

Notes on three species of *Pyxine* (lichenized Ascomycetes) from Tibet and adjacent regions

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Abstract: *Pyxine limbulata*, *P. microspora*, and *P. soredata* are reported for Tibet and adjacent Chinese regions. Notes on morphology (including photographs of thalli, apothecia, spores, and spermatia), TLC-data and a distribution map are presented. Semi-mature spores of *Pyxine limbulata* are optically compared with those of *Physcia aipolia*. As an appendix a list of *Pyxine* taxa collected in Nepal and housed in the herbarium GZU is provided.

Zusammenfassung: *Pyxine limbulata*, *P. microspora* und *P. soredata* werden für Tibet und angrenzende chinesische Gebiete angegeben. Anmerkungen zur Morphologie (mit Bildern von Thalli, Apothecien, Sporen und Spermation), TLC-Daten und eine Verbreitungskarte werden vorgelegt. Halbreife Sporen von *Pyxine limbulata* werden mit denen von *Physcia aipolia* optisch verglichen. Ein Anhang enthält eine Liste von *Pyxine*-Sippen, die in Nepal gesammelt worden sind und jetzt im Herbarium GZU liegen.

Key words: Physciaceae, China, Himalaya, chemistry, ascospore type

Introduction

Several regional revisions of the lichenized ascomycete genus *Pyxine* have already been published, most of which have included a more or less detailed descriptions of the generic characters. These include the works of SWINSCOW & KROG (1975a, 1988), KASHIWADANI (1977a), AWASTHI (1982), ROGERS (1986), KALB (1987, 2002), and ELIX (2009). Briefly, these characters can be summarized as follows:

Morphology/anatomy: Thallus foliose, rarely squamulose-areolate, greyish, olive to brownish grey, often with white pseudocyphella-like or maculoid structures, usually pruinose, loosely to firmly appressed to the substrate. Vegetative propagules absent or present (isidia, or soralia, or dactyls [= polysidiangia]). Lower surface black, attached with black (or marginally often greyish-white) branched rhizines (Fig. 5) or (when areolate) directly adnate to the substrate. Three types of apothecial development have been observed. 1. The hamathecium is permanently surrounded by a thalline exciple (= *physciaeformis*-type). 2. The

ascocarps start as a lecanorine apothecium, but with age the thalline exciple is pressed downwards and finally becomes excluded by the thickening of the parathecium (*cocoes*-type). 3. The exciple contains no or only a few algae from the start and the marginal cells of the exciple are heavily pigmented [K+ violet in sections] (= *obscurascens*-type). All three species presented here have apothecia belonging to the latter type. Disc black, whitish pruinose or pruina lacking. Epithecium bluish-black, K+ violet. Spores brown, 2-[rarely 4]-celled, with thickened walls, of the *Dirinaria*-type (but see 'remarks' under *Pyxine limbulata*).

Chemistry: Pruina of the cortex surface with hydrated calcium oxalate crystals (whewellite and weddellite). Cortex UV-, with atranorin, though often in very small amounts, chloroatranorin partly present, or UV+ golden yellow, with lichexanthone. Medulla always with various (often very characteristic) triterpenes, several taxa additionally with norstictic acid in the medulla (very seldom in the apothecia), and/or testacein, and/or yellowish pigments (occasionally more concentrated in the apothecia). The reddish naphthoquinone chiodectonic acid (also known as pyxiferin) and some other related substances occur in one species (*Pyxine coccifera*).

Note: Recently zeorin, 4-*O*-methylcryptochlorophaeic acid, cabraleadiol monoacetate, and methyl- β -orcinolcarboxylate were isolated from *Pyxine retirugella* (as *P. consocians*; see KATHIRGAMANATHAR et al. 2006: 218). As the mentioned substances were found together with atranorin and lichexanthone (hitherto not known to occur together in the same *Pyxine* taxon), we question whether their results might have been due to having actually sampled a mixture of different taxa (maybe including *Heterodermia*). The lack of norstictic acid and testacein indicates that at least *P. retirugella* was not part of the collection. If the reported co-occurrence of zeorin and 4-*O*-methylcryptochlorophaeic acid proves to be correct, and the thalli sampled prove to be a pure collection of *Pyxine*, then the material could contain one or two undescribed taxa.

At present there are approximately 75 species of *Pyxine* known worldwide, most of them occurring in tropical or subtropical regions. Regarding China, 11 taxa have been treated recently (HU & CHEN 2003), including *Pyxine berteriana*, *P. cocoes* [syn.: *P. connectens*], *P. consocians*, *P. copelandii*, *P. coralligera*, *P. endochrysin*, *P. limbulata* [syn.: *P. subolivacea*, *P. himalayensis*], *P. meissnerina*, *P. philippina*, *P. soreliata*, and *P. subcinerea*. Two additional species (*P. microspora* and *P. petricola* [syn.: *P. endoleuca*]) that were previously cited from the Hengduan Shan by ZAHLBRUCKNER (1930: 235) and HUE (1900: 83), respectively, were excluded by HU & CHEN (2003) from their species list (and key) due to unavailable material (note, that they were listed by WEI 1991: 217). However, KALB (1987: 54) confirmed the determination of *Pyxine microspora* from China. He (KALB 1987: 45) additionally identified *P. cognata* (leg. Handel-Mazzetti 879) amongst two specimens from Sichuan, which were listed by ZAHLBRUCKNER (1930: 234) under *P. cocoes*. Thus the number of Chinese taxa of *Pyxine* is currently 13 (or 14, if the occurrence of *P. petricola* is confirmed).

Regarding the Tibetan area and closely adjacent regions, HU & CHEN (2003) report three species of *Pyxine*, namely *P. berteriana* (Xizang: Zhangmu [the same specimen was published by WEI & JIANG 1986: 114]; Sichuan: Mt. Emei;

Yunnan: Zhongdian [Shangri-La]), *P. limbulata* (Yunnan: Mt. Yulong, Lijiang county, Zhongdian county, and Weixi county; Xizang: Chayu county), and *P. sorediata* (Yunnan: Lijiang county, Baoshan county; Sichuan: Mt. Emei). Note that *P. minuta* (probably a misidentification of *P. microspora*) and *P. philippina* are mentioned by APTRoot & FEIJEN (2002) for Bhutan and thus the latter is likely to occur also in the Tibetan region. For further species of *Pyxine* that might be expected in the study area due to their confirmed occurrence on the opposite side of the Himalayas in Nepal, we refer the reader to the appendix.

The present study, which is based on collections from Tibet and closely adjacent areas, provides new data on the distribution, spore morphology and chemistry of three species, i.e. *Pyxine limbulata*, *P. microspora*, and *P. sorediata*. Photos showing morphological characters of all three taxa are included. The appendix contains unpublished locality data from *Pyxine* specimens collected in Nepal and housed in GZU.

Materials and Methods

If not otherwise stated, all cited specimens are housed in GZU (herbarium of the Institute for plant sciences, University Graz, Austria). Part of the material was collected in the southeast Tibetan region during an expedition of the first author in 1994. Chemical analyses were conducted using standard methods of thin layer chromatography (CULBERSON & AMMANN 1979; ELIX et al. 1987). For a more effective separation of triterpenes, TLC-plates were run to a height of 15 cm.

The optical equipment comprised a dissecting microscope (Leica, Wild M3Z) and an optical microscope (Zeiss, Axioskop 50), including a digital camera (Zeiss, AxioCam MRc5) which fits to both mentioned units. In order to expand the depth of focus, in some pictures a stack of images (gained by the program 'Zeiss AxioVS40') was taken and subsequently processed with the public domain software 'CombineZP' (developed by Alan Hadley).

The species

Pyxine limbulata Müll.Arg.

Flora [Regensburg] 74: 112 (1891).

= *P. subolivacea* Zahlbr., in Handel-Mazzetti, Symb. Sinicae 3: 235 (1930).

= *P. meissneri* var. *vulnerata* Hue, Nouv. Arch. Mus., ser. 4(2): 83 (1900).

= *P. sibirica* Tomin, Bull. South. Ussuri Branch. State Russ. Geogr. Soc.: 221 (1926).

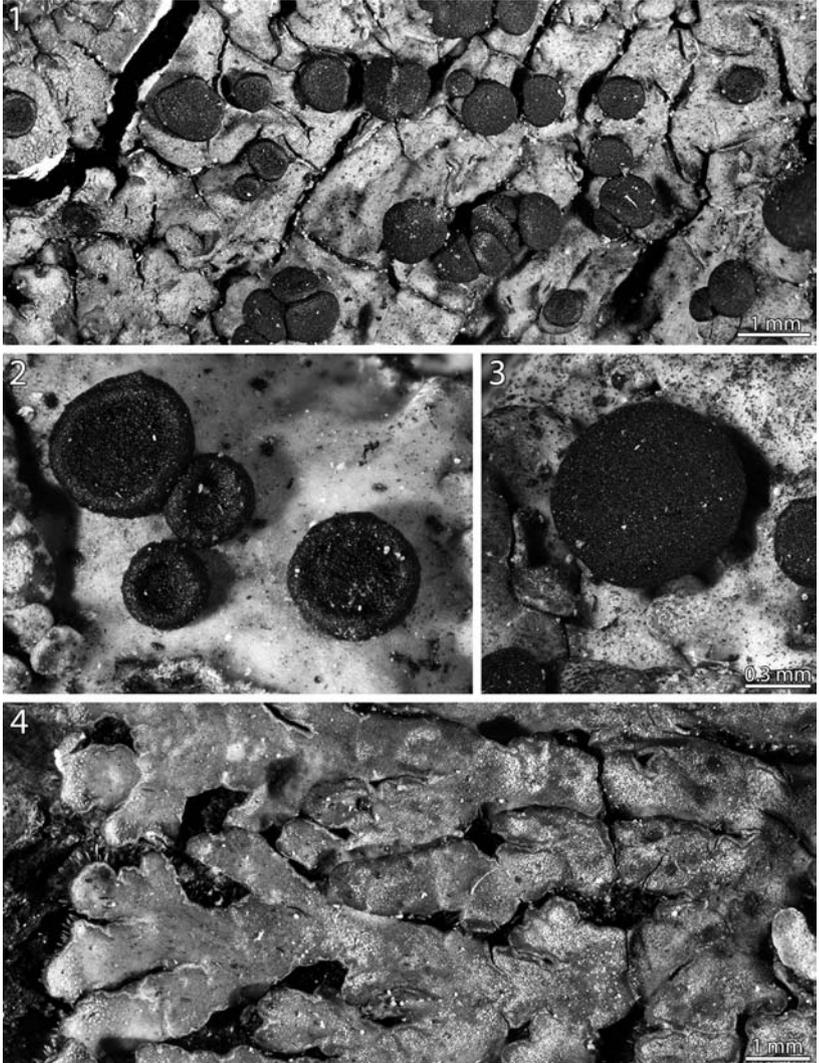
= *P. himalayensis* D.D.Awasthi, Phytomorphology 30(4): 371 (1982).

(Fig. 1–23)

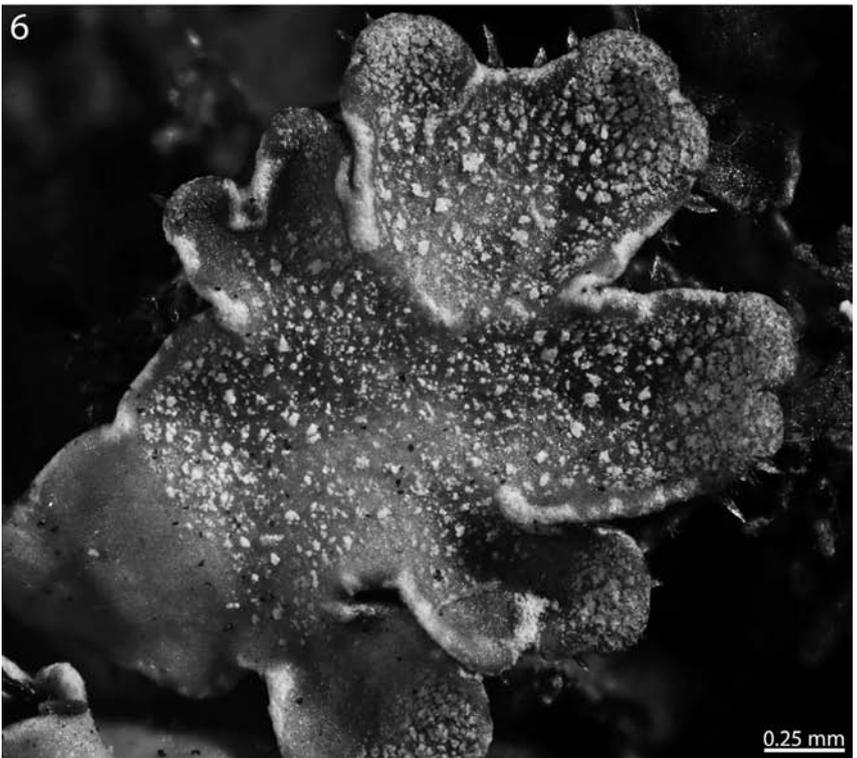
Description based on the material studied:

Thallus whitish to grey (herbarium specimens can be pale yellowish-brown), up to 14 cm in diam., consisting of radiating, elongated, unequally branched lobes (1.5–2(–3) mm wide), which are slightly concave at their growth tips and sometimes transversely cracked in central parts. Pseudocyphellae distinct, at the margin of the lobes (figs. 5, 6). *Lobes* when young often with spotted, white pruina (see fig. 6, but in some specimens, pruinose lobes are very scarce [e.g. Obermayer 06103]). Older portions of the lobes (=inner part of the thallus) epruinose or

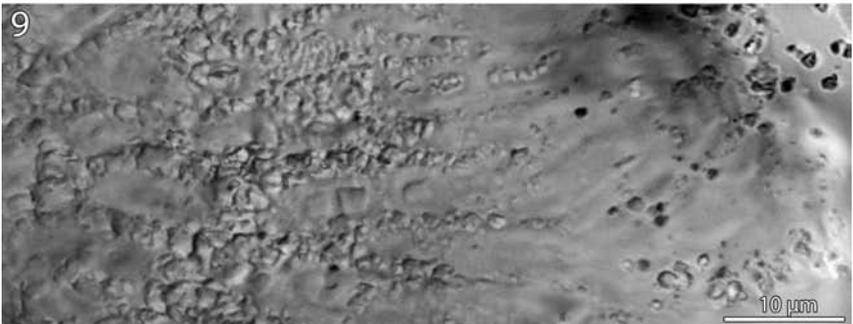
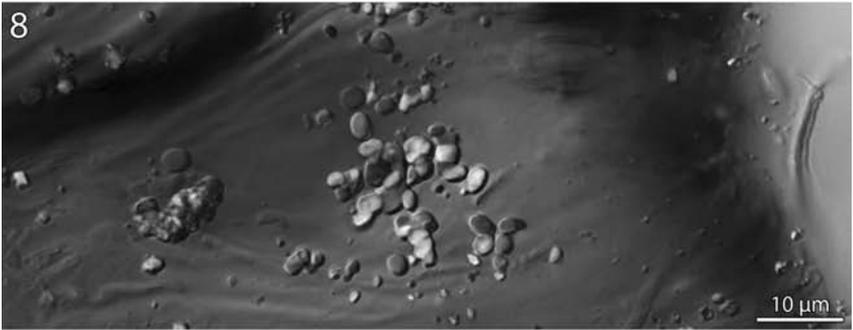
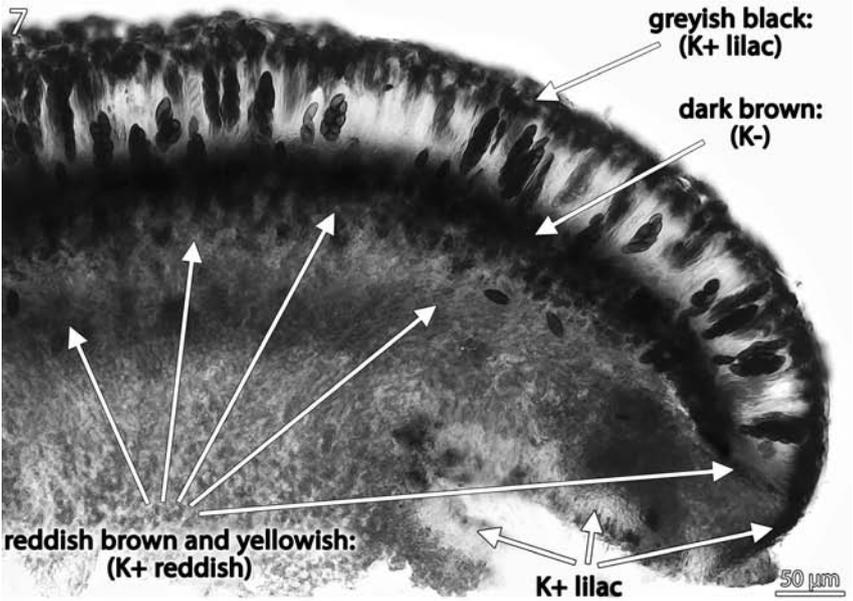
pruinose (in the latter case the pruina is not spotted but forms a thin, rather homogeneous whitish layer). *Soralia* or isidia-like structures absent (but from the radial margins of the main lobes, secondary lobules may be produced, these with a rather narrow base [less than 0.5 mm]). *Medulla* yellowish-ochraceous (a cut of the thallus shows a \pm homogenous colouration), colour sometimes very weak, KOH gives no colour reaction.



Figs 1–4. *Pyxine limbulata*. 1. Centre of a partly cracked thallus with apothecia (Obermayer 05882). 2. Distinctly marginate, flat apothecia (Obermayer 02955). 3. Slightly convex apothecium with inconspicuous margin (Obermayer 05882). 4. Radiating lobes at the thallus margin, with pruina at lobe tips and in the central part of the thallus (Obermayer 05882).



Figs 5–6. *Pyxine limbulata*. Pruinose lobe ends with distinct marginal pseudocypellae and bleached rhizines. 5. Obermayer 05882. 6. Obermayer 10506.



Figs 7–9. *Pyxine limbulata* (Obermayer 10506). 7. Cross section of an apothecium with colours before and after treatment with KOH. 8. Semi-crystalline grains in the hymenium after treatment with KOH (developed after some hours, seen in polarized light). 9. Grain-like structures along swollen hyphae in the apothecial margin after KOH application.

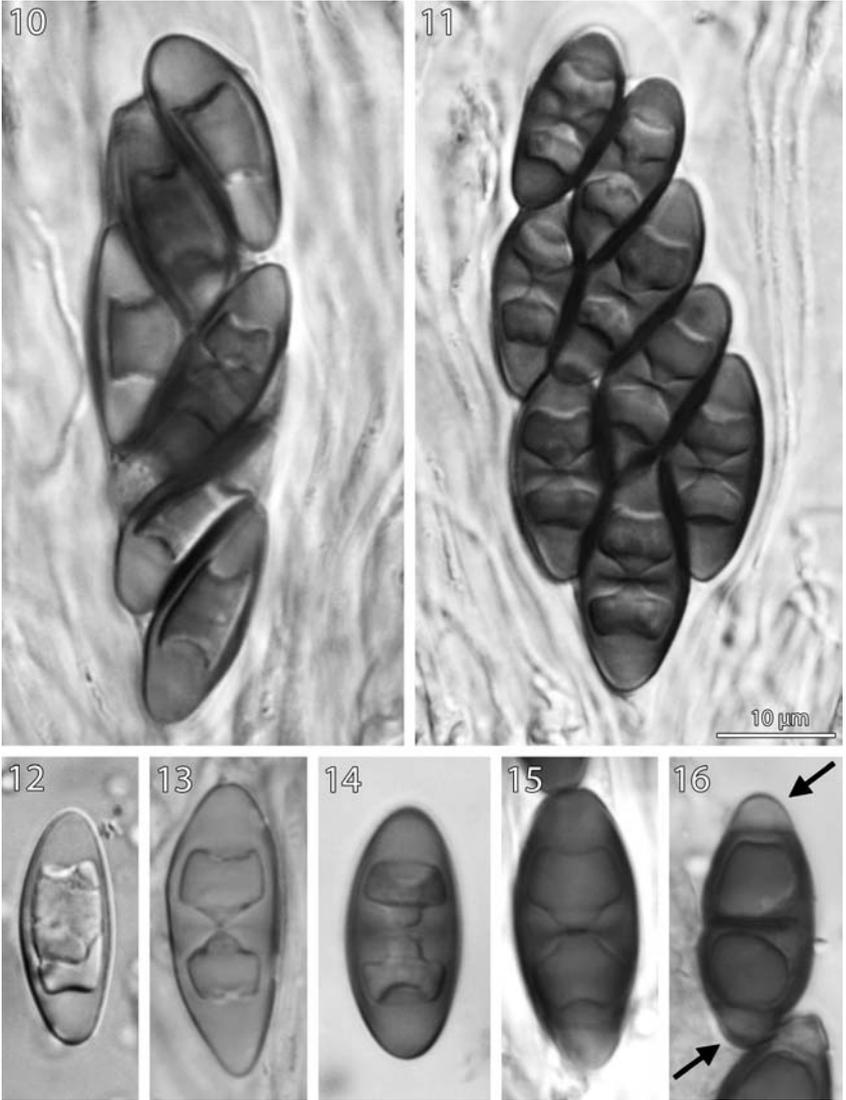
Apothecia mostly well developed, up to 2 mm in diam., either flat with a distinct and relatively thick margin (fig. 2), or (in older or regenerating specimens) strongly convex and without a visible margin (fig. 3). Epithecium greyish-black, KOH+ lilac. Hymenium colourless. Hypothecium developed as a rather thin, dark brown layer, KOH- (or a slightly intensifying brownish). Subhypothecial area (stipe) rather thick, reddish brown or yellowish brown in upper portions, KOH+ distinctly reddish-purple (not lilac), in lower portions almost colourless or faintly yellowish, KOH-. Basal part of the apothecium developed as all other parts of the medulla (colourless or slightly yellow in cross-section view, KOH-). *Ascospores* 17–25[27] x 7–9[11] μm (figs. 10–20), spore size variable in the same apothecium but hardly variable in the same ascus (e.g. *Obermayer 07044* shows many asci with spores measuring 18–20 x 7–8 μm and a few asci with spores measuring 23–25 x 8–9 μm), many spores (especially at mature and overmature stages) with paler tips at both ends (see figs. 16 & 20 and comments below).

Pycnidia laminal or submarginal (fig. 21), mostly sunken into the thallus, only the blackish, K+ lilac ostiole slightly raised. *Conidia* slightly asymmetric bifusiform with two unequal swellings, the broader one near the short stalk (where the conidium connects to the conidiophore) the smaller one apical (see figs 22 & 23 of the present paper and KALB 1987: 16 Abb. 4e), 3.5–4 x 1 μm [at the constricted area]–1.2 [at the swollen area].

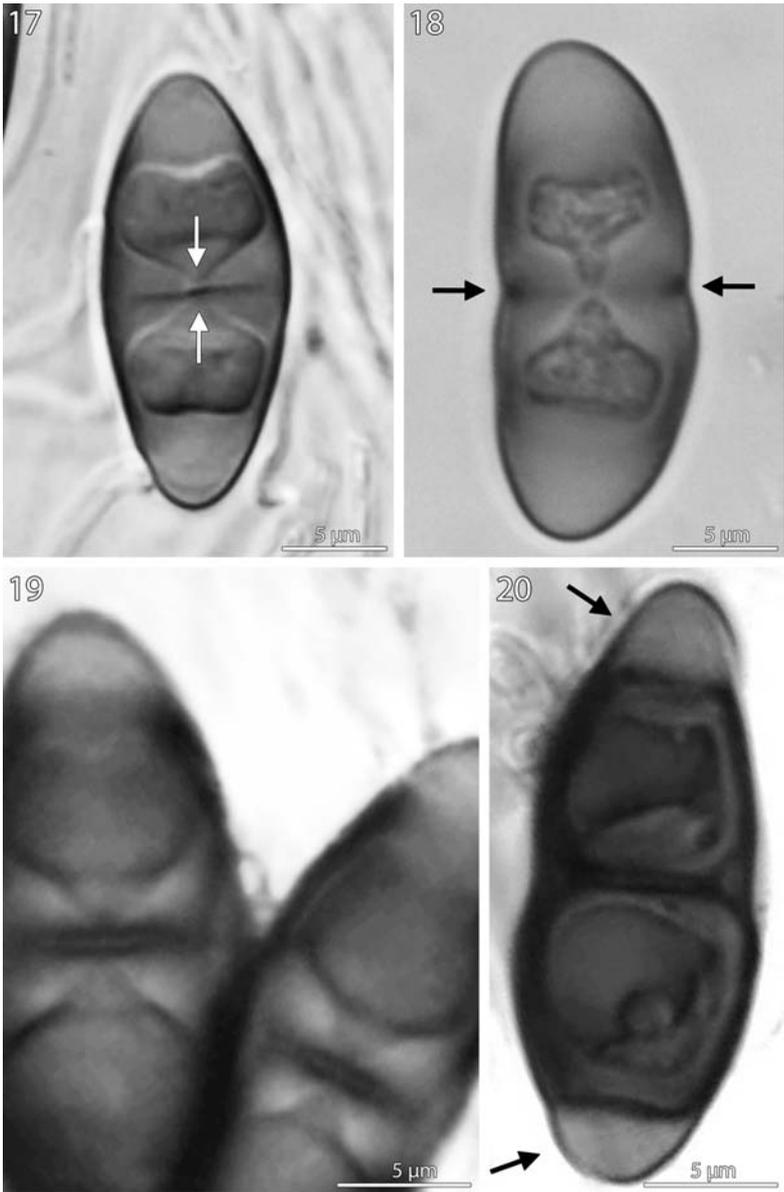
Chemistry (fig. 31): Atranorin always only in trace amounts (never found when only apothecia were used for TLC), at least 2 yellowish pigments (TLC-plate B' often shows 4 separate spots), a set of terpenoids (two or three of them in high concentrations), and unknown substances which are visible in long-wave UV after treatment with sulfuric acid.

Remarks: The spore type can be regarded as of the '*Dirinaria*-type' (MAYRHOFER 1982: 315 and Abb. 2 Fig. i), (figs. 12–16) clearly with 'type B' development (figs. 12–16)(GIRALT 1994: 319 and Fig. 2): Early in development apical thickenings are produced at both ends (fig. 12) and later on continue with a delayed septum formation (SCHEIDEGGER et al. 2001: 30, 36, and Fig. 4). Although the non-septate stage is generally seen prior to the deposition of pigments, it is sometimes seen in spores in early pigmented stages (already with the typical spore-end thickenings) (fig. 10).

In *Pyxine*, the cell wall thickenings of semi-mature spores at a first glance very much resemble those of the genus *Phycia*. But unlike *Phycia*, the spores of *Pyxine* lack a typical torus, which is a narrow, dark brown, ring-like structure encircling the outermost part of the septum (fig. 18). Instead, middle-aged spores of *Pyxine limbulata* (and probably of many other members of the genus) develop a darker coloured structure in the central parts of the septum. A closer view shows that the narrow, colourless innermost part of the septal wall, (which is seemingly the invagination of the so called 'intermediate layer' towards the spore centre; see also BUNGARTZ & NASH 2004: figs. 9, 15, 25) is covered on both sides with a darkly pigmented coat (pigmented proper wall; concerning terminology see e.g. NORDIN 1997). This double layer can be seen in the very centre of the septum, where it does not show any thickenings, and with a decrease in pigmentation from inner to outer portions in the subcentral areas (see especially figs 19 & 20), where the structure is thickly coated with endospore-material.

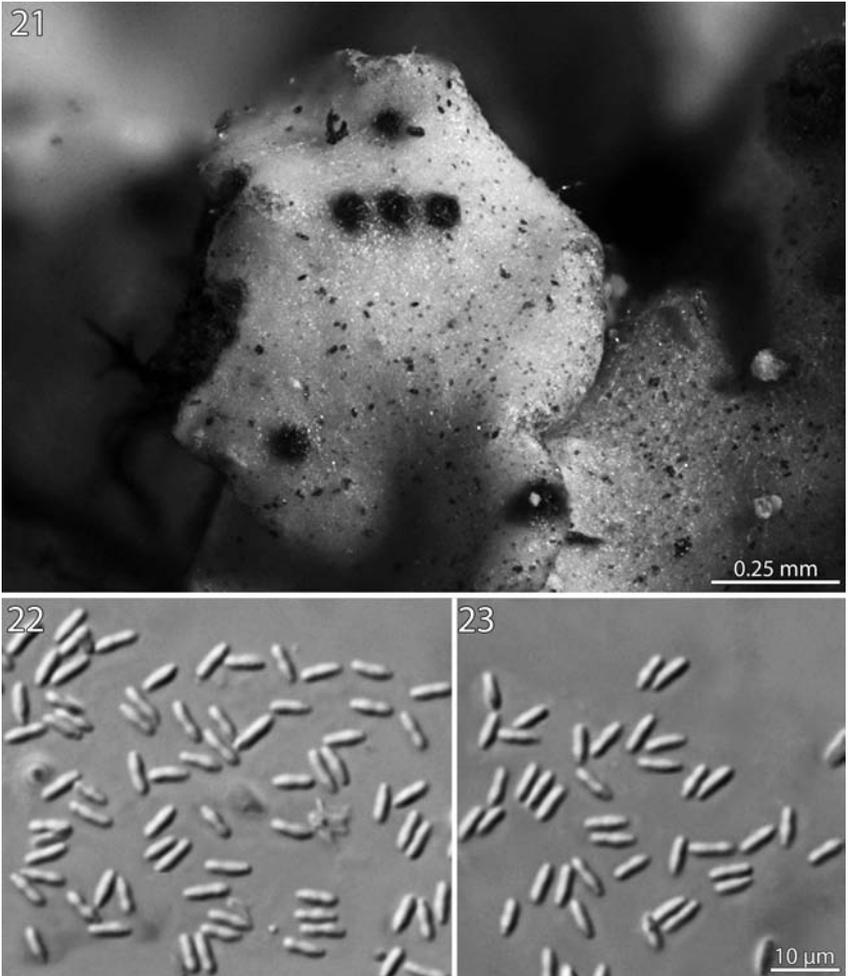


Figs 10–16. *Pyxine limbulata* (Miehe 94-179-31/3). Spores after treatment with KOH. **10.** Ascus with young (but already slightly coloured) spores, five spores with wall thickenings at both ends but still without a septum. **11.** Ascus with (semi-)mature spores of the 'Dirinaria-type'. **12–16.** Ontogenetic stages (and size differences) of spores, starting (left) with the youngest. Black arrows point to paler regions at both ends of mature spores.



Figs 17, 19 & 20. Spores of *Pyxine limbulata* after treatment with KOH (Miehe 94-179-31/3). **17.** Typical spore of the '*Dirinaria*-type'. White arrows point at the central and sub-central area with the strongest colouration of the septum. **19 & 20.** Strong magnification of the septal area showing the darker coloured proper spore wall at both sides of the septum and an uncoloured fissure ('middle lamella') in between. Black arrows point to paler regions at both ends of the mature spore. - **Fig. 18.** Spore of *Physcia aipolia* (Hafellner 51833) after treatment with KOH. Black arrows point to the 'torus', a ring-like, brown coloured structure girding the equatorial region.

Most semimature and mature spores have at both ends a paler region (see figs. 16 & 20) resembling early stages of germination. But as this 'decoloration' can also be seen in rather young stages, it seems to be an early developed character, namely a brownish coat covering the whole spore except the very ends (TEM-studies might reveal whether it is developed by denser pigmentation of the perispore or of the proper spore wall). In some stages, the brown coat itself seems to be thinner in the equatorial zone of the spore, which gives the spore an appearance rather similar to '*Bicineta*-type' (i.e. two broad, separated brown rings each of them girtling one spore cell). Note that with a close view, the spores of *Physcia aipolia* appear to have a similar coating (see fig. 18).



Figs 21–23. *Pyxine limbulata*. 21. Pycnidia (spermogonia) in laminal and submarginal position (Obermayer 10506). 22 & 23. Pycnospores (spermatia) slightly asymmetric bifusiform (Obermayer 02959).

All parts of the apothecium, especially the epithecium and outer part of the lecidine margin, both of which are KOH lilac, yield round droplets a few minutes after application of KOH. After some hours, these partly develop into crystal-like structures (see fig. 8). The KOH- inner part of the apothecial margin, as well as the medulla, shows irregularly shaped, non-crystalline granules covering the exterior of the hyphae (fig. 9).

Morphological and anatomical features of *Pyxine limbulata* were well characterized by ZAHLBRUCKNER (1930: 235; sub *P. subolivacea*; see below) and by KASHIWADANI (1977b: 161–163; fig. 4). This species is regarded as the esorediate counterpart of *P. sorediata* (SWINSCOW & KROG 1975a, 1975b, KASHIWADANI 1977b, KALB 1987). A close relationship between these two is supported by their sharing the same set of triterpenes, as well as the same yellow pigments (see fig. 31), which occur in the apothecia of *P. limbulata* and in the thallus (especially soredia) of *P. sorediata*. *Pyxine endochrysinina*, also with the same chemical profile, forms the third member of this group, morphologically distinguished by producing nodular to lobulate marginal isida-like structures.

Pyxine subolivacea, which was established based on type material collected close to the south-eastern border of the Tibetan area, was synonymized with *P. limbulata* by SWINSCOW & KROG (1975b: 127). Regarding the length of the spores, the present material (17–25[27] μm) ranges between those of the type of *P. limbulata* (given by MÜLLER 1891: 112 as 16–23 μm) and those of the type of *P. subolivacea* (given by ZAHLBRUCKNER 1930: 235 as 21–27 μm). Spore sizes of *Pyxine himalayensis*, which is also conspecific with *P. limbulata* (see notes above) was given by AWASTHI (1982: 372) as 16–23 x 6–8 μm . These values are confirmed by investigation of the type material.

The description of *Pyxine himalayensis* (AWASTHI 1982: 371) fits well within the present species, with the only difference being that the internal stipe of *P. himalayensis* should be "...incolorato, K- ...". In our material the lower, almost colourless part of the stipe is indeed K- (as is stated e.g. for *Pyxine sorediata*; see KALB 1987: 75). To observe the pigmentation of the stipe correctly, it is necessary to cut the apothecium exactly through the centre. Such an exactly medial section reveals that the type of *P. himalayensis* shows a reddish brown to ochre brown colour in the upper part of the stipe, reacting K+ dark wine red, while the lower part of the stipe is colourless and K-.

Ecology and Distribution: *Pyxine limbulata* is regarded as an Eastern Asian endemic (hitherto known from China [see HU & CHEN 2003] and Japan [see KASHIWADANI 1977b]) and has recently been found in South Korea (WEI & HUR 2007: 136). The present specimens were collected at altitudes between 2200 m and 4100 m and occur on deciduous trees, conifers, and even on rocks (Obermayer 02955).

Specimens examined (18): China. [Tibet.] Xizang Province: Himalaya Range, 275 km ESE of Lhasa, 60 km SW of Mainling, 29°02'N, 93°53–54'E, 3150–3250 alt., forest area close to a river, 9. VIII. 1994, leg. Obermayer 05727, 05746 (on *Rhododendron*), 05804 (on *Quercus*), 05881, 05882, 05884, 05879 (on *Picea*), 05854 (on *Prunus*). Tsangpo tributary, Nangxian to Mainling, Lower Lilung Chu (Camp 14), 29°2'N, 93°54'E, 3280 m alt., *Quercus-Pinus* (*Picea*?) forest on SW-facing slope, on bark, (veg.rec.no. 49), 9. VIII. 1994, leg. Mische 94-155-36/06 & Wündisch. Ibid., Lilung Chu Eastern branch (Camp 14 to High Camp), 29°2'N, 93°55'E, 3380 m alt., *Picea-Abies* forest with *Quercus* on gravel terrace, (veg.rec.no 59), on bark, 14. VIII. 1994, leg. Mische 94-179-31/03 & Wündisch. Himalaya Range, 280 km ESE of Lhasa, 40 km SW of

Mainling, 29°03'N, 93°56'E, 3900–4100 m alt., *Juniperus-Abies* forest, on *Abies*, 13. VIII. 1994, leg. Obermayer 06103. Nyainqêntanglha Shan, 360 km E of Lhasa, near the bend of the river Tsangpo, N-Side of Gyala Peri, 6 km S of Dongjug village, 29°56–58'N, 94°53–54'E, 3000 m alt., on *Populus*, 21. VIII. 1994, leg. Obermayer 06865. Nyainqêntanglha Shan, 370 km E of Lhasa, near the bend of the river Tsangpo, N-side of the mountain Gyala Peri, between the villages Tongjug and Tangmai, 30°01'N, 94°58'E, 2300–2400 m alt., deciduous trees along the river, on *Populus/Fraxinus*, 23. VIII. 1994, leg. Obermayer 07009, 07031, 07044, 07090. 55 km NNE of Nyingchi, river valley at the west side of Gyala Peri, 29°59'N, 94°53'E, 2500 m alt., deciduous trees along the river-bank, on *Salix*, 17. VIII. 1994, leg. Obermayer 06393, 10506. Sichuan Province: 210 km WSW of Chengdu, Daxu Shan Mts., in a canyon, 10 km NE of Kangding (=Dardo), 30°05'N, 102°03'E, 2200 m alt., on rocks, 22. VI. 1994, leg. Obermayer 02955

***Pyxine microspora* Vain.**

Philipp. J. Sci., sec. C, 8: 110 (1913).

(Fig. 24–26)

Description based on the material studied:

Thallus greyish to pale olivaceous, up to 3 cm in diam., consisting of radiating lobes at the margin and elongated, cracked areoles in the centre. Lobes/areoles very small (0.35–0.6 x 0.8–1.5[2.0] mm), attached directly without rhizines to the substrate (resembling *Caloplaca demissa* or esorediate parts of *Hyperphyscia adglutinata*). Inner part of the thallus with very small, pale coloured lobules (see fig. 24), sprouting from the margins of the areoles. Pruina mostly absent, but present in a few young radiating lobes (fig. 26). Pseudocyphellae laminal, sparsely developed. *Soralia* or isidia-like structures absent. Medulla white.

Apothecia rather small, 0.35–0.6[0.8] mm in diam., mostly appearing lecideine, but a few with (at least partly) a margin with the colour of the thallus, in very young stages bursting through the cortex, later constricted at the base, initially with a flat disc and a distinct margin, 60–80 µm in diam. (Fig. 25), old stages with a strongly convex disc and evanescent margin. Hypothecium brown, KOH-. Stipe dark brown in the upper part, pale brown or almost colourless in lower parts, KOH-. *Ascospores* poorly developed in the present material, only one spore found (2-celled, brown, 13 x 6 µm). Many ascus lumina in the present sample contain a brown mass sometimes with one to three septum-like structures. This lumen content thus resembles a single, large, brown, two- or four-celled spore.

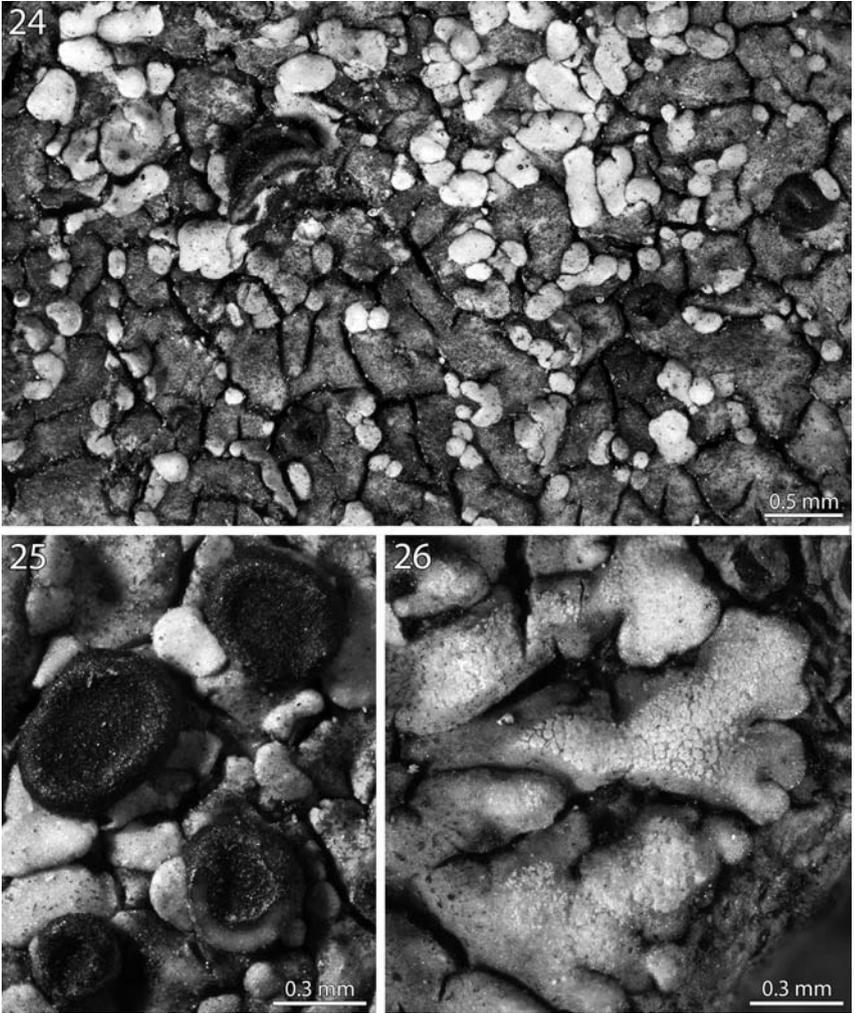
Pycnidia not seen.

Chemistry: lichexanthone (only in the thallus, not found in apothecia), triterpenes (fig. 31), and probably traces of testacein (see below).

Remarks: *Pyxine microspora* has been well characterized by SWINSCOW & KROG (1975a: 60), who state that "...the most notable feature of this species is the small size of all its parts ...". In addition to terpenes, an unknown pigment and lichexanthone, the authors (SWINSCOW & KROG 1988: 269) reported the occurrence of testacein in the thallus. This substance probably also occurs in our studied material (fig. 31 marked as 'T?'), but due to the availability of only a single specimen, we waived removing further material for more detailed TLC studies.

One TLC run of a portion of the thallus without apothecia showed traces of a substance running like norstictic acid, but the amount was too small to verify. A squash preparation of the thallus with KOH revealed a rather strong reddish

brown solution, but without any red crystals (which would be typical for norstictic acid). Though the greater portion of the present specimen is epruinose (SWINSCOW & KROG 1988: 60 characterized the taxon as being 'not pruinose'), some pruinose lobes are developed. (see fig. 26).



Figs 24–26. *Pyxine microspora* (Obermayer 02943). **21.** Centre of the slightly cracked thallus with young (pale coloured) sprouting lobes and a few apothecia (the larger one in the left corner is cut diagonally). **22.** Distinctly marginate apothecia. **23.** Out of the entire thallus, only a few lobe ends show a white pruina.

Pyxine minuta (type from Brazil), which is morphologically very similar to *P. microspora* (type from the Philippines), was regarded as a distinct species by

SWINSCOW & KROG (1975a: 60) because of its different set of triterpenes. KALB (1987: 67) additionally reported a statistically significant difference in the spore-length. AWASTHI (1982: 373), who has reported *P. minuta* from India treated *P. microspora* as its synonym. But the author mentioned that his cited material from the Indian Himalayas (Almora district) is chemically identical to an African specimen of *P. microspora* cited by SWINSCOW & KROG (1975a). Thus the reports for India obviously refer to *Pyxine microspora*.

The first (and hitherto only) report of *P. microspora* for China comes from ZAHLBRUCKNER (1930: 235), who cited three specimens collected by Handel-Mazzetti. Two locations are situated in southern Sichuan (Datjiaoku, HM 2694), [ca. 1700 m alt.]; Dechang ('Dötchang'), HM 1166, [ca. 1400 m alt.]). The third named village (Manhao, HM 5788 [ca. 160 m alt.]) is located in Southern Yunnan near the border with Vietnam. The correctness of the determination of two specimens housed in herbarium W (numbers 1166 and 9788; the latter not cited by Zahlbruckner) was verified by KALB (1987: 54).

Note that *Pyxine microspora* is missing in the key to Chinese *Pyxine* provided by HUE & CHEN (2003: 446).

The hitherto known specimens of our study area were found on rocks at rather lower altitudes between 900 m (see cited specimen below) and 1700 m (Handel-Mazzetti 2694). Further reports of *Pyxine microspora* come from the Philippines (type collection), Uganda (SWINSCOW & KROG 1975a: 60), India (AWASTHI 1982: 373; see discussion above), the Galapagos Islands (APTROOT & SPARRIUS 2009), Kiribati (ELIX & MCCARTHY 2008), Sambia (KALB 1987: 54), and Australia (KALB 1987: 54; KALB 2004: 316; ELIX 2009: 527). We have found the species (with more than trace amounts of testacein) also amongst specimens from Nepal (see 'Appendix' below).

Specimen examined. China. [Tibet.] Sichuan Province: 220 km SW of Chengdu, Daxiang Ling Mountains, 30 km WSW of Fulin (=Hanyuan), near Shimian, 29°24'N, 102°20'E, 900 m alt., on overhanging rock, 22. VI. 1994, leg. Obermayer 02943.

***Pyxine soredata* (Ach.) Mont.**

in Sagra, Hist. Cuba 9: 188 (1842).

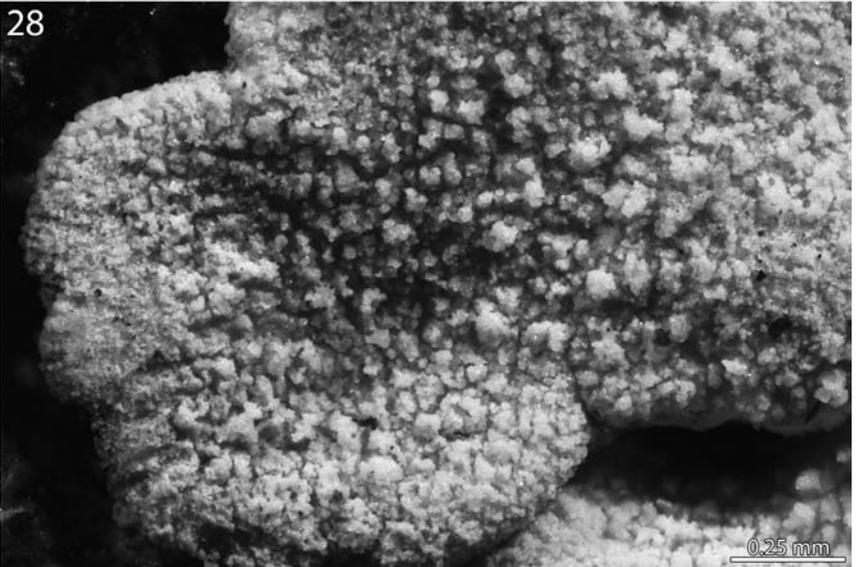
(Fig. 27–30)

Short description based on the studied material:

Thallus whitish to grey, consisting of radiating, elongated, unequally branched lobes (1.5–2.5 mm wide), which are slightly concave at their growth tips and sometimes transversely cracked in central parts. Young lobes often with spotted, white pruina. Pseudocyphellae developed as a white edging along the lobe margins (difficult to see at the pruinose lobe tips). *Soralia* whitish or bluish-grey, marginal (or along cracks), capitate to elongated, developed on older parts (=centre) of the thallus. *Medulla* yellowish-ochraceous (a cut of the thallus shows a ±homogeneous colouration).

Apothecia and *pycnidia* not seen (note, that in North America, 12 % of the collections were found fertile [IMSHAUG 1957: 260]).

Chemistry: 'limbulata-terpenes' and 'limbulata-pigments' (see under *P. limbulata*), atranorin (low concentrations in the thallus, higher concentrations in the soralia), two unknown depsides/depsidones (see fig. 31).

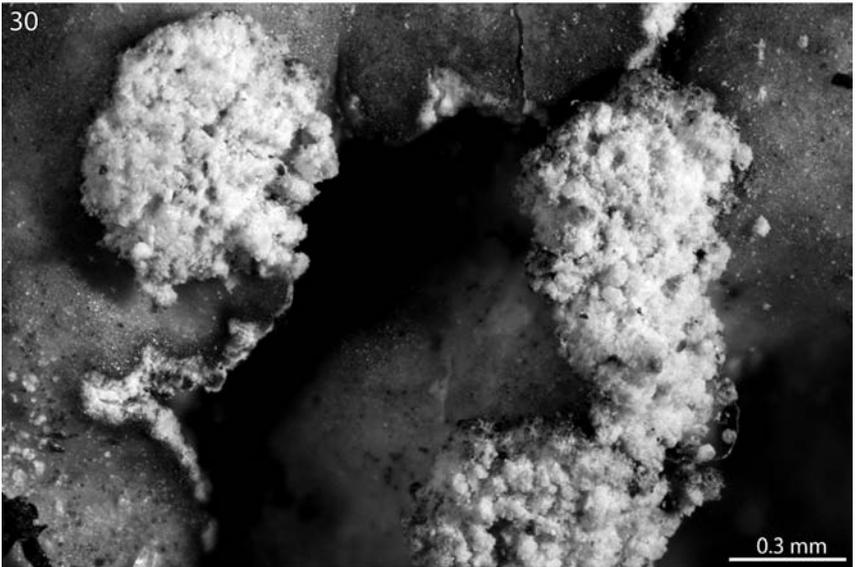
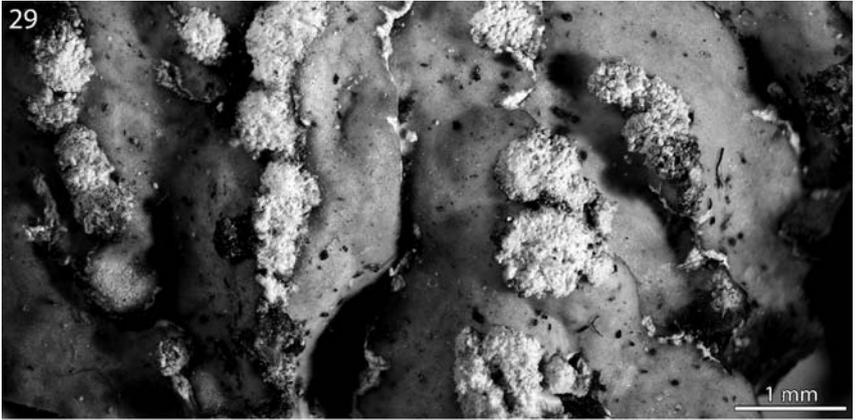


Figs 27–28. *Pyxine sorediata* (Obermayer 05726). **27.** Heavily pruinose lobe ends (left) and epruinose lobe centre with soralia (right). **28.** Strongly magnified structure of the pruinose.

Remarks: *Pyxine sorediata* (well characterized by ELIX 2009: 531) shows an almost worldwide distribution. In Asia, the taxon has recently been reported (under the status "rare") from the Lake Baikal region in Siberia (see BUDAJEVA 2007) and from South Korea (WEI & HUR 2007: 136). Occurrences of the present material ranged from 2200 to 3250 m in altitude.

The specimens we studied of *Pyxine sorediata*, the type species of the genus *Pyxine*, contain one unknown depside/depsidone ('U1': [3–4]/5/5) in medium to low concentration (and traces of a further unknown substances, 'U2'; see fig. 31).

Zeorin, which was reported by HU & CHEN (2003: 453), was not found in the material we sampled. For further notes see under *Pyxine limbulata*.



Figs 29–30. *Pyxine sorediata* (Obermayer 02949). **29.** Epruinose thallus with marginal soralia and marginal pseudocyphellae. **30.** Strongly magnified soralia and pseudocyphellae.

Specimens examined (3). **China.** [Tibet.] Xizang Province: Himalaya Range, 275 km ESE of Lhasa, 60 km SW of Mainling, 29°02'N, 93°53–54'E, 3150–3250 m alt., forest area close to a river, on *Rhododendron*, 9.VIII.1994, leg. Obermayer 05726; 5742 [hb Kalb]. Sichuan Province: 210 km WSW of Chengdu, Daxu Shan Mts., in a canyon, 10 km NE of Kangding (=Dardo), 30°05'N, 102°03'E, 2200 m alt., on rocks 22.VI.1994, leg. Obermayer 02949.

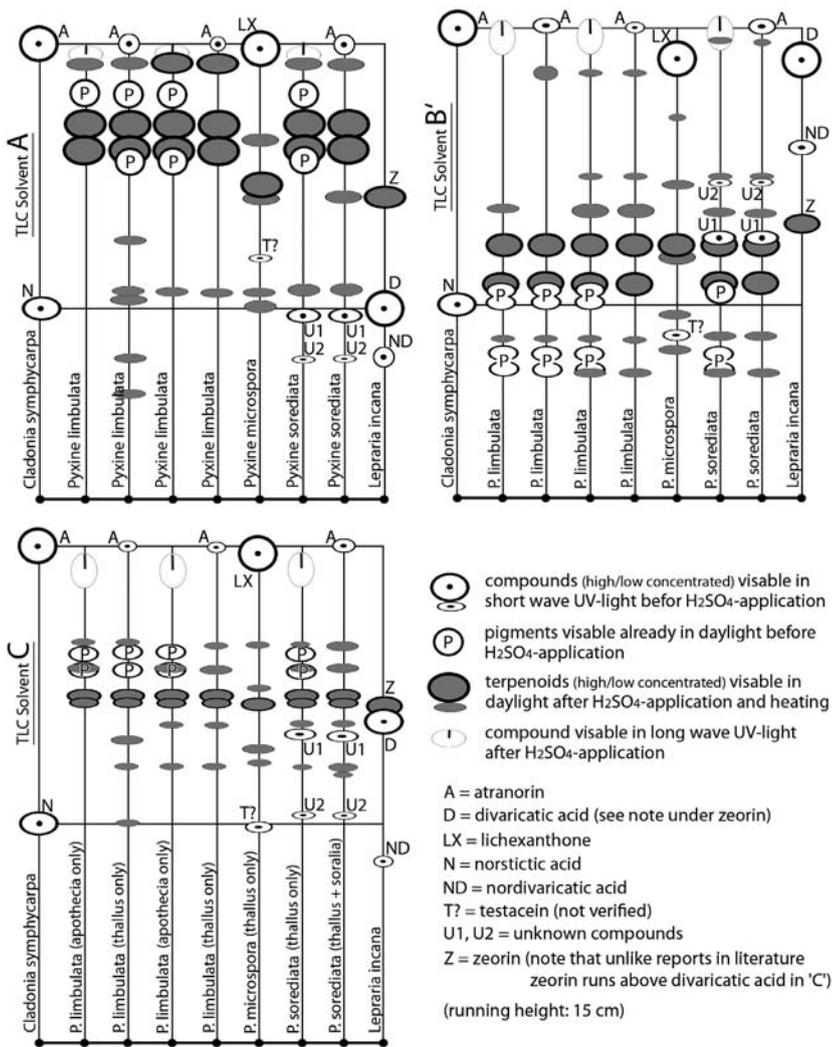


Fig. 31. Chromatograms of *Pyxine limbulata*, *P. microspora*, and *P. soreidiata*. *Lepraria incana* was used as source for zeorin as a standard (drawn chromatograms of other species of *Pyxine* compare e.g. KALB 1994: 62).

Appendix

The appendix comprises unpublished label data of *Pyxine* specimens from Nepal housed in GZU. Part of the material (mainly collected by the late Prof. Josef Poelt) has been cited by KALB (1987) for Nepal, but without detailed label information. Note, that *Pyxine nilgiriensis* was the only species of *Pyxine* cited by POELT (1990) for the Langtang Area in Nepal.

Pyxine asiatica Vain.

Nepal. Langtang Area, way from Syabru to Barkhu, humid N forest, dry W-exposed pine-forest, 2000–2500 m alt., 22. IX. 1986, leg. J. Poelt N86-L859.

TLC of the present sample: atranorin (maj.), testacein (maj.), triterpenes.

Pyxine cocoes (Sw.) Nyl.

Nepal. Kathmandu-Jawalakhel, 1400 m alt., an Straßenbäumen, VIII. 1962, leg. J. Poelt s.n.

Pyxine cognata Stirt.

Nepal. Langtang Area, slopes N above Thangsep (Thogsep), pasture, big rocks, 3200 m alt., 4. IX. 1986, leg. J. Poelt N86-L114. Kali-Gandaki-Gebiet, NW von Tukuche, Weg nach Podassedanda, Südhang mit schütterem Tränenkiefernbestand, 3300 m alt., in der Monsunwolkenzone, an einer alten Tränenkiefer [*Pinus wallichiana*], 28. III. 1979, leg. Kirschbaum s.n.

Note: One specimen of the collection of Handel-Mazzetti (no 879) from Sichuan cited by ZAHLBRUCKNER (1930: 234) under *Pyxine cocoes* was identified by KALB (1987: 45) as *P. cognata*.

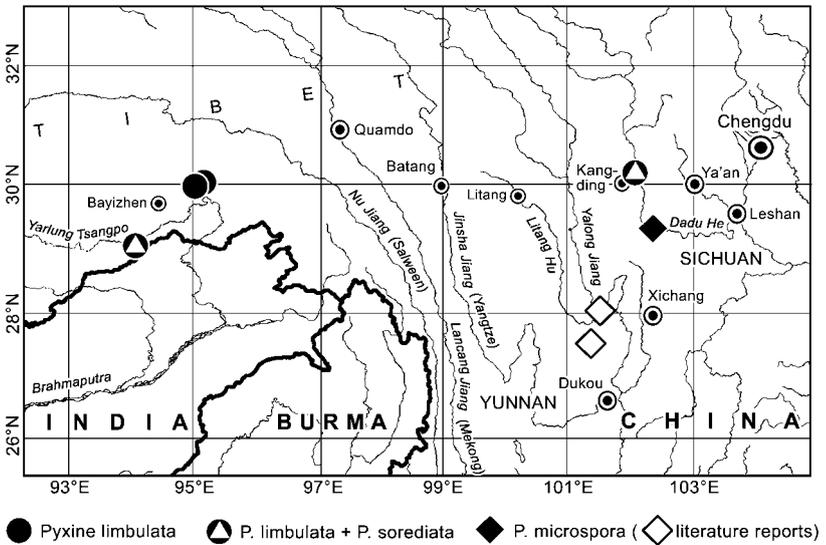


Fig. 32. Map of collection sites of *Pyxine limbulata*, *P. microspora* and *P. sorediata* in the Tibetan Area and adjacent regions.

Pyxine limbulata Müll.Arg.

Nepal. Khumbu, felsige Hänge südlich von Khumzung, 3800–3900 m alt., über Gneis, X. 1962, leg. J. Poelt L-364-dupl. Langtang Area, very humid mixed *Abies* forest on N-exposed slope S of Langtang Khola, S of Chamky, 3000–3200 m alt., 5. IX. 1986, leg. J. Poelt N86-L542.

Pyxine meissnerina Nyl.

Nepal. Chitaw district, 60 km W of Hetauda, NE part of Chitwan national park, 2 km S of Sauraha, thin deciduous forest, 27°35'N, 84°30'E, 214 m alt., 20. XII. 1979, leg. G. Thor 1592.

Pyxine microspora Vain.

Nepal. Langtang Area, Trisuli Ganga Valley, wet rocks on way from Bharku to Syabrubensi, 1500–1800 m alt., 27. VIII. 1986, leg. J. Poelt N86-L929.

TLC of the present sample: lichexanthone (maj.), microspora-triterpenes, testacein?(tr.)

Pyxine nilgiriensis D.D.Awasthi

Nepal. Langtang gorge, 1960 m alt., *Alnus-Lithoc.-Magnolia* forest, N-exposed, on bark of a branch fallen to the ground, veg.rec.no. 1041,1042, 18. X. 1986, leg. Miehe 14327.

Pyxine sorediata (Ach.) Mont.

Nepal. Langtang Area, forest on way from Ghora Tabela to Gumnachok to Lama Hotel, 3000–3500 m alt., 19. IX. 1986, leg. J. Poelt N86-L1102. Langtang Area, way from Syabru to Barkhu, humid forest, dry W-exposed pine-forest, 2000–2500 m alt., 22. IX. 1986, leg. J. Poelt N86-L865. Langtang Area, Schiabru, *Acer-Abies*-Wald, 3620 m alt., 21. X. 1986, leg. Miehe 14579a. Langtang Area, on way Khangjung to Sangsa (E of Khangjung), towards Pang Sang Lekh, 2800 m alt., 28. VIII. 1986, leg. J. Poelt N86-L1219. Langtang Area, Trisuli Ganga Valley, N below Dhunche and on way to Barkhu, 1800 m alt., 26. VIII. 1986, leg. J. Poelt N86-L968. Pokhara district, 16 km WNW of Pokhara, Naudanda, grass land with occasional trees, 1 km W of Naudanda, 28°20'N, 83°50'E, 1458 m alt., 12. XII. 1979, leg. G. Thor 1322. Prov. Taplejung, Weg Richtung Kangchendzönga, Strecke Dobhan to Nesum, 1300 m alt., an Silikatgestein, 8. XI. 1989, leg. Rettig 7359-dupl.

Additional specimen from Europe: [**Germany**], Böhmerwald, Kleiner Falkenstein, on *Fagus*, 14. IX. 1926, leg. Hilitzer (det. Nadvorník as *Physcia endochrysoides*; conf. J. Poelt as *Pyxine sorediata*).

Note: The envelope in GZU contains a small fragment of the thallus (less than one square centimetre). Recently KANZ et al. (2005: 362) drew attention to this interesting collection, which is the first and only report of *Pyxine sorediata* for Germany (originally reported by NÁDVORNÍK 1947: 122). The main part of the collection, which was not cited in the treatment of European *Pyxine* taxa (MOBERG 1983), is housed in the herbarium PRM (Zdeněk Palice in litt.).

Pyxine subcinerea Stirt.

Nepal. Langtang Area, on way Khangjung to Sangsa (E of Khangjung), towards Pang Sang Lekh, 2300 m alt., 28.VIII.1986, leg. J. Poelt N86-L1217. Pokhara district, 13 km NW of Pokhara, Naudanda, grass land with occasional trees, 28°20'N, 83°50'E, 1458 m alt., 12.XII.1979, leg. G. Thor 1567.

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