SHORT, NON-REFEREED PAPER

# USING MORPHOLOGICAL AND MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF WHITE GRUB SPECIES (COLEOPTERA: SCARABAEIDAE)

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## Abstract

Scarabs are of enormous ecological and economic importance, yet their taxonomy, morphology, ecology and species status are poorly known, especially in southern Africa and surrounding Indian Ocean islands. Morphological features are not always reliable, particularly for immature stages, therefore inclusion of DNA-based methods offer a more comprehensive means to identify species. This study investigates the utilisation of molecular methods for larval and adult white grubs in a number of specimens obtained from Mauritius, Tanzania, Madagascar, South Africa and Zimbabwe. Mitochondrial *co1* and *28S rRNA* from larval and adult specimens were used. Individuals were grouped into putative species using the nearest neighbor joining method. The morphological features based on available keys were also included to draw solid conclusions, as many of the species are not available in GenBank. For larval forms, the raster patterns, the spiracles, the head capsule, the mandibles and the tarsi were used to compare morphological features. Where no larval form was obtained, the exuviae were used instead. For the adult forms the clypeus, front and hind tarsi and the properties of the elytra were used.

Several species of white grubs were identified with a high tendency of area specificity, particularly in the oceanic and coastal regions. A variety of species were identified from the inland areas, necessitating the need to employ broad spectrum pest control strategies in the latter regions.

*Keywords:* taxonomy, ecology, morphology, *mt*DNA, ribosomal *r*RNA, white grub species

## Introduction

The application of molecular techniques in taxonomy is an important approach in identifying organisms that present difficulties in morphological identifications. Difficulties in organism identifications are caused by high similarities which cannot be easily deciphered by morphological examinations. Although identification keys have been developed, uncertainties are still common especially for many unexplored species from the tropics (Šípek and Ahrens, 2011). In white grubs, complications arise when matching larval and adult specimens due to the longer time that the white grubs spend in the larval form compared to short periods of adult life. To precisely match adult and larval forms, morphological examinations have to be

carried out using the same specimen (Dittrich *et al*., 2006; Arhens *et al*., 2011), which in many instances is not the case. Mitochondrial DNA (*mt*DNA) is a useful tool that can be used to overcome this obstacle in conjunction with morphological studies as there are not many species of white grubs that are found in the GenBank compared with those that are unknown.

The objectives of this study were to use molecular techniques to link (i) immature scarabaieds to their adult forms, (ii) link larval and pupal forms of scarab beetles and (iii) to identify the unknown scarabaied forms.

## Materials and Methods

Total genomic DNA was individually extracted from muscles of the heads and legs of adult and larval white grubs that were obtained from various sugar producing areas in southern Africa and Indian Ocean islands. One mitochondrial gene region, cytochrome c oxidase subunit 1 (*co1*) and a fragment of *28S r*RNA were sequenced for analysis. PCR and sequencing were performed using the primers **Pat** (5'tccaatgcactaatctgccatatta) and **Jerry** (5'caacattattttgatttttttgg) (Simon *et al*., 1994) for the *co1*, and primers **FF** (5'ttacacactccttagcggat) and **DD** (5'gggacccgtccttgaaacac) for the *28S r*RNA (Inward, 2003).

All sequences were manually edited in the BioEdit computer program to obtain consensus sequences. The nucleotide sequences were individually entered into BOLD, which automatically submitted them to NCBI for blasting on GenBank for species identity. The *co1* and 2*8S r*RNA nucleotides from the specimens and those retrieved from the GenBank were aligned using Multiple Alignment with Fast Fourier Transform (MAFFT) version 6, and sequence data was bootstrapped into 1000 replications (Katoh *et al*., 2002) A tree based on genetic distances was constructed by the neighbour joining method (NJ) (Saitou and Nei, 1987) which was read using Archaeopteryx applet v 0.955 (Zmasek and Eddy, 2001; Han and Zmasek, 2009).

## Results and Discussion

Using *mt*DNA *co1*, the authors successfully grouped adult specimens from Mauritius into three subfamilies (Dynastinae, Melolonthinae and Rutelinae) and four different species (Figure 1). Unknown larvae from South Africa were also successfully grouped into four genera and several species that are yet to be verified (Figure 2). Using *28S r*RNA, two unknown larvae and pupa from Madagascar were matched. A global search using *Blastn* indicated that they are *Paramorphochelus* sp (Randriamanantsoa *et al*., 2010) (Figure 3). Larval and adult forms of *Cochliotis* sp. from Tanzania (Evans *et al*., 1999), and *Oryctes* spp. were also successfully matched while *Heteronychus* sp. adults from Madagascar and Zimbabwe were also matched (Figure 3). These results form a foundation for a comprehensive identification of the species in which morphological features will be included to give a more accurate species identification and development of taxonomic keys. The preliminary results have provided scientific evidence to link immature and adult white grub specimens (Figures 2 and 3) indicating the relevance of the DNA region being sequenced. Molecular techniques are therefore an important tool in matching different forms of the scarabs and this will address the problem of having to depend exclusively on morphological features to identify species.

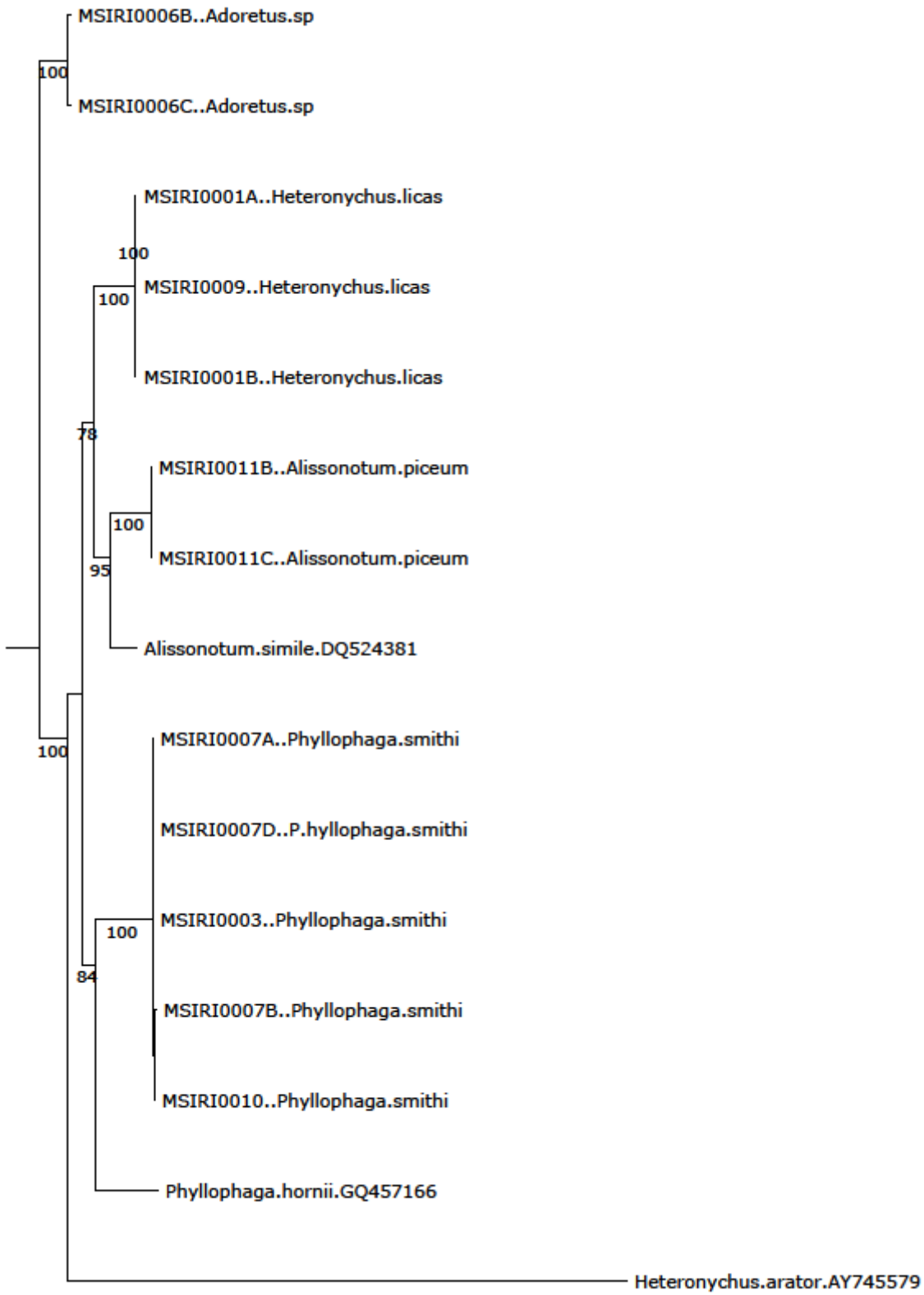


**Figure 1. NJ tree using *mt*DNA *co1* for specimens of adult scarabs from Mauritius (MSIRI). *A. simile* (DQ524381) and *P. hornii* (GQ457166) from the GenBank were incorporated into the tree. The dynastid, *Heteronychus arator* (AY745579) was used to root the tree. Numbers on branches show confidence levels. Clustal format alignment was run by MAFFT version 6 with bootstrap re-sampling set at 1000.**

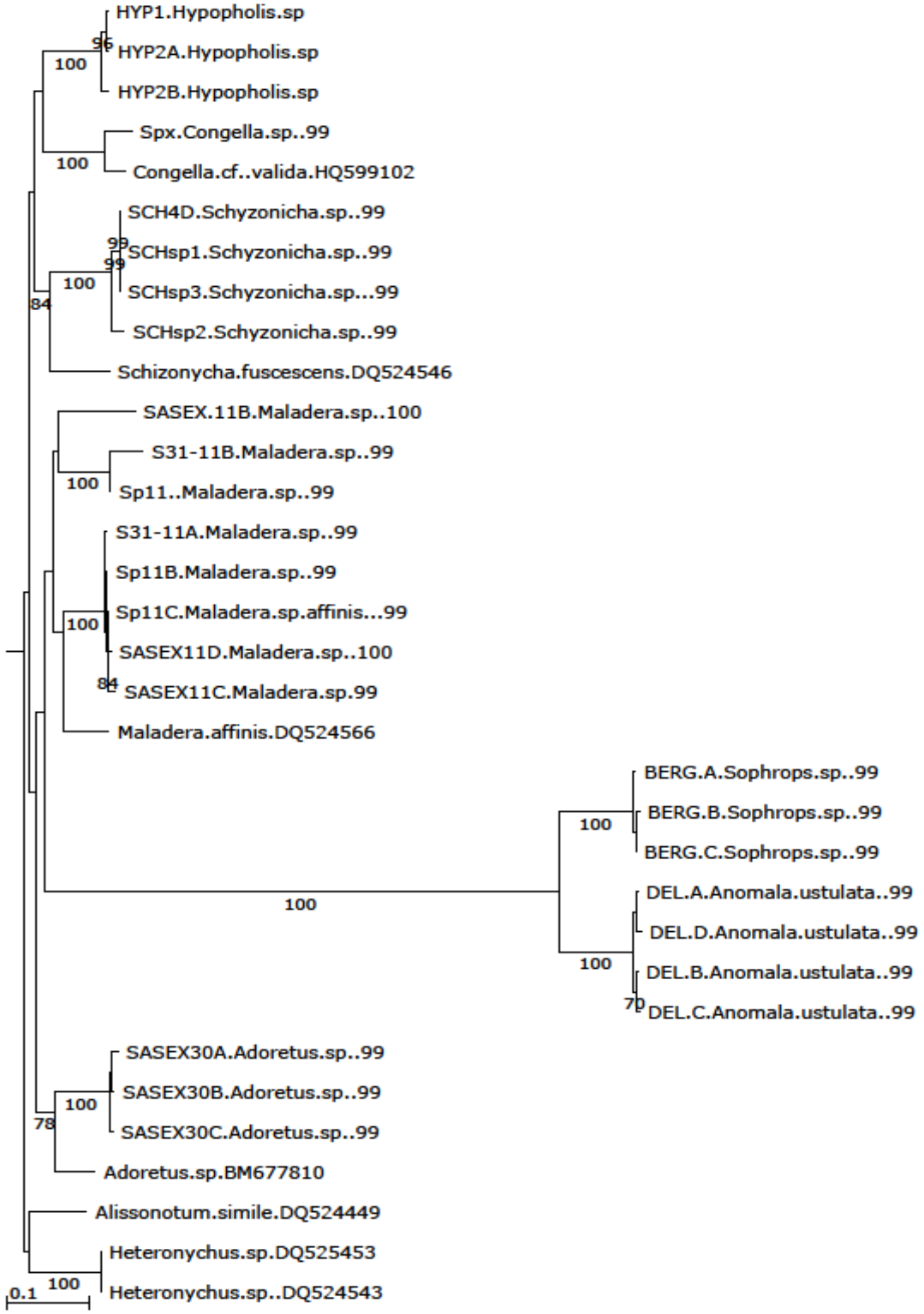


**Figure 2. NJ tree obtained using *mt*DNA *co1* for South African larval specimens including *Adoretus* sp (BM677810), *Congella.* cf *valida* (HQ599102), *Maladera affinis* (DQ524566) and *Schyzonicha fuscescens* (DQ524546) retrieved from the GenBank. The dynastids, *Heteronychus* sp (DQ525453 and DQ524543) and *Alissonotum* sp. (DQ4449) were used to root the phylogenetic tree. Numbers on branches show confidence levels while those in front of taxa names indicate percentage similarity with the given taxa. Clustal format alignment was done by MAFFT version 6. Bootstrap re-sampling was set at 1000.**

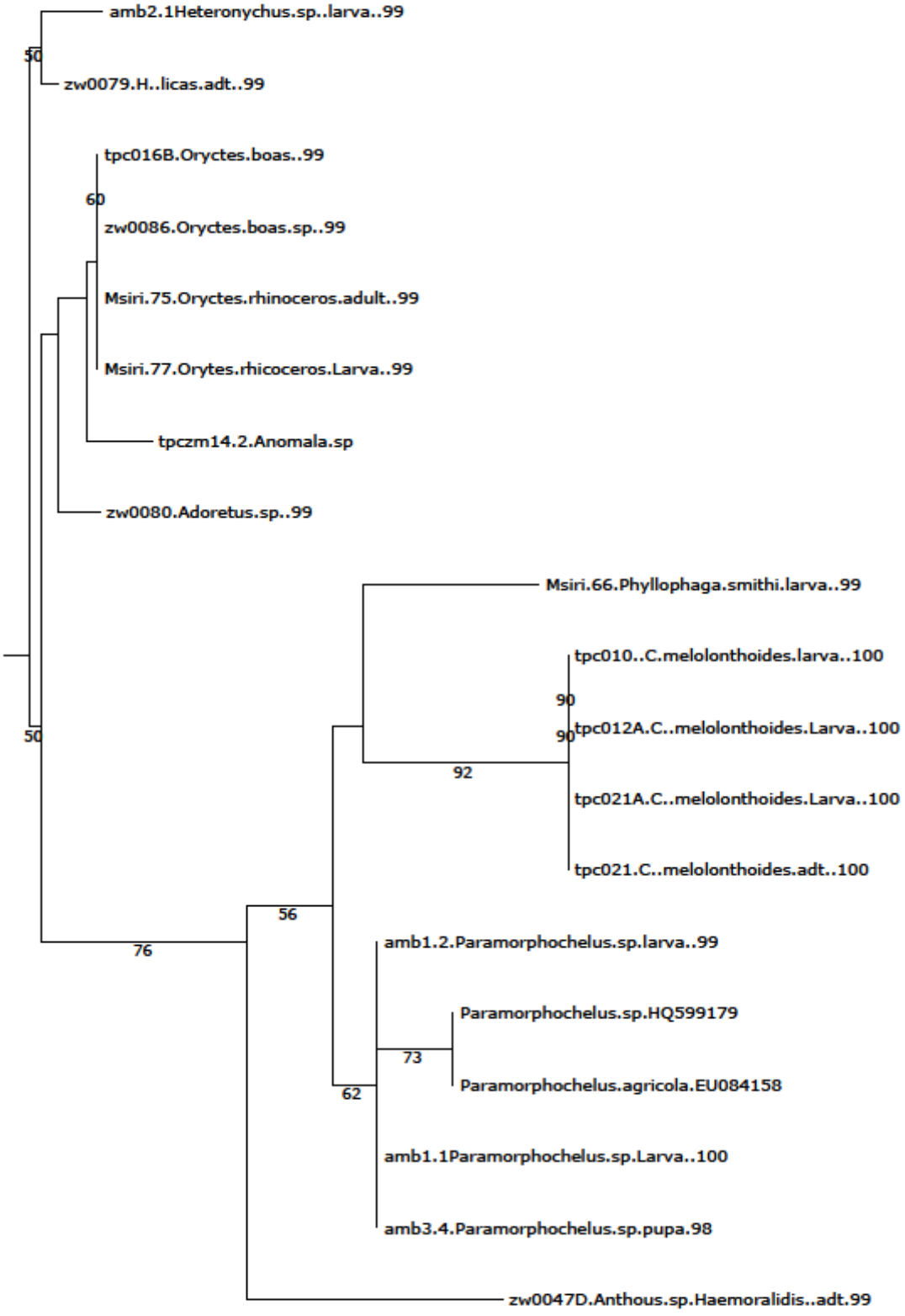


**Figure 3. NJ tree for Mauritius (Msiri), Madagascar (amb), and Tanzania (tpc) and Zimbabwe (zw) specimens based on the 28S ribosomal RNA (*r*RNA) The Elaterid, *Athous haemorallidis* (zw0047D) was used as an out-group to root the tree. Numbers of branches show confidence levels while those in front of taxa names indicate the percentage degree of similarity with the given taxa. Clustal format alignment was done by MAFFT version 6 with bootstrap re-sampling set at 1000.**

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