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PHYLOGENETIC EIGENVECTOR REGRESSION IN PALEOBIOLOGY

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ABSTRACT – Phylogenetic Eigenvector Regression (PVR) is a flexible comparative method that allows testing several hypotheses on phylogenetic signal and correlated evolution among traits. Selected phylogenetic eigenvectors extracted from a phylogenetic distance matrix among taxa allow representing their phylogenetic relatedness in a raw-data form (*i.e.* instead of a distance matrix) and can then be used as explanatory variables in statistical models aiming to estimate phylogenetic signal or phylogenetically corrected correlations. Because of the growing use of PVR by paleobiologists in recent times, here the main theoretical/statistical basis of the method and the developments made in the 15 years after its original proposition are reviewed, highlighting further potential applications in paleobiology. For the first time, a multivariate extension of the phylogenetic signal-representation curve for estimating phylogenetic signal is presented. Another innovation is to show how PVR can be used to assess morphological disparity in a phylogenetic context. A dataset of cranial morphology and function of 35 theropod dinosaur genera is used to illustrate the applications of PVR and how it can be used to answer four questions: (i) what are the phylogenetic patterns in theropod skull shape? (ii) is possible to tease apart the evolutionary models underlying variation in traits? (iii) how are evolutionary history, function, and diet related to variation in theropod skulls? (iv) what are the evolutionary components of morphological disparity in theropod skulls? Answering these questions provide a roadmap for using PVR to address a range of issues relevant to contemporary paleobiology research programs.

Key words: comparative methods, morphometrics, phylogenetic eigenvectors, Theropoda.

RESUMO – A Análise de Regressão por Autovetores Filogenéticos (PVR) é um método comparativo flexível que permite testar diversas hipóteses sobre sinal filogenético e evolução correlacionada de caracteres. Autovetores extraídos da matriz de distâncias filogenéticas representam a relação evolutiva entre os táxons de forma vetorial, sendo facilmente inseridos em análises de correlação ou modelos de regressão. Considerando o grande interesse recente de paleontólogos por essa abordagem, aqui foi revisado o PVR e apresentado os seus desenvolvimentos nos últimos 15 anos, com destaque para aplicações potenciais em paleobiologia. Apresenta-se, pela primeira vez, uma extensão multivariada de uma abordagem sequencial do PVR com o objetivo de avaliar modelos de evolução (a curva sinal-representação) e como os autovetores filogenéticos podem ser utilizados para avaliar a evolução da disparidade morfológica. A aplicação das diferentes técnicas foi demonstrada com dados de evolução do crânio em dinossauros terópodes, respondendo a quatro questões básicas: (i) qual o sinal filogenético na forma do crânio? (ii) é possível utilizar o PVR para avaliar os modelos de evolução relacionados a esse sinal? (iii) como a variação na história evolutiva, função e dieta estão relacionadas à variação na forma craniana? (iv) quais são os componentes evolutivos da disparidade no crânio dos terópodes? Ao responder a essas questões, é fornecido um roteiro sequencial de como o PVR pode ser aplicado para avaliar uma série de perguntas relevantes no programa de pesquisa atual em paleobiologia.

Palavras-chave: métodos comparativos, morfometria geométrica, autovetores filogenéticos, Theropoda.

INTRODUCTION

A fundamental goal of many paleontological research programs is to produce phylogenies of extinct taxa, which show how fossil species are related to each other and to living taxa. Phylogenies are not merely an end in themselves, and in recent years there has been an increasing interest in applying phylogenetic analyses in paleobiological studies (Pennel & Harmon, 2013). For example, phylogenies have become essential tools for evaluating patterns of diversification through time, allowing better estimation of speciation and extinction rates (*e.g.* Quental & Marshall, 2010; Stadler, 2013), and to test for phylogenetic patterns and correlated evolution between pairs of traits (Hunt, 2006; Hunt & Carrano, 2010; Slater *et al.*, 2012). In the later case, adding phylogeny is important for taking into account the non-independence among taxa (or phylogenetic autocorrelation) and, therefore, for controlling Type I error rates (Felsenstein, 1985; Hansen

& Martins 1996; Hansen *et al*., 2008). Statistically, this non-independence generated by evolutionary dynamics of traits during diversification creates phylogenetic autocorrelation of phenotypes among taxa, which is an unwelcome effect that may complicate our ability to understand patterns and rates of character evolution (Gittleman & Kot, 1990; Martins, 1996; Diniz-Filho, 2001; Revell *et al.*, 2008; see Blomberg & Garland, 2002 for a discussion on terminology).

There are several statistical approaches to estimate phylogenetic autocorrelation (*i.e.* phylogenetic signal) and to control for this when testing the correlation between traits (see Pavoine *et al*., 2007; Pavoine & Ricota, 2012; Munkmuller *et al.*, 2012; Hernandez *et al.*, 2013 for recent reviews). In a regression context, two analytical frameworks have been used to model traits that are phylogenetically patterned (Martins & Hansen, 1997). First, variables representing patterns of phylogenetic relationships among the species can be incorporated as explanatory variables or as covariables (depending on the goals) in a standard Ordinary Linear Regression (**OLS**) model, resulting (ideally) in a model with independent and homoscedastic residuals (because phylogenetic structure was already incorporated in the model). Second, the residual covariance structure can be modified to directly incorporate non-independence among taxa and describe their expected evolutionary relationships under a given evolutionary model (*e.g.* Brownian motion and Ornstein-Uhlenbeck) in a Phylogenetic Generalist Least-Squares (**PGLS**) approach (Martins & Hansen, 1997). In PGLS, the regression parameters are thus estimated already assuming the phylogenetic patterns in residual variation, whereas in OLS the variation among species is assumed to be independent.

Allowing for "phylogenetic" variables (*i.e.* the first framework) to study trait variation throughout a phylogeny or correlated evolution, in turn, can be achieved by different strategies. Considering a cross-species dataset with a trait as a response variable, one strategy is to extract eigenvectors from a phylogenetic distance matrix using a Principal Coordinate Analysis (see Legendre & Legendre, 2012; Bookstein, 2013; see also Ollier *et al.*, 2006) and, subsequently, incorporate some of the eigenvectors as explanatory variables that express the phylogenetic relationships among species or genera (see Diniz-Filho *et al.*, 1998, 2012a,b; Desdevises *et al.*, 2003; Peres-Neto, 2006; Kuhn *et al*., 2009; Peres-Neto *et al.*, 2012 - see also Borcard & Legendre, 2002; Griffith & Peres-Neto, 2006; Dray *et al.*, 2006; Bini *et al.*, 2009 for the use of this approach in a spatial context). In general, this approach has been called eigenfunction (spatial or phylogenetic) analysis (see Peres-Neto & Legendre, 2010; Peres-Neto *et al.*, 2012)

The strategy of regressing a trait on phylogenetic eigenvectors was originally proposed by Diniz-Filho *et al.* (1998) and was called Phylogenetic EigenVector Regression (**PVR**). The PVR can be used both to estimate the phylogenetic signal in a dataset (*i.e.* the coefficient of determination R^2 of the linear model of a trait regressed against the phylogenetic eigenvectors) and to study correlated evolution between multiple traits. For this last task, one can estimate a correlation between PVR residuals of two traits (for a similar reasoning, see Cheverud *et al.*, 1985; Gittleman & Kot, 1990) or add phylogenetic eigenvectors and other explanatory variables into a multiple or partial regression model (Desdevises *et al.*, 2003; Peres-Neto *et al.*, 2012). More recently, PVR approach started to be used to infer trait values (for species for which these values are unknown) based on their phylogenetic position, a procedure called phylogenetic imputation (Guenard *et al*., 2013; Swenson, 2014).

However, despite numerous empirical applications, the PVR approach (as well as its analogous spatial forms) has been criticized on several grounds, especially when its statistical performance is compared with other methods, such as PGLS (see Monteiro, 2013 for a recent review in the context of geometric morphometrics), or because it is usually described as a purely empirical approach, not relying on explicit models of trait evolution (*e.g.* Laurin, 2010; Freckleton *et al.*, 2011; Pennell & Harmon, 2013). Moreover, Rohlf (2001) pointed out that, because all eigenvectors would be necessary to describe the entirety of the phylogenetic relationships expressed by the distance matrix, selecting a few of them to model trait variation (a common procedure in any multivariate analyses) would lead to a underestimation of phylogenetic signal and to a high type I error rate when correlating traits.

Despite these criticisms, PVR is valid in empirical applications if model assumptions are not violated. For instance, a careful selection of phylogenetic eigenvectors, especially taking the residual phylogenetic autocorrelation into account, leads to accurate estimates of phylogenetic signal and satisfactory Type I error rates (Diniz-Filho *et al.*, 2012b). At the same time, the possibility of selecting eigenvectors that are structured across different parts of the phylogeny allows modeling more complex patterns that are not easily described by a single model across the entire phylogeny of a group. This is clear in the new extension of PVR, which consists in successively adding phylogenetic eigenvectors as explanatory variables to model trait variation. The relationship between the R^2 values of the successive PVR models (each one with an increased number of phylogenetic eigenvectors) and the cumulative eigenvalues (associated with the eigenvectors entering the models) exhibits different patterns under different evolutionary models. For instance, this relationship, called Phylogenetic Signal-Representation (**PSR**) curve, would be linear under a Brownian model of trait evolution (Diniz-Filho *et al.*, 2012a). Departures from linearity in the PSR curve can be straightforwardly associated with other evolutionary models. This extension gives PVR an explicit interpretation in terms of evolutionary models underlying trait variation and, at the same time, allows choosing eigenvectors for further linear modeling.

Over the past decade, PVR has been frequently used in paleobiological research (*e.g.* Cubo *et al.*, 2005, 2008, 2012; Spocter & Manger, 2007; Kriloff *et al.*, 2008; Pouydebat *et al.*, 2008; Pierce *et al.*, 2009; Piras *et al.*, 2010; Sakamoto *et al.*, 2010; Brusatte *et al.*, 2012; Close & Rayfield, 2012; Ercoli *et al.*, 2012; Prevosti *et al.*, 2012; Sakamoto & Ruta,

2012; Legendre *et al.*, 2013). Given the considerable usage of PVR by paleobiologists, here the main theoretical/statistical basis of the method and the developments made in the 15 years after its original proposition are reviewed, highlighting further potential applications in paleobiology. Understanding the basis of the PVR method and how to meet its assumptions is crucial for the correct application of the method. As an example of the application of the PVR model and its variations, an exemplar dataset of proxies for cranial form and function in theropod dinosaurs is used, which was originally presented by Brusatte *et al.* (2012). Using this dataset, the following specific questions are asked: (i) what are the phylogenetic patterns in theropod skull shape?; (ii) how can PVR help us tease apart the evolutionary models underlying variation in traits?; (iii) how are evolutionary history, function, and diet related to variation in theropod skulls?; and (iv) how can PVR be used to understand the evolutionary components of morphological disparity in theropod skulls? By addressing these questions, a step-wise guide for how PVR can be implemented (see Appendix 1) is provided, and used to address a range of issues pertinent to contemporary paleobiology research programs.

THE BASIC PVR MODEL

The PVR Model

The general idea of PVR is to model the variation in a trait (**Y**) as a function of eigenvectors extracted from a phylogenetic distance matrix (usually patristic distances, **D** hereafter), using a Principal Coordinate Analysis (**PCOA** – see Legendre & Legendre, 2012; Bookstein, 2013). The general goal of a PCOA is to transform a distance matrix into a series of vectors with coordinates that describe the relationship among observations (*i.e.* taxa in the phylogenetic context) in a continuous orthogonal space. Plotting these vectors allow a graphical inspection of the relationships expressed in the distance matrix. More specifically, these coordinates are the eigenvectors (**E**) resulting from the characteristic equation of a double-centered distance matrix (see Legendre & Legendre 2012, p. 426). Therefore, if the distances are phylogenetic (patristic), the eigenvectors from **D** (previously transformed) will ordinate the taxa in a phylogenetic space, in which each of the n-1 eigenvectors (where n is the number of taxa) express a different component of variation among taxa based on the phylogenetic distances between them (see below).

After selecting some of the eigenvectors (**E**), the PVR of a trait **Y** is given by a linear multiple regression model of the form:

$$\mathbf{Y} = \mathbf{E}\beta + \varepsilon$$

where **β** is a vector containing the partial regression coefficients and **ε** are the residuals. The coefficient of determination (R^2) of this model indicates the proportion of variation in **Y** that is "explained" by the part of the phylogeny that is being represented by the set of eigenvectors used, being thus an estimate of the phylogenetic signal.

The PVR model defined above can be understood under the reasoning originally proposed by Cheverud *et al.* (1985), in which the total variation (**T**) in a trait **Y** can be partitioned into phylogenetic (**P**) and specific components (**S**), containing variance in the trait that is accounted for by phylogenetic relationships and the variance that is independent of the phylogenetic relationships between taxa, respectively. The **P**-component is given by the estimated values of the regression (**Y'**), whereas the **S**-component is given by the model residuals [**ε** = (**Y** – **Y'**)]. Diniz-Filho *et al.* (2009) showed that the **S**-component is correlated with the difference between the observed and ancestral reconstructed values for the trait, strengthening its interpretation as phylogenetically-independent variance.

PVR is thus a multivariate ordination of phylogenies coupled with linear modeling. It can be implemented in any standard package for statistical and multivariate analyses and in several R packages (R Development Core Team 2012), including the specific package PVR. Other related functions can be implemented using packages *ape* (Paradis 2012) and *vegan* (Borcard *et al.*, 2011) (see also Appendix 1 for some R-script useful for helping PVR implementation).

Testing PVR Assumptions

It is assumed that the **S**-component is normally and independently distributed. Absence of phylogenetic autocorrelation in the residuals is critical for the validity of the PVR partition. This assumption can be tested by estimating Moran's I autocorrelation coefficients (Gittleman & Kot, 1990). Moran's I can then be used to estimate phylogenetic signal both in traits and model residuals (see Diniz-Filho, 2001; Pavoine *et al.*, 2007; Munkmuller *et al.*, 2012), and is given by:

$$I = \left(\frac{n}{S_0}\right)\left[\frac{\sum_{i=1}^{n}\sum_{j=1}^{n}(y_i - y)(y_j - \bar{y})w_{ij}}{\sum_{I=1}^{n}(y_i - \bar{y})^2}\right]$$

where n is the number of species, y_i and y_j are the trait values in the vector **Y** (or the elements of the residual vector ε) for the species i and j (with average $\bar{y}$), and w_{ij} are the elements of the weighting matrix **W**. Matrix **W** expresses the pairwise phylogenetic relationship between species, that can be given by **1 –D** (and if **W** is standardized to vary between 0 and 1, it becomes a "phylogenetic correlation" matrix, expressing the shared proportion of branch lengths from the root of the tree). Thus, values of Moran's I closer to 1.0 indicate that phylogenetically related species (*i.e.* close relatives, with high values for **W**) are more similar for trait **Y** (or residuals ε) than randomly chosen pairs of species, whereas the null expectation is given by $(n\text{-}1)^{-1}$. Differences from null expectation can be tested by Monte Carlo methods, especially for small (*i.e.* $n < 25$) sample sizes. For a given trait **Y**, a high value of Moran's I indicates thus a strong phylogenetic signal. However, statistical independence of the **S**-component (*i.e.* PVR residuals) is supported when Moran's I is close to zero,

indicating that, after applying PVR to partition trait variation, there is no phylogenetic structure in model residuals (*i.e.* residuals are independent).

It is common to estimate several Moran's *I* coefficients for a single variable, after partitioning the matrix **D** into several connection matrices $\mathbf{W}_1, \mathbf{W}_2, \mathbf{W}_3, \ldots \mathbf{W}_k$ whose values (0 or 1) depend on the level of phylogenetic relatedness between pairs of taxa considering a given interval of phylogenetic distance (Gittleman & Kot, 1990; Diniz-Filho, 2001). A value of 1 is attributed to pairs of taxa within a given phylogenetic distance class, otherwise a value o zero is attributed. Plotting Moran's *I* against the mid-point of the *k* distance class intervals forms a phylogenetic correlogram, indicating the distance at which phylogenetic signal can be detected and allowing the evaluation of more complex phylogenetic patterns in data.

An Application

For an initial illustration of the PVR model the data from Brusatte *et al.* (2012), a study of theropod dinosaur evolution that uses geometric morphometrics to quantify proxies for cranial skull shape variation and quantitative metrics of biting behavior to encapsulate cranial functional variation, is used. A comparative analyses was started by evaluating the phylogenetic signal in the first two principal components (GEOM1 and GEOM2 hereafter) from a morphometric analysis based on 13 two-dimensional skull landmarks for 35 theropod genera (see Brusatte *et al.*, 2012 for details). These first two principal components account for approximately 73.4% of the total morphometric variation in the skulls. As discussed by Brusatte *et al.* (2012, p. 369), the first principal component (GEOM1) reflects an anteroposterior shortening of the skull, a dorsoventral deepening of the snout and reorientation of the long axis of the naris from a horizontal to an oblique orientation. Variations along the second principal component (GEOM2) reflect a reduction in the area of the orbit, the development of a proportionally taller and shorter orbit and a deepening of the cheek region.

To analyze phylogenetic signal in the geometric morphometric data, eigenvectors of geometric morphometric data were extracted using the phylogeny of theropod dinosaurs that was utilized by Brusatte *et al.* (2012). This is an "informal" consensus tree showing the relationships of the 35 taxa based on a number of recent phylogenetic analyses (see Brusatte *et al.*, 2012 for further details). Branches of the phylogeny were time-calibrated using the approach proposed by Brusatte *et al.* (2008), utilizing code developed by Graeme Lloyd (see http://www.graemetlloyd.com/methdpf.html) (Figure 1). A total of 34 phylogenetic eigenvectors were extracted from the phylogenetic distance matrix via PCOA, out of which the first three and the first 24 eigenvectors represented 48.97% and 95% of the structure of the phylogenetic distance matrix, respectively (Figure 2). Notice that when performing the PCOA the phylogenetic distances were not squared (see the section below about the PSR curve for more detail).

The meaning of the phylogenetic eigenvectors can be straightforwardly understood by overlaying their values (*i.e.* the scores from the PCOA) on the phylogeny to see which group of taxa each one describes and differentiates (Figure 1). The eigenvectors with the largest eigenvalues will differentiate basal clades or taxa. For example, the first eigenvector (PHY1 hereafter) portrays the difference between the most phylogenetically distant taxa on the tree, with positive high values for the derived *Gallimimus-Velociraptor* clade, and high negative values for the paraphyletic array of basal lineages, from *Herrerasaurus* to *Majungasaurus* (and with intermediate values for the other taxa) (Figure 1). The second eigenvector (PHY2), on the other hand, separates the *Tarbosaurus-Dilong* clade (the tyrannosauroid theropods) from other taxa. The third eigenvector (PHY3) divides the basal taxa in the *Herrerasaurus* to *Majungasaurus* zone into two groups: the paraphyletic array of basal lineages (*Herrerasaurus* to '*Syntarsus'*) and the clade *Limusaurus* to *Majungasaurus* (the ceratosaurian theropods). The following eigenvectors (*i.e.* PHY4 onwards) will progressively describe less inclusive sets of taxon relationships nearer and nearer to the tips of the phylogeny.

Moran's *I* correlograms based on five distance classes with intervals defined with constant time-steps throughout the phylogeny were initially obtained for GEOM1 and GEOM2. This number and range of distance classes is someway arbitrary and robustness of correlogram in respect to number of classes will depend of shape of the phylogeny and number of species/taxa (see Diniz-Filho, 2001). A rule of thumb in spatial analysis is to have no more than five classes for 20-30 species. For both GEOM1 and GEOM2, there is high (*i.e.* around 0.4), positive, and significant autocorrelation in short phylogenetic distances, so that closely related taxa tend to be morphologically similar (Figure 3). This morphological similarity decreases as phylogenetic distances increase, and tends to stabilize when distances increase to about 120 million years (for GEOM1) and to about 200 million years (for GEOM2).

PVR was applied by regressing GEOM1 and GEOM2 against the first three eigenvectors PHY1, PHY2 and PHY3 (see Figure 1). The R^2 of the multiple regression model for GEOM1 was equal to 0.258 ($F = 3.56$; $p = 0.024$), with a significant partial regression coefficient for PHY3. For GEOM2, the coefficient of determination (R^2) was equal to 0.462 ($F= 8.87$; $p < 0.001$) and the partial regression coefficients associated to PHY1, PHY2 and PHY3 were all significant. Thus, both traits are phylogenetically structured (*i.e.* exhibit a significant phylogenetic signal), although patterns in GEOM2 are stronger than in GEOM1. This is also observed in the correlograms because, when compared to GEOM1, high Moran's *I* coefficients are detected for a larger phylogenetic distance (up to 200 million years) for GEOM2 (Figure 3).

Moreover, after using PVR to partition the total variation T into P and S components, there is still significant residual autocorrelation in the first distance class ($I = 0.27$; $p = 0.032$ for GEOM1; Figures 3A,B). Thus, the results indicate that these PVR models were not able to completely take phylogeny into account. With this exercise, our goal here was to show that more eigenvectors are needed to better model these two

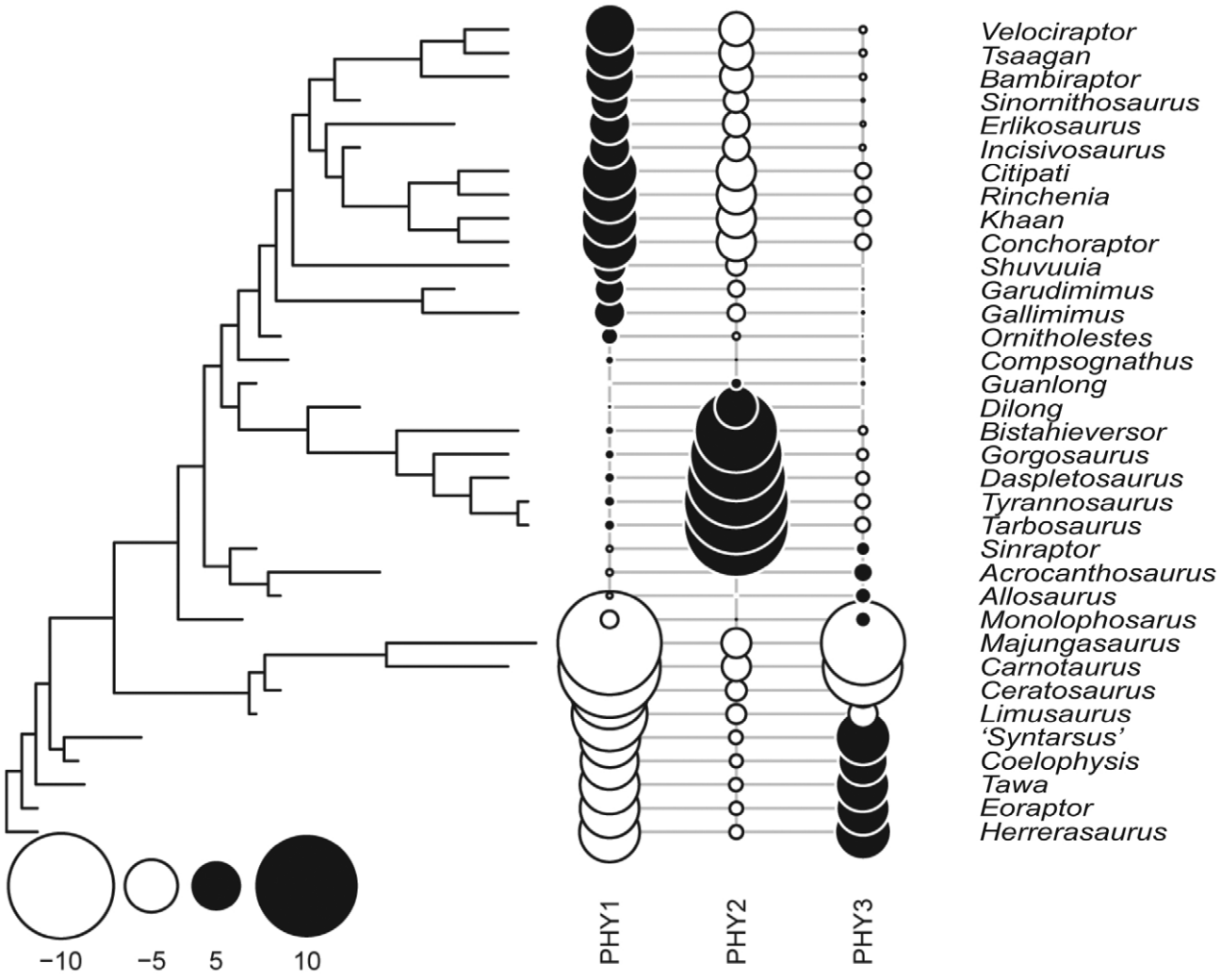

Figure 1. Phylogeny and the first three eigenvectors (PHY1, PHY2 and PHY3) for the 35 Theropoda genera.

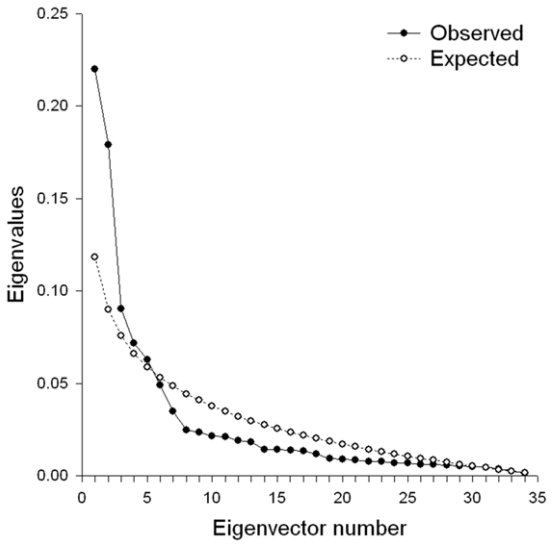

Figure 2. Scree plot of eigenvalues extracted from the phylogenetic distance matrix, as well as broken-stick expectation (comparing the two curves allow defining that five eigenvectors should be used in PVR, according to this criterion).

traits and to take the residual autocorrelation into account. For example, including the first 10 eigenvectors increase the R^2 of GEOM1 from 0.258 to 0.888, removing positive and significant autocorrelation from the residuals (in fact, it tends to create a negative autocorrelation in the first distance class of PVR residuals, equal to I = -0.36; p = 0.017) (Figure 4).

These first results are in line with Rohlf's (2001) criticisms, because both the amount of phylogenetic signal (the R^2) and the **S**-component (or residuals **ε**) given by PVR will depend on how many (and which) eigenvectors are used. Thus, it is necessary to establish criteria to define which eigenvectors should be used in PVR, an issue that is rarely discussed, despite its importance (see Blanchet *et al.*, 2008; Bini *et al.*, 2009; Safi & Pettorelli, 2010; Diniz-Filho *et al.*, 2012b).

THE ISSUE OF EIGENVECTOR SELECTION

Criteria to select eigenvectors should be goal-oriented. If the goal is to estimate phylogenetic signal, the critical issue is that residuals (the S-component) are independent (i.e. without significant phylogenetic autocorrelation) and, as

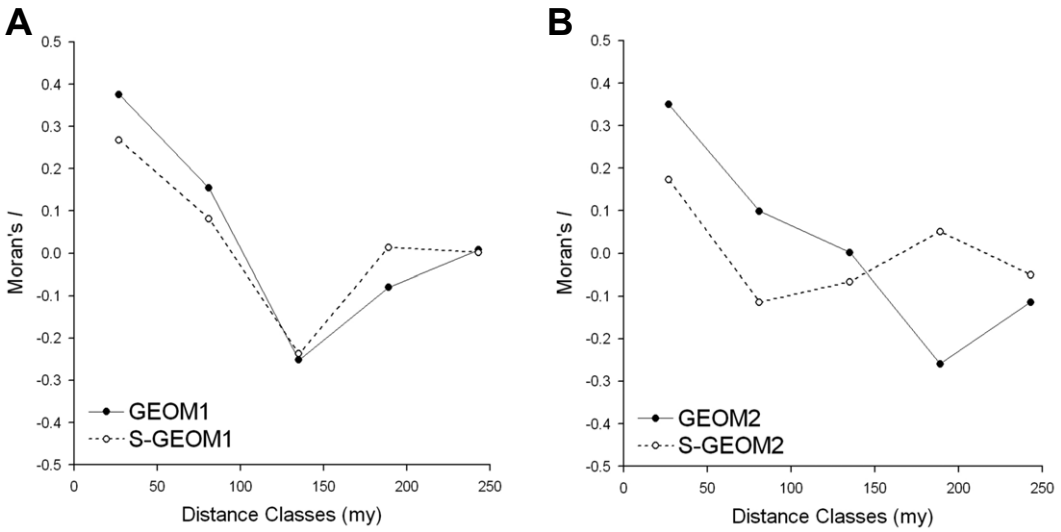

Figure 3. Moran's *I* correlogram for GEOM1 (**A**) and GEOM2 (**B**). The continuous (filled circles) and dashed (empty circles) lines represent, respectively, the results for the original variables and for the residuals from PVR models using the first three eigenvectors shown in Figure 1A.

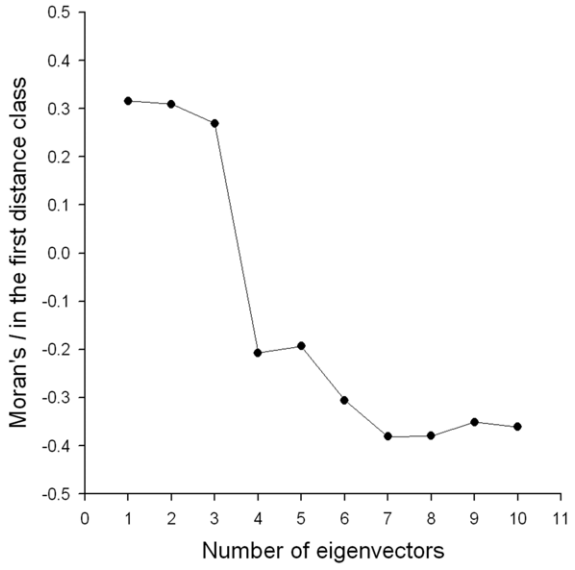

Figure 4. Residual autocorrelation analysis from PVR models as the number of eigenvectors used to model GEOM1 increases.

explained above, this is a testable assumption using Moran's *I* as a diagnostic tool. If residuals are independent, then the S-component contains the unique variation of a trait, and its correlation with other S-components or other variables (such as environmental variation, temperature, ecology, etc.) will be due to responses of taxa independently of ancestral states (see Diniz-Filho *et al*., 2009).

Methods to select eigenvectors can be divided into sequential and non-sequential criteria (Diniz-Filho *et al.*, 2012b). In the sequential criteria, eigenvectors are added one-by-one to the PVR model until elimination of the residual autocorrelation. Using GEOM1 as an example, the Moran's I coefficient in the first distance class for the S-component is not significant after adding the first four eigenvectors to the PVR model. With the increase of eigenvectors, Moran's *I* coefficients become increasingly negative and significant (indicating over-correction) (Figure 4). Thus, according to this sequential approach, GEOM1 should be modeled with the first 4 eigenvectors, revealing a much stronger phylogenetic signal (R^2= 0.831) in comparison with the result shown above (R^2= 0.258), which was obtained with the first 3 eigenvectors only.

Other criteria, including the non-sequential ones, could also be used, and results will vary in terms of R^2 and autocorrelation in the S-component (Table 1). For example, it is common in multivariate analyses to use the ordination axes that cumulatively explain a certain (subjective) threshold value of a large amount of variation (*e.g.* 90%). Using the theropod data, 24 eigenvectors should be used in the PVR according to this criterion, resulting in a phylogenetic signal (R^2) of 92%. However, this model strongly overcorrects for phylogeny and possesses a high negative autocorrelation in the first distance class (Moran's I = -0.46; p = 0.01). Thus, as indicated above, a much simpler model, including the first four eigenvectors, would suffice to correct for autocorrelation. One alternative would be the broken-stick criterion for selecting which eigenvectors are significantly different from null expectation (as originally proposed by Diniz-Filho *et al*., 1998; see also Legendre & Legendre, 2012). Based on this criterion only the first 5 eigenvectors should be used (see Figure 2), with a similarly high phylogenetic signal but a low residual autocorrelation (Moran's I = -0.19; p = 0.05).

Other non-sequential criteria can also be used (Table 1; see Diniz-Filho *et al.* 2012b for a discussion). First, one can use in the PVR model only the eigenvectors that are significantly correlated (using a Pearson's correlation coefficient or partial the regression coefficients) with the trait of interest. When this criterion is applied to model GEOM1, the first four eigenvectors should be used, coinciding in this case with the sequential criterion that minimizes the Moran's *I* coefficient presented above. Second, an information-

Table 1. Criteria used to select the eigenvectors for PVR, showing the number of eigenvectors (**k**) selected, the cumulative eigenvalues associated with selected eigenvectors, the coefficient of determination ($\mathbf{R^2}$) of the PVR models, the ratio between R^2 and the eigenvalues, and the residual autocorrelation as given by the Moran's I coefficient in the first distance class. Methods used to select the eigenvectors were based on the percentage of the representation of the phylogenetic distance matrix (Lambda 95%), the Broken-Stick model, the correlation between eigenvectors and the trait of interest (**Y**), Akaike information criterion (**AIC**) of the PVR models and the minimization of phylogenetic autocorrelation of the residuals (Moran's *I*).

Response	Criterion	*k*	Eigenvectors	Eigenvalues	R^2	R^2/Eigenvalue	Moran's *I*
			in PVR	(%)			
GEOM1	Lambda 95%	24	1 to 24	95.0	0.92	0.97	-0.46
	Broken-Stick	5	1 to 5	62.4	0.85	1.35	-0.19
	Correlation with Y	4	1,2,3,4	56.1	0.83	1.48	-0.21
	AIC	6	1-5,7	66.0	0.87	1.32	-0.24
	Min. Moran's *I*	3	2, 3, 4	36.1	0.80	2.21	0.05
GEOM2	Lambda 95%	24	1 to 24	95.0	0.87	0.92	-0.46
	Broken-Stick	5	1 to 5	62.4	0.48	0.76	0.14
	Correlation with Y	4	1-3,6	52.9	0.61	1.15	-0.06
	AIC	6	1-3,6-8	67.0	0.70	1.04	0.31
	Min. Moran's *I*	2	2,5	24.2	0.26	1.09	0.09

theoretic approach can be used by searching all possible combinations of eigenvectors (see Diniz-Filho *et al.*, 2008). In this case, because of the very high number of possible models incorporating the 34 eigenvectors, the search was restricted to the combinations of the first ten eigenvectors. In this case, the model would retain the first 7 eigenvectors, excluding eigenvector 6, for GEOM1. Finally, it is possible to use a procedure that automatically searches for the PVR model with the smallest number of eigenvectors and simultaneously minimizes Moran's *I* coefficients of the residuals (see Diniz-Filho *et al*., 2012b for details). This last option selects again the first four eigenvectors, but excludes the first eigenvector.

Despite variation in the results (Table 1), a key issue here is to define a parsimonious model with independent residuals. Paradoxically, selection based on the information theoretic approach tended to overcorrect for residual autocorrelation probably because the Akaike information criterion may itself be biased by phylogenetic autocorrelation (Diniz-Filho *et al.*, 2008). Minimization of phylogenetic autocorrelation of the residuals, albeit interesting, involves some subjectivity (the choice of a threshold; *e.g.* a Moran's *I* coefficient of 0.05). In general, the non-sequential approaches seem to be more useful (but see below), especially the one selecting the eigenvectors significantly correlated with the response variable. This is so because the eigenvectors selected by these approaches are representing parts of phylogeny that are indeed important to model trait evolution and diversification. Other possibility is to try several approaches (as shown in Table 1) and critically interpret resulting PVR models, checking for robustness in ecological or evolutionary conclusions.

For the following analyses in this paper, when necessary, eigenvectors were selected according to their correlation with the response variables (GEOM1 and GEOM2).

PHYLOGENETIC EIGENVECTORS AND EVOLUTIONARY MODELS

The Phylogenetic Signal-Representation (PSR) Curve

The idea of phylogenetic signal has recently been associated with evolutionary models and it may be interesting to define the amount of variation in a trait explained by phylogeny under a given evolutionary model (see Freckleton *et al.*, 2002; Blomberg *et al.*, 2003; Cooper *et al.*, 2010). In a first instance, it would be difficult to link the R^2 estimated by PVR with any evolutionary model defined in advance, especially because, although eigenvectors represent patterns of phylogenetic relationships among the species, they are just linear combinations of distances among taxa and do not have evolutionary meanings, and will depend on size and shape (in terms of how balanced or pectinate a tree is) of the phylogeny. Thus, the question is: how PVR can be interpreted in a "model-based" context?

The reasoning for answering this question starts by considering that when all eigenvectors are used in a given PVR model, then the coefficient of determination would be, by definition, equal to 1.0. On the other hand, as explained above, the entire phylogeny will be not considered by leaving out some eigenvectors (*i.e.* the sum of the relative eigenvalues associated with the eigenvectors used in the PVR model will be less than 100%; see Rohlf, 2001). However, under a linear model of trait divergence (usually approximated by a Brownian motion process) in which each change in the trait is explained by the phylogenetic divergence, a linear relationship between the cumulative eigenvalues (*i.e.* the amount of representation, by the eigenvectors used, of the phylogenetic relationships among the taxa) and the amount of variation explained by PVR (*i.e.* an estimation of phylogenetic

signal) is expected. Departures from this linear relationship will happen if a trait is evolving faster or slower than predicted by a Brownian model of trait evolution. Using this rationality, two of us (JAFDF and LMB), recently developed a method called Phylogenetic Signal-Representation curve (PSR; Diniz-Filho *et al.*, 2012a) by expanding the original PVR approach.

The PSR curve is obtained by fitting successive PVR models with the sequential addition of eigenvectors. The first model includes only the first eigenvector; the second model includes the first two eigenvectors, and so on. Then, the resulting R^2 values from PVR models are plotted against the cumulative eigenvalues (*i.e.* λ_1, $\lambda_1+\lambda_2$, $\lambda_1+\lambda_2+\lambda_3$, …,$\lambda_1+\lambda_2+\lambda_3+\ldots+\lambda_{n-1}$). As will be showed subsequently, the PSR curve can be interpreted in terms of evolutionary models (see Diniz-Filho *et al.*, 2012a).

The area between the observed PSR curve and a line with slope equal to 1, called the PSR area, can be used to measure the departure from a Brownian model of trait evolution (by convention, areas above the Brownian model are positive and areas below the curve are negative). For comparative purposes, it is possible to randomize the response variable and produce a null PSR curve. Also, it is possible to simulate any evolutionary model (*e.g.* Brownian motion, Ornstein-Uhlenbeck models, and so on – see Hansen & Martins, 1996) using, for example, the APE (Paradis *et al.*, 2004) for the R package, and build the PSR curve under this model.

PSR Curve and Cranial Evolution in Theropoda

The PSR curve for GEOM1 (Figure 5A) reveals that, up to the first three eigenvectors, the R^2 values are below the line with slope = 1, so the PVR model explains less than it is expected by a Brownian, linear model of trait evolution. In other words, although the first three eigenvectors represent nearly half of the variation in phylogenetic distances (48.97%; see Figure 2), they account for less than 26% of the variation in GEOM1. However, a conspicuous increase in the R^2 is detected with the addition of the fourth eigenvector. Thus, in this comparison, the evolution is occurring faster than expected by a Brownian model (see Blomberg *et al.*, 2003). The PSR curve for GEOM2 is much more linear and is only slightly below the line representing Brownian motion across its entire length.

The interpretation of these results can be made clearer by considering which aspect of the phylogeny is represented by the fourth eigenvector that greatly increases the amount of explanation (R^2) and causes a conspicuous deviation from the Brownian model in GEOWM1 (Figure 5B). The fourth eigenvector differentiates a clade of derived theropods (*Velociraptor* to *Sinornithosaurus*, the dromaeosaurid theropods) with an array of paraphyletic outgroup taxa, including *Incisivosaurus*, *Shuvuuia*, and the oviraptorosaurian theropods (*e.g. Khaan*, *Citipati*). Because the R^2 of the model that includes the first four eigenvectors is much higher than the one expected by the Brownian expectation, one can conclude that the morphological variation between these two derived clusters is much higher than expected by their relatively small phylogenetic distance. This signal reflects the earlier findings of Brusatte *et al.* (2012), who showed that the skulls of oviraptorosaurian theropods are highly morphologically aberrant compared to those of other theropods, including their close relatives. Perhaps surprisingly, the major differentiation in this eigenvector is not between oviraptorosaurs themselves and all other theropods, but rather between a paraphyletic cluster of oviraptorosaurs and a few outgroup taxa and a proximal clade (dromaeosaurids). This result may be due to the fact that the two outgroups that cluster with oviraptorosaurs, particularly *Shuvuuia*, are themselves highly morphologically aberrant theropods.

Diniz-Filho *et al.* (2012a) compared the results of the PSR curve with the Blomberg's *K*-statistic (Blomberg *et al.*, 2003), which is another method to estimate phylogenetic signal in data (also used in the original paper by Brusatte *et al.*, 2012). According to this statistic (calculated with Kembel's *et al.*, 2010 picante package), significant phylogenetic signal was detected for GEOM1 ($K = 0.66$) and GEOM2 ($K = 0.47$), because both differed statistically from null expectations. Both *K*-statistic values were less than the one, and therefore lower than expected under Brownian motion (K=1.0), suggesting that traits (in this case changes in theropod skull shape) are evolving slower than expected under this linear model. Similarly, the PSR areas were also "negative" (-0.035 and -0.069 for GEOM1 and GEOM2, respectively), corroborating the notion that traits are evolving at slower rates than expected under a Brownian motion model of trait evolution. Although the analyses were restricted to the first two principal components of morphological variation (*i.e.* those that present significant phylogenetic signal), it is possible to use all 22 principal components to compare PSR area and the Blomberg's *K*-statistic. Reinforcing the previous findings by Diniz-Filho *et al.* (2012a), there is a high correlation between PSR area and Blomberg's *K*-statistic ($r = 0.833$; $p < 0.01$).

Especially for GEOM1, the PSR curve does not suggest a simple departure from the Brownian expectation. The value of the PSR area (and of the Blomberg's *K*-statistic) suggests a rate of evolutionary divergence slower than expected by Brownian model only when comparing the deeper nodes in the phylogeny. The differences between the PSR curves of GEOM1 and GEOM2 reveal that these two dimensions of skull variation evolved under different processes, and that GEOM1 accelerates in the most derived theropods (especially in the restricted portion of the phylogeny including the peculiar oviraptorosaurs). Importantly, this illustrates the power of PVR in detecting patterns of phylogenetic non-stationarity and does not assume, during the modeling process, a unique pattern (and a unique underlying evolutionary model) across the entire phylogeny (Diniz-Fillho *et al.*, 2010; see also Eastman *et al.*, 2011).

Non-stationarity patterns can be also detected by the ratios between the R^2 and the cumulative eigenvalues associated with the eigenvectors used in each model (see Table 1). For instance, these ratios are usually above 1.0 for GEOM1 (*i.e.* the R^2 is higher than the amount of phylogenetic structure being represented), indicating the higher-than-expected

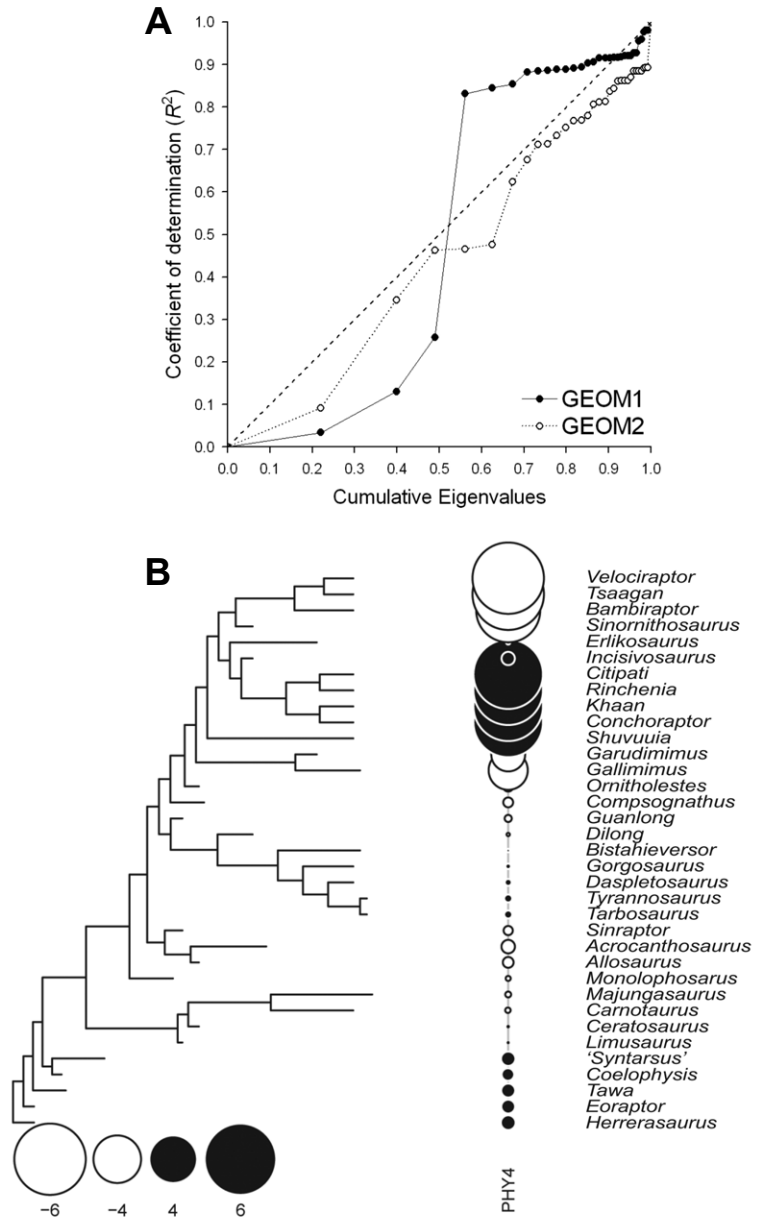

Figure 5. A, the Phylogenetic Signal-Representation curve for GEOM1 (continuous lines and filled circles) and for GEOM2 (dashed lines and empty circles); **B**, the taxa contrasted by eigenvector 4, which increases the ability of the PVR model in explaining variation in GEOM1.

differentiation in the derived theropods. For GEOM2, on the other hand, the ratios are usually around 1.0.

Finally, it is important to emphasize that the eigenvectors used in the PSR curve are extracted without squaring phylogenetic distances (which is the default option in most software for PCOA). The linear relationship between R^2 and eigenvalues under Brownian motion will appear only if distances are not squared, because the original distances in the phylogenetic distances matrix are preserved in the multivariate ordination space. Despite the large number of eigenvectors that are needed to account for patterns, this option, which is herein advocated, offers more interesting interpretation of eigenvectors by PSR curve in terms of evolutionary models.

CORRELATION BETWEEN TRAITS AND MULTIPLE REGRESSION MODELS

Correlating S-components

The PVR approach can also be used to test for correlated evolution between pairs of traits. Following the framework of autoregressive models originally proposed by Cheverud *et al.* (1985), the idea is to correlate the S components of two traits (*i.e.* the model residuals) because they express the part of variation in these traits that is independent of the phylogenetic relationships between taxa. This is actually a partial correlation between traits while holding phylogenetic eigenvectors constant, so the degrees of freedom should be properly calculated (Martins *et al.*, 2002).

As one example of a correlated evolution test, using the theropod dinosaur dataset, Brusatte *et al.* (2012) looked at the relationship between proxies for skull shape (which quantifies an aspect of cranial form) and quantitative metrics of biting behavior (which quantifies an aspect of cranial function). The biting behavior metric was calculated by evaluating the mechanical advantage, defined as the ratio of the muscle moment arm to the biting moment arm, for three major groups of cranial muscles (Sakamoto *et al*., 2010; Brusatte *et al.*, 2012). The standardized ratios were subjected to a principal component analysis, and the first three components (BIT1, BIT2 and BIT3 hereafter) can then be used as explanatory variables. The question is how morphometric variation is correlated with these biomechanical variables, within a phylogenetic context. Brusatte *et al.* (2012) provided some analyses, but here they are expanded using new methods.

Both GEOM1 and GEOM2 are uncorrelated to BIT1 (r = 0.07, $p > 0.05$ and r = -0.21, $p > 0.05$, respectively), suggesting poor correlations between cranial shape and biting function. However, these correlations are based on raw morphometric and mechanical advantage scores only, and therefore contain a mix of phylogenetically shared variation and independent variation, due to evolution and not phylogenetic non-independence, in each taxon. It is important, therefore, to decouple these components.

P- and S-components of GEOM1 and GEOM2 is estimated using the PVR models estimated with the eigenvectors significantly correlated with these variables (Table 1). The same procedure was used to estimate the P- and S- components of BIT1. S- components of GEOM 1 and BIT1 were uncorrelated (r = 0.015; $p > 0.05$), as discussed by Brusatte *et al.* (2012). After using this procedure, it was found a highly significant correlation between the S-components of GEOM2 and BIT1 (r = -0.53; $p < 0.01$) (Figure 6). Thus, although there is no correlation between morphometric variation along GEOM2 and biting function, there is a correlation among the unique components of these two traits, indicating that taxa that evolved morphologically along certain directions evolved to particular biting functions (independently of the other phylogenetically related taxa).

For comparison, it was also ran a PGLS model in R (package PGLS), regressing GEOM2 against BIT1 under the assumption of correlated residuals (following a Brownian model, for simplicity), which is analogous to the correlation among PVR S-components, because both approaches estimate the input correlation (see Martins *et al.*, 2002). The slope is significant at $p < 0.01$, and the r^2 of the model was equal to 0.388, supporting previous conclusions based on PVR. It is difficult to find particular explanations for this relationship (see Figure 7), but the pattern seems to be driven, or leveraged, by *Limusaurus*.

Thus, in summary, although the first dimension of cranial morphometric variation is not correlated with function after controlling for phylogenetic effects, an interesting pattern appears when using the specific component GEOM2, but this appears to be driven by a few taxa and does not represent a general pattern. Still, this nuance went unnoticed by Brusatte *et al.* (2012) who were using coarser methods. It is also possible that other more complex non-stationary patterns in phylogeny, localized in a few subclades, can describe this potential relationship (see Henderson, 2002; Foth & Rabaut, 2013), but investigating this in more detail is beyond the scope of this paper.

The Partial Regression Approach

Partial regression analysis can be used, according to Legendre & Legendre (2012), to measure the amount of variation in a response variable that can be attributed uniquely to two (or more) sets of explanatory variables (see also Safi & Pettorelli 2010 for a discussion of how to select eigenvectors in partial eigenvector regressions). In this context, and considering the structure of the dataset used in this paper, one can separate the response variables (*i.e.* GEOM1 and GEOM2) from two sets of explanatory variables (phylogenetic eigenvectors and BIT1). Following Desdevises *et al.* (2003), the idea is to estimate three coefficients of determination (R^2). The first one (R^2_{PHY}) is obtained by regression of the response variable (*i.e.* the trait of interest) on the phylogenetic eigenvectors (the usual coefficient of determination from PVR); the second (R^2_X) is estimated by regressing the response variables on the other explanatory variables (BIT1, BIT2 and BIT3 in our example), and a third one by regressing the response variable on the phylogenetic eigenvectors and the explanatory variables together (R^2_T). If phylogenetic eigenvectors and explanatory variables are independent, the total R^2_T will be the sum of the R^2_X and R^2_{PHY}. However, this is rarely the case and, thus, the unique contributions of the phylogeny (c) and of the explanatory variable X (a) can be obtained by:

$$a = R^2_X - b$$
$$c = R^2_{PHY} - b$$
$$b = R^2_X + R^2_{PHY} - R^2_T$$

where b is the shared influence of both sets of explanatory variables (X and the phylogeny) on the trait of interest. Desdevises *et al.* (2003), in the context of ecological components of variation, interprets this shared component b as expressing niche conservatism during the evolution of the response variable.

A form of partial regression was carried out by Brusatte *et al.* (2012), but it is expanded on the analyses here. For GEOM1, results from the partial regression analysis indicated that phylogeny is more important than function in explaining cranial morphological variation among theropod dinosaurs (Table 2; as pointed out by Brusatte *et al.*, 2012). However, for both GEOM1 and GEOM2, a high component b is detected, indicating that the phylogenetic components of biting function are intrinsically related to morphometric variation, which means that it is difficult to tease apart the effects of the two sets of explanations (phylogeny and function). However, patterns differ for GEOM1 and GEOM2 (which is expected, by considering their different evolutionary patterns according to PVR and PSR curve discussed above). For GEOM1, the overall explanation is

much higher (almost 90%), but the unique effects of phylogeny and biting function are relatively small. For GEOM2, the overall explanation is smaller, but the unique components are much higher and, more important, the shared component is relatively lower, revealing that it may be possible to decouple the effects of phylogeny and function on morphology. Indeed, this result agrees with the one based on the previous approach of correlating the **S**-components that revealed a correlation between GEOM2 and BIT1 (see Figure 6B).

MULTIVARIATE GENERALIZATIONS OF PVR AND PSR CURVE

Monteiro & Abe (1999) soon after the original development of PVR, proposed a multivariate generalization of the method and applied to geometric morphometric data (see also Giannini, 2003; Sakamoto *et al.*, 2010; Sakamoto & Ruta, 2012; Brusatte *et al.*, 2012). Sakamoto *et al.* (2010) pioneered its application in paleontological data and called it mPVR,

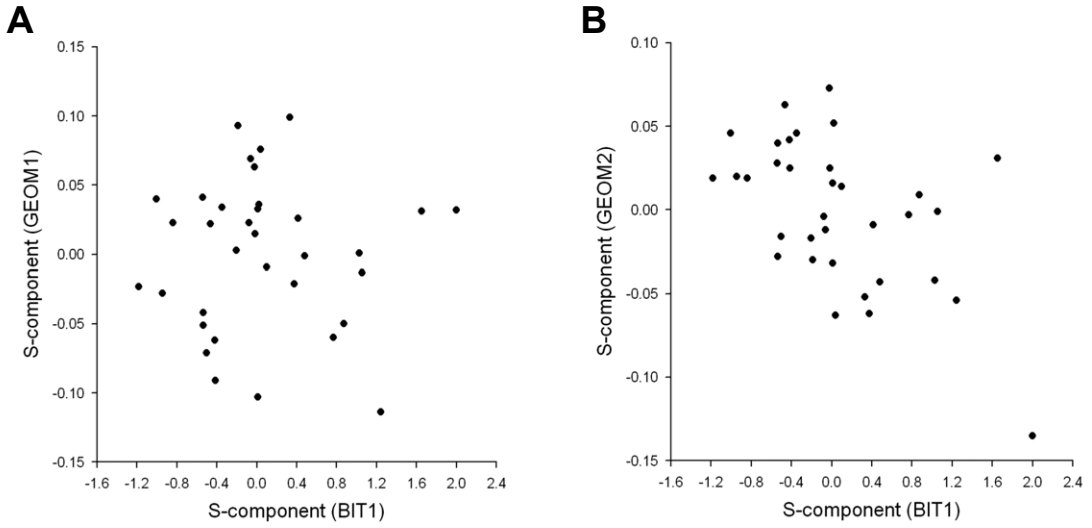

Figure 6. Relationships between the specific components of morphology (GEOM1 and GEOM2) and the specific component of BIT1 (**A** and **B**, respectively).

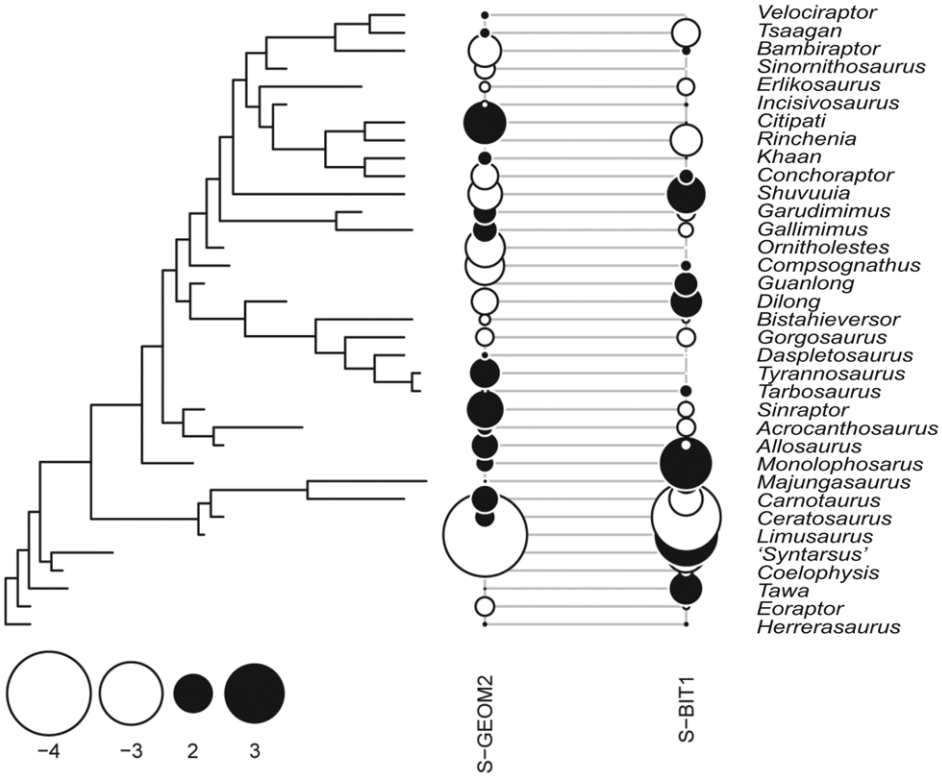

Figure 7. The phylogenetic patterns in the specific components of GEOM2 and BIT1 mapped on the phylogeny.

Table 2. Application of Desdevises *et al*.'s. (2003) approach to evaluate the effects of phylogeny and biting function on the morphological variation

Response	R^2 (PHY)	R^2 (BIT)	R^2 total	a	b	c
GEOM1	0.831	0.783	0.891	0.060	0.723	0.108
GEOM2	0.609	0.363	0.682	0.073	0.290	0.319
Multivariate	0.789	0.689	0.845	0.056	0.634	0.156

in the same framework described above. The idea is simply to replace the multiple regression by a multivariate analogue (canonical analyses). In ecological analyses, this multivariate form is implemented as a Canonical Correlation Analysis (CCoA) or Redundancy Analysis (RDA) (see Legendre & Legendre, 2012). Thus, all analyses described above can be generalized by using multivariate analyses.

Here an mPVR using GEOM1 and GEOM2 as response variables is performed. As explained above, each of these traits is explained by a different set of phylogenetic eigenvectors. The mPVR was performed using all all eigenvectors that were correlated with GEOM1 or GEOM2 (see Table 1) and thus our mPVR included eigenvectors 1, 2, 3, 4 and 6 as an explanatory matrix. The R^2 of the RDA was equal to 0.789, revealing a strong multivariate signal in both GEOM1 and GEOM2 (as already shown by univariate PVR models).

In this context, the PSR curve can also be generalized into a multivariate analysis (mPSR). Thus, instead of using the coefficients of multiple determination (from each successive PVR model), one should use the canonical coefficient of determination (see Sakamoto *et al*., 2010) and plot against the cumulative eigenvalues associated with the eigenvectors from PCOA. Results from this method (*i.e.* mPSR) suggested the same pattern portrayed by GEOM1 (Figure 8), indicating the non-stationarity of divergence among the derived theropods in the clade encompassing *Shuvuuia* to *Velociraptor*. Statistically, this occurs because the principal component of the trait matrix, which is the basis of RDA, is still strongly determined by the trait with strongest phylogenetic patterns. To support the interpretation of the mPSR curve under Brownian motion, 1000 simulations of the two traits under this process (see above) and recomputed the mPSR curve were performed. As in the univariate case, the Brownian mPSR curve is linear in relation to the eigenvalues.

Finally, a partial RDA (see Legendre & Legendre, 2012) can be used to estimate the effects of phylogeny, function (BIT1) and the intersection between these variables on the morphological variation. The canonical coefficients of determination associated to phylogeny and biting function were equal to 0.789 and 0.689, respectively. Of course, the mPVR with both sets of explanatory variables cannot be the sum of these two values, and the total canonical coefficient of determination was actually equal to 0.845, which suggest again a strong shared component. Results from the multivariate variance partitioning indicate that 63% of the morphometric variation was explained by phylogenetic component of biting function, with unique contributions of phylogeny and biting function being estimated as 15.6% and 5.6%, respectively. Because the different numbers of variables in the sets, it is preferable to use adjusted- R^2 in these analyses (Guenard *et al*., 2013), but in this particular case there was no qualitatively differences in the proportion among the three components (*a*, *b*, and *c*).

DIVERSITY AND DISPARITY BASED ON EIGENVECTOR REGRESSION

Diniz-Filho *et al*. (2011) showed that phylogenetic eigenvectors can be used to measure phylogenetic diversity, by summing the variance of groups of taxa in each eigenvector (*i.e*. this is the trace of the covariance matrix among eigenvectors for a group of species). This trace is closely correlated with Helmus *et al.*'s (2007) PSV (Phylogenetic Species Variability). This is analogous to evaluating the variability of groups of species for a given trait (because phylogenetic eigenvectors can be viewed as "traits", at different parts of the phylogeny). Thus, it is possible to expand the reasoning and use the PVR to decouple P- and S-components for a trait and evaluate if distinct groups of species have different variability, or disparity. Measuring disparity is a well-known field in paleobiology, and several metrics are available for achieving this (Foote, 1993; Wills *et al*., 1994; Ciampaglio *et al*., 2001; Erwin, 2007).

As measured using PVR, phylogenetic disparity is analogous to the patristic disparity metrics of Smith (1994), which calculate disparity based on the number of characters changing on a phylogeny, and not the phenetic approaches that quantify variation in observed anatomy only, which are currently standard in the paleontological disparity literature (*e.g.* Gould, 1991). In a sense, phylogenetic disparity measured using PVR is conceptually similar to quantitative measures of evolutionary rates, which use variables (*e.g.* continuous characters such as body size, or discrete characters) optimized a time-calibrated phylogeny to identify variation in the tempo of morphological evolution between groups of taxa (*e.g.* Thomas & Freckleton, 2011; Lloyd *et al*., 2012).

Brusatte *et al*. (2012) measured the morphological variability in skull shape in different subsets of theropods and made comparisons between groups to gauge whether certain groups were more or less variable than others. They used standard phenetic approaches and calculated disparity based on the range and variance of taxa on the principal component axes derived from the geometric morphometric analysis (GEOM1-5). To demonstrate how PVR can also be used to evaluate morphological disparity, one disparity comparison also made by Brusatte *et al.* (2012; Table 1) is presented: the disparity of large carnivorous theropods (*e.g. Tyrannosaurus rex*) and other theropods. This comparison was based on all four range and variance-based phenetic metrics of disparity used by Brusatte *et al.* (2012). The original values of GEOM1 and GEOM2 and their P- and S-components were used. Is the analysis and separated the 35 taxa into the two categories described above (*i.e.* large carnivores and the other theropods).

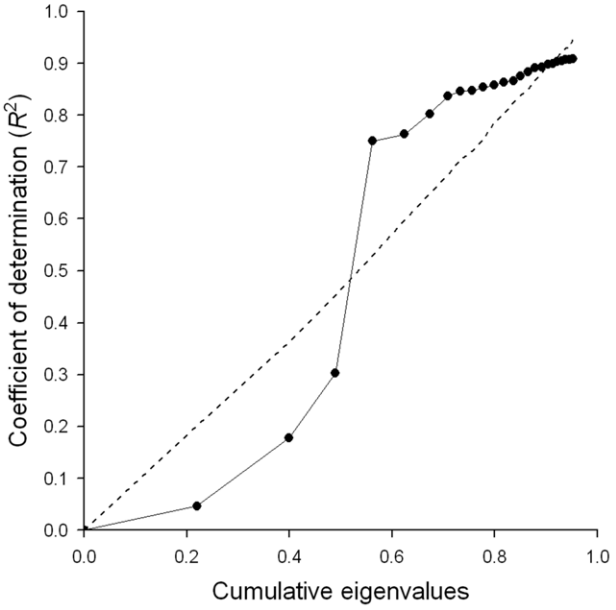

Figure 8. Multivariate PSR curve based on unadjusted R^2 of RDA for the two principal components of morphometric variation (filled circles) and the mean PSR curve for 1000 Brownian simulations over the phylogeny of Theropoda (dashed line).

Then, it was evaluated if the variability of the two groups differ for the total, phylogenetic, and specific components of the two morphometric variables, using both a univariate test (Levene's test for homogeneity of variance) and a multivariate test (Anderson's, 2006 test of multivariate dispersion, based on Euclidian distances within and among groups).

These analyses reinforce the conclusion of Brusatte *et al.* (2012) that large theropods are significantly less disparate than all other theropods. The new PVR-based disparity analyses show that multivariate distances based on GEOM1 and GEOM2 to the group centroid are significantly smaller for large carnivores (0.045) than for other theropods (0.122) ($F = 4.91$; $p = 0.033$). The differences in disparity are higher for the S-component ($F = 6.02$; $p = 0.019$) than for the P-component ($F = 4.19$; $p = 0.049$). Univariate Levene's test of the components of the two variables reveals that these patterns are mainly driven by a significant disparity in the S-component of GEOM1. This reinforces that small variation in large carnivores (which do not form a clade, but are members of multiple clades) is due to evolutionary convergence probably related to similar feeding styles and is not widely a phylogenetic inherited component.

CONCLUDING REMARKS

PVR is a widely used method in paleobiology, as it is a straightforward and powerful technique for removing phylogenetic non-independence when comparing traits (*e.g.* correlated character evolution tests) or assessing the phylogenetic structure in a dataset. Thus, the PVR method originally proposed by Diniz-Filho *et al.* (1998) was reviewed and applications discussed in the context of paleobiology using Brusatte *et al.*'s (2012) theropod dinosaur dataset. Moreover, improvements in the method and its multivariate generalizations, as well as the important (and usually neglected) issue of eigenvector selection, were discussed. Hopefully, calling attention to all these issues will improve the quality of applications of the PVR approach to test evolutionary and ecological hypotheses. Indeed, the application of the improvements in PVR and the PSR curve allowed us to detect a few more complex patterns that went unnoticed by Brusatte *et al.* (2012), especially the non-stationarity in skull shape evolution.

PVR and its recent expansions allow researchers to answer several questions related to trait evolution and diversification, even when the processes underlying them are non-stationary in the phylogeny. PVR is also easily generalized for the multivariate case (using RDA) and here for the first time a multivariate generalization of the PSR curve was showed. This paper also discussed how PVR can be used to evaluate different components of morphological disparity, revealing the flexibility of eigenfunction approaches. The PVR-based method for calculating phylogenetic disparity is an attractive method for identifying differences in trait variability among groups, with useful applications in many paleobiological studies attempting to identify differences in disparity or evolutionary rates among clades.

There are several methods for phylogenetic comparative analyses today; many of them based on the unifying framework of phylogenetic generalized least squares (PGLS). Although the results of these different methods may be comparable in most empirical cases (see also Pavoine & Ricotta, 2013 for a more theoretical reasoning supporting this similarity), there have been some discussions about the advantages of each approach and some criticisms of the eigenfunction analyses in general. Even if PGLS has better statistical performance under simple evolutionary models (*i.e.* see Martins *et al.*, 2002; Freckleton *et al.*, 2011; Pennell & Harmon, 2013) and also may provide a unifying framework (Monteiro, 2013), eigenfunctions are usually much easier to implement and are able to deal with more complex patterns (such as non-stationarity) that may be difficult to express in an "a priori" evolutionary model. In fact, rather than assuming an evolutionary model, such as Brownian motion or Ornstein-Uhlenbeck, and adding the expected covariance among taxa into model residuals (so that results are conditional to this model, see Martins & Hansen, 1997), the PVR uses the phylogenetic eigenvectors to empirically model the trait variation. In the end, it may be even possible to join the two frameworks, as recently proposed by Guenard *et al.* (2013). Finally, as an exploratory tool, the PSR curve developed from the original PVR approach, generalized here to a multivariate form, allows one to define the expectation of PVR under simple evolutionary models, as has been showed in the case of theropod dinosaurs, which in turn allows a better understanding of how traits evolve over different time scales.

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Appendix 1. The methods used in this paper can be implemented in several R packages, including mainly *PVR*, *ape* and *vegan*. Other packages, including *phylobase*, *motmot*, *ouch*, *geiger*, *pGLS*, *caper*, *adephylo*, *phytools*, *picante*, *syncsa*, can also be useful for several phylogenetic comparative analyses. Below is provided some simple R code for PVR and phylogenetic autocorrelation, based on simulated phylogeny and data, which allow implementing the analyses discussed in this paper.

```
#install these packages from CRAN
library(PVR)
library(ape)
library(vegan)
library(phylobase)
library(adephylo)

install.packages("packfor", repos="http://R-Forge.R-project.org")
library(packfor)

#SIMULATED DATA
#phylogenies and traits Y and X
phy <-rcoal(15) #simulated phylogeny with 15 species
plot(phy)
y <-rTraitCont(phy) #trait y simulated under Brownian motion for 15 species
x <- rTraitCont(phy) #trait x simulated under Brownian motion for 15 species

#plot trait on the phylogeny
plot_PHY <- phylo4d(phy, y)
par(mar=rep(.1,4))
table.phylo4d(plot_PHY,ratio.tree=0.25,var.label=1:36,cex.symbol=1,pch=15,box=F)

#THE PVR FUNCTION
#Eigenvector and eigenvalue extraction using PVRdecomp function
eigvec <-PVRdecomp(phy)
eigval <-eigvec@Eigen$values
sum(eigval)
eigp <-eigval/(sum(eigval))
eigpc <-cumsum(eigp)
eigpc <-eigpc*100
plot(eigpc, xlab="Eigenvalues",ylab="% of explanation", font=1, cex=1.0, pch=16,type="b")
#you can use the plot to select a few eigenvector for further modeling, in a sequential approach

#Basic PVR
#modelling trait y
PVR <- PVR(eigvec,trait=y,method="moran")
PVR@Selection$Id # look at selected axis using Moran's I
SCompY <-PVR@PVR$Residuals #extract the S-component that can be used for further analyses

#modelling trait x
PVRX <- PVR(eigvec,trait=x,method="moran")
SCompX <-PVRX@PVR$Residuals #extract the S-component that can be used for further analyses

cor(SCompX,SCompY) # correlation between the S-components of PVR for y and x

#testing autocorrelation in PVR's S-component with a single Moran's I across phylogeny
w <-vcv.phylo(phy) #calculates phylogenetic covariance/correlation
diag(w)<-0
Moran.I(y,w) # Moran's I in the original trait y
Moran.I(SCompY,w) # Moran's I in the PVR residuals, after taking phylogeny into account in y
#correlograms can be generated by breaking a distance matrix (obtained using the
#cophenetic function in ape) into several binary matrices akin to w-matrix used here,
#and calculating Moran's I using the function above

#The PSR curve
PSR <- PSR(eigvec,trait=y,null.model=TRUE,Brownian.model=TRUE,times=50)
plot(PSR)
#see below for a multivariate PSR curve using vegan

#Partial regression using PVR, regressing y against eigenvectors and x
PVRX <- PVR(eigvec,trait=y,envVar=x, method="moran")
Partition <-PVRX@VarPart #show the fractions 'a','b','c','d'
VarPartplot(PVRX)
```

```
#MULTIVARIATE PVR USING VEGAN
#If two data matrices ym and xm are created, it is possible to use a multivariate
#partition using the rda function in vegan

y1 <-rTraitCont(phy)
y2 <-rTraitCont(phy)
x1 <- rTraitCont(phy)
x2 <- rTraitCont(phy)

ym <-as.matrix(cbind(y1,y2))
xm <-as.matrix(cbind(x1,x2))

vectors <-eigvec@Eigen$vectors #extract the eigenvector matrix from PVRDecomp object
vec <-as.matrix(vectors)

prda<-rda(ym~vec+xm)
test <-anova(prda, by="margin") ## test fractions[a] and [c]
part<-varpart(ym,vec,xm) # creates a variance partition table

#MULTIVARIATE PSR CURVE
r2m <-numeric(ncol(vec))
for(i in 1:ncol(vec)){
     e <- as.matrix(vec[,1:i])
     rdam <-rda(ym~e)
     r2m[i] <-RsquareAdj(rdam)
}
plot(eigpc,r2)
# for a univariate PSR curve, replace the RDA above by a standard OLS model

# BASIC PVR USING APE AND VEGAN
phydist <-cophenetic(phy)
phydist <- (phydist)^(1/2) # for original PVR from 1998, skip this step
pcord <-pcoa(phydist,correction="none", rn=NULL)
vec2 <-pcord$vectors

#linear modeling of y and eigenvector selection based on stepwise
m <-lm(y~vec2)
step <-forward.sel(y,vec2,alpha=0.05)
vecsel <-vec2[,step[,2]] #separates which eigenvectors were selected
pvr2 <-lm(y~vecsel)
RsquareAdj(pvr2)$r.squared
Scomp2 <-pvr$residuals
Moran.I(Scomp2,w) #Moran's I in residuals
```