

The genus *Endocena* (*Icmadophilaceae*): DNA evidence suggests the same fungus forms different morphologies

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Abstract: Numerous recent studies of lichenized fungi have uncovered hidden genetic diversity within a single phenotypic entity (so-called ‘cryptic species’). Here we report the opposite situation with vastly different morphologies apparently deriving from the same genotype. *Endocena* is a monotypic genus known only from southern South America. The single reported species, the terricolous *E. informis*, is morphologically variable; the type and other collections from the west coast of Chile being subfruticose, whereas specimens from further south and east are almost crustose in form. A sorediate terricolous lichen that is frequent on the Falkland Islands was confirmed by phylogenetic analysis of ITS rDNA and mtSSU rDNA sequences as being congeneric with *E. informis* and, surprisingly, both taxa were recovered as congeneric with the recently described genus and species *Chirleja buckii*, which is morphologically distinct from both *E. informis* and the sorediate taxon. Consequently, the genus *Chirleja* is included in the synonymy of *Endocena* and the new combination *Endocena buckii* is proposed. Because *E. informis* and the sorediate specimens have a similar thallus structure that differs radically from that of *E. buckii*, the name *E. informis* var. *falklandica* is proposed for the sorediate taxon. Poorly developed, incipient apothecia are also described from both varieties of *E. informis*, the first time that these have been reported for *Endocena*. We also report two lichenicolous fungi from *E. informis* var. *informis*, which are the first reports of lichenicolous fungi occurring on this genus.

Key words: *Chirleja*, lichenized fungi, lichens, molecular systematics, phenotypic plasticity, southern South America, typification

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Introduction

Molecular investigation of lichenized-fungi has repeatedly shown that a single morphological ‘species’ is often comprised of several divergent genotypes, the so-called ‘cryptic species’ (e.g. Crespo & Pérez-Ortega 2009; Vondrák *et al.* 2009; Núñez-Zapata *et al.* 2011; Kraichak *et al.* 2015; Leavitt *et al.* 2016). Here we report the opposite situation, with the same genotype apparently responsible

for ‘species’ the morphologies of which are so different that they were described in different genera.

The genus *Endocena* was described by Crombie (1876 [1877]) from a collection made from Port Grappler on the west coast of Chile (49.4229°S, 74.2968°W) by R. O. Cunningham, who was the naturalist on board H.M.S. Nassau. Crombie included the single species *E. informis* Cromb. in his genus and, correctly, likened it to *Siphula* Fr., separating it from that genus by the thallus being hollow rather than solid (“Accedens ad Siphulas, facie fere conveniens, at thallo intus nonnihil cavo (vel parte axili medullae cava in toto thallo)”). The relationship of this monotypic genus to *Siphula* and its position within the *Icmadophilaceae* Triebel was confirmed by Stenroos *et al.* (2002).

Among the extensive lichen collections made by Henry Imshaug and co-workers from southern South America housed in the

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herbarium of Michigan State University (MSC: Fryday & Prather 2001), there are several collections from the Falkland Islands and Isla de los Estados that resemble *E. informis* but differ in being sorediate and having a thallus consisting of flat to convex areoles rather than the hollow pustules typical of *E. informis*. They are also chemically similar, both containing thamnolic acid. During recent fieldwork on the Falkland Islands by the first author, this entity was found to be frequent, growing among terricolous bryophytes on exposed peat or decaying stems of *Blechnum magellanicum* Mett., and was also discovered on Isla Grande de Tierra del Fuego by the third author.

The present study was initiated to confirm the placement of the new sorediate taxon in *Endocena* using molecular methods, but it revealed that both the new taxon and *E. informis* were congeneric with the recently described genus and species *Chirleji buckii* Lendemer & B. P. Hodk. *Chirleji buckii* is morphologically dissimilar to *Endocena informis* and resembles a species of the unrelated genus *Leprocaulon* Nyl. (*Leprocaulaceae*, *Leprocaulales*; Lücking *et al.* 2016). It is known only from the type collection from Seno Agostini in the Parque Nacional Alberto de Agostini in the Chilean section of Isla Grande de Tierra del Fuego (Lendemer & Hodkinson 2012).

Materials and Methods

Morphology

The study is based chiefly upon collections made by Henry Imshaug and co-workers during fieldwork in southern South America between 1968 and 1971 and now held in the herbarium of Michigan State University (MSC; Fryday & Prather 2001) and recent fieldwork on the Falkland Islands and southern Chile by the first and third authors, respectively. Apothecial characteristics were examined by light microscopy on hand-cut sections mounted in water. Thallus sections were investigated, and anatomical measurements made, in 10% KOH. Standard chemicals were used for spot-test reactions and thin-layer chromatography followed the methods of Orange *et al.* (2001).

Molecular methods

Nuclear ITS rDNA was amplified using the primers ITS1f (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and mitochondrial SSU rDNA using mrSSU1

(Zoller *et al.* 1999) and MSU7 (Zhou & Stanosz 2001). Protocols for DNA extraction, amplification and sequencing were the same as in Singh *et al.* (2015). The 18 new sequences were deposited in GenBank (Accession numbers KY495225–KY495242). Details of the specimens used in the phylogenetic analysis are given in Table 1.

Sequence selection and phylogenetic analyses

The new sequences were aligned with sequences of the *Icmadophilaceae* available in GenBank (*Dibaeis* spp., *Icmadophila* spp., *Siphula* spp. and *Thamnolia vermicularis* (Sw.) Schaer.), members of the *Ochrolechiaceae* (*Ochrolechia* spp.) and the species *Chirleja buckii*. Members of the *Coccotremataceae* (*Coccotrema* spp.) and *Ochrolechiaceae* (*Ochrolechia* spp.), which have been shown to be closely related to the *Icmadophilaceae* (Miadlikowska *et al.* 2014; Lücking *et al.* 2016) were used as outgroup. Gblocks v0.91b (Castresana 2000) was used to remove ambiguously aligned regions using the less stringent parameter settings allowing half gap positions. Model selection was performed using the Corrected Akaike Information Criterion (AICc) (Sugiura 2007) as implemented in jModelTest v2.1.1 (Darriba *et al.* 2012). The best fitting models (SYM+I+G for ITS and GTR+G for mtSSU) were used in the subsequent analysis in MrBayes v3.2.1 (Huelsenbeck & Ronquist 2001). Two parallel MCMCMC runs were executed, each using four chains and 1 000 000 generations, and sampling trees every 100th generation. A 50% majority-rule consensus tree was generated from the combined tree sample after discarding the first 25% of the trees as burn-in.

Results

The ITS alignment was 515 bp long (274 variable sites (VS)) and 412 bp after Gblocks (203 VS). The mtSSU alignment was 840 bp long (215 VS) and 726 bp after Gblocks (199 VS). Members of the *Icmadophilaceae* form a supported monophyletic group in the phylogenetic tree (Fig. 1). Relationships within the family are not fully supported by either marker but some remarkable patterns are observable. The mtSSU tree shows a supported basal position for *Thamnolia vermicularis* within *Icmadophilaceae*; a similar position is shown in the ITS tree but is not statistically supported. The ITS tree further confirms that, as previously shown (Platt & Spatafora 2000; Stenroos *et al.* 2002; Grube & Kantvilas 2006; Ludwig 2015), the genus *Siphula* is polyphyletic as currently understood. Ludwig (2015) further showed that the name *Nylanderella* Hue is available for

TABLE 1. Specimen data and GenBank Accession numbers for the collections used in the phylogenetic analysis of *Endocena* shown in Fig. 1. Newly obtained sequences are in bold.

Taxon	Location	Reference	Voucher	Lab Code	GenBank Accession numbers	
					ITS	mtSSU
<i>Coccotrema cucurbitula</i>	Argentina, Prov. Rio Negro	Schmitt <i>et al.</i> 2001	<i>Vobis</i> (ESS-20862)	–	AF329162	AF329161
<i>C. maritimum</i>	Canada, British Columbia	Schmitt <i>et al.</i> 2001	<i>Brodo</i> 30130 (CANL)	–	AF329165	AF329163
<i>C. pocillarium</i>	USA, Alaska	Schmitt <i>et al.</i> 2001	<i>Printzen</i> (ESS-20863)	–	AF329167	AF329166
<i>Dibaeis absoluta</i>	China, Hainan	Cao <i>et al.</i> 2013	HMAS-L 118073	–	KC414626	–
<i>D. absoluta</i>	China, Hainan	Cao <i>et al.</i> 2013	HMAS-L 118071	–	KC414625	–
<i>D. arcuata</i>	New Zealand	Ludwig 2015	OTA 063978	–	KP759335	–
<i>D. baemyces</i>	USA, Alaska	Spribile <i>et al.</i> 2014	<i>Spribile</i> 38948 (GZU)	–	KJ462265	KJ462397
<i>D. baemyces</i>	no location given	James <i>et al.</i> 2006	–	–	DQ782844	–
<i>D. baemyces</i>	Austria,	Lumbsch <i>et al.</i> 2004	<i>Obermayer</i> 7797 (UPS)	–	–	AY300883
<i>D. soreliata</i>	China, Hainan	Cao <i>et al.</i> 2013	HMAS-L 118090	–	KC414627	–
<i>Endocena buckii</i>	Chile, Tierra del Fuego	Lendemmer & Hodkinson 2012	<i>Buck</i> 57033 (NY)	–	JX673914	JX673913
<i>E. informis</i> var. <i>informis</i>	Falkland Islands, East Falkland	–	<i>Fryday</i> 10728	150550	KY495226	KY495234
<i>E. informis</i> var. <i>informis</i>	Falkland Islands, West Falkland	–	<i>Fryday</i> 10886	150551	KY495232	KY495235
<i>E. informis</i> var. <i>informis</i>	Chile, Región de los Lagos	–	<i>Pérez-Ortega</i> 3340	S1572	KY495227	KY495236
<i>E. informis</i> var. <i>informis</i>	Chile, Región de los Lagos	–	<i>Pérez-Ortega</i> 3336	S1573	KY495228	KY495237
<i>E. informis</i> var. <i>informis</i>	Chile, Región de Magallanes	–	<i>Pérez-Ortega</i> 2629	S1577	–	KY495238
<i>E. informis</i> var. <i>informis</i>	Chile, Región de Magallanes	–	<i>Pérez-Ortega</i> 2631	S1575	–	KY495242
<i>E. informis</i> var. <i>informis</i>	Chile, Región de Magallanes	–	<i>Pérez-Ortega</i> 2626	S1578	KY495229	KY495239
<i>E. informis</i> var. <i>informis</i>	Chile, Región de Magallanes	–	<i>Pérez-Ortega</i> 2624	S2239	KY495230	KY495240
<i>E. informis</i> var. <i>informis</i>	Chile, Región de Magallanes	–	<i>Pérez-Ortega</i> 2628	S2238	KY495231	KY495241
<i>E. informis</i> var. <i>falklandica</i>	Falkland Islands, Weddell Island	–	<i>Fryday</i> 10857	150184	KY495225	KY495233
<i>Icmadophila ericetorum</i>	USA, Alaska	Spribile <i>et al.</i> 2014	<i>Spribile</i> 36042 (GZU)	–	KJ462267	KJ462399
<i>I. ericetorum</i>	Sweden	Wedin <i>et al.</i> 2005	<i>Wedin</i> 6525 (UPS)	–	–	AY853327
<i>I. ericetorum</i>	no location given	Miadlikowska <i>et al.</i> 2006	–	–	–	DQ986897
<i>I. japonica</i>	Japan	Ohmura 2011	–	–	AB623070	–
<i>Ochrolechia androgyna</i>	Germany	Lumbsch <i>et al.</i> 2004	<i>Schmitt</i> (ESS21066)	–	–	AY300897
<i>O. frigida</i>	no location given	Schmull <i>et al.</i> 2011	–	–	HQ650675	–
<i>O. tartarea</i>	United Kingdom	Schoch <i>et al.</i> 2012	–	–	JN943620	–
<i>O. frigida</i>	Antarctica	Lumbsch <i>et al.</i> 2004	<i>Ott</i> (hb. Ott)	–	–	AY300898
<i>Siphula ceratites</i>	USA, Alaska	Resl <i>et al.</i> 2015	<i>Spribile</i> 38923(GZU)	–	KR017095	–
<i>S. ceratites</i>	no location given	Schmull <i>et al.</i> 2011	–	–	HQ650642	–
<i>S. decumbens</i>	New Zealand	Ludwig 2015	OTA 063984	–	KP984796	–
<i>S. fastigiata</i>	New Zealand	Ludwig 2015	OTA 062499	–	KP984797	–
<i>S. fastigiata</i>	Australia, Tasmania	Grube <i>et al.</i> 2004	–	–	–	AY648898
<i>Thamnomlia vermicularis</i>	New Zealand	Lord <i>et al.</i> 2013	OTA 59464	–	AY961605	–
<i>T. vermicularis</i>	Peru	Lord <i>et al.</i> 2013	OTA 58855	–	JQ409343	–
<i>T. vermicularis</i>	no location given	Lutzoni <i>et al.</i> 2004	–	–	–	AY584728
<i>T. vermicularis</i>	Sweden	Wedin <i>et al.</i> 2005	<i>Wiklund</i> 34 (UPS)	–	–	AY853345
<i>T. vermicularis</i>	Russia, Altai Republic	Resl <i>et al.</i> 2015	<i>Resl</i> 1136(GZU)	–	–	KR017348

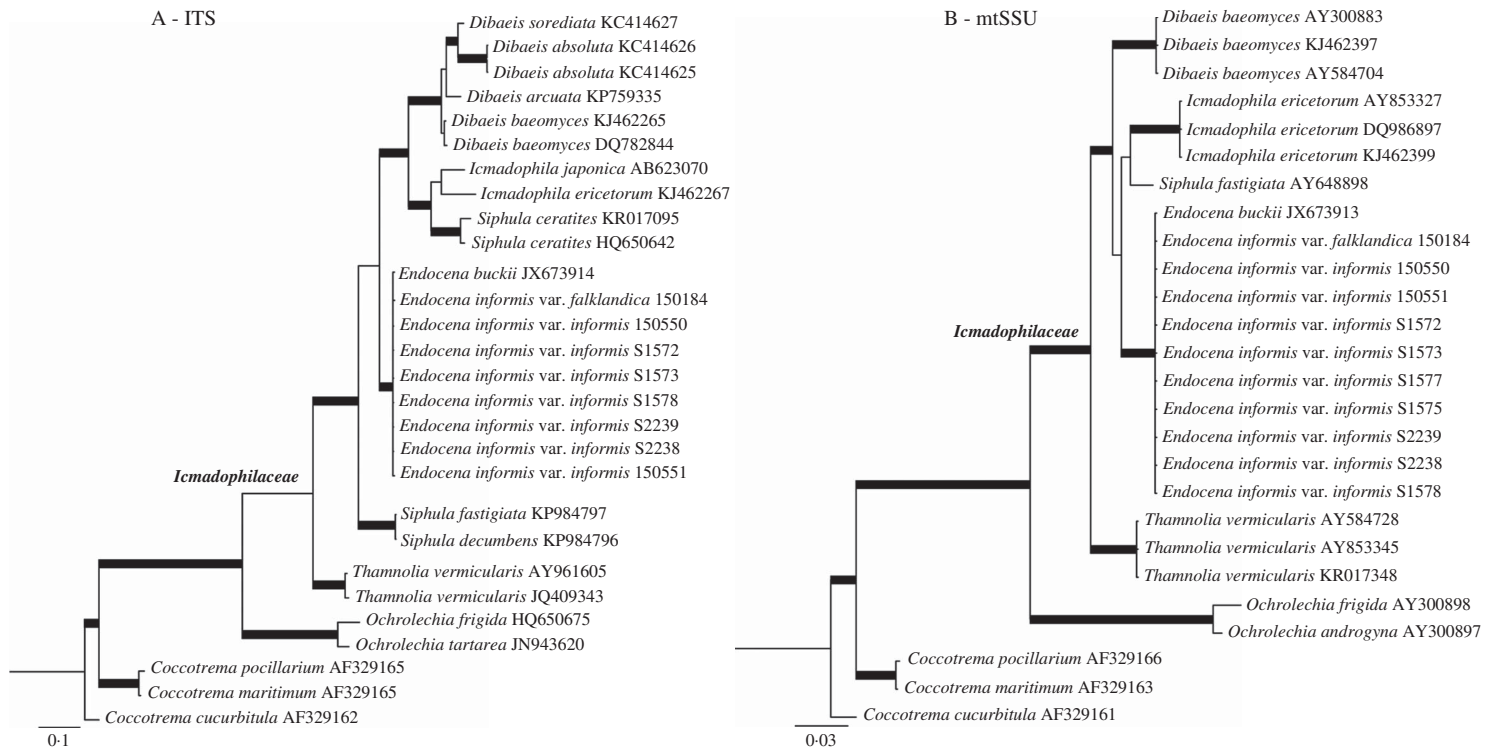


FIG. 1. Phylogenetic placement of the genus *Endocena*. Bayesian phylogeny based on ITS (A) and mtSSU (B) data. Thickened branches indicate posterior probabilities ≥ 95 . All sequences of *Endocena informis* are newly generated for the present study, all others are from GenBank.

the *S. decumbens* group. Representatives of *Endocena* and *Chirleja* form a supported monophyletic group within the *Icmadophilaceae* in both trees. Comparison of all sequences of *Endocena* and *Chirleja* reveals two variable positions in the ITS alignment and none in the mtSSU alignment.

Examination of the type (and only) collection of *Chirleja buckii* revealed it to be morphologically distinct from both the other two taxa, consisting of short, erect pseudopodetia with granular-sorediate phyllocladia and resembling a species of *Leprocaulon* (Fig. 2A & B). *Endocena informis* differs

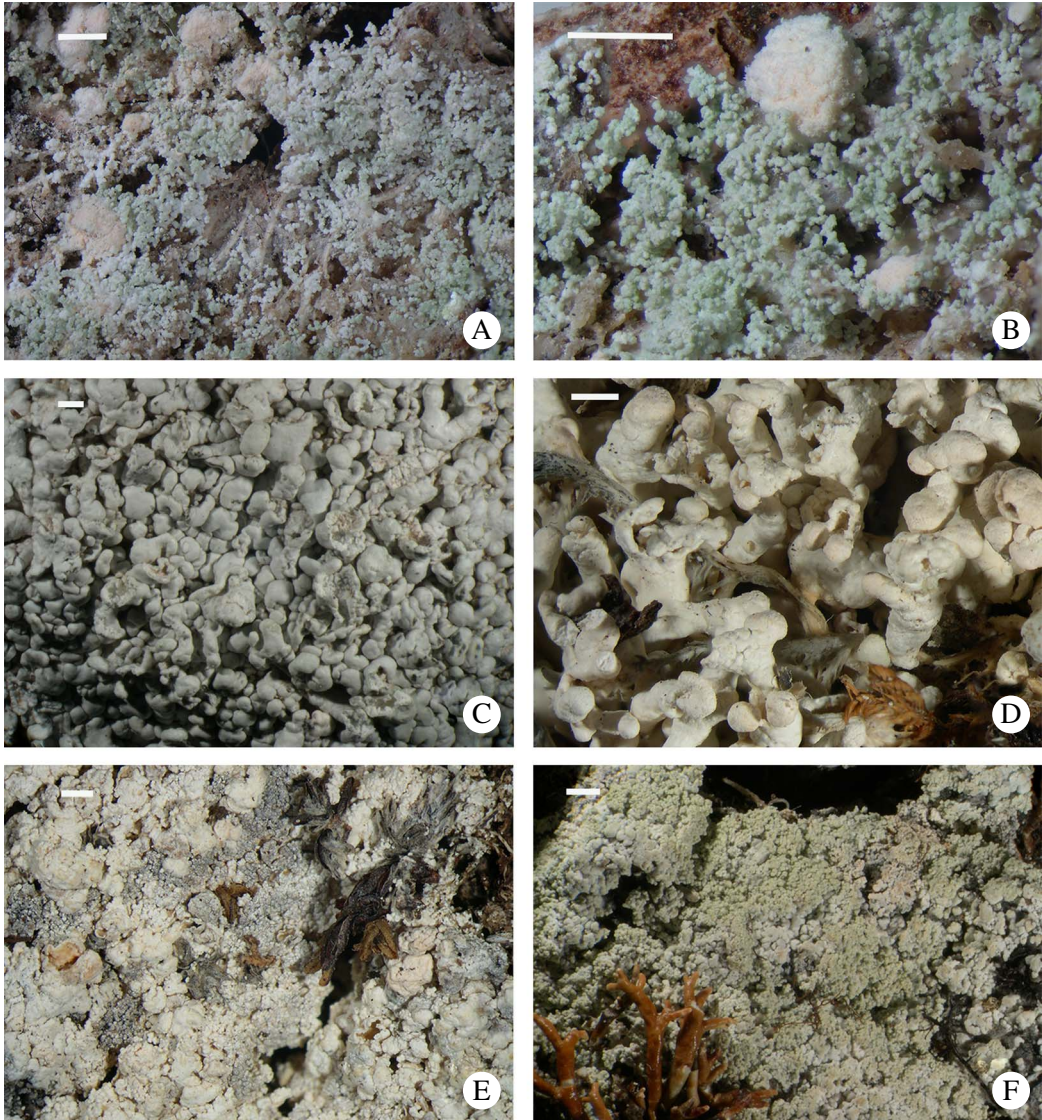


FIG. 2. A & B, *Endocena buckii* (Buck 57033—holotype), thallus with sporodochia. C & D, *E. informis* var. *informis*; C, ±crustose form (Fryday 11424); D, fruticose form (Imshaug 41064). E & F, *E. informis* var. *falklandica*; E, leprose form (Imshaug 52644); F, typical form with discrete soredia (Fryday 10741—holotype). Scales = 1 mm. In colour online.

TABLE 2. Comparison of *Endocena informis* var. *informis*, *E. informis* var. *falklandica*, and *E. buckii*.

	<i>E. informis</i>		
	var. <i>informis</i>	var. <i>falklandica</i>	<i>E. buckii</i>
Growth form	Crustose, subfruticose, or fruticose	Crustose	Dimorphic, leprose
Thallus structure	Elongate, hollow pustules	Areolate, hollow pustules absent	Short, erect pseudopodetia
Thallus colour	Shiny ivory white	Dull creamy white	Pale greenish-white
Soredia	Absent, but broken ends of pustules with granular-sorediate interior	Usually in discrete patches but occasionally covering complete thallus	Thallus and phyllocladia granular-sorediate
Apothecia	Incipient, poorly developed	Incipient, poorly developed	Not observed
Chemistry	Thamnolic acid	Thamnolic acid	Thamnolic acid
Distribution	Widely distributed throughout southern South America	Predominantly Falkland Islands	Southern Chile (known only from type collection)
Habitat	Terricolous, mainly over or among bryophytes	Terricolous, mainly on soil and peat hags	Underside of slanted tree trunk in wet <i>Nothofagus</i> forest

significantly from *Chirleja buckii* in having a thallus composed of elongate, hollow pustules (Fig. 2C & D). The thallus of the sorediate taxon is similar to *Endocena informis* but differs in being crustose, lacking hollow pustules and developing soredia that are usually in discrete patches but occasionally cover the complete thallus giving it a leprose appearance (Fig. 2E & F). A comparison of the main characteristics of the three taxa is given in Table 2.

Apothecia are reported for the first time from the genus (Fig. 3).

Discussion

The ITS and mtSSU sequences of the three taxa are so similar that, based solely on the molecular data, all the collections might be included in a single species (*E. informis*). However, despite this genetic uniformity the morphological variation within the collections is remarkable (Fig. 2, Table 2). Two entities having almost identical ITS rDNA sequences but having such contrasting morphology that it was felt necessary to describe them in different genera, indicates that the use of ITS as the universal DNA barcode marker for fungi (Schoch *et al.* 2012) is not useful for all groups. It is known that ITS does not work well for highly

speciose genera such as *Fusarium* (Al-Hatmi *et al.* 2016) or *Aspergillus* (Samson *et al.* 2014) because it cannot discriminate among closely related species. Genera in the *Icmadophilaceae* are not highly speciose but they could have either evolved recently or display characteristic low levels of molecular evolution.

Recent evidence suggests that the capacity for lichens to consistently harbour secondary fungi might have been underestimated in the past (U'Ren *et al.* 2012, 2014; Spribille *et al.* 2016) and one of the biggest current issues in lichen molecular taxonomy is that researchers fail to check which hyphae in their lichens are the source of their PCR products. This is especially relevant for sterile lichens such as *Endocena* where fruiting bodies are not available to be used as a source of DNA. Since we sequenced several collections of *E. informis*, we are confident that our PCR amplicons represent that species but, because *C. buckii* is known from only a single collection, there is a possibility that this is not the case for that species. However, we consider this unlikely because Lendemer & Hodkinson (2012) provided two identical sequences from the single specimen and the chemistry of *C. buckii* is consistent with its position in the *Icmadophilaceae*.



FIG 3. Apothecia of *Endocena informis* var. *falklandica* (Orange 19571). The lichenicolous fungus in the image appears to be different from the *Sphaerellothecium* sp. mentioned in the text, but it lacks fruiting bodies and its identity is uncertain. Scale = 1 mm. In colour online.

We consider that the morphological differences exhibited by the three entities are sufficient to recognize them as distinct taxa; as two species with one comprising two varieties. *Endocena informis* itself is morphologically variable and specimens from the extremes of its range appear quite distinct. However, they are united by the basic thallus form of hollow pseudopodetia/pustules and, because intermediates occur and the two forms are sympatric in southern Chile, occurring together in the same population (cf. discussion under *E. informis* var. *informis*), they are retained here in a single taxon. It is clear from the molecular data that *Chirleja* should be included in the synonymy of *Endocena* but, because the thallus structure of *C. buckii* and *E. informis* are very different, they are retained here as distinct species and the new combination *Endocena buckii* (Lendemer & B. P. Hodk.) I. Schmitt, Fryday & Pérez-Ortega is proposed. The thallus structure of the sorediate taxon is closer to that of the subcrustose form of *E. informis* (with which it is sympatric) than *E. buckii*, and also marginally closer in the molecular analysis, and so the name *E. informis* var. *falklandica* Fryday, I. Schmitt & Pérez-Ortega is proposed for that taxon.

Taxonomic Section

Endocena Cromb.

J. Linn. Soc., Bot. 15: 226 (1876) [1877] emend. Fryday, I. Schmitt & Pérez-Ortega; type: *E. informis* Cromb. *loc. cit.*

Chirleja Lendemer & B. P. Hodk., *N.Z. Jl Bot.* 50(4): 451 (2012) syn. nov.; type: *Chirleja buckii* Lendemer & B. P. Hodk., *loc. cit.*

(Figs 2, 3, 5 & 6)

Thallus crustose to subfruticose, composed of hollow pustules or pseudopodetia, or solid pseudopodetia with granular phyllocladia on a thin primary thallus in one species; sorediate or not. Sporodochia present in one species.

Apothecia very rare and poorly developed, sessile, up to 2 mm diam., irregular, strongly concave, margin flexuose, irregular; *disc* strongly concave, pale pink-buff. No functional hymenium seen (Fig. 3).

Chemistry. Thamnic acid by TLC.

Apothecia are reported here for the first time from the genus from four collections, two from *E. informis* var. *falklandica* and two from var. *informis* (see under ‘specimens

seen' for the respective taxa), but all are poorly developed (Fig. 3). They apparently first appear as thalline warts with a white, granular surface that expands and becomes pinkish as the white granules are dispersed on the expanding apothecium surface, which finally opens to reveal the apothecium disc.

Endocena buckii (Lendemer & B. P. Hodk.) I. Schmitt, Fryday & Pérez-Ortega comb. nov.

MycoBank No.: MB 819789

Chirleja buckii Lendemer & B. P. Hodk., *N.Z. Jl Bot.* 50(4): 451 (2012); type: Chile, Prov. Tierra del Fuego, Comuna Timaukel, Parque Nacional Alberto de Agostini, Isla Grande de Tierra del Fuego, S side of Seno Agostini, opposite Monte Buckland, 0–50 m elev., very wet *Nothofagus* forest with hepatic carpet on forest floor, on wood, 25.i.2011, *W. R. Buck* 57033 (NY 1726379)—holotype!; CONC—isotype).

(Fig. 2A & B)

Description adapted from Lendemer & Hodkinson (2012).

Primary thallus thin, shiny, varnish-like, white, with scattered pale green ecorticate granules; secondary thallus of prostrate to suberect pseudopodetia with ecorticate granules resembling those found on the primary thallus; pseudopodetia solid, typically simple though sometimes with irregular branching; *photobiont* green, coccoid; *cells* globose, 7–10 µm diam.

Apothecia not observed.

Sporodochia borne on both the primary and secondary thallus, often at the apices of the pseudopodetia, hemispherical, cream-coloured and usually with a distinctly pinkish hue, 0.5–1.5 mm diam.; *conidiophores* not apparent; *conidia* hyaline, formed from hyphae derived from the primary thallus or pseudopodetia that thicken and become constricted at irregular intervals; transversely septate, 1(–2)-celled, 5–10 × 4–7 µm.

This species is known only from the type collection which was growing on the underside of a tree in a wet *Nothofagus* forest in southern Chile (Fig. 4C).

Endocena informis var. informis Cromb.

J. Linn. Soc., Bot. 15: 226 (1876) [1877]; type: Chile, Port Grappler, 2.xii.1868, *R. O. Cunningham* (E 00429965)—holotype! (or, if not, lectotype, designated here. See discussion on typification below).

Siphula subtabularis Nyl. *Lich. Fueg. Patag.*: 3 (1888) syn. nov.; type: Argentina, Isla de los Estados, Port John, 1882, *C. Spegazzini* (H 9 503 897)—holotype).

(Fig. 2C & D)

Thallus ± crustose-placodioid (Fig. 2C) to subfruticose, or truly fruticose (Fig. 2D) as originally described by Crombie (1876 [1877]). Composed of crowded, branched lobes, up to 1.6 mm wide, but often all lobes irregularly isodiametric to shortly elongated, 0.7–2.8 × 0.7–1.0 mm; ivory white, matt, epruinose, convex, solid when young but soon developing a hollow interior, some lobe apices breaking open to reveal the hollow interior, inner surface rough or slightly granular and appearing sorediate.

Apothecia rare and poorly developed, observed on only two collections (*Imshaug* 40362, *Pérez-Ortega* 2631). See generic account above for description.

Conidiomata not observed.

Chemistry. K+ bright yellow, C–, Pd+ yellow, UV+ orange; thamnolic acid by TLC.

There are 117 collections of this taxon in MSC, with 32 from the Falkland Islands and 46 from Isla de los Estados. However, its range extends throughout southern Tierra del Fuego and northward along coastal Chile at least as far as Región de los Lagos (42°S). There is a distinct change in morphology along this latitudinal gradient, with collections from the west coast of Chile (including the type collection from Port Grappler, 49°26'S) having erect, subfruticose lobes (Figs. 2D, 5 & 6), whereas those from the southern part of the range (Tierra del Fuego, Isla de los Estados and the Falkland Islands) have decumbent lobes and appear almost crustose (Fig. 2C). The two forms are sympatric at Puerto Cutter (53°22.5'S, 72°25'W; Fig. 4A) on the Brunswick Peninsula and Seno del Almirantazgo (54.56989°S,

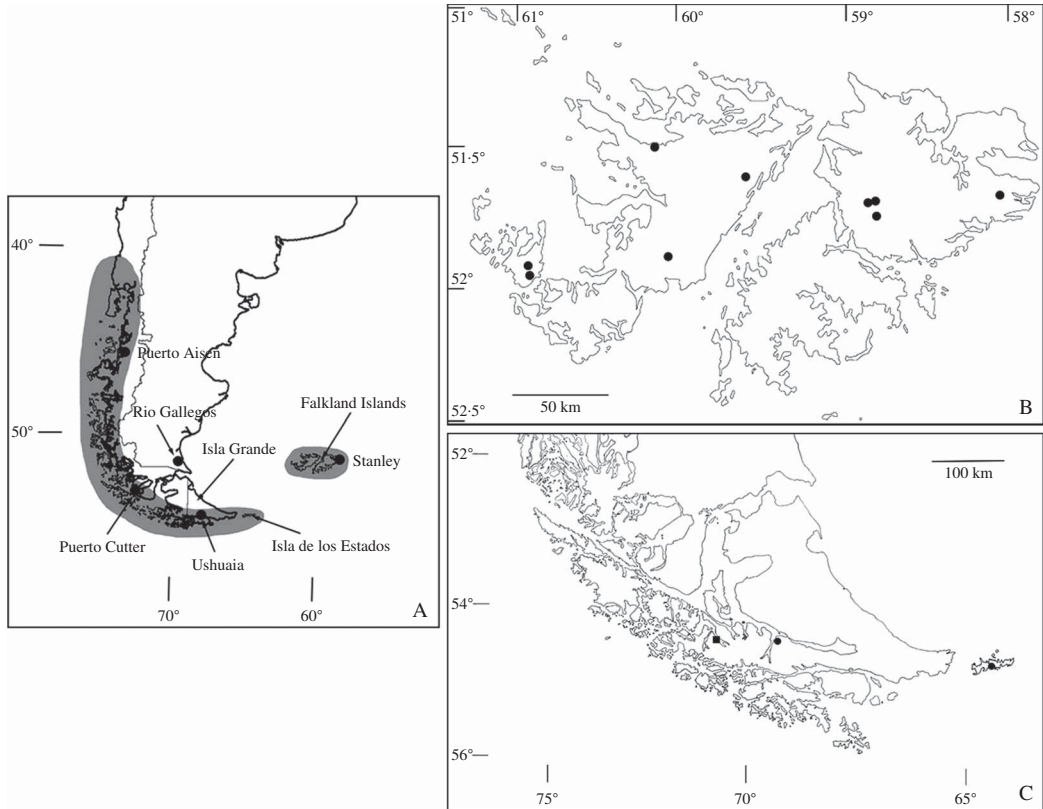


FIG. 4. Distribution of *Endocena* spp. A, projected distribution of *E. informis* var. *informis* from known collections; B & C, voucher collections of *E. informis* var. *falklandica* (●) and *E. buckii* (■).

69.13509°W) in southern Chile where both can be observed in a single collection, even appearing to be part of the same thallus. A range of intermediates also occurs indicating that only one taxon is involved, which is confirmed by the molecular data. The nature of the substratum appears to be an important driver of thallus morphology and this, in turn, is possibly explained as a response to the differences in precipitation between the hyperoceanic climate of the west coast of Chile, where annual precipitation is in excess of 2000 mm (e.g. Puerto Aisen: 2238.5 mm y^{-1} ; Fig. 4A), and the drier south coast of Isla Grande de Tierra del Fuego, Isla de los Estados and the Falkland Islands, which experiences less than 600 mm y^{-1} (e.g. Ushuaia: 529 mm y^{-1} ; Fig. 4A). It is hypothesized that this wetter climate promotes bryophyte growth which provides support for

the lobes of *Endocena informis* that consequently take on a fruticose habit.

The holotype specimen of *Siphula subtabularis* in H was annotated as *Endocena informis* Cromb. by Rolf Santesson in 1953, which is clearly correct. Because this specimen is in the herbarium where Nylander worked, it must be designated as the holotype. If a duplicate specimen is present in Spegazzini's herbarium in Universidad Nacional de La Plata (LPS) it is unlikely that Nylander saw it and so it could not be considered as the holotype.

Selected specimens examined. Argentina: Tierra del Fuego: Isla de los Estados, Puerto Basil Hall, on plateau on S side of Puerto Abrigado, -54.76666° , -64.25000° , *Astelia* bog, 1971, *Imshaug* 51325 & *Ohlsson* (MSC0008898); *ibid.*, Bahía Flinders, on ridge summit at head of W part of bay, -54.81667° , -64.60000° , exposed rock with small *Nothofagus antarctica*, 1971, *Imshaug* 53439 & *Ohlsson*,

(MSC0008836); *ibid.*, Bahía Capitan Canepa, on ridge on W side of N arm of bay, -54.83333° , -64.50000° , field-mark, 1971, *Imshaug* 52996 & *Ohlsson*, (MSC0008817); *ibid.*, Puerto Vancouver, on E side of bay, -54.78333° , -64.05000° , cliffs, 1971, *Imshaug* 52060 & *Ohlsson*, (MSC0008808); *ibid.*, Puerto San Juan, at head of bay, -54.75000° , -63.88334° , boggy slopes covered with *Marsippospermum* & occ. heath, 1971, *Imshaug* 51850 & *Ohlsson*, (MSC0008810); *ibid.*, Puerto Celular, summit of mountain on S side of cove, -54.80000° , -64.31667° , 1971, *Imshaug* 52489 & *Ohlsson*, (MSC0004835); *ibid.*, Puerto Cook, at SE corner of bay, -54.76667° , -64.01667° , alpine summit of mountain, 1971, *Imshaug* 51551 & *Ohlsson*, (MSC0004855); Isla Grande de Tierra del Fuego, Bahía Valentin, on E side of bay, -54.90000° , -65.43333° , open *Marsippospermum* bog with scattered *Nothofagus*, 1971, *Imshaug* 50378 & *Ohlsson* (MSC0008894); *ibid.*, Bahía Buen Suceso, on ridge behind bay, -54.80000° , -65.28333° , mosaic of dwarf *Nothofagus* groves and heath, 1971, *Imshaug* 50046 & *Ohlsson* (MSC0008892); *ibid.*, Paso Garibaldi, -54.66667° , -67.91666° , dry border of schistous rock wall, 1969, *Rovainen* 882 (MSC0008828); *ibid.*, to the E of Monte Olivia, Sierra de Sorondo, -54.71667° , -68.11667° , alpine region on summit of mountain, 1971, *Imshaug* 55586, 55602 & *Ohlsson* (MSC0004860, MSC0004861).—**Chile:** *Región de los Lagos:* Hualaihué, Fundación San Ignacio Huinay, $42^{\circ}22'39''S$, $72^{\circ}22'57''W$, 1128 m, peat bog and *Nothofagus* forest, 2014, *Pérez-Ortega* 3336, 3340 (MA); *Región de Magallanes:* Isla Grande de Tierra del Fuego, brazo suroeste del Seno Parry, $54^{\circ}40'32''S$, $69^{\circ}26'25''W$, 2–20 m, peat bog with granite boulders and *Nothofagus betuloides*, 2009, *Pérez-Ortega* 2628 (MAF), 2629, 2630 (epiphytic on *Nothofagus pumilio*), 2631 (with apothecia), 2632 (MA); *ibid.*, Seno del Almirantazgo, Bahía Blanca, $54.56989^{\circ}S$, $69.13509^{\circ}W$, 0–15 m, peat bog and *Nothofagus betuloides* forest, 2009, *Pérez-Ortega* 2624 (MAF), 2625, 2626 (MA); Brunswick Peninsula, Puerto Cutter, along shore N of copper mine, 1967, *Imshaug* 39416, 39437, 39446 & *Harris* (MSC0008787, MSC0008799, MSC0008788); *ibid.*, rock dome of Monte Condor, 1967, *Imshaug* 39480, 39482 & *Harris* (MSC0008789, MSC0008790); *ibid.*, coastal rocks at copper mine, 1967, *Imshaug* 39496, 39539 & *Harris* (MSC0008791, MSC0008792).—**Falkland Islands:** *East Falkland:* Stanley, N peak of Two Sisters, UTM: 21F VC 3073 ($-51^{\circ}41.150'$, $-58^{\circ}1.150'$), 800–900 ft (275 m), outcrops on summit ridge, 1968, *Imshaug* 40362 & *Harris* (MSC; with apothecia); Stanley Common, Two Sisters, -51.681705° , -58.003490° , 125 m, damp *Empetrum* heath, 2015, *Fryday* 10722 (MSC); *ibid.*, -51.68574° , -58.01652° , 280 m, 2015, *Fryday* 10728 (MSC); *ibid.*, -51.68574° , -58.01652° , 280 m, over bryophytes in crevice in rock face, 2015, *Fryday* 10730 (MSC); Mt Kent, east of road, before cliff, -51.67460° , -58.10640° , 425 m, over bryophytes among alpine boulder field, 2015, *Fryday* 10794, 10795, (MSC); Mt Osborne, between The Gap and summit, -51.691550° , -58.850976° , 400 m, over bryophytes on rock ledge, 2015, *Fryday* 11424. *West Falkland:* Port Howard, Mount Maria, lower end of Freezer Rocks, N-facing crags, -51.60989° , -59.55596° ,

230 m, crevice in rock face, 2015, *Fryday* 10886, 10888 (MSC).

Endocena informis var. falklandica
Fryday, I. Schmitt & Pérez-Ortega
var. nov.

MycoBank No.: MB 819788

Similar to *E. informis* var. *informis* but with a crustose thallus that develops soredia.

Type: Falkland Islands, East Falkland, Stanley Common, Two Sisters, -51.68171° , -58.00349° , 125 m, peat in damp *Empetrum* heath, 18 January 2015, *Fryday* 10741 (MSC (0195387)—holotype!).

(Figs 2E & F)

Thallus crustose, effuse white, composed of irregular, flat to convex areoles *c.* 0.3–0.7 mm diam., matt, epruinose. *Soralia* numerous, initially discrete but becoming confluent, greenish white when fresh, becoming cream-coloured with age; *soredia* 0.10–0.15 mm diam. *Photobiont* chlorococcoid; *cells* 10–15 μ m diam.

Apothecia rare and poorly developed, observed on only two collections (*Imshaug* 52644, *Orange* 19571; see 'Additional specimens seen'). See generic account for description.

Conidiomata not observed.

Chemistry. K+ yellow, C–, Pd+ yellow, UV+ orange; thamnolic acid by TLC.

Endocena informis var. *falklandica* is similar to *E. informis* var. *informis* but is readily separated from that taxon by having a crustose, areolate thallus rather than being composed of hollow pustules, and by the presence of soredia. In the field, it is easily distinguished by its dull creamy rather than shiny, ivory white thallus and also because it typically occupies a different ecological niche, being more strictly terricolous than var. *informis*. The new variety occurs predominantly among bryophytes on peat hags (the edge of an area from where the peat has been eroded away and that is consequently drier than surrounding areas) and moribund stems of *Blechnum magellanicum* rather than among other lichens and vascular plants over rocks, which is the most frequent

habitat of var. *informis*. In fact, *E. informis* var. *falklandica* more closely resembles *Ochrolechia frigida* f. *lapuensis* (Vain.) Coppins or *O. inequatula* (Nyl.) Zahlbr., but the thallus of these taxa is C+ red due to the presence of gyrophoric acid.

Endocena informis var. *falklandica* is very frequent on the Falkland Islands (Fig. 4B) but apparently less so in Tierra del Fuego, being known from only two collections (Fig. 4C; see below).

Additional specimens examined. **Argentina:** *Tierra del Fuego:* Isla de los Estados, Puerto Celular, plateau on N side of cove, 54°47'S, 64°19'W, mosaic of *Marsippospermum* bog and heath, 1971, *Imshaug* 52644 & *Ohlsson* (MSC0008826; with apothecia).—**Chile:** *Región de Magallanes:* Isla Grande de Tierra del Fuego, Seno del Almirantazgo, Bahía Blanca, 54°56'989"S, 69°13'509"W, 0–15 m, peat bog and *Nothofagus betuloides* forest, 2009, *Pérez-Ortega* 2623, 2627 (MA).—**Falkland Islands:** *East Falkland:* Mt Osborne, The Gap, UTM 21F UC 7171, 900–950 ft, *Cortaderia*-heath, 1968, *Imshaug* 39903, 39907 & *Harris* (MSC0195393, MSC0195394); *ibid.*, 51°43'44'S, 58°50'32'W, 170 m, on dead *Bolax* cushion in heathland, 2011, *A. Orange* 19559 (NMW); *ibid.*, 51°43'32'S, 58°50'71'W, 200 m, on peaty soil amongst collapsing bush of *Empetrum rubrum*, 2011, *A. Orange* 19571 (NMW C.2011.015.170; with apothecia); *ibid.*, -51°69'155", -58°85'098", 423 m, peat hag in grass heath, 2015, *Fryday* 11425 (MSC0195388); Port William, outcrops on N side of Gypsy Cove, 1969, *Imshaug* 41666 & *Harris* (MSC); Stanley Common, Two Sisters, -51°68'171", -58°00'349", 125 m, damp *Empetrum* heath, 2015, *Fryday* 10721 (MSC0195389). *Weddell Island:* Waterfall Valley, W of settlement, UTM 21F TC 2842, 750 ft, *Empetrum* heath, 1968, *Imshaug* 41857 & *Harris* (MSC0195395); *ibid.*, 400 ft, *Empetrum* heath, 1968, *Imshaug* 41867 & *Harris* (MSC0195396; TLC); *ibid.*, *Empetrum* heath between stream and stone run, 1968, *Imshaug* 41921 & *Harris* (MSC0195397); Circum Peak, NW side, -51°92'760", -60°92'950", 135 m, peat hag, 2015, *Fryday* 10854 (MSC0195390); between Waterfall Valley and Settlement, -51°90'000", -60°94'300", 145 m, peat in *Empetrum* heath, 2015, *Fryday* 10857 (MSC0195391). *West Falkland:* Hill Cove, NE base of French Peaks, UTM 21F TC 8188, 200 ft, stone run, 1968, *Imshaug* 41031 & *Harris* (MSC0195398; TLC); Fox Bay, NE from Sullivan House, UTM 21F TC 8952, 500 ft, outcrops on ridge, 1968, *Imshaug* 42337 & *Harris* (MSC0195399); Port Howard, between Freezer Rocks and Castle Rock, -51°60'989", -59°55'596", 230 m, *Empetrum* heath, 2015, *Fryday* 10891 (MSC0195392).

Distribution and ecology

The three taxa have different distributional ranges. *Endocena informis* var. *informis* is

apparently widely distributed throughout southern South America from the Falkland Islands, through Isla de los Estados and Isla Grande de Tierra del Fuego and northward along the west coast of Chile, at least as far as Región de los Lagos (42°S). It has rarely been reported from Argentina. Calvelo & Liberatore (2002) cite only Grassi (1950) for records of this species, who in turn cites only Zahlbruckner (1917) who reported it from a single collection from the Hornby Mountains on West Falkland. The only other previous reports of which we are aware are those of the type collection of *Siphula subtabularis* from Isla de los Estados (see above) and Stenroos *et al.* (2002) who use a collection from Tierra del Fuego for their phylogenetic analysis. Despite this, collections in MSC indicate that it is frequent on Isla de los Estados (46 collections) and in southern Isla Grande de Tierra del Fuego (18 collections). Its apparent absence from further north in Argentina can probably be explained by the low precipitation resulting from the area being in the rain shadow of the Andes. Annual precipitation in Rio Gallegos, for example, is 242.2 mm compared to 529 mm at Ushuaia (Fig. 4A). The var. *falklandica*, by contrast, although frequent on the Falkland Islands (Fig. 4B) is much less frequent in Tierra del Fuego, being known from only two collections, one from Isla de los Estados and the other from Seno del Almirantazgo in Chile (Fig. 4C). *Endocena buckii* apparently has an even more restricted range, being known from a single collection from Seno Agostini on the Chilean part of Isla Grande de Tierra del Fuego (Fig. 4C).

There is also a report of *E. informis* from Macquarie Island, Australia (Kantvilas & Seppelt 1992?), which is based on a collection made by the Australian Museum Macquarie Island Expedition of 1977–78 (Lowry *et al.* 1978) and further reported by Seppelt (1980). The lichens collected during this expedition were identified by D. J. Galloway (Filson 1981) but we have been unable to locate the voucher collection of *E. informis* in any herbarium and the species is not represented on the Australian Virtual Herbarium (AVH 2017). We consider this report to be extremely dubious. It is the only report of the species

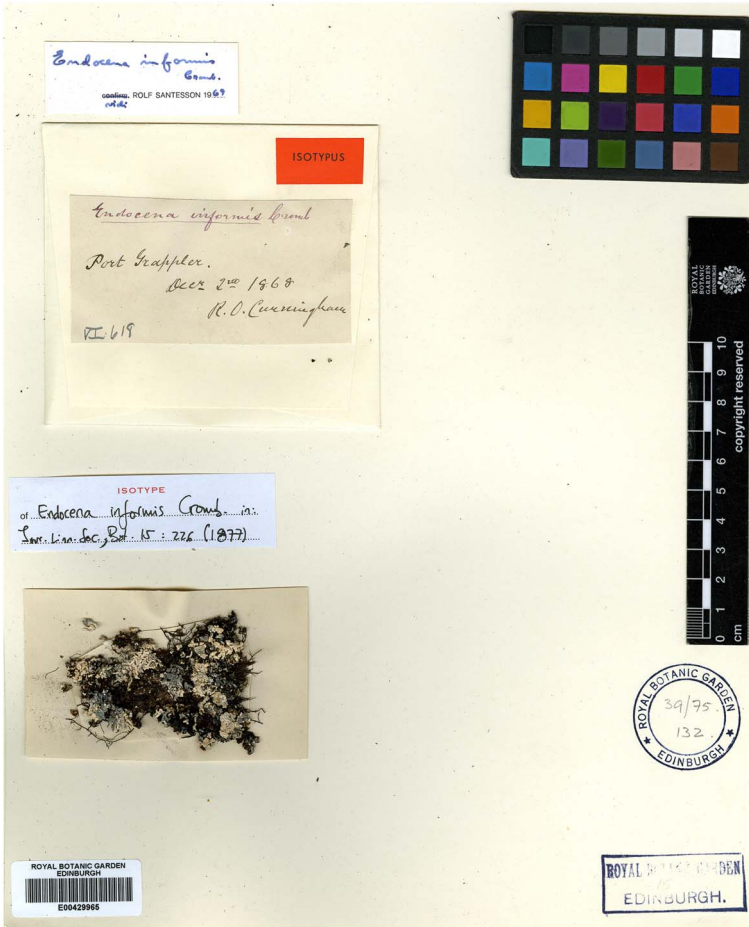


FIG. 5. Herbarium sheet in E with holotype of *Endocena informis*. In colour online.



FIG. 6. Holotype of *Endocena informis*. In colour online.

from outside of southern South America and, although a distribution pattern of SW Chile–Campbell Plateau is not unusual (Fryday & Coppins 2007), if the Macquarie Island report is correct it is strange that the species is not present among the over 4500 collections made by Imshaug and co-workers from Campbell Island and the Auckland Islands held in MSC (Fryday & Prather 2001). Filson (1981) mentioned the collection of *E. informis* by the Australian Museums Expedition but did not include the genus in his key to genera present on the island, which suggests that he also had doubts about its authenticity. We suspect that the specimen is actually a species of *Coccotrema*, *Pertusaria* or *Siphula*, most probably *P. tyloplaca* Nyl., which is frequent on Campbell Island and is also reported from Macquarie Island (Kantvilas & Seppelt 1992?).

The ecology of the three taxa included here in *Endocena* is also somewhat different. Although both varieties of *Endocena informis* are terricolous, primarily occurring in open heathland, the var. *informis* occurs mainly over or among bryophytes whereas the var. *falklandica* is more frequent on soil and peat hags. The single collection of *E. buckii* was made from the underside of a tree in a wet *Nothofagus* forest (Lendemer & Hodgkinson 2012). However, one collection of *E. informis* var. *informis* (Pérez-Ortega 2630) was also made from this substratum and it is possible that *E. buckii* may be a primarily terricolous species but has been overlooked in that habitat.

Typification of *Endocena*

Crombie (1876 [1877]) gives the collection details for *Endocena informis* as “Port Grappler, December 2nd 1868”. There are four co-types listed in the GBIF (2017) and Global Plants (2017a) databases: two in the Natural History Museum, London (BM) and one each in the Royal Botanic Garden Edinburgh (E) and the Botanische Staatssammlung München (M). There is also a collection in the Royal Botanic Gardens Kew (K) and two collections in the Nylander herbarium at the University of Helsinki (H-Nyl.).

The details of these collections are:

BM: Halt Bay, Chile, 21 April 1868 (BM001097291, BM001097292; Global Plants (2017b)); **E:** Port Grappler, Dec. 2nd 1868 (E00429965; Global Plants (2017c), RBGE 2017); **M:** Port Grappler, W coast of Patagonia, 1869, Ex Herb. Kew (M0103214; Global Plants (2017d)); **K:** Halt Bay, Chile, 21 April 1868 (K(M)233841); **H-Nyl.:** Mayne Harbour, W coast of Patagonia, April 1869 (H9211027); summit of hill, Mayne Harbour, 1869 (H 9211028).

Five of these collections, those in BM, E, M and one (H9211027) in H-Nyl, have been annotated “*Endocena informis* Cromb.” by Crombie and he also annotated the specimen in M and one of those in BM “gen. et sp. n”. The other collection in H-Nyl (H9211028) has no original annotation but was annotated “*Endocena informis*” by Santesson in 1953. The specimens in BM would originally have been housed in K and the label of the specimen currently in K, which was written by R. W. G. Dennis, was probably transcribed from the specimen in BM before it was transferred there in 1961. It is probable, but not certain, that this specimen was removed from the BM collection at the same time and retained in K as a separate voucher. Dennis annotated this specimen “*E. informis* var. *digitata* Cromb.” but this is an unpublished name.

The collections in BM, H-Nyl and K are clearly not type material because the date and locality are different from those cited by Crombie. In addition, either the date or the locality on the collection in M must be an error because HMS Nassau spent the early months of 1869 further south or on the east coast of Patagonia before leaving for England at the end of May (Cunningham 1871). Because the collection in E is the only specimen explicitly associated with the date and locality given in the protologue it is considered here to be the holotype (Figs 5 & 6). Because the specimens in BM, M and one of those in H-Nyl (H9211027) were annotated by Crombie they are part of the “original material” (ICNafp Art 9.3; McNeill *et al.* 2012) and are paratypes. The specimen in K is also probably a paratype but because there is

no annotation from Crombie, and Dennis did not note whether it was removed from one of the collections currently in BM, this cannot be stated for certain. Alternatively, it could be argued that the specimens in E and M are both potentially type material because they were annotated as having been collected at Port Grappler and Cunningham collected there only on December 2nd 1868 and so the date on the specimen in M must be an error. In this case, the specimen in E should be selected as the lectotype because it better represents the habitat of the species. Because of this uncertainty, the suggestion made by McNeill (2014) to provisionally designate a lectotype is taken above.

Lichenicolous fungi

Collections of *Endocena informis* var. *informis* sometimes show grey-coloured areas. In one case we identified a lichenicolous fungus (*Sphaerellothecium* sp.) as the cause of such coloration. Three species are known growing on species from the *Icmadophilaceae*: *S. icmadophilae* (R. Sant.) Zhurb. on *Icmadophila ericetorum* (L.) Zahlbr., *S. siphulae* Zhurb. on *Siphula ceratites* (Wahlenb.) Fr. and *S. thammoliae* Zhurb. on *Thammolia vermicularis* (Zhurbenko 2015). Our specimen shows intermediate characters and does not fit with any of these species. It shows superficial vegetative hyphae, scattered immersed to sessile ascomata, 60–70 µm in diameter, and ascospores 11–14 × 5–6 µm. The high specificity exhibited by *Sphaerellothecium* growing on members of *Icmadophilaceae* and the deviant characters shown by our specimen point to the presence of an undescribed species on *Endocena*. Unfortunately, the scarcity of the material prevented us from describing a new taxon. Furthermore, two specimens of *E. informis* var. *informis* were colonized by the plurivorous species *Spirographa fusispora*.

Spirographa fusispora (Nyl.) Zahlbr.

Specimens seen. **Chile:** *Región de los Lagos:* Hualaihué, Fundación San Ignacio Huinay, 42°22'39"S, 72°22'57"W, 1128 m, peat bog and *Nothofagus* forest, 2014, Pérez-Ortega 3336 (sub *Endocena informis* var. *informis*, MA); *Región de Magallanes:* Isla Grande de

Tierra del Fuego, brazo suroeste del Seno Parry, 54°40'32"S, 69°26'25"W, 2–20 m, peat bog with granite boulders and *Nothofagus betuloides*, 2009, Pérez-Ortega 2631 (sub *Endocena informis* var. *informis*, MA).

Sphaerellothecium Zopf sp.

Specimen seen. **Chile:** *Región de los Lagos:* Hualaihué, Fundación San Ignacio Huinay, 42°22'39"S, 72°22'57"W, 1128 m, peat bog and *Nothofagus* forest, 2014, Pérez-Ortega 3340 (sub *Endocena informis* var. *informis*, MA).

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