Holger Thüs / Matthias Schultz

Fungi · 1. Teil / 1st Part: Lichens

Süßwasserflora von Mitteleuropa

Freshwater Flora of Central Europe

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Contents

Introduction

Amphibious lichens are widely distributed but little recognized components of freshwater vegetation. In most assessments of freshwater organisms however, lichens are still neglected which is partly due to the fact that corresponding literature is widely scattered and a profound treatment of these lichens in Central Europe is still missing. This book is intended to provide a comprehensive tool for the determination of freshwater lichens in Central Europe for both, the non-specialized limnologist and the interested lichenologist. It further summarizes basic information on ecology and distribution. The book roughly covers an area ranging from the Alps to the south shores of the North and Baltic Sea and from the Western Carpathians in the east to the Vosgues-Mountains in the west. Amphibious lichens are found in springs, streams and rivers wherever hard and stable substrata as well as sufficient light are granted. In certain streams of cooler upland areas, lichens can represent the most diverse group of organisms covering virtually any rock surface (e.g. Frey 1922). On the other hand, they are very rarely found in lakes. Rock surfaces incrusted by silt, surface erosion by sediment particles or water with a pH below 5 can exclude any growth of lichens. Resulting from that, freshwater lichens are valuable long-term indicators of the specific structure of a given watercourse, as well as they indicate key factors of water chemistry (Nascimbene et al. 2007). Water pollution was – and certainly still is – the main reason for the dramatic decline of lichen growth especially along larger watercourses during the past decades. However, increasing water quality and reduced acidic impacts have enabled some species to recover even along the banks of large Central European rivers as for example the Rhine (Thüs 2006). Consequently, the potential use of lichens for bioindication purposes is regarded very high (Aptroot & Seaward 2003, Nascimbene et al. 2007, Thüs 2002). Unfortunately, the knowledge on ecology and indicator values of many freshwater lichens and basic floristic information that would allow to evaluate whether a certain species is endangered or not are still sketchy. Therefore, corresponding notes have been made only in cases where sufficient data were available. In spite of this, we hope that the present book will stimulate the recognition of freshwater lichens and promote the use of these organisms in matters of nature conservation.

Lichens do not form a natural group of organisms (Liu & Hall 2004, Lutzoni et al. 2001) but represent an exceedingly delightful lifestyle in higher fungi, characterized by the symbiotic association with one or more photosynthetic partner(s) which supply the lichenized fungus with organic metabolites (Honegger1998). More than 2000 species of lichen forming fungi occur in Central Europe, but only a much smaller number is found in and along watercourses. The lichen symbiosis is a nutrition strategy with an excellent performance under conditions characterized by frequent changes in water availability, the total lack of liquid water or extended periods of desiccation (Lange 2003a, b, Lange & Green 1997, Ried 1960a). On the other hand, ecosystems with permanent supply of liquid water are rather poor in lichen species (Ried 1960b). In Central Europe, roughly a dozen taxa of lichens are able to survive under permanent submersion. In the splash water zone of watercourses and in temporarily inundated micro-sites the species number is much higher, reaching more than 100 taxa in upland areas.

In the field, it is often difficult to assess with a single visit whether a stream or small river is permanent or ephemeral, and in large rivers the water level can change several meters. Due to the specific ecological needs of individual lichen species, the water regime of such water bodies can be assessed by analysing the composition of the lichen communities at a given site. The inundation-dependent zonation of the lichen vegetation can provide important information for the prediction of regularly occuring high water levels as well as for the assessment of extreme floodwaters in the past (Beschel 1954, Hale 1984, Marsh & Timoney 2005, Rosentreter 1984, Timoney & Marsh 2004, 2005). Furthermore, the analysis of amphibious lichen vegetation provides valuable information on physico-chemical properties of both, the water (Pentecost 1977, Nascimbene et al. 2007) and the surrounding air bound influences (Thüs 2002). The inclusion of lichens in the assessment of benthic communities is thus highly recommended for applied issues in nature conservation and ecology – but has received little interest among limnologists so far. The common practice of neglecting amphibious lichens in ecological studies is mainly due to the lack of a comprehensive floristic treatment of these organisms and the fact that amphibious lichen communities are often composed by representatives of taxonomically highly problematic genera.

This book is intended as a tool for the identification of all lichenized fungi in Central Europe which can be called freshwater lichens, either because they are adapted to freshwater habitats or because they are frequently observed in or at streams and rivers due to their exceptionally wide ecological amplitude. While the first group is reasonably defined, the second group is difficult to delimit and may also be described as facultative amphibious species. Many lichens can be found occasionally at the banks of watercourses at temporarily inundated microsites. Especially on carbonaceous rocks it may prove impossible to distinguish between hydrophilic communities (amphibious, regularly submersed or strongly influenced by splash water) on the one hand and hygrophilous communities (preferring humid sites, with either high air humidity or frequent access to liquid water) on the other. Local characteristics can strongly affect the composition of hygrophilous lichen communities, especially in the less frequently inundated zones. The composition of the freshwater lichen vegetation at a certain site furthermore largely depends on macroclimatic factors. Some species that are frequently found inundated in Northern or Western Europe, expose a less pronounced hydrophytic performance in Central Europe (compare species lists and lichen communities described by Gilbert 1996, Gilbert & Giavarini 1997 for the British Isles; Santesson 1939, Nordin 2002a for Sweden; Beschel 1954, Frey 1921, Keller 2000, Motyka 1926, Ried 1960b, Scheidegger & Keller 1994 and Thüs 2002 for Central Europe). In the Mediterranean region, high summer temperatures and periodic drought periods result in a specific composition of the lichen flora in streams especially at low altitudes. Consequently, the lichen vegetation along watercourses in the Mediterranean differs considerably from that in Central Europe (Pereira 1992). In Central Europe, freshwater lichens are best developed in rapidly flowing water whereas in stagnant water such as along rocky lake shores the species number remains low (e.g. Erichsen 1957). In Nordic countries and the British Isles instead, lake shores are important habitats for freshwater lichens (Gilbert & Giavarini 2000, Santesson 1939).

The present book comprises all specialized aquatic lichens occurring in Central Europe. However, it proved impossible to key out every species that potentially occurs among the facultative "guests" in freshwater ecosystems. Therefore, only the most frequent ones are included in the keys. If the user finds more facultative freshwater lichens than more or less specialized amphibious species, it can be concluded that the watercourse under study is either non-permanent or the collecting site is not much influenced by splash water or periodic inundation. The so-called "riparian" lichens are typically found in the vicinity of large water bodies, but outside the direct influence of inundation or water spray. These lichens benefit from more frequent fog events as well as from increased dew fall around large water bodies, but they are not specifically adapted to an amphibious life style, and therefore the present book offers only limited information on these lichens. For the identification of riparian as well as for facultatively amphibious lichens the user is refered to comprehensive determination books such as Wirth (1995), Clauzade & Roux (1985) or Purvis et al. (1992).

Ecology of Freshwater Lichens

Inundation tolerance versus inundation demand

Most freshwater lichens are inundation tolerant to a certain degree, but only a small number is really inundation demanding and appears to be highly sensitive to complete desiccation (Ried 1960a). Field observations, however, confirm that even lichens typically found in permanently inundated communities (*Verrucaria aquatilis*, *V. funckii*, *Hydropunctaria rheitrophila*) are able to survive limited periods of desiccation up to several weeks (!) at shaded micro-sites with high air humidity. Obviously, the circumstances under which desiccation occurs decide about the survival of the lichens from usually permanently submersed sites.

Aptroot & Seaward (2003) followed Santesson (1939) in his suggestion that possibly no freshwater lichen is completely restricted to submersed micro-sites and added the aspect that every freshwater lichen relies on a limited desiccation tolerance at least for the dispersal of the ascospores via the air. They concluded that the term "aquatic" should be avoided and instead proposed the term "amphibious". Our own observations of populations of *Verrucaria aquatilis*, *V. funckii* and *Hydropunctaria* (*Verrucaria*) *rheitrophila* from Central Europe over almost ten years however, leave no doubt that at least for these species the complete life cycle from the establishment of young thalli to the production of perithecia and ascospores takes place at micro-sites that remain inundated even during extended heat and drought periods. Similar observations were made by Hawksworth (2000) for *Collema dichotomum* and *Hydropunctaria* (*Verrucaria*) *rheitrophila*. Therefore, we see no reason to avoid the term "aquatic" for these organisms, even if they have the principal ability to survive short periods of time without liquid water irrespective of their ability to disperse ascospores via the air. In this book we use the term "**aquatic**" synonymously to "**growing submersed**" for lichens able to grow for more than a year under permanent submersion in the whole distribution area. In contrast, the term "**amphibious**" is used for species that are best developed in the splash water zone or at micro-sites that are regularly subject to desiccation for at least some weeks during the year and for species that occur permanently submersed only in rare cases and under special conditions (usually only in parts of their distribution range, e.g. in cooler upland streams or Nordic regions). A third group of lichens does not have any physiological adaptation to inundation, but benefits from higher air humidity in the vicinity of certain types of waterbodies. These species are referred to as "**riparian**" lichens. Widespread and common species in Central Europe such as *Lecanora muralis* fall under this category, but also rare and highly specialized taxa adapted to shaded situations with high air humidity such as *Lecidea ahlesii*. We have included a selection of species from the riparian flora in the keys as far as there is evidence for occasional records from temporarily inundated sites. A few of the species which are rare and often neglected are presented in more detail in order to stimulate their consideration in future studies. In general, a high frequency of riparian lichens at a certain site indicates that they are not regularly inundated and regional floristic surveys or determination books should be consulted. Finally, "**terrestrial**" species are those with a generally low submergence tolerance and which are usually not found in the direct vicinity of water bodies. This group includes all lichens growing in any kind of non-aquatic ecosystem (including rocks, living or dead plants or soil) and it should not mixed up with the term "**terricolous**" which refers to lichens growing on soil.

Substratum stability

Unlike many free living algae and mosses, freshwater lichens are rather slow growing organisms although large differences exist in growth rates among aquatic lichen species (Keller 2005). While some freshwater lichens can even be considered pioneer species (Krzewicka & Galas 2006), others specifically occur on immobile substrata that offer a hard and stable surface (Thüs 2002). The knowledge on the individual preferences of particular species regarding the substrate stability is very sketchy and only in cases where sufficient observations exist a corresponding note was added in the species description part of this book.

Silting tolerance

Most freshwater lichens are very sensitive against deposition of silt-like sediments of either organic or inorganic origin on the thallus surface (e.g. Erichsen 1957, Gilbert 1996, Gilbert & Giavarini 1997, Thüs 2002, 2006). Possibly, the establishment of ascospores is hindered by silted substrate surfaces, but no study addressed this aspect so far. A small number of species however, appears to be tolerant to moderate silting (e.g.: *Bacidina inundata*, *Placynthium tantaleum*, *Pyrenocarpon flotowianum*, *Leptogium subtorulosum*, *Verrucaria praetermissa*). In general, species with thin subgelatinous thalli (consistency like "solid jelly", becoming ± transparent when wet) are most sensitive to silting while thick areolate species are often less sensitive (Thüs 2002). Silting is difficult to quantify and general notes on the silt tolerance of a species were made only where sufficient field observations exist.

Nutrient supply

Experimental studies on nutrient demands and tolerance limits to high nutrient supply are very scant (Davis et al. 2000) and therefore, generalisations are premature. Field observations suggest a higher species diversity in oligotrophic environments. In eutrophic watercourses most lichen species are easily out-competed by cyanobacteria, algae and mosses. Only a few taxa such as *Verrucaria praetermissa* are able to survive even in polluted streams when the current is rapid and silting does not exceed a critical level. Possibly, lichen growth is often not limited simply by eutrophication (nutrient content) but by the silting effect of detritus which is related to high degree of saproby and directly affects the lichen vegetation. Detailed studies on direct or indirect effects of eutrophication versus saproby related silting of organic matter are still missing.

Light demands

The highest species diversity is usually found at well lit streams and rivers, but shade demanding species do exist (Pentecost 1977, Gilbert 1996, Gilbert & Giavarini 1997, Thüs 2002). Therefore, more or less shaded places should also be examined for a complete assessment of species richness. Shade demanding species can be found for example on the lower side or at the flanks of larger boulders or on small pebbles in the shade of weedy vegetation at the banks of a watercourse. Deep shade, however, is generally avoided by all freshwater lichens. Therefore, streams in dense thickenings of e.g. young spruce forests are usually completely devoid of freshwater lichens.

Preferences for acidity

Freshwater lichens have very specific responses to the pH value of their habitat, sometimes not only of the surrounding water but also of the substratum and in case of amphibious species also of the precipitation. Permanently submersed lichens are only affected by the pH value of the water body. In contrast, amphibious lichens in the splash water zone or at micro-sites with frequent changes of the water level are considerably influenced by air-borne acidic emissions (Thüs 2002).

The underlying processes for the different ranges of pH tolerance are still poorly understood. Specific acidity levels may have different effects on the fungal or algal partner of a lichen and certain developmental stages may also have different tolerance limits. Some general patterns however are obviously related to the kind of associated photobionts: freshwater lichens with cyanobacterial photobionts are best developed in neutral or slightly (?) alkaline waters while they are usually absent in waters below pH 5. The same pattern is observed for lichens with obligatory symbioses with *Dilabifilum*-like algae (*Verrucaria aquatilis*) or *Diplosphaera chodatii* and other *Stichococcus*-like green algae (most species of *Dermatocarpon*, *Polyblastia*, *Staurothele*, *Thelidium*, and *Verrucaria*). Freshwater lichens with “trebouxioid” photobionts (genera *Asterochloris*, *Myrmecia*, *Trebouxia*), *Trentepohlia* or *Heterococcus* tend to prefer neutral to acidic water. Species with a wider pH-tolerance (*Verrucaria hydrela*, *Hydropunctaria rheitrophila*) are able to associate with different photobionts (*Hydropunctaria rheitrophila*: *Dilabifilum*, *Heterococcus*; *Verrucaria hydrela*: *Dilabifilum*, *Diplosphaera*, *Elliptochloris*, *Heterococcus*) suitable for the respective pH-range in a specific environment.

Freshwater lichens are usually slow growing and – compared to other benthic cryptogams – rather long living organisms. The knowledge of these preference profiles makes inventories of freshwater lichen vegetation valuable for a rapid *in situ* assessment of pH-extremes occurring on the large time scale. They allow for a more integrative assessment compared to the short term indication by common indicators such as diatoms or other free living algae with a short life cycle. Furthermore, the separate analysis of the composition of lichens from submersed and amphibious sites gives a detailed impression of the acidic load transported by the water body and the air.

Interactions with other organisms and eutrophication

Fungi

Saprophytic or commensalistic filamentuos fungi are frequently growing among lichen thalli, colonising the cavities of over-aged perithecia or the cracks between the areoles of crustose species. Only few lichenicolous fungi are known to rely on freshwater lichens as their hosts (for details see Clauzade et al. 1989, Rambold et al. 1990, Molitor & Diederich 1997, Orange 2002). The external appearance of lichens may change considerably when being infected by lichenicolous fungi, often making a proper identification impossible. Infected lichen thalli are typically much more cracked and have brownish to blackish coloured areas on the thallus with a brittle consistency.

Algae

Epiphytic algae are often present in certain periods of the year, e.g. during mass developments of diatoms in spring. Long-living, non motile algae are much rarer on completely submersed lichens. On the other hand, the brownish cyanobacterium *Oncobrysa rivularis* can regularly be observed as an epiphyte on submersed *Verrucaria* thalli without causing any visible damage to the lichen. In alpine areas mostly reddish coloured cyanobacteria are sometimes covering crustose species of *Verrucaria* and *Staurothele* with a continuous layer. Free-living cyanobacteria with reddish sheaths are often found on moist rock and may resemble accompanying, juvenile thalli of *Pyrenopsis* which contain *Gloeocapsa*-like cyanobacteria as photobionts (=cyanobiont). Likewise, free-living Rivulariaceae can be observed growing abundantly on slack stones in the freshwater tidal zone along the river Elbe in and around Hamburg, Germany. These cyanobacteria form a blackish, irregular crust and may resemble young (or dying?) thalli of species of *Porocyphus* which contain Rivulariaceae as photobionts and are known to occur in the same habitat. The red alga *Hildenbrandia rivularis* and the lichen *Hydropunctaria rheitrophila* are frequently observed side by side at shady places in cooler and neutral to alkaline streams and lakes. Only rarely one of the two species is able to overgrow the other causing considerable changes in the colour of the lichen. Chlorophycean taxa such as *Stichococcus* spp., or *Trebouxia* spp. have also been observed as epiphytes on amphibious lichens. They are more frequent at low altitudes and in more or less nutrient rich waters. In the splash water zone of moderately eutrophic watercourses an epiphytic cover of lichens with a huge diversity of cyanobacteria and eukaryotic algae is rather frequent and can considerably influence the colouration and external appearance of the lichens.

Bryophytes

Bryophytes (Mosses and Liverworts) are generally strong competitors in shaded habitats. When they start overgrowing lichens the latter will soon die, probably due to shading or allelopathic effects. Mats of pleurocarpous mosses however, are favoured substrata for some foliose lichens (*Collema* spp., *Leptogium* spp.) and the membranaceous species *Lempholemma polyanthe*s. Shade tolerant crustose lichens can be found among the shoots of turf forming acrocarpous species.

Vascular Plants

Vascular plants are usually not competing with lichens for space but influence their occurrence by shading. Roots of Black Elder (*Alnus glutinosa*) and Willow (*Salix* spp.) are occasionally colonized by crustose freshwater lichens. In areas where larger boulders are rare, this substratum can be the most important habitat for amphibious lichens (Motijūnaitė 2003). Epiphytic growth of freshwater lichens on the roots of vascular plants is more often observed in the Alps and in North-Eastern Europe (Motiejūnaitė 2003, Pykkälä 2006) but is a rare phenomenon in most areas of Central Europe at low or moderate elevations (Thüs 2002).

Animals

Herbivores attack amphibious lichens preferably in the splash water zone where snails, springtails and other insects feed on algae from the thallus and the hymenium of the lichen ascomata. Determination of such thalli is often impossible and regeneration of grazed lichen thalli may result in manifold aberrations of the normal thallus appearance ("doubled" or threefold basal layer, changes in colour due to the absence or paucity of cortex, uneven surface etc.). The cushions of large, foliose lichens such as *Dermatocarpon* spp. may also play a role as resting sites for aquatic invertebrates in streams with strong current but detailed studies on the associated fauna of these lichens are still missing.

Phytosociology

Freshwater lichen vegetation can be arranged in characteristic and more or less colourful belts at the shores and banks of lakes and watercourses. This phenomenon has attracted the interest of phytosociologists for a long time and many relevées from various European regions exist. More frequent however, is a patchy, small-scale distribution of species with different hydration needs and the circumscription of homogenous relevées in the field is especially difficult in the splash water zone. Comprehensive overviews of the currently described phytosociological unities from Central Europe are described and discussed by Wirth

(1972) and Thüs (2002). For communities occurring on siliceous substrata detailed systems of phytosociological entities already exist, but for those from carbonaceous rocks (especially for the amphibious and rarely inundated communities) more relevées and systematic arrangements are still needed before a reasonable system can be proposed. Basic data for freshwater limestone communities have been collected by Pereira (1992) with a strong focus on the Mediterranean region and thus cannot be directly adopted for Central European rivers.

Collection and Storage of Herbarium Specimens

Aptroot & Seaward (2003) provide a good introduction of the methodology for collecting freshwater lichens, and their description is followed here. Usually, it is inevitable to collect specimens from the field for the determination of freshwater lichens. Even for identifications to genus level, microscopical characters have to be consulted by means of a compound microscope. In general, large thalli with reproductive structures (apothecia, perithecia, pycnidia, soralia or isida) should be selected for collection, because material without fruiting bodies or other dispersal structures is often impossible to determine. In the case of foliose species, it is possible to remove the thalli from the substratum by using a knife, but care has to be taken that the lower side is not damaged and possibly present rhizines are preserved during the collection process. Most freshwater lichens however, are crustose and tightly connected to their substratum. Some species are fast growing and colonize even small and moving pebbles. They are easily collected with their substratum, but the collection of loose pebbles will usually cover only a small fraction of the total species diversity. For a complete assessment of species, stable rocks flushed or splashed by water (e.g. in rapids) have to be studied as well as small pebbles, old wood or roots of Elder (*Alnus nigra*) and Widdow (*Salix* spp.). For the collection of the species from rocky substrates, the use of hammer and chisel is inevitable. Working with the chisel below water level in a current is sometimes difficult and periods with low water appear as the preferred time for inventories of freshwater lichens. A few species however (especially *Verrucaria aquatilis*, *V. funckii* and *Hydropunctaria rheitrophila*) live at sites that are permanently submerged and it may be necessary to examine rocks from at least half a meter below the current water level in order to find these desiccation sensitive species in larger streams even during low water periods.

Large boulders in rapidly running streams usually have smooth surfaces, thus making it difficult to place the chisel in the right position. In such cases it is useful to first make a furrow a few centimetres beside the lichen thallus using the chisel in vertical direction. In a second step the chisel is positioned at the flank of the furrow more or less parallel to the rock surface and a then a flat piece with the lichen can be chipped off the rock.

After collection, lichen samples should be dried as soon as possible in order to avoid moulding. For photobiont isolation, subsequent cultivation and detailed identification, the specimens must not dry out as some of the photobionts are very sensitive to desiccation. If it is impossible to dry the lichen sample directly in the field (e.g. during rainy weather), transportation of lichen samples should be done in plastic bags. For final storage in the herbarium the lichen thalli must be dried completely. It should be noted however, that rapid drying may cause the formation of wide cracks in the thalli of submersed lichens which may be problematic for determination, especially when the thallus surface is usually continuous. The paper capsules which are commonly used by lichenologists for epiphytic samples become rapidly soaken and instable when wet specimens of rock are transported. Small freezer bags with a volume of 1 litre are usually best suited since the plastic is more robust. An additional smaller set of 3-litre freezer bags is useful for transportation of large samples which is sometimes necessary when the rock is very hard and smaller samples are impossible to obtain. Each bag should contain only a single piece of rock and the bags have to be carried carefully in order to avoid damage of the thallus surfaces. Sample numbers and some basic collection data should be noted with a water resistant permanent pen directly on the freezer bag completed by detailed descriptions of the collection site in the field book (locality, distance from current water level, turbulence of the streaming, accompanying organisms, vegetation type in the surrounding etc.).

Preparation and Storage of Sections

Thallus dimensions, inner and outer thallus structure as well as fruit body morphology of the fungal symbiont are important characteristics for species identification. For the study of internal morphology sections of thallus and fruit bodies are necessary. Best results are obtained using a freezing microtome, but in most cases sections by hand with a razor blade will have to suit for determination purposes. Recommendable is the use of smaller blades which are used to remove horny skin. These blades are cheaper, usually sharper and less flexible compared to many common razor blades. Care should be taken to use only new, sharp blades and it is advisable to have a set of new blades at hand. Depending on the consistency of the lichen thallus, sections should be done with slightly moistened material. Usually it is recommendable to put a drop of distilled water on the lichen thallus, wait until it is absorbed and afterwards test several times for optimal stability of the lichen tissue during preparation. If the thallus is too dry, sections will be brittle and the surface of the sections becomes uneven. If the thallus is too wet, the thallus will be squeezed instead of being cut by the blade.

Finer structures of the fungal tissues and ascospores are sensitive to shrinking or swelling in different media. Therefore, all measurements should be taken in distilled water. If chemical tests are required, the application of e.g. caustic potash solution, Lugol's solution or Nitric acid should always be done after the measurements have been finished. Unstained sections can easily be stored dry on a slide covered by a coverslip. The coverslip can be fixed with small drops of nail varnish which are placed at the corners of the coverslip, while the section is embedded in a drop of water. The water prevents the varnish to flow under the coverslip and additionally serves as a distance keeper. After study, the water will evaporate from the open flanks between slide and cover slip and the dry sections can be stored without further treatment in a dry and tightly closed slide box. For re-examination a drop of distilled water is placed at the flank of the cover slip and the water is soaked under the coverslip via capillary forces.

Morphological Characteristics of Freshwater Lichens

Morphological structures relevant for identification of freshwater lichens are shortly described and illustrated in the glossary. For more comprehensive descriptions of thallus anatomy and function textbooks like Nash (2008) and the introductions of the books written by Wirth (1995, in German) or Purvis et al. (1992) and Brodo et al. (2001) are recommended.

Running water is a strong selective force which influences the ecophysiology as well as the mechanical constraints of the evolution of amphibious lichens. Consequently, lichens adapted to amphibious habitats are often very similar in morphology. Structures are optimised for minimum resistance to the current, e.g. most freshwater lichens are crustose, a much smaller number is foliose and only a few taxa with fruticose thalli occur in or at watercourses (Santesson 1939, Nordin 2002a). The reproductive organs are either perithecia which are often protected by a solid, carbonised cap, the involucrellum, or apothecia which are often immersed in the thallus or situated in thallus warts. Prominent and unprotected reproductive structures like soredia or sessile apothecia are rare exceptions in freshwater lichens. Lichen thalli are often differentiated in functional layers: a cortex (with cells of distinctly different shape compared to those in the algal layer) or pseudocortex (lacking photobionts, often with a special pigmentation but with fungal cells of similar size and shape as in the algal layer), a photobiont layer containing the algae, and a medullary layer which is usually composed of loosely interwoven hyphae. In terrestrial lichens, the medullary hyphae are often coated by water repellent substances, thus facilitating the mobility of gaseous components within the thallus (Honegger 1998). In the water saturated environment of freshwater lichens the air filled medullary layer is generally reduced or composed of a carbonised and tightly glued paraplectenchyma referred to as “black basal layer”.

Photobionts

The algal component of the lichen symbiosis was long regarded as a fundamental basis for both, large-scale systematics and for species separation, especially in freshwater lichens (Zahlbruckner 1921, Zschacke 1934, Servít 1954). However, the identification of the photobionts was often based on the study of the lichenized phenotypes alone. In some cases it is possible to determine lichen photobionts to genus level. However, in most cases the identification of the algae from lichen thallus sections will prove to be very difficult or virtually impossible – even to family level. The use of photobiont characters for species determination in lichens is thus much restricted by practical limitations. Recent studies on the specificity of the fungal-algal relationship by means of molecular markers revealed a limited flexibility in specific groups of lichen fungi, which is further depending on a variety of habitat specific factors (Beck 2002, Beck et al. 1998, 2002, Piercey-Normore & DePriest 2001, Guzow-Krzemínska 2006). Still, there are some general patterns, e.g. the obligatory association of all members of the Collemataceae, Lichinaceae and Placynthiaceae with prokaryotic, cyanobacterial photobionts or the limited submersion tolerance of lichens with *Trebouxia* s.str., *Asterochloris* or *Trentepohlia* photobionts in contrast to the generally hydrophilic and desiccation sensitive lichens with *Dilabifilum* or *Heterococcus* as photosynthetic partners. For many species however, more data are needed to accurately determine the specificity of the fungal-algal relationship and to achieve a better understanding of the reasons for site-specific differences. The ecophysiology of suitable lichen photobionts certainly plays an important role in the understanding of the ecological preferences of lichens, but still much field observations and laboratory experiments are needed in order to verify preliminary conclusions as outlined above, e.g. for the preferences of amphibious lichens for acidic water. We have provided information about the identity of lichen-associated photobionts only when these informations have been confirmed by our own observations in the study area or have been reproduced from reliable literature sources clearly referring to Central Europe and including detailed drawings and descriptions of the isolation process. In the keys we have attempted to make only little use of characters of the photobionts. In order to facilitate the distinction of relevant colour characters, examples of several photobionts in the thalli of amphibious lichens are given in colour plate 6.

A proper identification of a photobiont to species level usually requires the isolation of photobiont cells from the lichen thallus and the cultivation of the algae or cyanobacteria in culture media. For isolation of amphibious lichen photobionts only freshly collected material should be used which has to be kept in moist condition until the isolation procedure. For isolation a laminar flow bench and sterile conditions are required. In the laminar flow bench a stereo microscope is sterilised with ethanol. The surface of many amphibious lichens can be covered by epiphytic algae which are easily mistaken for photobiont cells when cultivation experiments are performed with entire thallus fragments. Therefore, it is recommendable to first decorticate approximately 5 mm^2 of the lichen by a horizontal section with a razor blade. In the decorticated area a small drop of sterile water is placed and the algae containing layer of the thallus is disrupted by means of a sterile preparation needle. The resulting dispersion of algal and fungal cells is soaked with the tip of a fine glass capillar (e.g. the tip of a Pasteur-pipette after careful elongation over a gas burner). The suspension is then transferred onto a Petri-dish containing a suitable culture medium. For green algae and Xanthophytes good results are received with Bold's Basal Medium with an additional drop of soil extract per litre. Axenic (bacteria free) cultures can be prepared either by repeated streaking of small cell groups to fresh media or by picking single cells from thallus fragments or pre-cultured thallus suspensions using a modified micro-manipulator (Beck & Koop 2001). Still a good starting point for identification is the comprehensive "Syllabus der Boden-, Luft- und Flechtenalgen" (Ettl & Gärtner 1995) which also includes instructions for isolation and cultivation of lichen photobionts.

Taxonomic Concept

The relatively uniform structure of the vegetative thallus makes quantitative characters of the reproductive structures a principal source of information for morphological species delimitation in many freshwater lichens. When using quantitative characters in the keys, care must be taken to obtain a sufficient sample size. For example, the variation in ascospore size should be assessed by measuring at least 10 ascospores, if necessary from different asci and ascomata.

This book is intended for use in ecological studies and we attempted to follow a strictly morphological concept for species identification. Recent results of molecular studies are commented where appropriate, but changes in species delimitation were only proposed if a morphology based distinction of the taxa is possible. In general, only those taxa were accepted which are corrobated by at least two independent characters from otherwise similar taxa. Ecological characters are helpful for identification purposes in some cases, but for proper species delimitation morphological, chemical – or if available – molecular characters are mandatory. In some cases such as the crustose genera of the Verrucariales we followed traditional genus concepts based on single characters which have been demonstrated to have evolved convergently in different lineages of the Verrucariales (Gueidan et al. 2007, 2008). Although the polyphyletic origin of genera such as *Verrucaria*, *Polyblastia* and *Thelidium* has become evident, new monophyletic entities are often difficult to distinguish by morphological characters alone and for several lineages a morphological circumscription has not been achieved so far. We have followed Gueidan et al. (2008) in the separation of the black dotted species of *Verrucaria* with small-sized ascospores as the new genus *Hydropunctaria* and the treatment of *Sporodictyon* at genus rank separated from the remaining species of *Polyblastia* (Savić et al. 2008). However, further changes in the delineation of the genera *Verrucaria*, *Polyblastia* and *Thelidium* are to be expected in the near future. Similarly, the cyanobacterial lichen genera *Porocyphus*, *Pterygiopsis*, *Pyrenocarpon*, *Pyrenopsis* and *Thelignya* are currently based on (sometimes still rather vague) morphological concepts (Henssen '1979'1980, Henssen & Jørgensen 1990, Schultz & Büdel 2002) and thus, changes are to be expected once sufficient molecular data have become available.

Photography

Only few of the taxa treated in this book have been illustrated elsewhere. Since photographs of the overall thallus habitus can contribute much towards identification, we provide images for almost all of the species treated here. However, we are aware that images can illustrate only a small proportion of the species variation. Furthermore, species of genera such as *Verrucaria* and *Thelidium* may look very much alike and can be distinguished only using microscopic characters. Therefore, the photographic illustrations are intended to serve as a supplement to the information provided in the species descriptions and keys.

Most of the lichens are illustrated by black and white photographs directly accompanying the descriptions in the text. Some colour images have been assembled at the end of the book to give an impression of the general appearance and colour for a selection of the species of *Ionaspis*, *Staurothele*, *Verrucaria* as well as some others.

With a few exceptions, we did not attempt to take photographs at the natural habitat of the species. Instead, we selected recently collected material for illustration whenever available. Many aquatic species exhibit a vivid green colour when studied in nature. Unfortunately, this fresh green colour fades rapidly once the lichen is dried, mounted and kept under herbarium conditions. On the other hand, the dark colour of many cyanobacterial lichens changes little with time so that old herbarium material is still suitable for photography.

A number of species occurs in terrestrial as well as in amphibious or riparian habitats. Sometimes, the external appearance of these lichens varies depending on the habitat. We therefore attempted to provide images based on material from habitats that the book is focused on (i.e. margins of rivers, lakes etc.).

Most images were produced using the following digital equipment: a Canon EOS 400D digital camera mounted on a Novoflex (Germany) bellows with Canon FD mount using an adapter (Kood, Japan Canon AF/FD, central correction lens removed) and fixed in a Novoflex (Germany) macro stand. A set of macrolenses was used for a magnification range of 1–7 times. Most images were taken using a Zeiss (Germany) Luminar 40 mm/1:4 lens. Additionally, Zeiss (Germany) Luminar 63 mm/1:4.5 and 25 mm/1:3.5 lenses and two Canon Macro lenses 35 mm/1:2.8 and 20 mm/1:3.5 were used. Images 15 & 160 (*Collema dichotomum*, *Dermatocarpon arnoldianum*) were taken with a Canon Macro Lens EF-S 60 mm/1:2.8 USM. For illumination a Schott (Germany) cold-light source was used. An image of white photo paper taken with the camera set to automatic white balance was used as reference for correction of light temperature to obtain natural light conditions.

Glossary

A

accessory lobule, small ± erect, simple or branched thallus lobe formed by outgrowing marginal isidia or originating from the margin or surface of larger, main lobes; term mostly used in *Collema* (Degelius 1954, 1974).

acicular, needle-shaped.

amphibious, temporarily inundated or ± frequently wetted by splash water.

amyloid, staining blue, blue-violet, orange or red in iodine (I).

anastomosing, repeatedly branched and reconnected.

apical dome, thickened inner part of the ascus wall in the ascus apex, in some species amyloid (staining I+ blue with iodine).

apothecial disc, the surface of the hymenium surrounded by the exciple.

apothecium, disciform to cup shaped ascoma (fruit body) with hymenium exposed at maturity.

aquatic, (in the sense used here) completely submersed, or very rarely subject to desiccation.

areolate, crustose thallus with deep cracks separating the thallus into mosaic-like fragments.

areole, one of the fragments in an areolate thallus.

ascoma, fruit body in which the asci are formed; in freshwater lichens either **perithecia** or **apothecia**.

ascospore, diaspore type, meiotically formed in an ascus.

ascus, cylindrical to sack-like, swollen hyphal cell in which ascospore formation takes place.

B

bacilliform, rod-shaped.

(black) basal layer, algal free tissue of compact fungal cells with brownish-black cell walls in the basal part of a crustose thallus.

biatorine, type of apothecial margin formed by a pale, not carbonised true exciple free of algae.

blastidium, rounded lichen propagule produced by the budding (yeast-like propagation) of a thallus.

C

C, saturated aqueous solution of calcium hypochlorite, $Ca(OCl)_2$. Commercially available for disinfection purposes and principal compound of common house-hold bleach products. Instable, proper reactivity should be checked regularly on lichens with known reaction (e.g. the common epiphyte *Lecanora expallens*, which should turn C+ yellow to reddish if the reagent is still in good condition). Caustic!

canaliculate, channelled.

carbonised, optically dense, brown-black appearance of fungal tissue with usually small cell lumina and thickened cell walls, containing large amounts of dark coloured phenolic substances.

cilia, hair-like outgrowths on the thallus surface or margin.

clavate, club-shaped.

conidium (conidiospore), mitotically produced spores, in freshwater lichens exclusively found in pycnidia.

cortex, outermost layer in heteromerous thalli composed of differentiated fungal hyphae, often with compact arrangement and special cortical pigments.

crenate, with toothed edges, teeth rounded.

crenulate, delicately crenate.

crustose, lichen growth form with thalli tightly attached to the substratum and lacking a lower cortex.

D

decumbent, resting on the substratum with the ends turned up.

E

effigurate, thallus margin consisting of ± radially arranged, elongated areoles.

epihymenium, often coloured layer above the hymenium (fig. 1).

epinecral layer, layer of dead fungal hyphae with indistinct, often collapsed lumina.

epithecium, upper part of hymenium.

eumuriform, spores having numerous horizontal and longitudinal septa.

euparaplectenchymatous, composed of ± isodiametric, strongly conglutinate hyphae; term refers to a type of proper exciple mostly in *Collema* (Degelius 1954, 1974).

euthyplectenchymatous, composed of parallel, elongated, not or weakly conglutinate hyphae; term refers to a type of proper exciple mostly in *Collema* (Degelius 1954, 1974).

exciple, tissue forming the wall of an ascoma (apothecium or perithecium).

excipulum proprium (excip. prop., proper or true exciple), margin of an apothecium composed of a fungal plectenchyme, pale or variously coloured, carbonised or not, texture various from loosely interwoven to strongly agglutinated, from reticulate to euthyplectenchymatous or paraplectenchymatous.

F

foliose, leaf-like growth form of lichens with the thallus attached to the substratum by rhizines, hapters or punctually by the lower surface, but never with the entire lower surface. Often with differentiated upper or lower cortex.

fruticose, shrub-like growth form of lichens with ± cylindrical, often furcate and erect thallus branches.

fusiform, spindle-like, with acute apices.

G

globose, with rounded outline.

H

halonate, ascospores surrounded by a thick transparent coat or perispore (often distinctly swelling in K).

hamathecium, sterile hyphae between asci and ascoma walls.

hapter, structure which is fixing the thallus to the substratum.

heterocyte, (often incorrectly referred to as “heterocyst”) special cells in some filamentous cyanobacteria, free-living or lichenized, characterized by a thick, multiply layered wall and enzymes able to fix atmospheric nitrogen; position basal (e.g. Rivulariaceae) or intercalary (*Nostoc*, *Stigonema*, *Scytonema*).

heteromerous, photobiont cells arranged in a distinct layer in the lichen thallus.

homoiomerous, photobiont cells not forming a distinct layer in the lichen thallus.

holdfast, structure for punctual attachment of the lichen thallus to the substratum.

hydrophytic, demanding frequent or constant supply of liquid water.

hygrophytic, demanding high air humidity or supply of liquid water.

hymenial algae, photobiont algae in the cavity of a perithecium.

hymenium, fungal tissue composed of interascal filament (e.g. paraphyses) and asci.

hypothecium, layer of fungal tissue below the hymenium.

I

I, chemical reagent, 0.3% aquaceous solution of 1 part iodine, I and 2 parts potassium iodine, KI. The final solution is instable, and the preparation of a stock solution (1 g I and 2 g KI diluted in 30 ml distilled water) is recommended for storage. The working solution is a freshly prepared 10% dilution of the stock solution.

involucrellum, conical tissue of compact cells surrounding the exciple (fig. 4). Sometimes fused with the exciple and only recognizable by a abrupt thickening of the perithecial wall.

isidium, lichen propagule covered by a distinct cortex and containing fungal and algal cells.

isodiametric, more or less equally sized in all directions.