

# Colipila, *a new genus in the Helotiales*

**Hans-Otto Baral, Guy Garcia, Mesfin Bogale, Matthew J. O'Hara & Wendy A. Untereiner**

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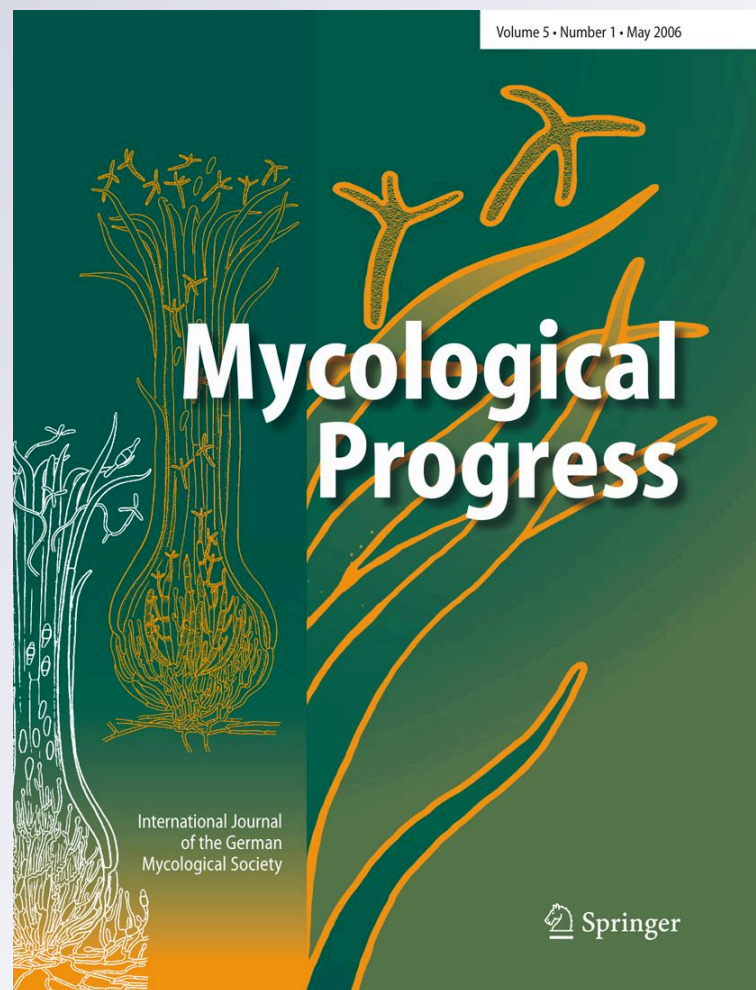
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## *Colipila*, a new genus in the *Helotiales*

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**Abstract** *Colipila*, a new member of the *Helotiales*, is erected for two previously undescribed lignicolous species resembling *Dasyscyphella* and *Lachnum* by macroscopy. Species of *Colipila* are characterized by their long, entirely smooth, hyaline, thin-walled, multi-septate, subulate to basally fusoid hairs that tend to be curved on the stipe and lower flanks, and dimorphic, partly strongly protruding paraphyses which closely resemble the hairs. The type species, *C. masduguana*, is recorded repeatedly in southern France on rotten decorticated branches and trunks of *Castanea sativa* on the moist forest floor in sub-Mediterranean regions with siliceous soils, but also once on *Quercus robur* in a temperate forest with calcareous soil. The second species, *C. pilatensis*, was found on wood of an unidentified member of *Rosaceae* in a calcareous region of the Northern Alps and is known only from the holotype. The phylogenetic position of *C. masduguana* within the *Helotiales* was not resolved based on the analysis of nuclear LSU ribosomal DNA sequences. A key to the species of *Colipila* is provided.

**Keywords** Ascomycota · *Albotricha* · *Dasyscyphella* · *Lachnum* · Phylogeny · Ribosomal DNA sequences

### Introduction

Ascomycetes on woody substrates that form comparatively large, entirely white, distinctly stalked, and entirely hairy apothecia generally belong to two well-known genera in the *Lachnaceae* (Order *Helotiales*), *Dasyscyphella* Tranzsch. and *Lachnum* Retz. The members of these genera have more or less cylindrical or gradually tapering hairs with distinctly granulate walls (in the former genus only in the lower part of the hair), and lanceolate paraphyses quite different from the hairs

During investigations of the mycoflora of the decayed wood in forests in France and Switzerland, we encountered two lignicolous, apothecial ascomycetes resembling species of *Dasyscyphella* and *Lachnum*. These two taxa could be distinguished microscopically from *Dasyscyphella* and *Lachnum* by their entirely smooth, somewhat spindle-shaped to subulate apothecial hairs, and dimorphic paraphyses that quite closely resemble the hairs in both shape and septation. Another distinctive feature of these species was the presence of partly distinctly curved hairs on stipe and lower flanks of the apothecia. A thorough comparison of the descriptions of the members of the *Hyaloscyphaceae* and *Lachnaceae* led us to conclude that these species could neither be assigned to a currently recognized genus nor to a described species. We therefore describe the new helotialian genus, *Colipila*, to accommodate these species. We also explored the phylogenetic position of *C. masduguana*, the type species of *Colipila*, based on the analyses of nuclear large subunit (LSU) ribosomal RNA gene sequences.

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## Materials and methods

### Materials examined

The majority of collections were examined in the fresh, living state in tap water (see Baral 1992), using a Zeiss Standard 20 microscope. The iodine reaction was tested with Lugol's solution (IKI= $\approx$ 1% I<sub>2</sub>, 2% KI, in H<sub>2</sub>O), without potassium hydroxide ( $\sim$ 3–5% KOH) pre-treatment; low concentrations were obtained by adding the reagent to a water mount and monitoring the diffusion of I<sub>2</sub>. Lugol's solution and Melzer's Reagent (MLZ=IKI+chloral hydrate 1:1) were also tested after treatment with KOH. The presence of gel was tested using Brilliant Cresyl Blue (CRB,  $\sim$ 1% in H<sub>2</sub>O) added to a water mount. Air-dried material was examined in H<sub>2</sub>O, or in 5% KOH to which Congo Red (CR,  $\sim$ 1% in H<sub>2</sub>O) was later added. Photographic images (macro- and microphotos) were obtained using a Nikon Coolpix E4500 or Nikon E4300, and all drawings were done by hand. Type material is deposited in the Botanische Staatssammlung München (M). Additional collections are held in the private herbaria of H.O. Baral (H. B.), G. Garcia (G.G.), and M. Hurtu (M.H.).

A pure culture of *C. masdugwana* (CBS 128287) was established from ascospores shot onto the surface of a plate containing 2% malt extract agar (MEA) (Gams et al. 1998). Cultures were maintained on modified Leonian's agar (MLA) (Malloch 1981). For micromorphological comparisons, this species was grown on filtered oatmeal agar (CBSOA) (Gams et al. 1998), MEA, MLA, and oatmeal agar (OA) (Tuite 1969) in 100-mm Petri plates. Plates were inoculated in triplicate using 2–3 mm<sup>2</sup> squares of agar cut from the actively growing edges of colonies on MLA and incubated at room temperature. Colony diameter was measured and descriptions of colony morphology were made at 7-day intervals for 21 days. Color descriptions are based on Kornerup and Wanscher (1978).

### DNA extraction and sequence analyses

DNA extraction from CBS 128287 and the amplification and sequencing of the LSU gene region were carried out as described previously (Bogale et al. 2010). Taxa included in the LSU dataset were selected based on previously published phylogenies and highly similar sequences recovered from GenBank using BLAST searches. Sequences were edited and assembled into larger consensus sequences using Sequencher 3.0 software (Gene Codes, Ann Arbor, USA) and alignments were generated using either ClustalX, version 2.0.12 (Larkin et al. 2007), or Multiple sequence Alignment based on Fast Fourier Transform (MAFFT), version 6 (Kato et al. 2002). Aligned sequences were corrected using Se-AL version 1.0 alpha 1 (Rambaut 1996).

Multiple base indels were reduced to single characters and all ambiguously aligned sequences were excluded.

The position of *Colipila masdugwana* within the *Helotiales* was inferred based on the analysis of sequences of 53 taxa (Table 1). The outgroup taxa were *Geoglossum glabrum*, *G. umbratile*, and *Trichoglossum hirsutum*. Phylogenetic relationships were inferred employing maximum parsimony (MP) method found in PAUP\* 4.0, v.4.0b 10 (Swofford 2003), using tree bisection-reconnection with the MulTrees option activated. Bootstrap support (BS) for branches was evaluated using heuristic searches from 1,000 random addition replicates and only groups with BS greater than 50% were retained in the bootstrap consensus. Bayesian analysis was performed using MrBayes, version 3.1.1 (Huelsenbeck and Ronquist 2001). This analysis employed TIM1 with an estimated proportion of invariable sites (I) and a gamma shape distribution of rates among site (G), which was determined as the best-fit model of sequence evolution using jModel Test (Posada 2008). Bayesian posterior probabilities (PP) were estimated using the Metropolis-coupled Markov chain Monte Carlo method by running four chains with 4,000,000 generations with rate categories and rates set to 6 and gamma, respectively, and using the program default priors for the remaining model parameters. Trees were sampled every 100th generation and those obtained before likelihoods converged were discarded. Sampled trees and parameters were summarized using the sumt and sump commands, respectively, in MrBayes.

## Results

**Abbreviations** \* = living state, † = dead state. Iodine reaction in IKI (=Lugol's solution): RB = red at a high concentration of iodine (I<sub>2</sub>) and blue at a low concentration of I<sub>2</sub> (hemiamyloid); rB = indistinctly dirty red at a high concentration of I<sub>2</sub>; BB = blue at either concentration of I<sub>2</sub>; VBs = refractive vacuolar bodies. Lipid content: 0 = without lipid bodies (LBs), 5 = maximum possible lipid content relative to ascospore volume. Values in { } indicate the number of collections (or pieces of substrate bearing the fungus) that were examined.

The dataset of aligned partial LSU sequences consisted of 930 characters of which 192 variable characters were parsimony-informative. A heuristic search of this data set produced 2 most parsimonious trees (MPT) 888 steps in length (L) with a consistency index (CI) of 0.467 and a retention index (RI) of 0.639; one of these trees is presented in Fig. 6 (see "Discussion"). In this phylogeny, strongly supported lineages corresponding to recognised families within the *Helotiales* (>75% BS, PP 0.95) included the *Dermateaceae* (BS 88%, PP 1.0), *Helotiaceae* (BS 77%, PP



**Table 1** Sources and accession numbers of the isolates used in this study

Taxon	nucLSU GenBank accession number	Reference
<i>Albotricha acutipila</i> (P. Karst.) Raitv.	AB481317	Hosoya et al. 2010
<i>Albotricha albotestacea</i> (Desm.) Raitv.	AB481303	Hosoya et al. 2010
<i>Antinoa strobilina</i> (Fr.) Velen.	EF596821	Unpublished
<i>Ascocoryne cylichnium</i> (Tul.) Korf	AY789394	Wang et al. 2005
<i>Bryoglossum gracile</i> (P. Karst.) Redhead	AY789420	Wang et al. 2005
<i>Bulgaria inquinans</i> (Pers.) Fr.	DQ470960	Spatafora et al. 2006
<i>Capitotricha bicolor</i> (Bull.) Baral	AY544674	Lutzoni et al. 2004
<i>Catenulifera rhodogena</i> (F. Mangelot) Hosoya	DQ227258	Untereiner et al. 2006
<i>Chloroscypha enterochroma</i> (Peck) Petrini	AY544656	Lutzoni et al. 2004
<i>Colipila masduguana</i> Baral & G. Garcia	HQ694501	This study
<i>Cudoniella clavus</i> (Alb. & Schwein.) Dennis	AY789373	Wang et al. 2005
<i>Cudoniella clavus</i>	DQ470944	Spatafora et al. 2006
<i>Cyathicula microspora</i> Velen.	EU940088	Baral et al. 2009
<i>Dasyscyphella longistipitata</i> Hosoya	AB481294	Hosoya et al. 2010
<i>Dasyscyphella montana</i> Raitv.	AB481299	Hosoya et al. 2010
<i>Dasyscyphella</i> sp. 1	AB481313	Hosoya et al. 2010
<i>Dermea acerina</i> (Peck) Rehm	DQ247801	Schoch et al. 2006
<i>Geoglossum glabrum</i> Pers.	AY789317	Wang et al. 2005
<i>Geoglossum umbratile</i> Sacc.	AY789315	Wang et al. 2005
<i>Hyaloscypha aureliella</i> (Nyl.) Huhtinen	EU940152	Baral et al. 2009
<i>Hyaloscypha daedaleae</i> Velen.	AY789415	Wang et al. 2005
<i>Hyaloscypha vitreola</i> (P. Karst.) Boud.	EU940155	Baral et al. 2009
<i>Hymenoscyphus fructigenus</i> (Bull.) Fr.	EU940157	Baral et al. 2009
<i>Hymenoscyphus scutula</i> (Pers.) W. Phillips	AY789431	Wang et al. 2005
<i>Hyphodiscus hymeniophilus</i> (P. Karst.) Baral	DQ227264	Untereiner et al. 2006
<i>Hyphodiscus incrustatus</i> (Ellis) Raitv.	GU727556	Bogale et al. 2010
<i>Incrucipulum longispineum</i> (Hosoya & Issh. Tanaka) Sasagawa & Hosoya	AB481325	Hosoya et al. 2010
<i>Incrucipulum radiatum</i> (Hosoya & Issh. Tanaka) Sasagawa & Hosoya	AB481322	Hosoya et al. 2010
<i>Lachnum nudipes</i> (Fuckel) Nannf.	AB481314	Hosoya et al. 2010
<i>Lachnum pudibundum</i> (Qué.) J. Schröt.	AB481298	Hosoya et al. 2010
<i>Lachnum soppittii</i> (Massee) Raitv.	AB481308	Hosoya et al. 2010
<i>Lachnum virgineum</i> (Batsch) P. Karst.	AY544646	Lutzoni et al. 2004
<i>Lasiobelonium lonicerae</i> (Alb. & Schwein.) Raitv.	AB481319	Hosoya et al. 2010
<i>Leotia lubrica</i> (Scop.) Pers.	AY789359	Wang et al. 2005
<i>Loramycetes macrosporus</i> Ingold & B. Chapman	DQ470957	Spatafora et al. 2006
<i>Microglossum rufum</i> (Schwein.) Underw.	DQ257359	Wang et al. 2006
<i>Microglossum viride</i> (Schr.) Gillet	AY789337	Wang et al. 2005
<i>Mitruha borealis</i> Redhead	AY789404	Wang et al. 2005
<i>Mitruha elegans</i> (Berk.) Fr.	AY789417	Wang et al. 2005
<i>Mitruha paludosa</i> Fr.	AY789423	Wang et al. 2005
<i>Mollisia cinerea</i> (Batsch) P. Karst.	DQ470942	Spatafora et al. 2006
<i>Neofabraea malicorticis</i> H.S. Jacks.	AY544662	Lutzoni et al. 2004
<i>Pezicula carpinea</i> (Pers.) Tul. ex Fuckel	DQ470967	Spatafora et al. 2006
<i>Phialocephala fortinii</i> C.J.K. Wang & H.E. Wilcox	AF269219	Jacobs et al. 2001
<i>Trichoglossum hirsutum</i> (Fr.) Boud.	AY544653	Lutzoni et al. 2004
<i>Trichopezizella otanii</i> J.H. Haines	AB481307	Hosoya et al. 2010
<i>Trichopezizella</i> sp. 1	AB481321	Hosoya et al. 2010
<i>Vibrissea flavovirens</i> (Pers.) Korf & J.R. Dixon	AY789426	Wang et al. 2005
<i>Vibrissea truncorum</i> (Alb. & Schwein.) Fr.	AY789402	Wang et al. 2005

**Table 1** (continued)

Taxon	nucLSU GenBank accession number	Reference
Fungal sp. M20	EU940093	Stenroos et al. 2010
Fungal sp. M147	EU940110	Stenroos et al. 2010
Fungal sp. M171	EU940118	Stenroos et al. 2010
Fungal sp. M288	EU940144	Stenroos et al. 2010

0.96), *Leotiaceae* (BS 99%, PP 1.0), *Geoglossaceae* (BS 100%, PP 1.0), and *Sclerotiniaceae* (BS 100%, PP 1.0). The *Hyaloscyphaceae* and *Lachnaceae* did not receive significant support and were not resolved as separate lineages. *Colipila masduguana* was positioned as sister to the lineage comprised of members of the *Helotiaceae*, but without significant support.

### Taxonomy

***Colipila*** Baral et Guy Garcia, gen. nov.

Mycobank MB 517661

Apothecia gregaria, modice ad longe stipitata, tota alba, rubrescentia, sicca pallide ochracea ad profunde rubrobrunnea, cyathea ad complanata, extus dense albopilosa. Asci cylindrico-clavati, 8-spori, apice rotundati vel modice conici, annulo apicali amyloideo, typo *Calycina*<sup>1</sup>), ex uncis nati. Ascosporeae anguste ellipsoideae vel fusoido-clavatae, rectae, hyalinae, aseptatae, nullas vel paucas guttulas minutas polaribus continentes. Paraphyses dimorphae, subcylindricae et ascos non superantes vel late lanceolatae et ascos valde superantes, apice obtusae, laeves, multiseptatae, in statu vivo eguttulatae. Excipulum ectale textura prismatica, cellulis magnis, tenuiter tunicatis. Pili cylindranei vel saepe subbasaliter inflati, multiseptati, recti vel interdum curvati, tenuiter tunicati, laeves, hyalini, in statu vivo eguttulati, sine excreto (interdum sparso), crystallis carentibus. Habitat ad lignum putridum humidum ramorum vel truncorum arborum frondosarum.

*Typus generis* *Colipila masduguana*

**Apothecia** gregarious, medium to long stipitate, entirely white, turning reddish with age, dry pale ochraceous to bright red-brown, cupulate to flattened, externally densely covered with white hairs. **Asci** cylindric-clavate, octosporous, rounded or medium conical at the apex, with an amyloid apical ring of the *Calycina*-type\*), arising from croziers. **Ascospores** narrowly ellipsoid or fusoid-clavate,

straight, hyaline, aseptate, without or with a few minute polar oil drops. **Paraphyses** dimorphic, subcylindrical and not protruding beyond the asci, or broadly lanceolate and strongly protruding, with obtuse apices, smooth, multi-septate, eguttulate when alive. **Ectal excipulum** composed of large, thin-walled prismatic cells. **Hairs** cylindrical or often inflated toward base, multiseptate, straight, on flanks and stipe sometimes curved, thin-walled, entirely smooth, hyaline, eguttulate when alive, without or sometimes with sparse exudate, not forming crystals.

*Habitat* Rotten wood of branches or trunks of angiosperm trees lying on the moist ground.

*Etymology* Named after the somewhat spindle-shaped hairs, especially in the vital state, which resemble a distaff (Latin *colus*).

### Key to the species of *Colipila*

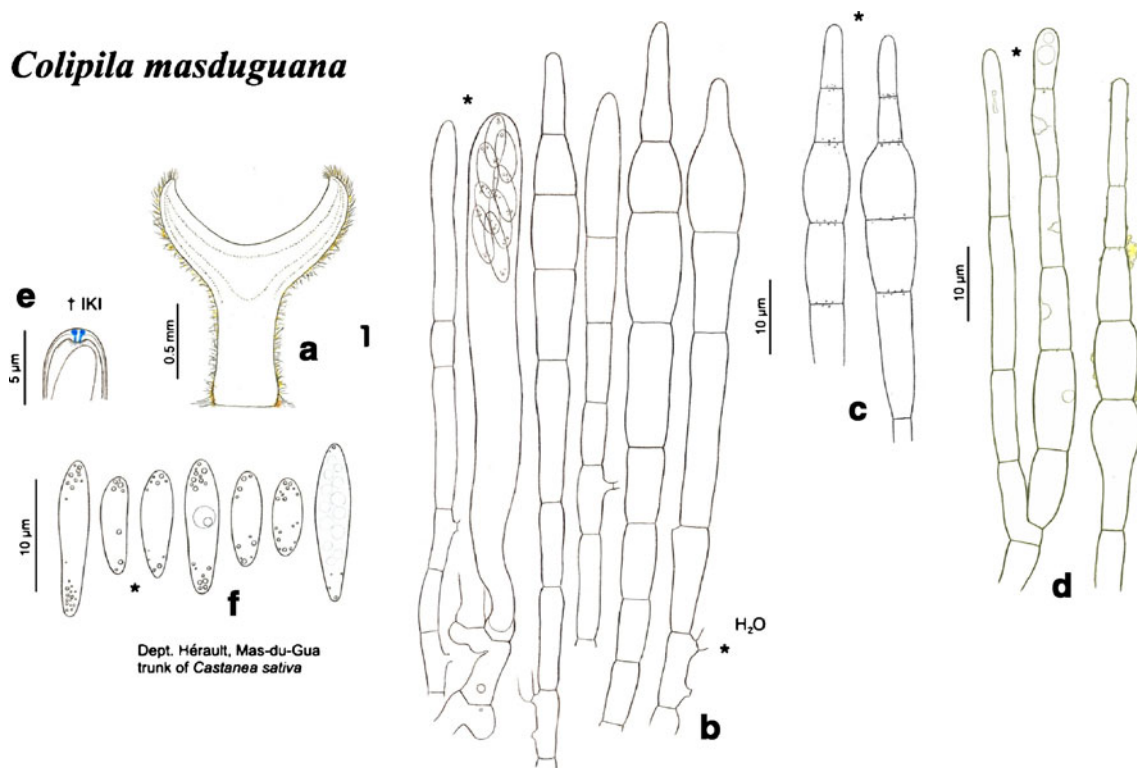
1. Asci \*56–72×6.7–7.5 μm (†42–58×5.3–6 μm), apical ring hemiamyloid (rB/RB), ascospores \*6.5–14×2.4–3.5 μm (†5.8–13×2–3.3 μm), lipid content 0.5–1.5, hairs at margin 40–90 μm long, apothecia often >0.8–1 mm diam, Dec.–March, wood of *Fagaceae* (*Castanea* and *Quercus*), sub-Mediterranean and temperate Europe ***C. masduguana***
2. Asci \*50–63×5.5–6 μm (†40–48×4.5–5.3 μm), apical ring euamyloid (BB), ascospores \*9–12×2–2.3 μm (†6–10.8×1.5–2 μm), lipid content 0–0.5, hairs at margin 60–150 μm long, apothecia <1 mm diam, June, unidentified wood (?*Rosaceae*), montaneous Central Europe ***C. pilatensis***

***Colipila masduguana*** Baral et Guy Garcia, spec. nov.  
Figs 1, 2, and 3

Mycobank MB 517662

*Description* Apothecia gregaria, (0.4)0.8–2.5(4) mm diam, extus dense breviter pilosa. Asci 51–72×6–8 μm in statu vivo, annulo apicali hemiamyloideo. Ascosporeae 6.5–14×2.4–3.5 μm in statu vivo, guttulas nonnullas minutas continentes, in statu germinato 0–1-septatae. Pili

<sup>1</sup> named by Verkley (1995: 186) "Type VIII (preliminary): 'Chlorociboria-Pezizella-Calycina'"



**Fig. 1** *Colipila masdugwana*. **1a, 1b, 1d–1fM** ..... ex H.B. 7458 (holotype). **1c** H.B. 8399. **1a** Apothecium in median section; **1b, 1c** asci and paraphyses; **1d** hairs on margin and flanks of apothecia; **1e**

ascus apex with amyloid apical ring; **1f** Free ascospore (showing a nucleus). Living state (\*) except for **1e**

45–90×3.5–8 μm. Habitat ad lignum putridum humidum *Castaneae sativae* vel *Quercus roboris*.

**Apothecia** when fresh (0.4)0.8–2.5(4) mm diam, entirely pure white, non-gelatinous, gregarious to partly fasciculate; disc slightly to strongly concave, finally flat, margin and exterior densely clothed with hyaline hairs; stipe 0.4–1×(0.1) 0.2–0.6(0.8) mm, cylindrical to often obconical, partly with bulbous base, densely fimbriate, without subiculum; apothecia rapidly turning light yellow and then fox-ochre to red-brown when cut or bruised, receptacle turning light to bright cream-ochraceous to yellow-orange-brown or deep red-brown with age, especially when dried, hairs remaining white or sometimes turning light yellow due to the presence of pigments in the excipular cells and bases of hairs. **Asci** \* (51)56–72×(6)6.7–7.5(8) μm {3}, †42–58×(5)5.3–6 μm {3}, 8-spored, pars sporifera \*20–27(29) μm long; †apex hemispherical to conical, containing a small apical ring staining moderately to strongly blue in IKI without KOH pre-treatment, at high I<sub>2</sub> very dirty reddish (hemiamyloid, rB {1} or RB {2}), *Calycina*-type; ascus base with a moderately long, thick, slightly flexuous stalk arising from croziers {4}. **Ascospores** \*(6.5)8–11(14)×(2.4)2.6–3(3.5) μm {3}, †(5.8) 7–11(13)×(2)2.2–2.7(3.3) μm {2}, obliquely biseriata, †flower ascospores sometimes uniseriate, subcylindrical to narrowly ellipsoid or slightly fusoid (rhomboid), partly heteropolar, ends obtuse, straight, containing ca. 2–10 LBs

0.1–0.2 μm diam, lipid content 0.5–1.5, uninucleate, without a sheath, surface not staining in CRB; mature ascospores 0–1-septate, frequently forming germ tubes in senescent apothecia. **Paraphyses** of two kinds (of equal frequency, Fig. 1b): (1) subcylindrical, sparsely septate, \*/†not protruding or protruding only slightly above the asci, \*2–4.5 μm, †1.5–4 μm wide at the widest part {2}, terminal cell \*/†15–40(50) μm long, and (2) broadly lanceolate-fusiform with partly±abruptly attenuated but not acute apices, densely and ±equidistantly septate, straight, protruding beyond mature asci by up to 16 μm, in KOH protruding by 5–18 μm, \*6–7.5(8.5) μm, †4–7 μm wide at the widest part {2}, constricted at the septa, especially at the widest part, terminal cell \*6–15 μm long; eguttulate (without VBs), but containing minute (0.1 μm diam), pale yellowish LBs at the septa; branched and anastomosing only near the base. **Subhymenium** 20–30 μm thick, composed of narrow ascogenous hyphae with croziers, intermingled with hyphae that give rise to paraphyses. **Medullary excipulum** in the center forming a moderately dense *textura intricata*, on flanks and in the stipe a dense *textura porrecta*, individual cells \*(35)50–80(95)×(2)6–10(15) μm, smooth, thin-walled, very indistinctly delimited from the ectal excipulum, ca. 50–70 μm thick on flanks. **Ectal excipulum** in the stipe and receptacle composed of cells \*(15)20–55×6–10(14.5) μm that form a thin-walled *textura prismatica* oriented at a 0–10° angle to

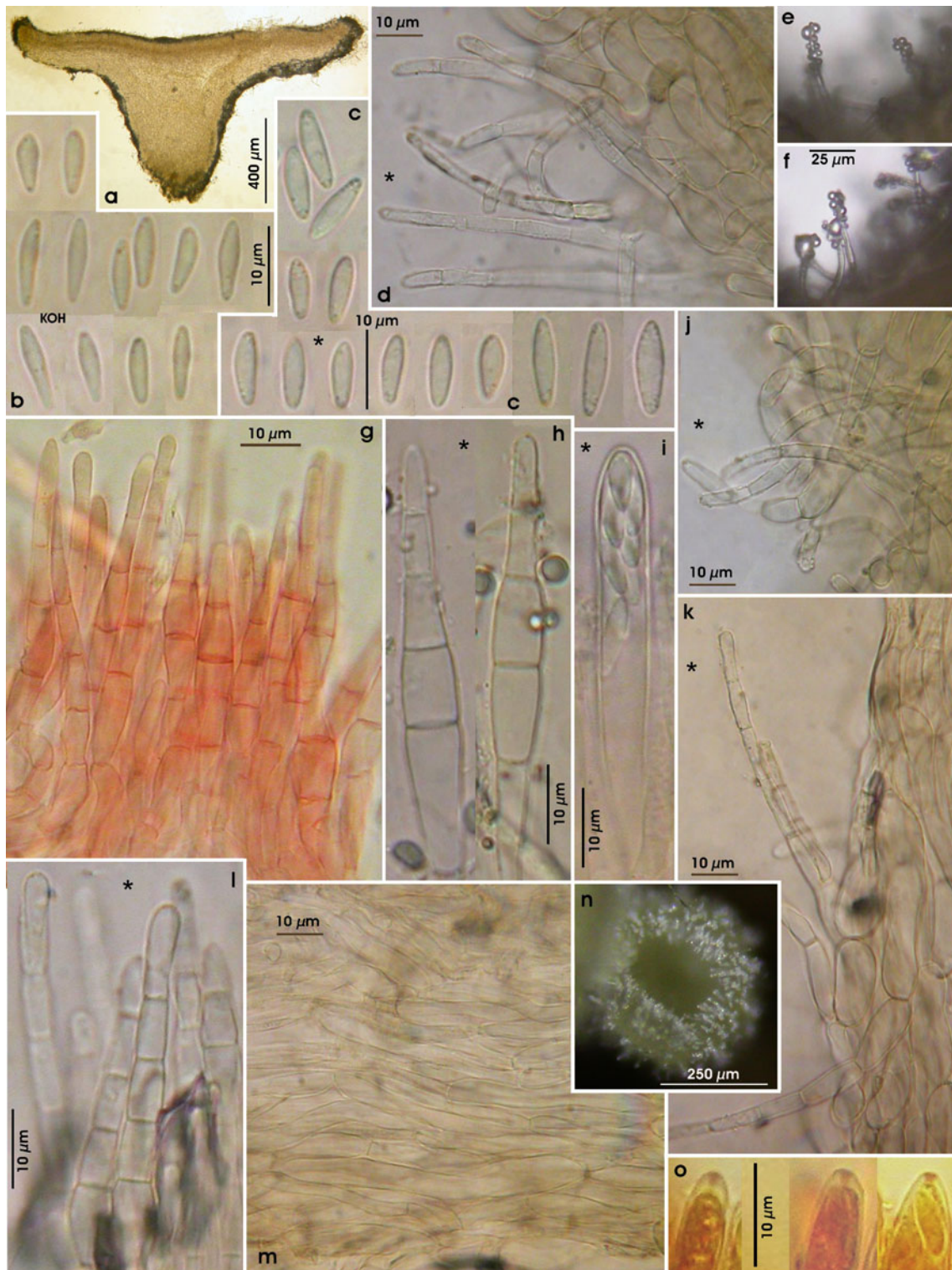




**Fig. 2** *Colipila masduguana*. **a, b** Cut logs of *Castanea* lying in leaf litter at the type locality (Mas-du-Gua) **c, d** apothecia of *C. masduguana* on lower surface of trunks; **e–o** fresh apothecia (**o** showing color change to red-brown); **p** dry apothecia: **a** 5.II.2006; **c, b**

7.I.2007; **d** G.G. 05031801; **e, f, i, j, l, p** G.G. 06123102=H.B. 8399; **g, h** G.G. 05021801; **k, m, o** M ..... ex H.B. 7458 (all from Olargues, Mas-du-Gua, except for (**d, g, h**): Saint-Gervais-sur-Mare, Combes)





**Fig. 3** *Colipila masduguana*. **a** Apothecium in median section; **b**, **c** free ascospores; **e**, **f** hairs with minute water drops; **d**, **j**, **k** curved hairs on stipe; **g**, **l** straight hairs on margin; **h** paraphyses; **i** ascus; **m** median section of ectal excipulum on flanks; **n** young apothecium, hairs

bearing minute water drops; **o** ascus apices. **a**, **c–f**, **h**, **j–n** Living state (in water; **n**, **e**, **f** in air); **g** in KOH+CR; **b** in KOH; **o** in IKI (dead state). H.B. 8399 (Mas-du-Gua) except for (**g**, **o**): H.B. 8403 (Vitrimont)

the surface, on lower flanks 40–70  $\mu\text{m}$  thick (here more of a *textura porrecta*), composed of cells  $*40\text{--}70(90)\times 5\text{--}11(15)$   $\mu\text{m}$  {3}, on upper flanks 30  $\mu\text{m}$  thick, composed of cells  $*20\text{--}40\times 7.5\text{--}11$   $\mu\text{m}$  {2}, at margin 15–20  $\mu\text{m}$  thick, composed of cells  $*11\text{--}23\times 7\text{--}10(14.5)$   $\mu\text{m}$  {1}; cells thin-walled, covered by a thin gelatinous sheath that stain lilac in CRB. **Hairs** on flanks and margin  $*(45)55\text{--}75(90)\times (3.5)4.5\text{--}7(8)$   $\mu\text{m}$  {2},  $\dagger(40)50\text{--}75(85)\times (3.5)4\text{--}6.5(8)$   $\mu\text{m}$  {2}, cylindrical or often subulate, widened and fusiform below, upwards gradually tapered towards a rounded and sometimes slightly inflated apex, terminal cell  $*(7.5)9\text{--}15(22.5)\times 2.5\text{--}3.5$   $\mu\text{m}$ ,  $\dagger 9\text{--}15\times 2.2\text{--}3$   $\mu\text{m}$ , hairs  $\pm$  straight near margin, straight to somewhat curved on flanks, of a similar size and shape but more frequently curved on the stipe; entirely smooth, thin-walled, (4)5–6(7)-celled {3},  $\pm$  equidistantly septate, cells at inflated part constricted at the septa (Fig. 1d), in the dead state less so (Fig. 3g), partly agglutinated as teeth in older apothecia. Exudate sparingly present as minute granules on hairs and among paraphyses, subhyaline to light yellow, dissolving in KOH and staining the medium pale yellow, bright violet in CRB. Rhomboid crystals absent. Tissues negative in IKI, KOH+IKI, and KOH+MLZ.

**Cultural characteristics** Ascospores germinating rapidly to form slowly growing colonies. Colonies on CBSOA 27–28 mm diam in 21 days, appearing waxy, aerial mycelium absent or scant and restricted to the colony centre, yellowish white (42A). Colony reverse yellowish white (4A2). Colonies on MEA 25–26 mm diam in 21 days, aerial mycelium sparse, restricted to the colony centre, cottony-lanose, orange white (5A2), mycelium towards the margin immersed, appearing waxy, orange red to greyish white (5B2–3). Colony reverse brown (5 F6–8) at the centre, becoming greyish white (5B3) towards the margin. Colonies on MLA 18–20 mm in 21 days, slightly furrowed, aerial mycelium dense, short, cottony lanose and covering the entire colony, orange grey to greyish orange (6B2–3), mycelium at the margin orange white (6A2). Colony reverse greyish orange to golden yellow (5B6–7) at the centre, becoming darker (5B4–5) towards the margin. Brownish orange (6 C7–8) pigment diffusing from the colony margin 5 mm into the surrounding agar. Colonies on OA 22–24 mm diam in 21 days, furrowed, aerial mycelium dense, short, lanose and covering the entire colony, greyish orange to greyish red (6–7B3), becoming orange white to orange grey (5A–B2) towards the margin, mycelium at the margin immersed, wrinkled, appearing waxy. Colony reverse brown to dark brown (7E–F7–8). Brown to dark brown (7E5–8) pigment diffusing from the colony margin 10 mm into the surrounding agar, darkest and most intensely colored 1–2 mm from the colony. Margin sharp and regular on CBSOA, MEA, and MLA, indistinct and irregular on OA.

Conidiogenous cells and conidia absent. Ascomatal initials not observed.

**Etymology** Named after Mas-du-Gua (France), the type locality.

**Habitat** On the lower surfaces of decorticated, ~2.5–20-cm-thick branches and trunks of *Castanea sativa* Mill. {9} and *Quercus robur* L. {1} lying on ground (and then partly hidden in leaf litter), on 0.2–0.7 mm deep strongly decayed, brown-rotted wood {10}, inner parts very little to medium decayed, surface sometimes blackened, sometimes fruiting in old beetle galleries, in sub-Mediterranean acidophilous forests of *C. sativa* and *Q. pubescens* Willd. at 330–710 m alt. (Dept. Hérault), also in temperate calcareous forests of *Carpinus betulus* L. and *Q. robur* at 250 m alt. (Dept. Meurthe-et-Moselle), December to March. Associated fungi include *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C. S. Ramamurthi et al. {1}, *Dasyscyphella montana* {1}, *D. nivea* (R. Hedw.) Raitv. (on separate branches) {1}, and *Hymenochaete rubiginosa* (Dicks.) Lév. {1}.

**Geology** Siliceous, rarely calcareous soil.

**Tolerance to desiccation** Many ascospores and some hair cells survive drying for up to two days.

**Holotype** FRANCE, **Dept. Hérault** (Languedoc-Roussillon), Commune de Saint-Vincent d'Olargues: Parc régional du Haut-Languedoc (Caroux-Espinouse), 1.6 km NW of Olargues, 0.8 km NE of Mas-du-Gua, Les Codouls, Ruisseau de Caudes, elevation 330 m, 43°34'09"N, 2°54'10"E, MEN 2543 C33, old *Castanea* plantation with *Corylus* L., *Juglans* L., *Quercus pubescens*, and *Sambucus nigra* L., on a fallen (cut) decorticated trunk of *Castanea sativa*, 18.I.2004, G. Garcia. Holotype (M-0140887, ex H.B. 7458), isotype: G.G. 04011104.

**Additional specimens examined** FRANCE, **Dept. Hérault**, Commune de Saint-Vincent d'Olargues: from the same trunk of *C. sativa* as the holotype, 11.I.2004, G. Garcia (G.G. 04011104); 25.I.2004, G. Garcia (G.G. 04012501); 20.II.2005, G. Garcia (not preserved); 31.XII.2006, G. Garcia (G.G. 06123102, H.B. 8399); 11.III.2007, G. Garcia (not preserved); 24.II.2008, G. Garcia (G.G. 08022401); same location but on a different trunk of *C. sativa*, 7.I.2007, G. Garcia (not preserved); 10 mS of the holotype site, on a branch of *C. sativa*, 11.III.2007, G. Garcia (G.G. 07031101); 100 m NW of the holotype site, 345 m, on a branch of *C. sativa*, 1.II.2004, G. Garcia (G.G. 04020103); 300 m NW of the holotype site, on a branch of *C. sativa*, elevation 365 m, 8.II.2004, G. Garcia (G.G. 04020803); 13.III.2005, G. Garcia (M-0140888, ex H.B. 7695; CBS 128287);



– Commune de Saint-Julien d'Olargues: 5.5 km NW of Olargues, 1.1 km WNW of Mauroul, S of entrance to dam tunnel, elevation 710 m, MEN 2443D42, on branch of *C. sativa*, 25.III.2007, G. Garcia (not preserved); – Commune de Combes: 3.5 km WNW of Lamalou-les-Bains, 0.7 km NW of Combes, Ruisseau du Vernet, near an old ranch, elevation 625 m, MEN 2543 C24, old *Castanea* plantation with *Alnus glutinosa* L., *Corylus avellana* L., *Ilex aquifolium* L., and *Quercus pubescens*, at the border of a rivulet, on cut trunk of *C. sativa*, 18.II.2005, G. Garcia (G.G. 05021801); 24.III.2006, G. Garcia (G.G. 06032404); on a branch and a cut trunk of *C. sativa*, 18.III.2005, G. Garcia (G.G. 05031801); – **Dept. Meurthe-et-Moselle** (Lorraine), SE of Nancy, W of Lunéville, Forêt de Vitrimont, elevation 250 m, *Quercus-Carpinus* forest with *Corylus* and *Crataegus*, on fallen decorticated branch of *Quercus robur*, with *Dasyscyphella nivea* (always on other branches), 14.XII.2006 and 6. I.2007, M. Hurlu (M.H. 0601071, H.B. 8403).

*Notes Colipila masduguana* is known primarily from the wood of *Castanea sativa* and, except for a single collection from north-eastern France near Nancy (on wood of *Quercus robur*, at a site with calcareous soil), all specimens were collected in southern France. The six collection sites in southern France are located at the southern border of the Massif Central in the Parc régional du Haut-Languedoc at altitudes between 330 and 710 m, on south-facing slopes situated in sub-Mediterranean forests with acidic soils, partly very close to the eu-Mediterranean belt but also close to the oro-Mediterranean zone. *Colipila masduguana* has so far only been found fruiting during the winter months.

All collection sites in southern France are south-exposed slopes or creeks, usually rather close to rivulets but sometimes away from water courses. They are located in the *Castanea* zone up to the border where the *Fagus* forests begin. For many years, old, diseased *Castanea* trees in the area have been cut down. The logs remain lying on the ground because this wood is not valued as firewood. Although frequently collected by one of us (G.G.), *C. masduguana* has never been found on *Quercus pubescens*, a common tree in that area. The identity of *Quercus* as a substrate at the site Forêt de Vitrimont is beyond doubt, because of the presence of broad radial rays that never occur in *Castanea*.

The single collection of *C. masduguana* on *Quercus robur* closely matches collections on *Castanea* both micro-morphologically and in the dimensions of the asci, ascospores, and hairs [asci  $\dagger 45\text{--}50 \times (5) 5.3\text{--}5.7 \mu\text{m}$ , ascospores  $* 6.5\text{--}10 \times 2.6\text{--}2.9 \mu\text{m}$ , hairs  $\dagger (40) 50\text{--}75 (85) \times (4) 5.5\text{--}6.5 (8) \mu\text{m}$ ]. Apart from the substrate, this collection also differs from those on *Castanea* in occurring in a temperate region with calcareous soil.

*Colipila pilatensis* Baral, spec. nov. Figs 4 and 5

MycoBank MB 517689

*Description* Apothecia gregaria, 0.3–0.9 mm diam, extus dense pilosa. Asci  $50\text{--}63 \times 5.5\text{--}6 \mu\text{m}$ , annulo apicali euamyloideo. Ascospores  $9\text{--}12 \times 2\text{--}2.3 \mu\text{m}$ , guttulas nullas vel paucas minutas continentes. Pili  $60\text{--}150 \times 4\text{--}7 \mu\text{m}$ . Habitat ad lignum putridum humidum (?Rosaceae).

**Apothecia** when fresh or rehydrated 0.3–0.9 mm diam, stipe  $0.4\text{--}0.5 \times 0.1\text{--}0.25 \text{ mm}$ , cylindrical, entirely white when fresh, receptacle pale to light reddish-brown when dry. **Asci**  $* 50\text{--}63 \times 5.5\text{--}6 \mu\text{m}$ ,  $\dagger (36) 40\text{--}48 (51) \times (4.2) 4.5\text{--}5.3 \mu\text{m}$ , apical ring strongly blue (euamyloid, BB), of the *Calycina*-type, arising from croziers. **Ascospores**  $* 9\text{--}12 \times 2\text{--}2.3 \mu\text{m}$ ,  $\dagger (6) 6.5\text{--}9.5 (10.8) \times (1.5) 1.7\text{--}1.8 (2) \mu\text{m}$ , subcylindrical to subfusoid-clavate, with up to 4 minute LBs in each half, lipid content 0–0.5. **Paraphyses** dimorphic (Fig. 4c), usually subcylindrical to lanceolate, not or only slightly protruding above the asci,  $* 3.5\text{--}5 \mu\text{m}$  ( $\dagger 2\text{--}4 \mu\text{m}$ ) wide at its widest part, terminal cell ca.  $* 30\text{--}33 \mu\text{m}$  long; rarely fusiform and protruding above the asci, up to  $* 8.5 \mu\text{m}$  wide in middle part, terminal cell ca.  $* 16\text{--}25 \times 3\text{--}5.5 \mu\text{m}$ . **Hairs** on flanks and margin  $* (60) 100\text{--}150 \times 4\text{--}7 \mu\text{m}$ ,  $\dagger 60\text{--}110 (150) \times 3.5\text{--}5 (7.3) \mu\text{m}$ , 5–7(10)-celled, straight or only very slightly curved, terminal cell  $\dagger 11\text{--}20 \times 2.2\text{--}2.8 (3.5) \mu\text{m}$ , in their lower part constricted at septa when alive (Fig. 4d), in the dead state wall of septum protruding instead (Fig. 4e–f); hairs on stipe ca.  $\dagger 50\text{--}75 (90) \times 3\text{--}4.5 \mu\text{m}$ , 4–6(8)-celled, straight or sometimes moderately curved to form a wide arc.

*Etymology* Named after Mount Pilatus (Switzerland), the type locality.

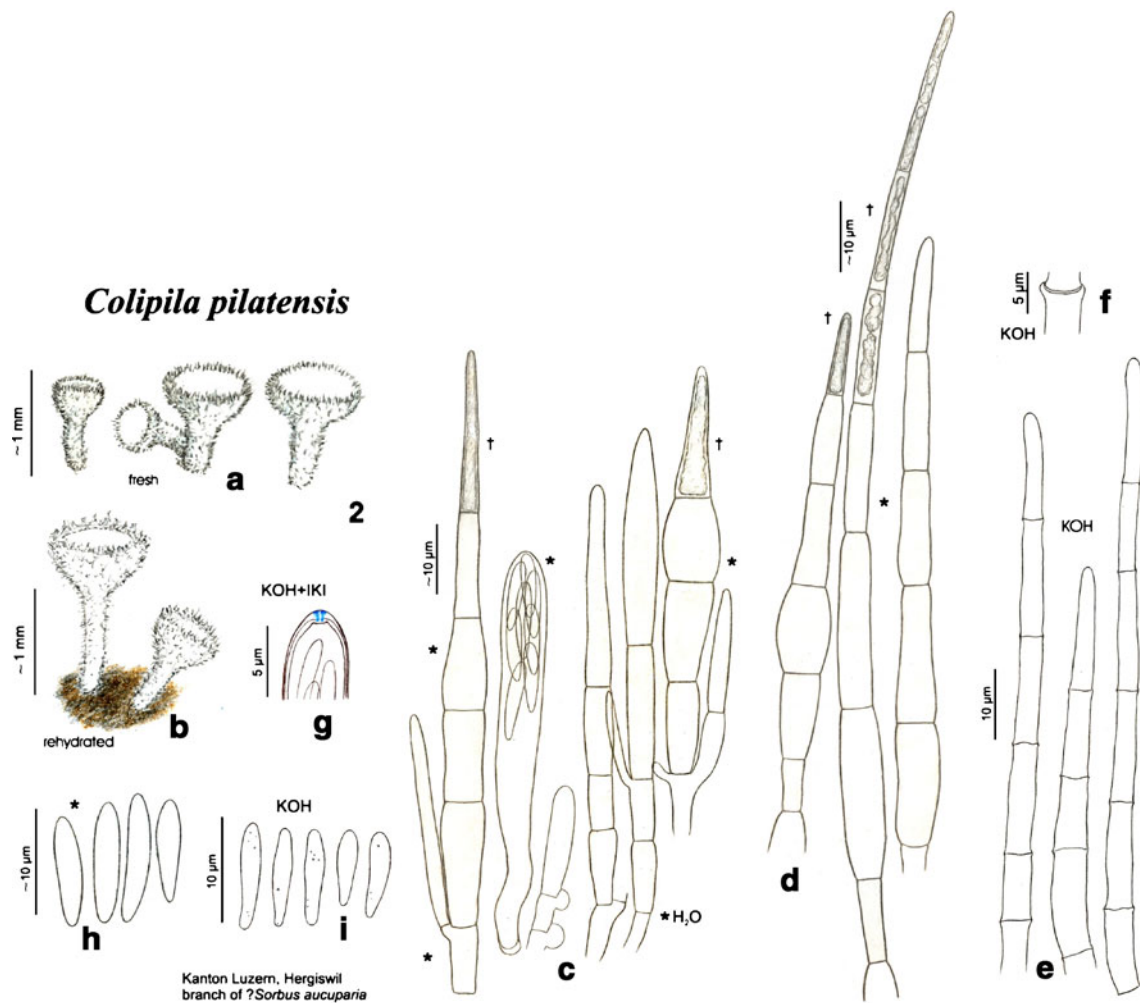
*Habitat* On a decaying branch of an unidentified woody plant (? *Rosaceae*, possibly *Sorbus aucuparia* L.), lying on moist ground in leaf litter under *Taxus baccata* L., in mountainous mixed forest composed of *Abies alba* Mill., *Betula* sp., *Fagus sylvatica* L., *Picea abies* (L.) H. Karst., and *Taxus baccata* with *Athyrium filix-femina* (L.) Roth. and *Dryopteris filix-mas* (L.) Schott, June. Not observed to be associated with other fungi.

*Tolerance to desiccation* Not tested.

*Holotype* SWITZERLAND, **Kanton Luzern**, 7 km SSW of Luzern, 1.9 km W of Hergiswil, Renggeli, eastern foot of Mount Pilatus, elevation 830 m, on the decaying branch of an unidentified woody plant, 6.VI.1985, H.O. Baral. Holotype (M-0140889, ex. H.B. 2909).

*Notes* The only known collection of *Colipila pilatensis* was made in June 1985 in the northern calcareous Alps south of





**Fig. 4** *Colipila pilatensis*. **a–i** M ..... ex H.B. 2909 (holotype). **2a**, **2b** Apothecia; **2c** asci and paraphyses; **2d**, **2e** hairs on margin and flanks of apothecia; **2f** detail of septum of a hair; **2g** ascus apex with

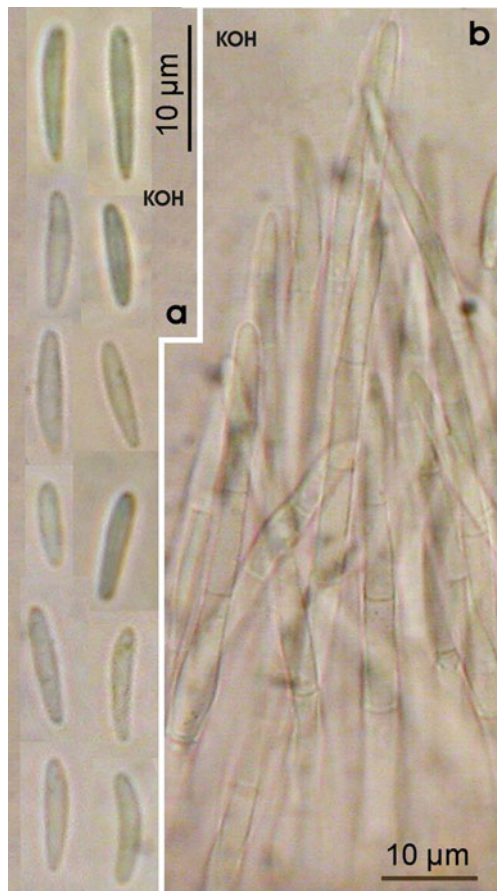
amyloid apical ring; **2h**, **2i** free ascospores. Living state (\*) except for (**2c**, **2d**) (†, terminal cells of paraphyses and hairs), (**2e–2g**)

Luzern at the border of a pasture and a mountainous mixed coniferous forest. The soil is formed of rocks crushed down from the peak region. The holotype consists of approximately 20 apothecia on a piece of wood of an unidentified, dicotyledonous plant that is probably a member of the *Rosaceae*, according to the wood anatomy, which clearly excludes members of *Fagaceae* and some other families. *Sorbus aucuparia* is rather frequent around the collection site, and pastures with *Malus* trees also occur in this area.

*Colipila pilatensis* was thought initially to be conspecific with *C. masduguana*, but it differs in possessing distinctly shorter and especially narrower asci with an euamyloid apical ring, and distinctly narrower ascospores. This species can apparently be differentiated from *C. masduguana* also by its somewhat longer and wider paraphyses and hairs, and smaller apothecia with narrower stipes. These differ-

ences are combined with ecological features: a deviating substrate, climatic region, geology, and phenology.

When examined in water, the terminal cells of many paraphyses and hairs of fresh specimens of *C. pilatensis* were dead. The cytoplasm of these dead cells appears highly refractive in contrast to the living lower cells (Fig. 4c, d). Although cells with refractive contents could be interpreted as being glandular in function, there are no observable differences between terminal and lower cells when all cells are alive (Figs. 1b, c, and 3d, h, j–l, *C. masduguana*), or when specimens are mounted in lethal agents such as KOH (Fig. 4e, *C. pilatensis*; Fig. 3g, *C. masduguana*). When the apothecia of *C. masduguana* are placed for several days in a moist box, the hairs bear small droplets of water which are best seen under the microscope (×400) by placing a fresh apothecium on a slide without adding water (Fig. 3e, f).



**Fig. 5** *Colipila pilatensis*. **a** Ascospores; **b** marginal hairs. **a**, **b** In KOH (M ..... , ex H.B. 2909, Kt. Luzern, Hergiswil, holotype)

## Discussion

### Morphological comparison with members of the *Lachnaceae*

The genus *Colipila* is characterized by white, distinctly stipitate (stipe longer than wide), hairy, somewhat delicate to robust, lignicolous apothecia that closely resemble those of wood-inhabiting members of *Lachnum* Retz. Species of *Colipila* differ from *Lachnum* mainly in two features: (1) the paraphyses are multiseptate, apically obtuse, and dimorphic (varying from subcylindrical and hardly protruding beyond the asci to broadly lanceolate and protruding strongly), and (2) the apothecial hairs resemble the paraphyses. These hairs are entirely smooth, multi-septate, thin-walled, basally±inflated, cylindrical to gradually tapered toward a rounded apex; those that occur on the flanks and stipes of apothecia are partly curved to form a wide arc. *Colipila* shares with *Lachnum* an ectal excipulum of large-celled, thin-walled *textura prismatica*. *Colipila* is further characterized by the absence of both refractive vacuolar bodies (VBs) and rhomboid crystals,

and by the distinct reddening of apothecia despite the absence of VBs. In *Lachnum*, the frequent reddening of apothecia is generally correlated with the presence of VBs (Baral 1992: 366).

The genus *Dasyscyphella* Tranzsch., which encompasses species that are closely related to *Lachnum* (Hosoya et al. 2010), appears at first glance to be intermediate between *Colipila* and *Lachnum*. However, like members of the genus *Lachnum*, and in contrast to *Colipila*, the paraphyses of species of *Dasyscyphella* are distinctly different from the hairs. The hairs of *Dasyscyphella* are warted only basally, and the upper part is gradually narrowed though partly inflated at the apex. These hairs bear more or less abundant quantities of hyaline to yellow resin on their surface, a feature that is absent in *Lachnum* and sparsely and inconsistently present in *Colipila*.

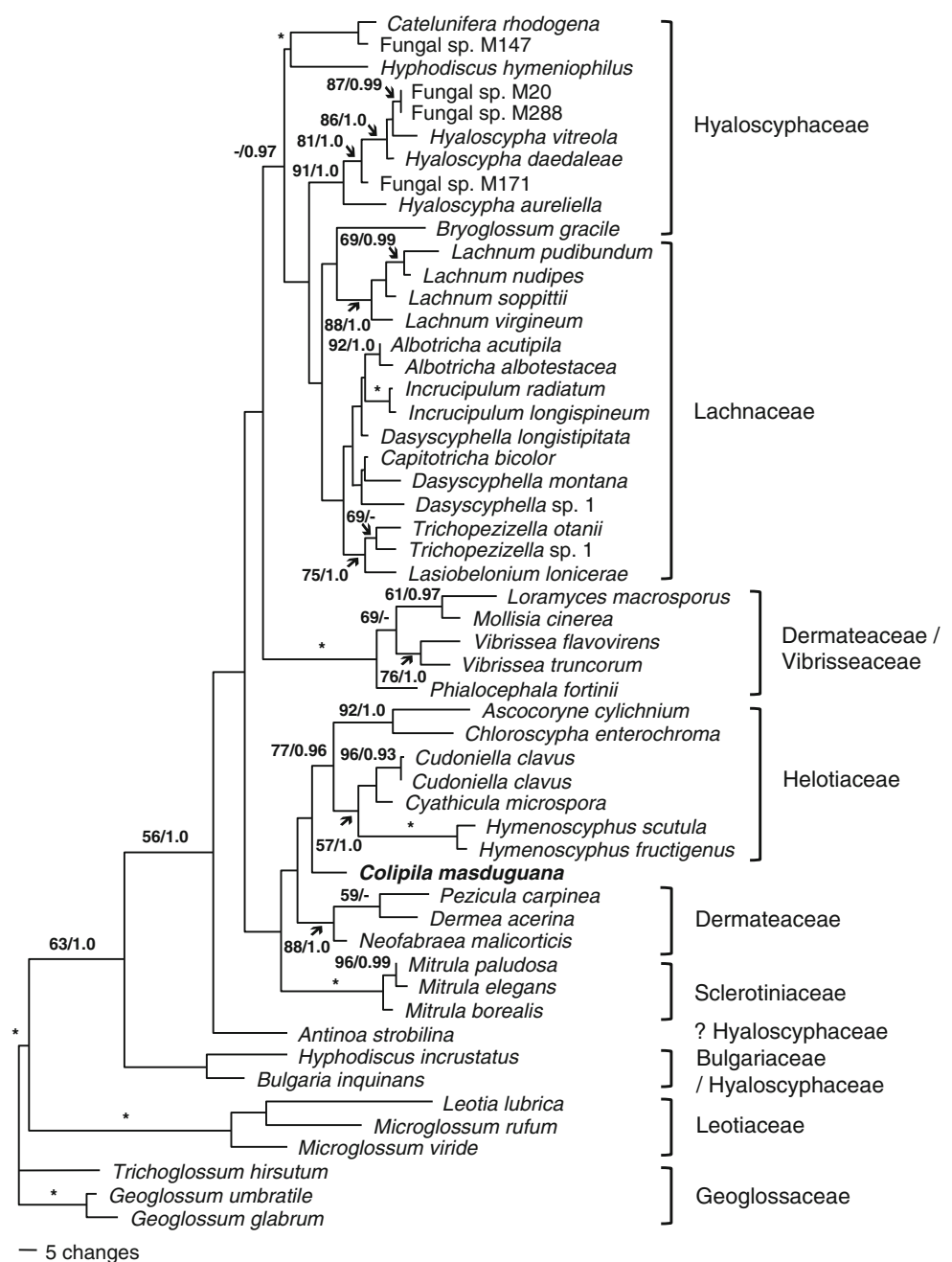
Species of *Colipila* are also similar microscopically to members of the genus *Albotricha* Raitv. sensu Raitviir (1970, 1973) and Zhuang (1998: 360), but differ in possessing longer apothecial stalks and dimorphic paraphyses. *Albotricha*, as circumscribed currently, is a polyphyletic taxon comprised of species that belong to different families. The type species, *A. acutipila*, has been included in *Dasyscyphella* (Weber 1992, as *D. acutipilosa* Baral & Weber), and it is possible that *A. laetior* (P. Karst.) Raitv. and *A. caduca* (Rehm) Raitv. & Sacconi also belong to this genus. *Albotricha albotestacea*, *A. kamtschatica* (Raitv.) Raitv., and *A. kurilensis* Raitv. somewhat resemble species of *Trichopeziza*. However, in the molecular study of Hosoya et al. (2010) *A. albotestacea* and *A. acutipila* were shown to be closely related species that were sister to *Incrucipulum* within the *Lachnaceae* and very distantly related to species of *Trichopeziza*.

*Albotricha fagicola* Ono & Hosoya (2001), a species with a light orange hymenium that occurs on leaves of *Fagus crenata* Blume, resembles *Colipila* in the shape of its hairs and lanceolate paraphyses. It differs in possessing densely warted hairs that are only smooth at the very apex and here strongly narrowed, and in the large crystals that are loosely attached to the hairs. Judging from the detailed original description, *A. fagicola* is close to *Dasyscyphus patulus* (Pers.) Sacc. sensu Dennis (1949: 37), a species belonging in *Capitotricha* (Raitv.) Baral. A further species, *A. miniata* (Kanouse) Raitv., has entirely smooth hairs and is, therefore, probably better placed in the genus *Psilachnum* Höhn. (see below).

### Morphological comparison with members of the *Hyaloscyphaceae*

*Colipila* is similar to the genus *Psilachnum* Höhn., a member of the *Hyaloscyphaceae* that includes species with smooth, lanceolate but also cylindrical paraphyses and hairs

**Fig. 6** Phylogenetic relationship of *Colipila masdugwana* and other members of the *Helotiales* inferred from the analysis of LSU sequence data. This is one of 2 MPT generated from a heuristic search of 930 characters (L=888, CI=0.467, RI=0.639). Bootstrap values (numbers in the first position) and Bayesian posterior probabilities (numbers in the second position) are provided above or adjacent to the branches. An asterisk indicates branches that received significant support (BS>97%, PP 1.0). Vertical bars indicate currently recognized families within the *Helotiales*



(Baral & Krieglsteiner 1985). Species of *Psilachnum* differ from *Colipila* in their smaller, more fragile apothecia with a shorter stipe. They also possess paraphyses and hairs with terminal cells that are considerably longer than the lower cells, and an ectal excipulum composed of much smaller cells. Moreover, the living paraphyses and hairs of *Psilachnum* contain slightly to highly refractive VBs that are responsible for color changes of the apothecia to yellow and red-brown (Baral, unpublished). The living asci of these species frequently contain a large, refractive globose, KOH-soluble guttule in their lower part. Neither of these vital characters have ever been observed in *Colipila*.

*Colipila* can also be compared with *Psilocistella* Svrček, a genus that encompasses *Hyaloscyphaceae* with smooth, septate, ±cylindrical hairs. However, the paraphyses of *Psilocistella* are cylindrical, and the rather small, ±sessile apothecia more closely resemble those of species of *Hyaloscypha* Boud.

*Phialina setigera* Huht. (Huhtinen 1990: 226) and *P. lacrimiformis* Hosoya (Hosoya & Otani 1997: 181) resemble *Colipila* in the morphology of the apothecial hairs, but differ in possessing paraphyses that are cylindrical and do not protrude beyond the asci (even in the living state), and small, rather short-stalked or sessile apothecia. Unlike *Colipila*,



species of *Phialina* Höhn. (= *Calycellina* Höhn.; see Baral & Krieglsteiner 1985) are characterized by VBs in the living paraphyses and hairs that Huhtinen (1990) reported as "bright yellow amorphous pigment". These vacuoles are often overlooked when dealing with herbarium specimens and were, perhaps therefore, not reported for *P. lacrimiformis*.

Species of *Colipila* are identified as *Lachnaster* Höhn. using Svrček's (1987) key to the European genera of *Hyaloscyphaceae* if the key character for fasciculate hairs is accepted. This monotypic genus, which is based on *L. gracilis* Höhn., is characterized by its long-stipitate apothecia that bear long, smooth, fasciculate hairs (Höhnel von Höhnel 1917: 349). However, the hairs and paraphyses of *L. gracilis* are described as much narrower (both 3 µm wide at the widest point) than those of *Colipila*, and the hairs as very slightly roughened basally. Höhnel (von Höhnel 1917) considered the teeth formed by peripheral fasciculate hairs to be a defining character of *Lachnaster*, but this has been observed by us to be a variable, age-dependent feature in inoperculate discomycetes. The protologue of *L. gracilis* is suggestive of a *Dasyscyphella* (R.P. Korf pers. comm.), and microscopically this species appears to be identical to *D. montana* Raitv. Re-examination of type material would be necessary to confirm if these two taxa are conspecific.

#### Phylogenetic considerations

*Lachnum*, which was treated until recently as a member of the family *Hyaloscyphaceae*, has been segregated with other genera possessing lanceolate paraphyses in the family *Lachnaceae* (Raitviir 2004: 7), based on a clear stratification of the hair wall (see also Leenurm et al. 2000). Hosoya et al. (2010) presented a narrower concept of the *Lachnaceae* based on the analysis of rDNA and RPB2 gene sequences, and restricted this family to species with either basally or entirely granulate hairs (i.e., *Albotricha* Raitv., *Brunnipila* Baral, *Dasyscyphella* Tranzsch., *Incrucipulum* Baral, *Lachnellula* P. Karst., and *Lachnum*). In the phylogeny inferred from three gene regions, lachnoid taxa with entirely smooth-walled hairs (i.e., species of *Lasiobelonium* Ellis & Everh., *Trichopeziza* Fuckel and *Trichopezizella* Raitv.) were positioned outside of a strongly supported group corresponding to the *Lachnaceae* (Hosoya et al. 2010).

We hypothesized that *Colipila* was a member of the *Lachnaceae* because of its macroscopical similarity to the members of this family, particularly the lignicolous species of *Lachnum*. And while our LSU phylogeny (Fig. 6) resolves many of the strongly supported lineages recognized by Hosoya et al. (2010), it does not position *C. masdugwana* as a close relative of *Lachnum* or the smooth-haired genera *Trichopezizella* and *Lasiobelonium*. A close relation to the latter two genera is also morphologically improbable because that group differs from *Colipila* in ±

sessile apothecia, ± thick-walled hairs, and an ectal excipulum of *textura globulosa*.

The presence of granulate apothecial hairs within the *Lachnaceae* is taxonomically important (Hosoya et al. 2010), and it is tempting to speculate that this character evolved recently in this lineage (i.e., *Lachnum* and its allies are derived from an ancestor with smooth hairs). However, because it is likely that many of the morphological characters used to delimit the *Lachnaceae* are homoplasious, (i.e., developed by convergent evolution) (Hosoya et al. 2010), accurately inferring the evolutionary history of the structure of hairs and paraphyses within the *Helotiales* will require the improved resolution of the phylogenetic relationships within this order.

Our analyses of LSU sequences did not identify a close phylogenetic relative of *Colipila masdugwana* and its position within the *Helotiales* remains unresolved. In contrast to lachnoid species with warted hairs, the paraphyses and the marginal hairs of *Colipila* resemble each other morphologically, as in other members of this order with rather primitive apothecia (e.g., *Calycina*, *Psilachnum*, or *Psilocistella*). If this resemblance is due to a similarity in the ontogenetical origin of these organs, then paraphyses and hairs (including the excipular cells from which they arise) can be considered to be homologous within the *Helotiales*. This homology is particularly striking when the cytoplasm of these organs contains the same types of cell organelles (VBs), (e.g., in *Calycellina*, *Calycina*, *Lachnum*, and *Psilachnum*). From this point of view, it can be hypothesized that genera possessing differently shaped apothecial hairs and paraphyses evolved from taxa resembling *Colipila* in which these two organs were morphologically similar. Testing this question will require the availability of gene sequences of taxa in which the hairs and paraphyses are morphologically similar, i.e., species of genera such as *Calycellina* (s.str.), *Calycina*, *Psilachnum*, and *Psilocistella*.

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