

Phylogenetic placement of *Sarcotrochila alpina*, the hitherto unknown teleomorph of *Rhabdocline laricis*

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Sarcotrochila alpina (type species of the genus *Sarcotrochila*) has long been known as a saprotroph colonising larch needles in litter. During a survey of mycobiota colonising needles in litter, we regularly observed apothecia of this species on larch needles cultivated in damp chambers, and isolated the fungus from its ascospores into culture. Analysis of ITS rDNA obtained from these cultures revealed a surprising connection to the anamorph-typified species *Rhabdocline* (= *Meria*) *laricis*, a weak pathogen of European larch. A new combination, *Rhabdocline alpina*, is proposed, reflecting their conspecificity and the priority of the epitheton *alpina*. A proposal to protect the much more often used generic name *Rhabdocline* against the older *Sarcotrochila* is planned. The morphology of the species in vital condition is presented, and for the two examined type specimens of *Orbilina retrusa* and *Hyalinia nostra* (both synonyms of *R. alpina*) in dead condition. A comparison of our findings with descriptions in the literature is presented.

Key words: *Meria laricis*, *Larix decidua*, phylogeny, vital characteristics, ascospore morphology.

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Koukol O., Lukáčová K., Baral H.-O. (2024): Fylogenetické postavení *Sarcotrochila alpina*, dosud neznámé teleomorfy od *Rhabdocline laricis*. – Czech Mycol. 76(1): 17–32.

Sarcotrochila alpina (typový druh rodu *Sarcotrochila*) je dlouho známá jako saprotrofní druh kolonizující modřínové jehlice v opadu. Při studiu mykobioty kolonizující jehlice v opadu jsme pravidelně pozorovali apothecia tohoto druhu na jehlicích modřínu, kultivovaných ve vlhkých komůrkách, a následně jej z askospor izolovali do kultury. Provedená analýza ITS rDNA získané z těchto kultur odhalila překvapivé spojení s anamorfním druhem *Rhabdocline* (= *Meria*) *laricis*, slabým patogenem modřínu evropského. Vzhledem k tomu, že se jedná o identický druh, je navržena nová kombinace *Rhabdocline alpina*, odrážející nejstarší dostupné epiteton *alpina*. Plánován je také návrh na konzervaci častěji používaného rodového jména *Rhabdocline* oproti staršímu jménu *Sarcotrochila*. Je uvedena morfologie druhu včetně vitálních znaků a také znaků na typových položkách druhů *Orbilina retrusa* a *Hyalinia nostra* (v obou případech jde o synonyma *R. alpina*). Pozorované znaky jsou srovnány s literárními údaji a diskutovány.

INTRODUCTION

The genus *Sarcotrochila* Höhn. (*Cenangiaceae*) was described by Höhnelt (1917) with *S. alpina* (Fuckel) Höhn. as the type species. This fungus, originally placed in *Naemacyclus* Fuckel as *N. alpinus* Fuckel, was described from needles of European larch [*Larix decidua* Mill. (as *Pinus larix* L.)] in St. Moritz (Switzerland) by Fuckel (1875), who also mentioned its occurrence close to Brno (Moravia, Czech Republic).

Currently, the genus *Sarcotrochila* contains seven species accepted by Stone et Gernandt (2005), who synonymised *Hemiphacidium* Korf with *Sarcotrochila* based on molecular data. All *Sarcotrochila* species colonise coniferous needles and form apothecia erumpent through the needle epidermis.

The yellow, convex, rounded to oval or elongated apothecia of *S. alpina* are found on litter needles bursting through the epidermis. The asci are 8-spored with unicellular, multiguttulate, oblong, partly constricted ascospores with a gelatinous sheath. The species has been recorded in North America and Europe on needles of several European, American and Asian larch species (Rehm 1908, Funk 1985, Jalkanen 2016). Search in the GBIF database (www.gbif.org, as retrieved in Nov 2023) revealed 67 observations, out of which 47 were preserved as fungarium specimens.

Just like many other saprotrophic microfungi, *S. alpina* has been often overlooked and the ecology of the species, including its life cycle, phylogenetic placement, and potential anamorph have not been studied intensively. Similarly, also sequence data of *Sarcotrochila* species are very scarce, and presently only *S. macrospora* Ziller et A. Funk and *S. longispora* (Ziller et A. Funk) J.K. Stone et Gernandt are represented by single sequences of ITS rDNA, obtained from the holotype collections. There are no sequences of the type of the genus, *S. alpina*, published in GenBank as yet.

During comparison of microfungi colonising European larch, Scots pine, Norway spruce, and Douglas fir litter needles in artificial plantations on the outskirts of Prague in early spring (A. Vicioso, unpubl. results), we found a fungus producing phacidioid apothecia on surface-sterilised larch needles cultivated on agar media. The same apothecia were also produced after several days of cultivation of litter needles in damp chambers. After preliminary identification as *S. alpina* based on morphology, we realised the gaps in knowledge of its life history and made targeted collections and performed cultivations of larch litter needles from various localities and habitats in the Czech Republic and Slovakia. Due to the presence of fresh collections, we succeeded to isolate the fungus into cultures, and extracted DNA from them.

The aims of our study were thus (1) to elucidate the phylogenetic placement of *S. alpina* based on analysis of three molecular markers (ITS and LSU rDNA,

RPB2), and (2) to provide a morphological description based on fresh collections and cultural characteristics.

MATERIAL AND METHODS

Litter needles of *Larix decidua* were collected into plastic bags from various localities across the Czech Republic and Slovakia, including managed forests and parks. The collected needles were placed in sterile damp chambers consisting of a glass Petri dish (18 cm in diam.) with moist cotton tissue and filter paper on the bottom. Chambers were kept at natural daylight and in a temperature range of 16–20 °C, and were periodically examined for fungi growing on the needles. Needles with developed apothecia of *S. alpina* were dried and deposited in the Fungarium of Charles University, Prague (PRC). We also included in our description and illustrations results from the examination of a fresh collection from Luxembourg and the re-examined type specimens of two old taxa placed in synonymy with *S. alpina* by Baral et al. (2020).

Isolation of *S. alpina* was carried out either from freshly developed ascomata on needles in damp chambers or from needles in Petri dishes with Malt extract agar (MEA; Oxoid, Hampshire, UK) and Potato carrot agar (PCA; Oxoid, Hampshire, UK). In the latter, needles were surface-sterilised prior to culturing according to Koukol et al. (2022). In only two cases, slow-growing white sterile mycelia, obviously not belonging to contamination, were obtained. In order to confirm the identity of these mycelia, DNA from pure cultures was extracted and three molecular markers, ITS and LSU rDNA, and fragments of a gene encoding the second largest subunit of the ribosomal polymerase (*RPB2*) were amplified using the procedure described by Delgado et al. (2020).

BLAST search for ITS rDNA indicated a close relationship of our isolates with members of the genus *Rhabdocline* Syd. Two datasets (ITS and LSU) were built with low species overlap. The ITS dataset (Tab. 1) included 30 sequences with 541 sites, out of which 212 were variable and 159 parsimony informative. The LSU dataset consisted of 36 sequences with 849 sites, out of which only 173 were variable and 131 parsimony informative. Model selection and settings for Maximum likelihood (ML) and Bayesian phylogenetic analyses followed Koukol et al. (2018). Analyses were performed using RAxML v8.2.10 (Stamatakis 2014) implemented on the CIPRES Science Gateway server (Miller et al. 2010) and MrBayes v3.2.6 (Ronquist et al. 2012), respectively. The *RPB2* marker was not represented by relevant sequences and was not used in further phylogenetic analyses.

RESULTS

Out of the 13 collections of litter needles, apothecia of *S. alpina* occurred in eight of them after cultivation in damp chambers for at least one week. Due to contaminations by other fungi outgrowing from the needles, only two isolates of *S. alpina* were obtained.

The two cultures were fully identical in all three molecular markers; no intra-specific variability was found. Phylogenetic analysis of ITS rDNA showed that sequences obtained from our isolates clustered within *Rhabdocline*. They showed a high similarity to several sequences of *Rhabdocline* (= *Meria*) *laricis* (Fig. 1), suggesting conspecificity. Only one sequence of *M. laricis* (accession no. U92298) was different and was placed between the *S. alpina* clade and the other *Rhabdocline* species.

Tab. 1. Species and sequences of ITS rDNA with their accession numbers and information on host and geographical occurrence obtained from GenBank and used to build the dataset.

Species	Voucher/ Strain	GenBank accession	Country of origin	Host	Reference
<i>Cenangium ferruginosum</i>	TAAM 198451	LT158471	Montenegro	<i>Pinus nigra</i>	Pärtel et al. (2016)
<i>Cenangium ferruginosum</i>	OULU 24434	LT158467	Germany	<i>Pinus sylvestris</i>	Pärtel et al. (2016)
<i>Chlorenchocelia torta</i>	H.B. 8415	LT158424	Taiwan		Pärtel et al. (2016)
<i>Chlorenchocelia versiformis</i>	TAAM 179803	LT158427	Estonia		Pärtel et al. (2016)
<i>Chlorenchocelia versiformis</i>	TU 119720	LT158479	USA, Tennessee		Pärtel et al. (2016)
<i>Chlorociboria aeruginascens</i>	TNSF 36241	LC425045	Japan, Niigata		Johnston et al. (2019)
<i>Ciboria batschiana</i>	TU 104222	LT158466	Estonia	<i>Quercus robur</i>	Pärtel et al. (2016)
<i>Crumenulopsis sororia</i>	TU 104504	LT158442	France	<i>Pinus sylvestris</i>	Pärtel et al. (2016)
<i>Heyderia abietis</i>	TAAM 165961	LT158426	Estonia	<i>Picea abies</i>	Pärtel et al. (2016)
<i>Heyderia pusilla</i>	TU 104257	LT158430	Estonia	<i>Pinus sylvestris</i>	Pärtel et al. (2016)
<i>Meria laricis</i>		U92298	USA, Washington	<i>Larix occidentalis</i>	Gernandt et al. (1997)
<i>Meria laricis</i>		U92299	Switzerland	<i>Larix decidua</i>	Gernandt et al. (1997)
<i>Meria laricis</i>	CBS 283.59	MH857866	United Kingdom		Vu et al. (2019)
<i>Meria laricis</i>	CBS 298.52	MH857046	Switzerland		Vu et al. (2019)
<i>Meria laricis</i>	CBS 216.31	MH855193			Vu et al. (2019)
<i>Piceomphale bulgarioides</i>	TAAM 165289	LT158483	Estonia	<i>Picea abies</i>	Pärtel et al. (2016)
<i>Piceomphale bulgarioides</i>	TAAM 198322	LT158469	Estonia	<i>Picea abies</i>	Pärtel et al. (2016)
<i>Rhaddoclina alpina</i>	PRC 9202	PP134997	Czech Republic	<i>Larix decidua</i>	this study
<i>Rhaddoclina alpina</i>	PRC 9258	PP134996	Czech Republic	<i>Larix decidua</i>	this study
<i>Rhaddoclina parkeri</i>		U92294	USA, Wyoming	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina parkeri</i>		U92295	USA, Wyoming	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina parkeri</i>		U92296	USA, Oregon	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina parkeri</i>		U92297	USA, Oregon	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina pseudotsugae</i> subsp. <i>epiphylla</i>		U92292	USA, Oregon	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina pseudotsugae</i> subsp. <i>pseudotsugae</i>		U92290	USA, Oregon	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina pseudotsugae</i> subsp. <i>pseudotsugae</i>		U92291	USA, Pennsylvania	<i>Pseudotsuga menziesii</i>	Gernandt et al. (1997)
<i>Rhaddoclina weirii</i> subsp. <i>oblonga</i>		AF260814	USA, Michigan		Catal M. and Adams G.C., unpublished
<i>Sarcotrochila longispora</i>	CBS 273.74	KJ663836	Canada, British Columbia	<i>Pinus contorta</i>	Crous et al. (2014)
<i>Sarcotrochila macrospora</i>	ATCC 26762	AY645900	Canada, British Columbia	<i>Pinus contorta</i>	Stone et Gernandt (2005)
<i>Sclerenocelia fraxinicola</i>	TAAM 198511	NR_154458	Germany	<i>Fraxinus excelsior</i>	Pärtel et al. (2016)

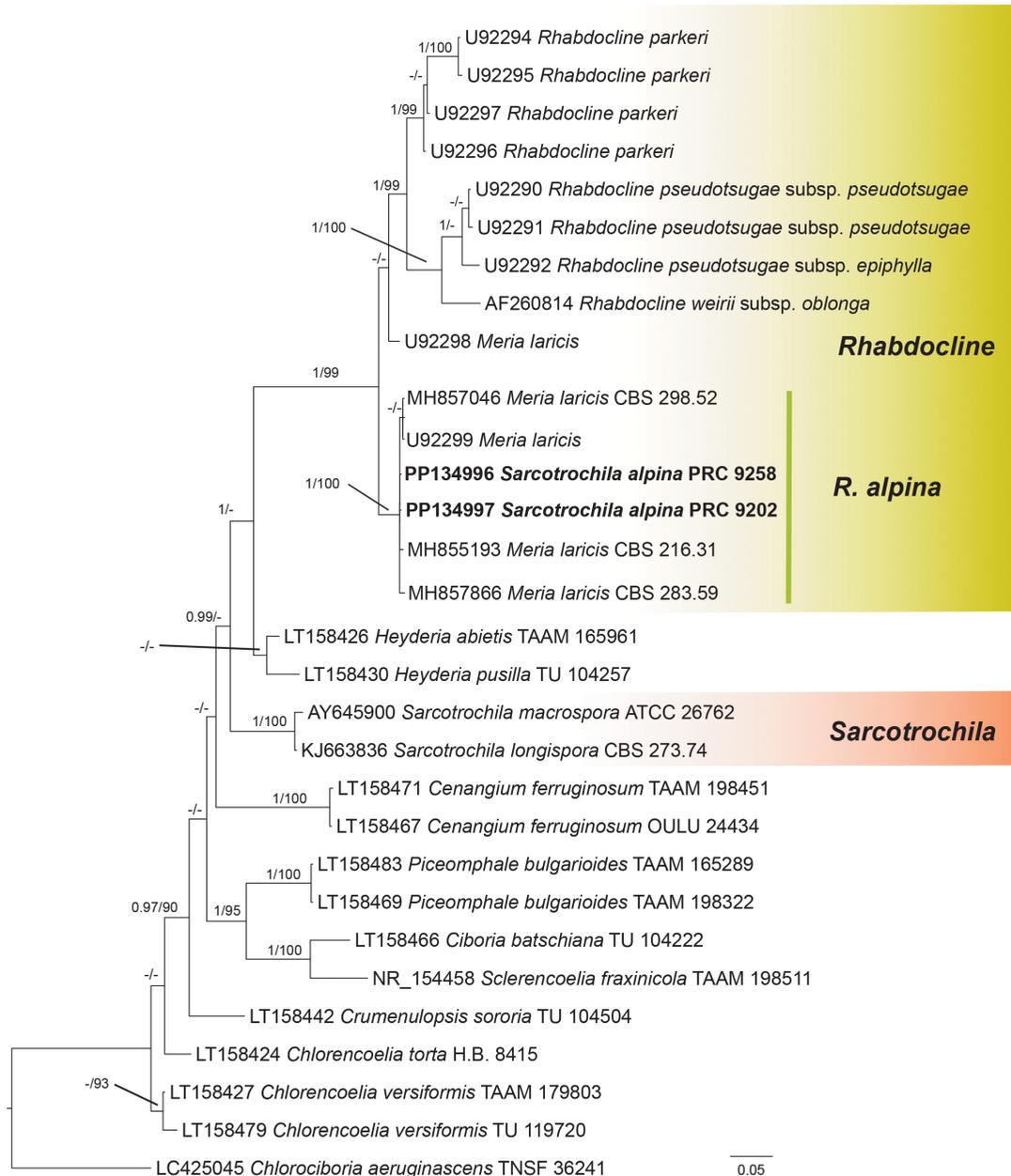


Fig. 1. Phylogenetic relationship of *Rhabdocline* within *Cenangiaceae*, with *Chlorociboria aeruginascens* (*Chlorociboriaceae*) as outgroup. The tree is inferred from Bayesian and Maximum likelihood analyses based on ITS rDNA. Fungal names are presented in their original form. Sequences highlighted in boldface were obtained in this study. Numbers above the branches represent statistical support PP > 0.95 / ML > 90.

Phylogenetic analysis of LSU rDNA (data not shown) could not further elucidate the phylogenetic placement within *Rhabdocline*, since there are presently no LSU sequences of any other member of this genus in GenBank. A well-supported *Sarcotrochila/Meria* clade was basal to other members of *Cenangiaceae*, but this placement should be considered with hesitation in view of the low number of variable sites in the dataset.

Our results from the ITS phylogeny and the connection with *R. laricis* were also supported by the *RPB2* gene region. The two sequences obtained in our study differed by only two transitions (T-C) and when aligned with the only available sequence of *M. laricis* (CBS 298.52, accession no. DQ470904), they differed in three transitions (T-C) not affecting the translation.

TAXONOMY

Following the phylogenetic placement of *S. alpina* as the teleomorph of *R. laricis*, the name of this species has to be changed following the concept of ‘one fungus, one name’. Johnston et al. (2014) suggested protecting *Rhabdocline* against *Meria* Vuill., which is an older generic name. *Sarcotrochila* (type *S. alpina*) pre-dates *Rhabdocline*, but in order to avoid numerous name changes in this well-established genus, we will propose *Rhabdocline* also for protection against *Sarcotrochila*. The correct name thus has to be based on the oldest available epitheton, which is *alpina*. Furthermore, when *Sarcotrochila* becomes synonymous, the remaining *Sarcotrochila* species must be combined into separate genera because of their phylogenetic distance to *Rhabdocline* spp. This will not necessarily cause new combinations, since most of them were described in different genera and only later combined into *Sarcotrochila* by Stone et Gernandt (2005).

***Rhabdocline alpina* (Fuckel) Koukol, comb. nov.**

Figs 2–5

Mycobank: MB851205

Basionym: *Naemacyclus alpinus* Fuckel, Jb. Nassau. Ver. Naturk. 29–30: 27, 1875 [1877–1878]

- = *Stegia alpina* (Fuckel) Rehm, Rabenhorst’s Kryptogamen-Flora, Pilze-Ascomyceten 1.3(29–31): 157, 1888
- = *Sarcotrochila alpina* (Fuckel) Höhn., Sber. Akad. Wiss. Wien, Math.-Naturw. Kl., Abt. I 126(4–5): 310, 1917
- = *Meria laricis* Vuill., C. r. hebd. Séanc. Acad. Sci., Paris 122: 546, 1896
- = *Rhabdocline laricis* (Vuill.) J.K. Stone, in Johnston, Seifert, Stone, Rossman et Marvanová, IMA Fungus 5(1): 106, 2014
- = *Peziza retrusa* W. Phillips et Plowr., Grevillea 4 (no. 31): 122, 1876
- = *Calloria retrusa* (W. Phillips et Plowr.) W. Phillips, Man. Brit. Discomyc. (London): 407, 1887
- = *Orbilina retrusa* (W. Phillips et Plowr.) Sacc., Syll. fung. (Abellini) 8: 630, 1889
- = *Mollisia retrusa* (W. Phillips et Plowr.) Sacc., Syll. fung. (Abellini) 8: 630, 1889
- = *Pseudopeziza retrusa* (W. Phillips et Plowr.) Masee, Brit. Fung.-Fl. (London) 4: 294, 1895
- = *Hyalinia nostra* Rehm, Anns mycol. 6(2): 117, 1908
- = *Orbilina nostra* (Rehm) Sacc. et Trotter, Syll. fung. (Abellini) 22(1): 724, 1913

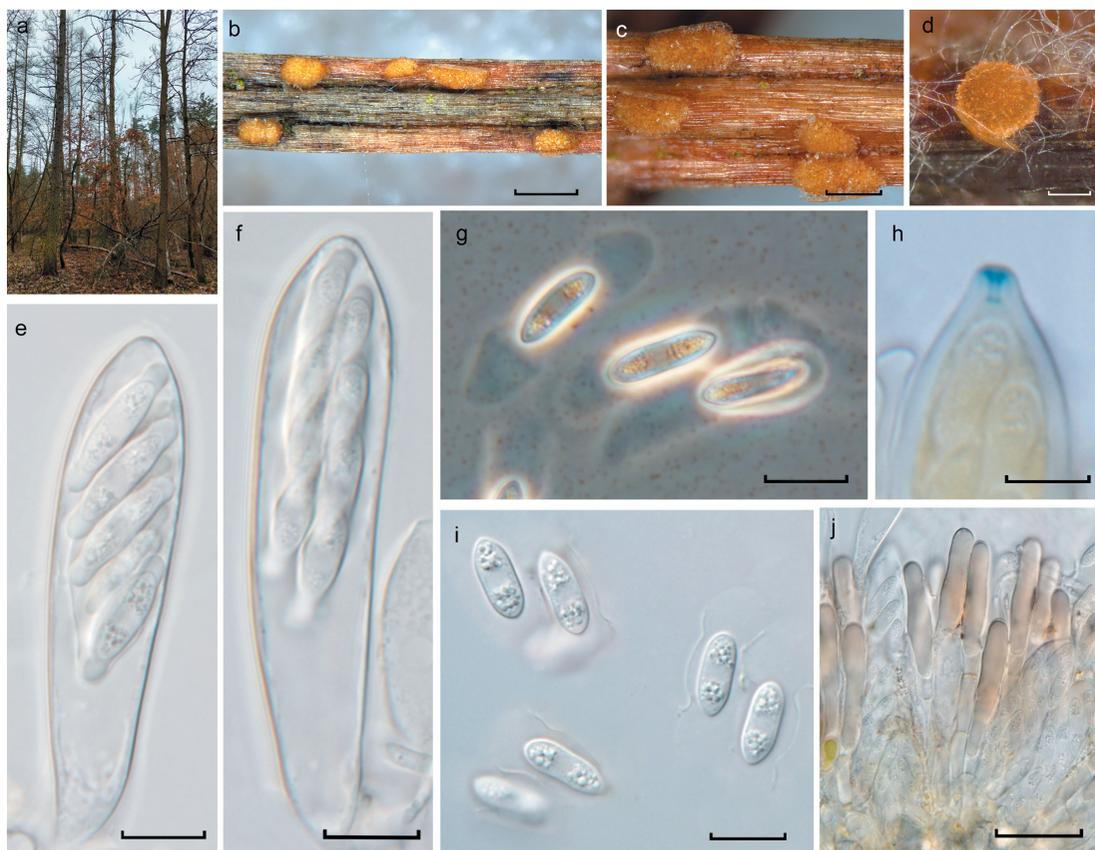


Fig. 2. *Rhabdocline alpina* (PRC9258). **a** – collection site, mixed forest with section of *Larix decidua* trees; **b–c** – ascomata on needles after cultivation in damp chamber; **d** – ascoma developing on surface-sterilised needle on MEA; **e–f** – mature asci with ascospores with compressed gelatinous sheath (preparation in water); **g** – ascospores with swollen gelatinous sheath in phase contrast; **h** – ascus apical ring blued in Melzer's reagent; **i** – ascospores with swollen sheath; **j** – paraphyses with ochraceous vacuolar bodies. Scale bars: **b** = 300 μ m; **c** = 200 μ m; **d** = 100 μ m; **e–g**, **i** = 10 μ m; **h** = 5 μ m; **j** = 20 μ m. Photos O. Koukol.

Description on natural substrate (all in living state, except for amyloid ring if not marked by †). Apothecia formed on both sides of the needle, scattered, immersed, erumpent by a single longitudinal slit in the host epidermis producing a small erect lobe (lid) on one side of the apothecium, disc yellow to light orange, circular to elliptical, 200–400 \times 120–270 μ m, slightly concave, without a clearly defined margin, lid closing the disc on drying. Asci inoperculate, club-shaped, 49–66.5 \times 9.5–13(15) μ m († 46–64 \times 8–13.5 μ m), 8-spored, spores obliquely biseriate, with strongly euamyloid (blue in IKI and Melzer's reagent) apical ring (†) of the *Calycina*-type with a minute basal protrusion, arising from crosiers.

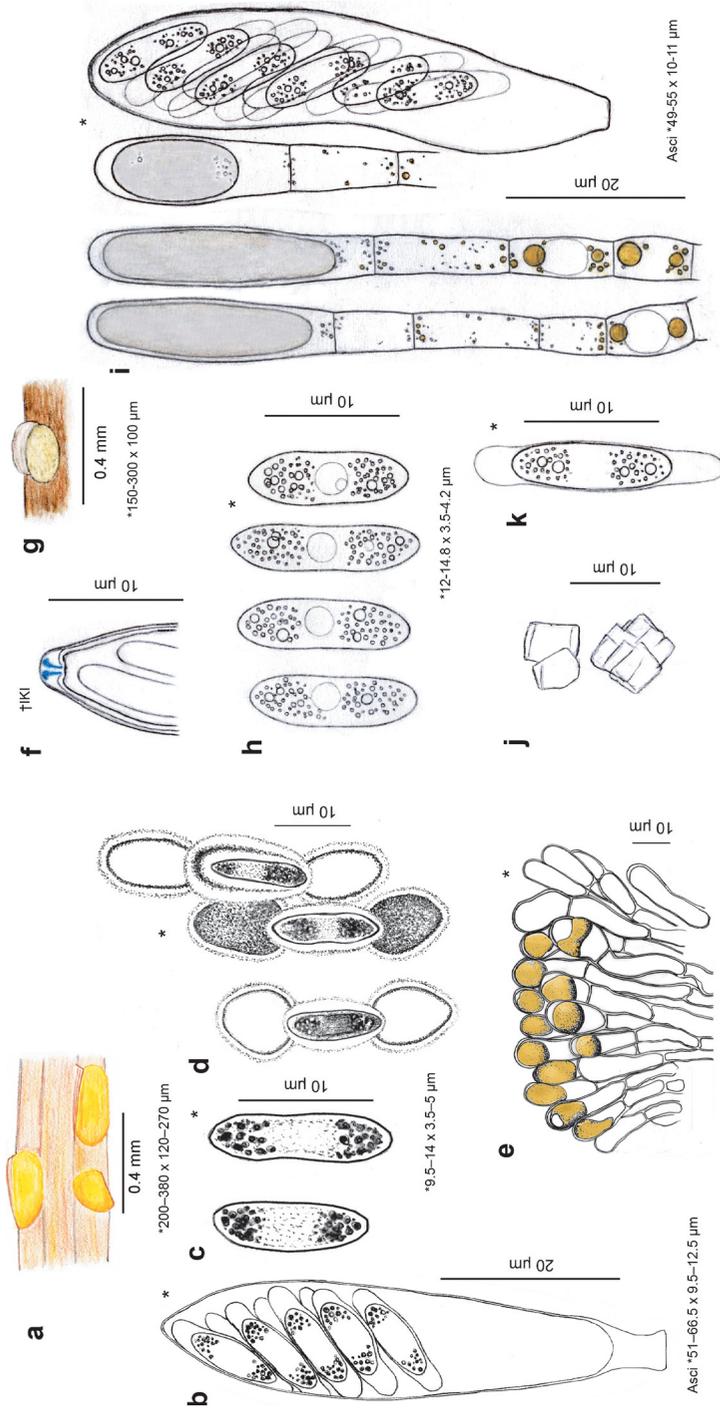


Fig. 3. Fresh collections of *Rhabdocline alpina* (a–e: PRC9202; f–k: H.B. 6109). **a, g** – apothecia with epidermis lid; **b, i** – mature asci, paraphyses with hyaline vacuolar bodies in apical cells and yellow lipid bodies in basal cells; **f** – apex of mature ascus with eumyloid apical ring; **k** – ascospore inside ascus, with gelatinous sheath; **d** – ascospores early after discharge from the ascus, gelatinous sheaths strongly swollen; **c, h** – ascospores after ejection, having lost their sheath, in h with central nucleus; **e** – marginal excipular cells with ochraceous vacuolar bodies in apical cells; **j** – refractive crystals in excipulum. Scale bars: a, g = 0.4 mm; b, i = 20 µm; c–f, h, j–k = 10 µm. Drawings K. Lukáčová (a–e), H.-O. Baral (f–k).

Ascospores cylindrical-ellipsoid, sometimes with slightly pointed ends and/or median constriction, smooth, hyaline, with numerous minute guttules (lipid bodies) in each half, and one central nucleus, (9)10.5–13(14.8) × 3.5–4.5(5) µm († 9–13 × 3–4 µm), on each pole with an evanescent, inamyloid, cap-like gelatinous sheath of 2–5 µm in length, swelling to 6.5–15 µm after spore ejection. Paraphyses with several septa in lower part, cells 5–15 × 4–5.5 µm († 3–4 µm wide), lowermost cells containing yellow-orange lipid bodies, apical cell 16–36 × (4.5)6–7(8) µm († 3–6 µm wide), containing large, elongated, hyaline to pale ochraceous, refractive vacuolar bodies of (15)19–29(35) µm in length, becoming fragmented and deep yellow-ochre in dead cells, and disappearing in KOH. Excipulum generally thin (ca 15 µm) and undifferentiated, of a pale ochraceous *textura prismatica-angularis*, cells † 6–18 × 5–7 µm, thick-walled (0.5–1.2 µm), containing abundant refractive crystals, at margin with swollen cells containing globose to short elongated, refractive vacuolar bodies.

Description in culture. Mycelium on MEA dense, compact, flat, initially white, later producing a green pigment and becoming darker. Mycelium on PCA flat, loose, white, no pigment produced. No anamorph was produced in culture within three months, but cultures surveyed after seven months revealed numerous minute sporodochia on PCA (Fig. 4c), and larger but less abundant sporodochia on MEA (Fig. 4g). The minute sporodochia contained oblong to oval conidia of 3–5.5 × 1.5–3(5) µm, whilst the larger sporodochia contained oblong conidia with a slight median constriction and truncate base, 9–11 × 3.5–4 µm. Conidiogenous cells not observed.

Notes. The morphological descriptions given for *R. laricis* in the literature confirm our observation in most respects. However, there are a few minor differences in the morphology of the ascospores. Firstly, their gelatinous sheaths (Fig 2 g, i) are not mentioned in the literature (Fuckel 1875, Rehm 1908, Höhnelt 1917, Korf 1962, Ellis et al. 1997) and have probably been overlooked due to their evanescence, especially when studying herbarium material. Secondly, we did not observe any kind of septation or pigmentation of the ascospores in any of our collections, although we studied the apothecia at full maturity. Likewise, neither Fuckel (1875) nor Rehm (1908) or Phillips et al. (1876) mention spore septa and pigmentation. However, Korf (1962) observed aged ascospores becoming faintly brown and “rarely develop one or three septa” in material collected in Engadin, Switzerland (Barb.-Boiss. 1059).

Baral et al. (2020: 1680, 1683) revised the types of *Hyalinia nostra* Rehm and *Peziza retrusa* W. Phillips et al., and found them to fit *S. alpina*. In the syntypes deposited in M, large, swollen polar sheaths were observed, though only inside the asci (Fig. 5). The much larger spore size given in the protologue of *P. retrusa* (17–19 × 5–6 µm) appears to be an error. Fuckel (1875) described the spores of

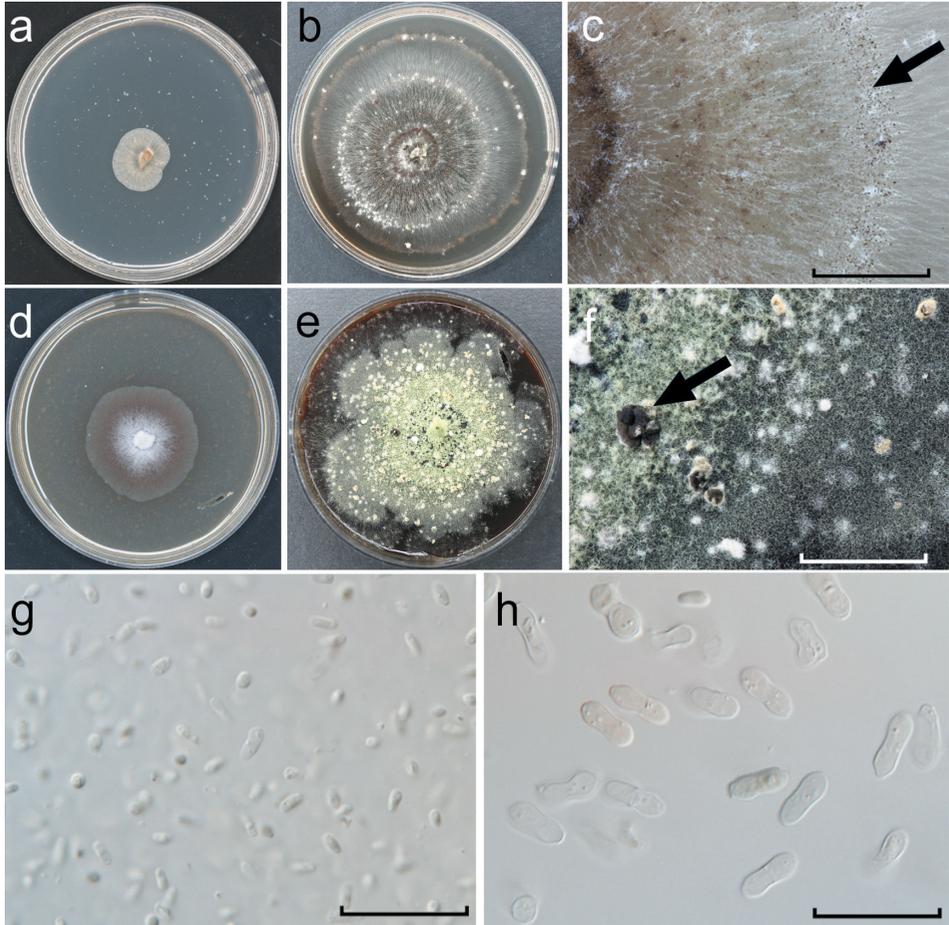


Fig. 4. *Rhabdocline alpina* in culture (CCF6728). **a** – colony on PCA after 2 weeks; **b** – the same colony after seven months; **c** – detail of mycelium with minute sporodochia (arrow); **d** – colony on MEA after 2 weeks; **e** – the same colony after seven months; **f** – detail of mycelium with larger sporodochia (arrow); **g** – microconidia; **h** – macroconidia. Scale bars: c, f = 5 mm; g, h = 20 µm. Photos O. Koukol.

Naemacyclus alpinus as biguttulate, certainly due to confluence of the lipid bodies (this change from multi- to biguttulate was also observed in the recent collection from Hessen). Refractive vacuolar bodies have been overlooked in the literature because of their disappearance in herbarium material, especially when using KOH as mounting medium. They are characteristic of *Cenangiaceae*, while consistently absent in e.g. *Cordieritidaceae* and *Chlorociboriaceae*. Crosiers have also often been neglected because of the difficulty of making adequate preparations, and were seen here only in the type of *Hyalinia nostra*. The numerous

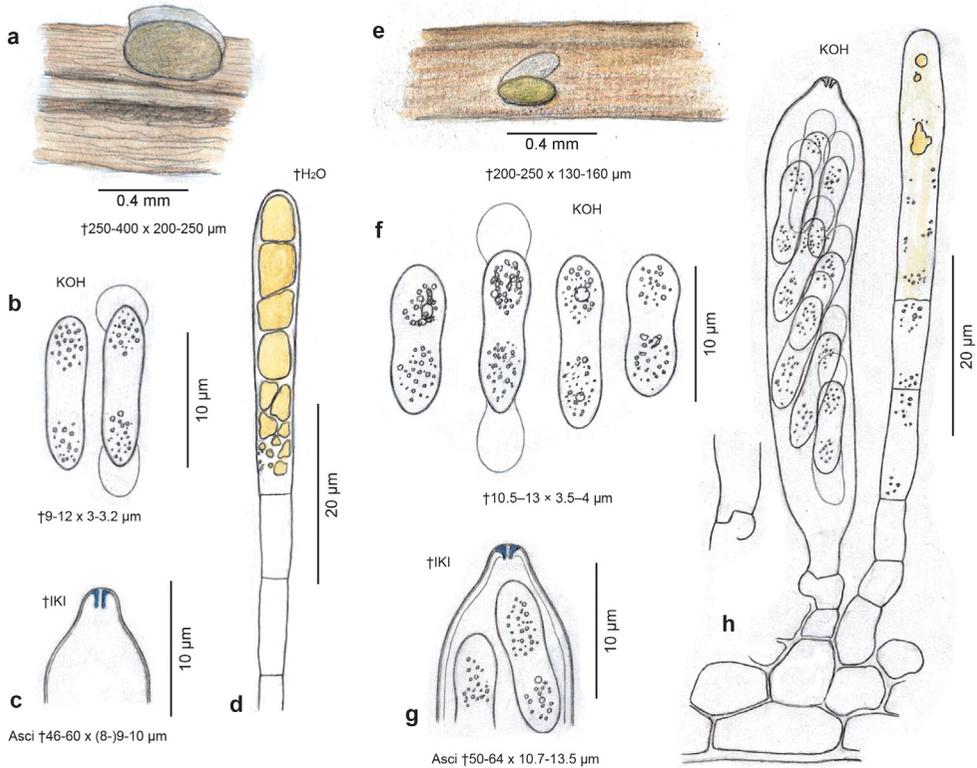


Fig. 5. *Rhabdocline alpina*, herbarium material [a–d: Phillips 126, M (H.B. 5052), type of *Peziza retrusa*; e–h: Rehm 1754, M (H.B. 5050), type of *Hyalinia nostra*]. **a, e** – apothecia with epidermis lid (rehydrated); **b, f** – ascospores inside and outside asci, some with gelatinous sheaths; **d, h** – mature ascus, paraphyses with hyaline vacuolar bodies in apical cells, in d with crosiers at base, and thick-walled basal excipular cells; **c, g** – apices of immature (c) and mature (g) asci with euamyloid apical rings. Scale bars: a, e = 0,4 mm; d, h = 20 µm; b–c, f–g = 10 µm. Drawings H.-O. Baral.

crystals observed in the basal excipulum of the type of *H. nostra* and in a fresh collection from England (H.B. 9544) were also mentioned by Höhnelt (1917).

Both types of conidia, which we observed in cultures after a prolonged period of cultivation, agree with the macro- and microconidia mentioned by Sherwood-Pike et al. (1986), with the exception that these authors observed concurrent production of both types in the same sporodochia. Our cultures were too old to observe also conidiogenous cells, which disintegrate rapidly according to Sherwood-Pike et al. (1986). Oblong and constricted macroconidia were also described on larch needles infected by the anamorphic pathogen (Funk 1985).

Specimens observed (all on needles of *Larix* in litter)

Czech Republic. Praha, Cholupický vrch Hill, mixed plantation of coniferous and broad-leaved trees, NE of Točná Airport, 302 m a.s.l., 49°59'20.4" N, 14°25'52.9" E, 12 March 2022, leg. A. Vicioso, det. O. Koukol AV03 (PRC 9258, living culture CCF 6727; GenBank: ITS = PP134996, *RPB2* = PP197242). – Praha, Hostivař, floodplain forest NE of Triangl marsh, 252 m a.s.l., 50°03'44.7" N, 14°31'25.2" E, 25 February 2023, leg. K. Lukáčová, det. O. Koukol KL-1 (PRC 9202, living culture CCF 6728; GenBank: ITS = PP134997, *RPB2* = PP197243). – Praha, Hostivař, tree plantation between Rabakovská and K Pérovně Streets, 263 m a.s.l., 50°03'59.5" N, 14°31'25.7" E, 25 February 2023, leg. K. Lukáčová, det. O. Koukol KL-2 (PRC 9203). – Ústí nad Labem Region, Duchcov, spoil tip W of Duchcov Castle, 241 m a.s.l., 50°36'13.0" N, 13°43'47.4" E, 20 March 2023, leg. M. Kabát, det. O. Koukol MK-2 (PRC 9259).

Germany. Hessen, Rhön, Tann, Schweidhof, ~550 m a.s.l., 2 May 2002, leg. et det. L. Kriegsteiner (not preserved). – Bayern, Oberbayern, München, park of Neufriedenheim Monastery, 535 m a.s.l., April 1908, leg. H. Rehm, det. H.-O. Baral [Rehm 1754, M, type of *Hyalinia nostra* (H.B. 5050)].

Great Britain. North Wales, Trefriw, ca 50 m a.s.l., May 1874, leg.? W. Phillips, det. H.-O. Baral [W. Phillips 126, M, type of *Peziza retrusa* (H.B. 5052)]. – South Yorkshire, Sheffield, Greno Wood, 275 m a.s.l., 18 May 2011, leg. et det. H.-O. Baral (H.B. 9544).

Luxembourg. Müllerthal, Schnellert, 300 m a.s.l., 7 May 1998, leg. et det. H.-O. Baral (H.B. 6109).

Slovakia. Bratislava Region, Malacky, Malé Karpaty Protected Landscape Area, forest W of Korenec hill, 421 m a.s.l., 48°17'33.2" N, 17°05'30.9" E, 19 March 2023, leg. K. Lukáčová, det. O. Koukol KL-8 (PRC 9205). – Košice Region, Nižný Klatov, Volovské vrchy Mountains, 537 m a.s.l., 48°44'52.3" N, 21°11'14.8" E, 4 March 2023, leg. K. Lukáčová, det. O. Koukol KL-5 (PRC 9204). – Štós-kúpele, planted park trees, 625 m a.s.l., 48°43'19.5" N, 20°45'46.9" E, 10 April 2023, leg. K. Lukáčová, det. O. Koukol KL-9 (PRC 9260). – Volovské vrchy Mountains, Štóske sedlo saddle, 778 m a.s.l., 48°43'19.1" N, 20°47'28.0" E, 10 April 2023, leg. K. Lukáčová, det. O. Koukol KL-10 (PRC 9261).

DISCUSSION

Our study shows that an apparently saprotrophic fungus growing on litter needles of various larch species, and long known as *Sarcotrochila alpina*, is conspecific with the anamorph-typified larch needle pathogen *Rhabdocline laricis*. This result was based primarily on an analysis of ITS rDNA, which showed placement of *S. alpina* in the *Rhabdocline* clade and conspecificity of the two taxa (Fig. 1). It is further supported by the morphology of the phenotype: *S. alpina* fits well into the concept of *Rhabdocline*, whose members produce very similar apothecia almost without stromatic or excipular tissue immersed in host tissue, and a similar type of ascospores (particularly *R. parkeri* Sherwood, J.K. Stone et G.C. Carroll and *R. weirii* A.K. Parker et J. Reid), including a thick gelatinous sheath (*R. weirii*; Funk 1985). The presence of a pycnidial anamorph producing unicellular phialoconidia connects *R. alpina* with *R. pseudotsugae* Syd., the type of the genus *Rhabdocline* (Sydow et Petrak 1922).

It is of special interest that this anamorph-teleomorph connection had been considered earlier, though not confirmed. Both the anamorph and teleomorph stages were described in the late 19th century, and repeatedly reported in the literature. Sherwood-Pike et al. (1986) predicted, based on the germination pattern of

conidia of *R. alpina* that “*Meria laricis* has an inconspicuous hemiphacidiaecous teleomorph which has been hitherto overlooked”. This opinion, made long before the use of molecular data, was confirmed in our study. On the other hand, Gernandt et al. (1997) supposed that *R. laricis* lost the ability to form the teleomorph state. They hypothesised that the connection to a deciduous conifer prevents ascocarps to develop, which requires at least one year just as in other *Rhabdocline* species. Indeed, the life cycle of some *Rhabdocline* species may span over several years, such as in *R. parkeri*, a pathogen of Douglas fir. According to Sherwood-Pike et al. (1986), the life cycle of *R. parkeri* starts with autumn infection of needles caused by conidia of the anamorph, followed by a latent phase lasting a few years with the fungus colonising needles as an endophyte. After natural senescence, the teleomorph appears on the needles in litter early in winter. Production of the teleomorph by *R. alpina* early in the growing season may thus be viewed as a specific adaptation to the colonisation of a deciduous conifer.

The teleomorph of *R. alpina* has only rarely been recorded in the literature, usually on fallen needles (without specification of natural or premature senescence due to a pathogen). Literature reports mention its occurrence on European larch, but Rehm (1908) recorded it (as *Hyalinia nostra*) on litter needles of *Larix kaempferi* (Lamb.) Carr. (= *Larix japonica* Carrière) near München, Germany.

Rhabdocline alpina has apparently only once been mentioned as a foliar pathogen in connection with the teleomorph. Ziller (1969) reported a severe blight of alpine larch (*Larix lyallii* Parl.) in British Columbia (Canada). He observed apothecia belonging to *Sarcotrochila alpina* on dead needles and suspected this species to cause “needle blight, mainly of seedlings and young trees”. Although these symptoms matched those caused by the anamorph (Jalkanen 2016), the two fungi were still not connected.

The name *Meria laricis* for the pathogenic anamorph was used until Gernandt et al. (1997) connected it with other members of the teleomorph-typified genus *Rhabdocline*, based on ITS rDNA. The Douglas fir needle pathogen *R. parkeri* was found to be the most closely related species, which was also confirmed in our study (Fig. 1). The new combination of *M. laricis* in *Rhabdocline* was proposed by J.K. Stone (in Johnston et al. 2014) only after cessation of the provision in Article 59 of the International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012). No sequence of any member of *Sarcotrochila* had been available until Stone et Gernandt (2005) obtained the first ITS rDNA sequences from the holotypes of *S. macrospora* and *S. longispora* (as *Hemiphacidium longisporum* Ziller et A. Funk). Based on a very small difference in this gene region (only three base pairs), they decided to synonymise *Hemiphacidium* under *Sarcotrochila*. In their phylogeny, the *Hemiphacidium/Sarcotrochila* clade formed the outgroup together with *Fabrella tsugae* (Farl.) Kirschst., but nevertheless indicated a close relationship to *Rhabdocline*.

Following our finding and the new combination, further nomenclatural changes are necessary. In a more recent study of *Cenangiaceae*, Pärtel et al. (2016) showed that *S. longispora* (as *H. longisporum*) clustered together with *S. macrospora* in a position distant from the *Rhabdocline* lineage. The use of the once abandoned genus *Hemiphacidium* may thus be reappraised. Most probably, *S. longispora* and *S. macrospora* are conspecific, but this cannot be decided on with certainty, since both species are represented by a single sequence in GenBank. However, if additional collections of these two species become available, a morphological study could also confirm the taxonomic value of the reaction of the ascus apical ring in iodine. When Ziller et Funk (1973) newly described these two species, they distinguished them by the different iodine reaction: the ring of *S. macrospora* became clearly visible (blue) in Melzer's reagent, whilst *H. longisporum* showed no reaction. However, Gernandt et al. (1997) saw a limited value in the apical ring reaction (*R. pseudotsugae* I–, *R. weirii* and *R. parkeri* I+), which did not match with their ITS rDNA phylogeny, and therefore concluded that “the possession of an amyloid ascus pore is an unreliable character for differentiation of species and subspecies” (p. 735).

During our morphological studies, we recorded only unicellular ascospores, although literature records also indicate presence of septate ascospores. Ziller (1969) even mentioned that Canadian collections of *S. alpina* had unequally two-celled, pale yellow ascospores. This observation was connected to a larch needle blight incidence in British Columbia (Canada). Together with the single ITS sequence (accession no. U92298, obtained from a fungus isolated from needles of *Larix occidentalis* Nutt. in Washington, USA) forming a distinct lineage within the *Rhabdocline* clade, this indicates that another, hitherto undescribed pathogenic species occurs in North America. Further research of native larch species such as *L. lyallii* could confirm this assumption.

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