The identity of *Cintractia disciformis*: reclassification and synonymy of a southern Asian smut parasitic on *Carex* sect. *Aulocystis*

Marcin Piątek

Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland; corresponding author e-mail: m.piatek@botany.pl

Abstract: The identity of a neglected smut fungus, *Cintractia disciformis*, described from *Carex hirtella* in the Western Himalaya, India is reassessed. The species is excluded from *Cintractia* and is confirmed as a distinct species of *Anthracoidea*. Two smuts, *A. nepalensis* on *Carex nakaoana* in Nepal, and *A. haematostomae* on *Carex haematostoma* in China, are similar morphologically and considered to be later heterotypic synonyms of *Cintractia disciformis*. The appropriate nomenclatural combination for this species, *Anthracoidea disciformis* comb. nov., is validated.

Article info: Submitted: 16 March 2012; Accepted: 29 May 2012; Published: 21 June 2012.

Key words: Anthracoidea

Anthracoldea Carex Cintractia Historical Collections Smut Fungi Taxonomy

INTRODUCTION

The smut fungus Cintractia disciformis was originally described from a plant identified as Carex hirtella (sect. Aulocystis) collected at Nipchang in Western Himalaya, India. Cintractia disciformis was first invalidly introduced without the mandatory Latin description that was required from 1 January 1935 until 31 December 2011 (Liro 1935). A few years later Liro (1939) provided the missing Latin diagnosis. The species has only occasionally been reported in the literature, for instance in connection with a second finding on Mt. Sawi in Indian Kashmir on a new host, Carex haematostoma (Ling 1949), or in monographic studies on smut fungi (Zundel 1953, Zambettakis 1978, Piepenbring 2000, Vánky 2007a, 2012, Gandhe 2011). Ling (1949) and Zambettakis (1978) prepared descriptions of the Kashmiri specimen of Cintractia disciformis. Other authors have either repeated the description from the protologue (Zundel 1953, Gandhe 2011), or not included one (Piepenbring 2000, Vánky 2007a, 2012). In a monograph of Anthracoidea, Zambettakis (1978) proposed a new combination "Anthracoidea disciformis (Liro) Zambett.", but without giving any indication of the basionym or any reference to the place of its valid publication, rendering the combination invalid (ICN, Art. 33.4), like all new combinations introduced in that work.

Vánky (2012) examined the type material of *Cintractia disciformis* in H, and concluded that it was an *Anthracoidea*. However, he did not accept the species or make any transfer to that genus as he noted that the host *Carex* was not *C. hirtella* according to an annotation by I. Kukkonen on the specimen. Vánky commented that "without the correct name of the host plant it cannot be identified".

Two smuts with similar phenotypic characteristics on related hosts in *Carex* sect. *Aulocystis* in the same geographical area of southern Asia (Himalaya Mts) were described several decades later, *Anthracoidea nepalensis* on *Carex nakaoana* in Nepal (Kakishima & Ono 1988) and *Anthracoidea haematostomae* on *Carex haematostoma* in China (Guo 2006). These two smuts were found to be conspecific by Vánky & Piątek (in Vánky 2007b) and that treatment is followed in the monograph of Vánky (2012). This work aims to clarify the taxonomic status of *Cintractia disciformis* and ascertain whether it is distinct from or conspecific with *Anthracoidea nepalensis* (incl. *A. haematostomae*).

MATERIALS AND METHODS

Sori and spore characteristics were studied using dried herbarium material deposited in H, IBAR and "H.U.V."¹. The specimens were examined either by light microscopy (LM) and scanning electron microscopy (SEM) or only by light microscopy (LM).

For light microscopy (LM), small pieces of sori were mounted in lactic acid, heated to boiling point and cooled, and then examined under a Nikon Eclipse 80i light microscope. LM micrographs were taken with a Nikon DS-Fi1 camera. Fifty spores were measured from each collection, using NIS-Elements BR 3.0 imaging software. Spore size ranges were assigned to one of the three groups distinguished by Savile (1952): (1) small-sized spores $- 13-21(-23) \times 9-17(-20) \mu m$;

¹The personal collection of Kálmán Vánky, "Herbarium *Ustilaginales* Vánky" currently held at his home (Gabriel-Biel-Straße 5, D-72076 Tübingen, Germany).

Non-commercial: You may not use this work for commercial purposes.

^{© 2012} International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

No derivative works: You may not alter, transform, or build upon this work. For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

(2) medium-sized spores – $15-25(-27) \times 10-21 \mu m$; (3) largesized spores – $18-33 \times 13-28 \mu m$. Unless otherwise stated, the spores were measured in plane view and measurements are adjusted to the nearest 0.5 μm .

For scanning electron microscopy (SEM), spores taken directly from dried herbarium samples were dusted onto 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*. 12 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).

RESULTS

Anthracoidea disciformis (Liro) M. Piątek, comb. nov.

MycoBank MB800481

(Fig. 1)

Basionym: Cintractia disciformis Liro, Myc. Fenn. fasc. 16, no. 110 (1939).

Synonyms: Cintractia disciformis Liro, Ann. Bot. Soc. zool.bot. Fenn. "Vanamo" 6: 6 (1935); nom. inval. (Art. 36.1).

Anthracoidea disciformis (Liro) Zambett., Bull. Soc. mycol. France 94: 166 (1978); nom. inval. (Art. 33.4).

Anthracoidea nepalensis Kakish. & Y. Ono, in Watanabe & Malla, *Crypt. Himal.* **1**: 128 (1988).

Anthracoidea haematostomae L. Guo, Fungal Diversity **21**: 83 (2006).

Sorus in one ovary of the inflorescence, black, ovoid, presumably around the achene, about 4 × 2.5 mm diam, composed of agglutinated spores, powdery on the surface, partly hidden by the perigynium and scales. Spores smallsized, flattened, disc-shaped, chestnut-brown to reddish brown, regular in shape and size, in plane view globose, subglobose or broadly ellipsoidal, 16.5-18.5(-19.0) × (13.5–)14.0–18.0 μm [av. ± SD, 17.6 ± 0.6 × 15.7 ± 1.2 μm, n = 50], in side view broadly ellipsoidal (8.5–)10.0–12.0 µm (measurements without hyaline caps), usually enclosed by prominent mucilaginous sheath visible as hyaline caps on the flattened sides, up to 1.5 µm wide; wall even, 1.5-2.0 µm, darker than the rest of spore; surface finely papillate in LM, spore profile finely serrulate, surface sparsely papillate in SEM, papillae up to 0.3 µm high (from SEM micrographs), interspaces smooth.

Specimens examined: China: Yunnan Province: Deqen, elev. 2700 m, on Carex haematostoma, Sept. 1935, C.W. Wang 70101 ("H.U.V." 20090, isotype of Anthracoidea haematostomae). – India: Darma, Nipchang, on Carex plectobasis (as "C. hirtella"), 31 Aug. 1884, J. F. Duthie (H s.n. – holotype of Cintractia disciformis). – Nepal: Bagmati Zone: Langtang, Kyangjin–Langshisa, elev. 3900 m, on Carex haematostoma (syn. C. nakaoana), 3 Sept. 1986, Y. Ono 86NE-223 (IBAR 0628 – isotype of Anthracoidea nepalensis); elev. 3800 m, on Carex haematostoma (syn. C. nakaoana), 3 Sept. 1986, Y. Ono 86NE-214 (IBAR 0619, paratype of Anthracoidea nepalensis); Kyangjin, elev. 3800 m, on Carex haematostoma (syn. C. nakaoana), 4 Sept. 1986, Y. Ono 86NE-234 (IBAR 0639 – paratype of Anthracoidea nepalensis).

Hosts and distribution: On members of Carex sect. Aulocystis: Carex digyna, C. haematostoma (syn. C. nakaoana), and C. plectobasis (syn. C. hirtella). Known from China, India, and Nepal.

Observations: While the host of *Cintractia disciformis* is uncertain according to the annotation by Ilkka Kukkonen on the holotype; re-identification of the specimen based on one inflorescence is difficult. However, the sedge definitely belongs to the section *Aulocystis*, and the length of perigynia (5.0–6.5 mm) indicates an affinity with *Carex plectobasis* (syn. *C. hirtella*) according to the available keys and descriptions in the Flora of Pakistan and Flora of China (eFloras, http://www. efloras.org). Guo (1994) has also reported *Anthracoidea nepalensis* on this host (as *Carex hirtella*).

The host of Anthracoidea nepalensis was reported as Carex nakaoana, but this species is now considered synonymous with *C. haematostoma* (Chlebicki 2002). Yet another host of this smut is *C. digyna* listed in Chinese reports of *A. nepalensis* (Guo 1994, as "digyne"). The host of *A. haematostomae* is *C. haematostoma.* Vánky (2007b) included the European sedge *C. sempervirens* in the list of hosts of *A. nepalensis*; this was evidently a mistake, and the species was not cited as a host in his subsequent monograph (Vánky 2012). *Carex sempervirens* has to be excluded from the host range of *A. disciformis.*

DISCUSSION

The internal structure of the sori is one of the main differentiating characteristics between Anthracoidea and Cintractia (Kukkonen 1963, Piepenbring 2000). Unfortunately this feature could not be examined in the holotype of Cintractia disciformis without destroying the specimen. However, the hyaline caps on the spores preclude a placement in Cintractia and support an affinity to Anthracoidea. Further, species of Cintractia are not known to occur on Carex, nor even members of the Cariceae (Piątek & Vánky 2007). The internal sorus structure of Anthracoidea nepalensis (Fig. 2), regarded here as a synonym, is typical of Anthracoidea species in that the spores are formed on the outer surface of the achene, and not within the U-shaped pockets embedded in the sterile stroma, a character of the genus Cintractia (Kukkonen 1963, Piepenbring 2000). This provides additional indirect evidence that Cintractia disciformis is a member of the genus Anthracoidea as indicated by Zambettakis (1978) and Vánky (2012).

The characteristics of the holotype of *Cintractia disciformis* are included in the species description presented above and shown in the illustrations (Fig. 1). The morphological details of specimens of *Anthracoidea nepalensis* I examined were: sori globose or ovoid surrounding the achenes, about 1.5–3.0 long and 1.5–2.5 mm wide, spores disc-shaped, chestnutbrown to reddish brown, globose, subglobose, rarely broadly ellipsoidal or somewhat subangulate, (15.0–)15.5–19.5(–20.5) × (12.5–)14.0–18.0(–19.0) µm, the flattened



Fig. 1. Anthracoidea disciformis (H s.n. – holotype). **A.** The holotype material. **B–C.** Enlarged sorus visible from both sides of inflorescence respectively. **D–E.** Spores in LM, median and superficial views respectively. **F–I.** Spores with prominent hyaline mucilaginous sheath on the flattened sides. Note small papillae on spore surface indicated by arrows on picture F. **J–K.** Spores in SEM. Note remnants of mucilaginous sheath on surface of spores illustrated on picture J and in central part of spore illustrated on picture K. **L.** Ornamentation of spore in SEM. Bars: A-C = 5 mm, D-J = 10 µm, K = 5 µm, L = 3 µm.

sides of spores rarely enclosed by a hyaline mucilaginous sheath, spore wall even, 1.0–1.5(–2.0) μ m, spore surface finely papillate, spore profile finely serrulate. The SEM

characteristics of spores of *A. nepalensis* (Kakishima & Ono 1988, Chlebicki 2002) agree well with those of *Cintractia disciformis*.



Fig. 2. Internal sorus structure of *Anthracoidea nepalensis* (IBAR 0619). **A.** Transverse section through the sorus. **B.** Enlarged area close to the achene surface. Abbreviations: n - rudimentary achene, e - dark layer of the remnants of the achene epidermis, h - layer of sporogeneous hyphae, s - layer of young hyaline spores, m - layer of gradually maturing dark spores. Bars: $A = 20 \mu m$, $B = 10 \mu m$.

The morphology of *Anthracoidea haematostomae* was investigated by Vánky & Piątek (in Vánky 2007b) to establish the synonymy between this species and *A. nepalensis*, although only the morphology of *A. nepalensis* was presented in the published results. However, the key morphological features of the material of *A. haematostomae* studied are: spores dark reddish brown, 17.5–22 × 15–20 µm; spore wall even, 1.5–2.5 µm thick, with hyaline caps, spore surface finely papillate, spore profile finely wavy. The spore ornamentation observed in SEM (Guo 2006) also agrees well with that of *Cintractia disciformis*.

The morphology of *Cintractia disciformis*, *Anthracoidea nepalensis* and *A. haematostomae* is very similar, and the only differences concern the hyaline mucilaginous sheath. This sheath was less developed in the material of *A. nepalensis*, and the spores are somewhat larger and the spore wall slightly thicker in *A. haematostomae* compared to *Cintractia disciformis*. However, these minor differences lie within the normal variability of a single *Anthracoidea* species (Kukkonen 1963, Denchev 1991, Piątek & Mułenko 2010, Savchenko *et al.* in press). Consequently, these three species names are considered as synonymous and the oldest available name, *Cintractia disciformis*, is therefore taken up as a new combination, that proposed by Zambettakis (1978) being invalid.

The disc-shaped, papillate spores of *Anthracoidea disciformis* are distinctive and rarely observed in other *Anthracoidea* species that have verruculose or rarely smooth spores. This feature readily differentiates this smut from four other *Anthracoidea* species infecting members of *Carex* sect. *Aulocystis* which all have verruculose spores (*viz. A. altera, A. misandrae, A. sempervirentis,* and *A. stenocarpae*). In the entire genus, only a few other *Anthracoidea* species have disc-shaped and papillate spores, for example *A. bistaminatae* (Guo 2006), *A. lindebergiae* (Vánky 1994), *A. mulenkoi* (Piątek 2006), *A. setschwanensis* (Guo 2002), *A. royleanae* (Guo 2006), *A. setschwanensis* (Guo 2007), *A. smithii* (Vánky 2007a), and *A. xizangensis* (Guo 2005), all of which infect *Kobresia*. Interestingly, most of these

Anthracoidea species occur in eastern and southern Asia. An exception is *A. lindebergiae*, which is widely distributed in arctic and alpine ecosystems of the Northern Hemisphere. Whether these *Anthracoidea* species are closely related and have evolved from a common ancestor is unclear and open to future studies.

This study demonstrates that a critical evaluation of historical names could prevent an unnecessary proliferation of names proposed for the same organism. Such taxonomical expertise appears even more urgent in the light of molecular initiatives, especially DNA Barcoding (Seifert 2008, Begerow *et al.* 2010, Schoch *et al.* 2012). In order to be most effective the molecular studies should be accompanied by a critical reassessment of as many historical names of fungal species as possible that can be linked to freshly collected specimens for use in molecular analyses (Lücking 2008, Hyde *et al.* 2010).

ACKNOWLEDGEMENTS

I thank the curators of H, "H.U.V." and IBAR for the loan of specimens, Anna Łatkiewicz (Kraków, Poland) for her help with the SEM micrographs, and David L. Hawksworth (Madrid, Spain / London, UK) and Roger G. Shivas (Dutton Park, Australia) for helpful comments on the manuscript. This study was supported by the Polish Ministry of Science and Higher Education (grant no. 2 P04G 019 28).

REFERENCES

- Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* 87: 99– 108.
- Chlebicki A (2002) Two cypericolous smut fungi (*Ustilaginomycetes*) from the Thian Shan and their biogeographic implications. *Mycotaxon* **83**: 279–286.

- Denchev CM (1991) Genus *Anthracoidea* Bref. (*Ustilaginales*) in Bulgaria – species and morphometric variability of the teliospores. *Fitologija* **41**: 51–60.
- Gandhe RV (2011) *Ustilaginales of India*. Dehra Dun: Bishen Singh Mahendra Pal Singh.
- Guo L (1994) *Anthracoidea* and allied genera in China (*Ustilaginales*). *Mycosystema* **7**: 89–104.
- Guo L (2002) Two new species of *Ustilaginomycetes* and a species new to China. *Mycotaxon* 82: 147–150.
- Guo L (2005) Two new species of *Anthracoidea* (*Ustilaginales*) from China. *Mycotaxon* **94**: 47–50.
- Guo L (2006) Six new species of *Anthracoidea* (*Ustilaginales*) from China. *Fungal Diversity* **21**: 81–92.
- Guo L (2007) *Anthracoidea setschwanensis* sp. nov. (*Ustilaginales*) and a new record of *Anthracoidea* from China. *Mycotaxon* **99**: 227–230.
- Hyde KD, Abd-Elsalam K, Cai L (2010) Morphology: still essential in a molecular world. *Mycotaxon* **114**: 439–451.
- Kakishima M, Ono Y (1988) Three species of smut fungi (Ustilaginales) from Nepal. In: Cryptogams of the Himalayas.
 Vol. 1. The Kathmandu Valley (M Watanabe, SB Malla, eds): 127–132. Tsukuba: National Science Museum.
- Kukkonen I (1963) Taxonomic studies on the genus Anthracoidea (Ustilaginales). Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo' 34(3): 1–122.
- Ling L (1949) Taxonomic notes on Asiatic smuts. I. Sydowia 3: 123–134.
- Liro JI (1935) Über neue, seltene und vermeinte Ustilagineen. Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo' 6(1): 1–18.
- Liro JI (1939) Mycotheca fennica. Die Etiketten. No. 301–600. Helsinki.
- Lücking R (2008) Taxonomy: a discipline on the brink of extinction. Are DNA barcode scanners the future of biodiversity research? *Archives des Sciences* **61**(2): 75–88.
- Piątek M (2006) *Anthracoidea mulenkoi (Ustilaginomycetes*), a new cypericolous smut fungus from Pakistan. *Nova Hedwigia* **83**: 109–116.

- Piątek M, Mułenko W (2010) The calcareous mires in South-East Poland are home to two rare *Anthracoidea* species. *Acta Mycologica* **45**: 175–184.
- Piątek M, Vánky K (2007) *Cintractia bulbostylidicola* sp. nov. (*Ustilaginomycotina*) from North America. *Nova Hedwigia* **85**: 187–194.
- Piepenbring M (2000) The species of *Cintractia* s.l. (*Ustilaginales*, *Basidiomycota*). *Nova Hedwigia* **70**: 289–372.
- Savchenko KG, Lutz M, Piątek M, Heluta VP, Nevo E. (in press) Anthracoidea caricis-meadii is a new North American smut fungus on Carex sect. Paniceae. Mycologia.
- Savile DBO (1952) A study of the species of *Cintractia* on *Carex*, *Kobresia*, and *Scirpus* in North America. *Canadian Journal of Botany* **30**: 410–435.
- Seifert KA (2008) Integrating DNA Barcoding into the Mycological Sciences. *Persoonia* **21**: 162–166.
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi. Proceedings of the National Academy of Sciences, USA* **109**: 6241–6246.
- Vánky K (1994) European Smut Fungi. Stuttgart: G. Fischer Verlag.
- Vánky K (2007a) Smut fungi of the Indian Subcontinent. *Polish Botanical Studies* **26**: 1–265.
- Vánky K (2007b) Taxonomic studies on Ustilaginomycetes 27. Mycotaxon **99**: 1–70.
- Vánky K (2012) *Smut Fungi of the World*. St Paul, MN: American Phytopathological Society Press.
- Zambettakis C (1978) Les Anthracoidea des Carex et les Ustilaginées aberrantes. Bulletin de la Société Mycologique de France 94: 109–260.
- Zundel GL (1953) The Ustilaginales of the world. Pennsylvania State College School of Agriculture Department of Botany Contribution **176**: xi+1–410.