**INTRODUCTION**

*Geological evolution of the Amazon Basin.* The geological history of lowland Amazonia is still poorly understood, in part because much of the Tertiary stratigraphic history has been removed by erosion and that which remains is difficult to study and access in a rainforest environment. The landscape evolution of Amazonia during the Neogene was influenced mainly by Andean tectonics and climate changes that controlled the distribution of sedimentary environments. The most important natural barrier has been the Purus Arch, a NW-SE trending tectonic structure located 300 km to the west of Manaus that separates the Solimões and Amazonas basins [1]. This feature was a Neoproterozoic basin filled by sandstones, mudstones and carbonates and tectonically inverted during Early Paleozoic times. Evidence of inversion includes inclination of Purus Group beds up to 10 degrees towards the south, as well as the onlap and pinch-out disposition of Paleozoic units on the Purus Arch. Additionally, the thickness of Phanerozoic rocks is dramatically reduced in the region of the Arch suggesting an uplifted block. This geometry indicates that the Purus Arch was a compartmentalized zone during the Phanerozoic evolution of the Solimões and Amazon basins, controlling, in part, the lateral migration of the depositional system [1,2]. A westward-flowing interbasin fluvial system is recorded in the Cretaceous Alter do Chão Formation [3], but with increased Neogene Andean uplift and tectonic subsidence in western Amazonia this regime gave way to isolated sedimentary basins east and west of the Purus Arch [1].

The long-accepted model postulating the formation of the Amazon River drainage in the Middle to Late Miocene relies relatively little on the Late Miocene (post-Pebasian) and Plio-Pleistocene stratigraphic record in the Solimões Basin [e.g., 3-7], hence it was the loss of the Miocene Lake Pebas and its aquatic environments that has been used as the signature evidence for the initiation of terrestrial environments and the establishment of the modern drainage system in western Amazonia (but see below). In contrast, the hypothesis that the Amazon River and its western drainage were established in Plio-Pleistocene times [2, 8, 9] relies heavily on evidence presented by stratigraphic sections of Late Miocene to Recent age.

The Lake Pebas aquatic environments of the Early and Middle Miocene of western Amazonia are recorded in the Solimões Formation (which extends eastward to the Purus Arch; 2) and the "Pebas Beds." These are overlain by weathered paleosols and then by an unconformity (termed the Ucayali Unconformity by Campbell et al.; 8), both of which are taken to signify the end of Lake Pebas followed by a period of nondeposition and erosion [2 and references cited therein; 8]; the unconformity is dated at slightly older than ~9.01 ± 0.28 Ma [8]. Because the unconformity has been postulated [8] to be correlatable to the eastern Solimões Basin [2], this is suggestive of the widespread disappearance of Lake Pebas and a period of nondeposition.

Sedimentary environments of upper Miocene and lower Pliocene deposits are dominated by thick beds of clays, silts, mudstones, and fine sandstones that thin eastward toward the Purus Arch [2, 8-10], and which are interpreted as indicating widespread lacustrine, swampy, and fluvial conditions across the Solimões Basin. Some authors describe a Late Miocene unconformity in the upper part of the Solimões formation in the Purus arch region, suggesting an uplifted zone that hindered a connection between the Solimões and Amazon Basins [2, 9].

Distributed widely across the Solimões Basin, above the unconformity, are presumably correlatable formations (Madre de Dios, Içá, Candelaria) that record, in their upper sections, an extensive aquatic environment consisting of fluvial, deltaic, and lacrustine settings [2, 8]. The comparability of this sedimentary record among distant localities has been interpreted as a "Lago
Amazonas mega-lake complex" [8], whose origin was driven by the rapid rise of the Andes and closure of the western drainage of Amazonia to the Pacific Ocean. The lake environment captured by the Madre de Dios Formation persisted at least until 3.12 ± 0.02 Ma based on radiometric dating [8] from ash deposits within the upper member of the Madre de Dios Formation along the Las Piedras River of lowland eastern Peru. Although the precise distribution of this lake system is unknown, the evidence suggests there would not have been widespread terra firme forests in western Amazonia at this time. Partially based on these stratigraphic data, Campbell et al. [8] postulated that Lago Amazonas was drained sometime after ~3.0 Ma as the eastern terminus of the lake breached the lip of the Solimões Basin and a transcontinental Amazon River was formed.

Thus, the establishment of the transcontinental drainage can be considered younger than the Late Miocene. The presence of the Plio-Pleistocene Iça formation overlying the Solimões formation, as well as the presence of Early Pliocene lateritic profiles in the Amazon Basin that are sandwiched by deposits of the same age [2], corroborate this. There is thus evidence for two distinct paleoenvironmental regimes, with a lacustrine system to the west of the Purus arch and a fluvial system restricted to the east of the arch [9]. This geology implies a young transcontinental Amazon River system that formed no earlier than latest Miocene [~6.5 Ma; 9] or, more probably, in Pliocene times [by ~2.5 Ma; 8].

Campbell et al. [8] also cite as evidence for a 3.0-2.5 Ma origin for the Amazon River a marked increase in the terrigenous sediment mass accumulation rate (TMAR) at 2.5 Ma, which is observed in several of the five Ocean Drilling Project cores from the Ceara Rise located 800 km off the mouth of the Amazon River [summarized in 11; see also 12]. Because of ocean currents, sea level fluctuations, different potential ages and chemical composition of sediment sources, and other factors, interpreting sedimentary patterns over time and across cores is complex. Generally, increases in TMAR after 10.0-8.0 Ma, along with changes in chemical composition, have been interpreted as signatures of a transcontinental Amazon River [e.g., 12-14], but the significance of these observations has been questioned [8].

Recently, Figueiredo et al. [15] studied two proprietary Petrobras wells in the Amazon Fan that are interpreted to show several pulses of increased sedimentation rate, one at ~12-11 Ma (increase of sedimentation rate to 0.05 m/ka), a second at ~6.9 Ma (0.3 m/ka), and a third at ~2.2 Ma (11.16 m/ka). Using data from the well cores, they propose a "precise" date for the origination of a transcontinental Amazon River at 11.8-11.3 Ma. In addition, they employed Sm-Nd and Pb-Pb isotopic ratios to age sediment sources, defining any sample grains aged at >1.6 Ga as being from the Amazon craton, and any sample <1.6 Ga as being Andean in origin. These new data reinforce the conclusion that sedimentation rates can vary over time and that assigning causation to patterns is complex. In both Ceara Rise and well-log 2 of Figueiredo et al. [15] there was a slight increase in sedimentation rate near 8.0 Ma and then a more substantial increase 3.5-2.5 Ma. Yet, each increase has been used to support alternative Amazonian paleogeographic models, and thus it could be argued the data are not definitive. The isotopic data presented by Figueiredo et al. [15] show a marked increase in young ages (< 1.6 Ga) from the Late Miocene onward but sampling was relatively sparse. More importantly, perhaps, it is not clear that a simple dichotomous age distribution for sediment source is appropriate or definitive since Amazon Basin cratonic detrital zircon ages vary significantly and include sediment sources of widely varying ages, including those less than 1.6 Ga [10].

The eastern edge of the proposed Lago Amazonas is still uncertain. Although the Purus Arch is generally considered the boundary for Lake Pebas [6, 7, 15], Rossetti et al. [2] and Campbell et al. [8], as well as others have argued that because the arch is buried deeply it would have had no
necessary direct effect on surface elevation. Campbell et al. [8] suggest instead that the eastern rim of Lago Amazonas lies somewhere to the east but they are not specific as to location.

**Rio Negro formation.** As discussed in the text, we postulate that the Negro area of endemism separated from the Napo area to the west by a paleoviccance event. We propose the location of this event, near the mouth of the Rio Japurá and the Rio Solimões, was an ancient paleoriver, probably the Rio Negro or a large tributary. There is strong evidence from interferometric synthetic aperture radar data for a west-to-east migration of the Rio Negro from a position ~150 km to the west of its current position at Manaus [16]. Given the SE-NW trends of current and ancient paleochannels [16], the E-W dextral strike-slip faulting patterns within the region of the Negro area of endemism [17], and the lower elevation of that area relative to surrounding regions [see topographic map (USGS Digital Series DDS-62-A) in 8], it is reasonable to postulate that the Rio Negro migrated from a position much further to the west. This hypothesis can be tested with additional analysis of digital elevation data and field geology examination.

**Amazon areas of endemism and Psophia distribution.** Areas of endemism within Amazonia have been recognized by several authors. Haffer [18-20], Haffer and Prance [21], and Cracraft [22] recognized seven areas of endemism for lowland birds (Belém, Pará, Rondônia, Inambari, Napo, Imeri, Guiana). Brown [23] has shown that these areas are common for birds, butterflies, and plants. Silva et al. [24] suggested that the Pará area is composed of two areas: Tapajós and Xingú. More recently, Borges [25] proposed that the distinct avifauna endemic to the lower Negro/Solimões interfluvium should be indicative of a new area of endemism (Rio Negro area), totalling 9 areas of endemism within Amazonia.

*Psophia* is one of the few avian genera endemic to Amazonia, with cranes (Gruidae) and limpkins (Aramidae) being distant living relatives [26, 27]. All 9 Amazonian areas of endemism [22, 24, 25, 28], are occupied by morphologically distinct taxa of *Psophia*, with only one instance in which the same taxon (*crepitans*) occupies two adjacent areas (Guiana and Imeri).

**METHODS**

**Morphological variation.** We examined specimens (study skins) housed at the American Museum of Natural History (120 specimens) and Museu Paraense Emílio Goeldi (80 specimens) from 95 different localities (see Appendix S1), representing all taxa within *Psophia*. For each specimen, mantle and wing color(s) were determined using Smithe [29, 30] as a standard color reference. Those two sets of plumage characters were also used previously by other authors to diagnose taxa within *Psophia* [28].

**Data collection.** DNA extraction from both tissue and skin samples was performed using the DNeasy Tissue Kit (Qiagen, Valencia, CA). For extractions from skin samples DTT (dithiothreitol) was added to the first incubation step, which was performed overnight. All material was irradiated with ultraviolet light before use, and negative controls were employed at all steps of the extraction procedure. For amplification of skin samples specific primers were designed to amplify fragments of 250-300 base pairs. For tissue samples the whole gene fragment was amplified in a single reaction to avoid amplification of pseudogenes, and internal primers were employed on the sequencing reactions. PCRs consisted of an initial denaturation at 94°C for 5 min, followed by 40
cycles of denaturation at 94°C for 30 s, annealing varying from 58°C to 54°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. For tissue samples, regular Taq DNA polymerase was used (Promega), while for the skin samples we used HotStar Taq DNA polymerase, and lower annealing temperatures (49°C-50°C). PCR products were purified with Perfectprep PCR cleanup kits (Eppendorf, Westbury, New York). Sequencing of purified PCR products was performed with Big Dye terminator kit (Applied Biosystems, Foster City, California). Cycle sequencing products were run on an ABI Prism 3100 automated DNA sequencer (Applied Biosystems). Both strands were sequenced for all regions.

**Sequence comparisons.** Sequence fragments were assembled and corrected using Sequencher 4.5 (Genecodes, Ann Arbor, Michigan). Alignment was performed manually using MacClade. Base composition was evaluated using PAUP* version 4b10 [31]. The incongruence length difference test was performed in PAUP* to determine if there was conflicting phylogenetic signal between the two mitochondrial genes, and also among the three codon positions. Saturation of nucleotide substitutions was evaluated through plots of the absolute number of transitions at the 1st, 2nd, and 3rd codon positions as a function of maximum likelihood distances under a best-fit evolutionary model.

**Phylogenetic analysis.** MP and ML analyses were performed using heuristic searches, tree bisection-reconnection (TBR) branching swapping, and 10 random additions of taxa. Support for the inferred nodes was obtained through non-parametric bootstrap (100 replicates for ML, 1000 for MP). Two independent BI analyses were run for 20 million generations each, both with one cold and three heated chains. Characters were partitioned by gene (cyt b and ND2) and by codon position, following the results obtained with Bayes factor. Parameters for the different partitions were unlinked. Sampled parameter values were evaluated using Tracer v.1.4 [32]. Log likelihood values reached stability (stopped improving) within the first 1 million generations thus as a conservative measure, the first 5 million generations of each run were discarded as burnin. Results of independent runs were very similar, so the remaining trees (after burnin) of each run were combined to obtain a majority rule consensus in PAUP. As it is difficult to determine if the Markov chain has reached stationarity by checking only the likelihood scores, and as convergence should be checked for all parameters, we used AWTY (33) to assess convergence in all Bayesian runs. This was done by uploading the tree files from the Bayesian analyses to the AWTY website (http://king2.scs.fsu.edu/CEBProjects/awty/) and generating cumulative plots of posterior probabilities of splits at different points of the MCMC runs, to check if they reach stationarity along the run (34).

**Population variation and structure.** To test whether the observed mismatch distributions deviate significantly from those expected under the population expansion model, the sum of square deviations (SSD) was computed in Arlequin. The Rogers and Harpending [35] model was used to calculate the time since population expansion by estimating the parameter τ, which was used to estimate the time since the expansion (t) using the equation t = τ/2u, where u is the total mutation rate per sequence per generation [36, 37]. Time since expansion was estimated using mutation rates for mtDNA derived from the results of the molecular clock analysis, and the rate of 2% divergence per million years. According to Sherman [38] individuals of *Psophia* reach sexual maturity at two years of age, but because of their complex social interactions, young individuals are unlikely to contribute to group reproduction until they reach a higher social position within the group. Due to
this social structure, we adopted three years as the average age at which individuals of *Psophia* might reproduce for the first time.

Further investigation of population demography was conducted using Extended Bayesian Skyline Plots [39]. MtDNA and Fib 7 sequences were analyzed together in BEAST to estimate current and past population size. Strict clock model was used with fixed rates of 2.1% (0.01 substitutions per site per million years; 40) and 1.34% (0.0067 substitutions per site per million years; from results of the RAG2 analysis) for mtDNA. The MCMC was run for 20 million generations, effective sample sizes were checked in Tracer and the plots were constructed in Excel using the .csv files for each taxon.

**RESULTS**

*Species and species limits.* It has long been recognized that these species-level units are diagnostically distinct from one another on the basis of morphology (bill shape and color, color of the feet and legs, and numerous plumage characters) [28, 41, 42]. It is worth stressing moreover that our observations indicate that *P. dextralis* and *P. interjecta* are also morphologically distinct from one another [contra 42, who concluded that *P. v. interjecta* consisted of “an individual variation of *P. v. dextralis*”]. Based on a larger series of specimens (Figure S2, Appendix 1), we found consistently that the upperparts of *dextralis* are a deeper dark brown than in *interjecta*, whereas the character originally chosen to diagnose the latter taxon (“blue apical spots to the wing-coverts”; 37), was conspicuously absent from *dextralis*. Even though Oppenheimer and Silveira [42] mention several individuals within the Xingu area of endemism possessing those two characters critical to the recognition of a distinct *interjecta*, they nevertheless interpreted those specimens as individual variants of *dextralis*.

Our new genetic data from mitochondrial DNA find that all eight lineages are reciprocally monophyletic and diagnosable on the basis of molecular characters. Thus, the eight species are distinct, with their pattern of morphological and genetic differences being consistent with restricted gene flow, long-term isolation, and evolutionary independence.

Over the years, application of the BSC has stimulated an array of speculation about gene flow among the taxa [28]. For example, Haffer [28] proposed that the taxa *viridis, dextralis, interjecta,* and *obscura* would intergrade along the upper reaches of the Tapajos, Xingu, and Tocantins rivers in eastern Amazonia, and earlier Hellmayr and Conover [41] implied that *interjecta* represents a hybrid population between *dextralis* and *obscura*. Similarly, the taxon *P. ochroptera* was suggested to represent a hybrid population between *P. crepitans* and *P. napensis* [28]. As a consequence of these suppositions linked to putative nonisolated "subspecies" within biological species, traditional taxonomy has been in a state of flux and subject to arbitrary decisions and statements [28, 38, 41-45], with, for example, *P. ochroptera* being alternatively lumped with both *P. crepitans* or *P. leucoptera*.

Our data significantly clarify many of these misinterpretations. *P. ochroptera* is the sister taxon of *P. crepitans* and both are phylogenetically distant from *leucoptera*, which is inconsistent with the hypothesis of extensive hybridization between *P. crepitans* and *P. leucoptera* in upper Amazonia. Haffer [28] grouped *P. ochroptera* under the polytypic *P. crepitans* (including *napensis*) and stated that *napensis* and *ochroptera* intergrade in the upper reaches of the Rio Negro. He referred to three specimens from Mt. Curicuriari, Brazil, housed at the AMNH, two of which he considered to be typical *napensis* and the third to be morphologically intermediate. The latter specimen was sequenced (N3, Table S1, Figs. 1, and S1) and it groups with other *napensis* with high support. Haffer [28] further suggested nominate *crepitans* and *napensis* also interbreed in the upper Rio
Orinoco region where morphologically intermediate specimens are found. In the present study, the westernmost locality for which *crepitans* was sampled is Cerro de la Neblina in southwestern Venezuela. The three specimens sequenced from that locality show haplotypes included in the *crepitans* clade, and do not exhibit any sign of differentiation from other *crepitans* that were sampled. Because *ochroptera* and *napensis* potentially meet somewhere in the upper reaches of the Japurá–Negro interfluvium in Brazil, where no major river barrier is found, and the ranges of *napensis* and *crepitans* apparently abut along the narrow upper Rio Negro in Brazil and Venezuela, opportunities for interbreeding between *napensis* and *ochroptera* or *crepitans* are greater in this part of Amazonia (Figs. 1, S1, and S2). The fact that the only *napensis* sequenced for BFib7 was a heterozygote with one allele being shared with *P. crepitans* (Fig. S4) could indicate the existence of some current gene flow among those taxa, but, due to the extensive non-monophyly of species in the Fib7 analysis (Fig. S1), ancestral polymorphism may be a more probable explanation.

**Data characteristics.** Within *Psophia*, there were no deviations in base composition for either of the genes sequenced. Most substitutions in cytochrome (cyt) *b* and ND2 were transitions at 3rd codon positions, as expected for protein coding genes. The mitochondrial gene sequences also lacked indels and stop codons in unexpected positions. No saturation was detected within the ingroup in any dataset, even when including only transitions at 3rd positions.

The ILD test did not detect incongruent phylogenetic signal between cyt *b* and ND2 (*p*=0.13), so that sequences of both genes were combined for the final analyses. Among the 62 individuals for which mitochondrial sequences were obtained, there were 42 distinct haplotypes. The final combined matrix was composed of 42 sequences for *Psophia*, two outgroups (*Aramus guarauna* and *Grus canadensis*) and a total of 2034 bp.

Average ML distance between the northern and southern clades is 3.4% (SD=0.6). Within the northern clade *napensis* is the sister taxon of *crepitans* + *ochroptera*, with both clades diverging by a mean genetic distance of 1.4% (SD=0.2). The clades *crepitans* and *ochroptera* are separated by the middle/lower portion of the Negro river and are 1.3% (SD=0.1) divergent. The splits within the southern clade are coincident with the position of the rivers Madeira (between *leucoptera* and *viridis* / *dextralis* / *interjecta* / *obscura*; ML distance = 2.1%; SD = 0.3), Tapajós (between *viridis* and *dextralis* / *interjecta* / *obscura*; ML distance = 1.6%; SD = 0.2), Xingu (between *dextralis* and *interjecta*; ML distance = 0.5%; SD = 0.1), and Tocantins (between *dextralis* / *interjecta* and *obscura*; ML distance = 0.8%; SD = 0.2).

In all Bayesian runs, effective sample sizes were larger than 200 for all parameters. AWTY cumulative plots indicate that stationarity was reached for all splits within *Psophia*.

**DISCUSSION**

**The Refuge Hypothesis.** The most important explanation for Amazonian diversity over the past 40 years has been the refuge hypothesis, which proposes that during cool-glacial cycles allopatry and differentiation of populations took place within forest refugia isolated by intervening dryland habitats, whereas during warmer, wetter interglacials those populations would then have became more cosmopolitan [47-51].

The hypothesis itself is based on the intersection of three primary sets of data [49, 52]: (1) gradients in species diversity: refuges are those areas that now harbor highest diversity and endemism, (2) gradients in rainfall: refuges are those areas with the highest current rainfall that, it is assumed, persisted in more arid times, and (3) geological and palynological evidence for dry environments (grasslands, savannas) in areas that now are occupied by wet forest.
The existence and importance of refuges in the Northern Hemisphere is well supported by biological data [53, 54], but comparable evidence for Amazonia is lacking. Multiple authors [e.g., 55-58] have critically discussed aspects of the refuge hypothesis and have pointed out that proposed refugia are not based on any physical geological or paleontological evidence. Thus, even though paleoenvironmental uncertainties about the extent and location of arid habitats are still debated [52, 56-59], current evidence supports extensive rainforest during glacial cycles [60]. The fact that Amazonia may have exhibited more arid conditions in some regions in the past does not constitute sufficient confirming evidence for refuges or for the hypothesis that repeated cycles of refuge formation drive species diversity. Climate models indicate that Amazonia remained forested in the LGM but that deciduous seasonally-dry vegetation would have expanded, particularly in the south [61]. These changes would not have resulted in scenarios proposed by the refuge hypothesis. At the same time, it is reasonable to entertain the hypothesis of some large-scale forest fragmentation due to climatically-driven environmental change. What form this may have taken is uncertain given the paucity of the palynological record in Amazonia [61]. Pennington et al. [62] propose dry-forests were much more widespread in Amazonia in the LGM, but our results for Psophia across southern Amazonia suggest that rainforest persisted there through the LGM and other glacial cycles. On the other hand, our results also show strong evidence of recent population expansion in the Guiana and Inambari areas of endemism, suggesting that distribution of rainforest within these areas may have been affected by the LGM. However, this contrasts with previous studies that did not show evidence for population expansion in most groups of small mammals in the Inambari area of endemism [63]. It also contrasts with the view that Inambari rainforests, in western Amazonia, which were close to the base of the Andes and thus very humid, would have been less affected by glacial cycles [56, 64]. Studies of the Guiana area [65, 66] suggest that cooling and downward migration of taxa may have been related to diversification in this region, but these studies do not propose reduction in forest cover. In both cases cooling, rather than forest fragmentation, could account for population contraction in Psophia, but population genetic evidence from additional taxa will be needed to identify general patterns and reconstruct glacial environments within different Amazonia areas of endemism.

Distributional data within Amazonia have been a common starting point for all hypotheses proposed to explain high species diversity, but by themselves do not discriminate among hypotheses. On the other hand, biogeographical patterns based on phylogenetic relationships are critical evidence as those are the empirical historical traces of speciation. The refuge hypothesis cannot make specific predictions about the history of speciation within any clade, and therefore does not establish a testable mechanistic link to the generation of diversity [57, 67].

**Generality of the model.** The model also can be used as a framework for comparisons across clades and for revealing other patterns, whether at older or younger temporal scales. However, because of multiple sources of error inherent in estimating temporal patterns of speciation within and among clades, such comparisons will often be imprecise. Another difficulty is that the time-scale of geological change thought to drive particular speciation events can be synchronous or asynchronous with respect to the time required to effect allopatry and differentiation. Thus, the evolutionary response of different taxa to the same vicariance event depends on the ecological context of each, and on population characteristics that influence rates of change [46]. Ideally, synchrony is best measured by congruence among biogeographic patterns in relation to specific geological events.

**How old are Amazonian species?** The time period over which the high species diversity found
within Amazonia was generated is still poorly understood. Because he associated speciation dynamics with cyclical, orbitally-driven climate change, Haffer [47, 52] saw speciation in Amazonia as primarily being a Pleistocene phenomenon. Proponents of a Late Miocene age for the origin of the Amazonian drainage and terrestrial ecosystem, especially in the west, reject the Quaternary as the major period of Amazonian diversification [68, 69]. As molecular phylogenies of Amazonian clades become available, this phylogenetic information has been used to test hypotheses about the origins of Amazonian diversity, relying on molecular dating analyses to infer species ages and diversification rates [68-72]. Older time-estimates for primary diversification are based largely on “crown clade” ages (i.e., usually genera) of sparsely-sampled molecular phylogenies rather than on analyses of speciation per se. Currently, we see many challenges to developing robust estimates of the timing of species diversification and briefly discuss these here.

The taxonomic problem: genera and species. As noted by many authors working in Amazonia, the taxonomy of even the best-known groups, like birds and mammals, needs to be revised and species limits re-evaluated before any conclusions can be drawn regarding species’ ages [73]. Using sampling within so-called “crown-groups” or genera inevitably overestimates ages of included species [74]. “Crown-groups” can even be more inclusive than a single genus, and a “genus” itself is only a collection of related species given a particular Linnaean rank and is not nonarbitrarily delineated.

The idea of a species can also lead to different estimates of ages. Thus, the application of the Biological Species Concept has resulted, at least in birds, in circumscribing collections of diagnosable taxonomic units (subspecies), which overestimates distributions and underestimates taxonomic diversity. Coupled with dating studies, the use of biological species can lead to an overestimate of species ages, which would imply an “old” Neotropical biota [73]. Most Neotropical polytypic species that have been studied in detail have been shown to include multiple distinct lineages, and, in some cases, the "species" are not even monophyletic [73, 75-82; this study]. This is the reason we adopted a phylogenetic species approach for *Psophia*. All diagnosably (taxonomically) distinct groups are recognized (as hypotheses), and their estimated ages pertain to the times of taxonomic diversification. This approach shows that diversification within *Psophia* is largely Pleistocene.

Sampling. Analyses of the timeline of diversification must be based on complete sampling of species within each studied group, as undersampling results in overestimation of species ages [83]. Many molecular phylogenetic studies currently available for Amazonian taxa rely on sampling few (one or two) individuals per species from few localities —often representing distributions that may encompass regions as large and disjunct as, for example, the whole Amazonian forest plus Atlantic and Central American Forests. Artifacts of sampling also frequently extend to studies of “crown groups” or genera. The more sparse the species-level sampling, the more ages will be overestimated.

Issues of dating. As noted above, concern for an appropriate species-level taxonomy as well as sampling are crucial for integrating biogeographic pattern and Earth history. It is also key that estimates of dating take into account these taxonomic and sampling criteria. Moreover, integrating biogeography and species’ origins with Earth history also requires an accurate temporal framework,
yet, precision in dating is a problem for both. At a large scale, linking Andean uplift to the temporal pattern of speciation in Amazonia is problematic, as the latter is likely to be more local (or regional) and due to younger geological events such as regional tectonics (e.g., faulting leading to river change) or climate-vegetation change that are the proximate causes of allopatry and speciation. These types of events need to be dated at more regional or local scales than at the level of Andean uplift itself. Likewise, dating of speciation events must be as accurate as possible, which is why we suggest using independent methods as cross-checks. Biogeographic and temporal congruence among taxa will also be important for validating links to Earth history.

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