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FULL PAPER

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Pinnaticoemansia, a new genus of Kickxellales, with a revised key to the genera of Kickxellales

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Abstract *Pinnaticoemansia coronantispora* gen. sp. nov. (Kickxellaceae, Kickxellales) is described and illustrated. This species is characterized by imparipinnate sporocladia (sporocladia arranged in pairs), sporangiospores with a three-lobed corona, and the germination pattern of sporangiospores with downward hyphal growth and repeated dichotomy. The key to the genera of Kickxellales by Kurihara et al. (2001) is revised based on the observation of the germination pattern of this fungus in comparison with that of Asellariales.

Key words Asellariales · Kickxellales · New genus · *Pinnaticoemansia coronantispora* · Taxonomy

Introduction

Kickxellales Kreisel ex R.K. Benj. is an order of Zygomycetes composed of one family that includes 11 genera. Most members of the order are saprobes and rare species (Benjamin 1959, 1979; Kwaśna et al. 1999; Benny et al. 2001). Almost all species have been isolated from mammal feces or soil, but some species have been found on dead bodies or feces of small insects (Kurihara et al. 2001, 2004). The order is characterized by the production of septa with a central pore that is filled with a plug persistent in acidic staining and KOH (2%–3%), a convoluted asexual reproductive structure that was named "coemansioid pattern" by Moss and Young (1978), and sporangiospores in monosporic sporangiola (Benjamin 1979). The habitat of the fungi also characterizes the order to which they belong; namely, they do not live in arthropodal host guts as opposed to Trichomycetes s. str. (=Harpellales Lichtw. & Manier. and Asellariales Manier ex Lichtw. & Manier), which has been regarded as a close relative of Kickxellales (Lichtwardt 1973a). We found a hitherto undescribed species of the order, and here we propose a new genus to accommodate the species.

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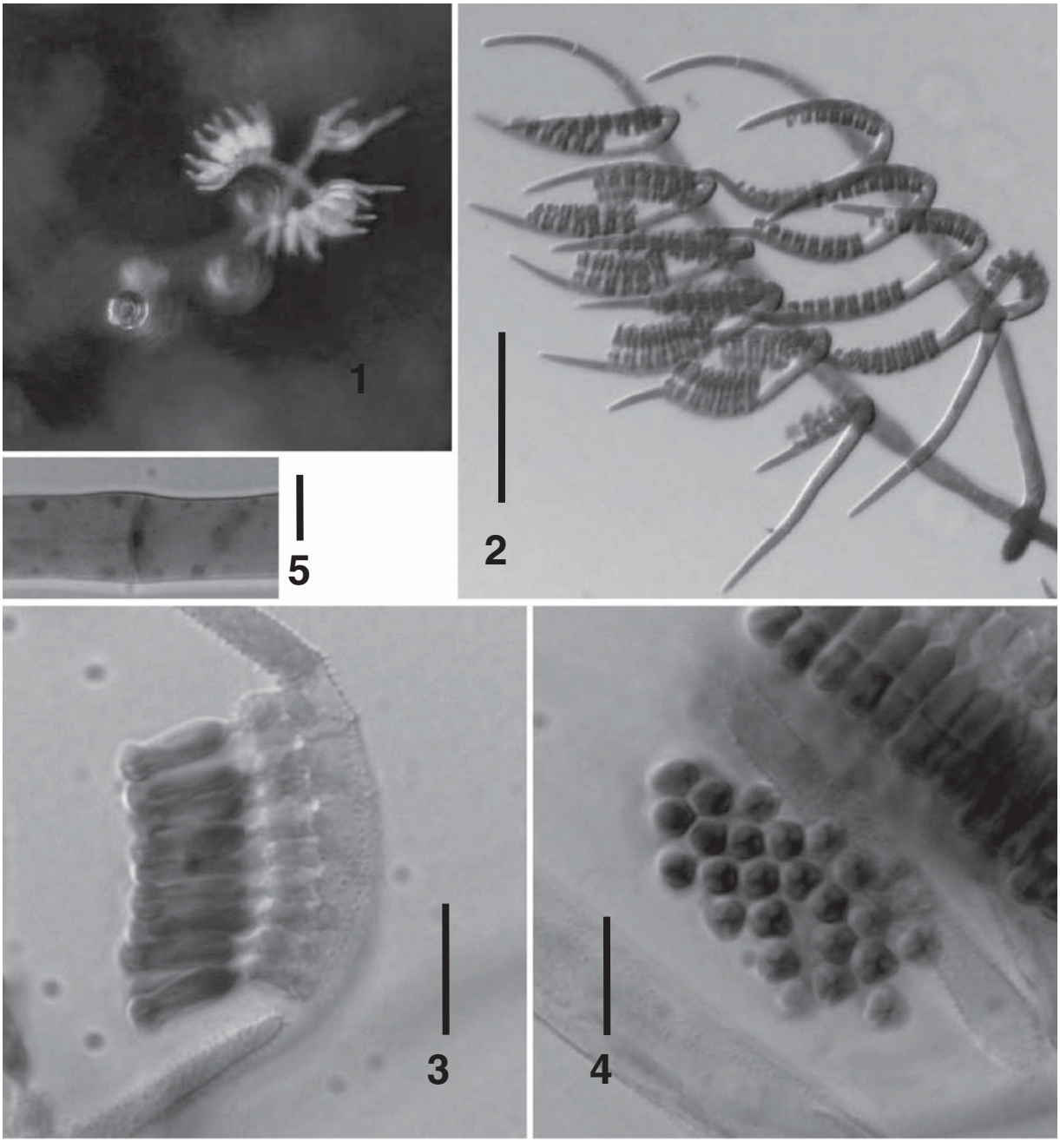
Materials and methods

The fungus described here was isolated from soil incubated in a moist chamber. The origin of the isolate seemed to be the feces of a dermapteran contained in the sample soil, although we were unable to identify it accurately. The isolate was incubated on Miura agar medium (LCA; Miura and Kudo 1970) at 25°C for 10 days. Preparation of slides and measurements of each morphological feature were as described by Kurihara et al. (2000). Growth and sporulation of the isolate was examined on LCA, half-strength malt extract-yeast extract agar medium (1/2ME-YE; Benjamin 1958), and ten-strength brain-heart infusion agar medium (BHI/10; Lichtwardt 1986).

Taxonomy

Descriptions

Pinnaticoemansia Kurihara & Degawa, gen. nov.
Sporangiophora erecta, septata, furcata. Sporocladia opposita, corniformia, asperula, stipitata, opposita. Pseudo-



Figs. 1–5. *Pinnaticoemansia coronantispora* (holotype). **1** A sporangiophore appeared in a moist chamber (not to scale). **2** Two fertile branches of a sporangiophore. **3, 4** A sporocladium from lateral view (**3**) and from ventral view (**4**). **5** A sporangiophore septum. *Bars* **2** 50 µm; **3–5** 10 µm

phialides lageniformes. Sporangiosporae ellipsoideae, hyalinae, coronatae deciduae, per sporangiola vestita. Zygosporae ignotae.

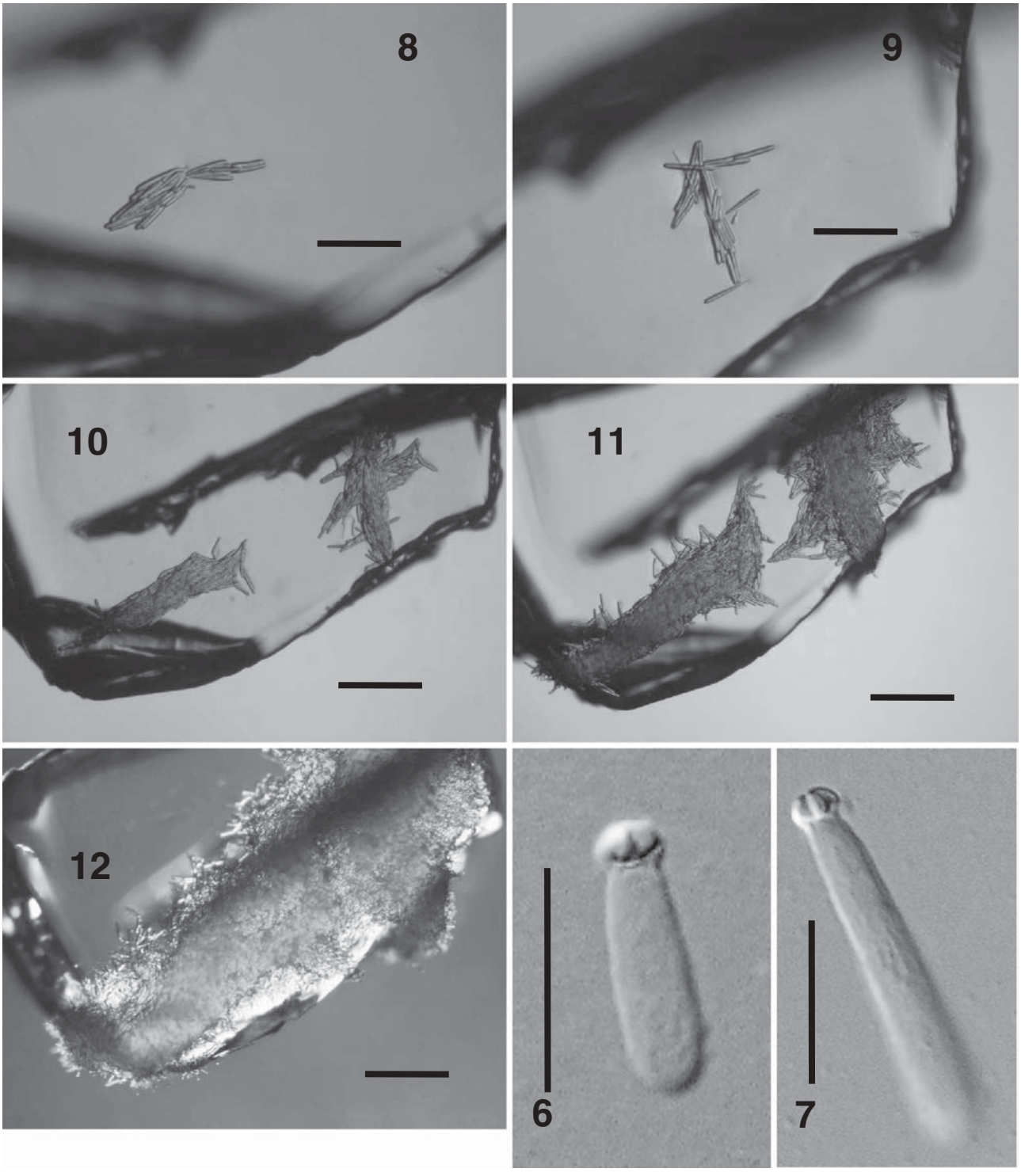
Species typica: *Pinnaticoemansia coronantispora* Kurihara & Degawa.

Etymology: From Latin *Pinnatus* (feather-like) and *Coemansia*, based on its feather-like arrangement of sporocladia and the resemblance of sporocladia to those of *Coemansia* Tiegh. & G. Le Monn.

Sporangiophores erect, septate, branched. Sporocladia opposite with single apical one, corniform, asperulate, stalked. Pseudophialides flask-shaped. Sporangiospores ellipsoid, colourless, coronate, deciduous, covered in sporangiola. Zygospores unknown.

Pinnaticoemansia coronantispora Kurihara & Degawa, sp. nov. Figs. 1–22

Coloniae in LCA luteolae. Sporangiophora erecta, septata, furcata, 5–12 sporocladia ferentia. Sporocladia corniformia, asperula, stipitata, opposita, 6–11-cellularia, praeter stipitem 40–68 × 4.5–7 µm. Pseudophialides lageniformes, 5–7 × 2.5–3.5 µm. Sporangiola 8–10 × 3–4 µm. Sporangiosporae ellipsoideae, hyalinae, 5.5–7 µm longae, coronam trilobam 1.5–3 × 3–4 µm ferentes. Zygosporae ignotae.

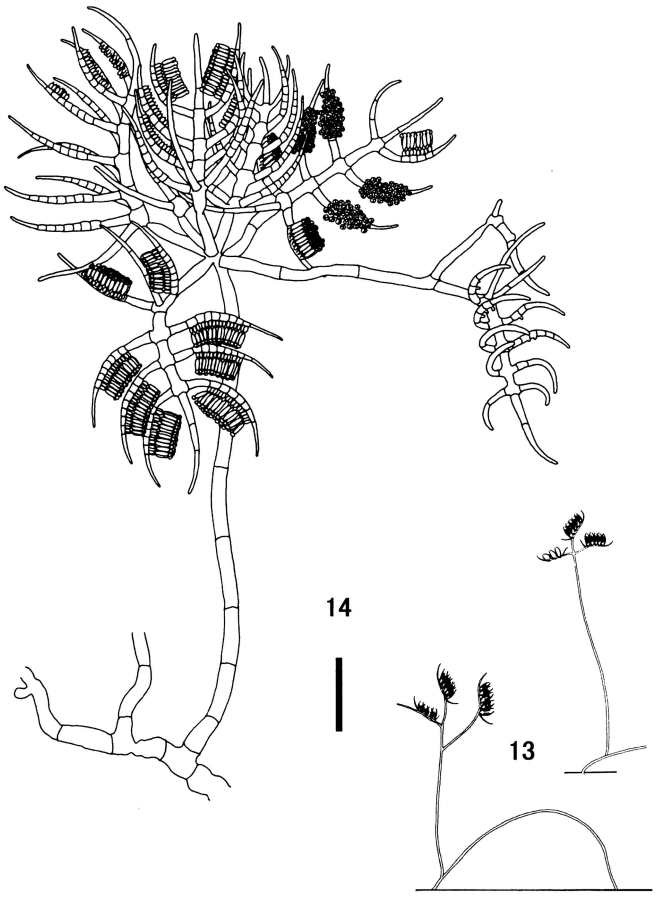


Figs. 6–12. *Pinnaticoemansia coronantispora* (holotype). **6** A sporangiospore with a corona. **7** A germinating sporangiospore. **8–12** Successive development of two raftlike colonies. **8** Colony A at 0 h. **9** Colony B at 0 h. **10** Colony A (*left*) and Colony B (*right*) at 17 h. **11** Colony A (*left*) and Colony B (*right*) at 29 h. **12** Two colonies were combined with each other at 51 h. *Bars* **6, 7** 10 µm; **8, 9** 100 µm; **10–12** 50 µm

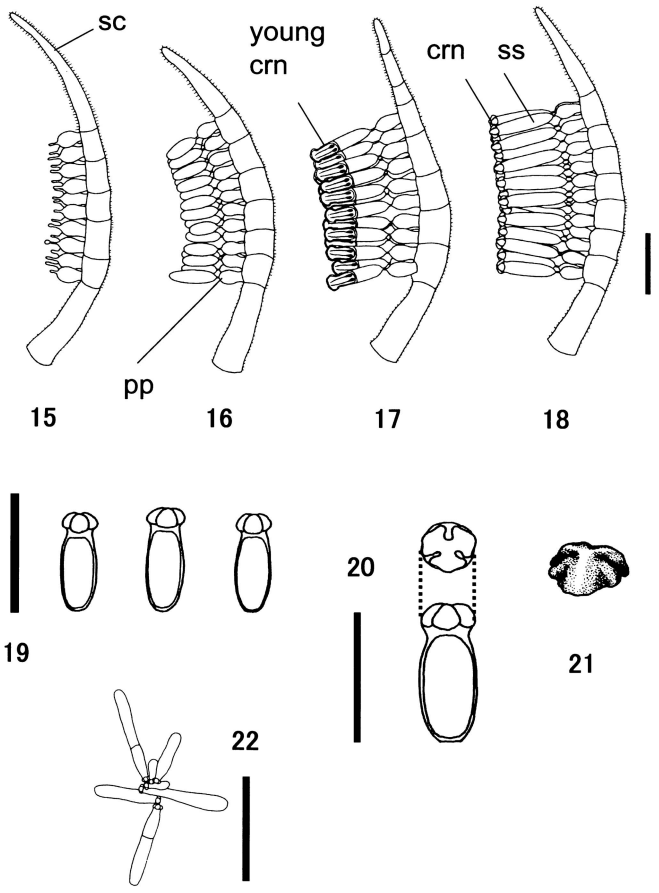
Holotypus: Japan, Honshu; Kushimoto, Wakayama Prefecture, from soil containing feces of a dermapteran, February 22, 2003, Y. Degawa leg., KPM-NC0012131-holotypus, NBRC 100470-cultura viva.

Etymology: From Latin *coronans* (coronate) and *-sporus* (-spored), referring to its coronate sporangiospores.

Colonies on LCA pale yellow. Vegetative hyphae colorless, septate. Sporangiophores erect, septate, branched, producing one to several fertile parts, bearing 5–12 sporocladia. Sporocladia arranged in pairs (opposite) with single apical one along branches of sporocladia, corniform, asperulate, composed of 6–11 cells excluding the stalk,



Figs. 13, 14. *Pinnaticoemansia coronantispora* (holotype). **13** Sketches of the habitat of two sporangiophores developed from stolons (not to scale). **14** A sporangiophore bearing almost mature sporangiospores. *Bar* 50 μm



Figs. 15–22. *Pinnaticoemansia coronantispora* (holotype). **15–18** Four successive stages of a sporangiospore and its corona development (*left to right*). **19** Three mature sporangiospores. **20, 21** Diagrams of a sporangiospore and its corona. **20** Diagram from vertical view and view from above. **21** Bird's-eye view diagram showing three ridges of corona. **22** A mass of germinating sporangiospores mounted with water. *sc*, sporocladium; *pp*, pseudophialide; *ss*, sporangiospore; *crn*, corona of a sporangiospore. *Bars* **15–20** 10 μm; **22** 50 μm

40–68 × 4.5–7 μm, with a stalk of 10–27 × 4.5–8 μm; the apical cell long, almost straight, sterile, 10–25.5 × 3–5 μm. Pseudophialides flask-shaped, 5–7 × 2.5–3.5 μm, sitting in rows on the fertile cells of sporocladia. Sporangiospores ellipsoid, colorless, 5.5–7 μm long, with a three-lobed corona of 1.5–3 × 3–4 μm, deciduous, covered by a liquid droplet at maturity, enveloped with colorless sporangiola of 8–10 × 3–4 μm. Zygospores unknown.

Notes. This fungus resembles species of the genus *Coemansia* in asexual reproductive structure in general, but it is readily distinguished from them by its imparipinnate arrangement of sporocladia (sporocladia arranged in pairs on branches of sporangiophores), coronate sporangiospores, and the germination pattern of sporangiospores with downward hyphal growth and repeated dichotomy (Fig. 23A–D). This fungus grew vigorously on LCA, 1/2 ME-YE, and BHI/10, and sporulated most abundantly on LCA.

Structure and germination pattern of sporangiospores

Coronas of sporangiospores were separated from sporangiospores by thick sporangiola wall (see Figs. 6, 19, 20). However, unlike the septa between pseudophialides and sporangiospores, no median plugs were observed between sporangiospores and coronas under a microscope. Coronas were readily stained with cotton blue in lactic acid during sporulation, but lost their stainability and became reflective at maturity. Coronas remain on vegetative hyphae for a long time even after sporulation of the new colony originated from the sporangiospore. When mounted with water, coronas often adhered to each other and made a spore mass (see Fig. 22). The phenomenon may be the result of the sticky nature of the coronas, which makes it possible to increase the inoculum potential of spores.

At sporangiospore germination, this fungus shows budding yeastlike growth on/under the surface of agar plates; sporangiospores swell and grow downward to become clavate, and then the clubs repeatedly split into two in the middle and germ from the sections to form raftlike colonies (see Figs. 7–12, 23A–D).

Key to the genera of Kickxellales, revised from that of Kurihara et al. (2001)

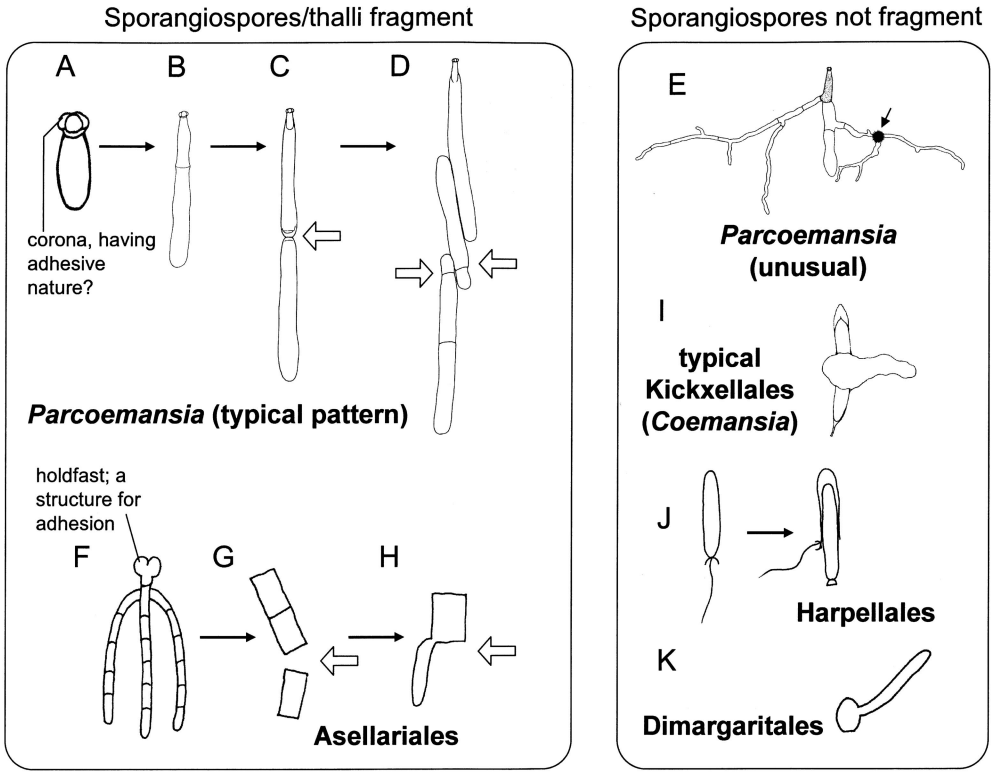


Fig. 23. Diagrams of spore germination patterns of *Pinnaticoemansia coronantispora* and its related taxa. **A–E** *P. coronantispora*. **A–D** Four successive stages of sporangiospore germination. **A** Mature sporangiospore before germination. **B** Sporangiospore swells and grows downward and turns to become clavate. **C** Sporangiospore is fragmented into two (*arrow*). **D** New portions grow out of the sections (*arrows*), and each fragment is repeatedly split into two in the middle and blasts out of the sections to form a raftlike colony. **E** An unusual germination in a slide preparation mounted with water. After swelling and downward growth of a sporangiospore, vegetative hyphae grow directly out of the swollen sporangiospore. This pattern appears to be a modification of the typical pattern of Kickxellales as the initiation of a new sporangiophore (*arrow*). **F–H** Three successive stages of sporulation of Asellariales as modified from Lichtwardt (1973b). **F** A thallus. **G** Thallus fragments (*arrow*) with each fragment turns into an arthrospore. **H** Germ tubes grow out of the section of the arthrospores (*arrow*). **I** A typical pattern of Kickxellales (*Coemansia*). Germ tubes grow out of the center of sporangiospores and grow laterally. **J** Harpellales. Sporangiospores (trichospores) strip off sporangiola and appendage(s), and turn into a thallus with a holdfast formed underneath. **K** Dimargaritales (Zygomycetes): another possible close relative of Kickxellales. Germ tubes grow out of sporangiospores

1. Sporocladia bearing sporangiospores on pedicels or directly on the sporocladia; pseudophialides not produced; zygospores ornamented where known 2
1. Sporocladia bearing sporangiospores on pseudophialides; zygospores smooth where known 3
2. Sporocladia "brioche à tête"-shaped or globose with a globose stalk, produced pleurogenously solitarily; sporangiospores globose, formed on a short pedicel on sporocladia, remaining dry at maturity . . . . . *Spiromyces* R.K. Benj.
2. Sporocladia lageniform with long necks, produced acrogenously in mass or pleurogenously when solitary; sporanigiospores fusiform, formed directly on sporocladia, covered by liquid at maturity . . . . . *Mycoëmilia* Kurihara et al.
3. Sporocladium usually composed of a single cell 4
3. Sporocladium composed of several cells 6
4. Only a single pseudophialide formed on a sporocladial element before senescence . . . . . .*Ramicandelaber* Ogawa et al.
4. Several to many pseudophialides formed on a sporocladial element 5
5. Sporocladium single, large, and lenticular . . . . . *Linderina* Raper & Fennell
   Sporocladia cylindrical, one- or sometimes two-celled, many on a sporangiophore vesicle . . . . . *Myconymphaea* Kurihara et al.
6. Sporangiola ovoid, length : width ratio not exceeding 2; sporangiospores dry at maturity . . . . . *Spirodactylon* R.K. Benj.
6. Sporangiola elongate, length : width ratio exceeding 2; sporangiospores wet at maturity 7
7. Sporocladia verticillate or umbellate, developing from a vesicle 8
7. Sporocladia racemose, scorpioid, or imparipinnate, not developing from a vesicle 9
8. Sporocladia without a stalk, borne verticillately; apical cell of sporocladia often furcate; sporangiola and sporangiospores fusiform . . . . . *Kickxella* Coem.
8. Sporocladia stalked, borne umbellately; apical cell of sporocladia simple; sporangiola alate; sporangiospores obclavate . . . . . *Martensiomyces* J.A. Mey.
9. Sporocladia opposite with a single terminal one on fertile branches of sporangiophores; sporangiospores

coronate . . . . . *Pinnaticoemansia* Kurihara & Degawa
9. Sporocladia alternate along sporangiophores or on fertile branches of sporangiophores; sporangiospores not coronate 10
10. Sporangiospores clavate with an aristate tip (acuminate) . . . . . *Dipsacomyces* R.K. Benj.
10. Sporangiospores fusiform or aciculate 11
11. Pseudophialides formed on the upper surface of the sporocladium . . . . . *Martensella* Coem.
11. Pseudophialides formed on the lower surface of the sporocladium . . . . . *Coemansia* Tiegh. & G. Le Monn.

Discussion

The coronate structure and germination pattern of sporangiospores of *P. coronantispora* have never been found in Kickxellales, and are quite different from those of other species of the order. However, we regard these two features as genus-level characteristics and classify this fungus into Kickxellales judging from its total morphological features and habitat. However, the coronate structure and germination pattern of this fungus are reminiscent of those of Asellariales (see Fig. 23F–H).

All species of Asellariales are obligate parasites of isopods or insects and live in the host gut (Lichtwardt 1986). The thalli of Asellariales are simple and with a structure called holdfast to adhere to the inner surface of the host gut. In asexual reproduction, the whole thalli are fragmented, and each fragment turns into an arthrospore, and the arthrospores then germinate out of the sections. This pattern resembles sporangiospore germination of *P. coronantispora* (Fig. 23A–D).

Three-lobed coronas of *P. coronantispora* also resemble the holdfasts of Asellariales. Holdfasts are composed of enlarged or differentiated basal cells and are divided longitudinally in some species (Lichtwardt 1986) (Fig. 23F). Coronas of *P. coronantispora* appear to have a sticky nature. Holdfasts of *Asellaria armadillidii* Tuzet & Manier ex Manier and some other species secrete mucilage, although holdfasts lacking prominent secreted structures are observed in most species (Lichtwardt 1986). Coronas of *P. coronantispora* might help sporangiospores to adhere to its substrata at spore dispersal as does the holdfast of Asellariales, although this has not been confirmed yet. No other kickxellalean species have such an accompaniment outside the sporangiospores as the coronas of *P. coronantispora*. The sporangiospores of *Dipsacomyces acuminosporus* R.K. Benj. have an acuminate outgrowth that is part of the sporangiospores (Young 1999). Such differences from other kickxellalean species in germination pattern and sporangiospore structure suggest the possibility that *P. coronantispora* has a habitat distinct from that of other kickxellaleans.

Asellariales has been regarded to be phylogenetically close to Harpellales (Manier 1973; Saikawa et al. 1997) based on its septal ultrastructure (Moss and Young 1978) and germination pattern of asexual spores (Lichtwardt 1973a). Also, because Harpellales has been regarded as a close relative of Kickxellales (Benny and Aldrich 1975; Moss and Lichtwardt 1976; Moss and Young 1978; Saikawa et al. 1997; O'Donnell et al. 1998; Tanabe et al. 2000; Sato 2001), Asellariales is presumed to be closely related to Kickxellales, although there has been no direct evidence to show the relationship between Kickxellales and Asellariales, largely because there are insufficient data for Asellariales (Benny 2001). The fungus we have described here might be one of the taxa that would be a missing link connecting the two orders. Further observation and DNA sequence analysis of this fungus may lead to the establishment of the phylogenetic relationships between these orders.

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