Hypogymnia caperatica, a new species from the Hengduan Shan area (easternmost Tibetan region), with notes on some rare taxa from the greater Tibetan region and Bhutan

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Abstract: OBERMAYER W., WITZMANN M. & McCune B. 2018: *Hypogymnia caperatica*, a new species from the Hengduan Shan area (easternmost Tibetan region), with notes on some rare taxa from the greater Tibetan region and Bhutan. – Herzogia **31**: 677–694.

Hypogymnia caperatica, which contains caperatic acid as a major lichen substance in the medulla, is described as new from the Hengduan Shan area (southeasternmost boundaries of the Tibetan plateau). The aliphatic acid caperatic acid is reported for the first time from the genus Hypogymnia. The occurrence of fatty acids in the genus Hypogymnia is generally discussed. The very rare lichen Hypogymnia sikkimensis, hitherto only known from two localities in Sikkim, as well as Hypogymnia diffractaica and H. hengduanensis are reported as new for the Kingdom of Bhutan. Hypogymnia capitata, known from the type and paratype specimens only, is reported from three further localities in the East Tibetan region.

Zusammenfassung: OBERMAYER W., WITZMANN M. & MCCUNE B. 2018: *Hypogymnia caperatica*, eine neue Art aus dem Hengduan-Shan-Gebiet (östlichste tibetische Region), mit Anmerkungen zu einigen seltenen Taxa aus dem tibetischen Großraum und Bhutan. – Herzogia **31**: 677–694.

Hypogymnia caperatica, die Caperatsäure als Hauptinhaltsstoff im Lagermark aufweist, wird aus dem Gebiet des Hengduan Shan (südöstlichste Randgebiete des Tibetischen Plateaus) neu beschrieben. Die Fettsäure Caperatsäure wird erstmals für die Gattung Hypogymnia angegeben und das Auftreten von Fettsäuren in dieser Gattung grundsätzlich diskutiert. Die bisher nur von zwei sikkimesischen Belegen bekannte Flechte Hypogymnia sikkimensis sowie H. diffractaica und H. hengduanensis werden für das Königreich Bhutan erstmals nachgewiesen. Hypogymnia capitata, die nur vom Typus- und Paratypusbeleg bekannt war, wird von drei weiteren, osttibetischen Fundorten angegeben.

Key words: Pruinose taxa, China, chemistry, apinnatic acid, fatty acid, lichenized fungi.

Introduction

Species of the lichen genus *Hypogymnia* contain a wide range of lichen metabolites of the following main structural classes: **orcinol depsides** (olivetoric acid), β -orcinol depsides (atranorin, chloroatranorin, barbatic [in former times sometimes confused with diffractaic acid], norbarbatic [=4-O-demethylbarbatic], diffractaic, elatinic, hypothamnolic, squamatic, baeomycesic [tr.] acids, 1'-methyl hypothamnolate), **orcinol depsidones** (alectoronic, physodic, oxyphysodic [=3-hydroxyphysodic, =conphysodic], 2'-O-methylphysodic, vittatolic acids), β -orcinol depsidones (physodalic, protocetraric, hypoprotocetraric, virensic acids), **usnic acid derivatives** (usnic, isousnic, placodiolic, pseudoplacodiolic acids), **terpenoids** (hopane-6 α ,22-diol [=zeorin]), various (yellowish to orange) unknown pigments (especially in the medulla

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just below the apothecia), further unknown substances (e.g. 'unknown C22', see McCune & Wang 2014: 64), and **aliphatic acids** (e.g. apinnatic acid complex). The new described species *Hypogymnia caperatica*, which morphologically resembles other pruinose, non-sorediate taxa of *Hypogymnia* (especially *Hypogymnia pruinosa*, but also *H. lijiangensis*, *H. pruinoidea*, *H. pseudopruinosa*, and *H. subpruinosa*) contains (beside atranorin and chloroatranorin) caperatic acid, a fatty acid, which has not yet been reported as a main lichen compound from the genus *Hypogymnia*. Additionally, some new reports of rare taxa of *Hypogymnia* from Bhutan and from Tibet and adjacent regions are given.

Material and methods

Habit photographs were taken with a CANON EOS 760D (equipped with a CANON macro lens EF 100 mm 1:2.8) and a LEICA Wild M3Z stereo-microscope (assembled with a ZEISS AxiocamMRc5 camera). To extend depth of field of the images, the program 'CombineZP' (an open source image processing software) was used. Close up pictures of thalli were stacked with the 'do stack' algorithm. Photos of spores and spermatia (=pycnospores) came from a ZEISS Axioskop microscope, equipped with the same camera as given above and were partly stacked with the 'pyramid weighted average' algorithm. Mean values of spore sizes are based on at least 20 measurements. For the identification of lichen substances, thin layer chromatography (TLC) was performed following a summary by ELIX (2014). Running height of the plates were 15 cm. For the detection of fatty acids, glass plates (Macherey-Nagel TLC Plates, ADAMANT UV254 with 0.25 mm silica gel layer) were dipped into (rather calcium-rich, comparatively 'hard') tap water for less than one second (instead of spraying). Pictures of TLC plates in transmission-daylight and under UV-light ('CAMAG UV Lamp 4'; 254 and 366 nm) were taken with the same CANON camera as mentioned above, using the lens CANON EFS 18–55 mm (with image stabilizer). If not otherwise stated, specimens are housed in GZU.

Specimens used for comparison of lichen substances (label-data are abridged; lichen compounds, which were important for comparison, are underlined): Alectoria ochroleuca (TLC: Usnic acid, diffractaic acid): Austria, Obermayer (13305). - Alectoria sarmentosa (TLC: Alectoronic acid, usnic acid): Austria, Obermayer (13611). - Cetrelia olivetorum (in a narrow sense) (TLC: Olivetoric acid, atranorin): Austria, Obermayer (13271). - Cladonia macilenta (TLC: Barbatic acid, thamnolic acid): Austria, Hafellner (66242). - Hypogymnia apinnata (TLC: Atranorin, chloroatranorin, fatty acids of the apinnatic acid complex): U.S.A., Spribille (38160; 39294) [MSC]. - Hypogymnia madeirensis (TLC: Atranorin, chloroatranorin, norbarbatic (=4-O-demethylbarbatic), physodic acids): Portugal, Madeira, Vezda, 'Lichenes Rariores Exsiccati 63', Feige (12359). - Hypogymnia physodes (TLC: Physodalic, oxyphysodic, physodic, protocetraric acids, atranorin, chloroatranorin): Austria, Obermayer (13090). - Hypogymnia vittata (TLC: Atranorin, physodic, oxyphysodic, vittatolic acids, unknown z1): Austria, Obermayer (1006). - Lecanora barkmaniana (TLC: Chloroatranorin, atranorin, zeorin): Austria, Berger (32774). – Parmotrema xanthinum s.l. (TLC: Usnic acid, gyrophoric acid, several unknown fatty acids in major and minor quantities [probably including apinnatic acid]): Uruguay, Osorio (9651; determined by Jack Elix). - U.S.A., 'Lichens of Eastern North America Exsiccati 195', Lendemer (3727). – Note: Due to the TLC results, the samples belong the chemically defined taxon P. madagascariaceum. – Platismatia glauca (TLC: Atranorin, chloroatranorin, caperatic acid syndrome): Austria, Obermayer (13785b). -Usnea hirta (TLC: Usnic acid, three acids of the murolic acid complex): Austria, Obermayer (12738).

Results

Hypogymnia caperatica Obermayer & McCune, species nova

(figures 1-4)

MycoBank number: MB 824621

Diagnosis: *Hypogymnia caperatica* morphologically resembles some other pruinose (esorediate and mostly non-isidiate) taxa of *Hypogymnia* from Asia, but differs in the medullary chemistry. It contains the aliphatic compound caperatic acid and related substances, whereas similar taxa named below con-

tain various depsidones, like alectoronic acid (in *H. pruinosa*), physodic, physodalic, oxyphysodic, protocetraric acids, and 2'-O-methylphysodic acids (in *H. lijiangensis*, *H. subpruinosa*, and the epruinose *H. laccata*), or physodic, oxyphysodic, and vittatolic acids (in *H. pruinoidea* and *H. pseudopruinosa*).

Type: China, Tibet, prov. Sichuan, Hengduan Shan, Shaluli Shan, 35km north-northeast of Batang, southeast of Yidun, 30°16'N, 99°28'E, elevation 4200–4300 m, pasture with schist outcrops and single *Juniperus* trees, on bryophytes above ground, 27 June 1994, Walter Obermayer (3493) [GZU].

Description: Thallus appressed (and often adnate) to the substrate, often rosettiform (up to 6cm in diameter), epruinose parts pale beige-brown to dark brown, pruinose parts whitish, without isidia or soredia or budding lobules. Lobes separate to contiguous to partly overlapping, branching of lobes isotomic dichotomous or irregular, black border on the lateral sides of the lobes often distinctly developed but sometimes also lacking; lobe width 0.8-1.5(-2.5) mm; upper surface of lobes smooth, partly strongly pruinose, often with an abrupt border to epruinose parts (figs. 1D; 4A,B), pruina on lobe tips eventually fine reticulate; lobe tips and axils partly with comparably big, circular perforations, margins of old perforations slightly curved inwards (well-developed circular perforations may occur even on the upper side of lobes, see figure 2A); lower surface of lobes (shiny) brownish, rugose, perforations difficult to see because just behind the tips the lobes are closely adnate to the substrate). Medulla hollow, ceiling of the cavity white at the foremost lobe tips (greyish behind the utmost tip area), floor of the cavity grayish to dark brown. **Apothecia** (figs. 1, 3, 4A) common, substipitate to stipitate, up to 1.5 cm in diameter, disc brown to (glossy) dark-brown, cortex of young apothecia smooth, of big (old) apothecia strongly rugose. **Hymenia** 35–40 µm, colourless, epihymenium brownish, paraphyses with internal dark brown cap at their slightly swollen tips. Asci 8-spored (figure 4D–F), $25-30 \times 12-14 \,\mu m$. **Spores** subglobose (very broadly ellipsoid) or almost globose, $(4.5-)5-6(-7)\times4.5-5.5(-6)\,\mu m$ (figure 4D–F). Spermatogonia (=pycnidia) black, when young sunken into the lobes and covered with the pruina of the lobes, older ones projecting from the thallus and epruinose, 125 µm in diameter (figure 4A,B). **Spermatia** (=pycnospores) cylindrical (rod-shaped) at the first glance, but mostly very weakly bottle shaped (swelling slightly submedial), $5.5-6.7 \times 1-1.2 \,\mu m$ (figure 4C).

Chemistry (figures 8,9): Atranorin and chloroatranorin (minor in apothecia, traces in thallus), caperatic acid (major) and related compounds in traces; unknown 'x1' (minor), unknown 'x2' (traces), and unknown 'x3' (traces). Medulla under UV366 without strongly white emitted light. – Note 1: 'x1' and 'x2'. were only detected in the holotype material. 'x1' can be seen as a minor spot in solvent system B', slightly below the height of diffractaic acid. 'x2' appears as a weak, ring-like spot in solvent system A (slightly above the starting line) and in C (at the height of oxyphysodic acid). 'x3' was found in the sample of the holotype, which contains a big apothecium. The spot shows a white fluorescence in short wave UV after running and resembles an unknown substance in *Alectoria ochroleuca* (see figs. 8,9 number 21). – Note 2: Interestingly, apothecia contain a significantly higher amount of atranorin (but not of chloroatranorin) as do sterile parts of the thallus (see figs. 8,9: sample number 6 with one big apothecium, sample number 5 with thallus only). A somehow similar situation of unequal concentration of lichen substances within one thallus was demonstrated e.g. for the genus *Cetrelia* (see Obermayer & Mayrhofer 2007: 240), where soralia were shown to contain much more atranorin than esorediate parts of the thalli.

Etymology: The epithet refers to the main medullary lichen substance caperatic acid.

Substrate: On bark of trees (*Picea* and *Salix*) and on bryophytes above ground.

Distribution and ecology: Occurs together (and even intermixed) with *Hypogymnia pruinosa* at high altitudes (between 3700 and 4700 m) near the timber line in the Hengduan Shan area.

Notes: Morphologically, *Hypogymnia caperatica* strongly resmbles *H. pruinosa*, the latter with the orcinol depsidone alectoronic acid instead of the fatty acid caperatic acid. In fact, one specimen in GZU (Obermayer 4024) was cited under *H. pruinosa* in an earlier study (McCune & Wang 2014: 58). Another specimen (Obermayer 3296) contains a mixture of both taxa (two thalli of *H. caperatica* and c. six thalli of *H. pruinosa*). Under UV366-light, *H. pruinosa* can easily be separated by the strong white fluorescence of the medulla due to alectoronic acid.

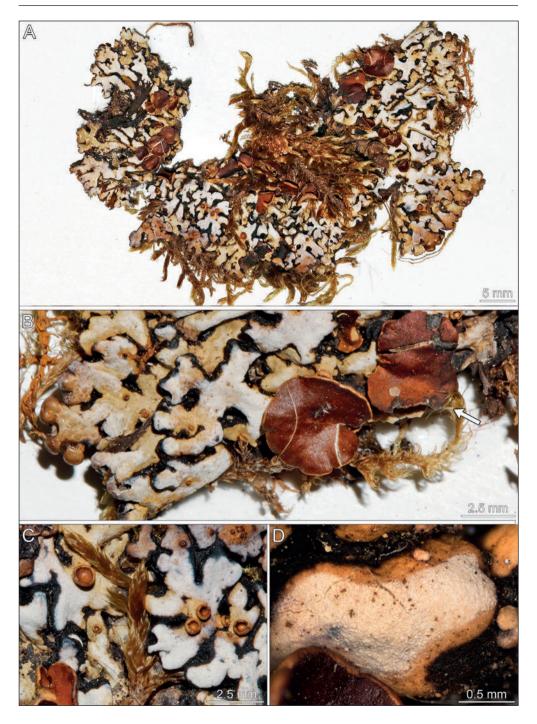


Figure 1: $Hypogymnia\ caperatica$, holotype (Obermayer 3493). – A) Habit. – B,C) Close-ups of the (in large part) pruinose thallus, in B) with large apothecia (white arrow points at an apothecium, which was used up for TLC investigation). – D) Strongly pruinose lobe with an abrupt border to epruinose parts.

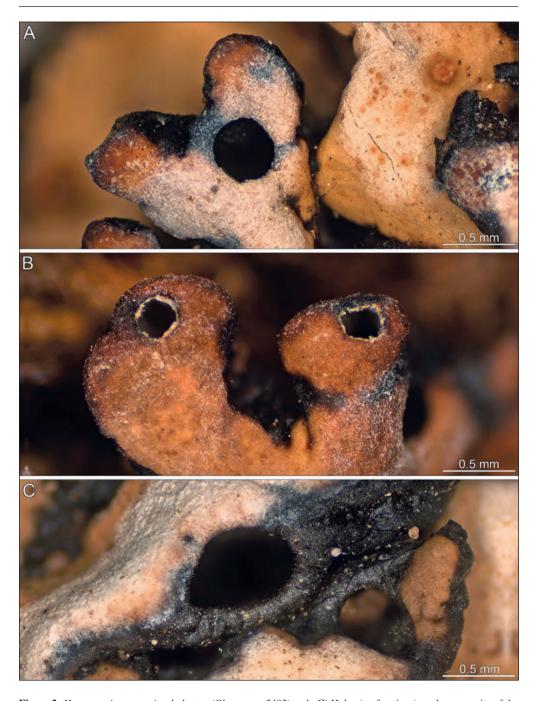


Figure 2: *Hypogymnia caperatica*, holotype (Obermayer 3493). - **A–C**) Holes (perforations) on the upper site of the thallus (A), near the lobe tips (B) and at the black bordered rim of the lobes (C).

The shape of ascospores of *Hypogymnia caperatica*, which are globose to subglobose, agree well with that of *H. pruinosa* pictured in the protologue (see WEI & JIANG 1980: 386, figure 1.1). However,

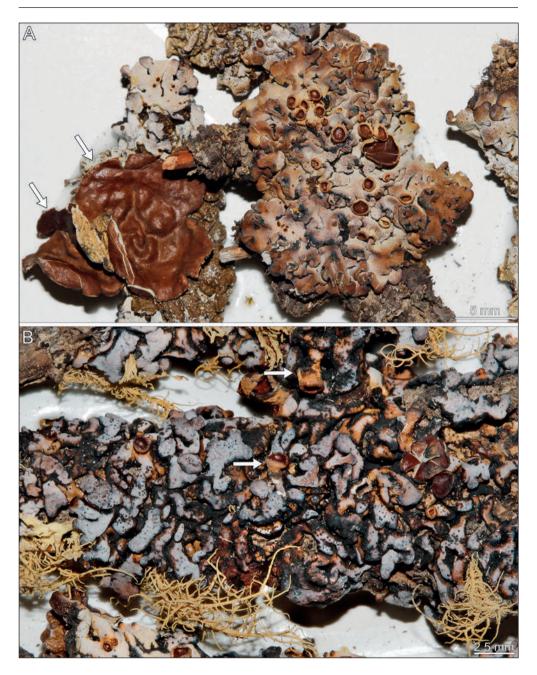


Figure 3: *Hypogymnia caperatica*; morphological diversity seen in paratypes. – **A)** Two huge apothecia (white arrows) reaching 1.5 cm in diameter (left side) and rosettiform thallus, with small and middle-sized apothecia (right side); Obermayer 3930. – **B)** Strongly pruinose thallus with black rimed lobes; white arrows point at two young, stipitate apothecia; (Obermayer 3323).

the spore size of *H. pruinosa* is given as "...3.12–4.16 μ m..." in the original description and thus would clearly differ from the spore sizes of our new described *H. caperatica* ((4.5–)5–6(–7) ×

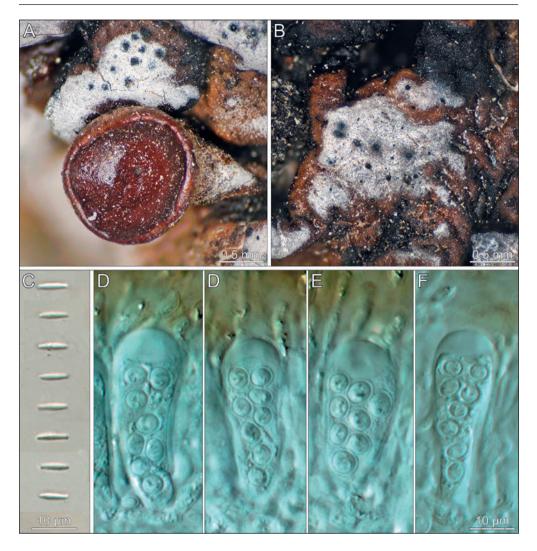


Figure 4: *Hypogymnia caperatica.* – **A,B)** Spermatogonia (in A together with one apothecium) partly covered with a thick white pruina (Obermayer 3323). – **C)** Spermatia (in KOH) with unusual morphology (cylindrical with a weak, submedial swelling versus weakly bifusiform in many other Hypogymnias). Orientation chosen after lateral illumination (thus, fracture points of spermatia are partly left and partly right) (Obermayer 3323). – **D–F**) Asci and spores (in KOH, slightly squeezed) (holotype, Obermayer 3493).

 $4.5-5.5(-6)\mu m$). Nevertheless, comparing spore sizes from several fertile specimens of *H. pruinosa*, we found, that only young or poorly developed spores are that small, but when spores are well developed and plentiful, they average $6-8 \times 4.5-5.5 \mu m$; more comparisons are needed.

The shape of spermatia (=pycnospores), which are very slightly bottle-shaped ('cylindrical' at the first glance) in *Hypogymnia caperatica* also agree with that in *Hypogymnia pruinosa*, whereas most other Hypogymnias (with the exception of e.g. *H. capitata*) have weakly bifusiform spermatia (see McCune & Wang 2014: 58). The width of the spermatia (in general distinctly broader than 1 μm) in *Hypogymnia caperatica* is unusual.

Isidia, which are cited to rarely occur in *Hypogymnia pruinosa* (McCune & Wang 2014: 58) and may be just regeneration lobes, were not found in *H. caperatica* (note: *H. pruinosa* is keyed out in McCune & Wang 2014: 32 as 'non-isidiate'). A black rim bordering the lobes of the new described *H. caperatica* is often clearly developed (figure 1C). This character is lacking in *Hypogymnia pruinosa* (McCune & Wang 2014: 58) but should be characteristic for e.g. *H. lijiangensis* (see key of pruinose taxa in Wei & Wei 2012: 784). The latter species and three other *Hypogymnia* taxa (i.e. *H. pruinoidea*, *H. pseudopruinosa*, and *H. subpruinosa*) also partly resemble *H. caperatica* in morphology, but likewise differ in medullary chemistry (summarized below).

All of these named taxa which are at least partially pruinose, were morphologically compared by WEI & WEI (2012) and by McCune & Wang (2014), the latter authors adding the main chemistry. Including the new described Hypogymnia caperatica, a comparison of the main medullary compounds of these 'pruinose taxa' (lacking soredia and isidia) reveals four chemical groups: 1) Physodic, physodalic, oxyphysodic, protocetraric, 2'-O-methylphysodic acids (in H. lijiangensis and H. subpruinosa [and the non-pruinose, but otherwise quite similar H. laccata]), 2) Physodic, oxyphysodic, vittatolic acids (in H. pruinoidea and H. pseudopruinosa), 3) Alectoronic acid (in H. pruinosa), and 4) Caperatic acid (in H. caperatica). Freshly collected material studied with molecular methods might solve the question of whether the named Hypogymnia taxa are 'good' species or (especially in the case of H. pru*inosa* and the newly described *H. caperatica*) should be regarded as chemical strains of one taxon only. Additional specimens examined: China, Tibet, prov. Xizang, 120km SSW of Quamdo (=Changtu), 10km S of Bamda, 30°09'N, 97°17'E, elevation 4500-4700 m, on Picea, 6 July 1994, Obermayer (3930). - Tibet, prov. Xizang, way from Bamda to Quamdo (=Changtu), 30°42'N, 97°15'E, elevation 4050 m, on Picea, 7 July 1994, Obermayer (4024). Notes: This specimen was cited by McCune & Wang (2014: 58) under Hypogymnia pruinosa. Another cited specimen (Obermayer 3215) proved to be the real H. pruinosa (with alectoronic acid, traces of atranorin, and minor quantities of unknown substances; see below). - Tibet, prov. Sichuan, Tibetan fringe mountains (=Hengduan Shan), Shaluli Shan, 18 km SSE of Litang, pass area between Litang and Cogsum, 29°52'25"N, 100°19'57"E, elevation 4100 m, *Picea* forest with *Salix*, on *Salix*, 11 August 2000, Obermayer (9636), [specimen with additional H. lijiangensis]. - prov. Sichuan, Shaluli Shan Mts., 30km NE of Batang, S of Yidun, 30°16'N, 99°25'E, elevation 3750-3800 m, on Picea, 25 June 1994, Obermayer (3296). Note: The specimen is intermixed with H. pruinosa. - prov. Sichuan, Shaluli Shan Mts., 30 km NNE of Batang, SSE of Yidun, 30°16'N, 99°25'E, elevation 4100-4150 m, S-facing slope with Quercus cf. aquifolioides, Juniperus tibetica and Picea spec., on Picea, 26 June 1994, Obermayer (3323). Note that several of these were previously determined in 2005 by Xin-Li Wei as H. pruinosa.

General remarks on the occurrence of fatty acids in the genus *Hypogymnia*

Until recently, the occurrence of aliphatic compounds in the genus *Hypogymnia* were not in the focus of chemical investigations, although authors were aware of their existence (see McCune & Wang 2014: 28). Early reports on lipids and fatty acids in *Hypogymnia* (*H. physodes*) come from Dertien et al. (1977). Beside listing a range of unnamed fatty acids, the authors made mention of behenic acid (= docosanoic acid), a saturated higher fatty acid on the frontier between fatty acids and wax acids), eicosadienoic acid, and of linoleic acid (the latter two are unsaturated omega-6 fatty acids). The named aliphatic acids probably play a role in the primary metabolism of the algal partner but cannot be regarded as "typical secondary lichen compounds". It was only in 2010 that a fatty acid as a major lichen compound was first reported from taxa of *Hypogymnia* (*H. wilfiana*, *H. apinnata*, and *H. occidentalis*; see Goward et al. 2010). Since then, the (newly named) fatty acid apinnatic acid (previously known from a few other lichens as 2-methylene-3-carboxy-18-hydroxynonadecanoic acid) has been reported by Goward et al. (2012) from five other Hypogymnias from North America: *H. verruculosa* (in 100% of investigated specimens), *H. protea* (85%), *H. dichroma* (75%), *H. salsa* (50%), and *H. austerodes* (12%).

Our TLC-studies on nine specimens of *Hypogymnia apinnata* from North America showed a more complex fatty acid profile for this species than suggested by Goward et al. (2012).

Instead of just one fatty acid, we observed four spots with fatty acid behavior on the plates, the spots occurring in at least three combinations of two to four spots. None of these spots occurred in all specimens. All spots of this complex (here named as 'apinnatic acid complex') are, if highly concentrated, pale pinkish-brown after sulfuric acid treatment and heating; some are weakly UV+ prior to that treatment. Their running heights are quite similar to fatty acid spots of the murolic acid complex (figure 10, numbers 5 and 6), and especially of the allopertusaric acid complex (figs. 8,9, numbers 3 and 4) all of which run at Rf-classes between 2 and 4 in solvent system A, between 3–4 and 5 in B', and between 3 and 4–5 in C. According to Goward et al. (2012: 89), the relative running heights of apinnatic acid on TLC plates are A18, B'27, C33. Based on these data, we tentatively assigned one spot as apinnatic acid in A and B' and with some more uncertainty in C (see figure 10: "a" and "a?").

As *Parmotrema xanthinum* is cited by ELIX (2014) as a source for apinnatic acid [(-)-2-Methylene-3(R)-carboxy-18(R)-hydroxynonadecanoic acid)], we used two samples of that species to prove its occurrence in *Hypogymnia apinnata*. Both specimens in GZU, determined as *Parmotrema xanthinum*, contained several, partly high concentrated fatty acids and additionally gyrophoric acid (the latter substance is diagnostic for the chemically separated taxon *P. madagascariaceum*). Due to the number of different fatty acids, the 'real' apinnatic acid could not be reliably assigned (note that unknown fatty acids are common in taxa of the genus *Parmotrema*; see e.g. Spielmann & Marcelli 2009). Figure 10 shows that some of the fatty acid spots in *Hypogymnia apinnata* and *Parmotrema xanthinum* s.l. differ significantly in their relative height on the TLC-plates. Protolichesterinic acid, which sometimes is cited for *Parmotrema xanthinum* s.str. (e.g. in Brodo 2016: 251) might be one of those spots. TLC studies of fatty acids are inherently limited and thus, we suggest that further studies using the methyl esters of these substances (abbreviated as FAMEs, see e.g. Vu et al. 2016) are necessary to clarify the identity of these fatty acids, which partly may be undescribed.

The identity of caperatic acid as the main medullary fatty acid in *Hypogymnia caperatica* was demonstrated using *Platismatia glauca* and *Flavoparmelia caperata* as controls for this aliphatic compound. On TLC plates, caperatic acid also appears as a complex, consisting of two (or three) spots (see e.g. figure 9 number 5, and OBERMAYER & RANDLANE 2012: 58, figure 5). It is best seen in solvent system B', where the main spot is conically narrowed to the base line (probably consisting of two spots) and a weak concentrated spot occurs below it.

Regarding the huge range of the Himalayas, the Tibetan plateau and the Hengduan Shan area in a broad sense (the latter including Wuliang Shan and Ailoa Shan in the south and Qionglai Shan and Min Shan in the east; see Tang 2015: 2–7) none of the following *Hypogymnia* taxa were reported to contain fatty acids as main medullary substance: *H. alpina*, *H. arcuata*, *H. austerodes*, *H. bitteri*, *H. bulbosa*, *H. capitata*, *H. congesta*, *H. crystallina*, *H. delavayi* (incl. *H. yunnanensis*), *H. diffractaica*, *H. flavida*, *H. hengduanensis*, *H. hypotrypa*, *H. irregularis*, *H. laccata*, *H. laxa*, *H. lijiangensis*, *H. macrospora*, *H. magnifica*, *H. metaphysodes*, *H. mundata*, *H. nitida*, *H. pendula*, *H. physodes*, *H. pruinosa*, *H. pseudobitteriana*, *H. subarticulata*, *H. subfarinacea*, *H. subpruinosa*, *H. tenuispora*, *H. thomsoniana*, *H. tubulosa*, *H. vittata* (the list above is compiled from recently published papers by Wei et al. 2010, McCune 2011, McCune et al. 2012, McCune 2012, Wei & Wei 2012, McCune & Wang 2014, McCune et al. 2015).

Notes on some rare taxa of *Hypogymnia* from the greater Tibetan region and Bhutan

Hypogymnia capitata McCune

(figure 6)

Characteristics in brief (excerpted from McCune & Wang 2014: 40): Lobes contiguous to separate, brownish, smooth then becoming rugose; lobe outline even; <u>soralia</u> terminal and <u>capitate</u>, lobe cavities with white ceilings and white to dark floors, lobes sparsely perforate below; <u>spermatia</u> are often typically <u>cylindrical</u> rather than weakly bifusiform (as in most species of *Hypogymnia*); <u>olivetoric acid</u> as a major medullary substance.

Chemistry of the studied specimen (listed below): Atranorin and chloroatranorin (minor or traces), olivetoric acid.

Notes: Hypogymnia capitata was described (see McCune & Wang 2014: 40) on the base of the holotype material (Chola Shan, near Manigango, at 4730 m) and one paratype specimen (Shaluli Shan, near Batang, at 4300 m), both housed in GZU. Pictures of the specimens are available at http://hypogymnia. myspecies.info/ (McCune 2014 onwards). The additional three specimens cited below come from a mountain area near the paratype locality (Miehe 94-29-5 H; see figure 6), from a pass area near Bamda (=Bangdazhen) (Obermayer 3916), and from mountains south of Litang (Obermayer 09682). All specimens contain olivetoric acid (major) and atranorin (minor). Unknown terpenoids were additionally found in the first named specimen. Physodalic and protocetraric acids, which are mentioned for the paratype material, were not detected. The morphological and chemical differences with Hypogymnia tubulosa and H. submundata (both taxa also with capitate soralia) are discussed by McCune & Wang 2014. Interestingly, the shape of spermatia (cited as "...cylindrical...") are similar to those of H. pruinosa (cited as "...rod-shaped...") and the newly described H. caperatica, and thus differ from the weakly bifusiform spermatia of most other Hypogymnia species. Regarding the chemical differences (physodic and oxyphysodic acids in H. tubulosa and H. submundata versus olivetoric acid in H. capitata), the situation is analogous to the chemically defined taxa of *Pseudevernia furfuracea* (var. furfuracea with physodic and oxyphysodic acids versus var. ceratea with olivetoric acid). According to the few available locality data, the species seems to be a typical element of rather high altitudes.

Specimens examined (in addition to the type and paratype material): CHINA, Tibet, prov. Xizang, 120 km SSW of Quamdo (=Changtu), 10 km S of Bamda, 30°09'N, 97°17'E, elevation 4600–4800 m, alpine meadows with *Kobresia*, on *Rhododendron*, 5.VII.1994, Obermayer (3916). - Prov. Sichuan, E Tibet, Litang to Batang, Jinsha (Yangtse) tributary, E of Yidun / Yarwa, below pass to Litang plateau, 30°20'N, 99°33'E, elevation 4500 m, lake shore communities, on bark, 1994-06-29, collected by G.Miehe (94-29-5 H) & U.Wündisch. - Prov. Sichuan, Hengduan Shan, Shaluli Shan, 50 km south of Litang, road from Cogsum to Sumdo, 29°33'05"N, 100°17'25"E, 4270 m alt., boulder field (big siliceous rocks), with *Potentilla, Caragana, Salix, Juniperus*, on *Juniperus*, 7.VIII.2000, Obermayer (09682).

Hypogymnia diffractaica McCune

(figure 7A,B)

Characteristics in brief (excerpted from McCune et al. 2003: 230–231): Lobes narrow, separate (slender), with arcuate lobe tips, often browning; <u>isidia absent</u>; <u>holes</u> in lower surface with <u>raised rim</u>; lower surface expanded and puffed out; <u>diffractaic</u> (major) and barbatic acids (minor) as medullary compounds.

Chemistry of the studied specimens: Atranorin (traces only, or not detected), diffractaic acid (major), unknown, slightly rose pigment "z1", probably related to placodiolic/pseudoplacodiolic acids (minor; in A slightly above zeorin, in B slightly below norstictic acid, in C slightly above diffractaic acid [see figs. 8,9, number 20]).

Notes: The closely related species *Hypogymnia hengduanensis* (see below) has an almost identical habit (pendulous narrow lobes and lax branching) and chemistry (diffractaic acid), but differs by the presence of globose to cylindrical isidia. Barbatic acid, which is cited in the protologue as minor compound in *H. diffractaica*, was not detected in the material examined by us. *Hypogymnia diffractaica* seems to range to slightly higher altitudes than *H. hengduanensis*. It was hitherto known from the Hengduan Shan (and adjacent areas) and is now reported as new for the Kingdom of Bhutan.



Figure 5: *Hypogymnia pruinosa* (Obermayer 3215). – A) Rosettiform thallus with two huge apothecia (up to 1.2 cm in diameter). – B) Close up of strongly pruinose lobes.

Specimens examined: Bhutan, Flor-Prov. C14 (Bumthang distr., Tsochen Chen, 27°45'N, 90°35'E, 4020 m alt., open *Abies densa* forest on E-facing slope (former grazing), 25.VI.2000, Miehe (00-132-20/03b). – **China**, Xizang,



Figure 6: *Hypogymnia capitata* (Miehe 94-29-5 H). – **A)** Marginal part of the thallus with four capitate (pseudo-) soralia. – **B–D)** Perforations (holes) on lobe tips from very young (B) to old stages (D). – **E)** Thumb-like lobe with capitate (pseudo-)soralium (soredia are not distinctly separated but still 'glue' together).

SE-Tibet, Gyala Peri W, Upper Bong Chu (Lang Chu), E of pass Nyingchi to Dongjuk, 29°37'N, 94°35'E, 4300 m, uppermost *Abies* forest on E-facing slope, on bark, 1994-08-26, Miehe (94-233-33/05) & Wündisch.

Hypogymnia hengduanensis Wei

(figure 7C)

Characteristics in brief (excerpted from Wei 1984 and McCune & Wang 2014: 43–44): Lobes erect to more often trailing (<u>budding lobules</u> occasional), branching pattern mostly isotomic dichotomous, upper surface smooth to weakly rugose; <u>isidia present</u>, cylindrical to coralloid or sometimes globose; <u>holes</u> in lower surface with <u>raised rim</u>; medulla of lobes hollow, ceiling and floor of cavity dark; with medullary <u>diffractaic acid</u> (and rarely barbatic acid); apothecia rare.



Figure 7: A,B) *Hypogymnia diffractaica* (Miehe 00-132-20/03b) with typical rimed holes (perforations) at lobe tips (B). – C) *Hypogymnia hengduanensis* (Miehe 00-441-26/09a) with isidia and budding lobules. – **D,E**) *Hypogymnia sikkimensis* (Miehe 00-441-26/09b) with isidiate upper surface of the lobes (D; isidia partly abraded) and one typical, huge circular perforation on the lower surface (E).

Chemistry of the studied specimens: Atranorin (traces only), diffractaic acid (major), unknown substances (including yellow pigments) as accessories, barbatic acid not detected.

Notes: Two specimens from the herbarium GZU (Obermayer 8724; 7411) have already been cited by McCune & Wang 2014: 44). The new samples cited below (except the Bhutan locality) were determined by Xin-Lin Wei (Beijing, 2005) on the basis of morphology and are confirmed here in part by TLC. Barbatic acid, which is cited in the protologue to occur in traces, was not detected in the material seen by us. The closely related species *Hypogymnia diffractaica* (see above) with almost identical habit and chemistry lacks isidia. *Hypogymnia hengduanensis*, a quite common species in Southeast Asia at comparably high altitudes is reported here as new for the Kingdom of Bhutan.

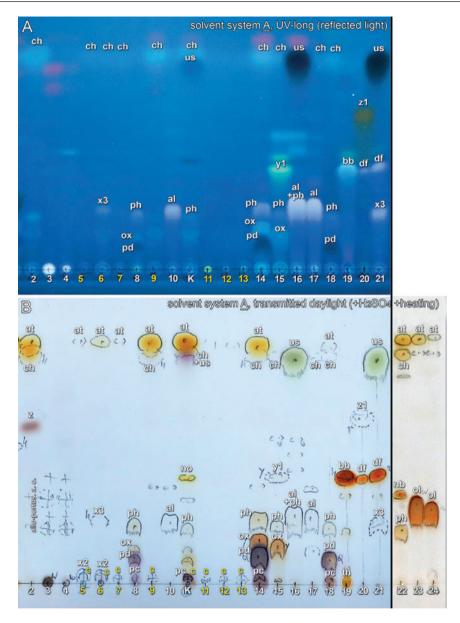


Figure 8: Chromatograms of several taxa of *Hypogymnia* in solvent system A. – **A**) Reflected UV-366-light. – **B**) Transmitted daylight after H₂SO₄ and heating. - 3,4 *Lepra albescens* (Berger 32777). - 5,6 *Hypogymnia caperatica* (holotype, Ob. 3493; 6 with one big apothecium). - 7,11–13 *H. caperatica* (7 Ob. 3323; 11 Ob. 4024; 12+13 Ob. 3930). - 14 *H. physodes* (Ob. 13090). - 15 *H. vittata* (Hafellner 68899). - 16 *Alectoria sarmentosa* (Ob. 13611). - 17 *H. pruinosa* (Ob. 3215). - 18 *H.* spec.1 (Ob. 3452). - 19 *Cladonia macilenta* (Hf. 66242). - 20 *H. diffractaica* (Miehe 94-233-33/05). - 21 *Alectoria ochroleuca* (Ob. 13305). - 22 *H. madeirensis* (Feige 12359). - 23 *Cetrelia olivetorum* s.str. (Ob. 13271). - 24 *H. capitata* (Miehe 94-29-5H). - Abbreviations: al = alectoronic acid, allo-pertus.a.c = allo-pertusaric acid complex, at = atranorin, bb = barbatic acid, c = caperatic acid, ch = chloroatranorin, df = diffractaic acid, nb = norbarbatic [=4-O-demethylbarbatic] acid, ol = olivetoric acid, ox = oxyphysodic [=3-hydroxyphysodic] acid, pc = protocetraric acid, ph = physodic acid, pd = physodalic acid, th = thamnolic acid, us = usnic acid, vi = vittatolic acid, x1 + x2 + x3 = unknown substances in *H. caperatica* (holotype), y1 = unknown substance in *H. vittata*, z = zeorin, z1 = unknown substance in *H. diffractaica*.

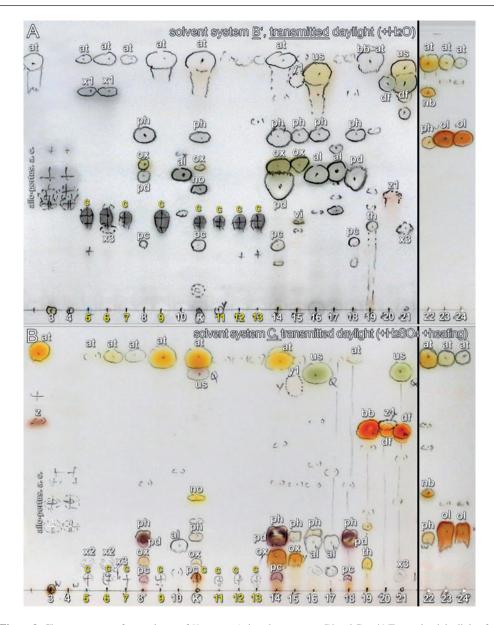


Figure 9: Chromatograms of several taxa of *Hypogymnia* in solvent system B' and C. – **A**) <u>Transmitted</u> daylight after 'watering' in B'. – **B**) <u>Transmitted</u> daylight after H₂SO₄ and heating in C. – 3,4 *Lepra albescens.* – 5,6 *Hypogymnia caperatica* (holotype, 6 with one big apothecium). – 7,11–13 *H. caperatica*. – 14 *H. physodes.* – 15 *H. vittata*. – 16 *Alectoria sarmentosa*. – 17 *H. pruinosa*. – 18 *H.* spec.1. – 19 *Cladonia macilenta*. – 20 *H. diffractaica*. – 21 *Alectoria ochroleuca*. – 22 *H. madeirensis*. – 23 *Cetrelia olivetorum* s.str. – 24 *H. capitata*. For collector numbers of each sample and for abbreviations of lichen substances see figure 8.

Specimen examined: BHUTAN, Flor-Prov. N22 (Upper Kuru Chu), Lhuntse distr., S of Singye Dzong, 27°58'N, 91°18'E, elevation 3950 m, open *Juniperus recurva* forest and scrub near treeline on sunny slope, 23.XI.2000, Miehe (00-441-26/09a; associated *Hypogymnia sikkimensis* has been separated under 00-441-26/09a [see below]). – **China**, Tibet, prov. Xizang, Nyainqêntanglha Shan, 345 km E of Lhasa, 20 km NE of Nyingchi, 5 km E of the pass, near the timber line, 20°38'N, 94°42'E, 4200–4300 m alt., *Juniperus-(Abies-)* forest with siliceous boulders, on

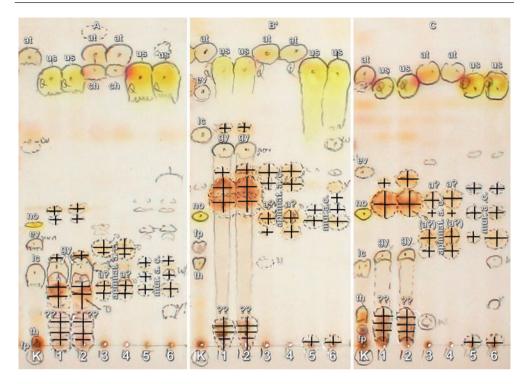


Figure 10: Characters of fatty acid complexes (highlighted by black crossing bars) in solvent systems A, B', and C. - K = mixture of *Cladonia fimbriata*, *Cladonia symphycarpia*, *Evernia prunastri*, *Hypocenomyce scalaris*, and *Thamnolia vermicularis* s.str. - 1,2 *Parmotrema xanthinum* s.l. (1 Osorio 9651; 2 Lendemer 3727). - 3,4 *Hypogymnia apinnata* (3 Spribille 39294; 4 Spribille 38160). - 5,6 *Usnea hirta* (5 Mayrhofer 13867; 6 Obermayer 12738). - Abbreviations: a = atranorin, apinnat.a.c. = apinnatic acid complex (a? = tentatively apinnatic acid s.str.), ch = chloro-atranorin, ev = evernic acid, fp = fumarprotocetraric acid, gy = gyrophoric acid, mur.a.c = murolic acid complex, no = norstictic acid, th = thamnolic acid, us = usnic acid.

Juniperus (covered with bryophytes), 26.VIII.1994, Obermayer (7428). - Tibet, prov. Xizang, 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 Abies, 21.VIII.1994, Obermayer (6954). - Prov. Sichuan, SE-Tibetan fringe mountains (=Hengduan Shan), Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougou glacier and forest park, path from the glacier-viewpoint to the glacier, 29°34'01"N, 101°58'48"E, 3150–3200 m alt., lateral margin area of the glacier with boulders, on *Betula utilis*, 28.VII.2000, Obermayer (8875).

Hypogymnia pruinosa Wei & Jiang

(figure 5)

Characteristics in brief (excerpted from WEI & JIANG 1980: 386–387, WEI & WEI 2012: 788, and McCune & Wang 2014: 58): Thallus appressed, often rosettiform, the lobe tips often with a <u>pronounced white pruinose patch</u>; lobes strongly inflated, but compressed and contorted from crowding; soredia lacking; isidia rare; spermatia <u>rod-shaped</u> <u>or</u> with a slight <u>submedial swelling</u>; <u>alectoronic</u> acid in the medulla (and thus UV366 white).

Chemistry of the studied specimen: Atranorin (traces or not detected), alectoronic acid (major), traces of unknown substances.

Notes: *Hypogymnia pruinosa* is easily separated from the morphologically similar *H. caperatica* by the strong UV+ white medulla (under UV366-light) due to alectoronic acid. See *Hypogymnia caperatica* for further discussion.

Specimens examined: CHINA, prov. Sichuan, Shaluli Shan Mts., 30 km NE of Batang, S of Yidun, 30°16'N, 99°25'E, elevation 3750–3800 m, on bryophytes (soil above marble outcrops), 25 June 1994, Obermayer (3215),

determined by Xinli Wei (2005). Note: This specimen has already been cited by McCune & Wang (2014: 58). The second cited specimen from GZU (Obermayer 4024) turned out to be the new *Hypogymnia caperatica* (see above). - ibidem, on *Picea*, Obermayer (3296). Note: The specimen contains both *Hypogymnia caperatica* (two thalli) and *H. pruinosa* (c. six thalli).

Hypogymnia sikkimensis G.P.Sinha & Elix

(figure 7D,E)

Characteristics in brief (excerpted from SINHA & ELIX 2003: 81–83 and McCune et al 604–605): Thallus grey to yellowish green, <u>isidiate</u>, linear-elongate lobes with adventitious budding; <u>large</u>, ellipsoidal, subapical <u>holes</u> in the <u>lower surface</u>; <u>usnic acid</u>, <u>isousnic acid</u>, atranorin, chloroatranorin, traces of placodiolic acid and pseudoplacodiolic acids; species similar to the two other usnic-acid-containing taxa *Hypogymnia flavida* and *H. hypotrypa*.

Chemistry of the studied specimen: Atranorin, chloroatranorin, usnic acid, isousnic acid, zeorin (traces). Note: Although not cited in the protologue (SINHA & ELIX 2003: 82; see also McCune et al. 2012), zeorin is mentioned as a minor compound on the isotype sheet in CANB (see: http://plants.jstor.org/stable/viewer/10.5555/al.ap.specimen.canb667300).

Notes: According to McCune et al (2012: 604–605) only two locations of *Hypogymnia sikkimensis* were hitherto known. Both, the type collection and one additional sample come from mountainous regions in Sikkim. Our studied specimen (being isidiate on the upper surface) does not show the yellowish colour diagnostic for usnic acid. Instead it has a greyish-brownish appearance, a feature also cited in the protologue ("...pale grey to grey or yellowish-grey..."). The species is reported as new for the Kingdom of Bhutan.

Specimen examined: BHUTAN, Flor-Prov. N22 (Upper Kuru Chu), Lhuntse distr., S of Singye Dzong, 27°58'N, 91°18'E, elevation 3950 m, open *Juniperus recurva* forest and scrub near treeline on sunny slope, 23.XI.2000, Miehe (00-441-26/09b; associated *Hypogymnia hengduanensis* has been separated under 00-441-26/09a [see above]).

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