Molecular validation of Sarcodon quercinofibulatus, a species of the S. imbricatus complex associated with Fagaceae, and notes on Sarcodon

Published version:

DOI:10.1007/s11557-012-0851-9

Alfredo Vizzini , Matteo Carbone, Fabrizio Boccardo and Enrico Ercole

"Molecular validation of **Sarcodon quercinofibulatus***, a species of the* **S.imbricatus** *complex associated with* **Fagaceae***, and notes on* **Sarcodon"**

Mycological Progress - *August 2013, Volume 12, Issue 3, pp 465-474,* 10.1007/s11557-012-0851-9

Molecular validation of Sarcodon quercinofibulatus, a species of the S. imbricatus complex associated with Fagaceae, and notes on Sarcodon

Alfredo Vizzini , Matteo Carbone, Fabrizio Boccardo and Enrico Ercole

Abstract

Morphological and molecular phylogenetic analyses revealed that Italian and Mexican collections of an unknown Sarcodon species of the S. imbricatus complex associated with Fagaceae (Castanea and Quercus), were assignable to Sarcodon quercinofibulatus, a species recently described from Spain. The species, characterized by a light brown-hazelnut coloured pileus surface eventually breaking into large and coarse scales, was recognized as independent from Sarcodon imbricatus and S. squamosus. S. aspratus, usually synonymized with S. imbricatus, is a different species. S. squamosus collections from montane and Mediterranean pine woodlands were shown to be conspecific. Four sections of Sarcodon (Sarcodon, Violacei, Squamiceps and Scabrosi) established by Maas Geesteranus (Verh K ned Akad Wet III, 65: 1–127, *1975*) only on morphological basis, are here confirmed as monophyletic.

Introduction

The genus Sarcodon Quél. ex P. Karst., typified by Hydnum imbricatum L., includes stipitate hydnoid fungi characterized by fleshy basidiomata, a soft to firm, brittle and not zonated, never duplex context, a brown spore print, a monomitic hyphal system, generative hyphae often inflated with or without clamp-connections, and irregularly tuberculate spores (Maas Geesteranus *1956*, *1971*, *1975*; Maas Geesteranus and Nannfeldt *1969*; Baird *1984*, *1986a*, *b*; Harrison and Grund *1987*; Jülich *1989*; Stalpers *1993*; Pegler et al. *1997*; Strid *1997*; Arnolds *2003*). According to recent molecular analyses, Sarcodon belongs to the so called thelephoroid clade (=Thelephorales Corner ex Oberw. partim), together with other stipitate hydnoid genera such as Hydnellum P. Karst., Phellodon P. Karst., Bankera Coker & Beers ex Pouzar; in these studies Sarcodon appears to be sister to Hydnellum (Bruns et al. *1998*; Larsson et al. *2004*; Binder et al. *2005*).

Most of the Sarcodon species are distributed in the Northern temperate Hemisphere (Maas Geesteranus *1971*, *1975*; Baird *1986b*; Stalpers *1993*; Pegler et al. *1997*), and are generally regarded as ectomycorrhizal partners of a range of woody angiosperms and gymnosperms, particularly within Fagaceae and Pinaceae. Ectomycorrhizae formed by this group of fungi are poorly known: those produced by S. imbricatus and S. leucopus with Picea abies were described by Agerer (*1991a*, *b*) and Mleczko et al. (*2011*), and represent the only ones to have been well investigated in the genus to date. Sarcodon as well as the other stipitate hydnoid fungi are extensively used in ecological-environmental research (Newton et al. *2002*; Van der Linde et al. *2008*, *2009*, *2010*; Hobbie and Agerer *2010*; Lindman *2010*; Mleczko et al. *2011*). Basidiome production of these fungi has been declining in northern Europe and North America over the past few decades (Arnolds *1989*, *2003*, *2010*; Otto *1990*, *1992*; Gulden and Hanssen *1992*; Hrouda *1999a*, *b*; Vesterholt et al. *2000*; Walleyn and Verbeken *2000*; Newton et al. *2002*); it is suggested that this negative trend is correlated with habitat loss and increasing airborne nitrogenous eutrophication (Arnolds *1989*, *2003*, *2010*; Vesterholt et al. *2000*; Walleyn and Verbeken *2000*). They are regarded as one of the most endangered groups of macrofungi in Europe (Hrouda *1999a*, *b*, *2005a*, *b*), resulting in their inclusion in European Red Data Lists (Walleyn and Verbeken *2000*; Nitare *2006*; Senn-Irlet et al. *2007*; Hrouda *2005a*, *b*).

Within Sarcodon, species identification relied primarily on the observation of a rather limited set of characters, such as presence/absence of clamp-connections, colour of the context in both pileus and stipe, arrangement of pileus surface, organoleptic features (smell and taste) and spore size (Harrison *1964*; Maas Geesteranus and Nannfeldt *1969*; Maas Geesteranus *1971*, *1975*; Harrison and Grund *1987*; Baird *1986a*, *b*; Stalpers *1993*; Pegler et al. *1997*; Strid *1997*). Infrageneric partition of the European taxa of Sarcodon was addressed by Maas Geesteranus (*1975*), who recognized six sections, viz sect. Sarcodon, Squamiceps Maas Geest., Scabrosi Maas Geest., Virescens Maas Geest., Violacei Maas Geest. and Velliceps Maas Geest.; these sections were since then usually accepted by later authors. Sect. Sarcodon encompasses the clamp-bearing species having a clearly areolated fissured-scaly pileus, whitish to light brown context without green tinges and only rarely turning red vinaceous when cut, and absence of farinaceous smell and taste. In this section, S. imbricatus is a species traditionally defined by a fleshy, dark brown pileus, breaking up into large pronounced scales, erect in the centre of pileus but adpressed and flat on its margin, and deeply fissured between the scales; it is known from Europe, Asia and North America, usually associated with Pinaceae (Picea and Pinus, Maas Geesteranus *1971*, *1975*; Stalpers *1993*; Pegler et al. *1997*), but reported also under hardwoods from North America (Coker and Beers *1951*; Smith and Smith *1973*; Baird *1986b*).

Recently, Johannesson et al. (*1999*), based on morphological data and analysis of the ITS sequence distances and RFLP-patterns of several S. imbricatus collections from northern Europe, showed that specimens growing under Picea abies and those under Pinus sylvestris are different taxa, thus recognizing two species: S. imbricatus s.s. (under Picea) and S. squamosus (Schaeff.) Quél. (under Pinus). These two species mainly differ in the shape, colour and ornamentation of the pileus and host preferences; however, until the contribution of Johannesson et al., Sarcodon squamosus was for a long time been confused with S. imbricatus, even though the former Hydnum squamosum was described already in the eighteenth century by Schaeffer (*1774*). Because of the confusion, almost all specimens collected in the 20th century have been identified as Sarcodon imbricatus, and an exact identification of the old samples is difficult in some cases. Collections surely attributable to S. squamosus are those of Phillips (*1981*) and Pegler et al. (*1997*).

After Johannesson et al. (*1999*), S. squamosus was found under Pinus sylvestris and/or P. nigra in different European countries (Dickson *2000*; Kotlaba and Pouzar *2000*; Schafer *2000*; Schmidt-Stohn *2001*; Arnolds *2003*; Hausknecht and Klofac *2004*; Papoušek *2004*; Hrouda *2005a*, *b*; Della Maggiora *2007*; Dollé et al. *2007*; Shiryaev *2008*; Kirk and Cooper *2009*; Pérez-De-Gregorio et al. *2011*), and under P. pinaster and P. laricio subsp. calabrica (Pinus nigra subsp. laricio) in Italy (Della Maggiora *2007*).

Cifuentes (*1996*), Patiño-Conde et al. (*2004*) and Patiño-Conde (*2006*), based on morphological and molecular data, reported a S. aff. imbricatus from Quercus in Quercus-Pinus forests in Mexico, suggesting the existence of a second species close to S. imbricatus.

Recently, Pérez-De-Gregorio et al. (*2011*) established, on morphological basis only, the new species Sarcodon quercinofibulatus (as S. quercinofibulatum) for Spanish specimens differing from S. imbricatus and S. squamosus only for growing under a non-coniferous ectomicorrhizal host, Quercus petraea.

Hereafter we refer to the closely similar species S. imbricatus, S. squamosus, S. quercinofibulatus and S. aff. imbricatus as the S. imbricatus complex.

On account of morphological analysis and nrITS sequence data, the main aims of this study can be summarized as follows: i) to investigate the identity and the phylogenetic placement of several Italian collections of a Sarcodon species belonging to this complex characterized by a pale brown tomentose pileus, squamose only at maturity, and associated with Castanea sativa; ii) to find out whether S. squamosus collections from Mediterranean pines are conspecific to those occurring in montane pine forests or not; iii) to test Maas Geesteranus' (*1975*) morphologically based taxonomy against molecular phylogenetic approach.

Materials and methods

Morphology

The description of the macroscopical features is based on fresh material. Micro-features are based on dried specimens, rehydrated in water or 5 % KOH, and then mounted in Congo red to observe the hymenium and pileipellis, and in water for spore dimensions and pigment localization. Spore size is expressed both as a range and mean value. Extreme measurements are indicated within parentheses; spore measurements include tubercles. The following abbreviations are used: [X, Y, Z] indicating that measurements were made on X spores, in Y samples from Z collections; Q = the spore quotient (length/width ratio); Qm = the average spore quotient. Author citations follow the Index Fungorum-Authors of Fungal Names (http:// www.indexfungorum.org/authorsoffungalnames.htm). Colour terms in capital letters (e.g. Raw Sienna) are those of Ridgway (*1912*). Herbarium acronyms follow Thiers (*2011*) except that JC refers to the personal herbarium of J. Carbó.

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of 12 herbarium specimens (Table 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy). Universal primers ITS1f/ITS4 were used for the ITS region amplification (White et al. *1990*; Gardes and Bruns *1993*). Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM MgCl2, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega). The PCR program was as follows: 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 visualized by staining with ethidium bromide. PCR products were purified and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). Sequence assembly and editing were performed using Geneious v5.3 (Drummond et al. *2010*). The sequences are deposited in GenBank under the accession numbers given in Table 1.

Table 1

Sarcodon sequences newly generated for this study and associated GenBank accession numbers

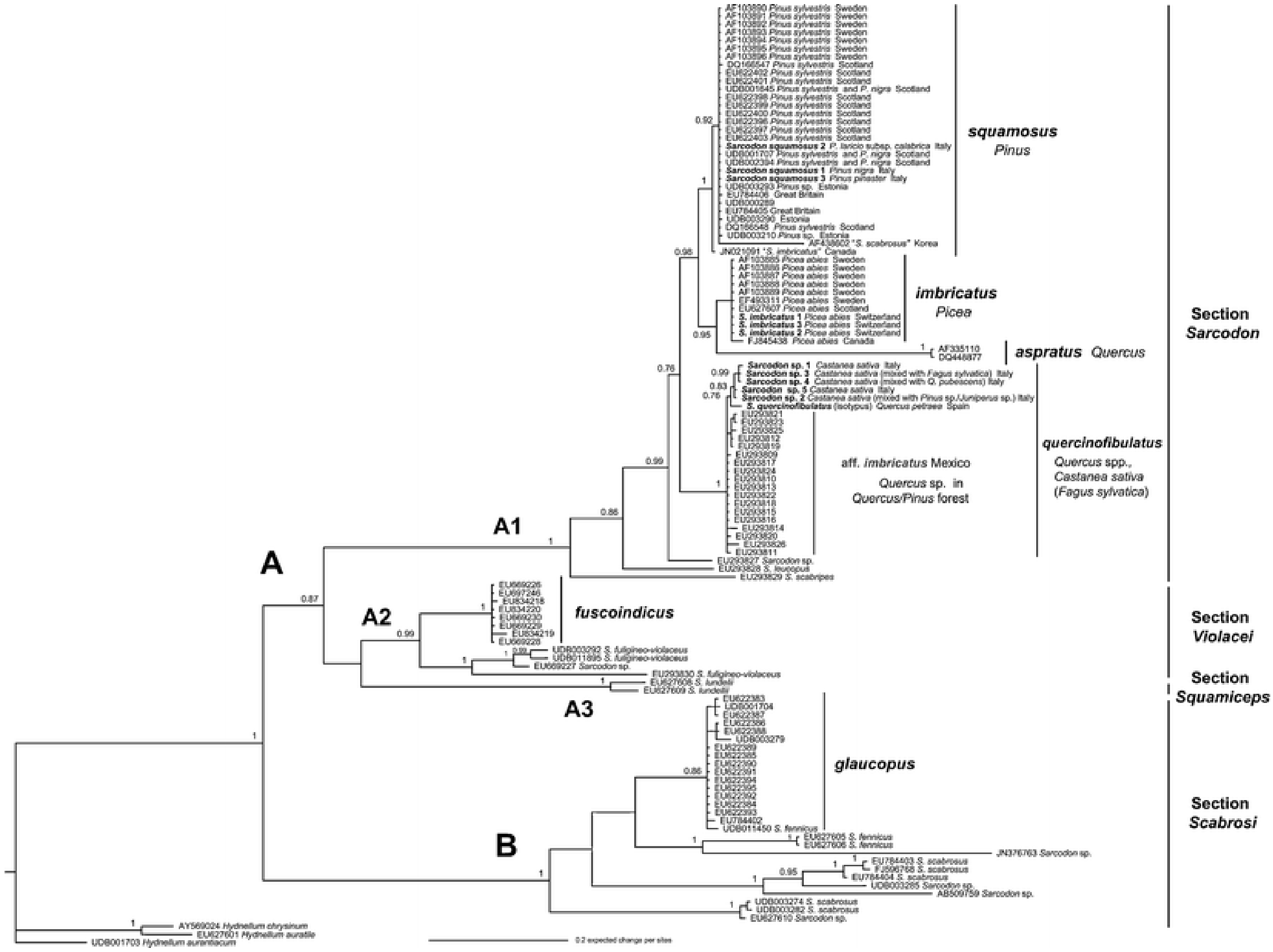
Species	**Collection data**	**Herbarium no.**	**ITS GenBank acc. no.**
S. imbricatus 1	Switzerland, Valais canton, Liddes, Chandonne, 18. 08. 2005, under Picea abies	MCVE 27380	JX271810
S. imbricatus 2	Switzerland, Ticino canton, Olivone, 14. 08. 2007, under Picea abies	MCVE 27381	JX271811
S. imbricatus 3	Switzerland, Ticino canton, Lucomagno, 25. 08. 2007, under Picea abies	MCVE 27382	JX271812
Sarcodon sp. 1	Italy, Liguria, Davagna (GE), Piancarnese, 18. 07. 2005, under Castanea sativa	MCVE 27374	JX271813
Sarcodon sp. 2	Italy, Liguria, Fontanigorda (GE), Casoni, 27. 09. 2005, under Castanea sativa (Castanea mixed forest with Pinus sp. and Juniperus sp.)	MCVE 27379	JX271814
Sarcodon sp. 3	Italy, Liguria, Fontanigorda (GE), Casoni, 14. 07. 2009, under Castanea sativa (Castanea mixed forest with Fagus sylvatica)	TUR-A 195698	JX271815
Sarcodon sp. 4	Italy, Lombardy, Varzi, Serra del Monte (PV), 21. 09. 2005, under Castanea sativa (Castanea mixed forest with Quercus pubescens)	TUR-A 195696	JX271816
Sarcodon sp. 5	Italy, Liguria, Savona, Sassello, 20. 07. 2009, under Castanea sativa	GDOR 1476	JX271817
S.	Spain, Girona, Puig Rodon, La Vall de Bianya,	JC-20090718.2	JX271818

Species	Collection data	Herbarium no.	ITS GenBank acc. no.
quercinofibulatus	18. 07. 2009, under Quercus petraea	(Isotypus)	
S. squamosus 1	Italy, Tuscany, Comano (MS), 07. 07. 1995, under Pinus nigra (P. nigra mixed forest with Castanea sativa)	TO AV 1195	JX271819
S. squamosus 2	Italy, Calabria, Gambarie (RC), 16. 10. 2003, under Pinus laricio subsp. calabrica	TO AV 258-S	JX271820
S. squamosus 3	Italy, Tuscany, Capannori (LU), Coselli, 25. 11. 2006, under Pinus pinaster	TO AV 623-S	JX271821

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were either generated in this study (Table 1) or retrieved from GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/ index.php?e=true) databases. Multiple sequence alignments for ITS fragments were generated using MAFFT (Katoh et al. *2002*) with default conditions for gap opening and gap extension penalty. The alignment was slightly edited using MEGA 5.0 (Tamura et al. *2011*). Phylogenetic analysis was performed using the Bayesian Inference (BI) approach. The BI was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist *2001*) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run over 10 million generations, under GTR + Γ evolutionary model. Trees were sampled every 1,000 generations resulting in an overall sampling of 10,001 trees; the first 2,500 trees were discarded as "burn-in" (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Only BPP values over 0.75 are reported in the resulting tree (Fig. 1). Branch lengths were estimated as mean values over the sampled trees. Pairwise % identity values of ITS sequences (P%IV) were calculated using MEGA 5.0 (Tamura et al. *2011*).

Fig. 1



Bayesian phylogram obtained from the ITS (I TS1-5.8S-ITS2) sequence alignment of Sarcodon spp. Hydnellum auratile, H. aurantiacum and H. chrysinum were used as outgroup taxa. BPP values over 0.75 are given above branches. Newly sequenced collections are in bold. Numbers (e.g. 1-5) refer to the Sarcodon collections reported in Table 1

Results

Molecular results

The amplification of the ITS regions was successful for the 12 specimens, yielding a PCR product of about 720 bp. The ITS data matrix comprises a total of 116 sequences (including 89 from GenBank and 15 from UNITE). The Sarcodon atroviridis sequence (EU293831) (Sarcodon sect. Virescentes) was not used since clearly of chimeric origin. This dataset is 882 base pairs long and contains 490 (55.6 %) variable sites. Of these, 402 (45.6 %) are parsimony-informative.

In the obtained Bayesian phylogram (Fig. 1), two major clades, A and B, were distinguished within Sarcodon. Clade A consists of three subclades (A1-A3). Clade A is supported by a BPP value of 0.87, while clade B by a BPP value of 1. A1, A2, A3 and B correspond quite well to the sections Sarcodon, Violacei, Squamiceps and Scabrosi, as traditionally delimited by Maas Geesteranus (*1971*, *1975*).

Section Sarcodon (BPP = 1), the focus of the paper, encompasses six species; S. squamosus, S. imbricatus, S. aspratus, S. quercinofibulatus, S. leucopus, Sarcodon sp. and S. scabripes.

Our five sequences of Sarcodon collections from Castanea sativa, clustered together with the sequence of S. quercinofibulatus (isotypus, specimen from Spain) (P%IV = 99.4) and 18 sequences relating to S. aff. imbricatus from Mexico, forming a well supported monophyletic clade (BPP = 1). The pairwise % identity value of the 24 sequences of this clade is 98.6. European sequences differ from the Mexican ones only by one polymorphic site (one mutation) in position 599 of the total alignment (T instead of A). The quercinofibulatus clade is sister to a clade formed by S. squamosus, S. imbricatus, and S. aspratus (BPP = 0.98).

The three sequenced collections of S. imbricatus from Switzerland clustered with GenBank sequences of the same fungal species. This clade clustered sister to two S. aspratus sequences, forming a well supported clade (BPP = 0.95). The three sequenced collections of S. squamosus collected under Mediterranean pines (P. pinaster and P. laricio subsp. calabrica) clustered with GenBank and UNITE sequences from specimens collected under montane pines (P. sylvestris and P. nigra); the squamosus clade was supported by BPP of 1.

Taxonomy

Sarcodon quercinofibulatus Pérez-De-Greg., Macau & J. Carbó, Rev. Catal. Micol. 33: 26 (2011)

Figs. 2, 3, and 4

Fig. 2

Sarcodon quercinofibulatus. Macromorphological features. a Basidiomata (TUR-A 195698); b, c pileus surface and hymenophore (MCVE 2737); d, e scales (MCVE 27376 and TUR-A 195695). Photos by M. Carbone. Scale bars (a,c,d,e) 5 cm, (b) 10 cm

Fig. 3

Sarcodon quercinofibulatus. Basidiomata. Drawing by F. Boccardo (GDOR 1476)

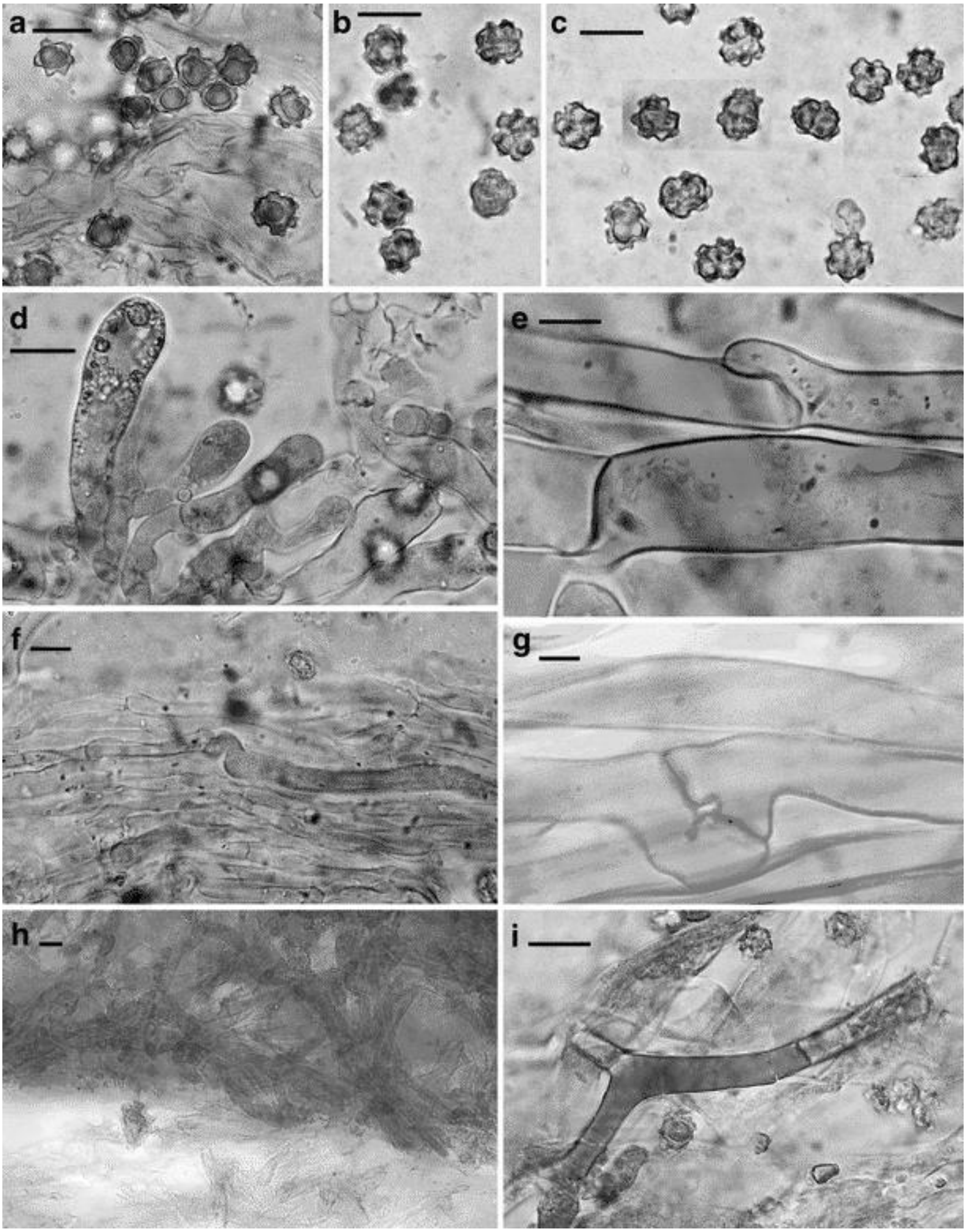


Fig. 4

Sarcodon quercinofibulatus. Micromorphological features (MCVE 27374). a-c Spores, d clamped hymenial elements, e clamp-connections in tramal hyphae, f hymenophoral trama with clamped thromboplerous hyphae, g medallion-type clamp connection, h pileipellis elements in water mount, i thromboplerous hyphae in the pileipellis. Photos by M. Carbone. Scale bars (a-i) 10 µm

Macrocharacters Pileus up to 20(23) cm in diam., convex to plane, slightly to strongly umbilicate in age; surface hazelnut coloured, light brown (Mars Yellow, Raw Sienna, Ochraceous-Tawny), slightly darker (Buckthorn Brown, Sudan Brown, Hazel) in age; at first velutinous to very slightly floccose, then cracked by fissures forming scales arranged concentrically, mainly triangular to truncated pyramidal in shape, at first concolorous then darker than the background; tips not or only slightly pointed upward to definitely vertical in the centre in mature specimens; margin strongly incurved for long time, straight only in mature specimens; spines up to 1.2 cm long in large and over-mature specimens, adnate-subdecurrent to definitely decurrent on the stipe (both the types can occur on the same basidiome), whitish to grayish, then brownish; stipe up to 8 cm long and up to 2 cm wide, cream coloured, slightly darker to sub-concolorous with the pileus, central to slightly eccentric, cylindrical to sub-bulbose, covered by small aborted spines, often with curved base; context uniformly coloured in all parts, whitish to very pale brownish; taste mild but with a very slightly bitter aftertaste, barely appreciable on the back of the tongue after prolonged chewing; smell agreeable, reminiscent of liquorice in young basidiomes, stronger and more or less unpleasant in old specimens.

Microcharacters Spores [120, 6, 6], (5.5–)6–8(–8.3) × (5.5–)6–7(–7.6) μm, on average 6.96 × 6.38 μm, Q = 1–1.27, Qm = 1.12, globose to subglobose, brownish, irregular in outline due to the presence of numerous exsculpate to 3-pointed tubercles; basidia (25–)30–40(–45) × 8–10 μm, club-shaped, slender clavate, 4-sporic, sterigmata up to 5 μm long, clamped; basidioles club-shaped, very numerous, clamped; cystidia absent; hymenophoral trama regular, composed of cylindrical hyphae, up to 6 μm wide, septate, thin-walled, hyaline, clamped, sometimes anastomosing; pileipellis composed of cylindrical hyphae, up to 10 μm wide, septate, thin-walled, clamped, brownish due to an epiparietal and intracellular pigment; suprapellis a cutis-subtrichoderm of prostrate hyphae with some bundles of hyphae rising upward; context consisting of hyphae up to 21(–24) μm wide, cylindrical, septate, branched, inflating, thin-walled, clamped; thromboplerous hyphae (=oleiferous hyphae sensu Clémençon *2004*) present; clamp connections abundant in all tissues; clamps of the medallion-type observed in the context.

Ecology and distribution Gregarious to subcespitose, on bare ground under broadleaved trees and strictly associated with Fagaceae: in Italy (Liguria and Lombardy) mainly under Castanea sativa, but also in mixed forests with Quercus pubescens, Fagus sylvatica, in one collection Pinus sp. and Juniperus sp. were nearby; in Spain collected under Quercus petraea, Q. humilis and Acer opalus, and one collection under Fagus sylvatica; in Mexico, under Quercus sp. in Quercus and Quercus/Pinus forests.

Collections examined Sarcodon quercinofibulatus. ITALY- Liguria, Davagna (GE), loc. Piancarnese, 18. 07. 2005, many basidiomes under Castanea sativa, leg. M. Carbone (MCVE 27374); ibidem, in a different part of the same forest, 28. 07. 2005, leg. M. Carbone (MCVE 27375); ibidem, 02. 09 2005, leg. M. Carbone (MCVE 27376); ibidem, in a different part of the same forest, 05. 09. 2005, leg. M. Carbone (TUR-A 195695); ibidem, in a different part of the same forest, 22. 09. 2005, leg. M. Carbone (TUR-A 195697). Uscio (GE), 10. 09. 2005, two basidiomes under Castanea sativa, leg. M. Carbone (MCVE 27377). Recco (GE), 20. 08. 2006, one basidiome under Castanea sativa, leg. M. Carbone (MCVE 27378). Fontanigorda (GE), loc. Casoni, 27. 09. 2005, many basidiomes under Castanea sativa but with some Pinus sp. and Juniperus sp. in the sorroundings, leg. M. Carbone (MCVE 27379); ibidem, many basidiomes, in the same forest but under Castanea sativa and Fagus sylvatica, 14. 07. 2009, leg. M. Carbone (TUR-A 195698). Lombardy, Varzi (PV), Serra del Monte, 21. 09. 2005, 3 basidiomes under Castanea sativa (Castanea mixed forest with Quercus pubescens), leg. M. Carbone (TUR-A 195696). Liguria, Savona, Sassello, 20. 07. 2009, under Castanea sativa, leg. F. Boccardo (GDOR 1476). SPAIN – Girona, La Vall de Bianya, Puig Rodon, 18. 07. 2009, under Quercus petraea, leg. J. Carbó, J. Galí, C. Miñarro, G. Mir, M.À. Pérez-De-Gregorio, and À. Torrent (JC-20090718.2, isotypus).

Additional collections examined Sarcodon imbricatus. SWITZERLAND - Valais canton, Liddes, loc. Chandonne, 18. 08. 2005, many basidiomes under Picea abies, leg. M. Carbone (MCVE 27380). Ticino canton, Olivone, 14. 08. 2007, many basidiomes under Picea abies, leg. M. Carbone (MCVE 27381). Ticino canton, Lucomagno, 25. 08. 2007, many basidiomes under Picea abies, leg. M. Carbone (MCVE 27382). Sarcodon squamosus. ITALY – Tuscany, Comano (MS), 07. 07. 1995, many basidiomes under Pinus nigra (P. nigra mixed forest with Castanea sativa), leg. Luciana Bonamini (TO AV1195); Calabria, Gambarie (RC), 16. 10. 2003, under Pinus laricio subsp. calabrica, leg. M. Della Maggiora (TO AV258-S); Tuscany, Capannori (LU), Coselli, 04. 12. 2005, under Pinus pinaster, leg. M. Della Maggiora (TO AV 623-S).

Discussion

Notes on the infrageneric classification of the genus Sarcodon

According to our phylogenetic analysis, two major clades are recognized within Sarcodon (Fig. 1): the clade A encompassing species with or without clamp-connections and characterized by the absence of blue-green tinges at stipe base; clade B consisting of species without clamp-connections and with blue-green tinges at stipe base. Even if a more extensive sampling of Sarcodon species is needed for a comprehensive revision of the whole genus, our preliminary molecular data support the infrageneric Sarcodon classification established by Maas Geesteranus (*1975*). Four clades corresponding to four out of the six sections recognized by Maas Geesteranus were recovered: section Sarcodon (type species S. imbricatus, pileus fissurate to squamose, context without both blue-green and reddish violaceous tinges, odour not farinaceous, presence of abundant clamp connections), supported by BPP = 1, consists of S. aspratus (Berk.) S. Ito, S. imbricatus, S. leucopus (Pers.) Maas Geest. & Nannf., S. quercinofibulatus, S. scabripes (Peck) Banker, Sarcodon sp. and S. squamosus; section Violacei [type species S. joeides (Pass.) Bataille, context reddish pink, violaceous, odour farinaceous, clamp-connections very rare or absent], supported by BPP = 0.99, consists of S. fuscoindicus (K.A. Harrison) Maas Geest. and S. fuligineo-violaceus (Kalchbr.) Pat.; section Squamiceps (consisting only of the type species S. lundellii, pileus scaly-areolated, context whitish to brownish, odour usually farinaceous, absence of clamp connections); finally, section Scabrosi [type species S. scabrosus (Fr.) P. Karst., areolate pileus, bluish-green tinges at stipe base, absence of clamp-connections], supported by BPP = 1, consists of S. glaucopus Maas Geest. & Nannf., S. fennicus (P. Karst.) P. Karst. and S. scabrosus. Our phylogenetic analysis highlighted that the sequences of S. scabrosus from Genbank and UNITE databases form two distinct and well-supported clades, suggesting that S. scabrosus, as traditionally defined (e.g. Maas Geesteranus *1975*; Baird *1986b*; Stalpers *1993*; Arnolds *2003*), is polyphyletic and probably consists of two independent taxa.

No sequences were available for sect. Velliceps [type species S. martioflavus (Snell, K.A. Harrison & H.A.C. Jacks.) Maas Geest., pileus surface strongly velutinous, context whitish to brownish, odour farinaceous, clamp-connections absent]; for sect. Virescentes [type species S. atroviridis (Morgan) Banker, basidiome entirely turning olive green on drying, with or without clamp-connections] the only sequence present in GenBank is chimeric and it was not used for phylogenetic purposes.

Sarcodon collections of the S. imbricatus complex associated with Fagaceae

Our specimens collected under Castanea sativa are clearly conspecific with S. quercinofibulatus from Spain associated with Quercus petraea (in one case with Fagus sylvatica) and with Mexican specimens collected under Quercus sp. in Quercus/Pinus forests

(Fig. 1). Spanish and Mexican collections show morphological features fitting well with the Italian ones (Cifuentes *1996*; Patiño-Conde *2006*; Pérez-De-Gregorio et al. *2011*, and our observations). S. quercinofibulatus is proven to be a taxon independent from the S. imbricatus/S. squamosus species-pair. This species seems mainly associated with Fagaceae, and shows a disjoint distribution pattern, although this could be due to insufficient collection data. Only the presence of one polymorphic site in ITS sequences distinguishes European collections from Mexican ones (see Results) and this difference is probably attributable to geographical segregation.

As far as we know, the first account on the existence of this species in Italy (Liguria), under Castanea sativa, can be found in Orsino and Dameri (*1989*) under the name Sarcodon imbricatus. The authors stated (translated from Italian): "this species deserves further studies, because the specimens collected under chestnut trees show some differences from the typical collections fruiting under conifers in mountain areas". Other records are also reported by Zotti and Orsino (*2001*), always as S. imbricatus. Our collections come from adjacent valleys, definitely sharing the same kind of habitat and climatic conditions. Coker and Beers (*1951*) and Baird (*1986b*) stated that in the eastern United States S. imbricatus is associated with frondose woods. Smith and Smith (*1973*) pointed out that it occurs throughout North America under both conifers and hardwoods. In the light of our present results we believe that further studies are required in order to ascertain if S. quercinofibulatus also occurs in broadleaved forests of northern and eastern United States.

S. imbricatus and S. squamosus are the two most similar species to S. quercinofibulatus. They appear to be quite identical under the microscope, but have a few morphological differences (Johannesson et al. *1999*; Kotlaba and Pouzar *2000*; Schafer *2000*; Schmidt-Stohn *2001*; Arnolds *2003*; Della Maggiora *2007*, and our observations): i) S. imbricatus s.s. is characterized by a brown pileus (paler than S. squamosus) with brown scales, a margin not remaining incurved for a long time, an always depressed, often infundibuliform or hollow centre with scales usually pointing almost straight upward; brown spines not decurrent and quite long; a stipe normally longer than the pileus diameter, cylindrical or with slightly bulbous base, not paler at the apex; a smell somewhat disagreeable, and a bitter taste; ii) S. squamosus is distinguished by a yellow-brown to vinaceous brown pileus with blackish brown scales, pileus margin remaining incurved, pileus centre not deeply depressed, scales usually smaller than S. imbricatus with those in the centre not or only slightly pointed upward; spines slightly decurrent, rather short and crowded, greyish, often with a greyish-blue tint when fresh; stipe short, about the same as the pileus diameter or shorter, paler at the apex and narrowing at the base; smell aromatic-spicy, taste not bitter. The habitat seems to be an additional distinguishing feature: as pointed out by Johannesson et al. (*1999*) the former is associated with Picea on calcareous soil, and the latter with Pinus on acid soil. According to our analysis (Fig. 1) specimens of S. squamosus collected under montane pines (P. sylvestris and P. nigra) are genetically identical to those collected under Mediterranean pines (P. pinaster and P. laricio). S. quercinofibulatus differs from the S. imbricatus/S. squamosus pair only by a paler pileus, at first tomentose then squamose only at maturity, and for its association with Fagaceae. The S. imbricatus complex seems to be a case of host-driven speciation processes (Rochet et al. *2011*).

S. aspratus from Japan, Korea and China (Kawagoe *1924*; Imazeki et al. *1988*; Huang *1998*; Kim et al. *2002*) at first was synonymized by Maas Geesteranus (*1960*, *1971*) with S. imbricatus, and then considered an independent species close to S. praestans Maas Geest. from New Guinea (Maas Geesteranus *1974*). According to our data (Fig. 1, P%IV = 94.1) S. aspratus is probably independent from S. imbricatus; it differs by having smaller pileal scales, a fragrant sweetish smell, and by growing under Quercus spp. (Kawagoe *1924*; Maas Geesteranus *1971*; Imazeki et al. *1988*; Kim et al. *2002*).

The presence of abundant clamp connections in all tissues is a striking feature which separates the S. imbricatus complex and the species belonging to Sarcodon sect. Scabrosi and Sarcodon sect. Squamiceps (Maas Geesteranus *1975*). S. scabrosus is, in fact, very common in Italy under Castanea sativa but is microscopically unclamped and macroscopically it shows purplish hues on the pileus, pale grayish-bluish at the base of the stipe, farinaceous smell and very bitter taste. Sarcodon regalis Maas Geest. is another species recorded from Castanea sativa woods but it is very different macroscopically (Calledda and Carbone *2006*). As pointed out by Pérez-De-Gregorio et al. (*2011*) the other known European species associated with broadleaved trees are unclamped. Among them, the only taxon macroscopically resembling Sarcodon quercinofibulatus is undoubtedly S. underwoodii Banker [incl. Sarcodon radicatus Banker and Sarcodon murrillii Banker fide Maas Geesteranus and Nannfeldt (*1969*) and Baird (*1986a*, *1986b*)]; for details about its distribution see also Banker (*1906*, *1913*), Coker and Beers (*1951*), Hall and Stuntz (*1972*), Maas Geesteranus (*1975*), Stalpers (*1993*), Bessette et al. (*1997*), and more recently Dollé et al. (*2007*) concerning its presence in Europe. Nevertheless, all these above mentioned species show very different macroscopic (general habit, colours, smell and taste) and microscopic (spores size) features, as can be appreciated in Harrison

(*1964*), Maas Geesteranus (*1971*, *1974*, *1975*), McNabb (*1971*), Hall and Stuntz (*1972*), Baird (*1984*, *1986a*, *b*), Breitenbach and Kränzlin (*1986*), Jülich (*1989*), Stalpers (*1993*) and Strid (*1985*, *1997*).

Acknowledgments

We are grateful to J. Carbó (Girona, Spain) and M. Della Maggiora (Pieve di Compito-Capannori, Lucca, Italy) for sending herbarium specimens, to Samuele Voyron (University of Turin, Italy) for providing helpful technical assistance, and to Matteo Gelardi (Rome, Italy) and Caroline Hobart (Sheffield, U.K.) for improving the English text. Lastly, thanks to J. Vauras (TUR-A Herbarium, Turku, Finland) and G. Robich (MCVE Herbarium, Venice, Italy) for housing many of the studied collections.

References

Agerer R (1991a) Ectomycorrhizae of Sarcodon imbricatus on Norway spruce and their chlamydospores. Mycorrhiza 1:21–30CrossRef

Agerer R (1991b) Sarcodon imbricatus. In: Agerer R (ed.) Colour Atlas of Ectomycorrhizae, Einhorn, Schwäbisch Gmünd, plate 66

Arnolds E (1989) Former and present distribution of stipitate hydnaceous fungi (Basidiomycetes) in the Netherlands. Nova Hedwigia 48:107–142

Arnolds E (2003) De stekelzwammen en pruikzwammen van Nederland en België. Coolia 46(3), suppl: 1–96

Arnolds E (2010) The fate of hydnoid fungi in The Netherlands and Northwestern Europe. Fungal Ecol 3:81–88CrossRef

Baird RE (1984) Study of the stipitate hydnums of the southern Appalachian Mountains. Genera: Bankera, Hydnellum, Phellodon, Sarcodon. Ph.D. dissertation, University of Tennessee, Knoxville

Baird RE (1986a) Type studies of North American and other related taxa of stipitate hydnums: Genera Bankera, Hydnellum, Phellodon, Sarcodon. Bibl Mycol 103:1–89

Baird RE (1986b) Study of the stipitate hydnums of Appalachian mountains. Bibl Mycol 104:1–156

Banker HJ (1906) A contribution to a revision of the North American Hydnaceae. Mem Torr Bot Club 12(2):99–194

Banker HJ (1913) Type studies in the Hydnaceae III. The genus Sarcodon. Mycologia 5:12–17CrossRef

Bessette AE, Bessette AR, Fischer DW (1997) Mushrooms of Northeastern North America. Syracuse University Press, Syracuse

Binder M, Hibbett DS, Larsson K-H, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Syst Biodivers 3(2):113–157CrossRef

Breitenbach J, Kränzlin K (1986) Champignon de Suisse Vol. 2. Verlag Mykologia, Luzern

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton DR, Kretzer A, Garbelotto M, Li Y (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272CrossRef

Calledda F, Carbone M (2006) Una "idnacea"non comune in Italia: Sarcodon regalis. Pagine di Micologia 26:27–30

Cifuentes BJ (1996) Estudio taxonómico de los generous hidniodes estipitados (Fungi: Aphyllophorales) en México. Tesis de Doctorado en Ciencias (Biología). Fac. De Ciencias, UNAM, Mexico DF

Clémençon H (2004) Cytology and plectology of the Hymenomycetes. Bibl Mycol 199:1–488

Coker WM, Beers AH (1951) The stipitate hydnums of the eastern United States. University of North Carolina Press, Chapel Hill

Della Maggiora M (2007) Sarcodon squamosus una specie comune ma poco conosciuta. Riv Micol 50(2):127–137

Dickson G (2000) A field key to British non-resupinate hydnoid fungi. Field Mycol 1(3):99–104CrossRef

Dollé B, Moingeon JM, Sugny D (2007) Sarcodon underwoodii, un taxon signalé pour la première fois en France. Bull Soc Mycol Fr 123(1):29–40

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2010) Geneious v5.3. Available from http://www.geneious.com/

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118PubMedCrossRef

Gulden G, Hanssen EW (1992) Distribution and ecology of stipitate hydnaceous fungi in Norway, with special reference to the question of decline. Sommerfeltia 13:1–58

Hall D, Stuntz DE (1972) Pileate Hydnaceae of the Puget Sound Area. II. Brown-Spored Genera: Hydnum. Mycologia 64(1):15–37CrossRef

Harrison KA (1964) New or little known North American stipitate hydnums. Can J Bot 42(9):1205–1233CrossRef

Harrison KA, Grund DW (1987) Preliminary keys to the terrestrial stipitate hydnums of North America. Mycotaxon 28(2):419–426

Hausknecht A, Klofac W (2004) Ergebnisse des Mykologisches Arbeitstreffens in Horitschon (Burgenland) im September/Oktober 2001. Österr Z Mykol 13:237–265

Hobbie EA, Agerer R (2010) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil 327(1–2):71–83CrossRef

Hrouda P (1999a) Hydnaceous fungi of the Czech Republic and Slovakia. Czech Mycol 51:99–155

Hrouda P (1999b) Hydnaceous fungi – changes in their occurrence and possible causes. In: Jankovský L, Krejčíř R, Antonín V (eds) Houby a les (sborník referátu). MZLU, Brno, pp 53–56

Hrouda P (2005a) Bankeraceae in Central Europe. 1. Czech Mycol 57(1-2):57–78

Hrouda P (2005b) Bankeraceae in Central Europe. 2. Czech Mycol 57(3-4):279–297

Huang N-L (1998) Colored illustration of macrofungi (mushrooms) of China. China Agricultural Press, Beijing

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755PubMedCrossRef

Imazeki R, Otani Y, Hongo T (1988) Fungi of Japan. Yama-kei Publ, Tokyo

Johannesson H, Ryman S, Lundmark H, Danell E (1999) Sarcodon imbricatus and Sarcodon squamosus - two confused species. Mycol Res 103(11):1447–1452CrossRef

Jülich W (1989) Guida alla determinazione dei funghi vol. 2. Edizioni Saturnia, Trento

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066PubMedCrossRef

Kawagoe S (1924) The market fungi of Japan. Trans Brit mycol Soc 10:201–206CrossRef

Kim HE, Koo CD, Kim JS, Park JI, Shin WS, Shin CS (2002) Ecological characteristics of below-ground ectomycorrhizal colony of Sarcodon aspratus in oak tree stands. J Kor For Soc 91:457–464

Kirk P, Cooper J (2009) The GB Checklist of Fungal Names. http://www.fieldmycology.net/GBCHKLST/gbchklst.htm

Kotlaba F, Pouzar Z (2000) Přehlížený druh našich lošáků - Sarcodon squamosus. Mykol Listy, Praha 74:5–7

Larsson K-H, Larsson E, Kõljalg U (2004) High phylogenetic diversity among corticioid Homobasidiomycetes. Mycol Res 108:983–1002PubMedCrossRef

Lindman J (2010) Evaluation of an ectomycorrhizal macrofungi as an indicator species of high conservation value pine-heath forests in northern Sweden. Master degree thesis in Biology, Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU, Umeå

Maas Geesteranus RA (1956) The stipitate Hydnums of the Netherlands. I. Sarcodon P. Karst. Fungus 26:44–60

Maas Geesteranus RA (1960) Notes on hydnums. Persoonia 1:341–384

Maas Geesteranus RA (1971) Hydnaceous fungi of the eastern old world. Verh K ned Akad Wet II 60(3):1–176

Maas Geesteranus RA (1974) Hydnaceous fungi of the eastern Old World. Supplement. Proc K Ned Akad Wet, Sect C 77:477–495

Maas Geesteranus RA (1975) Die terrestrischen Stachelpilze Europas. Verh K ned Akad Wet III 65:1–127

Maas Geesteranus RA, Nannfeldt JA (1969) The genus Sarcodon in Sweden in the light of recent investigations. Svensk Bot Tidskr 63(4):401–440

McNabb RFR (1971) Some new and revised taxa of New Zealand Basidiomycetes (Fungi). N Z J Bot 9:355–370CrossRef

Mleczko P, Zubek S, Kozak M (2011) Description of ectomycorrhiza and a new Central European locality of the rare hydnoid species Sarcodon leucopus (Pers.) Maas Geest. et Nannf. (Thelephorales, Basidiomycota). Nova Hedwigia 92(1–2):257–272CrossRef

Newton AC, Holden E, Davy LM, Ward SD, Fleming LV, Watling R (2002) Status and distribution of stipitate hydnoid fungi in Scottish coniferous forests. Biol Conserv 107(2):181–192CrossRef

Nitare J (2006) Åtgärdsprogram för bevarande av rödlistade fjälltaggsvampar (Sarcodon). Naturvårdsverket, Stockholm, Rapport 5609

Orsino F, Dameri RM (1989) Ricerca sulla flora della Liguria. 2. I macromiceti die castagneti delle alte Valli Scrivia e Polcevera (Apennino ligure). Webbia 43(2):355–386CrossRef

Otto P (1990) Die terrestrischen Stachelpilze der DDR-Taxonomie, Ökologie, Verbreitung und Rückgang. Dissertation, Martin-Luther-Universität, Halle-Wittenberg

Otto P (1992) Verbreitung und Rückgang der terrestrischen Stachelpilze Ostdeutschlands. Gleditschia 20:153–202

Papoušek T (2004) Velký fotoatlas hub z jižních Čech. České Budějovice

Patiño-Conde V. (2006) Relaciones filogenéticas del complejo Sarcodon imbricatus–S. squamosus. Maestría en Ciencias Biológicas (Sistemática). Facultad des Ciencias. Universidad Nacional Autónoma de México.

Patiño-Conde V, Cifuentes J, González D, Magallón S, León-Regagnon V (2004) Taxonomic boundaries of Sarcodon imbricatus sensu lato from Mexico. Annual Meeting, Mycological Society of America – poster. Asheville N.C., July 15-21

Pegler DN, Roberts PJ, Spooner BM (1997) British chanterelles and tooth fungi. Royal Botanical Gardens KEW, London

Pérez-De-Gregorio MÀ, Macau N, Carbó J (2011) Sarcodon quercinofibulatum, una nueva especie del género con hifas fibulíferas. Rev Catal Micol 33:25–30

Phillips R (1981) Mushrooms and other fungi of Great Britain and Europe. Pan Books, London

Ridgway R (1912) Color standards and color nomenclature. Washington, D.C., published privately (by the author)

Rochet J, Moreau P-A, Manzi S, Gardes M (2011) Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi Alnicola, Alpova and Lactarius (Basidiomycota) in Europe. BMC Evol Biol 11:40PubMedCrossRef

Schaeffer JC (1774). Fungorum qui in Bavaria et Palatinatu circa Ratisbonam nascuntur. Vol. IV, Regensburg

Schafer D (2000) Dyeing to resurrect an old species? Field Mycol 1(2):42–43CrossRef

Schmidt-Stohn G (2001) Sarcodon imbricatus und S. squamosus – zwei vermischte Arten. Boletus 24(1):48–53

Senn-Irlet B, Bieri G, Egli S (2007) Lista Rossa dei macromiceti minacciati in Svizzera. Ed. Ufficio federale dell'ambiente, Berna e WSL, Birmensdorf. Pratica ambientale n. 0718

Shiryaev A (2008) Diversity and distribution of thelephoroid fungi (Basidiomycota, Thelephorales) in the Sverdlovsk region, Russia. Folia Cryptog Estonica 44:131–141

Smith AH, Smith HV (1973) How to know the non-gilled mushrooms. Wm. C. Brown Company, Iowa

Stalpers JA (1993) The Aphyllophoraceous fungi I: keys to the species of the Thelephorales. Stud Mycol 35:3–168

Strid Å (1985) Svenska taggsvampar 6. Släktet Sarcodon. Jordstjärnan 6:5–10

Strid Å (1997) Sarcodon P. Karst. In: Hansen L, Knudsen H (eds) Nordic macromycetes, vol 3. Nordsvamp, Copenhagen, pp 313–315

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739PubMedCrossRef

Thiers B (2011) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Van der Linde S, Alexander IJ, Anderson IC (2008) A PCR-based method for detecting the mycelia of stipitate hydnoid fungi in soil. J Microbiol Methods 75:40–46PubMedCrossRef

Van der Linde S, Alexander IJ, Anderson IC (2009) Spatial distribution of sporocarps of stipitate hydnoid fungi and their belowground mycelium. FEMS Microbiol Ecol 69(3):344–352PubMedCrossRef

Van der Linde S, Alexander IJ, Anderson IC (2010) Do stipitate hydnoid fungi have the ability to colonise new native pine forest? Fungal Ecol 3:89–93CrossRef

Vesterholt J, Asman W, Christensen M (2000) Nitrogen deposition and decline of fungi on poor and sandy soils. Svampe 42:53–60

Walleyn R, Verbeken A (2000) Een gedocumenteerde Rode Lijst van enkele groepen paddestoelen (macrofungi) in Vlaanderen. Instituut voor Natuurbehoud, Brussel

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand D, Sninsky J, White T (eds) PCR Protocols, a guide to methods and applications. Academic, Orlando, pp 315–322

Zotti M, Orsino F (2001) The check-list of macrofungi in Liguria. Fl Medit 11:115–294