

**COPROTUS ARDUENNENSIS, A NEW SPECIES OF
COPROPHILOUS DISCOMYCETES (PEZIZALES, ASCOMYCOTA)**

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A new coprophilous species of *Coprotus* (Pezizales, Pyronemataceae) is described and reported from five localities of the Ardennes (Belgium). Within the genus, it belongs to a group of species mainly identified by the presence of carotenoids within the paraphyses. It could not be identified as any of the 25 species known, but is closest to *C. ochraceus*.

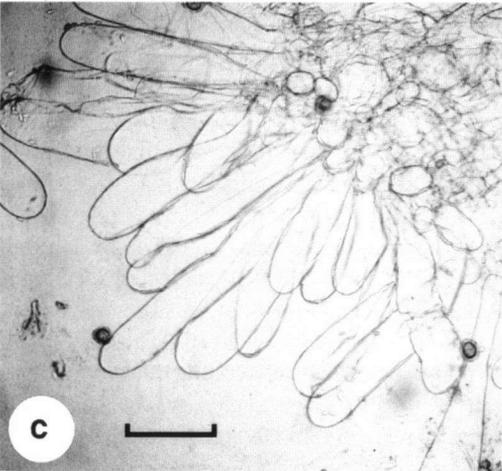
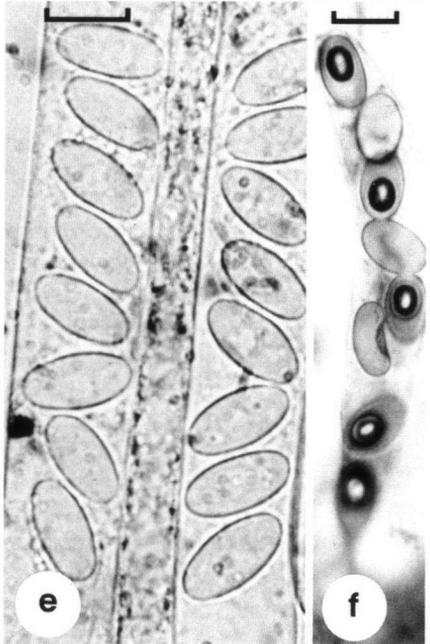
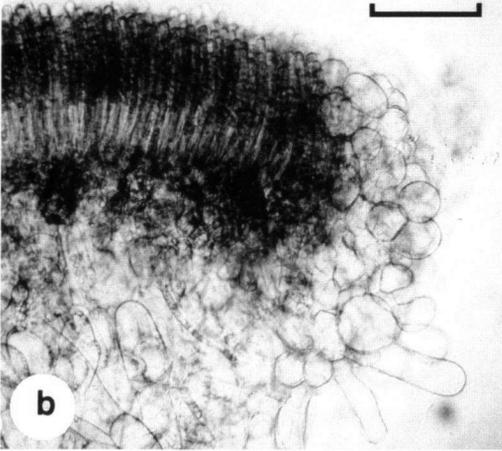
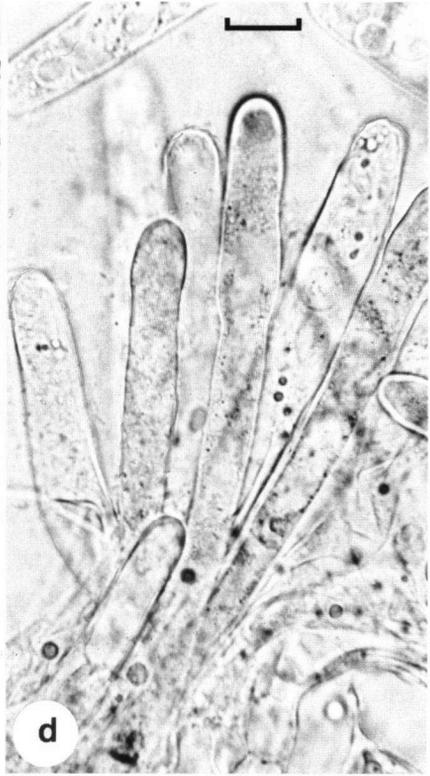
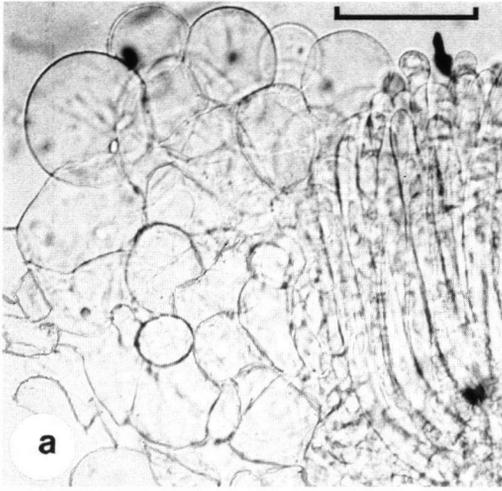
Coprotus Korf & Kimbr. was first suggested by Korf (1954) who later drafted the diagnosis of the genus to comprise mainly coprophilous species with non-amyloid 8-spored asci, smooth, hyaline ascospores and uncinuate paraphyses i.e. species traditionally placed within the old heterogeneous *Ascophanus* Boud. (Korf, 1958). Subsequently Kimbrough (1966), Kimbrough & Korf (1967) and Kimbrough et al. (1972) extended the limits of the genus to species with multisporied asci, some extracted from *Rhyarobius* Boud., admitting that their paraphyses are not strictly hooked and adding new discovered species. For an up-to-date circumscription of the genus, see Van Brummelen (1998: 427).

In the basic paper of Kimbrough et al. (1972) eighteen species were combined, shortly described and keyed. Moravec (1971), Bell & Kimbrough (1973), Jeng & Krug (1977), Thind et al. (1978), Gibson & Kimbrough (1980), Gené et al. (1993) and Wang (1994) added seven more species. No comprehensive study has been made to assess the validity of the 25 described species, which more often than not are quite obviously difficult to delimit (Kimbrough et al., 1972). For instance, a recent study on coprophilous Pezizales in Italy (Doveri et al., 2000) deals with nine taxa of *Coprotus* of which two could not be assigned to previously described species.

Before dealing with the new species hereafter described we produced a key (not shown here) to the best of our knowledge for taxa referred to *Coprotus*¹. This comparative approach confirmed that the informal groups outlined by Kimbrough et al. (1972) within the genus still hold but that specific delimitations are hard to pin down on the grounds of limited descriptions of most of the species involved. Nevertheless, the material described hereunder combines sufficient differential diagnostic characters to be distinguished as a separate species.

The dung collected was placed in moist chambers for several weeks knowing that *Coprotus* species are among the last discomycetes to appear. Freshly collected specimens were mounted in distilled water in which all measurements were made. Histological details have been studied from freehand sections. Cotton blue in lactic acid (CB) has only been tested for its effects on ripe ascospores with non-elastic walls developing de Bary bubbles artifact. Freehand drawings were made for the illustrations. Photomicrographs were made with light microscopy with a Leitz microscope using a 35 mm Olympus camera.

1) This key and comments on the species involved will be published in the near future.



Coprotus arduennensis J.R. De Sloover, spec. nov. — Figs. 1, 2

Apothecia discoidea, sessilia, dispersa; discus aurantius 0.5–1.5 mm diametro, scabriusculus ob protrudentes ascos. Superficies externa glabra, alba vel pallide lutea. Margo alba, parum prominens. Excipulum ectale textura globulosa, cellulis valde inflatis, 10–45 µm diam. praecipue ad marginem; cellulae marginales valde elongatae praesertim ad basim, usque 200 µm longae. Asci octospori 150–185 × 10–16 µm, late cylindracei vel cylindrico-clavati, inferne leviter attenuati. Ascosporae 12.5–15.5 × 6.5–7.5 µm, ellipsoideae, uniseriales, hyalinae, levigatae. Paraphyses cum multis parvis aurantiisque guttulis, cylindraceae omniquoque erectae, comparate crassiusculae, inferne septatae et raro ramosae, 6–9 µm diam., raro ad apicem leviter incrassatae (10 µm) obtusaeque.

In fimo fero porcino (*Sus scrofa*) crescens, in calluneto cum sphagnis (*Vaccinietum*).

Typus: *J. De Sloover 00C9*, Vielsalm, A Sacrawé, Belgium, 23.III.2000, (holotypus: herb. J. De Sloover; isotypus L 998.171-667).

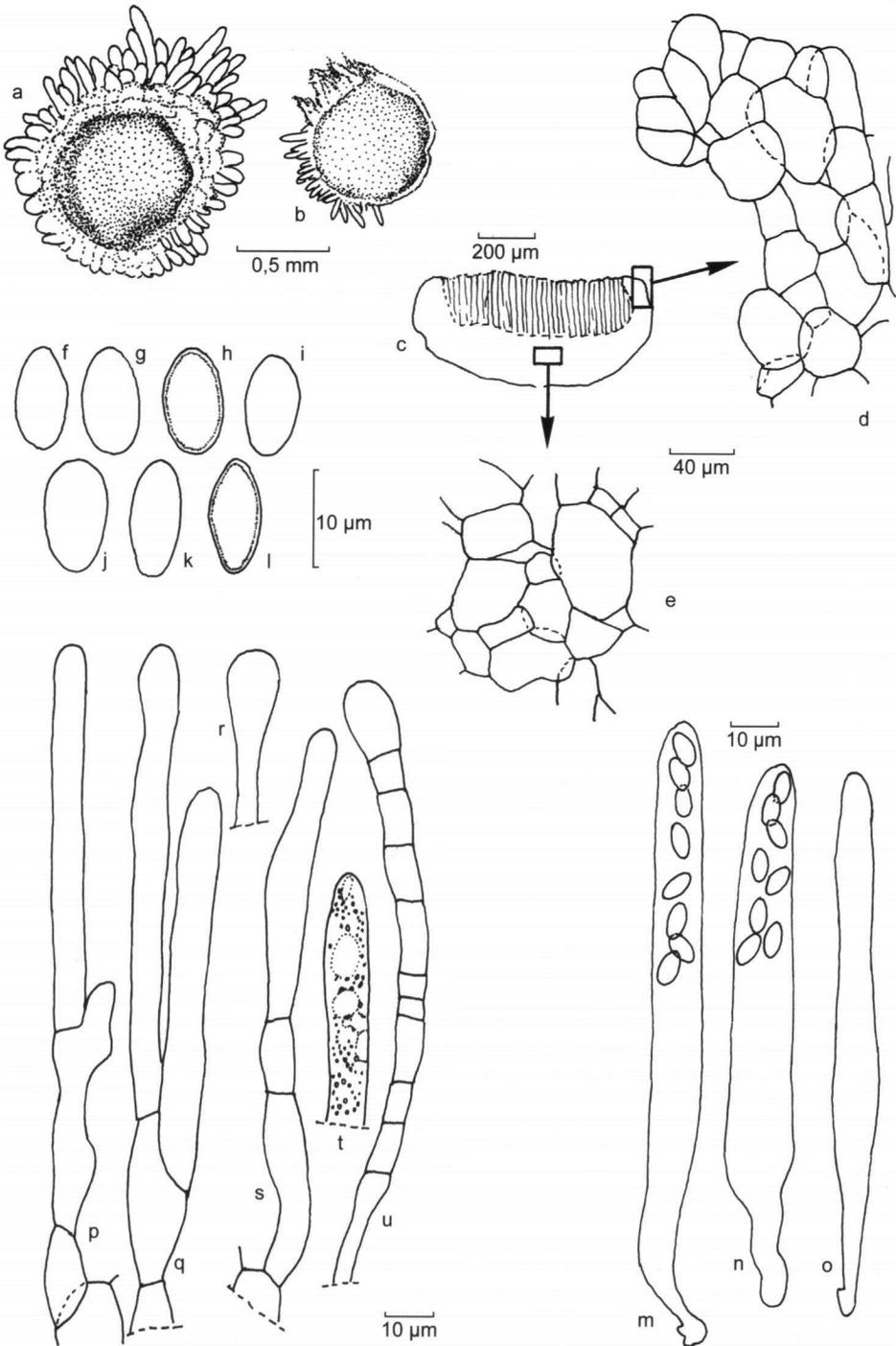
Etymology: from Latin, from the Ardenne country.

Apothecia discoid, superficial, scattered, sessile on an obconical base, 0.5–1.5 mm across, 0.5–0.7 mm high. Receptacle at first subglobular, then expanding and finally high saucer-shaped, light orange turning whitish when dry, smooth with a narrow and rough margin slightly raised above the disk. Disk flat, bright orange, roughened by the slightly protruding asci or paraphyses varying greatly in length, remaining deep orange upon drying. Hymenium 250–400 µm thick. Cortical excipulum 250–300 µm thick near the base, of closely compacted isodiametric globose cells 10–45 µm across, spreading into the lower and then the upper flank made of isodiametric, subglobular-subangular cells 30–40 µm across (textura globulosa to angularis), or oblong cells up to 200 µm long with thin hyaline walls near the margin. Margin made of inflated globular cells causing its rough bumpy appearance. Asci cylindrical or cylindrical clavate gently tapering upwards and downwards from a maximum width below the middle, (150–)160(–185) × (10–)12.8(–16) µm, rounded above, 8-spored (but often with only some of the spores fully developed or rarely 16-spored), the wall not blue in Melzer's reagent. Ascospores uniseriate or rarely biseriate, ellipsoid, hyaline, smooth, (12.5–)14.5(–15.5) × (6.5–)6.8(–7.5) µm. Paraphyses simple or branched below the long upper cell, strictly cylindrical and straight, rather thick, 6–9 µm wide from base to tip (10 µm), 3 or 4-septate (exceptionally more septate), the upper cell (60–)80(–100) µm long, with numerous tiny (up to 1 µm) orange plasmatic oil guttules and large vacuoles.

Habitat — On dung of wild boar in wet heathland.

Specimens examined. BELGIUM: Luxemburg Province, Vielsalm, A Sacrawé (alt. 585 m), on wild boar dung in wet *Vaccinietum* heathland, 23.III.2000, *J. De Sloover 00C9* (holotype of *Coprotus arduennensis*, herb. J. De Sloover; isotype L 998.171-667); Petit-Thiers, Grand Fond (alt. 440 m), on deer dung in wet *Molinietum*, 15.VII.2000, *J. De Sloover 00C106*. Liège Province, Büllingen, Holzwarche (alt. 640 m), on wild boar dung in peaty *Molinia* grassland with *Coprotus leucopocillum* Kimbrough et al., *Ascobolus michaudii* Boud., 04.VII.1999, *J. De Sloover 99C111*; Biron, on deer dung in a spruce afforested site, 15.X.2000, *J. De Sloover 00C139*. Namur Province, Oignies-en-Thiérache, Trieu des Cavaliers (alt. 360 m), on deer dung in birch-oak wood with *Ascozonus woolhopensis* (B. & Br.) Boud., *Ascobolus furfureus* Pers., *A. albidus* Crouan and *Thelebolus stercoreus* Tode, 20.VIII.2000, *J. De Sloover 00C114*.

Fig. 1. *Coprotus arduennensis*. a. Longitudinal median section through ripe apothecium: raised margin of bullate cells (left) and hymenium; b. median section through ripe apothecium: hymenium and ectal excipulum with bullate cells and elongated cells on the lower flank; c. lower flank excipulum with external elongated cells; d. paraphyses with granular content and refringent guttules; e. ascospores in two asci flanking one paraphysis; f. ascus with ascospores showing de Bary bubbles in CB. a–c, e: *De Sloover 00C9*, holotype; d & f: *De Sloover 99C111*). — Scale markers: a & c = 50 µm; b = 100 µm; d–f = 10 µm.



Criteria commonly used to delimit species of *Coprotus* are both quantitative and qualitative: apothecia colour and size, excipulum extent, ascus and ascospore form and size, paraphyses form and content. Depending on the relative importance given to these criteria the resulting classification may be quite different.

De Bary bubbles are consistently induced in *Coprotus* spores by the use of special mounting media and they have been regarded as one of their taxonomic features (Kimbrough et al., 1972). To our mind those artifacts created by mounting the spores, e.g. in cotton blue in lactic acid, must only be viewed as a clue to the thickness or rigidity of the spore walls (Baral, 1992): they are produced in the same conditions in quite different taxa such as Pyrommataceae like *Pulvinula* (pers. obs.) and *Pseudombrophila* (van Brummelen, 1995) or Thelebolaceae like *Coprotiella* (Jeng & Krug, 1976).

Quantitative criteria, like spore and ascus size, are highly variable in species of *Coprotus* from one sample or from one author to another. For *C. ochraceus* (H. & P. Crouan) J. Moravec for instance, ascospore length is reported as 13.5–17.5 μm (Aas, 1983) and as 15–18 (Moravec, 1971; Ellis & Ellis, 1998). Such data are difficult to handle where means are not mentioned. For *C. arduennensis* one of the collections (*J. De Sloover 99C111*) had smaller ascospores (9–)10.5(–13) μm , but agreed with the type in all other aspects: that apparently aberrant sample could perhaps indicate a slightly deviating taxon of which the delimitation is not yet clear.

Sizes of asci and apothecial diameter also vary greatly. Quantitative data are so variable, and simple statistical analysis should be considered essential when describing new taxa but it is far from being a common practice.

Presence vs. absence of carotenoid pigments was proposed by Kimbrough et al. (1972) as a first order qualitative criterion to found groups within the genus. Yellow to bright orange colours are frequently noticed in the hymenium of some species of *Coprotus*, particularly in the paraphyses in granules or oil droplets. Similar but duller shades have been detected both in the ascospores and in the excipulum cells and cell walls. It should be noted that the orange colour of the hymenium, as described for instance in *C. aurora*, is always the sign of carotenoid bound lipids in the paraphyses. On the other hand, yellow or yellowish shades are mostly linked to excipulum wall pigments or to refringent droplets, e.g. in paraphyses. These quite different origins of colour are not to be confused. In Table I the 26 known species are set out so that species sharing the same characteristics are brought together. The group with lipid bound orange-yellow pigments in paraphyses is limited to seven among the 26 species listed; most of them have ascospores with light yellowish contents, as well as a small amount of carotenoids in the excipulum mainly on cell walls. Obviously the greatest amounts of carotenoids are located in the paraphyses. It should be noted that this small group of seven species has eight-spored asci. Furthermore, the last nine species listed are completely devoid of any yellow pigments. They usually possess translucent to white apothecia except sometimes when drying. Here also are most of the multispored species.

Fig. 2. *Coprotus arduennensis*. a, b. Habit of fruit-bodies from above; c. median section of an apothecium showing the location of d and e; d. hypothecium cells (textura angularis); e. ectal excipular cells; f–l. ascospores with two (h, l) in optical section; m–o. asci with uniseriate to irregularly biseriate spores; p–u; paraphyses straight and thick, rarely branched towards basis (p, q) or clavate at apex (r), exceptionally septate (u) with small refractive golden yellow droplets (t); a, b: *De Sloover 00C114*; c–u: *De Sloover 99C111*.

Concerning pigmentation *Coprotus arduennensis* seems related to the group of the other six species with golden yellow droplets in the paraphyses, i.e. *C. breviascus*, *C. vicinus*, *C. aurora*, *C. luteus*, *C. baeosporus*, and particularly *C. ochraceus*.

In *Coprotus* the excipulum is as a rule poorly developed: this is particularly true in species like *C. disculus* (Thind et al., 1978), yet in a few others like *C. baeosporus* (Jeng & Krug, 1977) or *C. sarangpurensis* (Thind et al., 1978) it is rather well differentiated with medullary and ectal layers. Moreover, the hypothecium is absent or at least indistinct as reported, e.g. in *C. ochraceus* (Thind et al., 1978). Even though well differentiated, the excipulum thickness and the number of its layers are only reported for seven species out of 25, from two layers in *C. baeosporus*, up to a maximum of four in *C. vicinus* and up to five or six layers in *C. sexdecimsporus* (Kimbrough et al., 1972). Ectal excipulum thickness goes from 50–95 µm, while the medullary excipulum could reach 425 µm as reported in *C. ochraceus*. While basal and medullary cells are usually isodiametric or slightly elongated [8–30 µm, except in *C. ochraceus* where they may reach 55 µm (Thind et al., 1978)], cells of the ectal excipulum along the margins are clearly elongated in most species reaching up to 100 µm in *C. marginatus*. In this respect *C. dhofarensis* is the only one of its kind, where most top-

Table I. Location of carotenoids in selected cells or walls of species of *Coprotus*: (+) = in small amount, + = present, ++ = in large amount.

	Paraphyses with yellow oil gut- tules	Ascospores slightly yel- lowish	Excipulum walls and/or cells yellow
<i>C. lacteus</i> (Cooke & W. Phillips) Kimbr., Luck-Allen & Cain ⁵			
<i>C. glaucellus</i> (Rehm) Kimbr. ⁵			(+)
<i>C. marginatus</i> Kimbr., Luck-Allen & Cain ⁵			(+)
<i>C. granuliformis</i> (P. & H. Crouan) Kimbr. ⁵			(+)
<i>C. dextrinoideus</i> Kimbr., Luck-Allen & Cain ⁵			+
<i>C. dhofarensis</i> Gené, ElShafie & Guarro ⁸			+
<i>C. leucopocillum</i> Kimbr., Luck-Allen & Cain ⁵		(+)	
<i>C. duplus</i> Kimbr., Luck-Allen & Cain ⁵		(+)	(+)
<i>C. sexdecimsporus</i> (P. & H. Crouan) Kimbr. ⁵		(+)	(+)
<i>C. disculus</i> Kimbr., Luck-Allen & Cain ⁵		(+)	(+)
<i>C. breviascus</i> (Velen.) Kimbr., Luck-Allen & Cain ⁵ +		(+)	(+)
<i>C. vicinus</i> (Boud.) Kimbr., Luck-Allen & Cain ⁵ +		(+)	(+)
<i>C. aurora</i> (H. & P. Crouan) Kimbr., Luck-Allen & Cain ⁵		++	(+)+
<i>C. ochraceus</i> (H. & P. Crouan) Moravec ^{1, 5}	++	(+)	(+)
<i>C. arduennensis</i> J.R. De Sloover	++		
<i>C. luteus</i> Kimbr., Luck-Allen & Cain ⁵	++		
<i>C. baeosporus</i> Jeng & Krug ⁴	++		
<i>C. sphaerosporus</i> Gibson & Kimbr. ³			
<i>C. niveus</i> (Fuckel) Kimbr., Luck-Allen & Cain ⁵			
<i>C. rhyparobioides</i> (Heimerl) Kimbr. ⁵			
<i>C. winteri</i> (E. Marchal) Kimbr. ⁵			
<i>C. albidus</i> (Boud.) Kimbr. ⁵			
<i>C. sarangpurensis</i> K.S. Thind S.C. Kaushal ⁷			
<i>C. trichosurus</i> A.E. Bell & Kimbr. ²			
<i>C. uncinatus</i> Y.Z. Wang ⁹			
<i>C. subcylindrosporus</i> J.M. Moravec ⁶			

1) Aas, 1983; 2) Bell & Kimbrough, 1973; 3) Gibson & Kimbrough, 1980; 4) Jeng & Krug, 1976, 1977; 5) Kimbrough et al., 1972; 6) Moravec, 1971; 7) Thind et al., 1978; 8) Gené et al., 1993; 9) Wang, 1994.

cells on the upper flank of the ectal excipulum elongate upwards forming a fringe of long cells exceeding the surface of the hymenium. Here the marginal structure is far more developed than the one described for *C. marginatus*, but its cells look like the nearby paraphyses and the ones described here for *C. arduennensis*, while *C. dhofarensis* paraphyses have a size typical of those in *Coprotus*. *Coprotus arduennensis* shows a well-differentiated excipulum with globose isodiametric basal cells up to 45 µm, which are of about the same order of size as the largest ones that are known in the genus, in *C. ochraceus*. The sizes of cells at the excipular margin in *C. arduennensis* (exceeding 200 µm) are even larger than the largest reported thus far, in *C. marginatus*. The cells of the raised margin are also considerably enlarged in *C. arduennensis*. It appears that *C. marginatus*, *C. dhofarensis* and *C. arduennensis* form a group of three species where the marginal cells are particularly well developed in different ways.

Thus *C. arduennensis* shares the same characteristics with the six species indicated in Table I as having paraphyses with yellow guttules and especially the most richly pigmented *C. ochraceus*, *C. aurora*, *C. luteus* and *C. baeosporus*. Moreover, the pronounced yellow to orange colour of the apothecium is another feature that *C. arduennensis* has in common with *C. ochraceus* and *C. aurora*. The wide straight cylindrical paraphyses, the larger asci, the smaller ascospores and particularly the large swollen excipular cells are consistent, and sufficient to distinguish *C. arduennensis* from these other species.

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