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Australian marsupial species identification

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ABSTRACT

Wildlife crime, the illegal trade in animals and animal products, is a growing concern and valued at up to US$20 billion globally per year. Australia is often targeted for its unique fauna, proximity to South East Asia and porous borders. Marsupials of the order Diprotodontia (including koala, wombats, possums, gliders, kangaroos) are sometimes targeted for their skin, meat and for the pet trade. However, species identification for forensic purposes must be underpinned by robust phylogenetic information. A Diprotodont phylogeny containing a large number of taxa generated from nuclear and mitochondrial data has not yet been constructed. Here the mitochondrial (COI and ND2) and nuclear markers (APOB, IRBP and GAPD) are combined to create a more robust phylogeny to underpin a species identification method for the marsupial order Diprotodontia. Mitochondrial markers were combined with nuclear markers to amplify 27 genera of Diprotodontia. Data was analysed using a likelihood method. The combined data set resolved two suborders: Vombatiformes and Phalangeriformes. Phalangeriformes was subsequently split into two clades. The first clade contained the Macropodiformes and Burramyidae. The second clade contained Petauridae, grouping with Phalangeroidea. Of the markers tested, ND2 provided the greatest level of diagnostic accuracy and could be used as a forensic species identification tool for Diprotodonts, with appropriate validation.

1. Introduction

Wildlife crime is a deliberate illegal activity involving animals and plants, alive or dead (including their parts, products and derivatives) for which purposeful gain is the principle motive [1]. The Australian marsupial order Diprotodontia which includes the kangaroos, possums and gliders are purportedly targets of wildlife crime, being illegally exported overseas as part of the pet trade or for collections, as well as illegally hunted for sport, meat or skins. A number of Diprotodontid species are listed on Schedules I and II of CITES and are therefore subject to strict exportation/importation restrictions. Fundamental to the regulation of trade and harvesting of these species is an appropriate method of identification.

A key issue in wildlife forensics is defining the biological (taxanomic) units of concern, whether they are species or populations, or some other taxonomic unit. Species identification is underpinned by robust phylogenetic information regarding the relevant species. However, there continues to be much debate regarding the classification and phylogeny of the Diprotodontia and no significant research has combined mtDNA and nuclear data to create a more robust phylogeny to underpin a method of species identification.

2. Materials and methods

56 samples were obtained from mostly vouchered specimens, usually tissue (heart, liver), from various collections representing 10 families, 27 genera and 55 species of Diprotodontia, plus an additional two species as an outgroup. Taxonomic designations follow Meredith et al. [2]. All tissue samples were extracted either using standard phenol chloroform procedures or Qiagen DNeasy® Blood and Tissue Kit (Qiagen part # 69506), using the animal tissue bench protocol. Negative controls were used in all extractions.

Standard PCR reactions were performed for three nuclear markers: APOB, IRBP and GAPD. Mitochondrial markers targeted were COI and ND2. All sequencing was performed in both directions by the Australian Genome Research Facility using M13 primers. Run data was edited using SEQUENCHER version 4.1.4 (GeneCodes Corporation 1991–2002). Consensus sequences were aligned using the CLUSTAL module in MEGA version 4 with manual adjustments. Maximum likelihood analysis of concatenated data was performed

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Table 1
Classification of Diprotodontia using combined nuclear and mitochondrial DNA sequence data.

Order Diprotodontia
Suborder Vombatiformes
Family Phascolarctidae
Family Vombatidae
Suborder Phalangeriformes
Infraorder Phalangeromorphia
Superfamily Petauroidea
Family Petauridae
Family Pseudocheiridae
Family Acrobatidae
Family Tarsipedidae
Superfamily Phalangeroidea
Family Phalangeridae
Infraorder Macropodomorphia
Superfamily Burramyoidea
Family Burramyidae
Superfamily Macropodoidea
Family Potoroidae
Family Hypsiprymnodontidae
Family Macropodidae

using PHYML [3] on the Montpellier bioinformatics platform (http://www.atgc-montpellier.fr/phyml/).

3. Results and discussion

After removing ambiguous data the concatenated data set contained 3332 base pairs for each of 64 taxon. The likelihood analysis of the concatenated data combing the nuclear and mitochondrial markers displayed expected grouping of species within clades. Results are strongly supportive of a two suborder phylogeny of Vombatiformes and Phalangerida. Interestingly, the results supported the three distinct possum clades. The largest clade containing the superfamily Petauroidea (Petauridae, Pseudocheiridae, Acrobatidae and Tarsipedidae) and this clade is sister to a second clade Phalangeridae and a final clade Burramyidae, which grouped with the macropods. By combining nuclear and mitochondrial sequence DNA data, better resolution of this relationship can be obtained. It is important to note that the topology found in the concatenated data set was consistent with the topologies found in the single marker data sets of both the nuclear and mitochondrial DNA markers (data not shown here).

Therefore from these data a robust phylogeny has been produced. Table 1 displays the suggested phylogenic relationships for the Diprotodont species emerging from this study.

Before implementation of the testing and methods for species identification in Diprotodonts, further work should be conducted to thoroughly validate the methods. In addition, for each laboratory that implements species identification, verification studies must be conducted to ensure expected results are obtained in line with the previously obtained validation results. This ensures consistent results are obtained for the courts regardless of which scientist or laboratory conducts the analysis.

4. Conclusion

Presented here is a robust phylogeny developed for the first time using an extensive data set of nuclear and mitochondrial information in Diprotodontia. Of the markers tested, ND2 provided the greatest level of diagnostic accuracy combined with amplification ease for degraded samples, often encountered within wildlife forensics, and could be used as a species identification tool for Diprotodonts.

Conflict of interest

None.

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