

## SHORT COMMUNICATION

# *Aquacidia*, a new genus to accommodate a group of skiophilous temperate *Bacidia* species that belong in the Pilocarpaceae (lichenized ascomycetes)

A. Aptroot<sup>1</sup>, L. B. Sparrius<sup>2</sup>, P. Alvarado<sup>3</sup>

### Key words

*Lecanorales*  
*Ramalinaceae*  
riparian  
saxicolous lichens

**Abstract** – The new genus *Aquacidia* is proposed to accommodate three temperate *Bacidia* species that belong in the Pilocarpaceae and are not related to the type species *B. rosella*, which is in the Ramalinaceae. The phylogenetic position was clarified by an analysis of the mtSSU region showing a distinct lineage within the Pilocarpaceae. The following new combinations are proposed: *Aquacidia antricola* (Hulting) Aptroot (syn. *Bacidia antricola*, *B. carneoglauca*), *A. trachona* (Ach.) Aptroot, and *A. viridifarinosa* (Coppins & P. James) Aptroot. A key to *Aquacidia* species is provided.

**Samenvatting** – Het nieuwe korstmossengeslacht *Aquacidia* wordt beschreven en omvat drie soorten van het genus *Bacidia* (Ramalinaceae) die voorkomen in gematigde klimaatzones en die niet tot de familie Ramalinaceae blijken te behoren, maar tot een andere familie, namelijk de Pilocarpaceae. Ook zijn deze drie soorten niet verwant aan de typesoort van het genus *Bacidia*, *B. rosella*, of aan andere bekende genera in de Pilocarpaceae, waardoor voor die drie soorten het beschrijven van een nieuw genus nodig is. De fylogenetische positie van deze soorten is bepaald aan de hand van een mtSSU rDNA marker. De volgende nieuwe combinaties worden gemaakt: *Aquacidia antricola* (Hulting) Aptroot (syn. *Bacidia antricola*, *B. carneoglauca*), *A. trachona* (Ach.) Aptroot, en *A. viridifarinosa* (Coppins & P. James) Aptroot. Voor deze soorten is een sleutel opgenomen. In Nederland komen ze alledrie voor in diepe spleten van kalkarm gesteente op oude zee- en rivierdijken.

Published on 27 February 2018

## INTRODUCTION

*Bacidia* De Not. (Ramalinaceae C. Agardh) is one of the more species-rich lichen genera. Species are known from almost all substrates and habitats where lichens occur, but they are more abundant and diverse in sheltered habitats, such as tree bark, in moist areas of boreal, temperate, and especially tropical regions. A small group of temperate species of *Bacidia* was recently associated to the family Pilocarpaceae Zahlbr. (Andersen & Ekman 2005; Coppins & Aptroot 2009). Formal accommodation of this group into a different genus would help to solve the phylogenetic status of *Bacidia*.

One of these temperate species under consideration here, “*Bacidia*” *trachona* (Ach.) Lettau, has already been demonstrated to belong to the Pilocarpaceae based on mtSSU rDNA sequences (Andersen & Ekman 2005). We sequenced one more related species to investigate the phylogenetic position, viz. “*Bacidia*” *antricola*. Based on their strong similarity in fundamental morphological features, we hypothesized that they would cluster together in a well-supported group within the Pilocarpaceae. A third taxon, “*Bacidia*” *viridifarinosa* is considered the sorediate counterpart of “*B.*” *antricola* and was not sequenced.

<sup>1</sup> ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, the Netherlands; e-mail: andreaptroot@gmail.com

<sup>2</sup> BLWG, Beyerd 39, 4811 GZ Breda, the Netherlands;  
e-mail: sparrius@blwg.nl

<sup>3</sup> ALVALAB, Av. Bruselas 2 3B, 33011 Oviedo, Spain;  
e-mail: pablo.alvarado@gmail.com

corresponding author e-mail: sparrius@blwg.nl

## MATERIALS AND METHODS

Identification and descriptive work was carried out using an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections have been mounted in tap water, in which also all measurements were taken.

Newly sequenced specimens:

*“Bacidia” antricola*: Netherlands, Noord-Holland, Holysloot, on basalt, hidden between boulders, on dike along former sea, A. Aptroot 73454, 24 March 2015 (ABL), GenBank ALV4918;

*Psilolechia leprosa*: Netherlands, Gelderland, Malden, Mulderskop, on pebbles beneath disused railway track, A. Aptroot 73444, 9 January 2015 (ABL), GenBank ALV4917.

**DNA extraction, amplification and sequencing:** Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). A portion of each sample was blended with the aid of a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform: isoamyl alcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifugated for 10 min at 13,000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifugated again for 2 min and dried. It was finally resuspended in 200 µL ddH<sub>2</sub>O. PCR amplification was performed with the primers mrSSU1 and mrSSU3R (Zoller et al. 1999) for the mtSSU region (mitochondrial small subunit ribosomal DNA). PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 72 °C step for 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one of the PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

**Phylogenetic analyses:** BLAST (Altschul et al. 1997) was used to select the most closely related sequences of the family Pilocarpaceae and related families Scoliciosporaceae, Psilolechiaceae from INSD public databases. Sequences came mainly from Andersen & Ekman (2005). Species of the genera *Buellia* and *Physcia* (both Physciaceae) were used as outgroups. Sequences were first aligned in MEGA 5.0 (Tamura et al. 2011) software with its Clustal W application and then corrected manually. The final alignment included 330/786 variable sites. The aligned locus was loaded in PAUP\* 4.0b10 (Swofford 2001) and subjected to MrModeltest 2.3 (Nylander 2004). Model GTR+Γ was implemented in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0.2, sampling every 100<sup>th</sup> generation) until convergence parameters were met after about 100,000 generations (on which the Bayesian analysis is based), standard deviation having fell below 0.01. Significance threshold was set above 0.95 for posterior probability (PP).

## RESULTS

The only mtSSU sequence in GenBank of the type species of *Bacidia*, *Bacidia rosella* (AY300877, Lumbsch et al. 2004), is about 28% different from that obtained here from *“Bacidia” antricola*, confirming that both species belong to different families. *Bacidia rosella* was hence not included in the mtSSU phylogenetic analysis. The resulting tree (Fig. 1) has three major lineages

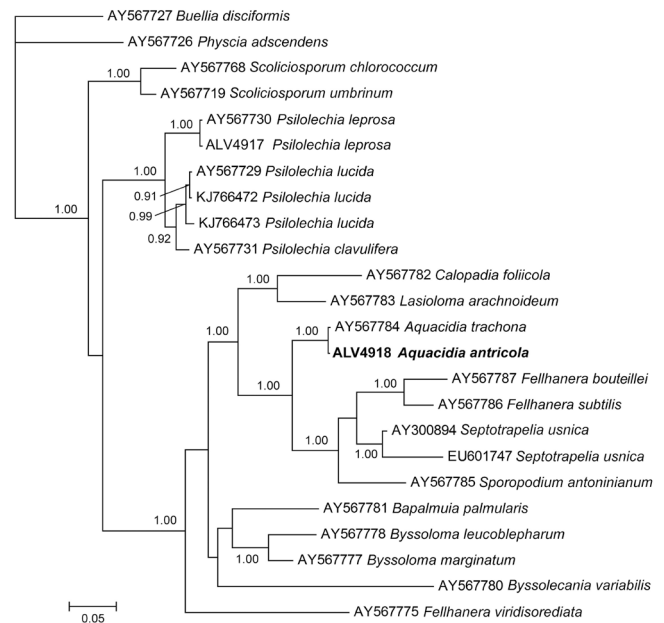


Fig. 1. Consensus phylogram obtained from Mr.Bayes after the analysis of a mtSSU alignment. Values next to nodes represent Bayesian PP, only those > 0.90 PP are annotated. Bold names come from new samples analyzed in the present study. Taxon names are preceded by the Genbank accession number.

with significant support: the monogeneric clades *Scoliciosporum* (Scoliciosporaceae) and *Psilolechia* (Psilolechiaceae), and a monophyletic lineage containing the Pilocarpaceae genera *Bapalmua*, *Byssolecania*, *Byssoloma*, *Calopadia*, *Fellhanera*, *Lasioloma*, *Septotrapelia*, and *Sporopodium*, as well as two of the *“Bacidia”* species that are the subject of our study. The three main phylogenetic lineages in our results (Fig. 1) match the findings of Andersen & Ekman (2005).

The mtSSU sequence of *Psilolechia leprosa* is closely related to a sequence which has also been identified as *P. leprosa* (AY567730), and to a lesser extent to those of other species of this genus, such as *P. lucida* and *P. clavulifera*. The mtSSU sequence of *“Bacidia” antricola* shows a significant relationship with the only sequence of *“Bacidia” trachona* (AY567784) in GenBank, but differs in about 14% of the nucleotide positions (mostly large insertions), supporting the idea that both represent phylogenetically related but independent species. These data suggests that (1) *“B.” antricola* is related to *“B.” trachona*, and (2) that both species differ significantly from the type species of this genus, *Bacidia rosella*, and hence deserve to be placed into a different genus. This conclusion seems to agree with Coppins & Aptroot's (2009) observations, who already noticed that *“B.” antricola*, *“B.” trachona* and *“B.” viridifarinosa* share many morphological characters and secondary metabolites. Hence, we propose here the creation of the new genus *Aquacidia* to accommodate these three taxa.

***Aquacidia*** Aptroot, gen. nov. — MycoBank 824168

**Diagnosis:** Pilocarpaceae with thalli extending; paraphyses branched, with widened, elongatedly clavate tips; ascospores bacillar, (0–)3(–5)-septate; pycnidia often present, often with open ostiole.

**Type:** *Aquacidia trachona* (Ach.) Aptroot (holotype)

Lichenized ascomycetes in the Pilocarpaceae. Thalli extending, often covering waste areas of substrate. Photobiont cells small

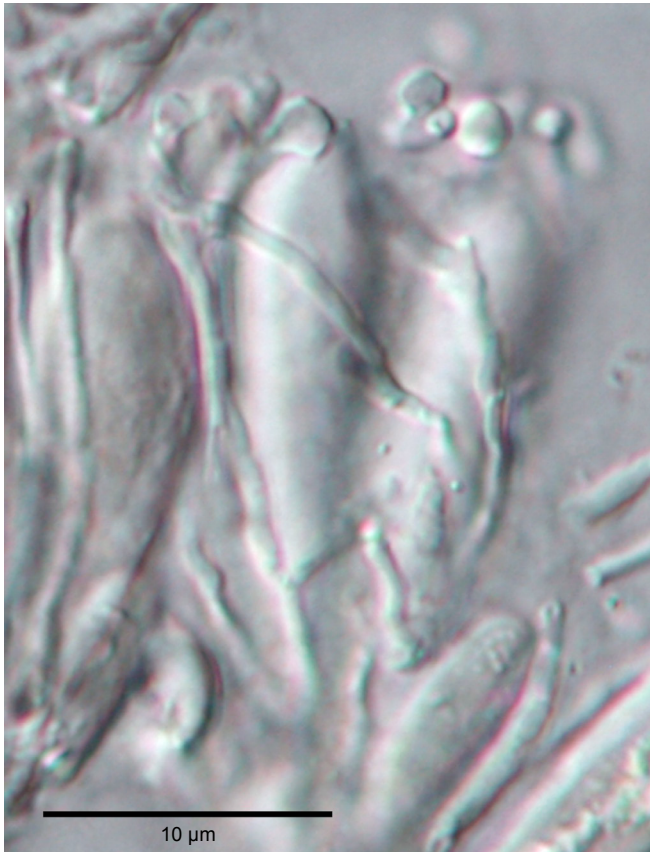


Fig. 2. *Aquacidia trachona* (Scotland, B.J. & A.M. Coppins 21699, E), paraphyses.

(less than 10 µm). Apothecia rarely present, lecideoid, flat, with persistent margin. Asci of the Pilocarpaceae-type. Paraphyses branched, with widened, elongatedly clavate tips. Ascospores eight per ascus, bacillar, hyaline, (0–)3(–5)-septate. Pycnidia often present, relatively large and conspicuous, often with wide open ostiole. Conidia oblong to bacilliform, often biguttulate or with a median constriction.

Secondary chemistry — Secondary metabolites always present; thallus with xanthenes (UV+ pink) or argopsin (Pd+ red); apothecia and pycnidia sometimes with anthraquinones (K+ purple).

Distribution and Ecology — So far three species are known, from Europe and North America. Species of this group occur on rocks, soil, tree bark and roots, close to rivers or lakes, often in wooded valleys (see e.g. Boom et al. 1998, Coppins & Aptroot 2009, Wirth et al. 2013). In The Netherlands, these species are frequent on sheltered rock (usually brick, granite or basalt) forming large colonies in deep crevices between the boulders on dikes along rivers and lakes (Herk et al. 2018). Frequently, two or three species can be found growing together.

Etymology — The name refers to the habitat of the species, which occur mostly on rocks near water.

***Aquacidia antricola*** (Taylor) Aptroot, comb. nov. — MycoBank 824169

Basionym: *Bacidia antricola* Hulting, Lich. Exkurs. Vestra Becking: 17 (1872).

*Variolaria torta* Taylor, in Mackay, Fl. Hibern. 2: 114 (1836); nom. rej. prop. by Svensson et al. (2017).

*Lecidea carneoglauc*a Nyl., Flora 56 (19): 295 (1873); *Bacidia carneoglauc*a (Nyl.) A.L. Sm., Monogr. Brit. Lich. 2: 155 (1911).

*Lecidea byssoboliza* Nyl., Flora 62: 206 (1879). — *Bilimbia byssoboliza* (Nyl.) A.L. Sm., Monogr. Brit. Lich. 2: 141 (1911). — *Bacidia byssoboliza* (Nyl.) H. Olivier, Bull. Géogr. Bot. 21: 170 (1911).

See Svensson et al. (2017) for details on the nomenclature on this taxon.

***Aquacidia trachona*** (Ach.) Aptroot, comb. nov. — MycoBank 824170; Fig. 2

Basionym: *Verrucaria trachona* Ach., Methodus, Suppl.: 16 (1803) — *Bacidia trachona* (Ach.) Lettau, Hedwigia 52: 133 (1912).

***Aquacidia viridifarinsa*** (Coppins & P. James) Aptroot comb. nov. — MycoBank 824171

Basionym: *Bacidia viridifarinsa* Coppins & P. James in Coppins et al., Lichenologist 24: 353 (1992).

**KEY TO THE SPECIES OF AQUACIDIA**

- 1. Apothecia and pycnidia dark, K+ purple; thallus Pd+ red, UV-negative . . . . . *A. trachona*  
 – Apothecia and pycnidia pallid, K-; thallus Pd-, UV+ pink 2
- 2. Thallus soresiate . . . . . *A. viridifarinsa*  
 – Thallus not soresiate . . . . . *A. antricola*

**DISCUSSION**

The new genus *Aquacidia* fits well in the Pilocarpaceae, both phylogenetically and morphologically. At the moment, 30 genera are accepted within this family, and up to ten of them are monotypic. The family is abundant and diverse in the tropics, especially on living leaves (Lücking 2008). In temperate regions, only a few species of a few genera occur, viz. of *Byssoloma* Trevis., *Fellhanera* Vězda, *Fellhaneropsis* Sérus. & Coppins, and especially *Micarea* Fr. Molecular and morphological data support Coppins & Aptroot's (2009) observations that the three *Aquacidia* species are closely related. The three species also share a similar ecology.

Preliminary phylogenetic studies of the family Pilocarpaceae (Ekman 2001; Andersen & Ekman 2005) showed that the genera *Fellhanera* and *Micarea* are polyphyletic in their current delimitation. Taxa within these two genera can be placed in other genera once the phylogenetic position of the type species of *Fellhanera* and *Micarea* becomes known. *Aquacidia* forms a morphologically distinctive genus closely resembling the *Fellhanera viridisorediata*-group (to which the type of the genus *Fellhanera*, *F. fuscatula* (Müll. Arg.) Vězda, most probably belongs) except for two features. (1) In *Aquacidia* the paraphyses are clavate (Fig. 2), where they are never apically thickened in *Fellhanera*. (2) The *Aquacidia* species always have a secondary chemistry of xanthenes or anthraquinones, while most *Fellhanera* species are devoid of secondary substances, and none have xanthenes or anthraquinones; only usnic acid and zeorin are occasionally present (Lücking 2008) in species that belong to the *Fellhanera bouteillei*-group.

In this paper, a morphologically distinct group of Pilocarpaceae was removed from *Bacidia*, but much more work is necessary to make *Bacidia* monophyletic. Nested within *Bacidia* are several

clusters of species that are currently placed in microsquamulose Ramalinaceae genera, with large genera such as *Bacidiopsis* Kalb and *Phyllopsora* Müll. Arg. These microsquamulose genera have either to be merged with *Bacidia* or both *Bacidia* and these genera have to be subdivided into several separate genera.

**Acknowledgements** – The curator of E. D.J. Harris, is warmly thanked for sending fertile specimens from all three species on loan.

## REFERENCES

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25: 3389–3402.
- Andersen HL & Ekman S. 2005. Disintegration of the Micareaeaceae (lichenized Ascomycota): a molecular phylogeny based on mitochondrial rDNA sequences. *Mycol. Res.* 109: 21–30.
- Boom PPG van den, Sérusiaux E, Diederich P, Brand A, Aptroot A & Spier L. 1998. A lichenological excursion in May 1997 near Han-sur-Lesse and Saint-Hubert with notes on rare and critical taxa of the flora of Belgium and Luxembourg. *Lejeunia* 158: 1–58.
- Coppins BJ & Aptroot A. 2009. *Bacidia* De Not. 1846. In: Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW & Wolseley PA, The lichens of Great Britain and Ireland: 189–207. The British Lichen Society, London.
- Ekman S. 2001. Molecular phylogeny of the Bacidiaceae (Lecanorales lichenized Ascomycota). *Mycol. Res.* 105: 783–797.
- Herk CM van, Aptroot A & Sparrius LB. 2018. Veldgids korstmossen. KNNV Uitgeverij, Zeist.
- Lücking R. 2008. Foliicolous lichenized fungi. *Fl. Neotrop.* 103: 1–866.
- Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M. 2004. Supraordinal phylogenetic relationships of Lecanoromycetes based on a Bayesian analysis of combined nuclear and mitochondrial sequences. *Mol. Phylogenet. Evol.* 31: 822–32.
- Murray MG & Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8: 4321–4325.
- Nylander JAA. 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Uppsala.
- Ronquist F & Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Svensson M, Ekman S, Klepsland JT, Nordin A, Thor G, Hirschheydt G von, Jonsson F, Knutsson T, Lif M, Spribille T & Westberg M. 2017. Taxonomic novelties and new records of Fennoscandian crustose lichens. *MycoKeys* 25: 51–86.
- Swofford DL. 2001. PAUP\*4.0b10: phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molec. Biol. Evol.* 28: 2731–2739.
- Wirth V, Hauck M & Schultz M. 2013. Die Flechten Deutschlands. Ulmer, Stuttgart.
- Zoller S, Scheidegger C & Sperisen C. 1999. PCR Primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming Ascomycetes. *Lichenologist* 31: 511–516.