

Feeding behaviour in a ‘basal’ tortoise provides insights on the transitional feeding mode at the dawn of modern land turtle evolution

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ABSTRACT

Almost all extant testudinids are highly associated with terrestrial habitats and the few tortoises with high affinity to aquatic environments are found within the genus *Manouria*. *Manouria* belongs to a clade which forms a sister taxon to all remaining tortoises and is suitable as a model for studying evolutionary transitions within modern turtles. We analysed the feeding behaviour of *Manouria emys* and due to its phylogenetic position, we hypothesise that the species might have retained some ancestral features associated with an aquatic lifestyle. We tested whether *M. emys* is able to feed both in aquatic and terrestrial environments. In fact, *M. emys* repetitively tried to reach submerged food items in water, but always failed to grasp them—no suction feeding mechanism was applied. When feeding on land, *M. emys* showed another peculiar behaviour; it grasped food items by its jaws—a behaviour typical for aquatic or semiaquatic turtles—and not by the tongue as generally accepted as the typical feeding mode in all tortoises studied so far. In *M. emys*, the hyolingual complex remained retracted during all food uptake sequences, but the food transport was entirely lingual based. The kinematical profiles significantly differed from those described for other tortoises and from those proposed from the general models on the function of the feeding systems in lower tetrapods. We conclude that the feeding behaviour of *M. emys* might reflect a remnant of the primordial condition expected in the aquatic ancestor of the tortoises.

Subjects Animal Behavior, Ecology, Evolutionary Studies, Zoology

Keywords Food uptake, Turtle, Evolution, Tetrapoda, Feeding kinematics, Transition to land

INTRODUCTION

Comprising more than 180 species, the cryptodiran taxon Testudinoidea represents the most diverse group of extant turtles (e.g., *Fritz & Havaš, 2007; Thomson & Shaffer, 2010*).

Submitted 14 March 2015
Accepted 20 July 2015
Published 11 August 2015

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Academic editor
Jérémy Anquetin

Additional Information and
Declarations can be found on
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DOI 10.7717/peerj.1172

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Traditionally, it contains three major extant groups, including the emydids, the geoemydids and the testudinids (tortoises) (Fig. 1). All molecular phylogenetic studies (Iverson *et al.*, 2007; Shaffer, 2009; Thomson & Shaffer, 2010; Barley *et al.*, 2010) confirm a sister group relationship of the testudinids and the geoemydids (Fig. 1). The relationship of Platysternidae to other turtle groups remains unresolved (Parham, Feldman & Boore, 2006), but most molecular studies support a closer relationship to emydids (e.g., Thomson & Shaffer, 2010; Crawford *et al.*, 2015). Palaeontological studies have shown that all testudinoids share aquatic ancestors, from which terrestrial species evolved (Danilov, 1999; Sukhanov, 2000; Joyce & Gauthier, 2004).

The majority of the Triassic stem turtles were terrestrial as indicated by the design and proportions of the limbs, which were adapted for terrestrial locomotion (for a comprehensive discussion see Joyce, 2015). With the emergence of modern turtles (Testudines) during the Jurassic period (e.g., Danilov & Parham, 2006; Sterli, 2010; Sterli & de la Fuente, 2011), a general transition of turtles into an aquatic environment occurred (Willis *et al.*, 2013). The invasion of aquatic environments induced diversification into several subgroups (see Joyce, 2007; Thomson & Shaffer, 2010). Due to the different physical properties (drag, viscosity, etc.) of air and water, the new environment required morphological and functional adaptations of the locomotion and feeding system to enable efficient swimming behaviour and aquatic food uptake (i.e., suction feeding) (Schumacher, 1973; Lemell *et al.*, 2002).

The sister group of all remaining Testudinoidea is the diverse and possibly paraphyletic extinct taxon †Lindholmemydidae (Fig. 1; Lourenço *et al.*, 2012), which contains genera such as †*Mongolemys* and †*Lindholmemyx* (Danilov, 1999; Joyce & Gauthier, 2004). Some poorly documented aquatic taxa such as †Haichemydidae and the †Sinochelyidae may perhaps also belong to †Lindholmemydidae. This group was recorded from aquatic sediments of the late Early Cretaceous and apparently had an amphibious lifestyle (Sukhanov, 2000). Among recent cryptodirans, semi-aquatic to semi-terrestrial lifestyle is typical for most emydids (plus platysternids) and geoemydids, as well as for some kinosternids (see Depeker *et al.*, 2006, but also Nakajima, Hirayama & Endo, 2014). The remaining extant species are entirely terrestrial (tortoises), or predominantly aquatic (see Joyce & Gauthier, 2004; Rasmussen *et al.*, 2011).

Among modern turtles, a transition from an aquatic to a semi-terrestrial or fully terrestrial habitat and the capacity to exploit terrestrial food sources has evolved independently within all three major testudinoid lineages (for overview see Summers *et al.*, 1998; Natchev *et al.*, 2009). At least eight emydid species are able to feed on land as well as under water (see Bels, Davenport & Delheusy, 1997; Bels *et al.*, 2008; Summers *et al.*, 1998; Stayton, 2011). During terrestrial feeding, such amphibious emydids use their jaws to grasp food items (jaw prehension). Similarly, all amphibious geoemydids studied to date also use jaw prehension in terrestrial food uptake (see Heiss, Plenk & Weisgram, 2008; Natchev *et al.*, 2009). In contrast, all testudinids studied so far use the tongue to touch the food items, a behaviour referred to as “lingual prehension” (see Wochesländer, Hilgers & Weisgram, 1999;

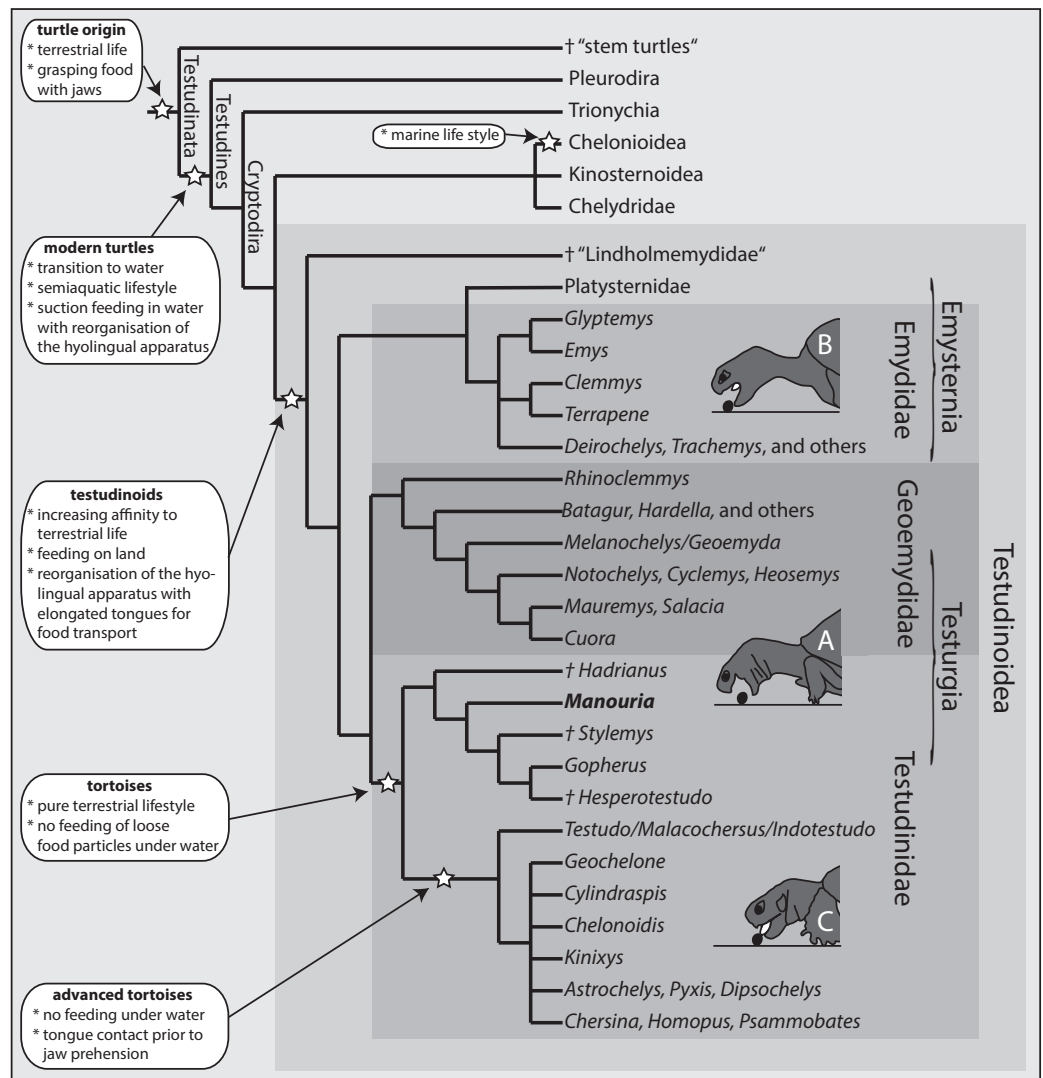


Figure 1 Phylogeny of turtle clades with a focus on Testudinoidea. Interrelationship following Thomson & Shaffer (2010). Major evolutionary changes are listed; for details see text. Three modes of terrestrial food uptake are illustrated. (A) Jaw prehension; the tongue is not protruded and is only used for food transport (Geoemydidae, Manouria). (B) Jaw prehension; the elongated tongue is protruded during prehension but does not contact the food and is only used for food transport (Emyridae). (C) Prior to jaw prehension, the tongue contacts the food (advanced tortoises).

Bels et al., 2008). According to Bels et al. (2008), lingual prehension is obligatory for all tortoises.

The tortoises show a clear tendency towards herbivory and emancipation from water as living and feeding medium (see Pritchard, 1979; Ernst & Barbour, 1989; Bonin, Devaux & Dupre, 2006). In fact, testudinids seem to have lost their ancestral ability to feed under water and exclusively rely on terrestrial trophic ecologies. Some predominantly terrestrial geoemydids are able to complete the whole feeding process on land and under water (Natchev et al., 2010). Similarly, testudinids with tendencies towards an

amphibious lifestyle might have retained the ancestral skill to feed underwater. Hence, information on bimodal feeding mechanisms in tortoises is of great importance to understand the evolution of terrestrial feeding mechanisms and subsequent evolution of the predominantly terrestrial lifestyle in tortoises.

The genus *Manouria*, being of the most ‘basal’ extant tortoises with a strong association to aquatic environments (*Høybye-Mortensen, 2004; Stanford et al., 2015*), constitutes a suitable model to study the feeding mechanisms in testudinids. Its partially aquatic feeding habit purported to be associated with the observed morphological extension of the palatines onto the triturating surface of the upper jaw (character 30 sensu *Gerlach, 2001*), a diagnostic feature common to geoemydid turtles. Another geoemydid-like feature is the unique existence of class II mental glands (*Winokur & Legler, 1975*).

The present study was conceived to provide a detailed analysis of the feeding behaviour in a species of the genus *Manouria*. *Manouria emys* is found in close association with water. Hence, we designed experiments to reveal whether this species is able to complete the entire feeding process under both aquatic and terrestrial conditions as some geoemydids do (see *Natchev et al., 2009; Natchev et al., 2010*).

Similar to all investigated testudinids, the Asian forest tortoise possesses a well developed tongue. The hyoid complex is predominantly cartilaginous (*Heiss et al., 2011*). On the base of the specific morphology of the feeding apparatus (elastic basis of the oropharynx and voluminous lingual structures) we suggest a poor suction feeding performance in case *Manouria* attempts to feed under water.

Wochesländer, Hilgers & Weisgram (1999), Wochesländer, Gumpenberger & Weisgram (2000) and *Bels et al. (2008)* stated that the feeding kinematics in all testudinids involve two common features: an obligatory lingual prehension and the split of the gape cycle in four main phases: slow open phase I (SOI); slow open phase II (SOII); fast open phase (FO); fast close phase (FC). In our experiments we test whether these kinematical elements are present in the feeding behaviour of *M. emys*. On the basis of our findings, we fine-tune the kinematical feeding models proposed for tortoises. The gained new data requires a re-evaluation of the concept on the function of the tongue in food uptake in tortoises. Having in mind the phylogenetical position of *M. emys* and the specifics of its feeding behaviour, we propose a hypothesis on the evolution of the terrestrial feeding among testudinids in particular and turtles in general. We discuss also the interrelationship between the diet and the feeding media in the course of turtle evolution.

MATERIALS AND METHODS

Ecological background

Both extant species of *Manouria*, the Asian forest tortoise *M. emys* and the impressed tortoise *M. impressa*, have a restricted distribution in Southeast Asia. *M. emys* has a narrow distribution in Bangladesh, India (Assam, Meghalaya, Mizoram, Nagaland), Myanmar, Thailand, Malaysia (East and West), and Indonesia (Kalimantan, Sumatra). The nominate subspecies, *M. emys emys*—the subject of this study, inhabits the southern part of the species range (*Fritz & Havaš, 2007; Stanford et al., 2015*).

M. emys inhabits tropical evergreen monsoon forests and exhibits high tolerance for soil moisture. It is commonly found reposing in wet areas, buried in mud or under the leaf litter where it may spend long periods of time. It is active even during rainy weather. Direct sun exposure and basking are not required. Furthermore, this species has a mostly crepuscular and nocturnal lifestyle (Ernst, Altenburg & Barbour, 2000; Vetter & Daubner, 2000; Stanford et al., 2015).

According to the available literature, the diet of *M. emys* includes plants, fungi, invertebrates, and frogs (Nutphand, 1979; Das, 1995; Lambert & Howes, 1994; Høybye-Mortensen, 2004). It has been reported to feed on plants in shallow mountain streams (Nutphand, 1979).

Experimental setting

Animal husbandry and experiments were in strict accordance with the Austrian Protection of Animals Act. The animals used in the present study were obtained commercially and kept at 12 h dark/light cycles in a large terrarium (150 × 100 cm ground area) with a permanently filtered water basin and spacious terrestrial area. The turtles were fed different fruits, vegetables, commercially obtained tortoise pellets, dead mice, as well as pieces of cattle heart and liver, offered on the terrestrial part of the terrarium. Carapace lengths in the three subadult experimental animals ranged between 109 and 135 mm with body masses between 234 and 236 g. For filming terrestrial feeding, the specimens were put in a dry glass cuvette (24 × 60 × 30 cm). When the food was offered on the floor of the cuvette, the tortoises often twisted their necks and rotated their heads in attempt to grasp the food item. The side movements made the filming of the animals in strict lateral view very difficult and the landmarks were not clearly visible during the sequence. By the use of forceps for food display we completely eliminated these problems and were able to shoot perfect lateral plans of the feeding turtles. The food in the feeding experiments was offered at a position which was similar to the position on which we offered the food in the terrarium where the tortoises were housed. The animals did not extend vastly their necks to reach the food items (see Appendix S1). The position of the offered food was completely “natural”. The tortoises needed to stretch their necks forwards rather than downwards, which did not impacted other kinematic patterns of the feeding cycles.

As food items we used small pieces of cattle heart measuring approximately 5 × 5 × 5 mm. The turtles were filmed from lateral aspect (with a reference grid 1 × 1 cm in the background) via the digital high-speed camera system Photron Fastcam-X 1024 PCI (Photron limited, Tokyo, Japan) at 500 fps with a highly light-sensitive objective AF Zoom—Nikkor 24–85 mm (f/2, 8-4D IF). Two “Dedocool Coolh” tungsten light heads with 2 × 250 W (ELC), supplied by a “Dedocool COOLT3” transformer control unit (Dedo Weigert Film GmbH, München, Germany) were used for illumination. We filmed and analysed the food uptake and the food transport cycles in eight feeding sequences for each specimen.

The setting for filming aquatic feeding of submerged food comprised the experimental aquarium filled with water to a level of 3 cm and presentation of food items in front of the



Figure 2 Selected frame from a high-speed video sequence (500 frs) of food transport in *Manouria emys*, showing the landmarks used for kinematic analyses. C, rostral tip of sagittal line of the carapace; Hy, hyoid at the basis of ceratbranchial I; LJ, tip of the lower jaw; P, posterior most point of crista supraoccipitale; TT, tip of the tongue; Tv, ventral most point of the tympanum at the position of the jaw joint; UJ, tip of the upper jaw; grid 10 × 10 mm. Abbreviations in [Appendix S1](#).

turtle's snout. In order to reduce the light intensity and for optimisation of the digitising process, the frame rate was reduced to 250 fps. As the tortoises were unable to grasp the food item in a total of 36 trials, the kinematics of the feeding apparatus had been analysed (see below) in nine selected representative feeding trials.

For both terrestrial and “aquatic feeding” sequences, horizontal (*X*-axis) and vertical (*Y*-axis) coordinates of relevant landmarks (see [Fig. 2](#)) were digitised frame by frame using “SIMI-MatchiX” (SIMI Reality Motion Systems, Unterschleißheim, Germany). Based on the displacement of the markers, we were able to calculate the gape amplitude (distance between the tip of the upper and lower beak), head movement (distance between the anterior tip of the carapace and the point “P” on [Fig. 2](#)), tongue movements (distance between the most ventral point on tympanum and the tip of the tongue when visible), and hyoid movements (distance between the point “P” on [Fig. 2](#) and the basis of the posterior ceratobranchial). To compare the kinematic feeding pattern of *M. emys* to those of other studied turtles and to understand the coordination between the elements of the feeding apparatus, these data were used for calculation of the following kinematical variables: duration of Slow open phase (SO); duration of Slow open phases I and II (SOI and SOII) when present; duration of fast open (FO); duration of maximum gape phase (MG); duration of fast close (FC); time to peak gape (TPG); total cycle duration (TCD); duration

of hyoid protraction (HyDD); duration of hyoid retraction (HyVD); duration of the total hyoid cycle (THC); hyoid retraction velocity (HyRV); duration of head protraction (HP); duration of head retraction (HR); duration of tongue protraction (TP); tongue retraction velocity; delay of the start of hyoid retraction relative to the tongue retraction start; delay of reaching peak gape relative to start of the hyoid retraction; delay of reaching peak gape relative to tongue retraction start (see [Table 1](#)).

Statistics

We tested for any differences among the frequency of occurrences of defined patterns both in food uptake (FU) and food transport (T), i.e., sequences with: missing split of the jaw opening in SO and FO; without detectable split of discrete SOI and SOII slow gape phase; lacking MG phase. In order to provide the comparisons, Chi-square test with Yates' correction was performed. Then we tested for possible existence of differentiation in kinematical variables in both feeding stages (FU and T). All variables were tested with the Shapiro–Wilk test for normal distribution. When the p -value was less than the chosen alpha level ($p < 0.05$), the null hypothesis was rejected and data were excluded from further analyses. In addition, all variables included in [Table 1](#) were tested with Levene's and Brown–Forsythe tests and then processed with Welch's ANOVA for heteroscedastic data. Tukey's honest significant difference test (HSD) was performed for post-hoc analyses when applicable.

Furthermore, in order to express the degree of individual differentiation among the studied specimens, a Canonical discriminant analysis (CDA) was performed. Standard descriptive statistics including mean, range, standard deviation ((SD) and confidential interval at 95% CI) were presented.

RESULTS

When feeding on land, the Asian forest tortoises always grasped food by the jaws. After food uptake, one to four transport cycles followed prior to oesophageal packing (see [Schwenk, 2000](#)). The tip of the tongue was barely visible during food uptake (see [Figs. 3B](#) and [3C](#)) indicating that the tongue was not protracted. By contrast, during transport cycles, the cyclic movements of the tongue were well visible as it was rhythmically pro- and retracted to transport the food item towards the oesophagus ([Fig. 5](#)).

When trying to feed under water ([Fig. 4](#) and at <http://figshare.com/s/5d9e23c8f4ec11e49cb306ec4b8d1f61>), *M. emys* submerged its head under the water level and protruded the gaping jaws toward the food item. The gape cycle was newer split in slow and fast jaw open phases. The tongue tip was not visible from the lateral aspect and the hyolingual complex did not protract prior reaching peak gape. No retraction of the hyoid complex was detected prior jaw closure. The gape cycle duration exceeded one and a half seconds and was 1.94 ± 0.36 s (mean \pm SD). Despite the unsuccessful attempts, the turtles repeatedly tried to catch the submerged food. In several events, we were able to detect that the food item was carried away by the bow wave induced by jaw closing.

The variables of the kinematical profiles are summarised in [Table 1](#). In the statistic tests, we found highly significant differences in sequences with and without both SOI and SOII

Table 1 Variables describing the feeding process in *Manouria emys*, present as means \pm SD; *n*, sample size. Abbreviations in Appendix S1.

Variable	Food uptake (FU)				Transport (T)			I vs. T	
	Individual 1 (<i>n</i> = 8)	Individual 2 (<i>n</i> = 8)	Individual 3 (<i>n</i> = 8)	p1	Individual 1 (<i>n</i> = 33)	Individual 2 (<i>n</i> = 20)	Individual 3 (<i>n</i> = 21)	p2	p3
SOI duration (s)	0.168 \pm 0.060 <i>n</i> = 2	0.618 \pm 0.231 <i>n</i> = 3	0.562 <i>n</i> = 1	n.c.	0.146 \pm 0.016 <i>n</i> = 18	0.126 \pm 0.014 <i>n</i> = 14	0.115 \pm 0.015 <i>n</i> = 11	0.378	0.068
SOII duration (s)	0.738 \pm 0.508 <i>n</i> = 2	0.453 \pm 0.294 <i>n</i> = 3	1.024 <i>n</i> = 1	n.c.	0.147 \pm 0.014 <i>n</i> = 18	0.187 \pm 0.027 <i>n</i> = 13	0.190 \pm 0.021 <i>n</i> = 11	0.187	0.072
FO duration (s)	0.450 \pm 0.060 <i>n</i> = 2	0.379 \pm 0.150 <i>n</i> = 3	0.694 <i>n</i> = 1	n.c.	0.122 \pm 0.009 <i>n</i> = 25	0.126 \pm 0.012 <i>n</i> = 15	0.102 \pm 0.006 <i>n</i> = 19	0.111	0.011*
MG duration (s)	0.079 \pm 0.017 <i>n</i> = 4	0.095 \pm 0.031 <i>n</i> = 4	0.166 \pm 0.044 <i>n</i> = 6	0.271	0.025 \pm 0.003 <i>n</i> = 6	0.033 \pm 0.004 <i>n</i> = 10	0.042 \pm 0.019 <i>n</i> = 4	0.318	0.001*
FC duration (s)	0.157 \pm 0.079 <i>n</i> = 8	0.105 \pm 0.036 <i>n</i> = 8	0.158 \pm 0.030 <i>n</i> = 8	0.024*	0.089 \pm 0.020 <i>n</i> = 33	0.186 \pm 0.040 <i>n</i> = 20	0.119 \pm 0.088 <i>n</i> = 21	0.155	0.010*
TPG (s)	0.943 \pm 0.144 <i>n</i> = 8	0.989 \pm 0.177 <i>n</i> = 8	1.784 \pm 0.137 <i>n</i> = 8	0.002*	0.408 \pm 0.021 <i>n</i> = 33	0.439 \pm 0.038 <i>n</i> = 20	0.403 \pm 0.028 <i>n</i> = 21	0.187	<0.001*
TCD duration (s)	1.139 \pm 0.148 <i>n</i> = 8	1.128 \pm 0.169 <i>n</i> = 8	2.073 \pm 0.144 <i>n</i> = 8	0.001*	0.499 \pm 0.020 <i>n</i> = 33	0.655 \pm 0.098 <i>n</i> = 20	0.510 \pm 0.030 <i>n</i> = 21	0.311	<0.001*
HDD duration (s)					0.281 \pm 0.025 <i>n</i> = 30	0.216 \pm 0.035 <i>n</i> = 14	0.169 \pm 0.019 <i>n</i> = 21	0.005*	
HVD duration (s)					0.176 \pm 0.011 <i>n</i> = 31	0.167 \pm 0.014 <i>n</i> = 17	0.149 \pm 0.009 <i>n</i> = 21	0.162	
THC duration (s)					0.456 \pm 0.028 <i>n</i> = 30	0.384 \pm 0.041 <i>n</i> = 14	0.317 \pm 0.022 <i>n</i> = 21	0.002*	
HRV velocity (cm/s)					0.718 \pm 0.059 <i>n</i> = 31	0.938 \pm 0.107 <i>n</i> = 17	0.551 \pm 0.071 <i>n</i> = 21	0.016*	
HP duration (s)	1.345 \pm 0.159 <i>n</i> = 8	1.204 \pm 0.246 <i>n</i> = 8	2.494 \pm 0.177 <i>n</i> = 8	0.001*	0.220 \pm 0.049 <i>n</i> = 32	0.864 \pm 0.132 <i>n</i> = 14	0.464 \pm 0.089 <i>n</i> = 10	0.001*	<0.001*
HR duration (s)	0.296 \pm 0.041 <i>n</i> = 8	0.487 \pm 0.086 <i>n</i> = 7	0.704 \pm 0.174 <i>n</i> = 8	0.052	0.236 \pm 0.027 <i>n</i> = 14	0.211 \pm 0.025 <i>n</i> = 13	0.316 \pm 0.065 <i>n</i> = 10	0.333	0.002*
TP duration (s)					0.165 \pm 0.008 <i>n</i> = 30	0.160 \pm 0.017 <i>n</i> = 19	0.133 \pm 0.025 <i>n</i> = 13	0.483	
TR velocity (cm/s)					7.459 \pm 0.550 <i>n</i> = 31	5.798 \pm 0.547 <i>n</i> = 20	6.562 \pm 0.595 <i>n</i> = 13	0.121	
Delay of HVD start relative to TR start (s)					-0.2011 \pm 0.026 <i>n</i> = 30	-0.039 \pm 0.231 <i>n</i> = 15	-0.082 \pm 0.025 <i>n</i> = 14	0.005*	
Delay of TPG relative to HVD start (s)					-0.007 \pm 0.007 <i>n</i> = 31	-0.016 \pm 0.008 <i>n</i> = 17	-0.032 \pm 0.005 <i>n</i> = 21	0.014*	
Delay of TPG relative to TR start (s)					-0.062 \pm 0.007 <i>n</i> = 31	-0.045 \pm 0.013 <i>n</i> = 20	-0.055 \pm 0.008 <i>n</i> = 14	0.521	

Notes.* Significant differences ($\alpha = 0.05$) among individuals in the ingestion phase (P1), in the transport phase (P2), and between both mode (P3); n.c., *p* value not calculated.

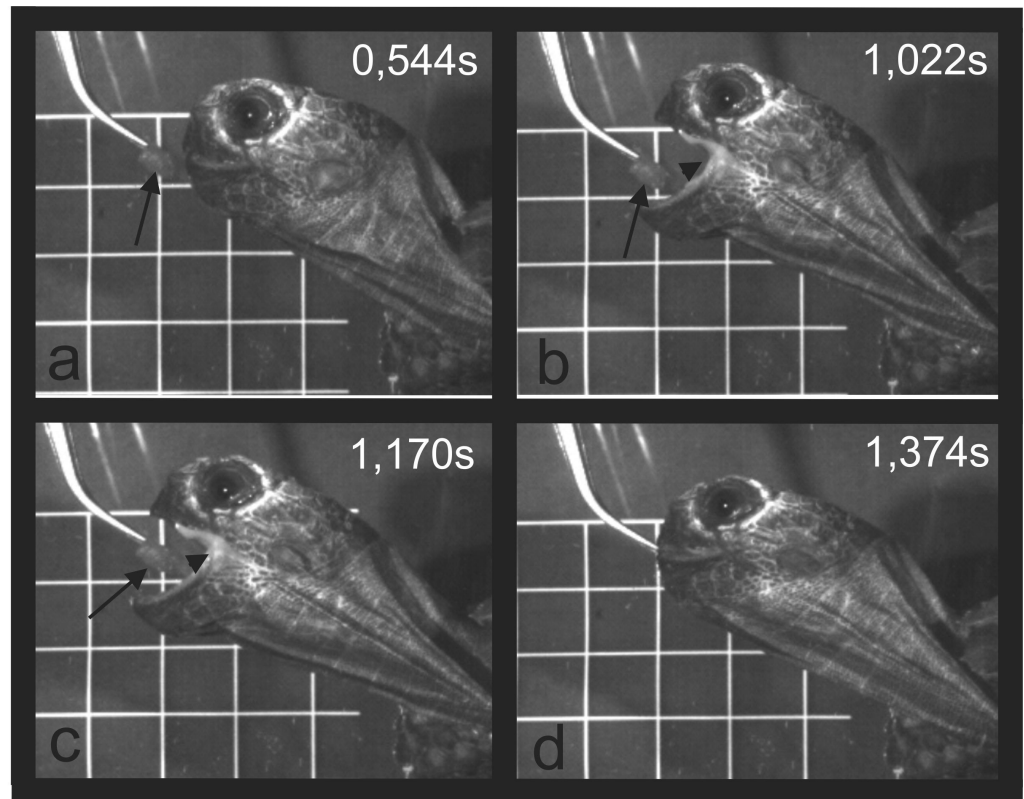


Figure 3 Selected frames and graphics (based on a high-speed video with 500 frs) represent the movements of jaws, hyoid and t head during terrestrial food uptake in *Manouria emys* when feeding on pieces of beef heart. (A) slow open phase end (lacking discrete SOI and SOII); (B) fast open end; (C) fast close start; (D) fast close end; arrows indicate the position of the food item; arrowheads represent the position of the tip of the tongue; grid 10 × 10 mm. Abbreviations in [Appendix S1](#).

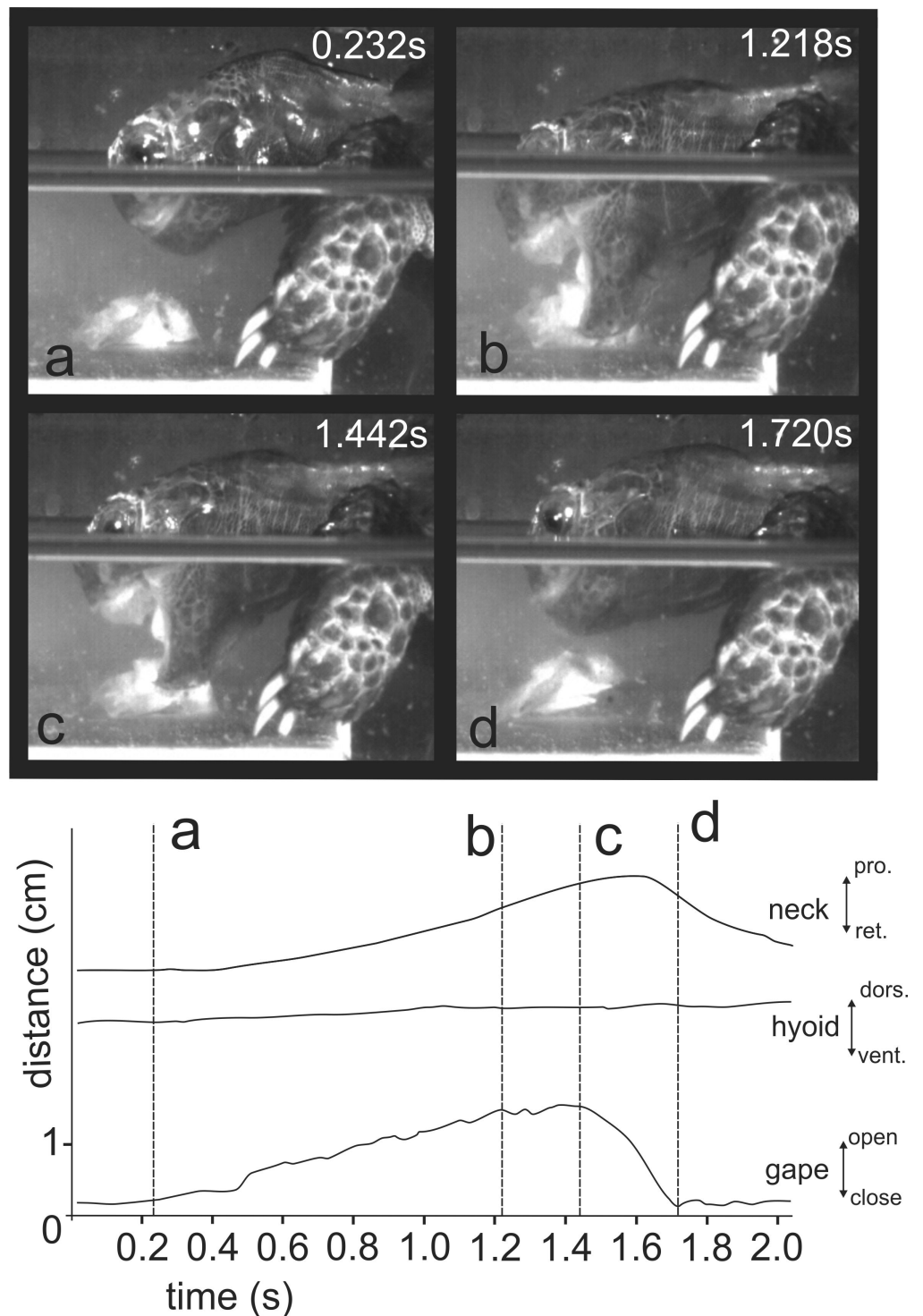


Figure 4 Selected frames and graphics (based on high-speed video with 250 frs) showing the movements of jaws, hyoid, and head during attempts of aquatic food uptake in *Manouria emys*. (A) start of the gape cycle; (B) end of jaw opening; (C) maximum gape end; (D) fast closure end; note the lack of movement of the hyoid complex during the whole cycle; grid 10 × 10 mm. Abbreviations in Appendix S1.

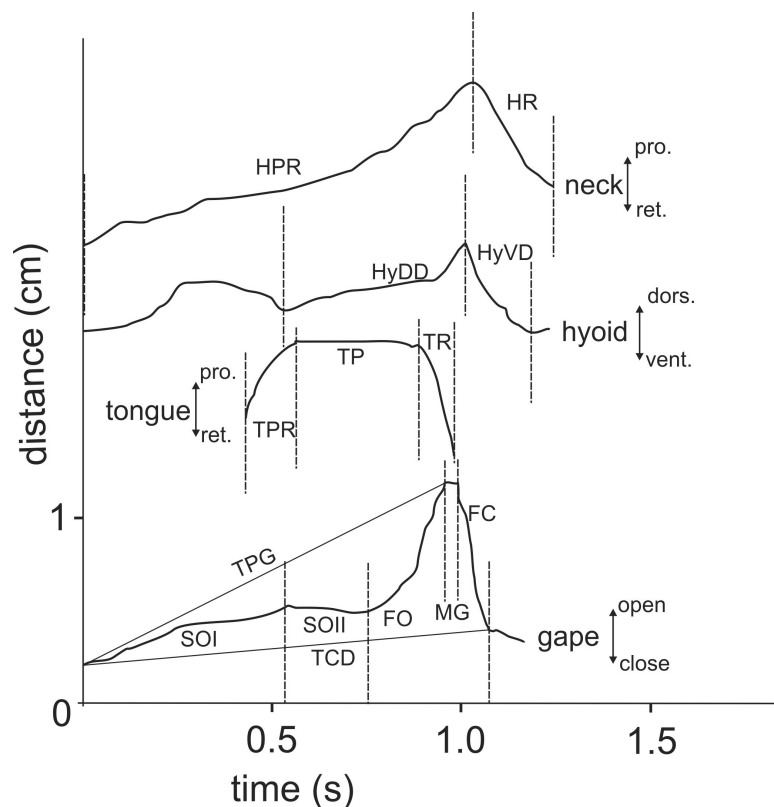


Figure 5 Selected graphics (based on a high-speed video with 500 fr/s) showing the movement patterns of jaws, hyoid, tongue and head during terrestrial food transport in *M. emys*; note the delay in hyoid ventral displacement relative to the start the retraction of the tongue tip, as well as the delay of both the tongue retraction and hyoid retraction relative to the start of the FO phase. Abbreviations in Appendix S1.

when food uptake and transport stages were compared ($\chi^2_{(1, N=98)} = 25.05, p < 0.001$). Similarly significant differences were observed when comparing food uptake and transport cycles in respect to sequences with and without slow jaw open phases as well as with and without maintaining jaw maximum gape—MG phase ($\chi^2_{(1, N=98)} = 6.10, p = 0.02$; $\chi^2_{(1, N=98)} = 6.52, p = 0.01$).

Nine of the variables which describe the food uptake process were detected to show significant differences between individuals (Table 1). In transport cycles, six out of 18 variables differed significantly amongst individuals (see Table 1). Seven out of nine variables differed significantly when testing for differences between grasping and transport cycles: fast jaw open duration (FO; $F_{\text{Welch}(1,43)} = 15.17, p = 0.011$); maximum gape (MG; $F_{\text{Welch}(1,26)} = 15.89, p = 0.001$); fast closing (FC; $F_{\text{Welch}(1,26)} = 7.86, p = 0.010$); time to peak gape (TPG; $F_{\text{Welch}(1,72)} = 46.78, p < 0.001$); total gape cycle duration (TCD; $F_{\text{Welch}(1,72)} = 52.50, p < 0.001$); head protraction duration (HP; $F_{\text{Welch}(1,67)} = 52.23, p < 0.001$); and head retraction duration (HR; $F_{\text{Welch}(1,47)} = 12.57, p = 0.002$).

When comparing three further parameters among the transport cycles in all three specimens (delay of HyVD start relative to TR start; delays of TPG relative to HyVD; delay

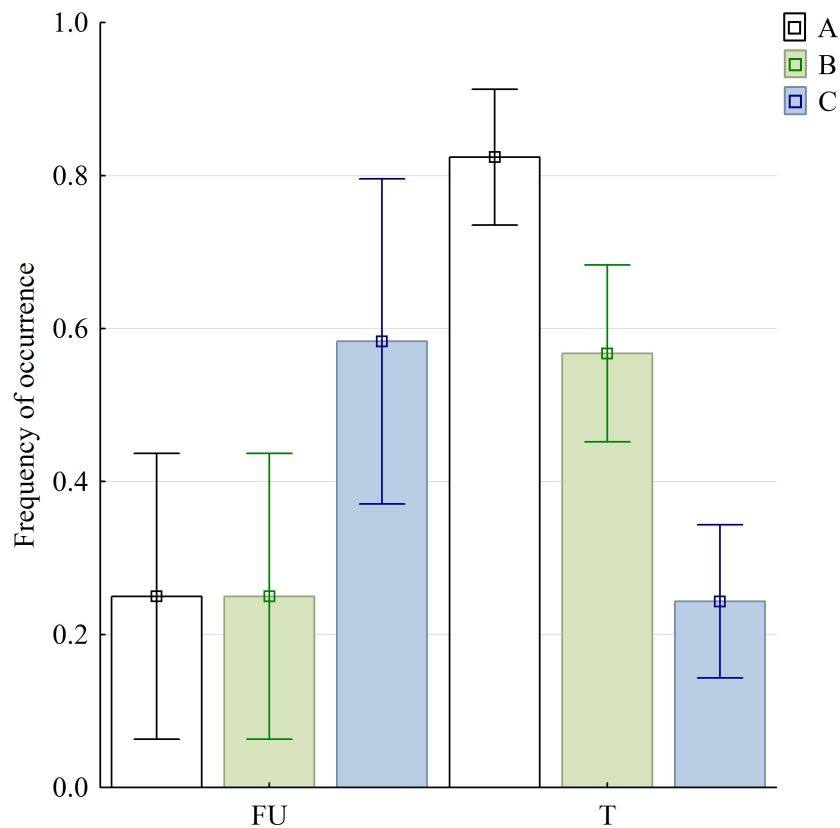


Figure 6 Graphical representation of three selected variables in food uptake (FU) and food transport (T) phases. Bars are denoted by their mean values and whiskers present the 95% CI; (A) sequences with discrete SO and FO phases; (B) sequences with no detectable split of discrete slow gape phase (SOI and SOII were inseparable); (C) sequences with lacking MG phase. Abbreviations in [Appendix S1](#).

of TPG relative to TR starts), statistically significant differences were found among all compared pairs ($F_{\text{Welch}(2,105)} = 41.58, p < 0.001$).

The performed canonical discriminant analysis (CDA) revealed the existence of substantial degree of individualism among the studied specimens ([Fig. 7](#)). However, only the first axis eigenvalue exceeded the level of acceptance, i.e., 1. First axis explained 70% of the total variance. Among the 18 studied variables only two (THC and HPR) showed higher correlation scores than 0.75. On the base of the CDA and the detected degree of individualism mentioned above, we can conclude that the patterns displayed by the studied specimens can be regarded as similar but not as uniform.

DISCUSSION

The Asian forest tortoise repetitively tried to feed on dispersed food items under water, which was an unexpected and hitherto unknown behaviour among tortoises. However, *M. emys* always failed to consume the submerged food. On land, *M. emys* grasped food with the jaws, just like all known aquatic or semiterrestrial turtles do, but not with the

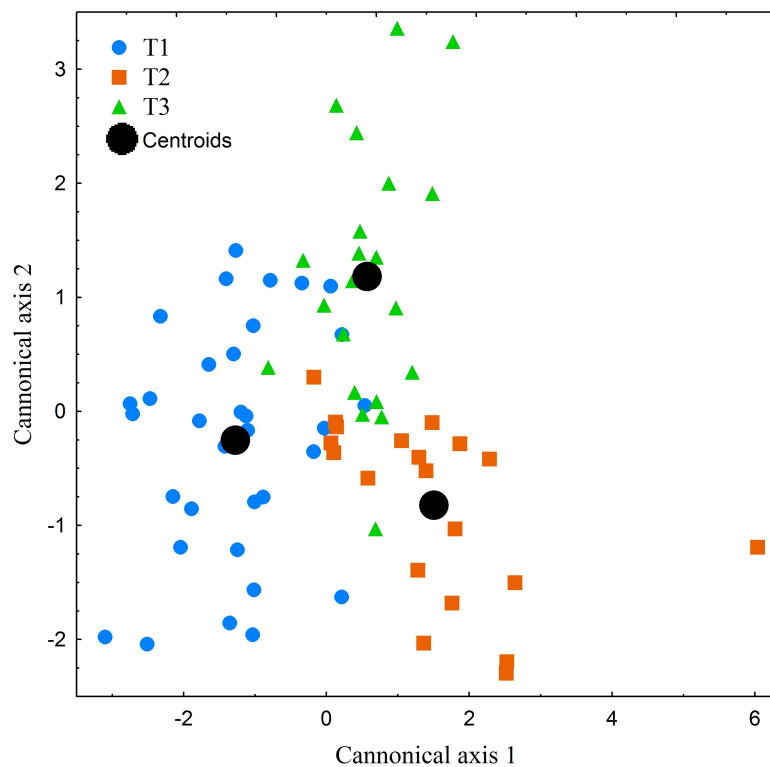


Figure 7 Canonical centroid plots of three *Manouria emys* specimens (T1–T3), centroid scores for each individual and measurement repetition in food transport phase.

tongue as formerly predicted for all tortoises. On the basis of our results we discuss several important evolutionary, behavioural, and functional aspects.

Evolution of food uptake among turtles

In general, most aquatic turtles combine a fast acceleration of the head towards the food or prey item and a suction feeding mechanism is induced by fast oropharyngeal volume expansion. In some extant turtles, a strong suction flow can be generated and prey is directly sucked into the oropharynx without contact with the jaws (e.g., *Chelus fimbriatus* (Lemell et al., 2002), *Apalone spinifera* (Anderson, 2009), *Pelodiscus sinensis* (N Natchev & I Werneburg, 2013, unpublished data)). However, most extant turtles cannot generate such strong suction flows and only compensate (“gulp”) the bow wave that otherwise would push small to moderately sized food items away from the fast approaching head. These species finally fix and grasp prey with the jaws (see Lauder & Prendergast, 1992; Lemell, Beisser & Weisgram, 2000; Aerts, Van Damme & Herrel, 2001; Natchev et al., 2009; Natchev et al., 2011). We consider the latter plesiomorphic behaviour for extant turtles.

Among extant turtles, the ability to complete the whole feeding process (including food uptake, food manipulation and transport, esophageal packing, and swallowing) on land has been tested and documented for only six species so far. All of them were members of Testudinoidea (Fig. 1; see also Summers et al., 1998; Bels et al., 2008; Natchev et al., 2009). The terrestrial mode of food uptake differs dramatically among and within

the three testudinoid subgroups (see *Bels, Davenport & Delheusy, 1997; Bels et al., 2008; Summers et al., 1998; Wochesländer, Hilgers & Weisgram, 1999; Natchev et al., 2009*, present study). Correspondingly, it appears as if terrestrial feeding re-evolved several times independently amongst turtles. Unfortunately, only limited experimental data are available on feeding mechanisms in emydids and geoemydids. Further functional and palaeontological investigations may help to sort out the issues on the evolution of the feeding behaviour and the morphology of the feeding apparatus in testudinoids.

Very limited information is available on feeding mechanisms employed by amphibious non-testudinoid turtles that occasionally exploit terrestrial food sources. *Weisgram (1985a)* and *Weisgram (1985b)* documented a kinosternid (*Claudius angustatus*) that caught prey on land and dragged it into water for transport and swallowing. *Natchev et al. (2008)* documented another kinosternid (*Sternotherus odoratus*) catching food on land, but failing to transport it through the oropharynx. Among extant turtles, successful food transport on land seems to be restricted to testudinoids. The development of enlarged and muscular tongues within this group (*Von Bayern, 1884; Werneburg, 2011*) represents adaptation to improved terrestrial food manipulation.

Based on experimental data, *Natchev et al. (2009)* described and summarised three categories of terrestrial food uptake modes among Testudinoidae: (A) Jaw prehension with retracted hyolingual complex, as observed in the geoemydid genus *Cuora* (*Natchev et al., 2009*); (B) Jaw prehension with slightly protracted hyolingual complex, as observed in emydids (*Bels, Davenport & Delheusy, 1997; Stayton, 2011*); (C) Lingual prehension—the tongue touches the food item prior to food uptake, as documented in all tortoises studied so far (*Wochesländer, Hilgers & Weisgram, 1999; Bels et al., 2008*). The food uptake mode of *M. emys*, however, differs substantially from that of all remaining tortoises (category C). In fact, the hyolingual complex in *M. emys* remained fully retracted during the food prehension on land, and the first contact with the food item was by the jaws. Accordingly, the feeding mechanism of *M. emys* should be assigned to category A, along with that of semi-aquatic geoemydids.

We now aim to construct a theoretical scenario on the evolution of terrestrial feeding mechanisms in turtles. Given the aquatic origin of all living turtles, the functional transition from aquatic to terrestrial feeding mechanisms could hypothetically have involved four stages, beginning with an exclusively aquatic feeding ancestor. In different lineages and stages, turtles may have left their aquatic environments for various reasons e.g., for exploiting new food niches. The species that retained predominantly aquatic life styles may grasp food by the jaws on land, but have to drag it into the water for further intraoral (hydrodynamic based) transport. In recent turtles, such behaviour was documented in the kinosternids *C. angustatus* (*Weisgram, 1985a; Weisgram, 1985b*) and *S. odoratus* (*Natchev et al., 2011*), as well as in the emydid *Trachemys scripta* (*Weisgram, 1985b; Weisgram, Dittrich & Splechtina, 1989*) and other emydids (see *Stayton, 2011*). Turtles of the second hypothetical evolutionary stage grasped food by the jaws, while the tongue was used for intraoral food transport on land. Such species would still have retained their underwater feeding ability by using hydrodynamic mechanisms. When

grasping food on land, the tongue remained retracted or was protracted without touching the food item. Among extant turtles, such a feeding mode is found in the geoemydid genus *Cuora* (Heiss, Plenk & Weisgram, 2008; Natchev et al., 2009; Natchev et al., 2010) and in some emydids (Bels, Davenport & Delheusy, 1997; Summers et al., 1998; Stayton, 2011). In the next theoretical evolutionary step (stage three), behavioural and morphological adaptations for terrestrial feeding were further advanced, increasing the efficiency of terrestrial food transport at the expense of the ability to use effective hydrodynamic mechanisms in water. Such species still grasped food items with their jaws on land (as typical for aquatic or semiaquatic turtles), but were no longer able to take up dispersed food if submerged, which features prominently in the present case of *M. emys*. Finally, in a fourth stage, turtles became fully terrestrial and their tongue was obligatorily involved in food uptake as documented in the tortoises *Testudo* (*Eurotestudo*) *hermanni boettgeri* (Weisgram, 1985b; Wochesländer, Hilgers & Weisgram, 1999), *Kinixys belliana*, *Geochelone elephantopus* and *G. radiata* (Bels et al., 2008).

Our investigations demonstrate that the ‘basal’ tortoise *M. emys* does not contact food with the tongue prior to jaw prehension on land. This shows that tongue to food contact is characteristic of advanced tortoises only. We consider the terrestrial feeding behaviour of *M. emys* as plesiomorphic and potentially inherited from its semiaquatic ancestors. On that basis, *M. emys* can be considered a transitional turtle in regard to secondary terrestriality.

We propose that hyolingual protrusion evolved in the lineage forming to advanced tortoises (Fig. 1). *Manouria emys* has a large tongue with massive intrinsic and extrinsic musculature (see Heiss et al., 2011). The advanced and complex lingual musculo-skeletal architecture allows the turtle to protrude the tongue outside the margins of the rhamphothecae (see Fig. 2). However, *M. emys* does not use lingual food prehension as typical for all other tortoises studied so far. In fact, it seems that the *Manouria* (and perhaps *Gopherus* (N Natchev, pers. obs., 2015)) “lineage” has retained the jaw prehension mechanism inherited from earlier aquatic ancestors. It seems that the tortoises, in general, evolved fleshy tongues which improve the food transport performance. The advanced tortoises only refined the behaviour of food uptake on land via lingual food contact prior to jaw closure (see Wochesländer, Hilgers & Weisgram, 1999; Bels et al., 2008).

Function of the protruded tongue in the testudinid’s food uptake

What would be the potential advantage of the obligatory lingual protrusion, found in the more derived tortoises? One possible explanation is that the tongue is used as a prehensile organ for food ingestion analogous to that found in other tetrapod groups (for overview see Schwenk, 2000; Schwenk & Wagner, 2001). However, for tortoises such interpretation might be put into question. By examining all published data available (Wochesländer, Hilgers & Weisgram, 1999; Bels et al., 2008), we were not able to find any convincing evidence that tortoises collect food with their tongues—they just touch it. In all published feeding sequences, the contact between the food and the tongue is clearly demonstrated—yet, in all cases, after the initial contact of the tongue with the food, the head moves forward and the food item is not dragged into the mouth by tongue retraction,

but is grasped by the jaws during the fast jaw closing (FC gape phase). Initial food ingestion in tortoises might not be considered “lingual prehension” (see [Schwenk, 2000](#); [Bels et al., 2008](#)) in the strict sense, but should be regarded as “jaw prehension following lingual contact”. This prompts the question: why is an obligatory contact of the tongue to the food present in extant tortoises (except in *Manouria* and also conceivably in *Gopherus*) during food uptake? In other words: why do tortoises apply a more complex and presumably more energetically expensive food uptake mechanism by including movements of the hyolingual complex in addition to the movements of the neck and jaws alone?

We propose that the lingual contact provides tactile information on the position of the food item and helps the advanced tortoises to compensate the “information gap” which occurs when the food is approached to a distance where it is out of sight. The eyes of tortoises are positioned laterally on the head ([Pritchard, 1979](#)) and the turtles are not able to permanently observe the position of the food item when the neck is protracted and the gape is positioned around it. The prolonged maximum gape (MG) phase found in most ingestion cycles of *M. emys* (see [Table 1](#), [Figs. 3](#) and [6](#)) might be the result of lack of lingual contact with the food surface. In all published sequences and kinematical profiles on food uptake in tortoises, there is a clear tendency toward a split of the gape cycle into slow open (SO) and fast open (FO) gape phases (see [Wochesländer, Hilgers & Weisgram, 1999](#); [Bels et al., 2008](#)). The lack of tongue protrusion might explain the lack of slow open (SO) and fast open (FO) split in the gape cycle of food uptake in the geoemydid *Cuora* (see [Natchev et al., 2009](#)). In most food uptakes analysed in *M. emys*, SO phases are not present and the gape increases gradually (see [Table 1](#) and [Fig. 6](#)). Similar as in *Cuora*, the lack of SO phases might be explained by the lack of tongue protrusion in food uptake (see [Natchev et al., 2009](#)).

Intraoral food transport on land

The execution of the transport cycles require coordination of the activities of contractile elements such as the jaw opening and closing muscles, head protracting and retracting muscles, intrinsic and extrinsic lingual muscles, as well as muscles that protract and retract the hyolingual complex as a whole unit ([Jones et al., 2012](#); [Werneburg, 2011](#); [Werneburg, 2013](#)). In contrast to this complicated choreography, the mode of food prehension in *M. emys* suggests less complex neuromotoric coordination between neck and jaw movements. Yet, the execution of the transport cycles is often more than twice shorter in duration (see [Table 1](#)). A possible explanation for the longer duration of food uptake cycles relative to transport cycles might be that during transport, the coordination centres of the muscle activities execution are permanently supplied with information concerning the position of the food item within the oropharynx and the proper movements can be executed precisely in a shorter time.

In his work on the feeding mechanisms in domestic animals, [Bels \(2006\)](#) established that a pre-programmed Generalised Cyclic Model (GCM)—very similar to those proposed from [Bramble & Wake \(1985\)](#)—is universally valid among the different groups of tetrapods. The feeding kinematics of the Asian forest tortoise differs in some aspects from those proposed by the GCM. The kinematics of the feeding system in *M. emys* seems to be

pre-programmed, but under permanent feedback control. The values of the gape and hyoid/hyolingual cycle patterns in the three specimens studied here show high degrees of variation, both concerning food uptake and food transport (see [Table 1](#) and [Figs. 5–7](#)). The slow open phases (SO) are not obligatory. The gape cycle often includes a phase of retaining maximum gape (see [Table 1](#), [Figs. 3](#) and [5](#)). In turtles, the maximum gape phase (MG) was described for the gape cycle in *Cuora* sp. ([Natchev et al., 2009](#); [Natchev et al., 2010](#)) and was confirmed for kinosternidae ([Natchev et al., 2011](#)). The presence of a prolonged maximum gape phase (MG) can be easily overlooked when the frame rate of the film sequence is not high enough (i.e., step between successive frames over 10 ms). Thus, it may be present in other turtles, but was not taken into account by the calculations of the kinematical profile (see [Stayton, 2011](#); [Nishizawa et al., 2014](#)).

The GCM presupposes that the start of hyoid retraction coincides with the start of fast open phase (FO). However, our calculations (see [Table 1](#) and [Fig. 5](#)) demonstrate that in *M. emys* the hyoid retraction in the food transport cycle starts shortly prior reaching peak gape. The same pattern was detected by the investigation of aquatic, semi-aquatic, but also predominantly terrestrial cryptodirans ([Natchev et al., 2008](#); [Natchev et al., 2009](#); [Natchev et al., 2010](#); [Natchev et al., 2011](#)).

Relations between the habitat preferences and the diet in turtles

In the evolution of the testudinids there is a clear shift not only in the habitat preferences (from aquatic to terrestrial), but corresponding shifts are also seen in dietary preferences. In that taxon it manifests in a tendency toward herbivory. Most of the recent tortoises rely on diets mainly composed of plant material (for overview see [Pritchard, 1979](#); [Ernst, Altenburg & Barbour, 2000](#); [Bonin, Devaux & Dupre, 2006](#)). The partly carnivorous lifestyle of *Manouria* sp. ([Bonin, Devaux & Dupre, 2006](#)) may be a relic of the carnivorous diet of the ancestor of the tortoises and supports the transitional status of the genus.

Apparently the feeding media (air vs. water) and the dietary shift had a large influence on the overall feeding behaviour of the testudinids (see [Bels et al., 2008](#)). The suction mechanism was lost and replaced by a jaw food prehension system (this study) or by “lingual prehension” ([Wochesländer, Hilgers & Weisgram, 1999](#); [Bels et al., 2008](#)). Probably, the switch to herbivory determined the reorganisations in the morphology of the jaw muscle system and the proportions of the skull in tortoises (see [Werneburg, 2011](#); [Werneburg, 2012](#); [Werneburg, 2013](#)). By feeding predominantly on plants (immobile items), the tortoises may prolong the duration of the food uptake cycles and have more time to adjust their prehension kinematics to every single feeding situation.

We propose that the habitat preferences and the diet change in turtles are firmly correlated (e.g., [Bels et al., 2008](#); [Werneburg, 2014](#)). The overall rigid design of the turtle ‘body plan’ hinder the animals to actively hunt for agile prey in terrestrial environments ([King, 1996](#)). We hypothesise that this statement is also valid for the terrestrial stem turtles and that these animals were predominantly herbivorous (sensu [King, 1996](#)). In aquatic turtles, in contrast, the buoyancy of the water overrides the shell-caused restrictions of mobility and also suction feeding can be applied for carnivorous feeding. Those advantages

might have been the key factor for the turtles to become aquatic (and carnivorous) in the Jurassic. There may be several reasons for the secondary terrestriality in tortoises and presumably one of the main reasons was the inter- and intraspecific concurrence for food resources.

CONCLUSIONS

We propose that the ancestral food uptake mode in tortoises was jaw based when feeding on land. During the shift from aquatic to terrestrial lifestyle, including a shift from aquatic to terrestrial feeding biology, the *Manouria* (and most likely also *Gopherus*) “lineage” had retained pure jaw prehension in food uptake. The hyolingual complex in that lineage exhibits the typical morphological features of tortoises that feed exclusively on land (see [Bramble, 1973](#); [Winokur, 1988](#); [Heiss et al., 2011](#)), such as an enlarged fleshy tongue with abundant papillae, a complex tongue musculature, a relatively small and mainly cartilaginous hyoid and hypoglossum. The evolutionary shift in the morphology of the hyolingual complex was apparently primed by the optimisation of the food transport behaviour and not for food uptake. We suggest that the involvement of the tongue during food uptake found in the derived extant tortoises serves as a tactile sensory tool for the localisation of the food item prior to jaw prehension. Thus, the tongue is not used as the main food collecting organ in modern tortoises and the food uptake mode represents a derived jaw prehension system.

ACKNOWLEDGEMENTS

We would like to thank Josef Weisgram, Andreas Wanninger, Patrick Lemell, Christian Beisser and Thomas Schwaha (Department for Integrative Zoology, University of Vienna) for providing material and suggestions for the executions of our experiments. Alexander Westerström contributed sorely to the revision of our manuscript. Stefan Kummer, Katherina Singer, Monika Lintner and Marion Hüffel are acknowledged for the careful housing of the animals. The reviewers provided helpful comments and suggestions to improve our paper.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The study was funded by FWF Austrian Science Fund, Project no. P20094-B17 on that EH and NN were employed and the Advanced Postdoc Mobility fund P300P3_158526 of the Swiss National Science Foundation granted to IW. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

FWF Austrian Science Fund: P20094-B17.

Swiss National Science Foundation: P300P3 158526.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Nikolay Natchev conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Nikolay Tzankov and Ingmar Werneburg analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Egon Heiss conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The animals were commercially obtained. As no invasive techniques were applied, no approval documents were required by the Austrian Protection of Animals Act at the time of the experiments.

Data Availability

The following information was supplied regarding the deposition of related data:

Figshare: figshare.com/s/5d9e23c8f4ec11e49cb306ec4b8d1f61 & figshare.com/s/1920fc6af4ed11e4972106ec4bbcf141.

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.1172#supplemental-information>.

REFERENCES

- Aerts P, Van Damme J, Herrel A. 2001. Intrinsic mechanics and control of fast cranio-cervical movements in aquatic feeding turtles. *American Zoologist* 41:1299–1310 DOI 10.1668/0003-1569(2001)041[1299:IMACOF]2.0.CO;2.
- Anderson NJ. 2009. Biomechanics of feeding and neck motion in the softshell turtle. In: *Apalone spinifera*. Rafinesque: Idaho State University. p. 89.
- Barley AJ, Spinks RC, Thomson HB, Shaffer. 2010. Fourteen nuclear genes provide phylogenetic resolution for difficult nodes in the turtle tree of life. *Molecular Phylogenetics and Evolution* 55:1189–1194 DOI 10.1016/j.ympev.2009.11.005.
- Bels VL. 2006. *Feeding in domestic vertebrates—from structure to behaviour*. Oxfordshire: CABI Publishing. p. 384.
- Bels VL, Baussart S, Davenport J, Shorten M, O’Riordan RM, Renous S, Davenport J. 2008. Functional evolution of feeding behaviour in turtles. In: Wyneken J, Godfrey MH, Bels V, eds. *Biology of turtles*. Boca Raton: CRC Press Taylor & Francis Group, 189–212.

- Bels VL, Davenport J, Delheusy V. 1997.** Kinematic analysis of the feeding behaviour in the box turtle *Terrapene carolina* (L.), (Reptilia: Emydidae). *Journal of Experimental Zoology* 277:198–212 DOI 10.1002/(SICI)1097-010X(19970215)277:3<198::AID-JEZ2>3.0.CO;2-J.
- Bonin F, Devaux B, Dupre A. 2006.** *Turtles of the world*. Baltimore: Johns Hopkins University Press. Translated by Pritchard PCH.
- Bramble DM. 1973.** Media dependent feeding in turtles [Abstract 1342]. *American Zoologist* 13.
- Bramble DM, Wake DB. 1985.** Feeding Mechanisms of Lower Tetrapods. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, eds. *Functional vertebrate morphology*, vol. 13. Massachusetts and London: Harvard University Press Cambridge, 230–261.
- Crawford NG, Parham JF, Sellas AB, Faircloth BC, Glenn TC, Papenfuss TJ, Henderson JB, Hansen MH, Simison WB. 2015.** A phylogenomic analysis of turtles. *Molecular Phylogenetics and Evolution* 83:250–257 DOI 10.1016/j.ympev.2014.10.021.
- Danilov I. 1999.** A new lindholmemydid genus (Testudines: Lindholmemydidae) from the mid-Cretaceous of Uzbekistan. *Russian Journal of Herpetology* 6(1):63–71.
- Danilov I, Parham JF. 2006.** A redescription of ‘Plesiochelys’ tatsuensis from the Late Jurassic of China, with comments on the antiquity of the crown clade Cryptodira. *Journal of Vertebrate Paleontology* 26(3):573–580 DOI 10.1671/0272-4634(2006)26[573:AROPTF]2.0.CO;2.
- Das I. 1995.** *Turtles and tortoise of India*. Bombay: Oxford University Press. p. 179.
- Depeker M, Berge C, Penin X, Renous S. 2006.** Geometric morphometrics of the shoulder girdle in extant turtles (Chelonii). *Journal of Anatomy* 208:35–45 DOI 10.1111/j.1469-7580.2006.00512.x.
- Ernst CH, Altenburg RGM, Barbour RW. 2000.** *Turtles of the World. World Biodiversity Database, CD-ROM Series, Windows, Version 1.2*. Amsterdam: Biodiversity Center of ETI.
- Ernst CH, Barbour RW. 1989.** *Turtles of the world*. Washington, D.C.: Smithsonian Institution Press.
- Fritz U, Havaš P. 2007.** Checklist of chelonians of the world. *Vertebrate Zoology* 57(2):149–368.
- Gerlach J. 2001.** Tortoise phylogeny and the ‘Geochelone’ problem. *Phelsuma* 9(suppl. A):1–24.
- Heiss E, Natchev N, Schwaha T, Salaberger D, Lemell P, Beisser C, Weisgram J. 2011.** Oropharyngeal morphology in the basal tortoise *Manouria emys emys* with comments on form and function of the testudinid tongue. *Journal of Morphology* 272:1217–1229 DOI 10.1002/jmor.10978.
- Heiss E, Plenk H, Weisgram J. 2008.** Microanatomy of the Palatal Mucosa of the semiaquatic Malayan Box Turtle, *Cuora amboinensis*, and functional implications. *Anatomical Records* 291(7):876–885 DOI 10.1002/ar.20695.
- Høybye-Mortensen K. 2004.** The tortoise *Manouria emys emys*: behaviour and habitat in the wild. M. Sc. Thesis, University of South Denmark, p. 100.
- Iverson J, Brown BRM, Akre TS, Near TJ, Le M, Thomson RC, Starkey DE. 2007.** In search of the tree of life for turtles. *Chelonian Research Monographs* 4:85–106.
- Jones MEH, Werneburg I, Curtis N, Penrose R, O’Higgins P, Fagan MJ, Evans SE. 2012.** The head and neck anatomy of sea turtles (Cryptodira: Chelonioidae) and skull shape in Testudines. *PLoS ONE* 7(11):e47852 DOI 10.1371/journal.pone.0047852.
- Joyce WG. 2007.** Phylogenetic relationships of Mesozoic turtles. *Bulletin of the Peabody Museum for Natural History* 48(1):3–102 DOI 10.3374/0079-032X(2007)48[3:PROMT]2.0.CO;2.
- Joyce WG. 2015.** The origin of turtles: a paleontological perspective. *Journal of Experimental Zoology, Part B, Molecular and Developmental Evolution* 324(3):181–193 DOI 10.1002/jez.b.22609.

- Joyce WG, Gauthier JA. 2004. Palaeoecology of Triassic stem turtles sheds new light on turtle origins. *Proceedings of the Royal Society London Series B—Biological Sciences* 271:1–5 DOI 10.1098/rspb.2003.2523.
- King G. 1996. *Reptiles and herbivory*. London: Chapman & Hall.
- Lambert FR, Howes JR. 1994. Ranging, breeding behaviour and food of Asian brown tortoise *Manouria emys* in Borneo. *Malayan Nature Journal* 48:125–131.
- Lauder GV, Prendergast T. 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. *Journal of Experimental Biology* 164:55–78 DOI 10.1016/0022-0981(92)90136-X.
- Lemell P, Beisser CJ, Weisgram J. 2000. Morphology and function of the feeding apparatus of *Pelusios castaneus* (Chelonia; Pleurodira). *Journal of Morphology* 244:127–135 DOI 10.1002/(SICI)1097-4687(200005)244:2<127::AID-JMOR3>3.0.CO;2-U.
- Lemell P, Lemell C, Snelderwaard P, Gumpenberger M, Wochesländer R, Weisgram J. 2002. Feeding patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). *Journal of Experimental Biology* 205:1495–1506.
- Lourenço JM, Claude J, Galtier N, Chiari Y. 2012. Dating cryptodiran nodes: origin and diversification of the turtle superfamily Testudinoidea. *Molecular Phylogeny and Evolution* 62:496–507 DOI 10.1016/j.ympev.2011.10.022.
- Nakajima Y, Hirayama R, Endo H. 2014. Turtle humeral microanatomy and its relationship to lifestyle. *Biological Journal of the Linnean Society* 112:719–734 DOI 10.1111/bij.12336.
- Natchev N, Heiss E, Lemell P, Stratev D, Weisgram J. 2009. Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. *Zoology* 112:113–127 DOI 10.1016/j.zool.2008.05.002.
- Natchev N, Heiss E, Lemell P, Weisgram J. 2008. Kinematic analysis of prey capture, prey transport and swallowing in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae) [Abstract 95]. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 150 DOI 10.1016/j.cbpa.2008.04.185.
- Natchev N, Heiss E, Singer K, Kummer S, Salaberger D, Weisgram J. 2011. Structure and function of the feeding apparatus in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). *Contributions to Zoology* 80:143–156.
- Natchev N, Lemell P, Heiss E, Beisser C, Weisgram J. 2010. Aquatic feeding in a terrestrial turtle: a functional-morphological study of the feeding apparatus in the Indochinese box turtle *Cuora galbinifrons* (Testudines, Geoemydidae). *Zoomorphology* 129:111–119 DOI 10.1007/s00435-010-0104-x.
- Nishizawa H, Tabata R, Hori T, Mitamura H, Arai N. 2014. Feeding kinematics of freshwater turtles: what advantage do invasive species possess? *Zoology* 117:315–318 DOI 10.1016/j.zool.2014.04.005.
- Nutphand W. 1979. *The turtles of Thailand*. Bangkok: Siam Farm Zoology Garden. p. 222.
- Parham JF, Feldman CR, Boore JL. 2006. The complete mitochondrial genome of the enigmatic bigheaded turtle (Platysternon): description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitochondrial and nuclear DNA. *BMC Evolutionary Biology* 6:1–11 DOI 10.1186/1471-2148-6-11.
- Pritchard PCH. 1979. *Encyclopaedia of turtles*. Neptune: TFH Publications, Inc.
- Rasmussen ARJ, Murphy C, Ompi M, Gibbons JW, Uetz P. 2011. Marine reptiles. *PLoS ONE* 6(11):e27373 DOI 10.1371/journal.pone.0027373.

- Schumacher GH. 1973.** The head muscles and hyolaryngeal skeleton of turtles and crocodilians. In: Gans C, Parsons TS, eds. *Biology of Reptilia*. New York: Academic Press, 101–199.
- Schwenk K. 2000.** *Feeding: form, function and evolution in tetrapod vertebrates*. San Diego-San Francisco-New York-Boston-London-Sydney-Tokyo: Academic Press.
- Schwenk K, Wagner G. 2001.** Function and the evolution of phenotypic stability: connecting pattern to process. *American Zoologist* **41**:552–563
DOI [10.1668/0003-1569\(2001\)041\[0552:FATEOP\]2.0.CO;2](https://doi.org/10.1668/0003-1569(2001)041[0552:FATEOP]2.0.CO;2).
- Shaffer HB. 2009.** Turtles (Testudines). In: Hedges SB, Kumar S, eds. *The time tree of life*. New York: Oxford University Press, 398–401.
- Stanford CB, Wanchai P, Schaffer C, Schaffer R, Thirakhupt K. 2015.** *Manouria emys* (Schlegel and Müller 1840)—Asian Giant Tortoise, Giant Asian Forest Tortoise. In: Rhodin AGJ, Pritchard PCH, Van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB, Mittermeier RA, eds. *Conservation biology of freshwater turtles and tortoises: a compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group, Chelonian research monographs*, vol. 5(8). 086
DOI [10.3854/crm.5.086.emys.v1.2015](https://doi.org/10.3854/crm.5.086.emys.v1.2015).
- Stayton CT. 2011.** Terrestrial feeding in aquatic turtles: environment-dependent feeding behavior, modulation and the evolution of terrestrial feeding in Emydidae. *Journal of Experimental Biology* **214**:4083–4091
DOI [10.1242/jeb.060574](https://doi.org/10.1242/jeb.060574).
- Sterli J. 2010.** Phylogenetic relationships among extinct and extant turtles: the position of Pleurodira and the effects of the fossils on rooting crown-group turtles. *Contributions to Zoology* **79**:93–106.
- Sterli J, de la Fuente MS. 2011.** Re-description and evolutionary remarks on the Patagonian horned turtle *Niolamia argentina* Ameghino, 1899 (Testudinata, Meiolaniidae). *Journal of Vertebrate Paleontology* **31**(6):1210–1229
DOI [10.1080/02724634.2011.615685](https://doi.org/10.1080/02724634.2011.615685).
- Sukhanov VB. 2000.** Mesozoic turtles of middle and Central Asia. In: Benton MJ, Shishkin MA, Unwin DM, Kurochkin EN, eds. *The age of dinosaurs in Russia and Mongolia*. Cambridge: University Press, 309–367.
- Summers AP, Darouian KF, Richmond AM, Brainerd EL. 1998.** Kinematics of aquatic and terrestrial prey capture in *Terrapene carolina*, with implications for the evolution of feeding in Cryptodire Turtles. *Journal of Experimental Zoology* **281**:280–287
DOI [10.1002/\(SICI\)1097-010X\(19980701\)281:4<280::AID-JEZ4>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-010X(19980701)281:4<280::AID-JEZ4>3.0.CO;2-K).
- Thomson RC, Shaffer HB. 2010.** Sparse supermatrices for phylogenetic inference: taxonomy, alignment, rogue taxa, and the phylogeny of living turtles. *Systematic Biology* **59**(1):42–58
DOI [10.1093/sysbio/syp075](https://doi.org/10.1093/sysbio/syp075).
- Vetter H, Daubner M. 2000.** *Das Schildkrötenlexikon auf CD*. Bergheim: L. Staackmann Verlag KG.
- Von Bayern LF. 1884.** *Zur Anatomie der Zunge—eine vergleichend-anatomische Studie*. München: Literarisch-Artistische Anstalt (Theodor Riedel).
- Weisgram J. 1985a.** Feeding mechanics of *Claudius angustatus* Cope 1865. In: Duncker HR, Fleischer G, eds. *Fortschritte der zoologie*. Stuttgart: Gustav Fischer Verlag, 257–260.
- Weisgram J. 1985b.** Zum Mechanismus der Nahrungsaufnahme bei Schildkröten. Eine vergleichend-funktionsanatomische Studie an *Claudius angustatus* Cope 1865, *Pseudemys scripta elegans* Wied 1839 und *Testudo hermanni hermanni* Gmelin 1789. D. Phil. Thesis, University of Vienna. 130 p.
- Weisgram J, Dittrich H, Splechna H. 1989.** Comparative functional study of the oral cavity in two turtle species. *Plzener Lekzion Sbornik Supplement* **59**:117–122.
- Werneburg I. 2011.** The cranial musculature in turtles. *Palaeontologia Electronica* **14**(2): 15a:99.

- Werneburg I. 2012.** Temporal bone arrangements in turtles: an overview. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **318**:235–249 DOI [10.1002/jez.b.22450](https://doi.org/10.1002/jez.b.22450).
- Werneburg I. 2013.** Jaw musculature during the dawn of turtle evolution. *Organismal Diversity and Evolution* **13**:225–254 DOI [10.1007/s13127-012-0103-5](https://doi.org/10.1007/s13127-012-0103-5).
- Werneburg I. 2014.** Konvergente Evolution herbivorer Landwirbeltiere [ein Review]. In: Maier W, Werneburg I, eds. *Schlüsselergebnisse der organismischen Makroevolution*. Zürich: Scidinge Hall Verlag, 295–331.
- Willis KL, Christensen-Dalsgaard J, Ketten DR, Carr CE. 2013.** Middle ear cavity morphology is consistent with an aquatic origin for Testudines. *PLoS ONE* **8**(1):e54086 DOI [10.1371/journal.pone.0054086](https://doi.org/10.1371/journal.pone.0054086).
- Winokur BM. 1988.** The buccopharyngeal mucosa of the turtles (Testudines). *Journal of Morphology* **196**:33–52 DOI [10.1002/jmor.1051960105](https://doi.org/10.1002/jmor.1051960105).
- Winokur RM, Legler JM. 1975.** Chelonian mental glands. *Journal of Morphology* **147**:275–291 DOI [10.1002/jmor.1051470303](https://doi.org/10.1002/jmor.1051470303).
- Wochesländer R, Hilgers H, Weisgram J. 1999.** Feeding Mechanism of *Testudo hermanni boettgeri* (Chelonia, Cryptodira). *Netherlands Journal of Zoology* **49**:1–13 DOI [10.1163/156854299X00010](https://doi.org/10.1163/156854299X00010).
- Wochesländer R, Gumpenberger M, Weisgram J. 2000.** Intraoral food transport in *Testudo hermanni* (Chelonia, Cryptodira)—a radiographic video analysis. *Netherlands Journal of Zoology* **50**:445–454 DOI [10.1163/156854200X00199](https://doi.org/10.1163/156854200X00199).