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Phylogenetic relationships of the marine gasteromycete *Nia vibrissa*

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Abstract: Phylogenetic relationships of the marine gasteromycete, *Nia vibrissa,* were investigated using four ribosomal DNA (rDNA) regions. Independent analyses of mitochondrial small-subunit (mt-ssu) rDNA, mitochondrial large subunit (mt-lsu) rDNA, nuclear small-subunit (nuc-ssu) rDNA, and nuclear large subunit (nuc-lsu) rDNA all suggest that *Nia vibrissa* is in the euagarics clade. The mt-lsu, nuc-ssu, and nuc-lsu datasets suggest that *Nia vibrissa* is closely related to the cyphelloid fungus, *Henningsomyces candidus,* but in all three datasets the monophyly of the *Nia-Henningsomyces* group is weakly supported. Analyses of mt-ssu rDNA suggest that the sister group of *Nia vibrissa* is *Schizophyllum commune,* but again the relationship is weakly supported. A combined analysis of all four rDNA regions strongly supports the sister group relationship of *Nia vibrissa* and *Henningsomyces candidus. Schizophyllum commune* and *Fistulina hepatica* form a strongly supported clade that is weakly supported as the sister group of the *Nia-Henningsomyces* clade. *Schizophyllum commune* and *Fistulina hepatica* both resemble cyphelloid fungi in some aspects of their hymenophore morphology, and it is plausible that they could be closely related to *Henningsomyces candidus.* Eighteen genera of terrestrial gasteromycetes were included in the analyses, but none are closely related to *Nia vibrissa,* which therefore represents an independent origin of the gasteroid habit in the homobasidiomycetes.

Key Words: cyphelloid fungi, *Fistulina hepatica, Henningsomyces candidus,* marine fungi, molecular systematics, *Schizophyllum commune*

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INTRODUCTION

Several lines of evidence suggest that fungi arose from flagellated, aquatic ancestors and, along with plants, were among the first eukaryotes to colonize the land (Pirozynski and Malloch 1975, Berbee and Taylor 1993). Ascomycetes and basidiomycetes radiated extensively in terrestrial ecosystems and now comprise about 85% (60 000 species) of the known fungi (Hawksworth et al 1995). A few groups of fungi have secondarily become aquatic, including four genera of marine basidiomycetes (Kohlmeyer and Kohlmeyer 1979, Hawksworth et al 1995). Marine fungi often display unique morphological adaptations to the aquatic environment, which makes it difficult to identify their closest relatives among the terrestrial fungi. A prime example is the taxonomically enigmatic marine gasteromycete, *Nia vibrissa.* Moore and Meyers (1959) described *Nia vibrissa* as a deuteromycete. However, Doguet (1967, 1968) demonstrated that *Nia vibrissa* develops basidia and clamp connections, and Brooks (1975) showed that *Nia vibrissa* has dolipore septa. These characters indicate that *Nia vibrissa* is a homobasidiomycete, but do not reveal its exact taxonomic position.

Nia vibrissa generates minute ([0.5] 1–3 [5] mm) subglobose, gasteroid fruiting bodies, which are sessile or, rarely, stalked. The color ranges from pale cream to white-orange and orange-brown (Doguet 1967, Kohlmeyer and Kohlmeyer 1979, Barata et al 1997). Basidiocarps grown in culture exhibit polymorphic peridial surfaces with or without peridial hairs (Schimpfhauser and Molitoris 1991). Slightly curved or bifurcate curled hairs project from the outer peridium in some collections, but are lacking in others. This considerable spectrum in habit of basidiocarps may indicate the presence of a multi-species complex (Jones and Jones 1993). Within a gelatinous gleba, the statismosporic basidia produce 4–8 ovoid basidiospores that have 4 lateral appendages and one terminal appendage (Doguet 1967). Both peridial hairs and basidiospore appendages appear to play a role in dispersal and colonization. Kohlmeyer and Kohlmeyer (1979) suggested that the peridial hairs trap air, allowing the basidiocarps to float after being detached from the substrate. It has also been suggested that the appendages of the basidiospores anchor spores to substrates (Webster 1959, Leightley

and Eaton 1979) or slow their sinking rate (Ingold 1975, 1976, Roselló et al 1993). Whatever the precise function of the peridial hairs and basidiospore appendages, *Nia vibrissa* is obviously very successful at dispersal. It is widespread in temperate and tropical localities in the Atlantic, Pacific, and Indian Oceans, and the Mediterranean Sea (Kohlmeyer 1983, Hyde 1986, Jones and Kuthubutheen 1989).

Nia vibrissa has been collected on diverse woody substrates, including driftwood, mangroves, and sunken ship timbers, as well as *Spartina* culms and rhizomes (Kohlmeyer and Kohlmeyer 1979). It has also been isolated by baiting with wooden test panels (Cuomo et al 1988, Grasso et al 1989) and feathers (Rees and Jones 1985). A second species, *Nia epidermoidea,* was detected by baiting with horsehair (Rosselló et al 1993), and a third species, *Nia globospora,* was described from continuously submerged *Spartina* culms (Barata et al 1997). Thus, *Nia* species exploit keratinic as well as lignocellulosic substrates. Like many saprotrophs, *Nia vibrissa* grows well in culture, and several studies have investigated the light, temperature, and salinity requirements for growth and fruiting body production (Doguet 1968, Schimpfhauser and Molitoris 1991, Jones and Jones 1993). Leightley and Eaton (1979) demonstrated a white rot pattern of wood decay in *Nia vibrissa* cultures (presence of phenol oxidase and laccase using the Käärig drop test confirmed by the Bavendamm test) and illustrated a decay micromorphology typical of terrestrial white rot fungi.

Different authors have suggested that *Nia* is closely related to several groups of terrestrial gasteromycetes. *Nia* has been classified in the Melanogastrales, either in the Torrendiaceae (Dring 1973) or Melanogastraceae (Doguet 1969, Kohlmeyer and Kohlmeyer 1979), the Nidulariales (Rosselló et al 1993), or in the Niaceae, among the gasteromycetes incertae sedis (Jülich 1981). Recently, several large molecular datasets have been developed that resolve phylogenetic placements of many homobasidiomycetes (e.g., Hibbett et al 1997, Bruns et al 1998, 2000, Moncalvo et al 2000). In the present study, we performed independent and combined analyses of four different rDNA regions to address the phylogenetic position of *Nia vibrissa.*

MATERIALS AND METHODS

Nia vibrissa strain M200 was collected in Apr 1990 at the west coast of Turkey (leg. Breuer A., det. Rohrmann S.). Cultures were grown on GPYS agar (2% agar, 0.01% glucose, 0.005% peptone, 0.001% yeast extract, 1L synthetic seawater; Molitoris and Schaumann 1986) at 23 C and 65% humidity in the dark. Strains were examined by light microscopy (Nikon YS2) in methylene blue. The culture and voucher specimen of *Nia vibrissa* (culture and herbarium accession number M200) used in this study are deposited in the herbarium of the University of Regensburg (REG).

Sequence data were generated from four rDNA regions: partial mitochondrial small subunit (mt-ssu) rDNA, bounded by primers MS1 and MS2, partial mitochondrial large subunit (mt-lsu) rDNA, bounded by primers ML5 and ML6, nearly complete nuclear small subunit (nuc-ssu) rDNA, bounded by primers PNS1 and NS8, and partial nuclear large subunit (nuc-lsu) rDNA, bounded by primers LR0R and LR5. In addition, mt-lsu and nuc-lsu sequences were generated for *Schizophyllum commune* (isolate Sco1, Germany, REG) and a mt-lsu sequence was generated for the cyphelloid wood-decay fungus, *Henningsomyces candidus* (isolate R.G. Thorn 156, DAOM accession number 195432). An unpublished nuc-lsu sequence of *Fistulina hepatica* (isolate TW351, Germany, REG) was provided by Tobias Wagner (University of Regensburg), and a published nuc-lsu sequence of *Henningsomyces candidus* isolate R.G. Thorn 156 was downloaded from GenBank accession number AF287864; Hibbett et al 2000). Protocols and primer sequences for amplification and sequencing have been described elsewhere (Vilgalys and Hester 1990, White et al 1990, Bruns and Szaro 1992, Hibbett 1996, Bruns et al 1998, Moncalvo et al 2000, http:–www.botany.duke.edu-fungi-mycolab-primers.htm; http:–plantbio.berkeley.edu-~bruns-primers.html). The new sequences have been deposited in GenBank (AF334747–AF334754).

The sequences from *Nia vibrissa, Schizophyllum commune, Henningsomyces candidus,* and *Fistulina hepatica* were manually aligned to four published datasets. The mt-ssu and nuc-ssu datasets are being published by Hibbett and Donoghue (in press) and include 127 and 111 sequences, respectively. Sampling in that study was biased toward wood decay fungi, especially members of the polyporoid clade (clade names used in this paper follow Hibbett and Thorn 2001), which was represented by 49 mt-ssu sequences and 34 nuc-ssu sequences. Nevertheless, the mt-ssu and nuc-ssu rDNA datasets each include representatives of all eight major clades of homobasidomycetes recognized by Hibbett and Thorn. The mt-ssu and nuc-ssu analyses were rooted with the heterobasidiomycetes *Auricularia, Dacrymyces,* and *Tremella.* The mt-lsu dataset was published by Bruns et al (1998), and includes 153 sequences, most of which are from ectomycorrhizal species, including 67 sequences from the bolete clade. Forty-four mt-lsu sequences represent unidentified species (e.g., *Tomentella* sp.) or direct amplifications from mycorrhizae. There are no representatives of the hymenochaetoid clade in the mt-lsu dataset, but all of the other major clades of homobasidiomycetes are represented. The mt-lsu analysis was rooted with *Sebacina.* The nuc-lsu dataset was published by Moncalvo et al (2000) and includes 156 sequences, of which 139 are in the euagarics clade. There are also representatives of the bolete, russuloid, and polyporoid clades. The nuc-lsu analysis was rooted with nine species of the russuloid clade. The alignments have been deposited in TreeBASE (accession number S628).

The four datasets were analyzed independently in PAUP* 4.0 (Swofford 1999) using equally-weighted parsimony. The analyses used a two-step search protocol that we have de-

scribed previously (Hibbett and Donoghue 1995). Step one involves 1000 heuristic searches with random taxon addition sequences, but keeping only two trees per replicate, and step two involves branch swapping on the shortest trees found in the first step. MAXTREES was set at 1000 in the second step. One-hundred bootstrap replicates were performed for each dataset, with one random taxon addition sequence each, TBR branch swapping, keeping up to ten trees per replicate, and MAXTREES set to 1000.

Combined analyses of all four rDNA regions were performed using 15 species of the euagarics clade, including those that could be closely related to *Nia* based on the independent analyses (FIGS. 1–5). *Fistulina hepatica,* which lacks a mt-lsu rDNA sequence, was the only species that was not represented by all four regions. The sequences and alignment used in the four-region analyses are derived from a study of higher-level relationships of homobasidiomycetes (Binder and Hibbett, unpubl). The new alignment has been deposited in TreeBASE (accession number S628). Based on the results of that study, the Hygrophoraceae (*Humidicutis marginata* and *Hygrophorus sordidus*) was used for rooting purposes. A parsimony analysis was performed using 1000 heuristic searches with TBR branch swapping and MAXTREES set to autoincrease. Bootstrapped parsimony analysis used 1000 replicate searches, each with one heuristic search, random taxon addition sequence and TBR branch swapping, keeping up to ten trees per replicate, and MAXTREES set to 10 000. A maximum likelihood analysis was also performed, using the HKY85 model of sequence evolution with empirical base frequencies, transition-transversion bias set to two, and site-to-site rate heterogeneity modeled on a discrete gamma distribution with four rate classes and alpha = 0.5. The maximum likelihood analysis used the most parsimonious trees as starting trees for a heuristic search with TBR branch swapping. Bootstrapped maximum likelihood analysis used 100 replicates with one random taxon addition sequence per replicate and TBR branch swapping.

RESULTS

Culture and strain specification.—Nia vibrissa strain M200 grew as a white, sparse aerial mycelium 2 wk after inoculation. Fully developed basidiocarps were obtained 10 wk later. Usually, 18–27 white-orange fruiting bodies 1–3 mm in diameter were counted per plate. Basidiocarps were usually sessile, but were occasionally stalked. There was no indication of spore release and peridia remained closed over a two year storage at 4 C. Hyphae projecting from the outer peridial layer showed slightly curved tips that were not bifurcated. Ovoid basidiospores (number per basidium not observed) were 8.5–11 × 10–15 μm. Lateral spore appendages ([3]–4) were 22–33 μm long and apical appendages were 25–47 μm long. Remnants of the pedicel ranged between 1.7–3.2 μm in length. Both morphological features and measurements match the description of *Nia vibrissa* in Kohlmeyer and Kohlmeyer (1979), but the shape of the peridial hairs, and size of spores and appendages differ from specifications in Doguet (1967) and Jones and Jones (1993).

Analyses of the mt-ssu dataset.—The MS1-MS2 PCR product of *Nia vibrissa* was 647 base pairs (bp) long. Excluding ambiguous regions (described in Hibbett and Donoghue 1995), the mt-ssu dataset included 418 aligned positions, of which 341 were variable and 269 were parsimony-informative. Phylogenetic analysis recovered 1000 trees (3119 steps, consistency index [CI] = 0.221, retention index [RI] = 0.550; FIG. 1). The strict consensus of all most parsimonious trees suggests that *Nia* is nested among 22 species of the euagarics clade sensu Hibbett and Thorn (2000). However, bootstrap support for the euagarics clade is weak (bootstrap < 50%; FIG. 1). The sister group of *Nia vibrissa* in the mt-ssu trees is *Schizophyllum commune,* but monophyly of the *Nia-Schizophyllum* clade is weakly supported (bootstrap < 50%; FIG. 1). The sister group of the *Nia-Schizophyllum* clade contains the "beefsteak fungus", *Fistulina hepatica,* which is a polypore that grows on live hardwoods, and *Typhula phacorrhiza,* which is a terrestrial clavarioid fungus. These nodes are also weakly supported (bootstrap < 50%).

Analyses of the mt-lsu dataset.—The ML5-ML6 PCR product of *Nia vibrissa* was 426 bp long. The mt-lsu dataset included 354 aligned positions, of which 200 were variable and 153 were parsimony-informative. Phylogenetic analysis recovered 1000 trees (851 steps, CI = 0.396, RI = 0.866; FIG. 2). In the strict consensus tree, *Nia vibrissa* is nested in a weakly supported (bootstrap < 50%) group of 31 species that represent the euagarics clade (in part). *Henningsomyces candidus* is weakly supported as the sister group of *Nia vibrissa* (bootstrap = 62%; FIG. 2). The *Nia-Henningsomyces* clade is nested in a weakly supported clade that includes eleven species of *Amanita.*

Analyses of the nuc-ssu dataset.—The PNS1-NS8 PCR product of *Nia vibrissa* was 1791 bp long. Excluding ambiguous regions (described in Hibbett and Donoghue in press), the nuc-ssu dataset included 1820 aligned positions, of which 737 were variable and 435 were parsimony-informative. Phylogenetic analysis recovered 1000 trees (3175 steps, CI = 0.337, RI = 0.524; FIG. 3). The strict consensus tree suggests that *Nia vibrissa* is nested in a weakly supported (bootstrap < 50%) clade that contains 22 species of the euagarics clade, plus the brown rot polypore *Laetiporus portentosus* (FIG. 3). Based on other analyses (Hibbett and Donoghue in press) and morphology, we suspect that the placement of *L. portentosus* in the euagarics clade is an artifact. The sister group of *Nia vibrissa* in the nuc-ssu trees is *Henningsomyces candidus,* and the sister group of the *Nia-Henningsomyces*

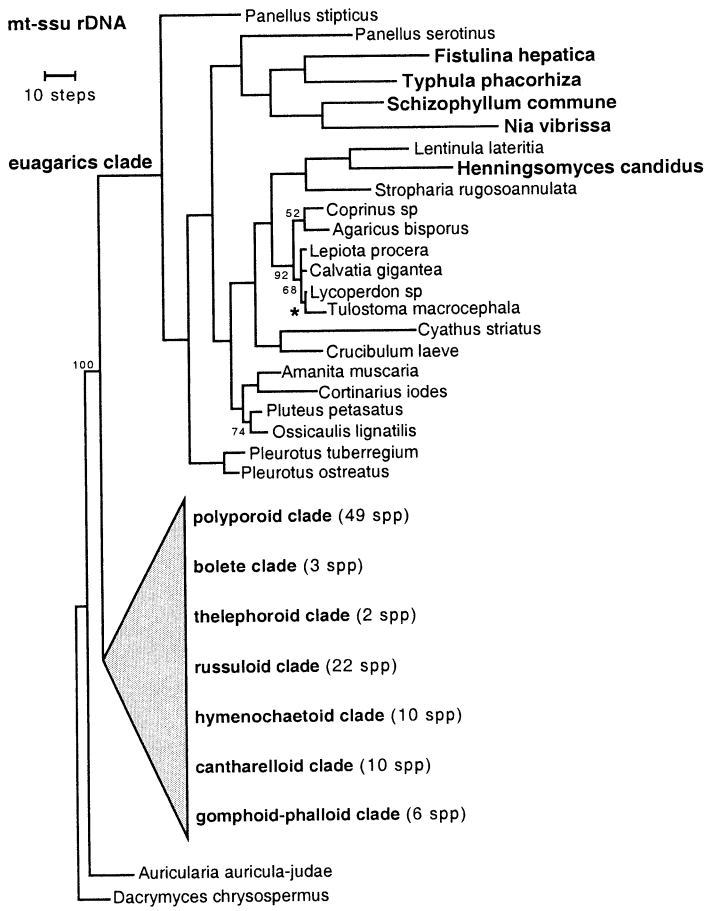

FIG. 1. Phylogenetic placement of *Nia vibrissa* inferred from mt-ssu rDNA sequences. Tree 1/1000 (3119 steps, CI = 0.221, RI = 0.550). The branch that collapses in the strict consensus tree is marked with an asterisk. The resolved portion of tree is the euagarics clade, with branch lengths drawn proportional to the number character state changes inferred (see scale bar). Bootstrap frequencies are shown below branches (values below 50% are not shown). The shaded triangle represents a paraphyletic assemblage of species that are not closely related to *Nia*. Trees deposited in TreeBASE show positions of all taxa.

clade is *Cortinarius iodes,* but these nodes are weakly supported (bootstrap < 50%; FIG. 3). *Fistulina hepatica* and *Schizophyllum commune* form a strongly supported (bootstrap = 93%) clade that is weakly supported as the sister group of a clade that contains *Agaricus bisporus* and *Amanita muscaria* (FIG. 3).

Analyses of the nuc-lsu dataset.—An 872 bp region of the LR0R-LR5 PCR product of *Nia vibrissa* was sequenced. The nuc-lsu dataset included 1193 aligned positions, of which 435 were variable and 307 were parsimony-informative. Phylogenetic analysis recovered 1000 trees (4254 steps, CI = 0.224, RI = 0.538; FIG. 4). The strict consensus tree suggests that *Nia vibrissa* is the sister group of *Henningsomyces candidus* (with 75% bootstrap support). The sister group of the *Nia-Henningsomyces* clade includes five species of *Amanita.*

Analyses of the combined dataset.—The combined four-region analysis included the two species that were resolved as the sister group of *Nia vibrissa* in one or more of the independent analyses (*Henningsomyces candidus* and *Schizophyllum commune*) as well as other taxa that appeared to be closely related to the *Nia* clade (*Cortinarius, Amanita, Fistulina, Typhula*). The dataset had an aligned length of 3559 bases, of which 81 bp were deemed to be ambiguously aligned and were excluded from the analysis. The remainder of the alignment included 840 variable positions, of which 408 were parsimony-informative. Parsimony analysis recovered six trees (1793 steps, CI = 0.603, RI = 0.315). Bootstrapped parsimony analysis strongly suggests that *Nia vibrissa* is the sister group of *Henningsomyces candidus* (bootstrap = 94%; FIG. 5). Four of the equally most parsimonious trees suggest that the sister group of the *Nia-Henningsomyces* clade is a strongly supported (bootstrap = 97%) group that contains *Schizophyllum commune* and *Fistulina hepaitca* (FIG. 5), but the other two trees suggest that *Amanita muscaria* is the sister group of the *Nia-Hen-*

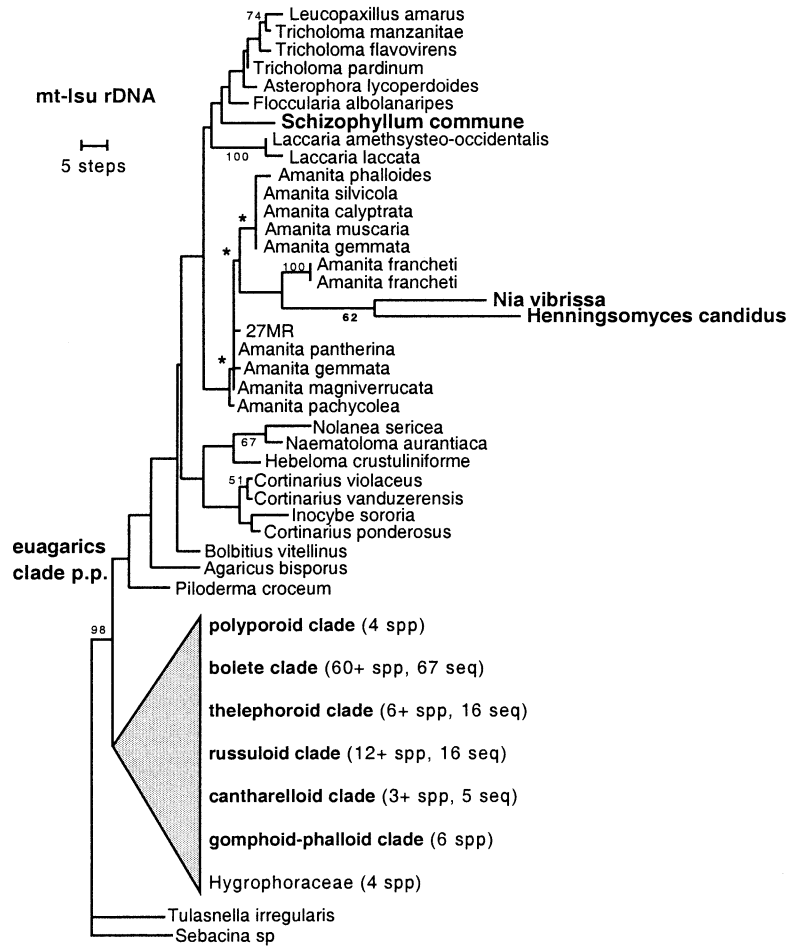

FIG. 2. Phylogenetic placement of *Nia vibrissa* inferred from mt-lsu rDNA sequences. Tree 1/1000 (851 steps, CI = 0.396, RI = 0.866). The mt-lsu sequence of *Agaricus bisporus* was published as *A. brunnescens,* and the sequence of *Floccularia albolanaripes* was published as *Armillaria albolanaripes* (Bruns et al 1998). Symbols as in FIG. 1.

ningsomyces clade. Maximum likelihood analysis recovered a tree (−logL = 13772.96731) that is consistent with one of the parsimony trees and that suggests the *Schizophyllum-Fistulina* clade is the sister group of the *Nia-Henningsomyces* clade (FIG. 5). Bootstrapped maximum likelihood analysis supported the *Nia-Henningsomyces* clade at 73% and the *Schizophyllum-Fistulina* clade at 95% (FIG. 5).

DISCUSSION

Independent analyses of all four rDNA regions agree that *Nia vibrissa* is in the euagarics clade, and three of the independent analyses suggest that its sister group is *Henningsomyces candidus* (FIGS. 1–4). The independent analyses provide only weak to moderate bootstrap support for the *Nia-Henningsomyces* clade, but the combined analyses (which included all the taxa that the independent analyses suggested might be closely related to *Nia vibrissa*) provide strong (94%, parsimony) to moderate (73%, maximum likelihood) bootstrap support for the *Nia-Henningsomyces* clade (FIG. 5).

The relationship between *Nia vibrissa* and *Henningsomyces candidus* could not easily have been predicted based on morphology. *Henningsomyces candidus* and *Nia vibrissa* both produce minute fruiting bodies on wood (the fruiting bodies of *Henningsomyces candidus* are about 0.3 mm wide; Agerer 1973), but there are no obvious anatomical features that unite the taxa. Both *Nia vibrissa* and *Henningsomyces candidus* produce hairs on the surfaces of their fruiting bodies, but in *Nia vibrissa* the hairs are curved and may be dichotomously branched, whereas in *Henningsomyces candidus* they are irregularly diverticulate. Nevertheless, the molecular data strongly suggest that *Henningsomyces candidus* and *Nia vibrissa* are closely related (FIG. 5).

The four independent datasets that were analyzed include 18 genera of gasteromycetes (*Alpova, Brauniellula, Calostoma, Chamonixia, Crucibulum, Cyathus, Gastroboletus, Gautieria, Geastrum, Hymenogas-*

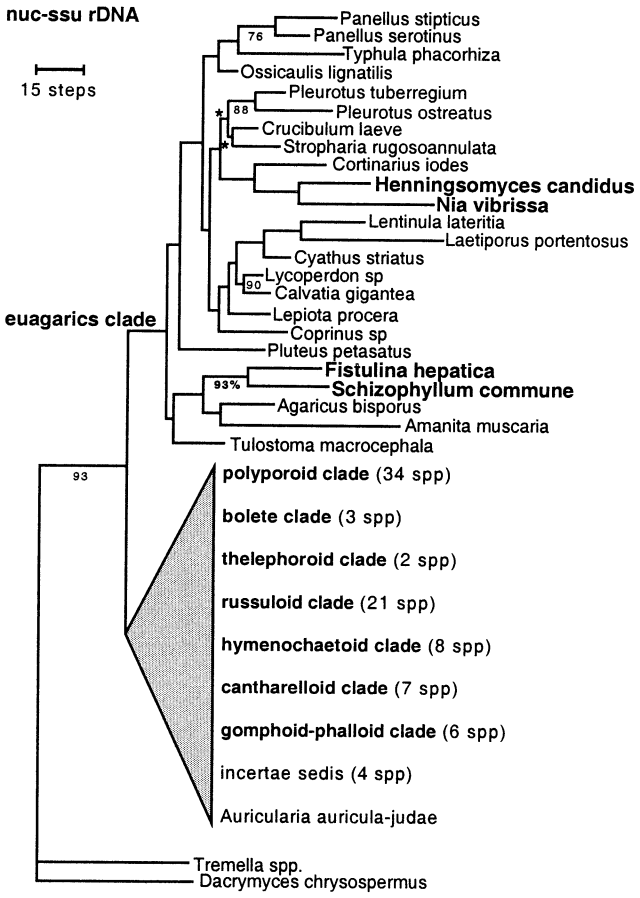

FIG. 3. Phylogenetic placement of *Nia vibrissa* inferred from nuc-ssu rDNA sequences. Tree 1/1000 (3175 steps, CI = 0.337, RI = 0.524). Symbols as in FIG. 1.

ter, Lycoperdon, Pisolithus, Pseudocolus, Rhizopogon, Scleroderma, Truncocolumella, Tulostoma, Sphaerobolus) and numerous genera of agarics that have secotioid relatives (e.g., *Agaricus, Coprinus, Entoloma, Laccaria, Russula*), but none were supported as close relatives of *Nia vibrissa*. Therefore, *Nia* represents an independent origin of the gasteroid habit in the homobasidiomycetes.

Cyphelloid-gasteroid transformations have not (to our knowledge) been suggested previously, but it is easy to envision how they could occur. In *Henningsomyces candidus* and many other cyphelloid taxa there is a narrow constriction at the apex of the tube (FIG. 6A, B), which would simply need to be closed to produce a gasteroid fruiting body that retains the basic structure of a cyphelloid fungus. Interestingly, there is a marine cyphelloid fungus, *Halocyphina villosa,* in which the hymenium is exposed in young fruiting bodies, but later becomes enclosed "by an elongation and interweaving of the hairs at the aperture of the basidiocarp" (Ginns and Malloch 1977, p 55–56). In other regards, *Halocyphina villosa* resembles *Henningsomyces candidus*: it produces minute (0.3–0.5 mm wide), white fruiting bodies with branched surface hairs, clavate basidia, and subglobose, asymmetric, inamyloid spores (Ginns and Malloch 1977). *Halocyphina villosa* occurs on periodically exposed mangrove roots (Ginns and Malloch 1977), whereas *Nia vibrissa* occurs on fully submerged substrates, and *Henningsomyces candidus* is terrestrial (it has also been collected on driftwood; S. A. Redhead, pers comm). Thus, *Halocyphina villosa* appears to be intermediate between *Nia vibrissa* and *Henningsomyces candidus* in both form and habitat. If *Nia vibrissa, Halocyphina villosa,* and *Henningsomyces candidus* are in the same clade, then this would suggest that the transition to a marine habitat preceded (and therefore could have triggered) the evolution of the gasteroid habit.

The sister group of the *Nia-Henningsomyces* clade is not resolved with confidence. Based on the independent analyses, which use different sets of species, plausible candidates include a group containing *Schizophyllum commune, Fistulina hepatica,* and *Typhula phacorrhiza* (suggested by mt-ssu rDNA, FIG. 1), *Amanita* spp. (suggested by mt-lsu rDNA and nuc-lsu rDNA, FIGS. 2, 4), and *Cortinarius iodes* (suggested by nuc-ssu rDNA, FIG. 3). Maximum likelihood analysis of the combined dataset suggests that the sister group of the *Nia-Henningsomyces* clade is the *Schizophyllum-Fistulina* clade, but parsimony analysis suggests it could be either the *Schizophyllum-Fistulina* clade or *Amanita muscaria* (FIG. 5). Neither the parsimony analysis or maximum likelihood analysis of the combined dataset suggest that *Typhula* or *Cortinarius* is very closely related to *Nia.*

Morphological and ecological characters and the maximum likelihood analysis suggest that *Amanita* is not closely related to the *Nia-Henningsomyces* clade. The former produces large, centrally stipitate, agaricoid fruiting bodies with a complex system of veils, whereas the latter produces minute cyphelloid or gasteroid fruiting bodies. Moreover, *Amanita* species are ectomycorrhizal, whereas *Nia vibrissa* and *Henningsomyces candidus* are both lignicolous. *Schizophyllum commune* and *Fistulina hepatica* are also lignicolous, and *Nia vibrissa, Henningsomyces candidus,* and *Schizophyllum commune* all produce or are associated with a white rot (Stalpers 1978, Leightley and Eaton 1979, Ginns and Lefebvre 1993). *Fistulina hepatica* is unusual in this group in that it produces a brown rot (Gilbertson and Ryvarden 1986). *Nia vibrissa* can exploit keratinic substrates, which has been shown by baiting for the fungus with feathers (Rees and Jones 1985) and by decay studies with horse tail hair media (Rosseló et al 1993). *Schizophyllum commune* is also suspected to be keratinophilic and is a facultative human pathogen (Kligman 1950, Donk 1964, Sigler et

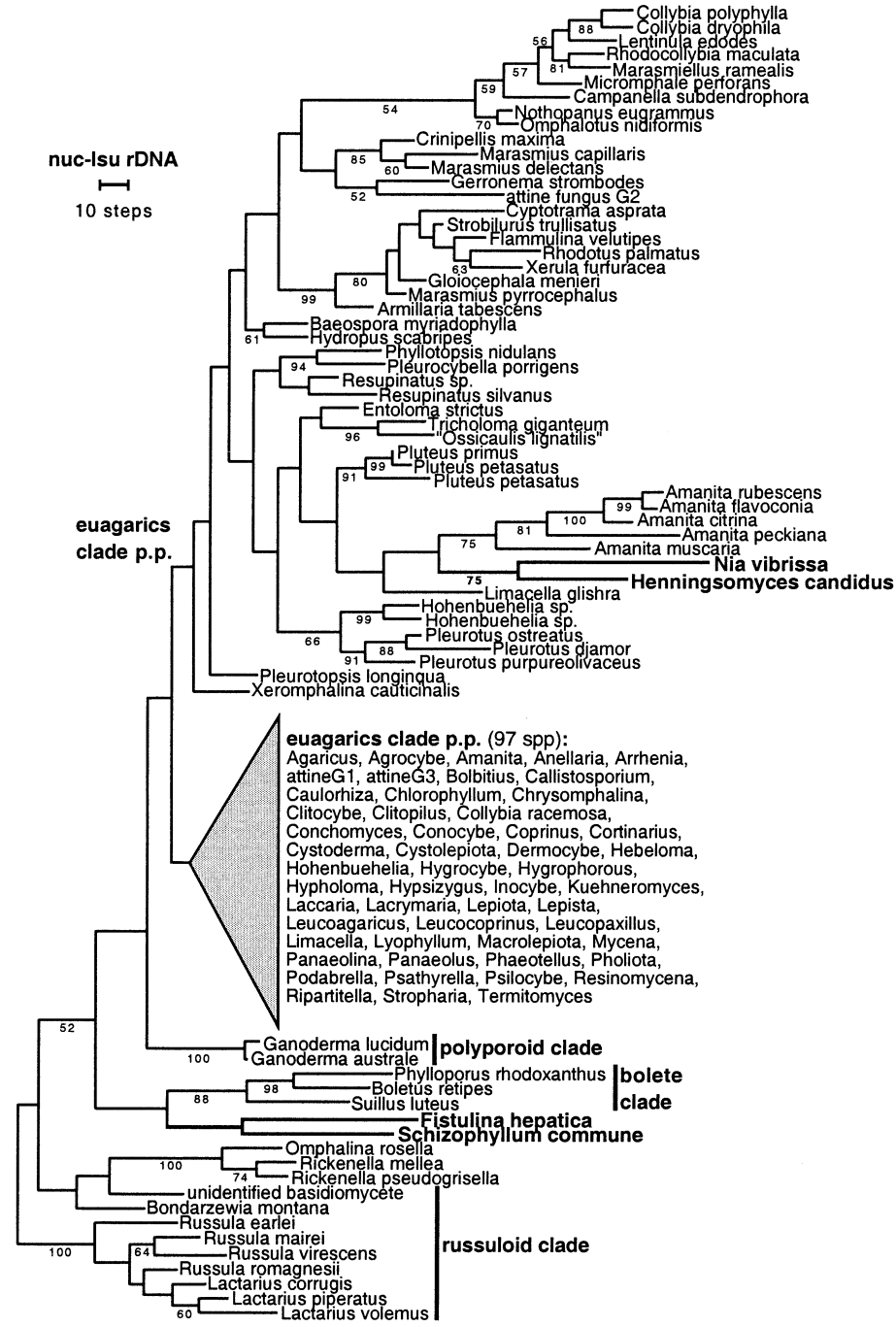

FIG. 4. Phylogenetic placement of *Nia vibrissa* inferred from nuc-lsu rDNA sequences. Tree 1/1000 (4254 steps, CI = 0.224, RI = 0.538). The sequence labeled *"Ossicaulis lignatilis"* was misidentified or mislabeled; its correct identity is uncertain (J.-M. Moncalvo, pers comm). Symbols as in FIG. 1.

al 1999). The genetic basis of keratin degrading activity in basidiomycetes is not well understood. If the mechanism is same in *Nia vibrissa* and *Schizophyllum commune,* then this would corroborate the view that they are closely related.

Our tentative inference that the *Nia-Henningsomyces* clade is the sister group of the *Schizophyllum-Fistulina* clade revives debate about the relationships among cyphelloid fungi, *Schizophyllum,* and *Fistulina.* Cyphelloid fungi have sessile or pendent, cup-shaped or tubular fruiting bodies, with smooth hymenophores (FIG. 6A, B). Although they have been nominally grouped in the family Cyphellaceae, they are widely regarded as a polyphyletic assemblage (Donk 1959, 1964, 1971, Agerer 1986, Singer 1986). A commonly held view is that some cyphelloid forms have

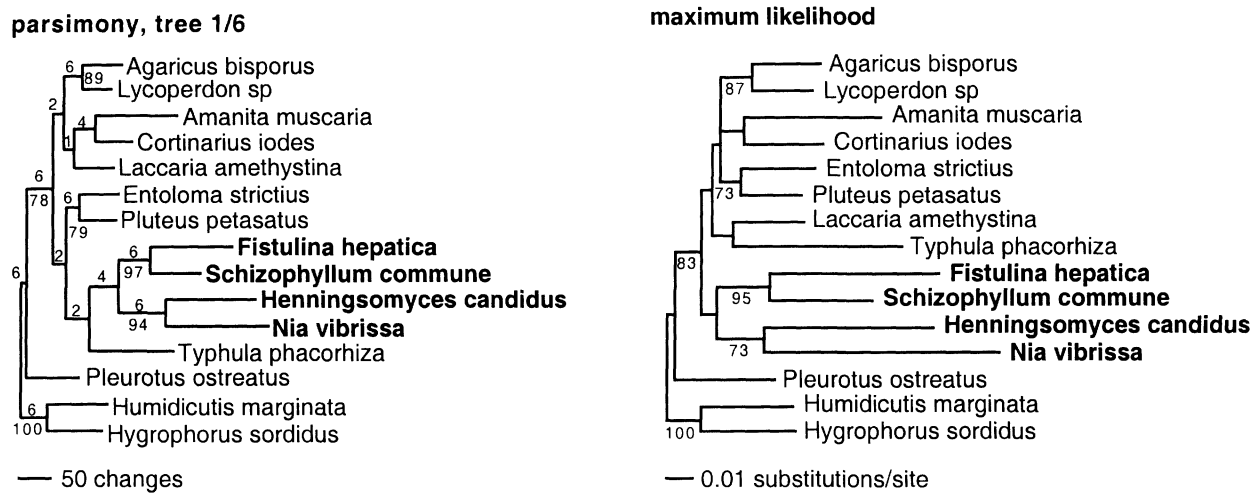

FIG. 5. Phylogenetic placement of *Nia vibrissa* inferred from parsimony analysis (one of six trees, 1793 steps, CI = 0.603, RI = 0.315) and maximum likelihood analysis (tree score: −logL = 13772.96731) of the combined, four-region dataset. Numbers above branches in the parsimony tree indicate frequency of groupings among equally parsimonious trees. Numbers below branches in both trees are bootstrap frequencies (values below 50% are not shown).

been derived by reduction from agaricoid ancestors (Donk 1959, 1964, 1971, Agerer 1978, Singer 1986), although Cooke (1961) suggested that cyphelloid fungi, which he united in the Porotheleaceae, are derived from corticioid ancestors. Our finding that *Henningsomyces candidus* is nested in the euagarics clade supports the view that some cyphelloid forms could indeed be "reduced agarics". However, this is the only cyphelloid taxon that we examined.

Schizophyllum and *Fistulina* have unusual fruiting bodies and their phylogenetic relationships have been obscure. Both genera resemble cyphelloid forms in some aspects. In *Schizophyllum,* the fruiting body is pendent and the elements of the hymenophore curl toward each other, enclosing the hymenium during dry periods (Essig 1922, Linder 1933). In tangential section, the adjacent pairs of hymenophore elements of *Schizophyllum* (Linder 1933) resemble individual fruiting bodies of *Henningsomyces* and other cyphelloid fungi, which also curl up when dry (Agerer 1986; FIG. 6C–E). Donk (1964) suggested that the fruiting body of *Schizophyllum* is essentially cyphelloid and he included *Henningsomyces* in the Schizophyllaceae, along with *Plicaturopsis,* which has a pileate fruiting body with a wrinkled hymenophore, and *Stromatoscypha,* which forms cyphelloid fruiting bodies on a well developed, membranous subiculum. In addition, molecular and morphological studies (Stalpers 1988, Nakasone 1996) suggest that *Auriculariopsis ampla,* which has a cupulate fruiting body with a smooth hymenophore, is closely related to *Schizophyllum commune.*

Fistulina produces large, fleshy fruiting bodies that superficially resemble those of polypores. However, the "pores" of *Fistulina* are actually composed of individual tubes of hyphae lined by a hymenium, which are initially formed on all surfaces of the fruiting body (Lohwag and Follner 1936; FIG. 6F). Each tube on the surface of the *Fistulina* fruiting body thus resembles an individual cyphelloid fruiting body. Singer (1986, p 843) suggested that the fruiting body of *Fistulina* could have been formed by an aggregation of cyphelloid fruiting bodies and the expansion of a subiculum, and he termed the pileus and stipe-like parts of the fruiting body a "protocarpic false carpophore". Singer further suggested that *Fistulina* and its putative cyphelloid relatives might be closely related to the Tricholomataceae. Based on the anatomy of the hymenophore, Lohwag and Follner (1936) classified *Fistulina* in the Cyphellaceae, while Bondarzew and Singer (1941) and Cooke (1961) classified *Fistulina* in its own family, Fistulinaceae, in the suborder Cyphellineae. In contrast, Donk (1959, 1964) and Agerer (1978) rejected the idea that *Fistulina* is closely related to cyphelloid fungi, although they commented on the similarities between *Fistulina* and the cyphelloid *Porotheleum* and *Stigmatolemma* (FIG. 6G, H).

Because of limited sampling and low bootstrap support it is not possible to draw firm conclusions about the composition or monophyly of the hypothesized *Nia-Henningsomyces-Schizophyllum-Fistulina* clade. Nevertheless, our results are consistent with the view that the unique fruiting bodies of *Schizophyllum commune* and *Fistulina hepatica* (and related taxa, like *Auriculariopsis ampla*) were ultimately derived from cyphelloid forms, which in turn were derived from agaricoid ancestors. Analyses with more taxa that may be in this group are needed. Such studies could elu-

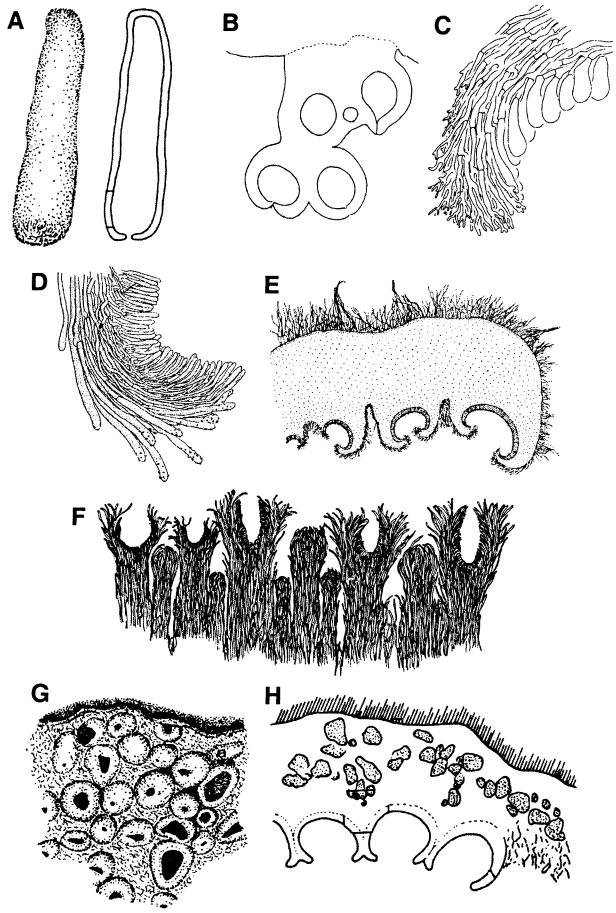

FIG. 6. Fruiting body morphologies of *Henningsomyces* and putatively related taxa. A. *Henningsomyces candidus,* habit and longitudinal section (after Agerer 1973, fig. 7). B, C. *Henningsomyces minimus* (after Agerer 1973, figs. 8, 10). B. Section through cluster of fruiting bodies. C. Detail of margin of fruiting body. D, E. *Schizophyllum* spp. (after Linder 1933, pl. 34, fig. 2, pl. 35, fig. 2). D. *Schizophyllum commune,* detail of margin of hymenophore. E. *Schizophyllum fasciatum,* tangential section through fruiting body. F. *Fistulina hepatica* hymenophore (after Lohwag and Follner 1936, fig. 1). G, H. *Stigmatolemma poriaforme* (after Agerer 1978, fig. 15). G. Habit. H. Section through cluster of fruiting bodies on membranous subiculum.

cidate novel pathways of morphological evolution in homobasidiomycetes, possibly involving the fusion and elaboration of cyphelloid fruiting bodies, and their transformation into aquatic gasteromycetes.

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