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Cortinarius spilomeoalpinus (Basidiomycota, Agaricales) a new alpine species of sect. *Spilomei* from South-Tyrolean *Dryas octopetala* habitats

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Abstract: During our long-term studies on *Cortinarius* species in alpine habitats, we discovered several collections of a *Cortinarius* species resembling *C. spilomeus*. We carried out comparative morphological studies and a phylogenetic analysis based on rDNA ITS sequences. We included also type material of *Cortinarius spilomeus forma dryadicola*. We confirmed that *Cortinarius spilomeoalpinus* is a distinct species which is typical for alpine *Dryas octopetala* habitats. *Cortinarius spilomeus forma dryadicola* is not part of *C. spilomeus* sensu stricto. It is conspecific with the later described *C. ferrusinus*, another taxon of sect. *Spilomei*, and we treat it as *forma* of it. Detailed descriptions are given for the alpine taxa, and a differential diagnosis and dichotomous identification key are provided.

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INTRODUCTION

Alpine taxa, specifically fungi found in habitats above the timberline, play a crucial role in their ecosystems. These fungi are not just incidental; they form symbiotic ectomycorrhizal relationships with typical alpine plants, enhancing nutrient uptake and promoting overall plant health. *Dryas octopetala*, *Salix herbacea*, *S. reticulata*, *S. retusa*, *Kobresia myosuroides*, and *Bistorta vivipara* are widespread alpine plants, and their associated ectomycorrhizal fungi have received much attention in recent years (e.g. Ryberg *et al.* 2009, Bjorbækmo 2010, Botnen *et al.* 2013, Arraiano-Castilho *et al.* 2021). The mutualistic interactions between plants and their associated fungi are crucial for maintaining the delicate balance in alpine environments, making these fungi essential contributors to biodiversity and ecosystem stability. However, our knowledge of their diversity and taxonomy is still at an early stage. For example, we still know little about how many of the ectomycorrhizal taxa we encounter in the alpine zone are strictly alpine and how many occur below the forest line, e.g. on similar hosts such as lowland *Salix* species. We use the term “alpine fungi” here to address habitat-specific arctic and alpine fungi. Those occurring only sporadically in alpine areas are not included. Species from the genera *Inocybe* and *Cortinarius* are the most frequent macrofungi forming ectomycorrhizas with *Dryas octopetala* or alpine dwarf *Salix* species (Brunner *et al.* 2017).

Starting from the pioneering work of Favre (1955) and Kühner (1955), many alpine *Cortinarius* taxa have been described in on different taxonomic ranks (*forma*, variety, species).

A nice overview of the diversity of alpine basidiome forming *Basidiomycota* can be found in the books of Jamoni (2008) or Armada *et al.* (2023). However, the identity and evolutionary history of many species remain difficult, and exhaustive, more thorough studies are needed to understand the taxonomy and thus, real diversity of alpine ectomycorrhizal fungi.

We have been collecting alpine *Cortinarius* species in the phantastic environment of the Italian Dolomites for decades. During our long-term studies, we discovered several collections of a *Cortinarius* species resembling *C. spilomeus*. We generated barcoding sequences from all available alpine vouchers. We carried out extensive literature research and included type material of *Cortinarius spilomeus forma dryadicola* in our study.

We were interested in the following main questions. Do alpine taxa of *Cortinarius* sect. *Spilomei* exist? Are temperate-montane species expanding into the alpine zone? Are European and American taxa distinct? And if there are distinct alpine taxa, how abundant are taxa of the sect. *Spilomei* in alpine areas?

MATERIALS AND METHODS

Collections and morphological analyses

Samples were collected in the Italian Alps. Detailed information on voucher provenance, ecology and GenBank numbers are provided (Table 1).

Fresh fungal collections were photographed in situ with a Nikon D5600 camera with AF Micro nikkor 60 mm objective

and documented. Macroscopic descriptions were routinely made from fresh basidiomes. Colours of the basidiomes were documented following the Methuen Handbook of Colour (Kornerup & Wanscher 1978). Macrochemical KOH reactions were tested on dried basidiomes (KOH 30 %).

Microscopic characters were taken from dried specimens. Microscopic data were documented with a Nikon camera DSFi1 in combination with the computer program NIS-Elements D 3.0. Measurements ($n \geq 30$) were made in 3 % KOH from basidiospores taken from spore deposits either on the apex of the stipe and on veil remnants. Observations of the pileipellis and the hymenium were made in water, KOH and Congo Red. Microscopic structures were drawn from photos. All specimens were examined by a microscope. Examined voucher material is deposited in the fungaria IBF, MCVE and the private fungarium Ferrari R.J.

DNA extraction and sequencing

In order to establish phylogenetic relationships, rDNA ITS sequences were produced as previously described (Peintner *et al.* 2001) using the primers ITS1 and ITS4 (White *et al.* 1990). The same primers were used for sequencing. Sequences were assembled and edited with Sequencher v. 4.1 (Gene Codes, Ann Arbor, Mich., USA) and BLAST-searches were conducted against UNITE (<http://unite.et.ee>) and the International Nucleotide Sequence Databases Collaboration (INSDC) databases. Sequences of closely related *Cortinarius* species were downloaded from GenBank (<http://ncbi.nlm.nih.gov/>) and UNITE. Sequences from type material were also included in the study (Table 1).

A total of 30 full ITS sequences from nine *Cortinarius* species were used for this study. Newly created sequences were submitted to GenBank (Table 1).

Data analysis

Evolutionary analyses involved 30 nucleotide sequences. The outgroup was selected based on published sister group relationships (Dima *et al.* 2021), and by including most closely related sequences from the type specimen based on own BLAST searches.

Sequences were automatically aligned in MEGA v. X (Kumar *et al.* 2018), which was also used to inspect the alignment. All positions with less than 95 % site coverage were eliminated. A total of 580 positions were included in the final dataset.

Maximum Likelihood (ML) analysis was conducted in Geneious Prime v. 2024.0.7 (<https://www.geneious.com>) using RAxML v. 8.2.11 (Stamatakis 2014). The evolutionary history was inferred by Maximum Likelihood with the GTR model with discrete Gamma distribution. It was run with Rapid Bootstrapping, searching for the best scoring ML tree, and 500 replicates.

The Bootstrap support values were also inferred using the Maximum Parsimony (MP) method. The MP tree was obtained in MEGA v. X (Kumar *et al.* 2018), using the Subtree-Pruning-Regrafting (SPR) algorithm, 500 replicates, 10 initial trees, search level 4, max. 50 trees retained.

Additionally, branch robustness was tested with Bayesian Inference in MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001). GTR was used as substitution model, and a gamma distribution of rate variation across sites was chosen. For prior probability settings defaults were kept. For the Markov Chain Monte Carlo

(MCMC) analyses, four chains were run for 2 M generations, with trees being sampled every 1000 generations. The analysis was stopped as the convergence diagnostic (average standard deviation of split frequencies) was below 0.05 after 10 M generations. From the sampled trees, 25 % were discarded as burn-in before summary statistics were calculated (using sump and sumt commands). Diagnostic plots, as well as the convergence diagnostics EES (Estimated Sample Size; min ESS around 10 K) and PSRF (Potential Scale Reduction Factor; 1.000 for all parameters), indicated stationarity.

For statistical evaluation, at least 30 basidiospores were measured. Basidiospore measurements are based on (min) mean \pm standard deviation (max): the range of spore length and width have been calculated as follows: mean-standard deviation–mean+standard deviation. Statistical analyses for comparing spore sizes between species were performed in R v. 4.4.1 (2024-06-14) (R Foundation for Statistical Computing, Vienna, Austria; <https://www.R-project.org/>) (R Core Team 2023), with spore width, length and Q. Variables were checked for normal distribution and equal variances. Based on data distributions, differences between species were analysed using a Kruskal-Wallis test followed by pairwise Wilcoxon tests with Bonferroni correction applied (package stats), performed at the 0.05 significance level. To improve the visualisation of the phylogenetic tree, Inkscape v. 1.3 (<https://inkscape.org/de/>) was used.

RESULTS

Phylogeny

The phylogenetic tree with the highest log likelihood (-1540.41) resulting from the ML analysis of the ITS sequences is congruent with the Bayesian consensus tree (Fig. 1). The phylogenetic analysis resulted in a well-supported (BS > 94 %) core clade of sect. *Spilomei*. The sect. *Spilomei* has no supported sister group relationship; the most closely related species are Southern Hemisphere taxa.

In our phylogeny, we were able to distinguish five well-supported taxa within sect. *Spilomei*. These include *C. spilomeus*, *C. ferrusinus* (with *C. spilomeus* f. *dryadicola*), *C. spilomeoalpinus* and the two recently described American species *C. magispilomeus* and *C. americanispilomeus*.

Cortinarius spilomeoalpinus is sister to *C. magispilomeus*, but clearly represents a distinct lineage. The sequence created for *C. spilomeus* f. *dryadicola* differ only by 1–2 bp from sequences of *C. ferrusinus*, and we consider them as ambiguous bases. Thus, there is no indication for the presence of a distinct alpine group in the *C. ferrusinus* lineage based on the available sequences.

Taxonomy

Cortinarius* sect. *Spilomei (Moenne-Loec. & Reumaux) Consiglio *et al.*, *Il Genere Cortinarius in Italia*, 4: 35. 2006.

Type species: Cortinarius spilomeus (Fr.) Fr., *Epicrasis Systematis Mycologici*: 287. 1838.

Based on current knowledge and the here presented data, sect. *Spilomei* consists of the following taxa: *C. spilomeus* from Northern Hemisphere coniferous forests, *C. magispilomeus*

and *C. americanospilomeus* from mixed forests in Canada, *C. ferrusinus* from European subalpine calcareous coniferous habitats, and *C. spilomeoalpinus* and *C. ferrusinus forma dryadicola* from European alpine calcareous habitats. We could not detect any statistically significant differences in spore size between the included species.

Cortinarius spilomeoalpinus R.J. Ferrari & Peintner, *sp. nov.*
MycoBank MB 855569. Figs 2, 3.

Etymology: *Spilomeoalpinus* refers to the resemblance and close relationship to taxa from the sect. *Spilomei* and to the alpine habitat.

Diagnosis: Pileus 7–35 mm diam, reddish brown, dry; stipe 15–30 mm long, slightly clavate, whitish-silky fibrillose. Universal veil forming rusty brown girdles on the stipe. Lamellae cream with lilac or pale blue hue when young. *Basidiospores* rusty brown, subglobose to broadly elyptic, strongly ornamented, 6.4–8.9 × 5.0–6.5 μm, Q = 1.3. In alpine *Dryas octopetala* habitats on calcareous ground. The ITS sequences of *Cortinarius spilomeoalpinus* differ by 37 base pairs from the closely related *C. spilomeus*.

Typus: **Italia**, Trentino-Alto Adige/Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Gran Fanes, 2 112 m a.s.l., 46°35.639580'S, 12°1.2732'W, *Dryas octopetala* and *Carex*

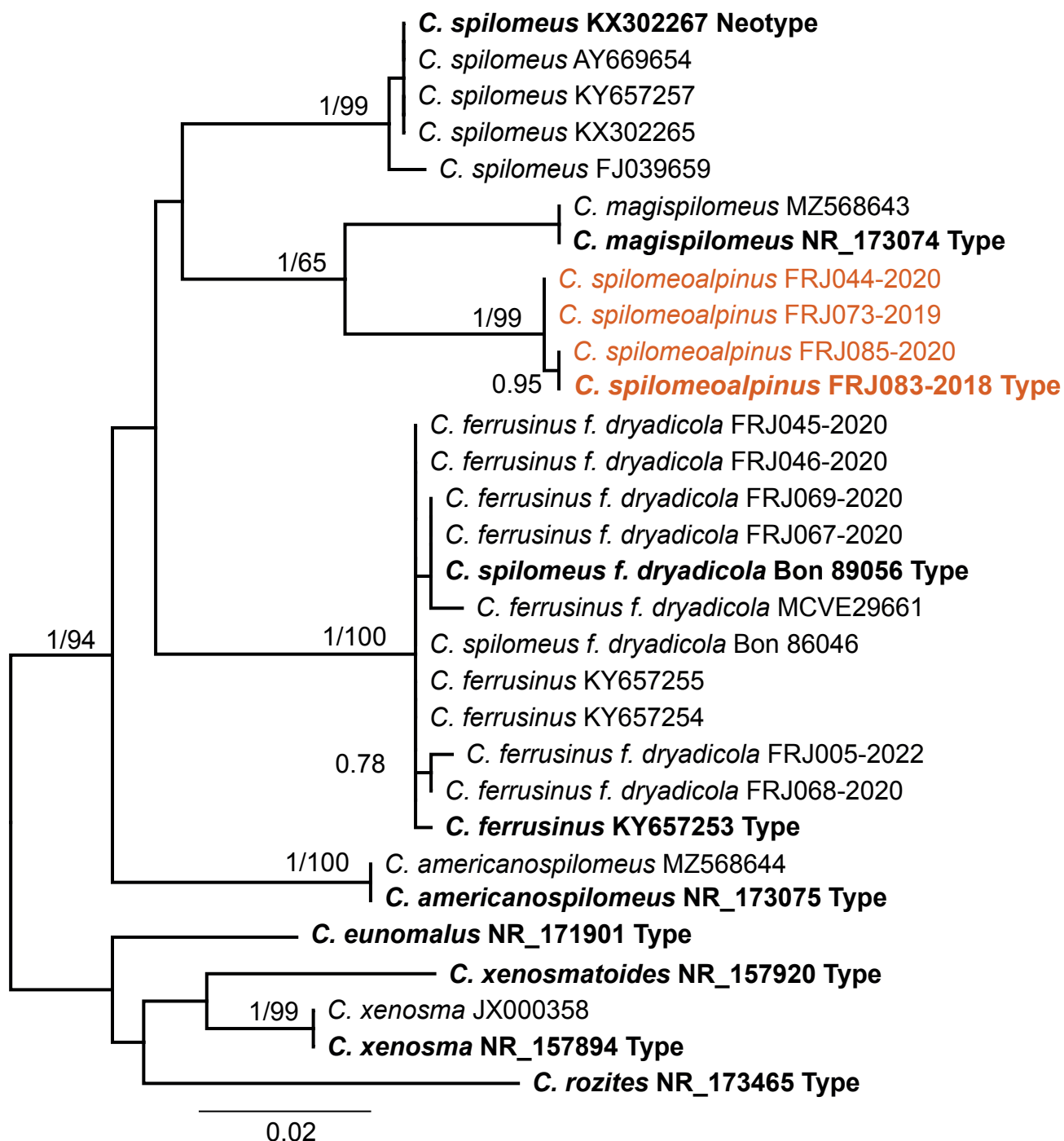


Fig. 1. Phylogenetic relationship of *Cortinarius* section *Spilomei* based on rDNA ITS sequences. Maximum Likelihood tree with Bayesian Posterior Probabilities / Maximum Parsimony Bootstrap support values above the branches.

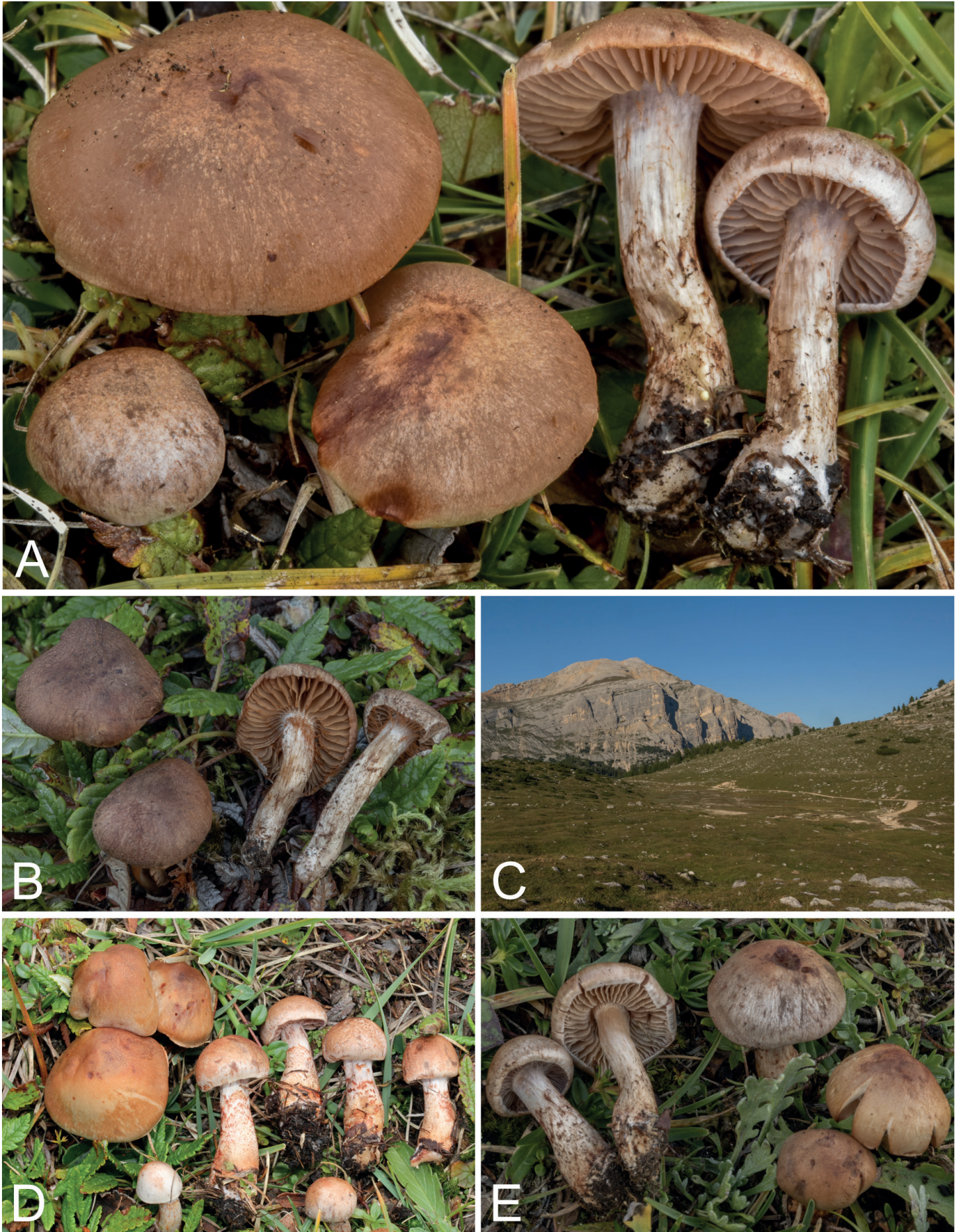


Fig. 2. **A.** *Cortinarius spilomeoalpinus* (holotype IBF20180234, isotype FR083-2018) in the natural environment with *Dryas octopetala*. **B.** Older basidiomata of *Cortinarius spilomeoalpinus* (FRJ0085-2020) in the natural environment with *Dryas octopetala*. **C.** Type locality, habitat of *Cortinarius spilomeoalpinus*. **D.** *Cortinarius ferrusinus* f. *dryadicola* (MCVE29661) in the natural environment with *Dryas octopetala*. **E.** Older basidiomata of *Cortinarius ferrusinus* f. *dryadicola* (FRJ069-2020). A–C, E, photo by Renato Jonny Ferrari; D, photo by Enrico Bizio.

firma on calcareous soil, 4 Sep. 2018, R.J. Ferrari (holotype IBF20180234, GenBank ITS accession number: OM432508); isotype FRJ83-2018.

Description: Pileus 7–35 mm, at first hemispheric then expanded with surging margin, initially cream-coloured (4A3) interspersed with greyish orange (6B5), gradually increasing copper red (7C8) +/- vivid, darker in the middle, in dry weather agate (7E8) - reddish orange (7B8) +/- silky especially when young, pale greyish brown (6E3) with a hue of silver (4E2) at the margin, scattered, evanescent, reddish orange (7B8) veil-like residues more evident in the discal area when young, rarely at the margin, surface smooth, hygrophonous. Lamellae sinuate to adnexed, slightly distant, initially cream (4A3) to orange white (5A2) suffused with violet white (18A2), soon ochraceous with irregular lighter edge, finally completely ochraceous. Stipe 15–30 × 3–5 mm with rounded base, pale violet (18A3) at apex, near lamellar junction especially when young, elsewhere +/- whitish then gradually becoming brownish orange (7C8) towards base, at manipulation and in more mature specimens, entirely covered with silky fibrillae. Universal veil distinct, forming brownish red (9C7) girdles best observed from 2/3 downwards, more evident towards base. Presence of white mycelium at base (Fig. 2). Context overall yellowish white (4A2), suffused with violet white (18A2), brownish yellow (5C8) in the pileus and at the base of the stipe. Smell faint of old potato skin, taste mild. Basidiospores rusty brown (6.4–)7.0–7.9(–8.9) × (5.0–)5.3–6.2(–6.5) μm (sub)globose to broadly elliptic, ornamentation strong, often with more pronounced ornamented apex, $Q = (1.1–)1.2–1.5(–1.6)$; $Q_{mw} = 1.3$ ($n = 31$, from holotype). Basidia tetrasporic hyaline to pale brownish yellow in 5% KOH, 20.0–39.5 × 7.5–10.5 μm, sterigmata up to 6.0 μm long and 2.2 μm wide, basidioles cylindrical 18.0–23.5 × 5–6 μm; Lamellar filament fertile, terminal cells 8.5–25.0 × 4.0–7.0 μm cylindrical-flexuous, clavate, pyriform sometimes lobed, lower than basidia, septate with a hemispherical apex, never emerging. Hyphae in lamellar trama partly inflated, up to 17.5 μm in diam. Pileipellis composed of brownish yellow hyphae; in the epicutis the hyphae are partly slightly incrustated, some totally hyaline, emerging and intertwined, sometimes grouped in scattered clumps, 4–9 μm diam with obtuse sometimes enlarged terminal cells; hypodermis poorly differentiated, composed of broader hyphae arranged horizontally and intertwined, swollen, cylindrical, sausage-like with a diameter of 8–16 μm. Clamp connections abundant in all tissues (Fig. 3). Chemical reactions (KOH 30%) negative.

Habitat and distribution: Known only from the alpine areas in the Italian area of the Dolomites, always in association with *Dryas octopetala*, sporulating in August to September.

Conservation status: Based on our observations this is a rare species linked to alpine *Dryas octopetala* habitats. The latter are threatened habitats in the Alpine range due to global warming.

Additional materials examined: **Italia**, Trentino-Alto Adige/Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Gran Fanes, 2112 m a.s.l., *D. octopetala* on calcareous soil, 46°35.639580'S, 12°1.2732'W, 30 Aug. 2019, R.J. Ferrari, FRJ073-2019, GenBank ITS accession number: PQ243142; *ibid.*, *D. octopetala* on calcareous soil, 46°35.639580'S, 12°1.2732'W, 15 Sep. 2020, R.J. Ferrari, FRJ085-2020, GenBank Acc. No.

PQ243141; *ibid.*, loc. Pici Ciemplac 2310 m a.s.l., *D. octopetala*, *Helianthemum oelandicum* subsp. *alpestre*, *Juniperus communis* and *Calluna vulgaris* on calcareous soil, 46°36.762'S, 11°59.700'W, 20 Aug. 2020, R.J. Ferrari, IBF20200078, FRJ044-2020, FRJ02-2020, GenBank ITS accession number: OM432507.

Notes: When comparing to taxa possibly also occurring in alpine habitats, *C. spilomeoalpinus* differs from *C. ferrusinus* by the more dull basidiome colours and less pronounced rusty red velar girdles not being in such clear contrast to the stipe surface. The stipe is clavate in *C. ferrusinus*, but only occasionally roundish at the base in *C. spilomeoalpinus*. There are no significant differences in spore size between *C. spilomeoalpinus* and *C. ferrusinus*. However, *C. spilomeus* can be distinguished from both, *C. ferrusinus* and *C. spilomeoalpinus*, based on the weakly to moderately verrucose basidiospores.

Comparison with other macro-morphologically similar European species of sect. *Spilomei* occurring in forests is based on literature only, as we have not been able to obtain type material for comparison, despite several requests.

Other taxa of sect. *Spilomei* occurring with conifers are *C. spilomeus* var. *subspilomeus*, *C. depauperatus*, and *C. depauperatus* var. *percoloratus*. *Cortinarius spilomeus* var. *subspilomeus* was neotypified in Atlas des Cortinaires Vol. IV (Bidaud *et al.* 1992) and described to have subglobose and densely warty basidiospores (5.5–)6–7(–7.5) × 5–6 μm.

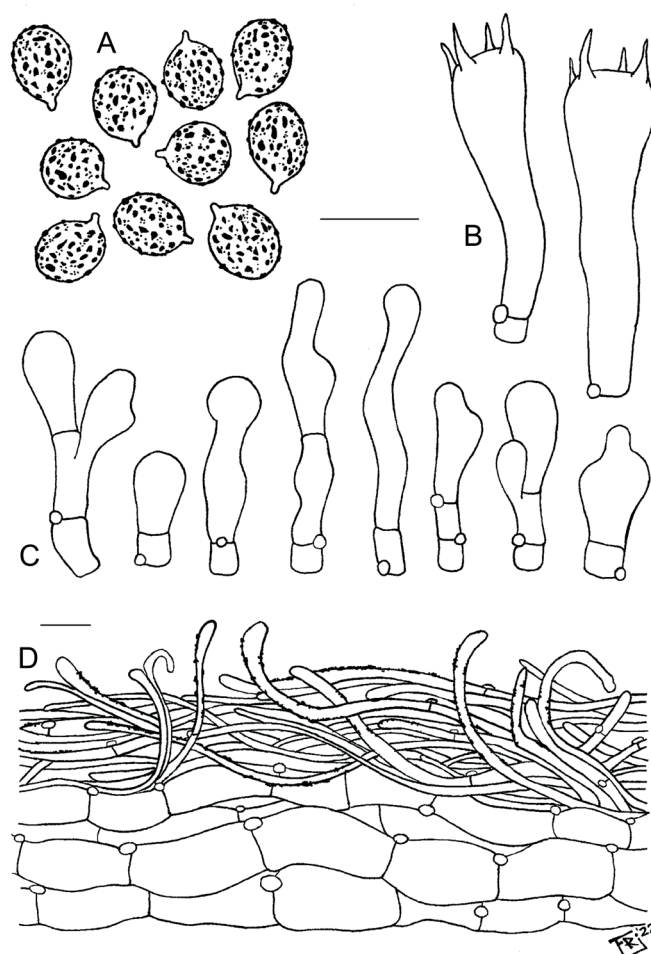


Fig. 3. Drawing of microscopic characters of *Cortinarius spilomeoalpinus* (holotype, IBF20180234, isotype FR083-2018). A. Basidiospores. B. Basidia. C. Terminal elements of the lamellar filum. D. Pileipellis. Scale bars = 10 μm.

Cortinarius depauperatus differs from most taxa of the section by the indistinctly punctate spores.

Additional species were described from other forest habitats: *C. floccosofibrillosus*, *C. ferrugineipes*, *C. pavonius*, *C. peronatorugosus*, and *C. peronatosericeus*. Type studies are urgently needed to clarify these epithets. Finally, Quèlet himself considered *Cortinarius lebretonii* as a variety of either *C. azureus* or of *C. spilomeus*. A sequence of a typical *Cortinarius lebretonii* is available in GenBank (JF907863). Based on BLAST searches, this taxon is clearly a member of sect. *Anomali*, not to sect. *Spilomei*.

Cortinarius ferrusinus Ballarà et al., *Moixero* 9: 32. 2017. MycoBank MB 820390.

Typus: Spain, Barcelona, Berguedà, Fígols, Ferrús, 1650 m a.s.l., in a calcareous *Pinus sylvestris* forest, 21 Sep. 2013, J. Ballarà (**holotype** personal herbarium of Josep Ballarà, JB-8106-13, **isotype** Museu Cerdà, Puigcerdà, Girona, 586/2017), GenBank ITS accession number: KY657254.

Description (based on Ballarà et al. 2017): *Basidiomata* rather slender. *Pileus* 15–30(–45) mm diam, hemispherical at first, then more convex or slightly umbonated, and flattened when mature, with an incurved, lobed and lacerate margin. *Pileus* colour beige, ochraceous to beige-chamois; surface smooth, dull, slightly hygrophanous, with small, brown-garnet to reddish universal veils remnants, also with whitish partial veil remnants on the pileus margin when young. *Lamellae* medium dense, broad, pale grey to purplish when young, then beige and finally deep beige to brownish, with paler, crenate edges. *Stipe* 30–50 mm long, 5–10 mm thick at the apex, 9–14 mm at the base, cylindrical to subfusiform, whitish to pale greyish, then pale beige; garnet-red universal veil copious, generating abundant girdles and flakes on the stipe surface. Base turning rusty red (like veil) with age. *Context* pale beige in the pileus, and greyish in the stipe, with purplish hues in the stipe cortex. *Smell* of slightly acidic honey. *Macrochemical reactions*: slowly positive reaction to guaiac, KOH reaction none, phenol-aniline, methol and NO₃Ag negative. *Basidiospores* are mostly ovoid, subspherical in profile, with medium-sized, moderately dense verrucose (6.5–)7–8(–8.5) × (5–)5.5–6.5 μm, Q = 1.15–1.25(–1.30). *Basidia* have 4 sterigmata, 25–23 × 7–9, cheilocystidia are lacking. The *epicutis*, hyphae are 3–9 μm wide, with fine parietal greyish pigment. Hypodermium well-developed, hyphae with increasing diameter up to 30 μm.

Habitat and distribution: *Cortinarius ferrusinus* was described from subalpine and altimontane coniferous forests on calcareous soil in part of Pre-Pyrenees, North of Catalonia (Spain) on 100–1800 m a.s.l., in association with *Pinus sylvestris*. *Cortinarius ferrusinus* (SH1018367.10FU) occurs also in other European countries (France, Italy, Spain, UK) in association with conifers and *Helianthemum*. A sequence-verified occurrence in calcareous pine forests from Scandinavia was also reported (TE Brandrud, pers. comm./unpubl. data). Searches in the GlobalFungi database (<https://globalfungi.com/>) confirm that it occurs in Scandinavia, and also indicate its occurrence in the Himalayas (Větrovský et al. 2020).

Conservation status: Based on the available sequence data referring to this species it is mainly linked to calcareous coniferous forest habitats. It is very likely widely distributed, but comparatively rare.

Cortinarius ferrusinus* f. *dryadicola (Bon) Peintner & R.J. Ferrari, **comb. nov.** MycoBank MB 360999. Fig. 2D, E.

Basionym: *Cortinarius spilomeus* f. *dryadicola* Bon, *Bull. Trimestriel Féd. Mycol. Dauphiné-Savoie* 32: 21. 1992. MycoBank MB 128979.

Typus: France, Vanoise, La Clusaz, 2200 m a.s.l., *Dryas* mixed with *Salix reticulata* and *Helianthemis*, 28 Aug. 1989, M. Bon (**holotype** LIP herbarium Bon 89056), GenBank ITS accession number: OM432506.

Description [based on Bon (1990) and complemented with our data]: *Pileus* 20–35(–50) mm, convex, not hygrophanous, fibrillose tomentose when young, later partly cracked, reddish-brown, reddish at disc, when drying more orange brown, sometimes with very faint violaceous colours. Veil indistinct or lacking. *Lamellae* adnate, distant, ochraceous brown with lilaceous reflects on margin at the outer part, then argillaceous ochraceous. *Stipe* 25–40 × 6–8(–10) mm, clavate or cylindrical, whitish, lilaceous at apex, with ochraceous base covered by brick-reddish to orange girlande-like velar scales and bands turning brown with age. *Context* whitish, pale, ochraceous shades at stipe base. *Smell* indistinct, *taste* mild. *Chemical reactions* negative (Phenol, Ammoniac, Formol, TL4). *Basidiospores* of the typus (Bon 89056) subglobose, obovoid, strongly verrucose, nearly spiny (6.2–)6.9–7.8(–8.5) × (4.7–)5.3–6.1(–6.3) μm, Q = (1.08–)1.17–1.43(–1.60) n = 30. *Cheilocystidia* lacking. *Pileipellis* is a cutis of interwoven hyphae with clavate terminal ends. *Mediopellis* pseudo-parenchymatous.

Key to the Central European species of *Cortinarius* sect. *Spilomei*

- | | |
|---|---|
| 1 In forests | 2 |
| 1* In alpine calcareous habitats with <i>Dryas</i> | 3 |
| 2 In forests, mainly <i>Picea</i> -dominated | <i>C. spilomeus</i> |
| 2* Subalpine-boreal calcareous <i>Pinus sylvestris</i> forests, or with <i>Helianthemum</i> | <i>C. ferrusinus</i> |
| 3 Vivid red velar girdles on pale stipe surface, spores strongly ornamented, sometimes nearly spiny | <i>C. ferrusinus</i> f. <i>dryadicola</i> |
| 3* Dull brownish red velar girdles, spores strongly ornamented but not spiny | <i>C. spilomeoalpinus</i> |

Table 1. Material sequenced for this study with ecological data, voucher number, fungarium and GenBank numbers.

Species	Voucher	GenBank Acc. No.	Country	Location	Associated plants
<i>Cortinarius spilomeoalpinus</i>	IBF20200078	OM432507	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2310 m	<i>Dryas octopetala</i> , <i>Helianthemum oelandicum</i> subsp. <i>alpestre</i>
	—	PQ243141	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Gran Fanes, 2112 m	<i>D. octopetala</i>
	—	PQ243142	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Gran Fanes, 2112 m	<i>D. octopetala</i>
<i>Cortinarius ferrusinus</i> f. <i>dryadicola</i>	IBF20180234 (Type)	OM432508	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Gran Fanes, 2112 m	<i>D. octopetala</i>
	IBF20200073	OM432499	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2250 m	<i>D. octopetala</i> , <i>H. oelandicum</i> subsp. <i>alpestre</i>
	IBF20200074	OM432500	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2250 m	<i>D. octopetala</i> , <i>H. oelandicum</i> subsp. <i>alpestre</i>
	IBF20200075	OM432501	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2250 m	<i>D. octopetala</i> , <i>H. oelandicum</i> subsp. <i>alpestre</i>
	IBF20200076	OM432502	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2250 m	<i>D. octopetala</i> , <i>H. oelandicum</i> subsp. <i>alpestre</i>
	IBF20200077	OM432503	Italy	Südtirol, Marebbe, Parco Naturale Fanes-Sennes-Braies, loc. Fanes, Pici Ciampiac, 2250 m	<i>D. octopetala</i> , <i>H. oelandicum</i> subsp. <i>alpestre</i>
	MCVE29661	—	Italy	Trentino, Primiero San Martino di Castrozza, Parco Naturale Paneveggio-Pale di San Martino, Campigol della Vezzana, 1950 m	<i>D. octopetala</i>
	Bon_86046	OM432505	France	Vanoise, Col du Joly	<i>D. octopetala</i> , <i>Salix reticulata</i>
	Bon_89056 (Type)	OM432506	France	Vanoise, La Cluseaz, 2200 m	<i>D. octopetala</i> , <i>S. reticulata</i>

Habitat and distribution: Alpine calcareous habitats mainly with *Dryas* and *Helianthemum ovatum*, sometimes also *Salix reticulata*, *S. retusa*, in France and Italy.

Additional materials examined: **France**, Vanoise, Col du Joly, *Dryas* mixed with *Salix reticulata*, 16 Aug. 1986, M. Bon, 86046, GenBank ITS accession number: OM432505. **Italy**, Trentino, Primiero San Martino di Castrozza, Parco Naturale Paneveggio-Pale di San Martino, Campigol della Vezzana, 1950 m a.s.l., alpine zone with *Dryas octopetala* intermixed with scattered *Larix decidua*, 8 Sep. 2018, E. Bizo, MCVE29661, GenBank ITS accession number: OM432504; Alto Adige/Südtirol, San Vigilio di Marebbe, Parco Naturale Fanes-Sennes-Braies, Fanes, Pici Ciampiac, 2200 m a.s.l., alpine zone with alpina *Dryas octopetala*, *Calluna vulgaris*, *Helianthemum oelandicum* subsp. *alpestre*, 20 Aug. 2020, R.J. Ferrari, FRJ045-2020, GenBank ITS accession number: OM432502; *ibid.*, 2250 m a.s.l., 20 Aug. 2020, R.J. Ferrari, FRJ046-2020, GenBank ITS accession number: OM432503; *ibid.*, 2250 m a.s.l., 25 Aug. 2020, R.J. Ferrari, FRJ067-2020, GenBank ITS accession number: OM432501, FRJ068-2020, GenBank ITS accession number: OM432499; *ibid.*, FRJ069-2020, GenBank ITS accession number: OM432500.

Notes: *Cortinarius spilomeus f. dryadicola* is conspecific with *C. ferrusinus*. The sequences of *C. spilomeus f. dryadicola* have 1–2 base pairs difference to the type of *C. ferrusinus*, but when considering all sequences, these are ambiguities, and these two taxa cannot be separated from each other. The descriptions are in accordance, and the calcareous habitat fits well into the species concept. It is very likely that this species made a host shift, thus expanding the habitat to the alpine area. We therefore consider *C. spilomeus f. dryadicola* as an alpine form of *C. ferrusinus*, growing in association with *Dryas octopetala*. It merely differs from typical *C. ferrusinus* by the alpine habitat and can be considered as a form of the latter.

Alpine forms of *C. ferrusinus* can be distinguished from *C. spilomeoalpinus* by the more vividly red colours of the velar bands contrasting to the pallid stipe surface when fresh. However, the stipe base often discolours rusty red in older specimen of *C. ferrusinus*. Thus, the distinction of *C. ferrusinus* and *C. spilomeoalpinus* based on macromorphological characters can be difficult if no young and fresh basidiomata are available. Both *C. ferrusinus* and *C. spilomeoalpinus* have strongly ornamented basidiospores, but in *C. ferrusinus f. dryadicola* they are often nearly spiny.

DISCUSSION

The sect. *Spilomei* is based on the type *Cortinarius spilomeus*, and consists of *Cortinari* with slender habitat, a reddish veil, and roundish spores. This section is part of the anomaloid group, which has no clear subgeneric affiliation (Soop et al. 2019).

Cortinarius spilomeus s. l. was usually recognised based on its characteristic red velar bands/girdles at the base of the stipe, in combination with slightly violet lamellae in young specimens which have a white lamellar edge. When considering reports from subalpine and alpine calcareous habitats, then *Cortinarius spilomeus* s. l. was reported to occur in a range of 670–1460 m, in

subalpine *Picea abies* forests in the National Park Berchtesgaden (Germany) (Schmid-Heckel 1985), and Favre (1960) reported it up to an altitude of 1900 m in the subalpine zone of the Suisse National Park. However, these reports very likely include other taxa of sect. *Spilomei*, like *C. ferrusinus* and maybe also *C. spilomeoalpinus*.

To clearly define and fix the epithet *Cortinarius spilomeus*, a neotype was defined based on voucher material from Sweden (GenBank ITS accession number KX302267). The basidiospores of this *C. spilomeus* s. str. are 5.0–7.5 × 5.0–6.5, Q = 1.2–1.3 (Dima et al. 2016). This neotype defines the species hypothesis (SH1017671.10FU) in UNITE. Based on this, *Cortinarius spilomeus* s. str. has a circumboreal distribution and was detected in association with *Picea abies*, *Tsuga heterophylla*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *Picea glehnii*. In Europe, it is usually associated to *Picea* in herbaceous sites, but also reported to *Pinus sibirica* in the Altai (Brandrud et al. 2019).

North America harbours distinct species of *Spilomei* (Liimatainen & Niskanen 2021), like *C. americanospilomeus* and *C. magispilomeus*. These taxa are typical for American *Tsuga*, *Abies*, or mixed coniferous forests. The basidiospores of *C. magispilomeus* are subglobose, moderately verrucose, 6–7 × 5–5.5 μm, Q = 1.15–1.30. *Cortinarius americanospilomeus* has subglobose, finely to moderately verrucose basidiospores, 6.5–7.5 × 5.5–6.5 μm, Q = 1.10–1.20 (1.30).

The habitat/host associations are remarkable uniform in sect. *Spilomei*, at least in Europe: the habitat ranges from boreal-montane to alpine. The boreal-montane species in Central Europe are typical conifer forest species. *Cortinarius ferrusinus* was described from calcareous subalpine sites and in association with *Pinus sylvestris*. Liimatainen & Ainsworth (2018) indicate *Helianthemum nummularium* as an additional possible plant host to *C. ferrusinus*. It has basidiomes with a tendentially warmer, ochre-reddish colouration, a “slightly acidic honey” odour, and larger, more ellipsoid basidiospores (6.5–)7–8(–8.5) × (5–)5.5–6.5 μm, Q = 1.15–1.25; based on our observations, this species can also occur in alpine areas with *Pinus mugo*, and in purely alpine habitats with *Dryas octopetala* and *Helianthemum ovatum*. In this latter case it can be addressed as *Cortinarius ferrusinus f. dryadicola*, differing only by habitat and host associations from the typical form.

Cortinarius spilomeoalpinus differs morphologically by smaller basidiomes, more dull colours of the stipe surface and less conspicuous velar girdles, and the brownish discolouration at the base of the stipe on handling and/or with age. Based on sequence data mining, this alpine species is quite rare, as we could not find a corresponding match in the databases. Thus, we must await more reliable data before being able to clearly state that this is an alpine species. Host relationship of ectomycorrhizal fungi and their geographical dislocation can be considered as important factors for speciation events (Hoeksma et al. 2018). Host shifts from other hosts to *Dryas* are very unlikely for this species, as we compared and sequenced all representatives of *Spilomei* occurring in the surrounding habitats, and could not detect any closely related similar species, but *C. ferrusinus* and *C. spilomeus*. This contrasts with the sister group relationship of *C. spilomeoalpinus* to the American *C. magispilomeus*, which might rather be supporting speciation due to geographic dislocation.

CONCLUSIONS

Here, we describe a new alpine *Cortinarius* species from sect. *Spilomei* and differentiate it from closely related subalpine-boreal taxa. This study shows that alpine habitats harbour a rich mycobiota. It also indicates that the diversity of alpine *Cortinarius* species might be higher than currently known. This gap of knowledge is probably caused by the difficulty to collect in remote habitats, and by the problems to distinguish species in these groups based on morphological characters. Furthermore, the often-harsh weather leads to rapid changes in basidiome structure and colours. Phylogenetic analyses allow deeper insights into the evolutionary history of these interesting and important ectomycorrhizal fungi. The availability of reliable reference sequences, if possible, from type specimen, allows for an unambiguous identification of these often so obscure taxa.

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