Taxonomy and phylogeny of European Gymnopus subsection Levipedes (Basidiomycota, Omphalotaceae)

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Key words
ITS neotype nomenclature translation elongation factor 1-alpha

Abstract The systematic integrity of European Gymnopus subsect. Levipedes is verified based on anatomic-morphological characters with support from DNA sequences of ITS and translation elongation factor 1-alpha genes. Seven species (G. alpinus, G. aquosus, G. dryophilus – including var. lanipes, G. erythropus, G. falafhus, G. hybridus, and G. ocior) belonging to this subsect are included. We clarify the concepts of G. dryophilus and G. ocior, which were occasionally confused in older literature. Due to unavailability of previously selected neotype of G. dryophilus the substitute neotype specimen is selected. Gymnopus dryophilus var. lanipes is confirmed as a variety; no important differences from nomenclotypical variability were detected. All discriminative characters used for identification of these species are discussed in detail. An identification key is also provided.

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INTRODUCTION

Gymnopus is a large agaricoid genus distributed world-wide circumscribing c. 300 species (Kirk et al. 2008). It is characterized in basidiocarps collybioid, rarely tricholomatoid or marasmioid with a pileus convex, plano-convex to applanate, radially arranged cylindrical hyphae, or interwoven, made up of irregular coralloid terminal elements (Dryophila-structure); hyphae never amyloid or dextrinoid (except for sect. Androsacei with dextrinoid context hyphae, at least in stipe apex), and clamp connections mostly present. Gymnopus sect. Levipedes subsect. Levipedes (type species: Gymnopus dryophilus) is characterized in having a pileipellis composed of cells inflated, lobed or coralloid (a Dryophila-type cutis), well-developed cheilocystidia, a smooth stipe, and hyphae only rarely becoming green in alkali or not (Antonín & Noordeloos 2010).

In Europe, seven species occur: G. alpinus, G. aquosus, G. dryophilus (with var. lanipes), G. erythropus, G. falafhus, G. hybridus, and G. ocior. The DNA-based phylogenetic relations among these species are insufficiently known. Mata et al. (2006) included several species of this subsection from various continents (some of them also from Europe), but in their comprehensive analysis did not cover all European taxa of subsect. Levipedes. Moreover, our preliminary results were in disagreement with some of their interpretation of G. ocior and its placement in the ITS phylogeny. Therefore, the aim of this paper is a phylogenetic and taxonomic revision of all European taxa of this subsect.

MATERIAL AND METHODS

Morphological dataset

The specimens studied, especially of the G. dryophilus complex, were selected from various regions of Europe. The macroscopic descriptions are based on fresh basidiocarps, if available, were made by the collectors. Microscopic features were studied under Olympus BX 50 light microscope from dried material mounted in H₂O, 5 % KOH solution, Melzer’s reagent and Congo Red. Microscopic characters were studied with emphasis on the most important morphological features for taxon delimitation – shape and dimensions of basidiospores, pileipellis structure and shape and dimensions of cheilocystidia. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. In each herbarium collection, 20 basidiospores were measured. Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do); colour abbreviations are according to Kornerup & Wanscher (1983), and for herbarium acronyms see Thiers (2012; accessed 10 Sept. 2012).

Molecular dataset

DNA extraction and PCR

The DNA was extracted from dried herbarium specimens. The specimens selected for DNA extraction and PCR are listed in Table 1. The two loci: ITS region of ribosomal RNA gene (ITS) and partial sequence of translation elongation factor 1-alpha gene (tefa) – were selected for the analysis. The DNA extraction and PCR of ITS was applied according to Tomšovský et al. (2010). For the amplification of tefa, the primer pair 983F/2218R was used (Rehner & Buckley 2005). PCR for the tefa locus was

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performed using a following touchdown PCR procedure: The amplifications were initiated with a 2 min denaturation at 94 °C. The annealing temperature in the first amplification cycle was 60 °C, which was subsequently incrementally reduced by 1 °C per cycle over the next 9 cycles. An additional 35 amplification cycles were then performed, each consisting of 30 s denaturation at 94 °C, a 30 s annealing step at 50 °C, and a 1 min extension at 72 °C, concluding with a 10 min incubation at 72 °C. In case of unsuccessful amplification of tefa gene, the nested PCR of this gene region was performed according to Tomšovský et al. (2010).

Phylogenetic analysis
The newly obtained sequences were augmented by those published by Lutzoni et al. (2004), Mata et al. (2006), and Antonín et al. (2012). Sequences of each individual locus were aligned using the MAFFT version 6 with selected Q-INS-i option algorithm (Katoh & Toh 2010). The sequences of Gymnopus confluens were selected as an outgroup.

The two separate phylogenetic analyses were performed – the first one including a two-gene dataset of both genetic markers (ITS and tefa) and the second one of newly obtained ITS data alone complemented with the respective sequences from GenBank (mostly published by Mata et al. 2006).

To determine whether the datasets of the different genetic markers (ITS and tefa) were in significant conflict, two methods were applied. The partition homogeneity test in PAUP* 4.0b10 (Swofford 2003) was used between the markers using 100 replicates and the heuristic general search option. The null hypothesis of congruence was rejected if p < 0.01. A test based on

Table 1  The specimens sequenced by the authors.

| Species          | Country       | Herbarium | Nr. | ITS            | tefa        |
|------------------|---------------|-----------|-----|----------------|-------------|
| G. alpinus       | Latvia        | CB 16251  | G69 | JX536168       | JX536191    |
| G. aquosus       | Czech Republic| BRNM 665362 | G3  | JX536172       | JX536192    |
| G. aquosus       | Czech Republic| BRNM 695556 | G6  | JX536173       | JX536193    |
| G. aquosus       | Czech Republic| BRNM 710027 | G30 | JX536170       | JX536194    |
| G. aquosus       | Czech Republic| BRNM 670755 | G36 | JX536171       | JX536174    |
| G. aquosus       | Czech Republic| BRNM 691431 | G37 | JX36169       |
| G. aquosus       | Sweden        | DUKE 193432 | G63 | JX536198       |
| G. aquosus       | Czech Republic| BRNM 734005 | G60 | JX36124        | JX536178    |
| G. aquosus       | Slovakia      | BRA 13021  | G1  | JX36140        | JX536195    |
| G. aquosus       | Czech Republic| BRNM 695586 | G4  | JX36143        | JX536196    |
| G. aquosus       | Czech Republic| BRNM 691279 | G5  | JX36145        |
| G. aquosus       | Slovenia      | BRNM 695317 | G8  | JX36146        | JX536197    |
| G. aquosus       | Switzerland   | BRNM 693554 | G9  | JX36147        | JX536198    |
| G. aquosus       | Slovakia      | BRNM 670778 | G10 | JX36141        | JX536199    |
| G. aquosus       | Czech Republic| BRNM 704894 | G11 | JX36156        | JX536210    |
| G. aquosus       | Italy         | BRNM 707149 | G12 | JX36157        | JX536204    |
| G. aquosus       | Czech Republic| BRNM 712600 | G31 | JX36158        | JX536206    |
| G. aquosus       | Slovakia      | BRNM 642393 | G34 | JX536142       |
| G. aquosus       | Czech Republic| BRNM 691282 | G38 | JX36138        |
| G. aquosus       | Czech Republic| BRNM 695739 | G42 | JX36144        |
| G. aquosus       | Czech Republic| BRNM 705601 | G43 | JX36155        |
| G. aquosus       | Czech Republic| BRNM 732938 | G49 | JX36149        | JX536202    |
| G. aquosus       | Czech Republic| BRNM 734758 | G98 | JX36148        | JX536201    |
| G. aquosus       | Norway        | BRNM 737692 | G44 | JX36154        |
| G. aquosus       | Germany       | BRNM 737691 | G61 | JX36139        |
| G. aquosus       | Sweden        | DUKE 193401 | G64 | JX36159        |
| G. aquosus       | Sweden        | DUKE 193429 | G65 | JX36150        |
| G. aquosus       | Sweden        | DUKE 193405 | G66 | JX36151        |
| G. aquosus       | Sweden        | DUKE 193406 | G67 | JX36152        |
| G. dryophilus (neotype) | Spain          | BRNM 670686 | G13 | JX36137        | JX536205    |
| G. dryophilus var. lanipes (isoneotype) | Spain | BRNM 714784 | G2  | JX36136        | JX536183    |
| G. dryophilus var. lanipes (isoneotype) | Czech Republic | BRNM 705224 | G14 | JX36131        | JX536180    |
| G. dryophilus var. lanipes (isoneotype) | Slovakian | BRNM 706885 | G15 | JX36134        | JX536182    |
| G. dryophilus var. lanipes (isoneotype) | Switzerland | BRNM 693553 | G18 | JX36135        | JX536181    |
| G. dryophilus var. lanipes (isoneotype) | Czech Republic | BRNM 666730 | G39 | JX36132        |
| G. dryophilus var. lanipes (isoneotype) | Czech Republic | BRNM 664995 | G40 | JX36133        |
| G. fagophillus    | Czech Republic| BRNM 707079 | G19 | JX36129        | JX536209    |
| G. fagophillus    | Czech Republic| BRNM 712422 | G22 | JX36125        | JX536210    |
| G. fagophillus    | Czech Republic| BRNM 707066 | G23 | JX36130        | JX536211    |
| G. fagophillus    | Czech Republic| BRNM 712407 | G24 | JX36126        | JX536212    |
| G. fagophillus    | Czech Republic| BRNM 691489 | G27 | JX36128        | JX536213    |
| G. fagophillus    | Slovakia      | BRNM 695747 | G41 | JX36127        |
| G. hybridus       | Italy         | BRNM 695773 | G26 | JX36177        | JX536208    |
| G. hybridus       | Czech Republic| Dvořák 393/07, BRNU | G58 | JX36176       | JX536207    |
| G. inusitatus (holotype) | Spain | SCM B-84056 | 057 | JN247553       |
| G. inusitatus (holotype of G. bisporus) | Spain | SCM B-84065 | 055 | JN247551       |
| G. inusitatus (holotype of G. catalonicus) | Spain | SCM B-84057 | 056 | JN247552       |
| G. inusitatus var. cystidiatus (holotype) | Hungary | BRNM 737257 | G45 | JN247550       |
| G. ocror         | Czech Republic| BRNM 697975 | G29 | JX36166        | JX536188    |
| G. ocror         | Slovakia      | BRNM 728586 | G2n | JX36165        | JX536185    |
| G. ocror         | Czech Republic| BRNM 728539 | G3n | JX36167        | JX536186    |
| G. ocror         | Czech Republic| BRNM 728540 | G4n | JX36161        | JX536187    |
| G. ocror         | Norway        | BRNM 737697 | G46 | JX36162        | JX536189    |
| G. ocror         | Norway        | BRNM 737695 | G47 | JX36163        | JX536190    |
| G. ocror         | Norway        | BRNM 737693 | G48 | JX36164        |
maximum agreement sub樹es (de Vienne et al. 2007) was further performed. Phylogenies were generated in MrBayes version 3.2.1 (Ronquist & Huelsenbeck 2003). The substitution models were selected prior to analyses using the MrModeltest 2.3 (Nylander 2008). For ITS+tefa dataset the GTR + I + G (General time reversible model + Proportion of invariant + Gamma) while for the ITS dataset GTR + G (General time reversible model + Gamma) were chosen. Markov chains were initiated from a random tree and were run for 5 000 000 generations; samples were taken every 100th generation. The number of excluded generations determined as burn-in was used by Tracer 1.5 (Rambaut & Drummond 2009); burn-in = 500 000 in both analyses. The Bayesian branch supports were assigned as posterior probabilities (PP) on the consensus trees. In addition, bootstrap branch support values (BS) were estimated in PAUP 4.0b10 using 1 000 replicate datasets with the random addition of sequences during each heuristic search.

Additional, phylogenetic analyses were carried out in PHYML estimating maximum likelihood phylogenies and run at the server Phylogeny.fr (Dereeper et al. 2008) using ‘A la Carte’ mode. The alignments were treated with Gblock, eliminating poorly aligned positions and ambiguous regions, and GTR substitution model was selected for both ITS and LSU datasets. Bootstrap branch support values (BP) were estimated in PHYML 3.0 (Guindon et al. 2010) under the maximum likelihood criterion using default 100 replicates. The alignments and phylogenograms from the Bayesian analyses were deposited in Treebase (ID 13361).

RESULTS
We obtained 58 new sequences of ITS and 36 of tefa gene regions. A partition homogeneity test and a test of maximum agreement sub树es allowed us to combine the ITS and tefa data. The lengths of datasets, likelihood values and model parameters of Bayesian and Maximum likelihood of both datasets are summarized in Table 2.

The molecular data (Fig. 1, 2) confirmed sequence homogeneity of G. alpinus, G. aquosus, G. dryophilus, G. erythropus, G. fagiphilus, G. hybridus, and G. ocior. However, four sequences obtained from the Genbank (AY256691, DQ450003, DQ449976-7) formed two unsupported groups proximal to G. ocior or G. aquosus in the ITS phylogram. Gymnopus dryophilus var. lanipes (labelled G13 in the phylogenetical trees) fell unambiguously within other sequences of G. dryophilus. Gymnopus hybridus is closely related to G. instustus placed in sect. Levipedes subsect. Alkalivirentes Antonín & Noordel. This indicates the current concept of subsections in sect. Levipedes (Antonín & Noordeloos 2010) may not follow phylogenetic relations of the species.

NOTES ON STUDIED TAXA
Gymnopus dryophilus complex
The recent identification of four European species of this complex is based on studies by Vilgalys & Miller (1987) and Vilgalys (1991), who distinguished four species based on anatomic-morphological studies and confirmed the distinctions using mating compatibility tests. The published key (Vilgalys & Miller 1987) relies on the pileus colour, presence of the basal stipe bulb, size of basidiospores and shape of cheilocystidia to distinguish species. See Antonín & Noordeloos (2010) for monographic details on all taxa.

Gymnopus alpinus (Vilgalys & O.K. Mill.) Antonín & Noordel.

Specimen examined. LATVIA, Kemer National Park, in a Sphagnum stand under Pinus sylvestris and Betula pendula, 23 Aug. 2006, M. Beran, CB 16251.

Notes — Gymnopus alpinus differs from other species of this complex by having a dark red-brown, only weakly hygrophanous pileus, 6.0–7.5 × 3.0–4.0 μm basidiospores, and 14–32 × 7.0–12 μm, clavate, simple, irregular to coralloid cheilocystidia. This species is a widespread but probably rare fungus with unknown distribution in Europe.

Gymnopus aquosus (Bull.: Fr.) Antonín & Noordel.

Specimens examined. CZECH REPUBLIC, White Carpathian Mts, Sidonie, Sidorie Nature Reserve, beech forest, under Fagus sylvatica, 14 May 2008, V. Antonín 08.07, BRNM 710027; Žďárské vrchy Mts, Cihkáž, Žáková hora National Nature Reserve, in fallen leaves of Acer pseudoplatanus and Acer pseudoplatanus and Fagus sylvatica, 2 July 2004, A. Vágner, BRNM 601431; Utěchov near Brno, Obrora forest, in fallen leaves of Quercus and Tilia, A. Vagner, BRNM 665362; Mokrá near Brno, Nad dlouhým (Sivický les) forest, under Carpinus and Picea abies, 21 May 2002, A. Vágner, BRNM 670755; Bílé Karpaty Mts, Suchov, Porážky Nature Reserve, 1 June 2005, A. Vagner, BRNM 695556.—SWEDEN, Uppsala, Ladugårdsparken, in moss, 16 June 1984, S. Ryman & R. Vilgalys RV 84/1999, DUKE 193407; ibid., 17 June 1984, S. Ryman & R. Vilgalys RV 84/200, DUKE 193408; ibid., amongst herbaceous matter, 17 June 1984, S. Ryman & R. Vilgalys RV 84/197, DUKE 193432; ibid., 17 June 1984, S. Ryman & R. Vilgalys RV 84/201, DUKE 193409; ibid., in moss, 17 June 1984, S. Ryman & R. Vilgalys RV 84/205, DUKE 193412; ibid., in moss, 17 June 1984, S. Ryman & R. Vilgalys RV 84/202, DUKE 193410.

Notes — Gymnopus aquosus is mainly characterized by having a hygrophanous, almost to the centre translucently striate, rather pale coloured, pale yellow to ochre pileus, pallescent

| Dataset/analysis | ITS+tefa / Bayesian analysis (MrBayes) | ITS+tefa / Maximum likelihood (PHYML) | ITS / Bayesian analysis (MrBayes) | ITS / Maximum likelihood (PHYML) |
|------------------|----------------------------------------|---------------------------------------|----------------------------------|----------------------------------|
| Dataset length/bp| 1294                                   | 1005                                  | 773                              | 531                              |
| Variable positions| 336                                   | 235                                   | 188                              | 121                              |
| Singleton positions| 171                                   | 140                                   | 109                              | 67                               |
| Log-likelihood   | -4169.583                              | -2942.619                             | -2581.831                        | -1592.603                        |
| Gamma shape parameter (alpha in Bayesian analysis) | 0.914                                 | 0.555                                 | 0.09735                          | 0.693                            |
| Proportion of invariant | 0.368                                 | 0.382                                 | N/A                              | N/A                              |
| f(A)             | 0.22600                                | 0.23292                               | 0.22900                          | 0.22748                          |
| f(C)             | 0.21200                                | 0.21714                               | 0.17900                          | 0.18814                          |
| f(G)             | 0.22300                                | 0.23595                               | 0.21400                          | 0.21315                          |
| f(T)             | 0.33900                                | 0.31399                               | 0.37800                          | 0.37123                          |
Fig. 1 The phylogram inferred from the Bayesian analysis of combined dataset tefa and ITS DNA sequences of Gymnopus spp. included in the study. Numbers at branches indicate Maximum likelihood, Bayesian bootstrap values and Bayesian posterior probabilities values higher than 50%. The bar indicates the number of expected substitutions per position.

Fig. 2 The phylogram inferred from the Bayesian analysis of ITS gene sequences of Gymnopus spp. included in the study. Numbers at branches indicate Maximum likelihood, Bayesian bootstrap values and Bayesian posterior probabilities values higher than 50%. The bar indicates the number of expected substitutions per position.
to almost white, a distinctly bulbous stipe base with pinkish-
ochraceous rhizomorphs, (5.0–)5.5–7.0 × 3.0–4.0–(4.25) μm basidiospores, and 16–51 × (5.0–)7.0–17 μm, clavate, capi-
tate and pedunculate, less frequently subcylindrical or fusoid, simple or coralloid cheilocystidia. It also appears very early in the season (from May, rarely late April). It grows in deciduous, rarely coniferous forests, but also among grass on road-sides in semi-open places, and is widespread all over Europe.

Gymnopus dryophilus (Bull.: Fr.) Murrill s.str.

Lectotypification. Bulliard, Herbin de la France: Historie des champignons de la France, pl. 434 A, B, E, nad F (C and D excluded), 1789; designated here.

Bulliard kept a very broad concept of this species. Excluded pictures C and D show darker, brown to dark brown coloured fungi which may represent other taxa of this species complex (G. ocior, G. alpinus?).

Vilgalys & Miller (1987) proposed the collection from Sweden (Uppsala, Stadsskogen, R. Vilgalys 84/181) as the neotype. However, this neotype specimen was not traced in herbaria BPI, DUKE, VPI, and NY (relocated VPI herbarium) (Halling, 2004). We added two of these sequences (DQ449963 – Duke29 and DQ449956 – TFB 3849, Scotland) included in the North American specimens of G. dryophilus var. lanipes. – S. Ryman & R. Vilgalys RV 84/190, DUKE 193401; ibid., in moss, 17 June 1984, S. Ryman & R. Vilgalys RV 84/203, DUKE 193429; ibid., in Sphagnum, 17 June 1984, S. Ryman & R. Vilgalys RV 84/193, DUKE 193403; ibid., in moss, 17 June 1984, S. Ryman & R. Vilgalys RV 84/198, DUKE 193406; ibid., 17 June 1984, S. Ryman & R. Vilgalys RV 84/204, DUKE 193411, neotype, selected here; ibid., 17 June 1984, S. Rym.

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Notes — Gymnopus dryophilus is characterized by having a pale coloured, orange-brown or ochraceous brown, later ochraceous brown, yellow ochraceous to pinkish ochraceous, hygrophanous, transversely striate pileus, white, cream to yellow lamellae (see Discussion), 5.0–7.0–(8.0) × (2.5–)3.0–4.0–(4.5) μm basidiospores, and 17–55 × 4.0–10 μm, (sub) cylindrical, narrowly clavate cheilocystidia, which are mostly coralloid, but also lobate or with apical projections. It grows mostly in deciduous, sometimes also coniferous forests, or in Sphagnum stands, and is widely reported from all over Europe and North America. All studied collections of fungi from this species complex from the alpine habitats represent this species.

Gymnopus dryophilus var. lanipes (Ortega et al. 2003) especially differs from the type variety by having a finely tomentose stipe. It is known from Mediterranean thermophilic forests, especially those containing Quercus ilex, Pinus halepensis, or Cistus. Molecular studies showed that the isoneotype specimen (BRNM 670686) agrees with sequences of G. dryophilus var. dryophilus. Therefore, the proposal to consider it a separate species (Vilia & Límona 2006) is not supported.

Gymnopus ochor (Pers.) Antonín & Noordel.

Specimens examined. CZECH REPUBLIC, České Švýcarsko National Park, Jeřišovce, Babylion, under Picea abies, Pinus sylvestris, 12 June 2010, V. Antonín 10.50, BRNM 728540; ibid., V. Antonín 10.49, BRNM 728539; České Švýcarsko National Park, Jeřišovce, Starý mýn, on a woolly musl in a garden, 29 May 2009, V. Antonín 09.19 and S. Komínková, BRNM 714622; Všeoteč, on a mulch, 22 May 2010, M. Míšek, BRNM 722586; Mokrá near Brno, Nad dlouhým (Sivíčky les) forest, under Larix decidua, Pinus sylvestris and Picea abies, 7 June 2006, A. Vagner, BRNM 699779. – NORWAY, Østfold Co., Sarpsborg, Hafrsfjordparken, 24 May 2010, Ø. Weholt, BRNM 737695; ibid., 12 June 2010, Ø. Weholt, BRNM 737696; Østfold Co., Fredrikstad, Skjåklin, Borge skytterhus, 25 June 2010, Ø. Weholt, BRNM 737697; Østfold Co., Fredrikstad, Veberg, 5 June 2010, Ø. Weholt, BRNM 737693; ibid., 21 June 2010, Ø. Weholt, BRNM 737694. – SWITZERLAND, Podunajské Biskupovce, Topolové, Topolové hony National Nature Reserve, alluvial forest, under Quercus robur, Acer campestre, Padus racemosa, and Corylus avellana, 9 June 2010, L. Nagy, Antonín 10.82, BRNM 728565.

Notes — Gymnopus ochor is especially characterized by having a non-transparent or only at margin transversely striate, dark red- or orange-brown pileus, pallescent to reddish yellow or pinkish brownish, whitish or yellowish lamellae (see Discussion), (5.0–)5.5–6.5–7.0 × (2.5–)2.75–3.5–(4.0) μm basidiospores, and 16–60 × 6.0–12 μm, clavate, subcylindrical or subutiiform cheilocystidia, often lobate, branched, coralloid or with (apical) projections. It occurs in both deciduous and
coniferous forests, on road margins and similar stands. It is a widespread fungus in Europe. The ITS phylogram of Mata et al. (2006) published in f. 4 of the respective publication depicted clade of *G. ocior* Europe with nested subclade of *G. dryophilus* North Am.*. After co-analysis of selected sequences from this work with our data we revealed sequences of 'Europe' in fact European specimens of *G. dryophilus*.

**Gymnopus erythropus** (Pers.: Fr.) Antonín, Halling & Noordeloos.

Specimens examined. **Czech Republic**, Český krás, Sv. Jan pod Skalou, J. Burej, BRNM 714784; Moravský kras, Ochoz near Brno, Hornek Nature Reserve, living stem of Crataegus, 3 Oct. 2001, A. Vánger, BRNM 666730; Litovelští Pomoraví, Litovel, Vrapad National Nature Reserve, stump of Quercus, 2 Aug. 2000, A. Vánger, BRNM 664995; Mokrá near Brno, Nad dlouhým (Sivický les) forest, stump of *Quercus petraea*, 7 Sept. 2005, A. Vánger, BRNM 705224. — **Slovakia**, Strážovské vrchy mts, Kláštorná, Slavcov hill, on soil under *Fagus sylvatica*, V. Antonín 07.235, BRNM 706885. — **Switzerland**, Graubünden, Tamins, Reichenau, Ilus Aults, on soil, on a pasture under *Pinus sylvestris*, 1 Oct. 2004, V. Antonín 04.232, BRNM 693593.

Notes — This species is especially recognizable by having a ± dark red-brown, shining stipe with typically red-brown coloured basal hairs, rather large basidiospores (15.0–5.0 × 9.0–4.5 μm), and rather narrow (13–40 × 5.0–10–13 μm), (sub)clavate, subfusoid, irregular to coralloid or apically mucronate cheilocystidia. It usually grows on dead wood or wood debris of various broad-leaved, rarely coniferous (*Picea*) forests, on road margins and similar stands. It is a widespread fungus in Europe, but details of occurrences are not known. However, (18–26 × (3.1–)5.2–6.6 μm), clavate to cylindrical, mostly irregular cheilocystidia. It mostly grows on fallen leaves of *Quercus*, less frequently on other broad-leaved tree litter, mostly in thermophilic forests, and is widely distributed especially in Central and Western Europe. For a detailed description, photograph and citations of other literature, see Antonín & Noordeloos (2010).

**Gymnopus fagiphilus** (Velen.) Antonín, Halling & Noordeloos.

Specimens examined. **Czech Republic**, České Svícarsko National Park, Jetřichovice, Babylon, fallen leaves and cupules of *Fagus sylvatica*, 3 Oct. 2007, V. Antonín 07.320, BRNM 707079; Orlické hory Mts, Horní Rokytnice, Černý důl Nature Reserve, fallen leaves of *Fagus sylvatica*, V. Antonín 07.310, BRNM 707068; Novohradské hory Mts, Pivnice u Pohorské Vsi, Žofínky prales National Nature Reserve, fallen leaves of *Fagus sylvatica*, 30 Sept. 2008, V. Antonín 08.245, BRNM 712407; Moravský kras, Vilémovice, Vývěry, Punkevny National Nature Reserve, between Suchý and Pustý žleb, decaying leaves of *Fagus sylvatica*, A. Vánger, BRNM 691489; ibid., MacBook Prahr, decaying leaves of *Fagus sylvatica*, V. Antonín, BRNM 712422. — **Slovakia**, Jaworníky Mts, Dolná Mariková, part Kátlina, fallen leaves of *Fagus sylvatica*, V. Antonín 05.196, BRNM 695747.

Notes — *Gymnopus fagiphilus*, known also as *Collybia konradiana* Singer or *Collybia fuscorupurea* sensus Konrad & Maublanc and Kühner & Romagnesi, is characterized by the moderately distant, pinkish brown or pinkish cream coloured lamellae, an apically glabrous, otherwise from a base upwards finely hairy stipe, rather large basidiospores (6.0–7.0 × 4.5–6.0 μm), and only small (16–26 × (3.1–)5.2–6.6 μm), clavate to cylindrical, mostly irregular cheilocystidia. It usually grows on fallen leaves of *Quercus*, less frequently on other broad-leaved tree litter, mostly in thermophilic forests, and is widely distributed especially in Central and Western Europe. For a detailed description, photograph and citations of other literature, see Antonín & Noordeloos (2010).

**Gymnopus hybridus** (Kühner & Romagn.) Antonín & Noordeloos.

Specimens examined. **Czech Republic**, Petrovice nad Orlicí, Obara forest, U Houkvice Nature Reserve, under Aesculus in an oak stand, 26 Sept. 1993, H. Deckor. Antonín, Antonín 93.272, BRNM 576770; Blíže Karpaty Mts, Suchov, Broulov National Nature Monument, 20 Sept. 2006, V. Antonín 06.100, BRNM 704957; Mokrá u Brna, Sivický les, alt. c. 380 m, fallen leaves of *Quercus*, 5 Oct. 2007, D. Dvořák 393/07, BRNU; Bilovice nad Svítavou, Hádecká planinka National Nature Reserve, alt. c. 405 m, broadleaved forest (*Tilia, Quercus, Carpinus*), 7 Oct. 2002, D. Dvořák 138/02, BRNU. — **Hungary**, Börzsöny Mts, Törökmező, under *Quercus* and Acer campestre, 27 Oct. 1994, V. Antonín 94.274, BRNM 599209. — **Italy**, Emilia-Romagna, Borgo val di Taro, Stadielle, on fallen leaves of *Quercus robur* and *Q. cerris*, 19 Oct. 2005, V. Antonín 05.230, BRNM 695773.

Notes — *Gymnopus hybridus* is easily distinguishable by having rather distant, cinnamon-brown lamellae, rather large basidiospores (6.2–7.4 × 9.6 × 3.5–4.8 μm), and only small (18–26 × (3.1–)5.2–6.6 μm), clavate to cylindrical, mostly irregular cheilocystidia. It mostly grows on fallen leaves of *Quercus*, less frequently on other broad-leaved tree litter, mostly in thermophilic forests, and is widely distributed especially in Central and Western Europe. For a detailed description, photograph and citations of other literature, see Antonín & Noordeloos (2010).

**DISCUSSION**

The DNA sequences brought light to morphological characters useful for identification of *Gymnopus* spp. in subsect. *Levipes*. Three taxa of subsect. *Levipes* in the *G. dryophilus* complex (*G. erythropus, G. fagillus, G. hybridus*) are clear and easily to identify. Therefore, the most important features of species belonging to the *G. dryophilus* complex are discussed below.

**Colour of lamellae**

In the *G. dryophilus* complex, the lamellae colour is used as an identification character. According to literature, lamellae are white to cream in *G. alpinus, G. aquosus*, and *G. dryophilus*, whereas yellowish to yellow, rarely whites in *G. ocior* (e.g. Vilgalys & Miller 1987, Vilgalys 1991). Our results show that the lamellae colour agrees with the literature (e.g. Hausknetch & Krisai-Greilhuber 2000, Gröger 2006, Antonín & Noordeloos 2010) Especially for the latter species, lamellae colour represents an important feature.

Our results show that the lamellae colour agrees with the literature (e.g. Hausknetch & Krisai-Greilhuber 2000, Gröger 2006, Antonín & Noordeloos 2010) in *G. alpinus* and *G. aquosus*. On the other hand, the variability is distinctly broader in *G. ocior* and *G. dryophilus*. In *G. ocior*, lamellae may be white to whitish when young and then pale cream coloured (e.g. BRNM 728540 and BRNM 728565) or yellowish when young to pale or light yellow (3A3–4) when old (e.g. BRNM 728539). A surprisingly broad variation was found in *G. dryophilus* — the lamellae are mostly white to pale cream when young to cream (e.g. BRNM 734758), yellowish white, pale or light yellow (3A2–3A4, 4A3; e.g. BRNM 732938, 642393, and 737691). Even a collection with entirely yellow (yellowish white, pale or light yellow) basidiocarps (pileus 3–4A4, lamellae 2A3, stipe 3–4A4), agreeing macroscopically with *G. ocior*, belongs here. As summarized, the yellow coloured lamellae may not unambiguously lead to *G. ocior*. The yellow coloured lamellae are also present in *G. junquilleus* R.H. Petersens & J.L. Mata (Mata et al. 2006), *G. subsulphureus* (Peck) Murrill (Vilgalys & Miller 1987, Vilgalys 1991).

**Basidiospores**

Basidiospores of all taxa of the *G. dryophilus* group are (broadly) ellipsoid, oblong, pip-shaped or ellipsoid-fusoid. Data on basidiospore measurements are summarized in Table 3. The studied specimen of *G. alpinus* showed slightly smaller basidiospores (6.0–7.5 × 3.0–4.0 μm) than mentioned in the literature (6.2–6.5 × 3.0–4.4 μm; Antonín & Noordeloos 2010). This size is in the lower limit of their variability. For studied specimens of *G. aquosus* the basidiospores size (15.0–)5.7–7.0 × 3.0–4.0(–4.25) μm) also generally agrees
The differences between single specimens are, however, rather great, and vary between (5.0–)5.5–6.0 × (2.5–)3.5–4.0 μm (Antonín & Noordeloos 2010). The small basidiospores were also found in the isoneotype specimen of G. dryophilus. The basidiospore variability in G. ocior (5.0–)5.5–6.5 × (2.5–)3.75–4.0 μm (Antonín & Noordeloos 2010).

**Cheilocystidia**

The shape of cheilocystidia is one of the most important identification characters. The first detailed studies of cheilocystidia in the recent literature were made by Vilgalys & Miller (1987). They distinguished inflated-clavate to subglobose or sphaero-pendunculate, frequently diverticulate (G. ocior), inconspicuous, diverticulate-filamentous (G. alpinus), inflated-clavate to sphaero-pendunculate, somewhat echinulate or lobate-diverticulate (G. aquosus), and sometimes inconspicuous, filamentous cheilocystidia with numerous diverticulate branches (G. dryophilus). However, the variation is even broader.

Cheilocystidia of G. alpinus of the studied specimen (Fig. 3) agree with those drawn by Vilgalys & Miller (1987) and Antonín & Noordeloos (2010). They are clavate, simple, irregular to coralloid. Gymnopus aquosus has clavate, capitate and pedunculate, less frequently subcylindrical or fusoid, simple or coralloid cheilocystidia (Fig. 4); some of them may even be similar to those of G. dryophilus. Clavate to sphaero-pendunculate cheilocystidia drawn by Vilgalys & Miller (1987) represent only the minority of ones found.

The typical shapes of G. dryophilus are (sub)cylindrical, narrowly clavate, mostly coralloid (Fig. 5). Their form agrees with figures by Vilgalys & Miller (1987) and Antonín &
Noordeloos (2010). However, we also usually find clavate, simple or slightly irregular cheilocystidia in most of the basidiocarps. The basic shapes of *G. ocior* cheilocystidia (Fig. 6) are clavate, subcylindrical, or subutriform, but, most of them are lobate, branched, with (apical) projections or coralloid. The form of cheilocystidia drawn by Vilgalys & Miller (1987) represents only a part on their variability. Antonín & Noordeloos (2010) mentioned that forms of *G. ocior* with yellow lamellae have more distinctly coralloid cheilocystidia. Our studies showed that these yellow forms actually belong to *G. dryophilus*, and the cheilocystidia fully support that placement. Nevertheless, we can also rarely find cheilocystidia of the *G. dryophilus* type in typical basidiocarps of *G. ocior*.

**Pileipellis**

The pileipellis is a Dryophila-structure in all discussed taxa. In *G. alpinus* it is composed of cylindrical to inflated, often irregularly coralloid terminal elements with lateral and terminal projections (a poorly developed Dryophila-structure as defined by Antonín & Noordeloos 2010). However, this type of pileipellis structure is useless for identification because of the variability. A poorly to well-developed Dryophila-structure is dependent on the age and development of basidiocarps, and also on the location on the pileus (centre, margin) where the structure is observed.
KEY TO THE EUROPEAN SPECIES OF GYMNOPSIS SUBSECTION LEVIPEDES

1. Stipe smooth except for basal rhizoids .......................... 2
2. Stipe entire pubescent, or glabrous only at apex and dis-
tinctly finely hairy from a base upwards (up to 2/3 of length) 7
3. Pileus not translucently striate or at margin only, uniformly
pale to dark (reddish, pinkish) brown .......................... 4
4. Spores 6.0–7.5 × (9.0–)3.5–4.5(–5.0) μm .......................... 5
5. Lamellae rather distant, cinnamon-brown; basidiospores
4. Spores (5.0–)5.5–6.5(–7.0) ............................................. 5
6. Lamellae white, yellowish to yellow ... G. ochraceus yellow, orange-brown, sometimes with darker centre
7. Lamellae pinkish brown or pinkish cream; stipe apically gla-
aeous yellow, orange-brown, sometimes with darker centre
8. Stipe never red-brown, never with red-brown basal hairs 3
9. Pileus distinctly translucently striate, (pale) yellow, ochra-
3. lamellae pale cream-coloured; smell sometimes unpleasantly foetid; basidio-
cystidia clavate, capitate and pedunculate, less frequently
3. spores (5.0–)5.5–6.0–12 μm, clavate, subcylindrical, or
8. subcylindrical, lobate, branched, coralloid or with (apical)
3. projections; lamellae white, yellowish to yellow ... G. ochr
8. ocr
9. Spores 6.0–7.5 × 3.0–4.0 μm; cheilocystidia 14–32
3. × 7.0–12 μm, clavate, simple, irregular to coralloid; lamellae
9. white ............................................. 5
5. Lamellae close, white, pale cream-coloured to yellow; basi-
diospores up to 7.0 × 4.25 μm; cheilocystidia distinct ... 6
6. Basidiospores (5.0–)5.5–7.0 × 3.0–4.0(–4.25) μm; cheilo-
cystidia clavate, capitate and pedunculate, less frequently
6. subcylindrical or fusoid, simple or coralloid; pileus pale yel-
5. low, usually without ochre or brown tinges, almost to centre
6. translucently striate, stipe often with distinctly inflated basal
5. part .................................................. 7
7. Spores 5.0–7.0(–8.0) × (2.5–)3.0–4.0(–4.5) μm; cheilocystidia
7. (sub)cylindrical, narrowly clavate, mostly coralloid; pileus
7. with ochre-brown tinges, especially at centre, translucently
7. striate up to half the radius; stipe more or less equal ....  7
8. G. dryophilus var. dryophilus
7. Lamellae whitish; stipe pubescent; basidiospores (5.0–)5.5–
7. × 2.75–3.5 μm; Mediterranean thermophilic forests, espe-
7. cially of Quercus ilex, Pinus halepensis, or Cistus. .......... 6
8. G. dryophilus var. lanipes
8. Lamellae pinkish brown or pinkish cream; stipe apically gla-
8. bourgeois, otherwise from a base upwards finely hairy; basidi-
8. ospores (6.0–)7.0–9.0 × (3.0–)3.5–4.5 μm; connected with
8. Fagus sylvatica ............................................. 9
9. G. fagiphilus

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