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Title: The sister group relationships of the largest family of lichenized fungi, Parmeliaceae (Lecanorales, Ascomycota)

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Abstract

Parmeliaceae is the largest family of lichen-forming fungi. In spite of its importance for fungal diversity, its relationships with other families in Lecanorales remain poorly known. To better understand the evolutionary history of the diversification of lineages and species richness in Parmeliaceae it is important to know the phylogenetic relationships of the closest relatives of the family. A recent study based on two molecular loci suggested that either *Protoparmelia* s. str. or a group consisting of *Gypsoplaca* and *Protoparmelia* s. str. were the possible sister group candidates of Parmeliaceae, but that study could not distinguish between these two alternatives. Here, we used a 4-locus phylogeny (nuLSU, ITS, *RPB1*, *MCM7*) to reveal relationships of Parmeliaceae with other potential relatives in Lecanorales. Maximum likelihood and Bayesian analyses showed that *Protoparmelia* is polyphyletic, with *Protoparmelia* s. str. (including *P. badia* and *P. picea*) being most closely related to Parmeliaceae s. str., while the *P. atriseda*-group formed the sister group to *Miriquidica*. *Gypsoplaca* formed the sister-group to the Parmeliaceae s. str. + *Protoparmelia* s. str. clade. Monophyly of *Protoparmelia* as currently circumscribed and *Gypsoplaca* as sister-group to Parmeliaceae s. str. were both significantly rejected by alternative hypothesis testing.

Keywords: Lichen-forming fungi, *Miriquidica*, phylogeny, *Protoparmelia*, secondary metabolites

1 Introduction

Parmeliaceae is the largest family of lichen-forming fungi, consisting of over 2700 species (Thell et al. 2012). The family includes well-known foliose and fruticose lichens, such as beard-lichens (*Usnea*) and species that are frequently used in monitoring of air pollution, e.g. *Parmelia sulcata* and *Flavoparmelia caperata* (Nimis et al. 2002; Crespo et al. 2004). The

species richness of a clade may be explained by the evolution of key innovative characters that confer adaptive advantages and lead to adaptive radiation (Sanderson and Donoghue 1994). One way to study key innovations is to compare evolutionary changes in sister clades. These traits affect the rate of lineage diversification and are expected to leave an imprint in the phylogeny of the affected group (Ree 2005). Thus one prerequisite for identifying such a character is elucidating the phylogenetic relationships of the group of interest (Ridley 1983; Pagel and Harvey 1988). Recently, a number of phylogenetic studies enhanced our knowledge of evolution and phylogenetic relationships within Parmeliaceae (Crespo et al. 2010, 2011; Thell et al. 2012). All genera currently accepted in Parmeliaceae in a wide sense (including the sometimes recognized Anziaceae, Alectoriaceae, Hypogymniaceae and Usneaceae) form a well-supported monophyletic group in all major published analyses (Persoh and Rambold 2002; Crespo et al. 2007 Arup et al. 2007). However, the relationships of Parmeliaceae with other groups in Lecanorales are poorly known. The study by Arup et al. (2007), based on mtSSU and nuLSU rDNA sequences focused on the sister group relations of Parmeliaceae within Lecanorales. This study (Arup et al. 2007) included all known potential relatives to Parmeliaceae (e.g. *Gypsoplaca*, *Japewia*, *Mycoblastus*, *Protoparmelia*, *Tephromela* and *Calvitimela*), and concluded that *Protoparmelia* and Gypsoplacaceae were the two most closely related groups, with high support. Arup et al. (2007) compared the probabilities of the three possible alternative topologies and concluded that Gyposoplacaceae was clearly unlikely to be the closest relative to Parmeliaceae s. str. However, these authors suggested their data were not sufficient to differentiate between the scenario where *Protoparmelia* was sister to Parmeliaceae, and where (*Protoparmelia*+*Gypsoplaca*) was the sister. Crespo et al. (2007) likewise showed a supported relationship of *Protoparmelia badia* as a close relative to Parmeliaceae, but here Gypsoplacaceae was not included. Furthermore, “*Lecidea*” *rubrocastanea* was proposed to be close to *Protoparmelia* (Spribille and Printzen 2007) but there was no support for this relationship. In a recent study (Pamong et al. 2011), which

included *Lecidea rubrocastanea*, *Protoparmelia*, *Maronina* and other Parmeliaceae genera, the authors showed that “*Lecidea*” *rubrocastanea* neither belongs to *Protoparmelia* nor to the Parmeliaceae but falls outside of both groups. Additionally, the genus *Maronina* was considered a morphologically close relative of *Protoparmelia* (Hafellner and Rogers 1990). However this genus has recently been synonymized within *Protoparmelia*, based on molecular and morphological data (see Papong et al. 2011).

Protoparmelia has indeed been suggested to be better classified within the Parmeliaceae by several authors (Miyawaki 1991; Henssen 1995; Lumbsch and Huhndorf 2010). The genus was resurrected by Hafellner (1984) for species previously included in *Lecanora* but differing in details of the amyloid staining of the ascus apex, brown pigmentation, and lack of atranorin. The genus was placed in Lecanoraceae. Subsequently, studies on apothecial anatomy showed the presence of a cupular exciple in the type species, *P. badia*, which is typical of Parmeliaceae (Miyawaki 1991; Henssen 1995). However, *Protoparmelia* as currently circumscribed has also been shown to be heterogeneous and chemically diverse (Ryan et al. 2004). For example, *Protoparmelia badia* and *P. picea* have lobaric acid (Hertel 1984) whereas *P. cupreobadia* and *P. atriseda* contain norstictic acid as major secondary metabolites. Further, some species in *Protoparmelia* have bacilliform conidia, while others have filiform conidia, similar to the genus *Miriquidica* (Ryan et al. 2004). Thus, the monophyly and circumscription of *Protoparmelia* remained uncertain.

Here we use an extended taxon sampling and a data set including four loci to address the following questions: i) Which lineage is most closely related to Parmeliaceae? ii) Is *Protoparmelia* monophyletic, and if not, what are the phylogenetic relationships of the lineages within the genus?

2 Materials and methods

2. 1. Sampling

A total of 54 taxa from four families were sampled: Cladoniaceae, Gypsoplacaceae, Lecanoraceae and Parmeliaceae s. str. Members of the Cladoniaceae were selected as outgroup for the analysis because previous studies suggested that these families are closely related to Parmeliaceae (Miadlikowska et al. 2006; Crespo et al. 2007). Details of the studied material, including GenBank accession numbers are shown in Table 1.

2. 2. DNA extraction and molecular methods

Genomic DNA was extracted from lichen thalli using the CTAB method (Cubero and Crespo 2002). PCR amplification was performed using general, previously published or taxon-specific primers for ITS2, *MCM7*, *RPB1* and nuLSU (Table 2). PCR reactions were carried out in a volume of 25 µl. Each reaction mix contained 2.5 µl buffer, 0.13 µl (= 0.65 U) Ex Taq polymerase 1.0 µl dNTP mix (2.5 mM each), 1.0 µl each (10 µM) of the primer set (forward and reverse), ca. 20 ng of template, and 16 µl H₂O. Reactions were performed with the following cycling conditions: initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 40 s, 72 °C for 1 min, and final elongation at 72 °C for 5 min. PCR products were checked for amplification on 1 % agarose gels. Bands of the expected size were extracted using the peqGOLD Gel Extraction Kit (PEQLAB Biotechnologie GmbH). These fragments were then labeled for cycle sequencing using Big Dye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems) and sequenced as follows: (1) 1 min 96 °C, (2) 26 cycles of 20 s 96 °C, 5 s 50 °C, and 2 min 60 °C. Products were purified using the Big Dye XTerminator Purification Kit (Life Technologies) and then detected on ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

2. 3. Phylogenetic analyses

Sequences were assembled using Geneious v. 5.4 (Drummond et al. 2011) followed by manual editing. Sequences from 54 species were aligned for each locus separately using MAFFT (Kato et al. 2005). Gaps were treated as missing data and ambiguously aligned parts were excluded. The program Gblocks v. 0.91b (Castresana 2000; Talavera and Castresana 2007) was used to remove poorly aligned regions.

We checked for congruence of the four loci by performing CADM analysis (Campbell et al. 2011). Data of congruent loci ($p < 0.001$) were concatenated. Maximum likelihood (ML) analysis was performed on the single-loci using RAxML v. 7.0.4 (Stamatakis 2006) using the default GTR+G model, the standard model implemented in RAxML, for all loci, with 1000 bootstrap (BS) replicates. Conflicts were considered significant if individuals group in a clade supported by $> 75\%$ ML BS support in data set from one locus, but in a different supported clade in data set from another locus. Maximum likelihood search was performed on the concatenated 4-locus dataset with RAxML-HP BlackBox v. 7.2.8 (Stamatakis et al. 2008) on the Cipres Scientific gateway v. 3.3 (www.phylo.org; Miller et al. 2010) using the default GTR+G model with data partitioning according to the different genes (www.phylo.org). For *RPB1* and *MCM7* data were also partitioned by codon position.

We used the Corrected Akaike Information Criterion (AICc) (Sugiura 1978; Hurvich and Tsai 1989) as implemented in JmodelTest v. 2.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012) to find the appropriate model for each locus. Bayesian tree inference was carried out, using the best fitting model, for both single-locus and 4-locus concatenated data set as implemented in MrBayes v. 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Two parallel MCMCMC runs were performed each using four chains and 5,000,000 generations, sampling trees every 100th generation. A 50 % majority rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25 % as burn-in (12,500 trees, likelihoods below stationary level).

We used alternative hypothesis testing to test whether our data are sufficient to reject a) monophyly of *Protoparmelia*, and b) *Gypsoplaca* forming a sister-group to Parmeliaceae s. str. The constrained and unconstrained trees were inferred using the program Tree-PUZZLE 5.2 (Schmidt et al. 2002) employing the GTR+I+G nucleotide substitution model. We used two methods to compare the different topologies: the Shimodaira – Hasegawa (SH) test (Shimodaira and Hasegawa 1999) and the expected likelihood weight (ELW) test (Strimmer and Rambaut 2002).

3 Results

3. 1. DNA sequences

We generated 43 new sequences for this phylogeny, including 13 *RPB1*, 16 *MCM7*, eight nuLSU and seven ITS sequences. The data sets include 118 sequences from previous publications by PARSYS working group (Crespo et al. 2007, 2010, Divakar et al. 2012) and 13 downloaded from GenBank. A total of 54 taxa were analyzed including 36 representatives of the family Parmeliaceae s. str., five *Protoparmelia* species, seven Lecanoraceae, five Cladoniaceae and the only representative of Gypsoplacaceae, i.e. *Gypsoplaca macrophylla*.

3. 2. Phylogenetic analysis

CDAM results showed no significant incongruence among loci, thus allowing concatenation. Only specimens with sequence information available for at least two loci were included in the analysis. The concatenated 4-locus data set contained 54 sequences of the following lengths: 689 bp for *RPB1*, 616 bp for *MCM7*, 851 bp for nuLSU rDNA and 534 bp for ITS rDNA. Total length of the concatenated alignment was 2693 bp. The ML tree for the concatenated data set is presented in Fig. 1.

Partition finder showed that the model of sequence evolution was different for each locus. According to JModelTest v. 2.1.1, the following best fitting models were used:

RPBI:TIM2ef+I+G; *MCM7*: HKY+G; nuLSU: GTR+I+G; ITS2: 012343+I+G. Bayesian analysis was performed using the best fitting model for each locus in the concatenated sequence. We did not find any differences in topology between RAxML and MrBayes trees obtained from individual and concatenated data sets. Therefore only the ML tree based on the concatenated data set is presented (Fig. 1). Nodes with ML BS equal to or greater than 70 % and Bayesian posterior probability (PP) greater than 0.94, were considered as strongly supported.

The family Parmeliaceae s. str. formed a well-supported monophyletic group (BS = 100 %, PP = 1.0). *Protoparmelia badia* and *P. picea* were found to be the sister-group to Parmeliaceae s. str. with strong support from both BS (72 %) and PP (0.97). Parmeliaceae s. str., together with *Protoparmelia* s. str. and *Gypsoplaca macrophylla*, formed a well-supported group with strong support from both ML (100 %) and PP (1.0), as was previously suggested by Arup et al. (2007). Three species of *Protoparmelia*, *P. atriseda*, *P. cupreobadia*, *P. phaeonesos*, formed a monophyletic group sister to members of the genus *Miriquidica* (BS = 100 %, PP = 1.0).

Alternative hypothesis testing using the ELW and SH-tests, showed that the following hypotheses can be rejected: i) *Protoparmelia* is monophyletic ($p < 0.001$ in both tests) and ii) *Gypsoplaca* is sister to Parmeliaceae s. str. ($p < 0.001$ in both tests).

4 Discussion

Phylogenetic analyses were performed to infer the sister-group relations of Parmeliaceae with other potential relatives groups/families within Lecanorales. Our multi-locus analyses focused on representatives of three lineages, Lecanoraceae, *Protoparmelia* spp. and Gypsoplacaceae.

Parmeliaceae s. str. forms a well-supported monophyletic group, confirming the results of earlier studies (Mattsson and Wedin 1999, Persoh and Rambold 2002, Arup et al. 2007, Crespo et al. 2007, 2010). Our analyses showed that two representatives of the genus

Protoparmelia, *P. badia* and *P. picea*, form the sister-group to Parmeliaceae s. str., similar to Arup et al. (2007) and Crespo et al. (2007). Furthermore, we conclusively showed that Gypsoplacaceae is sister to this group. Our results furthermore indicate that *Protoparmelia*, as currently circumscribed, is polyphyletic. Three species (*P. atriseda*, *P. cupreobadia* and *P. phaeoesos*) that have been placed in the section *Phaeonora* (Poelt and Leuckert 1991) based on morphological characters are found in a well-supported group sister to *Miriquidica*.

The phylogenetic relationships of the heterogeneous genus *Protoparmelia* have been matter of debate and, at present, *Protoparmelia* is placed within Parmeliaceae. Morphological and anatomical characters of this genus are difficult to interpret as they show similarity to both Lecanoraceae and Parmeliaceae. Interestingly, the two separate groups of *Protoparmelia* species as circumscribed in this study have different secondary metabolite profiles. The predominant compound found in *P. badia* and *P. picea* is lobaric acid (Hertel 1984), which is rare in Lecanoraceae. On the other hand, *P. atriseda*, *P. cupreobadia* and *P. phaeonesos* lack lobaric acid and the major compound found in *P. atriseda* and *P. cupreobadia* is norstictic acid. The relationship of the *P. atriseda*-group and *Miriquidica* is also supported by the presence of filiform conidia in both species groups.

It is interesting to note here that the sister groups of the largest family of lichenized fungi are species-poor; *Protoparmelia* s. str. has few species and Gypsoplacaceae is monotypic (Timdal 1990). Disparity in species richness of closely related clades is often explained by the evolution of key innovative characters. We need more detailed studies on morphological characters in the genus *Protoparmelia* to understand which characters are potentially involved in adaptive radiation of Parmeliaceae. This is beyond the scope of present study and will be subject of future investigations.

In conclusion, our multilocus phylogeny indicates that i) *Protoparmelia* s. str. forms a sister-group to Parmeliaceae s. str., ii) Gypsoplacaceae is the closest relative of the

Protoparmelia s. str.-Parmeliaceae s. str. clade, and iii) *Protoparmelia* is polyphyletic and the separation into two monophyletic lineages is supported by phenotypic characters.

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Figure Legends:

Fig 1 — Phylogeny of the Parmeliaceae and related taxa. This is a maximum likelihood tree based on a concatenated alignment of nuLSU rDNA, ITS rDNA, *RPB1*, *MCM7*. ML bootstrap support values > 80 %, based on 1000 replicates, are indicated below branches. Bold branches indicate posterior probabilities > 0.95 from a Bayesian analysis.

Table captions:

Table 1 Specimens used in this study including voucher information, and GenBank accession numbers. Herbarium acronyms follow Thiers (2012).

Table 2 Primers used in this study. For the genera *Protoparmelia* and *Miriquidica* we designed taxon-specific primers.

Table 1

Species	Voucher Information ^a	Accession number ^b			
		nuLSU	MCM7	RPB1	ITS
<i>Alectoria ochroleuca</i>	-	DQ899288	n/a	n/a	DQ979997
<i>Alectoria ochroleuca</i>	Sweden, Härjedalen, <i>Wedin 6542</i> (UPS)	n/a	XXX	DQ923677	n/a
<i>Austroparmelia pruinata</i>	-	EF042914	JX974675	GU994680	EF042905
<i>Cetraria islandica</i>	-	AY340539	JX974677	DQ923685	AF117995
<i>Cetraria nigricans</i>	Canada, Nunavut, <i>Westberg 2377</i> (LD)	JN000257	XXX	JN000287	AF254629
<i>Cetrariastrum andenze</i>	-	GQ919245	GQ272429	GU994690	GQ919269
<i>Cetrariastrum dulitens</i>	-	GQ919246	GQ272427	GU994691	GQ919270
<i>Cetrariella commixta</i>	Finland, Southern Finland, Haikonen 19093 (H)	JN000260	XXX	JN000290	AF451796
<i>Cetrariella delisei</i>	-	DQ923657	JX974679	n/a	DQ980005
<i>Cetrariella delisei</i>	Sweden, Jämtland, <i>Wedin 8465</i> (S)	n/a	n/a	XXX	n/a
<i>Cladia aggregata</i>	-	GQ500966	HM441287	n/a	GQ500917
<i>Cladia dumicola</i>	-	GQ500968	HM441281	n/a	GQ500915
<i>Cladia schizopora</i>	Australia, HTL 19994c (F)	GQ500952	HM441290	XXX	GQ500919
<i>Cladonia rangiferina</i>	-	AY300832	n/a	DQ915595	AY300881
<i>Emodomelanelia masonii</i>	-	GU994595	JX974681	GU994695	GU994549
<i>Everniastrum nepalense</i>	-	AY607783	n/a	EF092106	AY611071
<i>Everniopsis trulla</i>	-	EF108290	GQ272396	EF105429	EF108289
<i>Flavoparmelia marchantii</i>	-	GU994598	GQ272420	GU994698	DQ299905
<i>Flavoparmelia soredians</i>	-	AY584835	JX974684	EF092108	AY586562
<i>Gowardia nigricans</i>	-	DQ923649	n/a	n/a	DQ979996
<i>Gowrdia nigricans</i>	Norway, Troms, <i>Wedin 7297</i> (UPS)	n/a	XXX	DQ923676	n/a
<i>Gypsoplaca macrophylla</i>	-	DQ899298	n/a	n/a	n/a
<i>Gypsoplaca macrophylla</i>	USA, Utah, R.W. Rosentreter 15995 (F)	n/a	n/a	XXX	XXX
<i>Hypogymnia vittata</i>	-	DQ900637	n/a	DQ923689	DQ980012
<i>Hypogymnia vittata</i>	Sweden, Västerbotten, <i>Wedin 6814</i> (UPS)	n/a	XXX	n/a	n/a
<i>Hypogymnia physodes</i>	-	AY756338	n/a	AY756407	AF058036
<i>Hypogymnia physodes</i>	Sweden, Jämtland, <i>Wedin 6623</i> (UPS)	n/a	XXX	n/a	n/a
<i>Lecanora carpinea</i>	-	DQ787363	n/a	n/a	AY541248
<i>Lecanora hybocarpa</i>	-	EF105421	n/a	EF105430	EF105417
<i>Lecanora paramerae</i>	-	EF105422	n/a	EF105431	EF105418
<i>Lecanora sulphurea</i>	-	EF105423	n/a	EF105432	AF070030
<i>Melanelia hepatizon</i>	-	DQ923667	JX974678	DQ923692	DQ980016
<i>Melanelixia fuliginosa</i>	-	AY607801	JX974686	EF092116	AY611089
<i>Melanelixia subaurifera</i>	-	AY607811	JX126390	EF092120	AY611095
<i>Melanohalea elegantula</i>	Spain, Madrid, <i>Crespo s.n.</i> (MAF-Lich 10231)	AY607806	n/a	XXX	AY611094
<i>Melanohalea exasperata</i>	-	AY607793	n/a	EF092123	AY611081
<i>Menegazzia terebrata</i>	Sweden, Gästrikland, <i>Wedin 4392</i> (UPS)	DQ899304	XXX	DQ923694	DQ980019
<i>Metus conglomeratus</i>	Australia, Tasmania, H.T. Lumbsch 19982b (F)	GQ500958	HM441294	XXX	GQ500912
<i>Miriquidica complanata</i>	Poland, Karkonosze Mts, <i>Szczepańska 935</i> (herb. <i>Szczepańska</i>)	XXX	XXX	XXX	XXX
<i>Miriquidica garovaglii</i>	Slovakia, Karpaty Mts, <i>Szczepańska 538</i> (herb. <i>Szczepańska</i>)	XXX	n/a	XXX	XXX

<i>Miriquidica leucophaea</i>	Poland, Kossowska 448 (herb. Kossowska)	XXX	XXX	XXX	XXX
<i>Montanelia disjuncta</i>	Sweden, Lycksele Lappmark, Wedin 7143 (UPS)	DQ923666	JX974699	DQ923691	DQ980015
<i>Montanelia soledata</i>	-	GU994604	JX974704	GU994706	GU994556
<i>Myelochroa irrugans</i>	-	AY607815	JX974708	EF092128	AY611103
<i>Nephromopsis leucostigma</i>	Bhutan, Thimpu District, Søchting 9151 (LD)	JN000267	XXX	JN000295	AF451777
<i>Parmelina quercina</i>	-	AY607818	n/a	EF092136	n/a
<i>Parmelia saxatilis</i>	-	AY300849	JX974709	DQ923695	AF058037
<i>Parmotrema reticulatum</i>	-	AY584848	JX974712	GU994729	AY586577
<i>Protoparmelia atriseda</i>	USA, Washington, McCune, H. Ponzetti 26046 (OSU)	XXX	XXX	XXX	XXX
<i>Protoparmelia badia</i>	Austria, Kärnten, Hafellner, Muggia, Hafellner 68478 (GZU)	XXX	XXX	XXX	XXX
<i>Protoparmelia cupreobadia</i>	USA, Maine, Fryday 863 (MSC)	XXX	XXX	XXX	XXX
<i>Protoparmelia phaeonesos</i>	Norway, Buskerud, Rui, E. Timdal 11000 (O)	XXX	XXX	XXX	XXX
<i>Protoparmelia picea</i>	Norway, Sør-Trøndelag, Haugan 9612 (O)	XXX	XXX	XXX	XXX
<i>Pseudephebe pubescens</i>	-	AY607839	n/a	EF092148	n/a
<i>Relicina subnigra</i>	-	AY785267	n/a	EF092152	n/a
<i>Tuckermannopsis chlorophylla</i>	Sweden, Västerbotten, Wedin 6995 (UPS)	DQ923674	XXX	DQ923697	DQ980025
<i>Vulpicida pinastri</i>	-	DQ923675	JX974721	DQ923698	AF058039
<i>Xanthoparmelia conspersa</i>	-	AY578962	n/a	EF092155	n/a
<i>Xanthoparmelia tinctina</i> 1	Spain, Madrid, Crespo s.n. (MAF-Lich 6070)	AY578976	JX974720	XXX	AY581108

a Herbarium acronyms follow Thiers 2012.

b new sequences are presented in bold.

Table 2

Taxon	Locus	Primer name	Sequence	Reference
<i>Protoparmelia</i>	<i>RPB1</i>	gRPB1Af (FOR)	GADTGTCCDGGDCATTTTGG	Stiller and Hall (1997)
		fRPB1cR (REV)	CNGGCDATNTCRTTRTCCATRTA	Matheny et al. (2002)
		RPB1PPspf (FOR)	GTGCTTTGCTTCAGCAGTGCTC	This study
		RPB1PPspr (REV)	AGCGACGAACATTGCCGTTTCGCAC	This study
	<i>MCM7</i>	MCM7-709 (FOR)	ACIMIGITITCVGAYGTHAARCC	Schmitt et al. (2009)
		MCM7-1348 (REV)	GAYTTDGCACICCGGRTCWCCCAT	Schmitt et al. (2009)
		MCM7PPspf (FOR)	GAICGDTGIGGITRIGARRTITTIC	This study
		MCM7PPspr (REV)	GIIARRTAITCRTACATGKIRCC	This study
	nuLSU	AL1R (FOR)	GGGTCCGAGTTGTAATTTGT	Döring et al. (2000)
		LR6 (REV)	CGCCAGTTCTGCTTACC	Vilgalys and Hester (1990)
		LR5 (FOR)	TCCTGAGGGAAACTTCG	Vilgalys and Hester (1990)
		LROR (FOR)	ACCCGCTGAACTTAAGC	Vilgalys and Hester (1990)
		L3 (REV)	CCGTGTTTCAAGACGGG	Vilgalys and Hester (1990)
		NULSUPPspf (FOR)	GAAACCCCTTCGACGAGTCGAG	This study
		NULSUPPspr (REV)	AGATGGTTCGATTAGTCTTTTCG	This study
		ITS	ITS1-F (FOR)	CTTGGTCATTTAGAGGAAGTAA
	ITS2 (REV)		GCTGCGTTCCTCATCGATGC	White et al. (1990)
	ITS3 (FOR)		GCATCGATGAAGAACGCAGC	White et al. (1990)
	ITS4 (REV)		TCCTCCGCTTATTGATATGC	White et al. (1990)
	<i>Miriquidica</i>	<i>RPB1</i>	RPB1MIRIf (FOR)	CTACAGATGATATCAAGCTCATG
RPB1MIRIr (REV)			CATGAGCTTGATATCATCTGTAG	This study
<i>MCM7</i>		MCM7MIRIf (FOR)	CAATTTACTCCAATGACTGAATGTC	This study
		MCM7MIRIr (REV)	CATGCCGTCGCCTATCTCCTTAGTC	This study
ITS		ITSMIRIf (FOR)	TATCGAGTGGAGGGGCTTCGCTC	This study
		ITSMIRIr (REV)	TAACGTTTAGGCGGTTGTTGGC	This study

Fig 1