Palaeontology
The Palaeontological Association
www.palass.org

[Palaeontology, 2018, pp. 1–14]

DISCUSSION

TUATARA AND A NEW MORPHOMETRIC DATASET FOR RHYNCHOCEPHALIA: COMMENTS ON HERRERA-FLORES *ET AL.*

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It has recently been suggested that a ‘living fossil’ can be identified because it is both morphologically conservative and exhibits a significantly slower rate of morphological evolution compared to related lineages (Herrera-Flores *et al.* 2017*a*). As an exemplar, variation among known rhynchocephalians was investigated, and it was concluded that the New Zealand tuatara *Sphenodon punctatus* Gray is a living fossil species (Herrera-Flores *et al.* 2017*a*). We respond here to Herrera-Flores *et al.*, questioning the biological meaning and basis for describing a ‘living fossil’, and we draw attention to limitations in their previous investigation of morphological conservatism among rhynchocephalians. To assist future studies of dentary-shape-variation data among rhynchocephalians and morphological conservatism, we provide a new geometric morphometric dataset for tuatara.

THEORETICAL PROBLEMS WITH A ‘LIVING FOSSIL’

Charles Darwin (1859, p. 486) recognized that living species possessed seemingly primitive morphological features and might, ‘fancifully be called living fossils’. As a concept a ‘living fossil’ is an oxymoron that has been widely criticized in scientific and education literature (Schopf 1984; Freeman & Herron 2007; Mobley *et al.* 2009; Casane & Laurenti 2013; Futuyma 2013; Mathers *et al.* 2013; Grandcolas *et al.* 2014; Werth & Shear 2014; Bennett *et al.* 2017). Putative living fossils are typically extant taxa that are considered to have undergone little morphological and inferred ecological change from a temporally distant ancestor known from palaeontological evidence (Grandcolas *et al.* 2014; Grandcolas & Trewick 2016). The amount of change considered to be ‘little’ is subjective as it varies wildly among organisms due to different evolutionary rates, durations in the fossil record, and taxonomic paradigms employed (Vaux *et al.* 2016; Bennett *et al.* 2017). Also, limited morphological change over time might not indicate evolutionary conservatism, but trait fluctuation around a mean state due to constraining selection (Charlesworth & Lande 1982; Eldredge *et al.* 2005; Hunt 2007; Davis *et al.* 2014; Grandcolas *et al.* 2014; Pennell *et al.* 2014*a*). The morphology of fossils represents only a fraction of the genetic, behavioural, ecological, internal morphological and physiological diversity that has evolved in a lineage (Grandcolas *et al.* 2014; Vaux *et al.* 2016, 2017*a*).

Most species gain recognition as ‘living fossils’ because they belong to groups that contained greater species diversity in the past (see references in Grandcolas *et al.* 2014) but this reasoning is barren because taxon rarity on a lineage reflects rates of extinction. Indeed, almost all biodiversity is extinct. Phylogenetic relicts are not especially uncommon, and the perception of rarity and the timing of species origination are sensitive to subjective taxonomy (Forey *et al.* 2004; Haile-Selassie & Simpson 2013; Vaux *et al.* 2016). Phylogenetic relicts at all systematic levels from genera to phyla attract attention because they represent the sole living representatives of taxonomic clades known better in the fossil record (e.g. ginkgo Werth & Shear 2014; vampire squid Yokobori *et al.* 2007; hoatzin Mobley *et al.* 2009; red panda Mallick 2015). For example, humans could justifiably be considered to be living fossils under current taxonomy due to the extinction of ~16 other species within *Homo* (Schopf 1984).

doi: 10.1111/pala.12402

Some putative living fossils are inferred to have persisted over many millions of years as a single evolutionary lineage segment (e.g. *Ginkgo biloba*, see Werth & Shear 2014). However, a taxon that appears to straddle a long period of evolutionary time almost certainly represents inadequate sampling of the fossil record, where evolutionary change and multiple evolutionary lineage segments have simply not been documented thoroughly (Werth & Shear 2014; Vaux *et al.* 2016). Palaeontologists are acutely aware of this issue, and it is one reason why rare specimens are not always classified below the genus level and certain taxa are treated as merely placeholders (e.g. *Archaeopteryx* Rauhut *et al.* 2018; see discussion by Forey *et al.* 2004).

Morphological evolution is a crucial aspect of palaeontology and evolutionary biology, independent of any consideration of 'living fossils'. Many recent studies have taken model-based approaches to analyse morphological change through time, and such methods are appropriate to address morphological conservatism and the origin of relict lineages (e.g. paleoTS and Geiger; Hunt 2007; Harmon *et al.* 2008; Pennell *et al.* 2014*b*; Hunt *et al.* 2015).

TUATARA AS A 'LIVING FOSSIL'

The tuatara (*Sphenodon punctatus*) is among the most frequently cited animal phylogenetic relicts (Fraser 1993; Sues *et al.* 1994; Jones 2008; Jones *et al.* 2009*a*; Meloro & Jones 2012; Cree 2014; Grandcolas *et al.* 2014). It is often described as a living fossil (Hay *et al.* 2008; Mitchell *et al.* 2008; Benton 2014; Klein & Scheyer 2017), although fossil remains of tuatara are all fewer than 10 000 years old (Hay *et al.* 2008). Fossil jaw fragments from New Zealand Miocene deposits have been assigned to Sphenodontinae (a putative subgroup within Rhynchocephalia), but the material is insufficient to identify to genus level (Jones *et al.* 2009*a*). Given the brief fossil record of tuatara, it is not possible to infer morphological stasis for the evolutionary lineage it represents.

Herrera-Flores *et al.* (2017*a*) investigated morphological variation in the dentary bones of rhynchocephalian reptiles and concluded that tuatara have a conservative morphology among their extinct relatives. The authors focused especially on the comparison of morphological variation with inferred diet. A phenogram based on morphology (Herrera-Flores *et al.* 2017*a*, fig. 1), indicated that tuatara are equally related to fossil genera that might have eaten plants and insects (*Clevosaurus* Bonaparte & Sues; Fraser 1988), bivalves or crabs (*Oenosaurus* Rauhut *et al.*, 2012) and seaweed (*Ankylosphenodon* Reynoso, 2000). A principal component analysis (PCA) scatterplot was presented too (reproduced in Fig. 1; Herrera-Flores *et al.* 2017*a*, fig. 3) indicating that the tuatara occupies a region of morphospace shared with putatively distant relatives that are argued to have been omnivorous, insectivorous and piscivorous (Herrera-Flores *et al.* 2017*a*). Since tuatara were the 'seventh most similar to the average shape (out of 29 taxa)', it was concluded that the species is 'conservative' for dentary shape as well as diet (Herrera-Flores *et al.* 2017*a*).

Treating diet as a categorical variable is problematic, even for living species for which behavioural and dietary data are available from observation, as habitats and feeding opportunity can vary dramatically among individuals and time (Arthur *et al.* 2008; López-Alfaro *et al.* 2014; Williams *et al.* 2015; Sato *et al.* 2016). Tuatara themselves are primarily insectivorous (Walls 1981; Cree *et al.* 1999; Ussher 1999; cf. Herrera-Flores *et al.* 2017*a*) but also feed on crustaceans, reptiles and fish, as well as the eggs and juveniles of small seabirds (Cartland-Shaw *et al.* 1998; Cree *et al.* 1999; Ussher 1999). Many animal species also change dietary preference with ontogeny. For example, juvenile *Clevosaurus brasiliensis* Bonaparte & Sues may have been restricted to insectivory, but it is likely that adults also ate plants (de Vivar Martínez & Soares 2015). Classifying both *Sphenodon* and *Clevosaurus* as omnivores (Herrera-Flores *et al.* 2017*a*) risks oversimplifying real intraspecific diversity, and introducing inaccuracy where no controls or sampling restrictions are enforced for ontogeny. A compounding issue is the accuracy of dietary estimates for fossil taxa, and inferences about extinct rhynchocephalians have been criticized (de Vivar Martínez & Soares 2015). Studies of other reptiles also demonstrate that dentary morphology without additional evidence, such as preserved stomach contents or coprolites, can mislead interpretation of diet (e.g. some herbivory-adapted dinosaurs ate crustaceans and rotten wood; Chin *et al.* 2017).

Beyond diet estimated from dentary morphology, little consideration was given to other known differences among the rhynchocephalians sampled by Herrera-Flores *et al.* (2017*a*). Predominant habitat differs among species of Rhynchocephalia from terrestrial to aquatic (de Vivar Martínez & Soares 2015; Klein & Scheyer 2017; Bever & Norell 2017) and there is a considerable size range (Apesteguía *et al.* 2014; de Vivar Martínez & Soares 2015). Notably, although tuatara exhibit similar dentary shape to the earliest known species *Brachyrhinodon* (Triassic, 250–200 million years ago), body shape differs with tuatara having a larger trunk and shorter limbs (Fraser & Benton 1989). Overall, there are good reasons why the well-preserved relatives of tuatara, alive millions of years ago, have been interpreted as representing diversity spanning genera and higher ranks. Therefore, focusing on a single trait is unlikely to provide a reliable assessment of evolutionary change in general (McCormack *et al.* 2010; Funk *et al.* 2012), or estimation of phenotypic stasis (Hunt 2007).

LIMITATIONS OF THE MORPHOMETRIC APPROACH

Herrera-Flores *et al.* used a two-dimensional, landmark-based geometric morphometric method to analyse morphological variation in dentary bones sampled from the extant tuatara and 30 extinct Rhynchocephalian taxa. Principal component analysis (PCA) and a disparity analysis based on Procrustes distances were interpreted as evidence of morphological conservatism (Herrera-Flores *et al.* 2017*a*). They interpreted the tuatara dentary as being 'close to the centroid of morphospace', indicating 'that the morphology of the modern tuatara is conservative' and it is also an 'average rhynchocephalian'. Given this result, the species was interpreted as being morphologically 'average' and conservative among fossil relatives (Herrera-Flores *et al.* 2017*a*). This result appeared to fulfil one criterion for the proposed definition of a living fossil (Herrera-Flores *et al.* 2017*a*). In fact, closer examination of all five statistically significant principal components (PCs) for 31 taxon specimens reveals a range of the proximity to the centroid. In terms of ranked position, we found that the tuatara was 5th, 24th, 18th, 10th and 17th closest specimen to the centroid for PCs 1–5 respectively.

The geometric morphometric method used by Herrera-Flores *et al.* is statistically powerful, and similar landmark approaches have been used to investigate morphological variation in many different organisms (e.g. *Dactylorhiza* orchids, Shipunov & Bateman 2005; *Achinanthidium* diatoms, Potapova & Hamilton 2007; *Placostylus* snails, Dowle *et al.* 2015; Tanganyikan cichlid fish, Clabaut *et al.* 2006). However, the accuracy of statistical results is always dependent on the precision of the data used. The fossil record for rhynchocephalia is sparse, and so just one dentary bone per species was used to represent 240 million years of evolution (Herrera-Flores *et al.* 2017*a*). A single dentary bone was used in the final analysis for tuatara as well, even though this living species is represented by curated specimens in museums worldwide. Thus, the conclusion of morphological conservatism for tuatara was based on the shapes of one specimen per species.

Images used for traditional and geometric morphometric analysis need to be produced in a consistent manner with similar quality photographs, rather than a mixture of material. Researchers are aware that differences in lens distortion and camera resolution, and minor changes in the positioning or lighting of an object can all introduce non-biological variation (Arnqvist & Mårtensson 1998; Collins & Gazley 2017). Wherever possible experimental error is quantified (Dowle *et al.* 2015; Vaux *et al.* 2017*b*) because combining datasets generated by different authors can introduce further confounding variation (Arnqvist & Mårtensson 1998; Fruciano 2016; Fruciano *et al.* 2017). The mixture of images used by Herrera-Flores *et al.* (2017*a*), was a likely source of non-biological variation. Fourteen photographs of dentary bones belonging to tuatara were initially used, but all fossil rhynchocephalian material was digitized from images produced in previous literature (Herrera-Flores *et al.* 2017*a*). Of the 30 images used, 23 were illustrated reconstructions, rather than direct digital images of intact specimens (Gilmore 1909; Fraser 1988; Ortega *et al.* 2009; Apesteguía & Carballido 2014). Additional shape artefacts are likely to have been introduced through digitizing PDF copies of previously published photographs and reconstructions, rather than using original images (Herrera-Flores *et al.* 2017*b*). Thus, most of the data in the analysis represented species singletons obtained from re-scanned images of fossil reconstructions, with no controls for errors.

Landmark-based geometric morphometric analyses use fixed landmarks placed at biologically homologous positions that are anatomical structures of shared origin present among all individuals or taxa (Bookstein 1991; Øyvind & Harper 2008, pp 115–116; Webster & Sheets 2010; Gunz & Mitteroecker 2013). Semi-landmarks are used to describe the curves or surfaces of an object, where homology between samples is difficult to identify. To be comparable, a curve must be anchored by at least one landmark, with semi-landmarks slid between two landmarks (or semi-landmarks) along a sequence of points (Perez *et al.* 2006; Sheets *et al.* 2006; Gunz & Mitteroecker 2013). The placement of a semi-landmark upon a curve itself is arbitrary (Webster & Sheets 2010), and so it is crucial that placement procedures are consistent between samples if mathematical descriptions of curves are to be compared (Gunz & Mitteroecker 2013). This is usually achieved using a 'fan' or 'comb' that enforces equal angular or linear spacing along a curve (Sheets *et al.* 2006) or by placing points evenly around a curve (Collins *et al.* 2013). In the 'living fossil' analysis, semi-landmarks were anchored between fixed landmarks and slid using Procrustes distances. However, some of these semi-landmarks seem to have been placed on biologically homologous positions, akin to the treatment of fixed landmarks. It is therefore unclear how semi-landmarks were placed consistently and why some of the biologically homologous positions used for semi-landmarks were not treated instead as fixed landmarks (see Herrera-Flores *et al.* 2017*a*, *b*).

Each PC is a mathematically independent axis of variation among specimens, and therefore morphological differences represented by each PC are equally valid to investigate, but it is useful to reduce interpretations to a smaller number of 'meaningful' PCs. It is common practice for the broken-stick test to be applied to the eigenvalues of each PC to establish statistical significance (Jackson 1993; Bro & Smilde 2014) although there are many other component retention methods (Cangelosi & Goriely 2007). If more than the first two PCs are significant, the distribution of specimens on the further axes can potentially reveal additional relationships (Bro & Smilde 2014). Herrera-Flores *et al.* did not mention the number of statistically significant PCs for their dataset, and only presented scatterplots using PC1 (38.5% (38.8% in original paper)) and PC2 (15.1%). Using the broken-stick test implemented in the R package `vegan` (Oksanen *et al.* 2015; R Core Team 2016), we found that PCs 1–5 were all statistically significant in the dataset produced by Herrera-Flores *et al.* The additional PCs show different patterns of dentary-shape-variation with, for example, the combination of PC2 and PC5 (5.9%) revealing a more distant position of tuatara from the centroid of sampling (Fig. 1). Determining a meaningful distance from a centroid is largely subjective, and we agree with the authors that there is no methodological consensus for demonstrating morphological conservatism (Herrera-Flores *et al.* 2017*a*). However, all meaningful axes of variation need to be explored before an average can be considered.

Lastly, Herrera-Flores *et al.* also plotted a 'phylogeny' onto their PCA analysis as a phylomorphospace (Herrera-Flores *et al.* 2017*a*, fig. 3c). This approach risks circularity however, because the phylogeny (more accurately a phenogram) was derived from the same dentary-shape-variation data used to estimate the principal components. Separating derived, convergent and ancestral morphological traits in a phylogeny built from these same traits is not possible (de Queiroz 1996).

A NEW MORPHOMETRIC DATASET FOR TUATARA DENTARY BONES

We provide a new dataset for dentary shape and size variation in tuatara, adapting a previous protocol used by de Vivar Martínez & Soares (2015) to analyse dentary morphology in *Clevosaurus brasiliensis.* Sampling of further fossil species is required before morphological change can be investigated among other rhynchocephalians, and the new data from tuatara cannot be used alone to address the hypotheses considered by Herrera-Flores *et al.* In addition, the method presented here was developed to digitize tuatara, and it therefore may not be suitable for all rhynchocephalian dentary bones, and there is certainly room for modification and improvement. The intention of this method and new dataset is to assist with future investigations of morphological evolution in Rhynchocephalia. The new dataset also considers error associated with photography and landmark placement, and the variation associated with comparing left and right dentary specimens.

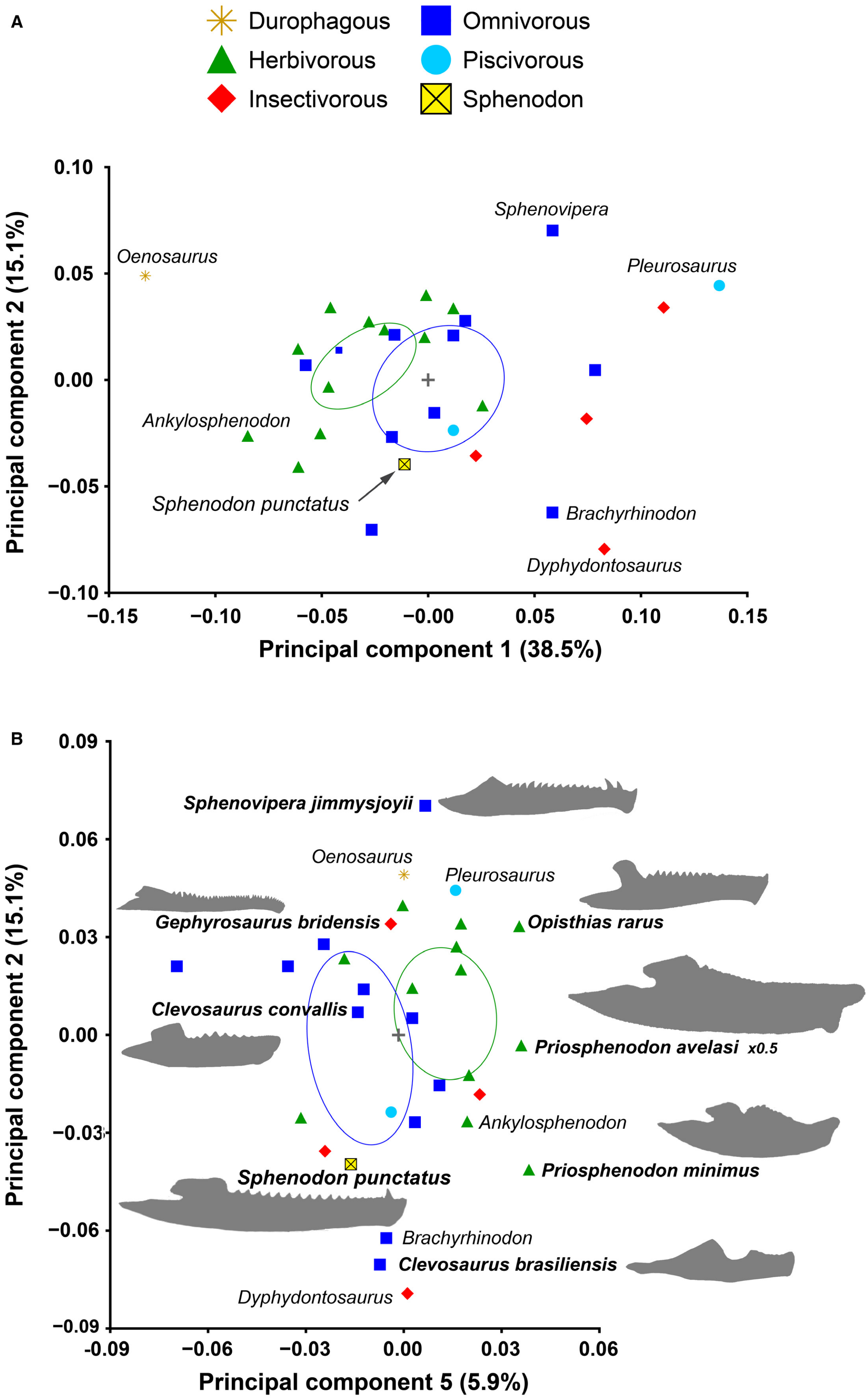
MATERIAL AND METHOD

Sampling

We sampled 86 dentary bones (44 left, 42 right) of *Sphenodon punctatus* held at the National Museum of New Zealand Te Papa Tongarewa (Vaux *et al.* 2018, table S1). Of these, 9 bones were of recent origin, collected from populations of tuatara living on small offshore islands, whereas 71 bones were Holocene fossils (<10 000 years old) collected at sites on the North Island and South Island (Hay *et al.* 2010; Fig. 2). Many of the sampled fossil bones came from geological sites or collections previously sampled for radiocarbon dating (^{14}C), DNA sequencing or morphological trait comparison (Hay *et al.* 2003; Hay *et al.* 2008; Jones *et al.* 2009*a*). As a result, 29 fossils from six sample collections ranged between 1130 and 5190 years old (Vaux *et al.* 2018, table S1), based on the radiocarbon dating of bones from the same provenance (Hay *et al.* 2008). We did not attempt to account for differing age of specimens or ontogeny.

We first investigated the potential for experimental error during photography and digitization by repositioning and re-photographing the left and right copy of the dentary from one individual multiple times (five times left, four times right), and then re-digitized one left image four more times. Combined with 79 observations from left and right dentaries taken from separate

FIG. 1. Shape variation among rhynchocephalian dentary bones illustrated with scatterplots of principal components (PCs) estimated by Herrera-Flores *et al.* (2017*a*). PCs 1–5 were statistically significant according to the broken-stick test. A, dentary shape variation between PC1 (38.5% of variation) and PC2 (15.1%; fig. 3 of Herrera-Flores *et al.* 2017*a*). B, PC5 (5.9%) and PC2; silhouettes of dentary bones are shown at the same scale for taxa labelled in bold. Specimens are grouped by hypothesized diet (following Herrera-Flores *et al.* 2017*a*, fig. 3), with 90% mean confidence ellipses shown for dietary groups of five or more specimens (i.e. taxa). A selection of taxa is labelled, including the tuatara *Sphenodon punctatus*, following the style of Herrera-Flores *et al.* (2017*a*, fig. 3). Each plot includes a grey cross for the geometric centre of variation (centroid) for the PCs illustrated. Note that *S. punctatus* is displaced from the centroid for both pairs of PCs. Image sources and dentary lengths: *Clevosaurus brasiliensis* (25 mm) de Vivar Martínez & Soares (2015); *Clevosaurus convallis* (23 mm) Säilä (2005); *Gephyrosaurus bridensis* (20 mm) Evans (2008); *Priosphenodon avelasi* (~100 mm) Apesteguía & Novas (2003); *Priosphenodon minimus* (~30 mm) Apesteguía & Carballido (2014); *Sphenodon punctatus* (~50 mm) Jones *et al.* (2009*b*); *Sphenovipera jimmysjoyi* (20 mm) Reynoso (2005). Colour online.


A
Durophagous
Omnivorous
Herbivorous
Piscivorous
Insectivorous
Sphenodon
0.10
0.05
0.00
−0.05
−0.10
Principal component 2 (15.1%)
Sphenovipera
Oenosaurus
Pleurosaurus
Ankylosphenodon
Sphenodon punctatus
Brachyrhinodon
Dyphydontosaurus
−0.15
−0.10
−0.05
−0.00
0.05
0.10
0.15
Principal component 1 (38.5%)
B
0.09
0.06
0.03
0.00
−0.03
−0.06
−0.09
Principal component 2 (15.1%)
Sphenovipera jimmysjoyii
Oenosaurus
Pleurosaurus
Gephyrosaurus bridensis
Opisthias rarus
Clevosaurus convallis
Priosphenodon avelasi x0.5
Ankylosphenodon
Priosphenodon minimus
Sphenodon punctatus
Brachyrhinodon
Clevosaurus brasiliensis
Dyphydontosaurus
−0.09
−0.06
−0.03
0.00
0.03
0.06
Principal component 5 (5.9%)

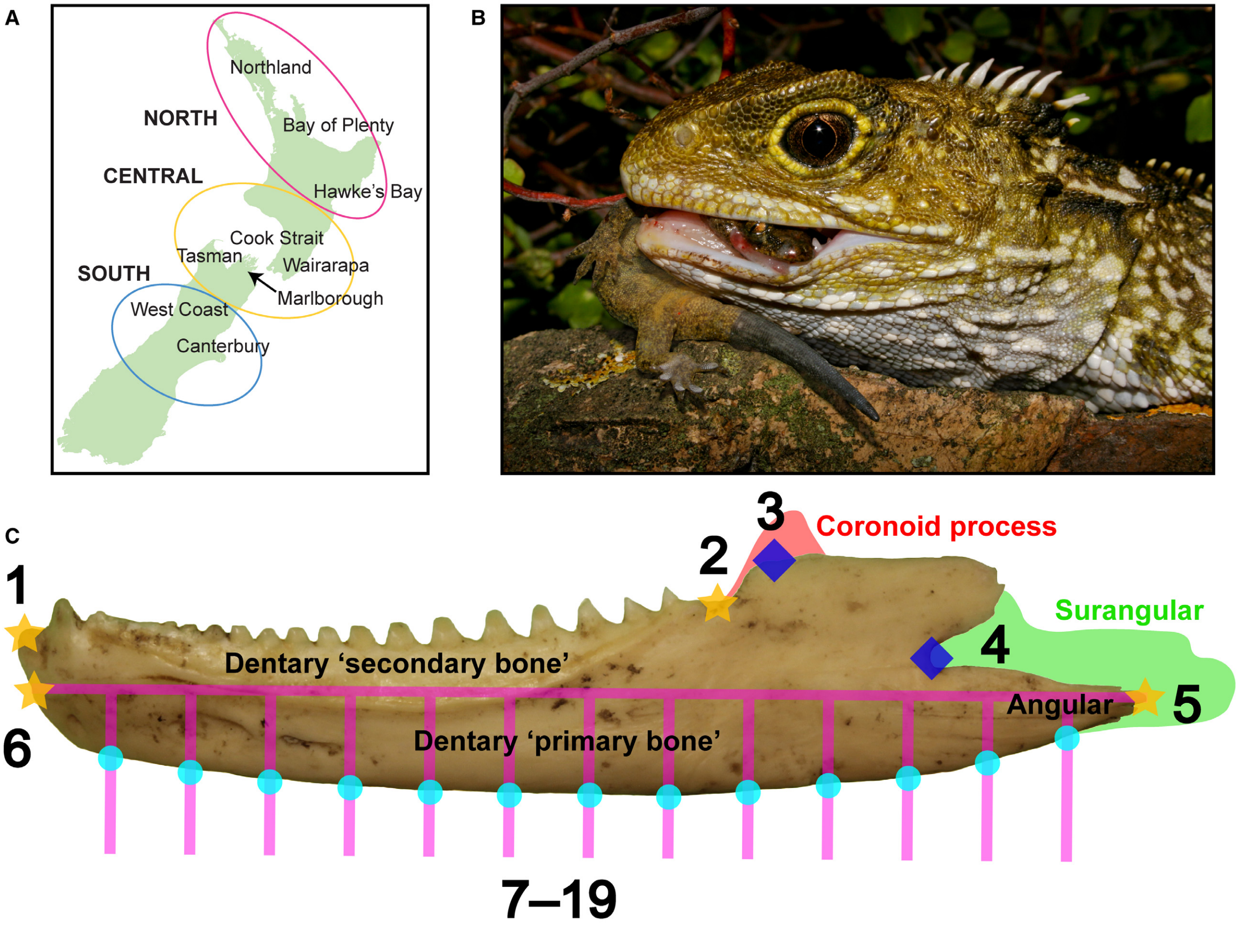


FIG. 2. Sampling and morphometric landmarks used for the analysis of tuatara dentary bones. A, sample regions for our morphometric sampling of tuatara dentary bones, grouped into three broad geographic regions: North, Central and South. B, a live tuatara, using its dentary to ingest a New Zealand common gecko *Woodworthia maculata* (Gray) (Photo: Tony Jewell). C, landmarks used to digitize morphological variation in the dentary bones of the tuatara; six fixed landmarks (stars and diamonds) are individually numbered; landmarks 1, 2, 5 and 6 (stars) were also used by de Vivar Martínez & Soares (2015) to digitize dentaries of *Clevosaurus brasiliensis*, whereas fixed landmarks 3 and 4 (diamonds) and semi-landmarks 7–19 (circles) are unique to this tuatara dataset. The 13 semi-landmarks used to describe the ventral edge of the dentary were identified using the comb overlaid onto the dentary. The dentary can be subdivided into 'primary' and 'secondary' bone, and it is fused with the angular bone. Although the majority of digitized specimens were dislocated, we label and illustrate the articulation of the dentary with the coronoid process and surangular bones. Colour online.

individuals, this yielded an *Error dataset* of 92 observations. A *Paired dataset* comprising our total sample of 86 dentaries, which included six pairs of left and right bones each taken from the same individual, was used to investigate differences between left and right bones.

Our *Main dataset* consisted of 80 extant and Holocene fossil tuatara dentary bones, randomly excluding either the left or right dentaries from individuals where both sides were available. For an initial inspection of variation in the *Main dataset*, specimens were classified into three broad geographic regions encompassing mainland and nearshore islands (Fig. 2A): (1) North: Hawke's Bay to Northland; (2) Central: the Wairarapa, the Cook Strait, Marlborough and Tasman; (3) South: Canterbury and West Coast.

Geometric morphometric method

Dentary bones were positioned on fine-grade silica sand of contrasting colour. Specimens were photographed in the external lateral view using a Canon EF-S 600D camera with an 18–55 mm IS II lens. All photographs were taken from a height of 54.5 cm using a Compact Action Manfrotto tripod (MKCOMPACTACN), with a 50 mm scale bar included in each digital image. All positioning, photography and subsequent digitization was conducted by one person to minimize experimenter error (Schilthuizen & Haase 2010). Images of right-sided bones were transformed horizontally to match the orientation of left specimens using Adobe Photoshop CS6. A virtual alignment comb was overlaid on each image in the same program so that semi-landmarks could be placed consistently (Fig. 2C). Digital images were organized into thin plate spline (TPS) files using `tpsUtil` (Rohlf 2013), with the order of specimens randomized to reduce potential experimental bias.

Geometric morphometric analysis followed a pipeline similar to that used by Vaux *et al.* (2017*b*) in a study of snail shell morphology. Six landmarks and 13 semi-landmarks were identified on each image using a Wacom Cintiq 22HD Pen Display tablet (Fig. 2). Data were scale-calibrated using `tpsUtil`, `tpsDig` (Rohlf 2013), and semi-landmarks were slid to minimize the Procrustes distance among individuals using the IMP programs `CoordGen7` and `Semiland7` (Sheets 2014), yielding *X–Y* coordinates for points digitized on the bones.

Biologically homologous positions were captured by six fixed landmarks (Table 1). The landmarks used were based on those previously applied to *Clevosaurus brasiliensis* jaw morphology (de Vivar Martínez & Soares 2015). Specifically, our landmarks 1, 2, 5 and 6 correspond to landmarks 5, 3, 1 and 8 of de Vivar Martínez & Soares (2015; Fig. 2C). Future authors adapting our set of landmarks for a larger dataset (sampling multiple species) would probably benefit from resampling the curve captured by our semi-landmarks, and reducing the number of semi-landmarks used, rather than using a comb.

TABLE 1. Definitions used for the geometric morphometric landmarks digitized for tuatara dentary bones.

Landmark	Definition
1	The most superior point on the anterior end of the dentary
2	The inflexion point of the dentary as it descends in front of the coronoid process, before the most posterior tooth
3	Most superior point of the dentary immediately after the curve of the dentary parallel to the coronoid process, which grows parallel with the coronoid process
4	Inflexion point of the dentary in the curve after the coronoid process and before the suture of the dentary, angular and surangular
5	Most posterior point of the dentary, and the suture of the dentary, angular and surangular
6	The most anterior-ventral point of the ‘secondary bone’ of the dentary. An explanation of this feature observed in tuatara was provided by Kieser *et al.* (2009), and de Vivar Martínez & Soares (2015)
7–19	Semi-landmarks describing the ventral curve of the dentary, anchored between landmarks 5 and 6

Specimen sampling was insufficient for a comprehensive statistical analysis of dentary shape or size variation within tuatara, but the shape variation captured by current sampling was examined using PCA ordination. Partial Procrustes superimposition and the generation of a covariance matrix using *X–Y* Procrustes coordinates was conducted using MorphoJ v.1.06c (Klingenberg 2011). Partial Procrustes superimposition is the preferred method when morphological variation is relatively small (Perez *et al.* 2006). It aligns and superimposes landmarks for all specimens to remove confounding variation due to differences in the size, translation (position), and orientation of objects (Webster & Sheets 2010; Gunz & Mitteroecker 2013; Monteiro 2013; Polly *et al.* 2013). Principal component analysis was conducted on the covariance matrix of superimposed landmarks (MorphoJ). The PCs reflect variation in the shape of objects, and centroid size represents size variation independent of shape. Statistically significant principal components were identified using the broken-stick test on eigenvalues, implemented in the R package `vegan` v.2.2-1 (Jackson 1993; Oksanen *et al.* 2015). Only the significant PCs were used in subsequent analyses. Procrustes variance among groups in the *Error dataset* was analysed using the R package `geomorph` (Adams & Otárola-Castillo 2013; R Core Team 2016) to estimate morphological disparity.

RESULTS

Using the photography and digitization *Error dataset* (92 observations), the broken-stick test identified PCs 1–4 as statistically significant. These PCs represent 51.8%, 17.4%, 9.5% and 6.0% of shape variation respectively. Based on PCA ordination, three error treatments (left photo repeat photographs, right photo repeat photographs and left digitization repeats) overlapped in morphospace and could not be separated using 90% mean confidence ellipses (Fig. 3). Comparison of Procrustes variance contributed by the error treatment groups was low and statistically insignificant (<5% of variation; Table 2). Left repeated photographs and digitizations contributed 4.4% and 2.6% of variation among all left-sided specimens, and right repeated photographs contributed only 0.9% of variation among all right-sided specimens (Table 2). In contrast, all remaining observations (i.e. separate individuals, probably reflecting true biological variation) contributed 93.0% and 99.1% of variation in left and right-sided comparisons respectively (Table 2). Given that left digitization repeats contributed an intermediate percentage of

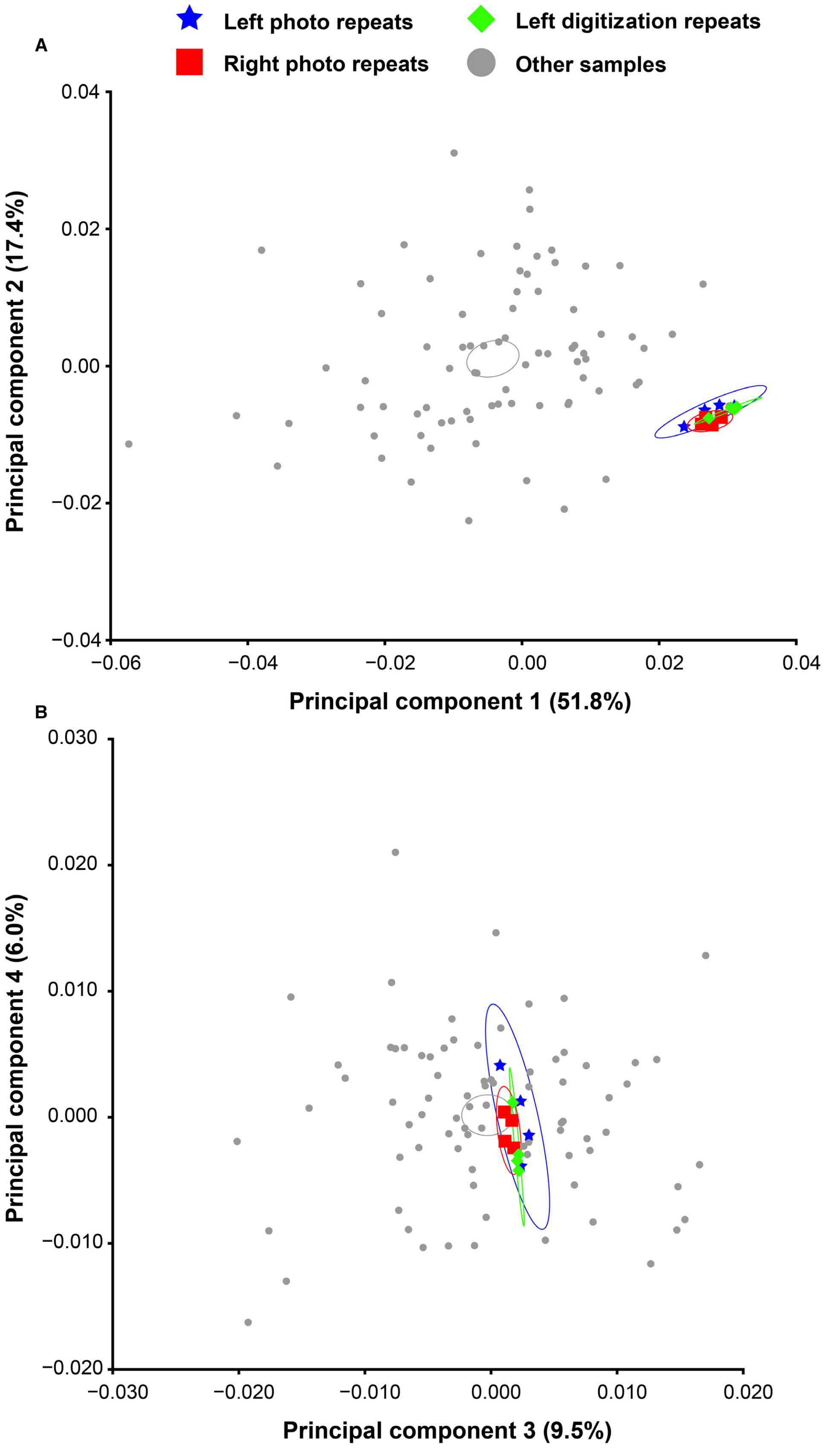

★ Left photo repeats
◆ Left digitization repeats
■ Right photo repeats
● Other samples
A
0.04
0.02
0.00
−0.02
−0.04
Principal component 2 (17.4%)
−0.06
−0.04
−0.02
0.00
0.02
0.04
Principal component 1 (51.8%)
B
0.030
0.020
0.010
0.000
−0.010
−0.020
Principal component 4 (6.0%)
−0.030
−0.020
−0.010
0.000
0.010
0.020
Principal component 3 (9.5%)

TABLE 2. Procrustes variance among error treatment groups.

Groups	Sampling	Procrustes variance	Percentage of left variance	Percentage of right variance
Left digitization repeats	4	1.38E-05	2.6	N/A
Left photographic repeats	5	2.38E-05	4.4	N/A
Right photographic repeats	4	6.06E-06	N/A	0.9
Left others	41	5.41E-04	93.0	N/A
Right others	38	6.56E-04	N/A	99.1

Morphological disparity estimated using Procrustes variance for photographic (five photographs taken from a left and four from a right dentary belonging to the same individual) and digitization repeats (another four digitizations taken from one left dentary photograph using the same individual). Variance for these treatments was compared to sampling of other tuatara individuals, using the respective side. The analysis was conducted using the R package `geomorph` (Adams & Otárola-Castillo 2013; R Core Team 2016).

variation to left and right photography repeats, it is likely that photography and digitization error contribute a similar level of insignificant confounding variation to a dataset. If future analyses use the same morphometric method to estimate experimental error, it should also be possible to account for inter-dataset variation.

Using our *Paired dataset*, containing 86 observations, which included six pairs of left and right bones taken from same individuals, the broken-stick test identified PCs 1–4 as statistically significant. These PCs represented 44.6%, 19.4%, 11.3% and 7.1% of variation among observations respectively. PCA ordination indicated that the distances within shape space between paired bones were mostly short (Vaux *et al.* 2018, fig. S1). Our sampling of known, paired left and right bones was limited to only six individuals, and therefore further statistical analyses without additional sampling are problematic, but we observed no consistent directional trend in shape space for any PC between left and right bones (Vaux *et al.* 2018, fig. S1). Size (based on centroid size) was also almost identical between paired left and right dentary bones. Our current results therefore indicate that left and right bones from the same individual are similar in shape and size, and their shape does not differ in a consistent manner among individuals.

For the *Main dataset*, containing 80 extant and Holocene fossil tuatara bones, with paired left or right dentaries randomly excluded, PCs 1–4 were identified as being statistically significant by broken-stick test. These PCs represented 43.6%, 19.3%, 11.6% and 7.3% of variation among individuals respectively. PCA ordination using 90% mean confidence ellipses did not reveal shape difference between left and right dentary bones (Vaux *et al.* 2018, fig. S2). PCA ordination did however indicate some shape variation among the sampled locations across all significant PCs (Fig. 4; Vaux *et al.* 2018, fig. S3). Modern and Holocene fossil specimens appeared to differ for PC1, however, this was not evident from the remaining statistically significant PCs (collectively 38.2%; Fig. 4). Based on landmark PC loading across all axes, differences in length of the dentary, particularly at the posterior portion where the 'primary' dentary articulates with the coronoid process and surangular bones, contributed the most to variation among specimens (Vaux *et al.* 2018, fig. S4).

DISCUSSION

The variation observed in our morphometric dataset indicates that future investigations of variation in tuatara and Rhynchocephalia should be fruitful. Using our new tuatara dentary morphometric method, we estimated that experimental error during photography and landmark digitization was statistically insignificant (<5% of variation among observations; Fig. 3; Table 2). We found no evidence of fluctuating asymmetry between left and right dentary bones (Fig. 3; Vaux *et al.* 2018, figs. S1, S2). If extrapolated to other sphenodonts or rhynchocephalians, this suggests that morphometric studies combining left and right sampling dentary bones (e.g. de Vivar Martínez & Soares 2015; Herrera-Flores *et al.* 2017*a*) are unlikely to be confounded by asymmetric variation. This is a useful finding because choosing between left and right bones is unlikely to be an option in the sparse rhynchocephalian fossil record, and because asymmetry is an ongoing topic of morphometric research (Klingenberg 2015). The fact that we observed little variation among modern and recent

FIG. 3. Error associated with photography and digitization compared to interspecific shape variation of tuatara dentary bones. Significant principal components (PCs) of shape variation among 92 sampled tuatara dentary bone photographs illustrated with scatterplots. 79 separate specimens were photographed, with 4 right and 5 left photographic repeats, and 5 left digitization repeats of an additional specimen. A, comparison of PC1 (51.8% of variation) and PC2 (17.4%). B, comparison of PC3 (9.5%) and PC4 (6.0%). Legend indicates error treatment. 90% mean confidence ellipses for groups are shown. Colour online.

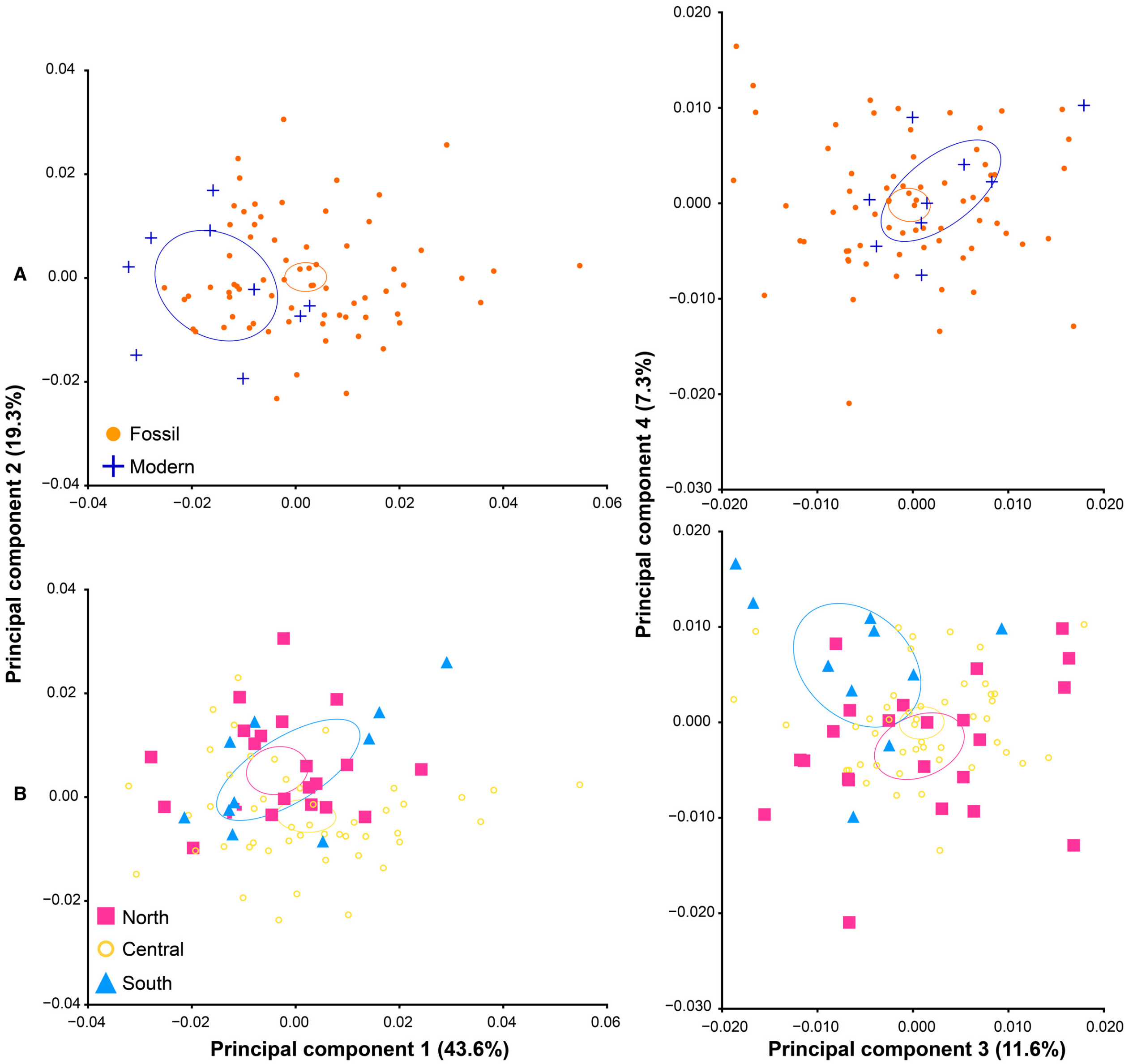


FIG. 4. Shape variation of tuatara dentary bones sampled across time (A) and space (B). Principal component (PC) scatterplots illustrate shape variation among 80 sampled tuatara dentary bones (Main dataset). Variation is shown for the comparison of PC1 (43.6% of variation) and PC2 (19.3%), as well as PC3 (11.6%) and PC4 (7.3%). 90% mean confidence ellipses for groups are shown. A, temporal origin: sampling of modern and Holocene fossils <10 000 years-old. B, spatial origin (see Fig. 2A). Colour online.

fossil (<10 000 years old) tuatara specimens, also indicates that modern and fossil material are similar enough to permit analyses of morphological evolution using programs such as paleoTS (Hunt 2007; Hunt *et al.* 2015), as soon as there are enough time point samples to model a series (e.g. if fossils older than 10 000 years are discovered). This finding means that topics such as morphological conservatism considered by Herrera-Flores *et al.* could be addressed in the future using geometric morphometric analyses.

Without additional sampling to permit more comprehensive statistical analyses, our current sample of 80 bones (*Main dataset*) is insufficient to determine the biological significance of dentary shape and size variation in tuatara. Nonetheless, our dataset permits the first geometric morphometric comparison of extant and fossil tuatara. Minor shape differences were observed among samples from modern and fossil geographical locations (Fig. 4; Vaux *et al.* fig. S3). PCA results indicated that modern and fossil specimens mostly differ for PC1 (43.6% of variation among samples), but samples overall could not be distinguished due to homogeneity across the remaining significant PCs 2–4 (collectively 38.2%; Fig. 4). Specimens coded into three broad geographical ranges (north, central, south) could not be clearly distinguished by PCA, although there was potentially a small visual difference between the north and central regions (Fig. 4). Notably, the four specimens sampled from Brothers Island were not separated from other individuals in shape space (Vaux *et al.* 2018, fig. S3), which suggests concordance with genetic data used to disregard the treatment of this population as a distinct lineage (Hay *et al.* 2010). Future studies with more numerous sampling should be able to test the distinction of groups using methods such as canonical variates analysis or Bayesian assignment (Fraley & Raftery 2002), and researchers could investigate statistically significant shape differences using programs such as TwoGroup (Sheets 2014).

CONCLUSION

The objective and approach of identifying a ‘living fossil’ is of limited evolutionary insight. We find that the previously available data (Herrera-Flores *et al.* 2017*a*) are insufficient to justify the statistical interpretation of tuatara as morphologically conservative among ancestors or a living fossil. However, with appropriate sampling and data, geometric morphometric methods provide powerful tools for testing explicit hypotheses about morphological evolution.

The new tuatara geometric morphometric dataset provided uses landmarks that should be compatible with the dentary bones of other rhynchocephalians (e.g. *Clevosaurus brasiliensis*; de Vivar Martínez & Soares 2015) and we have demonstrated that experimental error and fluctuating asymmetry are unlikely to be sources of confounding variation (at least for this exact method in tuatara). To thoroughly investigate other hypotheses in tuatara, such as morphological stasis and population-level variation, further sampling is required.

The variation observed in our new tuatara dataset adequately demonstrates why the reduction of an entire species to a single specimen for evolutionary analysis, as conducted by Herrera-Flores *et al.* (2017*a*), is inappropriate (see similar discussion by de Vivar Martínez & Soares 2015). Given the criticisms we highlight about the analysis and data used by Herrera-Flores *et al.*, we conclude that there is no advantage in using precision methods such a geometric morphometrics if the specimens used are deficient or suspect. We expect the dataset provided for tuatara to facilitate future morphometric analyses of Rhynchocephalia and efforts to investigate morphological conservatism.

Acknowledgements. We thank Thomas Schultz and the Museum of New Zealand Te Papa Tongarewa for providing us with access to the tuatara specimens, and Paulo R. Romo de Vivar Martínez (Federal University of Rio Grande do Sul) for sharing his morphometric dataset for *Clevosaurus brasiliensis*. We are grateful for helpful comments by Katie S. Collins (University of Chicago) and James S. Crampton (GNS Science/Victoria University of Wellington) regarding the geometric morphometric methods. Thank you to Tony Jewell for sharing the photograph of the live tuatara specimen. Lastly, we thank the Editor, Andrew Smith and our two anonymous reviewers for their generous and detailed feedback regarding the manuscript and the geometric morphometric methods.

DATA ARCHIVING STATEMENT

Data, and supplementary table and figures for this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.ms7q2q4

Editor. Hannah O’Regan

REFERENCES

ADAMS, D. C. and OTÁROLA-CASTILLO, E. 2013. geomorph: an R package for the collection of analysis of geometric morphometric shape data. *Methods in Ecology & Evolution*, **4**, 393–399.

APESTEGUÍA, S. and CARBALLIDO, J. L. 2014. A new eilenodontine (Lepidosauria, Sphenodontidae) from the Lower Cretaceous of central Patagonia. *Journal of Vertebrate Paleontology*, **34**, 303–317.

—— and NOVAS, F. E. 2003. Large Cretaceous sphenodontian from Patagonia provides insight into lepidosaur evolution in Gondwana. *Nature*, **425**, 609–612.

—— GÓMEZ, R. O. and ROUGIER, G. W. 2014. The youngest South American rhynchocephalian, a survivor of the K/Pg extinction. *Proceedings of the Royal Society B*, **2881**, 20140811.

ARNQVIST, G. and MÅRTENSSON, T. 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduces its impact on measures of shape. *Acta Zoologica Academiae Scientiarum Hungaricae*, **44**, 73–96.

ARTHUR, K. E., BOYLE, M. C. and LIMPUS, C. J. 2008. Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. *Marine Ecology*, **362**, 303–311.

BENNETT, D. J., SUTTON, M. D. and TURVEY, S. T. 2017. Evolutionary distinct “living fossils” require both lower speciation and lower extinction rates. *Paleobiology*, **43**, 34–48.

BENTON, M. J. 2014. *Vertebrate palaeontology*. 4th edn. Wiley-Blackwell. 472 pp.

BEVER, G. S. and NORELL, M. A. 2017. A new rhynchocephalian (Reptilia: Lepidosauria) from the Late Jurassic of Solnhofen (Germany) and the origin of the marine Pleurosauridae. *Royal Society Open Science*, **4**, 170570.

BOOKSTEIN, F. L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press. 435 pp.

BRO, R. and SMILDE, A. K. 2014. Principal component analysis. *Analytical Methods*, **6**, 2812.

CANGELOSI, R. and GORIELY, A. 2007. Component retention in principal component analysis with application to cDNA microarray data. *Biology Direct*, **2**, 2.

CARTLAND-SHAW, L. K., CREE, A., SKEAFF, C. M. and GRIMMOND, N. M. 1998. Differences in dietary and plasma fatty acids between wild and captive populations of a rare reptile, the tuatara (*Sphenodon punctatus*). *Journal of Comparative Physiology B*, **8**, 569–580.

CASANE, D. and LAURENTI, P. 2013. Why coelacanths are not 'living fossils'. *BioEssays*, **35**, 332–338.

CHARLESWORTH, B. and LANDE, R. 1982. Morphological stasis and developmental constraint: no problem for Neo-Darwinism. *Nature*, **296**, 610.

CHIN, K., FELDMANN, R. M. and TASHMAN, J. N. 2017. Consumption of crustaceans by megaherbivorous dinosaurs: dietary flexibility and dinosaur life history strategies. *Scientific Reports*, **7**, 11163.

CLABAUT, C., BUNJE, P. M. E., SALZBURGER, W. and MEYER, A. 2006. Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. *Evolution*, **61**, 560–578.

COLLINS, K. S. and GAZLEY, M. F. 2017. Does my posterior look big in this? The effect of photographic distortion on morphometric analyses. *Paleobiology*, **43**, 508–520.

—— CRAMPTON, J. S. and HANNAH, M. 2013. Identification and independence: morphometrics of Cenozoic New Zealand *Spissatella* and *Eucrassatella* (Bivalvia, Crassatellidae). *Paleobiology*, **39**, 525–537.

CREE, A. 2014. *Tuatara: Biology and conservation of a venerable survivor*. Canterbury University Press, Christchurch, New Zealand.

—— LYON, L. G., CARTLAND-SHAW, L. and TYRRELL, C. 1999. Stable carbon isotope ratios as indicators of marine versus terrestrial inputs to the diets of wild and captive tuatara (*Sphenodon punctatus*). *New Zealand Journal of Zoology*, **26**, 243–253.

DARWIN, C. R. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. 1st edn. John Murray, London. 502 pp.

DAVIS, C. C., SCHAEFER, H., XI, Z., BAUM, D. A., DONOGHUE, M. J. and HARMON, L. J. 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences*, **111**, 5914–5919.

DOWLE, E. J., MORGAN-RICHARDS, M., BRESCIA, F. and TREWICK, S. A. 2015. Correlation between shell phenotype and local environment suggests a role for natural selection in the evolution of *Placostylus* snails. *Molecular Ecology*, **24**, 4205–4221.

ELDREDGE, N., THOMPSON, J. N., BRAKEFIELD, P. M., GAVRILETS, S., JABLONSKI, D., JACKSON, J. B. C., LENSKI, R. E., LIEBERMAN, B. S., McPEEK, M. A. and MILLER, W. I. I. I. 2005. The dynamics of evolutionary stasis. *Paleobiology*, **31**, 133–145.

EVANS, S. E. 2008. The skull of a new eosuchian reptile from the Lower Jurassic of South Wales. *Zoological Journal of the Linnean Society*, **70**, 203–264.

FOREY, P. L., FORTEY, R. A., KENRICK, P. and SMITH, A. B. 2004. Taxonomy and fossils: a critical appraisal. *Philosophical Transactions of the Royal Society of London B*, **359**, 639–653.

FRALEY, C. and RAFTERY, A. E. 2002. Model-based clustering, discriminant analysis and density estimation. *Journal of the American Statistical Association*, **97**, 611–631.

FRASER, N. C. 1988. The osteology and relationships of *Clevosaurus* (Reptilia: Sphenodontida). *Philosophical Transactions of the Royal Society B*, **321**, 125–178.

—— 1993. A new sphenodontian from the Early Mesozoic of England and North America: implications for correlating early Mesozoic continental deposits. *New Mexico Museum of Natural History & Science Bulletin*, **3**, 135–139.

—— and BENTON, M. J. 1989. The Triassic reptiles *Brachyrhinodon* and *Polysphenodon* and the relationships of the sphenodontids. *Zoological Journal of the Linnean Society*, **96**, 413–445.

FREEMAN, S. and HERRON, J. C. 2007. *Evolutionary analysis*. 4th edn. Pearson Education Inc., Upper Saddle River, NJ, **707–709**, 800 pp.

FRUCIANO, C. 2016. Measurement error in geometric morphometrics. *Development Genes & Evolution*, **226**, 139–158.

—— CELIK, M. A., BUTLER, K., DOOLEY, T., WEISBECKER, V. and PHILLIPS, M. J. 2017. Sharing is caring? Measurement error and the issues arising from combining 3D morphometric datasets. *Ecology & Evolution*, **7**, 7034–7046.

FUNK, W. C., McKAY, J. K., HOHENLOHE, P. A. and ALLENDORF, F. W. 2012. Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, **27**, 489–496.

FUTUYMA, D. J. 2013. *Evolution*. 3rd edn. Sinaeur Associates, Sunderland, MA, **611–614**, 656 pp.

GILMORE, C. W. 1909. A new rhynchocephalian reptile from the Jurassic of Wyoming, with notes on the fauna of "Quarry 9". *Proceedings of the United States National Museum*, **37**, 35–42.

GRANDCOLAS, P. and TREWICK, S. A. 2016. What is the meaning of extreme phylogenetic diversity? The case of phylogenetic relict species. 99–115. *In* PELLENS, R. and GRANDCOLAS, P. (eds). *Biodiversity conservation and phylogenetic systematics*. Topics in biodiversity and conservation, **14**. Springer. 390 pp.

—— NATTIER, R. and TREWICK, S. A. 2014. Relict species: a relict concept? *Trends in Ecology & Evolution*, **23**, 655–663.

GUNZ, P. and MITTEROECKER, P. 2013. Semilandmarks: a method for quantifying curves and surfaces. *Hystrix*, **24**, 103–109.

HAILE-SELASSIE, Y. and SIMPSON, S. W. 2013. A new species of *Kolpochoerus* (Mammalia: Suidae) from the Pliocene of central Afar, Ethiopia: its taxonomy and phylogenetic relationships. *Journal of Mammalian Evolution*, **20**, 115–127.

HARMON, L. J., WEIR, J. T., BROCK, C. D., GLOR, R. E. and CHALLENGER, W. 2008. Geiger: investigating evolutionary radiations. *Bioinformatics*, **24**, 129–131.

HAY, J. M., DAUGHTERY, C. H., CREE, A. and MAXSON, L. R. 2003. Low genetic divergence obscures phylogeny among population of *Sphenodon*, remnant of an ancient reptile lineage. *Molecular Phylogenetics & Evolution*, **29**, 1–19.

—— SUBRAMANIAN, S., MILLAR, C. D., MOHANDESAN, E. and LAMBERT, D. M. 2008. Rapid molecular evolution in a living fossil. *Trends in Genetics*, **24**, 106–109.

—— SARRE, S. D., LAMBERT, D. M., ALLENDORF, F. W. and DAUGHTERY, C. H. 2010. Genetic diversity and taxonomy: a reassessment of species designation in tuatara (*Sphenodon*: Reptilia). *Conservation Genetics*, **11**, 1063–1081.

HERRERA-FLORES, J. A., STUBBS, T. L. and BENTON, M. J. 2017*a*. Macroevolutionary patterns in the Rhychocephalia: is the tuatara (*Sphenodon punctatus*) a living fossil? *Palaeontology*, **60**, 319–328.

—— —— —— 2017*b*. Data from: Macroevolutionary patterns in the Rhychocephalia: is the tuatara (*Sphenodon punctatus*) a living fossil? *Dryad Digital Repository*. https://doi.org/10.5061/dryad.568jh

HUNT, G. 2007. The relative importance of directional change, random walks, and stasis in the evolution of fossil lineages. *Proceedings of the National Academy of Sciences*, **104**, 18404–18408.

—— HOPKINS, M. J. and LIDGARD, S. 2015. Simple versus complex models of trait evolution and stasis as a response to environmental change. *Proceedings of the National Academy of Sciences*, **112**, 4885–4890.

JACKSON, D. A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology*, **74**, 2204–2214.

JONES, M. E. H. 2008. Skull shape and feeding strategy in *Sphenodon* and other Rhynchocephalia (Diapsida: Lepidosauria). *Journal of Morphology*, **269**, 945–966.

—— TENNYSON, A. J. D., WORTHY, J. P., EVANS, S. E. and WORTHY, T. H. 2009*a*. A sphenodontine (Rhynchocephalia) from the Miocene of New Zealand and palaeobiogeography of the tuatara (*Sphenodon*). *Proceedings of the Royal Society B*, **276**, 1385–1390.

—— CURTIS, N., O'HIGGINS, P., FAGAN, M. J. and EVANS, S. E. 2009*b*. The head and neck muscles associated with feeding in *Sphenodon* (Reptilia: Lepidosauria: Rhynchocephalia). *Palaeontologia Electronica*, **12**, 56 pp.

KIESER, J. A., TKATCHENKO, T., DEAN, M. C., JONES, M. E., DUNCAN, W. and NELSON, N. J. 2009. Microstructure of dental hard tissue and bone in the tuatara dentary, *Sphenodon punctatus* (Diapsida: Lepidosauria: Rhynchocephalia). *Frontiers of Oral Biology*, **13**, 80–85.

KLEIN, N. and SCHEYER, T. M. 2017. Microanatomy and life history in *Palaeopleurosaurus* (Rhynchocephalia: Pleurosauridae) from the Early Jurassic of Germany. *The Science of Nature*, **104**, 4.

KLINGENBERG, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353–357.

—— 2015. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. *Symmetry*, **7**, 843–934.

LÓPEZ-ALFARO, C., COOGAN, S. C. P., ROBBINS, C. T. and FORTIN, J. K. 2014. Assessing nutritional parameters of brown bear diets among ecosystems gives insight into differences among populations. *PLoS One*, **10**, e0128088.

MALLICK, J. K. 2015. *In situ* and *ex situ* conseration of Red Panda in Darjeeling district, West Bengal, India. 283–305. *In* GUPTA, V. K. and VERMA, A. K. (eds). *Animal diversity, natural history and conservation. Vol. 5*. Daya Publshing House, New Dehli, 445 pp.

MATHERS, T. C., HAMMOND, R. L., JENNER, R. A., HÄNFLING, B. and GÓMEZ, A. 2013. Multiple global radiations in tadpole shrimps challenge the concept of 'living fossils'. *PeerJ*, **1**, e62.

McCORMACK, J. E., HELED, J., DELANEY, K. S., PETERSN, A. T. and KNOWLES, L. L. 2010. Calibrating divergence times on species trees versus gene trees: implications for speciation history of *Aphelocoma* Jays. *Evolution*, **65**, 184–202.

MELORO, C. and JONES, M. E. 2012. Tooth and cranial disparity in the fossil relatives of *Sphenodon* (Rhynchocephalia) dispute the persistent 'living fossil' label. *Journal of Evolutionary Biology*, **25**, 2194–2204.

MITCHELL, N. J., KEARNEY, M. R., NELSO, N. J. and PORTER, W. P. 2008. Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proceedings of the Royal Society B*, **275**, 2185–2193.

MOBLEY, J. A., BREWER, D., ELPHICK, J., HOARE, B., UNWIN, M. and WOODWARD, J. 2009. Cuckoos and turacos. 186–187. *In* MOBLEY, J. A. (ed.) *Birds of the world. Vol. 3*. Marshall Cavendish, Singapore. 821 pp.

MONTEIRO, L. R. 2013. Morphometrics and the comparative method: studying the evolution of biological shape. *Hystrix*, **24**, 25–32.

OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R. B., SIMPSON, G. L., SOLYMOS, P., STEVENS, M. H. H. and WAGNER, H. 2015. vegan: Community Ecology Package. R package version 2.2-1. https://cran.R-project.org/package=vegan

ORTEGA, F., MALAFAIA, E., ESCASO, F., PÉREZ-GARCÍA, A. and DANTAS, P. 2009. Faunas de répteis do Jurássico Superior de Portugal. *Paleolusitana*, **1**, 43–56.

ØYVIND, H. and HARPER, D. A. T. 2008. *Paleontological data analysis*. John Wiley & Sons. 351 pp.

PENNELL, M. W., HARMON, L. J. and UYEDA, J. C. 2014*a*. Is there room for punctuated equilibrium in macroevolution? *Trends in Ecology & Evolution*, **29**, 23–32.

—— EASTMAN, J. M., SLATER, G. J., BROWN, J. W., UYEDA, J. C., FITZJOHN, R. G., ALFARO, M. E. and HARMON, L. J. 2014*b*. Geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics*, **30**, 2216–2218.

PEREZ, S. I., BERNAL, V. and GONZALEZ, P. N. 2006. Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *Journal of Anatomy*, **208**, 769–784.

POLLY, P. D., LAWING, A. M., FABRÉ, A. and GOSWAMI, A. 2013. Phylogenetic principal components analysis and geometric morphometrics. *Hystrix*, **24**, 33–41.

POTAPOVA, M. and HAMILTON, P. B. 2007. Morphological and ecological variation within the *Achinanthidium minutissimum* (Bacillariophyceae) species complex. *Journal of Phycology*, **43**, 561–575.

QUEIROZ, K. DE 1996. Including the characters of interest during tree reconstruction and the problems of circularity and bias in studies of character evolution. *The American Naturalist*, **148**, 700–708.

R CORE TEAM. 2016. *R: a language environment for statistical computing. Version 3.3.2.* R foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

RAUHUT, O. W. M., HEYNG, A. M., LÓPEZ-ARBARELLO, A. and HECKER, A. 2012. A new Rhynchocephalian from the Late Jurassic of Germany with a dentition that is unique amongst tetrapods. *PLoS One*, **7**, e46839.

—— FOTH, C. and TISCHLINGER, H. 2018. The oldest *Archaeopteryx* (Theropoda: Avialiae): a new specimen from the Kimmeridgian/Tithonian boundary of Schamhaupten, Bavaria. *PeerJ*, **6**, e4191.

REYNOSO, V. H. 2000. An unusual aquatic sphenodontina (Reptilia: Diapsida) from the Tlayua Formation (Albian), Central Mexico. *Journal of Paleontology*, **74**, 133–148.

—— 2005. Possible evidence of a venom apparatus in a Middle Jurassic sphenodontian from the Huizachal red beds of Tamaulipas, México. *Journal of Vertebrate Paleontology*, **25**, 646–654.

ROHLF, F. J. 2013. tpsUtil 1.58 and tpsDig 2.17. http://life.bio.sunysb.edu/morph/

SÄILÄ, L. K. 2005. A new species of the sphenodontian reptile *Clevosaurus* from the Lower Jurassic of South Wales. *Palaeontology*, **48**, 817–831.

SATO, H., SANTIN, L., PATEL, E. R., CAMPERA, M., YAMASHITA, N., COLQUHOUN, I. C. and DONATI, G. 2016. Dietary flexibility and feeding strategies of *Eulemur*: a comparison with *Propithecus*. *International Journal of Primatology*, **37**, 109–129.

SCHILTHUIZEN, M. and HAASE, M. 2010. Disentangling true shape differences and experimenter bias: are dextral and sinistral snail shells exact mirror images? *Journal of Zoology*, **282**, 191–200.

SCHOPF, T. J. M. 1984. Rates of evolution and the notion of "living fossils". *Annual Review of Earth & Planetary Sciences*, **12**, 245–294.

SHEETS, H. D. 2014. Integrated Morphometrics Package (IMP) 8. http://www3.canisius.edu/~sheets/morphsoft.html

—— COVINO, K. M., PANASIEWICZ, J. M. and MORRIS, S. R. 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Frontiers in Zoology*, **3**, 15.

SHIPUNOV, A. B. and BATEMAN, R. M. 2005. Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia. *Biological Journal of the Linnean Society*, **85**, 1–12.

SUES, D. H., SHUBIN, N. H. and OLSEN, P. E. 1994. A new sphenodontian (Lepidosauria: Rhynchocephalia) from the McCoy Brook Formation (Lower Jurassic) of Nova Scotia, Canada. *Journal of Vertebrate Paleontology*, **14**, 327–340.

USSHER, G. T. 1999. Tuatara (*Sphenodon punctatus*) feeding ecology in the presence of kiore (*Rattus exulans*). *New Zealand Journal of Zoology*, **26**, 117–125.

VAUX, F., TREWICK, S. A. and MORGAN-RICHARDS, M. 2016. Lineages, splits and divergence challenge whether the terms anagenesis and cladogenesis are necessary. *Biological Journal of the Linnean Society*, **117**, 165–176.

—— CRAMPTON, J. S., MARSHALL, B. A., TREWICK, S. A. and MORGAN-RICHARDS, M. 2017*b*. Geometric morphometric analysis reveals that the shells of male and female siphon whelks *Penion chathamensis* are the same size and shape. *Molluscan Research*, **37**, 194–201.

—— TREWICK, S. A. and MORGAN-RICHARDS, M. 2017*a*. Speciation through the looking-glass. *Biological Journal of the Linnean Society*, **120**, 480–488.

—— MORGAN-RICHARDS, M., DALY, E. E. and TREWICK, S. A. 2018. Data from: Tuatara and a new morphometric dataset for Rhynchocephalia: a response to Herrera-Flores *et al. Dryad Digital Repository*. https://doi.org/10.5061/dryad.ms7q2q4

VIVAR MARTÍNEZ, P. R. DE and SOARES, M. B. 2015. Dentary morphological variation in *Clevosaurus brasiliensis* (Rhynchocephalia, Clevosauridae) from the Upper Triassic of Rio Grande do Sul, Brazil. *PLoS One*, **10**, e0119307.

WALLS, G. Y. 1981. Feeding ecology of the tuatara (*Sphenodon punctatus*) on Stephen Island, Cook Strait. *New Zealand Journal of Ecology*, **4**, 89–97.

WEBSTER, M. and SHEETS, H. D. 2010. A practical introduction to landmark-based geometric morphometrics. *Quantitative Methods in Paleobiology*, **16**, 163–188.

WERTH, A. J. and SHEAR, W. A. 2014. The evolutionary truth about living fossils. *American Scientist*, **102**, 434–443.

WILLIAMS, A. J., ALLAIN, V., NICOL, S. J., EVANS, K. J., HOYLE, S. D., DUPOUX, C., VOUREY, E. and DUBOSC, J. 2015. Vertical behaviour and diet of albacore tuna (*Thunnus alalunga*) vary with latitude in the South Pacific Ocean. *Deep-Sea Research II*, **113**, 154–169.

YOKOBORI, S., LINDSAY, D. J., YOSHIDA, M., TSUCHIYA, K., YAMAGISHI, A., MARUYAMA, T. and OSHIMA, T. 2007. Mitochondrial genome structure and evolution in the living fossil vampire squid, *Vampyroteuthis infernalis*, and extant cephalopods. *Molecular Phylogenetics & Evolution*, **44**, 898–910.