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ORIGINAL ARTICLE

Kuehneola species (Phragmidiaceae, Pucciniales) on Vitaceae plants

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Abstract The type specimen of *Uredo cissi-debilis* on *Cissus debilis* from Côte d'Ivoire was found to bear aparaphysate telia, in which a chain of two to four teliospores was formed on a short pedicel. The teliospores were thin-walled and germinated in situ. Another fungus identified as *U. cissi-debilis* on *Cayratia gracilis* from Sierra Leone was also found to form teliospores similar to those of the *Cissus* rust fungus in paraphysate telia. The teliospore morphology and host relationships showed their taxonomic affinity to *Kuehneola vitis* on *Ampelocissus latifolia* distributed in South Asia. It was concluded that presence or absence of paraphyses in sori and spore morphology, in addition to the assumed host preference, distinguish between the three fungi. New names, *K. cissi-debilis* comb. nov. and *K. deightonii* sp. nov., were proposed for the *Cissus* fungus and the *Cayratia* fungus, respectively.

Keywords Africa · *Ampelocissus* · *Cayratia* · *Cissus* · Southeast Asia · *Vitis*

Introduction

Twenty-one species in nine teleomorphic genera and eight species in two anamorphic genera of rust fungi have been listed for plants of the Vitaceae (Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA; http://nt.ars-grin.gov/fungaldatabases/, accessed 25 Feb 2015). In the course of a taxonomic study on vitaceous phakopsoroid fungi (Phakopsoraceae, Pucciniales), telia were found among uredinia on the abaxial leaf surface in the type specimen of *Uredo cissi-debilis* Vienn.-Bourg. on *Cissus debilis* Planch. from Côte d'Ivoire (Viennot-Bourgin 1958). The telia were aparaphysate and consisted of shortly pedicellate teliospores composed of two to four thin-walled cells. Another specimen identified as *U. cissi-debilis* on *Cayratia gracilis* (Guill. & Perr) Suess. from Sierra Leone (unpublished) was also found to form two-celled or three-celled, thin-walled teliospores on a short pedicel in paraphysate telia formed among uredinia. The two fungal specimens were morphologically similar to *Kuehneola vitis* (E. J. Butler) P. Syd. & Syd. on *Ampelocissus latifolia* (Roxb.) Planch. distributed in South Asia (Butler 1912; Mundkur and Thirumalachar 1943).

This paper describes the morphology of the *Cayratia* and *Cissus* fungi and discusses their taxonomic distinctness from previously described rust species on vitaceous plants and the taxonomy of the genus *Kuehneola* and its allies.

Materials and methods

Specimens examined: listed under the name and description of each species. Roman numerals placed before a herbarium accession number denote the presence of uredinia (II) or telia (III) in a specimen.

Microscopic observation: Small sorus-bearing pieces were cut out from the herbarium specimens and they were thin-sectioned with a razor blade under a binocular dissecting microscope. Spores and paraphyses were scraped from sori. Thin sections, scraped spores and paraphyses were mounted on a microscopic slide and treated as described elsewhere (Ono 2000). The slide preparations were then examined both by bright-field and differential interference contrast microscopy

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(DIC) with an Olympus BH2 or Olympus BX51 microscope (Olympus, Tokyo, Japan) and measurements were made with an ocular micrometer. Twenty selected spores and paraphyses were measured for each specimen. To observe urediniospore germ pores, the spores were mounted in lactic acid on a slide glass and heated at boiling point for a few seconds, and a drop of lactophenol solution with aniline blue was then added onto the boiled spores.

Results

Key to *Kuehneola* species parasitic on Vitaceae plants

1. Paraphyses absent in sori; urediniospores subglobose, broadly obovoid or broadly ellipsoid, 15–20×10–15 μm in size; teliospores 2–4-celled, 25–39×9–13 μm in size…..… *Kuehneola cissi-debilis* (Vienn.-Bourg.) Y. Ono
1. Paraphyses present at periphery of sori ……..... 2
2. Paraphyses cylindrical, thin-walled, 30–46×7–12 μm in size; urediniospores subglobose, broadly obovoid or broadly ellipsoid, 17–25×14–22 μm in size; teliospores 2–3-celled, 24–40×10–15 μm in size…..… *Kuehneola deightonii* Y. Ono
2. Paraphyses broadly cylindrical, incurved, dorsally thick-walled (−3 μm), 26–50×9–15 μm in size; urediniospores subglobose, obovoid or ellipsoid, 17–25×11–20 μm in size; teliospores 2–5-celled, 28–65×7–14 μm…..... *Kuehneola vitis* (E. J. Butler) P. Syd. & Syd.

Taxonomy

Kuehneola cissi-debilis (Vienn.-Bourg.) Y. Ono, **comb. nov.** Fig. 1a, b; Fig. 2a.

MycoBank no.: MB811793

Basionym: *Uredo cissi-debilis* Vienn.-Bourg. Uredineana 5: 230, 1958.

Spermogonia and aecia: not observed. Uredinia: formed on abaxial leaf surface, solitary to densely grouped, subepidermal in origin, soon becoming erumpent surrounded by torn epidermis, aparaphysate. Urediniospores: formed singly on a short pedicel, appearing almost sessile, mostly subglobose, broadly obovoid or broadly ellipsoid, 15–20×10–15 μm in size (Fig. 1a); the wall thin, colorless, echinulate with no apparent germ pore. Telia: formed on abaxial leaf surface, replacing uredinia, densely grouped, slightly raised, waxy, becoming reddish or dark brown, aparaphysate. Teliospores: composed of 2–4 linearly arranged cells formed on a short pedicel (Figs. 1b and 2a); two-celled spores 25–32 μm long; three-celled spores 28–38 μm long; four-celled spores 34–39 μm long; each cell broadly ellipsoid, 12–17×9–13 μm in size; the wall thin and colorless; metabasidia and basidiospores not observed.

Holotype: on *Cissus debilis* Planch. (Vitaceae): République de Côte d'Ivoire, near Bongouanou, August–September 1951, G. Viennot-Bourgin (II+III, PC0167202).

Notes: The type specimen bore abundant telia among uredinia on the abaxial leaf surface. Many uredinia were also replaced by telia. Being covered by urediniospores, the tiny telia were easily overlooked even though they were slightly raised and tinted differently from the host epidermal cells. Viennot-Bourgin (1958) described three to five obscure germ pores scattered on the urediniospore wall.

Kuehneola deightonii Y. Ono, **sp. nov.** Fig. 1c–e; Fig. 2b.

MycoBank no.: MB811794

Differs from *K. cissi-debilis* in forming paraphysate sori and from *K. vitis* in forming evenly thin-walled paraphyses and shorter teliospore.

Spermogonia and aecia: not observed. Uredinia: formed on abaxial leaf surface, scattered or loosely grouped, subepidermal in origin, soon becoming erumpent, peripherally paraphysate. Paraphyses: cylindrical, straight or weakly incurved, basally united, evenly thin-walled, colorless, 30–46 μm high, 7–12 μm wide (Fig. 1c). Urediniospores: formed singly on a short pedicel, appearing almost sessile, mostly subglobose or broadly ellipsoid, obovoid, 17–25×14–22 μm in size (Fig. 1d); the wall thin, colorless, echinulate, with no apparent germ pore. Telia: formed on abaxial leaf surface, white, cottony, peripherally paraphysate. Paraphyses: as in uredinia. Teliospores composed of 2–3 linearly arranged cells formed on a short pedicel (Fig. 2b); two-celled spores 24–33 μm long; three-celled spores 34–40 μm long; each cell broadly ellipsoid, 10–18 (−20)×10–15 μm in size (Figs. 1e and 2b); the wall thin, colorless; germinating in situ to form four basidiospores on a four-celled metabasidium. Basidiospores: obovoid, 6–9×5–7 μm in size, the wall thin, colorless.

Holotype: on *Cayratia gracilis* (Guill. & Perr) Suess. (originally identified as *Cissus gracilis* Guill. & Perr.) (Vitaceae): Sierra Leone, Bonjema, Kori, 29 October 1954, F. C. Deighton (II+III, PURF15960); Isotype (II+III, IMI58574a).

Etymology: from F. C. Deighton, a dedicated mycologist on African fungi.

Kuehneola vitis (E. J. Butler) P. Syd. & Syd., Monogr. Ured. 3: 321, 1914. Fig. 1f–h; Fig. 2c.

≡ ***Chrysomyxa vitis*** E. J. Butler, Ann. Mycol. 10: 158, 1912.

≡ ***Cerotelium vitis*** (E. J. Butler) Arthur, Bull. Torrey Bot. Club 44: 509, 1917.

≡ ***Catenulopsora vitis*** (E. J. Butler) Mundk. & Thirum. Ann. Bot. 7: 218, 1943.

Spermogonia and aecia: not observed. Uredinia: minute, scattered or loosely grouped on abaxial leaf surface, subepidermal in origin, soon becoming erumpent, peripherally paraphysate. Paraphyses: broadly cylindrical, moderately

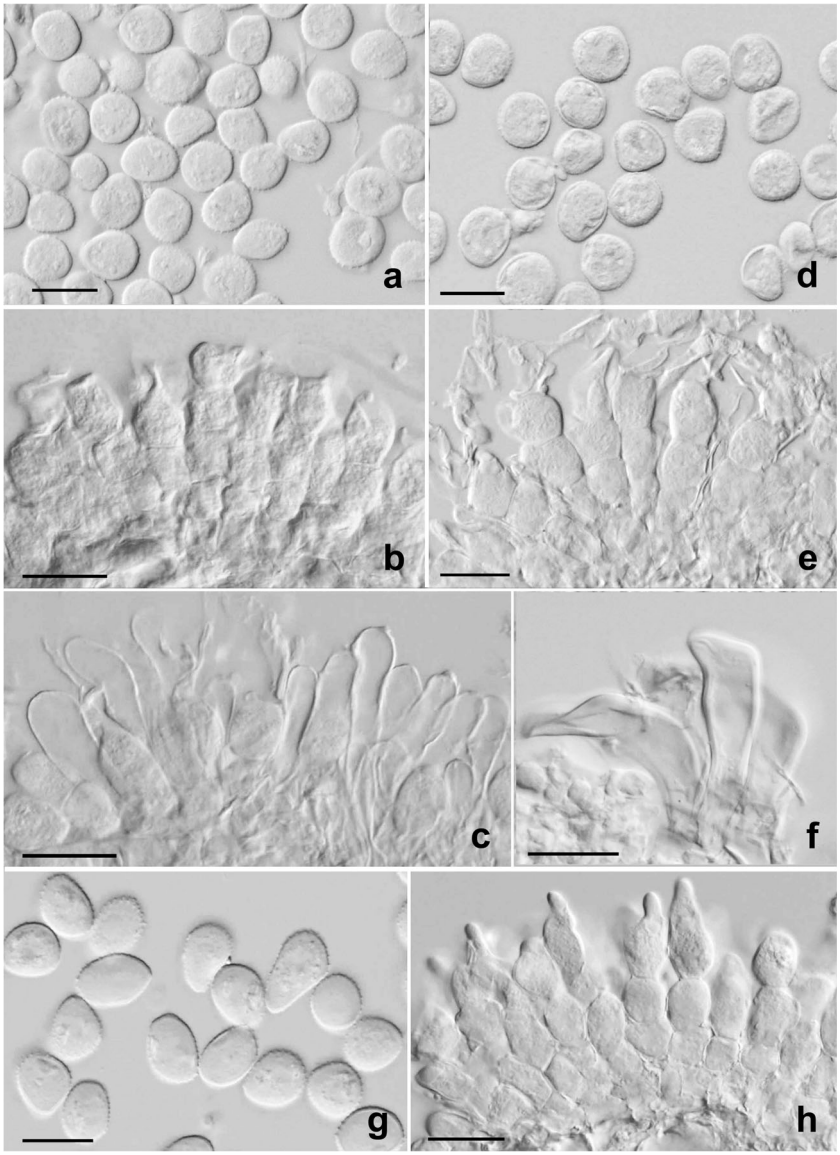

Fig. 1 *Kuehneola* species on Vitaceae plants. **a**, *K. cissi-debilis* (holotype, PC0167202). urediniospores; **b**, *K. cissi-debilis* (PC0167202, holoype). teliospores; **c**, *K. deightonii* (PURF15960, holoype). paraphyses; **d**, *K. deightonii* (PURF15960, holoype). urediniospores; **e**, *K. deightonii* (PURF15960, holoype). teliospores; **f**, *K. vitis* (PUR68020, isotype). paraphyses; **g**, *K. vitis* (IBAR7782). urediniospores; **h**, *K. vitis* (PURF15960, isotype). teliospores. *Bars* **a**–**h** 20 μm

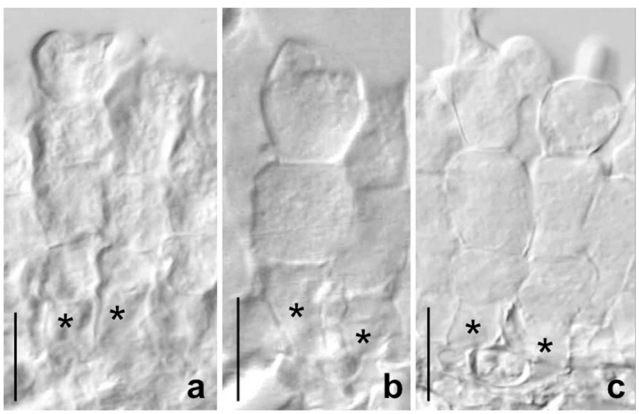

Fig. 2 Pedicellate teliospores of *Kuehneola* species on Vitaceae plants. **a**, *K. cissi-debilis* (PC050286, holotype); **b**, *K. deightonii* (PURF15960, holotype); **c**, *K. vitis* (PUR68020, isotype). Pedicels are indicated by asterisks (*). *Bars* **a**–**c** 20 μm

incurved, basally united, dorsally thick-walled (up to 3 μm thick), colorless, 26–50 μm high, 9–15 μm wide (Fig. 1f). Urediniospores: formed singly on a short pedicel, appearing almost sessile, mostly subglobose or broadly ellipsoid, obovoid, 17–25×11–20 μm in size (Fig. 1g); the wall ca. 2 μm thick, colorless, echinulate with no apparent germ pore. Telia: minute, loosely grouped on abaxial leaf surface, often replacing uredinia, white, cottony, peripherally paraphysate; paraphyses as in uredinia. Teliospores: composed of 2–5 linearly arranged cells formed on a short pedicel (Figs. 1h and 2c); two-celled spores 28–36 μm long; three-celled spores 33–53 μm long, four- or five-celled spores up to 65 μm long; each cell broadly ellipsoid, 10–19×7–14 μm in size; the wall thin and colorless; germinating in situ to form four basidiospores on a four-celled metabasidium. Basidiospores: obovoid, 6–9×5–7 μm in size; the wall thin, colorless.

Holotype: on *Ampelocissus latifolia* (Roxb.) Planch. (as "*Vitis latifolia*" in the original description), Bangladesh, Rangspur (as "Rangspur, Eastern Bengal" in the original description), 18 October 1909, S. N. Mitra (II+III, Herbarium Cryptogamae Indiae Orientalis, Delhi, India); Isotype in the Arthur Fungarium, Purdue University, USA (II+III, PUR68020).

Other specimen examined: on *A. latifolia*, India, Maharashtra, Ratnagiri, Dapoli, II, 14 October 1996, M. S. Patil (II, IBAR7782).

Notes: Describing *K. vitis*, Butler (1912) did not designate a holotype, but only mentioned "In foliis *Vitis latifoliae*, Dacca (A. L. Som) et Rangpur (S. N. Mitra), Indiae or." Mundkur and Thirumalachar (1943) designated the holotype as above and specified another specimen as "Dacca, October 7, 1910, leg. A. L. Som." They indicated the presence of spermogonia on the holotype specimen and a possible demicyclic life cycle of this species. Although Ragunathan and Ramakrishnan (1973) examined the holotype, they did not notice spermogonia on it. The isotype and an additional specimen examined in this study bore no structure like spermogonia.

Mundkur and Thirumalachar (1943) included two specimens on *Cissus adnata* Roxb. collected by E. J. Butler and Inayat in Noakhali, southeastern Bangladesh. Detailed observation was lacking for these specimens. Because these specimens were not available for the study, their taxonomic identity remains unsolved. Ragunathan and Ramakrishnan (1973) listed three specimens of *V. vinifera* L. as the host of this species. Because these specimens were also not available for study, their taxonomic identity was not determined. Unless the presence of teliospores is confirmed, the Southeast Asian *Phakopsora* species on *V. vinifera* (Pota et al. 2015) is easily mistaken as *K. vitis*.

Discussion

Distinction between *Kuehneola*, *Phragmidiella*, *Catenulopsora* and *Cerotelium*

Rust species forming uniseriate multiple cellular teliospores, which are thin-walled and laterally not adherent, are classified in *Chrysomyxa* Unger (Coleosporiaceae), *Cerotelium* Arthur, *Phragmidiella* Henn. (Phakopsoraceae) or *Kuehneola* Magnus (Phragmidiaceae) (Dietel 1928; Cummins 1959; Cummins and Hiratsuka 2003; Buriticá 1999). The family and genus assignment of a rust fungus forming this kind of teliospores is supplemented by the morphological types of spermogonium, aecium and urediniun, which the fungus produces during its life cycle. The host relationships in the life cycle are also considered to be important in some rust families and genera. If only telia and uredinia are known for a rust fungus to be classified, assignment of the species to a family and a genus is often arbitrary, except for *Chrysomyxa*, whose caeomatoid uredinia and the specific host relationships easily separate it from its morphological allies. This ambiguity of the generic classification is primarily due to different interpretations of the cell basal to a uniseriately arranged multiple cell. The basal cell may be interpreted as a meristematic cell from which one-celled teliospores are formed in basipetal succession; contrarily, it may be viewed as a pedicel on which an uniseriate, multicellular teliospore is formed by transverse septation. The two or more transversely septate cells on a pedicel are considered as integral parts of a single teliospore. This kind of teliospore was the characteristic of the genera *Kuehneola* (the type: *K. albida* (J.G. Kühn) Magnus on *Rubus*, Rosaceae) and *Phragmidiella* (the type: *P. markhamiae* Henn. on *Markhamia*, Bignoniaceae) (Hennings 1905). The two genera were distinguished by the presence (in *Kuehneola*) or absence (in *Phragmidiella*) of paraphyses in uredinia (Hennings 1905). It was also stressed that none of the species of *Kuehneola* occurred on the Bignoniaceae.

Subsequently, "pedicels" of teliospores of *Kuehneola* species became considered as meristematic sporogenous cells. Dietel (1912) said, "the so-called teliospores of *Kuehneola* are spore-chains, series of one-celled single spores, which are successively abstricted one after the other from the apex of common hypha, and remain united fast with one another" (cited from Arthur 1917). He restricted only species on rosaceous plants to accommodate in the genus and classified species of *Kuehneola* on non-rosaceous plants and of *Phragmidiella* in *Cerotelium* (Dietel 1928). Sydow and Sydow (1914) also defined *Kuehneola* as forming one-celled teliospores in elongated chains splitting apart upon maturity, and synonymized *Phragmidiella* under *Kuehneola*. They classified in the genus those species that occurred on non-rosaceous plants and which had been classified both in *Phragmiella* and *Kuehneola*. For them, taxonomic close host relationships were not important in defining rust genera.

Catenulopsora Mundk. was elected based on the type *C. flacourtiae* Mundk. & Thirum. on *Flacourtia* (Flacourtiaceae) (Mundkur and Thirumalachar 1943). In the type species, a laterally free, uniseriate multicellular teliospore developed from a teliospore initial arisen from a telial hymenium, from which a teliospore mother cell and a pedicel cell differentiated. Subsequently, the teliospore mother cell elongated and became transeversly septate to form a multicellular mature teliospore. Teliospore germination by continuous apical elongation was said to be unique to the genus (Mundkur and Thirumalachar 1943). However, teliospore germination (basidium production) by continuous apical elongation of teliospore cells was proven to be common in *Kuehneola*. Therefore, *Catenulopsora* was synonymized under *Kuehneola* (Thirumalachar 1960), despite

Thirumalachar and Mundkur (1949) again characterizing *Catenulopsora* by pedicellate multicellular teliospores and *Kuehneola* by sessile catenate teliospores.

Cummins (1959) explicitly defined *Kuehneola* as forming pedicellate, transversely multi-septate teliospores and *Phragmidiella* as forming one-celled, sessile and catenate teliospores. The same taxonomic treatment was followed by Buriticá (1999) and Cummins and Hiratsuka (2003), except for *Catenulopsora*, i.e., being treated as distinct in the former and synonymized under *Kuehneola* by the latter authors. The ontogeny of teliospores in species of *Kuehneola* and *Phragmidiella* has not been precisely determined, except for *K. flacourtiae* (Mundk. & Thirum.) Thirum.. (= *Catenulopsora flacourtiae* Mundk. & Thirum.). Photomicrographs in some published studies on *Kuehneola* species (Bagyanarayana and Rao 1985, Fig. 2; Ono 2012, Fig. 2) and in this study (Fig. 2), however, showed that a cell basal to linearly arranged cells in telia was narrower than, and firmly attached to, upper cells. This basal cell is morphologically comparable to the teliospore pedicel of *Hamaspora* (Monoson 1969; Cummins and Hiratsuka 2003) or *Fromeëlla* (McCain and Hennen 1990; Cummins and Hiratsuka 2003). Therefore, the generic definitions of *Kuehneola* and *Phragmidiella* by Cummins and Hiratsuka (2003) is followed in this study.

Species of *Cerotelium* form one-celled teliospores successively from the basal sporogenous cells on a telial hymenium as in *Phragmidiella*. Unlike *Phragmidiella* species, however, the teliospore chains of *Cerotelium* species are compacted, even if laterally not adherent, and the upper part of teliospores becomes easily separated and irregularly arranged upon maturity (Arthur 1917; Ono et al. 1992). In addition to the telial characteristics, association of *Milesia*-type, *Malupa*-type or *Physopella*-type of uredinia with telia makes the genus distict from *Phragmidiella* (Ono et al. 1992; Buriticá 1999).

Taxonomic changes of *Chrysomyxa vitis* on *Ampelocissus latifolia*

A rust fungus on *A. latifolia* was orignally described under the name of *Chrysomyxa vitis* E. J. Butler. Two to four thin-walled teliospores were arranged in a uniseriate laterally free column and echinulate urediniospores appeared to be formed in chains (Butler 1912). Butler was "unable to find any character which separate this fungus from the genus *Chrysomyxa*," even though he was well aware of the host difference between this species on the Vitaceae and other *Chrysomyxa* species occurring on Ericaceae and Pinaceae. Due to old and collapsed sori on the specimen he examined, however, Butler (1912) was unable to determine that the urediniospores were formed singly on a short pedicel. For similar reasons, Kühn (1883) might have erroneously described *K. uredinis* (Link) Arthur in the genus *Chrysomyxa* as *C. albida* J.G. Kühn.

Because of the changes in generic definitions due to different interpretations of teliospore ontogeny in *Kuehneola*, *Phragmidiella, Catenulopsora* and *Cerotelium* as briefly described above, *C. vitis* was classified differently later on. Sydow and Sydow (1914) classified the fungus on *A. latifolia* in the genus *Kuehneola*, as *K. vitis* (E. J. Butler) P. Syd. & Syd., a decision well supported by Butler's (1912) description and illustration. Arthur (1917) affirmed that the teliospores of *K. vitis* and *Kuehneola fici* E. J. Butler (Butler 1914) were produced in the same way as in *Cerotelium gossypii* (Lagerh.) Arthur, in which species one-celled teliospores successively formed from basal sporogenous cells were laterally adherent (Arthur 1917), and therefore transferred both species to *Cerotelium* as well. This was in contradiction to the descriptions and illustration in Butler (1912) that clearly indicated what Arthur (1917) circumscribed for *Kuehneola*. On the other hand, Mundkur and Thirumalachar (1943), when establishing *Catenulopsora*, determined that *K. vitis* formed a laterally free, uniseriate multicellular teliospore by transverse septation on a pedicel and transferred it to *Catenulopsora.* This taxonomic treatment was soon replaced by the reclassification of *C. vitis* in *Kuehneola* with nomenclatural revision as *K. vitis*, which name is accepted herein.

Morphology-based distinction of the rust genera and classification of species may not reflect their history of evolutionary diversification (Aime 2006). The presence or absence of teliospore pedicels and host restriction may have different meanings in different rust genera and families. Molecular systematic analyses (Maier et al. 2003; Wingfield et al. 2004; Aime 2006) indicated that the family Phragmidiaceae to which *Kuehneola* belongs is a well-circumscribed monophyletic taxon, most, if not all, species occurring on rosaceous plants. If *Kuehneola* were proven to be restricted to the species parasitic only on the plants of Rosaceae, other species on non-rosaceous plants would be classified in *Catenulopsora*, or even in *Phragmidiella* or *Cerotelium* with the generic definitions revised. Molecular phylogenetic analyses at a generic level with a number of species representing *Kuehneola*, *Phragmidiella,* and *Cerotelium* species incorporated will resolve the current difficulty in the taxonomy of the four genera and their possible allies, such as *Phakopsora, Mehtamyces* and *Newinia*.

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