Coscinocladium, an overlooked endemic and monotypic Mediterranean lichen genus of Physciaceae, reinstated by molecular phylogenetic analysis

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The position of the sterile western Mediterranean crustose-placodioid lichen generally known as *Lecanora lisbonenesis* has been investigated using mitochondrial SSU rDNA sequence data. It proves to belong to an independent genus of Physciaceae, for which the generic name *Coscinocladium* is available, and the earliest species name is *Variolaria gaditana*. Previous reports of apothecia in the species prove to be a result of mixtures with other lichen species. A lectotype is selected for *L. lisbonensis*, a neotype designated for *V. gaditana*, and the new combination *Coscinocladium gaditanum* made. The species is described, illustrated, its ecology discussed, and a distribution map provided.

KEYWORDS: Ascomycota, biogeography, *Buellia*, Europe, *Lecanora*, Lecanorales, *Mobergia*.

INTRODUCTION

The systematic placement of lichenized fungi in which no sexual stage is known has traditionally been based on thallus structure and form, supplemented by secondary chemistry. Numerous sterile and often widespread lichens have been placed in genera characterized by a particular sexual stage using these types of characters (e.g., Coppins & James, 1979). In some cases, subsequently discovered ascomata have led to placements being confirmed, or found to be erroneous. As lichenized fungi are wisely exempted from Art. 59, the option for a dual nomenclature is closed. Molecular methods, however, mean that as in other fungi for which no sexual stage is known, totally sterile lichenized species can be unequivocally placed in families or genera whose members have sexual stages (e.g., Arup & Grube, 1999; Ekman & Tønsberg, 2002).

This paper addresses the nomenclature and placement of a sterile crustose-placodioid lichen with a whitish, pruinose and sorediate thallus. The lichen is locally frequent on somewhat soft calcareous rocks in the western Mediterranean, and is currently generally referred to as *Lecanora lisbonensis* G. Samp. (Nimis, 1993; Llimona & Hladun, 2001; Fig. 1). The species was placed in *Lecanora* Ach. (Lecanoraceae) because of reported apothecia with simple colourless ascospores described by Sampaio (1921), but has also been referred to *Placodium* auct. non (Ach.) DC. (Lecanoraceae) as the thallus was placodioid by Klement (1965), and to *Buellia* De Not. (Physciaceae) by Werner (1976) who considered he had fertile material with brown muriform ascospores. In addition, the monotypic genus *Coscinocladium occidentale* Kunze (Kunze, 1846a) and the species name *Variolaria gaditana* Clemente (Clemente, 1807; Fig. 2) have been suggested to belong to the same species, notably by Tavares (1956, 1958). De Notaris was evidently shown material by Kunze, and concluded that this lichen belonged to an unpublished genus; Kunze accepted De Notaris' view and coined the generic name *Coscinocladium*. De Notaris added: "Botanicis occiden-

Fig. 1. *Coscinocladium gaditanum* (MAF 9855 - neotype).

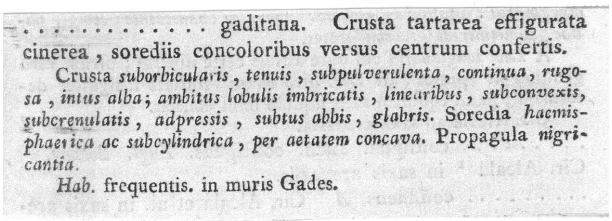
. . . . . gaditana. Crusta tartarea effigurata cinerea, sorediis concoloribus versus centrum confertis.
Crusta *suborbicularis, tenuis, subpulverulenta, continua, rugosa, intus alba; ambitus lobulis imbricatis, linearibus, subconvexis, subcrenulatis, adpressis, subtus abbis, glabris.* Soredia *hacmisphaerica ac subcylindrica, per aetatem concava.* Propagula *nigricantia.*
Hab. frequentis. in muris Gades.

Fig. 2. Original description of *Variolaria gaditana* (Clemente, 1807: 295).

talis hauc plantam lichenosam, accuratus observandam commendo"[1] (in Kunze, 1846a, b). However, these early species names were not taken up by later workers because of either uncertainties over their typification, or because a combination of Kunze's epithet into *Lecanora* was precluded by the existence of the name *L. occidentalis* (Lynge) Lynge (Lynge, 1940).

We endeavoured to follow De Notaris' admonishment 157 years later through collecting and studying fresh material of this taxon, examining its phylogenetic position by molecular methods, and also investigating and typifying the pertinent names suggested to belong to the species. As a result, we show that the species represents a hitherto unrecognised genus of Physciaceae, we fix the application of relevant species names by neo- and lectotypification, and we determine that the binomial that should be applied under the *Code* is *Coscinocladium gaditanum*.

MATERIALS AND METHODS

Taxa sampled. — For this study we used material or sequences from collections named as *Lecanora lisbonensis* (i.e., *Coscinocladium gaditanum*) and several other samples from different taxonomic groups. 19 specimens were collected specifically for DNA isolation, amplification and sequencing for this study, while sequences from 24 other taxa were downloaded from GenBank. Full details of the specimens and GenBank accession numbers are included in Appendix 1 and 2 (see online version of *Taxon*). Sequences from the mtSSU were used for estimating the taxonomic position of the target samples. Then, in order to identify the closest relationships further, nuclear ITS rDNA was also used to analyse relationships with the most similar sequences resulting from a BLAST search.

DNA extraction and PCR amplification. — Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen), with minor modifications as described by Crespo & al. (2001). Mitochondrial DNA amplification was undertaken with the primers NMS1 and NMS2 (Li & al., 1994) and SSU1 and SSU3R (Zoller & al., 1999), and nuclear ITS rDNA with ITS1F (Gardes & Bruns, 1993) and ITS4 (White & al., 1990).

Each PCR reaction contained the following ingredients: 29.75 µl dH_2O, 5 µl 10X PCR buffer where the $MgCl_2$ 2 mM was already included (Tris-HCl 75mM pH 9.0; KCl 50 mM; $(NH_4)_2SO_4$ 20mM), 1 µl dNTP 10 mM, 2.5 µl of a 10 µM dilution of each of the primers and 1.25 µl DNA Polymerase (1 unit/µl, Biotools). This cocktail was mixed with 8 µl of the DNA template. The PCR amplification for the mitochondrial gene ran 35 cycles: denaturation at 94ºC for 60 s, annealing at 57–58ºC for 60 s, and extension at 72ºC for 90 s. The reaction was carried out in an automatic thermocycler (Hybaid OmniGene). The PCR amplification of the ITS region ran 30 cycles: denaturation at 94ºC for 60 s, annealing at 54ºC for 60 s, and extension at 72ºC for 90 s. This reaction was carried out in an automatic thermocycler (Techne Progene).

The PCR products were purified through a Biotools Bioclean DNA Purification column kit, according to the manufacturer's specifications. Sequencing was performed on both strands using the ABI PRISM™ Dye terminator cycle Sequencing Ready Reaction kit (Applied Biosystems), with the PCR primers. The following cycling profile was used: denaturation for 3 min at 94ºC, 25 cycles of 10 s at 96ºC, 5 s at 50ºC and 4 min at 60ºC. The products for sequencing were electrophoresed on an ABI PRISM 377 DNA sequencer (Applied Biosystems).

Sequence analysis. — Sequences were compared with the assistance of Windows SeqMan (DNAStar) to check for reading errors, and, when possible, to resolve ambiguities. Sequences of SSU and ITS contain segments that are very variable. Since standard multiple alignment programs, such as Clustal (Thompson & al., 1994), become less reliable when sequences are highly divergent, we used an alignment procedure employing a linear Hidden Markov Model (HMM) as implemented in the software SAM (Hughey & Krogh, 1996; http://www.cse.ucsc.edu/research/compbio/sam. html). Regions that were not aligned with statistical confidence were excluded from the subsequent phylogenetic analysis.

The data were analysed using a Bayesian approach (Larget & Simon, 1999; Huelsenbeck & al., 2000). Posterior probabilities were approximated by sampling trees using a Markov Chain Monte Carlo (MCMC) method. The posterior probabilities of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. The

[1] *Transl.: Western [European] botanists are commended to study in more detail this lichen plant.*

program MRBAYES 2.01 (Huelsenbeck & Ronquist, 2001) was employed to sample the trees, and the analysis was performed assuming the general time reversible model (Rodriguez & al., 1990), including estimations of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) for the combined analyses. A run with one million generations starting with a random tree and employing 12 simultaneous chains for mitochondrial and eight for ITS sequences was executed; every 100th tree was saved into a file. We used the default settings for the priors on the rate matrix, branch lengths, gamma shape parameter, and the proportion of invariable sites. A Dirichlet distribution was assumed for the base frequency parameters, and an uninformative prior was used for the topology (default settings).

We plotted the log-likelihood scores of sample points against generation time, and considered that stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist, 2001). The initial 400 trees were discarded as burn-in before stationarity was reached. Using PAUP 4.0b10 (Swofford, 2002), majority-rule consensus trees were calculated from 9600 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Unlike nonparametric bootstrap values (Felsenstein, 1985), these are estimated probabilities of the clades under the assumed model (Rannala & Yang, 1996) and hence posterior probabilities equal to and above 95 are considered significant support. Phylogenetic trees were drawn using TREEVIEW (Page, 1996).

The polarity of the characters was assessed by outgroup comparisons using *Protoparmelia badia* and *Rhizoplaca bullata* in the mitochondrial tree. Due to its position as a member of the well-supported *Buellia* group in the mitochondrial tree, *Dirinaria applanata* was chosen as outgroup for the ITS rDNA region analysis.

RESULTS

Molecular studies. — MtSSU rDNA analysis. Insertions of varying lengths were present in *Dirinaria applanata* and *Heterodermia leucomela*. In addition, a small insertion of 14 bp was detected in *Lecanora muralis*, *Protoparmelia badia*, and *Rhizoplaca bullata*. A matrix of 704 aligned nucleotide position characters was used for the analysis, after 133 positions ambiguously aligned and all insertions were excluded.

Two major monophyletic groups were discovered (Fig. 3). The first comprises the three *Coscinocladium gaditanum* collections, and the species of *Anaptychia*, *Heterodermia*, *Phaeophyscia*, *Phaeorrhiza*, *Physcia*, *Physconia*, and *Rinodina* studied, together with *Buellia lindingeri* (99 posterior probability). And the second, groups *Amandinea*, *Calicium*, *Cyphellium*, *Diplotomma* (incl. *Diploicia*), *Dirinaria*, *Pyxine*, *Tholurna*, *Texosporium*, and all other *Buellia* species studied (100 pp).

Within the *Physcia* group, a well-supported monophyletic group includes the two species of *Anaptychia* as the sister group of a branch where *Phaeorrhiza sareptana* is basal in relationship with the three samples of *Physconia*.

Within the *Buellia* group, a well-supported clade joins *Diplotomma canescens* as the basal sister group of *Dirinaria applanata*, which is also the basal sister group of the three species of *Pyxine*.

Nuclear ITS rDNA sequences analysis. Sixteen samples, including *Mobergia calculiformis* and *Physcia tenella* var. *maritima*, the most similar sequences in the BLAST search, were included in the analysis; also other sequences belonging to other taxa in the *Physcia* group clade were included (see Fig. 3). A matrix of 526 nucleotides was obtained, of which 72 ambiguous positions were excluded in the analysis.

Three main groups were found (Fig. 4). Although without significant support (85 pp), *Coscinocladium gaditanum*, *Mobergia calculiformis* and *Rinodina sophodes* grouped in the same clade. *C. gaditanum* and *M. calculiformis* samples formed two independent monophyletic clades, in both cases with 100 pp.

Morphological studies. — Of the 11 collections under the name *Lecanora lisbonensis* in PO, the thalli of all conformed to that of *Coscinocladium gaditanum* as typified here. Three were found to have *Lecanora*-type apothecia intimately mixed with the sterile lichen, pushing between cracks in the placodioid lobes (PO 991L, 1563L and 2317L); all three have detailed annotations and sketches in Sampaio's hand, with dimensions of apothecia and ascospores conforming to those in the published account (Sampaio, 1921). The apothecia did not arise from a well-defined thallus and none were found to originate in the placodioid lobes. Squash mounts were made in water, and all three have ascospores 10.5–12 × 4.5–5(–6) µm and have thalline and apothecial characters which conform to *L. flotowiana* Spreng., as understood by Fröberg (1997). However, we recognize that the spore dimensions are in the upper end of the range given by Foucard (1990; 7–14 × 3–7 µm) and Purvis & al. (1992; (7–)8.5–14 × (3–)4–7 µm) as regards length in the *L. dispersa* group, to which *L. flotowiana* belongs, and that the complex is yet to be studied critically in the Mediterranean region.

The rare apothecia reported by Werner (1976) were quite different from those of *L. flotowiana*. According to Werner's original notes and drawings, which are now held by X. L., the collection he thought had apothecia

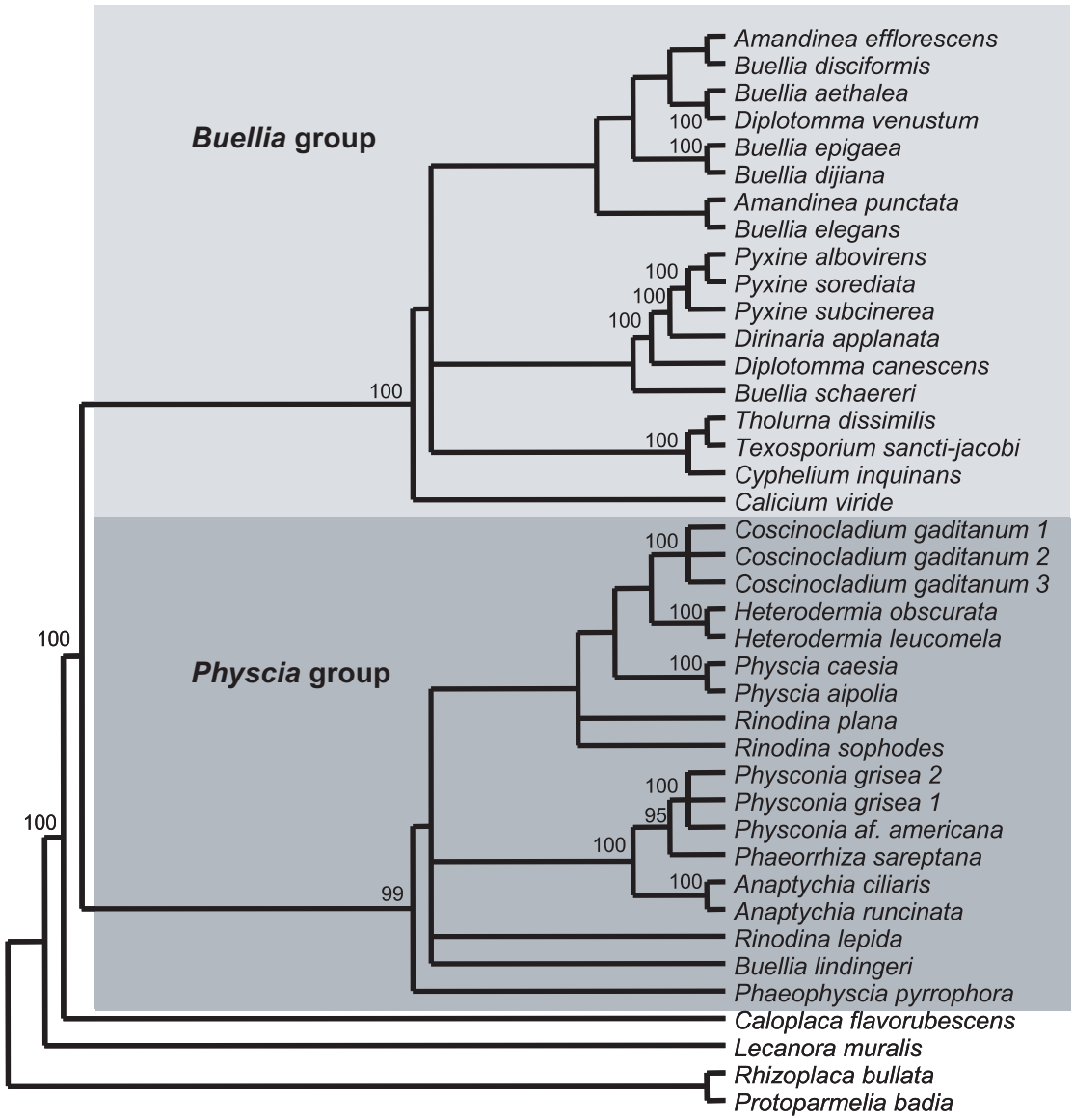

Fig. 3. Mitochondrial SSU rDNA majority-rule consensus tree based on 9600 trees from a B/MCMC tree sampling procedure. Posterior probabilities equal to or above 95 are given above the branches.

was from Rabat, and we located and examined this material (Morocco, Rabat, en contre-bas de l'hopitâl Marie-Feuillet sur sables agglomérées, avec *Caloplaca aurantia*, 8 April 1933, leg. R. G. Werner, BC-Werner); the notes, measurements and drawings Werner made correspond so closely to those he published that there is no doubt this is the collection on which he based his conclusion that the species belonged in *Buellia*. The specimen consists of a whitish thallus partially overgrown by *C. gaditanum*. We were unable to find any apothecium, but Werner's description of the apothecia, paraphyses and ascospores leads us to consider this may well be the species generally known in the Iberian peninsula as *Diplotomma ambiguum* (Ach.) Flagey or a closely related species, and not a lichenicolous fungus. Werner's transfer of Sampaio's epithet was therefore evidently based on this mixed collection. We note that the name *D. ambiguum* was placed as a synonym of *D. alboatrum* (Hoffm.) Th. Fr. by Nordin (2000), but we feel that his treatment may have been too broad.

Werner (1976) also mentioned pycnidia in a different collection of *C. gaditanum* (as "*L. lisbonensis*") which we also located and examined (Morocco, "Sidi Moussa, rochers maritimes calcaires, à 500 m de l'océan", Jan. 1932, leg. J. Gattefossé, BC-Werner). These seem to be genuine pycnidia of the species; according to Werner's drawings they are immersed, globose, with pleurogenous conidiogenous cells (Vobis, 1980: type VI) and cylindri-

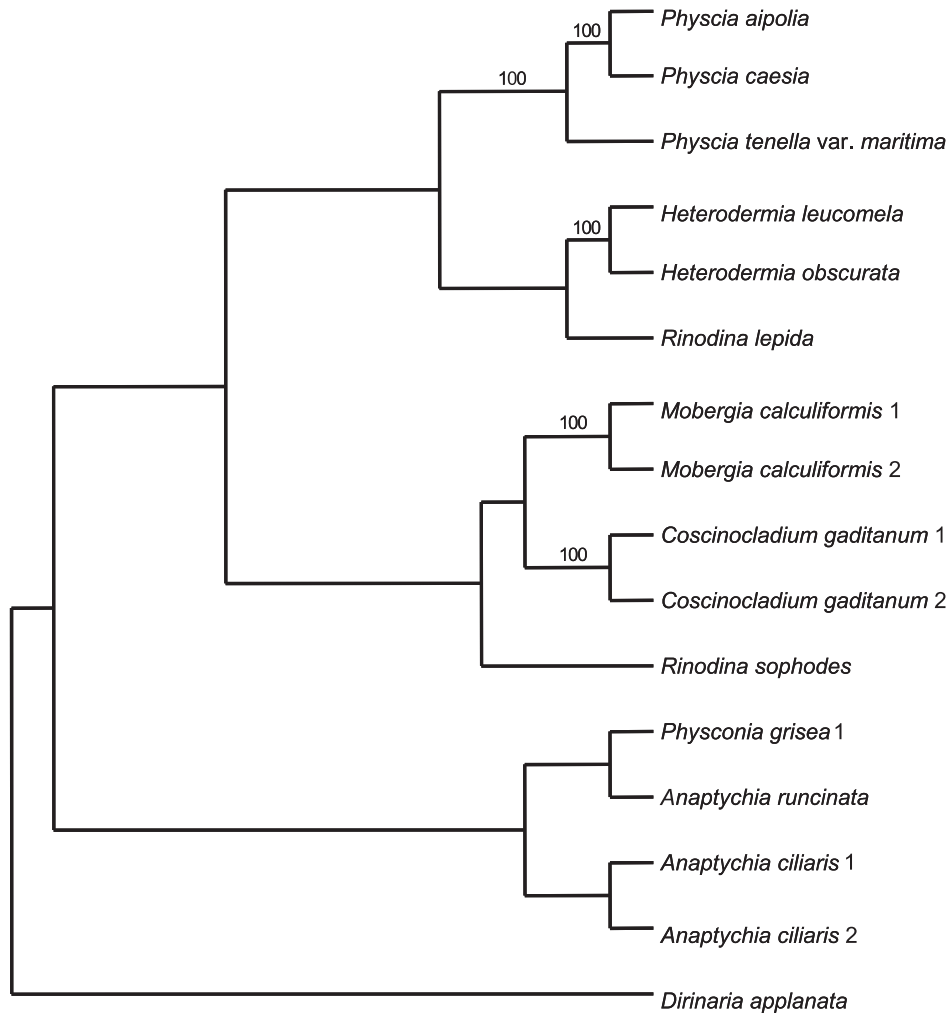

Fig. 4. Nuclear ITS rDNA majority-rule consensus tree based on 9600 trees from a B/MCMC tree sampling procedure. Posterior probabilities equal to or above 0.95 are given above the branches.

cal conidia he measured as 3.8–5 × 1.3 µm. These structures are very similar to those known in *Physcia* and *Physconia* (Vobis, 1980), and consistent with the relationships that emerged from our molecular studies.

TAXONOMY

Coscinocladium Kunze, Flora 29: 768. 1846.
Type species: *C. occidentale* Kunze [= *C. gaditanum* (Clemente) A. Crespo, Llimona & D. Hawksw.].

Coscinocladium gaditanum (Clemente) A. Crespo, Llimona & D. Hawksw., **comb. nov.**

≡ *Variolaria gaditana* Clemente, Ens. Veg. Andalucia: 295. 1807 [basionym]. – Neotypus (***hic designatus***): Spain, Andalucía, Cádiz, Calle Honduras, on walls of the old city, 15 Feb 2003, leg. A. Crespo, C. Bencomo & J. F. de Bobadilla (MAF 9855).

≡ *Pertusaria communis* var. *gaditana* (Clemente) Colm., Enum. Rev. Pl. Hisp.-lusit. 5: 833. 1889.

= *Coscinocladium occidentale* Kunze, Flora 29: 768. 1846. – Lectotypus (***hic designatus***): [Spain, Andalucía, Cádiz, 1844/45], "pl. Willkomm 987" (UPS L-82639).

= *Ricasolia cesatii* [var.] γ *plumbea* Bagl., Nuovo Giorn. Bot. Ital. 11: 70. 1879. – Holotypus: Italy, Sardinia, leg. Moris (destroyed fide Tavares, 1958).

≡ *Solenopsora cesatii* var. *plumbea* (Bagl.) Zahlbr., Cat. Lich. Univ. 5: 755. 1928.

= *Lecanora lisbonensis* G. Samp., Brotéria, sér. Bot. 19: 33. 1921. – Lectotypus (***hic designatus***): Portugal, [Estremadura], S. Martinho do Porto, rochedos marítimos, 12 Oct. 1917, leg. A. Ricardo Jorge (PO 1567L).

≡ *Psoroma lisbonense* (G. Samp.) G. Samp., Lich. Port. Exs. No. 85. 1923.
≡ *Placodium lisbonensis* (G. Samp.) Klem., Nova Hedwigia 9: 488. 1965.
≡ *Buellia lisbonensis* (G. Samp.) Werner, Bull. Soc. Bot. France 123: 438. 1976.

Etymology. — The generic name is based on the Greek words "*kóskinon*" (sieve) and "*kládos*" (branch). The name is spelled as "*Coscinacladium*" in several publications (e.g., Poelt, 1969; Nimis & Poelt, 1987) but we see no compelling orthographic reason to replace the "*o*" of the original publication. See below for the origin of the specific epithet "*gaditanum*".

Description. — *Thallus* crustose-placodioid, orbicular, forming rosettes 1–2(–3) cm diam, often confluent and forming extensive patches, frequently overgrowing other lichens, usually abundantly pruinose, whitish grey to almost white, with a bluish tinge around and in the central part. *Peripheral lobes* usually well-differentiated, 1–2(–3) × 0.5–1 mm, contiguous to laterally subimbricate, tips only slightly broadened, covered by a thick, white, coarsely crystalline pruina, the pruina sometimes poorly developed towards the apices and then with a whitish grey colouration. *Central parts* of the thallus irregular and often uneven, with minute fissures delimiting convex areoles. *Soralia* arising on the lobe surfaces, starting to burst out at the base of the lobes as protruding groups of soredia, later becoming well-circumscribed, discrete, not confluent even where they are denser in the older more central parts of the thallus, 0.2–0.25(–0.32) mm diam, sometimes empty of soredia (most probably after periods of heavy rain) and then appearing as concave circular depressions. *Soredia* spherical, 50–55(–90) µm diam, lead-grey to brownish grey, surface smooth, covered with thin pruina. *Cortex* covered and interspersed with coarse crystals, composed of hyphae arranged perpendicular to the surface with the upper 1–3 cells brownish and ca 3.5 µm wide, the soredia also covered with a layer of brownish angular cells individually 6–10 µm diam covered by a variably developed layer of minute hyaline crystals. *Ascomata* not known. *Conidiomata* (reported by Werner 1976; see above) pycnidial, immersed, globose. *Conidiogenous cells* pleurogenous (Vobis, 1980: type VI). *Conidia* short-cylindrical, simple, hyaline, 3.8–5 × 1.3 µm[1].

Chemistry. — Thallus and medulla K-, C-, KC-, PD-, and I-. Zeorin has been detected by t.l.c. (G. Paz-Bermúdez, specimen annotations in PO). We have been able to confirm this, we have also found an unknown compound in material from the Empúres (L'Escala, Catalonia). The unknown substance belongs to RF classes 5–6 in Solvent A, 6 in B, and 7 in C (Elix & Ernst-Russell, 1993), has a yellow fluorescence under 365 nm UV-light. A yellow or dull yellow-red fluorescence was also evident under the same UV light in most intact thalli checked.

Illustrations. — Tavares (1956: 134, pl. 1, Figs 1–2), Martellos & Nimis (2003, in colour), Fig. 1.

Typifications. — The original locality for *Variolaria gaditana* given by Clemente (1807, Fig. 2) was "frequentis, in muris Gades". "Gades" is the latinized version of "Gadir", the Phoenician name for what is now the city of Cádiz (Andalucía), reputedly founded around 1100 BC and the oldest city in Europe (Williams, 2000). No original material could be located amongst Clemente's material in MAD, but what is certainly the same species still grows on the walls of the old city in Cádiz and we therefore designate a collection from the original locality as neotype here. We also note that the original place of publication of Clemente's name was incorrectly indicated by Zahlbruckner (1927–1928) to have been in Acharius (1814: 133), despite Acharius having correctly cited Clemente's publication.

Interestingly, Kunze (1846a) gave the original locality of *Coscinocladium occidentale* as "In urbe Gades ad muros et saxa arenosa marina copiose". Despite the similarity in the localities, habitat, and actual description, Kunze did not mention Clemente's name at all here nor in the subsequently separately printed version of the work (1846b). Kunze's herbarium in LZ was destroyed in World War II, but three collections of this species were sent by Kunze to E. M. Fries and are now preserved in UPS. R. Santesson was in no doubt that they were the same as the species called *Lecanora lisbonensis* in Portugal, and he sent them on loan to Tavares (1956) who concurred; we also agree that the three are conspecific. UPS (L-82445) does not have the name on the original label but is from "ad rupes mari propinq. pr. Gades leg. Willkomm Kze."; UPS (L-82639) is labelled only "*Coscinocladium occidentale* m. pl. Willk. 987 Hispan. Kze"; and UPS (L-74641) has the information "987(98) Coscinoclad. (scrips. Kunze!) *Coscinocladium occidentale* Kze! (spec. originale!) SüdSpanien, leg. Willkomm, comm. Auerswald" and is from the "Herb. Rel. W. v. Zwackh". However, in this last collection, only "987(98) Coscinoclad." is in Kunze's hand, the notation "987(89)" being the entry (and collection) numbers in Kunze (1846a, b). All three are on the same friable rock and are probably parts of a single original collection. We select as lectotype that which has the binomial in Kunze's hand as it is well-developed (five fragments) even though Gades is not mentioned specifically.

In the original account of *L. lisbonensis*, numerous Portuguese localities were mentioned by Sampaio (1921)

[1] *We did not see any pycnidia, and Werner's observations are in need of confirmation.*

with varying degrees of detail, but it is unclear to what extent these were actual collections or field observations. Eight collections in PO were listed as "syntypes" in the catalogue of Sampaio's lichen types in PO compiled by Paz-Bermúdez & al. (2002). We do not, therefore, repeat that information here, but note that by an oversight material distributed in Lich. Portugal No. 85, which was not collected until January 1922, was stated to be a syntype, and that the two collection numbers "991bL" and "991aL" in their paper should be corrected to PO 5608bL and PO5608a respectively. A total of 11 specimens remain under this name in PO, but none of the pre-1921 packets are from Lisbon or its immediate vicinity. As lectotype we select a collection definitely cited in the protologue, which is well-developed (six rock fragments, one of which has no lichen) and lacks intermixed *Lecanora* apothecia (see above).

Ecology. — Usually on porous, soft, calcareous rocks, including cemented sand or sandstones, more rarely terricolous or on more compacted calcareous or other alkaline rocks (e.g., metabasite). Also frequent on mortar, plasterwork, and tiles in old buildings and walls. Mainly along the coast, halotolerant on the seashore. It generally occurs with species of *Aspicilia*, *Caloplaca*, *Diplotomma*, *Lecanora* and (or) *Verrucaria*; examples of relevé including the species are presented in Table 3.

Distribution. — The currently known distribution (Fig. 5) is certainly incomplete, but follows the western Mediterranean coast and also the Atlantic coast from Portugal (Beira Litoral) to Morocco (near Safi). The eastern limit appears to be around Linosa and Lampedusa in southern Italy (Nimis, 2003). It reaches Macaronesia, with a single record from the Selvages Islands (Tavares, 1958).

Identification. — *Coscinocladium gaditanum* could be confused with five superficially similar crustose-placodioid lichens. (1) *Diplotomma canescens* (Dicks.) Flotow (syn. *Diploicia canescens* (Dicks.) DeNot.; see Molina & al., 2002) which has thicker, more robust thalli with better developed marginal lobes, a more greenish-grey colour, gradually coalescing (not persistently discrete) soralia, and which is K+ yellow. (2) *Caloplaca teicholyta* (Ach.) J. Steiner with ash-grey thalli, shorter and less well-demarcated lobes, the central parts of the thallus being occupied by minute isidia with no soralia. (3) *Aspicilia radiosa* (Hoffm.) Poelt & Leuckert which has a grey thallus, usually with brown apothecia, no soralia, and is K+ red. (4) *Solenopsora candicans* (Dicks.) J. Steiner, with a purer white thallus, lacking soredia and pruina, PD+ red, and usually with apothecia producing 1-septate hyaline ascospores. And (5) *Buellia zoharyi* Galun which is always on soil, frequently sterile, but with pure white K+ red lobes.

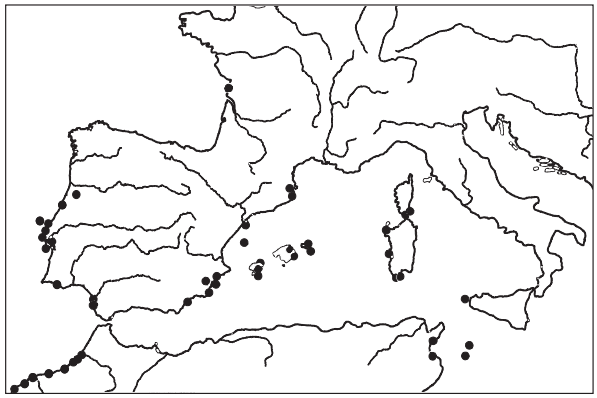

Fig. 5. The distribution of *Coscinocladium gaditanum*. Sources: Literature: Clemente (1807), Kunze (1846), Colmeiro (1867), Sampaio (1921), Werner (1955), Tavares (1956), Poelt (1958), Tavares (1958), Klement (1965), Poelt (1969), Werner (1976), Llimona (1980), Houmeau & Roux (1984), Llimona & Egea (1984), Nimis & Poelt (1987), Breuss (1988), Boqueras & al. (1989), Vězda (1989), Bricaud & Roux (1990), Nimis (1993), Nimis & al. (1994), Lumbsch & Feige (1995, 1996), Egea & Llimona (1997), Paz-Bermúdez & al. (2002), Nimis (2003), and unpublished records of A. Crespo, A. Gómez Bolea, and X. Llimona. Herbaria: HERBESS, PO, BC-Werner, BCN.

DISCUSSION

The mtSSU rDNA has proved to be sufficiently conservative to elucidate relationships of lichen taxa at the generic level (Crespo & al., 2001; Wedin & al., 2002). On the basis of the morphological habit of the thallus, the kind of photobiont, and the current systematic circumscription, the species was compared with other taxa from Lecanorales suborders Lecanorinae (Lecanoraceae and Physciaceae, including Caliciaceae; Wedin & Grube, 2002) and Teloschistinae (Teloschistaceae; Eriksson & al., 2001). The mtSSU rDNA majority rule consensus tree (Fig. 3) shows that *C. gaditanum* is not a member of Lecanoraceae, but belongs in the *Physcia* group (Wedin & al., 2002), along with species of *Anaptychia*, *Heterodermia*, *Phaeophyscia*, *Physconia*, etc.

The three samples of *C. gaditanum* grouped (100 pp), but no relationship between those samples and the others included was resolved in the mitochondrial tree. *Diplotomma canescens*, a morphologically similar species, is placed in the other monophyletic group (i.e., the *Buellia* group).

A well-supported clade (100 pp) included *Anaptychia* as the sister group of *Physconia* and *Phaeorrhiza*. A relationship between *Anaptychia* and *Physconia* had already been suggested by Poelt (1965) on morphological grounds and by Nordin & Mattson (2001) and Grube & Arup (2001) on molecular charac-

Table 1. Examples of relevés including *Coscinocladium gaditanum*. For explanation of the codings used see Egea & Llimona (1987).

	1	2	3	4	5
Slope (°)	**5**	**5**	**10**	**10**	**3**
Exposure	**SE**	**S**	**N**	**W**	**W**
Coverage (%)	**80**	**90**	**95**	**90**	**75**
Coscinocladium gaditanum	1.1s	1.1s	3.1s	1.1s	2.3s
Lecania turicensis	2.3s	2.3f	-	-	-
Aspicilia contorta var. *hoffmanniana*	-	-	2.3f	1.2f	-
Caloplaca flavescens	1.1f	4.3f	-	-	-
C. irrubescens	-	1.2f	-	5.4f	-
Xanthoria calcicola	-	-	3.3f	+s	-
Verrucaria macrostoma	2.3f	-	-	-	-
V. muralis	2.3f	-	-	-	-
Lecanora albescens	1.1f	-	-	-	-
Toninia aromatica	1.1f	-	-	-	-
Verrucaria nigrescens	+s	-	-	-	-
Caloplaca littorea	-	1.1s	-	-	-
Buellia sequax	-	1.1f	-	-	-
Lecidella elaeachromoides	-	-	1.1f	-	-
Lecanora campestris	-	-	1.1f	-	-
Clauzadea monticola	-	-	-	-	3.2f

1. St Jaume d'Enveija (Ebro delta, Catalonia): on mortar and tiles of an old hut, 1 m.s.m. (Boqueras & al., 1989).
2. Nova Tabarca island (Alacant, E Spain): on littoral metabasite, 4 m s. m., in the *Buellio-Caloplacetum littoreae* (Egea & Llimona, 1997).
3. Columbret Gran island (off Castelló, E Spain): on soft, eutrophicated insolated lava rock, 20 m.s.m. (Llimona, unpubl.).
4. Perdiguera island, Mar Menor (Murcia, SE Spain): on insolated surface of soft lava, 30 m s.m. (Llimona & Egea, 1984).
5. El Pilar, Formentera (Balearic Islands): on subhorizontal surface of porous limestone of walls separating fields, 90 m.s.m. (Llimona, unpubl.).

ters. *Dirinaria applanata*, a widespread tropical species with lecanorine apothecia, was included in the study as the genus has several placodioid species that have some superficial resemblance to *C. gaditanum*. However, *D. applanata* was included in the *Buellia* group. A well-supported clade (100 pp) places *Diplotomma canescens* as the basal sister group to *Dirinaria applanata* and the three species of *Pyxine*. Surprisingly, *Diplotomma canescens* did not nest with *D. venustum*, a result incongruent with previous ITS analyses (Molina & al., 2002) and indicating that further studies on the relationships of those species are required, perhaps utilising additional genes.

We conclude that *Coscinocladium* belongs to Physciaceae but is distinct from the other genera with which we have been able to compare it at the molecular level. However, it may be most closely allied to the relatively recently described Central and North American genus *Mobergia* H. Mayrhofer & Sheard (Mayrhofer & al., 1992). Unfortunately no mtSSU rDNA sequences are available for any species of that genus in GenBank and no fresh material was available for extraction. Nevertheless, the information from an ITS tree analysing also several species of the *Physcia* group, including *Mobergia calculiformis* and *Physcia tenella* var. *maritima* (the closest sequences in the BLAST search), does not contradict the hypothesis that *Coscinocladium* is a monophyletic independent genus. Moreover, both genera are not very similar morphologically.

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Appendix 1. Newly produced rnITS and mtSSU sequences.

Species	Country	Collector(s)	Voucher	GenBank acc. no. mt SSU	GenBank acc. no. nITS DNA
Anaptychia ciliaris 1	Spain	*Crespo & Cubero s.n.*	MAF 9796		AY449724
A. runcinata	Spain	*Llimona s.n.*	MAF 6789	AY464078	
Coscinocladium gaditanum 1	Spain	*Crespo & al. s.n.*	MAF 9855	AY464073	AY449720
C. gaditanum 2	Spain	*Crespo & al. s.n.*	MAF 9856	AY464074	AY449721
C. gaditanum 3	Spain	*Llimona s.n.*	MAF 9584	AY464075	
Diplotomma canescens	Spain	*N. Hladum & Gómez Bolea s.n.*	MAF 8656	AY464084	
D. venustum	Spain	*Llimona s.n.*	MAF 9587	AY464082	
Dirinaria applanata	Australia	*J. A. Elix 32649*	CANB	AY464079	AY449727
Heterodermia leucomela	Spain	*Crespo & al. s.n.*	MAF 7638	AY464072	AY449725
H. obscurata	Spain	*Crespo & al. s.n.*	MAF 7635	AY464071	AY449726
Lecanora muralis	Spain	*Crespo & Molina s.n.*	MAF 9853	AY464076	
				AY464085	
Phaeophyscia pyrrophora	China	*Crespo & al. s.n.*	MAF 9857	AY464083	
Physcia aipolia	Spain	*Crespo & al. s.n.*	MAF 7464	AY464069	AY449723
Physconia grisea 1	Austria	*Cubero s.n.*	CUB-GRAZ1	AY464077	AY449722
P. grisea 2	Spain	*Crespo s.n.*	MAF 9895	AY464067	
P. americana	Spain	*Crespo & al. s.n.*	MAF 9896	AY464068	
Pyxine sorediata	China	*Crespo & al. s.n.*	MAF 9851	AY464081	
P. subcinerea	Australia	*J. A. Elix 32652*	CANB	AY464080	
Rhizoplaca bullata	Spain	*Crespo & al. s.n.*	MAF 9894	AY464070	

Appendix 2. Sequences downloaded from GenBank.

Species	GenBank acc. no. mtSSU	GenBank acc. no. nITS DNA	Species	GenBank acc. no. mtSSU	GenBank acc. no. nITS DNA
Amandinea efflorescens	AY 143414		*Cyphelium inquinans*	AY 143404	
A. punctata	AY 143399		*Mobergia calculiformis* 1		AF 224359
Anaptychia ciliaris 2	AY 143400	AF 250782	*M. calculiformis* 2		AF 250796
A. runcinata		AF 226344	*Phaeorrhiza sareptana*	AY 143421	
Buellia aethalea	AY 143415		*Physcia caesia*	AY 143422	AF 540530
B. dijiana	AY 143416		*P. tenella* var. *maritima*		AF 224426
B. disciformis	AY 143401		*Protoparmelia badia*	AF 351179	
B. elegans	AY 143417		*Pyxine albovirens*	AY 143423	
B. epigaea	AY 143418		*Rinodina lepida*	AY 143424	AY 143413
B. lindingeri	AY 143419		*R. plana*	AY 143425	
B. schaereri	AY 143420		*R. sophodes*	AY 143426	AF 540550
Calicium viride	AY 143402		*Texosporium sancti-jacobi*	AY 143405	
Caloplaca flavorubescens	AY 143403		*Tholurna dissimilis*	AY 143407	