

Standard Paper

Thelopsis challenges the generic circumscription in the *Gyalectaceae* and brings new insights to the taxonomy of *Ramonia*

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Abstract

The genus *Thelopsis* was classified in the family *Stictidaceae* but its systematic position has never been investigated by molecular methods. In order to determine its family placement and to test its monophyly, fungal DNA of recent collections of *Thelopsis* specimens was sequenced. Phylogenetic analyses using nuLSU, *RPB2* and mtSSU sequences reveal that members of *Thelopsis* form a monophyletic group within the genus *Gyalecta* as currently accepted. The placement of *Thelopsis*, including the generic type *T. rubella*, within the genus *Gyalecta* challenges the generic circumscription of this group because *Thelopsis* is well recognized by the combination of morphological characters: perithecioid ascomata, well-developed periphysoids, polysporous asci and small, few-septate ellipsoid-oblong ascospores. The sterile sorediate *Opegrapha corticola* is also placed in the *Gyalectaceae* as sister species to *Thelopsis byssoidea* + *T. rubella*. Ascumata of *O. corticola* are illustrated for the first time and support its placement in the genus *Thelopsis*. The hypothesis that *O. corticola* might represent the sorediate fertile morph of *T. rubella* is not confirmed because the species is phylogenetically and morphologically distinct. *Thelopsis* is recovered as polyphyletic, with *T. melathelia* being placed as sister species to *Ramonia*. The new combinations *Thelopsis corticola* (Coppins & P. James) Sanderson & Ertz comb. nov. and *Ramonia melathelia* (Nyl.) Ertz comb. nov. are introduced and a new species of *Gyalecta*, *G. amsterdamensis* Ertz, is described from Amsterdam and Saint-Paul Islands, characterized by a sterile thallus with discrete soralia. *Petractis luetkemulleri* and *P. nodispora* are accommodated in the new genus *Neopetractis*, differing from the generic type (*P. clausa*) by having a different phylogenetic position and a different photobiont. *Francisrosea bicolor* Ertz & Sanderson gen. & sp. nov. is described for a sterile sorediate lichen somewhat similar to *Opegrapha corticola* but having an isolated phylogenetic position as sister to a clade including *Gyalidea praetermissa* and the genera *Neopetractis* and *Ramonia*. *Gyalecta farlowii*, *G. nidarosiensis* and *G. carneola* are placed in a molecular phylogeny for the first time. The taxonomic significance of morphological characters in *Gyalectaceae* is discussed.

Key words: *Arthoniales*, *Gyalectales*, lichen, multispory, phylogeny

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Introduction

Recent molecular studies have resolved the systematic position of sterile *Arthoniales* described from Great Britain. *Enterographa sorediata* Coppins & P. James was shown to be the sorediate morph of *Syncesia myrticola* (Fée) Tehler (Ertz *et al.* 2018a), while *Opegrapha multipuncta* Coppins & P. James and *Schismatomma quercicola* Coppins & P. James were both reclassified in the genera *Porina* and *Schizotrema* respectively (Ertz *et al.* 2019). One remaining species is *Opegrapha corticola* Coppins & P. James, a corticolous crustose lichen characterized by a thick grey-green to dull brown thallus and pale greenish fawn to ochraceous soralia often becoming ± patchily continuous in irregular and erose groups 2–3 mm wide (Fig. 1). This sterile species was suspected to be a sorediate morph of *Thelopsis rubella* Nyl. because both taxa often grow together in Great Britain

(Pentecost & James 2009). Recent specimens collected by the second author (NS) produced typical *Thelopsis* perithecia. These were apparently in the same thallus of *O. corticola* and some were close to patches of soralia but without obvious separation between the areas of thalli with soralia and those with perithecia. These latter specimens were generally similar to *Thelopsis rubella* (Fig. 2), supporting the hypothesis that *O. corticola* might be the sorediate morph of *T. rubella*. The spores, however, were much smaller than those typical of *T. rubella*, suggesting that *O. corticola* might be a separate species, having a normally sterile sorediate thallus. In order to test these hypotheses, lichen fungal DNA of specimens was sequenced. The first sequences obtained from *Opegrapha corticola* placed the species surprisingly in the genus *Gyalecta*, as currently circumscribed by Baloch *et al.* (2010, 2013b) and Lücking *et al.* (2019). No sequences of *Thelopsis* were available from GenBank, therefore recently collected specimens of *Thelopsis* (including the generic type *T. rubella*) were used to generate fungal DNA sequences.

Thelopsis [nom. cons.] is a species-poor but widespread genus occurring in temperate and tropical regions on bark and rocks. It is characterized by the combination of a crustose thallus with a

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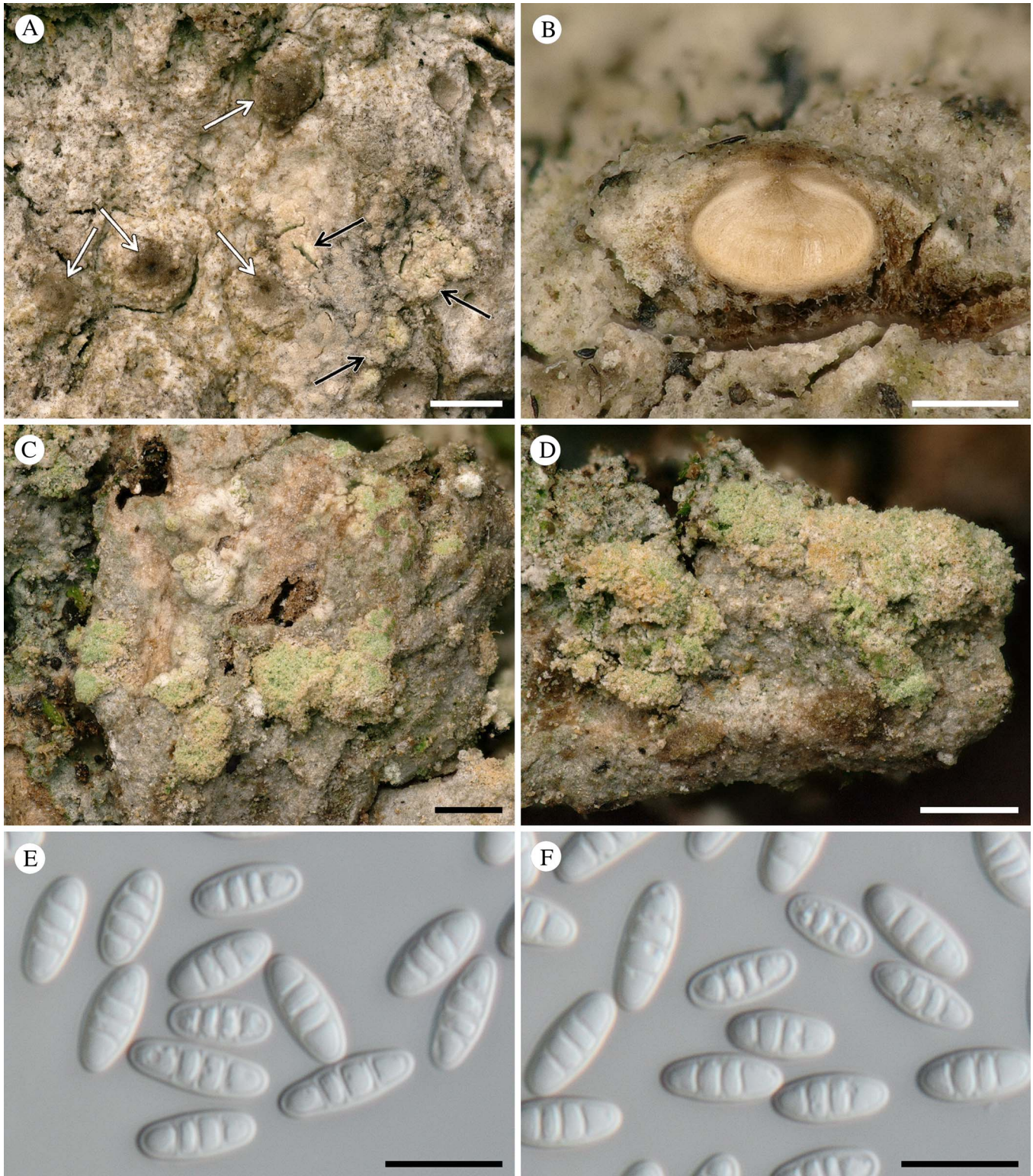


Fig. 1. *Thelopsis corticola*. A, thallus with perithecia (white arrows) and soralia (black arrows) (Sanderson 2053 & Cross). B, section through dry perithecium (Sanderson 1971). C, sterile sorediate thallus when \pm fresh (Sanderson 2002). D, detail of the soralia when \pm fresh (Sanderson 2002). E & F, ascospores in water (Sanderson 1971). Scales: A, C & D = 500 μ m; B = 250 μ m; E & F = 10 μ m. In colour online.

trentepohlioid photobiont; globose semi-gelatinous perithecia; short, stiff periphyses; long, unbranched paraphyses; unitunicate, uniformly thin-walled, polysporous asci with an I+ usually blue wall; simple, transversely septate or (sub-)muriform, hyaline

ascospores (Vězda 1968; Egea & Torrente 1996; Renobales *et al.* 1996; Aptroot *et al.* 1997; Breuss & Schultz 2007; Moon & Aptroot 2009; Rose *et al.* 2009). The genus is currently accepted in the family *Stictidaceae* (Eriksson 1999; Lücking *et al.* 2017),

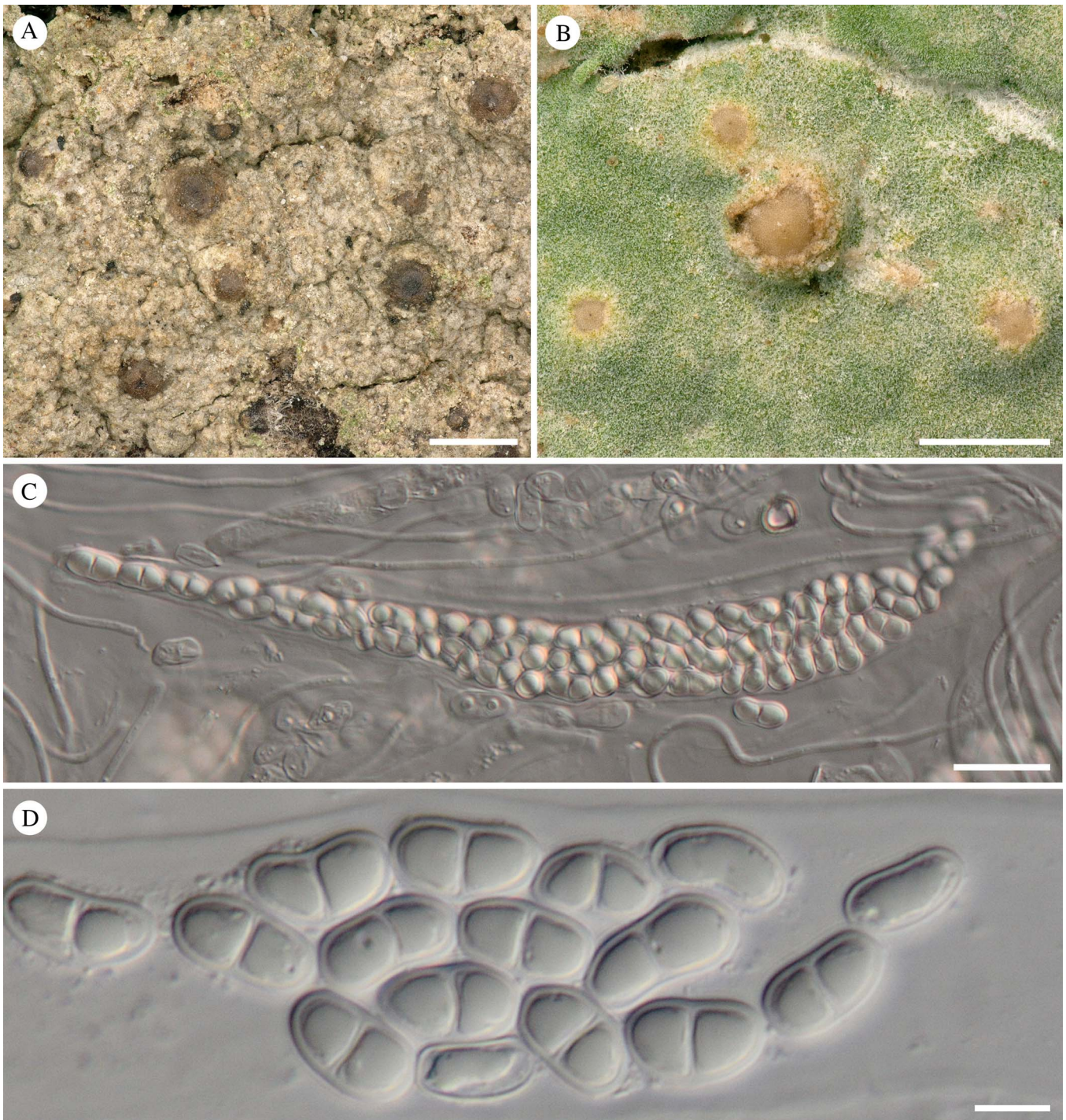


Fig. 2. *Thelopsis rubella* (A, Ertz 20377) and *T. byssoidea* (B–D, Ertz 17384). A, thallus with perithecia. B, byssoid thallus with four perithecia. C, ascus with mature ascospores, in water. D, ascospores, in water. Scales: A & B = 500 μm ; C = 20 μm ; D = 5 μm . In colour online.

but the systematic position has never been tested with molecular data. Vězda (1968) treated six species in his revision of *Thelopsis*. He suggested a close relationship with the genus *Ramonia* within the family *Thelotrema*aceae, but this genus is now accepted in *Gyalectaceae* (Lücking *et al.* 2017). *Ramonia* differs from *Thelopsis* by having urceolate apothecia with the exciple splitting radially and exposing the sunken hymenial disc, while *Thelopsis* has perithecia with the ostiole-opening remaining punctiform. Jørgensen & Vězda (1984) suggested a close relationship between

Thelopsis and *Topelia*. This latter genus differs from *Thelopsis* by having 8-spored asci and broadly ellipsoid muriform ascospores. *Topelia* and *Thelopsis* were placed in *Thelotrema*aceae s. lat. by Vězda (1968) and near *Stictis*, an ostropalean genus, by Sherwood (1977) and Eriksson & Hawksworth (1986). Jørgensen & Vězda (1984) intimated possible placement in the *Gyalectales* because of similarities (ascus type, paraphyses, iodine reaction and excipulum) to *Belonia*. However, they retained these genera in the *Ostropales* because *Belonia* differs by the elongated,

fusiform spores, no periphyses and the presence of small, yellowish oil droplets in the excipulum (characteristic of the *Gyalectales* according to Jørgensen *et al.* (1983)). Breuss & Schultz (2007) published an identification key to all known species of *Thelopsis* and accepted 10 species. With the recent description of new species from Brazil (Aptroot *et al.* 2014a), Cape Verde (van den Boom 2012) and South Korea (Moon & Aptroot 2009; Kondratyuk *et al.* 2016a, b, 2018), the genus now includes 16 accepted species. *Thelopsis* appears heterogeneous because it includes species having pale or entirely black perithecia, which are entirely (e.g. *T. isiaca* Stizenb.) or partially immersed in prominent thalline warts and possessing simple or septate ascospores. *Thelopsis africana* van den Boom was even described as having asci 'with a small ocular chamber, 1–', while periphysoids were not mentioned.

This paper aims to: 1) determine the systematic position of the genus *Thelopsis* and of *Opegrapha corticola*; 2) test whether *O. corticola* is the sorediate morph of *Thelopsis rubella* or a different species; 3) test the monophyly of the genus *Thelopsis*; 4) describe new taxa of sterile sorediate lichens belonging to *Gyalectaceae* as a result of sequencing material from Amsterdam Island and Great Britain.

Materials and Methods

Voucher specimens are deposited in the herbaria BR and BM. The external morphology was studied and measured using an Olympus SZX12 stereomicroscope. Macroscopic images were captured with a Keyence VHX-5000 digital microscope and a VH-Z20R/W/T lens. Hand-cut sections and squash preparations of thalli were mounted in water, a 5% aqueous potassium hydroxide solution (K), or in Lugol's iodine solution (1% I₂) without (I) or with K pretreatment (KI) and studied using an Olympus BX51 compound microscope.

Measurements refer to dimensions in water. Microscopic photographs were prepared using an Olympus BX51 compound microscope fitted with an Olympus SC50 digital camera. Colour reactions of the thallus were studied using K, household bleach (C), K followed by household bleach (KC), crystals of paraphenylenediamine dissolved in ethanol (PD) and long-wave UV (366 nm). Lichen secondary metabolites were investigated using thin-layer chromatography (TLC) in solvent C (Orange *et al.* 2010).

Molecular techniques

Well-preserved specimens lacking any visible symptoms of fungal infection, either freshly collected (less than one month, except for *T. melathelia* Nyl. which was a four-year-old herbarium specimen) or kept in the freezer and frozen less than one month after collection, were used for DNA isolation. Hand-cut sections of the hymenium (*Gyalecta carneola* (Ach.) Hellb., *G. farlowii* Tuck., *Petractis clausa* (Hoffm.) Kremp., *Porina leptalea* (Durieu & Mont.) A. L. Sm., *Thelopsis byssoidea* Diederich, *T. melathelia*, *T. rubella* and a fertile specimen of *Opegrapha corticola* (Sanderson 2053)) or a small number of soredia (*Gyalecta amsterdamensis* Ertz, *G. nidarosiensis* (Kindt) Baloch & Lücking, sterile *Opegrapha corticola*) were used for direct PCR as described in Ertz *et al.* (2015). The material was placed directly in microtubes with 20 µl H₂O. Amplification reactions were prepared for a 50 µl final volume, as detailed in Ertz *et al.* (2018b). A targeted fragment of c. 0.8 kb of the mtSSU rDNA was amplified using primers

mrSSU1 and mrSSU3R (Zoller *et al.* 1999), a fragment of c. 1 kb of the *RPB2* protein-coding gene was amplified using primers fRPB2-7cF and fRPB2-11aR (Liu *et al.* 1999), and a fragment of c. 1.1 kb at the 5' end of the nuLSU rDNA was amplified using primers LIC15R (Miadlikowska *et al.* 2002) and LR6 (Vilgalys & Hester 1990). Both strands were sequenced by Macrogen® using the amplification primers, and with the additional primer LR3 (Vilgalys & Hester 1990) for nuLSU. Sequence fragments were assembled with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'Megablast' searches to verify their closest relatives and to detect potential contaminations.

Taxon selection and phylogenetic analyses

Two matrices using the same three loci (nuLSU, mtSSU and *RPB2*) were assembled: a first dataset for placing the newly sequenced taxa in a phylogeny of the order *Ostropales* s. lat. (now split into *Graphidales*, *Gyalectales*, *Ostropales* s. str., *Thelenellales* and *Odontotrematales*; Kraichak *et al.* 2018; Lücking 2019), and a second dataset for providing a detailed phylogeny of *Gyalecta* s. lat. (= *sensu* Baloch *et al.* (2010, 2013b) and Lücking *et al.* (2019)).

The closest relatives of the new sequences based on BLAST searches were retrieved from GenBank. Additional taxa were selected mainly from Baloch *et al.* (2010), with others from Aptroot *et al.* (2014b), Dou *et al.* (2018), Fernández-Brime *et al.* (2011), Kauff & Lutzoni (2002), Lücking *et al.* (2019), Lumbsch *et al.* (2004), Lutzoni *et al.* (2001), Miadlikowska *et al.* (2014), Orange (2009), Pino-Bodas *et al.* (2017) and Yang *et al.* (2019), in order to include an exhaustive list of taxa belonging to different families of the *Ostropales* s. lat., and a wide array of taxa belonging to the *Gyalectaceae*. One nuLSU sequence of *Gyalecta leucaspis* (AF465462) was not included owing to its poor quality (including 31 'N' distributed throughout the sequence): the species groups with *G. ulmi* (Sw.) Zahlbr. in Kauff & Lutzoni (2002) and Orange (2009). The sequences of taxa listed in Table 1 were aligned using MAFFT v.7.402 (Kato *et al.* 2002) on the CIPRES Web Portal (Miller *et al.* 2010) and manually corrected for errors using Mesquite 3.04 (Maddison & Maddison 2015). Terminal ends of sequences, ambiguously aligned regions, and introns were delimited manually following Lutzoni *et al.* (2000) and excluded from the datasets.

The resulting matrix of *Ostropales* s. lat. consisted of 77 terminals, while the matrix of *Gyalecta* s. lat. consisted of 31 terminals. *Orceolina kerguelensis* (Tuck.) Hertel was used as the rooting taxon in the *Ostropales* s. lat. dataset, with *Coenogonium leprieurii* (Mont) Nyl., *C. luteum* (Dicks.) Kalb & Lücking and *C. pineti* (Ach.) Lücking & Lumbsch selected in the *Gyalecta* s. lat. dataset. The datasets of *Ostropales* s. lat. and *Gyalecta* s. lat. consisted of 2342 (860 for nuLSU, 597 for mtSSU and 885 for *RPB2*) and 2361 (851 for nuLSU, 646 for mtSSU, 864 for *RPB2*) unambiguously aligned sites, respectively. The datasets were deposited in TreeBASE as submissions 26711 and 26712, respectively.

Best-fit evolutionary models were estimated using the Akaike Information Criterion (AIC) as implemented in jModelTest v.2.1.6 (Darriba *et al.* 2012). For *Ostropales* s. lat., the GTR+I+G model was selected for the nuLSU, *RPB2*/1st position and *RPB2*/3rd position datasets, and the TVM+I+G model was selected for the mtSSU and the *RPB2*/2nd position datasets. For *Gyalecta* s. lat., the GTR+I+G model was selected for the nuLSU and mtSSU datasets, the TIM1+I+G model was selected for the

Table 1. Species names, voucher specimens and GenBank Accession numbers of taxa belonging to different families of the *Ostropales* s. lat. Newly generated sequences are in bold. * = outgroup.

Species	Voucher/Source	LSU	mtSSU	RPB2
<i>Absconditella sphagnum</i>	Czech Republic, <i>Palice</i> 11146 (S)	AY300824	AY300872	HM244777
<i>Acarosporina microspora</i>	CBS 338.39; AFTOL-ID 78	AY584643	AY584612	AY584682
<i>Carestiella socia</i>	Norway, <i>Wedin</i> 7194 (UPS)	AY661687	AY661677	HM244782
<i>Claviradulomyces dabeicola</i>	IMI 393994	GQ337897	GQ337898	-
<i>Coccomycetella richardsonii</i>	Sweden, <i>Baloch</i> SW068 (S); EB74	HM244761	HM244737	HM244785
<i>Coenogonium lepreurii</i>	<i>Kauff</i> pa04021998-522 (hb. Frank Kauff); AFTOL-ID 351	AF465442	AY584698	AY641032
<i>C. luteum</i>	<i>Ryan</i> 31430 (ASU); AFTOL-ID 352	AF279387	AY584699	AY641038
<i>C. pineti</i>	Italy, <i>Thor</i> 19164 (UPS)	AY300834	AY300884	HM244786
<i>Cryptodiscus cladoniicola</i>	Denmark, Faroe Islands, <i>Kocourkova</i> et al. (H)	KY661653	KY661675	-
<i>C. pallidus</i>	Sweden, Östergötland, <i>Baloch</i> SW174 (S)	FJ904680	FJ904702	HM244789
<i>Cyanodermella viridula</i>	Sweden, <i>E & C Baloch</i> SW129 (S)	HM244763	HM244739	HM244792
<i>Diploschistes cinereocaesius</i>	AFTOL-ID 328	DQ883799	DQ912306	DQ883755
<i>Fissurina insidiosa</i>	AFTOL-ID 1662	DQ973045	DQ972995	DQ973083
<i>Francisrosea bicolor</i>	Great Britain, <i>Sanderson</i> 2183 (BM)	MT830998	MT831487	-
<i>F. bicolor</i>	Great Britain, <i>Sanderson</i> 2200 (BR)	MT830999	MT831488	MT831991
<i>Geisleria sychnogonoides</i>	GESY7510	KF220304	KF220306	-
<i>Glomerobolus gelineus</i>	OSC 100192; AFTOL-ID 1349	DQ247803	DQ247783	-
<i>Graphis librata</i>	El Salvador, <i>Lücking</i> 28001	HQ639636	HQ639621	JF828945
<i>Gyalecta amsterdamensis</i>	Île Amsterdam, <i>Del Cano, Ertz</i> 21359 (BR)	MT831003	MT831492	MT831993
<i>G. amsterdamensis</i>	Île Amsterdam, <i>Jardin Météo, Ertz</i> 21404 (BR)	MT831004	MT831493	MT831994
<i>G. (Pachyphiale) carneola</i>	Norway, <i>Ertz</i> 22499 (BR)	MT831000	MT831489	-
<i>G. (Cryptolechia) carneolutea</i>	United Kingdom, <i>Hawksworth</i> s. n.	-	MK848680	-
<i>G. caudiospora</i>	<i>Wu</i> GZ17001 (LCU; holotype)	MH345767	-	-
<i>G. (Pachyphiale) fagicola</i>	Sweden, <i>Delin</i> L-163179 (UPS)	-	HM244753	HM244807
<i>G. (Petractis) farlowii</i>	Curaçao, <i>Ertz</i> 18328 (BR)	MT831001	MT831490	-
<i>G. flotowii</i>	Sweden, <i>Svensson</i> 679 (UPS)	HM244764	HM244740	HM244794
<i>G. friesii</i>	<i>Björk</i> 05-973 (UBC); AFTOL-ID 4926	KJ766566	KJ766400	-
<i>G. geoica</i>	Sweden, <i>Svensson</i> 664 (UPS)	HM244765	HM244741	HM244795
<i>G. (Belonia) herculina</i>	Czech Republic, <i>Palice</i> s. n. (F)	FJ941886	-	HM244779
<i>G. herrei</i>	<i>Nimis & Tretiach</i> 1993, 18438 (TSB)	AF465449	-	-
<i>G. hypoleuca</i>	Austria, <i>Hafellner</i> 63694 (UPS)	AF465453	HM244742	AY641060
<i>G. jenensis</i>	<i>Lutzoni</i> 98.08.17-6, (DUKE); AFTOL-ID 361	AF279391	AY584705	AY641043
<i>G. (Belonia) nidarosiensis</i>	Belgium, <i>Ertz</i> 23169 (BR)	MT831002	MT831491	MT831992
<i>G. (Belonia) russula</i>	Sweden, <i>Hermansson</i> 14140 (UPS)	HM244759	HM244735	HM244780
<i>G. schisticola</i>	<i>Gueidan & Miadlikowska</i> (DUKE); AFTOL-ID 1002;	-	KJ766401	KJ766974
<i>G. thelotremella</i>	<i>Nimis & Tretiach</i> 1996, 22375 (TSB)	AF465455	-	-
<i>G. truncigena</i>	Sweden, <i>Nordin</i> 5851 (UPS)	HM244766	HM244743	HM244796
<i>G. ulmi</i>	<i>Scheidegger</i> 30.05.1998 (DUKE); AFTOL-ID 362	AF465463	AY584706	AY641044
<i>Gyalidea hyalinescens</i>	AFTOL-ID 332 as 'hyalinus'	DQ973046	DQ972996	DQ973084
<i>G. praetermissa</i>	Sweden, <i>Svensson</i> 949 (UPS)	HM244768	HM244745	HM244798
<i>Ingvariella bispora</i>	Spain, <i>Llimona</i> s. n.; (BCN) Lich 17183	HQ659185	HQ659174	-
<i>Karstenia rhopaloides</i>	Denmark, <i>Laessøe</i> 12881 (S); EB100	FJ904685	FJ904707	HM244791

(Continued)

Table 1. (Continued)

Species	Voucher/Source	LSU	mtSSU	RPB2
<i>Myeloconis erumpens</i>	New Caledonia, <i>Lumbsch</i> 8233 (F)	KJ449338	KJ449328	-
<i>Neopetractis (Petractis) luetkemuelleri</i>	<i>Nimis & Tretiach</i> 2000 (TSB) - LSU; <i>Geletti & Tretiach</i> 1995 (TSB) - RPB2	AF465454	-	AY641061
<i>N. (Petractis) nodispora</i>	Great Britain, <i>Orange</i> 17559 (NMW)	FJ588711	-	-
<i>Odontotrema phacidiellum</i>	Sweden, <i>Gilenstam</i> 2625 (UPS)	HM244769	HM244748	HM244802
<i>O. phacidioides</i>	Morocco, <i>Palice</i> 11440 (S)	HM244770	HM244749	HM244803
<i>Orceolina kerguelensis*</i>	Kerguelen, <i>Poulsen</i> 456 (C)	AF274116	AF381561	DQ366256
<i>Ostropa barbara</i>	Sweden, <i>Wedin & Baloch</i> SW071 (S)	HM244773	HM244752	HM244806
<i>Petractis clausa</i>	<i>J. Hafellner</i> A 1/2 IAL3 96 (DUKE)	AF356662	-	-
<i>P. clausa</i>	Belgium, <i>Ertz</i> 23174 (BR)	MT831005	-	-
<i>Phlyctis agelaea</i>	<i>Nordin</i> 3028 (UPS)	AY853381	AY853332	-
<i>P. argena</i>	AFTOL-ID 1375	DQ986771	DQ986880	DQ992458
<i>Porina aenea</i>	Sweden, <i>Arup & Baloch</i> SW154 (S)	-	HM244754	HM244808
<i>P. byssophila</i>	Sweden, <i>Nordin</i> 5990 (UPS)	-	HM244755	HM244809
<i>P. lectissima</i>	Sweden, <i>Arup & Baloch</i> SW152 (S)	HM244774	HM244756	HM244811
<i>P. leptalea</i>	Belgium, <i>Ertz</i> 23175 (BR)	-	MT831494	MT831995
<i>Protothelenella sphinctrinoidella</i>	Antarctica, Livingston Island, <i>Lumbsch</i> 19031d (F)	AY607735	AY607747	-
<i>Ramonia (Thelopsis) melathelia</i>	Austria, <i>Ertz</i> 20503 (BR)	MT831006	MT831495	MT831996
<i>R. valenzueliana</i>	<i>Palice</i> 3178 (hb. <i>Palice</i>); as ' <i>Palice</i> 2336'	AY300871	AY300921	-
<i>Sagiolechia protuberans</i>	Sweden, <i>Nordin</i> 5893 (UPS)	HM244775	HM244757	HM244812
<i>S. rhexoblephara</i>	Sweden, 2002, <i>Palice</i> s. n. (hb. <i>Palice</i>)	AY853391	AY853341	-
<i>Schizoxylon albescens</i>	Sweden, <i>Gilenstam</i> 2696a (UPS), <i>Wedin</i> 7919 (UPS)	DQ401144	DQ401142	HM244813
<i>Sphaeropezia capreae</i>	<i>Gilenstam</i> 2560 (UPS); GG2560	AY661684	AY661674	-
<i>S. lyckselensis</i>	Sweden, <i>Gilenstam</i> 2651 (UPS); EB 2012a	JX266158	JX266156	-
<i>Stictis radiata</i>	<i>Jamie Platt</i> 222 (OSC, DUKE)	AF356663	AY584727	AY641079
<i>Thelenella antarctica</i>	Antarctica, Livingston Island, <i>Lumbsch</i> 19006a (F)	AY607739	AY607749	-
<i>Thelopsis byssoidea</i>	Thailand, <i>Ertz</i> 17384 (BR)	MT831007	MT831496	MT831997
<i>T. (Opegrapha) corticola</i>	France, <i>Ertz</i> 17602 (BR)	MT831008	-	MT831998
<i>T. (Opegrapha) corticola</i>	Great Britain, <i>Sanderson</i> 2188 (BM)	MT831009	-	MT831999
<i>T. (Opegrapha) corticola</i>	Great Britain, <i>Sanderson</i> 2202 (BM)	MT831010	-	MT832000
<i>T. (Opegrapha) corticola</i>	Great Britain, <i>Sanderson</i> 2053 (BM)	MT831011	-	MT832001
<i>T. rubella</i>	Belgium, <i>Ertz</i> 18094 (BR)	MT831012	MT831497	MT832002
<i>T. rubella</i>	Italy, <i>Ertz</i> 20377 (BR)	MT831013	MT831498	MT832003
<i>T. rubella</i>	Great Britain, <i>Sanderson</i> 2186 (BM)	MT831014	MT831499	MT832004
<i>Thelotrema lepadinum</i>	India, <i>Lumbsch</i> 19744i	JX421652	JX421365	JX420850
<i>Xyloschistes platytropa</i>	AFTOL-ID 4891	KJ766680	KJ766517	-

RPB2/1st position, the TVM + G model for the RPB2/2nd position and the TrN + G model for the RPB2/3rd position datasets.

Analyses for topological incongruence among loci were carried out for both the three-locus dataset of the *Ostropales* s. lat. and the three-locus dataset of *Gyalecta* s. lat. The single locus datasets were analyzed with a maximum likelihood (ML) approach using the program RAXML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller *et al.* 2010) with 1000 ML bootstrap iterations (ML-BS). The GTRGAMMA

model was used and node support was assessed running 1000 bootstrap replicates. We analyzed the three single locus datasets for their topological incongruence by assuming a conflict significant when two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values $\geq 70\%$ (Mason-Gamer & Kellogg 1996; Reeb *et al.* 2004). Based on this criterion, we detected partial conflict among the nuLSU and RPB2 datasets for *Gyalecta* s. lat. In the nuLSU tree,

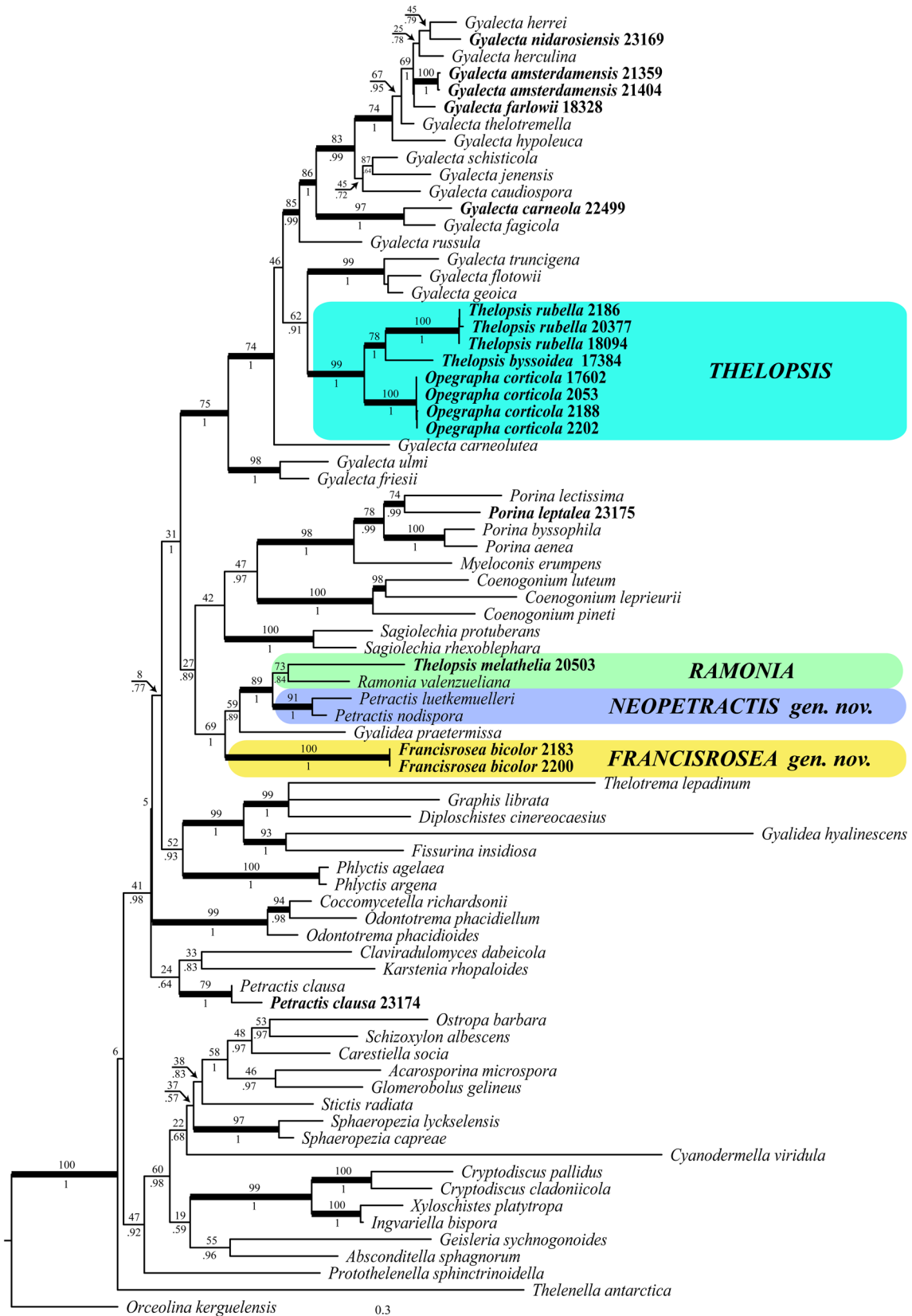


Fig. 3. Phylogeny of *Ostropales* s. lat. (with *Orceolina kerguelensis* as outgroup) based on a dataset of nuLSU, mtSSU and *RPB2* sequences that resulted from the RAxML analysis. Maximum likelihood bootstrap values are shown above internal branches and posterior probabilities obtained from a Bayesian analysis are shown below. Internal branches, considered strongly supported by both analyses, are represented by thicker lines. The newly sequenced samples are in bold and their names are followed by collection numbers of authors, which act as specimen and sequence identifiers. Lineages corresponding to the genera *Francisrosea*, *Neopetractis*, *Ramonia* and *Thelopsis* are highlighted. In colour online.

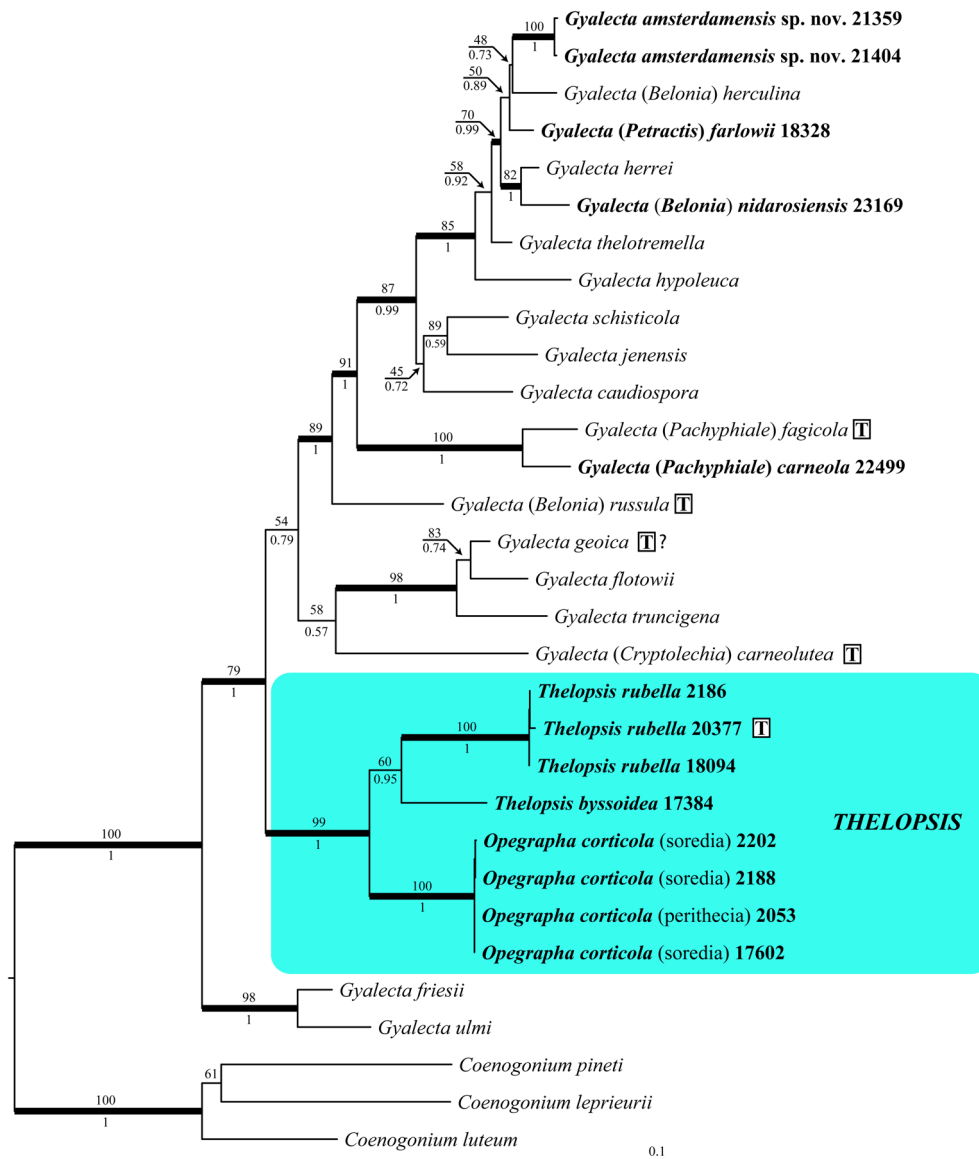


Fig. 4. Phylogeny of *Gyalectaceae* (with three species of *Coenogonium* as outgroup) based on a dataset of nuLSU, mtSSU and *RPB2* sequences that resulted from the RAXML analysis. Maximum likelihood bootstrap values are shown above internal branches and posterior probabilities obtained from a Bayesian analysis are shown below. Internal branches, considered strongly supported by both analyses, are represented by thicker lines. The newly sequenced samples are in bold and their names are followed by collection numbers of authors, which act as specimen and sequence identifiers. The lineage corresponding to the genus *Thelopsis* is highlighted, showing the genus nested in *Gyalecta* s. lat. Generic names in use before Baloch et al. (2013b) and Lücking et al. (2019), together with *Petraetis* (for *P. farlowii*), are shown in brackets. **T** indicates the type of the earlier genus name. In colour online.

Gyalecta ulmi was basal to the rest of *Gyalecta* s. lat. with a bootstrap support of 80%, while in the *RPB2* tree *G. ulmi* was nested in *Gyalecta* s. lat. with a bootstrap support of 88%, taxa of *Thelopsis* and the clade with *Gyalecta flotoiwii* + *G. geoica* + *G. truncigena* being at the base of the tree. Including or removing the *RPB2* of *G. ulmi* from both the single locus and the combined analyses had no impact on the topology of the trees, therefore the nuLSU, mtSSU and *RPB2* datasets were concatenated.

Bayesian analyses were carried out on the three-locus datasets under the selected models for five partitions (nuLSU, mtSSU, *RPB2*/1st, *RPB2*/2nd, *RPB2*/3rd positions), using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v.3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES Web Portal (Miller et al. 2010). For the *Ostropales* s. lat. dataset, two parallel

MCMCMC runs were performed each using four independent chains and 80 million generations, sampling trees every 1000th generation. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 120 002 post burn-in trees of the 160 002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. Similarly, for the *Gyalecta* s. lat. dataset, two parallel MCMCMC runs were performed each using four independent chains and 40 million generations, sampling trees every 1000th generation. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 60 002 post burn-in trees of the 80 002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. For both Bayesian analyses, Tracer v.1.7.1 (Rambaut et al. 2018) was used to ensure that stationarity was reached by plotting the log-likelihood values of the sample

points against generation time, making sure that the ESS values were much higher than 200. Convergence between runs was also verified using the PSRF (Potential Scale Reduction Factor), where values were all equal or close to 1.000.

In addition, a maximum likelihood analysis was performed using RAxML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller *et al.* 2010) with 1000 ML bootstrap iterations (ML-BS). The two three-locus datasets were divided into five partitions (nuLSU, mtSSU, *RPB2*/1st, *RPB2*/2nd, *RPB2*/3rd positions) with the GTRGAMMA model.

The ML trees did not contradict the Bayesian tree topologies for the strongly supported branches. Therefore, only the ML trees are shown with the ML-BS values added above the internal branches and the PP values added below (Figs 3 & 4). Internodes with ML-BS ≥ 70 and PP ≥ 0.95 were considered to be significant. Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results

Phylogenetic analysis

Forty-four new sequences (17 nuLSU, 13 mtSSU, 14 *RPB2*) were obtained for this study and 144 additional sequences (54 nuLSU, 52 mtSSU, 38 *RPB2*) were retrieved from GenBank (Table 1). The RAxML tree obtained from the combined three-locus analysis of the *Ostropales* s. lat. dataset is shown in Fig. 3. The main well-supported lineages are in accordance with the results obtained by Baloch *et al.* (2010) and Spribille *et al.* (2020: fig. 7). The *Ostropales* s. lat. are now split into *Graphidales*, *Gyalectales*, *Ostropales* s. str., *Thelenellales* and *Odontotrematales* (Kraichak *et al.* 2018; Lücking 2019), but Spribille *et al.* (2020: fig. 7) includes *Graphidales* and *Odontotrematales* in *Gyalectales*. We prefer to use *Ostropales* s. lat. which is more appropriate for the topology of our tree, the backbone of which is poorly resolved. The genus *Thelopsis* is recovered as polyphyletic. The type of the genus, *T. rubella*, forms a well-supported lineage with *T. byssoidea* and *Opegrapha corticola* within the genus *Gyalecta* sensu Baloch *et al.* (2010) and Lücking *et al.* (2019). *Thelopsis melathelia* is the sister species to *Ramonia valenzueliana* (Mont.) Stizenb., a relationship supported only by the RAxML analysis.

The RAxML tree obtained from the combined three-locus analysis of the *Gyalecta* s. lat. dataset is shown in Fig. 4. The generic names in use before Baloch *et al.* (2013b) and Lücking *et al.* (2019) are shown in brackets. The topology of this tree is in accordance with the results obtained by Lücking *et al.* (2019). Relationships within *Gyalecta* s. lat. are generally well supported. The analysis of this reduced dataset of *Gyalectaceae* resulted in 19 more unambiguously aligned sites than in the *Ostropales* s. lat. dataset, and in a slightly different placement of *Thelopsis* as sister to the clade from *Gyalecta carneolutea* to *G. amsterdamensis* (Fig. 4). In addition to the genus *Thelopsis* and *Opegrapha corticola*, other taxa are newly included in the phylogeny. *Gyalecta carneola* is the sister species of *Gyalecta fagicola* (Hepp ex Arnold) Kremp. *Gyalecta nidarosiensis* is sister to *G. herrei* Vězda. *Gyalecta farlowii* and the new species *G. amsterdamensis* cluster close to *G. herculina* (Rehm) Baloch *et al.*

The family *Gyalectaceae* is not monophyletic in our tree (Fig. 3). The genera *Francisrosea*, *Ramonia*, *Neopetractis* and '*Gyalidea*' *praetermissa* form a different lineage sister to the families *Sagirolechiaceae* + *Coenogoniaceae* + *Porinaceae*. However, this relationship is not supported. Miadlikowska *et al.* (2014) wrote

that '*Petractis nodispora* and *P. luetkemulleri* (Stictidaceae), *Gyalidea praetermissa* (Graphidaceae) and *Ramonia* sp. (*Gyalectaceae*) should be accommodated in different genera outside of their respective families'. Further studies using more loci and more taxa are needed to investigate whether this lineage might represent a different family or not. The topology is unresolved, and it is unclear what might be observed when more markers are added, and the amount of missing data is reduced. Merging the families *Coenogoniaceae*, *Gyalectaceae*, *Porinaceae* and *Sagirolechiaceae* into a single family is also possible, particularly as these families include few genera, a big contrast to the family *Graphidaceae*. In this context, the lineage with *Petractis clausa* is also orphaned and needs further studies. We could confirm the published nuLSU sequence of *P. clausa* by sequencing a second specimen (Fig. 3), but we were unsuccessful obtaining mtSSU and *RPB2* sequences.

Taxonomy

Francisrosea Ertz & Sanderson gen. nov.

Mycobank No.: MB 836494

A new genus in the family *Gyalectaceae*, distinguished by having an isolated phylogenetic position as sister to a clade including *Gyalidea praetermissa* and the genera *Neopetractis* and *Ramonia*, and characterized by an inconspicuous thallus with small discrete erumpent soralia lacking acetone-soluble secondary metabolites detectable by TLC.

Type species: *Francisrosea bicolor* Ertz & Sanderson.

Etymology. Named after Francis Rose for his outstanding contribution to the protection and study of the lichen flora of forests with a long historical continuity, in Great Britain.

Description. See specific description below.

Francisrosea bicolor Ertz & Sanderson sp. nov.

Mycobank No.: MB 836495

Distinguished from all known *Gyalectaceae* by a unique phylogenetic position as sister to a clade including *Gyalidea praetermissa* and the genera *Neopetractis* and *Ramonia*, and characterized by an inconspicuous thallus with small discrete erumpent soralia, pale greenish inside, orange-ochre at the surface, and by lacking acetone-soluble secondary metabolites detectable by TLC.

Type: Great Britain, V.C.11, New Forest, Busketts Wood, Little Stubby Hat, Grid Ref. SU30532 10627, *Quercus-Fagus-Ilex* pasture woodland, wound track on ancient *Fagus sylvatica*, 27 September 2016, Sanderson 2200 (BR—holotype!).

(Fig. 5A & B)

Thallus inconspicuous, immersed in the bark, only visible by the soralia. **Soralia** erumpent, first punctiform, later becoming \pm rounded to ellipsoid, erose, slightly convex and elevated above the substratum, 0.2–0.8(–1) mm diam., pale greenish inside, orange-ochre at the surface and mainly at the margins, discrete, scarcely distributed, rarely 1–4 becoming confluent and forming patches up to c. 1.5 mm across. **Soredia** without projecting hyphae, (25–)30–50(–70) μ m diam., composed of hyaline smooth hyphae 4–6(–7) μ m diam., I–, KI– and trentepohlioid cells 6–13 μ m diam. in short chains of 2–6(–8) cells. Crystals absent (polarized light).

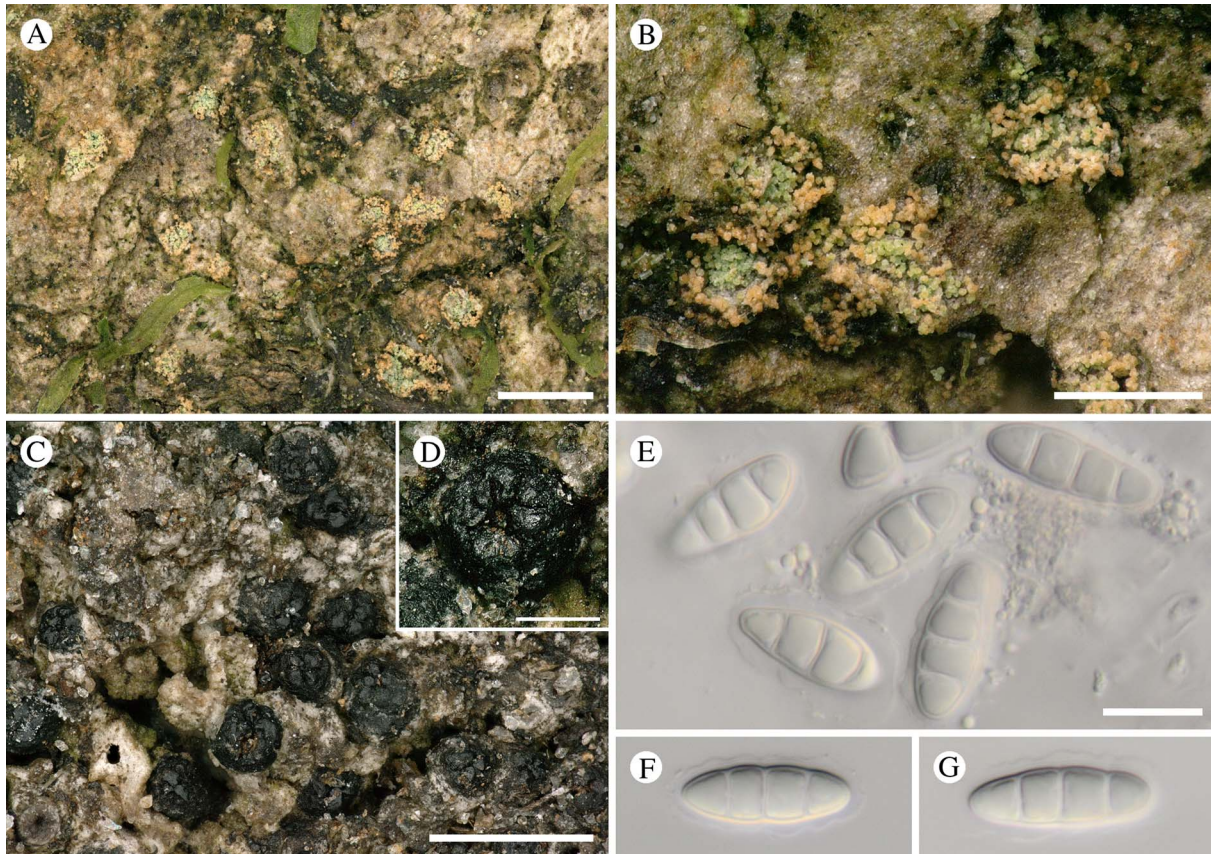


Fig. 5. *Francisrosea bicolor* (A & B, Sanderson 2200: specimens kept frozen since fieldwork) and *Ramonia melathelia* (C–G, Ertz 20503). A, inconspicuous thallus with discrete soralia. B, soralia. C, thallus with black perithecia. D, close-up of a perithecium showing the wrinkled surface. E–G, ascospores showing the thick gelatinous sheath, in water. Scales: A & C = 1 mm; B = 500 μ m; D = 250 μ m; E–G = 10 μ m. In colour online.

Apothecia and *pycnidia* unknown.

Chemistry. Soralia C–, K–, KC–, PD–, UV–. TLC: nil (small amount of lichen material used).

Etymology. The epithet refers to the two colours of the soralia.

Distribution and ecology. In the New Forest, this species has been recognized as distinct from *Thelopsis corticola* for some time, although the separation from *Porina multipuncta* (Coppins & P. James) Ertz *et al.* was not fully understood. However, beyond this area it continues to be confused with *T. corticola*. As such, the national distribution is not yet clearly known but it is widespread in old-growth *Fagus-Quercus-Ilex* pasture woodlands in the New Forest, Hampshire, where it has been recorded from 26 woods since 1992. Here it is most frequently found in wound tracks on senescent *Fagus sylvatica*, but has also been found in wound tracks on *Quercus robur*. There are often no associated lichens in the habitat but usually only algae crusts and a scattering of bryophytes such as *Metzgeria furcata* (L.) Dumort. and *Zygodon rupestris* Schimp. ex Lorentz. It has been noted as occasionally growing with typical wound track colonist lichens such as *Alyxoria varia* (Pers.) Ertz & Tehler, *Caloplaca ulcerosa* Coppins & P. James, *Opegrapha vulgata* (Ach.) Ach., *Porina aenea* (Körb.) Zahlbr. and *Strigula taylorii* (Carroll ex Nyl.) R. C. Harris, along with outlying thalli of species from adjacent stable communities such as *Agonimia octospora* Coppins & P. James, *Enterographa crassa* (DC.) Fée, *E. elaborata*

(Lyell ex Leight.) Coppins & P. James, *Pyrenula chlorospila* Arnold and *Porina rosei* Sérus. s. str. The distribution of *Francisrosea bicolor* outside of the New Forest is not known with any certainty but the species has been noted as causing confusion in the recording of *Thelopsis corticola* in Exmoor and North Wales and was recently definitively recorded in a wound track on an ancient *Quercus* at Rydal Park, Lake District, England. The latter indicates that it occurs much further north than most *Thelopsis corticola* records and may account for at least some of the outlying records of *T. corticola* north of its main southern English distribution. Examination of herbarium collections of *T. corticola* and potentially *Porina multipuncta* is likely to produce further records.

Discussion. *Thelopsis corticola* (Gyalectaceae) is similar to the new species in the ochre-coloured soralia, but having a more even colour with the deeper orange tints mostly absent and the internal green colouring paler and less often visible, and a thallus that is always visible at least near the soralia. The soralia are more often confluent, forming larger patches of 2–3 mm diam., and more dense with smaller soredia (up to 25 μ m in Sanderson 2202); the difference between soralia size is easily apparent in the field. *Porina multipuncta*, in the *Porinaceae*, differs in having a superficial thallus with numerous minute (0.1–0.3 mm) soralia that have a uniformly bright orange colour when fresh. *Zwackhia soreidifera* (P. James) Ertz has C+ pink-red soralia and belongs to the *Arthoniales*. *Caloplaca lucifuga* G. Thor (*Teloschistales*) has pale yellow to dirty yellow-orange-brown soralia that are K+ purple.

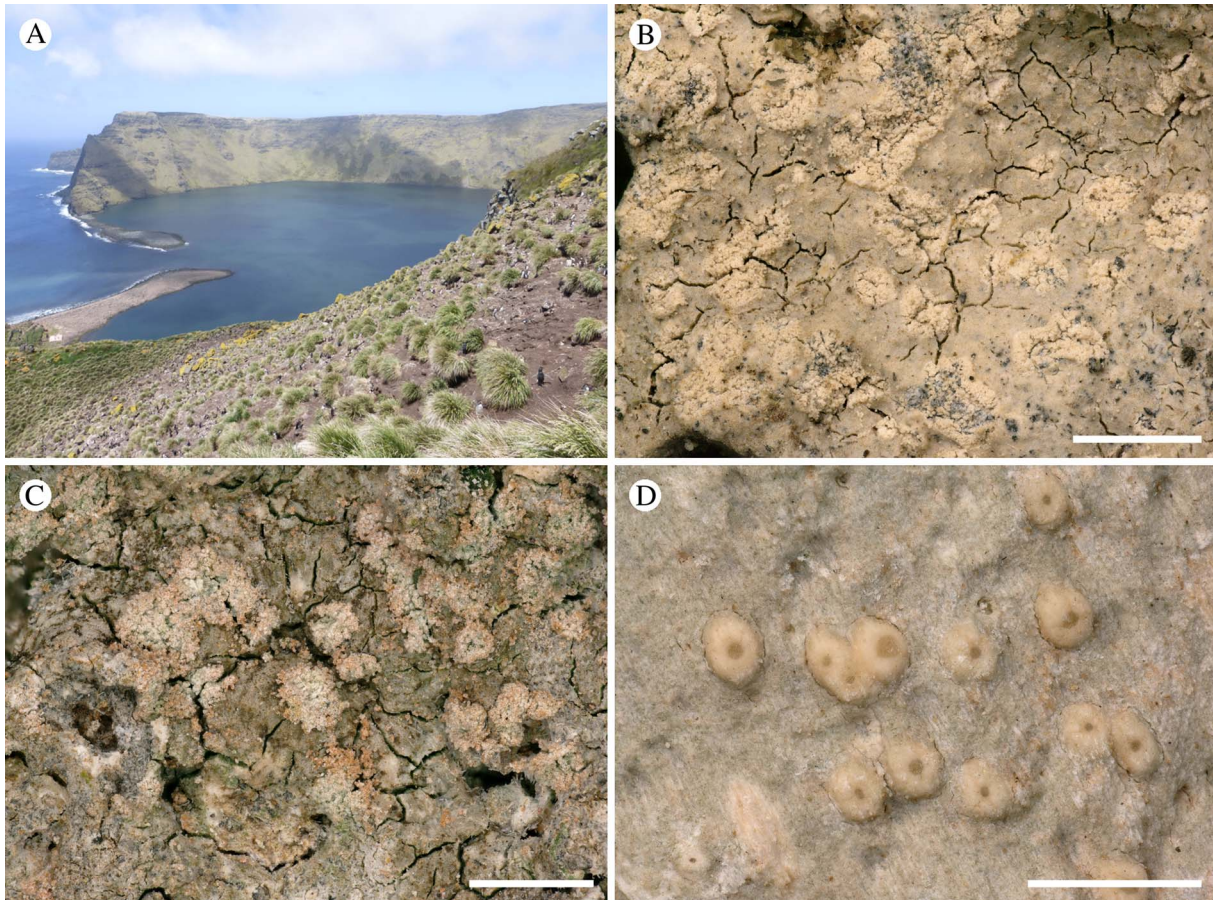


Fig. 6. *Gyalecta amsterdamensis* (B & C) and *G. farlowii* (D). A, crater of Île Saint-Paul, one of the localities of *G. amsterdamensis*. B, three-year-old herbarium specimen (Ertz 21404) showing thallus with soralia that have turned pale cream. C, specimen kept frozen since fieldwork (Ertz 21355) showing thallus with soralia having a pink-orange tinge. D, thallus with ascomata (Ertz 18328). Scales: B–D = 1 mm. In colour online.

Additional specimens examined. Great Britain: England: V.C.11, South Hampshire, New Forest, Busketts Wood, The Ridge, Grid Ref. SU31128 10988, 2016, *Sanderson* 2183 (BM); *ibid.*, New Forest, Gritnam Wood, Grid Refs SU282 067 & SU284 064, 1992, *Sanderson* 2745 (BM); *ibid.*, New Forest, Eyeworth Wood, Grid Ref. SU22517 15574, 2020, *Sanderson* 2746 (BM); *ibid.*, New Forest, Allum Green, Bramble Hill, Grid Ref. SU2775 0676, 2020, *Sanderson* 2747 (BM).

***Gyalecta amsterdamensis* Ertz sp. nov.**

Mycobank No.: MB 836496

A species of *Gyalecta* characterized within the genus by a smooth, rimose, saxicolous thallus with discrete soralia.

Type: Terres Australes et Antarctiques Françaises (TAAF), Île Amsterdam, Base Martin de Viviés, Jardin Météo, 37°47'57.2"S, 77°34'10.8"E, 50 m elev., paroi de roche volcanique ±abritée, 19 December 2016, Ertz 21404 (BR—holotype!; PC—isotype!).

(Fig. 6B & C)

Thallus saxicolous, crustose, forming patches of c. 0.5–5 cm diam., ±neatly delimited, continuous, distinctly rimose, smooth, matt, corticate, sorediate, c. 100–300 µm thick, pale greenish grey or pale greyish cream; calcium oxalate crystals absent; thallus in section not distinctly layered, mainly composed of more or less

loosely interwoven fungal hyphae, with photobiont cells scattered irregularly. *Prothallus* absent. *Soralia* discrete, not or rarely confluent, flat to slightly convex, with a pinkish orange tinge when fresh, fading to pale cream, often paler than the thallus, 0.2–0.8 (–1) mm diam.; soredia (17–)22–38 µm diam., formed of individual or short chains of photobiont cells surrounded by hyaline hyphae of (2.5–)3–4 µm diam., without projecting hyphae. *Photobiont* trentepohlioid, visible as individual globose cells, 9–15(–20) µm diam. or in short chains of c. 2–4 cells, with individual cells elliptical to rectangular, 9–20 × 7–13 µm.

Ascomata and *conidiomata* unknown.

Chemistry. Thallus and soralia K–, C–, PD–, UV–; hyphae I+ pale orange, KI–. TLC: traces of two UV+ red spots of high R_f (specimen Ertz 21404 tested in solvent C).

Etymology. The specific epithet refers to Amsterdam Island, the type locality.

Distribution and ecology. So far known only from the islands of Amsterdam and Saint-Paul (Fig. 6A), where it inhabits volcanic rock in rather open and ±sheltered conditions near the sea.

Discussion. The generic placement in *Gyalecta* s. lat. of this sterile species is confirmed by our phylogenetic analysis (Fig. 4). *Gyalecta amsterdamensis* is at present the only known member

of the genus having a rimose thallus with discrete soralia. The saxicolous *Gyalecta nidarosiensis* differs by having a thallus that is finely powdery-granular, without well-defined, discrete soralia. Among species from the Antarctic, *G. pezizoides* Vězda et al. has a leprose-granulose, yellowish brown thallus and grows on moss and soil (Vězda et al. 1992). Despite the rather rich material collected on the islands of Amsterdam and Saint-Paul, ascomata could not be found, nor observed in the field where the species was easily recognized by its thallus with discrete soralia having a pink-orange tinge. This colour fades to pale greyish cream in the herbarium, as known in other members of the genus. No other species of *Gyalectaceae* is known from Amsterdam and Saint-Paul Islands (Aptroot et al. 2011).

Additional specimens examined. Terres Australes et Antarctiques Françaises (TAAF): *Île Amsterdam*: Del Cano, 37°52'S, 77°32'E, 170 m elev., 2016, Ertz 21359 (BR); *ibid.*, 37°52'07.7"S, 77°32'30.4"E, 172 m elev., 2016, Ertz 21355 (BR). *Île Saint-Paul*: versant extérieur du cratère, crête de la Novara, non loin des terres chaudes à *Sphagnum*, 38°42'57.7"S, 77°31'07.1"E, 226 m elev., 2016, Ertz 21051 (BR).

***Neopetractis* Ertz gen. nov.**

Mycobank No.: MB 836497

Similar to *Petractis* but differing from the type of that genus (*P. clausa*, which associates with a cyanobacterial photobiont) in associating with a trentepohlioid photobiont.

Type species: *Neopetractis luetkemulleri* (Zahlbr.) Ertz.

Description (based mainly on the descriptions in Orange (2009) and Vězda (1965)). Thallus crustose, endolithic or semi-epilithic, continuous, rarely with fine cracks, smooth to minutely rugose, whitish grey or pale pink, ecorticate. Photobiont trentepohlioid.

Apothecia immersed, at first perithecioid, finally with a slightly to rather widely expanded disc; margin slightly raised, of the same colour as the thallus or slightly paler, with or without radial cracks, up to 0.5 mm diam.; disc beige-pink to pale brown, smooth, concave or flat, sunken below level of margin. **Exciple** thin, colourless or yellowish; cells angular, isodiametric to oblong. **Hymenium** colourless, I– or I+ faint blue, KI+ blue. **Hypothecium** thin, colourless. **Paraphyses** simple, apex not or slightly widened. **Asci** narrowly clavate, thin-walled, 8-spored, KI+ blue. **Ascospores** hyaline, ellipsoid, 3–5-transversally septate to submuriform (with 1–2 additional longitudinal septa), medium-sized (c. 16–25 × 5.5–10 µm), with a distinct gelatinous sheath, c. 2–4 µm thick.

Conidiomata pycnidia, immersed in the thallus; conidiogenous cells holoblastic, not proliferating; conidia colourless, simple or formed of irregular multicellular clusters.

Chemistry. No lichen substances detected by TLC.

Etymology. The name reflects its morphological similarity to the cyanolichen genus *Petractis*.

Discussion. *Neopetractis* differs from *Petractis* in having a trentepohlioid photobiont and from *Gyalecta* s. lat. in having ascospores with a thick gelatinous sheath. Orange (2009) described *P. nodispora*, which is the sister species of *P. luetkemulleri* in his

molecular study. In our phylogenetic tree, these two *Petractis* species form a lineage close to the genus *Ramonia* and are distantly related to *Petractis clausa*. Because of the different photobiont and the distinct phylogenetic position, both species are transferred to the new genus *Neopetractis* (see also general discussion below). *Petractis crozalsii* (B. de Lesd.) Clauzade & Cl. Roux is a species with non-halonate ascospores and is now considered to be a species of *Gyalecta* closely related to *Gyalecta hypoleuca* (Ach.) Zahlbr. (Roux et al. 2008), thus leaving *Petractis* as a monotypic genus. Both species of *Neopetractis* grow on calcareous rocks.

***Neopetractis luetkemulleri* (Zahlbr.) Ertz comb. nov.**

Mycobank No.: MB 836498

Gyalecta luetkemulleri Zahlbr. [as 'lütkemüller?'], *Österreichische Botanische Zeitschrift* 53, 178 (1903).—*Petractis luetkemulleri* (Zahlbr.) Vězda, *Preslia* 37, 137 (1965); type: Jugoslawien, Insel Hvar (Lesina), auf Kalkfelsen am Wege von Lesina nach Citavecchia, 1902, Lütkemüller (W—holotype, not seen).

***Neopetractis nodispora* (Orange) Ertz comb. nov.**

Mycobank No.: MB 836499

Petractis nodispora Orange, *Lichenologist* 41, 217 (2009); type: Great Britain, Wales, Glamorgan, Southerndown, Dunraven Park, Pant y Slade, national grid reference 21/8872.7330, 51° 26'50"N, 3°36'05"W, 30 August 2008, on vertical side of unshaded, north-west-facing limestone wall, Orange 17573 (NMW [C.2007.001.284]—holotype; AIX—isotype, not seen).

***Ramonia melathelia* (Nyl.) Ertz comb. nov.**

Mycobank No.: MB 836500

Thelopsis melathelia Nyl., *Flora, Regensburg* 47, 358 (1864).—*Verrucaria melathelia* (Nyl.) Leight., *Lich.-Fl. Great Brit.*, 447 (1871).—*Sagedia melathelia* (Nyl.) Jatta, *Syll. Lich. Ital.*, 553 (1900); type: [United Kingdom: Scotland], Ben Lawers, Jones s. n. (H-NYL 1427, lectotype *vide* Vězda (1968: 380); see <https://plants.jstor.org/specimen/h9504760>).

(Fig. 5C–G)

Discussion. Sequences obtained from a specimen surprisingly place *Thelopsis melathelia* as sister species to a specimen of *Ramonia valenzueliana*. This latter specimen was published by Lumbsch et al. (2004) as *Xerotrema* sp. and was later included as *Ramonia valenzueliana*, the type species of the genus, in the phylogeny of Rivas Plata et al. (2013). *Ramonia valenzueliana* shares with *Thelopsis*, the presence of periphysoids, multispored asci and small few-septate ascospores. *Thelopsis melathelia* differs from the type species of *Thelopsis* in having ascomata with a wrinkled surface, an excipulum with a darker outer layer all around and ascospores with a rather thick gelatinous sheath. These features support a closer relationship with the type species of *Ramonia* rather than with *Thelopsis* s. str. and *Gyalecta sensu* Lücking et al. (2019). The wrinkled ascomatal surface, dark excipulum and shape of ascospores also fits with *Ramonia* s. str. (= section *Ramonia sensu* Vězda (1966)). Therefore, the species is transferred to the genus *Ramonia*.

Specimen used for fungal DNA sequencing. Austria: Karnten, National Park Hohe Tauern, Glockner-Gruppe, above Hochtor, 47°05'04"N, 12°50'10"E, c. 2600 m, on soil-mosses in alpine vegetation, 2015, Ertz 20503 (BR).

Thelopsis corticola (Coppins & P. James) Sanderson & Ertz comb. nov.

MycoBank No.: MB 836501

Opegrapha corticola Coppins & P. James, *Lichenologist* **11**, 162 (1979); type: Ireland, V.C. H3, West Cork, 4 miles east of Baltimore, on poplar, 27 February 1965, P. W. James (BM—holotype; see <https://plants.jstor.org/specimen/BM000501110>).

(Fig. 1)

Description (of thallus partly from Coppins & James (1979)).

Thallus continuous, thin, smooth, matt, grey-green in shaded situations, becoming grey-brown in more exposed situations; soralia initially punctiform, scattered, becoming patchily contiguous in irregular and erose groups 2–3 mm wide, greenish fawn, pale grey-brown or ochraceous, fading to whitish grey in the herbarium.

Perithecia scattered, discrete, immersed in the thallus, sometimes visible as verrucae covered laterally by the thallus, with usually only the upper part of the perithecia visible, rarely the upper 1/3 emerging from the thallus, 0.4–0.6 mm diam., pale brownish to reddish brown or dark brown, often darker around the ostiole. *Excipulum* hyaline to pale yellowish, c. 30–40 µm thick laterally, becoming thicker around the ostioles, c. 60–70 µm, composed of hyphae with thick gelatinized walls, I–, K/I–. *Hymenium* hyaline, not interspersed, I+ orange-reddish, K/I+ blue (mainly the ascus walls). *Paraphyses* unbranched, (1.5–)2 µm, apex not widened, hyaline. *Periphysoids* simple or with short lateral branches, 20–40(–50) µm long. *Asci* over 100-spored, c. 150–180 × 12.5–20 µm; wall I+ reddish, K/I+ blue. *Ascospores* hyaline, ellipsoid-oblong, ends rounded, (2–)3-septate, 7.5–10.3–13 × 3–4.1–5 µm ($n = 40$), without a gelatinous sheath.

Discussion. Typical *Thelopsis* perithecia are described for the first time for *Opegrapha corticola*, a species previously known only as a sterile crustose sorediate lichen. DNA sequences obtained independently from both, the soralia of three specimens and the hymenium of one fertile specimen, clearly place the species in *Thelopsis* as defined here (Figs 3 & 4). *Opegrapha corticola* is similar to *Thelopsis rubella* but differs by having a sorediate thallus and distinctly smaller ascospores ((10–)12–16(–18) × 4–8 µm in *T. rubella* (Rose et al. 2009)). Perithecia of *O. corticola* are generally also duller and less reddish when wet than those of *T. rubella*, and when dry, are generally a bit more sunken into the thallus than is typical for *T. rubella*. Our morphological observations along with our phylogenetic results leave no doubt that *O. corticola* is a normally sorediate *Thelopsis* and that it is a separate taxon from *T. rubella*. Therefore, a new combination is made in *Thelopsis*.

Specimens used for fungal DNA sequencing (Sanderson 2053 is fertile, the others sterile; all on trunks of Quercus). **France:** Brittany: Concoret, château de Comper, 48°04'18"N, 2°10'23"W, 109 m elev., 2012, Ertz 17602 (BR).—**Great Britain:** Wales: V.C.48, Merionethshire, Nannau, The Deer Park, Tree NN274,

Grid Ref. SH74931 19550, 2015, Sanderson 2053 & Cross (BM). **England:** V.C.11, South Hampshire, New Forest, Sunny Bushes, Grid Ref. SU25949 14155, 2016, Sanderson 2188 (BM); *ibid.*, Matley Wood, Grid Ref. SU33395 07824, 2016, Sanderson 2202 (BM).

Additional fertile specimens examined (all on trunks of Quercus). **Great Britain:** **England:** V.C.11, South Hampshire, New Forest, Frame Wood, Grid Ref. SU35970 03286, 2013, Sanderson 1971 & Wessex Lichen Group (BM); V.C.8, South Wiltshire, Longleat Park, The Rookery, Grid Ref. ST80704 43776, 2015, Sanderson 2120 (BM).

Other specimens used for fungal DNA sequencing. Gyalecta (Pachyphiale) carneola. Norway: Hordaland: Tysnes, Hovdanes, Beltestadknappen, 59°59'43"N, 5°27'14"E, 2018, Ertz 22499 (BR).

Gyalecta farlowii. Netherlands Antilles: Curaçao: Westpunt, Playa Piskadó (Grandi), 12°22'12"N, 69°09'11"W, c. 12 m, limestone rocks, 2013, Ertz 18328 (BR).

Gyalecta nidarosiensis. Belgium: Yvoir, Champalle, grand affleurement rocheux au sud du village d'Yvoir, 50°19'00"N, 4°52'59"E, 167 m elev., limestone rocks, 2019, Ertz 23169 (BR).

Petractis clausa. Belgium: commune d'Anhée, à 500 m au nord-est du village de Foy, Bois de la Saute, sur le versant droit de la Molinee, juste en aval du confluent Molinee-Flavion, 50°17'46"N, 4°48'59"E, 150 m elev., paroi de calcaire compact, 2019, Ertz 23174 (BR).

Porina leptalea. Belgium: commune d'Anhée, à 500 m au nord-est du village de Foy, Bois de la Saute, sur le versant droit de la Molinee, juste en aval du confluent Molinee-Flavion, 50°17'46"N, 4°48'59"E, 140 m elev., trunk of *Carpinus*, 2019, Ertz 23175 (BR).

Thelopsis byssoidea. Thailand: Trat Prov.: Sapan Hin Waterfall, 12°06'09"N, 102°42'44"E, c. 30 m elev., tropical rainforest along a river, base of a big tree, 2012, Ertz 17384 (BR).

Thelopsis rubella. Belgium: Rochefort, grotte de Lorette, 50°09'17"N, 5°13'40"E, 220 m, on *Tilia*, 2013, Ertz 18094 (BR).—**Great Britain:** **England:** V.C.11, South Hampshire, New Forest, Sunny Bushes, Grid Ref. SU26175 14316, *Quercus-Fagus-Ilex* pasture woodland, base rich bark on old *Quercus petraea*, 2016, Sanderson 2186 (BM).—**Italy:** Genoa Prov.: Genoa, Pegli, Villa Doria, 44°25'47"N, 8°48'53"E, c. 55 m elev., park, on big trunk of *Quercus*, 2015, Ertz 20377 (BR).

Discussion

Should Thelopsis be merged with Gyalecta?

The placement of *Thelopsis* in the genus *Gyalecta* is surprising because *Thelopsis* is well recognized by the combination of the following morphological characters: perithecioid ascomata, well-developed periphysoids, polysporous asci ((30–)40–150(–300) spores), and small few-septate ellipsoid-oblong ascospores. *Thelopsis* is a further remarkable example of parallel evolution of perithecioid ascomata within *Gyalectaceae*, in addition to *Belonia*. No previous studies have mentioned the possibility that *Thelopsis* should be merged into *Gyalecta* and the genus was even considered to belong to the family *Stictidaceae* (Lücking et al. 2017). Only Jørgensen & Vězda (1984) intimated placement in the *Gyalectales* but they retained *Thelopsis* in the *Ostropales* (see Introduction). While the combination of morphological characters makes *Thelopsis* unique within *Gyalectaceae*, none of

the morphological features taken alone supports *Thelopsis* as being distinct from *Gyalecta*. Perithecioid ascomata are known in *Gyalecta* species formerly included in *Belonia* (e.g. the sequenced *G. herculina*, *G. nidarosiensis* and *G. russula*), but these taxa lack periphysoids (Jørgensen et al. 1983; Navarro-Rosinés & Llimona 1997). Henssen (1976) proved that periphysoids are present during ascomal ontogeny of several species of *Gyalectaceae*. In mature apothecia of *Gyalecta ulmi*, periphysoids are still present but restricted to the outermost margin, while in *Gyalecta jenensis* (Batsch) Zahlbr. the periphysoids remain short and imbedded in mucilage forming a rim along the inner boundary of the excipulum against the hymenium (Henssen 1976). In *Belonia*, *Cryptolechia* and *Pachyphiale*, these structures are reduced and generally visible only in young apothecia (Henssen 1976; Kauff & Büdel 2005, as 'lateral paraphyses'). However, the periphysoids if present are never as well developed as in *Thelopsis*, where they occupy a broad zone around the ostiole in mature ascomata. In the *Stictidaceae*, the genera *Carestiella* and *Schizoxylon* lack periphysoids but they have been recorded within the genus *Stictis*. Species of *Stictis* have periphysoids (Wedin et al. 2005, 2006), suggesting that the importance of this character (=presence vs absence of periphysoids) might have been overestimated for generic delimitation. These different genera were maintained until now (Lumbsch & Papong 2009; Fernández-Brime et al. 2011, 2018), however, and the generic delimitations in the *Stictidaceae* need further investigation. Because *Stictis* is a large and poorly known group with many species that are mainly tropical, short-lived and growing on debris of various sorts, it is much more likely that the genus will eventually 'fall to pieces' (Mats Wedin, personal communication), as suggested by the results of recent studies in the family (e.g. Fernández-Brime et al. 2018; Phukhamsakda et al. 2020).

Polyspory originated many times during the evolution of lichenized fungi (Reeb et al. 2004; Aptroot & Schumm 2012). It is usually not considered as a character deserving genus recognition in the *Gyalectaceae* (e.g. Vězda (1967) for *Ramonia* sect. *Ramonia*) or in the *Stictidaceae* (e.g. Baloch et al. (2013a) for *Sphaeropezia*). The inclusion of the genera *Pachyphiale* and *Cryptolechia* in *Gyalecta* renders polyspory a character important only at the species level in the *Gyalectaceae* (Baloch et al. 2010, 2013b; Lücking et al. 2019). *Thelopsis* shares the polysporous asci characteristic with *Cryptolechia* and *Pachyphiale* but does not group with them in our phylogenetic analyses. It differs from *Cryptolechia* and *Pachyphiale* in having perithecioid ascomata with periphysoids and generally more spores per ascus (e.g. 100–150 in *T. rubella*). Regarding ascospore shape and size, a large variation is observed in *Gyalecta*, from small few-septate ellipsoid spores to long, many septate and needle-shaped or muriform spores.

Therefore, individual morphological characters might not appear to prevent the separation of *Thelopsis* from *Gyalecta*. Yet we refrain from merging *Thelopsis* with *Gyalecta* for several reasons. The genus is well recognized by the combination of morphological characters (see above) and a wider combination of morphological characters has proved useful in refining genera more accurately in other groups such as the *Graphidaceae* (e.g. Frisch et al. 2006; Parnmen et al. 2013). Furthermore, the three species of *Thelopsis* (viz. *T. byssoidea*, *T. corticola* and *T. rubella*) included in our phylogeny of *Gyalecta* s. lat. (Fig. 4) form a well-supported monophyletic lineage lower down the tree. *Gyalecta friesii* Flot. ex Körb. and *G. ulmi*, which form a lineage outside *Gyalecta* + *Thelopsis*, might be transferred to another genus. They differ from the other *Gyalecta* species included in the

phylogeny by the larger ascomata with a distinctly constricted base and generally with a widely exposed hymenium at maturity, giving them some similarities with *Lecanora* species in the field. Moreover, in the framework of phylogenies of the *Ostropales* s. lat., the branch lengths also support the recognition of more than one genus within the broadly defined *Gyalecta* lineage, as can be seen for instance in Fig. 3 or in other published phylogenies (e.g. fig. 1 in Aptroot et al. (2014b)). Transferring *Thelopsis* to *Gyalecta* would also necessitate the introduction of new names for the well-established epithets of *T. rubella* and *T. corticola* because these epithets are already in use for other species in *Gyalecta*. This is a minor practical point but would not be welcomed by field lichenologists. For all these reasons, we see no gain in transferring *Thelopsis* species to *Gyalecta*. Instead, we suggest keeping *Thelopsis* as distinct pending further studies with a more exhaustive sampling of *Gyalectaceae*. The genera *Myeloconis* and *Trichothelium* are also maintained within a paraphyletic *Porina* for similar reasons.

Towards a refined generic concept of *Gyalecta*?

A wider combination of characters applied to a more exhaustive molecular analysis might lead to a revision of the generic classification in the *Gyalectaceae*, as pointed out by Lücking et al. (2019) who have already listed some promising morphological features (e.g. the nature of the paraphyses). In this context, the sequencing of the genus *Topelia* is of great interest because of its supposed close relationship with *Thelopsis* (Vězda 1968; Jørgensen & Vězda 1984). *Topelia* differs from *Thelopsis* by having eight muriform ascospores per ascus. However, Moon & Aptroot (2009) and Aptroot et al. (2014a) highlighted the existence of intermediate species between the genera *Thelopsis* and *Topelia* for the ascospore types: for example, *Thelopsis muriformis* Aptroot & K. H. Moon with truly muriform ascospores (Moon & Aptroot 2009), and *Thelopsis cruciata* Aptroot & M. Cáceres with cruciate septate ascospores (Aptroot et al. 2014a). They suggested that both genera are indistinguishable and should probably be merged.

Pachyphiale fagicola (Arnold) Zwackh is considered by Lücking et al. (2019) as the most crucial taxon regarding the generic concept in *Gyalectaceae* because it forms the longest branch in the tree, has the most deviating feature in the family besides *Belonia* and would involve splitting *Gyalecta* s. lat. into six different genera if *Pachyphiale* is maintained. The placement of *G. carneola* as the sister species of *G. fagicola*, both forming a well-supported lineage, suggests that the genus *Pachyphiale* could be resurrected from the synonymy of *Gyalecta* if a refined generic concept of *Gyalecta* s. lat. is justified. In that case, several other generic names need to be considered and are available for all the lineages of *Gyalecta* s. lat. (Fig. 4). According to Lücking et al. (2019: 292), the type species of *Gyalecta* is *G. geoica*, but a typification does not seem to have been published. The genus *Gyalecta* could thus possibly be restricted to the *G. truncigena*-*G. geoica* clade (Fig. 4). The genus *Secoliga* Norman (typification missing too?) appears to be available for the basal lineage formed by *G. friesii* and *G. ulmi*, *Cryptolechia* A. Massal. for its type *G. carneolutea*, *Belonia* Körb. for its type *G. russula*, and *Clathroporinopsis* M. Choisy (lectotype *G. nidarosiensis* fide Lücking et al. (2017)) and *Protoschistes* M. Choisy (lectotype *G. herculana* fide Lücking et al. (2017)) for the clade *G. caudiospora*-*G. amsterdamsis* (Fig. 4); a small number of other generic names are listed in MycoBank and need to be evaluated too, but this is beyond the scope of the present study.

Thelopsis byssoidea deviates from all known *Thelopsis* species by the distinct byssoid thallus (Fig. 2B–D), but our phylogenetic results confirm its placement within the *Thelopsis* lineage (Figs 3 & 4). The species is a nice example of parallel evolution of the byssoid thallus within genera known otherwise to have a more compact thallus, in addition to, for example, *Crocynia* in the *Phyllopsora* clade (Kistenich *et al.* 2018) and *Sagenidium* in the *Roccellaceae* (Ertz *et al.* 2015). Furthermore, *Gyalecta amsterdamensis* and *Thelopsis corticola* are the first examples of sorediate lichens confirmed in the *Gyalecta* s. lat. clade (Figs 3 & 4). It is evident that thallus morphology does not provide useful taxonomic information at the genus level in this group, at least for the byssoid and sorediate character states.

The genus *Ramonia* and polyphyly of *Thelopsis*

The placement of *Thelopsis melathelia* as sister species to *Ramonia valenzueliana* (Fig. 3) is interesting for our understanding of character evolution in the *Gyalectaceae*. Vězda (1966) emended *Ramonia* but recognized three groups within his enlarged concept of the genus. He admitted that these groups could be recognized as distinct genera because of a combination of important morphological differences. Therefore, the genus *Ramonia* appears clearly heterogeneous. The type species of *Ramonia*, *R. valenzueliana*, shares several important morphological similarities with *Thelopsis*, such as the ascum anatomy including the presence of periphysoids and multispored asci containing small ellipsoid ascospores, and Vězda (1968) has already suggested a close relationship between the genera. *Ramonia valenzueliana* differs from *Thelopsis* mainly by the type of ascum that slightly widens in a late stage, while in *Thelopsis* the ascum remains closed (Vězda 1968). However, the degree of opening of the ascum is variable within genera of *Gyalectaceae*, as illustrated for example by species of *Gyalecta* with perithecioid ascum (e.g. *G. herculina* and *G. nidarosiensis*; Jørgensen *et al.* 1983) that cluster with other *Gyalecta* species having a narrow ascum opening (e.g. *G. farlowii*, *G. herrei*, *G. hypoleuca* and *G. thelotremella*) (Fig. 4). The placement of *Thelopsis melathelia* as sister species to *Ramonia valenzueliana* suggests that other phenotypic characters might be used to predict phylogenetic relationships, such as the wrinkled ascum surface (smooth in *Thelopsis* s. str.), a darker excipulum all around the ascum and ascospores with a thick gelatinous sheath. The wrinkled ascum surface, dark excipulum and shape of ascospores also fits with *Ramonia* s. str. (= section *Ramonia sensu* Vězda (1966)), which led us to combine *Thelopsis melathelia* in *Ramonia* (see Taxonomy section). In this context, further molecular data are needed to investigate whether these morphological characters might predict closer affinities of other species of *Thelopsis* with *Ramonia*. *Thelopsis lojkana* Nyl. and *Topelia heterospora* (Zahlbr.) P. M. Jørg. & Vězda are two species that deviate from the core of their genus in having distinctly halonate ascospores. Further studies might prove *Thelopsis* to be more heterogeneous: *T. flaveola* Arnold deviates by its simple ascospores and *T. isiaca* by perithecia remaining entirely immersed in prominent thalline warts. *Ramonia* also needs to be investigated further, in particular regarding the three sections distinguished by Vězda (1966).

The genus *Petractis*

In his revision of *Petractis*, Vězda (1965) accepted five species, four of which he newly transferred from *Gyalecta* because these


taxa share the same structure and ontogeny of ascum. However, as stated by Orange (2009), the distinction of the genus from other genera of *Gyalectoid* lichens is unclear at present owing to uncertainties in the circumscription of the genus. The clade from *G. hypoleuca* to *G. amsterdamensis* (Fig. 4) includes three members that were treated as *Petractis* species by Vězda (1965): *P. hypoleuca* (Ach.) Vězda and *P. thelotremella* (Bagl.) Vězda were shown to be phylogenetically related to *Gyalecta* by Kauff & Lutzoni (2002), while our study shows that *P. farlowii* (Tuck.) Vězda also belongs here (Fig. 4). These three species share with *Gyalecta* the non-halonate ascospores and the trentepohlioid photobiont. The type species of *Petractis* (*P. clausa*) is not phylogenetically related to these *Gyalecta* species (Fig. 3). It differs morphologically by having a cyanobacterium (*Scytonema*) as photobiont, a fully endolithic thallus (vs 'pseudoeplithic' in the other species treated by Vězda (1965)), ascospores having a distinct gelatinous sheath and by a more fissured apothecial margin. It is therefore surprising that Vězda (1965) enlarged the concept of *Petractis* by transferring species from *Gyalecta*. However, he distinguished two groups within *Petractis*: 1) *P. clausa* and *P. luetkemuelleri*, with a similar ascum type (= in young stage, always covered by a radially fissured thallus) and ascospores having a notably thick (2–4 µm) gelatinous sheath; 2) *P. farlowii*, *P. hypoleuca* and *P. thelotremella* where a fissured ascum thallus cover is only occasionally observed and this only in specimens having a thin epilithic thallus with more protruding ascum, and the ascospores lacking a gelatinous sheath. The separation of these two groups is now supported by phylogenetic results. However, the first group has not been recovered as monophyletic because *P. luetkemuelleri* did not cluster with *P. clausa* in various phylogenetic studies (e.g. Kauff & Lutzoni 2002; Orange 2009; Miadlikowska *et al.* 2014; this study, Fig. 3). Both species differ, however, in their photobionts and the endolithic (*P. clausa*) versus epilithic or 'pseudoeplithic' (*P. luetkemuelleri*) thallus. As already shown by Orange (2009), *Petractis nodispora* is the sister species of *P. luetkemuelleri* (Fig. 3). In our phylogenetic tree, these two *Petractis* species cluster in a strongly supported lineage close to the genus *Ramonia* and are distantly related to *Petractis clausa*. *Ramonia* differs from *Petractis* notably in having periphysoids and polysporous asci. Since *Petractis luetkemuelleri* and *P. nodispora* differ morphologically and phylogenetically from *P. clausa* and *Ramonia*, they are transferred to the new genus *Neopetractis* (see Taxonomy section).

Conclusion

Our phylogenetic results shed light on the taxonomic significance of some morphological features in the family *Gyalectaceae* (e.g. degree of opening of the ascum, carbonization of ascum wall, byssoid/sorediate thallus, multispory, periphysoids, gelatinous sheath around the ascospores) and the placement of *Thelopsis* in *Gyalecta* challenges the generic circumscription in this genus. Fieldwork and sequencing also revealed a hidden diversity for the group among sterile sorediate specimens, resulting in the discovery of two new taxa: *Francisrosea bicolor* and *Gyalecta amsterdamensis*. Much remains to be done to improve our understanding of evolution within the *Gyalectaceae* and relatives, since the molecular data available at present are still limited.

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