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ORIGINAL ARTICLE

Intravertebral vs. intervertebral integration and modularity in the vertebral column of mammalian carnivorans

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Abstract

The vertebral column is a multicomponent structure whose organization results from developmental and functional demands. According to their distinct somitic origins, individual vertebrae exhibit intravertebral modularity between the centrum and neural spine. However, vertebrae are also organized into larger units called intervertebral modules that result from integration between adjacent vertebrae due to locomotory demands or from common developmental origins due to resegmentation. A previous hypothesis suggested that the boundaries of intervertebral modules coincide with changes in the patterns of intravertebral integration. Here, we explicitly test whether the patterns of modularity and integration between the centrum and neural spine (i.e., intravertebral) in the boundary vertebrae among previously defined intervertebral modules change with respect to those in the vertebrae within intervertebral modules. We quantified intravertebral modularity patterns and quantified the strength of intravertebral integration for each vertebra of the presacral region in 41 species of carnivoran mammals using 3D geometric morphometrics. Our results demonstrate a significant intravertebral modular signal between the centrum and neural spine in all post-cervical vertebrae, including the boundary vertebrae among intervertebral modules. However, the strength of intravertebral integration decreases at the boundary vertebrae. We also found a significant correlation between the degree of intravertebral integration and intervertebral integration. Following our results, we hypothesize that natural selection does not override the integration between the centrum and neural spine at the boundary vertebrae, a pattern that should be influenced by their distinct somitic origins and separate ossification centers during early development. However, natural selection has probably influenced (albeit indirectly) the integration between the centrum and neural spine in the vertebrae that compose the intervertebral modules.

KEYWORDS
centrum, integration, mammals, modularity, neural spine, vertebral column

1 | INTRODUCTION

The vertebral column is a complex morphological structure composed of serially homologous subunits called vertebrae that are involved in body flexion and locomotion by transmitting propulsive forces from the limbs and play an essential role in a multitude of biological aspects, including body posture, weight support, and acquisition of food (Böhmer, 2015). Morphological discontinuities among vertebrae have been used to subdivide the vertebral column into discrete series or regions that result from the expression fields of

Hox and other patterning genes (e.g., Burke et al., 1995; Mallo, 2021). However, recent studies on evolutionary integration and modularity among presacral vertebrae in different mammalian species, including carnivorans, demonstrate that their organization into modules, i.e., sets of tightly integrated vertebrae that are relatively independent of other vertebral sets, does not match the classic morphological regions (e.g., Buchholtz, 2007; Randau & Goswami, 2017a, 2017b; Jones, Benitez, et al., 2018; Jones et al., 2020; Martín-Serra et al., 2021; Smith and Angielzyk, 2022).

1.1 | Intervertebral modularity and integration in the carnivoran backbone

Martín-Serra et al. (2021) analyzed evolutionary integration and modularity of all presacral vertebrae of mammalian carnivorans taking phylogeny into account, and concluded the presence of three presacral modules (Figure 1a) that are presumably related to the motion capability of the presacral spine. The highly integrated anterodorsal and posterodorsal modules coincide with two regions with motion constraints to avoid compromising trunk ventilation and exceeding the extension of the posterior back, respectively. On the other hand, Martín-Serra et al. (2021) also demonstrated that boundary vertebrae, located between modules, are less integrated with other vertebrae of the module they belong to, and the diaphragmatic vertebra is the least integrated with other vertebrae (low covariation) of all the presacral axial system. Therefore, its inclusion in either of those modules is undefined (Figure 1a). This was interpreted by Martín-Serra et al. (2021) as related to the ability to move excessively that characterizes the 'Diaphragmatic joint complex' (Filler, 1986; Flower, 1885; Slijper, 1946). This reorganization of vertebral column regions has been linked to different locomotor strategies and, therefore, it probably reflects the action of natural selection towards different locomotory demands (e.g., Jones, Benitez, et al., 2018; Randau & Goswami, 2017b; Martín-Serra et al., 2021; Figueirido et al., 2021).

However, it has not been tested whether this pattern of intervertebral integration that is presumably related with functional performance is also significant when using more sensitive approaches to find regions, such as for instance, that used by Head and Polly (2015) who found 'cryptic' regionalization in apparently 'de-regionalized' spines. On the other hand, Jones, Angielczyk, et al. (2018) studied functional regionalization (and heterogeneity) in the vertebral columns of mammals and in non-mammalian synapsids and they revealed that once forelimb function diversified, the spine developed distinct regions with further differentiation leading to the high disparity observed today in mammals. Both approaches were based on a segmented linear regression approach, and it would be of interest to compare whether this approach identifies the same 'regions' as those obtained using more conventional analyses of integration/modularity, especially considering that the additional regions often recovered using segmented regression relate to functional performance, particularly in mammals.

1.2 | Intravertebral modularity and integration in carnivoran vertebrae

Vertebrae are composed of the centrum and neural spine, two vertebral parts (Figure 1b) with distinct somitic origins through segmentation of the sclerotome. While the vertebral body (centrum) originates from the ventral and central regions of the sclerotome, the neural arch, spinous process, pedicles, and transverse processes originate from the dorsal and postero-central regions, including cells of the somitocoel (e.g., Christ et al., 2007). A recent study on evolutionary integration and modularity within-species demonstrated that the centrum and neural spine of felids are two separate intravertebral modules (Randau & Goswami, 2017a).

1.3 | How does intervertebral and intravertebral integration relate?

We define intravertebral module as centra or neural spines that are relatively independent of each other in the same vertebra. Additionally, we define an intervertebral module as a set of vertebrae that closely covary with each other within that set, but that covary distinctly less with vertebrae outside it and especially in different such sets or modules. Taken this information together, previous evidence indicates that the distinct somitic origins influence the patterns of intravertebral integration and modularity, i.e., covariation between the centrum and neural spine, during early development (Randau & Goswami, 2017a). However, it has been proposed that the organization of this chain of repeated vertebrae into intervertebral modules is also influenced by natural selection in response to different locomotory demands (Randau & Goswami, 2017b; Martín-Serra et al., 2021).

Furthermore, Randau & Goswami (2017a) hypothesized that abrupt changes in the strength of integration between the centrum and neural spine coincide with the boundaries among traditional and functional regions (i.e., patterns of covariation between adjacent vertebrae [intervertebral covariation]) in felids (Felidae, Carnivora). These authors also proposed that a functional overprinting of developmental patterning may occur in these structures in order to maintain larger modular organization of the whole vertebral column, suggesting that intravertebral modularity may reflect larger-scale modularity of the felid vertebral column (Randau and Goswami, 2017a). These authors also suggest that further analyses in other datasets are needed to confirm their hypothesis. Moreover, whether a significant association between intravertebral and intervertebral integrations exists was not tested by Randau and Goswami (2017a).

Here, we use a dataset of 41 species belonging to 12 carnivoran families (Figure 2) including all presacral vertebrae to investigate the relationship between patterns of intravertebral (centrum vs. neural spine) and intervertebral integration (between adjacent vertebrae) at an evolutionary level (sensu Klingenberg, 2008) regardless of whether intra and intervertebral modularity and integration

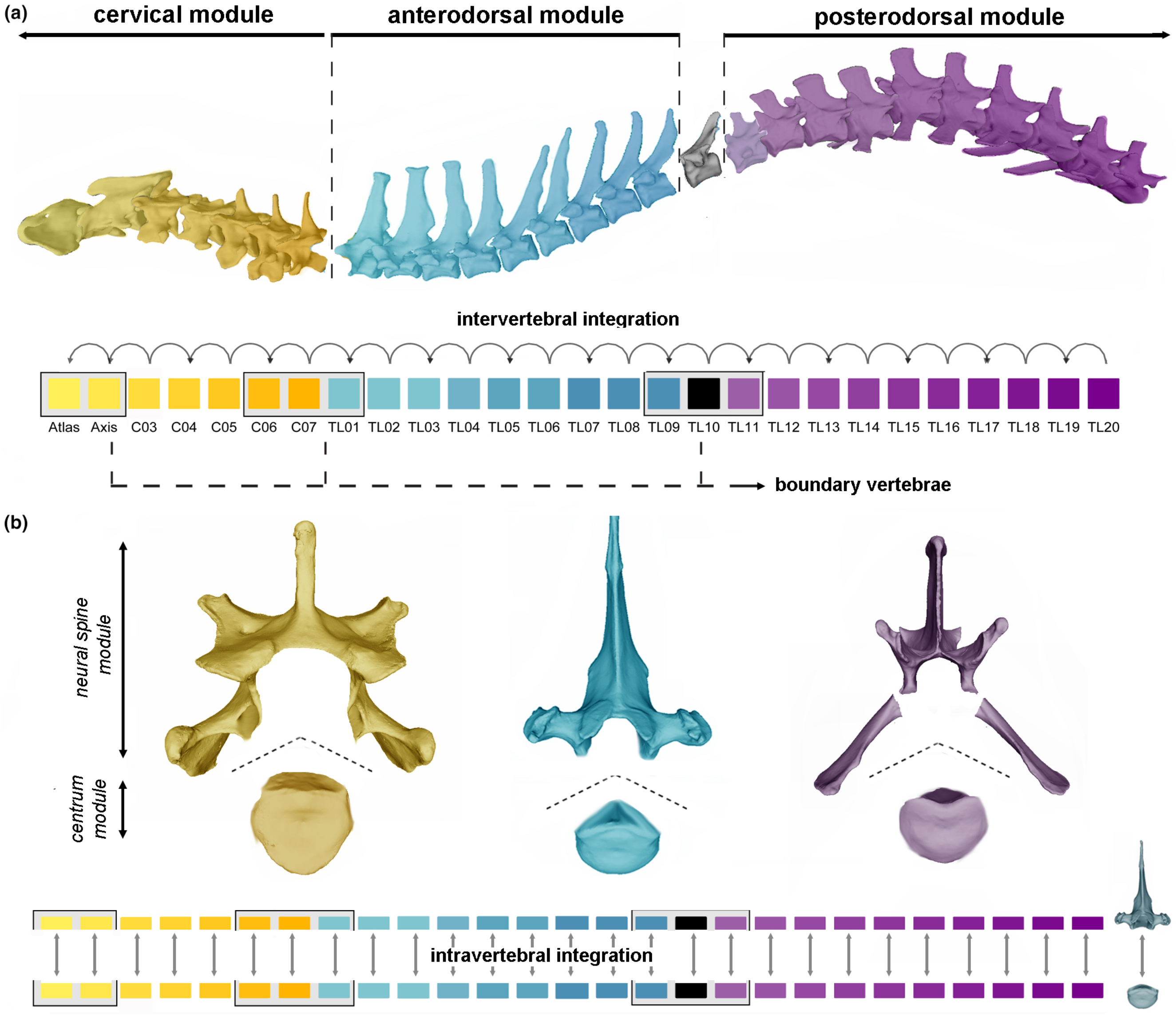

FIGURE 1 Intravertebral vs. intervertebral integration and modularity. (a) functional modules found by Martín-Serra et al. (2021) in the carnivoran column as a result of intervertebral integration analysis. Vertebrae within grey boxes represent boundary vertebrae between regions, and arrows indicate correlations between adjacent vertebrae to calculate intervertebral integration and modularity (see methods). (b) The two vertebral parts (i.e., the centrum and neural spine) subject to analyses of intravertebral modularity and integration.

patterns are driven by the distinct somitic origins of the centrum and neural spine or by natural selection to accomplish different locomotory demands, respectively.

We specifically address the following questions: (i) Are patterns of modularity between the centrum and neural spine (intravertebral) influenced by their distinct somitic origins in the entire order Carnivora, or is it only a unique property of felids, as Randau and Goswami (2017a) demonstrated? (ii) Is the strength of integration between the centrum and neural spine modified in all presacral vertebrae or only for some sets of vertebrae (i.e., either within-module or boundary vertebrae)? (iii) Is there a significant correlation between intra- and intervertebral integration across the presacral axial column?

2 | MATERIAL AND METHODS

2.1 | Collecting vertebrae

We used the dataset published by Martín-Serra et al. (2021), which comprises a set of 34 homologous landmarks that were digitized into 1107 three-dimensional models (Figure S1; Table S1). These landmarks were divided into those located on the spinous process and those located in the centrum (Figure 3).We sampled a single individual for each species, except for *Mustela putorius* and *Suricata suricatta*, which were sampled from two individuals, and were averaged for both species. The models comprised all presacral vertebrae (27 vertebrae; 7 cervicals, 20 thoracolumbars) of 41 carnivoran species

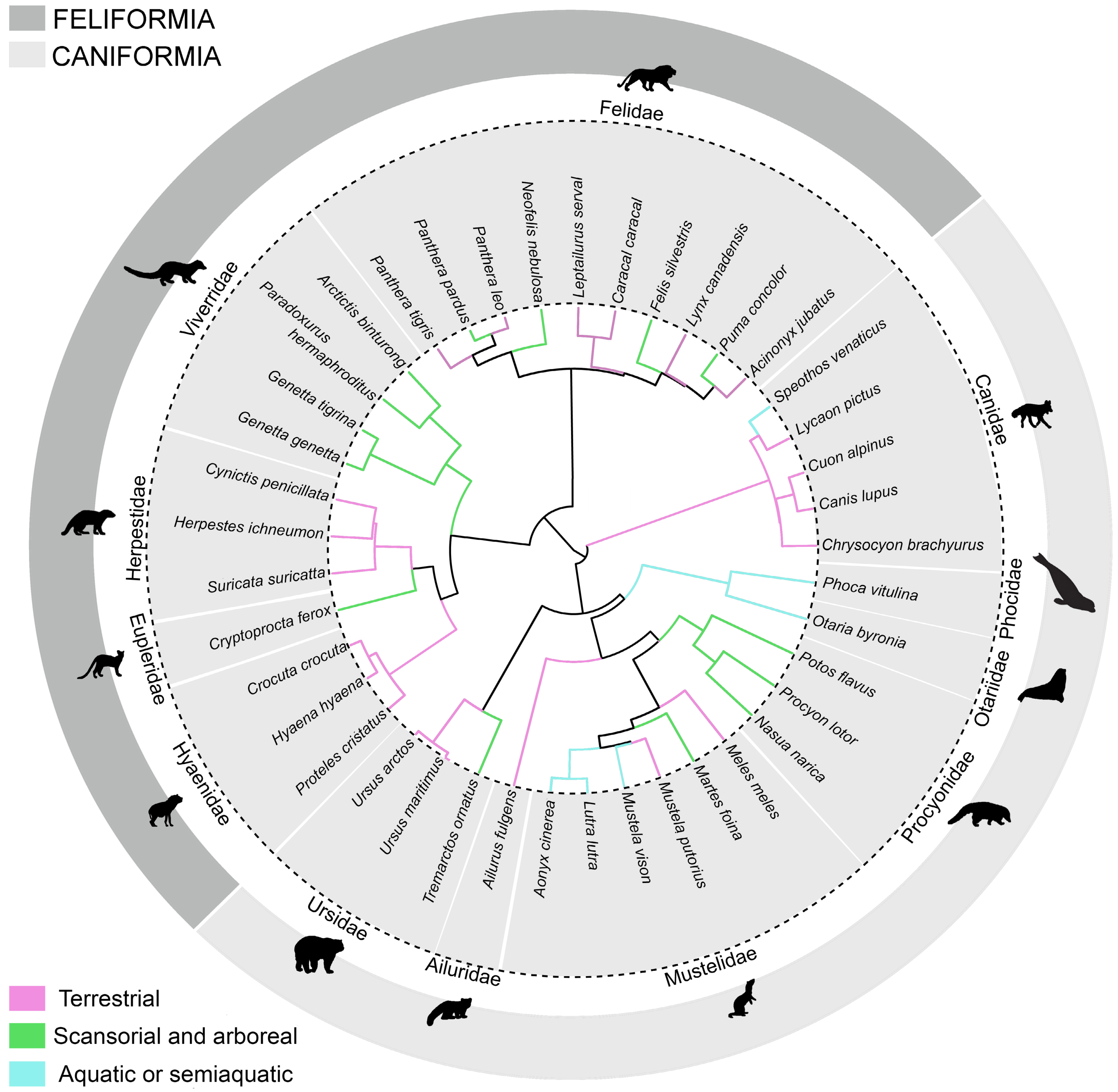

FIGURE 2 Phylogeny of the species included in this study. The tree topology and branch lengths were obtained from the supertree published by Nyakatura and Bininda-Emonds (2012). The classification into ecological categories was based on previously published sources (Table S1).

that were scanned with a medical CT or NextEngine® surface scanner (http://www.nextengine.com) and edited with Meshlab (Cignoni et al., 2008). It is worth mentioning that our landmarks do not cover the shape of the transverse processes of the lumbar vertebrae or rib articulation facets in the thoracic vertebrae because these structures are not present in all presacral vertebrae. However, our landmarks accounted for other traits of the boundary vertebrae. For example, the diaphragmatic vertebrae possess a prezygapophysis with a different orientation to the postzygapophysis, which is accounted for by digitized landmarks. Additionally, the anticlinal vertebra, which is another important boundary vertebra, is defined by the orientation of the spinous process, a morphological trait that is also captured by the landmarks. Although the digitized landmarks do not capture the shape of the rib articulation facets, the cervicothoracic boundary vertebrae are also characterized by other morphological traits. These traits, such as the height of the spinous process (Figure 1), do not escape quantification using digitized homologous landmarks.

2.2 | Sampling schemes

We followed the count procedures described by Martín-Serra et al. (2021) to consider different hypotheses of homology between vertebrae along the antero-posterior axis across taxa

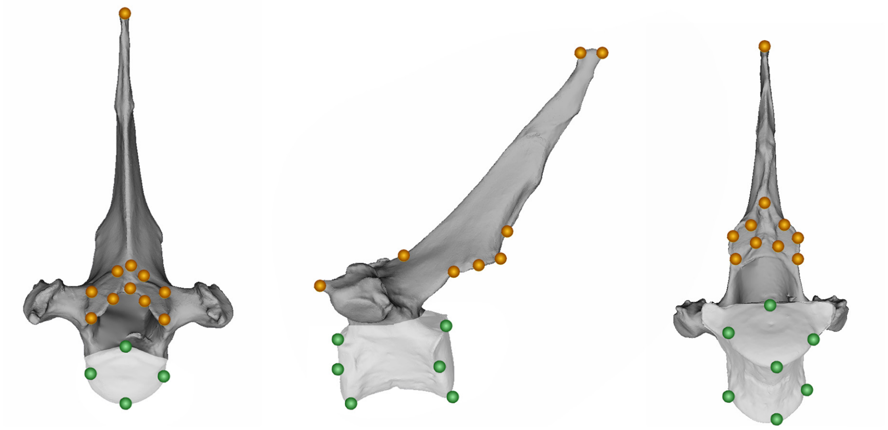

FIGURE 3 Landmarks digitized in this article using the thoracic vertebra of the cheetah (*A. jubatus*) as an example. Landmarks in green and orange represent those used to capture the shapes of the centrum and neural spine to test for intravertebral developmental modularity. For intervertebral analyses, both sets of landmarks were analyzed together in each vertebra. See also Figure S1.

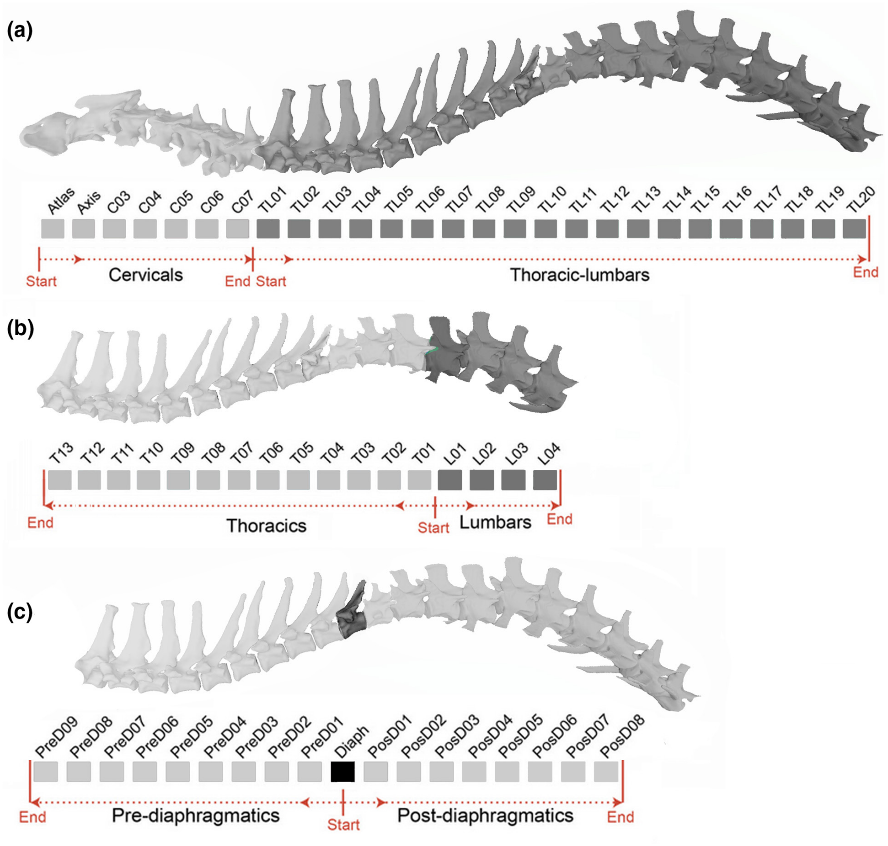

FIGURE 4 Scheme of the different counting methods used in this article (after Martín-Serra et al. 2021). (a) cervical plus joined thoracolumbar count. (b) thoracolumbar boundary count. (c) Diaphragmatic start count.

(Figure 4): (i) *A joined thoracolumbar count procedure*, in which we count cervical vertebrae and directly group with the thoracolumbar vertebrae as dorsal vertebrae; (ii) *A thoracolumbar boundary count procedure*, counting 13 thoracic vertebrae from the thoracic-lumbar transition in the cranial direction and 4 lumbar vertebrae in the caudal direction (the maximum number of vertebrae shared by all species); and (iii) *A diaphragmatic starting count procedure*, in which we counted from the diaphragmatic vertebra defined as the last thoracic vertebra, with vertebrosternal ribs and with pre- and post-zygapophyses oriented in horizontal and vertical planes, respectively. All diaphragmatic vertebrae are joined together regardless of their position, and the remaining vertebrae are established as pre- and post-diaphragmatic in cranial and caudal directions. We did not use the selected vertebrae procedure described by Jones, Benitez, et al. (2018) and applied by Martín-Serra et al. (2021) and Figueirido et al. (2021) because we were interested in testing the association between intravertebral and intervertebral integration. The procedure of Jones, Benitez, et al. (2018) analyzed a phylogenetically wider dataset (52 mammalian species) with variation in count across taxa, but they solely used five thoracolumbar vertebrae for which homology across species was clear. Therefore, the selected vertebrae procedure is based on the analysis of very few vertebrae ($N = 6$) and this precludes us to use this procedure for clustering vertebrae because it compromises the statistical significance of the results.

2.3 | Geometric morphometrics

The raw coordinates of the digitized landmarks were incorporated into *geomorph* (Adams et al., 2019). For each vertebral position, we performed a Procrustes alignment (Dryden & Mardia, 2016), accounting for bilateral symmetry (Klingenberg et al., 2002). Next, we performed phylogenetic regression analyses of Procrustes coordinates against log-transformed centroid size for each vertebra (Monteiro, 1999), i.e., phylogenetic Procrustes ANOVAs using log-transformed centroid size as the independent variable (sensu Collyer et al., 2015). We used the residuals of this phylogenetic regression as the allometry-free shape coordinates in subsequent analyses. The phylogeny included in this analysis was taken from Nyakatura and Bininda-Emonds (2012) and is shown in Figure 2.

2.4 | Modularity analyses

We tested for intravertebral modularity as quantified by Randau and Goswami (2017a), for the neural spine and centrum (Figure 1a), in all the presacral axial column vertebrae. The modularity hypothesis was tested using the procedure described by Adams (2016). These functions estimated the covariance ratio (CR) coefficient as a measure of the strength of integration between the hypothesized modules and compared it with a set of CR coefficients obtained from random distributions of modules (999 iterations). The modular hypothesis was accepted if the observed CR coefficients were lower than 95% of the random CR coefficients ($p < 0.05$).

2.5 | Integration analyses

We explored the strength of intravertebral integration using a phylogenetic two-block partial least squares analysis (2B-PLS) between the two hypothesized vertebral modules (Adams & Collyer, 2016; Rohlf & Corti, 2000). This analysis quantifies the degree of covariation between two sets of variables and provides a correlation coefficient (*r*PLS), to indicate the strength of integration (Zelditch & Goswami, 2021). It is worth mentioning that our dataset is high-dimensional (34 landmarks in 3D). Although we sampled 1107 vertebrae, they belong to 41 species (or even lower in the case of the analyses taking into account taxonomy and ecology; see below). Therefore, we urge readers to interpret the results of *r*PLS across taxa with caution, as the values could be upwardly biased. However, as both the sample size and the number of variables are constant in all analyses, the reported weaknesses of *r*PLS as an estimator of the strength of the covariation among datasets with different sample sizes and dimensionality (Adams & Collyer, 2016) should not be critical. Furthermore, we performed the analyses of integration between both hypothesized modules for all vertebrae, regardless of modularity test significance, to obtain comparable results between vertebrae.

2.6 | Significance correction

To detect false positives in both modularity tests and 2B-PLSs, we used the Benjamini and Hochberg (1995) for significance correction, as each analysis was independently computed for each vertebra. First, the procedure orders the p values from smallest to largest and assigns them a position, i, from one to m (the total number of p values included). Then, the formula $(i/m)*\alpha$ is applied for each, where α is set to 0.05. Finally, a new corrected significance level is established at the largest i, where the p value is equal to or lower than the formula value.

2.7 | Effects of ecology and phylogeny on intravertebral modularity and integration

To test whether species ecology affects the patterns of intravertebral integration/modularity between the centrum and neural spine, we divided taxa into terrestrial and arboreal based on their locomotory adaptations towards substrate use. We performed the same analyses for each ecological grouping (Figure 1; Table S1). The aquatic and semiaquatic taxa were excluded from the analysis because the low number of these species included in our sample size precluded us from performing statistical tests. Moreover, to test for possible modularity and integration differences between both carnivoran suborders (Figure 2), we repeated the modularity tests and 2B-PLS analyses for caniform and feliform species separately.

2.8 | Comparing intravertebral with intervertebral integration

To test the association between intravertebral integration (i.e., centrum vs. neural spine) and intervertebral integration (i.e., between adjacent vertebrae), we regressed the *r*PLS obtained from intravertebral integration with the *r*PLS values from intervertebral integration obtained by Martín-Serra et al. (2021) for each count procedure. For the latter, we used the average *r*PLS for each vertebra with its two immediately adjacent vertebrae (except for the first cervical and last lumbar vertebrae) as a proxy for intervertebral *r*PLS. Regressions were made using the reduced major axis (RMA) method. We also considered as boundary vertebrae, the atlas-axis complex and those vertebrae located at intermodule boundaries plus the anterior and posteriormost vertebrae, following the results of Martín-Serra et al. (2021). See also Figure 1.

3 | RESULTS

Using all sampling procedures, the two-module hypothesis between the centrum and neural spine was found to be significant for most vertebrae (Figures 5, S2; Table S2). Exceptions are the C01, C03-C07, TL03, TL09, and TL13 with the 'joined thoracolumbar-count' sampling procedure (Figures 5a, S2a; Table S2). Using other sampling procedures that consider different hypotheses of homology between vertebrae across taxa, the two-module hypothesis between the centrum and neural spine was significant in almost all vertebrae (Figures 5b,c, S2b,c; Table S2).

Despite this significant intravertebral modular pattern, the strength of integration between the centrum and neural spine varied (Figure 5, Figure S2; Table S2). The boundary vertebrae consistently showed decreased strength of integration between their centra and neural spines with respect to other vertebrae (Figure 5, Figure S2; Table S2). This was observed in C06-TL01 at the boundary between the cervical and anterodorsal modules, and in TL09-TL11 at the boundary between the anterodorsal and posterodorsal modules (Figure 5a, Figure S2; Table S2). It is worth mentioning that the integration between the centrum and the neural spine of the boundary vertebrae located between the cervical and the anterodorsal module is weaker than in the mid-cervical vertebrae (C03-C05) but not in the anterodorsal vertebrae (Figure 5a, Figure S2). Using other sampling procedures, the reduction in the strength of integration between the centrum and the neural spine at boundary vertebrae is consistent, i.e., T02-T04 for the 'thoracolumbar-boundary count' (Figures 4b, S2) and PreD01-PosD01 for the 'diaphragmatic-boundary count' (Figures 5c, S2; see also Table S2).

Moreover, this pattern was also observed separately for the suborders Caniformia and Feliformia (Figure 6, Figure S3; Table S2), and there were no significant differences in the intravertebral modularity signal between suborders (Figure 6, Figure S3; Table S2). On dividing the sample, into arboreal and terrestrial species, the strength of integration between the centrum and the neural spine at the boundary vertebrae was also reduced (Figure 7, Figure S4; Table S2). However, the modularity signal was blurred more frequently (in more vertebrae) in terrestrial species than in arboreal species (Figure 7, Figure S4; Table S2).

The bivariate regressions between the *r*PLS obtained for the centrum and neural spine of each vertebra (intravertebral) and the average of the *r*PLS obtained for each vertebra with its two immediate adjacent vertebrae (intervertebral) demonstrated a significant association for all count procedures (Figure 8; Table 1).

4 | DISCUSSION

4.1 | Modularity and integration between the centrum and the neural spine in Carnivora

Our results demonstrate that the centrum and neural spine are separate modules when the entire order Carnivora is analyzed at an evolutionary level, a pattern that should be influenced by the distinct somitic contributions (and separate ossification centers) of both vertebral parts (Figure 5, Figure S2; Table S2). However, the cervical vertebrae did not show a significant modularity signal between the centrum and neural spine, except for the axis (Figure 5, Figure S2; Table S2). This was also observed by dividing the samples based on taxonomy (Figure 6, Figure S3; Table S2) or ecology (Figure 7, Figure S4; Table S2). Moreover, cervical vertebrae also exhibited high values of integration between the centrum and the neural spine (Figure 5, Figure S2; Table S2).

Morphological integration is expected to produce phenotypic disparity that is more constrained along specific axes of variation (e.g., Felice et al., 2018). In contrast, modularity permits a more even exploration of trait space as a result of unconstrained axes of maximum covariation (e.g., Goswami et al., 2014; Goswami & Polly, 2010; Klingenberg, 2005). Therefore, the lack of intravertebral modularity signal concomitant with high intravertebral integration of the cervical vertebrae is probably related to their low among-species disparities (i.e., more similar morphologies across species) and their highest morphological disparity indices (i.e., greater evolutionary constraints than other post-cervical subunits), as reported by Figueirido et al. (2021).

Contrary to the cervical vertebrae, the posterodorsal vertebrae (i.e., post-diaphragmatic subunits) exhibit a significant modularity signal between the centrum and neural spine, but both modules are highly integrated (Figure 5, Figure S2; Table S2). This was also evident for both carnivoran suborders separately (Figure 6, Figure S3; Table S2) and for terrestrial and arboreal species (Figure 7, Figure S4; Table S2). Figueirido et al. (2021) demonstrated that the posterior thoracic and lumbar vertebrae exhibit high among-species disparities and low morphological disparity indices, suggesting that these post-diaphragmatic subunits exhibit different morphologies across species and relaxed evolutionary constraints. Although the modularity signal between the centrum and neural spine is significant in these vertebrae, the high integration between these two modules may permit the appearance of new morphotypes at constrained axes of maximum covariation. This could be related to the previously reported increasing evolvability and ecological plasticity of this region in mammals (e.g., Buchholtz, 2012; Figueirido et al., 2021; Jones, Benitez, et al., 2018; Wellik & Capecchi, 2003).

Almost all vertebrae belonging to the anterodorsal module had a significant modularity signal between the centrum and neural spine when the complete sample was analyzed (Figure 5, Figure S2; Table S2). However, this modularity signal for the analyses of both suborders (Caniformia and Feliformia) and both

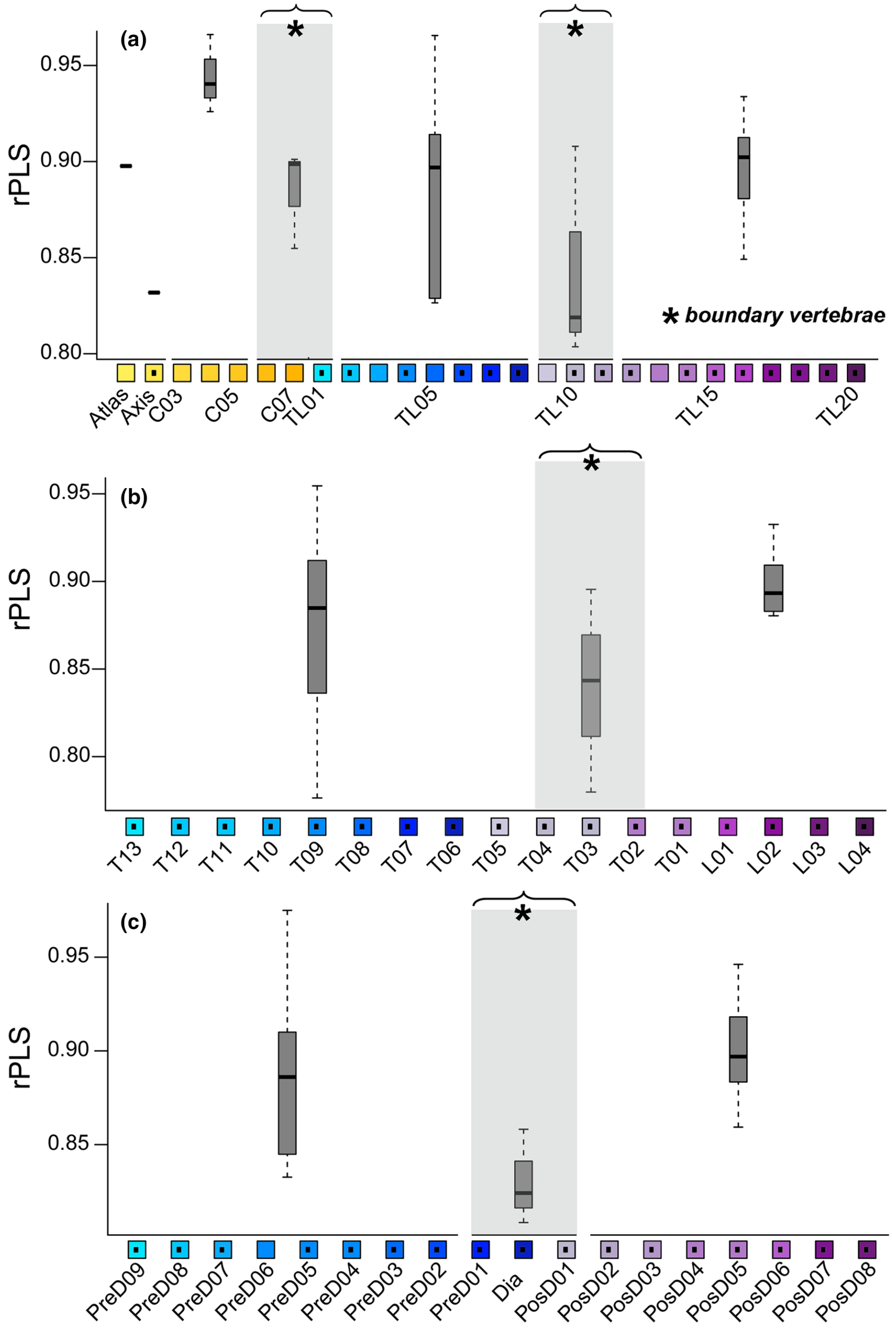

FIGURE 5 Results obtained from integration and modularity tests using all sampled species in the same analysis. (a) cervical plus thoracolumbar joined count; (b) thoracolumbar boundary count; (c) diaphragmatic start count. In all cases, the *x*-axis represents the vertebral position, and the *y*-axis represents the *r*PLS as a proxy for integration strength between the centrum and the neural spine. The horizontal line inside each box is the median of the *r*PLS computed for those vertebrae within intervertebral modules and boundary vertebrae (grey areas) following the three-intervertebral-modules hypothesis of Martín-Serra et al. (2021) for carnivorans. Box length is the interquartile range (IQR) and shows the difference between the 75th and 25th percentiles. Horizontal bars enclose values of 5–95%. Those vertebrae in which the modularity test between the centrum and the neural spine was significant are indicated by black dots within the square representing each vertebra (see also Figure S2 and Table S2).

ecological groupings (arboreal and terrestrial) was blurred to some degree (Figures 6, 7, Figure S3, and S4; Table S2). Nevertheless, we urge readers to interpret this result with caution because the sample size reduction in these analyses is likely to compromise statistical significance.

4.2 | Modularity and integration between the centrum and neural spine at boundary vertebrae

Our results do not demonstrate a lack of modularity signal between the centrum and the neural spine in vertebrae located

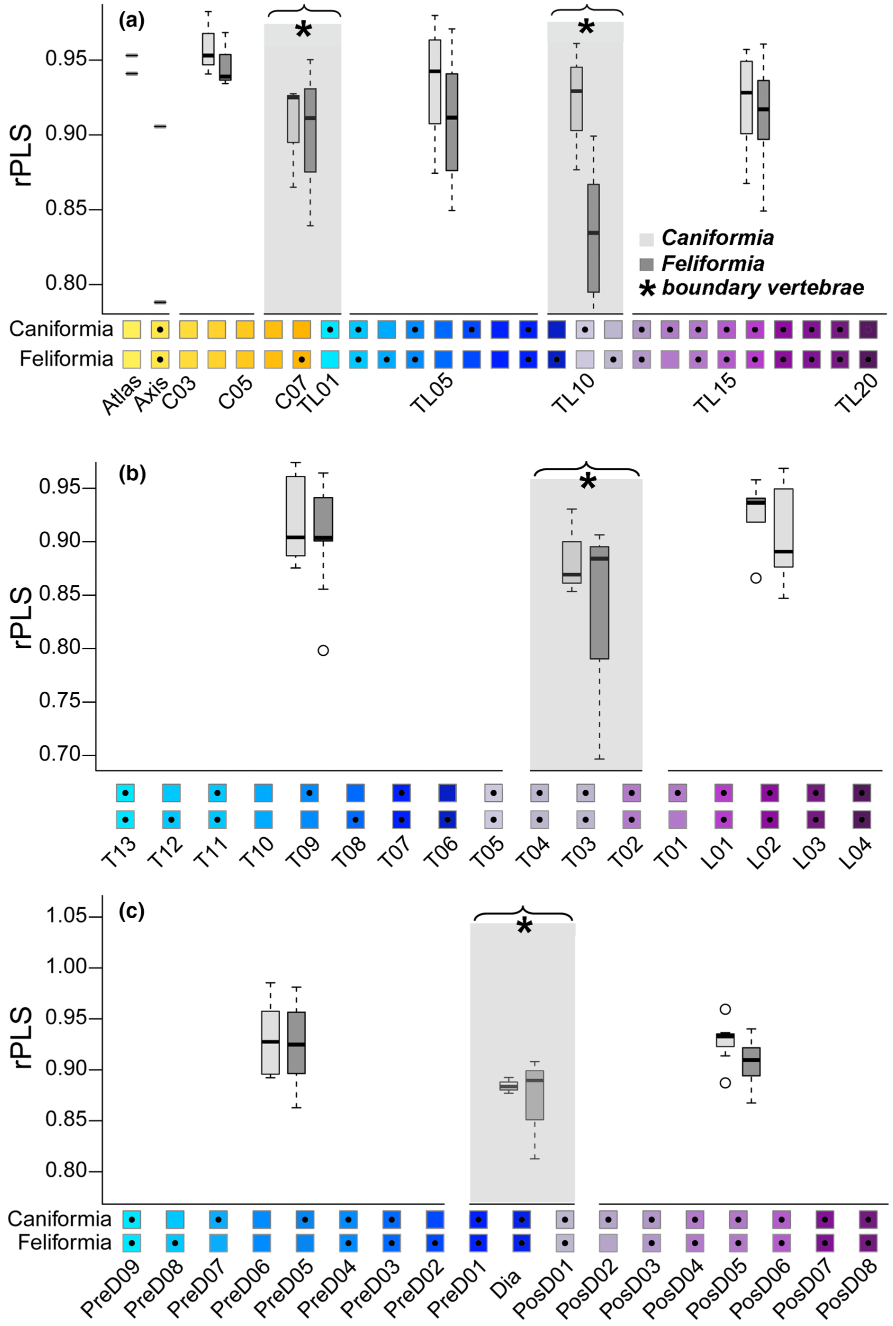

FIGURE 6 Results obtained from integration analyses and modularity tests for the suborders Caniformia and Feliformia. (a) cervical plus thoracolumbar joined count; (b) thoracolumbar boundary count; (c) diaphragmatic start count. In all cases, the *x*-axis represents the vertebral position, and the *y*-axis represents the *r*PLS as a proxy for modularity signal and integration strength between the centrum and the neural spine. The horizontal line inside each box is the median of the *r*PLS computed for those vertebrae within intervertebral modules and boundary vertebrae (grey areas) following the three-intervertebral-modules hypothesis of Martín-Serra et al. (2021) for carnivorans. Box length is the interquartile range (IQR) and shows the difference between the 75th and 25th percentiles. Horizontal bars enclose values of 5–95%. Those vertebrae in which the modularity test between the centrum and the neural spine was significant are indicated by black dots within the square representing each vertebra (see also Figure S3 and Table S2).

at the boundaries between functional intervertebral modules (Figures 5–7, S2–S4). The discrepancy in the modularity signal at boundary vertebrae between the results obtained by Randau and Goswami (2017a) and the results obtained here could be due to either the use of the within-species matrix by Randau and Goswami (2017a) or because it is a unique property of the felid spine. Although our sample size did not allow us to perform an intravertebral modularity test for only felids, our results obtained from the modularity analyses performed solely with the suborder Feliformia gave similar results to those of the analyses of the complete sample (Figure 6, Figure S3). On the other hand, the integration strength between the centrum and the neural spine

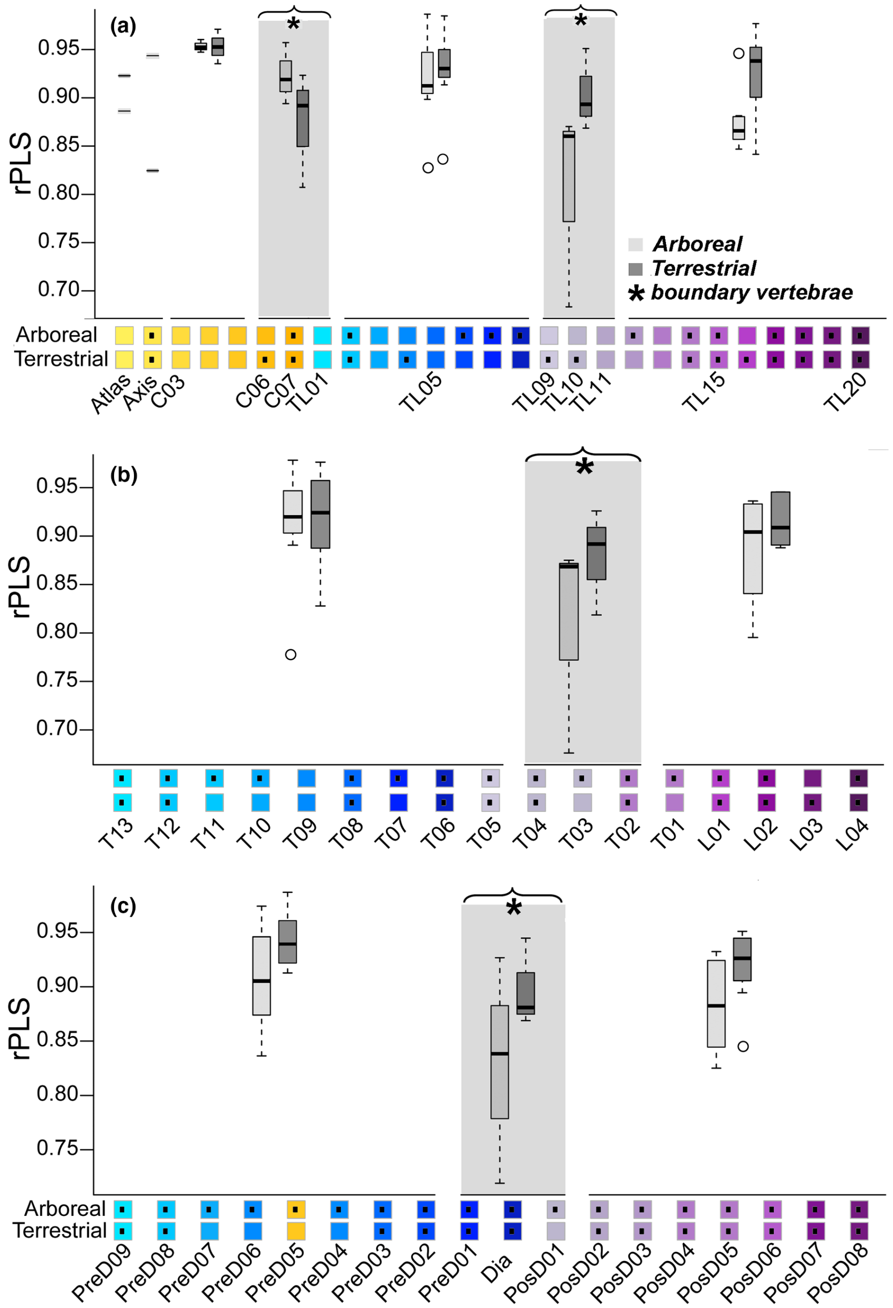

FIGURE 7 Results obtained from integration analyses and modularity tests for arboreal and terrestrial species. (a) cervical plus thoracolumbar joined count; (b) thoracolumbar boundary count; (c) diaphragmatic start count. In all cases, the *x*-axis represents the vertebral position, and the *y*-axis represents *r*PLS as a proxy for integration strength between the centrum and the neural spine. The horizontal line inside each box is the median of the *r*PLS computed for those vertebrae within intervertebral modules and boundary vertebrae (grey areas) following the three-intervertebral-modules hypothesis of Martín-Serra et al. (2021) for carnivorans. Box length is the interquartile range (IQR) and shows the difference between the 75th and 25th percentiles. Horizontal bars enclose values of 5–95%. Those vertebrae in which the modularity test between the centrum and the neural spine was significant are indicated by black dots within the square representing each vertebra (see also Figure S4 and Table S2).

of the boundary vertebrae, especially those of the diaphragmatic boundary, is lower than for those located within intervertebral modules (Figure 5–7, Figures S2–S4). This weaker intravertebral integration could be related to their low across-species disparities and high evolutionary constraints (Figueirido et al., 2021).

4.3 | Intravertebral vs intervertebral integration in the carnivoran spine

We found a significant positive correlation between the strength of intravertebral and intervertebral integration (Figure 8), which confirms that vertebrae with less integrated centra and neural spines

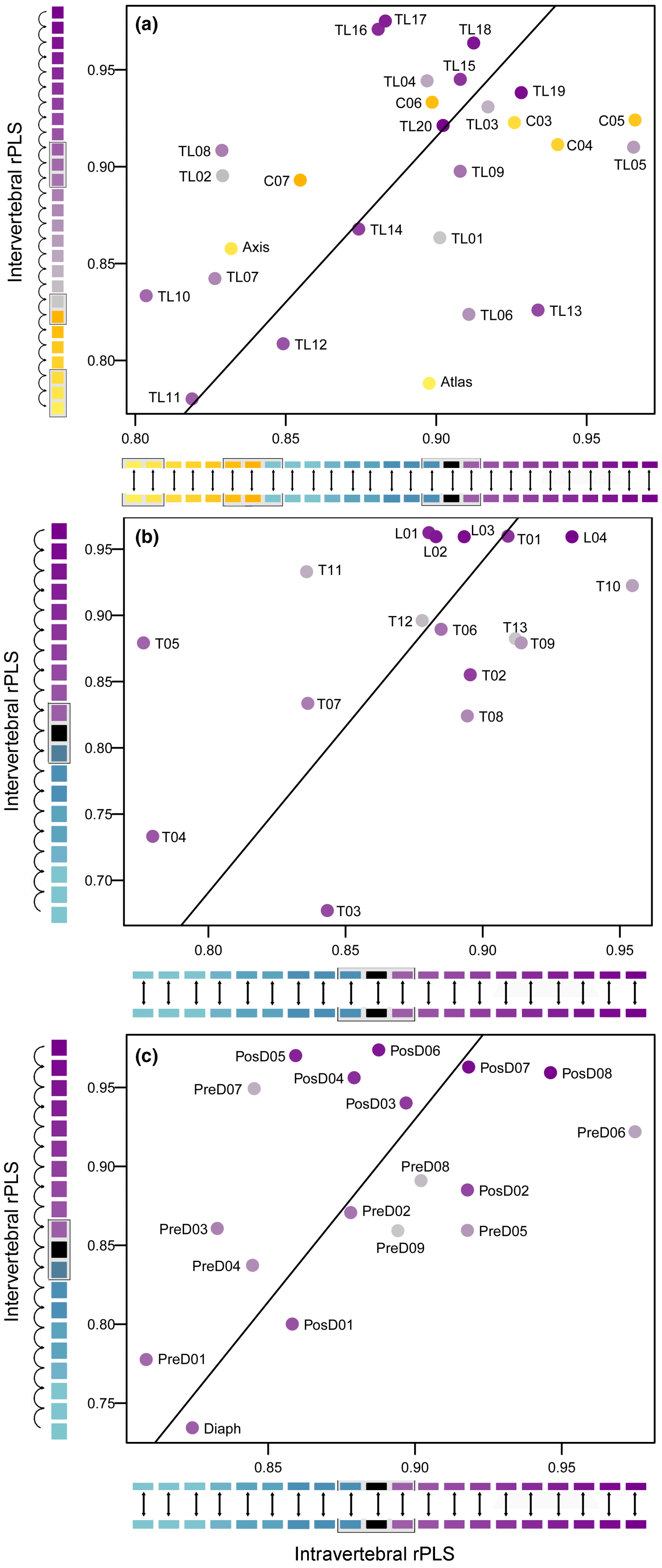
(a)
TL17
TL16
TL18
TL15
TL04
TL19
C06
TL20
TL03
C03
C05
C04
TL05
TL08
TL02
C07
TL09
TL14
TL01
Axis
TL07
TL10
TL06
TL13
TL12
Atlas
TL11
0.95
0.90
0.85
0.80
0.80
0.85
0.90
0.95
Intervertebral rPLS
(b)
L01
L03
T01
L04
L02
T11
T10
T12
T06
T13
T09
T05
T02
T07
T08
T04
T03
0.95
0.90
0.85
0.80
0.75
0.70
0.80
0.85
0.90
0.95
Intervertebral rPLS
(c)
PosD05
PosD06
PosD07
PosD08
PosD04
PreD07
PosD03
PreD06
PreD08
PosD02
PreD02
PreD03
PreD09
PreD05
PreD04
PosD01
PreD01
Diaph
0.95
0.90
0.85
0.80
0.75
0.85
0.90
0.95
Intervertebral rPLS
Intravertebral rPLS

TABLE 1 Results of the reduced major axis (RMA) regressions of mean intervertebral *r*PLS obtained from the data of Martín-Serra et al. (2021) on intravertebral *r*PLS obtained in this study.

Count procedure	*R* squared	*p*-value
Cervical + Thoracolumbar	0.166	0.016
Thoracolumbar boundary	0.256	0.017
Diaphragmatic starting	0.289	0.019

are also less integrated with their adjacent vertebrae and vice versa. This correlation entails the appearance of empty spaces in these bivariate plots (Figure 8) that show non-observed combinations of intra- and intervertebral integrations. Accordingly, vertebrae that are highly integrated with their adjacent regions do not exhibit weakly integrated centra and neural spines (Figures 8 and 9a), and vertebrae that are weakly integrated with their adjacent regions do not possess highly integrated centra and neural spines (Figures 8, 9d).

4.4 | The role of locomotor performance on intra- and intervertebral integration

Martín-Serra et al. (2021) found that vertebrae of the carnivoran spine had been reorganized into discrete modules (cervical, anterodorsal, and posterodorsal), and they cocnluded that the last two modules were probably influenced by natural selection in response to locomotory demands. Indeed, Jones, Benitez, et al. (2018) analyzed five thoracolumbar vertebrae in 52 mammalian species and they found that the vertebral regionalization in mammals has facilitated rapid evolution of the posterior trunk in response to natural selection for locomotion and static body support. On the other hand, Randau and Goswami (2017a) analyzed the presacral column of the family Felidae and concluded that patterns of modularity may also have permitted the posterior vertebral column to be more responsive to selection and achieve higher morphological disparity than other vertebral regions.

In this study, we also demonstrate that vertebrae within the modules found by Martín-Serra et al. (2021) possess highly integrated centra and neural spines. Strong integration between two adjacent vertebrae only occurs when the variation between the two vertebral parts is also integrated (Figures 8, 9b). Therefore, we hypothesize that the high integration found between the centra and the neural spines of vertebrae that compose each intervertebral module is an indirect byproduct of their high integration with adjacent vertebrae, which is, as interpreted by Martín-Serra et al. (2021), influenced by natural selection to stabilize column regions. In functional terms, this could be explained because axial stabilization across the anterodorsal module allows ventilation when moving rapidly and it also avoids excessive motion in the posterodorsal module (Martín-Serra et al. 2021). However, functional analysis of breathing in moving animals across different locomotory scenarios would be required to support this interpretation.

Moreover, it is worth noting that re-segmentation – i.e., the dual origin of the centrum from two adjacent somites (Ward et al., 2017) – could explain strong, developmental origin of among-vertebrae integration without the necessity to allude to natural selection. However, in our opinion, this developmental hypothesis to explain the observed integration between adjacent vertebrae seems unconvincing because our results indicate abrupt changes in the strength of among-vertebrae integration.

Martín-Serra et al. (2021) also found that the diaphragmatic vertebra is the least integrated vertebra of the presacral column of carnivorans and separates the anterodorsal and posterodorsal intervertebral modules (Figure 1b). The “diaphragmatic joint complex” of mammals concentrates the movement of the spine to be propagated through the anterior thorax to the lumbar vertebrae (Filler, 1986; Slijper, 1946). Based on this, Martín-Serra et al. (2021) hypothesized that the more relaxed limitations for the permissive motion of this area must be related to the absence of intervertebral integration. Our results also demonstrate that the weak integration between two adjacent regions only occurs in our sample when the centrum and neural spine variation is also weakly integrated (Figures 8, 9c). Furthermore, we also hypothesize that the weak integration between the centrum and neural spine of the diaphragmatic boundary vertebrae is associated with the weak integration found with its adjacent vertebrae.

The case of the diaphragmatic vertebra is interesting because its morphology is also transitional – it often has anterior zygapohyses that are similar to those of the thoracic vertebrae, whereas the posterior zygapophyses are more like those of the lumbar vertebrae. However, to date, it has not been tested whether the morphology of the diaphragmatic vertebrae itself is essentially divided between modules, i.e., whether its anterior portion is integrated with the anterodorsal module, while the posterior part is integrated with the posterodorsal module. This disjunction could explain the overall weak apparent integration of the diaphragmatic vertebra with the rest of the presacral vertebrae (and perhaps the other transitional vertebrae too). Moreover, it would be also interesting to investigate at the level of integration between the anterior and posterior landmarks within the diaphragmatic vertebra and within other vertebrae to quantify how they vary, e.g., whether the landmarks of within-module vertebrae are more integrated ‘front-to-back’ than in boundary vertebra.

In any case, based on our results, we hypothesize that natural selection has probably influenced (albeit indirectly) the pattern of integration between the centrum and neural spine in the vertebrae that compose the intervertebral modules but not at the boundary

FIGURE 8 Results obtained from the regressions between *r*PLSs obtained from intravertebral and intervertebral integration. Lines represent reduced major axis regression for r^2 and *p* value (see Table 1). (a) cervical plus thoracolumbar joined count; (b) thoracolumbar boundary count; (c) diaphragmatic start count. Grey areas highlights unobserved combinations of intravertebral and intervertebral integrations.

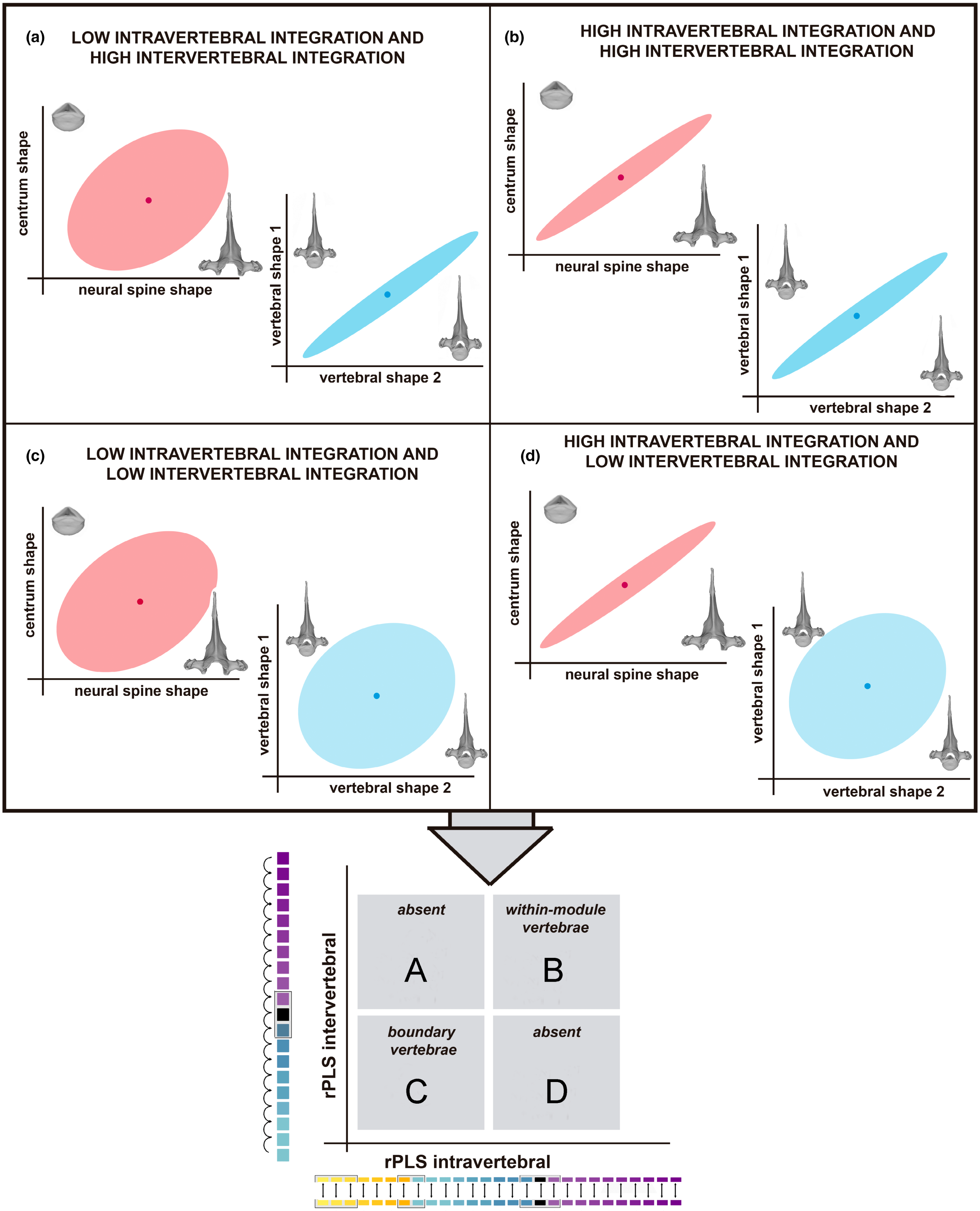

FIGURE 9 Observed and non-observed combinations of hypothetical intravertebral (red) and intervertebral integration (blue). (a) Low intravertebral integration and high intervertebral integration; (b) High intravertebral integration and low intervertebral integration; (c) Low intravertebral integration and low intervertebral integration; (d) High intravertebral integration and low intervertebral integration. The hypothetical bivariate graph at the bottom represents where the hypothetical combinations of intravertebral and intervertebral integrations plot in Figure 8.

vertebra, whose pattern ultimately is influenced by their distinct somitic origins and separate ossification centers.

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CONFLICT OF INTEREST

The authors declare that there is not conflict of interest of any kind. The authors confirm that the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

DATA AVAILABILITY STATEMENT

The data used in this paper have been previously published by our team in a recent publication (Martín-Serra, A., Pérez-Ramos, A., Pastor, F. J., Velasco, D., & Figueirido, B. (2021)). Phenotypic integration in the carnivoran backbone and the evolution of functional differentiation in metameric structures. Evolution letters, 5(3), 251–264; Dryad access: https://doi.org/10.5061/dryad.8931zcrq9.

ETHICS APPROVAL

This research does not involve human participants or experimental procedures with living beings.

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SUPPORTING INFORMATION

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