

Finnish records on the genus *Inocybe*. The new species *Inocybe saliceticola*

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Inocybe saliceticola, a new species of section *Marginatae*, is described from Finland. It is characterized by ochraceous pileus, bulbous stipe base and weakly nodulose spores. It grows with *Salix* in moist habitats. The holotypes of *I. alnea*, *I. dunensis*, *I. obtusiuscula*, *I. ochracea* and *I. straminipes* are treated. *I. straminipes* is reduced to a synonym of *I. salicis*. The synonymy of *I. ochracea* with *I. alnea* is supported by DNA analysis. The microscopical drawings of the type of *I. obtusiuscula* are given for the first time.

Key words: Agaricales, Basidiomycetes, Finland, *Inocybe*, *Salix*, taxonomy

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Introduction

The genus *Inocybe* is one of the largest genera of Agaricales in the Nordic countries. In the book *Funga Nordica* there are 154 species of *Inocybe* in the key (Jacobsson 2008). Several species of *Inocybe* are known to grow associated with *Salix*. From moist *Salix* thickets in Finland, the authors have collected a nodulose-spored species of *Inocybe*, which does not fit to any known taxon of *Inocybe*. We describe it here as a new species, *Inocybe saliceticola*. For comparison, we have studied the type specimens of *I. alnea* Stangl, *I. dunensis* P.D. Orton, *I. obtusiuscula* Kühner, *I. ochracea* Stangl, *I. salicis* Kühner and *I. straminipes* Romagn. In addition to morphology, ITS (Internal Transcribed Spacer) regions within ribosomal DNA were studied from some specimens. The variation in ITS is commonly regarded useful to separate agaric species. The differences between *I. saliceticola* and macroscopically fairly similar species are pointed out to help identifications.

Material and methods

The description of *Inocybe saliceticola* is based on ca. 20 specimens from Finland, most of which collected by the authors. Untypical specimens by spores (marked with * in chapter *Specimens examined*), and one with nearly glabrous stipe (**), were excluded from the description. The acronyms of the herbaria follow those used by Holmgren et al. (1990).

Macroscopical characteristics were noted and cross sections drawn from fresh fruit bodies. Given colour codes refer to Cailleux (1981) or Küppers (1999). Vegetation, especially trees and bushes, were observed at the growth sites. At four sites in the province Pohjois-Karjala (communes of Valtimo and Nurmes), samples were taken from soil surface in order to analyse pH and quantities of nutrients. The samples were analysed by Viljavuuspalvelu Ltd.

Microscopical characteristics were measured and drawn from dried material mounted in 10% NH₄OH solution at 1000 × magnification. Twenty mature spores were measured from each of 10 collections and from each of two fruit bodies of *I. saliceticola* holotype (totalled 240 spores). The number of 89 pleurocystidia were measured from 10 collections of *I. saliceticola*. Twenty spores were measured from each other *Inocybe* type studied. The spores were measured including the nodules, the cystidia lengths excluding the crystals, and the basidia lengths ex-

cluding the sterigmata. Given length \times width of spores and pleurocystidia is a 95 % confidence interval with underlined mean in center and range in parentheses. The Q value means the ratio of spore length to spore width (calculated for each spore).

The ITS sequence including 5.8S of rDNA was analysed from the holotype of *I. saliceticola*, another collection from the type site (Kokkonen & Vauras 23082F) and from four untypical collections to see, whether the untypical ones were within the species limits. Mika Toivonen (University of Helsinki) analysed two of these collections. Furthermore, ITS was analysed from the types of *I. obtusiuscula*, *I. alnea*, *I. ochracea* and *I. hirculus* for comparison. We also wanted to assure ourselves that *I. ochracea* is conspecific with *I. alnea*. The DNA was extracted from 0.2–2.3 mg of dried lamella by NucleoSpin Plant or NucleoSpin Tissue XS kit (Macherey-Nagel). The PCR primers were ITS1-F, ITS4-B, ITS2 or ITS3 (White et al. 1990, Gardes & Bruns 1993). The PCR was run by GeneAmp PCR system 9700 (PE Applied Biosystems) with PuRe Taq Ready-To-Go PCR beads (Amersham Biosciences). There were 30–36 cycles consisting of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. The PCR products were purified with GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences) and sent to Macrogen Inc. for sequencing. The primers ITS1, ITS4, ITS2 or ITS3 were used for sequencing. The sequences were edited by Seqman (DNASTar), aligned by ClustalW (www.ebi.ac.uk/clustalw/) and compared visually. Ambiguous bases were excluded from the comparisons.

***Inocybe saliceticola* Vauras & Kokkonen, spec. nova** – Figs. 1–5

Mycobank no.: MB 513002

Pileo 0.7–4 cm lato, potissimum flavo-brunneo. *Lamellis* usque ad 7.5 mm latis, mediocriter ventricosis. *Stipite* 0.7–6.2 cm longo, 1.5–6.5 mm crasso, pallido, omnino albo-pruinoso, basi frequenter marginato-bulboso. *Sporis* (7.2–)7.9–10.9(–11.6) \times (5.1–)5.7–7.1(–7.9) μ m, angulato-nodulosis. *Pleurocystidiis* 41–89 \times 12–23 μ m, nonnihil ventricosis. *Caulocystidia* per totam longitudinem stipitis praesentia. *Cum Salicibus*.

Holotype: **Finland**, Pohjois-Karjala, Nurmes, Salmi, E shore of lake Pahakala, moist shore forest, near *Salix phylicifolia*, *S. myrsinifolia*, *S. pentandra*, *S. cinerea*, *Betula*, *Alnus incana*, *Equisetum arvense*, *Lysimachia thyrsiflora*, *Filipendula ulmaria*, *Ranunculus repens*, *Calliargon cordifolium* and *Inocybe acuta*, Grid 27°E: 70392:6018, alt. ca. 103 m, 31.VII.2003 K. Kokkonen & J. Vauras 19738F, GenBank no.

FJ755796 (TUR-A; isotype in MICH).

Etymology: *saliceticola* (Latin), refers to the habitat of the species amongst bushes of *Salix*.

Pileus 7–40 mm in diameter, 4–11 mm high, conical to paraboloid when young, then conico-convex to applanate, prominently umbonate but sometimes only indistinctly umbonate, centre smooth, outwards radially fibrillose, rarely breaking up, no velipellis observed; yellow-brown (S30Y70M40, 67N) to pale brown (S30Y50–60M30), sometimes rather strongly contrasting with the centre, which is red-brown (S30Y60M50, 49P), dark red-brown (S60Y60M50–60) or grey-brown (S40Y70M50), outmost margin often the palest.

Lamellae up to 5 mm, rarely to 7.5 mm broad, moderately crowded, somewhat ventricose, narrowly adnate, when young pale grey (S20Y30M10), pale grey-brown (S20Y40M10–20, 91K), later grey-brown (S30Y50M20, Y60M50C30), edge minutely fimbriate, concolorous or paler.

Stipe 0.7–6.2 cm long, 1.5–6.5 mm wide, equal or slightly widening towards the base, with large, often emarginate, up to 11 mm wide bulb, solid, whitish, pale yellow-brown (ca. S00Y40M10), pale brown, pale red-brown (S10Y50M20), rarely grey-brown (ca. S20Y50M30), base white; white-pruinose all over, longitudinally striate.

Cortina not observed.

Context in pileus whitish to pale brownish-grey to pale yellowish, in stipe concolorous or slightly paler compared with the stipe surface, longitudinally striate. *Smell* and *taste* weak, fungoid.

Spores (7.2–)7.9–9.2–10.9(–11.6) \times (5.1–)5.7–6.3–7.1(–7.9) μ m, total range of mean values 8.6–10.4 \times 5.9–6.5 μ m, Q = (1.15–)1.25–1.46–1.7(–1.8), total range of mean Q values = 1.31–1.59; subangular, with few, obtuse nodules, apex at times elongated. *Basidia* 25–40 \times 9–14 μ m, clavate, 4-spored.

Pleurocystidia (41–)50–67–84(–89) \times (12–)13–17–23 μ m, narrowly to broadly ventricose with long or short neck, rarely clavate, apex crystalliferous, at times bent, base tapered or with pedicel, wall up to 4.5 μ m thick, yellowish. *Cheilocystidia* similar to pleurocystidia, but generally shorter and more ventricose, scattered



Figs. 1–2. Fruit bodies of *Inocybe saliceticola* in situ in type locality. – 1: Holotype. – 2: Collection *Kokkonen & Vauras 23082F*.

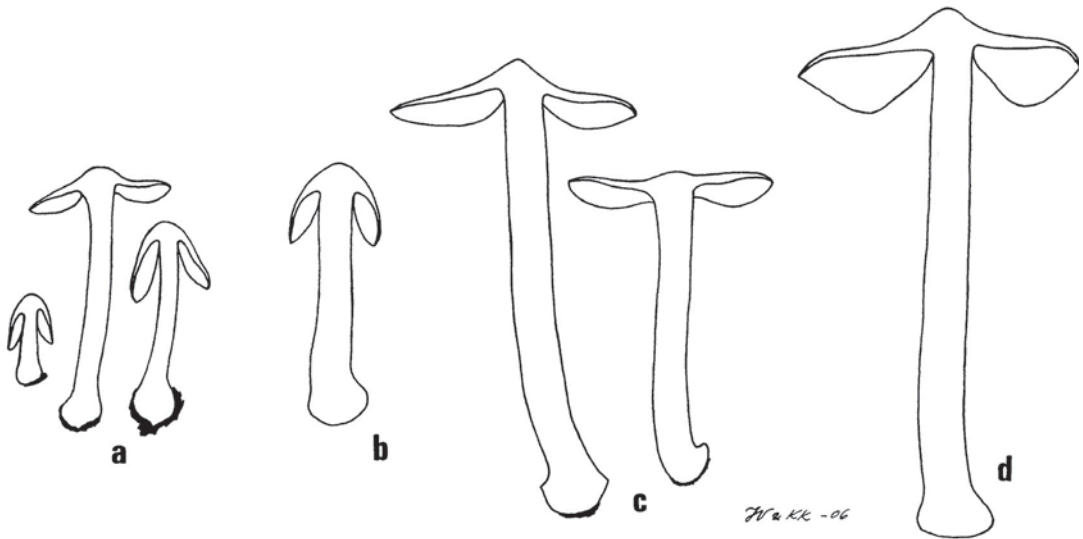


Fig. 3. Fruit bodies of *Inocybe saliceticola*, $\times 1$. – a) From *Vauras 12642F*, b) *Kokkonen 147/03*, c) part of holotype, d) *Kokkonen 281/03*, with untypical broad and ventricose lamellae

to rather frequent. *Paracystidia* clavate, thin-walled, fairly abundant.

Caulocystidia occurring down to base of stipe, similar to pleuro- and cheilocystidia, but shape more variable, up to 99 μm long. *Cauloparacystidia* clavate, wall thin to slightly thickened, rather abundant, often in clusters.

Ecology, distribution and phenology

Inocybe saliceticola is a mycorrhizal species of *Salix* in moist habitats. We have always collected it near willows, usually in alluvial shore thickets or shore forests, but also once in a moist, abandoned sand pit. At least *Salix phylicifolia* or *S. myrsinifolia* is a host, because no other arboreal plants were recorded at one site. Other usual companions on shores are *S. pentandra*, *S. cinerea*, *Alnus incana* and *Betula*.

Inocybe saliceticola grows on leaf litter or amongst mosses such as *Calliergon cordifolium*, *Sphagnum squarrosum* and *Mnium*. Typical vascular plants at the sites are *Lysimachia thyrsoiflora*, *Ranunculus repens*, *Galium palustre*, *Potentilla palustris* and *Calamagrostis canescens*. It shares the sites with e.g. *Inocybe acuta* Boud. (syn. *I. acutella* Bon) and *I. lacera* var. *helobia* Kuyper. Soil is at least moderately rich and varies from turf to mull to fine sand. Soil characteristics varied as follows: pH 4.9–5.3, Ca

668–1490 mg/l, K 146–226 mg/l, Mg 137–309 mg/l, P 3.5–7.7 mg/l.

The collections of *Inocybe saliceticola* date from late July to early October. At present, we know only localities from Finland and one locality from Sweden. In Finland, it has been collected from the eastern, southern and northern parts of the country, ranging from the hemiboreal to the northern boreal zone (Fig. 5). Most of the specimens were collected in the province of Pohjois-Karjala, where the species is not rare in suitable habitats.

Specimens examined: FINLAND. Varsinais-Suomi. Dragsfjärd, Holma, 27.IX.1995 *Vauras 10868*, Gen-Bank no. FJ755798 (TUR-A)*. Koski TI, Sulkalammi, 2.IX.1991 *Heinonen & Vauras 5995F* (TUR-A, GB). Kustavi, Puorenjärvi, 31.VII.1993 *Vauras 8015F* (TUR). **Uusimaa.** Espoo, Nuksio, 31.VIII.1994 *Kytövuori 94-115* (H). **Satakunta.** Luvia, Porsmusä, 10.IX.1997 *Vauras 12642F* (TUR-A). **Pohjois-Savo.** Kuopio, Kolmisoppi, 2.VIII.1986 *Vauras 2144* (TUR-A). **Pohjois-Karjala.** Ilomantsi, Särkkä, 30.VIII.1996 *Jakobsson 1979A* (TUR-A). Nurmes, Metsä-Vastimo, 20.VII.2002 *Kokkonen* (TUR), 9.VIII.2002 *Kokkonen* (TUR), 31.VIII.2002 *Kokkonen* (TUR), 31.VII.2003 *Kokkonen & Vauras 19739F* (TUR-A), 8.VIII.2003 *Kokkonen 106/03, 1058/03* (TUR), 30.VIII.2003 *Kokkonen 292/03, 294/03* (TUR); Pahakala, 26.VIII.2002 *Kokkonen* (TUR), 31.VII.2003 *Kokkonen & Vauras 19738F* (TUR-A, holotype, MICH, isotype), 4.VIII.2003 *Kokkonen 98/03* (TUR, MICH), 18.VIII.2003 *Kokkonen 147/03* (TUR), 27.VIII.2003 *Kokkonen 258/03, 266/03* (TUR), 14.VIII.2005 *Kokkonen & Vauras 23082F* (TUR-A, GB, H, L, OULU, UPS). Valtimo, Mahalanniemi, 18.IX.2001 *Kokkonen*,

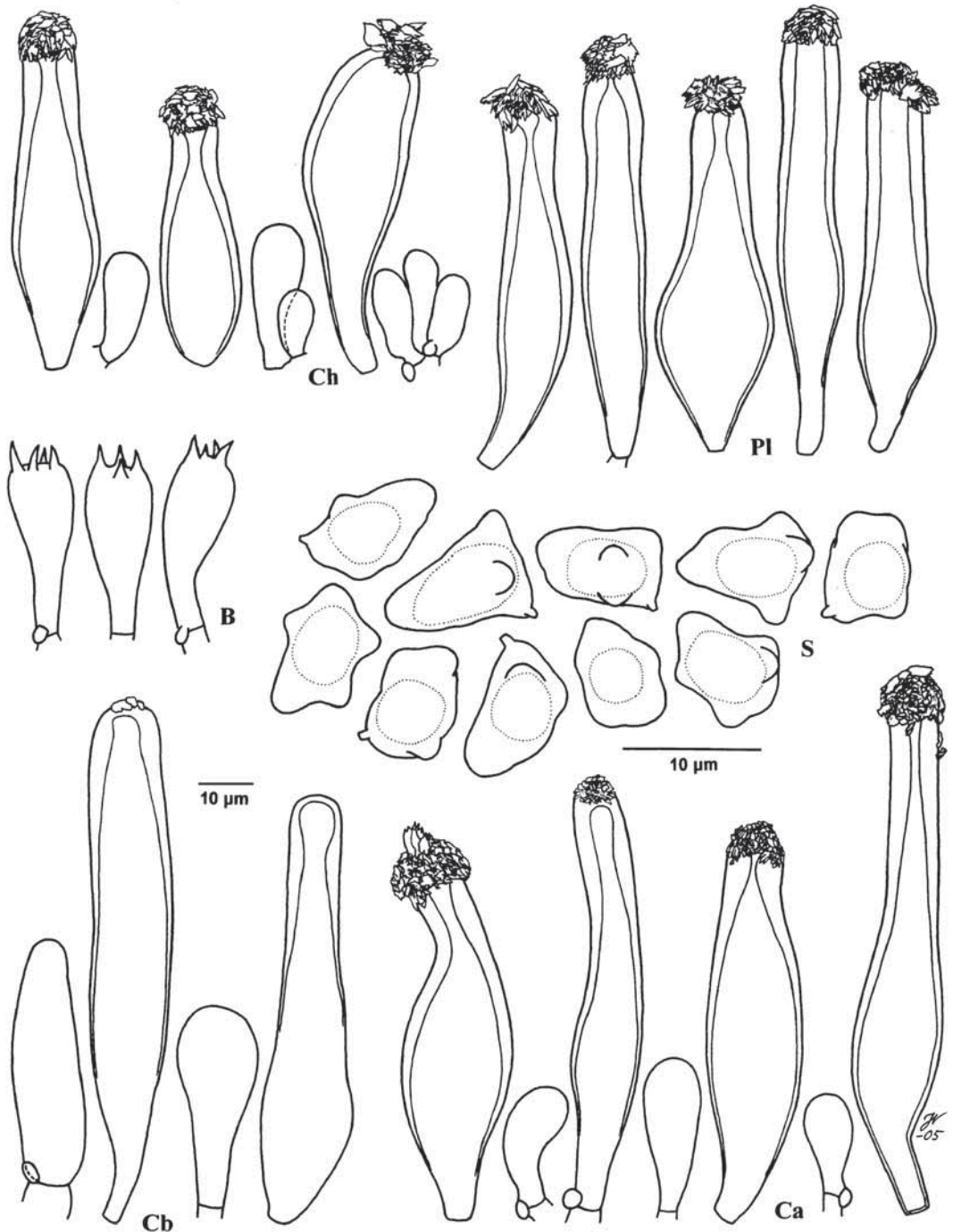


Fig. 4. Microscopical characters of *Inocybe saliceticola* (holotype). Symbols: B = basidia, Ca = caulocystidia and paracystidia at apex of stipe, Cb = caulocystidia and paracystidia at base of stipe, Ch = cheilocystidia and paracystidia, Pl = pleurocystidia, S = spores. Scales: spores $\times 2000$, others $\times 800$.



Fig. 5. Distribution of *Inocybe saliceticola* in NW Europe according to the specimens examined.

GenBank no. FJ755799 (TUR-A)**; 2.IX.2002 *Kokkonen* (TUR), 21.IX.2002 *Kokkonen* (TUR), 10.X.2002 *Kokkonen* (TUR-A)*, 1.VIII.2003 *Kokkonen & Vauras 19754* (TUR-A), 10.VIII.2003 *Kokkonen 124/03* (TUR), 29.VIII.2003 *Kokkonen 281/03, 1059/03* (TUR); Matkusjoki, 3.IX.2002 *Kokkonen* (TUR). **Kittilän Lappi.** Kittilä, Sirkka, 16.VIII.2006 *Kokkonen 169/06* (TUR-A). **Enontekiön Lappi.** Enontekiö, Kaaresuvanto, 20.VIII.2006 *Kokkonen 262/06* (TUR-A); Vähäniva, 8.VIII.1986 *Vauras 2193*, GenBank no. FJ755797 (TUR-A)*. **SWEDEN. Värmland.** Grava, Klarälven, 30.IX.1989 *Jansson* (GB), 23.VIII.1990 *Jansson* (GB).

The other type studies

Inocybe straminipes Romagn. – Fig. 6
Inocybe straminipes Romagn., Beih. Sydowia 8: 362. 1979. – Type: France, Coye-la-Forêt (Oise), 18 July 1952 *H. Romagnesi 52.46* (holotype PC).

The type consists of one small fruit body. Spores (10.3–)10.5–11.3–11.8(–11.9) × (7.4–)7.7–8.3–9.1(–9.3) μm, Q = 1.2–1.36–1.5. Pleurocystidia 53–60–66 × 17–22–26 μm (n=18), wall up to 4 μm thick, colourless. Basidia 4-spored, 26–36 × 10–15 μm (n=3). The stipe was not studied, because it was small and hard.

Inocybe dunensis P.D. Orton – Fig. 6
Inocybe dunensis P.D. Orton, Trans. Br. Mycol. Soc. 43: 277. 1960. – Type: England, Lancashire, Freshfield, 9 July 1956 *P.D. Orton 912* (holotype K).

The type consists of only two pieces of pileus. Spores 10.0–10.9–11.5(–12) × 6.5–7.0–7.5 μm, Q = 1.4–1.55–1.7. Pleurocystidia 52–57–62 × 20–25–27 μm (n=9), wall up to 4.5 μm thick, yellowish. Basidia often 2-spored, 27–29 × 11–14 μm (n=3).

Inocybe obtusiuscula Kühner – Fig. 7
Inocybe obtusiuscula Kühner, Doc. Mycol. 19 (no. 74): 23. 1988. – Type: France, Savoie, Région de Pralognan, Haute vallée de Champagny, Parc de la Vanoise, sous l'entrée du Cirque du Dard, avec *Salix herbacea*, alt. 2200 m, exp. N., 23 Aug 1960 *R. Kühner "K. 60-56"* (holotype G).

The type consists of ca. 4 fruit bodies. Spores (10.2–)10.7–12.4–14.3(–15.0) × 6.9–8.3–9.2(–10.5) μm, Q = (1.2–)1.25–1.49–1.7(–1.85), yellow-brown, thick-walled. Pleurocystidia 53–65–

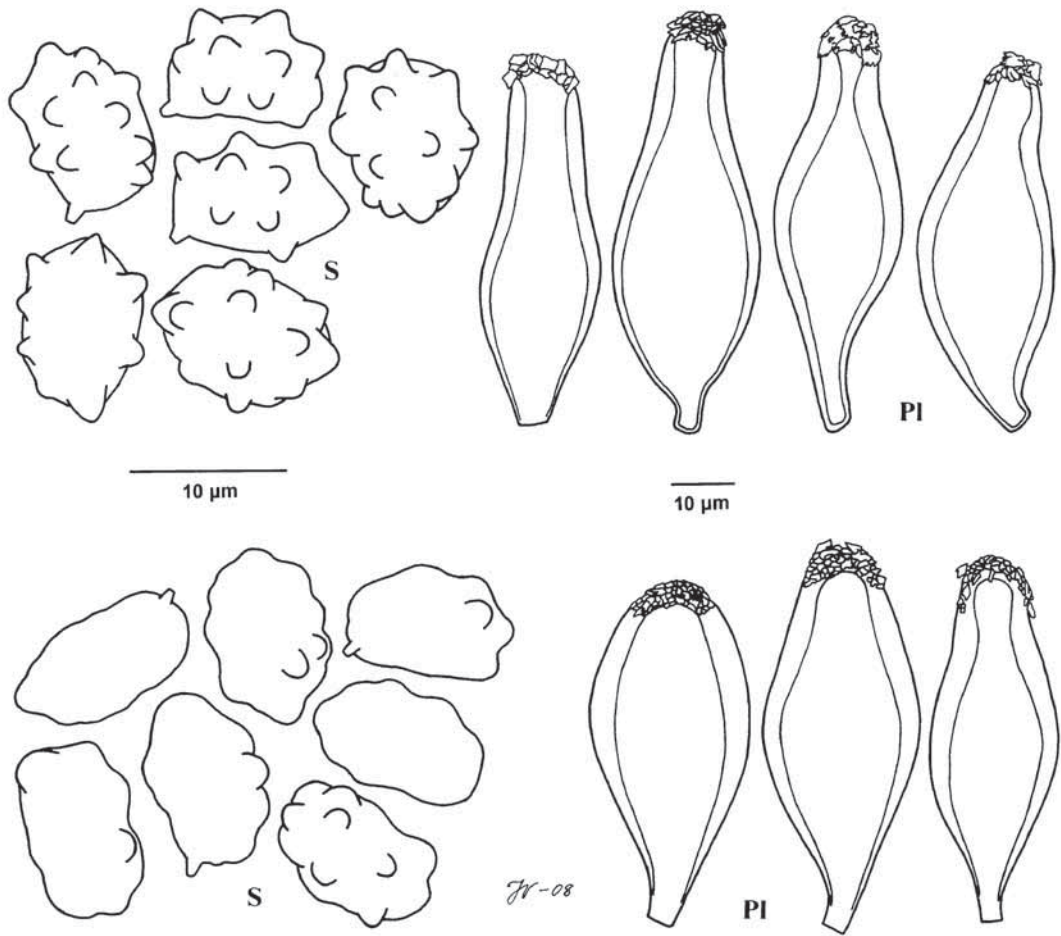


Fig. 6. Spores and pleurocystidia of holotype of *Inocybe straminipes* (above) and holotype of *I. dunensis* (below). For scales see Fig. 4.

$75 \times 14\text{--}18\text{--}26 \mu\text{m}$ ($n=10$), wall very slightly yellowish. Cheilocystidia $59\text{--}69 \times 18\text{--}27 \mu\text{m}$ ($n=3$), often yellow-brown inside. Caulocystidia from middle part of stipe up to $90 \mu\text{m}$ long, thick-walled, paracystidia often thick-walled. Basidia 4-spored, $31\text{--}36 \times 11\text{--}13 \mu\text{m}$ ($n=6$). GenBank no. FJ755800.

Inocybe alnea Stangl – Fig. 8
Inocybe alnea Stangl, Z. Mykol. 45: 151. 1979.
 – Type: Germany, Augsburg, “Gögginger Wäldchen”, 11 Nov 1968 *J. Stangl* (holotype M).

The type consists of ca. 4 fruit bodies. Spores $(8.8\text{--})9.3\text{--}10.1\text{--}11.0\text{--}11.1) \times 7.1\text{--}7.8\text{--}8.6\text{--}8.9) \mu\text{m}$, $Q = (1.15\text{--})1.2\text{--}1.30\text{--}1.35\text{--}1.45)$. Pleurocystidia $62\text{--}71\text{--}83 \times 15\text{--}17\text{--}20 \mu\text{m}$ ($n=9$), wall

up to $4 \mu\text{m}$ thick, very pale yellowish. Caulocystidia at stipe apex up to $73 \mu\text{m}$ long, thick-walled. Basidia $33\text{--}36 \times 10 \mu\text{m}$ ($n=6$). GenBank no. FJ755801 and FJ755802.

Inocybe ochracea – Fig. 8
Inocybe ochracea Stangl, Z. Mykol. 45: 150. 1979. – Type: Germany, Augsburg, “Gögginger Wäldchen”, 7 June 1968 *J. Stangl* (holotype M).

The type consists of one fruit body. Spores $(8.7\text{--})9.1\text{--}10.1\text{--}11.2\text{--}11.6) \times 6.8\text{--}7.4\text{--}8.0\text{--}8.3) \mu\text{m}$, $Q = (1.15\text{--})1.2\text{--}1.36\text{--}1.55\text{--}1.65)$. Pleurocystidia $52\text{--}69\text{--}75 \times 15\text{--}17\text{--}22 \mu\text{m}$ ($n=10$), wall up to $4 \mu\text{m}$ thick, yellowish. Caulocystidia at stipe apex up to $82 \mu\text{m}$, thick-walled. Basidia $27\text{--}37 \times 10\text{--}12 \mu\text{m}$ ($n=3$). GenBank no. FJ755803.

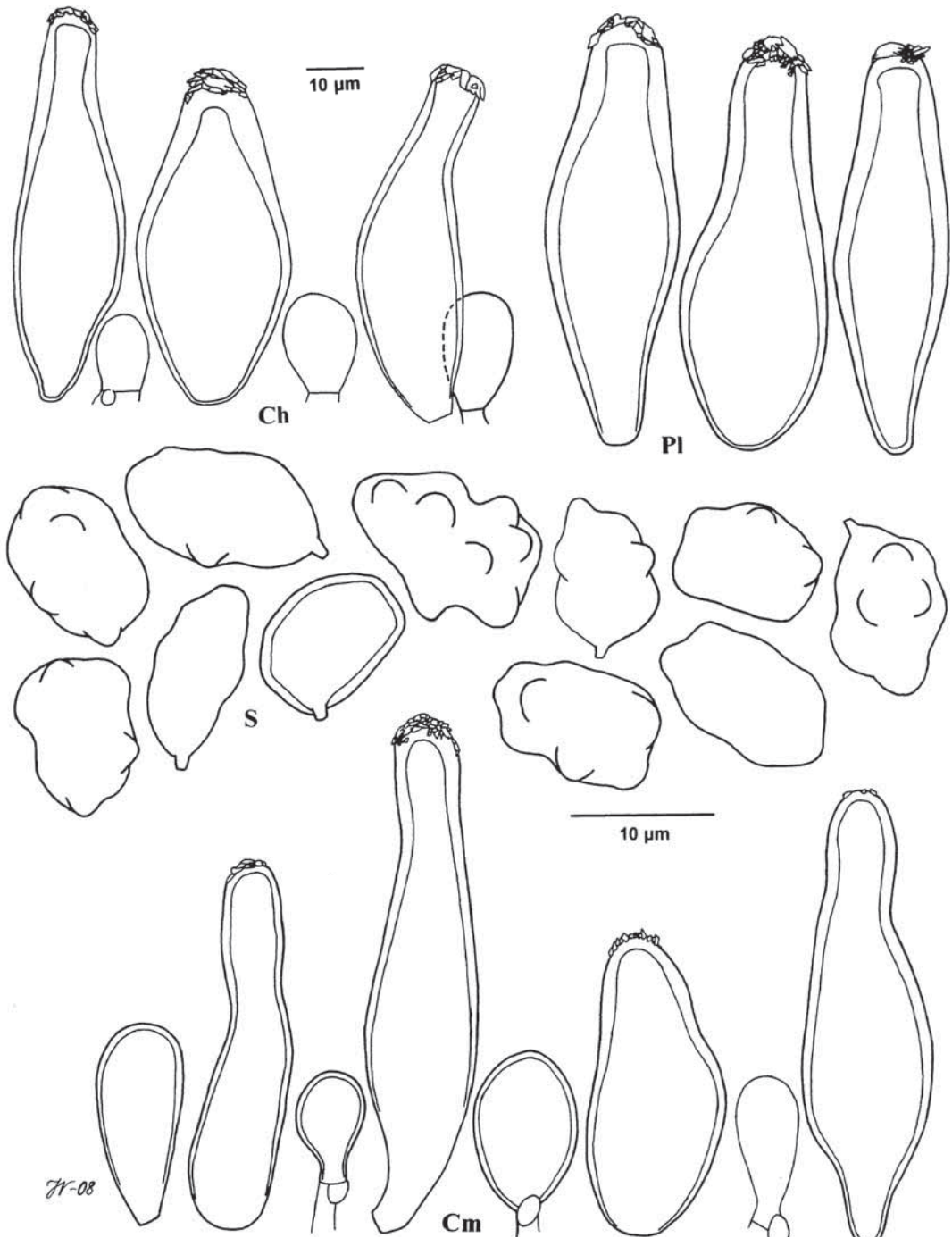


Fig. 7. Microscopical characters of *Inocybe obtusiuscula* (holotype). For symbols and scales see Fig. 4, but Cm = caulocystidia and paracystidia at middle part of stipe.

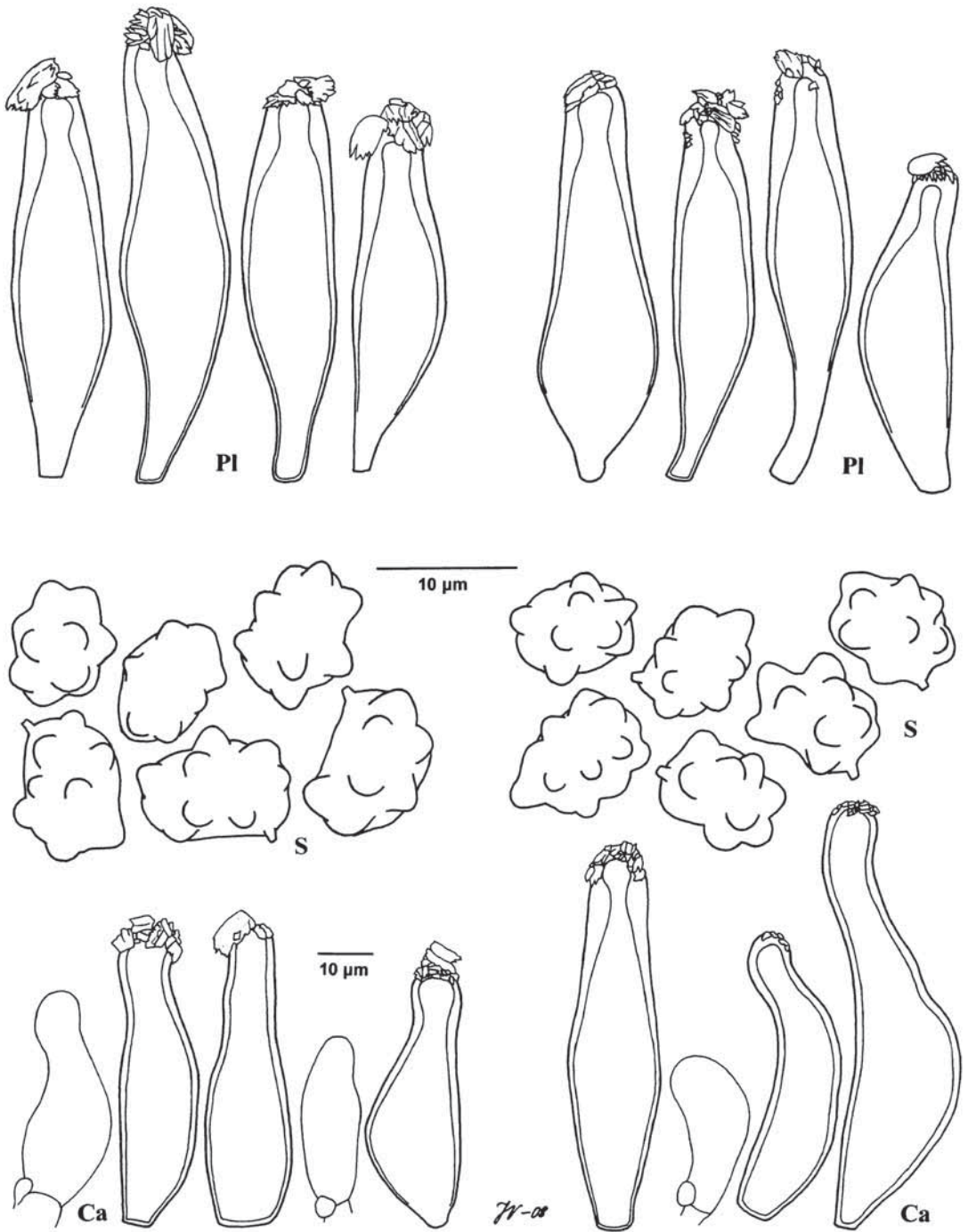


Fig. 8. Comparison of holotypes of *Inocybe alnea* (left) and *I. ochracea* (right). For symbols and scales see Fig. 4.

Differential diagnosis

Several *Inocybe* species have been reported to be associated with *Salix*. Of them *I. obtusiuscula* Kühner, *I. dunensis* P.D. Orton, *I. salicis* Kühner, *I. straminipes* Romagn., *I. salicis-herbaceae* Kühner, *I. substellata* Kühner, *I. praetervisa* Qué. and *I. mixtilis* (Britzelm.) Sacc., belonging to the section *Marginatae* and having ochraceous, fibrillose pileus, resemble *I. saliceticola* macroscopically to some extent. However, they differ by microscopical features. The last six of the above-mentioned species have strongly protruding nodules on spores. Further, *I. mixtilis* grows mainly with *Picea abies*, *Pinus sylvestris* and *Betula*, and *I. praetervisa* with at least *Betula* and *Quercus robur* in the boreal zone. They also prefer drier habitats. Their only records exclusively with *Salix* come from the alpine zone (Horak 1987, Bizio 1995, Bizio 1997, Bizio & Rossi 2002, Ferrari 2006). *I. salicis-herbaceae* and *I. substellata* are alpine species (Kühner 1988). Concerning the rest of mentioned species, we have studied the type specimens.

The holotype of *I. straminipes* proved to be identical with the holotype of *I. salicis* microscopically (Fig. 6). Kühner (1955) has illustrated *I. salicis* well. Conversely, the spores of *I. straminipes* drawn by Romagnesi (1979) in his description deviated from our view. On the ground of our observations, we thus reduce *I. straminipes* to a synonym of *I. salicis*. Having been described earlier, *I. salicis* has a priority. *I. salicis* seems to differ also ecologically from *I. saliceticola*. It has been found only once in Finland: from paludified river shore in Kiiminki in Oulun Pohjanmaa (specimen in OULU). The species is rare in the Nordic and Baltic countries, and has been collected mainly on seashore dunes.

The low-nodulated spores of the types of *I. dunensis* (Fig. 6) and *I. obtusiuscula* (Fig. 7) are distinctly larger and of somewhat different shape compared with *I. saliceticola*. In addition, cystidia of *I. dunensis* are shorter, and spores of *I. obtusiuscula* have thicker walls being thus darker. The DNA analysis confirmed that *I. obtusiuscula* is a clearly separate species from *I. saliceticola*: there were 46 base pair differences and 6 gaps (length 1–9 bases) within ITS1 sequence. The analysis of ITS2 of *I. obtusiuscula* failed. It is an alpine species (Kühner 1988). *I. dunensis* grows on fine sand, mainly at seashores, and has

not been found in Finland.

Also *Inocybe alnea* Stangl and *I. ochracea* Stangl resemble *I. saliceticola* macroscopically to some extent, but they are possibly not associated with *Salix*. According to our type studies, they have more protruding nodules on spores (Fig. 8) compared with *I. saliceticola*. Further, the ITS sequence of *I. alnea* and *I. ochracea* likewise differed by 60 base pairs and 19 gaps (length 1–11 bases) from *I. saliceticola* indicating separate species (when part of the conservative 5.8S of *I. alnea* failed). The microscopical features of *I. alnea* and *I. ochracea* proved to be nearly identical, having only slightly different spore sizes, and the DNA analysis supported their conspecificity: the ITS sequences were identical despite one base pair difference and a gap of two bases. Already Zitzmann (2002) regarded them conspecific, and chose the name *I. alnea* to remain. Stangl (1979) described these species in the same article. According to Stangl (1989), the abundant velipellis of *I. ochracea* and rimose pileus of *I. alnea* were the most distinct differences between these species.

Inocybe hirculus Vauras grows like *I. saliceticola* in moist habitats, preferring eutrophic fens (Vauras 1994). In one case both species were collected close to each other. Compared with *I. saliceticola*, *I. hirculus* differs e.g. by the woolly-fibrillose pileus, the stipe base lacking a bulb, and larger spores. The holotype of *I. hirculus* (GenBank no. FJ531872) differed by 50 base pairs and 18 gaps (length 1–17 bases) from the holotype of *I. saliceticola*, when ten bases were missing from the beginning of *I. hirculus* sequence.

Inocybe rivularis Jacobsson & Vauras grows in moist habitats, too. It differs e.g. by larger size, having the stipe pruinose at apex only, and dissimilar microscopical characters (Jacobsson & Vauras 1990).

Lastly, the prominence of nodules and the shape of spores varies rather much between *I. saliceticola* collections. Sometimes aberrant spores may have resulted from hard weather conditions. Three collections with atypical spores had an identical ITS area with the holotype despite one gap (1 base) in one collection. One atypical specimen with normal spores has a nearly glabrous stipe. It differed from the holotype by one base pair and one gap (1 base) in ITS.

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