

# *Phormidium ingrediens* sp. nova (Cyanobacteria, Oscillatoriales), a limestone- boring euendolithic inhabitant of flowing waters

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## Abstract

In the upper Danube river-system, but also in some more watercourses of the German lower mountains range, an endolithic filamentous cyanobacterium is a prominent compound of the phytobenthos. These filaments penetrate limestones in the river bed and form brush-like endolithic layers of about 0.5 mm in depth. There are similarities between this taxon and approximately 10 other filamentous Cyanophyceae in terms of the width of the trichomes, the geometry of the cells and the shape of the apical cells. However, none of these species allow for a direct and clear-cut identification, and a precise taxonomic determination has therefore been unresolved until today. In order to make progress in this matter, it is necessary to get a better insight into the boring behaviour of this inhabitant and into the endolithic arrangements of the filaments. For this purpose, special preparation methods and SEM examinations were applied. The results of these procedures as well as critical comparisons with taxonomic properties of similar phormidian taxa, and the special demands on physico-chemical water conditions, led to the conclusion that the taxon in question cannot be assigned to a known species, but must be considered as an independent species. It is here described as *Phormidium ingrediens* species nova.

## Kurzfassung

***Phormidium ingrediens* sp. nova  
(Cyanobacteria, Oscillatoriales), ein kalkbohrender  
euendolithischer Fließwasserbewohner**

In der oberen Donau und ihren Quellflüssen, aber auch in einigen anderen Wasserläufen der deutschen Mittelgebirge, gehört eine endolithische fadenförmige Phormidiacee zu den auffälligen Komponenten des Phytobenthos. Die Fäden dringen bis zu einer Tiefe von ca. 0,5 mm in die Kalksteine der dortigen Flussgerölle ein und verleihen ihnen eine auffällige, leuchtend blaugrüne Färbung. Obwohl dieses Phänomen schon länger bekannt ist, steht eine genaue taxonomische Identifizierung dieses Taxons noch aus. Die Gründe hierfür liegen in der Schwierigkeit der Abgrenzung gegenüber einer größeren Zahl sehr ähnlicher Formen und in der bisherigen Unkenntnis des Thallusaufbaus innerhalb der Gesteinsmatrix. Um das Bohrmuster und die Lagerbildung im Gestein sichtbar zu machen, wurden in einem speziellen, aus der Literatur bekannten Araldit-Ausgussverfahren die Bohrgänge mit Harz verfüllt und nach Herauslösen der Gesteinsmatrix als freies Gerüst

dargestellt. Es zeigte sich ein büstenartiges Lager, eine Form, die bisher bei anderen Vertretern dieser Gattung nicht beobachtet wurde. Zusätzlich zu dieser Charakteristik konnten spezifische autökologische Ansprüche dieses Taxons aufgezeigt werden. Diese korrespondierten mit den Substratvorkommen und lokalen wasserchemischen Gegebenheiten hinsichtlich Gesamthärte, Pufferkapazität, elektr. Leitfähigkeit und pH-Werten, wie sie vorwiegend in den Übergangszonen kalkarmer, wenig gepufferter Gewässer aus dem Urgesteinsbereich in geologisch bedingte kalkreichere Regionen anzutreffen sind. Besiedelt werden Muschelkalk unterschiedlicher Feinstruktur, Jurakalke, diagenetisch veränderte Devonkalke und Marmor. Die Resultate der REM-Untersuchungen, die besonderen ökologischen Ansprüche und die kritischen Vergleiche mit ähnlichen Phormidiaceen-Species lassen den Schluss zu, dass das untersuchte Taxon keiner bekannten Species zuzuordnen ist. Es wird hier neu beschrieben als *Phormidium ingrediens* species nova.

Key words: Limestone boring, Cyanobacteria, endolithic, rivers, *Phormidium*, new species.

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## 1 Introduction

In the upper Danube river and its headwaters, but also in some more water courses in regions of German lower mountains, an endolithic filamentous cyanobacterium is a prominent compound of the phytobenthos. The settled Danube-limestones are conspicuous, because in these localities a bright sort of rocks meets with an intensive blue-green colour of the cyanophyte (fig. 1). In other regions were rocks tend to a darker brown the settlements appear more inconspicuous.

The taxon, which causes this phenomenon, at first was mentioned by BACKHAUS (1968 a, b) sub *Lyngbya* spec. In the last decades, further observations of this river inhabitant have been reported from other regions including Austria (KANN

1978), Eifel (FRIEDRICH, oral commun), Frankenstein (STEINBERG, written commun), Sauerland (GUTOWSKI and FOERSTER, oral commun). Nevertheless, a satisfying and clear-cut identification of this organism has until now not been achieved. In the present examination of materials from known places it became clear that a definitive description of this taxon can not be achieved without a deeper critical taxonomic comparison with a number of phormidian species which show very similar cell dimensions, and without a detailed knowledge of the boring behaviour and of the resulting pattern of the endolithic layers. To make progress in this field, special preparations of the colonized carbonates were necessary, including embedding methods with synthetic resin, dissolving the rock matrix, and SEM treatments. Based on the forthcoming results an assessment of the taxon is intended.

Several investigations into limestone-boring were done earlier (GOLUBIĆ et al. 1970, GOLUBIĆ 1973, GOLUBIĆ et al. 1975, SCHNEIDER 1977, PENTECOST et RIDING 1986, SCHNEIDER & LE CAMPION-ALSUMARD 1999, ARP 1999, PENTECOST 2003, GARCIA-PICHEL 2006), but there are lacking examples for carbonate boring Phormidians.

## 2 Materials and methods

The regional distribution of the cyanobacterium in question is focused on the flowing waters of the middle and southern Black Forest, where as a consequence of changes in the geological subsoil limestones increasingly appear. For the present investigation settled limestones from the river Breg near the town of Hüfingen and from the river Elz above the town of Emmendingen were applied.

### 2.1 Observations with light optical microscope

Fresh bright blue-green colonized limestones were transported cooled and wet into the laboratory, where small notches were cut into them using a scalpel blade. The excavations, as thin as possible, were directly transferred to the microscope and examined without further preparation. The light optical microscope photographs were taken with a NIKON-Coolpix 995 digital camera (3,34 megapixel), connected with a CARL ZEISS Photomicroscope I. Sketching the drawings was supported by the use of the "Great ZEISS drawing apparatus".

### 2.2 SEM examinations

In order to obtain information about the boring procedure and the three-dimensional pattern of the endolithic thallus, two different steps of investigation were needed: (1) The inspection of fractured areas, (2) the release of resin-embedded endolithic parts of thallus from the limestone matrix.

#### 2.2.1 Preparation of open fractures

Densely colonized limestones, just taken from the river bed, were divided by hammer and chisel into little blocks and fixed in 4 % formaldehyde for 24 hours. They were then processed step by step through increasing alcohol-water and alcohol-acetone solutions until 100 % acetone was reached. Afterwards the objects were dried following the "critical point" -method in order to avoid any shrinkage. Only just before the SEM-procedure started, a little piece of dried limestone was broken up at a prepared position (sawing), treated according to the usual SEM-preparation methods, and examined immediately so as to prevent abrasions of the area in the interim.

#### 2.2.2 Embedding – casting technique

After fixing in 4 % formaldehyde, pieces of recently sampled limestones were treated following the method given by GOLUBIĆ et al. (1975): Putting the fixed objects into NaOCl (Clorox, MERCK) for 24-48 hours, rinsing with tap water and gradually increasing the acetone content to 100 %. During this process, negative pressure was temporarily applied (3 hours in an exsicator) in order to remove possible small air bubbles out of the pores. From acetone the objects were transferred into a covered vessel containing a 1:1 mixture of acetone : Araldite (compl.). Used Araldite: CARL ROTH, Karlsruhe, type 11 (CY212), 1:1 composed with hardening agent HY 964, incl. accelerator 964 (DY 064). The time in the acetone-resin solution lasted 6 hours in total. After 3 hours, the lid was taken away in order to start the evaporation process of the acetone. The following transfer into a pure complete Araldite-mixture was performed in little troughs moulded out of copper foil. In order to achieve the hardening of the resin, the troughs remained uncovered over night at room temperature and were then heated at 60 °C for 48 hours. From the hardened blocks the overlapping resin was sanded down and the stone broken up to provide open areas for acid treatment. The carbonate substrate was etched by 2 % hydrochloric acid. The remaining Araldite-framework of

the cyanophycean thallus was then coated with metal under vacuum, studied using SEM and depicted.

### 3 Results and discussion

The gravels and cobbles containing endolithic layers of the cyanophyte in focus mostly showed a smooth surface without overlaying parts of the thallus. Biogenic deposits of calcium carbonates were not observed. In sawed stones a peripheric  $\pm 0.5$  mm deep bluegreen band within the calcareous matrix became evident (fig. 2). After mashing of little pices from this zone, single filaments or bundles of filaments could be made visible, but without elucidating the construction of layers within the rock matrix.

The filaments had diameters of 3,5-4,5  $\mu\text{m}$ , extremes reached 3-5  $\mu\text{m}$ . They were remarkably straight and stiff, and only rarely bent. The lyngbya-like sheaths were thin, firm and colourless, not lamellated and could not be stained with chlorine-zink-iodide-solution.

The trichomes were bright blue-green, (2,2) 2,9-3,7 (4,4)  $\mu\text{m}$  wide, straight, neither attenuated at the ends nor capitate. Apical cells were gently rounded to obtuse conical, without calyptra. Over the whole length of the trichome the cells were uniformly isodiametric, only in zones of active cell division somewhat shorter or longer than broad, immediately after division 2,2-2,5  $\mu\text{m}$ , later on 3-4,4 (5)  $\mu\text{m}$  long, not constricted at the cross walls and not, or very slightly, granulated. The cross walls themselves were mostly hardly visible (figs 4-6). Within the cells the centroplasma was quite noticeably separated by a pale colour from the strong blue-green coloured peripheric chromatoplasma. This special feature is discussed by KANN (1978) to be a phenomenon of light conditions within the rock matrix.

Following the nomenclature of ANAGNOSTIDIS & KOMÁREK (1988), KOMÁREK & ANAGNOSTIDIS (2005), HINDÁK (2008), species of filamentous Cyanophyceae with a phormidian trichome morphology have to be transferred to the genus *Phormidium*, despite the presence of sheaths. The taxon in focus is in line with that. In the following text for the present it will be denoted as "*Phormidium spec.*".

Using the above mentioned Araldite- and SEM-applications the boring behaviour of the filaments and the framework of the endolithic thallus became visible (figs 7-14). Fig. 7 shows an overall

view of the distribution of the bore-canals within the rock matrix. The distances between the canals are in a range of only some  $\mu\text{m}$  and do not follow a preconceived pattern. It can be seen that the filaments penetrate the substrate directly without following micro-cracks or micro-channels revealing a clear euendolithic behaviour. The sheaths obviously were withdrawn during the boring process (fig. 8). Higher magnification shows that the bored tunnels were perfectly circular and the crystal lamellae immediately penetrated (figs 9, 10). The smooth insides of the boreholes indicate that the filaments filled the canals almost completely. The width of the boreholes of 3-4  $\mu\text{m}$  in the majority of cases corresponded with that of the filaments. These results show "*Phormidium spec.*" as an euendolithic life form.

The casting procedure left the canals filled with Araldite. After etching the substrate the tunnels remained in their original position, showing a forest of micro-pillars. In this view the brush-like form of the thallus appeared clearly (figs 11, 12). It can be concluded that the filaments invade the stones  $\pm$  parallel perpendicular to the surface. They reach a length of max. 300  $\mu\text{m}$ . At the surface of the stones the outside of the thalli seem to be abraded by gravel movings.

The front parts of columns appear slightly thickened and club-shaped with a scaly surface (Figs 13, 14). Here the cleavage planes of the calcite crystals are apparent in the same manner as they were depicted in a *Hormonema*-preparation by GOLUBIĆ et al. (1975, fig.12.7 B). The club-shaped swellings at the front could be a result of continuing boring activities at the top of the filaments, whereas a further penetration of the matrix is no longer possible as light-compensating borderline conditions had been reached.

#### 3.1 Comparison with similar species

It remains to be clarified how "*Phormidium spec.*", specified through the mentioned features, is related to taxa which are already represented in scientific literature. It is possible that the building of endolithic thallus described above is only one of several possible forms of life of a *Phormidium* species, which otherwise is known as a epilithic form from lotic waters.

Comparisons were done with species descriptions and remarks from the following literature: GEITLER (1932), ELENKIN (1949), DESIKACHARY (1959), PRESSCOTT (1962), STARMACH (1966), KANN (1978), JOHN et al. (2002), KOMÁREK et ANAGNOSTIDIS (2005).

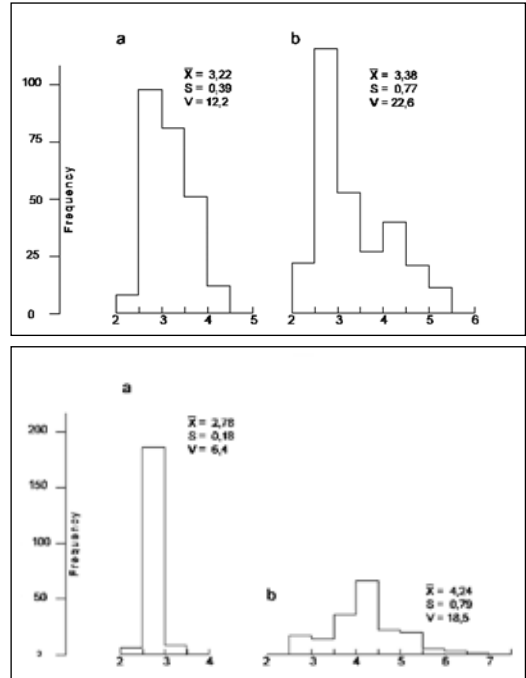
With reference to the cell dimensions, the cell morphology and the shape of apical cells, “*Phormidium spec.*” showed similarities with *Phormidium corium*, *P. inundatum*, *P. taylori*, *P. rimosum*, *P. incrustatum*, *P. umbilicatum*, *P. toficola*, but also *Leptolyngbya vandenberghenii*. Common to all are trichome diameters in the range of 3-5 (7)  $\mu\text{m}$ , more or less isodiametric cells and rounded or obtuse conical apical cells. As for “*Phormidium spec.*”, a conspicuous bright blue-green colour of the thallus is ascribed to most of them. For the present purpose they are separated into two groups: (1) species which do not deposit calcium carbonate, (2) species encrusting calcium carbonate. *Phormidium kuetzingianum* (KIRCHNER) ANAGNOSTIDIS et KOMÁREK 1988 and *Phormidium papyraceum* GOMONT ex GOMONT 1892 are not taken into consideration here (despite similar trichome diameters), because they are subaerophytes and settle entirely different biotopes. A brief overall view is given also in Table 1.

(1) Species that do not induce precipitation of calcium carbonate

#### *Phormidium corium* GOMONT 1892

Comparing “*Phormidium spec.*” and *P. corium* a far reaching similarity in the shape of apical cells becomes evident. They are both gently bulged to obtusely conically rounded. Additionally the trichome diameters seem to differ insignificantly. These aspects make it necessary to take both formes into a more detailed examination.

With regard to the cell dimensions frequency histograms and statistics for the cell width and cell length were established for both following the procedure of PENTECOST (2003). Fig. 15 shows the mean of cell width in “*Phormidium spec.*” to be 3,2  $\mu\text{m}$ , and the cell length 3,4  $\mu\text{m}$ . In fig.16 the same measurements for *Phormidium corium* are 2,8  $\mu\text{m}$  (width) and 4,2  $\mu\text{m}$  (length). To avoid a random choice of data, samples of three different locations were used each time. The different results of the cell dimensions may be the initial stage in distinguishing both taxa. The gap between them becomes greater if relating data given in the literature are taken in consideration. Very variable dimensions of trichome widths of *Phormidium corium* are listed, and only the smaller ones are compatible with those of “*Phormidium spec.*”. KANN (1978), with regard to her extensive material, indicates trichome diameters in the majority of > 4,5  $\mu\text{m}$ .



Figs 15, 16. Frequency histograms and statistics for the cell width (a) and cell length (b) of “*Phormidium spec.*” (fig. 15) and *Phormidium corium* (fig. 16), established from samples of three different locations,  $n = 200$  each time.

Even more the differences in the consistency of the thallus are striking. *P. corium* usually forms long, variously curved and densely entangled filaments (figs 17, 18) and a membranaceous or leather-like thallus. It is not plausible that precisely the cosmopolitan and probably ubiquitous *P. corium* should develop a totally different lifestyle as an endolith in the ecologically inconspicuous Black Forest streams, in contrast to its usual and typical behaviour as surface-living form. KANN (1978, p. 441) questioned whether *P. corium* could possibly be carbonate boring, but she simultaneously emphasized the resemblance and possible identification of her *P. corium*-specimens with the endolithic *Lyngbya spec.* mentioned by BACKHAUS (1968 b) as present in the Black Forest streams. The question about the boring ability of *P. corium* therefore should be taken up with some scepticism and answered in the negative. The negative assessment is strengthened considering the fact that in spite of many discoveries of this taxon, no other observations dealing with this aspect are

mentioned in the literature. Furthermore, the different reactions towards the staining of sheaths with chlorine-zinc-iodine-solution are of particular importance: *P. corium* reacts positively, “*Phormidium spec.*” negatively. At last we must consider the different autecology of both taxa: In contrast to the wide ecological valence of *P. corium*, as it can be deduced from its widespread occurrence, “*Phormidium spec.*” offers a distinct reference for streamwaters of specific physico-chemical character as shown further down.

*Phormidium inundatum* KUETZING ex GOMONT 1892

Just as said for *Phormidium corium* also for *P. inundatum* several remarks indicate a greater distance to “*Phormidium spec.*”: Greater dimensions of trichome widths and cell lengths, the positive staining with chlorine-zinc-iodine solution, the forming of membranaceous thalli and the colonizing of other biotopes than low scale chalk streams. Of additional particular importance may be the brief attenuation of the trichome ends as it is implied also for *Phormidium corium* (KOMÁREK & ANAGNOSTIDIS 2005), but is not found for “*Phormidium spec.*”. The noted granulations at the cross walls should not be a diacritical feature.

Looking at all their taxonomic criteria, *P. corium* and *P. inundatum* differ insignificantly, with the likely consequence that mix-ups and false assignments of samplings should be expected frequently. For example, even in the “Süßwasserflora von Mitteleuropa” (KOMÁREK & ANAGNOSTIDIS 2005), the same illustration appears for both *P. inundatum* and *P. corium* (figs 618 b and 660 d). Because of the occurrence as cosmopolitans as well as their similar autecological profiles, the combination of both taxa would be obvious. An identification with “*Phormidium spec.*” should be excluded for *P. inundatum* just as it is for *P. corium*.

*Phormidium taylori* (DROUET & STRICKLAND) ANAGNOSTIDIS 2001

The continuous straight line of the trichomes as well as the isogeometric shape of the cells combined with lowest diameters beginning at 4 µm initially make *P. taylori* appear to belong to the species group looking like “*Phormidium spec.*”. But in the majority cell diameters span 6-7 µm, and this aspect must be considered to be a significant difference in comparison to 2,9-4,2 µm of “*Phormidium spec.*”. In addition, slight constrictions at the cross walls are apparent. Finally, the benthic predominant occurrence in still waters

and the tuft-like structure of the thallus are to be taken into consideration. Taken together, the factors mentioned above make the identification of *P. taylori* with “*Phormidium spec.*” very doubtful.

*Phormidium rimosum* (KOMÁREK)

ANAGNOSTIDIS & KOMÁREK 1988

The following properties of *Phormidium rimosum* show similarities with “*Phormidium spec.*”: The potential chasmoendolithic habitation, the partly parallel-orientated filaments, the straight form of the trichomes without any attenuation, hardly visible cross-walls, and the slightly obtuse conical terminal cells. On the other hand, the differing features are clearly predominant: The total lower trichome widths of only 2,6-3,2 µm, the shape of the cells as distinctly shorter than wide, constrictions at the cross walls, which may be slight but obvious. Further differences are the conspicuously thickened sheaths and the presence of this species in wooden creeks, which are free of lime. With respect to these characteristics, the assignment of the carbonate-boring “*Phormidium spec.*” to *P. rimosum* should be excluded.

(2) Species with carbonate incrustations

*Phormidium incrustatum* GOMONT ex GOMONT 1892

*Phormidium toficol*a (NÄGELI) GOMONT ex GOMONT 1892

*Phormidium umbilicatum* (NÄGELI) GOMONT 1892

The mutual morphological differentiation of these three *Phormidium*-species from one another is difficult, because they are identical in essential features and differ only very slightly in the trichome widths. For *P. toficol*a and *P. umbilicatum*, the width-spans of 3-4,5 µm, respectively 3-4,8 µm are practically identical. They are embedded in the range of 3-6,5 µm for the trichomes of *P. incrustatum*. These aspects caused KANN (1973) to consider all three together as a taxonomic unity, since ELENKIN (1949) and STARMACH (1966) already pointed out these similarities. PENTECOST (2003), in his examination of *Phormidium incrustatum*, did not support this point of view. The taxonomic position of these three taxa, however, remains undecided until now since KOMÁREK & ANAGNOSTIDIS (2005) did not include *P. toficol*a into the revision of the phormidians and refer to *P. incrustatum* (p.425). For this reason, a separate discussion of the single taxa will be related to *P. incrustatum* only.

Table 1. Comparing determination-relevant taxonomic features of similar *Phormidium* species inclusive *Leptolyngbya vandenberghenii*.

Features	" <i>Phormidium</i> spec."	<i>Phormidium corium</i>	<i>Phormidium inundatum</i>	<i>Phormidium taylori</i>	<i>Phormidium rimosum</i>
Width of filaments µm	3,5 – 4,5				? – 3,7
Width of trichomes µm	2,9 – 3,7				2,6 – 3,2
Cell geometry	isodiametric	3 – 5,4 isodiametric - longer than wide	3 – 5 isodiametric, shorter or longer than wide	4 – 7 isodiametric (little shorter than wide)	mostly shorter than wide
Cell-length µm	3,5 – 5	3,4 – 6,4	3,3 – 8	2 – 7	1,4 – 3 (4,2)
Constrictions at the cross walls	no	no	no	no (-little)	no or little
Granulated at the cross walls	no or little	no	yes	no	no
Filament orientation	straight, stiff, parallel	long, curved, intertwined	± straight, entangled	long, parallel ± entangled	parallel – intertwined
Ending of trichome	straight, not attenuated	straight, not attenuated	briefly attenuated	straight, briefly attenuated	not attenuated
Apical cell	rounded, obtuse conical	rounded, obtuse conical	conical rounded	broad rounded	broad rounded
Calyptra	no	no	no	?	no
Sheaths	thin, firm, colourless, not lamellated	thin, firm, colourless, not lamellated	thin, mucilaginous	thin, stiff, colourless	thick, colourless
Stainable with chlor.-zinc-jod ?	no	yes	yes	?	?
Carbonate encrusting?	no	no	no	no	?
Carbonate boring?	yes	?	no	?	?
Form of thallus	brushlike within the rock matrix	leathery-membranaceous, substrate covering	membranaceous	?	thin, partly chasmoolithic

Contin. Tab. 1.

Features	<i>Phormidium incrustatum</i>	<i>Phormidium toficola</i>	<i>Phormidium umbilicatum</i>	<i>Leptolyngbya vandenberghenii</i>
Width of filaments $\mu\text{m}$	–	–	–	(2,5) 3,5 – 4
Width of trichomes $\mu\text{m}$	(3) 4 – 6,5	3 – 4,5	3 – 4,5	3 – 4,8
Cell geometry	shorter than wide (1/3 of cell-length)	isodiametric	isodiametric -	(isodiametric) –
Cell-length $\mu\text{m}$	2,3 – 5,6	2,3 – 5	shorter than wide	longer than wide
Constrictions at the cross walls	no (- little)	no	3 – 5	2,5 – 4 (-5)
Granulated at the cross walls	no	–	no	no (- little)
Filament orientation	straight – entangled	entangled	curved – parallel	straight, $\pm$ curved
Ending of trichome	straight, briefly attenuated	straight, briefly attenuated	straight, briefly attenuated	straight, briefly attenuated
Apical cell	hemispherical – conical rounded	obtuse – conical	obtuse – conical	rounded
Calyptra	?	no	no	no
Sheaths	thin, mucilaginous – lamellated (yellowish)	very thick, mucilaginous – lamellated	thick, diffluent	firm, colourless
Stainable with chlor.-zinc-iod.	no	no	yes	no
Ca-carbonate encrusting?	yes, very hard (yes)	yes, hard, petrified	yes, stony	yes
Carbonate boring?	wart-like	?	?	no
Form of thallus		spread (?)	wart-like	spread

PENTECOST (2003) indicates that *P. incrustatum*, known hitherto to only build up travertine deposits, during the beginning phase of settlement can also display a chalk-boring behaviour (reaching a depth of 100  $\mu\text{m}$ ). This ability brings it closer to "*Phormidium spec.*". In addition, possible small trichome widths as mentioned in the literature can overlap with those of "*Phormidium spec.*" (Table 1). However, the feature "width of the trichomes" needs a critical consideration in relation to *P. incrustatum*. In most of the common determination manuals as well as in the monographic examination by KANN (1973) the trichom widths of *P. incrustatum* are consistently indicated to be 4-5  $\mu\text{m}$ . Diverging from these remarks, KOMÁREK & ANAGNOSTIDIS (2005) increase the span to 3-6,5  $\mu\text{m}$ . The lower dimensions of 3  $\mu\text{m}$  may be a result of including the uncertain *P. toficola* and *P. umbilicatum*, or the result of single recordings in particular regions (such as Bihar, DESIKACHARY 1959, p. 269). For the characterization of *P. incrustatum*, they are considered to be of low value. This interpretation is confirmed by the results of PENTECOST (2003), who, based on the measuring of a great amount of *P. incrustatum*-trichomes, detected the predominant range of trichome width to be 5-6,5  $\mu\text{m}$  with a statistical mean of 5,7  $\mu\text{m}$ . Simultaneously, it was established that the cell lengths in general are 1/3 of the cell widths. These taxonomical key-characteristics are not compatible with the relationships of "*Phormidium spec.*", which has main trichome widths between 2,9 and 3,7  $\mu\text{m}$  only, combined with isodiametric cells.

*Leptolyngbya vandenberghenii* (SYMOENS)  
ANAGNOSTIDIS 2001

In relation to the geometry of the cells, outline of the trichome ends and the shape of apical cells, there may be prominent similarities between *Leptolyngbya vandenberghenii* and "*Phormidium spec.*". But the great difference between the trichome widths of both should exclude the mutual identification. The width of 2,3-3  $\mu\text{m}$  mentioned by SYMOENS is confirmed by JOHN et al. (2002) with reference to rivers in England. They are significantly lower than the span of 2,9-4,2  $\mu\text{m}$  established for "*Phormidium spec.*".

### 3.2 Ecological characteristics

The distribution of "*Phormidium spec.*" within the geologically widely diversified region of South-West-Germany gave indications of its ecological requirements. The local streams include waters

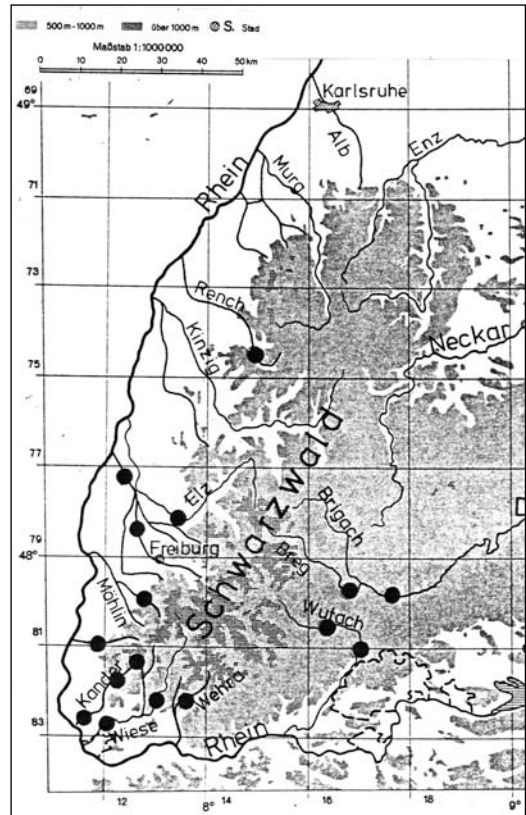


Fig.19. Places in the Black Forest where "*Phormidium spec.*" was found.

in pure granite- and gneiss-regions with corresponding "soft" water, rivers in granite-chalk transition zones to rivers in pure shell limestone and jurassic formations with corresponding extreme "hard" water (BACKHAUS 1973, Geologisches Landesamt B-W 1956). Within this scale of water hardness, the presence of "*Phormidium spec.*" was restricted to river sections containing freighted or occurring limestones and where the hardness did not exceed more than about 10 °dH. A low concentration of dissolved calcium with correspondingly low values of ABC (acid binding capacity), electric conductivity and pH seemed to play an important role. This leads to a particular map of distribution of "*Phormidium spec.*" in the Black Forest as shown in fig. 19. In streams with water hardness > 15 °dH and mean pH-values > 7,5 "*Phormidium spec.*" was missing, even though the same kinds of limestones were present. This



preference, deduced from regional conditions, could be a significant and further indication for the specific properties of "*Phormidium spec.*". The related physico-chemical water conditions in rivers of the Black Forest where "*Phormidium spec.*" was found, were recorded as: Total hardness: 1-8 (9,5) °dH (German degree of hardness); carbonate hardness: (0,1) 1-17 °dH; ABC: 0,14-2,5 mval (acid binding capacity); electr. conductivity: (35) 60-200 (335) µS/cm; pH: 6,5-7,5 (8).

The *Lyngbya spec.*, found by KANN (1978, p. 432) in the Irdningbach in Austria, probably is the same "*Phormidium spec.*" as present in the Black Forest streams. The Irdningbach is also situated in a region of waters with low calcium content and holds dispatched limestones in the river bed.

### 3.3 Conclusions

The ability of filamentous cyanophytes to deposit as well as to bore into limestone, as appears to have been demonstrated for *Phormidium incrustatum* by PENTECOST (2003), was already proven for *Schizothrix* by SCHNEIDER (1977) and SCHNEIDER & LE CAMPION-ALSUMARD (1999). This ability, however, seems to be restricted to few taxa only. After more than 100 years of cyanophyte research, however, more confirming observations of this phenomenon should have occurred, but this has not been the case. Limestone boring and calcification are two different processes not fully understood in the last details; and it is not yet clear how the first or second (contrasting) phase of activity is physiologically controlled (GEITLER 1960, SCHNEIDER & LE CAMPION-ALSUMARD 1999, GARCIA-PICHEL 2006). Active limestone boring by small filamentous species within narrow boreholes needs the ability for a specific intercellular Ca-transport (GARCIA-PICHEL 2006). This condition may be present in "*Phormidium spec.*". Whether this capacity is genetically fixed to distinct taxa and serves as distinguishing feature remains uncertain. Despite the wide distribution of "*Phormidium spec.*" combined with high abundances, no signs of a transition from endo- to epilithic forms of thallus were seen. This may be a sign of evidence for a clear demarcation of "*Phormidium spec.*" from the species definition subsumed for *Phormidium incrustatum*.

It may be possible that hormogonia of carbonate-encrusting species are also present in streams with low carbonate content, but in that environment they are not able to develop at full. However, if they meet carbonate rocks which can be bored, they take ad-

vantage of the endolithic lifestyle rather than not being able to develop outside. This scenario can be disproved by the ecological conditions in the headwater system of the upper Danube river. There, the carbonate content of the stream water increases after it passes from the gneiss- and granite-subsoil into Jurassic formations (BACKHAUS & SANDER 1967). The pressure for an endolithic way of life, therefore, should decrease, but no corresponding reactions were observed. Even in streams within pure Jura-rock strata of the south-western German mountain range "Schwäbische Alb" (such as Schmiecha, Lauchert), which have a hardness of water of 15-16 °dH, or in shell limestone streams with even a stronger hardness of up to 22 °dH, no epilithic thalli or travertines of "*Phormidium spec.*" were observed. These facts should be estimated as a further sign for the independence of "*Phormidium spec.*".

By which way the hormogonia (or filaments?) do force their entrance into the rock matrix against lotic flow conditions needs to be clarified by a specific examination. However, it seems to be plausible that this process can only be successful in combination with the presence of a sufficiently developed biofilm just in the function as starting support as shown by KEMMERLING et al. (2004) for comparable euendolithic settlements in rock biotopes.

### 3.4 Species description

Based on essential points of view as morphological differences from similar phormidians, the colonization of specific kinds of limestone, developing a typical, hitherto unknown brush-like thallus within the rock matrix, and the requirements of specific physico-chemical water conditions, "*Phormidium spec.*" is assessed to be a new species and is described here as

#### *Phormidium ingrediens nova species*

(Figs 20, 21)

Filamenta recta, circiter 300 µm longa, 3,5-4,5 µm crassa, lapides calcareos verticaliter perforantes, multis paralleliter adjuncta, aedificantes stratum usque ad 0,5 mm modo caespitis inversi vel peniculi in matrice peripherica lapidis; vaginae tenues, firmae, hyalinae, haud lamellosae, chlorocinco iodurato non coerulentes; trichomata (2,2) 2,9-3,7 (4,4) µm crassa, ad dissepimenta non constricta, non vel parum granulata, ad finem non attenuata, cellulae isodiametricae, (2,2) 2,9-4,7 (5) µm longae. Cellula apicalis rotunda vel conicalis; calyptra nulla; chromatoplas-

ma visitabiliter distincta a centroplasma, valide aeruginosa.

**Locus typicus:** Breg flumen (Danuvii fluv. flumen fontanum dextrum) ad oppidum Hüfingen (Donaueschingen), Germania.

**Habitatio:** Endolithice in lapidibus calcariis locatis in aquis fluentibus.

**Holotypus:** Materia deposita in herbario 'Botanischer Garten und Botanisches Museum Berlin, Germania, sub num.: B 40 0040626.

**Iconotypus:** Figura nostra 21, sub num.: B 40 0040628.

**Etymologia:** Nomen deductum a proprietate ingredi in materias calcareas.

Filaments straight, up to 300 µm long, 3,5-4,5 µm wide, penetrating limestones in the bed of rivers perpendicular to the surface, in great amounts densely arranged and in ±parallel, building up an approximately 0,5 mm thick thallus in the peripheral rock matrix looking like an inversed lawn or a brush. Sheaths thin, firm, hyaline, not lamellated, not stainable with chlorine-zinc-iodine-solution. Trichomes (2,2) 2,9-3,7 (4,4) µm wide, at the crosswalls not constricted, not or only slightly granulated, at the ends not attenuated. Cells isodiametric or – depending on the dividing process – somewhat shorter or longer than wide, (2,2) 2,9-4,7 (5) µm long. Apical cells rounded or obtuse conical, without calyptra; within the cells the stronger blue-green coloured peripheral chromatoplasma clearly distinct from the paler centroplasma.

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Fig. 1. Limestone with endolithic thallus of *Phormidium ingrediens* from the river Breg near the town of Hüfingen.

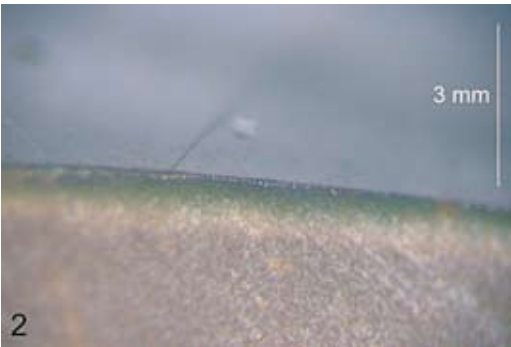


Fig. 2. Sawed limestone showing colonization of the periphery by the endolithic *Phormidium*.

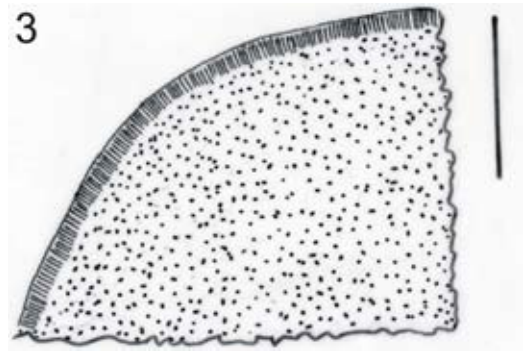
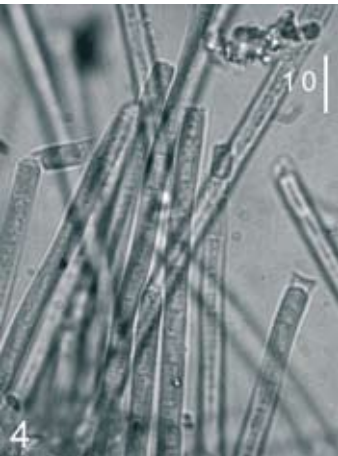
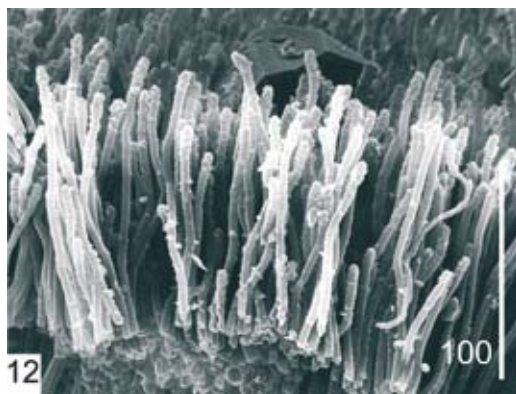
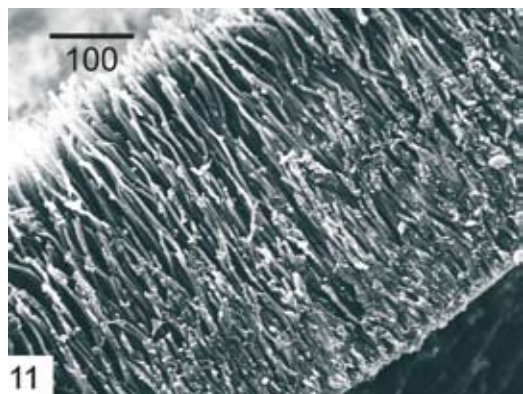
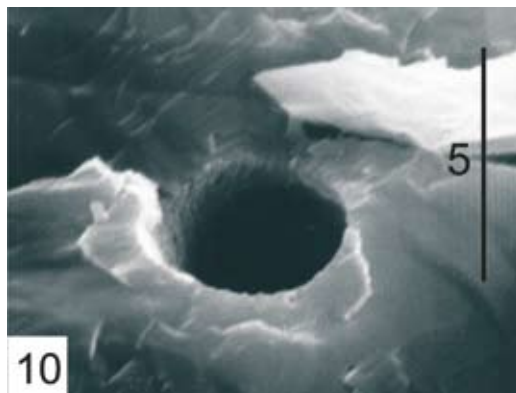
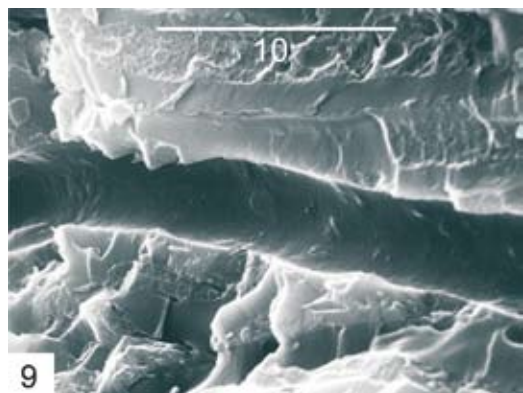
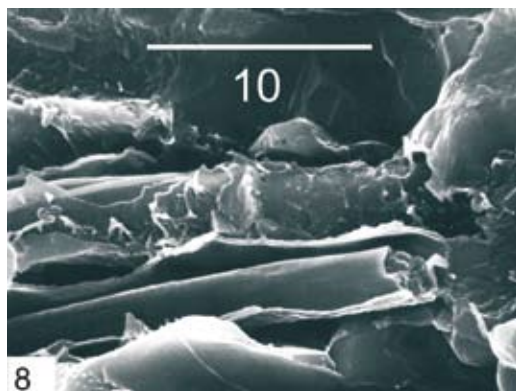
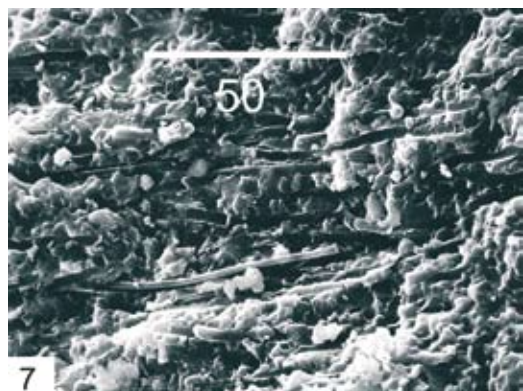


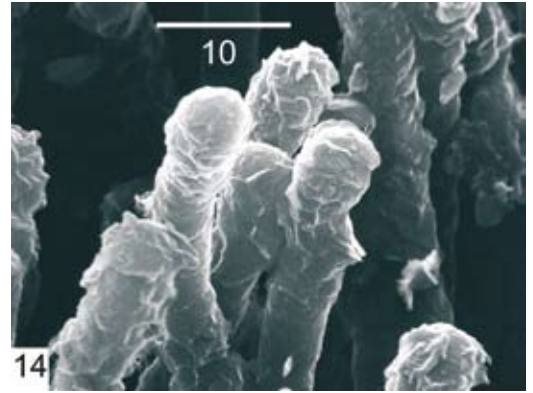
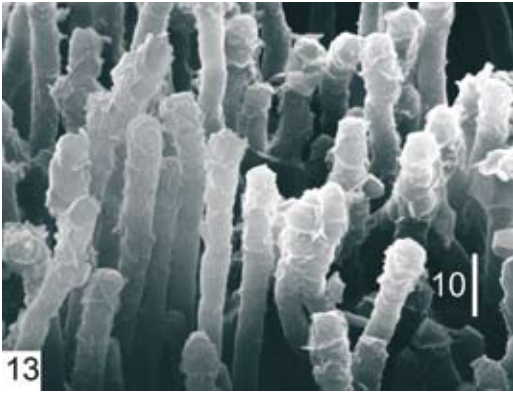
Fig. 3. Schematic design of colonization. Scale bar: 5 mm.



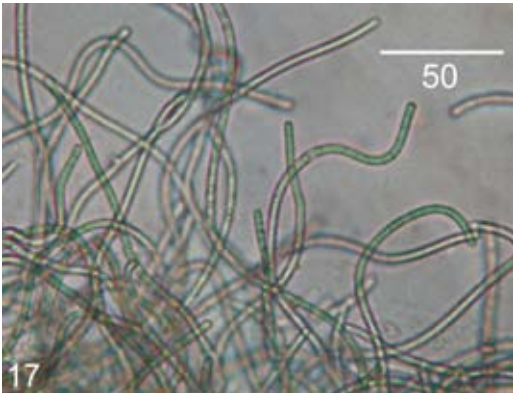
Figs 4-6. Endolithic *Phormidium*-filaments, prepared from limestones of the Black Forest-river Breg. Scale bars in µm.



Figs. 7-14. SEM of *Phormidium*-penetrated limestone before and after special treatment with embedding-casting method. – 7. Overview of broken stone; 8. Part with broken up sheath and fragment of a trichome within the stone matrix; 9. Broken up canal; 10. Cross section of borehole; 11-12. Overview of endolithic *Phormidium*-thallus in the peripheral zone of a shell limestone after resolving the stone matrix (vertical view). Scale bars in  $\mu\text{m}$ .



Figs 13-14. Front of Araldite-filled up boring canals showing the calcite crystal structure. Scale bars in  $\mu\text{m}$ .



Figs 17, 18. *Phormidium corium* from a rivulet in the southern Black Forest. Scale bars in  $\mu\text{m}$ .

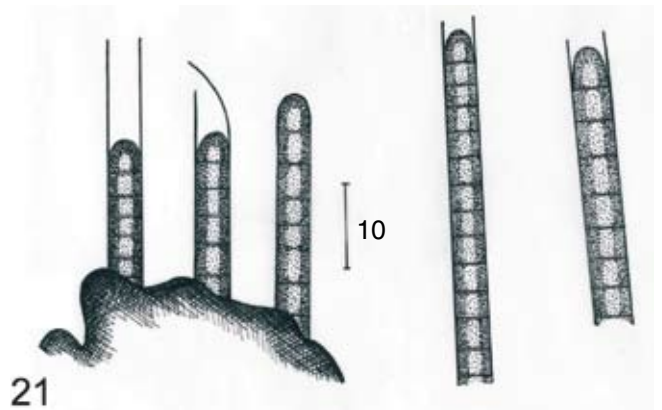


Fig. 20-21. *Phormidium ingrediens* from river Breg near Hüfingen, south-east edge of the Black Forest. Scale bar in  $\mu\text{m}$ .





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