

Secondary conidia observed in *Bartheletia paradoxa*

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Bartheletia paradoxa is an enigmatic microscopic basidiomycete restricted to leaves of ginkgo (*Ginkgo biloba*) in litter. This species forms a basal lineage to Agaricomycotina and its life cycle and ultrastructure are unique among members of this subdivision. To date, it has been recorded mostly in the litter of ginkgos planted as ornamental trees in gardens and parks. In this paper we report new collections of this fungal species and also a new phenotypic phenomenon in its life cycle, i.e. the formation of secondary conidia. They are oval to ovoid, hyaline and are formed terminally on stalks growing out of the conidia. The function of these secondary conidia as actively discharged ballistoconidia is discussed. They are supposed to enhance either short- or long-range dispersal of *B. paradoxa*.

Key words: ginkgo leaves, LSU and SSU rDNA, Agaricomycotina, *Bartheletiaceae*.

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Bartheletia paradoxa je podivný mikroskopický bazidiomycet vyskytující se pouze na listech jinanu (*Ginkgo biloba*) v opadu. Tento druh tvoří bazální linii v Agaricomycotina, přičemž jeho životní cyklus a ultrastruktura jsou jedinečné mezi zástupci tohoto pododdělení. Až doposud byl zaznamenán převážně v opadu jinanů vysazovaných jako okrasné stromy v zahradách a parcích. V této práci uvádíme nové sběry tohoto druhu a také nový fenotypový fenomén v životním cyklu – tvorbu sekundárních konidií. Jsou oválné až vejčité, hyalinní a tvoří se terminálně na stopkách vyrůstajících z konidií. Funkce těchto konidií jakožto aktivně odstřelovaných balistokonidií je diskutována; pravděpodobně napomáhají rozšiřování druhu *B. paradoxa* na kratší, případně i delší vzdálenosti.

INTRODUCTION

Bartheletia paradoxa Arnaud ex Scheuer was invalidly described (without a Latin diagnosis and holotype designation) by Arnaud (1954). The species has received very little attention ever since, until Scheuer et al. (2008) validated its description and provided a first comprehensive treatment of its life cycle,

ultrastructure and phylogenetic placement. According to Scheuer et al. (2008), most of the life cycle is completed in a rather short time (usually between October and early December). Thick-walled teliospores (resting spores, probasidia) undergo hibernation and aestivation and germinate in autumn with long-stalked, longitudinally septate phragmobasidia. The basidia produce numerous unicellular basidiospores which colonise freshly fallen ginkgo leaves. The mycelium develops in leaf parenchyma and produces the anamorph. Cushion-like conidiomata (sori) protrude the leaf epidermis and expose densely packed conidiophores producing numerous cylindrical slimy conidia infecting other leaves. The short phase of mass reproduction is terminated by formation of teliospores which replace the conidiophores in the conidiomata. Eventually, single spherical teliospores [mentioned by Arnaud (1954) as “globular cysts”] are also produced in the leaf tissue. The teliospores then remain dormant until the next autumn.

As far as we know, the distribution of *B. paradoxa* includes European countries (Austria, Denmark, France, Germany, Russia, Sweden, The Netherlands, United Kingdom), Korea and Japan (Kirschner & Okuda 2012). In autumn 2014, this species was collected in Prague (Czech Republic) and at several locations in Hesse (Germany). During morphological examination of the anamorph, formation of a hitherto unknown type of spores was observed. The main objective of this contribution is to present a description and discussion of the morphology and function of these spores.

MATERIAL AND METHODS

Freshly fallen ginkgo leaves were collected from litter under solitary trees in the Botanical Garden of the Faculty of Science, Charles University in Prague (Czech Republic; further abbreviated as CUP) and at several localities in Hesse (Germany). Leaves were observed under a dissecting microscope. Slides for microscopy were mounted in tap water, 3% KOH and Melzer reagent. Microscopic structures were examined and documented with differential interference contrast (Olympus BX-51 with digital camera, Quick Photo software, Olympus, Japan). Representative specimens were deposited in PRC (Herbarium of the CUP, Czech Republic) and in the private collection of the second author (abbreviated HLW in this study).

DNA was extracted from five mature conidial sori using a Zymo Research Fungal/Bacterial Kit (Zymo Research, Orange, USA). Sori were sampled under a dissecting microscope using a sterile needle and put directly into the lysis tube provided in the kit. Nuclear rDNA containing the 28S region was amplified with primer set NL1/NL4 (O'Donnell 1993) and the 18S region was amplified with primer set NS1/NS4 (White et al. 1990). The PCR products were viewed by means

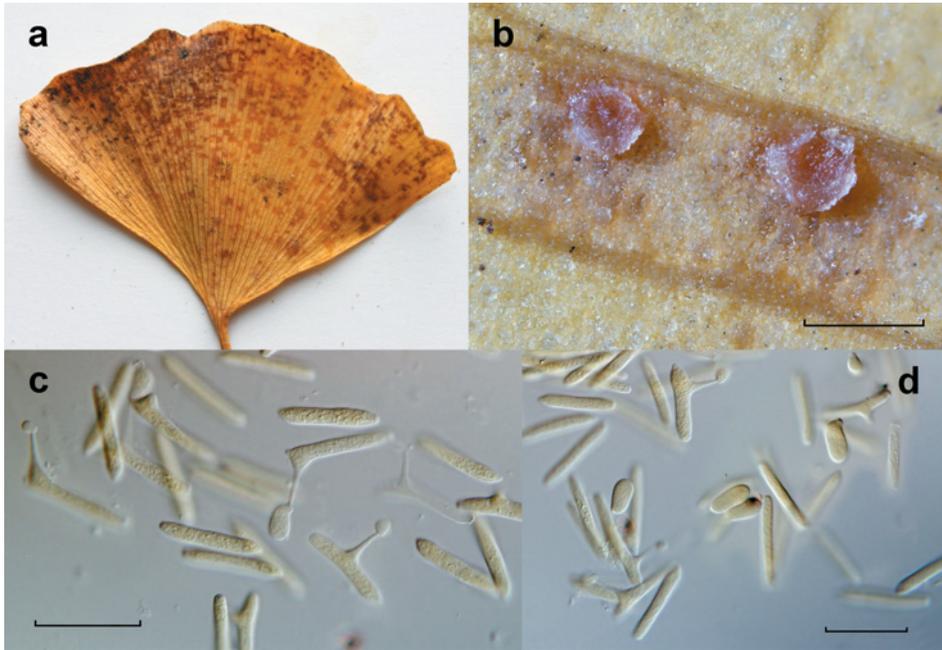


Fig. 1. *Bartheletia paradoxa* (PRC 3336): **a** – ginkgo leaf from fresh litter with numerous conidiomata; **b** – detail of two conidiomata protruding leaf epidermis (scale bar = 500 µm); **c, d** – conidia producing secondary conidia on top of a stalk, mature detached secondary conidia (scale bar = 20 µm). Photo O. Koukol.

of electrophoresis on 1% (w/v) TAE agarose gel, stained with ethidium bromide. The PCR products were purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). Both strands of the PCR fragments were sequenced with the primers used for amplification in the Sequencing Laboratory (Faculty of Science, CUP, Czech Republic).

RESULTS AND DISCUSSION

Phenotypic description and function of *Bartheletia* spores

In specimens from Germany, both anamorph and teleomorph were observed. In the specimen from the Czech Republic only the anamorph was recorded (Fig. 1). The morphology of the conidiomata with conidia and telia with teliospores matched the description given in Scheuer et al. (2008) and also in Kirschner & Okuda (2012). We do not describe it here, but only schematically depict the tenta-

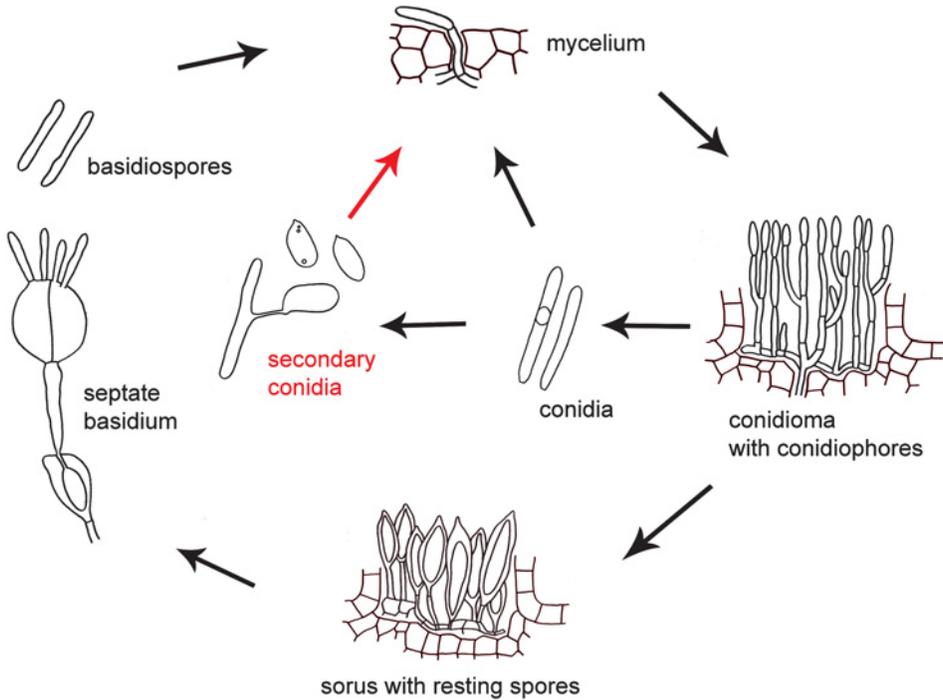


Fig. 2. Tentative life cycle of *Bartheletia paradoxa* growing in ginkgo leaves in litter (drawings from observation under a light microscope, size not to scale). The newly discovered secondary conidia and their supposed function are in red. The sorus with resting spores hibernates and aestivates for about 11 months, the rest of the life cycle is completed within one month. (Single teliospores in the leaf tissue were omitted for simplicity.) Drawing H. Lotz-Winter.

tive life cycle with a new type of spores observed in this study (Fig. 2). In the Czech specimen as well as in one collection from Germany (HLW 2097) conidia were observed to produce another type of spores. One-celled, thin-walled, hyaline, oval to ovoid secondary conidia, 8–12 µm long and 4–6 µm wide, were formed on a stalk (3)6–8 µm long growing laterally or subapically out of the conidium (Fig. 1c, d). The most striking feature is their asymmetrical shape in lateral view and the prominent lateral hilum on the basis resembling basidiospores of Agaricomycetes or ballistoconidia which can be found in yeast fungi belonging to all subphyla within the Basidiomycota (Boekhout et al. 2011, Wang & Wang 2015).

The function of the secondary conidia at *B. paradoxa* is not clear. Their active discharge has not yet been observed and the droplet mechanism (Kluyver & van Niel 1924) was not proven. Their production on top of a long stalk is also similar to blastoconidia known in *Fellomyces* and *Kockovaella* (Tremellomycetes) as well as *Kurtzmanomyces* (Agaricostilbomycetes), but these blastoconidia are

variable in shape, without active discharge, and stalks may proliferate to produce more blastoconidia (Sampaio et al. 1999). Without direct evidence of the active discharge, we assume that they serve as ballistoconidia. A future survey aimed at the function of the secondary conidia should also elucidate their ability to form further secondary conidia in repetition. Within basidiomycetes, repetitive germination was observed in ballistoconidia of *Bensingtonia ciliata* Ingold (Atractiellomycetes; Ingold 1986) and frequently in basidiospores of members of Pucciniomycetes (Mims & Richardson 1990).

Scheuer et al. (2008) assumed that conidia formed in slimy masses (and potentially also the basidiospores, due to the adhesive surface) may be responsible for a long range dispersal by adhering to insects or other animals. Presumably, the newly discovered secondary conidia may allow the fungus to actively colonise neighbouring leaves in the litter, but may also travel over longer distances by means of air currents. The secondary conidia may be easily overlooked and seem to be formed only temporarily, depending most probably on microclimatic conditions, i.e. humidity.

Molecular data

The sequence of 18S rDNA was identical to an unpublished sequence of *Bartheletia paradoxa* kindly sent by M. Grube (University of Graz, Austria). The identification of *B. paradoxa* based on phenotypic characteristics was also supported by molecular data. Together with the sequence of 28S rDNA they were deposited in GenBank with Accession Numbers LT560339 and LT560340, respectively.

Occurrence and site conditions

Ginkgo trees are commonly planted as ornamental trees in various public and private gardens and parks. Although *B. paradoxa* is restricted to this host, this fungus may not always be present. Three aged ginkgo trees are grown in the Botanical Garden of CUP, but *B. paradoxa* was found only under one of them, even though the trees are not more than 100 m apart. This seems to be caused above all by different maintenance of the sites and by microclimatic conditions as already suggested by Scheuer et al. (2008). The *B. paradoxa* tree is situated in a less maintained part of the garden, close to a wall with a dense growth of *Hedera helix*. Fallen ginkgo leaves remain in the litter for the whole year in the shade of lower branches of *H. helix* and the wall protects the environment from desiccation. The two trees without *B. paradoxa* are situated in the centre of a well-managed lawn, where fallen leaves are regularly removed during autumn. Therefore, the life cycle of *B. paradoxa* cannot be completed here, even when the leaves are infected by spores from a nearby tree.

Specimens examined

Czech Republic. Bohemia. Prague, Botanical Garden of the Faculty of Science, CUP, NE corner, in litter of *Ginkgo biloba* growing close to a wall surrounding the garden, 50°04'16.98" N, 14°25'22.90" E, alt. 223 m, 4 Dec 2014, leg. & det. O. Koukol (PRC 3336).

Germany. Hesse. Moerfelden-Walldorf, private garden, in litter of *G. biloba*, 50°00'33.92" N, 8°34'26.07" E, alt. 101 m, 4 Dec 2014, leg. & det. H. Lotz-Winter (HLW 2097). – Frankfurt am Main, Palmengarten, in litter of *G. biloba* in the „Goethegarten“, 50°07'29.87" N, 8°39'24.47" E, alt. 115 m, 27 Mar 2011, leg. & det. H. Lotz-Winter (HLW 2001). – Frankfurt am Main, Botanical Garden, in litter of *G. biloba* near the main entrance, 50°07'35.00" N, 8°39'28.26" E, alt. 118 m, 27 Mar 2011, leg. & det. H. Lotz-Winter (HLW 1023).

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