

Short title: Samoan *Hebeloma*

A Samoan *Hebeloma* with phylogenetic ties to the western Pacific

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Abstract: *Hebeloma ifeleletorum* is described as a new species from American Samoa. Based on analyses of ITS and combined nLSU-ITS datasets *H. ifeleletorum* clusters with but is distinct from described species that have been placed in the genus *Anamika* by some. The phylogenetic relationship of *H. ifeleletorum* to the genus *Anamika* from Asia and to other species from Australia and New Caledonia suggests that *H. ifeleletorum* has origins in the western Pacific.

Key words: *Agaricales*, *Anamika*, biogeography, Oceania, South Pacific

INTRODUCTION

The islands of the South Pacific have received scant attention from mycologists. At first glance, these islands seem too small to harbor much fungal diversity. Whereas that might be true for many of the tiny atolls scattered across the Pacific, some of the larger volcanic islands like those of the Samoan Archipelago hold unique and diverse plant communities. As a consequence they potentially also hold diverse fungal communities (Whistler 1992, 1994; Hawksworth 2001; Schmit and Mueller 2007). Thus far some familiar and widespread agarics such as *Chlorophyllum molybdites* are known from American Samoa along with one recently described species of *Inocybe* and another new species of *Moniliophthora* (Kropp and Albee-Scott 2010, 2012). Other than that, the Samoan mycobiota is poorly known and more undescribed species probably will be uncovered as work on the material collected in these islands continues. The purposes of this article are to propose a new species of *Hebeloma* from American Samoa and to assess its phylogenetic and geographic affinities.

MATERIALS AND METHODS

Notes were taken from fresh material in the field and a photograph was taken with a gray card as background. Microscopic study of dried material was done with a light microscope after mounting sections in Melzer's reagent, 10% NH_4OH and cotton blue. All measurements were made with material mounted in 10% NH_4OH . Color notations are from Munsell Soil Color Charts (Munsell Color 2000) and were taken from digital photographs made in the field. The microscopic measurements were made with oil immersion at 1000 \times and line drawings were done with the aid of a drawing tube. Basidiospores were photographed with a light microscope at 1000 \times with DIC illumination. The spore measurements cited are from the holotype and are derived from 20 spores and are reported as an average and a Q value with ranges, whereas measurements for the other cells are given as a size range. The scales proposed by Vesterholt (2005) were used to quantify spore ornamentation (O0–O4), loosening of the perispore (P0–P3), and dextrinoid reaction (D0–D4). The holotype was deposited in the Intermountain Herbarium (UTC) at Utah State University.

DNA was extracted with a modified CTAB miniprep after crushing a small sample of dried herbarium material in liquid nitrogen (Kropp et al. 1996) and target sequences were amplified with standard PCR protocols (White et al. 1990). The portion of the nuclear large ribosomal subunit (nLSU) in this study was amplified with primers LROR and LR5 (Moncalvo et al. 2000), and primers ITS4 and ITS5 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region. Direct sequencing of the amplified PCR products also was done using these primers with an ABI 3730 DNA analyzer. The new sequences generated were deposited in GenBank (ITS = KJ494930, nLSU = KJ494929).

Initial BLAST queries indicated that the species from Samoa had affinities to the genus *Anamika*, thus the aligned dataset of Yang et al. (2005) available at TreeBase (alignment S2122) was used as a base for assessing the phylogenetic position of the Samoan material. To generate the combined nLSU-ITS phylogram, the Samoan sequences were aligned onto this dataset with Clustal x (Thompson et al. 1997), and the alignments were adjusted by hand where necessary before a phylogenetic analysis was done with MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Taxon sampling for the ITS phylogram also was based on the dataset of Yang et al. (2005) obtained from TreeBASE, however the dataset was pruned to reduce the number of representatives for individual species and some taxa were added from Rees et al. (2013). The dataset was realigned before being analyzed. The realigned nLSU-ITS and ITS datasets were deposited in TreeBASE (alignments 16366 and 16333 respectively).

Tree searches were performed for both sets of sequences using a time reversible model of evolution (Rodriguez et al. 1990, Maddison 1994) and a gamma distribution with six substitution types and some invariant sites (GTR+G+I). The Bayesian simulations were conducted with eight active MCMC chains and with the temperature set at 0.2. The four MCMC simulations were done with 1 000 000 generations and with sampling every one-hundredth tree to approximate posterior probabilities. The potential scale reduction factors for all parameters in both analyses were 1.000–1.002, indicating that convergence had occurred. A strict consensus tree was calculated from the last 7500 sampled trees from a 10 000-tree dataset. Support measures for nodes with less than 70 percent posterior-probability support are not shown on the phylograms. TreeView (Page 1996) was used to visualize the consensus trees obtained from the simulation. The tree generated with the ITS sequences was rooted with species of *Naucoria*, whereas the tree generated from the combined nLSU-ITS sequences was rooted with species of *Gymnopilus*.

RESULTS

ITS analysis indicates that the Samoan species, *Hebeloma ifeleletorum*, falls into a well-supported clade comprising fungi that have been placed in the genus *Anamika* by Thomas et al. (2002) and Yang et al. (2005). In addition the results of the ITS analysis indicate that *Hebeloma ifeleletorum* is closely related to *Hebeloma (Anamika) indica*, *H. (Anamika) angustilamellata*, *H.(Anamika) lactariolens* and *H. youngii* as well as to a number of additional pleurocystidiate *Hebeloma* species. However it is phylogenetically distinct from each of these species (FIG. 1). The combined nLSU-ITS analysis produced results similar to those obtained with the ITS sequences alone (FIG. 2) and supports the recognition of a new species. In addition the nLSU-ITS and the ITS analysis also support the conclusion of Rees et al. (2013) that the clade comprising the species of *Anamika* is derived from within *Hebeloma* and should not be given generic recognition.

DISCUSSION

Because *Hebeloma* is an ectomycorrhizal genus, *Hebeloma ifeleletorum* is an unexpected element of the Polynesian mycobiota. Except for *Inocybe tauensis* from American Samoa (Kropp and Albee-Scott 2010) and *Tomentella* species associated with *Pisonia grandis* on coral cays in the Great Barrier Reef (Ashford and Allaway 1982, Chambers et al. 2005), few fungi from ectomycorrhizal genera have been reported from the tropical South Pacific. However, given the presence of ectomycorrhizal hosts such as *P. grandis* and *Intsia bijuga*, additional ectomycorrhizal fungi potentially will be found on these islands (Whistler 1992, 1994).

Samoan plant communities are thought to have Australasian connections (van Balgooy et al. (1996), but the geographical origins of the fungal populations found in Samoa are unknown. The meager data available so far indicate that these populations have a complex history. For example, Kropp and Albee Scott (2010) published evidence that *Inocybe tauensis*, the other putative ectomycorrhizal fungus reported from Samoa, originated in the Paleotropics of the western Pacific. On the other hand, another Samoan species, *Moniliophthora aurantiaca*, might have a Neotropical rather than a Paleotropical origin (Kropp and Albee-Scott 2012) and some of the fungi that are present such as *Chlorophyllum molybdites* appear to be Pantropical. In the case of *Hebeloma ifeleletorum* the phylogenetic and morphological evidence both point to a tight relationship with species that have been placed in the genus *Anamika*. The fact that all known *Anamika* species occur in Australia or Asia strongly suggests that *H. ifeleletorum* has connections to the western Pacific or India (Thomas et al. 2002, Yang et al. 2005, Rees et al. 2013).

Whether *Hebeloma ifeleletorum* is a recent arrival to Samoa or an endemic species is still an open question. Two of the specimens of *H. ifeleletorum* reported in this paper were collected within a native stand of *Intsia bijuga* in the National Park of American Samoa and a third

specimen was found with saplings of *Intsia bijuga* that had been planted along a trail within the park. The fact that one collection was found with planted trees initially raised the question of whether the fungus might have been transported with the trees from elsewhere because *Intsia bijuga* is such a widespread ectomycorrhizal species (Alexander and Högborg 1986). However it is known that the seed used to grow the planted trees was collected within the park (Tavita Togia, National Park of American Samoa, pers comm), eliminating the possibility that the fungus was imported recently with the planted trees. The question of whether this fungus is endemic to Samoa cannot be fully answered without further work on its geographical distribution.

Rees et al. (2013) concluded that the clade containing the species placed in the genus *Anamika* lacks sufficient support to merit generic status. This clade currently comprises *Hebeloma (Anamika) indica*, *H. (Anamika) angustilamellata* and *H. (Anamika) lactariolens* along with *H. youngii* and *H. ifeleletorum* (FIG. 1). The phylogeny presented here is consistent not only with the work of Rees et al. (2013) but also of Yang et al. (2005) in showing that the anamika clade is derived from within *Hebeloma*. As a result accepting *Anamika* as a genus would render *Hebeloma* polyphyletic. Furthermore the primary distinguishing morphological character for *Anamika* is the presence of pleurocystidia, which typically are lacking in *Hebeloma* species (Smith et al. 1983, Vesterholt 2005). Yet there are several Australasian *Hebeloma* species that are pleurocystidiate and cluster in a well-supported clade with the species of *Anamika* (FIG. 1). This indicates that more species could be added to *Anamika*, however the character is unreliable because two clade members, *H. victoriense* and *H. khogianum*, do not possess pleurocystidia (TABLE 1). The other salient morphological character given for *Anamika* by Thomas et al. (2002) is the presence of cavernae in the basidiospores. Unfortunately, with the exception of *Anamika indica*, none of the descriptions of the current *Anamika* species or of the *Hebeloma*

species that cluster with it in the pleurocystidiate clade clearly indicate whether their spores possess cavernae (TABLE 1). Based on the currently available phylogenetic evidence and the inconsistent presence of pleurocystidia and cavernae among the fungi that could be placed in *Anamika*, it is preferable to follow Rees et al. (2013) by placing all these species into *Hebeloma*. The Samoan species described here is placed in *Hebeloma*. However further phylogenetic work and studies on the distribution of cavernae among species in this group should be done before a final decision is made to retain or reject *Anamika*.

The phylogenetic analyses for this study (FIGS. 1, 2) indicate that *Hebeloma ifeleletorum* is closely related to *Hebeloma (Anamika) lactariolens*, *H. (Anamika) indica* and to *H. (Anamika) angustilamellata*. However, in addition to being phylogenetically divergent from *H. lactariolens*, *H. ifeleletorum* can be differentiated from it by its smooth rather than rugulose pileus surface, by the fact that it lacks a swollen stipe base and by its nondextrinoid spores. *Hebeloma indica* is morphologically similar to *H. ifeleletorum* but differs by having larger basidiospores, a solid stipe and versiform pleurocystidia rather than consistently rostrate pleurocystidia such as those found in *H. ifeleletorum*. *Hebeloma angustilamellata* is both phylogenetically distinct from *H. ifeleletorum* and differentiated from it by having much larger basidiomata, slightly rugulose pilei and extremely crowded narrow lamellae. Finally Rees et al. (2013) described an additional species, *Hebeloma youngii*, from Australia that falls within the anamika clade. *Hebeloma youngii* and *H. ifeleletorum* could be considered cryptic species in that they strongly resemble one another morphologically but are phylogenetically distinct. However, in addition to being phylogenetically separated, the two species also have sharply different ecological characteristics. *Hebeloma youngii* is associated with *Eucalyptus* and *Corymbia* in Australia where it was reported from decomposing marsupial remains (Rees et al. 2013), whereas *H. ifeleletorum* occurs

on soil and is associated with *Intsia bijuga* in American Samoa. There are relatively few reliable morphological differences between the two species. Although the basidiospores of *Hebeloma youngii* are strongly dextrinoid (D3) whereas the spores of *H. ifeleletorum* are not dextrinoid (D0). *Hebeloma youngii* also produces longer cheilocystidia than *H. ifeleletorum* (45–80 µm versus 25–55 µm for *H. ifeleletorum*).

TAXONOMY

Hebeloma ifeleletorum Kropp, sp. nov.

FIGS. 3, 4, 5

Mycobank MB808062

Holotype: USA. AMERICAN SAMOA: National Park of American Samoa, Tutuila Unit, clustered on ground along Mount Alava Trail with *Intsia bijuga*, 19 May 2009, B.R. Kropp 19-May-09-15 (HOLOTYPE UTC00235643).

Diagnosis: *Hebeloma ifeleletorum* differs from *H. lactariolens* by its smooth pileus and by lacking a swollen stipe base, from *Hebeloma indica* by having larger basidiospores and rostrate pleurocystidia and from *H. angustilamellata* by having much smaller basidiomata. It is phylogenetically and ecologically divergent from *Hebeloma youngii*.

Etymology: belonging to *Ifelele*, the Samoan name for the tree *Intsia bijuga*.

Pileus 3–32 mm diam, at first broadly conic with the margin slightly inrolled, later expanding and becoming convex, smooth, lacking an umbo; reddish brown to buff (5YR 4/6–7.5YR 8/4) at the pileus center, becoming pale buff to cream (10YR 8/3) toward the margin; a pale whitish veil is present before expansion. Lamellae 0.5–1.5 mm wide (dry material), narrowly attached, close, with even concolorous edge; pallid or pale pinkish brown when young, dull pinkish brown (5YR 7/4) at maturity, lamellulae 1–2 mm long. Stipe 9–39 × 2–7 mm, central, terete, equal or slightly enlarged toward the base, hollow, pliant; surface finely pruinose over the upper third of the stipe, otherwise lightly longitudinally fibrillose with faint annular

cortinate remains present, some fibrils and the remains of the cortina become brownish mostly from spore deposits; stipe light buff to cream (10YR 8/3–8/2), the base becoming reddish brown (5YR 4/4) at maturity. Dried spore print on white paper dull reddish brown (5YR 5/3). Odor faintly fungoid to acidulous. Context pallid, no color changes observed.

Basidiospores $9.0(10.6) 11.0 \times 5.5 (6.8)7.5 \mu\text{m}$, $Q = 1.55$, ornamented (O4) with a loosening perispore (P2) but cavernae not obvious under light microscopy, amygdaliform, not cyanophilous, not reacting in Melzer's reagent (D0). Basidia $20\text{--}27 \times 8\text{--}9 \mu\text{m}$, four-spored, clavate. Cheilocystidia $25\text{--}55 \times 7\text{--}14 \mu\text{m}$, numerous, hyaline, thin-walled, variable but mostly clavate to cylindrical or fusiform. Pleurocystidia $25\text{--}35 \times 8\text{--}11 \mu\text{m}$, not abundant, hyaline, thin-walled, mostly rostrate occasionally with an elongated tip. Pileipellis a layer of enlarged cylindrical to subglobose cells, $12\text{--}25 \mu\text{m}$ wide, with a thin superficial, slightly gelatinized layer; golden brown encrusting material present. Lamellar trama of parallel, smooth hyphae $4\text{--}8 \mu\text{m}$ wide. Stipe surface at apex a loosely woven covering of hyphae with numerous irregular mostly clavate cystidioid tips, $28\text{--}50 \times 5\text{--}12 \mu\text{m}$; at base covered with loosely woven hyphae, $3\text{--}6 \mu\text{m}$ wide, with some golden brown encrusting material. Clamp connections present. Habitat: clustered on the ground in association with *Intsia bijuga*.

Additional specimens examined: USA. AMERICAN SAMOA: National Park of American Samoa, Tutuila Unit, clustered on ground at Siufaga Ridge with *Intsia bijuga*, 4 Dec 2013, *B.R. Kropp 4-Dec-13-11* (UTC00266257); National Park of American Samoa, Tutuila Unit, clustered on ground at Siufaga Ridge with *Intsia bijuga*, 4 December 2013, *B.R. Kropp 4-Dec-13-12* (UTC00266258).

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LEGENDS

FIG. 1. Bayesian phylogram resulting from an analysis of an ITS dataset showing the position of *Hebeloma ifeleletorum* relative to the anamika clade and other pleurocystidiate species. Support measures are not shown for nodes with less than 70% posterior probability support. GenBank numbers are shown for the ITS sequences used in the analysis. Following Rees et al. (2013), *Anamika indica* = *Hebeloma indicum*, *A. lactariolens* = *H. lactariolens*, *A. angustilamellata* = *H. angustilamellatum*.

FIG. 2. Bayesian phylogram resulting from an analysis of a combined ITS-nLSU dataset showing the position of *Hebeloma ifeleletorum* within the anamika clade. Support measures are not shown for nodes with less than 70%

posterior probability support. Following Rees et al. (2013), *Anamika indica* = *Hebeloma indicum*, *A. lactariolens* = *H. lactariolens*, *A. angustilamellata* = *H. angustilamellatum*.

FIG. 3. Basidiomata of the holotype collection for *Hebeloma ifeleletorum* (UTC253643). Scale bar = 1 mm.

FIG. 4. Microscopic characters of *Hebeloma ifeleletorum*. S = basidiospores, B = basidia, Ch = cheilocystidia, Ca = caulocystidia, Pl = pleurocystidia. Scale bar = 10 μ m.

FIG. 5. Basidiospores of *Hebeloma ifeleletorum*. P = perispore. Scale bar = 10 μ m.

FOOTNOTES

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TABLE I. Salient morphological characters and origins of the species in the pleurocystidiate clade

| Taxon | Pleurocystidia | Cavernae ^b | Geographical origin |
|---|----------------|-----------------------|---------------------|
| <i>Anamika</i> ^a <i>angustilamellata</i> Zhu L. Yang & Z.W. Ge | + | 0 | China |
| <i>Anamika indica</i> K.A. Thomas, Peintner, M.M.Moser & Manim. | + | + | India |
| <i>Amanika lactariolens</i> (Clémenton & Hongo) Matheny | + | – | Japan |
| <i>Hebeloma aminophilum</i> R.N. Hilton & O.K. Mill. | + | 0 | Australia |
| <i>Hebeloma ifeleletorum</i> | + | – | Samoa |
| <i>Hebeloma khogianum</i> Bresinsky | – | 0 | New Caledonia |
| <i>Hebeloma subvictoriense</i> B.J. Rees | + | 0 | Australia |
| <i>Hebeloma victoriense</i> A.A. Holland & Pegler | – | + | Australia |
| <i>Hebeloma youngii</i> B.J. Rees | + | 0 | Australia |

^a *Anamika indica* = *Hebeloma indicum*, *A. lactariolens* = *H. lactariolens*, *A. angustilamellata* = *H. angustilamellatum*.

^b Cavernae indicated as + (present), – (absent), or 0 (not clearly given or illustrated in the species description). The authors of *H. victoriense* do not specifically describe cavernae but their basidiospore illustration shows them to be present (Holland and Pegler 1983).









