

## ***Camarographium koreanum* sp. nov., a new coelomycete from Korea**

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A new coelomycete found on the bark of twigs of *Cornus kousa* in Seoul, Korea is described on the natural host, and on three artificial media. The fungus is characterized by immersed pycnidial conidiomata 175–300(–450) µm diam and large, cylindrical macroconidia 52–62.5 × 17–19.5 µm, that become muriform and ultimately brown after secession from percurrently proliferating conidiogenous cells. It is placed in the genus *Camarographium* because of the pycnidial conidiomata and structure of the macroconidia.

Keywords: coelomycetes, *Cornus*, new species.

A distinctive coelomycete was collected by the second author during his visit to Seoul, Korea in 2004. The conidiomata were pycnidial and deeply immersed in bark of the host plant, *Cornus kousa* Bürger ex Miq. Microscopic examination revealed a number of features rarely seen in anamorphic fungi, indicating that the affinities of this fungus are with species of *Camarographium* Bubák. It was successfully isolated in pure culture, and sporulates abundantly on various media. The fungus is still only known from the type collection and, although no other collections were available to assess infra-specific variation, the morphological characters were regarded distinctive enough to propose it as a new species.

### **Material and Methods**

Isolates were obtained by transferring single conidia with a micromanipulator on to malt extract agar (MEA, Oxoid) with antibiotics (penicillin 0.3 g/L, streptomycin 0.8 g/L). After conidial germination, mycelia were transferred to oatmeal (OA) and cornmeal

(CMA) agars for further study. Media were prepared according to CBS List of cultures, Fungi and bacteria 35<sup>th</sup> ed. (2001). For study of the colony characteristics and morphological analysis, plates were incubated on the laboratory bench in diffuse daylight at room temperature, and in an incubator under near-ultraviolet light (12 h cycles) at 18°C. Colony colours were described according to Rayner (1970). Microscopic measurements were taken from material mounted in water, and drawings were made with a drawing tube. Herbarium specimens have been deposited in LE, L, and CBS, and ex-type cultures are maintained at the Centraalbureau voor Schimmelfcultures (CBS) in Utrecht, the Netherlands.

## Results

### Taxonomy and morphology

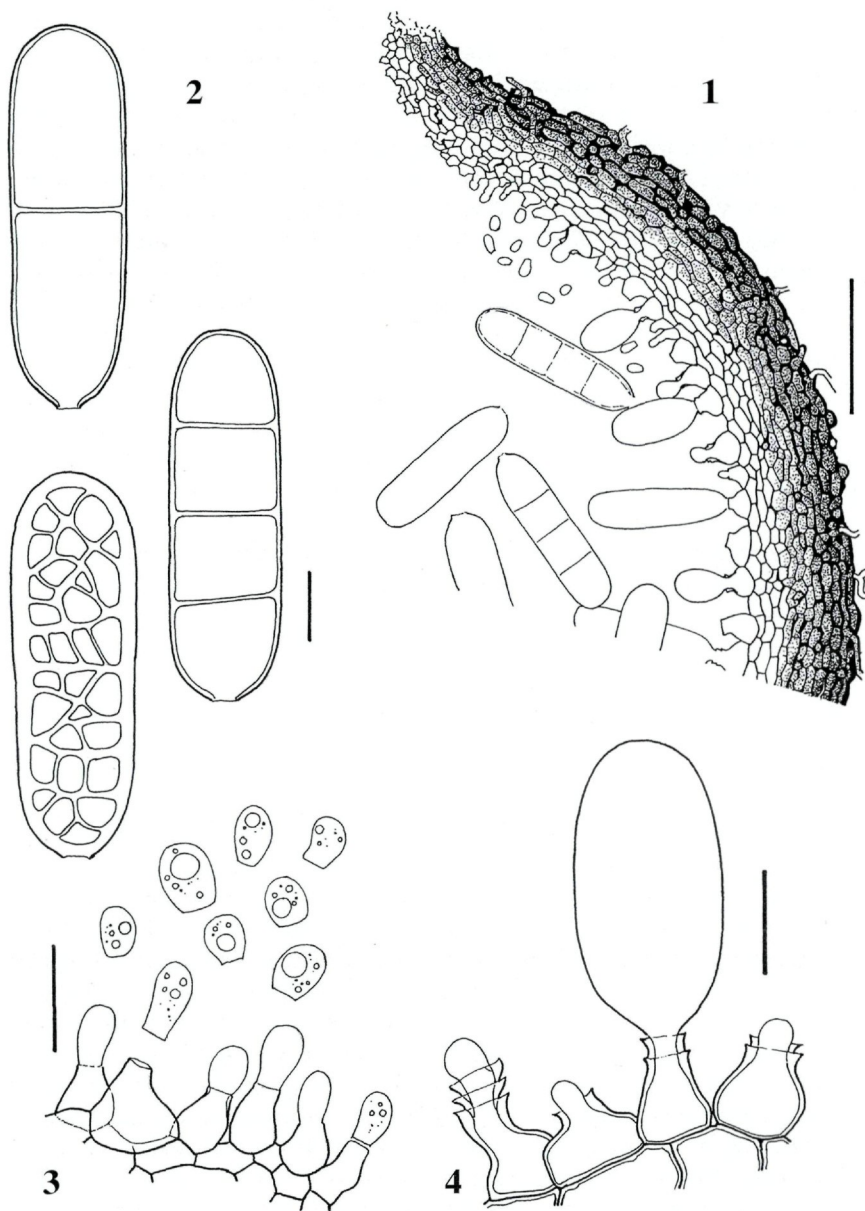
***Camarographium koreanum*** Verkley, Melnik & Crous, **sp. nov.** – Figs. 1–10.

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Conidiomata pycnidialia immersa, singularia vel aggregata, stromata nulla, 175–300(–450) µm diam, globosa vel paulo depressa, ostiolo centrali 25–45 µm diam. – Cellulae macroconidiogenae hyalinae, discretae, annellidicae, semel vel bis percurrentes, late ampulliformes vel doliiformes, 9–12(–14) × 8–10 µm; – macroconidia initio hyalina, tarde pallide olivaceo-brunnea, elongate ellipsoidea vel cylindrica, tempore liberationis continua et hyalina, deinde muriformia et distoseptata, 52–62.5 × 17–19.5 µm. – Cellulae microconidiogenae holoblasticae, non proliferentes; microconidia hyalina, subglobosa vel ellipsoidea, 4.5–6.5 × 3.4–5 µm.

Description of the fungus *in planta*. Conidiomata pycnidial, subperidermal, separate, single or in small clusters but without connecting stroma, completely immersed in the bark of the host, 175–300(–450) µm diam, globose or slightly depressed, with a central, 25–45 µm wide ostiolum, which is merely an inconspicuous and undifferentiated channel perforating the bark in a slightly raised area. – Conidiomatal wall composed of a 25–45(–55) µm thick outer layer of *textura angularis* with dark brown cells 5–10(–12) µm diam, the walls thickened up to 1 µm, and a 10–15 µm thick inner layer of *textura angularis-globulosa* of yellowish to hyaline, thin-walled cells, 4–7.5(–10) µm diam (this inner layer sometimes absent), giving rise to conidiogenous cells all over the inner wall surface.

Macroconidiogenous cells hyaline, discrete, holoblastic, annellidic, with 1 or 2 percurrent proliferations, broadly ampulliform to doliiform, the walls gradually thickened towards the apex, 9–12(–14) × 8–10 µm; – macroconidia initially hyaline, elongated-ellipsoidal to cylindrical, broadly rounded at the apex, rounded and with a 3.5–5 µm wide scar at the base, 1-celled and thin-walled and



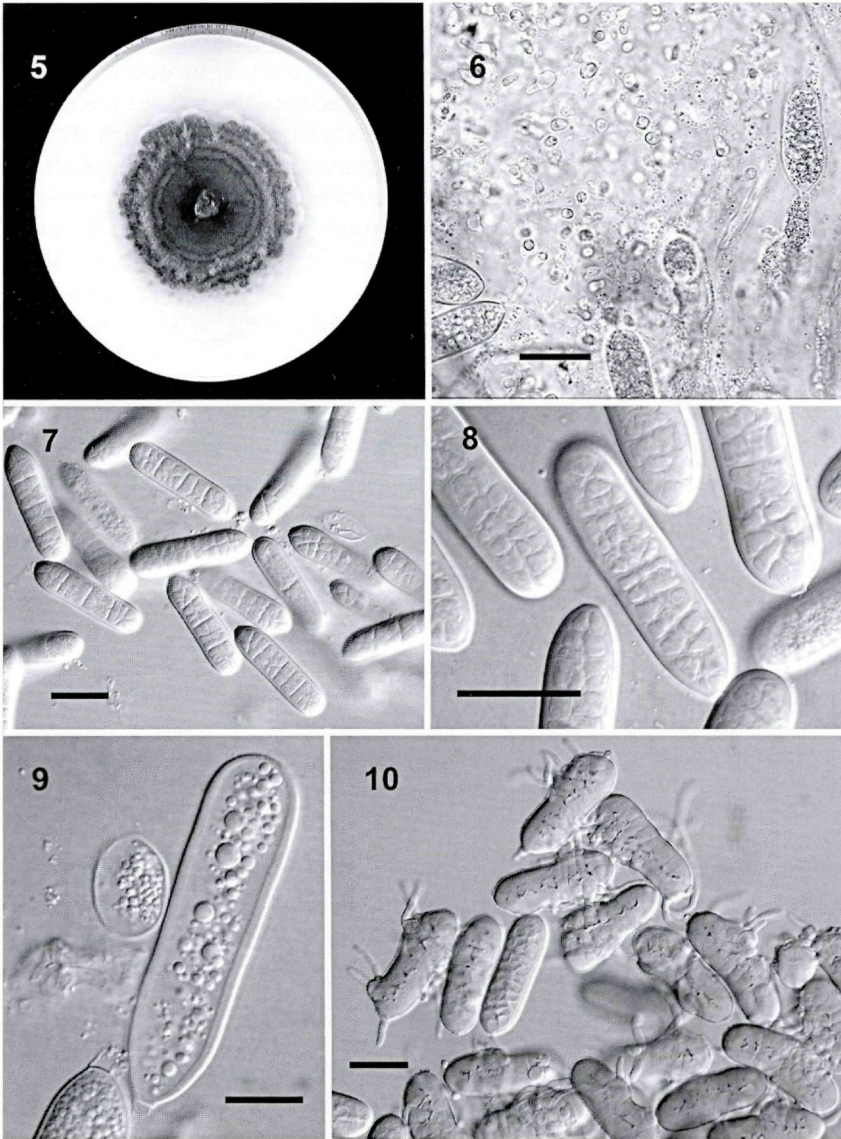
**Figs. 1-4.** *Camarographium koreanum* in planta. - 1. Part of a transverse section of a conidioma. - 2. Macroconidia in the 2-, 4-celled and muriform stages prior to the development of pigmentation in the outer wall. - 3. Microconidia and microconidiogenous cells. - 4. Macroconidiogenous cells. - From isotype CBS-H 14254. - Bars = 10  $\mu$ m, except in Fig. 1, bar = 50  $\mu$ m.



containing numerous small oil-droplets when seceded (in conidia dying at this stage, the inner conidial wall swells up to about 6  $\mu\text{m}$  thick); prior to release from the conidioma, conidia attaining an up to 1  $\mu\text{m}$  thickened outer wall, and becoming medianly 1-euseptate, then transversely 3-septate, and finally multi-celled because of the formation of numerous distosepta oriented in all directions; each cell filled with 1 or more relatively large oil-droplets, outer conidium wall slowly becoming pale olivaceous-brown, 52–62.5  $\times$  17–19.5  $\mu\text{m}$  (av.  $\pm$  SE: 55.3  $\pm$  2.0  $\times$  17.6  $\pm$  0.6; N=30); conidia germinating from one or several neighbouring cells that first swell up and often cause the outer conidium wall to tear open, after which germinating hyphae are formed that are 2.5–4.5  $\mu\text{m}$  wide and often swollen up to 7.5  $\mu\text{m}$  in width at their base. – Microconidiogenous cells mostly arising from the upper part of the conidiomatal wall, hyaline, discrete, holoblastic, non-proliferating, ampulliform to doliiform, 5–11  $\times$  3.5–5  $\mu\text{m}$ ; – microconidia hyaline, subglobose to ellipsoidal, rounded at the apex, broadly truncate at the base, with a few small oil-droplets, 4.5–6.5  $\times$  3.4–5  $\mu\text{m}$ .

Macroconidia in the hyaline stage are often extruded from the well-hidden conidiomata in conspicuous whitish masses.

Description of the fungus *in vitro*. Colonies on OA reaching 50–54 mm diam in 5 weeks, with a glabrous, colourless and lobed margin; immersed mycelium mainly colourless, in the centre somewhat buff or olivaceous, also with greenish olivaceous sectoring near the margin; surface mostly glabrous, but high tufts of grey-olivaceous to smoke-grey aerial mycelium formed in concentric patterns especially in the submarginal area; conidiomata abundantly formed submerged in the agar or on the agar surface in concentric rings; reverse in the centre grey-olivaceous, surrounded by pale olivaceous grey to pale greenish grey areas, margin buff. Colonies on CMA reaching 40–43 mm diam in 5 weeks, with an even, colourless to pale buff margin, covered with scarce whitish aerial hyphae; immersed mycelium mostly olivaceous, in the centre darker and almost olivaceous-black; colony surface in the centre mostly glabrous, but in a broad submarginal zone with a dense woolly grey-olivaceous mat, and closer to the margin with areas of glaucous blue-green aerial mycelium; conidiomata formed mainly on the agar surface in indistinct concentric patterns; reverse olivaceous-black in the centre, fading to greenish grey and glaucous-grey towards the margin. Colonies on MEA (Oxoid, 3%) reaching 48–52 mm diam in 5 weeks, with an even, slightly undulating buff submerged margin; immersed mycelium fully covered by a dense woolly to felty mat of aerial mycelium which is smoke-grey to oliveaceous-grey, but white near the margin; reverse mostly dark brick to sepia, with paler fawn to isabelline areas near the centre.



**Figs. 5-10.** *Camarographium koreanum* *in vitro*. – **5.** Colony on OA after 5 weeks incubation (18 °C, n-UV). – **6-10.** Conidia on OA. – **6.** Macro- and microconidia. – **7., 8.** Macroconidia, still hyaline. – **9.** Macroconidium just after secession, prior to septation. – **10.** Macroconidia with pigmented outer walls, most germinating. – From living culture CBS 117158. – Figs. 6-8, 10: bars = 25 µm; Fig. 9: bar = 10 µm.

Conidiomata developing on the agar surface, or submerged, globose, black, 150–350 µm diam, those on the agar surface densely clothed with very long, mostly darkly pigmented 7–10 µm wide hyphae with up to 1.5 µm thick walls, ostiolum barely developed. – Conidiomatal wall composed of a relatively thin outer layer of *textura angularis* with brownish to olivaceous cells 7–12 µm diam, the walls thickened up to 1 µm and incrustated with dark brown amorphous material, and a thicker inner layer of hyaline *textura angularis-globulosa* of thin-walled cells 5–10 µm diam, giving rise to conidiogenous cells all over the inner wall surface. – Macroconidiogenous cells as *in planta*, with 1 or 2 (rarely up to 4) percurrent proliferations, 9–12(–14) × 8–10 µm; – macroconidia as *in planta*, persistently hyaline, becoming olivaceous-brown only after prolonged incubation, 1-celled and thin-walled and containing numerous small oil-droplets when liberated; prior to release from the conidioma, conidia attaining an up to 1 µm thickened outer wall, becoming medianly 1-euseptate, then 3-septate, and finally multicelled as a result of the formation of numerous distosepta in all directions, each compartment filled with 1 or more large oil-droplets, 57–74 × 16–18.5 µm (aver. ± SE: 64.1 ± 4.4 × 17.3 ± 0.6 µm; N=30; on OA). – Microconidiogenous cells intermingled with macroconidiogenous cells, hyaline, discrete, holoblastic, non-proliferating, ampulliform to doliiform, 5–11 × 3.5–5 µm; – microconidia hyaline, subglobose to ellipsoidal, rounded at the apex, broadly truncate at the base, with a few small oil-droplets, 4.5–7 × 3.5–5(–5.5) µm.

Holotypus – KOREA: Seoul, Hongneung Arboretum, Korea Forest Research Institute, on dead twigs of *Cornus kousa* (Cornaceae), V. Mel'nik s.n., 10 Sep. 2004 (LE 226162, HOLOTYPE; L, isotype; CBS-H 14254, isotype); living ex-type cultures CBS 117158, 117159.

## Discussion

Although *C. koreanum* shows a combination of very distinctive morphological characters, its classification is not straightforward. The conidial septation and conidiogenesis of *C. koreanum* is reminiscent of the *Shearia* anamorphs of *Pleomassaria* Speng. (*Pleomassariaceae*, Pleosporales), but the conidia of species of that genus are fusiform, and have a basal or lateral mucilaginous sheath, remnants of a sheath surrounding the conidium body during conidiogenesis (Sutton 1980). Moreover, the conidiomata of *Shearia* species are pseudostromatic, while those of the present fungus are pycnidial on the host plant. In *Camarosporium* Schulz. fruitbodies are pycnidial, and the conidiogenous cells are similar in shape to those of the Korean fungus. The conidiogenous cells of *Camarosporium* species



also proliferate percurrently, but the conidia in members of that genus are not distoseptate and become very dark brown much sooner. In the genus *Camarographium*, conidia form septa in a similar fashion as in *C. koreanum*; they also are guttulate and become pale brown. Conidiomata in the type species of *Camarographium*, *C. stephensii* (Berk. & Broome) Bubák, could be interpreted as either pycnidia or eustromata with about equal justification. *Camarographium stephensii* is only known from petioles of *Pteridium aquilinum* (L.) Kuhn, on which the unilocular fruitbodies are aggregated in linear eustromata (Sutton 1980). On the basis of these similarities, we decided that the Korean fungus can best be accommodated in the genus *Camarographium*.

To date, three further pycnidial species are accepted in *Camarographium*: *C. atriplicis* Golovin, *C. fructicola* Frolov, and *C. indicum* S.S. Kelkar, V.G. Rao, A. Pande & V.P. Bhide. Sutton (1980) mentioned *C. abietis* (R. Wilson & R.B. Anderson) Grove and *C. metableticum* (Trail) Grove, but regarded these species not as congeneric with the type species. Recently they were referred to other genera: *C. abietis* is a synonym of *Myxocyclus cenangioides* (Ellis & Rothr.) Petr., for which Warmelo & Sutton (1981) erected a new genus, *Stegonsporiopsis* Warmelo & B. Sutton. For *C. metableticum*, the new genus *Amarenographium* O. Eriksson was erected (Eriksson 1982), on the basis of the morphology of the conidiophores, which are longer than in *C. stephensii* and the conidia, which are provided with mucilaginous appendages. *Camarographium indicum*, a species described from spines of *Acacia sphaerocephala* Cham. & Schlecht. in India, has much smaller conidia ( $10-18 \times 7-12 \mu\text{m}$ ), appearing more like those in species of *Camarosporium*. *Camarographium fructicola*, which was described from fruit of *Prunus domestica* Thunb. in Turkmenia, also has much smaller conidia ( $13-19 \times 5.6-7.5 \mu\text{m}$ ; Frolov 1968) than *C. koreanum*. *Camarographium atriplicis* Golovin was described from leaves of *Atriplex moneta* Bunge ex Boiss. in Tadzhikistan. The conidia of this species are hyaline,  $14.5-16 \times 4-7 \mu\text{m}$ , with 1-4(-5) transverse and 0-4(-5) longitudinal septa (Golovin 1950). The conidia of *C. stephensii* are comparable in length but much wider ( $40-52 \times 22-28 \mu\text{m}$ ) and more irregular in shape than those of the Korean fungus.

The conidiogenous cells in *Camarographium* were interpreted as enteroblastic phialidic by Sutton (1980), but it is now well-known that in many natural groups both phialides and percurrently proliferating conidiogenous cells can occur (Verkley 1999, Crous, Kang & Braun 2001). It is unknown whether the conidiogenous cells of the other *Camarographium* species can also proliferate percurrently. The relatively thick apical walls of the conidiogenous cells in *C. koreanum* resemble periclinal thickenings of typical

phialides, and the percurrent proliferations are much more distinct in culture than on the host plant. We therefore propose to place this interesting fungus in the genus *Camarographium*.

The key below is based on characters *in planta*.

### Key to *Camarographium* species

- 1 Conidiomata linear stromata, on petioles of *Pteridium aquilinum*, conidia 22–28  $\mu\text{m}$  wide . . . . . *C. stephensii*
- 1\* Conidiomata pycnidial, on other substrata, conidia less than 22  $\mu\text{m}$  wide . . . . . 2
- 2 Conidia 52–62  $\times$  17–19.5  $\mu\text{m}$ , immersed in bark of *Cornus kousa*.  
*C. koreanum*
- 2\* Conidia much smaller, on other substrata . . . . . 3
- 3 Conidia hyaline, 14.5–16  $\times$  4–7  $\mu\text{m}$ , on leaves of *Atriplex moneta*  
*C. atriplicis*
- 3\* Conidia brown, on other substrata . . . . . 4
- 4 Conidia 5.6–7.5  $\mu\text{m}$  wide, on fruits of *Prunus domestica* . . . . .  
. . . . . *C. fructicola*
- 4\* Conidia 7–12  $\mu\text{m}$  wide, on spines of *Acacia sphaerocephala* . . . . .  
. . . . . *C. indicum*

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