SHORT COMMUNICATION

MOLECULAR IDENTIFICATION OF SOUTH AFRICAN SUGARCANE WHITE GRUBS (COLEOPTERA: SCARABAEIDAE)

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Abstract

Several species of white grub are known to attack sugarcane in South Africa, many of which have not been identified. Correct identification of species is essential to develop sound control tactics. Morphological identification of larvae and adults is possible, but it is often difficult to link adults and larvae of the same species because of their distinct morphological differences. Complementary molecular diagnostic techniques are useful to match adults to larvae of the same species. A molecular analysis of the cytochrome oxidase *c* subunit I (COI) region of the mitochondrial genome was conducted on populations of white grub larvae and adults from various parts of the sugarcane growing regions. Results presented show it is possible to link larvae collected from soil in sugarcane fields with adults of the same species from light trap catches.

Keywords: Scarabaeidae, white grubs, mitochondrial DNA, COI, sugarcane, taxonomy

Introduction

Since the mid-1980s, sugarcane damage by scarabaeid (Coleoptera: Scarabaeidae) larvae has become prevalent in certain areas of the sugar industry, initiating investigations into their pest status, economic impact and control measures (Mansfield, 2004). In South Africa the pest species associated with sugarcane belong to the families Melolonthidae, Rutelidae, Dynastidae and Cetoniidae (Scholtz and Holm, 1996). Most of these scarabaeids, with the exception of one species, *Heteronychus licas* Klug, whose adults feed on young sugarcane shoot tissue, feed only as larvae on the sugarcane roots (Wilson, 1969). However, it is not known for certain how many species of white grubs attack sugarcane in South Africa, despite the importance of this information for developing controls. There are limitations using only morphological characteristics to identify larvae and adults, even of the same species, because they look so different to each other.

Although adults can be collected from light traps and identified using morphological differences, this does not accurately reflect Scarabaeids attacking sugarcane. Light traps attract a range of Scarabaeids, some from sugarcane, and others from other vegetation types (Sweeney, 1967). It would be better to collect the Scarabaeid larval stage causing damage, but the identity of many of these is unknown. To be identified, they need to be reared to adults, which could take up to a year. Larvae can be bred from eggs laid by identified adults, but adults occur for only short periods each year (Sweeney, 1967).

However, recent developments in deoxyribonucleic acid (DNA) technology have resulted in a range of molecular techniques for use in species identification (Gleeson *et al,* 2000). These complementary molecular diagnostic techniques are useful to match adults (from light traps) to larvae (from soil) of the same species. Mitochondrial DNA (mtDNA) has been widely used in taxonomic and population studies, and is well suited to identifying morphologically similar species because its mutation rate is high enough to provide numerous sequence differences between closely related species (Avise, 1994).

This paper demonstrates that mtDNA has the potential to match adults to larvae of the same species, and can separate different species from each other.

Materials and Methods

Taxon sampling

Eight Scarabaeid specimens, collected from the cane growing areas of South Africa, were used in this study.

Laboratory techniques

Eight specimens were used, of which six were processed by the Barcode of Life Data Systems (Hebert and Ratnasingham (2006) http://www.barcodinglife.org/views/login.php) and two were processed as outlined by King *et al.* (2002).

Analysis

DNA sequence chromatograms were edited and checked for base-calling errors using the Staden package (Staden, 1996). Sequences were automatically aligned using ClustalX (Thompson *et al,* 1997) and manually corrected using BioEdit (Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic analysis was performed by Maximum Parsimony using PAUP*4.0b10 (Swofford, 2002), using a heuristic search with 1000 random addition sequences. A 1000-replicate bootstrap analysis was performed to assess node support. P-distance was analysed, and specimens with a very small distance were considered.

Morphological parameters

Individuals were linked together by mitochondrial DNA similarity, and photographs of morphologically important features were considered and evaluated using a LEICA MZ16 Light Microscope.

Results

Maximum parsimony analysis

One hundred and seventy-seven variable sites were found in the 658 bp fragment, of which 164 sites were parsimony-informative. One strict parsimonious tree was recovered, with a length of 198 steps, CI of 0.9949 and RI of 0.9963. The strict consensus tree showed the presence of three species groups, each strongly supported (100%). The first species group

clustered an unidentified adult (DNA81) from Mtunzini, as well as an unidentified larva (DNA 0507) from Seven Oaks. The second species group clustered three unidentified larvae (DNA 0529, 0530, 0535) from Dalton, and *Temnorrhynchus* sp. adult (DNA 0583) from Richmond. The third species group clustered *Heteronychus licas* adult (DNA 0562) and an unidentified adult (DNA 40) together.

Morphological identification

Figure 1 shows larval raster patterns and adult specimens of unknown species 1 (A, B and C), those of *Temnorrhynchus* sp. (D, E and F), and an adult *H. licas* (G). The larvae of species 1 are differentiated from other Scarabaeid larvae by the oval raster shape, with 12-14 pali present on each side of the oval. Similarly, larvae of species 2 are differentiated by the setae, which become gradually longer towards the anal orifice.

Discussion

The maximum parsimony tree reconstructed using the eight sequences clearly separated the specimens into three distinct groups. Each group constitutes adults from a separate species and larvae with different morphological features. There is a strong bootstrap support for all three species groups that are formed. The sequence divergence within each group ranged from 0.00-1.09%, which is within the limit of intraspecific sequence divergence in mitochondrial DNA reported from different Coleopteran species (Cognato, 2004). In a study by Cognato (2004), the intraspecific sequence divergence between different species of Coleopterans was found to vary from 0.3-9.1%. However, mitochondrial DNA sequence divergence observed in this study is far below the limit of the sequence divergence reported between two sister coleopteran species (1.7-18.2%). From this it is clear that the sequence divergence observed within each group in our study is a sequence divergence that is expected to be found within closely related populations of a species. From the result of this experiment it is therefore possible to say that the unknown larvae grouped with the already identified/unidentified adults are from the same population.

Conclusions

Combining molecular and morphological data was found to be efficient in linking scarabaeid larvae with adults. Identification of the economically important larvae and linking them with adults will help in developing a reliable morphological key, which can be used by field workers. This contributes to the development of sound pest management strategies.

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REFERENCES

Avise JC (1994). *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.

Cognato AI (2004). Standard percent sequence difference for insects will not predict species boundaries. *J Econ Ent* 97: 1-17.

Gleeson D, Holder P, Newcombe R, Howitt R and Dugdale J (2000). Molecular phylogenetics of leafrollers: Application to DNA diagnostics. *New Zealand Plant Prot* 53: 157-162.

King H, Conlong DE and Mitchell A (2002). Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): Evidence from the mitochondrial cytochrome oxidase I and II genes. *Proc S Afr Sug Technol Ass* 76: 321-328.

Mansfield LR (2004). Ecology and control of Scarabaeid soil pests in South African inland sugarcane. MSc Thesis, University of Pretoria, Pretoria, South Africa.

Scholtz CH and Holm E (Eds) (1996). *Insects of Southern Africa*. University of Pretoria, Pretoria, South Africa.

Staden R (1996). The Staden sequence analysis package. *Molecular Biotechnology* 5: 233-241.

Sweeney C (1967). The Scarabaeoidea associated with sugar cane in Swaziland: An account of preliminary investigations into bionomics and control. August 1965-June 1967. Research Bulletin No. 16, Swaziland Ministry of Agriculture, Swaziland. 163 pp.

Swofford DL (2002). *PAUP* Phylogenetic analysis using parsimony *(And other methods)*. Sinauer Associates, Sunderland, Massachusetts, USA.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997). The ClustalX window interface: Flexible strategies for multiple sequence alignment aided by quality and analysis tools. *Nucleic Acid Resume* 24: 4876-4882.

Wilson G (1969). White grubs as pests of sugarcane. pp 237-254. In: Williams JR, Metcalfe JR, Mungomery RW and Mathes R (Eds), *Pests of Sugarcane*. Elsevier Publishing Company, Amsterdam, The Netherlands.

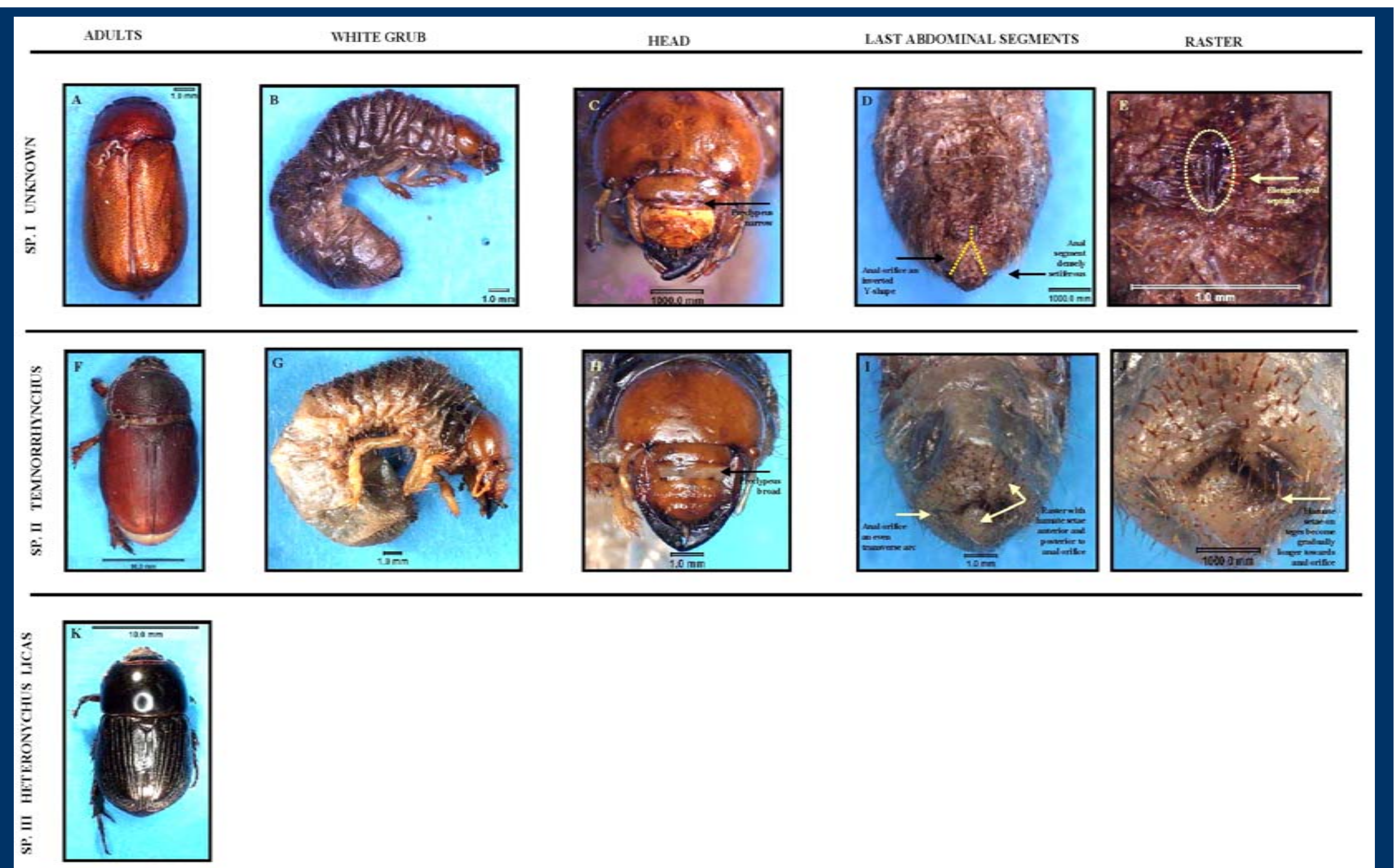

Figure 1. Morphological description of adults and raster pattern of larvae. A = Adult specimen of Scarabaeid DNA 81 10x magnification; B = Whole Scarabaeid larvae DNA 0507 8x magnification; C = Head capsule of Scarabaeid DNA 0507; D = Raster pattern of Scarabaeid DNA 0507 20x magnification; E = Raster pattern of Scarabaeid DNA 0507 57.5x magnification; F = Adult specimen of Scarabaeid DNA 0583 *Temnorrhynchus* sp. 5x magnification; G =Whole Scarabaeid larvae DNA 0530 8x magnification; H = Head capsule of Scarabaeid larvae DNA 0530 10x magnification; I = Raster pattern of Scarabaeid DNA 0530 *Temnorrhynchus* sp. 16x magnification; J = Raster pattern of Scarabaeid DNA 0530 *Temnorrhynchus* sp. 25x magnification; K = Adult specimen of Scarabaeid DNA 40 *Heteronychus licas* 5x magnification.