## *Entoloma aurorae-borealis* sp. nov. and three rare *Entoloma* species in the Sinuatum clade (subg. *Entoloma*) from northern Europe

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*Entoloma aurorae-borealis* is described as new to science and three rare or little known *Entoloma* species (*E. borgenii*, *E. eminens*, and *E. serpens*) from Norway are treated based on morphological and molecular evidences. In the ITS phylogeny presented here, all species belong to the Sinuatum clade, one of five well-supported lineages of subgenus *Entoloma* (= *Rhodopolia* & *Nolanidea*). *Entoloma aurorae-borealis* is only known from northern Norway, whereas *E. eminens* and *E. serpens*, apart from the here reported new records in Norway are known only from a few localities in Finland, and *E. serpens* also in NW Russia. *Entoloma borgenii* is a wide-spread, though rarely recorded species from alpine sites in north Norway as well as the arctic (Greenland, Svalbard) and a few places in northern and eastern Finland. The two arctic-alpine species (*E. aurorae-borealis* and *E. borgenii*) are associated with *Salix herbacea*, *E. serpens* grows with *Salix* spp. and probably also with *Betula* and *Populus* in boreal-boreonemoral (-alpine) areas, and *E. eminens* in Norway is associated with *Tilia* and *Corylus* in a boreonemoral area. In Finland it is apparently associated with boreal deciduous trees. Full descriptions are given, as well as notes on the variability, ecology and geographic distribution of each species.

Keywords: Agaricales, Basidiomycota, ITS phylogeny, Rhodopolia, taxonomy, 1 new species

The huge genus Entoloma sensu lato (Basidiomycetes, Tricholomatinae) contains at least 2000 species worldwide (Romagnesi 1941; Romagnesi & Gilles 1979; Horak 1980, 1982, 2008; Noordeloos 1992, 2004; Largent 1994; Baroni & Halling 2000; Baroni & Ortiz 2002; Henkel et al. 2011; Karstedt et al. 2007; Karstedt & Capelari 2010, 2013, 2017; He et al. 2013; Noordeloos & Gates 2012; Morozova et al. 2014). Traditionally it is considered one genus with around 15 subgenera, or split into smaller genera, that generally follow the subgenera in the first approach. Recent phylogenetic studies (Baroni & Matheny 2011, Co-David et al. 2009, Kokkonen 2015, Morgado et al. 2013, Morozova et al. 2014) show that, despite the fact that some of the subgenera form well-supported clades and could be raised to generic rank, other traditional (sub)genera and sections appear to be polyphyletic or paraphyletic. The current state of taxon- and geographic sampling do not allow us to create a stable classification yet. So for the time being, the genus is considered a single, large one by the current authors.

Subgenus *Entoloma* in the traditional sense (see e.g. Noordeloos 1981) is polyphyletic: on one hand there is a well-supported clade Rhodopolia s.l. comprising of two well supported subclades, viz. *Rhodopolia* (including *E. rhodopolium*, and the type species of the genus, *E. sinuatum*), and on the other, the *Nolanidea*, comprising *E. clypeatum* and related species. A second group of smaller clades round *E. prunuloides*, and *E. nitidum*, traditionally thought to belong to subg. *Entoloma*, however, are very distant, and need to be treated as a separate subgenus or group of subgenera (Morgado et al. 2013, Baroni et al. 2011). Interestingly, the Entoloma clade has a life strategy that is completely different from all other Entoloma clades/subgenera, viz. all species are mycorrhizal. The Rhodopolia clade forms a more or less traditional ectomycorrhiza, whereas the *Nolanidea* have developed a separate mycorrhizal type, of a more parasitic nature (Agerer & Waller 1993, Kasuya et al. 2010, Kobayashi & Hatano 2001, Sánchez-García & Matheny 2017). Furthermore, the Rhodopolia clade (which is our focus) is characterized by an association more or less completely with deciduous trees and shrubs, including a high number of boreal-alpine species being associated with mainly boreal-alpine woody plants, chiefly *Salix* spp., *Alnus* spp., and *Betula* spp., some also apparently with *Populus tremula* (Kokkonen 2015).

The *Rhodopolia* therefore form an ecologically interesting group for study, and their ecological requirements as well as host range may give us also a clue for distinguishing them. Particularly in the vulnerable (sub)arctic habitat, members of this group are common and diverse, comprising at least a dozen species, some of them not being formally described yet. The Rhodopolia have been subject of a recent thorough morphological, ecological and molecular study in Finland (Kokkonen 2015). She recorded a total of 20 species in Finland, of which nine were new to science. All species are molecularly well separated, but, according to the present information, some of them appear semi-cryptic. Further studies will certainly elucidate some of these semi-cryptic complexes when morphological characters and habitat preferences are critically studied on more collections in a wider geographic context, and in a multigene molecular approach. Currently the authors are involved in a large scale study of the Rhodopolia, including many samples and also type studies from the whole of Europe, including the European part of Russia.

The present paper deals with one new and three little known Rhodopolia species belonging to the Sinuatum clade (corresponding to the E. bisporigerum-E. paludicola-E. eminens clade in Kokkonen 2015, fig. 41). The data in this paper are derived from a Norwegian Entoloma project and in particular from (i) Entoloma studies in the Holmvassdalen nature reserve in Nordland county (see e.g. Noordeloos et al. 2017, Weholt et al. 2014) and (ii) monitoring of calcareous Tilia forests of SE Norway (see e.g. Brandrud et al. 2016) This paper includes one new, alpine species (E. aurorae-borea*lis*) and three species new to Norway (*E. borgenii*, *E.* eminens, and E. serpens), formerly known only from a few Finnish, Russian or subarctic localities (Kokkonen 2015, Kalinina 2018).

## **Materials and methods**

## Morphological study

All collections studied were photographed in the field, and much attention was paid to characterize the surrounding vegetation and ecology. The materials were described and dried. The macromorphological description is based on the field notes and photographs of the available material. Spores, basidia and cystidia were observed in squash preparations of small parts of the lamellae in 5 % KOH or 1 % Congo Red in concentrated NH<sub>4</sub>OH. The pileipellis was examined in a preparation of the radial section of the pileus in 5 %KOH. Microscopic measurements and line drawings were made using a Leica DMLS microscope at 1000× using an oil immersion objective and drawing tube. Basidiospore dimensions are based on 20 spores, and basidia dimensions on 10. Basidia were measured without sterigmata, and the spores without hilum. Spore length to width ratios are reported as Q. All materials were deposited in the herbarium of the Botanical Museum, University of Oslo (O).

## Molecular study

DNA extractions, PCR amplifications, and sequencing were performed in the Norwegian Barcode of Life (NorBOL) or as described in Weholt et al. (2014). Sequence editing and comparison with public databases followed Noordeloos et al. (2017). Newly generated sequences were submitted to Gen-Bank (Tab. 1).

Preliminary analyses on available Norwegian *Entoloma* ITS sequences in addition to those down-loaded from GenBank and UNITE were carried out in the framework of the Norwegian *Entoloma* project (data not shown). The monophyletic clade of subg. *Entoloma* (cf. subg. *Rhodopolia* in Kokkonen 2015) was highlighted from the dataset and was subjected to further analyses. The phylogenetic structure within the subgenus is shown as a cartoon tree in Fig. 1. A subset of sequences representing the Sinuatum clade where the focused species are clustered was analysed including 43 ingroup and two outgroup sequences of *E. rhodopolium*.

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). Indels were coded following the simple indel coding algorithm (Simmons et al. 2001) with the program FastGap 1.2 (Borchsenius 2009).

Tab. 1. ITS sequences of the studied <i>Entoloma</i> species including all newly generated sequences with accession numbers
MH234479–MH234495 used in the phylogenetic analysis of the Sinuatum clade. Country's abbreviations: NOR = Norway, FIN =
Finland.

Species	Voucher	Country	Sequence origin	Accession no.
E. aurorae-borealis	O-F-254652 / JL10-15	NOR, Nordland	This study	MH234485
$E.\ aurorae-boreal is$	O-F-76217 / JL118-16	NOR, Nordland	This study	MH234484
$E.\ aurorae-boreal is$	O-F-76218 / JL 67-16	NOR, Nordland	This study	MH234487
$E.\ aurorae-boreal is$	O-F-254651 / JL 85-14 (holotype)	NOR, Nordland	This study	MH234486
E. borgenii	KK 135/10	FIN	Kokkonen (2015)	LN850522
E. borgenii	KK 458/07	FIN	Kokkonen (2015)	LN850523
E. borgenii	TB 79-120	Greenland	Kokkonen (2015)	LN850524
E. borgenii	O-F-76219 / JL 51-16	NOR, Nordland	This study	MH234482
E. borgenii	O-F-76220 / JL 63-16	NOR, Nordland	This study	MH234483
E. eminens	KK 40/14	FIN	Kokkonen (2015)	LN850583
E. eminens	KK 417/12 (holotype)	FIN	Kokkonen (2015)	LN850584
E. eminens	O-F-248409 / TEB 97-11	NOR, Telemark	This study	MH234479
E. eminens	O-F-254653 / TEB 226-16	NOR, Telemark	This study	MH234481
E. eminens	O-F-254654 / TEB 699-17	NOR, Telemark	This study	MH234480
E. serpens	KK410-09 (holotype)	FIN	Kokkonen (2015)	LN850526
E. serpens	O-F-76212 / MP-1-240915	NOR, Østfold	This study	MH234494
E. serpens	O-F-254350 / MP-2-120914	NOR, Østfold	This study	MH234489
E. serpens	O-F-254353 / MP-5-060815	NOR, Østfold	This study	MH234493
E. serpens	O-F-254352 / MP-7-130915	NOR, Østfold	This study	MH234495
E. serpens	O-F-254351 / MP-E1-14	NOR, Østfold	This study	MH234490
E. serpens	O-F-253866 / EB 94/16	NOR, N-Trøndelag	This study	MH234488
E. serpens	O-F-303802 / GB 4713	NOR	This study	MH234491
E. serpens	O-F-249466 / TEB 748-13 / DB5281	NOR, Buskerud	This study	MH234492

Maximum Likelihood (ML) analysis was performed in raxmlGUI (Silvestro & Michalak 2012). Rapid bootstrap analysis with 1000 replicates and the GTRGAMMA substitution model for the DNA partitions (ITS1, 5.8S, ITS2) and the default set for binary (indel) were used.

Bayesian inference (BI) analyses were performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The nucleotide and indel characters were partitioned (ITS1, 5.8S, ITS2 and indels). The GTR + G model of evolution for the DNA partitions and the two-parameter Markov model for the indels were applied. Four Markov chains were run for 10,000,000 generations, sampling every 1000<sup>th</sup> generation. Burn in of 3000 trees (30 %) was established. The remaining 7000 trees were sampled and were combined in a 50 % majority rule consensus phylogram.

#### Results

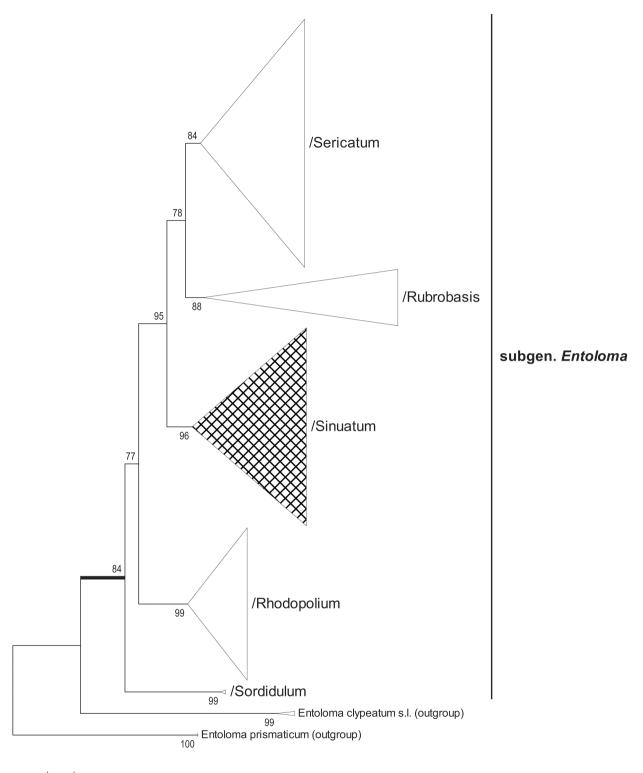
## Phylogenetic analysis

The final alignment including nucleotide + binary data was 1095 characters long (995 DNA + 122 binaries). Phylogenetic trees from both ML and BI analyses had congruent topologies. The ML tree is illustrated (Fig. 2), bootstrap values (BS) >70 % and Bayesian posterior probabilities (PP) >0.90 are indicated as evidence for strong support. All of the four species treated in this study, E. aurorae-borealis, E. borgenii, E. eminens, E. serpens, formed well-supported terminal clades (Fig. 2). The new species, E. aurorae-borealis seems to be sister to E. serpens differing by nine substitution and indel positions (similarity 98.8 %). Entoloma borgenii comes closer to E. bryorum and E. brunneipes, while E. eminens has a basal, quite isolated position within the Sinuatum clade. According to molecular, morphological and ecological evidences, we describe here E. auroraeborealis as new to science.

#### Taxonomy

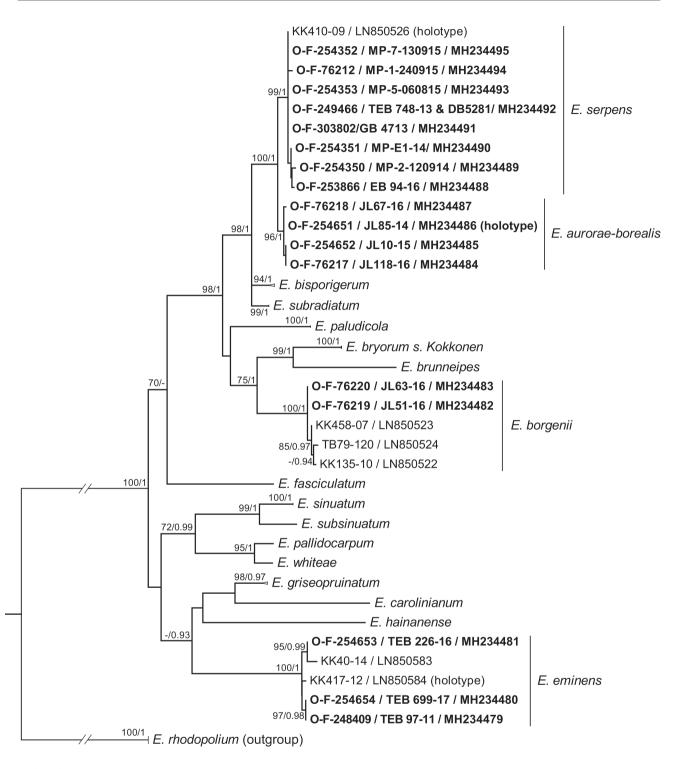
*Entoloma aurorae-borealis* Noordel., Weholt, Eidissen & Lorås, **spec. nov**. – Figs. 3, 7a–c MycoBank: MB 825140

E tymology. – The epithet refers to the Northern Light that shines often over Holmvass-



0.02

**Fig. 1.** Maximum Likelihood phylogenetic tree of the five main clades in *Entoloma* subg. *Entoloma* taken from the Norwegian *Entoloma* project. The Sinuatum clade is highlighted in checked pattern. The *E. clypeatum* complex and *E. prismaticum* were chosen as outgroup. RAxML bootstrap supports values (>70%) are shown at the branches. The bar indicates 0.02 expected change per site per branch.



## 0.02

**Fig. 2.** Maximum likelihood phylogeny (ITS) in the Sinuatum clade of *Entoloma* subg. *Entoloma* inferred from RAxML analysis. Newly generated sequences are highlighted in bold. Voucher numbers followed by personal herbarium numbers and GenBank accession numbers are given. Other than the four studied species are compressed. *Entoloma rhodopolium* was selected as outgroup. RAxML bootstrap supports values (>70%) and Bayesian posterior probabilities (>0.90) are indicated on the branches at nodes (ML/PP). The bar indicates 0.02 expected change per site per branch.

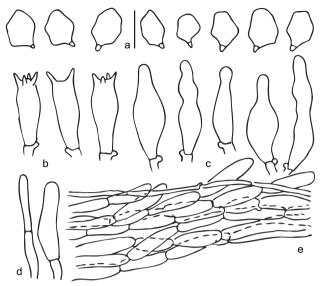


Fig. 3. Microcharacters of *Entoloma aurorae-borealis*. a. Spores, b. Basidia, c. Cheilocystidia, d. Caulocystidia, e. Pileipellis. Bar =  $10 \mu m$ .

dalen Nature Reserve, the *Entoloma* rich type locality of this species.

H o l o t y p e . – NORWAY. Nordland, Grane, Holmvassdalen Nature Reserve, 19 September 2014, J. Lorås, JL 85-14 (O-F-254651). GenBank: MH234486.

Description. - Pileus 30-60 mm broad, convex-umbonate at first then expanded with straight margin, with low, rounded umbo, sometimes within a shallow depression, probably not distinctly hygrophanous, reddish brown to very dark brown or almost black at centre, distinctly paler towards margin, almost glabrous to slightly rimose in marginal zone, somewhat rugulose at centre, sometimes radially splitting with age, translucently striate up to half the centre. - Lamellae emarginate, subsegmentiform, L = up to 40, 1 = 3-7, white, only at maturity with slight pink tinge. -Stipe  $25-75 \times 4-12$  mm, cylindrical or gradually broadened towards apex and base, grevish brown, colour more or less the same as margin of pileus, with scattered white fibrils lengthwise, not polished, occasionally somewhat scurfy towards apex. - Context. Smell none. Taste not noted.

S p o r e s 9.0–10.5 × 6.0–9.0 µm, Q = 1.1–1.4, subisodiametrical to heterodiametrical, Qav = 1.2, 5–7 angled in side view with rather pronounced angles. – B a s i d i a 22–30 × 6.0–9.0 µm, 2- and 4-spored. – L a m ella edge heterogeneous. – C h e i l o c y s t – i d i a very abundant, in clusters among the basidia,  $20-42 \times 5.0-12$  µm, versiform or (irregularly) clavate to lageniform or utriform, thin-walled. – H y m e – n o p h o r a l t r a m a more or less regular, made up of relatively short elements,  $40-120 \times 5-11 \mu m$ . – Pileipellis a cutis with transitions to a trichoderm, made up of cylindrical hyphae,  $4.0-12 \mu m$  wide, with cylindrical to subclavate terminal elements, up to 15  $\mu m$  wide, with intracellular and minutely incrusting pigment. – Caulocystidia scattered at apex of stipe, similar to cheilocystidia. – Clamp connections present in all tissues.

Habitat. – *Entoloma aurorae-borealis* has been recorded four times among stones in a groove, sloping approximately across a north-south axis, in an alpine area (altitude between 540 and almost 700 meters) with different levels of calcareous soil, partly sandy, in snow-bed vegetation with bryophytes, *Salix herbacea*, *Oxyria* sp. and *Athyrium distentifolium*.

Material examined. – NORWAY. Nordland, Grane, Holmvassdalen Nature Reserve, 19 September 2014, J. Lorås, JL 85-14 (holotype, O-F-254651); 22 September 2015, J. Lorås, JL 10-15 (O-F-254652); 13 September 2016, S.E. Eidissen, J. Lorås, JL 118-16 (O-F-76217); 4 October 2016, S.E. Eidissen, J. Lorås, JL 67-16 (O-F-76218).

Comments. - Entoloma aurorae-borealis belongs to the Rhodopolia clade of *Entoloma* subg. Entoloma. It is morphologically characterized by the somewhat rimose-rugulose surface of the pileus and presence of abundant cheilocystidia. Furthermore, the alpine habitat amongst Salix herbacea appears to be characteristic for the species. In the summer season hundreds of reindeer affect the soil and vegetation by grazing and trampling in the alpine zone of the Holmvassdalen area, which could have a positive effect on alpine fungi favoured by disturbance. Entoloma serpens Kokkonen is a sister species, and very similar, particularly with respect to the fibrillose-rimulose aspect of the cap (centre) surface. However, it differs in ecology, as it has been found mainly in more thermophilic Salix-Betula-Populus forests in Norway, including one find in low alpine Salix shrubs, but never in typical, open Salix herbacea snow beds. Entoloma serpens seems also on average to have somewhat darker pileus colour, and the spores are on average narrower and more distinctly heterodiametrical. Our preliminary phylogenetic and morphological study of the Rhodopo*lia* indicates that the presence of cheilocystidia may be a good separating morphological character, and this feature distinguishes E. aurorae-borealis from the most similar species, except *E. serpens*. It seems, however, that the cheilocystidia of the former are more abundant and well-developed. Among the arctic-alpine representatives of this group, Entoloma rimulosum Noordel. from Greenland comes

morphologically close to it with respect to the somewhat wrinkled-rugulose surface of the pileus, and the presence of differentiated cheilocystidia (Noordeloos 1984). However, ITS obtained from the holotype places *E. rimulosum* in the Nolanea clade, close to *E. calthionis*. Kokkonen (2015) placed *E. rimulosum* in the Sericatum clade, based on other, apparently misidentified collections from Greenland (T. Borgen and D. Boertmann, C).

**Entoloma serpens** Kokkonen, Mycol. Progr. 14: 24 (2015). – Figs. 4, 7d–e

Pileus 15–60 mm, campanulate-umbonate, then expanded with often a low to pronounced umbo; with subinvolute then straight margin, slightly hygrophanous, not or very obscurely translucently striate, sometimes more pronounced striate

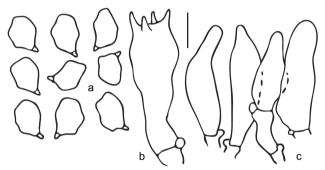


Fig. 4. Microcharacters of *Entoloma serpens*. a. Spores, b. Basidia, c. Cheilocystidia. Scale bar: 10 µm.

with age, when young with fibrillose-rimulose to sometimes scaly-tomentose centre, more glabrous to sometimes rugulose towards margin, sometimes with some minute hyaline-white scales/hairs visible; glabrescent with age, very dark greyish brown, even blackish brown when young, in maturity much paler grey-brown, somewhat pallescent on drying, umbo often persistently darker. – Lamellae L =about 40-50, 1 = 3-5, broadly adnate-emarginate, segmentiform to subventricose, up to 5 mm broad, pale greyish, then pink with subentire, concolorous edge. – Stipe  $30-100 \times 2-8$  mm, base clavate-radicate (up to 1.5 cm wide), straight, rarely flexuous, pale to moderately dark grey-brown, paler with age, fistulose, base whitish; fistulose-fibrillose, sometimes with white scales at apex. - Context dark to pale grey-brown, almost whitish in pileus when dry. Smell none to more farinaceous, and then distinctly so when cut, taste distinctly farinaceous.

Spores  $7.5-11.5 \times (6.0)6.5-7.5(8.2)$  µm, av spore  $8.8-9.4 \times 7.2-7.9$  µm Q = 1.2-1.6(1.8), Qav = 1.2-1.45, heterodiametrical, Qav = 5-7 angled in side view (n = 50 from 5 collections. - Basidia  $18-40 \times 8-15$  µm, mainly 4-spored. – Lamella edge usually heterogeneous, with scattered cystidia, sometimes in clusters along the edge, generally shorter than mature basidia. - Cheilocystidia  $20-45 \times 8-12$  µm, versiform, (irregularly) clavate to lageniform-utriform, thin-walled, often with strongly vacuolized content and thus distinguishable from young basidia. - Hymenophoral trama more or less regular, made up of relatively short elements,  $20-50 \times 6-15 \mu m$ . – Pileipellis a cutis with some more erect cylindrical-subclavate terminal hyphae; at umbo structure more trichodermal, with turfs of erect hyphae; cutis hyphae parallel-cylindrical, 4–11 µm wide; with intracellular and some also with distinct encrusting pigment. - Caulocystidia scattered at apex of stipe, similar to cheilocystidia. - Clamp connections present in all tissues.

Habitat. – Entoloma serpens is recorded mainly in rich to calcareous mixed deciduous forests, forest rims and parks in southern boreal and boreonemoral zone, but also one record so far from low alpine zone. One collection was from a Tilia forest rim with mainly Salix caprea and Corylus avellana, and together with typical Salix associates. Other records were from (grassy) garden/parks near Betula pendula, Populus tremula and (more rarely) Salix caprea. One record was from an Alnus forest, another collection from park was found near Fagus. The alpine record was found in herb-rich vegetation along a brook, with Salix herbacea, S. lapponum, S. lanata and Bistorta vivipara.

Collections examined. - NORWAY. Buskerud, Røyken, Bøsnipa, 20 September 2013, T.E. Brandrud & B. Dima, TEB 748-13/DB5281 (O-F-249466). Nord-Trøndelag, Levanger, Hårskallen, Øvre Forra Nature Reserve, 22 August 2016, E. Bendiksen, EB 94/16 (O-F-253866). Østfold, Fredrikstad, Hunnfeltet, 13 September 2015, M. Pettersen, MP-7-130915 (O-F-254352); 24 September 2015, M. Pettersen, MP-1-240915 (O-F-76212); Tofteberg, 18 October 2014, M. Pettersen, MP-E1-14 (O-F-254351); 6 August 2015, M. Pettersen, MP-5-060815, (O-F-254353); Vesten, 12 September 2014, M. Pettersen, MP-120914 (O-F-254350). Møre og Romsdal, Rindal, 29 August 2013, G. Bureid, GB 4713 (O-F-303802).

C o m m e n t s. – *Entoloma serpens* is characterized by the initially very dark (almost blackish brown) pileus with rimose to finely scaly umbo, fibrillose-scaly, greyish stipe and habitat in more or less calcareous forests and parks where it seems to be mainly associated with *Betula* spp., *Populus* 

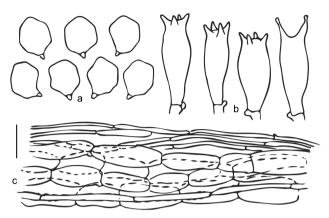
tremula and Salix spp. (mainly Salix caprea), but probably also with Alnus, once also indicated near Fagus. It differs from its sister species E. auroraeborealis mainly by habitat, possibly also by an on average darker pileus, apparently normally also less pronounced cheilocystidia. Entoloma serpens seems to be a more thermophilic species, with most finds from the boreonemoral zone, whereas E. aurorae*borealis* hitherto is found only in northern, alpine habitats with Salix herbacea. However, one find of *E. serpens* was from the low alpine, riparian willow belt including Salix herbacea, so it is possible that these species have an overlapping habitat range. With its initially rimose-fine scaly pileus, *E. serpens* may also resemble the frequently occurring *E. sericatum* which might have a fine, white hairy pileus (margin) when very young. Even though E. serpens on average seems to occur in drier habitats than *E*. sericatum, they can co-occur in Salix-Alnus-Betula mixed forests or parks. Entoloma serpens should then be possible to distinguish by a more umbonate, darker pileus, and presence of cheilocystidia.

Entoloma serpens furthermore differs from other dark Rhodopolia species (such as E. gerriae) in the initially rimose fine-scaly pileus umbo, and the presence of cheilocystidia in most cases. It reminds also of Entoloma rimulosum from Greenland, which has a similar dark colour, rimose pileus, encrusting and intracellular pigment (Noordeloos 1984). Kokkonen (2015) ranged this species among the synonyms of E. sericatum, but she did not study the type, only other collections labelled E. rimulosum belongs to subg. Nolanea, it is probably identical with E. calthionis, and not in the Rhodopolia, despite its strong resemblance to other small, subalpine species of subg. Entoloma.

Based on data available, *E. serpens* seems to be a northwestern species in Europe, and it seems to be much more frequent in Norway (seven records verified by sequencing) than in Finland (one record) and in NW Russia (Kalinina 2018).

**Entoloma borgenii** Noordel., Persoonia 12(3): 283 (1984). – Figs. 5, 7f

Pileus 10–30 mm, convex-umbonate to applanate, with rounded umbo or with shallow central depression, hardly hygrophanous, uniformly coloured dark sepia-brown or reddish brown, with darker blackish brown centre, not much paler towards margin, not or very obscurely translucently striate, appearing smooth, glabrous or slightly rimose near margin. – Lamella e moderately dis-



**Fig. 5.** Microcharacters of *Entoloma borgenii*. **a**. Spores, **b**. Basidia, **c**. Pileipellis. Scale bar: 10 μm.

tant, arcuate-subdecurrent, pale creamy brown then with pink tinge, with almost entire, concolorous edge. – Stipe up to  $45 \times 2-5$  mm, cylindrical or gradually broadened towards base, pale brown, almost the same colour as the lamellae, slightly striate with a few paler fibrils, but almost smooth. – Context. Smell not known.

S p o r e s 8.0–11.5(12) × 6.5–8.5 µm, average 9.0 × 8.0 µm, Q = 1.1–1.25–1.4, 6–7 angled in side-view. – B a s i d i a 22–40 × 7–15 µm, normally 4-spored, few 2-spored. – L a mella edge fertile, cheilocystidia absent. – H y m e n o p h o r a l t r a m a regular, made up of inflated, sausage-shaped elements, 79–200 × 6–20 µm. – Pileipellis a narrow ixocutis of a few, narrow, 2–4 µm wide cylindrical hyphae, situated above a subpellis of short, inflated elements, 50–160 × 7–20 µm, with abundant brown, intracellular pigment. Incrusted hyphae present, but sparse. – Stipitipellis a cutis of narrow, cylindrical hyphae, 4–11 µm, with pale brown, intracellular pigment. – Clamp connections abundant in all tissues.

Habitat. – *Entoloma borgenii* has been recorded twice among bryophytes and stones on somewhat calcareous ground, in the same area as *Entoloma aurorae-borealis* (in Holmvasdalen). One record was alpine with *Salix* spp., while the other was subalpine close to a brook (485 meters asl), with *Salix* sp. and *Betula pubescens* in the vicinity. About 10 meters lower down there is a small limestone cave.

Collections examined. – NORWAY. Nordland, Grane, Holmvassdalen Nature Reserve, 5 September 2016, J. Lorås, JL 51-16 (O-F-76219); 22 September 2016, J. Lorås, JL 63-16 (O-F-76220).

C o m m e n t s . – The specimens from Holmvassdalen fit well within the concept of *Entoloma borge*- *nii* from Greenland (Noordeloos 1984), which has also been recorded from alpine snow-beds and associated with *Salix lanata* thickets in the Hardangervidda, Norway (Noordeloos & Gulden 1989) and with *Salix polaris/Dryas* at Svalbard (Noordeloos & Gulden 2004). Kokkonen (2015) reported it from lowland old *Salix* thickets, mostly on shores of lakes and rivers, but her specimens slightly differed in ITS (2–4 substitution and indel positions), and differed macroscopically in having a deeply translucent pileus, and a paler stipe. These (small) differences may well be worth further study, as another taxon may appear to be involved.

# *Entoloma eminens* Kokkonen, Mycol. Progr. 14: 22 (2015). – Figs. 6, 7g

Pileus 50–120 mm, convex-umbonate, becoming applanate with expansion, sometimes with a low umbo, sometimes irregular, sometimes concen-

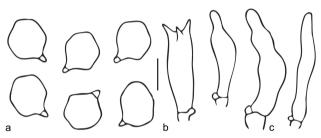


Fig. 6. Microcharacters of *Entoloma eminens*. a. Spores, b. Basidia, c. Cheilocystidia. Scale bar: 10 µm.

trically undulate; glabrous (fine hyaline-white hairs not seen, but could be present in young stages); hygrophanous, margin often faintly translucently striate with age; uniformly brownish grey, becoming paler grey-brown when dry and with age. - Lamellae L = up to 100, l = 3-9; up to 12 mm broad, adnate-emarginate, subventricose, pale greyish, then pink; edge more or less even. - Stipe long,  $6-120 \times 10-15$  mm, base clavate-radicate and often irregular (up to 20 mm wide), often fistulose and brittle; with a felty thick white hyaline tomentum at base (often 20-30 mm upwards), otherwise glabrous to faintly fibrillose and pruinose at apex; whitish to pale grevish, sometimes discolouring vellowish at base. - Context fairly brittle, greyish in pileus, whitish on drying, stipe whitish to pale grey. Smell more or less farinaceous, distinctly when cut, taste distinctly farinaceous.

Spores (7.5)8.0–10.0  $\times$  7.5–8.7 µm, Q = 1.05– 1.2, Qav = 1.15, 5–7-angled, subisodiametrical in side view. – Basidia 22–35  $\times$  7–10 µm, mainly 4-spored. – Lamella edge usually heterogeneous, with scattered cystidia, sometimes without observable cystidia. – Cheilocystidia 20–30 × 3–9 µm, irregularly cylindrical to narrowly lageniform, thin-walled, shorter than basidia and hard to find. – Hymenophoral trama regular, made up of short, cylindrical or slightly inflated elements, 25–95 × 5–14 µm. – Pileipellis a thin cutis of cylindrical, 3–7 µm wide hyphae, with scattered, ascending, cylindrical-subclavate terminal hyphae (some more erect). Pigment brown, intracellular, particularly in the subpellis and upper trama; some hyphae distinctly incrusted. – Clamp connections present in all tissues.

Habitat. – *Entoloma eminens* is recorded from Norway from a calcareous *Tilia* forest in the outer Oslofjord; three collections, all found near *Tilia* and *Corylus*, and at least two of them very unlikely to be in association with any other trees. Kokkonen (2015), reported four collections, including the type, from Eastern and Northern Finland, all from rich, moist, mixed *Picea abies*-dominated forests, with scattered deciduous trees, such as *Populus tremula* and *Alnus incana* in the type locality.

Collections examined.-NORWAY. Telemark, Bamble, Høgenheitunnelen W, 15 July 2011, T.E. Brandrud, TEB 97-11 (O-F-248409); 15 August 2016, T.E. Brandrud & B. Dima, TEB 226-16 (O-F-254653); 16 September 2017, T.E. Brandrud & B. Dima, TEB 699-17 (O-F-254654).

Comments. – Entoloma eminens is well characterized by the large and tall, but often hollow and brittle basidiomata, with a brownish grey, soon flattened and often irregular pileus and a pale stipe with often pronounced, whitish mycelial felt/tomentum basally and a farinaceous smell and taste. Also some other Rhodopolia species such as E. rhodopolium might possess a hirsute-tomentose stipe base, but they have a different (never mealy) smell, and are rarely so large. In the phylogeny it is sister to E. sin*uatum*, which differs not only in the colour of the cap and in yellow lamellae, but also lacks encrusting pigments in the pileipellis and cheilocystidia. It has some resemblance to Entoloma sinuatum sensu Cooke(1884–1886), Romagnesi(1978) and Noordeloos (1988), a species recorded a few times from Britain, the Netherlands and France, differing from *E. sinu*atum sensu auct. (syn. E. eulividum Noordel.) by the lack of yellow in the lamellae, and the more greyish pileus. Later, Noordeloos (1992) changed his mind and used the name *E. sinuatum* again for the wellknown yellow-gilled poisonous species, and the interpretation of Noordeloos (1988) became oblivious. However, judging from the descriptions of E. sinuatum (Romagnesi 1978, Noordeloos 1988) this spe-



**Fig 7.** Basidiomata of the *Entoloma* species studied: *E. aurorae-borealis* (**a-b.** JL 85-14 holotype, **c.** JL 67-16), *E. serpens* (**d.** MP-7-130915, **e.** EB 94/16), *E. borgenii* (**f.** JL 63-16), *E. eminens* (**g.** TEB699-17).

cies differs from *E. eminens* by the lack of incrustations in the pileipellis and the lack of cystidia. The French and Dutch collections were made in parkland with *Quercus* and *Tilia* on heavy, slightly calcareous, clayey soils.

## Discussion

Altogether four collections of the new species *Entoloma aurorae-borealis* were verified with sequencing and morphological evidences. They were collected in different spots in alpine *Salix herbacea* habitats in the Holmvassdalen nature reserve of northern Norway. Two collections of *E. borgenii* were also made at the same location. This species has a predominantly (sub)arctic distribution, and is known from Greenland, The Faeroes, and Svalbard (Noordeloos 1984, 2012, Noordeloos & Gulden 2004), and also from some lowland localities in northern and eastern Finland (Kokkonen 2015), although phylogenetically, the latter material deviates slightly.

Seven localities of *E. serpens* were recorded from boreonemoral to alpine habitats in Norway, apparently associated mainly with Salix species, but also with Betula, Populus and Alnus. The species was formerly known only from the type locality in Finland; a meadow with scattered Salix caprea trees (Kokkonen 2015, and from NW Russia (Kalinina 2018).). The species seems to have rather similar habitat requirements as the more frequently found Rhodopolia species E. majaloides and E. sericatum, being associated with a wide range of boreal deciduous trees, but with an apparent preference for Salix spp. These occur both in forests and in more secondary habitats such as forests rims, roadsides, parks and gardens. Entoloma serpens seems on average to be a more southern/boreonemoral species than the other two species, however, the alpine find from North Trøndelag indicates that the species might have a wider distribution also in northern areas.

Entoloma eminens is a tall and prominent Rhodopolia species that was collected and verified at three different subsites within a large calcareous Tilia forest in outer Oslofjord region. The specimens were all found near Tilia cordata and Corylus avellana (without Salix, Populus, Betula or Alnus), and were believed to be associated with these hosts at the site. Entoloma eminens was formerly known only from four Finnish collections from two calcareous, moist mixed forest areas. At the type locality the tree species Populus tremula, Alnus incana and Picea abies were possible hosts. The typical/major habitat for the species is still somewhat obscure. In Norway it is so far found only in boreonemoral (thermophilous) calcareous Tilia forests, whereas in Finland it is found in middle/northern boreal rich/ calcareous *Picea abies* forests with some boreal deciduous trees. Some of the most frequent *Rhodopolia* species such as *E. sericatum* are often found in (moist) coniferous forests, but always near a *Betula*, *Salix, Alnus* or *Populus* tree. In the same manner, it seems probable that *E. eminens* in *Picea abies* dominated forests is associated with broad-leaved trees, and not with *Picea*, which seems to be a very rare associate for *Rhodopolia* species in general. At the type locality *E. eminens* most probably was associated with *Populus tremula*. Although *E. eminens* is a rather large and striking species, it seems to be a rare species, whether being associated mainly with thermophilous or boreal broad-leaved trees.

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#### References

- Agerer R., Waller K. (1993) Mycorrhizae of *Entoloma saepium*: parasitism or symbiosis? *Mycorrhiza* **3**: 145–154.
- Baroni T.J., Matheny P.B. (2011) A re-evaluation of gasteroid and cyphelloid species of Entolomataceae from eastern North America. *Harvard Papers in Botany* 16(2): 293–310.
- Baroni T.J., Halling R.E. (2000) Some Entolomataceae (Agaricales) from Costa Rica. *Brittonia* 52: 121–135.
- Baroni T.J., Ortiz B. (2002) New species of *Oudemansiella* and *Pouzarella* (Basidiomycetes: Agaricales) from Puerto Rico. *Mycotaxon* 82: 269–279.
- Baroni T.J., Hofstetter V., Largent D.L., Vilgalys R. (2011) *Entocybe* is proposed as a new genus in the Entolomataceae (Agaricomycetes, Basidiomycota) based on morphological and molecular evidence. *North American Fungi* **6**(12): 1–19.

- Borchsenius F. (2009) *FastGap 1.2*. Department of Biosciences, Aarhus University, Denmark. Published online at: http:// www.aubot.dk/FastGap\_home.htm.
- Brandrud T.E., Evju M., Blaalid R., Skarpaas O. (2016) Nasjonal overvåking av kalklindeskog og kalklindeskogsopper. Resultat fra første overvåkingsomløp 2013–2015. – NINA Rapport 1297: 128.
- Co-David D., Langeveld D., Noordeloos M.E. (2009) Molecular phylogeny and spore evolution of Entolomataceae. *Persoo*nia 23: 147–176.

Cooke M.A. (1884–1886) Illustrations of British fungi 3. London.

- Gouy M., Guindon S., Gascuel O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**: 221–224.
- He X.-L., Li T.-H., Xi P.-G., Jiang Z.-D., Shen Y.-H. (2013) Phylogeny of *Entoloma* s.l. subgenus *Pouzarella*, with descriptions of five new species from China. *Fungal Diversity* **58**: 227–243.
- Henkel T.W., Aime M.C., Largent D.L., Baroni T.J. (2011) The Entolomataceae of the Pakaraima Mountains of Guyana 5: new species of *Alboleptonia*. *Mycotaxon* **114**: 115–126.
- Horak E. (1980) *Entoloma* (Agaricales) in Indomalaya and Australasia. *Beihefte Nova Hedwigia* **65**: 1–352.
- Horak E. (1982) *Entoloma* in South America. II. *Sydowia* 35: 75–99.
- Horak E. (2008) The fungi of New Zealand: Agaricales of New Zealand 1. Pluteaceae–Entolomataceae. Fungal Diversity Research Series 19: 1–305.
- Kalinina L.B. (2018) Agaricoid fungi (Basidiomycota) of Izhora Upland (Leningrad Region) I. The State Nature Reserve «Oak forests near Velkota Village». Novosti Sistematiki Nizshikh Rastenii 52(2): 359–372.
- Karstedt F, Capelari M. (2010) New species and new combinations of *Calliderma* (Entolomataceae, Agaricales). *Mycologia* 102: 163–173.
- Karstedt F., Capelari M. (2013) *Inocephalus* (Entolomataceae, Agaricales) from São Paulo State, Brazil. *Nova Hedwigia* **96**: 279–308.
- Karstedt F., Capelari M. (2017): A new species of *Entoloma* subgenus *Trichopilus* from Atlantic Forest Region (Brazil): *Sydowia* **69**: 23–28.
- Karstedt F., Capelari M., Stürmer S.L. (2007) A new combination and new records of *Pouzarella* (Agaricales, Entolomataceae) from Brazil. *Mycotaxon* 102: 147–153.
- Kasuya T., Takehashi S., Hoshino T., Noordeloos M.E. (2010) Entoloma aprile (Agaricales, Entolomataceae) new to Japan, with notes on its mycorrhiza associated with Populus maximowiczii in cool-temperate deciduous forests of Hokkaido. Sydowia 62(2): 205–223.
- Katoh K., Standley D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kobayashi H., Hatano K. (2001) A morphological study of the mycorrhiza of *Entoloma clypeatum* f. *hybridum* on *Rosa multiflora*. *Mycoscience* **42**: 83–90.
- Kokkonen K. (2015) A survey of boreal *Entoloma* with emphasis on the subgenus *Rhodopolia*. *Mycological Progress* 14: 1–116.
- Largent D.L. (1994) Entolomatoid fungi of the Pacific Northwest and Alaska. Mad River Press, USA.
- Morgado L.N., Noordeloos M.E., Lamoureux Y., Geml J. (2013) Multi-gene phylogenetic analyses reveal species limits, phylogeographic patterns, and evolutionary histories of

key morphological traits in *Entoloma* (Agaricales, Basidiomycota). *Persoonia* **31**: 159–178.

- Morozova O.V., Noordeloos M.E., Vila J. (2014) *Entoloma* subgenus *Leptonia* in boreal-temperate Eurasia: towards a phylogenetic species concept. *Persoonia* **32**: 141–169.
- Noordeloos M.E. (1981) *Entoloma* subgenera *Entoloma* and *Allocybe* in the Netherlands and adjacent regions with a reconnaissance of its remaining taxa in Europe. *Persoonia* **11**: 153–256.
- Noordeloos M.E. (1984) Entolomataceae (Agaricales, Basidiomycetes) in Greenland. I. The genus Entoloma. Persoonia 12: 263–305.
- Noordeloos M.E. (1988) Entolomataceae Kotl. & P. In: Bas C. et al (eds.) *Flora Agaricina Neerlandica 1*, pp. 77–177.
- Noordeloos M.E. (1992) *Entoloma* s.l. Fungi Europaei 5. Giovanna Biella, Saronno, Italy.
- Noordeloos M.E. (2004) *Entoloma* s.l. Fungi Europaei 5a. Edizione Candusso, Italy.
- Noordeloos M.E. (2012) *Entoloma* (Fr.) P. Kumm. In: Knudsen H., Vesterholt J. (eds.) Funga Nordica. Nordsvamp, Copenhagen, pp. 517–576.
- Noordeloos M.E., Gates G.M. (2012) The Entolomataceae of Tasmania. *Fungal Diversity Research Series*. 22. Springer Dordrecht, Heidelberg, New York, London.
- Noordeloos M.E., Gulden G. (1989) *Entoloma* (Basidiomycetes, Agaricales) of alpine habitats on the Hardangervidda near Finse, Norway, with a key including species from Northern Europe and Greenland. *Canadian Journal of Botany* **67**: 1727–1738.
- Noordeloos M.E., Gulden G. (2004) The genus *Entoloma* (Basidiomycetes, Agaricales) on Svalbard. In: Cripps C. (ed.) *Fungi in forest ecosystems: systematics, diversity, and ecology.* New York Botanical Garden, pp. 97–106.
- Noordeloos M.E., Dima B., Weholt Ø., Eidissen S.E., Lorås J., Brandrud T.E. (2017) *Entoloma chamaemori* (Entolomataceae, Bacidiomycota) – a new boreal species, with isolated phylogenetic position. *Phytotaxa* **298**(3): 289–295.
- Romagnesi H. (1941) Les Rhodophylles de Madagascar. Laboratoire Cryptogamie Museum National d'Histoire Naturelle.
- Romagnesi H. (1978) Quelques espèces méconnues ou nouvelles de Macromycetes VI. Bulletin de la Société Mycologique de France 94: 97–108.
- Romagnesi H., Gilles G. (1979) Les Rhodophylles des fôrets côtières du Gabon et de la Côte d'Ivoire. *Beihefte Nova Hedwigia* **59**: 1–649.
- Ronquist F, Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Sánchez-García M., Matheny P.B. (2017) Is the switch to an ectomycorrhizal statean evolutionary key innovation in mushroom-forming fungi? A case study in the Tricholomatineae (Agaricales). *Evolution* **71**(1): 51–65.
- Silvestro D., Michalak I. (2012) RaxmlGUI: a graphical frontend for RAxML. Organisms Diversity & Evolution 12: 335–337.
- Simmons M.P., Ochoterena H., Carr T.G. (2001) Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. *Sytematic Biology* 50(3): 454–462.
- Weholt Ø., Lorås J., Eidissen S.E. (2014) One new and one rare species of Entoloma from the Norwegian nature reserve Holmvassdalen. Österreichische Zeitschrift für Pilzkunde 23: 55–60.

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