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New Asian species of the genus *Anamika* (euagarics, hebelomatoid clade) based on morphology and ribosomal DNA sequences

Zhu L. YANG[1], Patrick B. MATHENY[2], Zai-Wei GE[1], Jason C. SLOT[2] and David S. HIBBETT[2]

[1] *Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming 650204, P. R. China.*
[2] *Department of Biology, Clark University, 950 Main Street, Worcester, Massachusetts 01610, USA.*
E-mail : fungi@mail.kib.ac.cn

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Two dark-spored agaric species from Asia are placed in the genus *Anamika* (*Agaricales* or euagarics clade). This result is supported by ITS and nLSU-rDNA sequences with strong measures of branch support, in addition to several morphological and ecological similarities. An inclusive ITS study was performed using a mixed model Bayesian analysis that suggests the derived status of *Anamika* within *Hebeloma*, thereby rendering *Hebeloma* a paraphyletic genus. However, the monophyly of *Hebeloma* cannot be rejected outright given ITS and nLSU-rDNA data. Thus, we propose two new Asian species in *Anamika*: *A. angustilamellata* sp. nov. from dipterocarp and fagaceous forests of southwestern China and northern Thailand; and *A. lactariolens* comb. nov., a Japanese species originally described in the genus *Alnicola*. A complete description of *A. angustilamellata*, including illustrations, is provided.

INTRODUCTION

The genus *Anamika* was recently described to accommodate a single species characterized by the unique combination of pleurocystidia, spore ornamentation type, and putative ectomycorrhizal association with *Dipterocarpaceae* in southern India (Thomas *et al.* 2002). Recently, several collections of agarics from dipterocarp and fagaceous forests in southern China and northern Thailand were observed to share morphological affinities with the genus *Anamika* and *Alnicola lactariolens* described from Japan (Clémençon & Hongo 1994). In order to confirm the generic and specific dispositions of these Asian materials, ITS-rDNA and nLSU-rDNA nucleotide sequences were generated and analyzed phylogenetically. These sequences were evaluated in light of recent molecular phylogenetic studies of *Anamika*, *Hebeloma*, *Alnicola*, and *Hymenogaster* by Aanen *et al.* (2000), Peintner *et al.* (2001), and Thomas *et al.* (2002).

MATERIALS AND METHODS

Specimens and morphological descriptions

Mature and developing basidiomata of *Anamika* were collected in summers in forests dominated by *Dipterocarpaceae* and *Fagaceae* in southwestern China and northern Thailand. The possible mycorrhizal hosts were recorded at the time of collecting. Spore prints were also attempted at the time of collection or upon arrival at the laboratory or hotel. Specimens were noted and/or photographed in the field. Colour standards used were Ridgway (1912) and Kornerup & Wanscher (1981). Colour names with first letters capitalized are from Ridgway (1912); colour codes of the form '5D5' which indicates the plate, row, and colour block are from Kornerup & Wanscher (1981). Noted material was dried using an electric drier, and then prepared and deposited in appropriate reference collections, including the Kunming Institute of Botany (HKAS).

5% KOH was used as a mounting medium for microscopic studies. The abbreviation [*n*/*m*/*p*] shall mean *n* basidiospores measured from *m* basidiocarps of *p* collections. Dimensions of basidiospores including the ornamentation are given using notation of the form (*a*) *b*–*c* (*d*). The range *b*–*c* contains a minimum of 90% of the measured values. Extreme values *a* and *d* are given in parentheses. Q refers to the length/breadth ratio of basidiospores; **Q** refers to the average Q of all basidiospores ± sample standard deviation. All line drawings of this study were made from fresh material.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from Chinese and Thai material of *Anamika* (HKAS 42927 and CMU 45194)

and the isotype of the Japanese *Alnicola lactariolens* (HC 88/95, LAU). Additional material also used in this study includes: *Hebeloma velutipes* (AFTOL-ID 980, WTU), *Agrocybe praecox* (AFTOL-ID 728, WTU), *Hypholoma sublateritium* (AFTOL-ID 597, Clark Fungal Herbarium), and *Stropharia ambigua* (AFTOL-ID 726, WTU). DNA was isolated with a SDS miniprep following the protocol of Wang, Binder & Hibbett (2002) or using the E.Z.N.A. Fungal DNA Kit (Omega Bio-tek, Doraville, GA). ITS1, the 5.8S rRNA, and ITS2 were amplified as a single amplicon using primers ITS1F and ITS4 (White *et al*. 1990, Gardes & Bruns 1993). nLSU-rDNA was amplified using primers LR0R and LR7 or LR5 (Vilgalys & Hester 1990). PCR products were purified using a QIAquick PCR purification kit (Qiagen Science, MD). Sequencing was performed using a Big-dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Sequencing primers for the ITS regions included ITS1F, ITS4, ITS2, and/or 5.8SR (White *et al*. 1990, Gardes & Bruns 1993, http://www.biology.duke.edu/fungi/mycolab/primers). Sequencing primers for the nLSU region included LR0R, LR7, LR3R, LR5, and LR16 (Vilgalys & Hester 1990, http://www.biology.duke.edu/fungi/mycolab/primers). Sequencing reactions were purified using Pellet Paint (Novagen, Madison, WI, USA) and were run on an Applied Biosystems 377 XL automated DNA sequencer. Sequence chromatograms were compiled with Sequencher 4.1 software (GeneCodes, Ann Arbor, MI). Both ITS and nLSU sequences were generated for the taxa enumerated above and have been deposited in GenBank: new ITS accession nos include: AY575917, AY575919, and AY818348–AY818352; and new nLSU accession numbers include AY646101, AY635774, AY646102, AY745703, AY575918, AY575919 (combined with ITS), and AY818353.

Phylogenetic analysis

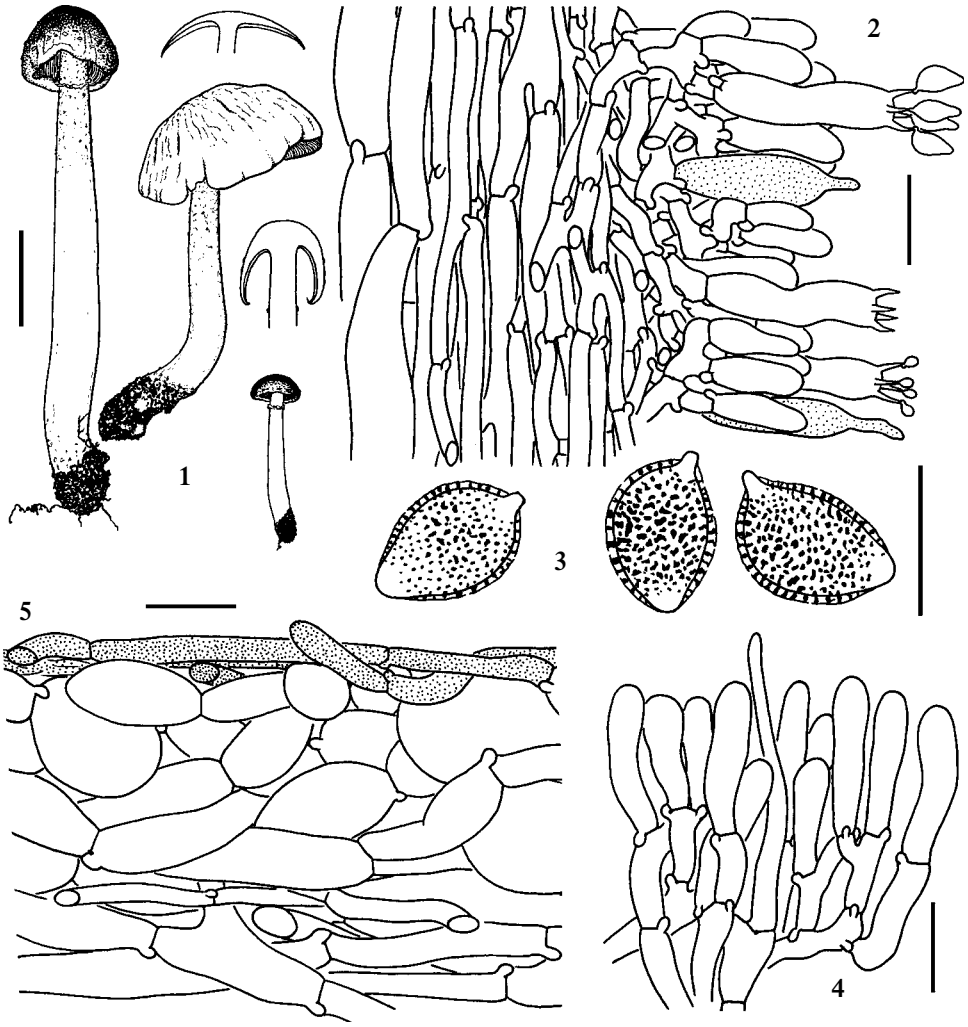
Our ITS sequences were aligned manually in MacClade 4.0 (Maddison & Maddison 2000) with the data set of Aanen *et al*. (2002), which was downloaded from TreeBASE (accession M613) (http://www.treebase.org/treebase/). We supplemented this alignment with ITS sequences available on GenBank of six *Alnicola* (=*Naucoria*) species, including one previously published in Martin & Moreno (2001), eleven *Hymenogaster* ITS sequences of Peintner *et al*. (2001), and the ITS sequence of *Anamika indica* (Thomas *et al*. 2002). This inclusive taxon sampling was necessary to evaluate the monophyly of *Anamika*, *Alnicola*, and *Hebeloma*. Characters too ambiguous to align were excluded. Gaps were scored as missing data. The alignment is available at TreeBASE (accession no. SN1827).

Both ITS and nLSU sequences were assembled manually into a data set with ITS and nLSU sequences of those taxa in Fig. 11 of Thomas *et al*. (2002) to evaluate further the position of *Anamika* in relation to *Hebeloma* and *Alnicola* using more characters than ITS could provide. These data were also used to evaluate the sister group to the hebelomatoid clade of Moncalvo *et al*. (2002) (alignment also deposited at TreeBASE as SN1827). To facilitate this alignment, the two *Cortinarius* taxa of Fig. 11 were excluded and *Gymnopilus* taxa were used instead as outgroups. All gaps were recorded as missing data.

Phylogenetic analyses of the inclusive ITS data set (75 taxa) were carried out in MrBayes 3.0 (Ronquist & Huelsenbeck 2003). Separate best-fit models to ITS1, the 5.8S rRNA, and ITS2 were estimated using Modeltest 3.0 (Posada & Crandall 1998, 2001). Separate models were then applied to the partitioned ITS data set in MrBayes using a Bayesian method of phylogenetic inference (Huelsenbeck *et al*. 2001, 2002, Archibald *et al*. 2003). A preliminary run of 1 M generations using six Metropolis-Coupled Monte Carlo Markov chains (default heating temperature set to 0.2) was done to estimate how many generations were required for likelihood scores to reach stationarity. This result then dictated our burn-in value for a second run of 2 M generations also using six chains.

The ITS and nLSU data set included 15 taxa. Phylogenetic relationships were estimated in PAUP* (Swofford 2004) under maximum parsimony (MP) and maximum likelihood (ML) criteria. A single model of nucleotide substitution was estimated by Modeltest 3.0 for the combined data set to implement ML analysis in PAUP* using a heuristic search strategy with the 'as-is' addition sequence and TBR branch-swapping. Data were combined since both ribosomal units are linked within a single array (Hillis & Dixon 1991). 1000 ML bootstrap replicates (Felsenstein 1985, Efron, Halloran & Holmes 1996) were performed using the as-is addition sequence and TBR-branch-swapping. Branch and bound was used with the furthest addition sequence under the MP criterion (character state optimization set to delayed transformation or DELTRAN). 1000 MP bootstrap replicates were done also using branch and bound. A mixed models Bayesian analysis was also performed on this data set running four chains for 1 M generations. Separate models were used for the ITS1, 5.8S, ITS2, and nLSU partitions. A total of 8000 trees, among 10 000 sampled, was used to calculate posterior probabilities.

To test whether alternate topologies of the ITS and nLSU dataset could be rejected, constrained analyses that enforced the monophyly of *Hebeloma*, but resolved no other nodes, were implemented in PAUP* under MP and Bayesian criteria. MP constrained trees were compared with our optimal MP trees using the Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999). 1000 RELL bootstrap replicates were performed. *P*-values less than 0.05 were considered significant. The same constraint trees were used as filters in PAUP* on trees derived from Bayesian analyses. The proportion of trees that resolved *Hebeloma* as monophyletic was



Figs 1–5. *Anamika angustilamellata*. **Fig. 1.** Basidiomata. **Fig. 2.** Lamellar trama, subhymenium, and hymenium with pleurocystidia (shown as shaded cells) and basidia at different stages of development. **Fig. 3.** Basidiospores. **Fig. 4.** Cheilocystidia. **Fig. 5.** Radial section of pileipellis with epicutis (shaded hyphae) and subcutis. Fig. 1 from the holotype; Figs 2–5 from CMU 45194. Bars: Fig. 1 = 3 cm; Figs 2, 4–5 = 20 μm; and Fig. 3 = 10 μm.

taken to represent the posterior probability of the monophyly of that particular group. If the proportion was less than 5%, the monophyly of the constrained group could be rejected ($P < 0.05$).

TAXONOMY

Anamika angustilamellata Zhu L. Yang & Z. W. Ge, **sp. nov.** (Figs 1–5)

Etym.: Named because of the narrow lamellae.

Pileus 3–10 cm diametro, primo hemisphaericus vel conico-convexus, brunneus vel luteo-brunneus, dein convexus vel plano-convexus, rugulosus, luteolus vel cremeus, saepe disco brunneo, reliquiis volvae coactis, flocculosis, albidis, glabrescentibus ornatus. Lamellae adnatae vel subdecurrentes, confertae, angustae, primo albidae, dein flavidae, flavae vel pallide aurantiacae, postremo brunneae, lamellulis vulgaribus. Stipes 5–12 × 0.5–1.5 cm, subcylindricus vel sursum attenuatus, primo albidus, cremeus vel flavidus, dein albidus vel griseolus, annulatus. Annulus minutulus, albidus, cremeus vel flavidus, superior, evanescens. Caro alba vel albida, amarella. Basidia subcylindrica, medio constricta, 4-sporigera. Basidiosporae in cumulo brunneae, (9.0–)9.5–11.0(–12.5) × (6.5–)7.0–8.5(–9.5) μm, amygdaliformes vel limoniformes, verrucosae, poris germinativis absentibus. Pleurocystidia versiformia vel subfusiformia, saepe mucronata vel rostrata. Cheilocystidia subcylindrica vel angusticlavata. Pileipellis ex epicute e hyphis repentibus, cylindraceis et hypocute e hyphis inflatis, subcellularibus composita. Fibulae praesentes.

Habitatio: terrestris in silvis, prope *Castanopsis*, *Dipterocarpus*, *Lithocarpus*.

Typus: **China**: *Yunnan Province*: Yingjiang County, Geduo, 18 July 2003, *Z. L. Yang 3753* (HKAS 42927 – holotypus).

Basidiomata (Fig. 1) medium to large-sized. *Pileus* 3–10 cm diam, hemispherical to conico-convex when young, becoming convex to plano-convex when mature; colour brown to yellow brown or 'Cinnamon-Brown' or somewhat paler (5D5-5D6-5E6-5E7) with paler margin when young, cream to yellowish or 'Cream Color' to 'Cream Buff' (4A2-A3) in age but often with a brownish tinge over the centre; surface usually finely and radially rugulose, slightly viscid when wet; margin incurved when young but expanded or reflexed in age;

veil remnants on surface as small whitish floccose patches that range from 1–2 mm diam, these randomly arranged, soon glabrescent; context white to whitish, not changing. *Lamellae* adnate to subdecurrent, crowded to very crowded, initially whitish but soon yellowish to yellow or pale orange (3A2-3A4 to 4A2-4A4), becoming light brown to brown or nearly 'Cinnamon-Brown' (6D4-6D6 to 6E4-6E6) when mature, narrow, 1–3 mm wide, lamellulae of 2–3 lengths, edges grayish or paler. *Stipe* 5–12 × 0.5–1.5(–2.0) cm, subcylindric or tapering upward, whitish, cream to yellowish or 'Cream Color' to 'Cream Buff' (4A2-4A3) when young, becoming whitish to grayish in age, covered with white to brownish fine squamules or fibrils; context white, solid; base of stipe not swollen. Annulus present, small, concolourous with surface of young stipe, superior, fugacious. Odour indistinct, taste slightly bitter. Spore print brown (7D5-7E5).

Lamellar trama (Fig. 2) composed of regularly arranged filamentous hyphae, 3–15 μm diam. *Subhymenium* (Fig. 2) 20–30 μm thick, composed of hyphal elements 3–6 μm wide. *Basidia* (Fig. 2) (25–)30–40 × 7–11 μm, subcylindric, usually with one median constriction, sometimes two, 4-spored, on occasion 2-spored, rarely 1-spored, sterigmata 4–6(–8) μm long, basal septa often clamped. *Basidiospores* (Fig. 3) [140/7/6] (9–)9.5–11.0(–12.5) × (6.5–)7–8.5(–9.5) μm [Q = (1.06–)1.20–1.47(–1.56), $\mathbf{Q} = 1.34 \pm 0.08$], amygdaliform to subamygdaliform in profile, nearly citriniform in face view, rusty ochraceous to yellowish brown in KOH, thick-walled, acyanophilous, possibly with a cavernous type of ornamentation because of the discontinuity of the epitunica under light microscope (oil immersion), germ pore absent but often with a conspicuous callus, apiculus small. *Pleurocystidia* (Fig. 2) scattered to abundant, versiform to subfusiform, 35–45 × 7–13 μm, often with a mucronate or rostrate apex, thin-walled, usually with yellowish to yellowish brown contents. *Cheilocystidia* (Fig. 4) numerous, mostly subyclindric to narrowly clavate, 25–55 × 6–10 μm, or occasionally lanceolate, 45–130 × 6–10 μm, rarely with a mucronate, rostrate or capitate apex, thin-walled, hyaline. *Pileipellis* (Fig. 5) consisting of an epicutis and hypocutis; epicutis composed of a thin layer with a few more or less repent filamentous hyphae, 3–7 μm diam, thin-walled, often with yellowish to brownish vacuolar pigment, at times with fine incrustations, also sometimes slightly gelatinized; hypocutis composed of subglobose (25–50 × 20–40 μm) to elliptic (30–50 × 15–25 μm) colourless cells, these thin-walled but at times with fine brownish pigment incrustations. *Veil remnants* on pileus filamentous, 2–7 μm diam, frequently branched, interwoven, sometimes anastomosing, hyaline, thin-walled or more or less so; annular hyphae similar to veil remnant hyphae on the pileus but at times with yellowish brown cell wall and contents. *Stipe trama* composed of longitudinally arranged, thin-walled hyphae, 2–15 μm diam. *Caulocystidia* 30–50 × 6–10 μm, scattered to clustered, subcylindric to narrowly clavate, often with yellowish to brownish vacuolar pigment, at times hyaline. *Clamp connections* present and frequent on all parts of the basidiomata.

Habitat: Solitary to scattered or gregarious on soil under *Castanopsis*, *Lithocarpus* (*Fagaceae*), and *Dipterocarpus* (*Dipterocarpaceae*); fruiting between July and November in tropical Yunnan, southwestern China and northern Thailand at 560–900 m elev. Possibly forming ectomycorrhizae with *Dipterocarpaceae* and *Fagaceae*.

Additional specimens examined: **China**: *Yunnan Province*: Mengla County, Menglun, Xishuangbanna Tropical Botanical Garden, 580 m, under cultivated pure stand of *Dipterocarpus zeylanicus*, 9 Aug. 1988, *Z. L. Yang* 328 (HKAS 21914); *loc. cit*., 31 Oct. 1989, *Z. L. Yang* 878 (HKAS 24855); Menglun, Hill Mangang, associated plants unknown, 800 m, 31 Oct. 1989, *Z. L. Yang* 894 (HKAS 24859); Menglun, Xishuangbanna Tropical Botanical Garden, 560 m, under *Castanopsis indica*, 2 Nov. 1989, *Z. L. Yang* 931 (HKAS 24858). – **Thailand**: *Chiang Mai Province*: Chiang Mai City, Doi Suthep, under *Dipterocarpus* sp., 19 July 2002, *R. Sanmee* (CMU 45194).

Notes: *Anamika angustilamellata* is characterized by its medium to large-sized basidiomata, finely rugulose pileus, narrow and crowded lamellae, and subcylindric to narrowly clavate cheilocystidia. It resembles *A. indica*., the type species of *Anamika* originally described from India under *Hoppea* (*Dipterocarpaceae*) (Thomas *et al*. 2002). *A. indica* differs by the smaller basidiomata, smooth pilei, broader lamellae, somewhat shorter basidiospores, and cheilocystidia frequently with a subcapitate, mucronate, or rostrate apex. *A. angustilamellata* also has velar remnants on the pileus when young and a bitter taste. These characters have not been ascribed to *A. indica*. *A. angustilamellata* can be distinguished from *A. lactariolens* (see below) by the larger basidiomata, narrower lamellae, even base of the stipe, and brown spore deposit. The sizes of the basidiospores of the two taxa are nearly the same.

Anamika lactariolens (Clémençon & Hongo) Matheny, **comb. nov.**

Basionym: *Alnicola lactariolens* Clémençon & Hongo, *Mycoscience* **35**: 25 (1994).

Notes: For a complete description of this fungus, see Clémençon & Hongo (1994). This velate species was first described in a *Quercus-Pinus* forest in Japan. *Anamika lactariolens* shares several characters in common with *Anamika*, namely the possession of pleurocystidia, verruculose basidiospores, lack of a pronounced gelatinous pileipellis, and Asian distribution. We also predict this species is ectomycorrhizal as is attributed to *An. indica* (Thomas *et al*. 2002). The noteworthy characters of *A. lactariolens*, however, are the purple brown spore deposit and conspicuous pseudoparenchymatous hypocutis. *Hebeloma*

sarcophyllum also exhibits a purplish brown spore deposit as pointed out by Clémençon & Hongo (1994). An examination of the isotype of *Alnicola lactariolens* at LAU, however, includes a spore deposit that is dark reddish brown or near 'Argus Brown' of Ridgway (1912). Pleurocystidia can be easily observed in squash mounts, are distinctly rostrate, and differentiated from the slenderly lageniform cheilocystidia.

Species of *Alnicola* lack pleurocystidia (Singer 1986), which further affirms the generic placement of *Al. lactariolens* elsewhere. The possession of pleurocystidia among the three species known in *Anamika* is a shared derived character (synapomorphy), similar to the evolution of pleurocystidia in other agaric groups, for example *Inocybe* (Kühner 1980, Matheny *et al.* 2002, Matheny 2005).

Specimen examined: **Japan**: *Shiga Prefecture*: Ôtsu City, Tomikawa, in *Quercus-Pinus* forest, 15 Aug. 1988, *T. Hongo & H. Clémençon HC 88/95* (TNS-F-237670 – holotype; LAU – isotype).

RESULTS

The inclusive ITS data set consisted of 75 taxa and 684 characters, of which sites 38–41 and 516–525 were excluded due to alignment ambiguities. A Tamura-Nei (TN) model including gamma-distributed substitution rates was estimated for the ITS1 partition; a Jukes-Cantor (JC) model with no rate heterogeneity was estimated for the 5.8S rRNA gene; and a Hasegawa-Kishino-Yano (HKY) model including gamma-distributed substitution rates was estimated for the ITS2 partition. Because MrBayes does not permit an implementation of the TN model, we chose the next most complex model, GTR or general time-reversible, for the ITS1 partition. According to Ronquist, Huelsenbeck & van der Mark (2005), Bayesian inference is relatively robust to slight over-parameterization of models. Adenine-thymine (AT) bias was not acute in estimates of base composition. A preliminary Bayesian analysis of 1 M generations established the Markov chains were reaching stationary likelihood scores after 100 000 generations. We then used this 10 % threshold as our burn-in value in a subsequent run using 2 M generations. The tree shown in Fig. 6 is a majority-rule consensus tree of 18 000 trees. Only posterior probabilities (PP) greater than or equal to 0.95 are shown.

Fig. 6 indicates strong support for the monophyly of a clade containing *Hebeloma*, *Alnicola lactariolens*, and the *Anamika* accessions (shown in large light grey box). A weakly supported clade containing eight other *Alnicola* ITS sequences and the *Hymenogaster* species is placed as the sister group of the *Hebeloma-Anamika-Alnicola lactariolens* clade. Eight *Alnicola* taxa form a poorly supported grade from which a poorly supported monophyletic *Hymenogaster* is derived. The isotype of *Alnicola lactariolens*, the two accessions of *An. angustilamellata*, and *An. indica* form a strongly supported monophyletic group (shaded in dark grey box). *Alnicola lactariolens* is the sister group of *Anamika indica* plus *A. angustilamellata*. The two accessions of *A. angustilamellata* form a monophyletic group. These results also suggest the paraphyly of *Hebeloma* with *Anamika* occupying a nested position within it. However, there is no strong support for any node that anchors *Anamika* at any position within *Hebeloma*. The entire backbone of this portion of the tree is not well-supported.

We assembled a combined data set of ITS and nLSU sequences of 15 taxa to determine if adding more characters might resolve the position of *Anamika*. This data set included 1646 sites. ITS positions 182–184, 462–464, and 486–493 were excluded due to alignment ambiguity. No sites within the nLSU partition were excluded. A general-time-reversible (GTR) model including a proportion of invariable sites with gamma-distributed substitution rates at the remaining sites was estimated as the best-fit model to the combined data. Fig. 7 shows the ML tree ($-\ln = 5089.44$). Branches that differ from the strict consensus of four MP trees are indicated with asterisks. However, none of these branches receive more than 50 % of the bootstrap proportions (BP). As in the ITS analysis, *Anamika* plus *Alnicola lactariolens* form a well-supported monophyletic group that is nested within *Hebeloma* with moderate support. This result is consistent with the strict consensus MP tree and a Bayesian 50 % majority-rule consensus tree although *Hebeloma* is completely unresolved. The arrangements of *Hypholoma* and *Stropharia*, on one hand, and the *Agrocybe praecox* accessions, on the other, are inconsistent depending on the phylogenetic method used. However, their positions are poorly supported no matter the method. That is, with these data the sister group of the clade comprising *Hebeloma*, *Alnicola*, and *Hymenogaster* (hebelomatoid clade of Moncalvo *et al.* 2002) cannot be determined.

When we constrained *Hebeloma* as a monophyletic group in the ITS and nLSU dataset, the resulting four constrained MP trees were only 3 steps longer than the four optimal MP trees. None of the constrained trees (monophyletic *Hebeloma*) were significantly worse ($P = 0.819$–0.452) than optimal trees (paraphyletic *Hebeloma*) using the Shimodaira-Hasegawa test. In addition, only 526 of 8000 Bayesian trees of ITS and nLSU data set support the monophyly of *Hebeloma* ($P = 0.066$). However, the inclusive ITS data set is even more decisive with only 11 of 18 000 Bayesian trees supporting the monophyly of *Hebeloma* ($P = 0.0006$). Nevertheless, there is no strong consensus to transfer *Anamika* and *Alnicola lactariolens* to *Hebeloma*. Thus, most data presented in this study, including morphological data, support the placement of our new species in the genus *Anamika* and the transfer of *Alnicola lactariolens* to *Anamika*.

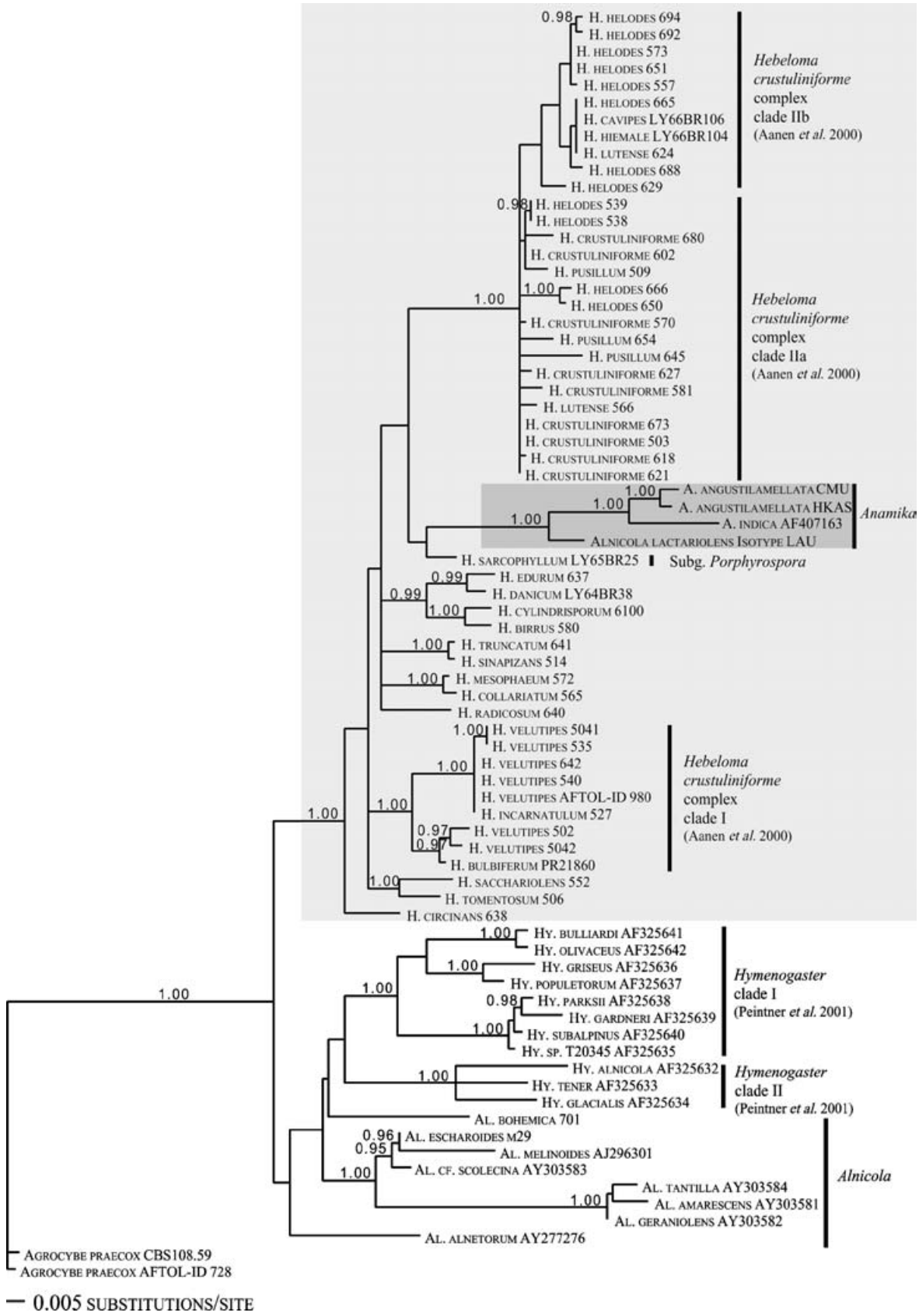


Fig. 6. The majority-rule Bayesian consensus tree of 18 000 trees using ITS sequence data and mixed models of DNA substitution. *Hebeloma*, *Alnicola lactariolens*, and three *Anamika* accessions form a monophyletic group in the light grey box. *Alnicola lactariolens* and the *Anamika* accessions form a monophyletic group in the nested dark grey box. Values above branches refer to the posterior probability of that clade. Only values equal or greater than 0.95 are shown.

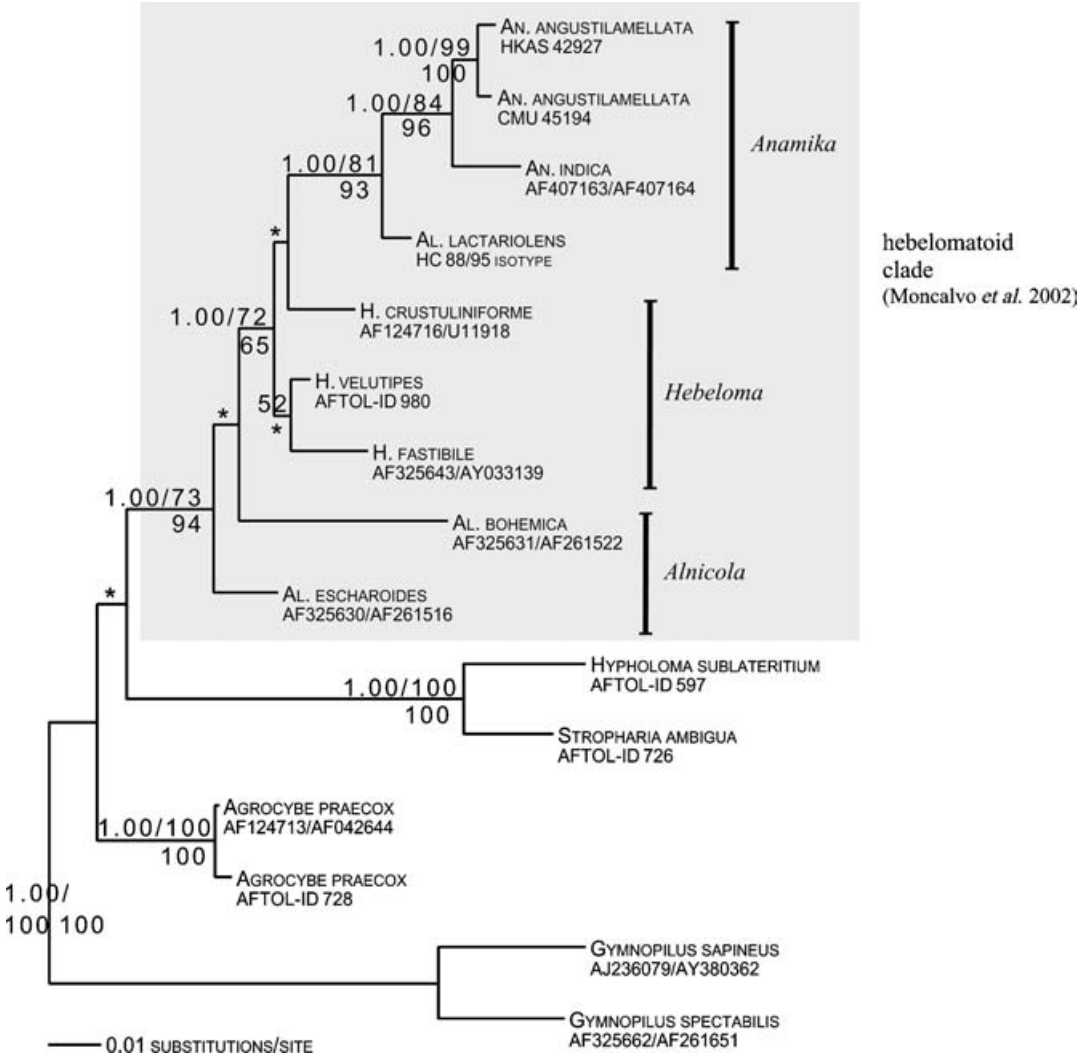


Fig. 7. The tree with the highest likelihood using ITS and nLSU sequences and maximum likelihood (ML) with a GTR model and rate heterogeneity parameters. The hebelomatoid clade is indicated in the grey box. The genera *Alnicola*, *Hebeloma*, and *Anamika* are indicated. ML and MP bootstraps greater than 50% are indicated above and below branches, respectively. Bayesian posterior probabilities >0.95 are indicated to the left of ML bootstrap values. Asterisks indicate that the branch is collapsed in the strict consensus of the four MP trees.

DISCUSSION

Additional Asian species of Anamika

The goals of this study were to evaluate the generic placement and specific level status for unidentified Asian material with morphological affinities to the dark-spored agaric genus *Anamika*, a monotypic genus originally described from India. Molecular phylogenies (Figs 6–7) strongly support the monophyly of *Anamika indica* (the type), two accessions of *An. angustilamellata*, and the isotype of *Alnicola lactariolens*. These three species constitute the anamika clade, which we refer to as the genus *Anamika*. Both Chinese and Thai material of *Anamika angustilamellata* and the isotype of the Japanese *Alnicola lactariolens* share with *Anamica indica* the possession of pleurocystidia, verruculose basidiospores, and lack of a pronounced gelatinous pileipellis, perhaps the most salient morphological characters of *Anamika*. Additionally, they are possibly ectomycorrhizal with plants of the *Dipterocarpaceae* and *Fagaceae*.

Fig. 6 also shows (but with <0.95 PP) a sister group relationship between *Hebeloma sarcophyllum* and *Anamika*. *Hebeloma sarcophyllum* is distinguished in part by its purplish (to reddish) brown spore deposit and is treated by Singer (1986) in subgenus *Porphyrospora*. However, Smith, Evenson & Mitchell (1983) believed spore deposit colour to intergrade too much among veiled and non-veiled species of *Hebeloma* to be used taxonomically. *H. sarcophyllum*, to our knowledge, does not possess pleurocystidia and is not strongly supported in the clade containing *Anamika*. For these reasons, we prefer not to transfer *H. sarcophyllum* to *Anamika*. Attention should also be brought to the rather robust *H. victoriense* described from the Melbourne area in Victoria, Australia; the authors

ascribe a vinaceous brown spore deposit to this species, but pleurocystidia are reported as absent (Holland & Pegler 1983).

Anamika *and the ambiguous status of* Hebeloma

Members of *Anamika* can be distinguished most easily from *Hebeloma* (Singer 1986) and *Alnicola* by the possession of pleurocystidia. However, both of these genera (and agarics in general) are very poorly known in China and elsewhere in Asia (Horak 1987). Unlike previous analyses (Aanen *et al*. 2000, Peintner *et al*. 2001, Thomas *et al*. 2002), our results using Bayesian and maximum likelihood methods raise the possibility that *Hebeloma* is not monophyletic (Figs 6–7). However, when *Hebeloma* is constrained to be monophyletic, this arrangement cannot be statistically rejected in all cases. Given that the weight of ITS and nLSU-rDNA evidence does not point conclusively to a paraphyletic *Hebeloma* at this time, we continue to recognize the genus *Anamika*, now known to include three Asian species: *A. angustilamellata*, *A. lactariolens*, and *A. indica*. If additional evidence strongly confirms *Hebeloma* to be paraphyletic, with *Anamika* nested within it, then *Anamika* might best be considered a synonym of *Hebeloma*.

Sister group relationships to the hebelomatoid clade are not strongly supported in this study although previous studies have shown parsimony bootstrap support $>50\%$ for *Agrocybe praecox* as the sister-group (Moncalvo *et al*. 2000, Thomas *et al*. 2002). Variable regions of alternative genes, for example, *rpb1* and *rpb2* (Matheny *et al*. 2002, Matheny & Ammirati 2003, Kropp & Matheny 2004, Wang *et al*. 2004, Matheny 2005) could be considered to help resolve the ambiguous status of *Hebeloma* in relation to *Anamika* as well as other groups within the hebelomatoid clade (Moncalvo *et al*. 2002), viz. *Alnicola* and *Hymenogaster*.

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