

## Gone with the wind: sequencing its type species supports inclusion of *Cryptolechia* in *Gyalecta* (Ostropales: Gyalectaceae)

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**Abstract:** *Cryptolechia carneolutea* is the type species of the genus *Cryptolechia*, a rare taxon classified as endangered in the UK, now largely confined to ancient *Fraxinus* trees. The only tree with abundant growth of the species in one of its strongholds, the Slapton Ley National Nature Reserve in Devon, was blown over in a storm in April 2017, making it possible to collect material for molecular studies and transplant specimens to other *Fraxinus* trees in the area. The results of the phylogenetic analysis revealed *C. carneolutea* to be nested within the genus *Gyalecta* (*Gyalectaceae*). This further supports a broad circumscription of *Gyalecta*, after also including species previously placed in *Belonia* and *Pachyphiale* as proposed in other recent studies. It might be possible to introduce an alternative genus concept in *Gyalectaceae*, not schematically based on ascoma type and ascospore number, but presently not enough data are available to proceed with such a novel classification. A review of the taxonomic concept of *Cryptolechia* demonstrates that this name was used inconsistently in the past, and its possible inclusion in *Gyalecta* was anticipated by other authors. As the majority of species presently classified in *Cryptolechia* had been placed in *Gyalecta* before, only five new combinations are required to provide formal inclusion of all taxa in the latter genus: *Gyalecta bicellulata* (Kalb) D. Hawksw. & Lücking comb. nov., *G. caudata* (Kalb) D. Hawksw. & Lücking comb. nov., *G. pittieriana* (Kalb et al.) D. Hawksw. & Lücking comb. nov., *G. saxatilis* (Vězda) D. Hawksw. & Lücking comb. nov. and *G. stellaris* (Müll. Arg.) D. Hawksw. & Lücking comb. nov. We also supersede the previous lectotypification of *Parmelia carneolutea* Turner with the discovery of the holotype specimen in BM.

**Key words:** Devon, *Fraxinus*, *Gyalecta canariensis*, Slapton Ley National Nature Reserve

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### Introduction

The genus known as *Cryptolechia* A. Massal. was instated in its current sense by Vězda (1969) under the name *Gyalectina* Vězda, based on *Parmelia carneolutea* Turner.

Vězda included eight species: *G. carneolutea* (Turner) Vězda, *G. myriadella* (Nyl.) Vězda, *G. nana* (Tuck.) Vězda, *G. plurilocularis* (Vain.) Vězda, *G. saxatilis* Vězda, *G. stellaris* (Müll. Arg.) Vězda, *G. subincolorella* (Nyl.) Vězda and *G. versicolor* (Müll. Arg.) Vězda. In the protologue, Vězda (1969) gave a Latin description of the genus but no diagnosis to distinguish it from other genera in *Gyalectaceae*, particularly *Gyalecta* Ach. and *Pachyphiale* Lönnr. Since Vězda listed *Biatorinopsis* sect. *Polyphragma* Müll. Arg. as a synonym, and Müller (1894) had defined that section as having 8–16 ascospores per ascus, it is probable that Vězda (1969) considered the number of ascospores the principal diagnostic feature, then separating *Gyalectina* from *Gyalecta* by polyspored asci and from *Pachyphiale* in having more than eight but a maximum of 16 ascospores per ascus.

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Hawksworth (1972) pointed out that the type of *Gyalectina*, *Parmelia carneolutea*, had previously been placed in the monospecific genus *Cryptolechia* by Massalongo (1853). According to Hawksworth (1972), Vězda had been aware of this but had argued that Massalongo (1853) had derived his concept of *Cryptolechia* from a specimen erroneously identified as *P. carneolutea* but which actually represented *Pertusaria carneopallida* (Nyl.) Anzi ex Nyl. (currently a synonym of *P. protuberans* (Sommerf.) Th. Fr.). This had led Zahlbruckner (1907) to include *Cryptolechia* as a synonym of *Ochrolechia* A. Massal., before he changed his mind and listed the name as a synonym of *Gyalecta* (Zahlbruckner 1924). When Vězda (1969) established *Gyalectina*, the *Code* was ambiguous as to the correct typification of a generic name when the name of the type species had been misapplied, and so he did not adopt *Cryptolechia* but considered it a synonym of *Pertusaria* (Vězda, in Ainsworth *et al.* 1971: 631). Subsequently, a provision was added to the *Code* to fix the typification of a generic name to the name of its type, not the specimen to which the name had been misapplied (Turland *et al.* 2018: Art. 7.3). As a result, Hawksworth & Dibben (1982) adopted *Cryptolechia* as the correct name for *Gyalectina* and provided the necessary combinations for the species previously placed in *Gyalectina*.

Prior to molecular phylogenetic studies, most workers accepted *Belonia* Körb., *Coenogonium* Ehrenb., *Cryptolechia*, *Dimerella* Trevis., *Gyalecta*, *Pachyphiale* and *Ramonia* Stizenb. as genera in *Gyalectaceae* (Poelt 1969; Purvis *et al.* 1992; Andrés 2003). In addition to *Belonia* (ascomata perithecioid) and *Coenogonium* (thallus filamentous), pre-molecular concepts separated these genera as follows:

- ascospores 1-septate,  $\leq 8$  per ascus = *Dimerella*
- ascospores 1-septate,  $> 8$  per ascus = *Ramonia*
- ascospores 3- to multiseptate or muriform,  $\leq 8$  per ascus = *Gyalecta*
- ascospores 3- to multiseptate,  $> 8$  per ascus = *Cryptolechia*, *Pachyphiale*.

While *Dimerella* was synonymized with *Coenogonium* and excluded from *Gyalectaceae* (Lücking & Kalb 2000; Kauff & Lutzoni 2002; Rivas Plata *et al.* 2006), both *Cryptolechia* and *Ramonia* later also accommodated species with 8-spored asci (Kalb 2007; Aptroot *et al.* 2015), rendering the traditional separation from *Gyalecta* difficult. The phylogenetic position of *Ramonia* within *Gyalectaceae* is uncertain; the available sequences have variously been identified as *Ramonia* sp. and *Xerotrema* sp., and their identity is unclear (Lücking *et al.* 2004; Schmitt *et al.* 2005; Wedin *et al.* 2005; Miadlikowska *et al.* 2014). The separation of *Cryptolechia* from *Pachyphiale* was obscure from the beginning. Zahlbruckner (1907) considered *Cryptolechia* to be based on *Lecanora* (i.e. *Pertusaria*) *carneopallida*, and so in *Gyalectaceae* he only distinguished *Pachyphiale* with polysporous asci and more than 1-septate ascospores, but gave the number of ascospores for that genus as 12 or more. The species of *Pachyphiale* accepted by Zahlbruckner at the time (i.e. *P. carneola* (Ach.) Arnold, *P. corticola* Lönnr. and *P. fagicola* (Arnold) Zwackh) had (8–)24–48 spores per ascus but, for unknown reasons, Zahlbruckner did not consider *Parmelia carneolutea* as belonging here. The latter was later included in *Pachyphiale* by Sampaio (1921) and in *Gyalecta* by Zahlbruckner (1924).

When Vězda (1969) erected *Gyalectina*, he apparently intended to separate species with  $\leq 8$  spores per ascus (*Gyalecta*), (8–)12–16 spores per ascus (*Gyalectina*) and (8–)24–48 spores per ascus (*Pachyphiale*). This is supported by his combination of *Gyalecta geoioides* Vain., a species with 24–48-spored asci, into *Pachyphiale* in the same work, but contradicted by his acceptance of *Lecidea subincolorella* Nyl., a species with 8-spored asci, in *Gyalectina*. Vězda's concept was nevertheless broadly accepted, although modified in subsequent works. Poelt (1969) distinguished the three genera as follows:

- *Gyalecta*: asci 8-spored, (transversely septate to) muriform; apothecia variously coloured
- *Gyalectina*: asci (8–)12(–16)-spored, transversely septate; apothecia yellowish

– *Pachyphiale*: asci (8–)16(–48)-spored, transversely septate; apothecia reddish brown.

Poelt (1969) thus added apothecial colour as an additional diagnostic feature to separate *Gyalectina* from *Pachyphiale*. This concept was adopted by Purvis *et al.* (1992) and Smith *et al.* (2009) for *Cryptolechia*, although a similar range of colour variation, from pale yellowish to red-brown, was accepted within the single genus *Gyalecta*. Other authors, such as Sipman (2005), relied solely on ascospore number. Kalb (2007) was the first to present a key to the species accepted in *Cryptolechia* at the time; however, the already weak separation between *Gyalecta*, *Cryptolechia* and *Pachyphiale* was further challenged by Kalb's (2007) combination of *Pachyphiale geocoides* (Vain.) Vězda (with 24–48-spored asci) into *Cryptolechia*, and by accepting a second species with 8-spored asci in that genus besides *C. subincolorella*, namely *C. caudata* Kalb. While Kalb (2007) did not discuss *Pachyphiale*, he realized the problem of generic concepts by stating: '*Cryptolechia* has its closest generic connections with *Gyalecta*, and there are indeed species, e.g. *C. geocoides*, which are habitually not distinguishable from species of *Gyalecta*. In these cases, I have used the spore number per ascus as the discriminating character as is also done in the literature [...]. Further results based on molecular genetic studies may reveal that *Cryptolechia* is only a later synonym of *Gyalecta* ...' (Kalb 2007: 308). Van den Boom & Vězda (2005) further added to the confusion when describing the new species *Gyalecta canariensis* van den Boom & Vězda, with (8–)12–16-spored asci, in the genus *Gyalecta*, not even discussing *Cryptolechia carneolutea* as a possibly similar taxon. Notably, *G. canariensis* was subsequently synonymized with that species (van den Boom 2010; but see below).

From the above considerations, it becomes obvious that there are no grounds to maintain the separation of *Cryptolechia* and *Pachyphiale* from *Gyalecta* using the number of ascospores per ascus or characters such as ascoma colour. This view is supported by molecular evidence: Baloch *et al.* (2010) found the genera

*Belonia* and *Pachyphiale* nested within *Gyalecta* and subsequently both were formally synonymized with the latter (Baloch *et al.* 2013). At that time, it seemed logical to also subsume *Cryptolechia* within *Gyalecta*, but the only sequences then available, from a specimen identified as *Cryptolechia* sp. from Kenya (Mangold *et al.* 2008) and later labelled *C. nana* (Rivas Plata *et al.* 2013), either clustered with the genus *Leptra* Scop. (mtSSU: EU075572) or represented a contaminant in *Dothideomycetes* (nuLSU: EU075620). To finally shed light on this conundrum, we generated sequence data for the mitochondrial small subunit rDNA (mtSSU) from recent collections of the type species, *C. carneolutea*. These collections were from a rich population on a tree recently blown over by a storm, c. 250 km west of the five localities in Sussex from which it was first described and illustrated (Turner 1808).

## Material and Methods

### Target species and studied material

*Cryptolechia carneolutea* is a rare but possibly widespread species, occurring in Ireland, England, France, Portugal (including the original material of its synonym, *Pachyphiale lecanorina* J. Steiner 1918), Italy, Macaronesia and Algeria, with scattered and perhaps doubtful records from Canada, the Caribbean, India and New Zealand (Watson *et al.* 1988; Wong & Brodo 1990; Purvis *et al.* 1992; Hafellner 1995; Coppins & Coppins 2002; Sparrius *et al.* 2002; Boom *et al.* 2009; Seaward 2010; Roux 2012; Balaji & Hariharan 2013; Ait Hammou *et al.* 2014; Aptroot & Stech 2018). *Gyalecta canariensis* from the Canary Islands (see above) has been considered to be another synonym (van den Boom 2010), but the orange-brown apothecial disc of the type material suggests it could represent a different taxon. The New Zealand material (Berggren 136 (S-L34083)) was annotated as questionable by Frank Kauff.

In the UK, *C. carneolutea* is classified as endangered, having experienced a size reduction of more than 50% over the last ten years, at least 20% within five years, and a continued decline is expected with fewer than 250 colonies (Woods & Coppins 2012). It was formerly more widespread on *Ulmus* trees but, following their loss due to Dutch elm disease (*Ophiostoma novo-ulmi*) in the 1970s, is now largely confined to ancient and mature *Fraxinus* trees that persist in the same localities (Watson *et al.* 1988). One of the strongholds of *C. carneolutea* is the Slapton Ley National Nature Reserve in Devon, where the species was particularly abundant on one ancient *Fraxinus* tree (Fig. 1A); this was the source tree from which the material used by Letrouit-Galinou

(1973, 1974), to study the ontogeny of the species, was collected in 1971 on an excursion during the First International Mycological Congress (IMC1) based in Exeter. Chicita F. Culbertson also collected a piece on the same excursion and found an unidentified substance, possibly a depside, by TLC (Hawksworth 1972). Unfortunately, that same tree was blown over in a storm in April 2017 (Fig. 1B), evidently weakened by the wood-rotting bracket fungus *Rigidoporus ulmarius*, a basidiome of which was found on the base of the fallen trunk on 13 April 2019. The third author (DLH) was, however, able to rescue some material from the fallen tree with the permission of Natural England and under the supervision of the Field Studies Council warden of the reserve, to serve as voucher for this molecular study: **United Kingdom: England:** South Devon (V.C. 3), Stokenham Parish, Slapton, Slapton Ley National Nature Reserve, northern margin of Slapton Ley east of The Causeway, SX(20)/822.441, alt. c. 2 m, on bark of fallen *Fraxinus*, 22 May 2017, *D. L. Hawksworth* s. n. (B 60 0300001, K(M) 251532). Further pieces of bark from the fallen tree were selected for transplanting the species to younger *Fraxinus* trees further along Marsh Lane, leading from The Causeway to Deer Bridge, also within the National Nature Reserve. The locality lies 244–266 km west of the five localities in Sussex from which the species was first described and illustrated (Turner 1808). Material from the same *Fraxinus*, or an almost adjacent *Fraxinus* that had already been lost in the 1980s, and collected by DLH and Peter W. James in 1970, is distributed in Vězda, *Lich. Sel. Exs.* no. 981 (1971).

### Molecular phylogenetic analysis

Sequence data for the mitochondrial small subunit rDNA (mtSSU) were generated from the material using the Sigma REExtract-N-Amp Plant PCR Kit (St Louis, Missouri, USA) for DNA isolation, following the manufacturer's instructions, except that 40 µl of extraction buffer and 40 µl dilution buffer were used. The mtSSU was amplified using the SSU1R and SSU3R primers (Zoller *et al.* 1999). The PCR reactions consisted of 6 µl distilled water, 0.1 µl of each PCR primer (10 µM), 3.5 µl of SIGMA REExtract-N-Amp<sup>TM</sup> PCR ReadyMix (Sigma-Aldrich) and 2 µl genomic DNA. The PCR cycling conditions were as follows: 95 °C for 5 min, followed by 94 °C for 45 s, 50 °C for 1 min, 72 °C for 1 min 30 s (35 cycles), and a single 72 °C final extension for 10 min. Attempts to generate nuLSU (nuclear large subunit rRNA gene) and *RPB2* (the second largest subunit of nuclear RNA polymerase II) sequences were unsuccessful. PCR samples were visualized on a 1% ethidium bromide-stained agarose gel under UV light and bands were gel-extracted, heated at 70 °C for 5 min, cooled to 45 °C for 10 min, treated with 1 µl GELase (Epicentre Biotechnologies, Madison, WI, USA) and incubated at 45 °C for at least 24 h. Samples were sequenced bi-directionally with the relevant PCR primers at Macrogen Inc. (Korea), and sequences were assembled in DNASTAR SeqMan 4.03 and submitted to GenBank (Table 1).

The mtSSU data obtained were first analyzed within a broad data set representing all families within Ostropomycetes, to elucidate the placement of *Cryptolechia* relative to *Gyalectaceae*. For this purpose, we assembled the new sequence (MK848680) with 728 selected sequences downloaded from GenBank (for Accession numbers see Supplementary Material S1 & S2, available online) in BIOEDIT 7.09 (Hall 1999), then subjected the data to automated alignment with MAFFT 7.244 (Katoh & Standley 2013) and subsequent manual inspection (Supplementary Material S1, available online). The phylogenetic tree was reconstructed by means of maximum likelihood, with 100 bootstrap pseudoreplicates, using RAXML 8.2.0 (Stamatakis 2015). After confirming that *Cryptolechia carneolutea* was placed in *Gyalectaceae* with support (see Supplementary Material S2, available online), we placed the newly generated mtSSU sequence in the context of a 4-gene phylogenetic framework of *Gyalectaceae*, using the additional markers nuLSU, *RPB1* and *RPB2* from sequences obtained from GenBank, with *Coenogonium* as an outgroup (Table 1). All sequences were again assembled in BIOEDIT 7.09 (Hall 1999) and the individual markers were first aligned separately using MAFFT 7.244 (Katoh & Standley 2013) and subsequently combined, after manually checking for topological conflict. Phylogenetic trees were again built by means of maximum likelihood (ML), with 1000 bootstrap pseudoreplicates each, using RAXML 8.2.0 (Stamatakis 2015).

### Results and Discussion

Our analysis resulted in six clades (A–F) within *Gyalectaceae*, with good to strong clade and backbone support (Fig. 2). The earliest diverging clade (A) included *Gyalecta friesii* and *G. ulmi*, followed by *Cryptolechia carneolutea* (B) as supported sister to a clade including *Gyalecta flotowii*, *G. geoica* and *G. truncigena* (C). The next two clades corresponded to *Belonia russula* (D) and *Pachyphiale fagicola* (E), followed by the last, strongly supported clade (F), which included *Gyalecta herrei*, *G. hypoleuca*, *G. jenensis* (type species), *G. schisticola*, *G. thelotremella* and *Belonia herculana* (Fig. 2). Thus, three strongly supported yet unrelated clades (A, C & F) represented taxa consistently classified in *Gyalecta*, and *Cryptolechia carneolutea* appeared nested within the latter, in a similar way to *Belonia* and *Pachyphiale* (Baloch *et al.* 2010, 2013).

Given the rather well-supported topology with rather long stem branches, we considered the possibility of recognizing more than one genus in this constellation while at the



FIG. 1. *Fraxinus* tree at Slapton Ley National Nature Reserve in Devon, source of *Cryptolechia carneolutea* specimens (B 60 0300001, K(M) 251532). A, the *Fraxinus* tree still standing, with David Streeeter and students (April 2013; photograph courtesy of Andy Pratt); B, the fallen trunk, with DLH in the foreground (May 2017; photograph by Thomas Pinches). In colour online.

same time applying traditional taxonomic concepts, i.e. separation of genera based on ascoma type (apothecioid vs. perithecioid) and ascospore number per ascus. In order to retain *Belonia* (D), *Cryptolechia* (B) and *Pachyphiale* (E) as separate genera, *Gyalecta* in its current sense would have to be split into at least three genera (A, C & F). Given the phenotypic variation in the three clades, this is currently difficult to justify as the species in the *G. truncigena* (C) and *G. jenensis* (F) clades in particular are highly similar overall (Figs 3 & 4). The clade formed by *G. friesii* and *G. ulmi* (A) deviates in the large apothecia compared to the other species (Figs 3 & 4), but that is hardly a character deserving recognition at the rank of genus; in other morphological characters, the two species are quite disparate. In addition, the features on which the separation of *Belonia*, *Cryptolechia* and *Pachyphiale* from *Gyalecta* was originally based, namely polysporous asci or

perithecioid ascomata, evolved independently several times in the family (Fig. 2). Considering that the characters used to define the various genera do not form coherent phylogenetic entities, and that *Cryptolechia* was circumscribed as including species with 8-, 12-, 16- and 24–48-spored asci (Kalb 2007), it is obvious that generic classification in *Gyalectaceae* cannot be based on these characters; *Cryptolechia* cannot be separated from *Gyalecta* and *Pachyphiale* on account of the number of ascospores per ascus. Furthermore, asci can develop more than eight spores by different methods (Hawksworth 1987) and it cannot be assumed without critical investigations that similar numbers of ascospores are produced through the same ontogenetic process.

It might be possible in the future to revise the generic classification in the family using characters other than those traditionally applied, such as the internal anatomy and

TABLE 1. GenBank Accession numbers of sequences used for the phylogenetic analysis. Voucher information for *Cryptolechia carneolutea* (in bold) is presented in the Material and Methods section.

Species	mtSSU	nuLSU	RPB1	RPB2
<i>Belonia herculana</i>	—	FJ941886	FJ941896	HM244779
<i>B. russula</i> 1	HM244735	HM244759	—	HM244780
<i>B. russula</i> 2	—	FJ941887	FJ941897	—
<i>B. russula</i> 3	AY648888	—	—	—
<i>Coenogonium leprieurii</i>	AY584698	—	—	AY641032
<i>C. luteum</i>	AY584699	—	—	AY641038
<i>C. pineti</i>	AY300884	—	KR017489	HM244786
<i>Cryptolechia carneolutea</i>	<b>MK848680</b>	—	—	—
<i>Gyalecta flotowii</i>	HM244740	HM244764	KC191655	HM244794
<i>G. friesii</i> 1	KJ766400	KJ766566	KJ766854	—
<i>G. friesii</i> 2	HQ659178	HQ659179	—	—
<i>G. geoica</i>	HM244741	HM244765	—	HM244795
<i>G. herrei</i>	—	AF465449	—	—
<i>G. hypoleuca</i> 1	HM244742	AF465453	KC191656	AY641060
<i>G. hypoleuca</i> 2	HQ659180	—	—	—
<i>G. jenensis</i> 1	KR017330	KR017187	KR017455	KR017509
<i>G. jenensis</i> 2	AY340493	AY340544	—	—
<i>G. jenensis</i> 3	—	AF279391	—	AY641043
<i>G. jenensis</i> 4	AY584705	—	—	—
<i>G. jenensis</i> 5	—	AF465450	—	—
<i>G. jenensis</i> 6	—	—	—	KF875528
<i>G. schisticola</i>	KJ766401	—	KJ766825	KJ766974
<i>G. thelotremella</i>	—	AF465455	—	—
<i>G. truncigena</i> 1	HM244743	HM244766	KC191657	HM244796
<i>G. truncigena</i> 2	—	AF465451	—	—
<i>G. truncigena</i> 3 (as <i>flotowii</i> in GenBank)	AY300889	—	—	—
<i>G. ulmi</i> 1	AY300888	AF465463	KC191658	AY641044
<i>G. ulmi</i> 2	AY584706	—	—	—
<i>G. ulmi</i> 3	—	—	JX000138	—
<i>Pachyphiale fagicola</i> 1	HM244753	—	KC020303	HM244807
<i>P. fagicola</i> 2	—	—	KC191663	—

ontogeny of the ascomata (Letrouit-Galinou 1973, 1974, 1977; Ryan & Nimis 2004; Kauff & Büdel 2005), combined with substratum ecology. A promising feature may be the nature of the paraphyses, which in *Gyalecta flotowii*, *G. geoica* (type species) and *G. truncigena* (clade C) are apically thickened and in *G. herrei* and *G. jenensis* (clade F) remain thin but extend substantially beyond the asci (Ryan & Nimis 2004). There are also correlations between ascoma size, ascospore septation and substratum ecology and the clades in the tree; for instance, clade A features large apothecia, clades D–F are uniform in their substratum preferences, and muriform ascospores are concentrated in clades C, D and F (Fig. 2).

However, these correlations are mostly partial and it is difficult to predict where non-

sequenced species will cluster in the phylogeny. *Gyalecta* s. lat. (including *Cryptolechia*) contains more than 60 taxa (Lücking *et al.* 2017), of which 75% have not yet been sequenced. Species traditionally classified in either *Gyalecta* or *Belonia* do not form monophyletic clades and given the heterogeneous assemblage of species treated in *Cryptolechia* (Kalb 2007) and *Pachyphiale* (Baloch *et al.* 2013), it is unlikely that these will cluster phylogenetically with the only currently sequenced taxa, *C. carneolutea* and *P. fagicola*. Anatomical and ontogenetic studies by Kauff & Büdel (2005), which included *Belonia herculana*, *B. russula*, *Cryptolechia carneolutea*, *C. subincolorella*, *Gyalecta ulmi*, *G. jenensis*, *G. hypoleuca*, *G. thelotremella*, *Pachyphiale carneola* and *P. fagicola*, provide few characters that would correlate with phylogenetically defined

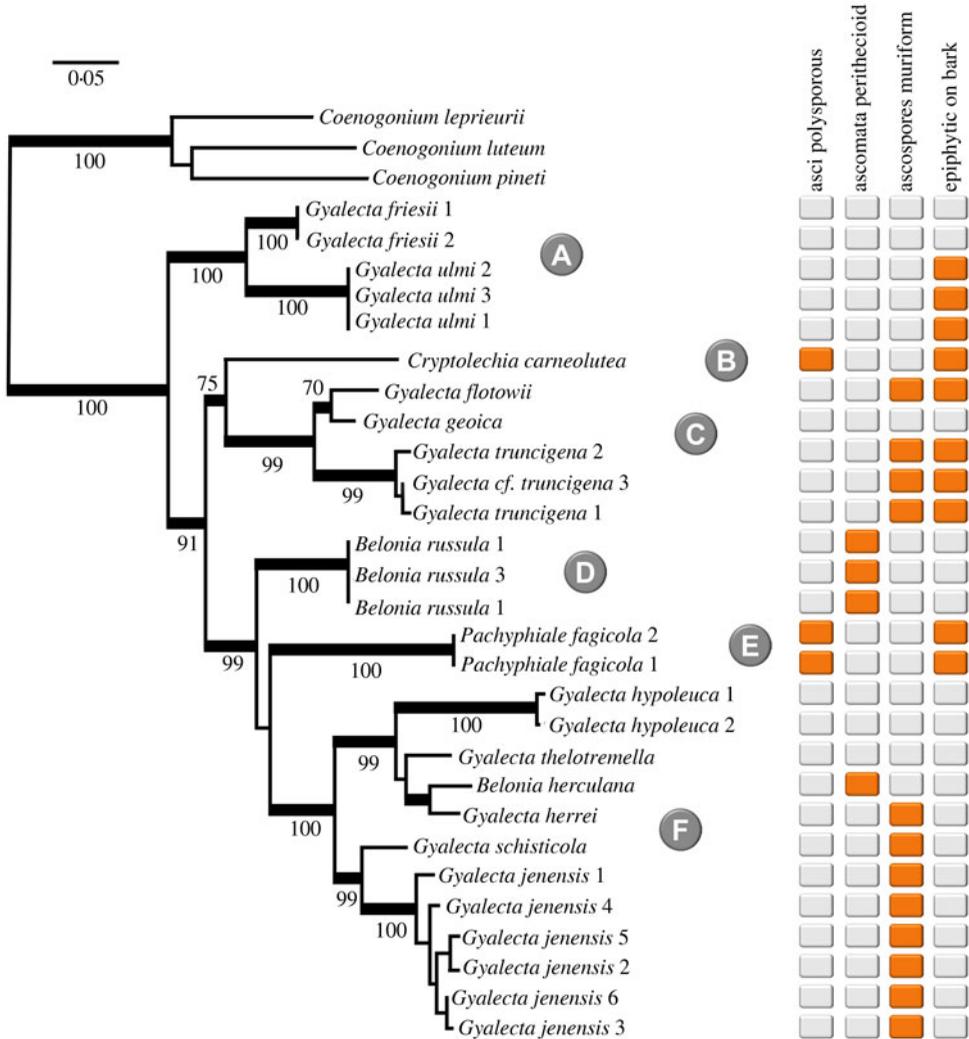


FIG. 2. Best-scoring maximum likelihood tree of *Gyalectaceae* based on four markers (mtSSU, nuLSU, *RPB1*, *RPB2*). Supported branches ( $\geq 70\%$ ) are thickened and bootstrap support values are given. Four main characters are mapped on the tree and clades under discussion are indicated (A–F). *Coenogonium* is used as an outgroup. GenBank Accession numbers for sequences are given in Table 1. In colour online.

clades. For example, the degree of ascoma opening was stated by these authors to be similar between *G. friesii* (clade A) and *C. carneolutea* (clade B), as well as between the two *Belonia* species (clades D & F). The lateral paraphyses (periphysoids) remain distinct in *G. jenensis* (part of clade F) but become reduced in *G. ulmi* (clade A), *C. carneolutea* (clade B) and *P. fagicola* (clade E). All species have a two-layered exciple (Kauff & Büdel 2005).

Comparison with the closely related family *Porinaceae* suggests that a broadly defined *Gyalecta* would not be conceptually different from a broadly defined *Porina* (McCarthy & Malcolm 1997). In the latter case, a much broader taxon sampling now allows the recognition of several genera based on characters previously not considered for generic delimitation (Sobreira et al. 2018). On the other hand, well-defined, monophyletic genera in the revised

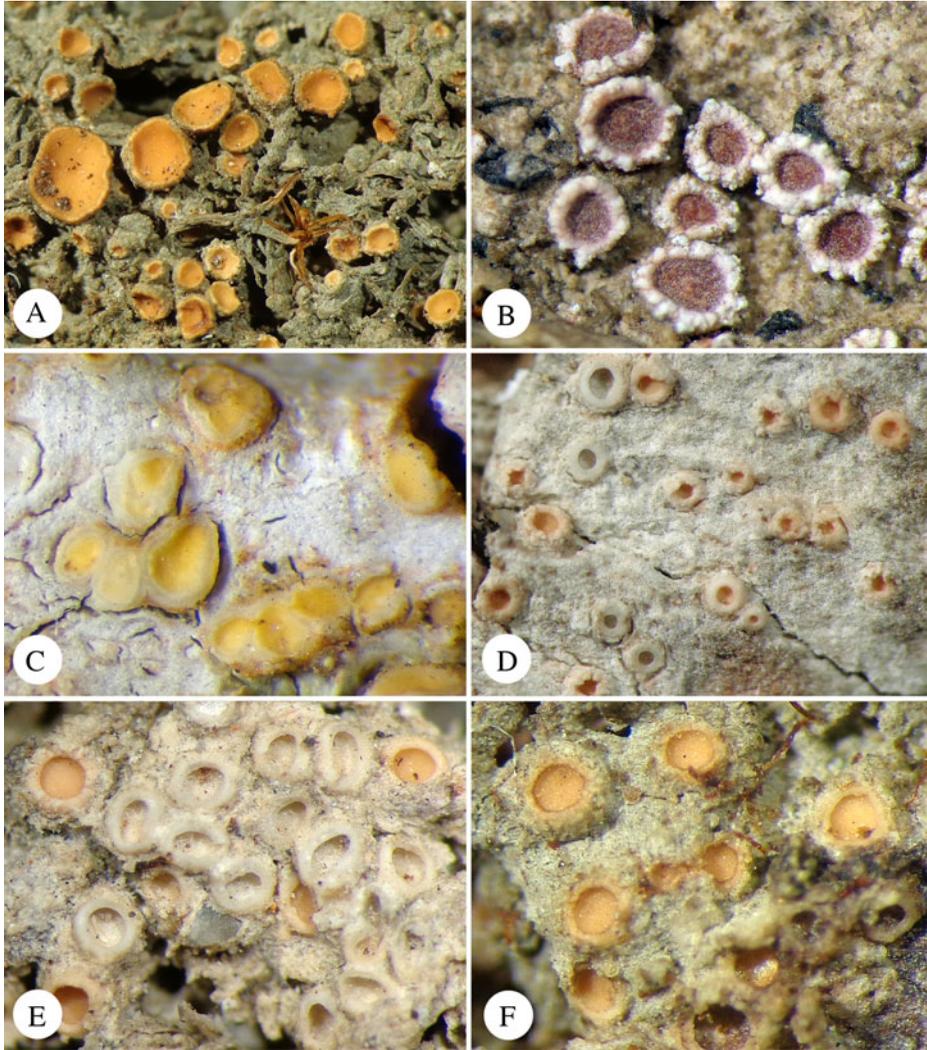


FIG. 3. Morphological variation in *Gyalectaceae* (clades A–C from Fig. 2). A, *Gyalecta friesii* (Greenland, *Alstrup* s. n., *Lich. Groenl. Exs.* 239, B); B, *G. ulmi* (Germany, s. col., s. n., B); C, *Cryptolechia carneolutea* (England, *Hawksworth* s. n., B); D, *Gyalecta flotowii* (Belgium, *Sérusiaux & Rose* 4948, B); E, *G. geoica* (Sweden, *Malme* s. n., *Lich. Suec. Exs.* 686, B); F, *G. truncigena* (France, *Sipman* 22777, B). In colour online.

*Graphidaceae*, such as *Graphis* s. str. (Lücking & Kalb 2018), *Ocellularia* s. str. (Rivas Plata *et al.* 2012) and *Thelotrema* s. str. (Rivas Plata *et al.* 2010), encompass phenotypic variation that is comparable with or even larger than that in a broadly defined *Gyalecta*. These genera all include species with exposed to completely concealed discs (analogous to *Gyalecta* vs. *Belonia*) and with 1- to 8-spored

asci (not polysporous but somewhat analogous to *Cryptolechia* and *Pachyphiale* vs. *Gyalecta*). Furthermore, other characters such as exciple carbonization (*Graphis*, *Ocellularia*) and ascospore amyloidity (*Thelotrema*) also vary. With between 100 and 400 species (Lücking *et al.* 2017), these genera are also much larger than a broadly defined *Gyalecta*, so there is no cogent argument as to why the

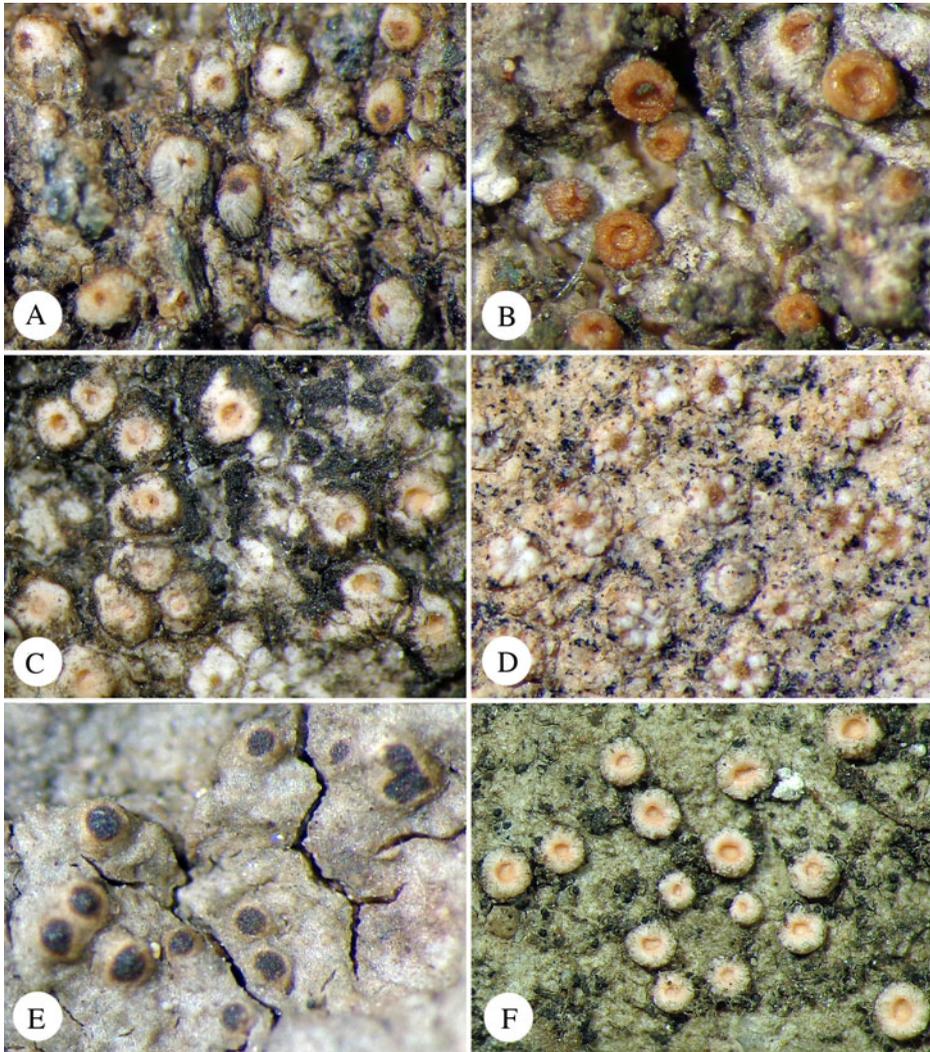


FIG. 4. Morphological variation in *Gyalectaceae* (clades D–F from Fig. 2). A, *Belonia russula* (Norway, Norman s. n., B); B, *Pachyphiale fagicola* (Sweden, Malme s. n., *Lich. Suec. Exs.* 147, B); C, *Gyalecta hypoleuca* (Hungary, Lojka s. n., *Lich. Regni Hung. Exs.* 156, B); D, *G. thelotremella* (Italy, Puntillo s. n., *Lich. Sel. Exs.* 2485, B); E, *Belonia herculana* (Slovakia, Vězda s. n., *Crypt. Exs. Mus. Hist. Nat. Vindob.* 4760, B); F, *Gyalecta jenensis* (France, Mies s. n., B). In colour online.

latter genus should not be treated in a broad sense.

The most crucial taxon regarding generic concepts in *Gyalectaceae* is not *Cryptolechia carneolutea*, which agrees in many aspects with *Gyalecta* s. lat. (Kauff & Büdel 2005; Kalb 2007), but *Pachyphiale fagicola*. The latter forms the longest branch in the tree (clade

E) although is nested with strong support between clades D and F (*Gyalecta* s. lat.). However, besides *Belonia*, it has the most deviating features in the family, including a much reduced thalline margin and apically hooked paraphyses (Itturiaga & Hawksworth 2004; Kauff & Büdel 2005; Smith *et al.* 2009). Accepting this lineage in a broadly

defined *Gyalecta* would render it logically consistent to accept *Cryptolechia* and *Belonia* also, whereas recognition of *Pachyphiale* as a separate entity would require a complete reassessment of *Gyalectaceae* and the recognition of at least six genera, almost none in line with traditionally defined genera.

The limited data available at present prevent the elaboration of a stable classification within *Gyalectaceae* that would recognize more than one genus. Therefore, we prefer to be consistent with the recent treatment that includes *Belonia* and *Pachyphiale* in a broadly defined *Gyalecta* (Baloch *et al.* 2013), and also subsume *Cryptolechia* into the synonymy of *Gyalecta*. However, we encourage further work on this family based on a broader taxon sampling and would certainly not be opposed to a more refined generic concept being developed in the future, should a much expanded data set justify this.

In order to synonymize *Cryptolechia* with *Gyalecta* today, the following eight names have to be transferred back into *Gyalecta*:

***Gyalecta carneolutea* (Turner)  
H. Olivier**

Basionym: *Parmelia carneolutea* Turner, *Trans. Linn. Soc. Lond.* 9: 145 (1808).

Synonyms: *Cryptolechia carneolutea* (Turner) A. Massal; *Lecania carneolutea* (Turner) Mudd

Type: UK, Sussex, *sine loc.*, 1805, W. Borrer (BM 000006709—holotype).

*Note.* This is the single specimen used by Turner to prepare the published plate and it agrees with the Slapton material. It seems probable that the localities in Sussex named in the original paper were given to him by Borrer, either in a letter or verbally when Borrer was invited to visit Turner in Great Yarmouth in 1805–1806 (Hawksworth & Seaward 1978). There is also an undated specimen noted just with ‘Anglia’ in S evidently sent by Turner to Acharius, which Vězda (1969) considered a lectotype, but there is no evidence that this material was used by Turner in preparing the original account and so it cannot be assumed to be ‘original material’. In addition, there is a single sheet of this taxon in Borrer’s herbarium in K (K(M) 136156), labelled in Borrer’s hand as

‘*Lecania carneolutea* (Turner)’, a combination not published until 1861 (Mudd 1861: 140), the year before Borrer’s death. The sheet has five specimens ‘on elm’, but with no locality or date or evidence they were ever seen by Turner. Vězda does not appear to have been aware of either the BM or the K specimens when preparing his account of this taxon.

***Gyalecta carneoluteola* Tuck.**

Synonym: *Cryptolechia carneoluteola* (Tuck.) Kalb

***Gyalecta geocoides* Vain.**

Synonym: *Cryptolechia geocoides* (Vain.) Kalb

***Gyalecta myriadella* (Nyl.) Hellb.**

Synonym: *Cryptolechia myriadella* (Nyl.) D. Hawksw. & Dibben

***Gyalecta nana* Tuck.**

Synonym: *Cryptolechia nana* (Tuck.) D. Hawksw. & Dibben

***Gyalecta plurilocularis* Vain.**

Synonym: *Cryptolechia plurilocularis* (Vain.) D. Hawksw. & Dibben

***Gyalecta subincolorella* (Nyl.) Zahlbr.**

Synonym: *Cryptolechia subincolorella* (Nyl.) D. Hawksw. & Dibben

***Gyalecta versicolor* (Müll. Arg.) Zahlbr.**

Synonym: *Cryptolechia versicolor* (Müll. Arg.) D. Hawksw. & Dibben

For the remaining five names, we provide the new combinations also required in *Gyalecta*:

***Gyalecta bicellulata* (Kalb) D. Hawksw. & Lücking comb. nov.**

Mycobank No.: MB 830755

Basionym: *Cryptolechia bicellulata* Kalb, *Sauteria* 15: 240 (2008).

***Gyalecta caudata* (Kalb) D. Hawksw. & Lücking comb. nov.**

Mycobank No.: MB 830756

Basionym: *Cryptolechia caudata* Kalb, *Biblioth. Lichenol.* 95: 309 (2007).

***Gyalecta pittieriana* (Kalb *et al.*)  
D. Hawksw. & Lücking comb. nov.**

Mycobank No.: MB 830757

Basionym: *Cryptolechia pittieriana* Kalb *et al.*, *Phytotaxa* 42: 39 (2012).

**Gyalecta saxatilis (Vězda) D. Hawksw. & Lücking comb. nov.**

Mycobank No. MB 830758

Basionym: *Gyalectina saxatilis* Vězda, *Folia Geobotanica Phytotax.* **4**: 445 (1969).

Synonym: *Cryptolechia saxatilis* (Vězda) D. Hawksw. & Dibben, *Lichenologist* **14**: 100 (1982).

**Gyalecta stellaris (Müll. Arg.) D. Hawksw. & Lücking comb. nov.**

Mycobank No. MB 830759

Basionym: *Patellaria stellaris* Müll. Arg., *Bot. Jahrb.* **20**: 274 (1894).

Synonym: *Cryptolechia stellaris* (Müll. Arg.) D. Hawksw. & Dibben, *Lichenologist* **14**: 100 (1982).

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SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <https://doi.org/10.1017/S0024282919000240>

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