

Molecular sequence data assess the value of morphological characteristics for a phylogenetic classification of species of *Cintractia*¹

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Abstract: The genus *Cintractia* s. l. (Ustilaginales, basidiomycetes) comprises about 40 species of smut fungi infecting Cyperaceae and Juncaceae. These organisms form a phenetic continuum with respect to their sets of morphological character states, which do not allow a clear delimitation of infrageneric groups. According to sequence data of the large subunit ribosomal DNA of 17 species of *Cintractia* s. l. and related taxa of other genera of smut fungi, *Cintractia* s. l. is polyphyletic. Five groups of species shown by sequence data are characterized morphologically as different genera: *Cintractia* s. s., *Tolyposporium*, and the new genera *Gymnocintractia*, *Leucocintractia*, and *Stegocintractia*. Species of *Cintractia* s. s. and *Tolyposporium* form one clade, which is supported morphologically by similar soral structure. In the phylogram based on large subunit rDNA sequence data presented here, *Cintractia* s. s. seems to be paraphyletic, but the bootstrap values of this topology are very low and members of *Cintractia* s. s. and *Tolyposporium* are morphologically easily distinguishable by the presence of irregularly roughly warty teliospores in balls in species of *Tolyposporium* and single, finely warty teliospores in species of *Cintractia* s. s. Species of *Gymnocintractia* and those of *Leucocintractia* are sister taxa, although the former are similar to species of *Stegocintractia* by the absence of a sterile stroma in the sori and foveolate teliospores, and the latter to those of *Cintractia* s. s. by the presence of a sterile stroma, peridia, and nonfoveolate teliospores. Distinctive morphological characteristics were, however, found, such as the presence of fungal peridia in sori of species of *Stegocintractia* and their absence in those of species of *Gymnocintractia*, teliospores with rough warts forming ridges in species of *Leucocintractia* and

finely warty ones in species of *Cintractia* s. s. Hosts of species of *Cintractia* s. s., *Gymnocintractia*, and *Leucocintractia* belong to the Cyperaceae, those of species of *Stegocintractia* and *Tolyposporium* to the Juncaceae.

Key Words: *Gymnocintractia*, *Leucocintractia*, LSU rDNA, molecular phylogeny, phenetic continuum, *Stegocintractia*, *Tolyposporium*, Ustilaginales

INTRODUCTION

The Ustilaginales Clinton emend. R. Bauer et Oberwinkler (Bauer et al 1997) are delimited by ultrastructural characteristics (Bauer et al 1997), molecular sequences of nuclear DNA of the ribosomal large subunit (Begerow et al 1997), and characteristics of teliospore development (Piepenbring et al 1998b). The phylogenetic relationship of the ca 20 genera within this order, however, is not well known. The present study focuses on species attributed to *Cintractia* s. l.

Ling (1950a, 1950b, 1951) revised 17 species of *Cintractia* (two of them as varieties) in three groups, i.e., species related to *C. axicola* (Berk.) Cornu, species related to *C. montagnei* (Tul. & C. Tul.) Magnus, and species related to *C. leucoderma* (Berk.) Henn. In a more comprehensive treatment of this genus (Piepenbring unpubl), 42 species are included in *Cintractia* s. l. They are characterized by dark masses of teliospores, which are mostly single, often flattened, and sometimes carry hyaline appendages. Host species belong to Cyperaceae and Juncaceae. The type species *C. axicola* has finely warty teliospores and sori with sterile fungal hyphae forming peridia and a sterile stroma with sporogenous pockets (e.g., Cornu 1883, Thirumalachar and Whitehead 1975). *Cintractia montagnei*, *C. luzulae* (Sacc.) Clinton and related species with foveolate teliospores are also included in *Cintractia* s. l. Some authors placed these species in *Ustilago* (e.g., Lehtola 1942, Boidol and Poelt 1963: 23, Vánky 1994), because they did not observe sterile fungal structures in sori of these species. The necessity of a taxonomic revision which may result in a new genus for species of the *C. montagnei* group was discussed by Savile and Parmelee (1953: 790), Scholz and Scholz (1988: 73), and Vánky (1994: 17). These authors did not present taxonomic

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conclusions because data about sorus structure, teliospore development, and teliospore germination were lacking for many species.

The morphological analysis, presented here and by Piepenbring (unpubl), however, does not allow a differentiation of phylogenetic lineages, because the distribution of morphological characteristics is too complex. There are no sharp limits between infrageneric groups—a common result as more species of a given relationship are known and well investigated (Oberwinkler 1991: 37). In order to develop a classification that is practical for the identification of genera and also reflects phylogeny (Stace 1978), it is desirable to combine morphological observations and molecular data. This is considered a good way to maximize the reliability of phylogenetic hypotheses (Hillis 1987: 24, McLaughlin et al 1995: S691). Studies of this kind are rare, because of the lack of reliable morphological and molecular data for the same taxa, especially among fungi (McLaughlin et al 1995: S688). The present publication represents an example for an integrative approach to phylogenetic morphological concepts of genera based on morphological and molecular data.

MATERIALS AND METHODS

Specimens of species of *Cintractia* s. l.: *C. amazonica* H. Sydow & Sydow on *Rhynchospora barbata* (Vahl) Kunth, MP 2008: AJ 236142; *C. axicola* (Berk.) Cornu on *Fimbristylis dichotoma* (L.) Vahl, HOLOTYPE (K), MP 10, MP 1168: AF 009847; *C. cubensis* M. Piepenbr. on *Rhynchospora microcephala* Britton, HOLOTYPE (MP 2252, in M): AJ 236153; *C. cyperi* Clinton on *Cyperus filiculmis* Vahl, ISOTYPE (BPI); *C. fimbriatylicola* Pavgi & Mundkur on *Fimbristylis spadicea* (L.) Vahl, MP 471, MP 1733, MP 2213: AJ 236143; *C. fimbriatylis-miliaceae* (Henn.) Ito on *Fimbristylis littoralis* Gaudich., HOLOTYPE of *C. pulchra* Ito (BPI), H.U.P. 1259; *C. gigantospora* Liro on *Rhynchospora alba* (L.) Vahl, MP 2335: AJ 236144; *C. hyperborea* (A. Blytt) Liro on *Luzula confusa* Lindeberg, HOLOTYPE (O); *C. junci* (Schwein.) Trel. on *Juncus tenuis* Willd., Ohio Fungi 66 (M), H.U.P. 690; *C. leucoderma* (Berk.) Henn. on *Rhynchospora barbata* (Vahl) Kunth, HOLOTYPE (K), ISOTYPE of *C. amicta* Cif. (H.U.V.); *C. aff. leucoderma* on *Rhynchospora holoschoenoides* (L. C. Rich.) Herter, MP 1518, MP 2026: AJ 236145; *C. lidii* Liro on *Juncus biglumis* L., HOLOTYPE (O); *C. limitata* Clinton on *Cyperus ligularis* L., MP 1594: AJ 236146; *C. cf. limitata* on *Cyperus rotundus* L., MP 1310: AJ 236147; *C. luzulae* (Sacc.) Clinton on *Luzula pilosa* (L.) Willd., MP 2340: AJ 236148; *C. major* (Desm.) Liro on *Rhynchospora alba* (L.) Vahl, Vánky, Ust. Exs. 879 (“*Ustilago intercedens*”), H.U.P.; *C. michellii* Vánky on *Fimbristylis schultzei* Boeck., H.U.V. 17666: AJ 236149; *C. montagnei* (Tul. & C. Tul.) Magnus on *Rhynchospora alba* (L.) Vahl, ISOTYPE (BPI), MP 2344: AJ 236150; *C. aff. montagnei* on *Rhynchospora globosa* (Kunth) Roemer & Schultes, MP 925: AJ 236151; *C.*

nova-guineae Zundel on *Rhynchospora cf. rugosa* (Vahl) Gale, HOLOTYPE (BPI); *C. pachyderma* Sydow on *Rhynchospora corniculata* (“?”) A. Gray, BPI 172059; *C. piluliformis* (Berk.) Henn. on *Juncus planifolius* R. Br., as “*Heterotolyposporium piluliforme* (Berk.) K. Vánky” in H.U.V. 15732: AF 009871; *C. portus-argenti* Cif. on *Rhynchospora* sp., Herb. Cif. 3448 (M); *C. samanensis* Cif. on *Rhynchospora fascicularis* (Michx.) Vahl ssp. *fascicularis*, MP 2106: AJ 236152; *C. scleriae* (DC.) Ling on *Rhynchospora triflora* Vahl, MP 93, MP 2074: AJ 236154; *C. spadicea* Liro on *Luzula alpinopilosa* (Chaix) Breistr., MP 2350: AJ 236155; *C. standleyana* Zundel on *Rhynchospora marisculus* Lindley & Nees, MP 1779; *C. aff. standleyana* on *Rhynchospora microcarpa* Baldwin ex Gray, DB 575; *C. taubertiana* (Henn.) Clinton on *Rhynchospora tenuis* Link, MP 1801, MP 2276: AJ 236156; on *Rhynchospora cf. tenuis* Link, MP 2133; *C. cf. taubertiana* (Henn.) Clinton on *Rhynchospora fascicularis* (Michx.) Vahl ssp. *fascicularis*, MP 2111; *Tolyposporium junci* (Schröter) Woronin on *Juncus bufonius* L., H.U.P. 1254, H.U.V. 17169: AF 009876; *Ustilago diplasiae* Henn. on *Diplasia karatifolia* C. L. Rich., ISOTYPE (HBG); *Ustilago solida* Berk., in Hooker, on *Schoenus apogon* Roemer & Schultes var. *apogon*, Vánky, Ust. Exs. 753 (“generic position uncertain”, H.U.P.). Many more specimens of *Cintractia* s. l. were studied for the comprehensive treatment of this genus (Piepenbring unpubl).

Additional specimens included in the sequence analysis: *Dermatosorus cyperi* Vánky on *Cyperus celluloso-reticulatus* Boeck., H.U.V. 15991: AJ 236157; *Farysia chardoniana* Zundel on *Carex polystachya* Swartz ex Wahlenb., MP 2062: AF 009859; *Melanotaenium endogenum* (Unger) de Bary on *Galium mollugo* L.: AJ 235294; *Melanotaenium euphorbiae* (Lenz) Whitehead & Thirum. on *Euphorbia heterophylla* L., H.U.V. 17733: AJ 236135; *Schizonella cocconii* (Morini) Liro on *Carex flacca* Schreber, DB 614: AJ 236158; *S. melanogramma* (DC.) Schröter on *Carex pilulifera* L., FO 37174: AF 009870; *Sporisorium ophiuri* (Henn.) Vánky on *Rottboellia cochinchinensis* (Lour.) W. Clayton, HB 20: AJ 236136; *S. ovarium* (Griff.) Vánky on *Urochloa fasciculata* (Sw.) R. Webster, MP 1871: AJ 236137; *S. sorghi* Ehrenb. ex Link on *Sorghum bicolor* (L.) Moench, MP 2036a: AF 009872; *Tolyposporium rhynchosporae-cephalotis* Vánky & T. Vánky on *Rhynchospora cephalotes* (L.) Vahl, H.U.V. 15199: AJ 236160; *Trichocintractia utriculicola* (Henn.) M. Piepenbr. on *Rhynchospora corymbosa* (L.) Britton, MP 2075: AF 009877; *Ustilago avenae* (Pers.) Rostrup on *Arrhenatherum elatius* (L.) Presl, GD 1292: AJ 236140; *U. hordei* (Pers.) Lagerh. (host not known): L 20286; *U. maydis* (DC.) Corda (host not known): L 20287; *U. nuda* (Jensen) Kellerman & Swingle on *Hordeum leporinum* Link, H.U.V. 17782: AJ 236139; *U. scitaminea* H. Sydow on *Saccharum* sp. cult., MP 541: AJ 236138; *U. trichophora* (Link) Körn. on *Echinochloa colonum* (L.) Link, MP 1898: AJ 236141; *Websdanea lyginiae* (Websdane et al.) Vánky on *Lyginia barbata* (Labill.) R. Br., H.U.V. 17900: AJ 236159.

DB (D. Begerow), FO (F. Oberwinkler), GD (G. Deml), HB (H.-J. Prillinger), and MP (M. Piepenbring) are collection numbers of specimens deposited in the corresponding private herbaria. H.U.V. is the abbreviation of the Herbarium Ustilaginales K. Vánky, H.U.P. of the Herbarium Ustilaginales Piepenbring. Further abbreviations correspond to

official herbaria. AF, AJ, and L numbers are GenBank accession numbers.

Soral morphology was analyzed by light microscopy (LM) on hand-cut sections in water and on semithin sections (SEMI, 1–1.5 μm thick) of material embedded for transmission electron microscopy (TEM, Piepenbring 1995). Teliospore germination was obtained from fresh spores dusted on water agar (WA). Germinating spores were observed by LM on sections of WA covered by a cover slip. Dry spores were fixed on double adhesive tape and sputter coated with gold-palladium (ca 20 nm) to study their ornamentation by scanning electron microscopy (SEM).

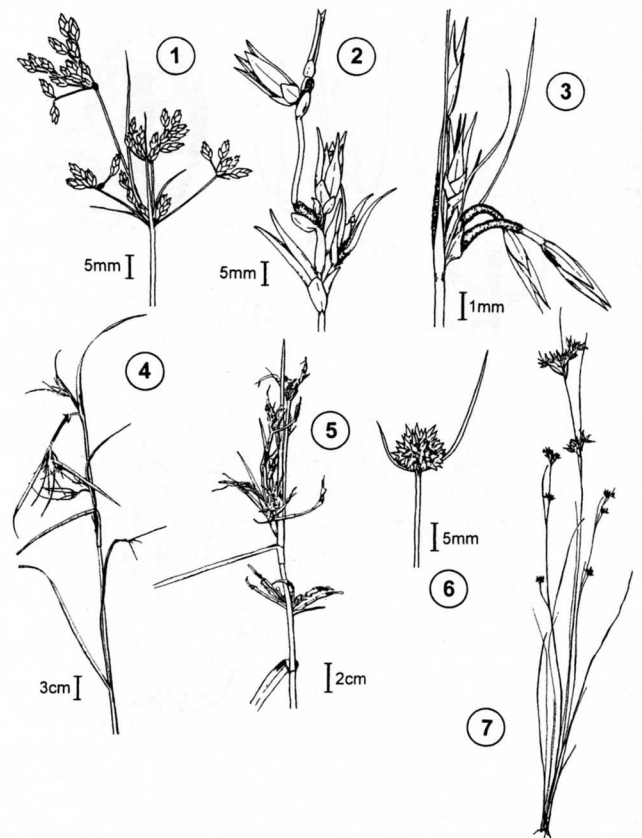
DNA was isolated from cultures or herbarium specimens following a modified version of the SDS method (Begerow et al 1997). An alignment of 551 bp was produced using the ClustalW algorithm of the Megalign package, Lasergene (DNASTAR Inc. 1997). The sequence data were analyzed with the PHYLIP package version 3.572 (Joseph Felsenstein 1995). We used neighbor joining and maximum parsimony methods to construct phylogenetic trees from LSU rDNA sequences. Neighbor joining analysis of a distance matrix produced by DNAdist (Kimura 2-parameter model, transition/transversion rate: 2.0) was applied with the default parameters of the program. Maximum parsimony analysis was performed by DNAPars (jumble option: 100) and Consens. Bootstrapping consisted of 1000 replicates for neighbor joining and of 100 replicates for maximum parsimony, using SeqBoot and Consens of the PHYLIP package with default parameters. The alignment is available upon request.

RESULTS

Morphology.—In the following, morphological characteristics and their states are described and illustrated for selected examples (for more complete documentation see Piepenbring unpubl). Numbers with small letters refer to character definitions used to describe the distribution of character states of 31 species of *Cintractia* s. l. (see FIG. 36).

Sori can develop locally, probably due to local infection (FIG. 1; 1a) or in groups (infection probably partly systemic, 1b), but not in all the respective organs of the host plant. When many organs, but not all of them, are used for soral development, the type of infection cannot be assessed (FIG. 2). The presence of sori in all the respective organs available in an infected plant is thought to be the result of systemic infection (FIGS. 3–7; 1c). Members of *Cintractia* s. s. infect their hosts mostly locally while species of the groups of *C. leucoderma*, *C. montagnei*, and *C. luzulae* show systemic or partly systemic infection.

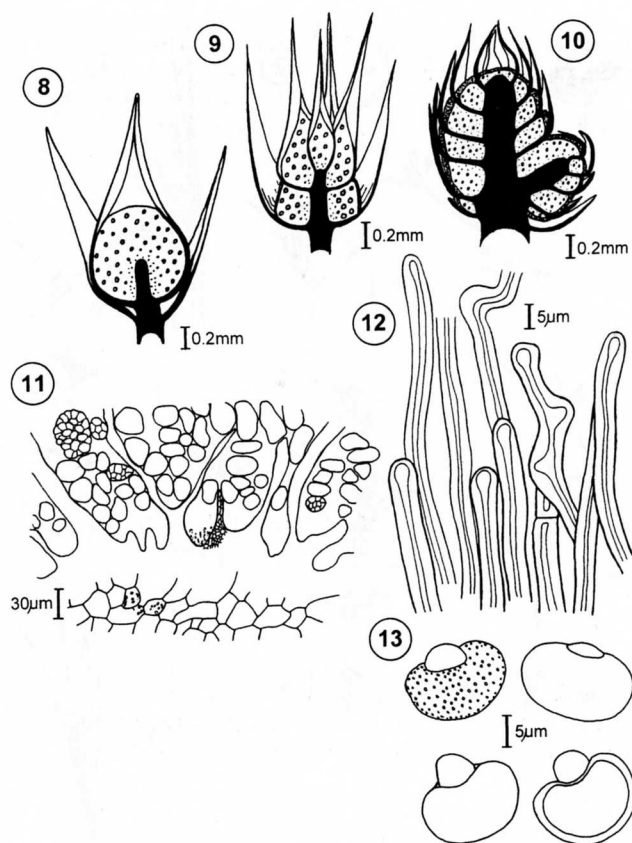
Sori can surround developed organs, like stems, pedunculi (FIGS. 1–5), ovaries or filaments (2a). Sori are formed at the base of internodes in species of the *C. axicola* group (FIGS. 1, 2) and in the middle parts of internodes in species of the *C. scleriae* group (FIGS. 4, 5). In *C. montagnei* and closely related species and



FIGS. 1–7. Plants infected by species of *Cintractia* s. l. 1. Sori of *C. axicola* at the bases of pedunculi in an inflorescence of *Fimbristylis dichotoma* (MP 1168). 2. Part of an inflorescence of *Juncus bufonius* infected by *Tolyposporium junci* (H.U.P.1254). 3. Part of an inflorescence of *Juncus tenuis* around the peduncles (Ohio Fungi 66). 4–5. Infected plants of *Rhynchospora* spp. with rudimentary inflorescences and sori partially covered by white peridia. 4. *R. holoschoenoides*, with sori of *C. aff. leucoderma* (MP 1518). 5. *R. corymbosa* with sori of *C. scleriae* (MP 93). 6. Inflorescence of *Rhynchospora barbata* infected by *C. amazonica* (MP 2008). 7. Several plants of *Rhynchospora tenuis* stunted by infection of *C. taubertiana* and one healthy, larger plant (MP 1801).

in *C. amazonica* sori develop at the apex of the axis of sterile spikelets (FIG. 8; 2b). In species of the *C. luzulae* group sori usually develop around the base of sterile glumes of spikelets (FIGS. 9, 10; 2c).

Teliospores develop in pockets of a sterile stroma made of sterile fungal hyphae in species of the *C. axicola* group, species of the *C. scleriae* group, and also in the spore ball forming species *U. solida*, *C. piluliformis*, and *T. junci* (FIG. 11; 3a). Teliospores of other species of *Cintractia* s. l. develop directly on the surface of the host organs (FIGS. 8–10; 3b). When young, sori of *C. axicola*, *C. scleriae*, and species closely related to them are covered by peridia made of thin gelatinized fungal hyphae (4a). Sori of species



FIGS. 8–13. Soral structures of species of *Cintractia* s. l. 8–10. Schematically drawn, longitudinal sections through sori in spikelets. Black: host tissue in section; dots and circles: young and mature teliospores; fine dots: peridia. 8. *C. gigantospora* (MP 2335). 9. *C. luzulae* (MP 2340). 10. *C. spadicea* (MP 2350). 11. Schematically drawn section through the sporogenous zone of *Tolyposporium junci*, showing from below upwards: host tissue with parasitic hyphae, sterile fungal stroma forming pockets and strands into the mass of teliospore balls of different ages. Details are only partially drawn (drawn from a SEMI, H.U.V. 17169). 12. Sterile hyaline hyphae from the peridium of *C. spadicea* (MP 2350). 13. Teliospores with appendages of *C. standleyana*, one drawn with ornamentation in surface view, two in surface view without ornamentation, and one in section (MP 1779).

of the *C. montagnei* group lack fungal peridia (FIG. 8; 4b), but are usually covered by glumes. *Cintractia luzulae* and closely related species have peridia (FIGS. 9, 10) of thick-walled, rarely septate hyphae, with walls thinner in the apical part than in the rest of the hyphae (FIG. 12; 4c).

Teliospores of most species of *Cintractia* s. l. are single (FIGS. 13–15, 18–23, 25, 26), in some species they form permanent balls (FIGS. 16, 17; 5a) or loose groups (FIG. 24; 5b). Balls are mixed with single sterile cells in *C. piluliformis*. Single teliospores are mostly flattened and can carry appendages (FIGS. 13, 20;

6a). The appendages are hyaline, as seen by LM, and lie individually on one or two sides of a flattened spore. Often, only a small percentage of the spores of a given species carries appendages.

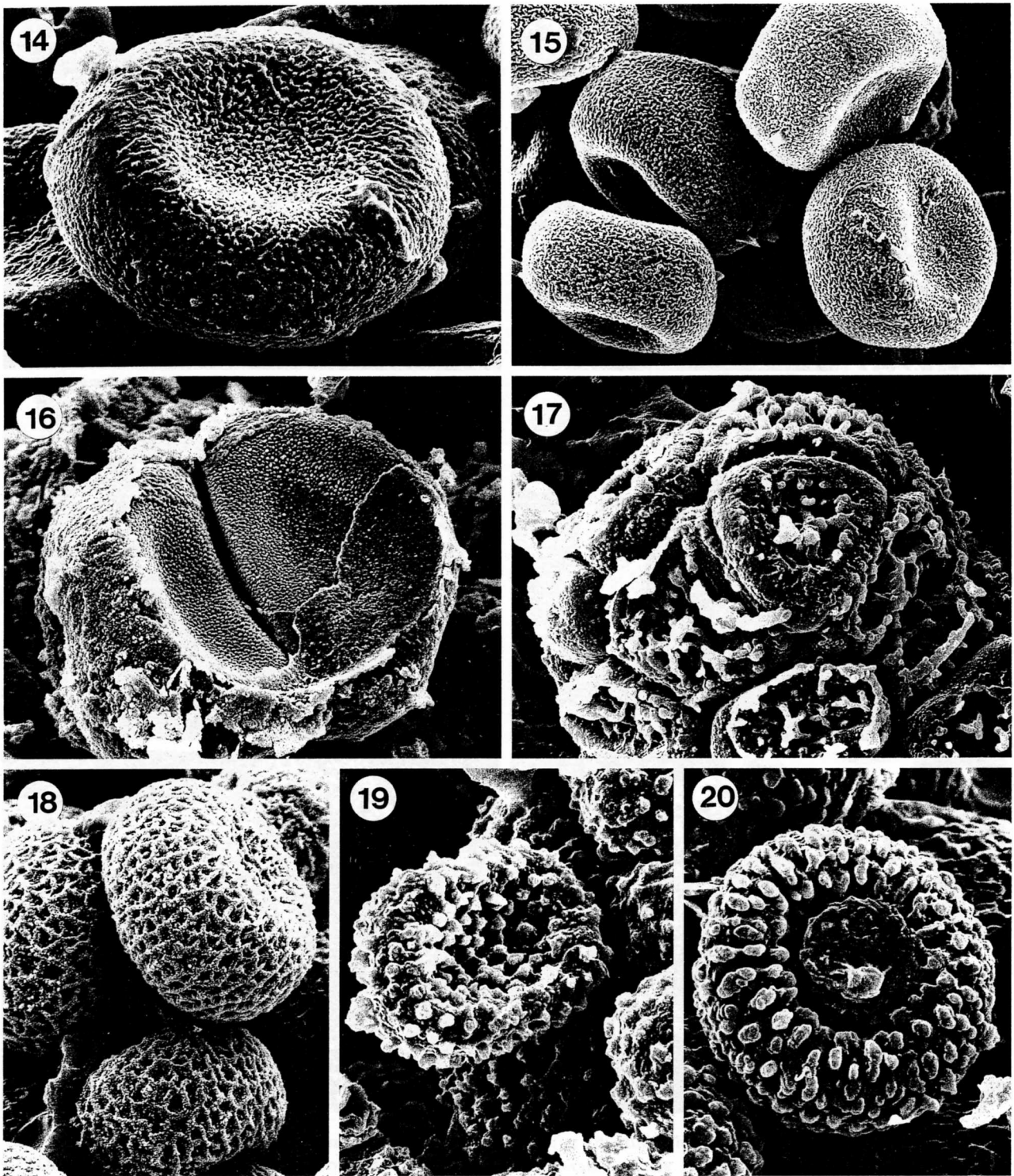
Teliospores are irregularly finely warty without foveolae (FIGS. 14–16; 7a), roughly irregularly warty (FIG. 17; 7b), irregularly reticulate (FIG. 18; 7c), regularly warty (FIGS. 19–21, 26; 7d), irregularly warty/reticulate with ridges at the sides (FIG. 22; 7e), foveolate without or with fine warts (FIGS. 23, 24; 7f) or foveolate with distinct warts (FIG. 25; 7g). In *C. cyperi* (FIG. 24), the foveolae are distinguishable only at high magnification as seen by SEM. As seen by LM, each teliospore of *C. hyperborea* has usually two light colored lines, considered as germ slits. These areas are collapsed, as seen by SEM (FIG. 26).

Recently collected teliospores of most species of *Cintractia* s. l. germinate on water agar after few days. The resulting basidia are mostly phragmobasidia with often four basidial cells (FIGS. 27–29). Only two basidial cells were observed for *C. cubensis* (Piepenbring 1999), more than four cells in basidia of *C. piluliformis* (as *Heterotolyposporium* sp., Vánky 1997: 153) and *C. samanensis* (Piepenbring 1999). Basidial cells can copulate by clamp connections or by copulation bridges (FIGS. 27, 29; 8a). Dikaryotic cells grow with hyphae which develop large dikaryotic conidia (e.g., Ingold 1995). Basidia with monokaryotic basidiospores also occur (FIG. 28; 8b) in these species. Other species exclusively develop basidia forming basidiospores without prior conjugation (FIGS. 30–32; 8b). FIG. 33 shows hyphal development of basidial cells, FIGS. 34 and 35 entire basidia growing as hyphae (8c).

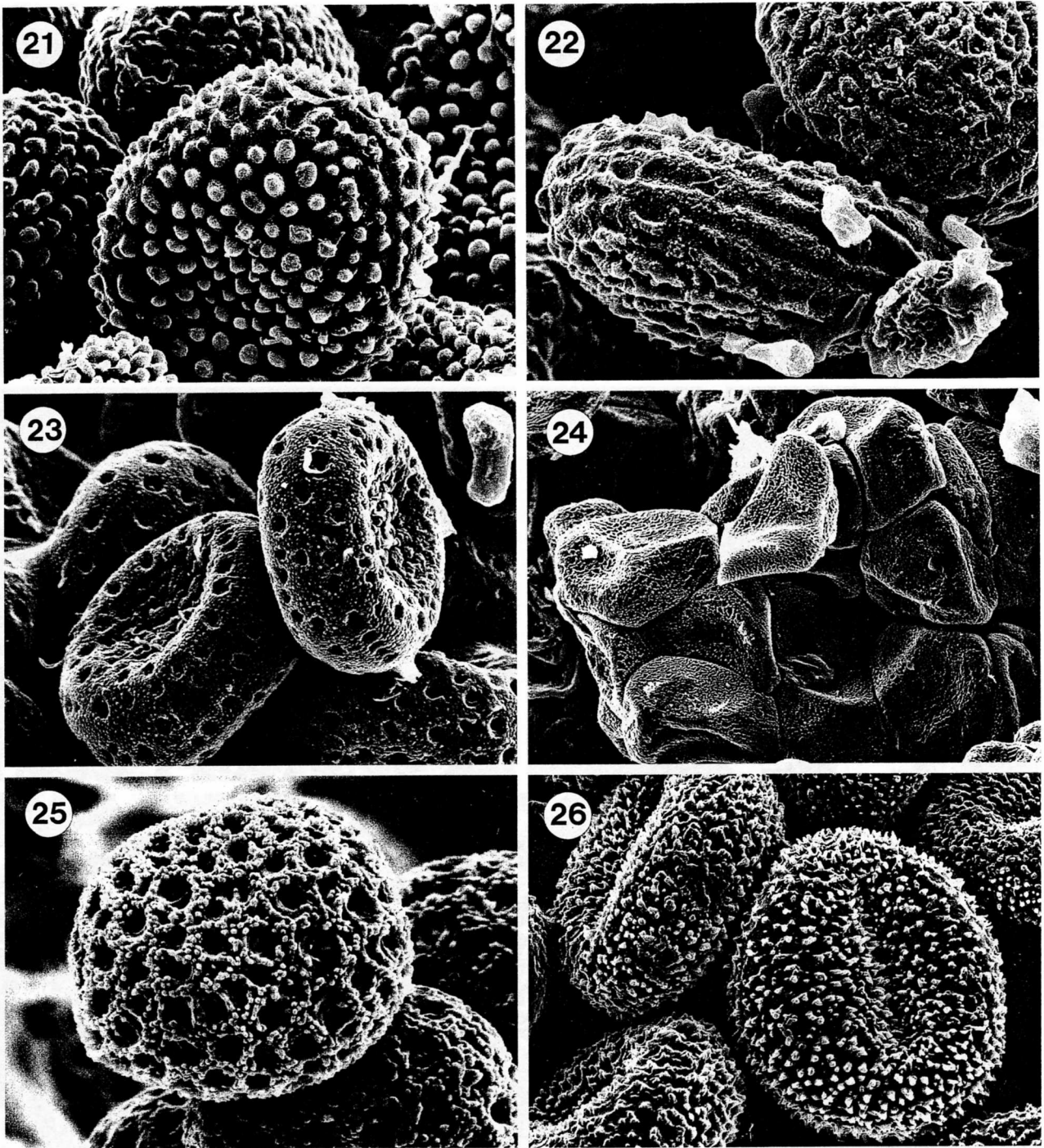
Host plants (9a, b) of species of *Cintractia* s. l. are known among Cyperaceae (*Cyperus*, *Dichronema*, *Diplasia*, *Fimbristylis*, *Kyllinga*, *Mariscus*, *Oreobolus*, *Psilocarya*, *Schoenus*, *Scirpus*, *Scleria*, *Rhynchospora*, and *Torulinium*) and Juncaceae (*Juncus* and *Luzula*).

States of 8 characteristics and host relationships are summarized for 31 species of *Cintractia* s. l. in FIG. 36. Selection of characteristics, character states, and the order of the species was influenced by the results obtained by molecular data (FIG. 37), i.e., we tried to identify characteristic morphological character states for phylogenetic groups.

Molecular data.—Phylogenetic trees (FIGS. 37, 38) are based on the analysis of 551 bp of LSU rDNA using the neighbor joining method (NJ, FIG. 37) and the maximum parsimony method (MP, FIG. 38). The latter yielded a strict consensus tree of 71 most parsimonious trees with 468 steps (CI: 0.7635–0.7657, RI: 0.4461–0.4483). In the two phylograms (FIGS. 37, 38), the topology of taxa is rather similar. There are three



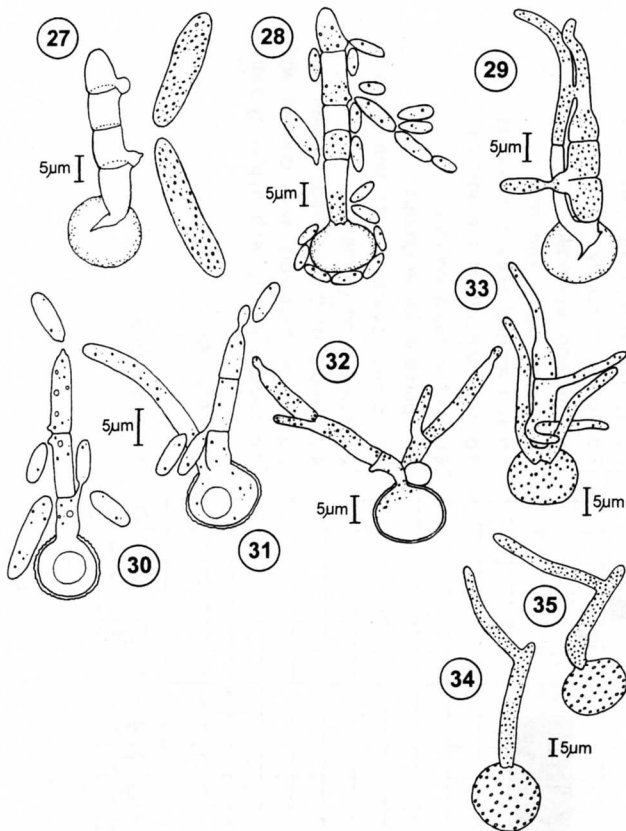
FIGS. 14–20. Teliospores of species of *Cintractia* s. l., as seen by SEM. 14. *C. axicola* (HOLOTYPE) $\times 4500$. 15. *C. amazonica* (MP 2008) $\times 2900$. 16. Teliospore ball of *Ustilago solida* with one cell broken off (Vánky, Ust. Exs. 753) $\times 3800$. 17. Teliospore ball of *Tolyposporium junci* (H.U.P. 1254) $\times 3200$. 18. *C. fimbriatylicola* (MP 471) $\times 4500$. 19. *C. fimbriatylis-miliaceae* (HOLOTYPE of *C. pulchra*) $\times 4750$. 20. *C. nova-guineae* (HOLOTYPE), teliospore with appendage $\times 3800$.



FIGS. 21–26. Teliospores of species of *Cintractia* s. l., as seen by SEM. 21. *Ustilago diplasiae* (ISOTYPE) $\times 2800$. 22. *C. leucoderma* (HOLOTYPE) $\times 4500$. 23. *C. aff. montagnei* (MP 925) $\times 4500$. 24. Group of teliospores of *C. cyperi* (ISOTYPE) $\times 1900$. 25. *C. spadicea* (MP 2350) $\times 4500$. 26. *C. hyperborea* (HOLOTYPE), teliospores with collapsed germ slits $\times 2900$.

clades formed by groups of species of *Cintractia* s. l. (including *T. junci*) and *C. fimbriatylicola* in an isolated position. The clade of the *C. axicola* group including *C. mitchellii*, *C. limitata*, and *C. amazonica* also contains the two spore ball forming species *T. junci* and *C. piluliformis*. Monophyly of the two latter

species is supported by a high bootstrap value, but their position in relation to the *C. axicola* group is not well resolved. There are no high bootstrap values for lineages among species of the *C. axicola* group, and for the relation of members of the *C. axicola* group to *C. fimbriatylicola*, *Trichocintractia utriculico-*



FIGS. 27–35. Teliospores of species of *Cintractia* s. l., which germinated with basidia on WA, as seen by LM. 27–28. *C. axicola* (MP 10). 27. Empty basidium with two large, probably dikaryotic conidia which developed after the conjugation of basidial cells by clamps. 28. Basidium with basidiospores. 29. *C. amazonica* (MP 2008). 30–31. *C. fimbri-stylicola* (MP 1733). 32. *C. standleyana* (MP 1779). Note the appendage close to the base of the basidium. 33. *C. taubertiana* (MP 1801), basidial cells grow with hyphae. 34–35. *C. luzulae* (MP 2340), teliospores germinating with hyphae.

la, and *Dermatosorus cyperi*. *Cintractia fimbri-stylicola* is placed separate from other examined species of *Cintractia* s. l. in the clade which includes species of the *C. axicola* group (FIG. 37). Another clade of species of *Cintractia* s. l. includes *C. montagnei* and related species, *C. scleriae*, and *C. aff. leucoderma*. In the NJ and MP trees (FIGS. 37, 38), this clade appears as a sister group of species of *Ustilago* and *Sporisorium*, all parasites of Poaceae, but this relationship received a bootstrap value of only 58% in FIG. 37. *Cintractia luzulae* and *C. spadicea* form a separate clade, located close to species of *Schizonella* and *Farysia*.

DISCUSSION

The pattern of morphological character states presented in the matrix in FIG. 36 does not show an obvious way to delimit infrageneric groups of *Cin-*

tractia s. l. Characteristics such as the presence of peridia and sterile stroma in the sori, local or systemic infection, teliospore ornamentation, and basidium morphology yield arguments for different groupings. *Cintractia amazonica* is related to the *C. axicola* group by the ornamentation of its teliospores and to the *C. montagnei* group by the morphology of its sori. The complex diversity of character sets exhibited by the different species represents a phenetic continuum of organisms (Sattler and Rutishauser 1997: 579). Based on sequence data (FIGS. 37, 38) however, morphological characteristics can be reevaluated and used for the definition of systematic groups.

The close relationship of the type species *C. axicola*, including *C. mitchellii*, and *C. limitata*, is morphologically evident by similar soral structure (1a, 2a, 3a, 4a) and teliospore ornamentation (7a). *Cintractia limitata*, known on numerous different species of *Cyperus* and related genera from tropical regions all over the world, probably includes several different species, as shown by the large genetic distance between *C. limitata* on the host of the type specimen of this species, *Cyperus ligularis*, (MP 1594) and the specimen called "*C. cf. limitata*" on *Cyperus rotundus* (MP 1310). *Cintractia amazonica* was included in the *C. montagnei* group based on the absence of sterile elements in its sori (3b, 4b) by Ling (1950a), but the fine, irregular warty ornamentation of its teliospores (7a) supports an inclusion in the *C. axicola* group, as suggested by molecular data. Peridia and sterile stroma are absent in *C. amazonica*, perhaps because there may not be enough space for these structures in the dense heads of spikelets of *Rhynchospora barbata* and because the sori are protected by glumes. The spore ball forming species *Ustilago solida* is included in the *C. axicola* group based on morphological characteristics such as the presence of sterile stroma (McAlpine 1910: 181, 184), peridia, and teliospores with fine irregular warts (3a, 4a, 7a), and because spore balls (5a) can evolve convergently (Bauer et al 1997: 1309). Sequence data of this species could not be obtained.

Tolyposporium junci and *C. piluliformis* belong to the lineage including species of the *C. axicola* group, as shown by molecular data and morphological aspects, like the presence of a sterile stroma and peridia in the sori (3a, 4a). Morphologically, the genus *Tolyposporium*, including *C. piluliformis* and *T. junci*, differs from species of the *C. axicola* group by teliospore balls with an irregularly roughly warty surface (5a, 7b). Sequence data presented in FIGS. 37, 38 suggest a paraphyletic origin of species belonging to the *C. axicola* group with species of the genus *Tolyposporium* located in the same clade. If this observation is confirmed by sequence data from other regions,

	1 a	1 b	2 a	3 a	4 a	6 a	7 a	8 a	8 b	5 a	7 b	7 c	7 d	1 c	7 e	2 b	3 b	4 b	5 b	7 f	2 c	4 c	7 g	8 c	9 a	9 b	
	infection local	infection partly systemic	sori around internodes or floral organs	sori with sterile stroma	peridia of gelatinized hyphae	spores at least rarely with appendages	spores finely warty	basidia with copulations	basidia with basidiospores	spores in balls	spores roughly, irregularly warty	spores irregularly reticulate	spores regularly warty	infection systemic	spores warty with ridges	sori around apex of spikelets	sori without sterile stroma	sori without peridia	spores in loose groups	spores foveolate with fine warts	sori around glumes	peridia of thick-walled hyphae	spores foveolate with distinct warts	basidia mostly with hyphal growth	host family	host genus	
C. axicola																									C	Fim	
C. limitata																										C	Cyp
C. mitchellii																										C	Fim
U. solida																										C	Sch
C. fimbristylicola																										C	Fim
C. fimbr.-miliaceae																										C	Fim
T. junci	?	?																								J	Jun
C. piluliformis	?	?																								J	Jun
C. scleriae																										C	Rhy
C. pachyderma																										C	Rhy
C. leucoderma																										C	Rhy
C. aff. leucoderma																										C	Rhy
C. portus-argenti																										C	Rhy
C. amazonica																										C	Rhy
C. montagnei																										C	Rhy
C. aff. montagnei																										C	Rhy
C. gigantospora																										C	Rhy
C. major																										C	Rhy
C. taubertiana																										C	Rhy
C. samanensis																										C	Rhy
C. cyperi																										C	Cyp
C. cubensis																										C	Rhy
U. diplasiae																										C	Dip
C. nova-guineae																										C	Rhy
C. standleyana																										C	Rhy
C. aff. standleyana																										C	Rhy
C. luzulae																										J	Luz
C. spadicea																										J	Luz
C. lidii																										J	Jun
C. junci																										J	Jun
C. hyperborea																										J	Luz

FIG. 36. Morphological characteristics (numbers), their character states (small letters), and host relationships of species of *Cintractia* s. l. (for description and illustration of characteristics see results). Bars indicate the presence of a character state in the respective species, "?" missing data. One species can show different states of one characteristic. Morphological characteristics of phylogenetic groups are shown by gray background. Names of type species of the genera belonging to *Cintractia* s. l. are written with bold letters. 9 a families: C = Cyperaceae, J = Juncaceae; b genera: *Cyperus*, *Diplasia*, *Fimbristylis*, *Juncus*, *Luzula*, *Schoenus*, *Rhynchospora*. If different genera are infected by the same species only the most important genus is mentioned.

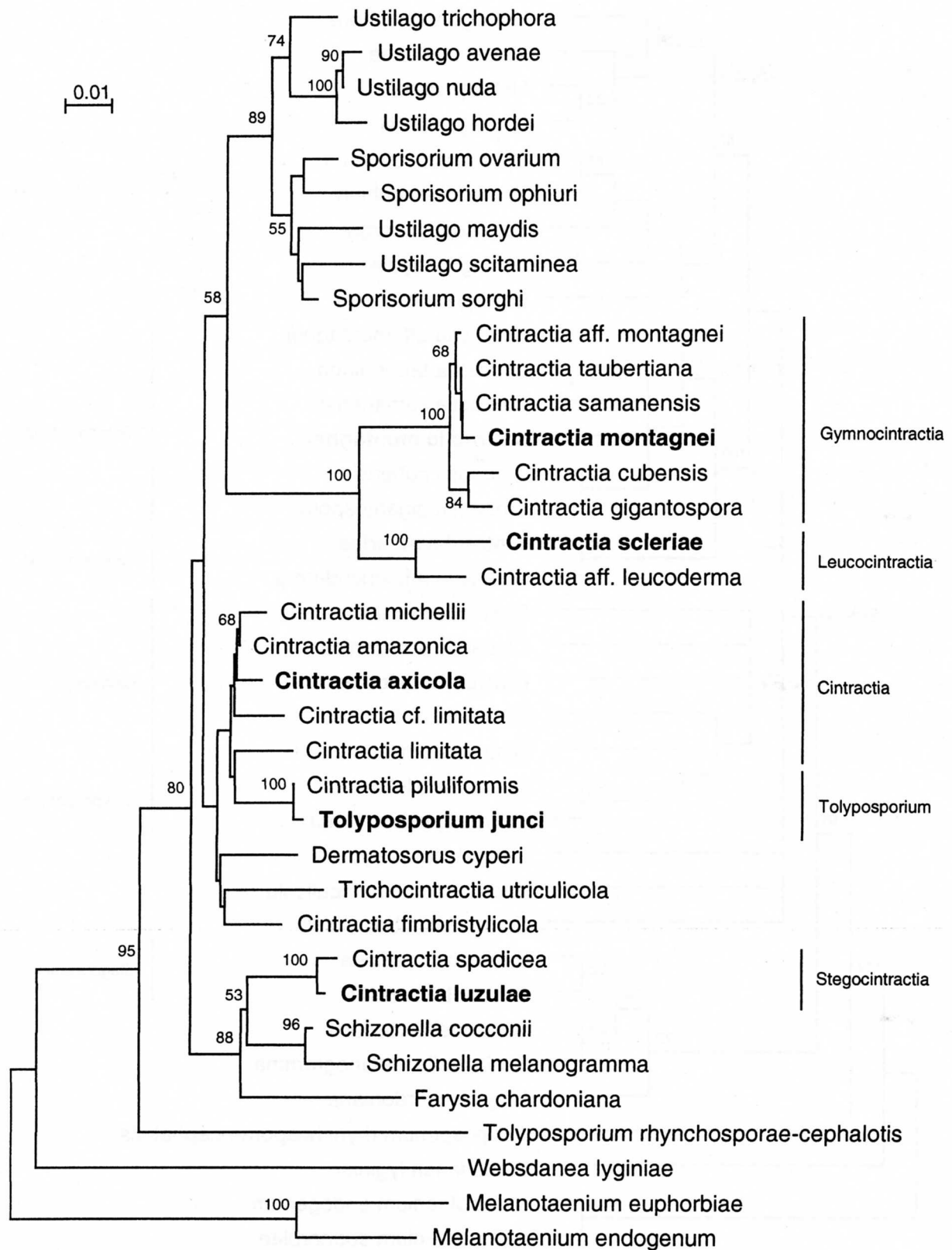


FIG. 37. Phylogram obtained by neighbor-joining analysis of LSU rDNA sequences of 33 species of Ustilaginales, using two species of Melanotaeniaceae as an outgroup. Bootstrap values greater than 50% of 1000 replicates are given. Type species of the genera belonging to *Cintractia* s. l. are written in bold.

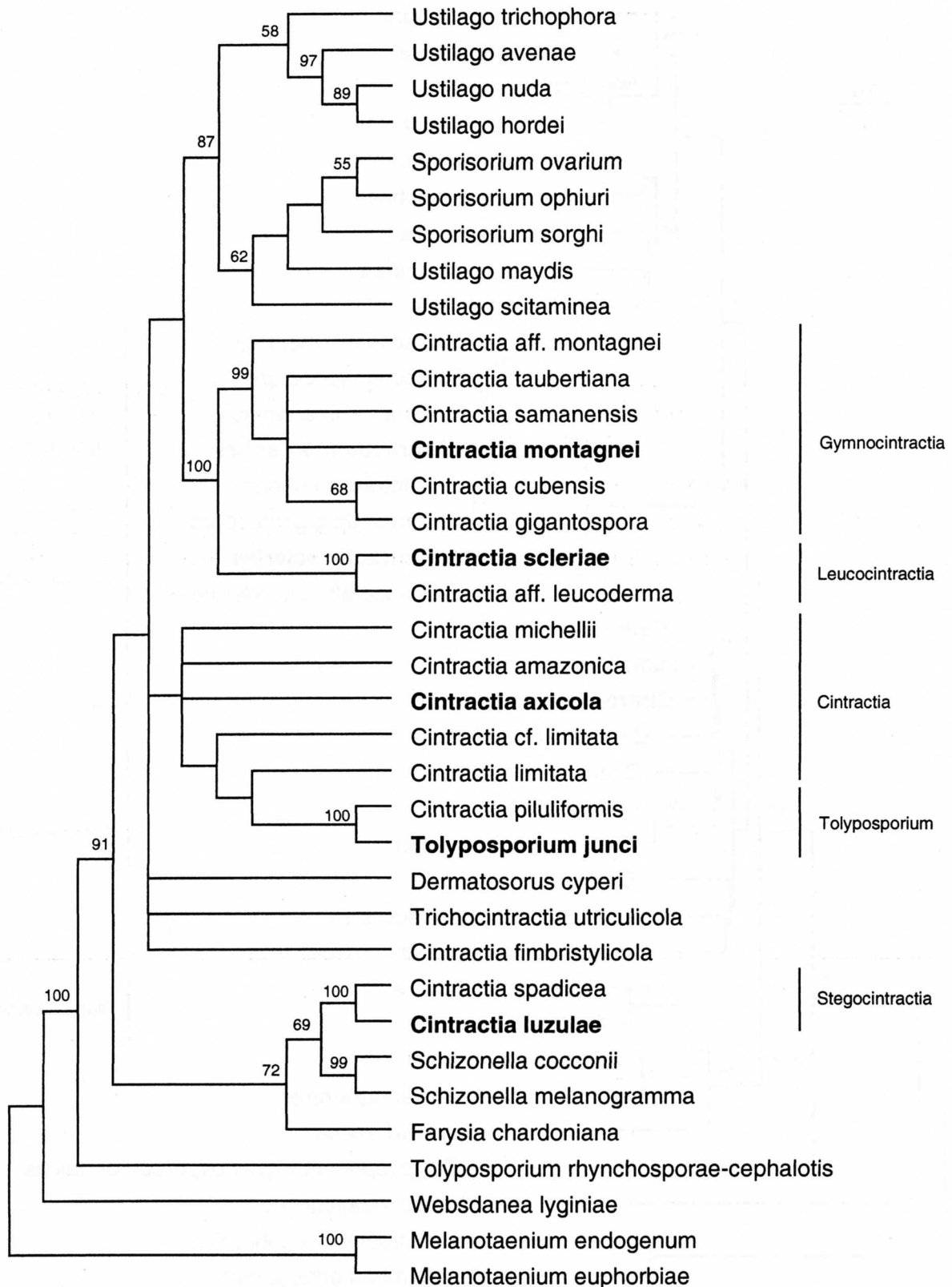


FIG. 38. Strict consensus tree (468 steps) of 71 most parsimonious trees obtained from parsimony analysis of LSU rDNA sequences of 33 species of Ustilaginales, using two species of Melanotaeniaceae as an outgroup. Bootstrap values greater than 50% of 100 replicates are given. Type species of the genera belonging to *Cintractia* s. l. are written in bold.

and if only monophyletic genera are accepted, the species of *Cintractia* s. s. would have to be included in the genus *Tolyposporium*, which was described earlier than *Cintractia*. This would be far from practical, because *Cintractia* s. s. and *Tolyposporium* are the only genera well established in the *Cintractia* s. l. relationship, and they are easily distinguishable by the presence of irregularly roughly warty teliospores in balls in species of *Tolyposporium* and single, finely warty teliospores in species of *Cintractia* s. s. The bootstrap values supporting the paraphyly are, however, very low and preliminary ITS sequence data (not illustrated) show a monophyletic origin of members of *Cintractia* s. s. Until further data support a more objective systematic conclusion, we refrain from including the species of *Cintractia* s. s. in *Tolyposporium*.

Species of the *C. axicola* group and *Tolyposporium* are closely related to *C. fimbriatylicola*, *Trichocintractia utriculicola*, and *Dermatosorus cyperi* in the NJ analysis (FIG. 37), but the relationships among these taxa are unresolved in the parsimony tree (FIG. 38). The position of *C. fimbriatylicola* in the NJ and MP trees cannot be explained by morphological data. The topology of the trees, however, is not very reliable with respect to this species, as shown by low bootstrap values. In addition, sequence data of the probably closely related species *C. fimbriatylis-miliaceae* are missing. These two species are provisionally maintained in the *C. axicola* group. *Trichocintractia utriculicola* was a species of *Cintractia* in the past, and was separated from this genus because of sterile, rarely septate hyphae inside the sori and small warts loosely connected to the teliospore walls (Piepenbring 1995). *Dermatosorus cyperi* develops teliospores ornamented by a high reticulum (Piepenbring et al 1998a) in balls with peripheral sterile cells inside ovaries of the host. A close relationship of *D. cyperi* with species of the *C. axicola* group or *Tolyposporium* cannot be supported by any morphological character known to the authors. For a phylogenetic interpretation of this relationship, sequence data of other morphologically closely related species, like those of *Testicularia* and *Kuntzeomyces*, are needed.

Species related to *C. montagnei*, i.e., *C. taubertiana*, *C. samanensis*, *C. cubensis*, and *C. gigantospora*, form a close relationship evident by molecular and morphological data, like the absence of sterile soral structures (3b, 4b) and foveolate spores (7f). Species of the *C. scleriae* group morphologically differ from species of the groups of *C. montagnei* and *C. axicola* by exclusively systemic infection (1c), sori around internodes but not at their bases (as in species of the *C. axicola* group), and teliospore ornamentation with rough irregular warts forming ridges (7e). A close relationship of the *C. montagnei* group and the *C.*

scleriae group is confirmed by high bootstrap values in both analyses, but an apomorphic morphological character supporting it was not found. Morphological data, like the presence of sterile stroma and peridia (3a, 4a), suggest that members of the *C. scleriae* group are more closely related to species of the *C. axicola* group, and members of the *C. montagnei* group to species of the *C. luzulae* group. Members of the two latter groups have sori without sterile stroma (3b) and usually foveolate teliospores (7f, 7g) in common.

The clade formed by the *C. montagnei* and the *C. scleriae* group is presented in FIGS. 37, 38 as a sister group of the species complex classified in *Ustilago* and *Sporisorium*. The respective bootstrap values are, however, rather low.

Without the help of molecular data, species related to *C. luzulae* would have been classified in the *C. montagnei* group, but they can be separated morphologically by sori around several glumes of sterile spikelets (2c), peridia of rarely septate, thick-walled hyphae (4c), and foveolate spores with distinct warts (7g). Similar nonseptate, thick-walled hyphae with thinner wall at the tips of the hyphae like those forming peridia in species of the *C. luzulae* group, are also present in sori of *Trichocintractia utriculicola*, but in this species they are located inside the sorus among the growing teliospores (Piepenbring 1995). No morphological characteristics could be found supporting a close relationship of the *C. luzulae* group with species of *Schizonella* and *Farysia*, as suggested in FIGS. 37, 38.

Species of each group described above are parasites of host species classified in the same family. Hosts of species of the *C. axicola* group, the *C. montagnei* group, and those of the *C. scleriae* group belong to the Cyperaceae, those of species of the *C. luzulae* group and *Tolyposporium* to the Juncaceae (9a). All the species shown to be rather closely related to species of the *C. axicola* group and the *C. luzulae* group in FIG. 37, belonging to *Dermatosorus*, *Trichocintractia*, *Schizonella*, and *Farysia*, infect members of the Cyperaceae. Coevolution of smut fungi and their hosts is evident. In accordance with the close relationship of smuts on Juncaceae with those on Cyperaceae, e.g., species of the *C. axicola* group and *Tolyposporium*, Juncaceae and Cyperaceae are known to be closely related based on morphological and molecular data (e.g., Kirpes et al 1996, Plunkett et al 1995, and citations therein).

TAXONOMY

In the following, the groups of species of *Cintractia* s. l. described above are presented as different gen-

era defined by morphological characteristics. For further new combinations and descriptions of species see Piepenbring (unpubl).

Cintractia Cornu 1883: 279

FIGS. 1, 6, 14–16, 18, 19, 27–31

Sori local or in groups; around the basal parts of pedunculi, around ovaries or stamina; young sori covered by peridia made of gelatinized hyphae; sori with sterile stroma forming sporogenous pockets. Teliospore mass agglutinated, superficially powdery; dark. Teliospores, when single, flattened; rarely with appendages; almost smooth to irregularly finely warty, as seen by SEM. Germination with phragmobasidia whose cells often conjugate.

Type species. *Cintractia axicola* (Berk.) Cornu

Further species included. *Cintractia amazonica* H. Sydow & Sydow, *C. limitata* Clinton, *C. mitchellii* Vánky, *C. oreoboli* Vánky & McKenzie, *Ustilago solida* Berk., in Hooker, *C. fimbristylicola* Pavgi & Mundkur, *C. fimbristylis-miliaceae* (Henn.) Ito.

Although *C. amazonica* has sori without sterile fungal structures, it is included here because of molecular data and irregularly finely warty teliospores (FIG. 15). *U. solida* differs by teliospores in balls (FIG. 16), but corresponds to members of this genus with respect to other characteristics. The ornamentation of teliospores of *C. fimbristylicola* (FIG. 18) and *C. fimbristylis-miliaceae* (FIG. 19) is not typical for this genus and molecular data show an isolated position of *C. fimbristylicola*. These two species are provisionally placed in the genus *Cintractia* s. s. *Cintractia limitata* is morphologically similar to *C. axicola*, so it is included in this genus, although molecular data are somewhat contradictory (FIGS. 37, 38). Further investigation is also necessary to confirm the paraphyly of *Cintractia* s. s. suggested by the molecular analysis.

Tolyposporium Woronin 1881: 577 FIGS. 2, 11, 17

Sori around few to numerous different organs of an infected plant; when around pedunculi, at their basal parts; young sori covered by peridia made of gelatinized fungal hyphae; sori with sterile stroma forming sporogenous pockets. Teliospore mass agglutinated, superficially powdery; dark. Teliospores in permanent balls; without appendages; irregularly roughly warty on the surface of the balls. Germination with phragmobasidia whose cells can conjugate.

Type species. *Tolyposporium junci* (Schröter) Woronin ex Schröter.

Further species included. *Cintractia piluliformis* (Berk.) Henn. differs from *T. junci* by the presence of sterile single cells among the balls. Other species of smut fungi with dark teliospores in balls hitherto designated to this genus are mostly not closely relat-

ed to *T. junci* (see *T. rhynchosporae-cephalotis* in FIGS. 37, 38). This is morphologically evident by sori without sterile fungal developments and different ultrastructure of the teliospore ornamentation (Piepenbring et al 1998a).

Gymnocintractia M. Piepenbr., Begerow et Oberwinkler, gen. nov. FIGS. 7, 8, 13, 20, 21, 23, 24, 32, 33

Sori in totis spiculis plantae vel in spiculis aggregatis; axium apices ante effectum organorum floris transformantes; sine structura fungali sterili. Sporarum massa basi agglutinata, pulveracea in parte superiore; obscura. Sporae singulares vel laxe connectae; si singulares leviter planae; saepe appendicibus hyalinis; foveolatae, visae per microscopium electronicum SEM inter foveas superficiei laevi vel subtiliter verrucosa, vel verrucosae.

Sori in all the spikelets of an infected inflorescence or in groups of spikelets; sori around the tips of the axes of sterile spikelets; without sterile fungal structure. Teliospore mass agglutinated at the base, powdery in the upper part; dark. Teliospores single or in loose groups; when single, flattened; often with hyaline appendages; foveolate with finely warty or smooth surface between the foveolae, as seen by SEM, or warty.

Typus generis. *Gymnocintractia montagnei* M. Piepenbr., Begerow et Oberwinkler, comb. nov.

Basionym. *Ustilago montagnei* Tul. & C. Tul., Ann. Sci. Nat. Bot., Sér. 3, 7: 88, 1847.

Synonyms. *Microbotryum montagnei* (Tul. & C. Tul.) Lév.-*Cintractia montagnei* (Tul. & C. Tul.) Magnus.; Type on *Rhynchospora rugosa* (= "*Rhynchospora glauca*" comp. Lindeberg 1959: 124; "*Schoenus* sp."), Algeria, La Calle, 2 Aug. 1841, M. C. Durieu de Maisonneuve (selected here: HOLOTYPE in PCI, ISO-TYPE BPI 195256!).

Etymology. Sori are not covered by peridia, naked (gr. gymnos).

Further species included. *Cintractia cubensis* M. Piepenbr., *C. cyperi* Clinton, *C. gigantospora* Liro (the basionym of the new combination will be *Ustilago rhynchosporae* Sauter), *C. major* (Desm.) Liro, *C. aff. montagnei*, *C. nova-guineae* Zundel, *C. samanensis* Cif., *C. standleyana* Zundel, *C. aff. standleyana*, *C. taubertiana* (Henn.) Clinton, *Ustilago diplasiae* Henn.

Leucocintractia M. Piepenbr., Begerow et Oberwinkler, gen. nov. FIGS. 4, 5, 22

Sori totos pedunculos inflorescentiae vel internodia caulis circumdantes; sori iuvenes peridio niveo tecti; stromate sterili cava sporogena formante. Sporarum massa agglutinata, superficialiter pulveracea; obscura. Sporae singulares; leviter planae; sine appendicibus; verrucis irregularibus, asperis, cristas in lateribus sporarum formantibus.

Sori around all the pedunculi of an inflorescence or around internodes of the stem (systemic infection); young sori covered by thick, conspicuously white peridia; with sterile stroma forming sporogenous pockets. Teliospore mass agglutinated, superficially powdery; dark. Teliospores single; flattened; without appendages; covered by irregular, rough warts forming ridges at the sides of the spores.

Typus generis. **Leucocintractia scleriae** (DC.) M. Piepenbr., Begerow et Oberwinkler, comb. nov.

Basionym. *Uredo scleriae* DC., in J. L. Poiret: Encyclopédie méthodique. Botanique. 8. Agasse, Paris, 1808: 228.

Synonyms. *Ustilago* ? *scleriae* (DC.) Tul. & C. Tul.; *Cintractia scleriae* (DC.) Ling.; Type on *Rhynchospora corymbosa* ("quelques espèces de *Scleria*"), French Guiana, Cayenne (G 555!).

Etymology. Shining white (gr. leucos = white) peridia are characteristic for members of this genus.

Further species included. *Cintractia leucoderma* (Berk.) Henn., *C. aff. leucoderma*, *C. pachyderma* Sydow, *C. portus-argenti* Cif.

Stegocintractia M. Piepenbr., Begerow et Oberwinkler, gen. nov. FIGS. 3, 9, 10, 12, 25, 26, 34, 35

Sori in totis spiculis plantae vel pedunculos inflorescentiae circumdantes; in spiculis bases glumarum steriliu circumdantes; iuvenes peridio ex hyphis hyalinis, raro septatis, parietibus crassis constante tecti; sine stromate sterili. Sporarium massa agglutinata, superficialiter pulveracea; obscura. Sporae singulares; sine appendicibus hyalinis; foveolatae, superficie inter foveas verrucosa, vel verrucosae.

Sori in all the spikelets of an infected inflorescence or around pedunculi (systemic infection); sori in spikelets surround the bases of sterile glumes; young sori covered by peridia made of rarely septate, thick-walled, hyaline hyphae; sori without sterile stroma. Teliospore mass agglutinated, superficially powdery; dark. Teliospores single; without hyaline appendages; foveolate and warty or warty.

Typus generis. **Stegocintractia luzulae** (Sacc.) M. Piepenbr., Begerow et Oberwinkler, comb. nov.

Basionym. *Ustilago luzulae* Sacc., Atti. Soc. Veneto-Trentina Sci. Nat. (Padova) 2: 121, 1873.

Synonym. *Cintractia luzulae* (Sacc.) Clinton.; Type on *Luzula forsteri* (Sm.) DC., Italy, "in sylva Cansiglio, agri Tarvisini", G. A. de Bérenger.

Etymology. Teliospore masses of species of this genus are covered (gr. stego = to cover) by peridia and glumes.

Further species included. *Cintractia hyperborea* (A. Blytt) Liro, *C. juncei* (Schw.) Trel., *C. lidii* Liro, *C. spadicea* Liro.

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