Speciation timing and neotropical biodiversity: the Tertiary–Quaternary debate in the light of molecular phylogenetic evidence

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Abstract

The evolutionary origin of extant species in the Neotropics, one of the most biodiverse regions of the world, has been widely debated. One hypothesis is that neotropical species emerged primarily during the Quaternary (the last ~2 million years), favoured by alternating glacial/interglacial climates. An opposite view proposes an older Tertiary origin linked primarily to palaeogeographical changes. Here, a thorough review of the available literature on DNA molecular dating shows that the Tertiary–Quaternary debate no longer makes sense. Indeed, the > 1400 neotropical species whose origin has been dated have appeared in a continual fashion since the late Eocene/early Oligocene (~39 million years before present) to the Quaternary. Palaeogeographical mechanisms of speciation are relatively well accepted, but diversification processes linked to climate are still controversial. These results are important to unravel both the origin of present-day biodiversity patterns at both local and global scales and the genetic and environmental mechanisms involved, which are two crucial aspects for suitable biodiversity conservation strategies.

Keywords: biodiversity, climate change, molecular phylogenetics, Neotropics, palaeogeography, speciation

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Introduction

The tempo and mode of origin of extant species and therefore of present-day biodiversity is still controversial. The debate has been largely focused on temperate regions, and the potential influence of the dramatic climatic changes occurred during the last ~2.5 million years, known as the Quaternary or Pleistocene glacial cycles (Bennett 2004). Some authors believe that Pleistocene glacial/interglacial alternation has been decisive in shaping the present-day temperate biota, while others think that modern species originated mostly in the Tertiary or earlier, but before the Quaternary glaciations (Hewitt 2000; Willis & Niklas 2004). A third option has been provided by Zink *et al*. (2004), who suggested continuous diversification trends, without significant differences

between Tertiary and Quaternary speciation rates. Knowing speciation timing, genetic mechanisms and forcing agents involved would provide fundamental clues for biodiversity conservation (Moritz 2002). Therefore, the research about the origin of present-day species is not only of intrinsic ecological and evolutionary interest but is also needed for the development of suitable biodiversity conservation strategies.

The Neotropics (Fig. 1) is among the most biodiverse regions in the world and, as such, is a preferred target for research about the origin of biological diversity. Some neotropical areas are under manifest danger of biodiversity loss and have been identified as biodiversity hotspots submitted to special conservation programs (Myers *et al*. 2000). For some time, the most popular proposal has been the refuge hypothesis, according to which the assumed aridity of neotropical lowlands during the Quaternary glaciations favoured forest fragmentation into islands in a sea of savannas and/or deserts, thus promoting allopatric speciation (Whitmore & Prance 1987; Hooghiemstra & van der Hammen 1998). The refuge hypothesis attracted

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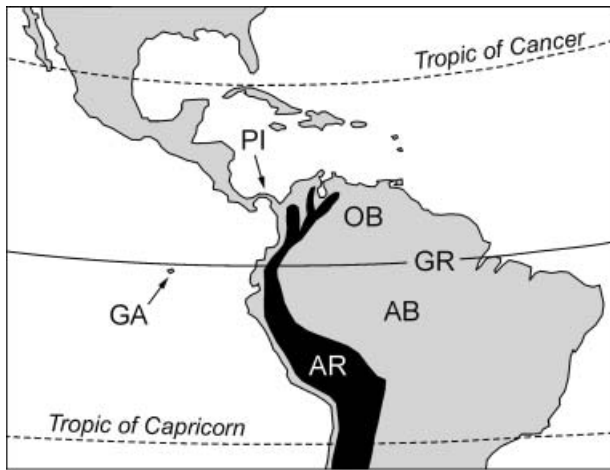
Fig. 1 Sketch-map of the neotropical region showing the main geographical features quoted in the text. AB, Amazon basin; AR, Andean range (in black); GA, Galápagos archipelago; GR, Guayana region; OB, Orinoco basin; PI, Panamá Isthmus.

the attention of many scientists and, for decades, it was considered the right explanation for the evolutionary consequences of Quaternary climate change. However, further palaeoecological evidence was controversial and this hypothesis is at present under serious criticism (Colinvaux *et al*. 2000; Bush & de Oliveira 2006). Such attacks of the refuge proposal led many researchers to abandon the idea of Quaternary speciation and to propose that neotropical species were older, in fact of Tertiary origin (e.g. Colinvaux & de Oliveira 2001). In this case, the main forcing agents suggested were related to palaeogeographical reorganizations linked to continental drift, such as the Andean orogeny, the closure of the Panama Isthmus or the flooding of the Orinoco and Amazon basins by epicontinental seas, among others (Nores 2004). This generated an ongoing debate about the Tertiary or Quaternary age of extant neotropical species.

Until recently, both Tertiary and Quaternary hypotheses have been supported primarily by geological and palaeoecological data, which provided indirect arguments in favour of either one or another. During the last decades, however, straightforward time measurements of diversification events have been possible thanks to the development of DNA-based molecular phylogenies that allow estimation of speciation timing (Nee *et al*. 1992; Harvey *et al*. 1994; Riddle 1996). At the beginning, this method was severely criticized (Ayala 1999) but further improvements, such as accommodating mutation rate heterogeneity and the calibration with radiometrically dated geological evidence, notably increased its reliability (Arbogast *et al*. 2002; Rutschmann 2006). Recent reviews conclude that DNA phylogenies are a very promising tool to investigate the historical construction of major biomes, especially those with high diversity and a poor fossil record (Pennington

et al. 2004b, 2006). However, despite the advent of such new powerful dating methods, the neotropical Tertiary–Quaternary controversy continues, due to the finding of contrasting diversification histories for different groups of organisms (e.g. Bush 2005; Rull 2006). The first attempt to gather phylogenetic data and draw a more or less general assessment was carried out by Moritz *et al*. (2000), who concluded that the diversification within 22 lineages of rainforest fauna (amphibia, reptiles, small mammals and birds) predates the Quaternary. Later, Pennington *et al*. (2004a) found higher Quaternary speciation in several tree genera from seasonally dry forests of Central America, and both Quaternary and pre-Quaternary speciation in the same genera from South America. More recently, Weir (2006) studied the data available for 27 avian taxa and observed higher Miocene diversification for lowland species and higher Quaternary speciation for highland taxa. Molecular phylogenetic studies of neotropical organisms have flourished during the last two decades, but a comprehensive review to provide solid support to the current hypotheses is still unavailable. This study attempts to fill the gap by providing a thorough analysis of the phylogenetic studies with quantitative dating of neotropical species published so far. It should be stressed that the study of diversification rates through time is beyond the scope of this paper which concentrates on the age of origin of living species and therefore of present-day biodiversity. To account for speciation rates, methods such as log-lineage plots and sister-taxon distances are more appropriate (Zink *et al*. 2004) and will be considered for future data management.

Methods

This paper is based on all literature dealing with phylogenetic analysis of living neotropical species published until 2006 in widely recognized journals included in the ISI's *Journal Citation Records* (Table 1). More than 300 references were analyzed in depth and a selection was performed based on the application of objective criteria. As the main objective was to know the time of origin of present-day species diversity, only studies dealing with species or lower taxonomic categories were used. The basic taxonomic unit considered was the Evolutionarily Significant Unit (ESU) (Ryder 1986). It has been emphasized that species alone are not enough to fully account for actual biological diversity, due to the ecological and evolutionary significance of intraspecific genetic variability (Agapow *et al*. 2004). ESUs– i.e., including species and subspecific categories that are reciprocally monophyletic, with significant allelic divergences, and with manifest ecological distinctiveness – have been considered more realistic biodiversity descriptors (Moritz 1994; Crandall *et al*. 2000). In this study, subspecific lineages have been treated as ESUs only if they are explicitly and unequivocally recognized as distinct genetic and

Table 1 List of journals used for the present study

American Journal of Botany
Annual Reviews of Ecology, Evolution and Systematics
Biochemical Systematics and Ecology
Biological Journal of the Linnean Society
BMC Evolutionary Ecology
Botanical Journal of the Linnean Society
Bulletin of the American Museum of Natural History
Cladistics
Development Genes and Evolution
Evolution
Evolution and Development
Evolutionary Ecology
Heredity
Insect Systematics & Evolution
International Journal of Primatology
Journal of Avian Biology
Journal of Biogeography
Journal of Evolutionary Biology
Journal of Experimental Zoology part B
Journal of Fish Biology
Journal of Mammalian Evolution
Journal of Mammalogy
Journal of Molecular Evolution
Journal of Zoological Systematics and Evolutionary Research
Mammal Biology
Marine Biology
Molecular Biology and Evolution
Molecular Ecology
Molecular Ecology Notes
Molecular Phylogenetics and Evolution
Nature
Organisms Diversity and Evolution
Philosophical Transactions of the Royal Society B
Plant Systematics and Evolution
Proceedings of the National Academy of Sciences, USA
Proceedings of the Royal Society B
Science
Studies on Neotropical Fauna and Environment
Systematic Biology
Systematic Botany
Systematic Entomology
Taxon
The American Naturalist
The Auk
The Condor
*Trends in Ecology and Evolution*
Zoological Journal of the Linnean Society

ecological/geographical entities in the source studies. This includes subspecies and disjunct geographical clades; minor differentiation at population or metapopulation level was not considered. Only studies providing quantitative estimations for the age of origin of involved species have been employed; surveys with qualitative, vague or incomplete dating information have not been included. The age of origin of a given ESU is considered here as the time at which it diverged from its closest relative. In Renner's (2005) terminology, this is the age of the 'crown node' containing the extant species. The use of 'stem lineages' to date diversification events is common in many original papers but has been avoided here. Finally, only surveys with original estimations of diversification timing have been selected; revisions and re-calculations based on the divergence rates of others were not considered. No taxonomic criterion was used, and any kind of organism was included, provided that their study fitted with the above-mentioned criteria. The final selection can thus be considered to be almost exhaustive, as well as taxonomically and chronologically unbiased.

In the final selection, only raw data provided by the authors in the form of numerical tables, time-constrained phylogenetic trees and explicit in-text numerical values were used, and no additional calculation was performed. In studies comparing two or more genetic divergence rates, those recommended by the author(s) have been selected. In the absence of author's pronouncement, the total age range derived from all calibrations considered was used. When different authors subdivide the same species into different sets of geographical clades, the more parsimonious solution adopted was to consider the option with less clades, in order to minimize the possibility of including nonsignificant genetic/ecological differences. In order to prevent taxonomic duplication, the following procedures were applied. In the case of two studies of the same species or ESU, both were included together if the results coincide, or separately if there was some age disagreement or uncertainty. The quantitative analysis, however, only uses their divergence ages if they coincide. If they disagree they are not used, unless the disagreeing taxa have been studied by the same author or research team, in which case the results of the more recent publication have been adopted.

A critical point to decide whether a species was originated during or before the Quaternary is the age of the Tertiary/Quaternary (or Pliocene/Pleistocene) boundary. According to classical lithological criteria, this boundary is placed at 1.8 million years before present [Million years ago (Ma)], while using magnetostratigraphy, the limit is located at 2.6 Ma (Walsh 2006). This would make a big difference in the context of the tempo of evolution of extant species, which is intimately related to the mechanisms and forcing factors involved. In this sense, Quaternary speciation is considered to be strongly linked to Pleistocene glaciations; hence, the age of initiation of these glacial cycles is the key point. Palaeoclimatic studies on marine sediment cores have shown that the onset of Northern Hemisphere glacial cycles was around 2.6 Ma (Raymo 1994), coinciding with the magneto-stratigraphic criterion. In order to avoid unnecessary terminological controversies, this study uses the informal terms 'Glacial' for the last 2.6 Myr, and 'pre-Glacial' for earlier Tertiary times. This focuses the debate on
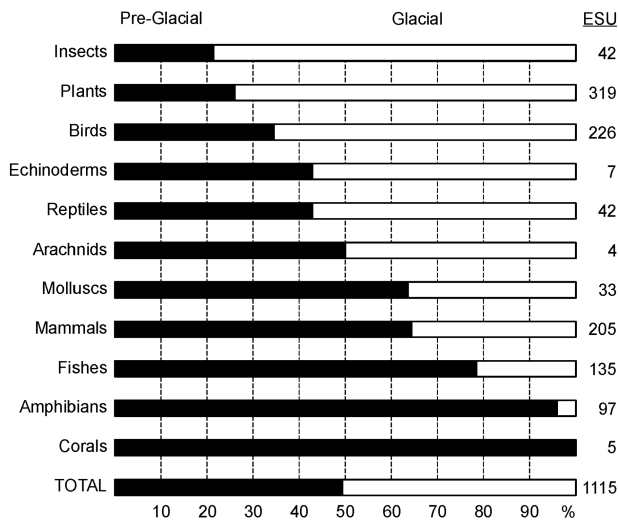
Fig. 2 Percentage of evolutionarily significant units (ESUs) originated either in the pre-Glacial (black bars) or in the Glacial (white bars) for each taxonomic group studied. The number of ESUs for each lineage is given in the right side. Note that the number of ESUs reported here (1115) is lower than the total number of ESUs analyzed (1404). This is due to the unavailability of quantitative age estimates for some of the ESUs considered (see Table S1, Supplementary material).

the potential influence on speciation of glacial/interglacial alternation rather than the stratigraphic concept of the Quaternary. The Tertiary interval considered began in the late Eocene (39 Ma, which is the age of the older species reported) and includes the Oligocene (initial age 36.0 Mya), the Miocene (25.2 Ma) and the Pliocene (5.2 Ma) (Berggren *et al*. 1995).

Results

The application of the conditions mentioned on the analyzed references resulted in a selection of 113 studies, containing 105 taxonomic groups (mostly genera) or lineages and 1404 ESUs. Raw data and details on the nature of surveys utilized, as well as the complete reference list, are provided in Table S1 (Supplementary material). Globally, around the half of ESUs studied so far were originated during the pre-Glacial (as described in the methods section) and the other half in the Glacial (Fig. 2). Individually, amphibians and fishes emerged primarily in the pre-Glacial, whereas insects, plants and birds arose mainly during the Glacial. Others are intermediate, but mammals show a pre-Glacial preference, while reptiles have more Glacial species. Studies on echinoderms, arachnids and corals are still too scarce for a sound assessment. It could be argued that the amount of Glacial speciation has been exaggerated because of the inclusion of subspecific categories as ESUs. However, numbers are not significantly different if subspecies and

geographical clades are removed. Indeed, from a total of 845 species left when these are removed, 51% originated in the pre-Glacial and the remaining 49% are of Glacial origin. In the case of vascular plants, there is also another potential source of bias towards younger ages, as some of the lineages analyzed have representatives in high Andean biomes (3000 m or higher), which are of relatively recent origin (Plio-Pleistocene). To test this effect, some groups (*Gentianella*, *Halenia*, *Hypochaeris*, *Tibouchina*, *Lupinus* and *Oreobolus*) were removed from the meta-analysis. Of the remaining 1277 ESUs, 56% were pre-Glacial while 44% originated during the Glacial period. Figure 3 shows that speciation leading to present-day ESUs has actually proceeded in a continual fashion since the late Eocene/early Oligocene (~39 Ma). The left side of each bar represents the age of the oldest extant species within the lineage or, in other words, the first diversification event within its 'crown group' (*sensu* Renner 2005). Therefore, the shape of the curve depicted by the left bar ends describes the trend of the initial diversification events of lineages. The major palaeo-geographical and palaeoclimatic events documented have been superimposed for a tentative test of their possible influence on the general diversification process (Fig. 3). Due to the monotonous trend of the speciation process, none of them seems to have had any differential impact on diversification with respect to the others.

Discussion

The available molecular phylogenetic evidence shows that the extant neotropical species and ESUs studied so far have originated in a continual manner since the late Eocene/early Oligocene until the Pleistocene. These data indicate that the Tertiary–Quaternary debate should be replaced by a more realistic view of present-day neotropical biodiversity as a mixture of species of different ages, likely originated through diverse mechanisms. This agrees with the results of Zink *et al*. (2004) for temperate avifauna, tentatively suggesting trends of more global nature.

Timing and speciation mechanisms are closely linked, as the more significant palaeogeographical reorganizations occurred in the Tertiary, whereas climatic changes were the norm during the Quaternary. The effects of Miocene and Pliocene palaeogeographical changes on speciation trends are relatively well-known and are related to the building and vanishing of bridges and barriers, thus changing migration and isolation patterns and favouring vicariance (Coyne & Orr 2004). There is little doubt that events such as the uplift of the Andes and the closure of the Panama Isthmus have played a significant role in shaping neotropical biodiversity patterns (Bush & Hooghiemstra 2005). The influence of climate, however, is still difficult to ascertain. So far, studies linking speciation with climate focused on the influence of Pleistocene glaciations, often through the
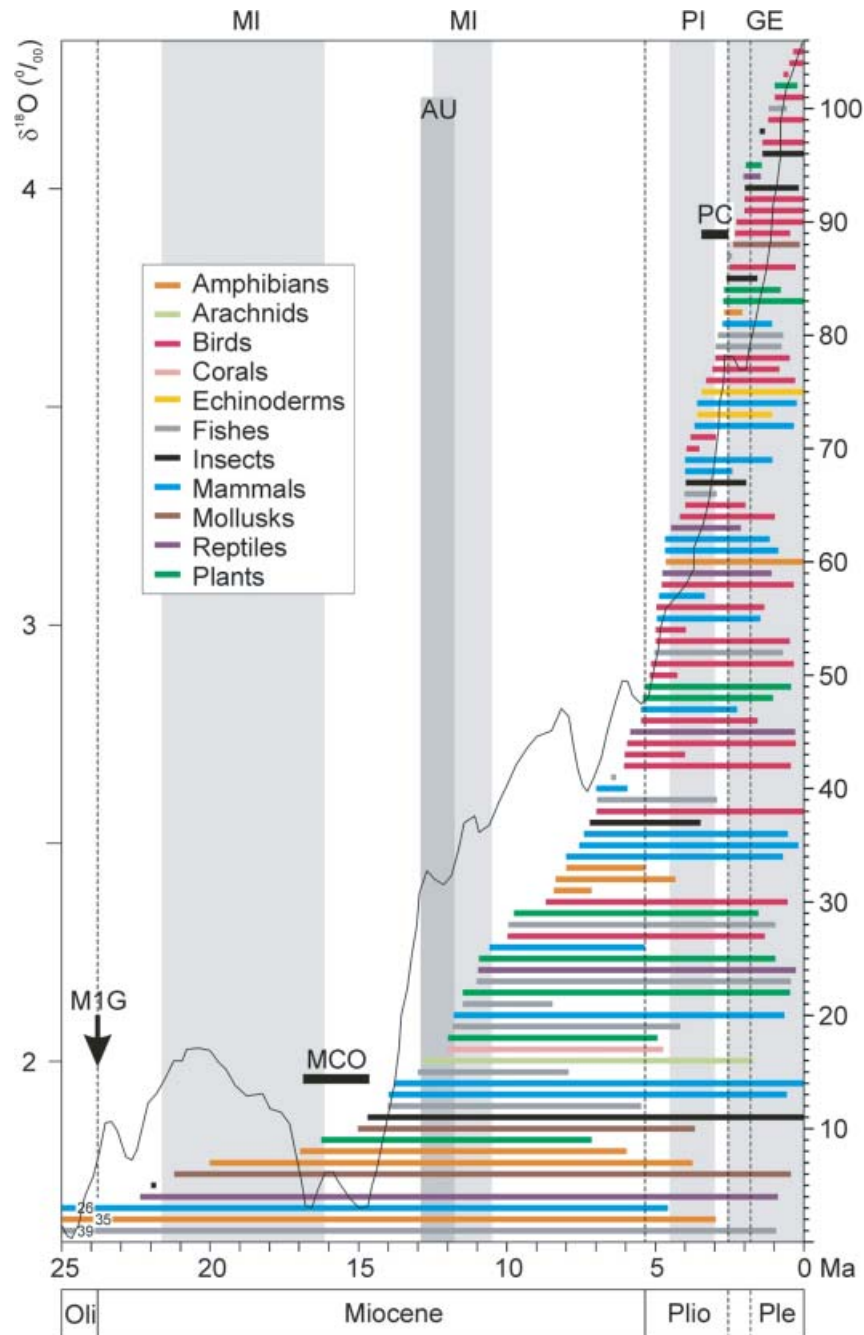
Fig. 3 Time range of the divergence of evolutionarily significant units (ESUs) for each lineage studied, according to raw data from the table provided as supplementary material (Table S1, Supplementary material). Bars represent the time interval between the origin of the older (left) and the younger (right) ESU within each group, and are sorted chronologically according to the emergence of the older ESU. Time in million years ago (Ma). Geochronological units: Oli, Oligocene; Plio, Pliocene; Ple, Pleistocene (Berggren *et al.* 1995). The two possibilities for the Pliocene/Pleistocene boundary are depicted (Walsh 2006). Singular periods and events are highlighted with grey areas: GE, Glacial Epoch; PI, formation of the Isthmus of Panamá (Bartoli *et al.* 2005); MI, Marine incursions into the Amazon basin (Lovejoy *et al.* 2006); AU, Major event of north-Andean uplift (Hoorn *et al.* 1995). The smoothed oxygen isotope deep-sea curve (solid black line) is represented in $\delta^{18}O$ units (Zachos *et al.* 2001). This parameter is largely (~70%) controlled by the ice volume of Antarctica and the Northern Hemisphere, hence, it is correlated with the intensity of continental glaciation and therefore with global cooling. M1G, Miocene-1 Glaciation; MCO, Miocene Climatic Optimum; PC, Pliocene Cooling (Zachos *et al.* 2001).

questionable refuge hypothesis. New proposals are needed to account for Pleistocene diversification. One possibility is the disturbance-vicariance hypothesis (Noonan & Gaucher 2006), according to which an oscillating cooling–warming trend can cause downward altitudinal migrations and spread of cool-adapted species in cool phases, followed by fragmentation and isolation of populations of these species in warm phases, ultimately resulting in adaptive radiation and allopatric/parapatric speciation (Colinvaux 1998; Rull 2005). An elegant example of such mechanism can be found in the harlequin toads of the genus *Atelopus*. It has been suggested that the Guayanan species of this genus derive from a common ancestor that, thanks to the Pliocene cooling, migrated from the Andes and crossed the Amazon basin (Fig. 1). Once in the Guayana region, the extant *Atelopus* species would have emerged by vicariance on isolated highland summits, favoured by Pleistocene climatic changes (Noonan & Gaucher 2005). Other mechanisms, as well as the coupling of two or more of them, are possible and this should be the target of future studies, especially in the case of aquatic organisms. The results shown here support the view of neotropical diversification as a complex process in which palaeogeographical and palaeoclimatic forces have been constantly interacting (Bush 1994). However, the surprisingly high amount of speciation found during the last 2.6 Myr, when climate changes have been the stronger environmental factor, is remarkable.

The main contribution of the present meta-analysis is to provide a different chronological scenario for the debate of the origin of extant neotropical biodiversity, but there are opportunities for continued improvement. As phylogenetic research is growing rapidly and the dating methods constantly improving, the conclusions of this study should be periodically revised and updated with new information. Potential improvements would include the re-analysis of evidence from older studies with more modern and robust dating techniques (Rutschmann 2006), the analysis of diversification rates by phylogenetic modelling (Zink *et al.* 2004) and the comparison timing of differentiation patterns under a geographical perspective (Mesoamerica, northern South America, the Andes etc.), in order to test the influence of the potential palaeogeographical and climatic factors involved. It would be also desirable to compare these results with other tropical regions of the world. Unfortunately, the scarcity of molecular phylogenetic studies at the species level in African and Asian tropics is a handicap (Pennington *et al.* 2004b; Plana 2004). The tempo and mode of speciation, as well as the Tertiary–Quaternary debate, is a common issue to other, extra-tropical regions, with important implications for conservation (Hewitt 2004). A classical example are the continuing arguments about the origin of the North-American avifauna (Johnson & Cicero 2004; Weir & Schluter 2004; Zink *et al.* 2004; Lovette 2005; Zink & Klicka 2006). More dated phylogenies would be also useful to address the problem of the latitudinal diversity gradient in terms of differences in speciation and extinction rates (Weir & Schluter 2007). It would be useful to establish a global database of dated phylogenies to help address the world-wide problems of biodiversity origin and conservation using a basis of firm data about speciation timing.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Raw data used in the study. Common and scientific names for organisms and organism groups considered are given in Columns 1 and 2. The area (Column 3) refers to the geographical range of the organisms considered in each study (CARIB, Caribbean;

GAL, Galápagos archipelago; MESO, Meso-America; NEO, The whole Neotropics; SA, South America). Columns 4 and 5 specify the actual DNA sequences employed (mt/chl, mitochondrial and chloroplastic; nuc, nuclear). GDR (Column 6) is the estimated genetic divergence rate in percentage per Myr. Columns 7 and 8 express the time range in Myr for the origin of the taxa considered, as represented by the age of the older (Column 7) and the younger (Column 8) ESU of the lineage. The corresponding geological age is given in Column 9, using the 2.6 Myr boundary for the beginning of the Pleistocene (Eo, Eocene; Mio, Miocene' Plio, Pliocene; Ple, Pleistocene; E, Early; L, Late). Taxa (Column 10) refers to the nature of the lower significant taxonomic category (Evolutionarily Significant Unit, ESU) considered in each study (S, species; SP, subspecies; GC, geographical clades). Column 11 (total) indicates the number of ESUs employed in each study. In the case of studies covering a wide geographical range, only neotropical ESUs were considered. Columns 12 and 13 give the number of ESUs originated either in preglacial (pG) or glacial (G) times (see text for details). When raw data from the original references are not sufficient to estimate these proportions, pG and G cells are blank. The sum of these two columns does not necessarily coincide with the total of Column 11, as not always has been possible to estimate the age of divergence of all the ESUs in a group. Reference numbers are given in Column 14

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