Molecular Phylogenetics and Evolution 50 (2009) 44–58

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny of the tribe Sphodrini (Coleoptera: Carabidae) based on mitochondrial and nuclear markers

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ARTICLE INFO

Article history:
Received 8 May 2008
Revised 4 September 2008
Accepted 23 September 2008
Available online 4 October 2008

Keywords:
Sphodrini
Carabidae
Coleoptera
Phylogeny
Evolution
Sensitivity analysis
Congruence
Alignment

ABSTRACT

A phylogenetic analysis of 6.4 kb of nucleotide sequence data from seven genes (mitochondrial cox1-cox2 and $\mathrm{tRNA}_{\mathrm{leu}}$, and nuclear Ef-1$\alpha$ C0, Ef-1α C1, 28S, and 18S) was done to reconstruct the phylogenetic relationships of the ground-beetle tribe Sphodrini. Gene regions of variable nucleotide length were aligned using both a secondary structure model, Clustal W, and a combination of the two. Sensitivity analysis was performed in order to explore the effect of alignment methods. The ribosomal and protein-coding genes were largely congruent based on the ILD test and partitioned Bremer support measures. MtDNA analysis provided high resolution and high support for most clades. The tribe Sphodrini and the related tribes Platynini, Pterostichini and Zabrini made up monophyletic clades, but the relationship between them was weakly resolved and sensitive to alignment strategy. Previously suggested relationships between subtribes of Sphodrini were not corroborated, and only the subtribe Atranopsina revealed high support as the sister clade to the other subtribes. The analyses clearly demonstrated the importance of exploring effects of alignment methods that may become particularly important in resolving polytomies and nodes with low support.

1. Introduction

The tribe Sphodrini is included in the subfamily Harpalinae of Erwin (1985), or the family Harpalidae of Deuve (1988), a large group of derived Carabidae that comprises around 19,500 species grouped in 37 (Lorenz, 2005) or 35 tribes (Erwin, 1985). Sphodrini includes about 823 species in 38 genera distributed mainly in the Holarctic region (Casale, 1988), with some additional species known from the East African region. According to the criteria used in the last Palearctic catalog (Hovorka and Sciaky, 2003) there are six subtribes: Atranopsina (101 spp), Calathina (179 spp), Dolichina (28 spp), Pristosiina (65 spp), Sphodrina (357 spp) and Synuchina (97 spp). These criteria are based on the classification proposed by Casale (1988).

The Sphodrini are distinguished from related tribes by the carinate posterior margin of prosternum, the gonostyli are usually armed by two or three spines (sensu Habu, 1978; Casale, 1988, etc.) but without a basal fringe of setae, and the asymmetric parameres of the male genitalia (the right one is often styloid). Members of the tribe are found in forests and open habitats of temperate areas but there are also a fair number of cave specialists. Life cycles vary notably (Matalin, 2007), but reproduction is more common in autumn (Luff, 1998).

The phylogenetic relationship of Sphodrini to other Harpalinae tribes is controversial. Jeannel (1942) suggested a close relationship between Sphodrini and Platynini, a hypothesis that has received general recognition on the basis of genitalia and external morphology (Lindroth, 1956; Liebherr, 1986; Casale, 1988). Recent catalogs (Hovorka and Sciaky, 2003; Lorenz, 2005) support also this relationship by admitting a subfamily Platyninae for both tribes. However, this hypothesis needs further assessment, as Arndt (1993) showed that platynines are polyphyletic with respect to larval characters and thus only a fraction of this tribe would be related to sphodrines. Recent analyses of 18SrDNA, 28SrDNA and *wingless* DNA sequences resulted in a polytomy comprising most representatives of Harpalinae (Sphodrini, Platynini, Pterostichini, Zabrini, etc.) (Maddison et al., 1999; Ober, 2002). Morphological data therefore provide the most reliable clues so far for Harpalinae relationships, indicating that Sphodrini and Platynini are likely related to Pterostichini and Zabrini (Erwin, 1985; Kryzhanovskij et al., 1995).

Relationships between the subtribes of Sphodrini are similarly controversial. Lindroth (1956) distinguished three lineages: Sphodri (Sphodrina, Calathina and *Dolichus*), Pristosiae (Pristosiina) and Synuchi (including Atranopsina), based mainly on characters in the male genitalia. Habu (1978) reclassified Sphodrini based on female genitalia, resulting in only two lineages—Sphodrina and Dolichina. Casale (1988) suggested a classification with six subtribes, Calathina, Sphodrina, Pristosiina, Dolichina, Synuchina,

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doi:10.1016/j.ympev.2008.09.023

and Atranopsina. He related Sphodrina and Calathina because both share a right styloid paramere and the gonostyli have a fovea with two short setae. He furthermore related Dolichina and Synuchina by sharing female gonostyli without fovea. Atranopsina and Pristosiina are supposedly more distantly related taxa, though Casale (1988, p. 126) considered the later closer to Calathina plus Sphodrina. Overall, the evolutionary history for Sphodrini is complicate and in need of new data to reconstruct the phylogeny and enable a more stable classification. We will therefore test the various hypotheses presented above using multiple sources of molecular data.

Previous molecular studies on Harpalinae (Maddison et al., 1999; Ober, 2002; Martinez-Navarro et al., 2005) have shown problems in solving their phylogenetic relationships. Some of them are possibly related to the low number of molecular markers applied. We are therefore increasing the number of mitochondrial and nuclear markers, and sample a large number of Sphodrini and outgroup taxa in the tribes Platynini, Pterostichini and Zabrini. The molecular data include partial sequences of two mitochondrial genes (cox1 and cox2), one nuclear protein-coding gene (Ef-1α), and two nuclear ribosomal genes (18S and 28S DNA). It is expected that the combination of mitochondrial and nuclear genes with different substitution rates will allow for a comprehensive phylogenetic analysis of Sphodrini.

In addition to increasing the amount of molecular data, we also consider several methodological problems, and particularly those associated with alignment of length variable regions. Morrison and Ellis (1997) showed that alternative alignments may result in alternative trees and concluded that phylogenies are often more sensitive to the alignment methods than to the tree reconstruction method used. Exploration of different alignment parameters is frequently neglected, e.g., by using default parameters in alignment software. Likewise, it may be also found that alignment ambiguous regions are simply excluded (Giribet and Ribera, 2000; Bleidorn et al., 2003; Kjer, 2004). However, these ambiguous regions can be aligned based on homologous secondary structures in the ribosomes which is more conservative than raw nucleotides, and that frequently yield more valuable phylogenetic information (Kjer, 1995; Morrison and Ellis, 1997; Xia et al., 2003; Kjer et al., 2007; Jordal et al., 2008). We are here exploring and comparing alignments based on rDNA secondary structure (Kjer et al., 2007) with a computer based alignment constructed in ClustalW. We are also exploring the impact of different parameter sets on the alignment by comparing the phylogenetic signal in the ambiguously aligned regions to unambiguous alignment regions, as previously emphasized by Wheeler (1995).

We aim to explore the phylogenetic relationships between Sphodrini and related tribes, and those within the tribe. More specifically, we are testing the hypotheses that (i) Platynini is the sister taxon of Sphodrini and (ii) the relationships between subtribes of Sphodrini are those depicted by Casale (1988). We also aim to test the effects of different alignment strategies on the reconstruction of phylogenetic relationships of these taxa.

2. Materials and methods

2.1. Taxa studied

Fifty-eight individuals belonging to 46 species and 29 genera representing the six subtribes of Sphodrini and related tribes have been sampled (Table 1). Available sequences in GenBank from six Sphodrini taxa were also included. The 14 studied genera of Sphodrini make up about 37% of described genera within the tribe. As initial outgroups we selected species from tribes currently accepted as putative closest relatives (Jeannel, 1942; Liebherr, 1986; Casale, 1988; Ober, 2002; Löbl and Smětana, 2003; Lorenz, 2005). Moreover, further analyses were made to search for related taxa within the subfamily Harpalinae using available 18S and 28S sequences from GenBank. Our preliminary phylogenetic analyses corroborated that Platynini, Pterostichini, Zabrini were the closest relatives to Sphodrini and have therefore been assigned as outgroups.

The individuals studied are kept in the collection of the Departamento de Zoología y Antropología Física (University of Murcia) at −20 °C in ethanol. Material of Nearctic Sphodrini were received on loan from the ESPM (UC-Berkeley) and will be subsequently returned.

2.2. Molecular markers

We studied a continuous fragment of the mitochondrial cytochrome oxidase I and II (cox1-cox2) genes (1675 bp) which includes part of cox1 gene (875 bp), the intervening tRNAleu (66 bp), and most of cox2 gene (734 bp). Within the ribosomal 28S rDNA we studied a partial sequence of about 974 bp length in the D2–D4 region. The most commonly amplified copy of the protein-coding gene Elongation Factor 1α (Ef-1α) lacked introns and consisted of 773 bp (named Ef-1α C0). A second paralogous copy included a single intron copy in position 753/754, similar to the Ef-1α C1 intron structure in other beetles (Jordal, 2002), and was studied in 19 taxa. EF-1α C0 is an intron free copy reported for the first time from Coleoptera, similar to that reported in Lepidoptera and Diptera (Jordal, 2002; Djernæs and Damgaard, 2006). The intron free copy of EF-1α is further distinguished from the intron-bearing copy in Carabidae by an average nucleotide sequence divergence of 9% similar to paralogous copies reported from other Coleoptera (Jordal, 2002). A nearly complete ribosomal 18S rDNA (2051 bp) sequence was studied in 16 taxa to assess outgroup relationships. All sequence data together comprised a total of 6.4 kb of nucleotide information. Sequences were deposited in GenBank under the accession numbers provided in Table 1.

2.3. Amplification and sequencing

DNA was extracted with QIAGEN Dneasy tissue kit (Qiagen, Hilden, Germany). Partial gene sequences were amplified by PCR using primers listed in Table 2. PCRs were made in a 25-µl volume. The mitochondrial cox1-cox2 fragment was amplified as described in Ruiz and Serrano (2006). The partial sequence of 28S rDNA (D2–D4 region) was amplified under standard PCR conditions (1 min at 50 °C annealing temperature). The nearly complete sequence of 18S rDNA (V1 to V9 domains) was obtained using four primer combinations as described in Shull et al. (2001). EF-1α sequences were amplified with a touchdown profile with primers Efs149 and Efa1043 (Normark et al., 1999), consisting of 31 cycles, each consisting of 94 °C denaturing for 30 s and 72 °C extension for 1 min. Annealing parameters in the first cycle were 58 °C during 1 min; temperature decreased by 2 °C in each of the next 6 cycles (it was then 46 °C in the seventh cycle), and was kept at 44 °C during the final 24 cycles. One microliter of the PCR product was used in a nested PCR with the primers Efs149 and Efa923 under standard PCR conditions (45 s at 50 °C annealing temperature). In some taxa it was found two bands of about 750 and 900 bp, corresponding to the two paralogous copies EF-1α C0 and EF-1α C1.

PCR products were purified with isopropanol and 5 M ammonium acetate. Double bands of EF-1α were purified directly from gel (MinElute Gel Extraction Kit, Qiagen, Hilden, Germany). Sequencing was performed in both directions using standard protocol for ABI BigDye(r) Terminator v3.1 Cycle sequencing kit (Applied Biosystems).