### Estimating the threat status of smut fungi

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Abstract. Conservation of fungal diversity needs to be integrated. Smut fungi are potentially a key group for illustrating the problems encountered when estimating the conservation status of microscopic fungi. Various difficulties in assessing the status of smut fungi are described. Examples are provided of threatened species of smut fungi, evaluated with IUCN criteria.

Key words: conservation of fungi, fungal diversity, smut fungi

### Introduction

Faced with the accelerated loss of biological diversity, conservation efforts to date have focused mainly on animals and plants, particularly vertebrate animals and vascular plants. Conservation of fungi has been seriously neglected, although exceptionally some activities have been proposed for estimation and conservation of larger fungi at continentaland global-levels (e.g., Koune 2001; Dahlberg & Croneborg 2003; Dahlberg 2005; Senn-Irlet *et al.* 2007).

In 2009, three new specialist groups of fungi were established by the IUCN Species Survival Commission, as follows: (1) Chytrids, Zygomycetes, Downy Mildews, Slime Moulds; (2) Cup-fungi, Truffles and their Allies; and (3) Rusts and Smuts. The two previously existing fungal groups for lichen-forming ascomycetes and larger basidiomycetes remained unchanged, although the latter was renamed Mushrooms, Brackets and Puffballs.

The present paper addresses the organisms covered by the specialist group for rusts and smuts. The main goals of that group are as follows: (i) estimation of species conservation status, and (ii) organization of global conservation of so-called "microscopic fungi", placed in basidiomycetes.

In the literature, fungi are commonly separated into macromycetes (larger fungi) and micromycetes (smaller fungi), but these two groups are arbitrary (Denchev 2005). Some authors include within the macromycetes not only basidiomycetes proper, cup fungi, and truffles, collectively recognised as larger fungi (those fungi whose basidiomata and ascomata are easily seen, i.e., larger than 1 cm), but also other species with smaller fruitbodies produced collectively in a structure large enough to exceed 1 cm (e.g., species of Clavicipitales). There are however, hundreds of such 'micromycetous' species of fungi. These include smut fungi, with larger structures (i.e., longer than 1 cm), e.g., galls of Entorrhiza spp. on roots of species of Juncaceae and Cyperaceae; galls of Melanopsichium pennsylvanicum Hirschh. and Liroa emodensis (Berk.) Cif. on species of Persicaria; sori of Farysia spp. in flowers of Carex; sori of some Sporisorium spp. involving the entire inflorescence of graminicolous hosts; sori of Ustilago trichophora (Link) Körn. on stems of Echinochloa. They also include smut fungi with conspicuous sori (i.e. up to 25 cm long), e.g. Ustilago maydis (DC.) Corda in female inflorescences of corn; Ustilago esculenta Henn. on stems of Zizania latifolia Turcz.

'Macromycetes' is not a taxonomic term and its arbitrary nature generates practical problems in investigation and conservation of fungal diversity. For example, current practice in many countries is the curious situation where the total fungal diversity is interpreted by governmental institutions and NGOs as only the diversity of macromycetes. As a result, many practical problems in preparing inventories of other taxonomical groups of fungi and fungus-like organisms have arisen. It is essential for conservationists to acknowledge that the study and conservation of fungal diversity within a particular site, in a particular country or at a global level, needs to consider all fungi and fungus-like organisms, instead of concentrating only on the arbitrarily-defined and visible 'macromycetes'. All fungal diversity as a collective whole constitutes an invaluable genetic resource and conservation strategies must accordingly be based on a full assessment of all fungi.

Conservation of microscopic fungi is not a new idea and has already been discussed by many authors (e.g., Vánky & Harada 1989; Simay 1991; Helfer 1993; Foitzik 1996; Ingram 1999, 2002; Cannon *et al.* 2001; Vánky 2004; Denchev 2005).

Conservation of microscopic fungi may be realised in several ways. Up to 1986, about 11 500 microscopic fungi, or less than 1 % of their estimated number, were being grown ex situ in culture collections (Staines et al. 1986). Unfortunately, the preservation of such collections needs well equipped laboratories and resources, which only a small number of countries are able to afford. Many obligate parasites, e.g. rust fungi and laboulbeniomycetes, have not yet successfully been grown in pure culture. Cultivation of some other groups, e.g. smuts and VAM-fungi, is possible but very difficult in practice, and not always successful (Rossman 1997). Even those species, which can be cultivated and preserved in a collection, are not having their full genetic diversity preserved ex situ. One or two cultures of a species represent only an insignificant part of the real genetic diversity of that species in nature (Rossman 1997).

The best strategy for conservation of microscopic fungi is thus conservation *in situ* – in their natural habitats, together with other organisms (Rossman 1997).

Some microscopic fungi are already represented in national red lists. For example, *A provisional red list of British fungi* (Ing 1992), included 50 species of rust fungi and 13 species of smut fungi, while *A provisional red list of phytoparasitic fungi* (*Erysiphales, Uredinales & Ustilaginales*) of Germany (Foitzik 1996), included 23 species of powdery mildew, 143 species of rust fungi, and 127 species of smut fungi.

The first step towards the conservation of "micromycetes" is to prepare guide-lines for application of the IUCN Red List Categories of threats to microscopic fungi. For this purpose, we need to test the application of these categories on a well recognized taxonomic group of microscopic fungi.

### Why the smut fungi may be a key group for understanding problems in estimating microscopic fungi?

We need a suitable taxonomic group with which to test and analyze the problems which are likely to hinder evaluation of "micromycetes".

Most species of smut fungi are suitable for long-term monitoring because: (1) their sori are visible or produce symptoms in their hosts which are visible; (2) they are easy to collect and preserve, and (3) they persist in most seasons and develop continuously over the years. While similar advantages also apply to the rust fungi, these considerations make clear that other "micromycetes", for example anamorphic fungi, may not be so suitable to serve as a pilot key group.

#### Nature and classification of smut fungi

Smut fungi are parasites mainly on flowering plants, both monocotyledonous and dicotyledonous. The sori of the smut fungi range from a few millimetres up to 25 cm in length. They may attack all kind of plant organs: roots, underground modifications of the stem, stems, leaves (including petioles), pedicels, bulbils, flowers, particular part(s) of the flower (gynoecium, anthers, filaments, glumes, etc.), the inflorescence and its axis, fruits, and seeds. Smut fungi are found throughout the world with many cosmopolitian species.

For many years, the smut fungi were considered as a particular taxonomic group of fungi, belonging to a single, distinctive and well defined order, Ustilaginales (Mordue & Ainsworth 1984; Durán 1987; Scholz & Scholz 1988; Vánky 1994; etc.). On the basis of molecular phylogenetical analyses (Bauer et al. 1998, 2006; Begerow et al. 1998, 2007; Swann et al. 1999; Hibbett et al. 2007; etc.), it has become clear however that the group is heterogenic. The smut fungi as classically understood have now been disposed in two subphyala: Ustilaginomycotina (with 3 classes: Entorrhizomycetes, Ustilaginomycetes, and Exobasidiomycetes) and Pucciniomycotina (with one class of interest, Microbotryomycetes) (Kirk et al. 2008; Vánky 2008a). Based on molecular evidence (Begerow et al. 1998; Hibbett et al. 2007; etc.), it has been demonstrated that phylogenetically, the Microbotryales are more closely related to rust fungi than to smut fungi of the Ustilaginomycetes. Bauer et al. (2000) and Vánky (2008a, b) defined the term "smut fungi" as a phylogenetically heterogeneous group of microscopic fungi having a similar life strategy and organization, specifically as plant parasites that develop teliospores as organs of dispersal and resistance. The germinating teliospores infect host plants, either directly or through the production of secondary spores. Vánky (2008a, b) applied this definition to include as smut fungi members of the ascomycetous genus Schroeteria G. Winter (with 6 known species, previously excluded from the smuts; comp. Nagler et al. 1989; Vánky 2002), and a second ascomycetous genus Restilago Vánky, with a single species, R. capensis. In that sense, the term "smuts" may be regarded as similar in use to terms like "yeasts" and "lichenized fungi", each applied to members of different orders of ascomycetous and basidiomycetous fungi.

#### Number of the smut fungi

The number of the basidiomycetous smut fungi is about **1650 species** belonging to **93 genera** (Vánky 2009). They

are the second largest group of basidiomycetous plant parasites after the rust fungi. The global inventory of smut fungi is far from completion and further studies will surely reveal hundreds of new species, as well as additional records to already described species currently known from only one or two localities.

### Identification of smut fungi

Traditionally, the taxonomy of the smut fungi is based on a limited number of characters connected mainly with (1) the structure, shape, size, and localization of the sori; (2) colour and consistence of the spore mass; (3) LM and SEM morphology; (4) presence of spore balls and if present, their morphology; (5) way of spore germination; and (6) host specialization. In the modern taxonomy, different additional characters (molecular, biochemical, and ultrastructural, e.g., septal pore morphology and host-parasite interaction types) are applied for identification and development of a more natural classification.

The usual approach to identification of smut fungi uses dried, non-living specimens. Standard tools are the stereomicroscope for observation of macroscopic features, the compound light microscope for observation of microscopic features, and the SEM for spore ornamentation.

### Importance of smut fungi

The idea that plant pathogens should be conserved is counterintuitive to plant pathologists, whose careers are dedicated to prevention or eradication of plant diseases, and to politicians (Ingram 1999, 2002). Conservation of smut fungi is therefore discussed from the perspective of the mycologist.

Smut fungi are significant components of most natural ecosystems. Co-evolution of pathogens with wild crop hosts has resulted in a wide range of disease resistance mechanisms, which have not always been used effectively in crop breeding (Allen *et al.* 1999; Cannon *et al.* 2001). Some species play a key role in the population dynamics of their hosts (e.g. the case of *Microbotryum violaceum* s. lat.) and the ecosystems they inhabit. Smut fungi may have practical value as research tools and model systems (Ingram 1999, 2002).

### Threats to smut fungi

The most serious threats to smut fungi worldwide are habitat loss and habitat degradation. Degradation reduces the quality of the habitat and sensitive species are lost. In general, the destruction of natural ecosystems is a threat not only for the plants but also for the parasitic fungi associated with them.

The threats to smut fungi may be local, national, and international. We have also to take into account that susceptibility varies among species.

### Estimation of the threat status of smut fungi

The problems which exist in assessing the threat status of microscopic fungi are similar to those known for macroscopic fungi.

# (1) Correct taxonomic base for identification and advanced stage of inventory in different world regions

In the first place, the assessed records must be taxonomically correctly identified. A lot of problems with the correct taxonomic base for identification of smut fungi will be resolved by publication of the world monograph by Vánky (2010). This book will be a necessary precondition for success of conservation activities in this group at a global level.

The level of inventory is a very important condition. Before development of a global Red list, preparation of regional taxonomic monographs of smut fungi, even articles with detailed taxonomic revisions of some critical genera or groups of related genera, must be stimulated. At that moment, we have a strongly uneven development of the studies in different world regions. For instance, there is no recent monograph of African smut fungi recorded since the monograph of Zambettakis (1970) and its supplement (Zambettakis 1980), nor is there a modern monograph of any African country. Piepenbring (2003) published a monograph of smut fungi in the Neotropics, includiung 227 species, but the species richness in that large region should be much higher. For particular South American countries, only monographs of Colombia (Molina-Valero 1980) and Argentina (Hirschhorn 1986) exist, both published more than 20 years ago. For many other regions of the World, we have to refer to old literature sources (e.g., Fischer 1953, for North America; Kakishima 1982, for Japan) or there may be no monographs of the smut fungi at all (e.g., for Turkey, the Near East, South-East Asia).

Because the smut fungi are identified mainly on dried material, visitations and revisions of dried reference collections, must be encouraged.

### (2) Accumulation of distribution records

A serious problem is the gap in knowledge about the distribution of species. The extent of that information varies among regions. Assessment of threat status depends on an accumulation of distribution records. In the case of smut fungi, it is a real problem because of the limited number of known records, especially for non-European regions. Field trips, collections, identifications, and preservation of specimens of smut fungi must be organized and well maintained. In that connection, revisions of specimens of smut fungi in world reference collections, made by experts, will yield new distribution records. For this purpose, it is

also very helpful to examine specimens of selected families of vascular plants in herbaria for presence of smut fungus infections.

Because of the uneven development of studies in different world regions, the interpretation of the threat status of a species, distributed in Europe with 2–4 localities, and another species, known in Africa from 2–4 localities, may be quite different. That is because some species have been rarely reported because they are rare while other species have been rarely reported but may be widespread, or at least, not meriting threatened status.

### (3) Parasitic specialization

Among the parasitic fungi (e.g. downy mildews, powdery mildews, rust fungi, smut fungi) there are species with wider (host family), and others, with narrow (host genus or even host species) specialization.

It is also possible for a parasite to have a narrow specialization but its principal host(s) to be rather widespread. On the other hand, narrow specialization may be on a host(s) with a limited or even local distribution. In this case, both the host and parasite species may be evaluated as critically endangered species possessing an extent of occurrence estimated as less than 100 km<sup>2</sup> and/or as species known from a single location. As a result, the parasite as well as the host plant, must be regarded as organisms requiring conservation.

A parasite may alternatively have a wide specialization but some of its hosts may be rare. For instance, *Schizonella melanogramma* (DC.) J. Schröt. is a wide-spread smut fungus which parasitizes many species of *Carex*. Attacked plants are usually sterile, i.e. without generative organs. *Schizonella melanogramma* is widely distributed in Bulgaria but among its Bulgarian hosts is *Carex rupestris* All., an *Endangered* plant species in Bulgaria known only from 2 localities in the Rila Mts and Pirin Mts. Because of its biology, *S. melanogramma* may be a factor in reducing the population of *Carex rupestris* in Bulgaria.

A given parasitic species may not or may attack threatened or near threatened plant species. In the latter case, the parasite could be an important factor in control of numbers as well as limitation of host distribution. It may even affect survival of the host population. Such a parasite attacking threatened plants may be a wide-spread species, but it may equally also be an endangered or vulnerable species. An example from Bulgaria is *Urocystis aquilegiae* (Cif.) Schwarzman, a *Critically Endangered* (CR) species at regional and European level, on *Aquilegia aurea* Janka, a Balkan endemic and *Near Threatened* (NT) species for Bulgaria.

An interesting question arises in the case of a threatened parasitic species on a threatened plant species, i.e. should the parasite be destroyed to protect the host or should it be protected together with the host species (cfr Helfer 1993)? The latter is surely the better conservation strategy.

## (4) Definition of the terms individual and population

Another problem in estimating smut fungi is clarification of the terms "individual" and "population" which have important places in the application of conservation categories. In some cases of parasitic species an explanation of the term 'individual' is necessary. For instance, in a case of local infection, one sorus and its mycelium may be an individual.

### (5) Difference in ease of collection as a factor affecting record numbers

The sori of some smut fungi are deeply embedded in leaf tissues (e.g. the doassansioid smut fungi) or may form galls in the roots (*Entorrhiza*). Such species are very difficult to collect. They seem to be rare but probably many are more common, but overlooked by collectors. In such cases, the principal question is whether the species is really rare, or simply rarely collected and recorded (Cannon *et al.* 2001).

### (6) Status of newly described species

Many newly described species are known only from the type locality. What time needs to elapse for such species to be considered worthy of conservation status? Arguably, a period of 3 years (if described from places where active field mycologists are more numerous, such as Europe) or 5 years (if described from other regions) or perhaps even longer if from poorly-surveyd regions, e.g. parts of north-western Australia or some African countries, should elapse before evaluation of such species can be considered possible.

### (7) Endemism

Because of the stage of inventory, the use of the term "endemism" in relation to smut fungi is debatable. In only a few cases, like that in the monograph of the Australian smut fungi (Vánky & Shivas 2008), has endemism been reasonably resolved.

### (8) What taxa can be assessed?

In plant conservation practice, rare subspecies are also estimated. In the modern taxonomy of the smut fungi, the category "subspecies" is not in use, but some varieties in use are apparently rare at global level. For instance, *Entorrhiza casparyana* var. *tenuis* Denchev & H.D. Shin is reported only from Austria, Costa Rica, Korea, and Romania (Denchev *et al.* 2007). The correct approach to rare varieties of smut fungi remains unresolved.

### (9) Cryptic species

During the last few years, as a result of molecular phylogenetic investigations, many cryptic species of fungi have been described. On the one hand, in mycology an explanation of the casus cryptic versus sibling species has not yet definitely given for related species without morphological differences. Cryptic and sibling species are very close in concepts. The term "cryptic species" should be used when, until recently, the species were not known to be distinct, while the term "sibling species" covers well recognized species with very few morphological differences. On the other hand, it is not clear how to apply IUCN criteria for cryptic species which seem to be rare. Molecular studies of old reference collection specimens are difficult (if not impossible), and that is an obstacle for complete revision of specimens when it is necessary to judge whether they are members of a particular cryptic species.

### (10) What IUCN categories and criteria should be predominantly used?

The main criterion applicable to smut fungi is 'B'.

To start, we have to give an idea of the approximate number of threatened species of smut fungi. Because the distribution of many species of smut fungi is poorly known, in many cases the category *Data Deficient* will be applied.

### (11) Lack of detailed information on threatened habitats of smut fungi

The available information on the threatened habitats of smut fungi species varies between regions; in general, there is lack of detailed information on threatened habitats of these fungi in most areas outside Europe.

### (12) Key habitats and their specific threats

The question of key habitats of smut fungi and their specific threats has never been discussed or analysed from the point of view of conservation. These issues need to be discussed and resulting recommendations listed in the framework of the current mandate of the IUCN SSC Rusts and Smuts Specialist Group.

### Method

Selection of species should be based on the following criteria:

- 1. The species must be threatened worldwide.
- 2. The species must be confined to a threatened habitat.
- 3. The species must have a narrow distribution range.

### Examples of threatened species

Evaluations are based on IUCN criteria (IUCN 2001).

Mundkurella kalopanacis Vánky

Family: Urocystidaceae

- Status: Critically Endangered CR B2ab(iii).
- **Distribution:** Korea (Mount Kongo, Sanseisan, Harigiri). Known only from one locality and only from the type collection, dated 1928.
- Habitat: a parasite on stems of *Kalopanax pictus* (Thunb.) Nakai (*Araliaceae*).

Threat: unknown.

- Conservation actions: none.
- References: Vánky (1990), Denchev & Kakishima (2007).

Mundkurella japonica Denchev & Kakishima

Family: Urocystidaceae

Status: Endangered — EN B2ab(iii).

- Distribution: Japan (Hokkaido, Honshu). Known from four localities: Ibaraki Prefecture, Kukizaki-machi, Forestry and Forest Products Research Institute; Hokkaido, Kamikawashicho, Nakagawa-machi, Kotohira; Hokkaido, Abashirishicho, Nishiokoppe-mura, Mt. Uenshiri; and Hokkaido, Ishikari-shicho, Chitose-shi, Shikotsu-Lake.
- Habitat: a parasite in fruits and clusters of compound umbels of *Kalopanax pictus* (Thunb.) Nakai (*Araliaceae*).

Threat: unknown.

Conservation actions: none.

References: Denchev & Kakishima (2007).

Thecaphora hedysari Vánky

Family: Glomosporiaceae

- Status: Endangered EN B2ab(iii).
- Distribution: Central Asia (Mongolia and Kyrgyzstan). Reported only two times: from the type locality (Mongolia, the Mongolian Altai Mountains; a collection from 1986) and from Kyrgyzstan (the Issyk-Kul hollow, W of Karakul Lake; a collection from 1965).
- Habitat: a parasite in seeds of *Hedysarum* spp. (*Fabaceae*). Known on two host plants: *Hedysarum ferganense* Korsh. and *H. kirghisorum* B. Fedtsch.

Threat: unknown.

- Conservation actions: none.
- References: Vánky (1991), Denchev & Karatygin (2009).

*Entorrhiza casparyana* (Magnus) Lagerh. var. *tenuis* Denchev & H.D. Shin

Family: Entorrhizaceae

- Status: Data Deficient DD.
- Distribution: Asia (Korea), Europe (Austria, Romania), and Central America (Costa Rica) – first collected in Romania in 1964, with subsequent collections from Costa Rica in 1992, Austria in 2002, and Korea in 2006, in all countries, from single localities.

Habitat: a parasite on the roots of *Juncus tenuis* Willd. (*Juncaceae*).

Threat: habitat loss and degradation.

Conservation actions: none.

References: Vánky (1985), Piepenbring (2003), Denchev (2004), Denchev *et al.* (2007), Denchev & Minter (2008a).

Urocystis dioscoreae Syd. & P. Syd.

Family: Urocystidaceae

Status: Data Deficient — DD.

- Distribution: Asia China, Japan, Pakistan, and Russia (Novosibirsk oblast, Primorskyi krai). At least some species of *Dioscorea*, its host genus, are themselves redlisted. The smut fungus seems to be rare in all countries from which it has been recorded.
- Habitat: a parasite in the leaf and petiole veins, and stem vascular system of *Dioscorea* species (*Dioscoreaceae*). Known host plants: *Dioscorea deltoidea* Wall. ex Griseb., *D. nipponica* Makino, and *D. tokoro* Makino.

Threat: unknown.

Conservation actions: none.

References: Sydow & Sydow (1909), Ito (1936), Yen (1937), Gutner (1941), Ling (1953), Wang (1964), Mirza (1968), Kakishima (1982), Govorova (1990), Azbukina & Karatygin (1995), Azbukina *et al.* (1995), Vánky (2007), Denchev & Minter (2008b).

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