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The genus *Volvariella* in Spain: *V. dunensis* comb. & stat. nov. and observations on *V. earlei*

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Abstract — *Volvariella nigrovolvacea* var. *dunensis* is raised to the species rank, and its delimitation from similar taxa is discussed. *Volvariella earlei* is recorded for the second time in Europe, and its taxonomy, nomenclature, and distribution are briefly discussed. A key to the Iberian species of *Volvariella* is provided.

Key words — *Agaricales*, *Agaricomycetes*, biodiversity, *Pluteaceae*

Introduction

In recent years the genus *Volvariella* Speg. (*Agaricales*, *Basidiomycota*) has been the subject of several regional (Justo & Castro 2004, Justo et al. 2005) or taxonomic (Vila et al. 1999) studies in the Iberian Peninsula (Spain, Portugal). After revising the collections deposited in several Iberian herbaria and identifying newly collected material, we have published an annotated checklist of the genus in our area (Justo & Castro 2010). According to our study 12 taxa of *Volvariella* occur in the Iberian Peninsula and Balearic Islands.

This paper presents additional taxonomic notes and observations resulting from our work on *Volvariella*. We raise *Volvariella nigrovolvacea* var. *dunensis* to species rank, based on morphological and ecological differences from related species, *V. nigrovolvacea* Kosina and *V. volvacea* (Bull.) Singer. *Volvariella earlei* is mentioned for the first time in our area and for the second time in Europe. Both species are fully described and their taxonomy and distribution are briefly discussed.

A key to all members of the genus *Volvariella* in the Iberian Peninsula and Balearic Islands is provided.

Material and methods

Standard methods for describing the basidiocarps were applied, using the terminology of Vellinga (1988) and Boekhout (1990). Color annotations for the macroscopic descriptions are from Munsell Color Company (2000). The notation [60, 2, 2] indicates that measurements were made on 60 basidiospores in 2 samples from 2 collections. At least 10 measurements per collection were performed for other microscopic features such as basidia (excluding sterigmata), cystidia, and pileipellis elements. Microscopical preparations were mounted in Congo Red, then the excess dye was removed and 5% KOH was added. The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient. Extreme measurements are indicated within parentheses. Herbarium acronyms follow Holmgren & Holmgren (1998) except "SCAT", which is used for the "Societat Catalana de Micologia" herbarium.

Taxonomy

1. ***Volvariella dunensis*** (Vila, Àngel & Llimona) Justo & M.L. Castro, **comb. & stat. nov.** Fig. 1

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Basionym: *Volvariella nigrovolvacea* var. *dunensis* Vila, Àngel & Llimona, Rev. Catalana Micol. 22: 131. 1999.

Pileus 35–100 mm, subglobose or campanulate when young, later plano-convex, without umbo; surface radially fibrillose, especially towards margin, sometimes radially fissurate; gray or bluish gray [approx. Mu. GLEY 2 4/1 "bluish gray", 5/1 "dark bluish grey"], with some brown or grayish-brown tint in older specimens; margin entire, not striate. Lamellae crowded, free, (broadly) ventricose; up to 10 mm broad; white when young, later pink, with white flocculose edges. Stipe 25–50 × 9–14 mm, cylindrical or narrowly clavate, with slightly broadened base (up to 20 mm); white; pubescent in young specimens, then glabrous. Volva saccate, membranous, irregularly lobed fragile; white, sometimes leaving small patches on pileus. Context in pileus white, with dark grey tints under pileipellis especially in older specimens; in stipe white. Smell fungoid. Taste not recorded. Spore print not recorded.

Basidiospores [60, 2, 2] 7–8.5 × 4.5–6 µm, avl × avw = 7.7–7.9 × 5.1–5.2 µm, Q = 1.3–1.7(–1.8), avQ = 1.5–1.55, ellipsoid to oblong. Basidia 20–35 × 7–15 µm, 4-spored, broadly clavate. Pleurocystidia (34–)50–95(–108) × (16–)20–45(–50) µm, clavate, (narrowly) utriform, obovoid; colorless; with thin, smooth walls; fairly abundant. Cheilocystidia 20–80 × 15–60 µm, clavate or utriform, without apical appendages, colorless; with thin, smooth walls; abundant and relatively crowded. Pileipellis a cutis made up of cylindrical elements (20–)

50–275 × 10–35(–50) µm, colorless or with brown intracellular pigment; with thin, smooth walls. Stipitipellis a cutis; hyphae 5–20 µm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. Caulocystidia 20–75 × 10–25 µm, clavate, utriform, lageniform, flexuous, sometimes with elongated or subcapitate apex, without internal septa, colorless or with brown pigment; with thin, smooth walls. Clamp connections absent in all tissues.

Ecology and distribution — In open dunes with most of the basidiocarp growing deeply buried in the sand. Known from two localities on the Mediterranean coast of Spain (Barcelona: Viladecans, Prat de Llobregat). January–February.

Collections examined—**Spain: Barcelona:** Viladecans (Baix Llobregat), in open dunes, 5.II.1998, J. Vila & F. Àngel, SCAT 3512 (Holotype); El Prat de Llobregat, El Pinar, in open dunes, 8.II.1997, F. Àngel, SCAT 3513.

Comments—*Volvariella dunensis* was first described as a variety of *V. nigrovolvacea*. However examination of the Spanish collections revealed important morphological and ecological differences that separate this taxon from *V. nigrovolvacea* as well as from the morphologically similar *V. volvacea*.

Volvariella nigrovolvacea is an obscure and little-known species originally described from grassy fields in the Czech Republic (Kosina 1974). Its main characteristics are the relatively large basidiomes (pileus 100–150 mm), fibrillose pileus, glabrous stipe, and a well-developed, saccate, grey-brown volva. As already noted by Boekhout (1990), the only difference from *V. volvacea* seems to be the glabrous stipe.

The original microscopical description of *V. nigrovolvacea* could be more complete, as Kosina (1974) provided data only for the spores ("7–8.5 × 4.5–5.5 µm") and cheilocystidia ("ampulliform, fusiform, colorless, 47–70 × 13–18 µm, rare"). The type collection is lost (Dr. Jan Holec, pers. com.), preventing further microscopic study. It seems likely that *V. nigrovolvacea* is a synonym of *V. volvacea*, but this should be confirmed by new collections of *V. nigrovolvacea* from the type locality.

Contu & La Rocca (1999) described a collection identified as *V. nigrovolvacea* from Sardinia from dunes under *Juniperus*. These authors described a taxon with relatively small basidiocarps (pileus 15–50 mm), which contrast with the larger basidiocarps (pileus 100–150 mm) mentioned in the original description of *V. nigrovolvacea* (Kosina 1974).

In the Sardinian collections the pleurocystidia are described as fusiform or utriform and the cheilocystidia as fusiform, sometimes mucronate. Because of the incomplete microscopical description of *V. nigrovolvacea*, and the differences in macroscopical and ecological characters it is uncertain whether the taxon described by Contu & La Rocca (1999) is really the same as the one described by Kosina (1974).

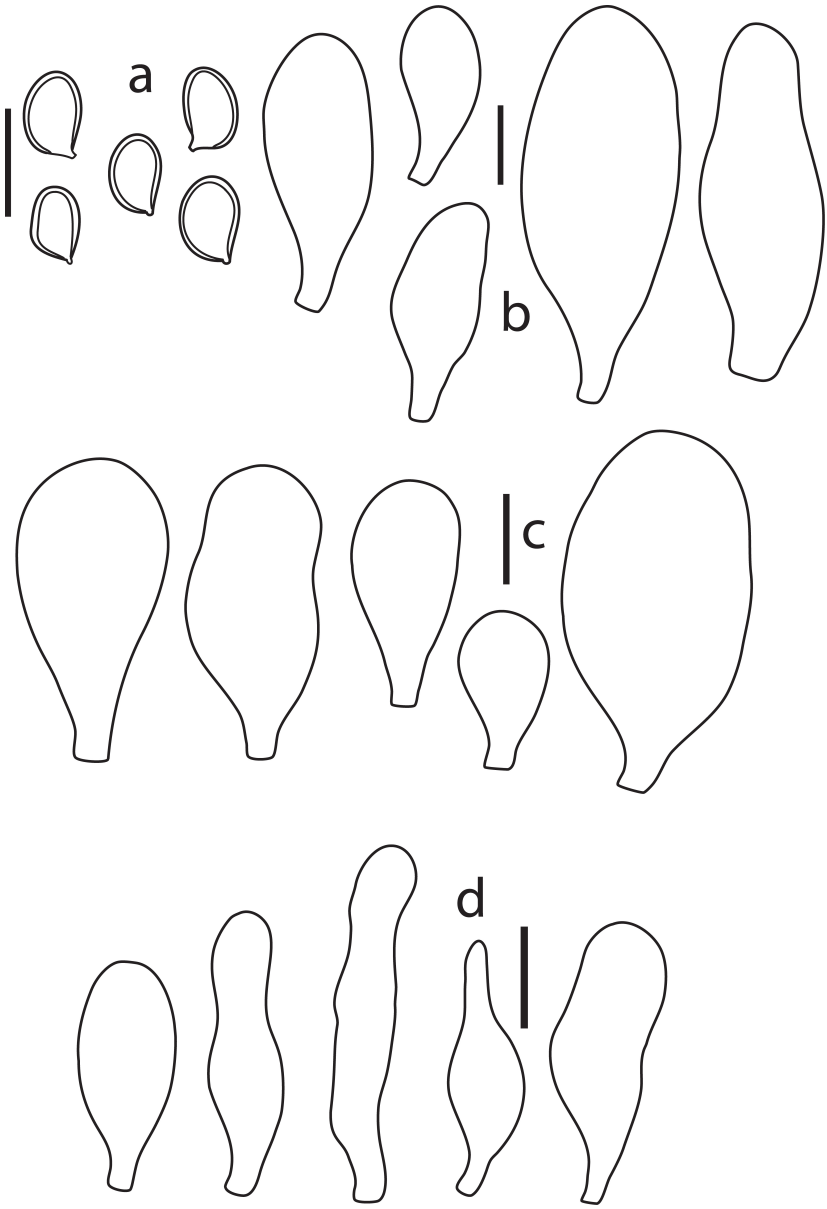

Fig 1. *Volvariella dunensis*.
a = spores; b = pleurocystidia; c = cheilocystidia; d = caulocystidia.
All from SCAT 3512 (Holotype). Scale bars a = 10 µm; b, c, d = 20 µm.

Although *V. dunensis* was first described as a variety of *V. nigrovolvacea,* these taxa differ in ecology and morphology of the volva and cheilocystidia. Moreover, the doubtful and uncertain status of *V. nigrovolvacea* contributed to our separating it from the well described and delimited *V. dunensis*.

Volvariella volvacea and *V. dunensis* resemble each other macroscopically, but *V. volvacea* has a well-developed grey-brown volva and fruits on organic-rich substrates (leaves, compost, sawdust) usually during summer and spring, at least in Europe (Boekhout 1990, Justo & Castro 2010). On the other hand, *V. dunensis* has a whitish, rather fragile volva and fruits on open dunes and is not directly associated with accumulations of organic matter during winter.

Cystidial shapes also differ in the two species. *Volvariella volvacea* has fusiform, lageniform, clavate or utriform pleurocystidia and cheilocystidia, usually with elongated apices, mucronate or with an apical flexuous appendage (data from the Spanish collections; Justo & Castro 2010). In *V. dunensis,* pleurocystidia and cheilocystidia are predominantly clavate, obovoid, or (narrowly) utriform, without elongated apices or appendages. The caulocystidia in *V. volvacea* are cylindrical to clavate, usually with 1–2 internal septa and measure 40–190 × 5–15 µm (data from the Spanish collections; Justo & Castro 2010), while in *V. dunensis* caulocystidia are predominantly clavate or utriform, have no internal septa, and measure 20–75 × 10–25 µm.

Vila et al. (1999), who compare *V. dunensis* with species of similar habitat, note that *V. arenaria* (Pat.) Singer, described from the Arabian Desert, has smaller basidiocarps (pileus ≤ 30 mm) and larger basidiospores (12–15 × 8–10 µm) while *V. psammophila* Singer, described from Argentina, has smaller basidiocarps (pileus ≤ 45 mm), smaller basidiospores (6.2–7.3 × 4.5–5.5 µm), and much narrower pleuro- and cheilocystidia (≤ 17 µm).

2. *Volvariella earlei* (Murrill) Shaffer, Mycologia 49: 550. 1957 FIG. 2

≡ *Volvariopsis earlei* Murrill, Mycologia 3: 282. 1911.

≡ *Volvaria earlei* (Murrill) Murrill, Mycologia 4: 332. 1912.

PILEUS 25–45 mm; hemispherical or conical when young, later plano-convex, slightly depressed at center in old specimens; surface glabrous or innately fibrillose, viscid at least in young specimens; white or ochraceous at center [Mu. 10YR 8/2–8/4]; margin translucently striate. LAMELLAE crowded, free, (broadly) ventricose, up to 6 mm broad; white when young, later pink, with white flocculose even edges. STIPE 30–50 × 2–6 mm, cylindrical, with slightly broadened base (up to 10 mm); white with some ochraceous tints [Mu. 10YR 8/2–8/3]; glabrous or pruinose. VOLVA saccate, membranous, 2–4 lobed, glabrous, white, up to 20 mm high. CONTEXT white or with some yellowish tints. SMELL not recorded. TASTE not recorded. SPORE PRINT not recorded.

BASIDIOSPORES [90, 6, 3] 11–16 × (7.5–)8–11 µm, avl × avw = 13.4–14.6 ×

9.1–9.7 Mm, Q = (1.25–)1.3–1.6(–1.7), avQ = 1.45–1.55 ellipsoid, more rarely broadly ellipsoid or oblong. Basidia 20–40 × 8–16 µm, 4-spored or 2-spored, rarely 1-spored, broadly clavate. Pleurocystidia absent. Cheilocystidia 30–70 × 10–35 µm, clavate, fusiform, lageniform or conical, usually each cheilocystidium with a flexuous apical appendage up to 40 µm long; with thin, smooth walls; abundant, crowded. Pileipellis an ixocutis made up of cylindrical hyphae, 5–15 µm wide, colorless; with thin, smooth walls; embedded in a gelatinous, colorless, matrix. Stipitipellis a cutis; hyphae 5–15 µm wide, cylindrical, colorless; with thin, smooth walls. Caulocystidia (not always present) 65–140 × 10–25 µm, cylindrical, hyaline or with brown pigment, sometimes with 1(–2) internal septa; with thin, smooth walls; scattered. Clamp connections absent in all tissues.

Ecology and distribution—In gardens, on soil. In Spain known from one locality (Madrid: Móstoles). June–July.

Collections examined—**Spain: Madrid:** Móstoles, Coimbra Park, in garden, 7.VI.1986, F.D. Calonge, MA-Fungi 16324; idem, 7.VII.1987, MA-Fungi 19490; idem, 20.VII.1989, MA-Fungi 22816.

Comments—*Volvariella earlei* is closely related to *V. gloiocephala* (DC.) Boekhout & Enderle, as both species have basidiospores over 12 µm long and a pileipellis as an ixocutis. However the two differ in several macro- and microscopical characters. *Volvariella gloiocephala* has medium-sized to relatively large basidiomes (pileus 50–150 mm), and has larger basidiospores (generally 13.0–16.5 × 8.0–9.3 µm, avQ = (1.5–)1.6–1.85), common and more or less clavate to fusiform pleurocystidia, and cheilocystidia that are sometimes apically papillate but not commonly rostrate (data from the Spanish collections; Justo & Castro 2010). *Volvariella earlei* produces smaller basidiomes (pileus 25–45 mm) with broader basidiospores, pleurocystidia that are absent (in the Spanish collections) or very rare (in North American collections, Shaffer 1957), and cheilocystidia that are usually rostrate.

Volvariella earlei, which was originally described from Cuba (Murrill 1911), has been reported thus far from the U.S.A (Coker 1947), Mexico (Vázquez et al. 1989), Africa (Heinemann 1975), and Sardinia (Contu 2006). The Spanish and the Italian collections were collected during late spring and summer (June–August) in artificially irrigated gardens, which suggests that *V. earlei* is a tropical species alien to Europe, but more research is needed to establish this with certainty.

The collections of *V. earlei* at MA herbarium were deposited under the name *Volvariella media* (Schumach.) Singer, but as Kosonen (1993) and Contu (2006) noted, the application of *Agaricus medius* Schumach., the basionym, is difficult to establish. In the original description, Schumacher (1803) described a small, whitish species that grows in coniferous forests (*Abies, Pinus*) during

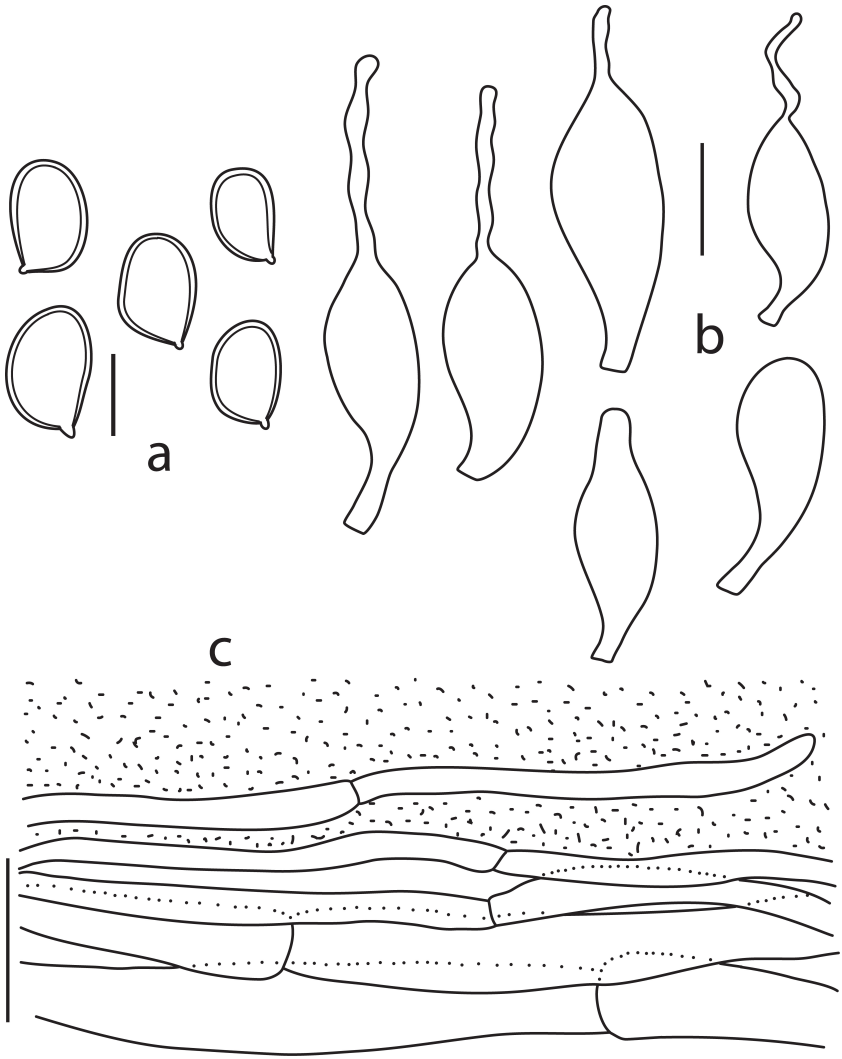

Fig 2. *Volvariella earlei.*
a = spores; b = cheilocystidia; c = pileipellis.
All from MA-Fungi 16324. Scale bars a = 10 μm; b, c = 20 μm.

the autumn (November) but provided no data on microscopic characters. Later authors have interpreted *Agaricus medius* in different ways: Bresadola (1929) described *Volvaria media* (Schumach.) Gillet, a nomenclatural synonym, as a small species with a gray, subtomentose volva and basidiospores of 7–9 ×

4–5 µm. Lange (1935) described a fungus that may correspond to *V. earlei* as described here based on its small basidiocarps, similar basidiospore size, and habitat on grassy fields, although some authors have argued that the species described by him is in fact just a small variant of *V. gloiocephala* (Contu 2006). Pilát (1959), who compared Bresadola's and Lange's different interpretations, proposed the name *Volvariella krizii* Pilát for the fungus described by Bresadola. Finally, Orton (1986) accounted for some British records that may represent *V. media* in the sense of Lange. In the Iberian bibliography there are three records under the names *Volvaria media* or *Volvariella media* (Torrend 1912, Rezende-Pinto 1943, Llimona et al. 1995). However none of them is provided with descriptions and/or cited herbarium collections. As we concur with Kosonen (1993) and Contu (2006) in considering *Agaricus medius* a doubtful name, we report the examined material as *V. earlei*.

Key to the species of *Volvariella* present in the Iberian Peninsula and Balearic Islands

1. Pileus viscid. Basidiospores > 12 µm long 2
1. Pileus not or only slightly viscid. Basidiospores < 12 µm long 3
2. Pileus 50–150 mm diam. Basidiospores with avQ = 1.6–1.85. Pleurocystidia common. Cheilocystidia rarely rostrate ..... ***V. gloiocephala***
2. Pileus 25–45 mm diam. Basidiospores with avQ = 1.45–1.55. Pleurocystidia absent or scarce. Cheilocystidia commonly rostrate .... ***V. earlei***
3. Growing on wood 4
3. Not on wood 6
4. Pileus 30–100 mm diam., without distinct squamules. Pileipellis elements septate, up to 145(–200) µm long ..... ***V. caesiotincta***
4. Pileus 50–200 mm diam., covered with distinct fibrillose squamules. Pileipellis elements rarely septate, up to 1000–1600(–2000) µm long 5
5. Pileus white, sometimes slightly yellowish in old specimens ..... ***V. bombycina* var. *bombycina***
5. Pileus yellow from the beginning ..... ***V. bombycina* var. *flaviceps***
6. Growing on basidiocarps of *Clitocybe nebularis* ..... ***V. surrecta***
6. Habitat different 7
7. Basidiospores with avw = 3.5–4 µm. avQ = 1.7–1.8 ..... ***V. murinella***
7. Basidiospores with avw = 4.4–5.3 µm. avQ = 1.3–1.55 8
8. Pileus covered with radial grey or grey-brown fibrils (at least in the center) 9
8. Pileus without radial grey or grey-brown fibrils 11
9. Pleurocystidia and cheilocystidia clavate, obovoid or (narrowly) utriform, without elongated apices. Caulocystidia clavate, utriform, lageniform, flexuous, without internal septa. Volva white, fragile. In open dunes ..... ***V. dunensis***

9. Pleurocystidia and cheilocystidia fusiform, lageniform, clavate or utriform, commonly with elongated apices and/or apical appendages. Caulocystidia cylindrical, with internal septa. Volva grey-brown, not fragile. In grasslands in or outside forests or in places with abundant organic matter 10

10. Pileus 30–50 mm diam. Volva glabrous, not covering more than the lower 1/3 of the stipe . . . . . ***V. taylorii***

10. Pileus 50–100 mm. Volva pubescent, usually covering more than the lower 1/3 of the stipe . . . . . ***V. volvacea***

11. Pileus up to 60 mm diam. Stipe pubescent even in old specimens ***V. hypopithys***

11. Pileus up to 30 mm diam. Stipe glabrous in old specimens . . . . . ***V. pusilla***

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