Taxonomy, ecology and distribution of Cortinarius rubrovioleipes and C. hinnuleoarmillatus (Basidiomycota, Agaricales) in Fennoscandia

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Two rare, orange to red-veiled species, *Cortinarius rubrovioleipes* Bendiksen & K. Bendiksen and *C. hinnuleoarmillatus* Reumaux, are studied morphologically and based on the molecular data (ITS-sequences). The distribution of species in Fennoscandia is mapped, their taxonomy and ecology discussed, and colour photographs provided. *C. rubrovioleipes* is reported for the first time since the description of the species. Also the relationship to *C. boulderensis* A.H. Sm. and *C. pseudobovinus* M. M. Moser & McKnight is discussed. The species are here placed in a new section *Boulderenses* Niskanen, Liimatainen & Kytöv. Short descriptions of North American *C. boulderensis* and *C. pseudobovinus* are provided based on the original descriptions and the type material. The nomenclature of species is confirmed by sequencing the type material.

Key words: Cortinarius, Telamonia, Fennoscandia, taxonomy, ITS, DNA, POY

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Introduction

Cortinarius rubrovioleipes Bendiksen & K. Bendiksen (Bendiksen & Bendiksen 1993) was described from Norway 1993 based on one collection and no documentation of further collections have surfaced since then. The North American species, C. boulderensis A.H. Sm. (Smith 1944) was mentioned to be its closest relative and based on the pigment chemistry Bendiksen and Bendiksen (1993) placed both of them in the section Armillati M. M. Moser. By having a hygrophanous cap, however, they also remind much of the species in C. armeniacus-group (Bendiksen & Bendiksen 1993).

Cortinarius hinnuleoarmillatus was described from France by Reumaux (Moënne-Loccoz &

Reumaux 1989). In Fennoscandia it was first found in 1986 in Yttereneby nature reserve area in Södertälje, Sweden and later in Hellasgården, Nacka, both regions in Södermanland (Santos et al. 2004). In Finland it is found in Varsinais-Suomi, Turku, Uusimaa, Espoo, and Pohjois-Savo, Kuopio (Kytövuori et al. 2005b). Reumaux and Moënne-Loccoz placed *C. hinnuleoarmillatus* in sect. *Hinnulei* Melot subsect. *Rubrovelati* (Moënne-Loccoz et al. 1990).

We studied the taxonomy of *C. rubrovioleipes* and *C. hinnuleoarmillatus* based on morphology and the ITS-regions of their rDNA. The distribution of the species is mapped and their taxonomy, ecology, and relationships are discussed.

Material and methods

The herbarium material of *Cortinarius hinnuleoarmillatus* and *C. rubrovioleipes* from Norway (O, BG, TRH, TROM), Sweden (S, GB, LD, UME, UPS), and Finland (H, TUR, OULU) was studied. Also, some specimens were gathered by the authors and some were found by chance among other species material. The type of *C. boulderensis* (Smith 17461, MICH 10323), *C. hinnuleoarmillatus* (PML no. 669, G 16160), and *C. rubrovioleipes* (Metsänheimo & Bendiksen 12.9.1984, holotype, O) were studied. Unexpectedly, *C. pseudobovinus* M.M. Moser & McKnight 1995 (Moser 1989/0300, IB), was found to be a close relative to *C. rubrovioleipes* and therefore included in our study. The acronyms follow those used by Holmgren et al. (1990).

Name interpretation used in the comments, when type material was not available, follows Cortinarius Flora Photographica (Brandrud et al. 1989–98, photo collections studied). Spore sizes given for these species are based on our own measurements.

Macroscopic characteristics are a combination of our own observations and the previously published descriptions from which we have seen the original material. Colour codes were not used, but colour photographs of *C. rubrovioleipes* and *C. hinnuleoarmillatus* are provided. For North American *C. boulderensis* and *C. pseudobovinus* the macroscopic characteristics are based only on original descriptions, but microscopic characteristics have been observed from the type collections.

Microscopic characteristics were examined with a light microscope (Leica DM/LS) and the measurements were made with an oil-immersion objective. Spores were drawn with a drawing tube at a magnification of 3000. They were examined from the surface view of pieces of gills of dried basidiomes, and measured (with an ocular micrometer) from the veil or from the top of the stipe, mounted in Melzer's reagent. Only mature, normally developed spores were measured, not unripe or abnormal spores.

Twenty spores, from collections marked with * in specimens examined, were measured of one fruitbody. The collections excluded from the measurements were e.g. in poor condition or contained only young fruitbodies. In presenting the variation of spore size, extreme measurements are in parenthesis. Length and width were measured from the same spore, and the length/width ratios (Q-value) were calculated for individual spores. The hyphae of the gill trama, basidia, and pileipellis were examined as in Kytövuori et al. (2005a).

Several collections of *C. hinnuleoarmillatus* and *C. rubrovioleipes* from different geographical areas (n=8, Table 1, marked with ^D in the lists of specimens examined) and type material of *Cortinarius rubrovioleipes*, *C. boulderensis*, *C. hinnuleoarmillatus* and *C. pseudobovinus* were sequenced. Also one sequence of previously published *C. brunneus* (Pers. : Fr.) Fr. var. *brunneus* (plate B07 in Brandrud et al. 1992, CFP587, S, neotype), *C. armillatus* (Fr. : Fr.) Fr. (plate B09 in Brandrud et al. 1992, CFP584, S), *C. hinnuleus* Fr. (plate A19 in Brandrud et al. 1989, CFP332, S), *C. armeniacus* (Schaeff. : Fr.) Fr (plate A46 in Brandrud et al. 1989, CFP809, S), and *C. sordidemaculatus* (PC0088382, PC, holotype) were used in analysis to get an idea of the systematic position of *C. rubrovioleipes*, *C. boulderensis*, *C. hinnuleoarmillatus* and *C.*

pseudobovinus inside subg. Telamonia. C. norrlandicus Brandrud (plate A26 in Brandrud et al. 1989, CFP526, S, isotype) was chosen for the outgroup species as in Kytövuori et al. (2005a).

Total DNA was extracted from a few milligrams of dried material (a piece of gill) using the NucleoSpin Plant kit (Macherey-Nagel). The primers ITS 1F and ITS 4B (Gardes & Bruns 1993) were used to amplify the ITS regions of the rDNA. PCR amplifications were performed in a 25 µl reaction mix with about 70 ng of extracted DNA, 1.0 U Phusion High-Fidelity DNA polymerase and 1X HF-buffer (Finnzymes, Finland), 200 µM of each dNTP, and 0.4 µM of each primer. PCR reactions were run on a PTC-200 Thermal Cycler (MJ Research, Inc.) with the following settings: denaturation for 30 s at 98°C, followed by 35 cycles of: 98°C denaturation for 10 s, annealing for 30 s at 50°C, and extension at 72°C for 30 s. The PCR products were purified using a GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences). Protocols for sequencing follow the method used in earlier study by the authors (Kytövuori et al. 2005a).

To get a rough idea of the closeness and relationships of *C. rubrovioleipes*, *C. boulderensis*, *C. hinnuleoarmillatus*, and *C. pseudobovinus*, the sequences were blasted against our personal sequence bank (contains sequences from about 300 species) and publicly available sequences and aligned using the ClustalW 1.8 program (Thompson et al. 1994) on the European Bioinformatics Institute server (http://www.ebi.ac.uk/clustalw/index.html). Differences between species were counted from pairwise alignments, which are usually fairly unambiguous in closely related species, thus making counting differences easy. Even though the method has limitations presenting differences between the taxa, it gives a rough idea of the closeness of the species relationship.

Alignments are seldom objective if there is a lot of variation in the DNA sequences, and ambiguous parts of the alignment have to be cut away before the analysis. To avoid these problems, the POY 3.0.6 program (Wheeler 1996), on the computers of CSC (Scientific computing, Espoo, Finland), was used for analyzing the sequences, because it does the alignment during the analysis. The analysis was run in parallel using 8 processors of 1.1 GHz each. The command line used for the analysis of POY was: poy -parallel -solospawn 7 -gap 2 -extensiongap 1 -random 150 -multirandom -norandomizeoutgroup maxtrees 5 -holdmaxtrees 100 -fitchtrees -spr -sprmaxtrees 1 -tbr -tbrmaxtrees 1 -checkslop 10 -ratchettbr 10 -multiratchet -ratchettrees 10 -treefuse -fusemaxtrees 20 -fuselimit 30 -indices -diagnose -impliedalignment names of the sequence files > name of the results file. Information about POY-commands and programs of different version can be found from web site: http://research. amnh.org/scicomp/projects/poy.php.

To quicken the analysis, the sequences were cut from conservative regions into four different regions: ITS 1, 5.8S, start of the ITS 2, and end of the ITS 2 (the sequence cut points are available upon request from the authors). Cut points were found by examining the Clustal alignment. The analysis was done without 5.8S, because it is identical in all the taxa and does not affect the results. In order to show the amount of evolutionary change accumulated on each branch, an implied alignment generated in POY was used to draw a phylogram in Winclada (Nixon 1999).

Table 1. Specimens used in the DNA study. The bolded GenBank numbers were generated in this study. AHS = A. H. Smith, CFP = Cortinarius Flora Photographica, EKB = E. Bendiksen & K. Metsänheimo, IK = I. Kytövuori, KS = K. Syrjänen, NM = N. Malmström, RT = R. Tuomikoski, TN = T. Niskanen. For acronyms of biological provinces see e.g. Hansen & Knudsen 1992: Nordic Macromycetes 2: 24, 25.

Species	Voucher	Herb.	Locality	Sequence no.	GenBank accession number
C. hinnuleoarmillatus Reumaux (holotype)	GK16160	G	France, Région de Semuy	491-04	DQ499464
C. hinnuleoarmillatus	F39953	S	Sweden, Sm, Nacka	711-05	DQ499461
C. hinnuleoarmillatus	TN03-093	Н	Finland, PS, Kuopio	243-03	DQ499460
C. hinnuleoarmillatus	KS-02.09.93	TUR	Finland, V, Turku	266-03	DQ499463
C. hinnuleoarmillatus	IK01-021	Н	Finland, U, Espoo	961-06	DQ499462
C. rubrovioleipes Bendiksen & K. Bendiksen (holotype)	EKB12.09.84	O	Norway, Oppl, Nordre Land	679-05	DQ497190
C. rubrovioleipes	RT-16.09.51	Н	Finland, ES, Punkaharju	066-03	DQ497192
C. rubrovioleipes	NM-18.09.60	Н	Finland, U, Porvoo	227-03	DQ497194
C. rubrovioleipes	IK97-1800	Н	Finland, PK, Ilomantsi	108-03	DQ497193
C. rubrovioleipes	IK04-031	Н	Finland, PH, Hankasalmi	960-06	DQ497191
C. boulderensis A.H. Sm. (holotype)	AHS17461	MICH	USA, Wash., Clallam Co.	708-05	DQ499466
C. pseudobovinus M.M.Moser & McKnight (holotype)	IB1989/0300	IB	USA, Wyoming, Teton Natl. Forest	677-05	DQ499465
C. sordidemaculatus Rob. Henry (holotype)	PC0088382	PC	France, Haut-Doubs	783-05	DQ139984
C. armeniacus (Schaeff. : Fr.) Fr.	CFP809	S	Sweden, Ång, Häggdånger	590-04	DQ117925
C. armillatus (Fr. : Fr) Fr.	CFP584	S	Sweden, Ång, Säbrå	542-04	DQ114744
C. brunneus (Pers. : Fr.) Fr. var. brunneus (neotype)	CFP587	S	Sweden, Ång, Säbrå	557-04	DQ117927
C. hinnuleus Fr.	CFP332	S	Sweden, Mpd, Torp	561-04	DQ117926
C. norrlandicus Brandrud (isotype)	S-F14270	S	Sweden, Ång, Häggdånger	237-03	DQ117928

Jackknife resampling (Farris et al. 1996) analysis was done using the POY 3.0.11 program with 1 processor of 2 GHz. The command line was: poy –jackboot –random 1000 –gap 2 –extensiongap 1 –norandomizeoutgroup – maxtrees 5 –holdmaxtrees 100 –fitchtrees –spr –sprmaxtrees 1 –tbr –tbrmaxtrees 1 –checkslop 10 –ratchettbr 10 –ratchettrees 10 –treefuse –fusemaxtrees 20 –fuselimit 30 names of the sequence files > name of the results file.

Results of DNA studies

The analysis resulted in one most parsimonious tree with the length of 403 steps (Fig. 1). The analysis took approximately 1 hour and, out of 150 replicates, the shortest tree was found 119

times. The use of ratchet or treefusing did not result in a shorter tree than SPR and TBR.

The clade containing *Cortinarius rubrovio-leipes* and *C. boulderensis* is supported by the jackknife value (=JK) of 69% and their nearest relative is *C. pseudobovinus* (JK < 50%). The near-identical sequences of *C. hinnuleoarmillatus* are supported by JK of 69% and the branch also including *Cortinarius hinnuleus* by JK of 67%.

Cortinarius rubrovioleipes and C. hinnuleoarmillatus have low intraspecific variation. Based on ITS-sequences and morphological data they seem to be well delimited species.

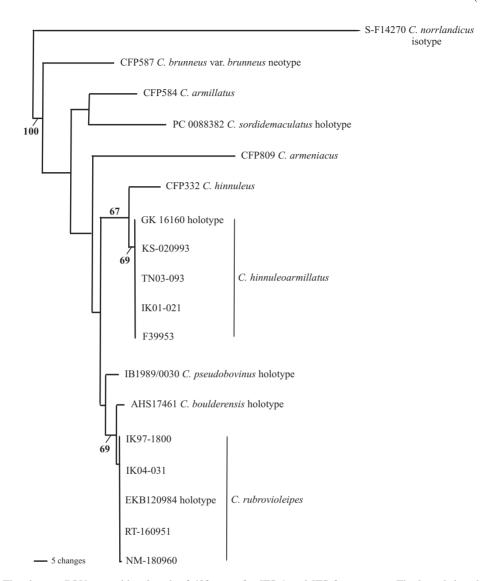


Fig. 1. The shortest POY tree with a length of 403 steps for ITS 1 and ITS 2 sequences. The branch lengths are proportional to the number of evolutionary steps. Jackknife values higher than 50% are shown above the respective branches.

Public gene bank sequences (GenBank: http://www.ncbi.nlm.nih.gov/ and UNITE: http://unite.zbi.ee/) were also checked, but neither a match nor even close sequences to *C. hinnuleoarmillatus*, *C. rubrovioleipes*, *C. boulderensis* or *C. pseudobovinus* were found, except for one locked sequence of *C. hinnuleoarmillatus* in UNITE (UDB000725). The sequence is from the collection *Santos F39953* (S), which we have also sequenced.

Cortinarius sect. Hinnulei Melot

1. *Cortinarius hinnuleoarmillatus* Reumaux – Figs. 3–5

Cortinarius hinnuleoarmillatus Reumaux, Bull. Féd. Myc. Dauphiné-Savoie 113: 24. 1989. – Type: France, Région de Semuy (Ardennes), sous feuillus humides, 29 Sept 1987 leg. anonyme n° 669 (holotype G).

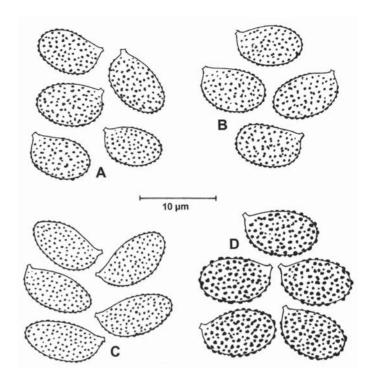


Fig. 4. Spores: A) Cortinarius rubrovioleipes (IK 97-1800, H); B) C. boulderensis (holotype, MICH); C) C. pseudobovinus (holotype, IB); D) C. hinnuleoarmillatus (holotype, G). Drawings T. Niskanen.

Illustrations: Atlas des Cortinaires, Les Cortinaires Hinnuloides pl. 2, p. 110, Jordstjärnan 25(3), p. 12.

Pileus: 3-5(6) cm, conical when young, later (plano-)convex with an umbo; surface smooth, glabrous, edge silky whitish fibrillose, especially when young, some orange-red veil remnants in the margin; orange to ochraceous brown, hygrophanous. Lamellae: distant (28 to 35 reaching the stipe), broad, emarginate, ochraceous brown with more or less obvious violet tint when young, cinnamon brown with age often retaining a violet tint, edge somewhat lighter, uneven. Stipe: 6-10 × 0.5–1.0 cm, cylindrical or slightly clavate, base somewhat radicating, fibrillose. Mycelium: white. Veil: orange-red, fairly abundant, often forming rings on the stipe. Context: in cap and stipe ochraceous, darker towards the base, whitish ochraceous when dry. Smell: radish or slightly earthy. Exsiccata: cap vivid yellow-brown to brown, orange veil still visible, in some fruitbodies violet tints also visible in gills, mycelium white.

Spores: 8.8–10.1(10.5) × 5.4–6.3 μm, Q=(1.49) 1.54–1.72(1.77) (80 spores, 4 collections, Fig. 4), \overline{X} =9.3–9.7 × 5.8–5.9 μm, \overline{X} Q=1.60–1.66, obovoid to (weakly) oblong ellipsoid, somewhat thick-

walled, fairly strongly dextrinoid, moderately to strongly and unevenly verrucose. Spores from the gill are usually somewhat longer (up to 10.9 um) and verrucosity is rather variable between the collections. The size in the original description is $(7.5)8-11(12) \times 6-6.5 \mu m$. Hyphae of the gill trama: in the overall view yellowish, finely to strongly scabrous. Basidia: 4-spored, 30-35 × 8–10 µm, hyaline or some with oil drops or granulose content. Pileipellis: with a fairly thin epicutis, hyphae 4-12 µm wide, with golden-yellow unevenly distributed granulose content or finely cross-striped incrusted or spotted; hypoderm well developed, with elongated elements about 40-55 × 18–25 μm, walls weakly thickened with faint cross-striped golden-yellow pigment. Clamp connections: present.

ITS-regions: (including 5.8S region) 507 bases long (total 5 sequences, Table 1). In one position of the ITS regions (in one sequence, F39953) two nucleotides were observed in one position (Y=C/T), otherwise there was no intraspecific variation. Difference to *C. hinnuleus* 12/13 base pairs.

Type material: The holotype includes a part of one fruitbody, which is in good condition.



Fig. 2. *Cortinarius rubrovioleipes*, Finland, Pohjois-Karjala, Ilomantsi commune, Mekrijärvi, 1997 Kytövuori 97-1800 (H). Photo I. Kytövuori.



Fig. 3. *Cortinarius hinnuleoarmillatus*, Sweden, Södermanland, Nacka commune, Hellasgården, 2004 Santos F39953 (S). Photo J. C. Santos.

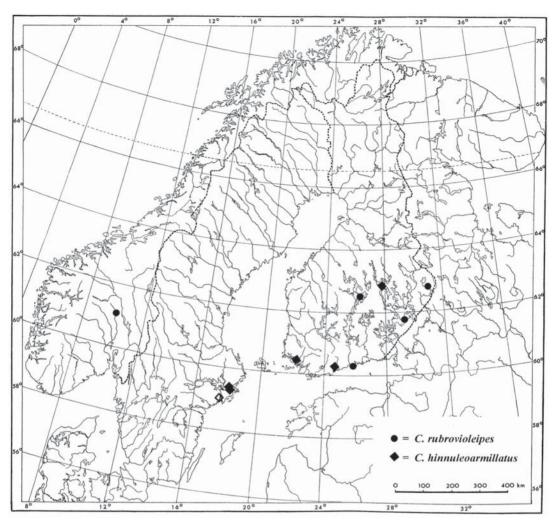


Fig. 5. Distribution of *Cortinarius rubrovioleipes* and *C. hinnuleoarmillatus* in NW Europe, according to the material examined. Circle = *C. rubrovioleipes*, square = *C. hinnuleoarmillatus* (collection examined), open square = *C. hinnuleoarmillatus* (collection not examined).

Ecology and distribution: Cortinarius hinnuleoarmillatus grows in nemoral to hemiboreal, eutrophic deciduous forests (Quercus, Corylus, Betula) often in moist, mull soil, solitary or cespitose. One collection, however, is found from central Finland, the northern part of the southern boreal zone (Ahti et al. 1968), under planted oaks. The most likely mycorrhizal partner for C. hinnuleoarmillatus is Quercus. Indicative of a long fruiting period the Fennoscandian material was collected between 24th July and 4th September.

Although *C. hinnuleoarmillatus* is a highly characteristic species, it has been found only in

a few localities (Fig. 5). This can be due partly to the fading colours of the veil, which makes its appearance more trivial when old. It is also possible that hidden collections exist under other names in the herbaria, e.g. *C. hinnuleus* or *C. helvolus* var. *medius*. In the Finnish agarics and boletes list (Kytövuori et al. 2005b) it has been suggested to belong to IUCN category DD. We propose it should be moved into category NT, because although it is rare in Finland it is fairly widely distributed in the southern parts of the country and grows also with planted oaks.

Comments: Cortinarius hinnuleoarmillatus is reminiscent of many other species in the section Hinnulei—the ochraceous brown cap, distant gills and earthy or radish smell make it very similar. Also genetically it seems to belong in the section Hinnulei, where Reumaux and Moënne-Loccoz first placed it (Moënne-Loccoz et al. 1990). The red-orange veil and bluish gills, however, make it very unique and in good condition it can not be mistaken. In poor condition, the remains of colourful veil can often still be seen in the cap margin and also the oblong ellipsoid spores help to distinguish it from most other Hinnulei species.

Soop's extreme form of C. helvolus var. medius (Fr.) Rob. Henry & Ramm with orange veil is probably C. hinnuleoarmillatus as he indicates in the later added comments of two papers (Soop 1990, Soop 1997 in http://karl.soop.org). In the original description (Henry & Ramm 1989) the veil of C. helvolus var. medius is noted as yellow but becoming ochraceous red with age, which is not typical for C. hinnuleoarmillatus. C. helvolus var. medius is also discussed and illustrated in Bidaud et al. (1997), planche 6, at least two fruitbodies on the left, where it looks very much like C. hinnuleoarmillatus. In all the descriptions the ellipsoid spores fit well with C. hinnuleoarmillatus, but the material has not been examined. Studies of this material are needed for further conclusions.

Specimens examined: SWEDEN. Södermanland: Nacka, Hellasgården, under Quercus on soil under oak in humid mixed deciduous forest, 4 Sept 2004 Santos F39953 P* (S); Stockholm, Sept 1908 Maire & Peltereau (S, as C. paragaudis). – FINLAND. Varsinais-Suomi: Turku, Ruissalo, W of Honkapirtti, mixed oak forest, 2 m from the field, 2 Sept 1993 Syrjänen 2.9.1993 P* (TUR, as C. sp.). Uusimaa: Espoo, Pirttimäki, under Quercus, between the road and the field, 22 Aug 2001 Kytövuori 01-021 P* (H, as C. cf. hinnuleus?). Pohjois-Savo: Kuopio, Karhonsaari, 24 July 1998 Ruotsalainen 4649b (KUO, as C. hinnuleus); under planted Quercus on mull soil, 30 July 2003 Niskanen et al. 03-093 P (H, as C. section Hinnulei). – FRANCE. Région de Semuy (Ardennes), sous feuillus, 29 Sept 1987 no 669 P* (holotype, G).

Cortinarius sect. Boulderenses Niskanen, Liimatainen & Kytöv. sect. nov.

Pileus hygrophanus. Velum universale coloratum, rubrum vel brunneum. Sporae ellipsoideae vel amygdaliformes. Typus sectionis: Cortinarius boulderensis A.H. Sm., Lloydia 7(3): 206. 1944. Pileus hygrophanous, veil coloured reddish to

brownish, spores ellipsoid to amygdaliform. All the species known so far grow in coniferous forest. (See also discussion.)

1. *Cortinarius rubrovioleipes* Bendiksen & K. Bendiksen – Figs. 2, 4, 5

Cortinarius rubrovioleipes Bendiksen & K. Bendiksen, Windahlia 20: 30. 1993. – Type: Norway, Oppland, Nordre Land, Aust-Torpa, north of Røste, in needle litter below large trees of *Picea abies*, 590 m a.s.l., 12 Sept 1984, *Metsänheimo & Bendiksen 1984-09-12* (holotype O, isotype OULU).

Pileus: 2-6 cm, convex when young, soon plano-convex with a low and broad umbo; surface smooth, glabrous, edge silky whitish fibrillose, especially when young, with some red veil remnants in the margin; (dark) red brown, hygrophanous. Lamellae: moderately crowded (30 to 40 reaching the stipe), emarginate, light greyish-ochraceous to light yellowish-brown when young, cinnamon-brown with age, middle thick, edge somewhat lighter, rather uneven. Stipe: $4.5-9 \times 0.7-1.3$ cm, cylindrical or slightly clavate, fibrillose, the whole length violet, Mvcelium: white, in a couple of exciccata red, but no notes of this in fresh conditions. Veil: vivid red, fairly abundant, in a continuous coat on the basal part or sometimes forming belts or zones. Context: in cap red brown, in stipe marbled yellowish-greyish brown, bluish in the cortex, whitish grey when dry. Smell: indistinct (no personal observations). Exsiccata: cap yellow brown to light vinaceous brown, red veil still visible on the stipe, in some fruitbodies also violet tints can be seen, mycelium white or pinkish to red.

Spores: $(7.6)7.8-8.8(9.0) \times (4.7)4.9-5.7(6.1)$ µm, Q=(1.39)1.46-1.67(1.69) (80 spores, 4 collections, Fig. 4), \overline{X} =8.1-8.5 × 5.1-5.6 µm, \overline{X} Q=1.52-1.59, ellipsoid, fairly thin-walled, dextrinoid to strongly dextrinoid, fairly finely verrucose. The size is bigger than in the original description (6.2)6.7-8.0 × 4.5-5.8 µm, \overline{X} =7.2 × 5.0 µm (30 spores). Hyphae of the gill trama: in the overall view yellowish, not incrusted or very finely scabrous. Basidia: 4-spored, 23-34 × 7-8 µm, hyaline or with yellowish granulose contents. Pileipellis: with a fairly thin epicutis (hyphae 4-10 µm wide) smooth to finely crossstriped incrusted; hypoderm well developed, with isodiametric to elongated elements about

 $40-65 \times 25-35$ µm, walls weakly thickened or with zebra striped yellow brown pigment. *Clamp connections:* present.

ITS-regions: (including 5.8S region) 505–506 bases long (total 5 sequences, Table 1). In one sequence (NM-180960) possible one base pair long length polymorphism was observed, otherwise there was no intraspecific variation. Four bases different to *C. boulderensis*, to *C. pseudobovinus* 9/10 bp.

Type material: The holotype includes parts of six fruitbodies. In most of them the cap cuticle is in poor condition. Also, most of the spores in the gills are immature and good spore deposits were not found. Therefore, the holotype was excluded from the spore size measurements.

Ecology and distribution: The type material collection was from oligotrophic submesic spruce forest of the *Myrtillus*-type. The Finnish material is from fairly similar habitat, except for one specimen (Tuomikoski 16.9.1951, H), which was collected from a planted *Abies sibirica* forest. Based on most of the collections *C. rubrovioleipes* seems to be a species of ordinary boreal spruce forests. All the collections were from quite late in the season, between the 12th and 25th September.

Despite its striking appearance and ordinary habitats, *C. rubrovioleipes* has rarely been found. The known distribution of the species is mapped in Fig. 5. In the Norwegian red list 1998 (Direktoratet for Naturforvaltning 1999), *C. rubrovioleipes* was classified as endangered (E). In Finland, the species might be rare but seems to grow in trivial habitats, so we propose that it would be placed in the IUCN-category NT.

Comments: Seen from above C. rubrovioleipes looks like a trivial brown Telamonia, but when picked up, the red veil and blue stipe make it a very eye-catching, unique looking species. When compared to other red-veiled species it differs from the members of the Armillati group with more slender appearance and hygrophanous cap. In C. caput-medusae H. Lindstr. the stipe becomes red, the veil is often olivaceous and the cap strongly innately fibrillose. In C. heterocyclus Soop the veil is not so obvious and its shade is more vinaceous red. Also, the blue colours are lacking from the latter two species. C. badiovinaceus M.M. Moser can be of equal size, but the veil is in scattered patches and vinaceous red, also the spores are ovoidly subglobose (6.8–7.4 \times 5.0–5.4 μ m). *C. spilomeus* has a greyish-brown cap, subglobose spores, and the veil appears as small red spots.

Bendiksen and Bendiksen (1993) considered C. rubrovioleipes to be closely related to North American C. boulderensis A.H. Sm. It was said to differ from the latter by having a wholly violet stem, different colour when dried, stouter fruitbodies, and a much stronger blue colour on a defined position on the chromatogram. When comparing our material to the type of C. boulderensis, we noticed no difference in exciccata color. Also the size of the fruitbodies was so variable that some C. rubrovioleipes were a comparable size as C. boulderensis, and we noticed no differences in spores. The ITS-sequences of C. boulderensis type differed by four base pairs, however, from C. rubrovioleipes sequences. Furthermore, previous studies (see Kytövuori et al. 2005a) have shown that intraspecific variation in ITS-sequences in *Telamonia* is very low, and at least some circumpolar species from different continents have identical sequences, e.g. C. traganus in Genbank (AF037224, Sweden & DQ367900, Canada). Therefore, we do not want to synonymise these taxa without further studies of North American C. boulderensis material.

Specimens examined: NORWAY. Oppland: Nordre Land, in needle litter below large trees of Picea abies, 12 Sept 1984, Metsänheimo & Bendiksen 12.9.1984^D (holotype, O). – FINLAND. Uusimaa: Porvoo, Gäddrag, coniferous forest on rocky ground, 18 Sept 1960 Malmström 18.9.1960^{D*} (H, collected as C. orellanus). Etelä-Savo: Punkaharju, Punkaharju, planted Abies sibirica forest, 16 Sept 1951 Tuomikoski 16.9.1951^{D*} (H, collected as C. paragaudis). Pohjois-Häme: Hankasalmi, spruce forest of the Myrtillus-type, 13 Sept 2004 Kytövuori 04-031^{D*} (H). Pohjois-Karjala: Ilomantsi, fairly young spruce heath forest with Pinus, Betula, Populus tremula and Salix spp., 25 Sept 1997 Kytövuori 97-1800^{D*} (H, S, collected as C. sp.).

2. Cortinarius boulderensis A.H. Sm. – Fig. 4

Cortinarius boulderensis A.H. Sm., Lloydia 7(3): 206. 1944. – Type: U.S.A., Washington, Olympic National Park, Olympic Hot Springs (Boulder Creek), under conifers, 30 Sept 1941. A.H. Smith 17461 (holotype MICH)

Illustrations: Lloydia 7(3), p. 207.

Cortinarius boulderensis is a medium-sized Telamonia with a reddish-brown to chocolatebrown, hygrophanous cap. Gills are grayish-lilac when young and cinnamom with age. The dull violet colour is also seen at the apex of the stipe, the base is brownish and slightly bulbous. Universal veil is testaceous and forms an annular band and scattered patches lower down or occasionally a sheat over the low third of the stipe. Odor and taste are not distinctive.

Microcharacters (from the type): Spores 7.8–8.5 (9.0) × 5.1–5.4 (5.6) μm, Q=(1.46)1.50–1.63(1.67) (20 spores, Fig. 4), \overline{X} =8.2 × 5.3 μm, \overline{X} Q=1.56, ellipsoid, fairly strongly dextrinoid, finely verrucose. The size is slightly bigger than in the original description, 7–8 (9) × 4–5.5 μm. Hyphae of the gill trama in the overall view yellowish, not incrusted or very finely scabrous. Basidia 4-spored, 23–25 × 7–8 μm, hyaline to yellowish granulose. Pileipellis with a thin epicutis, hyphae 4–10 μm wide, not incrusted or very finely scabrous and hyaline, hypoderm well developed with (isodiametric to) elongated elements about 40–60 × 20–30 μm, some with yellow granulose contents, walls weakly thickened.

ITS-regions: (including 5.8S region) 506 bases long (1 sequence, Table 1). Four base pairs different to *C. rubrovioleipes*, to *C. pseudobovinus* 11/12 bp.

Type material: The part of holotype includes three fruitbodies which are in good condition.

Ecology and distribution: Cortinarius boulderensis is a North American species, which is generally found in complex montane late-successional and old growth coniferous forests where it probably grows with trees belonging to the Pinaceae (Smith 1944, Castellano et al. 1999). There are 14 reported extant occurences in North America, of which 11 lie in currently protected areas (NatureServe 2006). Two photographs of *C. boulderensis* are presented in Castellano et al. (1999), but the photo on the right does not seem to represent this species.

Cortinarius boulderensis is uncommon to rare in the Pacific North-West America, where it is believed to be endemic (NatureServe 2006). However, one collection from Quebec, Canada as well as one from France have been reported in Bidaud et al. (1995). We have not seen the material and can not confirm these collections to be *C. boulderensis* or *C. rubrovioleipes*.

Comments: Cortinarius boulderensis, formerly placed in the serie C. armillatus-C. haematochelis (Smith 1944), is closely related to C. rubrovioleipes. We found no sure morphological

differences between the species, but they have a four base difference in the ITS-region, so we choose not to synonymise these taxa here (see also comments of *C. rubrovioleipes*).

Specimens examined: U.S.A. Washington: Olympic National Park, Olympic Hot Springs (Boulder Creek), under conifers, 30 Sept 1941 A.H. Smith 17461 D* (part of holotype, MICH).

3. Cortinarius pseudobovinus M.M. Moser & McKnight – Fig. 4

Cortinarius pseudobovinus M.M. Moser & McKnight, Mycotaxon 55: 326. 1995. – Type: U.S.A., Wyoming, Lake of the Woods, Union Pass, Teton National forest, under *Picea engelmannii* and *Pinus contorta*, 21 Aug 1989 *V. McKnight 89/300* (holotype IB).

Illustrations: Mycotaxon 55, p. 345.

Cortinarius pseudobovinus is a medium-sized Telamonia with a hygrophanous, greyish-brown cap which becomes dark umber brown. Gills are milk-coffee-brown, the stipe is clavate, silvery-grayish at the apex, and innately fibrillose. The greyish-brown to pale fuscous veil covers the lower half of the stipe in young fruitbodies, with age only one to several appressed to floccose belts can be present. Odor is lacking or sometimes slightly fungacious, taste is mild.

Microcharacters (from the type): Spores 8.6- $10.0 \times 4.7 - 5.1(5.4) \mu m$, Q=1.76-1.98 (20 spores, Fig. 4), \overline{X} =9.2 × 5.0 μ m, \overline{X} Q=1.84), weakly amygdaliform, fairly thin-walled, fairly faintly dextrinoid, brown, fairly finely to sometimes moderately, and densely verrucose. The size in the original description is $8-10.7 \times 4-5.3 \mu m$. Hyphae of the gill trama in the overall view brownish, fairly weakly but distinctly scabrous to small-spotted, granulose. Basidia 4-spored, 33- $38 \times 7-9 \mu m$, olivaceous brownish, some with oil drops or granulose content. Pileipellis with a fairly thin gelatinised epicutis, hyphae 6-13 µm wide, not incrusted or very finely scabrous, olivaceous-brown, hypoderm difficult to observe, with elongated, thin-walled elements about $40-50 \times 20-25 \mu m$.

ITS-regions: (including 5.8S region) 504 bases long (1 sequence, Table 1). Difference to *C. rubro-violeipes* 9/10 bp, to *C. boulderensis* 11/12 bp.

Type material: The holotype includes five fairly young fruitbodies and one mature. All of them are in good condition.

Ecology and distribution: Cortinarius pseudobovinus is a North American species which grows in coniferous forests under *Picea engelmannii* and *Pinus contorta* on calcareous soil (Moser et al. 1995, Moser 2001). All three collections reported by Moser (2001) are from Wyoming.

Comments: In the original description C. pseudobovinus was placed in the section Bovini M. M. Moser, because it is reminescent of his C. bovinus Fr. The latter has a similar type of fuscous universal veil and similar habit, but C. pseudobovinus differs by having more grey colors and elongate, elliptical spores (Moser et al. 1995). Based on ITS-regions, however, the closest known relatives are C. rubrovioleipes and C. boulderensis.

Specimens examined: U.S.A. Wyoming: Lake of the Woods, Union Pass, Teton National forest, under *Picea engelmannii* and *Pinus contorta*, 21 Aug 1989, *V. McKnight 89/300* D* (holotype, IB).

Discussion and conclusions

Cortinarius rubrovioleipes and C. hinnuleoarmillatus have low intraspecific genetic and morphological variation, and they seem to be clear species. It remains to be resolved if C. helvolus var. medius is a synonym to C. hinnuleoarmillatus and should C. rubrovioleipes be included in the intraspesific variation of C. boulderensis.

Based on our molecular analysis *Cortinarius boulderensis, C. rubrovioleipes* and *C. pseudobovinus* form a monophyletic group, although with low support (Fig. 1). Morphologically they share a combination of characteristics: mediumsized, hygrophanous cap, reddish to brownish veil, and amygdaliform to ellipsoid spores. We also know two, so far nameless species, belonging to this section (unpublished).

Cortinarius boulderensis and C. rubrovioleipes have traditionally been placed in the sect. Armillati, due to the red veil and/or anthraquinoid pigments (Smith 1944, Bendiksen & Bendiksen 1993). The pigment chemistry has been considered as an important characteristic in the infrageneric taxonomy of Cortinarius (e.g. Høiland 1983, Brandrud 1998). However, our molecular analysis shows that C. armillatus and C. boulderensis are not closely related. Also other recent Cortinarius studies have shown that e.g. in the subg. Phlegmacium the anthraquinoid containing species do not form only one monophyletic unit (Frøslev et al. 2005, Garnica et al. 2005). Morphologically the hygrophanous cap of *C. boulderensis* and *C. rubrovioleipes*, which is not typical for *C. armillatus* and allied species, supports the distinction of these species from the sect. *Armillati*.

North American *C. pseudobovinus* was placed by Moser at al. (1995) in the section *Bovini* M. M. Moser. In the previous study of Kytövuori et al. (2005a) *C. bovinus* (plate no. III Cortinarius 65 upper in Moser & Jülich 1990, IB 86/172) was shown to be related to *C. sordidemaculatus* Rob. Henry. Based on the genetic differences and phylogeny (Fig. 1) *C. pseudobovinus* and *C. sordidemaculatus* are not closely related. Thus the former can not belong to the section *Bovini*.

The sequences of *C. pseudobovinus*, *C. boulderensis*, and *C. rubrovioleipes* were also blasted against our own and publicly available sequences. The other *Telamonia* species known to contain anthraquinoid pigments, e.g. *C. anthracinus*, *C. colus*, and *C. bulliardii* were not found closely related. Furthermore, no other very close sequences were found, indicating that *C. pseudobovinus*, *C. boulderensis*, and *C. rubrovioleipes* form a monophyletic group inside the subg. *Telamonia*. A more detailed study of the red-veiled *Telamonia* species will be published soon by the authors.

Based on the information above, we therefore suggest a new section for *C. pseudobovinus*, *C. boulderensis*, and *C. rubrovioleipes*: *Cortinarius* sectio *Boulderenses* Niskanen, Liimatainen & Kytöv. sect. nov (see page 8).

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References

- Ahti, T., Hämet-Ahti, L. & Jalas, J. 1968: Vegetation zones and their sections in Northwestern Europe. Ann. Bot. Fennici 5: 169–211.
- Bendiksen, E. & Bendiksen, K. 1993: A new red veiled species of Cortinarius subgenus Telamonia, C. rubrovioleipes sp. nov. Windahlia 20: 29–34.
- Bidaud, A., Moënne-Loccoz, P. ja Reumaux, P. 1995:
 Atlas des Cortinaires, VII. Ed. Fédérat. Mycol.
 Dauphiné-Savoie. La Roche-sur-Foron. 199–237, 264–305, Pls. 145–168.
- Bidaud, A., Moënne-Loccoz, P. & Reumaux, P. 1997: Atlas des Cortinaires, Les Cortinaires Hinnuloides, Hors-Serien nº 1. – Ed. Fédérat. Mycol. Dauphiné-Savoie. La Roche-sur-Foron.
- Brandrud, T. E. 1998: Cortinarius subgen. Phlegmacium sect. Fulvi chemotaxonomy versus morphological taxonomy. Journal des J.E.C. 1: 4–9.
- Brandrud, T. E., Lindström, H., Marklund, H., Melot, J. & Muskos, S. 1989: Cortinarius Flora Photographica I (Swedish version). Cortinarius HB, Matfors. 38 pp. + 60 plates.
- Brandrud, T. E., Lindström, H., Marklund, H., Melot, J. & Muskos, S. 1992: Cortinarius Flora Photographica II (Swedish version). Cortinarius HB, Matfors. 40 pp. + 60 plates.
- Brandrud, T. E., Lindström, H., Marklund, H., Melot, J. & Muskos, S. 1994: Cortinarius Flora Photographica III (Swedish version). Cortinarius HB, Matfors. 35 pp. + 60 plates.
- Brandrud, T. E., Lindström, H., Marklund, H., Melot, J. & Muskos, S. 1998: Cortinarius Flora Photographica IV (Swedish version). Cortinarius HB, Matfors. 32 pp. + 60 plates.
- Castellano, M. A., Smith, J. E., O'Dell, T., Cázares, E. & Nugent, S. 1999: Handbook to strategy 1 fungal taxa from the Northwest Forest Plan. Gen. Tech. Rep. PNW-GTR-476. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 195 pp.
- Direktoratet for Naturforvaltning 1999: Nasjonal rødliste for truete arter i Norge 1998. Norwegian Red List 1998. DN-rapport 3: 1–161.
- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G. 1996: Parsimony jacknifing outperforms neighbor-joining. – Cladistics 12: 99–124.
- Frøslev, T., Matheny, P. & Hibbett, D. 2005: Lower level relationships in the mushroom genus Cortinarius (Basidiomycota, Agaricales): A comparison of RBP1, RPB2 and ITS phylogenies. – Mol. Phyl. Evol. 37: 602–618.
- Gardes, M. & Bruns, T. D. 1993: ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. – Mol. Ecol. 2: 113–118.
- Garnica, S., Weiß, M., Oertel, B. & Oberwinkler, F. 2005: A framework for a phylogenetic classification in the genus Cortinarius (Basidiomycota, Agaricales) derived from morphological and molecular data. – Can. J. Bot. 83: 1457–1477.

- Henry, R. & Ramm, E. 1989: Cortinaires rares ou nouveaux. Doc. Mycol. 20: 53–67.
- Holmgren, P. K., Holmgren, N. H. & Barnett, L. C. 1990: Index herbariorum 1. The herbaria of the world. 8th ed. – Regnum Vegetabile 120: 1–693.
- Høiland, K. 1983: Cortinarius subgenus Dermocybe.Opera Botanica 71: 1–113.
- Kytövuori, I., Niskanen, T., Liimatainen, K. & Lindström, H. 2005a: Cortinarius sordidemaculatus and two new related species, C. anisatus and C. neofurvolaesus, in Fennoscandia (Basidiomycota, Agaricales). – Karstenia 45: 33–49.
- Kytövuori, I, Nummela-Salo, U., Ohenoja, E., Salo, P. & Vauras, J. 2005b: Helttasienten ja tattien levinneisyystaulukko. Distribution table of agarics and boletes in Finland. In: Salo, P., Niemelä, T., Nummela-Salo, U. & Ohenoja, E. (eds.). Suomen helttasienten ja tattien ekologia, levinneisyys ja uhanalaisuus: 109–224. Suomen ympäristökeskus. Helsinki. Suomen Ympäristö 769.
- Moënne-Loccoz, P. & Reumaux, P. 1989: En marge de l'Atlas des Cortinaires (3). Bull. Féd. Myc. Dauphiné-Savoie 113: 23–26.
- Moënne-Loccoz, P., Reumaux, P. & Henry, R. 1990: Atlas des Cortinaires 1 (fasc.) – Ed. Fédérat. Mycol. Dauphiné-Savoie. Annecy. 22 pp.
- Moser, M. M. 2001: Rare, debated and new taxa of the genus Cortinarius (Agaricales). Fungi non delineati XV: 1–57.
- Moser, M. & Jülich, W. 1990: Farbatlas der Basidiomyceten 8. Gustav Fischer Verlag, Stuttgart. 38 pp. + 80 plates.
- Moser, M. M., McKnight, K. & Ammirati, J. F. 1995: Studies on North American Cortinarii I. New and interesting taxa from the greater Yellowstone area. – Mycotaxon 55: 301–346.
- NatureServe 2006: NatureServe Explorer: An online encyclopedia of life (web application). Version 4.7. NatureServe, Arlington, Virginia. Available http://www.natureserve.org/explorer.
- Nixon, K. C. 1999: Winclada (Beta) ver. 0.9.9 published by the author, Ithaca, NY.
- Santos, J., Toresson, H. G. & Taylor, A. 2004: Cortinarius hinnuleo-armillatus Reum. funnen i Sverige. Jordstjärnan 25(3): 10–12.
- Smith, A. H. 1944: New and interesting Cortinarii from North America. Lloydia 7(3): 163–235.
- Soop, K. 1990: Observationer av några intressanta Cortinarius-arter. Agarica 10/11 (19/20): 30–43. (commented version in http://karl.soop.org)
- Soop, K. 1997: Spindlingar med röd slöja. Jordstjärnan 18(1): 26–35. (commented version in http://karl.soop.org)
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994: Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. – Nucleic Acid Research 22: 4673–4680.
- Wheeler, W. C. 1996: Optimization alignment. The end of multiple sequence alignment in phylogenetics? – Cladistics 12: 1–9.