Two new species of *Inocybe*, section *Marginatae* (Agaricales, Cortinariaceae) from Japan

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Two new species of the section *Marginatae* are described, namely, *Inocybe populea* sp. nov. and *I. leptoclada* sp. nov., from western Japan and eastern Japan, respectively. *Inocybe leptoclada* is assigned to section *Marginatae*, even though it lacks a marginate bulb. *Inocybe populea* is placed in the section *Marginatae* because of the presence of a marginate (often indistinct) bulbous base of stipe and the caulocystidia throughout. The discoveries of *I. leptoclada* and *I. populea* provide further evidence that section *Marginatae* and section *Inocybe* [= Cortinatae] are poorly defined.

Key Words——Agaricales; Cortinariaceae; Inocybe leptoclada; Inocybe populea; Japan.

During taxonomic studies on the genus *Inocybe*, the authors encountered several apparently hitherto unknown taxa. Some of them have been reported by Kobayashi and Courtecuisse (1993) and Kobayashi (1993, 1995). In this paper two new species of *Inocybe* belonging in section *Marginatae* Kühner are described based on material collected from Japan.

Materials and Methods

The specimens cited in this paper are deposited in the herbarium of the Musée et Jardins Botaniques Cantonaux Lausanne (LAU), the Natural History Museum and Institute, Chiba (CBM-FB), the senior author's private herbarium (TAKK), Chiba, Japan, and the junior author's private herbarium in Lille, Faculty of Pharmacy Herbarium (LIP), France. The color notations used are those from Munsel color company (1988) or Kornerup and Wanscher (1981). For microscopic observations, dried specimens were rehydrated in 10% NH₄OH or 5% KOH and examined. Length measurements excluded the apiculus and sterigmata for spores and basidia respectively. The abbreviation Q is the ratio of spore length to spore width; N is the number of measured spores; IS is an index of slenderness, defined in Kobayashi and Courtecuisse (1993).

Thin-walled, clavate to pyriform elements are called paracystidia in this paper. Paracystidia are often catenate, and they do not originate from trama of lamellae. This term is taken from Huijsman (1955).

Taxonomy

Inocybe populea Takahito Kobay. & Courtec., sp. nov.

Figs. 1-3

Pileo 14–25 mm lato, subumbonato, luteo-brunneo, brunneo, fibrilloso, rimoso; lamellis adnatis, adnexis, luteo-brunneolis, aurario-brunneolis, brunneolis; stipite $32-40 \times 2.0-3.5$ mm, marginato-bulboso, solido, luteo, luteo-brunneo; carne luteo-alba; sporis $8.0-13.5 \times 6.5-11.0 \ \mu$ m, prominenter nodulosis, luteo-brunneis; basidiis $15-24(-26) \times 8-10(-12) \ \mu$ m, tetra-sporicis; pleurocystidiis $34-60(-71) \times 12-15(-19) \ \mu$ m, ventricosis, pachydermicis; cheilocystidiis $30-50 \times 11-20 \ \mu$ m, pachydermicis; caulocystidiis pachydermicis, apice praesentibus, base praesentibus. Holotypus: TAKK 1752-1 in LAU.

Pileus 14-25 mm broad, when young conical to hemispherial with involute margin, then subumbonate with straight margin, yellowish brown (10YR 5/4 to 5/6), brown (6E7 to 6E8) on umbo, surface fibrillose, rimose, almost smooth, with small recurved squamules on a narrow zone between umbo and margin, glabrous on umbo, shiny, velipellis absent. Lamellae subventricose, adnate to adnexed, subdistant to close, 0.5-1.5 mm apart at middle, sometimes with 1 lamellula, up to 0.5 mm apart at margin, when young yellow (10YR 7/6) to gravish yellow (4B6), then yellowish brown (10YR 5/ 6) to golden brown (5D7), brown (6D7 to 6E8) when aged, edge fimbriate to serrate, white to pallid. Stipe $32-40 \times 2.0-3.5$ mm, up to 4.0 mm broad at base, cylindrical, base marginately bulbous, often indistinctly marginate, solid, very pale brown (10YR 8/4) to brownish yellow (10YR 6/6) at apex, yellow (10YR 7/6) to yellowish brown (10YR 5/6 to 6/6) from middle to base, surface pruinose, shiny, striped, sometimes with brown fibrils. Cortina absent. Context fragile, in pileus yellowish white (4A1 to 4A2), 0.2-0.5 mm thick, at umbo up to 1 mm thick; in stipe white (4A1) to yellowish white (4A2)



Fig. 1. Inocybe populea.

A, Pleurocystidia [TAKK 1565]; B, Basidia [TAKK 1565]; C, Spores [TAKK 1565]; D, Cheilocystidia and paracystidia [TAKK 1565]; E, Basidioma [TAKK 97.10.10.03]; F, Caulocystidia at apex of stipe [TAKK 1565]; G, Caulocystidia at base of stipe [TAKK 1565]. Scale bars: A, B, D, F, G=20 μ m; C=10 μ m; E=25 mm.

at apex, yellowish white (4A2) to pale yellow (4A3) at middle to base. Odor none or indistinct. Taste indistinct. IS=19.1-42.1, on average 28.4. Spore print brown.

Chemical reactions on pileus: $FeCI_3 \cdot 6H_2O$ (20%) immediately olive-green, becoming olive black within 20 min, KOH (5%) negative, NH₄OH (10%) negative, aniline negative, phenol (2%) first negative, becoming pinkish within 20 min; on lamellae: FeCl₃·6H₂O (20%) first pale olive, becoming olive-green within 20 min, KOH (5%) negative, NH₄OH (10%) negative, aniline negative, phenol (2%) first negative, becoming pinkish within 20 min; on stipe: FeCl₃·6H₂O (20%) first pale olive, becoming olive green within 20 min, KOH (5%) negative, NH₄OH (10%) negative, aniline negative, phenol (2%) first negative, becoming pinkish within 20 min.



Fig. 2. Basidiomata of *Inocybe populea* [TAKK 1752: type]. Scale bar=20 mm.

Spores 8.0-13.5 \times 6.5-11.0 μ m, on average 9.3- $11.3 \times 7.5 - 9.4 \,\mu m$, N=90, Q=1.1-1.3, angular in all views, with prominent nodules, in one side 7-13 nodules visible. Basidia $15-24(-26) \times 8-10(-12) \mu m$, short clavate to clavate, 4-spored, almost hyaline to pale lemon. Pleurocystidia as metuloids, $34-60(-71) \times 12-15(-19)$ μ m, ventricose to narrowly utriform with a tapering base, walls up to 5.4 μ m thick, yellowish in KOH. Cheilocystidia similar to pleurocystidia. Paracystidia on edges of lamellae often catenate, total length up to 97 μ m long, often with yellow to yellowish brown intracellular pigment, terminal cells $19-39 \times 10-22 \mu m$, ellipsoid to ovoid, thin-walled. Hymenophoral trama subregular, made of hyphae 9.7–19.1 μ m in diam. Caulocystidia as metuloids, descending to base; at apex of stipe $28-48 \times$ 12–17 μ m, broadly ventricose, elongate, sometimes ovoid, lacking pedicel, walls up to 4.8 μ m thick, yellowish in KOH, intermixed with cauloparacystidia often catenate, total length up to 45 μ m, almost hyaline to slightly yellow, terminal cells $16-31 \times 11-24 \ \mu m$, ovoid to clavate, in clusters abundant. Pileipellis a cutis, duplex, with the uppermost layer up to $35 \,\mu m$ deep, subregular to regular, composed of agglutinated hyphae



Fig. 3. Inocybe populea [TAKK 1565-1]. Spores and pleurocystidium (SEM). Scale bar = 20 μm.



Fig. 4. Spores of *Inocybe salicis* [TAKK 1701]. Scale bar= 10 μm.

2.5-4.2 μ m in diam, walls yellowish in KOH, the subtending layer up to 28 μ m deep, of subregular hyphae 4.4-6.4 μ m in diam, walls brownish in KOH. Clamp connections present in all tissues, but not at all septa.

Collections examined: Japan, Shiga Pref.: Gorhocho, under *Salix* sp. and *Cedrus deodara* (Roxb.) Loud., [6 July 1992, coll. Takahito Kobayashi, TAKK 1752-1 in LAU (holotype), TAKK 1752-2, TAKK 1752-3, TAKK 1752-4 in LIP and TAKK 1752-5 in CBM-FB (isotypes); 6 July 1992, coll. T. K., TAKK 1758]; same locality, under *Populus nigra* Linn. [14 Oct. 1991, coll. T. K., TAKK 1565-1, TAKK 1565-2, TAKK 1565-3 and TAKK 1565-4 in LIP; 6 July 1992, coll. T. K., TAKK 1753; 10 Oct. 1997, coll. T. K., TAKK 97.10.10.03].

Inocybe populea belongs to subgenus Inocybe section Marginatae. This fungus is related to Inocybe salicis Kühner, which differs in its weakly nodulose spores (Fig. 4). The senior author studied two European specimens of I. salicis collected in Denmark (S Jylland, Soro island on W coast of Jylland, on dunes on W shore of Soro, S of Lakolk, near Salix repens, 21 Sept. 1988, Vauras 3319F duplicate in TAKK) and France (8 June 1986, RC 86.06.08.01 in LIP and TAKK 1701). They matched the description by Kühner (1956), having weakly nodulose spores. Inocybe Iasseri Dennis also resembles I. populea, but can be separated by its spiny spores.

Inocybe leptoclada Takahito Kobay. & Courtec., sp. nov. Figs. 5, 6

Pileo usque ad 22 mm lato, convexo, plano-convexo, albo, ochroleuco, fibrilloso, rimuloso; lamellis adnexis, pallido-brunneolis; stipite 19–30×1.2–2.0 mm, aequalibus, solido, albo, cremeo, pallido-vinaceo; carne alba; sporis 6.3–9.3×4.2–6.7 μ m, polyhedris, nodulosis, luteo-brunneis; basidiis 21–27×6.5–8 μ m, tetra-sporicis; pleurocystidiis 40–69×11–17 μ m, clavatis, obclavatis, pachydermicis; cheilocystidiis 37–57(–63)×12–14(–19) μ m, pachydermicis; caulocystidiis pachydermicis, apice



Fig. 5. Inocybe leptoclada.

A, Pleurocystidia [TAKK 683]; B, Basidia [TAKK 683]; C, Spores [TAKK 683]; D, Cheilocystidia and paracystidia [TAKK 683]; E, Basidiomata [TAKK 683 and TAKK 743]; F, Caulocystidia and cauloparacystidia at apex of stipe [TAKK 683]; G, Caulocystidia and cauloparacystidia at base of stipe [TAKK 683]. Scale bars: A, D, F, G=20 μ m; B, C=10 μ m; E=10 mm.

praesentibus, base praesentibus. Holotypus: TAKK 683-1 in CBM-FB.

Pileus up to 22 mm, when young conical with involute margin, then convex to plano-convex, white to ochroleucous, fibrillose, smooth, sometimes covered with pale brown fibrillose small scales, subrimose towards margin, silky when dry. Lamellae subventricose, adnexed, crowded to close, when young cream, then reddish orange to pale brown, edge farinaceous, whitish or concolorous. Stipe $19-30 \times 1.2-2.0$ mm, equal, base sometimes swollen, solid, white to cream, with light pink tint, surface pruinose, silky. Cortina absent. Context white, with light pink tint. Odor strong, spermatic. Taste indistinct.

Chemical reactions on pileus: FeCl₃. $6H_2O$ (20%) black within 1 h, KOH (30%) negative; on lamellae:



Fig. 6. Basidiomata of *Inocybe leptoclada* [TAKK 683: type]. Scale bar=20 mm.

 $FeCl_3\cdot 6H_2O$ (20%) negative, KOH (30%) negative; on stipe: $FeCl_3\cdot 6H_2O$ (20%) negative, KOH (30%) pale yellow within 15 min.

Spores 6.3–9.3×4.2–6.7 μ m, on average 7.6–8.1× 4.9-6.0 μ m, N=90, Q=1.2-1.5, polyhedral in outline, often with a pronounced apical nodule. Basidia 21-27 \times 6.5-8 μ m, narrowly clavate, clavate, cylindrical, 4spored, almost hyaline. Pleurocystidia as metuloids 40- $69 \times 11-17 \ \mu m$, obclavate to ovoid, with a rounded to truncate base and a cylindrical neck, the wall up to 3.4 μ m thick, slightly yellowish in KOH (5%), mixed with paracystidia which are thin-walled, terminal cells 9-14 imes7–10 μ m, subglobose to clavate. Cheilocystidia as metuloids $37-57(-63) \times 12-14(-19) \mu m$, obclavate to utriform, with a truncate to rounded base and a cylindrical neck, the wall up to 3.4 μ m thick, slightly yellowish in KOH, mixed with paracystidia which are napiform to obovoid, terminal cells $13-17 \times 7-13 \mu m$. Hymenophoral trama subregular, made of hyphae 4.2–5.6 μ m in diam. Caulocystidia present as metuloids, down to the base; at apex of stipe $29-55 \times 10-13 \,\mu$ m, narrowly utriform to cylindrical, with a truncate base and a cylindrical neck, the wall up to 3.6 μ m thick, slightly yellowish in KOH; at base of the stipe $32-58 \times 11-13 \,\mu$ m, cylindrical to obclavate. Cauloparacystidia frequent and often in clusters,



Fig. 7. Spores of *Inocybe senkawensis* [TNS 244749]. Scale bar = 10 μm.

at apex of stipe terminal cells $7-18 \times 7-8 \mu m$, obovoid to clavate, at base of stipe terminal cells $12-19 \times 8-10 \mu m$. Pileipellis a cutis, the uppermost layer up to $60 \mu m$ deep, pale yellow in NH₄OH, of subregular hyphae $2.6-5.2 \mu m$ in diam, the subtending layer up to $52.5 \mu m$ deep, yellowish brown in NH₄OH, of regular to subregular hyphae $4.6-8.0 \mu m$ in diam. Clamp connections present.

Collections examined: Japan, Kanagawa Pref.: Honatsugi, in *Quercus* and *Pinus* forest, [3 July 1988, TAKK 661; 24 July 1988, TAKK 683-1 in CBM-FB: holotype, TAKK 683-2 in TMI: isotype; 21 Aug. 1988, TAKK 743]; Jike, mixed forest, 18 June 1989, TAKK 828; Notsuda, mixed forest, 2 July 1989, CBM-FB 5236 (=TAKK 838); Chiba Pref., Hasunuma-mura, Hasunuma-kaihin-no-mori, on sand soil in *Pinus thunbergii* Parl. forest, 27 June 1999, coll. Takahito Kobayashi & K. Oosaku, TAKK 99.6.27.8.

Inocybe leptoclada belongs to section Marginatae sensu Singer (1986).

This fungus is related to *Inocybe senkawensis* Y. Kobayasi, from which it differs in the thinner wall of the metuloids and the pink surface of the stipe. The senior author's examination of the authentic material (Japan, Tokyo, Senkawa, 6 Aug. 1950, TNS-244749) revealed that its spores have rounded nodules, not polyhedral (Fig. 7). *Inocybe leptoclada* is close to *I. putilla* Bres., which is distinguished by its absence of caulocystidia at the base and its grayish brown pileus. *Inocybe leptoclada* is close to *I. trechispora* (Berk.) P. Karst., which differs in the strongly bulbous base of stipe and the absence of pink tint of stipe (Berkeley, 1860). The senior author's examination of the holotype (England, 6 Oct. 1840, coll. M. J. Berkeley, K) revealed that its pleurocystidia are narrowly ventricose with a long neck.

Discussion

Various interpretations have been made of the *Margina*tae and *Inocybe* as section (Moser, 1978; Singer, 1986; Kühner and Lamoure, 1989) or supersection (Kuyper, 1986; Stangl, 1989). Doubts have been raised about the reliability of some key defining characters of these groups, and the morphology of *I. leptoclada* and *I. populea* add weight to these doubts.

The section *Marginatae* was established by Kühner (1933) as characterized by 1) the absence of a cortina on the stipe, where the surface is entirely covered with caulocystidia, and 2) the bulbous-marginate stipe base. On the other hand, the section *Inocybe* [=*Cortinatae* Kühner & Boursier] is recognized by 1) presence of a cortina, 2) stipe not pruinose all over the surface but only at the apex or even fibrillose, woolly or scaly at the apex, 3) (generally) brownish color of the stipe, 4) often non bulbous-marginate stipe base, 5) context often inodorous or smell indistinct.

Singer (1986) emended the section Marginatae to include the intermediate species with the section Inocybe, emphasizing that the marginate bulbous base is not always present and the caulocystidia occur descending at least to the middle of the stipe length or down to the base. Kobayashi (1995) described a new species typically according with Singer's emendation of the section Marginatae, namely, Inocybe fastuosa Takahito Kobay. which lacks caulocystidia at the extreme base of the stipe and has no marginate bulb. Inocybe leptoclada has thick-walled caulocystidia (metuloids) down to the base of stipe but lacks a marginate bulb. The stipe base of *I. populea* is often indistinctly marginate. An intermediate Inocybe species between sections *Marginatae* and *Inocybe* [=*Cortinatae*] was described by Jacobsson and Vauras (1989), namely, Inocybe rivularis Jacobsson & Vauras: it lacks caulocystidia from the middle to the base, and the cortina is very slight and Therefore, the characters of the caulofugacious. cystidia distribution and the presence or absence of cortina seem not to be strictly reliable to define the sections of the subgenus *Inocybe* [=*Clypeus* Britz.].

Kuyper (1986) temporarily assigned these taxa to informal groups, viz., supersection *Marginatae* and supersection *Cortinatae*, respectively, and he also assigned some members of the subgenus *Inocibium* (Earle) Singer with metuloids to the supersections. The supersections *Marginatae* and *Inocybe* were accepted by Stangl (1989). However, the present authors do not agree with Kuyper's (1986) treatment. The caulocystidia distribution and the presence or absence of cortina have been overemphasized as taxonomic criteria. At the very best, could this pattern represent a practical but artificial tool to organize categories within this subgenus, devoid of true taxonomic, systematic or phylogenetic value.

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