Commentary

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A recent addition to the Pluteoid clade, which is subordinate to Melanoleuca, is the agaricoid genus Kinia.

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INTRODUCTION

Kinia priver-nensis is the typical species of Kinia Consiglio, Contu, Setti & Vizzini (Consiglio et al., 2008), a recently discovered monospecific genus. The species is distinguished by a distinct set of features that includes a tricholomatoid habit, ornamented, non-amyloid spores, long, hygrophoroid, siderophilous basidia with micro-type granulations (refer to Clémençon 1978, 2004), bilateral gill tracta, and the absence of clamp-connections in the hyphae of the entire basidioma as well as well-differentiated cystidia.

The taxon is unmistakably a member of the extinct Tricholomataceae sensu R. Heim ex Pouzar, but it does not meet the description of any of the genera that are currently recognized in the Agaricales, and its familial relationships are still somewhat unclear based only on micromorphological evidence.

The siderophilous basidia, angular-verrucose spores, and Kinia privernensis shares characteristics with the genus Gerhardtia Bon (Bon 1994, 1999; Contu & Consiglio 2004), a member of the Lyophyllaceae (Kühner) Jülich (Frøslev et al. 2003, Saar et al. 2009) in that it lacks clamp connections. It is differentiated by its non-divergent gill trama and shorter collybioid basidia. Clearly amyloid spore warts are seen in other species, such as Melanoleuca Pat. and Lyophyllopsis Sathe & Daniel (Singer 1986), which exhibit decorated basidiospores without clamps and somewhat siderophilous basidia. Some genera, such as Amanitaceae R. Heim ex Pouzar, Pluteaceae Kotl. & Pouzar, and Hygrophorus Fr. in the Hygrophoraceae Lotsy, have distinct hymenophoral tramas and are well-characterized, distinct from Kinia (Singer 1986, Reijnders & Stalpers 1992).

The current paper's goal was to use molecular approaches to examine the evolutionary relationships of Kinia within the Agaricales.

Techniques

Using a Zeiss DSM 950 SEM, electronic micrography was created in response to Moreno et al. (1995).

The new combination and subgenus have been added to MycoBank (http://www.mycobank.org/).

PCR amplification, DNA extraction, and DNA sequencing

Following the manufacturer's instructions, 1 mg of a dried herbarium specimen from the Kinia prvernensis type collection was used to obtain genomic DNA using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy).

For the LSU rDNA amplification, universal primers LR0R/LR6 (Vilgalys & Hester 1990; Vilgalys lab, unpublished, http: //www.botany.duke.edu/fungi/mycolab) were employed.

The PE9700 thermal cycler was used to conduct amplification reactions.

(Perkin-Elmer, Applied Biosystems) using the following final concentrations or total amounts in a 25 µl reaction mixture: Five nanograms of DNA, one PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), one microgram of each primer, 2.5 milligrams of MgCl2, 0.25 milligrams of each dNTP, and half a Taq polymerase unit (Promega) are required. The following was the PCR program:

For the ITS and LSU primers, the temperature ranges are as follows: 2 minutes at 72 °C for 35 cycles, 10 minutes at 72 °C for 1 cycle, and 30 seconds at 94 °C and 45 seconds at 50 °C. The results of the PCR were separated on a 1.0% agarose gel and made visible by ethidium bromide staining. DiNAMYCODE srl (Turin, Italy) purified and sequenced the PCR products.

Pairwise comparison and evolutionary analysis

Using the blastn technique, the sequence acquired in this work was compared to the GenBank sequence database (http://www.ncbi.nlm.nih.gov/Genbank/). We either created or downloaded the sequences used in the phylogenetic analysis from GenBank. Sequences were chosen based on the blastn

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results in accordance with the findings of previous Agaricales phylogenetic investigations (Moncalvo et al. 2002, Matheny et al. 2006). Using the default settings, multiple alignments were carried out with CLUSTAL W (Thompson et al. 1994), and BioEdit version 5.0.9 (Hall 1999) was used for manual optimization. A maximum-likelihood cladogram with 1,000 rapid bootstraps was created using RAxML (Stamatakis et al. 2005) in accordance with the GTR+G base substitution model, with Lyophyllum decastes serving as the outgroup. Inference using Bayesian Inference (BI) and the posterior MrBayes (Ronquist & Huelsenbeck 2003) was used to perform the probability distribution of trees, and the following settings were used: Lyophylum decastes is the outgroup; Lset nst = 6 rates = gamma; samplefreq = 1,000; other parameters = default. To confirm the analysis's stationarity, the sump burnin = 250 was employed. For each tree sampled throughout the Bayesian analysis, summary statistics were generated using the sumt command and the corresponding burnin settings. FigTree v1.1.2 was used to display and update the consensus tree (Rambaut 2010).

Findings and conversation

The alignments and phylogenetic tree were uploaded to TreeBASE (www.treebase.org) with accession number 10613, and the LSU rDNA K. privernensis sequence was submitted to GenBank with accession number FN825672.

The topology produced by Bayesian and maximum likelihood analysis was the same (Fig. 1). as defined by Matheny et al. (2006), with Kinia evidently grouping within Melanoleuca species of the Pluteoid clade. A preliminary study of ITS sequences (data not shown) also supports this result.

Four families of agaricoid and gasteroid fungi comprise the Pluteoid clade: Amanitaceae, Pleurotaceae Kühner, Limnoperdonaceae, and Pluteaceae. G.A. Escobar, as well as a few solitary and single genera, such as Cantharocybe H.E. Bigelow & A.H. Sm. and Tricholomopsis Singer.

Except for a few ectomycorrhizal Amanita Pers. species and their sequestrate allies (Torrendia Bres. and Amargrendia Bougher & T. Lebel), the majority of the Pluteoid clade's taxa are saprophybic and many of them develop evident cystidia (e.g., Pluteus Fr., Volvariella Speg., Hohenbuehelia Schulzer, Tricholomopsis, and Melanoleuca). Within the Pleurotaceae, Pleurotus (Fr.) P. Kumm. and Hohenbuehelia, possess the capacity to feed on nematodes (Thorn et al. 2000).

Given that their basidiomata have comparable habits, clamps, siderophilous granulation of the micro-type in the basidia, and decorated basidiospores, Kinia's placement within Melanoleuca is not entirely surprising. Consequently, we suggest the novel combination:

comb. nov. MycoBank 518313 Melanoleuca privernensis (Consiglio, Contu, Setti & Vizzini) Consiglio, Setti & Vizzini Rivista di Micologia 51(4): 293 (2008); Kinia privernensis Consiglio, Contu, Setti, & Vizzini.

However, the characteristics that make Melanoleuca so abnormal are the presence of decorated spores (Fig. 2) with inamyloid warts, lengthy basidia, and bilateral hymenophoral trama (Fig. 3).

Kinia (Consiglio, Contu, Setti & Vizzini) is a subgenus of Melanoleuca; Consiglio, Setti & Vizzini, stat. nov. MycoBank 518312 Genus Kinia Consiglio, Contu, Setti, & Vizzini, Rivista di Micologia 51(4): 292 (2008), is the basionym.

Typus : Consiglio, Contu, Setti, & Vizzini Kinia privernensis.

Melanoleuca now includes taxa with both amyloid and nonamyloid spores thanks to Kinia. However, another monophyletic genus of the Pluteoid group, Amanita, also has this condition and has long been acknowledged.

Three taxa in the Pluteoid clade—Kinia, Pluteaceae, and Amanitaceae—have bilateral gill tramas, which may serve as a useful phylogenetic marker.

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