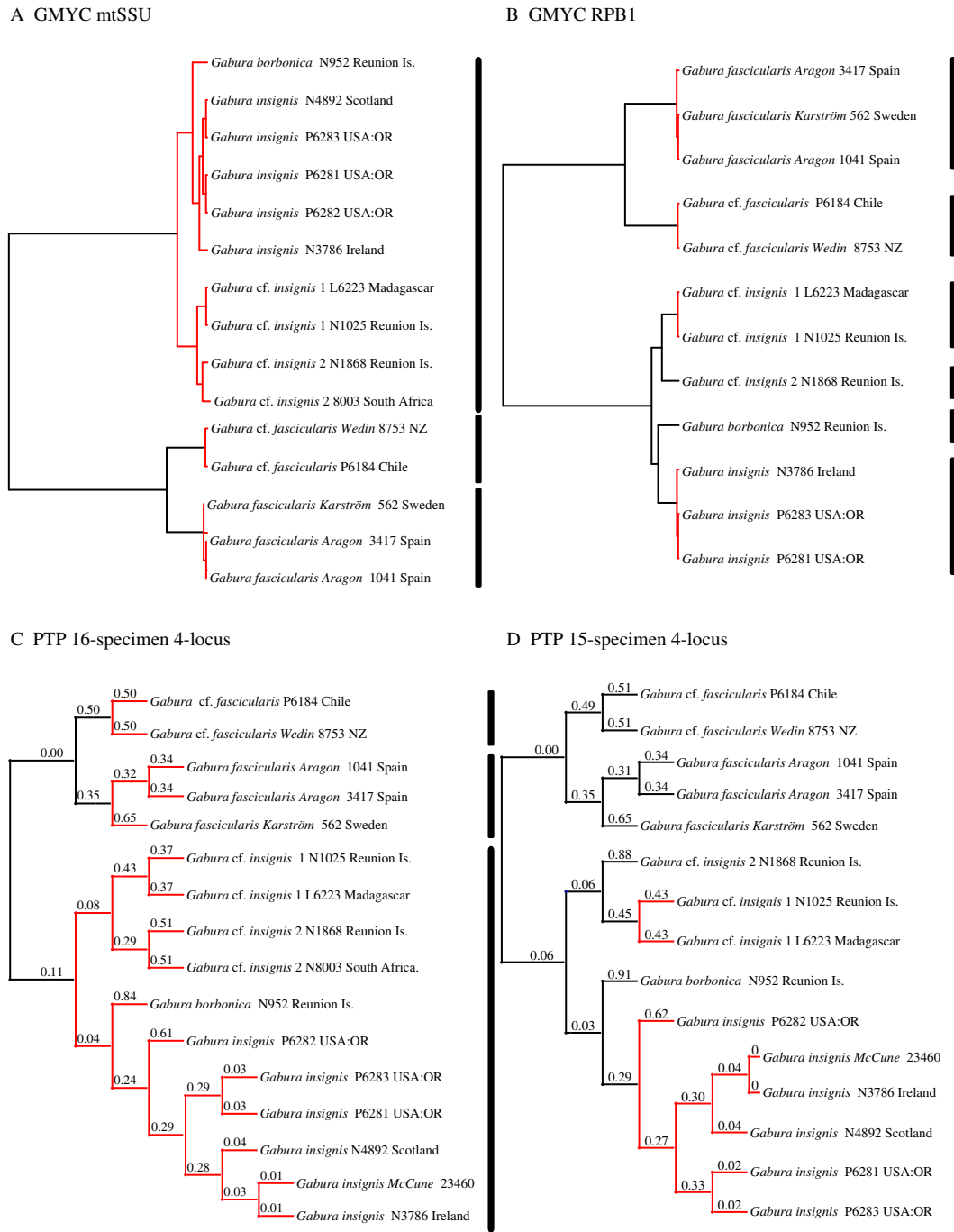


Fig. 3. Species discovery results for the genus *Gabura* based on GMYC analyses on the chronograms of mtSSU (A) and *RPBI* (B), and PTP analyses on the four-locus phylogram with 16 specimens (C) or 15 specimens (D). Red branches represent intraspecific branching events, and black branches interspecific branching events. Vertical bars to the right of the trees summarize the best ML species delimitations.

as suggested earlier by Jørgensen & Palice (2016) (Fig. 1). However, several lines of evidence suggest that each lineage represents a distinct species. First, there is almost no genetic differentiation among specimens of *G. insignis* from Oregon, Ireland and Scotland, suggesting that this species has low intraspecific genetic variation (Table 2). Furthermore, if the four lineages reflect a biogeographical structure within a species, we would expect to see all populations from Reunion Island clustered together. Instead, *G. borbonica* shares a most recent common ancestor with European and North American representatives of *G. insignis*,

while specimens from other lineages of *G. cf. insignis* from Madagascar, Reunion Island and South Africa form the most early-diverging lineage in this clade (Fig. 1). Branch lengths between these four lineages are similar to the branch lengths between species in the closely related genus *Steinera* (Fig. 1), suggesting a similar level of genetic differentiation between these specific-level lineages. Furthermore, a large difference between intraspecific and interspecific variation in all loci suggests the presence of a barcoding gap (Table 2) between these four putative species (with the exception of the mtSSU sequences of *G. cf.*

Table 2. Comparison of intraspecific variation (the maximum number of substitutions/indels between two sequences of the same species) and interspecific variation (the minimum number of substitutions between two sequences of two distinct species) in four loci of lineages of the *Gabura insignis* clade. Indels were treated as a fifth character state, except in ITS where they were very frequent. For ITS, the number including indels is indicated (not in parentheses). NA (not applicable) is indicated when only one sequence was available for a given species.

Locus	Intraspecific variation (Maximum no. of substitutions + indels)					
	<i>G. insignis</i>	<i>G. borbonica</i>	<i>G. cf. insignis</i> 1	<i>G. cf. insignis</i> 2		
ITS	1	NA	NA	3		
nuLSU	1	NA	0	NA		
mtSSU	1	NA	0	1		
<i>RPB1</i>	0	NA	0	NA		
Locus	Interspecific variation (Minimum no. of substitutions + indels)					
	<i>G. insignis</i> vs. <i>G. borbonica</i>	<i>G. insignis</i> vs. <i>G. cf. insignis</i> 1	<i>G. insignis</i> vs. <i>G. cf. insignis</i> 2	<i>G. borbonica</i> vs. <i>G. cf. insignis</i> 1	<i>G. borbonica</i> vs. <i>G. cf. insignis</i> 2	<i>G. cf. insignis</i> 1 vs. <i>G. cf. insignis</i> 2
ITS	19 (32)	18 (24)	20 (31)	15 (30)	19 (29)	17 (19)
nuLSU	6	8	7	6	8	8
mtSSU	3	5	5	7	7	2
<i>RPB1</i>	14	14	11	14	11	7

insignis 1 and *G. cf. insignis* 2, which are more similar). As mentioned, *G. fascicularis* also seems to be composed of two lineages (Fig. 1), probably representing distinct species with a similar strong biogeographical pattern: one clade in Europe (Spain and Sweden) and the other in the Southern Hemisphere (Chile and New Zealand). We also noticed subtle morphological differences in the examined collections although, as clearly demonstrated by the original description of *Leptogium insigne* (Jørgensen & Tønsberg 2010), considerable variation had already been observed. The monophyletic group of *G. fascicularis*, *G. borbonica*, *G. insignis* and other accessions here referred to as *G. cf. insignis* 1 and 2 is morphologically heterogeneous. *Gabura fascicularis* has a subfoliose thallus with numerous erect lobules, abundant and crowded apothecia with very thick rugose thalline margins, and vermiform, twisted ascospores, 10–15-septate, 50–95 × 5–6 µm, whereas all *G. borbonica*, *G. insignis* and *G. cf. insignis* 1 and 2 accessions develop subfoliose to granular thalli, often very crumpled, usually with indistinct lobes and produce granular, yellowish to brownish soralia, but never apothecia. Therefore, all the characters of apothecium development and structure, ascus apex and ascospores identified by Ojaló & Wedin (2013) to demonstrate the phylogenetic affiliation of the *Collema fasciculare* group with the *Arctomiaceae*, are not applicable to the *G. insignis*/*G. borbonica* complex. Despite these morphological differences, we refrain from describing a new genus to accommodate these taxa because their sister relationship with *G. fascicularis* is strongly supported and our sampling did not include many poorly explored territories. Species potentially related to *G. fascicularis* (*Collema leptosporum*, *C. papuanorum* and *C. uviforme*; see Degelius 1974) share a similar morphology. Interestingly, *G. borbonica* is the most morphologically distinct taxon within the *G. insignis* group, and easily distinguished from its sister species, *G. insignis* (see Taxonomic Treatment).

The current sampling suggests a complex pattern of genetic diversity within *G. insignis*, regardless of the ultimate delimitation of this species. Three distinct phylogenetic lineages occurring in the Indian Ocean are present on Reunion Island. The genetic diversity found in this island alone is higher than the diversity between collections from Europe and North America. The only lineage that has been sampled outside of the Mascarenes and Africa (i.e. in Europe and the Pacific Northwest of North America), *G. insignis* s. str., is one of the most recently diverged lineages within the *G. insignis* clade. If we were to assume that our sampling reflects the global population structure of the taxon, most of its genetic diversity was detected in the Indian Ocean, suggesting that this is the most probable origin and diversification centre for this group. Under this scenario, a single dispersal event might have led to the spread of the group to Europe and the Pacific Northwest of North America. However, the recent discovery of *G. insignis* in the British Isles, one of the best-explored areas in the world, underlines the deficiencies in our estimation of lichen distribution ranges. It is unclear whether the specimen from Ecuador reported by Jørgensen & Palice (2016) belongs to *G. insignis*, *G. borbonica*, one of the two unnamed early diverging lineages, or if it represents a new distinct lineage.

We refrain from describing new species for the two early diverging lineages in the *G. insignis* group because they are each represented only by two relatively small, and morphologically cryptic specimens. A lack of obvious diagnostic characters is the reason why these lichens might be overlooked and thus their distributions should be much broader than currently known.

Transferring *Arctomia insignis* and *A. borbonica* to the genus *Gabura* solves the problem of morphological and anatomical heterogeneity in the genus *Arctomia* (following Henssen (1969)) raised by Jørgensen & Palice (2016). As circumscribed, the

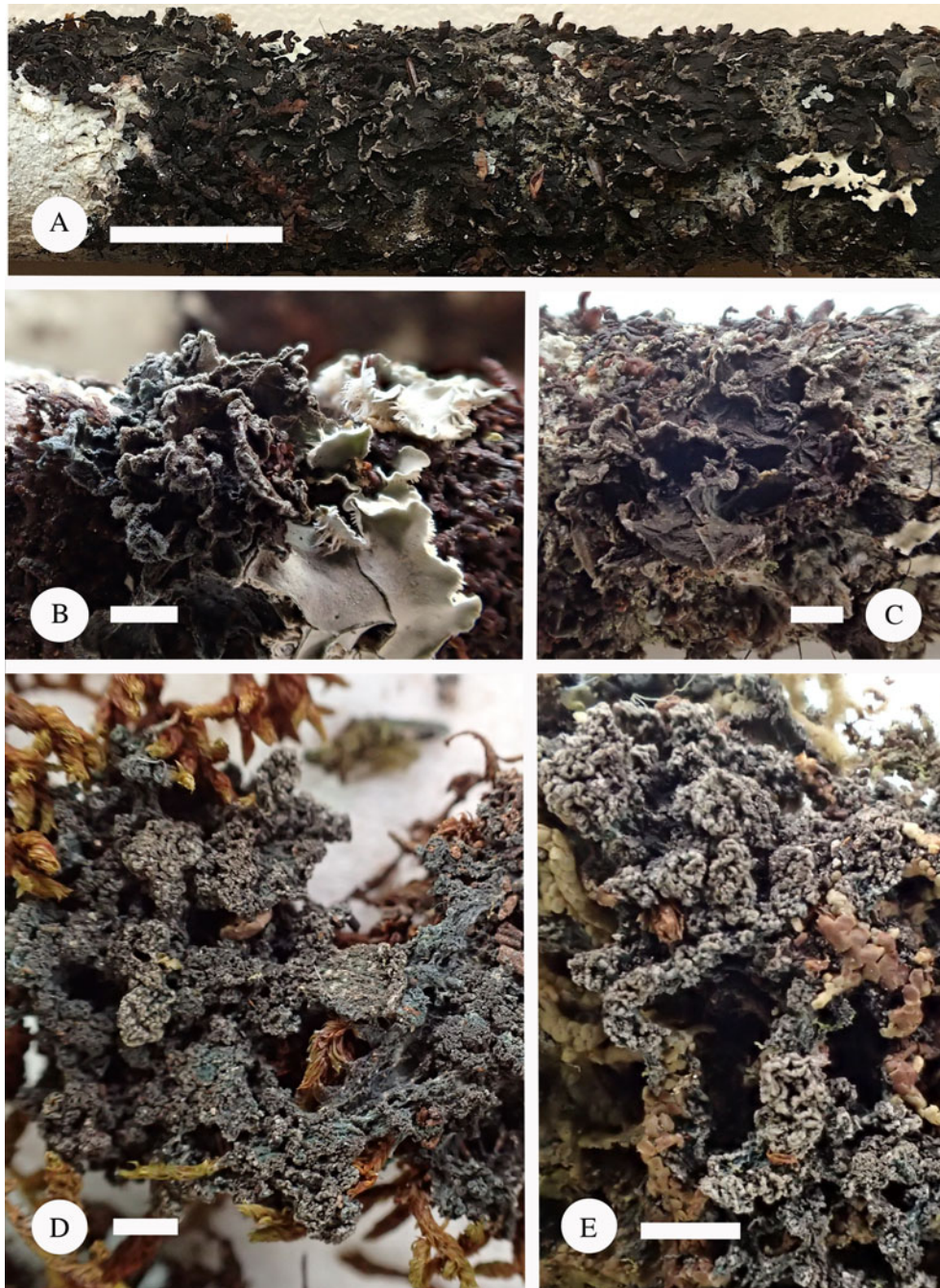


Fig. 4. *Gabura borbonica* and *G. cf. insignis* 1. A–C, type of *G. borbonica*. Conspicuous dark lobes (almost black) with a thick brownish blue sooredioid margin are typical for this species. The white thallus in B is *Leioderma erythrocarpum* (Delise ex Nyl.) D.J. Galloway & P.M. Jørg. D & E, *G. cf. insignis* 1, from Reunion Island (DNA 1025). Lamellate thallus with proliferation of verruciform masses (D) that become aggregated and erect (lobes) with dark bluish to brownish sooredioid margins. Scales: A = 1 cm; B–E = 2 mm.

monophyletic *Arctomia* s. str. currently encompasses two species, *A. delicatula* and *A. teretiusscula*. All species of the *Collema fasciculare* group transferred to *Arctomia* by Otálora & Wedin (2013) probably represent *Gabura*, but DNA evidence is required before applying any taxonomic changes to this complex group. The two subantarctic species, *Arctomia latispora* Øvstedal and *A. subantarctica* Øvstedal (Øvstedal & Gremmen 2001, 2006), were transferred to the genus *Steinera* (Ertz *et al.* 2017). Based on the reconstructed phylogeny (Fig. 1), *Arctomia interfixa* does not belong to *Arctomia* s. str. and a new genus should be erected to accommodate this species.

Taxonomic Treatment

Gabura borbonica (Magain & Sérusiaux) Magain & Sérusiaux *comb. nov.*

Mycobank No.: MB 883418

Basionym: *Arctomia borbonica* Magain & Sérus., *Myckeys* 4, 16 (2012); type: Reunion, Forêt de Bébour, track starting at gîte de Bélouve toward Piton des Neiges, 21°4'49"S, 55°31'24"E, 1850 m, 9 November 2009, N. Magain & E. Sérusiaux s. n. (holotype LG).

(Fig. 4A–C)



Fig. 5. *Gabura insignis* (A–F) and *Steinera* sp. (G & H). A, collection of *G. insignis* from USA (Oregon; DNA 6282). B, collection from Ireland (DNA 3786); circled area enlarged in E. C, collection from UK (Scotland; DNA 4892); circled area enlarged in F. D, collection from USA (Oregon; DNA 6281); white thallus is a fragment of *Peltigera*. E, collection from Ireland (DNA 3786); dark brownish lobes with yellowish punctiform soredioid masses are typical of this specimen but can be absent in other accessions of *G. insignis*. F, collection from UK (Scotland; DNA 4892). G & H, *Steinera* sp. (Tim Wheeler & Peter Nelson 2507); G, thallus and H, ascospores. Scales: A–C = 1 cm; D = 2 mm; E & F = 1 mm; H = 10 μ m.



Fig. 6. *Gabura* cf. *insignis* 2 from South Africa (DNA 8003). A, thallus rather similar to *G.* cf. *insignis* 1, with flat dark brown lobes. B, Lobes mainly hidden by the exuberant development of soredioid masses, forming soredioid margins and rarely well-delimited soralia. Scales: A & B = 2 mm.

Thallus up to 1 cm across, with distinct *c.* 1.5–2 mm large lobes, brownish black when dry, with a well-developed linear soredioid margin which may become bluish; lobes with strongly developed wrinkles, somewhat scrobiculate, with extremities erect and corrugated.

Gabura insignis (P. M. Jørg. & Tønsberg) Magain & Sérusiaux *comb. nov.*

Mycobank No.: MB 883419

Basionym: *Leptogium insigne* P. M. Jørg. & Tønsberg, *Biblioth. Lichenol.* **104**, 242 (2010); type: USA, Oregon [incorrectly reported as Washington in protologue], Lane Co., near mouth of Gwynn Creek on Pacific Ocean, 44°17'N, 124°06'W, 10 m, on leaning *Alnus* trunk in forest, 23 February 1996, B. McCune 23460 (holotype OSC (seen by TS), isotype BG, not seen).

Synonym: *Arctomia insignis* (P. M. Jørg. & Tønsberg) Ertz (as '*insigna*'), in Ertz *et al.*, *Phytotaxa* **324**(3), 231 (2017).

(Fig. 5A–F)

Short description for the European collections (Ireland and Scotland): thallus with distinct and erect lobes, *c.* 1.0–1.2 mm across, dark brown, without any bluish tinge, sometimes with punctiform and rarely linear soralia which are yellowish to dark brown, never bluish and not forming a linear soredioid margin; main lobes flat but can be overgrown by erect lobes and soredioid masses in some parts of the thallus.

Notes. In Europe, *Gabura insignis* is difficult to see in the field as thalli are minute (*c.* 2–5 mm across) and never abundant, unlike in western North America. It could be mistaken for a poorly developed *Leptogium brebissonii*, but it has not been found in the collections filed under that name in E (B. J. Coppins, personal

communication) or in LG. Interestingly, it was not found in our large collections of *Peltigerales* from Macaronesia (Canary Islands, Madeira and the Azores). This unexpected discovery demonstrates that our understanding of lichen biodiversity is still incomplete, and more fieldwork is necessary even in well-researched territories such as the British Isles.

Specimens examined. **Ireland:** Kerry: S of Killarney, near the Muckcross Lake, trees in parkland conditions, 52°01'05"N, 09°30'15"W (coordinates centred on Muckcross Castle), alt. 30 m, ii 2014, E. Sérusiaux s. n. (LG DNA 3786).—**Great Britain:** Scotland: V.C. 104, Isle of Skye, Dunvegan Park, park with various trees, incl. exotic species, 57°26.49'N, 06°35.17'W, alt. 20 m, vi 2015, E. Sérusiaux s. n. (LG DNA 4892).

Gabura cf. *insignis* 1

(Fig. 4D & E)

Flat and very thin thallus blade up to 2–3 cm long, conspicuous, with bloated lobes and soredioid masses; at first dark bluish green becoming dark brown; soredioid masses occurring on small aggregated and wrinkled lobes, starting as punctiform on lobe margins and becoming more developed over the erect parts of lobes.

Specimens examined. **Madagascar:** Prov. Diego Suarez, Antsiranana, Joffreville, Parc National de la Montagne d'Ambre, 12°31'37.5"S, 49°10'18.8"E, 1068 m, x 2014, E. Sérusiaux s. n. (LG DNA 6223).—**Reunion:** au-dessus de la Réserve de Mare Longue, près du gîte B. Brice, 21°20'23"S, 55°41'55"E, alt. 650 m, 10 xi 2009, N. Magain & E. Sérusiaux s. n. (LG DNA 1025).

***Gabura cf. insignis* 2**

(Fig. 6A & B)

Thallus up to 1 cm across, with rather flat dark brown lobes, almost hidden by the exuberant (compared to the other species) development of soredioid masses present on the margins and over the wrinkles of lobes, forming soredioid margins, and rarely well-delimited soralia.

Specimens examined. **Reunion:** Réserve naturelle de la Roche Ecrite, piste vers le sommet, 20°58'6"S, 55°26'26"E, alt. 1500 m, 4 xi 2009, N. Magain & E. Sérusiaux s. n. (LG DNA 1868).—**South Africa:** **Western Cape:** Cape Town, trail to Table Mountain from Kirstenbosch National Botanical Garden, 33.988°S, 18.424°E, 6 vi 2019, R. Vargas (BOL, DUKE DNA NM8003).

Steinera sorediata

Specimen examined. **New Zealand:** **South Island:** Otago, Mt Cargill (type locality), boulders below summit, 2009, D. Galloway 5984a (GZU).

***Steinera* sp.**

(Fig. 5G & H)

Specimens examined. **Chile:** **Region X:** north of Lago General Pinto Cabrera in subalpine Lenga forest and up above treeline at the base of Volcan Yates, 41°48.982'S, 72°21.074'W, P. Nelson & T. Wheeler 2479, 2483, 2507 (hb. Nelson).

Data Accessibility. Alignments have been deposited in TreeBase and can be accessed at <http://purl.org/phylo/treebase/phyloids/study/TB2:S25138>

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