Received: 10 September 2019 | Revised: 3 December 2019 | Accepted: 12 December 2019
DOI: 10.1002/jmor.21093

RESEARCH ARTICLE

Journal of morphology WILEY

Is variation in tail vertebral morphology linked to habitat use in chameleons?

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Funding information
Tournesol Mobility Grant; FWO, Grant/Award Number: #3G006716

Abstract

Chameleons (Chamaeleonidae) are known for their arboreal lifestyle, in which they make use of their prehensile tail. Yet, some species have a more terrestrial lifestyle, such as *Brookesia* and *Rieppeleon* species, as well as some chameleons of the genera *Chamaeleo* and *Bradypodion*. The main goal of this study was to identify the key anatomical features of the tail vertebral morphology associated with prehensile capacity. Both interspecific and intra-individual variation in skeletal tail morphology was investigated. For this, a 3D-shape analysis was performed on vertebral morphology using µCT-images of different species of prehensile and nonprehensile tailed chameleons. A difference in overall tail size and caudal vertebral morphology does exist between prehensile and nonprehensile taxa. Nonprehensile tailed species have a shorter tail with fewer vertebrae, a generally shorter neural spine and shorter transverse processes that are positioned more anteriorly (with respect to the vertebral center). The longer tails of prehensile species have more vertebrae as well as an increased length of the processes, likely providing a greater area for muscle attachment. At the intra-individual level, regional variation is observed with more robust proximal tail vertebrae having longer processes. The distal part has relatively longer vertebrae with shorter processes. Although longer, the small size and high number of the distal vertebrae allows the tail to coil around perches.

KEYWORDS
Chamaeleonidae, grasping ability, morphology, prehensility, vertebrae

1 | INTRODUCTION

Tails in vertebrates can serve various purposes, from direct tail-induced propulsion to indirect support for keeping balance, and social signaling. In some lineages, the tail has evolved to allow animals to grasp and hold onto objects. This adaptation is referred to as prehensility and is often seen in arboreal vertebrates. A prehensile appendage assists a wide range of vertebrate taxa (from fish to mammals) in their climbing and swinging from branches, or firm grasping onto the substrate including in chameleons (Bergmann, Lessard, & Russell, 2003; Zippel, Glor, & Bertram, 1999), scincid lizards (Etheridge, 1967), marsupials (Dalloz, Loretto, Papi Cobra, & Vieira, 2012), New World monkeys (German, 1982; Organ, 2010), carnivores (Youlatos, 2003), and syngnathid fishes (Neutens et al., 2014). In these species, the caudal vertebrae and muscular system are modified, allowing increased flexibility and gripping strength. Even some invertebrates, such as brittle stars, have articulated "vertebral ossicles" in their prehensile arms that act in a similar fashion (LeClair, 1996; LeClair & LaBarbera, 1997).

Previous studies on the musculoskeletal morphology of prehensile tails have uncovered some recurrent patterns. In prehensile lineages it has been shown that the tail is longer and that the vertebral column in the tail shows regional differences. In prehensile lineages the distal vertebrae show several structural modifications that have been suggested to be related to its use in grasping compared to non-prehensile lineages including a greater robustness and expanded transverse processes (Bergmann et al., 2003; Bergmann & Russell, 2001; German, 1982; Herrel, Measey, Vanhooydonck, & Tolley, 2012; LeClair, 1996; Neutens et al., 2014; Organ, 2010; Youlatos, 2003; Zippel et al., 1999). Chameleons (Chamaeleonidae), one of the most distinctive families of squamates, have prehensile tails (along with other unique features such as independently moving eyes, a ballistic tongue, and syndactylous feet; Gans, 1967; Estes, de Queiroz, & Gauthier, 1988; Frost & Etheridge, 2001). The combination of their grasping feet and tail allows them to firmly hold onto branches, adding stabilization while climbing or feeding, an advantage for their arboreal lifestyle (Bickel & Losos, 2002; Losos, Walton, & Bennett, 1993; Peterson, 1984). Arboreal chameleons use their tail as an anchoring structure while performing acrobatic maneuvers and as a stabilizer for the body when projecting their tongue during food acquisition (Harkness, 1977).

While the shared presence of grasping hands and feet in all taxa likely is an adaptation towards a branch-dwelling lifestyle, a terrestrial lifestyle is considered to represent the ancestral state in chameleons (Tolley, Townsend, & Vences, 2013). Some taxa did not evolve a prehensile tail and maintained a more terrestrial lifestyle (e.g., *Brookesia*, *Rieppeleon*, and *Rhampholeon*; Figure 1; Tolley et al., 2013). Interestingly, even within otherwise typically arboreal and prehensile genera, species have shifted towards a more terrestrial lifestyle such as *Chamaeleo namaquensis* (Smith, 1831) and *Bradypodion occidentale* (Hewitt, 1935) (Herrel, Measey, Vanhooydonck, & Tolley, 2011; Riedel, Böhme, Bleckmann, & Spinner, 2015). Though terrestrial chameleons usually have not entirely lost their prehensile capabilities (some still climb in trees and shrubs), their tails may have another prominent function. *Brookesia*, for example, use their shortened tail more or less as a "fifth-leg" or walking stick for stability (Boistel et al., 2010). Although classifying chameleons dichotomously into "arboreal" or "terrestrial" is a simplification of the actual ecological variation, in this study the genera *Brookesia*, *Rieppeleon*, and *Rhampholeon* as well as the species *C. namaquensis* and *B. occidentale* will be referred to as "terrestrial" to explore whether a more terrestrial lifestyle has induced changes in tail vertebral morphology. Also, for pragmatic reasons, we consider basal terrestrial species as "primarily terrestrial" and the terrestrial species nested within more derived arboreal clades as "secondarily terrestrial." In turn, *Archaius tigris* (Kuhl, 1820), a species most closely related to the terrestrial genus *Rieppeleon*, is entirely prehensile and likely to have independently become adapted to an arboreal lifestyle, convergent to other arboreal lineages (Tolley et al., 2013). Considering the more plesiomorphic terrestrial condition for the lineage towards *Archaius*, we labeled this species as secondarily arboreal'.

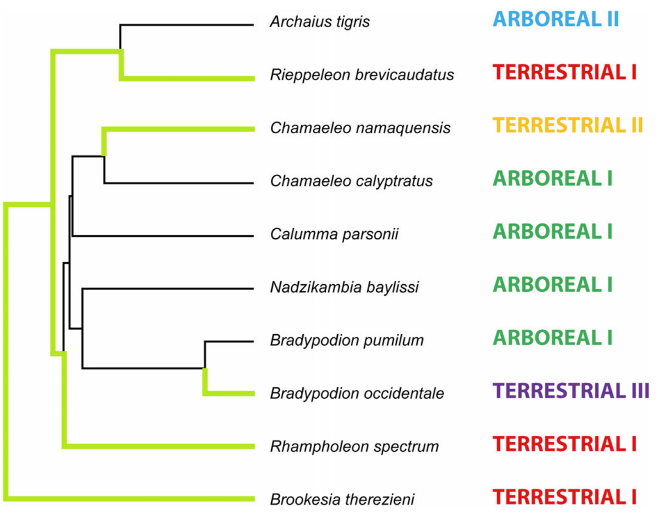

FIGURE 1 Phylogenetic relationships based on Tolley et al. (2013) including the species sampled for the study. Nonprehensile lineages are shown in green, prehensile lineages are shown in black. The group of each species is given behind the name

To date, most of the research on chameleon tails has focused on the overall anatomy (Ali, 1947; Zippel et al., 1999) or the external morphological variation in relation to habitat use (Hopkins & Tolley, 2011). Though there has been some research on the caudal musculature in chameleons (Ali, 1947; Bergmann et al., 2003; Zippel et al., 1999), little is known on how the skeletal structure may differ in relation to tail function or habitat use. Therefore, this study focuses on the shape variation in the tail vertebrae in order to explore whether vertebral shape patterns relate to habitat use, and by inference prehensile function, in chameleons. To be able to identify traits that could be considered functionally related to prehensility, we compare the observed variation with that of other prehensile vertebrates. Extensive research has been done on the prehensility of New World monkey tails. Organ and Lemelin (2009) determined that the proximal vertebrae in prehensile species are shorter, but have longer neural and transverse processes as well as more developed zygapophysial joints than the distal vertebrae. Having shorter vertebrae with a higher aspect ratio adds to their robustness. For our definition of robustness, we follow Organ (2010) who described it as "width-to-length ratio at the proximal end of the vertebral body" (p. 732). Organ (2010) also pointed out that in New World monkeys the proximal region of the tail has longer processes whereas the distal region has more numerous but smaller vertebrae that increase their ability to coil.

The goal of this study was to quantify morphological variation in relation to habitat use in chameleons by comparing the anatomy and shape of caudal vertebrae of arboreal and terrestrial species. Considering the difference in habitat (arboreal vs. terrestrial) and associated tail use within the Chamaeleonidae, we expect their tail vertebrae to have evolved traits convergent on those observed in New World monkeys. Not only do we expect to find this pattern of convergence at the level of the individual vertebrae, but also at the level of regional variation between the different parts of the tail. As a longer tail is required to wrap around objects, coiling multiple times around a perch to increase surface friction, we also expect arboreal species to have longer tails with more vertebrae. Indeed, having a high number of small distal vertebrae can be expected to increase flexibility. We expect there to be shape variation between the distal and proximal region of the tail, with distal vertebrae being smaller and having shorter processes. We also expect the vertebral processes to be longer (greater muscle attachment sites and increased lever length) and zygapophyseal joints to show increased intervertebral mobility in arboreal species compared to more terrestrial species.

2 | MATERIALS AND METHODS

2.1 | Specimens

For the initial analysis we included at least one specimen for 11 of the 12 identified genera of the family Chamaeleonidae, with the exception of the genus *Palleon*. After this analysis, *Trioceros melleri* was removed from further analyses as it was an outlier in our analyses due to the extremely enlarged neural spines that support the tail crest in this species. While we can expect the neural spine to have an important function for muscle attachment, in the case of *T. melleri* the large increase of the neural spine length is more likely related to sexual selection with the tail functioning as an ornament. The specimens were either obtained from museum collections of the Muséum national d'Histoire naturelle (MNHN), the Bayworld Oceanarium and Museum (PEM) or from the personal collection of Anthony Herrel (AH). *Trapelus pallidus* (Reuss, 1835) (AH0134) was also added to the analysis as an out-group. For the Chamaeleonidae we used the following species: *Brookesia therezieni* (Brygoo & Domerque, 1970) (AH0103), *Rhampholeon spectrum* (Buchholz, 1874) (AH0209), *C. namaquensis* (AH0143), *Rieppeleon brevicaudatus* (Matschie, 1892) (AH0102), *B. occidentale* (AH0146), *Chamaeleo calyptratus* (Duméril & Bibron, 1851) (AH0138), *Furcifer pardalis* (Cuvier, 1892) (AH0141), *Kinyongia fischeri* (Tilbury et al., 2006) (AH0133), *A. tigris* (MNHN2221), *Nadzikambia baylissi* (Branch & Tolley, 2010) (BOR21128), *T. melleri* (Tilbury & Tolley, 2009) (AH0169), *B. pumilum* (Gmelin, 1789) (AH0147) and *Calumma parsonii* (Cuvier, 1824) (MNHN1993 0104).

Chameleons vary in their lifestyles and it is difficult to unambiguously categorize them as having a more or less arboreal lifestyle. For the genera *Chamaeleo*, *Furcifer*, *Bradypodion*, *Kinyongia*, *Nadzikambia*, *Trioceros*, and *Calumma* most of the species can be considered as having an entirely arboreal lifestyle with the tails being fully prehensile, and rarely or never leaving the forest canopy other than for egg laying. As such, we grouped them as "Arboreal I." *A. tigris* is a fully prehensile and arboreal species, closely related to the nonprehensile genus *Rieppeleon* (Tolley et al., 2013). Hence, *A. tigris* is put in a separate group ("Arboreal II") to explore whether it has convergently evolved traits observed in the Arboreal I species. *Brookesia*, *Rieppeleon*, and *Rhampholeon* are closely related, nonprehensile taxa that have a less arboreal lifestyle, though they use low shrubs or trees to some degree. They are grouped as "terrestrial I." There are also two species that show a largely terrestrial lifestyle, although phylogenetically nested within fully arboreal and prehensile genera (i.e., *C. namaquensis* and *B. occidentale*). They are grouped in "terrestrial II" and "terrestrial III," respectively.

2.2 | Visualization and segmentation

All specimens were μCT-scanned at the center for X-ray Tomography at Ghent University (UGCT). To do so, a tube voltage between 70 and 120 kV was used to generate between 1,201 and 2,401 projections over 360°. The pixel pitch of the detector varied between 200 and 400 μm and the resulting voxel sizes between 26 and 120 μm. Most of the variation in set-up is due to the large size difference between specimens, the smallest ones having a tail of <1 cm and the largest ones >30 cm.

3D-visualizations of the vertebrae were generated and digitally segmented in Amira (v. 5.6). 3D-surfaces of each individual vertebra were generated and used to digitize 3D-coordinates of anatomical landmarks (Amira v. 5.6). To smooth out small irregularities in the 3D-surfaces, allowing a more accurate landmark placement, three smoothing iterations at a level of 0.6 were applied to the surface models. Individual tail vertebrae were segmented up to the most distal end of the tail, as long as a reliable segmentation of each vertebra was possible. Reliable segmentations for proper landmarking were possible for more than 50% of the total number of tail vertebrae in each species, and even more than 70% in most of the specimens (except for *N. baylissi*, *K. fischeri*, and *F. pardalis*; Table S1).

2.3 | Morphometrics

Landmarks were placed onto the segmented surface of each caudal vertebra using the "landmarks" tool in Amira. Caudal vertebra one was identified as the first vertebra after the last sacral vertebra, with the last sacral vertebra being characterized by its transverse process articulating with the ilia, thus connecting the spine to the pelvis. The transverse processes of the caudal vertebrae do not have this articulation.

To study the shape variation of the tail vertebrae, 26 homologous landmarks were placed on each vertebra (Figure 2). Their positions were chosen to quantify the overall shape of the vertebra and its processes. Landmarks were placed around the articulation surface of the vertebral centrum at the dorsal-most point (1), lateral-most points both left and right (2, 3) and the ventral most point (4). They were also placed on the prezygapophysial process at the anterior bases of the left and right prezygapophysial process (5, 6) and at the most anterior point at the distal tip (7, 8). On the right and left transverse processes landmarks were placed on the anterior base, the most anterior point at the distal tip, and the posterior-most base (9, 10, 11 on the right side, 12, 13, 14 for left). On the neural spine landmarks were placed on the anterior base, the anterior-most tip and the posterior base (15, 16, 17). On the postzygapophysial process also the anterior-most extremities were landmarked, both right (18) and left (19). At the dorsal point of the spinal cord foramen, formed by neural arches (20) another landmark is placed, as well as anteriorly to the posterior articulation surface of the vertebrae centrum at the base of the postzygapophyses at the right side (21) and left side (22). Finally, also landmarks are placed around the posterior articulation surface of the vertebrae centrum, at the dorsal-most point (23), the lateral-most points (right side 24, left side 25) and at the ventral-most point (26).

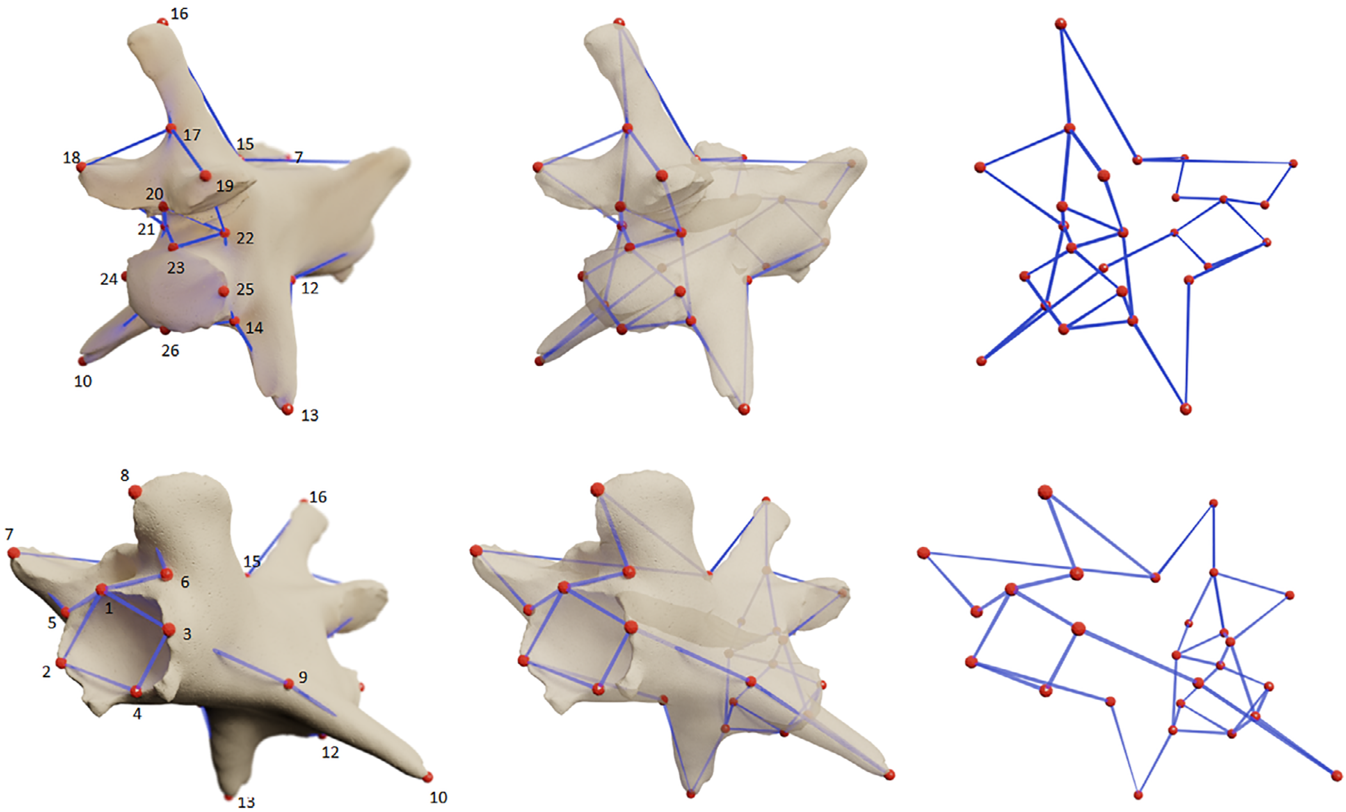

FIGURE 2 Visualization of the landmark positions on the vertebrae. Upper row shows a posterior oblique viewpoint of the right side, the lower row an oblique anterior view of the left side. Red dots show the position of the landmarks, blue lines connect these landmarks into a wireframe used visualize shape variation in the analyses

2.4 | Statistics

Data files containing the 3D-coordinates were reorganized into a TPS-format. The 3D-data were uploaded in MorphoJ 1.6d (Klingenberg, 2011) and considered as symmetrical shapes (left–right symmetry). A Procrustes analysis was performed, translating, rotating and uniformly scaling the landmark configurations of the vertebrae. Principal component analyses (PCA) were carried out using the covariance matrices, to explore the position of the different species in morphospace. For the interspecific variation, all species except for *F. pardalis*, *K. fischeri*, and *T. melleri* were included in the PCA (see the results section). On the PCA-data a multivariate normality test was performed to check whether the data is normally distributed. This turned out not to be the case, so a nonparametric test (One-Way PERMANOVA) was performed in PAST (v 3.22; Hammer, Harper, & Ryan, 2001) to test whether the variation between species was significant. Additionally, a pairwise NPMANOVA in which the significance level is determined by permutations was performed on the data to test for a significant difference between individual species. The *p* values were adjusted using a sequential Bonferroni correction.

Allometry was tested by plotting the PC-loadings against centroid size. This was done in MS Excel (v. Microsoft Office Professional Plus 2016) which also allowed us to calculate the coefficient of determination (R^2).

For further statistical analysis, we created a reduced dataset allowing us to compare the vertebrae from different species while taking positional homology into account. For this we selected the vertebrae situated at 1, 10, 20, 30, 40, 50, and 60% of the total number of vertebrae, yielding seven vertebrae per specimen. A list of which vertebrae and species we ended up using for the reduced dataset can be found in the supplementary data (Table S2). Beyond 60% most specimens did not have a high enough resolution for segmentation and accurate landmarking. Yet, these data should give a useful representation of both the proximal and distal part of the tail as this includes both proximal and distal vertebrae. If we are to consider the first 25 vertebrae in the tail of arboreal species as proximal vertebrae (see Section 3), this constitutes an average of 45% of the total amount of vertebrae. Measuring up to the 60% point of the total tail length would mean that at least two distal vertebrae per species are included in the analysis providing a good representation of both proximal and distal part of the tail. *K. fischeri* and *F. pardalis* were left out of this dataset as less than 60% of their tail vertebrae could be landmarked (Table S1). With four remaining fully prehensile and arboreal species, the "arboreal I" group is still well represented. Finally, a phylogenetic MANOVA was performed on the PC-scores to explore whether ecological groups were still different when taking into account their phylogenetic history. This was done in R (R v.3.5.32019) using the "geiger"-package (Harmon et al., 2005) and the "phytools"-

package (Revell, 2012). For this, the specimens were assigned to two groups, prehensile (arboreal I and II) and nonprehensile (terrestrial I, II, and III) species. Based on the outcome of the phylogenetic MANOVA a linear discriminant analysis (LDA) was performed to explore the major axes of differentiation between prehensile and nonprehensile species. The LDA allows us to visualize the variation driving the difference between these two groups.

3 | RESULTS

3.1 | Variability in vertebral number

We found that the number of tail vertebrae in arboreal chameleons is significantly larger than for terrestrial species (Figure 3). The Box and Whisker plot shows the distribution and how arboreal II and terrestrial II and III groups lie within the range of variation. This illustrates that arboreal species have a much higher number of vertebrae. *A. tigris* has a lower number of vertebrae compared to most other primarily arboreal species, but still higher than most terrestrial species. The same holds for *B. occidentale*, being a secondarily terrestrial species, yet having a higher number of vertebrae than most terrestrial species, but still fewer than the majority of arboreal species. *C. namaquensis* has a low number of vertebrae, typical to that observed in most terrestrial species.

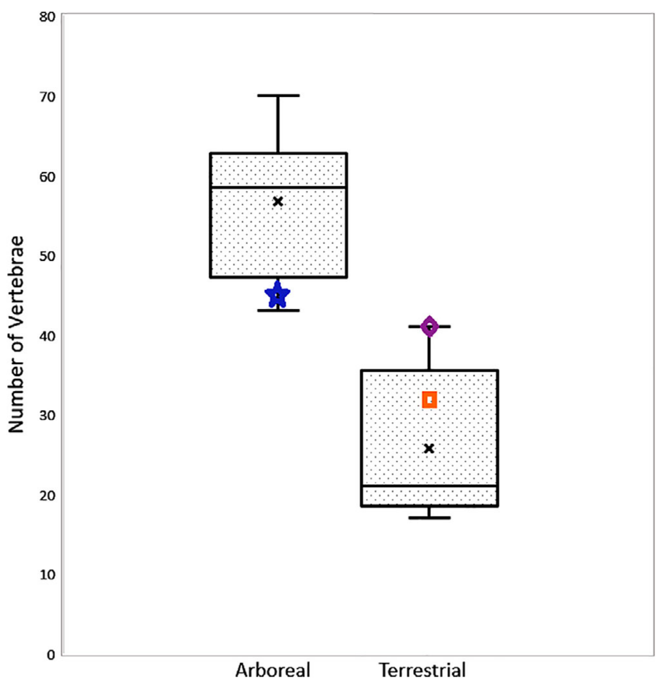

FIGURE 3 Box and Whisker plot depicting the difference in the number of vertebrae between the typical arboreal groups and terrestrial groups. Also indicated are the positions of *C. namaquensis* of the terrestrial II group (indicated with an orange square), *Bradypodion occidentale* (terrestrial III, purple diamond) and *A. tigris* (arboreal II, blue star)

3.2 | Patterns in vertebral shape variation

The out-group *T. pallidus* occupied a distinct part of the morphospace different from the Chamaeleonidae (Figure 4a). For the analysis, we focus on the shape variation explained by PCs 1–4 as these together explain most of the variation (78.9%). Principal component 1 (explaining 42.15% of the total variation) represents regional variation within the tail, showing that both arboreal and terrestrial species follow the same trend through vertebrae 1–25. That number also corresponds to the average number of caudal vertebrae in terrestrial chameleons. In the PCA graph with the full dataset this can be better visualized. However, after vertebra 25 the species with longer tails appear to cluster (Figure 4a). Shape variation along PC1 showed a strong allometric signal (R^2 = 0.99; p < .001). Positive PC1 scores show that the proximal-most vertebrae have longer transverse processes pointing distally, taller zygapophysial joints, and shorter vertebrae (Figure 5). Distal vertebrae have negative PC1 scores, corresponding with the distal-most vertebrae having shorter transverse processes pointing proximally, shorter prezygapophysis but slightly taller postzygapophysis. The vertebral bodies are also longer (Figure 5).

The variation represented by PC2 (22.23%) is largely based on either lifestyle or phylogenetic relationships. The terrestrial I species *R. brevicaudatus* and *B. therezieni* group separate from most other species. However, the terrestrial I *R. spectrum* groups with the other arboreal I species.” The terrestrial II *C. namaquensis*, and to a lesser degree the arboreal I species and *R. spectrum* (terrestrial I), show positive PC2 scores, whereas the other terrestrial I species *B. therezieni* and *R. brevicaudatus* are associated with negative PC2 scores (Figure 4(b)). *A. tigris* leans towards the other primarily terrestrial species, having negative PC2 scores. *B. occidentale* (terrestrial III) lies on the negative side of the PC2 axis, lower than the other arboreal taxa. Principal component 2 mainly represents variation in the transverse processes, being pointed more distally in *C. namaquensis* and more proximally for the terrestrial I group (except for *R. spectrum*) (Figure 5). Principal components 3 and 4 (respectively 9.25 and 5.27% of the variation) also show some grouping (Figure 4(c)). Especially *A. tigris* groups separately from the rest, showing shorter transverse processes that point downwards. Whereas *R. spectrum* still groups largely with the arboreal species, *B. therezieni* and *R. brevicaudatus* show shapes with a neural spine being positioned more distally. The terrestrial II and III species *C. namaquensis* and *B. occidentale* have an extremely large, laterally pointing, transverse process (Figure 5).

The results of the PERMANOVA showed significant differences between species (p = .0001; 10,000 permutations). The pairwise test and sequential Bonferroni test also showed that there was a significant difference between species.

3.3 | Phylogenetic patterns in shape variation

A phylogenetic MANOVA was performed on the reduced dataset to check whether differences between groups were due to ecology or

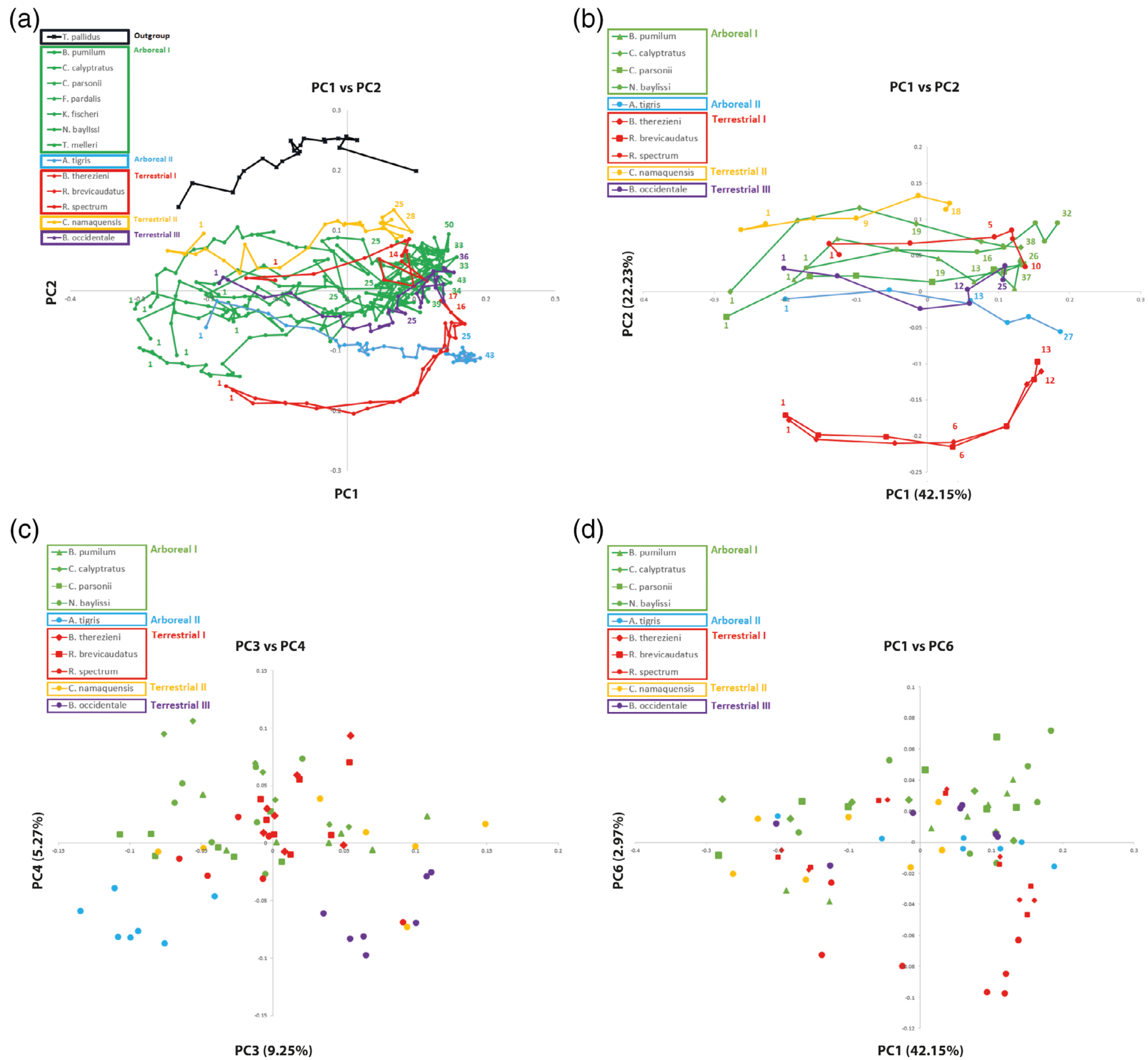

FIGURE 4 Lifestyle is indicated by color and each point represents a single vertebra. Green depicting arboreal I, blue arboreal II, red terrestrial I, yellow terrestrial II and purple terrestrial III. Plot A (PC1 vs. PC2) includes the out-group *Trapelus* pallidus with the full dataset. In A the first, last and 25th vertebrae are indicated by number to visualize the grouping of the distal vertebrae. Plot B (PC1 vs. PC2) shows the reduced dataset, as well as plot C (PC3 vs. PC4) and D (PC1 vs. PC6). Plot A shows the full dataset, plots B, C, and D have the reduced dataset to help visualize trends. In plots A and B a trendline has been added to help visualize the regional trend that can be found in the dataset

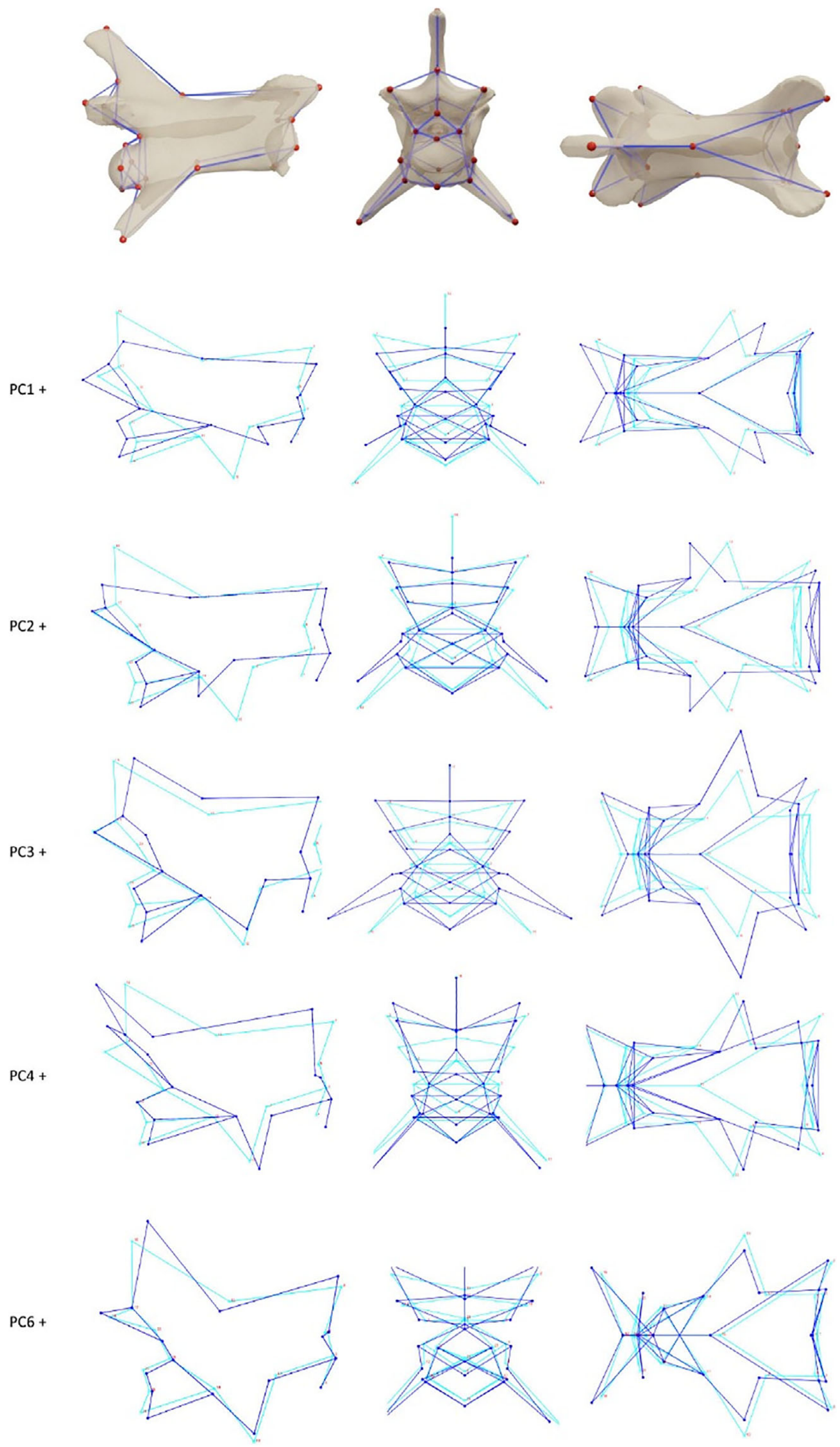

FIGURE 5 wireframes from the PCA depicting the shape variation in the vertebrae. The light blue wireframes show the consensus of all the vertebrae combined; the dark blue show the maximum aspects of shape change along the positive PC-axis using the value of the furthest most vertebrae along the axis. The wireframes for individual PCs shown here are the right lateral view (left), frontal view (middle), and dorsal view (right)

rather driven by phylogeny. The analysis shows that the variation in the vertebrae at 30, 40, and 50% show a significant difference irrespective of phylogeny (Table 1). The LDA performed on the shape of the vertebrae at 30, 40, and 50% showed that the classification between prehensile and nonprehensile species is 100% correct without overlap between groups. The wireframes show that most of the variation based resides in the transverse processes and neural spine, which are larger in nonprehensile, more terrestrial species. In addition, the vertebrae of nonprehensile species are shorter (Figure 6).

4 | DISCUSSION

Our data suggest that the tail vertebra shape differs between the arboreal and terrestrial species and that this variation can be linked to habitat use in addition to regional variation within the tail. Our hypothesis that there are differences in vertebral number and shape between arboreal and terrestrial species, as well as regional shape variation between the distal and proximal region of the tail, was thus supported by our analyses. When considering the observed patterns

TABLE 1 results of a phylogenetic MANOVA performed on the reduced dataset, p-levels are given in the table below. The analysis is performed per vertebrae on the first 7 PCs, as well as on PC1-7 overall. Asterisks indicate significant differences

Vertebrae (%)	Overall (PC1-7)	PC1	PC2	PC3	PC4	PC5	PC6	PC7
1	0.073	0.21	0.54	0.001*	0.53	0.375	0.65	0.064
10	0.11	0.16	0.13	0.001*	0.12	0.939	0.032*	0.72
20	0.10	0.27	0.16	0.013*	0.21	0.693	0.46	0.15
30	0.0090*	0.63	0.19	0.20	0.46	0.28	0.58	0.37
40	0.042*	0.27	0.32	0.11	0.86	0.95	0.018*	0.86
50	0.034*	0.27	0.52	0.31	0.87	0.941	0.006*	0.89
60	0.22	0.32	0.35	0.35	0.50	0.723	0.051	0.70

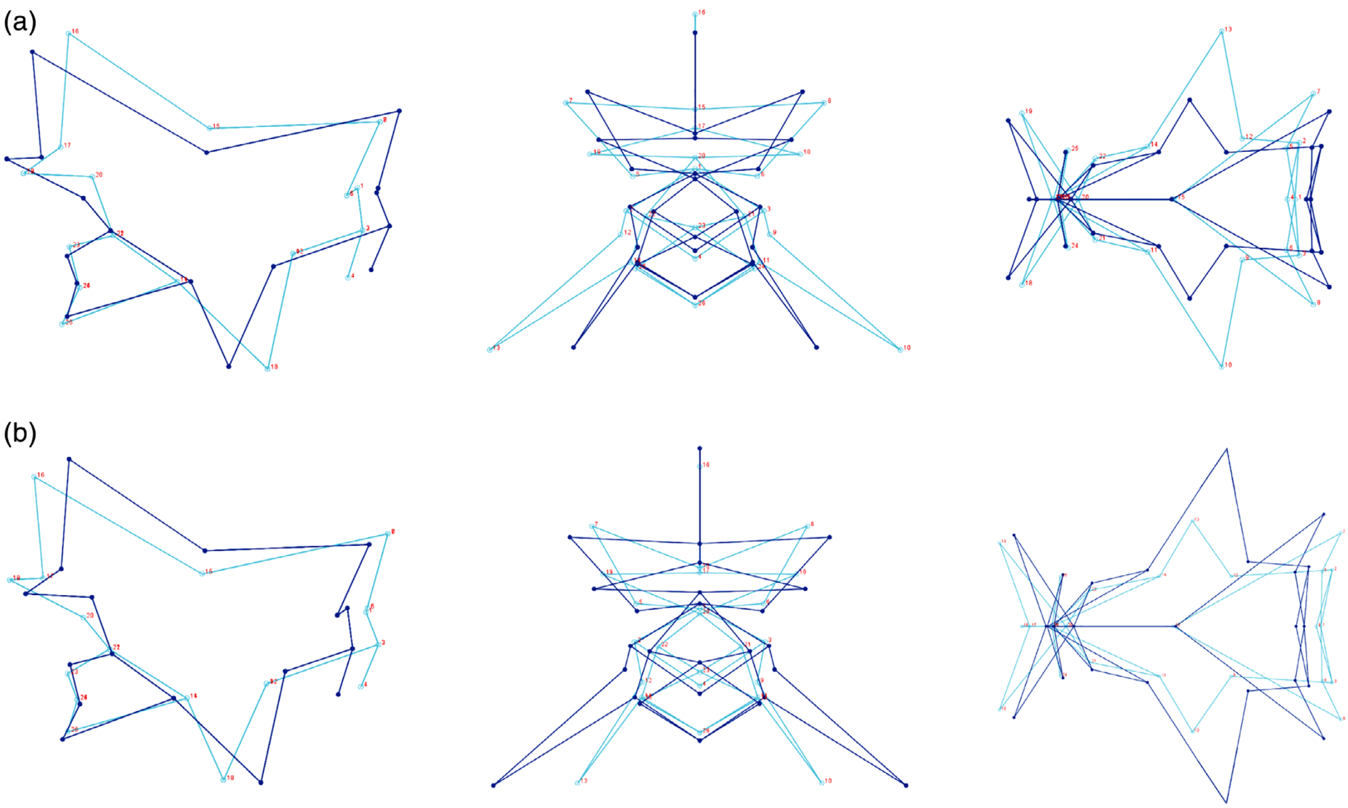

FIGURE 6 Wireframes showing the morphological variation explained by the LDA, here showing the variation between the consensus shape and that seen in prehensile species (a) and nonprehensile species (b). The light blue wireframes show the consensus of all vertebrae combined; the dark blue show the aspects of shape change based on the average value per group, related to prehensility according to the LDA. This row shows the lateral view (left), frontal view (middle), and dorsal view (right)

in shape variation with known muscle insertion sites, at least some traits could be considered as functionally relevant, promoting prehensile capacity in the prehensile species.

4.1 | Relating prehensility to tail length and the number of vertebrae

Arboreal chameleons have more vertebrae in their tails (Figure 3). This has been suggested previously to be correlated with prehensility across prehensile lineages of vertebrates (Organ, 2010; Youlatos, 2003). In arboreal species, after vertebra 25 the more distal vertebrae have roughly the same shape according to the PCA, after which the data points cluster and show little shape variation (Figure 4a). While overall the tail vertebrae in prehensile chameleons show a similar pattern to those in the tails of prehensile New World monkeys, they do not appear to have a distinctive transitional vertebra, as seen in platyrrhine monkeys or prehensile-tailed carnivores (Organ, 2010; Youlatos, 2003). Transitional vertebrae are the last vertebrae of the proximal part of the tail with a shape intermediate between proximal and distal vertebrae. New World monkeys have 1–3 transitional vertebrae that divide the tail in a proximal and distal part. Chameleons have a gradual transition between regions. However, while they do not have fixed transitional vertebrae, their most proximal and distal vertebrae do show morphological differences reminiscent of the proximal and distal vertebrae of New World monkeys. In both chameleons and New

World Monkeys, the proximal vertebrae are shorter while having longer processes, whereas the distal vertebrae are longer with their processes reduced. Terrestrial chameleons do not have any vertebrae that resemble those seen in the distal part of the arboreal species. Instead, they have an average of 26 vertebrae of which the morphology resembles that of the proximal vertebrae in arboreal species. The arboreal chameleons in our study have an average of 56.8 tail vertebrae which gradually transition from a proximal morphology into one typically seen in the distal portion of the tail. This happens usually around vertebrae 21–26, as can be observed in the PCA (Figure 4a). The most proximal vertebrae of arboreal species follow a trend similar to that observed in terrestrial species. The more distal vertebrae in arboreal species tend to group in the PCA showing a more similar shape, while the terrestrial species do not possess any vertebrae with this shape (Figure 4b). This appears one of the most important and distinctive differences between the arboreal and terrestrial species. Arboreal chameleons use the highly flexible distal end of their tail for holding on to the substrate. The extra tail length due to the multitude of smaller distal vertebrae allows them to coil the tail around the substrate, likely adding friction (Shapiro, 1995; Ward, 1993).

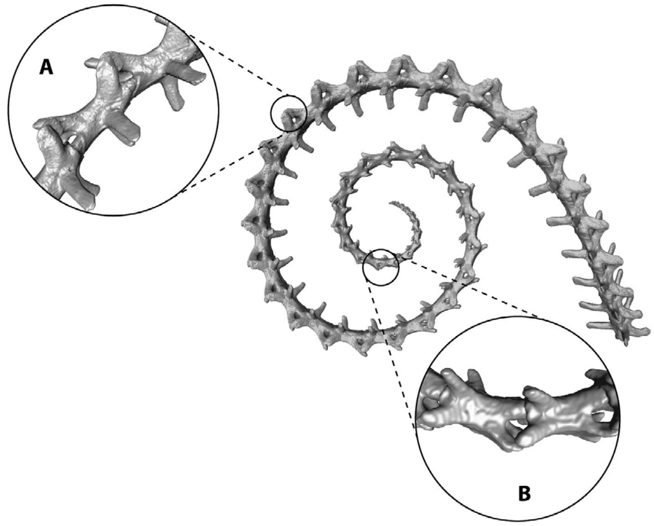

FIGURE 7 The orientation of the zygapophysial joints changes regionally towards the distal end. The further distal the more the processes extend further while the orientation becomes more horizontal. The image zooms in on an area typical for the proximal part of the tail (a) and an area typical for the distal part of the tail (b)

4.2 | Relating prehensility to regional shape variation

According to the PCA, the out-group, *Trapelus pallidus*, occupied a distinct part of the morphospace compared to the Chamaeleonidae (Figure 4a). This indicates that the Chamaeleonidae, including the terrestrial species, show a shared basic vertebral shape different from that in other lizards. This shows that the phylogenetic signal is stronger than the typical lifestyle signal, as the terrestrial chameleons do not cluster with the terrestrial and nonprehensile *T. pallidus*. It is also important to keep in mind that although the ancestral state of the habitat of chameleons has been suggested to be terrestrial, they did already have adaptations in their feet which that allowed them to grab onto fine twigs and branches, assisting them in climbing. Yet, whether these ancestral chameleons already showed adaptations in the tail that could have assisted them in climbing remains unknown. The main morphological variation is regional variation, as shown by PC1, which further showed a high level of allometry. Most regional shape variation can thus be attributed to size. Proximal vertebrae are shorter, but have longer neural spines, transverse processes, and zygapophyses than the distal vertebrae. This gives them a higher aspect ratio, which results in an increased robustness following Organ and Lemelin (2009). Another factor relating to robustness can be observed by looking at PC2, which shows that the arboreal groups, in general, have vertebrae which are shorter than wide, making them more robust. This follows the pattern observed in New World monkeys (German, 1982; Organ, 2010). However, it is important to note that while it could be tempting to link this to prehensility, distal vertebrae being narrower and relatively longer is something also seen in nonchameleonid lizards and various mammals. Previous studies have compared the aspect ratio of prehensile tails to nonprehensile tails in monkeys and found that the vertebrae in prehensile tails are relatively more robust compared to those in nonprehensile species (Organ, Teaford, & Taylor, 2009). However, we cannot say with certainty whether this is the case in our study as we do not have enough nonchamaeleonid comparison material. The robustness of the vertebrae would be interesting to test in the future to see if the same holds up comparing chamaeleonids to other squamates. Also, studies have been done linking the flexibility of vertebrae with aspect ratio and intervertebral disc size (Buchholtz & Schur, 2004). Measuring the intervertebral disc width and relating this to vertebrae length would also be an interesting avenue for future research.

As has been previously described for New World monkeys, the relatively larger zygapophyses in the proximal vertebrae could allow movement in the sagittal plane but may limit movement in other planes (Shapiro, 1993, 1995; Ward, 1993). This has also been described by Zippel et al. (1999) who mentions that in the proximal part, due to the overlapping arrangement of the zygapophyses, there are restrictions in movement except in the dorsoventral plane. Towards the distal part of the tail the zygapophyseal joints extend further than in the proximal part while the orientation becomes more horizontal (Figure 7). This orientation of the zygapophyseal joints in the proximal part of the tail makes the structure sturdier. Further distal along the tail the zygapophyseal joints are further reduced in size or even entirely absent, likely increasing the potential for movement in the lateral plane as there is less restriction by these joints. The highly flexible distal part of the tail is where the coiling occurs while the proximal part of the tail remains rigid (Zippel et al., 1999). The vertebrae have shorter processes, which could indicate that they are less restricted in their movement. However, as the processes also serve as muscle attachment sites, the shorter input levers would imply a reduction in the output force needed for a powerful gripping.

Most of the variation observed concerns the transverse process as reflected by the PCA. In the arboreal species, it points distally whereas in terrestrial species it is proximally directed. The hypaxial muscles of the tail (Ilio-caudalis and Infero-caudalis) are responsible for the coiling of the tail and required for lateral and ventral flexion (Zippel et al., 1999). These are attached to the transverse processes, hence their importance in relation to prehensility. In the proximal region the transverse processes are longer than in the distal part of the tail. In terrestrial species the transverse processes are strongly reduced. The longer transverse processes in the distal end of the tail in arboreal species likely allow them to have a more force efficient lever system. This might help explain the higher force output in the distal part, enough to suspend the entire body weight using just the tail (German, 1982; Organ, 2010). However, to understand how both the length and orientation of the various vertebral extremities relate to prehensility, further biomechanical analyses and testing are required.

4.3 | Relating prehensility to arboreal and terrestrial lifestyles

According to the LDA the primarily terrestrial species have narrower but relatively longer vertebrae following the notion that less-prehensile animals may have less robust vertebrae. The more robust vertebrae in arboreal species might make them able to resist higher mechanical loads during bending and torsional moments associated with tail prehension and suspension (German, 1982). However, it is not clear whether these geometric considerations on vertebral shape, considering both the robustness of the vertebrae and the length and orientation of the processes, are reflected in the efficiency in which the muscle force is converted into skeletal movement. It could also be that this only reflects muscle attachment size. In primarily terrestrial species the neural spines and zygapophyses are also longer than in arboreal species. The secondarily terrestrial *C. namaquensis* has much larger and laterally pointing transverse processes, as also observed in *B. occidentale*.

R. spectrum (terrestrial I) groups with the arboreal species in the PCA. Based on the phylogeny by Tolley et al. (2013), *B. therezieni* and *R. brevicaudatus*, have branched off earlier in the phylogeny than *R. spectrum*, the latter being more closely related to the prehensile species (Figure 1). This could help explain why in the PCA it appears to be more similar to the arboreal species. Following our data, this could indicate morphological adaptations could have occurred in vertebrae shape after the branching of *Brookesia* and *Rieppeleon*, with *R. spectrum* inheriting traits typical for that branch but not necessarily required for prehensility. However, according to the Phylogenetic MANOVA the variation explained by PC6 is significant and highest in the 30–50% area of the vertebrae. However, this is the area that is in arboreal species shows the vertebrae transitioning from a proximal morphology to a distal one. This area in *R. spectrum* resembles the proximal vertebrae as in the other terrestrial species. As both *R. spectrum* and the other terrestrial I species do not have any vertebrae resembling the distal vertebrae in arboreal species, it can be expected that these show most variation when comparing terrestrial with arboreal species (Figure 4d).

The main adaptation for prehensility appears to be having a multitude of highly similar vertebrae at the distal end, enabling the tail to coil around an object to increase friction. With the reduced extremities in the distal vertebrae one could expect there to be less restriction of movement in the various directions as compared to the proximal part. The proximal part of the tail in both terrestrial and arboreal species allows more area for muscle attachment; however, terrestrial species do not appear to have any vertebrae following the pattern typically seen in the distal vertebrae, but exclusively have vertebrae similar to the proximal ones of arboreal species. Arboreal species have up to 70 vertebrae, where distal vertebrae show a more conserved shape variation. Species which are secondarily terrestrial, like our terrestrial II and III groups, do not possess these distal vertebrae and as a result have shorter tails. *Bradypodion pumilum* ecotypes can be used as an example that when a species adapts to a more terrestrial lifestyle, their tails are shorter too. Herrel et al. (2011) describe how the forested ecotype has longer tails and more tail vertebrae than the fynbos ecotype (shrub- or heathland vegetation).

One thing that all terrestrial groups have in common is a longer neural spine. The neural spine functions as the attachment site for the m. transverso-spinalis and the m. interspinalis (Zippel et al., 1999). These muscles assist both in the uncoiling of the tail, as well as stabilizing the tail while walking. These dorsally located muscles keep the tail dorsally flexed and extended, which can assist in balancing the animal. Furthermore, a much variation appears to be situated in the transverse processes, being shorter in terrestrial species and longer in arboreal ones. However, to understand how this may relate to function we would need to study the vertebrae morphology in relation to the musculature and test function, for example through multi-body dynamics testing.

5 | CONCLUSION

The general structure of tail in chameleons can be linked to habitual tail-use behavior. Prehensile tails appear to be mechanically better designed to withstand bending and torsional stresses as they are more robust. Moreover, arboreal species have long distal tails that are flexible, allowing them to coil their tail around perches. The average number of vertebrae in arboreal species is higher compared to more terrestrial species. In arboreal species a gradual shift to the shape typical for distal vertebrae occurs around the 26th vertebrae. Terrestrial species appear to have longer neural spines and longer zygapophyses. However, how this shape variation is linked to function and mechanical performance remains to be investigated.

AUTHOR CONTRIBUTIONS

A.M.L.: CT scanning and processing of the data, morphometric and statistical analysis, writing; A.O.: analyses and processing of the CT data; B.D.K.: assisted in the laboratory, preparation of the specimens; A.H.: study design, project supervision, advice on the methods, specimens, writing; D.A.: study design, project supervision, advice on the methods, writing.

ACKNOWLEDGEMENTS

We would like to thank Iván Josipovic, Matthieu Boone for their assistance at the CT scanning facility; Jens De Meyer, Robert A. Paauwe for feedback; Gill Watson & Werner Conradie of the Port Elizabeth Museum at Bayworld, South Africa and Nicolas Vidal of the Muséum

National D'Histoire Naturelle at Paris, France for access to specimens. This project was funded by the FWO project #3G006716 and a Tournesol Mobility Grant. We would also like to thank the two anonymous reviewers for their constructive comments as we believe they have resulted in significant improvements to the paper. The authors declare there is no conflict of interest.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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How to cite this article: Luger AM, Ollevier A, De Kegel B, Herrel A, Adriaens D. Is variation in tail vertebral morphology linked to habitat use in chameleons? *Journal of Morphology*. 2019;1–11. https://doi.org/10.1002/jmor.21093