Molecular Phylogenetics and Evolution 99 (2016) 235–246

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Gondwanian relicts and oceanic dispersal in a cosmopolitan radiation of euedaphic ground beetles ☆

Carmelo Andújar [a,b,*], Arnaud Faille [c], Sergio Pérez-González [d], Juan P. Zaballos [d], Alfried P. Vogler [a,b], Ignacio Ribera [e]

[a] Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK
[b] Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK
[c] Zoologische Staatssammlung München, Münchhausenstraße 21, 81247 Munich, Germany
[d] Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain
[e] Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Passeig Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain

ARTICLE INFO

Article history:
Received 26 October 2015
Revised 18 February 2016
Accepted 11 March 2016
Available online 26 March 2016

Keywords:
Anillini
Endogean beetles
Tectonic vicariance
Molecular clock calibration
Molecular phylogeny

ABSTRACT

Anillini are a tribe of minute, euedaphic ground beetles (Carabidae) characterized by the loss of eyes, loss of wings and high levels of local endemism. Despite their presumed low dispersal, they have a nearly cosmopolitan distribution, including isolated islands such as New Zealand and New Caledonia. We used a time calibrated molecular phylogeny to test, first, if the tribe as currently understood is monophyletic and, second, whether the time of divergence is compatible with an early vicariant diversification after the breakup of Gondwana. We sequenced portions of 6 mitochondrial and 3 nuclear genes for 66 specimens in 17 genera of Anillini plus 39 outgroups. The resulting phylogenetic tree was used to estimate the time of diversification using two independent calibration schemes, by applying molecular rates for the related genus *Carabus* or by dating the tree with fossil and geological information. Rates of molecular evolution and lineage ages were mostly concordant between both calibration schemes. The monophyly of Anillini was well-supported, and its age was consistent with a Gondwanian origin of the main lineages and an initial diversification at ca. 100 Ma representing the split between the eyed *Nesamblyops* (New Zealand) and the remaining Anillini. The subsequent diversification, including the split of the Nearctic *Anillinus* and the subsequent splits of Palaearctic lineages, was dated to between 80 and 100 Ma and thus was also compatible with a tectonic vicariant origin. On the contrary, the estimated age of the New Caledonian blind *Orthotyphlus* at ca. 30 ± 20 Ma was incompatible with a vicariant origin, suggesting the possibility of trans-oceanic dispersal in these endogean beetles.

1. Introduction

Soils have been described as one of the remaining biotic frontiers (André et al., 1994), harboring an astonishing amount of life that may hold more than 1/4 of species diversity on Earth (Decaëns et al., 2006). A poorly known portion of this diversity is found in the deep layers of the soil (Jeffery et al., 2010), a complex and heterogeneous environment characterized by the absence of light, a water-saturated atmosphere and buffered temperature fluctuations (Eisenbeis and Wichard, 1987). Species inhabiting this environment (euedaphics *sensu* Eisenbeis and Wichard, 1987; endogeans *sensu* Giachino and Vailati, 2010) show varying degrees of adaptation to the conditions underground, with a general trend to size reduction, depigmentation, shortening of extremities, loss of eyes and loss of flight capacity (Eisenbeis and Wichard, 1987; Gisin, 1943).

For many euedaphic arthropods, and particularly beetles, taxonomic studies have shown a clear pattern of high diversity and micro-endemicity. Illustrative cases are found within the Leptotyphlini (Staphylinidae; Fancello et al., 2009), Bothrideridae (Dajoz, 1977), Reicheini (Carabidae; Casale, 2009) and Anillini (Carabidae; Jeannel, 1963; Giachino and Vailati, 2011; Pérez-González and Zaballos, 2013; Sokolov and Kavanaugh, 2014; Giachino, 2015). Phylogenetic community analyses based on molecular data have also shown that the adaptation to the deep soil layers promotes community phylogenetic clustering and high levels of local endemicity (Andújar et al., 2015). In other groups such as

☆ This paper was edited by the Associate Editor Francesco Frati.

* Corresponding author at: Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK.
E-mail address: candujar@um.es (C. Andújar).

http://dx.doi.org/10.1016/j.ympev.2016.03.013
1055-7903/

collembolans and annelids, thought to include few species with wider distributions, recent molecular work has also revealed high cryptic diversity and strong geographical structure (Cicconardi et al., 2013).

Despite this general trend to form geographically isolated entities, some euedaphic groups show transcontinental or even worldwide distributions when considering larger clades. This is the case of the endogean ground beetles of the tribe Anillini (subfamily Trechinae), which are distributed through all continents but Antarctica, including isolated islands such as Madagascar, New Zealand, New Caledonia, Hawaii, Galapagos, the Seychelles and some Mediterranean islands. In the last global taxonomic revision of Anillini, Jeannel (1963) divided the then 139 known species in two main "divisions" (Phanerodontes and Aphaenodontes) and 11 morphological lineages, a classification subsequently modified by Jeanne (1973) and extended to 12 lineages by Vigna-Taglianti (1973). Currently, approximately 520 species of Anillini are known (JPZ, unpublished data). All known Anillini are flightless, and only four species retain eyes, of which two belong to the genus *Nesamblyops* from New Zealand; the other two are *Microdipnodes tshuapanus* Basilewsky 1960 and *Cryptorites scotti* Jeannel 1950, both from central Africa and placed by Jeannel in the large lineage of *Microtyphlus*, distributed over most of Africa, Europe and Australia.

How the Anillini have reached their current distribution is intriguing. For other taxa with subterranean species and similar continent-wide distributions, such as the Molopini ground beetles (Casale and Ribera, 2008) or the Leptodirini cave beetles (Fresneda et al., 2011), it has been shown that the traditionally recognized taxa were polyphyletic due to the inclusion of unrelated lineages exhibiting similar morphologies resulting from convergence. In the case of Anillini, Jeannel (1937, 1963) proposed the monophyly of the group and a Gondwanian origin, a proposal that has been generally accepted by other authors, although sometimes questioned (e.g. Erwin, 1982). Based on this hypothesis, the Anillini would have diversified following tectonic vicariance after the breakup of Gondwana, with subsequent dispersion only possible through temporary land bridges from sea level changes and tectonic movements or by overseas dispersion among continents and islands.

We conducted a molecular phylogenetic analysis of Anillini including 9 of the 12 main lineages proposed by Jeannel (1963) and Vigna-Taglianti (1973), and representatives of the American, Palaearctic, African, New Zealand and New Caledonian fauna, to test whether (i) the tribe Anillini as currently understood is monophyletic; (ii) the main lineages proposed by Jeannel form monophyletic entities; (iii) the estimated ages of divergence are compatible with a Gondwanian origin of Anillini and the vicariant diversification after the breakup of Gondwana; and (iv) the Mediterranean taxa and the species from New Zealand and New Caledonia show divergence times compatible with the tectonic scenario. Studying the phylogeny, biogeography and lineage ages in Anillini will provide a better understanding of the systematics and evolution of the group, and ultimately on the processes driving the high biodiversity of euedaphic mesofauna.

2. Material and methods

2.1. Sampling

We included sequences from 66 specimens of 50 species, covering 17 of the 68 described genera and nine of the 12 main lineages of Anillini (Jeannel, 1963; Vigna-Taglianti, 1973). These taxa include several representatives within each of the main groups of Anillini proposed by Jeannel (1937) (Scotodipnines and Anillines), Jeannel (1963) (Phanerodontes and Aphaenodontes) and subtribes proposed by Jeanne (1973) (Typhlocharina, Scotodipnina and Anillina). Most genera (11) are from the west Palearctic region (from the Iberian Peninsula and Morocco to Turkey), with further exemplars from USA, Mexico, Tanzania, New Caledonia and New Zealand. We newly sequenced 54 specimens from 14 genera, whereas sequences for the remaining taxa were retrieved from Genbank, including *Nesamblyops* from New Zealand. Thirty-eight additional taxa were included as outgroups, including Bembidini, Tachyini, Trechini, Zolini, Xystosomina and Pogonini, which represent major lineages within the subfamily Trechinae that includes Anillini. *Ildobates neboti* Español, 1966 (Carabidae, Harpalinae, Zuphiini) was used to root the tree (Ribera et al., 2006; Hunt et al., 2007). Specimens were generally collected from soil samples using extraction methods (Berlese, 1905; Normand, 1909) or sifting forest litter and directly placed in absolute ethanol, either by the authors or provided by collaborators (see Tables S1 and S2 in Appendix S1 for details).

2.2. DNA extraction and sequencing

DNA was extracted non-destructively from whole specimens using Qiagen extraction kits (Hilden, Germany). Voucher specimens and DNA aliquots are kept in the IBE (CSIC-UPF, Barcelona) and NHM (London). Four DNA fragments were PCR amplified and sequenced, including: (i) the 3′ end of the *cox1* gene (≈753 bp); (ii) the 3′ end of the *rrnL* gene plus the complete *trnL* and the 5′ end of *nad1* (≈779 bp); (iii) a fragment of the *SSU* nuclear ribosomal gene (≈630 bp); and (iv) a fragment of the *LSU* nuclear ribosomal gene (≈1000 bp). PCRs were made using PuReTaq Ready-To-Go PCR beads (GE Healthcare, UK) or Biotaq Polymerase (Bioline, London, UK), with 39 cycles using 48–52 °C as the annealing temperature. The primers used for each gene fragment are given in Table S3 in Appendix S1. Additional sequences for the above markers and for two additional gene fragments were retrieved from Genbank, including: (v) the 3′ end of *cox1*, *trnL* and the initial portion of *cox2* (≈641 bp); and (vi) the nuclear internal transcriber spacer 2 (*ITS2*) (≈448 bp). Altogether, 203 new sequences were generated (194 for the ingroup) and 109 were obtained from public repositories (10 for the ingroup) (Andújar et al., 2011; Contreras-Díaz et al., 2007; Faille et al., 2010, 2013, 2012, 2011; Maddison and Ober, 2011; Maddison et al., 1999; Maddison, 2012; Ribera et al., 2006; Sokolov, 2007). Sequence accession numbers are provided in Table S1 in Appendix S1.

2.3. Alignment and dataset concatenation

Sequences were aligned using the online version of MAFFT 6.240 (Katoh and Toh, 2008a; Katoh et al., 2002), with the L-INS-i algorithm for the protein coding genes and Q-INS-i for ribosomal fragments (Katoh and Toh, 2008b). The correct translation to amino acids was checked in MEGA 6 (Tamura et al., 2013). Two datasets were generated: (a) the *all taxa* data set included all ingroup (Anillini) and outgroup (Trechinae + *Ildobates*) taxa for the six gene fragments (105 specimens, 4570 nt); and (b) the *ingroup* dataset included *cox1*, *rrnL*, *SSU* and *LSU* sequences for only the ingroup taxa (66 specimens, 3210 nt). The *all taxa* data set included some combined conspecific sequences from different studies (Table S1 in Appendix S1). The gene fragments *cox1-trnL-cox2* and *ITS2* were only sampled for the outgroup taxa of the *all taxa* data set.

2.4. Phylogenetic analyses

All phylogenetic and calibration analyses were conducted using the CIPRES Science Gateway (Miller et al., 2010). Data matrices