

***Hypotrachyna altorum* sp. nov., a new lichen from the cloud forests of Réunion Island, Indian Ocean**

Didier MASSON

*386 rue des Flamboyants, F-40600 Biscarrosse, France,
email: di.masson@wanadoo.fr*

Abstract – *Hypotrachyna altorum* (Ascomycota, Parmeliaceae) is described as a new corticolous lichen species from the montane cloud forests of Réunion Island (Mascarene archipelago). It is similar to the Brazilian species *H. silvatica* (Lyngé) Hale from which it differs by the size and shape of the ascospores, medullary pigmentation, habitat and distribution. The new taxon is briefly compared to other *Hypotrachyna* species with similar secondary metabolites.

***Acacia heterophylla* / Ascomycota / *Hypotrachyna silvatica* / Mascarene Islands / Parmeliaceae / taxonomy / tropical montane cloud forest**

Résumé – La nouvelle espèce *Hypotrachyna altorum* (Ascomycota, Parmeliaceae) est décrite des forêts des nuages de l'île de La Réunion (archipel des Mascareignes). Ce lichen corticole ressemble à l'espèce brésilienne *H. silvatica* (Lyngé) Hale dont il se distingue par la taille et la forme de ses ascospores, la pigmentation de sa médulle, son habitat et sa répartition. Le nouveau taxon est brièvement comparé aux autres *Hypotrachyna* contenant des métabolites secondaires semblables.

***Acacia heterophylla* / Ascomycota / forêt néphéliphile / *Hypotrachyna silvatica* / îles Mascareignes / Parmeliaceae / taxinomie**

INTRODUCTION

Hypotrachyna (Vain.) Hale is a genus of parmelioid lichens characterized by cell walls containing isolichenan, a pored epicortex, dichotomously branched lobes with subtruncate to truncate apices, lack of pseudocyphellae and marginal cilia, dichotomously branched rhizines and bifusiform conidia (Crespo *et al.*, 2010b; Elix, 1993; Sipman *et al.*, 2009). Recent molecular data show that *Hypotrachyna* belongs to a monophyletic clade together with the genera *Cetrariastrum*, *Everniastrum* and *Parmelinopsis* (Crespo *et al.*, 2010b, 2011). In its current circumscription, *Hypotrachyna* is a polyphyletic genus, even after the segregation of several mainly Asian species in the new genus *Remototrachyna* from the core of the genus (Divakar *et al.*, 2006, 2010b). According to phylogenetic studies, it appears that morphological characters such as presence/absence of marginal cilia, rhizine ramifications etc., traditionally used in the circumscription of *Hypotrachyna*, are not congruent with the molecular data

(Divakar *et al.*, 2006). More studies are thus needed to clarify the classification of the species belonging to the *Hypotrachyna* clade (Crespo *et al.*, 2010b, 2011).

Hypotrachyna (sensu Crespo *et al.*, 2010b) is a large genus with about 200 species. Tropical America is the main centre of diversity with ca. 145 taxa (Lumbsch *et al.*, 2011; Sipman *et al.*, 2009; Yáñez-Ayabaca & Eliasaro, 2009). Most *Hypotrachyna* species can be found in moist habitats in tropical montane regions, some also occurring in temperate oceanic areas (Alstrup *et al.*, 2010; Azevedo Rodrigues *et al.*, 2007; Boom & Ertz, 2012; Hale, 1975; Krog & Swinscow, 1979; Louwhoff, 2001; Louwhoff & Elix, 2002; Masson, 2005; Sipman *et al.*, 2009). Moist habitats preferred by most species may represent a key driver for the accelerated rate of evolution observed within the *Hypotrachyna* clade (Lumbsch *et al.*, 2008).

Located 680 km east of Madagascar and 170 km west-south-west of Mauritius, Réunion is a rather young mountainous volcanic island (2.1 million years old) with a surface area of 2512 km² (Montaggioni & Nativel, 1988). Tropical montane cloud forests are well preserved and spread from ca. 1000 to 2000 m a.s.l. (Blanchard, 2000). The genus *Hypotrachyna* is well-represented among the rich lichen flora present in this habitat, but still poorly known despite some old (Abbeyes, 1961; Hue, 1899; Nylander, 1859) and recent studies (Boom *et al.*, 2011; Krog, 2000). Among our collections made in 2003 and 2005 were several *Hypotrachyna* specimens which were initially identified, with some hesitation, to *H. silvatica* (Lyngé) Hale. A similar specimen, collected by K. Kalb from the same island and studied by J. A. Elix was determined as *H. brasiliiana* (Nyl.) Hale (J. A. Elix pers. com., 01-VI-2006). Subsequent detailed study of our material and comparison with *H. silvatica* and *H. brasiliiana* supported the recognition of a separate species, described as new to science in this paper.

MATERIAL AND METHODS

Morphological characters were examined under an Olympus SZ3060 stereomicroscope (9-40 × magnification) and anatomy of the specimens was studied under a Zeiss PrimoStar trinocular compound light microscope (magnification up to 1000 ×). Anatomical sections were made with a razor blade and mounted in tap water. For comparative reason, all ascospores were measured in tap water in the dead hydrated state (Baral, 1992) and outside the asci. For each taxon, 30 mature non-deformed spores in a single well-developed apothecium per thallus (= one individual) were measured in four or five thalli. For the other anatomical structures, five random measurements were also carried out in three individuals after staining in lactic cotton blue. The definition and terminology of the apothecial layers follow Letrouit-Galinou (1970) and Roux *et al.* (1993); that is to say that the hypothecium (hyaline layer) is discriminated as a distinct layer between the subhymenium s.str. above and the cupule of the excipulum proprium below. Statistics for spore measurements are given as arithmetical mean value (in italics and underlined) plus/minus 1.96 × standard deviation (rounded to the nearest 0.5 µm for the length and width values); values in parentheses represent exceeding observed minimum or maximum values. Q represents length/width ratio of the spores. Statistics for the other anatomical measurements are given as arithmetical mean value (in italics) between the observed minimum and maximum values (in parentheses).

Secondary metabolites were studied by colour reaction (spot tests), by UV light and by thin-layer chromatography (TLC) according to the standard procedure and with the solvent systems A, B and C (Orange *et al.*, 2001).

Photograph of the thallus was made with an Epson Perfection 1260 scanner, those of the ascospores with a Canon PowerShot A640 digital camera. The codes used for colours follow Online Auction Color Chart (2004). Bioclimates of the localities are determined according to Rivas-Martínez & Rivas-Sáenz (2009); climatic data used stem from Jumaux *et al.* (2011).

Specimens are deposited in B (acronyms according to Index Herbariorum), G, REU, UPS and the author's private herbarium. Specimens of similar species from SP, UPS and the private herbarium of K. Kalb were studied for comparisons. Photographs of some type specimens were also examined from the botanical database at the Swedish Museum of Natural History (Krypto-S, 2011).

DESCRIPTION

Hypotrachyna altorum D.M. Masson, sp. nov.

Figs 1-2

Mycobank: MB 564486

Diagnosis: *Thallus corticolus*, 4-7(10) × 2-4 cm, *plerumque arte adpressus; lobi sublineares*, ab 0.4 ad 2 mm lati, cum *subtruncatis apicibus; superior facies eburnea*, levis nitensque ad extremas partes, sed tenuiter rugosa impolitaque in media parte, non maculata; *thallus pustularum, dactylorum, lobularum, isidiorum et sorediorum expers; medulla alba, raro aliquibus in locis aurantiaca; rhizinae in inferiore thalli facie a densis usque ad mediocriter densas, ab 1 ad 3(4) dichotome ramosae. Apothecia frequentia, cum disco usque ad 6 mm diametro. Sporae a large ellipsoïdalibus usque ad subglobulosas, (7)7.5-9.0-10.5(11) × (5)5.5-6.6-8 μm, Q = (1.07)1.12-1.38-1.65(1.80). Cortex superior lichexanthonum continens, medulla protocetraricum acidum, vioxanthinum et aliquando skyrinum continens.*

Etymology: From the Latin *altorum*: of the Heights. The specific epithet relates to the mountainous parts of Réunion, locally named "les Hauts" (the Heights), where the species thrives.

Type material: FRANCE: La Réunion: **Saint-Paul**: route forestière des Tamarins des Hauts-sous-le-Vent, entre la sommière Éperon et la sommière la Saline, 21°05'08"S-55°21'37"E, alt. 1735 m, orientation générale ouest, sur branches de *Acacia heterophylla* dans une tamarinaie cultivée pâturée, 10-IV-2003, D. Masson 974.0272 (holotype in G; isotypes in B, UPS and herb. D. Masson)

Description: **Thallus**: foliose, corticolous, 4-7(10) × 2-4 cm, usually closely appressed to the substrate. **Lobes**: sublinear, contiguous to imbricate, dichotomously to subdichotomously branched, 0.4-2 mm wide, sinuous in the lobe axils, plane to convex, with entire and eciliate margins, subtruncate and slightly revolute apices, sometimes darkening at lobe tip. **Upper surface**: ivory white to yellowish grey (oac7 or oac900), more yellowish in the herbarium, epruinose, without pseudocyphellae, emaculate, smooth and shiny near the apex, finely rugulose and dull towards the centre, occasionally somewhat plicate or papillate in the centre, lacking pustules, dactyls, lobules, soredia and isidia. **Medulla**: generally white throughout, but sometimes with scattered orange (oac789 or oac810)

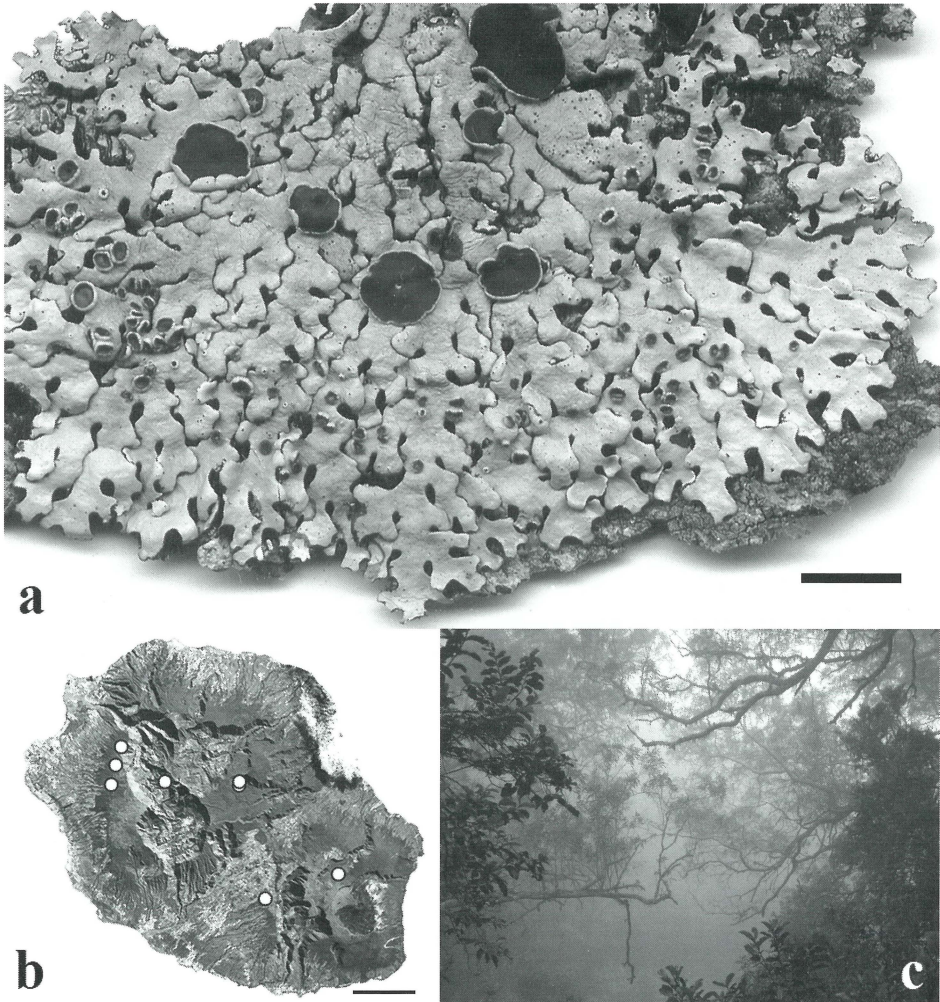


Fig. 1. *Hypotrachyna altorum*. **a**: habit (part of holotype, G); scale bar = 5 mm. **b**: known distribution in Réunion Island according to the specimens examined; scale bar = 10 km. **c**: montane forest of *Acacia heterophylla* in fog (type locality), the typical habitat.

patches. **Lower surface**: black, more or less shiny, smooth to somewhat rugulose, with or without a narrow (ca. 0.4 mm) chestnut brown margin. **Rhizines**: usually black, sometimes dark brown near the lobe margin, shiny, moderately dense to dense, more sparse centrally, often slightly projecting beyond the lobe margins and forming a narrow marginal fringe, ca. 0.3-0.7(1) mm long, 1-3(4) times dichotomously branched. **Apothecia**: common, laminal, sessile or occasionally substipitate, up to 6 mm in diameter; disc dark purple brown (oac523 or oac622), more or less glossy, first concave then undulate contorted with age (rarely radially split); thalline exciple thin and smooth; margin frequently crenate; epihymenium (5)-7.7-(10) μm high; hymenium (34)-42.3-(51) μm high; subhymenium (10)-12.9-(18) μm high; hypothecium (12)-15.3-(18) μm high; plectenchymatous cupule of

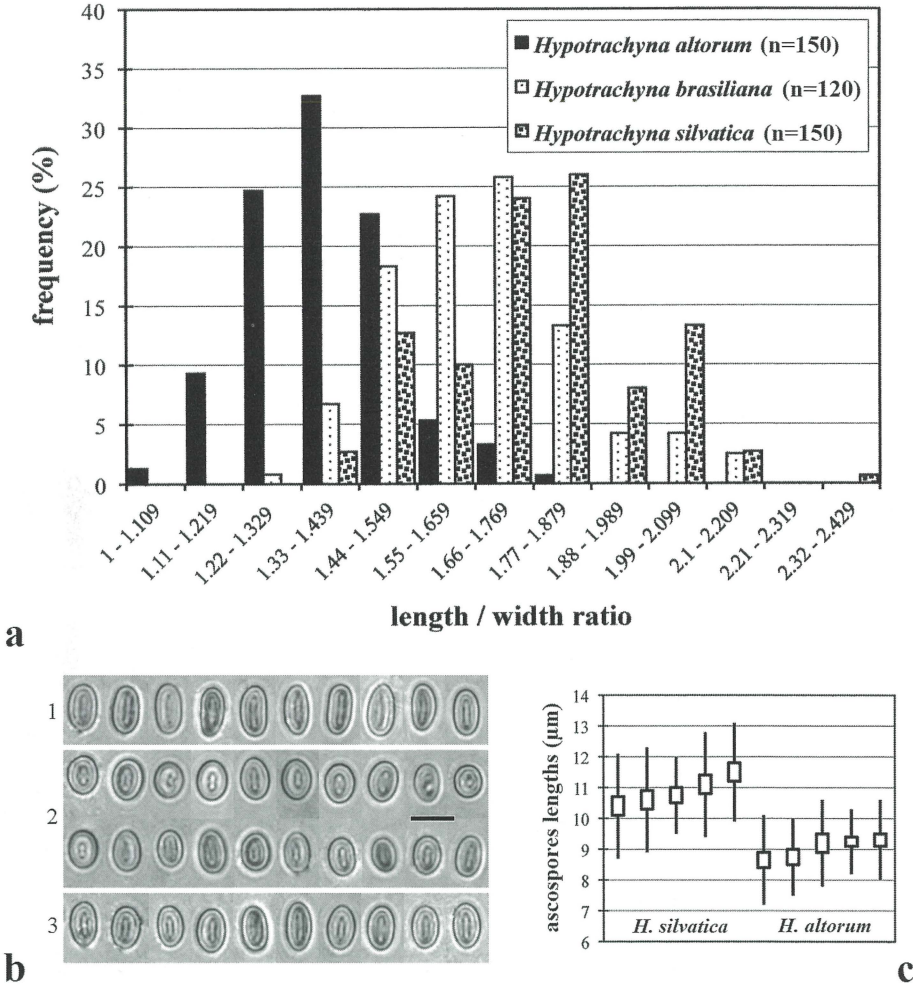


Fig. 2. Comparative morphology of the ascospores of *Hypotrachyna alatorum*, *H. brasiliiana* and *H. silvatica*. **a**: distribution of the Q values (length/width ratios). **b**: 1, *H. silvatica* spores (UPS 154735); 2, *H. alatorum* spores (upper row: 974.1597, lower row: isotype, B); 3, *H. brasiliiana* spores (UPS 014413); scale bar = 10 μ m. **c**: ascospores lengths in *H. alatorum* and *H. silvatica* individuals, confidence intervals for means at the 95% level (± 1.96 SE) are indicated by boxes, confidence intervals for single spores at the 95% level (± 1.96 SD) are indicated by straight lines.

excipulum proprium with thin cell walls, (32)-35.6-(44) μ m high. **Upper cortex**: paraplectenchymatous, (18)-20.7-(25) μ m thick. **Algal layer**: continuous, (28)-35.3-(40) μ m thick; photobiont trebouxoid. **Medulla**: (73)-85.1-(100) μ m thick. **Lower cortex**: paraplectenchymatous, (18)-22.2-(25) μ m thick. **Ascospores**: 8 per ascus, simple, colourless, broadly ellipsoidal to subglobose, rarely broadly ovoidal, (7)7.5-9.0-10.5(11) \times (5)5.5-6.6-8 μ m, Q = (1.07)1.12-1.38-1.65(1.80) (n = 150), epispore 1 μ m thick. **Pycnidia**: frequent, laminal to submarginal, black, immersed. **Conidia**: weakly bifusiform, 6-8 \times ca. 1 μ m.

Chemical spot tests and fluorescence: upper cortex K-, C-, KC-, P+ pale orange yellow, UV+ yellow; medulla K-, C-, KC- or KC+ pink, P+ orange, UV-; pigmented medulla K+ purple. *Secondary metabolites* (TLC): upper cortex with lichexanthone; medulla with protocetraric acid, viioxanthin (= pigmentosin B) and \pm skyrin.

Distribution, habitat and ecology: *Hypotrachyna altorum* is currently only known from Réunion Island (Fig. 1b). All specimens were collected between 1550 and 1815 m a.s.l. in natural montane cloud forests dominated by the tree *Acacia heterophylla* Willd. or in *A. heterophylla* cultivated forests (Fig. 1c). The bioclimatic features of the localities can be summarized as follows: bioclimate: pluvial tropical, thermotype belt: upper mesotropical ($333 \leq It \leq 385$), ombrottype belts: from low humid to ultrahyperhumid ($9.0 \leq Io \leq 37.1$). *Hypotrachyna altorum* grows on the rhytidome of branches, less often boles, of *Acacia heterophylla* which is its only known phorophyte so far. This endemic tree, locally named “Tamarin des Hauts”, needs moderate temperatures (mean annual temperatures between 11 and 17°C), a minimal annual rainfall of 1500 mm and always high hygrometry (Slimak *et al.*, 1999). Its light foliage and loose crown allow branches and trunk to be well lit and ventilated, so numerous corticolous chlorolichens can be found there, notably *Usnea* spp. and parmelioid lichens. Among them, *Hypotrachyna orientalis* (Hale) Hale, *Parmotrema reticulatum* aggr., *Parmelinopsis damaziana* (Zahlbr.) Elix & Hale and especially *Hypotrachyna* aff. *pseudosinuosa* (Asahina) Hale were found adjacent to *H. altorum* thalli. Collected material shows that specimens of *H. altorum* are frequently infested by a lichenicolous mite burrowing within the thallus and feeding on the medulla. Once the medulla disappeared, the upper cortex darkens and erodes, leaving irregular cavities with black underlying lower cortex and often containing whitish to brownish faecal pellets. Those structures may be misinterpreted as pustules.

Additional material examined: FRANCE: **La Réunion: La Possession:** cirque de Mafate, plaine des Tamarins, 21°04'44"S-55°26'37"E, alt. 1760 m, sur écorce de *Acacia heterophylla* dans une tamarinaie naturelle pâturée, 27-VII-2005, *D. Masson* 974.1763 (REU); **La Possession:** cirque de Mafate, plaine des Tamarins, 21°04'48"S-55°26'39"E, alt. 1765 m, sur écorce de *Acacia heterophylla* dans une tamarinaie naturelle pâturée, 27-VII-2005, *D. Masson* 974.1781; **Le Tampon:** Notre-Dame de la Paix, 21°14'24"S-55°35'05"E, alt. 1570 m, sur écorce de *Acacia heterophylla* dans une tamarinaie naturelle pâturée, 23-VII-2005, *D. Masson* 974.1678; **Saint-Benoît:** forêt de Bébour, sentier des Tamarins, 21°04'54"S-55°32'38"E, alt. 1555 m, sur tronc de *Acacia heterophylla* en tamarinaie cultivée, 19-VII-2005, *D. Masson* 974.1532; **Saint-Benoît:** forêt de Bébour, sentier des Tamarins, 21°05'03"S-55°32'45"E, alt. 1550 m, sur branches de *Acacia heterophylla* en tamarinaie cultivée, 19-VII-2005, *D. Masson* 974.1531, 974.1538; **Saint-Paul:** Dennemont, 21°03'39"S-55°22'11"E, alt. 1790 m, sur écorce de *Acacia heterophylla* dans une tamarinaie cultivée pâturée, 02-VIII-2005, *D. Masson* 974.1906; **Saint-Paul:** route forestière des Tamarins des Hauts-sous-le-Vent, 21°05'10"S-55°21'37"E, alt. 1735 m, sur branches de *Acacia heterophylla* dans une tamarinaie cultivée pâturée, 02-VIII-2005, *D. Masson* 974.1929, 974.1930; **Saint-Paul:** les Hauts de Bois de Nèfles, 21°01'38"S-55°22'43"E, alt. 1600 m, sur branche de *Acacia heterophylla* dans une tamarinaie naturelle, 31-VII-2005, *D. Masson* 974.1877; **Sainte-Rose:** ESE du Piton des Grands Bois, 21°12'25"S-55°41'31"E, alt. 1815 m, sur branche de *Acacia heterophylla* dans une tamarinaie naturelle pâturée, 20-VII-2005, *D. Masson* 974.1597.

Additional material examined for comparison: *Hypotrachyna brasiliiana* (Nyl.) Hale: BRAZIL: **Bahia:** Serra das Mangabeiras, etwa 30 km nach Seabra, an

sehr feuchten und zumeist schattigen Felsüberhängen in einem Cerrado, 1000 m, 17-VII-1980, K. Kalb 30997, 30998 (herb. Kalb). Minas Gerais: Carassa, 1885, E.A. Wainio (*Lichenes Brasilienses Exsiccati n°1184*, UPS L-014413). Rio de Janeiro: Serra da Mantiqueira, Itatiaia, zwischen Registro do Picú und Agulhas Negras, direkt unterhalb der Agulhas Negras, an hellen, trockenen Granitfelsen, 2400 m, 23-VII-1979, K. Kalb & G. Plöbst 9960, 9961 (herb. Kalb). São Paulo: Serra Taquari, Itirapina, etwa 50 km südwestlich von Piraçununga, an einem freistehenden Sandsteinfelsen, 550 m, 16-VI-1979, K. Kalb & G. Plöbst 14048 (herb. Kalb). *Hypotrachyna crustacea* (Lynge) Marcelli & Jungbluth: BRAZIL: São Paulo: Município de Mogi-Guaçu, Reserva Biológica de Moji-Guaçu, Fazenda Campininha, sobre tronco de árvore, 03-IV-1999, M.P. Marcelli & M. Falco 33207, 33248, det. P. Jungbluth (SP 380363, SP 380364). *Hypotrachyna silvatica* (Lynge) Hale: BRAZIL: Mato Grosso: Santa Anna da Chapada, 14-X-1902, G.O.A. Malme s.n. (UPS L-154735); Serra da Chapada, Buruti, in silva clara, fere "cerrado", 25-VI-1894, G.O.A. Malme (*Lichenes Austroamericani ex Herbario Regnelliano n°87*, UPS L-046782). São Paulo: Serra do Córrego Fundo, bei Vassununga (Gemeinde Sta. Rita do Passo Quatro), in einem hellen, trockenen Cerrado, 550 m, 15-VI-1979, K. Kalb & G. Plöbst 10470, 10472 (herb. Kalb); Fazenda 13. de maio, 18 km nördlich von Botukatu und 14 km östlich von São Manuel, in einem Cerradão, 550 m, 09-XI-1979, K. Kalb & G. Plöbst 10471, 14079 (herb. Kalb).

DISCUSSION

So far five *Hypotrachyna* species are known to contain lichexanthone in the upper cortex and protocetraric acid as the only major secondary metabolite in the medulla ± pigments: *H. brasiliiana* (Nyl.) Hale, *H. crustacea* (Lynge) Marcelli & Jungbluth, *H. malmei* (Lynge) Hale, *H. silvatica* (Lynge) Hale and *H. subaffinis* (Zahlbr.) Hale. All occur in South America, more precisely in Brazil, where they form a complex of taxa still not thoroughly understood. The discovery in the Old World of specimens belonging to that genus with such chemistry is thus rather unexpected.

With an adnate, corticolous thallus lacking vegetative propagules, narrow and sublinear lobes with subtruncate apices and a white medulla occasionally somewhat orange pigmented, *Hypotrachyna altorum* is morphologically similar to *Hypotrachyna silvatica*. This corticolous Brazilian lichen typically inhabits the cerrado vegetation (Eliasaro *et al.*, 1998; Jungbluth, 2006; Mistry, 1998; Sipman *et al.*, 2009) under mostly pluviseasonal tropical (rarely pluvial tropical) bioclimates with a thermotype belts range from upper infratropical to low mesotropical and an ombrotype belts range from low subhumid to low humid (data determined from Rivas-Martínez & Rivas-Sáenz, 2009); therefore under warmer and dryer conditions than those found by *H. altorum* in the montane cloud forests of Réunion. According to Sipman *et al.* (2009) and Sipman (pers. comm.), *H. silvatica* contains alectoronic acid as a further medullary substance together with protocetraric acid. Nevertheless, neither Hale (1975) nor Eliasaro *et al.* (1998) mentioned that secondary metabolite; our own TLC fail to detect it too in the specimens of *H. silvatica* examined (including a topotype). *Hypotrachyna altorum* is therefore chemically close to *H. silvatica*. However the

Table 1. Comparison of the ascospores measurements of *Hypotrachyna altorum*, *H. brasiliiana* and *H. silvatica*. Statistical tests (two-tailed Z tests) show that the length and width means of *H. altorum* spores are highly significantly different from those of *H. brasiliiana* and those of *H. silvatica*. Q values show that spores of *H. altorum* are distinctly more globose.

Ascospores measurements	<i>H. silvatica</i> (n = 150)	<i>H. altorum</i> (n = 150)	<i>H. brasiliiana</i> (n = 120)
length (µm)	9- <u>10.9</u> -12.5(13) (*)	(7) <u>7.5-9.0-10.5</u> (11) (*) (**)	8- <u>9.6</u> -11(11.5) (**)
two-tailed Z test	(*) $\alpha < 10^{-9}$	(**) $\alpha < 10^{-9}$	
width (µm)	5- <u>6.2</u> -7(7.5) (*)	(5) <u>5.5-6.6-8</u> (*) (**)	5- <u>5.8</u> -6.5(7) (**)
two-tailed Z test	(*) $\alpha < 10^{-6}$	(**) $\alpha < 10^{-6}$	
Q (=length/width)	1.41- <u>1.77</u> -2.13(2.40)	(1.07) <u>1.12-1.38-1.65</u> (1.80)	(1.29)1.33- <u>1.66</u> -1.99(2.10)

medulla of *H. silvatica* is usually orange-ochre in its lower part and the pigments are varied: euplectin, vioxanthin, pigmentosins and skyrin (Sipman *et al.*, 2009; pers. obs.). On the contrary, the medulla of *H. altorum* is generally white throughout with only occasional, scattered orange patches of skyrin. Virescic acid occurs as trace in the specimens of *H. silvatica* examined by us as well as those examined by Eliasaro *et al.* (1998) but seems to be totally absent in *H. altorum* (TLC). The main difference between *H. altorum* and *H. silvatica* lies in the size and shape of ascospores (Fig. 2). In the specimens examined (Tab. 1) and in accordance with the protologue (Lynge, 1914) and Eliasaro *et al.* (1998), the spores of *H. silvatica* are not only distinctly longer but also narrower than those of *H. altorum*. As a result, the length/width ratio (Q) is clearly greater in *H. silvatica*: ascospores of *H. altorum* are broadly ellipsoidal to subglobose, those of *H. silvatica* ellipsoidal. Recent molecular phylogenetic studies showed that the taxonomic value of ascospore characters, especially size and shape, has been underestimated in parmelioid lichens at the generic (e.g. Crespo *et al.*, 2010a, 2011) as well as specific levels (e.g. Argüello *et al.*, 2007; Divakar *et al.*, 2010a; Núñez-Zapata *et al.*, 2011). Differences noticed in ascospore measurements together with the slightly different medullary chemistry, the disjunct distributions and the somewhat distinct ecological requirements strongly suggest that the Réunion material is not conspecific with *H. silvatica* but represents a distinct species.

The other four Brazilian *Hypotrachyna* species with lichexanthone, protocetraric acid and \pm pigments as major secondary metabolites are clearly distinct from *H. altorum* (Eliasaro *et al.*, 1998; Hale, 1960; Lynge, 1914; Marcelli *et al.*, 2007; Sipman *et al.*, 2009; Vainio, 1890; pers. obs.). *Hypotrachyna brasiliiana*, the type species of the genus *Hypotrachyna*, lacks vegetative propagules like *H. altorum*, but is a saxicolous lichen with larger and more loosely adnate thallus, broader lobes (1.5-10 mm vs 0.4-2 mm), additional virescic acid as minor medullary substance and longer, narrower, and therefore more ellipsoidal ascospores (Tab. 1; Fig. 2). *Hypotrachyna subaffinis* is a sorediate species with a more complex medullary chemistry (physodic, 4-O-methylphysodic and colensoic acids as additional minor substances) and with ellipsoidal ascospores. Finally, the very similar (most probably conspecific) *Hypotrachyna crustacea* and *H. malmei* [including *H. minima* (Lynge) Hale] differ from *H. altorum* in having dactyls on the upper surface and apothecial margins, a pigmented lower medulla and more ellipsoidal ascospores.

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