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Knoxdaviesia with *K. serotectus* (Van der Linde & Jol. Roux) Z.W. de Beer & M.J. Wingf. a new world record from the gut of red palm weevils

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Abstract

During surveys of the mycota inhabiting guts of insect species in Assiut area, Egypt, two interesting isolates were obtained from two different gut samples (out of 11 investigated) of the red palm weevil (*Rhynchophorus ferrugineus* Olivier). These isolates were identified phenotypically as *Knoxdaviesia serotectus* (≡ *Gondwanamyces serotectus*). The isolates were deposited in the Assiut University Mycological Centre Culture Collection and given AUMC numbers, AUMC 10299 and AUMC 10293. Brief descriptions and photos are presented. This species is recorded here for the first time worldwide from the gut of red palm weevils.

Key words – Assiut – *Gondwanamyces* – insect gut – *Rhynchophorus ferrugineus*

Introduction

This investigation lies in the domain of environmental mycology that clearly characterizes the laboratory of mycology in the Department of Botany and Microbiology and Assiut University Mycological Centre, Egypt. It is an extension of the extensive surveys performed in different environments in Egypt: in soil, air, phyllosphere and phylloplane, rhizosphere and rhizoplane, carposphere and carpoplane, seeds and grains and food materials, which extended for approximately 50 years. The achievements were very fruitful with regard to the unprecedented broad knowledge of fungi in Egypt, which was culminated by the establishment of a large Culture Collection of fungi embraced by the Mycological Centre, Assiut University. Only a small percentage of the total fungal species on earth are known, suggested to be 1.62 million (Hawksworth 1991), 9.9 million (Cannon 1997), 2.27 million (Hawksworth 2001), or 3.5–5.1 million (O'brien et al. 2005). However, it is likely that the true number of fungal species on the planet is a seven-digit number, and may even be an order of magnitude higher (Bass & Richards 2011). Mycologists are strongly urged to work to search for new species or records in the different ecosystems around them.

The genus *Knoxdaviesia* M.J. Wingf., P.S. van Wyk & Marasas was described for *K. proteae*, the asexual state of a species described at the same time as *Ceratocystiopsis proteae* M.J. Wingf., P.S. van Wyk & Marasas (Wingfield et al. 1988). Marais et al. (1998) proposed the sexual morph genus *Gondwanamyces* Marais & M.J. Wingf. with *G. proteae* (M.J. Wingf., P.S. Van Wyk &

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Marasas) Marais & M.J. Wingf. as type species, to accommodate two ophiostomatoid species *Ceratocystiopsis proteae* (Wingfield et al. 1988) and *Ophiostoma capense* M.J. Wingf. & P.S. van Wyk (Wingfield & van Wyk 1993). These species are unique in having *Knoxdaviesia* asexual states and differ from each other in their ascospore morphology, and both are sensitive to cycloheximide, a typical of *Ceratocystis*. These species were transferred to the new genus as *Gondwanamyces proteae* and *G. capense* (M.J. Wingf. & P.S. van Wyk) Marais & M.J. Wingf. The genus *Gondwanamyces* is characterized by black, globose to subglobose perithecia, with relatively long necks, tapered towards the apex, asci evanescent and hyaline, ascospores hyaline, aseptate, with or without a sheath, asexual state *Knoxdaviesia* forming phialoconidia holoblastically from obovoid conidiogenus cells. Later, sequence data showed that they could be accommodated in the asexual state *Custingophora* (Kolarik & Hulcr 2009).

Custingophora Stolk, Hennebert & Klopotek, was introduced in 1968 by Stolk & Hennebert (1968) with *C. olivacea* Stolk, Hennebert & Klopotek as the type species, isolated from compost in Germany. *Custingophora* species have mononematous conidiophores that terminate in obovoid conidiogenous cells with distinct collarettes and conidia in drops of slime. The sequence data from the large subunit (LSU) ribosomal RNA gene suggested that species of *Gondwanamyces* and *C. olivaceae* are phylogenetically related and their morphological similarity suggests that *Gondwanamyces* and *C. olivaceae* have a shared ancestry and speculate that the divergence between the ancestral state of *Knoxdavesia* and *Custingophora* occurred (Viljoen et al. 1999). Until recently *Gondwanamyces* spp. were known only from southern Africa either specific to the infructescences of *Protea repens* L. (*G. proteae*) or in the floral parts of many *Protea* sp. (*G. capense*). However, Kolarik & Hulcr (2009) described two more species, *Custingophora cecropiae* M. Kolarik and *G. scolytodis* M. Kolarik from galleries in the sapwood of *Cecropia angustifolia* Trecul in Costa Rica. *C. cecropiae* from Costa Rica is associated with a scolytid beetle, *Scolytus unipunctatus* Blandford (Kolarik & Hulcr 2009). The discovery of *Gondwanamyces* on native trees in the Neotropics in Central America calls to question a hypothesis that these fungi are specific to the southern hemisphere (Roets et al. 2009).

In November 2011, two more species of *Gondwanamyces* species (*G. serotectus* Van der Linde & Jol. Roux and *G. ubusi* Van der Linde & Jol. Roux) were identified both were associated with damage caused by beetles (*Cossonus* sp.) to native *Euphorbia ingens* trees from South Africa in their decline and death phase. These authors transferred also *Custingophora cecropiae* to *Gondwanamyces* as *G. cecropiae* (M. Kolarik) Van der Linde, Jol. Roux & M.J. Wingfield.

In June 2012, one more species from South Africa, *Gondwanamyces wingfieldii* Roets & Dreyer was described as the first species of the genus collected from the infructescences of *Protea* (*P. kaffra*) from outside the boundaries of the Cape Floral Kingdom of South Africa, that *Protea* species has one of the widest distributions of all *Protea* spp. and extends from the KwaZulu-Natal Drakensberg in South Africa northwards into tropical Africa (Roets et al. 2012).

In November 2013, a new species of *Custingophora*, *C. blanchettei* Marinc., Z.W. de Beer, M.J. Wingf., C.A. Perez was described from Uruguay, near Maldonado, on soft wood of a *Phytolacca dioica* (Marincowitz et al. 2013).

Species of *Gondwanamyces* and their *Custingophora* asexual states form a strongly supported monophyletic clade that is sister to the Ceratocystidaceae. The morphology of the asexual morphs of *Gondwanamyces* is distinctive supporting a new family, Gondwanamycetaceae Réblová, W. Gams & Seifert in the Microascales to accommodate species of this genus (Réblová et al. 2011).

Viljoen et al. (1999) showed that the asexual state genus *Custingophora* was closely related to *Gondwanamyces*, and Kolařík & Hulcr (2009) subsequently suggested that *Knoxdaviesia* and *Custingophora* should be treated as synonyms. This suggestion was rejected by Van der Linde et al. (2012), but De Beer et al. (2013) concur with the separate treatment of these genera and applied one fungus one name principles accepted at the 18th International Botanical Congress held in Melbourne during July 2011 (Hawksworth 2011, Hawksworth et al. 2011) under which *Knoxdaviesia*, the oldest name, has priority over *Gondwanamyces*. *Knoxdaviesia* was thus redefined to accommodate the asexual morph species previously treated in *Gondwanamyces* and

provide new combinations where needed and nine *Knoxdaviesia* species were treated (*Knoxdaviesia capensis* M.J. Wingf. & P.S. van Wyk, *K. cecropiae* (M. Kolařík) Z.W. de Beer & M.J. Wingf., *K. scolytodis* (M. Kolařík) Z.W. de Beer & M.J. Wingf., *K. proteae* M.J. Wingf., P.S. van Wyk & Marasas, *K. serotectus* (Van der Linde & Jol. Roux) Z.W. de Beer & M.J. Wingf., *K. suidafrikana* (Morgan-Jones & R.C. Sinclair) Z.W. de Beer & M.J. Wingf., *K. ubusi* (Van der Linde & Jol. Roux) Z.W. de Beer & M.J. Wingf., *K. undulatistipes* (Pinnoi) Z.W. de Beer & M.J. Wingf. and *K. wingfieldii* (Roets & Dreyer) Z.W. de Beer & M.J. Wingf. Also, *Custingophora* is presently known only from its asexual state *C. olivacea* Stolk, Hennebert & Klopotek (De Beer et al. 2013).

Materials & Methods

During research of filamentous and yeast fungi in Egypt in guts of three insect species, namely honey bees (*Apis mellifera* L.) (Apidae, Hymenoptera) and black beetles (*Pterostichus melanarius* Illiger) (Carabidae, Coleoptera), and to our knowledge, for the first time worldwide from the gut of adult red palm weevils, (RPW) (*Rhynchophorus ferrugineus* Olivier) (Curculionidae, Coleoptera) (Fig. 1), yeasts and filamentous fungi were isolated more frequently in guts of RPW than in honey bees and black beetles (Moubasher et al. 2016, in prep.). Two filamentous isolates related to dematiaceous hyphomycetes were recovered on yeast malt extract agar, (YM) (Wickerham 1951) at 28°C from the guts of two different gut samples of RPW. For identification, these isolates were grown on malt extract agar (MEA) and potato dextrose agar (PDA) (Samson et al. 2004). The plates were incubated at 25°C for 7–15 days. For micro-morphological observations, microscopic mounts were made in lactophenol cotton blue.

Fig. 1 – Date palm tree (left) and red palm weevil: *Rhynchophorus ferrugineus* (right).

Results

Identification could designate the two isolates to *Knoxdaviesia serotectus* (≡ *Gondwanamyces serotectus*).

Knoxdaviesia serotecta (J.A. van der Linde & Jol. Roux) Z.W. de Beer & M.J. Wingf., in de Beer, Seifert & Wingfield, CBS Biodiversity Series 12: 298 (2013). Figs. 2–3

≡ *Gondwanamyces serotectus* J.A. van der Linde & Jol. Roux, Mycologia 104: 578 2012.

Facesoffungi number: FoF 03252.

The current strains are similar in their macro and micro-morphology to *Knoxdaviesia serotectus*. In brief, colonies fast growing on PDA than on MEA at 25°C, conidiophores arising from finger-shaped rhizoids, conidiophores dark-brown, 50–150 × 5 µm, wider at the base (6–7

µm), slightly thinner at the apex (5 µm), 2–4-septate, terminating into globose vesicles (*Aspergillus*-like), 7–10 µm diameter, vesicles bearing 4–10 conidiogenous cells, conidiogenous cells with distinct collarettes, often proliferating into secondary conidiophores bearing conidiogenous cells, conidia rounded at base and apex, oblong, hyaline, aseptate, 8–10 × 4–5 µm, conidia darken after 2 weeks, sexual state not observed.

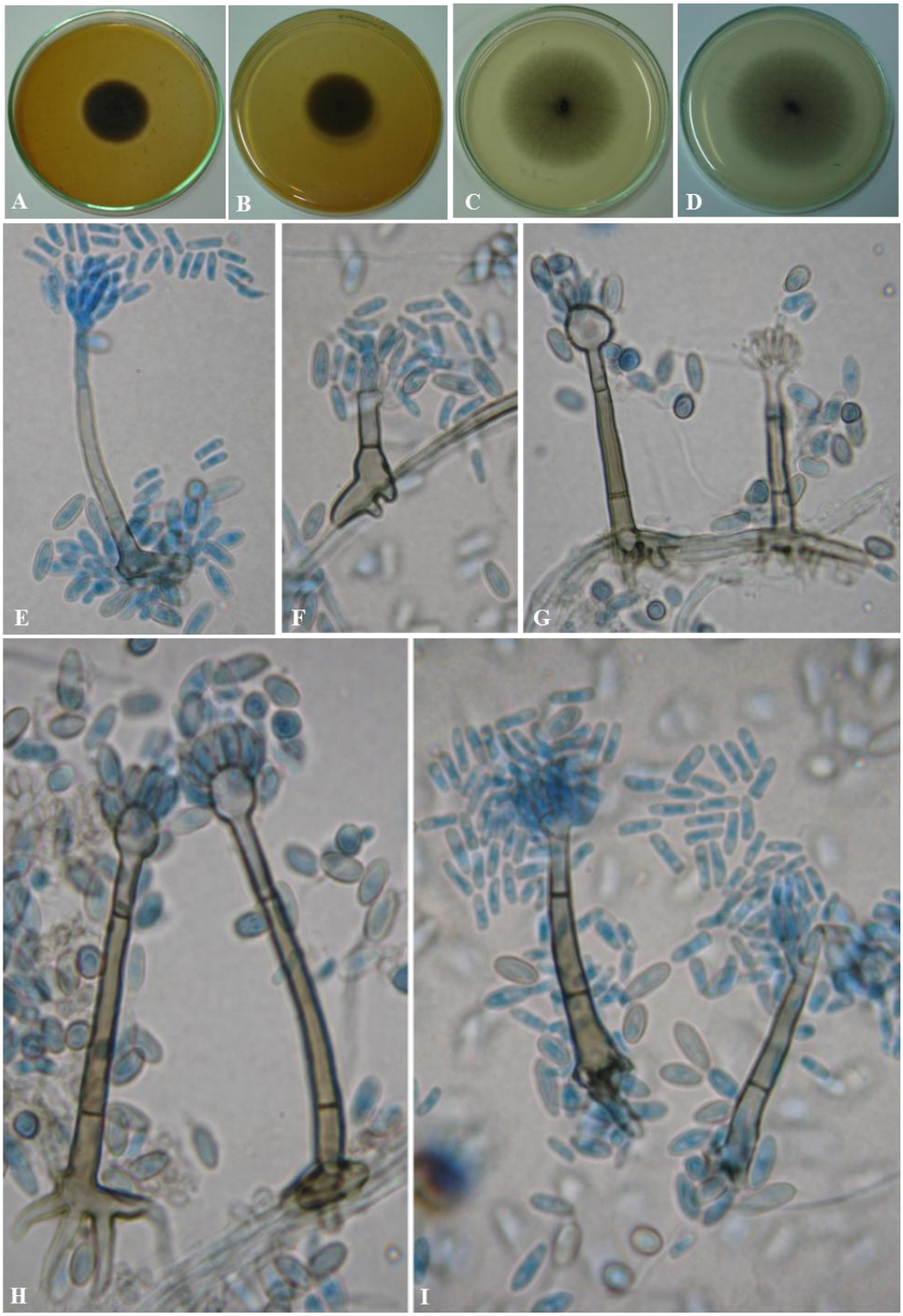

Fig. 2 – *Knoxdaviesia serotectus* (AUMC 10299). A Culture obverse on MEA. B Reverse on MEA. C Obverse on PDA. D Reverse on PDA. E-I Foot cells with finger-shaped rhizoids, dark brown, septate conidiophores ending with vesicles, bearing conidiogenous cells and aseptate, cylindrical slimy phialoconidia.

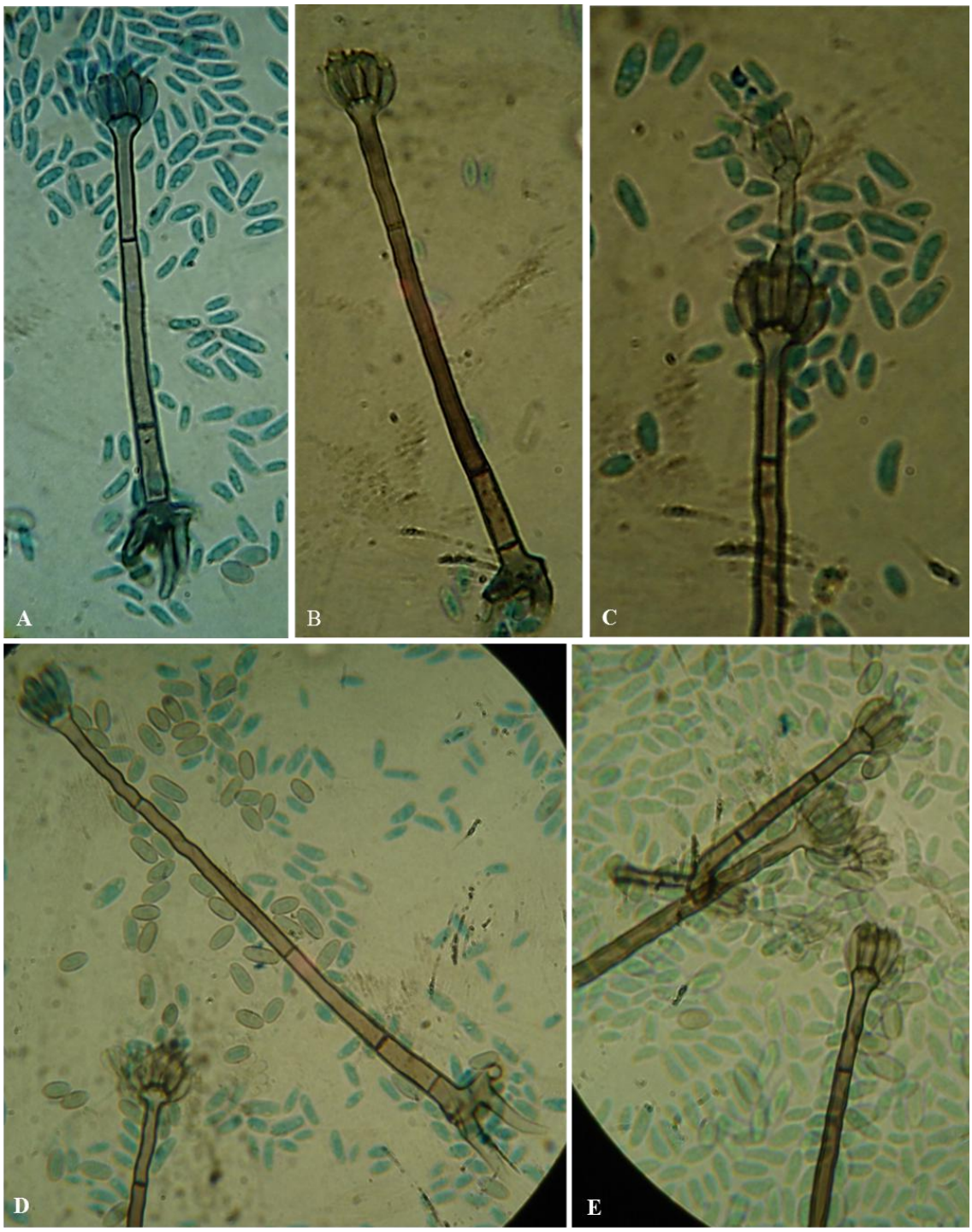

Fig. 3 – *Knoxdaviesia serotectus* (AUMC 10299). A, B, D. Foot cell with finger-shaped rhizoids giving rise to brown, 1–4 septate conidiophores. C Proliferation arising from the vesicle. E Subapical proliferation and proliferation from the vesicle.

Ecology

The present strains were isolated on July 2013 from the gut of *Rhynchophorus ferrugineus* (kindly supplied by the Faculty of Agriculture, Assiut University). This is the second record after its first description by Van der Linde et al. (2012) from discolored plant material from *Euphorbia ingens* and from the bodies of the weevil *Cossonus*, but the first from the gut of red palm weevils. These strains were deposited at the Culture Collection of Assiut Universiy Mycological Centre and given numbers AUMC 10299 and AUMC 10293.

Discussion

Knoxdaviesia serotectus (as *Gondwanamyces serotectus*) was first isolated from discolored plant material from *Euphorbia ingens*, as well as from the bodies of the weevil *Cossonus* from South Africa, Limpopo Province in May 2009 and known only from four strains (Van der Linde et al. 2012). The present two strains were isolated from the gut of red palm weevils on July 2013 and this is the second record after its first description by Van der Linde et al. (2012).

Van der Linde et al. (2012) evaluated the pathogenicity of the *Knoxdaviesia* species they described including *K. serotectus* and found that both produced lesions on healthy succulent branches of *Euphorbia ingens* in contrast to the control inoculation in which internal lesion development and discoloration was absent. This suggests that these fungi and the insects that carry them could play a role in the decline of *E. ingens* in Limpopo Province.

The association of a *Knoxdaviesia* sp. with insects is not unexpected given that *K. proteae* and *K. capensis* occur in insect-infested *Protea* infructescences and initially were thought to be vectored by one or more of the insects in this niche (Wingfield et al. 1988). The insect species *Genuchus hottentottus* Fabricius collected from *Protea repens* infructescences was confirmed as carrier of *K. proteae* using PCR-based method and this suggests that there may be a symbiotic relationship between the plants, vector insect, and (or) the fungus (Roets et al. 2006). However, *K. proteae* DNA and ascospores were identified on diverse lineages of arthropods including beetles (*Euderes lineicolis* and *Genuchus hottentottus*), bugs (*Oxycarenus maculates*), a psocopteran species and five mite (*Acari*) species but based on isolation frequency, a mite species in the genus *Trichouropoda* appears to be the most common vector of *K. proteae* (Roets et al. 2006, 2011).

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