Caloplaca austrocitrina (Teloschistaceae) new for South America, based on molecular and morphological studies

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Abstract. Specimens of *Caloplaca* belonging to the group of *C. citrina* (Hoffm.) Th. Fr. were collected in different localities in the provinces of Buenos Aires (Argentina) and studied using morphology and nrITS data. They were identified as *C. austrocitrina* Vondrák, Riha, Arup & Søchting, a species newly described from the Black Sea area, but also found in other European countries. These findings are the first mention of this species for South America and broaden its distribution range considerably. The results also show that the specimens from Argentina are genetically very similar to the European ones and that the variation in this gene within the species seems to be very low.

Keywords. Argentina, *Caloplaca citrina*, ITS, lichen, phylogeny.

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Species of the *Caloplaca citrina* (Hoffm.) Th. Fr. group are the most common and widespread lichens on buildings of architectural heritage and concrete structures in the Buenos Aires Province, Argentina (Rosato 2006). Recently, this group was revised for northern Europe by Arup (2006) who used molecular data and correlated them with morphological and chemical data of Scandinavian samples. He found morphological differences but he did not find chemical diversity: all the species have parietin and other minor compounds. Therefore he confirmed that these species belong to chemosyndrome A of Teloschistaceae as described by Søchting (1997). Arup (2006) recognized five species that previously had been included in the *C. citrina* group. Søchting et al. (2008) reported a further species, *C. alaskensis* Wetm., morphologically similar to this sorediate group, but this species belongs elsewhere (Arup, unpubl. data). These studies were followed by an extended study of the *C. citrina* group in SE Europe, especially around the Black Sea (Vondrák et al. 2009). They reported another five species from the group: *C. arcisproxima* Vondrák, Riha, Arup & Søchting, *C. austrocitrina* Vondrák, Riha, Arup & Søchting, *C. communis* Vondrák, Riha, Arup & Søchting, *C. confusa* Vondrák, Riha, Arup & Søchting and *C. limonia* Poelt & Nimis.

Grassi (1950) and Calvelo and Liberatore (2002) noted in their catalogues that Cengia Sambo (1930) cited *C. citrina* from Argentina, but there were no revisions of the group in the country. In this study, selected specimens belonging to the *C. citrina* group from different heritage buildings and concrete structures from the Buenos Aires Province were

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DOI: 10.1639/0007-2745-113.1.124

revised in order to identify them with morphological and molecular techniques.

Materials and Methods

Lichens belonging to the *Caloplaca citrina* group were collected mainly in the central and SW part of Buenos Aires province (Argentina) as well as the southern coast of the Río de la Plata. Some of the samples were selected and revised under stereo and light microscope. Samples obtained by scraping were excluded from this study; only samples with the substratum were revised. Thin Layer Chromatograph (TLC) of all the specimens was also performed and samples of *Xanthoria parietina* were used as controls according to Culberson (1972). The vouchers are deposited at LPS.

DNA-extraction, PCR-amplification and sequencing. PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA was made without an extraction step, but using Direct PCR as described by Arup (2006). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The PCR settings used followed the manufacturer's recommendations.

Products were cleaned using E.Z.N.A. Cycle-Pure Kit (Omega Bio-Tech). PCR cycling parameters included an initial hold at 94°C for 5 min, then denaturating at 94°C for 1 min, annealing at 56°C for 1 min., decreasing 1°C per cycle for the first 6 of the 39 cycles (touchdown), and extension at 72°C for 3 min. Both complementary strands were sequenced with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) using the primers mentioned above, and run on an ABI PRISM 3100 Genetic Analyzer.

A total of 26 ingroup taxa from the *Caloplaca citrina* group and two outgroup taxa were aligned by hand. (For a list of the specimens and GenBank accession numbers see **Table 1.**) The alignment included the ITS1, 5.8S and ITS2 regions for a total of 542 bases. *Caloplaca holocarpa* and *C. pyracea* were used as outgroups.

Phylogenetic analyses. A phylogenetic analysis was carried out using PAUP*4.0b10 (Swofford 2002) under the maximum parsimony optimality criterion. The characters were given equal weight and gaps were treated as missing data. A heuristic search was performed using tree bisection-reconnection (TBR) branch-swapping. Bootstrap proportions were estimated using 1000 bootstrap replicates, each with 10 random addition sequence replicates. Because test runs showed large numbers of trees in some replicates the limit of maximum number of trees was set to 2000 per random addition sequences replicate.

Results

ITS sequences were obtained for three specimens from Argentina (LPS 48214, LPS 48215 and LPS 48219) and aligned with those of various species of the *Caloplaca citrina* group. The alignment contained 676 characters of which 89 were parsimony-informative. Only constant characters were excluded from the analysis. The analysis resulted in six equally parsimonious trees of 170 steps (CI=0.812, RI=0.875, HI=0.188) that differed slightly from each other only in the branch lengths and not in their topology (**Fig. 1**). Although ITS data support the monophyly of most taxa, the relationships among them remain ambiguous. The three samples from Argentina are identical to two sequences of *C. austrocitrina* and differ only by one substitution from sequence of the Russian specimen (**Fig. 1**).

Morphology. The thallus of the Argentinean specimens (**Fig. 2**) is areolate, yellow to orange-yellow, with flat to slightly convex areolae, which are irregular in shape, 0.5–1.0 mm in diam. and densely covered with soredia. The soralia start at the margin of the areolae but very often most of the surface is covered by soredia.

The apothecia are zeorine, yellow to orange with orange to reddish orange discs, 0.3–0.6 mm in diam. The asci are clavate, octosporous; and the spores are polardiblastic, 10–14 × 4–6 μm with the septum 4.5–5.0 μm wide. For a full description see Vondrák et al. (2009).

Chemistry. The TLC results were the same for the samples and the controls The compounds detected by TLC in the Argentinean samples are identical to those of the control (i.e., *Xanthoria parietina*): the main substance is parietin, with other minor substances: fallacinal, emodin, teloschistin and parietinic acid. These secondary metabolites are also found in the *Caloplaca citrina* complex (Arup 2006)

Table 1. Sequences used in the analyses, with location, collector, collection number, and GenBank accession numbers. Specimens in bold were newly produced for the current study.

Species	Specimen	Accession number
C. arcis	Austria, *Arup L97514*	DQ173213
C. arcis	Sweden, *Frödén & Ekman 949*	DQ173214
C. arcis	England, *Arup L92118*	DQ173215
C. arcis	Bulgaria, *Vondrak JV3036*	EU563395
C. arcisproxima	Greece, *Vondrak JV4125*	EU563413
C. arcisproxima	Ukraine, *Vondrak JV5473*	EU563425
C. austrocitrina	Russia, *Vondrak JV5474*	EU563426
C. austrocitrina	Ukraine, *Vondrak JV5476*	EU563427
C. austrocitrina	Czech Rep., *Vondrak JV991*	EU563450
C. austrocitrina	Argentina, *Rosato* LPS 48214	**GQ338422**
C. austrocitrina	Argentina, *Rosato* LPS 48219	**GQ338423**
C. austrocitrina	Argentina, *Rosato* LPS 48215	**GQ338424**
C. citrina	Sweden, *Arup L03065*	DQ173222
C. dichroa	Austria, *Arup L03054*	DQ173228
C. dichroa	Sweden, *Arup L04005*	DQ173229
C. flavocitrina	Sweden, *Arup L03052*	DQ173219
C. flavocitrina	Sweden, *Arup L99002*	DQ173221
C. flavocitrina	Sweden, *Arup L03080*	DQ173220
C. flavocitrina	Sweden, *Arup L03203*	**GQ338425**
C. flavocitrina	Sweden, *Bergquist s.n.*	**GQ338426**
C. holocarpa	Austria, *Hafellner 44063*	AF353945
C. limonia	Italy, *1991 Poelt* (isotype)	EU563467
C. limonia	Czeck Rep., *Vondrak JV2515*	EU563391
C. limonia	Bulgaria, *Vondrak JV3388*	EU563400
C. limonia	Bulgaria, *Vondrak JV3438*	EU563407
C. pyracea	Sweden, *Foucard s.n.*	AF353945

that belongs to chemosyndrome A (Søchting 1997). Thallus and apothecia are K+ purple red.

Ecology. The ecology of *Caloplaca austrocitrina* in the Buenos Aires area is very similar to that of European samples. The species often grows on cement mortar (defined as a hardened mix of cement, sand and water to distinguish it from other kind of mortars, such as lime mortar) and concrete in cities and villages. It often occurs near water, both next to rivers, lakes and the sea.

Specimens studied. ARGENTINA. PROV. BUENOS AIRES: Azul: Estancia San Rufino, rocky outcrop near the "pirca"(stone wall), on "tosca" (calcareous rock), 2006, *Ribot & Rosato*, (LPS 48220); Castelli: Canal 15, on cement mortar, 2003, *Ribot & Rosato* (LPS 48207); General Lavalle: ruta 11, auxiliar bridge 200 m from the sea, on cement mortar, 2002, *Traversa et al.* (LPS 48208); Magdalena: flow control canal of Salado River, cement mortar, 2003, *Traversa* (LPS 48209), flow control canal of Salado River, on quartzite, 2003, *Traversa* (LPS 48210), flow control channel over Río Salado, cement mortar, 2003, *Traversa* (LPS 48218), flow control channel over Río Salado, on white quartzite, 2003, *Traversa* (LPS 48219); Arditi: railroad bridge; on brick, 2004, *Traversa et al.* (LPS 48211), railroad bridge, on cement mortar, 2004, *Traversa et al.* (LPS 48212); Bartolomé Bavio: railroad bridge, on brick, 2004, *Traversa et al.* (LPS 48213); Tandil: Tandil city, dam, base of the drain, 2000, *Traversa et al.* (LPS 48214), water drain at the side of the road, blocks of cement mortar, 2000, *Traversa et al.* (LPS 48215); Tornquist: Town Hall, on white cement mortar, 2002, *Traversa* (LPS 48216); Verónica: Punta de Indio, Estancia "Luis Chico," on serpentinite column, 2001, *Traversa & Rosato* (LPS 48217).

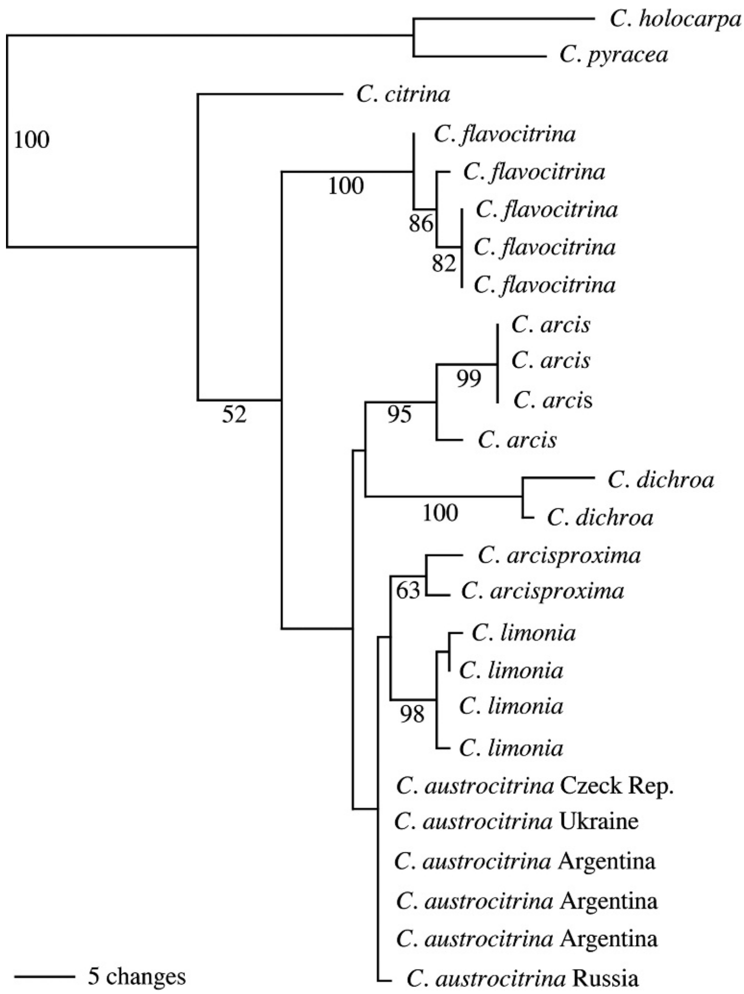

Figure 1. One of six most parsimonious trees inferred from the phylogenetic analysis of nrDNA ITS sequences obtained for *Caloplaca austrocitrina* and related species. Numbers below branches are bootstrap values.

Discussion

Caloplaca austrocitrina, recently described from the Ukraine (Vondrak et al., in press), is reported from the Buenos Aires area, Argentina, based primarily on specimens referred to as *C. citrina* (Hoffm.) Th. Fr. (Rosato 2006). The morphology of the specimens from Argentina (**Fig. 2**) corresponds well with that of the European ones, but the areolae seem to be slightly smaller and thinner on average in the material from Buenos Aires. The European material also seems to have more soredia that often cover large parts of the areolae. Morphologically *C. austrocitrina* is most similar to *C. flavocitrina* (Nyl.) Oliv., but differs in the soredia that often cover larger parts or all the areolae and by areolae that are smaller, and more crustose than squamulose.

In Europe *C. austrocitrina* is known also from Austria, Bulgaria, Germany, Greece, the Czech Republic, Romania, Russia and Slovakia (Vondrák et al. 2009). It will be interesting to follow the increasing knowledge about the true distribution of the species. Up to now, it has been collected in South America and Europe, so it is likely that it will be found also on other continents. The Argentinian specimens share nearly (except for a single substitution in the Russian specimen) identical ITS sequences with conspecific European samples. Compared to other species of the genus the variation in the ITS locus is unusually low, especially

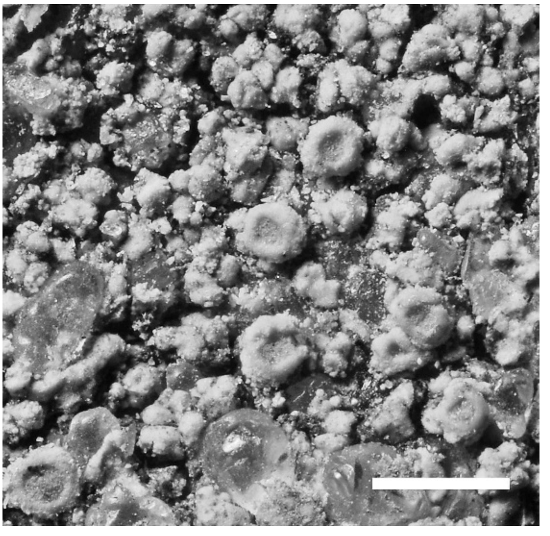

Figure 2. Thallus and apothecia of *Caloplaca austrocitrina* seen under stereomicroscope (from LPS 48216).

considering the distance between Europe and the South American populations. It is, however, too early to speculate about the reason for this before we know more about the real distribution of the species.

Acknowledgments

We thank the Director and colleagues of LEMIT for collecting the samples and to Conicet for the support to do this work.

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ms. received March 20, 2009; accepted September 12, 2009.