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Uprooting phylogenetic uncertainty in coalescent species delimitation: A meta-analysis of empirical studies

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Abstract Phylogenetic and phylogeographic studies rely on the accurate quantification of biodiversity. In recent studies of taxonomically ambiguous groups, species boundaries are often determined based on multi-locus sequence data. *Bayesian Phylogenetics and Phylogeography* (BPP) is a coalescent-based method frequently used to delimit species; however, empirical studies suggest that the requirement of a user-specified guide tree biases the range of possible outcomes. We evaluate fifteen multi-locus datasets using the most recent iteration of BPP, which eliminates the need for a user-specified guide tree and reconstructs the species tree in synchrony with species delimitation (= unguided species delimitation). We found that the number of species recovered with guided versus unguided species delimitation was the same except for two cases, and that posterior probabilities were generally lower for the unguided analyses as a result of searching across species trees in addition to species delimitation models. The guide trees used in previous studies were often discordant with the species tree topologies estimated by BPP. We also compared species trees estimated using BPP and *BEAST and found that when the topologies are the same, BPP tends to give higher posterior probabilities [*Current Zoology* 61 (5): 866–873, 2015].

Keywords BPP, Species delimitation, Phylogeography, Species tree

Phylogeographic inference is the foundation for ecological and evolutionary studies; it helps establish the evolutionary units that are used to measure diversity and to develop conservation plans (Agapow et al., 2004). Those studies rely on the accurate quantifications of biodiversity to draw taxonomically relevant conclusions (Frankham et al., 2012). Consequently an accurate delimitation of species is imperative for these efforts (Wiens, 2007). In recent years, an increasing number of studies have aimed to delimit species in large species complexes and/or diagnose cryptic species. The current framework takes two separate steps in which species assignments are used to (1) reconstruct a species tree and (2) determine the boundaries between these species. In both steps, the results are contingent upon the accuracy of initial species assignments – culminating in a result that tests support for the initial assignments, not the species.

A "species" comprises much more than just a genetically distinct lineage, making species delimitation both a complex and a controversial endeavor (Bauer, 2011; Fujita and Leaché, 2011). To address this problem, integrative taxonomy approaches advise including multiple lines of evidence to test species delimitations and to account for the unique evolutionary histories of different taxa (de Queiroz, 2007; Padial et al., 2010; Yeates et al., 2011, Leaché et al., 2009; Padial et al., 2009; Minoli et al., 2014). New methods that implement different sources of evidence in a common analytical framework are becoming available (Solís-Lemus et al., 2015), though many studies still rely exclusively on molecular data (reviewed by Carstens et al., 2013). Relying solely on molecular data to delimit species is potentially problematic; one challenge associated with using multi-locus datasets to delimit species is incorporating discrepancies between gene trees (Fujita et al., 2012). Each gene tree reflects an independent evolutionary history, which may or may not coincide with the species tree (Knowles and Carstens, 2007). Thus, species delimitation based on individual gene trees is prone to inaccuracy (DeSalle et al., 2005; Carstens et al., 2013). Departing from the previous paradigm of estimating species trees using concatenated datasets, coalescent-based methods now account for the independent evolutionary histories of individual genes (Edwards, 2009), increasing the statistical rigor and objectivity of species delimitation (Fujita et al., 2012).

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The current molecular methods used to delimit species fall into two categories: species validation and species discovery (Carstens et al., 2013). Validation methods test pre-defined hypotheses of species boundaries (Camargo et al., 2012; Grummer et al., 2014; Leaché et al., 2014), while discovery methods estimate the number of species supported by molecular sequence data (Jones et al., 2014) or by a phylogenetic tree (Pons et al., 2006). Methods using the multispecies coalescent, both discovery- or validation-based, typically require the user to define species assignments prior to analysis. Although there is flexibility in how individuals are assigned to species (e.g., based on geography, current taxonomy, morphology, or a particular gene tree), these assignments greatly limit the number of species delimitations examined (Carstens et al., 2013). One popular method for species delimitation, Bayesian Phylogenetics and Phylogeography (BPP; Yang and Rannala, 2010), allows the user to assign individuals to species or populations based on independent lines of evidence, but it is reliant on a user-inputted guide tree for species delimitation. Some studies have raised the concern that an erroneous guide tree may bias the results of species delimitation or lead to the over-splitting of species (Leaché and Fujita, 2010; Olave et al., 2014). However, a recent simulation study has found BPP is capable of overcoming some types of guide tree errors (Zhang et al., 2014).

Unlike previous versions of BPP, BPP3 (Yang and Rannala, 2014; Yang, 2015) can simultaneously estimate the species tree and delimit species, and these “unguided” analyses eliminate the need for a user-specified guide tree. These unguided analyses could help bypass any uncertainties or errors propagated by reconstructing a species tree and then delimiting species in two separate steps. In the present study, we investigate the impact of phylogenetic uncertainty on species delimitation by comparing the discordances between guided and unguided species delimitations. We use BPP3 to reanalyze 15 datasets from published studies, which originally based their species delimitation results on the assumption of an accurate guide tree. We compare (1) species delimitations obtained from guided and unguided analyses, (2) species trees estimated using BPP3 to the guide trees used in the original studies, and (3) BPP3 species trees to *BEAST species trees.

1 Materials and Methods

1.1 Sampling

We reanalyzed 15 datasets published between 2010 and 2014, which implemented previous versions of BPP that relied on a user-specified guide tree. The datasets span a wide range of organisms including plants, fungi, reptiles, and insects (see Table 1 for details). With permission from the original authors, we compiled the three original input files used for BPP analyses: a control file that specifies parameters for the analyses, a data file that includes sequence data, and an Imap file linking

Table 1 Species delimitation studies included in the analysis

Author	Organism	Genus	Species[1]	Samples[2]	Loci[3]	Phased	Guide tree source
Barrett & Freudenstein, 2011	Coralroot orchids	*Corallorhiza*	5	36	5	no	Parsimony[4]
Burbrink et al., 2011	Kingsnakes	*Lampropeltis*	3	90	3	yes	MrBayes
Camargo et al., 2012	Lizards	*Liolaemus*	4	80	4	yes	*Beast
Domingos et al., 2014	Naked-toed geckos	*Gymnodactylus*	12	90	2	no	*Beast
Fuchs et al., 2011	Tiny greenbuls	*Phyllastrephus*	4	209	4	yes	*Beast
Grummer et al., 2014	Bunchgrass lizards	*Sceloporus*	10	68	6	yes	*Beast
Joerger et al., 2012	Acochlidian sea slugs	*Pontohedyle*	12	40	3	no	RaxML
Leache & Fujita, 2010	Forest geckos	*Hemidactylus*	4	102	5	yes	*Beast
Levitta et al., 2011a	Rock-shield lichen	*Xanthoparmelia*	21	155	6	no	*Beast
Levitta et al., 2011b	Rimmed navel lichens	*Rhizoplaca*	10	43	4	no	*Beast
Miralles &Vences, 2013	Skinks	*Madascincus*	10	126	4	no	MrBayes[4]
Satler et al., 2013	Trapdoor spiders	*Aliatypus*	6	27	6	no	*Beast
Setiadi et al., 2011	Fanged frogs	*Limnonectes*	3	31	2	no	MrBayes[4]
Welton et al., 2013	Monitor lizards	*Varanus*	10	100	4	yes	*Beast[4]
Yang & Rannala, 2010	Fence lizards	*Sceloporus*	5	17	29	yes	*Beast[4]

[1] Number species or populations in the original study. [2] Total number of samples (specimens) included in the species delimitation analysis. [3] Total number of loci used during the species delimitation analysis. [4] Guide tree estimated with a dataset that differed from the one used to delimit species.

each sequence to a species assignment. In order to ensure comparability between the new BPP3 analyses and the original analyses, we conserved all original parameters and avoided modifying the control and Imap files, except for minor changes necessary to run the new version of the program. If nuclear haplotypes were recovered using the program PHASE (Stephens et al., 2001), the same nuclear haplotypes were used in the new analyses.

1.2 Unguided species delimitation

We used BPP version 3.0 for joint species delimitation and species tree inference (Yang and Rannala, 2014; Yang, 2015). We used the guide tree from the original studies as the starting tree. Priors for parameters (population sizes, θ, and divergence times, τ), as well as the priors for species delimitation models (speciesmodelprior = 0 or 1) were consistent with those used in the original study. We used the same MCMC parameters (e.g., burn in, sample frequency, and number of samples) as the original study. We ran four replicates of each dataset with different random starting seeds and compared posterior probability (PP) distributions to ensure that the replicates converged. For analyses that failed to converge, we reran each replicate five times longer.

To evaluate the influence of the guide tree on species delimitation, we compared the number of species estimated by the original “guided” studies to the new unguided analyses. We compared the posterior probability distributions for the total number of species, independent of the species relationships. We considered outcomes with PP values ≥ 0.95 to be highly supported.

1.3 Species tree comparison to *BEAST

We compared species trees estimated using BPP3 to species trees estimated with *BEAST. In cases where *BEAST species trees were estimated in the original studies, we used the original *BEAST trees in our comparisons. For studies that did not estimate a species tree with *BEAST, or for studies that estimated a *BEAST tree with a dataset that differed from the one used to delimit species (i.e., different number of individuals or different loci), we estimated a species tree using *BEAST v 1.8.0 (Drummond et al., 2012). We used the same loci used in BPP3 analyses, and individuals were assigned to species according to the BPP Imap file. Models and priors for each gene (and correct inheritance scalars for mitochondrial and nuclear DNA) were assigned following the information available in the original publications. When information on nucleotide substitutions models was not included in the original publications, we selected substitution models using jModeltest (Posada, 2008) according to the AIC. We applied an uncorrelated lognormal relaxed molecular clock and a Yule process prior. We ran two replicates for each analysis and included the same number of MCMC generations and sampling as the original publication if the information was available, otherwise, we ran analyses for one million generations. We assessed convergence visually in Tracer v 1.5 (Rambaut and Drummond, 2009), combined replicate runs with LogCombiner v1.8.0, and summarized the posterior distribution of trees with TreeAnnotator v1.7.4 (Rambaut and Drummond, 2012). We constructed a maximum clade credibility (MCC) tree separately per run using TreeAnnotator v1.8.1 (Drummond et al., 2012). We compared species tree topologies and posterior probabilities for each node among replicates. If topologies were identical or lacked any strong discordance (conflicting branches with strong support > 0.95) we combined the samples of trees from the two runs and summarized the combined set of trees using TreeAnnotator.

2 Results

Unguided species trees estimated with BPP3 are summarized in Fig. 1. Of the 15 datasets reanalyzed in this study, only two converged using the original MCMC parameters from the guided analyses (e.g., burn in, sample frequency, and number of samples). The remaining studies converged after we increased the length of the MCMC five-fold.

2.1 Guided vs. unguided species delimitation

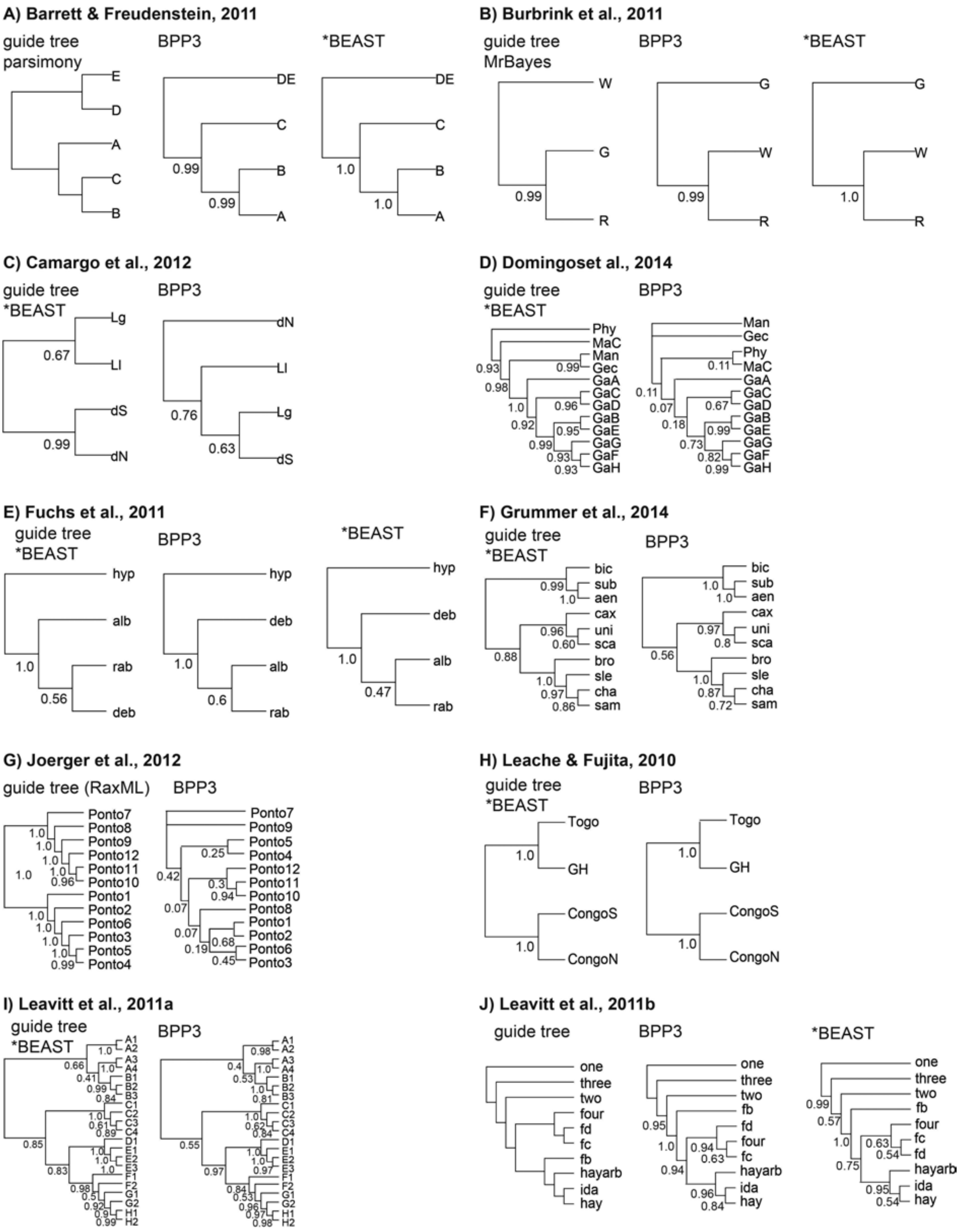
Species delimitations for the 15 studies are shown in Table 2. The number of species estimated using guided and unguided species delimitation was the same for all but two studies (Table 2). The unguided species delimitation analyses of the Fuchs et al. (2011) and Setiadi et al. (2011) datasets both estimated an additional species compared to the original study. For the analyses that estimated the same number of species, the posterior probability for the number of species is generally lower for the unguided analyses (Table 2, Fig. 2A).

2.2 Guide trees vs. species trees

Species tree topologies estimated with BPP3 were mostly discordant with the guide trees assumed in the original studies (Fig. 1). Four of the 15 studies obtained identical results, and each of those correspond to studies that used *BEAST to estimate the guide tree (Fig. 1).

2.3 Comparisons between *BEAST and BBP species trees

Species tree topologies estimated with *BEAST and BPP were the same for eight of 12 studies, whereas different topologies were estimated for four data sets (Fig. 1). We could not run *BEAST on three data sets that did not include at least one individual per species for all loci. We compared the posterior probabilities for shared bipartitions estimated with BPP and *BEAST (Fig. 2B). The support values for species relationships were typically different; BPP tended to provided higher support than *BEAST (Fig. 2B).



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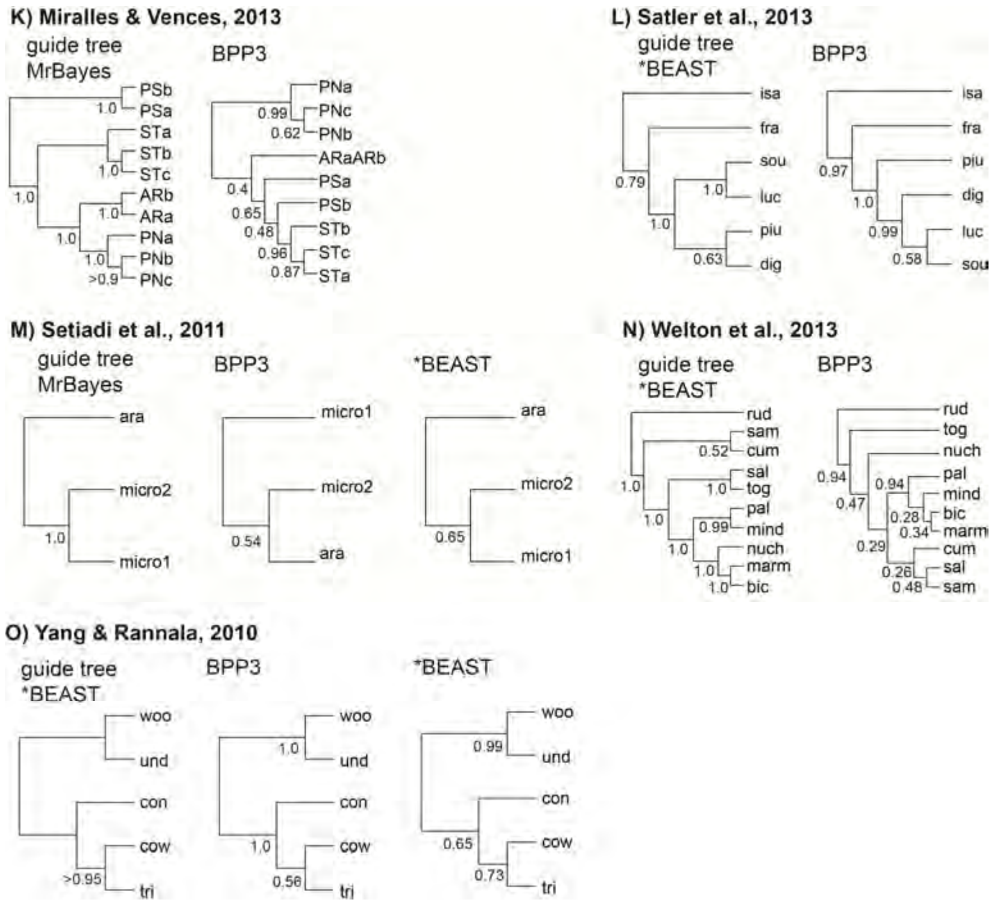

Fig. 1 A summary of tree topologies from the 15 empirical studies, including the guide tree topology used in the original publication, the unguided species tree from BPP3, and the *BEAST species tree

Table 2 Species delimitation comparison between guided and unguided analyses

Study	Guided		Unguided	
	# species[1]	Posterior	# species	Posterior
Barrett & Freudenstein, 2011	4	>0.98	4	0.99
Burbrink et al., 2011	3	>0.97	3	0.99
Camargo et al., 2012	4	1.0	4	1.0
Domingos et al., 2014	12	0.89-0.91	12	0.91
Fuchs et al., 2011	3	1.0	4	1.0
Grummer et al., 2014	10	>0.95	10	0.99
Joerger et al., 2012	12	>0.95	12	0.70
Leache & Fujita, 2010	4	1.0	4	1.0
Levitta et al., 2011a	21	>0.95	21	0.99
Levitta et al., 2011b	10	>0.95	10	0.88
Miralles & Vences, 2013	9	1.0	9	0.78
Satler et al., 2013	6	0.99	6	0.93
Setiadi et al., 2011	2	>0.99	3	0.99
Welton et al., 2013	10	–[2]	10	0.42
Yang & Rannala, 2010	5	1.0	5	0.95

[1] Number of species delimited in the original studies. [2] Posterior was not recorded in the publication.

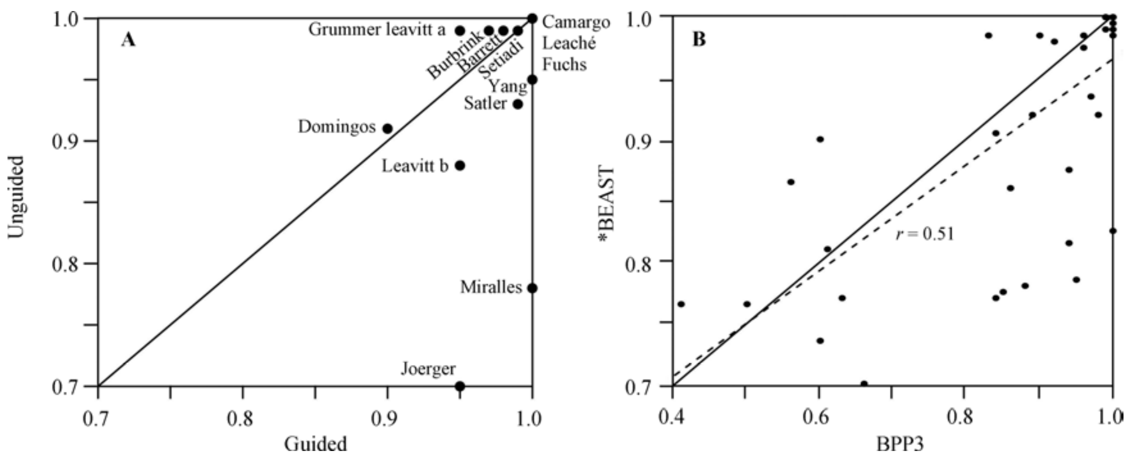

Fig. 2 Comparison of (A) the posterior probabilities for the number of species estimated using guided and unguided species delimitation, and (B) the posterior probabilities for shared bipartitions estimated with BPP3 (unguided species delimitation) and *BEAST

The dash line corresponds to the regression line, and r corresponds to coefficient of correlation.

3 Discussion

Phylogeography links phylogenetics, geographic distributions, and population genetics to provide a spatially explicit snapshot of biodiversity, and is contingent upon an accurate assessment of species (Hickerson et al., 2010). The integrative nature of phylogeography has been key to the success of the field (Knowles, 2009). With roots in phylogenetics and population genetics, phylogeography and species delimitation have both become statistically rigorous fields of study that facilitate cross-disciplinary hypothesis testing. For many groups of organisms, especially cryptic species and/or species complexes, the distinctions between phylogeography and species delimitation can be difficult to define. Studying the phylogeography of a species is analogous to delimiting species; the geographically structured populations discovered via phylogeographic inference may represent independent evolutionary lineages. However, estimating phylogeny and delimiting species can be difficult, and even circular, because it has often been necessary to assume one (i.e., phylogeny or delimitation) prior to estimating the other.

Unguided species delimitation (Yang and Rannala, 2014) gets around the problem of having to pre-define the phylogenetic relationships for putative species that are yet to be delimited. In this regard, unguided species delimitation has a clear advantage over guided species delimitation for phylogeographic studies. Guided species delimitation requires pre-established phylogenetic hypotheses to evaluate the support for species delimitation models, and the only models explored are constrained to subtrees of the assumed guide tree (Yang and Rannala, 2010). Our reanalysis of 15 empirical datasets suggests that unguided species delimitation typically supports the same number of species as the guided approach (Table 2), and the joint estimation of the species tree topology is an added benefit that makes the assumption of a guide tree unnecessary.

In our reanalysis of 15 empirical datasets, the topology of the guide tree matched the topology of the species tree reconstructed by BPP3 (i.e., unguided species delimitation) in only 4 of the 15 studies. In these four cases, the guide tree was estimated using *BEAST. However, other studies within our sample had discordant guide trees and species trees despite also estimating the guide tree using *BEAST. It has been repeatedly noted that inaccurate guide trees may result in erroneous species delimitations (Carstens et al., 2013), and our results suggest that discordance between the guide tree and species tree topologies in some cases constrained the number of species delimited by BPP when a guide tree was required. It is worth mentioning that even though four studies obtained different topologies using a *BEAST guide tree, those studies used different datasets for estimating the guide tree versus delimiting species. For example, some guide trees were estimated with different genes. Studies that obtained guide trees using parsimony, maximum likelihood, Bayesian concatenated analyses or other unreported sources produced species trees that were discordant with the starting guide tree (Fig. 1) (Barrett and Freudenstein, 2011; Burbrink et al., 2011).

In 12 out of the 14 studies that reported posterior probabilities for the number of species, the number of delimited species matched between guided and unguided analyses, although the posterior probability for the number of species was generally lower for the unguided approach (Fig. 2A). Considering that unguided species delimitation simultaneously estimates the species tree and the number of species, it is not surprising that the posterior probability for the number of species is reduced. Uncertainty in the species tree topology could be responsible for the discordance in the number of delimited species in the Fuchs et al. (2011) and Setiadi et al. (2011) datasets. When the guide tree is incorrect, or when the species composition violates the assumptions of the model (for example, by having migration or recombination), the probability for the number of species is biased (Zhang et al., 2014). Unguided species delimitation eliminates the constraints of the guide tree; as a result, the phylogenetic uncertainty of the guide tree is not propagated to species delimitation.

Our results suggest that species tree topologies estimated with the unguided version of BPP are often concordant with topologies estimated using *BEAST (Fig. 2B). BPP and *BEAST both implement the multi-species coalescent model to infer species trees (Yang and Rannala, 2003), and both provide estimates of the divergence times and the effective population sizes of current and ancestral populations. However, *BEAST and BPP have different capabilities for implementing priors; some can be specified in both programs, whereas others are exclusive to one and without an equivalent in the other. For example, BPP is limited to the Jukes-Cantor nucleotide substitution model, which may be inappropriate for many datasets. While increasing the complexity of models in *BEAST has advantages, often these models slow down the search of parameter space during the MCMC, resulting in a promising analysis that may have difficulty converging.

We do not think that inadequate MCMC mixing was responsible for the discordance that we found for the empirical studies that produced conflicting species trees for BPP and *BEAST. We repeated our analyses with different starting seeds, and the independent analyses converged on similar posterior distributions. Instead, it is more likely that the differences in the priors used for BPP and *BEAST analyses are responsible. It is not possible to conduct analyses with the same exact priors, because these methods have different implementations of the multi-species coalescent model. For example, a new assignment-free species delimitation method was recently developed for *BEAST (Jones et al, 2014), but instead of using reversible-jump MCMC to explore species delimitation models it uses an approximation in the form of a modified birth-death prior for the species tree. We recommend that future users of unguided species delimitation should also estimate a species tree with *BEAST, or some other coalescent-based approach, to provide additional comparisons between different species tree estimation methods.

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