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Machiel E. Noordeloos
Genevieve M. Gates

The Entolomataceae of Tasmania

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Photograph was taken at Kermandie Falls, May 2010.
From *left to right*: David Ratkowsky, Genevieve Gates, Machiel Noordeloos, and Michael Pilkington.
Sitting: Fernanda Karstedt.

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The Entolomataceae of Tasmania

by Machiel E. Noordeloos & Genevieve M. Gates

with photographs by the authors and with additional photographs by Michael Pilkington and with line drawings by Anita Walsmit-Sachs

Abstract

This book is the result of 14 years of collecting Entolomataceae in the native forests of Tasmania, Australia. Although initially involving only the Tasmanian residents Genevieve Gates and David Ratkowsky, who made twice- or thrice-weekly forays into the forests throughout the year, the project was subsequently joined by agaric specialist Machiel Noordeloos from the Netherlands and by fungi photographer Michael Pilkington from the United Kingdom. The international character of the project is further evidenced by the earlier contributions of American mycologist Tim Baroni to the Tasmanian *Rhodocybe* species which form the basis of the chapter on the now-expanded concept of *Clitopilus*, and a visit of several months in 2010 by Brazilian Ph.D. candidate Fernanda Karstedt, who helped to formulate the keys to the *Entoloma* species. Consequently, several thousand well-annotated collections were found during this inventory and form the basis of this monographic treatment of the *Entoloma* and *Clitopilus* of Tasmania. The resulting 90 *Entoloma* species and 10 *Clitopilus* species are well documented with standardized descriptions, line drawings of fruit bodies and diagnostic microscopic characters, and, when available, with colour photographs. Thanks to the intensive search, it was possible to illustrate most species in colour. Dichotomous keys facilitate identification of the species. The species concept used is morphologically based; in several cases, however, identification to species level is supported by molecular data.

The Entolomataceae mycota of Tasmania appears to be fairly unique, as 73 out of 90 species of *Entoloma* and 5 out of 10 *Clitopilus* species are new to science, with the majority of the remaining species shared with New Zealand. Only a few taxa have characteristics that match those of European species, and might have been introduced from Europe.

The large number of observations enabled the authors to use a statistical analysis of the phenological data, resulting in the recognition of five distinct fruiting patterns. Some species appear preferably in winter and spring, others in the summer and autumn months, where groups can be distinguished with a rather wide fruiting

spectrum, encompassing eight months, whereas others have a typical autumnal appearance in the months of April–June.

The introductory part contains chapters focussed on the taxonomy, phylogeny, and biogeography of Entolomataceae in Tasmania, in which the current state of knowledge is discussed. There are chapters dedicated specifically to the study of Entolomataceae, giving instructions how to collect, document, and preserve specimens for identification, and a well-illustrated chapter on characters and character states that are used in Entolomataceae identification and taxonomy. The introductory part concludes with a chapter dedicated to the ecology, distribution, and phenology of the Tasmanian Entolomataceae, based on the very many observations during this study.

Full references to the cited literature are given, as well as an index of species names and synonyms.

Acknowledgments

This book is dedicated Dr. David Ratkowsky for his support, assistance during field trips and editing the final text, and to Johan van Dongen, for his support and patience.

Other persons played an important role in the production of this book. We are especially grateful to Michael Pilkington for his photographs, and also to Anita Walsmit-Sachs, who prepared the line drawings for publication.

We also wish to make special mention of the following individuals:

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Fernanda Karstedt, Instituto de Botânica de São Paulo, Brazil, for assisting with the development of the keys, useful comments and photographs;

Mayo Kajitani of Forestry Tasmania, for the preparation of Figure 1.11;

Kim and Peter Eastman of Forester Road, Scottsdale, for their collection and photographs of *E. albomagnum*;

Sarah Lloyd and Ron Nagorcka for the collection of *E. percrinitum* from their property at Black Sugarloaf, Birralee.

Many *Entoloma* collections were made from frequent visits to the Donnellys Road, Geeveston, property of Drs. Laurie Bishop and Fiona Lewis, whom we thank.

Microscopic photographs were made with a Nikon Coolpix 950, a gift from Dr. Tim Baroni of the State University of New York, Cortland, to G.M. Gates.

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Dr. Brian Spooner of the Herbarium KEW, United Kingdom, kindly assisted M.E. Noordeloos during a visit to the herbarium in November 2010 to study the types of Greta Stevenson.

We thank the following institutions: the Schools of Plant Science and Agricultural Science at the University of Tasmania, Forestry Tasmania, the Cooperative Research Centre for Forestry, CSIRO Sustainable Ecosystems and the Tasmanian Herbarium (HO), for logistic support.

Author Biographies

Dr. Machiel E. Noordeloos was born in The Hague, The Netherlands, in 1949. He grew up in the outskirts of an expanding post-war town, with lots of opportunities to explore the plant life of the polders and nearby coastal dunes. Already as a small child he got interested in nature, particularly botany, and started collecting and making a herbarium. In 1967, he started his biology education at Leiden University and was trained as a mycologist by Dr. C. Bas working on a revision of *Marasmius* in the Netherlands. In 1981, he got his Ph.D. on a dissertation on the taxonomy and geographic distribution of *Entoloma* sensu lato in Europe. From 1987 to 1991 he was head of the Mycology Department of the Plant Protection Service in Wageningen where he studied plant pathogenic fungi and their ecology. From 1991 until his retirement in 2011, he was staff member and group leader of the Department for Plants and Cryptogams of the Netherlands and Europe at the National Herbarium of the Netherlands in Leiden. He is editor-in-chief of the series Flora Agaricina Neerlandica and has published many papers and books on various groups of Agaricales, including Entolomataceae, Marasmiaceae, and Strophariaceae, with a focus on Europe and Australia. In 2009, he was awarded the Clusius Medal of the Hungarian Mycological Society. He is an honorary member of the Dutch Mycological Society.

Dr. Genevieve Gates (née Piscioneri) was born in Pyramid Hill, Victoria, Australia, in 1952. Her father, an irrigation engineer, moved the family from the hot, dry, dusty Mallee of Victoria to the cooler climes of the island of Tasmania in 1959 where she continued her education, culminating in a B.Sc. degree majoring in botany and zoology at the University of Tasmania in 1974. She worked for several years as a laboratory technician at the Department of Agriculture at the University before becoming a full time mother. In 1998, in the middle of raising her three sons, she was drawn back to her botanical studies and became very interested in the taxonomy of Tasmanian fungi, particularly the family Entolomataceae. In 2009, she was awarded a Ph.D. in mycology and forest ecology for her study which investigated the macro-fungal assemblages associated with wood, soil, and litter in the wet eucalypt forests of southern Tasmania. Currently, she is an honorary research associate at UTAS.

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Chapter 1
Introduction

Abstract The introductory part gives a general introduction to the book, illustrating how the book came into being, based on 14 years of intensive collecting on a year-round basis by Genevieve Gates and David Ratkowsky, and how Machiel Noordeloos became involved in the project. The second chapter gives an account of the current state of knowledge of the taxonomy, phylogeny and biogeography of Entolomataceae, a species-rich agaric family with more than 1,500 described species worldwide, and their position in the Tasmanian mycota. In the book, special attention is paid to modern species concepts, and the infrageneric classification is considered in the light of current phylogenetic knowledge. A large chapter is devoted to methods used to study the family, from collecting to describing and conserving, as well as microscopic techniques, to facilitate identification. A fully illustrated guide to the characters used and their character states facilitate the use of the keys and help in the understanding of the descriptions. The last chapter of the introductory part, co-authored by David Ratkowsky, deals with the ecology, distribution and phenology of the Entolomataceae. The forest types in which this family has been studied are described. A map of Tasmania is given, showing the predominant forest types and the sites at which species of Entolomataceae were found. The size of the symbol reflects the number of species of the family found at each site. A dataset of about 4,000 collections forms the basis of an analysis of the fruiting pattern of *Entoloma* species in Tasmania. Statistical methods have been applied in an attempt to reveal differences between the monthly fruiting patterns. As a result, five distinct groups could be distinguished. Another database, containing information from ca. 1,000 forays, enabled a comparison to be made among Entolomataceae, ectomycorrhizal macrofungi, and soil-borne saprobic agarics, using the total number of records for each month as a percentage of the annual total. The results indicate that species of *Entoloma*, of which the majority are supposedly non-ectomycorrhizal, have a different emergence pattern from that of other saprobic agarics. Full references to the cited literature are given.

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General Introduction

This work started in 1998, when Genevieve Gates and David Ratkowsky began an inventory of the mycota in the forests of Tasmania on a regular basis, foraying throughout the year, and collecting as much data as possible about the taxonomy and distribution of macrofungi. This resulted in a number of reports and publications (Gates and Ratkowsky 2003, 2004a, b, 2005; Gates et al. 2005, 2009b; Ratkowsky and Gates 2002a, b, 2005, 2009).

Genevieve soon developed a particular interest in the family Entolomataceae and made throughout the years two and a half thousand well-annotated and documented collections, plus another 2,000 collections of now familiar species as voucher specimens from around Tasmania. When she realized how many of the taxa were unidentifiable, she took the initiative to contact known experts in this field, viz. Timothy J. Baroni, and Machiel E. Noordeloos. Her contact with Baroni resulted in a study of the genus *Rhodocybe* in Tasmania (Baroni and Gates 2006), and with Machiel Noordeloos the current project was undertaken and focused on a monographic treatment of the Entolomataceae of Tasmania. Since 2002, Machiel has jointly collected with Genevieve in Tasmania during visits on an almost yearly basis, each visit lasting several weeks. On some of these visits, they were accompanied by Michael Pilkington, a photographer friend from the United Kingdom who specializes in photographing fungi and who has provided photos for this monograph. This collaboration has led to a series of preliminary accounts of the new taxa encountered (Gates and Noordeloos 2007; Gates et al. 2009a; Noordeloos and Gates 2009).

The current publication is an account of all work spent on the study of the Tasmanian Entolomataceae to date, including also the results of Baroni and Gates (2006). We present keys and full descriptions of 90 *Entoloma* species, some with more than one variety, and 10 taxa belonging to the emended genus *Clitopilus*. We are well aware of the fact, however, that this surely is not the last word on this agaric family in Tasmania. More species will be discovered and described, and further molecular-phylogenetic studies will help also to understand more of the variability of species, and will make it possible, eventually, to make a sounder phylogeny-based classification. See also the remarks on species concept, below.

Taxonomy, Phylogeny and Biogeography of the Entolomataceae

Definition of the Entolomatoid Fungi and the Current State of Knowledge

The euagaric family Entolomataceae Kotl. & Pouzar is very species-rich. It is composed of more than 1,500 species and occurs worldwide from arctic to tropical habitats (Baroni 1981; Gates and Noordeloos 2007; Gates et al. 2009a; Horak 1980,

2008; Largent 1977, 1994; Noordeloos 1988, 1992, 2004; Noordeloos and Gates 2009; Noordeloos and Hausknecht 2007; Romagnesi and Gilles 1979). Recent molecularly based phylogenetic studies have revealed that the family is monophyletic, and sister to the Lyophyllaceae (Matheny et al. 2006; Moncalvo et al. 2002; Co-David et al. 2009).

The Entolomataceae is highly variable in terms of sporocarp morphology (tiny to large pleurotoid, omphalioid, collybioid, mycenoid, and tricholomatoid, as well as sequestrate), and micromorphology (spore shape, pileipellis structures, pigmentation types, presence and shape of cystidia, etc.; see Noordeloos 2004). Lifestyles are equally varied. Most species are saprotrophic on soil, wood or moss, but some are parasitic on other mushrooms (Noordeloos 2004) or plants or are ectomycorrhizal (Antibus et al. 1981; Agerer and Waller 1993; Agerer 1997; Kobayashi and Hatano 2001; Montecchio et al. 2006). The family traditionally is divided into three main agaricoid genera: *Rhodocybe* Maire, *Clitopilus* (Fr. ex Rabenh.) P. Kumm. and *Entoloma* (Fr.) P. Kumm. sensu lato. The latter genus is sometimes split into more genera (e.g. 13 genera, including *Entoloma* sensu stricto, *Nolanea*, *Leptonia*, *Inocephalus*, *Trichopilus*, *Claudopus*, etc.; see Largent 1994). Additionally, three smaller non-agaricoid genera have been distinguished on the basis of habit, namely, the monotypic *Rhodocybella* T.J. Baroni & R.H. Petersen (with a cyphelloid habit), *Rhodogaster* E. Horak (secotioid) and *Richoniella* Costantin & L.M. Dufour (gasteroid).

It is no surprise that Entolomataceae, being such a large and highly variable family, raised questions that analysis of morphological characters alone cannot answer, either due to scarcity of characters and/or difficulty in interpreting the significance of the characters. Co-David et al. (2009) applied molecular phylogenetic methods in an attempt to clarify the inter-generic relationships within the Entolomataceae, as well as the evolution of the characteristic spore morphology within the family.

The entolomatoid fungi form morphologically a rather heterogeneous group of agarics and secotioids that share a unique character in the spore wall. The genus *Entoloma* has spores with an internal skeleton of connected ribs. When viewed under the light microscope they have an angular appearance. In addition, the spores are pink to pinkish brown in mass which, usually, is easily noticed by the pinkish colour on the lamellae. The two other genera, *Clitopilus* and *Rhodocybe*, have a similar structure in the spore wall. In *Clitopilus* this structure is present in the form of longitudinal ribs, which are usually clearly visible under the microscope. When seen from above, the spore appears angular. In *Rhodocybe*, the internal structure consists of isolated warts or bumps, which cause the irregular warted outline of the spores under the microscope. Co-David et al. (2009) found that in the three-gene phylogeny they reconstructed, *Clitopilus* is nested within *Rhodocybe*. This had also been suggested earlier in a paper by Moncalvo et al. (2004). As a result it was decided to merge the two genera into one. For nomenclatural reasons, *Clitopilus*, being the eldest name, has priority for the merged genus. However, a recent study by Baroni and Matheny (2011) suggests that the emended genus *Clitopilus* may be broken up again into *Clitopilus* sensu stricto, the genera *Rhodophana* and *Clitopilopsis*, and an emended concept of *Rhodocybe* sensu stricto.

The study of Co-David et al. (2009) also demonstrated that the gasteroid genera *Richoniella* and *Rhodogaster* should be combined with genus *Entoloma*. Baroni and Matheny (2011) confirmed the polyphyly of gasteroid *Richoniella*, and also transferred the cyphelloid genus *Rhodocybella* to the genus *Entoloma* where it was placed in the basal Entoloma clade close to *E. pluteisimilis* and *E. zuccherellii*. Thus the emended concept of the genus *Entoloma* now encompasses agaricoid, gasteroid, and cyphelloid basidiocarp types.

Current Taxonomy

Species Concept

This monograph essentially uses a morphological species concept, and not a phylogenetic one, because current knowledge of the phylogenetic position of most species does not allow us to use a phylogenetic species concept. We therefore follow Kuyper (in Bas et al. 1988) in defining a species on two supposedly independent characters in which it differs from similar (related) taxa. In the long taxonomic practice of the Leiden agaricologists, this has proved to be a practical and feasible approach.

Similarity in morphology may easily lead to the use of European or North American names, as has been done frequently in the past. However, our increasing knowledge of other groups makes it clear that many agaric species do not have a world-wide distribution, but are often geographically restricted (Geml et al. 2004, 2006, 2008; Nuytinck et al. 2006; Matheny et al. 2009). Ongoing phylogenetic studies in *Entoloma* also show that the Tasmanian species often form a clade of their own, sister to a clade with another geographic origin.

Infrageneric Classification

When the project started, we initially classified the Tasmanian species as best we could in the taxonomic framework of Noordeloos (2004), which essentially goes back to the earlier classifications by Romagnesi (1937, 1974). Increasing insight into the phylogeny of *Entoloma*, however, made it necessary to adjust the current classification considerably, although, since the studies currently are not finished, a final, phylogenetically-based infrageneric classification cannot be presented. The recently published phylogenies of Entolomataceae (Baroni and Matheny 2011; Co-David et al. 2009) led to the following new taxonomic insights:

Subgenus *Entoloma* as conceived by Noordeloos (2004) is polyphyletic. The /prunuloides clade, which contains the type species *E. prunuloides*, is phylogenetically distant from the /rhodopolioid clade, which includes *Entoloma sinuatum* and *E. rhodopolium*. As a result, subgenus *Entoloma* must be emended, excluding the *rhodopolium*-group, which can better be considered a subgenus in its own right,

characterized among other things by the mainly tricholomatoid habit, and possibly also an ectomycorrhizal life mode. The emended subgenus *Entoloma* now not only includes species from the old subgenus, like *E. prunuloides*, *E. bloxamii*, *E. nitidum* and related species, but also section *Turfosa* (= section *Trachyospora*), and surprisingly also species with a differentiated pileipellis (trichoderm, hymeniderm and so-called calliderm), including the type species of the genus *Calliderma* (Romagn.) Largent. Good supporting morphological characters for the emended subgenus *Entoloma* are the often relatively small, weakly angled, relatively thin-walled, isodiametric to subisodiametric spores, presence and abundance of clamp-connections, intracellular pigments, and a usually well-developed bi-layered pileipellis, with inflated elements in the subpellis. Recently, Baroni et al. (2011) created the new genus *Entocybe* for part of the /prunuloides clade, mainly based on the occurrence of pustulate-angular spores, encompassing former section *Turfosa* (= *Trachyospora*) and *Entoloma nitidum*. Whether this generic concept will stand in future phylogenetic analyses remains to be seen.

Subgenus *Leptonia*, traditionally split up in three sections, viz. *Leptonia*, *Cyanula*, and *Griseorubida* (Noordeloos 2004), is polyphyletic. Section *Leptonia* belongs to the /nolanea-claudopus clade, *Cyanula* and *Griseorubida* to the /inocephalus-cyanula clade.

Claudopus, considered by many mycologists as a genus in its own right (e.g. Largent 1974; Horak 1980, 2008), based on the pleurotoid habit, is within the /nolanea-claudopus clade, and pleurotoid species turn out to be mixed with omphalioid and collybioid species in the same clade. This proves that the pleurotoid habit cannot be considered a justification for generic rank. Furthermore, species with a pleurotoid habit are also found in the /inocephalus-cyanula clade.

Pouzarella is strongly supported as a monophyletic clade within the large genus *Entoloma* with a highly specialized morphology (Noordeloos 1979; Baroni and Matheny 2011), and can best be considered at a subgeneric rank.

Trichopilus also falls out as a good monophyletic group within the bigger /inocephalus-cyanula clade, uniting species with capitate cheilocystidia and a fibrillose or subsquamulose pileus.

Calliderma, an assemblage of species with a velvety-cracked pileal surface, which consists of a hymenidermal or pallisade-like layer, is also polyphyletic. The type species *E. callidermum* is in the /prunuloides clade, close to our *E. indigoticoumbrinum.*

Species assigned to *Alboleptonia* appear in different clades in the phylogeny. *Entoloma sericellum* is sister to "*Trichopilus porphyrescens*", but the morphologically very similar *Entoloma albidosimulans* from Tasmania is placed in the /inocephalus-cyanula clade. *Entoloma cephalotrichum* (P.D. Orton) Noordel., however, is in the /nolanea-claudopus clade. This proves that the genus *Alboleptonia* is polyphyletic.

Richoniella pumila, a secotioid species with cuboid spores, from Australia and New Zealand, is within the /inocephalus-cyanula clade, and has therefore been incorporated in the genus *Entoloma*. Since the epithet *pumila* already was given to an *Entoloma* species, it has been renamed *Entoloma gasteromycetoides* Noordel. & Co-David.

Some Tasmanian taxa, such as *Entoloma camarophyllus* in our analyses are in a big unresolved group within the /inocephalus-cyanula clade, and cannot be classified satisfactorily at the moment.

In the synopsis in the taxonomic part of this book the species are arranged according to these new insights. We want to stress, however, that it is still a preliminary classification that surely will be emended in the future due to ongoing morphological and phylogenetic research.

The apparent monophyletic status of some of the clades (e.g. /pouzarella, /inocephalus-cyanula, /entocybe) may tempt one to distinguish them at a generic level. However, the existing phylogenetic reconstructions are far from resolved, and more work has to be done to get a better view of the status of many clades. Therefore, we stick to the broad concept of *Entoloma* s.l. bearing in mind what Romagnesi in Romagnesi and Gilles (1979) wrote on this subject: "one should not be surprised by the difficulty of delimiting sections, let alone subgenera. It happened often that a new collection made our classification questionable, and it is extremely difficult to define natural groups, because all characters seem to mix. Therefore, at this stage, and without any doubt for a long time to come, it is perfectly vain to elevate one of these taxa to the rank of genus". Despite the introduction of molecular characters and phylogenetic methods, this still seems to be true.

Biogeographical Considerations

Although the family Entolomataceae has received considerable attention in the past decades, not many publications deal with the Australian Entolomataceae. Grgurinovic (1997), in her account of the macrofungi of South Australia, devotes a chapter to the Entolomatales, including 2 species of *Rhodocybe* and 10 of *Entoloma*. However, only one of these species, viz. *Entoloma viridomarginatum* (Cleland) E. Horak, has been recorded from Tasmania with certainty. Recently, the North American *Entoloma* expert David L. Largent started an investigation of the Entolomatoid fungi of Queensland and New South Wales, together with a number of Australian mycologists (Largent and Abell-Davis 2011; Largent et al. 2011a, b). While exploring the Tasmanian Entolomataceae, it became clear that many species appeared to be undescribed. Some of them could be identified with the works of Horak (1973, 1980, 2008), but others had to be published as new species (Gates and Noordeloos 2007; Gates et al. 2009a; Noordeloos and Gates 2009, and this book).

The current state of knowledge, despite intensive collecting by the authors in Tasmania, and by E. Horak in New Zealand, is still insufficient to make reliable statements about endemism. Of the more than 100 taxa in the current treatise, at least 15 are also found in New Zealand, including several species that Horak (2008) cited as endemic to New Zealand. With further work more species in common can be expected. Some of our species may well occur on mainland Australia in similar

habitats (Largent and Abell-Davis 2011; Largent et al. 2011a, b), and in addition we have unpublished information that this is the case with some of our species. Eventually, detailed biogeographical studies in a larger area, including the *Nothofagus* zone of South America (i.e. Patagonia), will make it possible to decide whether or not certain species are endemic.

Materials and Methods

The species descriptions in this work are all based on extensive collecting by the authors. Colours are matched with Methuen's Handbook of Colour (Kornerup and Wanscher 1978) (e.g. 6A2 or 19E5–19F5), and Munsell Soil Colour Charts (Munsell 1975) (e.g. 2.5Y 8 or 10YR 7/6). Photos were taken in the field by Genevieve Gates with a Nikon Coolpix 5000 and by Machiel Noordeloos with a Nikon D90. Michael Pilkington used a Canon 5D Mark II, in conjunction with CombineZ stacking software. Microscopic observations were made with Olympus and Leica microscopes, using standard techniques. Fresh material was observed in water to determine the true colour and characteristics of the pigmentation. Fresh and dried material were mounted in 5 % KOH or 10 % ammonia or a Congo Red solution to observe, draw and measure microscopic details (for details see section "Microscopic Techniques").

How to Study the Entolomataceae

Although the study of the Entolomataceae requires the same techniques as for other genera of Agaricales, some experience and skill are needed with the microscope, since microscopic characters are essential for identification in many cases. A good microscope equipped with an oil-immersion objective is therefore necessary.

How to Collect

Good material facilitates identification. The following recommendations are made:

- Collect fresh specimens only, preferably both young and mature fruit bodies. Be sure to collect the entire mushroom, including the stipe base and any adherent rhizomorphs.
- Be careful not to mix up different species. Different *Entoloma* species can grow together on quite a small spot. Make a note of the habitat: type of vegetation, dominant trees, shrubs, herbs and mosses. Characterise, if possible, the soil type.

- Be aware of colour changes during transport and storage. In many taxa the colour can change rapidly, not only because of the sometimes hygrophanous nature of the pileus, but also subtle colours (especially blue, violaceous, pink etc.) may change or disappear. The best way is to make notes in the field, compare the fresh material with a colour code, and take a colour photograph of the fruit bodies (including their undersides) in the field.
- Note the smell and taste in the field and compare it with the smell and taste after transport in a small container and after dissecting the fruit body. Temperature may affect these characters. Also, odour changes with the age of the fruit body.
- Transport the fruit bodies of each collection separately in small boxes, carefully packed with some moist mosses or other soft material, so that they arrive home relatively hydrated and undamaged. Do not collect small species in a basket.

What to Do at Home

If you want to name the species at a later stage after they have been dried, or if you want to save your material to deposit in your herbarium, make a full description of all macroscopic characters that may disappear with drying. Make a sketch of the fruit body using colour pencils. This is very useful even if you took a photograph. No matter how good your colour photo may be, diagnostic characters often cannot be verified with certainty from it. Making a standard description for all your collections makes critical comparison easier and facilitates identification.

How to Make a Macroscopic Description

There are several ways of doing this, but it is important to do it always in the same way. Some people use pre-printed forms, other people write the description down for each collection. One of the advantages of a pre-printed form is that you do not easily forget to note a character. When making a description without a form, always use the same order of characters. This also helps you to remember the relevant and important morphological features. The authors generally use the following order in their descriptions in this book:

Pileus [size in mm, shape, margin, centre, hygrophanity, translucency, colour when moist, colour when dry, surface structure]. Lamellae [insertion (attachment) on stipe, shape, spacing, width and colour of sides, shape and colour of edge, other characteristics, e.g. L = number of entire lamellae, l = number of short lamellae (lamellulae), veins on sides (transvenose) or veins between lamellae (intervenose)]. Stipe [dimensions in mm, length x width, shape: cylindrical, compressed etc., shape of base e.g. swollen, tapering etc., colour and surface structure e.g. glabrous, fibrillose-striate, squamulose etc., colour of basal tomentum]. Fruit body context [colour, consistency, stuffed, fistulose]. Odour, taste and spore print colour.

Drying and Conservation of the Material

To make sure that your freshly collected specimens can be studied in a dried state, it is important to dry them as quickly as possible after collecting and describing. Use a drying method with good ventilation and a moderate temperature, preferably not above and not too much below 40 °C. Store the material after drying in envelopes with a label. On this label write with permanent ink or type the following data: Species name, collector, locality, date, and collecting number. In case you keep your notes and descriptions separately, give all items the same collecting number. Store your herbarium in a cool, dry place. Beware of insects. Freezing for 3 days at –25 °C has been proved to be a good conservation method.

Microscopic Techniques

For the study of the various microscopic characters the following methods are recommended:

Spores, Hymenial Structures (Basidia, Cystidia) and Clamp-Connections

Stain with Congo Red (1 % Congo Red in concentrated ammonia):

- Fresh material: put a fragment of the lamella including the edge in Congo Red, let it stain for a few minutes then observe.
- Dried material: similarly put a fragment of the lamella in Congo Red, let it stain for a few minutes. It may be necessary, particularly with old or badly dried material, to warm it a bit with a small flame. Remove the excess stain with filter paper, and replace it with a 3–10 % KOH solution. Observe in this medium.

For both fresh and dried material, be careful not to press the cover slip too much, as you want to leave the fragment intact. Observe first with low magnification to locate the structures you want to make visible. If you cannot see them properly, push gently on the cover slip until the hymenial elements lie free in the medium. The KOH makes this process easier.

Pileipellis Structure and Pigmentation

- Fresh material: make a radial section of the pileipellis, and observe in water to see the true colour of the pigments, and also make another mount in a saturated sugar or salt solution to make the intracellular pigment more visible.
- Dried material: make a radial section of the pileipellis, and observe in 10 % ammonia. Do not use KOH, except on very old material that does not respond well to the ammonia treatment.

Be careful not to press the cover slip too much, so as not to change the orientation of the hyphae. When you have determined the structure of the pileipellis, you may press a bit to have a close look at the size and shape of the hyphal elements, pigmentation, and for the presence of clamps.

The Characters Used for the Delimitation of Taxa in *Entoloma*

Macroscopic Characters

Habit types are illustrated in Figs. 1.1 and 1.2.

Habit

Habit types are often used to characterise higher taxa (subgenera and sections), and are frequently used in the keys in this monograph. Five main types are distinguished (see Figs. 1.1 and 1.2).

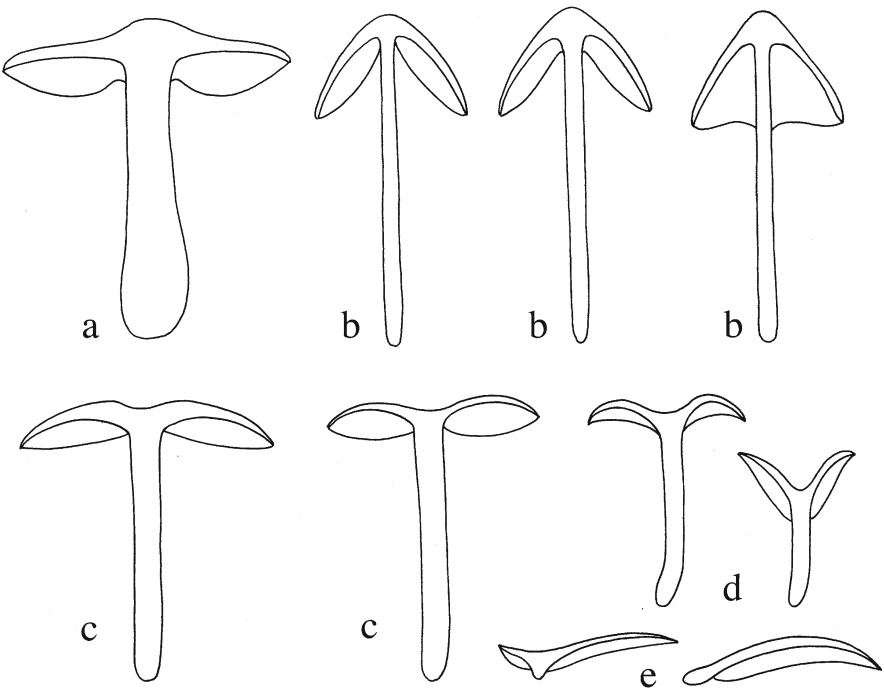

Fig. 1.1 Habit types

Fig. 1.2 Habit types

a – tricholomatoid: resembling a *Tricholoma* with convex, umbonate pileus, free to adnate-emarginate lamellae, a relatively thick stipe, and thick context.
b – mycenoid: resembling a *Mycena* with conical or campanulate pileus, free or adnate lamellae and a relatively long and slender stipe and thin context.
c – collybioid: resembling a *Collybia* with convex or plano-convex pileus with blunt or slightly depressed centre; lamellae adnate-emarginate or almost free.
d – omphalioid: resembling an *Omphalia* (or *Clitocybe*) with depressed to infundibuliform pileus and decurrent lamellae.
e – pleurotoid: resembling a (small) *Pleurotus* or *Crepidotus* with a lateral stipe, often reduced or absent.

Colour

Colour is one of the important diagnostics in describing fruit bodies. It is essential to know how the colour changes from young to mature to overmature, and from moist to dry. Many species are hygrophanous, i.e. the pileus changes colour from moist to dry. Usually this goes in a pattern of radial streaks starting from the centre of the pileus towards the margin. Many other species are not hygrophanous in this way, but become more or less equally paler on drying. They are called weakly hygrophanous. Colour changes may also occur when the specimen is dried for conservation. This is different to being hygrophanous. For changes involved with age, young, mature and overmature fruit bodies must be compared. In particular, the species of subgenus *Leptonia* show remarkable differences. In this subgenus one must

be careful to look for blue tinges in the pileus and lamellae of young specimens. The colour of the stipe may change quickly, and, for example, lose the blue or violaceous tinge even immediately after being removed from the substrate. Many *Leptonia* species have a grey-blue or violaceous stipe when young, fading to grey or even grey-brown with age. Good collecting and colour notation in the field may be of considerable help.

Surface Structures

The surfaces of the pileus and stipe must be studied and noted for the use of the keys. The following types are found (see Fig. 1.3):

a. glabrous – bald.
b. fibrillose – with fibrils that usually are arranged radially. They can be innate, as is seen in most glabrous species, or superficial, where the fibrils are lying on the surface, often giving a silvery impression. In descriptions of the stipe you may find the description 'stipe silvery striate', which means that the surface is covered with silvery, shining, superficial fibrils.
c. velutinous (velvety, felted, tomentose) – covered with fine short hairs.
d. squamulose – covered with minute, appressed or somewhat erect scales.
e. micaceous – covered with glistening particles.
f. radially veined – covered with low vein-like elevations (as in *Pluteus* species).
g. squamules – scales.
h. squarrose – with relatively coarse, erect scales.

In addition (not depicted):
pruinose – dusted with a fine bloom.
rugulose – minutely wrinkled, rough.

Lamella Edge and Face

Quite a few species have a lamella edge that has a colour different from the faces, usually brown or blue. In addition, many taxa have an irregular lamella edge, varying from finely fimbriate to coarsely toothed. Staining or mottling may also occur on the edges and faces of the lamellae. Intervenose ribbing may also occur on the lamella faces.

Stipe Surface

The structure of the stipe surface is often characteristic and of diagnostic value, particularly in *Nolanea* and *Cyanula*. The following main types are distinguished (see Fig. 1.4):

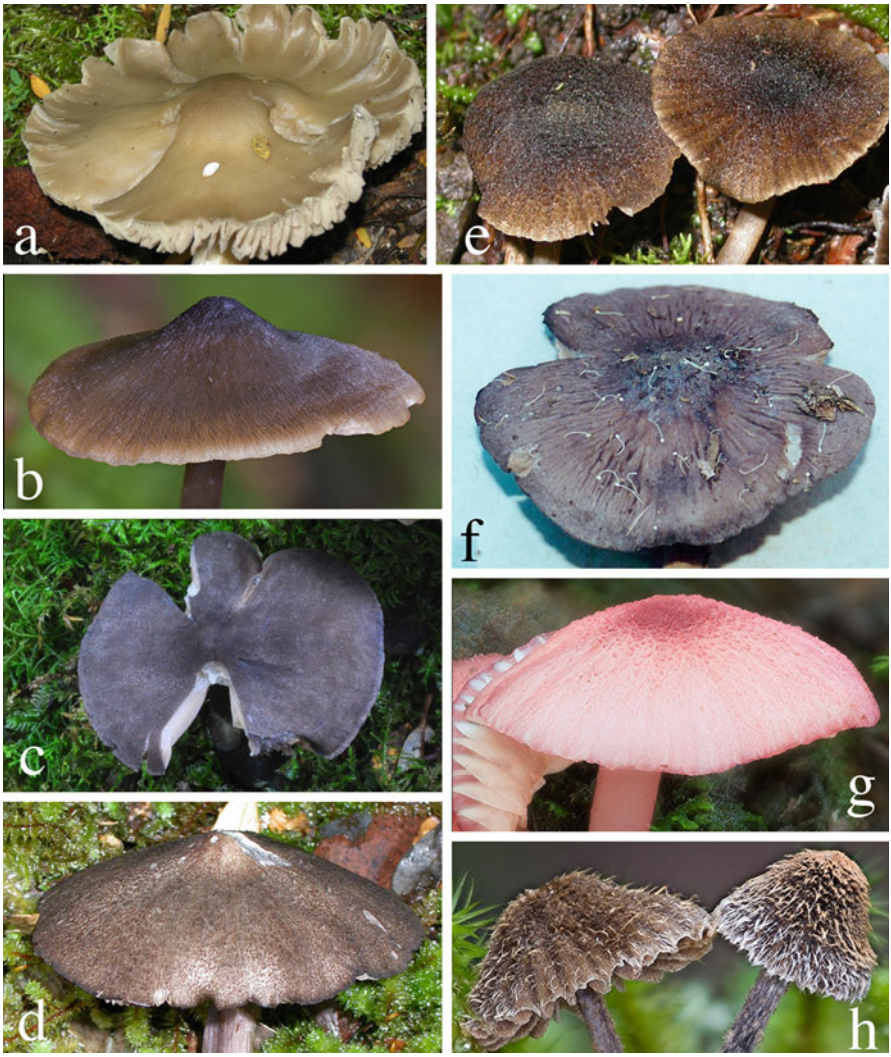

Fig. 1.3 Surface structures of pileus. (**a**) Glabrous. (**b**) Fibrillose. (**c**) Velutinous. (**d**) Minutely squamulose. (**e**) Micaceous. (**f**) Radially veined. (**g**) With raised squamules in central part. (**h**) Squarrose

Microscopic Characters

Spores

The spores of all *Entoloma* species are angular, as seen from all views under the microscope (see Figs. 1.5a, b, c, d, e, f, g). It is very easy to make a preparation to study the spores. A small part of the lamella, gently pressed under the cover slip and

Fig. 1.4 Stipe surface. (**a**) Glabrous and polished. (**b–d**) Longitudinally fibrillose-striate (covered with paler, glistening fibrils, sometimes twisted). (**e**) Pruinose. (**f–g**) Squamulose. (**h**) Hairy-squarrose with erect hairs

observed in water (with fresh material) usually will reveal a large number of spores. This is in contrast to genera such as *Marasmius* and *Collybia*, where spores can be very sparse. Since the spores of most *Entoloma* species are relatively thick-walled and slightly straw-coloured using light microscopy, they can be fairly easily observed. Because of the relatively thick wall, it is also easy to study dried material. Soak a very small piece of lamellae (0.5×0.5 mm) in a 10 % ammonia solution for approximately 10 min and observe under the microscope. Normally you will find as many spores as you want, or even more!

Spore Size and Shape

For measurements only choose spores in your preparation that lie in profile (i.e. side) view. Measure at least 10 spores to see the variation in length and width. It is useful to calculate the length/width ratio of individual spores (Q), and its average, as this

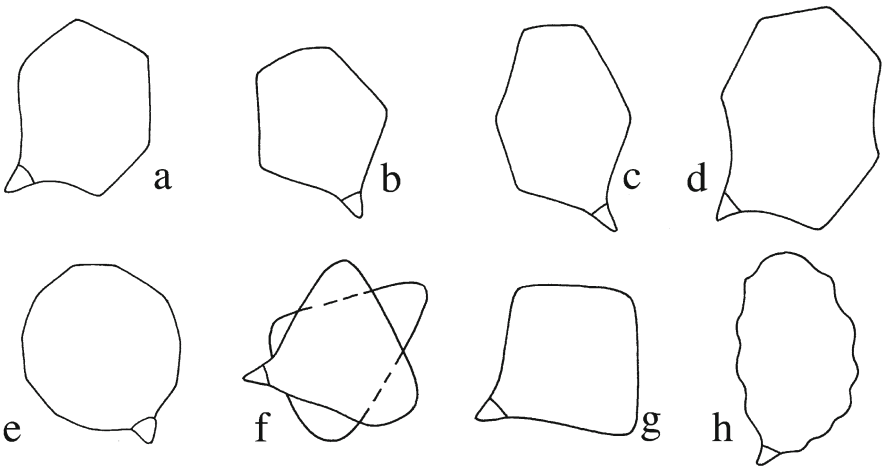

Fig. 1.5 Spore shape. (**a–g**) Examples of *Entoloma* spore shapes

is often used in the keys. Do not include the apiculus, which is the triangular-shaped point of attachment of the spore to the sterigma of the basidium.

The overall shape of the spores, in combination with the number of angles in side view, and the thickness of the spore wall are important diagnostic characters. When the Q value is 1.0–1.1, the overall shape is called **isodiametric** (Figs 1.5b, e), and when the Q value is larger, **heterodiametric** (Figs 1.5a, c, d)**.** A special shape is the so-called **cuboid** spore (Fig. 1.5g), which has a rectangular shape in side view, and in three-dimensional views resembles a cube. **Cruciform** spores (Fig. 1.5f) have a very complex structure, more or less derived from the cuboid type, where the upper part of the spore is twisted along the side axis. Some species have spores with many angles which are blunt and these are called **nodulose** spores (Fig. 1.5h). The number of angles is counted in side view, i.e. in the same position as when the size is measured. The apiculus is included as one of the angles. Furthermore, it is important to note whether the angles are sharp (pronounced) or weak, and the relationship to the wall thickness.

Clamp-Connections

Basidiomycetes typically possess clamp-connections, which are often formed, and are visible, on the septae of the hyphae, and also at the base of the hymenial elements, such as the basidia and cystidia. In *Entoloma* they are usually easy to find and are fairly large. It is far more difficult, in some cases, to establish the absence of clamps. Many species in subgenus *Entoloma* have abundant clamps, almost on every septum in the hymenium, but also in the trama and cutis layers. However, in subgenus *Nolanea*, for example, clamp-connections are often only present in the hymenium, and rare or absent in the pileipellis and subpellis. Many species in subgenus *Cyanula* do not have clamp-connections at all.

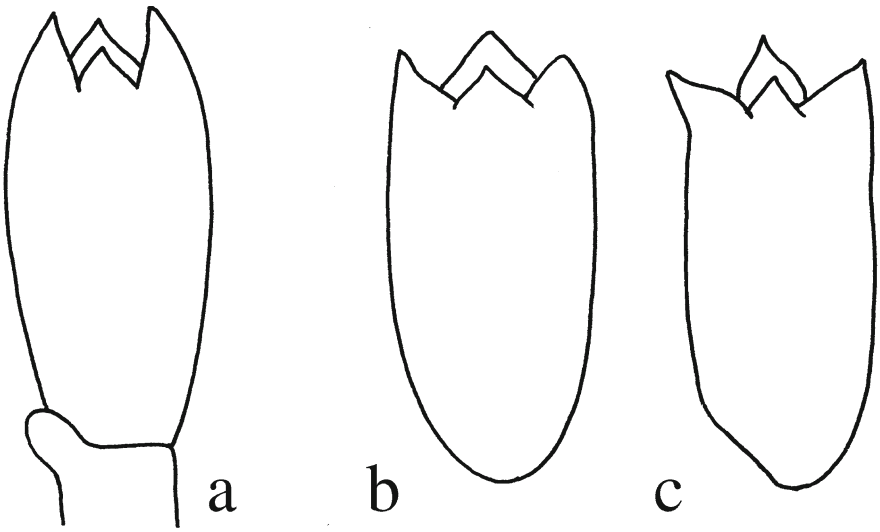

Fig. 1.6 Basidial shape

In our keys the statement 'clamps present' always means: clamp-connections present in the hymenium, at the base of basidia. Therefore, for this character it is sufficient to look for clamp-connections in a preparation of a small piece of the lamella. To facilitate the finding of clamp-connections, it is recommended to stain the hyphal wall with Congo Red as described above. Having done so, there are two possibilities (see Fig. 1.6):

1. Clamp-connection can clearly be observed at the base of the basidium (a).
2. There are no clear clamp-connections at the base of the basidium (b, c). In this case there are two possibilities: there are no clamp-connections or they are not visible as complete clamps. In many species of *Entoloma* mature basidia have lost the basal clamp because it has become part of a new basidium or has degenerated. Therefore, it is important to look for young basidia to see the clamp-connections. The base of a mature basidium is also important. Clampless species have basidia with an equally rounded base (b), whereas basidia that did have a basal clamp that degenerated usually show a slight flattening at the base (c).

Basidia

In *Entoloma* most species have 4-spored basidia, with only a few species having exclusively 2-spored basidia. An important diagnostic feature is the presence or absence of a clamp at the base (see Fig. 1.6a). The size of the basidia usually ranges from 20–40 × 8–12 µm. Members of section *Dysthales* have basidia that range from 40–60 × 10–20 µm. Basidia with brown, granulose, intracellular pigment may occur