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Lecania falcata, a new species from Spain, the Canary Islands and the Azores, close to *Lecania chlorotiza*

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Abstract: *Lecania chlorotiza* and *L. falcata*, described here as new from Spain/Navarra, the Canary Islands and the Azores, do not belong to *Lecania* s. str. They belong to a strongly supported clade comprising *Bacidia, Bacidina, Scutula* and *Toninia* when examined with maximum parsimony, maximum likelihood and Bayesian inferences using mtSSU, nuLSU and nuITS sequences. This clade represents the *Bacidiaceae* and is included in the *Ramalinaceae* s. lat. Most genera included in that family need further work before a new genus can possibly be described for *Lecania chlorotiza* and *L. falcata*.

Key words: *Bacidia, Bacidiaceae, Bacidina,* phylogeny, *Ramalinaceae, Scutula*, taxonomy, *Toninia*

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Introduction

The simple, almost strictly automatic and dichotomous, taxonomic system generalized by Zahlbruckner for lichenized fungi in his classical Catalogue (Zahlbruckner 1921–1940; reprint issued in 1951) denied any evolutionary concepts. This monumental work has further obliterated several earlier studies on the systematics of many crustose species with tiny ascocarps. Fortunately, it has already been disputed by major contributions such as Santesson (1952) in his monograph of foliicolous lichens, and Poelt & Vězda (1977, 1981) in their keys to many European genera and species. The introduction of much more carefully examined morphological, anatomical and chemical characters, such as in Coppins (1983) for the genus *Micarea*, and Lücking (2008) for the foliicolous representatives of the *Bacidiaceae* and *Ramalinaceae*, could provide a more comprehensive description of many genera, and sharpened delimitation of species and distinction of closely related species. A major breakthrough, albeit much disputed immediately after its publication, was the use of hamathecium features, and especially ascus structure, as a radical set of characters to reorganize and delimit the genera and families (Hafellner 1984).

Production of large datasets of sequences for single or several loci and their processing with modern statistical techniques within a phylogenetic framework led to a thorough reassessement of taxa at all levels, including at the highest level within the Pezizomycotina (Miądlikowska *et al.* 2006). Generic circumscription is also a major issue at stake, including for crustose species producing lecideoid or biatorine apothecia and historically assigned to heterogeneous genera such as *Bacidia, Catillaria* and *Lecidea*. Indeed, few studies using molecular data and statistical analysis within an evolutionary framework are dedicated to assignment of such species at generic level: Stenroos *et al.* (2009) for *Lecidea margaritella*, now included in the newly described genus *Puttea*, Sérusiaux *et al.* (2010) for *Lecidea doliiformis* now referred

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to *Micarea* and *Catillaria alba* now referred to *Biatora*, and more recently Schmull *et al.* (2011) who examined the phylogenetic position of 25 species of *Lecidea* s. lat. and 22 putatively allied species. These species were resolved in many and very diverse positions within the Lecanoromycetidae, and outside of it. A new order (*Lecideales*) could be circumscribed for *Lecidea* s. str. and species of *Porpidia*, and a single species was transferred to a more appropriate genus (*Lecidea pullata* to *Frutidella*).

The genus *Lecania* is characterized (Smith *et al.* 2009) by its sessile, first flat then often becoming convex apothecia, a proper margin usually well developed, a thalline margin usually present, rarely excluded, hamathecium of thick, simple, conglutinated paraphyses, sometimes sub-moniliform, or with 1–2 terminal pigmented cells, with a dark cap, asci of the *Bacidia*- or *Biatora*- type, 8- or 16-spored, ascospores simple or with 1–7 septa, mostly being 1-septate, thin-walled, conidiomata frequent with curved conidia. The phylogeny of the genus was investigated by Reese Næsborg *et al.* (2007) using sequences from mtSSU rDNA, nuITS DNA and RNA polymerase II second large subunit (RPB2); phylogenetic analyses were carried out using Bayesian inference, maximum likelihood and maximum parsimony methods. The genus was clearly demonstrated to be polyphyletic: a clade supported in the Bayesian analysis including the type species [*L. fuscella* (Schaer.) A. Massal.] was referred to as *Lecania* s. str. and is nested within a larger one, also supported in the Bayesian analysis, and including other species currently assigned to *Lecania* (*L. furfuracea, L. naegelii*), all accessions of *Bilimbia* (*B. lobulata, B. microcarpa* and *B. sabuletorum*) and of *Thamnolecania* (*L. brialmontii*, *L. gerlachei* and *L. racovitzae*), *Bacidia fuscoviridis*, as well as several other species currently referred to other genera (*Cliostomum tenerum*, *Catillaria aphana* and *C. scotinodes*). Three species are excluded from the latter clade: *Lecania baeomma,* a usually sterile species, easily distinguished by its bluish soralia, growing on rocky seashores in Norway and the British Isles, *L. glauca*, a sorediate species from the Antarctic with a pseudoparenchymatous exciple, and rather large apothecia and ascospores, and finally *Lecania chlorotiza*. The last species is resolved in a strongly supported clade with *Bacidia rosella, Catillaria modesta* and *Toninia cinereovirens*, all representatives of the *Bacidiaceae* s. str. (Andersen & Ekman 2005; Sérusiaux *et al.* 2010).

A new species close to *Lecania chlorotiza* was detected in the material available to us from continental Spain, the Canary Islands and the Azores and is described in this paper (Fig. 1). Its study provides an opportunity to further assess the generic assignment of the rare *L. chlorotiza*.

Material and Methods

The material assembled for this study primarily consists of all collections available to us of both species targeted here (*Lecania chlorotiza* and *L. falcata* sp. nov.). Both species have been collected in the field by the authors and were identified following Smith *et al.* (2009). Further detailed studies of material of *L. chlorotiza* from SW England, where the type specimen comes from, were crucial to understanding the variation of that species. For inclusion in our DNA datasets, we also gathered recent collections in both genera *Bacidia* and *Bacidina* (*sensu* Ekman 1996 and Spribille *et al.* 2009) (Table 1).

Well-preserved lichen specimens lacking any visible symptoms of fungal infection were used for DNA isolation. Extraction of DNA and PCR amplification were performed following the protocol of Cubero *et al.* (1999). The primers used were the following: ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) for nuITS; mtSSU1 and mtSSU3R (Zoller *et al.* 1999) for mtSSU; and LR0R, LR3R, LR3, LR5R and LR6 (following the suggestions available on www.lutzonilab.net/primers) for nuLSU. Amplicons were sequenced by Macrogen®. Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to BLAST searches to detect potential contaminations.

We first assembled a matrix with mtSSU sequences of all species of *Lecania* included in the detailed study of Reese Næsborg *et al.* (2007), and related species, to test the phylogenetic position of *Lecania falcata* sp. nov., assumed to be close to *L. chlorotiza*. *Sphaerophorus globosus* was chosen as the outgroup following Reese Næsborg *et al.* (2007). As the position of *Lecania falcata* in the same clade as *L. chlorotiza* was confirmed, we assembled a second matrix with nuLSU, nuITS and mtSSU sequences of species resolved as members of that clade, including data retrieved from GenBank as well as sequences produced for this study. *Biatora pallens* and *Ramalina farinacea* were chosen as the outgroup as they belong to the basal groups of the clade containing all other species of

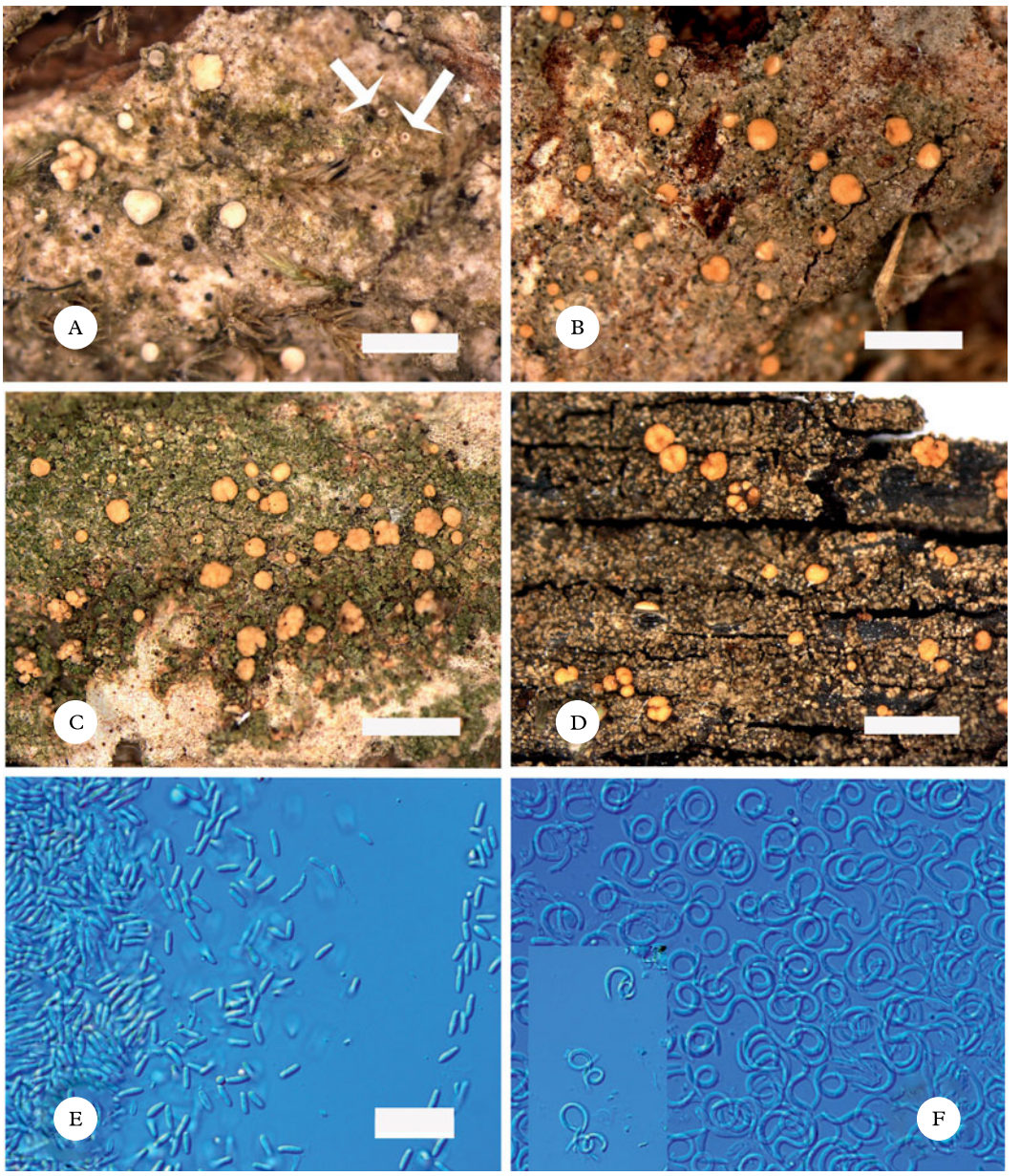

FIG. 1. *Lecania chlorotiza* and *L. falcata*. A–D, thalli with apothecia. A & B, *L. chlorotiza*; A, *B. J. Coppins* s.n. (LG) with arrows pointing to two empty pycnidia; B, *A. M. Brand* 35163 (hb. Brand). C & D, *L. falcata*; C, *P. van den Boom* 37919 (LG, isotype); D, *A. M. Brand* 13600 (hb. Brand). E & F, conidia; E, *L. chlorotiza*, *A. M. Brand* 35163 (hb. Brand); F, *L. falcata, P. van den Boom* 37919 (LG, isotype), insert with strongly curved conidia under unpressed cover slip. Scales: A–D = 1 mm; E & F = 20 μm.

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TABLE 1. *Species, specimens and DNA references used in this study, with their respective voucher information. GenBank accessions in bold refer to sequences produced for this study. All others were retrieved from GenBank*

Species name	Voucher collection	GeneBank Accessions Numbers		
		nuLSU	nuITS	mtSSU
Bacidia arceutina	Switzerland, *van den Boom* 41117 (LG DNA 579)	**JQ796842**	**JQ796851**	**JQ796829**
B. rosella	Sweden, *Ekman* 3117 (BG)	AY300829	AF282086	AY300877
B. rubella 1	Switzerland, *van den Boom* 41103 (LG DNA 578)	**JQ796843**	**JQ796852**	**JQ796830**
B. rubella 2	Switzerland, *van den Boom* 41259 (LG DNA 581)			**JQ796831**
B. schweinitzii 1	USA, *Lutzoni* 0047624 (DUKE) AFTOL-ID 642	DQ782911		DQ972998
B. schweinitzii 2	*Wetmore* 72619 (MIN)		AF282080	
B. sipmanii	Tenerife, *Sérusiaux* s.n. (LG DNA 361)	**JQ796844**	**JQ796853**	**JQ796832**
Bacidina arnoldiana 1	Poland, *Kukwa* 0047731 (DUKE) AFTOL-ID 1845	DQ986798		DQ986810
B. arnoldiana 2	*Ekman* 3157 (BG)		AF282093	
B. chloroticula 1	Norway, *Tønsberg* 18642 (BG)	AF282098		
B. chloroticula 2	Liechtenstein, *van den Boom* 41297 (LG DNA 580)			**JQ796833**
B. delicata	France, *Sérusiaux* s. n. (LG DNA 369)	**JQ796845**	**JQ796854**	**JQ796834**
B. egenula 1	Belgium, *van den Boom* 39669 (LG DNA 487)			**JQ796835**
B. egenula 2	Belgium, *van den Boom* 39665 (LG DNA 489)	**JQ796846**		**JQ796836**
B. neosquamulosa 1	Netherlands, *van den Boom* 41056 (LG DNA 490)	**JQ796847**	**JQ796855**	**JQ796837**
B. neosquamulosa 2	Netherlands, *van den Boom* 39692 (LG DNA 491)	**JQ796848**	**JQ796856**	**JQ796838**
B. phacodes	Sweden, *Ekman* 3414 (BG)		AF282100	AY567725
B. sulphurella	Switzerland, *van den Boom* 41263 (LG DNA 577)			**JQ796839**
Biatora ligni-mollis	France, *Sérusiaux* s.n. (LG DNA-D 25)			GU138665
B. meiocarpa	Norway, *Tønsberg* 28317a (BG)			AM292710
B. pallens	Norway, *Nordin* 5640 (UPS)			AM292709
B. vernalis	No data, *Tønsberg* 23757 (BG)			DQ838753
B. veteranorum	France, *Sérusiaux* s.n. (LG DNA-D 24)			GU138664
Bilimbia lobulata	Norway, *Rui & Timdal* 9169 (O)			AM292712
B. microcarpa	Canada, *Westberg* 1294 (LD)			AM292714
B. sabuletorum	Norway, *Ekman* 3091 (BG)			AY567721
Catillaria scotinodes	Scotland, *Coppins* 18298 & *O'Dare* (E)			AM292720
Cliostomum tenerum	Norway, *Klinkenberg & Jørgensen* (UPS)			AM292722
Crocynia pyxinoides	AFTOL-ID 111 (nuLSU and mtSSU) or USA, *Harris* 39717, NY (nuITS)	AY584653	AF517920	AY584615
Lecania aipospila	Norway, *Reese Næsborg* 148 & *Ekman* (UPS)			AM292723
L. atrynoides	Sweden, *Arup* L03286 (LD)			AM292724
L. baeomma	Norway, *Reese Næsborg* 149 & *Ekman* (UPS)			AM292725
*L. belgica**	Belgium, *van den Boom* 30770 (hb. van den Boom)			AM292746
L. brialmontii	Antarctica, *Convey* 121 (AAS)			AM292765
L. chlorotiza	Scotland, *Coppins* 19211 (E)		AM292679	AM292766
L. cyrtella	Sweden, *Ekman* 3017 (BG)			AM292767

Table 1. *Continued*

Species name	Voucher collection	GeneBank Accessions Numbers		
		nuLSU	nuITS	mtSSU
L. cyrtellina	Sweden, *Tibell* 23416 (UPS)			AM292730
L. dubitans	USA, *Hutchinson* ID-933-08 (hb. McCune)			AM292732
L. erysibe	Scotland, *Coppins* 17537 (E)			AM292733
L. falcata 1	Tenerife, *Sérusiaux* s. n. (LG DNA 1372)	**JQ796849**		**JQ796840**
L. falcata 2	Tenerife, *Sérusiaux* s. n. (LG DNA 1374)	**JQ796850**		**JQ796841**
L. falcata 3	Tenerife, *van den Boom* 37919 (holotype; LG DNA 316)		**JQ796857**	
L. furfuracea	Czech Rep., *Palice* 5595 (hb. Palice)			AM292734
L. fuscella	Sweden, *Arup* L03046 (LD)			AM292735
L. gerlachei	Antarctica, *Søchting* US7688 (BG)			AM292736
L. glauca	Antarctica, *Søchting* US7595 (BG)			AM292738
L. hutchinsiae	Scotland, *Coppins* 17549 (E)			AM292739
L. inundata	Scotland, *Coppins & Coppins* 19139 (E)			AM292740
L. naegelii	Austria, *Vondrák* 247 (CBFS)			AM292741
L. nylanderiana	Sweden, *Tibell* 23168 (UPS)			AM292742
L. rabenhorstii	Sweden, *Reese Næsborg* 53 (UPS)			AM292743
L. sambucina	Sweden, *Tibell* 23458 (UPS)			AM292744
L. turicensis	Spain, *van den Boom* 30865 (hb. van den Boom)			AM292748
Lecidea sphaerella	Slovakia, *Guttová et al.* 4646 (hb. Palice)			AM292749
Mycobilimbia pilularis	Norway, *Ekman* 3454 (BG)			JQ922247
M. tetramera	Norway, *Anonby* 856 (BG)			AM292750
Ramalina farinacea	Sweden, *Ekman* s.n. (BG)			AM292752
Scutula krempelhuberi	Sweden, *Ekman* 6356 (UPS)			AY567789
S. miliaris	Sweden, *Ekman* 6850 (UPS)			AY567790
Sphaerophorus globosus	Canada, *Brodo* 30171A (UPS)			AY256762
Toninia cinereovirens	Norway, *Haugan & Timdal* 7953 (O)	AY756365	AF282104	AY567724
T. sedifolia 1	*Knutsson* 97-407 (BG)		AF282120	AY300918
T. sedifolia 2	Canada, *Lutzoni & Miądlikowska* 0047744 (DUKE) AFTOL-ID 213	DQ973039		DQ972987

* = sp. 1 in Reese Næsborg *et al.* (2007); see Reese Næsborg & van den Boom (2007)

Lecania (Reese Næsborg *et al.* 2007). Altogether, we generated 29 new sequences, including five for *Lecania falcata* sp. nov. (Table 1).

For both matrices, the sequences were first aligned using MAFFT (online version available at http://mafft.cbrc.jp/alignment/server/), and eventually adjusted manually using MACCLADE v. 4.05 (Maddison & Maddison 2002). Sets of characters to be excluded from phylogenetic analyses were constructed 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.

The first matrix included 58 accessions (two for *Lecania falcata* sp. nov.) and 718 characters representing the loci mtSSU, and is deposited in TreeBASE under the accession number 12482. An unweighted maximum parsimony (MP) analysis was performed in PAUP* 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. A first heuristic analysis was performed using NNI (Nearest Neighbor Interchange) branch swapping, with 1000 replicates and saving 10 trees at each step, the functions Steepest Descent and MulTrees being in effect. A second analysis was performed with the 10 000 trees saved using TBR (Tree Branch Swapping), with a maximum of 200 trees saved at each step, the function Steepest Descent being inactivated. A 50% consensus tree was produced, and the strength of support for individual branches was estimated using bootstrap values (MPBS) obtained from 500 heuristic bootstrap pseudoreplicates. As four taxa (*Bacidina phacodes,* both species of *Scutula* and *Toninia cinereovirens*) have three identical deletions (20 bp at positions 304–323, 1bp at position 392, 5bp at positions 550–554), a second analysis was performed with gaps treated as a fifth character, all other parameters being identical.

Models of evolution for the maximum likelihood and Bayesian analysis were selected based on the Akaike Information Criterion (Posada & Buckley 2004) as implemented in Mr. Modeltest v2.3 (Nylander 2004). The selected model corresponds to the GTR model of nucleotide substitution (Rodríguez *et al.* 1990), including a proportion of invariable sites and a discrete gamma distribution of six rates categories. The maximum likelihood analysis was performed using GARLI (Zwickl 2006, version 0.951 for OS X) with gaps treated as missing data, and a single most likely tree was produced. Support for the branches was estimated using bootstrap values (MLBS) from 100 pseudoreplicates (all parameters identical to the original ML search).

Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004). Priors values were set to default and gaps were treated as missing data. Four parallel runs were performed, each using four independent chains (three heated and one cold chain), with a single tree saved every 100th generation for a total of 6 000 000 generations. The incremental heating scheme was set to default. We used TRACER v1.4.1 (Rambaut & Drummond 2007) to plot the log-likelihood values of the sample points against generation time, and determine when stationarity was achieved. Consequently the first 6 000 trees sampled 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 TreeFig v1.3.1 (Rambaut 2009).

The second matrix was assembled with nuLSU, nuITS and mtSSU sequences of 17 species belonging to the *Bacidiaceae*, plus *Biatora pallens* and *Ramalina farinacea* as the outgroup. The dataset is incomplete for several taxa: both outgroup species and both species of *Scutula* are represented only by mtSSU sequences; *Bacidina egenula* and *B. chloroticula* lack ITS sequences; finally *Bacidina phacodes* and *Lecania chlorotiza* lack LSU sequences. Furthermore, sequences from different origins have been assembled under a single entry for the following species: *Bacidia schweinitzii, Bacidina arnoldiana, B. chloroticula* and *Toninia sedifolia*. For this dataset, sequences from two collections of *Lecania falcata* have been assembled; they were gathered at the very same location (references to LG DNA data: 316 and 1372).

The second matrix included 20 accessions (one for *Lecania falcata* sp. nov.) and 3690 characters representing the three loci nuLSU, nuITS and mtSSU, and is deposited in TreeBASE under the accession number 12482. The matrix was submitted to the same three analyses, always with gaps treated as missing data. The selected model corresponds to the GTR model of nucleotide substitution (Rodríguez *et al.* 1990), including a proportion of invariable sites and a discrete gamma distribution of six rates categories. The maximum likelihood analysis performed using GARLI (Zwickl 2006, version 0.951 for OS X) produced a single most likely tree. Incongruence between single-gene matrices was tested with maximum likelihood analysis using GARLI for each partition. A conflict was considered significant if a clade was supported with bootstrap support >75% in a one-locus analysis and not in the other two. A further test for conflict was performed with ITS and LSU concatenated in a partition versus mtSSU in another. No conflict was detected and therefore the available sequences for the three loci were concatenated.

For analysis of both matrices, branches support were considered as significant when Maximum Parsimony Bootstrap (MPBS) > 75%, Maximum Likelihood Bootstrap (MLBS) > 75% and Posterior Probabilities (PP) > 0·95.

Results

The first matrix included 58 accessions (two for *Lecania falcata* sp. nov.) and 718 characters representing the loci mtSSU. Fifty-five characters are excluded from the analysis (*i.a.* an intron of 20 bp at positions 565–584 in *L. falcata*), 381 are constant, 59 are parsimony non-informative and 223 are potentially parsimony-informative. The single most

likely tree has a ln likelihood score of −5375·527542. The second matrix included 20 accessions (one for *Lecania falcata* sp. nov.) and 3690 characters representing the three loci nuLSU, nuITS and mtSSU. A total of 2090 characters are excluded from the analysis, including the whole ITS1 and almost all ITS2, and the second part of nuLSU for which available sequences were very much incomplete. Of the remaining 1600 characters, 1182 are constant, 151 are parsimony non-informative and 267 are potentially parsimony-informative.

The matrix with mtSSU sequences of all species of *Lecania* (Reese Næsborg *et al.* 2007) and related species, including *Lecania chlorotiza* and *L. falcata* sp. nov., retrieved the topology obtained by those authors with 3 loci (mtSSU, nuITS and RPB2) (Fig. 2): a clade supported only in MrBayes (MPBS and MLBS < 70%; PP = 1·0) includes most accessions of *Lecania* (including the type species of the genus, *L. fuscella*), with *L. glauca* as sister to all other species, and several species currently assigned to other genera (e.g. species of *Bilimbia*, *Cliostomum tenerum, Lecidea sphaerella, Mycobilimbia pilularis*). Two species of *Lecania* are excluded from that clade: *L. baeomma* which is resolved as sister to *Ramalina farinacea* (with strong support only in MrBayes), and *L. chlorotiza* which is resolved, together with *L. falcata* sp. nov., into a strongly supported clade (MBPS = 98%; MLBS = 96%; PP = 1·0) representing the *Bacidiaceae* s. str. The latter is resolved into two strongly supported clades: one with all species referred to *Bacidia* (MPBS = 100%; MLBS = 100%; PP = 1·0), and the second with all other species (MPBS = 97%; MLBS = 99%; PP = 1·0). The latter clade further provides strong support for two genera: 1) *Scutula* with two species (MPBS = 100%; MLBS = 100%; PP = 1·0), and 2) *Bacidina* with five species (MPBS = 100%; MLBS = 100%; PP = 1·0) but excluding its type species, *B. phacodes*. *Toninia*, represented here by two species (*T. cinereovirens* and *T. sedifolia*), is not resolved as a monophyletic group. *Lecania chlorotiza* and *L. falcata* sp. nov. are also not resolved in a monophyletic group.

In a second analysis on the same matrix (tree not shown), a maximum parsimony run with gaps scored as a fifth character gave prominence to the three indels autapomorphic of the group formed by *Bacidina phacodes, Toninia cinereovirens* and both species of *Scutula*. Indeed, a strongly supported clade (MPBS = 100%) comprising only these four species is resolved at an unsupported position within a supported clade (MPBS = 75%), including all other accessions of *Bacidina* and *Toninia sedifolia*. This clade further provided support (MPBS = 70%) to a clade comprising *Bacidina phacodes* and *Toninia cinereovirens*. All these branches collapsed in maximum likelihood and MrBayes analysis.

The second matrix assembled species of a clade strongly supported in the first analysis and representing the *Bacidiaceae* s. str., and thus comprised species of *Bacidia*, *Bacidina*, *Scutula*, *Toninia*, *Lecania chlorotiza* and *L. falcata* sp. nov. Three loci are included here: mtSSU, nuLSU and nuITS (Fig. 3). The *Bacidiaceae* s. str. is resolved as a strongly supported clade (MPBS = 100%; MLBS = 100%; PP = 1·0). All accessions of *Bacidia* (*B. arceutina, B. rosella, B. rubella, B. sipmanii* and *B. schweinitzii*) are resolved in a strongly supported clade (MPBS = 100%; MLBS = 100%; PP = 1·0), sister to all other accessions, also resolved in a strongly supported clade (MPBS = 96%; MLBS = 97%; PP = 1·0). The 50% majority rule consensus tree produced by the Bayesian analysis resolves the latter clade into two branches: a clade comprising *Lecania chlorotiza* and *L. falcata* sp. nov. supported only in MrBayes (PP = 0·99), sister to a supported clade (MPBS = 87%; MLBS = 80%; PP = 1·0) comprising other accessions, for example both species of *Scutula* (clade strongly supported: MPBS = 100%; MLBS = 100%; PP = 1·0), all species of *Bacidina*, except *B. phacodes* (clade strongly supported: MPBS = 100%; MLBS = 100%; PP = 1·0), the topological position of others (*Bacidina phacodes*, *T. cinereovirens* and *T. sedifolia*) being unsupported.

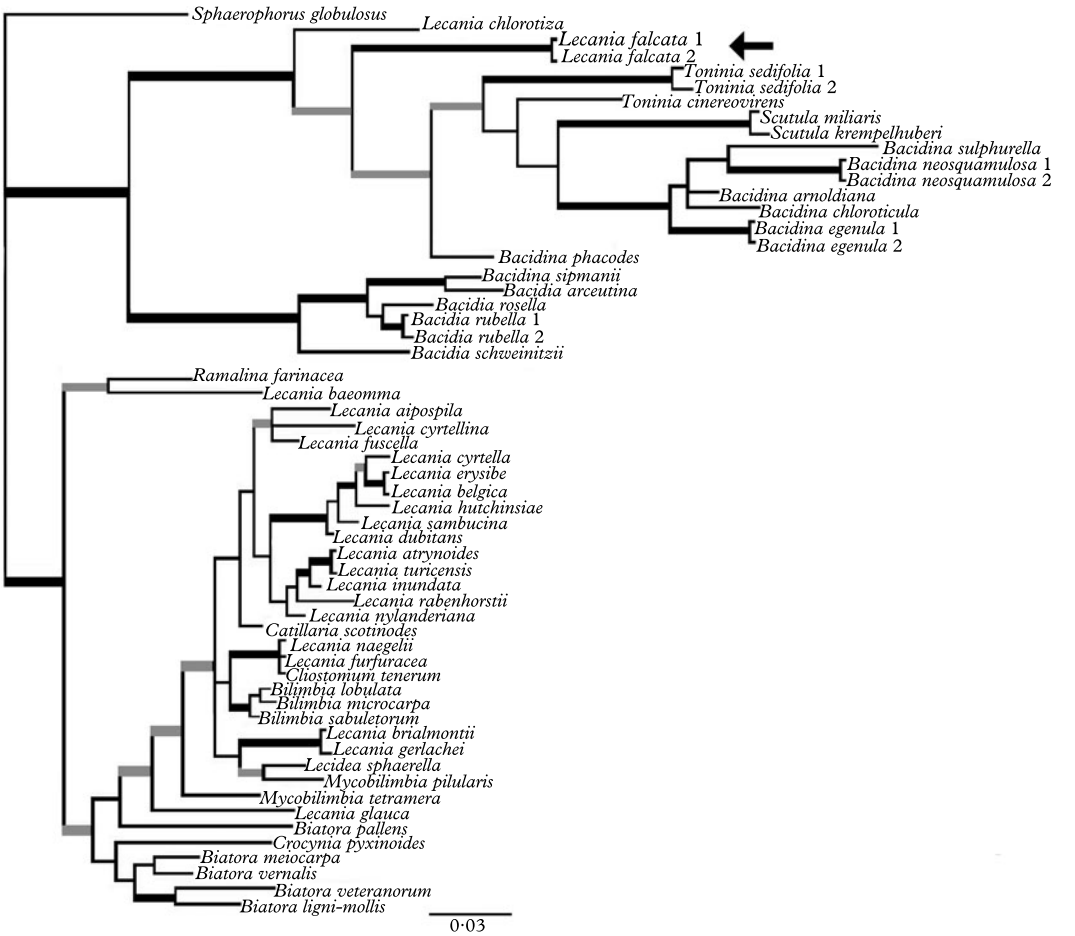

FIG. 2. One locus (mtSSU) 50% majority rule consensus tree produced by the Bayesian analysis and representing the phylogenetic relationships of *Lecania chlorotiza* and *L. falcata* sp. nov. (arrow) within the *Ramalinaceae* sensu lato. Branches supported by MPBS and MLBS > 75% and Bayesian posterior probabilities > 0·95 are in black; those supported only by Bayesian posterior probabilities > 0·95 are in grey.

Discussion

In a pioneering molecular study of the *Bacidiaceae*, Ekman (2001) pointed out that the relationships between this family and the *Ramalinaceae* were "definitely in need of scrutiny". Their close relationship was eventually demonstrated by Andersen & Ekman (2005) and by Reese Næsborg *et al.* (2007). Currently both families are united under the name *Ramalinaceae* C. Agardh (=*Bacidiaceae* W. Watson), which encompasses 38 genera (Lumbsch & Huhndorf 2010). Inclusion of the *Crocyniaceae*, as proposed by Miądlikowska *et al.* (2006), and of the *Megalariaceae*, as proposed by Schmull *et al.* (2011), would add four other genera. Most of those genera have never been studied within a molecular phylogenetic context, and further their relationships with the *Pilocarpaceae* Zahlbr. are not settled. An example is *Eugeniella* Lücking *et al.*, a recent segregate of corticolous and foliicolous species around the neotropical *Bacidia psychotriae* (Müll. Arg.) Zahlbr., which was assigned to the *Pilocarpaceae* (Lücking 2008) and eventually trans-

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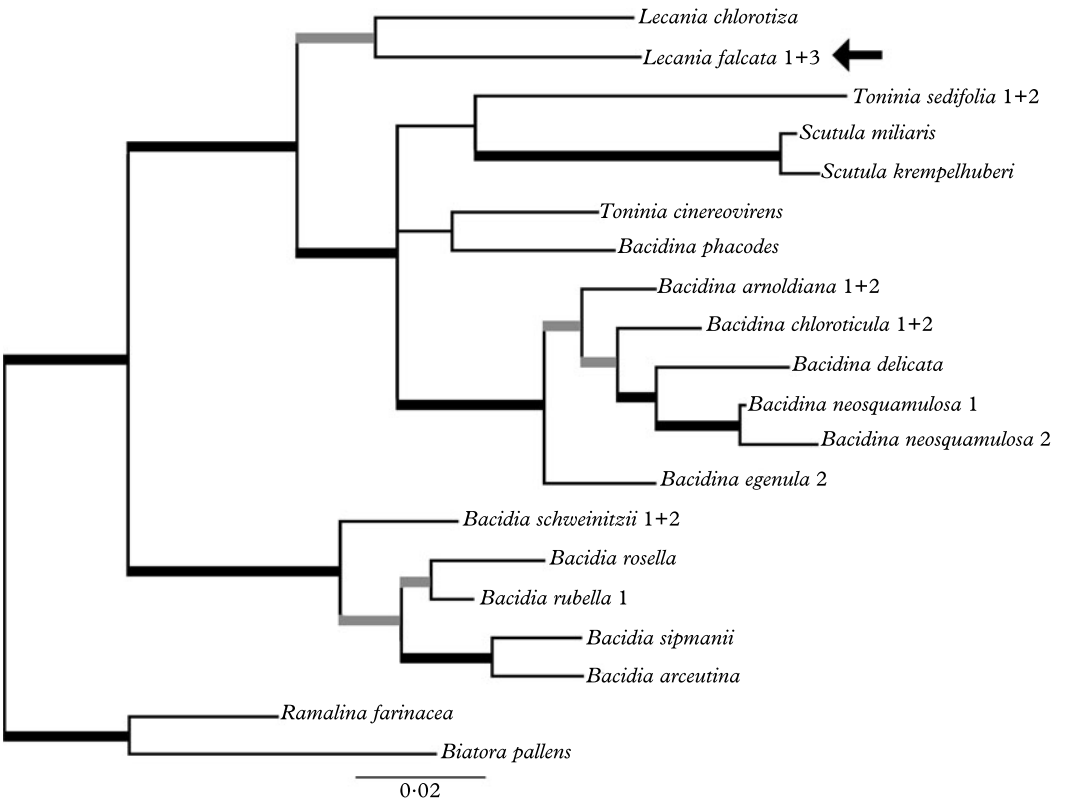

FIG. 3. Three loci (nuLSU, nuITS and mtSSU) 50% majority rule consensus tree produced by the Bayesian analysis and representing the phylogenetic relationships of *Lecania chlorotiza* and *L. falcata* sp. nov. (arrow) within the *Bacidiaceae sensu stricto*. Branches supported by MPBS and MLBS > 75% and Bayesian posterior probabilities > 0·95 are in black; those supported only by Bayesian posterior probabilities > 0·95 are in grey.

ferred to the *Ramalinaceae* without new data or analysis (Lumbsch & Huhndorf 2010). The present study does not contradict the concept of the *Ramalinaceae* as circumscribed by Lumbsch & Huhndorf (2010), but provides further support for a clade comprising *Bacidia, Bacidina, Scutula, Toninia*, *Lecania chlorotiza* and *L. falcata* sp. nov., thus representing the *Bacidiaceae* s. str.

The genus *Lecania*, as currently circumscribed in floras (van den Boom & Ryan 2004; Smith *et al.* 2009), is again demonstrated as polyphyletic, and the clade referred to *Lecania* s. str. by Reese Næsborg *et al.* (2007) is resolved outside of the *Bacidiaceae* s. str. (Fig. 2). The discovery of an undescribed species, close to *Lecania chlorotiza*, and the analysis of its phylogenetic position inferred from mtSSU sequences, did not detect a well-supported clade for them within the *Bacidiaceae* s. str. Indeed, only the two species currently assigned to *Scutula*, and the species assigned to *Bacidia* (including the type species *B. rosella*) are strongly supported, as shown by Ekman (2001) with a much larger species sampling but on the basis of ITS sequences only. Furthermore, all species representing *Bacidina* are resolved as a strongly supported group which does not include the type species of the genus (*B. phacodes*). Our results (Fig. 3), however, do not exclude the possibility that the latter actually belongs to that genus.

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The phylogenetic tree inferred from three loci sequences provided some support for the recognition at genus level for *L. chlorotiza* and *L. falcata* sp. nov. (Fig. 3). Indeed, the Bayesian analysis could detect strong support for a clade comprising these two taxa. Absence of such support in the other two optimization analyses (maximum parsimony and maximum likelihood) may be due to an incomplete dataset, the latter being very sensitive to missing data (Simmons 2012). With the existing data, no genus name published seems to be available. We refrain from describing a new genus for these two species as most genera currently assigned to the *Ramalinaceae* by Lumbsch & Huhndorf (2010) have never been assessed with molecular data and modern statistical optimization methods. Analysis of a much larger sampling throughout the whole family *Ramalinaceae* (including *Bacidiaceae*) is therefore needed before such a decision can be taken. A comprehensive and fully supported generic classification of many crustose species with tiny ascocarps remains a long-term goal (see the case of *Lecidea rubrocastanea* in Spribille & Printzen 2007).

The Species

In this section, we refer to Coppins (1983) and van den Boom & Brand (2008) for the description and terminology of conidia (microconidia, mesoconidia, macroconidia and leptoconidia). In particular, the term macroconidia refers here to slightly to strongly curved, 0–3-septate and 1·0–3·0 μm wide conidia, whereas the term leptoconidia refers to filiform, often curved, non-septate, and 0·6–1·0 μm wide conidia.

Lecania chlorotiza (Nyl.) P. James

in Coppins, James & Hawksworth, *Lichenologist* **24:** 367 (1992). Basionym: *Lecidea chlorotiza* Nyl., *Flora* **49:** 85 (1866); type: England, "ad corticem ulmi prope Clifton in Anglia (Larbalestier, 1865)" (H-Nyl!)

(Fig. 1A & B, E)

Thallus never larger than 1–2 cm across, diffuse, mostly developed in fissures of the bark, greenish to greenish brown or grey, thin, rather granulose, made of small granules (50–100 μm diam.), rarely smooth, without cortex, nor prothallus. *Photobiont* belonging to the *Chlorococcaceae*, cells 5–9 μm, thin-walled, without visible haustoria but closely surrounded by hyphae.

Apothecia rarely abundant, up to 0·30–0·45 mm diam., and 0·10–0·14 mm high, pale orange, sometimes with the disc more orange than the margin, with constricted base, flat when young and slightly to distinctly convex when mature. *Excipulum* 30–50 μm thick, chondroid, made up of radiating and connected thin hyphae, with marginal cells slightly but distinctly broadened (up to 3·5–4·5 μm); *hymenium* 25–32(–35) μm; *paraphyses* simple, 1·0–1·5 μm thick, with apical cell slightly broadened (up to 2·5 μm); *asci* 8-spored, 20–28 μm long, clavate, of the *Bacidia*-type (with rather broad and rounded axial mass, and not a narrow cone as in *Bacidia rosella*; see Fig. 2 in Hafellner 1984: 261); *ascospores* narrowly ellipsoid, 1-septate (rarely simple), 10·3–11·1 × 1·9–2·4 μm.

Pycnidia of two types: 1) abundant, immersed in thallus or emergent, 60–120 μm diam. and 40–100 μm high; wall 10–20 μm thick, of conglutinated hyphae; conidiogenous cells parietal, closely packed, *c.* 8·0 × 1·8 μm; mesoconidia bacilliform, simple, with rounded ends, 4·3–5·1 × 1·0–1·5 μm; 2) not common, very small (20–25 μm diam.), completely immersed in the thallus; microconidia narrow, curved, 8·4–9·6 × 0·6 μm.

Chemistry. No crystals or pigment observed (Meyer & Printzen 2000); no lichen substances detected by microcrystallizations (Huneck & Yoshimura 1996).

Ecology and distribution. On shaded and base-rich bark, sometimes inside hollow trunks, or under well-developed aerial roots, reported on *Ulmus, Fraxinus, Salix* and old *Quercus* in Smith *et al.* (2009). Reported by the same authors from throughout Britain, France, Denmark, Norway and Spain, and also from Portugal by van den Boom (2005)

and Luxembourg by Ertz *et al.* (2008). The species thus has an Atlantic distribution in Europe and is not known elsewhere.

Selected specimens examined. **Great Britain:** *England*: **V.C. 3,** South Devon: Dartmoor, 6 km NW of Bovey Tracey, 240 m, old *Fraxinus* at edge of garden, sheltered, 1996, *A. M. Brand* 35146 (hb. Brand); *ibid.*, 15 km W of Exeter, Teign Valley, 1 km W of Steps Bridge near foot bridge, 100 m, base of old *Quercus*, 1996, *A. M. Brand* 35163 (hb. Brand); *ibid.*, Buckland, valley of River Webburn, 90 m, old *Quercus* at stream in wooded valley, 1996, *A. M. Brand* 35358 (hb. Brand). **V.C. 4,** North Devon: Abbeyford woods, on trunk of *Fraxinus*, 4 iii 2010, *B. J. Coppins* s. n. (LG). *Scotland*: **V.C. 104,** North Ebudes: Eigg, on *Ulmus*, 2000, *B. J. Coppins* 19211 (E).—**France:** *Dépt. Pyrénées Atlantiques*: SE of Arthez, 370 m, on *Quercus* at shore of stream (on limestone), 2003, *A. M. Brand* 48402 (hb. Brand).—**G. D. Luxembourg:** *Gutland*: Vogelsmühle, vallon du Halerbaach, sur *Fagus* dans une chênaie-hêtraie, 2000, *P. Diederich* 14028 & *J. M. Cepeda* (hb. Diederich, LG).—**Portugal:** *Alto Douro*: W of Villa Real, Amarante, N-slope with *Pinus* and *Quercus* wood, roots of *Quercus* under overhang, N face, 1999, *P. van den Boom* 22947 (hb. van den Boom, LG).

Lecania falcata van den Boom, Brand, Coppins, Magain & Sérus. sp. nov.

MycoBank No: MB 564696

Similis speciei *Lecania chlorotiza* sed cum conidiis majoribus et valde curvatis vel sigmoideis.

Typus: Canary Islands, Tenerife, Puerto de la Cruz, 'Jardin Botanico', 100 m, N 28°24·58′W 16°32·15′, 100 m, on *Erythrina corallodendron*, 14 May 2007, *P. & B. van den Boom* 37916 (LG—holotypus; E, hb. v.d. Boom—isotypi).

(Fig. 1C, D & F)

Thallus forming large thalli, covering several dm^2 in the type locality, but obviously less exuberant in natural conditions, diffuse, on bark of living trees or on dead standing wood, vividly green when living and moistened, or greyish or yellowish brown, pale green, thin to rather thick (0·2–0·3 mm thick), made of tiny granules (50–150 µm diam.) sometimes aggregated and thus forming tiny scurfy to eroded squamules up to 200–250 µm across, without cortex or prothallus. *Photobiont* belonging to the *Chlorococcaceae*, cells 5–10 µm, thin-walled, in rounded aggregates, without visible haustoria but closely surrounded by hyphae.

Apothecia abundant, 0·3–0·5 mm diam., and 0·13–0·20 mm high, pale orange to pale brownish, with constricted base, flat when young and slightly to distinctly convex when mature, sometimes becoming aggregated or tuberculate and thus somewhat deformed, margin slightly prominent in young apothecia, becoming excluded in older ones. *Excipulum* 40–50 µm thick, chondroid, made of radiating hyphae, with rather large cells (3–4 µm) and marginal cells identical; *hymenium* 40–45 µm; *paraphyses* simple, *c.* 1·5 µm thick, with apical cell slightly broadened (up to 2·5 µm); *asci* 8-spored, 30–37 µm long, clavate, of the *Bacidia*-type (with rather broad and rounded axial mass, and not a narrow cone as in *Bacidia rosella*); *ascospores* narrowly ellipsoid, 1-septate, 10·3–11·7 × 2·3–2·5 µm.

Pycnidia of two types: 1) sometimes abundant, immersed in thallus or emergent, rounded, 140–160 µm diam., 40–100 µm high; wall 7–10 µm thick, of conglutinated hyphae; conidiogenous cells parietal, closely packed, 6–8 × 1·8–2·1 µm; macroconidia rather polymorphic but of a single type, long, strongly and repeatedly curved sigmoid, tapering towards proximal ends, simple or 1(–2)-septate, 12·3–26·4 × 1·0–1·2 µm; 2) very rare, immersed in the thallus, less than 50 µm diam.; microconidia narrow, curved, 8·5–12·0 × 0·6–0·8 µm.

Chemistry. No crystals or pigment observed (Meyer & Printzen 2000); no lichen substances detected by microcrystallizations (Huneck & Yoshimura 1996).

Taxonomic notes. Lecania falcata can be distinguished from *L. chlorotiza* by several characters: 1) bacilliform mesoconidia never produced but long and strongly curved macroconidia always found; 2) excipulum without distinctly swollen outer cells; 3) hymenium slightly higher (40–45 µm vs. 25–35 µm in *L. chlorotiza*), and asci longer (30–37 µm vs. 20–28 µm in *L. chlorotiza*). The thallus of *Lecania falcata* is also better developed and more conspicuous, with granules and minute, ±eroded squamules in optimal conditions.

Ecology and distribution. *Lecania falcata* is certainly a rare species. It is known from two natural, prestigious sites (NE of La Palma and the Foz de Arbayún in Navarra), and

in two botanical gardens (Tenerife in the Canary Islands and San Miguel in the Azores). It is abundant in the botanical garden of Puerto de la Cruz on the northern side of Tenerife but was carefully looked for, in vain, in both large stands of laurisilva on the same island, during further field excursions in 2009 and 2010. Interestingly, it was found in similar ecological conditions in the Azores archipelago, on the island of San Miguel. It further occurs in the most diverse laurisilva in the NW of the rather young island of La Palma in the Canary Islands, an area where many new and rare lichen species have also been detected, such as *Byssoloma kalbii, Gyalectidium membranaceum, Porina ocoteae* and *Strigula brevis* (Sérusiaux *et al.* 2007). In continental Spain (Navarra), it occurs in the most famous gorge on the southern flank of the Pyrenees, the Foz de Arbayún, where other interesting species are reported, such as *Megalaria grossa, M. laureri, Phaeographina buxi* (on twigs on *Buxus*), *Porina rosei*, and two species of the mainly foliicolous and tropical genus *Gyalectidium*, *G. setiferum* (Sérusiaux 1993) and *G. puntilloi* (Ferraro *et al.* 2001).

Additonal specimens studied. **Portugal:** *Azores*: San Miguel, Ponta Delgada, jardín José do Couto, on trunk, 2007, *J. Etayo* 24089 & *E. Ros* (hb. Etayo).—**Spain:** *Canary Islands*: Tenerife, Puerto de la Cruz, 'Jardin Botanico', same locality as the type, also found on other trees such as *Chrysophyllum* sp. or on the 'trunk' of *Livistona australis*, 14 v 2007 (hb. van den Boom 37917 and 37914); *ibid. P & B. van den Boom* 37915 (hb. v.d. Boom); same locality as the type, abundant on *c.* 30 tree boles, v 2010, *E. Sérusiaux* s. n. (LG, E, hb. Brand, hb. van den Boom); La Palma, 3·5 km WSW of Los Sauces, N-slope of Barranco del Agua, 530 m, on wood of dead *Ocotea foetens* in laurisilva, 1986, *A. M. Brand* 13600 (hb. Brand). *Navarra*: Foz de Arbayún, le long du Rio Salazar, 550 m, fourrés de *Buxus* et futaie à *Quercus rotundifolia* sur le flanc gauche des gorges, sur *Quercus*, 12 vii 1989, *E. Sérusiaux* s. n., *P. W. James, R. Rose & J. Etayo-Salazar* (LG); *ibid.*, 500 m, on bark, 1993, *J. Etayo & P. van den Boom* 16549 (hb. v.d. Boom).

Further notes

Albeit belonging to a different and strongly supported clade in molecular phylogenetic analysis, *L. chlorotiza* and *L. falcata* do not feature clear and diagnostic morphological or anatomical differences with *Lecania* s. str. (*sensu* Reese Næsborg *et al.* 2007). In most species of *Lecania* s. str., a thalline margin is clearly visible in young apothecia, but several species (especially *L. hutchinsiae* and *L. sylvestris*) have apothecia with an evanescent margin. The latter two species, however, feature characters distinct from those of both *L. chlorotiza* and *L. falcata*: thallus smooth or almost so, sometimes with scattered granules, hamathecium with conglutinated paraphyses, sometimes sparingly branched and apical cells enlarged to 2·5–5·0 µm, often pale but sometimes dark brown pigmented, and ascospores wider (3·0–4·5 µm vs. 2·3–2·5 µm in *L. chlorotiza* and *L. falcata*). Furthermore, in *Lecania* s. str. (*sensu* Reese Næsborg *et al.* 2007), conidia (when produced) most usually belong to the leptoconidia-type (*sensu* van den Boom & Brand 2008): slighty curved, non-septate, *c.* 10–25 × 0·8–1·0 µm (van den Boom 1992; Smith *et al.* 2009). A remarkable exception is *Lecania cyrtellina*, a species resolved with strong support in *Lecania* s. str. and which produces macroconidia crescent-shaped, 0–1-septate, 10–15 × 1·5–2·0 µm.

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