Major Radiations in the Evolution of Caviid Rodents: Reconciling Fossils, Ghost Lineages, and Relaxed Molecular Clocks

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Abstract

Background: Caviidae is a diverse group of caviomorph rodents that is broadly distributed in South America and is divided into three highly divergent extant lineages: Caviinae (cavies), Dolichotinae (maras), and Hydrochoerininae (capybaras). The fossil record of Caviidae is only abundant and diverse since the late Miocene. Caviids belongs to Cavioida sensu stricto (Cavioida s.s.) that also includes a diverse assemblage of extinct taxa recorded from the late Oligocene to the middle Miocene of South America ("eocardiids").

Results: A phylogenetic analysis combining morphological and molecular data is presented here, evaluating the time of diversification of selected nodes based on the calibration of phylogenetic trees with fossil taxa and the use of relaxed molecular clocks. This analysis reveals three major phases of diversification in the evolutionary history of Cavioida s.s. The first two phases involve two successive radiations of extinct lineages that occurred during the late Oligocene and the early Miocene. The third phase consists of the diversification of Caviidae. The initial split of caviids is dated as middle Miocene by the fossil record. This date falls within the 95% higher probability distribution estimated by the relaxed Bayesian molecular clock, although the mean age estimate ages are 3.5 to 7 Myr older. The initial split of caviids is followed by an obscure period of poor fossil record (referred here as the Mayoan gap) and then by the appearance of highly differentiated modern lineages of caviids, which evidentially occurred at the late Miocene as indicated by both the fossil record and molecular clock estimates.

Conclusions: The integrated approach used here allowed us identifying the agreements and discrepancies of the fossil record and molecular clock estimates on the timing of the major events in cavioid evolution, revealing evolutionary patterns that would not have been possible to gather using only molecular or paleontological data alone.

Introduction

Estimating the timing of evolutionary diversification events is the field of major interaction between paleontology and molecular biology. During the last two decades, the alternative evolutionary timescales for different taxonomic groups were the focus of intense debates between paleontologists and molecular biologists [1–6]. The divergence estimates from molecular data do not always coincide with the first appearances in the fossil record, as molecules often estimate dates too old, and the fossil record underestimates the actual dates. Recent attempts to reconcile the two sources of information [7–10] aimed to increase the interaction and reciprocal illumination between these two sources, rather than highlighting the conflict or disagreement between paleontological and molecular clock estimates. The integration of both sources of evidence can reveal patterns on the time and mode of the evolutionary history of a group that would not be evident using only fossils or DNA sequences.

Rodents provide an interesting case for analyzing the interaction between fossils and molecules, as this is a diverse group with a relatively complete fossil record. Rodents are the most diverse group of mammals at present, which include more than 2256 species representing 41% of all mammals [11] and their evolution has been intensively studied in recent years [12–20]. South American rodents belong to Caviomorpha [21] and evolved during the last 45 million years from hystricognath forms that invaded South America (most likely from Africa) during the Paleogene [13,20,22–29]. Caviomorphs underwent an extraordinary evolutionary radiation that made this group the rodent clade with the greatest morphological and ecological disparity, including the broadest range of body size within Rodentia [30–34]. Examples of the diversity of caviomorph rodents are porcupines (Erethizontoidea), coypus, degus, and spiny rats (Octodontioidea),...
viscachas, chinchillas, and pacaranas (Chinchilloidea), and capybaras, maras, caviies (or ‘guinea pigs’), and pacas (Cavioida) [34,35].

Within caviomorphs, Cavioida is crucial for understanding the diversification of South American rodents given that it includes the greatest morphological disparity (e.g., digitigrades [36–39]), inhabits a broad range of ecosystems (e.g., semiaquatic, open grasslands, dry steppes, forest edges, wetlands, rocky ledges [40]), displays diverse levels of sociality [41], and includes the largest living rodent [35,40,42].

Different authors, however, have variously interpreted the taxonomic content of Cavioida. The most inclusive and traditional conception of the group includes four families: Dasyproctidae (agouties), Cuniculidae (paca), Caviidae (caviies and maras), and Hydrochoeridae (capybaras) [34,35,43–45]. Patterson and Wood [46] recognized Cavioida sensu stricto (Cavioida s.s.) clustering the extant Caviidae and Hydrochoeridae together with a diverse assemblage of primitive taxa of the extinct family Eocardiidae, given the presence of unique dental and mandibular modifications (e.g., heart-shaped occlusal surface, hypsodonty, reduced lateral deflection of the angular process). Recent phylogenetic analyses of this group based on morphological characters [47] corroborated the monophyly of Cavioida s.s. but retrieved a paraphyletic arrangement of “eocardiids” as successive sister taxa of the crown-group comprised of caviies, maras, and capybaras.

Regarding the relationships of the extant lineages, the availability of DNA sequences of extant species of Cavioida s.s. has provided a wealth of new data to understand more thoroughly the relationships of the group, such as the close affinities of the capybara (Hydrochoerus) and caviies (i.e., Cavia) that have been corroborated in broad-scale phylogenetic analyses of hystricognath rodents [13,18,20]. The most detailed molecular phylogenetic analysis of cavioid rodents [41] retrieved a deeply nested position of Hydrochoerus within the multiple representatives of caviides and maras used in that study, being the sister group of the Rock cavy, Kerodon. This result was incongruent with traditional morphological classification schemes, but has been subsequently corroborated by phylogenetic studies based on morphological characters [47–49].

Therefore the clade Caviidae can be applied to the cluster of the crown-group of three major living lineages: caviies (Caviinae), maras (Dolichotinae), and capybaras (Hydrochoerinae). These three major lineages of extinct caviides are well differentiated from a morphological and ecological perspective. Caviies are usually small-bodied taxa that inhabit a variety of environments (e.g., open grassland, forest edge, swamps) and they feed on diverse types of plants. Maras, instead, are much larger, adapted to cursorial habits with elongated hind limbs, and exclusively inhabit arid areas with coarse grass or scattered shrubs. Capybaras are not only the largest rodents alive but are also characterized by their highly apomorphic dentition (e.g., multilaminated molariforms with extremely deep flexus/ids) and inhabits densely vegetated areas around freshwater bodies [50]. Here we follow the taxonomic proposal of recognizing Caviidae as the crown-group formed by these three distinct living lineages [34,51]. Cavioida sensu stricto represents the clade formed by Caviidae and its stem group (the more basal and paraphyletic “eocardiids”), and Cavioida as an even more inclusive group that also includes Cuniculidae and Dasyproctidae, the two other lineages leading to the extant pacas and agouties.

The major focus of this contribution is the analysis of the timing and diversification patterns in the evolutionary history of Cavioida s.s. and its crown-group Caviidae, using the information of the fossil record and molecular clock estimates (evaluating the impact of different calibration constraints based on a critical use of the available fossil record). The fossil record of Cavioida s.s. includes a large diversity of extinct species recorded in South America since the late Oligocene (24.5–29 Ma). The fossil evidence indicates that Cavioida s.s. diversified after the arrival of rodents in South America [52,53], formed an important component of the vertebrate fauna, and occupied a broad range of ecological niches during the rest of the Cenozoic Era. Traditionally, the early evolution of the group was interpreted as a case of gradual transformation [52,54,55] starting from the low-crowned and mesodont primitive forms recorded in the Oligocene and ending with the diversification of high-crowned euhyodont forms that appear in the fossil record at the early Miocene (15.7–16.5 Ma). Fossil members of the crown-group Caviidae, however, appear later in the fossil record, by the late middle Miocene (ca. 11.8–13.5 Ma) and became morphologically and taxonomically diverse since the late Miocene (ca. 6.1–9.07 Ma). The recent morphological phylogenetic studies of these extinct and extant forms [47–49] have implications for understanding the tempo and mode of the evolution of Cavioida s.s. Previous hypotheses on the group, including the traditional interpretation of gradual evolution, need to be revisited within an explicit phylogenetic context.

Furthermore, the availability of molecular data for species of Caviidae also allowed exploring the divergence time of this clade using different approaches to the molecular clock [13,20,56–59], some of which proposed the divergence time of Caviidae occurred up to 25 Ma, several million years before the first appearance of the group in the fossil record. The availability of molecular and morphological phylogenies with extensive taxon sampling of fossil taxa, and the extensive fossil record of Cavioida s.s. provide an interesting test-case to evaluate the congruence between divergence estimates based on the fossil record and the molecular clock.

Here we present new phylogenetic results based on a morphological dataset that expands previously published evidence [47–49] and a molecular dataset of four genes. The results of a simultaneous parsimony analysis of morphological and molecular data and a Bayesian analysis of the molecular partition are used to evaluate the diversification patterns in the evolutionary history of Cavioida s.s. Finally, we compare the timing of these events as inferred from the fossil record evidence and through the use of relaxed molecular clocks [60], assessing the possible causes of conflict on divergence times inferred from the available paleontological evidence as well as from the molecular clock estimates. This offers a more complete understanding of the evolutionary tempo and mode of Cavioida s.s.

Results

The phylogenetic analyses conducted here are highly congruent in terms of the retrieved topologies. The most parsimonious trees (MPTs) of the parsimony analysis of the combined dataset (including the morphological partition and all scored fossil taxa) differ in the interrelationship of some fossil forms of stem cavioids (e.g., Eocarida spp., Schistomyx, Matamux) and in the alternative positions of the fragmentary crown caviid taxon Allocavia (see Document S1.doc). The reduced strict consensus tree of the MPTs pruning Allocavia resolves the interrelationships of the three major lineages of Caviidae: Caviinae, Dolichotinae, and Hydrochoerinae (Figs. 1–2). This tree is identical in terms of the interrelationships of the living lineages of Cavioida to the topology obtained in the Bayesian analysis of the molecular partition. Furthermore, if all fossils are excluded from the combined analysis, a single most parsimonious tree is found with identical topology as the one from
the Bayesian analysis. Almost all nodes of this topology have high values of support (Fig. 3). In the Bayesian analysis all nodes had posterior probabilities that varied between 0.80 and 1.0. Similarly, if only the extant taxa are included in the parsimony analysis, the resampling support values (bootstrap, jackknife) of most nodes within Caviidae range between 80% and 100%, except for Cavinae that is 60% (Fig. 3).

The calibration of the obtained phylogenetic hypotheses against time reveals the presence of three major phases in the evolutionary history of Cavioidea s.s. The first two of them are radiations exclusively revealed by fossil forms that are placed basally within Cavioidea s.s., prior to the origin of the crown-group Caviidae. These radiations are evident when the phylogenetic trees stemming from the parsimony analysis of the combined dataset are calibrated against geological time and characterize the early evolution of this group from the late Oligocene to the middle Miocene (Fig. 1). The third phase started in the middle to late Miocene and involves the diversification of the crown-group Caviidae (Figs. 1, 4). Given the presence of extant lineages of Caviidae, the timing of the diversification events of this third stage can be approached using both the fossil record of crown caviid and molecular clock estimates.

The evidence that leads to the recognition of these diversification events is summarized here in chronological order, discussing the timing inferred for these events and the evolutionary novelties that appeared at these times. The inferences made upon the fossil record evidence are presented first and the results of the molecular clock estimates are provided subsequently.

Diversification Patterns Inferred from Fossils and Ghost Lineages

**Phase 1: Radiation of basal Cavioidea s.s.** The most ancient records of Cavioidea s.s. come from late Oligocene beds of Patagonia (Deseadan SALMA [South American Land Mammal
Figure 2. Nodal support values of the parsimony combined phylogenetic analysis of morphological and molecular dataset using TNT. Three values are indicated for each node, the first of them is the Bremer support value, the second is the absolute frequency of each node in the bootstrap replicates and the third is the absolute frequency in the jackknife replicates (see Document S1 for further details).

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Two taxa are known from this age: *Asteromys punctus* [49,52,63] and *Chubutomys simpsoni* [52]. Both taxa are only known by fragmentary specimens composed by mandibular fragments. The relatively limited fossil record of Deseadan age does not reveal by itself the presence of a radiation during this time. However, when the phylogenetic hypotheses obtained in the parsimony analysis of combined dataset are calibrated against the geological age of fossil taxa, a basal cavioid radiation is revealed by the presence of four ghost lineages that must have originated in the Deseadan age (in addition to the two species recorded for this age; Fig. 1). These four ghost lineages are present in all the most parsimonious trees (MPTs) and extend the minimum age of origin of the lineages leading to *Luantus initialis*, *Luantus minor*, *Chubutomys leucoreios*, and the clade formed by *Luantus propheticus* and more derived cavioids back to the late Oligocene (Deseadan SALMA). All these forms appear later in the fossil record, in early Miocene beds referred to the Colhuehuapian SALMA (18–20.5 Ma; [61,62]). Therefore, the calibrated phylogenies extend the evolutionary origins of these lineages at least 4 Myr before their first appearance in the fossil record. The extent of these ghost lineages is caused by the relatively derived phylogenetic position of the Oligocene taxon *Chubutomys simpsoni* (and the paraphyly of the genus *Luantus*; Fig. 1). The character support for such a derived position of *Chubutomys simpsoni* is, however, moderately low (as it takes two extra steps to place it basally within Cavioida s.s. and bootstrap and jackknife frequencies of the two nodes basal to *Chubutomys simpsoni* are approximately 40% and 60%; Fig. 2; see Document S1).

The discovery of a basal cavioid radiation in the late Oligocene contrasts with previous interpretations about the early evolution of Cavioida s.s. [52,55,64]. These traditional hypotheses postulated that the early phases of cavioid evolution proceeded through gradual changes from the late Oligocene to the early Miocene, reflected in the progressive appearance of *Asteromys* and the species of *Luantus* in the fossil record. Our phylogenetic analysis rejects this interpretation, placing *Chubutomys simpsoni* deeply nested within basal cavioids given the presence of apomorphic features of its dentition (e.g., protohypsodont condition of the teeth and the absence of mesossetid in early ontogenetic stages). This implies a previously undetected radiation of all basal lineages of Cavioida s.s. that must have occurred at least by the late Oligocene. The optimization of character transformations in these basal nodes of Cavioida s.s. indicates that major evolutionary novelties must have appeared in this late Oligocene radiation, including changes from a mesodont to a protohypsodont dentition and other dental modifications such as the appearance of cement and enamel discontinuities and the loss of fossettes/ids during ontogeny.

The ghost lineages provide a minimum estimate for the age of these basal nodes of Cavioida s.s., so it is possible that the actual diversification of this group predated the Deseadan SALMA (24.5–29 Ma). The pre-Deseadan record of fossil rodents in South America however provides relevant information to evaluate this possibility. The youngest pre-Deseadan rodent assemblage is
known from La Cantera horizon of Central Patagonia (Upper Puesto Almendra Member, Sarmiento Formation; 29.5–31.1 Ma; [62]), which has yielded numerous rodent specimens but none belonging to Cavioidea s.s. (see also Node 2 in Calibrated Nodes). The La Cantera rodents include plesiomorphic taxa of Cavioidea (Dasyproctidae?) and representatives of Octodontoidae and Chinchilloidae [65]. Earlier faunal assemblages with fossil rodents from South America are limited to Tinguiririca in Chile (31.5–37 Ma; [29,61]) and possibly the Santa Rosa fauna of Peru (estimated as late Eocene or Oligocene; [66]). The fossil rodents from these localities are also plesiomorphic forms of Cavioidea. Furthermore, the recently discovered rodent fauna from the Yahuarango Formation (ca. 41 Ma; middle Eocene; [28]) has yielded the oldest known South American rodents, which are basal forms of Caviomorpha that are not closely related to Cavioidea. The absence of Cavioidea s.s. in all these pre-Deseadan rodent faunas is
compatible with the hypothesis of a basal radiation of this group close to or in the late Oligocene.

Phase 2: Radiation of euhypsodont cavioids. The euhypsodont condition represents the presence of teeth with continuous growth (lacking a root) and was one of the major evolutionary novelties in the history of Cavioida s.s. The most ancient records of euhypsodont cavioids come from the early Miocene Santa Cruz Formation of Central Patagonia (Santacrucian SALMA; 15.7–16.5 Ma; [61,67–69]). This unit has yielded thousands of specimens of cavioids, including the three youngest records of protohypsodont species (L. taldens, Phanomys mixtus, P. velatus) and the five oldest euhypsodont species of Cavioida s.s. (Eocavia montana, E. fissa, E. excavata, Schizonyx s.s., S. volans). The sudden appearance of multiple euhypsodont lineages at this time suggests the occurrence of an evolutionary radiation that not only includes the five above mentioned taxa but also three ghost lineages leading to slightly younger taxa of the Colloncuran (SALMA; 15.8–15.5 Ma; [70]). 1) Matiamys elegans lineages leading to slightly younger taxa of the Colloncuran includes the five above mentioned taxa but also three ghost lineages. The morphological data provide strong support for the Santacrucian radiation of euhypsodont cavioids. The node formed by Eocavia and more derived cavioids and the node formed by Phanomys and more derived cavioids (see Fig. 1) have bootstrap and jackknife frequencies between 82% and 85% (Fig. 2; see Document S1). The Bremer support for these nodes is only moderate (Bremer = 2) but forcing any of the presantacrucian cavioids (e.g., L. prophycticus, L. minor) within this clade results in markedly suboptimal topologies (i.e., at least ten extra steps).

The exceptional Santacrucian fossil record and the sudden appearance of a high diversity of euhypsodont cavioids can be interpreted in two alternative ways. On the one hand, the fossil record could be actually capturing the early offshoots of a major radiation, characterized by the acquisition of a key evolutionary innovation (euhypsodonty), which has been interpreted as an adaptation to grazing. The acquisition of the euhypsodont condition may have been a critical innovation at this time given the major environmental changes recorded in Patagonia, which includes high volcanic activity related to the uplift of the Andes (Quechua phase; [71]); a change from grasslands and woodlands biomes, and a general drop in humidity and temperature [72,73].

On the other hand, the exceptional Santacrucian fossil record could cause a Lagerstätten effect: the sudden first appearance of multiple lineages merely due to the presence of favorable conditions for fossilization. In this way, some lineages may have existed before the Santacrucian but were not captured by the fossil record just because the preservation potential of rodents in older sediments was unfavorable. A critical analysis of the cavioid fossil record in sediments older than the Santa Cruz Formation, however, lends support to the existence of a major radiation during the Santacrucian SALMA. The Pinturas Formation contains a sedimentary sequence that is slightly older than the Santa Cruz Formation, dated at 17.7 Ma [67]. The rodent fossil record in this unit is extremely rich, being the second-best record from the Oligocene-mid Miocene of Patagonia (after the Santacrucian). Out of the thousands of rodent specimens known from the Pinturas Formation, all the cavioid taxa are protohypsodont and there is no record of euhypsodont species in this large sample [55,74]. Based on the high quality of the rodent fossil record of the Pinturas Formation, we believe that the sudden appearance of multiple euhypsodont species at the Santa Cruz Formation represents a true evolutionary radiation. Furthermore, the numerous cladogenetic events inferred to occur during the Santacrucian in the calibrated phylogeny (Fig. 1) are restricted to a very specific part of the cavioid tree, rather than distributed in different parts of the phylogeny. As noted by Calvin and Forey [75], such scenarios are more likely to result from a genuine radiation event. If the sudden diversity increase were due to a Lagerstätten effect, there would be no expectation to find all the new lineages clustered in one region of the phylogenetic tree.

Phase 3: Diversification of Caviidae. The oldest caviid known is Prodocichotis pridiana [76] from the La Venta section of Colombia (La Victoria and Villa Vieja formations; Laventan SALMA, middle Miocene, 11.8–13.5 Ma; [77]). This taxon has been traditionally referred to either Dolichotini [76] or Caviinae [78] given the presence of derived similarities shared with these two lineages (e.g., presence of the nasolacrimal foramen in the maxilla). However, the morphological data used in the combined phylogenetic analysis presented here unequivocally places P. pridiana as the sister group of the third major lineage of caviids: Hydrochoeriinae (including Kerodon, Fig. 1). This position is supported by the presence of two unambiguous synapomorphies (i.e., p4 with three lobes [character 45] and frontals not convex [character 59]). This position is however weakly supported given that P. pridiana can be positioned as the sister group of dolichotines with a single extra step or basally within Caviidae with two extra steps. Similarly, bootstrap and jackknife frequencies of the node joining P. pridiana and Hydrochoeriinae range between 5% and 7% (Fig. 2; see Document S1).

Slightly older fossils recorded in deposits of Colloncuran age represent successive sister taxa of Caviidae (e.g., Guionys, Microcardiodon; see Fig. 1) so that based on the available information P. pridiana is the most ancient Caviidae. The phylogenetic position of P. pridiana implies that the diversification of Caviidae in its three major lineages must have occurred, at least, by Laventan times (11.8–13.5 Ma; [69,79]). After this time, the caviid fossil record has an extensive gap (of at least 2.7 Myr) until the late Miocene Arroyo Chasigués Formation of Central Argentina (Chasicoan SALMA; 6.1–9.07 Ma), in which derived specimens of the three modern lineages of caviids are recorded (i.e., Caviinae, Dolichotinae, Hydrochoeriinae; see Calibrated Nodes). The only caviid remain that fill the extensive gap between the Laventan and Chasicoan records is a single isolated tooth from the Río Frías Formation (Mayoan SALMA) that is possibly related to basal hydrochoeriines [80] (see Calibrated Nodes).

Therefore, the known fossil record lacks adequate information for thoroughly understanding the timing and radiation of modern lineages of crown caviids. The basic evolutionary pattern that can be inferred from the available fossil record is that: a) the initial split of Caviidae in three major lineages must have occurred at least 11.8–13.5 Ma (based on the Laventan record of P. pridiana and its phylogenetic position) and b) that the three modern and morphologically distinct lineages of Caviidae (Hydrochoeriinae, Dolichotinae, Caviinae) were already present, abundant, and diverse about 6.1–9.07 Ma (based on the derived caviid fauna of late Miocene Arroyo Chasigués Formation and the ghost lineages that can be inferred from their phylogenetic positions; see Fig. 1).

Molecular clock estimates

Understanding the tempo and mode of the diversification of Caviidae is especially interesting because this is the time when the three major living lineages acquired their distinctive morphologies, modes of life, body sizes, and social behaviors [35,40,41]. As noted above the fossil record lacks enough information to provide a complete picture of this critical time in the evolutionary history of
Caviid rodents. The DNA sequences of extant caviids can provide insights on the timing of these evolutionary events that can be compared with the pattern emerging from fossil-based datings.

Previous molecular clock estimates on the time of diversification of Caviidae resulted in disparate dates [13,20,56,59], ranging from slightly older (14 Ma) to markedly older (25 Ma) than the age of the oldest caviid (*P. pridiana*; 11.9–13.5 Ma). These estimates, however, were based on different DNA sequences and molecular clock methods so that it is difficult to compare their reliability and determine the causes of their differences. Given the multiple nodes that are paleontologically dated by the phylogenetic study presented here (Fig. 1), we tested the influence of the choice of calibration constraints (see Calibrated Nodes), as well as the use of alternative prior probability distributions for the age of these calibrations on relaxed molecular clock estimates (see Calibrated Nodes and Table 1). The results of the 30 analyses conducted using different calibration constraints indicate a moderate to high level of rate heterogeneity depending on the calibration constraints used. The four independent MCMC runs conducted for each of the 30 analyses yielded extremely similar results in terms of the parameters of interest: topology and ages of diversification of the nodes of Caviidae (Tables 2, 3) and the ESS of the parameters of interest (ages of Caviidae and major lineages of caviids) are well above 200, indicating strong convergence. The extent of the 95% HPD of node ages depends on the prior distribution and the calibration constraints used, but all of them indicate a considerable degree of uncertainty in the relaxed molecular clock (see Table 3).

The results of the analyses involving different number of calibration constraints and their prior probability distributions are summarized below for the time of diversification of Caviidae and the diversification of the major modern lineages of Caviidae (see also Tables 2 and 3).

### Four calibration constraints.

Owing to the incorporation of fossils in the phylogeny, these are the most tightly constrained analyses of the diversification timing of Caviidae (Fig. 4). The estimate of the time of diversification of Caviidae using gamma prior distribution yields a mean estimate of 18.8 Ma (leftmost blue circle in row 4 of Fig. 4; Table 2) and a 95% HPD ranging between 12.66 and 24.77 Ma (Table 3) in the four independent MCMC runs (ESS = 480). As in most analyses, when a normal prior distribution is used the estimates are slightly younger, yielding a mean age of 17 Ma (rightmost blue circle in row 4 of Fig. 4; see Table 2) and 95% HPD ranging from 10.75 to 24.43 Ma (Table 3). In both cases, the age of the oldest fossil caviid (*P. pridiana*; Laventan age; purple column in Fig. 4) is younger than the mean age estimate but falls within the 95% HPD (blue bar on tree of Fig. 4; Table 3).

The initial diversification of Caviinae and the split between Hydrochoerinae and Dolichotinae are inferred to occur two to five million years after the initial diversification of Caviidae, with mean gamma estimates of 16.37 and 14.42 Ma for these two clades and normal estimates of 14.08 and 12.30 Ma (Fig. 4). An interesting pattern in these estimates is the coincident time of diversification of three major modern and morphologically distinct lineages of Caviidae (Fig. 4): Dolichotinae, Hydrochoerinae (node 3), and the caviine lineage leading to the genus *Galea*. The age of one of these nodes was constrained by a prior distributions (node 3; see Calibrated Nodes) but the ages retrieved for the diversification of Dolichotinae (yellow circles in row 4 of Fig. 4) and *Galea* (red circles in row 4 of Fig. 4) are highly congruent, with mean ages of 6.91 and 6.79 (normal distribution of calibration constraints; see Table 2) and 8.25 and 7.96 (gamma distribution of calibration constraints; see Table 2). The estimated ages of these nodes lie within the range of ages of the Chasicoan sediments, in which the oldest members of caviines, dolichotines, and hydrochoerines are recorded.

### Three calibration constraints.

These analyses sequentially exclude one of the four calibration constraints (see Calibrated Nodes and analyses 3 to 10 in Table 1), to test their influence in inferring the time of diversification of Caviidae. The estimate of the initial diversification of Caviidae excluding node 1, node 2, or node 3 (central blue circles in row 3 of Fig. 4) yield similar results as the estimate with four calibration points (mean age ranging between 15.48 and 20.01 Ma and 10.05–26.64 Ma 95% HPD; see Tables 2, 3). However, when the caviine calibration constraint (node 4) is excluded, the retrieved age are markedly older (leftmost blue circles in row 3 of Fig. 4), and the age of the oldest fossil caviid (*Prodolichotis pridiana*) lies outside the 95% HPD (15.9–27.28 Ma; see Table 3).

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### Table 1. Parameters used to set the prior distribution of ages in each of the 30 analyses of Bayesian relaxed molecular clock in BEAST v. 1.6.

| Analysis Distribution | Node 1 | Node 2 | Node 3 | Node 4 |
|-----------------------|--------|--------|--------|--------|
| 1                     | Normal | 34.5, 1.8 | 26.8, 1.4 | 7.6, 0.9 | 4.65, 0.4 |
| 2                     | Gamma  | 2, 2.85, 3.15 | 2.5, 1.05, 2.45 | 1.5, 1.85, 6.1, 2, 0.76, 4 |
| 3                     | Normal | 34.5, 1.8 | 26.8, 1.4 | 7.6, 0.9 | TP |
| 4                     | Gamma  | 2, 2.85, 3.15 | 2.5, 1.05, 2.45 | 1.5, 1.85, 6.1, 2, 0.76, 4 |
| 5                     | Normal | 34.5, 1.8 | 26.8, 1.4 | TP | 4.65, 0.4 |
| 6                     | Gamma  | 2, 2.85, 3.15 | 2.5, 1.05, 2.45 | TP | 2, 0.76, 4 |
| 7                     | Normal | 34.5, 1.8 | TP | 7.6, 0.9 | 4.65, 0.4 |
| 8                     | Gamma  | 2, 2.85, 3.15 | TP | 1.5, 1.85, 6.1, 2, 0.76, 4 |
| 9                     | Normal | TP | 26.8, 1.4 | 7.6, 0.9 | 4.65, 0.4 |
| 10                    | Gamma  | TP | 2.5, 1.05, 2.45 | 1.5, 1.85, 6.1, 2, 0.76, 4 |
| 11                    | Normal | 34.5, 1.8 | 26.8, 1.4 | TP | TP |
| 12                    | Gamma  | 2, 2.85, 3.15 | 2.5, 1.05, 2.45 | TP | TP |
| 13                    | Normal | 34.5, 1.8 | TP | 7.6, 0.9 | TP |
| 14                    | Gamma  | 2, 2.85, 3.15 | TP | 1.5, 1.85, 6.1 | TP |
| 15                    | Normal | 34.5, 1.8 | TP | TP | 4.65, 0.4 |
| 16                    | Gamma  | 2, 2.85, 3.15 | TP | TP | 2, 0.76, 4 |
| 17                    | Normal | TP | 26.8, 1.4 | 7.6, 0.9 | TP |
| 18                    | Gamma  | TP | 2.5, 1.05, 2.45 | 1.5, 1.85, 6.1 | TP |
| 19                    | Normal | TP | 26.8, 1.4 | TP | 4.65, 0.4 |
| 20                    | Gamma  | TP | 2.5, 1.05, 2.45 | TP | 2, 0.76, 4 |
| 21                    | Normal | TP | TP | 7.6, 0.9 | 4.65, 0.4 |
| 22                    | Gamma  | TP | TP | 1.5, 1.85, 6.1 | 2, 0.76, 4 |
| 23                    | Normal | 34.5, 1.8 | TP | TP | TP |
| 24                    | Gamma  | 2, 2.85, 3.15 | TP | TP | TP |
| 25                    | Normal | TP | 26.8, 1.4 | TP | TP |
| 26                    | Gamma  | TP | 2.5, 1.05, 2.45 | TP | TP |
| 27                    | Normal | TP | TP | 7.6, 0.9 | TP |
| 28                    | Gamma  | TP | TP | 1.5, 1.85, 6.1 | TP |
| 29                    | Normal | TP | TP | TP | 4.65, 0.4 |
| 30                    | Gamma  | TP | TP | TP | 2, 0.76, 4 |

The parameters given for normal distributions are the mean and standard deviation, and the parameters given for the gamma distributions are the shape, scale, and offset. Uncalibrated nodes are marked with TP, representing the tree priors used in each analysis for the age of uncalibrated nodes. DOI: 10.1371/journal.pone.0048380.t001
As with the estimates of the diversification of Caviidae, the estimates on the time of diversification of the modern lineages of Caviidae are highly sensitive to the exclusion of node 4 from the calibration set. Excluding any of the other three calibration constraints (nodes 1, 2, or 3) the mean ages of these nodes fall within the Chasicoan SALMA (see Table 2). However, when node 4 is excluded from the calibration set, the estimated age of these nodes is older. This is especially marked when a gamma distribution is used, yielding mean age estimates that predate the Chasicoan age (leftmost red and yellow circles in row 3 of Fig. 4; Table 2). Interestingly, the mean ages inferred for node 4 (Microcavia + Cavia) when this node is not calibrated is remarkably old, with mean ages of 14.78 and 17.45 Ma and 95% HPD (Table 3) lying outside the Chasicoan (significantly predating the appearance of derived caviines in the fossil record) This suggests that either this caviine lineage has a higher evolutionary rate than the other caviids or that its fossil record failed to capture more than 60% of their evolutionary history. These two hypotheses are plausible explanations but more data and research is needed for testing them.

Two calibration constraints. These analyses retrieved disparate results on the time of diversification of Caviidae (see analyses 11 through 22 in Table 1). Out of the 12 conducted analyses, six of them yielded 95% HPD age estimates for the initial diversification of Caviidae that are older than the first fossil caviid and one of them yielded 95% HPD age estimates that are younger than the first fossil caviid (see Table 3). The six analyses that excluded the caviine calibration constraint (node 4 in Fig. 4; see Calibrated Nodes) yielded the oldest age estimates for the initial diversification of caviids (Table 2). The inclusion of the caviine calibration point (node 4) in combination with the calibration of the basal nodes of Cavioidea (node 1 or node 2) yielded mean age estimates (central blue circles in row 2 of Fig. 4) that are similar to those of the analysis with four constrained nodes (Table 2). The 95% HPD of these analyses included the age of appearance of fossil caviids (Table 3). However, when the two calibration constraints were node 4 and node 3, the estimated mean age and 95% HPD resulted exceedingly young (Table 3) in comparison with the caviid fossil record (mean age estimates ranging between Table 2.

| Analysis | Node 1   | Node 2   | Node 3   | Node 4   | Caviidae | Galea   | Dolichotinae | Caviomorpha |
|----------|----------|----------|----------|----------|----------|---------|--------------|-------------|
| 1        | 32.827   | 27.149   | 7.653    | 4.827    | 17.002   | 6.787   | 6.912        | 38.914      |
| 2        | 33.189   | 27.629   | 9.118    | 7.774    | 18.790   | 7.961   | 8.254        | 39.706      |
| 3        | 31.904   | 27.576   | 8.487    | 14.778   | 20.527   | 9.238   | 8.936        | 38.800      |
| 4        | 32.953   | 29.486   | 13.175   | 17.455   | 23.228   | 10.732  | 10.958       | 41.748      |
| 5        | 32.856   | 27.236   | 9.376    | 4.825    | 18.888   | 7.252   | 7.959        | 38.688      |
| 6        | 33.155   | 27.871   | 11.485   | 8.646    | 20.069   | 8.677   | 9.267        | 40.226      |
| 7        | 33.417   | 29.163   | 7.663    | 4.830    | 17.121   | 7.055   | 6.946        | 39.429      |
| 8        | 33.775   | 30.416   | 9.206    | 7.706    | 19.300   | 8.211   | 8.391        | 40.801      |
| 9        | 27.637   | 25.906   | 7.646    | 4.858    | 15.479   | 6.474   | 6.604        | 33.406      |
| 10       | 27.534   | 25.886   | 9.481    | 9.379    | 17.656   | 7.807   | 8.078        | 34.000      |
| 11       | 31.740   | 28.395   | 14.162   | 17.221   | 22.803   | 10.545  | 11.008       | 40.187      |
| 12       | 33.116   | 30.100   | 15.022   | 18.288   | 24.151   | 11.202  | 11.685       | 42.566      |
| 13       | 33.230   | 30.216   | 8.485    | 15.300   | 21.487   | 9.675   | 9.186        | 40.193      |
| 14       | 34.215   | 31.946   | 14.166   | 18.822   | 24.974   | 11.645  | 11.734       | 44.091      |
| 15       | 33.578   | 29.653   | 9.520    | 4.838    | 18.783   | 7.524   | 7.681        | 39.783      |
| 16       | 33.881   | 30.835   | 12.258   | 8.764    | 21.257   | 9.008   | 9.847        | 41.472      |
| 17       | 27.213   | 25.662   | 8.633    | 13.857   | 18.793   | 8.587   | 8.401        | 33.932      |
| 18       | 27.898   | 26.227   | 11.967   | 15.650   | 20.645   | 9.553   | 9.780        | 36.340      |
| 19       | 27.818   | 26.030   | 8.637    | 4.856    | 16.687   | 6.569   | 6.996        | 33.792      |
| 20       | 27.645   | 25.964   | 11.181   | 10.186   | 18.682   | 8.380   | 8.784        | 34.455      |
| 21       | 10.138   | 9.486    | 5.381    | 4.984    | 7.535    | 3.396   | 3.745        | 13.104      |
| 22       | 13.422   | 12.576   | 6.691    | 7.207    | 9.992    | 4.619   | 4.971        | 17.574      |
| 23       | 34.202   | 32.086   | 15.801   | 19.353   | 25.491   | 11.819  | 12.256       | 44.411      |
| 24       | 35.371   | 33.234   | 16.539   | 20.225   | 26.512   | 12.370  | 12.845       | 46.475      |
| 25       | 28.198   | 26.585   | 13.061   | 16.028   | 21.110   | 9.794   | 10.165       | 36.668      |
| 26       | 28.292   | 26.549   | 13.118   | 16.088   | 21.171   | 9.817   | 10.187       | 36.971      |
| 27       | 15.299   | 14.390   | 7.262    | 8.667    | 11.482   | 5.278   | 5.519        | 19.984      |
| 28       | 15.889   | 14.897   | 7.393    | 8.956    | 11.860   | 5.526   | 5.743        | 20.881      |
| 29       | 7.885    | 7.412    | 3.657    | 4.545    | 5.926    | 2.733   | 2.828        | 10.279      |
| 30       | 8.605    | 8.094    | 3.957    | 4.884    | 6.428    | 3.007   | 3.074        | 11.255      |

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and red circles in row 2 of Fig. 4; Table 2). The use of only the two mean ages that fall within the Chasicoan SALMA (central yellow calibration constraints (nodes 1 or 2) the estimates on the

noticeable when a gamma distribution is used in the prior

yellow circles in row 2 of Fig. 4; Table 2). Again, this is particularly

the inferred ages of several nodes are markedly older, with most

caviidae mentioned above (Fig. 4). As for the estimates on the age

Caviidae

Table 3. 95% HPD for the age estimates (in Ma) for the age of nodes of interest based on the integration of the four independent MCMC runs made for each of the 30 different Bayesian relaxed molecular clock analyses.

| Analysis Node 1 | Node 2 | Node 3 | Node 4 | Caviidae | Galea | Dolichotinae | Caviomorpha |
|-----------------|--------|--------|--------|----------|-------|-------------|-------------|
| 1               | 29.180 | 26.444 | 24.459 | 29.9     | 5.83  | 9.398       | 4.06  5.615 |
| 2               | 31.546 | 36.337 | 28.497 | 31.332   | 6.133 | 13.268      | 4.167 12.85 |
| 3               | 28.695 | 35.426 | 25.068 | 29.996   | 6.706 | 10.278      | 9.206 19.887|
| 4               | 31.539 | 35.671 | 25.911 | 32.756   | 7.796 | 17.106      | 11.445 22.279|
| 5               | 29.31  | 36.378 | 24.589 | 29.879   | 2.62  | 17.211      | 4.029 5.609 |
| 6               | 31.539 | 36.631 | 24.911 | 31.461   | 4.74  | 17.554      | 4.243 14.526|
| 7               | 29.775 | 36.991 | 20.128 | 35.249   | 5.987 | 9.441       | 4.071 6.565 |
| 8               | 31.529 | 38.647 | 22.373 | 36.7     | 6.129 | 13.499      | 4.244 13.056|
| 9               | 23.561 | 33.413 | 23.091 | 28.787   | 5.974 | 9.404       | 4.086 5.652 |
| 10              | 25.015 | 31.572 | 24.588 | 28.2     | 6.19  | 12.983      | 4.587 14.333|
| 11              | 28.846 | 34.762 | 26.048 | 30.781   | 9.678 | 18.495      | 12.478 21.399|
| 12              | 31.556 | 35.979 | 26.411 | 33.527   | 10.479| 19.463      | 15.039 22.907|
| 13              | 29.572 | 32.924 | 24.795 | 35.508   | 6.53  | 13.068      | 4.818 23.624|
| 14              | 31.544 | 39.448 | 27.418 | 38.309   | 7.25  | 18.451      | 12.03 25.183|
| 15              | 30.007 | 37.257 | 21.362 | 35.832   | 2.262 | 17.345      | 4.064 25.626|
| 16              | 31.571 | 38.821 | 23.922 | 37.678   | 5.168 | 19.098      | 4.193 15.32 |
| 17              | 23.372 | 31.437 | 22.822 | 28.516   | 6.757 | 10.389      | 8.642 18.701|
| 18              | 25.089 | 32.188 | 24.644 | 29.021   | 7.469 | 15.241      | 10.539 20.285|
| 19              | 23.561 | 33.545 | 23.211 | 28.806   | 2.684 | 15.915      | 4.061 35.632|
| 20              | 25.068 | 31.815 | 24.578 | 28.347   | 4.187 | 16.294      | 4.464 15.007|
| 21              | 7.194  | 14.999 | 6.739  | 13.7     | 3.897 | 7.325       | 4.242 5.742 |
| 22              | 10.143 | 18.069 | 7.924  | 16.439   | 6.102 | 8.079       | 5.121 9.454 |
| 23              | 30.657 | 37.779 | 27.712 | 36.648   | 10.866| 20.686      | 14.169 24.431|
| 24              | 31.588 | 42.768 | 27.858 | 42.184   | 10.94 | 23.897      | 13.961 28.007|
| 25              | 24.731 | 32.124 | 23.836 | 29.301   | 9.216 | 17.322      | 11.644 20.078|
| 26              | 25.372 | 32.619 | 24.584 | 29.716   | 2.635 | 17.233      | 10.55 21.112|
| 27              | 9.935  | 21.803 | 9.269  | 20.207   | 5.515 | 9.121       | 5.291 12.658|
| 28              | 10.781 | 25.699 | 10.215 | 23.521   | 6.1  | 10.682      | 5.426 15.611|
| 29              | 5.772  | 10.499 | 5.486  | 9.784    | 2.438 | 5.191       | 3.725 5.318 |
| 30              | 6.147  | 12.885 | 5.815  | 11.973   | 2.474 | 6.438       | 4.025 6.644 |

The lower and upper bound of the 95% HPD are given for each analysis separated by a semicolon.
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7.53 and 9.99 Ma, using a normal and gamma distribution; rightmost blue circles in row 2 of Fig. 4; Table 2). The conducted analyses also retrieved highly variable results on the time of diversification of the major modern lineages of Caviidae mentioned above (Fig. 4). For the estimates on the age of Caviidae, when the cavein calibration (node 4) was excluded, the inferred ages of several nodes are markedly older, with most mean estimates of most nodes dating over 9.5 Ma (leftmost red and yellow circles in row 2 of Fig. 4; Table 2). Again, this is particularly noticeable when a gamma distribution is used in the prior probability of the calibration constraints. When node 4 is included in the calibration set in combination with the basal most calibration constraints (nodes 1 or 2) the estimates on the diversification ages of the major lineages of Caviidae retrieved mean ages that fall within the Chasicoan SALMA (central yellow and red circles in row 2 of Fig. 4; Table 2). The use of only the two most recent constraints (nodes 3 and 4) yields mean ages that are much younger than the Chasicoan age (rightmost yellow and red circles in row 2 of Fig. 4; see Table 2). In most of these estimates, however, the 95% HPD is notably large and extends for more than 10 Myr for both normal and gamma prior distributions; see Table 3) covering from the Pleistocene to the Miocene (thus completely including the Chasicoan age).

One calibration constraint. These analyses also retrieved disparate results on the timing of caviid evolution (analyses 23 through 30 in Table 1). All analyses that used the calibration of the two basal nodes of Cavioidea (node 1 and node 2; see Calibrated Nodes) retrieved remarkably old mean ages (leftmost blue circles in row 1 of Fig. 4; Table 2) with 95% HPD that are older than the first appearance of caviids in the fossil record (Table 3). Conversely, when node 4 is used as the single calibration point, the 95% HPD for the time of the initial diversification of caviids is younger than the first appearance of this group in the fossil record (rightmost blue circles in row 1 of Fig. 4; see Table 3). Finally, node 3 is the only calibration point that retrieves mean ages close to the age of the most ancient caviid P. frioida (central blue circles in row 1 of Fig. 4; Table 2) and 95% HPD that includes this age (Table 3).
The estimates on the time of diversification of the major modern lineages of Caviidae are also highly sensitive to the choice of a single calibration constraint. When any of the two basal calibrations were used (node 1 and node 2), the mean ages of the Dolichotinae and Galea nodes are notably old (ranging from 9.79 to 12.84 Ma with both normal and gamma prior distributions; four leftmost red and yellow circles in row 1 of Fig. 4; Table 2). Furthermore, using these calibrations, the 95% HPD of the age of two other modern lineages of caviids (nodes 3 and 4) are in most cases exceedingly old (i.e., older than the Chasicoan SALMA; Table 3). Conversely, when the caviine age (node 4) is used as the only calibration constraint, the mean ages and the 95% HPD for the time of diversification of modern caviid lineages are younger than their first appearance in the fossil record at the Chasicoan SALMA (rightmost yellow and red circles in row 1 of Fig. 4; Table 3). Finally, as in the case of the age of Caviidae, when node 3 is the only calibration constraint the mean ages lie close to the upper bound of the Chasicoan age (5.3 to 5.7 Ma; see Fig. 4 and Table 2) and the 95% HPD of all the modern lineages includes the Chasicoan age (Table 3).

Summary of molecular clock estimates. These results indicate a considerable degree of rate heterogeneity among groups of Cavioida. The two oldest and most basal calibration constraints (node 1 and node 2; see Calibrated Nodes) lead to inferences of a much slower rate of evolution than when the caviine calibration constraint (node 4) is used, and rate estimates derived from calibrations of node 3 are intermediate between these two extremes. Consequently, estimates on the age of Caviidae and on the radiation of the major modern lineages of caviids based only on the basal calibrations (or basal nodes and node 3) are much older than those retrieved when node 4 (or node 4 and node 3) is used for calibrating the relaxed molecular clock (Fig. 4). This sensitivity to the choice of calibration constraint is also reflected in the 95% HPD that show variable results depending on the calibrate node/s. These distributions, however, converge to a similar result as the number of calibrated nodes increases, as exemplified for the 95% HPD of the age of Caviidae (Fig. 5). Based on these results, the use of four calibration points better accommodates the rate heterogeneity of the group as a whole, because these bracket the nodes of interest (Caviidae and the principal modern lineages of Caviidae). Therefore, we will discuss below the molecular clock inferences based on four calibration points (row 4 of Fig. 4) in terms of their agreement with the diversification pattern inferred from the fossil record of early caviids.

Discussion

The integration of the morphological and molecular data in the phylogenetic analyses conducted here and the temporal information from the fossil record and molecular clock estimates reveal the basic diversification patterns during the early evolution of Cavioida s.s and the crown-group Caviidae. The cumulative number of lineages (counting those leading to both extinct and living taxa) plotted across time reveals the diversification events of this group inferred from fossils and the molecular clock (Fig. 6). This plot contrasts the timing of each diversification event inferred from the age of first appearance of fossil taxa (red curve in Fig. 6) and from the molecular clock estimates (blue curve in Fig. 6). This diversification plot highlights the agreement and discrepancies of the results presented in this study.

Diversification of Cavioida s.s

The morphological data of extinct species and their stratigraphic record document two early diversification phases: the Deseadan and the Santacrucian radiations (1 and 2 in Fig. 6). These radiations can only be inferred from the fossil record, as the only lineage that survived the Miocene was the one leading to Caviidae.

As noted above, the Deseadan radiation (1 in Fig. 6) involves the first record of Cavioida s.s. (24.5–29 Ma; late Oligocene; Deseadan SALMA) and is recognized mainly by the four ghost lineages of forms that appear later in the fossil record (early Miocene; Colhuehuapian SALMA). Only two species are known up to date from this age, which provide the minimum estimate for the age of basal nodes of Cavioida s.s. Although a more gradual diversification of this group might have occurred before the Deseadan SALMA, the older rodent assemblages at Tinguiririca and La Cantera lack members of this clade. An initial radiation during the late Oligocene fits the available fossil data and the morphological phylogeny.

The Santacrucian radiation (2 in Fig. 6) is the earliest record of euhypsodont cavioids (15.7–16.5 Ma; early Miocene; Santacrucian SALMA). It is mainly recognizable from the Patagonian fossils of a
remarkably abundant and diverse rodent fauna. Fossils offer direct evidence of multiple euhypsodont lineages present at this time, unlike the Deseadan event, which was mainly inferred by the evidence for ghost lineages. However, as in that case, stratigraphic evidence—the lack of euhypsodont taxa in the well-sampled rodent faunas of older sediments of Patagonia (e.g., “Pinturan” or Colhuehuapian ages)—identifies this as a discrete evolutionary event rather than an artificial aggregation of diversity owing to taphonomic processes (e.g., Lagerstatte effect).

**Diversification of Caviidae**

The diversification of the crown-group Caviidae (phase 3 in Fig. 6) involved the appearance of lineages that survived until today. Both the fossil record (red curve in Fig. 6) and inferences from the molecular clock (blue curve in Fig. 6) help to illuminate this event. Three distinct periods are identifiable: the initial split of Caviidae in three major lineages (3a in Fig. 6), followed by an obscure period with poor fossil record (Mayoan gap; Fig. 6), and then by the diversification and establishment of the morphologically and ecologically distinct modern lineages of Caviidae (3b in Fig. 6). Whereas the molecular clock estimates differ from the information provided by the fossil record regarding the timing of the initial split of Caviidae (3a), both sources of information agree on the timing and characteristics of the two subsequent periods (Mayoan gap and 3b; see Fig. 6).

**Initial split of Caviidae**

This event involved the split between the caviine, dolichotine, and hydrochoerine lineages. The minimum age of this event is dated stratigraphically by the appearance of *Prodolichotis pridiana* (11.8–13.5 Ma; middle Miocene; Laventan SALMA), as shown by the diversification plot (3a in red curve of Fig. 6). Molecular clock inferences, however, yielded a mean age estimate that predates this age by 3.5 to 7 million years (17 and 18.8 Ma using normal and gamma priors, respectively), represented by the shifted position of this event in the diversification curve of molecular clock dates (3a in blue curve of Fig. 6). The black arrow marks the discrepancy between the timing of the initial diversification of Caviidae based on the paleontological record (red curve) and the molecular clock estimates (blue curve). doi:10.1371/journal.pone.0048380.g006

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Figure 6. Diversification plot of cumulative number of cavioid lineages (leading to both extinct and extant species) against geological time. The red curve represents the number of lineages based on the simultaneous analysis of morphological and molecular data of both extinct and extant taxa (as shown in Fig. 4). The uncertainty in the geological age of each fossil creates a maximum and minimum times of divergence and is represented by the breadth of the diversification events on the red curve. The blue line represents the timing of diversification events of crown caviid lineages based on mean age estimates of the molecular clock estimates. The most relevant SALMAs are highlighted and the numbers 1, 2, 3a, and 3b represents the Deseadan radiation (1), the Santacrucian radiation (2), the initial diversification of Caviidae (3a), and the diversification of modern and morphologically well differentiated lineages of caviids during the Chasicoan (3b). The black arrow marks the discrepancy between the timing of the initial diversification of Caviidae based on the paleontological record (red curve) and the molecular clock estimates (blue curve). doi:10.1371/journal.pone.0048380.g006
The molecular clock estimates, therefore, indicate there is a high probability that the fossil record is currently missing the first few million years of caviid evolution, although the breadth of the 95% HPD and the possible alternative positions in slightly suboptimal trees of some key fossils indicates the two sources of information are not yielding entirely incompatible estimates. Further studies on some of these fossils (e.g., *Prodolichotis pridiana*) and new remains of incompletely known taxa (e.g., *Guianomys unica*), as well as new sequences to base molecular clock estimates on a more extensive dataset are all necessary steps to solve this apparent discrepancy (see also *Reconciling molecular dating with the fossil record*).

Mayoan Gap

After the Laventan age, the caviid fossil record has an extensive gap that stretches over the Mayoan SALMA (Fig. 1) and extends until the late Miocene. During the late Miocene (Chasicoan-Huayquerian SALMAs; see Calibrated Nodes) the oldest and already well differentiated members of the three modern clades of Caviidae (Caviinae, Dolichotinae, Hydrochoerinae) appear in the fossil record. Only two isolated cavioid teeth are known from the Mayoan SALMA (Rio Frias Formation) and they have been recently interpreted [80] either as a close relative of the crown-Mayoan SALMA (Río Frias Formation) and they have been already well differentiated members of the three modern clades of Caviidae until the late Miocene. During the late Miocene (Chasicoan-gap that stretches over the Mayoan SALMA (Fig. 1) and extends further studies on some of these fossils (e.g., Prodolichotis pridiana) and new remains of incompletely known taxa (e.g., Guianomys unica), as well as new sequences to base molecular clock estimates on a more extensive dataset are all necessary steps to solve this apparent discrepancy (see also *Reconciling molecular dating with the fossil record*).

Diversification of modern caviid lineages

As noted above, the Arroyo Chasico Formation records multiple lineages of derived caviids [53], which represent the earliest record of the clearly distinct body plans, body size ranges, apomorphic morphological traits, and probably the ecological roles of modern lineages of Caviidae (e.g., Dolichotinae, Hydrochoerinae, Caviinae). The Chasicoan radiation has been recognized on the basis of the diverse fossil fauna recorded in central Argentina [53,81], a pattern that is corroborated here based on the combined phylogenetic analysis and the calibration of these events stratigraphically (Fig. 1) as well as the molecular clock estimates (Fig. 4). The radiation of modern and well differentiated caviid lineages (3b in Fig. 6) is inferred to occur during the Chasicoan SALMA both by the paleontological dates and by the mean age estimates using a relaxed molecular clock. The timing of this event in the cumulative diversity curve based on fossil dates (3b of red curve in Fig. 6) largely coincides with the cumulative diversity curve inferred from molecular clock estimates (3b of blue curve in Fig. 6). The sudden appearance of multiple lineages with markedly different morphological and ecological characteristics suggests the Chasicoan was indeed a key stage in caviid evolution, which resulted in the establishment of the distinct body plans of extant forms through an adaptive radiation.

Reconciling molecular dating with the fossil record

Discrepancies of paleontological and molecular dates are relatively common and often, molecular clock estimates yield ages that are much older than the first appearance of taxa in the fossil record. This has led to heated debates on the relative merits of both approaches [1,3,5,82,83]. Such differences can be attributed to deficiencies of both the methods of molecular clock estimation and/or to the quality of the fossil record.

Molecular clock: potential biases

Several authors have noted molecular clock methods can suffer from biases that produce exceedingly old divergence dates [5,7,84–86]. These include the presence of extreme rate heterogeneity that cannot be accounted by current methods (e.g., gamma distribution, invariant sites), such as the possible inadequacy use of homogeneous Markov models of nucleotide evolution due to the existence of heterotachy [87–89], which would affect branch length estimation and therefore molecular clock estimates. Although the use of relaxed clocks has been proposed as a way to at least ameliorate some of these problems [60] and current methods provide molecular clock estimates with credibility intervals, the extent of the above noted problems in empirical datasets is difficult to assess.

It is worth noting that the credibility intervals of relaxed Bayesian molecular clock estimates (95% HPD) can be of substantial duration, and many times they do overlap with the first appearance datum of fossil taxa, as in the case of the initial diversification of Caviidae and the Laventan age. In many instances, therefore, the discrepancies between the molecular clock and the fossil record disappear when then 95% HPD are considered.

One of the interesting outcomes of the 30 different molecular clock analyses conducted here is the identification of the sensitivity of the results to the inclusion or exclusion of node 4 (Microcavia+Cavia). A striking difference exists between the age inferred by the molecular clock when this node is not calibrated and the first appearance of members of this clade in the fossil record. As noted above, the molecular clock estimates are likely too old and suggest the fossil record of caviine rodents would be missing 60% of its evolution. We suggest it is more likely this caviine lineage has a higher evolutionary rate in comparison with other cavioid rodents (at least for these genes). Caviines have a reduced body size (and related life-history traits such as shorter generation times) in comparison with other cavioid rodents (e.g., dolichotines, hydrochoerines), providing another case of correlation between high evolutionary rates and small body size if this hypothesis is correct. More data are needed to test this correlation, as well as to provide reliable molecular clock estimates for Caviinae. New data must include both a more extensive taxon sampling among caviines for these sequences and further studies on fossil caviines to provide alternative calibration points within this clade.

The fossil record: potential biases

The fossil record is inherently incomplete and can certainly fail to capture representatives of two lineages soon after they split from their most recent common ancestor,-underestimating the age of a clade’s initial divergence [90]. Most importantly, in addition to being incomplete, the fossil record also has well-recognized biases that can systematically fail to capture the earliest members of the lineage of interest (selective incompleteness). The most obvious biases of the fossil record that explain the poor record of some lineages are those related to intrinsic biological causes (e.g., low fossilization potential of their body parts, small body size) or related to extrinsic causes due to the incompleteness of the
sedimentary record. The latter include the absence/rarity of sedimentary rocks from a given period of time (i.e. non-depositional hiatuses), from the geographic region where the lineage might have originated (i.e. biogeographical biases), and/or from the ecosystem where the organisms lived (i.e. environments with low sedimentary rates). It is therefore critical to consider these possible biases when divergence dates from the fossil record are being compared with molecular clock estimates.

The case of the diversification of Caviidae allows exploring some of these potential biases of the fossil record, as their record is remarkably good in comparison with other terrestrial vertebrates from the Cenozoic of South America. The molecular clock mean age estimates on the initial diversification of Caviidae is up to seven million years older than the date documented by fossils, placing the basal split of Caviidae at the “Pinturan”/Santacrucian boundary (3a of blue curve in Fig. 6). The fossil record of cavioid rodents during these ages is remarkably abundant, including hundreds of cavioid specimens representing at least ten different species. This high diversity and abundance of cavioid actually represents the basal euhyopsodont radiation (2 in Fig. 6) and none of these fossils can be identified as members of the crown-group Caviidae.

The well sampled “Pinturan”/Santacrucian cavioid fauna could be used as evidence rejecting the molecular clock estimates on the time of diversification of Caviidae. However, the adequacy of the cavioid fossil record to reject the molecular dates should be evaluated considering its completeness not only through time but also through space. Mapping the geographic distribution and the number of fossil species of rodents during the Oligocene-Pleistocene in South America reveals an uneven geographic coverage of the fossil record in this continent (Fig. 7). The highly fossiliferous “Pinturan”/Santacrucian deposits are restricted to southern South America (Patagonia; southernmost red/yellow areas in Fig. 7) and therefore the high degree of certainty that crown caviids were absent from Patagonia during “Pinturan”/Santacrucian may not be valid for all of South America. Interestingly, the oldest fossil caviid (P. pridiana) comes from northern South America (Colombia; grey arrow in Fig. 7), a region completely lacking a pre-Laventan rodent fossil record. If the crown-group Caviidae actually originated at low latitudes in South America about 18 Ma, the available fossil record could not reflect this evolutionary event, as all the fossiliferous rocks from this age are restricted to the southern South America (Patagonia; see Fig. 7).

This biogeographic bias actually occurs for the entire rodent fossil record of the Cenozoic of South America, which despite being remarkably abundant is heavily biased towards fossils found at higher latitudes (Fig. 7). This geographic bias of the terrestrial fossil record of the Cenozoic of South America is extensive, at least to some degree, to all groups of fossil mammals and therefore should be considered when studying the evolutionary events of multiple fossil groups. Our current knowledge on the diversity of rodent fossils is remarkably good in mid- to high latitudes of South America. Numerous species are known from highly fossiliferous and extensive outcrops located in the southern half of South America (especially in Patagonia). These geological units have been known for over a century and intensive collecting efforts have been conducted on fossil mammals since the initial discovery of these outcrops [91]. In contrast, the rock availability and exposures in the Neotropics is much more restricted and collecting efforts conducted at higher latitudes of South America [92–96] have not been as intensive as in Patagonia.

As stated above, an incomplete fossil record is caused not only by non-depositional hiatuses but also involves the incomplete representation of the geographical distribution of a clade. We have no doubts that the fossil record can and should be used to refine and clarify the chronology of diversification events. However, its limitations and biases need to be borne in mind, particularly when we have to evaluate its conflict with other methodologies, such as a molecular clock. Ignoring the limitations and uncertainties of the fossil record could be as dangerous as ignoring the uncertainties associated with molecular clock estimates (e.g., 95% HPD of relaxed Bayesian clock estimates).

Conclusions

Estimating the divergence time of clades based on phylogenetic studies is the area of most intense interaction (and conflict) between paleontology (providing fossils with dates) and molecular systematics (providing molecular clock estimates), as both areas provide information for understanding the tempo and mode of the evolution of a group. Recent efforts and progress have been made to incorporate different kinds of uncertainties to both molecular clock methods [60] and paleontological dating of divergence times.
Six crown caviid taxa (four fossils, two extant) and ten new species were gathered from a recent study through the incorporation of new sources of evidence into an integrated approach. Combined Parsimony Analysis (morphology + DNA) and molecular data do not match. Our study integrates morphological and molecular data gathered from extant and extinct taxa into a phylogenetic analysis of Cavioida s.s. These analyses result in a global picture on the evolutionary history of Cavioida s.s., including the origins and diversification of Caviidae, one of the most remarkably disparate lineages of living rodents. Three major evolutionary phases are recognized in the history of the group. The first two were radiations of basal forms that acquired the dental hallmarks of the groups: the appearance of protohypsodont forms in the Deseadan radiation of Cavioida s.s. and of euhyopsodont cavioids in the Santacrucian radiation, the evidence coming mostly from the Patagonian fossil record. The third phase was the diversification of Caviidae, which seems to have occurred in two temporally discrete episodes, the initial split of the group in three major lineages and the subsequent diversification of its modern clades, which are highly differentiated morphologically and ecologically.

A general agreement exists on the divergence dates estimated from molecular data and the fossil record. Molecular clock estimates place the origin of most modern lineages of caviids close to the Chasicoan, which coincides with the earliest appearance in the fossil record of the modern caviid lineages, which are characterized by remarkably distinct body plans, body size ranges, and ecological roles (dolichotines, hydrochoerines, and caviines).

However, the timing of the initial diversification of Caviidae was detected as the major discrepancy. The initial split of Caviidae is inferred to occur at Laventan times by paleontological evidence or perhaps as much as 7 million years earlier using a relaxed molecular clock. However, the uncertainties of the paleontological and molecular estimates reveal that more data is needed to solve this apparent conflict between the fossil record and the molecular clock.

From a paleontological point of view, a more extensive knowledge of pre-Laventan faunas are critical to clarify the time and place of the initial diversification of Caviidae. Although the record of pre-Laventan faunas (i.e., Fraisian, Colloncuran) is geographically extensive, rodent faunas from these ages are all restricted to the southern half of South America (Chile, Argentina, and Bolivia), limiting our ability to localize the group’s origin.

Finally, increasing the amount of molecular data (taxon and gene sampling) is also needed to achieve a more robust phylogenetic framework for caviid evolution and to generate more robust molecular clock inferences. The prospective integration of new sources of evidence into an integrated approach will be unavoidable steps for understanding the evolutionary history of Cavioida s.s.

**Methods**

**Combined Parsimony Analysis (morphology + DNA)**

**Morphological data.** The morphological dataset was expanded from a recent study through the incorporation of six crown caviid taxa (four fossils, two extant) and ten new morphological characters. The morphological dataset included fossil and living representatives of Cavioida s.s. that were selected as the ingroup. The dataset include all the known stem-group fossil taxa (“eocardiids”), at least one representative of each extant genus of Caviidae, and nine extinct species of caviids (see Document S2). Outgroup taxa included representative species of Dasyproctidae, Cuniculidae, and Echimyidae, the latter of which was used to root the topologies (see Document S2). The character sampling is based on 69 cranio-mandibular and 27 dental characters (see Document S3).

**Molecular data.** The DNA sequences of extant caviid species were gathered from GenBank for two nuclear and two mitochondrial genes: exon 10 of growth hormone receptor (Ghr), intron 1 of transfhyretin (Tth), 12 subunit ribosomal RNA (12s), and cytosome b (cyb). Sequences of these genes were available for nine extant representatives of Caviidae and the three outgroup taxa (see Document S2 for GenBank accession numbers). Sequences of two of the outgroup taxa (Proechimys and Dasyprocta) have been assembled from two different species of each genus (see Document S2). These sequences have been successfully used by previous authors to resolve relationships of cavimorph and/or cavioid rodents [13,18,26,40] and were therefore thought to provide adequate levels of divergence for conducting molecular clock estimates.

**Combined dataset.** The phylogenetic dataset consisting of the 96 morphological characters and the 4014 characters of the four genes (Ghr, Tth, 12s, cyb) is available as Dataset S1 and also at DataDryad http://datadryad.org/ (doi:10.5061/dryad.v5p71).

**Data analysis.** The DNA sequences of each of the four genes (12s, cyb, Ghr, and Tth) were compiled from several previous analyses (see Document S2), and were aligned using CLUSTAL X [99] using the default values of gap opening (10/100) and gap extension (0.1). The leading and trailing gaps were replaced with missing entries. After alignment, the leading and trailing ends of the sequences with no homologous sequences in other species were deleted. The alignment resulted in 961 base pairs (bp) for 12s gene, 1140 bp for cyb gene, 1099 bp for Tth gene, and 814 bp for Ghr gene.

The combined dataset of the 96 morphological characters was concatenated with the DNA sequences of the four genes (12s, cyb, Ghr, and Tth), scoring fossil taxa with missing entries for the DNA partitions. This dataset contained a total of 40 taxa and a total of 4110 characters. An equally weighted parsimony analysis was conducted treating gaps as missing data in TNT 1.1 [100,101]. The heuristic search consisted in 1000 replicates of a Wagner tree with random addition sequence of taxa and followed by TBR branch swapping, collapsing zero-length branches under the strictest criterion. After this procedure, a final round of TBR branch swapping to find all most parsimonious trees (MPTs).

The results of the cladistic analysis (Fig. 1) are congruent with previous phylogenetic hypotheses [41,47–49] and with the topology obtained in the Bayesian analysis of the molecular partition (see below). Further details on the parsimony analysis are given in the Document S1.

**Ghost Lineages and Calibrated Phylogenies**

The timing and mode of evolutionary history of a group can be inferred by integrating the temporal information of the fossil record and the phylogenetic trees [102]. These two sources of temporal information are independent and can be compared to measure their congruence and to infer the existence of “ghost lineages” [103,104], which extend the temporal range of a lineage prior to its appearance in the fossil record based on information from its sister lineage. Calibrating the phylogenetic trees that contain fossil taxa with the chronosтратigraphic information (geologic time) provides minimal ages of divergence for each node.
of tree. We calibrated the phylogenetic trees using a script for TNT (see Script S1) and identify ghost lineages following the methodology proposed by previous authors, considering the age of first appearance of each terminal taxon in the fossil record as the only relevant temporal information [105].

Molecular Clock Estimates
Bayesian analyses were conducted on the molecular data in BEAST v. 1.6 [106], performing a simultaneous estimation of the topology and divergence dates using a relaxed molecular clock. The four genes (12s, cyb, Ghr, and Tih) were input as separate partitions for the Bayesian analyses, model selection was performed using AIC (Akaike Information Criterion) [107] and hLRT (hierarchical Likelihood-Ratio Test) as implemented in Modeltest v. 3.1.2 [108]. The MCMC (Monte Carlo Markov Chain) was run using unlinked substitution models (GTR+I for all genes except for cyb that used GTR+I+F), a linked clock model (uncorrelated lognormal relaxed clock), and tree priors assuming a Yule process. Four independent MCMC runs of 10,000,000 generations (sampling every 1000 generations) were run independently for each of the 30 analyses we conducted using different calibration constraints (see below). Results of the four independent MCMC runs were integrated and summarized for checking convergence using the BEAST v. 1.6 package [106] and Tracer 1.5 [109]. The topologies sampled from the MCMC were summarized using TreeAnnotator v. 1.6.2 [106] selecting the maximum clade credibility tree. This tree (Fig. 4) is identical to the one obtained using the majority rule consensus of the trees sampled in the MCMC runs and also recover the same relationships of extant lineages as we recovered from the parsimony analysis of the combined dataset (Figs. 1, 3), indicating relationships of extant lineages as we recovered from the sampled in the MCMC runs and also recover the same maximum clade credibility tree. This tree (Fig. 4) is identical to the summarized using TreeAnotator v. 1.6.2 [106] selecting the maximum clade credibility tree. This tree (Fig. 4) is identical to the one obtained using the majority rule consensus of the trees sampled in the MCMC runs and also recover the same relationships of extant lineages as we recovered from the parsimony analysis of the combined dataset (Figs. 1, 3), indicating the phylogenetic signal of the data is robust to the assumptions of the analysis (see Document S1).

Calibration constraints. Bayesian approaches to molecular clock estimates, as implemented in BEAST v. 1.6, allows using different prior probability distributions to calibrate selected node ages (calibration constraints). As noted by several authors in recent years [98,110–115], the choice of calibration points is a critical step in molecular clock studies but is rarely discussed at length. We have explored the calibration of up to four nodes located both above and below the nodes of interest (diversification of caviid lineages). We performed 30 different analyses varying the number of calibrated nodes (from 1 to 4 calibrated nodes) and using two different prior distributions used for calibrating the age of these nodes (normal and gamma distributions; see below and Table 1). The analyses are numbered from 1 to 30, starting with the run with four constrained nodes that used a normal (analysis 1) or gamma (analysis 2) distribution. Analyses 3 to 10 used only three calibration constraints. Analyses 11 to 22 used two calibration constraints. Analyses 23 to 30 used a single calibration constraint. Prior distributions of the ages of these four nodes were defined based on the available chronostratigraphic information of the fossil record, considering the phylogenetic placement of fossils in the phylogenetic analysis (see Fig. 1) and the uncertainties in the age of the fossiliferous sediments. Two different prior distributions were alternatively used (Normal and Gamma), which represent different levels of confidence on the true absence of a given lineage in sediments older than its first appearance in the fossil record.

Normal distributions were centered on midpoint age of the period of time to which the fossil-bearing formation has been referred. The standard deviation was set so that the 95% probability distribution reached the upper and lower bound of the age of the lithostratigraphic unit (see Calibrated Nodes and Table 1). This prior distribution makes a strong assumption on the absence of representatives of the calibrated node in older sediments, which may be appropriate in some cases but not in others [90].

Gamma distributions were used to produce asymmetric probability distributions that place the highest prior probability along the interval of time represented by the lithostratigraphic unit that has yielded the oldest member of the clade being calibrated. The long tail of the distribution extends towards older ages, gradually decreasing the probability (maximum soft-bound), and including in the 95% prior probability distribution the age of the most recent sediments in which representatives of the node being calibrated are absent, but numerous remains of its stem-group or other caviid lineages are known (see Calibrated Nodes and Table 1). The presence of abundant remains of outgroup taxa is a taphonomic-preservation control using ecological/taxonomic equivalents [116] and resembles the criteria advocated for calibrating nodes by some authors [98,112,114,115,117].

The 30 exploratory MCMC runs were conducted using different combinations of these distributions for the four calibration points and varying the number of nodes calibrated with the fossil record (from one to four calibrations; Table 1). The evidence used to define the prior distribution of ages of the four calibrated nodes is given in Calibrated Nodes and further details on the parameters used and the results obtained are given in Table 1.

Calibrated Nodes
Node 1 (Cavioidae; see Figs. 1, 4). This node represents all cavioids and includes representatives of Dasyproctidae, Cuniculidae, and Cavioidae sensu stricto. The most ancient fossil that has been assigned to this clade is *Andemys tenanai*, known from a mandibular fragment found in the Tinguiririca fauna from the Abanico Formation of Central Chile [24,29,118], dated at 31.5–37.5 Ma [61]. Its affinities have never been tested in a phylogenetic analysis, but the combination of plesiomorphic and apomorphic features identified in previous studies [24,29]; mesodont crowns but with a deep hypoflexid that would have projected nearly to the lingual margin of the molars when unworn) indicates this specimen can be tentatively regarded as a basal member of Dasyproctidae and therefore useful in calibrating the age of the node Cavioidae.

Calibrating this node with *Andemys tenanai* [29] from the Tinguiririca fauna involves two different sources of uncertainty. First, there is an uncertainty in the radiometric dates of the Abanico Formation. Wyss et al. [24] provided Ar/Ar dates of the fossiliferous horizons and concluded that the fossils are at least as old as 31.5 Ma but the deposition of lower levels of the unit must have started about 37.5 Ma. Therefore, the 31.5–37.5 Ma uncertainties should be taken into account when calibrating the age of this node. Second, as noted above, this fossil is the among the oldest cavioid rodent known from South America, being the earliest member of this large clade of caviomorph rodents that evolved after the arrival of ancestral hystricognaths to this continent. The timing of the arrival of rodents to South America is, however, poorly constrained given the scarce rodent fossil record prior to the Tinguirirican deposits. The recent discovery of the oldest rodent fauna in the Yahuarango Formation [27] lacks cavioid rodents and has been radioisotopically dated by Ar/Ar at 43.44±2.5 Ma, suggesting an upper bound for the origin of Cavioidae. This finding lies within the range of previous molecular clock estimates for the initial diversification of caviomorph rodents (after their arrival to South America) that ranged between 30.7 and 53 Ma [20,56,59]. Therefore, the actual time of diversification of Cavioidae is uncertain due to the poor record of Eocene caviomorphs and the error associated to radioisotopic dates of the
oldest rodent faunas, as there is a minimum of 3.5 my and a maximum of 14.5 my between their appearance of cavioids in the fossil record (Tinguiririca) and the oldest rodent record from South America, in which cavioids are absent (Yahuarango).

We have explored the use of two different prior probability distributions to account for these sources of uncertainty while calibrating this node. The first approach uses a normal distribution whose 95% probability density encompasses the radioisotopic ages published for Tinguiririca (31.5–37.5 Ma). This distribution largely ignores the second source of uncertainty (i.e., lack of adequate early fossil record of Caviomorpha and Cavioida) and places a strong belief in that the dasyproctid from Tinguiririca is actually very close (in time) to the divergence time of Cavioida. The second approach uses a gamma distribution, with a hard minimum bound at 31.5 Ma and its long tail extends the 95% probability density back to 45 Ma, representing the upper bound of the available dates of oldest record of caviomorphs from South America [27], as well as the inferred date for the diversification of Caviomorpha obtained in the most densely sampled molecular clock analysis [59].

Finally, given the scarcity of the available material to constrain the age of this node and the lack of an explicit phylogenetic analysis including Andemys termasi [24,29] Tinguiririca mandible, we have also tested the use of this fossil for calibrating the deepest node of our phylogenetic tree (Fig. 4). The results, however, are largely similar in terms of the molecular clock estimates for most nodes within Cavioida and therefore we conducted all analyses using Andemys termasi to calibrate Cavioida.

Node 2 (Cavioida s.s. + Cuniculus paca; see Figs. 1, 4). This node of the phylogenetic tree represents all forms of Cavioida s.s. plus the lineage of the family Cuniculidae. The fossil record of Cuniculidae is extremely poor and only starts in the Pleistocene [119,120]. Cavioida s.s., in contrast, has an extremely rich fossil record [53], especially in the southern region of South America (Patagonia). The most ancient definitive members of Cavioida s.s. are Asteromys punctus and Chabutomys simpsoni, both known from few specimens found in the late Oligocene beds of Patagonia (Deseadan SALMA) of the Sarmiento Formation [49,121]. The phylogenetic position of both taxa within Cavioida s.s. is strongly supported in by the morphological data of the phylogenetic analysis presented here (Fig. 1), as in previous phylogenetic studies [47–49]. In our analysis, the two most basal nodes of Cavioida s.s. have high Bremer support (i.e., 4 and 5) and bootstrap and jackknife frequencies above 96% (Fig. 2; see Document S1).

The age of Deseadan deposits that yielded Asteromys and Chabutomys is therefore critical for calibrating this node. Specimens of Asteromys were found in the localities Cabeza Blanca and Laguna de los Machos [49] and the type Chabutomys simpsoni only in the former locality. These fossils were found together with a mammalian faunal assemblage characteristic of the Deseadan age [52]. No radiometric dates are available for these two localities but other Deseadan beds have been dated between 24.5 and 29 Ma [61–62]. The range of radiometric ages obtained for other Deseadan localities is the first source of uncertainty that should be taken into account for calibrating this node. The possibility that these early cavioid fossils are younger than the cladogenetic event that separated the cuniculid lineage from the lineage of Cavioida s.s. is the second source of uncertainty that should be considered. Pre-Deseadan rodent assemblages from Patagonia are known from La Cantera locality, which lacks forms of Cavioida s.s. [65]; see below and has been dated between 29.5 and 31.1 Ma [62].

As with the calibration of Node 1, we explored the use of two different prior probability distributions for this node. The first was a normal distribution whose 95% probability density encompasses the range of radioisotopic ages published for the Deseadan SALMA (24.5–29 Ma); ignoring the second source of uncertainty. The second approach uses a gamma distribution, which puts a hard minimum bound at 24.5 Ma and its long tail extends the 95% probability density back in time to the age of the youngest rodent assemblage that lacks forms of Cavioida s.s. (i.e. 29.5–31.1 Ma; [62]). Although we have explored both strategies we believe the second option more accurately represents the uncertainties of the early fossil record of cavioid rodents.

Node 3 (Kerodon + Hydrochoerus; see Figs. 1, 4). This node of the molecular phylogeny represents all forms of Hydrochoerinae (including the lineage leading to the Rock cavy Kerodon). The fossil record of Kerodon is only known from scarce material of the late Pleistocene of Brazil [122–124]. Fossil representatives of the lineage leading to Hydrochoerus (see Fig. 1), in contrast, are relatively abundant in some Miocene-Pliocene deposits of South America [125]. The oldest definitive records of this lineage are found in the Arroyo Chasico Formation of Central Argentina (Chasicoan SALMA) and include Cardiatherium chasicoense [126,127], Cardiomys cavinus [128], and Procardiomys martinoi [129]. The phylogenetic position of Cardiomyus cavinus and Cardiatherium chasicoense within Hydrochoerinae is supported by the morphological data of the phylogenetic analysis presented here (Fig. 1). Although the clades within Hydrochoerinae have low to moderate Bremer support values and bootstrap and jackknife frequencies are below 60% (Fig. 2; see Document S1), forcing the exclusion of both Cardiomys and Cardiatherium outside Hydrochoerinae requires five extra steps in the parsimony analysis, demonstrating their inclusion within Node 3 is relatively well supported by the morphological data.

The age of Chasicoan deposits yielding this diverse assemblage of hydrochoerines is important for calibrating this node. The stratigraphic levels that contain these taxa belong to the upper section of the Arroyo Chasico Formation (Lithofacies 3 sensu Zárate [130] or Las Barrancas Member sensu Ugarte [131]. The age of these levels is certainly younger than 9.07 Ma, which is the age of radiometric dates of the underlying lithofacies 1 and 2 of the Arroyo Chasico Formation [130]. Unfortunately, radiometric dates for the fossiliferous levels are lacking, but Deschamps [132] considered that lithofacies 3 of the Arroyo Chasico Formation can be bistratigraphically correlated with the Loma de Las Tapias Formation (northernwestern Argentina), which has a radiometric date of 7±0.9 Ma. A conservative approach, therefore, is to consider the age of the fossiliferous levels of the Arroyo Chasico Formation as ranging between 6.1 and 9.07 Ma.

Although these fossils from the Chasico Formation are the oldest well preserved and definitive record of hydrochoerines, it is difficult to determine if earlier members of this node were present before the deposition of this unit. In fact, the fossil record of cavioid rodents has a noticeable gap before the Chasicoan. The only older rodent remains that could be related to hydrochoerines come from the Mayoan SALMA (11.5 Ma; [133]) and consists of a single isolated tooth from the Río Frias Formation that has been referred with doubts to Cardiomys [50,134]. This isolated tooth, however, lacks diagnostic features to determine their phylogenetic placement either as part of Node 3 or as a close relative of this clade. The caviid fossil record before the Mayoan is found in the Mayan SALMA (11.5 Ma; [69]) and consists of the basalmost members Caviidae, which are definitively placed outside Hydrochoerinae (e.g., Pro dolichotis pridania; see below and Fig. 1), placing a maximal bound for the origin of this node.

As with the other nodes, we explored two different prior probability distributions for the age of Node 3. The first used a
normal distribution whose 95% probability density encompasses the range of radiocarbon ages that bracket the fossiliferous levels of the Arroyo Chasico Formation (6.1–9.07 Ma); this ignores the phylogenetic uncertainty of the fragmentary remains with possible hydrochoerine affinities in older sediments (e.g., Mayoan SALMA). The second used a gamma distribution, with a hard minimum bound at 6.1 Ma and a long tail to extend the 95% probability density back to the age of the Lartian SALMA, which is the youngest rodent assemblage that is well sampled and lacking forms that could potentially belong to Node 3 (i.e., 11.8–13.5 Ma). As in previous cases, we have explored both approaches but consider the second option better represents the uncertainties in the fossil record of early hydrochoerines.

**Node 4 (Microcavia + Cavia; see Figs. 1, 4).** This node of the molecular tree represents all forms of Caviinae closer to Microcavia or Cavia than to Galea. The earliest fossil members of Cavia come from the San Andrés Formation (late Pleocene; [135]) and are based on scarce mandibular material. In contrast, fossils referred to Microcavia or other genera possibly related to Microcavia are relatively abundant in some late Miocene to early Pleocene deposits of South America [124,136]. The phylogenetic analysis performed here includes four fossil taxa that have been regarded as primitive members of Caviinae: Allocavia chasicoense, Paleocavia impar, Dolichotis minuscula, and Microcavia chapatmalensis [124,134,137,138]. The last three taxa are retrieved as more closely related to the extant Microcavia australis than to Cavia and provide adequate information for calibrating this node.

*M. chapatmalensis* is recovered as the sister taxon of *M. australis* in the phylogenetic analysis (Fig. 1) and comes from the Chapadmalal Formation of Central Argentina (Chapadmalan SALMA). The sister-group relationship of these two species of *Microcavia* are well supported by the morphological data of our phylogenetic analysis. The support values for the node of the genus *Microcavia* are low (Bremer = 2 and bootstrap and jackknife frequencies around 60%; Fig. 2; see Document S1), but this is due to the unstable behavior in suboptimal (or bootstrap) trees of some fragmentary taxa (e.g., Allocavia). Forcing *M. chapatmalensis* to be positioned outside the Node 4 requires a minimum of five extra steps demonstrating the strong support for its inclusion within this node. The minimum age of the Chapadmalal Formation has been dated at 3.27 Ma using K-Ar radioisotopes [139], whereas the maximum age of these levels is usually regarded as 4 Ma [140,141]. Fragmentary remains found in older sediments of Monte Hermoso Formation (Montehermosan SALMA) and Aconquija Formation (late Miocene – early Pleocene) have been referred to *Microcavia* [142,143] but these cannot be identified at the species level and lack unambiguous synapomorphies of this genus. These remains could belong to the stem of *Microcavia* (i.e., being part of Node 4) or have an even more basal position, but more complete remains are needed to place them confidently.

*Dolichotis minuscula* was recovered in a basal polytomy within the lineage leading to the genus *Microcavia* together with *Paleocavia impar* (Fig. 1). Numerous and well-preserved remains of *Dolichotis* are known from the Chapadmalal Formation of Central Argentina ([134,144]; 3.27–4 Ma). No remains of this taxon have been found in older deposits (e.g., Monte Hermoso Formation). In contrast to the strong support for the position of *Microcavia chapatmalensis*, the position of *Dolichotis* is only poorly supported, as it takes a single extra step to place this taxon basally within Caviinae and only 20% of the bootstrap and jackknife trees place this taxon within Node 4.

*Paleocavia impar* is known from multiple specimens with skull and mandibles found in the Monte Hermoso Formation [145]. As in the case of *Dolichotis*, the inclusion if *Paleocavia impar* within Node 4 is not strongly supported. Topologies with a single extra step places this taxon more basally within Caviinae and only 33% of the bootstrap and jackknife trees place *Paleocavia* allied with the genus *Microcavia*. The maximum age of this unit has been determined as 5.3 Ma based on radiometric dates [146] and the minimum age was estimated as 4 Ma [140,141]. The presence of *Paleocavia* in older sediments (Huayquerian SALMA; 5.3–6.1 Ma; [147]) of the Ituzaingó Formation [148] and of the Cerro Azul Formation [149] have been reported in faunal lists of these units, but these specimens have not been described and we cannot test at the moment their phylogenetic affinities. If the presence of *Paleocavia* in the Ituzaingó and Cerro Azul formations were confirmed, it would push the first appearance of Node 4 back to the Huayquerian, but for the moment the Montehermosan record of *Paleocavia* is the oldest record of Node 4 (*Microcavia+Cavia*).

The rodent fossil record of the older sediments of the Chasico Formation (Chasicoan SALMA; 6.1–9.07 Ma; see above) provides confident information to place a maximal bound for the origin of this node. The Chasicoan fossil record contains remains of caviine-like caviids (e.g., Allocavia) as well as numerous forms of Dolichotinae and Hydrochoerinae [53,81,150]. All these forms are recovered in the parsimony analysis outside the derived Node 4 of *Caviinae* (see Fig. 1). None of the hundreds of rodent remains known from Chasicoan deposits can be allied to the node formed by *Microcavia* and *Cavia* (Node 4).

As with other nodes, we explored two different prior probability distributions for the age of Node 4 based on the position of fossil taxa in the most parsimonious trees of our analysis. The first used a normal distribution whose 95% probability density encompasses the range of radiocarbon ages that bracket the fossiliferous levels of the Monte Hermoso Formation (4.5–3 Ma); this ignores the uncertainty associated with the possible presence of *Paleocavia* in older sediments (i.e., Huayquerian). The second approach used a gamma distribution, with a hard minimum bound at 4 Ma and a long tail that extended the 95% probability density back to the Chasicoan, which is the youngest well-known rodent assemblage lacking taxa that belong to Node 4 (i.e., 6.1–9.07 Ma). As in previous cases, we have explored both approaches but believe the second option better represents the uncertainties in the fossil record of early caviines.

Finally, given the oldest member of this clade (*Paleocavia, Montehermosan SALMA*) is only poorly supported within Node 4, we have also tested alternative calibrations for this node. We have conducted exploratory runs of the Bayesian analysis calibrating this node with the age of *Microcavia chapatmalensis* (Chapadmalan SALMA), which is the only fossil of this clade that is robustly supported by the morphological data of the phylogenetic analysis (see above). The results of this analysis are largely similar in terms of the molecular clock estimates for Caviidae (mean age = 17.5 Ma) and place the diversification of dolichotines and Galea within the Chasicoan SALMA. Therefore, the estimates of interest for our purposes do not seem to be sensitive to the alternative ages that can potentially be used for calibrating Node 4.

**Supporting Information**

**Document S1 Supporting information of the phylogenetic analysis conducted on the combined dataset.** (DOC)

**Document S2 List of taxa used for the Phylogenetic Analysis and GenBank accession numbers.** (DOC)
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Author Contributions
Conceived and designed the experiments: MEP DP. Performed the experiments: MEP DP. Analyzed the data: MEP DP. Contributed reagents/materials/analysis tools: MEP DP. Wrote the paper: MEP DP. Conducted the morphological phylogenetic study: MEP.

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