
The agaricoid genus *Kinia* is a new member of the Pluteoid clade subordinate to *Melanoleuca*

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Based on maximum likelihood and Bayesian LSU rDNA analyses, the recently described monospecific genus *Kinia* is reduced to a subgenus of the genus *Melanoleuca*, a genus exhibiting phylogenetic affinities with members of the Pluteaceae within the Pluteoid clade. The new combination *Melanoleuca privernensis* and the new subgenus *Kinia*, are introduced.

Key words – Agaricomycetes – Amanitaceae – Pluteaceae – bilateral gill trama – taxonomy

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Introduction

Kinia Consiglio, Contu, Setti & Vizzini (Consiglio et al. 2008) is a recently described monospecific genus typified by *Kinia privernensis*. It is characterized by a unique and rather peculiar suite of characters such as a tricholomatoid habit, non-amyloid, ornamented spores, long hygrophoroid and siderophilous basidia with granulations of the micro-type (sensu Cléménçon 1978, 2004), bilateral gill trama, and a lack of both clamp-connections in the hyphae of the whole basidioma and well-differentiated cystidia.

The taxon is clearly a member of the former Tricholomataceae sensu R. Heim ex Pouzar, but it does not fit the description of any of the currently described genera in the Agaricales and, based on micromorphological data only, its familial affiliations remain rather cryptic.

The angular-verrucose spores, siderophilous basidia and the lack of clamp connections

in *Kinia privernensis* are features shared 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) which, however, is distinguished by having shorter collybioid basidia and a non-divergent gill trama. Other genera with slightly siderophilous basidia and ornamented basidiospores lacking clamps, such as *Melanoleuca* Pat. and *Lyophyllopsis* Sathe & Daniel (Singer 1986), have clearly amyloid spore warts. Genera with a divergent hymenophoral trama are well characterized and different from *Kinia*, namely Amanitaceae R. Heim ex Pouzar, Pluteaceae Kotl. & Pouzar, and *Hygrophorus* Fr. in the Hygrophoraceae Lotsy (Singer 1986, Reijnders & Stalpers 1992).

The aim of the present paper was to investigate the phylogenetic relationships of *Kinia* within the Agaricales by the application of molecular techniques.

Methods

Electronic micrography was made under a Zeiss DSM 950 SEM following Moreno et al. (1995).

The new subgenus and the new combination are deposited in MycoBank (<http://www.mycobank.org/>).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of a dried herbarium specimen from the *Kinia privernensis* type collection by using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. Universal primers LR0R/LR6 (Vilgalys & Hester 1990; Vilgalys lab, unpublished, <http://www.botany.duke.edu/fungi/mycolab>) were used for the LSU rDNA amplification.

Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of *Taq* polymerase (Promega). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C for both ITS and LSU primers, 2 min at 72 °C for 35 cycles, 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. The PCR products were purified and sequenced by DiNAMYCODE srl (Turin, Italy).

Sequence alignment and phylogenetic analysis

The sequence obtained in this study was compared to the GenBank sequence database (<http://www.ncbi.nlm.nih.gov/Genbank/>) using the blastn algorithm. Sequences included in the phylogenetic analyses were either generated by us or obtained from GenBank. Based on the blastn results, sequences were selected according to the outcomes of other phylogenetic studies of Agaricales (Moncalvo et al. 2002, Matheny et al. 2006). Multiple alignments were performed with CLUSTAL W (Thompson et al. 1994) using default settings and manually optimized with BioEdit version 5.0.9 (Hall

1999). Using RAxML (Stamatakis et al. 2005), a maximum-likelihood cladogram with 1,000 fast bootstraps was constructed following the GTR+G base substitution model using *Lyophyllum decastes* as outgroup. Bayesian inference (BI) based upon the posterior probability distribution of trees was performed with MrBayes (Ronquist & Huelsenbeck 2003) with the following settings: outgroup *Lyophyllum decastes*; Lset nst = 6 rates = gamma; mcmc ngen = 1,000,000 samplefreq = 1,000; other settings = default. The sump burnin = 250 was used to verify the stationarity of the analysis. The sumt command with respective burnin values was used to produce summary statistics for trees sampled during the Bayesian analysis. The consensus tree was visualized and edited with FigTree v1.1.2 (Rambaut 2010).

Results and discussion

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

Both maximum likelihood and Bayesian analyses resulted in the same topology (Fig. 1), with *Kinia* clearly clustering within *Melanoleuca* species of the Pluteoid clade as delimited by Matheny et al. (2006). This result is also confirmed by a preliminary analysis of ITS sequences (data not shown).

The Pluteoid clade includes four families of agaricoid and gasteroid fungi: the Pluteaceae, Amanitaceae, Pleurotaceae Kühner and Limnoperdonaceae G.A. Escobar, plus some single and isolated genera as *Tricholomopsis* Singer and *Cantharocybe* H.E. Bigelow & A.H. Sm. Many taxa of the Pluteoid clade develop evident cystidia (e.g. *Pluteus* Fr., *Volvariella* Speg., *Hohenbuehelia* Schulzer, *Tricholomopsis* and *Melanoleuca*) and most taxa are saprobic, with the exception of many ectomycorrhizal *Amanita* Pers. species and their sequestrate allies (*Torrencia* Bres. and *Amarrendia* Bougher & T. Lebel). *Pleurotus* (Fr.) P. Kumm. and *Hohenbuehelia*, in the Pleurotaceae, are able to prey upon nematodes (Thorn et al. 2000).

The placement of *Kinia* within *Melanoleuca* is not completely surprising since their

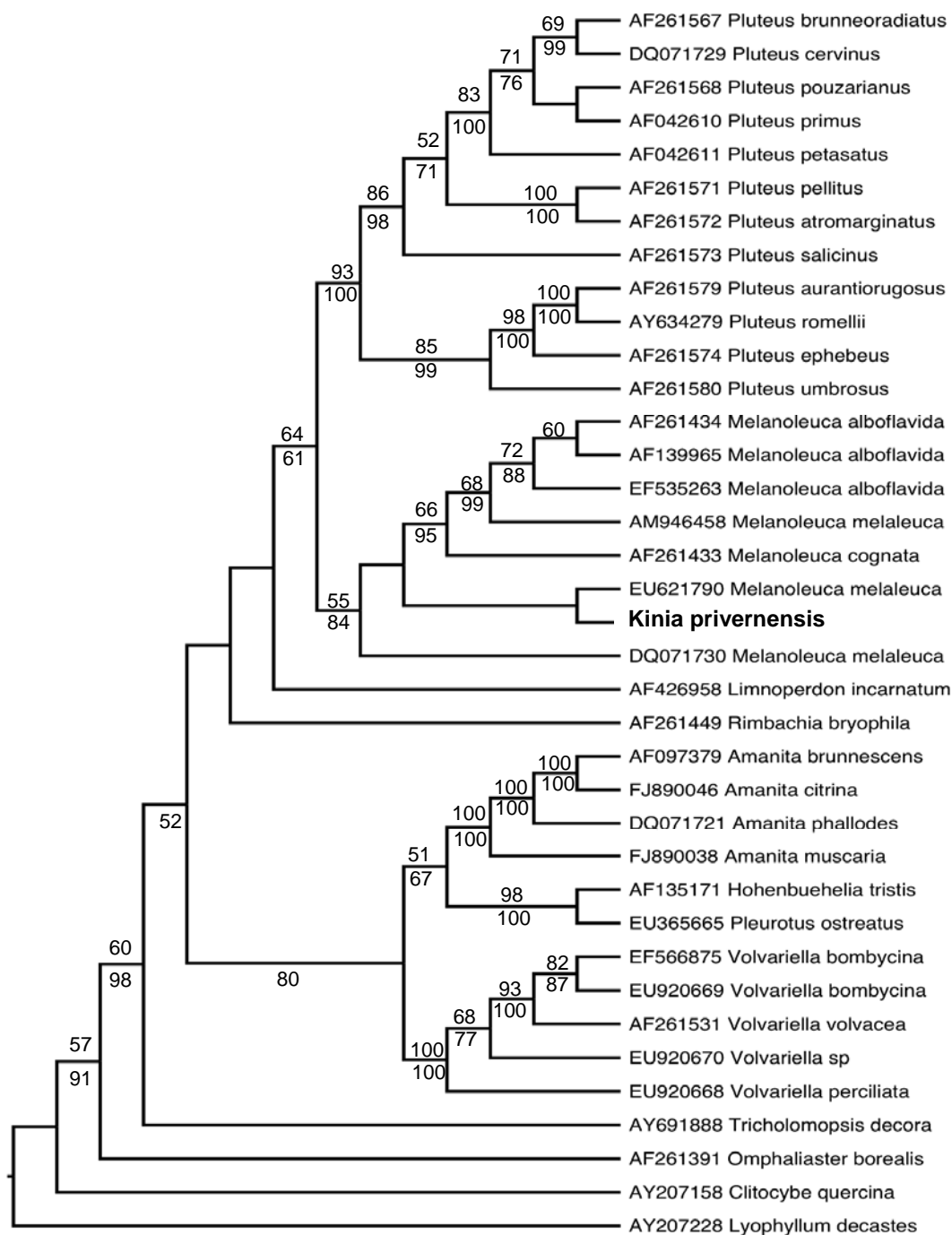


Fig 1. – The Pluteoid clade. Maximum-likelihood cladogram inferred from 940 bp LSU rDNA region alignment. Bootstrap values superior to 50 are shown above branches. Posterior probability values coming from the Bayesian inference over 50 are shown below branches. *Lyophyllum decastes* was used as outgroup.

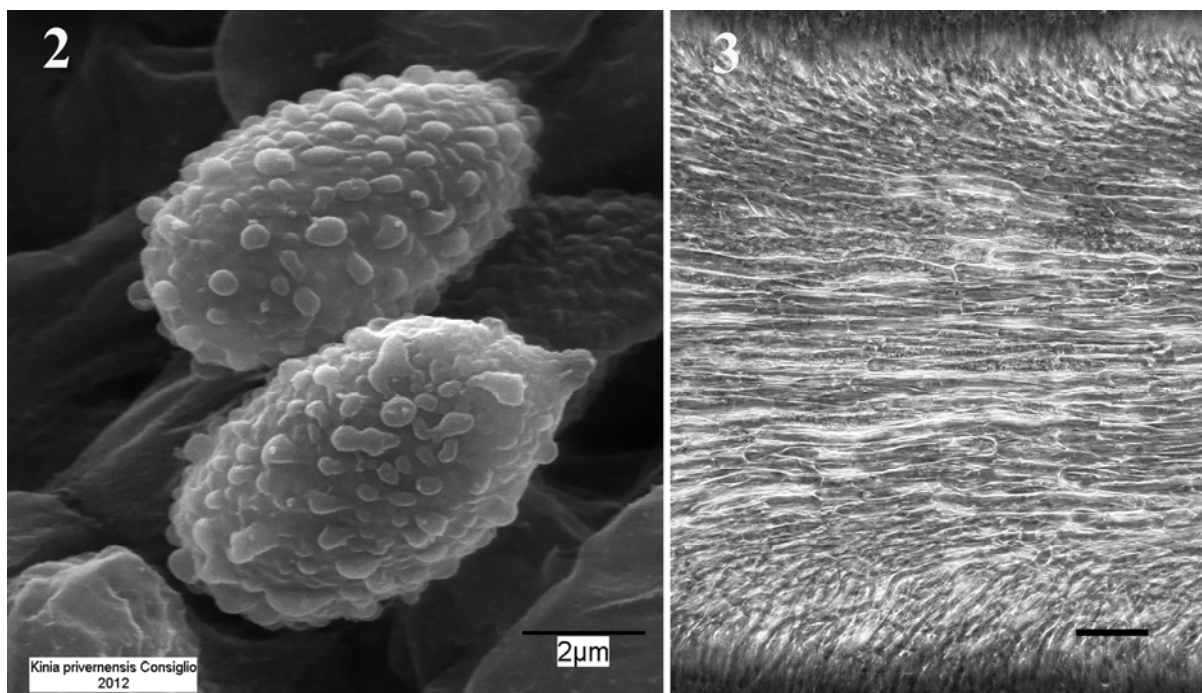
basidiomata share a similar habit, have clamps, siderophilous granulation of the micro-type in the basidia, and ornamented basidiospores. As a consequence, we propose the new combination:

Melanoleuca privernensis (Consiglio, Contu, Setti & Vizzini) Consiglio, Setti & Vizzini, **comb. nov.**

Mycobank 518313

Basionym: *Kinia privernensis* Consiglio, Contu, Setti & Vizzini, *Rivista di Micologia* 51(4): 293 (2008).

The presence of ornamented spores (Fig. 2) with inamyloid warts, long basidia and bilateral hymenophoral trama (Fig. 3), however, are characters so aberrant in *Melanoleuca*



Figs 2–3 – *Melanoleuca privernensis*. **2** Spores. **3** Gill trama (bar = 20 μ m).

as to warrant the establishment of a new subgenus:

Melanoleuca* subgenus *Kinia (Consiglio, Contu, Setti & Vizzini) Consiglio, Setti & Vizzini, **stat. nov.**

MycoBank 518312

Basionym: Genus *Kinia* Consiglio, Contu, Setti & Vizzini, *Rivista di Micologia* 51(4): 292 (2008).

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

With the inclusion of *Kinia*, *Melanoleuca* encompasses taxa with amyloid and non-amyloid spores. However, such a situation is also present, and has been long accepted in *Amanita*, another monophyletic genus of the Pluteoid clade.

The presence of a bilateral gill trama in three taxa (namely *Kinia*, Pluteaceae and Amanitaceae) of the Pluteoid clade could be a good phylogenetic marker.

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