Artikel: How to arrange the diversity of a tropical lichen family? : Systematics and generic concepts in the lichen family Graphidaceae

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How to arrange the diversity of a tropical lichen family?

Systematics and generic concepts in the lichen family Graphidaceae

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Short introduction to lichens in general

The famous botanist Carl von Linné considered the lichens to be just «rustici pauperrimi» – the poor trash of vegetation – and he did not even distinguish families or genera of lichens but classified them all in one genus – «lichen». Admittedly, most lichens have rather inconspicuous greyish or greenish thalli but some are producing yellow, orange or red pigments, and the variability of their growth forms (crustose, foliose, shrub-like or even barbe-like) and their fruiting bodies (round or elongate, open or closed, exposed on podetia or hidden in the thallus) is fascinating. In the 19th century many botanists became interested in this group of organisms and started to investigate them in more detail – such as the two famous botanists of Geneva Augustin Pyramus de Candolle and Jean Müller Argoviensis.

But it took quite a lot of time until their real nature was revealed: It was in 1869 when Simon Schwendener discovered that lichens are not one organism but composed of two – a fungus and an algae living together in a well organised symbiosis. However several decades had to pass before this new discovery was accepted by most lichenologists. The mycobiont (the fungal partner) is the more dominant part of this symbiosis, being responsible for the growth form and being able to reproduce sexually while the algal partner (photobiont) is less specific and restricted to vegetative reproduction. Therefore systematic and classification of lichens always refer to the fungal partner.

Lichen systematics

It was an important aim of lichenology not only to describe new species but also to «put in order» this great variability and diversity – i. e. to develop a hierarchical system. This system should reflect similarities and possible relationships between taxa. But soon the question arose on which characters the system should be based on. If we look back in the history of lichenology we see that the point of view what are important characters for the lichen systematic changed considerably from time to time. Very often the development of new techniques lead to the discovery of new characters which were considered to be essential for a «modern» system.

The first lichen systems were mostly based on the growth form of the thallus: Crustose thalli were considered to be less evolved («primitive»); foliose and shrub like thalli high evolved.

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However some time later the fruiting body (the ascocarp) thus the reproductive part of the lichen came into the focus of the lichenological research. While first the texture or character of the margins (black, round, with or without algae) were observed, later also microscopic features were investigated in detail – and for a long time spore characters were most important for the systematic of lichens.

At the beginning of the 20th century the chemical exploration of the lichens started: The so called lichen acids and their chemical constitution were discovered and had a great impact on the systematic and taxonomy of lichens. At the end of the last century another new character, the ascus type, came into the focus of fungal (and lichenological) research while during the last 5-10 years molecular genetics provided new tools for lichen systematic studies. Nowadays differences of DNA sequences are used to investigate the relationships of lichens and to reconstruct phylogenetic trees.

Each new approach led to a better understanding of the relationships between taxa and improved the current phylogenetic system. Yet the perfect system still does not exist.

Special part – The lichen family Graphidaceae

With this history of lichen systematic in mind I want to focus now on my special subject, the family Graphidaceae.
Typical members of the Graphidaceae are characterised by whitish to greyish crustose thalli with black, «carbonised», elongate fruiting bodies («lirellae») (Fig. 1). The Graphidaceae are also called «script-lichens» and the probably best known species is *Graphis scripta*. Most species of the family favour the bark of trees as substrate, only very few are growing on rock or soil. The family Graphidaceae comprises more than 1000 species and the great majority of them occur in tropical rain forests or subtropical forests. So the tropics are the centre of diversity for this family – and we find there a great diversity and variability of ascocarp morphology and shape: There are species with fissure-like fruiting bodies having inconspicuous, pale, uncarbonised margins (Fig. 2A) or species with very prominent ascocarps and black, carbonised margins (Fig. 2B). The fruiting bodies of other species have uncarbonised but well developed margins with striation (Fig. 2C), or the ascocarps are clustering together and are located in special parts of the thallus (so called «stromata», Fig. 2D, E, F). We also find species with conspicuous red or orange coloured ascocarps (Fig. 2H, J) or species with more or less round fruiting bodies (Fig. 2I, K), a feature that is quite uncommon within the family Graphidaceae.

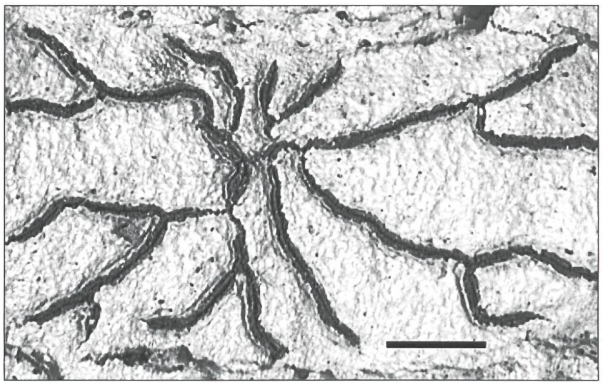


Fig. 1: Graphis duplicata, *a typical member of the lichen family Graphidaceae, with black (carbonised) elongate fruiting bodies, bar = 1 mm.*

And the question was how to arrange this great variability in a systematic order. What is a suitable natural system that reflects phylogenetic relationships between taxa and how can we distinguish genera?

Generic concepts in the family Graphidaceae

If we look back on the early 19th century several competing, parallel generic concepts existed. However these concepts were not very clear or concise and many lichenologists did not follow any of these but placed all species with elongate ascocarps in the genus *Graphis*. It was finally Jean Müller Argoviensis (1880, 1882) who developed a «modern» system based on spore characters for the Graphidaceae. This system was widely accepted by most lichenologists and also adopted by Zahlbruckner (1926) in Engler & Prantls important oeuvre «Die natürlichen Pflanzenfamilien». Müller Argoviensis (loc. cit.) considered the spore colour and the spore septation as most important characters for the delimitation of genera. He distinguished species with hyaline (colourless) or brown spores, having transverse septa only or transverse and longitudinal septa (muriform spores). Based on these differences Müller Arg. described four main genera: *Graphis*, *Graphina*, *Phaeographis* and *Phaeographina* (Fig. 3). His system allows a quite easy determination of the genera but it has several drawbacks and does not fulfil the criteria for a natural system, as the following examples will show

1. Hyaline spores may become brown when degenerating and several species have (wrongly) been placed in brown spored genera as degenerated spores were present in the type specimen.

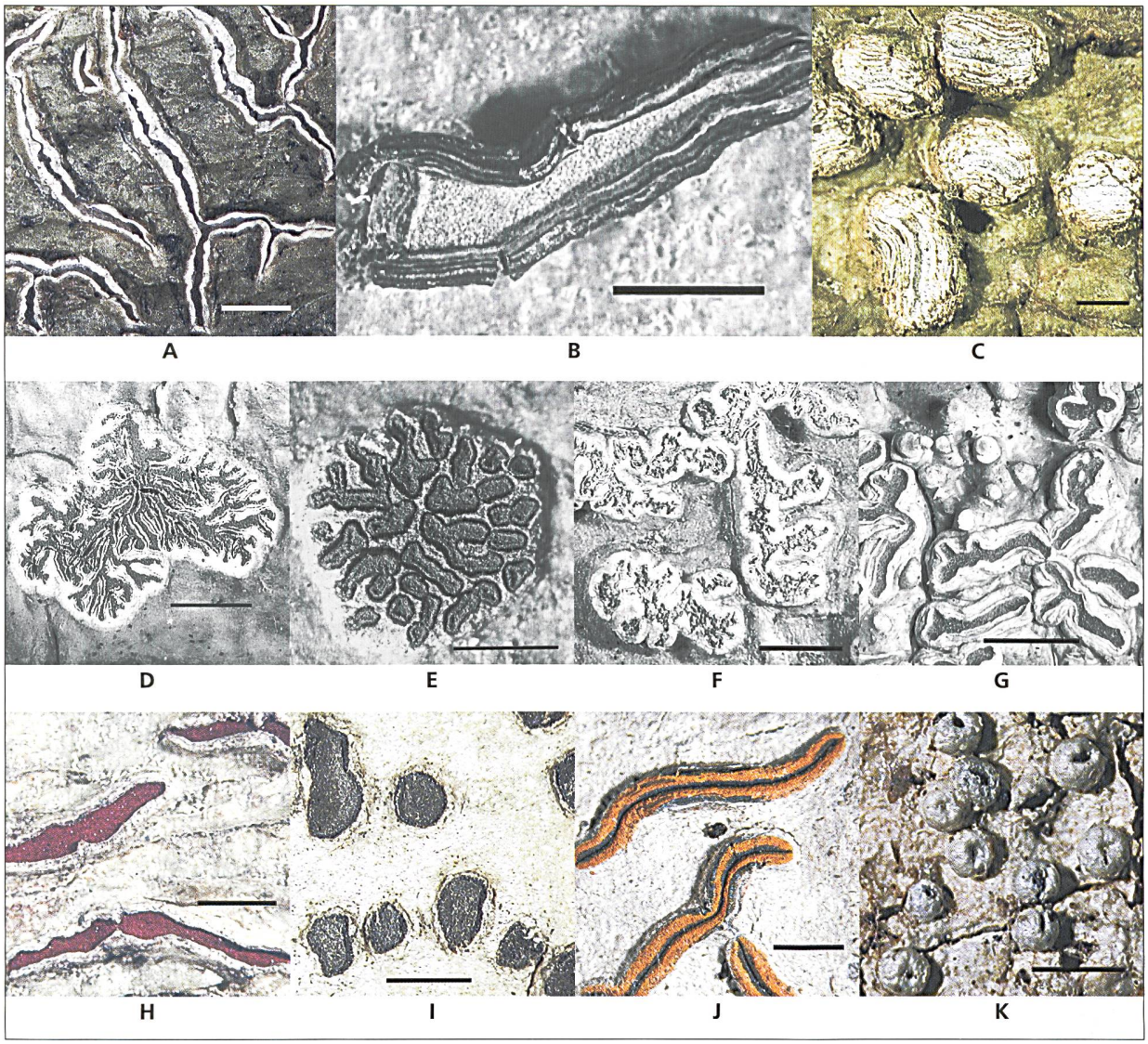


Fig. 2: Ascocarp variability within the family Graphidaceae.
A: Fissurina dumastii; *B:* Platygramme australiensis; *C:* Acanthothecis subclavulifera; *D:* Sarcographa cinchonarum; *E:* Glyphis cicatricosa; *F:* Sarcographa difformis; *G:* Platythecium dimorphodes; *H:* Thecaria montagnei; *I:* Phaeographis lobata; *J:* Graphis chrysocarpa; *K:* Graphis muscicola; *bar = 1 mm.*

2. The presence of one additional longitudinal septum changes the generic affiliation of species. Therefore many species with similar or corresponding morphological characters (but different spore septation) have been placed in different genera (Fig. 4).

3. Spore based genera are inhomogeneous in respect of other morphological characters. Fig. 5 shows species placed in the genus Graphis – they all have hyaline transversely septate spores but differ considerably in respect of morphology and anatomy of the fruiting body.

But even when these problems became evident, no alternative concepts were developed and the spore based system survived for more than 100 years.

Therefore I started to study the species of the family Graphidaceae (with main focus on the old generic names and their types) in order to develop a new, more natural generic system.
A special emphasis was laid on the examination of morphological and anatomical characters as well as on the analysis of the chemical compounds.

Results and new generic concepts (Staiger 2002)

In the course of my investigations several characters turned out to be constant within species groups and so suitable for the generic delimitation e. g.:

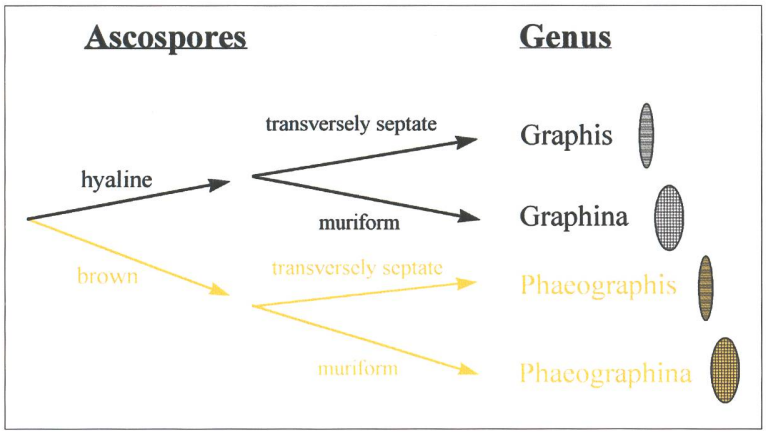


Fig. 3: The system of spore genera by Müller Arg. (1880, 1882).

- The exciple morphology with characters like carbonisation, texture or overall shape.
- The paraphyses-type with differences in pigmentation and branching of the tips as well as the nature of the cell wall (e. g. jelly-like, thick, thin).
- The ascospore-type i. e. the colour, the presence of wall thickenings and the colour reaction with iodine (brown spores turn red with iodine, hyaline spores turn blue-violet with iodine or show no reaction).

Homogenous species groups in respect of these characters or character combinations could be detected and used as a basis for a new generic division of the Graphidaceae. Three of these genera are introduced here in detail:

The genus* Fissurina *(Fig. 6):

The species placed in this genus are characterised by fissure-like, inconspicuous fruiting bodies. Only some species develop more prominent, slightly thickened margins. The exciple is usually poorly developed, and the spores are round to oval with jelly-like, often thick outer spore wall (halo).

The genus* Hemithecium *(Fig. 7)

Hemithecium-species have pale, often cream-coloured lirellae with crenate or flaky margins and concealed discs. The uncarbonised exciple is well developed and we could observe that in the same exciple new hymenia can develop several times resulting in multiple striations of the lateral exciple.

The genus* Platygramme *(Fig. 8)

Species belonging in the genus *Platygramme* have well developed, carbonised margins and open discs with greyish-white pruina. Cross sections through ascocarps (Fig. 8C) reveal that the carbonisation is usually restricted to the apical part of the fruiting body. In the lateral part of the exciple often crystals (Ca oxalate) are embedded.

Molecular genetic studies

Although most genera could be well circumscribed with the characters described above, some doubts remained in respect of the classification of other

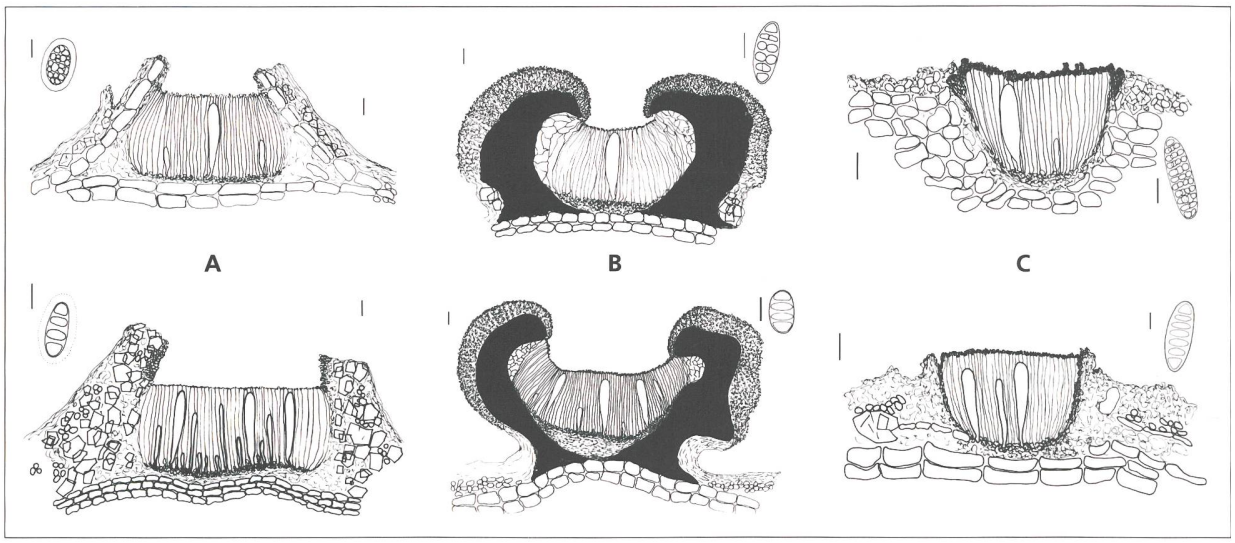


Fig. 4: Species-pairs with similar morphological characters being placed in different genera: A: Graphina cingalina *(above) and* Graphis dumastii *(below); B:* Graphina oryzoides *(above) and* Graphis afzelii *(below); C:* Graphina anguina *(above) and* Graphis hypolepta *(below); bar = 20 µm (cross sections through ascocarps) or bar = 10 µm (ascospores).*

species or species groups. Yet, these classification problems could neither be solved with «classical» microscopic methods nor with the analysis of secondary chemistry. Meanwhile in lichen systematics molecular genetic approaches are state of the art. These methods offer the possibility to integrate additional, independent, non-morphological characters and to develop phylogenetic hypothesis. Therefore we conducted a molecular study based on sequence data of two ribosomal genes: The mitochondrial small subunit ribosomal DNA (mtSSU) and the nuclear large subunit ribosomal DNA (nLSU). The detailed results of this study will be published in a forthcoming paper (Staiger et al. in prep.). I want to present here three examples how the molecular data gave answers to remaining questions:

1. When describing *Hemithecium* we were faced with the problem that the exciple morphology is very homogenous within this genus (see fig. 7), whereas two different ascospore types are present: Some species have hyaline, I+ blue ascospores while others have brown ascospores turning red with iodine (I+ red). In all other genera of the family the ascospore type is homogenous, indicating that this is an important generic character.

Is the exciple morphology more important than the ascospore type for the generic delimitation?

The phylogenetic tree based on the molecular data gave an interesting result:

One species of *Hemithecium* with hyaline spores (*H. implicatum*) is grouped together with *Graphis scripta* and other *Graphis*-species having hyaline spores but black, carbonised ascocarps. A second species of *Hemithecium* with brown ascospores clusters in a branch with all other species having brown ascospores of the same type. So the genus *Hemithecium* is not monophyletic. The molecular study clearly shows that the ascospore type is an important character in the phylogeny of the Graphidaceae while the excipular carbonisation seems to be of less relevance.

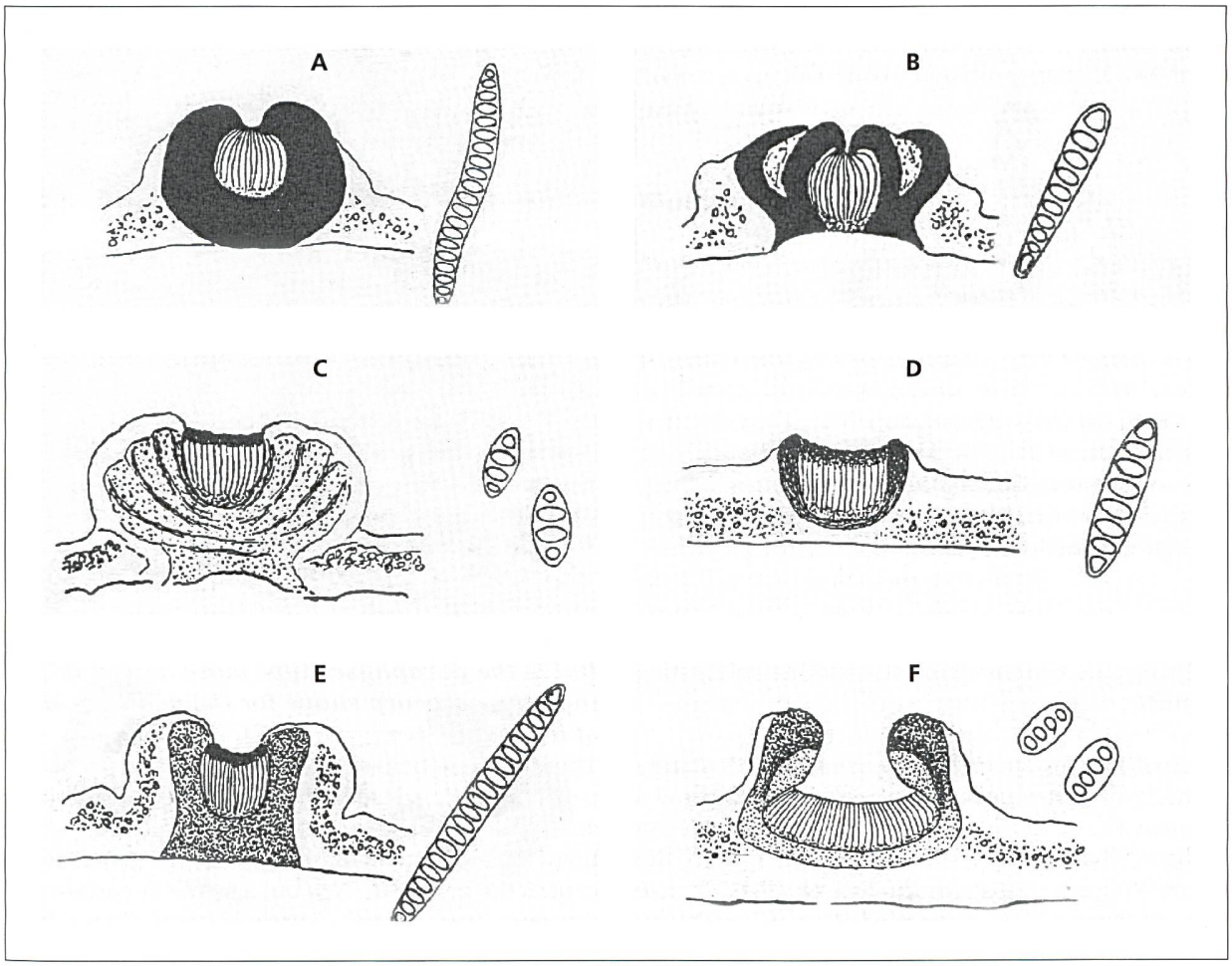


Fig. 5: Inhomogeneity of spore genera – the genus Graphis. *(drawings by Redinger 1936). A:* Graphis regularis; *B:* Graphis schizographa; *C:* Graphis bogoriensis; *D:* Graphis hypolepta; *E:* Graphis aphanes; *F:* Graphis javanica.

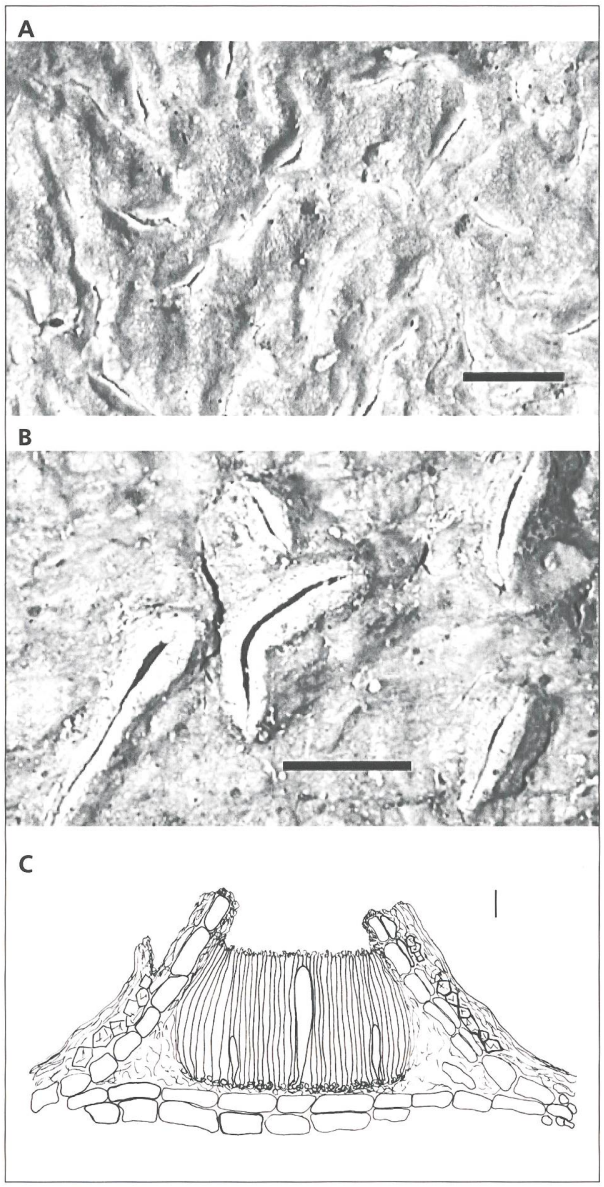


Fig. 6: Fruiting bodies of Fissurina-species*: A:* Fissurina columbina *with fissure-like, inconspicuous lirellae, bar = 1 mm; B:* Fissurina subcontexta *with slightly thickened margins, bar = 1 mm; C: Cross section through ascocarp of* Fissurina cingalina, *bar = 20 µm.*

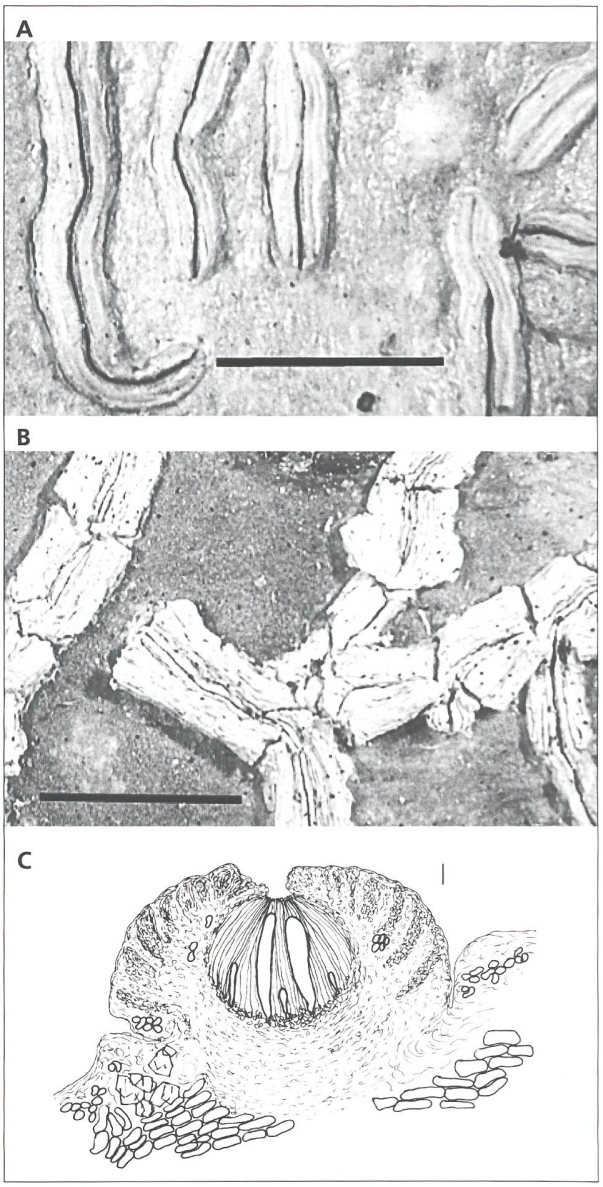


Fig. 7: Fruiting bodies of Hemithecium-species*: A:* Hemithecium chlorocarpum, *bar = 1 mm; B:* Hemithecium chrysenteron *with prominent, flaky margins, bar = 1 mm; C: Cross section through ascocarp of* Hemithecium balbisii *with distinct striation of the lateral exciple, bar = 20 µm.*

2. The genus *Glyphis* provides another interesting example:

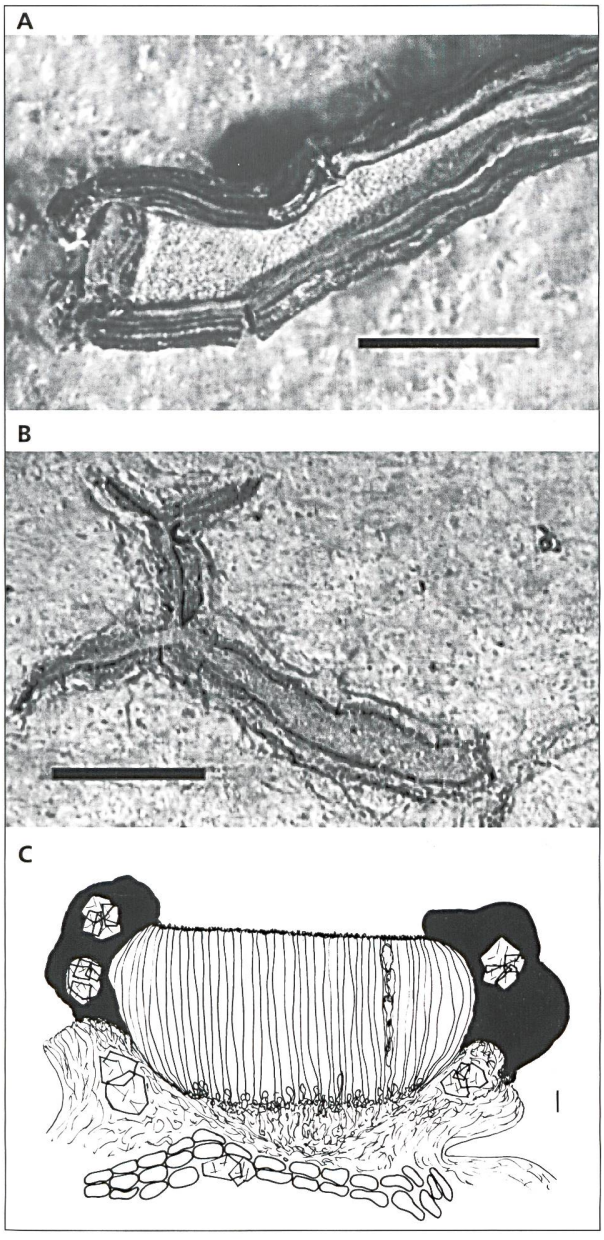
When examining species of this genus we constantly found a very unique paraphyses type with brown granular tips and thick jelly-like walls. However the ascocarp-shape differs considerably within the genus: *Glyphis substriatula* has elongate lirellae and slit-like, narrow, concealed discs (fig. 9A); in *Glyphis cicatricosa* the fruiting bodies are clustering together in a stroma (fig. 9B) and *Glyphis scyphulifera* has open, round (!) ascocarps (fig. 9C).

But is the paraphyses type more important than the ascocarp shape for the delimitation of a genus?

The phylogenetic tree shows that all species of the genus *Glyphis* – despite their differences in ascocarp shape – cluster together in one well supported branch. This result confirms not only the generic concept of the genus *Glyphis* but also the importance of the paraphyses type as generic character in the family Graphidaceae. It also indicates that lirelliform fruiting bodies may have evolved several times in the phylogeny of the Graphidaceae.

3. A difficult subject in systematic studies is the correct assignment of homologous characters. Superficial similarities may pretend homologies. In the family Graphidaceae we could find two species with carbonised ascocarps having very conspicuous «powdery» coverings:

Fig. 8: Fruiting bodies of Platygramme-species: *A:* Platygramme australiensis *with prominent, carbonised margins and open pruinose disc, bar = 1 mm; B:* Platygramme reticulata, *bar = 1 mm; C: Cross section through ascocarp of* Platygramme australiensis *with apically carbonised margins containing large crystals, bar = 20 µm.*



Dyplolabia afzelii (fig. 10) has a white covering containing the lichen substance lecanoric acid, while the covering of *Graphis chrysocarpa* (fig. 11) is orange and contains an antrachinone pigment.

Both species share several morphological similarities (fig. 12): The exciple is convergent and carbonised; the conspicuous covering contains not only granula of the lichen substances but also hyaline hyphae that develop from the carbonised part of the exciple below (and not from the thallus!).

But are these coverings really the result of one evolutionary «invention» (i. e are they homologous) or is it also possible that they have evolved twice in the phylogeny of the Graphidaceae i. e. the similarities are the result of a convergent evolution?

The phylogenetic analysis reveals that the two species are placed in very distant branches: *Dyplolabia afzelii* clusters together with *Fissurina*-species at the basal branch of the tree whereas *Graphis chrysocarpa* is grouped together with other *Graphis* species having no excipular cover. So despite the apparent similarities both species are not closely related; the coverings of *Graphis chrysocarpa* and *Dyplolabia afzelii* are no homologous characters but the result of two independent developments.

Summary

New generic concepts for the family Graphidaceae could be proposed based on detailed morphological studies of ascocarp features like exciple anatomy, paraphyses type and ascospore type (Staiger 2002). Additional molecular genetic investigations resulted in new insights in the phylogeny of the Graphidaceae and so offered the possibility to verify the new generic concepts. In addition the importance of morphological characters for the generic delimitation as well as their homology could be (at least partly) clarified. Three examples are presented:

1. The genus *Hemithecium* as previously characterised based on excipular morphology is not monophyletic as species with different ascospore types (brown/hyaline) cluster in different branches. Thus indicates that the ascospore type is of great relevance in the phylogeny of the Graphidaceae.

2. The genus *Glyphis*, characterised by a uniform paraphyses type is monophyletic despite great differences in ascocarp shape. So the paraphyses turned out to be crucial for the generic delimitation within the family.

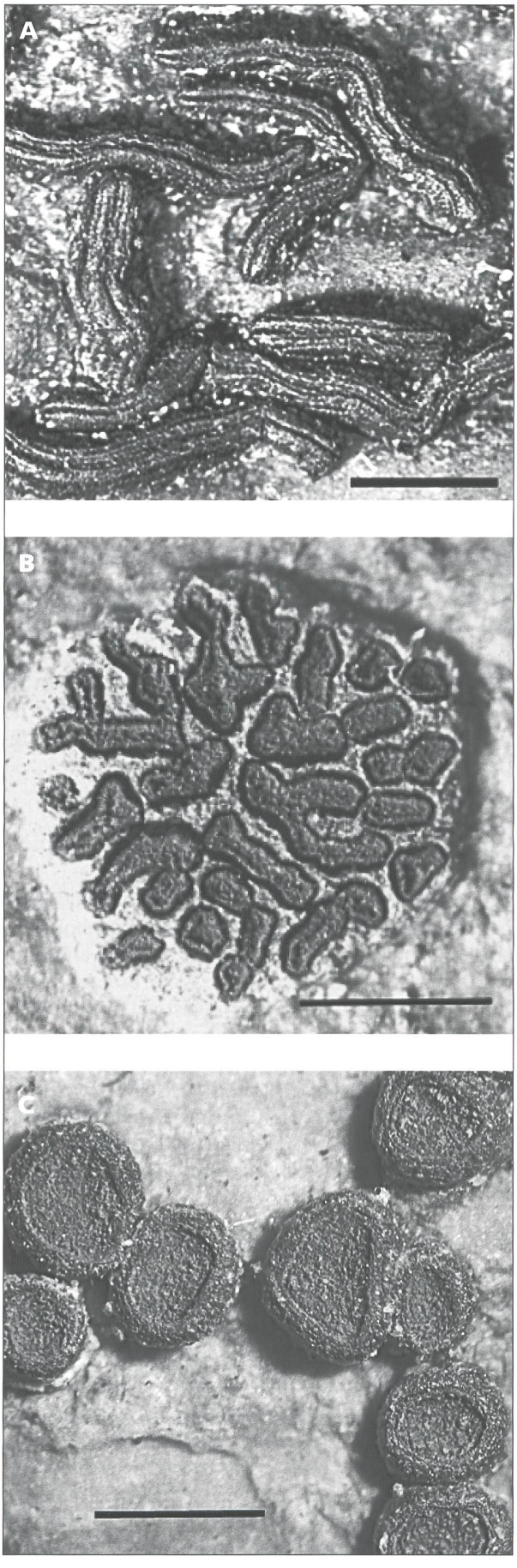


Fig. 9: Glyphis-*species with different ascocarp shapes. A:* Glyphis substriatula; *B:* Glyphis cicatricosa; *C:* Glyphis scyphulifera. *Bar = 1 mm.*

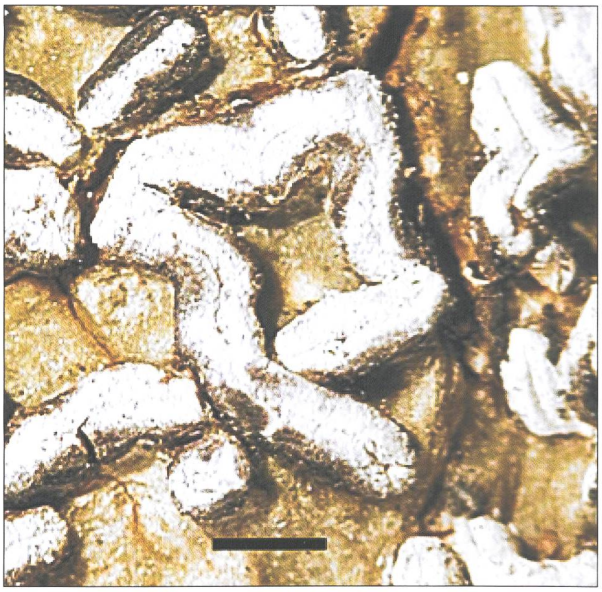


Fig. 10: Dyplolabia afzelii, *bar = 1 mm.*

Fig. 11: Graphis chrysocarpa, *bar = 1 mm.*

3. The conspicuous ascocarp coverings present in *Dyplolabia afzelii* and *Graphis chrysocarpa* are no homologous characters but must have evolved at least two times in the evolutionary history of the Graphidaceae.

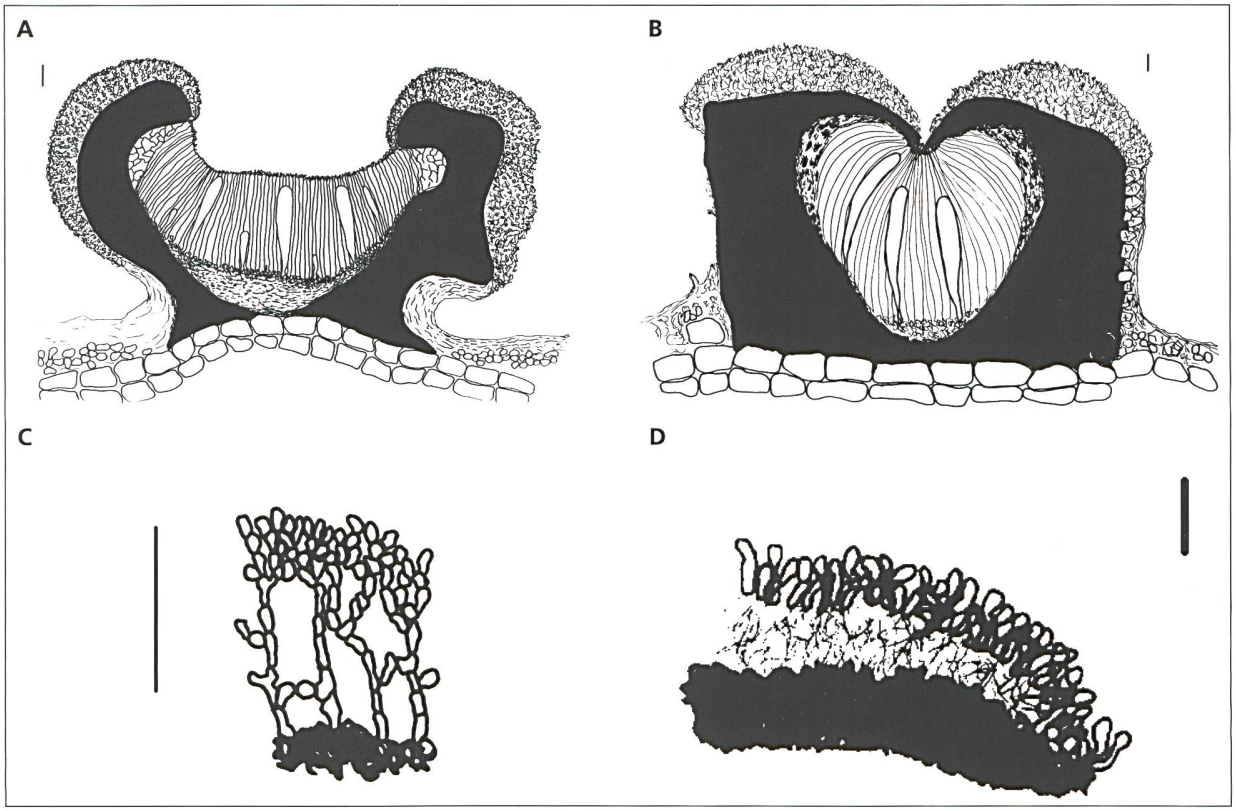


Fig. 12: Cross sections through ascocarps of Dyplolabia afzelii *(A) and* Graphis chrysocarpa *(B); details of the excipular coverings of* Dyplolabia afzelii *(C) and* Graphis chrysocarpa *(D) after removal of lichen acids and pigment granula. Bar = 20 µm.*

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