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Unravelling the *Entoloma politum* complex

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Abstract. *Entoloma politum* is a common lowland species in northern Europe. It has been described to have two close species with a more intense smell, *E. nitriolens* and *E. pernitrosum*. To clarify the taxonomy of these three species, the types of *E. nitriolens* and *E. pernitrosum*, as well as many recent collections were examined by ITS sequences and morphologically. *Entoloma nitriolens* was found to be a sibling species of *E. politum*, whereas *E. pernitrosum* was confirmed as a synonym of *E. politum*. *Entoloma nitriolens* and *E. politum* are described based on own material. They grow in similar habitats, but *E. nitriolens* prefers colder climates and calcareous ground.

Key words: *Agaricales*, alpine, boreal, distribution, Fennoscandia, molecular systematics, nomenclature, Switzerland

Introduction

Some species of *Entoloma* subg. *Entoloma* (*Agaricales*, *Entolomataceae*) are characterized by a nitrous smell. The best known of them are *E. nidorosum* (Fr.) Quél. and *E. politum* (Pers.: Fr.) Donk. Both occur commonly in moist boreal habitats in Europe. Further, a few subg. *Entoloma* species have occasionally been observed to smell faintly nitrous, such as *E. rhodopolium* (Fr.) P.Kumm., *E. uvidicola* Kokkonen and *E. paludicola* (P.D. Orton) Romagn. (Kokkonen 2015, 2021). *Entoloma nidorosum*, *E. rhodopolium* and *E. uvidicola* are usually easy to separate from *E. politum* and *E. paludicola* by a larger size and a fibrillose, pale stipe, and *E. paludicola* differs from *E. politum*, for example, by more heterodiametrical spores. In the past, two very similar species with *E. politum* were described mainly based on an intense nitrous smell: *E. nitriolens* (Kühner) Trimbach and *E. pernitrosum* (P.D. Orton) Trimbach. The interpretations of these three species have varied among mycologists, but currently *E. nitriolens* and *E. pernitrosum* are commonly considered synonyms of *E. politum*.

A morphologically and genetically close species to *E. politum* had been collected by the author in boreal and alpine habitats. The aim of this study was to clarify the identity of this species by comparing it to the types of *E. nitriolens* and *E. pernitrosum*, as well as to explore similar species to *E. politum*.

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Materials and methods

Specimens and morphological studies

The holotype of *E. nitriolens* was loaned from the herbarium G and the holotype of *E. pernitrosum* was loaned from K. All other examined *E. nitriolens* and most *E. politum* specimens were collected by the author, mainly in Finland. Additionally, several *Entoloma* specimens of TUR and TUR-A were examined. Excluding the types, all examined specimens are deposited in TUR or TUR-A. A photograph of *Agaricus politus* Pers. herbarium sheet L 0115327 was sent from L. The *A. politus* specimens of Herbarium Fries were observed in UPS.

The macromorphological descriptions are based on notes from fresh basidiomata. Vegetation was noted at the collecting sites. The micromorphological structures were examined in 10% NH_4OH from dried material. The sizes of spores and basidia are given as length × width. In the species descriptions, the range of the spores is in parentheses and between the parentheses is the 90% confidence interval. Concerning the types, the interval is the range. The mean is in italics. The Q value is the ratio of spore length to spore width. Basidial lengths excluded sterigmata. Stipitipellis was examined from the stipe apex. The morphological descriptions were based on sequenced specimens.

Molecular and phylogenetic methods

The internal transcribed spacer (ITS) DNA sequence was analyzed from all examined specimens excluding the authors collections 852/03, 18 Oct. 2005 and 304/08. Additionally, partial DNA sequences of the RNA polymerase II subunit (RPB2) were analyzed from two *E. nitriolens* and two *E. politum* specimens. DNA was extracted by NucleoSpin Tissue XS or Plant II kits (Macherey-Nagel). The PCR was run using a GeneAmp PCR system 9700 (PE Applied Biosystems), with Illustra PuReTaq Ready-To-Go PCR Beads (Cytiva) or MyTaq Red Mix (Meridian Bioscience). The PCR procedure consisted of 5 min at 94°C, 30–38 cycles of 1 min at 94°C, 1 min at 51–55°C and 1 min at 72°C, and final extension 7 min at 72°C. The PCR primers were ITS1-F, ITS4-B (Gardes & Bruns 1993), ITS2, ITS3, ITS4 (White et al. 1990), fITS7tel, ITStel1 (Kokkonen 2020), RPB2-6F and RPB2-7R (Liu et al. 2000). The purified PCR products were sequenced by Macrogen Europe (Amsterdam, the Netherlands). The sequencing primers were ITS1 (White et al. 1990), ITS4, ITS2, ITS3, fITS7tel, ITStel1, RPB2-6F and RPB2-7R. The sequences were edited by Seqman (DNAStar). The sequences obtained from the types and high-quality sequences showing intraspecific divergence were submitted to GenBank. Their accession numbers are in Table 1.

An ITS phylogenetic tree of subg. *Entoloma* containing *E. nitriolens* and *E. politum* was prepared. In addition to new sequences, some sequences were taken from GenBank (Bjorbækmo et al. 2010; Kokkonen 2015, 2021; Reschke et al. 2022a). The sequences were aligned by MAFFT 7.0 (Katoh 2013) and the alignment was adjusted manually in AliView (Larsson 2014). A maximum likelihood tree (ML) was run by raxmlGUI 2.0.10 (Edler et al. 2021) with thorough bootstrap, 1,000 bootstrap replicates, and GTRGAMMA model. The tree was edited in TreeGraph 2 (Stöver & Müller 2010).

Results

Entoloma nitriolens (Kühner) Trimbach, Docums Mycol 11(44): 3. 1981. (Figs 1–2)

Basionym: *Rhodophyllus nitriolens* Kühner, Bull. Soc. Mycol. Fr. 93(4): 453. 1977.

Type: Switzerland, Graubünden, Val S-charl, Valbella, with *Salix reticulata*, *S. retusa* and *Saxifraga caesia*, calcareous ground, alt. 2,300 m, 24 Aug. 1966, R. Kühner K. 66-120, the Type bag specimen (G00126522 – lectotype!, designated here, MBT 10012897). – ~10 entire basidiomata and small pieces. Spores 7.1–*8.2*–9.2 × 6.4–*7.2*–8.0 µm, Q = 1.04–*1.15*–1.26 (n = 20), subisodiametrical or isodiametrical, rarely broadly heterodiametrical, usually with rather weak angles. Basidia 4-spored. Cheilocystidia absent. Pileipellis hyphae with brown internal pigment, smooth in a small piece examined. Clamps present.

Description. Pileus 0.6–3.0 cm in diameter; convex or applanate, center often depressed, with or without a small umbo, at times margin narrowly fringy; pale grey brown, rather dark brown with a yellow brown margin, or dark brown, center darker, specimens from the alpine zone darker than from the boreal zone; smooth or slightly fibrillose, dry or viscid, hygrophanous, usually translucently striate. Lamellae adnate or subdecurrent; rather distant to distant; pale grey or pale grey brown when young, then pale grey brown having sometimes a pink tinge; edge even or somewhat uneven, concolorous. Stipe 2.2–5.0 cm long, 1.5–4 mm wide; equal or somewhat tapering downwards, rarely base somewhat broadening; whitish, pale grey or pale grey brown, rarely dark brown in the upper part (pileus also dark, in the alpine zone), base greyish or whitish; smooth or faintly fibrillose. Context concolorous

Table 1. Accession numbers of the *Entoloma* sequences submitted to GenBank and origins of the specimens.

Species	Specimen	Origin	Accession number	
			ITS	RPB2
Entoloma nitriolens	Kokkonen 508/21	Finland	OQ799367	OQ791972
	Kokkonen 283/18	Finland	OQ799368	–
	Kokkonen 296/16	Sweden	OQ799369	–
	Kokkonen 171/20	Finland	OQ799370	OQ791971
	Kokkonen 176/20	Finland	OQ799371	–
	lectotype, R. Kühner K.66-120 Type	Switzerland	OQ799372	–
Entoloma politum	Kokkonen 340/09	Finland	OQ799349	–
	Kokkonen 141/02	Finland	OQ799350	–
	Kokkonen 195/20	Finland	OQ799351	OQ791969
	Kokkonen 173/06	Finland	OQ799352	–
	Kokkonen 64/15	Slovenia	OQ799353	–
	Kokkonen 142/02	Finland	OQ799354	–
	Kokkonen 115/19	Sweden	OQ799355	OQ791970
	S. Huhtinen 83/372	Scotland	OQ799356	–
	J. Vauras 1983	Finland	OQ799357	–
	Kokkonen & J. Vauras 4 Sept. 2005	Finland	OQ799358	–
	M. Toivonen & I. Kytövuori 14-310	Finland	OQ799359	–
	Kokkonen 600/12	Finland	OQ799360	–
	Kokkonen 200/08	Finland	OQ799361	–
	Kokkonen 28 Aug. 2005	Finland	OQ799362	–
	E. Campo 7 Nov. 2020	Italy	OQ799363	–
	E. pernitrosum holotype, P.D. Orton 528	England	OQ799364	–
	Kokkonen 147/02	Finland	OQ799365	–
	Kokkonen 22/18	Finland	OQ799366	–

Figure 1. *Entoloma nitriolens*. A − KK 508/21, Metsähallitus; B − KK 296/16; C − KK 283/18, Metsähallitus; D − KK 171/20; E − KK 176/20.

with the surface or somewhat paler. Smell nitrous, rarely indistinct. Taste indistinct.

Spores (7.0)7.6−*8.6*−9.5(11.0) × (6.4)6.8−*7.6*−8.8(9.9) µm, range of mean values 8.2−8.8 × 7.2−8.1 µm, Q = (1.00)1.02−*1.13*−1.24(1.28), range of mean Q values 1.09−1.15 (120 spores from 6 collections); subisodiametrical or isodiametrical, often roundish and with rather weak angles. Basidia 4-spored, 32−*39*−49.5 × 10.5−*12*−14 µm (n = 43). Cheilocystidia absent. Pileipellis hyphae with brown internal pigment diffusely and sometimes also as flecks, clumps or small clusters of granules, smooth or some hyphae slightly to moderately encrusted; terminal cells cylindrical, clavate or fusoid. Stipitipellis: terminal cells cylindrical, clavate, with one or a few constrictions near the apex, or fusoid. Clamps abundant.

Habitat and distribution (sequenced collections). Calcareous or eutrophic moist forests, fens and brooksides with mixed stand or *Salix* bushes in the boreal zone, calcareous sites with, e.g., *Salix reticulata* in the alpine zone, arctic zone; associated with *Salix*, *Dryas octopetala* (GenBank HQ445607, as *E.* cf. *alpicola*, Bjorbækmo et al. 2010), and likely *Alnus*; northern boreal and alpine zones of Finland, Sweden, Switzerland (type), Norway, Svalbard (Bjorbækmo et al. 2010; GenBank MZ869001, as *E. lactarioides*, Reschke et al. 2022a; BOLD, four sequences as *E. alpicola*, Brandrud et al. 2018), Iceland (UNITE, UDB05329818, as *Entoloma* sp., Tedersoo et al. Global soil samples), USA, Alaska (BOLD, as *E. lactarioides*, iNat16874968).

Comments. Authentic material of *Entoloma nitriolens* includes four larger bags, of which two contain basidiomata: the 66-120 Type bag containing ~10 basidiomata and the 66-120B bag containing ca. two basidiomata in pieces in addition to a nº 8 small bag with a section of pileus. The 66-120B is regarded as a separate collection, because it could not be confirmed that it was growing together with the 66-120 Type. The Type bag specimen was selected as a lectotype due to the notation as a type and the larger material.

The ITS sequences of *E. nitriolens* including the type deviated from the *E. politum* sequences only by 7−8 bases and 2 indels, and their RPB2 sequences were identical excluding one heterozygous site in each *E. nitriolens* sequence. Within the species, two *E. nitriolens* sequences deviated from others by one base, but intermediates occurred. Despite the genetic proximity with *E. politum*, *E. nitriolens* is supported as a separate species in the phylogenetic analysis of the ITS sequences (Fig. 4). Furthermore, the species are considered sibling species due to the observed differences in morphology, habitat requirements and distribution.

Kühner (1977) described *E. nitriolens* as similar to *E. politum*, but the latter would lack a nitrous smell. Since then, these species have been confused with each other.

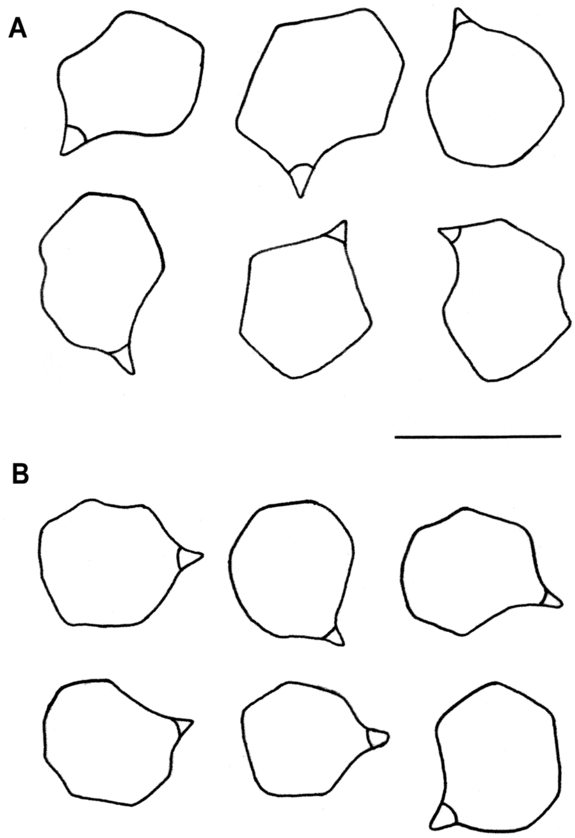

Figure 2. Spores. A – *E. politum*, KK 115/19; B – lectotype of *E. nitriolens*. Scale = 10 µm

Noordeloos (1981) reduced *E. nitriolens* to a synonym of *E. politum*. Horak (1993) did not accept the synonymization and reported several new *E. nitriolens* collections from Switzerland and France. Nowadays, *E. nitriolens* is treated as a synonym of *E. politum*. The extensive protologue of Kühner includes descriptions of collections from the alpine zones of France, Switzerland, Norway and Sweden, as well as lowlands of France. His lowland collections likely represent *E. politum*, which also smell nitrous according to the present knowledge. *Entoloma nitriolens* occurs in higher altitudes and latitudes than *E. politum*, although their ranges overlap. All genetically confirmed specimens of *E. nitriolens* are from boreal, alpine and arctic sites. In the boreal zone, it is much rarer than *E. politum*, and its distribution appears to concentrate on the arctic and alpine zones. By comparison, *E. politum* is probably absent from the arctic and high alpine zones and its distribution extends to southern Europe. *Entoloma nitriolens* favors or demands calcareous ground. In addition to the type, all examined collections were from calcareous or eutrophic sites. The species were once found at the same boreal site.

The habitus of the *E. nitriolens* specimens varied between the boreal and alpine sites, the alpine specimens being darker. In the boreal zone, the stipe appeared paler compared with *E. politum*. Further, the pileus was more shallowly depressed compared to the usual *E. politum*. Both smelled more or less nitrous, but the odor of *E. nitriolens* was never observed to turn farinaceous when crushed, nor tasted farinaceous as sometimes with *E. politum*. *Entoloma politum* is gregarious, whereas *E. nitriolens* grew solitarily or in small groups. Their microscopic features are similar, but the spores of the *E. nitriolens* collections were on average more isodiametrical or more roundish compared to most of the *E. politum* collections (Fig. 2). *Entoloma nitriolens* may also be confounded with *E. paludicola*, which sometimes has a faint nitrous smell (Kokkonen 2015). However, the spores of *E. paludicola* are mostly heterodiametrical. In the alpine and arctic zones, *E. alpicola* (J. Favre) Noordel. is a stouter species and possibly conspecific with *E. majaloides* (Kokkonen 2015).

Additional specimens examined. FINLAND. Koillismaa. Kuusamo, N of Pikku Kumpuvaara, eutrophic paludified forest with *Betula*, *Picea abies*, *Salix caprea* and *Alnus incana*, among *Mnium*, 25 Aug. 2021, Kokkonen 508/21, SW of Siirtola, eutrophic brookside, under *Salix phylicifolia*, among *Sphagnum* and *Mnium*, 19 Aug. 2018, Kokkonen 283/18. Inarin Lappi. Utsjoki, Tsuomasvarri, alpine zone, margin of eutrophic fen, near *Salix glauca*, *S. phylicifolia* and *Betula nana*, 24 Aug. 2020, Kokkonen 171/20, eutrophic depression, near *Salix herbacea*, *S. reticulata*, *S. myrsinites* and *S. glauca*, Kokkonen 176/20. SWEDEN. Jämtland. Strömsund, near Torpen, eutrophic fen with *Alnus incana*, *Betula* and *Picea abies*, among *Sphagnum* and *Mnium*, 20 Aug. 2016, Kokkonen 296/16.

Entoloma politum (Pers.: Fr.) Donk, Bull. Bot. Gardens Buitenzorg 18: 158. 1949. (Figs 2–3)

Basionym: *Agaricus politus* Pers., Syn. meth. fung.: 465. 1801.

Type: *Agaricus politus* Pers. Syn. f., no locality, no date, Herb. Persoon, Herb. Lugd. Bat. No.910.255-345 (L 0115327 – lectotype!, designated here, MBT 10012898, as holotype in Noordeloos (1992)). — Two basidiomata fastened on sheet, partially moldy, dark brown, pileus ~2.2 cm wide, stipe ~4–5.5 cm long and 1.5–3 mm wide (from the photo of L).

= *Entoloma pernitrosum* (P.D. Orton) Trimbach, Docums Mycol 11(44): 5. 1981.

Basionym: *Leptonia pernitrosa* P.D. Orton, Trans. Br. Mycol. Soc. 43: 297. 1960.

Type: United Kingdom, England, Dorset, Studland, 21 Nov. 1955, Orton no. 528 (K 264849 – holotype!). — Specimen with one pileus. Spores 8.0–*9.3*–11.0 × 6.7–*7.9*–9.1 µm, Q = 1.10–*1.19*–1.36 (n = 20), usually subisodiametrical. Basidia 28–35 × 9.5–12 µm (n = 4). Cheilocystidia absent. Pileipellis hyphae hyaline or yellowish, smooth or slightly encrusted; an observed terminal cell clavate. Clamps present. Spores depicted well by Orton (1960).

Description. Pileus 0.55–5.7 cm in diameter; when young convex or applanate with a papilla, later depressed to infundibuliform, low convex, or applanate, margin often undulate; grey brown, dark brown, yellow brown, rarely white or yellowish; smooth or rarely scarcely fibrillose, hygrophanous, translucently striate. Lamellae usually rather broad; adnate or subdecurrent; moderately crowded or rather distant, rarely transvenose; when young pale grey brown or whitish, then darker grey brown or pinkish, in the white form first white and then pinkish; edge even or somewhat uneven, concolorous. Stipe 1.2–9.2 cm long, 1–10 mm wide; equal or slightly tapering downwards, rarely somewhat broadening at the base, rarely flattened;

Figure 3. *Entoloma politum*. A – KK 115/19; B – KK 128/10; C – KK 280/09; D – KK 19/18, Metsähallitus; E – a white form, KK 42/08; F – KK 64/15.

usually slightly paler than the pileus, more greyish, or concolorous, base and/or apex at times whitish; smooth, polished, often with translucent, transverse stripes, rarely delicately longitudinally striate. Context concolorous with the surface or brown grey in pileus. Smell spontaneously slightly nitrous, 'entolomaceous' (perhaps a mixture of faint nitrous and farinaceous) or indistinct, when crushed slightly farinaceous or indistinct. Taste slightly farinaceous, 'entolomaceous' or indistinct.

Spores (7.0)7.7–*8.8*–10.0(11.0) × (5.5)6.2–*7.4*–8.5(9.8) µm, range of mean values 8.4–9.3 × 6.8–8.0 µm, Q = (1.00)1.08–*1.20*–1.34(1.47), range of mean Q values 1.13–1.26 (240 spores from 12 collections); subisodiametrical or heterodiametrical, usually with rather strong angles. Basidia mainly 4 or very rarely mainly 1–2-spored, 22–*35*–49 × 9–*11*–14 µm (n = 98). Cheilocystidia absent. Pileipellis hyphae with brown internal pigment diffusely and sometimes also as flecks, smooth or some hyphae slightly encrusted. Stipitipellis: terminal cells cylindrical, clavate, with one or a few constrictions, or somewhat fusoid. Clamps abundant.

Habitat and distribution. Moist habitats with *Salix* or *Alnus*: lakesides, riversides, brooksides, moist forests, swamps, marshes; collected with *S. phylicifolia*, *S. myrsinifolia*, *S. cinerea*, *S. trianda*, *A. incana* and *A. glutinosa*; the examined specimens from the temperate to the subarctic zone; confirmed from the alpine zone

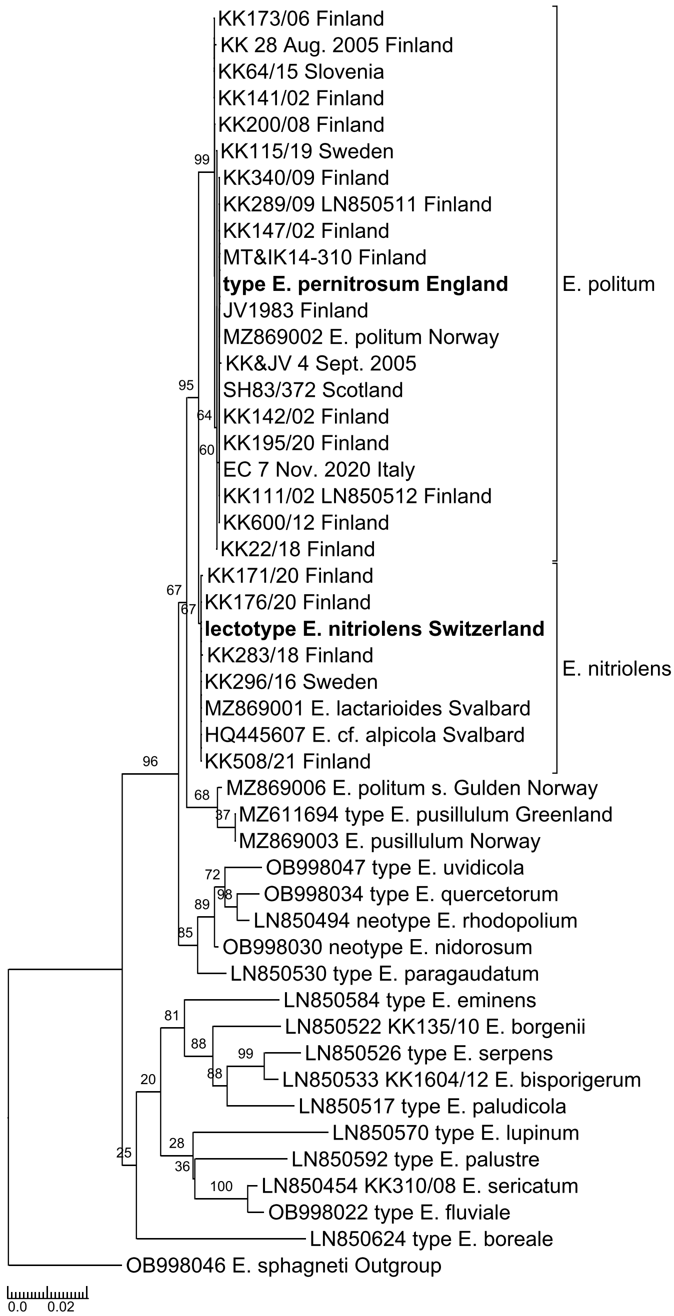

Figure 4. A maximum likelihood tree of ITS sequences comprising species of *Entoloma* subg. *Entoloma*. The sequences are represented by voucher records or when retrieved from GenBank by accession numbers. The types of *E. nitriolens* and *E. pernitrosum* are highlighted. ML bootstrap values are on branches.

(Brandrud et al. 2018), but probably absent from the arctic and high alpine zones with dwarf *Salix*; reported from southern to northern Europe, Morocco (Malençon & Bertault 1970), North America (Largent 1994).

Comments. Persoon (1801) did not designate a type for *Agaricus politus*. In the protologue, the shape of the pileus and the color are mentioned to vary, which implies that several specimens were used for the description. Although the lectotype lacks a collection date, there are annotations and "*Agaricus politus* S" written by the author (handwriting compared to Burdet n.d., Auxilium ad Botanicorum Graphicem, https://www.ville-ge.ch/musinfo/bd/cjb/auxilium/index.php). This indicates that the specimen was probably used for Synopsis methodica fungorum. It is the only *A. politus* specimen in Herbarium Persoon (Roxali Bijmoer personal communication). According to the protologue, the species has a more or less umbilicate, ~3.7 cm (1.5 unc.) wide pileus, subdecurrent lamellae, and 7.5 cm (3 unc.) long stipe. The type basidiomata agree with the description on the whole. Singer (1961) examined this specimen by microscope. The reported spore size 7−10.3 × 5.8−8.5 µm and the spore drawings agree with *E. politum* as perceived today. In the sanctioning work of Fries (1821), the species is described similarly as in Persoon (1801), but the habitat is grassy marshes in contrast to the *Fagus* forests. Later, Fries (1838) wrote that the species is odorous in *Fagus* forests and odorless in marshes. Odor was not mentioned by Persoon. Herbarium Fries (UPS) has two specimens and a picture of *Agaricus politus*, which all appear to be from a later period than the sanctioning description. They originate from W.G. Lasch: one specimen from a moist forest in Poland, a picture of var. *sylvaticus* from Germany, and a specimen of var. *sylvaticus* from an unknown locality. In the current and past usage, *E. politum* has been a species which grows in moist places, usually with *Salix* or *Alnus* (e.g., Bresadola 1929; Lange 1940; Kühner & Romagnesi 1953; Moser 1978; Noordeloos 1981; Ludwig 2007; Kokkonen 2015; Brandrud et al. 2018). It is treated accordingly in this article. *Fagus* has seldomly been mentioned in the literature: "mostly moist *Fagus* forests" by Kummer (1871), "under *Fagus* and *Picea*" by Bon & Chevassut (1973), and a *Fagus-Picea* forest along with the *Salix* sites by Krieglsteiner (2003). *Entoloma politum* as perceived today, may grow in moist places in *Fagus* forests, but it may also be confounded with small basidiomata of *E. nidorosum* and *E. rhodopolium*, as well as *E. paludicola*. Of them, *E. rhodopolium* commonly occurs in *Fagus* forests. Whether the *E. politum* lectotype represents the current *E. politum*, would require a DNA analysis of the old type for the confirmation. The type basidiomata have dark brown stipes, which indicates *E. politum* rather than *E. rhodopolium* or *E. nidorosum*, but the color may have changed when drying and the stipe color is not mentioned in the protologue. Since the lectotype is in a relatively good condition, an epitype is not designated. Further, the current identity of *E. politum* is widely accepted. The current usage of *E. politum* is urged to maintain consistency.

Since Fries, *E. politum* has been reported to be odorless (Bresadola 1929; Kühner & Romagnesi 1953; Malençon & Bertault 1970; Moser 1978) or have a varying smell from nitrous to absent (Orton 1960; Noordeloos 1981; Ludwig 2007; Vesterholt 2009; Brandrud et al. 2018), or becoming sometimes farinaceous (Largent 1994; Ludwig 2007). Further, the ability to sense nitrous smell has been reported to vary among people (Noordeloos 1981). Orton (1960) described a new species, *E. pernitrosum*, which was described to have a more persistent nitrous smell, paler stipe and narrower spores than *E. politum*. It was reduced to a paler form of *E. politum* by Noordeloos (1981). Contrary to the current view, Ludwig (2007) considered *E. pernitrosum* as a distinct species and reported the only difference to be encrusted pileipellis hyphae. The DNA analysis of the *E. pernitrosum* type confirms that *E. pernitrosum* is conspecific with *E. politum* as perceived today. Its ITS sequence was identical with many *E. politum* sequences excluding two ambiguous bases. All *E. politum* sequences differed from each other at most by two bases, but intermediate sequences occurred. At one very variable site, 16 sequences were heterozygous having both adenine and guanine, while 12 sequences had only guanine and 7 sequences only adenine. The specimens having only adenine were interestingly intermediate to *E. nitriolens* based on one or two bases. The different states of this site were not geographically separate and occurred sometimes at the same locations.

The smell, color and spore morphology of *E. politum* may vary considerably. The spores are usually subisodiametrical, but in some collections most spores are heterodiametrical. In lowland moist habitats, the most similar species are *E. nitriolens* and *E. paludicola*. Both of them are rarer than *E. politum*. A comparison with *E. nitriolens* is presented above. *Entoloma paludicola* has a similar habitus, but its basidiomata are usually smaller and the spores are usually more heterodiametrical with the mean Q values 1.22−1.29 as measured in six specimens (Kokkonen 2015). *Entoloma lactarioides* Noordel. & Liiv is likely conspecific on the basis of the short type sequence and the morphology (Noordeloos 1992; Reschke et al. 2022a). *Entoloma politum* sensu Gulden (Brandrud et al. 2018) is close to or conspecific with *E. pusillulum* Noordel. according to the published sequences (Reschke et al. 2022a). They are probably restricted to arctic and alpine habitats. They are genetically rather close to *E. politum* and *E. nitriolens*, but the published data is too scarce for a morphological comparison.

Additional specimens examined. FINLAND. Varsinais-Suomi. Aura, riverside *Salix* thicket, 18 Oct. 2005, Kokkonen. Etelä-Häme. Nokia, Penttilä, a trickle in *Picea abies* forest, 11 Sept. 2007, L. Hammar, Pitkäniemi, under *Alnus* at lakeside, 14 Sept. 1992, L. Kosonen. Pohjois-Häme. Jyväskylä, Tourula, damp grass-herb forest with *Betula*, *Populus tremula*, *Salix* and *Picea*, 4 Sept. 2014, M. Toivonen & I. Kytövuori 14-310 (as cf. *politum*). Pohjois-Savo. Kuopio, Antikkalanrinne, near *Salix caprea* and *S. phylicifolia* in moist mixed forest, 25 Sept. 2010, Kokkonen 128/10, Nilsiä, Pieni-Tarpinen nature reserve, near *Salix myrsinifolia* and *Alnus* on lake shore, 22 Sept. 2008, Kokkonen 408/08, Puijo nature reserve, Peipposenrinne, under *Alnus* in moist mixed forest, 2 Oct. 2016, Kokkonen 857/16. Pohjois-Karjala. Ilomantsi, Mekrijärvi, near *Alnus incana*, *Prunus padus* and *Betula* by lake shore, 7 Sept. 1985, J. Vauras 1983. Nurmes, Joki-Vastimo, riverside *Salix* thicket, 22 Sept. 2002, Kokkonen 144/02, Metelinmäki, lakeside *Salix* thicket, 23 Sept. 2002, Kokkonen 145/02, Metsä-Vastimo, lakeside *Salix* thicket, 16 Sept. 2009, Kokkonen 289/09, Pahakala, lakeside moist forest with e.g., *Salix* and *A. incana*, 26 Aug. 2002, Kokkonen 142/02, 7 Oct. 2002, Kokkonen 111/02, 149/02, 28 July 2008, Kokkonen 42/08, Saramo, lakeside *Salix* thicket, 29 Sept. 2003, Kokkonen 852/03, Valtimo, Haapakylä, Heinälampi, riverside *Salix* thicket, 12 Aug. 2002, Kokkonen 141/02, 24 Sept. 2002, Kokkonen 146/02, 147/02, Mahalanniemi, lakeside *Salix* thicket, 29 Aug. 2002, Kokkonen 148/02, 15 Sept. 2009, Kokkonen 280/09, 22 Sept. 2009, Kokkonen 340/09, Kalliojärvi, lakeside *Salix* thicket, 13 Sept. 2008, Kokkonen 304/08. Kainuu. Kuhmo, NW of Koirakangas, under *S. phylicifolia* at brookside, 8 Sept. 2018, Kokkonen 19/18, 22/18, brookside with *S. phylicifolia*, *A. incana*, *Pinus sylvestris* and *Betula* 405/18, under *Salix* at brookside, 407/18. Perä-Pohjanmaa. Ylitornio, Roomisaari, riverside *Salix trianda* forest, 7 Sept. 2012, Kokkonen 600/12. Koillismaa. Kuusamo, Oulanka National Park, Nurmisaarenrinne, moist *Salix* thicket, 4 Sept. 2005, Kokkonen & J. Vauras, SW of Siirtola, near *S. phylicifolia* at paludified brookside, 27 Aug. 2020, Kokkonen 195/20. Kittilän Lappi. Kittilä, near the main village, near *S. phylicifolia* in a moist depression in sheep pasture, 28 Aug. 2008, Kokkonen 200/08, Sirkka, riverside *Salix* thicket, 16 Aug. 2006, Kokkonen 173/06. Inarin Lappi. Utsjoki, Pulmankijoki, on path near *S. phylicifolia* by the river, 28 Aug. 2005, Kokkonen. ITALY. Pordenone. Celante – Castelnuovo del Friuli, moist place under *Salix* sp. and *Populus nigra*, 7 Nov. 2020, E. Campo. SLOVENIA. Southeast

Slovenia. Kostel, Slavski Laz, moist forest, near e.g., *Alnus glutinosa* and *Corylus avellana*, 28 Sept. 2015, Kokkonen 64/15. SWEDEN. Uppland. Uppsala, Vårdsätra, moist depression in a mixed forest, near *Salix cinerea* and *S. caprea*, 8 Oct. 2019, Kokkonen 115/19. UNITED KINGDOM. Scotland. Perthshire, Kindrogan Field Centre, unvegetated ground under *Symphoricarpos*, 25 Sept. 1983, S. Huhtinen 83/372, det. M. Noordeloos (as f. *pernitrosum*).

Discussion

In this study, *Entoloma nitriolens* was found to be a distinct species close to *E. politum*. Both are morphologically quite variable, but they differ slightly from each other. Kühner (1977) considered smell to be the major difference between them, which was not confirmed despite the slight differences. *Entoloma pernitrosum* had been described to have a stronger smell than *E. politum* (Orton 1960), too, besides being paler, and it was confirmed to be conspecific. The appearances and the colors of *E. nitriolens* and *E. politum* differed slightly from each other. Additionally, the color of *E. politum* was rarely white, as in some other species of subg. *Entoloma*.

The species were delimited by ITS. The RPB2 sequences of *E. nitriolens* and *E. politum* were identical excluding the ambiguous bases, indicating that RPB2 alone is sometimes unsuitable for a recognition of *Entoloma* species. Since Co-David et al. (2009), RPB2 and ITS have commonly been used in multigene phylogenetic analyses in *Entolomataceae*, but closer comparisons of their suitability for species delimitation or phylogenetic inference have been few. Within *Entoloma*, both ITS and RPB2 were reported to be well suited for sequestrate species (Kinoshita et al. 2012), species of subg. *Claudopus* (He et al. 2019) and species of subg. *Nolanea* (Reschke et al. 2022b). Further, they have been used together for the species delimitation in subg. *Entoloma* (syn. subg. *Rhodopolia*), where ITS was considered to be better than RPB2 (Kokkonen 2015; Kondo et al. 2017).

The habitat requirements and distributions of *E. nitriolens* and *E. politum* differed, which supported the separation of the species. The differences probably result from allopatric speciation. *Entoloma nitriolens* is adapted to a colder climate and a calcareous soil. It occurs from the boreal to the arctic and alpine zones. *Entoloma politum* is more southern and less demanding. At least *E. nitriolens* is a circumpolar species. No sequences of *E. politum* from North America are deposited in the public databases, but it has been reported from the USA states of North Carolina, Oregon and Washington comprising the synonyms *Entoloma maculatum* Hesler and *Entoloma triviale* (Kauffman) Largent (Noordeloos 1988; Largent 1994). Due to the confusion of *E. nitriolens* and *E. politum* with each other or with other species, their exact ranges remain unclear.

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