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ScienceDirect
Molecular Phylogenetics and Evolution 44 (2007) 436–449
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae)

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Received 28 August 2006; revised 21 February 2007; accepted 23 February 2007
Available online 2 March 2007

Abstract

DNA sequences provide a universal character system in taxonomy for associating all developmental stages of organisms, but ambiguity arises due to genetic variation within species. The problem is compounded where target groups are less well studied or incompletely represented in DNA databases. Here we investigate the utility of DNA for larval–adult species associations within chafer (Coleoptera: Scarabaeidae) communities from four sites in the tropical lowlands of Nepal. We sequenced *ca.* 1600 bp of mitochondrial *cox1* and *rrnL* and 700 bp of nuclear 28S rRNA from 250 larval and adult specimens. Individuals were grouped into putative species using statistical parsimony analysis and population aggregation analysis (PAA), whereby specimens from each locality were grouped according to the presence of diagnostic nucleotides. In addition, species membership was determined based on shifts in branching rates on clock-constrained trees to detect the putative transition from speciation to population coalescence patterns. Using these two methods we delineated between 48 and 56 groups, of which 16–20 were composed of larval and adult individuals. Nuclear and mtDNA-based groups were highly congruent; variation of 28S rRNA within groups was very low, while one widespread 28S rRNA genotype was universally found in a paraphyletic group of five mtDNA clusters. Linnean names could be assigned to 19 groups, and hence between 86.1% and 92.7% of larval specimens could be associated to species by their membership in clearly delineated groups that contained fully identified adults. The remaining larvae were delineated as five species, four of which could be assigned to *Anomala* or *Adoretus* based on their phylogenetic position. We conclude that the sequence variation was highly structured in this complex assemblage of chafers and that any given individual (larva or adult) can be readily associated with a particular DNA group using the criterion of diagnosability. The association of different developmental stages therefore becomes a matter of determining the extent of the DNA-based groups, rather than matching of sequences from adult and larval individuals. This indicates the need for a purely sequence-based taxonomic system when associating different life stages via DNA.

Keywords: Species delimitation; DNA barcoding; Integrative taxonomy; Molecular identification; White grubs; Biodiversity; Biocontrol; Nepal

1. Introduction

The application of DNA data in taxonomy and species diagnosis has aroused a great deal of controversy, but there is general agreement that genetic information is useful for associating different developmental stages of organisms and for identifying partially preserved specimens unsuitable for morphological study (Vences et al., 2005; Wheeler, 2004; Will et al., 2005). DNA data provide a character system universal to all life stages with the potential to overcome the problems of working with different semaphoronts. A DNA-based approach has already been used to associate different developmental stages in order to identify agricultural pests and invasive species (Ball and Armstrong, 2006; Harper et al., 2005; Miller et al.,

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doi:10.1016/j.ympev.2007.02.024

1999; Rao et al., 2006; Scheffer et al., 2006), forensically important insects (Wells and Sperling, 2001), larval parasitoids (Agusti et al., 2005) and endangered species in their early life stages (DeSalle and Birstein, 1996). Initial attempts have also been made to survey larval or mixed larval and adult assemblages with DNA methods (Barber and Boyce, 2006; Paquin and Hedin, 2004).

As presently implemented, reliable adult–larval associations require authenticated sequences against which queries can be tested, and analytical methods that discriminate clearly against other entries. DNA-based identifications of larvae therefore generally rely on reference taxa previously identified by other means, usually the rearing of samples and morphological identification of adults, and a corresponding DNA database to which larval sequence data are matched (e.g. Rao et al., 2006). In this sense, the assignment of different developmental stages to a particular species is similar to the notion of "DNA surveillance" (Baker et al., 2003) or "DNA barcoding" (Hebert et al., 2003a) in species identification which requires the development of a test database against which identifications are made (Dalebout et al., 2004; Teletchea et al., 2005).

The great diversity of natural lineages, however, would indicate that the current methods used to associate larvae and adults of the same species may be problematic where target groups are less well studied or incompletely represented in DNA databases. Equally, high genetic variation within species and the possible existence of closely related subgroups can result in ambiguity of species association. In those cases species associations conducted via DNA sequences have remained unsatisfactory (e.g. Paquin and Hedin, 2004; Scheffer et al., 2006). Fundamentally, the problem of DNA-based species association results from the fact that species circumscriptions are based on morphological information, and hence any linkage of life stages using DNA is indirect because the diagnostic characters for species recognition are different from those on which the species circumscription is based.

To avoid these difficulties, a number of recent studies have concluded that the association of different developmental stages requires increased efforts in "integrated taxonomy" (Will et al., 2005) and, where possible, rearing studies that link larvae directly with the adult-based species delimitation (Barber and Boyce, 2006; Paquin and Hedin, 2004; Scheffer et al., 2006). Although intuitively appealing, this integrated approach is not necessarily a solution to the problem of associating unknowns. Strictly, morphological characters in larvae and adults can only be associated if they are scored on the same specimen. This requires scoring of larval characters on individuals that are then reared through for scoring of adult characters. However, for most groups it is only practical to work on dead larval material and hence larval and adult characters are scored on different cohorts of specimens. The linking of these two cohorts as members of a particular species is usually indirect, e.g. by co-occurrence of larvae and adults in a particular locality, or by rearing of offspring from adults that have been positively identified. DNA characters are independent of life stage, and therefore could provide a more direct link by scoring the same characters on adults and larvae.

Such direct link for establishing species membership using DNA data could be achieved if the groups were defined by the DNA sequences themselves. Newly sequenced individuals of any life stage could be integrated directly into this taxonomic system, and this grouping would be based on the same procedures that were used to establish the species delineation in the first place rather than by indirect means. With the recent expansion of methods for quantitative species delimitation (Sites and Marshall, 2003) formal analyses of species limits are now possible. Among others, these methods rest on the notion of "diagnostic" character changes that are unique to a hypothesized entity. Their presence in all individuals of a (set of) population(s) and absence in all other populations would confirm the coherence of the group and, *vice versa*, the lack of recombination with other groups (Brower, 1999; Cracraft, 1983; Davis and Nixon, 1992). These methods are readily implemented in an algorithmic fashion, usually by testing the variation within and between populations which are then "aggregated" to circumscribe the species (Sites and Marshall, 2003). Initial groups are hypothesized based on morphological, geographical, ecological, or reproductive information, and this can be applied equally to sets of larval and adult individuals which may be considered separate populations for the tests of species limits. The subsequent aggregation analysis based on DNA (as the character system applicable to larval and adult stages) may or may not corroborate this initial assumption of separate species. In addition, it is possible to determine species limits directly from the sequence variation without the need to apply population aggregation methods, by exploiting the dynamics of lineage branching in a phylogenetic tree (Fontaneto et al., 2007; Pons et al., 2006).

Two important assumptions are that DNA sequence data form groups or clusters in such a way as to make group delineation straightforward, and that these groups correspond to species using evolutionary criteria. Here we build a DNA-based taxonomy as a test case for associating adults and larvae in a species-rich assemblage of chafers (Coleoptera: Scarabaeidae) and their larvae ("white grubs"). Chafers are a highly diversified lineage of herbivorous beetles whose adults feed mainly on leaves of trees and shrubs, and whose subterranean larvae feed on roots and soil organic matter. They are common in cultivated tropical soils and include many important pests of crop species and turf grass (Jackson, 1992). Differences in pest status and numerous biological traits, such as host associations, soil preference, phenology and susceptibility to parasites, requires accurate species identification for the application of control measures. However, species identification of larval stages is difficult because of the limited taxonomic work done to date and the frequent lack of distinguishing features (Miller et al., 1999). We studied