biology letters

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Biol. Lett. 2010 **6**, 740-743 first published online 12 May 2010
doi: 10.1098/rsbl.2010.0322

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Published online 12 May 2010

Assisted walking in Malagasy dwarf chamaeleons

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Chamaeleons are well known for their unique suite of morphological adaptations. Whereas most chamaeleons are arboreal and have long tails, which are used during arboreal acrobatic manoeuvres, Malagasy dwarf chamaeleons (*Brookesia*) are small terrestrial lizards with relatively short tails. Like other chamaeleons, *Brookesia* have grasping feet and use these to hold on to narrow substrates. However, in contrast to other chamaeleons, *Brookesia* place the tail on the substrate when walking on broad substrates, thus improving stability. Using three-dimensional synchrotron X-ray phase-contrast imaging, we demonstrate a set of unique specializations in the tail associated with the use of the tail during locomotion. Additionally, our imaging demonstrates specializations of the inner ear that may allow these animals to detect small accelerations typical of their slow, terrestrial mode of locomotion. These data suggest that the evolution of a terrestrial lifestyle in *Brookesia* has gone hand-in-hand with the evolution of a unique mode of locomotion and a suite of morphological adaptations allowing for stable locomotion on a wide array of substrates.

Keywords: locomotion; tail; stability; synchrotron X-ray phase-contrast imaging; kinematics; chamaeleon

1. INTRODUCTION

Tails are an often overlooked but important component of locomotion in lizards and other terrestrial vertebrates (Jusufi *et al.* 2008). Tails may provide stability, weight support or act as a balancing organ when running bipedally (Snyder 1949; Emmons & Gentry 1983; Jusufi *et al.* 2008). Many groups of arboreal vertebrates have independently evolved prehensile tails that are used for both support and locomotion (Emmons & Gentry 1983). Among arboreal lizards, chamaeleons are well known for their unique morphological adaptations including grasping feet, independently movable eyes, projectile tongues and prehensile tails (Gans 1967; Harkness 1977; Herrel *et al.* 2000). In most chamaeleons, the long, prehensile tail is not engaged during walking (Peterson 1984; Higham & Jayne 2004), but is used in the execution of acrobatic manoeuvres or to hold onto branches (Zippel *et al.* 1999).

Malagasy dwarf chamaeleons (genus *Brookesia*) are small terrestrial lizards with relatively short tails. Moreover, the tail in *Brookesia* has been observed to be placed on the substrate during locomotion. Although it has been suggested that the tail in these chamaeleons may function as an additional foot (Glaw & Vences 2007), it remains unknown whether the tail helps generate propulsion or acts as a stabilizer by providing additional support. Maintaining stability on terrestrial or broad substrates in small chamaeleons like *Brookesia* may be compromised by the highly specialized grasping feet. Consequently, the tail may effectively be used to improve balance. Here, we study locomotion in a Malagasy ground chamaeleon of the genus *Brookesia* while walking on substrates of different diameter. Additionally, we use X-ray synchrotron phase-contrast microtomography to visualize and study the anatomy of the tail and inner ear in these animals in comparison with closely related arboreal species (*Calumna tigris* and *Anolis carolinensis*), to better understand the morphological specializations associated with this unique mode of locomotion.

2. MATERIAL AND METHODS

(a) *Animals*

Brookesia thieli ($N = 7$) and *B. superciliaris* ($N = 2$) were obtained from the pet trade, kept in a vivarium at a temperature varying between 18°C and 23°C, and were fed crickets and *Drosophila* flies dusted with calcium and vitamin supplements three times a week. Water was provided ad libitum and animals were misted daily.

(b) *Experimental video protocol*

Observations and video recordings of *B. thieli* and *B. superciliaris* moving on different substrates were obtained using three synchronized digital cameras (Prosilica GE 680; Allied Vision Technologies, Stadtroda, Germany) set at 10 fps. All substrates were 30 cm long and consisted of a 30 cm wide-wood platform, wooden dowels of 12.40 and 3.84 mm diameter, respectively, and an iron wire of 1.38 mm in diameter. Video sequences were analysed using a custom MATLAB routine (Loco 3.3) to determine the three-dimensional kinematic parameters and the frequency and amplitude of the movements of the feet and tail. To evaluate the contribution of tail placement to stability, the three-dimensional coordinates of hands, feet and tail were projected on the horizontal plane and used to calculate the triangle of support. Next, the centre of mass (COM) was calculated based on a three-dimensional reconstruction of an entire animal (see below) and projected onto the horizontal plane to investigate whether tail placement affected the position of the COM relative to the triangle of support (electronic supplementary material, figure S2).

(c) *X-ray synchrotron phase-contrast microtomography*

We used the ID19 long (150 m) imaging beamline of the European Synchrotron Radiation Facility (Grenoble, France) with large spatial coherence (Du Pasquier *et al.* 2007; Guigay *et al.* 2007; Van der Meijden *et al.* 2007). We used a monochromatic beam with a bandwidth of $\Delta E/E$ of 10^{-4} obtained with a double Si111 Bragg monochromator. We used a detector composed of a 10 μm thick gadolinium oxide scintillator and an optical system coupled to a cooled charge-coupled FReLoN camera (Labiche *et al.* 2007). We acquired tomographic data from complete adult female *B. thieli* (energy: 20.5 KeV; propagation distance: 600 mm), one *C. tigris* (collection MNHN 1989.2872; 25 KeV, 700 mm) and one *A. carolinensis* (personal collection AH; 25 KeV, 800 mm). The effective pixel size at the converter screen position was 7.46 μm, resulting in a field of view of 15×14.11 mm^2. The dataset for a complete sample consists of between two and five scans of 1500 projections taken over 180° with vertical displacements between each scan with a small overlap for scan alignment. The reconstruction was performed using the filtered back-projection algorithm

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2010.0322 or via http://rsbl.royalsocietypublishing.org.

Received 12 April 2010
Accepted 22 April 2010

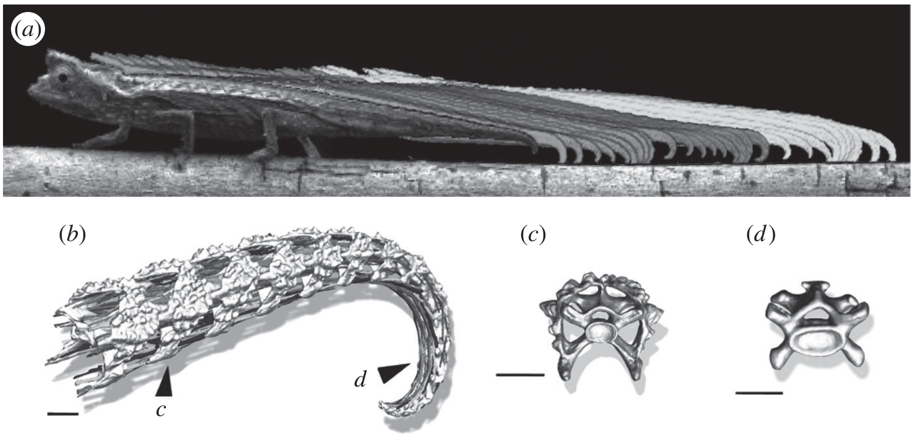

Figure 1. Tail-assisted walking in the chamaeleon *B. thieli*. (*a*) Representative video images illustrating how the tail curls and contacts the substrate (each colour illustrates a successive contact phase) during locomotion on a large branch. (*b*) Volume rendering of the tail in *Brookesia* showing the gradual change in morphology from proximal to distal with a decrease in vertebral ornamentation, and an increase in the development of the ventral tail tendons. Arrow heads point towards a proximal and distal vertebra illustrated in (*c*) and (*d*), respectively. These changes ensure increased mobility of the distal part of the tail. Scale bars, (*b*–*c*) 1 mm; (*d*) 0.25 mm.

Table 1. Summary of movement parameters for *B. thieli* walking on different substrates. Avg, average; s.d., standard deviation; ct. dur., contact time duration; cycle, cycle length duration; freq, frequency; d.f. duty factor.

		fore limb				hind limb				tail			
substrate		ct. dur. (s)	cycle (s)	freq. (Hz)	d.f. (%)	ct. dur. (s)	cycle (s)	freq. (Hz)	d.f. (%)	ct. dur. (s)	cycle (s)	freq. (Hz)	d.f. (%)
wire	avg	10.6	13.3	0.08	79	8.8	12.7	0.08	69				
	s.d.	3.2	3.7	0.02	4	2.2	2.2	0.01	9				
narrow	avg	13.0	16.4	0.08	77	12.5	16.3	0.08	75				
branch	s.d.	7.0	8.9	0.05	5	7.2	8.3	0.03	6				
wide	avg	10.3	13.7	0.08	79	10.7	13.1	0.08	81	3.0	6.1	0.19	49
branch	s.d.	3.4	3.9	0.02	2	3.1	3.4	0.02	4	2.0	2.0	0.07	18
ground	avg	5.4	6.5	0.17	77	4.5	6.7	0.17	67	1.0	3.8	0.34	27
	s.d.	2.4	2.8	0.05	5	2.0	2.6	0.05	10	0.9	2.3	0.13	12

(PyHST software, European Synchrotron Research Facility). Three-dimensional processing and rendering was obtained after semi-automatic segmentation of the skeleton, skull, dermal bones and parts of the nervous system (brain, inner ear, eye; see electronic supplementary material, movie S2) using gensurface in Amira 4.1.1 (Mercury Computer Systems, Chelmsford, MA, USA). We extracted the tail comprising the 15 posteriormost vertebrae to study the vertebral and tendon architecture. Geometrical and quantitative measurements on the sample were performed with ImageJ (available from http://rsb.info.nih.gov/ij).

3. RESULTS

Observations based on video recordings show that on broad substrates, the tail tip is flexed ventrally and brought into contact with the substrate on the dorsal side of the extreme tip touching the ground (figure 1 and electronic supplementary material, figure S1). Subsequently, the tail is extended, lifted and repositioned onto the ground. Tail positioning on the substrate is coordinated with movements of the feet. Depending on the type of substrate, a single step cycle is typically associated with two ground–tail contact phases (table 1). Moreover, higher frequencies of tail placement are associated with the broadest locomotor substrate (table 1). Interestingly, whereas the tail retains the movements associated with tail placement on narrow perches, it only rarely touches the substrate (electronic supplementary material, movie S1). Projection of the position of the feet and tail on the substrate shows that tail placement doubles the surface area of the polygon of support (on average 207.3%). Projection of the COM onto the substrate in cycles with and without tail placement (electronic supplementary material, figure S2) shows that the tail is placed on the substrate in cases where the COM is positioned far forward, and near the edge of the support polygon.

Synchrotron X-ray phase-contrast microtomography shows that the tail of *B. thieli* consists of 20 vertebrae in contrast to the 50 typically observed in arboreal chamaeleons (Zippel *et al.* 1999). The mobile region of the tail is restricted to the distal part only, in contrast to arboreal species, and shows two morphological specializations (figure1 and electronic supplementary material, figure S3 and movie S2): the terminal nine caudal vertebrae lack a bony shield, thus increasing vertebral mobility, and the ventro-medial tendons are strongly developed.

Our video recordings show that *Brookesia* move slowly with locomotor movements typically interrupted

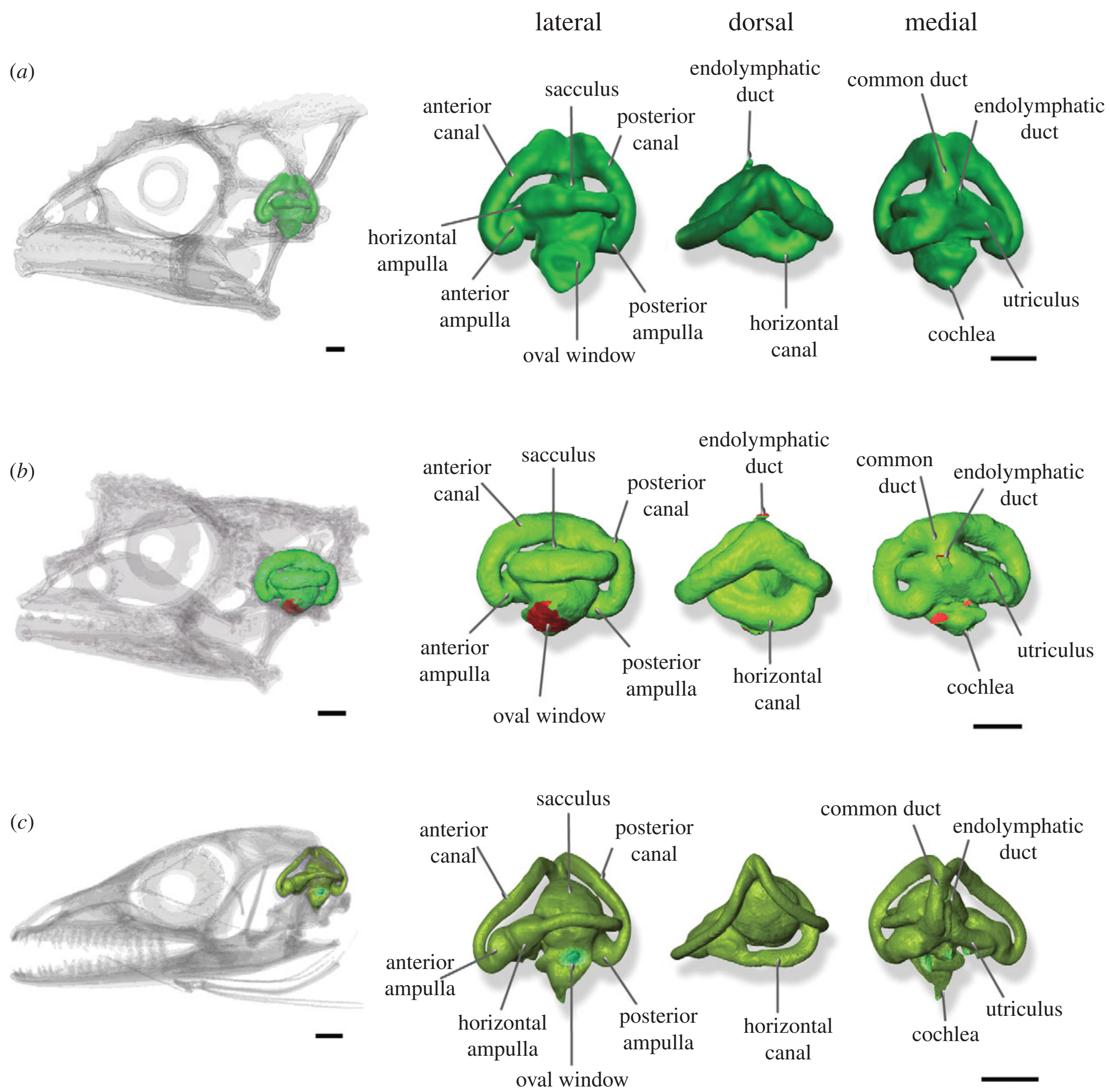

Figure 2. Volume rendering of the inner ear in the arboreal chamaeleon, (*a*) *C. tigris*, (*b*) *B. thieli* and (*c*) *A. carolinensis*, a typical arboreal Iguanian lizard. Illustrated is the position of the inner ear *in situ*, and lateral, dorsal and medial views of the inner ear in all species. Note how the semicircular canals are flattened and oblong in *B. thieli* compared with both other species. Interestingly, the horizontal canal in the two chamaeleons is orientated horizontally only when the lizard elevates its head. Scale bars, 1 mm.

by rocking motions during which the head is elevated. The three-dimensional reconstruction of the inner ear based on synchrotron microtomography demonstrates that the semicircular canals are oriented horizontally only when the head is elevated (figure 2). The shape of the vestibular system is characterized by well-developed semicircular canals with a curve extending ventrally (for the anterior and the posterior ones) and medially (for the horizontal canal). However, the semicircular canals in *Brookesia* are rather oblong and flattened in the vertical plane, in contrast to what is observed for an arboreal chamaeleon (*C. tigris*) or other arboreal lizards (figure 2).

4. DISCUSSION

Our observations suggest an important contribution of the tail during locomotion on broad substrates in dwarf chamaeleons of the genus *Brookesia*. Moreover, our data indicate that tail placement is restricted to broad substrates and improves stability on these substrates that can no longer be grasped by the prehensile feet, by significantly increasing the polygon of support.

The well-developed ventral tendon system suggests specializations for active bending of the tail tip. Given the lack of well-developed dorsal tendons that would be needed to extend the tail if it were to be used to generate propulsion, and the lack of tail-substrate contact on narrow substrates, we suggest that the tail most probably provides support and stability only. The inner ear of *Brookesia* is morphologically unique and comparative data suggest that it may be optimized to detect weak accelerations, probably important to ensure postural stability. Indeed, the semicircular canals are very different from those observed for arboreal chamaeleons, such as *C. tigris* or other arboreal lizards such as *A. carolinensis*, and resemble those of other slow-moving terrestrial vertebrates such as turtles and tuatara (Walsh *et al.* 2009).

Our observations suggest distinct morphological adaptations to the tail and sensory systems allowing these animals to exploit terrestrial habitats, despite the presence of specialized grasping feet. These data suggest an important role of the tail in locomotion on substrates where balance may be compromised. Given our observations for the Malagasy ground chamaeleons, it would be interesting to study chamaeleons of the genus *Rhampholeon*, an independent radiation of small ground-dwelling chamaeleons (Raxworthy *et al.* 2002), to test whether a similar tail-assisted locomotor mechanism is present in animals facing similar constraints on locomotion.

All experiments were approved by the Animal Care and Use Committee at the National Natural History Museum (Paris, France).

We thank Prof. Dany Azar for a critical reading of a previous version of the manuscript and are grateful to the CEMIM team (MNHN, Paris, material from MNHN was kindly loaned by Ivan Ineich). Supported by a European Synchrotron Radiation Facility Postdoctoral Project through allocation of beam time and ANR-06blan013202 (Locomo).

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