DNA-based identification of Lepidoptera associated with citrus in South Africa

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A number of insects, primarily Lepidoptera, cause damage to citrus in South Africa. A major limitation to the management and control of these pests is their correct identification. The aim of this study was to develop a database of gene sequences to aid in the identification of these Lepidoptera. Multiple specimens of 12 species were sequenced for the ~650 bp of the cytochrome oxidase I gene. These sequence data were supplemented and validated using sequences available in public databases. Results showed that each species could be unambiguously identified, and neighbour-joining analysis based on K2P distances formed highly supported, distinct clusters for each species, *i.e.* the maximum intraspecific genetic distance was less than that of the minimum interspecific genetic distances. Thus, this data set provides a molecular means to successfully identify the most important Lepidoptera associated with citrus in South Africa.

Key words: DNA barcode, Tortricidae, Noctuidae, *Ectomyelois ceratoniae, Thaumatotibia leucotreta.*

INTRODUCTION

Citrus fruit is a major source of revenue for South Africa, and in 2012 the country ranked second in the exportation of fresh citrus worldwide (CGA 2013). However, this value would be greater were it not for the wide range of pests that attack citrus, which can reduce yields if not controlled properly and/or pose a phytosanitary risk. Globally, citrus has been documented as a host for approximately 875 insects and mites (Smith & Peña 2002). Of these, approximately 100 are found in South Africa, including scale insects, mealybugs, aphids, whiteflies, leafhoppers, mites, beetles, thrips, fruit flies and various moth and butterfly species (Bedford *et al.* 1998; Smith & Peña 2002). The high number of pests reflects the fact that South Africa has a favourable climate, including tropical and subtropical regions (Urquhart 1999).

Several lepidopteran species are associated with citrus in South Africa (Bedford 1998). Of these, only one is considered to be a major pest – *Thaumatotibia leucotreta* (Meyrick, 1913) (Tortricidae, false codling moth). Minor or sporadic primary lepidopteran pests of citrus include *Papilio demodocus* (Esper, 1798) (Papilionidae, citrus swallowtail), *Pa. nireus lyaeus* (Linnaeus, 1758) (Papilionidae), *Pa. dardanus* (Brown, 1776) (Papilionidae), *Lozotaenia capensana* (Walker, 1863) (Tortricidae, apple leaf roller), *Ectomyelois ceratoniae* (Zeller, 1839) (Pyralidae, carob moth), *Phyllocnistis citrella* (Stainton, 1856) (Gracillaridae, citrus leaf miner), *Prays citri* (Milliére, 1873) (Yponomeutidae, citrus flower moth), *Choristoneura occidentalis* (Walsingham, 1891) (Tortricidae, citrus leaf roller), *Helicoverpa armigera* (Hübner, 1809) (Noctuidae, cotton bollworm), and *Ascotis selenaria reciprocaria* (Denis & Schiffermüller, 1775) (Geometridae, citrus looper). Fruit-piercing moths associated with citrus include *Serrodes partita* (Fabricius, 1775) (Noctuidae), *Ophiusa tirhaca* (Cramer, 1773) (Noctuidae), *Eudocima divitosa* (Walker, 1869) (Noctuidae), *Eudocima materna* (Linnaeus, 1767) (Noctuidae), *Oraesia emarginata* (Fabricius, 1794) (Noctuidae), *Pericyma atrifusa.* (Hampson, 1902) (Noctuidae) and *Pe. mendax* (Walker, 1858) (Noctuidae). Secondary citrus pests include the fruit-sucking moths *Achaea echo* (Walker, 1858) (Noctuidae), *A. finita* (Guenée, 1852) (Noctuidae), *A. infinita* (Guenée, 1852) (Noctuidae), *A. lienardi* (Boisduval, 1833) (Noctuidae), and *Sphingomorpha chlorea* (Cramer, 1777) (Noctuidae), and *Hypanua xylina* (Distant, 1898) (Noctuidae) (Bänziger 1969; Bedford *et al.* 1998; Newton 1998; Smith & Peña 2002; Timm 2008; Rentel 2013). In addition, a number of lepidopteran species have been historically associated (perhaps erroneously) with citrus, although they do not have pest status. Such species include *T. batrachopa* (Meyrick, 1908) (Tortricidae, macadamia nut borer), which has only been recorded once on citrus (Bedford *et al.* 1998; Newton 1998),

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