A new species of *Colacogloea* with zygoconidia*

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Kirschner, R. & F. Oberwinkler (2000). A new species of *Colacogloea* with zygoconidia. – Sydowia 52 (2): 195–203.

A fungus isolated from bark beetles infesting conifers in Germany is described as the new species *Colacogloea papilionacea* R. Kirschner & Oberw. It differs from the known species of *Colacogloea* by the production of zygoconidia. Colacosomes were detected by studies with transmission electron microscopy and the host fungus was assigned to the ascomycetes.

Keywords: *Colacogloea*, colacosomes, heterobasidiomycetous fungi, mycoparasitism, simple septal pores.

The genus *Colacogloea* Oberw. & Bandoni was segregated from the heterogeneous genus *Platygloea* J. Schröt. by Oberwinkler & al. (1990) mainly because of the presence of colacosomes in *Colacogloea peniophorae* (Bourdot & Galzin) Oberw. & Bandoni (= *Platygloea peniophorae* Bourdot & Galzin). Colacosomes are organelles known in certain mycoparasitic heterobasidiomycetous fungi (Bauer & al., 1997; Kirschner & al., 1999) and are visible by transmission electron microscopy showing an electron-dense core that protrudes through the cell walls of the parasite and the host fungus (Bauer & Oberwinkler, 1991). Two species were hitherto assigned to the genus, *C. peniophorae* and *C. bispora* (Hauerslev) Oberw. & R. Bauer, both growing within the hymenia of corticioid basidiomycetes (Oberwinkler & al., 1999). During a survey of fungi associated with bark beetles in Central Europe, a species of *Colacogloea* was found to be carried by some conifericolous bark beetles.

Material and methods

Bark containing old galleries without beetles was peeled of from a standing trunk of *Pinus sylvestris* L. Bark samples containing bark beetles were peeled off from felled stems of *Picea abies* (L.) Karst. A few additional adult beetles were caught from the air by hand.

* Part 186 of a series "Studies in Heterobasidiomycetes"

Beetles were individually placed into Petri dishes containing autoclaved pieces of inner bark of *Picea abies* embedded in 4% water agar. Conidia of the fungus developing in these Petri dishes were aseptically transferred to Petri dishes containing 2% Difco malt extract agar (MEA) and Difco corn meal agar (CMA). Inocula of this fungus were transferred from pure cultures to cultures of *Hormonema dematioides* Lagerb. & Melin and *Phialophora americana* (Nannf.) S. J. Hughes. Transmission electron microscopy (TEM) was conducted as described in Kirschner & Oberwinkler (1998).

Results

In each Petri dish containing a single bark beetle, a mixed culture developed, composed of different microorganisms originating from propagules carried by the beetle. In 7 cultures among some thousands, gelatinous masses were formed by aggregation of spirally coiled hyphae. The lumen enclosed by the hyphal coils contained remnants of decayed hyphal cells (Fig. 1) that were in some cases connected with intact cells. The wall of these cells was composed of an outer electron-opaque layer and an inner electron-transparent layer (Fig. 2). The septa between the cells exhibited simple pores associated with Woronin bodies (Fig. 2). Using TEM, colacosomes and simple septal pores were detected within the hyphae of the fungus producing the coils (Figs. 1, 3, 4). Old septal pores became occluded by secondary wall material (Fig. 5). The colacosomes were arranged at the inner surface of the hyphal coils (Fig. 1). In the original mixed cultures, the masses of hyphal coils were associated with slimy masses of zygoconidia (Fig. 6). Zygoconidia were aseptically transferred to new media to establish pure cultures. Spiral coils were inconspicuous and scattered in pure cultures and did not aggregate in gelatinous masses as in the original cultures. After transfer of inocula to cultures of *H. dematioides* and *P. americana*, hyphae did not coil around hyphae of these fungi. Masses containing coiled hyphae

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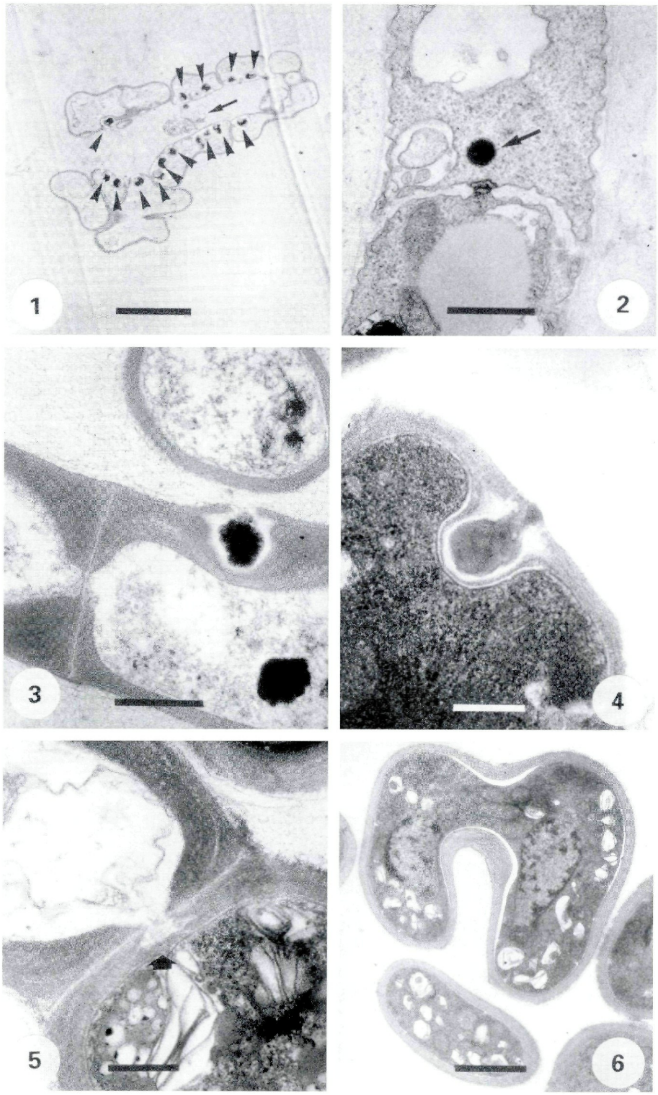
Figs. 1–6. *Colacogloea papilionacea*, ex-type culture, seen by TEM. – 1. Section through hyphal coils. Colacosomes arranged at the inner surface of the hyphal coils (arrowheads). Remnants of the host hypha in the centre of the lumen enclosed by the hyphal coils (arrow). – Scale bar = 3 µm. – 2. Longitudinal section through a host hypha showing a simple septal pore and an associated Woronin body (arrow). Scale bar = 0.5 µm. – 3. Simple septal pore and one colacosome of *C. papilionacea*. – Scale bar = 0.5 µm. – 4. Colacosome of *C. papilionacea*. Scale bar = 0.25 µm. – 5. Longitudinal section through a hypha of *C. papilionacea* with a simple septal pore that is on one side occluded by secondary cell wall material (arrow). – Scale bar = 0.25 µm. – 6. Logitudinal section through a zygoconidium. – Scale bar = 1.0 µm.



and zygoconidia, however, were also found *in situ* in old beetle galleries in the bark of *Pinus sylvestris*.

The zygoconidia developed by fusion of simple, ellipsoid, 3–5 µm long and 1.5–2 µm broad conidia. The conidia bud off in pairs from bent, filiform, transversely septate conidiophores with a length of 20–38 µm and width of 2–3 µm (Figs. 7, 8). The conidiogenous cells were 3–15 µm long and connected by enlarged clamps.

In some of the original cultures and in pure cultures on CMA, auricularioid basidia were also produced (Fig. 9). The basidia formed clusters of two to several basidia, but no hymenia, and developed without any probasidia. – Basidia developed 1 or 3 septa prior to the production of sterigmata up to 20 µm long and 3 µm thick (Fig. 9). Basidiospores were cylindrical or slightly allantoid, with an inconspicuous basal apiculus, and produced secondary spores (Fig. 10). The basidiospores and secondary spores measured 6–18 × 3–5 µm. In one of the original mixed cultures, basidia were found to be supported by the same hyphal system as the conidiophores (Fig. 11).

In pure cultures on CMA and MEA, a unicellular dikaryotic stage was dominant (Figs. 12, 13). The colonies on CMA were white with a powdery surface after a growth of one month, with small, slimy glistening areas. Colonies on MEA, however, were cream coloured, slimy, and had a brain-like wrinkled centre with a powdery surface. On MEA atypical conidiophores exhibited a retarded fusion and detachment of simple conidia (Fig. 14).

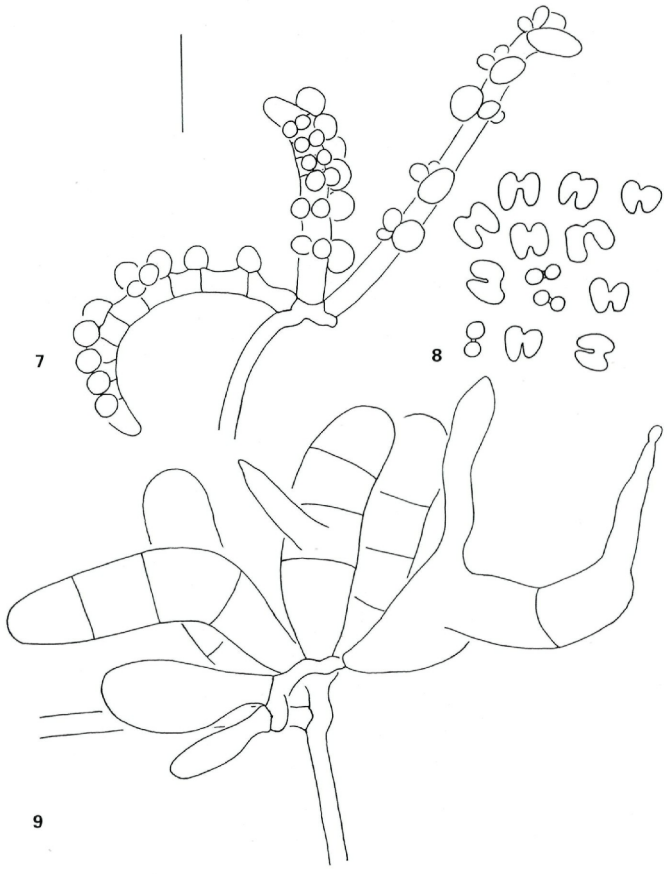
Colacogloea papilionacea R. Kirschner & Oberw. **sp. nov.** – Figs. 1–14

Fructificationes absentes. Hyphae fibulatae, 2 µm diametro. – Basidia transversaliter septata, bicellularia vel quadricellularia, 26–30 × 7–8 µm. Basidiosporae et sporae secundariae allantoideae vel cylindricae, 6–18 × 3–5 µm. Conidiophora hyalina, filiformia, curvata, septata, fibulis inflatis, 20–38 × 2–3 µm. Cellulae conidiogenae intercalares vel terminales. Conidia ellipsoidea, 3–5 × 1.5–2 µm, ante dehiscentiam zygoconidia formantia. Hospes fungus ascomycetum.

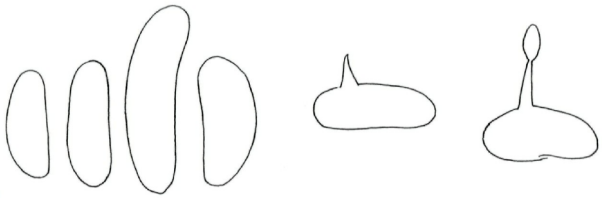
Holotypus. – RoKi 618, dried culture, isolated from the bark beetle *Ips typographus* (L.) infesting a stem of *Picea abies*, Germany, Hessen, Darmstadt-Eberstadt, 5. 4. 1996, leg. R. Kirschner, in TUB.

Etymology. – The Latin epithet papilionacea, butterfly-like, refers to the shape of the zygoconidia.

Basidiocarps not developed. – Hyphae with clamps, 2 µm diam. – Basidia auricularioid, with 1–3 septa, 26–30 × 7–8 µm. – Basidiospores and secondary spores allantoid or cylindrical, 6–18 × 3–5 µm. – Conidiophores hyaline, filiform, curved, septate, with enlarged clamps, 20–38 × 2–3 µm. – Conidiogenous cells



Figs. 7–9. *Colacogloea papilionacea*. – 7. Conidiophores (originating from an individual of *Pityogenes chalcographus* (L.) from the same stem of *Picea abies* as the ex–type culture). – 8. Zygoconidia in lateral and end view (ex–type culture). – 9. Basidia (originating from an individual of *Ips typographus*, different from the ex–type culture, but from the same stem of *Picea abies*). – Scale bar = 10 µm.



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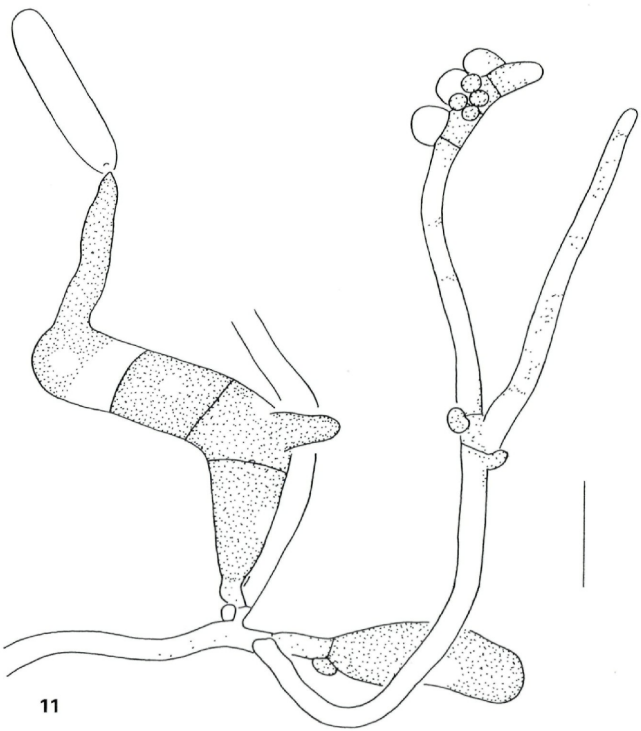

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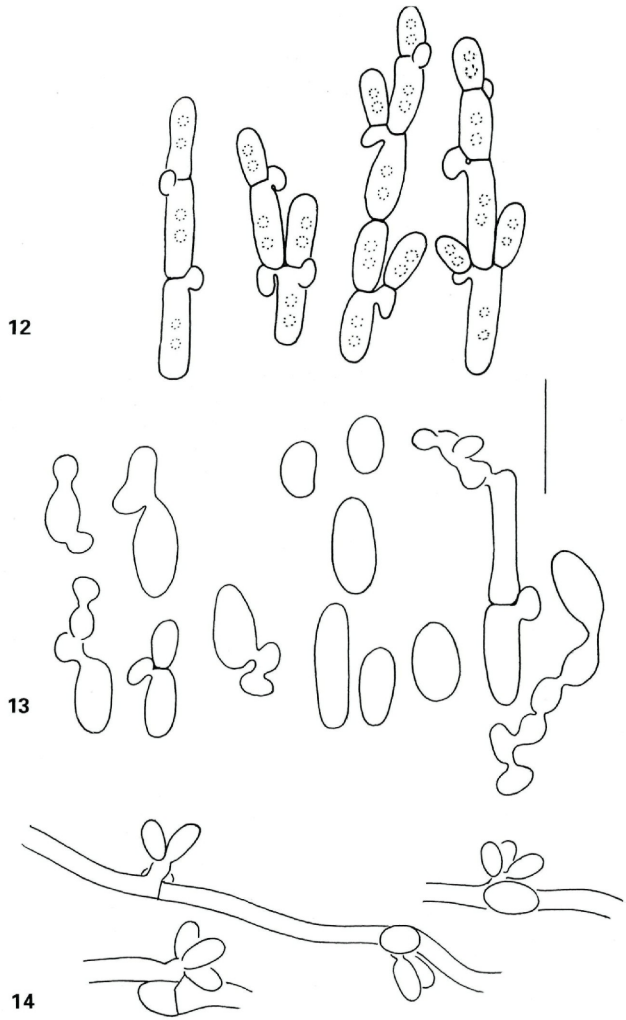
10. Basidiospores and secondary spores (from the same culture as in 9). – 11. Mature and young basidia and conidiophores arising from the same hypha (from the same culture as in 9). – Scale bar = 10 µm.



12. Unicellular stage grown on CMA (nuclei indicated with dotted lines) (ex–type culture). – 13. Unicellular stage grown on MEA (ex–type culture). – 14. Conidiophores developed on MEA (ex–type culture). – Scale bar = 10 μm.

intercalar or terminal. – Conidia ellipsoid, 3–5 × 1.5–2 µm, forming zygoconidia prior to dehiscence from the conidiogenous cells.

Material examined. – (not deposited in a collection because of the scant material or absence of the teleomorph). – cultures originating from 3 individuals of *Ips typographus* (L.) and 3 individuals of *Pityogenes chalcographus* (L.) all removed from the same stem of *Picea abies*, Germany, Hessen, Darmstadt-Eberstadt, 5. 4. 1996; culture originating from 1 individual of *Orthotomicus laricis* (F.), caught from the air, 20. 04. 1996, colonies in old beetle galleries in the bark of *Pinus sylvestris* (only anamorph and hyphal coils), Germany, Hessen, Darmstadt-Eberstadt, 22. 4. 2000, leg. R. Kirschner.

Discussion

The fungus was only carried by a few bark beetles and is, therefore, assumed not to be regularly associated with bark beetles. The auricularioid basidia, colacosomes and simple septal pores justify the placement of this species in the genus *Colacogloea*. *C. peniophorae* differs from this species by producing another type of conidiophore. Basidiocarps do not develop in *C. bispora*, but in contrast to *C. papilionacea*, coiled basidia and apparently vesiculate cells are produced, and no conidial stages were found in *C. bispora* (Oberwinkler & al., 1999). The two species hitherto assigned to *Colacogloea* are parasites developing within basidiocarps of corticioid fungi (Oberwinkler & al., 1990; 1999), whereas the ultrastructure of the septal pores and cell wall indicates the ascomycetous nature of the fungal host of the new species. In descriptions of other species of *Platygloea s. l.*, no conidiophores producing zygoconidia similar to those described here were reported. A species designated as *Achroomyces* sp. by Roberts (1997) was reported to produce zygoconidia. The basidia of this species, however, arise from distinct probasidia (Roberts, 1997).

Zygoconidia were described in anamorphs of species of *Christiansenia* Hauerslev (Oberwinkler & Bandoni, 1982), *Tremella* Pers. (Hauerslev, 1999), *Trimorphomyces* Bandoni & Oberw. (Oberwinkler & Bandoni, 1983), and *Zygogloea* P. Roberts (Roberts, 1994), and in species of the anamorph genera *Anastomyces* W. Wu, B. Sutton & Gange (Wu & al., 1997), *Papilionospora* V. G. Rao & B. Sutton (Rao & Sutton, 1975), and *Syzygospora* G. W. Martin (Oberwinkler & Bandoni, 1982). Mycoparasitic behaviour was reported in members of all these genera, with the sole exception of *Papilionospora*. Teleomorphs are not known for species of *Anastomyces* and *Papilionospora*. The anamorph found in the new species of *Colacogloea* cannot be accommodated in any of these genera because of the exclusively terminal conidiogenous cells and the lack of clamps in species of *Anastomyces* and *Papilionospora*. Among the holomorphic species with

zygoconidia, auricularioid basidia are only known in *Zygogloea* which differs from *Colacogloea* by developing tremelloid haustoria (Roberts, 1994).

Acknowledgments

We thank R. Bauer and M. Wagner-Eha for help with the TEM, F. Albrecht for help with the photographic work, J.-P. Sampaio for his suggestion on using CMA for culturing, R. Bauer for critically reading the manuscript, and S. Wood and I. Reinhold for correcting the English text. The study was supported by the DFG-projects "Organismische Interaktionen in Waldökosystemen" and KI 697/1-2.

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(Manuscript accepted 13th June 2000)