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Anthracoidea obtusatae (Anthracoideaceae, Ustilaginales), a new smut fungus on *Carex obtusata* (Cyperaceae) from Central Asia

TEODOR T. DENCHEV[1,2], CVETOMIR M. DENCHEV[1,2*], DOMINIK BEGEROW[3] & MARTIN KEMLER[2,3]

[1] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin St., 1113 Sofia, Bulgaria*
ttdenchev@gmail.com; https://orcid.org/0000-0002-7242-3307
[2] *IUCN SSC Rusts and Smuts Specialist Group, Sofia, Bulgaria*
[3] *Universität Hamburg, Institute of Plant Science and Microbiology, Organismic Botany and Mycology, Ohnhorststraße 18, 22609 Hamburg, Germany*
dominik.begerow@uni-hamburg.de; https://orcid.org/0000-0002-8286-1597
martin.kemler@uni-hamburg.de; https://orcid.org/0000-0002-0738-4233
**Corresponding author: cmdenchev@yahoo.co.uk; https://orcid.org/0000-0001-6301-1629*

Abstract

Anthracoidea species are highly host-specific smut fungi. Their host range is restricted to a single sedge species or sedge species belonging to the same or closely related sections. The species on *Carex obtusata* was referred to as *Anthracoidea caryophylleae*. However, the hosts of *A. caryophylleae* were reduced to carices in *Carex* sect. *Mitratae*, in its traditional circumscription. In the most recently proposed classification of *Carex, C. obtusata* has been placed within the Capitata Clade of *C.* subgen. *Euthyceras*. In order to clarify the taxonomic status of the *Anthracoidea* species on *C. obtusata*, we conducted a molecular study of specimens on *C. obtusata* and *C. caryophyllea*, using LSU rDNA sequences. It was found that the *Anthracoidea* species on *C. obtusata* is distinct from *A. caryophylleae*. A new species, *Anthracoidea obtusatae* on *C. obtusata*, is described and illustrated from Russia, based on a specimen from the Altay Mts. Its phylogenetic placement and affinities in *Anthracoidea* are analyzed. *Anthracoidea obtusatae* is distributed in Central Asia—the Northern Tian Shan Range (Zailiysky Alatau Range and Ketmen Ridge) and the Russian and Mongolian Altay Mts. A morphological examination of a specimen of *Anthracoidea* on *C. obtusata* from Canada found that its spore morphology did not match that of *A. obtusatae*. Additional collections and molecular study are required to clarify the taxonomic status of the North American *Anthracoidea* species on *C. obtusata*.

Key words: Altay Mts, *Anthracoidea*, Anthracoideaceae, *Carex*, Cyperaceae, new species, phylogeny, smut fungi, taxonomy, Tian Shan Range

Introduction

The genus *Anthracoidea* Bref. is the largest genus of the Anthracoideaceae, and at the same time, the largest genus of smut fungi on host plants belonging to the Cyperaceae (Vánky 2011a, Denchev & Denchev 2016, Denchev *et al.* 2020, 2021a). *Anthracoidea* is a cosmopolitan genus comprising 112 species (Denchev *et al.* 2021a). The smut fungi in this genus are characterized by sori that form globose to broadly ellipsoidal or ovoid, black, hard bodies around aborted nuts of cyperaceous plants. In *Carex* L. (Cyperaceae), the sori are scattered in female spikes or in female flowers of mixed spikes, depending on the *Carex* species. The sori are covered initially by a thin peridium, which later ruptures to expose the spore mass. The spore mass is firmly agglutinated at first and at maturity becomes powdery on the surface of the sorus. The spores are formed singly and are usually flattened. The infection is local and confined to individual flowers (Kukkonen 1963, Vánky 2013, Denchev & Denchev 2016, Denchev *et al.* 2020, 2021a). *Anthracoidea* species are considered to be restricted to host plants belonging to the same or closely related sections of *Carex*, whereby host specificity of *Anthracoidea* species is regarded to be a result of homothallism and coevolution with their hosts (Kukkonen 1963, Vánky 1979).

During an examination of specimens of sedges in the herbarium of the Berlin Botanic Garden and Botanical Museum, Germany carried out by two of the authors (T.T.D. & C.M.D.), a specimen of *Carex obtusata* Lilj. (B 10 0240197) from the Russian Altay Mts. infected by a smut fungus belonging to the genus *Anthracoidea* was found.

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The aim of the present study is to clarify the taxonomic status of the *Anthracoidea* species on *Carex obtusata*. A combined approach, using host specialization, comparative morphology, and molecular data, revealed a new species, *A. obtusatae*. This smut fungus is described and illustrated herein and its phylogenetic placement and affinities in *Anthracoidea* are analyzed.

Material and methods

DNA extraction, PCR amplification, and sequencing

For DNA extraction, one *Anthracoidea* sorus was used. The sample was milled in the Fastprep-24™ Sample Preparation Instrument (MP Biomedicals), using two steel beads. Genomic DNA was isolated using the my-Budget Plant DNA Kit™ (Bio-Budget Technologies GmbH, Germany), according to the manufacturer's protocol (protocol 1: "Isolation of DNA from plant material using lysis buffer SLS"). PCR using GoTaq™ Master Mix (Promega, U.S.A.) with the primer combination LR0R/LR6 (Vilgalys & Hester 1990, Moncalvo *et al.* 1995) was performed to amplify the LSU rDNA region, which is the standard molecular marker for *Anthracoidea* (e.g., Hendrichs *et al.* 2005, Denchev *et al.* 2021a). Standard thermal cycling conditions with an annealing temperature of 52 °C were used for amplification. Five µl of PCR products were purified using a modified ExoSAP protokol (1 : 5 diluted in ddH_2O; New England Biolabs, U.S.A.). Amplicons were sequenced in both directions with the BigDye™ Terminator Cycle Sequencing Kit V3.1 (Applied Biosystems, U.S.A.) on an ABI 3130xl Genetic Analyser at the Faculty of Chemistry and Biochemistry, Ruhr-Universität Bochum, Germany. Subsequently, forward and reverse reads were quality checked and assembled in Geneious 10.2.6 (Biomatters Ltd, New Zealand). Sequences were deposited in the NCBI nucleotide database (see Table 1 for accession numbers).

Phylogenetic analysis

The newly generated *Anthracoidea* sequences and representative sequences downloaded from GenBank (Table 1) were aligned with the l-ins-i option in MAFFT using the online server version (Katoh *et al.* 2019). The best nucleotide substitution model was determined using ModelTest-NG (Darriba *et al.* 2020) implemented in raxmlGUI 2.0 (Edler *et al.* 2021). RaxML-NG (Kozlov *et al.* 2019), also implemented in raxmlGUI, was used for inferring a maximum likelihood phylogeny with 1000 thorough bootstrap replicates. FigTree v1.4.3 (Rambaut 2012) was used for visualization of the phylogeny.

Morphological examination

Dried specimens from B, C, DAOM, K(M), NY, and SOMF (herbarium codes according to Thiers 2023+) were examined using a light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The measurements of spores are given as min–max (extreme values) (mean ± 1 standard deviation). The spore length range is assigned to one of the groups distinguished by Denchev *et al.* (2020: 11): very small-sized, small-sized, medium-sized, and large-sized. For SEM, spores were attached to a specimen holder by double-sided adhesive tape and coated with gold in an ion sputter. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a JEOL SM-6390 scanning electron microscope. The description of spore ornamentation is in accordance with Denchev *et al.* (2013). The description below is based on the specimen examined. The shapes of spores are arranged in descending order of frequency.

Results

Phylogenetic analysis

ModelTest-NG determined GTR+I+G as the best substitution model for the dataset. The phylogenetic analysis inferred similar species relationships as previous studies. All *Anthracoidea* sequences clustered together and formed a well-supported clade. The *Anthracoidea* specimen on *Carex obtusata* did not cluster together with *A. caryophylleae* on *C. caryophyllea*, but formed an independent lineage that was sister clade to all other *Anthracoidea* species (Fig. 1).

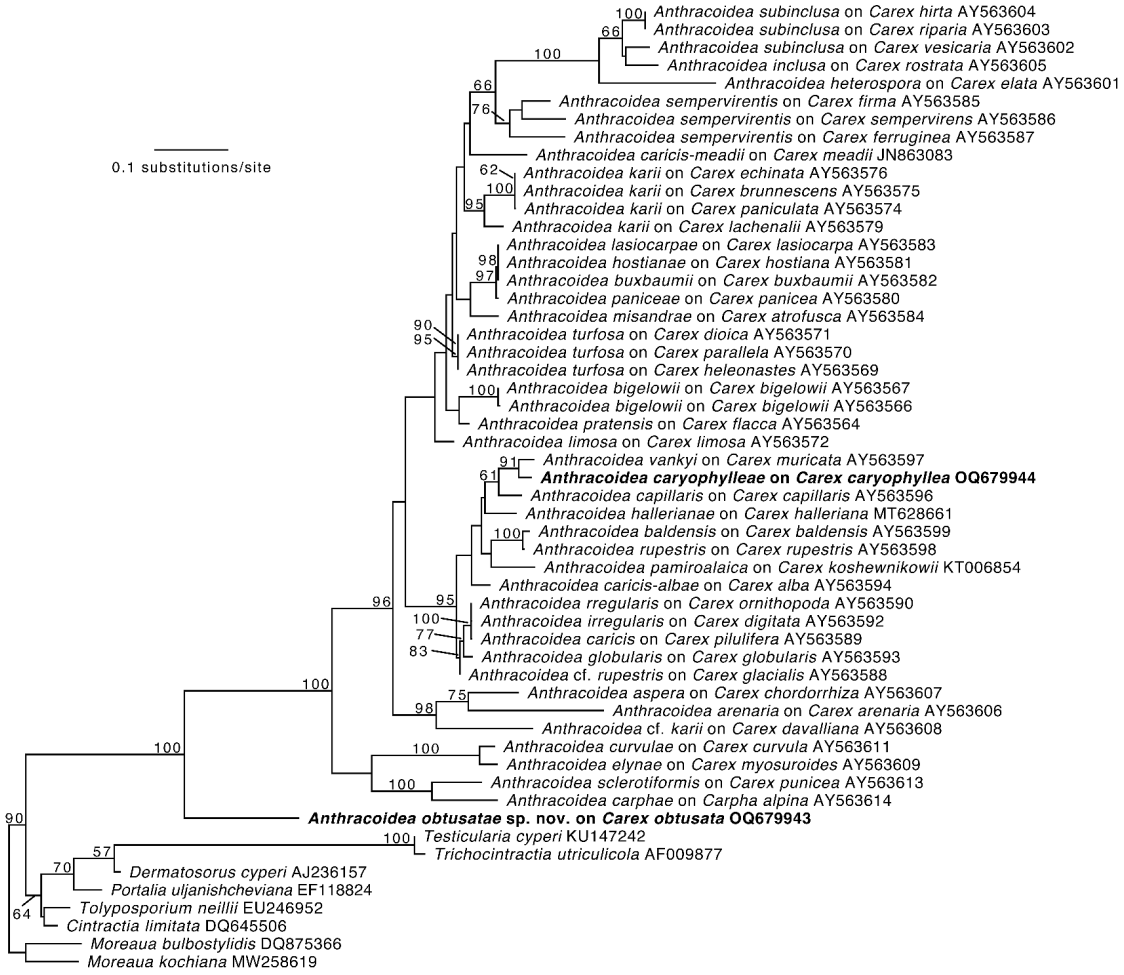


FIGURE 1. RAxML phylogeny of species in the genus *Anthracoidea* based on a MAFFT alignment of partial rDNA LSU sequences. Bootstrap values ≥ 50 are shown above branches. The phylogenetic tree was rooted with *Moreaua bulbostylidis* and *M. kochiana* (according to Denchev *et al.* 2021b).

Morphology

Anthracoidea species have very few diagnostic morphological characteristics. The morphology of the sori bears no diagnostic value, with the exception of very few species (e.g., *A. intercedens* Nannf., *A. pseudofoetidae* L. Guo and *A. subinclusa* (Körn.) Bref.; Vánky 2011a, Denchev *et al.* 2020). The most important characteristics are spore-based: sizes, shape (in plane view, since most species have flattened spores), and wall thickness and ornamentation (pattern and height). Characteristics of less taxonomic significance include internal swellings, light-refractive areas, and protuberances. Their presence and frequency may vary between different collections of one species, but due to the scarcity of morphological characteristics, their careful examination and use in combination with the diagnostic morphological features is still very important (Denchev *et al.* 2021a).

The morphological description of the studied smut fungus on *Carex obtusata* was based on examination of a specimens from the Altay Mts (Russia). The specimen was characterized by small-sized spores (12.5–20 µm long), irregularly rounded, suborbicular, broadly elliptical, elliptical or ovate (as seen in plane view), with an unevenly thickened, 0.8–2.0(–2.3) µm thick spore wall, with 1–3(–4), well visible internal swellings. The wall was smooth; in SEM—sparsely punctate.

Taxonomy

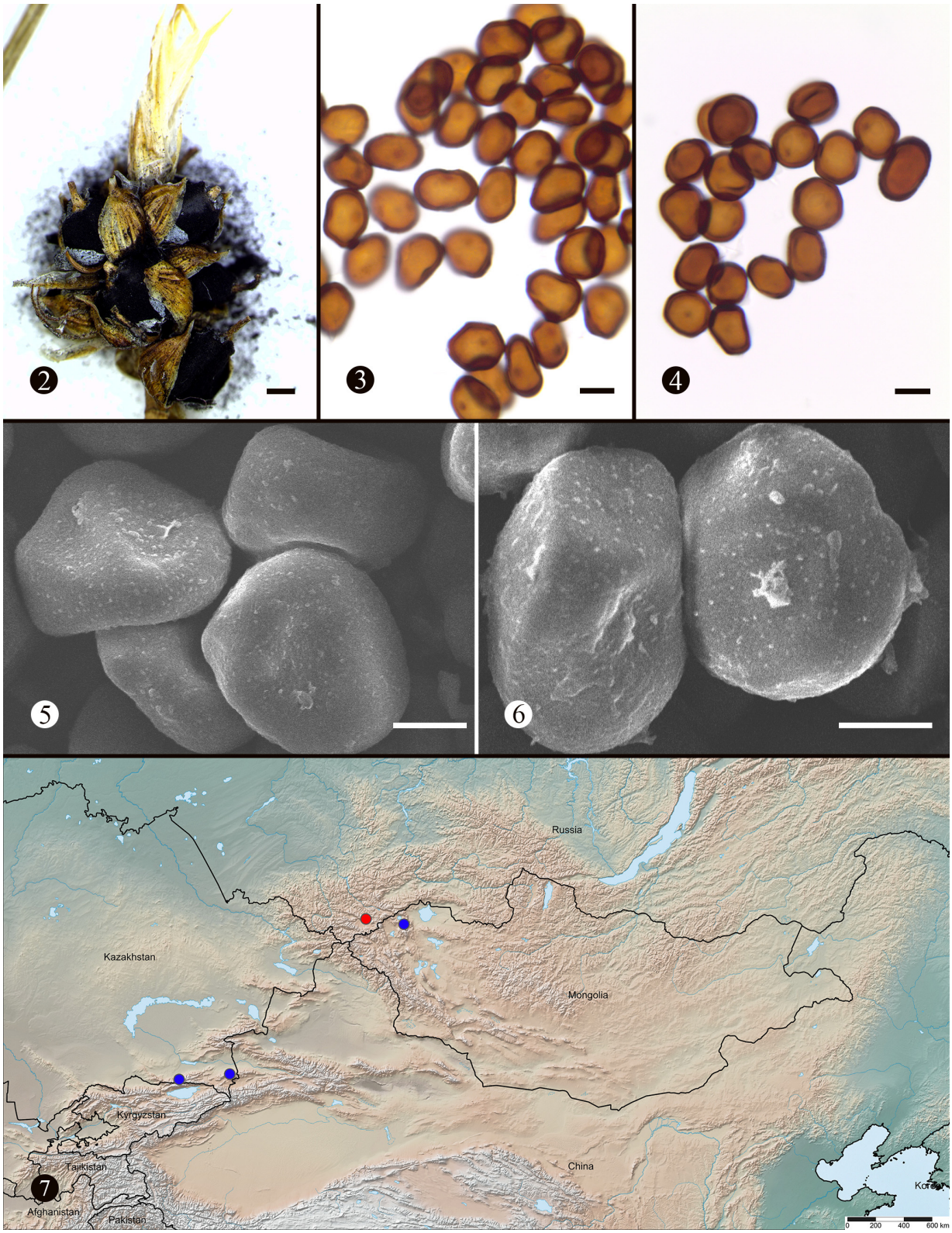
Based on the host specialization, morphology, and phylogenetic data, we propose a new species of *Anthracoidea* on *Carex obtusata*.

Anthracoidea obtusatae T. Denchev, Denchev, Begerow & Kemler, *sp. nov.* (Figs 2–6)

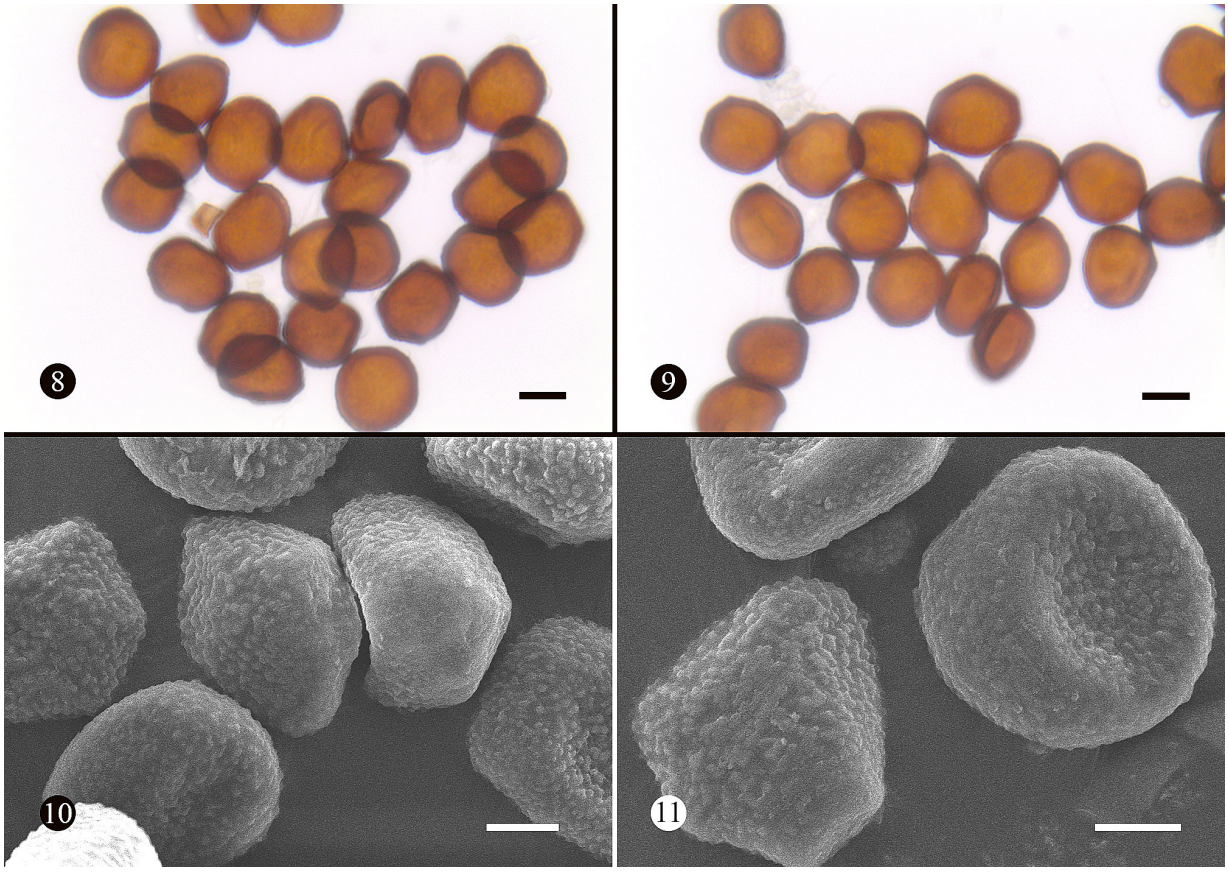
Index Fungorum number: IF 900382

Type:—On *Carex obtusata* Lilj. (Cyperaceae). RUSSIA. Altay Republic: Altay Mts, Kosh-Agachskiy Rayon, 19 km ENE Kosh-Agach, valley of the Kokorya River, 50°05′56″N, 88°53′35″E, elev. 2060 m, 8 August 2008, leg. B. Martins & M. Schnittler 2468, comm. C.M. Denchev & T.T. Denchev (SOMF 30400, holotype).

LSU rDNA GenBank accession no.:—OQ679943.



FIGURES 2–7. *Anthracoidea obtusatae sp. nov.* on *Carex obtusata*. 2. Habit (from holotype). 3–4. Spores in LM (from holotype). 5–6. Spores in SEM (from holotype). 7. Geographic distribution of *A. obtusatae* (generated with Simple-Mappr, Shorthouse 2010): type locality indicated by a red circle, literature records – by blue circles. Scale bars: 2 = 1 mm, 3–4 = 10 µm, 5–6 = 5 µm.



FIGURES 8–11. *Anthracoidea* sp. on *Carex obtusata* from Canada (DAOM 28184). 8–9. Spores in LM. 10–11. Spores in SEM. Scale bars: 8–9 = 10 µm, 10–11 = 5 µm.

Diagnosis:—*Anthracoidea obtusatae* differs from the *Anthracoidea* species on hosts in the *Carex* Capitata Clade by having small-sized spores while *A. nardinae* and *A. rupestris* possess medium-sized spores.

Etymology:—The epithet is derived from the host plant, *Carex obtusata*.

Infection local. *Sori* in some female flowers, around aborted nuts as ovoid or ellipsoidal, hard bodies, 1.3–2.5 mm long, initially covered by a thin, greyish peridium that later flakes away exposing a black spore mass, powdery on the surface. *Spores* small-sized, flattened, in plane view irregularly rounded, suborbicular, broadly elliptical, elliptical or ovate, in plane view (12.5–)13.5–19(–20) × (10.5–)11.5–15.5(–17) (15.7 ± 1.3 × 13.2 ± 1.0) µm (n = 300), in side view 12–14.5 µm thick, medium to dark reddish brown; wall unevenly thickened, 0.8–2.0(–2.3) µm thick, with 1–3(–4), well visible internal swellings, light refractive areas and protuberances absent; smooth. In SEM, sparsely punctate, ornaments up to 0.1 µm high. *Spore germination* unknown.

Known host and distribution:—On Cyperaceae: *Carex*, Capitata Clade of *C.* subgen. *Euthyceras*: *Carex obtusata*, Central Asia (Kazakhstan, Mongolia, Russia) (Fig. 7).

Literature records (specimens not seen):—On *Carex obtusata*. MONGOLIA. Uvs Province: Mongolian Altay Range, Turgen-uul, Burgastayn-gol valley, 15 km S of Uureg-nuur Lake, at approximately 49°53′N, 1 August 1964, leg. M. Schmiedeknecht 2239a (as '*Cintractia/Anthracoidea caricis*') (Schmiedeknecht & Puncag 1966, Braun 1999).—KAZAKHSTAN. Northern Tian Shan Range, Zailiysky Alatau Range, Big Almaty Gorge, 43°03′50.6″N, 76°59′05.1″E, elev. 2480 m, 3 September 2018, leg. U.K. Jetigenova *s.n.* (as '*Anthracoidea caricis*') (AA; Sypabekkyzy *et al.* 2020).—Northern Tian Shan Range, Ketmen Ridge, Komirchi, elev. ca. 2800 m, 6 August 1946, leg. N. Rubtsov & E. Stepanova *s.n.* (as '*Cintractia caricis*') (Schwartzman 1960).

Additional specimens examined:—*Anthracoidea caricis-pauciflorae* (Lehtola) Kukkonen on *Carex pauciflora* Lightf. FINLAND. Satakunta, Kankaanpää, Luomajärvi, Vääräneva, 18 August 1935, leg. L.E. Kari, in *Fungi Exsicc. Fenn. 220* (K(M), isoparatype).—*Anthracoidea caryophylleae* Kukkonen on *Carex caryophyllea* Latourr. BULGARIA. Pernik Province: above Kralev Dol village, 42°33′42.0″N, 23°05′04.4″E, elev. 845 m, 9 May 2014, leg. C.M. Denchev & T.T. Denchev 1414 (SOMF 30399); LSU rDNA accession no. OQ679944.—*Anthracoidea externa* on *Carex filifolia* Nutt. U.S.A. Wyoming: Buffalo, August 1808, leg. Williams & Griffiths, in *D. Griffiths, West American Fungi*

305 (NY, isotype).—*Anthracoidea rupestris* on *Carex rupestris* All. GREENLAND. West Greenland: Ilulissat (as 'Jakobshavn'), 69°13′N, 51°06′W, 19 July 1892, leg. G.H. Sørensen *s.n.*, comm. C.M. Denchev & T.T. Denchev (C-Greenland herb., s.n.).—West Greenland: Sisimiut, 31 July 1947, leg. T. Sørensen *s.n.*, comm. C.M. Denchev & T.T. Denchev (C-Greenland herb., s.n.).—*Anthracoidea* sp. on *Carex obtusata*. CANADA. Yukon: Mt. Caribou, 5 mi. N of Carcross, 17 August 1949, leg. J.M. Gillett 4550 & D.A. Mitchell, det. D.B.O. Savile (DAOM 28184, as '*Cintractia caricis* var. *intermedia* Savile', Savile 1952: 425; a paratype of *Anthracoidea caryophylleae*, Kukkonen 1963: 55).

Comments:—*Carex obtusata* is distributed in North, Central, and East Europe, the Caucasus, Siberia, Russian Far East, Central Asia to NE China, Alaska, Canada, and the Rocky Mts (Egorova 1999, Murray 2002, Koopman 2022). It is an Eurasiatic–amphi-Beringian–North American (western) species (Egorova 1999, Elven *et al.* 2018).

Only two smut fungi are known to infect *C. obtusata*: *Anthracoidea/Cintractia caricis* (Savile 1952, Fischer 1953, Zundel 1953, Schwartzman 1960, Jørstad 1962, Sypabekkyzy *et al.* 2020) and *Anthracoidea caryophylleae* (Kukkonen 1963, Nannfeldt 1979, Vánky 2011a). In the past, *Anthracoidea/Cintractia caricis* was considered in a very broad sense (e.g. Fischer 1953, Zundel 1953, Schwartzman 1960). Vánky (2011a: 26–27) listed many carices that were cited in the literature or preserved in herbaria as hosts of *Anthracoidea caricis*. Currently, the hosts of *A. caricis* are reduced to members of *Carex* sect. *Acrocystis* (Vánky 2011a, Denchev *et al.* 2021a). Similarly, the hosts of *A. caryophylleae* are reduced to carices in *C.* sect. *Mitratae*, in its traditional circumscription (Denchev *et al.* 2013). To test the hypothesis that *Carex obtusata* is not a host of *A. caryophylleae*, we carried out a molecular study of a specimen of *A. caryophylleae* on *Carex caryophyllea* Latourr. (a type host of this smut fungus) from Bulgaria. The data obtained confirmed that *Anthracoidea obtusatae* is a distinct species from *A. caryophylleae* (Fig. 1, Table 1).

From Europe, *Anthracoidea* on *Carex obtusata* had been reported only once (Zundel 1953: 23, as '*Cintractia caricis*'), based on the following specimen: Germany, Berlin, Pichelswerder, 30 May 1869, leg. Vatke *s.n.* (HBC, as '*Ustilago urceolorum* (DC.) Tul. & C. Tul.', q.e. *Anthracoidea caricis* (Pers.) Bref.). Scholz & Scholz (1988: 45), however, noted that both the fungus and the host plant of this specimen were misidentified, instead of *A. caryophylleae* on *Carex supina* Wahlenb.

From North America, *Anthracoidea* on *Carex obtusata* is known from Alaska (Jørstad 1962), Canada—Yukon (Savile 1952, as '*Cintractia caricis* var. *intermedia*'; Nannfeldt 1979, as '*Anthracoidea caryophylleae*') and Saskatchewan (Fischer 1953, as '*Ci. caricis*'), and U.S.A.—Montana, Galcier National Park (DAOM, as '*Anthracoidea caryophylleae*'). We examined a specimen of *Anthracoidea* on *Carex obtusata* from Yukon (DAOM 28184) and found that its spore morphology does not agree with that of *Anthracoidea obtusatae*. The Canadian specimen differs by having (i) larger spores, (15.5–)16.5–21(–22.5) × (13.5–)14.5–18(–19) (19.0 ± 1.4 × 16.2 ± 1.1) µm (n = 100), (ii) higher warts, up to 0.35 µm high, and (iii) fewer and less conspicuous internal swellings (Figs. 8–11). Additional collections and molecular study are required to further clarify the taxonomic position of the North American *Anthracoidea* species on *Carex obtusata*.

Carex obtusata has been traditionally included in a monotypic section, *C.* sect. *Obtusatae* (Tuck.) Mack. (Egorova 1999, Murray 2002) of *Carex* subgen. *Psyllophora* (Degl.) Peterm. A recently proposed classification of *Carex* places this sedge within the Capitata Clade of *Carex* subgen. *Euthyceras* Peterm. (Roalson *et al.* 2021). The Capitata Clade is an informal group of species that includes 11 carices with a single, terminal androgynous spike: *C. arctogena* Harry Sm., *C. argunensis* Turcz. ex Trevir., *C. capitata* Sol., *C. microglochin* Wahlenb., *C. monostachya* A. Rich., *C. motuoensis* Y.C. Yang, *C. nardina* (Hornem.) Fr., *C. obtusata*, *C. oreophila* C.A. Mey., *C. runssoroensis* K. Schum., and *C. rupestris* (Roalson *et al.* 2021). Two *Anthracoidea* species have been previously reported on carices in the Capitata Clade: *A. nardinae* (Kukkonen) Nannf. on *Carex nardina*, and *A. rupestris* Kukkonen on *Carex rupestris* (Vánky 2011a, Denchev *et al.* 2020). *Anthracoidea obtusatae* differs from *A. nardinae* by having small-sized spores versus medium-sized spores, (16–)17–22(–23) (19.2 ± 0.9) µm long, for *A. nardinae* (after Denchev *et al.* 2020). *Anthracoidea obtusatae* can be distinguished from *A. rupestris* by having (i) small-sized spores versus medium-sized spores, (16–)17–23(–26) (20.3 ± 1.5) µm long, for *A. rupestris*, (ii) thinner spore wall, 0.8–2.0(–2.3) µm thick versus 1–3(–3.8) µm thick for *A. rupestris*, and (iii) sparsely punctate wall ornamentation, with ornaments up to 0.1 µm high, versus densely spaced, minutely verruculose ornamentation for *A. rupestris* (data for *A. rupestris* based on our study of Greenlandic specimens in C-Greenland herb.).

In the most recent infrageneric classification of *Carex*, Roalson *et al.* (2021) applied combinations of informally named clades that have the potential to be described as formal sections, but that are not currently recognized as such, and formally named sections to reflect the current state of knowledge of *Carex* phylogeny. The Capitata–Schiedeanae Clade comprises species mainly with unbranched terminal spikes (excl. *C.* sect. *Schiedeanae*), and it is divided into five named clades/sections (Roalson *et al.* op.cit.). In these phylogenetic clades, there are additional carices with known *Anthracoidea* species on them: *A. breweri* Salo & Vánky on *Carex breweri* Boott and *C. subnigricans* Stacey,

A. caricis-pauciflorae (Lehtola) Kukkonen on *Carex pauciflora* Lightf., *A. externa* (Griffiths) Kukkonen on *Carex filifolia* Nutt., and *A. kenaica* (Savile) Piątek on *Carex micropoda* C.A. Mey. (Kukkonen 1963, Nannfeldt 1979, Vánky 2011a, b, Denchev *et al.* 2020, Denchev & Denchev 2022).

TABLE 1. Specimens and NCBI nucleotide database accession numbers used for phylogenetic analysis (newly generated sequences indicated in boldface).

Species	Host	LSU rDNA accession no.	Reference
A. arenaria	*Carex arenaria*	AY563606	Hendrichs *et al.* 2005
A. aspera	*C. chordorrhiza*	AY563607	Hendrichs *et al.* 2005
A. baldensis	*C. baldensis*	AY563599	Hendrichs *et al.* 2005
A. bigelowii	*C. bigelowii*	AY563566	Hendrichs *et al.* 2005
A. bigelowii	*C. bigelowii*	AY563567	Hendrichs *et al.* 2005
A. buxbaumii	*C. buxbaumii*	AY563582	Hendrichs *et al.* 2005
A. capillaris	*C. capillaris*	AY563596	Hendrichs *et al.* 2005
A. caricis	*C. pilulifera*	AY563589	Hendrichs *et al.* 2005
A. caricis-albae	*C. alba*	AY563594	Hendrichs *et al.* 2005
A. caricis-meadii	*C. meadii*	JN863083	Savchenko *et al.* 2013
A. carphae	*Carpha alpina*	AY563614	Hendrichs *et al.* 2005
A. caryophylleae	***Carex caryophyllea***	**OQ679944**	**this study**
A. curvulae	*C. curvula*	AY563611	Hendrichs *et al.* 2005
A. elynae	*C. myosuroides*	AY563609	Hendrichs *et al.* 2005
A. globularis	*C. globularis*	AY563593	Hendrichs *et al.* 2005
A. hallerianae	*C. halleriana*	MT628661	Denchev *et al.* 2021a
A. heterospora	*C. elata*	AY563601	Hendrichs *et al.* 2005
A. hostianae	*C. hostiana*	AY563581	Hendrichs *et al.* 2005
A. inclusa	*C. rostrata*	AY563605	Hendrichs *et al.* 2005
A. irregularis	*C. ornithopoda*	AY563590	Hendrichs *et al.* 2005
A. irregularis	*C. digitata*	AY563592	Hendrichs *et al.* 2005
A. karii	*C. paniculata*	AY563574	Hendrichs *et al.* 2005
A. karii	*C. brunnescens*	AY563575	Hendrichs *et al.* 2005
A. karii	*C. echinata*	AY563576	Hendrichs *et al.* 2005
A. karii	*C. lachenalii*	AY563579	Hendrichs *et al.* 2005
A. cf. *karii*	*C. davalliana*	AY563608	Hendrichs *et al.* 2005
A. lasiocarpae	*C. lasiocarpa*	AY563583	Hendrichs *et al.* 2005
A. limosa	*C. limosa*	AY563572	Hendrichs *et al.* 2005
A. misandrae	*C. atrofusca*	AY563584	Hendrichs *et al.* 2005
A. obtusatae	***C. obtusata***	**OQ679943**	**this study**
A. pamiroalaica	*C. koshewnikowii*	KT006854	Piątek *et al.* 2015
A. paniceae	*C. panicea*	AY563580	Hendrichs *et al.* 2005
A. pratensis	*C. flacca*	AY563564	Hendrichs *et al.* 2005
A. rupestris	*C. rupestris*	AY563598	Hendrichs *et al.* 2005
A. cf. *rupestris*	*C. glacialis*	AY563588	Hendrichs *et al.* 2005
A. sclerotiformis	*C. punicea*	AY563613	Hendrichs *et al.* 2005
A. sempervirentis	*C. sempervirens*	AY563586	Hendrichs *et al.* 2005
A. sempervirentis	*C. firma*	AY563585	Hendrichs *et al.* 2005
A. sempervirentis	*C. ferruginea*	AY563587	Hendrichs *et al.* 2005
A. subinclusa	*C. riparia*	AY563603	Hendrichs *et al.* 2005
A. subinclusa	*C. vesicaria*	AY563602	Hendrichs *et al.* 2005

.....continued on the next page

TABLE 1. (Continued)

Species	Host	LSU rDNA accession no.	Reference
A. subinclusa	*C. hirta*	AY563604	Hendrichs *et al.* 2005
A. turfosa	*C. dioica*	AY563571	Hendrichs *et al.* 2005
A. turfosa	*C. heleonastes*	AY563569	Hendrichs *et al.* 2005
A. turfosa	*C. parallela*	AY563570	Hendrichs *et al.* 2005
A. vankyi	*C. muricata*	AY563597	Hendrichs *et al.* 2005
Cintractia limitata	–	DQ645506	Matheny *et al.* 2007
Dermatosorus cyperi	*Cyperus cellulosoreticulatus*	AJ236157	Piepenbring *et al.* 1999
Moreaua bulbostylidis	*Bulbostylis capillaris*	DQ875366	Begerow *et al.* 2006
M. kochiana	*Schoenus nigricans*	MW258619	Denchev *et al.* 2021b
Portalia uljanishcheviana	*Scirpoides holoschoenus*	EF118824	González *et al.* 2007
Testicularia cyperi	–	KU147242	Kijpornyongpan *et al.* 2018
Tolyposporium neillii	*Isolepis nodosa*	EU246952	Vánky 2008
Trichocintractia utriculicola	*Rhynchospora corymbosa*	AF009877	Begerow *et al.* 1998

Anthracoidea obtusatae can easily be differentiated from these species by spore size and other features. *Anthracoidea breweri* (after Vánky 2011b) has medium-sized to large-sized spores, 20–27 µm long; evenly thickened spore wall, ca. 1.5 µm thick, without internal swellings; and presence of distinctly and densely minutely verruculose ornamentation. *Anthracoidea caricis-pauciflorae* (our study of *Fungi Exsicc. Fenn. 220*, K(M), isoparatype) has large-sized spores, (18.5–)19.5–28(–30.5) (23.3 ± 2.1) µm long; spore wall without internal swellings; light refractive areas common (often with more than one); and minutely to moderately verruculose ornamentation, warts 0.2–0.4 µm high. *Anthracoidea externa* (our study of *D. Griffiths, West American Fungi 305*, NY, isotype) has medium-sized spores, (16–)17–21.5(–23) (18.8 ± 1.5) µm long, with more regular shape (in plane view orbicular or broadly elliptical); absence of internal swellings; presence of a well developed hyaline sheath. *Anthracoidea kenaica* (after Denchev & Denchev 2022) has medium-sized spores, (16–)17–21(–23) (19.3 ± 1.1) µm long; evenly or almost evenly thickened spore wall, 0.8–1.2(–1.5) µm thick, presence of a thin hyaline sheath around some spores.

Five other species in the Capitata–Schiedeanae Clade, *C. capillacea* Boott, *C. capitata* L., *C. leptalea* Wahlenb., *C. microglochin* Wahlenb., and *C. rara* Boott, are recorded as hosts of *Anthracoidea caricis* (see Vánky 2011a: 26), but as was stated above, currently, the host range of *A. caricis* includes only sedges in *Carex* sect. *Acrocystis*, and the phylogenetic placement of the *Anthracoidea* species on these carices remains unresolved.

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References

Begerow, D., Bauer, R. & Oberwinkler, F. (1998) Phylogenetic studies on nuclear large subunit ribosomal DNA sequences of smut fungi and related taxa. *Canadian Journal of Botany* 75: 2045–2056. https://doi.org/10.1139/b97-916

Begerow, D., Stoll, M. & Bauer, R. (2006) A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. *Mycologia* 98: 906–916. https://doi.org/10.1080/15572536.2006.11832620

Braun, U. (1999) An annotated list of Mongolian phytoparasitic micromycetes. *Schlechtendalia* 3: 1–32.

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: a new and scalable tool for the

selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37: 291–294. https://doi.org/10.1093/molbev/msz189

Denchev, T.T. & Denchev, C.M. (2016) *Anthracoidea caricis-reznicekii* (*Anthracoideaceae*), a new species on *Carex reznicekii*, and *A. eburneae,* a new record for the USA. *Phytotaxa* 244: 69–79. https://doi.org/10.11646/phytotaxa.244.1.5

Denchev, T.T. & Denchev, C.M. (2022) *Anthracoidea kenaica*—a new record from Russia. *Mycotaxon* 137: 353–358. https://doi.org/10.5248/137.353

Denchev, T.T., Denchev, C.M., Michikawa, M. & Kakishima, M. (2013) The genus *Anthracoidea* (Anthracoideaceae) in Japan and some adjacent regions. *Mycobiota* 2: 1–125. https://doi.org/10.12664/mycobiota.2013.02.01

Denchev, T.T., Knudsen, H. & Denchev, C.M. (2020) The smut fungi of Greenland. *MycoKeys* 64: 1–164. https://doi.org/10.3897/mycokeys.64.47380

Denchev, T.T., Denchev, C.M., Koopman, J., Begerow, D. & Kemler, M. (2021a) Host specialization and molecular evidence support a distinct species of smut fungus, *Anthracoidea hallerianae* (Anthracoideaceae), on *Carex halleriana* (Cyperaceae). *Willdenowia* 51: 57–67. https://doi.org/10.3372/wi.51.51105

Denchev, T.T., Martín, M.P., Kemler, M. & Denchev, C.M. (2021b) Additions to the smut fungi of the Iberian Peninsula. *Anales del Jardín Botánico de Madrid* 78: e109 [pp. 1–13]. https://doi.org/10.3989/ajbm.2589

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2021) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12: 373–377. https://doi.org/10.1111/2041-210X.13512

Egorova, T.V. (1999) *The sedges (Carex L.) of Russia and adjacent states (within the limits of the former USSR)*. St. Petersburg State Chemical-Pharmaceutical Academy, St. Petersburg & Missouri Botanical Garden Press, St. Louis, 773 pp.

Elven, R., Murray, D.F., Razzhivin, V.Y. & Yurtsev, B.A. (Eds.) (2018) *Annotated checklist of the Panarctic Flora (PAF). Vascular plants*. Natural History Museum, University of Oslo. Available from: https://panarcticflora.org/ (accessed: 22 March 2023).

Fischer, G.W. (1953) *Manual of the North American smut fungi.* Ronald Press Co., New York, 343 pp.

González, V., Vánky, K., Platas, G. & Lutz, M. (2007) *Portalia* gen. nov. (Ustilaginomycotina). *Fungal Diversity* 27: 45–55.

Hendrichs, M., Begerow, D., Bauer, R. & Oberwinkler, F. (2005) The genus *Anthracoidea* (Basidiomycota, Ustilaginales): a molecular phylogenetic approach using LSU rDNA sequences. *Mycological Research* 109: 31–40. https://doi.org/10.1017/S0953756204001686

Jørstad, I. (1962) Parasitic fungi collected in Alaska by Olav Gjærevoll. *Kongelige Norske Videnskabers Selskabs Skrifter (Trondheim)* 1962 (4): 1–22.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kijpornyongpan, T., Mondo, S.J., Barry, K., Sandor, L., Lee, J., Lipzen, A., Pangilinan, J., LaButti, K., Hainaut, M., Henrissat, B., Grigoriev, I.V., Spatafora, J.W. & Aime, M.C. (2018) Broad genomic sampling reveals a smut pathogenic ancestry of the fungal clade Ustilaginomycotina. *Molecular Biology and Evolution* 35: 1840–1854. https://doi.org/10.1093/molbev/msy072

Koopman, J. (2022) *Carex Europaea*. 3rd edn. *The genus Carex L. in Europe.* Vol. 1. *Accepted names, hybrids, synonyms, distribution, chromosome numbers*. Margraf Publishers, Weikersheim, Germany, 808 pp.

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Kukkonen, I. (1963) Taxonomic studies on the genus *Anthracoidea* (Ustilaginales). *Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo'* 34 (3): 1–122.

Matheny, P.B., Gossmann, J.A., Zalar, P., Kumar, T.K.A. & Hibbett, D.S. (2007) Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. *Canadian Journal of Botany* 84: 1794–1805. https://doi.org/10.1139/b06-128

Moncalvo, J.-M., Wang, H.-H. & Hseu, R.-S. (1995) Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* 87: 223–238. https://doi.org/10.1080/00275514.1995.12026524

Murray, D.F. (2002) *Carex* section *Obtusatae* (Tuckerman) Mackenzie. *In*: Flora of North America Editorial Committee (Eds.) *Flora of*

North America, north of Mexico. Magnoliophyta: *Commelinidae* (*in part*): *Cyperaceae*. Vol. 23. Oxford University Press, New York, pp. 555.

Nannfeldt, J.A. (1979) *Anthracoidea* (Ustilaginales) on Nordic Cyperaceae-Caricoideae, a concluding synopsis. *Symbolae Botanicae Upsalienses* 22 (3): 1–41.

Piątek, M., Lutz, M., Nobis, M. & Nowak, A. (2015) Phylogeny and morphology of *Anthracoidea pamiroalaica* sp. nov. infecting the endemic sedge *Carex koshewnikowii* in the Pamir Alai Mts (Tajikistan). *Mycological Progress* 14: 120. https://doi.org/10.1007/s11557-015-1140-1

Piepenbring, M., Begerow, D. & Oberwinkler, F. (1999) Molecular sequence data assess the value of morphological characteristics for a phylogenetic classification of species of *Cintractia*. *Mycologia* 91: 485–498. https://doi.org/10.1080/00275514.1999.12061042

Rambaut, A. (2012) *FigTree v1.4*. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed: 17 March 2023).

Roalson, E.H., Jiménez-Mejías, P., Hipp, A.L., Benítez-Benítez, C., Bruederle, L.P., Chung, K.-S., Escudero, M., Ford, B.A., Ford, K., Gebauer, S., Gehrke, B., Hahn, M., Hayat, M.Q., Hoffmann, M.H., Jin, X.-F., Kim, S., Larridon, I., Léveillé-Bourret, É., Lu, Y.-F., Luceño, M., Maguilla, E., Márquez-Corro, J.I., Martín-Bravo, S., Masaki, T., Míguez, M., Naczi, R.F.C., Reznicek, A.A., Spalink, D., Starr, J.R., Uzma, Villaverde, T., Waterway, M.J., Wilson, K.L. & Zhang, S.-R. (2021) A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. *Journal of Systematics and Evolution* 59: 726–762. https://doi.org/10.1111/jse.12722

Savchenko, K.G., Lutz, M., Piątek, M., Heluta, V. & Nevo, E. (2013) *Anthracoidea caricis-meadii* is a new North American smut fungus on *Carex* sect. *Paniceae*. *Mycologia* 105: 181–193. https://doi.org/10.3852/12-074

Savile, D.B.O. (1952) A study of the species of *Cintractia* on *Carex*, *Kobresia*, and *Scirpus* in North America. *Canadian Journal of Botany* 30: 410–435. https://doi.org/10.1139/b52-032

Schmiedeknecht, M. & Puncag, T. (1966) Ustilaginales aus der Mongolischen Volksrepublik. Ergebnisse der Mongolisch-Deutschen Biologisehen Expeditionen seit 1962, Nr. 17. *Feddes Repertorium* 73 (3): 170–183. https://doi.org/10.1002/fedr.4910730304

Scholz, H. & Scholz, I. (1988) Die Brandpilze Deutschlands (Ustilaginales). *Englera* 8: 1–691. https://doi.org/10.2307/3776736

Schwartzman, S.R. (1960) Smut fungi. *In*: *Cryptogamic flora of Kazakhstan*. Vol. 2. Izdatel'stvo Akademii Nauk Kazakhskoi SSR, Alma Ata, 369 pp. [In Russian]

Shorthouse, D.P. (2010) *SimpleMappr, an online tool to produce publication-quality point maps*. Available from: http://www.simplemappr.net (accessed: 26 March 2023).

Sypabekkyzy, G., Rakhimova, Y.V., Yermekova, B.D., Kyzmetova, L.A. & Assylbek, A.M. (2020) Smut fungi of Zailiysky Alatau: species composition, host range and distribution features. *Eurasian Journal of Ecology* 62 (1): 87–98. https://doi.org/10.26577/EJE.2020.v62.i1.09

Thiers, B. (2023 [continuously updated]) *Index herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's virtual herbarium. Available from: http://sweetgum.nybg.org/science/ih/ (accessed 26 March 2023).

Vánky, K. (1979) Species concept in *Anthracoidea* (Ustilaginales) and some new species. *Botaniska Notiser* 132: 221–231.

Vánky, K. (2008) Taxonomic studies on Ustilaginomycetes – 28. *Mycotaxon* 106: 133–178.

Vánky, K. (2011a) *Smut fungi of the world*. APS Press, St. Paul, 1458 pp.

Vánky, K. (2011b) Seven new species of smut fungi (Ustilaginomycotina). *Mycologia Balcanica* 8: 97–104. https://doi.org/10.5281/zenodo.2550638

Vánky, K. (2013) *Illustrated genera of smut fungi*. 3rd edn. APS Press, St. Paul, 288 pp.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990

Zundel, G.L. (1953) The Ustilaginales of the World. *Pennsylvania State College, School of Agriculture, Department of Botany. Contributions* 176: i–xi + 1–410.