

Lundquistia is a synonym of *Sporisorium* (Ustilaginomycetes)

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Abstract. Phylogenetic analysis of four species of *Lundquistia* revealed the genus to be polyphyletic. Morphological characters and phylogenetic relationships demonstrate that *Lundquistia* should be reduced to synonymy with *Sporisorium*. Three new combinations are proposed: *Sporisorium dietelianum*, *S. duranii*, and *S. mexicanum*. *Sporisorium fascicularis* is considered a synonym of *S. panici-leucophaei*.

Key words: *Lundquistia*, molecular biology, smut fungi, *Sporisorium*, taxonomy

Introduction

The genus *Lundquistia* Vánky (2001: 371) was proposed for a peculiar smut fungus that formed spore balls in the host parenchymatic tissue between vascular fascicles of the stem, leaves, and inflorescence of *Poaceae* (type *L. fascicularis* Vánky on *Digitaria brownii* (Roem. & Schult.) Hughes, Australia). Spore balls in *Lundquistia* differentiated within the mass of sporogenous hyphae, without sterile cells around them, which is typical for *Sporisorium*. A study of several similar smut fungi, collected in Mexico and Ethiopia, showed that sterile cells were sometimes present, and the circumscription of the genus was slightly emended (Vánky 2004b: 160). The new combinations *Lundquistia dieteliana* (Henn.) Vánky and *L. duranii* (Vánky) Vánky were made and *L. mexicana* Vánky was described. Additionally, *L. fascicularis* Vánky was found to be synonymous with *Sporisorium panici-leucophaei* (Bref.) M. Piepenbr., and the new combination *L. panici-leucophaei* (Bref.) Vánky was made.

Recently, Stoll *et al.* (2005) published the results of their molecular phylogenetic analyses of numerous *Ustilago* and *Sporisorium* species, including species of *Macalpinomyces*, *Melanopsichium*, *Moesziomyces*, and the type of *Lundquistia*

fascicularis. They concluded that *Lundquistia fascicularis* is a *Sporisorium* species, and introduced the new name *S. fascicularis* (Vánky) M. Stoll, Begerow & Oberw. However, the phylogenetic relationships of the remaining *Lundquistia* species are unknown. We initiated a combined morphological and molecular study to more fully understand the relationship between *Lundquistia* and *Sporisorium*.

Materials and Methods

Sorus and spore characteristics were studied using either freshly collected material or dried herbarium specimens, preserved in different herbaria or in Herbarium Ustilaginales Vánky (H.U.V.). Herbarium names are abbreviated according to *Index Herbariorum* (Holmgren *et al.* 1990).

For light microscopy (LM), dried teliospores were rehydrated in a droplet of lactophenol, on a microscope slide, by gently heating to boiling point under a cover glass. For scanning electron microscopy (SEM), dried teliospores were dusted on double-sided adhesive tape, mounted on a specimen stub, sputter coated with gold-palladium *ca* 20 nm, and examined in a scanning electron microscope at 10 kV.

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For molecular analysis, three specimens were chosen for sequencing: *Lundquistia dieteliana* (H.U.V. 20 560), *L. mexicana* (H.U.V. 20 498, type specimen), and *L. panici-leucophaei* (H.U.V. 7090). Material of *L. duranii* was unavailable. DNA was extracted from dried material or living cultures using a DNeasy Plant Mini Kit (QIAGEN) according to the manufacturers instructions. The ribosomal DNA internal transcribed spacer (ITS) region were amplified by the polymerase chain reaction using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Products were purified with a QIAquick PCR Purification Kit (Qiagen) and sequenced directly using ABI Prism BigDye technology (Applied Biosystems). The sequences have been deposited in GenBank under accessions AY998100-AY998102. They were aligned with sequences from approximately 50 species of *Ustilago*, *Sporisorium*, *Macalpinomyces*, *Melanopsichium*, and *Lundquistia* obtained by Stoll *et al.* (2003, 2005), using ClustalX (Thompson *et al.* 1997). A minimum evolution tree was constructed with MEGA2 (Kumar *et al.* 2001) using the Kimura-2-parameter method, a complete deletion of gapped sites and 1000 bootstrap replicates.

Results and Discussion

The minimum evolution tree (Fig. 1) is very similar to those trees published by Stoll *et al.* (2003, 2005). It reveals *Lundquistia* to be polyphyletic with respect to *Sporisorium* and *Ustilago*. The ITS sequence from *L. panici-leucophaei* (H.U.V. 7090) was almost identical to both the *S. panici-leucophaei* sequence obtained by Stoll *et al.* (2003) and the *L. fascicularis* sequence obtained by Stoll *et al.* (2005), further supporting that these taxa are conspecific. However, this taxon is not closely related to the other two *Lundquistia* species sequenced in this study. *Lundquistia mexicana* belongs in the same well supported “*Sporisorium*” clade as *L. panici-leucophaei*, but at the same time is only distantly related. *Lundquistia dieteliana* falls outside any of the major *Sporisorium* or *Ustilago* clades, but rather clusters with *U. maydis*. Despite having high bootstrap support, the ITS sequences of these two taxa are difficult to align, indicating only a distant relationship. *Lundquistia dieteliana* is certainly far removed from the other *Lundquistia* species included in the analysis. The close relationship of *L. dieteliana* with *U. maydis* is explained by the fact that they parasitise host plants of two closely related genera, *Tripsacum* and *Zea* respectively, of the tribe Tripsacinae, subfam. Andropogoneae.

Given that *Lundquistia* is a polyphyletic assemblage of a small number of species, we agree with the conclusion reached by Stoll *et al.* (2005) based on sequence data for *L. fascicularis*. That is, *Lundquistia* not a tenable genus and should be considered a synonym of *Sporisorium*, with which it shares several morphological similarities. Five smut fungi have so far been described or transferred to the genus *Lundquistia*, representing four species, which are now placed into

Sporisorium, a genus of which description is complemented with the soral characters of *Lundquistia*.

***Sporisorium dietelianum* (Henn.) Vánky & Cunnington, comb. nov.**

Basionym: *Ustilago dieteliana* Hennings, Hedwigia 37: 268, 1898. — *Lundquistia dieteliana* (Henn.) Vánky, 2004b: 163. — Type on *Tripsacum dactyloides* (L.) L., Mexico, near Mexico City, Oct 1896, E.W.D. Holway; isotypes in Sydow, Ust. no. 152 (H.U.V. 3621!).

Sori on the top of sterile shoot as long, twisted tubes or bands, 2-5 mm × 40-140 cm, partly enclosed by leaf sheaths, infecting inner leaves and the peduncle of the inflorescence (sometimes remnants of raceme branches are present on the top of the sori). The teliospores develop in the parenchymatic tissues between the veins. At maturity, the host tissues rupture longitudinally and the dark brown, semiagglutinated to powdery spore mass and sterile cells are scattered leaving behind fascicles of long filaments of vascular tissues. **Teliospores** globose, subglobose, broadly ellipsoidal, rarely ovoid, 8-11 (–12) × 9-13 µm, yellowish brown; wall even, ca 1 µm thick, conspicuously verrucose, spore profile wavy. **Sterile cells** in small groups, single cells variable in shape and size, from subglobose to irregular, with 1-2 flattened sides, 6-13.5 × 6-17 µm, hyaline; wall even, ca 0.5 µm thick, smooth.

On Gramineae: *Tripsacum dactyloides* (L.) L., *T. latifolium* Hitchc., *T. laxum* Scribn. & Merr.

Distribution: N. America (Mexico), C. America (El Salvador, Guatemala), West Indies (Cuba).

***Sporisorium duranii* (Vánky) Vánky & Cunnington, comb. nov.**

Basionym: *Ustilago duranii* Vánky, Mycotaxon 89: 77, 2004a. — *Lundquistia duranii* (Vánky) Vánky, 2004b: 165. — Type on *Andropogon semiberbis* (Nees) Kunth, Mexico, Chihuahua State, Creel, Monte Gallegos (Padre Gallegos), above Urique, 12 Oct 1969, R. Durán (WSP 58 571!).

Sori in the uppermost 1-2 leaves of sterile shoots, destroying and replacing the parenchymatous tissues between the leaf-veins with a black, semiagglutinated to powdery mass of loose spore balls and teliospores. Sori protruding from the last healthy leaf-sheath, 2-3 mm wide, 6-8 cm long, first covered by the epidermis which ruptures, liberating the spore masses which are scattered, leaving behind fascicles of vascular tissues as long, often curled filaments. Only the 1-2 cm long tip of infected leaves remains intact. **Teliospores** when mature single, globose, subglobose, ovoid, ellipsoidal, 10.5-13.5 × 11-15 (–16) µm, medium dark yellowish brown; wall even or slightly uneven, 1-2.5 µm thick, provided with densely situated, rather high, confluent warts forming irregular rows and a fine, incomplete or complete reticulum; spore profile roughly wavy to denticulate.

On Gramineae: *Andropogon semiberbis* (Nees) Kunth.

Distribution: N. America (Mexico). Known only from the type collection.

Sporisorium mexicanum (Vánky) Vánky & Cunnington, comb. nov.

Basionym: *Lundquistia mexicana* Vánky, Fungal Diversity 17: 161, 2004b. — Type on *Andropogon gerardii* Vitman, Mexico, Durango State, 57 km WSW of Durango, Hwy no. 48, alt. 2538 m, 19 Nov 2003, T. & K. Vánky (H.U.V. 20 498; isotypes in Vánky, Ust. exs. no. 1202). Paratype on *Schizachyrium mexicanum* (Hitchc.) A. Camus, Mexico, Mexico State, 18.5 km W of Toluca, Hwy no. 1, alt. 2812 m, 6 Dec 2003, C. & K. Vánky (H.U.V. 20 526; isoparatypes in BPI and IMI).

Sori on the top of sterile shoots as dark, long, slender, bent bodies composed of numerous vascular bundles and between them spore masses and sterile cells destroying the parenchymatous tissues. No peridium, no true columella. At maturity the host tissues disintegrate and the dark brown, semiagglutinated to powdery mass of teliospores and sterile cells is successively liberated leaving behind a 2-5 mm wide, 15-40 cm long, twisted and curled fascicle of numerous, filiform vascular bundles. **Teliospores** globose to subglobose, 8-10.5 × 8-11 µm, medium dark yellowish brown, provided with 1.5 µm high, anastomosing warts forming an irregular, labyrinthiform or incompletely and irregularly reticulate pattern. Warts in optical median view acute. **Sterile cells** single, in short chains or in small, irregular groups. Single cells subglobose or ellipsoidal with flattened contact sides, 7-13 µm long, subhyaline to pale yellowish brown; wall even, ca 0.5 µm thick, smooth. **Spore germination** (Vánky 2004b: 161 + fig. 2) results in 4-celled basidia (often in 3+1 arrangement), measuring 2-2.5 × 15-25 µm, on which laterally and terminally, on sterigmata, ovoid basidiospores are produced measuring 1.5-2.5 × 4-5.5 µm. The basidiospores bud like yeast cells giving rise to yeast colonies.

On Gramineae: *Andropogon gerardii* Vitman, *Schizachyrium mexicanum* (Hitchc.) A. Camus.

Distribution: N. America (Mexico). It is known from only the type collections.

Sporisorium panici-leucophaei (Bref.) M. Piepenbring, 1999: 465.

Ustilago panici-leucophaei Brefeld, 1895: 114. — *Sphacelotheca panici-leucophaei* (Bref.) G.P. Clinton, 1906: 28. — *Lundquistia panici-leucophaei* (Bref.) Vánky, 2004b: 167. — Type on *Panicum leucophaeum* H.B.K. (= *Digitaria insularis* (L.) Fedde), Brazil, Rio de Janeiro, coll. E. Ule (HBG).

Ustilago bonariensis Spegazzini, 1909: 287. — *Sphacelotheca bonariensis* (Speg.) Ciferri, 1931: 56. — *Sorosporium bonariense* (Speg.) Zundel, 1953: 54 (as “*bonariensis*”). — *Sporisorium bonariense* (Speg.) Vánky, nom. herb. — Lectotype (design. by Zundel, 1953: 54) on *Panicum spectabile* Nees (= *Echinochloa polystachya* (H.B.K.) Hitchc.; no healthy host plant to check host identity), Argentina, near Buenos Aires, Jan 1904, C. Spegazzini (LPS 3018!) (syn. by Vánky, 2004b: 167).

Sphacelotheca viegasiana Zundel, 1939: 588. — Type on *Trichachne sacchariflora* (Raddi) Nees (= *Digitaria insularis*

(L.) Fedde), Brazil, Est. São Paulo, Campinas, Terreno baldio, 5 Oct 1935, A.P. Viégas 2554 (BPI 195 095!; isotype BPI 195 097!) (syn. suggested by Vánky, 2000: 208, confirmed by Piepenbring, 2003: 121).

Sorosporium lindmanii Zundel, 1943: 173. — Type on *Oplismenopsis najada* (Hack. & Arechav.) Parodi, Paraguay, in the forests on the banks of the river Riacho Mbopi, 11 Sep 1893 (not “19 Nov 1893”), C.A.M. Lindman B 331 (S; isotype H.U.V. 1714!) (syn. by Vánky, 2004b: 167).

Ustilago garcesii Zundel, 1945: 372 (as “*Garcesi*”). — Type on *Paspalum saccharoides* Nees ex Trin., Colombia, Valle, Palmira, Estacion Experimental, 13 Dec 1940, C. Garces O. (= C.G. Orejuela), Fungi of Colombia 1281 (BPI 160 488!) (syn. by Piepenbring, 2002: 110, but host identification doubtful, confirmed).

Lundquistia fascicularis Vánky, 2001: 373. — *Sporisorium fasciculare* (Vánky) M. Stoll, Begerow & Oberwinkler, 2005: 354 (as “*fascicularis*”). — Type on *Digitaria brownii* (Roem. & Schult.) Hughes, Australia, New South Wales, NW of Hermidale, between Cobar and Nyngan, 1 Mar 1971, D.A. Campbell (DAR 58 832; isotype H.U.V. 19 444!) (syn. by Vánky, 2004b: 167).

Sori comprise the uppermost leaves of sterile shoots, also the floral stem and often also a more or less developed inflorescence or only part of it, long cylindrical, often twisted and tapering towards the apex, 0.2-0.8 × 4-15 cm, usually partly enclosed by healthy leaf sheaths. In the sori, the parenchymatous tissues between the veins are replaced by the dark brown, semiagglutinated or granular-powdery mass of spore balls and teliospores disclosed by the rupture of the covering epidermis, leaving behind the typical fascicles of vascular bundles. **Spore balls** globose, subglobose, ellipsoidal, oblong to slightly irregular, 20-70 × 25-90 µm, yellowish to dark brown, composed of many teliospores, when young rather permanent, later separating. **Teliospores** globose, subglobose, broadly ellipsoidal to slightly subpolyhedrally irregular, 5.5-8 × 6.5-9.5 µm, yellowish brown; wall evenly thick (ca 0.5 µm), finely, moderately densely punctate-verruculose, spore profile smooth to just finely wavy; in SEM finely verrucose-echinulate. **Sterile cells** absent.

On Gramineae: *Digitaria brownii* (Roem. & Schult.) Hughes, *D. horizontalis* Willd., *D. insularis* (L.) Fedde (*Trichachne insularis* (L.) Nees, *Panicum lanatum* Rottb., *P. leucophaeum* H.B.K., *T. sacchariflora* (Raddi) Nees), *Echinochloa polystachya* (H.B.K.) Hitchc. (*Panicum spectabile* Nees), *Oplismenopsis najada* (Hack. & Arechav.) Parodi (*Panicum najadum* Hack. & Arechav.), *Panicum elephantipes* Nees, *Trichachne californica* (Benth.) Chase (*Panicum californicum* Benth., *Digitaria californica* (Benth.) Henr., *Trichachne saccharata* (Buckl.) Nash).

Distribution: Australia, N. America (Mexico, USA), West Indian Antilles (Cuba, Dominican Rep., Haiti, Jamaica, Martinique, Puerto Rico, Virgin Islands), C. America (Guatemala), S. America (Argentina, Brazil, Colombia, Paraguay).

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