Assisted walking in Malagasy dwarf chamaeleons

Renaud Boistel1,2,3,*, Anthony Herrel1, Gheylen Daghrous1, Paul-Antoine Libourel1, Eodie Boller2, Paul Taftoreau2 and Vincent Bels1

1CNRS UMR 7179 ‘Mécanismes adaptatifs: des organismes aux communautés’, Départ. EGB, Museum National d’Histoire Naturelle, 57 rue Cuvier, case postale 55, 75231 Paris cedex 5, France
2European Synchrotron Radiation Facility, 6 route Jules Horowitz, B.P. 220, F-38043 Grenoble, France
3IPHEP-UMR CNRS 6046–UFR SFA Université de Poitiers, 1983; Jusufi running bipedally (Snyder 1949; Emmons & Gentry 1983). Among arboreal lizards, vertebrates (Jusufi 2008). Tails may provide stability, weight support or act as a balancing organ when walking on terrestrial 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, with light/dark cycles of 12 h. They were fed crickets and 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 ΔE/E ≈ 10−5 obtained with a double Si111 Bragg monochromator. We used a detector composed of a 1 μm thick gadolinium oxide scintillator and an optical system coupled to a cooled charge-coupled FRLeO N 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 mm2. 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 http://rsbl.royalsocietypublishing.org.
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 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.

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>Hind limb</th>
<th>Tail</th>
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<td>cycle (s)</td>
<td>freq. (Hz)</td>
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<tr>
<td></td>
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</table>

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.

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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 chameleon (*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 chameleons 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 chameleons, 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 *Rhampoleon*, 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).

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