New combinations in *Clitocybula*: a study of cystidiate *Pseudoomphalina* species (Basidiomycota, Agaricomycetes)

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Malysheva E. F., Morozova O. V. & Contu M. (2011) New combinations in *Clitocybula*: a study of cystidiate *Pseudoomphalina* species (Basidiomycota, Agaricomycetes). – Sydowia 63 (1): 85–104.

The morphology and phylogeny inferred using molecular data from the nrITS region of species traditionally classified in the genus *Pseudoomphalin*a (Basidiomycota, Agaricomycetes) are investigated. The present work is mainly focused on two cystidiate species, viz. *Pseudoomphalina flavoaurantia* and *P. lignicola*, which were originally described from Italy and Russia, respectively. Morphological examination of type collections and phylogenetic analyses showed these species to be closely related to each other and sister to a representative clade of *Clitocybula* species in a larger clade that is recognized as *Clitocybula*. A neotype for *Pseudoomphalina lignicola* is designated and the new combinations, *Clitocybula flavoaurantia* and *Clitocybula lignicola* are proposed.

Key words: Tricholomataceae, Marasmiaceae, taxonomy, ITS, phylogeny

The genus *Pseudoomphalina* (Singer) Singer comprises saprotrophic species usually with omphalinoid appearance. This genus is relatively uncommon and infrequently noticed in the field. Most of the species belonging to this genus are characterized chiefly by dark yellow-brown color of the pileus as well as by ellipsoid or subglobose, amyloid basidiospores, a lack of true cystidia and by the presence of intraparietal or incrusting pigment in the pileipellis. According to Singer (1986), who originally introduced the genus *Pseudoomphalina* (Singer 1956) with *Omphalia kalchbrenneri* Bres. [≡ *Pseudoomphalina kalchbrenneri* (Bres.) Singer] as type species, it differs from the related genus *Omphalina* Quél. only by having amyloid basidiospores. Further research and descriptions of new taxa under *Pseudoomphalina*, namely *P. lignicola* Lj. N. Vassiljeva (Vassiljeva 1973) and *P. flavoaurantia* Contu along with the section *Cystidiatae* (Contu 2003), expanded the application of the genus to include substantial variation in morphological characteristics (bright color of basidiomata, presence of large

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well-developed pileo-, cheilo- and caulocystidia) and suggested its close relation to some species of *Clitocybula* (Singer) Métrod. The members of the genus *Clitocybula* [type species: *C. lacerata* (Scop.: Fr.) Singer] are characterized by their clitocyboid to collybioid habit, globose to subglobose amyloid basidiospores, regular hymenophoral trama (sometimes with interwoven hyphae) and lignicolous habitat. The main distinguishing features of these genera in Singer's sense (Singer 1975, 1986) are habitat and kind of pigmentation comprising, besides intracellular pigment, also intraparietal and incrusting pigments in *Pseudoomphalina*.

Pseudoomphalina as a whole has been classified for a long time in the Tricholomataceae R. Heim ex Pouzar, tribe *Leucopaxilleae* Singer (Singer 1975, 1986). Recently, the classification of *Pseudoomphalina* is in flux and it seems to be uncertain as it varies in different taxonomic systems (Kuyper 1995, Knudsen 2008, Kirk *et al.* 2008). Unfortunately, recent molecular phylogenetic studies of the *Agaricales* based on several genes that were performed by Lutzoni (1997), Moncalvo *et al.* (2002), Matheny *et al.* (2006) and Redhead *et al.* (2002) have not included *Pseudoomphalina* in the analyses. At the present time, there are approximately five (Kirk *et al.* 2008) to 15 recognized species of *Pseudoomphalina* in the world (http://www.indexfungorum.org/) and five among them occur in Europe (Contu 2003). Recent detailed investigations of *Pseudoomphalina* including observations of type material (Ballero & Contu 1993, Contu 2003, Consiglio *et al.* 2006, Knudsen 2008) clarified knowledge of its species diversity and ecology in Europe. The generic position of some species, however, is still debated and their relationships with other species and genera remain unknown and difficult to discern (Bon 1997, Contu 2003). *Pseudoomphalina* species with well-developed cystidia are especially problematic in this regard.

Pseudoomphalina lignicola, originally described from the Primorsky Territory by Lj. N. Vassiljeva (Vassiljeva, 1973), is widely distributed all over the Western and Eastern Siberia and the Russian Far East, but is unknown in Europe and in other parts of the world. However, the description of *P. lignicola* published by Vassiljeva in 1973 is brief and could have been more detailed, and, unfortunately, the holotype material has apparently been lost. The study of our collection revealed that *P. lignicola* is very similar to the European species *Pseudoomphalina flavoaurantia* (Contu 2003), known only from Italy.

The purpose of this paper is to clarify the taxonomy of *P. lignicola* and *P. flavoaurantia* and to examine phylogenetic relationships of these species with other European *Pseudoomphalina* species and representatives of other closely related genera. This was accomplished by a morphological study based on several collections of the two species from different regions as well as phylogenetic analysis based on nrITS region sequence data.

Materials and Methods

Morphological study

The studied collections of *Pseudoomphalina lignicola* consist of material collected by the authors in Altaj (Siberia, Russia) as well as selected specimens from the Mycological Herbarium of the Komarov Botanical Institute (LE) (Saint Petersburg) and the Institute of Biology and Soil Science (VLA) (Vladivostok), including the designated neotype specimen from the Russian Far East. All collections of *P. flavoaurantia* and *P. pachyphylla* (Fr.: Fr.) Knudsen together with one *P. kalchbrenneri* (Bres.) Singer specimen originated in Italy: one part of the studied specimens (including the type of *P. flavoaurantia*) is kept in the Museo Civico di Storia Naturale «Giacomo Doria» (Genova, Italy) and the other part includes exsiccata housed in the private mycological collections of Prof. Dr. G. Consiglio and M. Contu.

New collections from this study were preserved with standard methods. The macroscopic description was based on examination of the fresh material and the analysis of the photos. The dried material was studied using standard microscopic techniques. Observations, measurement and drawings were variously made on preparations of basidioma mounted in 5 % KOH, Melzer's reagent and 1 % Congo Red with Micmed 2-2 and AxioImager A1 microscopes. Basidiospore dimensions are based on observation of 30 basidiospores per collection and cystidia dimensions – on observation of at least 10 structures per specimen. The designations used for the basidiospore measurements are: Q = range of spore length to width ratios; Q* = mean value for Q.

Molecular analysis

Taxon sampling. Twelve specimens of *Pseudoomphalina lignicola* and three of *P. flavoaurantia* (including type) examined in the morphological study were selected for molecular analysis. Other European representatives of *Pseudoomphalina* as well as *Clitocybula* and *Pseudoclitocybe* (Singer) Singer were also selected for analysis. We generated a total of 25 ITS sequences for this study.

Six additional ITS sequences of other clitocyboid/omphalinoid genera were retrieved from GenBank based on BLAST results (http://www.ncbi.nlm.nih.gov/BLAST/) with *Pseudoomphalina* species as well as the data of the Matheny *et al.* (2006) paper.

An overview of all taxa studied is given in Tab. 1, which shows the names of species, GenBank accession numbers, Herbarium numbers and collection particulars.

DNA extraction, PCR and sequencing. DNA was extracted from herbarium material using CTAB extraction buffer with following steps of consecutive addition of chloroform-isoamyl alcohol

Table 1. – Species and collections/sequences used for phylogenetic analyses.

Species	Origin of collections	Location and date of collection	GenBank accession no.
Clitocybe gibba	GenBank	No data	GU188436
C. gibba	GenBank	No data	AB301608
Clitocybula atroalba	GenBank	No data	DQ192179
C. flavo-aurantia	GDOR, holotypus	Italy: Sardegna, Diga del Liscia, lato Luras, loc. Carana, stazzo Podda (OT), 20 Sep 2002	HM191744
C. flavo-aurantia	GDOR /D	Italy: Sardegna, loc. Stazzo Montesu Tempio Pausania, 15 Oct 2002	HM191743
C. flavoaurantia	LE 262757	Italy: Sardegna, prov. Olbia-Tempio P., ad locun dictum Golfo di Marinella, 26 Oct 2008	HM191745
C. lacerata	LE 6639	Russia: Primorsky Territory, Khasansky District, Kedrovaya Pad Nature Reserve, bank of Kedrovaya River, 16 Sep 1985	HM191746
C. lacerata	LE 262743	Russia: Altaj Republic, Altajsky Nature Reserve, vicinities of Yajlyu, 14 Aug 2008	HM191748
C. lacerata	LE 262744	Russia: Altaj Republic, vicinities of Bijka, 16 Aug 2008	HM191747
C. lignicola	LE 262727	Russia: Altaj Republic, Altajsky Nature Reserve, bank of Kamga River, 18 Aug 2008	HM191731
C. lignicola	LE 6625	Russia: Krasnoyarsky Territory, Turukhansky District, 10 Aug 1979	HM191732
C. lignicola	LE 262728	Russia: Altaj Republic, vicinities of Bijka, 16 Aug 2008	HM191733
C. lignicola	LE 201190	Russia: Buryatia Republic, Pribajkal'sky District, valley of Selenga River, 1 Aug 1997	HM191734
C. lignicola	LE 262738	Russia: Altaj Republic, Altajsky Nature Reserve, vicinities of Yajlyu, bank of Atkichu River, 15 Aug 2008	HM191738
C. lignicola	LE 253926	Russia: Altaj Republic, vicinities of Bijka, 16 Aug 2008	HM191741
C. lignicola	LE 262737	Russia: Altaj Republic, Altajsky Nature Reserve, bank of Malyj Mionok River, 17 Aug 2008	HM191742
C. lignicola	VLA M-19.178	Russia: Jewish Autonomous Region, broad-leaved forest, 18 Aug. 2002	HM191740

Species	Origin of collections	Location and date of collection	GenBank accession no.
C. lignicola	VLA M-19218	Russia: Jewish Autonomous Region, Bastak Reserve, Sopka Dubovaya, 21 Aug 2003	HM191739
C. lignicola	VLA M-20.989	Russia: Jewish Autonomous Region, Bastak Reserve, head-stream of Ikura River, 10 Aug 2006	HM191735
C. lignicola	VLA M-20.825	Russia: Jewish Autonomous Region, Bastak Nature Reserve, valley of Bastak River, 21 Aug 2004	HM191736
C. lignicola	VLA M-10763	Russia: Primorsky Territory, vicinities of Vladivostok, Bogataja Griva, 19 Aug 1997	HM191737
C. oculus	GenBank	No data	DQ192178
Omphalina mutila	GenBank	No data	FJ770399
O. mutila	GenBank	No data	FJ770394
Pseudoclitocybe cyathiformis	LE 262726	Russia: Saint Petersburg, Duderhof Hills, broad-leaved forest, on wood, 23 Sep 2005	HM191729
P. cyathiformis	LE 258346	Russia: Sverdlovsk Region, Visimsky Nature Reserve, 24 Sep 2004	HM191730
Pseudoompha–lina kalch–brenneri	LE 262742	Russia: Leningrad Region, Gogland Island, 7 Aug 2007	HM191752
P. kalch-brenneri	LE 262748	Italy: Toscana, prov. Grosseto, loc. Oasi di Patanella, 9 Dec 2006	HM191753
P. pachyphylla	LE 262747	Italy: Sardegna, Aglientu, loc. Rena Majore, 29 Oct 2000	HM191749
P. pachyphylla	LE 262750	Italy: Sardinia, prov. Olbia-Tempio P., Aglientu, ad locun dictum Rena Majore, 9 Feb 2008	HM191750
P. pachyphylla	LE 262756	Italy: Lazio, prov. Latina, loc. Sabaudia, 17 Nov 2006	HM191751

mixture, then isopropyl alcohol-3M sodium acetate solution for precipitation, 70 % ethanol for washing and finally CTAB buffer or water for dissolution. The alternative method of extraction DNA was using Qiagen DNA easy Plant Mini Kit. The ribosomal ITS1-5.8S-ITS2 region was amplified by PCR with the fungal specific primers ITS1F and ITS4B (http://www. biology.duke.edu/fungi/mycolab/primers.htm). The PCR products were purified using Qiagen QIAquick Gel Extraction Kit and PCR Purification Kit. Sequencing of this

strand was performed with ABI model 3130 Genetic Analyzer (Applied Biosystems) using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) with the same primers. The raw data were processed using Sequencing Analysis 5.3.1 (Applied Biosystems).

Alignments and phylogenetic analysis. The sequences obtained were compared with those available in GenBank using the BLAST algorithm. The ITS sequences were aligned with several programs and web tools: ClustalX (Thompson *et al.* 1997) with different settings for reaching the best alignment, T-Coffee (www.tcoffee.org), Muscle (www.ebi.ac.uk/Tools/muscle/index.html) with the default settings and MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) with Q-INS-i option. Different alignments obtained were compared using AltAVisT web tool (http://bibiserv.techfak.uni-bielefeld.de/altavist). Ambiguously aligned ITS sequences or multiple base gaps were excluded from further analysis. The final alignment was corrected manually using BioEdit (Hall 1999).

Phylogenetic reconstructions were performed with maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BA) analyses. In all analyses *Clitocybe gibba* (Pers.: Fr.) P. Kumm. was selected as an outgroup based on alignment and preliminary parsimony analysis. Besides, *C. gibba* is an appropriate outgroup because of its location between two large clades including the majority of clitocyboid/omphalinoid taxa (Tricholomatoid clade and Mycenoid clade) in the work of Matheny *et al.* (2006).

A MP analysis was performed using PAUP*4.0.b10 (Swofford 2002). One hundred heuristic searches were conducted by stepwise addition with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithm. One tree was held at each step during stepwise addition. Parsimony bootstrap analysis was performed with 1000 replicates. Clades with only a support ≥ 50 % were retained. Gaps were treated as missing characters.

For ML analysis, the appropriate nucleotide substitution model was determined using ModelTest Web Server (http://darwin.uvigo.es). The TVM+I+G model with – lnL= 3660.1128 was chosen based on Akaike Information Criterion (AIC) and was then used for performing ML analysis.

Bayesian analysis was performed using MrBayes 3.1 (Ronquist & Huelsenbeck 2003) for two independent runs, each with 2 300 000 generations with sampling every 100 generations, with GTR model and four chains. Posterior probability (PP) value ≥ 0.95 are considered as significant.

Genetic distances were estimated in the program Mega4 (Tamura *et al.* 2007) using the JC69 nucleotide substitution model with gamma distributed rate among sites ($\alpha = 1.0$).

Results

Molecular phylogeny

The ITS sequence dataset, including 5.8S gene, contains 31 sequences, 615 characters long (gaps included), with 20 variable and 284 parsimony informative sites. The MP analysis yielded 702 MPTs of 708 steps (l = 720, CI = 0.6681 and RI = 0.8908). Using the best model of evolution for analyzed datasets, ML analysis resulted in two trees with –lnL score 3660.11274. For the Bayesian analysis, the General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rate variation across sites was used.

The topologies in all analyses were almost identical. The bootstrap 50 % majority-rule consensus tree yielded from MP analysis is shown in Fig. 1 and a ML/BA tree is presented in Fig. 2. Four distinct clades were recovered from all analyses. Most of the clades found in the MP analysis have a high bootstrap support (close to or equal 100 %) with the exceptions of two clades. Clades with posterior probability value of 95 % or higher are generally congruent with clades with a bootstrap support ≥ 70 % and are presented in all MP, ML and BA trees. Individual clades A, B, C and D corresponding apparently to the genus rank comprise the groups of closely related species: clades A and B consist of the representatives of *Pseudoclitocybe* and *Omphalina*, respectively; a highly supported clade C contains *Pseudoomphalina kalchbrenneri* and *P. pachyphylla*; the fourth major clade D contains three species of *Clitocybula* together with *Clitocybula lignicola* and *C. flavoaurantia* and is subdivided into two additional subclades. The level of sequence divergence between any pair of sequences in the *Clitocybula*-clade varies from 0 % (identical collections inside *C. lignicola* subclade and *C. flavoaurantia* group) to a maximum percentage of 28.2 %. Clade C was recovered as basal to A and D clades in all topologies. A clade with *Pseudoclitocybe* and clade D received a rather low BS (60 %) in the MP analysis and a low PP value (0.80) in BA analysis.

Taxonomy

Clitocybula lignicola (Lj. N. Vassiljeva) E. F. Malysheva & O. V. Morozova, **comb. nov.** – Figs. 3, 4, 6.

MycoBank no.: MB 518337

Basionym. – *Pseudoomphalina lignicola* Lj. N. Vassiljeva, Agaricovye Shlyapochnye Griby (por. Agaricales) Primorskogo Kraya: 153. 1973.

Neotype of *Pseudoomphalina lignicola* (designated here): Sakhalin Island, vicinities of Novo-Aleksandrovsk, flood plain forest with *Alnus hirsuta*, 20 Sept 1960, *leg.* and *det.* Lj. N. Vassiljeva (VLA M-1462).

Type Study

Original diagnosis of *Pseudoomphalina lignicola*:

"Pileo juniore convexo, dein umbilicato, infundibuliformi brunneo-ochraceo, pubescenti, hygrophano, 1.5–2.5 cm, margine striato; lamellis valde decurrentibus,

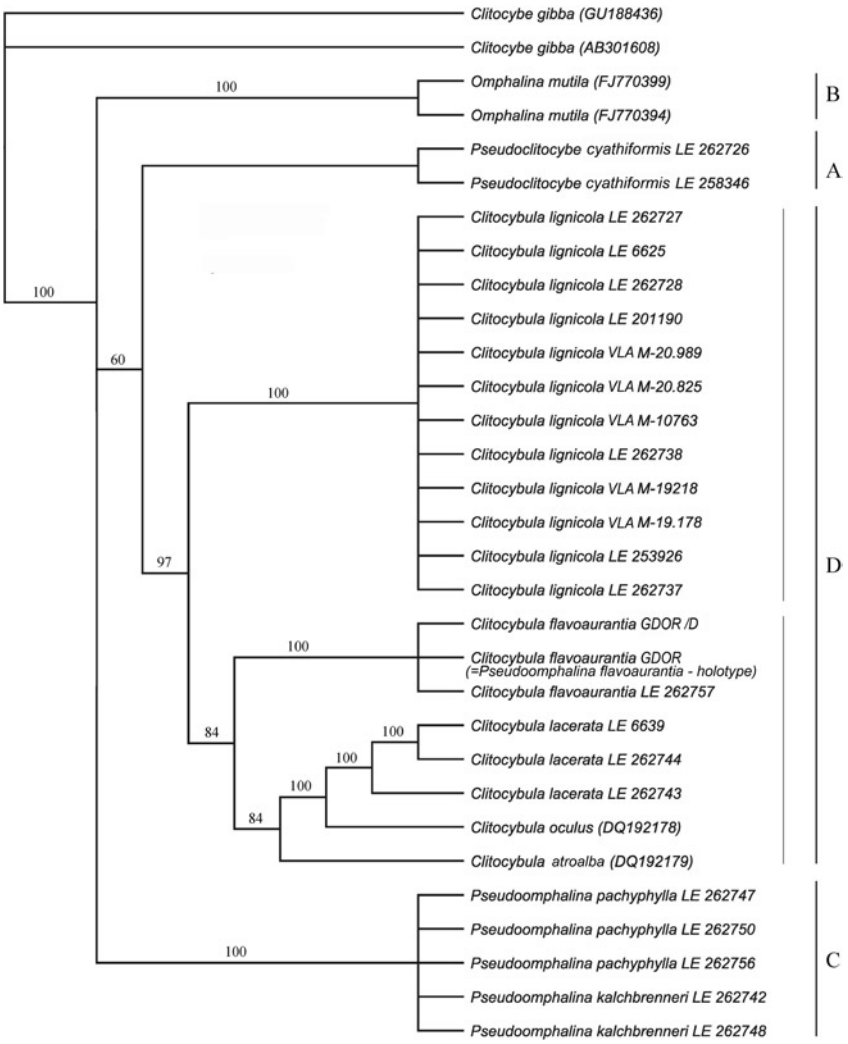

Fig. 1. – A Bootstrap 50 % majority-rule consensus tree of 702 most parsimonious trees, based on ITS data. Bootstrap support > 50 % is indicated above branches. Collection accession numbers for studied collections are listed after taxon names. Accession numbers of sequences retrieved from GenBank are given in brackets.

angustis, confertis, cremeo-ochraceis; stipite tubuloso, pubescenti, apice-pilei concoloribus, infra atro-brunneo, ad basin setuloso. Sporis globosis, glabris vel paulum scabrosis, rotundatis, amyloideis 6–8 × 5–7 µm; basidiis 25–32 × 4-5 µm; trama regulari; cute pilei e hyphis parallelibus, flavescentibus".

Data based on the study of the neotype specimen:

Pileus 26–30 mm, infundibuliform, hygrophanous, margin slightly involute, reddish brown or ochraceous with rust tinge. Lamellae distant, deeply decurrent

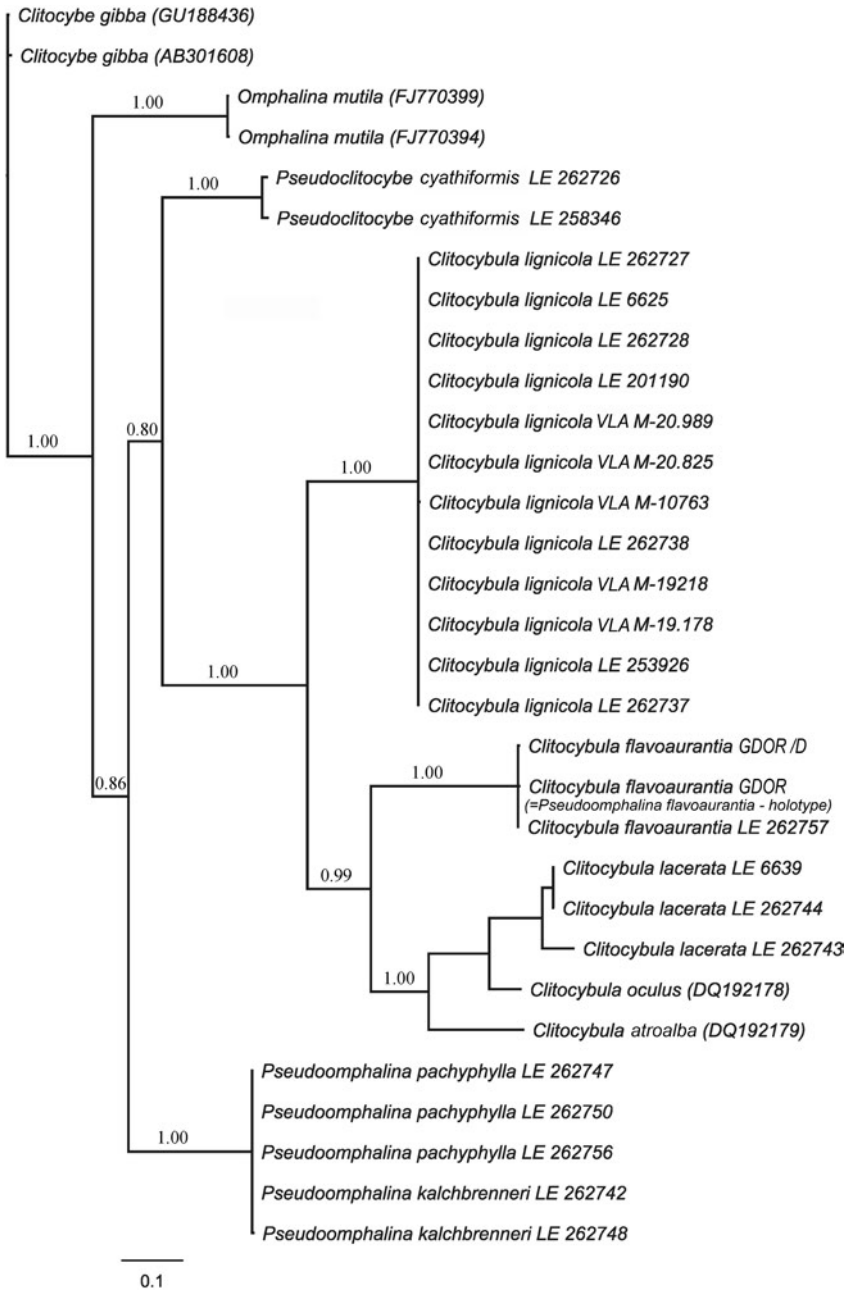

Fig. 2. – A phylogram derived from ML and Bayesian analyses based on ITS data. PP values are shown above branches.

on stipe, thin, cream-colored or ochraceous. Stipe 35–40 × 2–3 mm, cylindrical, shining in upper part, ochre-yellow.

Basidiospores 5.7–8.0 × 5.5–6.2 µm, Q = 1.0–1.3, Q* = 1.2, broadly ellipsoid or almost globose to globose, thin-walled, hyaline, strongly amyloid. Basidia 18–26 × 8–9 µm, 4-spored, clavate. Cheilocystidia 35–70 × 6–9 µm, numerous, fusoid or lageniform with long mucronate apex (3–4 µm wide), sometimes almost cylindrical, hyaline, thin-walled. Pleurocystidia absent. Pileipellis a cutis, made up of radially arranged pigmented, thin- or slightly thick-walled cylindrical and inflated hyphae 6–9 µm wide; pigment intracellular and intraparietal. Pileocystidia 30–68 × 8–16 µm, very abundant, similar in shape to cheilocystidia or clavate, thick-walled, weakly pigmented by yellow-brown intracellular pigment. Caulocystidia 30–75 × 5–11 µm, numerous, variable in shape, mainly similar to cheilocystidia, hyaline, thin-walled. Clamp connections numerous and present in all tissue (Fig. 3).

The author Lj. Vassiljeva based the description of the species on material collected from Russian Far East, Primorsky Territoryj, Ussurijsky District, Suputinsky (= Ussurijsky) Reserve, valley of Suputinka River, mixed forest, on a decaying wood of coniferous tree, 16 September 1961. The holotype material mentioned in the protologue was not found in the Mycological Herbarium of the Institute of Biology and Soil Science and it seems to be lost. However, original material collected by the author is kept in VLA. So, we selected one of these specimens as neotype. Unfortunately, we could not obtain ITS data for our study from this collection.

Description of the species based on the material collected recently:

Pileus 8–40 mm, clitocyboid or omphaloid, usually distinctly infundibuliform with deeply depressed center, glabrous or slightly pruinose to minutely scaly at centre, hygrophanous, margin straight to slightly flexuous and involute, slightly to strongly translucently striate up to one half a radius when moist, variable in color: ochraceous, umbrinous, clay color, brightly color, orange, or lightly red-brown. Lamellae distant, thin, deeply decurrent on stipe, cream-colored or yellowish. Stipe 25–60 × 2–5 mm, cylindrical or broadened towards base, pruinose or minutely pubescent, hygrophanous, yellowish at upper part and more dark at base, ochraceous, yellowish or brightly orange, with tomentose base. Smell indistinct. Spore print white.

Basidiospores 6.1–9.0(10) × 5.3–7.0 µm, Q = 1.0–1.4, Q* = 1.2, broadly ellipsoid or almost globose, thin-walled, hyaline, strongly amyloid. Basidia 20–35 × 7–9 µm, 2- or 4-spored, clavate. Cheilocystidia 27–70 × 5.5–13.0 µm, numerous, forming sterile heteromorphic edge, variable in shape, mostly fusoid with mucronate apex 2.7–5 µm wide (sometimes bifurcated or branched) or sublageniform or lageniform with long neck, hyaline, thin- or slightly thick-walled. Pleurocystidia absent; trama regular. Pileipellis a cutis made up of radially arranged hyaline or pigmented, thin- or slightly thick-walled cylindrical and inflated hyphae 5.0–13.5 µm wide; pig-

ment intracellular and intraparietal. Pileocystidia 25–90 × 8–18 µm, very abundant, fusoid or lageniform with very long and often capitate apex, clavate, almost cylindrical, thick-walled (wall up to 2.5 µm wide), hyaline or pigmented by brown intraparietal or slightly incrusting pigment. Caulocystidia 27–126 × 5.5–16.0 µm, numerous, variable in shape, mostly similar to pileocystidia, hyaline, thin- or slightly thick-walled. Clamp connections present in all tissues.

Habitat. – On trunks or decayed wood of coniferous, more rarely deciduous trees.

Distribution. – Russia (Western Siberia: Altaj Republic; Eastern Siberia: Krasnoyarsk Territory, Irkutsk Region, Buryatia Republic; Far East: Amur Region, Jewish Autonomous Region, Khabarovsk and Primorsky Territories, Sakhalin Island).

Material examined. – Russia. Altaj Republic: Ongudaj District, the bank of Bolshoj Ilgumen' River, coniferous forest, on wood, 3 Aug 2008, *leg.* O. Morozova (LE 253931); Altajsky Nature Reserve, vicinities of Yajlyu, bank of Atkichu River, forest with *Pinus sibirica*, *Picea* and *Betula*, on decaying wood of coniferous tree, 15 Aug 2008, *leg.* E. Malysheva (LE 262738, LE 253925, LE 262735); Altajsky Nature Reserve, bank of Malyj Mionok River, forest with *Salix* and *Alnus*, on decaying wood of deciduous tree, 17 Aug 2008, leg. E. Malysheva (LE 262737, LE 253929, LE 262734); Altajsky Nature Reserve, bank of Kamga River, flood plain forest (*Alnus*, *Salix*, *Betula*), on decaying wood, 18 Aug 2008, *leg.* E. Malysheva (LE 262727); Ulagan District, vicinities of Bijka, forest with *Betula*, *Picea* and *Abies*, on decaying wood of *Betula*, 16 Aug 2008, *leg.* A. Kovalenko (LE 262728); ibid., forest with *Betula*, *Picea* and *Abies*, on decaying wood, 16 Aug 2008, *leg.* E. Popov (LE 253926); ibid., coniferous forest, on decayed wood of coniferous tree, 16 Aug 2008, *leg.* A. Kiyashko (LE 262741). Krasnoyarsk Territory: Turukhansky District, forest with *Betula* and *Pinus sibirica*, 10 Aug 1979, *leg.* E. Nezdojminogo (LE 6625). Irkutsk Region: bank of Bajkal Lake, vicinities of Listvennichnoje, coniferous forest, on wood of *Picea*, 16 Aug 1947, *leg.* B. Vassil'kov (LE 6624). Buryatia Republic: Pribajkal'sky District, valley of Selenga River, forest with *Larix*, on wood of *Larix sibirica*, 1 Aug 1997, *leg.* A. Petrov (LE 201190). Amur Region: Tygda, bank of Ulagan River, on stump of Larix, 19 Aug 1958, *leg.* Lj. Vassiljeva (VLA M-1463). Jewish Autonomous Region: broad-leaved forest, 18 Aug 2002, *leg.* E. Bulakh (VLA M-19.178); Bastak Nature Reserve, Sopka Dubovaya, broad-leaved forest, 21 Aug 2003, *leg.* E. Bulakh (VLA M-19218); ibid., valley of Bastak River, broad-leaved forest, 21 Aug 2004, *leg.* E. Bulakh (VLA M-20.825); ibid., headstream of Ikura River, broad-leaved forest with *Pinus sibirica*, 10 Aug 2006, *leg.* E. Bulakh (VLA M-20.989). Khabarovsk Territory: Komsomolsky Nature Reserve, basin of Kamenka River, coniferous forest with *Abies* and *Picea*, on wood, 21 Jun 1986, *leg.* E. Bulakh (VLA M-1464). Primorsky Territory: Sikhote-Alin Nature Reserve, Tayezhnaya River, mixed forest, on decayed wood, 29 Aug 1996, *leg.* O. Morozova (LE 262733); Shkotovsky District, Khualaza Mountain, forest with *Abies* and *Picea*, on decaying stump of coniferous tree, 22 Jun 1964, *leg.* M. Nazarova (VLA M-1460); Ussurijsky Nature Reserve, mixed forest, on decaying wood of coniferous tree, Aug 1969, *leg.* M. Nazarova (VLA M-1457); Ussurijsky Nature Reserve, Sopka Grabovaya, *Pinus sibirica* forest, on decaying wood in soil, 9 Jun 1970, *leg.* M. Nazarova (VLA M-1458); Lazovsky Nature Reserve, valley of Perekatnaya River, mixed forest, on root and stump of coniferous tree, 10 Jun 1994, *leg.* S. Laptev (VLA M-1465); Muravyov-Amursky Peninsula, basin of Bogataya River, Sopka Korejskaya, on wood, 16 Nov 1955, *leg.* Lj. Vassiljeva (VLA M-22001); vicinities of Vladivostok, Emar bay, broad-leaved forest, 28 Sept 1989, *leg.* E. Bulakh (VLA M-1455); vicinities of Vladivostok, Bogataja Griva, 19 Aug 1997, *leg.* E. Bulakh (VLA M-10763); Khasansky District, Kedrovaya Pad

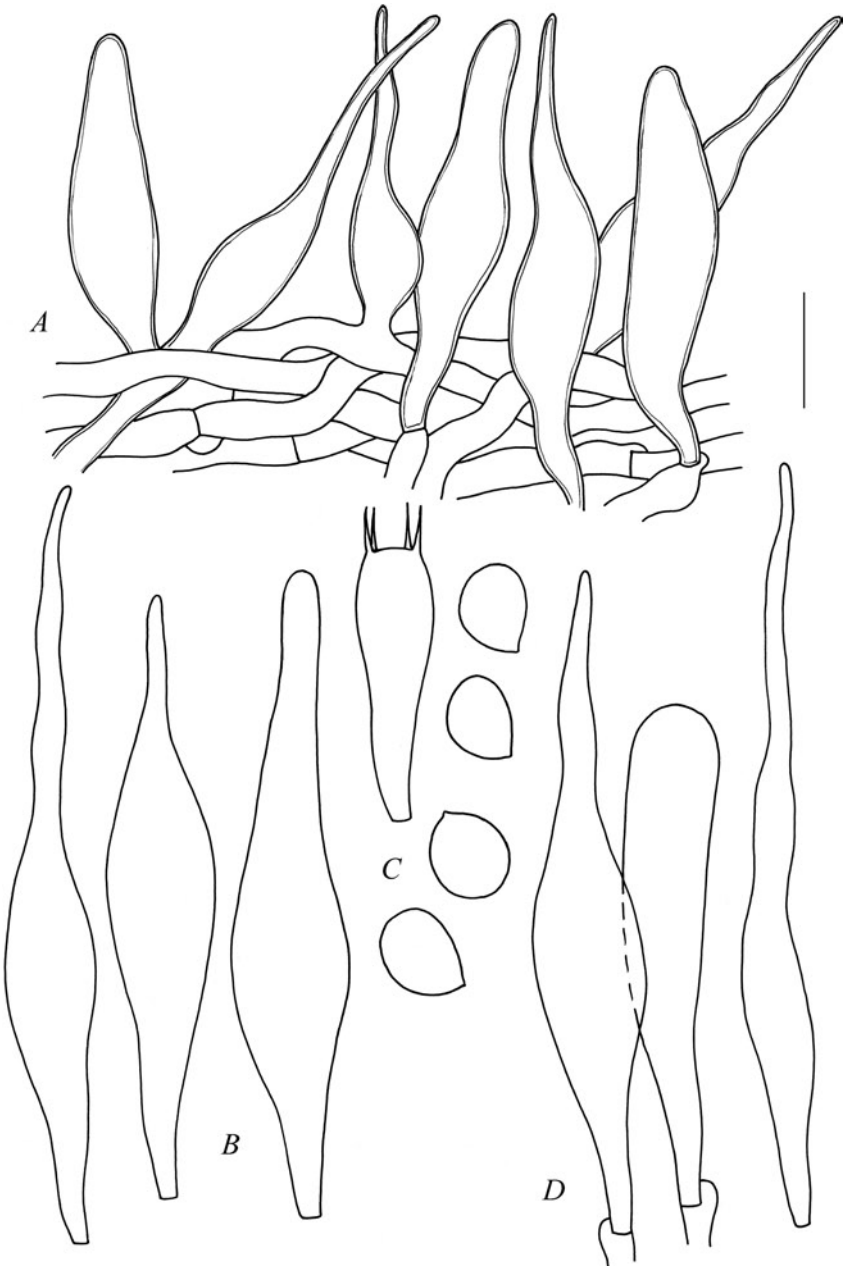

Fig. 3. – *Pseudoomphalina lignicola* (neotype, VLA M-1462): ***A*** – elements of pileipellis with pileocystidia, ***B*** – cheilocystidia, ***C*** – basidium and spores, ***D*** – caulocystidia; scale bar for C = 10 µm; for A, B, D = 15 µm.

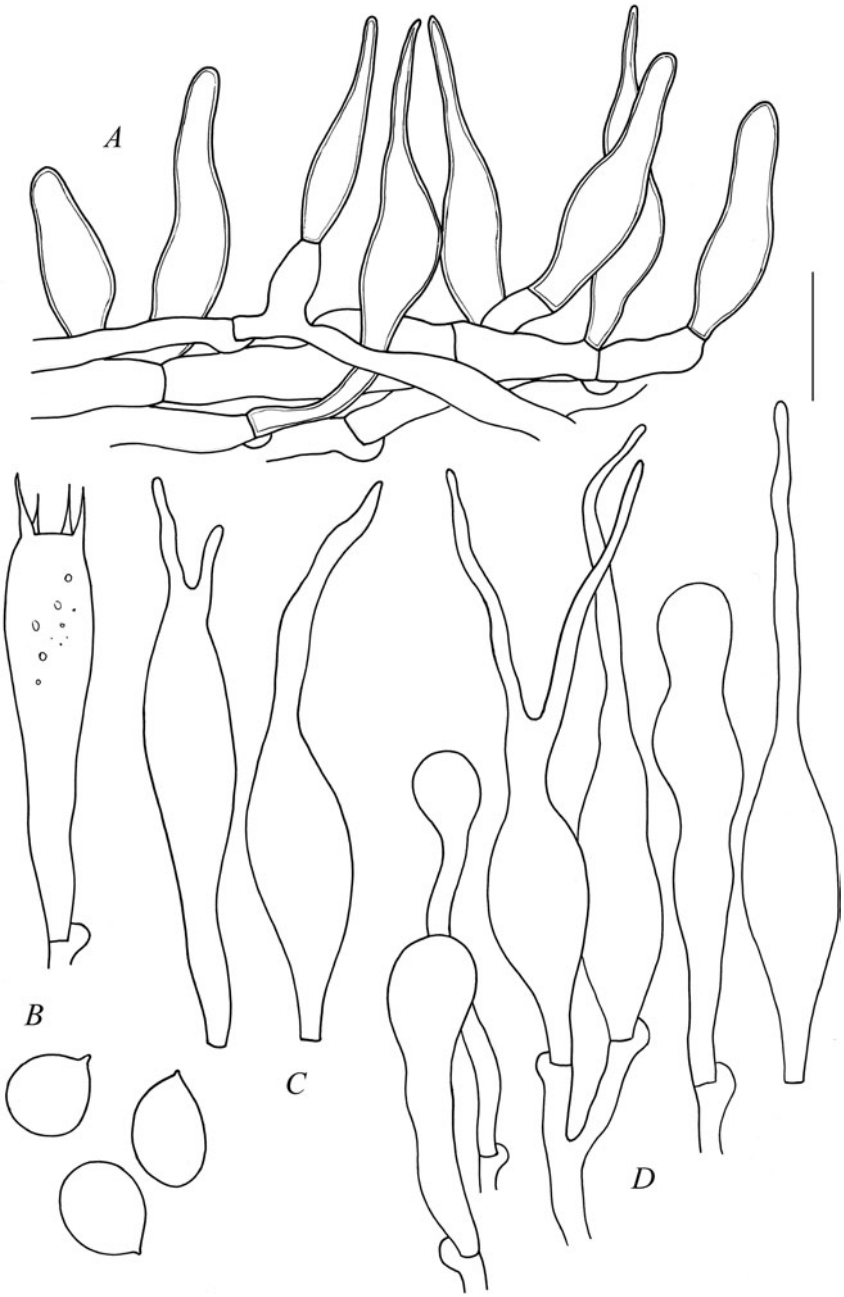

Fig. 4. – *Clitocybula lignicola* (LE 262728): ***A*** – elements of pileipellis with pileocystidia, ***B*** – basidium and spores, ***C*** – cheilocystidia, ***D*** – caulocystidia; scale bar for *B* = 10 µm; for *A, C, D* = 20 µm.

Nature Reserve, on wood, 26 Sept 1955, *leg.* Lj. Vassiljeva (VLA M-1453); Kedrovaya Pad Nature Reserve, valley of Kedrovaya River, broad-leaved forest, on wood in soil, 28 Jul 1979, *leg.* E. Bulakh (VLA M-1454).

Comments. – Based on molecular evidence, the correct taxonomical position of "*Pseudoomphalina lignicola*" is in the genus *Clitocybula* and, as a consequence, the new combination is necessary. Different collections of this species differ significantly in shape and color of the pileus. At first glance, they can be divided into two distinct groups in the field according to their appearance: group 1 with strongly hygrophanous, infundibuliform, ochre or beige, pale pileus, and group 2 with brightly orange, weakly hygrophanous pileus (Fig. 6). Contrary to macromorphological subdivision, all collections have similar microstructures and are inseparable from each other under light microscope. Moreover, they form one monophyletic group with an internally unresolved topology because all sequences are almost 100 % identical.

All phylogenetic analyses carried out clearly supported the placement of this species inside a monophyletic clade containing three other species of *Clitocybula*. This clade received a very high BS index (97 %) in MP analysis and the highest PP value (1.00) in the BA topology. Twelve collections of *C. lignicola* involved into analysis formed a distinct subclade under clade D: their genetic distance to *C. lacerata* is slightly lower (23.6 %) than to *C. flavoaurantia* (27.1 %).

Vassiljeva stressed in the protologue the high morphological similarity of this species to "*Gerronema postii* (Fr.) Singer" (≡ *Loreleia postii* (Fr.) Redhead, Moncalvo, Vilgalys & Lutzoni). However, based on molecular evidence presented in a recent study on omphalinoid genera by Redhead *et al.* (2002), *Loreleia* as well as *Rickenella* species clustered together within the *hymenohaetoid* clade and were not closely related to other groups of *Agaricales*. Therefore, we have not included these taxa in the current molecular study.

Clitocybula flavoaurantia (Contu) E. F. Malysheva, O. V. Morozova & Contu, **comb. nov.** – Figs. 5, 7.

MycoBank no.: MB 518338

Basionym. – *Pseudoomphalina flavoaurantia* Contu, Micol. Veg. Medit. 18(1): 65. 2003.

Pileus 2–20 mm, clitocyboid or omphalinoid, convex to campanulate-convex and bearing a low umbo in young stages, usually distinctly infundibuliform with deeply depressed center, glabrous not pruinose, not scaly, not or very scarcely hygrophanous, entirely translucently striate in fresh basidiomata, margin straight to slightly flexuous and involute, variable in color: brown in young stages, fulvous, orange or lightly reddish-yellow, usually with darker centre, especially in young basidiomata. Lamellae distant, thin, deeply decurrent on stipe, white. Stipe 15–40 × 2–5 mm, cylindrical or broadened to-

wards base, pruinose, hyaline at upper part and brown towards the base, which is strigose due to white hairs. Context very thin, subcartilaginous, hyaline. Smell indistinct to somewhat alkaline. Spore print white.

Basidiospores 6.2–7.8 × 4.8–7.0 µm, Q = 1.1–1.5, Q* = 1.3, broadly ellipsoid, broadly amygdaliform, ovoid or almost globose, thin-walled, hyaline, strongly amyloid. Basidia 30–40 × 6–10 µm, 4-spored, clavate. Cheilocystidia 40–70 × 12–20 µm, numerous, forming sterile edge, variable in shape, lageniform with long neck, rarely forked, sublageniform or fusoid, thin-walled. Pleurocystidia absent or rare, similar to cheilocystidia. Trama irregular. Pileipellis a cutis made up of radially arranged hyaline or slightly pigmented, thin-walled cylindrical or inflated hyphae up to 12 µm wide; pigment intracellular and intraparietal. Pileocystidia 50–90 × 10–15 µm, rare, clavate, fusoid to lageniform with long apex, thin-walled, hyaline or pigmented by brown intraparietal or minutely incrusting pigment. Caulocystidia 40–95 × 6–20 µm, numerous, variable in shape, similar to pileocystidia, clavate, fusoid or lageniform with long neck, hyaline, thin-walled. Clamp connections present in all tissue.

Habitat. – On sandy soil in grasslands.

Distribution. – Italy (Sardinia).

Material examined. – Italy, Sardegna, Diga del Liscia, lato Luras, loc. Carana, stazzo Podda, 20 Sept 2002, *leg.* M. Contu (GDOR, holotype); Sardegna, loc. Stazzo Montesu Tempio Pausania, 15 Oct 2002, *leg.* M. Contu (GDOR/D); Sardegna, prov. Olbia-Tempio P., ad locum dictum Golfo di Marinella, sandy grassland, near the coast, on acid soil, 26 Oct 2008, *leg.* M. Contu (LE 262757).

Comments. – As a further consequence of our molecular studies, the transfer of "*Pseudoomphalina flavoaurantia*" to the genus *Clitocybula* is also necessary. According to phylogenetic analysis, the position of this species in one clade together with other *Clitocybula* species, including the type species of *Clitocybula*, *C. lacerata*, is unambiguous.

Discussion and Conclusion

According to the trees depicted in Figs. 1 & 2, *Clitocybula lignicola* groups with *C. flavoaurantia* and other species of *Clitocybula* in a highly supported clade. The percentage of sequence divergence in this clade does not exceed an acceptable level of intrageneric divergence (28.2 %).

Morphologically, *Clitocybula lignicola* and *C. flavoaurantia* are mainly characterized by remarkable bright colored basidiomata with orange pileus and orange-yellow stipe in combination with presence of well-developed cystidia and amyloid basidiospores. The shape of cystidia in both species is almost identical including rare forms with branched apices. So, morphological distinction of both species from each other can be reduced to a few characters: the structure of pileipel-

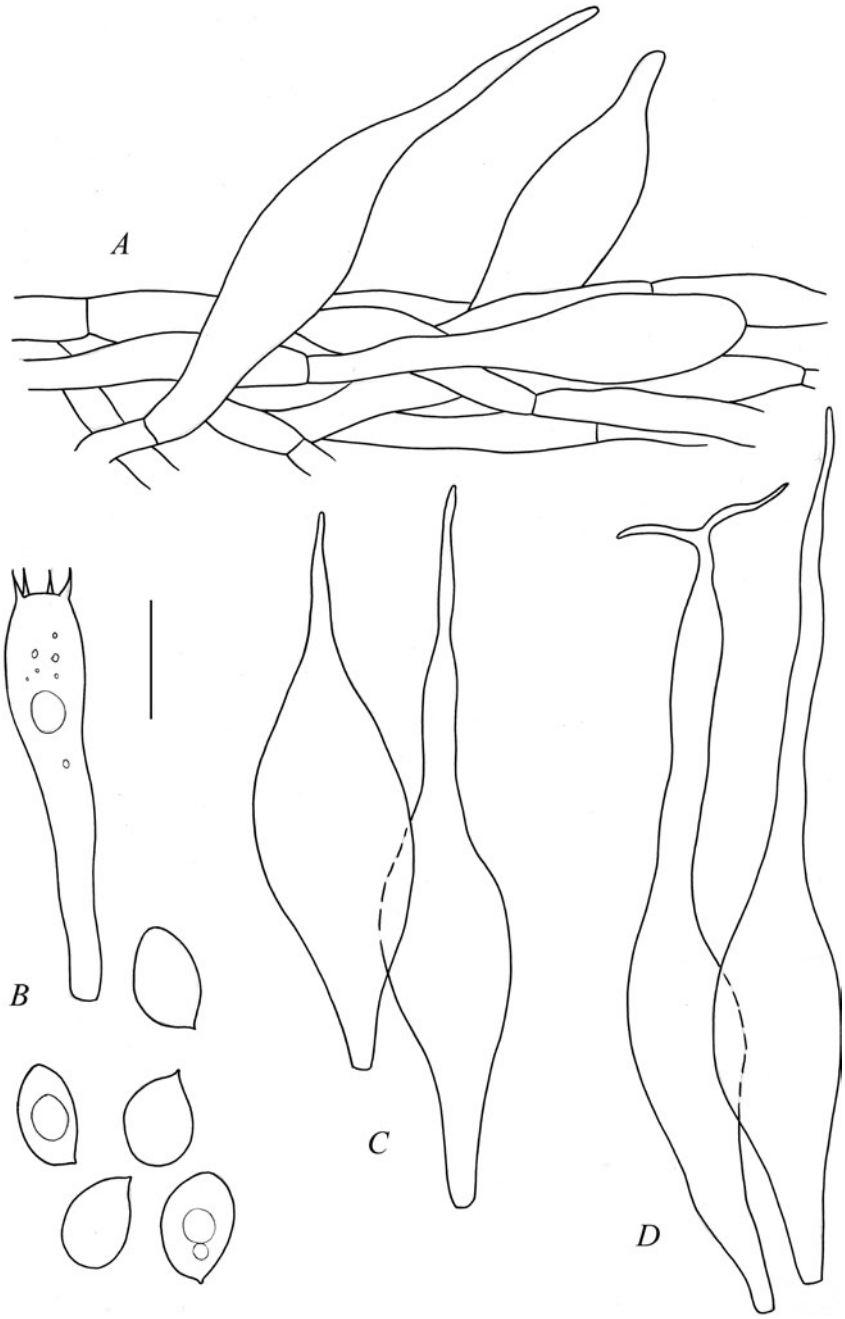

Fig. 5. – *Clitocybula flavoaurantia* (LE 262757): ***A*** – elements of pileipellis with pileocystidia, ***B*** – basidium and spores, ***C*** – cheilocystidia, ***D*** – caulocystidia; scale bar 10 µm.

Fig. 6. – Two morphotypes of *Clitocybula lignicola* basidiomata: **a**, **b** with dark, and **c**, **d** with pale pileus; scale bar 1 cm for all.

lis with a palisade layer of pileocystidia in *C. lignicola*, and scattered ones in *C. flavoaurantia*; and smaller, partially amygdaliform basidiospores in collections of *C. flavoaurantia*. The relationship between these species was also confirmed by molecular data.

The genus *Clitocybula* includes, besides *C. lignicola* and *C. flavoaurantia*, several cheilocystidiate species [among them *C. oculus* (Peck) Singer included in our analysis] and differs primarily from *Pseudoomphalina* by having well-developed cystidia and their preferred lignicolous habitat. Recent molecular studies recognized *Clitocybula* as a

Fig. 7. – Basidiomata of *Clitocybula flavoaurantia*; scale bar 1 cm.

group closely related to *Hydropus* Kühner ex Singer and *Megacollybia* Kotl. et Pouzar within the *hydropoid* clade (Moncalvo *et al.* 2002). Both *C. flavoaurantia* and *C. lignicola* are apparently close to *C. taniae* Vila (Vila 2002) when compared to other representatives of the genus because the latter species is also characterized by orange color of pileus and similar micromorphology.

Two additional taxonomical characters for distinguishing *C. lignicola* and *C. flavoaurantia* are their ecology and geographical distribution. All collections of *C. lignicola* have been found mostly in coniferous, rarely deciduous, forests on the same woody substrate. Morphological variations of basidiomata were identified as belonging to one single species and were not characterized by any ecological or geographical borders. This species is known only from the Russian territory and has a rather wide distribution: there are several of our own collections from Siberia (Altaj) together with numerous specimens collected by previous researchers from the Russian Far East and Siberia. *Clitocybula flavoaurantia* is so far only known from Italy. Thus, the distributions of these species do not overlap. Moreover, the habitat of *C. flavoaurantia* on sandy soil among grass is considerably different from that of *C. lignicola*.

Some authors (Knudsen & Hansen 1991, Ballero & Contu 1993) stressed the similarity of *Pseudoomphalina* with *Pseudoclitocybe* and *Clitocybula* in some morphological characteristics. The phylogenetic reconstructions, based on ITS markers, revealed that these genera form different well-supported clades in all trees, and *Pseudoclitocybe* oc-

curred as sister-clade to *Clitocybula,* but with low support. The genus *Pseudoomphalina,* represented in our study by several European collections of *P. kalchbrenneri* and *P. pachyphylla*, forms one basal monophyletic group. The internal topology of this group remained unresolved in all analyses. The percentage of sequence divergence between the pair of sequences inside this group is very low (0.2 %) providing a strong evidence for the identity of all collections. Based on this conclusion, *P. kalchbrenneri* and *P. pachyphylla* might be considered as conspecific. To address such taxonomical questions, further investigations based on more material and other molecular markers would be very helpful.

Acknowledgements

We thank Prof. Dr. A. Kovalenko, Dr. N. Psurtseva, Dr. V. Malysheva and Dr. A. Kiyashko (Komarov Botanical Institute, Saint Petersburg) for help in the field work and kind permission to use their collections. We are very grateful to Prof. Dr. G. Consiglio for useful advices and loan of specimens. Our gratitude is extended to Dr. E. Bulakh (Institute of Biology and Soil Science, Vladivostok) and Dr. R. Poggi (Museo Civico di Storia Naturale «Giacomo Doria», Genova) for the loans of collections used in this study. Financial support for this work was provided by the State Contract N P1300 "Phylogeography and trends of evolution of basidiomycetes and myxomycetes model groups" and the Russian Foundation for Basic Research (project N 10-04-01189-a).

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(Manuscript accepted 7 December 2010; Corresponding Editor: R. Pöder)