Molecular Phylogenetics and Evolution 70 (2014) 137–151

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Hidden diversity in the Andes: Comparison of species delimitation methods in montane marsupials

Thomas C. Giarla [a,b,*], Robert S. Voss [c], Sharon A. Jansa [a,b]

[a] Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108, USA
[b] J.F. Bell Museum of Natural History, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108, USA
[c] Division of Vertebrate Zoology (Mammalogy), American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA

A R T I C L E I N F O

Article history:
Received 30 April 2013
Revised 17 September 2013
Accepted 20 September 2013
Available online 2 October 2013

Keywords:
Species delimitation
Cryptic species
Anonymous loci
Multilocus power analysis
Didelphidae
Thylamys

A B S T R A C T

Cryptic genetic diversity is a significant challenge for systematists faced with ever-increasing amounts of DNA sequence data. Computationally intensive coalescent-based analyses involving multiple unlinked loci are the only currently viable methods by which to assess the extent to which phenotypically similar populations (or metapopulations) are genetically distinct lineages. Although coalescent-based approaches have been tested extensively via simulations, few empirical studies have examined the impact of prior assumptions and dataset size on the ability to assess genetic isolation (evolutionary independence) using molecular data alone. Here, we consider the efficacy of two coalescent-based approaches (BPP and SpeDeSTEM) for testing the evolutionary independence of cryptic mtDNA haplogroups within three morphologically diagnosable species of Andean mouse opossums (*Thylamys pallidior*, *T. sponsorius*, and *T. venustus*). Fourteen anonymous nuclear loci, one X-linked nuclear intron, and one mitochondrial gene were analyzed for multiple individuals within each haplogroup of interest. We inferred individual gene trees for each locus and considered all of the nuclear loci jointly in a species-tree analysis. Using only the nuclear loci, we performed "species validation" tests for the cryptic mitochondrial lineages in SpeDeSTEM and BPP. For BPP, we also tested a wide range of prior assumptions, assessed performance of the rjMCMC algorithm, and examined how many loci were necessary to confidently delimit lineages. Results from BPP provided strong support for two independent evolutionary lineages each within *T. pallidior*, *T. sponsorius*, and *T. venustus*, whereas SpeDeSTEM results did not support splitting out mtDNA haplogroups as distinct evolutionary units. For most tests, BPP was robust to prior assumptions, although priors were shown to have an effect on both the strength of lineage recognition among *T. venustus* haplotypes and on the efficiency of the rjMCMC algorithm. Comparisons of results from datasets with different numbers of loci revealed that some cryptic lineages could be confidently delimited with as few as two loci.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The current debate regarding species concepts (reviewed by Hausdorf, 2011) shows no sign of abating, but most biologists agree that speciation is an ongoing process, and that contemporary populations of organisms represent evolutionary lineages at different stages of distinctiveness (De Queiroz, 2007). For some organisms, the delineation of independent evolutionary lineages seems straightforward due to the presence of diagnostic morphological characters, which can be expected to evolve either by genetic drift after a long history of isolation or by divergent selection on readily observable traits (Lande, 1976). However, for many organisms—perhaps especially animals that do not rely on visual mating cues (Bickford et al., 2007)—species recognition based solely on morphological differences can be problematic if diagnostic traits are subtle, occur in commonly overlooked anatomical structures (e.g., soft tissues), or simply are not present.

A proliferation of cryptic diversity in a given area could be caused by geographical or ecological factors unique to that region. For example, mountainous areas might harbor more cryptic lineages than lowland areas because rugged terrain and altitudinal zonation of habitats could limit dispersal and thus create more opportunities for allopatric divergence, especially in combination with dynamic climate-change regimes (Roy, 1997; Weir, 2006; Kozak and Wiens, 2006; Brumfield and Edwards, 2007; Ribas et al., 2007). In particular, if the ecological niches of allopatric montane populations remain similar (Wiens and Graham, 2005), reliable phenotypic indicators of lineage divergence may not evolve.

* Corresponding author. Address: Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, Room 100, St. Paul, MN 55108, USA.

E-mail address: giarla@umn.edu (T.C. Giarla).

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2013.09.019

In situations like this, morphological traits may not be sufficient to detect recently diverged (or incipiently diverging) species.

Advances in DNA sequencing technologies over the past 20 years have allowed systematists to uncover cryptic genetic diversity at a rapid pace, challenging all researchers who study biological processes at or above the species level to contend with unexpected numbers of putative lineages and candidate species (Beheregaray and Caccone, 2007; Bickford et al., 2007). Mitochondrial DNA (mtDNA) markers have been especially effective for revealing cryptic genetic diversity within animal systems because of the mitochondrial genome's high mutation rate and rapid coalescence time (Moore, 1995). However, the exclusive use of mtDNA in phylogenetic and phylogeographic work has been criticized (Ballard and Whitlock, 2004; Edwards and Bensch, 2009; Galtier et al., 2009), primarily because single-gene trees can deviate from historical patterns of population branching due to the stochastic nature of coalescence (gene-tree/species-tree incongruence; Degnan and Rosenberg, 2009). Nonetheless, studies that include mtDNA are still common because mtDNA is typically quite variable for most animal taxa, is easy to amplify and sequence, and may be a "leading indicator" of isolation (Zink and Barrowclough, 2008). Many studies that included both mtDNA and multiple unlinked nuclear DNA (nDNA) markers have reported striking examples of cytonuclear discordance (Toews and Brelsford, 2012). The discordance observed between trees inferred from mtDNA and nDNA could be caused by phylogenetic error, incomplete lineage sorting, or lateral gene transfer (Funk and Omland, 2003), so studies based on both types of data are necessary to disentangle the evolutionary processes that resulted in observed patterns.

The purpose of this study is to evaluate the evolutionary independence of morphologically cryptic mtDNA haplogroups within a genus of Neotropical marsupials based on data from 15 nuclear loci and one mitochondrial locus. Didelphid marsupials of the genus *Thylamys* are found in a variety of ecoregions in central and southern South America, but they primarily inhabit arid and semiarid open habitats. Three species—*T. pallidior*, *T. sponsorius*, and *T. venustus*—are principally montane (with some populations inhabiting areas up to 4000 m above sea level in the Andes) and occur in Peru, Bolivia, Chile, and Argentina. In a previous study, we identified multiple allopatric mtDNA haplogroups within each of these species, but we were unable to find morphological characters that could consistently distinguish conspecific haplogroups (Giarla et al., 2010). Nevertheless, the deep mtDNA divergence we observed among conspecific haplogroups (2.5% to 5.4% at the cytochrome *b* locus) suggests that up to seven independent lineages might be present (Giarla et al., 2010).

A variety of methods have recently been proposed to assess the evolutionary independence of putative lineages using DNA sequence data; here we focus on two of the more widely used approaches. The first, Bayesian Phylogenetics and Phylogeography (BPP; Yang and Rannala, 2010), provides a Bayesian approach to "species delimitation" in which both phylogenetic uncertainty and stochastic lineage coalescence are taken into account when testing predefined splits on a single proposed species tree. BPP does this by simultaneously estimating a distribution of genealogies for each locus and fitting that distribution to various permutations of the species tree. The permutations allow the program to test different species tree models, from the assumption of a single-taxon, panmictic population that incorporates all of the putative species to the maximally resolved guide tree with each putative species as a tip. By contrast, SpeDeSTEM (Ence and Carstens, 2011) is a software pipeline based on the multilocus species-tree method STEM (Kubatko et al., 2009), in which various permutations and combinations of subpopulations within putative species are assessed for genetic independence using previously estimated gene trees. SpeDeSTEM, unlike BPP, relies on a set of previously estimated gene trees being in place before "species limits" are assessed, so phylogenetic error is not incorporated directly into the analysis. This approach, however, has the added benefit of being computationally efficient, which becomes especially important if many sequences or loci are included.

Tests of "species limits" (in effect, genetic isolation) using both of these coalescent-based approaches have the potential to validate or refute cryptic lineage diversity that only receives weak support from traditional morphological approaches or that depends on single-gene phylogenetic analyses. Here, we use both approaches to examine the coalescent history of putative lineages within *T. pallidior*, *T. sponsorius*, and *T. venustus* in order to assess their genetic independence. In addition to the evolutionary implications of our results, various aspects of the lineage recognition process were considered and tested in this study, including: (1) Do different coalescent-based "species validation" approaches yield similar results based on the same data? (2) How does the choice of priors affect Bayesian lineage recognition? (3) How many loci are necessary to confidently recognize distinct lineages? And (4), to what extent do partitions based on mtDNA haplotype membership correspond to real evolutionary units?

2. Materials and methods

2.1. Nuclear marker design

Primer pairs for nearly 40 anonymous nuclear loci were developed for testing within *Thylamys* species. A genomic library of DNA fragments ranging in length from 500 to 1500 bp was developed following a modified version of the genomic library generation protocol of Glenn and Schable (2005). First, whole genomic DNA was extracted from tissue sample NK22949 (*Thylamys venustus*) using a DNeasy Blood and Tissue Kit (Qiagen Inc.). Genomic DNA was digested with restriction enzymes *Xmn1* and *Rsa* and run on an agarose gel. Portions of the gel corresponding to fragment lengths between 500 and 1500 bp were excised and purified using a QIAquick Gel Extraction Kit (Qiagen Inc.). Double-stranded linkers were ligated to the size-selected fragments, and PCR was used to amplify the fragments. The resulting amplified fragment library was cloned into *E. coli* cells using a pGEM-T Vector System (Promega Inc.). After growing overnight, dozens of bacterial colonies containing inserts were picked with sterile toothpicks and immediately added to a PCR reaction mixture (12.5 μL GoTaq Green Master Mix [Promega Inc.], 1.0 μL of 10 μM M13F primer solution, 1.0 μL of 10 μM M13R primer solution, and 10.5 μL water) for colony PCR (5 min. of initial melting at 95°; followed by 35 cycles of melting at 95° for 30 s, annealing at 55° for 30 s, and extension at 72° for 1.5 min; and a final extension for 3 min at 72°). PCR products were run on an agarose gel, with 700–1000 bp fragments preferentially selected for further development. Selected PCR products were cleaned using Exonuclease I and Shrimp Alkaline Phosphatase (Hanke and Wink, 1994) and sequenced in both directions on an ABI 3730 at the University of Minnesota's Biomedical Genomics Center.

Sequences were assembled in Geneious version 5 (Drummond et al., 2010) and cloning vector regions were trimmed. For 35 genomic regions, forward and reverse primers were designed using the Primer3 (Rozen and Skaletsky, 2000) software plug-in in Geneious with the goal of selecting primers that would amplify products between 500 and 800 bp long. All primers were designed to be approximately 30 bp long in order to achieve a higher amplification success rate and fewer instances of non-specific amplification (Belfiore, 2011). Primers were tested on individuals from each of the three taxonomic species under consideration in this study: *Thylamys pallidior*, *T. sponsorius*, and *T. venustus*. If amplification

Download English Version:

https://daneshyari.com/en/article/5919355

Download Persian Version:

https://daneshyari.com/article/5919355

Daneshyari.com