Taxonomy and phylogeny of Megasporaceae (lichenized ascomycetes) in arid regions of Eurasia

Dissertation

zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I ó Biowissenschaften ó

der Martin-Luther-Universität
Halle-Wittenberg,

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Halle (Saale), 25.09.2018

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تقدیم به همسرم

به پاس قدردانی از قلبی آکنده از مهربانی و عشق

که برایم خانه ای از آرامش و آسایش را فراهم کرده

دلت زنده باشد به فرهنگ و هوش

به بد در جهان تا توانی مکوش

خرد همچو آبست و دانش زمین

بدان کاین جدا و آن جدا نیست زین

فردوسی

May thy heart live by prudence and good senses;

Do thou thine utmost to avoid all ill.

Knowledge and wisdom are like earth and water;

And should combine.

Firdowsi Tusi

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Extended summary:

The present work is a taxonomic study within the family Megasporaceae; an overview is given on history, phylogeny, taxonomy, description, delimitation and taxonomic revision of some Megasporaceae genera and species.

The genus *Aspiciliella* is reintroduced on the basis of phylogenetic and morphological studies; thus six genera are now recognized in the family Megasporaceae: the five previously accepted genera *Aspicilia* A.Massal., *Megaspora* (Clauzade & Cl.Roux) Hafellner & V.Wirth, *Lobothallia* (Clauzade & Cl.Roux) Hafellner, *Circinaria* Link and *Sagedia* Ach., and the genus *Aspiciliella* M.Choisy postulated in this work.

Molecular investigation based on three genetic markers (nrITS, nuLSU und mtSSU) of samples from a wide geographical range including Iran, Caucasia, Greece and Macaronesia revealed a strongly supported clade in a sister position to the other genera of the family Megasporaceae (PP = 1.00; MP/ML BS = 100/100). Morphological and chemotaxonomic surveys showed that the genus is characterized by a thallus that is crustose, rimose-areolate, partially continuous, K+ red; a green, olive-green to greenish-brown N+ light green epihymenium; 8-spored asci, ellipsoid, colourless, simple ascospores and small (7ó 11 m long) conidia. The genus *Aspiciliella* M.Choisy (type: *A. intermutans*) contains two species previously placed in *Aspicilia*, and a new species, *A. portosantana* Sipman & Zakeri. The new combination *A. cupreoglauca* (B.de Lesd.) Zakeri, Divakar & Otte is proposed.

The material previously reported under *A. intermutans* proved to be taxonomically heterogenous. Phylogeny at species level (species boundaries) for the *A. intermutans* complex was studied by use of the markers previously used for the recognition of this genus (nrITS, nuLSU and mtSSU), and the DNA replication license factor MCM7 for 79 samples from Europe and Asia, predominantly from Iran, Caucasus, Greece and Eastern Europe. Delineating species boundaries in closely related species or species complexes often requires a range of independent data sets and analytical methods. We used a combination of phylogenetic strategies and a variety of empirical, sequence-based species delimitation approaches to infer species boundaries in this group. This includes the poisson tree processes (PTP) model, the automatic barcode gap discovery (ABGD), the general mixed Yule coalescent model (GMYC), and the multispecies coalescent approach *BEAST and BPP. Additionally, different species delimitation scenarios were compared

using Bayes factors species delimitation analysis. Furthermore, morphological, chemical, ecological and geographical features of the sampled specimens were examined. We identified a total of six species-level lineages in the *A. intermutans* complex using inference from multiple empirical operational criteria. Generally, we found little corroboration between morphological and ecological characters with our candidate species, while secondary metabolite data could not support our candidate species. Our study of the *A. intermutans* species complex indicates that the genus *Aspiciliella*, as currently circumscribed, is more diverse in Eurasia and consequently in other parts of the world than previously recognised.

In another work, species-level phylogeny for the genus *Megaspora* was investigated using nrITS sequence data. The corticolous species *Megaspora cretacea* is described as new for science. The phylogenetic analysis revealed that *M. cretacea* clustered within the *Megaspora* clade as a sister species to *M. rimisorediata* with high support. The genus *Megaspora* comprises three species now.

Furthermore, a lectotype for *Aspicilia reticulata* and a neotype for *Aspicilliella cupreoglauca* were designed, and *A. reticulata* was synonymised with *A. cupreoglauca*. Some new records from Megasporaceae from Armenia are reported.

Key words: Lichenized fungi, Caucasus, Iran, Mediterranean, Macaronesia, *Aspicilia*, *Aspiciliella*, combined analysis, integrated taxonomy and species delimitation.

Ausführliche Zusammenfassung:

Die vorliegende Arbeit ist eine taxonomische Untersuchung innerhalb der Familie Megasporaceae; es werden Historie, Phylogenie, Taxonomie, Umschreibung und Abgrenzung der Familie Megasporaceae und der Gattungen und Arten dieser Familie dargestellt. Darüber hinaus ist die Arbeit ein Beitrag zu einer taxonomischen Revision einiger Megasporaceae-Gattungen und -Arten.

Die Gattung *Aspiciliella* wird aufgrund phylogenetischer und morphologischer Studien wieder eingeführt; somit werden in der Familie Megasporaceae jetzt sechs Gattungen anerkannt: die fünf zuvor akzeptierten Gattungen *Aspicilia* A.Massal., *Megaspora* (Clauzade & Cl.Roux) Hafellner & V.Wirth, *Lobothallia* (Clauzade & Cl.Roux) Hafellner, *Circinaria* Link und *Sagedia* Ach. sowie die in dieser Arbeit wieder postulierte Gattung *Aspiciliella* M.Choisy.

Molekulare Untersuchungen auf der Grundlage dreier genetischer Marker (nrITS, nuLSU und mtSSU) von Proben mit geographisch weit gestreuter Herkunft (Iran, Kaukasien, Griechenland und Makaronesien) zeigten für letztere Gruppe eine hohe Unterstützung für einen *Cladus* in einer Schwesterposition mit den übrigen Gattungen der Familie Megasporaceae. Morphologisch und chemisch ist die Gattung charakterisiert durch einen krustigen, zusammenhängenden, rimos-areolierten, K+ roten Thallus; ein grünes, olivgrünes bis grünlich-braunes, N+ hellgrünes Epihymenum; 8-sporige Asci; ellipsoide, farblose, einzellige Ascosporen und sehr kleine (7ó11 m) Konidien. Die Gattung *Aspiciliella* M.Choisy (Typus: *A. intermutans*) beinhaltet zwei Arten, die zuvor in *Aspicilia* platziert wurden und eine neue Art, *A. portosantana* Sipman & Zakeri. Entsprechend wurde die Neukombination *A. cupreoglauca* (B.de Lesd.) Zakeri, Divakar & Otte vorgenommen.

Das bisher unter *A. intermutans* geführte Material erwies sich als taxonomisch uneinheitlich: Die Phylogenie auf Speziesebene (Artengrenzen) für den *A. intermutans*-Komplex wurde unter Verwendung der vorher für die Erkennung dieser Gattung benutzten Marker (nrITS, nuLSU und mtSSU) und des DNA-Replikationslizenzfaktors MCM7 für 79 Proben aus Europa und Asien, überwiegend aus dem Iran, dem Kaukasus, Griechenland und Ost-Europa untersucht. Die Abgrenzung von nahe verwandten Arten in Artenkomplexen wie dem *A.-intermutans*-Komplex erfordert aufgrund der Spärlichkeit taxonomisch verwertbarer Merkmale unter den traditionell verwendeten morphologischen

und chemischen Merkmalen oft eine Reihe von unabhängigen Datensätzen und Analysemethoden. Es wurde eine Kombination aus phylogenetischen Strategien und einer Vielzahl von empirischen sequenzbasierten Methoden zur Abgrenzung der Arten verwendet. Dazu gehören das *poisson tree processes* (PTP) model, die *automatic barcode gap discovery* (ABGD), das *general mixed Yule coalescent model* (GMYC) und der Koaleszenz-Multispezies-Ansatz in * BEAST und BPP. Zusätzlich wurden verschiedene Artenabgrenzungsszenarien mit *Bayes factors species delimitation* verglichen. Darüber hinaus wurden morphologische, chemische, ökologische und geographische Merkmale der Proben untersucht. Wir konnten insgesamt sechs Abstammungslinien auf Artenebene im *A. intermutans*-Komplex identifizieren, die aus mehreren empirischen operationellen Kriterien abgeleitet wurden. Im Allgemeinen fanden wir wenig Übereinstimmung zwischen morphologischen und ökologischen Eigenschaften unserer Kandidatenspezies, während Sekundärmetabolitdaten unsere Kandidatenspezies gar nicht unterstützen konnten. Unsere Untersuchung des *A. intermutans*-Artenkomplexes zeigt, dass die Gattung *Aspiciliella*, wie sie derzeit umschrieben ist, in Eurasien vielfältiger ist, als wir erwartet hatten.

In eine andere Arbeit wurde die Phylogenie auf Speziesebene für die Gattung *Megaspora* unter Verwendung von nrITS-Sequenzdaten untersucht. Die rindenbewohnende *Megaspora cretacea* wurde als neue Art beschrieben. Phylogenetische Analysen zeigten, dass *M. cretacea* innerhalb der *Megaspora*-Clade mit hoher Unterstützung als Schwesterspezies zu *M. rimisorediata* gruppiert ist. Die Gattung *Megaspora* umfasst somit jetzt drei Arten.

Ferner wurden ein Lectotypus für *Aspicilia reticulata* und ein Neotypus für *Aspicilliella cupreoglauca* ausgewählt und *A. reticulata* wurde mit *A. cupreoglauca* synonimisiert. Einige Neufunde von Megasporaceae aus Armenien werden mitgeteilt.

Schlüsselwörter: Flechtenförmige Pilze, Kaukasus, Iran, Mittelmeerraum, Makaronesien, *Aspicilia*, *Aspiciliella*, kombinierte Analyse, integrierte Taxonomie und Artenbegrenzung.

Abkürzungsverzeichnis:

°C	Grad Celsius
ABL	Adviesbureau voor Bryologie en Lichenologie
B	Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin
BS	*bootstrap values*
C oder $Ca(OCl)_2$	Hypochloritlösung
cm	Zentimeter
CR	private herbarium of Claude Roux
DC/ TLC	Dünnschichtchoromatographie/ *thin layer chromatography*
dNTP	Desoxyribonukleosidtriphosphate
engl.	Englisch
GLM	Herbarium des Senckenberg Museums für Naturkundemuseum Görlitz
GZU	Herbarium der Karl-Franzens-Universität Graz
H	Herbarium der University of Helsinki
HPLC	Hochleistungsflüssigkeitschromatographie
ITS	*nuclear ribosomal internal transcribed spacer 1*, *5.8S* und *internal transcribed spacer 2*
K oder KOH	Kaliumhydroxid
M	Botanische Staatssammlung München
m/z	Massenzahl/Ladungzahl
MCM7	*DNA replication licensing factor MCM7*
min	Minute
ML	*maximum likelihood*
mL	Milliliter

mm	Millimeter
MP	*maximum parsimony*
MS	Massenspetrometrie
mtSSU	kleine Untereinheit der mitochondrialen ribosomalen DNA
nuLSU	große Untereinheit der nuklearen ribosomalen DNA
PCR	*polymerase chain reaction*
PL	*Západo eské muzeum*
PP	*posterior probability*
PRA	*Institute of Botany of the Czech Academy of Sciences*
PRC	*Charles University in Prague*
psi	*Pounds per square inch*
S	Herbarium des *Swedish Museum of Natural History*
s.lat.	*sensu lato*
typ. cons.	*typus conservandus*
UV	Ultraviolett
l	Mikroliter

Kapitel 1:

Allgemeine Grundlagen, Hintergründe und relevante Studien

1. **Einleitung zur Geschichte und Taxonomie**
2. **Einleitung zur allgemeine Methodik**
3. **Material und Methoden**

1.1 Einleitung zur Geschichte und Taxonomie

Die Familie Megasporaceae ist in den Trockengebieten Eurasiens weit verbreitet und es wurden zahlreiche Taxa beschrieben; nur für die Nordhemisphäre wurden etwa 400 Arten angegeben (Smith et al. 2009). Das taxonomische Verständnis dieser Flechtengruppe ist ungenügend und aufgrund der vielen ungelösten taxonomischen und auch nomenklatorischen Probleme besteht die Notwendigkeit einer gründlichen Revision (Clauzade & Roux 1984; Hafellner 1991; Nordin et al. 2007).

Die Taxonomie von Flechten basierte in der Vergangenheit zum großen Teil auf morphologischen Merkmalen (Wirtz et al. 2008). Obwohl phänotypische Merkmale wertvolle Informationen zum Verständnis der Artengrenzen liefern, können molekulare Sequenzdaten einen direkten genetischen Nachweis des Abstammungsstatus liefern, unabhängig davon, ob sich Abstammungslinien in phänotypischen Merkmalen unterscheiden, die für menschliche Beobachter offensichtlich sind (Fujita et al. 2012; Adams et al. 2014). Daher finden auch in der Flechtentaxonomie zunehmend molekulare Methoden Anwendung. In den letzten zwanzig Jahren haben molekulare Phylogenien zu erheblichen Verschiebungen der Taxonomie flechtenbildender Pilze auf allen taxonomischen Ebenen geführt (Lumbsch 2000; Lumbsch 2006; Printzen 2010; Molina et al. 2011; Jaklitsch et al. 2016).

Analytische Verbesserungen bei DNA-basierten Ansätzen unterstützen in wachsendem Maße die Erkennung der Artenvielfalt bei lichenisierten Pilzen (Leavitt et al. 2015). Dies eröffnet neue Möglichkeiten auch für die Revision einer Familie wie den Megasporaceae, deren taxonomische Gliederung allein mit klassischen phänotypischen Merkmalen großen Schwierigkeiten unterliegt. Durch die Auswertung von morphologischen, ökologischen, biogeographischen und chemischen Daten kombiniert mit molekularen phylogenetischen Analysen hat sich die Identifizierung der Artengrenzen erheblich verbessert.

Mein Vorhaben zielt darauf ab, zur Lösung einiger taxonomischer und phylogenetischer Fragestellungen in dieser Familie durch chemische, molekulare und morphologische Untersuchungen beizutragen.

Diese Dissertation konzentriert sich auf folgende Arbeiten:

1. ***Taxonomy and phylogeny of Aspiciliella, a resurrected genus of Megasporaceae, including the new species A. portosantana*****:** Das Ziel dieser Studie war es, die taxonomische Position dieser Taxa zu untersuchen und phylogenetische Beziehungen dieser Flechtenproben innerhalb der Megasporaceace aufzuklären.

2. ***Discovering cryptic species in the Aspiciliella intermutans complex (Megasporaceae, Ascomycota) using gene concatenation and coalescent-based species tree approaches*****:** Hier wollen wir die Artengrenzen im *A. intermutans* - Komplex mit einer Kombination aus phylogenetischen Strategien und einer Vielzahl von empirischen sequenzbasierten Methoden untersuchen.

3. ***Neotypification of Aspiciliella cupreoglauca and lectotypification and synonymization of Aspicilia reticulata (Megasporaceae, Ascomycota)*****:** Die unterschiedliche Nutzung der Namen *A. reticulata*, *A. intermutans* und *A. cupreoglauca* in der Literatur hat viel Verwirrung gestiftet; diese Studie klärt die korrekte Anwendung dieser Namen. *Aspiciliella reticulata* wird als Synonym für *A. cupreoglauca* erkannt.

4. ***A new corticolous Megaspora (Megasporaceae) species from Armenia*****:** Ziel war es, eine neue Spezies in der artenarmen Gattung *Megaspora* (bisher nur zwei Arten bekannt) zu beschreiben, die in Armenien neben den anderen beiden Arten dieser Gattung vorkommt, und ihre Position in der Gattung zu klären.

5. ***First inventory of lichens and lichenicolous fungi in the Khosrov Forest State Reserve, Armenia*****:** Das Ziel dieser Studie war die Erforschung der Flechtenvielfalt des Khosrov Forest State Reserve nach unserer Exkursion in diesem Gebiet.

6. ***Additions to the lichenized and lichenicolous mycobiota of Armenia*****:** Das Ziel dieser Studie war die Erforschung der Flechtenvielfalt von Armenien nach unserer Exkursion in verschiedene Gebiete im diesem Land.

1.1.1 Zur Geschichte und Taxonomie der Gattung *Aspicilia* s. lat. und der Familie Megasporaceae

Aspicilia A. Massal. (Pertusariales, Lecanoromycetes, lichenized Ascomycota) wurde als Gattung von Massalongo im Jahre 1852 beschrieben. Typus ist *Aspicilia cinerea*, die 1767 von Linnaeus als *Lichen cinereus* beschrieben wurde.

Die systematische Position von *Aspicilia* s.lat. hatte lange Zeit ein sehr wechselvolles Schicksal. Die Arten von *Aspicilia* s. lat. wurden nach der Beschreibung der Gattung im Jahre 1852 im 20. Jahrhundert für lange Zeit als Subgenus von *Lecanora* Körb. oder als eigene Gattung in der Familie Lecanoraceae betrachtet, wie in den Arbeiten von Steiner (1898; 1917), Zahlbruckner (1928), Poelt (1958) und in der umfangreichsten Bearbeitung von *Aspicilia* durch Magnusson (1939; 1940; 1951).

Besonders aus dem östlichen Mittelmeerraum und den Trockengebieten Asiens wurden frühzeitig zahlreiche Taxa erwähnt: Steiner gibt in seiner Arbeit zur griechischen Flechtenflora (1898; 1917) 19 Arten von Aspicilien (unter der Gattung *Lecanora*) für Griechenland und in einer Arbeit über Flechten aus Mesopotamien und Kurdistan sowie Syrien und Prinkipo (1910) 9 Arten an, beschrieb 7 neue Arten und eine Reihe neuer Variatäten und Formen und nahm drei Neukombinationen vor. Hue bringt in einer Arbeit hauptsächlich aus Asien (1910), *Aspicilia* als eigene Gattung, beschreibt über 20 neue Arten und nimmt viele neue Kombinationen vor. Zahlbruckner (1940) behandelt die Aspicilien in der Gattung *Lecanora* als Sektion *Aspicilia* und verzeichnet 61 Arten mit vielen Formen und Variatäten, die bis 1937 veröffentlicht wurden. In einer Arbeit über die *Aspicilia gibbosa*-Gruppe aus Skandinavien und Nordeuropa beschreibt Magnusson (1939) 51 neue Taxa. Magnusson (1940) behandelt in einer Arbeit über Aspicilien aus Zentralasien insgesamt 114 Arten und beschreibt 23 neue Taxa (Arten und Variatäten) und 7 neue Funde.

In den 70er Jahren setzte sich die Ansicht durch, dass *Aspicilia* mit *Lecanora* nicht näher verwandt sei (Poelt 1974; Roux 1977; Santesson 1984; Clauzade & Roux 1984, 1987; Hafellner 1984) und *Aspicilia* wurde wieder als eigenständige Gattung behandelt (Oxner 1971). Oxner bearbeitet insgesamt 116 Arten mit einem Schlüssel und Beschreibung aller angegebenen Arten, was als die neueste umfangreiche Arbeit über Aspicilien zu betrachten ist.

Poelt (1974) schlug die neue Familie Aspiciliaceae vor, um die taxonomische Distanz zwischen *Aspicilia* und *Lecanora* deutlich zu machen.

Hafellner (1984) stellte die Platzierung von *Aspicilia* s. lat. erneut in Frage und ordnete sie den Hymeneliaceae zu, einer nicht eng mit den Lecanoraceae verwandten Familie. Später zog er dies selbst wieder in Zweifel (Hafellner 1989) und zeigte die offensichtlichen Unterschiede zwischen *Hymenelia* und *Aspicilia*.

Lumbsch et al. (1994) schlugen die neue Familie Megasporaceae für die Art *Megaspora verrucosa* (Clauzade & Cl.Roux) Hafellner vor, eine Art die vorher innerhalb als *Aspicilia* klassifiziert wurde (Clauzade & Roux 1984), und gruppierten diese Familie zusammen mit den Pertusariaceae in die Ordnung Pertusariales. Diese Familie wurde später durch molekulare Methoden bestätigt und akzeptiert (Schmitt et al. 2006; Lumbsch et al. 2007; Nordin et al. 2010).

Wedin et al. (2005) konnten mit neuen Methoden molekular-phylogenetischer Untersuchungen auf der Basis von nuLSU und mtSSU zum ersten Mal phylogenetisch zeigen, dass *Aspicilia* näher mit den Pertusariales verwandt ist als mit den Hymeneliaceae.

In der Arbeit von Miadlikowska et al. (2006) wurden auf Basis von ribosomalen RNA-Markern (NuSSU, nuLSU, mtSSU) und Protein-kodierenden Genen (RPB1, RPB2), die Ergebnisse von Wedin et al. 2005 bestätigt und gezeigt, dass *Aspicilia* eine sehr gut unterstützte Schwestergruppe von *Ochrolechia* ist, eingeschlossen in die Pertusariaceae.

Im Konzept von Lumbsch et al. (2007) und Schmitt et al. (2006) wurden die drei Genera *Aspicilia, Ochrolechia* und *Pertusaria* phylogenetisch eindeutig getrennt und *Aspicilia* in die Megasporaceae, *Ochrolechia* in die Ochrolechiaceae und *Pertusaria* in die Pertusariaceae eingeordnet. Die von Lumbsch et al. (1994) vorgeschlagene Familie Megasporaceae wurde von Lumbsch et al. (2007) mit einer Drei-Gen-Analyse (nuLSU, mtsu, RPB1) als eine gut unterstützte monophyletische Gruppe mit den Gattungen *Aspicilia, Megaspora* und *Lobothallia* (Clauzade & Cl.Roux) Hafellner bestätigt und akzeptiert.

Nordin et al. (2010) bestätigte die Monophylie der Megasporaceae und schlug eine Teilung der Familie in fünf Gattungen auf der Grundlage von mtSSU und nuLSU vor: die drei von Lumbsch et al. (2007) anerkannten Gattungen sowie die beiden wiedererrichteten Gattungen *Circinaria* Link und *Sagedia* Ach. Nach diesem Konzept ergab sich somit folgende Gliederung der Megasporaceae:

É ***Aspicilia*** A. Massal. in Ric. Auton. Lich. Crost.: 36. 1852.

➢ Typusart: *Aspicilia cinerea* (L.) Körb. typ. cons.

É ***Circinaria*** Link in Neues J. Bot. 3: 5. 1809.

➢ Typusart: *Urceolaria hoffmannii* (Ach.) Ach.

É ***Lobothallia*** (Clauzade & Cl.Roux) Hafellner in Acta Bot. Malac. 16: 139. 1991.

➢ Typusart: *Lobothallia alphoplaca* (Wahlenb.) Hafellner

É ***Megaspora*** (Clauzade & Cl.Roux) Hafellner & V.Wirth in Wirth, Die Flechten Baden-Württembergs: 511. 1987.

➢ Typusart: *Megaspora verrucosa* (Ach.) Hafellner & V. Wirth

É ***Sagedia*** Ach. in Kongl. Vetensk. Akad. Nya Handl. 30: 164. 1809.

➢ Typusart: *Sagedia zonata* Ach.

Die neueste Studie von Miadlikowska et al. (2014) mit kombinierten Datensätzen von drei ribosomalen RNA- (nuSSU, nuLSU und mtSSU= und zwei Protein-codierenden Genen (RPB1 und RPB2) zeigt die pylogenetische Platzierung der Megasporaceae in der Ordnung Pertusariales zwischen anderen Familien wie Ochrolechiaceae, Coccotremataceae, Pertusariaceae und Icmadophilaceae. In dieser Studie wurde gezeigt, dass die Megasporaceae eng mit den Ochrolechiaceae verwandt sind.

Von den anderen neuesten Forschungen im Bereich der Megasporaceae, die in den letzten Jahren publiziert wurden, sind jene von Paukov et al. (2014; 2015; 2016; 2017), Owe-Larsson et al. (2011), Nordin et al. (2010; 2011; 2015), Sohrabi et al. (2013a; 2013b) und Roux et al. (2016a; 2016b) zu nennen, welche verschiedene Arten von dieser Familie untersucht haben.

1.1.2 Morphologie der Vertreter der Familie Megasporaceae

Taxa von Megasporaceae sind mit grünen Algen lichenisiert und die Mehrheit der Arten ist saxicol.

Die meisten von ihnen wachsen krustos, aber auch mehrere fruticose und subfoliose bis umbilicate Arten sind bekannt. Die Arten können am Substrat festgewachsen sein oder nicht. Letzteres tritt bei den ausschließlich terricolen (vagranten) Arten auf; manche Arten können in beiden Formen auftreten (erratische Arten) (Büdel & Wessels 1986; Lumbsch & Kothe 1988).

Arten von Megasporaceae haben oft tief in den Thallus eingetauchte Apothecien, aber in einigen Taxa sind die Apothecien erhöht und bilden große flache Scheiben, die meistens bereift sind. Die Asci sind dickwandig mit einer stark verdickten apikalen Kappe mit I + Reaktion und haben (1-) 4-6 oder 8 Sporen pro Ascus. Die Ascosporen sind einzellig, hyalin und sehr variabel (in ihrer Größe) zwischen den verschiedenen Arten. Konidien sind bekannt aus vielen Arten und variabel in der Größe zwischen 7 und 40 m (Cannon & Kirk 2007; Nash 2008).

Der Vergleich der aktuellen phylogenetischen Hypothese mit den klassischen morphologie-basierten Klassifikation zeigt, dass die Thallus-Form, die Anzahl der Sporen pro Ascus, die Größe der Ascosporen, die Konidienlänge und manchmal die Anwesenheit einiger chemischer Substanzen Merkmale für die Erkennung der molekular-phylogenetisch postulierten Gattungen liefern.

Die Hauptmerkmale der Gattungen der Megasporaceae sind im Kapitel 5 angegeben.

1.1.3 Ökologie und Verbreitung der Familie Megasporaceae

Die Arten der Familie Megasporaceae zeigen eine breite Palette von Substratpräferenzen und ökologischen Anpassungen und haben eine weltweite Verbreitung. Sie sind bipolar hauptsächlich in ariden gemäßigten oder polaren Klimazonen verbreitet (Nash III 2007). Die Arten sind Bestandteile in der Flechten-Vegetation auf exponierten Felsen in einer Vielzahl von Biomen von heißen Wüsten bis zur arktischen Tundra (Nordin 2015). Während die Mehrheit der Arten saxicol ist, wachsen einige Taxa auch auf dem Boden (terricol), insbesondere Vertreter der Gattung *Circinaria* (siehe z. B. Rosentreter 1993;

Sohrabi et al. 2011, 2013), und nur wenige auf Rinde und Holz (epiphytisch), speziell Arten der Gattung *Megaspora*.

Die saxicolen Arten wachsen auf verschiedenen Gesteinsarten, von calciumreichen Gesteinen bis zu den eisenreichen, nährstoffarmen silikatischen Gesteinen, wobei sie insgesamt vorwiegend auf sauren Gesteinen auftreten. Einige Arten dieser Familie wachsen gelegentlich auf hartem Holz, Nadelbäumen und bearbeitetem Holz, oft mit morphologischen Eigenschaften, die etwas von saxicolen Formen abweichen (Rico et al. 2007; Owe- Larsson et al. 2007). Sie sind aus einer Vielzahl von Habitaten bekannt, von salzigen hypermaritimen Spritzzonen der Küsten bis hin zu Süßwassermontanströmen und Seen (Nash III 2007), und von Wüstenböden bis zu hohen Gipfeln.

1.1.4 Hintergrund dieser Arbeit

Die beschriebenen Arten (auch Varitäten und Formen) der Megasporaceae in ihrer Abgrenzung zu anderen Taxa, ihrer Variabilität, ihrem Vorkommen und ihrer Verbreitung, sind im Allgemeinen schlecht bekannt. Auch in artenreicheren Gebieten wie Iran und Armenien wurde die Familie Megasporaceae nicht besonders gut untersucht und es sind nur einige Arten in wenigen Veröffentlichungen gennant (Szatala 1957, Riedl 1979, Seaward et al. 2004 and 2008, Harutyunyan et. al 2011). Es bedarf noch einer umfassenderen Bearbeitung dieser Familie mit modernen Methoden, um die existenten Taxa zu charakterisieren und die in der Literatur verwendeten unzähligen Namen korrekt zu benutzen.

Auf der Basis von Aufsammlungen hauptsächlich aus verschieden Gebieten in meiner Heimat Iran (Nord-Ost- bis Nord-West- und Zentral- Iran) und auch während der internationalen lichenologischen Exkursion in Armenien (Fig. 1 & 2) wurde eine komplexe Untersuchung mit morphologischen, chemischen und molekularen Methoden über die Familie Megasporaceae durchgeführt, um einen Überblick über das gesammelte Material aus diesen Gebieten auf einer möglichst umfangreichen Basis zu gewinnen.

Es zeigte sich, dass einige Proben in den molekularen Studien eine sehr distinkte und monophyletische Gruppe repräsentierten, und nach morphologischen Untersuchungen wurde klar, dass diese Proben trotz großen morphologischen Unterschiede der Farbe und Form des Thallus die gleichen anatomische Merkmale wie *A. intermutans* haben und alle K+ rot reagieren und Norstictinsäure als Inhaltsstoff besitzen. Daher wurde als erstes Ziel gesetzt, herauszufinden zu welchen Arten diese Belege genau gehören.

Es sind auch ein paar andere interessante Materialien vom diesen Gebieten gefunden wurden, die teilweise bearbeitet wurden oder später bearbeitet werden sollen.

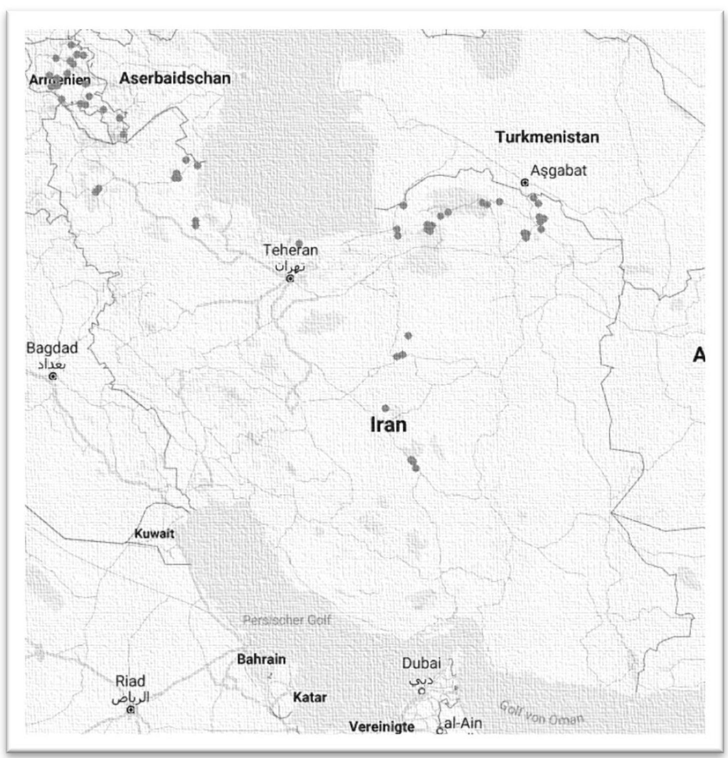

Fig. 1: Die Karte vom Iran und Armenien mit besuchten Orte, gezeigt mit Punkten

Fig. 2: Natürliche Landschaften in: A- Armenien; B- in Nord-Ost Iran (Nord-Khorasan); C- Nord Iran (Mazandaran)

1.1.5 Zur Geschichte und Taxonomie der *Aspicilia-intermutans*-Gruppe

Aspicila intermutans ist eine thermophile Art, die im Mittelmeergebiet weit verbreitet ist und überwiegend auf horizontalen Oberflächen freiliegender Gesteine wächst (Sipman & Raus 1999). Die Thallusmorphologie des Materials ist sehr variabel, und benachbarte Flechten bilden oft ein Mosaik unterschiedlich aussehender Individuen (Sipman & Roux 1999). Dies legt nahe, dass genetische Unterschiede auftreten und dass mehr als eine Art oder ein Komplex von meist unbeschriebenen Arten beteiligt sein können (Sipman & Roux 1999; Zakeri et al. 2017).

Das Taxon *Aspicilia intermutans* wurde 1872 von Nylander unter dem Namen *Lecanora intermutans* für die Wissenschaft beschrieben (Nylander 1872). Nylander gibt in der Beschreibung der Art an, dass sie aussieht wie *Aspicilia cinerea*, aber größere Sporen von 23-34 × 9-15 µm und weit kleinere Konidien von 7-9 x 1 µm hat (Fig. 3). Von Arnold (1887) wurde die Art in die Gattung *Aspicilia* übertragen. Verschiedene Autoren zu Ende des 19. und im 20. Jahrhundert behandelten sie mal als *Aspicilia*, mal als *Lecanora* und unterschieden einige Varietäten; *Lecanora intermutans* var. *reticulata* J. Steiner 1898; *Lecanora intermutans* var. *intermutans* Nyl. 1872; *Lecanora intermutans* var. *turgida* J. Steiner 1907. Gelegentlich wurde *A. intermutans* als Varietät anderer Arten (*A. cinerea*, *A. reticulata*) behandelt: *Lecanora cinerea* var. *intermutans* (Nyl.) H. Olivier, *Expo. Syst. Descr. Lich. Ouest Fr.* 1: 304 (1897), *Aspicilia cinerea* var. *intermutans* (Nyl.) Boistel, *Nouv. Flore Lich.*, Edn 2: 145 (1903) und *Aspicilia reticulata* var. *intermutans* (Nyl.) Szatala, in Gyelnik, *Lichenotheca parva* 4: no. 72 (1935)). Aktuell ist die Unterscheidung von *A. intermutans* und *A. cinerea* generell akzeptiert. *A. cinerea* kann leicht durch die Länge der Konidien (11-16 µm) und das Auftreten der Apothecien unterschieden werden. In *A. cinerea* sind die Apothecien häufiger und die Scheiben sind niemals bereift, im Gegensatz zu *A. intermutans* mit kurzen Konidien und bereiften Scheiben und Areolen.

Aspicilia reticulata Kremp. 1869 ist ein Name, der in Zusammenhang mit *A. intermutans* häufig benutzt wurde und das so bezeichnete Material gehörte auch nach Beschreibungen von verschiedenen Autoren nach meiner Ansicht ohne Zweifel in die Gruppe *intermutans*. Nylander 1886 schreibt, dass *A. reticulata* zur gleichen Art wie *Lecanora intermutans* gehört, und davon nur eine Form mit etwas hellerer Farbe ist. Oxner (1971) benützte *A. reticulata* als Bezeichnung für den ganzen Komplex, und unterschied *A. intermutans* auf

der Varietäts-Ebene. Sipman & Raus (1999) listen *A. reticulata* var. *contortoides* (J.Steiner) Szatala in Sipman & Raus (1995) unter *A. intermutans*. Die Unsicherheit und unklare Zuordnung dieser Taxa bei verschiedenen Autoren haben mich veranlasst, mehr darüber zu recherchieren. Die Ergebnisse von dieser Recherche sind detaillierter im Kapitel 4 eingegeben.

Choisy (1932) beschrieb eine neue Gattung *Aspiciliella* nur für die Art *A. intermutans* (*Aspiciliella intermutans* (Nyl.) M. Choisy), die Art wurde aber danach immer wieder in andere Gattungen übertragen, sogar als *Squamaria reticulata* var. *intermutans* (Nyl.) Szatala, *Annls mycol.* 14: 318 (1960) *Urceolaria intermutans* (Nyl.) Motyka, *Porosty* (Lublin): 193 (1996).

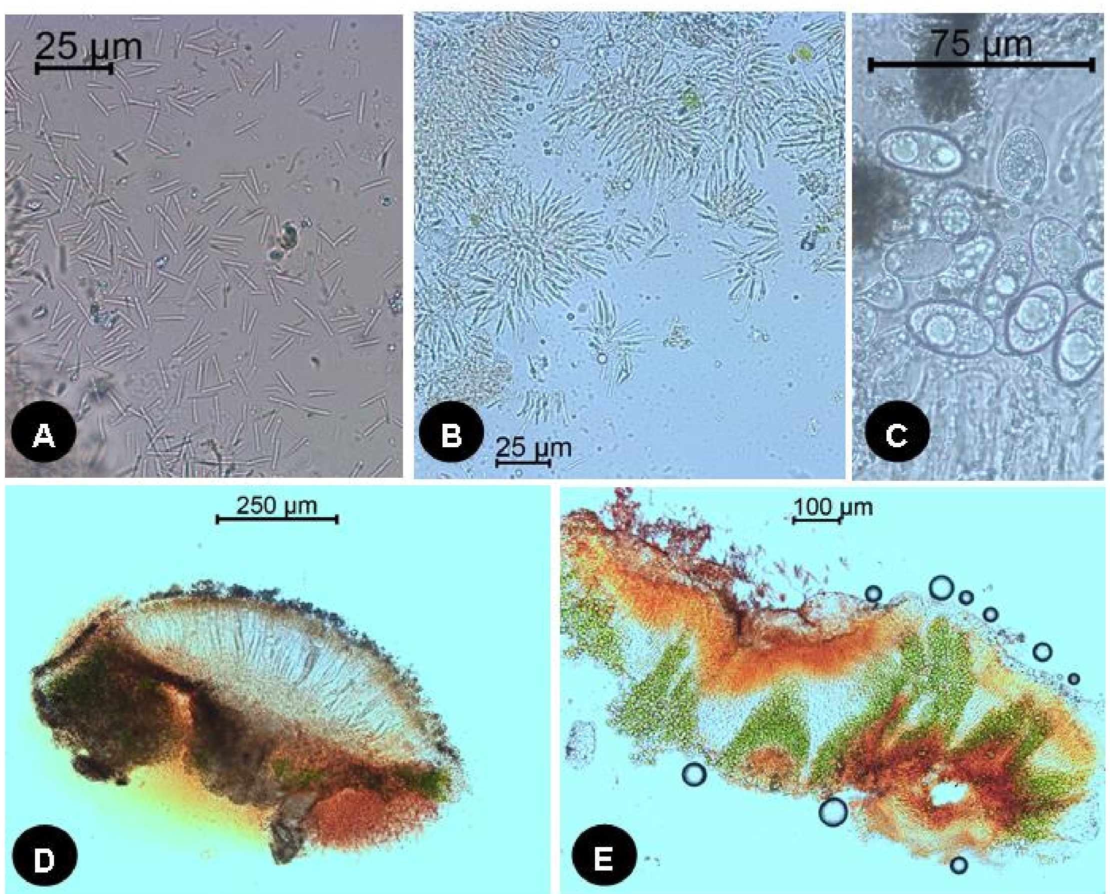

Fig. 3: Ein Beleg von *Aspiciliella intermutans*; A- Filiform gerade Konidien; B- Konidiphoren; C- Ellipsoide Ascosporen; D- Querschnitt des Apotheciums nach Behandlung mit K; E- Querschnitt von Kortex und Medulla nach Behandlung mit K

Viele Aufsammlungen dieser Gruppe sind vor allem aus Iran (Zakeri et al. 2017; Seaward et al. 2008), Armenien (Zakeri et al. 2017; Gasparian et al. 2015, 2016), Griechenland und Frankreich bekannt; sie wird auch aus Italien angegeben (Nimis & Martellos 2008). In Mitteleuropa ist *A. intermutans* selten und lokal verbreitet wie in Ungarn (Verseghy 1994)

und der Slowakei (Fa kovcová et al. 2014). Ihre Gesamtverbreitung erreicht Großbritannien (Fletcher et al. 2009), Kanarische Inseln (Hafellner 1995), Tunesien (z. B. Pitard & Bouly de Lesdain 1909), Syrien (John et al. 2004), den Ural (Paukov et al. 2014), USA (McCune et al. 2014).

1.1.6 Zur Geschichte und Taxonomie der Gattung Megaspora

Megaspora (Clauzade & Cl.Roux) Hafellner & V. Wirth wurde erstmals als Subgenus von *Aspicilia* A. Massal eingeführt (Clauzade & Roux 1984), aber kurz danach auf Gattungsebene erhoben, mit zunächst nur der einen, hauptsächlich bodenbewohnenden, schwerpunktmäßig arktisch-alpinen Art *M. verrucosa* (Ach.) Hafellner & V.Wirth, (Wirth 1987). *Megaspora verrucosa* ist aus Europa, Afrika, Asien, Nord- und Südamerika, Neuseeland und der Antarktis bekannt (Smith & al. 2009). *Megaspora* und *Aspicilia* waren die ersten zwei unterschiedenen Gattungen in der neu postulierten Familie Megasporaceae (Lumbsch et al. 1994). *Megaspora*-Arten haben im Unterschied zu *Aspicilia*-Arten sehr große bis zu 60 Mikrometer lange, dickwandige Ascosporen und in den Thallus eingetauchte Apothecien. Genetische Untersuchungen zeigen, dass *Megaspora* eng verwandt mit der Gattung *Circinaria* ist (Nordin et al. 2010).

Valadbeigi et al. (2011) beschrieben die neue Art *Megaspora rimisorediata* Valadbeigi & A. Nordin aus Iran (hauptsächlich auf Baumrinde, aber auch auf kalkhaltigen Gesteinen und Moosen) als zweite Art in dieser Gattung. Sie wurde inzwischen von Südosteuropa und Kaukasus (Armenien) bis Zentralasien, d. h. Iran und China nachgewiesen (Gasparyan & Sipman 2013; Gasparyan et al. 2015; Kondratyuk et al. 2016).

Vor kurzem wurde zusätzlich eine andere neue Art *M. Iranica* M. Haji Moniri & S. Y. Kondr. für diese Gattung aus dem Iran beschrieben (Haji Moniri et al. 2017).

1.2 Einleitung zur allgemeinen Methodik

Generell werden verschiedene Methoden, unter anderem chemische und molekulare, zur Identifizierung und Klassifizierung von Flechten benutzt.

Chemie und molekulare Marker spielen eine wichtige Rolle in der Taxonomie von Flechten, die anhand ihrer Morphologie nicht einfach zu bestimmen sind oder in schlecht entwickelten Exemplaren morphologisch schwierig zu identifizieren sein können. Diese Flechten können dann einfacher durch ihre sekundären Metabolite, die oft in erheblicher Quantität vorkommen, und ihre molekularen oder phylogenetischen Verhältnisse, unterschieden und klassifizert werden.

1.2.1 Chemische Untersuchungen

Zur Identifizierung von Flechten werden verschiedene chemische Untersuchungen durchgeführt. Am häufigsten verwendete chemotaxonomische Techniken sind der Tüpfeltest und die Dünnschicht-Chromatographie (TLC), aber auch Hochleistungs-Flüssigkeitschromatographie (HPLC), Gaschromatographie und Massenspektrometrie (GCMS) wurden zu diesem Zweck verwendet (Karunaratne et al. 2005).

Tüpfeltest: Der Tüpfeltest ist eine der ältesten chemische Methoden in Flechten, Kaliumhydroxid (KOH) zusammen mit Hypochloritlösung ($Ca(OCl)_2$) waren die ersten verwendeten Chemikalien zur Identifizierung von Flechten durch Nylander (1866). Dieser Test wird in einem bestimmten Teil des Thallus (Kortex oder/ und Medulla) mit einer bestimmten Chemikalie durchgeführt und die Reaktion wird durch Beobachtung der Farbänderung überwacht. Diese Methode liefert keine detaillierten chemischen Informationen wie TLC oder HPLC, aber sie ist praktisch, schnell, sehr sparsam und benötigt sehr wenig Flechtenmaterial ohne Extraktionsverfahren.

DC (Dünnschichtchromatographie): DC (engl. TLC, thin layer chromatography) ist eine Methode für die physikalisch-chemische Trennung von Substanzen und gehört zu den Flüssigchromatographien. Der schwedische Chemiker Wachmeister führte in der Zeit von 1952 bis 1956 Papier- Chromatographie für die Identifikation der Flechtensubstanzen und von deren Hydrolyse-Produkten ein (Nash 2010). Dünnschichtchromatographie ist zurzeit die am weitesten verbreitete Methode zur Identifizierung von Flechten-Produkten. Diese Technik ist relativ kostengünstig und verbessert erheblich die Geschwindigkeit und

Sicherheit der Erkennung von Flechten-Substanzen (Nash 2010). In diesem Verfahren werden Flechtenstoffe mittels Acetons extrahiert und auf die DC-Fertigplatten mittels Mikropipetten aufgetragen. Die Platten wurden dann in einer sogenannten Primerkammer zur Verbesserung der Ergebnisse vorbehandelt und danach mit entsprechenden Laufmitteln bearbeitet. Durch das Laufmittel werden verschiedene Substanzen aus der Startzone gelöst und weiter transportiert. Die einzelnen Substanzen laufen im Verhältnis zur Laufmittelfront unterschiedlich weit.

Nach der Bearbeitung mit Laufmitteln werden die Platten zunächst mit kurzwelligen UV-Licht (254 nm) und langwelligem UV-Licht (366 nm) beobachtet. Danach wird die Platte mit Wasser besprüht für die Identifikation von Fettsäuren und am Ende wird die Platte mit 10% H_2SO_4 im Trockenschrank für ca. 10 Minuten bei 110 °C entwickelt (Culberson & Ammann 1979; Orange et al. 2001).

Hochleistungsflüssigkeitschromatographie gekoppelt mit Massenspektrometrie (HPLC-MS): Diese Methode ist eine Flüssigkeitschromatographie gekoppelt mit einem massensensitiven Detektor (Massenspektrometer).

Die HPLC (engl. *high performance liquid chromatography*) ist eine Form der Flüssigkeitschromatographie, die mit hohen Drücken arbeitet und dadurch ein hohe Auflösung erzielt. Wie bei allen Chromatographien werden auch bei der HPLC Stoffgemische aufgetrennt. Setzt man Normalphasen- oder Umkehrphasensäulen als stationäre Phasen ein, so werden die Stoffe (Moleküle) aufgrund ihrer Hydrophobizität voneinander getrennt. Aufgrund von Polaritätsunterschieden zwischen mobiler und stationärer Phase werden die Analyte in Abhängigkeit von ihrer Polarität unterschiedlich stark bzw. lange von der stationären Phase gebunden. Die in dieser Arbeit verwendete RP-HPLC (*reversed phase HPLC*) besitzt eine polare mobile Phase (bestehend aus einem Gemisch von (A) wässrigem Ameisensäure- Puffer 0.1% und (B) Acetonitril) und eine unpolare stationäre Phase. Als stationäre Phase wird in der Kinetex PFPSäule ein oberflächenmodifiziertes Kieselgel eingesetzt, an welches Pentafluorphenyl gebunden ist.

Die Auftrennung der Analyte erfolgt anhand ihrer Retentionszeit. Polare Moleküle haben eine geringere Retentionszeit als unpolare. Es wurde eine Gradiententrennung angewendet, d. h. während des HPLC-Laufes wurde die Zusammensetzung des Eluenten geändert. Dadurch wurde die Auflösung der Chromatographie optimiert.

Die Massenspektrometrie (MS; engl. *mass spectrometry*) ist die Detektion von Atom- oder Molekülmassen. Sie wird zur Charakterisierung von unbekannten chemischen Verbindungen eingesetzt, die inonisiert werden können. Deshalb eignet sich dieses Verfahren zur Identifikation von unbekannten Flechten-Inhaltstoffen.

In der HPLC-MS dient die Flüssigkeitschromatographie der Trennung der Analyte und die Massenspektrometrie der Detektion und Identifikation der Molekularmassen der unbekannten Substanzen. Das Massenspektrometer ist mit einem Elektrosprayionisator gekoppelt, der die Bestandteile der Analytlösung durch ein elektrisches Feld in die entsprechenden Ionen überführt, die dann mittels Massenspektrometer detektiert werden können. An der Spitze der Metallkapillare entsteht ein Überschuss gleichartig geladener Ionen, welche sich gegenseitig abstoßen und feine Aerosole bilden. Stickstoff als Trägergas vernebelt die Lösung und fördert die Verdampfung des Fließmittels. Aufgrund der Verdampfung des Lösungsmittels verringert sich die Tropfengröße bei gleichzeitiger Zunahme der Dichte des elektrischen Feldes auf der Tropfen-Oberfläche. Wenn ein bestimmter Radius unterschriten wird, zerfallen die Tropfen wegen der Abstoßung von gleichartigen Ladungen (Coulomb-Explosionen) in kleinere Tröpfchen. Geeignete Analyte ionisieren nun in diesen winzigen Tröpfchen. Die Art der Spannung, die an der Kapillare angelegt wird, bestimmt die Ladung der Ionen, die erzeugt werden. Durch eine positive Spannung (engl. *positive mode*) werden positive geladene Ionen erzeugt und durch eine negative Spannung (engl. *negative mode*) negativ geladene Ionen. Alle ionisierten Verbindungen werden nun im Quadropol separiert und danach detektiert.

1.2.2 DNA Marker

Die ITS-Region wurde häufig für die Zwecke der Identifizierung und Klassifizierung von verschiedenen Taxa von Pilzen einschließlich Studien über die Familie Megasporaceae verwendet. Es wurde für die Regionen des ribosomalen Cistrons gezeigt,, dass ITS die höchste Wahrscheinlichkeit einer erfolgreichen Identifizierung für Pilzbereich aufweist (Schoch et al. 2012; Divakar et al. 2015).

Die ITS-Regionen werden als die variabelsten Regionen innerhalb des gesamten Clusters anerkannt und sind daher für phylogenetische Analysen auf infragenerischer und sogar infraspezifischer Ebene geeignet (siehe auch Högnabba 2007; Kelly et al. 2011). Aber nur auf ITS oder einen einzelnen anderen Marker für die Identifizierung von Arten und

insbesondere für die Arten-Abgrenzung zu setzen, wurde kritisiert (Dupuis et al. 2012); die Verwendung zusätzlicher unabhängiger Loci reduziert stochastische Fehler und erscheint eher geeignet zur Abgrenzung von Arten (Chen et al. 2008). Die große Untereinheit der ribosomalen rDNA (nrLSU) ist weniger variabel und bietet nützliche phylogenetische Informationen auf höheren taxonomischen Ebenen (Ordnungen, Familien, Gattungen). Die Region mtSSU ist ein vergleichsweise weniger variabler Marker als ITS und stärker Variabel als nrLSU und wurde auch häufig für Studien auf Gattungs- und Artebene (auch für kryptische Arten) in Pilzen und lichenisierten Pilzen benutzt, wie in Miadlikowska et al. (2006), Schmitt et al. (2006), Lumbsch et al. (2007) Nordin et al. (2010) für taxonomische Untersuchungen an Pertusariales und Megasporaceae. *DNA replication licensing factor* MCM7 ist ein proteincodierender Marker, der auch vergleichsweise weniger variabel ist als ITS und phylogenetisch informativer als LSU. Das MCM7-Gen ist ein wertvoller phylogenetischer Marker für sowohl phylogenetische Analysen auf niedriger als auch auf höherer Ebene innerhalb der Ascomycota, insbesondere wenn es in Kombination mit dem LSU-Gen verwendet wird (Raja et al. 2011). MCM7 wurde für zahlreiche Studien für kryptische Arten in Kombination mit anderen Markern benutzt (Singh et al. 2015; Spribille et al. 2011; Leavitt 2011a; Del-prado et al. 2016).

1.2.3 Molekulare Methoden für die Abgrenzung von Arten

Molekulare Sequenzdaten und phylogenetische Inferenz bieten nur dann eine starke Unterstützung für die Unabhängigkeit der Abstammungslinie, wenn Divergenzereignisse zwischen Spezies relativ weit zurückliegen und Ahnenpolymorphismen an Probenorten vollständig sortiert wurden (Leavitt 2016). Die Abstammungslinien können durch einen Barcoding-Ansatz mit einem einzigen Locus oder durch Ansatz der Verkettung von Sequenzdaten aus mehreren Loci identifiziert werden, können jedoch manchmal nicht durch phänotypische Merkmale identifiziert werden. Die unterstützten Abstammungslinien auf Artenebene durch genealogische Konkordanz können auch als verdiente formale Anerkennung bezeichnet werden, wenn die Kandidatenspezies nicht durch unabhängige Daten wie phänotypische Merkmale unterstützt werden (Avise & Ball 1990).

Bis vor kurzem waren Speziesabgrenzungen unter Verwendung von molekularen Daten auf monophyletische oder diagnostische Zustände (z. B. feste Unterschiede) als wichtige Kriterien zur Identifizierung von Spezies begrenzt (Sites & Marshall 2004). Aber trotz einer dramatischen Zunahme der Zahl der verfügbaren Methoden zur Abgrenzung von

Arten mit phylogenetischen Studien oder dem üblichen Ansatz der Verkettung von Sequenzdaten aus mehreren Loci kann die Erkennung der Abgrenzung von eng verwandten Taxa in den Anfangsstadien der Divergenz schwierig sein, da es in der Regel Diskordanzen zwischen Genbäumen und Speziesbäumen in verwandten Taxa und Genen gibt, die sich in ihrer evolutionären Geschichte erheblich unterscheiden können, was zu Problemen bei der Artenunterscheidung führen kann (Stewart et al. 2014; Kubatko & Degnan 2007).

Die Erfassung verschiedener Eigenschaften, die Spezies begrenzen (z. B. reziproke Monophylie, phänotypische Diagnostizierbarkeit usw.) ist nicht deckungsgleich; daher können unterschiedliche Artenabgrenzungskriterien zu inkongruenten Abgrenzungen von Artengrenzen führen (De Queiroz 2007; Carstens et al. 2013). Außerdem können Arten, die Produkte von Artbildungsereignissen sind, selbst nach späterer Speziation neue Arten hervorbringen und können die Umschreibung von Arten schwierig machen (Leavitt et al. 2016).

Genealogische Konkordanz zeigt ihre Stärke in tieferen Phylogenieereignissen und phänotypisch kryptischen Abstammungslinien, die schon seit Langem isoliert sind, aber sie erweist sich als schwach bei der Identifikation neuerer evolutionärer Linien, die noch nicht gut isoliert sind.

Es wird deshalb betont, dass taxonomische Entscheidungen in eng verwandten Artengruppen einschließlich der Fälle, in denen traditionell umschriebene Arten innerhalb einer einzigen Gruppe von eng verwandten Exemplaren gewonnen werden, auf strengen statistischen Tests basieren sollten, anstatt einfache heuristische oder qualitative Beurteilungen bei der Verwendung von molekularen Sequenzdaten (Leavitt et al. 2016).

Gegenwärtig gibt es in der Systematik eine Diskussion hinsichtlich der Verwendung strenger statistischer Ansätze; die koaleszenz-basierten und die "species treeõ-Ansätze haben sich als gutes Werkzeug für die Abgrenzung von Arten in lichenisierten Pilzen erwiesen (Leavitt et al. 2011a, b, 2013a, b; Del-prado et al. 2016; Parnmen et al. 2012). Das zentrale Ziel der koaleszenzbasierten Ansätze besteht darin, unabhängig sich entwickelnde Linien zu identifizieren, die jeweils eine Spezies darstellen.

Häufig verwendete Methoden zur Artenabgrenzungen umfassen die automatische Barcodelücken-Entdeckung (ABGD) [Puillandre et al. 2012], Poisson-Tree-Prozesse (PTP) -Modell [Zhang et al. 2013] und das allgemeine gemischte Yule-Koaleszenzmodell

(GMYC) [Monaghan et al. 2009; Pons et al. 2006]. Es gibt auch verschiedene Algorithmen, Artenbäume zu rekonstruieren; z. B. *BEAST und SpedeSTEM [Ence & Carstens 2011], Die Abschätzung der Artenbaum- und Artenabgrenzung mittels Koaleszenzmethoden für eng verwandte Taxa hat sich als sehr nützlich erwiesen und wurde für lichenisierte Pilze in einigen Artenkomplexen wie den *Parmotrema reticulatum*-*Parmotrema pseudoreticulatum*-Komplex, den *Rhizoplaca melanophthalma*-Komplex und den *Cladia*-*aggregata*-Komplex verwendet (Leavitt et al. 2011a, b, 2013a, b; Del-prado et al. 2016; Parnmen et al. 2012).

1.3 Material und Methoden

1.3.1 Material:

In dieser Studie wurde von der Autorin gesammeltes Material sowie Herbarmaterial verwendet. Das eigene Material wurde hauptsächlich in Armenien (2015) und Iran (2015/2016) gesammelt. Für Herbarstudien wurde Material aus B, GZU, PRA, PRC, PL, CR, ABL, H, M, S und GLM verwendet. Die selbst gesammelten Proben aus Iran und Armenien sind in GLM hinterlegt.

Alle untersuchten Proben in dieser Arbeit stammen aus gemäßigten, trockenen und semiariden Gebieten des westlichen Eurasiens und Mittelmeerraumes. Die Höhenamplitude reicht vom Tiefland des Kaspischen Meeres und Mittelmeeres bis zu den hohen Lagen des Aragaz (Armenien) und Svalanes (Nordwest-Iran).

1.3.2 Morphologische Untersuchungen:

Es gibt einige wichtige Merkmale, die morphologisch oft sehr ähnlichen Taxa der Megasporaceae zu unterscheiden: die Farbe und Form des Thallus, die Größe des Thallus, die Dicke der Rinde und des Markes, die Anwesenheit von Isidien und Soralen, die Größe und die Form der Sporen und Conidien, der Habitus und die geographische Verbreitung.

Die in dieser Arbeit betrachteten Arten wurden zunächst aufgrund der Morphologie, anhand von Farbe und Form des Thallus, Größe und die Form der Sporen und Conidien nach Ähnlichkeit zugeordnet, dann wurde untersucht, inwiefern sich die morphologisch unterscheidbaren Einheiten mit molekular ausgeschiedenen zur Deckung bringen lassen.

Allgemeine Beobachtungen der äußeren Morphologie wurden unter Verwendung eines Leica M165 C Stereomikroskops, das mit einer Leica DFC485 Digitalkamera verbunden war, durchgeführt. Detaillierte Beobachtungen der Thallusanatomie, Asci, Ascosporen und Conidiosporen wurden auf handgeschnittenen Abschnitten von Thallus, Apothecien und Pyknidien in Leitungswasser mit einem Leica DM 2500 P Lichtmikroskop, das an eine Leica MC 190 HD Digitalkamera angeschlossen war, untersucht.

Messungen von Ascosporen (8-40 für jede Spezies, minimale Länge-maximale Länge × minimale Breite-maximale Breite) und Conidien (40 für jede Spezies, minimale Länge-maximale Länge) wurden bei × 400 und × 1000 facher Vergrößerung durchgeführt. Für die

Feststellung der Dicke des Exipulums wurde der ascusfreie Bereich neben dem Hymenium von der Außenwand des letzten Ascus bis zur Grenze des Excipulums gemessen.

1.3.3 Chemische Untersuchungen:

Die Substanzen wurden nach dem Tüpfeltest zunächst mittels DC auf der DC-Platte detektiert. Anschließend wurden zur Absicherung der Befunde und um unidentifizerte Substanzen zu bestimmen oder die Eigenschaften zu prüfen, HPLC-Methoden benutzt.

1.3.3.1 Tüpfeltest:

In dieser Arbeit wurden die Chemikalien K (KOH), C ($Ca(OCl)_2$), KC (erst K und dann C auf dieselbe Stelle) für den Nachweis von sekundären Inhaltstoffen benutzt. Andere Chemikalien wie HNO_3 und IKI wurden an mikroskopischen Präparaten verwendet. N (HNO_3) wurde für epihymeniale Pigmente benutzt und IKI wurde für die Feststellung einer amyloiden Reaktion verwendet.

1.3.3.2 Dünnschichtchromatographie (DC)

Die Chemie der Proben wurde mittels Dünnschichtchromatographie (DC) unter Verwendung der Lösungsmittel A, B und C nach Orange et al. (2001) und Culberson & Ammann (1979) auf DC-Fertigplatten (SIL G-25 UV254, Schichtdicke 0,25 mm Kieselgel mit Fluoreszenz-Indikator UV 254 von Firma ROTH, Größe der Platten 20 x 20 cm und 5 x 10 cm) untersucht.

1.3.3.3 Hochleistungsflüssigkeitschromatographie gekoppelt mit Massenspektrometrie (HPLC-MS)

Für die chromatographische Trennung wurden eine Kinetex 2.6u PFP 100 Å, 150 x 2.10 mm-Säule verwendet. Die mobile Phase bestand aus einem wässrigen Ameisensäure-Puffer 0.1% (A) und Acetonitril (B). Die Trennungen erfolgten bei konstanten 45°C mit einem Stufengradienten. Der Gradient begann mit 30 % (B) für 1 min, dann ansteigend auf 85 % (B) innerhalb von 19 min. Dieses Gemisch wurde für 1 min gehalten, um stark unpolare Analyte zu eluieren. Die Säule wurde mit einer konstanten Fließgeschwindigkeit von 0.35 ml min^{-1} betrieben. Zur HPLC wurde ein das Chromatographiesystem 1200 von Agilent Technologies (Waldbronn, Deutschland) mit einem Agilent Dioden-Array SL 1200.-Detektor eingesetzt. Mittels Hochleistungsflüssigkeitschromatographie wurden Substanzen voneinander getrennt, identifiziert und quantifiziert.

Als massensensitiver Detektor wurde eine Ionenfalle (Ion TrapSeries 6300) der Firma Agilent Technologies (Waldbronn, Deutschland) benutzt. Als dazugehörige Ionenquelle wurde eine Atmosphärendruck-Ionisierung-Elektrospray-Ionisation (APCI/ESI)-Multimode eingesetzt, welche im ESI-Modus betrieben wurde. Substanzen wurden in dem Wellenlängenbereich von 100 bis 600 m/z detektiert. Es wurde folgende Methode verwendet:

Tabelle 1: Verwendete HPLC-Methode mit MS Kopplung (HPLC-MS Methode)

	Zeit (min)	Puffer %
Ameisensäure 0.1%	0	70
	1	70
	24	15
	25	15
Acetonitril	Zeit (min)	Puffer %
	0	30
	1	30
	24	85
	25	85
Injektionsvolumen	2 l	
Flussrate	0,35 mL min-1	
Max. Druck	400 Bar	
Lauflänge insgesamt	20 min	
Temperatur des Säulenofens	45 °C	
Wellenlänge für die Auswertung	210, 215, 230, 250, 280, 310 nm	

Tabelle 2: MS-Methode

Mode	Positiv oder Negativ
Nebulizer	35 psi
Dry-Gas	13 L min-1
Dry-Temperature	365 °C
Scan	100-600 m/z
Target-Masse	200

1.3.4 Identifizierung der Substanzen:

Die Identifizierung geschah mit Hilfe von Standards, die vorher bearbeitet und identifiziert wurden, durch Vergleich mit den in der Literatur angegebenen R_f-Werten, durch Vergleich mit Substanzen derselben Molekularmasse in anderen Proben mit bekannten Inhaltsstoffen und aufgrund ihrer biosynthetischen Verwandtschaft mit anderen Substanzen unter Nutzung des Programms WINTABOLITES (Mietzsch-Lumbsch et al. 1996). Dazu wurden Flechten mit bekannten Inhaltstoffen aus dem Herbar GLM benutzt.

1.3.5 DNA Marker:

Für die phylogenetische Analyse in dieser Arbeit wurden molekulare Untersuchungen auf der Grundlage dreier genetischer Marker, dem š*nuclear ribosomal internal transcribed spacer 1*, *5.8S und internal transcribed spacer* 2" (ITS), der großen Untereinheit des nuklearen ribosomalen DNA (nuLSU), der kleinen Untereinheit der mitochondrialen ribosomalen DNA (mtSSU) und *DNA replication licensing factor* MCM7 durchgeführt.

In der Kapitel 5 wurden ITS1-5.8S-ITS2-rDNA-Sequenzen von Proben in der molekularen Studie verwendet. Zur Amplifikation der ITS1-5.8S-ITS2-Region wurden die Primer ITS1-F (Gardes & Bruns 1993) mit ITS4 (White et al. 1990) oder ITS1-LM (Myllys et al. 1999) kombiniert mit ITS2-KL (Lohtander et al. 1998) verwendet.

Molekulare Untersuchungen auf der Grundlage der Kombination von drei genetischen Markern: nrLSU, ITS und mitochondrialem SSU wurden für die molekular-taxonomische Untersuchung der Familie Megasporaceae und der Gattung *Aspiciliella* im Kapitel 2 verwendet. Zur Amplifikation der mtSSU-Region wurden die Primer mtSSU1 und mtSSU3R (Zoller et al. 1999) verwendet und für nrLSU wurden LR0R und LR5 (Vilgalys & Hester 1990) verwendet.

Molekulare Untersuchungen auf der Grundlage der Kombination von vier genetischen Markern: nrLSU, ITS, mtSSU und MCM7 wurden für molekular-taxonomische Untersuchungen mittels *coalescent approaches* der Gattung *Aspiciliella* im Kapitel 3 verwendet. Zur Amplifikation der MCM7 Region wurden die Primer Mcm7-1348rev (Schmitt et al. 2009) und X_mcm7_F (Leavitt 2010) verwendet.

-Extraktion, PCR-Amplifikation, Alignment, phylogenetische und koaleszenzbasierte Methoden und Analysen werden in Kapitel 2, 3 und 5 detailliert beschrieben.

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Kapitel 2:

Taxonomy and phylogeny of *Aspiciliella*, a resurrected genus of Megasporaceae, including the new species *A. portosantana*

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Published By: **Herzogia 30: 166 ó176.**

(Format is based on journal guidelines)

Abstract:

The genus *Aspiciliella* M.Choisy (type: *A. intermutans*) is resurrected to accommodate two species previously placed in *Aspicilia*, and a new species, *A. portosantana* Sipman & Zakeri. The new combination *A. cupreoglauca* (B.de Lesd.) Zakeri, Divakar & Otte is proposed. Molecular investigation based on three genetic markers, the nuclear ribosomal internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (ITS) region, the nuclear large subunit (nuLSU) and the mitochondrial small subunit (mtSSU) ribosomal DNA, of samples from a wide geographical range including Iran, Caucasia, Greece and Macaronesia revealed a strongly supported clade in a sister position to the other genera of the family Megasporaceae (PP = 1.00; MP/ML BS = 100/100). Morphological and chemotaxonomic surveys showed that the genus is characterized by a thallus that is crustose, rimose-areolate, partially continuous, K+ red; a green, olive-green to greenish-brown N+ light green epihymenium; 8-spored asci, ellipsoid, colourless, simple ascospores and very small (7ó11 µm long) conidia. An identification key to the species of the genus is provided.

Zusammenfassung:

Die Gattung *Aspiciliella* M.Choisy (Typus: *A. intermutans*) wird wieder eingeführt für zwei Arten, die zuvor in *Aspicilia* platziert wurden und eine neue Art, *A. portosantana* Sipman & Zakeri. Die Neukombination *A. cupreo- glauca* (B.de Lesd.) Zakeri, Divakar & Otte wird vorgenommen. Molekulare Untersuchung auf der Grundlage dreier genetischer Marker, dem šnuclear ribosomal internal transcribed spacer 1, 5.8S und internal transcribed spacer 2" (ITS), der großen Untereinheit der nuklearen ribosomalen DNA (nuLSU) und der kleinen Untereinheit der mitochondrialen ribosomalen DNA (mtSSU), an Proben von geographisch weit gestreuter Herkunft, umfassend Iran, Kaukasien, Griechenland und Makaronesien, zeigte eine hohe Unterstützung für einen Clade in einer Schwesterposition mit den übrigen Gattungen der Familie Megasporaceae (PP = 1.00; MP / ML BS = 100/100). Morphologisch und chemisch ist die Gattung charakterisiert durch einen krustigen, zusammenhängenden, rimos-areolierten, K+ roten Thallus; ein grünes, olivgrünes bis grünlich-braunes, N+ hellgrünes Epihymenum; 8-sporige Asci, ellipsoide, farblose, einzellige Ascosporen und sehr kleine (7ó11 µm) Konidien. Ein Bestimmungsschlüssel für die Arten der Gattung wird bereit- gestellt.

Key words: Lichenized fungi, *Aspicilia*, combined analysis, integrated taxonomy, western Eurasia, Mediterranean, Macaronesia.

Introduction

Aspicilia A.Massal. is a diverse genus including approximately 230 species (Kirk et al. 2008, Nordin et al. 2010). Lumbsch et al. (2007) placed *Aspicilia* and two other genera, *Megaspora* (Clauzade & Cl.Roux) Hafellner & V.Wirth and *Lobothallia* (Clauzade & Cl.Roux) Hafellner, in a monophyletic family Megasporaceace based on a three-gene analysis (nuLSU, mtSSU and RPB1). Nordin et al. (2010) confirmed the monophyly of Megasporaceae and proposed a division of the family into five genera based on mtSSU and nuLSU: the three genera estab- lished by Lumbsch et al. (2007) as well as the two resurrected genera *Circinaria* Link and *Sagedia* Ach.

During the investigation of samples collected in the Caucasus, Iran, Greece and Macaronesia we found a group of samples with K+ red thallus and distinct morphology belonging to Megasporaceace that did not fit with any of the above mentioned, previously described genera.

The aim of this study was to investigate the taxonomic position and to establish phylogenetic re- lationships of these lichen samples among Megasporaceace taxa. In order to reach the goal, we sequenced ITS, nuLSU and mtSSU rDNA as they have been shown to be useful to resolve phy- logenetic relations in this group of lichenized fungi (Lumbsch et al. 2007, Nordin et al. 2010).

Materials and Methods

Morphology and anatomy of the specimens were observed by using a Leica M165 C stereo- microscope connected to a Leica DFC485 digital camera, and with a Leica DM 2500 P com- pound light microscope connected to a Leica MC 190 HD digital camera.

Detailed observations of thallus anatomy, asci, ascospores and conidiospores were made on hand-cut sections of thallus, apothecia and pycnidia mounted in tap water. Measurements of ascospores (40 for each species; minimum lengthómaximum length × minimum widthómaxi- mum width) and conidia (40 for each species; minimum lengthó

maximum length) were made at ×400 and ×1000 magnifications. The specimens used in this study were deposited in the Herbaria of GLM and B. The chemistry of the specimens was investigated by thin layer chro- matography (TLC) using solvents B and C (Orange et al. 2001) and by spot tests using KOH, C and I (Nylander 1866) directly on the lichen thalli.

Molecular study: Total DNA was extracted from freshly collected material according to Park et al. (2014) with some modifications: We used a 1 × 1 mm^2 piece of medulla and three 2.5 mm sterile beads and mixed it for 2 minutes with beadmill; added 300 µl of KCl extraction buffer to the samples, and inverted strongly by hand approximately 20 times; added 300 µl RotiR-C/I (chloroform/isoamyl alcohol at a ratio of 24:1), and inverted gently approximately 20 times. We centrifuged the sample for 1 min at 12,000 rpm at room temperature (RT), transferred the upper aqueous layer to a new 1.5 ml micro- centrifuge tube, and added 180 µl of chilled isopro- panol, mixed by very gentle inversion, centrifuged for 1 min at 12,000 rpm at RT and discarded the supernatant. We washed the resulting pellet with 300 µl of chilled 70 % ethanol and dried the pellet at 50~65 °C with Eppendorf Thermomixer and resuspend the pellet in 30 µl of TE buffer (1×) at 50~65 °C with Eppendorf Thermomixer for 5 min.

PCR amplifications and sequencing: the primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) was used for the PCR amplifications of the ITS region. For PCR am- plification of the nuLSU region the primers LR0R, LR7 and LR5 (Vilgalys & Hester 1990), and for the mtSSU region the primers mrSSU1 and mrSSU3R (Zoller et al. 1999) were used. PCR amplifications were performed in a 12.5 L volume containing 2 L undiluted DNA, 0.5 L of each primer (10 mm), 6.4 L of sterile water, 1 L dNTP (2 mm), 1 L sóbuffer, 1 L MgCl , 0.1 L Taqópolymerase (Peqlab). Thermal cycling parameters were initial denatura- tion for 5 min at 95 °C, followed by 30 cycles of 30 sec at 95 °C, 30 sec at 54 °C, and 1 min at 72 °C. Following the last cycle a final extensions for 3 min at 72 °C was included. For the

primer pairs LR0R, LR7 and LR5 the annealing temperature was set to 64 °C and for mtSSU1 and mtSSU3R to 59 °C. The amplification products were visualized by electrophoresis on 1 % agarose gels and stained with ethidium bromide, and were purified with Exosap (USB). Both DNA strands of the PCR product were sequenced on an ABI 3730 by the Bik-F Laboratory Center in Frankfurt am Main. A number of ITS, nuLSU and mtSSU sequences from five gen- era of Megasporaceae (Nordin et al. 2010) were

downloaded from NCBI GenBank (http:// www.ncbi.nlm.nih.gov/genbank/). Two samples of *Ochrolechia* were selected as outgroup, since this has been shown to be closely related to Megasporaceae (Miadlikowska et al. 2014)

Alignment and phylogenetic analyses: Voucher information is provided in Table 1. The sequences were aligned by the program Muscle V4 (Edgar 2004) on a web server (http:// www.ebi.ac.uk/Tools/msa/muscle/) with the default settings; output tree: none, output order: aligned. The aligned sequences were adjusted manually using the PhyDE software (Müller et al. 2005). Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to eliminate poorly aligned positions, applying settings allowing for smaller final blocks, gap position within the final blocks and less strict flanking position (Castresana 2000).

MrModeltest (Nylander 2004) was used separately for each locus to determine the most appropriate model of nucleotide evolution using the AIC. GTR + I + G was found to be the best-fitting model for nuLSU and ITS, and HKY+ I + G for mtSSU. Bayesian inference (BI) of phylogeny with Markov chain Monte Carlo sampling was performed on the concatenated dataset including 2344 unambiguously aligned nucleotide positions. Bayesian analyses were conducted with MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003). Two independent runs, each with four heated MetropolisóCoupled MCMC chains (õtemperatureö 0.2) were initiated and run for 1000000 generations, with tree and parameter sampling every 100 generations. Burnóin was set to discard the initial 25 % of samples. Convergence among the parameters of the both runs were assessed in Tracer v1.5 following the criteria of effective sample size (ESS) values above 200. Consensus tree was generated after discarding the initial 25 % trees. Maximum parsimonious trees (MP) were reconstructed by the MEGA7 software (Kumar et al. 2016) using the heuristic search option with 100 random sequence additions and tree bi- section and reconstruction (TBR) as the branchóswapping algorithm. Alignment gaps were treated as missing and all characters were unordered and of equal weight. The robustness of the trees obtained was evaluated by 1000 bootstrap replications with ten random sequence additions. Maximum likelihood (ML) analyses were performed for the single loci separately and for the concatenated dataset using the program RAxML v8.1.11 (Stamatakis 2014) at the CIPRES Science Gateway server (http://www.phylo.org/portal2/), and implementing the ÷GTRGAMMAø model, with locus-specific model partitions. Nodal support was evaluated us- ing 1000 bootstrap

pseudoreplicates with the same model settings. The conflict among single gene tree was assessed using the tree topology and following the criteria of nodes bootstrap support equal or above 70 % in one but below this value in other tree. No conflicts were found and thus all the three datasets were concatenated.

Results

Phylogeny

A total of 71 new DNA sequences (ITS, nuLSU and mtSSU) was generated for this study and aligned with sequences obtained from GenBank (Table 1). The matrix of the data sets had 660, 820 and 863 unambiguously aligned nucleotide positions for ITS, nuLSU and mtSSU, respectively.

The maximum parsimony analysis of the concatenated dataset resulted in two most parsimo- nious trees with 744 steps, consistency index (CI) = 0.567, retention index (RI) = 0.780 and rescaled consistency index (RC) = 0.442 for all sites. The majority rule consensus tree of the maximum parsimony analysis was congruent with the tree obtained by Bayesian and maxi- mum likelihood phylogenetic inference. Therefore, only the majority rule consensus tree of the Bayesian analysis is shown (Fig. 1) with posterior probabilities (PP) of Bayesian analysis and bootstrap support (BS) of Maximum Parsimony and Maximum Likelihood analyses.

The phylogenetic trees resulting from the three different analyses confirmed the monophyly of the family Megasporaceace and the previously described genera within this family with a high posterior probability and bootstrapping values (PP = 1.00; MP/ML BS = 100/100).

All phylogenetic analyses (BI, ML and MP) resolved a distinct clade within Megasporaceace, with a high confirmation (PP = 1.00; MP/ML BS= 100/100) in a sister position with other clades currently treated as genera. Samples of *Aspicilia cupreoglauca* formed a monophyletic clade with high support values (PP = 1.00; MP/ML BS= 99/100). The samples of a so far undescribed taxon composed also a monophyletic clade with high support values (PP = 1.00; MP/ML BS= 100/100) and the *A. intermutans* samples grouped in a monophyletic clade with high Bayesian posterior probability (PP=1.00), but relatively low support in MP (BS=76) and ML (BS=77).

Table 1: Voucher specimens and NCBI GenBank accession numbers of the ITS1-5.8S-ITS2, nrLSU and mtSSU se- quences used in the phylogenetic analyses. New sequences in bold.

Taxon	Locality, Voucher	GenBank acc. No		
		nrLSU		ITS
Aspicilia cinerea	Sweden, Dalarna, Hermansson 13275 (UPS)	HM060733	HM060695	EU057899
Aspicilia cinerea	Sweden, Östergötland, Nordin 5542 (UPS)	HM060734	HM060696	ó
Aspicilia cuprea	USA, California, Owe-Larsson 9112 (UPS)	HM060745	HM060707	ó
Aspicilia cyanescens	USA, California, Owe-Larsson 9151 (UPS)	HM060745	HM060707	ó
Aspicilia dudinensis	Sweden, Torne Lappmark, Nordin 6036 (UPS)	HM060748	HM060710	ó
Aspicilia epiglypta	Sweden, Västergötland, Nordin 6303 (UPS)	HM060756	HM060718	EU057907
Aspicilia indissimilis	Sweden, Nordin 5943 (UPS)	HM060746	HM060708	EU057909
Aspicilia laevata	Sweden, Tibell 23659 (UPS)	HM060730	HM060692	EU057910
Aspiciliella cupreoglauca	Greece, Sipman & Raus 61847 (B)	**KY576954**	**KY576930**	**KY618843**
Aspiciliella cupreoglauca	Greece, Sipman & Raus 62345 (B)	**KY576955**	**KY576931**	**KY618844**
Aspiciliella cupreoglauca	Greece, Sipman & Raus 62440 (B)	**KY576956**	**KY576932**	**KY618845**
Aspiciliella cupreoglauca	Greece, Sipman & Raus 62682 (B)	**ó**	**KY576933**	**KY618846**
Aspiciliella cupreoglauca	Greece, Sipman & Raus 62704 (B)	**KY576957**	**KY576934**	**KY618847**
Aspiciliella intermutans	Iran, Zakeri 49267 (GLM)	**KY576020**	**KY576019**	**KY596018**
Aspiciliella intermutans	Iran, Zakeri 49268 (GLM)	**KY576943**	**KY576919**	**KY596005**
Aspiciliella intermutans	Iran, Zakeri 49269 (GLM)	**KY576949**	**KY576925**	**KY596013**
Aspiciliella intermutans	Iran, Zakeri 49270 (GLM)	**KY576950**	**KY576926**	**KY596014**
Aspiciliella intermutans	Iran, Zakeri 49271 (GLM)	**KY576951**	**KY576927**	**KY596015**
Aspiciliella intermutans	Armenia, Zakeri 39727 (GLM)	**KY576941**	**KY576917**	**KY596006**
Aspiciliella intermutans	Armenia, Zakeri 39729 (GLM)	**KY576942**	**KY576918**	**KY596007**
Aspiciliella intermutans	Armenia, Zakeri 40474 (GLM)	**KY576944**	**KY576920**	**KY596008**
Aspiciliella intermutans	Armenia, Zakeri 40494 (GLM)	**KY576945**	**KY576921**	**KY596009**
Aspiciliella intermutans	Armenia, Zakeri 40501 (GLM)	**KY576946**	**KY576922**	**KY596010**
Aspiciliella intermutans	Armenia, Zakeri 40503 (GLM)	**KY576947**	**KY576923**	**KY596011**
Aspiciliella intermutans	Armenia, Zakeri 40719 (GLM)	**KY576948**	**KY576924**	**KY596012**
Aspiciliella intermutans	Azerbaijan, Otte 38706 (GLM)	**KY576952**	**KY576928**	**KY596016**
Aspiciliella intermutans	Azerbaijan, Otte 38755 (GLM)	**KY576953**	**KY576929**	**KY596017**
Aspiciliella intermutans	Greece, Sipman & Raus 61911 (B)	**KY576958**	**KY576935**	**KY618848**
Aspiciliella intermutans	Greece, Sipman & Raus 62681 (B)	**KY576960**	**KY576937**	**KY618850**
Aspiciliella portosantana	Portugal, Sipman 62854 (B)	**KY576961**	**KY576938**	**KY618851**
Aspiciliella portosantana	Portugal, Sipman 63019 (B)	**KY576962**	**KY576939**	**KY618852**
Aspiciliella portosantana	Portugal, Sipman 63025 (B)	**KY576963**	**KY576940**	**KY618853**
Circinaria calcarea	Sweden, Nordin 5888 (UPS)	HM060743	HM060705	EU057898
Circinaria desertorum	Russia, Astrakhan, Owe-Larsson 9814 (UPS)	HM060727	HM060689	ó
Circinaria emiliae	Kazakhstan, Atyrau, Kulakov 3798 (UPS)	HM060729	HM060691	ó
Circinaria hispida	Turkey, Malatya, Candan 11 (ANES)	HM060760	HM060722	HQ406806
Lobothallia farinosa	France, Rhône-Alpes, Roux 25286 (UPS)	HM060761	HM060723	ó
Lobothallia melanaspis	Sweden, Jämtland, Nordin 6622 (UPS)	HM060726	HM060688	HQ259272
Lobothallia radiosa	Switzerland, Lumbsch ó	DQ780306.1	DQ780274	ó
Megaspora verrucosa	Turkey, Prov. Çorum, Kinalioglu 1679 (B)	JQ797497	JQ797482	ó
Megaspora verrucosa	Sweden, Jämtland Nordin 6495 (UPS)	HM060725	HM060687	ó
Ochrolechia sp.	AFTOL-ID 318 ó	DQ986777	DQ986886	HQ650639
Ochrolechia subplicans	USA, Alaska, Spribille 38211 (GZU)	KR017182	KR017358	KR017121
Sagedia mastrucata	Sweden, Lycksele Lappmark, Nordin 5481 (UPS)	HM060737	HM060699	EU057914
Sagedia mastrucata	Norway, Troms, Nordin 5708 (UPS)	HM060736	HM060698	EU057913
Sagedia simoensis	Sweden, Own-Larsson 9000 (UPS)	HM060739	HM060701	EU057926
Sagedia zonata	Norway, Troms, Owe-Larsson 8942 (UPS)	HM060738	HM060700	EU057949

Taxonomy

As a result of our phylogenetic study, we propose the resurrection of the genus *Aspiciliella* M.Choisy to accommodate the species representing the distinct phylogenetic lineage within Megarosporaceae. The main distinguishing characteristics of the genera of Megarosporaceae are listed in Table 2. *Aspiciliella* is morphologically similar to *Aspicilia*, but differs in the size of its conidia and the shape of ascospores. In addition, norstictic acid

was found in all species, which is also known in some species of *Aspicilia*. Therefore the chemical feature alone is not useful to distinguish both groups. Our phylogenetic study based on three markers confirmed that *A. intermutans* and *A. cupreoglauca*, previously assigned to *Aspicilia* based on their general appearance, represent a distinct lineage in Megasporaceae and form a clade together with a species described as new in this paper.

Since Choisy in Werner (1932) refers only to: *õ*spores ovales et í paraphyses gélifiéesö as characteristics of his new genus *Aspiciliella* (comprising then only *A. intermutans*), we provide below an emendated diagnosis and description of the features of *Aspiciliella*:

Aspiciliella M.Choisy

Generic type: *Aspiciliella intermutans* (Nyl.) M.Choisy in Werner (1932): Contribution á la flore cryptogamique du Maroc V. ó Cavanillesia 5(5): 1576174 (Fig. 2A)

Basionym: *Lecanora intermutans* Nyl., Flora (Regensburg) 55: 354 (1872).

Aspicilia intermutans (Nyl.) Arnold, Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien 37: 98 (1887).

Syntypes: Ad saxa arenaria prope St. Laon, leg. Richard (H; annotated as lectotype by Cl. Roux whom we follow here); ad granitica prope Brestum, leg. Crouan (not seen).

Epitype (hic designatus): Armenien, Ararat, Vedi, Urtsadzor, Khosrove Forest state Reserve, Alt. ca 1390 m. co-ord.: 39°59'07"N, 44°53'51"E. 17 Jun 2015, Z. Zakeri 40501 (GLM).

Diagnostic important characters: *Aspiciliella* differs from *Aspicilia* in having small conidia, spores that are always ellipsoid and in producing norstictic acid.

Description: Thallus crustose, rimose-areolate, partially continuous, K+ red; photobiont chlorococ- coid. Apothecia pale brown to dark grey to black, proper exciple always present, apothecia rarely surrounded by an additional thalline margin. Epihymenium green to olive green to greenish-brown, N+ changing to light green. Exciple hyaline. Hypothecium and subhymenium colourless, I+ blue to rusty red. Hymenium colourless, I+ blue to rusty red. Asci 8-spored, of *Aspicilia*-type. Ascospores ellipsoid, colourless, simple. Conidia straight, 7611 µm long.

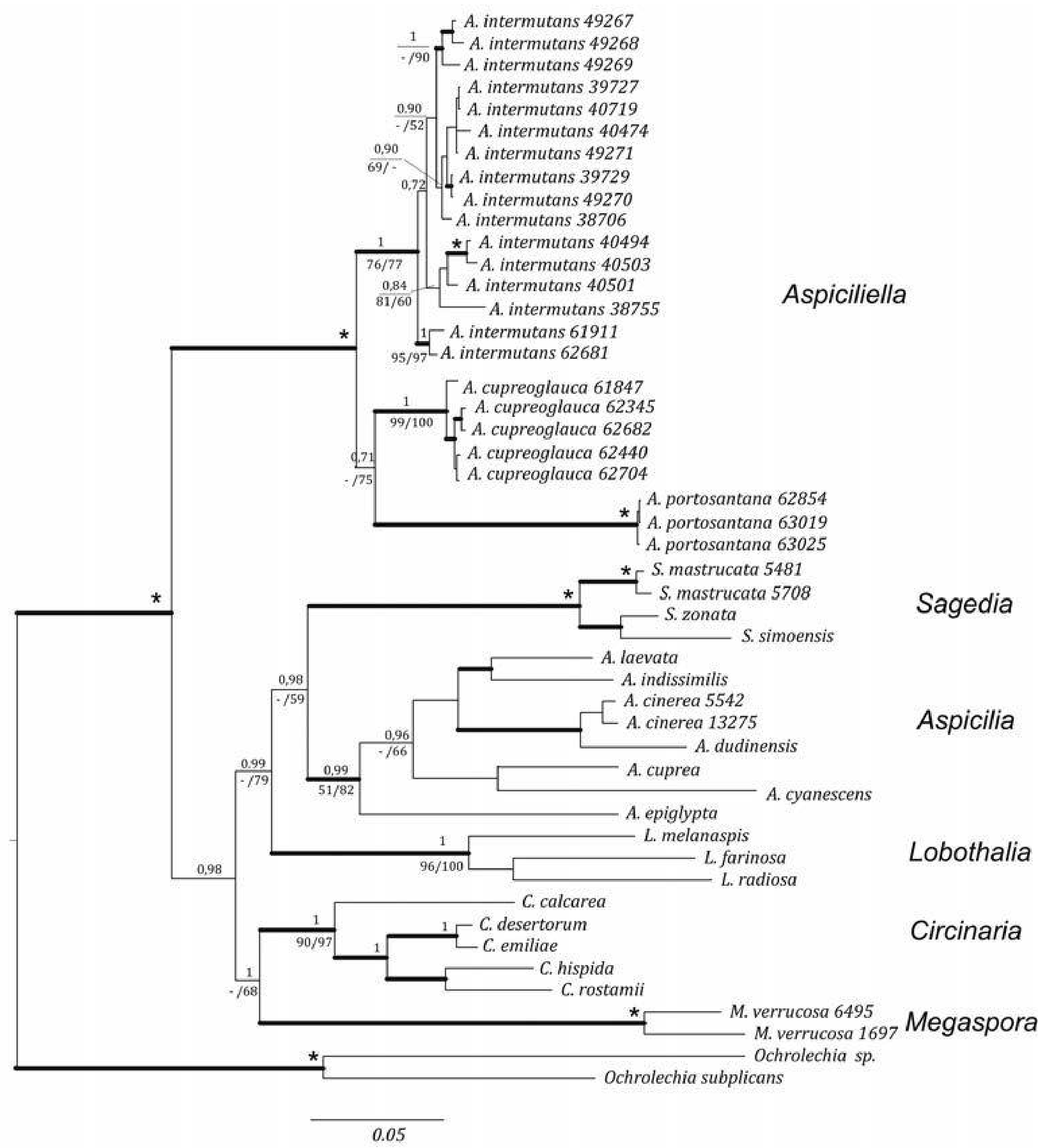

Fig. 1: Phylogenetic relationships of the family Megasporaceace with the genus *Aspiciliella* inferred by Bayesian analy- sis of the combined data sets of the three genetic marker: nrITS, nrLSU and mtSSU using mid-point rooting. Bayesian posterior probabilities are shown above the branches and MP/ML bootstrap values × 50 are shown below the lines adja- cent to the branches. Branches that received strong support in RAxML (bootstrap values ×70 %) and Bayesian inference (posterior probabilities ×0.95) are in bold and an asterisk above a branch shows 100 % for all three supports.

Chemistry and spot tests: Thallus KOH+ red, Có, UVó. TLC: norstictic acid and sometimes connor- stictic and stictic acids.

Distribution: The investigated material suggests that the genus is widespread in the Mediterranean and extends at least as far east as Iran and as far southwest as Macaronesia. Reports from *A. intermu- tans* from elsewhere may need verification.

Table 2: The main distinguishing characteristics of the genera of Megasporaceae (based on Nordin et. al. 2010, Zakeri et. al. 2016 and the present study)

Character	*Aspiciliella*	*Aspicilia*	*Circinaria*	*Lobothallia*	*Sagedia*	*Megaspora*
Ascospores	ellipsoid, 8 per ascus	ellipsoid, rarely globose, 8 per ascus	broadly ellipsoid to globose, 2ó6(ó8)	ellipsoid, 8 per ascus	ellipsoid, 8 per ascus	ellipsoid, 4 ó 8 per ascus
Spore size	22ó34 × 16 ó22 µm	10 ó27 × 8 ó19 µm	18 ó36 × 12ó26 µm	8 ó18 × 5 ó12 µm	14 ó25 × 7ó14 µm	27ó 60 × 18 ó 42 µm
Conidia length	7ó11 µm	11ó 40 µm	6 ó12 µm	3 ó 8 µm	8 ó12 µm	8 ó12 µm
Chemistry						
Aspicilin	ó	ó	±	ó	ó	ó
Substictic acid	ó	±	ó	ó	ó	ó
Norstictic acid	+	±	ó	±	ó	ó

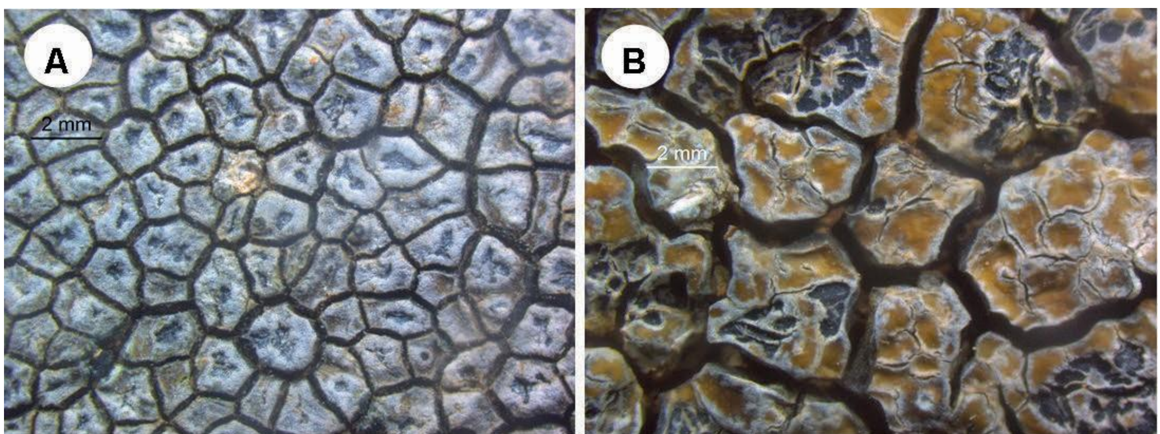

Fig. 2: **A** ó *Aspiciliella intermutans*, thallus with apothecia and pycnidia. **B** ó *Aspiciliella cupreoglauca*, thallus with apothecia.

New combination:

Aspiciliella cupreoglauca (B.de Lesd.) Zakeri, Divakar & Otte, **comb. nov.** [MycoBank MB821506] (Fig. 2B)

Basionym: *Aspicilia cupreoglauca* B.de Lesd., Bull. Soc. bot. Fr. 57: 32 (1910).

Type: Prémian vers Langlade, 500 m. alt., sur des schistes, 1909, leg. Abbé Soulié (not seen).

New species:

Aspiciliella portosantana Sipman & Zakeri, **sp. nov.** [MycoBank MB821509] (Fig. 3)

Diagnosis: Thallus grey to greenish grey, thin, 0.2ó 0.3 mm thick; apothecia aspicilioid, usually nu- merous; ascospores 8/ascus, hyaline, ellipsoid, 22ó28 × 17ó23 µm, conidia small, straight, 6 ó10 µm. Differs from the very similar *Apiciliella intermutans* by the thinner, pale grey thallus without or with weakly differentiated pale rims of the thallus areoles, and most clearly by the ITS and/or mtSSU sequences.

Type: Portugal, Madeira Islands, Porto Santo: SW Part, Pico de Ana Fereiera. Alt. ca 220 m. co-ord.: 16°22,2'W, 33°02,7'N. On volcanic rock outcrops on exposed, bare ridge and on stone walls along abandoned fields; 4 March 2016, H. Sipman 63019. (holotype: B 600154592; isotype: GLM).

Description: Thallus rimose-areolate to verrucose, (1ó) 2ó 8 (ó10) cmin diam., (0.1ó)0.3 ó 0.4(ó 0.6) mm thick; areoles angular to irregular, flat to ± convex, sometimes rounded (0.2ó)0.5 ó1(ó1.5) mm in diam.,

contiguous and separated by distinct cracks; thallus edge distinct; prothallus present, well developed along the thallus edge, fimbriate or forming a narrow brown to black zone, 0.1ó 0.4(ó 0.8) mm wide; surface usually grey, sometimes partly pale dark green to greenish grey; cortex: even, one-layered, co- vered with crystals; medulla white, containing crystals of calcium oxalate, Ió ; algal layer continuous; photobiont chlorococcoid, cells ± spherical to subspherical, (6 ó)8 ó12(ó14) µm in diam. **Apothecia** aspicilioid, usually common, (0.1ó)0.2ó 0.4(ó 0.6) mm in diam., 1ó2(ó3) per areole, round or elongated or irregular; disc black, flat, rarely concave, usually with a thin, white pruina; thalline margin usually elevated and often prominent in older apothecia, rarely flat in younger apothecia, concolorous with thallus, usually with a white to gray rim in greenish parts of thallus; exciple (8 ó)15 ó20(ó25) µm wide, I+ blue entirely and then changing to rusty red; epihymenium olive-brown to brown, sometimes olive, with crystals, N+ light green (caesiocinerea-green), K+ red; hymenium hyaline, I+ persistent blue to rusty-red, (80 ó)110 ó150(ó180) µm; paraphyses submoniliform to moniliform, with (1ó)2ó3(ó 4)

± globose to sometimes subglobose, rarely subcylindrical upper cells, 3 ó5(ó 6) µm wide; subhymeni- um pale (20 ó)25 ó50(ó 60) µm thick, I+ blue and then changing to rusty red. **Asci** clavate, 80 ó130 × 20 ó30 µm, 8-spored. **Ascospores** hyaline, simple, subglobose, 22ó28 × 17ó23 (n=40). **Pycnidia** rare, immersed, single or aggregated in swollen areoles, with a black, punctiform to rarely elongated ostiole; conidia filiform, straight, (5 ó)7ó9(ó 11) µm.

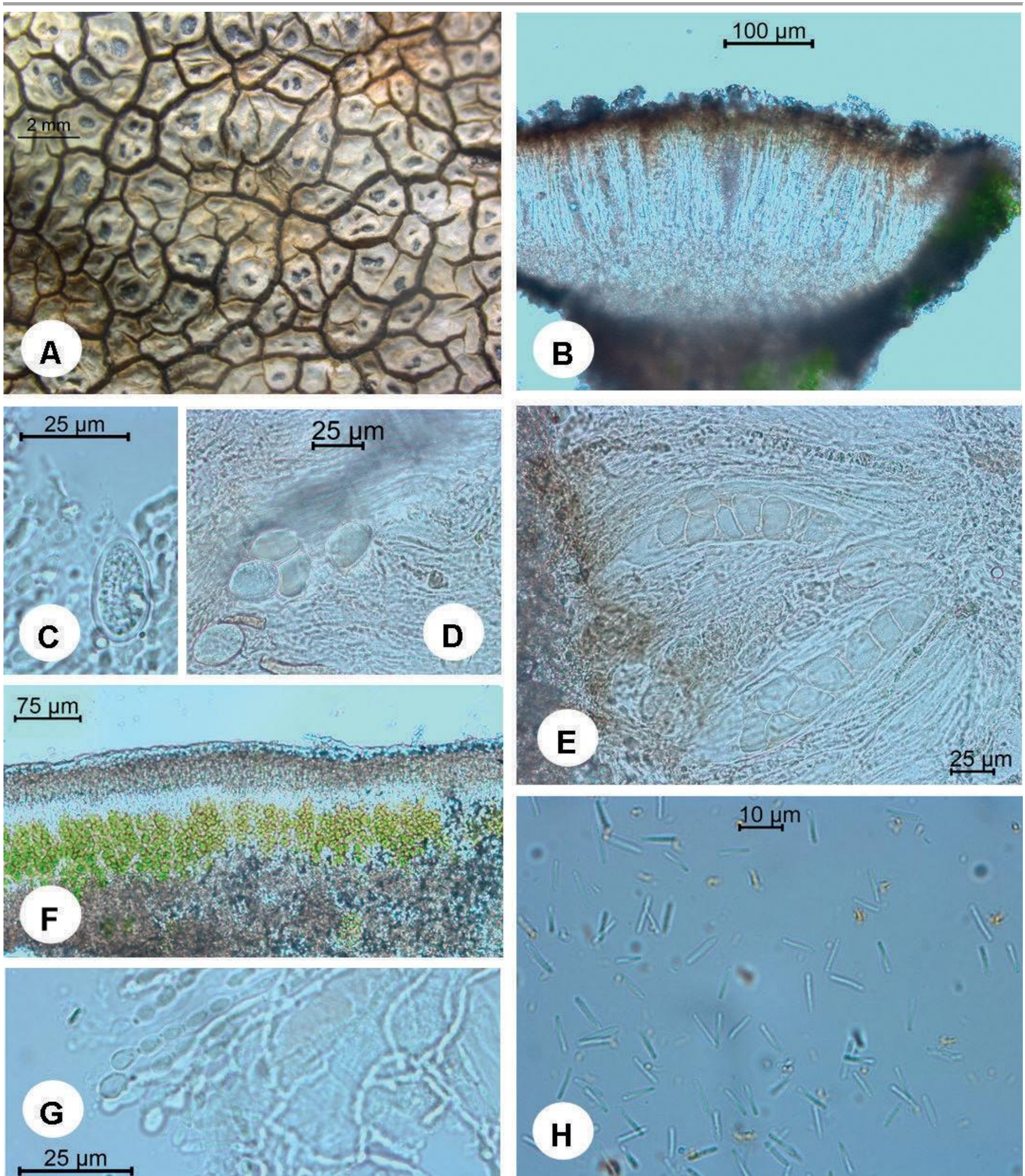

Fig. 3: *Aspiciliella portosantana*, holotype. **A** ó Thallus with apothecia; **B** ó Cross section of apothecium; **CóD** ó Ellipsoid ascospores; **E** ó Ascus, showing 8 ascospores per ascus; **F** *ó* Cross section of cortex and medulla; **G** ó Moniliform paraphysoids with 3ó6 globose uppermost cells; **E** ó Filiform straight conidia.

Chemistry and spot tests: Cortex and medulla Ió, K+ red, Có. TLC: Norstictic acid and connorstictic acid.

Etymology: The speciesøepithet refers to its occurrence in Porto Santo.

Ecology and distribution: On volcanic rock outcrops on exposed, bare ridge and on stone walls along abandoned fields and on steep SW-facing slope, at 220 ó250 m. The species is known only from the island Porto Santo near Madeira (Macaronesia).

Additional specimen examined: Portugal, Madeira Islands, Porto Santo: SW Part, Pico de Ana Fereiera. Alt. ca. 220 m. co-ord.: 16°22,2'W, 33°02,7'N. On volcanic rock outcrops on exposed, bare ridge and on stone walls along abandoned fields, 4 March 2016, H. Sipman 63025. Portugal, Madeira Islands, Porto Santo: E Part, along trail to Pico das Urzes (Pico Branco). Alt. ca. 250 m. co-ord.: 16°18,4'W, 33°05,56'N. On volcanic rock outcrops on steep SW-facing slope (537), 29 February 2016, H. Sipman 62854.

Notes: The new species is characterized by a pale grey to greenish grey, very thin thallus, (0.1ó) 0.2ó 0.3 mm thick, without or with indistinct pale marginal lines of the areoles. *Aspiciliella intermutans* has a yellowish grey thallus with pronounced or even confluent pale rims of the areoles and the thallus is usually thicker than that of *A. portosantana* (0.3 ó1.5 mm thick). *Aspiciliella cupreoglauca* is distin- guished from the closely related new species by having a brown thallus and larger areoles (larger than 2 mm) with white lines on the surface and along its margin.

Key to the species of *Aspiciliella*

1 Thallus pale brown to dark brown, areoles large (2.5 ó5 mm) with pale lines on surface and along the margins.. ***A. cupreoglauca***

1* Thallus grey to dark grey or yellowish- or greenish grey, areoles smaller (1ó2.5 mm). Without or with pale lines only along the margins...................................... **2**

2 Thallus grey, dark grey to greenish grey; pale marginal lines usually absent .... ***A. portosantana***

2* Thallus grey to pale yellowish- or brownish grey, pale marginal lines present, these often wide and covering most of the areoles .... ***A. intermutans***

Discussion

According to our ongoing studies, õ*Aspicilia intermutans*ö in its current understanding in- cludes most probably a complex of mostly undescribed species which is to be

resolved in a subsequent study especially with a broader sampling in the Oriental and Mediterranean re- gions (Zakeri, work in progress).

Acknowledgments

The first author would like to thank Harrie Sipman and Ali Bagherian Yazdi for generously providing taxonomic advice and helpful discussions, Christiane Ritz and Ulrike Damm for their worthy help and support. The authors grate- fully thank the curators of the herbaria H and B for the loan of specimens. We are particularly indebted to T. Spribille for his hint to the previously published genus name *Aspiciliella* M.Choisy for the group confirmed here as a distinct genus. Financial support from the Ungerer Foundation is gratefully acknowledged.

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Manuscript accepted: 23 May 2017. Communicated by: Christian Printzen

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Kapitel 3:

Discovering cryptic species in the *Aspiciliella intermutans* complex (*Megasporaceae*, *Ascomycota*), first results using gene concatenation and coalescent-based species tree approaches

Zakieh Zakeri*, Volker Otte, Harrie Sipman, Ji í Malí ek, Paloma Cubas,
Víctor J. Rico & Pradeep K. Divakar

(In prepration to submit)

Abstract:

Sample identifications in lichen forming fungi has been a challenge especially due to a few taxonomically relevant features and limitations of traditionally used morphological and chemical characters to identifying closely related taxa. Delineating species boundaries in closely related species or species complexes often requires a range of independent data sets and analytical methods. Here we aim to examine species boundaries in a mainly Mediterranean widespread saxicolous lichen forming fungi, the *Aspiciliella intermutans* complex (*Megasporaceae*). We gathered DNA sequences of the nuclear ribosomal internal transcribed spacer (nITS), the nuclear large subunit (nuLSU) and the mitochondrial small subunit (mtSSU) ribosomal DNA, and DNA replication licensing factor MCM7 from 80 samples from Europe and Asia, mostly from Iran, Caucasia, Greece and eastern Europe. We used a combination of phylogenetic strategies and variety of empirical, sequence-based species delimitation approaches to infer species boundaries in this group. This includes, poisson tree processes (PTP) model, the automatic barcode gap discovery (ABGD), the general mixed Yule coalescent model (GMYC), and the multispecies coalescent approach *BEAST and Bayesian Phylogenetics and Phylogeography (BPP) program. Additionally, different species delimitation scenarios were compared using Bayes factors species delimitation analysis. Furthermore, morphological, chemical, ecological and geographical features of the sampled specimens were examined. We identified a total of six species-level lineages in *Aspiciliella intermutans* complex using inferences from multiple empirical operational criteria. We found little corroboration between morphological and ecological characters with our candidate species, while secondary metabolite data do not support our candidate species. Our study of the *A. intermutans* species-complex indicates that the genus *Aspiciliella*, as currently circumscribed, is more diverse in Eurasia and consequently other part of world than we expected.

Keywords: crustose lichens, taxonomy, phylogeny, species delimitation, cryptic species.

Introduction

The lichen forming fungal family *Megasporaceae* Lumbsch, Feige & K. Schmitz is a monophyletic group, belonging to *Ascomycota* (Nordin et al. 2010). Its representatives are characterized by their mostly crustose thallus, urceolate or lecanorine type of apothecia, world-wide distribution and predominantly saxicolous, over bryophytes, but also terricolous and lignicolous habit (Nordin et al. 2010, Nordin 2015, Nash III 2007, Owe-Larsson et al. 2007, Zakeri et al 2016). Some members of *Megasporaceae* Lumbsch (e.g. *Aspicilia cinerea* (L.) Körb., *Circinaria caesiocinerea* (Nyl. *ex* Malbr.) A. Nordin, Savi & Tibel, *C. calcarea* (L.) A. Nordin, Savi & Tibel, *C. contorta* (Hoffm.) A. Nordin, Savi & Tibel, *Lobothallia radiosa* (Hoffm.) Hafellner) are widely distributed and also very common in best known European lichen flora and they significantly contribute to the saxicolous communities on various types of rocks. Nevertheless, the taxonomic concept of many species remains unclear and many lichenologists consider this family dauntingly difficult.

Miadlikowska et al. (2014) placed *Megasporaceae*, in the order *Pertusariales* M. Choisy *ex* D. Hawksw. & O. E. Erikss. with other families like *Ochrolechiaceae* R. C. Harris *ex* Lumbsch & I. Schmitt, *Coccotremataceae* Henssen *ex* J. C. David & D. Hawksw., *Pertusariaceae* Körb. *ex* Körb. and *Icmadophilaceae* Triebel. Further, in this study, authors showed that *Megasporaceae* is closely related to *Ochrolechiaceae.*

The currently accepted taxonomy of *Megasporaceae* includes six genera: *Aspicilia* A. Massal., *Circinaria* Link, *Lobothallia* (Clauzade & Cl. Roux) Hafellner, *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth, *Sagedia* Ach. and *Aspiciliella* M. Choisy *in* Werner (Jaklitsch et al. 2016, Zakeri et al. 2017). The old generic name *Aspiciliella* (Werner 1932), was reintroduced in a phylogenetic publication based on three markers, ITS, mtSSU and nuLSU (Zakeri et al. 2017). The study confirmed that *Aspiciliella intermutans* (Nyl.) M. Choisy *in* Werner and *A. cupreoglauca* (B. de Lesd.) Zakeri, Divakar & Otte, previously assigned to *Aspicilia* based on their general appearance, represent a distinct lineage (*Aspiciliella*) in *Megasporaceae* and form a monophyletic clade together with the proposed new species *A. portosantana* Sipman & Zakeri (Zakeri et al. 2017). The representatives of the genus *Aspiciliella* are distinguished in having rimose-areolate thallus, partially continuous, K+ red; chlorococcoid photobiont. Apothecia pale brown to dark grey to black, proper exciple always present, apothecia rarely surrounded by an additional thalline margin. Epihymenium green to olive green to greenish-brown, N+

changing to light green. Exciple hyaline. Hypothecium and subhymenium colorless, I+ blue to rusty red. Hymenium colourless, I+ blue to rusty red. Asci 8-spored of *Aspicilia*-type. Ascospores ellipsoid, colourless, simple. Conidia straight, 7ó11 m long (Zakeri et al. 2017, Zakeri et al. 2018).

Lecanora intermutans Nyl. was described in 1872 from France (Nylander, 1872). Nylander (1872) indicated in the original description that the species looks like *Aspicilia cinerea*, but has larger spores 23-34 × 9-15 µm and smaller conidia 7-9 x 1 µm. Later, this species was transferred to the genus *Aspicilia* (Arnold 1887) and more recently to *Aspiciliella* (Werner 1932, Zakeri et al. 2017). *Aspiciliella intermutans* is widely distributed, especially in the Mediterranean Region. Collections are known especially from Iran, Armenia, France and Greece. It is also very common in Italy (Nimis & Martellos 2008) and the Iberian Peninsula (Llimona & Hladun 2001). In Central Europe, *A. intermutans* is rare and locally distributed. Its overall distribution reaches Great Britain (Fletcher et al. 2009), Canary Islands (Hafellner 1995), Tunisia (e.g. Pitard & Bouly de Lesdain 1909), Syria (John et al. 2004), Ural Mountains (Paukov et al. 2014) and U.S.A. (McCune et al. 2014).

Our previous study has shown that *Aspiciliella intermutans* is a monophyletic lineage but has some well supported clades inside, and may represent a complex of unrecognized species (Zakeri et al. 2017). This assumption is further supported by a very large genetic and morphologic variability among all samples of this group, what pushed us to work more on this group to find out the phylogenetic relationship and explore the probable cryptic speciation in this complex, if any. Previous studies have repeatedly shown the presence of cryptic species in numerous species complexes of mainly foliose and fruticose lichens (e.g. Leavitt et al. 2011a, b, 2013a, b, Del-Prado et al. 2016, Parnmen et al. 2012). In many groups organisms, including lichenized fungi, new species are still being discovered using morphological data but species boundaries are still in a state of flux because finding appropriate character sets and analytical tools for empirical species delimitation are among the greatest taxonomic challenges (Crespo & Pérez-Ortega 2009, Lumbsch & Leavitt 2011). Recently, the growing number of methods for analyzing DNA sequence data in a coalescent-based framework are capable to critically evaluate species diversity in fungi (Leavitt et al. 2015). The coalescent-based species delimitation methods have otherwise not been used until now in *Megasporaceae* species, particularly in studies of *A. intermutans* complex. We used a combination of phylogenetic strategies to delimit the

species in *Aspiciliella intermutans* complex applying coalescent-based approaches and other recently developed DNA-based methods.

Commonly used methods for species delimitation include the automatic barcode gap discovery (ABGD) (Puillandre et al. 2012), poisson tree processes (PTP) model (Zhang et al. 2013), and the general mixed Yule coalescent model (GMYC) (Monaghan et al. 2009; Pons et al. 2006). We used *BEAST (Ence & Carstens 2011) to reconstruct species trees. Estimating the species tree and species delimitation using coalescent methods for closely related taxa have proven very useful and have been used for lichenized fungi in some species complexes, e.g. in *Parmotrema reticulatum* (Taylor) M. Choisy, *Parmotrema pseudoreticulatum* (Tav.) Hale, *Rhizoplaca melanophthalma* (DC.) Leuckert and *Cladia aggregata* (Sw.) Nyl. (Leavitt et al. 2011a, b, 2013a, b, Del-Prado et al. 2016, Parnmen et al. 2012).

The objective of this study was thus to define species boundaries within the *A. intermutans* complex by implementing single- and multi-locus phylogenetic analyses, and coalescent-based species delimitation methods. In order to reach the goal, we gathered 90 samples from Asia and Europe and generated DNA sequences of internal transcribed spacer region (ITS) the nuclear large subunit (nuLSU), the mitochondrial small subunit (mtSSU) ribosomal DNA and DNA replication licensing factor MCM7 (MCM7) as they have been shown to be useful to resolve phylogenetic relations in this group of lichenized fungi (e.g. Lumbsch et al. 2007, Nordin et al. 2010, Zakeri et al. 2016, 2017).

Materials and Methods

Specimen sampling and phenotypic study

The specimens examined were mostly collected by the authors from distant geographic regions in Iran, Armenia and Czech Republic and also specimens were obtained from the following herbaria: B, GZU, PRA, PRC, PL, CR and GLM. Sequence data of specimens tentatively referred as *Aspiciliella intermutans* were analyzed in 66 ITS, 51 MCM7, 61 nuLSU and 73 mtSSU sequences. Five samples of *Aspiciliella cupreoglauca* were selected as outgroup, since this has been shown to be closely related to *Aspiciliella intermutans* (Zakeri et al. 2017).

Morphology and anatomy of the specimens were observed, and tabulated further, by using a Leica M165 C stereomicroscope connected to a Leica DFC485 digital camera, and with a Leica DM 2500 P compound light microscope connected to a Leica MC 190 HD digital camera. Detailed observations of thallus anatomy, asci, ascospores and conidiospores were made on hand-cut sections of thallus, apothecia and pycnidia mounted in tap water.

The chemistry of the specimens was investigated, and tabulated further, by thin layer chromatography (TLC) using solvents B and C (Orange et al. 2001) and by spot tests using KOH, C and I (Nylander 1866) directly on the lichen thalli.

DNA extraction, PCR amplifications and sequencing

Total DNA was extracted from freshly collected material according to Park et al. (2014) but with some modifications as described in Zakeri et al. (2016, 2017).

The primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) was used for the PCR amplifications of the ITS region. For PCR amplification of the nuLSU region the primers LR0R, LR7 and LR5 (Vilgalys & Hester 1990), for the mtSSU region the primers mrSSU1 and mrSSU3R (Zoller et al. 1999), and for the MCM7 region the primers Mcm7-1348rev (Schmitt et al. 2009) and X_mcm7_F (Leavitt 2010) were used. PCR amplifications were performed in a 12.5 L volume containing 2 L undiluted DNA, 0.5 L of each primer (10 mm), 6.4 L of sterile water, 1 L dNTP (2 mm), 1 L sóbuffer, 1 L MgCl2, 0.1 L Taqópolymerase (Peqlab). Primers details and PCR conditions are summarized in Table 1. Thermal cycling parameters were initial denaturation for 5 min at 95 °C, followed by 30 cycles of 30 secs at 95 °C, 30 secs at 54 °C for amplifying ITS, 64 °C for nuLSU, 59 °C for mtSSU, 58 °C for MCM7, and 1min at 72 °C, a final extension step of 3 min at 72 °C was added, after which the samples were kept at 4 °C. The amplification products were visualized by electrophoresis on 1% agarose gels and stained with ethidium bromide, and was purified by adding 2 L ExoSAP-ITÎ (Exonuclease 1-shrimp alkaline phosphatase, USB) to 5 L of the PCR products, followed by a heat treatment of 15 min at 37 °C and 15 min at 80 °C.. Both DNA strands of the PCR product were sequenced on an ABI 3730 by the Bik-F Laboratory Center in Frankfurt am Main.

Sequence alignment

Sequence fragments generated for this study were assembled and edited using BioEdit version 7.0 (www.mbio.ncsu.edu/BioEdit/BioEdit.html). Sequence identity was confirmed using the mega-BLAST search function in GenBank

(https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were aligned by the program MAFFT v.7 (Katoh et al. 2009) on a web server (https://mafft.cbrc.jp/alignment/server/), implementing the E-INS-I alignment algorithm for nuLSU and G-INS-I alignment algorithm for all other markers, ÷200PAM/K = 2ø scoring matrix and with an offset value of 0.0, with the remaining parameters set to default values. Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to delimit and exclude ambiguously aligned positions, applying settings allowing for smaller final blocks, gap position within the final blocks and less strict flanking position (Castresana 2000).

Table 1: Primers used for PCR amplification and sequencing of the nuclear ribosomal internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (ITS) region, the nuclear large subunit (nuLSU), the mitochondrial small subunit (mtSSU) ribosomal DNA and DNA replication licensing factor MCM7.

Marker	**Primer name**	**Forward primer sequence**	**Annealing Temperature (°C)**	**Reference**
ITS	ITS1F	5'-CTTGGTCATTTAGAGGAAGTAA-3'	54 °C,	(Gardes & Bruns 1993)
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'	54 °C,	(White et al. 1990)
nuLSU	LR0R	5'-ACC CGC TGA ACT TAA GC-3'	64 °C	(Vilgalys & Hester 1990)
	LR7	5'- TAC TAC CAC CAA GAT CT-3'	64 °C	(Vilgalys & Hester 1990)
	LR5	5'- ATCCTGAGGGAAACTTC-3'	64 °C	(Vilgalys & Hester 1990)
MCM7	Mcm7-1348rev	5'-GAYTTDGCIACICCIGGRTCWCCC AT-3'	58 °C	(Schmitt et al. 2009)
	X_mcm7_F	5'-CGT ACA CYT GTG ATC GAT GTG-3'	58 °C	(Leavitt 2010)
mtSSU	mtSSU1	5'-AGCAGTGAGGAATATTGGTC-3'	59°C	(Zoller et al. 1999)
	mtSSU3R	5'-ATGTGGCACGTCTATAGCCC-3'	59°C	(Zoller et al. 1999)

Phylogenetic analyses

Maximum likelihood (ML) analyses were performed for the single loci separately and for the concatenated dataset using the program RAxML v.8.1.11 (Stamatakis 2014) at the CIPRES Science Gateway server (http://www.phylo.org/portal2/), and implementing the ÷GTRGAMMAø model, with locus-specific model partitions. Nodal support was evaluated using 1000 bootstrap pseudoreplicates with the same model settings. The conflict among single gene tree was assessed using the tree topology and following the criteria of nodes bootstrap support equal or above 70% in one but below this value in other tree. No conflicts were found (except one specimen) and thus all the datasets were concatenated.

A heuristic search for the maximum likelihood (ML) bootstrap tree with simultaneous inference of the optimal partitioning scheme and substitution models for each data partition was also performed using the online version of IQ-TREE (Nguyen et al. 2015, Chernomor et al. 2016, Kalyaanamoorthy et al. 2017) suggesting seven initial partitions (Group I intron, ITS1, 5.8S rDNA, ITS2, mtSSU, nuLSU and MCM7). Branch support was estimated with the ultrafast bootstrap algorithm (Minh et al. 2013) based on 1000 bootstrap replicates and using a maximum of 1000 iterations and a minimum correlation coefficient of 0.99 as a stopping rule.

We used the Markov Chain Monte Carlo approach implemented in MrBayes v.3.2.2 (Ronquist & Huelsenbeck 2003) to infer phylogenetic trees applying the partitioning scheme inferred with IQTREE and slightly simplified substitution models, because most of the models inferred by IQ-TREE are not implemented in MrBayes. See Table 2 for details on locus partitions and substitution models. Two independent runs, each with four heated MetropolisóCoupled MCMC chains (õtemperatureö 0.2) were initiated and run for 1000000 generations, with tree and parameter sampling every 100 generations. Convergence among the parameters of the both runs was assessed in Tracer v 1.6 following the criteria of effective sample size (ESS) values above 200. Consensus tree was generated after discarding the initial 25% trees.

Table 2: Details on locus partitions and substitution models.

	Group I intron	**ITS1**	**5.8S rRNA**	**ITS2**	**nuLSU**	**MCM7**	**mtSSU**
No. taxa	61	69	69	69	65	51	77
Position	1-183	184-414	415-553	554-725	726-1798	1799-2305	2306-3205
Substitution model	TIM2e+G4	TIM2e+G4	TVMe+I+G4	K2P+I+G4	TPM3+I+G4	TNe+I	F81+I+G4

Species delimitation analyses

In order to infer the most likely species numbers in our *Aspiciliella intermutans* dataset, we chose three commonly used methods for single-locus DNA-based species delimitation, the automatic barcode gap discovery (ABGD)) (Puillandre et al. 2012), a Bayesian implementation of the Poisson tree processes (bPTP) (Zhang et al. 2013), and the general mixed Yule coalescent model (GMYC) (Monaghan et al. 2009, Pons & Barraclough 2006), and two coalescent-based models *BEAST (Heled & Drummond 2010) and BPP v.3.2 (Yang & Rannale 2014) to analyze the four loci concatenated dataset.

ABGD is an automatic procedure that sorts the sequences into hypothetical species based on the barcode gap. This method automatically finds the distance where the barcode gap is

located (Puillandre et al. 2012). The ABGD method was carried out for the ITS, mtSSU and MCM7 datasets using the Web interface at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. Default parameters were chosen using Kimura 2-parameter (K2P) distances that correct for transition rate bias (relative to transversions) in the substitution process. The default for the minimum relative gap width was set to different values between 0.1 and 0.15. Our exploratory analyses showed that the ITS region is very variable in the *A. intermutans* group and the nuLSU is very conserved (results not shown) and thus they were not used for the final single-locus species delimitation analyses.

The PTP is an operational criterion of a gene coalescent view of the phylogenetic species concept (Baum & Shaw 1995) with the fundamental assumption that the number of substitutions between species is significantly higher than the number of substitutions within species (Leavitt et al. 2010, Drummond & Rambaut 2007). The PTP can use both ultrametric and non-ultrametric trees as input. We ran a PTP species delimitation analysis in the bPTP web server (http://species.h-its.org/) (Zhang et al. 2013). The ML phylogeny obtained with RAxML of the ITS, MCM7 and mtSSU data set, was used as the input trees to run bPTP species delimitation analysis. We ran the PTP analysis for 100,000 MCMC generations, with a thinning value of 100 and a burn-in of 10%. The tree was rooted and outgroup taxa were removed to improve species delimitation.

GMYC infers species boundaries using the differences of the branching rates in an ultrametric phylogenetic tree. In the single-threshold version of the method the switch from speciation to coalescence is supposed to be unique (Pons et al. 2006), while in the multiple thresholds version the initial species partition can be recursively re-analyzed to further split or join species (Monaghan et al. 2009). For the analyses, the ML tree obtained from a RAxML search using the ITS, MCM7 and mtSSU was used as initial tree to infer an ultrametric tree using the program BEAST v.1.8.0 (Drummond & Rambaut 2007), and applying the same locus specific substitution models as in MrBayes analysis. We implemented an uncorrelated relaxed lognormal clock (Drummond et al. 2006), and selected a Yule tree prior. Default values were used for remaining priors. MCMC analysis was run for a total of 10 million generations, sampling every 1000 steps. Convergence of each analysis was evaluated in Tracer 1.4.1 (Rambaut & Drummond 2007), and analyses were interrupted when ESS values exceeded 200. After excluding the first 25% trees as burn-in, a consensus tree was calculated. The consensus tree was then used to infer species

delimitation with the GMYC method, using both the single and the multiple thresholds methods. The five outgroup samples (*A. cupreoglauca*) were excluded from the data set.

We used the coalescent-based hierarchical Bayesian model *BEAST implemented in BEAST v.1.8.0 to estimate species tree following our proposed species delimitation scenarios. *BEAST incorporates the coalescent process and the uncertainty associated with gene trees and nucleotide substitution model parameters and estimates the species tree directly from the sequence data (Drummond & Rambaut 2007).

Species assignments are required **a priori** for *BEAST analyses. To designate species in *BEAST, we used four different scenarios (4, 5, 6 and 9 species) for assigning individuals to a ÷candidate speciesø based on well supported groups in the phylogenetic tree obtained from concatenated dataset and the 9 candidate species obtained from the single-locus species delimitation ABGD analysis (see the results). We used lognormal distributions for the relaxed uncorrelated rates for all loci, we selected a Yule process and gamma-distributed population sizes for the species-tree prior and a piecewise linear population size model with a constant root. Default values were used for remaining priors. Two independent Markov chain Monte Carlo (MCMC) analyses were run for a total of 100 million generations, sampling every 1000 generations and excluding the 12500 trees as burn-in. we assessed MCMC convergence and determined burn-ins by examining ESS values and likelihood plots in the program Tracer version 1.6. The posterior probabilities of nodes were computed from the sampled trees (excluding burn-in samples) using TreeAnnotator 1.8.0 (Drummond & Rambaut 2007).

We used multispecies coalescent model implemented in the program BP&P v.3.2 (Yang & Rannala 2014) to estimate the marginal posterior probability of speciation for each species scenario (4, 5, 6 and 9 species). BP&P incorporates coalescent theory and phylogenetic uncertainty into parameter estimation; and the posterior distribution for species delimitation models is sampled using a reversible-jumpMarkov Chain Monte Carlo (rjMCMC) method. We used the unguided species delimitation algorithm (÷A11ø Yang 2015). This algorithm explores different species delimitation models and different species phylogenies, with fixed specimen assignments to populations. The program attempts to merge populations into one species, and uses the nearest neighbour interchange (NNI) or subtree pruning and regrafting (SPR) algorithms to change the species tree topology (Yang & Rannala 2014). We performed analyses using algorithms 0 and 1 using three prior distributions on ancestral population sizes ( ) assigned to gamma distributions of G (2, 10),

G (2, 100) and G (2, 1000), combined with root age (0) assigned G (2, 2000). Rates were allowed to vary among loci (locus rate = 1), and the analyses were set for automatic fine-tune adjustments. Each reversible-jump Monte Carlo (rjMCMC) analysis was run for 100 000 generations, sampling every generations and specified a burn-in of the first 8 000 generations. Each analysis was run twice to confirm consistency between runs. Speciation probabilities greater than 0.95 were considered supported species delimitations.

Since different species delimitation analyses supported different scenarios for *Aspiciliella intermutans* complex, the most likely hypothesis of species boundaries was assessed using Bayes factor delimitation (BFD) test (Grummer et al. 2014). Bayes factor uses a Bayesian coalescent-based reconstruction of species trees to compare the different species delimitation models, calculating the marginal likelihood and does not require a guide tree. We calculated marginal likelihood estimates (MLEs) for four species delimitation scenarios (4, 5, 6 and 9) species. For each scenario, we reconstructed a species tree using *BEAST v.1.8.0. The analyses were run with the same substitution models used in MrBayes, a birth-death model for the species tree prior; population size model set to piecewise linear and constant root. The MCMC chain was run for 20,000,000 generations, sampled every 1000th, and the first 25% of each run was discarded as burn-in. MLEs were estimated using path sampling (PS) and stepping-stone sampling (SS) in *BEAST, with 100 path steps, a chain length of 100,000 generations and likelihoods saved every 100 generations. Bayes factor was calculated as 2 × (marginal likelihood of the best model (model1) - marginal likelihood of alternative model (model2). Positive values of Bayes factor support the best model against alternative models.

Results

Phylogenetic analyses

We generated a data set of a seven-locus data matrix consisting of 3205 nucleotide positions. The matrix of the data sets had 183, 231, 139, 172, 1073, 507 and 900 unambiguously aligned nucleotide positions for Group I intron, ITS1, 5.8s, ITS2, nuLSU, MCM7 and mtSSU respectively. The ITS PCR product obtained was around 800 bp. The larger sizes of ITS were due to the presence of insertions of about 200 bp identified as Group I introns (Gutierrez et al. 2007) at the 3ø end of the SSU rDNA. Group I introns were present in all the samples of *Aspiciliella intermutans* complex. Table 2 summarized

the best-fitting models of evolution for each locus. The multilocus data set was based on 79 individuals. A total of 218 new DNA sequences (ITS, nuLSU, mtSSU and MCM7) were generated for this study and were aligned with sequences obtained from GenBank (Table 3). The partitioned ML analysis of the concatenated data matrix yielded the optimal tree with Ln likelihood value = -12042.961 The mean LnL value of the two parallel runs of the Bayesian analysis for the seven combined loci was -12512.24 with a standard deviation of ±2.17.

Since the topologies of the trees estimated from Bayesian methods and ML (in Cipres and IQ tree servers) did not have any well-supported conflict, only ML topologies from IQ tree are shown with bootstrap and posterior probability values indicated on the nodes (Fig. 1). In the concatenated tree topology, specimens of *A. intermutans* splitted in several groups (named hereafter A, B, C and D), in some cases with low support especially in Bayesian analyses (Fig. 1). In all the groups, subclades with strong support were also found.

Species delimitation analyses

As the monophyletic groups obtained in the concatenated tree were not reciprocally monophyletic in the single gene trees (results not shown), except the clades A1, A2, A3 and A4, which were recovered in mtSSU gene tree and A3 was recovered in MCM7, we run species delimitation analyses to clarify which clades could represent candidate species.

ABGD analyses applied to MCM7 and mtSSU dataset detected 9 candidate species in both of them, which were further used as candidate species in the multispecies coalescent species delimitation. Whereas PTP analyses applied to the MCM7 and mtSSU dataset detected 10 and 11 candidate species respectively, which do not overlap well to each other or with the results of other methods. The GMYC approach employing the multiple and single threshold on MCM7 and mtSSU dataset suggests also different candidate species as ABGD and PTP.

Since the results of different single-locus species delimitation analyses were not congruent, we used the main monophyletic groups obtained from concatenated dataset analysis in IQ tree and the 9 candidate species obtained from ABGD analysis, as species scenarios for subsequent analyses. We tested a total of 4 different species delimitation scenarios: the 4 (A1+A2+A3+A4, B, C and D), 5 (A1+A2+A3, A4, B, C and D), 6 (A1+A2, A3, A4, B, C and D) and 9 species (A1, A2, A3, A4, B, C, D1, D2 and D3) (Fig. 1).

The results of the multispecies coalescent species delimitation method BP&P with different algorithms 0 and 1 are summarized in Table 4a and 4b. The two algorithms in BP&P consistently distinguished 4ó6 independent evolutionary lineages. While the 4, 5 and 6 species topologies had a speciation probability of >0.96 threshold in the runs with algorithm 1 and 0 (values ranged between 0.96 ó 1.0), the 9 species topology was only supported (0.95) with algorithm 1 and a prior distribution on ancestral population size (theta) with a gamma distribution of G (2, 1000).

Table 3: Voucher specimens and NCBI GenBank accession numbers of the ITS1-5.8S-ITS2, nrLSU, mtSSU and MCM7 sequences used in the phylogenetic analyses. New sequences in bold.

Taxon	Country, Voucher	nrLSU	mtSSU	ITS	MCM7
A. cupreoglauca	Greece, *Sipman & Raus 61847* (B)	KY576954	KY576930	KY618843	----
A. cupreoglauca	Greece, *Sipman & Raus 62345* (B)	KY576955	KY576931	KY618844	----
A. cupreoglauca	**Greece, *Sipman & Raus* 59778 (B)**	**MH290743**	**MH349000**	**MH290788**	----
A. cupreoglauca	**Greece, *Sipman 59898* (B)**	**MH248842**	**MH248890**	----	----
A. cupreoglauca	**Greece, *Sipman 60010* (B)**	**MH248843**	**MH248891**	**MH255584**	----
A. intermutans	Iran, *Zakeri 49267* (GLM)	KY576020	KY576019	KY596018	----
A. intermutans	Iran, *Zakeri 49268* (GLM)	KY576943	KY576919	KY596005	----
A. intermutans	Iran, *Zakeri 49269* (GLM)	KY576949	KY576925	KY596013	----
A. intermutans	Iran, *Zakeri 49270* (GLM)	KY576950	KY576926	KY596014	----
A. intermutans	Iran, *Zakeri 49271* (GLM)	KY576951	KY576927	KY596015	----
A. intermutans	Armenia, *Zakeri 39727* (GLM)	KY576941	KY576917	KY596006	----
A. intermutans	Armenia, *Zakeri 39729* (GLM)	KY576942	KY576918	KY596007	----
A. intermutans	Armenia, *Zakeri 40474* (GLM)	KY576944	KY576920	KY596008	----
A. intermutans	Armenia, *Zakeri 40494* (GLM)	KY576945	KY576921	KY596009	----
A. intermutans	Armenia, *Zakeri 40501* (GLM)	KY576946	KY576922	KY596010	----
A. intermutans	Armenia, *Zakeri 40503* (GLM)	KY576947	KY576923	KY596011	----
A. intermutans	Armenia, *Zakeri 40719* (GLM)	KY576948	KY576924	KY596012	----
A. intermutans	Azerbaijan, *Otte 38706* (GLM)	KY576952	KY576928	KY596016	----
A. intermutans	Greece, *Sipman & Raus 61911* (B)	KY576958	KY576935	KY618848	----
A. intermutans	Greece, *Sipman & Raus 62681* (B)	KY576960	KY576937	KY618850	----
A. intermutans	**Greece, *Sipman & Raus 62695* (B)**	**MH257199**	**MH257235**	**MH210647**	**MH257270**
A. intermutans	**Greece, *Sipman & Raus 61847* (B)**	**MH257198**	**MH257234**	**MH210648**	**MH257261**
A. intermutans	**Greece, *Sipman & Raus 62653* (B)**	**MH257200**	**MH257236**	**MH210649**	**MH257262**
A. intermutans	**Greece, *Sipman & Raus 62704* (B)**	**MH257201**	**MH257237**	**MH210650**	**MH257263**
A. intermutans	**Greece, *Sipman & Raus 62252* (B)**	**MH257202**	**MH257238**	**MH210651**	**MH257264**
A. intermutans	**Greece, *Sipman & Raus 62271* (B)**	**MH257203**	**MH257239**	**MH210652**	**MH257271**
A. intermutans	**Greece, *Sipman & Raus 62105* (B)**	**MH257204**	**MH257240**	**MH210653**	**MH257265**
A. intermutans	**Greece, *Sipman & Raus 62439* (B)**	**MH257205**	**MH257241**	**MH210654**	**MH257272**
A. intermutans	**Greece, *Sipman & Raus 62614* (B)**	**MH257206**	**MH257242**	**MH210655**	**MH257273**
A. intermutans	**Greece, *Sipman & Raus 62663* (B)**	**MH257207**	**MH257243**	**MH210656**	**MH257274**
A. intermutans	**Greece, *Sipman & Raus 61933* (B)**	**MH257208**	**MH257244**	**MH210657**	**MH257266**
A. intermutans	**Armenia, *Zakeri 39702* (GLM)**	----	**MH257209**	**MH210658**	**MH257267**
A. intermutans	**Armenia, *Zakeri 39711* (GLM)**	----	**MH257210**	**MH210659**	**MH257245**
A. intermutans	**Armenia, *Zakeri 40475* (GLM)**	**MH257184**	**MH257211**	**MH210660**	**MH257246**

A. intermutans	Armenia, *Zakeri 40480* (GLM)	MH257185	MH257212	MH210661	MH257268
A. intermutans	Armenia, *Zakeri 40486* (GLM)	MH257186	MH257213	MH210662	----
A. intermutans	Armenia, *Zakeri 40487* (GLM)	MH257187	MH257214	MH210663	MH257247
A. intermutans	Armenia, *Zakeri 40499* (GLM)	MH257188	MH257215	MH210664	MH257248
A. intermutans	Armenia, *Zakeri 40519* (GLM)	----	MH257216	MH210665	----
A. intermutans	Armenia, *Zakeri 40529* (GLM)	MH257189	MH257217	MH210666	----
A. intermutans	Armenia, *Zakeri 40562* (GLM)	MH257190	MH257218	MH210667	MH257249
A. intermutans	Armenia, *Zakeri 40725* (GLM)	----	MH257219	MH210668	----
A. intermutans	Armenia, *Zakeri 40741* (GLM)	MH257191	MH257220	MH210669	MH257250
A. intermutans	Armenia, *Zakeri 40745* (GLM)	MH257192	MH257221	MH210670	----
A. intermutans	Armenia, *Zakeri 40760* (GLM)	----	MH257222	MH210671	MH257269
A. intermutans	Armenia, *Zakeri 40763* (GLM)	MH257193	MH257223	MH210672	MH257251
A. intermutans	Iran, *Zakeri 49965* (GLM)	MH257194	MH257224	MH210673	MH257252
A. intermutans	Iran, *Zakeri 49967* (GLM)	----	MH257225	MH210674	MH257253
A. intermutans	Iran, *Zakeri 49960* (GLM)	MH257195	MH257226	MH210675	MH257254
A. intermutans	Iran, *Zakeri 49959* (GLM)	MH257196	MH257227	MH210676	----
A. intermutans	Iran, *Zakeri 49966* (GLM)	----	MH257228	MH210677	MH257256
A. intermutans	Iran, *Zakeri 49964* (GLM)	----	MH257229	MH210678	MH257257
A. intermutans	Iran, *Zakeri 49961* (GLM)	----	MH257230	MH210679	MH257258
A. intermutans	Azerbaijan, *Otte 38734* (GLM)	MH257197	MH257231	MH210680	----
A. intermutans	Iran, *Zakeri 49963* (GLM)	----	MH257232	MH210681	MH257259
A. intermutans	Iran, *Zakeri 49962* (GLM)	----	MH257233	-----	MH257260
A. intermutans	Iran, *Zakeri 50003* (GLM)	----	----	----	MH257255
A. intermutans	Romania, *™ϑun 82* (hb. ™ϑun)	MH248846	MH248875	----	----
A. intermutans	Slovakia, *Palice 15987* (PRA)	MH248857	MH248871	MH255575	MH293579
A. intermutans	France, *Roux 25790* (CR)	MH248863	MH248869	MH255576	MH293580
A. intermutans	France, *Roux 25602* (CR)	MH248849	MH248870	----	MH293588
A. intermutans	France, *Roux 26741* (CR)	MH248847	MH248874	MH255573	MH293576
A. intermutans	Armenia, *Oganesian* (GZU280656)	MH248845	MH248877	MH255572	MH293590
A. intermutans	Armenia, *Harutyunyan & Mayrhofer* (GZU280743)	MH248858	MH248878	----	MH293591
A. intermutans	Spain, *Sipman 45379* (B)	MH248844	MH248868	----	MH293582
A. intermutans	Italy, Sardinia, *Nöske 368* (B)	MH248862	MH248879	----	MH293583
A. intermutans	Greece, *Sipman 59825* (B)	MH248859	MH248882	MH255571	MH293570
A. intermutans	Greece, *Sipman 59827* (B)	MH248860	MH248881	MH255569	MH293571
A. intermutans	Greece, *Sipman 59969* (B)	MH248861	MH248873	MH255570	MH293572
A. intermutans	Czech Republic, *Peksa* (PL)	MH248850	MH248889	----	MH293592
A. intermutans	Ukraine, *Vondrák 7340* (PRA)	MH248848	MH248876	MH255567	MH293573
A. intermutans	Ukraine, *Vondrák 5204* (PRA)	----	MH248880	MH255577	MH293574
A. intermutans	Bulgaria, *Vondrák 2151* (PRA)	MH248865	MH248872	MH255568	MH293575
A. intermutans	Czech Republic, *Lenzová 255* (PRC)	MH248854	MH248885	MH255579	MH293593
A. intermutans	Czech Republic, *Lenzová 254* (PRC)	MH248851	MH248886	MH255578	MH293589
A. intermutans	Czech Republic, *Lenzová 253* (PRC)	MH248856	MH248883	MH255582	MH293587
A. intermutans	Czech Republic, *Lenzová 25* (PRC)	MH248852	MH248887	MH255581	MH293577
A. intermutans	Czech Republic, *Palice 14789* (PRA)	MH248853	MH248884	MH255583	MH293569
A. intermutans	Czech Republic, *Palice 11394* (PRA)	MH248855	MH248888	MH255580	MH293578

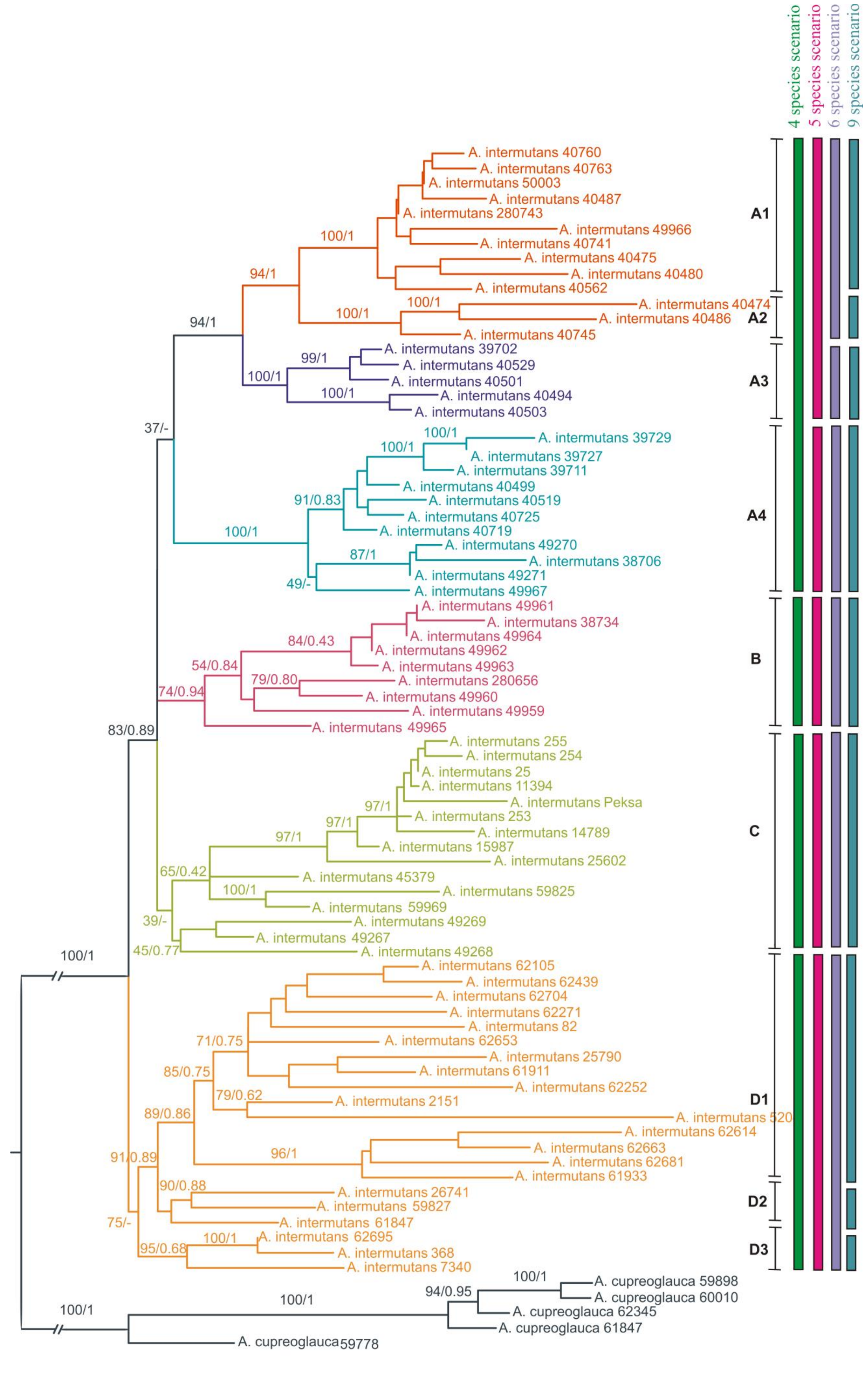
4 species scenario
5 species scenario
6 species scenario
9 species scenario
A1
A2
A3
A4
B
C
D1
D2
D3
A. intermutans 40760
A. intermutans 40763
A. intermutans 50003
A. intermutans 40487
A. intermutans 280743
A. intermutans 49966
A. intermutans 40741
A. intermutans 40475
A. intermutans 40480
A. intermutans 40562
A. intermutans 40474
A. intermutans 40486
A. intermutans 40745
A. intermutans 39702
A. intermutans 40529
A. intermutans 40501
A. intermutans 40494
A. intermutans 40503
A. intermutans 39729
A. intermutans 39727
A. intermutans 39711
A. intermutans 40499
A. intermutans 40519
A. intermutans 40725
A. intermutans 40719
A. intermutans 49270
A. intermutans 38706
A. intermutans 49271
A. intermutans 49967
A. intermutans 49961
A. intermutans 38734
A. intermutans 49964
A. intermutans 49962
A. intermutans 49963
A. intermutans 280656
A. intermutans 49960
A. intermutans 49959
A. intermutans 49965
A. intermutans 255
A. intermutans 254
A. intermutans 25
A. intermutans 11394
A. intermutans Peksa
A. intermutans 253
A. intermutans 14789
A. intermutans 15987
A. intermutans 25602
A. intermutans 45379
A. intermutans 59825
A. intermutans 59969
A. intermutans 49269
A. intermutans 49267
A. intermutans 49268
A. intermutans 62105
A. intermutans 62439
A. intermutans 62704
A. intermutans 62271
A. intermutans 82
A. intermutans 62653
A. intermutans 25790
A. intermutans 61911
A. intermutans 62252
A. intermutans 2151
A. intermutans 520
A. intermutans 62614
A. intermutans 62663
A. intermutans 62681
A. intermutans 61933
A. intermutans 26741
A. intermutans 59827
A. intermutans 61847
A. intermutans 62695
A. intermutans 368
A. intermutans 7340
A. cupreoglauca 59898
A. cupreoglauca 60010
A. cupreoglauca 62345
A. cupreoglauca 61847
A. cupreoglauca 59778
100/1
94/1
94/1
100/1
100/1
99/1
100/1
100/1
37/-
100/1
100/1
91/0.83
100/1
87/1
49/-
84/0.43
54/0.84
79/0.80
74/0.94
83/0.89
97/1
97/1
97/1
65/0.42
100/1
39/-
45/0.77
100/1
71/0.75
85/0.75
79/0.62
89/0.86
96/1
91/0.89
90/0.88
75/-
100/1
95/0.68
100/1
94/0.95
100/1
100/1
0.006

Fig. 1: Maximum likelihood tree (IQ tree analysis) showing phylogenetic relationships among *Aspiciliella intermutans* candidate species based on the ITS, nrLSU, MCM7 and mtSSU concatenated dataset. Bootstrap values and Bayesian posterior probabilities (BS/PP) are shown above their respective branches. The 6 candidate species within the *A. intermutans* complex obtained in the species delimitation analysis based on BP&P and Bayes factor are highlighted by different color.

The Bayes factor delimitation results are provided in Table 5. BFD test proposed the 6-species delimitation scenario model as the best scenario over other models tested i.e. 4, 5 and 9 species delimitation scenarios. The 6-species scenario corresponds to the well supported clades: A1+A2, A3, A4, B, C and D. Clades C and D were nor strongly supported in the concatenate gene tree. However, these were supported by *BEAST species tree (Fig. 2).

Table 4a: Species delimitations (excluding outgroup) and their posterior probabilities. Prior distributions on ancestral population sizes (theta) assigned to different gamma distributions (G) combined with root age (tau) assigned to G (2, 2000).

Species scenarios	Ancestral population sizes (theta)					
	Algorithm 0			Algorithm 1		
	G (2,10)	G (2-100)	G (2,1000)	G (2,10)	G (2,100)	G(2,1000)
4-species	**0.99**	**0.99**	**1.00**	**0.99**	**1.00**	**0.96**
5-species	**0.99**	**1.00**	**1.00**	**0.99**	**0.99**	**1.00**
6-species	**0.99**	**1.00**	**1.00**	**0.99**	**1.00**	**1.00**
9-species	0.38	0.67	0.82	0.59	0.71	**0.95**

Table 4b: Delimited species & their posterior probabilities.

Species scenarios		Algorithm 0			Algorithm 1		
		theta G (2,10)	theta G (2,100)	theta G (2,1000)	theta G (2,10)	theta G (2,100)	theta G (2,1000)
4-species	**A**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	B	**0.99**	**0.99**	**1.00**	**0.99**	**1.00**	**0.99**
	C	**0.99**	**0.99**	**1.00**	**0.99**	**1.00**	**0.99**
	D	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	outgroup	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	D+outgroup	**0.0001**	**-**	**-**	**-**	**-**	**-**
	B+C	**0.00006**	**0.00001**	**-**	**0.0019**	**-**	**0.03**
5-species	**A1+A2+A3**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	A4	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	B	**0.99**	**1.00**	**1.00**	**0.99**	**0.99**	**1.00**
	C	**0.99**	**1.00**	**1.00**	**0.99**	**0.99**	**1.00**
	D	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	outgroup	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	B+C	**0.0011**	**-**	**-**	**0.0023**	**0.00007**	**-**

6-species	**A1+A2**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	A3	**0.99**	**1.00**	**1.00**	**0.99**	**1.00**	**1.00**
	A4	**0.99**	**1.00**	**1.00**	**0.99**	**1.00**	**1.00**
	B	**0.99**	**1.00**	**1.00**	**0.99**	**1.00**	**1.00**
	C	**0.99**	**1.00**	**1.00**	**0.99**	**1.00**	**1.00**
	D	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	outgroup	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	B+C	**0.0016**	**-**	**-**	**0.0018**	**-**	**-**
	A4+C	**0.0008**	**-**	**-**	**0.0009**	**-**	**-**
9-species	**A1**	**1.00**	**1.00**	**0.99**	**0.99**	**0.99**	**1.00**
	A2	**0.97**	**0.99**	**0.99**	**0.96**	**0.99**	**0.99**
	A3	**1.00**	**0.99**	**1.00**	**0.99**	**0.99**	**1.00**
	A4	**0.98**	**0.99**	**0.99**	**0.98**	**0.99**	**1.00**
	B	**0.92**	**0.99**	**1.00**	**0.98**	**0.99**	**1.00**
	C	**0.65**	**0.99**	**0.99**	**0.95**	**0.99**	**0.97**
	D1	**0.99**	**0.77**	**0.89**	**0.97**	**0.88**	**1.00**
	D2	**0.68**	**0.67**	**0.83**	**0.63**	**0.71**	**0.95**
	D3	**0.40**	**0.89**	**0.96**	**0.62**	**0.83**	**0.95**
	outgroup	**1.00**	**0.99**	**0.99**	**1.00**	**0.99**	**0.99**
	D1+D2	**-**	**0.22**	**0.13**	**0.01**	**0.11**	**-**
	D2+D3	**0.20**	**0.09**	**0.03**	**0.29**	**0.16**	**0.02**
	C+D3	**0.29**	**0.001**	**0.0005**	**0.01**	**0.005**	**0.0009**
	A3+A2	**-**	**0.0006**	**-**	**0.0007**	**0.00005**	**-**
	A4+A2	**0.01**	**0.0005**	**0.0003**	**0.01**	**0.0009**	**-**
	B+D3	**0.006**	**0.0003**	**-**	**0.01**	**0.0003**	**-**
	D1+B	**0.003**	**0.0003**	**-**	**0.004**	**-**	**-**
	C+D2+B	**0.05**	**0.0002**	**0.00003**	**0.03**	**0.001**	**0.01**
	C+D2	**-**	**0.0001**	**0.0009**	**-**	**0.002**	**0.001**
	C+B	**0.001**	**0.0001**	**-**	**-**	**0.005**	**-**
	A3+D3	**-**	**0.00008**	**-**	**-**	**-**	**-**
	B+D2	**0.03**	**0.00007**	**0.0009**	**0.002**	**0.002**	**-**
	D2+ outgroup	**-**	**0.00003**	**-**	**-**	**0.00003**	**0.0005**
	A2+D2	**-**	**-**	**-**	**-**	**-**	**0.0004**

Table 5: Marginal likelihood and Bayes factor values for alternative species delimitation scenarios; Marginal-likelihood estimates and Bayes factor testing results (2lnBf) BF = 2 x (model1-model2); the model receiving the best marginal-likelihood score for each estimation method is indicated by a 2lnBf; score = N/A, and its associated marginal likelihood is in bold.

Model	**Marginal Likelihood estimates (MLE)**	**MLE 6 species (model 1) -MLE i species (model 2)**	**2lnBF = 2(MLE 6 species - MLE i species)**
9 species	-13865,84647	129,0528333	258,1056665
6 species	**-13736,79364**	N/A	
5 species	-13765,60161	28,80797094	57,61594187
4 species	-13756,53862	19,74498099	39,48996197

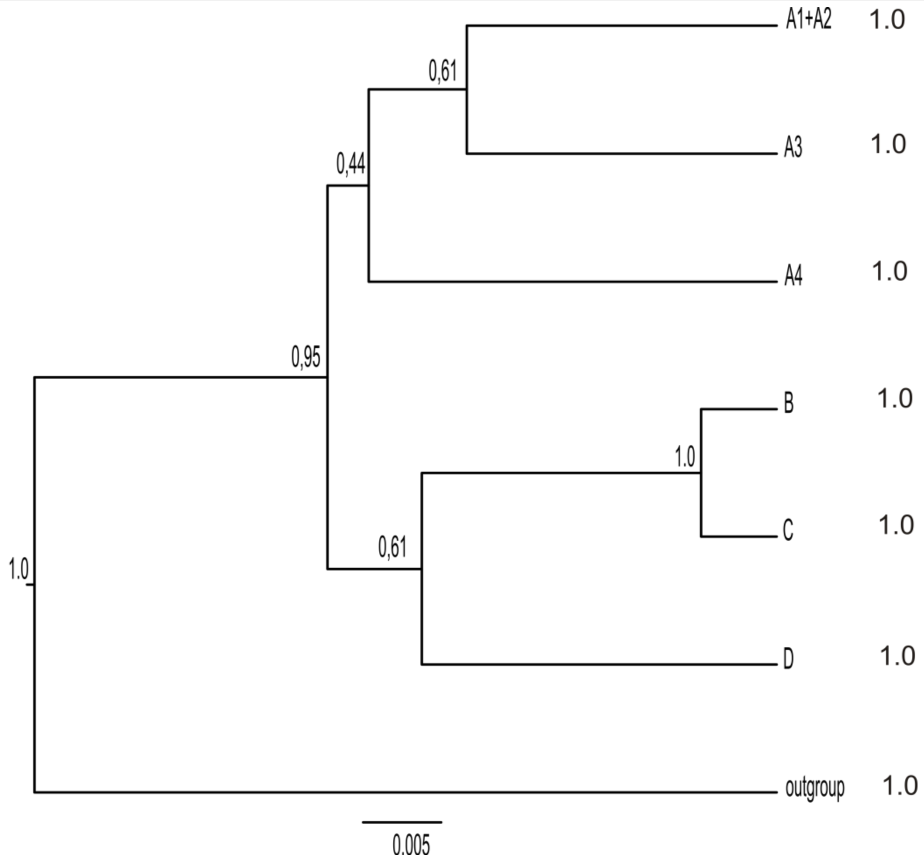

Fig. 2: *BEAST species tree of *Aspiciliella intermutans* complex; consensus tree from analyses of *BEAST for 6 species scenario. Posterior probabilities at nodes indicate support in the *BEAST analyses. The posterior probability of each delimited species calculated by BP&P is indicated in front of each putative species.

Phenotypical examination of *Aspiciliella intermutans* samples

Chemistry. Two chemotypes were detected by TLC: 1) with norstictic and a traces of connorstictic acids; 2) with norstictic, stictic and traces of cryptostictic acids. Similar results of a chemical analysis of *A. intermutans* from Greece were published by Sipman & Raus (2002). The second chemotype was only detected in some samples of clade D, and the first chemotype was found in all clades. The two chemotypes detected inside the *A. intermutans* complex do not discriminate putative lineages.

Morphology. No distinct morphological features were observed to segregate candidate species, except in areoles colour and form. Clades A1+A2 and B are characterized by a thick thallus and larger areoles with brown to red brown color, in contrast to the thinner thallus and smaller areoles with gray to light brown color of the samples in clades A3 and

A4. However, in samples from different parts of Europe, grouped in clades C and D, all these characters were totally intermixed. Although, the variability in thallus form and colour of *A. intermutans* complex was considerable, these appeared more or less homogeneous in the samples from Iran and Armenia. Consequently, we tempt to not consider them taxonomically relevant in this group.

Substrate preferences and Distribution

Aspiciliella intermutans complex occurs on siliceous or volcanic rocks in open habitats. They grow on substrates with high (e.g. basalt) to low pH (e.g. andesite, granite and quartzite rocks). They often grow on exposed, more or less horizontal faces of outcrops. Clades C and D include specimens with broad distributions, collected from geographically different regions; however, specimens collected in western north Iran, Armenia and Azerbaijan were recovered in clades A1, A2, A3, A4 and B.

Discussion

The frequent occurrence of cryptic species has repeatedly been shown in macrolichen species complexes, examples includes *Cladia* aggregate (Parnmen et al. 2012), *Dictyonema glabratum* (Lucking et al. 2014), *Letharia columbiana* (Altermann et al. 2014), *Parmelia saxatilis* (Molina et al. 2011), *Parmotrema reticulatum* (Del-Prado et al. 2016) and *Rhizoplaca melanophthalma* (Leavitt et al. 2013). However, this has been poorly described in microlichen species complexes (see e.g. *Graphis scripta*, Kraichak et al. 2015). This could most probably be due to less attention paid to microlichens or the thought that microlichen species usually encompass differential features, especially ascomatal.

Our study uncovers cryptic species diversity in the widespread Mediterranean microlichen species, the *Aspiciliella intermutans*, and adds another example of species complexes to the microlichens. We show that *A. intermutans* as currently described is not monophyletic but include several cryptic lineages. Our multispecies coalescent species delimitation approaches and BFD test supported the occurrence of at least six candidate species in *A. intermutans* complex. Our results are in accordance with a previous study showing different genetic clusters within *A. intermutans* group (Zakeri et al. 2017).

Since putative species were not supported unambiguously by all independent datasets, a multilocus data set from *A. intermutans* candidate was analyzed. mtSSU topology recovered most lineages identified in the combined analyses, that is suggesting that strong phylogenetic signal from mtSSU may have been dominating the analysis of the concatenated genes. The distinctiveness of the different monophyletic clades in concatenated phylogenetic tree is clear but phylogenetic analyses alone are insufficient (lack of strong support in some clades) to draw conclusions on species boundaries in this complex, therefore we used a combination of methods to address problems in species delimitation in this complex.

Although species delimitation analyses insisted the presence of different species lineages in *A. intermutans* complex, but we follow a conservative approach as suggested by *BEAST and BP&P, which is sensitive enough to detect evolutionarily distinct lineages at very shallow timescales (Yang & Rannala 2010). This method lump species together when they are exchanging genes even at low frequencies (Zhang et al. 2011).

Therefore, based on all of the available evidence; the inference of multispecies coalescent-based species tree for *A. intermutans* complex using *BEAST, BP&P, and Bayes Factor, we could circumscribe six candidate species within this species complex.

We examined also chemistry, morphology, substrate preferences and geographic distribution of samples. Generally, we found little corroboration between morphological and ecological characters with our candidate species, and also secondary metabolite data provided only very limited support for the putative species. However, that is a case as in other cryptic lineages of lichenized-fungi. The presence of cryptic species in lichen-forming fungi without any recognizable phenotypical characterization of the clades has been demonstrated in some studies (Lumbsch & Leavitt 2011).

We did not try to extract DNA of the *A. intermutans* type material from St. Laon (1872), because the material was too old for our method to obtaining sequence. However, we obtained DNA from an *A. intermutans* sample collected 160 km northwest of St. Laon, and the sample is grouped in clade G together with samples from other parts of Europe, so we suggest the candidate species G (clade G) as putative species for *A. intermutans* s. str. and all other clades as putative different cryptic species for *A. intermutans* complex.

Characters of the clades (putative species in *Aspiciliella intermutans* complex)

The phenotypic and geographic features corresponding to the different clades are also summarized in Table 6.

Clades A1+A2. Was recovered as a well-supported lineage (BS = 94/PP = 0.1), sister to the clade A3 with strong nodal support (BS = 94/PP = 0.1) they show some differences in ecological and morphological patterns. The samples A1 developed a gray-brown to light-brown thallus with pale marginal lines and large (0.3-2 mm) and irregular areoles; some areoles come over others and show a superimposed structure. They are commonly found on rocks, from medium to higher elevation, 1800-3044 m, in North and North-West of Iran and in Armenia.

Clade A3. Was recovered as a well-supported lineage (BS = 100/PP = 0.1). The samples developed a gray thallus, which at the edge of the thallus is light brown with pale marginal lines and in the middle totally gray and pruinose, areoles are smaller (0.3-1 mm) than samples in A1+A2 clades and they do not show an irregular structure. They are commonly found on rocks, from lower to medium elevation, 1390-1850 m, in Armenia.

Clade A4. Was recovered with high nodal support (BS = 100/PP = 0.1), and include samples morphologically similar to A3 clade samples, but preferring substrates with lower pH. The samples were collected from Azerbaijan, Armenia and Northwest of Iran, at medium elevation, 1300-1900 m.

Clade B. Was recovered with low nodal support (BS = 74/PP = 0.89), and corresponds to the samples collected from Azerbaijan, Armenia, Northwest and Northeast of Iran, at medium to high elevation, 1885-1992 m. The samples are morphologically similarity to those in A1 clade, but preferring substrates with higher pH.

Clade C. Was recovered with low nodal support (BS = 39), and is represented on mostly basaltic rocks, at low to medium elevations, 0-1030 m, from Czech Republic, Slovakia, Greece, France and Spain; and on andesite rocks, in medium to high elevations, 1305-1898 m, from Northwest of Iran.

Clade D. Was recovered with medium support (PP = 75), and it is sister to the clade with the remaining *Aspiciliella intermutans* samples (BS = 83/PP = 0.89). The samples have a wide morphological variation and are collected from Romania, Ukraine, Bulgaria, Italy, France and mostly from Greece (North Aegean Region), at low elevations, 2-760 m,

mostly on granite rocks. Some samples in this clade show a chemotype with norstictic acid, stictic acid and a trace of cryptostictic acids, what we could only found inside of this clade.

Table 6: The phenotypic and geographic features corresponding to the different clades in *Aspiciliella intermutans* complex based on result of BP&P and Bayes factor on concatenated phylogenetic tree. ADD chemotypes

Clades	Geographical region	No. of samples (74)	Altitude (m)	Substrate	Thallus form	Thallus color	Chemo-types
A1+A2	Armenia, Northwestern and North of Iran	13	1800-3044	Mostly basaltic and andesite rocks	Large and irregular areoles (0.3-2 mm)	Gray-brown to light-brown with pale marginal lines	1
A3	Armenia	5	1390-1850	Granite	Small and regular (0.3-1 mm)	Gray and pruinose	1
A4	Armenia, Northwestern Iran and Azerbaijan	11	1300-1600 (one sample 1900 m)	Andesite, quartz-andesite, granites	Small and regular (0.3-1 mm)	Gray and pruinose	1
B	Armenia, Northwestern and Northeastern Iran and Azerbaijan	9	1885-1992	Mostly quartz-lamprophyre and granites	Large and irregular areoles (0.3-2 mm)	Gray-brown to light-brown with pale marginal lines	1
C	Czech Republic, Slovakia, Greece, France, Spain and Northwestern Iran	15	0-1898	Mostly basalts and andesite	0.3-1,5 mm	Gray to dark-gray	1
D	Romania, Ukraine, Bulgaria, Italy, France and Greece (mostly)	21	2- 760	Mostly granite	No distinctive features	No distinctive features	1 and 2

Our study of the *A. intermutans* species complex indicates that the genus *Aspiciliella* and the *A. intermutans* complex are more diverse in Eurasia than expected. Our sampling was more intensive in Armenia, Iran, Greece and the eastern part of Europe. While four of the six known species-level lineages within *A. intermutans* complex are found in Armenia and Iran, only two broadly distributed lineages were found in samples from Greece and also other part of Europe (Fig. 3). Based on our recent result we suggest that the Caucasian region could be the main ecological region for speciation in this complex.

Ultimately, rigorous sampling in other regions, such as west and central Europe and Anatoly will be needed to more accurately assess the distribution patterns of all lineages within this group in Eurasian region. Additionally, understanding the general geographic

distribution of the candidate species identified in this study requires robust data from a broader geographic sampling, which may or may not correlate to additional lineages within the *A. intermutans* species-complex.

The authors plan a detailed taxonomic revision for the *A. intermutans* species-complex in the near future, with more taxonomic and morphological sampling to characterize boundaries between candidate species.

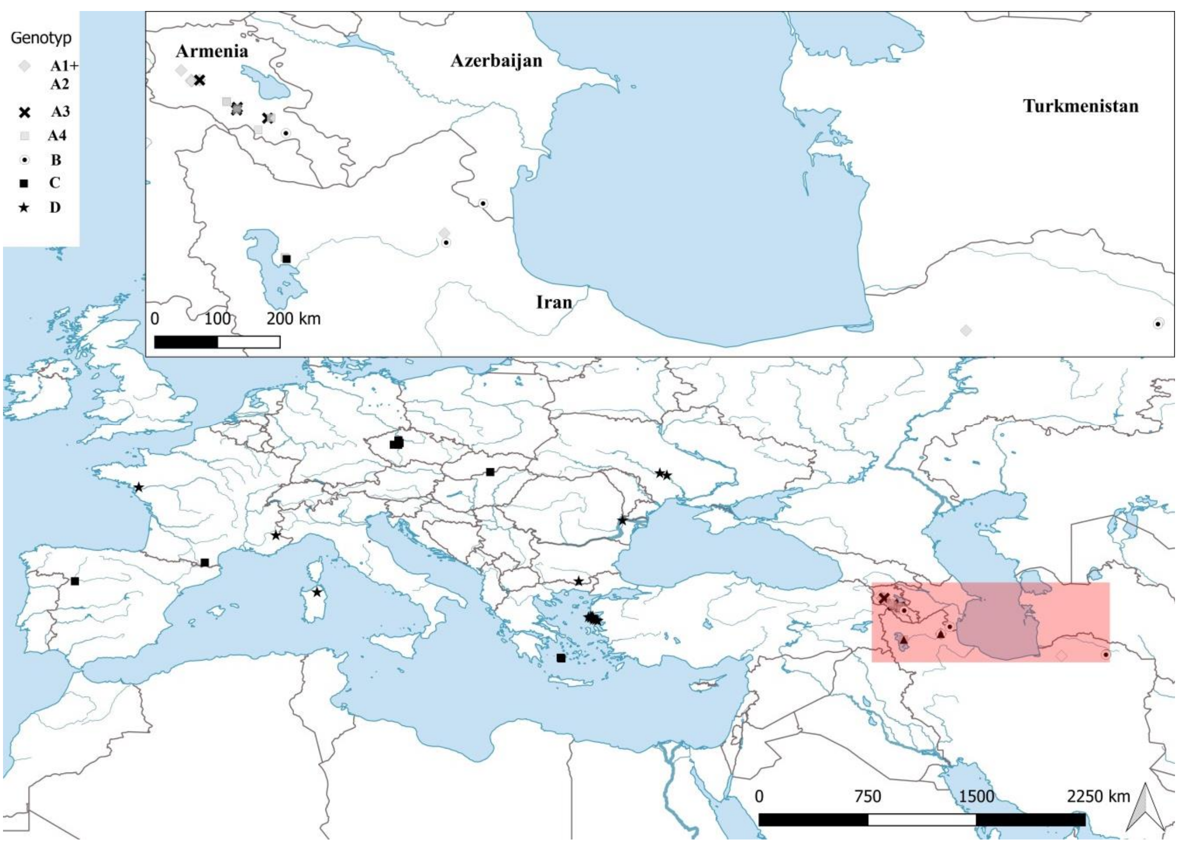

Fig. 3: Geographical distribution of *Aspiciliella intermutans* complex exanimated samples in this study. Symbols indicate the different clades.

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Kapitel 4:

Neotypification of *Aspiciliella cupreoglauca* and lectotypification and synonymization of *Aspicilia reticulata* (Megasporaceae, Ascomycota)

Zakieh Zakeri*[1], Harrie Sipman[2], Alexander Paukov[3], Volker Otte[1]

(Accepted in Lichenologist)

(Format is based on journal guidelines)

The genus *Aspiciliella* M.Choisy, as currently circumscribed, comprises three accepted species: *A. intermutans* (Nyl.) M.Choisy, *A. cupreoglauca* (B.de Lesd.) Zakeri, Divakar & Otte, and *A. portosantana* Sipman & Zakeri (Zakeri et al. 2017).

A name used for lichens in the *Aspicilia intermutans* group is õ*A. reticulata* Kremp.ö. It was used, e.g., in the Handbook of lichens of the USSR (Oxner 1971) as the name for the whole complex, with *A. intermutans* treated at the varietal level. This became a basis for floristic records of õ*A. reticulata*ö in literature, which may often refer to other *Aspiciliella* species. Our paper aims to establish the identity of *A. reticulata*.

The species name *Aspicilia reticulata* was first mentioned by Arnold (1869) referring to Rehm in litt. and to a specimen collected by Metzler in 1867 near Hyères (France). However, not accompanied by a description, this name was not validly published at this place. Nylander (1886, p. 466) referred to this material as follows: õ*Asp. reticulata* Rehm. Arn. Tirol 1869, p. 610 (sine ulla definitione) non differt nisi ut forma thallo pallescente a Lecanora intermutante vulgari in Gallia praesertim maritima, sed ambae etiam in eodem specimine simul obviae conspiciunturö. It is evident that Nylander did not intend to provide a validating description here, but only gives some comments (õObservationesö) on the material not accepted as a new taxon by him. A validating description was given only by Steiner (1898), on the varietal level, as *Lecanora intermutans* var. *reticulata*, in a paper dedicated to the lichens of Greece. The type material cited by Steiner is, on the one hand, a specimen from Greece (õauf Schiefer der höchsten Spitze des Godamanö, leg. Nider) on which his description is based. It only bears pycnidia, no apothecia; but, according to Steiner õgehört die Flechte sicher hierherö. On the other hand, Steiner refers to a specimen of Metzlerøs collection from Hyères, indicated as õOriginalmaterialö, and seen by him in the herbarium of Vienna University, which made him sure concerning the identity of Niderøs material. Thus, both collections represent syntypes, and a lectotype is to be chosen. Interestingly, Steiner refers to the brown colour of Metzlerøs material (õOriginalmaterialö), which is in direct conflict with Nylanderøs remark. Steiner, who cites Nylander, explains this conflict with darkening (õNachdunkelnö).

Finally, Steiner (1919) raised the taxon to species level. By referring to his name given on the varietal level in Steiner (1898), he made the type of his name previously validly published on varietal level also the type of his new combination *Lecanora reticulata* (J.Steiner) J.Steiner.

Synonymy

Aspicilia reticulata Rehm in Arnold, Verh. K. K. zool.-bot. Gesellsch. Wien 19: 610 (1869), nom. nudum

Lecanora intermutans var. *reticulata* Rehm in Arnold ex J. Steiner, Sitzungsber. Kaiserl. Akad. Wiss., Wien, Math.-Naturwiss. Cl., Abt. 1. 107: 142 (1898)

Lecanora reticulata (Rehm in Arnold ex J.Steiner) J.Steiner, Verh. Zool.-Bot. Ges. Wien 69: 84 (1919)

Lectotypification

One might argue to choose the Greek material as the lectotype, on which Steinerøs valid description is based. On the other hand, this material was said to be sterile. We therefore choose Metzlerøs material from Hyères as the lectotype, which is named õOriginalmaterialö by Steiner, and which gave him that idea of the taxon that led him to identification of his Greek material with this entity. Metzlerøs material is represented also by duplicates in other herbaria, besides that of Vienna University. Having studied the material from WU we are able to confirm that this is the very specimen studied by Steiner, which is obvious from his handwriting indicating the name (*Lecanora intermutans* var. *reticulata*) and from measurements of conidia and hymenium. Here we designate this specimen (WU 887) as a lectotype of *Lecanora intermutans* var. *reticulata* Rehm in Arnold ex J. Steiner and thus also of *Lecanora reticulata* (Rehm in Arnold ex J.Steiner) J.Steiner (Isotypes: H-Nyl 25458 in herbarium H, L4960 in herbarum S, M-0102336 in herbarium M).

The identity of Aspicilia reticulata

The type of *A. reticulata* accurately represents the taxon commonly known as *Aspicilia cupreoglauca*, and the characters in the description of *A. reticulata* agree with those of *A. cupreoglauca*. Also, the type localities of these two entities are situated in a relative proximity in Mediterranean France. Bouly de Lesdain (1910) described *Aspicilia cupreoglauca* based on a collection by Abbé Soulié from Hérault. According to B. Dennetière (pers. comm.), the herbarium of Bouly de Lesdain was destroyed in Dunkerque during the World War II (see also Grummann 1974) and no duplicates of *A. cupreoclauca* type material were traced in PC. In order to fix the use of the name *Aspicilia cupreoglauca*

in concordance with the original description and with the general use of this name, we choose a neotype of *A. cupreoglauca* below.

Since *A. cupreoglauca* was described as a species in 1910, and the specific epithet *reticulata* was validly published only in 1919 when Steiner raised his *L. intermutans* var. *reticulata* to species level, the epithet *cupreoglauca* has priority over the epithet *reticulata* on species level. Hence we synonymize the related names here.

Aspiciliella cupreoglauca (B. de Lesd.) Zakeri, Divakar & Otte [MycoBank MB821506]

Basionym: *Aspicilia cupreoglauca* B. de Lesd., Bull. Soc. bot. Fr. 57: 32 (1910).

Type: Hérault, Prémian vers Langlade, 500 m. alt., sur des schistes, 1909, leg. Abbé Soulié (**apparently destroyed in Dunkerque**).

Neotype (designated here): Greece, North Aegean Region, Lesbos: between Vatoussa and Antissa along provincial road, alt. 180 m. 26° 01.07' E; 39° 14.05' N. On volcanic rock cliff in river gorge; 7 Oct. 2015, H. Sipman & Th. Raus 62440 (neotype: B 60 0200002; isoneotype: GLM). GenBank acc. no. nrLSU: KY576956; mtSSU: KY576932; ITS: KY618845

= ***Lecanora reticulata* (J.Steiner) J.Steiner**, Verh. Zool.-Bot. Ges. Wien 69: 84 (1919) syn nov.

Lectotype: Hyeres, 1867, Metzler nr. 32B in litt. (lectotype: WU 887; Isotypes: H-Nyl 25458 in herbarium H, L4960 in herbarum S, M-0102336 in herbarium M).

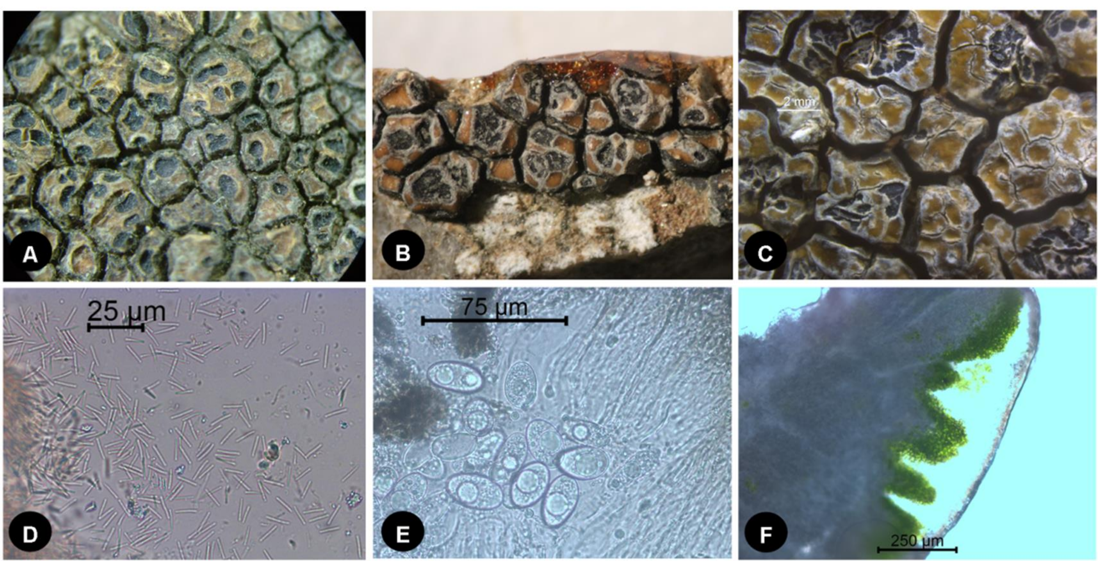

Fig. 1: **A** ó *Aspicilia reticulata* lectotype collected from Hyeres, WU 887 (Metzler 1867- nr. 32B in litt.); **B** ó *Aspicilia reticulata* type material from Herbarium, H-Nyl 25458; **C-F** ó *Aspiciliella*

cupreoglauca, neotype. **C** ó Thallus with apothecia; **D** ó Filiform, straight conidia; **E** ó Ascus; **F** ó Cross section of cortex and medulla.

Acknowledgments

The authors gratefully thank the curators of the herbaria H and M for the loan of specimens. Financial support from the Ungerer Foundation is gratefully acknowledged. AP would like to thank Andreas Beck (M) and Bruno Dennetière (PC) for their hospitality and possibility to work in the herbaria.

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Kapitel 5:

A new corticolous *Megaspora* (*Megasporaceae*) species from Armenia

Zakieh Zakeri , Arsen Gasparyan & André Aptroot

Published By: **Botanic Garden and Botanical Museum Berlin (BGBM)**

(Format is based on journal guidelines)

Abstract:

The corticolous species *Megaspora cretacea* is described as new for science. The species is characterized by a thick, cretaceous thallus and a pale bluish, rather coarse soredia covering most of the thallus. It grows on *Junipe- rus* bark in open arid woodlands in Armenia. A key to the three species included in the genus *Megaspora* is presented. Phylogenetic analysis based on nrITS sequences revealed that *M. cretacea* clustered within the *Megaspora* clade as sister species to *M. rimisorediata* with high support.

Key words: lichens, *Megasporaceae*, *Megaspora*, taxonomy, new species, sorediate, *Juniperus*, South Caucasus, Armenia, Khosrov Forest State Reserve, ITS

Introduction

According to recent phylogenetic studies, *Megaspo- raceae* Lumbsch is monophyletic (Nordin & al. 2010). They are mostly saxicolous crustose lichens (Valadbeigi & al. 2011). In Armenia, they are among the more com- mon lichen families, in species diversity but especially in abundance, covering large parts of most siliceous rock faces and also present on limestone. The genus *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth is closely related to the genus *Circinaria* Link (Nordin & al. 2010). It is an exception within the family, in that it is predominantly corticolous, with two species on trees, one of which is also occasionally terricolous. Both cur- rently accepted species, *M. rimisorediata* Valadbeigi & A. Nordin and *M. verrucosa* (Ach.) Hafellner & V. Wirth (Valadbeigi & al. 2011), occur in Armenia (Gasparyan & Sipman 2013; Harutyunyan & al. 2011). During a lichenological excursion to Armenia, organ- ized by the second author, we collected a sorediate crus- tose lichen at the bases of trees of *Juniperus polycarpos* K. Koch in the Khosrov Forest State Reserve. The terri- tory of the Reserve was already considered as a protect- ed area in the fourth century c.e. by the Armenian king Khosrov Kotak (Khanjyan 2004). In 1958, the Khosrov Forest was officially declared as a state reserve (Anony- mous 2008). The natural landscapes of phryganoid veg- etation, open arid forests and montane steppes have high biological diversity and are recognized as a priority area for conservation. So far, 1849 species of vascular plants (including 24 endemic species) and 176 lichenized and lichenicolous fungi have been registered in the reserve (Anonymous 2008; Gasparyan & al. 2015).

While in the field it was not possible to recognize the collected specimens as representatives of *Megaspora- ceae*; rather they gave the impression of a species of the *Caloplaca albolutescens* (Nyl.) H. Olivier / *C. teicholyta* (Ach.) J. Steiner group or, less likely, a species of *Lepraria* Ach., but subsequent examination of the material revealed a few black apothecia immersed in the thallus, with large, thin-walled ascospores and a greenish epihymenium, sug- gesting *Megasporaceae*.

In the framework of a phylogenetic study of Asian *Megasporaceae*, the first author sequenced the material and found that it clusters inside *Megaspora* as a sister species to *M. rimisorediata.* Therefore, we describe it as a new species in this genus.

Megaspora rimisorediata has a restricted distribu- tion. It was described from Iran (Valadbeigi & al. 2011) and later found also in S Armenia (Gasparyan & Sipman 2013;

Gasparyan & al. 2015). *Megaspora verrucosa* has been reported from Europe, Africa, Asia, North and South America, New Zealand and Antarctica (Smith & al. 2009).

Currently, Armenia is the centre of diversity of the genus, with all three currently known species present. The new species has been reported from two localities. Further comprehensive studies are required to explore distributional and ecological patterns of the new species.

Material and methods

Identification and descriptive work was carried out in Soest and BGBM using an Olympus SZX7 stereomicro- scope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water, in which also all measurements were taken. The specimens from this study are preserved in ABL and B (herbarium codes after Thiers 2016+). The chemistry of the type specimen was investigated by thin-layer chromatography (TLC) using solvent A (Orange & al. 2001).

DNA extraction ô We used nuclear ITS1-5.8S-ITS2 rDNA sequences of specimens in the molecular study because it has been shown that among the regions of the ribosomal cistron, the internal transcribed spacer (ITS) region has the highest probability of successful identification for a range of fungi (Schoch & al. 2012; Divakar & al. 2015). Total DNA was extracted from freshly collected material according to Park & al. (2014). We followed the instructions given in that paper except for the following steps: we used a 1×1 mm^2 piece of medulla and mixed it with bead- beader without liquid nitrogen; instead of chloroform we used Roti®-C/I (chloroform/isoamyl alcohol at a ratio of 24:1); and at the end we used only 30 L TE buffer instead of 100 L because of the low quantity of DNA.

PCR amplifications and sequencing ô The primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990) was used for the PCR amplifications. PCR ampli- fications were performed in a 12.5 L volume contain ing 2 L undiluted DNA, 0.5 L of each primer (10 mM), 6.4 L of sterile water, 1 L dNTP (2 mM), 1 L s-buffer, 1 L MgCl2, 0.1 L Taq-polymerase. Thermal cycling pa- rameters were initial denaturation for 5 min at 95 °C, fol- lowed by 30 cycles of 30 sec at 95 °C, 30 sec at 54 °C, and 1 min at 72 °C; following the last cycle a final extensions for 3 min at 72 °C was included. Amplification product was viewed by electrophoresis on 1% agarose gels and stained with

ethidiumboromide and was purified by add- ing 2 L ExoSAP-ITÎ (Exonuclease 1-shrimp alkaline phosphatase) to 5 L of the PCR products, followed by a heat treatment of 15 min at 37 °C and 15 min at 80 °C. The PCR product was sequenced in both directions by Bik-F Laboratory in Frankfurt am Main. For the reconstruction of a phylogenetic tree, all ITS sequences of *Megasporaceae* from Valadbeigi & al. (2011) were used as well as seven accessible sequences of *Megaspora* from NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Two sequences were obtained from the new species and submitted to the NCBI GenBank (Table 1). The sequences were aligned through the Muscle V4 program web server (Edgar 2004) with the default settings. The aligned sequences were ad- justed manually in PhyDE software (Müller & al. 2010). Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/ Gblocks_server.html) was used to eliminate ambiguously aligned positions, applying settings allowing for smaller final blocks, gap position within the final blocks and less strict flanking position (Castresana 2000).

Phylogenetic analyses ô MrModeltest (Nylander 2004) was used to determine the most appropriate model us- ing AIC, with GTR + I + G found to be the best-fitting model of nucleotide evolution. Bayesian inference of phylogeny with Markov chain Monte Carlo sampling was performed on the Bayesian inference of phylog- eny with Markov chain Monte Carlo sampling was per- formed on the 477 unambiguously aligned nucleotide positions. Bayesian analyses were conducted with Mr- Bayes v. 3.2.2 (Ronquist & Huelsenbeck 2003) using the GTR model of nucleotide substitution including a proportion of invariable sites and a discrete gamma distribution with six rate categories. Two independent runs, each with four Metropolis-Coupled Markov Chain Monte Carlo chains and a temperature of 0.2 were initi- ated and run for 1 000 000 generations, with tree and parameter sampling every 100 generations. Burn-in was set to discard 25 % of samples. Maximum parsimonious trees (MPs) were reconstructed in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) using the heuristic search option with 100 random se- quence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Alignment gaps were treated as missing and all characters were unordered and of equal weight. The robustness of the trees obtained was evaluated by 1000 bootstrap repli- cations with ten random sequence additions. Molecular Evolutionary Genetics Analysis software (MEGA version 7.0) was used to reconstruct the Maximum Like- lihood phylogenetic tree based on the GTR + I + G model (Nei

& Kumar 2000; Kumar & al. 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A GTR model of nucleotide substitu- tion including a proportion of invariable sites and a discrete gamma distribution with five rate categories (GTR + I + G) were used in Maximum Likeli- hood approach.

Table 1. Voucher specimens and NCBI GenBank accession numbers of the ITS sequences used in the phylogenetic analyses.

Taxon	**Locality, Voucher**	**GenBank acc. no.**
Aspicilia cinerea	Sweden, Dalarna, *Hermansson 13275* (UPS)	EU057899
Aspicilia indissimilis	Sweden, *Nordin 5943* (UPS)	EU057909
Aspicilia laevata	Sweden, *Tibell 23659* (UPS)	EU057910
Circinaria calcarea	Sweden, *Nordin 5888* (UPS)	EU057898
Circinaria contorta	Sweden, *Nordin 5895* (UPS)	EU057900
Circinaria leprosescens	Sweden, *Nordin 5906* (UPS)	EU057911
Lobothallia melanaspis	Norway, *Own-Larsson* 8943a (UPS)	JF825524
Lobothallia radiosa	Sweden, *Nordin 5889* (UPS)	JF703124
Megaspora cretacea	**Armenia, *Aptroot 73835* (B)**	**KX253974**
Megaspora cretacea	**Armenia, *Gasparyan 600199170* (B)**	**KX253975**
Megaspora rimisorediata	Iran, *Valadbeigi 2250* (TARI)	JF825525
Megaspora rimisorediata	China, *Xinjiang, XJU 20116002*	KT443790
Megaspora rimisorediata	China, *Xinjiang, XJU 20136001*	KT443789
Megaspora rimisorediata	China, *Xinjiang, XJU 20111617*	KT443788
Megaspora rimisorediata	China, *Xinjiang, XJU 91815043*	KT443787
Megaspora verrucosa	Austria, *Trinkaus* (GZU)	AF332121
Megaspora verrucosa	Austria, *Hafellner 48544 & Ivanova* (GZU)	AF332122
Megaspora verrucosa	China, *Xinjiang, XJU 200753*	KT443786
Megaspora verrucosa	China, *Xinjiang, XJU 20000724*	KT443785

Megaspora verrucosa	USA, Colorado, *St. Clair C54042* (BRY)	KC667053
Sagedia mastrucata	Norway, Troms, *Nordin 5708* (UPS)	EU057913
Sagedia simoensis	Sweden, *Own-Larsson* 9000 (UPS)	EU057926
Sagedia zonata	Sweden, *Nordin 5932* (UPS)	EU057949
Ochrolechia parella	France, Brittany, *Feige* (ESS-20864)	AF329174

Results

Phylogeny

The maximum parsimony ana- lysis resulted 12 most parsi- monious trees with 513 steps, consistency index (CI) = 0.591, retention index (RI) = 0.690, rescaled consistency index (RC) = 0.407 and homoplasy index (HI) = 0.409. The Maximum Likelihood analysis re- sulted a tree with the highest log likelihood (-2014.3274). Majority rule consensus tree for maximum parsimony analysis was congruent with the tree obtained by Bayesian and maximum likelihood phylogenet- ic inference. The majority rule consensus tree of Bayesian analysis is shown here (Fig. 1) with posterior probabilities of Bayesian analysis and bootstrap numbers of Maximum Parsimony and Maximum Likelihood analysis.

The molecular phylogenetic results confirmed affilia- tion of the new species to the genus *Megaspora*. It is clus- ters in a phylogenetic tree in *Megaspora*, as sister to *M. rimisorediata* (PP = 1; MP/ML BS= 100/100). The phy- logenetic trees resulting from the three different analyses also confirmed *Megaspora* clade as a monophyletic group even after adding the new species samples (*M. cretacea*) with a high posterior probability and bootstrapping val- ues (PP = 1; MP/ML BS = 99/99). Monophyly of species *M. verrucosa* and *M. rimisorediata* were confirmed with a high supporting values (PP = 1; MP/ML BS = 100/100 for *M. verrucosa* and PP = 0.94; MP/ML BS = 99/98 for *M. rimisorediata*).

Taxonomy

Megaspora cretacea Gasparayan, Zakeri & Aptroot, **sp. nov.** ó MycoBank #817072 ó Fig. 2AóC.

Holotype: Armenia, Ararat, Vedi, Urtsadzor, Khosrov For- est State Reserve, 40°00'42"N, 44°54'04"E, 1600 m, on *Juniperus polycarpos* bark, 17 Jun 2015, *A. Aptroot 73835* (B 600200932; isotypes: ABL, GLM).

Diagnosis ô *Megaspora* with thallus whitish grey, cre- taceous, fully sorediate with soredia c. 0.1 mm in diam.; apothecia sparse, immersed; ascospores 4 per ascus, broad- ly ellipsoid, 27ó31 × 18ó21 m, hyaline, thin-walled.

Description ô *Thallus* whitish grey, crustose, ecorticate, to 0.2 mm thick, irregularly delimited to almost lobate, occupying areas up to 5 cm in diam. *Medulla* white, cretaceous. *Soralia* covering most of thallus surface, pale bluish grey; soredia c. 100 m in diam. *Photobiont* chlorococcoid. *Apothecia* sparse, dispersed, immersed in thallus, round, 0.3ó0.5 mm in diam.; disc black, concave; margin black, raised above disc, incurved, c. 0.1 mm wide, with some crenations. *Hymenium* IKI+ blue, c. 150 m high, not inspersed with oil droplets. *Subhymenium* hyaline. *Epihymenium* greenish, colour unchanged in KOH. *Hypothecium* hyaline. *Paraphyses* 2ó2.5 m thick, not branched. *Asci* clavate, 125ó140 × 25ó31 m. *Ascospores* 4 per ascus, broadly ellipsoid, 27ó31 × 18ó21 m, hya line, thin-walled (less than 1 m). *Pycnidia* not observed. *Conidia* not observed.

Chemistry ô Thallus KOH-, C-, Pd-, UV-. TLC: No li- chen substances detected.

Distribution and ecology ô The species is known from two separate localities within the Khosrov Forest State Reserve, Armenia. It occurs on bases of trees of *Juniperus polycarpos* K. Koch in arid, open, montane for- ests. The forest ecosystems in the Khosrov Forest State Reserve, at 1400ó2300 m, are generally dominated by oak trees (*Quercus macranthera* Fisch. & C. A. Mey. ex Hohen.) and sparse juniper (*J. polycarpos*) formations, accompanied by *Fraxinus excelsior* L., *Sorbus aucu- paria* L., and species of *Acer* L. and *Pyrus* L. (Khanjyan 2004).

Etymology ô The epithet is derived from word *cretaceus* (resembling chalk) in reference to the colour and texture of the thallus.

Additional specimen examined ô Armenia: Ararat, Vedi, Urtsadzor, Khosrov Forest State Reserve, 39°59'07"N, 44°53'51"E, 1390 m, on *Juniperus polycarpos* bark, 17 Jun 2015, *A. Gasparyan* (B 600199170).

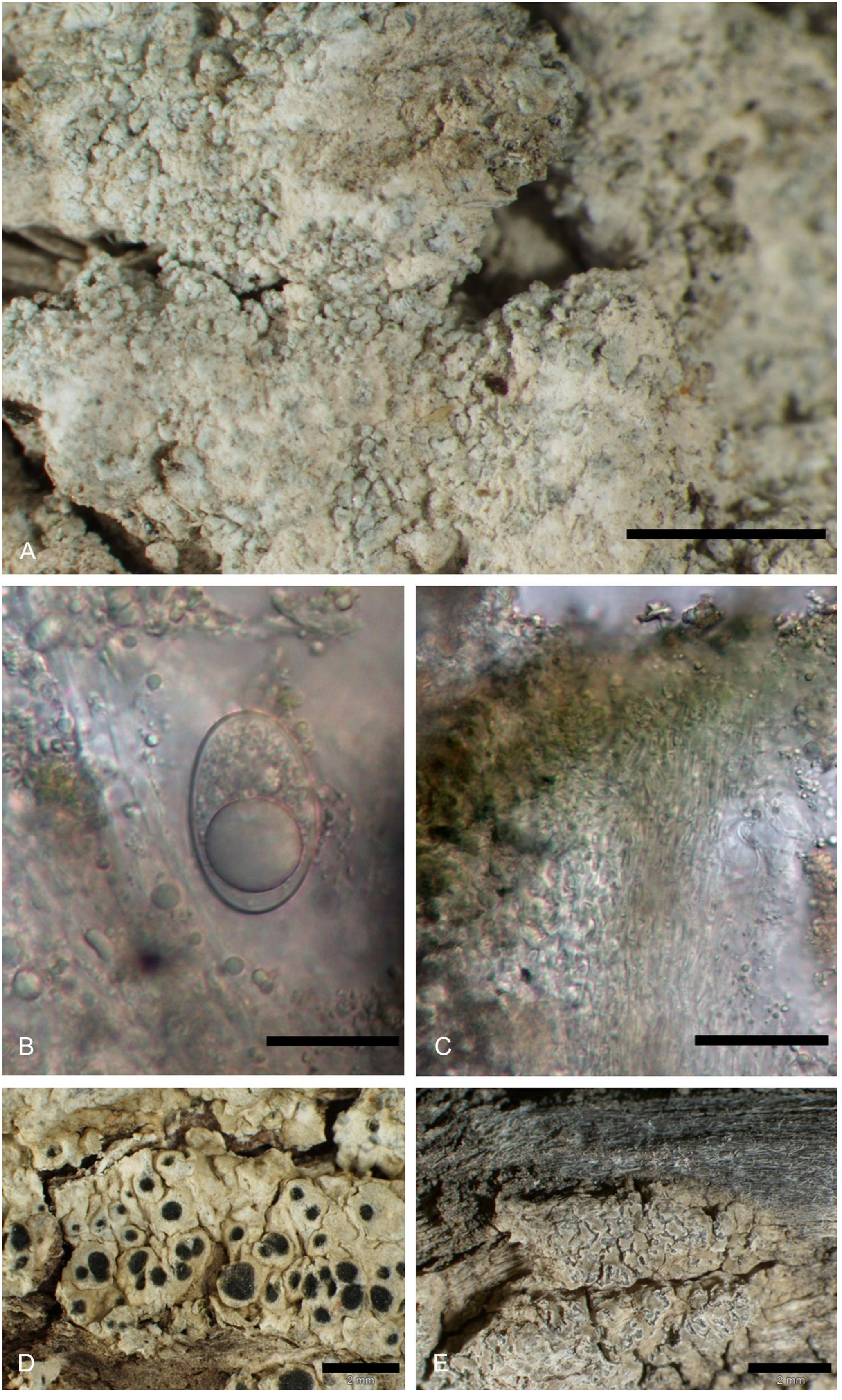
A
B
C
D
E

Fig. 2. AóC: *Megaspora cretacea*, holotype; A: thallus with soredia and apothecia; B: ascospore; C: hymenium (with excipulum at left). ó D: *M. verrucosa* thallus with apothecia. ó E: *M. rimisorediata* thallus with net of cracks and soredia. ó Scale bars: A, D, E = 2 mm; B, C = 20 m.

Discussion

Megaspora cretacea is a morphologically distinctive spe- cies, from which the two other species of the genus, *M. verrucosa* (Fig. 2D) and *M. rimisorediata* (Fig. 2E), can be separated as follows (Table 2): *M. verrucosa* has no soredia, whereas *M. cretacea* and *M. rimisorediata* are both sorediate; the closely related *M. rimisorediata* dif- fers from *M. cretacea* by the presence of a dense net of elongate cracks over the thallus, dark bluish green sore- dia, branched paraphyses and larger ascospores.

Table 2. The main distinguishing characteristics of *Megaspora cretacea*, *M. rimisorediata* and *M. verrucosa*.

Characteristics	***Megaspora cretacea***	***Megaspora rimisorediata***	***Megaspora verrucosa***
Thallus	Whitish grey, irregularly delimited to almost lobate	Ochraceous to bluish grey, dense net of cracks over the thallus	White to grey-white, continuous to areolate to verrucose
Soredia	Pale bluish grey, granules *c.* 100 m diam., covering most of the thallus	Dark bluish green, granules 50ó70 m diam., produced on sides of elongate cracks	Absent
Hymenium	not inspersed, 150 m	not inspersed, up to 150 m	inspersed at times, 200ó250 m
Paraphyses	Unbranched	Branched and anastomosing	Branched but not anastomosing
Asci	125ó140 × 25ó31 m	145 × 46 m	200ó230 × 45ó50 m
Ascopores /ascus	4	4ó8	8
Ascospores	27ó31 × 18ó21 m	35ó42 x 23ó27 m	30ó60 × 21ó42 m
Substrate	Bark of *Juniperus* sp.	Bark of *Juniperus* sp., *Quercus* sp.	On soil, mosses, plant remains on calcareous rocks, bark.

Key to the species of *Megaspora*

1. Soredia absent . . . . ***M. verrucosa***

ó Soredia present. **2**

2. Thallus ochraceous to bluish grey with a dense net of elongate cracks; soredia produced along sides of elongate cracks, dark bluish green . .. . ***M. rimisorediata***

ó Thallus whitish grey, irregularly delimited to almost lobate; soredia covering most of thallus, pale bluish grey . . . . ***M. cretacea***

Acknowledgements

The authors would like to express their gratitude to the staff of the Khosrov Forest State Reserve for kind support during field work and to the Ministry of Nature Protec- tion for permission to collect the specimens. The authors are also grateful to staff and volunteers of the Young Bi- ologists Association NGO, especially Hripsime Atoyan, Vanuhi Hambardzumyan and Maria Antonosyan for field assistance during the excursion. A.G. acknowledges fi- nancial support from the DAAD (Deutscher Akademi- scher Austauschdienst, German Academic Exchange Service) and the project õDeveloping Tools for Conserv- ing the Plant Diversity of the Transcaucasusö financed by the Volkswagen Foundation. A.A. thanks the Stich- ting Hugo de Vries-fonds for a travel grant. Leo Spier is thanked for performing TLC. The authors also thank Anders Nordin and Robert Lücking for their reviews of an earlier version of this paper.

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Kapitel 6:

First inventory of lichens and lichenicolous fungi in the Khosrov Forest State Reserve, Armenia

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Published By: **Fl. Medit. 25: 105-114.**

(Format is based on journal guidelines)

Abstract

In 2015, an international lichenological excursion to Armenia was organized by the Young Biologists Association NGO and Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area. One of the main goals of this excursion was to study lichen diversity of the Khosrov Forest State Reserve. As a result of this inventory, 176 species of lichenized and lichenicolous fungi have been found in the protected area. Out of these, 49 are reported for the first time from Armenia: *Acarospora versicolor*, *Agonimia tristicula*, *Anema decipiens*, *Arctomia fascicularis*, *Arthonia intexta*, *A. phaeophysciae*, *Aspicilia* cf. *glomerulans*, *A. inter- mutans*, *Bacidina arnoldiana*, *Bagliettoa calciseda*, *Bilimbia sabuletorum*, *Blennothallia crispa*, *Chrysopsora testacea*, *Collema subflaccidum*, *Diploschistes gypsaceus*, *Endocarpon pusillum*, *Gonohymenia nigritella*, *G. schleicheri*, *Gyalolechia juniperina*, *Immersaria iranica*, *Lecania rabenhorstii*, *Lecanora barkmaniana*, *L. juniperina*, *L. semipallida*, *Leprocaulon microscopicum*, *Llimoniella phaeophysciae*, *Lobothallia recedens*, *Peccania coralloides*, *Peltula euploca*, *Physconia thorstenii*, *Piccolia ochrophora*, *Placidium lacinulatum*, *Placopyrenium fuscellum*, *Psorotichia schaereri*, *Rinodina colobina*, *R. obnascens*, *Scytinium gelatinosum*, *S. turgidum*, *Solenopsora holophaea*, *Thermutis velutina*, *Toninia candida*, *T. squalida*, *Tremella phaeophysciae*, *Usnea lapponica*, *U. wasmuthii*, *Verrucaria dolosa*, *V. macrostoma*, *Xanthoparmelia protomatrae* and *X. tinctina*.

Key words: biodiversity, lichenology, protected areas, new records, oak and juniper forests, South Caucasus.

Introduction

The area of the Khosrov Forest State Reserve has already been recognized as a protect- ed area in the fourth century for hunting and conservation reasons by the Armenian king Khosrov Kotak. In 1958, the Khosrov Forest has officially been established as a State Reserve (Khanjyan 2004). The aim of this protected area is to preserve oak and juniper forest ecosystems and montane vegetation that supports numerous threatened species of plants and animals. The state reserve is under strict protection, corresponding to IUCN category I. The reserve area covers 29196 ha territory in the central part of the Ararat province in Southern Armenia (Fig. 1) (Anonymous 1999). It is situated between the slopes south of Geghama and north-west of Urts and north-east of the Yeranos mountain ranges, at an altitude varying between 900 and 2500 m. The climate is dry continental with annual precipitation of 350 to 800 mm (Anonymous 2008).

The area is known by its exceptionally rich biodiversity and natural landscapes of frigid vegetation, open arid forests and montane steppes (Fig. 2). Currently 1849 species of vascular plants (including 24 endemic species) and 283 animal species are known from the reserve (Anonymous 2008). The semi-arid and frigid formations occur on foothills and the lower mountain belt, where dominated a vegetation of *Artemisia fragrans, Salsola ericoides, S. dendroides*, etc. The forest ecosystems (1400-2300 m) are generally dominated by oak trees (*Quercus macranthera*) and sparse juniper (*Juniperus polycarpos*, etc.) formations, accompanied by *Fraxinus excelsior*, *Sorbus aucuparia*, *Acer*, *Pyrus*, etc., species (Khanjyan 2004). The riverine forest vegetation is predominated by *Fraxinus, Populus, Salix,* etc., species. Various grasses, such as the feather grass (*Stipa stenophylla*, *S. capil lata*, etc.), tragacanth (*Astragalus microcephalus* and *A. lagurus*) motley grass steppe, etc., dominate in the montane steppes (Anonymous 2008).

Currently, 464 lichens and 2 lichenicolous fungi species are known from Armenia (Harutyunyan & al. 2011; Gasparyan & Sipman 2013; Gasparyan & al. 2014). In the protected area, mainly higher plants, fungi and animals have been investigated and monitored. There are only a few previous lichen records known from the reserve. No comprehensive lichenological surveys have been carried out in the area till date, which could be important for our understanding of lichen diversity in Armenia.

This paper presents the results of the first inventory of lichens in the Khosrov Forest State Reserve, which has been carried out during an international lichenological excursion to

Armenia. The excursion was organized in the frame of the OPTIMA Iter Lichenologicum ini- tiative by the Young Biologists Association NGO (Armenia) and OPTIMA (Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area).

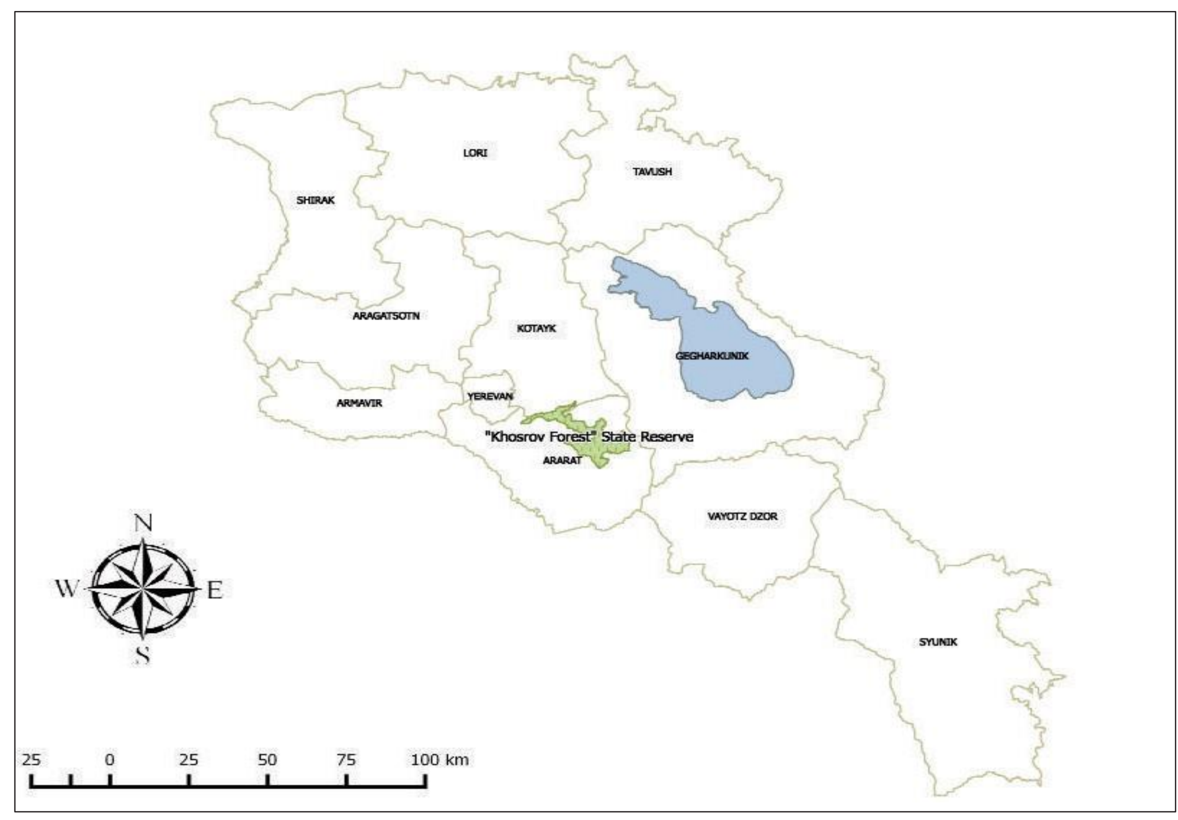

Fig. 1. The map of the Republic of Armenia including marzes (provinces), and location of the Khosrov Forest State Reserve.

Fig. 2. Natural landscapes in the Khosrov Forest State Reserve (photo by Víctor J. Rico).

Material and methods

During the excursion from 17 to 18 of June, 2015, a total of six localities were visited by the participants of the excursion in the Khosrov Forest State Reserve. The specimens have been collected and identified with routine methods. Secondary metabolites have been studied by thin-layer chromatography (Orange & al. 2010). Additionally, in order to confirm the identification based on morphological features, internal transcribed spacer (ITS) of some *Parmeliaceae* and *Physciaceae* specimens have been sequenced (extracts num- bered and kept in MAF-Lich.) and compared with available ITS sequences in GenBank (NCBI). Voucher specimens are deposited in the herbaria ABL, B, F, GLM, MACB, MAF- Lich. and PAL.

List of visited localities

1.- ARMENIA: ARARAT: Vedi, Urtsadzor, Khosrov Forest State Reserve, 39º59ǿ07öN 44º53ǿ51öE, 1390 m, 17-VI-2015, **a)** on *Ulmus* sp., **b)** on calcareous soil, **c)** on siliceous volcanic rocks, **d)** on limestone, **e)** on *Juniperus* sp., **f)** on bark, mixed forest of *Quercus*, *Juniperus* and *Acer*.

2.- ARMENIA: ARARAT: Vedi, Urtsadzor, Khosrov Forest State Reserve, 40º00ǿ42öN 44º54ǿ41öE, 1600 m, 17-VI-2015, **a)** on *Fraxinus excelsior*, **b)** on *Juniperus* sp., **c)** on siliceous volcanic rocks **d)** on limestone, **e)** on aquatic siliceous rocks, **f)** on rocks, **g)** on *Salix* sp., **h)** on *Quercus* sp., **i)** on *Acer monspessulanum*, **j)** on bark, mixed forest of *Quercus*, *Juniperus* and *Acer*.

3.- ARMENIA: ARARAT: Vedi, Urtsadzor, Khosrov Forest State Reserve, around a stream, 40º01ǿ14öN 44º55ǿ00öE, 1700 m, 17-VI-2015, **a)** on *Fraxinus excelsior*, **b)** on *Quercus macranthera*, **c)** on siliceous volcanic rocks, **d)** on soil, **e)** on *Juniperus* sp., **f)** on limestone, **g)** on bark, mixed forest of *Quercus*, *Juniperus* and *Acer*.

4.- ARMENIA: ARARAT: Vedi, Urtsadzor, Khosrov Forest State Reserve, top of the hill, 40º01ǿ20öN 44º54ǿ33öE, 1850 m, 17-VI-2015, **a)** on *Quercus macranthera*, **b)** on bark, mixed forest of *Quercus*, *Juniperus* and *Acer*.

5.- ARMENIA: ARARAT: Goght, Khosrov Forest State Reserve, entrance from Garni, riverside Azat, 40º06ǿ25öN 44º45ǿ16öE, 1300 m, 18-VI-2015, **a)** on *Quercus macran-*

thera, **b)** on *Populus* sp., **c)** on quartzitic rocks, **d)** on siliceous volcanic rocks, **e)** on *Fraxinus excelsior*, **f)** on *Juglans regia*, **g)** on *Cornus* sp., **h)** on soil, **i)** on limestone, **j)** on bark, mixed forest of *Quercus*, *Juniperus* and *Acer*.

6.- ARMENIA: ARARAT: Vedi, Urtsadzor, Khosrov Forest State Reserve, abandoned vil- lage, 40°01ø10.7öN, 44°54ø46.3öE, 1760 m, 17-VI-2015, **a)** on *Malus* sp.

Results and discussion

Overall, 172 species of lichenized and four species of lichenicolous fungi were found in the Khosrov Forest State Reserve. Species are listed in alphabetic order. Species names are followed by the locality or localities numbers, letter corresponding to the substrate and in case of lichenicolous fungi the host lichen species in brackets. Some specimens of *Anaptychia*, *Melanelixia*, *Melanohalea*, *Parmelia*, *Parmelina* and *Physconia*, have been sequenced, in those cases the DNA extraction number is included, in brackets, after the letter corresponding to the substrate. Forty five taxa of lichens and 4 taxa of lichenicolous fungi are new records for Armenia and marked in the list with (*), 14 of the new records represent new records for genera in Armenia and these have been marked using (**). With these new records, the total number of lichens and lichenicolous fungi known from Armenia reaches 513 species, 507 lichens and 6 lichenicolous fungi.

List of species

Acarospora assimulans Vain. 1c

Acarospora cervina (Ach.) A. Massal. 1d

Acarospora fuscata (Nyl.) Th. Fr. 5d

Acarospora insolata H. Magn. 5d

Acarospora veronensis A. Massal. 1c, 5d

**Acarospora versicolor* Bagl. & Car. 3f, 5d

***Agonimia tristicula* (Nyl.) Zahlbr. ó 2d, 3c

Alyxoria varia (Pers.) Ertz & Tehler ó 2a

Anaptychia ciliaris (L.) A. Massal. 2j (DNA 4965), 3g (DNA 4966)

Anaptychia roemeri Poelt ó 1d, 3c

***Anema decipiens* (A. Massal.) Forssell 1d, 2d

**Arctomia fascicularis* (L.) Otálora & Wedin 2a

**Arthonia intexta* Almq. (in *Lecidella elaeochroma* apothecia) 2j

**Arthonia phaeophysciae* Grube & Matzer (on *Phaeophyscia* cf. *hirsuta*) ó 2a, 5b

Aspicilia cinerea (L.) Körb. 2c

Aspicilia contorta subsp. *hoffmanniana* R. Sant. 1d

Aspicilia desertorum (Kremp.) Mereschk. 1cd, 2c, 5d

**Aspicilia* cf. *glomerulans* (Poelt) Poelt 5d

**Aspicilia intermutans* (Nyl.) Arnold 2c

Aspicilia reticulata Kremp. ó 3c

Athallia pyracea (Ach.) Arup *ó* 1ae, 2ae, 3be, 4a

**Bacidina arnoldiana* V. Wirth & V zda 2e

***Bagliettoa calciseda* (DC.) Gueidan & Cl. Roux 2d

**Bilimbia sabuletorum* (Schreb.) Arnold 2d

**Blennothallia crispa* (Hudson) Otálora, P.M. Jørg. & Wedin 3c

Calogaya biatorina (A. Massal.) Arup, Frödén & Søchtink 1d

Calogaya polycarpoides (J. Steiner) Arup, Frödén & Søchtink 3a

Calogaya pusilla (A. Massal.) Arup, Frödén & Søchtink 2d

Caloplaca cerina (Hedw.) Th. Fr. ó 1a, 2ah

Caloplaca demissa (Körb.) Arup & Grube 3c

Caloplaca monacensis (Leder.) Lettau ó 1ae, 2a, 3b

Candelariella antennaria Räsänen ó 2e, 4a

Candelariella aurella (Hoffm.) Zahlbr. 2d

Candelariella vitellina (Ehrh.) Müll. Arg. 1d, 2d

***Chrysopsora testacea* (Hoffm.) Choisy ó 1d

Circinaria calcarea (L.) A. Nordin, S. Savi & Tibell 1d, 2ad, 5d

Cladonia pocillum (Ach.) O. J. Rich 5a

Collema flaccidum (Ach.) Ach. 2a

**Collema subflaccidum* Degel. ó 3b, 4a

Dermatocarpon miniatum (L.) W. Mann 2c, 3f, 5d

Dimelaena oreina (Ach.) Norman ó 3c

**Diploschistes gypsaceus* (Ach.) Zahlbr. ó 2f, 3f

Diploschistes muscorum (Scop.) R. Sant. 5h

Diploschistes scruposus (Schreb.) Norman 5d

Diplotomma hedinii (H. Magn.) P. Clerc & Cl. Roux 2d

Enchylium tenax (Sw.) Gray 2d

**Endocarpon pusillum* Hedw. 3f, 5d

Evernia prunastri (L.) Ach. 3g

Flavoplaca flavocitrina (Nyl.) Arup, Frödén & Søchtink 2c

Glypholecia scabra (Pers.) Müll. Arg. ó 1d

***Gonohymenia nigritella* (Lettau) Henssen 1d

**Gonohymenia schleicheri* (Hepp) Henssen 3c

Gyalolechia flavovirescens (Wulfen) Søchtink, Frödén & Arup 5d

**Gyalolechia juniperina* (Tomin) Søchtink, Frödén & Arup ó 1b

Immersaria cupreoatra (Nyl.) Calat. & Rambold ó 1c, 2c

**Immersaria iranica* Valadb., Sipman & Rambold ó 2c

Lathagrium cristatum (L.) Otálora, P.M. Jørg. & Wedin 1bd, 5d

Lecania cyrtella (Ach.) Th. Fr. 3a

Lecania fuscella (Schaer.) A. Massal. - 2a, 3a

**Lecania rabenhorstii* (Hepp) Arnold 2d

Lecanora argopholis (Ach.) Ach. 1c, 5d

*_Lecanora barkmaniana_ Aptroot & Herk ó 3a

Lecanora bicincta Ramond - 3c

Lecanora bolcana (Pollinii) Poelt 2c, 3c

Lecanora carpinea (L.) Vain. ó 1a

Lecanora chlarotera Nyl. ó 1a

Lecanora garovaglii (Körb.) Zahlbr. ó 1c, 2c, 3c, 5d

*_Lecanora juniperina_ liwa _ó_ 1ae, 2ab, 3be

Lecanora muralis (Schreb.) Rabenh. ó 1d, 2d, 3c, 5d

Lecanora percrenata H. Magn. 1d, 5d

Lecanora rupicola (L.) Zahlbr. 2c, 3c

*_Lecanora semipallida_ H. Magn. 2d, 5d

Lecanora wetmorei liwa ó 2a, 4ab, 5abj

Lecidella elaeochroma (Ach.) M. Choisy ó 2aj, 3a, 5j

Lecidella euphorea (Flörke) Hertel _ó_ 1a, 2a, 3ag

Lecidella patavina (A. Massal.) Knoph & Leuckert 3c

Lepraria finkii (de Lesd.) R. C. Harris 3c

Lepraria nivalis J. R. Laundon 2d, 3c, 5d

**_Leprocaulon microscopicum_ (Vill.) Gams ó 3c

Leptogium saturninum (Dicks.) Nyl. 2a, 3a

**_Llimoniella phaeophysciae_ Diederich, Ertz & Etayo (on _Phaeophyscia_ cf. _hirsuta_) ó 1a, 5b

Lobothallia alphoplaca (Wahlenb.) Hafellner - 3c, 5d

Lobothallia praeradiosa (Nyl.) Hafellner 1cd, 2c

Lobothallia radiosa (Hoffm.) Hafellner ó 3c, 5d

*_Lobothallia recedens_ (Taylor) A. Nordin, S. Savi & Tibell 2c

Megaspora rimisorediata Valadb. ó 1e, 2b

Melanelixia glabra (Schaer.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch 1af (DNA 5053), 2aij (DNA 5055), 3abeg (DNA 5054), 5g

Melanelixia subargentifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch ó 1af (DNA 5065, 5067), 2abij (DNA 5068, 5070), 3aeg, 4ab (DNA 5069), 5fj (DNA 5063)

Melanohalea elegantula (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch ó 2j, 3g (DNA 5170), 4ab (DNA 5059)

Melanohalea exasperata (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch ó 1f, 2agi, 4a, 6a

Parmelia sulcata Taylor - 3g (DNA 5003, 5004)

Parmelina tiliacea (Hoffm.) Hale ó 2acj (DNA 4961, 4962, 4963, 4988, 4989, 4990, 4991, 5002), 3g (DNA 4992, 4993), 4b (DNA 4994, 4995, 4996), 6a

**Peccania coralloides* (A. Massal.) A. Massal. 3c

Peltigera canina (L.) Willd. 2c

Peltigera elisabethae Gyeln. 3cd

Peltigera ponojensis Gyeln. 2c

Peltigera praetextata (Flörke) Vain. 2c

Peltigera rufescens (Weiss) Humb. 3cd

**Peltula euploca* (Ach.) Ozenda & Clauzade 5d

Phaeophyscia ciliata (Hoffm.) Moberg ó 2g

Phaeophyscia cf. *hirsuta* (Mereschk.) Essl. *ó* 1ae, 2ab, 5b

Phaeophyscia nigricans (Flörke) Moberg *ó* 1ae, 3b, 5a, 6a

Phaeophyscia orbicularis (Neck.) Moberg 1a, 2aefi, 3a, 4ab, 5aef; 6a

Phaeophyscia sciastra (Ach.) Moberg ó 2f, 3c

Physcia adscendens (Fr.) H. Olivier ó 1a, 2ef, 3, 3a, 4b, 5ag

Physcia aipolia (Humb.) Fürnr. *ó* 1af, 2aej, 3ag

Physcia biziana (A. Massal.) Zahlbr. 1af, 2ai, 4a, 5ae

Physcia caesia (Hoffm.) Fürnr. ó 3c

Physcia dimidiata (Arnold) Nyl. ó 1ad, 2abf, 3e, 4a, 5f

Physcia dubia (Hoffm.) Lettau 2c

Physcia stellaris (L.) Nyl. ó 1af

Physcia tenella (Scop.) DC. ó 1f, 2f

Physconia detersa (Nyl.) Poelt ó 1f, 3af (DNA 5009)

Physconia distorta (With.) J. R. Laundon 1a, 2d, 3ab, 5bf

Physconia enteroxantha (Nyl.) Poelt ó 2i, 3bc, 5g

Physconia grisea (Lam.) Poelt 2a

Physconia perisidiosa (Erichsen) Moberg 2a, 3bg, 4a, 5j (DNA 4973)

**Physconia thorstenii* A. Crespo & Divakar 1f (DNA 4972, 5010), 2j

***Piccolia ochrophora* (Nyl.) Hafellner ó 1a

**Placidium lacinulatum* (Ach.) Breuss - 3f

Placidium rufescens (Ach.) A. Massal. 1d, 3cd

Placocarpus schaereri (Fr.) Breuss *ó* 1d, 5d

**Placopyrenium fuscellum* (Turner) Gueidan & Cl. Roux 2d

Placynthium nigrum (Huds.) Gray 2d, 3c

Pleurosticta acetabulum (Neck.) Elix & Lumbsch 2a

***Psorotichia schaereri* (A. Massal.) Arnold 1d

Punctelia borreri (Sm.) Krog 1f

Pyrenodesmia variabilis (Pers.) A. Massal. ó 1bc, 2d, 5d

Ramalina farinacea (L.) Ach. 3a

Ramalina pollinaria (Westr.) Ach. - 3g

Rhizocarpon disporum (Hepp) Müll. Arg. 2c, 3c

Rhizocarpon geographicum (L.) DC. ó 3c

Rhizoplaca chrysoleuca (Sm.) Zopf 3c

Rhizoplaca melanophthalma (DC.) Leuckert 1c

Rhizoplaca peltata (Ramond) Leuckert & Poelt ó 1c, 2c, 5d

Rinodina colobina (Ach.) Th. Fr. *ó* 1e, 2a

Rinodina immersa (Körb.) J. Steiner 2cd

Rinodina obnascens (Nyl.) H. Olivier 2c

Rinodina pyrina (Ach.) Arnold ó 3e

Romjularia lurida (Ach.) Timdal 2d

Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt 5d

Sarcogyne regularis Körb. 2d

Scytinium gelatinosum (With.) Otálora, P.M. Jørg. & Wedin 2adf, 3ac, 5d

Scytinium lichenoides (L.) Otálora, P.M. Jørg. & Wedin 2afj, 3e

Scytinium turgidum (Ach.) Otálora, P.M. Jørg. &Wedin 3c

**Solenopsora holophaea* (Mont.) Samp. ó 2d, 3c

Squamarina cartilaginea (With.) P. James 2c

Staurothele areolata (Ach.) Lettau 3c

Staurothele fuscocuprea (Nyl.) Zschacke 2d

Tephromela atra (Huds.) Hafellner ó 5a

**Thermutis velutina* (Ach.) Flot. 1c, 1d, 2c

Thyrea confusa Henssen 1d, 2d

Toninia candida (Weber) Th. Fr. 2d

Toninia cinereovirens (Schaer.) A. Massal. 1d (Fig. 3)

Toninia sedifolia (Scop.) Timdal 3d, 5d

Toninia squalida (Ach.) A. Massal. ó 3c

**Tremella phaeophysciae* Diederich & M. S. Christ. (on *Pheophyscia orbicularis*) - 4b

Usnea lapponica Vain. 3g

Usnea substerilis Motyka 3g

Usnea wasmuthii Räsänen ó 3g

Verrucaria dolosa Hepp 2e

Verrucaria hochstetteri Fr. 2d

**Verrucaria macrostoma* DC. 1d

Verrucaria muralis Ach. 2d

Verrucaria nigrescens Pers. 2de, 5d

Xanthocarpia lactea (A. Massal.) A. Massal. 2d

Xanthomendoza fulva (Hoffm.) Søchting, Kärnefelt & S.Y. Kondr. ó 5b

Xanthomendoza ulophyllodes (Räsänen) Søchting, Kärnefelt & S.Y. Kondr. 2a, 3b, 4a, 5b

Xanthoparmelia conspersa (Ach.) Hale 5d

Xanthoparmelia loxodes (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch 3c

**Xanthoparmelia protomatrae* (Gyeln.) Hale 5d

Fig. 3. *Toninia cinereovirens* and *Thermutis velutina* on limestone in the Khosrov Forest State Reserve (photo by Maaike Vervoort).

Xanthoparmelia pulla (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch ó 1c, 2c, 3c, 5dh

Xanthoparmelia somloensis (Gyeln.) Hale 3c, 5dh

**Xanthoparmelia tinctina* (Maheu & A. Gillet) Hale ó 1c, 2c, 3cd, 5d

Xanthoparmelia verruculifera (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch 5d

Xanthoria parietina (L.) Th. Fr. 5a, 5f, 5g

Acknowledgments

The authors would like to express their gratitude to the staff of the Khosrov Forest State Reserve for kind support during field work and to the Ministry of Nature Protection for permission to collect the specimens. We are also very thankful to staff and volunteers of the Young Biologists Association NGO, especially Hripsime Atoyan, Vanuhi Hambardzumyan and Maria Antonosyan for field assis- tance during the excursion. The first author is especially grateful to Drs Harrie J. Sipman and Jan Vondrak for support in the identification of some specimens, as well as acknowledges financial sup- port from DAAD (German Academic Exchange Service) and the project õDeveloping Tools for Conserving the Plant Diversity of the Transcaucasusö financed by the Volkswagen Foundation. AC, VJR, PKD and EA, acknowledges financial support from the Spanish Ministerio de Economía y Competitividad projects CGL2011-25003 and CGL2013-42498-P. ARB acknowledges financial sup- port to Ministry of Economy and Competitiveness, Spain, project CGL2013-41839-P. We are also thankful to Maaike Vervoort for permission to publish the photograph in Fig. 3.

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Kapitel 7:

Additions to the lichenized and lichenicolous mycobiota of Armenia

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Published By: **Herzogia 29: 692ó705.**

Format is based on journal guidelines

Abstract:

Two hundred and thirty-three lichenized and lichenicolous fungi are reported here from Armenia. Eighty-nine are new records for the country. Fifty-three taxa were found on basaltic rocks of the ancient megalithic monument õZorats Karerö. The new combination *Protoparmeliopsis bolcana* (Pollini) Lumbsch is introduced.

Zusammenfassung:

Es werden 233 Flechten und lichenicole Pilze aus Armenien dokumentiert, darunter 89 Erstnachweise für das Land. 53 Taxa wurden auf Basaltsteinen des prähistorischen Megalithmonumentes šZorats Karerõ gefunden. Die Neukombination *Protoparmeliopsis bolcana* (Pollini) Lumbsch wird vorgenommen.

Key words: Ascomycetes, biodiversity, Caucasus, lichen-forming fungi, new records, õZorats Karerö megalithic monument.

Introduction

The Republic of Armenia is a mountainous country in the South Caucasus (Transcaucasia). The variety of landscapes, geographic features, climatic and altitudinal zonation support a unique biological diversity. The lowest elevation is 375 m, near the river Debed and the highest elevation is 4090 m on Mt. Aragats. Annual average precipitation is about 600 mm and var- ies between 250 and 1000 mm. Annual average temperature ranges between 2.7 °C and 14 °C (Anonymous 1999). Currently about 3800 species of vascular plants, 4213 species of fungi, including six lichenicolous fungi, 428 species of algae and 399 species of bryophytes are known from Armenia (Anonymous 2015). The known lichen flora of Armenia consists 515 taxa (Harutyunyan et al. 2011, Gasparyan & Sipman 2013, Gasparyan et al. 2014, 2015, 2016, Zakeri et al. 2016).

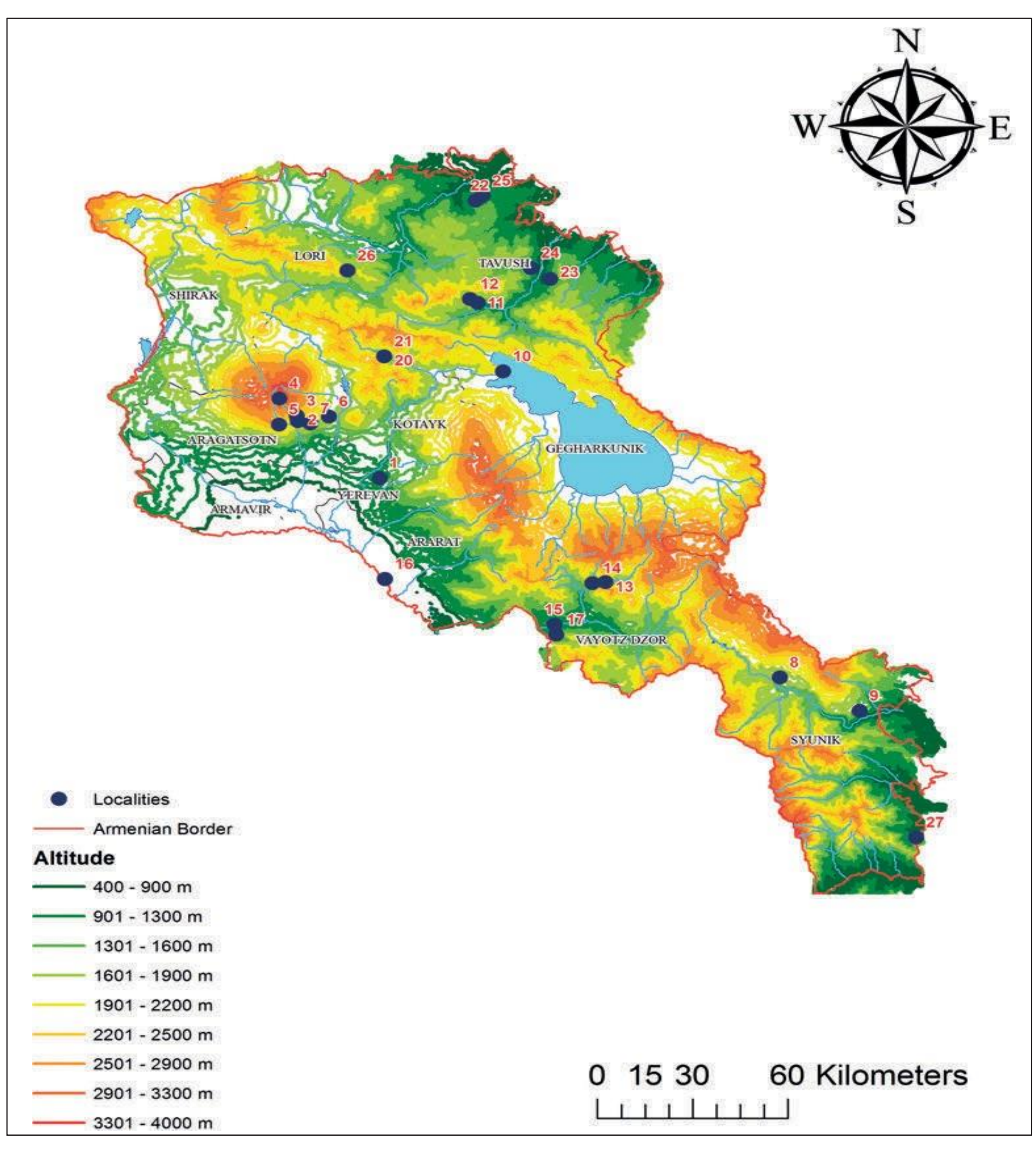

Fig. 1: The altitudinal gradient map of the Republic of Armenia including names of provinces and visited localities (1ó27).

On 15 ó24 June 2015, in the frame of the õOPTIMA Iter Lichenologicumö initiative, the Young Biologists Association NGO (Armenia) together with the Organization for the Phyto- Taxonomic Investigation of the Mediterranean Area (OPTIMA) organized an International Lichenological Excursion to Armenia. During the excursion, material has been collected from various natural ecosystems and historical sites. Several localities were visited in the Vayots Dzor province with rocky habitats and montane xerophyte vegetation and localities at different elevations of Mt. Aragats. The vegetation is dominated by steppes, sparse oak forests (*Quercus macranthera*) and montane alpine meadows. We also visited several protected areas, including temperate deciduous forests of the Dilijan and Sevan National Parks.

Fig. 2: Locality 8. Basalt rocks of õZorats Karerö megalithic monument (photo: P. K. Divakar).

In addition to natural habitats, lichens were also studied in the õKhor Virapö monastery, õAmberdö fortress and õZorats Karerö or õCarahunjeö (Armenian Stonehenge), a megalithic, 7500 years old monument considered by some authors as an astronomic observatory (Herouni 2004). It consists of more than 200 basalt stones located in the Syunik province of Armenia (González-García 2014) [Fig. 2]. This paper summarizes new records and records of poorly known species of lichenized and

lichenicolous fungi for Armenia. In addition, lichens found at the megalithic monument õZorats Karerö are listed.

Material and methods

The field studies were carried out in 19 localities by all authors spread across 10 provinces of Armenia (Fig. 1) from 16 to 23 June 2015. In addition, 8 localities were visited only by the first author in 2014 and 2015. Secondary metabolites were identified by spot tests and thin layer chromatography (TLC) following Orange et al. (2010). Additionally, in order to confirm the identification of cryptic taxa, internal transcribed spacer of nuclear rDNA (ITS) were analysed in some collections of *Anaptychia*, *Melanelixia*, *Melanohalea*, *Montanelia*, *Parmelia*, *Parmelina*, *Parmotrema* and *Physconia.* We compared such ITS sequences (ex- tracts numbered and kept in MAF), with available ITS sequences in GenBank (NCBI) using BLAST. DNA extraction, PCR, and cycle sequencing were done following Núñez-Zapata et al. (2015). Voucher specimens are deposited in ABL, B, F, GLM, MACB, MAF and PAL.

List of visited localities

1. Yerevan: Yerevan Botanical Garden, 40º12'45''N, 44º33'24''E, 1250 m alt., 15 óVIó 2015.
2. Aragatsotn: Byurakan, Mts. Aragats, H20 road, before diversion to Amberd castle, 40º23'55''N, 44º15'07''E, 2200 m alt., 16 óVIó2015.
3. Aragatsotn: Byurakan, Mts. Aragats, H20 road to lake Kari, after diversion to Amberd castle, 40º24'54''N, 44º14'51''E, 2470 m alt., 16 óVIó2015.
4. Aragatsotn: Byurakan, Mts. Aragats, around lake Kari, 40º28'21''N, 44º10'58''E, 3200 m alt., 16 óVIó2015.
5. Aragatsotn: Byurakan, Mts. Aragats, road connecting H20 to M3, 40º23'11''N, 44º16'58''E, 2040 m alt., 16 óVIó 2015.
6. Aragatsotn: Byurakan, Mts. Aragats, road connecting M3 to H20, 40º24'48''N, 44º22'03''E, 1800 m alt., 21óVIó 2015.
7. Aragatsotn: Byurakan, Mts. Aragats, road connecting M3 to H20, 40º23'26''N, 44º17'58''E, 2020 m alt., 21óVIó 2015.

8. Syunik: Sisian, at the north border of Zorats Karer, 39º33'12''N, 46º01'46''E, 1760 m alt., 20 óVIó2015.
9. Syunik: Shinuhayr, 39º26'25''N, 46º19'11''E, 1530 m alt., 20 óVIó2015.
10. Gegharkunik: Sevan, Sevan National Park, Sevanavank, 40º33'50''N, 45º00'48''E, 1950 m alt., 22óVIó2015.
11. Tavush: Dilijan, Dilijan National Park, H50 road to Haghartsin Monastery, 40º47'21''N, 44º55'07''E, 1250 m alt., 22óVIó2015.
12. Tavush: Dilijan, Dilijan National Park, around Haghartsin Monastery, 40º48'08''N, 44º53'28''E, 1440 m alt., 22ó VIó2015.
13. Vayots Dzor: Shatin, Yeghegis, 39º52'14''N, 45º23'26''E, 1600 m alt., 23 ó VIó 2015.
14. Vayots Dzor: Shatin, Yeghegis, 39º52'03''N, 45º20'38''E, 1530 m alt., 23 óVIó2015.
15. Vayots Dzor: Areni, Amaghu valley along Noravank Monastery road, 39º43'54''N, 45º12'15''E, 1050 m alt., 19ó VIó2015.
16. Ararat: Khor Virap Monastery graveyard, 39º52'50''N, 44º34'42''E, 800 m alt., 19ó VIó2015.
17. Vayots Dzor: Areni, Amaghu valley along Noravank Monastery road, 39º41'54''N, 45º12'35''E, 1265 m alt., 23 ó VIó2015.
18. Syunik: Tatev, near Tatev monastery, 39º22'49''N, 46º14'58''E, 1525 m alt., 20 óVIó 2015.
19. Aragatsotn: Amberd fortress, 40º22'28''N, 44º16'47''E, 2131 m alt., 21óVIó2015.
20. Kotayk: Artavaz, 40º36'46''N, 44º34'18''E, 1855 m alt., 04 óVIIó2015.
21. Kotayk: Tsaghkadzor, 40º36'46''N, 44º34'18''E, 1883 m alt., 27óVIIó2014.
22. Tavush: Koghb, Noyemberyan forestry, 41°07'35''N, 44°54'39''E, 1160 m alt., 23 ó VIIó2015.
23. Tavush: H36 road to Berd, 40°52'05''N, 45°11'28''E, 1446 m alt., 18 óVIIó2014.
24. Tavush: Getahovit, 40°54'15''N, 45°06'68''E, 877 m alt., 18 óVIIó2014.
25. Tavush: Zikatar State Sanctuary, N 41°08'31''N, 44°55'73''E, 1024 m alt., 23 óVIIIó 2014.
26. Lori: Pushkino pass, H23 road to Gargar, 40°53'42''N, 44°25'55''E, 1695 m alt., 05 ó

VIIó2014.

27. Syunik: Shikahogh State Reserve, 39°00'77"N, 046°30'62"E, 1700 m alt., 09 óIVó 2014.

Results

The list of taxa found is provided in alphabetic order. For each species, the locality number and substrate (for lichenicolous fungi host lichen) are listed. For sequenced specimens, GenBank accession numbers are given in brackets, after the letter corresponding to the substrate. The new country records are marked with an asterisk (*) and, records representing new genera with two asterisks (**). Lichenicolous fungi are marked with (#). Important synonyms for species with recent taxonomic changes are provided. The list of lichens reported from õZorats Karerö is separetely presented.

Abbreviations of the substrates: **A** *Acer* sp.; **aq** aquatic siliceous rock; **bas** basaltic rock; **C** *Carpinus betulus*; **cal** calcareous volcanic rock; **con** ó concrete; **F** *Fagus orientalis*; **Frax** ó *Fraxinus excelsior*; **J** *Juniperus* sp.; **Jug** *Juglans* sp.; **lim** limestone cliff; **M** *Mespilus* sp.; **m** ó mosses; **P** *Pinus sylvestris*; **Po** *ó Populus* sp.; **Q** *Quercus macranthera*; **sil** siliceous volcanic rock; **ter** ó soil; **tr** ó tree; **t** ó twig; **U** ó *Ulmus* sp.; **vor** ó volcanic rock.

List of taxa

Acarospora assimulans Vain. ó 3sil; 6sil; 10sil; 14sil.

Acarospora bornmuelleri J.Steiner ó 14bas.

Acarospora hospitans H.Magn. ó 6sil; 13sil.

Acarospora impressula Th.Fr. ó 6sil; 9cal.

Acarospora insolata H.Magn. ó 6sil.

Acarospora molybdina (Wahlenb.) Trevis. ó 2sil; 6; 13sil; 14sil; 16sil.

Acarospora strigata (Nyl.) Jatta ó 14bas.

Acarospora versicolor Bagl. & Car. ó 9cal.

Acrocordia cavata (Ach.) R.C.Harris ó 11F.

Agonimia tristicula (Nyl.) Zahlbr. ó 10cal; 15lim.

Anamylopsora pulcherrima (Vain.) Timdal ó 13vor.

Anaptychia ciliaris (L.) Körb. ó 10U; 11t; 12Q (GenBank accession no. KX457686), t.

Anaptychia roemeri Poelt ó 15A, sil; 17lim.

***Anaptychia ulotrichoides** (Vain.) Vain. ó 17m.

Anema decipiens (A.Massal.) Forssell ó 17lim.

****Anema tumidulum*** P.M.Jørg. et al. ó 15lim; 17lim.

****#Arthonia epiphyscia*** Nyl. ó 2bas (on *Physcia dubia*).

****#Arthonia hertelii*** (Calat. et al.) Hafellner ó 6vor (on *Circinaria elmorei*).

#Arthonia phaeophysciae Grube & Matzer ó 13Jug.

****Arthonia ruana*** A.Massal. ó 11F.

****#Arthonia varians*** (Davies) Nyl. ó 2bas (on *Lecanora rupicola*).

****Aspicilia cupreoglauca*** B.de Lesd. ó 2bas; 3sil.

Aspicilia intermutans (Nyl.) Arnold ó 2bas; 6bas.

****Aspicilia verrucigera*** Hue ó 17lim.

****Bacidina caligans*** (Nyl.) Llop & Hladun 3ter.

Bacidia polychroa (Th.Fr.) Körb. ó 11A.

Bacidia rubella (Hoffm.) A.Massal. ó 11F;12C.

Bacidina arnoldiana V.Wirth & V zda ó11sil; 12sil.

Bagliettoa calciseda (DC.) Gueidan & Cl.Roux ó 12lim; 17lim.

Blennothallia crispa (Weber ex F.H.Wigg.) Otálora et al. [syn. *Collema crispum* Weber ex F.H.Wigg.] ó 17ter.

****Buellia asterella*** Poelt & Sulzer ó 17lim.

Buellia disciformis (Fr.) Mudd ó 11F.

Buellia griseovirens (Turner & Borrer ex Sm.) Almb. ó 11F, 21tr.

Calogaya arnoldii (Weddell) Arup et al. [syn. *Caloplaca arnoldii* subsp. *obliterata* (Pers.) Gaya] ó 19vor.

Calogaya schistidii (Anzi) Arup et al. [syn. *Caloplaca schistidii* (Anzi) Zahlbr.] ó 17m.

Caloplaca atroflava (Turner) Mong. ó 9cal.

Caloplaca chlorina (Flot.) Sandst. ó 10sil; 13Jug, sil.

Caloplaca epithallina Lynge ó 3sil (on *Rhizoplaca*).

Caloplaca inconnexa (Nyl.) Zahlbr. ó 6sil; 9cal.

Caloplaca peludella (Nyl.) Hasse ó 2sil.

Caloplaca stillicidiorum (Vahl) Lynge ó 3ter.

Caloplaca teicholyta (Ach.) J.Steiner ó 16sil.

#Chaenothecopsis ochroleuca (Körb.) Tibell & K.Ryman ó 12 m (on *Lecanora thysanophora*).

Chrysothrix candelaris (L.) J.R.Laundon ó 11F; 12Q.

Circinaria caesiocinerea (Nyl. ex Malbr.) A.Nordin et al. ó 2bas, sil; 12sil; 13sil; 14sil.

Cladonia cf. ***cariosa*** (Ach.) Spreng. ó 6ter.

Cladonia foliacea (Huds.) Willd. ó 10sil, ter.

Cladonia rangiformis Hoffm. ó 10sil, ter.

Cladonia symphycarpa (Flörke) Fr. ó 2sil, ter; 6sil, ter.

Collema flaccidum (Ach.) Ach. ó 12C, Jug.

****Collolechia caesia*** (Fr.) A.Massal. ó 17lim.

****Cyrtidula quercus*** (A.Massal.) Minks ó 4Q.

Dimelaena oreina (Ach.) Norman ó 2bas; 3sil; 14sil, bas.

Diploschistes diacapsis (Ach.) Lumbsch ó 13vor.

****Dirina massiliensis*** Durieu & Mont. ó 12lim.

Enchylium ligerinum (Hy) Otálora et al. [syn. *Collema ligerinum* (Hy) Harm.] ó 12Jug.

Endocarpon adscendens (Anzi) Müll.Arg. ó 3sil; 6sil; 10ter; 14sil, bas; 17lim.

Flavoplaca flavocitrina (Nyl.) Arup et al. [syn. *Caloplaca flavocitrina* (Nyl.) H.Olivier] ó 10con; 11sil; 13sil.

****Flavoplaca oasis*** (A.Massal.) Arup et al. [syn. *Caloplaca oasis* (A.Massal.) Szatala] ó 9cal; 17lim.

Flavopunctelia flaventior (Stirt.) Hale ó 11F.

Glypholecia scabra (Pers.) Müll.Arg. ó 6ter; 9cal; 17lim.

****Gyalolechia fulgens*** (Sw.) Arup et al. ó 17lim.

*****Heteroplacidium compactum*** (A.Massal.) Gueidan & Cl.Roux ó 17lim.

****Immersaria athroocarpa*** (Ach.) Rambold & Pietschm. ó 13sil; 14sil.

Immersaria cupreoatra (Nyl.) Calat. & Rambold ó 2sil; 6sil, vor; 13sil.

Immersaria iranica Valadb. et al. ó 2sil, bas; 3sil; 5vor.

****Lathagrium auriforme*** (With.) Otálora et al. [syn. *Collema auriforme* (With.) Coppins & J.R.Laundon] ó 10sil; 15lim.

Lathagrium fuscovirens (With.) Otálora et al. [syn. *Collema fuscovirens* (With.) J.R.Laundon] ó 10sil.

****Lecania croatica*** (Zahlbr.) Kotlov ó 11F; 12M, 22F.

Lecania erysibe (Ach.) Mudd ó 2sil; 4Q; 12lim.

Lecania rabenhorstii (Hepp) Arnold ó 9cal; 12lim; 17lim.

****Lecania tavaresiana*** Clauzade & V zda ó 9cal.

****Lecanora albellula*** (Nyl.) Th.Fr. ó 7P.

Lecanora bicincta Ramond ó 5sil; 13sil; 14sil.

Lecanora dispersoareolata (Schaer.) Lamy ó 2bas.

****Lecanora expallens*** Ach. ó 11F, 22F.

****Lecanora gangaleoides*** Nyl. ó 9sil.

Lecanora horiza (Ach.) Linds. ó 12C, F.

****Lecanora klauskalbii*** Sipman ó 2sil; 3sil; 6sil.

Lecanora pulicaris (Pers.) Ach. ó 11t.

Lecanora rupicola (L.) Zahlbr. ó 2bas; 6vor.

Lecidea atrobrunnea (DC.) Schaer. ó 2bas; 3sil; 6vor. (Fig. 3)

Lecidea confluens (Weber) Ach. ó 3sil; 6vor; 10sil.

Lecidea lapicida (Ach.) Ach. ó 2sil.

***Lecidea plana** Kremp. ó 6sil.

***Lecidea promiscua** Nyl. ó 2bas.

***Lecidella carpathica** Körb. *ó* 2bas; 3sil; 6vor; 14bas.

Lepraria incana (L.) Ach. ó 13m.

Leproplaca cirrochroa (Ach.) Arup et al. [syn. *Caloplaca cirrochroa* (Ach.) Th.Fr.] *ó* 12lim.

Leproplaca xantholyta (Nyl.) Nyl. [syn. *Caloplaca xantholyta* (Nyl.) Jatta] ó 17lim.

***Leptogium byssinum** (Hoffm.) Zwackh ex Nyl. ó 3sil; 17lim.

Leptogium saturninum (Dicks.) Nyl. ó 11F.

****Lichinella nigritella** (Lettau) Henssen ó 13vor; 14bas; 17lim.

Lichinella stipatula Nyl. ó 10sil.

***Lobothallia chadefaudiana** (Cl. Roux) A.Nordin et al. ó 17lim.

***Lobothallia cheresina** (Müll.Arg.) A.Nordin et al. ó 17lim.

***Lobothallia farinosa** (Flörke) A.Nordin et al. ó 17lim.

Melanelixia epilosa (J.Steiner) A.Crespo et al. ó 5Q, 10U (GenBank accession no. KX457697), Po; 12F (GenBank accession no. KX457696), M.

Melanelixia glabratula (Lamy) Sandler & Arup ó 11F (GenBank accession no. KX457695); 12F (GenBank accession no. KX457703).

Melanelixia subargentifera (Nyl.) O.Blanco et al. ó 5Q; 10Po; 11F; 12F (GenBank accession no. KX457700), Frax, M.; 13Q (GenBank accession no. KX457701).

Melanelixia subaurifera (Nyl.) O.Blanco et al. ó 11F; 12M, t.

Melanohalea elegantula (Zahlbr.) O.Blanco et al. ó 3sil; 5Q; 7Q; 12Q.

Melanohalea exasperata (De Not.) O.Blanco et al. ó 5Q; 7Q; 10U (GenBank accession no. KX457698), 15sil.

Fig. 3: Locality 2. *Rhizocarpon geographicum*, *Rhizoplaca chrysoleuca*, *R. melanophthalma*, *Lecidea atrobrunnea*, *Aspicilia cinerea*, *Physcia albinea*, *Candelariella vitellina* (photo: Maaike Vervoort).

Melanohalea exasperatula (Nyl.) O.Blanco et al. ó 5Q, 11F, 12M.

Myriolecis perpruinosa (Fröberg) liwa et al. [syn. *Lecanora perpruinosa* Fröberg] ó 2sil; 13Jug.

****Myriolecis semipallida*** (H.Magn.) liwa et al. [syn. *Lecanora semipallida* H.Magn.] ó 14sil.

****Parmelia squarrosa*** Hale ó 20sil (GenBank accession no. KX457692).

Parmelina carporrhizans (Taylor) Hale ó 11F (GenBank accession no. KX457684).

Parmelina pastillifera (Harm.) Hale 12t, Q (GenBank accession no. KX457685).

Parmotrema perlatum (Huds.) M.Choisy ó 11F (GenBank accession no. KX457691).

Parmotrema reticulatum (Taylor) M.Choisy ó 11F (GenBank accession no. KX457693 and KX457694), 22F.

Parmotrema stuppeum (Taylor) Hale ó 11F.

Peccania coralloides (A.Massal.) A.Massal. ó 17lim.

Peltigera lepidophora (Nyl.) Bitter ó 3ter.

Peltigera monticola Vitik. ó 10sil, ter.

Peltigera polydactylon (Neck.) Hoffm. ó 11ter.

Pertusaria albescens (Huds.) M.Choisy & Werner ó 11F.

Pertusaria amara (Ach.) Nyl. ó 11F.

Pertusaria flavicans Lamy ó 9cal.

Pertusaria leioplaca DC. ó 11F, 22F, 23tr.

Phaeophyscia hirsuta (Mereschk.) Moberg ó 12C.

Phaeophyscia insignis (Mereschk.) Moberg ó 1Q; 12C.

Phaeophyscia pusilloides (Zahlbr.) Essl. ó 11F, 22F.

Physcia albinea (Ach.) Nyl. ó 2sil; 3sil. (Fig. 3)

Physcia dimidiata (Arnold) Nyl. ó 5Q; 14sil.

Physcia magnussonii Frey ó 2bas; 6vor.

Physconia distorta (With.) J.R.Laundon ó 11F; 12F (GenBank accession no. KX457687) and Q; 13Q.

Physconia grisea subsp. ***lilacina*** (Arnold) Poelt ó 14sil.

Physconia perisidiosa (Erichsen) Moberg ó 12Q; 14bas (GenBank accession no. KX457688).

Physconia thorstenii A.Crespo & Divakar ó 5Q; 10U (GenBank accession no. KX457689 and KX457690).

Placocarpus schaereri (Fr.) Breuss ó 14bas (also on *Protoparmeliopsis garovaglii*).

Placopyrenium trachyticum (Hazsl.) Breuss ó 3sil.

Placynthium garovaglii (A. Massal.) Zahlbr. ó 17lim.

Placynthium posterulum (Nyl.) Henssen ó 15lim.

Placynthium nigrum (Huds.) Gray ó 9cal; 12lim.

****Porina aenea*** (Wallr.) Zahlbr. ó 11F, 24F.

Protoparmeliopsis admontensis (Zahlbr.) Hafellner [syn. *Lecanora admontensis* Zahlbr.] ó 14bas.

Protoparmeliopsis garovaglii (Körb.) Arup et al. [syn. *Lecanora garovaglii* (Körb.) Zahlbr.] ó 2bas; 6sil; 9sil; 14sil.

Protoparmeliopsis laatokkaensis (Räsänen) Moberg & R.Sant. [syn. *Lecanora laatokkaensis* (Räsänen) Poelt] ó 2sil.

Psora vallesiaca (Schaer.) Timdal ó 17lim.

Psorotichia schaereri (A.Massal.) Arnold ó 17lim.

Punctelia jeckeri (Roum.) Kalb ó 11F.

Punctelia subrudecta (Nyl.) Krog ó 11F, 25F.

****Pycnothelia papillaria*** (Ehrh.) L.M.Dufour ó 17lim.

****Pyrenula laevigata*** (Pers.) Arnold ó 11F.

Pyrenula nitida (Weigel) Ach. ó 11F, 22F.

Pyrenula nitidella (Flörke ex Schaer.) Müll.Arg. ó 11F.

Rhizocarpon disporum (Nägeli ex Hepp) Müll.Arg. ó 12sil; 14sil, bas.

Rhizocarpon geminatum Körb. ó 14bas.

****Rimularia furvella*** (Nyl. ex Mudd) Hertel & Rambold ó 14sil.

Rinodina bischoffii (Hepp.) A.Massal. ó 17lim.

Rinodina guzzinii Jatta ó 2sil.

Rinodina pyrina (Ach.) Arnold ó 11F, 22F.

Rinodina sophodes (Ach.) A.Massal. ó 11F, 22F.

Romjularia lurida (Ach.) Timdal ó 3sil; 10ter; 15lim.

Rostania occultata (Bagl.) Otálora et al. [syn. *Collema occultatum* Bagl.] ó 12t.

Sarcogyne regularis Körb. ó 17lim.

Scytinium gelatinosum (With.) Otálora et al. [syn. *Leptogium gelatinosum* (With.) J.R.Laundon] ó 15lim.

Scytinium lichenoides (L.) Otálora et al. [syn. *Leptogium lichenoides* (L.) Zahlbr.] ó 11F.

***Scytinium parvum** (Degel.) Otálora et al. [syn. *Collema parvum* Degel.] ó 9cal.

Scytinium turgidum (Ach.) Otálora et al. [syn. *Leptogium turgidum* (Ach.) Leight.] ó 10cal.

***Solorina octospora** Arnold ó 3ter.

****#Spiloma auratum** Sm. ó 12lim, 27Q (on *Dirina massiliensis*).

Squamarina cartilaginea (With.) P.James ó 17lim, ter.

Staurothele areolata (Ach.) Lettau ó 2bas; 3sil; 6bas; 10sil.

****Synalissa symphorea** (Ach.) Nyl. ó 15lim; 17lim.

****Thelidium papulare** (Fr.) Arnold ó 12lim.

Toninia candida (Weber) Th.Fr. ó 17lim.

Toninia cinereovirens (Schaer.) A.Massal. ó 14sil, bas; 15lim; 17lim.

Toninia diffracta (A.Massal.) Zahlbr. ó 17lim.

Toninia opuntioides (Vill.) Timdal ó 17lim.

Toninia philippea (Mont.) Timdal ó 17lim.

***Toninia physaroides** (Arnold) H.Olivier ó 17ter.

Toninia squalida (Ach.) A.Massal. ó 17lim.

Usnea fulvoreagens Räsänen ó 12M.

Usnea lapponica Vain. ó 11F (TLC: salacinic acid); 12F (TLC: salacinic acid).

Usnea wasmuthii Räsänen ó 11F (TLC: salacinic acid).

Variospora aurantia (Pers.) Arup et al. [syn. *Caloplaca aurantia* (Pers.) Hellb.] ó 9cal; 12lim.

***Verrucaria aquatilis** Mudd ó 13aq.

***Verrucaria elaeina** Borrer ó 11sil.

Verrucaria hochstetteri Fr. ó 17lim.

Verrucaria macrostoma Dufour ex DC. ó 9cal; 17lim.

Verrucaria** cf. **praetermissa (Trevis.) Anzi ó 13aq.

Verrucaria sphaerospora Anzi ó 3sil; 6vor; 9cal; 14sil.

****#Verrucocladosporium dirinae*** K.Schub. et al. ó 12lim (on *Dirina massiliensis*).

*****Verrucula latericola*** Nav.-Ros. & Cl.Roux ó 6sil; 13sil; 14sil; 16sil.

Verruculopsis lecideoides (A.Massal.) Gueidan & Cl.Roux ó 6sil.

Xalocoa ocellata (Fr.) Kraichak et al. [syn. *Diploschistes ocellatus* (Fr.) Norman] ó 13sil; 15lim; 17lim.

Xanthocarpia crenulatella (Nyl.) Frödén et al. [syn. *Caloplaca crenulatella* (Nyl.) H.Olivier] ó 4sil; 6sil.

Xanthocarpia marmorata (Bagl.) Frödén et al. [syn. *Caloplaca marmorata* (Bagl.) Jatta] ó 17lim.

Xanthomendoza huculica (S.Y.Kondr.) Diederich ó 10Po; 12C, Q.

Xanthoparmelia camtschadalis (Ach.) Hale ó 3ter.

Xanthoparmelia chlorochroa (Tuck.) Hale ó 2ter.

Xanthoparmelia delisei (Duby) O.Blanco et al. ó 5sil¸9sil; 13sil; 14sil.

Xanthoparmelia loxodes (Nyl.) O.Blanco et al. ó 2sil; 3sil.

Xanthoparmelia luteonotata (J.Steiner) O.Blanco et al. ó 13sil.

Xanthoparmelia plittii (Gyeln.) Hale ó 9sil.

Xanthoparmelia protomatrae (Gyeln.) Hale ó 2bas.

Xanthoparmelia tinctina (Maheu & A.Gillet) Hale ó 2bas; 10sil; 13sil; 14sil, bas.

Xanthoparmelia verruculifera (Nyl.) O.Blanco et al. ó 3sil; 13vor; 14bas.

Zwackhia viridis (Ach.) Poetsch & Schied. [syn. *Opegrapha viridis* (Ach.) Ach.] ó 11F, 25F.

List of the lichens from megalithic monument õZorats Karerö (locality 8)

Acarospora assimulans Vain.

Acarospora cervina (Ach.) A.Massal.

Acarospora fuscata (Nyl.) Th.Fr.

Acarospora insolata H.Magn.

Acarospora molybdina (Wahlenb.) Trevis.

Acarospora versicolor Bagl. & Car.

Anaptychia ciliaris (L.) Körb.

Aspicilia cinerea (L.) Körb.

****Athallia saxifragarum*** (Poelt) Arup et al. [syn. *Caloplaca saxifragarum* Poelt]

Candelariella vitellina (Ehrh.) Müll. Arg.

Circinaria caesiocinerea (Nyl. ex Malbr.) A.Nordin et al.

Circinaria elmorei (E.D.Rudolph) Owe-Larss. et al. s. lat. [as *Aspicilia desertorum* (Kremp.) Mereschk. in our samples]

Cladonia foliacea (Huds.) Willd.

Dermatocarpon miniatum (L.) W. Mann

Dimelaena oreina (Ach.) Norman

Flavoplaca flavocitrina (Nyl.) Arup et al. [syn. *Caloplaca flavocitrina* (Nyl.) H.Olivier]

Immersaria cupreoatra (Nyl.) Calat. & Rambold

Lecanora argopholis (Ach.) Ach.

Lecanora dispersa (Pers.) Röhl.

Lecanora rupicola (L.) Zahlbr.

Lecidella carpathica Körb.

Lobothallia praeradiosa (Nyl.) Hafellner

Megaspora verrucosa (Ach.) Hafellner & V.Wirth

****Melanelixia fuliginosa*** (Fr. ex Duby) O.Blanco et al.

Melanohalea elegantula (Zahlbr.) O.Blanco et al. (GenBank accession no. KX457702).

****Montanelia tominii*** (Oxner) Divakar et al. (GenBank accession no. KX457699).

Phaeorrhiza nimbosa (Fr.) H.Mayrhofer & Poelt

Physcia caesia (Hoffm.) Fürnr.

Physcia dubia (Hoffm.) Lettau

Physconia detersa (Nyl.) Poelt

Physconia grisea (Lam.) Poelt

Physconia muscigena (Ach.) Poelt

Placidium squamulosum (Ach.) Breuss

Protoparmeliopsis bolcana (Pollini) Lumbsch [syn. *Lecanora bolcana* (Pollini) Poelt]

Protoparmeliopsis garovaglii (Körb.) Arup et al. [syn. *Lecanora garovaglii* (Körb.) Zahlbr.]

Ramalina capitata (Ach.) Nyl.

Ramalina polymorpha (Lilj.) Ach.

Rhizocarpon disporum (Nägeli ex Hepp) Müll.Arg.

Rhizocarpon geographicum (L.) DC.

Rhizoplaca chrysoleuca (Sm.) Zopf

Rhizoplaca melanophthalma (DC.) Leuckert

Rufoplaca arenaria (Pers.) Arup et al. [syn. *Caloplaca arenaria* (Pers.) Müll.Arg.]

Rusavskia elegans (Link) S.Y.Kondr. & Kärnefelt

Tephromela grumosa (Pers.) Hafellner & Cl.Roux

Verrucula cf. ***latericola*** Nav.-Ros. & Cl.Roux

Xanthoparmelia conspersa (Ach.) Hale

Xanthoparmelia delisei (Duby) Essl.

Xanthoparmelia loxodes (Nyl.) O.Blanco et al.

Xanthoparmelia pulla (Ach.) O.Blanco et al.

Xanthoparmelia somloensis (Gyeln.) Hale

Xanthoparmelia stenophylla (Gyeln.) Ahti & D.Hawksw.

Xanthoparmelia tinctina (Maheu & A.Gillet) Hale

Xanthoparmelia verruculifera (Nyl.) O.Blanco et al.

Discussion

In this paper, we report 233 species of which 83 lichen forming and 6 lichenicolous fungi are new records to Armenia. Among them, 15 genera are also new to Armenia. With these new records, the total species number of lichens and lichenicolous fungi known from Armenia is 604, 592 lichenized and 12 lichenicolous fungi. In addition, 144 rare or poorly known species, which have been previously reported from only few localities and/or represents old records, are also listed here. In total, 53 lichen and lichenicolous fungi taxa (including 3 new records for Armenia) have been observed on the megalithic monument õZorats Karerö (locality 8). These results indicate that the species diversity of lichenized and lichenicolous fungi in Armenia remains poorly known and additional records can be expected from intense sampling efforts. Most of the new records belong to species that are known from adjacent areas of the eastern Mediterranean and western Asia. According to Feuerer (2015; http://www.lichens.uni-hamburg.de/lichens/portalpages/index_index.htm) about 800 species are estimated to occur in Armenia. A recent lichen checklist for neighbouring Iran (Seaward et al. 2008) lists 590 lichen species and estimates suggest that the number of species in this country is also closer to 1000 (Feuerer, loc. cit.).

New combination

Recent phylogenetic analyses demonstrated that the genus *Lecanora* as traditionally circum- scribed was polyphyletic (Zhao et al. 2016) and consequently, two genera were resurrected and one recircumscribed to accommodate species clustering within three monophyletic, well sup- ported clades: 1) *Myriolecis* to accommodate the *Lecanora dispersa* group and *Arctopeltis*; 2) *Protoparmeliopsis* for the *L. muralis* group; and 3) *Rhizoplaca* in an extended circumscrip- tion including a few placodioid species occurring in North America. Whereas numerous com- binations were published by Zhao et al. (2016), *Lecanora bolcana* was not transferred to *Protoparmeliopsis* due to an oversight. Hence, the necessary new combination is proposed here:

Protoparmeliopsis bolcana (Pollini) Lumbsch, **comb. nov.** [MycoBank 817743]

Lecidea bolcana Pollini, Giornale di Fisica, Chimica, Storia Naturale, Medicina ed Arti, Pavia (Milan) 9: 178 (1816) [basionym]. ó Ind. Loc.: [Italy: Verona:] õHabitat in Basalthen adque lapides calcareous in monte Bolcaö. *Lecanora bolcana* (Pollini) Poelt, Mitteilungen der Botanisches Staatssammlung in München 19 ó20: 505 (1958).

Acknowledgements

The authors are grateful to staff and volunteers of the Young Biologists Association NGO, especially Hripsime Atoyan, Vanuhi Hambardzumyan and Maria Antonosyan for field assistance during the excursion. The first author is especially thankful to Harrie Sipman for support in the identification of specimens, as well as acknowledges financial support from the DAAD (German Academic Exchange Service) and the project õDeveloping Tools for Conserving the Plant Diversity of the Transcaucasusö financed by the Volkswagen Foundation. AC, VJR, PKD and EA acknowl- edge financial support from the Spanish Ministerio de Economía y Competitividad projects CGL2011ó25003 and CGL2013 ó 42498 óP; we are also grateful to P. Cubas and B. Roca Valiente (Madrid) for helping with the molecu- lar work. ARB acknowledges financial support of the Ministry of Economy and Competitiveness, Spain, project CGL2013 ó 41839 óP. We are also thankful to Maaike Vervoort for permission to publish the photograph in Fig. 3.

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Kapitel 8:

Synthese

Die Erhaltung der biologischen Vielfalt gehört zu den großen Herausforderungen im Zeitalter des globalen Wandels. Für die Bewältigung dieser Aufgabe sind eingehende Kenntnisse über diese Vielfalt, ihre Existenzbedingungen, ihre geographische Verteilung und eventuelle Risiken für ihre Fortexistenz essentiell. Erste Grundbedingung ist hierbei die taxonomische Kenntnis von Lebewesen.

Flechten sind eine vielfältige Gruppe von Organismen, die in fast allen terrestrischen Umgebungen verbreitet sind, von den Tropen bis zu den Polarregionen (Nash 2008). In den letzten Jahren hat sich das Verständnis der Evolution von Flechten und ihrer phylogenetischen Affinitäten auf der Basis umfangreicher Arbeiten zur Flechtensystematik mittels molekularer Methoden wesentlich verbessert. Dadurch konnten Nachweise des Abstammungsstatus, unabhängig von phänotypischen und chemischen Merkmalen gezeigt werden.

Phylogenetische Studien der Ascomycota haben ergeben, dass es eine enorme Vielfalt in flechtenbildenden Ascomyceten gibt. In der Studie von Lücking et al. (2009) wurde geschätzt, dass es weltweit etwa 28.000 Arten von Flechten gibt. Daher spielt die Anwendung molekulare Methoden in der Flechtentaxonomie, besonders auch in der in dieser Arbeit untersuchte Familie Megasporaceae mit bisher ungenügendem taxonomischem Verständnis, in jüngerer Zeit eine wichtige Rolle.

Die Nutzung von strengen statistischen Tests, welche zum Beispiel auf der koaleszenz-basierten Methode und den "species treeõ-Ansätzen basieren, versprechen besonders bei taxonomisch eng verwandten Artengruppen sehr hilfreich zu sein. Zum Beispiel, wenn die Identifikation und Beschreibung der Arten, die in den Anfangsstadien der Divergenz sind, wegen den Diskordanzen zwischen Genbäumen und Speziesbäumen in verwandten Taxa und Genen schwierig wird. Unsere Arbeit zeigt ein Beispiel für die Nutzung dieser Methoden, die unser Verständnis in dieser allein mit Morphologie und Multilocus-Phylogenetik schwierig zu identifizierte Gruppe verbessern konnten.

Die Hauptergebnisse aus den molekularen Untersuchungen der Familie Megasporaceae in dieser Arbeit legen eine signifikant höhere taxonomische Vielfalt von *Aspiciliella*-Arten in den untersuchten Gebieten nahe als bisher bekannt.

Die Familie Megasporaceae ist in Eurasien weit verbreitet, unsere Probenahme waren aber auf Armenien, Iran, Griechenland und den östlichen Teil Europas konzentriert. Unsere Studie zeigt eine große Vielfalt bei Proben aus Armenien und West Iran in den beiden

bearbeiteten Genera *Aspiciliella* und *Megaspora*. Während in Armenien und Iran sechs Arten-Kandidaten für *Aspiciliella* und vier Arten in *Megaspora* (drei davon neu für die Wissenschaft) nachgewiesen konnten, wurden in allen anderen untersuchten Gebieten in Europa nicht so viele Gruppen und Arten gefunden; zwei Arten-Kandidaten für *Aspiciliella* und zwei Arten in *Megaspora*.

Aufgrund unserer Ergebnisse kommen wir zu dem Schluß, dass der Kaukasus ein artenreiches Gebiet und die wichtigste Region für die Artbildung für die zwei in dieser Studie bearbeiteten Genera ist. Daher ist die Beobachtung und der Schutz der kaukasischen Region, als Heimat vieler vielleicht endemischer Taxa dieser Familie, sehr wichtig.

8.1 Flechtensubstanzen:

Die Chemie innerhalb der Megasporaceae ist nicht sehr variabel und ein hoher Anteil der bekannten Arten der Familie ist frei von mit Aceton extrahierbaren Flechtenstoffen. Es konnten einige Substanzen mittels DC und HPLC-MS innerhalb dieser Arbeit angesprochen werden; Depsidone aus der Norstictinsäure-Gruppe: Stictinsäuren, Norstictinsäure, Connorstictinsäure und Salazinsäure. Lecanorsäure konnte erstmalig für die Familie Megasporaceae nachgewiesen werden, ferner wurden 2 unidentifizerte Substanzen mit unbekannter Struktur festgestellt. Die Ergebnisse hinsichtlich dieser unidentifizerten Substanzen, die zu noch nicht beschriebene Arten gehören, werden wir später publizieren.

8.2 Taxonomie und Systematik:

Insgesamt ergaben vorliegende Untersuchungen folgende taxonomische Befunde:

Wiedererrichtete Gattung:

Aspiciliella M.Choisy

Generischer Typus: *Aspiciliella intermutans* (Nyl.) M.Choisy in Werner (1932): Contribution á la flore cryptogamique du Maroc V. ó Cavanillesia 5(5): 1576174

Basionym: *Lecanora intermutans* Nyl., Flora (Regensburg) 55: 354 (1872).

Aspicilia intermutans (Nyl.) Arnold, Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien 37: 98 (1887).

Syntypen: Ad saxa arenaria prope St. Laon, leg. Richard; ad granitica prope Brestum, leg. Crouan.

Lectotypus: Sur grès à St.-Laon (Vienne), 2 October 1868, J. Richard (H-Nyl. 25439!).

Neukombination:

Aspiciliella cupreoglauca (B.de Lesd.) Zakeri, Divakar & Otte, **comb. nov.** [MycoBank MB821506]

Basionym: *Aspicilia cupreoglauca* B.de Lesd., Bull. Soc. bot. Fr. 57: 32 (1910).

Typus: Hérault, Prémian vers Langlade, 500 m. alt., sur des schistes, 1909, leg. Abbé Soulié (anscheinend in Dunkerque zerstört).

Neotypus (**designated here**): Greece, North Aegean Region, Lesbos: between Vatoussa and Antissa along provincial road, alt. 180 m. 26° 01.07' E; 39° 14.05' N. On volcanic rock cliff in river gorge; 7 Oct. 2015, H. Sipman & Th. Raus 62440 (neotype: B 60 0200002; isoneotype: GLM). GenBank acc. no. nrLSU: KY576956; mtSSU: KY576932; ITS: KY618845

= ***Lecanora reticulata*** (J.Steiner) J.Steiner, Verh. Zool.-Bot. Ges. Wien 69: 84 (1919) syn nov.

Lectotypus (**designated here**)**:** Hyeres, 1867, Metzler nr. 32B in litt. (lectotype: WU 887; Isotypes: H-Nyl 25458 in herbarium H, L4960 in herbarum S, M-0102336 in herbarium M).

Neue Arten:

Aspiciliella portosantana Sipman & Zakeri, **sp. nov.** [MycoBank MB821509]

Typus: Portugal, Madeira Islands, Porto Santo: SW Part, Pico de Ana Fereiera. Alt. ca 220m. co-ord.: 16°22,2'W, 33°02,7'N. On volcanic rock outcrops on exposed, bare ridge and on stone walls along abandoned fields; 4 March 2016, H. Sipman 63019. (holotype: B 600154592; isotype: GLM).

Megaspora cretacea Gasparayan, Zakeri & Aptroot, **sp. nov.** [MycoBank MB817072]

Typus: Armenia, Ararat, Vedi, Urtsadzor, Khosrov Forest State Reserve, 40°00'42"N, 44°54'04"E, 1600 m, on *Juniperus polycarpos* bark, 17 Jun 2015, *A. Aptroot 73835* (B 600200932; isotypes: ABL, GLM).

Neue Nachweise für Armenien:

Aspicilia cf. *glomerulans* (Poelt) Poelt

Aspicilia verrucigera Hue

Lobothallia chadefaudiana (Cl. Roux) A.Nordin et al.

Lobothallia cheresina (Müll.Arg.) A.Nordin et al.

Lobothallia farinosa (Flörke) A.Nordin et al.

Lobothallia recedens (Taylor) A. Nordin, S. Savi & Tibell

Aspiciliellia intermutans (Nyl.) Arnold

Aspiciliella cupreoglauca B.de Lesd.

8.3 Phylogenetische und morphologische Studie:

In einer Drei-Gen-Analyse (nrLSU, mtSSU, RPB1) von Lumbsch et al. (2007) bildeten die Megasporaceae, umfassend *Megaspora*, *Lobothallia* und *Aspicilia*, eine monophyletische Gruppe mit einem hohen Unterstützungswert. Basierend auf einer umfangreichen Stichprobe und Analyse von zwei Loci (nrLSU und mtSSU) wurde die Monophylie von Megasporaceae auch von Nordin et al. (2010) mit fünf Gattungen *Aspicilia*, *Lobothallia*, *Megaspora*, *Circinaria* und *Sagedia* angenommen.

Die in vorliegender Arbeit präsentierte molekulare Untersuchung auf der Grundlage dreier genetischer Marker: ITS, nuLSU und mtSSU zeigt die Gattung *Aspiciliella* als einen distinkten *Clade* innerhalb der Megasporaceae.

Die Ergebnisse unserer Studie zeigen die Position der Gattung *Aspiciliella* und die Monophilie der Familie mit 6 Gattungen: den vorher anerkannten fünf Genera *Aspicilia*, *Megaspora*, *Lobothallia*, *Circinaria* und *Sagedia* und die restitutierte Gattung *Aspiciliella* M. Choisy.

Unsere phylogenetische Studie bestätigte, dass *A. intermutans* und *A. cupreoglauca*, die zuvor auf der Grundlage ihres allgemeinen Erscheinungsbildes zu *Aspicilia* gestellt wurden, eine eigene Abstammungslinie in den Megasporaceae darstellen und zusammen mit einer neuen Art, die in dieser Arbeit beschrieben wurde, einen *Clade* bilden, den wir als *Aspiciliella* bezeichnen.

Die Gattung *Aspiciliella* wurde durch Choisy in Werner (1932) nur für den Art *Aspiciliella intermutans* ganz kurz ohne weitere Angaben aufgestellt nur unter Verweis auf ovale Sporen und gelifizierte Paraphysen.

Arten von *Aspiciliella* sind nach unserer Beurteilung krustos, rimose-areolate, teilweise kontinuierlich; der Photobiont ist chlorococcoid. Die Apothecien sind blassbraun bis dunkelgrau bis schwarz, richtiges Eigenrand immer vorhanden, Apothecien selten von einem zusätzlichen Thallusrand umgeben. Das Epihymenium ist grün bis olivgrün bis grünlich-braun, N + wechselt es zu hellgrün. Das Excipulum ist hyalin, Hypothecium und Subhymenium sind farblos, I + blau bis rostrot. Das Hymenium ist farblos, I + blau bis rostrot. Die Asci sind 8-sporig, vom *Aspicilia*-Typ. Die Ascosporen sind ellipsoid, farblos, einfach, die Conidien gerade, 7611 m lang.

Der Thallus von *Aspiciliella*-Arten wird durch KOH rot gefärbt; die C- und UV-Reaktionen sind negativ. Alle Arten in dieser Gattung haben Norstictinsäure und manchmal Connorstictin- und Stictinsäure als sekundäre Inhaltstoffe.

Die Vertreter der Gattung *Aspicilliella* sind morphologisch ähnlich zu *Aspicilia*, unterscheiden sich aber in der Größe der Konidien und der Form und Größe der Ascosporen. Die bei *Aspiciliella* konstant vorhandene Norstictinsäure ist in der Gattung *Aspicilia* nur bei einigen Arten von *Aspicilia* bekannt. Deshalb können chemische Merkmale neben der Größe der Konidien nützlich sein, um beide Gruppen zu unterscheiden.

Die neue Art innerhalb *Aspicilliella*, *A. portosantana*, ist charakterisiert durch einen grauen bis grünlichgrauen, dünn (0,2- 0,3 mm dick) Thallus; aspicilioide, normalerweise zahlreiche Apothecien,; 8Ascosporen/Ascus, hyalin, ellipsoid, 22-28 × 17-23 m, Konidien klein, gerade, 6-10 m. Sie unterscheidet sich von der sehr ähnlichen *Apiciliella intermutans* durch den dünneren, hellgrauen Thallus ohne oder mit schwach differenzierten hellen Rändern der Thallusareolen und am deutlichsten durch die ITS und / oder mtSSU Sequenzen.

In der Studie zur Auflösung des *Aspiciliella intermutans*-Komplexes mit einer Kombination aus phylogenetischen Analysen und einer Vielzahl von empirischen, sequenzbasierten Methoden zur Abgrenzung von Arten und Untersuchung der morphologischen, chemischen, ökologischen und geographischen Daten konnten insgesamt sechs Abstammungslinien auf Artenebene identifiziert werden. Im Allgemeinen

fanden wir wenig Kongruenzen zwischen morphologischen, chemischen und ökologischen Charakteren unserer Kandidatenspezies. Das Vorhandensein von kryptischen Spezies in flechten-bildenden Pilzen ohne erkennbare phänotypische Charakterisierung der *Clades* wurde bereits in einigen Studien nachgewiesen (Lumbsch & Leavitt 2011).

In Kapitel 4 wurde die phylogenetische Position des Genus *Megaspora* und der dazu gehörigen Arten unter Verwendung von nrITS-Sequenzdaten dargestellt. Es wurde die neue Art *M. cretacea* vorgestellt. Phylogenetische Analysen zeigten, dass *M. cretacea* innerhalb der *Megaspora-Clade* als Schwesterspezies zu *M. rimisorediata* mit hoher Unterstützung gruppiert ist.

M. cretacea ist charakterisiert durch einen weißlich-grauen, kreidigen Thallus voller Soredien (c. 0,1 mm im Durchmesser); eingesenkte Apothecien; 4 Ascosporen pro Ascus, die breitellipsoid, 27 - 31 × 18 - 21 m, hyalin und dünnwandig sind. Die Gattung *Megaspora* wird jetzt in vier Arten unterteilt.

8.4 Nomenklatur:

In Kapitel 4 wurde die Identifizierung der korrekten Anwendung der *A. reticulata* und *A. cupreoglauca* vorgenommen. Es wurde eine Neotypifikation für *A. cupreoglauca* und eine Lectotypifikation für *A. reticulata* durchgeführt. Außerdem wurde die Konspezifität von *A. reticulata* mit *A. cupreoglauca* festgestellt und Synonymisierung vorgenommen; der Name *A. cupreoglauca* wird für dieses Taxon akzeptiert.

Ausblick

Aufgrund dieser Studie mit morphologischen, chemischen und molekularen Methoden wird deutlich, dass diese Familie noch weitere einer weiteren Bearbeitung im Bereich von Chemie und Molekulargentik braucht und voraussichtlich Arten neu zu beschreiben sind. Schon durch eine vergleichsweise kleine Stichprobe aus armenischem und iranischem Material konnte festgestellt werden, dass es viele neue, nicht bearbeitete und nicht dokumentierte Arten in diesen Gebieten gibt.

Ich gehe davon aus, dass nicht nur das vorhandene Material dieser Familie in Herbarien, sondern auch Material in bisher nicht untersuchten Gebieten neue Informationen bringen werden und das Verständnis dieser Familie verbessern können. Besonders Arbeiten hinsichtlich der Art-Begrenzung innerhalb einiger morphologisch plastischer Gruppen werden zweifellos neue Erkenntnisse bringen.

Aufgrund des verbreiteten Vorkommens und der molekularen Variabilität im *Aspiciliella intermutans*-Komplex in verschiedenen geographischen Regionen im weiteren Mediterranraum, insbesondere in Iran und Armenien, ist es ist notwendig, mehr Material davon aus anderen, noch nicht bearbeiteten Gebieten dieser Region, z. B. Anatolien und Westeuopa zu untersuchen, um eine bessere systematische Vorstellung von diesem Komplex zu erhalten. Außerdem könnte die Nutzung weiterer Gen-Loci weiterhelfen, einen besser aufgelösten Baum in diesem Komplex zu erzeugen. Ich wurde auch die Nutzung einer fortgeschritten Methode in der Morphologie; nämlich der Geometrische Morphometrie für weitere Studien in diese Familie vorschlagen. Bei dieser Methode wird eine Vielzahl von Messpunkten am zu untersuchenden Objekt platziert, um die Form des Objekts in seiner Gesamtheit zu erfassen. Diese Methode kann für Struktur mit festen Formen, z. B. Sporen, Konidien oder Asci benutzt werden und die möglichen subtilen morphologischen Unterschiede zwischen den Gruppen zeigen.

Erklärung über den persöhnlichen Anteil an den Publikationen

Chapter 2:

Zakeri, Z., Divakar, P.K. & Otte, V. 2017. Taxonomy and phylogeny of *Aspiciliella* a resurrected genus of Megasporaceae, including a new species *A. portosantana.* ó Herzogia 30: 166-176

Material-Collection	Zakieh Zakeri (100%) + Herbal Material
Lab-work	Zakieh Zakeri (100%)
Analysis	Zakieh Zakeri (90%); P. K. Divakar (advisory 10%)
Writing	Zakieh Zakeri (90%); other coauthors (10%)

Chapter 3:

Zakeri, Z., Otte, V., Sipman, H., Malí ek, J., Paloma, C. D., Victor, R. & Divakar, P.K. (In prepration to submit). Discovering cryptic species in the *Aspiciliella intermutans* complex (*Megasporaceae*, *Ascomycota*), first results using gene concatenation and coalescent-based species tree approaches

Material-Collection	Zakieh Zakeri (100%)+ Herbal Material
Lab-work	Zakieh Zakeri (70%); Ji í Malí ek (30%)
Analysis	Zakieh Zakeri (80%); P. K. Divakar (20%)
Writing	Zakieh Zakeri (75%); P. K. Divakar (20%); other coauthors (5%)

Chapter 4:

Zakeri, Z., Sipman' H., Paukov, A., Otte, V. (accepted in Lichenologist). Neotypification of *Aspiciliella cupreoglauca* and lectotypification and synonymization of *Aspicilia reticulata* (Megasporaceae, Ascomycota).

Data-Collection	Zakieh Zakeri (80%); other coauthors (20%)
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Analysis	-----
Writing	Zakieh Zakeri (60%); Volker Otte (30%); other coauthors (10%)

Chapter 5:

Zakeri Z., Gasparyan A. & Aptroot A. 2016: A new corticolous *Megaspora* (*Megasporaceae*) species from Armenia. ó Willdenowia 46: 245ó251

Material-Collection	other coauthors (100%)
Lab-work	Zakieh Zakeri (100%)
Analysis	Zakieh Zakeri (100%)
Writing	Zakieh Zakeri (60%); other coauthors (40%)

Chapter 6:

Gasparyan A., Aptroot A., Burgaz A. R. , Otte V. , Zakeri Z. , Rico V. J. , Araujo E. , Crespo A. , Divakar P. K. & Lumbsch H. T. 2015: First inventory of lichens and lichenicolous fungi in the Khosrov Forest State Reserve, Armenia. ó Fl. Medit. 25: 105ó 114.

Material-Collection	All coauthors
Lab-work	----
Analysis	----
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Chapter 7:

Gasparyan, A., Aptroot, A., Burgaz, A. R., Otte, V., Zakeri, Z., Rico, V. J., Araujo, E., Crespo, A., Divakar, P. K. & Lumbsch, H. T. 2016. Additions to the lichenized and lichenicolous mycobiota of Armenia. ó Herzogia 29: 692ó705.

Material-Collection	All coauthors
Lab-work	----
Analysis	----
Writing	Zakieh Zakeri (5%); other coauthors (95%)

Halle (Saale), den
Unterschrift

Additional publications during PhD study period:

Zakeri, Z., Elix, J.A. & Otte· V. 2017. Degradation of alectoralic acid in the lichen genus *Usnea.* Lichenologist 49 (5).

Lebenslauf

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Praktische Erfahrungen:

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Publikation:

2018	Zakeri, Z., Sipman' H., Paukov, A., Otte, V. (accepted in lichenologist). Neotypification of *Aspiciliella cupreoglauca* and lectotypification and synonymization of *Aspicilia reticulata* (Megasporaceae, Ascomycota).
2017	Zakeri, Z., Elix, J.A. & Otte, V. (2017). Degradation of alectoralic acid in the lichen genus *Usnea.* Lichenologist 49(5).
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2016	Zakeri Z., Gasparyan A. & Aptroot A. (2016). A new corticolous *Megaspora* (*Megasporaceae*) species from Armenia. - Willdenowia 46: 245ó251.
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Kongress, Seminar und Excurtion Teilnahmen:

20-22.04.2018	õ*Study on the Aspiciliella intermutans complex (Megasporaceae, Ascomycota) to identify cryptic species using gene concatenation and coalescent-based species tree approachesö* (Poster), BLAM symposium- Frankfurt, Germany
6-10.03.2018	õ*Hidden genetic diversity in the lichen forming fungal Aspicilliella intermutants-complex (Megasporaceae, Ascomycota)ö* (Poster), The 5th YNHM 2018- Paris, France
17-19.11.2017	59. Phylogenetisches Symposium 2017ó "Phylogeny in the post-genomic era", Berlin, Germany
19-21.05.2017	Senckenberg Young Scientists Retreat 2017, Frankfurt, Germany

18-20.11. 2016 — õ*Wide-range study on some strictly saxicolus lichens (Megasporaceace) suggests a new genus with a couple of speciesö*(Poster), 58. Phylogenetisches Symposium 2016 ó šEvolution meets Ecologyõ,Leipzig, Germany

01-05.08. 2016 — õ*A new corticolous species of the genus Megaspora from Armenia*ö (Poster), The 8th IAL Symposium- Helsinki, Finland

14-25.06. 2015 — International lichenological excursion to Armenia

25-27.04.2014 — õ*Chemotaxonomic study on the lichen genus Usnea (lichenized Ascomycetes, Parmeliaceae) in the Caucasusö* (Poster), Senckenberg Young Scientists Retreat 2014- Görlitz, Germany

10-13.05.2006 — Ferdowsi University of Mashhad 2006, (Poster in Persisch)- Mashhad, Iran

Danksagung

An dieser Stelle möchte ich mich herzlich bei allen bedanken, die mich bei der Anfertigung dieser Arbeit unterstützt haben.

Dr. Volker Otte danke ich für seine Betreuung und die Begutachtung meiner Doktorarbeit und dafür, dass er während meines Studiums eine großartige Anleitung, Möglichkeiten, Geduld und unzählige bunte Kommentare lieferte und mir so viel Lehre für Leben und Studium gegeben hat.

Prof. Dr. Karsten Wesche danke ich für die Übernahme der Begutachtung dieser Arbeit und seine Beratung und für Kommentare zu meinen Manuskripten.

Prof. Dr. habil. Martin Röser und Dr. Andre Aptroot danke ich für die Übernahme der Begutachtung dieser Arbeit.

Bedanken möchte ich mich auch bei Herrn Dr. René Ullrich vom IHI Zittau, Dr. Harrie J.M. Sipman vom BGBM, Dr. Pradeep K. Divakar von CUM, Prof. Dr. Jack Elix (Australian National University, Canberra) und Dr. Andre Aptroot (Adviesbureau voor Bryologie en Lichenologie, Soest, Niederlande) für ihre Hilfsbreitschaft während meiner Arbeit, die freundliche Unterstützung und Mitarbeit und für die Erweiterung meiner Fachkenntnisse.

Ich danke allen Labormitarbeitern des IHI Zittau und des Senckenberg Museums für Naturkunde Görlitz und auch allen meinen Kollegen in der Abteilung Botanik am Senckenberg Museum, besonders Dr. Christiane Ritz und Dr. Ulrike Damm, für ihre Sständige Hilfsbreitschaft bei den Problemen während der Arbeit.

Ich danke auch meinen lieben iranischen Freunden für ihre liebvolle Freundschaft und verschiedene schöne Texte, Gespräche und Gefühle, die in mein tägliches Leben reinbringen, meiner lieben Ergül für ihre mütterlichen Gefühle und meiner Agata für ihre schwesterlichen Gefühle und auch das Beisammensein während meines einsamen Lebens in einem fremden Land, dass mir das Gefühl gegeben hat, dass ich bei meiner Familie bin, meinen lieben Senckenberg-Freundinnen Yun und Oyuka, die in den letzten 2 Jahren immer in jeder Zeit für mich da waren und während der Abwesenheit meines Ali mir das Leben hier einfacher und bunter gemacht haben.

Ein ganz besonderes Dankeschön gilt meinem lieben Ali, mit dessen fachkundiger, mentaler und physischer Unterstützung so wertvolle und effektive Arbeit und schöne Momente während gemeinsamer Geländearbeit möglich geworden sind und dafür, dass er mich während meines gesamten Studiums so intensiv in stressigen Stunden ermutigt hat und für so viel Liebe, Lehre, Selbstbewusstsein und Kraft, die er mir im Leben gibt, dass ich mich so glücklich fühle und auch für sein vollkommenes Vertrauen, das mich stärker und unabhängiger machte als zuvor.

Zu guter Letzt habe ich am meisten und für immer meinen Eltern zu danken, für ihre bedingungslose Liebe und Sorge, die mich immer wie ein Kind fühlen lassen, für ihre endlose Unterstützung während meines Studium und auch für Beisammensein während einiger Geländearbeiten, dass ich mich zuhause und so glücklich bei ihnen gefühlt habe.

Eigenständigkeitserklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel **õTaxonomy and phylogeny of Megasporaceae (lichenized ascomycetes) in arid areas of Eurasiaš** bisher weder der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den
Unterschrift:
Zakieh Zakeri