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Evolution of South American Paucituberculata (Metatheria: Marsupialia): adaptive radiation and climate changes at the Eocene- Oligocene boundary

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ABSTRACT

The Eocene-Oligocene boundary (EOB) marks a period of remodeling in the metatherian faunas of South America. Paucituberculata was one of the groups that successfully diversified as the climate and environment conditions changed, and they became, during the first part of the Neogene, an important component of micromammal assemblages. Among paucituberculatans, the non-pichipilid palaeothentoids (NPP) has been recognized as the clade that diversified most widely in post-EOB times. Here we explore the evolutionary response of the NPP to the climatic-environmental changes around the EOB, by analysing the temporal patterns of disparity, taxonomic diversity and body mass in a phylogenetic context. To asses the magnitude of the NPP radiation comparisons based on these macroevolutionary parameters were done with its sister-group Pichipilidae, and its next closest relative, the Caenolestidae. In all considered parameters, NPP reached values significantly higher than the remaining paucituberculatans clades. From its initial diversification in the middle Eocene, taxonomic diversity increased through time, but it was decoupled from disparity across the EOB, and from the late Oligocene to early Miocene. The Oligocene emerges as the key period in NPP evolution, which is evidenced by a significant and concordant expansion of disparity and taxonomic diversity, suggesting evolution into empty ecospace.

Introduction

Metatherians (crown-clade Marsupialia plus their stem relatives; Rougier et al. 1998) evolved in South America throughout Cenozoic, achieving an important taxonomic and ecological diversity (Goin et al. 2016). Paucituberculata, Sparassodonta, Didelphimorphia, Microbiotheria, Polydolopimorphia, as well as diverse 'basal ameridelphians' (e.g., Pucadelphyidae and Protodidelphidae, Oliveira and Goin 2011) are among the main groups currently recognized that occurred in this continental area. From one or more immigrants lineages from North America, probably in a late Cretaceous – earliest Paleocene dispersal event (Pascual and Ortiz-Jaureguizar 2007; Goin et al. 2012; Woodburne et al. 2014), metatherians radiated in South America, occupying a wide range of ecological roles such as small (< 100g) to medium (100g – 500g) insectivores, omnivores and frugivores (didelphimorphians, microbiotherians, polydolopimorphians and paucituberculatans; Zimicz 2011, 2014; Abello et al. 2012), and small (< 7 kg) to large (above 15 kg) mesocarnivores and hypercarnivores (sparassodonts; Prevosti et al. 2013). The evolutionary history of these groups was marked by the impact of deep climatic and environmental changes, both global and regional, which appears to have driven major macroecological processes, such as radiations, extinctions and turnovers (Pascual and Ortiz-Jaureguizar 2007; Goin et al. 2012, 2016). For living marsupials, it has been stated that ambient temperature strongly influence several of their life history variables, such

as feeding preferences, reproductive strategies and distribution (see Goin et al. 2016). At a large temporal-scale, global temperature variations over the Cenozoic such as warming and cooling trends and thermal spikes (e.g. the Mid-Eocene Climatic Optimum, c.a. 42Myr BP; Hansen et al. 2013) appears to have been closely related to the major changes in the evolution of South American groups (Goin et al. 2010, 2016). Atlantic transgressions and regressions, such as those of the Paleocene and Miocene (Malumian and Nañez 2011), together with the Andean uplift, were among the major geological events that shaped the paleogeography of South America, having effects on regional climate and landscape evolution (Ortiz-Jaureguizar and Cladera 2006; Ramos and Ghiglione 2008; Hoorn et al. 2010). There were the isolation of Antarctica and the formation of the Antarctic Circumpolar Current, with consequent drops of global temperatures near the Eocene-Oligocene boundary (EOB), the geological and climatic events that deeply affected metatherian assemblages of South America (Goin et al. 2010, 2016). At that time, metatherian fauna suffered a large turnover, with many lineages becoming extinct (e.g. basal 'ameridelphians' and polydolopid polydolopimorphians) and others radiating (e.g. paciuberculatans and sparassodonts) (Goin et al. 2010, 2016).

Having arose in the early Eocene at the latest, and survived into the present, paucituberculatans are one of the oldest surviving lineages of South American metatherians. Thus, throughout the last 50 M.a., their evolution is likely to have

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been strongly influenced by diverse environmental changes such as those mentioned above. In fact, the EOB climatic event appears to have marked a turning point in their evolutionary history (Abello 2013; Abello et al. 2015).

Paucituberculatans are currently grouped into two main clades: Caenolestoidea, which includes the extant shrew opossums and their extinct relatives (Caenolestidae), and Palaeothentoidea, which groups the remaining extinct paucituberculatans into three main clades, Pichipilidae, Abderitidae and Palaeothentidae (Goin et al. 2009; Abello 2013; Rincón et al. 2015; Engelman et al. 2016). The caenolestids are generalized species, small sized, mostly insectivorous and terrestrials (Kirsch and Waller 1979; Patterson and Gallardo 1987; Martin and González-Chávez 2016; Figure 1A). On the other hand, the palaeothentoids includes the most specialized paucituberculatans, some of them dentally convergent on diprotodontian
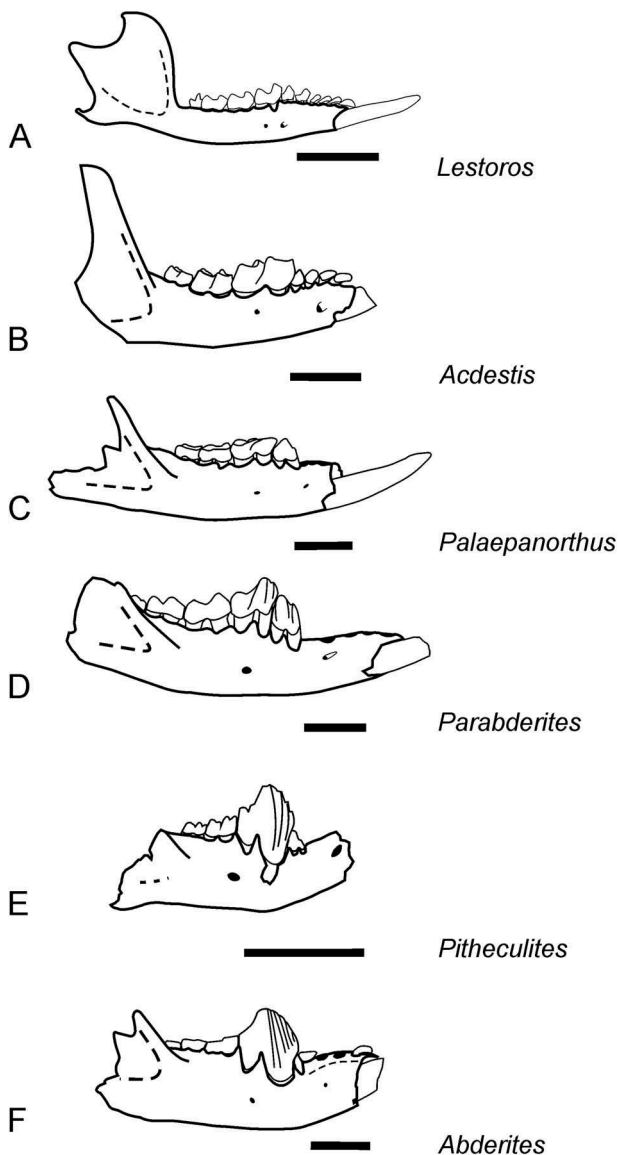

Figure 1. Comparative paucituberculatan mandible and lower dental morphology. Schematic drawings are based on the following specimens: A, USNM 194,383 (*Lestoros inca*); B, MACN 2038 and MACN 5560 (*Acdestis spegazzinii*); C, MLP 82-V-2–108 (*Palaepanorthus primus*); D, MPEF-PV 5834 (*Parabderites bicrispatus*); E, MPEF-PV 5833 (*Pitheculites minimus*); F, MPEF-PV 5847 (*Abderites crispus*). Scale bar = 5 mm.

marsupials (burramyds and petaurids; Marshall 1980; Ortiz-Jaureguizar 2003). In particular, the abderitids developed a plagiaulacoid dentition (i.e., one or more teeth transformed into large laterally compressed blades with cutting serrated margins (Simpson 1933; Figure 1D-F) similarly developed in certain multituberculates (e.g., the cimolodontan *Ptilodus*), primates (e.g., *Carpodaptes*) and diprotodontians (e.g., potoroids and *Burramys*). Studies on diet and body size indicated that the non-pichipilid palaeothentoids (NPP) were diverse, including small- to medium-sized insectivores, medium-sized frugivores and small- to -large-sized (> 500g) insectivore-frugivore species (Dumont et al. 2000; Abello et al. 2012; Zimicz 2012). It was proposed that this diversity, both ecological and taxonomic, expanded near the EOB in association with the climatic-environmental changes that took place in South America after the separation from Antarctica (Goin et al. 2010; Abello 2013; Rincón et al. 2015), but the key features of this radiation have only been subject to preliminary analyses (Abello et al. 2015, 2016). Studies on adaptive radiations state that significant increases in taxonomic and morphological diversity (disparity *sensu* Foote 1993a) occur as a lineage diversifies into different ecological niches (Simpson 1953; Foote 1994; Schluter 2000), and different models of radiations have been proposed depending on the relationships between both components of diversity (Foote 1993b; Benton et al. 2014). Large-scale, temporal patterns of disparity and taxonomic diversity are critical to understanding the macroevolutionary processes involved in generating biodiversity (Foote 1994, 1997; Erwin 2007). In addition, because body size its an important predictor of species ecology, as it is correlated with a host of biological variables (Damuth and MacFadden 1990), the analysis of its evolution can account for the pattern of niche occupation during diversification.

The main purpose of this contribution is to analyze the evolution of the NPP clade in terms of taxonomic diversity, disparity and body mass evolution (used as a proxy for body size), and to evaluate how climatic-environmental changes at the EOB could have be related to the observed pattern of changes in these macroecological parameters. Additionally, comparisons based on these evolutionary aspects will be done with their sister-group Pichipilidae, and its next closest relative, the Caenolestidae (Abello 2013). We expect that our results will allow a better understanding on paucituberculatan macroevolution, and contribute to future studies on mammal community interactions and biodiversity construction during the Cenozoic of South America.

Material and methods

Extinct paucituberculatan species are mainly known from fragmentary cranial remains, such as mandibles, maxillae and isolated upper and lower teeth, while postcranial and more complete cranial remains are scarce (Abello & Candela 2010; Forasiepi et al. 2014; Figure 1). Thus, phylogenetic relationships of paucituberculatans, as well as aspects of their paleobiology, such as diet and body mass, have been mainly inferred from dental morphology (Dumont et al. 2000; Abello et al. 2012; Zimicz 2012; Abello 2013; Rincón et al. 2015; Engelman et al. 2016). Here we present a revised phylogeny of the group, and body mass estimations for most

paucituberculatan species. The new hypothesis is used as a phylogenetic context to analyze body size evolution, and changes in diversity, disparity and morphospace occupation of the NPP during their Eocene-to-Miocene radiation.

Abbreviations

Species acronyms

Aaisen, *Abderites aisenense*; Acrispus, *Abderites crispus*; Aclemai, *Acdestis lemairei*; Acmad, *Acdestis maddeni*; Acowenii, *Acdestis owenii*; Aili, *Antawallathentes illimani*; Ameridio, *Abderites meridionalis*; Aquimsa, *Antawallathentes quimsacruza*; Bardh, *Bardalestes hunco*; Bardsp, *Bardalestes* sp.; Cae, *Caenolestes fuliginosus*; Cchubu, *Carlothentes chubutensis*; Evoh, *Evolestes hadrommatos*; Evosp, *Evolestes* sp.; Fsorex, *Fieratherium sorex*; Lest, *Lestoros inca*; nov. 1, Sp. nov 1; nov. 2, Sp. nov 2; nov. 3, Sp. nov 3; nov. 4, Sp. nov 4; nov. 5, Sp. nov 5; Palara, *Palaeothentes aratae*; Palinter, *Palaeothentes intermedius*; Pallem, *Palaeothentes lemoinei*; Palmarsh, *Palaeothentes marshalli*; Palmigue, *Palaeothentes migueli*; Palmin, *Palaeothentes minutus*; Palpas, *Palaeothentes pascuali*; Palpri, *Palaepanorthus primus*; Palreli, *Palaeothentes relictus*; Palserra, *Palaeothentes serratus*; Pbicris, *Parabderites bicrispatus*; Pboli, *Palaeothentes boliviensis*; Pcard, *Perulestes cardichi*; Pcent, *Pichipilus centinelus*; Pchenche, *Pitheculites chenche*; Pfrai, *Perulestes fraileyi*; Phono, *Phonocdromus gracilis*; Pilan, *Pilchenia antiqua*; Pilinter, *Pilchenia intermedia*; Pilu, *Pilchenia lucina*; Pminimus, *Pitheculites minimus*; Pminus, *Parabderites minusculus*; Posbor, *Pichipilus osborni*; Prig, *Pichipilus riggsi*; Prothi, *Pitheculites rothi*; Ptrip, *Pliolestes tripotamicus*; Pven, *Pliolestes venetus*; Quiroga, *Quirogalestes almagaucha*; Rhyn, *Rhyncholestes raphanurus*; Rio, *Riolestes capricornicus*; Stilo, *Stilotherium dissimile*; Sasa, *Sasawatsu mahaynaq*; Trothi, *Trelewthentes rothi*; Tsimp, *Titanothentes simpsoni*; Titano. *Titanothentes* sp.

Institutional abbreviations

MACN-A, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (Ameghino collection), Ciudad Autónoma de Buenos Aires, Argentina; MLP, Museo de La Plata, La Plata, Argentina; MPEF-PV, Museo Paleontológico Egidio Feruglio (palaeovertebrate collection), Trelew, Argentina; USNM, United States National Museum, Smithsonian Institution, Washington, D.C., United States.

Dietary habits and body size of paucituberculatans

For dietary categories (i.e., frugivore, insectivore-frugivore and insectivore) and body size categories (i.e., small< 100g, medium = 100g – 500g, large > 500g) used in this work, see discussion in Abello et al. (2012).

Paucituberculatan phylogeny and temporal calibration

The revised phylogeny of paucituberculatans is based on a data matrix modified from those of earlier works (Abello 2013; Rincón et al. 2015; Engelman et al. 2016). As in the analysis of Abello

(2013), a reduced matrix was constructed from a total matrix (SM1) consisting of 63 species and 82 discrete cranio-dental characters by the removal of three problematic taxa (SM2). Both matrices were subjected to a cladistic analysis using maximum parsimony, as implemented in TNT 1.5-beta (Goloboff et al. 2003). The search parameters to recover the most parsimonious trees are detailed in the SM2. Multiple most parsimonious trees were summarised using strict consensus.

To obtain a timescaled phylogeny, we followed the methodology of Brusatte et al. (2008a) and Lloyd (2016). We applied the command *timePaleoPhy* from the R package *paleotree* (Bapst 2012), set with the 'equal' method corresponding to that used in Brusatte et al. (2008a). This method proceeds by increasing the root divergence time by a predefined amount and then adjusting the zero-length branches in such a way that time on early branches is re-apportioned out along those later branches equally. Branches times were adjusted in order relative to the number of nodes separating the edge from the root, going from the shallowest branches to the deepest branches. For terminal taxa (cladogram tips), we used the absolute ages detailed in SM3, depicted as ranges between a First Appearance Date (FAD) and a Last Appearance Date (LAD). When no ranges were available, we introduced an extremely close hypothetical LAD of 10,000 years more recent than the actual age *datum*. Given the limitations of our dataset, we preferred this method instead of others, more sophisticated ones such as the 'cal3' by Bapst (2012), because we purposely intended to minimize the number of assumptions involved in the model, therefore decreasing the incertainty introduced by modelization (for example estimation of branching and extinction rates). In addition, some nodes were dated taking into account additional information from the fossil record. Such is the case of the records of *Perulestes* sp. and *Sasawatsu* sp. in the middle Eocene of Peru (Antoine et al. 2016) which places the origins of the NPP clade at a minimum age of 41 Ma ago, the records of an Acdestinae indet. in the late Oligocene of Patagonia (Bown and Fleagle 1993; Abello 2007), placing the origin of Acdestinae clade at least at 27.75 Ma., and *Abderites* sp. in the late Oligocene of Peru (Antoine et al. 2016) placing the origin of the *Abderites* clade at least at 26.56 Ma. ago. The absolute ages of terminal taxa, as well as references about their geographic and temporal distribution, are shown in SM3.

Taxonomic diversity through time

Previous analyses of paucituberculatan taxonomic diversity were based on a simple counting of the stratigraphic record of species during discrete intervals of time (e.g., Abello 2013; Rincón et al. 2015; Engelman et al. 2016). This traditional taxic approach leads to underestimate of the age of clades and palaeodiversity (Norell 1993; Lane et al. 2005). Therefore, in order to enhance taxonomic richness measures we estimated the diversity as predicted by phylogeny, using a calibrated phylogeny of paucituberculatans (see above) and correcting observed temporal ranges of taxa by considering ghost lineages (Norell 1992, 1993).

For the NPP, diversity was calculated for four time bins of comparable duration: middle-late Eocene (40 Ma to 33.9 Ma

~ 6 Ma), early Oligocene (33.9 Ma to 27.82 Ma ~ 6 Ma), late Oligocene (27.82 Ma to 23.03 Ma ~ 5 Ma), and early Miocene (23.03 Ma to 16 Ma ~ 7 Ma). Phylogenetically estimated diversity metrics (i.e. taxic count + ghost lineages) were then plotted against time.

Disparity analysis

Disparity refers to morphological diversity or variety (Gould 1991; Foote 1993a, 1997). It can be described by diverse parameters or indices, most of them focused on quantification of pairwise distances of studied taxa (Foote 1997; Ciampaglio et al. 2001), and visualized by plotting them in a morphospace reflecting distance information (Wills 2001). Disparity indices are used to evaluate morphological dissimilarity between taxa within groups, and to detect and measure patterns of morphological change over geological time.

To quantify the morphological diversity among paucituberculatans we followed a general protocol described by Lloyd (2016) and implemented by the R package *Claddis* (Lloyd 2016). Both R package and a brief tutorial are available in GitHub (https://github.com/graemetlloyd/Claddis). Discrete morphological characters, derived from a cladistic data-set (see Phylogenetic Analysis), were used to construct a matrix of pairwise inter-taxon distances. Hypothetical ancestors were included in the matrix in order to phylogenetically correct measures of disparity, as proposed by Brusatte et al. (2011). Ancestral morphologies as implied by phylogeny, were then reconstructed using both accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) optimization methods. Since no differences arose from these, we used ACCTRAN optimization results only. The distance matrix was based on Generalized Euclidean Distance metric (GED; Wills 1998), as our preferred distance measurement (see SM4.7). The distance matrix was calculated using the command *MorphDistMatrix* of the *Claddis* package. Given that GED can cope with missing entries leading to non-comparable pairwise characters (Lloyd 2016), we test for the presence of non-comparable pairs (which would introduce bias) by comparing the GED data matrix with the matrix of comparable characters looking for non-coincidences. We did not find any non-comparable taxon-taxon pairs. Taxa and nodes showing redundant information were pruned before calculation of the metrics. In addition, in order to compare GED results with other available distance metrics, we determinated the pairwise distance between taxa and reconstructed ancestors with three other metrics: Raw Euclidean Distance (RED), Gowers's Coefficient (GC), and Maximum Observable Rescaled Distance (MORD) (see SM4.7). Since GED matrix is suitable for Principal Coordinates Analysis (PCo – Multi Dimensional Scaling; Wills 1998), this ordination method was used for morphospace construction, using the command *cmdscale* from the R package *stats* (R Core Team 2017). To obtain triangular distance matrices for calculations, we used the commands *upperTriangle* (R package *gdata*; Warnes et al. 2015) and *as.dist* (R core package *stats*).

Among the diverse disparity measures that have been proposed (Foote 1997; Ciampaglio et al. 2001) we chose as our pre-ordination metric the Weighted Mean Pairwise Distance (WMPD; distances weighted by the ratio of comparable characters), and two metrics that require ordination and are therefore computed on PCo scores: Sum of Variances and Sum of Ranges (SV and SR, respectively; see Lloyd 2016). WMPD has proved to be robust to sample size and for data sets with common missing entries (Foote 1994; Ciampaglio et al. 2001; Lloyd 2016), therefore it considered appropriate for the present analysis. Post-ordination metrics such as product of variances and ranges have been used in many studies of disparity in both invertebrates and vertebrates (e.g. Wills et al. 1994; Wills 1998; Brusatte et al. 2008b; Benson et al. 2012), but they can over-amplify differences within groups, and therefore we preferred to use SV and SR as considering them more conservative. Variance-based measures express the dispersion of points in the morphospace, and therefore they are more appropriate where the concern is with the average dissimilarity between forms (Wills et al. 1994; Wills 2001). Range-based measures, on the other hand, express morphospace size (i.e., total extent of morphological variability, Wills et al. 1994); being insensitive to the distribution of points, they are free from biases caused by taxonomic practice (differential lumping or splitting of taxa). For SV and SR, only those PCo eigenvectors accounting for most of variability were used (based on cumulative variance histograms or scree-plots – see SM4.4), in order to avoid downsizing effect introduced by lower-than 0.5 variances (Lloyd 2016). Interquartile intervals were calculated by an *ad hoc* programmed R function for jack-knifing on the original taxa-character matrices, and are provided in SM4.3 (R script for jack-knife is included in SM4.6).

Disparity metrics were calculated for the following systematic groups: Paucituberculata as a whole, Caenolestidae, Pichipilidae and NPP (SM4). As for diversity, disparity measurements were estimated for four time bins: middle-late Eocene, early Oligocene, late Oligocene and, early Miocene (SM4).

In order to assess the statistical significance of differences between successive time bins and since GED and PCo scores had proven to show non-normal distribution (SM4.5), non-parametric (Mann-Whitney U test, Kruskal-Wallis rank sum tests and Wilkoxon pairwise tests) tests were used both on raw GED and PCo scores on one hand, and on jack-knifed resampled distributions of WMPD, SV and SR (Table 2) on the other. R commands *kruskal.test* and *wilcox.test* were used (core package *stats*).

Body size evolution

Body mass estimation of terminal taxa
Body mass was estimated from the area measurement (maximal length x width) of the lower second molar (m2) and maximal length of the third lower molar (m3), using linear regression equations derived from living marsupials and presented by Dumont et al. (2000) and Gordon (2003), respectively. We found that Gordon`s equation provided, among other available equations (e.g. Dumont et al. 2000; Myers 2001), the most accurate body mass estimation for living caenolestids (SM5, Table 1) and thus we assumed this would be the best predictive equations to estimate body mass in extinct caenolestids, as well as other taxa with a generalized molar pattern, such as basal paucituberculatans and outgroups. On the other hand, Dumont

Table 1. Disparity and diversity metrics.

Disparity values compared by groups			
	Weighted Mean	Sum of Variances	Sum of Ranges
Paucituberculata	4.502	28.302	68.726
Caenolestidae	3.475	6.617	18.757
Pichipilidae	3.575	7.136	15.495
NPP	3.815	8.794	22.388

Disparity of NPP			
Time bin	Weighted Mean	Sum of Variances	Sum of Ranges
middle-late Eocene	3.1487	6.1598	13.3774
early Oligocene	3.3154	5.9964	14.7705
late Oligocene	4.1295	8.1169	17.7205
early Miocene	3.7266	6.4750	16.4894

Taxonomic diversity of NPP			
Time bin	taxic estimate	ghost lineages	phylogenetic estimate
middle-late Eocene	3	11	14
early Oligocene	2	18	20
late Oligocene	6	22	28
early Miocene	19	10	29

Taxonomic diversity of Abderitids (A) and Palaeothentids (P)			
Time bin	taxic estimate	ghost lineages	phylogenetic estimate
early Oligocene		A (4); P (9)	A (4); P (9)
late Oligocene	A (1); P (3)	A (9); P (13)	A (10); P (16)
early Miocene	A (6); P (13)	A (2); P (8)	A (8); P (21)

Table 2. Statistical test for significant differences in disparity metrics between time-bins.

tests on GEDs			

Mann-Whitney U test of distances between succesive time bins

	U	p-value	estimate
Eocene vs early Oligocene	380	0.9735	0.0043
early vs late Oligocene	603	**0.0016**	−0.7480
late Oligocene vs Miocene	3828	0.0896	0.3094

Kruskal-Wallis test of distances between succesive time bins

	chi-squared	df	p-value
Eocene vs early Oligocene	0.001	1	0.9736
early vs late Oligocene	9.8676	1	**0.0016**
late Oligocene vs Miocene	2.8801	1	0.0896

Wilcoxon pairwise rank sum test of distances between time bins

	Eocene	early Oligocene	late Oligocene
early Oligocene	0.9801	–	–
late Oligocene	0.0547	**0.0102**	–
Miocene	0.2699	0.1695	0.2699

Wilcoxon pairwise rank sum test of jack-kniffed WM between time bins

	Eocene	early Oligocene	late Oligocene
early Oligocene	0.5360	–	–
late Oligocene	0.0001	**0.0000**	–
Miocene	0.0000	0.0000	**0.0000**

tests on PCO scores

Kruskal-Wallis test of PCO scores between succesive time bins

	chi-squared	df	p-value
Eocene vs early Oligocene	0.47	1	0.4901
early vs late Oligocene	1.44	1	0.2297
late Oligocene vs Miocene	0.02	1	0.8778

Wilcoxon pairwise rank sum test of jack-kniffed SV between time bins

	Eocene	early Oligocene	late Oligocene
Early Oligocene	0,9789	–	–
Late Oligocene	0,0002	**0,0000**	–
Miocene	0,9789	0.0239	**0,0000**

Wilcoxon pairwise rank sum test of jack-kniffed SR between time bins

	Eocene	early Oligocene	late Oligocene
Early Oligocene	0.0907	–	–
Late Oligocene	0.0001	**0,0000**	–
Miocene	0,0001	0,0000	**0.0002**

et al. (2000) equation was applied for the remaining, more derived species, as it was done in previous studies (Abello et al. 2010; Rincón et al. 2015; Engelman et al. 2016). In only two basal paucituberculatans, with no preserved m2 or m3 (*Bardalestes hunco* and *Bardalestes* sp.), the equation of Gordon (2003) generated from M2 maximum length was used. For each regression equation, smearing estimates, a correction factor to deal with the logarithmic detransformation bias (Smith 1993), was calculated and multiplied for each estimated value of mean body mass (SM5, Table 2). Body masses of extant caenolestids were taken from the published literature (Kirsch and Waller 1979; Albuja and Patterson 1996; Patterson 2007).

Mapping of body mass

In order to analyze the evolution of body mass we chose to map this continuous character on paucituberculatan phylogeny by linear parsimony (Swofford and Maddison 1987) using TNT 1.5-beta (Goloboff *et al.* 2003). Then, and following Giannini et al. (2012) and Amador and Giannini (2016), the resulting pattern of change of body masses was evaluated by a node-by-node descriptive approach in the framework proposed by Gould and MacFadden (2004). In this way, four kinds of body size changes could be identified: autapomorphic gigantism and nanism, and phyletic gigantism and nanism. Autapomorphic changes are recognized as those occurring in a single branch of a clade, while phyletic ones are those occurring in nested clades (see Gould and MacFadden 2004). The persistence of a phyletic pattern was assessed, as proposed by Amador and Giannini (2016), considering the order of phyletic change as the number of consecutive branches composing the phyletic pattern. In the context of body size evolution of paucituberculatans, we considered a species to be a giant if its body mass resulted more than 200% increase with respect to the reconstructed ancestor, and a nanism if its body mass represented more than two-fold size decrease with respect to the reconstructed ancestor.

Phylogenetical autocorrelation of body size

To test for phylogenetical autocorrelation of body mass, we performed two different analyses, one for Paucituberculata as a whole and the other for the NPP only. Given that body-mass estimates were not available for all taxa considered, several of them had to be pruned from the matrices in order to perform these analyses (*Riolestes capricornicus*, *Fieratherium sorex* and *Pliolestes venetus*). Most available tests for phylogenetical autocorrelation require fully dichotomous trees and/or branch lengths (see Paradis 2006; Münkemüller et al. 2012). However, the phylogenetic hypothesis considered in this work resulting in a cladogram including several polytomies. Tools for solving polytomies by random splitting of daughter branches can be considered as rendering artificial, spurious phylogenetic hypotheses. In addition, we consider that estimating divergence times from fossil record imply ad-hoc statistic and evolutionary assumptions (for instance, inference of 0-length internal branches and/or estimation of branching-extinction rates; see Ollier et al. 2006; and Paradis 2006; Münkemüller et al. 2012; for a review of methods). Therefore, in this study we preferred to use tests not relying on branch lengths but only on cladogram

topology, and also those allowing polytomies. Such analytical tools are the Orthonormal Decomposition by Ollier et al. (2006) and the Abouheif's C test (Abouheif 1999).

All tests were performed in R, using different tools from the *ape* (Paradis et al. 2004), *ade4* (Dray and Dufour 2007), *adephylo* (Jombart et al. 2010), *geiger* (Harmon et al. 2008), and *phylosignal* (Keck et al. 2016) packages.

Orthonormal decomposition

The Orthonormal Decomposition test (in the *ade4* package) operates by building a matrix of orthobases (i.e. orthonormal vectors describing the topology of the tree) and then analyzing the dependence of the variance of the studied variable (log-transformed body size) on that set of vectors by four non-parametric statistics. Monte Carlo permutations of the vector matrix against the studied variable allow construction of a null-model of no-correlation and confidence intervals (at alpha 0.05) for the statistics. The R2Max (maximal R2) peaks when a significant portion of variance dependence is detected at a single node (otherwise dependence is dispersed along several nodes). The Dmax (maximal deviation), corresponding to the Kolmogorov-Smirnov statistic, tests if the variable studied could be considered a random sample from a uniform distribution. The SkR2k (sum of k-nth R2) describes the trend of the variance distribution to be skewed toward the tree's tips or root. Finally, the SCE (sum of cumulative errors) depicts the averaged variation.

Abouheif's c-mean

the Abouheif's C index can be viewed as a special case of the spatial correlation Moran's I index. It was performed to test the correlation between the studied variable (log-transformed body size) and a matrix of phylogenetic proximities with non-null diagonal (see Pavoine et al. 2008) describing the cladogram topology. We calculated the proximity matrix using the 'oriAbouheif' method of the command *proxTips* (*adephylo* R package), as discussed in Pavoine et al. (2008), and then we used this matrix for the command *Moran.I* of the *ape* R package. The null-hypothesis is the absence of correlation (C-mean equal 0) and its significance is tested against a distribution built on permutations (see SM5).

Coupling between disparity and body mass

In order to search for putative correlation between morphological disparity and body size variability, several statistical tests were performed. Pearson correlation tests between both maximum GED and log-transformed body mass, and between first PCo scores and log body mass, at a 0.05 significance level were calculated using the *cor.test* command of the *stats* R core package. In addition, linear regression of both maximum GED and first PCo scores on log-transformed body mass were calculated using the *lm* commands of the *stats* R core package.

Results

Paucituberculatan phylogeny

The strict consensus trees (SCT) and branch support values resulting from the analyses of reduced and total matrixes using
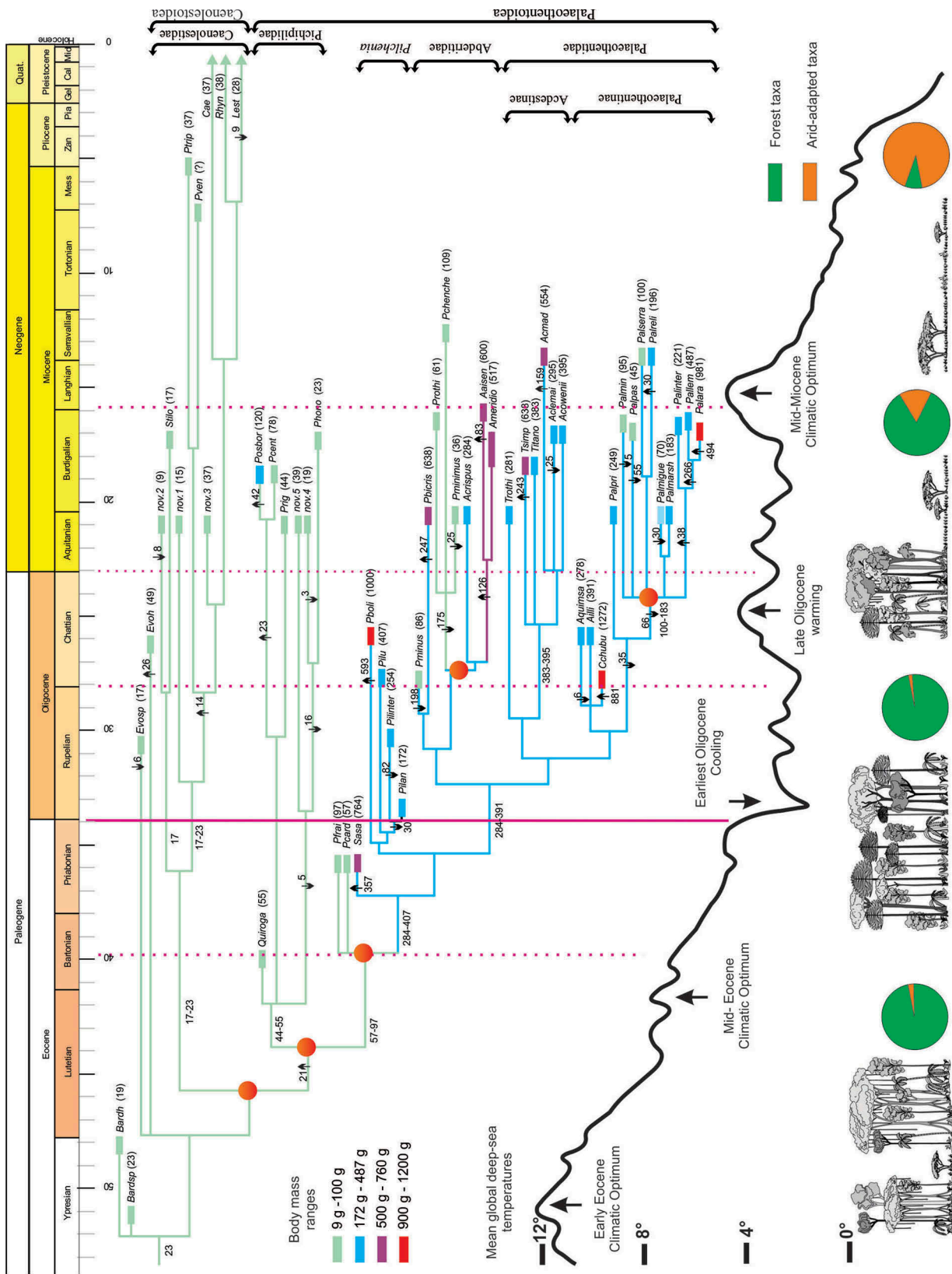
Figure 2. Phylogeny and body size evolution of paucituberculatans. Time-calibrated phylogeny based on relationships recovered in the strict consensus of 15 MPTs produced by the analysis of the reduced matrix under implied weights. Heavy lines = known temporal ranges; thin lines = missing ranges. Names on the right denote major clades. Evolution of body size is based on body mass optimization using linear parsimony; reconstructed values of body mass (g) are shown below branches and for terminal taxa are indicated in brackets. Arrows denote increases or decreases (number of changes occurred are indicated next to each arrow). Colored branches represent different body mass ranges (legend on graph). Red circles indicates nodes with most significant autocorrelation between body mass and cladogram topology as detected by Orthonormal Decomposition (see Figure 6). Eocene–Oligocene boundary is marked by a solid vertical line, remaining boundaries among time bins are marked by dashed vertical lines. Mean global deep-sea temperatures and climatic events are after Zachos et al. (2001) and Hansen et al. (2013). Landscape sketches representing major structures of the Patagonian vegetation and average percentages of forest and arid-adapted taxa were taken from Barreda and Palazzesi (2007) (Reproduced with permission of the copyright owner). For identity of species acronyms, see Abbreviations.

equal weighting and implied weighting are given in the SM2. Analysis of the reduced matrix yielded consensus trees with a higher number of nodes than those of the total matrix, providing more information about the evolution of paucituberculatans. The topology of the SCT resulting from the analysis of the reduced matrix under implied weighting differs only from that obtained using equal weighting in that the *Pilchenia* clade was fully resolved. In the following, the SCT topology recovered in this latter analysis (Figure 2) will be discussed in relation to recent hypotheses of paucituberculatan phylogeny.

As a result of this work, and in agreement with previous hypotheses (Abello 2013; Rincón et al. 2015; Engelman et al. 2016) Paucituberculata encompasses two main groupings, Caenolestoidea and Palaeothentoidea, and several basal taxa (Figure 2): *Fieratherium sorex, Bardalestes* spp. and *Evolestes* spp. However, and in contrast with earlier analyses (Abello 2013; Engelman et al. 2016), *Riolestes capricornicus* was excluded from Paucituberculata. On the other hand, *Evolestes hadrommatos* and *Evolestes* sp. were recovered, resembling the phylogeny of Engelman et al. (2016), as the paucituberculatan taxa most closely related to the Caenolestoidea +Palaeothentoidea clade.

The general topology of the Caenolestoidea clade is similar to that obtained by Abello (2013) and Engelman et al. (2016), and only differs in recovering *Stilotherium* as the sister-group

to remaining caenolestoids. The Palaeothentoidea comprises two main clades: Pichipilidae and NPP. The Pichipilidae topology was improved by the recovering of the *Pichipilus* clade and the monophyly of *Phonocdromus gracilis*, sp. nov 5 and sp. nov 4. Similarly to earlier mentioned studies, within the NPP clade *Perulestes* spp. are sister to the remaining taxa, and *Sasawatsu* is the sister-group to a clade that clusters *Pilchenia* spp., abderitids and palaeothentids. Among the NPP, Abderitidae was recovered with the same topology as that resulting in previous analyses: *Parabderites* (*Abderites + Pitheculites*). For the remaining NPP, the greatest differences with previous hypotheses (see, e.g. Rincón et al. 2015) are focused on the phylogenetic relationships among palaeothentines and *Pilchenia* spp. *Pilchenia* species were recovered as a clade, sister to Palaeothentidae + Abderitidae, as in Abello (2013) and Engelman et al. (2016, analysis under equal weighting), and not as a part of Palaeothentidae as in Engelman et al. (2016, analysis under implied weighting) and Rincón et al. (2015). Our Palaeothentidae topology largely agrees with that of Engelman et al. (2016, analysis under equal weighting); it includes two main groupings, Acdestinae and Palaeothentinae, and within the latter *Palaepanorthus primus* is the sister-group to *Palaeothentes*. In contrast, our results recovered a *Antawallalestes -Carlothentes* clade, which was positioned as sister-group to the *Palaepanorthus-Palaeothentes* clade.
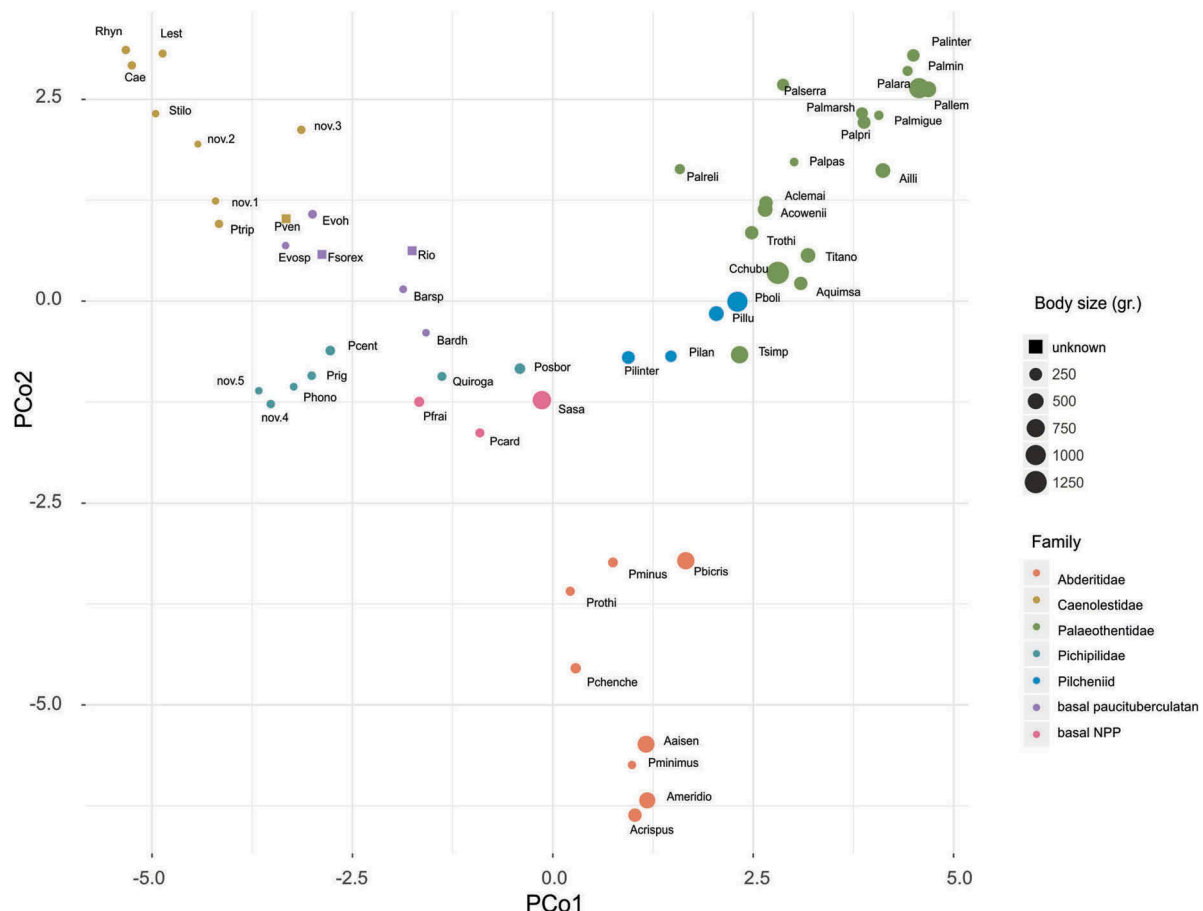

Figure 3. Morphospace occupation for paucituberculatans based on the first two principal coordinate axes. Major paucituberculatan groupings (Caenolestidae, Pichipilidae, *Pilchenia*, Abderitidae, Palaeothentidae) and basal taxa (basal paucituberculatans and basal NPP) are differently colored (legend on graph). Size of circles that represent each species indicates the reconstructed body mass (legend on graph). For identity of species acronyms, see Abbreviations.
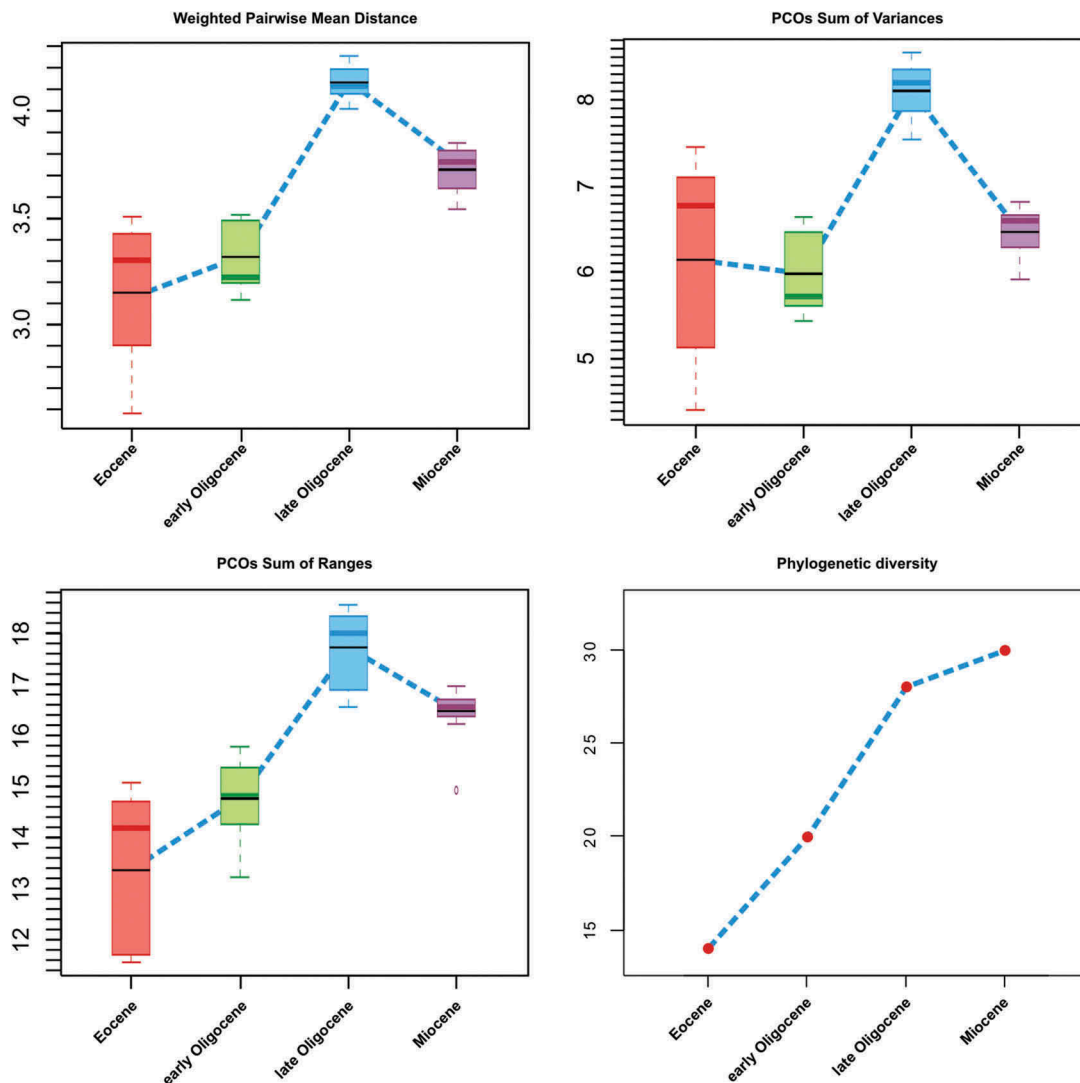
Figure 4. Phylogenetically corrected disparity and diversity metrics of non-Pihipilidae palaeothentoids through time. Interquartile intervals built on jack-knifed resampled distributions.

Diversity analysis

With thirty-five recognized species, the NPP is the taxonomically richest paucituberculatan clade (SM3). Otherwise, caenolestids group thirteen species, seven of which are living (Ojala-Barbour et al. 2013), and pichipilids include eight extinct species. The fossil record of both caenolestids and pichipilids is best in the Miocene, therefore long ghost lineages have emerged from their inferred origins in the middle Eocene (Figure 2). In contrast, the NPP are relatively well recorded since the late Eocene and showed an increase of phylogenetically corrected diversity over time. Their diversity strongly increased from the early to late Oligocene, and later on, during the early Miocene, showed the highest value. Comparing the NPP clades, palaeothentids were more diverse than abderitids in all time bins, and both groups had their highest diversity values in different times: abderitids in the late Oligocene, and palaeothentids in the early Miocene (Table 1). From the counting of the recorded NPP species and ghost lineages in each time-bin (Table 1), it can be seen that taxic diversity estimates for the Eocene and Oligocene resulted in a large underestimate of diversity values with respect to those calculated taking into account the phylogenetic information.

Disparity analysis

Non-Pichipilidae palaeothentoids (NPP) were significantly more disparate than caenolestids and pichipilids (Tables 1 and 2; Figure 3). Regarding disparity differences of the NPP over geological time, Euclidean distances show a marked disparity increase from the early Oligocene to the late Oligocene, and a drop from the late Oligocene to the early Miocene (Figure 4). Statistical tests performed on Euclidean distances and jack-knifed WMs (see Table 2) showed no significant differences between Eocene and early Oligocene data, and some found no significant differences between late Oligocene and Miocene data either. In contrast, significant differences between early and late Oligocene data were found, indicating that pre-ordination disparity has changed through Oligocene. In addition,
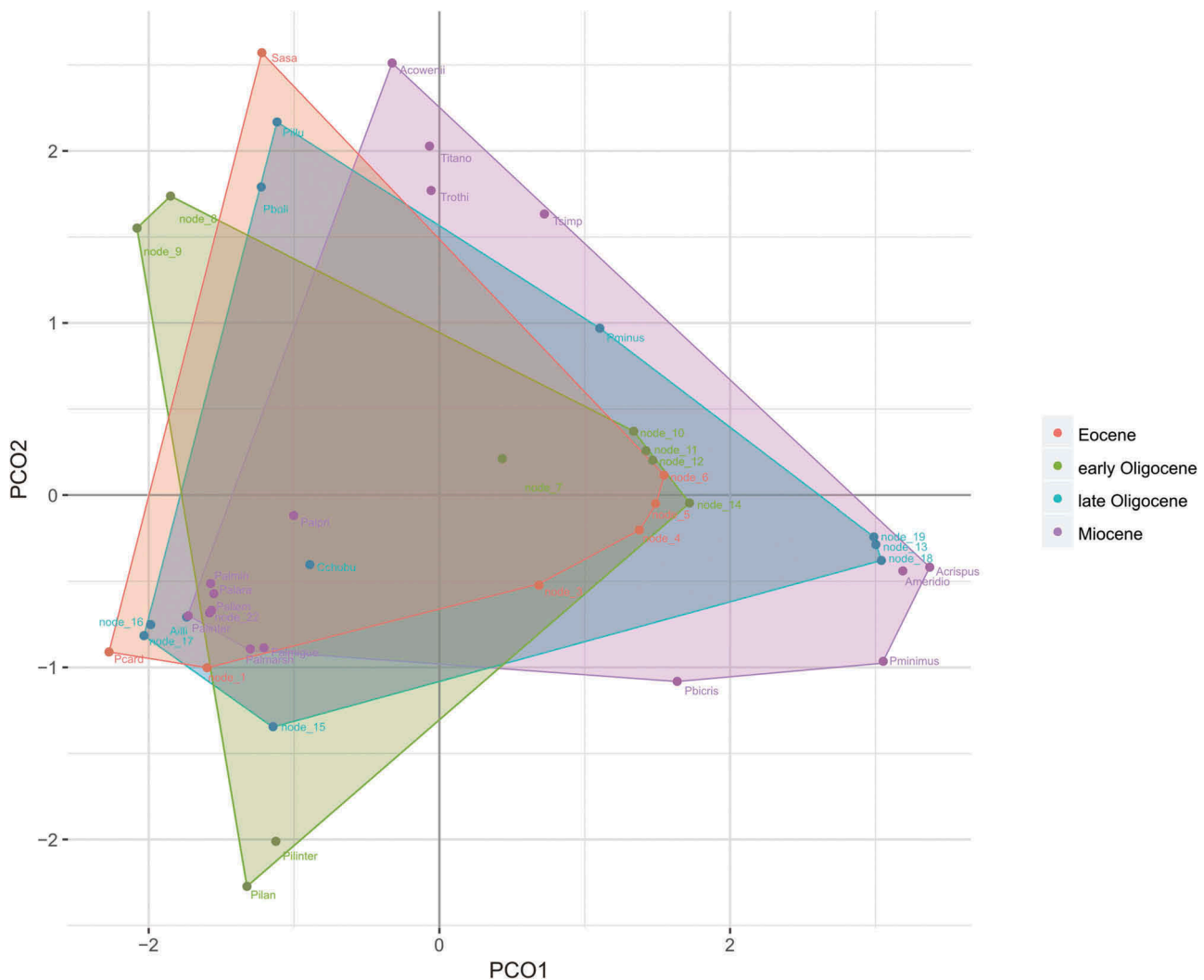
Figure 5. Morphospace occupation by non-Pichipilidae palaeothentoids over time. Plots are based on the first two axes from the PCo. Minimal morphospace occupation by taxa and reconstructed ancestors of each time bin are indicated by differently colored convex hulls (legend on graph). For identity of species acronym, see Abbreviations.

some tests found significant differences between jack-knifed late Oligocene and Miocene WMs (Table 2). Results of pre-ordination metrics based on RED and GC are mostly coincident with those found with GED. In contrast to GED, RED and GC recovered a significant increase between Eocene and early Oligocene, but a similar disparity trajectory as GED in latter time-bins: a significant disparity increase between early Oligocene and late Oligocene, and a decrease between late Oligocene and early Miocene (see SM4.7).

Analyzing post-ordination distances based on GED, SV behaved similar to SR, suggesting an increase of disparity from early to late Oligocene. This can be recognized as an expansion and dispersion of the occupied morphospace (see Figure 5). On the other hand, SR (similarly to WMPD) shows a small increase from the late Eocene to the early Oligocene but, in contrast to WMPD and SV, there is an increase from the late Oligocene to the early Miocene. However, statistical tests performed on PCo scores did not find significant differences between these successive time-bins (Table 2). On the other hand, tests performed on distributions of SVs and SRs

generated by jack-knifing of the matrices rendered significant differences between both early and late Oligocene and between late Oligocene and Miocene (Table 2).

Evolution of body size

Estimated body masses of paucituberculatans range from 9 g for the caenolestid sp. nov. 2, to 1272 g in *Carlothentes boliviensis* (SM5, Table 2). Caenolestids and pichipilids includes small species (mean = 40g), except for the medium-sized pichipilid *P. osborni* of 120 g. During their evolutionary history, caenolestids and pichipilids remained small-sized (Figure 2). However, certain phyletic changes can be seen, particularly among pichipilids, in which a succesive increase in size is seen in the *Pichipilus* clade, while a decrease occurs in the clade that groups *Phonocdromus gracilis*, sp. nov 5 and sp. nov 4. In contrast to the mentioned paucituberculatans, the NPP have a significatively broader range of body masses (SM5, Figure 1). Among the smallest species are the abderitids *Parabderites minusculus* and *Pitheculites* spp. (36 g
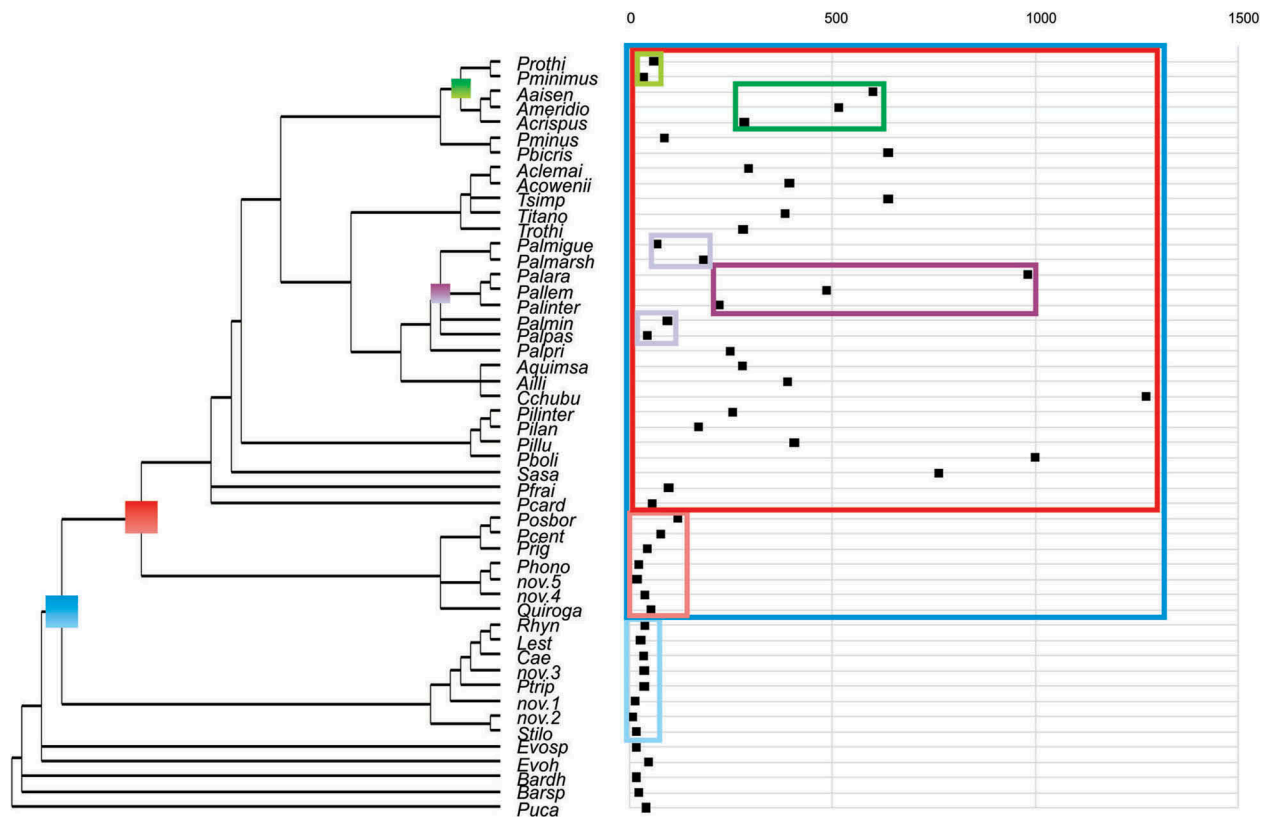
Figure 6. Paucituberculatan reconstructed body masses plotted on the cladogram (using the function *dotchart.phylog* of the R package *ade4*) highlighting nodes with most significant autocorrelation signal as detected by orthonormal decomposition (see SM5). En each highlighted node, distribution of body masses in each of its descendant clades is depicted in different shades of color to enhance visualizations of evolutionary patterns of size variability.

to 109 g), and the palaeothentids *Palaeothentes pascuali* and *P. minutus* (45g and 95g respectively). The largest NPP include *Pilchenia boliviensis* (1000g), *C. chubutensis* and the palaeothentid *Palaeothentes aratae* (900g). In the earliest cladogenesis of the NPP, there is a sharp change of body masses from 57g-97g to 284g-407g, at the node that clusters *Sasawatsu, Pilchenia*, abderitids and palaeothentids. With a 292% increase, this represent one of the largest changes in paucituberculatan body size evolution. Afterwards, from medium-sized ancestors (284g–407g), all the NPP major clades underwent increases and/or decreases in body masses. The *Pilchenia* clade includes one large species, *P. boliviensis*, and a group of smaller species, ranging from 407g in *P. lucina* to 172g in *P. antiqua*, which exhibits a trend of size decrease. Among abderitids, a phyletic trend of increased body mass is present in the *Abderites* clade, while its sister-group *Pitheculites* clusters some of the smaller NPP. The evolutionary change leading to *Pithecultites* involved a net decrease of 175g (260% decrease), and thus represents the second largest decrease among paucituberculatans after that of the autapomorphic nanism represented by the abdertitid *P. minusculus* (−198 g or 330% decrease). Similar to abderitids, palaeothentids include a diverse size range of paucituberculatans. Acdestines do not exhibit trends in body mass changes, and include only medium- to large-sized species. Among the largest species were *Acdestis maddeni* (554g) and *Titanothentes simpsoni* (638g). On the other hand, palaeothentines include an autapomorphic giant, *C. chubutensis* (+ 881 g or 225% increase) and several of the smaller

NPP, which are grouped in the *Palaeothentes* clade (e.g. *P. pascuali*). A trend towards body size decrease is present in the lineage leading to *Palaeothentes*. Within *Palaeothentes*, a successive size increase along three branches is seen in the *P. intermedius* (*P. aratae-P. lemoinei*) clade, thus representing a third-order case of phyletic giantism. In this trend, *P. aratae* achieves its larger size accumulating 798g from the *Palaeothentes* node, which represents a total increase of 536%.

Significant phylogenetic autocorrelation was detected by the Orthonormal Decomposition and Abouheif C methods, implying that body size variability was significantly linked to phylogeny, i.e. that closely related species have a greater probability of having similar body size values. Orthonormal Decomposition detected significant autocorrelation in several nodes illustrated in Figure 6. Abouheif C was significantly different from the null-model in both Paucituberculata and the NPP clades, supporting those results (SM5). In addition, Orthonormal Decomposition allows a finer exploration of autocorrelation. For each significant orthobase describing a given node with high autocorrelation, the same pattern could be recognized: one descendant clade showing high variability in body mass, while the other descendant clade shows a constrained diversity in body mass, therefore suggesting that members of the latter clade share an inherited pattern of body size (Figure 6). Concerning non-parametric statistics tested on orthobases, all four of them were significantly different from the uniform null-model for the entire Paucituberculata tree (see SM5), indicating that strong autocorrelation was focused on a few basal nodes, influencing derived branches.

When applied exclusively to the NPP tree, R2Max was the only statistic that was not significantly different from the null-model, indicating that the autocorrelation was not concentrated on a few basal nodes but spread over several more derived ones (see Ollier et al. 2006). In addition, graphical tools accompanying R implementation of Orthonormal Decomposition allow exploration of those nodes with stronger signal, which are depicted in Figure 6 and in SM5 (summarising information of most significant nodes over the orthobases matrix). Most significant nodes are those joining caenolestids and the remaining paucituberculatans on one hand, and those joining pichipilids and NPP on the other. Following these most significant nodes, there are those joining species within *Palaeothentes*; and joining the *Abderites* and *Pitheculites* clades.

Correlation between disparity (depicted as GEDs and PCo scores from the pruned matrix) and body size as tested herein did not find a significant relationship. Therefore, we may conclude that there is no direct link between morphological disparity and body size. Indeed, evolutionary changes in body size as detected by means of cladogram mapping and autocorrelation statistic show no coupling with greater changes in disparity as suggested by our analyses. Instead, greater changes in disparity occur between the early and late Oligocene, while significant evolutionary changes in body size appear to occur mainly during the middle Eocene and late Oligocene-Miocene (see Figure 2).

Discussion

Climates and environments in south america during the eocene and the origin of the NPP

During the Eocene, global temperatures were high and greenhouse conditions persisted up to the end of this period (Zachos et al. 2001, 2008; Hansen et al. 2013). No major Atlantic transgressions occurred, except for that which partially flooded southern Patagonia during the late middle Eocene (Malumian and Nañez 2011), and the most intense uplift pulses of the Andes had not yet begun (Hoorn *et al.* 2010; Ramos and Ghiglione 2008). Paleobotanical and palynological evidence indicate that vegetation was of tropical and subtropical type, widespread in South America, reaching middle and high latitudes with a high floral richness (Wilf et al. 2005; Jaramillo et al. 2006). During the early Eocene there was a predominance of the floristic elements indicating hot and humid climates in southern South America. The climatic reconstructions derived from this flora indicate subtropical temperatures and bi-seasonal precipitations (Villagrán and Hinojosa 2005; Barreda and Palazzesi 2007), although other studies suggest somewhat lower precipitation for the flora of central-western Patagonia (Wilf et al. 2013). From the middle Eocene to the early Oligocene, an expansion of the *Nothofagus* forests indicates a progressive replacement of the subtropical flora by a more temperate one (Barreda and Palazzesi 2010). Climatic seasonality is recorded (Barreda and Palazzesi 2010) and there are a few indicators of xeric environments (Villagrán and Hinojosa 2005; Barreda and Palazzesi 2007). Notwithstanding, during the middle Eocene phytolithic associations show the predominance of forest environments and

the first evidence of mega-thermic grasses in central Patagonia, although these grasses had a low abundance (Zucol et al. 2010; Strömberg et al. 2013). These paleoenvironmental inferences derived from the flora coincide with those derived from mammalian faunas. During the early and middle Eocene, mammal communities were dominated by browsing types, with a low diversity of mixed feeders and 'grazers' (i.e., mammals with hypsodont/hypselodont molar morphologies that may have fed on grasses and/or vegetation with exogenous grit). The browsers, together with the insectivore and the omnivore-insectivore types, allow us to infer the existence of subtropical forest environments, developed under hot and humid climates (Ortiz-Jaureguizar and Cladera 2006). Finally, during the late Eocene mammalian communities continue to be dominated by browsers, but the diversity of mixed feeders and 'grazers' increased. This rise in diversity of these two groups suggests a similar increase of open environments (Ortiz-Jaureguizar and Cladera 2006). The NPP arose in this paleoenvironmental context, approximately 43–45 Ma ago (middle Eocene, Figure 2). At the time, they expanded their taxonomic diversity and disparity, as expected in an initial radiation (Foote 1993b). Mammal assemblages where early NPP have been found, show that they coexisted with other small-sized mammals such as basal 'ameridelphians' (see Goin et al. 2016) and polidolopimorphian metatherians, and the first caviomorph rodents and primates registered in South America (Frailey and Campbell 2004; Goin and Candela 2004; Bond et al. 2015; Antoine et al. 2016). The first NPP were small-sized, as were members of their sister-group Pichipilidae and the most basal caenolestids, but early in their history they became larger. Approximately between 38 and 39 Ma, a threefold increase in body size occurred in the evolution of the NPP (see Figure 2). This change is concurrent with a functional and taxonomic turnover in metatherian assemblages identified at least in southern South America (Goin et al. 2016). This turnover has been related to an abrupt fall in precipitation and the establishment of relatively warm but more arid conditions from the early late Eocene onwards (Goin et al. 2016).

The EOB and the evolution of the NPP

The isolation of Antarctica due to the widening of the Tasman Sea and the full opening of the Drake Passage by the Eocene–Oligocene boundary (EOB), produced a ring of cold marine waters around the continent. Under the influence of this ring, glaciation began in the Antarctic continent, and this glaciation marked (at least in mid- and high latitudes located north and south of the Equator) the end of the last phase of the so called Greenhouse World and the beginning of the Icehouse World (Hansen et al. 2013). Additionally, the global cooling recorded during the EOB has been explained by other two concurrent factors: a higher albedo, and the progressive closing of tropical oceanic gateways (Lawver and Gahagan 2003; Livermore et al. 2005). The flora established since the late Eocene in the middle and high latitudes of South America, was characterized by a peculiar mixture of elements with a current Austral-Antarctic distribution, together with others of Neotropical and Pantropical distribution, and others with a wide geographical distribution. This peculiar conformation of temperate-cold and warm elements

led to its denomination as *Paleoflora Mixta* ('Mixed Paleoflora', Troncoso and Romero 1998). The climatic estimates derived from this flora, as well as the palynological assemblages recorded in deposits of the Fuegian Andes (Roselló et al. 2004) allow us to infer annual average temperatures and annual average rainfalls much lower than those of the early and middle Eocene (Villagrán and Hinojosa 2005), similar to those of the current dry forests of the Holdridge life zones (e.g., Lugo et al. 1999). The mentioned environmental changes appear to have triggered a major biotic turnover (Flynn et al. 2003; Goin et al. 2010), named the *Bisagra Patagónica* (the 'Patagonian Hinge'; Goin et al. 2010; 2012, 2016, in press), which represents in South America a regional expression of the Terminal Eocene Event. Among metatherians, this resulted in the demise of groups that had flourished during the Paleocene and Eocene, such as basal 'ameridelphians', Caroloameghiniidae (Didelphimorphia; Goin et al. 2016), Glasbiidae, Bonapartherioidea and Polydolopidae (Polydolpimorphia; Goin and Candela 2004; Chornogubsky 2010). Other metatherians groups radiated, giving rise to more modern lineages; among which there were the argyrolagoid polydolopimorphians (but see, Beck 2017), borhyaenid and hathliacynid sparassodontans (Forasiepi 2009), microbiotheriid microbiotherians (Goin and Abello 2013), and palaeothentoid paucituberculatans, which formed taxonomically different assemblages towards the end of the Paleogene and during the Neogene. The taxonomic diversity of metatherians sharply dropped near the EOB and, from an ecological point of view a functional turnover occurred, with its most notable aspects being the diversification of carnivorous (sparassodontans) and granivorous (argyrolagoid) types, and the decline of frugivores (Zimicz 2012; Goin et al. 2016). In this context, paucituberculatans were one of the metatherian groups that diversified more widely. However, across the EOB both components of diversity may be decoupled as taxonomic diversity increased while most disparity metrics evidenced non-significant change (Figure 4, Table 2, SM4.7). Occupied morphospace shows no significant range change between the Eocene and early Oligocene time bins (Figures 4 and 5; Table 2). These results may be due to morphologies that appeared in the initial diversification of the Abderitidae+Palaeothentidae clade, during the early Oligocene, compensating for the morphological diversity lost by the disappearance of *Sasawatsu* and *Perulestes* species at the end of the Eocene (Figure 5). On the other hand, the increasing taxonomic diversity of the NPP during the early Oligocene is mostly explained by the early cladogenesis of abderitids and palaeothentids (Figure 2). These paucituberculatans were successful in comparison to basal members of the NPP as they diversified during the Oligocene (see below) and became an important component of the micromammal assemblages during the Miocene (Marshall 1990; Bown and Fleagle 1993; Abello 2007; Rincón et al. 2015, Engelman et al. 2016).

Evolution of the NPP in the aftermath of the EOB climatic events

As mentioned in the previous paragraphs, the South American flora of the early Oligocene was similar to that of the late Eocene, with a progressive replacement of warm flora by a more temperate one, suggesting the existence of forests developed under abundant rainfalls (Barreda and Palazzesi 2010). Late Oligocene plant communities in Patagonia were characterized by a marked presence of groups with herbaceous-shrubby habits (Barreda and Palazzesi 2010), while xerophytic species would have occupied coastal salt marsh environments and open patches in internal areas, probably as a consequence of volcanic activity (Barreda and Palazzesi 2007). However, closed forests were still present in extra-Andean Patagonia (Barreda and Palazzesi 2010). This allows us to infer the establishment of a marked floristic contrast between the continental and coastal environments (Barreda and Palazzesi 2007).

The climatic-environmental inferences derived from mammals are congruent with botanical ones. The early Oligocene land-mammal communities were dominated by the browsers, but mixed-feeders and 'grazers' were more diverse than during the Eocene (Ortiz-Jaureguizar and Cladera 2006). This is compatible with the existence of wooded savannas, developed within a low annual rainfall regime. In the late Oligocene, and for the first time during the Cenozoic, the land-mammal communities become dominated by 'grazers', although browsers and frugivores still showed a high diversity (Ortiz-Jaureguizar and Cladera 2006). The predominance of mammals with hypsodont/hypselodont molars suggests that the open habitats had a wide areal distribution in central Patagonia during the late Oligocene. This new distribution of trophic types is compatible with a wooded savanna vegetation, although somewhat more open than that of the early Oligocene (Pascual *et al.* 1996).

From the early to the late Oligocene, the taxonomic diversity of NPP continued to increase, and, in contrast to the previous time interval, disparity experienced a significant increase, reaching its highest values towards the late Oligocene (Figure 4, Table 1). The concordant expansion of both components of the diversity suggests a gradual occupation of morphospace with no apparent constraint on morphological diversity (Erwin 2007). This pattern could be explained by the evolution of the NPP in an empty ecospace. Considering the ecospace hypothesis (see Erwin 1993, 1994), the release of ecological interactions following the extinctions at the EOB, as well as the availability of new resources, would have been key for novel morphologies emerged among NPP to become successful, allowing subsequent diversification. As expressed above, several metatherian lineages disappeared at the end of the Eocene (e.g. basal 'ameridelphians') or at the beginning of the Oligocene (e.g. polydolopids). Polydolopids and abderitids have a similar plagiaulacoid type of dentition and it has been proposed that they must have had a similar ecological role (Ortiz-Jaureguizar 2003). They potentially coexisted during the early Oligocene, as the reconstructed earliest lineages of abderitids were coeval with the last recorded polydolopid. However, the last registered polydolopids were relatively large metatherians, the largest one having a body mass of 3.5 kg (Zimicz 2012; Goin et al. in press) represented the end of a trend to size increase in this group (Goin et al. 2016), while the reconstructed body mass of the first abderitids was between 284–391g (Figure 2). Therefore, abderitids and polydolopids hardly competed then nor did abderitids opportunistically replaced polydolopids; rather it appears that abderitids

diversified because new ecological opportunities were generated by the vacancy left by the demise of other small-sized marsupials (e.g. Caroloameghiniidae, Zimicz 2012), and/or due to the availability of new resources. In extant marsupials with dental shearing complexes similar to those of abderitids (e.g. the burramyid *Burramys*, the hypsiprymnodontid *Hypsiprymnodon* and the potoroid *Bettongia*; Figure 1D-F), these dental modifications have been observed to be well-adapted to deal with diverse food items bearing hard coverings (e.g., insects and seeds), and they are associated with different dietary types, ranging from insectivore-frugivore and mostly frugivorore to fungivore-omnivore (Dimpel and Calaby 1972; Seebeck and Rose 1989; Strahan 1995). Regarding these ecological data, it is possible that, as was suggested by Dumont et al. (2000), abderitids were dietary generalists since their derived dental complex would allow them to have access to a wide range of food items. Thus, as climatic-environmental conditions were changing, such dental modifications could have been advantageous for the abderitids to exploit new food resources.

In contrast to the lophodont molars and the specialized plagiaulacoid dentition of abderitids, palaeothentids developed more crested molars, with crests not particularly transverse as in abderitids, and an enlarged first lower molar with a less emphasized shearing crest (Marshall 1980; Bown and Fleagle 1993; Abello 2013; Figure 1B-C). Among palaeothentids, the derived characters of acdestines that differentiate them from their sister group, the palaeothentines, include a longer shearing crest in the first lower molar, a quite reduced third lower premolar and a marked decrease in size from the first to the last molar (Bown and Fleagle 1993; Abello 2013; Figure 1C). Therefore, besides that of abderitids, two dental morphologies each characterizing the major clades of palaeothentids, appeared during the early Oligocene. Inferred diets for abderitids range from insectivore-frugivore, in the small *Pitheculites minimus*, to frugivore in the larger *Abderites meridionalis*. On the other hand, reconstructed dietary preferences of palaeothentids were broader, also including insectivory (Dumont et al. 2000), and probably also the opportunistic consumption of small vertebrates (Engelman et al. 2016). The various feeding habits reconstructed for palaeothentids coupled with the fact that they lacked a well-developed shearing complex led to Dumont et al. (2000) to consider palaeothentids as dietary specialists. Despite the fact that the ecological diversity of palaeothentids is mostly known from the Miocene fossil record, it can be inferred that it began to expand, as did their taxonomic diversity, during the Oligocene. By the late Oligocene, palaeothentids showed roughly twice the phylogenetically corrected diversity of abderitids (Table 1). Taking into account the climatic-environmental changes occurred near the EOB, it seems probable that, as in abderitid evolution, changing ecological conditions could have shaped the palaeothentid radiation, promoting extensive cladogenesis and ecological divergence among lineages.

From the Miocene, the tectonic activity becomes more important in the continent, as a consequence of the increase in the subduction rate between the South American and Nazca plates and the progressive raising of the Andes. The orographic change associated with this greater tectonic activity had a remarkable climatic and biotic impact in South America: it generated the marked contrast existing between the wet western and arid eastern environments of the southernmost part of the continent, as a result of the differentiation of the Andean region as a barrier to the wet winds from the Pacific Ocean (see Villagrán and Hinojosa 2005; Ortiz-Jaureguizar and Cladera 2006; Pascual and Ortiz-Jaureguizar 2007). During the early Miocene, the geological-geographical features of the continent were similar to those of the late Oligocene. However, at the end of this period, an explosive volcanism registered from central Patagonia to the Strait of Magellan injected into the atmosphere a high amount of volcanic ash, which gave rise to the thick deposits of tuffs that represents this time in Patagonia. Also, during this time a new Antarctic glaciation was recorded, and a well-defined climatic seasonality was established in Patagonia (Ortiz-Jaureguizar and Cladera 2006). Until the final part of the early Miocene, sea levels remained relatively low and temperatures remained relatively constant (Markgraf et al. 1996).

The flora of the early Miocene showed pulses of advance and retreat of the indicator communities of arid and humid climates (Barreda and Palazzesi 2010). The phytoliths continued to indicate a predominance of the arboreal vegetation, but with an increase of grasses of open zones. This has been interpreted as an indicator of the enduring existence of forests with open areas with grasses, or the existence of temporary variations due to seasonality, aridity or other environmental factors that damage the forests at certain times of the year and favour grasses (Strömberg et al. 2013). The early Miocene land-mammal fauna was similar to that of the late Oligocene, i.e., dominated by the 'grazers' but with a high diversity of browsers and frugivores. The main difference between both faunas is the greater number of mixed-feeders in the early Miocene, which indicates that during this time there was a balance between the open habitats and the forests, probably represented by park-savannah formations. The record of primates and other climate-sensitive vertebrates (such as frogs and anteaters) in latitudes as high as southern Patagonia, suggests that warm and humid climates, as well as forested formations, were well developed in Patagonia (see Kay et al. 2012). Together with these records, the diversity of some mammals adapted to open vegetation and drier climates [e.g., relative richness of dasypodid xenarthrans (see Vizcaíno et al. 2006, 2012), some caviomorph rodents such as eocardiids and dasyproctids (e.g., Candela et al. 2012)] suggest the existence of climatic events of greater aridity or complex environments represented by a mixture of closed and open plant formations (Pascual *et al.* 1996; Ortiz-Jaureguizar and Cladera 2006; Kay et al. 2012).

From the late Oligocene to the early Miocene, the taxonomic diversity of NPP continued increasing while disparity did not exhibit a clear change or even decreased, thus becoming again decoupled in some extent (Figure 4, Table 2, SM4.7). This pattern could be partially explained by the extinction of the *Pilchenia* and the *Antahuallathentes-Carlothentes* clades during the late Oligocene (Figure 2). However, the macroevolutionary pattern of both components of diversity from the late Oligocene to the Miocene is also compatible with the NPP having reached

certain constraints in their morphological evolution. It has been stated that in certain models of evolution, cladogenesis may progress up to some limits to form are reached, after which new lineages emerge as variations of the primary morphotypes filling in the gaps of the morphospace (Foote 1994; Wesley-Hunt 2005; Brusatte et al. 2010; Benton et al. 2014). Considering this model, the high Miocene taxonomic diversity of NPP can be seen as the result of a lineage proliferation within certain morphological bounds set in the Oligocene (i.e., those characterizing abderitids, palaeothentines and acdestines, see above). As shown by the morphospace occupation, Miocene species of the *Abderites-Pitheculites* and *Palaeothentes* clades constitute clumps. It appears that new lineages of NPP emerged in Miocene times by evolving minor differences in dental morphology and accentuated body size differences (Figure 2). This could be the case of *Palaeothentes* cladogenesis, a clade that includes almost half of the NPP species known from the Miocene. *Palaeothentes* species are quite similar dentally and they mainly differ in body size. In the late early Miocene of southern South America (Santa Cruz Formation; Vizcaíno et al. 2012), five *Palaeothentes* species coexisted ranging in body mass from 45 g (*P. pascuali*) to 981 g (*P. aratae*). Except for the frugivore *P. aratae*, remaining species were insectivores (*P. pascuali, P. minuntus, P. intermedius*, and *P. lemoinei*; Dumont et al. 2000). It was suggested that body size differences evolved among insectivore species of *Palaeothentes* allowing niche separation as occur in extant insectivore assemblages of dasyurid marsupials and soricid placentals (Abello et al. 2012). In addition, considering the diverse small-sized non carnivorous metatherians coexisting in Santacrucian assemblages, it was proposed that certain niche partitioning in the vertical dimension could not be ruled out. In this sense, in addition to the terrestrial curso-saltatorial *P. minutus* and *P. lemoinei* (Abello & Candela 2010; Forasiepi et al. 2014), NPP species may have had other substrate uses as arboreal or scansorial habits. This differential use of space would have allowed the coexistence of several marsupial species minimizing competition, as happens in extant ecosystems (Charles-Dominique et al. 1981; Charles Dominique 1983; Vieira and Monteiro-Filho 2003; Ernest 2013).

Conclusions

The Eocene-Oligocene boundary marks a period of remodeling of the South American metatherian assemblages. The changes in climatic-environmental conditions that occurred since then affected groups differently; while some of them declined and went extinct, others such as non-pichipilid paucituberculatans, diversified probably by the exploitation of new ecological opportunities.

Non-pichipilid paucituberculatans experienced a wide radiation, with an extensive cladogenesis and ecological diversification, which can be recognized by contrast with the evolutionary history of its sister clade Pichipilidae and the Caenolestidae. In all macroevolutionary parameters analyzed, i.e., taxonomic diversity, disparity and body mass range, the NPP reached values significantly higher than those of the remaining paucituberculatan clades.

Tracking the taxonomic diversity and disparity of NPP through time, was observed that each analyzed transition among time bins (middle-late Eocene to early Oligocene; early Oligocene to late Oligocene; late Oligocene to early Miocene) was characterized by different macroevolutionary patterns. From the initial radiation in the middle Eocene, taxonomic diversity increased through time, although it was decoupled from disparity from the middle-late Eocene until the early Oligocene, and then, from the late Oligocene until early Miocene. The Oligocene was the most important time in the evolution of the NPP, since this was when a significant and concordant expansion in disparity and taxonomic diversity occurred. This evolutionary response appears to have been triggered by the evolution in an empty ecospace. At that time, three different dental morphologies emerged, each one characterizing the main NPP clades: Abderitidae, Palaeothentinae and Acdestinae, and major cladogenetic events occurred in the *Abderites-Pitheculites* and Palaeothentidae clades. In addition, at the end of this epoch, and during the Miocene, they evolved a wide range of body masses that reflects an important ecological diversification. The increased taxonomic diversity from the late Oligocene to early Miocene and concurrent decrease in disparity during this temporal transition was probably the result of a wide diversification constrained within a given morphology. Among the NPP, the abderitid and palaeothentid clades followed different patterns of diversification. Palaeothentids were more speciose than abderitids at any time, and the latter reached their highest taxonomic diversity earlier (late Oligocene) than palaeothentids (early Miocene). These different patterns may be due to, as was proposed earlier (Dumont et al. 2000), the condition of dietary generalist (abderitids) or specialist (palaeothentids) of the species of each major NPP group.

Disclosure statement

No potential conflict of interest was reported by the authors.

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