Variation of morphological characters in the lichenicolous ascomycete genus *Abrothallus*

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The morphological variation of 65 samples of the exclusively lichenicolous ascomycete genus *Abrothallus* was studied by means of multivariate statistical approach (discriminant analysis). The samples were analysed in order to estimate the possible taxonomic implications of the characters frequently mentioned in literature. Colour of the epihymenium and hypothecium, shape of the ascomata, pruinosity of the ascomata, and Lugol reaction of the hyphae appeared to be the most distinguishing features for group recognition.

Key words: *Abrothallus*, Ascomycetes, host specificity, morphological variation, taxonomy

**Introduction**

The ascomycete genus *Abrothallus* (Ascomycota, incertae sedis) was introduced by de Notaris (1845) to accommodate *A. bertianus*. However, the genus was originally described as a lichen because of the misinterpretation of the host thallus. A few years later it was confirmed to be a lichenicolous fungus (Montagne 1851). Still, even in subsequent studies, *Abrothallus* was included either in *lichenes athalli* (Tulasne 1852) or *microlichens* (Lindsay 1857, 1869).

The question about the systematic position of *Abrothallus* has remained unclear due to the fact that the genus has no clear similarities with any other genera. A relationship with the family Phacidiaceae (Saccardo 1889) or with the orders Arthoniales (Jatta 1911, Bellemere *et al*. 1986) and Patellariales (Keissler 1930, Nannfeldt 1932) has been proposed. Still, in modern classification systems of Ascomycota (Kirk *et al*. 2001, and [http://www.umu.se/myconet/M10a.html](http://www.umu.se/myconet/M10a.html)), the position of *Abrothallus* remains uncertain.

Various taxa have been introduced in the genus, many of which have later been transferred to other genera, such as *Arthonia*, *Clypeococcum*, *Dactylospora*, and *Phacopsis* (e.g. Zahlbrucker 1924, 1931, Hawksworth 1977, Hafellner 1979, Triebel & Rambold 1988). According to the current circumscription, the genus comprises ca. 20 species, known on a wide range of hosts, especially Parmeliaceae (e.g. *Melanohalea*, *Melanelixia*, *Parmelia*, *Platismatia*, *Usnea*, etc.), but also Lobariaceae (*Sticta*, *Nephroma*, *Pseudocyphellaria*), Ramalinaceae (*Ramalina*), Stereocaulaceae (*Stereocaulon*) and Cladoniaceae (*Cladonia*). The genus is cosmopolitan, known from the Arctic (Alstrup & Hawksworth 1990)
Abrothallus is a rather well-defined genus morphologically: (1) globose or almost globose immarginate ascomata, sometimes with golden or green pruina, (2) bitunicate asci with four to eight ascospores, (3) brown, 2- to 4-celled, warted asymmetric ascospores, (4) ramified-anastomosed paraphyses, and (5) the epihymenium layer with granulose pigments which often dissolve in potassium hydroxide (KOH) (Figs. 1 and 2). In many cases, pycnidia of the Vouauxiomyces type have been found mixed with ascomata. Already Tulasne (1852) proposed that the pycnidia represent an imperfect state of Abrothallus.

Despite the clear circumscription of the genus, the subgeneric division has been a subject of dispute (Lindsay 1857, Kotte 1909, Keissler 1930, Santesson 1960) and of different interpretations (Hawksworth 1983, Clauzade et al. 1989, Santesson 1993, Santesson et al. 2004) for a long time: both broad (e.g. Keissler 1930, Hawksworth 1983, Santesson 1993) and narrow definitions of species have been used (e.g. Kotte 1909, Clauzade et al. 1989, Santesson et al. 2004). The confusion is caused by different levels of significance attributed to the relevant characters by various authors. For example, Kotte (1909) emphasized the importance of the iodine reaction of the vegetative hyphae, dimensions of ascospores and conidia as well as preference to a certain host as diagnostic features for species delimitation. In contrast, Keissler (1930) denied the taxonomical meaning of the iodine reaction and host preference, using characters such as presence of greenish pruina over ascomata, colour of epihymenium and reaction of hymenium with
KOH (K) instead. In the most recent complex treatments of lichenicolous fungi (Clauzade et al. 1989, Nash et al. 2004), the authors favoured the narrow species concepts proposed by Kotte (1909), applying, however, some additional characters used by Keissler (1930), such as pruinosity of ascomata, hymenium reaction with K, etc.

This study is a first step towards an assessment of the status of some 2-celled taxa of Abrothallus regarding the generic characters and their relative importance. The aim is to elucidate the host specificity of the genus and the value of the characters frequently used in the literature.

**Material and methods**

**Material**

Dried herbarium specimens (C, H, S, TU, UPS, UGDA-L) were used for the analysis of morphological features. The quantity of the material from different hosts and the availability of healthy specimens were the main criteria for material selection. In the final analysis, 65 specimens from eight different hosts were exploited. The main criterion for group separation was host specificity: (1) specimens on Hypogymnia physodes (in the further text Hyp), (2) on Melanohalea spp. (Mel), (3) on Parmelia spp. (Par), (4) on Platismatia spp. (Pla), (5) on Sticta spp. (Sti), (6) on Usnea spp. (Usn), (7) on Vulpicida spp. (Vul), (8) on Xanthoparmelia spp. (Xan).

**Microscopy**

Character examination was carried out with the stereomicroscope TECHNIVAL 2 (Carl Zeiss Jena) (magnification 50×) and with the light microscope Olympus CX41 (magnification 1200×). Routine methods of light microscopy were used: cross-sections were made with a razor blade, at first the sections were mounted in tap water and later in ca. 10% KOH (K) or Lugol’s solution (I, Fluka 62650). The iodine reaction of the vegetative hyphae was examined without pre-treatment with K. The procedure was performed twice to avoid the amylloid reaction remaining unnoticed. All measurements were made in the water medium. Microphotographs were taken with the digital camera Olympus Camedia Z4040.

**Characters**

Morphological characters for analysis were selected according to two criteria: (1) they were proposed as diagnostic in earlier studies (Kotte 1909, Hawksworth 1983, Clauzade et al. 1989, Diederich 1989), and (2) they were relatively easy to observe. The number of characters was higher initially but some of them (e.g. hymenium height) were not used later. The reaction with K, which has been considered to be important in species delimitation, was excluded because of rather constant positive greenish reaction shown on most of the studied specimens. The only difference was observed in its intensity (see also Calatayud & Barreno 1995).

As the colour of the epihymenium ranges from brown to dark red, this character was divided in two clearly distinguishable, but not transitional, character states. The shape of the ascomata was visually appraised by the dominance of either a flattened or a globose type. The ascomata of almost half of the examined specimens were mixed with the pycnidia of the Vouauxiomyces type. In some cases only one or two pycnidia were found. In other cases, mainly in older herbarium specimens, the conidiomata were empty. Therefore, in only two groups, Vul and Xan, the conidiomata were sufficiently mature to make an adequate number of measurements of conidia.

The dimensions of ascomata, ascospores and conidia were measured at least in ten replications, the dimensions of asci in two to five replications. The width of each ascospore cell was measured and treated as two separate characters. The width of the ascus was measured from the broadest point. Altogether sixteen characters were quantitative, eight characters were qualitative, and two were calculated ratios.

List of the characters used and their abbreviations:

ASCD = diameter of the ascoma (mm),
ASLEN = length of the ascospore (µm),
ASWI1 = width of the broader cell of the ascospore (µm),
ASWI2 = width of the narrower cell of the ascospore (µm),
ASRA = ratio of ascospore length to the width of the broader cell,
ASCLEN = length of the ascus (µm),
ASCWI = width of the ascus (µm),
ASCRA = ratio of ascus length to ascus width,
HYMCO = colour of the epihymenium (0 = dark red, 1 = brown),
LUG = reaction of sterile hyphae with Lugol’s solution (0 = negative reaction, 1 = positive reaction),
ASCP = pruinosity of the ascomata (0 – without green pruina, 1 – with green pruina),
HYPCOL = colour of the hypothecium (0 = dark brown, 1 = brown, 2 = light brown),
ASCS = shape of the ascomata (0 = globose, 1 = flattened),
CONID = presence/absence of conidiomata (0 = absence, 1 = presence),
CONLEN = length of the conidium (µm),
CONWI = width of the conidium (µm).

Statistical methods

In order to test the concordance of the conventional and predicted identifications of individuals, classificatory discriminant analysis (DA) was applied with Statistica 6.0 (Statsoft Inc. 2001). Data were analysed with standard methods of discriminant analysis. As characters 1 to 14 were observed in all studied specimens, they were selected for testing with this multivariate statistical method. Two separate discriminant analyses were performed. At first, the classification matrix was calculated where all specimens were grouped according to their host species. In the second classification matrix, two groups of specimens on Parmelia spp. were segregated according to the reaction of the hyphae with Lugol solution: Par+ specimens with a positive blue reaction and Par− with a negative reaction.

Means, standard deviations, minimum and maximum values were calculated for each specimen and for each quantitative character. Mean values of quantitative characters (ASLEN, ASWI1, ASWI2, ASRA, ASCD, ASCLEN, ASCWI) were compared pairwise with Student’s t-test. Comparison of the characters CONLEN and CONWI was applied only for the groups Vul and Xan.

Results

Summary of the distribution of the qualitative characters is presented in Table 1 and the descriptive statistics of the quantitative characters in Table 2.

Table 1. Distribution of qualitative characters. For abbreviations of group names and characters, see Material and methods; pos. = positive, neg. = negative, d. brown = dark brown, l. brown = light brown, n = number of studied specimens.

<table>
<thead>
<tr>
<th>Character</th>
<th>Hyp (n = 4)</th>
<th>Mel (n = 7)</th>
<th>Par (n = 18)</th>
<th>Pla (n = 10)</th>
<th>Sti (n = 4)</th>
<th>Usn (n = 6)</th>
<th>Vul (n = 9)</th>
<th>Xan (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYMCO red</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>brown</td>
<td>0</td>
<td>7</td>
<td>18</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>LUG neg.</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pos.</td>
<td>0</td>
<td>6</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>CONID abs. absence</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>presence</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>ASCP without with</td>
<td>4</td>
<td>5</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>with</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HYPICOL d. brown brown</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>l. brown</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ASCS globose flattened</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

| n | 4 | 7 | 18 | 10 | 4 | 6 | 9 | 7 |
The results of the first classificatory discriminant analysis (DA) showed a classification accuracy of 88% (Table 3). According to DA, 8 of a total of 65 individuals were re-classified into another group. The lowest rate of classification accuracy was observed for Vul (67%), in which two misclassified specimens were mixed with Xan and the other one with Par. The distinction of Hyp, Mel, Usn, Pla and Sti from the rest was obvious: the proportion of correctly classified specimens was 100%.

The proportion of correctly classified specimens in the second analysis was rather similar to that in the first analysis (86%), and nine specimens were re-grouped (Table 4). The observed classification was entirely in accordance with

**Table 2.** Mean values and standard deviations of qualitative characteristics of analyzed groups (mean ± S.E.). For abbreviations of characters and groups, see Material and methods.

<table>
<thead>
<tr>
<th>Character</th>
<th>Hyp (n=4)</th>
<th>Mel (n=7)</th>
<th>Par (n=18)</th>
<th>Pla (n=10)</th>
<th>Sti (n=4)</th>
<th>Usn (n=6)</th>
<th>Vul (n=9)</th>
<th>Xan (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCD (mm)</td>
<td>0.24 ± 0.07</td>
<td>0.24 ± 0.07</td>
<td>0.28 ± 0.09</td>
<td>0.28 ± 0.08</td>
<td>0.44 ± 0.13</td>
<td>0.26 ± 0.11</td>
<td>0.23 ± 0.06</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>ASLEN (µm)</td>
<td>12.96 ± 1.14</td>
<td>12.32 ± 1.65</td>
<td>14.16 ± 1.49</td>
<td>13.55 ± 1.3</td>
<td>15.14 ± 1.44</td>
<td>10.93 ± 1.08</td>
<td>11.49 ± 1.19</td>
<td>13.7 ± 1.46</td>
</tr>
<tr>
<td>ASW1 (µm)</td>
<td>5.28 ± 0.76</td>
<td>4.68 ± 0.58</td>
<td>5.39 ± 0.69</td>
<td>5.15 ± 0.56</td>
<td>6.19 ± 1.44</td>
<td>4.7 ± 0.5</td>
<td>4.68 ± 0.52</td>
<td>5.36 ± 0.63</td>
</tr>
<tr>
<td>ASW2 (µm)</td>
<td>4.42 ± 0.66</td>
<td>3.94 ± 0.55</td>
<td>4.4 ± 0.68</td>
<td>4.16 ± 0.49</td>
<td>5.12 ± 0.54</td>
<td>3.87 ± 0.37</td>
<td>3.87 ± 0.55</td>
<td>4.46 ± 0.6</td>
</tr>
<tr>
<td>ASRA</td>
<td>2.5 ± 0.38</td>
<td>2.66 ± 0.38</td>
<td>2.67 ± 0.42</td>
<td>2.66 ± 0.34</td>
<td>2.46 ± 0.24</td>
<td>2.34 ± 0.27</td>
<td>2.48 ± 0.36</td>
<td>2.58 ± 0.38</td>
</tr>
<tr>
<td>ASCLEN (µm)</td>
<td>45.47 ± 5.47</td>
<td>42.07 ± 6.26</td>
<td>48.45 ± 6.96</td>
<td>45.53 ± 6.64</td>
<td>54.84 ± 7.63</td>
<td>45.03 ± 7.4</td>
<td>40.64 ± 5.4</td>
<td>41.85 ± 6.2</td>
</tr>
<tr>
<td>ASCWI (µm)</td>
<td>12.59 ± 1.92</td>
<td>11.48 ± 1.17</td>
<td>11.96 ± 1.68</td>
<td>11.42 ± 1.26</td>
<td>13.32 ± 1.62</td>
<td>10.08 ± 1.18</td>
<td>11.02 ± 1.77</td>
<td>12.15 ± 1.68</td>
</tr>
<tr>
<td>ASCRA</td>
<td>3.76 ± 0.88</td>
<td>3.7 ± 0.65</td>
<td>4.15 ± 0.91</td>
<td>4.05 ± 0.85</td>
<td>4.2 ± 0.75</td>
<td>4.56 ± 1.07</td>
<td>3.8 ± 0.85</td>
<td>3.5 ± 0.69</td>
</tr>
<tr>
<td>CONLEN (µm)</td>
<td>6.17 ± 0.84</td>
<td>10.51 ± 2.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONWI (µm)</td>
<td>4.2 ± 0.54</td>
<td>3.78 ± 0.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Classification matrix I. Rows: observed classifications. Columns: predicted classifications. The number in brackets after the group name corresponds to the number of specimens.

<table>
<thead>
<tr>
<th>Group</th>
<th>% correct</th>
<th>Hyp</th>
<th>Mel</th>
<th>Par</th>
<th>Pla</th>
<th>Sti</th>
<th>Usn</th>
<th>Vul</th>
<th>Xan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyp (4)</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mel (7)</td>
<td>100</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Par (18)</td>
<td>78</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pla (10)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sti (4)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Usn (6)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vul (9)</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Xan (7)</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table 4.** Classification matrix II. Rows: observed classifications. Columns: predicted classifications. The number in brackets after the group name corresponds to the number of specimens.

<table>
<thead>
<tr>
<th>Group</th>
<th>% correct</th>
<th>Hyp</th>
<th>Mel</th>
<th>Par+</th>
<th>Par−</th>
<th>Pla</th>
<th>Sti</th>
<th>Usn</th>
<th>Vul</th>
<th>Xan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyp (4)</td>
<td>100</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mel (7)</td>
<td>86</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Par− (8)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Par+ (10)</td>
<td>70</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>0</td>
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<td>Pla (10)</td>
<td>80</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sti (4)</td>
<td>100</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Usn (6)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vul (9)</td>
<td>78</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Xan (7)</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>
the predicted classification for four groups (Hyp, Par, Sti, Usn) out of nine. The lowest rate of classification accuracy was detected for Par+ (67%), in which one specimen was grouped with Mel and two specimens with Pla.

In both analyses, five characters out of 14 used appeared to be statistically significant in distinguishing the groups of *Abrothallus*. The most important features in group separation were HYMCO (colour of the epihymenium), HYPCOL (colour of hypothecium), ASCS (shape of the ascomata), LUG (Lugol reaction) and ASCP (pruinosity of ascomata) (Table 5). In the second analysis, where the Par specimens were further grouped according to the presence or absence of amyloid reaction of the hyphae, the value of the character LUG in group separation was higher than in the first analysis. According to the results of DA, the quantitative characters were statistically insignificant.

The colour of the epihymenium (HYMCO) had the highest discriminative ability in both analyses. This two-mode appraised character showed no variation within species: dark red colour of the epihymenium occurred in all specimens of *Usn* and *Hyp*, separating them from the rest possessing a brown epihymenium (Table 1). The intensity of hypothecium pigmentation (HYPCOL) varied from light brown to dark brown between and within the groups. Still, there were two groups for which the sharing of pigmentation was clear: *Xan* with a remarkably dark brown and *Pla* with a light brown hypothecium. The shape of the ascomata (ASCS), which was estimated to be either flattened or globose, showed also significant variation within the groups. Still, at least in some cases, there was a clear tendency towards having either one or the other type: only flattened ascomata in *Sti* and *Pla* and only globose ascomata in *Usn*.

The reaction of vegetative hyphae with Lugol reactive has been widely used as a taxonomic character in mycology. The present study confirms this: a positive blue reaction of hyphae always occurred for *Sti, Usn, Vul* and *Xan*, but never for *Hyp*. However, the distinction was not so clear for *Mel, Pla* and *Par*: the hyphae of almost half of the *Par* specimens and most of the *Mel* and *Pla* specimens, except one, turned blue with the Lugol reagent (Table 1). For the second DA, two distinct *Par* groups were segregated

| Table 5. Summary of two classificatory discriminant analyses (DA): importance of characters in the identification of specimens. Abbreviations: ns = non-significant. DA 1: Wilks’ $\lambda = 0.001272$, approx. $F_{98,287} = 5.4766$, $p < 0.001$, DA 2: Wilks’ $\lambda = 0.00034$, approx. $F_{112,313} = 5.9453$, $p < 0.001$. |
|-----------------|-------|-------|-------|-------|-------|-------|-------|
| Character | DA 1 | DA 2 |
| ASCD | 0.70962 | ns | 0.60232 | ns |
| ASLEN | 1.1226 | ns | 1.31024 | ns |
| ASWI1 | 1.09973 | ns | 0.98963 | ns |
| ASWI2 | 1.33476 | ns | 1.29700 | ns |
| ASRA | 1.30739 | ns | 1.25804 | ns |
| ASCLEN | 1.09432 | ns | 0.44519 | ns |
| ASCWI | 0.92131 | ns | 0.39128 | ns |
| ASCRA | 0.98329 | ns | 0.40057 | ns |
| HYMCO | 53.83 | 0.001 | 39.45176 | 0.001 |
| LUG | 5.06838 | 0.001 | 22.05440 | 0.001 |
| CONID | 0.46198 | ns | 0.34019 | ns |
| ASCP | 4.14821 | 0.01 | 3.17484 | 0.01 |
| HYPCOL | 5.93686 | 0.001 | 4.01028 | 0.01 |
| ASCS | 5.17762 | 0.001 | 4.61596 | 0.001 |

| Table 6. The pairwise comparison of mean values (ascospore length/ascospore width/diameter of ascomata) by Student’s t-test. The significance levels of different characters are separated with oblique line. Significance levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = non-significant. |
|-----------------|-------|-------|-------|-------|-------|-------|-------|
| Character | Xan | Vul | Usn | Sti | Pla | Par | Hyp |
| Mel | ***/***/ns | ***/ns/ ns | ***/ns/ ns | ***/***/ *** | ***/***/ *** | ***/***/ *** | ns/ ***/ *** |
| Xan | ***/***/ns | ***/***/ *** | ns/ ***/ ns | ***/ ***/ *** | ***/***/ *** | ***/ ***/ *** | ns/ ***/ *** |
| Vul | ***/***/ *** | ***/ ***/ *** | ns/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ns/ ***/ *** |
| Usn | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ns/ ***/ *** |
| Sti | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ns/ ***/ *** |
| Pla | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ns/ ***/ *** |
| Par | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ***/ ***/ *** | ns/ ***/ *** |
according to the presence or absence of this reaction. In this case, all Par specimens with a negative reaction (Par–) were correctly classified, contrary to the specimens with a positive blue reaction (Par+), which were mixed with Mel and Pla (Table 4).

Finally, a character with rather a high discriminative power was also the presence or absence of greenish pruina on ascomata (ASCP). Four groups (Hyp, Usn, Vul and Xan) out of the eight were characterized by absence and one group (Sti) by presence of this character (Table 1). The situation was more complicated with Mel, Par and Pla, in which pruinose ascomata were common but not constant. It is notable that greenish pruina was best developed on younger ascomata, but were not always observed on older ones.

According to DA, quantitative characters such as dimensions of ascomata, ascospores and asci were insignificant for group separation (Table 5). Still, for some groups the t-test showed a statistically significant (p < 0.05) distinction (Table 6). The specimens of Sti differed from all others in the quantitative characters ASCD, ASLEN, ASWI1 and ASWI2: the mean values of both ascomata and ascospores were higher than the corresponding values of the rest of the taxa (Table 2 and Fig. 3A–C). There occurred also intrinsic distinction between Usn and Vul regarding the ascospore dimensions: the ascospores in these groups were on average shorter than those in the other groups (Table 2 and Fig. 3A) but there was no significant distinction between Vul and Usn.
Although the size of conidia has been considered to be a distinctive character, at least for some taxa, in the studied material only Vul and Xan possessed enough mature conidiomata to test their discrepancy. According to the t-test, the distinction of the conidia between these two groups was statistically significant: the conidia of Xan were longer and more slender in comparison with those of Vul, which were shorter and thicker (Table 2 and Fig. 4).

Discussion

Abrothallus, containing exclusively lichenicolous taxa, is a rather well known and widespread genus whose taxonomic relationships are unclear. Despite the distinctness of the genus, its subgeneric division is still a subject of interpretation. For this preliminary study, eight conventionally identified groups were tested to estimate host specificity and the value of the characters used in previous studies (Kotte 1909, Keissler 1930, Hawksworth 1983, Clauzade et al. 1989, Diedrich 2004), showed more variation than expected. The presence of greenish pruina, which has been considered to be one of the main diagnostic characteristics in Abrothallus (Keissler 1930, Hawksworth 1983, Clauzade et al. 1989), seems to be mainly applicable to younger ascomata (see the note in Hawksworth 1983). Moreover, in older herbarium specimens, the pruina might be swept off. Problems related to the pruina as a taxonomic character have also been pointed out in studies of lichenized fungi (Heidmarsson 1996). Hence, even if the character itself seems to be advantageous, one has to be careful when applying it in taxonomic studies.

Colour reactions with iodine solutions as diagnostic markers have been routinely used in the systematics of non-lichenized and lichenized fungi (reviews in Baral 1987, Common 1991). In Abrothallus systematics, the reaction of vegetative hyphae with Lugol reactive has been applied to subgeneric division since the publication by Kotte (1909). At the same time, exploitation of amyloid reaction in separation of Abrothallus species has been questioned in some earlier studies (Schaechtelin & Werner 1927, Keissler 1930), partly because of the difficulties with observation (Schaechtelin & Werner 1927).

Considering the studied material, the presence or absence of amyloid reaction seems to be a useful feature in taxon delimitation. There was one exception: the group Par included a more or less equal number of specimens with a positive reaction and specimens with a negative reaction (Table 1), which may indicate that more than one Abrothallus species can grow on the host genus Parmelia. Therefore, a second discriminant analysis, where the specimens of Par were further grouped according to the presence or absence of this reaction, was performed. Based on this analysis, all specimens of Par with a negative reaction (Par–) plus one specimen of Mel and one of Pla formed a distinct entity. Although the group Par with a positive reaction (Par+) showed low
resolution, a similar trend was observed: the misclassified specimens were grouped into _Mel_ and _Pla_, and _vice versa_ (Table 4).

The data on diaspore (ascospores, conidia) dimensions vary in literature because of the different species concepts used (e.g. Kotte 1909, Hawksworth 1983, Diederich 1989) or because of overly generalized data (Clauzade _et al._ 1989). Both make comparison of the variation rates of diaspores with corresponding literature data difficult or even impossible. Although, according to DA, the dimensions of ascospores and ascomata were insignificant for grouping, yet comparison of the mean values revealed some significant trends. Based on the presented data, the length, width and the length/width ratio of ascospores serve as useful distinguishing characters for the specimen groups _Sti_, _Usn_ and _Vul_ (Table 6).

It has been supposed that the conidiomata referred to as the anamorph genus _Vouauxiomycetaceae_ represent an asexual stage of _Abrothallus_ (Tulasne 1852, Kotte 1909, Galløe 1950, Nordin 1964, Hawksworth 1981, Wedin 1994). However, this evidence is based on the frequent co-occurrence of ascomata and conidiomata and has not yet been proved by additional culture experiments. The anamorph may also appear regularly without the teleomorph (Hawksworth 1981, Kondratyuk 1996). Considering literature data (Kotte 1909, Hawksworth 1981, 1983, Clauzade _et al._ 1989) as well as the present data, the characters originating from the imperfect state of _Abrothallus_ are also acceptable in delimitation of taxa. In the present study, the distinction between _Vul_ and _Xan_ was more evident by the size (and shape) of conidia than by the characters of ascomata.

**Conclusions**

The analyses based on 65 samples from eight host genera showed a clear tendency towards host specificity in the exclusively lichenicolous genus _Abrothallus_. This finding, however, was not so clear for specimens growing on _Parmelia (Par)_ , _Melanohalea (Mel)_ and _Platismatia (Pla)_ . Recent achievements in elucidating phylogenetic relationships within the lichen family Parmeliaceae (e.g. Crespo & Cubero 1998, Blanco _et al._ 2004) indicate rather a high affinity between the above lichen genera. Theoretically, the phylogeny of host taxa may also elucidate the phylogeny of their parasites.

The usefulness of several anatomical-morphological characters (i.e. pruinosity of ascomata, amyloid reaction of hyphae, colour of the epihyphenium) for taxon separation was supported by discriminant analysis. In addition, for recognition of _Abrothallus_ on _Sticta_ spp. (_Sti_), _Vulpicida_ spp. (_Vul_) and _Usnea_ spp. (_Usn_), dimensions of ascospores proved statistically significant. For distinguishing between _Abrothallus_ on _Vulpicida_ spp. (_Vul_) and _Abrothallus_ on _Xanthoparmelia_ spp. (_Xan_), the characters of the anamorph appeared to be better indicators than the characters of the teleomorph.

The obtained results are in good agreement with the contemporary standpoint about the high host-specificity of the lichenicolous fungi (Lawrey & Diederich 2003). However, further detailed research of the complicated taxonomy of _Abrothallus_ requires more material from different host lichens and more specific characters (e.g. DNA sequences, anamorph characters) as well as aspects of pathogenicity.

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Appendix. Examined specimens


