

Shivasia gen. nov. for the Australasian smut *Ustilago solida* that historically shifted through five different genera

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Abstract: The generic position of the enigmatic smut fungus *Ustilago solida* is evaluated applying molecular phylogenetic analyses using ITS and LSU rDNA sequences as well as light and scanning electron microscopical investigations of several collections of this species. *Ustilago solida* has previously been included in five different genera (*Ustilago*, *Urocystis*, *Sorosporium*, *Cintractia*, and *Tolyposporium*), however, molecular analyses revealed that this smut does not belong to any of these genera and represents a distinct ustilaginean lineage. The closest known phylogenetic relative of *Ustilago solida* is *Heterotolyposporium lepidospermatis*, the type species of the monotypic genus *Heterotolyposporium*. Both smuts differ considerably in both LSU sequences and in several morphological traits, such as the structure of sori and the characteristics of spore balls. Accordingly, the new genus *Shivasia* is described to accommodate *Ustilago solida*. This smut infects different *Schoenus* species (*Cyperaceae*) in Australia and New Zealand. The description of *Shivasia* increases the number of endemic smut genera in Australasia to ten. Compared to all other continents the number of endemic smut genera is exceptionally high, which may point at fast evolving characters and/or may be caused by the regional history, including the long-term geographic isolation of Australasia.

Key words:

Basidiomycota
Biogeography
Molecular phylogenetics
Plant pathogens
Schoenus
Ustilaginales

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INTRODUCTION

The order *Ustilaginales*, currently classified within the subclass *Ustilaginomycotina* (Bauer *et al.* 2006), contains the largest number of smut fungi, both in terms of the number of genera and species, among all ustilaginomycotinous orders. Forty-five genera are currently included in the *Ustilaginales* (Begerow *et al.* 2007 – excluding *Pseudozyma*, Vánky 2008a – excluding *Thecaphora*; Vánky 2011, Vánky & Lutz 2011), but this number is likely to increase since at least some of these genera are undoubtedly polyphyletic (Stoll *et al.* 2005), and several smut species with unusual characteristics could still be wrongly classified. All members of *Ustilaginales* share a similar ultrastructure (Bauer *et al.* 1997), which accordingly is useless for generic classification. However, molecular phylogenetic analyses proved to be useful in the *Ustilaginales*. Indeed, molecular phylogenetic analysis has been crucial in the generic placement of several ustilaginean species with ambiguous morphological characteristics (Piepenbring *et al.* 1999, Cunnington *et al.* 2005, Vánky *et al.* 2006, Bauer *et al.* 2007, González *et al.* 2007) and has also helped to elucidate the generic placement for several smuts from other orders (Vánky *et al.* 1998, Bauer *et al.* 1999, 2001a, 2005, Castlebury *et al.*

2005, Bauer *et al.* 2007, 2008, Chandra & Huff 2008, Vánky *et al.* 2008a, b, Lutz *et al.* 2012).

The generic classification of an enigmatic smut growing in the ovaries of several *Schoenus* species in Australasia, which had been described under the name *Ustilago solida* by Berkeley (*in* Hooker 1859), troubled several generations of smut researchers. This smut was originally described from the ovaries of “*Chaetophora imberbis*”, now *Schoenus apogon*, collected in Tasmania. Berkeley remarked that “This species connects *Ustilago* and *Sporisporium* [sic!]”. Consecutively, it was moved to *Urocystis* (Fischer von Waldheim 1877), *Sorosporium* (McAlpine 1910), and *Cintractia* (Piepenbring 2000), in each of these genera constituting a discordant element according to the current generic concepts (Vánky 2002). Recently, Vánky (2009) transferred *Ustilago solida* to *Tolyposporium*. Indeed, the spore balls of *Ustilago solida* show some resemblance to spore balls of *Tolyposporium junci* (type species), *T. isolepidis*, *T. neillii*, and *T. piluliforme*, which form a monophyletic group according to phylogenetic analyses presented by Lutz (*in* Vánky 2008b). This taxonomic decision could suggest that *Ustilago solida* reached the appropriate genus. However, since molecular data were still lacking for this species, it could not be excluded that the phenotypic similarity of spore balls may have resulted from

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Table 1. List of examined specimens of *Shivasia solida*.

Host	Locality, date, collectors	GenBank acc. no.	Reference specimens
<i>Schoenus apogon</i>	AU: Tasmania, Penquite, 21 Dec. 1845, R.C. Gunn	–	holotype K(M) 171338, isotype DAR 59818
<i>Schoenus apogon</i>	AU: Tasmania, Hobart, 4 Nov. 1894, L. Rodway	–	H.U.V. 17613
<i>Schoenus apogon</i>	AU: Victoria, Port Campbell Natl Park, 30 Oct. 1966, G. Beaton 114	–	H.U.V. 17483
<i>Schoenus apogon</i>	NZ: Auckland, Waikumete Cemetry, 1 Sep. 1976, S. Bowman & W.S.M. Versluys	–	H.U.V. 16467
<i>Schoenus apogon</i>	NZ: Auckland, Waikumete Cemetry, 26 Oct. 1989, E.H.C. McKenzie	LSU: JF966729	H.U.V. 15059, H.U.V. 15060, KRAM F-49115
<i>Schoenus apogon</i>	AU: Tasmania, 170 km NE of Hobart, 8 Mar. 1996, C. Vánky & K. Vánky	ITS: JF966731, LSU: JF966730	epitype H.U.V. 17649, isoeopitype TUB 20001
<i>Schoenus latelaminatus</i>	AU: Victoria, between Moora Channel and Mairstrack, 18 Jan. 1969, A.C. Beauglehole 30303	–	H.U.V. 20072
<i>Schoenus maschalinus</i>	NZ: Wellington, Upper Hutt, 13 Nov. 1952, A.J. Healy	–	H.U.V. 16477
<i>Schoenus nitens</i> var. <i>concinus</i>	NZ: Wanganui, Himatangi, 29 Jan. 1932, H.H. Allan	–	H.U.V. 18757
<i>Schoenus pauciflorus</i>	NZ: Canterbury, near Cass, Kettlehole Bog, 1 Feb. 1990, K. Vánky	–	H.U.V. 16755
<i>Schoenus tesquorum</i>	AU: New South Wales, Sydney, Enfield Sate Park, Drevers Road, 28 Nov. 1996, J. Dickins	–	H.U.V. 20073

morphological convergence, which is quite often observed in different smut fungi.

The present work aims to clarify the generic position of *Ustilago solida* applying molecular phylogenetic analyses using rDNA sequences as well as light and scanning electron microscopical investigation of several collections of this fungus.

MATERIALS AND METHODS

Specimen sampling and documentation

The specimens examined in this study are listed in Table 1. The voucher specimens have been deposited in DAR, K, KRAM F, and H.U.V. The latter abbreviation refers to the personal collection of Kálmán Vánky, “Herbarium *Ustilaginales* Vánky” currently held at his home (Gabriel-Biel-Straß 5, D-72076 Tübingen, Germany). Nomenclatural novelties were registered in MycoBank (www.MycoBank.org, Crous *et al.* 2004). The genotype concept follows the proposal of Chakrabarty (2010).

Morphological examination

Sorus structure, spore ball development, mature spore balls and spore characteristics were studied using dried herbarium specimens. For soral studies, young sori from herbarium specimens were rehydrated by briefly boiling in distilled water, and fixed with 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature. Following six transfers in 0.1 M sodium cacodylate buffer, samples were 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, samples were dehydrated in acetone, using 10 min changes at 25 %, 50 %, 70 %, 95 %, and three times in 100 % acetone. Samples were embedded in Spurr’s plastic

and sectioned with a diamond knife. Semi-thin sections were transferred to a microscope slide, stained with new fuchsin and crystal violet, mounted in Entellan under a cover slip, and studied by light microscopy (LM) at various magnifications.

For LM, spore balls and spores were dispersed in a droplet of lactophenol on a microscope slide, covered with a cover slip, gently heated to boiling point to rehydrate the spores and to eliminate air bubbles, and examined at 1000× magnification. For examination of spore ball development, sori were boiled in a mixture of lactophenol with cotton blue and distilled water, and hand sectioned with a razor blade under a stereomicroscope. Pieces of host tissues from the basal part of the sori and very young spore balls were transferred into a droplet of lactophenol with cotton blue and covered with a cover slip. Gentle pressure was applied until the host tissue became flat. Air bubbles were eliminated by gently heating to boiling point.

For scanning electron microscopy (SEM), spore balls and spores were mounted on carbon tabs and fixed to an aluminium stub with double-sided transparent tape. The stubs were sputter-coated with carbon using a Cressington sputter-coater and viewed under a Hitachi S-4700 scanning electron microscope, with a working distance of ca. 11 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of Jagiellonian University, Kraków (Poland).

DNA extraction, PCR, and sequencing

Genomic DNA was isolated directly from the herbarium specimens. For methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data see Lutz *et al.* (2004). ITS 1 and ITS 2 regions of the rDNA including the 5.8S rDNA (ITS, about 780 bp) were amplified using the primer pair M-ITS1 (Stoll *et al.* 2003) and ITS4 (White *et al.* 1990).

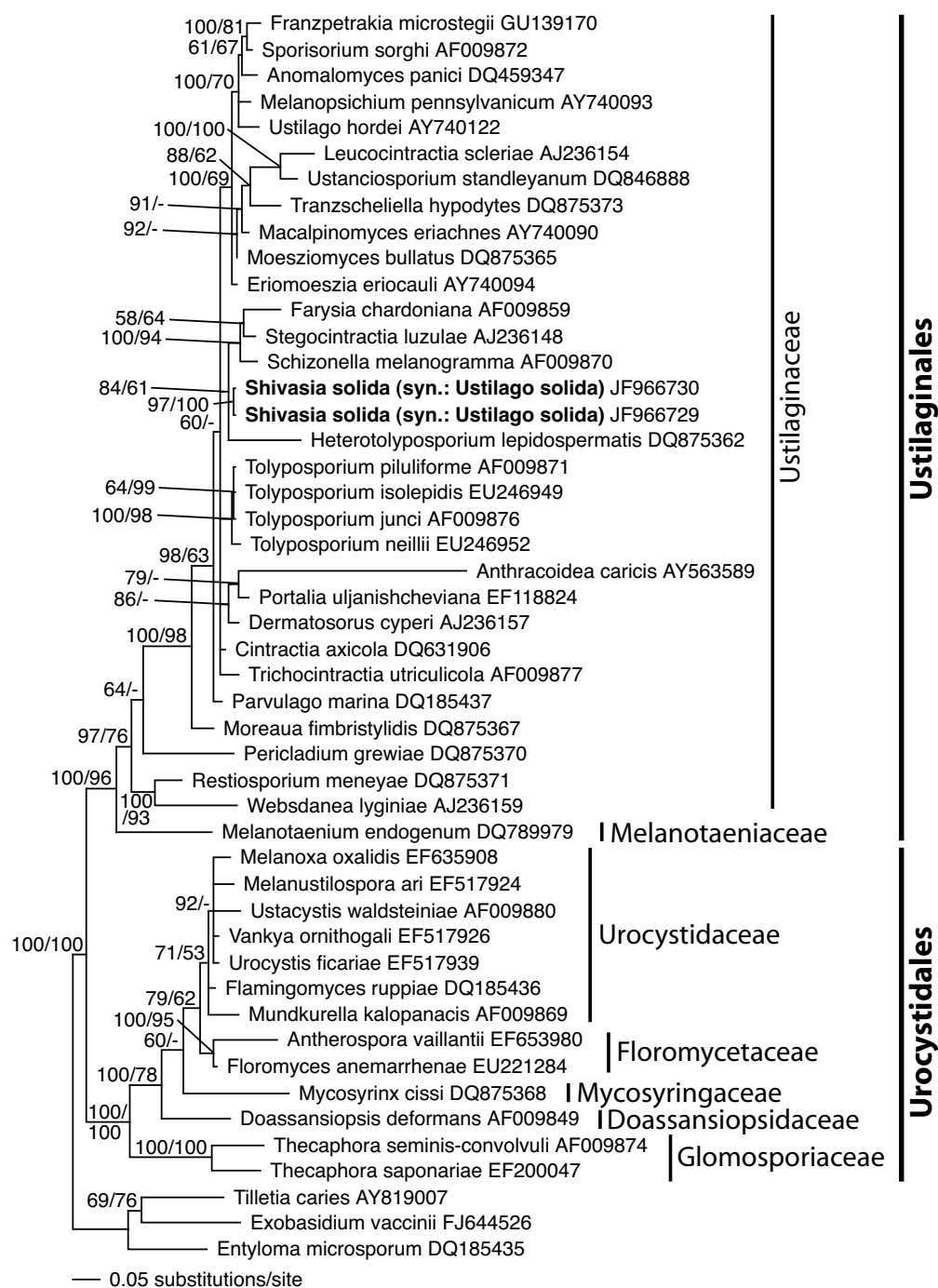


Fig. 1. Bayesian inference of phylogenetic relationships within the sampled *Ustilaginomycetes*: Markov chain Monte Carlo analysis of an alignment of LSU sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and an estimated proportion of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 50 % majority-rule consensus tree is shown computed from 45 000 trees that were sampled after the process had reached stationarity. The topology was rooted with the exobasidiomycetous species *Entyloma microsporium*, *Exobasidium vaccinii*, and *Tilletia caries*. Numbers on branches before slashes are estimates for *a posteriori* probabilities, numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. The taxonomic concept used here follows Bauer *et al.* (2001b).

The 5'-end of the nuclear large subunit ribosomal DNA (LSU, about 650 bp) was amplified using the primer pair NL1 and NL4 (O'Donnell 1993). PCR primers were also used for cycle sequencing. For amplification the annealing temperature was adjusted to 45 °C. DNA sequences determined for this study were deposited in GenBank. GenBank accession numbers are given in Table 1 and Fig. 1.

Phylogenetic analyses

The *Ustilago solida* specimens examined in this study are listed in Table 1. For molecular phylogenetic analyses the following sequences from GenBank were additionally used (Begerow *et al.* 1997, Piepenbring *et al.* 1999, Castlebury *et al.* 2005, Hendrichs *et al.* 2005, Stoll *et al.* 2005, Matheny *et al.* 2006, Vánky *et al.* 2006, Bauer *et al.* 2007, Begerow *et al.*

al. 2007, González *et al.* 2007, Vánky & Lutz 2007, Bauer *et al.* 2008, Vánky & Lutz, *in* Vánky 2008b, Vánky *et al.* 2008b, Kottke *et al.* 2010, Lutz *et al.* 2012): *Anomalomyces panici* DQ459347, *Antherospora vaillantii* EF653980, *Anthracoidea caricis* AY563589, *Cintractia axicola* DQ631906, *Dermatosorus cyperi* AJ236157, *Doassansiopsis deformans* AF009849, *Entyloma microsporium* DQ185435, *Eriomoeszia eriocauli* AY740094, *Exobasidium vaccinii* FJ644526, *Farysia chardoniana* AF009859, *Flamingomyces ruppii* DQ185436, *Floromyces anemarrhenae* EU221284, *Franzpetrakia microstegii* GU139170, *Heterotolyposporium lepidospermatis* DQ875362, *Leucocintractia scleriae* AJ236154, *Macalpinomyces eriachnes* AY740090, *Melanopsichium pennsylvanicum* AY740093, *Melanotaenium endogenum* DQ789979, *Melanoxa oxalidis* EF635908, *Melanustilospora ari* EF517924, *Moesziomyces bullatus* DQ875365, *Moreaua fimbristylidis* DQ875367, *Mundkurella kalopanacis* AF009869, *Mycosyrinx cissi* DQ875368, *Parvulago marina* DQ185437, *Pericladium grewiae* DQ875370, *Portalia uljanishcheviana* EF118824, *Restiosporium meneyae* DQ875371, *Schizonella melanogramma* AF009870, *Sporisorium sorghi* AF009872, *Stegocintractia luzulae* AJ236148, *Thecaphora saponariae* EF200047, *T. seminis-convolvuli* AF009874, *Tilletia caries* AY819007, *Tolyposporium isolepidis* EU246949, *T. juncei* AF009876, *T. neillii* EU246952, *T. piluliforme* AF009871, *Tranzscheliella hypodytes* DQ875373, *Trichocintractia utriculicola* AF009877, *Urocystis ficariae* EF517939, *Ustacystis waldsteiniae* AF009880, *Ustanciosporium standleyanum* DQ846888, *Ustilago hordei* AY740122, *Vankya ornithogali* EF517926, and *Websdanea lyginiae* AJ236159.

To elucidate the phylogenetic position of the *Ustilago solida* specimens their LSU sequences were analysed within a dataset covering all urocystidalean and ustilaginalean genera of which sequences were available in GenBank. If present in GenBank the respective type species were used. Additionally, all *Tolyposporium* LSU sequences available in GenBank and the LSU sequence of *Thecaphora saponariae* (type species of *Sorosporium*) were added.

Sequence alignment was obtained using MAFFT 6.853 (Katoh *et al.* 2002, 2005, Katoh & Toh 2008) using the L-INS-i option. To obtain reproducible results, manipulation of the alignment by hand as well as manual exclusion of ambiguous sites were avoided as suggested by Giribet & Wheeler (1999) and Gatesy *et al.* (1993), respectively. Instead, highly divergent portions of the alignment were omitted using GBlocks 0.91b (Castresana 2000) with the following options: 'Minimum Number of Sequences for a Conserved Position': 25, 'Minimum Number of Sequences for a Flank Position': 25, 'Maximum Number of Contiguous Non-conserved Positions': 8, 'Minimum Length of a Block': 5, and 'Allowed Gap Positions' to 'With half'.

The resulting alignment (new number of positions: 586; 21 % of the original 2790 positions; number of variable sites: 312) was used for phylogenetic analyses using a Bayesian Approach and Maximum Likelihood (ML). A Bayesian approach using a Markov chain Monte Carlo (MCMC) technique was used as implemented in the computer program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Four incrementally heated simultaneous Markov chains were run over 5 M generations using the

general time reversible model of DNA substitution with gamma distributed substitution rates and an estimated proportion of invariant sites, random starting trees and default starting parameters of the DNA substitution model as recommended by Huelsenbeck & Rannala (2004). Trees were sampled every 100th generation resulting in an overall sampling of 50 001 trees. From these, the first 5 001 trees were discarded (burnin = 5 001). The trees sampled after the process had reached stationarity (45 000 trees) were used to compute a 50 % majority rule consensus tree to obtain estimates for the *a posteriori* probabilities of groups of species. This Bayesian approach of phylogenetic analysis was repeated five times to test the independence of the results from topological priors (Huelsenbeck *et al.* 2002). ML analysis (Felsenstein 1981) was conducted with the RAxML 7.2.8 software (Stamatakis 2006), using raxmlGUI (Silvestro & Michalak 2012), invoking the GTRCAT and the rapid bootstrap option (Stamatakis *et al.* 2008) with 1000 replicates.

Trees were rooted with the exobasidiomycetous species *Entyloma microsporium*, *Exobasidium vaccinii*, and *Tilletia caries*.

RESULTS

Morphology

The morphological characteristics of *Ustilago solida* are included in the species description and depicted in illustrations (Figs 2–4).

Phylogenetic analyses

The LSU sequences of the two *Ustilago solida* specimens analysed were identical. Compared to the LSU of *Heterotolyposporium lepidospermatis* DQ875362 they differed in 51 positions (9.75 %) in 36 different sections.

BLAST searches (Altschul *et al.* 1997) for the ITS sequence of *Ustilago solida* H.U.V. 17649 revealed closest similarity to *Tolyposporium isolepidis* EU246950 and *T. neillii* EU246951. However, ITS sampling of ustilaginaceous species in GenBank is limited (e.g., there is no *Heterotolyposporium lepidospermatis* ITS sequence available) and sequences differ to a great extent (e.g., compared to the ITS of *Tolyposporium isolepidis* and *T. neillii* the ITS sequence of *Ustilago solida* differed in 211 positions (27.51 %) in 123 different sections). Thus, molecular phylogenetic analyses of the available ITS sequences, similar to the LSU analyses, supported the ustilaginaceous affiliation of *Ustilago solida* but did not resolve the placement within the *Ustilaginaceae* (data not shown).

The different runs of the Bayesian phylogenetic analyses that were performed and the ML analyses yielded consistent topologies. To illustrate the results, the consensus tree of one run of the Bayesian phylogenetic analyses is presented (Fig. 1). Estimates for *a posteriori* probabilities are indicated on branches before slashes, numbers on branches after slashes are ML bootstrap support values.

In all analyses the two *Ustilago solida* specimens formed a cluster within the *Ustilaginaceae sensu* Bauer *et al.* (2001b), which was revealed in a group consisting of *Heterotolyposporium lepidospermatis*, and the cluster of

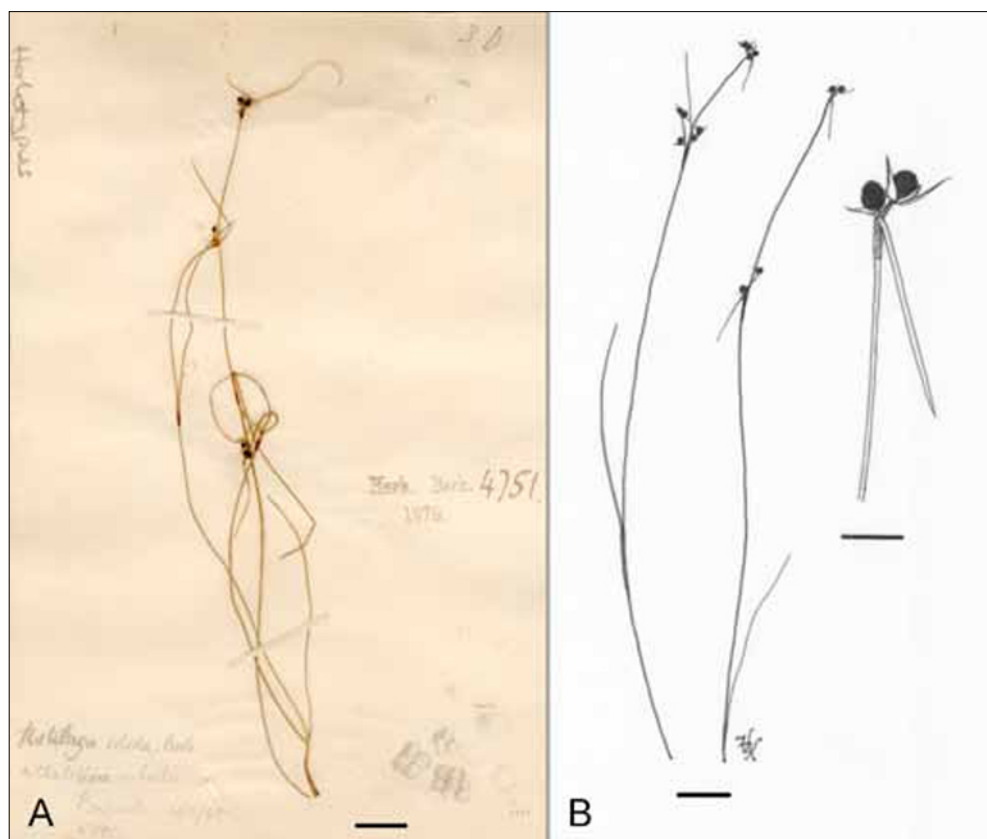


Fig. 2. *Shivasia solida* on *Schoenus apogon*. **A.** Holotype of *Ustilago solida* [K(M) 171338]. **B.** The habit of infected plants and two enlarged sori (H.U.V. 15059). Bars: A = 1 cm, B = 1 cm and 3 mm, respectively.

Farysia chardoniana, *Schizonella melanogramma*, and *Stegocinctria luzulae*. A closer relation of the *Ustilago solida* specimens to any of the genera *Cintractia*, *Sorosporium* (here: *Thecaphora saponariae*, the type species of *Sorosporium*), *Tolyposporium*, *Urocystis* or *Ustilago* was not revealed by any of the phylogenetic analyses.

TAXONOMY

Molecular phylogenetic analyses, sorus, spore ball and spore morphology, as well as the type of spore germination, supplemented by host plant taxonomy give evidence that a new genus should be erected to accommodate *Ustilago solida*.

Shivasia Vánky, M. Lutz & M. Piątek, **gen. nov.**

Mycobank MB800821

Etymology: This genus is named in the honour of Roger Graham Shivas, a multi-talented Australian mycologist, interested in plant parasitic microfungi, author of two books and over 130 scientific papers, in which over 160 new species and several new genera are described. Roger is a sharp-eyed, ardent collector who collected not only in Australia but also in Bolivia, Burma, China, India, Indonesia, Malaysia, New Zealand, Norway, Papua New Guinea, Philippines, South Africa, Thailand, and Vietnam.

Type species: *Shivasia solida* (Berk.) Vánky, M. Lutz & M. Piątek 2012.

Sori in the flowers of *Schoenus* (Cyperaceae) forming hard, globoid, black bodies composed of spore balls and spores on the surface of innermost floral organs developed in sporogenous hyphae within U-shaped pockets, at first covered by a fungal peridium. *Spore balls* few to many-spored, composed of spores only, enclosed by a subhyaline mucilaginous layer. *Spores* pigmented (brown). *Sterile cells* absent.

Shivasia solida (Berk.) Vánky, M. Lutz & M. Piątek, **comb. nov.**

Mycobank MB800822

(Figs 2–4)

Basionym: *Ustilago solida* Berk., in Hooker, *Flora Tasmaniae* **2**: 270 (1859).

Synonyms: *Urocystis solida* (Berk.) A.A. Fisch. Waldh., *Aperçu Syst. Ustil.*: 38 (1877).

Sorosporium solidum (Berk.) McAlpine, *Smuts of Australia*: 185 (1910).

Cintractia solida (Berk.) M. Piepenbr., *Nova Hedwigia* **70**: 310 (2000).

Tolyposporium solidum (Berk.) Vánky, *Mycotaxon* **110**: 320 (2009).

Type: [**Australia: Tasmania**]: “Herb. Berk. 4751, 1879. *Ustilago solida*, Berk. on *Chaetospora imberbis* Penquite 21/12/45 h°581.” [original label of holotype specimen, Fig. 2a] (K(M) 171338 – holotype; DAR 59818 – microscope slide ex-holotype). – **Australia: Tasmania**: 170 km NE of Hobart, on *Schoenus apogon*, 8 March 1996, C. Vánky & K. Vánky (H.U.V. 17649 – **epitype designated here**; TUB 20001 – isoepitype).

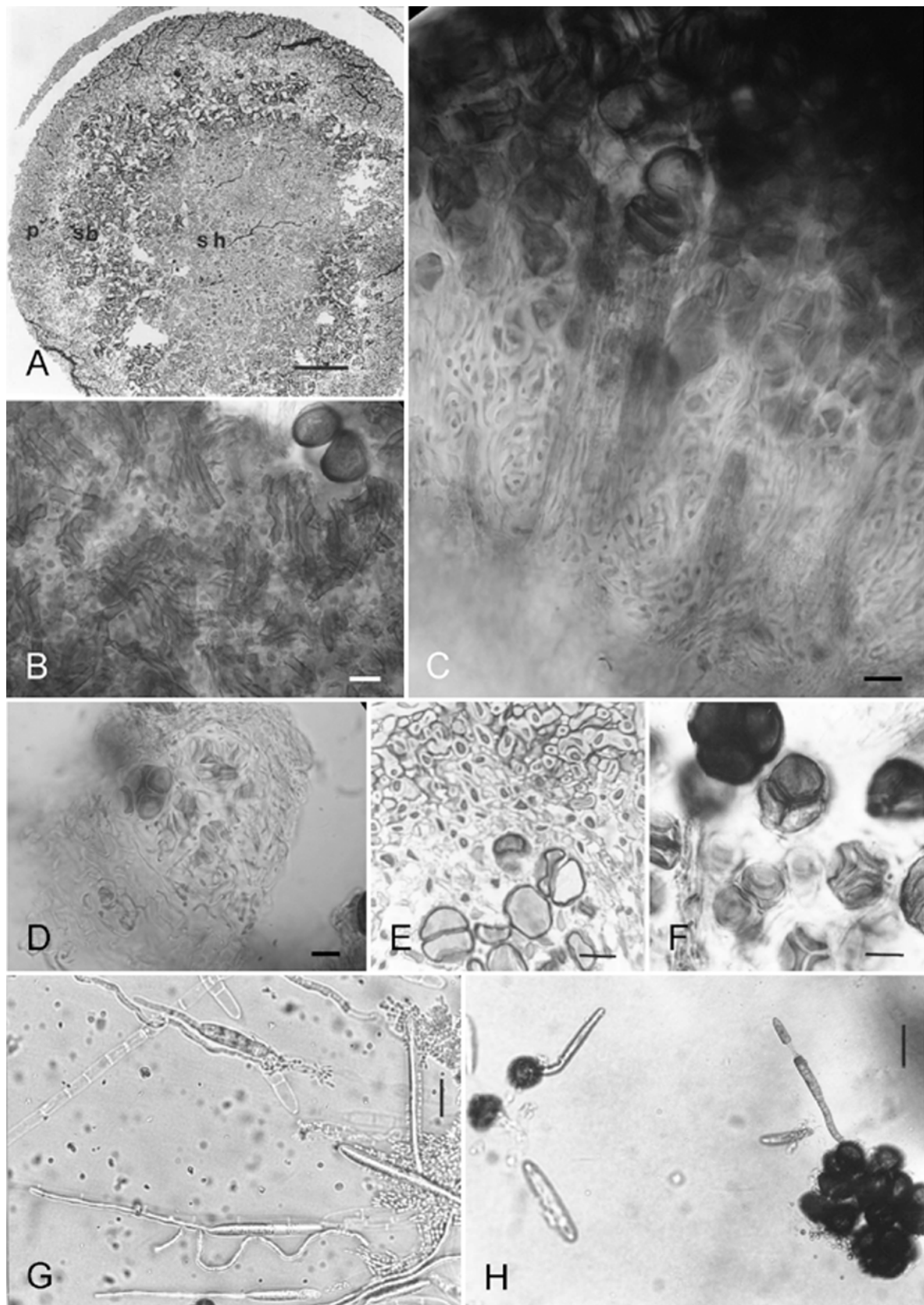


Fig. 3. *Shivasia solida* on *Schoenus apogon*. **A.** Embedded, stained, semi-thin section of a sorus (H.U.V. 17649), sh = sporogenous hyphae, sp = spore balls, p = peridium. **B.** Fungal cells of the peridium covering the sori, formed of thick-walled, sterile hyphae (H.U.V. 15059). **C.** Spore ball formation in sporogenous fungal layer on the surface of innermost floral organs, in U-shaped pockets, hand sectioned, stained with cotton blue in lactophenol (H.U.V. 15059). **D–E.** Young spores and spore balls covered by fungal cells of the young peridium, embedded in plastic, sectioned and stained with new fuchsin and cristal violet (H.U.V. 15059). **F.** Spore balls in different developmental stages, hand sectioned, stained with cotton blue in lactophenol (H.U.V. 15059). **G–H.** Spore germination in water, at room temperature, in 3–5 days (H.U.V. 15059). Bars: A = 100 μ m, B–H = 10 μ m.

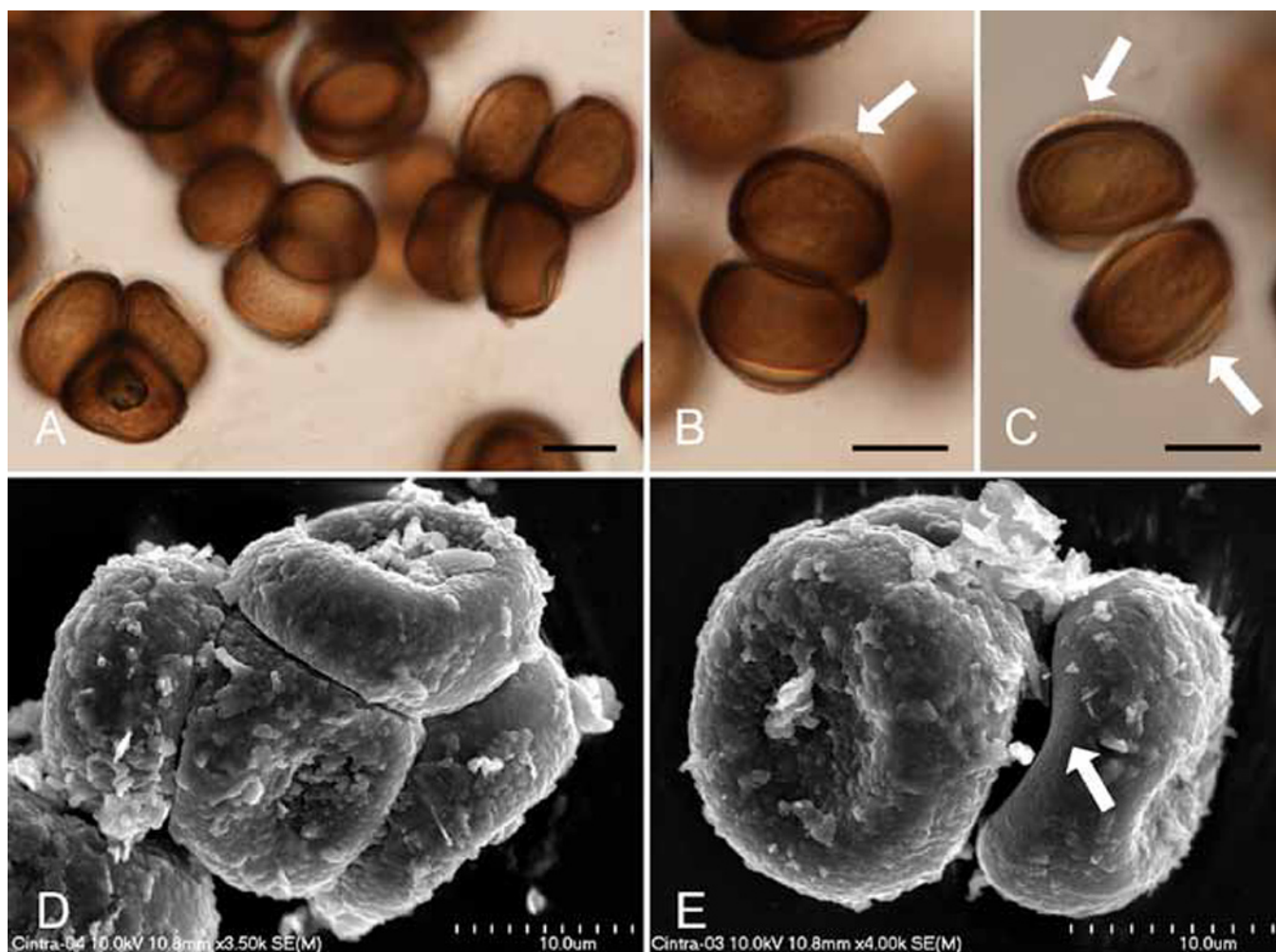


Fig. 4. *Shivasia solida* on *Schoenus apogon* (KRAM F-49115). **A–C.** Spore balls and spores seen in LM, note mucilaginous layer (subhyaline caps) around spores marked by arrows. **D–E.** Spore balls and spores seen in SEM, note that spores are enclosed by remnants of mucilaginous layer that form (pseudo-)ornamentation, while the spore surface is verruculose as marked by arrow. Bars = 10 µm.

Sori (Figs 2–3) in all flowers of an inflorescence, comprising the innermost floral organs, visible between the glumes as black, globose to ovoid bodies, 1–2 mm diam, rarely also on the stems, then fusiform, at first covered by a thick, whitish brown fungal peridium of thick-walled, sterile hyphae that early flakes away exposing the compact mass of spore balls with spores, powdery on the surface. *Spore balls* (Fig. 4) usually irregular or globoid to ellipsoidal, composed of 2–15 spores, loose but rather permanent, 25–55(–70) × 20–40 µm, reddish brown, enclosed by subhyaline mucilaginous layer. *Spores* (Fig. 4) subglobose, ovoid, elongate or irregular, flattened on one or two sides, 15–20 × 12–16 µm, yellowish to pale reddish brown; wall uneven, 0.5–1.5 µm thick, smooth to rough, in SEM finely, densely, irregularly verruculose and covered by remnants of the mucilaginous layer which form irregularly warty (pseudo-)ornamentation. *Spore balls and spores* produced on the surface of host tissues in hyaline, sporogenous fungal layer within radially arranged, U-shaped pockets (Fig. 3C–F). *Spore germination* (Fig. 3G–H; on water, at room temperature, in 3–5 d) results in long, aseptate basidia on which apically elongated, cylindrical basidiospores are produced that germinate by filaments.

The ITS/LSU epigenotype sequences are deposited in GenBank with the accession numbers JF966731/JF966730, respectively.

Hosts: On different *Schoenus* species (*Cyperaceae*): *S. apogon*, *S. calyptratus*, *S. carsei*, *S. cruentus*, *S. latelaminatus*, *S. maschalinus*, *S. nanus*, *S. nitens* var. *concinus*, *S. pauciflorus*, *S. tesquorum*, and *Schoenus* sp. (Table 1, Vánky & McKenzie 2002, Vánky & Shivas 2008).

Distribution: The genus and species are restricted to south-eastern Australasia: south-east Australia, including Tasmania, and north-west New Zealand (Fig. 5, based on the specimens included in Table 1 as well as in Vánky & McKenzie 2002, and Vánky & Shivas 2008).

DISCUSSION

In the present study molecular phylogenetic analyses and morphological data were used to clarify the systematic position of *Ustilago solida*. The molecular analyses revealed that this smut does not belong to any genus in which it

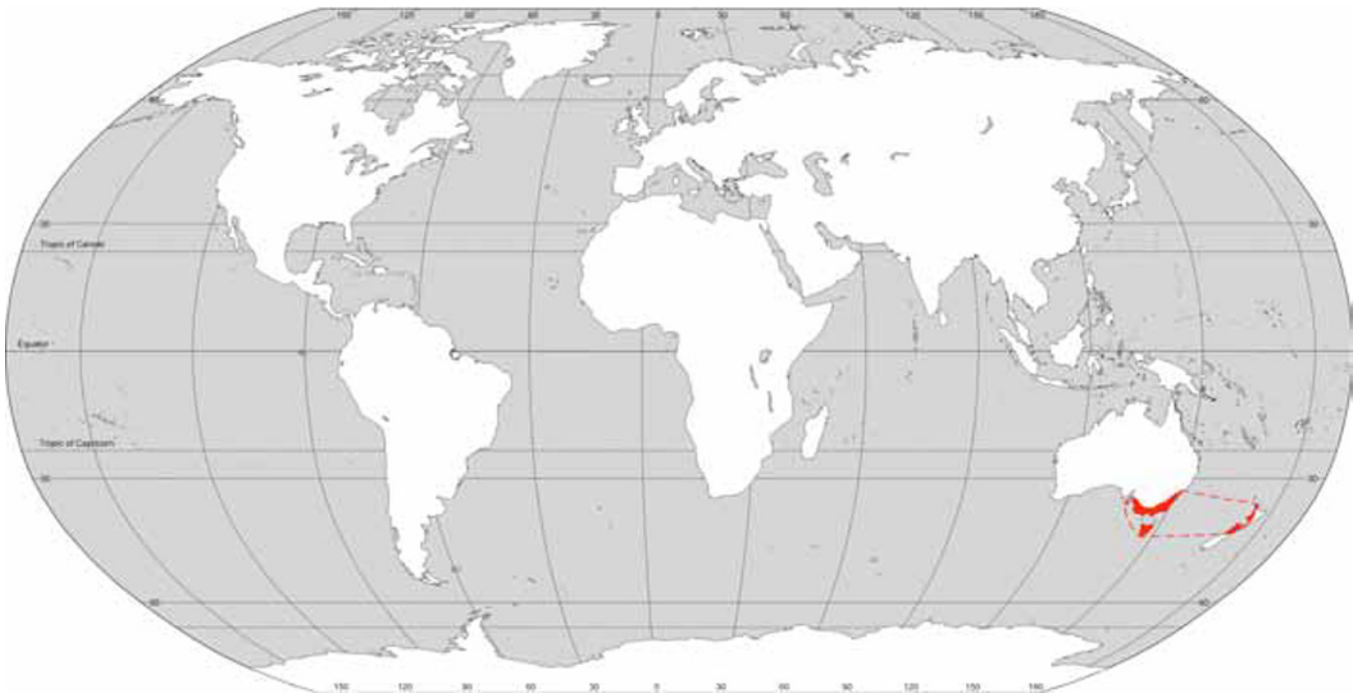


Fig. 5. Global distribution of *Shivasia solida*.

was included during the last 150 years. The morphological characteristics also contradict the placement of this species in *Ustilago*, *Urocystis*, *Sorosporium* as well as in *Cintractia*.

In the past the generic concept of *Ustilago* was very broad (Zundel 1953), but now this genus is restricted to smuts infecting poacean hosts, producing sori in vegetative or generative host plant organs, and having sori composed only of single spores, never forming spore balls (Vánky 2002). The species included in the genus *Urocystis* infect a wide range of host species from both mono- and dicotyledonous families, and are characterised by the production of spores in spore balls, which are usually enclosed by a complete or incomplete layer of sterile cells (Vánky 2002). The genus *Sorosporium* included in the past many, often unrelated smuts having spore balls (Zundel 1953), but the generic type, *Sorosporium saponariae*, has features of the genus *Thecaphora* with which it was merged based on both morphological and molecular phylogenetic analyses (Vánky 1998a, b, Vánky & Lutz 2007, Vánky *et al.* 2008a). *Thecaphora* (incl. *Sorosporium*) is restricted to smut fungi parasitic on dicotyledonous host plants, having sori with a granular-powdery spore ball mass that is yellowish, pale brown or dark reddish-brown, but never black or almost black (Vánky 2002, Vánky *et al.* 2008a). *Cintractia* species infect cyperaceous host plants forming single spores only (Vánky 2002, Piątek & Vánky 2007). The placement of *Ustilago solida* in *Cintractia* was in any case considered as provisional (Piepenbring 2000).

The most surprising result of the molecular analyses is that *Ustilago solida* is also not a member of the genus *Tolyposporium*, with which it shares many morphological traits, including the formation of sori in the floral organs and stems, sporulation within U-shaped pockets formed on the sterile stroma, and the development of spore balls (Piepenbring 2000, Vánky 2002). However, there are morphological differences between *Ustilago solida* and *Tolyposporium* that support the affiliation

to different phylogenetic lineages. *Ustilago solida* differs from the type species of *Tolyposporium* (i.e. *T. junci*) in spore wall ornamentation (rough or finely verruculose vs. irregularly, coarsely warty) and most notably in that spore balls of *U. solida* are enclosed by a mucilaginous layer that in light microscope is seen as subhyaline cap on the outer sides of spores, and in scanning electron micrographs as an irregularly warty (pseudo-) ornamentation. A mucilaginous layer around the spore balls is absent in *Tolyposporium*. This is in line with the observation of Piepenbring (2000) who used these two characteristics to differentiate *Tolyposporium* from *Cintractia solida*. It is worth noting that these two diagnostic features of *Tolyposporium* are easily applied to that three remaining species currently classified in this genus (*T. isolepidis*, *T. neillii*, and *T. piluliforme*).

The molecular analyses revealed that the closest phylogenetic relative of *Ustilago solida* may be *Heterotolyposporium lepidospermatis*, the type of the monotypic genus *Heterotolyposporium*. However, these two smuts differ considerably in LSU base sequences as well as in several morphological characteristics. In particular, the structure of the sori could hardly be considered congeneric. *Heterotolyposporium lepidospermatis* develops sori in the inflorescence of *Lepidosperma ensiforme*, with powdery spore masses replacing all central floral organs. A peridium is lacking and the sori are covered only by the outermost 3–4 glumes (Vánky 1997, 2002). This contrasts with the sori of *Ustilago solida* that are coated, at least in young stages, by a peridium, and the innermost floral organs are covered by a sterile stroma with U-shaped pockets within which spore balls are produced. The characteristics of the spore balls that differentiate *Ustilago solida* and *Tolyposporium* also distinguish *Ustilago solida* and *Heterotolyposporium*. The presence of two kinds of spores, originally used as the main diagnostic feature of *Heterotolyposporium* (Vánky 1997), is systematically irrelevant at the genus level since this character

Table 2. Endemic smut genera in particular continents.

Continent	Endemic smut genera
Africa	<i>Eriosporium</i> , <i>Geminago</i> , <i>Talbotiomyces</i>
Australasia	<i>Anomalomyces</i> , <i>Centrolepidosporium</i> , <i>Farysporium</i> , <i>Fulvisporium</i> , <i>Heterotolyposporium</i> , <i>Macalpinomyces</i> , <i>Pseudotracya</i> , <i>Restiosporium</i> , <i>Shivasia</i> , <i>Websdanea</i>
Asia	<i>Ahmadiago</i> , <i>Floromyces</i> , <i>Franzpetrakia</i> , <i>Georgefischeria</i> , <i>Liroa</i> , <i>Phragmotaenium</i> , <i>Zundeliomyces</i>
Europe	<i>Doassinga</i> , <i>Flamingomyces</i> , <i>Melanustilospora</i> , <i>Parvulago</i>
North America	<i>Clintamra</i> , <i>Exoteliospora</i> , <i>Planetella</i> , <i>Salmacisia</i> , <i>Tilletiaria</i>
South America	<i>Kuntzeomyces</i> , <i>Oberwinkleria</i> , <i>Uleiella</i>

evolved convergently in *Tolyposporium piluliforme*, which is only distantly related to *Heterotolyposporium* (Piepenbring *et al.* 1999, Vánky & Lutz, in Vánky 2008b).

Both genetic and morphological features reveal *Ustilago solida* as a unique smut that should be accommodated in a distinct genus, described here as *Shivasia*. This is in line with the current generic concept in smut fungi where distinct phylogenetic lineages distinguishable by morphological and/or ecological characters are referred to distinct genera (e.g., Piepenbring *et al.* 1999, Vánky *et al.* 2006, 2008b, Lutz *et al.* 2012). The close phylogenetic relation of *Heterotolyposporium* and *Shivasia* is reflected in the close phylogenetic relation of their host genera. Both *Lepidosperma* and *Schoenus* are placed in the same tribe *Schoeneae* within the *Cyperaceae* (Verboom 2006, Simpson *et al.* 2007, Muasya *et al.* 2009). Thus co-speciation of parasites and hosts may have played a role in the evolution of these two smuts.

In addition to resolving the phylogenetic and systematic placement of *Ustilago solida*, this study reports an emerging number of smut genera currently known exclusively from Australasia. Based on the present knowledge, and considering the relatively uniform and stabilized generic concept in smut fungi, they may be treated as endemic to this ecozone. The high rate of endemic smuts in Australia has been discussed at length by Shivas & Vánky (2003) and less extensively also by Vánky & Shivas (2008). Amongst the 309 species reported from the continent (Vánky & Shivas 2008, Barrett *et al.* 2009, McTaggart & Shivas 2009a, b, Shivas & McTaggart 2009, Vánky 2009, Shivas *et al.* 2010, Piątek & Shivas 2011, Shivas *et al.* 2011, Crous *et al.* 2012) about half are endemic. Six genera were considered to be endemic to Australia by Vánky & Shivas (2008), namely *Anomalomyces*, *Centrolepidosporium*, *Farysporium*, *Fulvisporium*, *Pseudotracya*, and *Websdanea*, but in fact *Farysporium* should be deleted as a strictly Australian endemic since it also occurs in New Zealand. This removal is balanced by adding *Heterotolyposporium* because, in the present circumscription, that genus has only one species, *H. lepidospermatis*, known from only one locality in Tasmania. Four additional genera are endemic to Australasia, being present in both Australia and New Zealand (*Farysporium*, *Restiosporium*, and *Shivasia*) or in Australia and Papua New Guinea (*Macalpinomyces*). Until recently *Macalpinomyces* included many unrelated smuts from different parts of the globe, but recent molecular analyses (Stoll *et al.* 2005) narrowed this genus to the type species *M. eriachnes* that occurs exclusively in Australasia. Thus, ten endemic genera occur in the Australasian ecozone and this number is not comparable with any other continent since all

of them have lower numbers of endemic smut genera (Table 2). The high number of unique smut genera may point to rapidly evolving characters and/or may result from the regional history, including the long geographic isolation of Australasia which started with the break-up of Gondwana and the initial separation of Australia and Tasmantia (incl. New Zealand) terranes from Antarctic terranes about 96 Myr and 84 Myr ago, respectively (McLoughlin 2001).

Shivasia solida has been reported on ten different *Schoenus* species. Recent molecular analyses of different smut genera (Lutz *et al.* 2005, Carris *et al.* 2007, Vánky & Lutz 2007, Bauer *et al.* 2008, Lutz *et al.* 2008, Kemler *et al.* 2009, Piątek *et al.* 2011, Lutz *et al.* 2012, Piątek *et al.* 2012, Savchenko *et al.* 2012) revealed that such polyphagous species are usually complexes of morphologically similar cryptic species that are often restricted to single host plant species. However, in the present study sequences were obtained only from specimens on *Schoenus apogon*, while repeated attempts to obtain sequences from specimens on two other hosts (*Schoenus pauciflorus*, H.U.V. 16755, and *Schoenus tesquorum*, H.U.V. 20073) failed. Thus, the question whether the collections on other *Schoenus* species than *Schoenus apogon* are genetically identical or cryptic species exist within what we now consider as one species is left open for future studies. To stabilise the taxonomy of *Shivasia solida* and to make future genetic comparisons possible an epitype is selected here based on the sequenced specimen collected on the same host (*Schoenus apogon*) in the same geographical area (Tasmania) as the holotype. This is in concordance with recent recommendations for epitypifications (Hyde & Zhang 2008).

The genus *Schoenus* shows greatest species diversity in Australasia. This is reflected by the inhabiting smut fungi – amongst seven smuts known on this genus worldwide, five occur in Australasia (Vánky & Websdane 1995, Vánky 2009). The two extralimital species are *Anthracoidea andina* in southern South America (Argentina: Tierra del Fuego) and *Moreaua kochiana* in Central Europe (Italy and Switzerland). The *Schoenus* smuts belong to three genera, *Anthracoidea*, *Moreaua* and *Shivasia*, that are not closely related, suggesting that at least three independent colonisation events took place onto this host plant genus in the course of evolution.

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