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ORIGINAL ARTICLE

Hypotrachyna penduliloba and *Remototrachyna pandani*, two new species in the hyperdiverse lichen family Parmeliaceae from Réunion in the Mascarene Archipelago

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Abstract Extensive exploration of lichen diversity in the tiny and remote tropical island of Réunion (Mascarene Archipelago, Indian Ocean) yielded two new species in the hyperdiverse lichen family Parmeliaceae. Morphological, anatomical and chemical characters and molecular inferences from 3 loci (ITS, nuLSU and mtSSU) strongly support their assignment to *Hypotrachyna* (subgenus *Longilobae*) and *Remototrachyna*. *Hypotrachyna producta* is further assigned to *H.* subgenus *Longilobae* and is newly reported for Réunion as well as *Remototrachyna costaricensis*.

Keywords Lecanoromycetes · Molecular phylogeny · Species diversity

Introduction

The Parmeliaceae family represents one of the most well-known lichen families, being easily recognized by any informed naturalist or ecologist, with representatives used in biodiversity assessment, and environmental and climate monitoring world-wide (examples in Aragón et al. 2013; Hauck and Javkhlan 2009; Normann et al. 2010; Saipunkaew et al. 2007). It is a large, subcosmopolitan and very diverse family with 79 genera and over 2700 species currently recognized (Thell et al. 2012), of which the basal radiation occured between 60 and 74 MA ago (Amo de Paz et al. 2011). This group has been extensively studied worldwide by several researchers for more than five decades (Crespo et al. 2010). However, some regions, especially the remote tropics, require further study. This includes Réunion, a small (2512 km^2) and remote island in the Mascarene Archipelago in the Indian Ocean. So far, 54 species representing 12 genera of the Parmeliaceae were recorded from the island by van den Boom et al. (2011).

Remototrachyna, is a recent segregate of this family based on molecular and morphological data. Species are distinguished in having a pored epicortex, broad, sub-irregular lobes with rounded apices, short, mostly dichotomously branched rhizines, scleroplectenchymatous cupulate exciple, and large ellipsoid ascospores (Divakar et al. 2010). It shows the centre of diversity on the Indian subcontinent and in South East Asia. So far, the genus was unknown from Réunion. More recently, the genus *Hypotrachyna* was reclassified and five subgenera are recognized viz. *Cetrariastrum*, *Everniastrum*, *Longilobae*, *Parmelinopsis*, and *Sinuosae* (Divakar et al. 2013). Species are characterized by a pored epicortex, narrow, sublinear to linear elongate lobes, with truncate apices, dichotomously branched rhizines, oval-ellipsoid ascospores and bifusiform conidia. So far, 19 species are reported from Réunion under the genus names *Hypotrachyna* and *Parmelinopsis* (Masson 2012; van den Boom et al. 2011), but a revision of species of the *Hypotrachyna* clade occuring on that island is in progress by the first author.

Traditionally, morphological and chemical features have been used for species delimitation in lichens in general and Parmeliaceae in particular. In some cases, it has been a matter of debate as morphotypes (distinguished by the means of reproduction, either with apothecia or not, and by means of

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vegetative dispersion, with soralia or isidia) and chemotypes (distinguished by the secondary metabolites produced in the medulla) were widely recognized as appropriate traits, diagnostic at the species level (Culberson and Hale 1973; Elix et al. 1986; Poelt 1972). Nonetheless, in the last decade, important additional molecular data have emerged for accurate species assessment and taxonomic re-evaluation, and are frequently used for species delimitation in Parmeliaceae, especially in the parmelioid groups (see the review by Crespo and Pérez-Ortega 2009 and Lumbsch and Leavitt 2011; see also Leavitt et al. 2011, 2012, 2013). Along with detailed morphological and chemical analysis, we use here a three-loci dataset (ITS, nuclear LSU and mitochondrial SSU) to clarify the taxonomic status of four species in the parmelioid genera *Hypotrachyna* and *Remototrachyna* from Réunion, two presumably undescribed (*H. pendululoba*, *R. pandani*) and two new to the lichen mycota of the island (*H. producta*, *R. costaricensis*).

Materials and methods

Material was collected during several field trips to Réunion in 2003, 2005, 2009, 2012 and 2013. Morphological and chemical characters were assessed following the protocol described by Masson (2012). In particular, ascopore measurements were made in tap water in the dead hydrated state (Baral 1992) and statistics are given as the arithmetical mean value (in italics and underlined) plus/minus (±) 1.96×the standard deviation (SD; rounded up to the nearest 0.5 μm); values in parentheses represent observed minimum and maximum values and Q represents the length/width ratio. Statistics for the other anatomical measurements are given as the arithmetical mean value (in italics) between the observed minimum and maximum values (in parentheses). Definition and terminology of the apothecial layers follow Ferencova (2012). Secondary metabolites were studied by thin layer chromatography (TLC) under the standard procedure with the solvent systems A, B, C, E and G (Orange et al. 2010). The codes used for colours follow Online Auction Color Chart (Online Auction Color Chart Company 2004). Bioclimates of the localities were determined according to Rivas-Martínez and Rivas-Sáenz (2009). Material preserved in the private herbarium of the first author is referred to as "h".

Accessions from GenBank have been retrieved to assess the identity and phylogenetic relationships of our material. We here provide detailed descriptions of all species dealt with in this paper, including previously described ones; we indeed suspect that many cryptic species are still to be detected within Parmeliaceae and, thus, detailed descriptions should be most useful for further studies.

Well-preserved lichen specimens lacking any visible symptoms of fungal infection were sampled for DNA isolation. Extraction of DNA and polymerase chain reaction (PCR) amplification were performed following the protocol of Cubero et al. (1999). The following primers were used: (a) for ITS: ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990); (b) for mtSSU: mrSSU1 and mrSSU3R (Zoller et al. 1999); (c) for nuLSU, as suggested at http://www.lutzonilab.net/primers: LIC2044, LR0R, LR3R, LR3, and LR6. Amplicons were sequenced by Macrogen® or by the GIGA sequencing platform of the University of Liège. Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to MEGABLAST searches to detect potential contamination. The sequences were aligned manually using MacClade 4.05 (Maddison and Maddison 2002). Ambiguous regions were delimited using the online version of GBlocks v 0.91b (Castresana 2000) at http://molevol.cmima.csic.es/castresana/Gblocks.html, allowing for gap positions within the final blocks, and carefully checked manually.

We assembled two matrices. Matrix 1 was assembled to detect the phylogenetic affinities within *Hypotrachyna* as recircumscribed by Divakar et al. (2013), and the second one for the same purpose with the genus *Remotrachyna* (Divakar et al. 2010). Both matrices included sequences of three loci, nuLSU, ITS and mtSSU for representative species of both genera, including the material under study and collected in Réunion (Table 1).

Congruence between the three loci partitions in both matrices was assessed, with datasets considered congruent if relationships characterized by bootstrap proportions for maximum likelihood (ML) or posterior probabilities above 70 % or 0.95, respectively, were identical or at least not in direct conflict among the inferences from individual loci. Since all partitions were shown to be congruent, they were concatenated. The two matrices are deposited in TreeBASE under the accession numbers 16641 and 16642, respectively, for the *Hypotrachyna* matrix and the *Remototrachyna* one.

For each matrix, phylogenetic relationships were reconstructed based on ML and Bayesian inferences. We used RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) for the ML analysis on the CIPRES gateway (Miller et al. 2010). We used the GTRGAMMA model for searching of the final most likely tree and for bootstrapping; the model includes a parameter (Γ) for rate heterogeneity among sites, and no parameter for estimating the proportion of invariable sites was included. Support for each branch was evaluated using the "fast bootstrap" with 1000 pseudoreplicates. Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MC^3) in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). No prior values were assumed. Model selection was based on Divakar et al. (2010, 2013). Four parallel runs were performed, each using four independent chains (three heated and one cold chain), with a single tree saved every 1000th generation for a total of

Table 1 Specimens including locality details and GenBank accession numbers of the three markers, ITS, nuLSU and mtSSU. Newly generated sequences for this study are in bold face

Species	Locality	Collector(s)	Voucher specimen	GenBank acc. no.		
				ITS	mtSSU	nuLSU
Matrix 1						
Hypotrachyna afrorevoluta	Canary Island: Tenerife	*Crespo* s/n	MAF-Lich 10409	DQ279529	DQ287839	EU562681
H. andensis	Bolivia: Camacho	*Flakus & Rodríguez* 16907	KRAM-L	KF380886	KF380965	KF380922
H. bogotensis	Chile: X Región	*Pérez-Ortega* 316 DNA 2254	MAF-Lich	KF380889	KF380969	KF380926
H. booralensis	Australia: Queensland	*Lumbsch* s/n	MAF-Lich 13969	DQ279493	DQ287801	EU562682
H. caraccensis	Bolivia: Nor Yungas	*Flakus & Rodríguez* 16878	KRAM-L	KF380890	KF380970	KF380927
H. cirrhata	Peru: Quebrada Parón	*Lumbsch* 19342r	MAF-Lich 13976	DQ279487	DQ287795	EU562674
H. denshirrhizinata	Bolivia: Nor Yungas	*Flakus & Rodríguez* 17003	KRAM-L	—	KF380975	KF380935
H. dubitans	Peru: Ancash	*Lumbsch, Wirtz & Ramírez* 19366	F (MAF-Lich 15621)	GQ919270	GQ919217	GQ919246
H. endochlora	Great Britain: Scotland	*Coppins* s/n	MAF-Lich 10178	AY 611072	AY611130	AY607784
H. halei	Bolivia: Nor Yungas	*Flakus & Rodriguez* 16897	KRAM-L	KF380898	KF380981	KF380939
H. horrescens	Spain: La Coruña	*Carvallal* s/n	MAF-Lich 9913	AY581085	AY582321	AY578951
H. kaernefeltii	Peru: Ancash	*Lumbsch, Wirtz & Ramírez* 19334	F (MAF-Lich 15620)	GQ919269	GQ919217	GQ919245
H. imbricatula	Brazil: São Paulo	*Benatti & Cintra* 3159	SP	KF380901	KF380983	KF380942
H. laevigata	Great Britain: Scotland	*Coppins* s/n	MAF-Lich 10177	AY611074	AY611132	AY607786
H. lipidifera	Peru: Quebrada Cojup	*Lumbsch* 19309b	MAF-Lich 13966	DQ279488	DQ287796	EU562675
H. livida 1	Brazil: São Paulo	*Benatti & Cintra* 3211	SP	KF380904	KF380986	KF380945
H. livida 2	Bolivia: Aniceto Arce	*Flakus* 18756	KRAM-L	KF380905	KF380987	KF380946
H. longiloba 1	Peru: Cusco	*Holgado, Mamani & Delgado* DNA 2198	MAF-Lich	KF380907	KF380989	KF380948
H. longiloba 2	Bolivia: Nor Yungas	*Flakus & Rodríguez* 16333	KRAM-L	KF380908	KF380990	KF380949
H. microblasta	Bolivia: Nor Yungas	*Flakus & Rodríguez* 16970	KRAM-L	KF380909	KF380991	KF380950
H. minarum	Spain: Cádiz	*Crespo* & al. s/n	MAF-Lich 7639	AY581086	AY582322	AY579852
H. neodissecta	South Africa: W Cape	*Crespo* & al. s/n	MAF-Lich 13986	DQ279510	DQ287820	EU562689
H. nepalensis	India: Uttarakhand	*Divakar* s/n	GUH 02-000924	AY611071	AY611129	AY607783
H. osseoalba	China: Yunnan	*Crespo, Blanco & Argüello* s/n	MAF-Lich 10390	DQ279512	DQ287822	EU562690
H. partita	Bolivia: Camacho	*Flakus & Rodríguez* 17699	KRAM-L	KF380910	KF380993	KF380952
H. penduliloba (type)	**Réunion**	***Masson***	**974.4087, LG DNA S3287**	**KP098555**	**KP098557**	**KP098559**
H. physcioides	China : Yunnan	*Crespo, Blanco & Argüello* s/n	MAF-Lich 10391	DQ279513	DQ287823	EU562691
H. polydactyla	Kenya: W province	*Divakar & Lumbsch* s/n	MAF-Lich 15518	GQ919283	GQ919231	GQ919258
H. producta	**Réunion**	***Masson***	**974.3907, LG DNA S3258**	**KP098554**	**KP098556**	**KP098558**
H. prolongata	Bolivia: Nor Yungas	*Flakus & Rodríguez* 17011	KRAM-L	KF380913	KF380996	KF380955
H. pseudosinuosa	China: Yunnan	*Crespo, Blanco & Argüello* s/n	MAF-Lich 10392	DQ279516	DQ287826	EU562692
H. rhizodendroidea	China: Yunnan	*Aptroot* 55665	ABL	DQ279489	DQ287797	EU562676
H. rockii	Peru: Quebrada Parón	*Lumbsch* 19342l	MAF-Lich 13965	DQ279524	DQ287834	EU562693
H. showmanii	USA: Pennsylvania	*Lendemer* 18060	NY 01080325	KF380916	KF380999	KF380958
H. sinuosa	Great Britain: Scotland	*Coppins* s/n	MAF-Lich 10179	AY611076	AY611133	AY607788
H. subfatiscens	Australia: Queensland	*Louwhoff, Molina & Elix* s/n	MAF-Lich 6878	AY611108	AF351174	AY607821
H. sorocheila	China: Yunnan	*Crespo, Blanco & Argüello* s/n	MAF-Lich 10375	DQ279490	DQ287798	EU562677
H. vexans	China: Yunnan	*Aptroot* 56597	ABL	DQ279491	DQ287799	EU562678
Parmeliopsis hyperopta	Spain: Madrid	*Blanco* s/n	M181F-Lich 108	AY611109	AY611167	AY607823
Matrix 2						
Bulbothrix goebelii	South Africa: W Cape	*Lumbsch* s/n	MAF-Lich 13985	DQ279484	DQ287791	EU562673
B. decurtata	South Africa: W Cape	*Ertz* 12878	BR	GQ919263	GQ919211	GQ919238
Remototrachyna adducta	China: Yunnan	*Crespo* & al. s/n	MAF-Lich 13988	DQ279483	DQ287790	EU562672

Table 1 (continued)

Species	Locality	Collector(s)	Voucher specimen	GenBank acc. no.		
				ITS	mtSSU	nuLSU
R. awasthii	India: Tamil Nadu	*Divakar, Lumbsch & Upreti* s/n	MAF-Lich 15614	GQ919271	GQ919219	GQ919247
R. ciliata	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10185	AY785273	AY785280	AY785266
R. costaricensis	Costa Rica: Volcán Arenal	*Molina* s/n	MAF-Lich 10211	AY785269	AY785276	AY785262
R. costaricensis	**Réunion**	***Masson***	**974.4056, LG DNA S3253**	**KP098542**	**KP098546**	**KP098550**
R. crenata	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10377	DQ279495	DQ287804	EU562683
R. aff. *crenata*	India: Karnatka	*Divakar, Lumbsch & Upreti* s/n	MAF-Lich 15616	GQ919275	GQ919223	GQ919250
R. dodapetta	India: Tamil Nadu	*Divakar, Lumbsch & Upreti* s/n	MAF-Lich 15612	GQ919276	GQ919224	GQ919251
R. flexilis	India: North Sikkim	*Divakar* s/n	MAF -Lich 13974	DQ279499	DQ287808	—
R. incognita 1	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10385	DQ279506	DQ287815	EU562687
R. incognita 2	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10384	DQ279507	DQ287816	—
R. infirma 1	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10386	DQ279508	DQ287817	—
R. infirma 2	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10210	AY785271	AY785278	AY785264
R. aff. *infirma*	India: Karnataka	*Divakar, Lumbsch & Upreti* s/n	MAF-Lich 15611	GQ919278	GQ919226	GQ919254
R. kingii	India: Tamil Nadu	*Divakar, Lumbsch & Upreti* s/n	MAF-Lich 15610	GQ919280	GQ919228	GQ919255
R. koyaensis	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10388	DQ279509	DQ287819	EU562688
R. pandani (type)	**Réunion**	***Masson***	**974.4433, LG DNA S3349**	**KP098544**	**KP098548**	**KP098552**
R. pandani 1	**Réunion**	***Masson***	**974.4107, LG DNA S3286**	**KP098543**	**KP098547**	**KP098551**
R. pandani 2	**Réunion**	***Masson***	**974.4257, LG DNA S3350**	**KP098545**	**KP098549**	**KP098553**
R. rhabdiformis	India: North Sikkim	*Divakar* s/n	MAF-Lich 15617	GQ919284	—	—
R. scytophylla	China: Yunnan	*Crespo, Blanco & Arguello* s/n	MAF-Lich 10410	DQ279525	DQ287835	EU562694

6,000000 generations. The incremental heating scheme was set by default. We used TRACER v1.6 (Rambaut et al. 2013) to plot the log-likelihood values of the sample points against generation time, and to determine when stationarity was achieved. Consequently the first 600,000 generations were deleted as the burn-in of the chain. A majority-rule consensus tree with average branch lengths was constructed for the remaining trees using the sumt option of MrBayes. Phylogenetic trees were visualized using FigTree v1.2.3 (Rambaut 2009). Branches support values were considered significant when ML bootstrap (MLBS) was>70 % and Bayesian posterior probabilities (PP) were>95 %.

Results and discussion

Phylogenetic analyses

Six sequences of nuLSU, ITS and mtSSU were newly generated for this study and the GenBank accession numbers are provided in Table 1. Matrix 1 was composed of nuLSU, ITS and mtSSU sequences for 38 representative species of the genus *Hypotrachyna*, with *Parmeliopsis hyperopta* as the outgroup, following Divakar et al. (2013). A total of 1910 characters were included, 290 being potentially parsimony-informative. The single most likely tree had a likelihood score of –8381.828239. Matrix 2 was composed of nuLSU, ITS and mtSSU sequences for 21 examples of the genus *Remototrachyna*, with *Bulbothrix decurtata* and *B. goebelii* as the outgroup (Divakar et al. 2010): these two species are representative of the two different clades forming the paraphyletic genus *Bulbothrix*, resolved as being a sister to *Remototrachyna*. A total of 1696 characters were included, 217 being potentially parsimony-informative. The single most likely tree had a likelihood score of –5490.597347.

The phylogenetic affinities within *Hypotrachyna* sensu Divakar et al. (2013) could be confirmed, except for the subgenus *Cetrariastrum* that is poorly supported in both optimization analyses; the subgenus *Longilobae* is well resolved and a sister to all other subgenera, and the position of *H. producta* and our new species, here described as *H. penduliloba*, within the subgenus is strongly supported (Fig. 1). Both species share the same chemistry and can be distinguished by their morphology; furthermore, they differ in 11 bp substitutions in ITS1, 1 indel in 5.8S, and 4 indels plus 9 bp substitutions in ITS2 (data obtained from two thalli, one for each species).

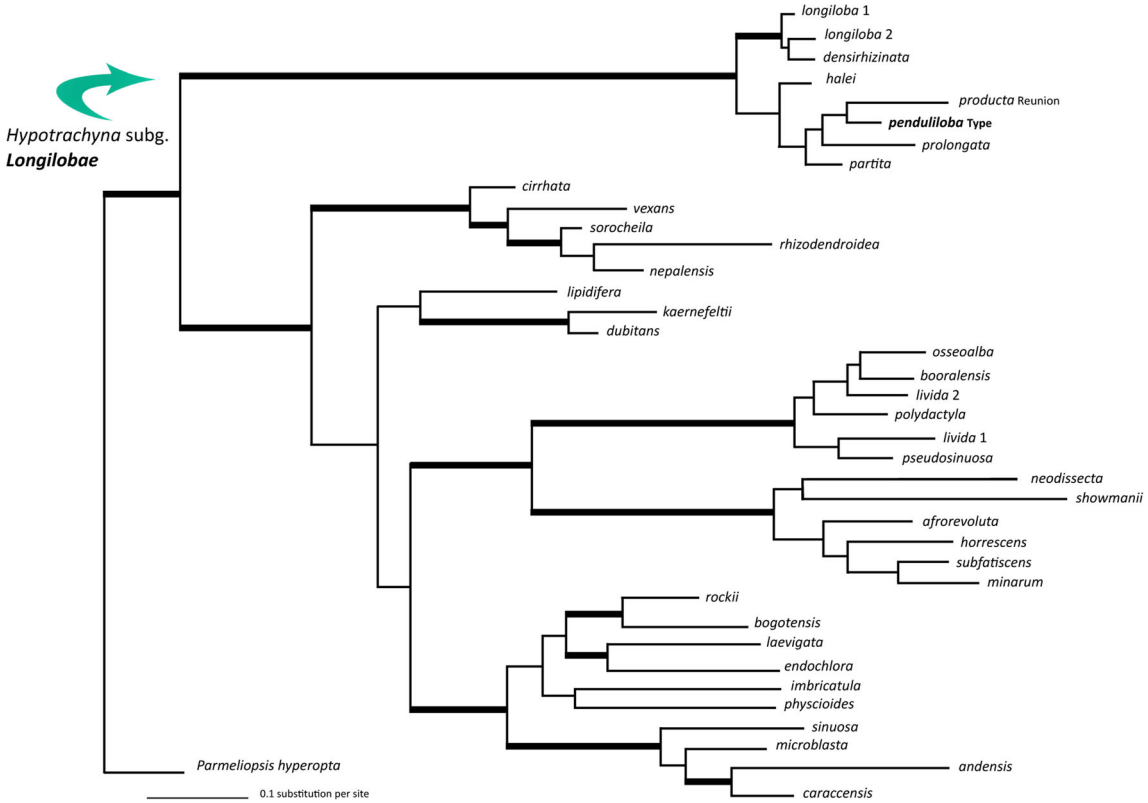

Fig. 1 Phylogenetic tree of the lichen genus *Hypotrachyna* including *H. penduliloba* sp. nov. and *H. producta*, both from Réunion material. *Arrow* points to subgenus *Longilobae*. 50 % consensus tree from MrBayes optimization, inferred from a three-loci matrix (ITS, nuLSU, mtSSU). Thickened branches with ML support>70 % and Bayesian posterior probabilities>0.95

Except for the strongly supported clade formed by *H. longiloba* and *H. densirhizinata*, all other nodes within the subgenus are not supported.

The genus *Remototrachyna* was retrieved as strongly supported, with *R. costaricensis*, including our sample from Réunion, as a strongly supported clade sister to all other accessions (Divakar et al. 2010). Our new species, here described as *R. pandani*, comes next, resolved as a sister to all other taxa (Fig. 2), this node being strongly supported in the Bayesian analysis (ML-bootstrap=72 %; PP=1.0). The barcode ITS of three different collections displays some variation as there are five variable positions in ITS1, two in 5.8S, and one in ITS2. A much larger variation was detected between a GenBank accession of *R. costaricensis* (collected in Costa Rica) and our single collection from Réunion as they differ by 14 bp substitutions plus 1 indel in ITS1, and 17 plus 1 indel in ITS2.

Taxonomy

Hypotrachyna penduliloba D.M. Masson & Sérus., *sp. nov.* (Fig. 3)

Mycobank MB 810751

Diagnosis Hypotrachyna species belonging to subgenus *Longilobae*; thallus large (9–15×6–10 cm), loosely adnate and partially free hanging; lobes sublinear to linear, 0.4–3 mm wide, internodes 1–7 mm long; upper surface more or less white-maculate; soralia linear and terminal at first, then occasionally slightly spreading on the upper surface and subterminal; soredia subgranular, (30)–*42.9*–(70) μm in diameter; medulla thin (28–78 μm thick), white with anziaic acid; upper cortex with atranorin and chloroatranorin.

Type France, Réunion, Le Tampon, le Volcan, trail to Piton Textor, 21°10′47″S, 55°38′13″E, 1870 m, on mossy branches of *E. reunionensis*, small patch of *E. reunionensis*-montane thicket in windward montane rainforest, 27 Aug. 2012, D. Masson 974.4087 (LG, holotype; G, isotype; GenBank Acc. for ITS: KP098555).

Description Thallus foliose, epiphytic, (7)9–15×(4)6–10 cm, loosely adnate and partially free hanging, membranaceous and fragile (Fig. 3a). *Lobes* sublinear to linear, separate to loosely imbricate, dichotomously to subdichotomously branched, 0.4–3 mm wide, internodes 1–7 mm long, sinuous or V-shaped axils, planar or sometimes slightly concave or convex, with entire and eciliate margins, subtruncate and occasionally slightly revolute apices. *Upper surface* whitish grey (oac123), almost white in central part, often brownish at lobe tip, more yellowish in the herbarium, with a narrow black marginal rim

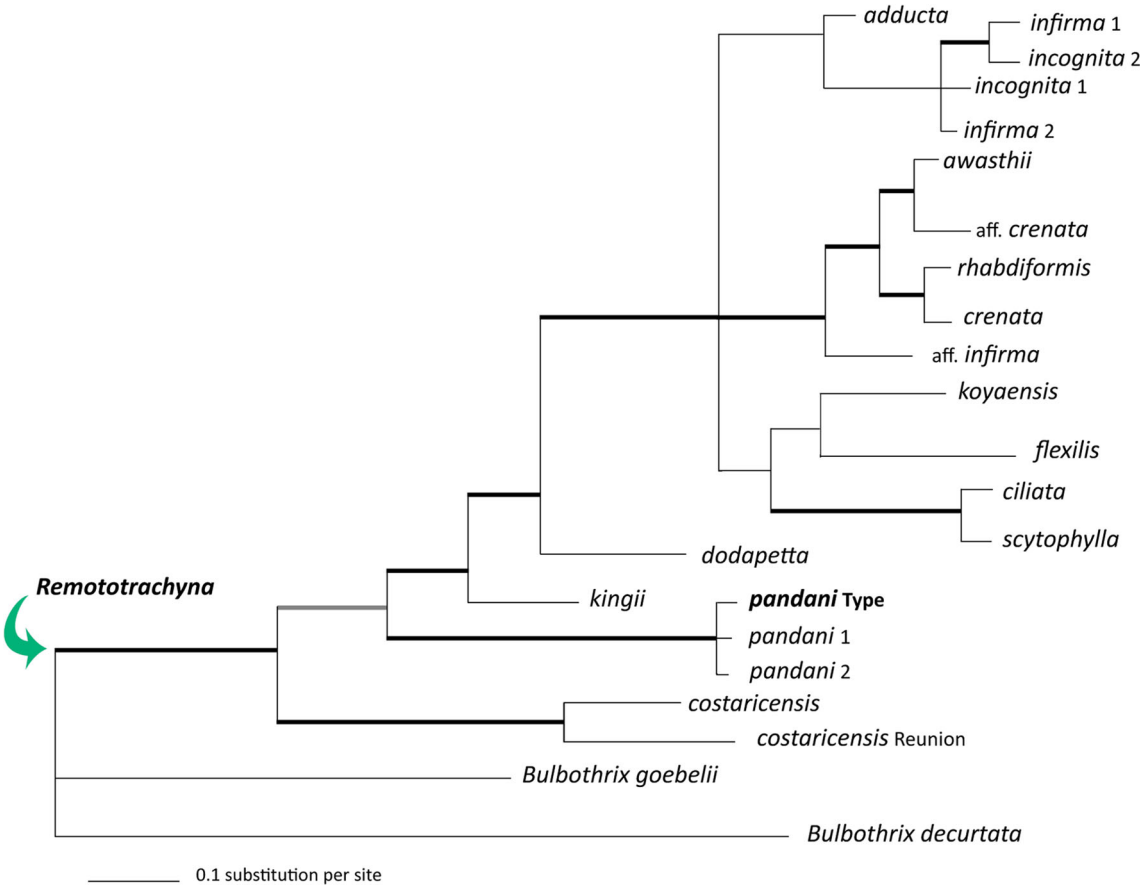

Fig. 2 Phylogenetic tree of the lichen genus *Remototrachyna* including *H. pandani* sp. nov. from Réunion and material of *R. costaricensis* from the same island. 50 % consensus tree from MrBayes optimization, inferred from a three-loci matrix (ITS, nuLSU, mtSSU). Thickened branches with ML support>70 % and Bayesian Posterior Probabilities>0.95; thickened branch in grey with support only in the Bayesian analysis (PP>0.95)

due to extension of the lower cortex along the edge, epruinose, without pseudocyphellae, more or less white-maculate, smooth and shiny near the apex, shallowly rugulose and rather dull towards the centre, sorediate, lacking pustules, dactyls and isidia. *Lobules* normally absent but marginal adventitious ones sometimes present in decaying parts. *Soralia* linear and terminal at first, the sorediate lobe tip becoming somewhat involute with the edge sinuate (Fig. 3b–c), then occasionally slightly spreading on the upper surface (Fig. 3d) and subterminal, the sorediate lobe tip becoming somewhat revolute. *Soredia* subgranular, (30)–*42.9*–(70) μm in diameter (n= 150, from 5 specimens, SD=7.4 μm). *Medulla* white throughout. *Lower surface* black, more or less shiny, smooth to slightly rugulose, generally with a marginal zone (ca. 0.5–1.5 mm wide) chestnut brown at the non-sorediate lobe apices, whitish or buff variegated at the sorediate ones. *Rhizines* usually black, sometimes dark brown near the lobe tip, shiny, sparse to moderately dense, projecting beyond the lobe margins and forming a marginal fringe, ca. 0.4–1(1.5) mm long, dendroid, (2)3–5 times dichotomously or trichotomously branched. *Apothecia* and *pycnidia* not seen. *Upper cortex* palisade plectenchymatous, not fragile, (15)–*20.1*–(30) μm thick. *Algal layer* continuous or discontinuous, (13)–*18.8*–(25) μm thick. *Medulla* thin, (28)–*44.7*–(78) μm thick. *Lower cortex* paraplectenchymatous, (13)–*15.7*–(25) μm thick.

Chemistry Spot tests and fluorescence: upper cortex K+ yellow, C-, KC-, P+ faintly yellow, UV-; medulla K-, C+ red, KC+ red, P-, UV-. *Secondary metabolites* (TLC): upper cortex with atranorin and chloroatranorin; medulla with anziaic acid.

Etymology The specific name relates to the rather elongated lobes that often hang down.

Distribution and ecology So far known from six localities in the central part of Réunion, on the southern side of the Piton des Neiges massif and the northwestern side of the Piton de la Fournaise massif (Fig. 3f). The localities lie between 1600 and 1870 m above sea level (a.s.l.) where the bioclimatic features can be summarized as follows: bioclimate: pluvial tropical; thermotype belt: upper mesotropical (332≤It≤393); ombrotype belts: from upper humid to ultrahyperhumid (11.6≤Io≤31.6). All thalli of *H. penduliloba* were found among mosses or on moss mats on bark of the endemic tree

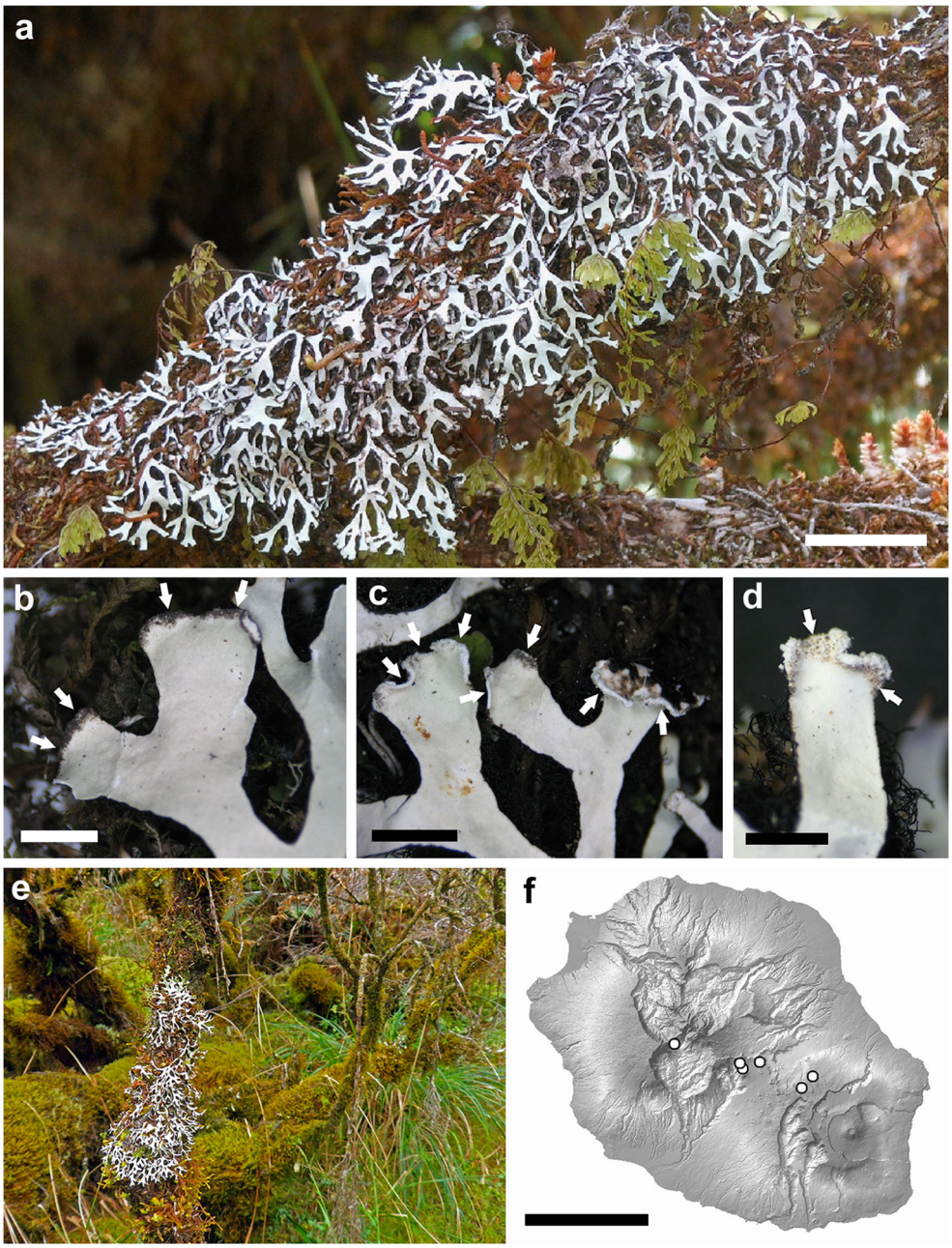

Fig. 3 **a–f** *Hypotrachyna penduliloba*. **a** Habit in the field (Masson 974.4361). **b–d** Linear soralia (pointed out with *arrows*) in various stages of development. **b** Plane lobe tips with young terminal soralia (Masson 974.4417). **c** Sinuous and involute lobe tips with terminal soralia (Masson 974.4420). **d** Sinuous lobe tip with subterminal soralium slightly spreading on the upper surface (Masson 974.4058). **e** Isotype in its natural habitat, a small patch of *E. reunionensis*-montane thicket in a cloud forest with abundant epiphytic moss mats. **f** Known distribution plotted on a topographic map of Réunion Island. *Scale bars*: **a** 2 cm, **b–c** 2 mm, **d** 1 mm, **f** 20 km

heather *Erica reunionensis* E.G.H. Oliv. [= *Philippia montana* (Willd.) Klotzsch] in *E. reunionensis*-montane thicket or montane rainforest (Fig. 3e). The filmy fern *Hymenophyllum inaequale* (Poir.) Desv. frequently grows intermixed with *H. penduliloba* thalli. *Hypotrachyna penduliloba* can be described as an aero- and substrato-hygrophilous, ombrophilous, moderately photophilous, acidophilous lichen.

Comments *Hypotrachyna penduliloba* belongs to the subgenus *Longilobae* within *Hypotrachyna* species but its phylogenetic relation within this subgenus remains unresolved (Fig. 1). Subgenus *Longilobae* includes all *Hypotrachyna* species with anziac acid as the major medullary secondary metabolite as well as atranorin and chloroatranorin in the upper cortex, namely: *H. halei*, *H. partita*, *H. producta* and *H. prolongata* (Divakar et al. 2013; present work). Besides the similar chemistry, they share sublinear lobes and a scarcity of sexual reproduction; apothecia are unknown in most species, except for *H. prolongata* in which they occur occasionally and produce only immature spores (Sipman et al. 2009). *Hypotrachyna partita* Hale and *H. prolongata* (Kurok.) Hale are both isidiate-lobulate and are not known outside the Americas. The neotropical *H. halei* Sipman, Elix & T.H. Nash lacks vegetative propagules (Flakus et al. 2012; Sipman et al. 2009). *Hypotrachyna producta* Hale is more widespread, being also known from outside America (see below). It is a

sorediate species like *H. penduliloba* but the soralia are subapical-subcapitate and their ontogeny are quite different from those of *H. penduliloba*. In *H. producta*, the lobe tip first becomes revolute and, very often, even cucullate, and eventually the soralium develops progressively on the convex dorsal side (Fig. 4b–c). This development pattern is very frequent amongst the sorediate *Hypotrachyna* species. In *H. penduliloba*, in contrast, soredia appear at the lobe tip margin and form a linear soralium restricted to the lobe tip (Fig. 3b) that can become somewhat involute with a sinuate margin (Fig. 3c). Ultimately the soralium may slightly spread over the upper surface (Fig. 3d), with the sorediate lobe tip becoming somewhat revolute. To our knowledge, this kind of soredia development has not been described in the genus *Hypotrachyna*.

The lobes of *H. penduliloba* are often distinctly elongated and pendulous. This growth form is unusual among the *Hypotrachyna* species of Réunion even if some specimens of *H. laevigata* (Sm.) Hale may develop a mixture of linear and sublinear lobes. Linear, free-hanging lobes with relatively long internodes rarely occur in East Africa (Krog and Swinscow 1979) or in Papua New Guinea (Louwhoff and Elix 2002) but are more frequent among the representatives of that genus in the mountains of Central and South America (Hale 1975; Sipman et al. 2009). According to Sipman (2002), this lobe configuration might be an adaptation to high humidity, allowing a more rapid drying of the thallus and, thus, maintaining the equilibrium between alga and fungus in humid conditions. Interestingly, *H. penduliloba* thalli from the Piton de la Fournaise localities, with approximately 4–5 m average annual rainfall (Jumaux et al. 2011), have clearly more elongated lobes than those from the Piton des Neiges localities, with ca. 2–4 m average annual rainfall.

Additional specimens examined **France**, Réunion, Cirque de Cilaos, track to Taïbit pass, 21°06′50″S, 55°26′28″E, 1600–1700 m, on trunk of *Erica*, thickets with *Hypericum lanceolatum* and *Sophora denudata*, 13 Nov. 2009, N.

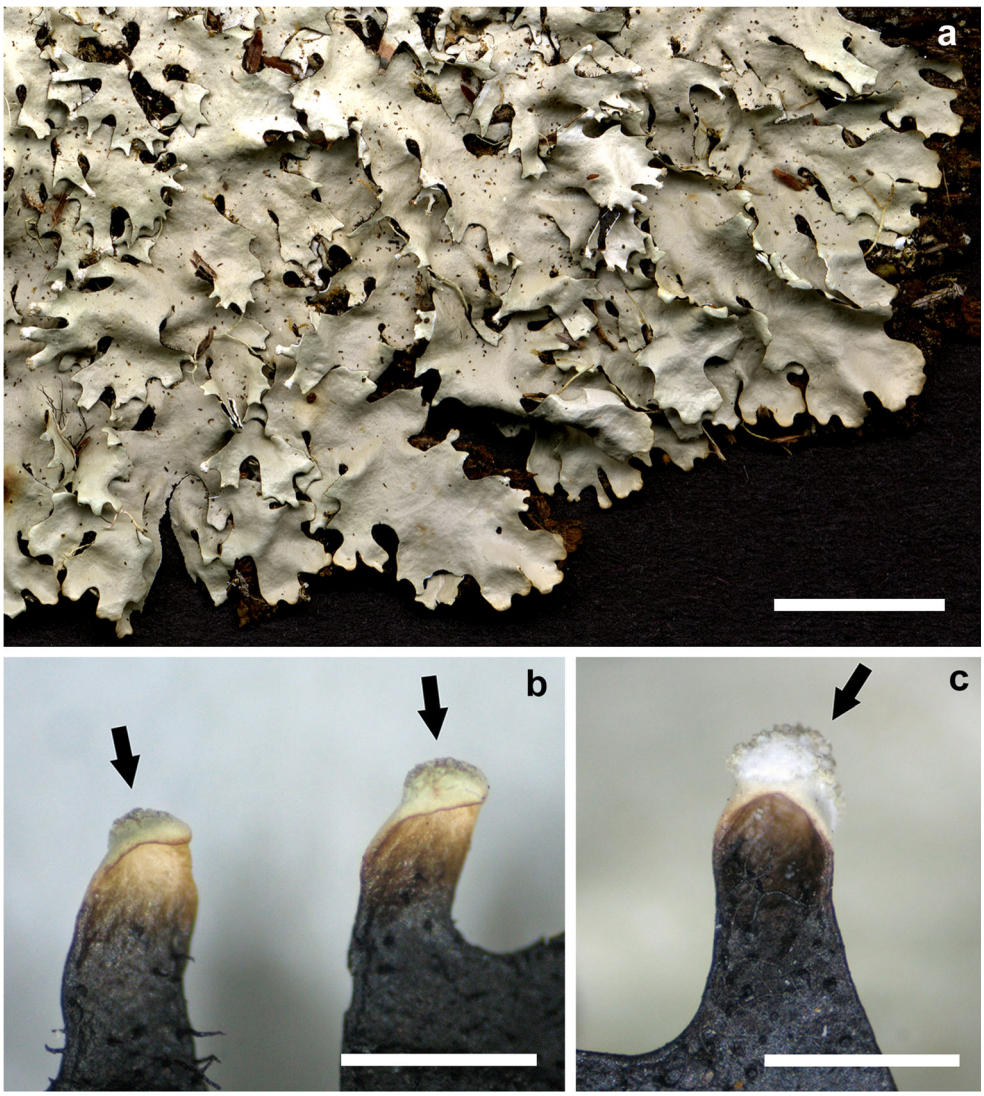

Fig. 4 **a–c** *Hypotrachyna producta* (Masson 974.3907). **a** Habit. **b** Young soralia (*arrows*) on the upper side of revolute lobe tips. **c** Cucullate lobe tip with well developed soralium (*arrow*). *Scale bars*: **a** 1 cm, **b–c** 1 mm

Magain & E. Sérusiaux s.n. (LG); La Plaine-des-Palmistes, trail to Piton des Cabris, 21°09′47″S, 55°39′17″E, 1655 m, on mossy branch of *E. reunionensis*, *E. reunionensis*-montane thicket in a small valley, 21 Aug. 2013, D. Masson 974.4361 (h); Le Tampon, Plaine des Cafres, trail to Bébour, 21°08′34″ S, 55°34′18″E, 1600 m, on mossy branchlet of *E. reunionensis*, somewhat human-disturbed *E. reunionensis*-montane thicket, 26 Aug. 2012, D. Masson 974.4058 (h); Le Tampon, forêt de la Plaine des Cafres, GR R2 trail, 21°09′03″ S, 55°32′42″ E, 1740 m, on mossy boles of old *E. reunionensis*, *E. reunionensis*-montane thicket, 23 Aug. 2013, D. Masson 974.4416, 974.4417, 974.4418 (h, REU); Le Tampon, forêt de la Plaine des Cafres, GR R2 trail, 21°08′58″S, 55°32′38″ E, 1755 m, on mossy branch of *E. reunionensis*, *E. reunionensis*-montane thicket, 23 Aug. 2013, D. Masson 974.4420 (h).

Hypotrachyna producta Hale, Smithson. Contr. Bot. 25: 56 (1975) (Fig. 4)

Description Thallus foliose, corticolous, 10×8 cm, adnate to moderately adnate (Fig. 4a). *Lobes* sublinear, contiguous to imbricate, dichotomously to subdichotomously branched, 1–5 mm wide, internodes 1–3 mm long, sinuous axils, planar or concave, with entire and eciliate margins, subtruncate to more or less rounded apices. *Upper surface* whitish grey (oac81 or oac123), sometimes brownish at the extreme lobe tip, more yellowish in the herbarium, narrow black marginal rim due to extension of the lower cortex along the edge, epruinose, without pseudocyphellae, in places faintly white-maculate, shallowly rugulose throughout, shiny near the apex and rather dull towards the centre, sorediate, lacking pustules, dactyls, lobules and isidia. *Soralia* subcapitate, subapical, forming dorsally at the cucullate tip of short lateral lobes (Fig. 4b–c). *Soredia* subgranular, (30)–*43.5*–(60) μm in diameter (*n*=50, from one specimen, SD=6.2 μm). *Medulla* white throughout. *Lower surface* black, more or less shiny, smooth to slightly rugulose, generally with a marginal chestnut brown zone (ca. 0.5–2 mm wide) at the lobe apices. *Rhizines* are usually black, sometimes dark brown near the lobe tip, shiny, fragile, sparse to dense, in places projecting beyond the lobe margins and forming a marginal fringe, ca. 0.3–1 mm long, dendroid, 2–5 times dichotomously to irregularly branched. *Apothecia* and *pycnidia* absent. *Upper cortex* palisade plectenchymatous, (15)–*17.2*–(20) μm thick. *Algal layer* more or less continuous, (20)–*22.8*–(25) μm thick. *Medulla* (40)–*52.8*–(73) μm thick. *Lower cortex* paraplectenchymatous, (13)–*14.4*–(18) μm thick.

Chemistry Spot tests and fluorescence: upper cortex K+ yellow, C-, KC-, P- or P+ faintly yellow, UV-; medulla K-, C+ red, KC+ red, P-, UV-. *Secondary metabolites* (TLC): upper cortex with atranorin and chloroatranorin; medulla with anziaic acid.

Distribution and ecology Known from Réunion from a single thallus collected at an elevation of 1735 m in a montane cloud forest [leeward mountain rainforest according to Strasberg et al. (2005)] covering a southeast facing slope above Îlet des Salazes in the Cilaos cirque. The bioclimatic features of the locality are: bioclimate: pluvial tropical; thermotype belt: upper mesotropical (It=372); ombrotype belt: upper humid (Io=10.8). The thallus thrived on bark of an old tree heather *Erica reunionensis*.

Specimens examined **Colombia**, Huila, La Plata, Vereda La Candelaria, headwaters of Rio La Candelaria, 2300 m, *Blechnum-Sphagnum* bog with scattered shrubs, 01 Oct. 1984, J. Aguirre C. & H.J.M. Sipman 6151, 6165 (B). **Costa Rica**, San José, E of Cerro Buenavista, Cerro de la Muerte, along the Panamerican Highway, 9°33′N, 83°46′W, 3400 m, epiphyte, ca. 2-m tall scrub with rock outcrops, 23 March 1985, H.J.M. Sipman 20.938 (B). **France**, Réunion, Cilaos, above Îlet des Salazes, 21°06′33″S, 55°26′46″E, 1735 m, corticolous on branch of old *E. reunionensis*, leeward montane rainforest, 20 Aug. 2012, Masson 974.3907 (h).

Comments Hypotrachyna producta is characterized by sublinear lobes with subapical, subcapitate soralia on short lateral lobes, atranorin and chloroatranorin in the cortex and anziaic acid in the medulla (Hale 1975; Krog and Swinscow 1979; Sipman et al. 2009). Known from southeastern USA and the Neotropics, New Zealand (Sipman et al. 2009), and Kenya and Uganda in Africa (Krog and Swinscow 1979), *H. producta* is a new addition to the lichen flora of Réunion as well as the Mascarene Archipelago. The phylogenetic position of *H. producta* remains unresolved within the subgenus *Longilobae* in our molecular study (Fig. 1).

Remototrachyna costaricensis (Nyl.) Divakar & A. Crespo, Am. J. Bot. 97: 586 (2010) (Fig. 5)

Description Thallus foliose, corticolous, 11×9 cm, moderately adnate. *Lobes* irregular, contiguous to imbricate, 1.5–5 mm wide, convex or concave, rarely planar, with entire and eciliate margins, subtruncate to more or less rounded apices, sinuous axils. *Upper surface* whitish grey (oac123), rarely brownish at the extreme lobe tip, more yellowish in the herbarium, narrow black marginal rim due to extension of the lower cortex along the edge, patchily distinctly white-pruinose in the central parts of the lobes, without pseudocyphellae, in places faintly white-maculate, rugulose throughout, more or less shiny near the apex, dull towards the centre, isidiate, lacking pustules, dactyls, lobules and soredia. *Isidia* laminal, rarely submarginal, unevenly distributed, subglobose at first, then irregularly cylindrical or slightly inflated, more or less branched and coralloid (to 0.6 mm high), epruinose, apices syncorticate, brown, eciliate. *Medulla* white throughout. *Lower surface* black, more or less shiny, rugulose, with a marginal chestnut brown

Fig. 5 *Remototrachyna costaricensis* habit (Masson 974.4056). *Scale bar* 1 cm

zone (ca. 1–2 mm wide) at lobe apices. *Rhizines* black, paler near the tip, shiny, moderately dense to dense, in places projecting beyond the lobe margins and forming a marginal fringe, ca. 0.2–0.5 mm long, dendroid. *Apothecia* absent. *Pycnidia* frequent, laminal towards lobe tips, black, immersed. *Conidia* not seen. *Upper cortex* palisade plectenchymatous, (15)–*20.2*–(25) μm thick. *Algal layer* more or less continuous, (23)–*24.2*–(25) μm thick. *Medulla* (58)–*64.2*–(70) μm thick. *Lower cortex* paraplectenchymatous, (18)–*19.4*–(23) μm thick.

Chemistry Spot tests and fluorescence: upper cortex K+ yellow, C-, KC-, P- or P+ faintly yellow, UV-; medulla K-, C-, KC-, P-, UV-. *Secondary metabolites* (TLC): upper cortex with atranorin and chloroatranorin; medulla with fatty acids of the constipatic acid complex.

Distribution and ecology Known in Réunion from a single thallus collected at 1600 m elev. on a mossy branch of the endemic shrub *Hubertia tomentosa* Bory, near a gully, in a somewhat human-disturbed *Erica reunionensis*-montane thicket. Bioclimate of the locality is pluvial tropical in the upper mesotropical thermotype belt (It=375) and the hyperhumid ombrotype belt (Io=25.9).

Specimen examined **France**, Réunion, Le Tampon, Plaine des Cafres, trail to Bébour, Bras Clair, 21°08′34″S, 55°34′18″ E, 1600 m, corticolous on branch of *Hubertia tomentosa*, *Erica reunionensis*-montane thicket near a gully, 26 Aug. 2012, D. Masson 974.4056 (h).

Comments Both morphological and chemical characters of the specimen from Réunion match well with *Remototrachyna costaricensis* (detailed descriptions in Louwhoff and Elix 2002 and Sipman et al. 2009). Furthermore, in the present phylogenetic tree it clustered with another accession of *R. costaricensis* from Costa Rica. The monophyly of the species is strongly supported (Fig. 2). The development of pruina on the upper surface as well as maculation seem rather variable in this species: pruina was absent and maculation was more or less present in specimens from Papua New Guinea (Louwhoff and Elix 2002), slight pruina near the tips and usually strong maculation in tropical America (Sipman et al. 2009), occasional pruina and faint maculation in specimens from Ecuador (Yánez-Ayabaca 2009), locally dense pruina and faint maculation in specimens from Brazil (Canêz 2005), and pruina in the central parts of the lobes and faint maculation in the specimen from Réunion. Thalli with fatty acids of the constipatic acid complex in the medulla are known from Mexico to Brazil in America (Nash et al. 2002; Sipman et al. 2009), Kenya and Tanzania in East Africa (Krog and Swinscow 1979), Papua New Guinea (Louwhoff and Elix 2002) and Réunion. Specimens with protolichesterinic acid as the major medullary substance and/or with an undetermined fatty acid (possibly caperatic acid) are also mentioned [as *Parmelia costaricensis* Nyl. or *Hypotrachyna costaricensis* (Nyl.) Hale] from several localities such as India (Patwardhan and Prabhu 1977; Divakar and Upreti 2005), Malaysia (Sipman 1993), Australia (Elix 1994), New Zealand (Galloway 1985), East Africa (Krog and Swinscow 1979), Brazil (Eliasaro et al. 1998), Ecuador (Yánez-Ayabaca 2009), Azores (Arvidsson 1990), southeast USA (Harris 1993), etc. Further detailed studies are needed to confirm that they all belong to *R. costaricensis*. Indeed, cryptic speciation may be hidden under a single epithet as an impressive variation in the barcode ITS has been detected between an accession retrieved from GenBank (material collected in Costa Rica) and our collection from Réunion: 14 bp substitutions plus 1 indel in ITS1 and 17 plus 1 indel in ITS2. Although no detailed study has been conducted on the genetic distances within this genus, the threshold to distinguish species boundaries as highlighted by Del-Prado et al. (2010) for several genera in the Parmeliaceae is crossed.

Remototrachyna pandani D.M. Masson & Sérus., *sp. nov.* (Fig. 6)

Mycobank MB 810752

Diagnosis Species belonging to the genus *Remototrachyna* according to apothecium anatomy and molecular inferences; thallus corticolous of medium size (6–9×3–5 cm), adnate to tightly adnate; lobes sublinear to subirregular, 1–3 mm wide; upper surface often white-pruinose; soralia subcapitate, subapical on short lateral lobes; soredia farinose, (20)–*27.3*–(40) μm in diameter; apothecia rare, up to 4 mm in diameter, margin crenate and sorediate; ascospores broadly ellipsoidal to ellipsoidal, (8)8.5–*10.1*–11.5(12)×5–*6.0*–7(7.5) μm; medulla

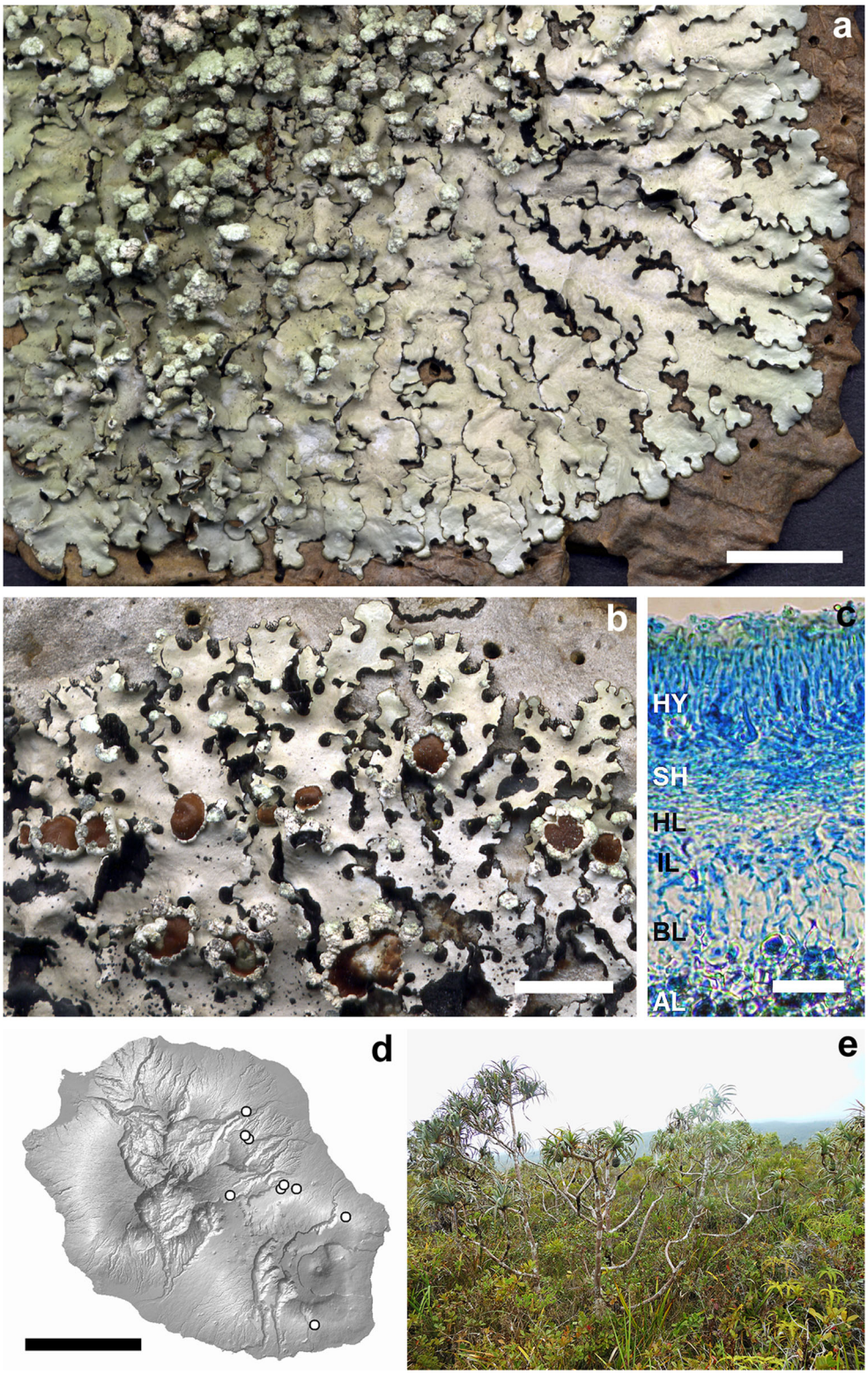

Fig. 6 **a–e** *Remototrachyna pandani*. **a** Habit (part of holotype). **b** Thallus with apothecia (Masson 974.4447). **c** cross-section through the apothecium in lactic cotton blue, HY = hymenium, SH = subhymenium, HL = hyaline layer, IL = intermediate layer, BL = basal layer, AL = algal layer (holotype). **d** Known distribution plotted on a topographic map of Réunion. **e** *Pandanus* submontane wet thicket with the endemic tree *Pandanus montanus*, the most frequent phorophyte (type locality). *Scale bars*: **a** 1 cm, **b** 5 mm, **c** 20 μm, **d** 20 km

white, with protocetraric acid and often an undetermined pigmentosin; upper cortex with atranorin and chloroatranorin.

Type France, Réunion, La Plaine-des-Palmistes, Ancienne Nationale, 21°06′36″S, 55°39′29″E, 775 m, on branches or trunks of *Pandanus montanus*, somewhat human-disturbed *Pandanus* submontane wet thicket, 24 Aug. 2013, D. Masson 974.4433 (LG holotype; G, UPS, herb. D. Masson, isotypes; GenBank Acc. for ITS: KP098544).

Description Thallus foliose, corticolous, (4)6–9(13)×3–5(8) cm, adnate to tightly adnate (Fig. 6a). *Lobes* separate to subimbricate, sublinear to subirregular, subdichotomously to irregularly branched, sinuous axils, (0.5)1–3(4) mm wide, more or less planar near tips, uneven in central parts, with eciliate, sinuous, occasionally somewhat involute margins, subrotund to subtruncate apices. *Upper surface* whitish grey to yellowish grey (oac30, oac67, oac81, oac123), more yellowish in the herbarium, at times dark olive-greenish at the lobe tip, narrow black marginal rim due to extension of the lower cortex along the edge, often (68 % of the thalli) with laminal, densely pruinose patches, mostly more or less scattered in young parts but sometimes extending to all parts and covering large areas, without pseudocyphellae, emaculate or faintly white-maculate, smooth and shiny near the apex, rather dull and usually transversely cracked towards the centre, sorediate, lacking pustules, dactyls and isidia. *Lobules* normally absent but marginal, short (up to 1 mm long), more or less spatulate ones sometimes present. *Soralia* subcapitate, subapical on short lateral lobes, very rarely laminal, shortly pedicellate and elevated at maturity, often coalescing, evenly distributed or mainly developing in the central parts of the thallus, occasionally with a yellowish tint. *Soredia* farinose, (20)–*27.3*–(40) μm in diameter (*n*=150, from 5 specimens, SD=5.5 μm). *Medulla* white throughout. *Lower surface* black, shiny, rugulose, with a marginal zone (ca. 0.5–1.5 mm wide) cinnamon brown at lobe apices. *Rhizines* black, more or less shiny, moderately dense, projecting beyond the lobe margins and forming a marginal fringe, ca. 0.2–0.7(1) mm long, dendroid, (1)2–5 times dichotomously to irregularly branched. *Apothecia* rare (fertile thalli found in two localities out of nine), laminal, sessile or rarely substipitate, up to 4 mm in diameter, disc purple brown (oac601), more or less glossy, first concave or plane, rarely convex, finally undulate contorted and somewhat radially split, margin crenate and soon sorediate (Fig. 6b), epihymenium (5)–*6.6*–(10) μm high, hymemium (33)–*37.5*–(45) μm high, subhymenium (15)–*20.0*–(25) μm high, proper exciple of type I (Ferencova 2012), hyaline layer (10)–*14.4*–(18) μm high, intermediate layer (13)–*16.9*–(20) μm high, cortex-like basal layer with hyphae predominantly vertically arranged, interwoven, made up of cells with thick walls and elongated and flexuous lumen, (23)–*33.1*–(43) μm high (Fig. 6c). *Ascospores* 8 per ascus, simple, colourless, broadly ellipsoidal to ellipsoidal, (8)8.5–*10.1*–11.5(12)×5–*6.0*–7(7.5) μm, Q=(1.33)1.35–*1.70*–2.05 (*n*=60), epispore ca. 1 μm thick. *Pycnidia* not seen. *Upper cortex* palisade plectenchymatous, (10)–*16.5*–(28) μm thick. *Algal layer* continuous, (13)–*18.6*–(23) μm thick. *Medulla* (40)–*53.5*–(65) μm thick. *Lower cortex* paraplectenchymatous, thin, (8)–*11.5*–(15) μm thick.

Chemistry Spot tests and fluorescence: upper cortex K+ yellow, C-, KC-, UV-; medulla K-, C-, KC± pink, P+ orange, UV-. *Secondary metabolites* (TLC): upper cortex with atranorin and chloroatranorin; medulla with protocetraric acid and often an undetermined pigmentosin (R_f A: 50–54, B: 28–30, C: 59–64, G: 75)

Etymology Named after the most frequent phorophyte, the endemic tree *Pandanus montanus* Bory.

Distribution and ecology So far known from nine localities in the windward part of Réunion from both the Piton des Neiges massif and the Piton de la Fournaise massif (Fig. 6d). The bulk of localities lies between 685 and 945 m elev. where the bioclimatic features can be summarized as follows: bioclimate: pluvial tropical; thermotype belts: upper thermotropical and lower mesotropical (478≤It≤540); ombrotype belts: from lower hyperhumid to ultrahyperhumid (18.0≤Io≤50.0). One locality differs by upper elevation (1385 m) and upper mesotropical thermotype belt (It=376). The main habitat type is the *Pandanus* submontane wet thicket (Fig. 6e) but *Remototrachyna pandani* can also thrive in the windward submontane or montane rainforests. The endemic screw-pine *Pandanus montanus* Bory is the principal phorophyte (86 % of the observations); *R. pandani* was also found on several other trees species such as *Monimia rotundifolia* Thouars. It grows on bark of branches or trunks, or even on adventitious roots of *P. montanus*. *Remototrachyna pandani* seems to be an aero-hygrophilous, ombrophilous, fairly photophilous, moderately thermophilous lichen.

Comments Amongst the 19 species of *Remototrachyna* currently known (Divakar et al. 2010; Flakus et al. 2012), *R. pandani* is the only sorediate one with protocetraric acid as the main medullary extrolite. Five other *Remototrachyna* species contain protocetraric acid in the medulla but three of them, *R. consimilis* (Vain.) Flakus, Kukwa & Sipman, *R. koyaensis* (Asahina) Divakar & A. Crespo and *R. sipmaniana* Kukwa & Flakus, are isidiate and the other two, *R. adducta* (Nyl.) Divakar & A. Crespo and *R. aguirrei* (Sipman, Elix & T.H. Nash) Flakus, Kukwa & Sipman lack vegetative propagules. Further, *R. adducta*, *R. aguirrei* and *R. koyaensis* have longer (>12 μm) and larger (>7 μm) ascospores; *R. aguirrei*, *R. koyaensis* and *R. sipmaniana* have wider lobes: 2–7 mm (Sipman et al. 2009), 2–8 mm (Divakar and Upreti 2005; Louwhoff and Elix 2002) or 4–10 mm (Hale 1975), and 5–8(10) mm (Flakus et al. 2012) wide, respectively. In overall morphology and chemistry, *R. pandani* closely resembles *Hypotrachyna pseudosinuosa* (Asahina) Hale and the two are likely to be easily confused. According to Divakar et al. (2010) the major morphological-anatomical character separating *Remototrachyna* from

Hypotrachyna is the ascoma anatomy, especially the structure of the proper exciple (see also Ferencova 2012; Flakus et al. 2012). Unfortunately *H. pseudosinuosa* seems to be very rarely fertile and the detailed anatomy of its apothecia is still unknown. *H. pseudosinuosa* has been described from Japan (Asahina 1951) and has been mentioned from various parts of the world ever since (see Masson 2005 for a review). However, the morphological variability observed throughout its range may reflect cryptic speciation that requires further research (Louwhoff and Elix 2002; our observations). The loci ITS, nuLSU and mtSSU of two *H. pseudosinuosa* specimens from China were examined by Divakar et al. (2010, 2013) and it appears that they belong to the *Hypotrachyna* sensu stricto clade; they are thus clearly distinct from the new species *R. pandani*.

Additional specimens examined **France**, Réunion, Bras-Panon, Plaine des Lianes, 21°01′50″S, 55°35′39″E, 880 m, on bark of *Pandanus montanus*, *Pandanus* submontane wet thicket, 25 Jul. 2005, D. Masson 974.1744 (h); Bras-Panon, Plaine des Lianes, 21°02′05″S, 55°35′45″E, 865 m, on branch of *Pandanus montanus*, *Pandanus* submontane wet thicket, 29 Aug. 2012, D. Masson 974.4121 (h); La Plaine-des-Palmistes, l'Ancienne Nationale, 21°06′35″S, 55°39′32″E, 770 m, on branches of *Pandanus montanus*, somewhat human-disturbed *Pandanus* submontane wet thicket, 24 Aug. 2013, D. Masson 974.4431, 974.4432 (h, REU); La Plaine-des-Palmistes, Ligne Deux Mille en Dessous, 21°07′00″S, 55°39′05″E, 870 m, on branches of *Pandanus montanus*, somewhat human-disturbed *Pandanus* submontane wet thicket, 24 Aug. 2013, D. Masson 974.4446, 974.4447 (h); Saint-André, forêt communale, 20°59′39″S, 55°35′33″E, 770 m, on trunk of an undetermined tree, windward submontane rainforest, 28 Jul. 2005, D. Masson 974.1807 (h); Saint-Benoît, Saint-François les Hauts, Sainte-Marguerite trail, 21°06′57″S, 55°40′42″E, 685 m, on branch and trunk of *Pandanus montanus*, *Pandanus* submontane wet thicket, 28 Aug. 2012, D. Masson 974.4099, 974.4107 (h); Saint-Benoît, Piton de Bébour, 21°07′33″S, 55°33′53″E, 1385 m, on branch of *Monimia rotundifolia*, windward montane rainforest, 07 Apr. 2003, D. Masson 974.0125 (h); Saint-Philippe, Saint-Philippe forest, trail to Piton Ravine Basse Vallée, 21°19′47″S, 55°42′12″E, 945 m, on root of *Pandanus montanus*, windward submontane rainforest, 16 Aug. 2013, D. Masson 974.4257 (h); Sainte-Rose, Mourouvin forest, Réservoirs trail, 21°09′37″S, 55°45′36″E, 880 m, on branch of *Pandanus montanus*, somewhat human-disturbed *Pandanus* submontane wet thicket, 15 Aug. 2013, D. Masson 974.4231 (h).

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