

Entoloma graphitipes – a species new to Northern Europe

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Entoloma graphitipes var. *graphitipes* is here reported from one location in Holmvassdalen Nature Reserve in the county Nordland, Norway. The species was first found in Germany in 1982, and then five times in Spain. So far Holmvassdalen is the only location in Northern Europe where it has been reported. Comparison of ITS sequences from the Norwegian material and the holotype of *E. graphitipes* f. *cystidiatum* support the morphological identification. Details of macro- and microscopical features as well as ecology are presented.

Key words: Agaricales, Holmvassdalen Nature Reserve, Norway, ITS

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Introduction

Holmvassdalen Nature Reserve, located in the southern part of Nordland county in Norway, covers approximately 6000 hectares and is one of the largest in Norway. It was established in 2008 by the Norwegian Ministry of Environment. The old spruce forest in the reserve is of long continuity, as it has never been clearcut (Lorås & Eidissen 2012). So far more than 130 red-listed species of fungi are recorded in the area following the Norwegian Red List (Brandrud et al. 2010), of which 40 belong to the genus *Entoloma* (Fr.) P. Kumm. (Weholt et al. 2015). A mix of different habitats exists in Holmvassdalen, with rich fens and tall- and low-herbs, mostly surrounded by calciphilous spruce woodlands (*Picea abies*). However, some areas are dominated by nutrient-poor soils where *Vaccinium myrtillus* L., among other plants, is widespread. A river, Holmvasselv, flows across the deep valley and keeps moisture in the air relatively constant. In

addition, diluted minerals coming from the calcareous hillsides fertilize the soil continuously. This process seems essential to the occurrence and distribution of several *Entoloma* species in the nature reserve (Lorås & Eidissen 2011).

In the period 2007–2014 hundreds of specimens of *Entoloma* were collected in Holmvassdalen Nature Reserve, mainly by Siw Elin Eidissen and Jostein Lorås. The first author studied a large number of dried *Entoloma* specimens under light microscope, identifying one collection as *Entoloma graphitipes* E. Ludw. which was later confirmed with sequencing.

Material and methods

For microscopic studies an Olympus CX31 light microscope was used. The dried material was observed in 10 % NH₄OH. Measurements were done with a 100× oil immersion objective. The line drawings were made by hand.

The collected specimens are deposited in the Norwegian Natural History Museum (O).

DNA was extracted from a dry specimen, grinding a portion of it with a micropestle in 600 μ L CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65°C. A similar volume of chloroform: isoamyl alcohol (24:1) was added and carefully mixed with the sample until it formed an emulsion. It was then centrifuged for 10 min at 13.000 g. The DNA in the supernatant was precipitated with 400 μ L of isopropanol, by centrifuging 15 min at the same speed. The pellet was then washed in cold 70% ethanol, centrifuged again for 2 min, and air-dried. It was finally resuspended in 200 μ L ddH₂O. PCR amplification of the ITS region was performed with the primers ITS1F and ITS4 (White et al. 1990), using a program with a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 72 °C step 10 min. PCR products were checked in a 1% agarose gel, and positive reactions were sequenced with primer ITS4. Chromatograms were checked searching for putative reading errors that were corrected when possible. The resulting ITS sequences were compared with those available in public INSDC databases through BLAST algorithm (Altschul et al. 1990).

Taxonomy

Results showed a 99% similarity (606/609 bp) with several *E. graphitipes* records from Vila et al. (2014), including the holotype. This species shows a moderate degree of genetic variability in ITS region, maybe associated also with the observed morphological variations which led to the proposition of *E. graphitipes* f. *cystidiatum* F. Caball., Vila & Català by these authors. In the present work, we report it as new to Norway, showing further genetic and morphological variability.

Entoloma graphitipes var. *graphitipes*

– Figs. 1–3

Pileus ca 16 mm broad, depressed, navelled, radially striate, margin somewhat irregular, not hygrophorous, brownish. *Lamellae* light brown, decurrent to subdecurrent, thick and distant, about 20 attached to the stipe, lamellulae present, edge brown. *Stipe* ca 26 \times 1.5 mm, cylindrical, longitudinal slightly striate, pruinose towards apex, otherwise smooth, brown. *Spores* isodiametrical to subisodiametrical, 8.3–9.5 \times 7.1–8.3 μ m, mean 9.1 \times 7.8 μ m, Q = 1.11–1.26, Q_m = 1.17 (n= 12), mostly with 6, often weak angles,

sometimes the perimeter almost roundish. *Basidia* tetrasporic, clampless. *Pileipellis* a cutis composed of thin hyphae, 4–8 μ m wide, incrustations abundant. *Clamp connections* absent.

Specimen examined: NORWAY. Nordland. Grane municipality, Holmvassdalen Nature Reserve, 65°19'3" N, 13°18'5" E, humid soil in old spruce-dominated, calcareous forests, 13.VIII.2014 *Eidissen & Lorås JL74-2014* (O-F76067, GenBank no. KU127249).

The macrocharacters fit well with previous descriptions, with exception of the size of pileus and stipe. The previous finds of *Entoloma graphitipes* refer to pilei with 6 mm smaller diameter and with stipes at least 11 mm shorter. The specimens from Holmvassdalen are also described as fragile.

E. graphitipes E. belongs to subgenus *Cladopus*, and is part of the *Entoloma rusticoides* (Gillet) Noordel. -group (Vila et al. 2014). The group comprises 12 taxa in Europe, including *E. graphitipes* var. *cystidiatum* F.Cabal., Vila & Català. The original description of this variety is based on two collections from Spain. Our find showed no cystidia, and should consequently be named *E. graphitipes* var. *graphitipes*. The most common species in the group is *E. rhodocalix* (Lasch: Fr.) M.M.Moser. Other species recorded from Norway are all rare, i.e. *E. phaeocyathus* Noordel., *E. chelone* Noordel. & E.Horak and *E. rusticoides*. *Entoloma chelone* is originally described from Norway, with the type from Mo, Ortfjell, Nordland county (Noordeloos 1987).

Previously Vila et al. (2007) had misidentified *E. graphitipes* as *E. flocculosum* (Bres.) Pacioni, another species in the group. However, this mistake was corrected in a later paper on the *E. rusticoides* -group (Vila et al. 2014). The pictures in both of the abovementioned issues documented specimens with a somewhat pruinose pileus, probably the reason it at first was misinterpreted as *E. flocculosum*. The original description gives the pileus centre as somewhat felted, but

Fig. 1. *Entoloma graphitipes* at the habitat in Holmvassdalen Nature Reserve. Photo: J. Lorås.

Fig. 2. Copy of original drawings of *Entoloma graphitipes* by Erhard Ludwig.



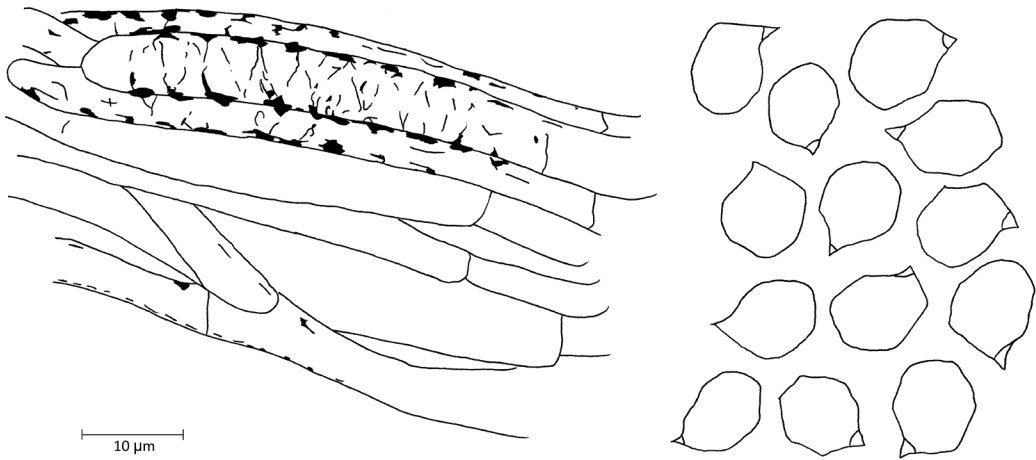


Fig. 3. *Entoloma graphitipes*. Pileipellis with encrusted hyphae and spores. Drawing: S.E. Eidissen.

not squamulose. Vila et al. (2014) described the species as slightly hygrophaneous, while in the original description Ludwig (2007) described it as strongly hygrophaneous. Apparently both the degree of pruina and hygrophaneity may vary, but the pruinose or felted pileus centre should be present. The species is definitely hygrophaneous, although not as strongly as *E. phaeocyathus*.

E. graphitipes var. *graphitipes* may microscopically be separated from others in the group by the lack of cheilocystidia and clamps in basidia and hyphae. Like in *E. rusticoides*, the pileipellis hyphae show a distinctly encrusted epiparietal pigment. Macroscopically, the most similar species is *E. rhodocalix*, but it may be separated by a paler stipe. Ludwig (2007) gives the taste as farinaceous, while Vila et al. (2014) call it “fungic”.

Distribution and ecology

Entoloma graphitipes was first recorded from Germany in 1982 (Ludwig 2007). Later on, five finds were recorded from Spain, collected during the period 1991-2007 (Vila et al. 2014). These samples were found among mosses, grasses and lichens, on sand or basic soil. Whether the finds in sandy soil also could be calcareous is unknown, but possible. None of the mosses and plants in the habitats were identified, except for

the *Satureja montana* growing near one collection.

In Holmvassdalen Nature Reserve three fruitbodies were found at the same location, and were confirmed to match *Entoloma graphitipes*. The basidiomata were found in a thick carpet of bryophytes, close to the edge of an almost dried-out small pond in a moist shady groove, sloping across a northwest-southeast axis, and surrounded by old spruce-dominated, calcareous forests (Fig. 4). A stream has gradually dug out a groove, which produces a relatively constant humid microclimate. The water flushes from a belt of limestone, but is probably becoming slightly more acid as it runs down the slope, mostly due to fallen conifer needles.

The carpet of bryophytes consists of three dominating species, i.e. *Plagomnium ellipticum* (Brid.) T. Kop., *Calliergonella cuspidata* (Hedw.) Loeske and *Drepanocladus aduncus* (Hedw.) Warnst. All three prefer rather nutrient-rich habitats occurring in basic soils with a pH higher than 7 (Weibull 2008, Hedenäs 2014a, b). Dominant vascular plants bordering the moss layer, among others, are *Filipendula ulmaria* (L.) Maxim., *Rubus saxatilis* L. and *Geranium sylvaticum* L., all preferring slightly basic conditions (Nilsson 1995). In September 2015 the average pH value in the pond was 7.2, suggesting a slightly basic habitat for *Entoloma graphitipes*. This agrees well with measures obtained from a soil sample



Fig. 4. The exact habitat in dry season. Photo: J. Lorås.



Fig. 5. In wet summers the moss layer is constantly flooded. Photo: J. Lorås.

taken beneath the moss layer, which also showed a pH around 7.

Like several other species in the genus *Entoloma*, *E. graphitipes* seems to need relatively moist substrates to form fruiting bodies. However, water level fluctuates seasonally in the pond due to different rainfall patterns from one year to another. The summer of 2014 was exceptionally dry, but the dense layer of bryophytes growing in the pond retained a significant moisture level. In comparison, the summer of 2015 was extremely wet and the moss layer was constantly flooded (Fig. 5).

We believe that the pond is usually filled up, because *Equisetum sylvaticum* L. is growing numerous around, suggesting a relatively constant humid habitat. In addition, the lichen *Alectoria sarmentosa* (Ach.) Ach. a species demanding high humidity (Nitare 2000) is growing on an old spruce just above the pond.

Compared to previous records, the find from Holmvassdalen has a noticeably larger pileus and stipe. The habitat may have an effect although most earlier finds were also associated to basic soils. It is not fully clear if they were linked to humid microclimate. Most of the collections were made on naked, sun-exposed and sandy soil. The find in Holmvassdalen is remarkable different from that.

Three other *Entoloma* species are recorded in the groove, namely *E. poliopus* (Romagn.) Noordel. var. *poliopus*, *E. mutabilipes* Noordel. & Liiv and *E. longistriatum* (Peck) Noordel. These are relatively common species and do not have the same specialized habitat demands (Noordeloos 1992, 2004) as *E. graphitipes*. In conclusion, the find of *E. graphitipes* in Holmvassdalen Nature Reserve provide useful new information about the species morphology, chorology and ecology.

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