

***Sarcodon fennicus*, a boreo-montane stipitate hydroid fungus with a remarkable smell**

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Key words: *Thelephorales*, *Bankeraceae*, *Sarcodon amygdaliolens*, stipitate hydnums, ecology. – New record, Funga of Austria.

Abstract: New Austrian finds of the critically endangered *Sarcodon fennicus* are presented and illustrated. The determination was confirmed morphologically as well as genetically. *Sarcodon fennicus* differs from the recently described, very similar Mediterranean species *S. amygdaliolens* by slightly smaller spores and the habitat in montane coniferous forest.

Zusammenfassung: Österreichische Neufunde des vom Aussterben bedrohten *Sarcodon fennicus* werden vorgestellt und illustriert. Die Bestimmung wurde sowohl morphologisch als auch genetisch bestätigt. Von der kürzlich neu beschriebenen, sehr ähnlichen mediterranen Art *S. amygdaliolens* unterscheidet sich *S. fennicus* durch etwas kleinere Sporen und das Habitat im montanen Nadelwald.

In 2017, two *Sarcodon* collections with an intense smell of bitter almonds were found in Austria. The collections were readily recognized in the field as rare species belonging to the *Sarcodon scabrosus* group on account of their grey-green flesh in the stipe base. Additionally, the peculiar smell of the fresh fruitbodies suggested a close relationship to *Sarcodon amygdaliolens* L. RUBIO-CASAS, L. RUBIO-ROLDÁN & S. CATALÀ. This species was only recently described from Spain, and is so far only known from the Mediterranean region (RUBIO-CASAS & al. 2011). The altitude of the collections from Austria, at around 1000 m s. m., thus raised doubts about the possible identification as *S. amygdaliolens*, despite the conclusion by RUBIO-CASAS & al. (2011) that *S. amygdaliolens* was the only species in the *S. scabrosus* group with this particular smell. The spore measurements and the habitat (high montane *Picea* forest) indeed fit better for *Sarcodon fennicus* (P. KARST.) P. KARST., which may also possess a smell of bitter almonds according to MAAS GEESTERANUS & NANNFELDT (1969) (which is critically discussed by RUBIO-CASAS & al. 2011). The recent Austrian col-

lections were studied anatomically and their ITS was sequenced, which confirmed the identification as *S. fennicus* as well as the conclusions by MAAS GEESTERANUS & NANNFELDT (1969). *Sarcodon fennicus* and *S. amygdaliolens* can therefore not be separated by their smell alone. However, the habitat and the somewhat different spore size can be used to distinguish these species by using traditional methods.



Fig. 1. *Sarcodon fennicus*, habitus, WU 39715, CH2017090101.

Material and methods

Morphology: An Olympus CH-2 light microscope (1000× magnification, oil immersion) was used to study the microscopic features and for the drawings. The spore photos were made using an Olympus BX51 light microscope with an Olympus DP72 camera and a SEM of the type Jeol 6610. Sections were made by hand with a sharp razor blade using fresh material. Tap water was used as a medium for all microscopic measurements as well as the microscopic drawings. The spores were measured in living state by selecting mature, brown spores from a longitudinally sectioned spine in a squash mount. The selection of the measured spores was random apart from choosing coloured spores with pronounced ornamentation. The spores were measured in increments of quarters of a micron using a measuring eyepiece (10×). Microscopic drawings were made free-hand and without drawing mirror. The light microscopy photos of the spores were made in KOH (10 %) using dried material. A Canon EOS 50 D camera was used for the habit photos.

DNA analysis: Samples for genomic DNA analysis were taken from the hymenium of fresh herbarium specimens and ground in a mixer mill. DNA was extracted with GeneJet Plant Genomic DNA Purification Kit (Thermo Fischer Scientific) and KAPA2G Robust PCR Kit (Merck KGaA, Darmstadt) mastermix following the manufacturer's protocol. Amplification and sequencing of the nrITS region followed MENTRIDA & al. (2015). Using the NCBI BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>), the ITS sequence was compared to the most similar sequences to confirm the taxonomic

classification. Sequences are deposited in GenBank with the accession no. MK474928 (WU 39715) and MK474929 (GJO 93975, WU 39729).

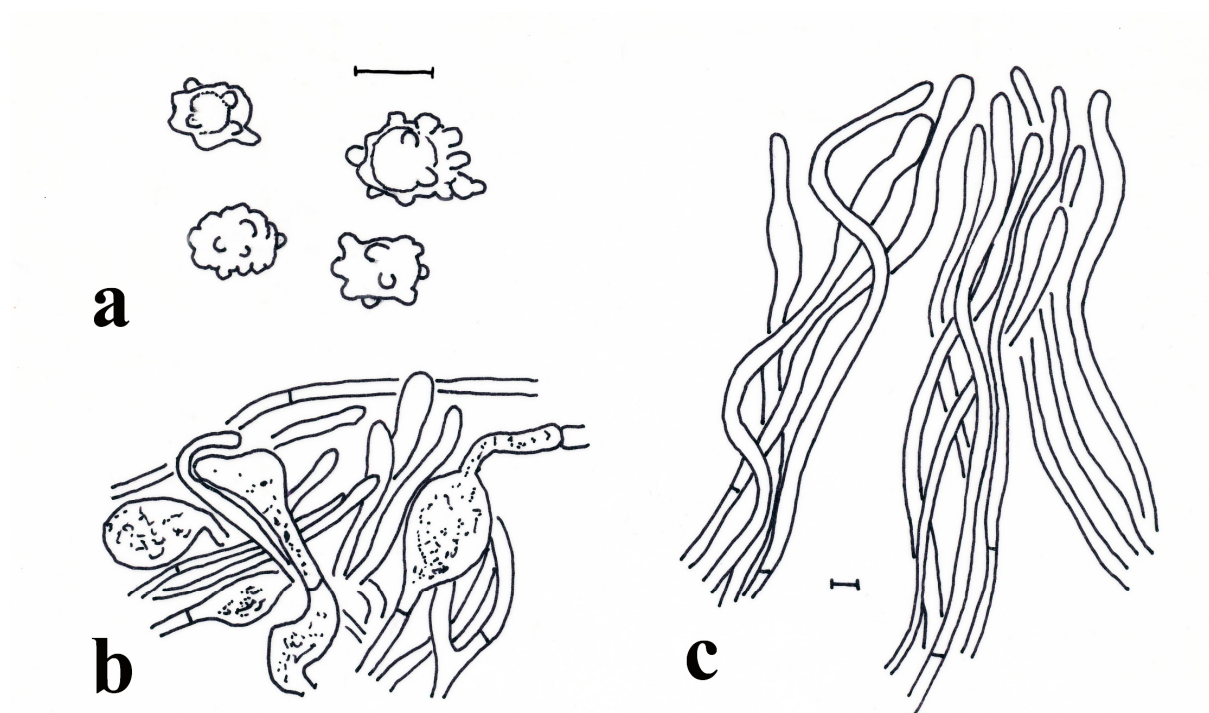


Fig. 2. *Sarcodon fennicus*, a spores; b pileus cuticle from the centre of the pileus with inflated end cells and intercalary cells filled with an oily substance; c pileus cuticle from the tomentose area at the cap margin, WU 39715, CH2017090101. Bars a 5 μ m, b–c 10 μ m.

Material examined: Austria, Carinthia, Sankt Veit an der Glan, Hüttenberg, Knappenberg, forest E of Obersemlach, south-facing slope, 46° 55' 15" N, 14° 33' 45" E, 1115 m s. m., leg. K. OBERHUBER, 31. August 2017, WU 39714, CH2017083101 (pers. fungarium of CHRISTOPH HAHN); – leg. E. BREIDLER, U. ÖSTERLE, C. HAHN, I. KRISAI-GREILHUBER, 1. September 2017, WU 39715, CH2017090101.

– Styria, Murtal, vicinity of Obdach, 47° 04' 05" N, 14° 41' 32" E \pm 2.5 km, 930–1110 m s. m. (estimated range), 19. August 2017, GJO 93975 (part-collection WU 39729).

Results

Sarcodon fennicus (P. KARST.) P. KARST., Revue Mycol. Toulouse 9: 10 (1887) – Figs. 1–4

\equiv *Sarcodon scabrosus* (FR.) P. KARST. var. *fennicus* P. KARST., Bidrag till Kännedom av Finlands Natur och Folk 37: 104 (1882)

\equiv *Hydnum fennicum* (P. KARST.) SACC., Sylloge Fungorum 6: 433 (1888)

\equiv *Phaeodon fennicus* (P. KARST.) HENN., Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen: I. Tl., 1. Abt.: *Fungi* (*Eumycetes*): 149 (1898)

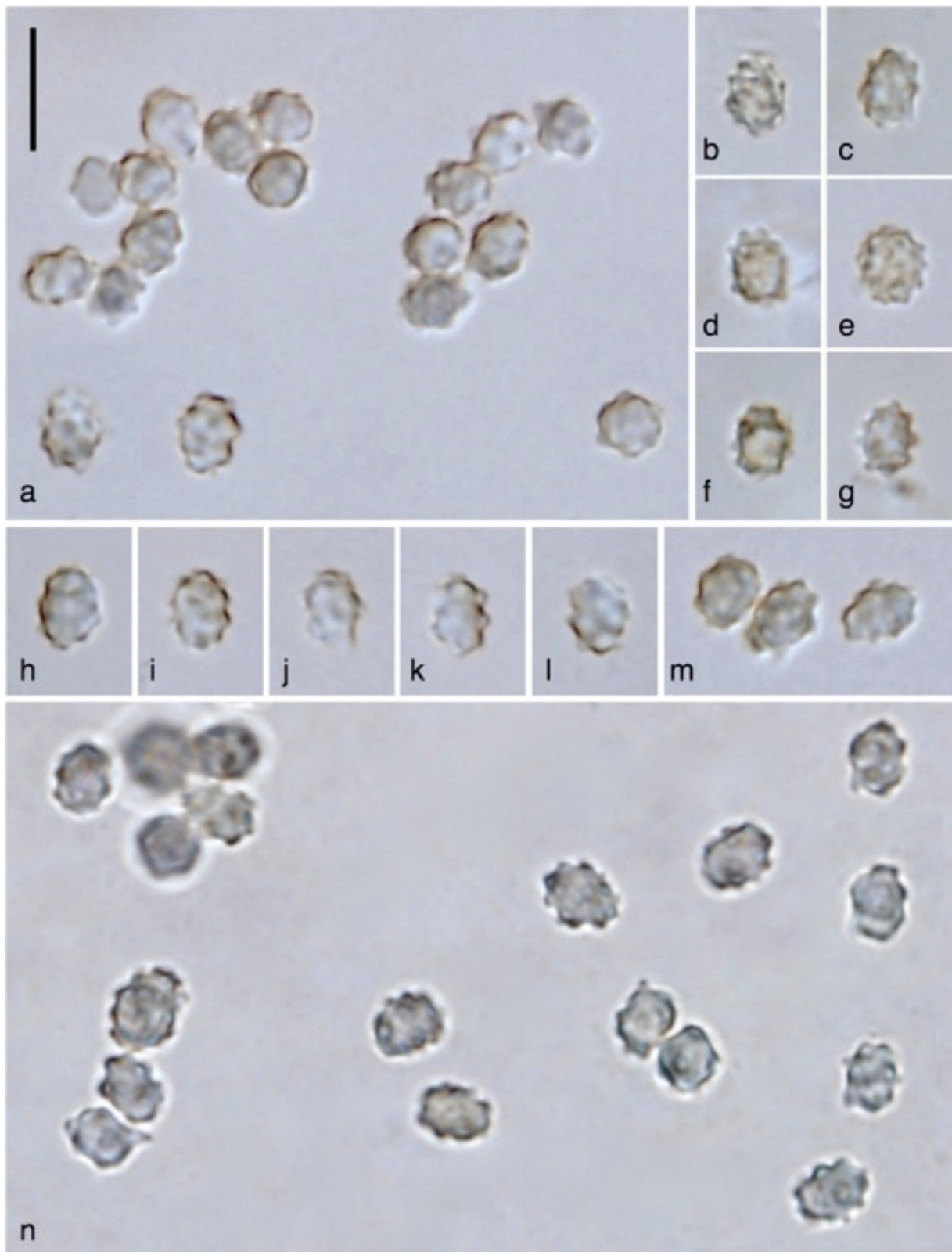


Fig. 3. *Sarcodon fennicus*, spores, a, h–n WU 39715, b–g WU 39714. Bar: 10 μ m for all photos.

Description:

Pileus: up to 9 cm in diam., surface tomentose, terrycloth-like, ragged (especially towards the centre), towards the margin radially shaggy to scaly, at the margin almost smooth (fibres completely appressed/ingrown, with fine fibrous scales under the mag-

nifying glass); colour a mix of different shades of brown: scales cinnamon to reddish-brown, between the scales more ochraceous to yellow-brown (reminiscent of *Tylopilus felleus* in terms of colour), in some parts also with vinaceous shades.

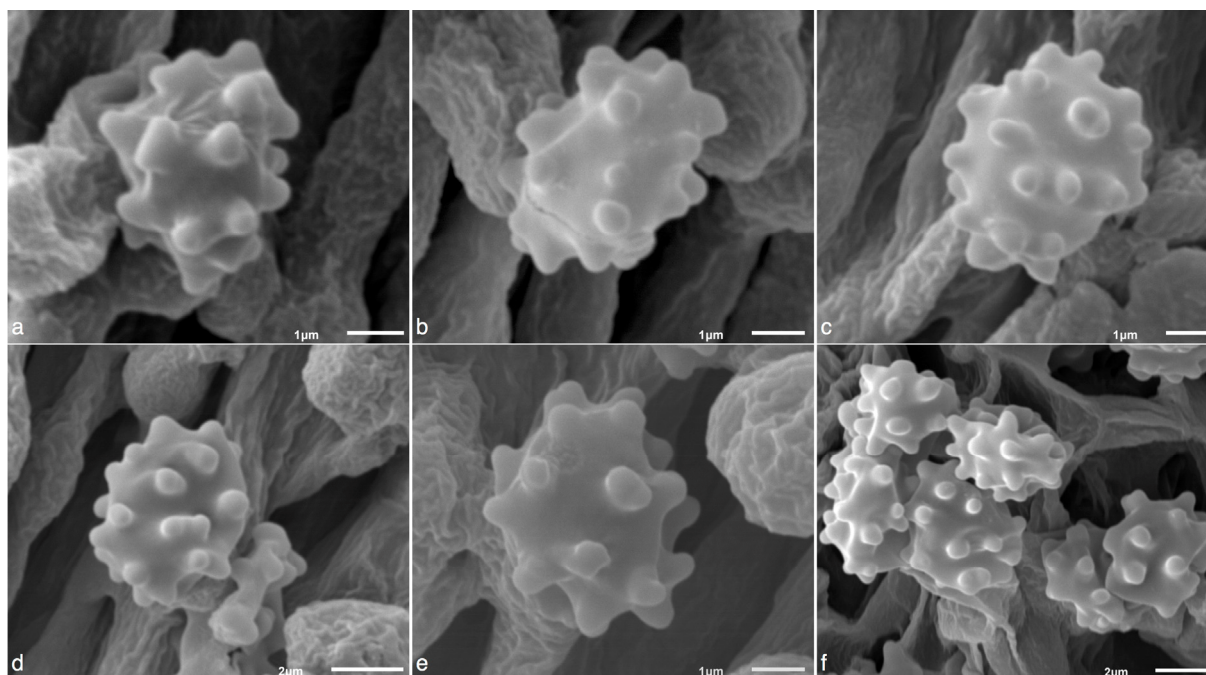


Fig. 4. *Sarcodon fennicus*, SEM photographs of spores, a–e WU 39714, f WU 39715.

S p i n e s : up to 6 mm long, strongly decurrent, circular at base; white at apex, brown at base; remarkably light coloured, almost shimmering white when observed from below.

S t i p e : short and compact, tapering towards the base, up to 3 × 3 cm, ochraceous brown (like the stipe colour of *Tylopilus felleus*), base with dirty whitish mycelium and cream-coloured rhizomorphs; surface with fine protuberances (magnifying glass!) to somewhat terrycloth-like.

C o n t e x t : hardly to clearly zonate, in the stipe base conspicuously dirty grey-green, in other parts of the stipe and the pileus dirty cream-coloured, in some parts greyish, partly turning somewhat yellowish when drying, after a few hours somewhat ochraceous to partly orange. Fresh context in KOH (10 %) at first yellow, then ochre and finally orange. Smell of freshly cut context first of cucumber, then intensively of bitter almond, especially in the hymenophor, after a while with a smell of a mixture of bitter almond and celery. Taste persistently very bitter.

B a s i d i o s p o r e s : light brown to amber in water in light microscope, ellipsoid with large, partly almost bifurcate warts and a conspicuous apiculus, $5.25\text{--}7.25 \times 4\text{--}5.5 \mu\text{m}$, (Figs. 2a, 3, 4).

B a s i d i a : 4-spored, $30\text{--}45 \times 5\text{--}6.5 \mu\text{m}$, with up to 5 μm long sterigmata.

H y m e n i a l c y s t i d i a : not observed.

P i l e u s c u t i c l e : terrycloth-like, tomentose parts of the pileus margin (Fig. 2c) consisting of hyphal bundles composed of 4–6(–9) μm wide, agglutinated hyphae; cells up to 200 μm long, somewhat widened towards the ends, with conspicuous yellow to amber content; pileus cover of the pileus centre (Fig. 2b) a mixture of hyphae: partly identical to those of the margin, partly 15–30 μm wide inflated to irregularly

clavate end cells filled with an oily substance, as well as similarly inflated intercalary cells.

Pileus trama: a mixture of regular, 5–15 µm wide hyphae, 3–6 µm wide thrombopleres and strongly inflated, up to 30 µm wide, short-celled elements with partly almost spherical cells due to the formation of secondary (unconstricted) septa.

Rhizomorphs: consisting of narrow, 2–3 µm wide, strongly incrustated hyphae (incrustations dissolving in KOH 10 %); up to 4.5 µm wide hyphae present in the centre. No chlamydospores observed.

Hyphal structure: monomitic, no clamp connections observed, septa simple.

Ecology: The locality in Carinthia is a montane coniferous forest at 1115 m s. m. with *Picea abies* (L.) H. KARST., *Larix decidua* MILL., *Pinus sylvestris* L. and several *Fagus sylvatica* L. trees on a south-facing slope. The soil consists of primary rock and the accompanying fungal species like *Cortinarius mucosus* (BULL.) J. KICKX F. and *Lactifluus oedematopus* (SCOP.) KUNTZE as well as *Vaccinium* dwarf shrubs are characteristic for acidic soil. However, the scattered occurrences of *Gomphus clavatus* (PERS.) GRAY or *Ramaria longispora* MARR & D. E. STUNTZ indicate a somewhat higher base-richness. It was not possible to retroactively clarify if the base-richness was locally increased despite the surrounding area generally being rather acidic.

The Styrian collection was brought to the attention of one of the authors during a larger foray in Obdach. Unfortunately, the person who originally collected the material could not be traced, therefore the exact location and ecology are unknown; however, the ecology might be comparable to that of the Carinthian material, which was collected about 20 km SW of Obdach, based on the altitude and similar prevalence of *Picea* forests.

Distribution: According to HROUDA (2018) *S. fennicus* is now an almost extinct species, which in Central Europe recently was confirmed only for Baden-Württemberg, Tyrol (three specimens), Czechia and Bohemia. In addition to the new findings presented here, two further Austrian collections from a locality in Styria from ca. 1900, which were revised by P. HROUDA, are in the herbarium GJO (<https://herbarium.univie.ac.at/index.htm>). In the recent Austrian Red List (DÄMON & KRISAI-GREILHUBER 2017) it is considered as extinct. This has to be corrected to the category Critically Endangered (1).

Genetic analysis: The nrITS sequences generated of WU 39715 and WU 39729/GJO 93975 were 100 % identical with the sequences EU627605 and EU627606 in Genbank originating from a Scottish *Sarcodon fennicus* basidioma (VAN DER LINDE & al. 2008) and with three sequences of Norwegian specimens in UNITE (UDB036811, UDB036633, UDB03628; <https://unite.ut.ee/analysis>). The sequence of WU 39714 also proved to be *S. fennicus* but only with 94 % similarity. This specimen could only be sequenced unidirectionally and the quality of the sequence obtained was low. All other *Sarcodon* sequences showed only similarities around 85 %.

4. *S. scabrosus* (Fr.) QUÉL. SCHAEFF., t. 271.

Hatten mörkt rostbrun, i början päronformig, först filtluden, sedan småfjällig.

Barrskog, m. r. Skand. (Småland, Östergötland).

Hatten m. köttig, kompakt, äldre ofvan flat, undertill m. kullrig, n. bugtbräddad, först filtluden, sedan af små, tätt sittande af hopfildadt ludd bildade fjäll knottig, inuti hvit, omkr. 12 cm. bred; foten trind eller sammantryckt, af rudimentära taggar prickad, gråaktig, nedtill afsnålmande, vid basen rundad och svartaktig, m. kort (knappt 3 cm. hög); taggarne likformiga, syllika, mörkt rostbruna, i spetsen hvitaktiga. — *Hydnum* Fr.

Var. *fennicus* KARST.

Hatten slutl. söndersprucken till rutor, 7–10 cm. bred; foten af hattens färg, men ljusare, vid basen blekt grå- eller svartblå, 3–6 cm. hög, 2–3 cm. tjock; taggarne hvitaktiga, äldre rostbrunaktiga; sporerne 4–5 mmm. i diam.

Barrskog, m. r. 9. Finl. (Mustiala).

Fig. 5. Original description of *Sarcodon scabrosus* var. *fennicus* (KARSTEN 1882: 104).

Discussion

Sarcodon fennicus versus *Sarcodon amygdaliolens*:

The remarkable smell of bitter almonds of the recent collections was very noticeable in the field. RUBIO-CASAS & al. (2011) describe *S. amygdaliolens* as a new species from Spain with such an intensive smell. It grows with *Pinus pinaster* AIT. and *P. sylvestris* on acidic soils, and is so far only known from the Mediterranean region. The great similarities with *S. fennicus* are striking and discussed in detail by RUBIO-CASAS & al. (2011). The authors mention that the smell of bitter almonds was a good field character of their new species, while *S. fennicus* is described as having a mealy smell (cf. RUBIO-CASAS & al. 2011: 54, Tab. 2).

However, some sources also attribute a smell of bitter almonds to *S. fennicus*, e.g. NATURARKIVET (2018) where it is named “Marsipanstorpigg” (“marzipan tooth fungus”). The smell of marzipan or bitter almonds is also mentioned as a character of *S. fennicus* by LABBÉ (2018) and NITARE & HÖGBERG (2012). RUBIO-CASAS & al. (2011) hypothesize that the attribution of an almond smell to *S. fennicus* resulted from an erroneous reference in MAAS GEESTERANUS & NANNFELD (1969) to a note by LUNDELL (on the label of a specimen of *S. fennicus* identified by him: Bohuslän, Uddevalla, 25. IX. 1944, leg. ISRAELSON, det. LUNDELL; UPS – see MAAS GEESTERANUS 1956: 22). RUBIO-CASAS & al. (2011: 52) consequently consider references of such a smell in the literature as doubtful: “En la bibliografía existente se ha hecho referencia a este peculiar olor, así, MAAS GEESTERANUS & NANNFELDT [sic!] (1969) ya comentan que LUNDELL describe el olor de *S. fennicus* como una mezcla entre almendras amargas y tierra mojada. Probablemente esta característica se haya podido atribuir erróneamente por más autores a este taxón, cuando en la descripción original del mismo no aparece mencionado, siendo otra de las razones por las que *S. amygdaliolens* ha pasado desapercibida hasta ahora”.

The basis for their hypothesis is the lacking information regarding the smell both in the original description of *S. scabrosus* var. *fennicus* (KARSTEN 1882: 104, see Fig. 5) as well as in the detailed description by KARSTEN (1887: 10, see Fig. 6), which elevates this taxon to species level. MAAS GEESTERANUS (1975) neither mentions a smell in his treatment of the European hydnums.

RUBIO-CASAS & al. (2011) also demonstrate genetically – based on the ITS – that *S. amygdaliolens* is a distinct species from *S. fennicus*. The sequences used were not generated from the type specimen but are apparently accepted as authentic by the authors. The recent collections from Austria therefore help to test and to refute the hypothesis that a smell of bitter almonds only occurs in *S. amygdaliolens* s. str., since these collections clearly belong to *S. fennicus* based on the currently available ITS sequences. Consequently, the smell cannot be used to differentiate between these two species. It still remains unclear, however, if a smell of bitter almonds is always present in *S. fennicus*; close attention should be paid to this feature in future collections.

Differences to other *Sarcodon* species:

Some *Sarcodon* species are macroscopically and anatomically similar to *S. fennicus* and *S. amygdaliolens*, apart from the striking smell by which this species pair can be readily recognized in the field.

The combination of grey-green context in the stipe base and absence of clamp connections leads directly to *Sarcodon* sect. *Scabrosi* MAAS GEESTERANUS within the European taxa (cf. MAAS GEESTERANUS 1975). *Sarcodon scabrosus* (FR.) P. KARST. is anatomically similar and also has an extremely bitter taste. MAAS GEESTERANUS (1975) distinguishes *S. fennicus* and *S. scabrosus* based on the pileus colour (ochraceous to yellow-brown in the former, red-brown to purple-brown in the latter) and spore ornamentation (warts blunter, coarser and less clearly rounded in *S. scabrosus*, warts somewhat smaller and well-rounded in *S. fennicus* – see MAAS GEESTERANUS 1975: 67, figs. 42, 47, 48). The specimens from Carinthia show some dark red-brown tinges – especially in the pileus centre – in addition to generally more ochraceous-brown shades, therefore the differentiation solely based on pileus colour seems difficult and the spore ornamentation should also be studied for reliable identification.

The taste of *Sarcodon glaucopus* MAAS G. & NANNF. is far less bitter, the decurrent spines are whitish only when young, purple-brown when older, and the spores are somewhat smaller (up to $6 \times 4.5 \mu\text{m}$; see MAAS GEESTERANUS 1975). The taste of *S. glaucopus* collections from Bavaria was tested by one of the authors and the differences were confirmed – it tastes more mealy and only slightly bitter in contrast to the extremely bitter taste of *S. fennicus*. HROUDA (1999, 2018) also mentions differences between these species in the reaction of the context to KOH: blue-green in *S. glaucopus* (and also *S. scabrosus*) and no reaction in *S. fennicus*. This was not confirmed for the Carinthian collections. However, the reaction to KOH was yellow at first and finally orange (see above), not blue-green, thus this reaction could still be useful for differentiation.

Sarcodon lepidus MAAS G. grows in lowland deciduous forests. The spore ornamentation is more pronounced and thus more similar to that of *S. scabrosus* (see MAAS GEESTERANUS 1975). In addition, the pileus is almost smooth and the taste is comparatively mild (see HROUDA 2018).

SARCODON FENNICUS Karst.

Syn. *Sarcodon scabrosus* (Fr.) Quél. var. *Fennicus* Karst. Haltsv. II, p. 104.

Pileus carnosus, fragilis, inaequalis, primitus flocculoso-squamulosus, demum diffractus, rufescente testaceus, dein obscurior, margine undulato lobatoque, 5–10 cm. latus, carne alba. Stipes sat validus, inaequalis, deorsum attenuatus, flexuosus vel curvatus, glaber, pileo concolor, basi acutata leviter albotomentellus, extus intusque subcoerulescente vel nigrescente cinereus, 2–8 cm. longus. 1–3 cm. crassus. Aculei decurrentes, aequales, subulati, ex albido fuscii, circa 4 mm. longi. Sporae ellipsoideo-sphaeroideae vel subsphaeroideae, asperae, fusciscentes, longit 4–6 mmm., crassit. 3–5 mmm.

— Locis sabulosis in silva mixta haud procul a Mustiala Fenniae.

Sapore valde acerbo a *S. scabroso* (Fr.) affini mox dignoscitur, qua nota ad *S. acrem*. Quél. in Bull. de la Soc. bot. de Franc. Tom. XXIV, séance. du 22 oct. 1877. p. 324–XXX II, Pl. VI, f. I, accedit, a qua vero colore distat.

Fig. 6. Description of *Sarcodon fennicus* (KARSTEN 1887: 10), which provides the new combination of *S. scabrosus* var. *fennicus* to species level due to the mention of the synonym and the same epithet. The text contains further information supplementing the protologue.

Sarcodon regalis MAAS G. can easily be distinguished by the violet context in the stipe base and the fruity smell (see MAAS GEESTERANUS 1975).

Discrepancies with the original description:

KARSTEN (1882) – see Fig. 5 – does not mention a smell of bitter almonds in the original description of *S. scabrosus* var. *fennicus*, even though, if present, it is a clear distinguishing feature to *S. scabrosus*. The later and more comprehensive description by KARSTEN (1887) (see Fig. 6) also lacks information regarding the smell. It can only be speculated whether KARSTEN (1882, 1887) simply did not test/note the smell or whether the original material actually lacked it, since he does not mention any smell at all.

The spore measurements of 4–5 µm and 4–6 × 3–5 µm given by KARSTEN (1882, 1887) deviate significantly from the present concept of *S. fennicus*. The revision of the neotype of *S. fennicus* (Finland: Valkiari prope Mustiala. IX.1886. KARSTEN no. 2389. – MAAS GEESTERANUS & NANNFELDT 1969) by BAIRD (1986), however, revealed spore measurements of 6.7–7.2(–8.0) × 5.0–6.0(–6.5) µm, which are in better accordance with modern descriptions of *S. fennicus*.

The concordance of the current interpretation of the name *S. fennicus* with the original description by KARSTEN is unlikely to be determined with certainty unless the neotype of *S. fennicus* is successfully sequenced. Until then, the current interpretation of *S. fennicus* (e.g. HROUDA 1999, 2018; MAAS GEESTERANUS 1975; NITARE & HÖGBERG 2012; RUBIO-CASAS & al. 2011) will be retained and supplemented by the smell of bitter almonds and the reaction of the context to KOH.

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