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Crepidotus crocophyllus includes *C. nephrodes*

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Abstract—Many species of the agaric genus *Crepidotus* are distinguished based on single, potentially variable morphological characters. Numerous collections of *Crepidotus* belonging to a species complex that includes *C. crocophyllus* and *C. nephrodes* were examined from Central and Eastern Europe and North America. Morphological observations were supplemented by phylogenetic analysis of nuclear ribosomal 28S DNA. Our results indicate that *C. crocophyllus* is a broadly-distributed temperate fungus with variable pigmentation. The fungus is re-described and a taxonomic discussion provided.

Key words—*Agaricales,* taxonomy

Introduction

Several collections of an interesting species of *Crepidotus* (Fr.) Staude (*Crepidotaceae, Agaricales*) made by one of us (SR) in Slovakia were identified as *C. crocophyllus* (Berk.) Sacc., although these collections were notably different in lamellae color from the description of Pilát (1948a). This prompted additional field observation of basidiomata development in specimens from Slovakia related to this taxon and the examination of numerous collections from North America and Eastern Europe. Published description of the North American *Crepidotus nephrodes* (Berk. & M. A. Curtis) Sacc. by Hesler & Smith (1965) describes it as very similar to *C. crocophyllus*, differing mainly in pigmentation of the lamellae—pallid in *C. nephrodes* and bright orange in *C. crocophyllus*. Previous mating intercompatibility studies between many North American isolates from *Crepidotus* subsection Fulvofibrillosi Hesler & Smith (Aime, 2004) showed that several morphological species placed here (mainly following Hesler

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& Smith, 1965), including *C. nephrodes*, were conspecific but the taxonomy of this group was not addressed in that work, and isolates of *C. crocophyllus* were not obtained for mating studies. This has led us to evaluate the relationship of *C. crocophyllus* and *C. nephrodes*, in North America and Europe, by examination of collections, sequencing data, field data of basidiomata development, and a re-evaluation of the phenotypic characters previously used to define taxa in this complex.

This paper provides: (1) a taxonomic revision of *Crepidotus crocophyllus*; (2) a discussion and an expanded description for this taxon; (3) a discussion of the morphological characters previously used to segregated species in this complex; (4) descriptions of basidiomata development in the field, and (5) a delimitation of the taxon supported by phylogenetic analysis.

Nomenclature and taxonomy

First to present a concise taxonomic and nomenclatural treatment of *Crepidotus* was Pilát (1948a) in the Czech edition of his monograph. Here he treated *C. crocophyllus*, *C. applanatus* var. *kuzyanus*, *C. fulvifibrillosus* and *C. dorsalis* as synonyms of *C. applanatus* var. *crocophyllus*. However, in the French edition of his monograph (Pilát, 1948b) the name *C. applanatus* var. *fulvifibrillosus* was used and only *C. fulvifibrillosus*, *C. applanatus* var. *kuzyanus* and *C. dorsalis* were listed in synonymy; the description was unchanged.

Originally (Pilát, 1940) the name *Crepidotus applanatus* var. *kuzyanus* Pilát was invalidly published (ICBN, Art. 32, 37). Several collections held at PRM (23481, 23482, 23483, 23484, 28616) were identified by Pilát under that name. He later 'renamed' those collections as *C. applanatus* var. *crocophyllus* (Pilát, 1948a) and/or *C. applanatus* var. *fulvifibrillosus*. (Pilát, 1948b).

Hesler & Smith (1965) in their monograph of North American members of *Crepidotus* distiguished two closely related taxa, *C. crocophyllus* and *C. nephrodes*. After studying type specimens they concluded that *C. dorsalis* was conspecific with *C. crocophyllus,* and that *C. fulvifibrillosus* was conspecific with their *C. nephrodes*.

Hesler & Smith's concept was followed by Lazebníček (1970) who treated Pilát's collections (originally labelled as *Crepidotus applanatus* var. *kuzyanus*) from the Eastern Carpathians as *C. nephrodes*. This was probably the reason he did not mention or comment on any of Pilát's names, such as *C. applanatus* var. *kuzyanus*, *C. applanatus* var. *crocophyllus* and *C. applanatus* var. *fulvifibrillosus*.

Senn-Irlet (1995), in her European monograph, accepted *C. crocophyllus* as an autonomous taxon and she listed in the synonymy *C. dorsalis, C. fulvifibrillosus, C. applanatus* var. *fulvifibrillosus* and *C. appalanatus* var. *crocophyllus*. She studied specimens collected by Lazebníček, but not those of Pilát. The identity of American taxa, including *C. nephrodes,* was not treated in that monograph.

Material and Methods

Material. Fresh material was examined immediately and the macroscopic structures recorded. Color designations follow Kornerup & Wanscher (1974). Microscopic structures were recorded from material rehydrated in 80% EtOH and water. Thin

sections were mounted in 10% NH_4OH, 5% KOH, and/or Congo red and viewed under oil immersion. Microscopic measurements are approximated to within 0.75 µm and were estimated as 5-95 percentiles of 39 measurements per collection. Terminology of microscopic features follow Vellinga (1988). Designations for herbaria are from Holmgren & al. (1990).

Field studies. Our field observations took place on Sihot' Island, Podunajská nížina lowland (lat. 48°09′19″–48°08′33″, long. 17°01′00″–17°03′30″, 136.5–140 m, south-western Slovakia, city of Bratislava, municipal part Karlova Ves). Fruiting bodies growing on the upper surface of a fallen, decorticated trunk of *Negundo aceroides* were monitored, and growth and morphology recorded every second day over a one week period in June 1999.

Morphometric analysis. Delimitation of *Crepidotus crocophyllus* and *C. nephrodes* were based on Hesler & Smith (1965) and on Pilát's (1940, 1948a, 1948b) and Senn-Irlet (1995) delimitation of taxa related to *C. crocophyllus*. Specimens from Europe and the U. S. A. originally identified as *C. crocophyllus* and *C. nephrodes* were also considered. Additionally, comparative studies of various of the concepts in *Crepidotus* used in this work followed Hesler & Smith (1965).

Phylogenetic analysis. Nine species of *Crepidotus* were analyzed to represent the genotypic diversity in the genus, including North American samples of *C. crocophyllus* and *C. nephrodes,* a Slovak isolate of *C. crocophyllus,* and *C. malachius* the known sister-species to *C. nephrodes* (Aime, 2001). Sequences of the 28S ribosomal DNA (nLSU) were obtained from GenBank as accession numbers AF139946, AF205670, AF205674, AF205677, AF205679, AF205690, AF205707, AF367936, AF367939, and AF367958 (Aime, 2001, 2004; Moncalvo & al., 2002; Thorn & al., 2000). Two sequences from the genus *Simocybe* were selected as outgroups for rooting the tree, as this genus has been previously shown to be a sister genus to *Crepidotus* (Aime, 2001). Sequence alignments were constructed by eye in Se-Al ver. 2.0a11 (Rambaut, 1996-2002). The nLSU data matrix of 16 taxa and 892 characters was then exported to Paup* 4.0b10 (Swofford, 2002) for analysis. Maximum parsimony (MP) analyses were conducted as heuristic searches with 100 random addition replicates and TBR branch swapping. Support for the branching topologies was evaluated by bootstrap analysis derived from 1,000 replicates with 10 random addition replicates each. Maximum likelihood (ML) analyses were conducted by the quartet puzzling method in Paup* 4.0b10 with 10,000 puzzling steps.

Results

Field observations

Our field observation on Sihoť Island, Podunajská nížina lowland, started on 16 June 1999 and were continued until 20 June 1999. Gross size and changes in the color of lamellae and pileus surface of 19 basidiomata of *Crepidotus crocophyllus* growing on the upper side of wood of a fallen rotten trunk of *Negundo aceroides* were monitored every second day.

June 16: Basidiomata 0.3–1.1 × 0.4–2.6 cm, lamellae yellowish orange (4A8), melon (5A6) or melon with yellowish orange tint at the edge of the pileus; pileus surface

cinnamon (6D6) to rust (6E8).

June 18: Basidiomata 1.6–2.4 × 1.8–3.9 cm, lamellae of all basidiomata corn (4B5) without any yellowish-orange or melon colouration. Colour of basidiomata not exposed to rain has not changed. Exposed basidiomata have lost their initial brownish fibrils, appearing cream (4A3). Two new basidiomata grew on the trunk: 0.8–0.9 × 1.1–1.7 cm, lamellae yellowish-orange (4A8) and pileus surface cinnamon (6D6).

June 20: Only five fruitbodies (of 19 previous) with distinct characters survived: 2–2.3 × 2.3–2.9 cm, colour of lamellae and pileus surface were unchanged. Lamellae of the remaining decaying basidiomata were honey yellow (5D6). Basidiomata that appeared 16–18 June had yellow ochre (5C7) lamellae, colour of pileus surface unchanging.

Phylogenetic analyses

Nine *Crepidotus* taxa were analyzed, representing a cross-section of the genetic variation already found within the genus in other studies (Aime, 2001). *Crepidotus nephrodes* and *C. crocophyllus* from North America and Slovakia form a single monophyletic lineage, sister to *C. malachius* (Berk. & M. A. Curtis) Sacc. (Fig. 1). A total of 892 equally-weighted characters were aligned across all bases. Of these, 71 were parsimony-informative and 773 were constant. The MP analyses produced a single most parsimonious solution of 204 steps with a CI of 0.696 and RI of 0.684. Both MP and ML analyses produced trees with identical branching topologies.

Taxonomy

Crepidotus crocophyllus (Berk.) Sacc., Syll. Fung. 5:886, 1887.

≡ *Agaricus crocophyllus* Berk., London J. Bot. 6:313, 1847.

≡ *Crepidotus applanatus* var. *crocophyllus* (Berk.) Pilát, Atlas Hub Evrop. 6: 33, 1948.

= *Agaricus nephrodes* Berk. & M.A. Curtis, Ann. Mag. Nat. Hist., ser. 2, 12:442, 1853.

≡ *Crepidotus nephrodes* (Berk. & M.A. Curtis) Sacc., Syll. Fung. 5:882, 1887.

= *Crepidotus fulvifibrillosus* Murrill, North Amer. Flora 10:153, 1917.

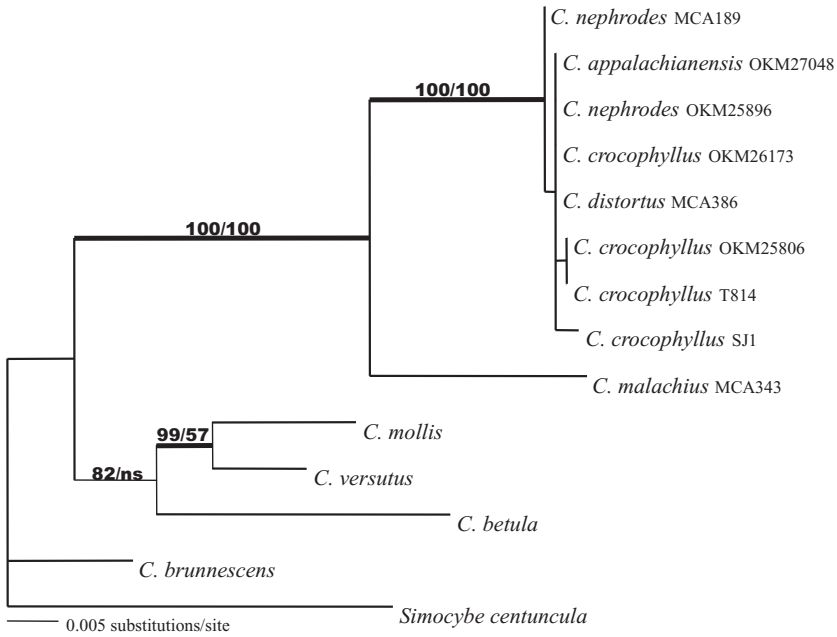
≡ *Crepidotus applanatus* var. *fulvifibrillosus* (Murrill) Pilát, Atlas Champ. Eur. 6: 35, 1948.

= *Agaricus dorsalis* Peck, Rep. (Annual) New York State Mus. Nat. Hist. 24: 69, 1972.

≡ *Crepidotus dorsalis* (Peck) Sacc., Syll. Fung. 5:883, 1887.

The description, based on fresh and herbarium specimens, expresses our taxonomic concept of the species and variability of its characters.

Pileus 7–78 × 5–55 mm, flabelliform, rounded flabelliform, spathuliform, reniform, conchate or irregularly circular, at first hemispherical, convex, soon plano-convex, later applanate, laterally or dorsally attached to the substratum, non-hygrophanous. **Margin of pileus** at first involute, soon inflexed, later straight, plane, smooth. **Pileus surface** at first squamulose, soon fibrillose, fibrils brownish orange (6C8), cinnamon (6D6), rust (6E8), or light yellow (4A4), at point of attachment tomentose and cream (4A3), maize (4A6), or butter yellow (4A7). **Pileipellis** a cutis composed of 4.5–7.5 μm thick, cylindrical, thin-walled, hyaline hyphae. Squamules formed by 6–19 μm thick, cylindrical, thin-walled, incrusted, hyaline to coloured, ascending hyphae. Their terminal cells are 7.5–18 × 41.5–97.5 μm, narrowly utriform, conical, cylindrical or



fusiform, at apex subcapitate or billed, thick-walled, without incrustations, coloured. **Pileus trama** up to 5 mm wide, thicker below point of attachment, whitish, composed of 7.5–14 μm thick, cylindrical, short-celled hyphae. **Lamellae** L = 10–32, l = 1–3, up to 6 mm wide, subventricose, ventricose, crowded, adnexed, narrowly adnate, at first yellowish orange (4A8), melon (5A6), apricot (5B6) or carrot orange (6B7), soon yellow ochre (5C7) or brownish orange (6C8), but also light yellow (4A4) or corn (4B5), later honey yellow (5D6) or cinnamon (6D6). Edges fimbriate, whitish. **Basidiospores** (4.5) 5–7 μm, globose to subglobose, baculate, yellowish. **Spore print** honey yellow (5D6). **Basidia** 6–7.5 × 22.5–30 μm, 4-spored, cylindrical or narrowly clavate, thin-walled. **Cheilocystidia** 28.5–57 × 6–9 μm, clavate, cylindrical, less flexuose, often narrowly lageniform, narrowly utriform or utriform, at apex capitate, subcapitate or very rarely branched, rarely septate, thin-walled, hyaline. **Clamp connections** present. **Stipe** only in very young fruitbodies recognisable, 1–1.5 × 1–2 mm, lateral and reduced, squamulose, brownish orange (6C8), soon absent.

Specimens studied – Slovakia: Záhorská nížina lowland, Brodské, boggy forest, on decaying trunk of Quercus sp., 6 June 1971, A. Dermek (BRA); Brodské, 8 May 1972, A. Dermek (BRA); Jakubov, Nature Reserve of Šmolzí (Jakubov Feld), flood-plain forest, 200 m, on rotten trunk of *Alnus?,* 24 Sept. 1993, P. Škubla (BRA); Moravský Svätý Ján, ca 4.7 km W from the St. Ján Krstiteľ church, flood-plain forest (Salicion albae), 153 m, on wood of fallen, rotten trunk of *Populus nigra,* 24 July 2001, V. Kučera & K. Janovicová (SLO). Podunajská nížina lowland, Bratislava – municipal part Devín, Sedláčkov ostrov Island, flood-plain forest, ca 135 m, on bark of fallen, decaying trunk of *Fraxinus* sp.,

2 July 1997; on wood of fallen, decaying trunk of *Fraxinus* sp., 2 Sept. 1998; on wood of fallen, decaying trunk of *Negundo aceroides,* 6 Aug. 1998; on wood of fallen, rotten trunk of *Negundo aceroides,* 16 July 1999, (all specimens) S. Ripková (SLO); Bratislava – municipal part Karlova Ves, Sihoť Island, flood-plain forest, ca 135 m, on wood of fallen, decaying branch of *Fraxinus* sp., 7 Aug. 1997, 4 Sept. 1999; on wood of fallen, decaying trunk of *Fraxinus* sp., 11 Aug. 1997, 16 Oct. 1997; on wood of fallen, decaying branch of *Negundo aceroides,* 18 Aug. 1997, 22 Sept. 1998; on wood of fallen, decaying trunk of *Negundo aceroides,* 19 Aug. 1999; on wood of fallen, rotten trunk of *Negundo aceroides,* 23 Sept. 1997, 7 June 1999, 16 June 1999, 18 June 1999, 20 June 1999, 24 Aug. 1999, 21 July 1998; on wood of fallen, decaying trunk of *Populus* sp., 7 Oct. 1998, 9 June 1999, 7 Oct. 1999; on wood of fallen, rotten trunk of *Populus* sp., 24 Aug. 1999, 2 Aug. 2001; on wood of fallen, rotten branch of deciduous tree, 1 June 1999 (all specimens) S. Ripková (SLO); same locale, on fallen trunk of *Populus* sp., 13 May 1993, S. Adamčík (BRA); Bratislava – municipal part Podunajské Biskupice, deciduous forest (*Acer, Cornus, Fraxinus, Populus, Quercus, Robinia, Salix, Swida, Ulmus*) at the SW Slovnaft's refinery edge, Q 7869c, ca 135 m, on wood of fallen, rotten trunk of *Fraxinus* sp., 2 Aug. 2002, S. Ripková (SLO). Strážovské Vrchy Mts., Nitrianske Rudno, valley of the Rudnianka stream, 330 m, on dead branch of Salix caprea, 14 July 1984, J. Kuthan [as *C. mollis*] (BRA). Vihorlatské vrchy Mts., valley of the Dielový potok stream, ca 3 km SW from a church in Podhoroď, ca 250 m, on bark of fallen, decaying trunk of Salix caprea, 12 June 2002, S. Ripková (SLO). Bukovské vrchy Mts., Kalná Roztoka (district of Humenné), ridge of Nastaz Mts., on the slope of Kalidlo hill, Nature Protected Area of Havešová, 650 m, on fallen trunk of *Fagus sylvatica,* 21 June 1991, J. Terray [as *C. applanatus*] (BRA); Kalná Roztoka, on fallen branch of *Fagus sylvatica,* 18. Sept. 1995, S. Adamčík [as C. mollis] (BRA). **Bulgaria:** Nature Protected Area of "Ropotamo", Reserve of "Veliov vir", valley of the Ropotamo river, 30 m, on decaying trunk of *Fraxinus* or *Ulmus*, 15 June 1972, J. Kuthan (BRA). **Ukraine:** Eastern Carpathians, mixed virgin forest (*Abies alba, Fagus sylvatica*), on the ridge of Menchul [Menčul] Mts., between the Kuzy and Bredecel rivers, near the town of Dilove [Trebušany], 800-1200 m, on *Fagus sylvatica,* Aug. 1934, A. Pilát (PRM 23483); same locale (PRM 23481); Eastern Carpathians, forest at the Kuzy river, near the town of Vel. Bichkiv [Veľký Bočkov], 350-1000 m, on Padus avium, July 1933, A. Pilát (PRM 23482); same locale, on *Fagus sylvatica* (PRM 23484); Eastern Carpathians, mixed virgin forest (*Abies alba, Picea excelsa, Fagus sylvatica* etc.), valley of the Liščenka river, near the town of Dilove [Trebušany], 800-1000 m, on *Fagus sylvatica,* Aug. 1936, A. Pilát (PRM 28616) [all specimens as *C. applanatus* f. *kuzyanus*]

Discussion

Aime (2004) showed that some members of *Crepidotus* in subsection *Fulvifibrillosi* produced hyphae of variable pigmentation in culture, ranging from hyaline to deep orange. This variation in pigmentation mirrors that which we have found in fruitbody development from a single thallus in the field. It was also demonstrated *via* mating studies that collections identified as *C. nephrodes* and *C. crocophyllus* were intercompatible in North America, although she did not treat the taxonomy of this group. Our analysis of the sequencing data suggests that *C. crocophyllus* in Central Europe is also most likely part of this same biological species although we were unable to obtain single spore isolates of the Slovak collections in order to test mating compatability. Nevertheless, all available data suggest that *C. crocophyllus* is a common fungus in hardwood forests throughout North America and Europe that is variably pigmented throughout its range.

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