



LICHENS AS BIOINDICATORS IN FRESHWATER ECOSYSTEMS - CHALLENGES AND PERSPECTIVES

NASCIMBENE J.^{1*}, NIMIS P.L.¹, THÜS H.²

¹*Dipartimento di Scienze della Vita, Università di Trieste, via Giorgieri, 10 – 34100 Trieste.*

²*Life Sciences Department, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom*

*Corresponding author: Telephone: +39 0405588820; e-mail: jnascimbene@units.it

(RECEIVED 28 JANUARY 2013; RECEIVED IN REVISED FORM 28 MARCH 2013; ACCEPTED 28 MARCH 2013)

ABSTRACT – This paper summarizes information on freshwater lichens in relation with their potential for bioindication, mainly pointing to ecological concepts and issues of practical relevance for promoting their inclusion in routine biomonitoring practices, thus contributing to a full implementation of the EU Water Framework directive. Results highlight the sensitiveness of freshwater lichens to some factors which cannot be technically measured by singular visits, and have relevance for human planning purposes and environmental impact and risk assessment. However, a full inclusion of freshwater lichens in monitoring practices would benefit from further ecological research testing the influence of potentially meaningful ecological drivers and developing statistically robust sampling methods. This would allow the development of standard guidelines applicable across Europe according to the policies of the EU Water Framework directive. On the taxonomical side, further DNA-based revisions and the creation of a European checklist of freshwater lichens should provide the basis for developing modern identification tools. Finally, it is suggested that the use of freshwater lichens in biomonitoring may be improved by model studies based on comparative trials of full, quantitative, species inventories at different spatial scales and by parallel simplified approaches with selected indicator species and morphological groups.

KEYWORDS: BIOMONITORING, DNA-BARCODING, EU WATER FRAMEWORK DIRECTIVE, FRESHWATER LICHENS, MACROPHYTES, TRAIT-BASED FUNCTIONAL GROUPS.

INTRODUCTION

Lichens are the symbiotic phenotype of specialised fungi which live in stable and highly organised associations with algae or cyanobacteria. These intricate miniature ecosystems appear in the environment as more or less sharply delimited units, forming composite thalli which are easily visible to the naked eye. The fungal component of most lichen thalli soon produces fruiting bodies which remain permanently on the thallus and facilitate the identification at any time of the year. In freshwater habitats, lichens are found wherever suitable solid substrata, alkaline to moderate acidic water, sufficient light, and low to moderate silting occur. Species composition and richness of freshwater lichen communities depend on ecological variables which are largely overlapping with factors of high importance for the management of freshwater

bodies (Thüs & Schultz, 2008). Moreover, some chemical factors such as pH, conductivity, alkalinity, silica and Mg contents are known to influence the occurrence of several aquatic species (i.e. Pentecost, 1977; Gilbert & Giavarini, 1997; Nascimbene et al., 2007).

In European streams and rivers, species richness in the permanently submerged parts of a stream bed rarely exceeds five to ten species at a given site (Gilbert, 1996; Gilbert & Giavarini, 1997; Ried, 1960a; Thüs, 2002). In unpolluted sites, including the splash water and temporarily but regularly inundated zones of the river banks can easily bring the number of freshwater lichens to 20-30, out of a pool of ca 150 species in Europe which have a more or less strong affinity to freshwater habitats (Nordin, 2002; Thüs & Schultz, 2008). At a worldwide scale, ca. 200 species are known from this habitat (Hawksworth, 2000), although a substantial

number of species, particularly in the tropics, may not have been described yet (Thüs, unpubl.).

These figures are smaller than those for other photoautotrophic organisms such as bryophytes, diatoms or green algae. In a comparative study including green algae, diatoms, lichens, and bryophytes in 40 springs of the Italian Alps, Nascimbene et al. (2011) found 69 species of green algae, 110 species of diatoms, 29 species of lichens, and 62 species of bryophytes. Interestingly, they have shown that the patterns of occurrence of these groups of organisms are not, or only weakly, correlated. As a consequence, lichens should be regarded as an independent functional group with potential for bioindication for factors which are not easily covered by other organisms. However, in most assessments of freshwater organisms, lichens are still neglected even if they are included in the concept of “macrophyte” which is addressed by the EU Water Framework directive providing the main policies for freshwaters monitoring.

This situation is at least in part due to the fact that, in spite of their limited number, freshwater lichens are often difficult to identify by non-specialists. Taxonomical problems are still a constraint for advances in ecological studies and for a wide use of freshwater lichens as bioindicators. In this perspective, the evaluation of patterns of lichen diversity in terms of species traits is a recent, promising approach (Ellis & Coppins, 2006; Johansson et al., 2007) that could be useful for large-scale comparisons when species-based evaluations might be hindered by uneven or poor levels of taxonomic knowledge (Giordani et al., 2009). Species traits (e.g. photobiont type, growth form, reproductive strategy) are indicative of lichen community adaptation to environmental conditions (Diaz & Cabido, 2001), providing relevant ecological information. For example, recent studies demonstrated that photobiont partner and thallus growth-form may control the community structure, determining large-scale patterns of diversity (Ellis & Coppins, 2006, 2010; Ellis et al., 2007; Marini et al., 2011). Current studies in this field are mainly devoted to forest epiphytic lichens (e.g. Giordani et al., 2012) or to the rapid assessment of air quality (e.g. Davies et al., 2011), while for freshwater lichens this approach is still poorly developed (e.g. Thüs, 2002) and statistically not tested.

This study aims at summarizing information on freshwater lichens in relation with their potential for bioindication, mainly pointing to ecological concepts and issues of practical relevance for promoting the inclusion of freshwater lichens in routine biomonitoring practices contributing to a full implementation of the EU Water Framework directive. In particular, we aim at elucidating the sensitiveness of freshwater lichens to the main ecological factors related with management practices, and the potential interpretation of their response in terms of trait-based functional groups. Problems related with taxonomic knowledge and species

identification are also addressed.

ECOLOGICAL FACTORS RELEVANT TO FRESHWATER LICHENS

Water level fluctuations

The largest diversity of lichens occurs in habitats which face harsh changes in water availability, where lichen communities can become dominant growing other photoautotrophic organisms. This is the case of hot and cold deserts, steep cliffs or particularly poor soils, but also of fast-flowing currents of streams and rivers. Most lichens, however, are symbiotically associated with only a handful of algal taxa, the genera *Trebouxia*, *Asterochloris* and *Trentepohlia* (all Chlorophyta) being the most common photobionts. The closest free-living relatives to lichenised taxa in these algal genera are living in habitats which are exposed to the air for most of the time, and their physiology is likely to be optimised for carbon uptake from the air. It has been shown that the carbon balance of lichens associated with *Trebouxia* or *Asterochloris* algae can become negative when the thalli are soaked with water (Lange & Green, 1996, 1997), giving them very limited inundation tolerance. Photobionts are important for the inundation tolerance of a lichen (Hawksworth, 2000) and although the morphology of the symbiotic system can largely influence the performance of lichens under temporarily high water conditions (Lange et al., 1999), most lichens from terrestrial and even from semi-aquatic habitats with non specialised photobionts are eventually literally “drowning” when exposed to extended periods of submergence (Ried, 1960b). Only a few groups of lichenised fungi, and particularly certain lineages within the family Verrucariaceae, have evolved a compatibility with a much wider spectrum of algae, including typical aquatic genera such as *Dilabifilum* or *Heterococcus*. These lichens are not only tolerant to permanent inundation, but also have a limited desiccation tolerance (Thüs et al., 2011). Species of the family Verrucariaceae occur in nearly all European freshwater lichen communities (Thüs, 2002), being often the dominant or the only family occurring in the permanently submerged zone (Gilbert, 1996; Gilbert & Giavarini, 1997; Keller & Scheidegger, 1994; Nascimbene et al., 2009; Ried, 1960a, b; Santesson, 1939; Thüs, 2002).

The result of the algal and fungal inundation and desiccation tolerances is a clear zonation pattern of different lichens on rocks and trees in streams and rivers (Beschel, 1954; Hale, 1984; Gregory, 1976; Luther, 1954; Ried, 1960; Rosentreter, 1984; Santesson, 1939). This permits to use the photobiont type as a functional trait for evaluating the response of

freshwater lichens to water level fluctuations. A similar approach was used for epiphytic lichens by Marini et al. (2011), demonstrating that the response of lichens to environmental factors may depend on the type of photobiont involved in the symbiosis.

Streambed stability

Substrate stability is one of the main habitat features for lichen colonization on rocks, most species requiring stable and large stones whose surfaces are not subject to erosion (Thüs, 2002). This could account for differences in species richness that were found between siliceous and calcareous rocks in freshwater habitats of the Italian Alps (Nascimbene & Nimis, 2006; Nascimbene et al., 2007), the former hosting a richer lichen biota. Siliceous rocks represent a more stable substrate due to their high resistance to erosion; their wrinkled surfaces harboring lichen diaspores and enhancing thallus development. On the contrary, calcareous rocks suffer of both mechanical and chemical erosion, the thalli being often thin and incomplete. However, lichen colonization on stable calcareous stones may be surprisingly rapid, as demonstrated in a study aiming at evaluating the effectiveness of freshwater lichens in colonizing newly constructed stone structures in low-elevation small streams subject to habitat restoration (Nascimbene et al., 2009). Substrate stability was also considered as the basis of the high suitability of bolder and cobble springs, compared with gravel springs, for both *Verrucaria elaeomelaena* and *V. funckii* (Nascimbene et al., 2007).

Light conditions

Most freshwater lichens are related to well lit conditions, resulting in low species diversity in shaded rivers, even if some shade-demanding species are known to occur in freshwaters, e.g. *Porina* spp. (Pentecost, 1977). This factor may however interact with other ecological variables driving freshwater lichen patterns, such as the extent of submersion period. In particular, shading enhances the tolerance of species to desiccation by increasing air humidity. For example, *Verrucaria funckii* is very desiccation-sensitive if air humidity is low, while it can survive above the water level if air humidity is constantly high (Ried, 1960a; 1960b), which indicates that shaded conditions may compensate for shorter submersion periods.

Silting

Silting can severely limit lichen growth, both because of its

interference with light penetration and because of its mechanical effect when covering the thalli (Thüs, 2002). As a consequence, most freshwater lichens are very sensitive to the deposition of silt-like sediments (both organic and inorganic) on their surface (Gilbert, 1996; Gilbert & Giavarini, 1997), and only a few species are tolerant to moderate silting, such as *Bacidina inundata*, and *Verrucaria praetermissa* (Thüs, 2002). A tolerance scale to this factor may be also based on species' functional traits, lichens with a subgelatinous thallus being extremely sensitive, and those with a thick areolate thallus being more tolerant.

Acidity

Freshwater lichens are sensitive to the pH of their habitat. Species of permanently submerged sites mainly reflect water pH, while amphibious species also reflect substrate and precipitation acidity (Thüs, 2002). The effect of pH in driving lichen patterns is not yet explicitly demonstrated, but for example Nascimbene et al. (2007) found significant differences in pH between siliceous springs hosting *Verrucaria funckii* and those where this species was not found. In general, at pH below 5 freshwater lichens are absent, but there is a tolerance range among different species that could be related with the type of photobiont (Thüs & Shultz, 2008). Species with *Dilabifilum*-like (i.e. *Verrucaria aquatilis*) and *Stichococcus* or *Diplosphaera*-like green algae (i.e. several species of *Staurothele*, *Thelidium*, and *Verrucaria*) are associated with alkaline waters, while species with trebouxoid algae (*Trentepohlia* and *Heterococcus*) are usually associated with neutral-acidic waters. The wide tolerance of some species to pH is likely to be connected with their capability of changing the photobiont type depending on environmental conditions, such as in the cases of *Verrucaria hydrela* and *Hydropunctaria rheitrophila* (Thüs & Shultz, 2008).

In the perspective of biomonitoring, both the sensitiveness of single species and the response of the whole group of freshwater lichens in term of photobiont type are suitable indicators of pH conditions of watercourses. Furthermore, since lichens are slow-growing and long-living organisms, their inclusion in biomonitoring practices may provide time-integrated information on pH conditions, which cannot be gathered using more popular indicators with a short life-cycle, such as diatoms or other free-living algae.

Eutrophication

Eutrophication is known to exert a strong influence on lichen communities, causing species loss and composition shifts toward assemblages composed by tolerant species (Gilbert,

1996; Gilbert & Giavarini, 1997; Thüs, 2002). Some field observations suggest that a higher species richness of freshwater lichens may be expected in oligotrophic sites, but experimental studies clearly addressing this issue are still lacking. In eutrophic watercourses, lichens are outcompeted by faster-growing organisms such as plants, algae and bryophytes, even if some species (e.g. *Verrucaria praetermissa*) are known to be able to survive in eutrophicated sites. Eutrophication is likely to interact and partially overlap with other drivers, such as silting, and therefore it is not as easy to clearly evaluate the response of freshwater lichens in terms of eutrophication as for epiphytic communities (Thüs, 2002).

TAXONOMIC CHALLENGES

Freshwater lichens belong to several genera, but the most frequent genus, occurring in almost all freshwater bodies is *Verrucaria*. This genus, in its current morphological circumscription, is simple to identify but highly polyphyletic (Gueidan et al., 2009) and the taxonomic position of several species still awaits further research. The morphological characters used in the identification keys are often either not clear (e.g. not visible in all specimens of a given species or with largely overlapping ranges in quantitative measurements), or deserve a critical review. In general, crustose Verrucariales are exceedingly poor in taxonomically useful characters and species-level identification by morphological characters is challenging, due to cryptic and semi-cryptic speciation (e.g. Orange, 2012 for *Hydropunctaria* spp.). Furthermore, many morphological characters are highly variable and largely dependent on environmental influences (Santesson, 1939; Keller, 1996; Thüs, 2002).

Taxonomic problems are a constraint for advances in ecological studies, for effective species protection, and for the use of freshwater lichens in biomonitoring. For this reason, specialists are trying to clarify the taxonomical relationships among genera and species belonging to critical groups, testing the usefulness of morphological characters against molecular data (e.g. Thüs & Nascimbene, 2008; Gueidan et al. 2009; Orange, 2012). The results indicate that for the correct identification of some of the newly circumscribed phylogenetic species, sequencing of selected marker areas of the genome (e.g. the nuclear ITS) in some specimens can become necessary (Orange, 2012). Although the costs for the extraction of DNA from fungal tissues and the sequencing of marker genes are constantly decreasing, the selection of tissue for molecular barcoding from freshwater lichens is not trivial, due to the frequent occurrence of contamination from other fungi, the presence

of intricate mosaics of different lichens, and the low quantity and poor quality of DNA from the often very thin and damaged thalli of aquatic lichens. For these reasons, a preliminary morphology-based identification continues to be essential, even if DNA-barcoding is employed for precise identification. In practical case-studies, one can critically examine whether the extra cost and effort for a DNA-based verification of species identification does provide significant additional ecological information. Alternatively, the effectiveness and robustness of using morphologically less ambiguously circumscribed species aggregates, or even non related species groups with similar functional traits, need to be tested.

PERSPECTIVES FOR BIOMONITORING

Organisms suitable for bioindication should have a combination of characters including high specificity for an ecological niche which is of practical relevance for human beings (e.g. for factors which are difficult or expensive to measure with technical devices), and a good balance between identification effort and relevance of results. How does this apply to freshwater lichens? These organisms are characterized by a high diversity in ecological niche differentiation, particularly for a limited number of factors which cannot be technically measured by singular visits, and have relevance for human planning purposes and environmental impact and risk assessments, e.g.: inundation patterns (Beschel, 1954; Gilbert, 1996; Gilbert & Giavarini, 1997, 2000; Keller & Scheidegger, 1994; Nordin, 2002), pH-variation in water and air over long time periods (Thüs, 2002), and waterbed stability (e.g. Krzewicka & Galas, 2006; Nascimbene et al., 2009; Thüs, 2002).

However, a full inclusion of freshwater lichens in monitoring practices would benefit from further ecological research. The ecological information on freshwater lichens summarized in the previous sections is mainly based on descriptive or semi-quantitative studies (e.g. Nascimbene et al., 2007), often associated with floristic or taxonomic research (Gilbert, 1996; Gilbert & Giavarini, 1997, 2000; Keller & Scheidegger, 1994; Motiejunajte, 2003; Thüs, 2002; Thüs & Nascimbene, 2008), while studies explicitly testing the influence of potentially meaningful ecological factors, and standard methodologies for ecological experiments are still lacking. Positive recent exceptions are provided by e.g. Glavich (2009) and Nascimbene et al. (2009). However, the study by Glavich (2009) was designed for estimating conservation priorities and not bioindication of environmental variables at a given site. The study of Nascimbene et al. (2009) was carried out in structurally rather homogenous

small streams with low species diversity, and the results could not be generalized to large rivers where environmental conditions are more heterogeneous.

Future research on freshwater lichens should therefore address these challenges by testing statistically robust sampling methods even in structurally highly heterogeneous (e.g. with rapids, waterfalls, alternating with meandering areas with pebble banks, etc.) freshwater systems of different size across Europe, also including Mediterranean areas which are currently neglected.

This target may be achieved by improving the collaboration with limnologists and using already available ecological databases to design hypothesis-based studies. On the taxonomic side, further DNA-based revisions and the creation of a European checklist of freshwater lichens should provide the basis for developing modern identification tools, such as user-specific keys on demand (Nascimbene et al., 2010) based on innovative software (e.g. Martellos, 2010). Finally, the use of freshwater lichens in biomonitoring may be improved by model studies based on comparative trials of complete, quantitative species inventories at different spatial scales and on parallel simplified approaches with selected indicator species (Nascimbene et al., 2007; Nascimbene et al., 2009) and morphological groups, as suggested by Thüs (2002).

These studies should include streams of different size in various climatic zones, and should compare effort and outcome for the different existing methodologies, allowing the development of standard guidelines applicable across Europe according to the policies of the EU Water Framework directive.

REFERENCES

- Beschel R., 1954. Die Stufung der Flechtenvegetation an Inn-Ufermauern in Innsbruck. *Phyton* 5, 247-266.
- Davies L., Bell J.N., Bone J., Head M., Hill L., Howard C., Hobbs S.J., Jones D.T., Power S.A., Rose N., Ryder C., Seed L., Stevens G., Toumi R., Voulvoulis N., White P.C., 2011. Open Air Laboratories (OPAL): a community-driven research programme. *Environmental Pollution* 159, 2203-2210.
- Diaz S., Cabido M., 2001. Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution* 16, 646-655.
- Ellis C.J., Coppins B.J., 2006. Contrasting functional traits maintain lichen epiphyte diversity in response to climate and autogenic succession. *Journal of Biogeography* 33, 1643-1656.
- Ellis C.J., Coppins B.J., 2010. Integrating multiple landscape-scale drivers in the lichen epiphyte response: climatic setting, pollution regime and woodland spatial-temporal structure. *Diversity & Distribution* 16, 43-52.
- Ellis C.J., Coppins B.J., Dawson T.P., Seaward M.R.D., 2007. Response of British lichens to climate change scenarios: trends and uncertainties in the projected impact for contrasting biogeographic groups. *Biological Conservation* 140, 217-235.
- Gilbert O.L., 1996. The lichen vegetation of chalk and limestone streams in Britain. *Lichenologist* 28, 145-159.
- Gilbert O.L., Giavarini V.J., 1997. The lichen vegetation of acid watercourses in England. *Lichenologist* 29, 347-367.
- Gilbert O., Giavarini V., 2000. The lichen vegetation of lake margins in Britain. *Lichenologist* 32, 365-386.
- Giordani P., Brunialti G., Benesperi R., Rizzi G., Frati L., Modenesi P., 2009. Rapid biodiversity assessment in lichen biomonitoring surveys: implications for quality assurance. *Journal of Environmental Monitoring* 11, 730-735.
- Glavich D.A., 2009. Distribution, rarity and habitats of three aquatic lichens on federal land in the U. S. Pacific Northwest. *The Bryologist* 112, 54-72.
- Gregory K.J., 1976. Lichens and the determination of river channel capacity. *Earth Surface Processes* 1, 273-285.
- Gueidan C., Savic S., Thüs H., Roux C., Keller C., Tibell L., Prieto M., Heidmarsson S., Breuss O., Orange A., Fröberg L., Amtoft Wynns A., Navarro-Rosinés P., Kristinsson H., Krzewicka B., Pykälä J., Lutzoni F., 2009. The main genera of *Verrucariaceae* (Ascomycota) as supported by recent morphological and molecular studies. *Taxon* 58, 184-208.
- Hale M.E., 1984. The lichen line and high water levels in a freshwater stream in Florida. *The Bryologist* 87, 261-265.
- Hawksworth D.L., 2000. Freshwater and marine lichen-forming fungi. *Fungal Diversity* 5, 1-7.
- Johansson P., Rydin H., Thor G., 2007. Tree age relationships with epiphytic lichen diversity and lichen life history traits on ash in southern Sweden. *Ecoscience* 14, 81-91.
- Keller C., 1996. Infrasppezifische Variabilität - ein Thema in der Systematik der Süßwasser-Verrucarien (Verrucariales, Ascomycotina). *Mycologia Helvetica* 8, 73-80.
- Keller C., Scheidegger C., 1994. Zur Verbreitung von Wasserflechten in Abhängigkeit zur jährlichen Überflutungsdauer im Flüelatal (Schweiz, Kanton Graubünden). *Herzogia* 10, 99-114.

- Krzewicka B., Galas J., 2006). Ecological notes on *Verrucaria aquatilis* and *V. hydrela* in the Polish Tatry Mountains. In: A. Lackovičová, A. Guttová, E. Lisická, P. Lizoň (Eds.) Central European Lichens - Diversity and Threat, pp. 193-204. Mycotaxon, Ltd., Ithaca and Institute of Botany, Slovak Academy of Sciences, Bratislava.
- Lange O.L., Green G.A., 1996. High thallus water content severely limits photosynthetic carbon gain of central European epilithic lichens under natural conditions. *Oecologia* 108, 13-20.
- Lange O.L., Green G.A., 1997. High thallus water contents can limit photosynthetic productivity of crustose lichens in the field. In: R. Türk, R. Zorer (Eds.): Progress and Problems in Lichenology in the Nineties. *Bibliotheca Lichenologica*, pp. 81-99. J. Cramer, Berlin, Stuttgart.
- Lange O.L., Green G.A., Reichenberger H., 1999. The response of lichen photosynthesis to external CO₂ concentration and its interaction with thallus water-status. *Journal of Plant Physiology* 154, 157-166.
- Luther H., 1954. Über Krustenbewuchs an Steinen fließender Gewässer, Speziell in Südfinnland. *Acta Botanica Fennica* 55, 1-61.
- Marini L., Nascimbene J., Nimis P.L., 2011. Large-scale patterns of epiphytic lichen species richness: photobiont-dependent response to climate and forest structure. *Science of the Total Environment* 409, 4381-4386.
- Martellos S., 2010. Multi-authored interactive identification keys: The FRIDA (FRiendly IDentificAtion) package. *Taxon* 59, 922-9.
- Motiejunaite J., 2003. Aquatic lichens in Lithuania. Lichens on submerged alder roots. *Herzogia* 16, 113-121.
- Nascimbene J., Nimis P.L., 2006. Freshwater lichens of the Italian Alps: a review. *International Journal of Limnology* 42, 27-32.
- Nascimbene J., Thüs H., Marini L., Nimis P.L., 2007. Freshwater lichens in springs of the eastern Italian Alps: floristics, ecology and potential for bioindication. *International Journal of Limnology* 43, 285-292.
- Nascimbene J., Thüs H., Marini L., Nimis P.L., 2009. Early colonization of stone by freshwater lichens of restored habitats: a case study in northern Italy. *Science of the Total Environment* 407, 5001-5006.
- Nascimbene J., Martellos S., Nimis P.L., 2010. An integrated system for producing user-specific keys on demand: an application to Italian lichens. In: P.L. Nimis, R. Lebbe (eds), *Tools for identifying Biodiversity: Progress and Problems*, pp. 151-156. ISBN 978-88-8303-295-0, EUT, Trieste.
- Nascimbene J., Spitale D., Thüs H., Cantonati M., 2011. Congruencies between photoautotrophic groups in springs of the Italian Alps: Implications for conservation strategies. *Journal of Limnology* 70 (Suppl. 1), 3-8.
- Nordin A., 2002. Du Rietz's lichen collections 1956-1965 from riverbanks and shores of lakes in connection with planned water regulations. *Thunbergia* 32, 1-26.
- Orange A., 2012. Semi-cryptic marine species of *Hydropunctaria* (*Verrucariaceae*, lichenized Ascomycota) from north-west Europe. *Lichenologist* 44, 299-320.
- Pentecost A., 1977. A comparison of the lichens of two mountain streams in Gwynedd. *Lichenologist* 9, 107-111.
- Ried A., 1960a. Stoffwechsel und Verbreitungsgrenzen von Flechten I. Flechtazonierung an Bachufern und ihre Beziehungen zur jährlichen Überflutungsdauer und zum Mikroklima. *Flora* 148, 612-638.
- Ried A., 1960b. Stoffwechsel und Verbreitungsgrenzen von Flechten. II. Wasser- und Assimilationshaushalt, Entquellungs- und Submersionsresistenz von Krustenflechten benachbarter Standorte. *Flora* 149, 345-385.
- Rosentreter R., 1984. The zonation of mosses and lichens along the Salmon River in Idaho. *Northwest Science* 58, 108-117.
- Santesson R., 1939. Über die Zonationsverhältnisse einiger Seen im Anebodagebiet. *Meddelanden från Lunds Universitets Limnologiska Institution* 1, 1-70.
- Thüs H., 2002. Taxonomie, Verbreitung und Ökologie silicicoler Süßwasserflechten im außeralpinen Mitteleuropa. *Bibliotheca Lichenologica* 83, 1-214.
- Thüs H., Nascimbene J., 2008. Contributions toward a new taxonomy of Central European freshwater species of the lichen genus *Thelidium* (*Verrucariales/Ascomycota*). *Lichenologist* 40, 1-23.
- Thüs H., Schultz M., 2008. Freshwater flora of Central Europe. Vol. 21/1, Fungi, 1st part Lichens. Heidelberg: Spektrum.
- Thüs H., Muggia L., Pérez-Ortega S., Favero-Longo S.E., Joneson S., O'Brien H., Nelsen M.P., Duque-Thüs R., Grube M., Friedl T., Brodie J., Andrew C.J., Lücking R., Lutzoni F., Gueidan C., 2011. Revisiting photobiont diversity in the lichen family *Verrucariaceae* (Ascomycota). *European Journal of Phycology* 46, 399-415.