Dermocybe, section *Sanguineae*: A look at species relationships within the *sanguinea* complex*

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Dermocybe marylandensis Ammirati, sp. nov. is described and discussed in relation to *D. sanguinea* var. *sanguinea, D. sanguinea* var. *vitiosa*, and *D. sierraensis*. A phylogenetic tree for selected *Dermocybe* species based on DNA sequence data is presented. It is suggested that the section *Sanguineae* could represent more than one line of evolution.

Keywords: Agaricales, *Cortinarius*, PCR, phylogenetic inference using parsimony, taxonomy.

For over a century the name *Dermocybe sanguinea* (Wulf. in Jacq.: Fr.) S. F Gray has been used in North America for speciemens from conifer forests with blood red pileus, lamellae and stipe (Ammirati, 1989, 1972; Smith, 1939; Kauffman, 1932, 1918; Peck, 1873). In addition to *D. sanguinea*, some provisional species in the *D. sanguinea* complex were noted by Ammirati (1972). In the 1970s collecting in the western mountains by J. Ammirati, A. H. Smith and H. D. Thiers, and in eastern North America by J. Ammirati produced several new species (Ammirati, 1989; Ammirati & Smith, 1984), including *Dermocybe sierraensis* Ammirati (1989) and *D. marylandensis* Ammirati, sp. nov. described below. The anthraquinone pigments of these North America taxa were reported by Keller and Ammirati (1983).

In this paper *Dermocybe sanguinea* var. *sanguinea, D. sanguinea* var. *vitiosa, D. sierraensis* and *D. marylandensis* are compared in terms of macroscopical, microscopical, ecological and chemical characteristics. In addition a preliminary phylogeny based on DNA

* This paper is dedicated to Professor M. Moser on the occasion of his seventieth birthday.

sequence data is presented for selected species of *Dermocybe*, including *D. sanguinea* var. *sanguinea* and *D. marylandensis*.

Materials and methods

Descriptive Studies

All microscopic descriptions were made from sections or pieces of tissue taken from dried sporocarps and mounted in 3% KOH. Capitalized color notations are from Ridgway (1912). Colors with the following type of designation, 7/A8, are from the Kornerup & Wanscher (1978). All collections are deposited in the University of Washington Herbarium (WTU) unless otherwise noted by use of abbreviations from *Index Herbariorum*.

Scanning Electron Microscopy (SEM)

Photographs of basidiospores were taken with a JEOL scanning electron microscope. Basidiospores were coated with gold/palladium (60/40) in a sputter-coater to an approximate thickness of 200–300 Å.

PCR Amplification and Sequencing

DNA was extracted using a CTAB extraction method (Rogers & al., 1989; Rogers & Bendich, 1994, 1988, 1985) from the following collections: *Dermocybe semisanguinea* (Fr.: Fr.) Moser [JFA 1650 (MICH), JFA 7908], *D. sanguinea* [JFA 8533, JFA 9748], *D. malicoria* (Fr.) Ricken [JFA 10074], *D. marylandensis* [JFA 7935], *D. crocea* (Schaeff.: Fr.) Moser [JFA 9732], *D. cinnamomea* (L.: Fr.) Wünsche [JFA 9762], and *Cortinarius limonius* (Fr.: Fr.) Fr. [JFA 9796]. See Tab. 1 for collection data. An aliquot of 10 ng was subjected to PCR amplification using equal concentrations of primers ITS4 and ITS5 (White & al., 1990). The thermal cycling program (using a Thermal Controller, MJ Research) was: 95° C for 2 min, then 35 cycles of 1 min @ 95° C, 2 min @ 55° C and 2 min @ 72° C. This was followed by a 10 min incubation @ 72° C. The amplified products were then subjected to restriction enzyme digestion, agarose gel electrophoresis and mapping using the following enzymes: *AluI, AvaII, ClaI, DdeI, EcoRI, HaeIII, HhaI, HinfI, MspI, Sau3AI, StuI, Sau96AI and TaqI.*

Asymmetric PCR (McCabe, 1990) was used to generate single-stranded DNA templates for sequencing. Primers ITS2, ITS3, ITS4 and ITS5 (White & al., 1990) were used in PCR reactions under the same conditions as above, except a 20:1 ratio of the first primer to the

Tab. 1. *Dermocybe* and *Cortinarius* collections for DNA extraction, PCR Amplification and Sequencing.

Species	Collection number	Site of Collection	Collector
C. limonius	JFA 9796	Tiers, Italy	J. Ammirati
D. cinnamomea	JFA 9762	Absam-Eichat, Austria	J. Ammirati & G. Keller
D. crocea	JFA 9732	Igls, Austria	J. Ammirati & M. Moser
D. malicoria	JFA 10074	Easy Pass Trailhead, North Cascades, Washignton, USA	J. Ammirati & M. Moser
D. marylandensis	JFA 7935	Gainesville, Florida, USA	J. Ammirati
D. sanguinea	JFA 8533	Redmond, Washington, USA	J. Ammirati
D. sanguinea	JFA 9748	Gnaldenwald, near Speckbacher	J. Ammirati & R. Pöder
D. semisanguinea	JFA 1650	Marquette, Michigan, USA	J. Ammirati & I. Bartelli
D. semisanguinea	JFA 7908	Macey Lake Bog, Ontario, Canada	J. Ammirati & C. Ovrebo

second primer was used. This amplified one strand to a greater extent than the other. The amplified templates were extracted with chloroform and precipitated in 0.3 M ammonium acetate and 70% ethanol. DNA sequencing was then carried out using the dideoxynucleotide chain termination methods (Sanger & al., 1977). Sequencing reactions were labeled with ^{35}S-dATP Annealing was at 65° C to avoid denaturation of the double stranded amplification products carried over from PCR amplification.

Phylogenetic Analysis

Branch and bound as well as heuristic searches using PAUP (Phylogenetic analysis using parsimony, version 3.0o, Swofford, 1986) were used on both restriction enzyme and sequencing data sets. The strengths of the branches were statistically tested by bootstrap analysis using 1000 replications.

Description of *Dermocybe marylandensis*

Dermocybe marylandensis Ammirati, sp. nov.

= *Cortinarius marylandensis* G. Keller & J. F Ammirati (‚nom. prov.'). Mycotaxon 18 (2): 364. 1983.

= *Cortinarius marylandensis* J. F Ammirati and A. H. Smith (‚nom. prov.'). McIlvianea 6 (2): 54. 1984.

Pileus 19–55 mm latus, primo rotundatus, disco obtuso, demum obtuse umbonatus vel plano-convexus, umbone lato, vel plano-umbonatus, extremus incurvatus vel decurvatus, superficie, praecipue disci, dense et subtiliter fibrilloso-tomentoso, margine plerumque magis fibrilloso, interdum extremo squamulis fibrillosis induto, humido vel sicco, lente hygrophano, colore brunneo-rubra in brunneo-aurantiacum vel ferrugineum vel surde pallideque cinnamomeum vergente, disco quam margo magis rubro manente. Contextus pilei tenuis, aliquid fragilis, coloribus superficialibus tinctus, in stipite farctus, demum excavatus, cum pilei contextu subconcolor, cortice cum superficie concolori. Lamellae densae, adnexae, sinuatae, vel uncinatae, interdum in lineam decurrentem desinatae, pilei colori similes, e sporis ferrugineae vel ferrugineo-brunneae desinentes. Stipes 23–65 mm longus, 1.5–9.5 mm crassus, supra subaequalis, basi subdilatata, apice sericeo nitidoque, coloribus pilei tinctus vel sub apice cum pileo concolori, mycelio basali pallide vinaceo-roseo vel roseo-alutacea. Cortina roseo-rubra vel cum stipite concolor. Basidiosporae (6.6–)7.4–8.1 (–8.5–9.3) × (4.1–)4.4–4.8 (–5.6) µm, oblique observatae ellipticae vel anguste ellipticae, antice ellipticae vel lato-ellipticae, plus minusve verruculosae, in medio lixivio pallide luteo-brunnea vel luteo-brunneae. Basidia sterigmatibus 4 ornata.

Gregarii vel dispersi in silvis ex angiospermis variis vel cum *Pinus* commixtis, e mense Junio ad Septembrem vel usque ad Novembrem fructiferentes.

Holotypus: Devil's Mill Hopper, prope Gainesville, comitatu Alechua, civitatis Floridae, J. F Ammirati 7936, in herb. WTU conservatus.

P i l e u s 10–55 mm broad, rounded with an obtuse disc at first, becoming obtusely umbonate to plano-convex with a broad umbo or plano-umbonate, outer margin incurved to decurved, sometimes nearly plane or at times more or less irregular to upturned or slightly recurved, margin sometimes split or somewhat lobed, surface densely and finely fibrillose-tomentose especially on the disc, margin usually more fibrillose, sometimes with small fibrillose scales on the outer margin, moist to dry, slowly hygrophanous, the margin often lighter in color than the disc when fully expanded, color at first ±8C/7 (brownish red) to Claret Brown to Morocco Red or Brick Red or sometimes Ocher Red to Etruscan Red, becoming paler shades of these brownish red colors or Terra Cotta to 7/C7 (brownish orange) or sometimes developing Ferruginous to dull light Cinnamon colors, disc usually remaining more reddish than the margin, but margin usually retaining at least some reddish to vinaceous color. Odor usually fungoid to slightly raphanoid. Taste fungoid or slightly raphnoid to slightly bitterish. – C o n t e x t of pileus thin, up to about 2.5 mm on the disc, solid, somewhat fragile, tinted with surface colors, flushed pale reddish or faded to pinkish white, vinaceous white or mottled whitish, in stipe stuffed becoming hollow, somewhat fibrous, in pith more or less concolor with the pileus context, cortex concolor with the stipe surface, darkening somewhat after cutting or handling. – L a m e l l a e adnexed to sinuate or uncinate, at times with a slight

decurrent line or tooth, some seceding in age, close, lamellulae usually three, up to 5 mm broad, more or less ventricose mature, moderately thin, edges even to uneven or wavy, color similar to the pileus surface or near Victoria Lake to Morocco Red or Claret Brown becoming ferruginous or shaded rusty brown from basidiospores. – Stipe 23–65 mm long, 1.5–9.5 mm at apex, ± equal above, ± enlarged at base, apex ± silky and shiny, tinted with pileus colors or ± pinkish red, below the apex ± Pompeian Red, Etruscan Red to ± Brick Red or concolor with the pileus surface, sometimes streaked with dark red fibrils, usually darkening where handled, basal mycelium pale vinaceous pink to pinkish or pinkish buff. – Cortina pinkish red or ± concolorous with the stipe apex. – Pleurocystidia absent, but with occasional long, clavate-mucronate (unisterigmate) elements. – Cheilocystidia 12.4–19.7 × 5.8–9.5 µm, ± common, sometimes intermixed with basidia, clavate to broadly clavate or even more enlarged, some irregularly shaped and/or with tapered ends, in KOH as basidia. – Lamella trama subparallel to interwoven, hyphae 2.9–29 µm in diameter, cylindrical to inflated, in KOH hyaline, pale pinkish, pale purplish pink or with dense reddish pigment or granules; Subhymenium of narrow, compactly interwoven hyphae. – Pileus cuticle usually well defined, hyphae ± interwoven, ± radially oriented, 3.7–22.2 µm, cylindrical to enlarged, end cells upturned in places but not cystidiate, with thin- to some thicken walls, some encrusted, in KOH hyaline, pale grayish to pale brownish, pale pinkish to pale purplish pink or with ± dense dull reddish to brownish purple or light purplish pink pigment. – Pileus subcuticle in KOH often appearing as a pale brownish zone between the cuticle and the trama, hyphae similar in size and shape to the pileus trama but sometimes with a considerable number of enlarged elements and appearing somewhat cellular. – Pileus trama interwoven, hyphae ± radially oriented, 3.7–33.2 µm, cylindrical to enlarged, some encrusted, in KOH as the lamellar trama. – Clamp connections present. – Basidiospores (6.6–)7.4–8.1(–8.5–9.3) × (4.1–)4.4–4.8(–5.6) µm, in profile elliptic to narrowly elliptic, in face view elliptic to ± broadly elliptic, ± verruculose, in KOH pale- to light yellow-brown to light brown. – Basidia 20.4–29.9 × 5.1–8.8 µm, tetrasterigmate, broadly clavate to narrowly clavate, in KOH hyaline, pale pinkish or with dark reddish pigment or granules.

Habit and habitat – Gregarious to scattered in mixed hardwoods (*Quercus, Carya, Fagus, Liriodendron*) or with *Pinus* and hardwoods together. Fruiting from June to September or as late as November. Distribution, as presently known, from the southern to the eastern United States.

Collections examined U.S.A: Delaware: Castle County. J. F. Ammirati 6549, near Wilmington, collected with D. Simons. Florida: Alachua County. J. F. Ammirati 7935, 6 miles N.W of Gainesville, University of Florida Horticulture Station; J. F Ammirati 7936 (Holotype), Devil's Mill Hopper, near Gainesville, collected with Dave Malloch and Walt Sundberg; J. F. Ammirati 7940, Sugarfoot Hummock, near Gainesville. Maryland: Prince George's County. J. F. Ammirati 6258, 4601 Wicomico Avenue, Beltsville. Virginia: King George County. J. F. Ammirati 6481, King George, collected by Joe Salvino.

Discussion and comparison of taxa

Some basic taxonomic features and pigmentation data for *Dermocybe sanguinea* var. *sanguinea, D. sanguinea* var. *vitiosa, D. sierraensis* and *D. marylandensis* are presented in Tab. 2. Basidiospore measurements for all of the taxa are similar and not diagnostic. Basidiospores of all species are ± verruculose and with the scanning electron microscope the ornamentation size and pattern is similar to that of *D. marylandensis* (Fig. 1). Cheilocystidia have been reported in *D. sanguinea* var. *sanguinea* (Ammirati, 1989; Høiland, 1984) and occur in *D. marylandensis* as well. They have not been seen in *D. sierraensis* and material of *D. sanguinea* var. *vitiosa* was not available for examination. However, Høiland (1984) reported cheilocystidia in Nordic collections. Cheilocystidia are not well differentiated in these taxa (varying from clavate to broadly clavate to vesiculate and at times are somewhat irregularly shaped) and their number and distribution do not seem to be very constant Therefore they do not appear to be very helpful taxonomically. The pileus subcuticle (hypodermium) of *D. sanguinea* often contains inflated or enlarged hyphae and sometimes apppears ± differentiated from the pileus cuticle and trama (Høiland, 1984). In *D. marylandensis* the pileus subcuticle is differentiated in a similar manner. The pileus subcuticle in *D. sierraensis* is not well differentiated; material of *D. sanguinea* var. *vitiosa* was not available for study. This charateristic requires further evaluation in the *Sanguineae* but likely will not be diagnostic.

The coloration of the sporocarps is helpful in differentiating the above *Dermocybe* species. *Dermocybe sanguinea* var. *sanguinea* typically has blood red pileus, lamellae and stipe but with the basal mycelium either orange-yellow, ochraceous, or ochraceous tinted with pink or orange (Ammirati, 1989; Moser, 1983). Høiland (1984) reported a broader range of colors for the basal mycelium of var. *sanguinea,* 'the base with bright rose, carmine or orange-red mycelial felt' His concept of var. *sanguinea* includes *D. punicea* (Orton) Moser. The basal mycelium in *D. marylandensis*, *D. sierraensis* and *D. sanguinea* var. *vitiosa* are typically rose to vinaceous or pinkish to pinkish buff with no development of ochraceous or orangish colors in

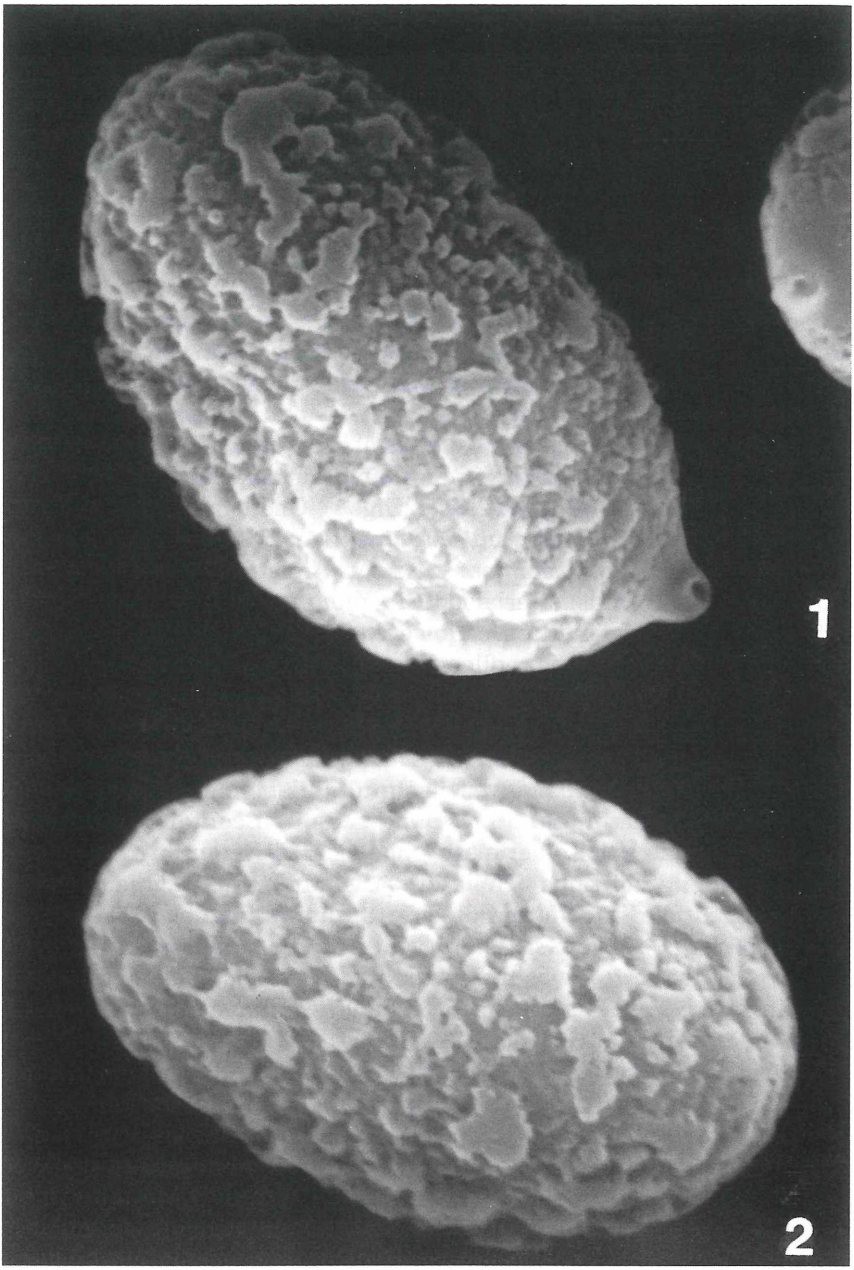

Fig. 1. – *Dermocybe marylandensis*. SEM photographs of basidiospore ornamentation, × 15000 (JFA 6258).

populations studied to date (Ammirati, 1989; Moser, 1983). The lamellae of *D. marylandensis* range in color from red to brownish red when fresh, those of *D. sierraensis* are typically red, and those of *D. sanguinea* var. *vitiosa* are brown-red or browner than in var. *sanguinea*. Stipe colors (excluding basal mycelium) for all species tend to be similar to their pileus surface colors, however, in var. *vitiosa* the apex is very lightly colored and often decidely whitish.

The coloration of veil fibrils on the stipe is another interesting feature of these species. In *D. marylandensis* they are concolorous with the stipe surface or may be darker red in color. In *D. sanguinea* var. *sanguinea* the color of the veil fibrils ranges from carmine to blood red or orange to somewhat ochraceous. The orange to ochraceous coloration is sometimes more characteristic of the fibrils nearer to the stipe base. *D. punicea* was distinguished in part from *D. sanguinea* by Orton (1958) on the basis of the ochraceous to golden brown cortina forming zones near the stipe apex. It is found in deciduous woods or mixed woods. Material matching the description of *D. punicea* (JFA 6482) was found in mixed woods in Virginia, USA during July. The specimens had a rusty ochraceous to rusty golden brown cortina and rusty ochraceous fibrils on the stipe and over the pileus surface. The coloration of the veil and the habitat would appear to make this a distinct taxon in North America. Its relationship to other species in this group is currently being investigated. For now it is placed under *D. punicea*. For *D. sanguinea* var. *vitiosa* the color of the veil has not been reported. In *D. sierraensis* the veil is concolorous with the stipe surface.

The pileus surface of *D. marylandensis* is fibrillose to silky on the margin with some small fibrillose scales in certain specimens; the disc is often more fibrillose tomentose, especially in younger stages. Its pileus is slowly hygrophanous especially on the margin and fades much more extensively than typical material of *D. sanguinea* var. *sanguinea*. Older specimens of *D. marylandensis* often can be very ferruginous having a coloration similar to some North American specimens in the *D. cinnabarina* complex. The latter of course differs significantly in its pigmentation as well as other features (Ammirati, 1972; Keller & Ammirati, 1983).

The ecology and distribution of *D. marylandensis* is very different from *D. sanguinea* var. *sanguinea* in North America. The former occurs in either hardwood forests or forests with a mixture of pine and hardwoods, habitats similar to that for members of the *D. cinnabarina* group. Records indicate that it occurs from Texas to the southeastern United States and northward at least to Massachusetts. The distribution of *D. sanguinea* var. *sanguinea* closely follows the pattern of more northern conifer forests but is most frequent in the Pacific Northwest and from the Great Lakes region to Maine and into Canada. Specimens tentatively named *D. punicea* have been found

Tab. 2. – Comparison of *Dermocybe sanguinea* var. *sanguinea*, *D. sanguinea* var. *vitiosa*, *D. sierraensis* and *D. marylandensis*.

Characteristics	*sanguinea* var. *sanguinea*	*sanguinea* var. *vitiosa*	*sierraenssis*	*marylandensis*
Habitat	conifer forests	conifer forests	*Pinus contorta* high elevations	hardwoods, *Pinus* and hardwoods
Distribution	North America, Europe	Europa	California	southern to eastern United States
Basidiospore measurements	6.5-9 × 4.5-5	*6-9 × 4-5µm	7.7-9.1 × 4.8-5.5 µm	7.4-8.1 × 4.4-4.8µm
Pileus color	deep red to brownish red	brown-red to light brown	brownish orange to brownish red-orange	brownish red to ferruginous
Basal mycelium of stipe	ochraceous or ochraceous tinted orange or pink	rose	vinaceous	pale vinaceous pink, pinkish or pinkish buff
Pigment** (color)				
Emodin (yellow)	++++		++	
Emodin-glucoside (yellow)	++++		+++	
Dermoglaucin (brown)	++	+	++	++
Dermoglaucin-glycoside (brown)	+++	+	++	++
Dermocybin (purple)	++++	++	+++	+++
Dermocybin-glucoside (purple)	++++	+++	+++	++
Endocrocin (yellow)	-	(+)		+
Endocrocin-glycoside (yellow)	-			
Dermolutein (yellow)	+	++	+	++
Dermolutein-glycoside (yellow)	+		(+)	+
Dermorubin (pink)	++	+	++	++
Dermorubin-glycoside (pink)	+	+	+	+

* Moser (1983) for *D. sanguinea*. ** Pigment data for *Dermocybe sanguinea* var. *vitiosa* from Keller in Moser (1983); otherwise from Keller & Ammirati (1983). - indicates pigment not detected; (+) trace amounts; +, ++, +++, ++++ indicate relative intensity.

only in Virginia associated with mixed hardwoods. *Dermocybe sanguinea* var. *vitiosa* occurs in conifer forests in Europe, and *D. sierraensis* has only been found in lodgepole pine forests in the high Sierra Nevada Mountain Range of California.

The pigmentation of *Dermocybe marylandensis, D. sierraensis,* and *D. sanguinea* var. *sanguinea* was reported by Keller & Ammirati (1983) and that of *D. sanguinea* var. *vitiosa* by Keller (1982) and Moser (1976) (Tab. 2). Høiland (1984) also studied the pigments in *D. sanguinea* and an overview of the anthraquinonic pigments for all species is presented in Gill & Steglich (1987). *Dermocybe sierraensis* and *D. marylandensis* have pigmentation similar to *D. sanguinea*. The former has a lower intensity of emodin and dermocybin than *D. sanguinea* but the pigment patterns have the same specificity (Keller & Ammirati, 1983). *Dermocybe marylandensis* in contrast to *D. sanguinea* var. *sanguinea* and *D. sierraensis* lacks emodin (Keller & Ammirati, 1983) a feature it has in common with *D. sanguinea* var. *vitiosa* (Keller, 1982; Moser, 1976). This presents the possibility two chemical groups in the *sanguinea* complex, one containing *D. sanguinea* var. *sanguinea* and *D. sierraensis* and another composed of *D. marylandensis* and *D. sanguinea* var. *vitiosa*. According to Høiland (1984) *D. punicea* has the same pigmentation as *D. sanguinea* var. *sanguinea*. Also, Høiland (1984) listed *D. sanguinea* var. *vitiosa* as a synonym of *D. phoenicea* stating that it was a dark red smaller form of the latter with pigmentation almost identical to it.

Results and discussion of DNA restriction map and sequence data

Restriction map and sequence data indicate that the *Dermocybe* species presented here form a coherent group, separate from *Cortinarius*. Of the 30 restriction sites scored, from 10 to 12 site differences were found between the *Dermocybe* species and *Cortinarius limonius*. *Dermocybe marylandensis* was the species closest to *C. limonius* (10 site differences). Among the *Dermocybe* species, from 2 to 8 site differences were found (data not shown). The closest species to *D. marylandensis* were *D. semisanguinea* and *D. malicoria* (2 site differences each). Using a branch-and-bound search, five equally parsimonious trees were found. In each case *Cortinarius* was well separated from all species of *Dermocybe*. The placement of *D. marylandensis* and *D. malicoria* varied somewhat, although both were closer to each other than they were to other species. *Dermocybe semisanguinea* was also placed close to these two species, while the other species were all clearly separated, with *D. crocea* and *D. cinnamomea* always on the same clade. The sequence data mirrored these relationships (Fig. 2). The resolution and strength of the results,

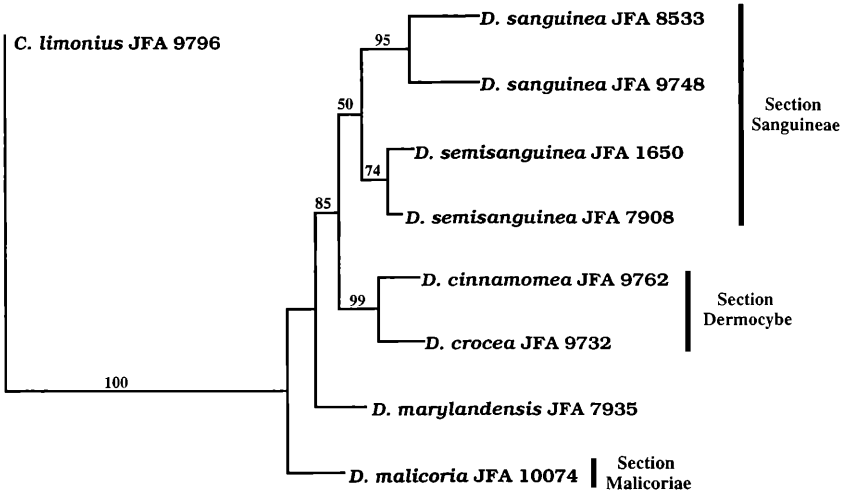

Fig. 2. – Unrooted phylogenetic tree for *Dermocybe* species based on DNA sequence data of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene. The length is 78 steps. The phylogram was generated using the branch and bound, as well as the heuristic search modes in PAUP (Swofford, 1986). Only one most parsimonious tree was generated from the sequence data. The values above the branches are percentages based on frequencies in which the branch appeared in 1000 bootstrap replications. Values below 50 are not shown.

however, were greater with the sequence data. A total of 744 nucleotide positions were scored. Due to gaps, the total number of nucleotides per species averaged 720. Again, all of the *Dermocybe* species clustered in a coherent group separate from *Cortinarius limonius*. Both results are consistent with *Dermocybe* being a monophyletic group. The branches of the phylograms (using PAUP) indicate that the sections *Sanguineae, Dermocybe* and *Malicoriae* form coherent subgroups for the most part. Data from Høiland (1984) and other data (not shown) indicate that some members of the *Sanguineae* are variable and occasionally do not fit well in the group. This will be discussed in a later paper. All branches of the phylogram (Fig. 2) were supported in bootstrap analysis. Furthermore, when other species within these groups were added to the analyses, the same relationships were found (data not shown). In all instances *Dermocybe marylandensis* clustered closest to *D. malicoria* (section *Malicoriae*).

The branch separating *Cortinarius* from *Dermocybe* was supported in 100% of the bootstrapped data sets. Separation of the members of section *Sanguineae* from the section *Dermocybe* was supported in 85% of the bootstrapped sets, while separation of the clades within the two sections were supported in 50 to 99% of the bootstrapped data sets. The branch separating *D. marylandensis* from *D. malicoria* was supported

well below the 50% level, indicating either a close relationship between the two, or insufficient data to distinguish between the two species.

Conclusions

The DNA restriction and sequence data strongly support other data (pigmentation, coloration and ecological) showing that *Dermocybe marylandensis* is clearly a distinct species from *D. sanguinea* var. *sanguinea*. DNA studies of *D. sierraensis* are in progress and we have plans to compare *D. sanguinea* var. *vitiosa* with North American taxa. Based on pigmentation the latter should be related to *D. marylandensis*. This, however, remains to be seen. There are several taxa to evaluate in such an analysis, including the *D. semisanguinea* complex as well as *Cortinarius* (*Dermocybe*) *fervidus* Orton and *D. phoenicea* (Bull. ex Mre.) Moser and it varieties. Also one would include *D. idahoensis* (Ammirati & Smith) Ammirati and *Cortinarius* (*Dermocybe*) *sommerfeltii* Høil., considered by Høiland (1984) to be part of the *Sanguineae*. Preliminary DNA data from *D. idahoensis* (not shown here) groups it with *D. malicoria* and *C. fervidus*. In this paper a close relationship is shown between *D. marylandensis* and *D. malicoria,* with *D. sanguinea* var. *sanguinea* distinctly separate from these species. It will be interesting to see whether or not there is more than one line of evolution leading to red and/or red-brown *Dermocybe* species. It may be that the traditional section *Sanguineae* is polyphyletic.

Acknowledgments

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References

Ammirati, J. F (1972). The section *Dermocybe* of *Cortinarius* in North America. – Ph. D. Thesis, University of Michigan, Ann Arbor, Michigan, 282 pp.

— (1989). *Dermocybe,* subgenus *Dermocybe,* section *Sanguineae* in Northern California. – Mycotaxon 34 (1): 21–36.

— & A. H. Smith (1984). *Cortinarius* II: A preliminary treatment of species in the subgenus *Dermocybe,* section *Sanguinei* in North America north of Mexico. – McIlvainea 6 (2): 54–64.

Gill, M. & W. Steglich (1987). Pigments of fungi (Macromycetes). – Prog. Chem. Organ. Nat. Prod. 51: 1–317.

Høiland, K. (1984). *Cortinarius*, subgenus *Dermocybe*. – Opera Bot. 71: 1–113.

Kauffman, C. H. (1918). The Agaricaceae of Michigan. – Michigan Geol. Biol. Surv. Publ., Biol. Ser. 1: 314–442.

— (1932). *Cortinarius* Fries. – North American Fl. 10: 292–348.

Keller, G. & J. F Ammirati (1983). Chemotaxonomic significance of anthraquinone derivatives in North American species of *Dermocybe*, section *Sanguineae*. – Mycotaxon 18:357–377.

Kornerup, A. & J. H. Wanscher (1967). Methuen Handbook of Colour, 2nd. – ed. E. Methuen, London. 243 pp.

McCabe, P. C. (1990). Production of single stranded DNA by asymmetric PCR. – In: Innis, M. A., D. H. Gelfand, J. J. Sninsky and T. J. White (eds.). PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, pp. 76–83.

Moser, M. (1976). Die Gattung *Dermocybe* (Fr.) Wünsche (Die Hautköpfe). Schweiz. Z. Pilzk. 54 (10): 145–150.

— (1983). Keys to Agarics and Boleti. – R. Phillip (G. Fischer), Stuttgart, 535 pp.

Peck, C. H. (1873). Report of the Botanist. – New York State Bot. Rep. 23: 27–135.

Ridgway, R. (1912). Color Standards and Color Nomenclature. – Published by the author, Washington, D.C., 43 pp., 53 plts.

Rogers, S. O. & A. J. Bendich (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. – Plant Mol. Biol. 5: 69–76.

— & A. J. Bendich (1988). Extraction of DNA from plant tissues. – In Gelvin, S. B. & R. A. Schilperoort (eds.). Plant Molecular Biology Manual, Kluwer Academic Publ., Boston. A6: 1–10.

— & A. J. Bendich (1994). Extraction of DNA from plants, algae and fungi. – In Gelvin, S. B. & R. A. Schilperoort (eds.). Plant Molecular Biology Manual, Kluwer Academic Publ., Boston. D1: 1–8.

—, S. A. Rehner, C. Bledsoe, G. J. Mueller & J. F Ammirati (1989). Extraction of DNA from Basidiomycetes for ribosomal DNA hybridizations. – Can. J. Bot. 67: 1235–1243.

Sanger, F., S. Nicklen & A. R. Coulsen (1977). DNA sequencing with chain terminating inhibitors. – Proc. Natl. Acad. Sci. 74: 5463–5467.

Smith, A. H. (1939). Studies in the genus *Cortinarius* I. – Contrib. Univ. Mich. Herb. 2: 5–42.

Swofford, D. L. (1986). PAUP: Phylogenetic analysis using parsimony, version 3.0o. – Illinois Natural History Survey, Champaign, Illinois.

White, T. J., T. D. Bruns, S. Lee & J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. – In Innis, M. A., D. H. Gelfand, J. J. Sninsky & T. J. White (eds.). PCR Protocols: A Guide to Methods and Applications. Academic Press, New York. pp. 315–322.

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