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Pseudohepatica duidensis, a new lichen from the venezuelan Amazonas

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Abstract: The species *Pseudohepatica duidensis* Marcano, Palacios & Morales is described from the Venezuelan Amazonas. Special attention has been paid to the morphology and the chemistry (e.g. polysaccharides and secondary compounds), using scanning electron microscopy (SEM), thin-layer chromatography (TLC, HPTLC) and iodine reagent tests. *Pseudohepatica duidensis* is characterized by a very small (to 1 mm long), yellowish-green thallus, non-pored epicortex, yellowish lower surface with soredial protuberances, paraplectenchymatous upper cortex consisting of strongly sclerotic cells, cell walls with iodine reactions characteristic of lichenan and gyrophoric acid as relevant chemical constituent.

Resumen: Se describe la especie *Pseudohepatica duidensis* Marcano, Palacios & Morales procedente del Amazonas Venezolano. Una atención especial es puesta en la morfología y en la química (e.g. polisacáridos y compuestos secundarios), utilizando microscopía electrónica de barrido (SEM), cromatografía de capa fina (TLC, HTLC) y pruebas con reactivos de yodo. *Pseudohepatica duidensis* está caracterizada por presentar un talo pequeño (hasta 1 mm de largo), verdo-amarillento, epicortex no-poreado, superficie inferior amarillenta mostrando protuberancias sorediales, y cortex superior paraplectenquimatoso consistiendo de células fuertemente esclerotizadas, paredes celulares con reacciones de yodo características de liquenina, y ácido girofórico como constituyente químico relevante.

INTRODUCTION

Pseudohepatica P. M. Jørg. is a so far monotypic lichen genus, not known to produce apothecia or pycnidia, with a thallus resembling a leafy hepatic, containing substances in the acetate-polymalonate pathway. This genus was described by Jørgensen (1993) from the Guayana Highlands, growing on living tree bark and roots in a microenvironment of scarce light on the summits of table mountains known as Tepuis (1200-1600 m). *Pseudohepatica* only has a superficial similarity to any other known lichen genus (Jørgensen 1993). The type species, *Pseudohepatica pachyderma* P. M. Jørg., was described from Auyantepui, Bolívar state whereas the new species described here, *P. duidensis*, was collected in a little-studied region, the Cerro Duida (Tepui) in the Amazonas state. The Cerro Duida region exhibits an important diversity and endemism in the lichen flora viz. Cladoniaceae (Ahti 1987, 1991), *Stereocaulon* (Marcano et al. 1995a), *Coccocarpia* (Marcano et al. 1995b), *Bulbothricella* Marcano et al. (endemic genus, Morales et al. 1995, Marcano et al. 1996) and *Leprocaulon* (Marcano et al. 1997).

MATERIALS AND METHODS

The total number of individuals examined chemically and anatomically in this study was 78. Thin-layer chromatography (TLC) analysis was carried out according to Culberson (1972), Culberson & Johnson (1976), Culberson et al. (1981), White & James (1985) and HPTLC according to Arup et al. (1993). The lichens *Relicina abstrusa* (Vain.) Hale, *Umbilicaria subcalvescens* Sipman, *U. nylanderiana* (Zahlbr.) H. Magn. and *Hypotrachyna caraccensis* (Taylor) Hale were used as controls, as was authentic material of norstictic and stictic acids, atranorin and lichexanthone, which were obtained by isolation (Morales & Marcano 1992; Marcano & Morales 1994). Spot tests showed that the lichen substances were often unevenly deposited in the thallus. Samples were therefore taken from various parts of the cortex and medulla. All three solvent systems mentioned by Culberson (1972) were used in these comparisons.

Freezing microtome sections of thalli were mounted in lactophenol / cotton blue and examined by light microscopy (LM). In addition, fragments of thalli were studied by scanning electron microscopy (SEM). This procedure was found to be especially useful for the study of the upper and lower surface and cortex. Material was first air-dried, coated with gold under vacuum and observed with an S-2500 Hitachi microscope at 10KV.

The polysaccharides present in the cell walls were determined using iodine reagents according to Common (1991): SIKI (= 10% sulfuric acid + iodine, potassium iodide solution), Melzer´s Reagent, 1.5% LPIKI (= lactophenol + iodine + potassium iodide solution) and 1.5% IKI (= iodine + potassium iodide solution). The reactions were identified by inverted light.

THE SPECIES

Pseudohepatica duidensis V. Marcano, E. Palacios-Prü et A. Morales sp. nov.

Thallus humicola, nitidus, viridoflavescens, dorsiventralis, vermiculatus, parvus (usque ad 1 mm longus), dichotome vel irregulariter ramosus; pagina superior epicorticata, glabra; pagina inferior sorediata; pycnidia et apothecia non visa. Acidum gyrophoricum et materia chemica ignota continens.

Typus: Venezuela, Amazonas state, Cerro Duida-Marahuaca, north face, south of Culebra, humicolous, 1200-1500 m, 19-2-96, leg. *L. Galiz, V. Marcano* and *A. Morales* 968 (herb. lich. *V. Marcano*-holotypus ; VEN-isotypus).

(Figs 1 and 2)

Etymology: The name of the species refers to the locality where the lichen was collected.

Thallus ascending, prostrate to erect, humicolous, shiny, yellowish-green, 0.8-1.0 mm long, dorsiventral. Branches elongate, 0.2-0.3 mm wide, 55-60 µm thick, dichotomously to irregularly branched, with irregularly sinuous margins.

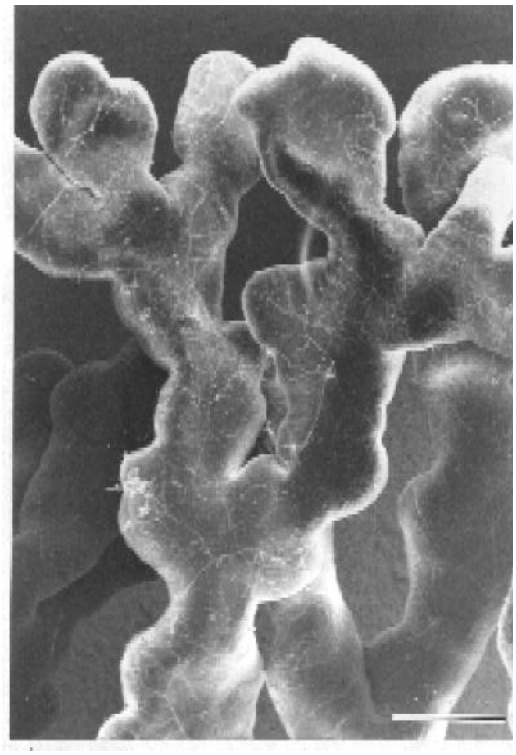


Fig. 1. *Pseudohepatica amazonensis* (SEM). View of the upper surface (holotype). Scale = 275 µm.

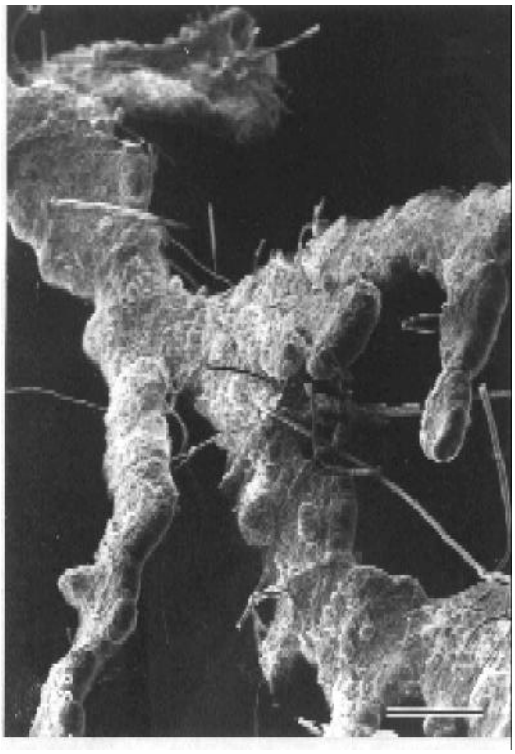


Fig. 2. *Pseudohepatica amazonensis* (SEM). View of the lower surface (holotype). Scale = 155 µm.

Upper surface convex, smooth, shiny at the apices, sometimes concave at the tip, lacking soredia and isidia; epicortex non-pored, sinuous, smooth, variously with microperforations, 0.4-0.6 µm in diameter; cortical tissue composed by a sclerenchymatous paraplectenchyma, consisting of strongly sclerotic cells and very few visible lumina, 30-35 µm thick. Lower surface concave, yellowish-green, with soredial protuberances; photobiont green, *Trebouxia*-like, 7-8 µm wide, included in a medullary pachydermatous tissue, 25-35 µm thick; lower cortical tissue poorly developed, 10-12 µm thick; midrib yellowish, with yellowish-brown, furcate-branched hyphae, enveloping green-algae, which arise from the interior of the lower surface; epicortical fissures and perforations on lower surface, elongated, irregular, 6 -14(-22) µm long, 3-5 µm wide.

Apothecia and *pycnidia* unknown.

Chemistry: Cell walls with iodine reactions characteristic of lichenan (SIKI + red, Melzer´s Reagent + orange, 1.5% LPIKI - and 1.5% IKI + red); medulla K -, C+ pink to orange, KC -, PD -; UV-; HPTLC, TLC: Gyrophoric acid, substance 1 (probably a norlichexanthone derivative) and substance 2 (probably a xanthone) (Table 1).

Ecology and distribution: Pseudohepatica duidensis is so far known only from the areas around Cerro Duida-Marahuaca in the Venezuelan Amazonas, where it was collected in closed primary forest at high elevations (1200-1500 m). *Pseudohepatica duidensis* was found confined to shaded and wet habitats, growing mostly over decaying organic matter, sometimes in places external to small caves and on root-soils. It was also found growing on musci, liverworts, crustaceous lichens and rarely on bark. Dead thalli

Table 1. T. L. C. and microchemical reactions data for the lichen *Pseudohepatica breviformis* Marcano et al. ***A***, Benzene / dioxan / acetic acid (180 / 45 / 5). ***B***, Hexane / diethylether / formic acid (130 / 100 / 20). ***C***, Toluene / acetic acid (200 / 30).

Compound	TLC			Spot Test		
	Rf (***A***)	Rf (***B***)	Rf (***C***)	K	C	PD
Atranorin (standard)	0.75	0.78	0.79	Yellow	—	Yellow
Norstictic acid (standard)	0.39	0.29	0.31	Orange	—	Orange
Lichexanthone (standard)	0.72	0.72	0.75	—	Orange	—
Gyrophoric acid	0.24	0.42	0.24	—	Pink	—
Substance 1	0.78	0.82	0.80	—	Orange	—
Substance 2	0.72	0.72	0.74	—	Orange	—

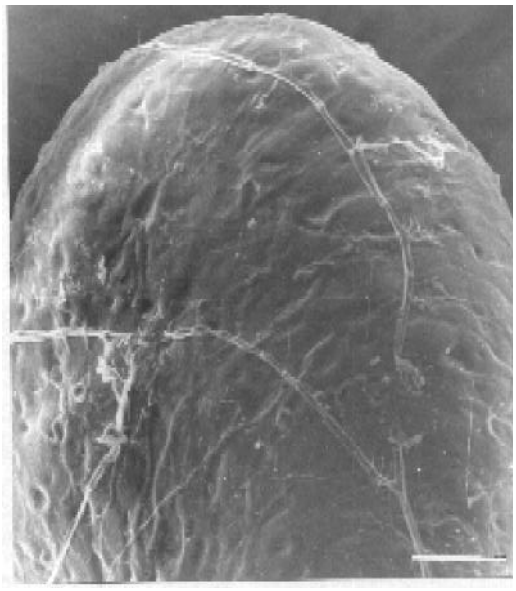
affected by a Coelomycete-like fungus were observed on a humicolous substrate with free-living green algae. Bacteria (coccoid) and microscopic filamentous fungi (Hyphomycetes) are also seen growing on both surfaces of the lichen, and appear to cause no damage to the tissue. Further, the presence of another filamentous fungus which emerges from the interior of the lichen is observed crossing the medullary tissue parallel to the length of the thallus without causing apparent damage to the host. The presence of bacteria and other fungi in *P. duidensis* could indicate that the cortical lichen substances have no inhibitor effect on their growth.

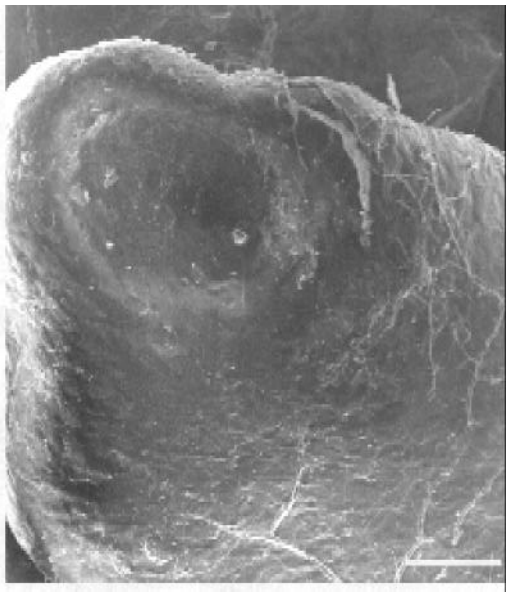
DISCUSSION

Pseudohepatica duidensis is morphologically very similar to *P. pachyderma* (Jørgensen 1993). Both species lack apothecia and pycnidia. However, *P. duidensis* has a yellowish-green and shorter thallus (to 1 mm long and 0.3 mm wide), with a yellowish lower surface, whereas *P. pachyderma* has a greenish-beige, longer thallus (to 15 mm long and 1 mm wide), and a whitish lower surface. In addition, *P. duidensis* is usually humicolous, muscicolous, lichenicolous and occasionally corticolous, whereas *P. pachyderma* is predominantly corticolous. The difference between the two species is clearly seen with use of the presence or absence of soredial protuberances. *Pseudohepatica duidensis* specimens have soredial protuberances on the lower surface (Fig. 2) whereas *P. pachyderma* lacks soredia on both surfaces (Jørgensen 1993).

Pseudohepatica duidensis was found to show several shapes in the branch tips, but a convex shape is dominant in the examined specimens (Figs 3 and 4). The upper surface shows a very distinct, compact, smooth, non-pored epicortex, 0.5-0.6 µm thick (Figs 3 and 4) with microperforations which are more or less circular in shape (Fig. 5) and 0.4-0.6 µm in diameter. However, the distribution of these microperforations is not constant on the thallus and they seem not to have an epicortical origin *sensu* Hale (1973, 1976, 1981). The lower surface shows epicortical fissures (Fig. 6) or perforations under the midrib (Fig. 7). The midrib is composed of anastomosing hyphae that arise from the interior of the lower surface, forming a net with variable density and thickness that covers the lower perforations. This midrib shows a similarity to veins or the venation of leaves.

Pseudohepatica duidensis shows anatomical features very similar to *P. pachyderma* (Jørgensen 1993), viz. a sclerenchymatous, paraplectenchymatic upper cortex, 30-40 µm thick, consisting of strongly sclerotic cells, with narrow lumina, while the lower cortex is poorly developed. Although *P. duidensis* is currently found in closed primary forests, this strongly sclerenchymatous





Figs. 3 and 4. Upper surface of *P. amazonensis* showing a non-pored epicortex (SEM).
3. Tip of branch showing convexity. Scale= 20 µm. 4. Tip of branch showing a small depression over its surface. Scale = 50 µm.

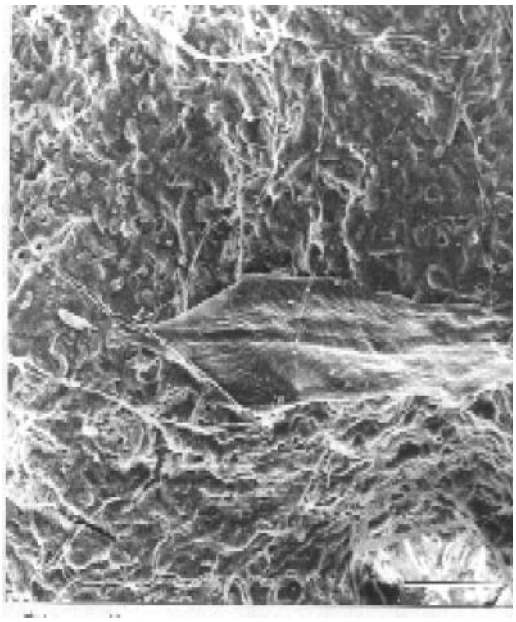


Fig. 5. Upper surface of *P. amazonensis* showing microperforations (SEM). Scale = 29 µm.

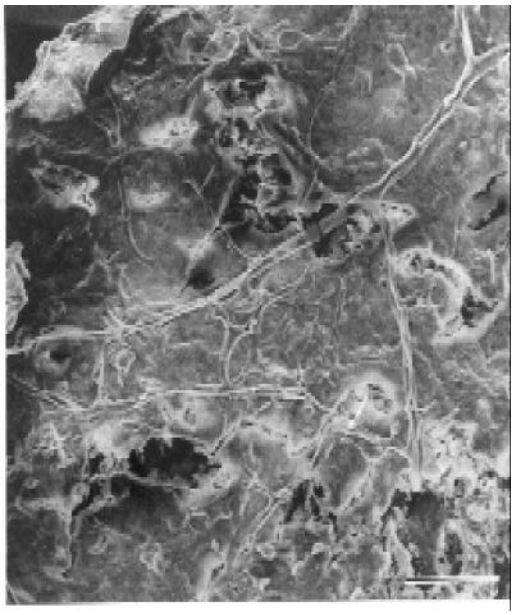
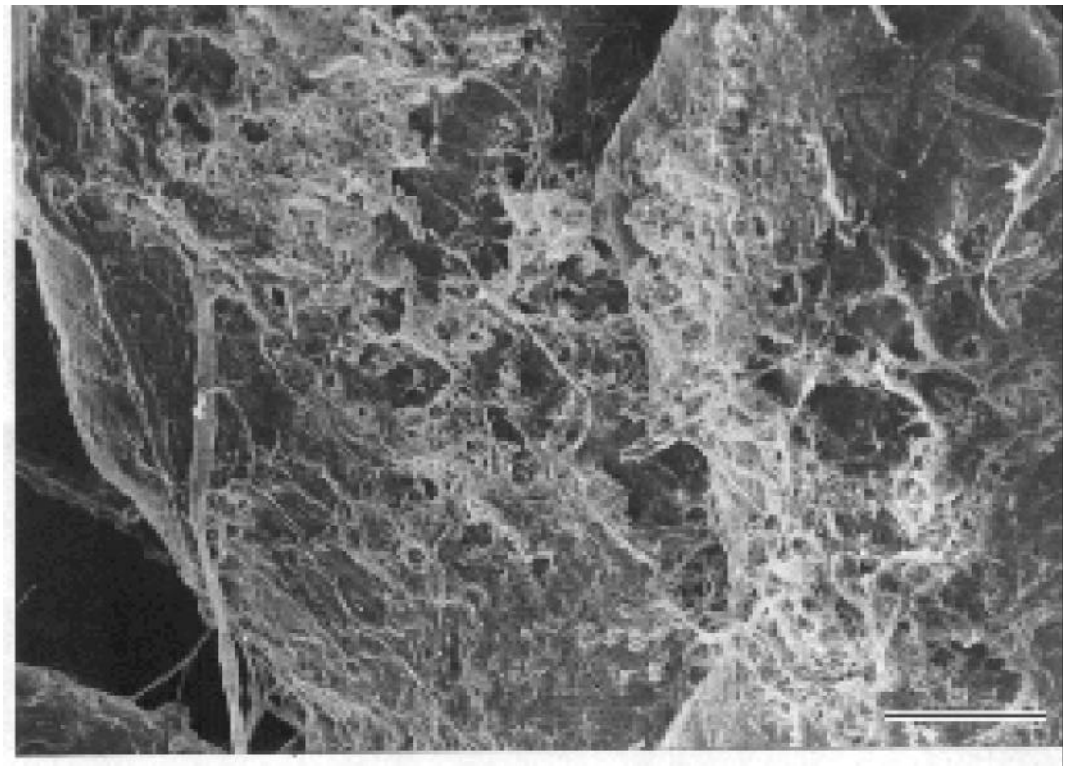


Fig. 6. Lower surface of *P. amazonensis* showing fissures and perforations (SEM). Scale = 25 µm.



Figs. 7 Characteristics of the midrib from the lower surface of *P. amazonensis* (SEM). View of midrib. Scale = 50 µm.

upper cortex could be an adaptation to open habitats with intense radiation during arid phases, as existed in the Guayana region before about 5000-6000 years before present (Rull 1991). The anatomical structure observed in *Pseudohepatica* is also very similar to that observed in *Concamerella* (Parmeliaceae) (Culberson & Culberson 1981, Elix 1993), which has strongly sclerotic cells in the upper cortex, with narrow and very few visible lumina.

The data obtained by HPTLC show the presence of three substances in *P. duidensis*: gyrophoric acid and two unidentified substances (Table 1). The TLC data and spot test reactions of gyrophoric acid are identical with those of controls obtained by isolation (Morales & Marcano 1992; Marcano & Morales 1994): *A*, 24, *B*, 42, *C*, 24; short wave UV + before H_2SO_4 spray bright blue, long wave UV + after H_2SO_4 spray green, visible after H_2SO_4 spray strong-pale yellow, gray halo; K-, C+ pink, KC-, PD-. The unknown substance 1 was reported by Jørgensen (1993) in *P. pachyderma* and constitutes probably a norlichexanthone derivative whereas the unknown substance 2 has Rf values and spot test reactions similar to lichexanthone (*A*, 0.72; *B*, 0.72 and *C*, 0.74; K -, C + orange, PD -; UV + yellow), but we do not know really if this substance is a lichexanthone derivative or lichexanthone properly. Gyrophoric acid is present in most parmeliaceous genera (e.g. *Xantho-parmelia, Relicina, Bulbothrix, Pseudoparmelia, Parmelina, Hypotrachyna*) (Elix 1993). It is also found in other taxa of Lecanorales (e.g. Acarosporaceae, Lecideaceae, Umbilicaria-ceae) (Culberson 1969, Culberson et al. 1977, Marcano 1994).

The substances present in *P. pachyderma* are all lichexanthone derivatives. TLC analysis showed the presence of four substances

(Jørgensen 1993) of which only one has been detected in traces in *P. duidensis* (substance 1, see Table 1). Two of these substances were identified by Leuckert *in* Jørgensen (1993) by mass spectrometry as probably norlichexanthone derivatives (ortho-methyldichlororlichexanthone and ortho-methylmonochloronorlichexanthone or unknown isomers thereof). In contrast, *Pseudohepatica duidensis* contains gyrophoric acid and two unidentified substances, both probably lichexanthone derivatives.

The polysaccharide content in the cell walls is useful taxonomically for phylogenetic units above species or genus level (Baral 1987, Common 1991, Elix 1993). Apparently it concerns conservative features in the evolution of fungi, which are significant at the upper levels of their classification. Imshaug (1981), Common (1991) and Elix (1993) have demonstrated the utility of this criterion mainly in the Parmeliaceae. An analysis carried out in the cell walls of *P. duidensis* shows the presence of iodine reactions characteristic of lichenan (SIKI + red, Melzer´s Reagent + orange, 1.5% LPIKI - and 1.5% IKI + red). The lichenan is the commonest polysaccharide in the cell walls of the Parmeliaceae *sensu lato* (Common 1991, Elix 1993). Iodine reactions characteristic of lichenan have not been found outside the Parmeliaceae (Common 1991). This could be a strong indication that the Parmeliaceae *sensu lato* are a natural group which can be recognized by it. The presence of iodine reactions characteristic of lichenan in *Pseudohepatica* suggests that it could be more closely related to the Lecanorales and particularly to the Parmeliaceae than to any other known lichen taxon. Other lichen families as Ramalinaceae, Umbilicariaceae, Opegraphaceae, Roccellaceae, Graphidaceae, Teloschistaceae, Verrucariaceae, Pertusariaceae and other families of the Lecanorales have very different iodine reactions, characteristic of other polysaccharides (Shibata 1973a and b, Baral 1987, Common 1991). However, the presence of iodine reactions characteristic of lichenan in the Parmeliaceae is not a certain proof that *Pseudohepatica* belongs in the Parmeliaceae, since only small parts of the lichen system have been scanned for this character so far.

The genus *Pseudohepatica* differs from all other lichens by its special shape resembling a leafy hepatic. Jørgensen (1993) suggests that the hepatic-like morphology of this lichen and the presence of UV + substances (lichexanthone derivatives) could be related to the UV-vision of insects and represent a case of mimicry or a lichen-insect interaction. However, we rather interpret these characters as a case of morphological convergence where the hyphae would determine the special shape from a specific ecophysiological adaptation to the microenvironment as has occurred in other lichenized fungi (Rogers 1990). In lichens, it is very difficult to find a case of mimicry as the one supposed by Jørgensen and most reports in the literature about mimicry in lichens are hypotheses without solid evidence (Marcano et al. 1995c).

Additional specimens examined: **Venezuela**: *Amazonas*: Cerro Duida-Marahuaca, north face, south of Culebra, 1200-1500 m, *L. Galiz, V. Marcano* and *A. Morales* 641, 837a and b (herb. lich. *V. Marcano, TFAZ*).

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