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Cortinarius hildegardiae and *C. mariekristinae* spp. nov., two new species in the phlegmacioid clade Humolentes (sect. *Calochroi* s. l.)

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Cortinarius hildegardiae and *C. mariekristinae* are described as new to science. They are phlegmacioid species with more or less yellow pilei, yellow lamellae, and extractable, greenish yellow antraquinonic pigments. They belong to the Humolentes s. l. clade. *Cortinarius hildegardiae* resembles *C. humolens* and *C. osloensis*, but differs from the former in the more vivid greenish yellow pileus margin and veil remnants on bulb margin when young. It differs from its phylogenetic sister species *C. osloensis* by smaller spores and different habitat and distribution. *Cortinarius mariekristinae* occupies a sister position to *C. humolens*, but differs from this and the often co-occurring *C. osloensis* e.g. by the larger spores. Species descriptions are provided, with emphasis on micromorphological characters. Their phylogenies based on nrDNA ITS sequences are presented.

Keywords: taxonomy, species descriptions, European distribution, ITS phylogeny, spore morphology, 2 new taxa.

The present paper treats two new phlegmacioid species of *Cortinarius* (Pers.) Gray in the Calochroi clade with yellow anthraquinonoid pigments. The phlegmacioid species containing such pigments have traditionally been classified in sect. *Fulvi* M.M. Moser (see e.g. Brandrud et al. 1989–2018), here termed fulvoid species. However, according to phylogenetic studies (see e.g. Frøslev et al. 2007, Garnica et al. 2016), the fulvoid species do not form a monophyletic group, but are in a number of subclades intermixed within the large Calochroi clade, referrable to section *Calochroi* M.M. Moser & Horak sensu Frøslev et al. (2005, 2007). This wide concept of sect. *Calochroi* is now applied by most authors (see e.g. Bellanger 2015, Garnica et al. 2016). The two new species treated here belong to the Humolentes (sub)clade, which is composed of both of fulvoid and non-fulvoid species.

The section *Calochroi* s. l. is a very large, but morphologically well delineated and phylogenetically distinct clade among the phlegmacioid taxa (see Garnica et al. 2003, 2005, 2009, 2016; Peintner et al. 2004; Frøslev et al. 2005, 2007). The species in sect. *Calochroi* s. l. (sensu Frøslev et al. 2005, 2007) can be distinguished from those in related groups by the combination of (i) basidiomata with abruptly bulbous stipes, (ii) simplex pileipellis consisting of a thick, strongly gelatinous, easily separable epicutis and (iii) amygdaloid–citriform spores with coarse, crust-like, almost net-like ornamentations (cf. Brandrud et al. 1989–2018). The Humolentes clade falls within this morphological framework, and with species that often have yellow(-ochre) colours, including pale (greenish) yellow to wax-yellow lamellae, a large marginated bulb, usually whitish context, often saffron-coloured spots following insect damages, negative microchemical KOH-reactions, and comparatively large spores, >10 µm long. Parts of the Humolentes clade as here applied were formerly referred to as Pseudoglaucopodes clade (Garnica et al. 2009, 2016).

Material and methods

Morphological study

A total of 24 collections of the two new species were examined in both fresh and dried condition, as well as a number of collections of the most similar and most closely related taxa of the Humolentes clade, e.g. *C. humolens*, *C. osloensis*, and *C. lavandu-*

lochlorus. Collections made by the first author (abbreviated TEB in the text) are deposited in the Botanical Museum of the University of Oslo (O). Materials collected by the second and third authors (abbreviated SSt and DB) are deposited in the Botanische Staatssammlung München (M) and in the Eötvös Loránd University, Budapest, Hungary (ELTE), respectively. Herbarium abbreviations follow Index Herbariorum (Thiers [continuously updated]).

The taxonomic descriptions are based on the material studied by the authors. The measurements of macromorphological characters are based on expanded, but never old (and then often aberrant) basidiomata. Macrochemical reagents applied were 2 % and 40 % KOH. The terminology of characters follows Brandrud et al. (1990, 2018a).

Microscopical structures were observed partly from fresh material mounted in H_2O, often with a drop of 40 % KOH subsequently added, and partly from dried material mounted in H_2O and then KOH. Measurements and photographs of basidiospores were made in L4 solution according to Clémençon (Clémençon 1972, Erb & Matheis 1983). When impossible to obtain a sufficient quantity of spores from the stipe, the cortina or spore deposits on microscope slides, a spore preparation from the lamellae was examined. Since the deviations of values between these methods within the same collection were found to be insignificant, all measurements of each collection were combined.

Basidiospore measurements were made at 1000× magnification with a calibrated optical micrometer or on a flat screen with the program ProgRes® CapturePro from Jenoptik. The measurements are based on at least 30 spores from each collection; numbers in square brackets refer (in this order) to the number of collections they originate from, the number of basidiomata and the number of spores measured, respectively. Spore measurements are given as follows: length range × width range. Q values were calculated as follows: Q = length divided by width. To exclude aberrant spores the given values in the text are based only on spores within the 95 % confidence interval (Tab. 1).

The photo micrographs of the spores are created with the method of "focus-stacking" (Schmidt-Stohn 2011). About twenty to thirty shots with the Jenoptik ProgRes® C10 plus digital camera, each with different focus (each step ca. 0.2 µm), are combined to the final picture with the Helicon Focus 6.5 program. For the correction and the final arrangement of the spores on the plates Adobe Photoshop CS5 was used. In these pictures, each spore can reliable be identified with its individual pattern of ornamentation.

Phylogenetic study

DNA extraction, polymerase chain reaction (PCR) and sequencing procedures followed Brandrud et al. (2018b) and Holec et al. (2018). Primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) were used to amplify the ITS region of the ribosomal RNA gene. Newly generated sequences were combined with data from Frøslev et al. (2007), Garnica et al. (2016) and other published sequences from GenBank, focusing on the Humolentes s.l. lineage within sect. *Calochroi* (Tab. 2). Sequences were aligned with the online version of MAFFT v. 7 using the E-INS-i algorithm (Katoh & Standley 2013). Alignment was checked and edited with SeaView 4 (Gouy et al. 2010). The phylogenetically informative indels of the ITS region were coded with FastGap 1.2 (Borchsenius 2009) following the simple indel coding algorithm (Simmons et al. 2001). The D1/D2 part of the LSU region was kept for those GenBank sequences, where it was available in order to improve the robustness of our phylogenetic analysis.

The final ITS+LSU comprised 34 samples of 1261 characters including gaps. Indels were coded as presence/absence data and used as separate partition (34 additional binary characters) in the phylogenetic analyses resulting in a final dataset of 1295 characters. The concatenated ITS+LSU+binary data set was subjected to Maximum Likelihood phylogenetic analyses in the raxmlGUI (Silvestro & Michalak 2012) implementation of RAxML (Stamatakis 2014) using the GTRGAMMA substitution model for the nucleotide partitions and the default setting for binary (indel) data. Rapid bootstrap analysis with 2,000 replicates was applied for testing branch support. Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The alignment was partitioned and the GTR + G model of evolution was applied for the ITS+LSU and the two-state Markov model for the indel characters. Four Markov chains and two independent runs were performed for 10,000,000 generations, sampling every 1,000 steps. Burn-in was set to 30 %. From the post burn-in trees, 50 % majority rule consensus tree and posterior probabilities (PP) were calculated. Tree topologies of both ML (Fig. 1) and BI analyses were checked visually and no incongruence was observed. Phylogenetic trees were edited in MEGA7 (Kumar et al. 2016) and Adobe Illustrator CS4.

Tab. 1. Spore dimensions of *Cortinarius hildegardiae* and *C. mariekristinae*.

Species	n coll.	n spec.	n spores	L×W (all) + *MV*; µm	L×W 95 %–variation w/ MV; µm	Q L/W (all) + *MV*	Q L/W 95%–var. w/ MV
C. hildegardiae SSt14-207 (holotype)	1	1	55	9.5–10.6–12.2 × 5.3–5.8–6.5	9.6–10.6–11.7 × 5.3–5.8–6.3	1.65–1.84–2.10	1.65–1.84–2.06
C. hildegardiae SSt13-128	1	3	129	9.4–10.7–11.7 × 5.1–6.0–6.7	9.7–10.7–11.7 × 5.4–6.0–6.6	1.62–1.80–2.01	1.62–1.80–1.98
C. hildegardiae SSt13-218 / TEB932-13	1	1	50	9.6–10.4–11.1 × 4.9–5.4–5.9	9.6–10.4–11.1 × 5.0–5.4–5.8	1.69–1.93–2.12	1.72–1.93–2.12
C. hildegardiae TEB743-12 / DB4804	1	2	41	10.3–11.2–12.4 × 6.0–6.6–7.1	10.2–11.2–12.2 × 6.2–6.6–7.0	1.49–1.70–1.87	1.54–1.70–1.86
C. hildegardiae TEB929-17	1	2	64	8.1–10.0–11.1 × 5.4–6.0–6.7	8.9–10.0–11.1 × 5.5–6.0–6.7	1.40–1.66–1.89	1.44–1.66–1.88
C. hildegardiae DB5620	1	2	71	8.4–9.7–10.6 × 5.0–5.8–6.5	8.7–9.7–10.7 × 5.2–5.8–6.4	1.42–1.66–1.88	1.44–1.66–1.88
C. hildegardiae DB5996 / TEB751-15	1	1	31	9.8–10.7–11.6 × 5.8–6.2–6.9	9.8–10.7–11.5 × 5.7–6.2–6.8	1.54–1.71–1.87	1.54–1.71–1.89
C. hildegardiae DB6615	1	1	62	9.9–11.0–12.1 × 5.8–6.5–7.2	10.0–11.0–12.0 × 5.8–6.5–7.1	1.51–1.70–1.93	1.52–1.70–1.88
C. hildegardiae CR5822-2017	1	2	78	9.2–10.6–11.9 × 5.6–6.4–7.2	9.6–10.6–11.6 × 5.8–6.4–7.0	1.47–1.66–1.97	1.48–1.66–1.84
				8.1–10.5–12.4 × 4.9–6.1–7.2	**9.1–10.5–11.9 × 5.3–6.1–6.9**	**1.40–1.74–2.12**	**1.48–1.74–2.00**
	9	**15**	**581**	**10.5 × 6.1**		**1.74**	
C. mariekristinae TEB413-14 (holotype)	1	1	49	11.6–12.5–13.9 × 6.8–7.4–8.0	11.3–12.5–13.6 × 6.8–7.4–8.0	1.50–1.68–1.86	1.50–1.68–1.86
C. mariekristinae TEB261-14 / DB5377	1	1	30	11.8–12.8–14.0 × 7.0–7.7–8.6	11.6–12.8–14.0 × 6.9–7.7–8.4	1.56–1.67–1.76	1.55–1.67–1.79
C. mariekristinae TEB379-15 / DB5771	1	1	30	11.3–12.5–13.3 × 7.0–7.5–7.9	11.7–12.5–13.4 × 7.1–7.5–7.9	1.52–1.68–1.82	1.54–1.68–1.82
C. mariekristinae TEB539-15	1	1	50	11.6–12.6–13.5 × 6.9–7.6–8.0	11.7–12.6–13.6 × 7.1–7.6–8.0	1.50–1.67–1.83	1.53–1.67–1.81
C. mariekristinae TEB347-17	1	1	38	11.2–12.7–13.8 × 7.0–7.5–8.5	11.5–12.7–13.9 × 6.9–7.5–8.2	1.44–1.69–1.93	1.50–1.69–1.88
C. mariekristinae TEB439-17	1	1	40	11.5–13.0–14.0 × 6.8–7.5–8.3	11.7–13.0–14.3 × 6.6–7.5–8.4	1.50–1.73–1.97	1.47–1.73–2.00
C. mariekristinae TEB502-17	1	1	31	10.8–12.0–13.2 × 7.2–7.7–8.3	10.9–12.0–13.1 × 7.1–7.7–8.2	1.43–1.55–1.72	1.41–1.55–1.69
C. mariekristinae GS19-10-2012	1	3	96	11.3–12.9–15.6 × 6.6–7.5–8.4	11.5–12.9–14.3 × 6.9–7.5–8.1	1.55–1.73–1.93	1.57–1.73–1.89
				10.8–12.7–15.6 × 6.6–7.5–8.6	**11.5–12.7–13.9 × 6.9–7.5–8.1**	**1.43–1.69–1.97**	**1.49–1.69–1.89**
	8	**10**	**364**	**12.7 × 7.5**		**1.69**	

Tab. 2. *Cortinarius* sequences used in the phylogenetic analysis of this study.

Species	Specimen voucher	Origin	ITS accession	Reference
C. caroviolaceus	TUB012695	Italy	EU056944	Garnica et al. (2009)
C. hildegardiae	JMT-17100310	France	MK659894	this study
C. hildegardiae	DB6615	Hungary	MK659895	this study
C. hildegardiae	SSt12-132	Germany	MK659896	this study
C. hildegardiae	DB5620	Hungary	MK659897	this study
C. hildegardiae	AL14/324	Hungary	MK659898	this study
C. hildegardiae	SSt13-128	Germany	MK659899	this study
C. hildegardiae	SSt13-218 / TEB932-13	France	MK659900	this study
C. hildegardiae	TEB743-12 / DB4804	Germany	MK659901	this study
C. hildegardiae	TEB744-12	Germany	MK659902	this study
C. hildegardiae	CR5822-2017	Italy	MK659903	this study
C. hildegardiae	DB5996 / TEB751-15	Italy	MK659904	this study
C. hildegardiae	SSt14-207, holotype	Germany	MK659905	this study
C. hildegardiae	SSt14-210	Germany	MK659906	this study
C. hildegardiae	TEB929-17	Spain	MK659907	this study
C. humolens	CFP1281, isotype	Italy	DQ663322	Frøslev et al. (2007)
C. humolens	TUB012723	Germany	EU056955	Garnica et al. (2009)
C. lavandulochlorus	GE 10.021, holotype	France	HQ843177	Eyssartier (2011)
C. lavandulochlorus	TUB011677	Germany	EU655675	Garnica et al. (2009)
C. mariekristinae	TEB502-17	Norway	MK659908	this study
C. mariekristinae	TEB439-17	Norway	MK659909	this study
C. mariekristinae	TEB347-17	Norway	MK659910	this study
C. mariekristinae	TEB153-06	Norway	MK659911	this study
C. mariekristinae	TEB539-15	Norway	MK659912	this study
C. mariekristinae	GS19-10-2012	Germany	MK659913	this study
C. mariekristinae	TEB261-14 / DB5377	Norway	MK659914	this study
C. mariekristinae	TEB413-14, holotype	Norway	MK659915	this study
C. mariekristinae	TEB379-15 / DB5771	Norway	MK659916	this study
C. osloensis	TEB559-04, holotype	Norway	DQ996975	Frøslev et al. (2006)
C. osloensis	DB6652	Hungary	MK659917	this study
C. pseudoglaucopus	IB 1999/192	Italy	EU056951	Garnica et al. (2009)
C. rapaceoides	AB05-10-103	France	DQ663417	Frøslev et al. (2007)
C. saporatus	TUB011880	Germany	AY669570	Garnica et al. (2005)
C. sulfurinus	TUB011908	Germany	AY669572	Garnica et al. (2005)

Results and discussion

Phylogeny

Both ML and BI analyses, applying ITS and LSU as molecular markers, recovered the two new species with maximum support (100/1.00) within the Humolentes s.l. clade. The intraspecific variability in the ITS region is low in *C. hildegardiae* (maximum of 2 nucleotides), and zero in *C. mariekristinae*. Both new species have a clear barcoding gap (ca. 3 % dissimilarity) towards the closest species in our phylogeny. *Cortinarius hildegardiae* is a well-supported (96/1.00) sister species to *C. osloensis*, while *C. mariekristinae* is sister to

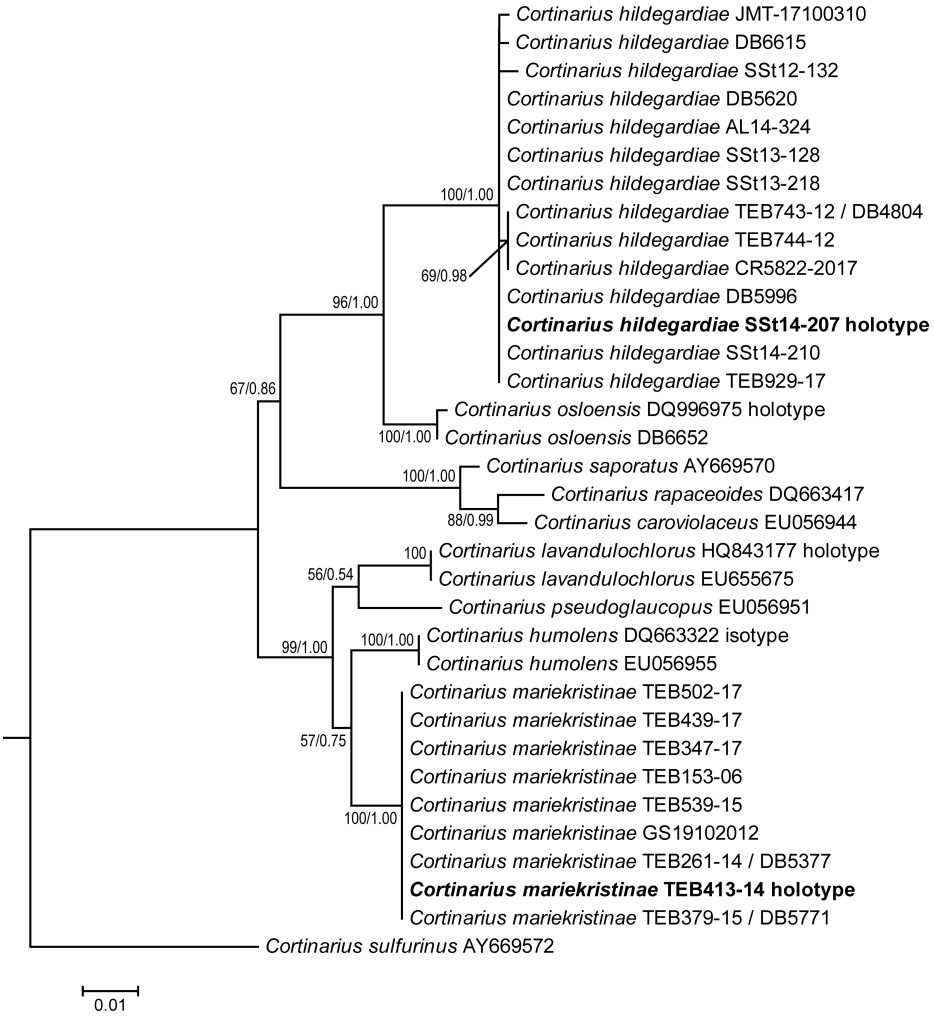

Fig. 1. Phylogenetic relationship of the Humolentes s.l. clade based on maximum likelihood analysis of nrDNA ITS and LSU sequences with coded gaps as additional source of information. Maximum likelihood bootstrap support values and Bayesian posterior probabilities given at branches. GenBank numbers given only for sequences taken from public databases. For newly generated sequences specimen vouchers given. Holotype sequences of the two new species highlighted in boldface. Bar indicating 0.01 expected change per site per branch.

C. humolens but the relationship was weakly supported (57/0.75).

The Humolentes clade (Humolentes s. l.) as here applied, consists of three well-supported lineages; the *C. humolens* lineage (incl. *C. mariekristinae*), the *C. osloensis* lineage (incl. *C. hildegardiae*) and the *C. saporatus*-*C. caroviolaceus* lineage. The two former lineages consist of morphologically very similar, mainly fulvoid taxa, but according to present molecular data do not form a monophyletic group. The *C. humolens* lineage/subclade was formerly named as Pseudoglaucopus clade by Garnica et al. (2009, 2016). However, since most of the species in this lineage/subclade as well as the three lineages taken together (clade Humolentes s. l.) possess yellow colours and anthraquinonic pigments, we find the name 'Humolentes clade' more informative, referring to a representative and rather frequent, yellow species with anthraquinonic pigments (*C. pseudoglaucopus* lacks these yellow pigments).

Taxonomy

Cortinarius hildegardiae Schmidt-Stohn, Brandrud & Dima, **spec. nov**. – Figs. 2–5, 10
MycoBank no.: MB 830268

Etymology. – In honour of Hildegard Badekow-Schmidt-Stohn, the wife of the second author of the paper.

Holotype. – GERMANY. Mecklenburg-Vorpommern, Plauer See, 27 October 2014, *leg*. G. Schmidt Stohn, SSt14-207 (M-0302848). GenBank: MK659905.

Description. – Pileus 4–12 cm, (hemi-) sphaerical, then plano-convex, glutinous, glabrous; incurved margin with small drop-like spots or not; sometimes innately fibrillose from darker, radial fibrils, sometimes also with dark brown strikes due to apressed grass/moss remnants; margin or sometimes entire pileus when young pale ochre yellow with an initial distinct greenish yellow-lemon yellow tinge, centre becoming (spotwise) discoloured ochre brown or more reddish brown with age due to oxidation (resembling *C. sulfurinus*, *C. quercilicis*), often distinctly bicoloured, sometimes persistently uni-coloured ochre yellow, when sheltered under leaves or a moss carpet; becoming more or less saffron brown tinged where eaten by snails. Universal veil remnants indistinct to rather abundant, and then leaving whitish, large patches at pileus centre. – Lamellae crowded (L = 70–100), 4–10 mm broad, pale greenish (-greyish) yellow, straw yellow to wax yellow, then ochraceous brown with an olivaceous tinge; edge often slightly to distinctly crenulate. – Stipe 4–8 × 1–2(2.5) cm, with an often large, marginate bulb (up to 4 cm wide), greyish white (to yellowish white) when young, then whitish, with age tinged ochre yellow from base upwards. Universal veil on the bulb margin when young distinct to abundant and volva-like, slightly viscid, distinctly greenish yellowish (contrasting with the whitish stipe above and the pure white mycelium below), with age slightly brownish. Cortina fairly abundant, whitish. Basal mycelium white, with whitish to yellow mycelial strands. – Context whitish, when young with watery greyish or yellowish grey tinged hygrophanous spots in stipe apex, rarely faintly bluish tinged. Outside and inside of bulb sometimes becoming saffron yellow spotted (especially where eaten), sometimes more brown-spotted. Smell weak, indistinct when cut, but distinctly earthy or dust-like on the lamellae. – Macrochemical reactions 2 % and 40 % KOH negative (pale brownish) in context. – Exsiccata ochre brown. – Extractable pigments not studied, but pigment topology, colours and oxidation behaviour clearly indicate presence of the same pigment as in the sister species *C. osloensis*; the greenish yellow flavomannin-6,6',8-trimethylether (FTM).

Basidiospores [9, 15, 581], 9.1–11.9 × 5.3–6.9 µm (MV = 10.5 × 6.1 µm), Q = 1.48–2.00 (MV = 1.74), amygdaloid-citriform, more or less strongly and coarsely net-like verrucose, warts more or less prominent, distinctly to hardly visible in the outline of the spores, suprahilar plague more or less distinct, apiculus rather smooth. – Basidia 9–11 µm wide, 4-spored, some with yellowish content. – Lamella edge more or less fertile. – Lamella trama of 4–20 µm wide, mainly hyaline hyphae. – Universal veil out of 3–8(10) µm wide hyphae, mainly hyaline, some with intracellular, yellow (brown) granules or lumps. – Pileipellis simplex, a cutis of 2–5 µm wide, loosely erect-entangled hyphae, gelatinous at surface, mainly hyaline, the basal part of cutis out of 4–7(9) µm wide hyphae, partly in subparallel, interconnected bundles; pigment mainly intracellular, initially pale (greenish) yellow, then yellow brown, granular, with KOH oleiferous; pigment from exsiccata usually yellowish brown with KOH. – Trama beneath pileipellis hyaline.

Habitat. – In calcareous or base-rich frondose forests, mainly associated with *Quercus* spp.; in *Q. ilex* forests, *Q. pubescens-Q. cerris* forests, mixed *Q. robur-Fagus sylvatica* forest (the type locality being a park) but also more or less calcareous *F. sylvatica* forests, once also in a *Tilia platyphyllos* stand with *Q. pyrenaica* along river. Also recorded in montane conifer forests under *Abies alba* in shallow, strict calcareous soil on mossy ground; found mainly in *Phlegmacium*-rich sites.

Distribution. – Widespread in European Mediterranean-nemoral-montane regions, but extremely rare. So far only known from two sites in Germany, Italy, Hungary, France and one site in Switzerland and Spain. Present records are restricted to SW, W and C European regions with calcareous *Quercus-Fagus* and *Abies* forests, but the species probably follows such habitats also further east, e.g. in the little investigated Carpathians-Balkan mountains and the Caucasus.

Material examined. – FRANCE. Languedoc-Roussilon, Hérault, Bédarieux, *Quercus ilex*, 23 October 2013, *leg*. T.E. Brandrud, G. Schmidt-Stohn, SSt13-218 (M-0302850), TEB932-13 (O); Grand Est, Alsace, Langensoultzbach, *Fagus*, *Quercus*, 3 October 2017, *leg*. J.-M. Trendel, JMT-17100310. GERMANY. Mecklenburg-Vorpommern, Plauer See, *Quercus robur*, 6 November 2012, *leg*. G. Schmidt Stohn, SSt12-132 (M-0302849); 4 October 2013, *leg*. G. Schmidt Stohn, SSt13-128 (M-0302851); 27 October 2014, *leg*. G. Schmidt Stohn, SSt14-207 (holotype, M-0302848); 27 October 2014, *leg*. G. Schmidt Stohn, SSt14-210 (M-0302852); Baden Württemberg, Baar, Flötzlingen, *Abies alba*, 28 September 2012, *leg*. T.E. Brandrud, B. Dima, G. Saar, TEB743-12 (O) / DB4804 (ELTE), TEB744-12 (O). HUNGARY. Budapest, Mt Széchenyi-hegy, *Quercus cerris*, *Q. pubescens*, 2 October 2014, *leg*. L. Albert, AL14/324 (L. Albert, pers. herb.); 13 October 2014, *leg*. B. Dima, L. Albert, DB5620 (ELTE); Pest County, Tahi, *Fagus sylvatica*, 4 October 2017, *leg*. B. Dima, DB6615 (ELTE). ITALY. Marche, Pesaro & Urbino, Fossombrone, Mt Paganuccio, *Quer-*

Figs. 2–9. Basidiomata of *Cortinarius hildegardiae*: **2.** SSt14-207 (holotype). **3.** SSt14-210 **4.** AL14/324. **5.** CR5822-2017, and *C. mariekristinae*: **6–7.** TEB413-14 (holotype) / DB5448. **8.** TEB379-15 / DB5771. **9.** TEB539-15.

cus ilex, 30 October 2015, *leg*. B. Dima, T.E. Brandrud, G. Schmidt-Stohn, DB5996 (ELTE), TEB751-15 (O); Trentino, Ruffrè, Bivio, *Abies alba*, *Picea abies*, *Fagus sylvatica*, 24 September 2017, *leg*. C. Rossi, CR5822-2017 (M-0302853). SWITZERLAND. Neuchâtel, south of Valangin, *Abies alba*, *Fagus sylvatica*, 5 October 1998, *leg*. T.E. Brandrud, H. Marklund, TEB172-98 (O). SPAIN. Cantabria, Potes NW, Camalenos, Los Llanos, *Quercus pyrenaica, Tilia platyphyllos*, 2 November 2017, *leg*. U. Winkler, T.E. Brandrud, G. Schmidt-Stohn, TEB929-17 (O).

Comments. – The ITS sequences are rather homogenous with only 1–2 nucleotides intraspecific variation. Three samples originating from *Abies*-dominated habitats differ by one nucleotide from other collections associated with deciduouos trees. The closest relative of *C. hildegardiae* is *C. osloensis* differing by 21–22 substitution and indel positions (96.55–96.39 % similarity).

Cortinarius hildegardiae is characterized by its initially pale greenish yellow colours on pileus (margin), lamellae and veil on bulb margin, contrasting white stipe and context. Like related taxa, it has an earthy-raphanoid smell, and a negative KOH-reaction. Phylogenetically, the species comes very close to *C. osloensis*, but differs (i) in more vivid greenish yellow tinges on pileus margin and veil at bulb margin when young, (ii) darker brown discolouration of pileus with age and exposure (iii) smaller spores and (iv) habitat (*C. osloensis* is only found in calcareous *Tilia cordata* forests). Although more distant phylogenetically (see Fig. 1), the species might also look very similar to *C. humolens* Brandrud, which has very similar spores, and may co-occur in the same habitat. However, when young, *C. hildegardiae* is distinguished by the pure greenish yellow to lemon yellow tinges on the pileus and bulb margin. *Cortinarius humolens* has a more greyish-green to greyish-olivaceous tinge when young. *Cortinarius hildegardiae* and *C. mariekristinae* are often difficult to distinguish macroscopically, but the former is on average slightly more greenish yellow on pileus (margin) when young, and becomes more brownish oxidized (often bicoloured). Furthermore, the latter has more frequently bluish tinges or spots on pileus apex. Microscopically, these are easily separated by the much larger spores of *C. mariekristinae* (see below). The related *C. lavandulochlorus* Eyssart. may also resemble it, but usually has some lilac tinges on lamellae when young.

There are a number of fulvoid species with greenish yellow colours on the pileus, veil and lamellae that can be confused with *C. hildegardiae*; such as *C. citrinus* J.E. Lange ex P.D. Orton and *C. splendens* Rob. Henry, or the taxa in the Elegantiores and Sulfurini clades. However, these species are normally more vividly coloured, with yellow pigments also in (parts of) the context, and usually some reddish KOH reaction in coloured parts of the basidiomes or on basal mycelium (in the Sulfurini clade).

The greenish yellow pigment found in *C. hildegardiae* is extractable, and according to microtopology and oxidation behaviour (hardly changing colour with age or with KOH), this is very probably the same, anthraquinonoid pigment (flavomannin-trimethylether) as found in other, yellow-gilled taxa in Humolentes. This is the most highly methylated, most stable, non-oxidized, flavomannin-pigment found in phlegmacioid cortinarii.

Cortinarius hildegardiae seems to occupy mainly two kinds of habitats; thermophilous, calcareous Mediterranean to southern nemoral *Quercus-Fagus* forests, and montane, strongly calcareous *Abies* forests. The *Abies*-associated variant seems to have slightly larger spores than the thermophilous *Quercus-Fagus* forest variant. The four measured collections of the *Abies* variant has an average spore size of 10.9 × 6.6 µm, whereas the specimens from the *Quercus* variant have MV = 10.3 × 6.0 µm. The *Abies*-associated variant apparently also shows a subtle differentiation in ITS-phylogeny (one nucleotide; Fig. 1). More material is needed to see if this morphological and phylogenetic differentiation is constant. A similar kind of habitat differentiation is seen in some other phlegmacioid taxa, such as *C. dibaphus* and *C. olidoamarus*. Also *C. subgracilis* has a similar kind of habitat preferences, and in this case, a morphological difference between *Abies* and southern *Quercus* variants is noted (Brandrud et al. 2018a).

Cortinarius mariekristinae Brandrud & Dima, **spec. nov.** – Figs. 6–9, 11
MycoBank no.: MB 830269

Etymology. – Dedicated to Marie Kristine Brandrud, the daughter of the first author of the paper.

Holotype. – NORWAY. Oslo, Malmøya, *Tilia cordata*, 8 September 2014, *leg*. B. Dima, T.E. Brandrud, TEB413-14 (O-F-255622). GenBank: MK659915.

Description. – Pileus 4–9 cm, (hemi-) spherical, then plano-convex, glutinous, glabrous, incurved margin with or without small drop-like spots; outer half pale ochre yellow with an initial distinct greenish yellow-lemon yellow tinge (at least margin), centre pale ochre brown, with age pale ochraceous yellow with ochraceous brown cen-

Fig. 10. Basidiospores of *Cortinarius hildegardiae*. Voucher numbers of the examined collections indicated below each series of photos.

tre; becoming more or less saffron brown tinged where snail eaten. Universal veil remnants sparse and indistinct or sometimes leaving pale ochraceous appressed scales at pileus centre. – Lamellae crowded (L = 60–90), 3–8 mm broad, pale (greenish/greyish) yellow, straw yellow to wax yellow, soon ochraceous brown with an olivaceous tinge; edge crenulate. – Stipe 4–7 × 0.8–1.5 cm, with a marginate bulb (up to 3.5 cm wide), bulb often rather sharply marginate, flattened; often greyish white when young, faintly to distinctly greyish blue (-lilac) at apex, soon entire greyish white, but sometimes with persistent lilac blue spots. Universal veil on the bulb margin sparse to distinct, slightly viscid, when young distinctly greenish yellowish (contrasting the whitish stipe above and the pure white mycelium below), with age more indistinctly whitish to pale ochraceous brown. Cortina fairly abundant, whitish. Basal mycelium white, a few pale greenish yellow mycelial strands observed. – Context whitish, when young with watery greyish hygrophanous spots in stipe (apex), apex also with lilac bluish spots. Bulb inside and outside often with pronounced saffron yellow spots, especially where eaten. Smell distinctly to strongly earthy, raphanoid, especially from the lamellae. – Macrochemical reactions 2 % and 40 % KOH negative (pale brownish) in context. Exsiccata ochre brown. – Extractable pigments not studied, but pigment topology, colours and oxidation behaviour clearly indicate presence of the same pigment as in the sister species *C. humolens*; the greenish yellow flavomannin-6,6',8-trimethylether (FTM).

Basidiospores [8, 10, 364], 11.5–13.9 × 6.9–8.1 µm (MV = 12.7 × 7.5 µm), Q = 1.43–1.97 (MV = 1.69), amygdaloid-citriform, strongly and coarsely, net-like verrucose, warts usually distinctly visible in the outline of the spores, suprahilar plague more or less distinct, apiculus smooth. – Basidia 9–12 µm wide, 4-spored, some with yellowish content. – Lamella edge ± fertile. – Lamella trama out of 4–20 µm wide, mainly hyaline hyphae. – Universal veil out of 2–6(–8) µm wide hyphae, mainly hyaline (pale yellow with KOH), some with intracellular, yellow granules or lumps, with KOH brown content becoming oleiferous (or granulate-cracked); at surface hyphae somewhat gelatinous. – Pileipellis simplex, a cutis out of 2–4 µm wide, loosely erect-entangled hyphae, gelatinous at surface, mainly hyaline (pale yellow with KOH); basal part of cutis out of 3–7(9) µm wide hyphae; pigment mainly intracellular, pale (greenish) yellow to yellow brown, granular, with KOH yellow brown content becoming oleiferous (also in exsiccata). Trama beneath pileipellis hyaline. Pileipellis and universal veil at bulb margin of 12-year old exsiccata become slightly pinkish yellow brown with 20 % KOH.

Habitat. – In calcareous *Tilia cordata* (-*Corylus avellana*) forests, mainly in thin soil on limestone benches or in gravelly scree soil.

Distribution. – Mainly known from SE Norway from the Oslofjord region (7 localities) and from one locality in Germany, Rhein valley (see Gminder & Saar 2013 and Saar & Schmidt Stohn 2018).

Material examined. – GERMANY. Baden-Württemberg, Kaiserstuhl-Limberg, *Tilia cordata*, 19 October 2012, *leg*. G. Saar, GS19-10-2012 (M-0302854). NORWAY. Akershus, Asker, Ormodden, *Tilia cordata*, 20 September 2006, *leg*. T.E. Brandrud, TEB153-06 (O); Bjerkås NR (Bjerkås IV), *Tilia cordata*, 4 September 2017, *leg*. T.E. Brandrud, B. Dima, TEB439-17 (O). Buskerud, Røyken, Bøsnipa, *Tilia cordata*, 24 September 2015, *leg*. T.E. Brandrud, B. Dima, TEB539-15 (O); Lillelien Ø, *Tilia cordata*, 30 August 2017, *leg*. E. Bendiksen, TEB347-17 (O). Oppland, Gjøvik, Biri, Eriksrud NR, *Tilia cordata*, 31 August 2014, *leg*. B. Dima, T.E. Brandrud, TEB261-14 (O), DB5377 (ELTE); Oslo, Malmøya, *Tilia cordata*, 8 September 2014, *leg*. B. Dima, T.E. Brandrud, TEB413-14 (holotype, O-F-255622), DB5448 (isotype, ELTE); 16 September 2015, *leg*. B. Dima, T.E. Brandrud, TEB379-15 (O), DB5771 (ELTE); Telemark, Bamble, Tangvall NR, *Tilia cordata*, 6 September 2017, *leg*. B. Dima, TEB502-17 (O).

Comments. – All ITS sequences are identical, no intraspecific variability was detected. The closest relative of *C. mariekristinae* is *C. humolens* differing by 13–18 substitution and indel positions (97.86–97.04 % similarity).

Cortinarius mariekristinae is characterized by the combination of initially greenish yellow pileus (margin), lamellae and veil, lilac bluish spots at stipe apex and remarkably large spores. The large spores distinguish it from all similar taxa. The species is macromorphologically very similar to the co-occurring *C. osloensis*, but can be distinguished by the more vivid greenish yellow veil and pileus margin when young and the often lilac bluish tinges at stipe apex, apart from the larger spores. The very dark spore powder sticking to the abundant cortina remnants on stipe surface gives this a superficial resemblance to the also co-occurring *C. caesiocortinatus*. However, the latter has more or less subglobose spores and never yellowish tinges on the lamellae.

Phylogenetically, *C. mariekristinae* is the sister species of *C. humolens*, but differs from it in the initially pure greenish yellow veil and pileus margin, the (much) larger spores and a different habitat. Macroscopically, this in fact looks most similar to *C. hildegardiae*, but has on average a paler, less oxi-

Fig. 11. Basidiospores of *Cortinarius mariekristinae*. Voucher numbers of the examined collections indicated below each series of photos.

dized pileus centre when mature, and furthermore, *C. hildegardiae* only exceptionally shows bluish tinges on the stipe.

Cortinarius mariekristinae is apparently a very rare species, with its major populations in the calcareous *Tilia* forests of the Oslofjord-Mjøsa region of SE Norway. Even though we have been studying the Humolentes group in many parts of Europe including extensive sequencing, we have only been able to document this one from a single site outside SE Norway; at the foothills of the Rhein valley (Gminder & Saar 2013, as *C. osloensis*, Saar & Schmidt-Stohn 2018, as *C.* sp.). The species shares this mainly Oslofjord *Tilia cordata* forest distribution with the related *C. osloensis*. Based on a recent finding, *C. osloensis* is now also confirmed from a single site outside SE Norway (calcareous *Tilia cordata* forest in Hungary, see Fig. 1, Tab. 2). Other species such as *C. tiliae* are also mainly confined to the old, relictual *Tilia* forests of SE Norway. We believe that these more or less *Tilia*-associated calciphilous taxa formerly had a much wider distribution in Europe when *Tilia* forests were abundant after the ice-age. In conjunction with the subsequent decline of these *Tilia* forests and expansion of other forest-forming trees on limestone, such as *Fagus sylvatica* and *Carpinus betulus*, these *Tilia* associated species apparently have disappeared from most areas, and are now confined to smaller, calcareous relicts. Some of these relicts are present mainly in SE Norway but also elsewhere, e.g. in the Czech Republic (Chytrý & Sádlo 1997), and both *C. mariekristinae* and *C. osloensis* should be searched for there.

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