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Disentangling the taxonomic structure of the *Lepidoderma chailletii-carestianum* species complex (Myxogastria, Amoebozoa): genetic and morphological aspects

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Summary

Phylogenetic relationships within the taxonomically difficult species complex *Lepidoderma chailletii-carestianum* (Myxogastria, Amoebozoa) were investigated by partial sequences of three independent genetic markers (18S rRNA, elongation factor 1 alpha, and cytochrome c oxidase subunit 1 genes). The resulting phylogenies were largely congruent in their topologies. Together with the analysis of morphological traits of fruiting bodies, the results supported the hypothesis that *L. chailletii* and *L. carestianum* are two separate valid species. Within the morphospecies *L. chailletii*, two clades were found that are quite divergent from *L. chailletii sensu stricto*, one of them clustering closely with *L. alpestroides* but morphologically differing from it. The second part of the cytochrome oxidase subunit 1 gene showed significant variation due to RNA editing; this mitochondrial genetic marker is likely useful for barcoding and phylogenetic reconstructions in dark-spored myxomycetes.

Key words: Amoebozoa, Myxogastria, myxomycetes, molecular phylogenetics, nivicolous myxomycetes, slime molds, cryptic speciation

Introduction

Myxomycetes (or Myxogastria), also called plasmodial slime molds, represent a monophyletic group of protists within Amoebozoa (Adl et al. 2005, 2012; Ruggiero et al., 2015) characterized by the alternation of amoeboflagellate and plasmodial vegetative stages and by the ability to form complex spore bearing structures called sporocarps (Schnittler et al., 2012). Since the sporocarps can be easily preserved in herbaria and show more morphological characters than available for most other groups of protists, the taxonomy of this group is based predominantly on the morphological species concept (Clark, 2000; Schnittler and Mitchell, 2000). However, the classical taxonomy based solely on morphological traits appears to produce a lot of para- and polyphyletic taxa, as revealed

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in recent studies utilizing molecular phylogenetic data (e.g., *Physarum*: Nandipati et al., 2012; Liceida and Trichiida: Fiore-Donno et al., 2013; Clastodermataceae: Kretzschmar et al., 2015; *Perichaena*: Walker et al. 2015; *Tubifera*: Leontyev et al., 2015). Another problem is an often presence of several genetically distinct lineages within morphologically circumscribed species. By the use of independent markers it could be shown in several cases that these lineages are likely to be reproductively isolated, thus confirming experiments with some cultivable members of the Physarales (see Clark and Haskins, 2010). This cryptic diversity seems to be more the rule than the exception for myxomycetes, and these putative biospecies may or may not show subtle differences in fruit body morphology. Examples include two studies with 18S rRNA genes showing multiple ribotypes, sometimes with geographical restriction (*Physarum pseudonotabile*, Novozhilov et al., 2013a; *Badhamia melanospora*, Aguilar et al., 2013), and several studies using multiple markers (*Lamproderma columbinum* and *L. puncticulatum*, Fiore-Donno et al., 2011; *Trichia varia*, Feng and Schnittler, 2015; *Meriderma* spp., Feng et al., 2016; *Hemitrichia serpula*, Dagamac et al., 2016 and *Physarum albescens*, Shchepin, unpublished). Most of the latter studies revealed at least some of the putative biospecies to differ by their spore ornaments, but in no case such characters were sufficient to allow an unambiguous separation of specimens.

Many taxonomically difficult (i.e., morphologically variable) morphospecies are still waiting for being studied using genetic markers, and among them is the species complex of two dark-spored nivicolous myxomycetes, *Lepidoderma chailletii* Rostaf. and *L. carestianum* (Rabenh.) Rostaf. Due to often indistinct morphological differences, the validity of *L. chailletii* was questioned by Lister (1911) who suggested considering it as a variety of L. carestianum. Kowalski (1971) synonymized the two taxa, but in recent monographs *L. chailletii* and *L. carestianum* are usually treated as separate valid taxa (Lado, 2005–2016; Poulain et al., 2011). However, several other taxa described independently are now put into synonymy: *L. granuliferum* (W. Phillips) R.E. Fr. with *L. carestianum* (Lado and Ronikier, 2008); *L. didermoides* Kowalski and *L. aggregatum* Kowalski with *L. chailletii* (Lado, 2005–2016). In addition, the recently described *Lepidoderma alpestroides* Mar. Mey. and Poulain is often difficult to separate morphologically from *L. chailletii*.

So far, the few partial 18S rRNA gene sequences of *L. chailletii, L. carestianum* and other *Lepidoderma* species obtained in some recent studies did not show any clear separation between these species, but raised even more questions (Novozhilov et al., 2013b; Kamono et al., 2013). Here we present first results on phylogenetic relationships within the *Lepidoderma chailletii-carestianum* species complex obtained using a combined molecular phylogenetic and morphological approach, with an emphasis on genetic diversity within *L. chailletii* morphospecies.

Material and methods

DNA extraction and sequencing

Specimens of sporocarps deposited in the mycological herbarium of the Komarov Botanical Institute, Laboratory of Systematics and Geography of Fungi (LE), were used for genetic analysis, totaling 37 specimens determined morphologically as *L. chailletii* and 5 specimens determined as *L. carestianum* (Table 1). Determinations were performed according to Poulain et al. (2011). Sporocarps were frozen at −20°C and crushed using a TissueLyser LT homogenizer (QIAGEN) equipped with a steel ball. DNA was extracted with the PureLink Plant Total DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The purified DNA was used for PCR amplification of partial sequences of three independent genetic markers: 18S rRNA gene (SSU, primers S1/SU19R or S3bF/S31R), translation elongation factor EF-1 alpha (EF1A, primers PB1F/PB1R) and cytochrome c oxidase subunit 1 (COI, also referred to as cox1, primers COIF1/COIR1) genes (Table 2). Sequencing was performed using Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) on an ABI 3130 sequencer (Applied Biosystems).

Phylogenetic analysis

Chromatograms of new sequences were manually checked for base calling errors in BioEdit 7.2.5 (Hall, 1999). In total, 42 SSU, 23 EF1A and 19 COI partial gene sequences were obtained and submitted to GenBank under the accession numbers listed in Table 1. This data set was complemented with SSU sequences from *Lepidoderma* spp. available in GenBank (Table 3), including *L. alpestroides*,

Table 1. Herbarium specimens studied and accession numbers of obtained nucleotide sequences submitted to GenBank

Sample, LE	SSU	EF1A	COI	Location	Lat (N), Lon (E)
Lepidoderma chailletii					
284732	KY123401			German Alps	N/A
284811	KY123402			German Alps	N/A
285156	KY123403	KY123379		Northern Caucasus	43.43222, 41.69889
285244	KY123404		KY123359	Northern Caucasus	43.43083, 41.70528
285716	KY123405	KY123380		Northern Caucasus	43.43225, 41.69928
285948	KY123406			Leningrad region	60.40006, 30.40736
285955	KY123411	KY123400		Leningrad region	60.40006, 30.40736
285962	KY123412			Leningrad region	60.39869, 30.3985
289753	KY123416	KY123381	KY123360	Khibine mts	67.64489, 33.65708
289759	KY123434	KY123382	KY123361	Khibine mts	67.66667, 33.66667
289768	KY123433			German Alps	N/A
289795	KY123417	KY123383		Khibine mts	67.67567, 33.57781
291611	KY123413			German Alps	N/A
296490	KY123414	KY123384		German Alps	47.45804, 11.08732
296574	KY123415			German Alps	47.45310, 11.08098
296753	KY123418		KY123362	Northern Caucasus	43.43225, 41.69928
296754	KY123419	KY123385	KY123363	Northern Caucasus	43.43225, 41.69928
296818	KY123420	KY123386	KY123364	Khibine mts	67.64508, 33.63561
297153	KY123421	KY123387	KY123365	Khibine mts	67.69319, 33.58958
297213	KY123422	KY123388	KY123366	Khibine mts	67.67375, 33.67533
297277	KY123423			Valamo island	61.38394, 30.96303
297301	KY123424			Valamo island	61.40942, 31.02367
299175	KY123408			Valamo island	61.40942, 31.02367
299176	KY123410	KY123389		Valamo island	61.40942, 31.02367
299932	KY123409			Valamo island	61.38394, 30.96303
299957	KY123407			Valamo island	61.40942, 31.02367
305731	KY123425	KY123390		Chunatundra mt	67.65581, 32.61589
305839	KY123426			Chunatundra mt	67.65728, 32.61583
305854	KY123427	KY123394	KY123370	Chunatundra mt	67.66044, 32.60289
305861	KY123428	KY123395	KY123371	Chunatundra mt	67.66044, 32.60289
305864	KY123429			Chunatundra mt	67.66044, 32.60289
305867	KY123430		KY123372	Chunatundra mt	67.66044, 32.60289
305868	KY123431		KY123373	Chunatundra mt	67.66044, 32.60289
305945	KY123432	KY123399	KY123374	Khibine mts	67.66758, 33.66953
305946	KY123435	KY123396	KY123375	Khibine mts	67.68333, 33.66667
305947	KY123436	KY123398	KY123376	Khibine mts	67.68333, 33.66667
305952	KY123437	KY123397	KY123377	Khibine mts	67.68333, 33.66667
Lepidoderma carestianum					
284700	KY123438			German Alps	N/A
285143	KY123439	KY123378		Northern Caucasus	43.41667, 41.7
305765	KY123440	KY123391	KY123367	Chunatundra mt	67.65, 32.6
305798	KY123441	KY123392	KY123368	Chunatundra mt	67.65, 32.6
305805	KY123442	KY123393	KY123369	Chunatundra mt	67.65, 32.61361

L. crustaceum, L. granuliferum, L. peyerimhoffii and *Diderma fallax*. The latter was included because of its high genetic and morphological similarity with *L. peyerimhoffii* (Novozhilov et al., 2013b). Three sequences from the model species *Physarum polycephalum* (X13160.1, AF016243.1 and L14779.1) were chosen as outgroups for SSU, EF1A and COI sequence sets, correspondingly, since this species belongs to Physaraceae, the sister group to Didymiaceae, and it was the only dark-spored myxomycete with all three markers already sequenced at the beginning of this study. Sequences were aligned with MAFFT 7.294 (Katoh and Standley, 2013) using the E-INS-i strategy for SSU and the G-INS-i strategy for protein-coding genes. For the SSU alignment 28 poorly aligned and gappy positions were eliminated using GBlocks 0.91b (Castresana, 2000) with default parameters. Since mitochondrial gene transcripts in myxomycetes are subjected to insertional RNA editing (Traphagen et al., 2010), the codon structure of COI sequences was restored by aligning to the mature mRNA of *P. polycephalum* (L14769.1) and *Didymium iridis* (GU182127). The final alignments of 75 SSU, 24 EF1A and 20 COI sequences contained 333, 756 and 627 positions, correspondingly. A multigene alignment was created with sequences of all three markers of 15 specimens, again with GBlocks filtering applied to SSU sequences, and totaled 1703 positions.

Maximum likelihood analyses (ML) were performed using IQ-Tree 1.4.3 (Nguyen et al., 2015) with 1000 ultrafast bootstrap replicates (Minh et al., 2013). The substitution models for the ML reconstruction were selected independently for each marker and for each codon position in protein-coding genes with a Model Test algorithm implemented in IQ-Tree under the Bayesian information criterion. Bayesian analyses (BI) were carried out with MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003) with 2 runs and 4 million generations, trees sampled every 100 generations. The EF1A and COI alignments were partitioned by codon positions with unlinked model parameters and ratepr option set to "variable". All trees were discarded as burn-in before the two runs reached a standard deviation of split frequencies less than 0.01.

Morphological analysis

Air-dried sporocarps of 24 *L. chailletii* and 5 *L. carestianum* specimens were studied with a Zeiss Axio Imager A1 light microscope with differential interference contrast. For microscopy, sporocarps were preserved as permanent slides in polyvinyl lactophenol. The freeware program CombineZP (www.hadleyweb.pwp.blueyonder.co.uk) was used to create stacked images under a Stemi 2000 dissection microscope with AxioCam MRc5 camera. Microscopic measurements were made with AxioVision 4.8.0.0 software (Carl Zeiss Imaging Solutions GmbH). Spore diameter and ornamentation were determined for 20 spores per specimen, for each of the specimens examined.

Results

Phylogenetic analysis

The SSU phylogeny of *Lepidoderma* species revealed six distinct clades (A–F) by both ML and BI analyses (Fig. 1). Most of the sequences from *L. chailletii* and *L. carestianum* fall into two different clades (A and B, correspondingly), and the only available sequence of *L. granuliferum* appeared within *L. carestianum* clade B. Two *L. carestianum* sequences obtained from GenBank (AM231296.1 and HE614609.1) appear in clade A, indicating taxonomical misidentification; one sequence was already reassigned to *L. chailletii* (Kamono et al., 2013). Several *L. chailletii* sequences form two additional and quite divergent clades (C and D), with clade C closely related to a clade with all specimens of *L. alpestroides* (E). The clade F includes all *L. peyerimhoffii* and D. fallax sequences as well as one available sequence of *L. crustaceum*.

The EF1A and COI phylogenies (Figs 2, 3), as well as three-gene phylogeny constructed from the concatenated sequences (Fig. 4), available for only a subset of *L. chailletii* and *L. carestianum* specimens of clades A–C, showed the same separate A, B and C clades with high posterior probability and bootstrap support of major branches.

The alignment of COI sequences demonstrates the presence of 22 sites with insertional RNA editing in clades B and C, but only 21 in clade A. Of those, 21 are single-nucleotide insertion sites (20 for clade A); the remaining site is a two-nucleotide insertion. Compared to *P. polycephalum* (L14779.2), these three *Lepidoderma* clades possess 5 insertion sites that are absent in *P. polycephalum* and share all other sites with it (except for the one which is absent in clade A). The COI sequence of *L. chailletii* sample LE285244 is very divergent from all other *L. chailletii*

Table 2. Primers used in this study

Name	Sequence	Specificity, authors
S1	AACCTGGTTGATCCTGCC	SSU of dark-spored myxomycetes, Fiore-Donno et al., 2008
SU19R	GACTTGTCCTCTAATTGTTACTCG	
S3b F	TCTCTCTGAATCTGCGWAC	SSU of dark-spored myxomycetes, Hoppe and Schnittler, 2015
S31 R	AATCTCTCAGGCCCACTCTCCAGG	SSU of dark-spored myxomycetes, Dahl et al., unpublished
PB1F	ACCCGTGAGCACGCTCTCCT	EF1A of dark-spored myxomycetes, Novozhilov et al., 2014
PB1R	CGCACATGGGCTTGGAGGGG	
COIF1	CTGCWTTAATTGGTGGBTTTGG	COI of bright-spored myxomycetes, Feng and Schnittler, 2015
COIR1	ACGTCCATTCCKACWGTRTAC	

and *L. carestianum* sequences and lacks 3 insertional editing sites that are found in all other sequences, but the specimen shows a SSU sequence typical for clade A.

Morphology

The following short descriptions focus on morphological variation within the clades found, with differences referring to the comprehensive description given for clade A.

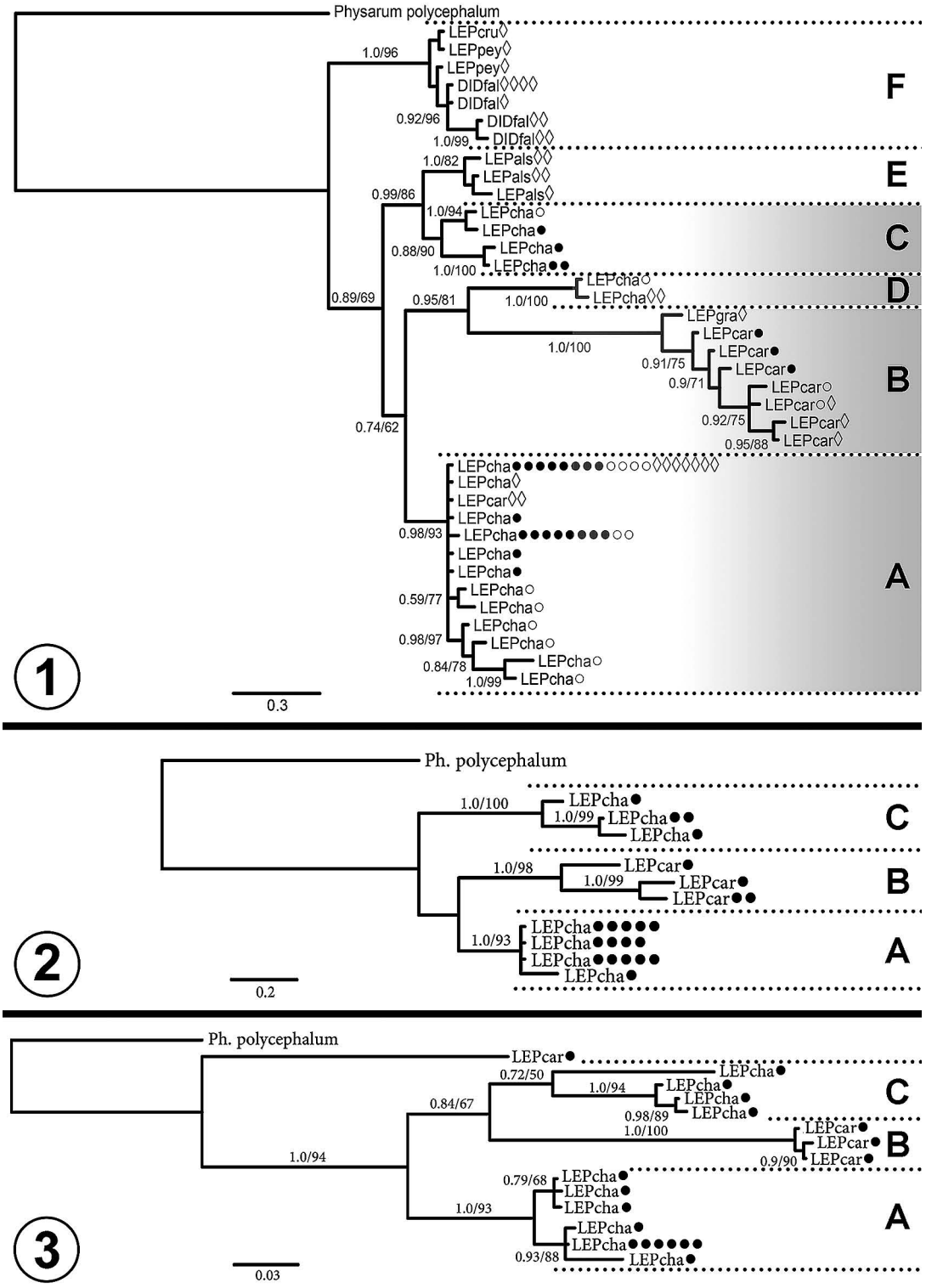
***Lepidoderma chailletii*, clade A**. Sessile sporocarps, elongate or of irregular shape, sometimes short, slightly branching plasmodiocarps (Figs 5, 6), densely grouped, almost hemispherical in cross-section, slightly constricted at the bottom, usually 1–3 mm in length. Peridium dark brown to black, with white or pale cream lime scales. Lime scales usually glossy, varying in size and density from loosely scattered flakes to a contiguous crust. Hypothallus, if present, thin, transparent and loosely covered with lime scales. Columella well-developed in some specimens, pale yellow, but often absent. Capillitium dark-brown, moderately branching and anastomosing, consisting of long threads about 1 μm diameter, almost smooth, with nodules up to 2 μm in diameter. Spores dark-brown by transmitted light, globose, covered irregularly with large spines, mean diameter (11)–12–(14.5) μm (Figs 7, 14 A); in a few spores up to 17.5 μm.

***Lepidoderma chailletii*, clade C**. Fructifications differ from those of clade A in shorter sporocarps and a smaller (sometimes absent) greyish-white columella. Lime scales never form a contiguous crust in the studied specimens (Figs 8, 9, 14 C).

L. alpestroides Mar. Mey. and Poulain, which is genetically closely related to this clade in SSU phylogeny, differs in several traits. Fructifications of *L. alpestroides* are elongate flattened plasmodiocarps with bright creamy-white lime crust on the peridium and spores are with mean diameter (12.5)–14–15–(17) μm (Poulain et al., 2011).

Table 3. Accession numbers of nucleotide sequences downloaded from GenBank

Species	GenBank accession #
Lepidoderma chailletii	AM231296.1, HE614609.1, JQ898098.1, JQ900774.1, SSU0026, SSU0027, SSU0028, SSU0029, SSU0030, SSU0025, SSU0038, SSU0039
Lepidoderma carestianum	JQ812618.1, SSU0035, SSU0036
Lepidoderma crustaceum	HE614619.2
Lepidoderma peyerimhoffii	JQ812627.1, JQ898099.1
Lepidoderma alpestroides	JQ031998.1, SSU0021, SSU0022,SSU0023, SSU0024
Lepidoderma granuliferum	SSU0034
Diderma fallax	JQ812628.1, JQ812629.1, JQ898089.1, KR029657.1, KR029658.1, KR029659.1, KR029660.1, KR029661.1
Physarum polycephalum	X13160.1, AF016243, L14769.1, L14779.1
Didymium iridis	GU182127.1



Figs 1–3. Consensus phylogenetic trees for *Lepidoderma species* (ML + BI) rooted with *Physarum polycephalum*. The number of geometric symbols indicates the number of specimens for a genotype. White diamonds are for sequences retrieved from GenBank; circles indicate specimens sequenced in this study: white – for SSU only, black – for two or three markers. Support is indicated for branches with Bayesian posterior probability > 0.7 and bootstrap support values > 50. 1 – SSU phylogeny, clades including sequences of *L. chailletii* and *L. carestianum* are shaded in grey; 2 – EF1A phylogeny; 3 – COI phylogeny. *Abbreviations of species are*: DIDfal – *Diderma fallax*, LEPals – *Lepidoderma alpestroides*, LEPcar – *L. carestianum*, LEPcha – *L. chailletii*, LEPcru – *L. crustaceum*, LEPgra – *L. granuliferum*, LEPpey – *L. peyerimhoffii*.

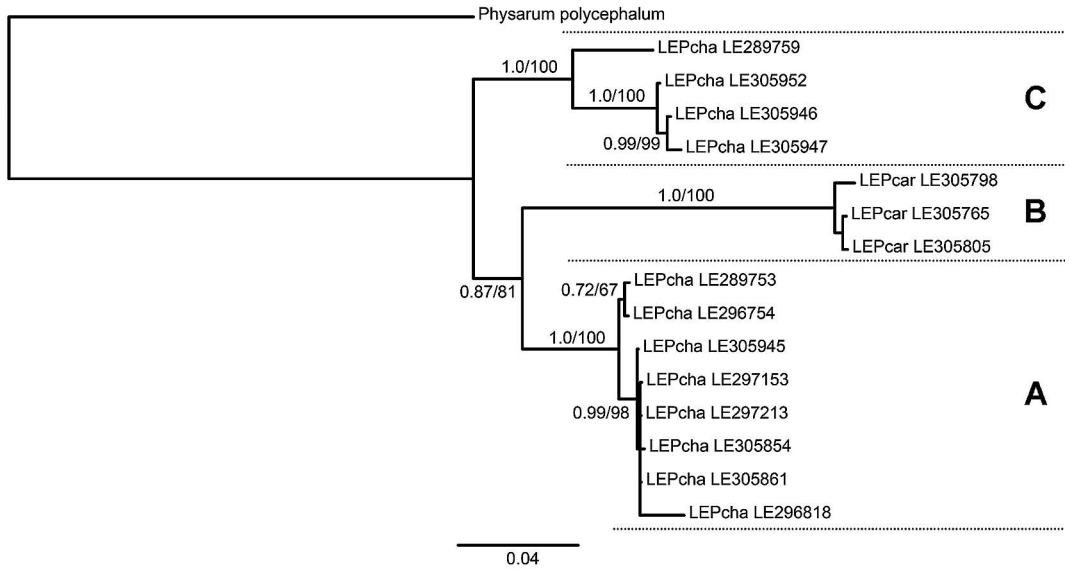


Fig 4. Three-gene phylogeny of *L. chailletii-carestianum* species complex rooted with *Physarum polycephalum*. Support is indicated for branches with Bayesian posterior probability > 0.7 and bootstrap support values > 50. For abbreviations see Figs 1–3.

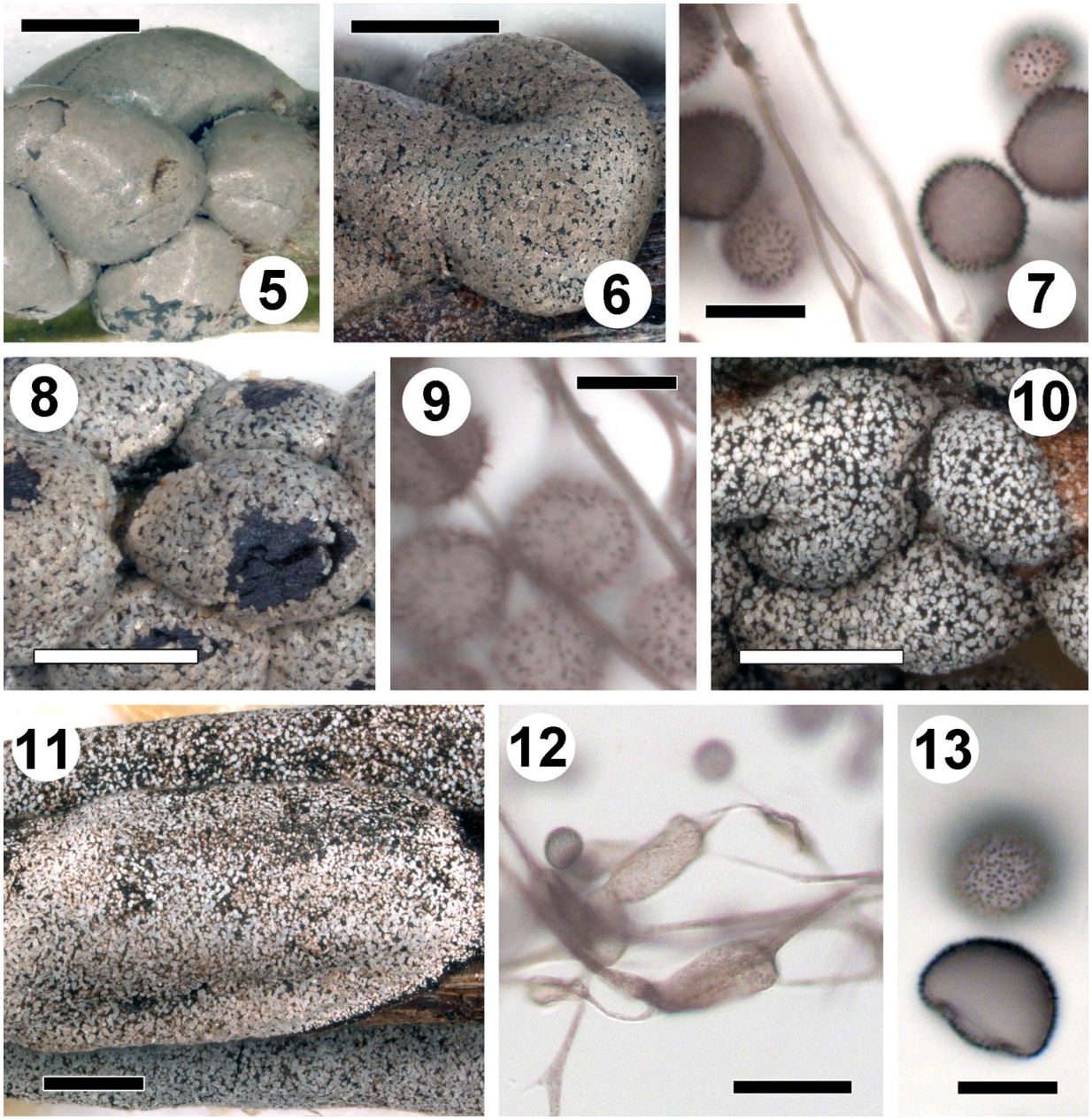
***Lepidoderma chailletii*, clade D**. The slight differences of the single available specimen in comparison to those of clade A include the more flattened sprocarps and short plasmodiocarps that are not constricted at the bottom and matt bright white lime scales (Fig. 10). A columella is absent. Spore size and ornamentation and features of capillitium do not differ from those of clade A.

***Lepidoderma carestianum*, clade B**. Prominent differences include the worm-like, flattened plasmodiocarps that are up to 10 mm long, sometimes branching. Peridium dark brown or black, densely or loosely covered with matt yellowish or whitish-grey lime scales. Capillitium dark-brown, consisting of intensively branching and anastomosing hollow tubes of irregular diameter varying approximately from two up to 10 μm, densely covered with minute warts and bearing huge nodules, sometimes up to 20 μm in width and 50 μm in length. Spores dark-brown by transmitted light, globose, densely covered with small spines, mean diameter (14.5)–15.5–(16.5) μm (Figs.11–13, 14 B), with a few spores reaching 25 μm.

Discussion

The myxomycete genus *Lepidoderma* consists of several dark-spored species, their amount varying from 9 to more than 20 according to different authors, not including forms and varieties (Lado, 2005–2016). Nearly all species are nivicolous, i.e. form their fructifications at the edge of melting snow banks in spring. Until now, there were no special studies of the phylogenetic structure of this genus, and the few partial 18S rRNA gene sequences of *L. chailletii, L. carestianum* and other *Lepidoderma* species obtained in some recent studies did not elucidate the relationships between them (Novozhilov et al., 2013b; Kamono et al., 2013).

The phylogenies obtained in this study include three independent genetic markers (extrachromosomal SSU, nuclear chromosomal EF1A and mitochondrial COI, see Feng and Schnittler, 2015 for location and inheritance modes). Together with the observed macro- and microscopic morphological traits, our data support the hypothesis that *L. chailletii* and *L. carestianum* represent two separate species. However, sequences of five specimens determined morphologically as *L. chailletii* form another distinct clade (C) for all three markers, which is genetically closely related to *L. alpestroides* but morphologically clearly differs from it. Moreover, other three specimens of *L. chailletii* fall into one more separate clade D, which is quite divergent from the main *L. chailletii* clade A but, unfortunately, only SSU sequences are available for them. As such, if *L. alpestroides* is maintained as a species, *L. chailletii* in its present circumscription



Figs 5–13. Morphological characters of *Lepidoderma* specimens. 5–7 – Fruiting bodies, spores and capillitium of *L. chailletii* clade A (LE305839, LE305946, LE297153); 8–9 – fruiting bodies, spores and capillitium of *L. chailletii* clade B (LE305952, LE289759); 10 – fruiting bodies of *L. chailletii* clade C (LE296574); 11–13 – fruiting bodies and capillitium with nodules and spores of *L. carestianum* clade B (LE285229, LE305798). Scale bars: 5, 6, 8, 10, 11 – 1 mm; 7, 9, 13 – 10 µm; 12 – 50 µm.

will not form a monophyletic clade.

We thus consider the clade A which includes most of *L. chailletii* specimens as *L. chailletii* s. str. For the other two *L. chailletii* clades (C and D), we could not find any distinct morphological characters that could help to separate them from *L. chailletii* s. str., their variation in morphological traits lies within that of *L. chailletii* s. str.; therefore, they can be seen as "cryptic species" of *L. chailletii*. Further studies using scanning electron microscopy to visualize spore ornamentation patterns and sequencing the genetic markers of other *Lepidoderma* species are needed to clarify their taxonomical status. It is possible that one of these clades is *L. aggregatum,*

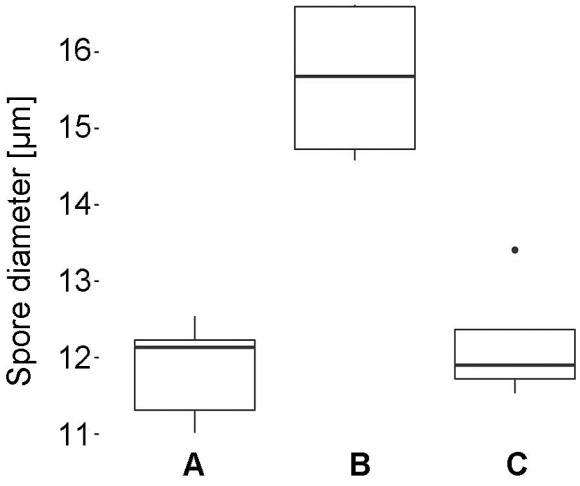


Fig. 14. Boxplot representing spore sizes within specimens of *Lepidoderma chailletii* clades A and C and *L. carestianum* (clade B). Calculations were performed for five herbarium specimens from each phylogenetic clade.

another nivicolous species which has not yet been sequenced and is synonymized with *L. chailletii* by some authors (Lado, 2005–2016). The sequence of *L. granuliferum* from GenBank appeared within *L. carestianum* clade, seemingly confirming its synonymy, but this taxon should as well be carefully investigated including multiple specimens.

All *L. peyerimhoffii* and *D. fallax* sequences obtained from GenBank cluster together into one clade. Given that and their morphological similarity noted previously (Novozhilov et al., 2013b), it is reasonable to reevaluate *D. fallax* and *L. peyerimhoffii* under the hypothesis that these taxa constitute a single polymorphic *Lepidoderma* species. Similar to the results of Nandipati et al. (2012) for the genera *Badhamia* and *Physarum*, this study underlines the need of a re-evaluation of generic delimitations, in this case for *Diderma* and *Lepidoderma*. The presence of one *L. crustaceum* sequence from GenBank in the clade formed by *D. fallax* and *L. peyerimhoffii* is most probably the result of incorrect taxonomical determination, since this species differs clearly in its morphology.

Most current phylogenetic studies of myxomycetes employ only one or two genetic markers that are usually partial SSU and EF1A sequences. However, the usage of more markers is becoming essential in modern phylogenetics to make phylogenies more reliable since the evolutionary trajectories may differ considerably between genes, even within one genome (Folk et al., 2016; Scornavacca and Galtier, 2016). In this study, the first half of the cytochrome c oxidase subunit 1 gene (COI) was evaluated as phylogenetic marker for dark-spored myxomycetes with the primers COIF1 and COIR1 developed previously by Feng and Schnittler (2015) for bright-spored species. Employing this marker as well for myxomycetes would bring these protists closer to other groups of organisms: COI is the barcode for animals and the default marker adopted by the Consortium for the Barcode of Life (http://www.barcodeoflife.org/) for all groups of organisms (Purty and Chatterjee, 2016; Coissac et al., 2016). We found that primers COIF1/COIR1 work well for both dark-spored and bright-spored species and they allowed to amplify fragments about 610 bp length in *Lepidoderma*, in which 162 positions were variable (two times more than in the studied EF1A fragment of 756 bp length), mostly at the third and the second positions of a codon. This marker has an additional informative feature: myxomycetes are unique in having insertional RNA editing of mitochondrial transcripts (Mahendran et al., 1991; Traphagen et al., 2010). The unedited mitochondrial genes have a broken (incomplete) codon structure and vary in length between species, although the length of the mature mRNA is conservative, lending evidence for a mechanism that inserts the additional bases during transcription. Aligning the gene sequence on the mature mRNA sequence can help to find these insertional editing sites that appear as gaps in the DNA sequence alignment. In the present study we have found that the clade including *L. chailletii* s. str. is additionally supported by the absence of one insertional editing site which is present in all other studied sequences from *Lepidoderma* spp. and in *Physarum polycephalum*. In contrast, five editing sites found in *Lepidoderma* spp. were absent in *P. polycephalum*. Thus, the second half of the COI gene can serve as an informative additional marker in further phylogenetic studies of both major clades of myxomycetes and, besides the commonly used SSU gene (Adl et al., 2014), may be suitable for barcoding myxomycetes.

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