



A molecular reappraisal of *Abrothallus* species growing on lichens of the order Peltigerales

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Abstract

Species of the genus *Abrothallus* (Abrothallales, Dothideomycetes) are obligately lichenicolous (lichen-inhabiting) and grow on a wide variety of foliose and fruticose lichens. Bayesian Interference (BI) and Maximum Likelihood (ML) analyses of two gene loci—rDNA ITS and TEF- α —were used in order to infer the phylogenetic relationships among lineages of *Abrothallus* associated with hosts from the order Peltigerales (Lecanoromycetes). We found that the clade is subdivided into 13 lineages each of which can be delimited also by phenotypic criteria. Seven new species (*Abrothallus boomii*, *A. canariensis*, *A. doliiformis*, *A. eriodermæ*, *A. ertzii*, *A. etayoi* and *A. nephromatis*) are described, two of which are known only by their asexual stage. *Abrothallus welwitschii* is lectotypified, and the original description is complemented. *Vouauxiomyces brattii* and *Epinephroma kamchatica* are combined within *Abrothallus*.

Key words: Abrothallales, *Epinephroma*, host-specificity, lichenicolous fungi, taxonomy

Introduction

Lichenicolous fungi represent a non-taxonomical group of fungi that are obligately associated with lichens (Lawrey & Diederich 2003). Species from the genus *Abrothallus* represent the earliest records (Acharius 1814 as *Endocarpon parasiticum* Ach., Sommerfelt 1826 as *Lecidea parmeliarum* Sommerf.), illustrations (Smith 1808 as *Lichen parasiticus*) and descriptions (de Notarius 1845, *Abrothallus bertianus* De Not.) of lichen-inhabiting fungi. The genus is easily recognizable in the field by its black roughly globose apothecioid ascomata usually covered with greenish pruina. Anatomically the genus is characterized by bitunicate asci with four to eight, 2- to 4-celled, brown, asymmetric ascospores with clear ornamentation, usually oriented with the wider cell placed upwards within the ascus. In some species the ascospores split into part-spores within the ascus (Etayo 2002, Hawksworth 1990, Suija *et al.* 2011, Wedin 1994). The sexual morph of *Abrothallus* is often accompanied with the asexual morph of *Vouauxiomyces*-type (Hawksworth 1981, Pérez-Ortega *et al.* 2011).

Despite being known for a long time, the phylogenetic adscription of the genus has remained unresolved until recently (Pérez-Ortega *et al.* 2014) as no clear synapomorphies with any other known genera have been found. Pérez-Ortega *et al.* (2014) showed that the genus belongs to Dothideomycetes and described the new order Abrothallales in the Pleosporomycetidae. *Abrothallus* represents one of the few genera in this class with apothecioid ascomata. The genus has been considered to be cosmopolitan with a broad host range including members of foliose and fruticose lichens from families Cladoniaceae, Lobariaceae, Nephromataceae, Pannariaceae, Parmeliaceae, Ramalinaceae and Stereocaulaceae. Twenty six (26) species have tentatively been accepted in the genus (*Index Fungorum* (<http://www.indexfungorum.org/>), Etayo & van den Boom 2006, Etayo 2010) from more than 30 macrolichen genera (Suija, pers. obs.).

Historically the species concept of the genus has been a topic of controversy, and host-specificity as species delimiting criterion has been used assuming either narrow (Kotte 1909, Santesson *et al.* 2004) or wide host selection (Hawksworth 1983, Keissler 1929, Santesson 1993). Preliminary multi-gene analysis (Pérez-Ortega *et al.* 2014) has shown that the host-specificity in *Abrothallus* is more pronounced at the host family/order level, allowing us to

discovered in the future, which will lead to the proposal of additional taxa. We also expect that extended sampling will enable us to shed light on the evolutionary processes (host switching, co-evolutionary processes, geographical barriers, etc.) that promote speciation in this group of lichenicolous fungi.

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References

- Acharius, E. (1814) *Synopsis methodica lichenum, sistens omnes hujus ordinis naturalis detectas plantas, quas, secundum genera, species et varietates disposuit, characteribus et differentiis emendatis definivit, nec non synonymis et observationibus selectis illustravit auctor.* Svanborg, Lund. 392 pp.
- Alstrup, V. & Hawksworth, D.L. (1990) The lichenicolous fungi of Greenland. *Meddelelser om Grønland, Bioscience* 31: 1–90.
- Crittenden, P.D., David, J.C., Hawksworth, D.L. & Campbell, F.S. (1995) Attempted isolation and success in the culturing of a broad spectrum of lichen-forming and lichenicolous fungi. *New Phytologist* 130(2): 267–297.
<http://dx.doi.org/10.1111/j.1469-8137.1995.tb03048.x>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772.
<http://dx.doi.org/10.1038/nmeth.2109>
- de Notaris, G. (1845) *Abrothallus* novum lichenum genus. [ser. 2] *Memorie della Reale Accademia delle Scienze di Torino* 10: 351–355.
- Diederich, P. (2004) *Abrothallus*. In: Nash, T.H. III, Ryan, B.D., Diederich, P., Gries, C. & Bungartz, F. (Eds.) *Lichen Flora of the Greater Sonoran Desert Region*, Vol. 2. Lichens Unlimited, Arizona State University, Tempe, Arizona, pp. 626–630.
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797.
<http://dx.doi.org/10.1093/nar/gkh340>
- Etayo, J. (2002) Aportación al Conocimiento de los Hongos Liquenícolas de Colombia. *Bibliotheca Lichenologica* 84, J. Cramer, Berlin, Stuttgart. 154 pp.
- Etayo, J. (2010) Hongos liquenícolas de Perú: Homenaje a Rolf Santesson. *Bulletin de la Société Linnéenne de Provence* 61: 1–46.
- Etayo, J. & Diederich, P. (1996) Lichenicolous fungi from the western Pyrenees, France and Spain. III. Species on *Lobaria pulmonaria*. *Bulletin de la Société des Naturalistes Luxembourgeois* 97: 93–118.
- Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
<http://dx.doi.org/10.1111/j.1365-294x.1993.tb00005.x>
- Goward, T. & Thor, G. (1992) Notes on the lichens and allied fungi of British Columbia. I. *Bryologist* 95(1): 33–37.
<http://dx.doi.org/10.2307/3243782>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hawksworth, D.L. (1981) The lichenicolous Coelomycetes. *Bulletin of the British Museum. Botany* 9: 1–98.
- Hawksworth, D.L. (1983) A key to the lichen-forming, parasitic, parasymbiotic and saprophytic fungi occurring on lichens in the British

- Isles. *Lichenologist* 15: 1–44.
<http://dx.doi.org/10.1017/s0024282983000031>
- Hawksworth, D.L. (1990) Notes on British lichenicolous fungi: VI. *Notes from the Royal Botanic Garden Edinburgh* 46(3): 391–403.
- Hawksworth, D.L. & Miadlikowska, J. (1997) New species of lichenicolous fungi on *Peltigera* in Ecuador and Europe. *Mycological Research* 101(9): 1127–1134.
<http://dx.doi.org/10.1017/s0953756297003778>
- Holien, H. & Tønsberg, T. (1994) The 10th meeting of the Nordic Lichen Society in Nord-Trøndelag, Norway, 1993. *Graphis Scripta* 6(2): 67–75.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Ihlen, P.G. & Wedin, M. (2005) Notes on Swedish lichenicolous fungi. *Nova Hedwigia* 81(3–4): 493–499.
<http://dx.doi.org/10.1127/0029-5035/2005/0081-0493>
- ICN (2012) *International Code of Nomenclature for algae, fungi, and plants*. Available from: <http://www.iapt-taxon.org/nomen/main.php> (accessed July 2013).
- Keissler, K. von (1929) *Die Flechtenparasiten Deutschlands, Österreichs und der Schweiz mit Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete*. Akademische Verlagsgesellschaft m. b. H., Leipzig. 240 pp.
- Kotte, I. (1909) Einige neue Fälle von Nebensymbiose (Parasymbiose). *Zentralblatt für Bakteriologie und Parasitenkunde* II 24: 74–93.
- Kondratyuk, S.Y. (1996) New species of *Pronectria*, *Vouauxiomyces*, *Wentiomyces* and *Zwackhiomyces* from Australasia. *Muelleria* 9: 93–104.
- Kondratyuk, S.Y. & Galloway D.J. (1995) Lichenicolous fungi and chemical patterns in *Pseudocyphellaria*. In: Knoph, J.-G., Schrüfer, K. & Sipman, H.J.M. (eds.) *Studies in Lichenology with Emphasis on Chemotaxonomy, Geography and Phytochemistry. Festschrift Christian Leuckert. Bibliotheca Lichenologica*, J. Cramer, Berlin, Stuttgart, pp. 327–345.
- Lawrey, J.D. & Diederich, P. (2003) Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* 106(1): 81–120.
[http://dx.doi.org/10.1639/0007-2745\(2003\)106\[0080:lfiab\]2.0.co;2](http://dx.doi.org/10.1639/0007-2745(2003)106[0080:lfiab]2.0.co;2)
- Liu, Y.J., Whelen, S. & Hall, B.D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026092>
- Martínez, I. & Hafellner, J. (1998) Lichens and lichenicolous fungi on Peltigerales in the Iberian Peninsula and the Canary Islands. *Mycotaxon* 69: 271–310.
- McGee, M. (2003) *Abrothallus welwitschii* in California on *Sticta limbata*. *Bulletin of the California Lichen Society* 10(2): 39.
- Millanes, A.M., Truong, C., Westberg, M., Diederich, P. & Wedin, M. (2014) Host switching promotes diversity in host-specialized mycoparasitic fungi: uncoupled evolution in the *Biatoropsis-Usnea* system. *Evolution* 68(6): 1576–1593.
<http://dx.doi.org/10.1111/evo.12374>
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, LA, pp. 1–8.
<http://dx.doi.org/10.1109/gce.2010.5676129>
- Molina, M.C., DePriest, P.T. & Lawrey, J.D. (2005) Genetic variation in the widespread lichenicolous fungus *Marchandiomyces corallinus*. *Mycologia* 97(2): 454–363.
<http://dx.doi.org/10.3852/mycologia.97.2.454>
- Montagne, C. (1851) Cryptogamia Guyanensis seu plantarum cellularium in Guyana gallica annis 1835-1849 a cl. Leprieur collectarum enumeratio universalis. suite. *Annales des Sciences Naturelles, Botanique* 16: 47–81.
- Montagne, J.F.C. (1856) *Sylloge generum specierumque cryptogamarum, quas in variis operibus descriptas iconibusque illustratas, nunc ad diagnosum reductas, nonnullasque novas interjectas, ordine systematica exposuit*. Parisiis. XXIV, 498 pp.
<http://dx.doi.org/10.5962/bhl.title.5403>
- Nilsson, R.H., Abarenkov, K., Veldre, V., Nylinder, S., De Wit, P., Brosche, S., Alfredsson, J.F., Ryberg, M. & Kristiansson, E. (2010) An open source chimera checker for the fungal ITS region. *Molecular Ecology Resources* 10(6): 1076–1081.
<http://dx.doi.org/10.1111/j.1755-0998.2010.02850.x>
- Pérez-Ortega, S., Suija, A. & de los Rios, A. (2011) The connection between *Abrothallus* and its anamorph state *Vouauxiomyces* established by Denaturing Gradient Gel Electrophoresis (DGGE). *Lichenologist* 43(3): 277–279.
<http://dx.doi.org/10.1017/s0024282911000089>
- Pérez-Ortega, S., Suija, A., Crespo, A. & de los Rios, A. (2014) Lichenicolous fungi of the genus *Abrothallus* (Dothideomycetes: Abrothallales ordo nov.) are sister to the predominantly aquatic Jahnulales. *Fungal Diversity* 64(1): 295–304.

- <http://dx.doi.org/10.1007/s13225-013-0269-y>
- Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25: 1253–1256.
<http://dx.doi.org/10.1093/molbev/msn083>
- Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98.
<http://dx.doi.org/10.3852/mycologia.97.1.84>
- Ronquist, F., Huelsenbeck, J.P. & van der Mark, P. (2005) MrBayes 3.1 Manual. Available from: http://mrbayes.csit.fsu.edu/mb3.1_manual.pdf
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology* 61(3): 539–542.
<http://dx.doi.org/10.1093/sysbio/sys029>
- Santesson, R. (1993) *The lichens and lichenicolous fungi of Sweden and Norway*. SBT-förlaget, Lund. 240 pp.
- Santesson, R., Moberg, R., Nordin, A., Tønsberg, T. & Vitikainen, O. (2004) *Lichen-forming and Lichenicolous Fungi of Fennoscandia*. Museum of Evolution, Uppsala University, Uppsala, Sweden. 359 pp.
- Schaechtlin, J. & Werner, R.G. (1927) Développement et biologie de l'*Abrothallus parmiliarum* Smft. *Bulletin de la Société mycologique de France* 42: 233–243.
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Bolchacova, E., Voigt, K., Crous, P.W., Miller, A.N., Wingfield, M.J., Kõljalg, U. & Tedersoo, L. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS* 109(16): 6241–6246.
<http://10.1073/pnas.1117018109>
- Smith, J.E. (1808) *English Botany XXVII*. R. Taylor and Co., Shoe-Lane, Fleet-Street London, 148 pp.
- Sommerfelt, S.C. (1826) *Supplementum florae lapponicae*. Borgianis et Gröndahljanis Christiania, 362 pp.
<http://dx.doi.org/10.5962/bhl.title.49579>
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22(21): 2688–2690.
<http://dx.doi.org/10.1093/bioinformatics/btl446>
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57(5): 758–771.
<http://dx.doi.org/10.1080/10635150802429642>
- Stenroos, S., Laukka, T., Huhtinen, S., Döbbeler, P., Myllys, L., Syrjänen, K. & Hyvönen, J. (2009) Multiple origins of symbioses between ascomycetes and bryophytes suggested by a five-gene phylogeny. *Cladistics* 26(3): 281–300.
<http://dx.doi.org/10.1111/j.1096-0031.2009.00284.x>
- Sugiura, N. (1978) Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics A* 7: 13–26.
- Suija, A. (2006) Variation of morphological characters in the lichenicolous ascomycete genus *Abrothallus*. *Annales Botanici Fennici* 43: 193–204.
- Suija, A., Pérez-Ortega, S. & Hawksworth, D.L. (2011) *Abrothallus halei* (Ascomycota, incertae sedis), a new lichenicolous fungus on *Lobaria* species in Europe and North America. *Lichenologist* 43(1): 51–55.
<http://dx.doi.org/10.1017/s002428291000054x>
- Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577.
<http://dx.doi.org/10.1080/10635150701472164>
- Tulasne, L.-R. (1852) Mémoire pour servir à l'histoire organographique et physiologique des lichens. *Annales des Sciences Naturelles, Botanique* 17: 5–128.
<http://dx.doi.org/10.5962/bhl.title.58656>
- Wedin, M. (1994) New and noteworthy lichenicolous fungi from southernmost South America. *Lichenologist* 26(3): 301–310.
- Werth, S., Millanes, A.M., Wedin, M., Scheidegger, C. (2013) Lichenicolous fungi show population subdivision by host species but do not share population history with their hosts. *Fungal Biology* 117(1): 71–84.
<http://dx.doi.org/10.1016/j.funbio.2012.11.007>
- White, T.J., Bruns, T.D., Lee, S.B. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D., Sninsky, J.H. & White, T.J. (Eds.) *PCR protocols – a guide to methods and applications*.

Academic Press, New York, pp. 315–322.

Zhurbenko, M.P. & Dillman, K. (2010) *Polycoccum hymeniicola* comb. nov. (Dacampiaceae) and other interesting lichenicolous fungi from southeastern Alaska. *Bryologist* 113(2): 260–266.

<http://dx.doi.org/10.1639/0007-2745-113.2.260>

Zhurbenko, M.P., Himmelbrant, D.E., Kuznetsova, E.S. & Stepanchikova, I.S. (2012) Lichenicolous fungi from the Kamchatka Peninsula, Russia. *Bryologist* 115(2): 295–312.

<http://dx.doi.org/10.1639/0007-2745-115.2.295>

Zhurbenko, M.P. & Yakovchenko, L.S. (2014) *Sagediopsis vasilyevae* sp. nov. and other lichenicolous fungi from Zabaikal'skii Territory of Russia, Southern Siberia. *Folia Cryptogamica Estonica* 51: 121–130.

<http://dx.doi.org/10.12697/fce.2014.51.14>

Zopf, W. (1897) Ueber Nebensymbiose (Parasymbiose). *Berichte der Deutschen Botanischen Gesellschaft* 15: 90–92.