Phialocephala trigonospora, a new hyphomycete species associated with conifericolous bark beetles

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A new species of the hyphomycete genus *Phialocephala* was isolated from conifericolous bark beetles and their galleries in Germany. The species differs from all known species of this genus by its conidia which have a triangular shape. The development of the conidia was investigated with scanning and transmission electron microscopy.

Keywords: *Phialocephala*, fungi, Scolytidae, conifers, conidial development

The hyphomycete genus *Phialocephala* was erected by Kendrick (1961). Its species are characterized by a pigmented stipe, a terminal sporogenous head formed by penicillately arranged metulae and phialides, and one-celled conidia accumulated in a slimy head. The known species were keyed out by Onofri & al. (1994), two further species were described by Kowalski & Kehr (1995).

Within the genus *Phialocephala*, there is only one report on *P. phycomyces* (Auersw.) Kendrick from the galleries of several species of bark beetles [Mathiesen-Käärik, (1953), as "*Scopularia phycomyces*" (Auersw.) Goid.], a poorly investigated habitat of fungi. These bark beetles (Coleoptera, Scolytidae) were collected from stems of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) (Mathiesen-Käärik, 1953). *Leptographium* Lagerb. & Melin is a similar genus containing species which are constantly associated with bark beetles (Harrington, 1988; Wingfield & Gibbs, 1991). The two genera differ by the annellidic conidiogenesis in *Leptographium* and the phialidic conidiogenesis in *Phialocephala* (Kendrick, 1961). Species of both genera were re-evaluated with detailed examinations of the conidial development by Wingfield & al. (1987). Annellations of the conidiogenous cells are often hardly visible by light microscopy. Therefore, the conidiogenesis of the new species was studied by scanning and transmission electron microscopy (SEM and TEM).

Material and methods

During a survey of microfungi in bark beetle galleries in Germany, bark of *Pinus sylvestris* and *Picea abies* containing bark beetle imagines and their galleries was collected in September, 1994, near Rohrbach (Odenwald), in June, 1995, near Grasellenbach (Odenwald), and in April and August, 1996, near Darmstadt-Eberstadt at the western border of the Odenwald (Hessen).

The beetles were identified as *Dryocoetes autographus* (Ratz.), *Hylurgops palliatus* (Gyll.), *Ips typographus* (L.) and *Orthotomicus laricis* (F.). Living beetles were individually placed in Petri dishes containing autoclaved pieces of inner spruce bark embedded in water agar. Fungi growing out from the beetles in mixed cultures were examined with the help of a dissecting microscope. Conidia of the fungi were aseptically removed with a fine needle from conidiophores growing in the mixed cultures on agar and also from conidiophores found in a bark beetle gallery. The conidia were placed on 2% MEA (Difco malt-extract). The fungus described herein was grown in diffuse daylight at room temperature (approx. 22 C). Measurements and drawings were made from freshly prepared material mounted in water.

For studies by SEM and TEM, pieces of the cultures were cut out of the agar, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for several days, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for one hour and washed with distilled water. The material for SEM was dehydrated in an ethanol series and critical point dried. After coating with gold palladium, the fungus was examined by using a Cambridge Stereoscan 250 MK 2 scanning electron microscope.

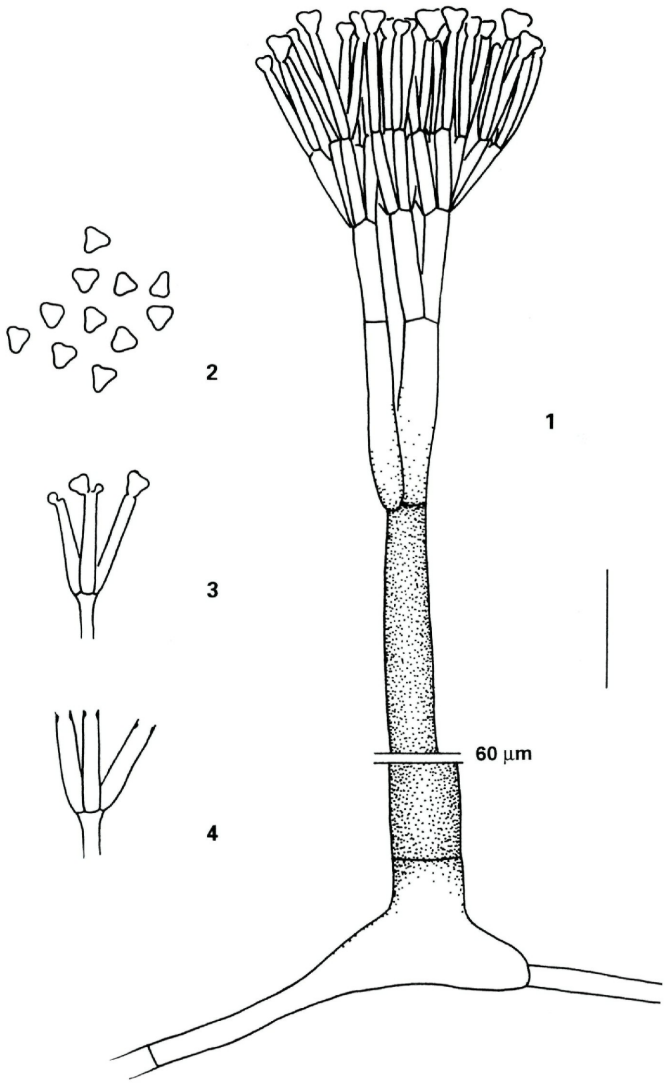
The material for TEM studies was stained in 1% uranyl acetate, washed with water, dehydrated in an acetone series and embedded in ERL (Spurr, 1969). Sections of 60 nm were cut with a diamond knife, mounted on copper grids coated with Formvar, and stained in lead citrate (Reynolds, 1963) for 10 min. The sections were examined with a Zeiss EM 109 transmission electron microscope.

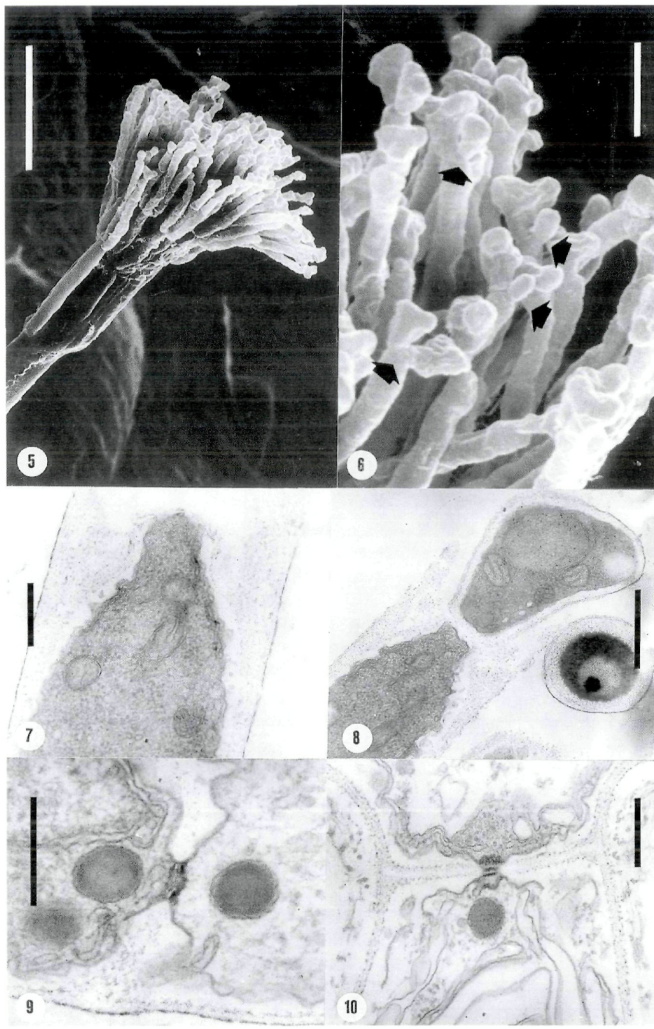
Results and discussion

Phialocephala trigonospora Kirschner & Oberwinkler sp. nov. – Figs. 1–10.

In agaro maltoso coloniae tarde crescentes, post 30 dies 15–20 mm diam., cremeae. Mycelium aerium perpaucum. Mycelium immersum septatum, hyalinum,

Figs. 1–4. *Phialocephala trigonospora*, CBS 100161 (ex type culture). – 1. Conidiophore. – 2. Conidia. – 3. Young phialides. – 4. Old, collapsed phialides with apical wall-thickenings. – Scale bar = 10 µm.





hyphae 1 µm diam. Conidiophora macronemata, solitaria, erecta, cum apparato conidiogeno 70–185 µm longa. Stipes brunneus, 2–6-septatus, ad basim 5–9 µm latus, sub apparato conidiogeno 3–5 µm latus. Apparatus conidiogenus ex 2–3 seriebus metularum compositus, ad basim brunneus vel hyalinus. Metulae primariae 10–21 × 2–4 µm, metulae secundariae 8–12 × 2 µm, metulae tertiariae 7–9 × 1.5–2 µm. Cellulae conidiogenae phialidicae, cylindricae, hyalinae, sine lato collo, 8–15 × 1–1.5 µm. Conidia triangularia, plana, unicellularia, 2–2.5 µm longa, 1 µm crassa, hyalina, in capitulum mucosum ad apicem conidiophori aggregata, capitulum mucosum, album, 20–200 µm diam.

Holotypus: CBS 100161 (cultura sicca). In cuniculis *Orthotomici laricis* in cortice interno *Pini sylvestris*, Germania, Odenwald, Rohrbach, 300 m, 5. 9. 1994, R. Kirschner.

Culturae vivae: CBS 100161, CBS 100162.

Colonies on malt extract agar slowly growing, reaching a diameter of 15–20 mm in 30 days, cream coloured. Aerial mycelium poorly developed. Immersed hyphae septate, hyaline, 1 µm diam. – Conidiophores macronematous, single, erect, including conidiogenous apparatus 70–185 µm long. Stipe brown, 2–6 septate, 5–9 µm wide at the base, 3–5 µm thick below the conidiogenous apparatus. – Sporogenous heads brown or hyaline at the base, consisting of 2–3 series of metulae. Primary metulae 10–21 × 2–4 µm, secondary metulae 8–12 × 2 µm, tertiary metulae 7–9 × 1.5–2 µm. – Conidiogenous cells monophialidic, cylindrical, hyaline, without deep collarettes, 8–15 × 1–1.5 µm. – Conidia one-celled, triangular, 2–2.5 µm in face view, flat, 1 µm in side view, hyaline, aggregated in a white slimy mass of 20–200 µm diam. at the apex of the conidiophore.

Habitat. – Galleries of bark beetles (*Dryocoetes autographus*, *Hylurgops palliatus*, *Ips typographus*, *Orthotomicus laricis*) of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*).

Known distribution. – Germany, Odenwald.

Etymology. – Named after the triangular outline of the conidia.

This species belongs to the genus *Phialocephala* Kendrick because of the pigmented, mononematous stipe with a head composed of series of metulae and phialides which produce one-celled conidia in a slimy mass (Figs. 1, 5). In old, collapsed conidiogenous cells, apical wall-thickenings are visible by light microscopy indicating a phialidic conidial development by a succession of replacement wall-building apices according to Minter & al. (1983) (Fig. 4). The TEM

Figs. 5–10. *Phialocephala trigonospora*, CBS 100162, seen by SEM and TEM. – 5. Conidiophore, SEM. – 6. Conidiogenous head, phialides often with two conidia attached to the distal ends (arrows), SEM. – 7–8. Longitudinal sections through phialides, TEM. – 9–10. Hyphal septa with pores, Woronin bodies and pore occlusions, TEM. – 9. Septal pore with interrupted electron-transparent bands. – 10. Septal pore with continuous electron-transparent bands in the pore occlusion. – Scale bars: 5 = 10 µm, 6 = 2 µm, 7 = 0.2 µm, 8 = 0.5 µm, 9 = 0.25 µm, 10 = 0.2 µm.

photographs show that the phialides have small collarettes and apical wall-thickenings (Figs. 7, 8). Annellations of the conidiogenous cells were not found.

The lack of a deep collarette at the opening of the phialides (Figs. 3, 4) is not typical for the genus, but three further species, *P. humicola* Jong & Davis, *P. phycomyces*, and *P. queenslandica* Matsushima also lack a deep collarette (Onofri & al., 1994). A further detail of the conidiogenesis in *P. trigonospora* was detected by light microscopy and SEM. After the development of the first conidium, a second conidium develops laterally at the tip of the same phialide while the first conidium is still attached (Figs. 3, 6). This mode of conidiogenesis is similar to that of *P. gabalongii* Sivasithamparam (1975) and *P. humicola* (Onofri & al., 1994). *P. trigonospora* differs from all known species of the genus by the triangular shape of its conidia (Fig. 2).

Tab. 1. – *Phialocephala trigonospora* infestation of bark beetles collected from *Picea abies* and *Pinus sylvestris* in 1995 and 1996. Samples from trees without *P. trigonospora* are not included.

Tree no.	Tree species	Bark beetle species	No. of beetles infested with *P. trigonospora*	% of beetles infested with *P. trigonospora*	Total no. of beetles examined per tree
1	*P. abies*	*Dryocoetes autographus*	2	29%	7
1	*P. abies*	*Ips typographus*	4	18%	22
2	*P. abies*	*Hylurgops palliatus*	1	6%	18
3	*P. sylvestris*	*Orthotomicus laricis*	10	23%	44
4	*P. sylvestris*	*Orthotomicus laricis*	2	7%	30
5	*P. sylvestris*	*Orthotomicus laricis*	3	25%	12
6	*P. sylvestris*	*Orthotomicus laricis*	1	2%	45

Woronin bodies, typical for ascomycetes, are associated with the septal pores of *P. trigonospora* (Figs. 9, 10). In some septa, the pores are occluded by up to four transversal electron-dense bands alternating with up to three electron-transparent bands (Fig. 10). The electron-transparent bands are continuous (Fig. 10) or centrally interrupted (Fig. 9). The electron-transparent bands are not continuous with the edges of the pore because the edges are surrounded by a continuous plasmalemma (Fig. 9). Similar septal pore occlusions are known from several ascomycetes (Curry & Kimbrough, 1983). The question whether the interrupted electron-transparent bands are a preceeding stage of the continuous ones (Hammill, 1974) or whether both kinds are parts of the same pore but sectioned at different sectional planes (Trinci & Collinge, 1973) is not yet resolved (Markham, 1994). This septal type, called "*Neurospora* septal type" by Curry &

Kimbrough (1983), was reported from different orders of the ascomycetes and does not allow a systematic conclusion.

P. trigonospora was isolated from 23 bark beetles and one gallery from only one area (Hessen, Odenwald). The fungus was not found in other investigated areas in South Germany (Kirschner, unpublished). *P. trigonospora* was isolated from four different species of bark beetles infesting *Pinus sylvestris* and *Picea abies*, but was obtained only from a small number of beetle individuals collected per tree (Tab. 1). In some samples, *P. trigonospora* was not found at all. Therefore, the fungus probably has a local distribution and is not consistently associated with bark beetles.

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