New data on the rare *Entoloma moserianum*

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Abstract: *Entoloma moserianum* is a very rare species described by the Dutch mycologist M. E. NOORDELOOS in 1983. It has been found in a few European countries like The Netherlands, Germany, Austria, and Italy. A recent finding in northern Italy has allowed us to analyse a lot of basidiomata and to notice some further characters not reported in the original diagnosis regarding the reactivity to several chemical compounds, microscopical and ecological features. Finally, a phenetic analysis of all the 11 species belonging to subg. *Entoloma* sect. *Entoloma,* based on 18 macro-, microscopical and ecological features and the principles of numerical taxonomy, has been carried out. Astonishing overall phenotypic relationships have been disclosed.

Zusammenfassung: *Entoloma moserianum* ist eine sehr seltene Art, die von dem niederländischen Mykologen M. E. NOORDELOOS im Jahr 1983 beschrieben wurde. Sie wurde bisher in einigen europäischen Ländern wie Italien, Österreich, Deutschland und den Niederlanden gefunden. Eine neue und reichliche Aufsammlung aus Norditalien erlaubte die Beobachtung von Merkmalen, die in der originalen Diagnose nicht beschrieben wurden, wie makrochemische Reaktionen, mikroskopische Merkmale und ökologische Angaben. Schließlich wurde eine phenotypische Analyse aller 11 Arten von subg. *Entoloma* sect. *Entoloma,* beruhend auf 18 mikro-, makroskopischen und ökologischen Merkmalen und auf den Prinzipien der numerischen Taxonomie, durchgeführt, die bemerkenswerte phänotypische Übereinstimmungen aufzeigte.

Riassunto: *Entoloma moserianum* è una specie piuttosto rara descritta dal micologo olandese M. E. NOORDELOOS nel 1983. Per quanto a nostra conoscenza è stata ritrovata in alcuni paesi europei quali l'Olanda, la Germania, l'Austria e l'Italia. Una recente e abbondante raccolta effettuata in Nord Italia ci ha permesso di notare delle caratteristiche non segnalate nella diagnosi originale concernenti la reattività a diversi composti chimici, la presenza di taluni elementi microscopici e annotazioni sull'ecologia. Infine viene riportata un'analisi di raggruppamento eseguita su tutte le 11 specie del sottogenere *Entoloma* sezione *Entoloma* basata su 18 caratteri macro-, microscopici ed ecologici, nonché sui principi della tassonomia numerica. Sorprendenti relazioni fenotipiche complessive vengono messe in evidenza.

Mycology has been undergoing an outstanding progress due to the application of molecular biology techniques: We look at these scientific developments with great enthu-

siasm, nevertheless, we think many aspects concerning the morphological taxonomy still need further investigations. Morphological studies are still necessary, especially on rare or uncommon taxa like *E. moserianum*.

Table 1. Data matrix.

	Diameter of pileus (min.)	Diameter of pileus (MAX)	Length of stipe (min.)	Length of stipe (MAX.)	Diameter of stipe (min.)	Diameter of stipe (MAX)	Length of spore (min.)	Length of spore (MAX)	Breadth of the spores (min.)	Breadth of the spores (MAX.)	Pileipellis: cutis	Pileipellis: transition to a trichoderm	Presence of cystidia	Yellowish lamellae in young specimens	Smell	Base of stipe differently coloured	Habitat: grasslands	Habitat: woods
Entoloma prunuloides	20	70	30	80	3	12	6,5	9	6,5	8	1	0	c	0	0	0	1	0
Entoloma sinuatum	30	250	40	150	5	35	8	11	7	9,5	1	0	0	1	0	0	0	1
Entoloma bloxamii	30	80	35	70	10	35	7	9	6,5	8	1	0	0	0	0	0	1	0
Entoloma nitidum	20	50	30	100	2	7	6,5	9	5,5	8	1	0	0	0	0	0	0	1
Entoloma moserianum	20	120	40	120	5	14	9	12	8	9,5	1	0	1	0	0	0	1	1
Entoloma rubellum	25	80	50	90	7	9	6,5	8	5,5	8	1	0	0	0	0	0	0	1
Entoloma viridans	30	40	40	60	6	9	7,5	9,5	6,5	8	1	0	0	0	0	0	0	1
Entooma luteobasis	25	50	60	80	8	14	6	8	6	7	1	0	0	0	0	1	0	1
Entoloma olidum	35	60	35	60	6	10	8,5	11	7	9	1	1	0	0	1	0	0	1
Entoloma myochroum	50	100	80	95	10	18	7	10	6,5	9	1	0	0	0	0	0	0	1
Entoloma alcedicolor	22	25	30	35	3	4	7	8,5	6	8	1	1	0	0	1	0	0	1

Material and methods

Photographs of the basidiomata were taken in the field by an Olympus FE-340 digital camera. Microphotographs were taken by a Nikon Coolpix 5400 digital camera and a Nikon Eclipse E-200 LM. Fragments of fresh material for microscopical analysis were stained with Congo red (Titolchimica, Rovigo, Italy) or mounted directly in water, while dried material was previously hydrated in 5% KOH.

We followed the taxonomic arrangement of NOORDELOOS (1992, 2004, 2009). Abbreviations of the authors of fungal names and acronyms of herbaria follow KIRK (2008) and HOLMGREN & HOLMGREN (1998).

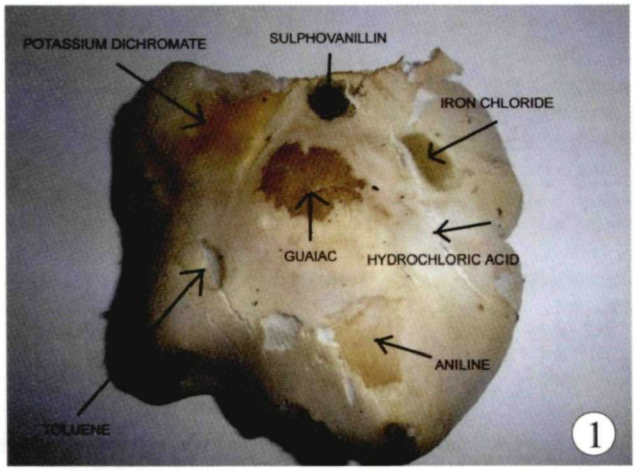
For the phenetic analysis two programs have been executed under Windows Vista: Microsoft Excel 2003 and ClustanGraphic (WISHART 2006).

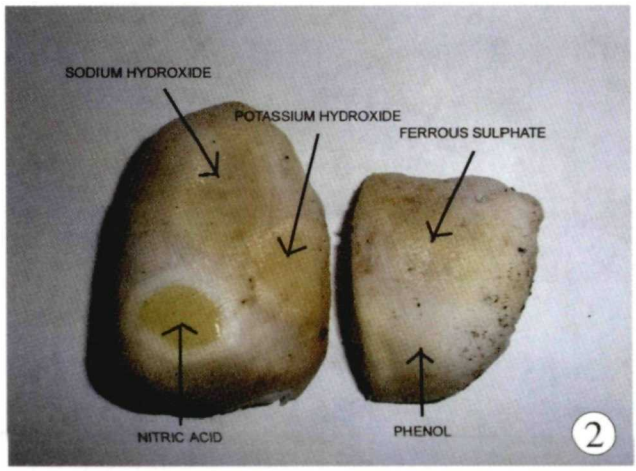
The principles of numerical taxonomy we followed are clearly explained in ALDENDERFER & BLASHFIELD (1984), FIELDING (2007), ROMESBURG (2004), SNEATH & SOKAL (1973), SOKAL & SNEATH (1963). This kind of numerical analysis is composed of five consecutive steps:

– creation of a data matrix (Table 1) containing mixed data types (continuous and binary values), i.e., a table of n rows and m columns in Excel listing the species (rows) and the variables or attributes of those species (columns) obtained from NOORDELOOS' descriptions. We did not take into account the colour of the basidiomata because the software is not able to codify nominal variables.

– standardization of the continuous variables like the size of pileus, stipe, spore.

– calculus of a similarity matrix by Gower's similarity coefficient.





Figs. 1, 2. Macrochemical reactions on pileus surface. – Phot. ELISEO BATTISTIN. – Fig. 3. *Entoloma moserianum* in situ. – Phot. NORBERTO RIGHETTO.

– graphical representation by phenograms of the clusters' linkages got by several agglomerative hierarchical clustering methods like average linkage also known as UPGMA, probably the most frequently used clustering strategy (SNEATH & SOKAL 1973), weighted average linkage method and complete linkage method.
– estimate of the goodness of the results through the calculus of the cophenetic correlation coefficient (r), which assesses how much the phenogram faithfully portraits the similarities in the resemblance matrix, and the determination of the optimal number of clusters by a statistical method, a bootstrap validation test without replacement and 500 random trials.

We tested the following chemical compounds (Titolchimica, Rovigo, Italy) on the pileus surface of fresh basidiomata: potassium hydroxide 30%, sodium hydroxide 30%, nitric acid 65%, hydrochloric acid 10%, ferrous sulphate (powder), α-naphtol 4%, aniline, potassium dichromate 10%, ferrous chloride, toluene 2% and guaiac.

Collection examined: Italy: Veneto, Vicenza, Spagnago di Cornedo Vicentino, terricolous under deciduous trees, 23. 7. 2009, leg. ELISEO BATTISTIN & NORBERTO RIGHETTO (MCVE 24415).

Results and observations

One reagent, i.e., nitric acid produced a clearly positive, lemon-yellow macrochemical reaction after about 15 min from its application on fresh specimens; the other were inert on the whole (Figs. 1, 2).

In case of doubtful classification this simple macrochemical reaction can contribute significantly to get a certain identification of the species.

With regard to its habitat (Fig. 3) we collected it on a humus-rich calcareous soil in a definitely heliophilous broadleaved wood – it occurs in deciduous forest (*Quercus*) on relatively open, sunlit places according to NOORDELOOS (1992) – located at about 200 m s. m. consisting of *Ulmus minor* MILL., *Corylus avellana* L., *Acer campestre* L. and *Robinia pseudacacia* L. Many tufts of *Ruscus aculeatus* L. were all around the mushrooms.

According to our experience (BATTISTIN & RIGHETTO 1998) it appears in April, May, June, July and October, so it makes sense to reckon that it is neither a strictly thermophilous nor cryophilous taxon.

By handling some basidiomata we noticed under lens that the apex of many stipes was minutely pruinose: a microscopical check revealed bundles of hyphae perpendicular or almost so to the longitudinal axis of the stipe. The terminal elements are cylindrical and have an obtuse or subcapitulate apex, and are morphologically almost identical to cheilocystidia (Fig. 4). Their size is 35-64 × 5-9 µm. This observation should be the first report of such a feature.

Finally, we performed an agglomerative hierarchical clustering analysis of the 11 species included in subg. *Entoloma* sect. *Entoloma* according to NOORDELOOS' taxonomy.

It is necessary to state first that cluster analysis is a type of multivariate statistical analysis whose aim is to divide a set of objects (species in this case) into relatively homogeneous subsets based on inter-object similarities (KACHIGAN 1991).

The outcome suggests that this section is phenotypically rather homogeneous: two clustering methods, average linkage (r = 0.87), the reference method, and weighted average linkage (r = 0.83) show that all the species belong to one cluster (Figs. 5, 6), while the third, i.e., complete linkage (r = 0.78) recognizes four clusters (Fig. 7). It is worth noting another element of homogeneity: The phenotypic relationship among species,

i.e., the topology of the phenograms, is identical (Figs. 5-7) in all of the three phenograms. It would be very interesting to acquire molecular data and compare them to the phenotypical ones.

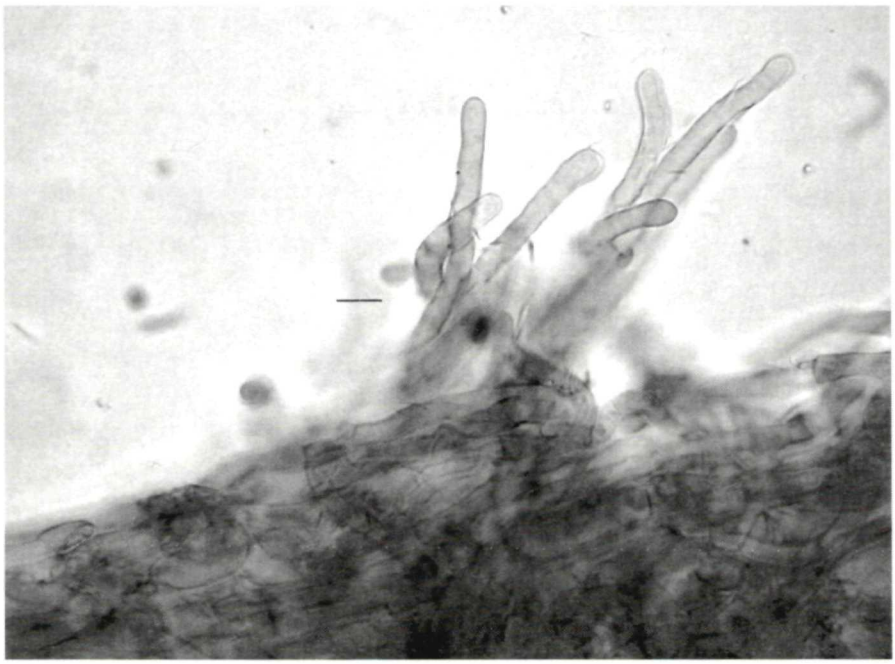


Fig. 4. A cluster of terminal hyphae of the stipitipellis perpendicular to the longitudinal axis of the stipe. – Bar: 9 µm. – Phot. ELISEO BATTISTIN.

In any event, aside from the colour of the basidiomata, unexpected overall phenotypical resemblances among species were disclosed like those between *Entoloma nitidum* QUÉL. and *E. rubellum* (SCOP.) GILLET, *E. prunuloides* (FR.) QUÉL. and *E. bloxamii* (BERK. & BROOME) SACC., *E. olidum* NOORDEL. & T. BORGEN and *E. alcedicolor* ARNOLDS & NOORDEL. or *E. moserianum* NOORDEL. itself and *E. sinuatum* (BULL.) P. KUMM.

With regard to *E. moserianum* and *E. sinuatum* they seem to be a little bit isolated within the phenograms and by comparing them to NOORDELOOS' taxonomy that couple of species could perhaps represent the subsect. *Sinuata* deprived of *Entoloma luteobasis* and *E. olidum.* In our opinion it is a little difference which does not affect the substantial agreement between NOORDELOOS' taxonomic arrangement and the outcome of the numerical taxonomy.

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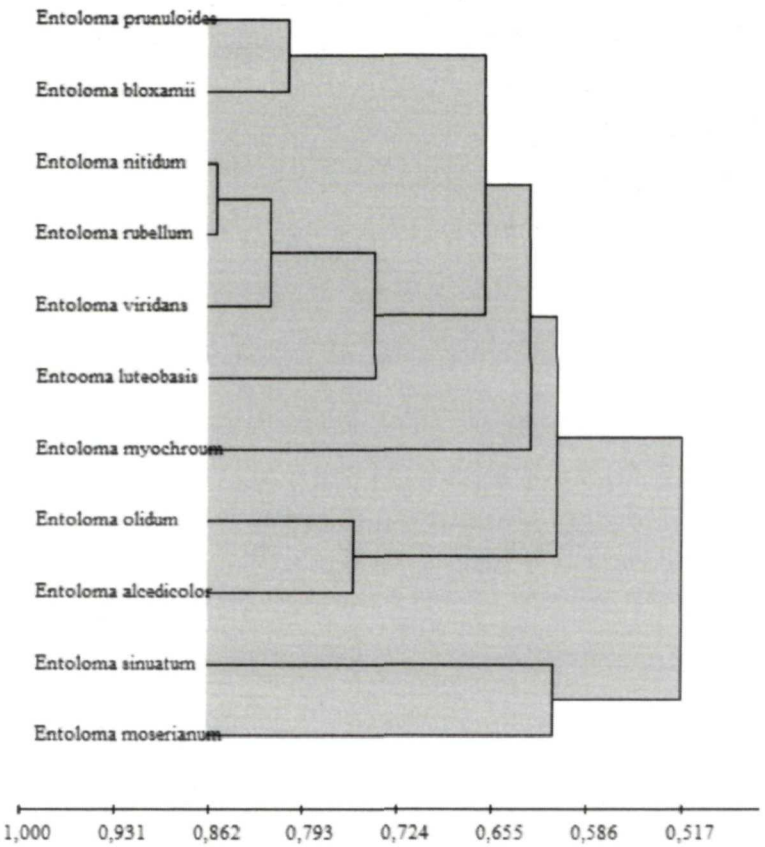
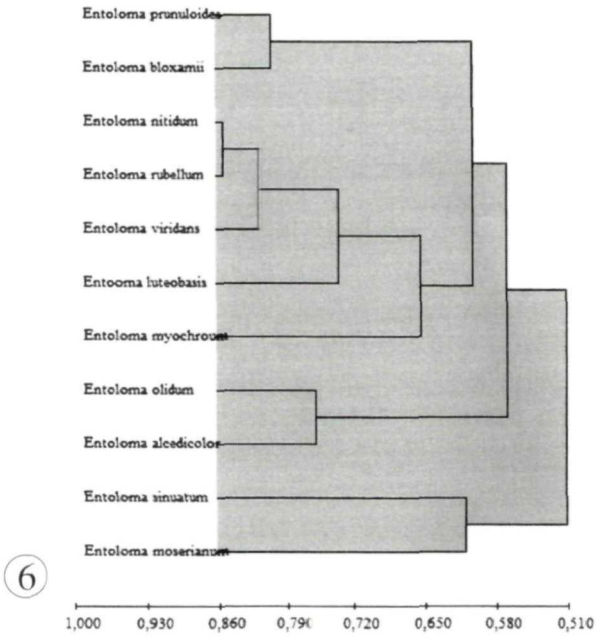


Fig. 5. Average linkage phenogram. Cophenetic correlation coefficient r = 0.87. Clusters: 1 (al the 11 species included in section *Entoloma*).



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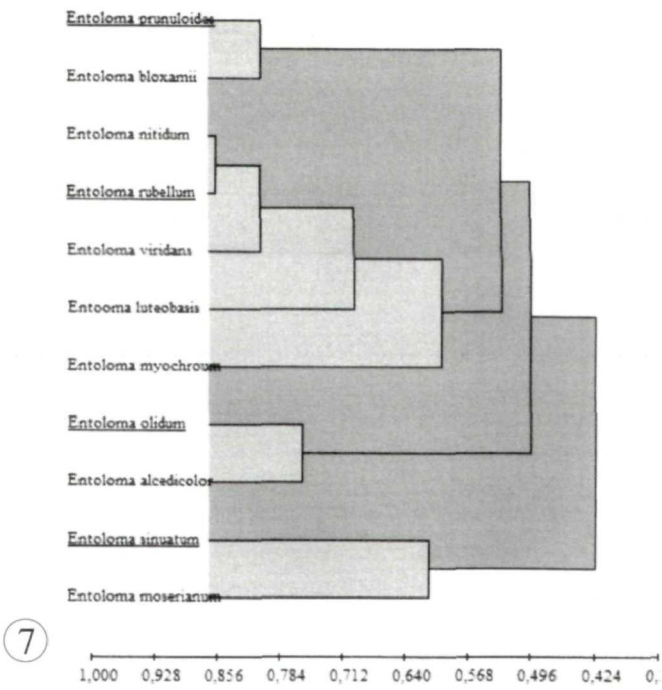

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Fig. 6. Weighted average linkage phenogram. Cophenetic correlation coefficient r = 0.83. Clusters: 1 (all the 11 species included in section *Entoloma*). – Fig. 7. Complete linkage phenogram. Cophenetic correlation coefficient r = 0.78. Clusters: 4 (*E. prunuloides* and *E. bloxamii*), (*E. nitidum, E. rubellum, E. viridans*, *E. luteobasis* and *E. myochroum*), (*E. olidum* and *E. alcedicolor*), (*E. sinuatum* and *E. moserianum)*.