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A common new species of *Inocybe* in the Pacific Northwest with a diagnostic PDAB reaction

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Abstract: A species of *Inocybe* common in Washington, Oregon and British Columbia is documented and described as new. The species, *I. chondroderma*, is characterized by these features: pileus with a fulvous disk and ochraceous to chamois margin, presence of a cortina, densely mycelioid stipe base, smooth spores and fall phenology. The most reliable and distinctive feature of the species is a blue-green or turquoise reaction in response to application of a solution of *p*-dimethylaminobenzaldehyde (PDAB), indicating the presence of what is most likely an indole alkaloid. PDAB use provides a quick and diagnostic character easily implemented in a laboratory setting. ITS sequences from recent collections of *I. chondroderma* and from materials collected in the 1940s in Washington and Oregon fully match numerous mislabeled sequences from specimens in British Columbia and Oregon. The species is most closely related to an unclarified taxon from Colorado and Japan (*I.* cf. *chondroderma*) and a rare European species, *I. submudipes*. Nine different species names in *Inocybe* and one in *Hebeloma* attributed to *I. chondroderma* based on GenBank BLASTN searches of the ITS locus match with 99–100% similarity, reinforcing concerns about taxonomic inaccuracies in public DNA sequence databases. A complete morphological description, illustrations and phylogenetic assessment are provided.

Key words: Agaricales, barcoding, herbarium, indole alkaloid, *p*-dimethylaminobenzaldehyde, phylogenetic taxonomy

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Introduction

During fungal surveys in western Oregon, Washington and British Columbia we regularly encountered a species of *Inocybe* typically between October and December. Identity of these collections initially was tenuous, and specimens appeared to resemble most closely descriptions of two European species *I. posterula* (Britzelm.) Sacc. and *I. auricoma* (Batsch) J.E. Lange (Kuyper 1986, Stangl 1989) and an eastern North American species, *I. submuricellata* G.F. Atk. (Kauffman 1924). As part of a routine application of *p*-dimethylaminobenzaldehyde (PDAB; also known as Ehrlich's reagent) to fresh basidiocarp tissue, we observed that the unidentified species consistently exhibited a blue-green or turquoise reaction in PDAB solution (Lennox 1979) (Fig. 1A) and that this was the only species of *Inocybe* to do so.

Unsatisfied with the tentative taxonomic resolution, we suspected the species in question matched the description of ''*I. chondroderma*'', a provisionally named species in an unpublished manuscript by Stuntz from 1965. In his doctoral thesis at Yale University, Stuntz (1940) provided a description of a species common in western Washington, to which he applied the name *I. agglutinata* Peck. Later at the University of Washington, he assembled 21 collections that he recognized as a distinct species and provisionally labeled ''*Inocybe chondroderma*''. Among his unpublished notes, Stuntz remarked that the species represented what he earlier had referred to as *I. agglutinata* and *I. submuricellata*. We were satisfied with the determination as ''*I. chondroderma*'' using Stuntz's unpublished work based on the following combination of morphological features: the presence of smooth basidiospores, caulocystidia absent from the lower part of the stipe, fulvous-colored pileus with a paler margin, and pallid to buff or pale yellow stipe. To test this hypothesis, we obtained numerous collections that Stuntz referred to ''*I. chondroderma*'' and sequenced the ITS region of five of these to compare with numerous materials studied by us in fresh condition and which exhibited the positive PDAB reaction. Here, we report the conspecificity of historical collections with our own and numerous mislabeled GenBank ITS sequences and produce a formal description for *I. chondroderma*.

Materials and methods

Herbarium materials.—Morphological features of fresh specimens were compared with guides to describe color

FIG. 1. The PDAB reaction and basidiomata of *Inocybe chondroderma*. A. Blue-turquoise pigment exuded into solution of PDAB (*g2021212h1-4*, PNWMS). Scale bar is 5 mm. B. *Inocybe chondroderma* basidiomata (*g2041013h2-18*, PNWMS). Scale bar = 1 cm. Photos by L.L. Norvell.

(Ridgway 1912, Munsell Soil Color Charts 1954). Sections of fresh or dried material were made in mounts of 10% NH_4OH or 3% KOH. Radial sections of the pileus were made to examine the pileipellis, and small sections of lamellae were squashed to examine basidia, pleurocystidia and cheilocystidia. The distribution of caulocystidia along the stipitipellis was examined by preparing sections from the apex of the stipe, center of the stipe and just above the stipe base. Spores and cystidia were observed and measured under an oil lens objective on a Nikon Eclipse 80i light microscope. Mean values of spore length, width and quotient (Q values) are italicized. The total number of spores measured and the number of collections sampled are reported as ''n = X/Y''. Line drawings were prepared with aid of a drawing tube. Collections are deposited at WTU, TENN and the Pacific Northwest Mycology Service (PNWMS at 6270 NW Skyline Boulevard, Portland, Oregon 97229-1309; www.pnw-ms.com). Herbarium abbreviations above are made with reference to *Index herbariorum* (Thiers continuously updated) except for PNWMS.

PDAB reaction.—*p*-dimethylaminobenzaldehyde (PDAB) was prepared in a 2% solution by dissolving 0.5 g of PDAB in 19.1 mL 95% ethanol, to which 5.9 mL hydrochloric acid was added (Largent 1977, Lennox 1979). The solution was stored in a 30 mL amber glass dropper bottle at room temperature and will maintain its efficacy indefinitely if not exposed to prolonged light (Largent 1977). To perform a macrochemical test, a small wedge of pileus or stipe tissue was excised from a fresh basidiocarp and placed in a clean porcelain spot plate. One milliliter PDAB solution was poured over the chip. A positive result was noted when a turquoise or blue-green pigment leached from the flesh chip into solution. The reaction typically occurs within several seconds. A negative reaction was judged if no pigment was emitted into solution or if lamellae slowly turned faint pinkish purple (Libonati-Barnes 1981). Dried basidiocarp tissue of *I. chondroderma* (*PBM1798*, *PBM2028*, *Stz4771*, *Stz6188*) of various ages (< 24 h, 12 y, 13 y, 62 y, 64 y) also was tested after rehydration in 250 mL sterile ddH_20.

DNA extraction, PCR and sequencing.—DNA was extracted with an E.Z.N.A. Fungal DNA kit (Omega Bio-Tek, Norcross, Georgia). For materials collected in the 1940s, we used an E.Z.N.A HP Fungal DNA kit, which isolates DNA with a combination of a CTAB method and spin column technology. DNA isolation procedures followed instructions provided by the manufacturer. The two internal transcribed spacers and intervening 5.8S ribosomal RNA gene (hereafter referred to as ITS) were amplified and sequenced following protocols outlined in Judge et al. (2010). For historical materials the two spacers were sequenced separately as in Ammirati et al. (2007).

Phylogenetic analyses.—Selected ITS sequences were assembled into a single matrix after a BLASTN (Zhang et al. 2000) query at NCBI. Guidelines for sequence selection included an E value of 0.0, query coverage of at least 63%, and maximum identities greater than 83%. Sequences were aligned with Clustal X 2.0.9 with default parameters (Larkin et al. 2007). Manual modifications were needed to improve the initial Clustal alignment. ITS1 only sequences were inserted later and aligned manually.

The final data matrix included 81 taxa and 748 sites, but 56 sites were excluded before phylogenetic analysis due to homology assessment ambiguities (692 sites included). A model of molecular evolution was determined with ModelTest 3.7 (Posada and Crandall 1998). Phylogenetic analyses were conducted in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML 7.0.3 (Stamatakis 2006). One thousand rapid bootstrap replicates were performed in RAxML. The number of steps to burn in MrBayes was determined by following chain convergence diagnostics in this program (i.e. when the average standard deviation of split frequencies reached < 0.01 reflecting the similarity between trees sampled in two independent runs). Posterior probabilities > 0.95 and bootstrap values ≥ 70% were considered significant. Trees were rooted using the midpoint rooting method in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Twenty-five new ITS sequences have been deposited at GenBank (GU949569–GU949592, JX258831). The ITS alignment, Bayesian consensus tree and RAxML bipartitions tree file are available online at http://www.bio.utk.edu/matheny/Site/Alignments_%26_Data_Sets.html.

RESULTS

Molecular phylogeny.—The model of molecular evolution best fit to the ITS alignment is a TVM + I + G model according to the Akaike information criterion (AIC). This model accepts unequal base frequencies, one rate for transitions (A-G, C-T), and unique rates for the four possible transversions (nst = 6). The proportion of invariable sites (I) was estimated at 0.2696. The gamma distribution shape parameter was estimated at 0.5841. In MrBayes the average standard deviation of split frequencies reached < 0.01 after 1 453 000 steps (sampling trees and other parameter values every 1000 steps). We thus let the analysis continue to 2 000 001 steps, from which we pooled the last 547 trees sampled (for a total of 1094 trees) to calculate posterior probabilities and produce a 50% majority rule consensus tree. Thus, we discarded 1454 samples, including the random starting tree, from the beginning of each chain.

A Bayesian cladogram of the ITS data is presented (FIG. 2). Twenty-two tips that correspond to *I. chondroderma* were recovered as a strongly supported monophyletic group and indicate this species' geographic range is British Columbia, Washington and Oregon. Four tips that we refer to *I.* cf. *chondroderma* are unresolved in the Bayesian cladogram but were recovered as monophyletic with 70% bootstrap support in a likelihood analysis (see insert phylogram in box of FIG. 2) and as the sister group to *I. chondroderma*. *Inocybe* cf. *chondroderma* occurs in both Colorado and Japan, where in Japan it associates with the mycoheterotrophic orchid *Epipogium aphyllum* Sw. (Roy et al. 2009). Sister to *I. chondroderma* and *I.* cf. *chondroderma* is a European sample of *I. subnudipes* Kühner from Sweden (Ryberg et al. 2008). These three taxa form a robust monophyletic group.

Molecular taxonomy.—BLASTN results of the ITS sequence (ITS1 + 5.8S rRNA + ITS2) of *I. chondroderma* GU949574 (BLASTN performed on 29 Apr 2012) produced 20 matches at 100–99% sequence similarity. Of these matches, nine different specific epithets in *Inocybe* and one in *Hebeloma* have been applied to what are two molecular operational taxonomic units (TABLE I).

Taxonomic utility of the PDAB reaction.—Numerous species of Agaricales exhibit a positive PDAB reaction of fresh basidiocarp tissues. Lennox (1979) reported that all species of *Lyophyllum* sampled by her produce a blue-green reaction similar to *I. chondroderma* illustrated here (FIG. 1). Our survey of selected Agaricales confirms this result but also reveals that the blue-green or turquoise PDAB reaction occurs in several unrelated species such as *Agrocybe erebia* (Fr. : Fr.) Kühner ex Singer (*PBM2760*, *PBM2762*, *PBM3348* [TENN]), *Hebeloma album* Peck sensu Bruchet (*PBM1361* [WTU]), *Psathyrella spadicea* (P. Kumm.) Singer (*PBM2049*, *PBM2073* [WTU]; *JCS100905B* [CUW]), *P. piluliformis* (Bull. : Fr.) P.D. Orton (*PBM2091* [WTU]), and an unidentified species of *Crepidotus* (*PBM2979*, *PBM2973* [TENN]) that shares affinities with *C. alabamensis* Murrill. Turquoise changes in PDAB, also observed for *Tricholoma odorum* Peck (*PBM2234*, *PBM2831* [CUW]) and *T. platyphyllum* (Murrill) Murrill (*PBM2013* [WTU]), are preceded by a violet or pinkish phase that transitions to turquoise. Recently dried (24 h) basidiocarp tissue of *I. chondroderma* did not produce the reaction as dramatically as the fresh tissues, and none of the dried materials aged 12–64 y produced a reaction at all. *Inocybe chondroderma* is the only species of Inocybaceae in which a turquoise reaction to PDAB has been observed.

Other species of Agaricales exhibit positive reactions but produce different colored pigments either in solution or on the basidiocarps. These include *Russula occidentalis* Singer (Woo 1993) and species of *Tricholoma*, stirps *Virgatum* (Ovrebo 1989). We have observed a reddish pigment exude into PDAB solution for what is *T. palustre* A.H. Sm. (as *T. aestuans* [Fr.] Gillet) (Clark Ovrebo pers comm) in Matheny et al. (2006)] and what may represent *T. pullum* Ovrebo (*PBM2505*, *PBM2837* [CUW]), as well as a change to magenta on the stipe flesh of *T. serratifolium* Peck. In *Inocybe* we observed a salmon flush to the surface of the pileus in *I. pudica* Kühner (*PBM1386*, *PBM2236*, *PBM2265* [WTU]). Collections that correspond closely to *I. straminipes* Romagn. and *I. nigrescens* G.F. Atk. (*I. xanthomelas* group) produce an intense yellow pigment in solution of PDAB (*PBM1812*, *PBM1813*, *PBM2056*, *PBM2246*, *PBM2282*, *PBM2283* [WTU], *AWW269*, *PBM2782* [TENN].

TAXONOMY

Inocybe chondroderma Stuntz ex Matheny, Norvell et Giles, sp. nov. FIGS. 1, 3

MycoBank MB800270.

Etymology: (Gk) *chondro-* (prefix) = grain or cartilage; (Gk) *-derma* (suffix) = skin; named in reference to the hygrophanous-corneous pileus surface.

Pileus 10–50 mm diam, conical to parabolic to obtusely conical or campanulate; umbo entirely absent or with a distinct or prominent obtuse umbo often by early age; margin decurved or deeply so; edge slightly inrolled when very young, remaining so in age but straight and undulating at times, occasionally with appendiculate veil material when young; surface dry or slightly lubricous or soapy when moist, not viscid, shiny, subhygrophanous, at center glabrous

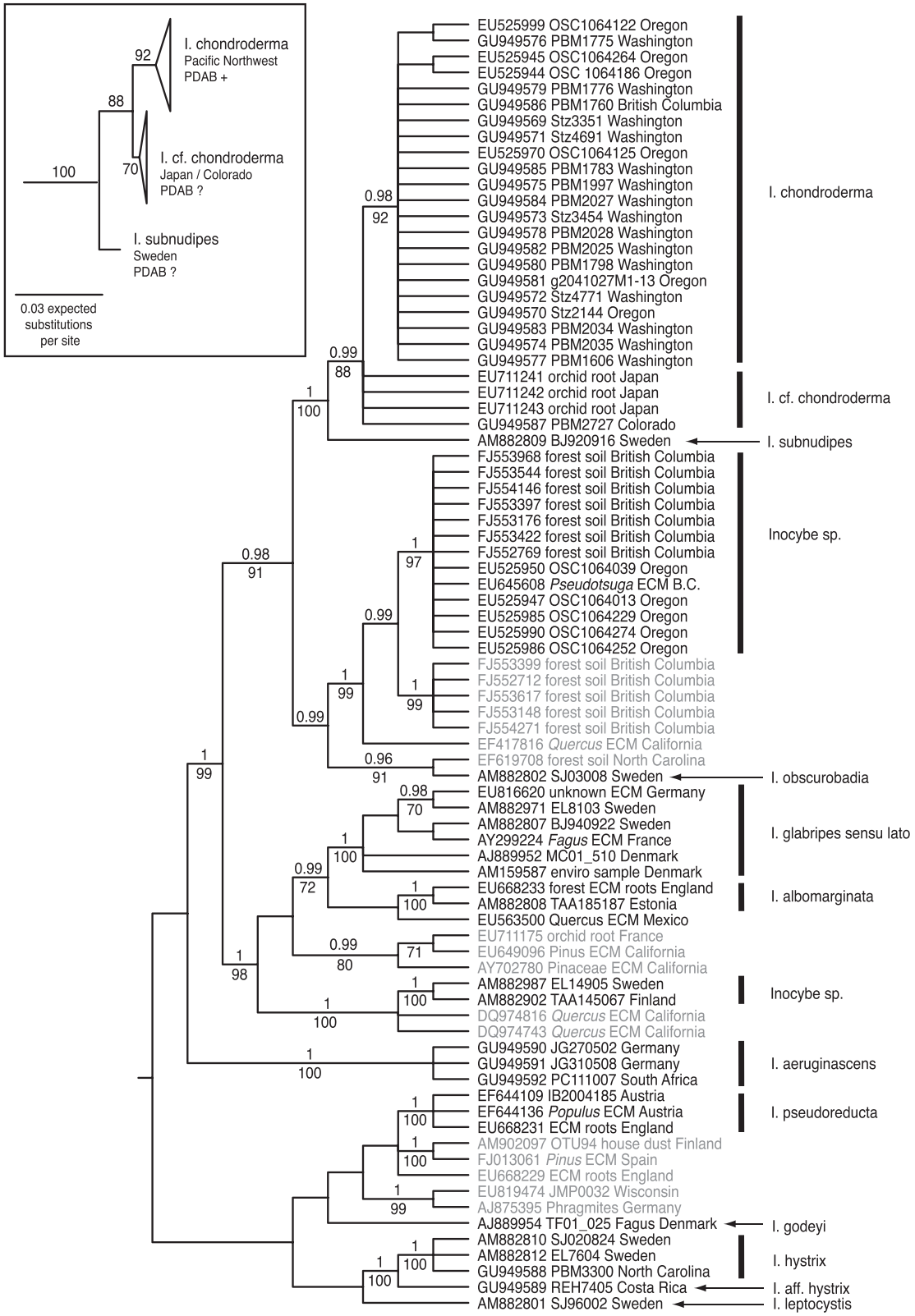

FIG. 2. A 50% majority rule consensus cladogram produced by a Bayesian analysis of ITS sequences. Clades or tips associated with specimen-voucher data are labeled with vertical black bars or horizontal black arrows. Clades or tips composed exclusively of environmental sequences bear gray-shaded tip labels. Posterior probabilities > 0.95 are indicated above branches, and maximum likelihood bootstrap values ≥ 70% are indicated below branches. The tree was mid-point rooted. *Inocybe* cf. *chondroderma* is monophyletic with 70% bootstrap support in a likelihood phylogenetic analysis (see insert phylogram in box) but unresolved in the Bayesian cladogram.

TABLE I. Results from BLASTN query of ITS sequence GU949574 *Inocybe chondroderma* on GenBank (GB) 29 Apr 2012. Sequences ≥ 97% similarity are shown

GenBank accession no.	GenBank species name	Max score	Query coverage	E value	Max identity	Geography	Annotated name
HQ604100	*I. auricoma*	1147	100%	0.0	100%	BC[a]	*I. chondroderma*
HQ604101	*I. fuscidula*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604098	*I.* cf. *sindonia*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604095	*I. posterula*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604096	*I. sindonia*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604097	*I. auricoma*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604094	*I.* cf. *sindonia*	1147	100%	0.0	100%	BC	*I. chondroderma*
HQ604103	*I. posterula*	1144	100%	0.0	99%	BC	*I. chondroderma*
HQ604099	*I. abietis*	1144	100%	0.0	99%	BC	*I. chondroderma*
HQ604093	*I. kauffmanii*	1144	100%	0.0	99%	BC	*I. chondroderma*
HQ604092	*I. sindonia*	1142	100%	0.0	99%	BC	*I. chondroderma*
EU525999	*Inocybe* sp. (originally as *Hebeloma mesophaeum*)	1142	100%	0.0	99%	Oregon	*I. chondroderma*
HQ604102	*I. sindonia*	1138	100%	0.0	99%	BC	*I. chondroderma*
EU525945	*I. pyriodora*	1138	100%	0.0	99%	Oregon	*I. chondroderma*
EU525944	*I. sororia*	1138	100%	0.0	99%	Oregon	*I. chondroderma*
EU525970	*I. pudica*	1136	100%	0.0	99%	Oregon	*I. chondroderma*
EU711242	Uncultured Inocybe isolate	1118	100%	0.0	99%	Japan	*I. chondroderma*
GU949587	*I.* aff. *subnudipes*	1114	100%	0.0	99%	Colorado	*I.* cf. *chondroderma*
EU711241	Uncultured Inocybe isolate	1114	100%	0.0	99%	Japan	*I.* cf. *chondroderma*
EU711243	Uncultured Inocybe isolate	1112	100%	0.0	99%	Japan	*I.* cf. *chondroderma*
AM882809	*I. subnudipes*	1046	99%	0.0	97%	Sweden	
FN550925	*I. subnudipes*	1037	99%	0.0	97%	Sweden	

[a] BC: British Columbia.

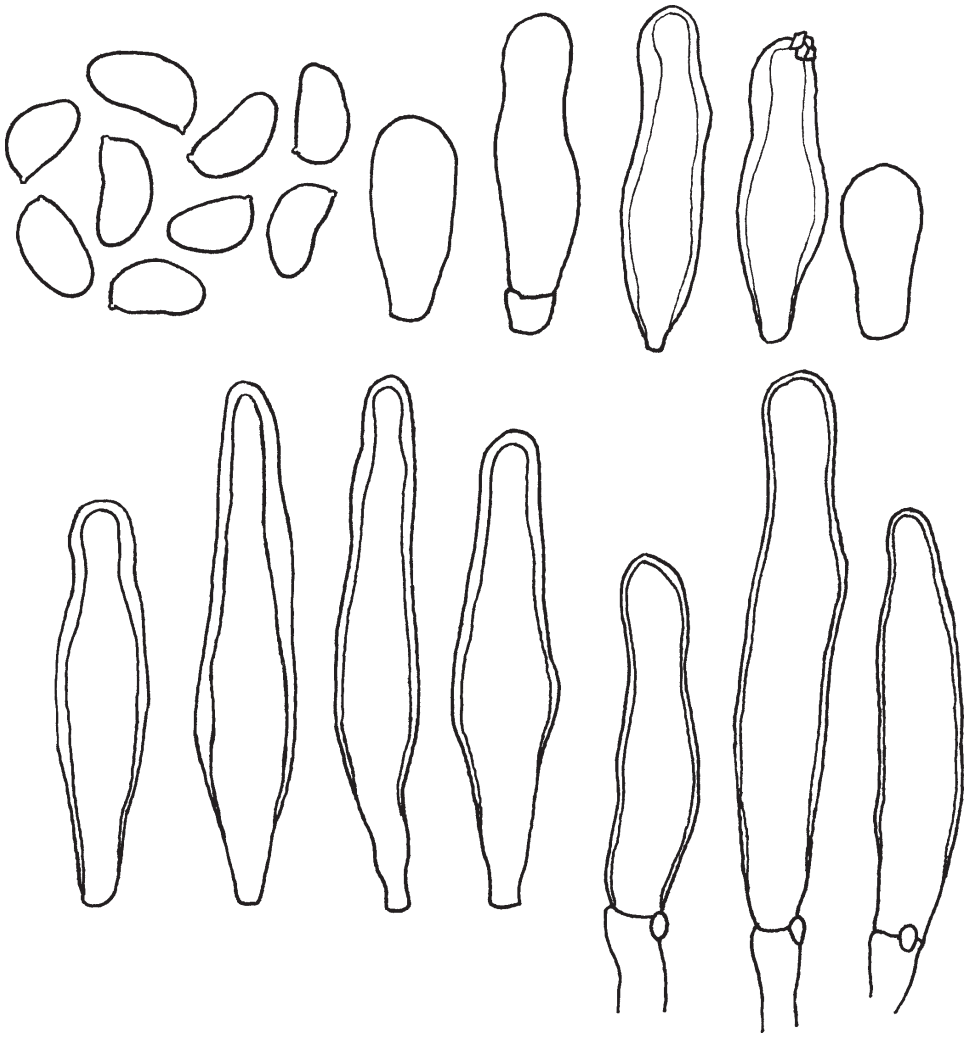

FIG. 3. Microscopic features of *I. chondroderma* (holotype, *Stz4771,* WTU). Upper left, basidiospores; upper right, cheilocystidia; lower left, four pleurocystidia; lower right, three caulocystidioid cells. Bars = 10 μm.

to more or less glabrous toward the margin when young (exceptionally minutely scaly), finely fibrillose in age; fibrils weakly diverging, usually not rimose or at most rimulose in age; disk fulvous or ''Buckthorn Brown'' or strong brown to brownish yellow (10YR 6/6–5/6, 7.5YR 5/6–4/4, 2.5Y 7/6), usually darker than the margin except when young, at times with a pallid or pale brown velipellis that imparts a light gray to pale brown (10YR 7/2–/3) color, the margin ''Chamois'', ''Honey Yellow'' , or ''Isabella Color'' or light olive brown, light yellowish brown, to pale yellow (10YR 6/6–7/4, 2.5Y 5/4–6/4–7/4), often bicolorous but this not always evident, center may fade from fulvous (10YR 5/6) to brownish yellow (10YR 6/6) with the margin fading from light olive brown (2.5Y 5/4) to light yellowish brown (2.5Y 6/4), eventually the center and margin may fade to pale yellow (2.5Y 7/4) with shades of light olive brown (2.5Y 5/4) at the edge of the margin; context white or pallid, unchanging where bruised or cut, ≤ 5 mm thick under the disk, not confluent with stipe context; odor spermatic to weakly so, flavor not remarkable; chip of flesh producing a turquoise pigment in solution of PDAB. Lamellae close, about 40 L with several tiers of lamellulae, narrowly adnate, seceding in age, rounded toward the stipe, ventricose, ≤ 4 mm deep, grayish brown (10YR 7/3) when young to pale brown (10 YR 6/3), becoming yellowish brown (10YR 5/4) or light olive brown (2.5Y 5/4) to dark yellowish brown (10YR 4/4), ''Dresden Brown'', or olive brown in age; edges pallid and fimbriate. Stipe 25–80 mm × 4–7 mm at apex, terete, rarely compressed, enlarged or slightly bulbous at the base but not marginate, ≤ 10 mm at widest point; cortina pallid and fugacious; surface pruinose under a hand lens, if at all, or at the extreme apex or upper one-sixth to one-eighth, in some collections not obviously pruinose at apex, elsewhere fibrillose to scurfy-fibrillose or glabrescent below; the

fibrillose layer at times forming a pallid vesture, with lower part usually densely covered with white patches of mycelium, rarely browning; color above pallid with isabelline or ''Chamois'' (10YR 6/4) undertone or streaks beneath a spreading white fibrillose vesture, developing pale yellow (near 5Y 7/4–2.5Y 8/4) undertones more evident with age, at extreme base pallid; context solid, pale grayish in areas.

Basidiospores 7.0–*8.5*–10.0(–11.5) ± 0.85 × (4.0–) 4.5–*4.8*–5.5(–6.0) ± 0.37 μm (n = 71/5), smooth, amygdaliform to subamygdaliform with bluntly pointed or conical apices, some noticeably swaybacked or near reniform in profile, apiculus distinct, in deposit yellowish brown (10YR 5/3) to dark yellowish brown (10YR 4/4). Basidia 25–31 × 7–8 μm, four-sterigmate, clavate to cylindrico-clavate, hyaline. Pleurocystidia 50–68 × 10–14 μm, fusiform, apices occasionally crystalliferous at apex, thick-walled; walls 1.0–2.5 μm thick, hyaline. Cheilocystidia similar to pleurocystidia but usually shorter and/or thin-walled, mixed with clavate paracystidia. Stipitipellis with caulocystidioid cells at the apex or extreme apex, these thick-walled or thin-walled; crystals present or absent; irregularly cylindrical to fusiform; apices on occasion subcapitate; occasionally mixed with shorter, cylindrical terminal cells. Pileipellis a cutis of brownish yellow to yellowish brown hyphae, these cylindrical, mostly 7–15 μm diam. Clamps present.

Habit, habitat and distribution: Scattered singly on soil under conifers, *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga heterophylla* (Raf.) Sarg., *Thuja plicata* Donn ex D. Don (Douglas-fir, western hemlock, western red cedar respectively) or under *Arbutus menziesii* Pursh. (Pacific madrone), fruiting in the fall, late September to December, rarely August, mainly on the west side of the Cascade divide in Oregon, Washington and British Columbia.

Holotype: Washington, Mount Rainier National Park, Longmire, Powerline Trail, leg. D.E. Stuntz, 25 Sep 1948 (*Stz4771*) (WTU). Isotype: *TENN066992*.

Additional specimens examined: CANADA. BRITISH COLUMBIA: Vancouver Island, Lizard Lake, Pacific Northwest Key Council Meeting, 30 Oct 1999, *S. Clark*, *PBM1760* (WTU). UNITED STATES OF AMERICA: OREGON: Clackamas County, five miles up Salmon River, near Welches, 13 Oct 1946, *D.E. Stuntz*, *Stz2144* (WTU). Benton County, Green Peak density management series transects, 60 y old conifer forest of *Ps. menziesii* and *Ts. heterophylla*, 13 Oct 2004, *L.L. Norvell*, *g2041013h2-18* (PNWMS); 27 Oct 2004: *g2041027h2-14* and *g2041027m1-13* (both PNWMS); 9 Nov 2004: *g2041109m1-28*, *g2041109m2-16*, *g2041109u1-15*, *g2041109u1-16*, and *g2041109u2-12* (all PNWMS); 26 Oct 2005: *g2051026h2-7* (PNWMS). Polk County, 30 y old BLM reserve of *Ps. menziesii* and *Ts. heterophylla* near Pedee west of Monmouth, 14 Nov 2001, *L.L. Norvell*, *a2011114y1-35* (PNWMS). WASHINGTON: Island County, Mutiny Bay Heights, 26 Nov 1972, *F. van de Bogart*, *FVB1646* (det. Stuntz) (WTU). King County, Sammamish Plateau, Hazel Wolf Wetlands, *P.B. Matheny*—11 Nov 1999: *PBM1783* (WTU); 18 Oct 2000: *PBM1997* (WTU); Pine Lake State Park, *P.B. Matheny*, 11 Nov 1999: *PBM1798* (WTU); 5 Nov 2000: *PBM2034*, *PBM2035*, and *PBM2047* (all WTU); University of Washington campus, Parrington Hall, *D.E. Stuntz*—29 Oct 1949: *Stz5887* (WTU); 24 Oct 1950: *Stz6089* (WTU); same locality as previous but location on UW campus unspecified, under *Arbutus menziesii*, 24 Oct 1950, *D.E. Stuntz*, *Stz6100* (WTU); University of Washington campus, Parrington Hall, under *A. menziesii*, 7 Nov 1951, *D.E. Stuntz*, *Stz6462* (WTU). Kitsap County, Seabeck, 6 Nov 1999, *S. Clark*, *PBM1775* (WTU; TENN066993) and *PBM1776* (WTU). Mason County, Mission Creek, Tahuya State Forest near Hood Canal, 29 Oct 2000, *P.B Matheny*, *PBM2025*, *PBM2027*, *PBM2028* (WTU); Shelton, near Harstene Island Bridge, on Pickering Road, in second-growth conifer forest, 12 Dec 1999, *E. Duffield*, *PBM1823* (WTU). Okanogan County, Okanogan National Forest, W. Chewuck River Road, 30 Mile Campground, under *Pseudotsuga menziesii* and *Picea*, 21 Aug 1999, *P.B. Matheny*, *PBM1606* (WTU). San Juan County, Friday Harbor Biological Station, 3 Nov 1951, *D.E. Stuntz*, *Stz6474* (WTU). General locations unknown (place names according to Stuntz collection labels): Mile Bridge Flat, 12 Oct 1942, *D.E. Stuntz*, *Stz3351* (WTU); Twin Bridges Camp area, 12 Oct 1942, *D.E. Stuntz*, *Stz3454* (WTU); date and collector not recorded, *Stz4691* (WTU); Rainier, 5 Nov 1950, *D.E. Stuntz*, *Stz6188* and *Stz6189* (WTU); north of Hawk Pr., 12 Nov 1950, *D.E. Stuntz*, *Stz6201* (WTU); St Patrol., 10 Nov 1951, *M. McKenny* & *D.E. Stuntz*, *Stz6496*, *Stz6502*, and *Stz6537* (WTU); 27 Oct 1970, *J.W. Lennox*, *JWL1031* (det. Stuntz) (WTU).

Comments: Inocybe chondroderma first was described in the literature from the Pacific Northwest as *I. agglutinata* in a doctoral dissertation by Stuntz (1940), who in the same work referred another collection to *I. submuricellata*. In his unpublished notes, Stuntz later indicated that both names actually encompassed what he had come to recognize as *I. chondroderma*, a name he suggested for a new species in what would remain an unpublished work in 1965. Stuntz's 1940 description of *I. submuricellata*, however, describes an entirely pruinose stipe, an observation at odds for the *I. chondroderma* stipe, which bears a cortina and is pruinose only at the (extreme) apex or not at all. However, we have not examined or sequenced either of these very early collections.

It is evident that *I. chondroderma* is widely misidentified in the Pacific Northwest (TABLE I). More discussion of this topic follows below. However, the species as typically observed features a subtly bicolorous pileus—fulvous or tawny at the center and buff or isabelline toward the margin. These colors may change slightly as the pileus dries. The occasional soapy or lubricous pileus surface may cause some to interpret this as viscid, which could explain referring the specimen to *I. agglutinata* or *Hebeloma*. Both

Stuntz and we noted that the stipe base may be white mycelioid. This feature—when combined with other traits such as habitat, fall phenology, relatively small smooth basidiospores (some of which are ''sway-backed'' or near reniform in appearance), presence of a cortina, restriction of caulocystidioid cells to the stipe apex, and overall coloration—should provide clues as to its identity. The easiest diagnostic feature, however, is the turquoise reaction of fresh basidiocarp tissue in PDAB.

We label two additional taxa most closely related to *I. chondroderma* as *I.* cf. *chondroderma* and *I. subnudipes* (FIG. 2). The ITS sequence of the only specimen-based collection of the former (PBM2727 from Colorado) differs from those of *I. chondroderma* at six positions (99% similarity). The spores of this collection are very similar to those of *I. chondroderma* as well. It may be that *I. chondroderma* comprises a separate but cryptic population with a distribution known to date in Colorado and Japan. However, the ML analysis recovers *I.* cf. *chondroderma* as a monophyletic group together with environmental sequences from root tips of the ghost orchid, *Epipogium aphyllum*, from Japan. Additional field collections together with PDAB results are needed to determine whether *I. chondroderma* has a wider distribution outside the Pacific Northwest. No previously described species from Japan match our description of *I. chondroderma* (Kobayashi 2002, Kobayashi 2009 and references therein).

Inocybe subnudipes, described originally by Kühner (1955) from France, is not widely reported from Europe and was regarded by Kuyper (1986) as a dubious taxon. Moënne-Loccoz et al. (1990) document this species in detail including a color icon. This species is morphologically and ecologically similar to *I. chondroderma* based on descriptions, but the spores lack the characteristic swayback outline in the illustration by Moënne-Loccoz et al. (1990). ITS sequences of Swedish material differ by 3% (Ryberg et al. 2008) from *I. chondroderma*. Because the PDAB reaction is not known for *I. subnudipes*, and given its phylogenetic autonomy and European distribution, we prefer to recognize it as separate from *I. chondroderma*. *Inocybe subnudipes* recently has been reported from northern Europe (Jacobsson 2008, Ryberg et al. 2008).

DISCUSSION

Inaccurate taxonomic annotation of DNA sequences of Inocybe on GenBank.—Several studies have pointed to the alarming problem of taxonomically misidentified DNA sequences in public data repositories such as GenBank (Bridge et al. 2003, Vilgalys 2003, Nilsson et al. 2006, Bidartondo et al. 2008). Indeed, about 20% of taxonomic entries are probably misidentified to the species level (Nilsson et al. 2006). Direct third-party annotations of sequences is still not possible without first-party permission, although recently the addition of biological quality filters and a process of verification applied by GenBank staff to sequences have been added to the pre-submission phase (Benson et al. 2012).

Here, *Inocybe chondroderma*, a common species in the Pacific Northwest, has been identified as at least 10 different species of *Inocybe* and *Hebeloma* (the last a GenBank record now updated to ''*Inocybe* sp.'') based on ITS BLASTN comparisons on GenBank (TABLE I). Many environmental sequences (FIG. 2) were grossly mislabeled to order (as Boletales, see FJ series of GenBank accession numbers). We ourselves attempted to fit the European names *I. posterula* and *I. auricoma* to what is now described as *I. chondroderma*. However, application of the numerous names to what is ultimately a single species (TABLE I) suggests that collections of *Inocybe* deserve more careful scrutiny with respect to specimen and sequence annotation. *Inocybe chondroderma* may well exhibit color and texture variation under unusual environmental conditions that leads to identifications under various names; the bicolorous nature of the pileus, for example, is not always evident. Nonetheless, we have demonstrated that a macrochemical, PDAB, produces a reliable and consistent diagnostic feature that readily aids identification of *I. chondroderma*. We are unaware whether *I.* cf. *chondroderma* and *I. subnudipes*, the two taxa most closely related to *I. chondroderma*, also exhibit a similar PDAB reaction. Two species of *Hebeloma*, *H. velutipes* Bruchet (*PBM2277* [WTU] and *H. olympianum* A.H. Sm., V.S. Evenson and Mitchel (*PBM2060* [WTU]), tested negative with PDAB, although *H. album* sensu Bruchet produced a positive turquoise reaction.

We also address the likelihood that any of the 10 species (TABLE I) actually represent *I. chrondroderma*. (i) As of this writing, the only available sequences of *I. auricoma* from GenBank were from British Columbia, with none sampled from Europe where it is reported as rare but widespread (Kuyper 1986). Morphologically *I. auricoma* in Kuyper (1986) and Heim (1931) is distinguished from *I. chondroderma* by its smaller habit and association with angiosperms (e.g. *Quercus* and *Alnus*), while Stangl (1989) records *I. auricoma* rarely with conifers. Given that *I. auricoma* is based on an epithet described in 1783 without a type, lacks the characteristic swayback or somewhat reniform spore according to the literature and is rarely collected in Europe, we prefer not to apply the name to *I. chondroderma*. Examination of two collections of *I.*

auricoma from L (*Kuyper 1603* and *Huijsman* s.n.) confirms the difference in spore morphology, and our ITS sequence (JX258831) of the Huijsman collection suggests *I. auricoma* is most closely related to *I. flocculosa* (Berk.) Sacc. (90–91% sequence similarity). (ii) Ryberg et al. (2008) nest *I. posterula* in the *I. geophylla* group, remote from *I. chondroderma*. *Inocybe posterula* exhibits a yellowish pileus lacking the fulvous to isabelline pileus tones found in *I. chondroderma* and appears to be the same as a Finnish collection of *I. xanthodisca* Kühner (*JV2527F*, TURA, unpubl), both of which nest in the *I. geophylla* clade (Ryberg et al. 2008). Our sequences of *I. chondroderma* do not nest in the *I. geophylla* clade. (iii) A third species, *I. sindonia*, is distinguished from *I. chondroderma* by its overall paler coloration, caulocystidia distributed below the center of the stipe, and molecular affiliation with *I. flocculosa*, *I. violaceocaulis*, *I. pusio* and *I. queletii* sensu Matheny in *Inocybe* clade Ic as recognized in Kropp et al. (2010). (iv) *Inocybe abietis* Kühner is described with an ochraceous brown to brownish yellow pileus in the protolog (Kühner 1955) but without a partial veil (cortina). Kuyper (1986), who was unable to locate the type, considered the identity of the species doubtful but similar to *I. glabrescens* Velen. Matheny et al. (2002) and Matheny (2005) applied the name *I. abietis* to what is now recognized as *I. praecox* Kropp, Matheny & Nanagy. (Kropp et al. 2010), a species also found in the Pacific Northwest but which fruits in the spring and bears caulocystidia below the center of the stipe. In contrast, *I. chondroderma* fruits in the fall, possesses a partial veil and has caulocystidioid cells restricted to the (extreme) stipe apex. (v) *Inocybe kauffmanii* A.H. Sm., a species known only from western North America (Kropp et al. 2010), readily differs by its cream to isabelline pileus and long, entirely pruinose stipe. The remaining BLASTN results are surprising because (vi) *I. pudica* Kühner, (vii) *I. pyriodora* (Pers.) P. Kumm. (currently accepted name *I. fraudans* [Britzelm.] Sacc.), (viii) *I. fuscidula* Velen., (ix) *I. sororia* Kauffman and (x) *Hebeloma mesophaeum* (Pers.) Quél., now labeled ''*Inocybe* sp.'', are grossly different in outward appearance and/or odor compared to *I. chondroderma*. In addition, forest soil samples from British Columbia (B.C.) cluster into two monophyletic groups (one of which is labeled *Inocybe* sp.), the taxonomy of which is unclear; all these ITS sequences are currently mislabeled as ''uncultured Boletales'' on GenBank (Hartmann et al. 2009). Specimens at OSC in *Inocybe* sp. that cluster with one of the two B.C. clades are mislabeled as *I.* cf. *lanuginosa* and *I. sororia*. The taxonomic identity of this clade is not known, and we have not examined the OSC collections labeled as such.

Although there are regional works that aid identification of fruit bodies of Inocybaceae in North America, they are inadequate. For example, Nishida (1989) presents a comprehensive examination of California Inocybaceae but lacks species descriptions and applies many European names that are likely inaccurate in some cases (see Kropp et al. 2010). Stuntz's unpublished 1965 work includes numerous tentative provisional names but is not widely available, and the keys are difficult to use for inexperienced workers. Recent monographs of various *Inocybe* species groups that include previously undocumented taxa (Matheny and Kropp 2001, Kropp and Matheny 2004, Cripps et al. 2010, Kropp et al. 2010) are narrow in taxonomic scope. No synthetic treatment referring to all North American taxa has been published since Kauffman (1924). Until such a revision is completed, we recommend caution and lowered expectations when applying names from European or Asian monographs and floras to North American Inocybaceae (Petersen 1975, Norvell and Exeter 2004) based on morphology alone. Recent taxonomic revisions presenting both morphological and molecular characterizations (e.g. section *Rimosae* sensu lato in Larsson et al. 2009 and an element of section ''*Cortinatae*'' in Kokkonen and Vauras 2012), however, should provide helpful comparisons with North American taxa.

PDAB as a chemotaxonomic reagent.—Use of macrochemicals for taxonomic purposes in agaricology has had a long history (Watling 1969, Singer 1986). PDAB is a chemical of medical importance (Bailly et al. 1967, Brown et al. 1973) that has been used to detect presence of various alkaloids, particularly indole alkaloids. The reaction of alkaloids with PDAB also has been termed the van Urk reaction (Calatayud and Hernandez 1991). The chemical is useful in the taxonomy of mushroom-forming fungi and has aided in identification of species of *Russula*, *Agrocybe* sensu lato (viz. *A. erebia*), *Tricholoma* (see also Ovrebo 1989), *Psathyrella*, *Crepidotus* and *Inocybe*. However, the reaction only occurs on fresh or very recently dried (< 24 h) materials. Many species of the Lyophyllaceae also produce a turquoise reaction (Lennox 1979), similar to what we observe in *I. chondroderma*. The extent to which the positive reaction is an important higher-level character state within the Lyophyllaceae has not been explored.

The blue-green or turquoise reaction of fresh tissues from fruit bodies of *I. chondroderma* suggests the presence of an indole alkaloid. In their chemotaxonomic evaluation of species of *Inocybe*, Robbers et al. (1964) report that collection *I.* 1774 (*Stz1774*) produced blue with PDAB in a paper chromatographic

test and lacked muscarine. Unfortunately, in our request for materials of *I. chondroderma* from WTU, we did not receive collection *Stz1774*. Nevertheless, Stuntz did indicate in his unpublished notes that this collection is representative of *I. chondroderma*, which also received the nickname the ''Brady indole *Inocybe*'', in reference to L.R. Brady, a co-author of the Robbers et al. (1964) study. In this work the authors suggest the unknown alkaloid of *I.* 1774 might be a hydroxylated indole compound.

Several questions remain to be addressed regarding the PDAB reaction: Is the blue-green reaction to PDAB by many species of Agaricales the same biochemical compound? Is this compound indeed an indole alkaloid? Do other color reactions with PDAB indicate presence of alkaloids or other secondary metabolites?

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