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Typification of *Octaviania rubescens* (*Paxillineae, Boletales*) and phylogenetic hypotheses for genus *Alpova*

Alfredo Vizzini[1]

Dipartimento di Biologia Vegetale, Università di Torino, Viale Mattioli 25, 10125 Torino, Italy

Mirca Zotti

DIP.TE.RIS, Polo Botanico "Hanbury", Università di Genova, Corso Dogali 1/M, 16136 Genova, Italy

Svengunnar Ryman

Museum of Evolution, Botany Section, University of Uppsala, Norbyvägen 16, 752 36 Uppsala, Sweden

Stefano Ghignone

Istituto per la Protezione delle Piante, UOS Torino-Consiglio Nazionale delle Ricerche (CNR), Viale Mattioli 25, 10125 Torino, Italy

Abstract: There are no usable herbarium specimens for the type of *Alpova rubescens*; therefore a lectotype and an epitype are designated hereby to preserve current usage of the name. As lectotype the Vittadini's Fig. XII E/Tab. IV (from Monographia Tuberacearum) was selected. The epitype chosen for *A. rubescens* is a recent large collection from Liguria (Italy). Furthermore to investigate the phylogenetic position of *A. rubescens* among *Alpova* spp. comparative 28S-rDNA gene sequence analyses were conducted. The new combination *Rhizopogon alexsmithii* (Trappe) Vizzini & Zotti is proposed.

Key words: *Agaricomycetes*, hypogeous *Boletales*, *Melanogaster*, *Paxillineae*, *Rhizopogon*, *Suillineae*, 28S–rDNA, taxonomy

INTRODUCTION

Genus *Alpova* was erected by Dodge (1931) to accommodate the single North American species *A. cinnamomeus* C.W. Dodge. Zeller (1939) studied the basidiome development of this fungus. Initially monotypic, *Alpova* became one of the largest genera of hypogeous *Agaricomycetes*. Since its creation the taxonomic position and the circumscription of *Alpova* has been much debated, owing to its strong relationships with genera *Rhizopogon* Fr. emend. Tul. & C. Tul. and *Melanogaster* Corda. In fact most of the accepted species of *Alpova* have been segregated from these genera by Smith and Zeller (1966), Trappe (1975), Beaton et al. (1985) and Liu et al. (1990).

The foremost contribution toward a better circumscribing of genus *Alpova* was by Trappe (1975) in his extensive monographic treatise. Trappe (1975) established that *A. cinnamomeus* had been described earlier as *Rhizopogon diplophloeus* Zeller & C.W. Dodge and recombined it as *A. diplophloeus* (Zeller & C.W. Dodge) Trappe & A.H. Sm. The species is characterized by having a hypogeous to sometimes emergent habit, a strict ectomycorrhizal association with *Alnus* spp., solid, gelatinous gleba that turns reddish brown when exposed to the air, smooth, thin-walled, small, ellipsoid to oblong, hyaline to pale brown spores, presence of clamp connections, lack of a hymenial palisade and a layer of swollen cells in the peridium. Trappe (1975) proposed a possible *Rhizopogon-Alpova-Melanogaster* evolutionary lineage, with a key to separate the three genera. He established as the main distinguishing features the presence or absence of a palisadic hymenium, of gel-filled glebal chambers and of clamp connections. *Rhizopogon* is characterised by a true palisadic hymenium, non-gelified glebal chambers and lack of clamp connections, according to Trappe, while *Alpova* lacks a true hymenium, has gelified glebal chambers and generally shows clamps. *Melanogaster* differs in the dark brown, thick-walled spores and a distinct hilar pore. So he emended genus *Alpova* by transferring there (as subgenus *Alpova* sect. *Rhizopogonella* [A.H. Sm.] Trappe) some species from genus *Rhizopogon* subgenus *Rhizopogonella* A.H. Sm., based on the absence of a true palisade-hymenium and to the gelatinous matrix filling the glebal cells at maturity. Trappe (1975) accepted 15 species, mainly distributed in the northern hemisphere.

Beaton et al. (1983) enlarged the concept of the genus by tentatively including three new Australian *Eucalyptus*-associated sequestrate species with hyaline, smooth, obovoid spores and non-gelified glebal chambers. However these species do not fit well the concept of *Alpova* as described in Trappe's monograph (1975). Afterward Bougher and Lebel (2002) transferred the Australian species to their new genus *Amarrendia* that has close relationship to *Amanitaceae* (see also Tulloss 2009). Three other species were added by Liu et al. (1990) from China, one from Spain (*A. pseudostipitatus*, Calonge and Siquier 1998) and one from Argentina (*A. austroalnicola*, Nouhra et

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[1] Corresponding author. E-mail: alfredo.vizzini@unito.it

al. 2005). In the latest edition of the Dictionary of the Fungi (Kirk et al. 2008) 20 *Alpova* species are recognized.

Despite these contributions the delimitation between *Alpova* and *Rhizopogon/Melanogaster* is still far from clear. Molecular data now have demonstrated that the morphological features formerly considered reliable markers in defining taxa of hypogeous fungi can be inadequate for that purpose. Based on preliminary molecular investigations, *Alpova* seems to be a polyphyletic genus (Grubisha et al. 2001, Jarosch 2001, Nouhra et al. 2005).

Among all *Alpova* species the rarely collected *A. rubescens* (Vittad.) Trappe (= *Octaviania rubescens* Vittad.) seems to be a key taxon due to some of its morphological features (peridiopellis and basidiospores) shared with both *Melanogaster* and *Alpova*. The aim of this paper, on the basis of a recent collection (40 basidiomes) of *A. rubescens* from Liguria (Italy), is to typify the taxon (for which original specimens are lacking or scarce and doubtful) and to provide a macromicromorphological analysis coupled with 28S-rDNA phylogenetic investigations on *Alpova* species.

MATERIALS AND METHODS

Morphology.—Macroscopic and microscopic characters were described from fresh specimens with a stereo microscope (Leica M 205 C) and compound microscope (Axioscope, Zeiss) respectively. The observations of microscopic features are based on material mounted in distilled water, in lactic acid plus acid fuchsine, in 5% potassium hydroxide and in Congo red. For spores and other structures at least 30 individuals were measured.

These abbreviations are used: Qm is the average spore quotient (length/width ratio); Met is short for the Methuen Handbook of Color (Kornerup and Wanscher 1967). All examined material was deposited and kept at GDOR (Herbarium of the Museo Civico di Storia Naturale Giacomo Doria, Mycologia Section, Genova, Italy). Authorial citations are according to the IPNI authors Website and the Index Fungorum authors of fungal names Website.

Herbarium abbreviations follow Holmgren and Holmgren (1998). Color comparisons were made with Kornerup and Wanscher (1967) and designated (Met 7A-B 2), which indicates plate, row and color blocks.

Geographic coordinates of the area, where the species was found, were acquired by a GPS receiver (Garmin eTrex Summit), according to WGS 84 standard.

DNA extraction, PCR amplification and DNA sequencing.—Genomic DNA was isolated from 1 mg dried herbarium specimen of *Alpova rubescens* (GDOR 55166 M.S.N.G.) with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to manufacturer instructions. Primers LR0R/LR6 (Vilgalys and Hester 1990; Vilgalys lab unpubl, http://www.botany.duke.edu/fungi/mycolab) were used for 28S-rDNA amplification. Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 μL reaction mixture with these final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20mM Tris/HCl pH 8.4, 50 mM KCl), 1 μM each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega). The PCR program was 3 min at 95 C for 1 cycle; 30 s at 94 C, 45 s at 50 C, 2 min at 72 C for 35 cycles, 10 min at 72 C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and viewed by staining with ethidium bromide. PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Torino, Italy). The sequences were assembled and edited with the phred/phrap/consed software suite (http://www.phrap.org). A primer pair was designed to resequence a fragment spanning positions 520–620, corresponding to the overlapping region between LR0R/LR6's sequence results, to confirm unsure reads. The sequence has been deposited in GenBank.

Sequence alignment and phylogenetic analysis.—*Alpova rubescens* sequence first was aligned manually to TreeBase study No. S2162/M4103 (Desjardin et al. 2008) to establish its relationships within the *Boletales*. Sequences from *A. alexsmithii* (EU669329 and EU669327), *A. diplophloeus* (AF352035 and AF071454), *A. olivaceotinctus* (AF352036) and *Alpova trappei* (AF071456) were retrieved from GenBank and manually added. *A. austroalnicola* sequences (AY377574 and AY377575), even if available in the public database, which were not included in this dataset because they were too short.

Phylogenetic analyses were performed on a Linux cluster in Dpt. Informatics at University of Torino. The model of evolution used in the phylogenetic analyses was determined with Modeltest 3.7 (Posada and Crandall 1998). Bayesian metropolis-coupled Markov chain Monte Carlo analysis was performed with the GTR+I+G model of evolution as implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Markov chains were run 10 000 000 generations with the sampling frequency set every 1000th generation; all other parameters were used at the default settings. Burn-in value set to 25% of sampled trees was used to calculate the parameters and trees summarization. Maximum likelihood (ML) searches also were conducted with PAUP* 4.0b10 (Swofford 2000) under a GTR+I+G model of sequence evolution determined with the Akaike information criterion as calculated by Modeltest with the starting tree(s) obtained via stepwise addition, randomized addition sequence and tree-bisection-reconnection (TBR) as branch-swapping algorithm.

To better resolve the phylogenetic placement of *A. rubescens* among *Alpova* species a smaller dataset of LSU from all *Alpova* spp. (*A. alexsmithii*, *A. austroalnicola*, *A. diplophloeus*, *A. olivaceotinctus* and *Alpova trappei*) and related species was obtained from GenBank. The sequence of *Boletus edulis* EU522822 was included as outgroup. Sequences were aligned with MUSCLE (with parameter −maxiters 5 −sv −weight1 henikoffpb −weight2 henikoffpb −diags1 −diags2) (Edgar 2004), and the resulting 912 bp long alignment was slightly edited with JalView 2.3 (Waterhouse et al. 2009).

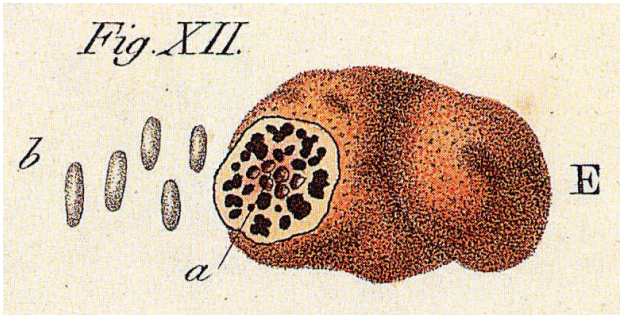


FIG. 1. *Octaviania rubescens* (Fig. XII E/Tab. IV, from Monographia Tuberacearum, lectotypus). a. Sectioned basidiome. b. Spores.

Neighbor joining (NJ) and ML searches both were performed with PAUP*. The Kimura two-parameter (K2P) method for superimposed mutations were used for NJ analyses. Robustness of the internal branches was evaluated by bootstrap analysis (1000 runs). ML analysis (ML) was performed with the GTR + I + G model and the same parameters used before. Bayesian inference was obtained with MrBayes using the same parameters described above. The phylogenetic trees were viewed and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree). The alignments and phylogenetic trees were deposited in TreeBase (www.treebase.org) under study accession No. S10550 and also are available from the authors upon request. Searches for the most similar sequences in sequence databanks were performed by the NCBI BLAST algorithm (Altschul et al. 1997).

TAXONOMY

Alpova rubescens (Vittad.) Trappe, Beihefte zur Nova Hedwigia 51:294 (1975)

Octaviania rubescens Vittad., Monographia Tuberacearum: 18 (1831)

Hyperrhiza rubescens (Vittad.) Rabenh., Deutschlands Kryptogamenflora 1:293 (1844)

Melanogaster rubescens (Vittad.) Tul. & C. Tul., Fungi Hypogaei: Histoire et Monographie des Champignons Hypogés: 96 (1851)

Lectotypus (selected here): Vittadini, Monographia Tuberacearum, Fig. XII E/Tab. IV (1831) FIG. 1

Epitypus (designated here): Italy, Liguria, Savona, Cairo Montenotte, Cascinazza, in a *Fagus sylvatica* forest, 02 Oct. 2008, leg. S. Roveta, GDOR 55166 M.S.N.G.

Description of the epitype (Ligurian collection) FIG. 2a–g

Basidiomes (1.5)3.5(6) cm diam, globose to subglobose-ellipsoid, usually regular to gibbous, mottled, sometimes lobed or slightly flattened, reniform, without rhizomorphs (FIG. 2a, b). Peridium thick and tough, not or hardly separable from the gleba, dull, initially reddish brown (M 6–7 D–E 7–8), bruising purplish brown with age (M 8 E 7–8, 9 E 7–8), smooth. Gleba initially white, becoming reddish at maturity (FIG. 2c). Chambers from spherical to oval, 200–1100 µm, at first empty becoming filled and gelatinized with slimy brownish contents. Tramal-plates whitish, irregular, 20–1000 µm thick. Odor strongly alliaceous, persistent and reminiscent of *Tuber magnatum*. Peridiopellis variable, 300–1400 µm thick, externally red-brown, yellow toward the inner part (FIG. 1d, e); exoperidium composed of more or less interlaced and filamentous hyphae (3–10 µm diam) characterized by club-shaped enlargements, with brown parietal pigment; endoperidium consisting of irregular globular and polyhedral cells (10–25 µm). Hyphae of the tramal-plates are 6–8 µm diam and present clamp connections. Spores(9–)10–13.5(–14) × 4–5 µm, on average 11.53 × 4.36 µm, Qm = 2.64, narrowly oblong, ellipsoid or cylindrical, orthotropic, thin-walled, hyaline or pallid yellow-green (M 2 A 3–4 B 5–4) in KOH, with homogeneous contents or with 1–2 guttules, with an obtuse, rounded poreless apex and a broadly truncate base, sometimes irregularly constricted (FIG. 2f), often with a ribbon-like 2–6 µm long, abruptly tapering hyaline vestigial appendage of sterigma (FIG. 2g). Basidia not forming a true hymenium, but borne in fascicles or tufts, soon collapsed, clavate, 20–45 × 7–10 µm, 4–8-spored.

Habitat. Terrestrial, hypogeous, in small groups of 4–7 basidiomes, under *Fagus sylvatica* L., October.

Material examined. Cascinazza, Cairo Montenotte (Savona, northwestern Italy), 8.399363°E, 44.393336°N (WGS-84, GPS coordinates in decimal degrees), 780 m in a beech wood of tall deciduous trees, 2 Oct 2008 (GDOR 55166 M.S.N.G.). The area where fungi were found extends about 1200 m^2; more than 40 basidiomes were collected.

DNA sequencing and analyses.—The primer pair LR0R/LR6's successfully amplified target DNA from the *Alpova rubescens* GDOR 55166 M.S.N.G. specimen. Sequence assembly yielded 1125 bp long fragment. The sequence was deposited in GenBank (accession No. GQ477440). It shares 86–94% similarity with previously published sequences of *Alpova* species. The lower similarity was found with *Alpova alexsmithii* EU669329 (86% on 909 bp stretch), whereas the higher similarity was found with *Alpova diplophloeus* AF071454 and *Alpova trappei* AF071456 (94% on 894 bp stretch).

In BLASTN analyses the *A. rubescens* 28S-rDNA sequence as query, *Alpova* species or related genera (*Rhizopogon*, *Melanogaster*) were not found among the first 100 sequences producing significant alignments. The top three most similar sequences retrieved by BLAST were *Paxillus vernalis* AY645059

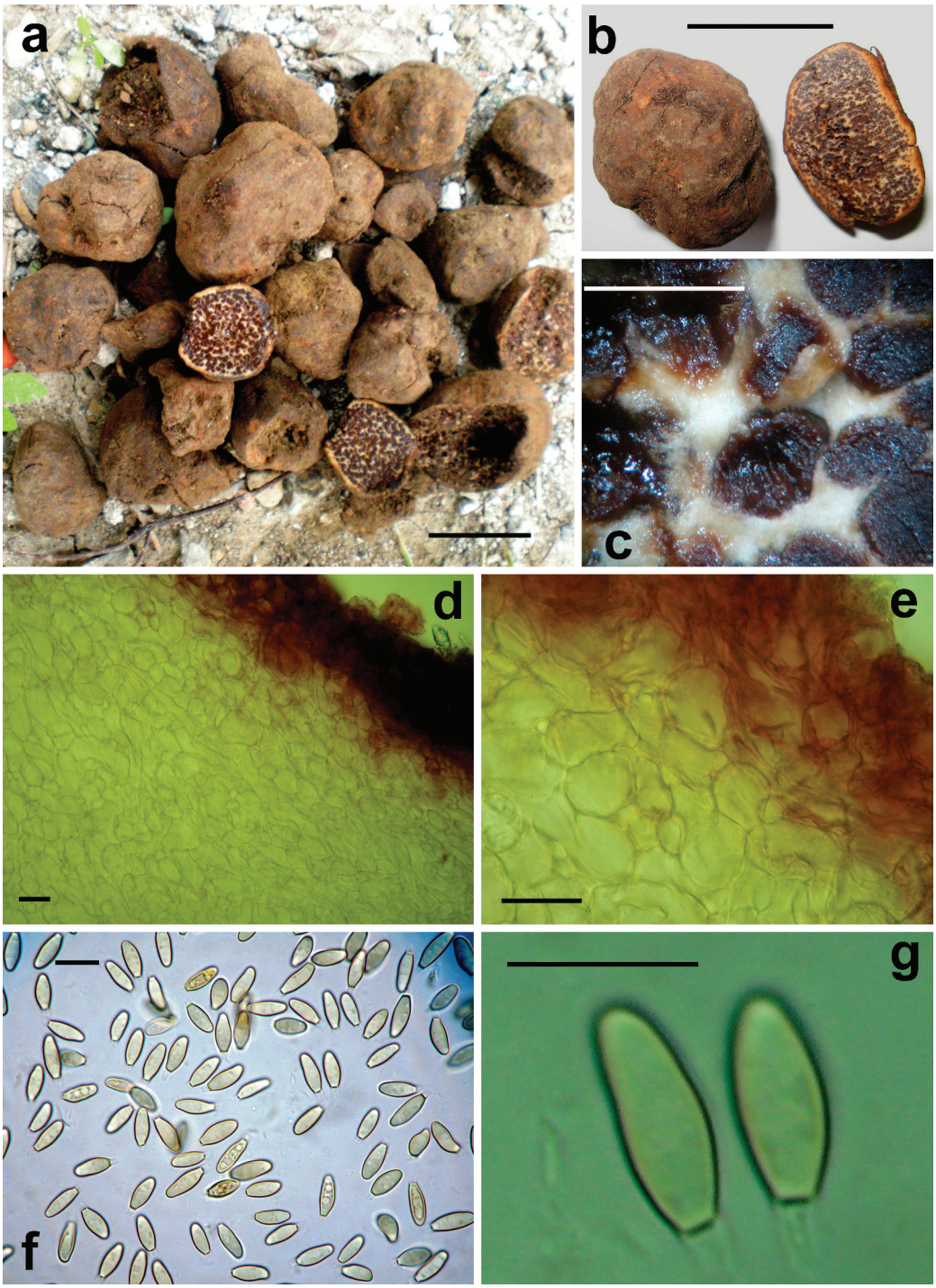


FIG. 2. *Alpova rubescens* (epitypus). a, b. Basidiomes. c. Gleba: chambers and tramal plates. d, e. Peridiopellis. f, g. Spores. Bars: a, b = 30 mm; c = 1 mm; d, e = 20 µm; f, g = 10 µm.

(score 1692, query coverage 98%, e-value 0.0, max ident. 94%), *Hydnomerulius pinastri* DQ534667 (score 1673, query coverage 98%, e-value 0.0, max ident. 94%) and *Boletinellus merulioides* AY684153 (score 1638, query coverage 98%, e-value 0.0, max ident. 94%). *Alpova trappei* AF071456 only ranks at position 250 (score 1377, query coverage 79%, e-value 0.0, max ident. 94%).

The first phylogenetic study aimed to place *A. rubescens* and closest species in the context of the boletaceous genera. *A. rubescens*, *A. diplophloeus*, *A. alexsmithii* Trappe, *A. olivaceotinctus* (A.H. Sm.)

Trappe and *A. trappei* Fogel partial 28S-rDNA sequences were added manually to TreeBase study No. S2162/M4103 (Desjardin et al. 2008), chosen because it is recent and includes many species. Bayesian inference consensus tree (10 000 000 generations, FIG. 3) of the dataset shows the *A. rubescens* placement among the *Paxillineae* (0.58 posterior probability) and not among *Suillineae*, although its relation to the *A. trappei* group or to the *A. diplophloeus* remains unresolved. These phylogenetic relationships also are confirmed by ML analysis, which also draws a link between *A. rubescens* and the group formed by *A. trappei* and *Melanogaster* species (data not shown).

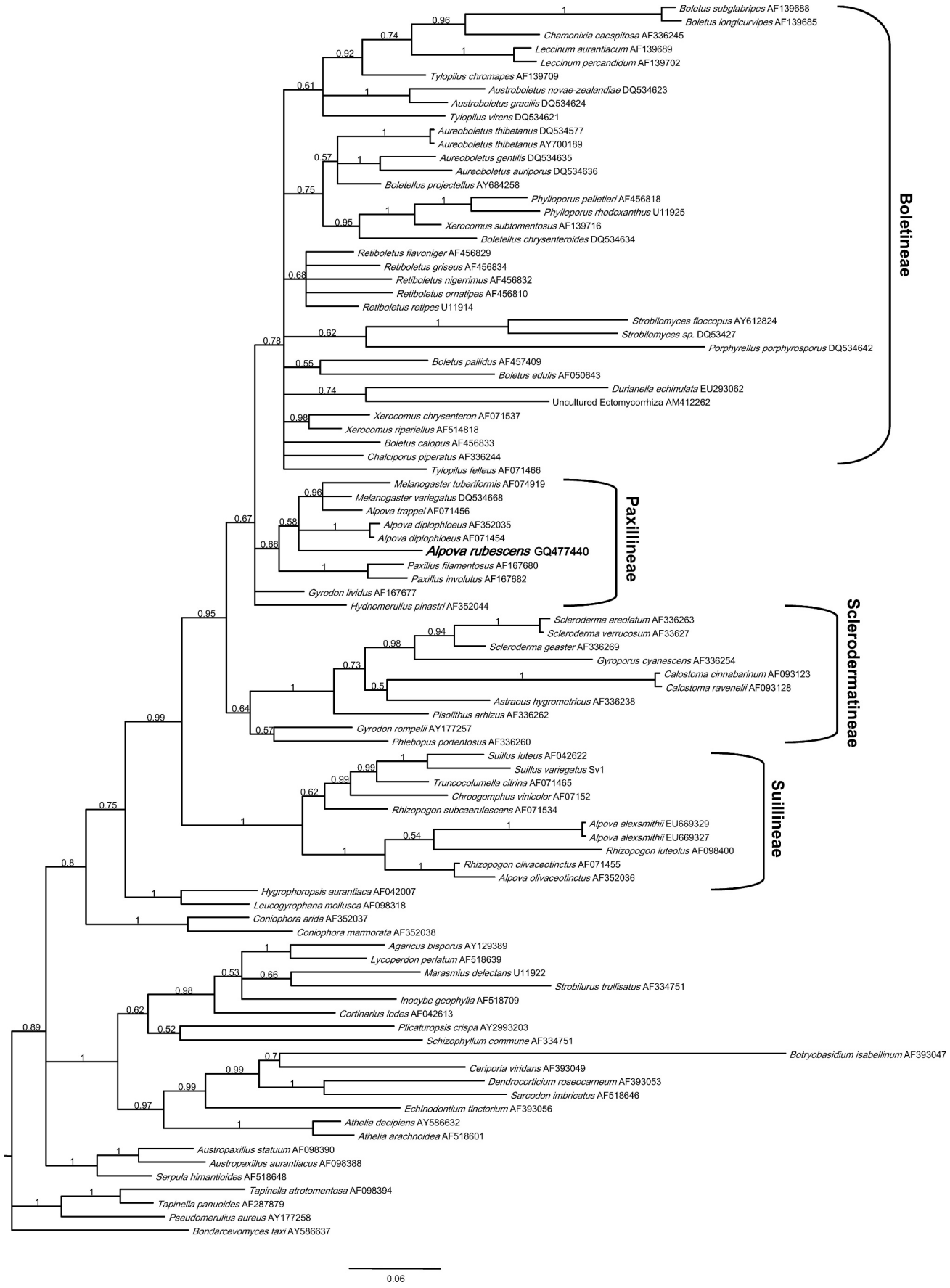
A second dataset of 16 taxa, encompassing *Alpova* spp. sequences and other related species, was built to better resolve *A. rubescens* placement among genus *Alpova*. NJ (FIG. 4), Bayesian inference and maximum likelihood analyses (data not shown) showed a congruent tree topology. In this topology *A. rubescens* clusters as a sister group of the paxilloid clade, including the *A. diplophloeus–A. austroalnicola* and *Melanogaster* species–*Alpova trappei* complexes, but no boostrap or posterior probabilities values supports such grouping. *A. alexsmithii* and *A. olivaceotinctus* cluster in the *Suillineae* together with *Rhizopogon* and *Suillus*.

DISCUSSION

Distribution and typification of Octaviania rubescens.—While conducting taxonomic studies on *Alpova* we realized that the typification of this European species is necessary to advance morphological and phylogenetic knowledge. Since the original description, *A. rubescens* has been collected rarely (e.g. Knapp 1954, Svrček 1958, Tabarés and Rocabruna 1991, Calonge and Pasabán 1993, Martín and Llimona 1994, Ruini 1995, Montecchi and Sarasini 2000, Kreisel 2001, Gori 2005, Tkalčec et al. 2005, Rubio et al. 2006). It is well characterized in having long spores and growing under *Fagaceae* (*Quercus*, *Castanea* and *Fagus*).

In the protolog Vittadini (1831) listed two collection localities for *Octaviania rubescens* (''In sylvis circa Carbonara in Laumellina, et prope Torre d'Isola in agro ticinensi obvius''), but he did not designated a type. Over the years Vittadini's collections of hypogeous fungi were dispersed in numerous herbaria; it is well known that he sent specimens to several contemporaries such as the Tulasne brothers in Paris and Fries in Uppsala. Mattirolo (1907) enlisted the cooperation of several herbarium directors to reassemble Vittadini's material in his home institution at TO (Herbarium Orti Botanici Taurinensis, University of Torino). Vittadini's original specimens are preserved at TO and PAD, according to Stafleu and Cowan (1986); so we checked the *O. rubescens* collections at these herbaria. At TO the collection cited by Mattirolo (1907) and studied by Lange (1956) is lacking. At PAD there is a specimen named *O. rubescens* but without collecting data or any indication on its origin (Rossella Marcucci, curator, pers comm), and so it cannot be assured with certainty whether that specimen represents original material. Therefore we also decided to investigate the other putative original collections reported by Lange (1956) at PC and UPS. At PC the mixed Vittadini's collection of *Octaviania rubescens* (reported as *Melanogaster*), cited by Lange (1956) as present in Tulasne's herbarium, is unobtainable (no reply from the curator after repeated requests). However we located a collection (F-166083, Herb. Fries; FIG. 5) in the herbarium of Uppsala University (UPS) surely made by Vittadini and consisting of four specimens. Microscopy analysis of this material revealed that the largest basidiome, to the left in the picture (FIG. 5a), has brown and thick-walled spores 9.5–11(–12.5) × 5–5.5 μm, and in our opinion it could represent a *Melanogaster*. The three other basidiomes (FIG. 5b, c, d) have light-colored and slender spores, 9.5–12 × 3.5–4 μm, and they are surely *O. rubescens*. However we think that it is not advisable to select these specimens as a lectotype because (i) the material is scarce and part of a mixed collection and is inadequate for accurate taxonomic interpretation and (ii) it cannot be assured with certainty that Vittadini's specimens of *O. rubescens* were collected before the species was described. Given the impossibility of definitely selecting a lectotype from any of Vittadini's herbarium material, we decided to designate Vittadini's Fig. XII E/Tab. IV (in Monographia Tuberacearum, 1831), which definitely was drawn after original material, as the lectotype for this species and at the same time to select our recent Italian collection as the epitype. A longitudinally sectioned basidiome and five basidiospores are illustrated (Plate XIIE) (FIG. 1). Our collection was chosen as epitype because it agrees well with the original diagnosis, as well as with recent descriptions by several authors (Knapp 1954, Trappe 1975, based on Mattirolo's collections; Ruini 1995, Montecchi and Sarasini 2000, Gori 2005). In addition we are convinced that a modern and large collection of well known origin will assure further microscopic and molecular investigations on this species.

Molecular speculation on the phylogenetic position of A. rubescens.—Phylogenetic investigations of *Rhizopogon*/*Boletales* relationships by Grubisha et al. (2001)


Boletus subglabripes AF139688
Boletus longicurvipes AF139685
Chamonixia caespitosa AF336245
Leccinum aurantiacum AF139689
Leccinum percandidum AF139702
Tylopilus chromapes AF139709
Austroboletus novae-zealandiae DQ534623
Austroboletus gracilis DQ534624
Tylopilus virens DQ534621
Aureoboletus thibetanus DQ534577
Aureoboletus thibetanus AY700189
Aureoboletus gentilis DQ534635
Aureoboletus auriporus DQ534636
Boletellus projectellus AY684258
Phylloporus pelletieri AF456818
Phylloporus rhodoxanthus U11925
Xerocomus subtomentosus AF139716
Boletellus chrysenteroides DQ534634
Retiboletus flavoniger AF456829
Retiboletus griseus AF456834
Retiboletus nigerrimus AF456832
Retiboletus ornatipes AF456810
Retiboletus retipes U11914
Strobilomyces floccopus AY612824
Strobilomyces sp. DQ53427
Porphyrellus porphyrosporus DQ534642
Boletus pallidus AF457409
Boletus edulis AF050643
Durianella echinulata EU293062
Uncultured Ectomycorrhiza AM412262
Xerocomus chrysenteron AF071537
Xerocomus riparíellus AF514818
Boletus calopus AF456833
Chalciporus piperatus AF336244
Tylopilus felleus AF071466
Boletineae
Melanogaster tuberiformis AF074919
Melanogaster variegatus DQ534668
Alpova trappei AF071456
Alpova diplophloeus AF352035
Alpova diplophloeus AF071454
Alpova rubescens GQ477440
Paxillus filamentosus AF167680
Paxillus involutus AF167682
Gyrodon lividus AF167677
Hydnomerulius pinastri AF352044
Paxillineae
Scleroderma areolatum AF336263
Scleroderma verrucosum AF33627
Scleroderma geaster AF336269
Gyroporus cyanescens AF336254
Calostoma cinnabarinum AF093123
Calostoma ravenelii AF093128
Astraeus hygrometricus AF336238
Pisolithus arhizus AF336262
Gyrodon rompelii AY177257
Phlebopus portentosus AF336260
Sclerodermatineae
Suillus luteus AF042622
Suillus variegatus Sv1
Truncocolumella citrina AF071465
Chroogomphus vinicolor AF07152
Rhizopogon subcaerulescens AF071534
Alpova alexsmithii EU669329
Alpova alexsmithii EU669327
Rhizopogon luteolus AF098400
Rhizopogon olivaceotinctus AF071455
Alpova olivaceotinctus AF352036
Suillineae
Hygrophoropsis aurantiaca AF042007
Leucogyrophana mollusca AF098318
Coniophora arida AF352037
Coniophora marmorata AF352038
Agaricus bisporus AY129389
Lycoperdon perlatum AF518639
Marasmius delectans U11922
Strobilurus trullisatus AF334751
Inocybe geophylla AF518709
Cortinarius iodes AF042613
Plicaturopsis crispa AY2993203
Schizophyllum commune AF334751
Botryobasidium isabellinum AF393047
Ceriporia viridans AF393049
Dendrocorticium roseocarneum AF393053
Sarcodon imbricatus AF518646
Echinodontium tinctorium AF393056
Athelia decipiens AY586632
Athelia arachnoidea AF518601
Austropaxillus statuum AF098390
Austropaxillus aurantiacus AF098388
Serpula himantioides AF518648
Tapinella atrotomentosa AF098394
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Pseudomerulius aureus AY177258
Bondarcevomyces taxi AY586637
0.06

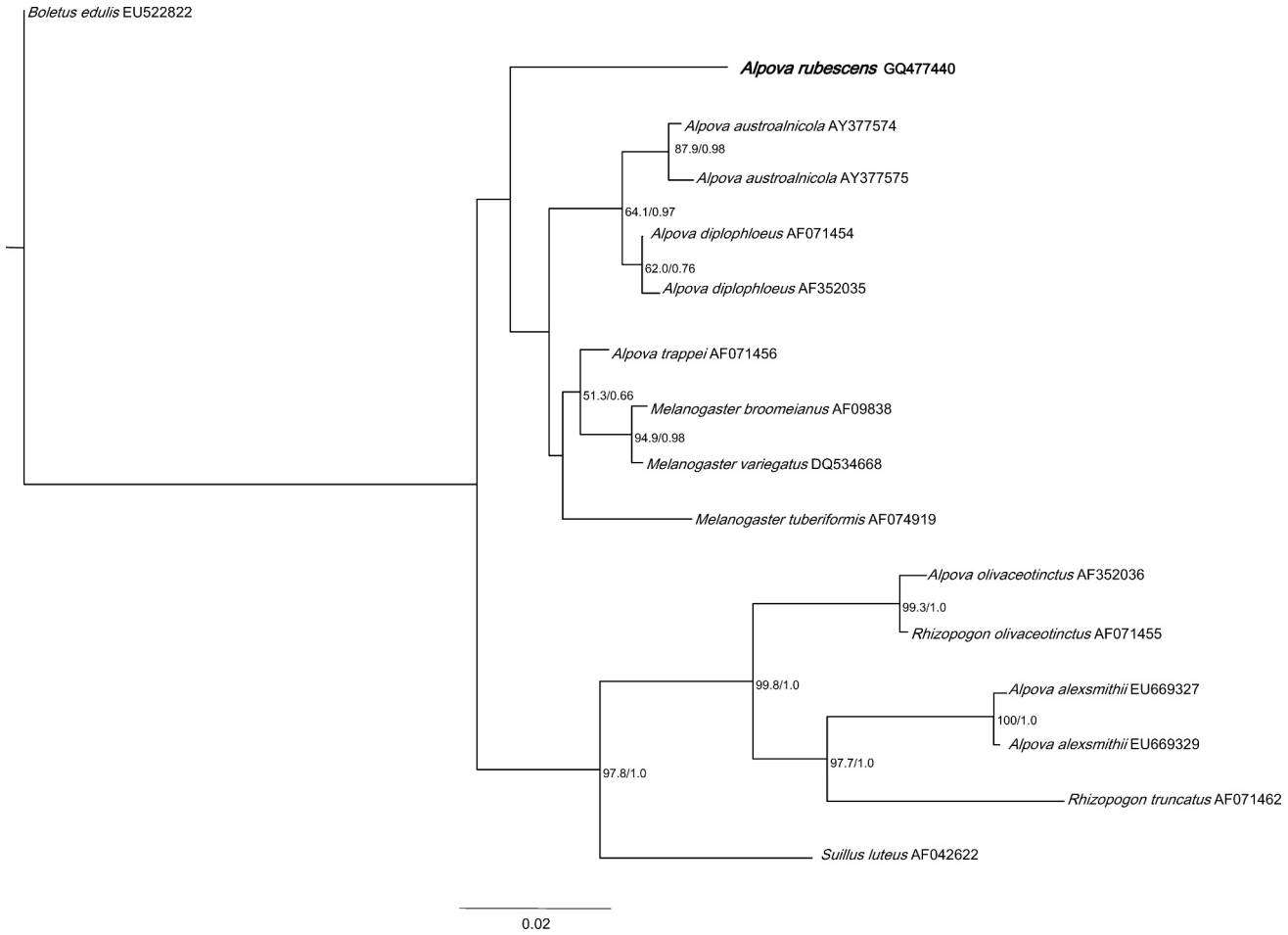

FIG. 4. Evolutionary relationships of *A. rubescens* and related species inferred by neighbor joining. The Kimura two-parameter model was used for pairwise distance measurement. Bootstrap values above 50% are indicated (1000 replicates) at nodes, followed by Bayesian posterior probability where available. *Boletus edulis* EU522822 was used as outgroup taxon to root the tree. Bar = Kimura distance.

and Jarosch (2001) have shown that *Alpova diplophloeus* relates to the boletoid radiation (corresponding to *Boletineae* + *Paxillineae* sensu Jarosch 2001 and Binder and Hibbett 2006) or to the *Paxillineae* in the *Melanogastraceae* respectively whereas the *Rhizopogon*-related *A. olivaceotinctus*, species placed by Trappe (1975) in *Alpova* subgen. *Alpova* sect. *Rhizopogonella*, clusters in the suilloid radiation (*Suillineae*) (Bruns et al. 1998, Kretzer and Bruns 1999, Grubisha et al. 2001, Jarosch 2001, Jarosch and Besl 2001) in the *Rhizopogonaceae*. On the basis of these data Grubisha et al. (2001) suggested that *Alpova* might not be monophyletic but recognized the need for new studies including more taxon sampling; in addition they transferred *A. olivaceotinctus* back to *Rhizopogon*, where it originally had been described as *R. olivaceotinctus* A.H. Sm. (Smith and Zeller 1966).

The present study aimed to investigate the position of *A. rubescens* in the *Boletales* and to compare phylogenetic relationships among *A. rubescens* and all *Alpova* species for which sequences are available in GenBank. Based on our results (FIGS. 3, 4), *Alpova* is clearly polyphyletic. *A. olivaceotinctus* and *A. alexsmithii*, placed by Trappe in sect. *Rhizopogonella*, fall within the *Suillineae*, whereas *A. rubescens*, *A. diplophloeus*, *A. austroalnicola* and *A. trappei* cluster

←

FIG. 3. Consensus tree derived from Bayesian phylogenetic inference of *A. rubescens* (Vittad.) Trappe 28S ribosomal RNA partial gene, obtained in this study, manually aligned to the existing TreeBase study No. S2162/M4103 (Desjardin et al. 2008). Numbers above branches are posterior probabilities derived from 10 000 Markov chain Monte Carlo sampled trees. The tree is unrooted. Bar = posterior probability.

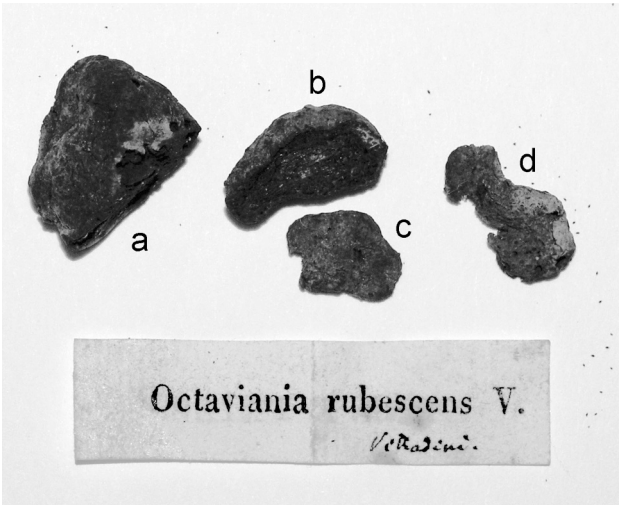

FIG. 5. *Octaviania rubescens*. Vittadini's mixed collection (Herb. Fries) located at UPS. a. *Melanogaster* sp. b, c, d. *O. rubescens*.

within the *Paxillineae*. As regards *A. diplophloeus* and *A. austroalnicola*, the two *Alnus*-associated species, they seem to be closely related and appear as sister group of the clade comprising *A. trappei* and *Melanogaster* spp. (FIG. 4).

A. rubescens occupies a distinct position: in our phylogenetic trees (FIGS. 3, 4). It clearly is not related to *A. diplophloeus*, type of genus *Alpova* (Trappe 1975), or to other *Alpova* species. These weak relationships are never supported by bootstrap or posterior probability values. *A. rubescens* may represent a new phyletic line of hypogeous *Paxillineae* probably distinctive enough to warrant the erection of a new genus. Hence the concept of *Alpova* will have to be reverted to the strict sense of its original description (Dodge 1931) and therefore intended and used for the species characterized by small spores lacking persistent rests of sterigmata, peridiopellis with a layer of inflated, swollen elements and close ectomycorrhizal association with *Alnus*. This concept would include only two morphologically similar species, *A. diplophloeus* and *A. austroalnicola*. *A. rubescens* shares peculiar characters of both *Melanogaster* (large spores with long rests of sterigmata, strong odor, and association with *Fagaceae*) and *Alpova* s.s. (pale spores, pseudoparenchimatic endoperidium), supporting its basal position to both the genera.

These data and speculations here are considered provisional because too few taxa have been investigated sufficiently and more species and specimens still have to be studied and sequenced to make a more accurate interpretation or a definitive statement. An increased taxon sampling is needed to improve phylogenetic inference on the polyphyly of the nonsuilloid *Alpova* species.

A. alexsmithii has to be transferred to *Rhizopogon*; the molecular evidence also is supported by its *Rhizopogon*-like features as the strict association with conifers and the presence of a rudimental hymenium, as inferred from Trappe (1975). It is likely that in future studies additional *Alpova* species from *Alpova* subg. *Alpova* sect. *Rhizopogonella* will have to be removed from that genus and placed in genus *Rhizopogon* as well.

We propose this new combination:

Rhizopogon alexsmithii (Trappe) Vizzini & Zotti, comb. nov.

MycoBank MB515248

Basionymum: *Alpova alexsmithii* Trappe, Beih. Nova Hedwigia 51:298 (1975).

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