DIVERSITY OF THE GENUS CALOPLACA (LICHENIZED FUNGI) ON SEASHORE ROCKS OF THE WESTERN BLACK SEA COAST

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1. Introduction

Coastal habitats influenced by salt spray are inhabited by specific organisms which are adapted to high salinity. Many examples can be seen among vascular plants, mosses and also lichens. Coastal lichens have been studied worldwide (e.g. Fletcher 1975 a, b, LaGreca et al. 2005, McCarthy 1991, Smith & Gremmen 2001); however, many sea shores are lichenologically still very poorly known. The upper-most littoral zone and the supralittoral zone of siliceous sea shore rocks can be divided into three zones according to the colours of the predominant lichens (Knowles 1913): the black zone – the lower-most belt mainly covered by diverse *Verrucaria* species and *Lichina confinis*; the orange zone – with prevailing species of *Caloplaca* and *Xanthoria*; and the grey zone – with many grey or brown lichen species.

The genus *Caloplaca* dominates the orange zone of seashores and is one of the most species-rich within lichen genera. Recently, over 800 species have been estimated to exist worldwide (Kärnefelt et al. 2002). This genus is characterized by its polarilocular spores, asci of *Teloschistes*-type, and a usual presence of yellow to red pigments from the anthraquinone group. Some recent work has found phylogenetic heterogeneity within *Caloplaca* (Gaya et al. 2003, Kärnefelt 1989, Søchting & Lutzoni 2003). Some *Caloplaca* species, namely those from the section *Gasparrinia*, should be included in *Xanthoria* (Søchting & Lutzoni 2003), and some species of *Fulgensia* should be placed in *Caloplaca* (Gaya et al. 2004).
The genus *Caloplaca* contains both clearly characterized, invariable species as well as taxonomically difficult groups of species. Some groups have been investigated in detail, e.g. *C. citrina* group (Arup 2006, Wetmore 2001), *C. ferruginea* group (Magnusson 1944, Wetmore 1996), *Gasparrinia* group (Nordin 1972, Wetmore & Kärnefelt 1998), *Pyrenodesmia* group (Wetmore 1994, Wunder 1974), and others. Most of these works are based only on morphological and anatomical characters. The chemical characteristics are used in relatively few recent studies on taxonomy of *Caloplaca*, most notably the analyses of anthraquinones (e.g. Søchting & Fröberg 2003) and insoluble pigments in acetone (Wetmore 1994, Tretiach et al. 2003). The first study on taxonomy at the species level using molecular methods has been carried out by Arup (2006) on the *C. citrina* group. However, more molecular data (mainly on ITS1, 5.8S, and ITS2 regions) on many *Caloplaca* species exist, but are unpublished (Arup, Fröden & Søchting, unpublished data).

Many species of *Caloplaca* were originally described as being restricted to seashore rocks. However, although new seashore species are described from European seashores regularly (Laundon 1992, Navarro-Rosinés & Roux 1993, 1995, Nimis & Poelt 1987, Roux & Navarro-Rosinés 1992, Sipman & Raus 2002, Tavares 1956), the taxonomy of coastal *Caloplaca* species is rarely studied in detail (Arup 1992, 1993 a, b, 1997).

The maritime habitats around the Black Sea are isolated from Atlantic seashores and also from the Mediterranean, which is connected with the Black Sea only by Bosporus. This situation is favorable for the evolution of endemic Black Sea organisms via allopatric speciation, including lichen species. However, the Black Sea shores are lichenologically still

very poorly known. The genus *Caloplaca* was extensively investigated in the northern Ukraine, as seen in several lichenofloristic papers (Khodosovtsev 2001, 2002, 2003). In fact, two endemic species of maritime rocks, *Caloplaca geleverjae* Khodosovtsev & S. Kondr. and *C. karadagensis* Khodosovtsev & S. Kondr. were described from coastal rocks of the Crimean peninsula (Khodosovtsev et al. 2003). Several records of *Caloplaca* are known from the western Bulgarian part (Vězda 1975, Vondrák & Slavíková 2006). However, we have no data from coastal rocks in the eastern, Russian and Georgian parts of this region, and very little data on the occurrence of *Caloplaca* in the southern Turkish part (Yazici & Aslan 2003). In Romania, coastal rocks are rare, due to unsuitable substrata and relief, and no data on *Caloplaca* are known from that part of the Black Sea (Ciurchea 2005).

2. Questions to be solved

1. Which species of *Caloplaca* occur on sea shore rocks of the western Black Sea coast and is the *Caloplaca* lichenoflora of the western Black Sea similar to that found on the sea shores in western and northern Europe and in the Mediterranean?
2. Which is the distribution and ecology of western Black Sea *Caloplaca* species?

3. Methods

Suggested solution of the problem

1. DNA sequences will be obtained to reveal relationships to other, allied species. The ITS1, 5.8S, and ITS2 regions of nrDNA will be used.
   For the delimitation and characterization of the species, morphological, anatomical, and chemical investigations will be carried out. In fertile species, the principal anatomical characteristics are ascospore characters, and shape of paraphyses and excipular hyphae. In the predominantly sterile species, the characteristics are restricted to characters of vegetative reproduction, cortex arrangement, etc. Chemical characteristics are mainly based on the composition of anthraquinones.
2. Two additional collecting trips are planed in 2007 and 2008 to obtain detailed data on the ecology and the distribution of respective species in the studied area. The factors affecting the occurrence of particular species in maritime habitats, such as substrate and aspect preferences and sensitivity to salinity (Fletcher, 1973) will be investigated.

DNA-extraction, PCR-amplification and sequencing

Extraction using DNeasy Plant Mini Kit (Qiagen).

PCR-amplification of ITS regions of the nuclear rDNA using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990).

Purification using QIAquick PCR purification Kit (Qiagen)

Sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystem) using the primers mentioned above, and run on an ABI PRISM 3100 Genetic Analyzer.

Molecular data analysis

Sequences will be aligned by hand with sequences from GenBank. The alignment will include only the ITS1, 5.8S, and ITS2 regions. The phylogenetic analysis will be carried out using PAUP*4.0b10 (Swofford 2002).

Chemical analysis

Anthraquinones will be analysed using HPLC-MS chromatography as described in Søchting (1997).
Pigments insoluble in acetone will be analysed following Meyer & Printzen (2000).

Microscopical examinations
Microscope work will be done using traditional methods (hand-made sections examined in water, KOH, and lactophenol cotton blue at a magnification of ×1000).

4. Time schedule

2006 (not supported by this grant)
Taxonomic work on the *Caloplac citrina*/ *marina*/ *microthallina* group. For this part of work, support from the SYNTHESYS grant (DK-TAF) is being pursued.

2007
Taxonomic work on the species of the *Gasparrinia* group, with a focus on the problematic taxa *C. thallincola*/ *aegaea* and *C.* aff. *scopularis*.
A collecting trip to the Bulgarian Black Sea coast to investigate more sites, and obtain more material, will be organized. This trip will be focusing on gathering data on the ecology of these species.

2008
Taxonomic work on the heterogenous group of species with a thallus devoid of the yellow-orange-red pigments, anthraquinones. This group includes the species *Caloplaca albolutescens*, *C.* aff. *chlorina*, *C.* aff. *furax*, and *C. fuscoatroides*.
A second collecting trip to the Turkish Black Sea coast is planned. This trip will be also focused on gathering data on ecology of the treated species.
Preliminary results of this project will be presented at the 6th IAL symposium.

2009
Taxomomic work on the problematic species *C. inconnexa* var. *nesodes* (= *C. necator*?) and *C.* aff. *ferrarii*.
Work on the "taxonomically clear" species (i.e., dealing with the data on their ecology and distribution only).

During the years 2007-2009, papers on the worked up groups will be submitted for publication. The flash point of the work will be a revision of *Caloplaca* species occurring on maritime rocks of the western Black Sea.

5. Research Team

Principal investigator: Jan Vondrák
The principle investigator is active in the field of taxonomy and nomenclature of the lichen genus *Caloplaca* (see CV for more details). He will receive a basic knowledge in molecular techniques on lichens during a 3-week stay at the University of Copenhagen in October, 2006 (advised by Prof. Ulrik Søchting).

Co-worker: Jaroslav Šoun
Participation in the respective project: working on the *Gasparrinia* group.

J. Šoun is currently working on the taxonomy of the *Caloplaca aurantia* group (a part of the *Gasparrinia* group).

Co-worker: Pavel Hrouzek
Participation in the respective project: performing chemical analyses of anthraquinones (HPLC-MS).
P. Hrouzek is currently working on investigations of cyanobacterial toxins using HPLC-MS chromatography. He received specific knowledge in analyses of anthraquinones in lichens during a recent collaboration with the principle investigator on the project: *Caloplaca soralifera*, a new sorediate species of *Caloplaca* (*Teloschistaceae*) from Europe.

6. Supporting circumstances

Work already done on this project
Sampling for chemical and molecular analyses, and morphological and anatomical observations:
In 2004 and 2005, three collecting trips were carried out to collect samples of *Caloplaca* from the study area. 120 samples of rich lichen material were obtained, ten of which are expected to be issued as exsiccates.
Study area and number of collecting sites:
Area of study contains the Black Sea coast in Romania (2 sites), Bulgaria (9 sites) and the European part of Turkey (4 sites). The sites cover the area more or less equitably, representing various substrata (siliceous and hard limestone rocks, soft calcareous sediments, and also concrete).

Rough determination of the obtained material:
Collected material was roughly determined following keys and descriptions of species from the Mediterranean and Atlantic coasts of Europe. 28 species were distinguished:
Caloplaca albolutescens, *C. arcis*, *C. aurantia*, *C.* aff. *biatorina*, *C.* aff. *chlorina*, *C. coronata*, *C. crenulatella*, *C. decipiens*, *C. demissa*, *C.* aff. *ferrarii*, *C. flavescens*, *C.* aff. *flavocitrina*, *C.* aff. *furax*, *C. fuscoatroides*, *C. grimmiae*, *C. holocarpa* agg., *C. inconnexa* var. *nesodes* (= *C. necator*?), *C.* aff. *lithophila*, *C.* aff. *marina*, *C.* aff. *microthallina*, *Caloplaca polycarpa*, *C. rubelliana*, *C. saxicola* agg., *C.* aff. *scopularis*, *C. teicholyta*, *C. thallincola* (= *C. aegea*?), *C. velana* agg., *C. viridirufa* (= *C. aractina*).

DNA data were obtained from several samples of the group *Caloplac citrina*/ *marina*/ *microthallina* (performed by Dr Arup, Lund University). These data indicate that there are several species that are morphologically similar to western European ones, but they sometimes differ greatly genetically. On the other hand, there are also samples that differ morphologically to some degree, but have DNA sequences similar to species from other parts of Europe.

Collaborations
Investigations on anatomy and morphology will be performed at the home institution of the principal investigator (University of South Bohemia, Faculty of Biological Sciences).
The HPLC analyses of anthraquinones will be carried out at the Academy of Sciences, Institute of Microbiology, Section of Photosynthetic Microorganisms (Třeboň, CZ).
The molecular laboratory work (isolation, PCR, purification, and sequencing) will take place at the University of Copenhagen in cooperation with Prof. Ulrik Søchting.
Analyses of the molecular data will be done with the help of Dr Ulf Arup (Lund University).

7. Literature

Arup, U. 1992: Caloplaca marina and C. rosei, two difficult species in North America. - *The Bryologist* **95(2)**: 148-160.

Arup, U. 1993a: Caloplaca flavogranulosa sp. nov. and C. citrina, two sorediate species on seashore rocks in western North America. - *The Bryologist* **96(4)**: 598-603.

Arup, U. 1993b: Caloplaca luteominia and C. bolanderi in western North America. - *The Bryologist* **96(3)**: 463-470.

Arup, U. 1997: Caloplaca maritima, a misunderstood species in western Europe. - *Lichenologist* **29(6)**: 503-512.

Fletcher, A. 1973: The ecology of maritime (supralittoral) lichens on some rocky shores of Anglesey. - *Lichenologist* **5**: 401-422.

Fletcher, A. 1975a: Key for the identification of British marine and maritime lichens. I. Siliceous rocky shore species. - *Lichenologist* **7**: 1-52.

Fletcher, A. 1975b: Key for the identification of British marine and maritime lichens. II. Calcareous and terricolous species. - *Lichenologist* **7**: 73-115.

Ciurchea M. 2005: *Catalog of lichens in Romania.* http://lichens.duci.ro/id26.htm (last visit: april 2006)

Gardes, M. & Bruns, T. D. 1993: ITS primers with enhanced specifity for basidiomycetes. Application for the identification of mycorrhizae and rusts. - *Molecular Ecology* **2**: 113-118.

Gaya, E., Lutzoni, F., Zoller, S. & Navarro-Rosinés, P. 2003: Phylogenetic study of Fulgensia and allied Caloplaca and Xanthoria species (Teloschistaceae, lichen-forming Ascomycota). - *American Journal of Botany* **90(7)**: 1095-1103.

LaGreca, S., Lay, E., Greene, D., Kneiper, E. & Lincoln, M. 2005: The lichens and bryophytes of the Boston Harbor islands. - *Northeastern Naturalist* **12/3**: 77-98.

Kärnefelt, I. 1989: Morphology and phylogeny in the Teloschistales. - *Cryptogamic Botany* **1**: 147-203.

Kärnefelt, I., Kondratyuk, S., Søchting, U., Frödén, P. & Arup, U. 2002: Two new species of Caloplaca (Teloschistaceae) from the Southern Hemisphere. - *The Bryologist* **105(3)**: 301-309.

Khodosovtsev, O. Y. 2001: [A new for Ukraine species of the genus Caloplaca Th. Fr. (Teloschistaceae).] - *Ukrayinskyi Botanichnyi Zhurnal* **58(4)**: 460-465. (in Ukrainian)

Khodosovtsev, O. Y. 2002: [A new for Ukraine and rare species of the genus Caloplaca Th. Fr. (Teloschistaceae) from southern Ukraine.] - *Ukrayinskyi Botanichnyi Zhurnal* **59(3)**: 321-329. (in Ukrainian)

Khodosovtsev, O. Y. 2003: [A new for Crimea and Ukraine species of the lichens from siliceous outcrops.] - *Ukrayinskyi Botanichnyi Zhurnal* **60(1)**: 70-78. (in Ukrainian)

Khodosovtsev, O. Y., Kondratyuk, S. Y. & Kärnefelt, I. 2003: New species of Caloplaca from Crimean Peninsula (Ukraine). - *Ukrayinskyi Botanichnyi Zhurnal* **60(3)**: 293-297.

Knowles, M. C. 1913: The maritime and marine lichens of Howth. - *Scientific Proceedings, Royal Dublin Society* **14**: 79-143.

Laundon, J. R. 1992: New British species of Caloplaca. - *Lichenologist* **24(1)**: 1-5.

McCarthy, P. M. 1991: Notes on Australian Verrucariaceae (Lichenes): 2. - *Muelleria* **7(3)**: 317-332.

Meyer, B. & Printzen, C. 2000: Proposal for a standardized nomenclature and standardization of insoluble lichen pigments. - *Lichenologist* **32**: 571-583.

Navarro-Rosinés, P. & Roux, C. 1993: Caloplaca tavaresiana Nav.-Ros. et Roux sp. nov., espèce nouvelle de lichen du littoral de la région méditerranéenne. - *Nova Hedwigia* **57(1-2)**: 169-177.

Navarro-Rosinés, P. & Roux, C. 1995: Caloplaca navasiana Nav.-Ros. et Roux sp. nov., espèce nouvelle de lichen du littoral Méditerranéen. - *Cryptogamie, Bryologie-Lichénologie* **16(2)**: 89-97.

Nimis, P. L. & Poelt, J. 1987: The lichens and lichenicolous fungi of Sardinia (Italy): an annotated list. - *Studia Geobotanica* **7 (Supplement 1)**: 1-269.

Nordin, I. 1972: *Caloplaca sect. Gasparrinia in Nordeuropa. Taxonomiska och Ekologiska Studier*. - Skriv Service AB, Uppsala.

Roux, C. & Navarro-Rosinés, P. 1992: Caloplaca egeana Roux et Nav.-Ros. sp. nov. kaj Caloplaca veneris Roux et Nav.-Ros. sp. nov., du novaj likenspecioj de la mediteranea marbordo. - *Bulletin de la Société Linnéenne de Provence* **43**: 97-103.

Sipman, H. & Raus, T. 2002: An inventory of the lichen flora of Kalimnos and parts of Kos (Dodecanisos, Greece). - *Willdenowia* **32**: 351-392.

Smith, V. R. & Gremmen, N. J. M. 2001: Photosynthesis in a sub-Antarctic shore-zone lichen. - *New Phytologist* **149(2)**: 291-299.

Søchting, U. 1997: Two major anthraquinone chemosyndromes in Teloschistaceae. - *Bibliotheca Lichenologica* **68**: 135-144.

Søchting, U. & Fröberg, L. 2003: Caloplaca asserigena, the correct name for a small lichen with a unique chemistry. - *In:* Jensen, M (ed.): *Lichenological Contributions in Honour of G.B. Feige*. Bibliotheca Lichenologica, J. Cramer, Berlin, Stuttgart, pp. 47-51.

Søchting, U. & Lutzoni, F. 2003: Molecular phylogenetic study at the generic boundary between the lichen-forming fungi *Caloplaca* and *Xanthoria* (Ascomycota, Teloschistaceae). - *Mycological Research* **107**: 1266-1276.

Swofford, D. L. 2002: PAUP*. *Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10.* - Sunderland, MA: Sinauer Associates.

Tavares, C. N. 1956: Notes lichenologiques--IX. - *Rev. da Fac. de Cien. de Lisboa 2. ser. (C)* **5(1)**: 123-134.

Tretiach, M., Pinna, D. & Grube, M. 2003: Caloplaca erodens [sect. Pyrenodesmia], a new lichen species from Italy with an unusual thallus type. - *Mycological Progress* **2**: 127-136.

Vězda, A. 1975: *Lichenes selecti exsiccati* - Instituto Botanico Academiae Scientiarum Cechoslovacae, Pruhonice prope Pragam. Fasc. LV (no. 1351-1375). XII.

Vondrák, J. & Slavíková-Bayerová, Š. 2006, in press: Contribution to the lichenized and lichenicolous fungi in Bulgaria. II, the genus Caloplaca. - *Mycologia Balcanica*

Wetmore, C. M. 1994: The lichen genus Caloplaca in North and Central America with brown or black apothecia. - *Mycologia* **86(6)**: 813-838.

Wetmore, C. M. 1996: The Caloplaca sideritis group in North and Central America. - *The Bryologist* **99(3)**: 292-314.

Wetmore, C. M. 2001: The Caloplaca citrina group in North and Central America. - *The Bryologist* **104(1)**: 1-11.

Wetmore, C. M. & Kärnefelt, E. I. 1998: The lobate and subfruticose species of Caloplaca in north and central America. - *The Bryologist* **101(2)**: 230-255.

White, T. J., Bruns, T. D., Lee, S. & Taylor, J. 1990: Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenies. *In:* Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (eds.): *PCR Protocols: a Guide to Methods and Applications (eds.).* San Diego: Academic Press, pp. 315-322.

Wunder, H. 1974: Schwartzfrüchtige, saxicole Sippen der Gattung Caloplaca (Lichenes, Teloschistaceae) in Mitteleuropa, dem Mittelmeergebiet und Vorderasien. - *Bibliotheca Lichenologica* **3**, 1-186.

Yazici, K. & Aslan, A. 2003: Lichens from the regions of Gümüshane, Erzincan and Bayburt (Turkey). - *Cryptogamie, Mycologie* **24**: 287-300.