# Monstruosities under the Inkap Mushrooms

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**Four different Inkcaps were isolated from horse dung and tested for growth on different medium. In addition to normal-shaped mushrooms, three of the isolates formed fruiting body-like structures resembling the anamorphs of *Rhacophyllus lilaceus*, a species originally believed to be asexual. Teleomorphs of this species were later found and are known as *Coprinus clastophyllus*, respectively *Coprinopsis clastophylla*. The fourth of our isolates also forms mushrooms but most of them are of crippled shape. Well-shaped umbrella-like mushrooms assigns this Inkcap to the clade *Coprinellus*. ITS sequencing confirmed that the first three strains and the *Rhacophyllus* type strain belong to the genus *Coprinopsis* and that the fourth isolate belongs to the genus *Coprinellus*.**

## 1. Introduction

Inkcaps are a group of about 200 basidiomycetes whose mushrooms usually deliquesce shortly after maturation for spore liberation (see Fig. 1). Until recently, they were compiled under the one single genus *Coprinus*. However, molecular data divided this group into four new genera: *Coprinus*, *Coprinopsis*, *Coprinellus* and *Parasola* (Redhead et al. 2001).

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**Figure 1.** Mushrooms of *Coprinopsis cinerea* strain AmutBmut (about 12 cm in size) formed on horse dung, the natural substrate of the fungus. Left. Adolescent fruiting body at the stage of cap expansion. Right. Aging mushroom at autolysis

The edible, very tasty *Coprinus comatus* (Fig. 2) is the type species of the new genus *Coprinus* (corresponding to the former *Coprinus* subsect. *Coprinus* http://www. grzyby.pl/coprinus-site-Kees-Uljee/species/Coprinus.htm). Only two other species, *Coprinus sterquilinus* and *Coprinus spadiceisporus*, are known in this genus that clusters within the Agaricaceae. A typical characteristic of these species is a central cord suspended inside the stipe (Redhead 2000).

**Figure 2.** Young, not fully developed fruiting body of *Coprinus comatus* (Shaggy Mane, Laywer's Wig) with the typical ring at the stipe left from rupturing the veil at the start of expansion of the pileus (about 10 cm in size)

The three other newly defined genera all belong into the family of Psathyrellaceae. *Coprinopsis* forms the largest genus with more than hundred defined species (Hopple and Vilgalys 1999, Redhead et al. 2001, Keirle et al. 2004) and includes well known species such as *Coprinus cinereus* (Dungheap Inkcap, Dunghill Mushroom; Fig. 1), *Coprinus lagopus* (Woolly Inkcap, Hare's Foot), *Coprinus atramentarius* (Common Inkcap, Alcohol Inkcap) and *Coprinus stercoreus*, now termed *Coprinopsis atramentaria*, *Coprinopsis cinerea*, *Coprinopsis lagopus* and *Coprinopsis stercorea*, respectively (Redhead et al. 2001). These species scatter over the formerly defined *Coprinus* section *Coprinus* subsect. *Atramentarii* and *Lanatuli* and section *Veliformis* subsect. *Narcotici*, respectively. A typical character of the subsect. *Lanatuli* is an easily removed hairy veil composed of sausage-shaped elements. In contrast, species of subsect. *Atramentarii* have a scarce veil. Typical veil elements of the subsection *Narcotici* are persistent, warty cells and mushrooms have a strong gas smell (Orton and Watling 1979, Breitenbach and Kränzlin 1995). Other *Coprinopsis* species not listed here were grouped into *Coprinus* section *Coprinus* subsect. *Alachuani* or into *Coprinus* section *Veliformis* subsect. *Nivei* (http://www.grzyby.pl/coprinus-site-Kees-Uljee/ species/Coprinus.htm).

*Coprinellus* is the second largest group with more than 50 defined species (Redhead et al. 2001), most of which belong to the *Coprinus* section *Pseudocoprinus* subsect. *Setulosi*. Other members of the genus *Coprinellus* are found in the *Coprinus* section *Veliformis* subsect. *Domestici* and *Micacei*. Well known members of this genus are *Coprinus disseminatus* (Fairy Bonnet, Little Helmet), now *Coprinellus disseminatus*, from section *Pseudocoprinus* subsect. *Setulosi* and *Coprinus micaceus* (Glistening Inkcap), now *Coprinellus micaceus*, from section *Veliformis* subsect. *Micacei*, respectively. Species of the section *Pseudocoprinus* subsect. *Setulosi* have either no veil or very fine veils and hair-like structures (setulae or setae) on stipe and pileus. Veils of species of the section *Veliformis* subsect. *Micacei* are made up of small granular flocks and stipes may be smooth or pruinose due to surface covering by crystals (http://www.grzyby.pl/coprinus-site-Kees-Uljee/species/Coprinus.htm).
Fruiting bodies of *Coprinellus* species are often very delicate by a less fleshly cap and stipes are often brittle (Breitenbach and Kränzlin 1995).

**Figure 3.** *Coprinellus xanthothrix* fruiting body (about 5 cm in size) formed on beech saw dust

*Parasola* is the smallest of the three new genera within the Psathyrellaceae with currently 18 defined species. The type species is *Parasola plicatilis* (Pleated Inkcap), previously *Coprinus plicatilis* (Breitenbach and Kränzlin 1995, Redhead et al. 2001) grouped in the *Pseudocoprinus* subsect. *Glabri* like most other Parasola species. *Parasola auricoma* (formerly *Coprinus auricomus*) is the only species forming *Pseudocoprinus* subsect. *Auricomi* (http://www.grzyby.pl/coprinus-site-Kees-Uljee/ species/Coprinus.htm).

The new classification of the Coprinii agrees well with Ulje's *Coprinus* key based on macro- and micro-morphologies of fruiting bodies and spores (http://www.grzyby.pl/ coprinus-site-Kees-Uljee/species/Coprinus.htm). Nevertheless, species are often difficult to recognize beyond doubt and, most likely, many species are still not characterized. Next to mushroom and spore morphology, the habitat of a fungus can add to correct identification (Breitenbach and Kränzlin 1995). The Coprinii can be found growing on a wide variety of substrates, such as soil, dung of herbivores, living or dead wood, straw, leaf litter and other plant debris and organic litter. Mostly, the Inkcaps are fimicolous, meaning that they grow on dung. Here we present some isolates from horse dung with unusual fruiting body morphologies.

## 2. Materials and Methods

### 2.1. *Strains and culture conditions, microscopy and DNA techniques*

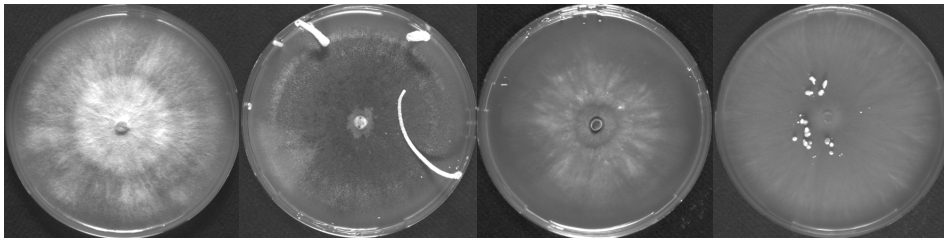
Four different *Coprinus* sp. isolates (sp. 1 to sp. 4) were obtained from horse dung from different localities close to Mainz, Germany (sp. 1 from Ober-Olm in year 2000; sp. 2 from Ginsheim-Gustavsburg in year 2003 and sp. 3 and 4 from Ingelheim am Rhein in year 2003). Isolates 3 and 4 came from the same collection of horse apples. *C. cinerea* strain AmutBmut (Swamy et al. 1984, Kertesz-Chaloupková et al. 1998) and *Coprinus xanthothrix* strain c144 (kindly supplied by Timothy James, Duke University) served in comparison of fruiting structures. The *C. clastophyllus* type strain was obtained from the CBS (473.70). Strains were cultivated on MEA (20 g malt extract, 1 g peptone, 20 g glucose, 10 g agar), solid YMG/T (Granado et al. 1997), solid corn meal-horse dung extract (HDE) according to Esser (2000), solid BSM (Hüttermann and Volger 1973) and sterilized horse dung either within a fungal growth chamber at constant 28°C in dark, ventilated boxes or in the laboratory at room temperature under normal day-night light conditions. Light microscopy was performed with a Zeiss Axiophot photomicroscope equipped with a Soft Imaging ColorView II digital camera. Lysomeres from caps were isolated with a scalpel and dispersed in distilled water. Samples were observed in light microscope at 20 x magnification. For observation of mycelia in the microscope, freshly grown agar pieces were squeezed on a glass slide. Aerial spores were harvested by pouring water over the surface of grown cultures. Genomic DNA was isolated by the protocol of Zolan and Pukkila (1986), ITS sequences amplified with primers ITS1 and ITS4 (Gardes & Bruns 1993) for sequencing at the Institute of Forest Genetics, Georg-August-University Göttingen.

## 3. Results and Discussion

### 3.1. *Culture and mycelial characteristics of the four newly isolated strains*

Cultivating on different agar media (YMG/T, MEA, BSM and HDE)

showed for all four new strains that HDE is best for mycelial growth in terms of speed of growth (Fig. 4). Strain sp. 1 was more dense and fluffy than the other three strains. Typical for strains sp. 2, sp. 3 and sp. 4 was thin growth within the agar in form of hyphal strands and very little or no aerial mycelium. Mycelia of the strains were observed. Strain sp. 1 had no clamp



cells at hyphal septa, unlike the three other strains (Fig. 5).

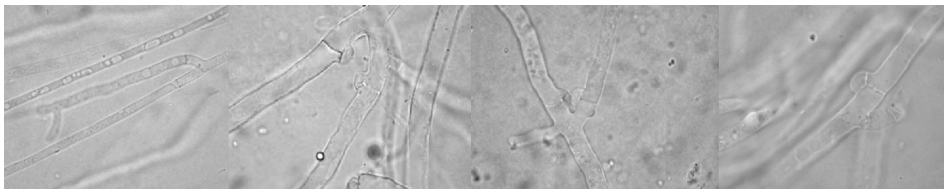


**Figure 4.** Colony morphology of isolates sp. 1, sp. 2, sp. 3 and sp. 4 (from left to right) on HDE medium grown at 28°C in the dark. Note the "etiolated stipe" structures on the plates of isolate sp. 2 (fully developed), sp. 3 (starting to develop), and sp. 4 (still growing)

**Figure 5.** Isolate sp. 1 had no clamp cells at hyphal septa in contrast to isolates sp. 2, sp. 3 and sp. 4 (shown from left to right)

Regularly in HDE cultures of strains sp. 2, sp. 3 and sp. 4, multi-cellular structures with long stipe and poorly developed caps appeared (Fig. 4) that

resembled etiolated stipes from *C. cinerea* strain AmutBmut (Fig. 6). Furthermore, cultures of the three isolates gave rise to white and black sclerotia (not shown). In the black forms, an outer melanized rind and an inner medulla were identified.

**Figure 6.** Etiolated stipes (also called dark-stipes) from *C. cinerea* strain AmutBmut that have an underdeveloped cap and an extra long stipe and form when the fungus has not enough light

### 3.2. *Fruiting abilities of the four new isolates*

In *C. cinerea*, etiolated stipes are formed when the fungus obtained a short light signal and then stayed in the dark. To correctly follow the sequence of developmental events leading to mature fruiting bodies with basidiospores, *C. cinerea* needs alternating light and dark phases synchronized to the normal day/night rhythm (Lu 1974, 2000, Kües 2000). Since the multi-cellular structures of sp. 2, sp. 3 and sp. 4 formed in the dark (Fig. 4) suggested that these came from aberrant fruiting body development possibly due to lack of light, new HDE cultures were incubated at room temperature on a bench in the laboratory under a natural day/night light regime.

Under the day/night light regime, production of multi-cellular, etiolated stipe-like structures with underdeveloped caps did not occur. Instead, the

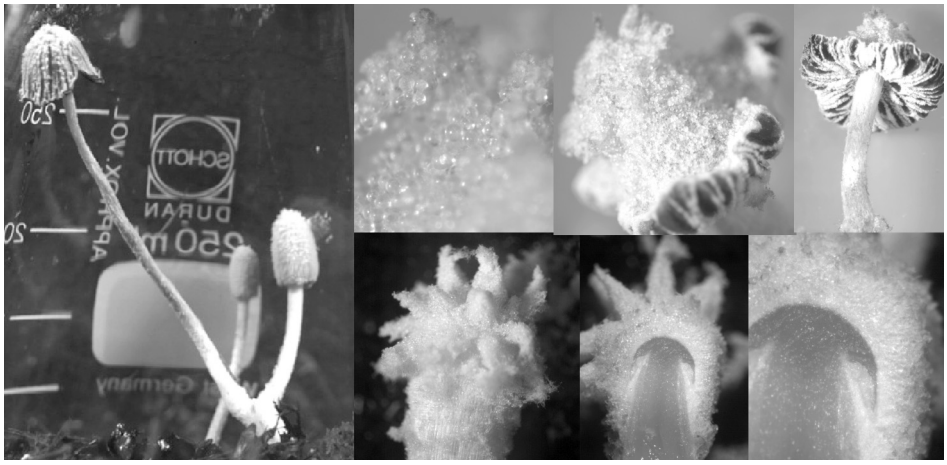



**Figure 7.** Fertile mushrooms of isolate sp. 4 on horsedung (photo at the left). At the right, upper panel of pictures: Masses of large round bodies are covering sterile fruiting-body-like structures of isolate sp. 4 (left). Transition structures form gills that partially carry basidiospores and are partially covered with large round bodies (middle). The more basidiospore, the less the amounts of large round bodies (right). At the right lower panel: Sterile fruiting-body like structures from the *Coprinopsis clastophylla* type strain are also covered by masses of large round bodies. The same structure is shown once intact (left) and longitudinal cut at two different enlargements (middle and right)

three strains regularly gave rise to many sterile fruiting body-like structures as well as some normal umbrella-shaped, fertile fruiting bodies carrying basidiospores (Fig. 7). Cultures obtained from germination of such basidiospores always gave rise to mycelium with clamps cells again able to form sterile and fertile fruiting structures (not shown). Cap tissues including the lamellae and partially the stipe of the sterile structures were covered by masses of large, round, glittering bodies as if cap and stipe have been dusted with icing sugar (Fig. 7). To different degrees, fertile structures had also such bodies (Fig. 7). Clemençon (1997) referred to unusual large round bodies on mushroom caps as lysomeres, Patouillard (1901) as bulbils.

Lysomeres observed by Clemençon (1997) and Reynders and Malençon (1969) contain many small cells and are probably different structures from what we see here. Lysomeres have been observed in the past in an anamorphic species forming sterile mushroom-like structures. This anamorph was originally called *Rhacophyllus lilacinus* (Berkeley and Broome 1871; Redhead et al. 2000). Later, an isolate of the species was observed to form also fertile

fruiting bodies. The sexual form was recognized as a *Coprinus* and the teleomorph obtained the name *Coprinus clastophyllus* (Maniotis 1964), respectively now described by Maniotis (1964) as *Coprinopsis clastophylla* (Redhead et al. 2001). The type strain of *C. clastophylla* was obtained from the CBS. This strain rarely formed fruiting bodies with basidiospores but very commonly sterile fruiting-body-like structures that resembled those of isolates sp. 2, sp. 3 and sp. 4 (Fig. 7). ITS sequencing confirmed the four strains to belong to one species.

We also observed the behavior of HDE cultures of isolate sp. 1 on the bench in the laboratory. This strain regularly produced many strangely shaped mushrooms of about 3 to 4 cm in size (Fig. 8). Usually, they carried basidiospores (Fig. 8) that germinated into monokaryons that constitutively produced oidia (Fig. 8). Typically, the stipe and often also the cap of the mushrooms split during development and often, inner stipe tissues shot up and appeared to pierce the cap during upward growth. As a result, mushroom caps look like "picked helmets". Amongst those and other misshaped mushrooms, there were always a few on a plate that look like a typical Inkcap (Fig. 8). These classically shaped mushrooms were delicate with thin, hollow stipes and fine umbrellas with little inner pileus trama. Brittle, hollow stipes and delicate caps are typical for *Coprinellus* species such as *Coprinellus xanthothrix* (Fig. 3). ITS sequences groups isolate sp. 1 into the genus *Coprinellus* (not shown).

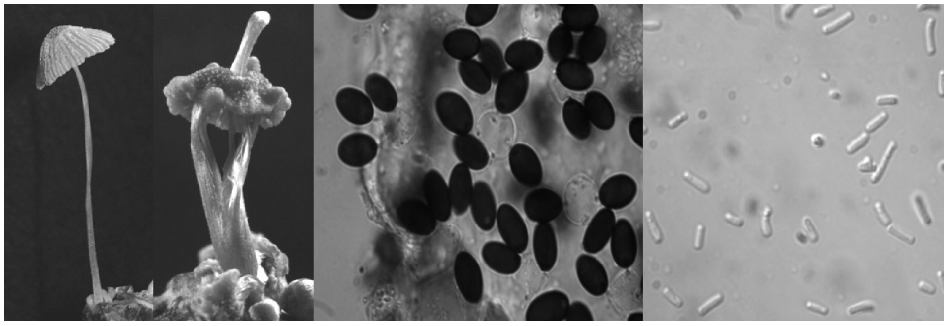


**Figure 8.** Classical umbrella-shaped mushroom of isolate sp. 1, a monstrous mushroom of the "picket

helmet"-type, basidiospores of isolate sp. 1 and oidia formed on monokaryons obtained from germination of basidiospores (from left to right)

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