

## *Tubariomyces*, a new genus of Inocybaceae from the Mediterranean region

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**Abstract:** The taxonomic position of *Inocybe inexpectata* is reconsidered on the basis of new molecular and morphological data from four published records. Up to three species can be recognized to which the name *I. inexpectata* has been applied. In addition to distinct morphological features present in this lineage it is proposed here as the new genus *Tubariomyces*. A complete taxonomical description of the genus and the species *Tubariomyces inexpectatus* and *T. hygrophoroides* sp. nov. also is provided.

**Key words:** *Inocybe inexpectata*, Mallocybella clade, phylogeny, *Tubariomyces hygrophoroides*

### INTRODUCTION

Recent molecular phylogenetic analysis and molecular clock estimations of the evolutionary history of Inocybaceae Jülich has shown that the family is composed of at least seven unique major lineages that appear to share a common palaeotropical ancestor (Matheny 2009, Matheny et al. 2009). Four of these major clades, namely *Inocybe sensu stricto*, *Pseudosperma*, *Mallocybe* and *Inosperma*, are widespread and have received considerable taxonomic attention (Kuyper 1986, Stangl 1989, Kobayashi 2002, Esteve-Raventós et al. 2003, Ryberg et al. 2008, Larsson et al. 2009). However Inocybaceae is poorly known in the tropics, regions of the southern hemisphere, much of Asia and southern Europe. The geographically more restricted clade *Auritella* recently was described as a new genus of seven species known only from Africa and Australia (Matheny and Bougher 2006). The lineage informally labeled

*Nothocybe* is apparently a relict major lineage from southern India, but it is supported currently by only a single collection.

The species *Inocybe inexpectata* Villarreal, Esteve-Rav., Heykoop & Horak was reported to resemble genus *Tubaria* (W.G. Sm.) Gillet on the basis of its macroscopic features (Villarreal et al. 1998), namely habit, small size, basidiocarps color and decurrent lamellae. The trichodermal pileipellis resembles that of *Phaeomarasmium* or *Flammulaster*, but the presence of suballantoid to boletoid spores, necropigmented basidia and deposits at the apex of cheilocystidia suggested a placement within *Inocybe*. The name *Mallocybella* was informally proposed as a relict major clade within *Inocybe* including *I. inexpectata* and another not clarified species from Zambia (Matheny et al. 2009). The phylogenetic position and composition of the *Mallocybella* clade are uncertain and incomplete because molecular data are scarce and collections geographically disparate. Fortunately the Mediterranean species *I. inexpectata* has been collected abundantly (two collections of more than 50 specimens), making it possible to evaluate its morphological variability. The type collection of *I. inexpectata* was collected in Toledo, Spain (Villarreal et al. 1998) in a *Quercus ilex* ssp. *ballota* (Desf.) Samp. clearing with *Tuberaria guttata* (L.) Fourr, a member of the ectomycorrhizal family Cistaceae (Comandini et al. 2006). A second collection from the same location (topotypus) was obtained a few years later (stored as AH 25500 at University of Alcalá, Spain). The next citation of *I. inexpectata* was published by Hermosilla and Sánchez (1998). This sample was collected in Fontecha, Álava (northern Spain) in a *Quercus ilex* ssp. *ballota* (Desf.) stand with *Cistus albidus* and *C. salvifolius*. Moreau et al. (2007) provided the third citation of this species from Mucchiu Biancu, Corse (France) in a *Halimium* sp. (Cistaceae) stand with ecological features similar to the previous Spanish collections. A new record of *I. inexpectata* was reported by Monedero García and Fernández Sasía (2009) from Hermosilla, Burgos (northern Spain). It was collected in an arenicolous *Quercus ilex* ssp. *rotundifolia* stand with *Cistus laurifolius*, *C. salvifolius* and *Lavandula stoechas*. In turn the *Inocybe* sp. BB6018 collection from Zambia sequenced by Matheny et al. (2009) probably is associated with plant species in *Phyllanthaceae* and/or *Fabaceae* families from a dry tropical climate. This species shares some features with *Inocybe inexpectata* such as a characteristic small size and abundant

caulocystidia (Buyck and Matheny pers obs). These phenotypic characters appear to unite these two members of the Mallochybella clade although it was not strongly supported by molecular data (Matheny et al. 2009).

Thus far no direct morphological and molecular comparison has been performed among multiple *I. inexpectata* collections. The purpose of the current study was to do so and evaluate its putative recognition as a distinct genus.

#### MATERIALS AND METHODS

**Morphological study.**—Microscopic slides of dried material from all published collections of *I. inexpectata* were prepared with 5–10% NH<sub>4</sub>OH and Congo red in 1% ammonia. Spore measurements are quoted according to Heinemann and Rammeloo (1985). Drawings were made with the aid of a Zeiss drawing tube under an oil-immersion objective. Colors of fresh and dry basidiomes were compared with reference colors in Munsell (1994). Abbreviations CEH, PAM and RFS make reference respectively to the private herbaria of Carlos Enrique Hermosilla, Pierre-Arthur Moreau and Roberto Fernández-Sasia.

**DNA extraction, amplification and sequencing.**—Four collections (TABLE I) were used in the molecular analyses. A MasterPure™ Plant Leaf DNA Purification Kit (Epicentre Biotechnologies, Madison, Wisconsin) was employed to extract total DNA from samples according to the manufacturer's specifications. A total of 1 µL water-resuspended DNA was added to each 50 µL PCR mixture with this composition: 1 u EcoTaq DNA polymerase with 1× EcoTaq Buffer (Ecogene), MgCl<sub>2</sub> 2 mM, and 0.2 mM of each of the dNTPs. Primers targeting the 5' end of the nuclear ribosomal large subunit (LR1 from van Tuinen et al. 1998, LR7 from Vilgalys and Hester 1990) and the 6–7 conserved domains of the *rpb2* gene (bRPB2-6F and bRPB2-7R from Matheny 2005) were added at 0.5 µM each. Internal transcribed spacer (ITS) sequences from *I. inexpectata* samples were obtained with the primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The forward primer NSII (Martin and Rygiewicz 2005) was employed with some samples. PCR reactions were performed under a program consisting of a hot start at 95 C for 5 min, five cycles at 94 C, 50 C and 72 C (45, 30 and 45 s respectively), followed by 30 cycles at 94 C, 54 C and 72 C (45, 30 and 45 s respectively) and a final 72 C step 10 min. PCR products were checked in 1% agarose gels before purification with an UltraClean PCR Clean-up DNA purification kit (Mo Bio Laboratories, Carlsbad California). The same PCR primers were used for sequencing. Chromatograms and sequences were compared visually in MEGA4 to detect ambiguous nucleotides and reading frame displacements. Consensus sequences have been deposited at GenBank. (Accession numbers, as well as those from the other sequences used in this study, are listed in TABLE I.)

**Sequence alignment and phylogenetic analyses.**—Sequences of *rpb2* and LSU produced for this study were added to an

alignment of *rpb1*, *rpb2* and LSU sequences from Matheny et al. (2009). We pruned this dataset to 56 taxa and 2853 included nucleotide sites for phylogenetic analysis with the RAXML BlackBox online server (<http://phylobench.vital-it.ch/raxml-bb/>) (Stamatakis et al. 2008) under the maximum likelihood (ML) criterion. This service uses RAXML 7.2.3 released by A. Stamatakis in Oct 2009. The data were partitioned by codon position for *rpb1* (positions 1–678) and *rpb2* (679–1386) and by the LSU gene region (1387–2853) after removal of ambiguously aligned regions including introns. Seven distinct data partitions were analyzed with joint branch-length optimization. A GTR model of nucleotide substitution was invoked for each partition, including parameters for invariant sites and gamma distributed rate heterogeneity following Matheny (2005). One hundred rapid bootstrap inferences and thereafter a thorough ML search were performed. *Phaeo-marasmius proximans* was used as outgroup per Matheny (2005). Bootstrap values >70% were considered significant.

#### PHYLOGENETIC RESULTS

Both the 5' end of nLSU and domains 6–7 of *rpb2* were successfully amplified and sequenced from all specimens, except *rpb2* from sample CEH2885. Sequences from six collections of *I. inexpectata* sensu lato and *Inocybe* sp. BB6018 formed a monophyletic group with 95% bootstrap support (FIG. 1). The entire cluster is distinct phylogenetically from other non-pleurocystidiata major lineages of Inocybaceae (i.e. lacking or “depauperate” in pleurocystidia). European exemplars of *I. inexpectata* formed three lineages (FIGS. 1, 2), one of which is characterized as a new species in the new genus *Tubariomyces* described below.

#### TAXONOMY

***Tubariomyces* Esteve-Rav. & Matheny, gen. nov.**  
Mycobank MB518073

Basidiomata minuta, tubaria similis (“tubarioid”), lamellata, non sequestrata, non rubescentia; pileus velutinus, furfuraceus, tomentosus vel floccoso-squamulosus; cortina praedita vel nulla; cheilocystidia tenuitunicata, non metuloidea, subcylindrica vel subclavata, elongata; caulocystidia numerosa, cheilocystidia similia; basidia necropigmentata; basidiosporae laeves, pallide luteae vel ochraceo-luteae, inamyloideae, apiculus plerumque indistinctus; pileipellis tricoloromphalea; hyphis fibulatis; habitat in silvis mediterraneis praecipue Cistacearum.

***Species typicae.*** *Tubariomyces inexpectatus* (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny

Basidiomata tubarioid, lamellate, non-sequestrate; pileus convex to plano-convex, velutinous, furfuraceous, tomentose to squamulose; cortina fugacious; context not reddening; cheilocystidia thin-walled, not crystalliferous, subcylindrical to subclavate, usually

TABLE I. Collections and GenBank accessions numbers used in the phylogenetic analysis of *Tubariomyces*

Species	Collection <sup>a</sup>	rpb1	rpb2	nLSU-rRNA
<i>Auritella aureoplumosa</i>	Wat23132 (E)	—	—	AY635766
<i>A. chamaecephala</i>	PBM2212 (PERTH)	—	AY635781	AY635765
<i>A. dolichocystis</i>	T24844 (WTU)	AY351797	AY337371	AY380371
<i>A. geoaustralis</i>	H7344 (PERTH)	AY351818	AY333774	AY380395
<i>A. serpentinocystis</i>	T25080 (WTU)	AF389547	AY333773	AY038325
<i>Crepidotus</i> cf. <i>applanatus</i>	PBM717 (WTU)	AY333303	AY333311	AY380406
<i>C. versutus</i>	PBM856 (WTU)	EU555440	AY33312-13	AY820890
<i>Flammulaster</i> sp. PBM1871	PBM1871 (WTU)	AY333308	AY333315	AY380408
<i>Inocybe adaequata</i>	JV16501F (WTU)	AY351791	AY333771	AY380364
<i>I. alabamensis</i>	PBM1892 (WTU)	AY536282	AY536281	AY536280
<i>I. ayangannae</i>	MCA1231 (WTU)	AY239028	AY337364	AY239018
<i>I. calamistrata</i>	JV11950 (WTU)	—	AY333763	EU555452
<i>I. calospora</i>	JFA12539 (WTU)	AF389535	AY333765	AY038313
<i>I. candidipes</i>	BK24079907 (UTC)	AY239029	AY337366	AY239019
<i>I. catalaunica</i>	JV9448 (WTU)	AY351808	AY337385	AY380383
<i>I. chelanensis</i>	PBM2314 (WTU)	AY239031	AY337369	AY239021
<i>I. corydalina</i>	TURA6488 (WTU)	AF389536	AY337370	AY038314
<i>I. dulcamara</i>	SAT9923301 (WTU)	AY351799	AY388644	AY380373
<i>I. erubescens</i>	JV9070F (WTU)	—	—	EU569846
<i>I. flocculosa</i>	PBM2392 (WTU)	AY351801	AY337375	AY380375
<i>I. geophylla</i>	JV6374 (WTU)	AY351803	AY333777	AY380377
<i>I. godeyi</i>	JV14914F (WTU)	AF389538	AY337378	AY038316
<i>I. heimii</i>	JV14932F (WTU)	AY351804	AY337380	AY380379
<i>I. hystrix</i>	RS31493 (WTU)	AY351805	AY337381	AY380380
<i>I. lacera</i>	PBM1462 (WTU)	AF389540	AY337383	AY380381
<i>I. lanatodisca</i>	SAT9929901 (WTU)	AY351807	AY333769	AY380382
<i>I. lanuginosa</i>	PBM956 (WTU)	AY351807	AY337384	AY038319
<i>I. leptocystis</i>	JV10412 (WTU)	AY351813	AY337386	AY380384
<i>I. leucoblema</i>	JV16437 (WTU)	AY333302; AY349177; AH013036	AY3333	AY333310
<i>I. maculata</i> (ocher form)	PBM2446 (TENN)	EU569862	EU569863	AY745700
<i>I. misakaensis</i>	96204 (PC) / Wat24830 (E)	EU569872	EU569873	EU569874
<i>I. napipes</i>	PBM2376 (WTU)	AY239034	AY337390	AY239024
<i>I. praetervisita</i>	PBM1021 (WTU)	AF389544	AY337392	AY038322
<i>I. relicina</i>	JV10258 (WTU)	AF389546	AY333778	AY038324
<i>I. rimosa</i> group	BK18089706 (UTC)	EU600845	EU600846	EU600847
<i>I. rimosa</i> group	BK28080513 (UTC)	EU600848	EU600849	EU600850
<i>I. rimosa</i> group	PBM2574 (TENN)	EU307857	EU307858	EF561633
<i>I. sp.</i> BB6018	BB6018 (PC)	—	EU600886	EU600887
<i>I. sp.</i> PBM525	PBM525 (WTU)	AF389543	AY333775	AY038321
<i>I. sp.</i> PC96073	96073 (PC)	—	EU600869	EU600870
<i>I. sp.</i> ZT9250	ZT9250 (ZT)	—	EU600904	EU604546
<i>I. stellatospora</i>	PBM963 (WTU)	AF389550	AY337403	AY038328
<i>I. tahquamenonensis</i>	PBM1142 (WTU)	AY351824	AY337407	AY380399
<i>I. terrigena</i>	JV16431 (WTU)	AY333301	AY333309	AY380401
<i>I. tubarioides</i>	PBM2570 (TENN)	EU307856	EU307855	AY732211
<i>I. unicolor</i>	PBM1481 (WTU)	AY351827	AY337409	AY380403
<i>Phaeomarasmium curcuma</i>	JFA11323 (WTU)	AF389551	—	AY038329
<i>P. proximans</i>	PBM1951 (WTU)	AY333307	AY333314	AY380410
<i>Simocybe serrulata</i>	PBM2536 (TENN)	DQ447940	DQ484053	AY745706
<i>Tubaria confragosa</i>	PBM2105 (WTU)	DQ447944	DQ408113	AY700190
<i>T. vinicolor</i>	JFA12905 (WTU)	DQ536419	DQ536418	DQ536415
<i>Tubariomyces hygrophoroides</i>	PAM05112008 (LIL)	—	GU907090	GU907094
<i>T. hygrophoroides</i>	2885 (CEH)	—	—	GU907093
<i>T. inexpectatus</i>	20390 (AH)	—	—	EU569855
<i>T. inexpectatus</i>	25500 (AH)	—	GU907088	GU907091

TABLE I. Continued

Species	Collection <sup>a</sup>	rpb1	rpb2	nLSU-rRNA
<i>Tubariomyces</i> sp_1	0805 (RFS)	—	GU907089	GU907092
<i>Tubariomyces</i> sp_2	BB6018 (PC)	—	EU600886	EU600887

<sup>a</sup> Herbarium abbreviations per Thiers B. (continuously updated). Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden Virtual Herbarium. <http://sweetgum.nybg.org/ih/>.

elongate; caulocystidia present, similar to cheilocystidia; basidia necropigmented; basidiospores smooth, pale yellow-brown to ochraceous in water and hyaline alkaline mounts; apiculus indistinct or small; pileipellis a trichodermium; clamp-connections present. Associated with *Cistaceae*. Holotype: *Tubariomyces inexpectatus* (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny.

**Etymology.** Due to its similarity in habit to genus *Tubaria* (W.G. Sm.) Gillet.

**Distribution.** Mediterranean Europe and probably northern Africa.

**Commentary.** The decurrent lamellae (“tubarioid habit”) and presence of caulocystidia along the surface of the stipe make this genus morphologically distinct from other genera and major clades of

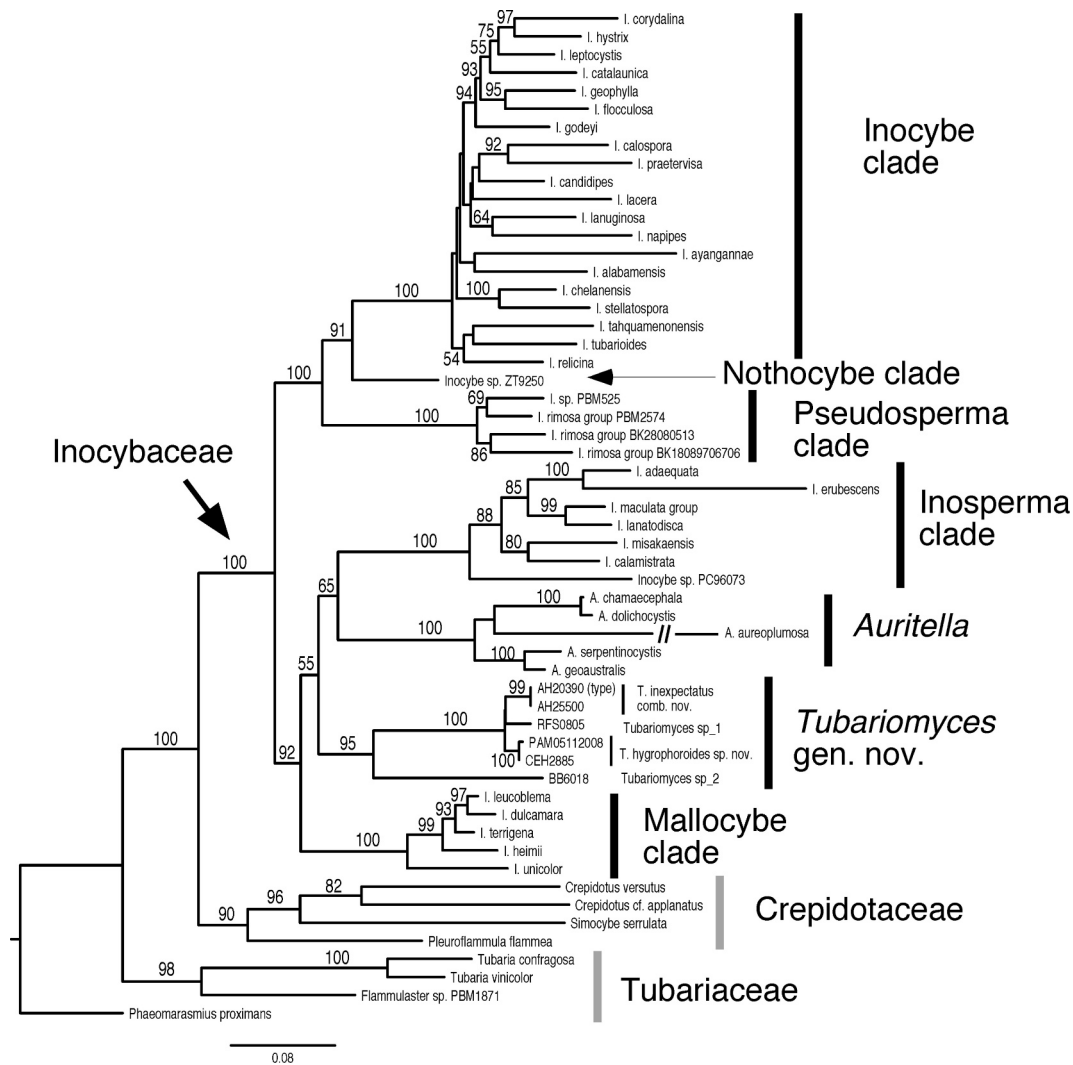


FIG. 1. Maximum likelihood (ML) phylogram from RAxML software. Sequences of *rpb2* and LSU produced for this study were added to a previous alignment of *rpb1*, *rpb2* and LSU sequences from Matheny et al. (2009). Provisional names from Matheny (2009) for the seven major clades or lineages in Inocybeaceae are provided with *Auritella* and now *Tubariomyces* indicated as genera. Numbers above branches represent ML bootstrap proportions.

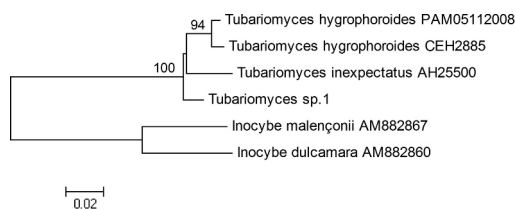


FIG. 2. Neighbor joining cladogram of a 500 bp alignment of the complete 5.8S nuclear ribosomal subunit and partial ITS1 and ITS2 from the different samples in *Tubariomyces* included in this study: *T. inexpectatus* AH25500 (GU907095), *Tubariomyces* sp. RFS0805 (GU907096), *T. hygrophoroides* PAM05112008 (GU907097) and *T. hygrophoroides* CEH2885 (GU907098). Tree reconstruction was performed with MEGA4 software (Tamura et al. 2007). Numbers above branches represent bootstrap proportions from 5000 replicates. Only values >70% are shown. *Inocybe* cf. *dulcamara* (AM882860) and *I. malençonii* (AM882867) from Ryberg et al. (2008) were used as outgroups.

Inocybaceae. The first impression reminded us of a small *Tubaria* species (especially *T. inexpectatus*), although both genera are micromorphologically different. Most species of the Mallocybe clade show adnate to emarginate lamellae, except for a few species, such as *I. heimii* Bon or *I. agardhii* (N. Lund) P.D.Orton, which sometimes exhibit subdecurrent lamellae. However, species of the Mallocybe clade are typically larger and lack the characteristic caulocystidia of *Tubariomyces* species.

***Tubariomyces inexpectatus*** (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny, comb. nov. FIG. 3A

MycoBank MB 518325

≡ *Inocybe inexpectata* M. Villarreal, Esteve-Rav., Heykoop & E. Horak, *Mycol. Res.* 102(4):479. 1998 (basonym).

Pileus 4–16 mm, convex, hemispherical to plano-convex or slightly depressed at maturity, without umbo; margin incurved when young, later straight and usually somewhat sinuose, not striate, without any remnants of veil; surface dry, velutinous, furfuraceous to slightly squamulose or floccose; brown-argillaceous, sienna to beige-brown or yellowish-brown (7.5YR 6/8, 5/6–5/8 to 10YR 6/8, 5/6–5/8); not or slightly hygrophanous. Lamellae arcuate-decurrent to triangular when young, deeply decurrent at maturity, close to moderately close (L = [20–]24–28; l = 1), first beige to cream-brownish, finally nearly ochraceous; edge floccose, white or paler than the surface. Stipe 11–23 × 1–2 mm, cylindrical, central, more rarely somewhat subeccentric, usually sinuose, solid, at first whitish to pale beige, then becoming beige-brownish, finally darker but always paler than the pileus; surface finely fibrillose-pruinose throughout. Cortina present in young specimens, fugacious. Context whitish to ochraceous in the pileus, concolorous to the surface in the stipe, unchanging. Odor and flavor none.

Basidiospores (9.5–)10.3–12.2–14 × 3.4–4–4.8 (–5.2) μm; Q = (2.3–)2.4–3.1–3.7 (n = 36), cylindrical to suballantoid (“boletoid”), sometimes with central constriction, smooth, thin-walled, not dextrinoid, without germ pore, yellowish in KOH. Basidia 25–37 × 5–11 μm, subcylindrical to narrowly clavate, predominantly four-spored, sterigmata up to 7(–8) μm in those scarcely two-spored, with yellowish necropigment. Cheilocystidia (29–)40–76 × 7–13(–18) μm, forming a nearly sterile band, variable in shape and size, predominantly cylindrical, flexuose, sublageniform to narrowly clavate, with obtuse rounded apex, exceptionally subcapitate, rarely septate, hyaline or with yellowish content, often covered apically by a refringent resinous matter. Pleurocystidia absent. Caulocystidia present, abundant throughout the

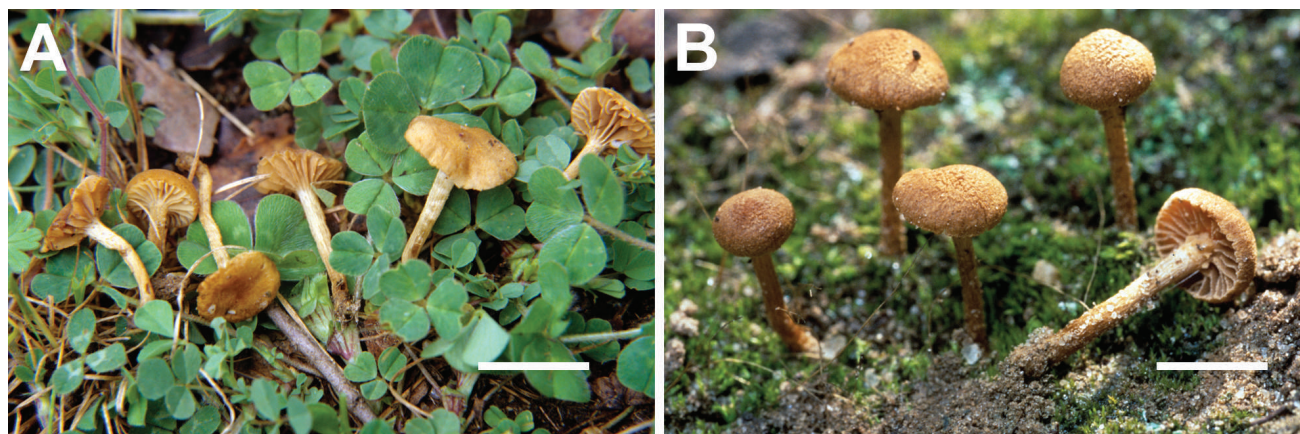


FIG. 3. A. *Tubariomyces inexpectatus* (AH25500, photo by Fernando Esteve-Raventós). Bar = 15 mm. B. *Tubariomyces hygrophoroides* (CEH1916, photo by Carlos Enrique Hermosilla). Bar = 10 mm.

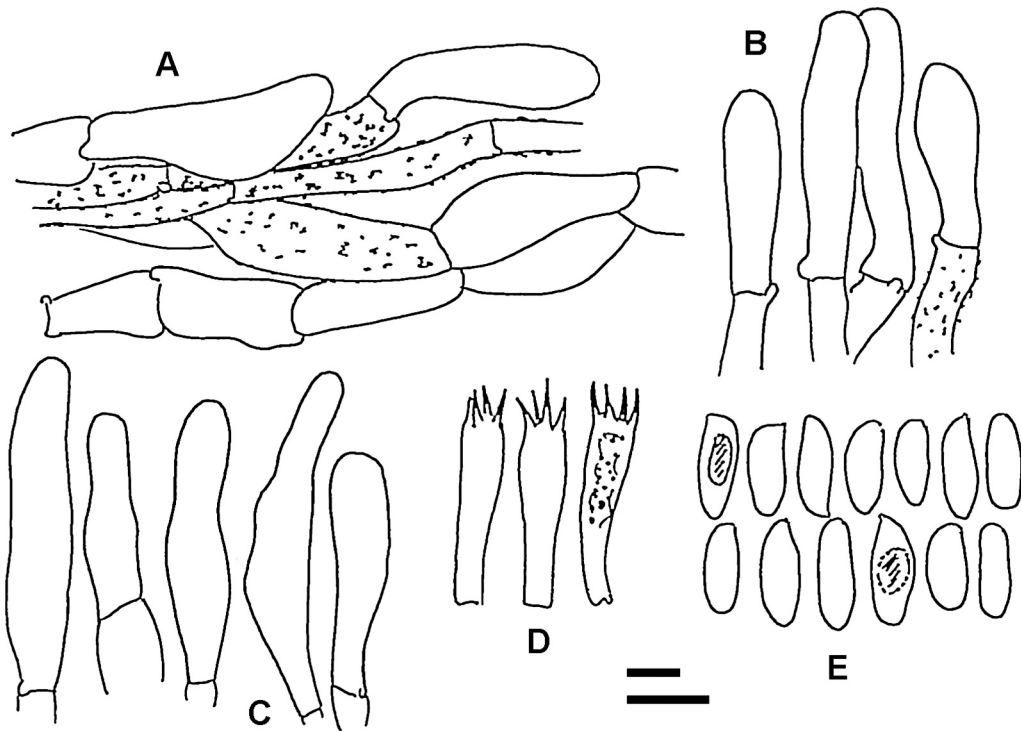


FIG. 4. *Tubariomyces hygrophoroides* (PAM05112008 in LIP, Holotypus). A. Pileipellis. B. Caulocystidia. C. Cheilocystidia. D. Basidia. E. Basidiospores. Bars = 10  $\mu$ m (large bar only for spores).

stipe, similar to hymenial cystidia or even longer, sometimes septate. Pileipellis a trichoderm with abundant caulocystidioid cylindrical to claviform terminal cells, 25–75  $\times$  6.5–13  $\mu$ m, sometimes packed in clusters, usually covered apically by amorphous yellow deposits; encrusting yellowish pigments in the hardly differentiated subpellis, sometimes intracellular pigment also present. Clamp connections present.

In open areas, on acid sandy soil, gregarious (more than 50 specimens in the holotypic collection), near *Tuberaria guttata* (Cistaceae), in Mediterranean evergreen oak forest (*Quercus ilex* subsp. *ballota*); Spain, spring (April).

*Specimens examined.* SPAIN. TOLEDO: La Iglesiasuela, El Bonal, 30T UK525575, 440–460 m. 4 Apr 1996, leg. M. Villarreal and M.A. Jiménez, (HOLOTYPE, AH 20390), (ISOTYPE, ZT 5496). *Ibidem*, 13 Apr 1996, leg. Villarreal and M.A. Jiménez, (AH 20391). *Ibidem*, 24 Apr 2000, leg. M. Villarreal and F. Esteve-Raventós, (AH 25500).

*Commentary.* *Tubariomyces inexpectatus* is known from two abundant collections that were made in the same locality fruiting among *Tuberaria guttata* (Cistaceae), a herbaceous annual plant that occurs in acidic sandy soils in open Mediterranean woodlands. This plant is known to form ectomycorrhizae with some truffles such as *Terfezia* spp., among others, and was known as *Helianthemum guttatum* (Comandini et al. 2006). It also is known that some agarics (e.g. *Cortinarius*, *Inocybe*) may establish ectomycorrhizae

with some *Helianthemum* spp. in subalpine areas of Europe, but these plants (like *Cistus* or *Halimium*), are biannual or perennials.

*Tubariomyces inexpectatus* originally was considered an *Inocybe* species (Villarreal et al. 1998), although the authors considered its inclusion in this genus as debatable on account of the deviating macro- and microscopical characters, which were “unexpected” for this genus. It then was compared with *I. malençonii* R. Heim, which also has similar long and narrow spores. However this species belongs to the *Mallocybe* clade (Matheny et al. 2009).

*T. inexpectatus* can be recognized morphologically by the strongly decurrent lamellae in mature specimens (sometimes suggesting an “omphalinoid” habit), the mucoid yellow deposits that cover the apex of the cystidia, and the typical trichodermoid pileipellis.

***Tubariomyces hygrophoroides*** Esteve-Rav., P.A. Moreau & C.E. Hermos., sp. nov. FIGS. 3B, 4A–E  
Mycobank MB518074

= *Inocybe inexpectata* sensu C.E. Hermos. & Jul. Sánchez, Est. Mus. Ci. Nat. Alava 13:65. 1998.

= *Inocybe inexpectata* sensu P.A. Moreau et al., Bull. FAMM 31:14. 2007.

Pileus 4–10 mm latus, convexus vel conico-convexus, leviter obtuse umbonatus, siccus, totus lanoso-

velutinus, furfuraceus vel subsquamulosus in senectute, aurantio-brunneus vel ochraceo-brunneus. Lamellae valde distantes (L = 18–22), arcuato-decurrentes, ab initio pallide ochraceae, deinde cinnamomeo-brunneae, acie crenulatus, leviter pallidior. Stipes 5–10 × 1–2 mm, cylindricus vel attenuatus versus basim, ab initio ochraceus, deinde ochraceo-argillaceus, floccoso-fibrillosus usque ad basim. Velum fugax. Caro alba vel ochracea in pileo, pallid brunnea in stipite; odor et sapor indistincti.

Basidiosporae (9.2–)9.7–13.1–16.5 × 4.4–5.1–5.8(–6) μm; Q = (2.1–)2.2–2.55–2.9(–3.2) (n = 36), cylindricae vel cylindrico-fusiformiae, laeves, flavae in KOH, non amyloideae. Basidia 34–42 × 6–9.5 μm, tetrasporigera, plena necropigmenti. Cheilocystidia 30–60 × 9–16 μm, diversiformia, non metuloidea, cylindrical, clavata vel sublageniformia. Pleurocystidia nulla. Caulocystidia presentia usque ad basim stipitis, cheilocystidia similia. Pileipelli subtrichodermium ex hyphis terminalibus 5–15(–18) μm latis, pigmento incrustato formata. Hyphis fibulatis.

Inter Cistaceae (Cistus, Halimium) in silvis arenosis. Corsica. Holotypus in LIP (Herb. P.A. Moreau n°05112008), Paratypus in Herb. C.E. Hermosilla n°02885, Isoparatypus in AH 36455.

*Etymology.* Taken from the agaricalean genus *Hygrophorus*, due to the distant and arcuate-decurrent lamellae.

Basidiomata gregarious, sometimes growing in small fascicles of up to four specimens. Pileus 4–10 mm, convex, hemispherical to broadly conical-convex, initially obtusely umbonate, hardly expanding on adult specimens; margin incurved when young, remaining so in adult specimens, slightly crenate to sinuose, not striate, covered by fragile and fugacious whitish veil remnants; surface dry, lanose-velutinous to velvety-furfuraceous (reminding us of *Mallocybe* species), often cracked concentrically and giving it a tessellate to subsquamulose aspect; brown-orange to brown-ocher, paler to ochraceous toward the margin (5YR 6/6-6/8 to 7.5YR 6/6-6/8, 7/8); not or slightly hygrophanous. Lamellae slightly arcuate with a decurrent tooth when young, persisting more or less so at maturity, distant (L = 18–22; l = 1), first beige to cream-brownish, finally nearly cinnamon-brown; edge floccose, white or paler than the surface. Stipe 5–10 × 1–2 mm, cylindrical or tapering toward the base, central, sometimes curved or sinuose, solid, at first whitish to pale beige, then becoming beige-brownish, finally darker but always paler than pileus; surface finely fibrillose throughout, especially at the lower part. Cortina present in young specimens, fugacious. Context whitish to ochraceous in the pileus, concolorous to the surface in the stipe, especially at the base unchanging. Odor and flavor not remarkable.

Basidiospores (9.2–)9.7–13.1–16.5 × 4.4–5.1–5.8 (–6) μm; Q = (2.1–)2.2–2.55–2.9(–3.2) (n = 36), cylindrical to cylindrical-fusiform (“boletoid”), sometimes with slight suprahilar depression, smooth, thin-walled, one-guttulate, not dextrinoid, without germ pore, yellowish in KOH solutions. Basidia 34–42 × 6–9.5 μm, subcylindrical to narrowly clavate, predominantly four-spored, sterigmata up to 7 μm, with yellowish necropigment. Cheilocystidia numerous, 30–60 × 9–16 μm, forming a nearly sterile band, variable in shape and size, predominantly cylindrical, flexuose, sublageniform to narrowly clavate, with obtuse rounded apex, exceptionally subcapitate, rarely septate, hyaline or with yellowish content, thin-walled. Pleurocystidia not observed. Caulocystidia present, abundant throughout the stipe, similar to hymenial cystidia (25–50 × 8–15 μm), sometimes septate, yellowish parietal or encrusting pigment, thin-walled, exceptionally walls –0.5 μm thick. Pileipellis a subtrichoderm of prostrate hyphae with suberect, numerous cylindrical to subclaviform terminal cells, 30–50 × 5–15(–18) μm, with pale yellowish parietal or slightly encrusting pigment; subpellis formed by shorter and broader articles (5–20 μm), showing abundant encrusting yellowish pigment. Clamps connections present.

In sandy open areas gregarious to subfasciculate, in littoral or inland dunes near *Cistaceae* bushes (*Cistus albidus*, *C. salvifolius*, *Halimium halimifolium*); known fructifying only in autumn (November).

*Specimens examined.* FRANCE, CORSICA: Corse du Sud, Monaccia d’Aullène, Réserve Naturelle de Mucchiu Biancu. Littoral dunes with *Halimium halimifolium*, 20 Nov 2005, leg. P.A. Moreau, PAM05112008. (HOLOTYPE, LIP). SPAIN, ÁLAVA: Fontecha, 30T VN93, 500 m, basin of Rio Ebro, inland dunes under *Cistus albidus* and *C. salvifolius*, 1 Nov 1997, leg. C.E. Hermosilla, J. Sánchez & J. Roa, CEH02885, (Herb. C.E. Hermosilla, duplicate in AH 36455).

*Commentary.* Apart from phylogenetic differences (FIG. 1), *Tubariomyces hygrophoroides* differs morphologically from *T. inexpectatus* mainly by the less decurrent and much more distant lamellae. It was reported as *I. inexpectata* by Hermosilla and Sánchez (1988) and Moreau et al. (2007). However there are differences in pileipellis structure, which is less trichodermoid in *T. hygrophoroides*, hence giving it a more lanose-velvety aspect (“dulcamaroid”). We have not observed in *T. hygrophoroides* the typical abundant mucoid deposits that so characteristically cover the apex of cystidia in *T. inexpectatus*. In addition *T. inexpectatus* has been found only under *Tuberaria*, an annual *Cistaceae* plant, and only in springtime. In contrast *T. hygrophoroides* was collected only under stouter and perennial *Cistaceae* such as *Cistus* or *Halimium* in autumn.

Based on available data, the medium quotient ( $Q_m$ ) obtained following Heinemann and Rammeloo (1985) method ( $n = 36$ ) differs significantly for both species; in *T. inexpectatus*,  $Q_m$  is considerably higher (3.1) with respect to that of *T. hygrophoroides* (2.55). However some two-spored basidia have been observed in both taxa.

ARTIFICIAL KEY TO SPECIES OF *TUBARIOMYCES* IN EUROPE

1. Lamellae subdistant or normally spaced ( $L = [20-24-28]$ ), strongly decurrent (“omphalinoid”); pileipellis a typical trichoderm with erect terminal cells; cystidia covered with abundant yellow-brown mucoid deposits; vernal species under *Cistus* or *Tuberaria* (*Cistaceae*) . . . . . 2
1. Lamellae distant ( $L = 18-22$ ), arcuate-decurrent or nearly straight with decurrent tooth (“tubaroid”); pileipellis a subtrichoderm with more prostrate elements; cystidia not covered apically with mucoid deposits; autumnal species under *Cistus* and *Halimium* (*Cistaceae*), perennial shrub plants . . . . . *Tubariomyces hygrophoroides* sp. nov.
  2. Under or near *Tuberaria guttata* (*Cistaceae*), annual herbaceous plant . . . . . *Tubariomyces inexpectatus*
  2. Under *Cistus* (*Cistaceae*), perennial shrub, genetically similar to voucher specimen RFS0805 . . . . . *Tubariomyces inexpectatus* sensu Monedero García & Fernández Sasia

DISCUSSION

Molecular, morphological and ecological data support the recognition of at least four taxa within *Tubariomyces*: (i) the type species *T. inexpectatus*, (ii) a new species supported by samples CEH2885 and PAM05112008 named *T. hygrophoroides*, (iii) RFS0805, and (iv) BB6018 (PC) from Zambia. The ambiguous sample RFS0805 should be regarded as *Tubariomyces* sp., not *T. inexpectatus*, because no characteristic features could be identified though ITS, nLSU and *rpb2*. The collection of new specimens could help ascertain its taxonomical relationship with other *Tubariomyces* species; hence we refrain from describing it formally. The fourth species from Zambia (BB6018) awaits formal description. At present it is known only from a single collection.

Genus *Tubariomyces* is equal to the earlier referred clade Mallocybella (Matheny et al. 2009). However we prefer to name the group differently in the formal sense due to similar gross morphological characters with genus *Tuberaria*, a group distinguished in part by convergent similar traits, namely small habit and subdecurrent to decurrent lamellae.

The formal recognition of *Auritella* and now *Tubariomyces* as distinct genera is consistent with

results where the species in genus *Inocybe* are regarded as members of an independent family, Inocybaceae (Matheny et al. 2006). Three distinct genera are recognized in the sister clade Crepidotaceae, and thus we think there are sufficient morphological and molecular differences among the major clades in Inocybaceae to support (or at least not to prohibit) such a practical arrangement. While we understand genus *Inocybe* is rendered paraphyletic by this study, the remaining lineages in family Inocybaceae show a degree of genetic differentiation similar to both *Auritella* and *Tubariomyces* and could be treated in the same way (i.e. raised to generic rank) once they are reviewed properly (Matheny 2009).

ACKNOWLEDGMENTS

The authors acknowledge Dr J. Rejos, Curator at AH for great help in requesting collections from different herbaria. M. Villarreal and C.E. Hermsilla shared interesting data and illustrations of some collections treated in this contribution. P.A. Moreau (Université Lille) kindly placed at our disposal the Corsican collection of *Tubariomyces hygrophoroides* and personal comments about this collection. This work was financed by the Spanish Ministry of Education and Culture for FPU grant AP2006-00890, the Consejería de Agricultura y Medio Ambiente de la Junta de Comunidades de Castilla-La Mancha for Research Projects 2004X802, PAI08-0240-5097 and the Empleaverde Truficultura project from the Fundación Diversidad (cofinanced by the European Social Fund and the FGUA Cátedra de Medio Ambiente).

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