Resolving genetic relationships in manna group of lichens from genus Aspicilia

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As in many lichen-forming fungi, species of Aspicilia genus are widely distributed all over the world, but no reports exist about their phylogenetic relations based on molecular biological methods. In the current study the phylogenetic relations of some Aspicilia species mainly manna group of lichens were investigated. The ITS rDNA sequence information of 12 samples from six species were generated. The samples examined were collected from different provinces of Anatolia and all the sequences were aligned with the other allied groups; Pertusaria sp., Thamnolia sp., Dibaeis sp., Diploschistes sp., Ochrolechia sp. and Lecanora sp. sequence data obtained from GenBank. The phylogenetic tree obtained by minimum evolution analysis displayed two major branches. One of the branches with its six members (Aspicilia contorta subsp. contorta, A. contorta subsp. hoffmanniana, Aspicilia hispida, Aspicilia fruticulosa, Aspicilia desertorum Aspicilia calcarea) composed solely of Aspicilia samples from Anatolia. Three of the manna groups of lichens were placed in this branch of the tree. The other related taxa from Ostropomycetidae and Lecanoromycetidae took place in the other branch of the tree with Aspicilia samples from Anatolia. Results displayed that manna group of lichens, although do not represent taxonomical unit tend to form a group especially apperent by the Neigbour Joining analysis with Anatolian samples. Also the sequence information from Anatolian samples displayed that Aspicilia genus is phylogenetically closer to the orders and families from Ostropomycetidae subclass rather than Lecanoromycetidae which support the placement of Hymeneliaceae within Ostropomyctidae.

Key words: Aspicilia, phylogenetic relations, ITS, Anatolia

INTRODUCTION

The genus Aspicilia belongs to Hymeneliaceae within the subclass Ostropomycetidae with photobiont chlorococoid which have one shape. Most of the species of this genus grow on calcareous and acidic rocks and most of the taxa prefer temperate and artic sites. The members are weakly cracked to distinct areolate, scattered to whole thallus. Some of the species of this genus are placodoid with radiating marginal lobes which appear chalky white, grayish, greenish or brownish. Some posses isidia and soredia. They have characteristic ascomata apothecia aspicilioid which is mostly immersed but occasionally emergent. Their epithecium pigment is from brown to green with N and K chemical tests. They have 4 to 8 spored asci with cylindrical to clavate shape. Their ascospores are simple which have ellipsoid to globose shape and colorless and also contain thin-wall. They often contain -orcinol depsidones such as norstictic and stictic acids; others have fatty acids or triterpenes. In genus Aspicilia radical changes in growth form are very common, such as some taxa may display extreme transition within the same population or even changes within the same thallus could be observed (Elenkin, 1901; Weber, 1967). For example, for Aspicilia desertorum which is a manna group of lichen, transformation to crustose form of lichen could arise under micro-environme-
ntal conditions of temperature and small melt water (Kunkel, 1980), although the significance of this phenomenon in general taxonomy of *Aspicilia* is not clear.

Manna group of lichens are unique in *Aspicilia* genus with their many specific characteristics. The use of Manna lichens as food is probably very old. The practice has survived into modern times chiefly in impoverished environments, such as deserts, steppes, and tundra, means beyond the limits of cultivation; elsewhere, lichens are occasionally reported as a famine food. *Lecanora esculenta* has been identified with the ‘manna’ of the Hebrews, although the lichen has not been observed in Sinai, either in situ or in the form of windborne deposits. However this in itself is not decisive for explicit reports of such deposits elsewhere in western Asia do not go back beyond the 15th century (Donkin, 1981). One of the manna group lichens, generally known as *L. esculenta* (Elenkin, 1901) has been placed within *Aspicilia*, and now known as *Aspicilia esculenta*. Mereschkovsky (1911) was the first to try an account of the *A. esculenta* group and to distinguish further taxa among the vagrant. In addition to some taxa known for a long time, namely *A. esculenta* (Pall.) Flagey, *Aspicilia fruticulosa* (Eversm.) Flagey, *A. desertorum* (Kremp.) Mereschk, Hafellner et al. (2004) described two further species (*Aspicilia hispida* Mereschk, and *Aspicilia lacunose* Mereschk) and some infraspecific taxa at the rank of forma or variety, one of which was later raised to species level (*A. desertorum* var. *aspera* Mereschk.) (Hafellner et al., 2004).

Genetic concept within Lecanoraceae has been slightly changed although the knowledge is still based on the pioneer work of Eigler (1969). Later some of the taxa were excised by Hafelner (1984) and *Aspicilia* is now defined as a separate genus, in the family Hymenochaetae (Hafellner, 1994; Poelt, 1974) which takes place within subclass Ostropomycetidae (Wedin et al., 2005).

The current research investigates the phylogenetic relationships of the manna group of lichens from *Aspicilia* grown in Anatolia and related groups. The purpose of the present investigation is to clarify the phylogenetic relationships of some *Aspicilia* species especially manna group of lichens grown in Anatolia and to provide a clear phylogenetic framework as a basis for detailed phylogenetic and population genetic investigations. The manna lichens; *A. fruticulosa*, *A. hispida*, *A. desertorum* and other *Aspicilia* species (*A. calcarea* and *A. contorta*) collected from various parts of Anatolia were used in the study. The phylogenetic relationships were analyzed based on ITS sequence data. The sequence data aligned with 5 *Lecanora* sp., one *Pertusaria* sp., one *Thamnolia* sp., one *Dibaeis* sp., three *Diploschistes* sp. and two *Ochrolechia* species obtained from GenBank were shown in Table 1. The samples were dried at room temperature and foreign matters were removed prior to grinding. The lichen samples are stored in the Herbarium of Anadolu University (Anadolu University, Department of Botany, Eskisehir, Turkey). Some of the lichen materials were provided from previously collected and stored material of Anadolu University Herbarium. The collection localities were as shown in Table 1.

### DNA extraction

DNA extraction was performed according to the protocol defined by Cansaran et al. (2006). In particular: lichen herbarium material (0.1 g) was ground to a fine powder in liquid nitrogen. Pre-warmed extraction buffer [50 mM Tris-HCl (pH 8), 50 mM EDTA, 0.8 M LiCl, 1% CTAB, 2% PVPP (addition of PVPP is optional)] in the amount of 1 ml was added to the samples and ground once more in the buffer. After the samples were taken to the 1.5 mL Eppendorf tubes, 0.2% mercaptoethanol was added. The solution was incubated in 65°C water bath for 15 min. Following these incubation periods, samples were cooled to room temperature, 0.5 ml chloroform/isoamyl alcohol (24:1:v/v) was added and mixed well (no vortex). Then, samples were centrifuged at 17,000 g (14,000 rpm) for 2 min, and the supernatant was transferred to a fresh tube (~0.8 ml). Equal volume of isopropanol was added to the supernatant and mixed gently by inversion several times. Incubation of the samples for at least 15 min on ice increased the efficiency of DNA yield. The samples were then centrifuged for 2 min at 17,000 g (14,000 rpm). Supernatant was discarded and 1 ml % 70 ethanol was added. The samples were then centrifuged for 1 min at 17,000 g (14,000 rpm). The pellet was once more washed with 70% ethanol optionally and air-dried until all ethanol was removed. The obtained nucleic acids as a pellet were dissolved in an appropriate amount of TE buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA) (30 - 60 µL). The nucleic acids dissolved in TE buffer, were treated with 1 µl of ribonuclease A (10 mg/mL) and stored at -20°C until use.

DNA was quantified via spectrophotometric measurement of UV absorption at 260 nm (Spectrocid UV-200). DNA was also quantified by means of agarose gel electrophoresis with ethidium bromide fluorescence and a 100 bp DNA ladder was used (Promega) as the DNA size marker.

### PCR amplification and sequencing

Internally transcribed spacer region of rDNA gene cluster was amplified with the primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). ITS1F primer was designed specifically for fungal sequences at the 3' end of small subunit gene of the rDNA, overlapping with ITS5, whereas ITS4 was described as a universal primer corresponding to the 5' end of the large subunit gene (Gardes and Bruns, 1993).

Different parameters were tested for optimization of PCR reactions. PCR amplification for sequence analysis were performed in a 50 µl volume containing 200 ng of genomic DNA, 5 µL of 10 x reaction buffer, 2.5 mM MgCl2, 20 µM dNTPs, 0.2 µM of each of the primers, and 1 U Taq DNA polymerase (Promega). Amplification was performed in a Techne Progene thermal cycler (Techne Cambridge Limited). The reactions were heated in an initial step of 94°C for 2 min and then subjected to 35 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 1 min 45 s. After the last cycle, the temperature was maintained at 72°C for 8 min for final extension step. The amplification products were analysed by electrophoresis in 1.2% agarose gel containing ethidium bromide and the product sizes were determined on agarose gels along with a known nucleotide size marker (100 bp ladder, Promega). The PCR products were sequenced by the cycle sequencing method using dye terminator cycle sequencing kit (Amersham Pharmacia, Amersham Pharmacia).
Table 1. Collection area of *Aspicilia* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection area</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspicilia contorta</em> subp hoffmanniana</td>
<td>Malatya, Hekimhan, Midemâ açı (Mollaibrahim) village, northern slopes, 23.07.2004, 1205 m, 38°43’20.5 N-38°04’22.4 E (leg. M. Candan) ANES 10200</td>
</tr>
<tr>
<td><em>Aspicilia contorta</em> subp hoffmanniana</td>
<td>Elazî, Harput, North of Serince village, calcareous rocks, 04.08.2004, 1580 m, 38°43’56 N-39°17’18 E (leg. M. Candan) ANES 10201</td>
</tr>
<tr>
<td><em>Aspicilia contorta</em> subp hoffmanniana</td>
<td>Adiyaman, Varlik village, South of Yo urtul Mezra, 30.07.2004, 1160 m, 37°54’05 N-38°17’47 E (leg. M. Candan) ANES 10202</td>
</tr>
<tr>
<td><em>Aspicilia contorta</em> subp contorta</td>
<td>Kütahya, North of Dumlupınar, 03.07.2001 (leg. et det. V. John) ANES 7391</td>
</tr>
<tr>
<td><em>Aspicilia calcarea</em></td>
<td>Adiyaman, Kahta, Southeast of Karaku Tümüli, Calcereous rocks, 28.07.2004, 830m, 37°52’34.6 N-38°35’35 E (leg. M. Candan) ANES 10203</td>
</tr>
<tr>
<td><em>Aspicilia calcarea</em></td>
<td>Adiyaman, Besni, Southwest of Çilbo azı village, 27.07.2004, 900 m, 37°44’08 N-37°48’06.3 E (leg. M. Candan) ANES 10204</td>
</tr>
<tr>
<td><em>Aspicilia calcarea</em></td>
<td>Sivas, Kangal, Hekimhan-Sivas 46. km, 23.07.2004, 1530 m, 39°04’01.2 N-37°41’43.4 E (leg. M. Candan) ANES 10205</td>
</tr>
<tr>
<td><em>Aspicilia calcarea</em></td>
<td>Malatya, Do an ehir, West of Kurucuova village, 30.07.2004, 1410 m, 37°59’26 N-38°03’42.4 E (leg. M. Candan) ANES 10206</td>
</tr>
<tr>
<td><em>Aspicilia desertorum</em></td>
<td>Elazî, Harput, North of Serince village, 04.08.2004, 1580 m, 38°43’56 N-39°17’18 E (leg. M. Candan) ANES 10207</td>
</tr>
<tr>
<td><em>Aspicilia desertorum</em></td>
<td>Elazî, Sivrice- Hazar Mountain, 05.08.2004, 1225 m, 38°27’36 N-39°23’10 E, (leg. M. Candan)</td>
</tr>
<tr>
<td><em>Aspicilia hispida</em></td>
<td>Malatya, Darende, Southeast of Zengibar Castle, 24.07.2004, 1105 m, 38°03’59.9 N-37°30’07 E (leg. M. Candan) ANES 10209</td>
</tr>
<tr>
<td><em>Aspicilia fruticulosa</em></td>
<td>Sivas, Kangal, Hekimhan-Sivas 46. km, 23.07.2004, 1530 m, 39°04’01.2 N-37°41’43.4 E (leg. M. Candan) ANES 10210</td>
</tr>
</tbody>
</table>

USA) according to the manufacturer’s protocol and OpenGeneÒ automated DNA sequencing system (Visible Genetics, Canada).

Sequence analysis

The amplified fragments with the primers ITSF1 and ITS4 comprising 3’ end of small subunit gene, ITS 1, the 5.8 S gene, ITS 2 and the 5’ terminus of the large subunit gene, were sequenced. Ambiguities arising from reading errors were resolved by comparing the complementary strands with the programme Clustal W 1.83. The sequences were also aligned using the same programme and adjusted visually. Ambiguous aligned sites were excluded from analysis and gaps were treated as missing data. ITS sequences for 12 other different samples from *Aspicilia* genus and for 5 different species of *Lecanora*, one *Pertusaria*, one *Thamnolia*, one *Dibaeis*, three *Diploschistes* and two *Ochrolechia* species obtained from GenBank (Table 2) were included in to multiple alignments (Figure 1). A sequence matrix of 866 nucleotide positions were analysed and (conserved, 52; variable, 671) 539 parsimony informative sites were detected. All data were analysed by MEGA 3 (Kumar et al., 2004) and a bootstrapped dendogram was generated.

RESULTS

A total of six species of Manna group of lichens and related species belonging to *Aspicilia* genus were inclu-ded to the analysis. Nucleotide sequences generated from *Aspicilia* samples were deposited to GenBank (Table 2). The sequence data yielded from ITS region of the species were aligned with sequences of related taxa obtained from GenBank. The target sequences generated by PCR amplification were sequenced in both directions. The sequencing reactions for species of *Aspicilia* yielded align-nable sequences of 866 nucleotides long. Within these
Table 2. The aligned species, localities and their GenBank accession numbers.

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank accession No.</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspicilia calcarea</td>
<td>DQ401563</td>
<td>Turkey-Malatya</td>
</tr>
<tr>
<td>Aspicilia calcarea</td>
<td>DQ401562</td>
<td>Turkey-Sivas</td>
</tr>
<tr>
<td>Aspicilia calcarea</td>
<td>DQ401561</td>
<td>Turkey-Adıyaman2</td>
</tr>
<tr>
<td>Aspicilia calcarea</td>
<td>DQ401560</td>
<td>Turkey-Adıyaman1</td>
</tr>
<tr>
<td>Aspicilia contorta subp contorta</td>
<td>DQ401559</td>
<td>Turkey-Afyon</td>
</tr>
<tr>
<td>Aspicilia contorta subp hoffmanniana</td>
<td>DQ401558</td>
<td>Turkey-Adıyaman</td>
</tr>
<tr>
<td>Aspicilia contorta subp hoffmanniana</td>
<td>DQ401557</td>
<td>Turkey-Elazig</td>
</tr>
<tr>
<td>Aspicilia contorta subp hoffmanniana</td>
<td>DQ401556</td>
<td>Turkey-Malatya</td>
</tr>
<tr>
<td>Aspicilia desertorum</td>
<td>DQ401568</td>
<td>Turkey-Elazig</td>
</tr>
<tr>
<td>Aspicilia desertorum</td>
<td>DQ401567</td>
<td>Turkey-Elazig1</td>
</tr>
<tr>
<td>Aspicilia fruticulosus</td>
<td>DQ401570</td>
<td>Turkey-Sivas</td>
</tr>
<tr>
<td>Aspicilia hispida</td>
<td>DQ401571</td>
<td>Turkey-Malatya</td>
</tr>
<tr>
<td>Dibaeis baemoyces</td>
<td>DQ782844</td>
<td>USA</td>
</tr>
<tr>
<td>Diploschistes hensseniae</td>
<td>AJ458291</td>
<td>Australia</td>
</tr>
<tr>
<td>Diploschistes thunbergianus</td>
<td>AJ458290</td>
<td>Australia</td>
</tr>
<tr>
<td>Diploschistes scapusus</td>
<td>AJ458287</td>
<td>Germany</td>
</tr>
<tr>
<td>Lecanora achariana A.L. Sm.</td>
<td>AF070019</td>
<td>Sweden</td>
</tr>
<tr>
<td>Lecanora garovaglia (Kölber) Zahlbr</td>
<td>AF189718</td>
<td>Austria</td>
</tr>
<tr>
<td>Lecanora novomexicana H. Magn., U 363</td>
<td>AF159945</td>
<td>Arizona</td>
</tr>
<tr>
<td>Lecanora pruinosa Chaub.</td>
<td>AF070018</td>
<td>Italy</td>
</tr>
<tr>
<td>Lecanora reuteri Schaer</td>
<td>AF070026</td>
<td>Austria</td>
</tr>
<tr>
<td>Ochrolechia parella</td>
<td>AF329174</td>
<td>Germany</td>
</tr>
<tr>
<td>Ochrolechia juvenalis</td>
<td>AF640957</td>
<td>USA</td>
</tr>
<tr>
<td>Perusaria erubescens</td>
<td>DQ219303</td>
<td>Antarctica</td>
</tr>
<tr>
<td>Thamnolia subuliformis</td>
<td>AY961605</td>
<td>New Zealand</td>
</tr>
</tbody>
</table>

These sites 52 were conservative and 671 were variable whereas 539 sites were parsimony informative. When transitional changes were compared transversional ones, bias towards transversional changes was observed, with the transition pair value of 80 versus transversional value of 122. When the base compositions were analysed the range values of 16.9 – 33.0; 20.4 – 33.1; 19.0 – 27.1 and 19.4 – 28.3 were observed for bases T(U), C, A, G respectively.

Nine most parsimonious trees were generated by aligning the sequence data also with allied groups. One of these trees generated by minimum evolution is shown in Figure 1. The trees yielded similar topology showing only slight rearrangements within the groups. Analysis with maximum parsimony and Neighbor joining revealed trees with similar topology with slight differences among the groups and within groups.

DISCUSSION

For the past two centuries the classification of lichenized genera, was guided primarily by morphological and chemical characters. As lichen thalli manifest a number of easily observable morphological characters, this kind of classification was relatively easy and straightforward. Lichens with a more complicated organization such as foliose or fruticose growth usually show more thallus characters. Although those anachronistic approaches are still largely sufficient and provide well-definition of foliose and fruticose genera, they remain relatively insufficient for some large crustose genera. In spite of their heterogeneity, due to low number of synapomorphic characters, it is not always possible to separate them from the large complexes (Grube et al., 2004).

Aspicilia is one of the examples of large and crustose genus from Hymeneliaceae family, which is the member of the subclass Ostropomycetidae. The classification of Hymenoloiid lichens has been an argument among lichenologists throughout the history. Körber (1855) originally circumscribed the Hymeneliaceae on the basis of 'pseudogymnocarpic' apothecial development and a double excipulum, forming a link between Lecanora and Lecidea Arch., and including Hymenelia, Petractis Fr. and Thelotrema Arch. In 1984 Hafellner regarded Hymeneliaceae as a 'still poorly understood family' and put a question mark especially to Aspicilia (Hafellner, 1984). In 1989 Hafellner totally excluded Aspicilia and closely related
Figure 1. Phylogenetic relations of 12 Aspicilia species from Anatolia and other Pertusaria, Thamnolia, Dibaeis, Diploschistes, Ochrolechia and Lecanora species. One of the nine equally parsimonious trees by minimum evaluation analysis is shown. Numbers at the nodes are bootstrap frequencies above 50%.

The current study comprises the first document about phylogenetic relationships of manna group of lichen from Aspicilia. The sequence data obtained were also aligned with related groups, with Ochrolechia sp., Diploschistes sp., Pertusaria sp., Dibaeis sp., Thamnolia sp., and Lecanora sp. Alignment that included representatives of other genera provided assessment of the relationship of Aspicilia genus in a larger phylogenetic context. Aspicilia was separated from Lecanora as a genus of its own. Reeb et al. (2004) according to the results of their study with nuclear ribosomal genes (SSU and LSU) and protein coding genes RPB2, redefined the subclass Ostropomycetidae. Also they showed that Pertusariales + Icmadophiliaceae is sister to Ostropales + Baeomycetales + Hymeneliaceae clade rather than Lecanoromycetidae (Reeb et al., 2004). The data obtained from this study also support the concept that Aspicilia should be regarded as a genus of its own.

In the current study, the revealed sequence data from 12 Aspicilia taxa were aligned with related taxa from Ostropomycetidae and samples from Lecanoromycetidae. The phylogenetic tree obtained by minimum evolution analysis displayed two major branches. One of the

genera from Hymeneliaceae (Lutzoni and Brodo, 1995). Eriksson and Hawksworth (1993) in a relatively recent study formed a list by including nine genera in the Hymeneliaceae that also consist Aspicilia. Lutzoni and Brodo (1995) reclassified the species groups, classified under Hymenelia and Ponaspis in relation to Eiglera and Aspicilia, and declared that the status of Aspicilia was not changed particularly due to lack of data and is still classified within Hymeneliaceae (Lutzoni and Brodo, 1995).

Wedin et al. (2005) investigated Ascomycota with combined nLSU rDNA and mtSSU rDNA sequence datasets. Aspicilia and Icmadophiliaceae were remained within Pertusariales, but these relationships lack jackknife support and only low (86) posterior probability. Exclusion of these two groups, remaining Pertusariales as monophyletic has resulted in trees only four steps longer than the most parsimonious. Although Icmadophiliaceae should be treated in Pertusariales, it formed an (unsupported) sister group to the Aspicilia. Hymenelia lacturis formed together with Tremolocia atrata as unsupported sister group to Arctomiaceae and Moelleropsis humida. These results suggested the polyphyletic nature of Hymene-liaceae.
Figure 2. Neighbor joining analysis inferred from ITS region sequences.

branches with its six members (Aspicilia contorta subsp. contorta, A. contorta subsp. hoffmanniana, A. hispida, A. fruticulosa, A. desertorum. A. calcarea) composed solely of Aspicilia samples from Anatolia. Three of the manna groups of lichens were placed in this branch of the tree. The other related taxa from Ostropomycetidae and Lecanoramycetidae took place in the other branch of the tree with Aspicilia samples from Anatolia. Although manna group of lichens share few common morphological characters and do not represent a taxonomical unit results suggest close relations in phylogenetic context.

A. hispida from eastern province of Malatya and A. fruticulose from middle Anatolian province of Sivas and which were all manna group of lichens composed a group on the tree that is supported with a 100% bootstrap value. Two samples of A. desertorum another manna group of lichen; collected from eastern city of Elazi generated close genetic relation to each other. Although A. desertorum samples remained relatively close to other manna group of lichens (A. hispida, A. fruticulosa, A. desertorum.) they constitute another group with samples of A. calcarea species (60% support) which is not defined as a manna group of lichens. On the other hand these two species A. desertorum and A. calcarea use the same type of substrate; means both grow on calcareous rocks.

A. contorta, like A. calcarea is a species close to manna group. A. contorta subsp. contorta from Afyon province and three A. contorta subsp. hoffmanniana from eastern provinces generated another sister group with a 100% bootstrap value. On the other hand although regarded as a sister group A. contorta subsp. contorta from Afyon province remained in a different branch with a rather longer branch length. The data is consistent to the morphological data when main characters distinguishing A. contorta subsp. contorta and A. contorta subsp. hoffmanniana are considered. Rico (1999) reported the differences, in thallus, areole, apothecia, ascospore size, between two subspecies (Rico, 1999). Although Eckman and Fröberg (1988) have identified a heterogeneous large group of intermediates between A. contorta subsp. contorta and A. contorta subsp. hoffmanniana and declared the hybrids between the parental forms might be the origin of these intermediates, we did not have enough data with respect to sample number and intermediates, to test this concept.

Hafellner et al. (2004) also discussed morphological variation in Aspicilia under steppe-like environmental conditions and thought that only the entire Aspicilia contorta/calcarea group was worth to be considered as a distinct taxon or it could well be a subgenus within Aspicilia.

The monophyletic nature and lower genetic diversity revealed as branch length for the related genera were prominent. The data for related genera obtained from GenBank comprised of particularly orders from Ostropomycetidae and also Lecanoramycetidae. Pertusaria erubescens from Pertusariaceae remained close to the Aspicilia species from Anatolia while the other members of Pertusariaceae formed another group relatively far apart. On the other hand samples from Thelotremataceae and Icmadophylaceae remained closer to Aspicilia species compared to the species from Lecanoromycetidae. Results obtained from the samples collected from Anatolia show that Aspicilia genus is phylogenetically closer to the orders and families from Ostropomycetidae subclass rather than Lecanoromycetidae. These results support the study of Wedin et al. (2005) which strongly suggest the placement of Hymeneliaceae and also Pertusariales and Icmadophylaceae within Ostropomycetidae.
Samples from Anatolia analysed with Neighbor Joining analysis without aligning with the related groups, revealed species regarded as manna as one group, apart from A. calcarea and A. contorta which were defined as related species to manna group of lichens (Figure 2).

In a sense, it could be interpreted that Aspicilia sp. from Anatolia might represent a group of its own with characteristics unique to that ecology. Aspicilia species grown in Anatolia manifested greater genetic diversity revealed as longer branch length, with a polyphyletic nature (Figure 1). The higher genetic diversity observed, might be the reflection of rich flora and high potential of germplasm consisting almost 9000 different species found in Anatolia with more endemic species than whole Europe continent. But this interpretation should be confirmed with a wide spectrum study including Aspicilia sp. from different continents throughout the world.

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