

The genus *Testicularia* (Ustilaginomycetes)

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Received 1 October 2006 / Accepted 18 October 2006

Abstract. The genus *Testicularia* is studied. In addition to the two known species, *Testicularia cyperi* and *Testicularia minor*, a new species, *Testicularia africana* is described on *Rhynchospora corymbosa* from Guinea.

Key words: new species, smut fungi, *Testicularia*, *Testicularia africana*, Ustilaginomycetes

Introduction

Klotzsch (1832: 202) described the genus *Testicularia*, with its type *T. cyperi* Klotzsch, on *Rhynchospora* sp., from North America. The genus is characterised by large, sac-like swellings in some spikelets of the host plants in the Cyperaceae (*Rhynchospora*), covered by a thick, whitish, fungal peridium, filled with a black, agglutinated or granular mass of spore balls, intermixed with long, radially arranged fascicles of sterile fungal cells. The spore balls are composed of a peripheral layer of dark, thick-walled spores and a central mass of thin-walled, light-coloured, sterile, fungal cells. Spore germination is of *Ustilago*-type. Host-parasite interaction is by intracellular hyphae, coated by an electron-opaque matrix. Mature septa are poreless (comp. Vánky 2002: 146–147).

Juel (1897: 9) described *Testicularia cyperi* var. *minor* Juel, type on *Rhynchospora* sp. (tribe *Psilocaria*), from Brazil, characterised by smaller sori and spore balls. Ling (1951: 109) rose Juel's variety to species level as *Testicularia minor* (Juel) L. Ling.

Scrutinising the phanerogamic herbarium of the W. Szafer Institute of Botany in Kraków (KRAM), the junior author found some smutted samples of *Rhynchospora corymbosa* collected in Guinea. The fungus turned out to represent a still unknown species of *Testicularia*, which is described below.

Materials and Methods

Spore ball, spore and sterile cell characteristics were studied using dried herbarium specimens. For light microscopy (LM), spore balls were rehydrated in a droplet of pure lactophenol or in lactophenol with cotton blue, on a microscope slide, by gently heating to boiling point. Some of the spore balls were squashed with a lancet prior to examination. For study of the sorus structure and spore ball development, young, rehydrated sori in hot water were fixed with 2 % glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.2 for several days. After six transfers in 0.1 M Na-cacodylate buffer, the material was postfixed in 1 % osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1 % aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, the material was dehydrated in an acetone series, embedded in Spurr's plastic and sectioned with a diamond knife. Semi-thin sections were stained with new fuchsin and crystal violet, mounted in 'Entellan' low viscosity resin and studied in a light microscope. For scanning electron microscopy (SEM), spore balls were placed on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with gold-palladium, c. 20 nm, and examined in a SEM at 10 kV.

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Results and Discussion

Study of the genus *Testicularia* resulted in the recognition of three species of which one is a new species. The recognised species are:

1. *Testicularia cyperi* Klotzsch, Linnaea 7: 202, 1832.

Type on *Rhynchospora* sp., N America (no closer data), herb. Hooker, K (n.v.); isotype BPI 711 939 (n.v.).

Millieria herbatica Peck, Annual Rep. New York State Mus. 31: 40, 1879. — Type on *Rhynchospora macrostachya* Torr., USA, New York, Wading River, no date, leg. E.S. Miller; isotypes in Ellis, N. Amer. fgi. no. 805, HUV 2039!

Sori (Fig. 1) comprise some spikelets of an inflorescence, forming conspicuous, subglobose, ellipsoidal or ovoid swellings, 5–20 mm long, covered by a thick, whitish peridium composed of hyaline, firmly united fungal cells, variable in shape and size, subglobose, ellipsoidal, elongated or irregular, 7–16 × 8–25 µm; wall variable in thickness, 1.5–6.5 µm wide, often with gelatinised external layer. The surface of the peridium is granular. At maturity, the peridium ruptures irregularly at the apex, becoming sac-like, and the black, agglutinated or granular-powdery mass of spore balls is exposed. The sori comprise all flowers of a spikelet, leaving intact the basal glumes and rarely also the tip of the distal floral envelopes. In some sori there is a well-developed, irregular columella, the axis of the spikelet. In young sori, between the spore balls, more or less radially arranged sterile hyphae can be seen. **Spore balls** (Figs 4–5) subglobose, ovoid to irregular, (60–) 70–200 (–260) × 75–320 (–350) µm, dark chestnut-brown, opaque, composed of an external, often incomplete layer of spores and an internal mass of sterile, parenchymatous cells. **Spores** (Figs 4–5) tangentially slightly flattened, in side view elliptic or slightly irregular, 9–13.5 µm wide, in plane view circular or subcircular, 13–16 (–17) µm in diam, reddish- to chestnut-brown; wall uneven, 1–3 µm, thinner on the flattened, outer surface, smooth, in SEM finely, densely granular-verruculose. **Spore germination** (Fig. 3) results in phragmobasidia measuring 3–4 × 30–45 µm, composed of 3–6 cells. On the basidia, on sterigmata, laterally and terminally, narrowly ellipsoidal to subfusiform basidiospores are produced measuring (1.5–) 2–3 × 8–16 µm. Basidiospores one- or two-celled, germinating by similar but smaller secondary and tertiary spores often on sterigmata, and also producing yeast colonies in nutrient media. **Sterile cells** (Figs 4–5) irregularly polyhedral, 5–12 × 6–13.5 µm, thin-walled (c. 0.3 µm), yellowish to brownish tinted, smooth.

On Cyperaceae: *Rhynchospora careyana* Fernald, *R. corniculata* (Lam.) A. Gray (*R. laxa* Vahl), *R. macrostachya* Torr. ex A. Gray; North America (USA).

For host plant range and geographic distribution see Zundel (1953: 116), and Vánky (1998: 11). Report of *T. cyperi* by Zambettakis (1970: 680) on *Rhynchospora corymbosa* from W Africa (Sierra Leone) most probably refers to the new species *T. africana*.

Sorus structure and spore ball building in *Testicularia* (*T. minor*) was described and beautifully illustrated by Juel (1897: 10–11, Plate II, Figs 6–10). According also to our observations (Fig. 2), on the surface of the host tissue (spikelet axis), which is permeated by parasitic fungal mycelia, a hyaline mass of sporogenous hyphae can be seen. In this mass, dispersed small spore ball initials appear close to the surface of the host tissue. These increase in size, are pushed towards the periphery, and differentiate into spore initials on the surface of a central mass, of which the sterile cells develop. During maturation, the spore initials increase in size, become pigmented and more or less thick-walled, depending also on the species. On the surface of the sori (not shown on Fig. 2), in a very early stage of soral development, a thick fungal layer, the peridium is formed. Its cells are firmly united by the partially gelatinised outer layer of the wall. In young sori, thin, sinuous fascicles of long, sterile, fungal cells can be seen between the spore ball initials and young spore balls, arranged more or less radially, extending from the host plant tissue to the peridium. In mature sori these cells are slightly irregular, 2.5–4 µm wide, with a 0.5–0.8 µm thick, smooth wall, without transversal walls. Their remnants are present between and on the surface of the mature spore balls (Figs 5, 9).

2. *Testicularia minor* (Juel) L. Ling, Lloydia 14: 109, 1951.

Testicularia cyperi Klotzsch var. *minor* Juel, Bih. Kongl. Svenska Vetensk.-Akad. Handl. 23: 9, 1897. — Type on *Rhynchospora* sp., Brazil, Rio Grande do Sul, Pôrto Alegre, c. 30°03' S, 51°10' W, 6 Oct 1892, leg. G.O.A. Malmé, S!

Sori in some spikelets of an inflorescence, similar to those of *T. cyperi* but smaller, measuring 2–4 × 3–6 mm, covered by a scurfy-verruculose peridium. **Spore balls** (Figs 6–7) globose, subglobose or ellipsoidal, 50–90 × 50–110 µm, composed of an external layer of spores which separate easily, and a central mass of sterile fungal cells. **Spores** (Figs 6–7) globose, subglobose, ovoid, ellipsoidal, not or only slightly flattened, 10.5–20 × 11–21.5 (–25) µm, yellowish brown; wall even, 0.5–0.8 µm thick, smooth, in SEM finely granular. **Sterile cells** subglobose, ellipsoidal or slightly irregular, collapsed in old specimens, 8–25 µm long, subhyaline to pale yellow; wall even, 1–1.5 µm thick, smooth.

On Cyperaceae: *Rhynchospora corymbosa* (L.) Britton, *R. gigantea* Link, *Rhynchospora* sp.; Antilles (Cuba, Puerto Rico), S. America (Bolivia, Brazil, Colombia). For host plant range and geographic distribution see also Piepenbring (2003: 138).

Testicularia minor differs from *T. cyperi* by smaller sori and spore balls, by larger spores and larger, rounded sterile cells with thicker wall.

3. *Testicularia africana* Vánky & M. Piątek, sp. nov.

Typus in matrice *Rhynchospora corymbosa* (L.) Britton, *W. Africa, Guinea, Reg. Macenta, ad oram lacus parvi prope urbem Macenta*, 28.XI.1962, leg. S. Lisowski. **Holotypus** in KRAM F-55901!, **isotypus** in Herbario Ustil. Vánky, H.U.V. 21 265!

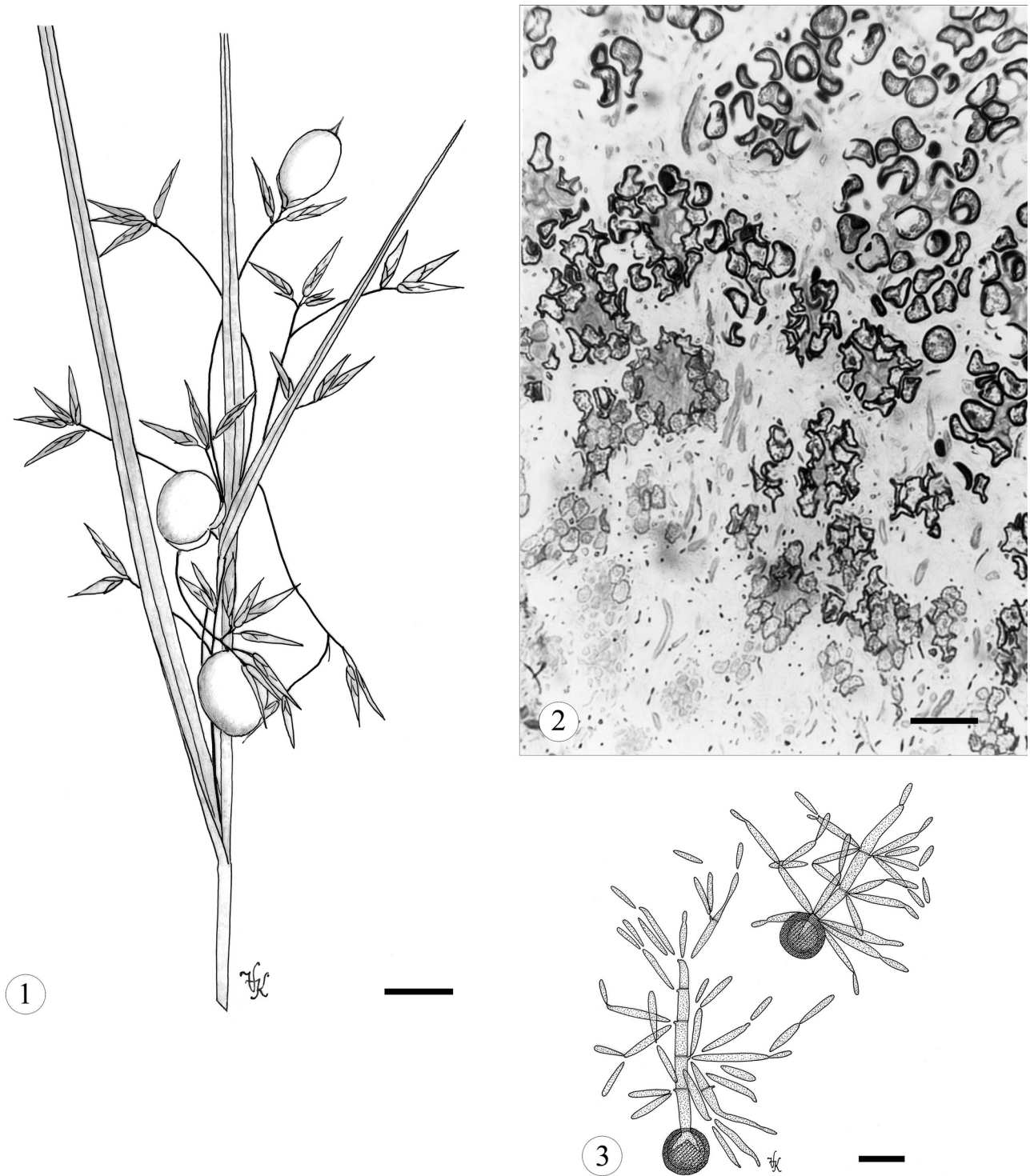
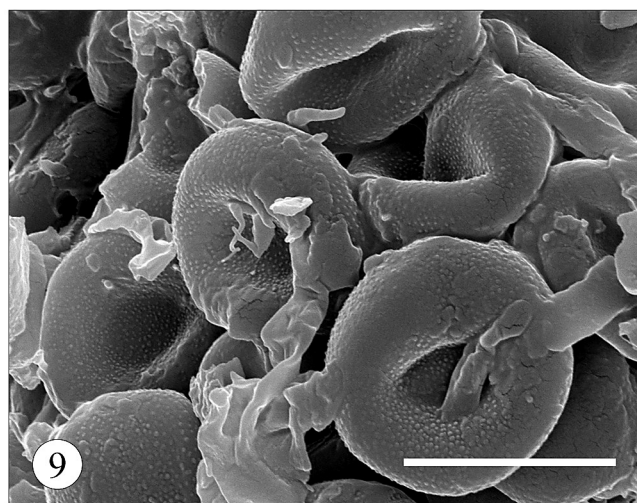
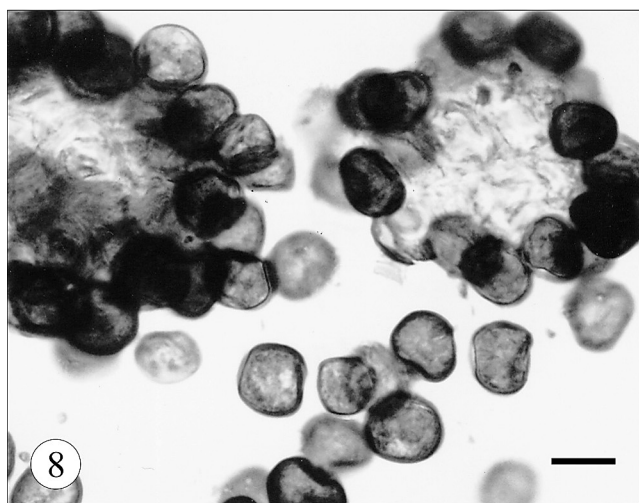
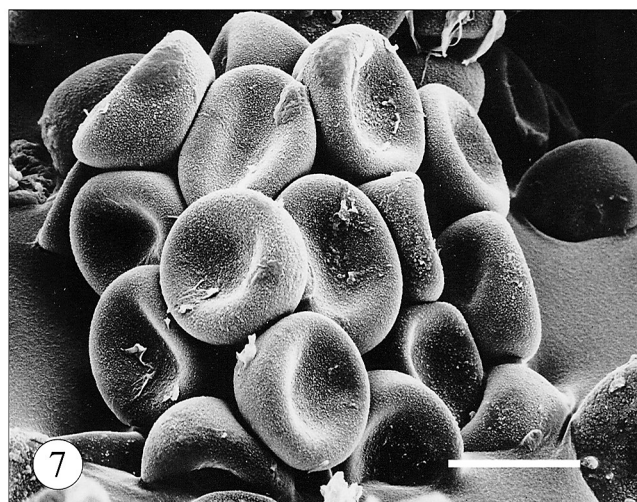
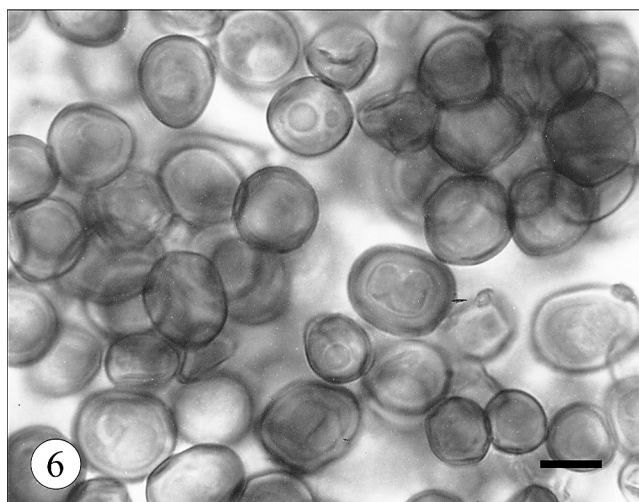
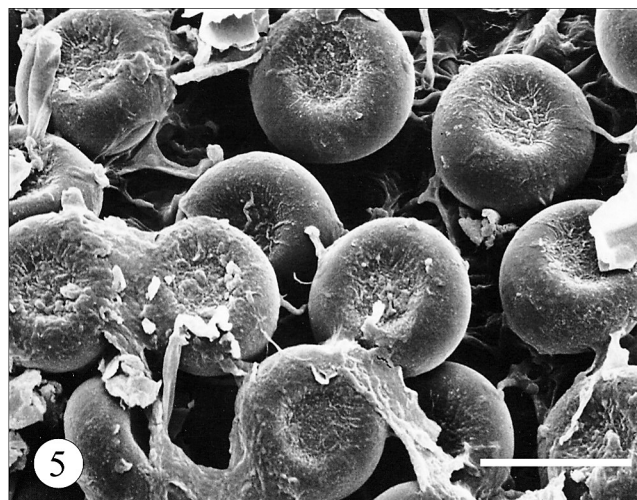
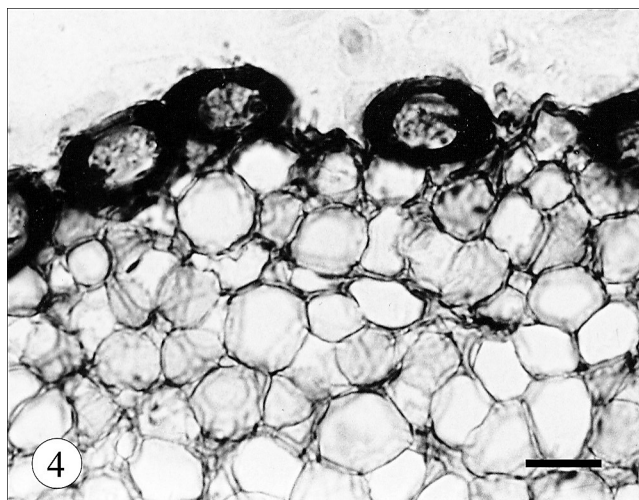


Fig. 1. Sori of *Testicularia cyperi* in some spikelets of *Rhynchospora macrostachya* (from isotype of *Milleria herbatica*, H.U.V. 2039). Habit. One sorus with remnants of floral envelopes as an acute tip. Bar = 1 cm. **Fig. 2.** Semi-thin, stained section through a young sorus of *Testicularia minor* (type), showing spore balls, spores, and sterile cells in different developmental stages. Very young stages in the lower left corner, more developed, but still immature spore balls towards the top right corner (for details see text). Bar = 25 μ m. **Fig. 3.** Germinating spores of *Testicularia cyperi* from *Rhynchospora careyana* (on MYP, at room temp., in 3 days), with phragmobasidia and narrowly ellipsoidal basidiospores. These give rise to secondary spores, often on sterigmata, and are also producing yeast cells (for details see text). Bar = 10 μ m



Figs 4-5. Spore balls, spores and sterile cells of *Testicularia cyperi* on *Rhynchospora careyana* (Vánky, Ust. exs. no. 1032, H.U.V. 18 603). Fig. 4. T.S. of a part of a spore ball with an incomplete, peripheral layer of dark, thick-walled spores, and a central mass of thin-walled, empty, sterile, parenchymatous cells, in LM. Fig. 5. A spore ball with loosely situated spores, remnants of sterile hyphae as debris, and between the spores parts of some sterile cells, in SEM. Bars = 10 μ m. Figs 6-7. Spore balls and spores of *Testicularia minor* on *Rhynchospora* sp. (type), in LM and in SEM. The central mass of relatively few sterile cells is hidden by the compact outer layer of spores. Bars = 10 μ m. Figs 8-9. Spore balls, spores and sterile cells of *Testicularia africana* on *Rhynchospora corymbosa*, in LM and in SEM (type). In Fig. 8, the spore balls are slightly squashed, to reveal the central mass of hyaline, sterile cells. Bars = 10 μ m

Sori in spiculis nonnullis inflorescentiae, similes illis *Testiculariae cyperi* Klotzsch, sed minores, 1,5-3 mm in diametro, peridio crasso, albido, cum superficie granulosa cooperti. *Glomeruli sporarum* globosi, subglobosi, ovoidei, ellipsoidales, 40-90 × 50-110 µm, e strato externo aliquantum laxo sporarum pressu separabilium et e massa centrale cellularum fungaliū sterilium compositi. *Sporae* parum depressae, in visu laterali ellipticae, 8-10,5 µm latae, in aspecto plano circulares vel ellipticae, 10,5-12,5 × 11,5-14,5 µm, flavidobrunneae; pariete levī, 0,5-1 µm crasso, cum areis 2 rotundis tenuioribus medianis laterum depressarum, 3-4 µm in diametro (pori germinationis?), in SEM subtiliter, dense verruculoso. *Cellulae steriles* globoideae, ellipsoidales vel irregulares, 6,5-13,5 × 8-17 µm, subhyalinae; pariete 0,5-1 µm crasso, levī.

Sori in some spikelets of an inflorescence, similar to those of *Testicularia cyperi* but smaller, 1.5-3 mm in diameter, covered by a thick, whitish peridium with granular surface. *Spore balls* (Figs 8-9) globose, subglobose, ovoid, ellipsoidal, 40-90 × 50-110 µm, composed of a rather loose external layer of spores which separate by pressure, and a central mass of sterile fungal

cells. *Spores* (Figs 8-9) slightly flattened, in side view elliptic, 8-10.5 µm wide, in plane view circular or elliptic, 10.5-12.5 × 11.5-14.5 µm, yellowish brown; wall smooth, 0.5-1 µm thick, with two rounded thinner areas in the middle of the flattened sides, 3-4 µm in diam. (germ pores?), in SEM finely, densely verruculose. *Sterile cells* (Fig. 8) globoid, ellipsoidal or irregular, 6.5-13.5 × 8-17 µm, subhyaline; wall 0.5-1 µm thick, smooth.

On Cyperaceae: *Rhynchospora corymbosa* (L.) Britton.

Distribution: W Africa (Guinea, ?Sierra Leone). Known with certainty only from the type collection. Zambettakis (1970: 680) reported *T. cyperi* on *Rhynchospora corymbosa* from Sierra Leone. No specimen is available for study in PC (Mouchacca, J., pers. comm.). Probably it represents *T. africana*. The description in Zambettakis (l.c.) is obviously taken from the literature for *T. cyperi*.

Testicularia africana differs from *T. minor* especially in the smaller size of the spores and sterile cells, and from *T. cyperi* in smaller sori, smaller spore balls, and larger, rounded sterile cells with thicker wall.

Key to the species of *Testicularia*

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|----|---|--------------------|
| 1 | Sori up to 20 mm long; spore balls 75-320 (-350) µm long; spore wall 1-3 µm thick; sterile cells polyhedral | <i>T. cyperi</i> |
| 1* | Sori smaller; spore balls 50-110 µm long; spore wall 0.5-1 µm thick; sterile cells globoid or ellipsoidal | 2 |
| 2 | Spores 11-21.5 (-25) µm long | <i>T. minor</i> |
| 2* | Spores 11.5-14.5 µm long | <i>T. africana</i> |

Acknowledgements. The authors are grateful to Dr. S. Tóth (Gödöllő, Hungary) for providing the Latin diagnosis, to Dr. R. Berndt (Zürich, Switzerland) for reviewing the manuscript and for useful suggestions, to C. Vánky, Mrs. M. Eha-Wagner and Mr. K.-H. Hellmer (Tübingen, Germany) for technical assistance in preparation of the illustrations, of semi-thin sections and TEM pictures, respectively. This study was partly supported by the Polish Ministry of Education and Science for the years 2005-2008, grant no. 2 P04G 019 28.

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