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Is *Anaptychia* monophyletic? A phylogenetic study based on nuclear and mitochondrial genes

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The phylogeny of the lichen genera *Anaptychia* (Physciaceae, Euascomycetes) and *Physconia* was studied using nucleotide sequences of the mitochondrial ribosomal small subunit (mtSSU rDNA) and the internal transcribed spacers of the nuclear ribosomal repeat (ITS). Phylogenetic analyses of the combined data set demonstrated that all included eight *Anaptychia* species formed a monophyletic group, as did the species of *Physconia*. The two gene regions revealed qualitatively similar relationships within the genus.

Key words: *Anaptychia*, ITS, lichen, mtSSU rDNA, phylogeny, *Physconia*

Introduction

The genus *Anaptychia* (Physciaceae, Lecanorales) of the class Lecanoromycetes contains about 15 lichen species worldwide (Moberg 1994, Chen & Wang 1999, Kulakov 2003). Initially Kurokawa (1962) recorded 88 *Anaptychia* species in his monograph of the genus. However, Poelt (1965) considered that the genus should be subdivided into two genera, *Anaptychia* and *Heterodermia*, based on, e.g., spore types. Later on Culberson (1966) emphasized that not only morphological characters but also secondary chemistry supports recognition of the two genera. Despite those opinions Kurokawa (1973) kept *Heterodermia* within *Anaptychia* and treated *Anaptychia* and *Heterodermia* as subgenera. In recent phylogenetic studies (Lohtander *et al*. 2000, Grube *et al*. 2001, Helms *et al*. 2003) *Anaptychia* was found to be rather distantly related to *Heterodermia*. Instead it forms a sister group to the genus *Physconia*, while *Heterodermia* appeared as closely related to the genus *Physcia*.

Anaptychia is characterized by brown, thin-walled, 1-septate spores of *Physconia* type and prosoplechtenchymatous upper cortex, while the spores of *Heterodermia* have thick walls of *Pachysporaria* type (Poelt 1965). The species of *Heterodermia* contain atranorin (K+ yellow cortex), while *Anaptychia* either lacks that substance or contains it in very low concentrations (K–). *Physconia* is characterized by the sclero- or paraplectenchymatous, pruinose upper cortex and squarrose rhizinae (Moberg 2002).

In the papers by Lohtander *et al*. (2000) and Grube *et al*. (2001), *Anaptychia* appeared as paraphyletic, but only few specimens were included in the analyses and the studies were based on one gene region (ITS of the nuclear ribosomal region) only. In another study (Cubero *et al*. 2004) *Anaptychia* and *Physconia* each formed monophyletic groups, but while the genus *Physconia* was extensively sampled, only two species of *Anaptychia* were included in the study.

We wanted to find out, whether *Anaptychia* and *Physconia* formed distinct monophyletic groups in an analysis based on two independent gene regions (ITS and mtSSU rDNA). We also wanted to clarify whether the topology of the molecular tree would be supported by some of the morphological characters present in *Anaptychia* (e.g. colour and the structure of the upper cortex).

Material and methods

Biological material

Herbarium specimens and fresh material from several geographic regions were included in the study. Altogether we were able to obtain DNA sequences from eight species of *Anaptychia* (*see* Table 1).

Molecular techniques

The total DNA of lichen samples was extracted using the QIAamp Tissue Kit (Qiagen) according to the manufacturer's instructions with slight modifications described in Lohtander *et al*. (2000). The ITS sequences and the small subunit of the mitochondrial ribosomal repeat (mtSSU rDNA) were obtained from 56 lichen samples including 16 *Anaptychia* specimens and 24 *Physconia* specimens, using fungal specific primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990) for the ITS region, as well as mtSSU1-KL/mtSSU2-KL (Lohtander *et al*. 2002) for the mitochondrial rDNA. The rest of the ITS sequences were obtained from the GenBank. No mtSSU region was sequenced for such specimens (specimens marked with an asterisk in the Fig. 1). PCR-reactions were performed using Amersham Pharmacia Biotech Inc. Ready to Go PCR beads following a procedure described in Lohtander *et al*. (2000). The PCR profile for each reaction was 60 sec at 95° (denaturation), 60 sec at 55° (for the mtSSU rDNA region) or 60° (for the ITS region; annealing), and 60 sec at 72° (extension) followed by 7 min at 72°. The PCR products were purified with the PCR Purification Kit of Qiagen. Both strands of the amplified DNA products were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems). Sequence reactions were purified using the AutoSeq™ G-50 columns of Amersham Pharmacia Biotech Inc. The purified samples were run either on an ABI Prism 377 automated sequencer or on an ABI Prism 310 genetic analyser (PE Biosystems).

Alignment and phylogenetic analysis

Sequences were aligned using the ClustalW

Table 1. Voucher specimens. The specimen numbers refer to those in Fig 1.

No.	Species	Herb.	Collection	Location	GenBank# ITS	GenBank# mtSSU
1	*Xanthoria parietina*	–	from GenBank		AF224347	AY143408
2	*Heterodermia erinacea*	UPS	*Moberg 12212*	Chile Coquimbo	EF582746	EF582789
3	*H. leucomelos*	UPS	*Moberg 12236*	Chile Maule	EF582747	EF582790
4	*Physcia alnophila*	H	*Urbanavichus B-04535*	Russia Buryatia	EF582748	EF582791
5	*P. alnophila*	S	*Lohtander 399*	Finland Ks	AF224381	EF582792
6	*P. aipolia*	UPS	*Moberg 12008*	Sweden Ångermanland	AF224391	EF582793
7	*P. caesia*	H	*Urbanavichus C-01566*	Russia Adygeya	EF582749	EF582794
8	*P. caesia*	UPS	*Hansen exs. 782*	Greenland Cass Fjord	EF582750	EF582795
9	*P. dubia*	UPS	*Moberg 10653*	Romania Cluj	AF224421	EF582796
10	*P. dubia*	S	*Lohtander 420*	Finland Le	AF224411	EF582797

continued

Table 1. Continued.

No.	Species	Herb.	Collection	Location	GenBank# ITS	GenBank# mtSSU
11	*P. adscendens*	H	*Haikonen 22251*	Finland Sb	EF582751	EF582798
12	*P. tenella*	S	*Tehler 8057*	Sweden Uppland	AF224424	EF582799
13	*P. tenella*	S	*Lohtander 650*	Finland N	AF224425	EF582800
14	*Phaeophyscia nigricans*	UPS	*Moberg 12046*	Sweden Uppland	AF224375	EF582801
15	*P. constipata*	UPS	*Löfgren 12.11.1998*	Sweden Västmanland	AF224374	EF582802
16	*P. ciliata*	LECB	*Himelbrant K-04-23*	Russia SW Kamchatka	EF582752	EF582803
17	*P. endococcina*	UPS	*Moberg 12253*	Chile Araucaria	EF582753	EF582804
18	*Physconia grisea*	UPS	*Mayrhofer 13888*	Italy Toscana	EF582754	EF582805
19	*P. grisea*	UPS	*Moberg 12031*	Sweden Gotland	EF582755	EF582806
20	*P.* sp.	H	*Ahti & Timofeev 64412*	Russia Sakha Rep.	EF582756	EF582807
21	*P. elegantula*	–	from GenBank		AY368119	–
22	*P. elegantula*	UPS	*Nordin 5192*	USA Arizona	EF582757	EF582808
23	*P. perisidiosa*	UPS	*Elvebakk 96:520*	Chile Ultima Esperanza	EF582758	EF582809
24	*P. venusta*	UPS	*Motiejunaite 2983*	Poland Olsztyn Co.	EF582759	EF582810
25	*P. venusta*	–	from GenBank		AY368147	–
26	*P. perisidiosa*	S	*Lohtander 600*	Finland N	AF224367	–
27	*P. detersa*	S	*Lohtander 306*	Finland N	EF582760	EF582811
28	*P. detersa*	H	*Ahti & Timofeev 64412f*	Russia Sakha Rep.	EF582761	EF582812
29	*P. distorta*	H	*Ahti 59430*	Russia Karel. Rep.	EF582762	–
30	*P. distorta*	H	*Urbanavichus 16*	Russia N. Caucasus	EF582763	EF582813
31	*P. distorta*	S	*Lohtander 313*	Finland N	EF582764	EF582814
32	*P. servitii*	UPS	*Thulin et al. 9144c*	Somalia Sanaag	EF582765	–
33	*P. subpulverulenta*		from GenBank		AY368144	–
34	*P. detersa*	LECB	*Himelb. & Kuznets. K-397*	Russia SW Kamchatka	EF582766	EF582815
35	*P. servitii*	–	from GenBank		AY368143	–
36	*P. enteroxantha*	H	*Urbanavichus T-0172*	Russia Tatarstan	EF582767	EF582816
37	*P. enteroxantha*	S	*Lohtander 302*	Finland N	AF224370	–
38	*P. enteroxantha*	H	*Urbanavichene T-0199*	Russia Tatarstan	EF582768	EF582817
39	*P. grumosa*	H	*Urbanavichus B-0425*	Russia Buryatia	EF582769	EF582818
40	*P. grumosa*	H	*Urbanavichus B-04332*	Russia Buryatia	EF582770	EF582819
41	*P. grumosa*	LECB	*Himelbrant k-382*	Russia SW Kamchatka	EF582771	EF582820
42	*P. muscigena*	UPS	*Moberg 12044*	Sweden Gotland	AF224369	–
43	*P. muscigena*	LECB	*Kuznetsova k-345*	Russia C Kamchatka	EF582772	EF582821
44	*"P. distorta"*	–	from GenBank		AY498686	–
45	*P. leucoleiptes*	–	from GenBank		AY368133	–
46	*P. kurokawae*	–	from GenBank		AY368131	–
47	*P. leucoleiptes*	MIN	*Lendemer et al. 2657*	USA New Jersey	EF582773	–
48	*P. muscigena*	–	from GenBank		AY368135	–
49	*P. muscigena*	H	*Urbanavichus 20*	Svalbard	EF582774	EF582822
50	*P. americana*	–	from GenBank		AY368111	–
51	*P. americana*	–	from GenBank		AY368114	–
52	*P. isidiigera*	–	from GenBank		AY368129	–
53	*P. isidiigera*	–	from GenBank		AY368130	–
54	*Anaptychia runcinata*	UPS	*Mayrhofer 13978*	Scotland Moray Dist.	EF582775	EF582823
55	*A. runcinata*	S	*Odelvik & Karlsson 00243*	Sweden Södermanland	EF582776	EF582824
56	*A. bryorum*	–	from GenBank		AF250781	–
57	*A. bryorum*	UPS	*Söchting 8378*	Bhutan Thimphu	EF582777	EF582825
58	*A. palmulata*	UPS	*Moberg 113336*	USA Tennessee	EF582778	EF582826
59	*A. palmulata*	H	*Ahti 58054*	USA Alabama	EF582779	EF582827
60	*A. isidiata*	LECB	*Himelbrant K-04-10*	Russia SW Kamchatka	EF582780	EF582828
61	*A. isidiata*	H	*Kashiwadani 45830*	Japan Hokkaido	EF582781	EF582829
62	*A. elbursiana*	–	from GenBank		AY368145	–
63	*A. elbursiana*	MIN	*Wetmore 81482*	USA Montana	EF582782	EF582830
64	*A desertorum*	UPS	*Moberg & Nordin K21:12*	Kazakhstan Vost. Kazakh.	EF582783	EF582831
65	*A. desertorum*	H	*Urbanavichus 5 A02*	Russia Orenburg Reg.	EF582784	EF582832
66	*A. crinalis*	S	*Wetmore 83986*	USA Michigan	EF582785	EF582833
67	*A. crinalis*	UPS	*John exs. 131*	Turkey Trabzon	EF582786	–
68	*A. ciliaris v. melanosticta*	S	*Odelvik & Karlsson 00411*	Sweden Södermanland	EF582787	EF582834
69	*A. ciliaris*	S	*Lohtander 498*	Sweden Södermanland	AF224366	
70	*A. ciliaris*	S	*Lohtander 301*	Finland N	AF224365	–
71	*A. cilaris*	H	*Haikonen 24158*	Finland Ab	EF582788	EF582835

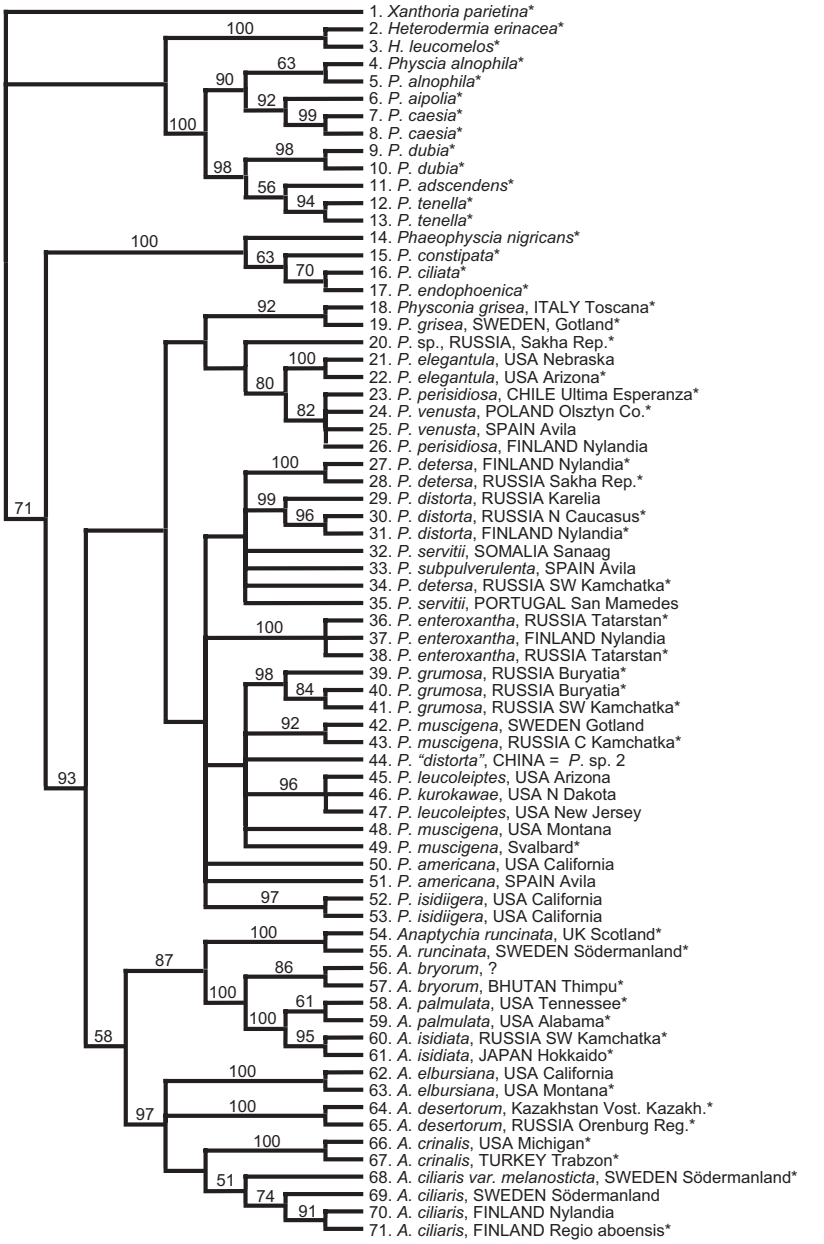

Fig. 1. A strict consensus of 3826 equally parsimonious trees based on ITS and mtSSU sequence data. Bootstrap support > 50% is shown at nodes. An asterisk indicates a specimen having both ITS and mtSSU rDNA regions sequenced. Otherwise only ITS region is present in the data set.

(Thompson *et al*. 1994) alignment program with default parameter settings. The phylogenetic trees were obtained by using the heuristic search option in PAUP 4.0b10 (10 replicates), with random addition sequence (Swofford 2000). Support for each node was estimated using bootstrapping (5000 repetitions), as implemented in PAUP. Gaps were coded as missing data due to long indels in the sequence data. Some species from other genera of Physciaceae (including *Heterodermia*) were included in the study and *Xanthoria parietina* was used as outgroup.

In addition to the combined analysis, the data sets were also analysed separately in order to compare the information provided by the different gene regions. Fifty specimens, having both the ITS and mtSSU rDNA regions sequenced, were included into these analyses.

Results

The combining of all available molecular information from all the specimens (two gene regions and 71 specimens altogether) resulted in a data set with 1855 characters (ITS 631 char. and mtSSU rDNA 1224 char.) of which 412 were informative (209 informative characters in ITS; 209 in mtSSU rDNA). The phylogenetic analysis resulted in 3826 equally parsimonious trees with a length of 1371 steps and CI of 0.52.

In the strict consensus tree (Fig. 1) *Anaptychia* and *Physconia* formed a monophyletic group with 93% bootstrap support. As in previous studies (Lohtander *et al*. 2000, Grube *et al*. 2001, Helms *et al*. 2003) *Heterodermia* was found more closely related to *Physcia* than to *Anaptychia* (Fig. 1). The phylogeny of *Physconia* appeared as similar to that in Cubero *et al*. (2004). However, there was less than 50% support for the *Physconia* group.

Anaptychia formed a monophyletic sister group to *Physconia* but that grouping had only 58% support. *Anaptychia* consisted of two major groups (*see* Fig. 1). One of the groups included *A. runcinata* as a sister group to a clade consisting of *A. bryorum*, *A. palmulata* and *A. isidiata* (syn. *A. isidiza*). This clade formed a sister group to a clade consisting of *A. elbursiana*, *A. desertorum* (syn. *A. ulotrichoides*; Urbanavichus & Kulakov 2008), *A. crinalis* and *A. ciliaris*.

There were four specimens of *A. ciliaris* and although collected from Fennoscandia only, they had a lot of variation in their sequences. The maritime *A. ciliaris* var. *melanosticta* appeared as a sister group to the rest of the *A. ciliaris* specimens, and is possibly an acceptable taxon (not accepted by Moberg 2002, for instance).

The separate analyses (ITS and mtSSU rDNA, respectively) including 50 specimens (trees not shown) had rather similar tree topologies. In the ITS analysis (tree not shown) *Anaptychia* and *Physconia* formed a monophyletic group, but that grouping had no support. Furthermore, *Anaptychia* formed a monophyletic group with a bootstrap support less than 50%. The tree based on the mtSSU rDNA data had 74% support for the group including *Anaptychia* and *Physconia*, and 60% support for the *Anaptychia* group. All *Physconia* specimens excluding *P. muscigena* formed a monophyletic group in the mtSSU rDNA tree and had 77% support. The combined analysis (tree not shown; note that this tree is not the same than that in Fig. 1, since only 50 specimens, including both gene regions, are included) resulted in a tree that had a better resolution and support than any of the trees previously mentioned. *Anaptychia* and *Physconia* formed a monophyletic group with 95% support, while *Anaptychia* had a bootstrap support of 68%. In the combined tree *Physconia* appeared as monophyletic, but lacked support.

Discussion

The combined ITS and mtSSU rDNA sequence data sets contained enough information to reveal the phylogenetic relationships of *Anaptychia* and *Physconia* despite of the fact that *Physconia* did not form a monophyletic group in the separate analyses based on ITS and mtSSU rDNA data sets, respectively (trees not shown). Both *Anaptychia* and *Physconia* formed monophyletic groups in the small (tree not shown) and in the large (Fig. 1) combined data set. The phylogeny of the genus *Physconia* is already thoroughly treated in the paper of Cubero *et al*. (2004) and it will not be further discussed in this paper. However, specimen 20 (*P.* sp. 1; Fig. 1) that appeared as a sister group to *P. elegantula*, *P. perisidiosa* and *P. venusta* did not correspond to any *Physconia* species described so far and is clearly an undescribed species (G. P. Urbanavichus unpubl. data).

Anaptychia formed two large groups in the phylogenetic analysis (Fig. 1). The tree topology (Fig. 1) correlates rather well with some morphological characters in *Anaptychia*. The group including *A. runcinata*, *A. bryorum*, *A. palmulata* and *A. isidiata* (specimens 54–61; Fig. 1) corresponds to the series *Aquilae* of Kurokawa (1973), which is characterized by dark brown to greenish olive thalli and smooth upper surface of the cortex. According to Kurokawa (1973) *A. isidiata* might have derived from *A. palmulata* by the formation of isidia. Our results are not in conflict with that hypothesis, since *A. isidiata* and *A. palmulata* form a monophyletic group.

The other group (Fig. 1) is comprised of specimens having an irregularly thickened upper

cortex. *Anaptychia elbursiana* and *A. desertorum* belong to the section *Protoanaptychia* (Kurokawa 1973, Urbanavichus & Kulakov 2008), having a greyish-white thallus. *A. ciliaris* of the series *Ciliares* groups together with *A. crinalis*. Also both *A. setifera* and *A. ethiopica* are probably closely related to *A. ciliaris* (*see* Kurokawa 1973, Swinscow & Krog 1976), but unfortunately we had no fresh specimens of these species and therefore could not confirm their relatedness to *A. ciliaris* by using molecular methods.

There was much variation in the DNA sequences of *A. ciliaris*. Kurokawa (1962) recognized five forms of *A. ciliaris* (*A. ciliaris* f. *ciliaris*, *A. ciliaris* f. *agriopa*, *A. ciliaris* f. *verrucosa*, *A. ciliaris* f. *melanosticta* and *A. ciliaris* f. *nigrescens*), based on differences in lobe width, the abundance of pycnidia and colours of the verrucae, but he reduced the forms to synonyms in 1973. The sequence variation may correspond to some formae of Kurokawa, but several specimens of each form must be sequenced to confirm that.

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