

## Morphological and molecular data support *Lichina intermedia* as a distinct austral-marine species in the *L. pygmaea* group

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**Abstract:** Morphological characteristics and analyses of molecular sequence data (ITS, mtSSU) indicate that the austral-marine lichen *Lichina pygmaea* var. *intermedia* is distinct from the chiefly European marine species *L. confinis* and *L. pygmaea*. It is thus proposed to treat var. *intermedia* as a separate species. *Lichina intermedia* differs from *L. confinis* chiefly in the distinctly corticated branches, and deviates from *L. pygmaea* in the shorter and thinner branches. Diagnostic differences between the three species are summarized and distribution patterns discussed.

**Key words:** distribution, lichen, *Lichina*, Lichinomycetes, phylogeny, taxonomy

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### Introduction

The genus *Lichina* C. Agardh (*Lichinaceae*, Lichinomycetes) contains two groups of dwarf-fruticose cyanolichens which are well distinguished by their deviating ecology and ascoma ontogeny, the latter being a cardinal character for genus separation in the *Lichinaceae*. The *Lichina pygmaea* group consists of *L. confinis* (O. F. Müll.) C. Agardh and *L. pygmaea* (Lightf.) C. Agardh. Both are well-known species that occur along the European coasts and form apothecia from a web of generative hyphae. In contrast, members of the *L. willeyi* group are non-marine, temperate to tropical and sub-antarctic lichens mainly distributed in the Southern Hemisphere producing pycnoascoms, a fundamentally different pathway of ascoma formation (Henssen 1969). Therefore, the monophyly of the genus *Lichina* is currently being evaluated as part of a comprehensive investigation of the phylogeny and character evolution in the Lichinomycetes (M. Schultz & M. Prieto, unpublished data). Not surprisingly, preliminary results suggest

that the species of the *L. pygmaea* and *L. willeyi* groups are only distantly related. While *L. confinis* and *L. pygmaea* are closely related (Schultz & Büdel 2003), the taxonomic status of the austral *L. pygmaea* var. *intermedia* C. Bab. remains uncertain due to its characters which partly overlap with those of the two European species. In fact, the original material collected by David Lyall (1817–1895) in Otago, New Zealand had been examined by various botanists including the prolific phycologist William Henry Harvey (1811–1866). Harvey was inclined to treat it as a new species. Babington (in Hooker 1855), however, discussed the subtle differences in detail and eventually saw more affinities with *L. pygmaea*. Later, *Lichina* samples from New Zealand and Australia were assigned to either of the two European species, undoubtedly due to their partly overlapping characters (Nylander 1888; Hue 1890; Wilson 1891, 1893; Müller Argoviensis 1894; Hellbom 1896; Zahlbruckner 1941; Galloway 1985, 2007; Kantvilas 1989, 1994; Filson 1996). Likewise, coastal *Lichina* specimens have been filed under both species names in Australian and New Zealand herbaria. The *Atlas of Living Australia* ([www.ala.org.au/](http://www.ala.org.au/); searched 14 September 2016) lists 99 records of *L. confinis* and 49 records of *L. pygmaea* showing a very similar

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geographical distribution area. It ranges from SE Australia and Tasmania to New Zealand and the surrounding islands. All these records obviously represent the same taxon. Interestingly, none of the more recent references considered var. *intermedia* to be a distinct taxon and placed it in synonymy instead, usually with *L. confinis* (Galloway 1985, 2007). The present study aims to clarify the taxonomic status of *L. pygmaea* var. *intermedia* and to investigate the phylogenetic relationships within the *L. pygmaea* group.

## Material and Methods

### DNA extraction, amplification and sequencing

DNA was extracted using innuPREP Plant DNA Kit (Analytik Jena) according to the manufacturer's instructions. Amplifications were performed using 1:10 diluted DNA. PCR amplifications for the nuITS 1 & 2 and 5.8S region were performed using a master mix containing 6.65 µl H<sub>2</sub>O, 1.25 µl buffer, 0.75 µl 10% DMSO, 0.15 µl hot start Taq, 0.35 µl of 10 mM primer ITS1 (White *et al.* 1990) and ITS4 (Gardes & Bruns 1993) and 1 µl template DNA, resulting in a final reaction volume of 12.5 µl. PCR amplifications of the mtSSU region were performed using Illustra™ Hot Start Mix RTG PCR beads (GE Healthcare, UK) according to the manufacturer's instructions and using the primers mrSSU1 and mrSSU3 (Zoller *et al.* 1999). PCR settings for ITS amplification were 15 min at 95 °C Taq activation followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 50 °C, 1 min amplification at 72 °C and a final elongation step of 10 min at 72 °C. PCR settings for mtSSU amplification were 14 min at 99 °C Taq activation followed by 38 cycles of 30 s denaturation at 95 °C, 30 s annealing at 56 °C, 1 min amplification at 72 °C and a final elongation step of 5 min at 72 °C. PCR products were purified using spin columns (Geneaid Gel/PCR DNA Fragments Extraction Kit). The purified PCR products were sequenced using the primers ITS1 and ITS4 for forward and reverse strands. Sequence chromatograms were visualized using FinchTV 1.4.0 (Geospiza Inc.) and only minimally edited. Consensus sequences were produced using BioEdit (Hall 1999). Vouchers and GenBank accession numbers of the sequences generated are given in Table 1.

### Alignment and phylogenetic analysis

Multiple sequence alignments for both gene regions of the *Lichina* species were calculated using MUSCLE online version implemented in the EMBL-EBI web pages ([www.ebi.ac.uk/Tools/msa/muscle](http://www.ebi.ac.uk/Tools/msa/muscle)) including the outgroup taxa *Gloeohheppia erosa* Henssen and *Psorotichia lutophila* Arnold. The program Gblocks 0.91b (Castresana, 2002) was used to exclude ambiguously aligned regions employing less

stringent settings according to Talavera & Castresana (2007). Mean genetic distances of sequences of each of the three taxa, as well as between the three taxa, were calculated using MEGA7 (Kumar *et al.* 2016). Uncorrected *P*-distances were corrected by maximizing the composite likelihood (i.e. the sum of pairwise log-likelihoods of a DNA distance matrix built of phylogenetically related sequences). Maximizing the composite likelihood has been shown to be an accurate estimate of the pairwise distances and the related substitution parameters under the Tamura-Nei model (Tamura & Nei 1993; Tamura *et al.* 2004). In order to account for gapped alignment positions the option pairwise deletion was chosen from the MEGA7 menu (MEGA7 help files).

Individual phylogenetic analyses of each of the genes did not show any supported conflict and thus the nuITS and mtSSU alignments were combined. The final alignment consisted of 1440 sites divided into two partitions (637 ITS, 803 mtSSU). The program MrModeltest v.2.3 (Nylander 2004) was used to select a best-fit substitution model according to the Akaike Information Criterion (AIC) for each of the two partitions. The GTR + G model (Rodríguez *et al.* 1990) was selected for both partitions and model parameters were set accordingly for phylogenetic inference in MrBayes 3.2 (Ronquist *et al.* 2012): a gamma distributed rate variation across sites with four categories and an estimated proportion of invariable sites. All parameters were unlinked across partitions and rates allowed to vary under a flat Dirichlet prior. The Bayesian inference was set to two runs each with four chains starting from random trees and running for 5 million generations, saving a tree every 100th generation. The first 25% of the sampled trees were discarded as burn-in for each run after checking that stationarity and convergence of the two runs had been reached by plotting the log likelihoods of each saved tree against the number of generations and by checking that the average standard deviation of split frequencies fell below 0.01. A majority-rule consensus tree with branch lengths and posterior clade probabilities was calculated from the remaining 75 002 trees pooled from both runs using the sumt option of MrBayes. The consensus tree was visualized and edited using FigTree 1.4.2 (Rambaut 2012).

### Morphological techniques

Lichen material collected by the author and obtained from various herbaria was studied using standard techniques. External morphology was investigated using a Wild M5 stereomicroscope. Habit photographs were taken with a Canon EOS 400D digital camera and a Zeiss Luminar 40 mm lens mounted on bellows.

Anatomical details in fresh and semi-permanent cryotome sections stained with lactophenol cotton blue were studied using an Olympus BX51 compound microscope set to differential interference contrast (DIC). Images were taken with an Olympus XC50 digital camera. Anatomical measurements displayed in Table 2 were made using Olympus cellSens imaging software and are given as (minimum–mean minus 1SD–mean–mean plus 1SD(–maximum).

TABLE 1. *Taxa, vouchers, origin and GenBank Accession numbers of newly obtained sequences.*

Taxon	Voucher	Origin	GenBank Acc. no.	
			ITS	mtSSU
<i>Gloeoheppia erosa</i>	Feuerer, 1 Dec 2012 (HBG)	Spain, Canary Islands, Tenerife, El Medano	KX984082	KX984058
<i>Lichina confinis</i> 1	Schultz 07198 (HBG)	Germany, Fehmarn, Orth	KX984084	KX984068
<i>L. confinis</i> 2	Schultz 07195 (HBG)	Germany, Fehmarn, Staberhuk	KX984085	KX984069
<i>L. confinis</i> 3	Schultz 13122 (HBG)	Russia, Karelia, White Sea, island Kostyan	KX984086	KX984070
<i>L. confinis</i> 4	Schultz 13122 (HBG)	Russia, Karelia, White Sea, island Kostyan	KX984087	KX984071
<i>L. confinis</i> 5	Schultz 04015a (HBG)	France, Brittany, Dept. Finistère, Raguénès	KX984088	KX984072
<i>L. confinis</i> 6	Schultz 04015a (HBG)	France, Brittany, Dept. Finistère, Raguénès	KX984089	KX984073
<i>L. confinis</i> 7	Schultz 05548 (HBG)	Sweden, Gotland, Ireviken, Fornborg	KX984090	KX984074
<i>L. confinis</i> 8	Schultz 05512 (HBG)	Sweden, Gotland, Hoburgen	KX984091	-
<i>L. intermedia</i> 1	Galloway (CHR528471)	New Zealand, Southland, Curio Bay	KX984100	KX984075
<i>L. intermedia</i> 2	Galloway (CHR528480)	New Zealand, Northland, E of Cable Bay	KX984103	KX984077
<i>L. intermedia</i> 3	de Lange 9093 & de Lange (AK312435)	New Zealand, Auckland, island Aotea, Oruawharo Bay	KX984104	KX984078
<i>L. intermedia</i> 4	Galloway (CHR528480)	New Zealand, Northland, E of Cable Bay	KX984102	KX984079
<i>L. intermedia</i> 5	Galloway (CHR528471)	New Zealand, Southland, Curio Bay	KX984101	KX984076
<i>L. intermedia</i> 6	de Lange & Sawyer (AK304799)	New Zealand, Chatham Isl.	KX984107	-
<i>L. intermedia</i> 7	de Lange 9075 & de Lange (AK312432)	New Zealand, Auckland, island Aotea, Whangaparapara Harbour	KX984105	KX984080
<i>L. intermedia</i> 8	de Lange 9093 & de Lange (AK312435)	New Zealand, Auckland, island Aotea, Oruawharo Bay	KX984106	KX984081
<i>L. pygmaea</i> 1	Büdel (hb. Büdel)	France, Brittany, Dept. Finistère, Roscoff	KX984092	KX984060
<i>L. pygmaea</i> 2	Schultz 04069 (HBG)	France, Brittany, Côte d'Armor, Cap Fréhel	KX984096	KX984061
<i>L. pygmaea</i> 3	Schultz 04076 (HBG)	France, Brittany, Dept. Ille-et-Vilaine, Dinard	KX984094	KX984062
<i>L. pygmaea</i> 4	Schultz 04075 (HBG)	France, Brittany, Dept. Côte d'Armor, Dahouët	KX984093	KX984063
<i>L. pygmaea</i> 5	Schultz 04069 (HBG)	France, Brittany, Côte d'Armor, Cap Fréhel	KX984095	KX984064
<i>L. pygmaea</i> 6	Schultz 17140 (HBG)	Portugal, Algarve, Praia da Ingrina	KX984097	KX984065
<i>L. pygmaea</i> 7	Schultz 17141 (HBG)	Portugal, Algarve, Praia da Ingrina	KX984098	KX984066
<i>L. pygmaea</i> 8	Schultz 04013 (HBG)	France, Brittany, Dept. Morbihan, Quiberon	KX984099	-
<i>Psorotichia lutophila</i>	Palice 1959 (PRA)	Czech Rep., E Bohemia, Labe valley	KX984083	KX984059

## Material studied

*Selected additional specimens examined.* *Lichina antarctica* Cromb.: **France:** French Southern and Antarctic Lands: Kerguelen Islands, Observatory Bay, ii 1875, A. E. Eaton (BM000022209-syntype).

*Lichina confinis:* **Norway:** Sogn og Fjordane: SW Voss, Lavik, 1986, G. Ernst 1425 (HBG-011530); Ortnevik, 1986, G. Ernst 1405 (HBG-011527).—**Sweden:** Bohuslän: Tjörn, 1884, P. J. Hellbom (Arnold, exs. 1137b) (HBG). Gotland: Ireviken, Fornborg, 2013, M. Schultz 05548 (HBG); Hoburgen, beach boulders, 2013, M. Schultz 05512 (HBG).—**Russia:** Karelia: Distr. Kandalakscha, White Sea Kandalakscha Bay, Kostyan Island, 1997, M. Schultz 13122 (HBG).—**Ireland:** Howth Coast, viii 1913, M. C. Knowles (HBG).—**Great Britain:** Scotland: V.C.97, Westernness: coastal rocks, 1 vi 2005, T. Feuerer (HBG).—**France:** Brittany: Dept. Finistère, S Raguénès, c. 500 m W of Île Raguénès, 1999, M. Schultz 04015a (HBG). Normandy: Cherbourg, S. R. Lenormand (HBG); Granville, S. R. Lenormand (HBG); Noirmoatier (Vendée), Viaud-Grand-Maraais, ad scopulos marinos (Claudel & Harmand, *Lich. Gall.*

*Praec.* no. 203) (HBG).—**Denmark:** Syddanmark: bei Kollund, 5 x 1909, C. F. E. Erichsen (HBG); (Aabenraa) Apenrade, an Strandblöcken bei Warnitzwik, 6 ix 1913, C. F. E. Erichsen (HBG).—**Germany:** Schleswig-Holstein: Friedrichsort b. Kiel, O. W. Sonder (HBG); Kielerhaven, Schrewenborn, 1827, E. F. Nolte (ex Kieler Provinzherbar) (HBG); Hohwacht, 30 viii 1933, C. F. E. Erichsen (HBG); Fehmarn, N of Staberhuk, 2010, M. Schultz 07195 (HBG); Orth, harbour basin, 2010, M. Schultz 07198 (HBG). Mecklenburg-Vorpommern: Rügen, Stubbenkammer, J. Münter (Rabenhorst, *Lich. Europ.* no. 782) (HBG); Rügen, F. K. L. Rudolphi (HBG).—**Romania:** Constanta: Caliacra, in saxosis calcareis maritimis indundatis ad Cap-Caliacra, 26 xii 1935, O. Klement & P. Crezoiu (*Lich. Romaniae Exs.* no. 19) (HBG).—**Italy:** Liguria: ad rupes emersas in litore Liguriaee occiduae, L. Dufour (Rabenhorst, *Lich. europ.* no. 225) (HBG). *Latina:* sugli scogli alle spiagge di Terracina, 1850, E. Fiorini-Mazzanti (*Erbar. Crittogam. Ital.* no. 279, Massal., *Miscell. Lich.* 52) (WU—type of *L. elisabethae* A. Massal.).—**Canada:** Newfoundland and Labrador: Newfoundland, Marysvale near Brigus, on marine rocks, 1953, I. M. Lamb 7579 (FH 00453035).

TABLE 2. Main diagnostic characters of marine species of *Lichina*.

Diagnostic characters	<i>intermedia</i>	<i>confinis</i>	<i>pygmaea</i>
Branch shape	usually cylindrical, sometimes flattened	(sub)cylindrical	flattened, rarely (sub)cylindrical towards tips
Branch length (mm)	1.5–6.0	0.5–2.5	1.5–10.0(–15.0)
Terminal branch width (mm)	(0.08–)0.12–0.16–0.20(–0.25)	(0.06–)0.07–0.09–0.11(–0.12)	(0.16–)0.19–0.24–0.29(–0.32)
Intercalary branch width (mm)	(0.10–)0.14–0.21–0.28(–0.35)	(0.08–)0.10–0.12–0.14(–0.14)	(0.20–)0.26–0.33–0.40(–0.48)
Basal branch width (mm)	(0.16–)0.17–0.24–0.31(–0.38)	(0.10–)0.12–0.15–0.18(–0.20)	(0.25–)0.34–0.43–0.52(–0.64)
Cortex at branch tips (µm)	distinct, (5.5–)7–15(–20)	absent or loose, 3–5(–6.5)	distinct, 9.5–20.0(–25.0)
Cortex at branch sides (µm)	distinct, (4–)5.5–12(–17)	absent or loose, 3–5(–5.5)	distinct, (7–)8–12(–15)
Hymenium height (µm)	270–400	190–220	250–400
Spore size (µm)	(15.0–)17.8–21.4–25.0(–28.5) × (7.0–)9.0–11.1–13.2(–15.0) (n = 82)	(13.5–)15.2–16.9–18.6(–21.0) × (9.0–)10.5–11.9–13.3(–14.5) (n = 44)	(17.5–)22.4–25.5–28.6(–31.5) × (10.5–)11.8–13.2–14.6(–16.5) (n = 38)
Distribution	SE Australia, Tasmania, New Zealand and surrounding islands	coastal Europe, Iceland to Greenland, NE North America	coastal W to NW Europe to NW Africa
Ecology	rocky sea coast upper intertidal zone, often among barnacles	rocky sea coast supralittoral zone, Baltic Sea eulittoral to lower supralittoral zones	rocky sea coast upper intertidal zone, often among barnacles

*Lichina intermedia*: **New Zealand**: Northland: Bay of Islands, south end of Poroporo Island, 7 i 1980, B. W. & G. C. Hayward (AK 165892); Moturoa Island, easternmost embayment on south coast, 1990, A. E. Wright 10360 (AK 200720). **Auckland**: Rodney Co., Leigh, on maritime rock, 1972, B. W. & G. C. Hayward H34.4 (AK 155063); Aotea (Great Barrier Island), Oruawharo Bay, Medlands Beach, Memory Island (Rock Stack), 2010, P. J. de Lange 9093 & T. J. de Lange (AK 312435); Whangaparapara Harbour, Whangaparapara Wharf, 2010, P. J. de Lange 9075 & T. J. de Lange (AK 312432); Rangitoto Island, viii 1933, H. H. Allan (W 1935-2393); Waiheke Island, i 1932, L. B. Moore (W 1935-2399). **Marlborough**: Ship Cove, 41°06'S, 174°14'E, i i 1992, B. W. Hayward (AK 205342). **Dunedin**: Dunedin, Saint Calan, 1934, J. S. Thomson ZA75 (W 1935-2393). **Chatham Islands**: Chatham (Rekohu) Island, Western Reef, 2006, P. J. de Lange CH2254 & J. W. D. Sawyer (AK 304799). **Campbell Island**: west shore of Garden Cove, 1970, R. C. Harris 5246 (MSC).—**Australia**: *South Australia*: Kangaroo Island, Cape St Albans, on intertidal rocks, 2015, G. Kantvilas 389/15 (HO 580954). *Victoria*: Waratah Bay, basalt, 1968, C. C. Bratt 68/1430 (HO 46489). *Tasmania*: Kingston Beach near Hobart, 1965, C. C. Bratt 2959 (HO 46531, MIN, SBBG); Lime Bay Nature Reserve, c. 1 km N of Plunkett Point, on subtidal basalt stone, 2016, G. Kantvilas 238/16 (HO 584310, dupl. HBG-024439).

*Lichina pygmaea*: **Norway**: Hordaland: Bømlo, Olvanda, 3 viii 1937, R. Santesson (Magnusson, *Lich. Sel. Scand. Exs.* no. 297) (HBG). *Sogn og Fjordane*: Askvoll hd., Smelvaer, 1951, K. Faegri (ex hb. Bergen Museum) (HBG).—**Ireland**: **V.C. H39**, coast of Co. Antrim at Ballycastle, ix 1922, M. C. Knowles (HBG).—**Great Britain**: *England*: **V.C. 3**, Devon, Torbay, M. Wyatt (Algae Damnon. no. 155) (HBG); A. W. Griffith, ded. W. H. Harvey 1835 (HBG). *Jersey*: viii 1875, L. King (HBG).—**France**: *Brittany*: Dept. Morbihan, Côte Sauvage, Quiberon Peninsula, S of Pointe du Percho, 1999, M. Schultz 04013 (HBG); Dept. Finistère, Raguénès, 1999, M. Schultz 04011 (HBG); S of Raguénès, c. 500 m W of Île Raguénès, 1999, M. Schultz 04019a (HBG); Roscoff, Perharidy, Enez Jacopin, 2 vi 2010, B. Büdel (dupl. HBG); Dept. Côtes d'Armor, Côte d'Émeraude, SE of Cap Fréhel, Point de la Guette, 12 vi 2007, D. & S. Schultz (hb. M. Schultz 04069, HBG); Dahouët, near Le Val-André, 3 vii 2012, D. & S. Schultz (hb. M. Schultz 04075, HBG); Dept. Ille-et-Vilaine, Dinard, 29 vi 2012, D. & S. Schultz (hb. M. Schultz 04076, HBG). *Normandy*: Cherbourg, S. R. Lenormand (HBG); Cherbourg (Hohenacker, Algae mar. sicc.) (HBG).—**Portugal**: sine loco, F. M. J. Welwitsch (BM). *Faro*: Algarve, Praia da Ingrina, 2013, M. Schultz 17140, 17141 (HBG).

**Results**

**Phylogeny**

Forty-nine sequences have been produced for the present phylogenetic analysis (Table 1).

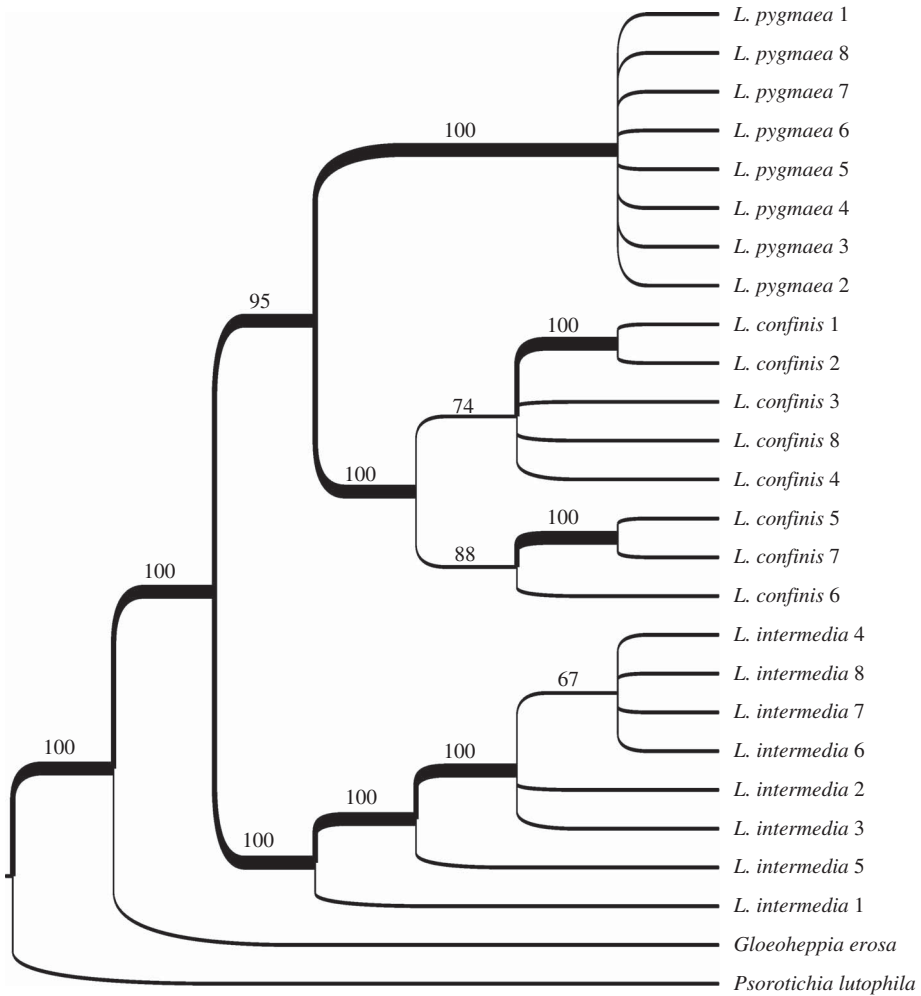


FIG. 1. Phylogeny of the *Lichina pygmaea*-group. 50% majority-rule consensus tree obtained from Bayesian phylogenetic analysis of ITS and mtSSU sequence data. Bold clades indicate significant posterior probability support. Outgroup taxa are *Gloeohheppia erosa* and *Psorotichia lutophila*.

The *Lichina pygmaea*-group forms a highly supported clade further divided into three subclades, each of which are likewise highly supported representing *L. confinis*, *L. pygmaea* and *L. pygmaea* var. *intermedia* (Fig. 1). Both *L. confinis* and *L. pygmaea* are closely related and form a sister clade with *L. pygmaea* var. *intermedia*. Mean distances in nuITS were highest within *L. pygmaea* var. *intermedia* when compared with the other two species (Table 3). However, the mean distance of *L. pygmaea* var. *intermedia* to *L. confinis* was

12 times greater than the intraspecific distance, and the distance to *L. pygmaea* was 18 times greater. Mean distances in mtSSU were similar within *L. pygmaea* var. *intermedia* and *L. confinis*. The mean distances, however, of *L. pygmaea* var. *intermedia* to *L. confinis* were 35 times greater than within *L. pygmaea* var. *intermedia*, and the distance to *L. pygmaea* was 38 times greater. *Lichina pygmaea* var. *intermedia* shows considerable genetic divergence from the other two species and hence should be recognized at species level.

TABLE 3. *ITS* and *mtSSU* mean genetic distances within and among marine/maritime species of *Lichina* corrected by maximum composite likelihood.

	Mean genetic distances within and among species					
	nuITS 1 & 2 and 5.8S ( <i>n</i> = 24)			mtSSU ( <i>n</i> = 21)		
	<i>confinis</i>	<i>intermedia</i>	<i>pygmaea</i>	<i>confinis</i>	<i>intermedia</i>	<i>pygmaea</i>
<i>confinis</i>	0.003021			0.000787		
<i>intermedia</i>	0.083947	0.006808		0.021281	0.000605	
<i>pygmaea</i>	0.093406	0.120420	0.0	0.006379	0.022989	0.0

## Taxonomy

The main diagnostic characters distinguishing *L. intermedia* (Fig. 2) from *L. confinis* (Fig. 3) and *L. pygmaea* (Fig. 4) are summarized in Table 2. A full, updated description of *L. intermedia* is provided below. Descriptions of *L. confinis* and *L. pygmaea* follow Poelt (1969), Blum *et al.* (1975), Clauzade & Roux (1985), Jørgensen (2007), Fletcher & Purvis (2009) and Wirth *et al.* (2013).

### *Lichina intermedia* (C. Bab.)

#### M. Schultz *stat. nov.*

MycoBank No.: MB 819915

Basionym: *Lichina pygmaea* var. *intermedia* C. Bab. in Hook. f., *Flora Nov.-Zeland.*, 2: 311 (1855).

Type: (New Zealand) On rocks, Otago [=Dunedin], fertile, March, Dr. Lyall (BM—holotype!); New Zealand, Southland, Curio Bay, 25 October 2009, D. J. Galloway (CHR-528471—epitype, designated here; HBG-024418—isoepitype).

(Fig. 2)

*Thallus* blackish, usually pale brownish-olivaceous at attachment point, dwarf-fruticose, forming carpets up to several cm in size, cushions intermediate between larger *L. pygmaea* and smaller *L. confinis*, branches cylindrical (Fig. 2B) to sometimes distinctly flattened (Fig. 2A), furcate, ascending to erect, 1.5–6.0 mm long, tips 0.08–0.25 mm thick, intercalary sections 0.07–0.35 mm thick, basal sections 0.07–0.38 mm thick. *Cortex* distinct, hyaline, small-celled paraplectenchymatous, 5.5–20.0 µm thick at branch tips, 4–17 µm thick at branch sides; *photobiont layer* 30–85(–100) µm thick with reticulate to fountain-like hyphal pattern and short, contorted cyanobiont chains

belonging to *Rivulariaceae* (Fig. 2D); central hyphal strand (50–)90–150 µm thick, compact, hyphae parallel to interwoven, fountain-like towards photobiont layer, cells distinctly elongated (Fig. 2C & D).

*Apothecia* terminal, (sub)globose, 0.4–0.7 mm diam.; *disc* reddish to yellowish brown, punctiform to concave and somewhat opened, often empty (Fig. 2A); thalline margin smooth, persisting, 60–75 µm thick. *Hymenium* 270–400 µm high, KOH/IKI+ weakly bluish green; *paraphyses* slender, septate, branched, tips hardly thickened, pale; *exciple* distinct, hyaline, 25–75 µm thick, small-celled (sub)paraplectenchymatous. *Asci* prototunicate, narrowly clavate, 8-spored, wall thin, non-amyloid, apical structures absent. *Ascospores* hyaline, simple, broad ellipsoid, (15.0–)17.8–21.4–25.0 (–28.5) × (7.0–)9.0–11.1–13.2(–15.0) µm (*n* = 82), wall soon thickened.

*Pycnidia* terminal, (sub)globose, 175–200 µm diam., ostiolum pale brownish, wall hyaline, becoming convoluted with age; *conidia* terminally formed, ellipsoid, 3.0 × 1.5 µm; *conidiophores* simple, elongated.

*Habitat and distribution.* Grows on intertidal rocks often associated with barnacles and *Hydropunctaria maura* (Wahlenb.) C. Keller *et al.* s. lat., with a distribution from SE Australia, Tasmania to New Zealand and surrounding islands; potentially also elsewhere in the Southern Hemisphere along cold rocky seashores.

## Discussion

Some morphological variation was observed among the more than 50 specimens of

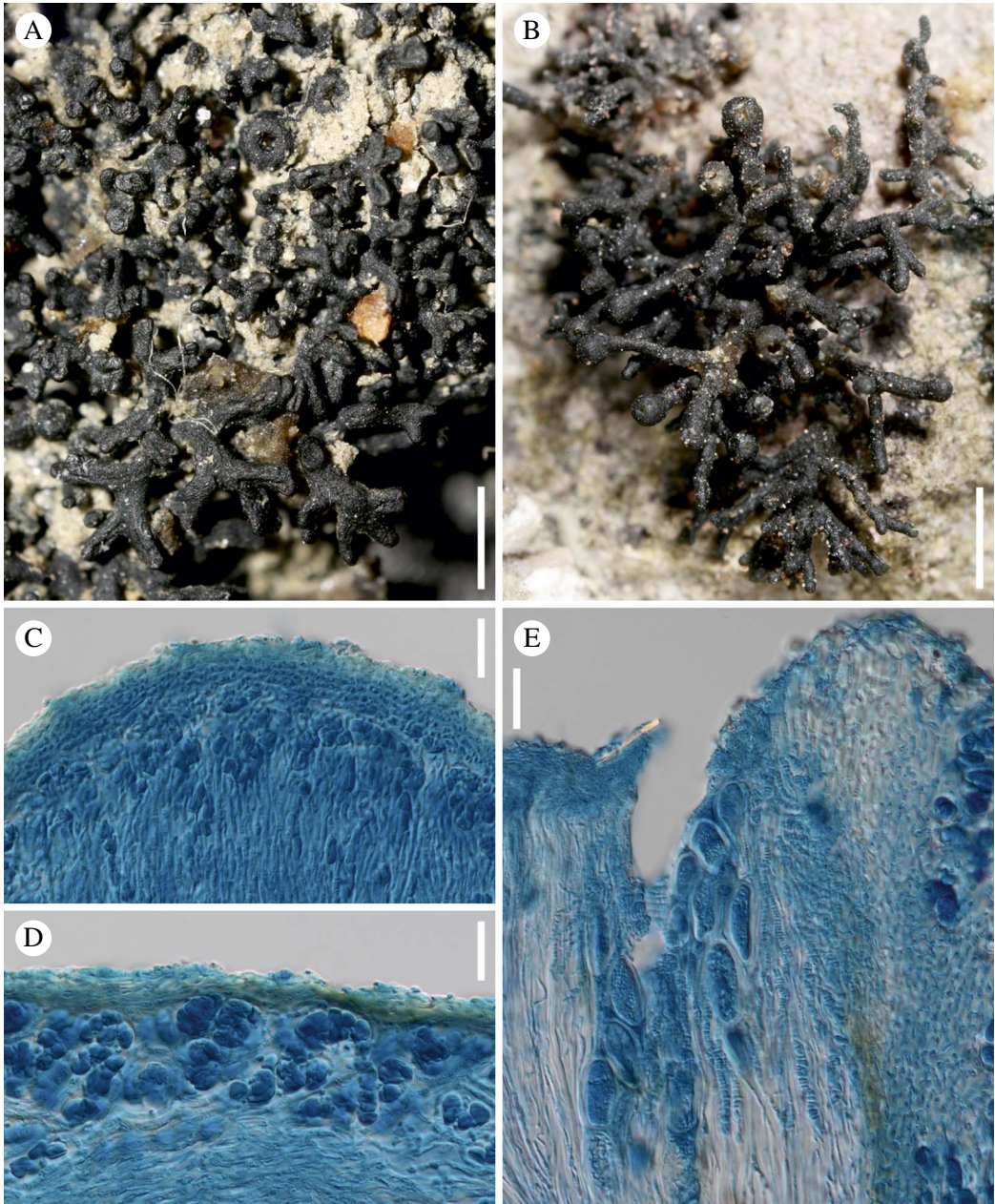


FIG. 2. *Lichina intermedia*. A, thallus with short, mostly flattened branches and terminal apothecia (holotype); B, thallus with more elongated, cylindrical branches (AK 175212); C, section of lobe tip with distinct cortex (top), contorted *Rivularia* cyanobiont chains (middle) and compact hyphae of central strand (bottom) (holotype); D, as C but section of lobe side showing thinner cortex; E, section of apothecium with slender paraphyses (left), mature ascospores (middle) and proper exciple (right) (holotype). Scales: A & B = 1 mm; C–E = 20  $\mu$ m.

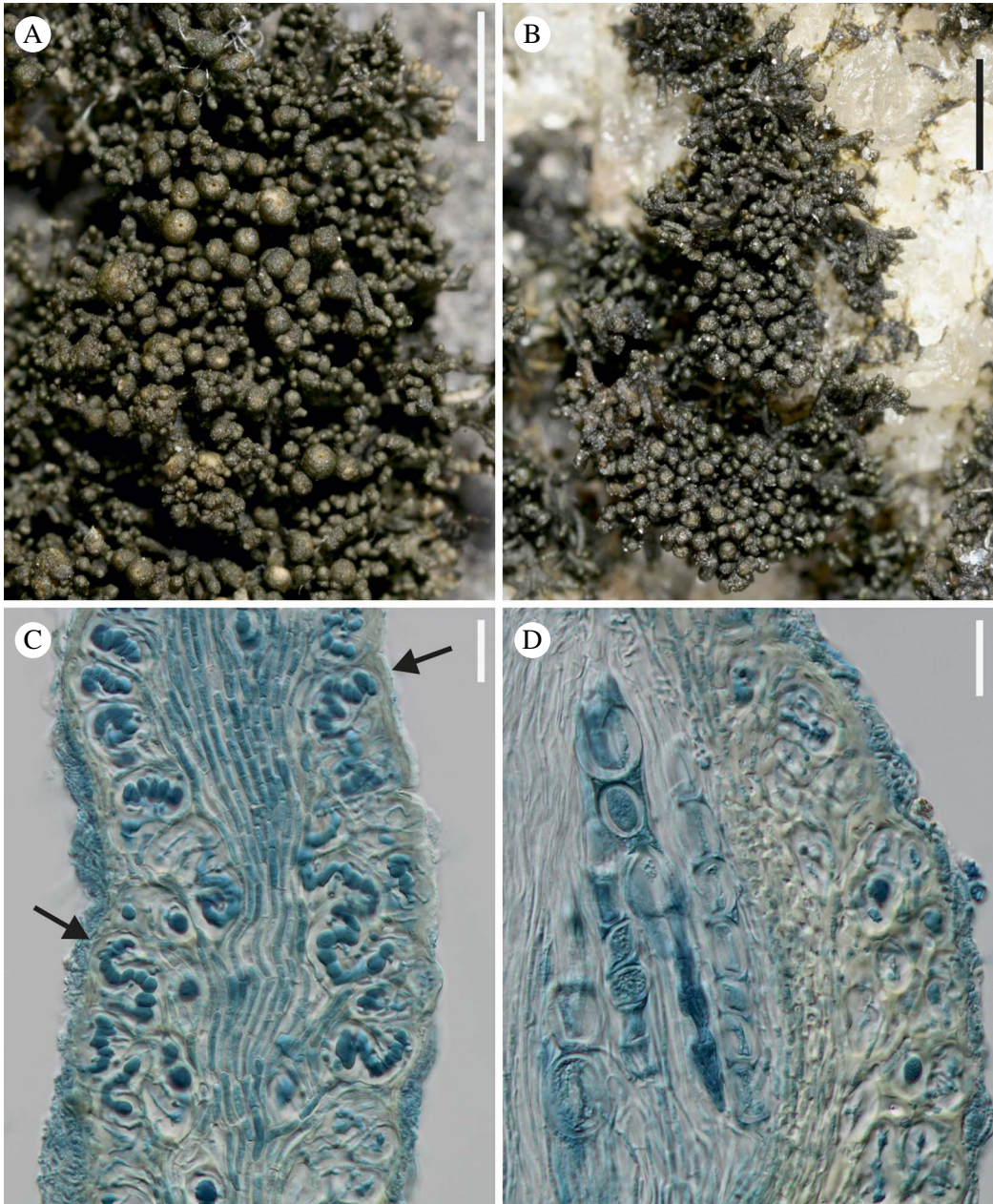


FIG. 3. *Lichina confinis*. A, thallus cushions with densely aggregated, cylindrical lobes and terminal pycnidia (Schultz 07198); B, central thallus branches densely packed, marginal branches prostrate, irregularly furcate to caespitose (Schultz 13122); C, median branch section with loose hyphae forming poor cortex (arrow), twisted *Rivularia* cyanobiont chains and compact, fountain-like central hyphal strand (Schultz 04015a); D, hymenium with paraphyses and thick-walled ascospores (left, centre), pale proper exciple (centre) and poorly corticated thalline exciple (right) (Schultz 04015a). Scales: A & B = 1 mm; C & D = 20  $\mu$ m.



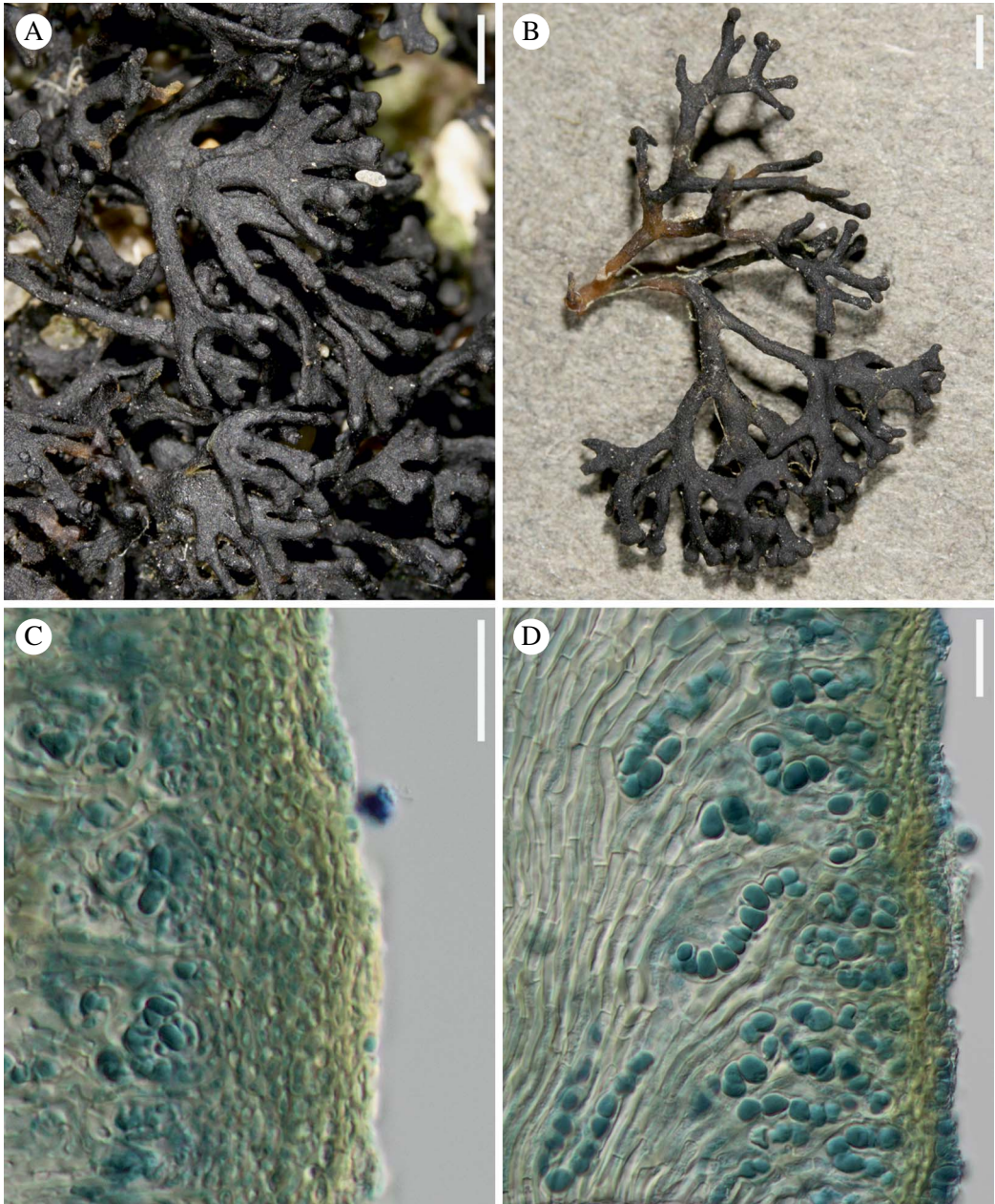


FIG. 4. *Lichina pygmaea*. A, thallus branches distinctly flattened, repeatedly furcate to palmately divided (Schultz 17140); B, thallus branches regularly furcate, narrow and subcylindrical towards tips with terminal, globose pycnidia (Schultz 04079); C, median section of lobe tip with distinct cortex composed of densely packed, small-celled hyphae (Schultz 04011); D, section of lobe side showing compact, fountain-like central hyphal strand (left), contorted *Rivularia* cyanobiont chains (middle) and cortex of 3–5 rows of densely packed, small-celled hyphae (Schultz 04011). Scales: A & B = 1 mm; C & D = 20  $\mu$ m.

*L. intermedia* studied. Thalli with cylindrical branches were dominant and these samples appeared as somewhat larger forms of *L. confinis* from Europe. This might have been the reason for recent authors to synonymize var. *intermedia* under *L. confinis*. On the other hand, the rarely observed forms with flattened branches (including the holotype) superficially resembled small *L. pygmaea*. However, the variation in lobe shape observed in *L. intermedia* did not correlate with the geographical origin of the samples studied or with the phylogenetic relationships of the sequences observed. Yet the differences between the species in branch size, cortex structure and thickness, ascospore size and ecology fit very well with the phylogenetic tree topology showing three highly supported clades representing three distinct species. This is further illustrated by the mean genetic distances observed in both gene loci (Table 3). The mean genetic distances were considerably higher between the three species than within each of the species, suggesting sufficient genetic divergence between the species. The overall similar distances among the species in mtSSU compared with the nuITS also indicate a largely congruent evolution of both gene loci. Taking all the evidence into consideration, the three marine/maritime species of *Lichina* treated here are each well characterized, morphologically, genetically and geographically.

Various sources report *L. confinis* (explicitly or implicitly including *L. pygmaea* var. *intermedia* as a synonym) to be a cosmopolitan species (Ozenda & Clauzade 1970; Blum *et al.* 1975; Galloway 1985, 2007; Litterski 1992; Nimis 1993; Fletcher & Purvis 2009). However, the results presented here clearly indicate that *L. confinis* occurs only in the Northern Hemisphere (chiefly Europe) whereas the Southern Hemisphere species, *L. intermedia*, is so far known only from SE Australia and New Zealand. These results have implications for species and habitat conservation. For example, the gene pools of both *L. confinis* and *L. intermedia* populations have to be considered much smaller and less diverse than previously thought.

Such observations are relevant, for example, in national census recordings and endangered species assessments. In fact, *L. confinis* is already red-listed as highly threatened in Germany (Wirth *et al.* 2011). It used to be very common along the German Baltic Sea coast but has declined significantly in recent times due to pollution of the shallow coastal waters. The status of *L. intermedia* in Australia and New Zealand also has to be re-evaluated now that the taxon is ranked at species level. It is an example of the taxonomic work needed to accurately describe species diversity, distribution and ecology. A further example is "*L. canariensis* ad int." which has been reported recently by Ortiz-Álvarez *et al.* (2015), who found that populations of *Lichina* in the Canary Islands probably represent a distinct, undescribed taxon. Focusing on the eastern North Atlantic coasts they further showed that *Lichina* species use highly differentiated pools of *Rivularia*-cyanobionts specific to geographical origin and littoral zones, and suspected that ecological speciation has likely occurred.

The *L. pygmaea* group shows a peculiar global distribution pattern. The most striking observation is certainly the absence of *Lichina* in suitable habitats in coastal western North America, raising questions about speciation history. *Lichina* is also absent from Japan, the Russian Far East and apparently also coastal South Africa. Although *L. confinis* and *L. pygmaea* were reported from Chile and South America (Nylander 1858; Galloway & Quilhot 1999; Galloway 2007), the records seem somewhat dubious and should be validated by consulting herbarium material. However, another member of the *L. pygmaea* group was found on maritime rocks of Livingstone Island just north of the Antarctic Peninsula (*Søchting* 7850, dupl. HBG). This lichen is very close to *L. confinis*. It differs significantly in the smaller, globose to subglobose ascospores (and is hence called here "*L. sphaerospora* ad int."). More material is needed to clarify if this is yet another austral *Lichina* species. Finally, it should be mentioned that *Lichina antarctica* described from the Kerguelen Islands does not belong to the

*L. pygmaea* group. The ascomata in the BM syntype are pycnoascocarps and hence the species is a member of the *L. willeyi* group which falls outside the genus *Lichina* and is very closely related to *Porocyphus* instead (M. Schultz & M. Prieto, unpublished data). To conclude, the taxonomic diversity of the *L. pygmaea* group is still incompletely known and there seems to be at least one undescribed species occupying a similar ecological niche to *L. confinis* in the north. The geographical distribution of these species and their speciation history still remain to be studied in more detail.

I wish to dedicate this paper to D. J. Galloway (1942–2014), foremost New Zealand lichenologist. I approached him in an early phase of this study and he showed great interest. He generously collected fresh material, sent duplicates to me and offered help in arranging the loan of material from AK. We had occasional contact about the progress of my studies. Finally, I received an email from David a few weeks before he passed away. He asked about *Lichina intermedia* and offered further help. I am happy to have shared the essence of the results presented here with him.

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