

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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Almost all extant land turtles are highly associated with terrestrial habitats and the few tortoises with high affinity to aquatic environment are found within the genus *Manouria*. *Manouria* belongs to a clade which forms the sister taxon to all remaining tortoises and is suitable to be used as a model for studying evolutionary transitions from water to land within modern turtles. We analysed the feeding behaviour of *M. emys* and due to its phylogenetic position, we hypothesise that the species might have retained some ancestral characteristics associated to aquatic lifestyle. We tested whether *M. emys* is able to feed both in aquatic and terrestrial environments as mud turtles do. In fact, *M. emys* repetitively tried to reach submerged food items in water, but always failed to grasp them and 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 in 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 profile 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 tortoises.

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

4

5 Abbreviated title: **Feeding ethology in *Manouria emys***

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17

18 **INTRODUCTION**

19

20 By comprising more than 180 species, the cryptodiran taxon Testudinoidea represents the most
21 diverse group of extant turtles (e.g., Fritz and Havaš, 2007; Thomson and Shaffer, 2010; Turtle
22 Taxonomy Working Group 2014). Traditionally, it contains three major extant groups, including the
23 mud turtles Emydidae (marsh turtles), the Geoemydidae (pond, river and wood turtles), and the land
24 turtles Testudinidae (tortoises) (Fig. 1). All molecular phylogenetic studies confirm a sister group
25 relationship of Testudinidae and Geoemydidae (Iverson et al., 2007; Shaffer, 2009; Thomson and

26 Shaffer, 2010; Barley et al., 2010) and Emydidae evolved independently from the former groups (Fig.
27 1). How Platysternidae are related to other turtle groups is uncertain (Fig. 1; reviewed by Parham et al.
28 2006), but most molecular studies provide evidence for a closer relationship to emydids (e.g., Shaffer
29 and Thomson, 2010, Crawford et al., 2015). Paleontological studies revealed that all testudinoids had
30 an aquatic ancestor and that terrestrial forms evolved secondarily (Danilov, 1999; Sukhanov, 2000;
31 Joyce and Gauthier, 2004). The only pure terrestrial living turtle group are the tortoises.

32 Amphibious to terrestrial lifestyles and the capacity to exploit terrestrial food sources had evolved
33 independently within all three major testudinoid lineages (for overview see Summers et al., 1998;
34 Natchev et al., 2009). At least eight emydid species are able to feed on land as well as under water (see
35 Bels et al., 1997, 2008; Summers et al., 1998; Stayton, 2011). During terrestrial feeding events, such
36 amphibious emydids use their jaws to grasp food items (jaw prehension). Similarly, all amphibious
37 geoemydids studied to date also use jaw prehension in terrestrial food uptake (see Heiss et al., 2008;
38 Natchev et al., 2009). By contrast, all testudinids studied so far use their tongue to grasp food items -
39 behaviour referred to as lingual prehension (see Wochesländer et al., 1999; Bels et al., 2008).
40 According to Bels et al. (2008), lingual prehension is obligatory for all tortoises. Tortoises show a
41 broad variety in their feeding ecology, with a clear tendency towards herbivory and emancipation from
42 water as living and feeding media (see Pritchard, 1979; Ernst and Barbour, 1989; Bonin, Devaux and
43 Dupre, 2006). In fact, testudinids seem to have lost their ancestral ability to feed under water and to
44 exclusively rely on terrestrial trophic ecologies. However, some predominantly terrestrial geoemydids
45 are able to fulfil the whole feeding process on land and under water (Natchev et al., 2010). Similarly,
46 testudinids with tendencies towards an amphibious lifestyle might have retained the ancestral capacity
47 to feed underwater. Accordingly, information on ambiguous feeding mechanisms in tortoises are
48 important to understand the evolution of terrestrial feeding mechanisms and subsequently the evolution
49 of the predominantly terrestrial lifestyle in tortoises. *Manouria*, as one of the ‘basal’-most extant

50 tortoises with strong association to water (Høybye-Mortensen, 2004; Stanford et al., 2015), provides a
51 promising candidate to study ambiguous feeding mechanisms in land turtles. Its partially aquatic
52 feeding habit was supposed to be associated with the observed morphological extension of the palatine
53 onto the triturating surface of the upper jaw (character 30 sensu Gerlach, 2001), a diagnostic feature
54 common to geoemydid (=batagurid) turtles. Another geoemydid-like feature is the unique existence of
55 class II mental glands (Winokur and Legler, 1975).

56 The present study was designed to provide a detailed analysis of the feeding behaviour in
57 *Manouria*. *Manouria emys* is closely related to water; hence, we provide experiments to reveal whether
58 this species is able to fulfil the whole feeding process in both media water and air as analogy to
59 geoemydids. The kinematical data of the feeding mechanism are compared with data available from
60 other tortoises. The development of the classical tetraphasic models of feeding kinematics proposed by
61 Bramble and Wake (1985) in lower tetrapods was largely based on kinematics of the feeding apparatus
62 of tortoises. According to Wochesländer et al. (1999, 2000) and Bels et al. (2008), the feeding
63 kinematics in tortoises sticks exactly to the generalised cyclic model (GCM) which was suggested to be
64 common for all lower tetrapods (Bramble and Wake, 1985). Accordingly, the second goal of this study
65 is to test if the GCM can also be applied to one of the most ‘basal’ recent tortoises, i.e. *Manouria*.
66 Based on the newly gained information, we formulate hypotheses concerning the evolution of the food
67 prehension mode within turtles. Additionally, we test and fine tune the classical feeding models in
68 tetrapods and discuss on their general validity.

69

70 MATERIALS AND METHODS

71

72 Ecological background

73 Both species of *Manouria*, the Asian forest tortoise *M. emys* and the impressed tortoise *M.*
74 *impressa*, have a restricted distribution in Southeast Asia. *M. emys* has a narrow distribution in
75 Bangladesh, India (Assam, Meghalaya, Mizoram, Nagaland), Myanmar, Thailand, Malaysia (East,
76 West), and Indonesia (Kalimantan, Sumatra). The nominate subspecies, *M. emys emys* - the subject of
77 this study, inhabits the southern part of the species range (Fritz and Havaš, 2007; TTWG, 2014,
78 Stanford et al., 2015).

79 *M. emys* inhabits tropical evergreen monsoon forests and tolerates high soil moisture. It is
80 commonly found reposing in wet areas, buried in mud or under the leaf litter where it may spend hours
81 or days. It is active even at rainy weather and not depending of sun basking, the species is mostly
82 crepuscular and nocturnal (Ernst, Altenburg, and Barbour, 2000; Vetter and Daubner, 2000, Stanford et
83 al., 2015).

84 According to the available literature, the diet of *M. emys* includes plants (mostly aquatic), fungi,
85 invertebrates, and frogs (Nutphand, 1979; Das, 1995; Lambert and Howes, 1994, Høybye-Mortensen,
86 2004). It has been reported to feed on plants in shallow mountain streams (Nutphand, 1979).

87

88 Experimental setup

89 The animals used in the present study were obtained commercially and kept at 12 h dark/light
90 cycles in a large terrarium (150x100 cm ground area) with a permanently filtered water basin and
91 spacious terrestrial area. The turtles were fed different fruits, vegetables, commercially obtained
92 tortoise pellets, dead mice, as well as pieces of beef heart and liver, offered on the terrestrial part of the
93 terrarium. The three subadult experimental animals had a carapace length between 109 and 135 mm
94 and weighted between 234 and 265 g. For filming terrestrial feeding, the specimens were put in a dry
95 glass aquarium (24x60x30 cm) and the food items were offered via forceps in front of the animals. As
96 food items, we used small cattle heart pieces measuring approximately 4x4x6 mm. The turtles were

97 filmed in left lateral view (with a reference grid 1x1 cm in the background) via the digital high-speed
98 camera system Photron Fastcam-X 1024 PCI (Photron limited; Tokyo, Japan) at 500 fps with a highly
99 light-sensitive objective AF Zoom - Nikkor 24-85 mm (f/2,8-4D IF). Two “Dedocool Coolh” tungsten
100 light heads with 2 x 250 W (ELC), supplied by a “Dedocool COOLT3” transformer control unit (Dedo
101 Weigert Film GmbH; München, Germany) were used for illumination. We filmed and analysed the
102 food uptake and the food transport cycles in eight feeding sequences for each specimen.

103 For filming aquatic feeding on submerged food, we filled the experimental aquarium with a water
104 level of 3 cm and offered food in front of the turtle’s snout. For reducing the light intensity and for
105 optimisation of the digitising process, the frame rate was reduced to 250 fr/s. As the tortoises were not
106 able to grasp the food item in a total of 36 trials, the kinematics of the feeding apparatus were analysed
107 (see below) in nine selected representative feeding trials. Horizontal (X-axis) and vertical (Y-axis)
108 coordinates of relevant landmarks (see Fig. 2) were digitised frame by frame using “SIMI-MatchiX”
109 (SIMI Reality Motion Systems; Unterschleißheim, Germany). On the base of the displacement of the
110 markers, we were able to calculate the gape amplitude (distance between the tip of the upper and lower
111 beak), head movement (distance between the anterior tip of the carapace and the point “P” on Figure
112 2), tongue movements (distance between the most ventral point on tympanum and the tip of the tongue
113 when visible), and hyoid movements (distance between the point “P” on Figure 2 and the basis of the
114 posterior ceratobranchial). These data were used to calculate the following kinematical variables:
115 duration of Slow open phase (SO); duration of Slow open phase I and II (SOI and SOII) when present;
116 duration of fast open (FO); duration of maximum gape phase (MG); duration of fast close (FC); time to
117 peak gape (TPG); total cycle duration (TCD); duration of hyoid protraction (HDD); duration of hyoid
118 retraction (HVD); duration of the total hyoid cycle (THC); hyoid retraction velocity (HRV); duration
119 of head protraction (HP); duration of head retraction (HR); duration of tongue protraction (TP); tongue
120 retraction velocity; delay of the start of hyoid retraction to the tongue retraction start; delay of reaching

121 peak gape relative to start of the hyoid retraction; delay of reaching peak gape relative to tongue
122 retraction start (see Table 1).

123

124 Statistics

125 Differences in food uptake and food transport cycles respectively with presence/absence of split in
126 jaw opening in SO and FO phases; split of SO phase in SOI and SOII phases; occurrence of MG phase
127 were tested with Yates corrected Chi-square test. Datasets for the studied variables were tested with
128 Shapiro-Wilk test for normal distribution. When the p-value was less than the chosen alpha level
129 ($p < 0.05$), the null hypothesis was rejected and data were excluded from further analyses. In addition,
130 variables were tested with Levene's and Brown-Forsythe tests and then processed with Welch's
131 ANOVA for heteroscedastic data. Tukey's honest significant difference test (HSD) was performed for
132 post-hoc analyses when applicable. Standard descriptive statistics including mean, range, and standard
133 deviation (SD) were performed. Canonical discriminant analysis was also performed, in order to
134 observe the individual differentiation of all measured variables in the transport phase.

135

136 RESULTS

137

138 When fed on land, the Asian forest tortoises always grasped their food by the jaws. After food
139 uptake, one to four transport cycles followed prior to oesophageal packing (see Schwenk, 2000). The
140 tip of the tongue was barely seen during food uptake (see Fig 3b-c) indicating that the tongue was not
141 protracted. By contrast, during transport cycles, the tongue was well visible as it was rhythmically pro-
142 and retracted to transport the food item towards the oesophagus (Fig. 5).

143 When trying to feed under water (Fig. 4), *M. emys* submerged its head under the water level, and
144 by protruding the head, the animals were trying to position the gaping mouth around the food item. The

145 gape cycle was never split in slow and fast jaw open phases. The tongue tip was not visible in lateral
146 view and the hyolingual complex did not protract prior reaching peak gape. No retraction of the hyoid
147 complex was detected prior jaw closure. The gape cycle duration exceeded for one and a half seconds
148 and was 1.94 ± 0.36 s (mean \pm SD) in all digitised sequences. Despite the lack of success, the turtles
149 had repeatedly tried to catch the submerged food. In several events, we were able to detect that the food
150 item was carried away by the bow wave induced by jaw closing.

151 The variables of the kinematical profiles are summarised in Table 1. In the statistic tests, we found
152 highly significant differences in sequences with and without both slow open phase I (SO I) and slow
153 open phase II (SO II) (see Bramble and Wake, 1985) when food uptake and transport phases were
154 compared ($\chi^2_{(1, N=98)}=25.05$, $p < 0.001$). Similarly significant differences were observed when
155 comparing food uptake and transport cycles in respect to sequences with and without slow jaw open
156 phases as well as with and without maintaining jaw maximum gape (MG phase) ($\chi^2_{(1, N=98)}=6.10$,
157 $p=0.02$; $\chi^2_{(1, N=98)}=6.52$, $p=0.01$).

158 When testing the variables of the feeding kinematics (Table 1), nine variables, which describe the
159 food uptake process, were detected to show significant differences between individuals for time to peak
160 gape duration (TPG; $F_{\text{Welch}(2,21)}=5.03$, $p=0.024$), total gape cycle duration (TCD; $F_{\text{Welch}(2,21)}=12.68$,
161 $p=0.001$), fast closing duration (FC, $F_{\text{Welch}(2,21)}=10.34$, $p=0.002$) and head retraction duration (HR;
162 $F_{\text{Welch}(2,21)}=13.86$, $p=0.001$). In transport cycles, six out of 18 variables differed significantly amongst
163 individuals: hyoid dorsal displacement duration (HDD; $F_{\text{Welch}(2,62)}=6.32$, $p=0.005$); total hyoid cycle
164 duration (THC, $F_{\text{Welch}(2,62)}=7.46$, $p=0.002$); hyoid retraction velocity (HRV, $F_{\text{Welch}(2,66)}=4.66$, $p=0.016$);
165 head protraction duration (HPR; $F_{\text{Welch}(2,53)}=11.47$, $p=0.001$); the delay in the start of hyoid ventral
166 displacement (HVD) relative to the start of tongue retraction (TR) ($F_{\text{Welch}(2,55)}=6.38$, $p=0.005$); the
167 delay of time to peak gape (TPG) relative to the start of hyoid ventral displacement (HVD) ($F_{\text{Welch}(2,66)}=4.74$, $p=0.014$). When testing for differences between grasping and transport cycles, seven out of

169 nine variables differed significantly: fast jaw open duration (FO; $F_{\text{Welch}}(1,43)=15.17$, $p=0.011$);
170 maximum gape duration (MG; $F_{\text{Welch}}(1,26)=15.89$, $p=0.001$); fast closure (FC; $F_{\text{Welch}}(1,26)=7.86$, $p=0.010$);
171 time to peak gape (TPG, $F_{\text{Welch}}(1,72)=46.78$, $p<0.001$); total gape cycle duration (TCD; F_{Welch}
172 $(1,72)=52.50$, $p<0.001$); head protraction duration (HP; $F_{\text{Welch}}(1,67)=52.23$, $p<0.001$); and head retraction
173 duration (HR; $F_{\text{Welch}}(1,47)=12.57$, $p=0.002$).

174 When comparing three further parameters among the transport cycles in all three specimens (delay
175 of HVD start relative to TR start; delays of TPG relative to HVD; delay of TPG relative to TR starts),
176 statistically significant differences were found among all compared pairs ($F_{\text{Welch}}(2,105)=41.58$, $p<0.001$).

177 In order to visualize the kinematical variability of transport cycles amongst individuals a canonical
178 discriminant analysis (CDA) was performed (Fig. 6). . In total, both axes explained 100% of the total
179 variance among individuals (70% explained by the first axis). Seven out of 18 variables loaded
180 positively to the first canonical axis (SO II, FC, TPG, TGC, HDD, HRV, HP - durations and delay of
181 TPG relative to TR). Respectively, six variables loaded positively to the second canonical axis (FC,
182 TPG, THR, THC, HR, and TR). Differences between hyoid retraction velocity (HRV) and tongue
183 retraction velocity (TRV) was highly statistically significant ($F_{(2,60)}=5.39$, $p<0.001$, $V_{\text{HRV}}=1,79\pm 1.21$
184 vs. $V_{\text{TRV}}=6.85\pm 2.80$).

185

186 DISCUSSION

187

188 The present contribution shows that *M. emys* repetitively tried to feed on dispersed food items
189 under water, which was a surprising and unknown behaviour among tortoises. However, the species
190 always failed to uptake the submerged food. On land, *M. emys* grasped food by the jaws, just like all
191 known aquatic turtles do in terrestrial situations, but not with the tongue as formerly predicted for all

192 tortoises. On the base of our results we discuss several important evolutionary, behavioural, and
193 functional aspects.

194

195 **Evolution of food uptake among turtles**

196 All, or almost all stem turtles of the Triassic were terrestrial as indicated by the anatomy and
197 proportions of the limbs, which were adapted for terrestrial locomotion (for a comprehensive
198 discussion see Joyce, in press). With the rise of modern turtles (Testudines) during the Jurassic period
199 (e.g., Danilov and Parham, 2006; Sterli, 2010; Sterli and de la Fuente, 2011), a general transition of
200 turtles into an aquatic environment took place (Willis et al. 2013). The invasion of aquatic
201 environments induced an immense radiation and diversification into several subgroups related to the
202 great evolutionary success of turtles (Joyce, 2007; Thomson and Shaffer, 2010). Due to the different
203 physical properties of air and water, the new environment required morphological and functional
204 adaptations of the locomotion- and feeding system to enable efficient swimming behaviour and aquatic
205 food uptake (i.e. suction feeding) (Schumacher, 1973; Lemell et al., 2002).

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

216 Among extant turtles, the ability to fulfil the whole feeding process (including food uptake, food
217 manipulation and transport, oesophageal packing, and swallowing) on land was tested and documented
218 for only six species so far. All of them were members of the taxon Testudinoidea (Fig. 1; see also
219 Summers et al., 1998; Bels et al., 2008; Natchev et al., 2009). The terrestrial mode of food uptake
220 differs dramatically among and within the three testudinoid subgroups (see Bels et al., 1997, 2008;
221 Summers et al., 1998; Wochesländer et al., 1999, 2000; Natchev et al., 2009, present study).
222 Accordingly, it seems that terrestrial feeding re-evolved several times independently amongst turtles.
223 Unfortunately, only limited experimental data are available on feeding mechanisms in emydid and
224 geoemydid species and accordingly, palaeontological evidence and functional indications can help to
225 address such questions. The sister group to all remaining Testudinoidea is the diverse and possibly
226 paraphyletic extinct taxon †Lindholmemydidae (Fig. 1; Lourenço et al., 2012), which contains genera
227 such as †*Mongolemys* and †*Lindholmemyd* (Danilov, 1999; Joyce and Gauthier, 2004). Some poorly
228 known aquatic taxa such as †Haichemydidae and the †Sinochelyidae may perhaps also belong to
229 †Lindholmemydidae. This group is first known from aquatic sediments of the late Early Cretaceous
230 and apparently had an amphibious lifestyle (Sukhanov, 2000). Among cryptodirans, amphibious
231 lifestyle is retained in most living emydids (plus platysternids) and geoemydids, as well as in
232 kinosternids (see Depecker et al., 2006, but also Nakajima et al., 2014). All other taxa are fully
233 terrestrial (tortoise), freshwater inhabitants or marine turtles (see Joyce and Gauthier, 2004; Rasmussen
234 et al., 2011).

235 Very limited information is available on feeding mechanisms employed by amphibious non-
236 testudinoid turtles that occasionally exploit terrestrial food sources. Weisgram (1985a) documented a
237 non-testudinoid turtle (*Claudius angustatus*) that caught prey on land and dragged it into water for
238 transport and swallowing. Natchev et al. (2008) documented another non-testudinoid (*Sternotherus*
239 *odoratus*) catching food on land, but failing to transport it through the oropharynx. Among extant

240 turtles, successful food transport on land seems to be restricted to testudinoids, because they have
241 evolved enlarged and muscular tongues (von Bayern, 1884; Werneburg, 2011,).

242 Natchev et al. (2009) described and summarised three categories of terrestrial food uptake modes
243 among Testudinoidae, based on experimental data: (A) Jaw prehension with retracted hyolingual
244 complex: the tongue is not protracted towards the food, as observed in the geoemydid genus *Cuora*
245 (Natchev et al., 2009). (B) Jaw prehension with slightly protracted hyolingual complex: a protraction of
246 the tongue is detectable during food uptake but the tongue does not touch the food, as observed in
247 emydids (Bels et al., 1997; Stayton, 2011). (C) Lingual prehension: The tongue touches the food item
248 prior to food uptake and possibly carries the food item into the mouth, as documented in all tortoises
249 studied so far (Wochesländer et al., 1999, Bels et al., 2008). The food uptake mode of *M. emys*,
250 however, differs substantially from that of all remaining tortoises (category C). In fact, the hyolingual
251 complex in *M. emys* remained fully retracted during the food prehension on land, and the first contact
252 with the food item was by the jaws. Accordingly, the feeding mechanism of *M. emys* should be
253 assigned to category A, next to semiaquatic geoemydids. In contrast to semiaquatic geoemydids, *M.*
254 *emys* was unable to grasp submerged food. We consider that the adaptations in the morphology of the
255 feeding apparatus (see Heiss et al., 2011) and the feeding motorics of this species are adapted for
256 terrestrial feeding .However, unlike other tortoises, *M. emys* is documented to actually feed in water
257 (Nutphand, 1979) but only on rigid water plants (equivalent to feeding plants on land) and not on loose
258 submerged food items as tested in our study.

259 The closest extant sister taxon to *Manouria* is that of *Gopherus sp* (Thompson and Shaffer, 2010) -
260 the desert tortoises. Like all other tortoises, the desert tortoises posses a fleshy tongue (Winokur, 1988).
261 There is no published data on the feeding kinematics of *Gopherus*, but personal observations have
262 shown that *Gopherus* grasps food by the jaws (N.N. pers. obs.). Accordingly, preliminary observations
263 imply that the terrestrial food uptake mechanism in *Gopherus* is similar to the feeding modes from

264 categories A or B (see above). Therefore, both *Gopherus* (preliminary observations) and *Manouria*
265 (this study) seem to share the plesiomorphic behaviour of jaw prehension with all amphibious turtles
266 studied so far.

267 Analyzing the feeding behaviour in extant turtles, we now aim to reconstruct a theoretical scenario on
268 the evolution of terrestrial feeding mechanisms in turtles. The ancestors of all living turtles were
269 aquatic animals. The functional evolution from aquatic to terrestrial feeding mechanisms could
270 theoretically have involved four stages, starting from exclusively aquatic feeding ancestor. In different
271 lineages, turtles may have left the water for example in search for food. The species that retained
272 predominantly aquatic life style grasped food by the jaws and brought it to water for further intraoral
273 (hydrodynamic based) transport. Such behaviour is still found in the kinosternids *C. angustatus*
274 (Weisgram, 1985a; Weisgram, 1985b) and *S. odoratus* (Heiss et al., 2010; Natchev et al., 2011), as
275 well as in the emydid *Trachemys scripta* (Weisgram, 1985b; Weisgram et al., 1989) and other emydids
276 (see Stayton, 2011). Turtles of the second theoretical evolutionary stage grasped food by the jaws and
277 the tongue was used for intraoral food transport on land. Such species had not lost the ability to feed
278 underwater by using hydrodynamic mechanisms. When grasping food on land, the tongue remained
279 retracted or was protracted without however touching the food item. Among extant turtles, such
280 feeding mode is found in the geoemydid genus *Cuora* (Heiss et al., 2008; Natchev et al., 2009, 2010)
281 and in some emydids (Bels et al., 1997; Summers et al., 1998; Stayton, 2011). In the next theoretical
282 evolutionary step (stage three), behavioural and morphological specializations for terrestrial feeding
283 advanced further, increasing the efficiency of terrestrial food transport at the cost of the capability to
284 use effective hydrodynamic mechanisms in water. Such forms still grasped food items with their jaws
285 on land (as typical for aquatic or semiaquatic turtles), but were no longer able to take up dispersed food
286 if submerged. Such a situation is still present in *M. emys*. Most importantly, stage three could have
287 marked the “point of no return” in the evolution of terrestrial feeding in turtles. Finally, in a fourth

288 stage, turtles became fully terrestrial and their tongue was obligatorily involved in food uptake as
289 documented in the tortoises *Testudo (Eurotestudo) hermanni boettgeri* (Weisgram, 1985b;
290 Wochesländer et al., 1999; 2000), *Kinixys belliana*, *Geochelone elephantopus* and *G. radiata* (Bels et
291 al., 2008).

292 According to Bels et al. (2008), tortoises exhibit very stereotypical kinematical patterns in food
293 ingestion and transport. These authors predicted obligatory lingual prehension for initial food uptake, a
294 split of the gape cycle in slow open (SO) and fast open (FO) phases and start of the tongue retraction at
295 the beginning of the fast open phase (see also Bramble and Wake, 1985). Specifically, the authors
296 demonstrated these elements of the feeding behaviour in the tortoises *G. radiata*, *G. elephantopus*, and
297 *K. belliana*. In these species both in food uptake and in food transport, the gape cycle starts with a slow
298 opening of the jaw to approximately one third of the maximum gape angle (slow open phase I; SOI).
299 The slow open phase I is followed by a prolonged slow open phase II (SOII), where the gape angle
300 remains similar as at the end of slow open phase I. During both slow open phases, the hyolingual
301 complex protracts and reaches its rostral most position, with the tongue protruding. At the end of slow
302 open phase II starts the fast opening of the jaws (fast open phase; FO) and the initiation of the hyoid
303 retraction. Immediately after the peak gape is reached, the jaws start to close fast (FC phase) and
304 during this gape phase the hyoid retraction is finished. Wochesländer et al. (1999, 2000) reported
305 similar patterns in the feeding kinematics of *T. hermanni boettgeri*. These four species were the only
306 tortoises in which feeding behaviour was studied on the base of high-speed sequence analyses - still,
307 the generalized gape cycle model (GCM) of Bramble and Wake (1985) was considered to apply to all
308 tortoises (Bels et al., 2008).

309 In contrast, our investigations demonstrate that the 'basal' tortoise *M. emys* does not contact food
310 with the tongue prior to jaw prehension on land. This shows that tongue to food contact is
311 characteristic for advanced tortoises only. We consider the terrestrial feeding behaviour of *M. emys* as

312 plesiomorphic and potentially inherited from its semiaquatic ancestors. On that base, *M. emys* can be
313 considered a transitional turtle in regard to secondary terrestriality.

314 Almost all tortoises are predominantly terrestrial animals. They feed exclusively on land and
315 protrude their tongues toward the food for initial contact during food uptake. We propose that
316 hyolingual protrusion evolved in the lineage leading to advanced tortoises (Fig. 1). *Manouria emys* has a
317 large tongue with massive intrinsic and extrinsic musculature (see Heiss et al., 2011). The advanced
318 and complex lingual musculo-skeletal architecture permits the turtle to protrude the tongue outside the
319 margins of the rhamphothecae (see Fig. 2). The food transport in *M. emys* is totally lingual based, as
320 cyclical tongue movements bring the food to the posterior pharynx. However, *M. emys* does not use
321 lingual food prehension as typical for all other tortoises studied so far. In fact, it seems that the
322 *Manouria* (and *Gopherus*, see above) “lineage” has retained the jaw prehension mechanism inherited
323 from its aquatic ancestors. Tortoises in general evolved fleshy tongues which improve the food
324 transport performance and advanced tortoises only refined the behaviour of food uptake on land via
325 lingual food contact (see Wochesländer et al., 1999; Bels et al., 2008) prior to jaw closure.

326

327 **Function of the protruded tongue in testudinid food uptake**

328 What could be the potential advantage of the obligatory lingual protrusion during food uptake
329 found in the more derived tortoises? One possible explanation is that the tongue is used as a prehensile
330 organ for food ingestion as found in other tetrapod groups (for overview see Schwenk, 2000; Schwenk
331 and Wagner, 2001; Heiss et al., 2014). However, this historical explanation might be put into question.
332 By examining all published data available (Wochesländer et al., 1999, Bels et al., 2008), we were not
333 able to find any hard evidences that tortoises collect food with their tongues - they just touch it. In all
334 feeding sequences, the contact between the food and the tongue is clearly demonstrated - still, in all
335 cases, after the initial contact of the tongue to the food, the head moves forward and the food item is

336 not dragged into the mouth by tongue (or hyolingual) retraction, but is grasped in the jaws during fast
337 jaw closing (FC gape phase). Hence, in extant tortoises, the tongue is not the main organ that is
338 catching (collecting) the food, but these are in fact the jaws. Accordingly, initial food ingestion in
339 tortoises might not be considered “lingual prehension” (see Schwenk, 2000; Bels et al., 2008) in the
340 strict sense, but rather as “jaw prehension following lingual contact”. This is a crucial difference,
341 which sets the question: why do extant tortoises except *M. emys* (and *Gopherus*) obligatory protrude
342 their tongues during food uptake? In other words: why do tortoises apply a more complex and
343 presumably more energetically expensive food uptake mechanism by including movements of the
344 hyolingual complex besides the movements of the neck and jaws alone?

345 We propose that the lingual contact provides tactile information on the position of the food item
346 and helps the advanced tortoises to compensate the “information gap” which occurs when the food is
347 approached to a distance where it is lost by the sight. The eyes of tortoises, similar to those of most
348 other turtles, are positioned laterally on the head (Pritchard, 1979) and the turtles are not able to
349 observe permanently the position of the food item when the neck is protracted and the gape is
350 positioned around it. The prolonged maximum gape (MG) phase found in most ingestion cycles of *M.*
351 *emys* (see Tble 1, Figure 3 and Figure 6) might be the result of lack of lingual contact to the food
352 surface. In all published sequences and kinematical profiles on food uptake in tortoises, there is a clear
353 tendency toward a split of the gape cycle into slow open (SO) and fast open (FO) gape phases (see
354 Wochesländer, 1999; Bels et al., 2008). The split of the gape cycle in slow and fast phases is associated
355 with the movements of the tongue (for overview see Bramble and Wake, 1985). The lack of tongue
356 protrusion might explain the lack of slow open (SO) and fast open (FO) split in the gape cycle of food
357 uptake in the geoemydid *Cuora* (see Natchev et al., 2009). In most food uptakes analysed in *M. emys*,
358 SO phases are not present and the gape increases gradually (see Table 1 and Figure 6). This fact may

359 be conditioned by the same factors as in the investigated geoemydids, namely a lack of tongue
360 protrusion in food uptake (Natchev et al., 2009).

361

362 **Intraoral food transport on land**

363 The intraoral transport of *M. emys* is entirely lingual based. The cycle duration is much shorter
364 than that of food uptake (see Table 1 and results), despite the involvement of the hyoid and the tongue
365 in the complex rhythmic motoric of transport mechanism. The execution of the transport cycles
366 demand complicated coordination of the activities of the contractile elements such as the jaw opening
367 and closing muscles, head protracting and retracting muscles, intrinsic and extrinsic lingual muscles, as
368 well as muscles that protract and retract the hyolingual complex as a whole unit (Jones et al., 2012;
369 Werneburg, 2011, 2013).

370 In opposite to this complicated choreography, the mode of food prehension in *M. emys* suggests
371 less complex neuromotoric coordination only between neck and jaw movements. The execution of the
372 transport cycles is at least more than 100 % shorter in duration (see Table 1). A possible explanation
373 for the longer duration of food uptake cycles relative to transport cycles might be that during transport
374 the coordination centres of the muscle activities execution are permanently supplied by feed-back
375 information concerning the position of the food item and the proper movements can be executed
376 precisely in a shorter time.

377 Alternative interpretation of our results might be that the food uptake cycle duration is longer, as
378 the movements have to be permanently fine-tuned according to the position of the food. The transport
379 cycles might be shorter, because of pre-programmed neuromotor control of rhythmic movement
380 patterns. Wochesländer et al. (1999, 2000) and Bels et al. (2008) hypothesised that the feeding
381 kinematical patterns in tortoises adhere exactly to those of the generalized cycle model (GCM)
382 proposed by Bramble and Wake (1985). In his work on the feeding mechanisms in domestic animals,

383 Bels (2006) established that the pre-programmed GCM is universally valid along different groups of
384 tetrapods, including amphibians, sauropsids, and mammals. On the base of our results, the feeding
385 kinematics of the ‘basal’ extant Asian forest tortoise differs significantly from those of the GCM. The
386 kinematics of the feeding system in *M. emys* seems to be though pre-programmed, under permanent
387 feed-back control. The values of the gape and hyoid/hyolingual cycle patterns in the three specimens
388 studied here show high degrees of variation, both concerning food uptake and food transport (see Table
389 1 and Figures 5-7). The slow open phases (SO) are obligatory neither for food uptake, nor for food
390 transport gape cycles. The gape cycle often includes a phase of retaining maximum gape (see Table 1,
391 Figure 3, 5). In turtles, the maximum gape phase (MG) was described for the gape cycle in *Cuora sp.*
392 (Natchev et al., 2009, 2010) and was confirmed for other turtles (Natchev et al., 2011). The presence of
393 a prolonged maximum gape phase (MG) can be easily overseen when the frame rate of the film
394 sequence is not high enough (i.e., step between successive frames over 10 milliseconds) In this case the
395 durations of the time to peak gape, or the fast close phase may be miscalculated.

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

401 We observed a clear delay in the offset of depression of the oropharyngeal basis (which is a good
402 indicator for the start of hyoid retraction in lower vertebrates (Schwenk, 2000) relative to the initial
403 retraction of the tip of the tongue (see Table 1). The tongue tip retraction velocity is much higher (see
404 Table 1) than the velocity of the mouth base depression. This fact indicates that there is an initial
405 caudal retraction of the tongue tip (probably by contraction of the intrinsic lingual muscles) prior to the
406 movement of the hyolingual complex as a whole unit. Another explanation might be that, the

407 hyolingual complex is retracted caudally and the depression of the oropharyngeal base is a result of the
408 repositioning of the voluminous tongue.

409

410 **CONCLUSIONS**

411

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

424

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433

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574

575 **FIGURE CAPTIONS**

576

577 **Figure 1.** Phylogeny of turtle clades with a focus on Testudinoidea. Interrelationship following Shaffer
578 and Thomson (2011). Those authors assign Platysternidae as sister taxon to Emydidae; here we show
579 different hypothesis for the position of this species. Major evolutionary changes are listed; for details
580 see text. Three modes of terrestrial food uptake are illustrated. **A)** Jaw prehension; the tongue is not
581 protruded and is only used for food transport (Geoemydida, *Manouria*). **B)** Jaw prehension; the
582 elongated tongue is protruded during prehension but does not contact the food and is only used for food
583 transport (Emydidae). **C)** Prior to jaw prehension, the tongue contacts the food (advanced tortoises).

584

585 **Figure 2.** Selected frame from a high-speed video sequence (500 fr/s) of food transport in *Manouria*
586 *emys*, showing the landmarks used for kinematic analyses: **C**, rostral tip of sagittal carapace, **Hy**, hyoid
587 at the basis of ceratbranchial I; **LJ**, tip of the lower jaw; **P**, posteriormost point of crista
588 supraoccipitale; **TT**, tip of the tongue; **Tv**, ventral most point of the tympanum at the position of the
589 jaw joint; **UJ**, tip of the upper jaw; grid 10x10 mm.

590

591 **Figure 3.** Selected frames and graphics (based on a high-speed video with 500 fr/s) represent the
592 movements of jaws, hyoid and t head during terrestrial food uptake in *Manouria emys* when feeding on
593 pieces of beef heart; **a**, slow open phase end (lacking discrete SOI and SOII); **b**, fast open end; **c**, fast
594 close start; **d**, fast close end; arrow indicates the position of the food item; arrowheads represent the
595 position of the tip of the tongue; grid 10x10 mm.

596

597 **Figure 4.** Selected frames and graphics (based on high-speed video with 250 fr/s showing the
 598 movements of jaws, hyoid, and head during attempts of aquatic food uptake in *Manouria emys*; **a**, start
 599 of the gape cycle; **b**, end of jaw opening; **c**, maximum gape end; **d**, fast closure end; note the lack of
 600 movement of the hyoid complex during the whole cycle; grid 10x10 mm. dors., – dorsal; vent. –
 601 ventral; pro – protraction; ret – retraction.

602

603 **Figure 5.** Selected graphics (based on a high-speed video with 500 fr/s) showing the movement
 604 patterns of jaws, hyoid, tongue and head during terrestrial food transport in *M. emys*; note the delay in
 605 hyoid ventral displacement relative to the start the retraction of the tongue tip, as well as the delay of
 606 both the tongue retraction and hyoid retraction relative to the start of the FO phase. Abbreviations: **FC**,
 607 fast closure; **GCM**, generalised cyclic model; **HDD**, hyoid dorsal displacement; **HPR**, head
 608 protraction; **HR**, neck retraction; **HRV**, hyoid retraction velocity; **HVD**, hyoid ventral displacement;
 609 **MG**, maximum gape; **PG**, peak gape; **SO**, slow open phase lacking discrete SOI and SOII; **SO I**, slow
 610 open phase I; **SO II**, slow open phase II; **T**, transport; **TCD**, total cycle duration; **THC**, total hyoid
 611 cycle; **TP**, tongue plateau; **TPG**, time to peak gape; **TPR**, tongue protraction; **TR**, tongue retraction.

612

613 **Figure 6.** Frequencies of occurrence of sequences with absence of selected variables in food uptake
 614 (FU) (light grey bars) and food transport (T) (black bars) phases, expressed as: percentages missing
 615 any possible split in slow and fast open gape phases - SO and FO (A); cycles in which SO phase was
 616 detectable, but the split of discrete slow gape phase in SOI and SOII is missing (B); lack of MG phase
 617 (C).

618

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

621 **TABLES AND TABLE LEGENDS**

622

623 **Table. 1:** Variables describing the feeding process in *Manouria emys*, present as means \pm SD; n,
624 sample size; *, significant differences ($\alpha=0.05$) among individuals in the ingestion phase (P1), in the
625 transport phase (P2), and between both mode (P3); n.c., p value not calculated.

Variable	Food uptake (FU)				Transport (T)				I vs. T	
	individual 1 (n=8)	individual 2 (n=8)	individual 3 (n=8)	p1	individual 1 (n=33)	individual 2 (n=20)	individual 3 (n=21)	p2	p3	
SOI duration [s]	0.168±0.060 n=2	0.618±0.231 n=3	0.562 n=1	n.c.	0.146±0.016 n=18	0.126±0.014 n=14	0.115±0.015 n=11	0.378	0.068	
SOII duration [s]	0.738±0.508 n=2	0.453±0.294 n=3	1.024 n=1	n.c.	0.147±0.014 n=18	0.187±0.027 n=13	0.190±0.021 n=11	0.187	0.072	
FO duration [s]	0.450±0.060 n=2	0.379±0.150 n=3	0.694 n=1	n.c.	0.122±0.009 n=25	0.126±0.012 n=15	0.102±0.006 n=19	0.111	0.011*	
MG duration [s]	0.079±0.017 n=4	0.095±0.031 n=4	0.166±0.044 n=6	0.271	0.025±0.003 n=6	0.033±0.004 n=10	0.042±0.019 n=4	0.318	0.001*	
FC duration [s]	0.157±0.079 n=8	0.105±0.036 n=8	0.158±0.030 n=8	0.024*	0.089±0.020 n=33	0.186±0.040 n=20	0.119±0.088 n=21	0.155	0.010*	
TPG [s]	0.943±0.144 n=8	0.989±0.177 n=8	1.784±0.137 n=8	0.002*	0.408±0.021 n=33	0.439±0.038 n=20	0.403±0.028 n=21	0.187	<0.001*	
TCD duration [s]	1.139±0.148 n=8	0.128±0.169 n=8	2.073±0.144 n=8	0.001*	0.499±0.020 n=33	0.655±0.098 n=20	0.510±0.030 n=21	0.311	<0.001*	
HDD duration [s]					0.281±0.025 n=30	0.216±0.035 n=14	0.169±0.019 n=21	0.005*		
HVD duration [s]					0.176±0.011 n=31	0.167±0.014 n=17	0.149±0.009 n=21	0.162		
THC duration [s]					0.456±0.028 n=30	0.384±0.041 n=14	0.317±0.022 n=21	0.002*		
HRV velocity [cm/s]					0.718±0.059 n=31	0.938±0.107 n=17	0.551±0.071 n=21	0.016*		
HP duration [s]	1.345±0.159 n=8	1.204±0.246 n=8	2.494±0.177 n=8	0.001*	0.220±0.049 n=32	0.864±0.132 n=14	0.464±0.089 n=10	0.001*	<0.001*	
HR duration [s]	0.296±0.041 n=8	0.487±0.086 n=7	0.704±0.174 n=8	0.052	0.236±0.027 n=14	0.211±0.025 n=13	0.316±0.065 n=10	0.333	0.002*	
TP duration [s]					0.165±0.008 n=30	0.160±0.017 n=19	0.133±0.025 n=13	0.483		
TR velocity [cm/s]					7.459±0.550 n=31	5.798±0.547 n=20	6.562±0.595 n=13	0.121		
delay of HVD start relative to TR start [s]					-0.2011±0.026 n=30	-0.039±0.231 n=15	-0.082±0.025 n=14	0.005*		
delay of TPG relative to HVD start [s]					-0.007±0.007 n=31	-0.016±0.008 n=17	-0.032±0.005 n=21	0.014*		
delay of TPG relative to TR start [s]					-0.062±0.007 n=31	-0.045±0.013 n=20	-0.055±0.008 n=14	0.521		

626

627 **APPENDIX**

628

629 List of die abbreviations in the text in alphabetic order:

630 C – Carapax rostral tip;

631 cbI – Ceratobranchiale I;

632 ch – Corpus of the hyoid;

633 chII – Ceratohyale II;

634 cm – Centimetre;

635 crh – Cornu hyale;

636 dors. – Dorsal;

637 ep I – Epibranchiale I;

638 FC – Fast Close;

639 GCM – Generalised Cyclic Model;

640 hg – Hypoglossum;

641 HDD – Hyoid dorsal displacement;

642 HP – Head protraction;

643 HPR – Head protraction;

644 HR – Head retraction;

645 HRV – Hyoid retraction velocity;

646 Hy – Hyoid at the basis of ceratbranchial I;

647 HVD – Hyoid ventral displacement;

648 I – Ingestion (food uptake);

649 LJ – Lower jaw tip;

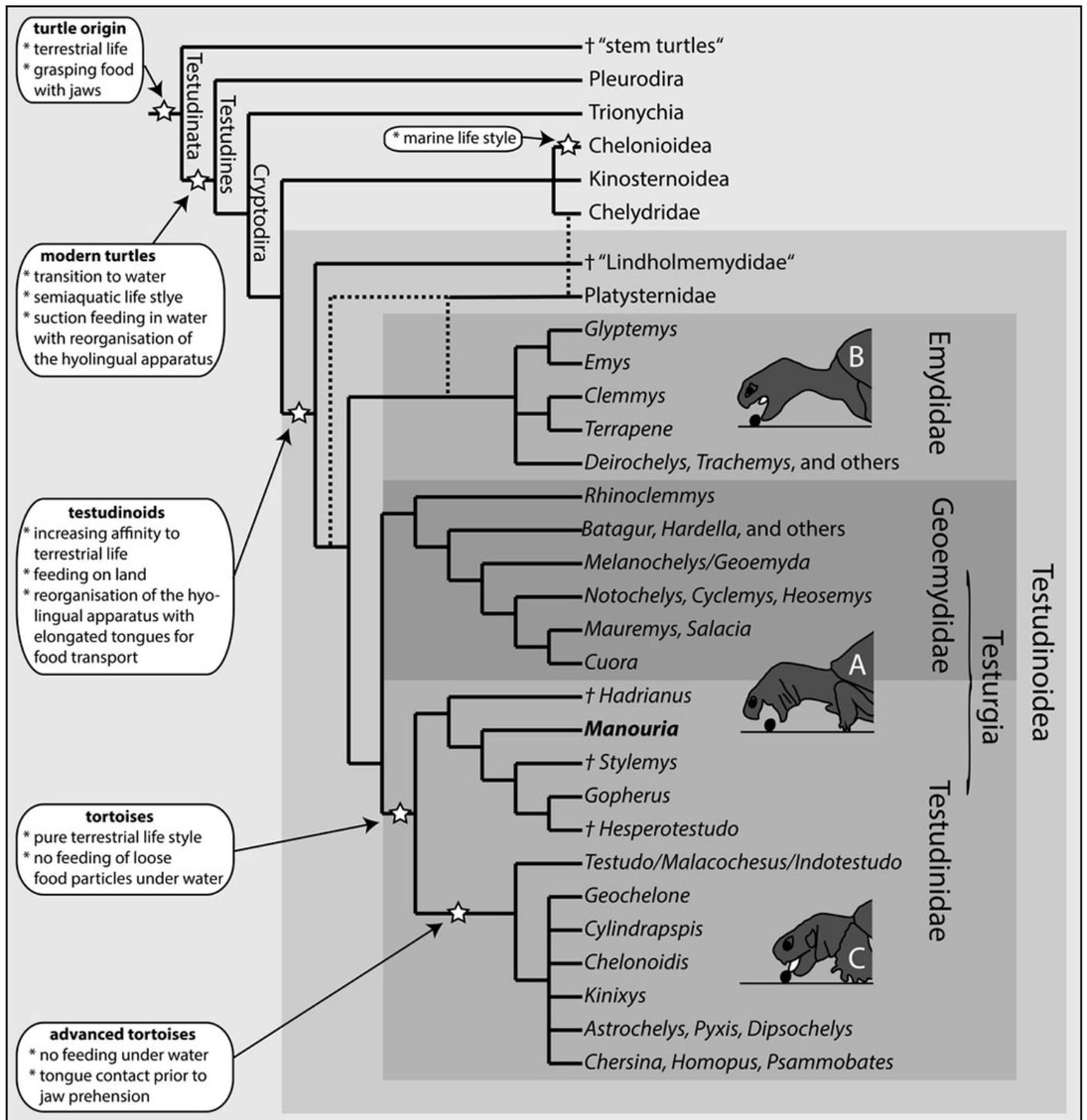
650 MG – Maximum gape;

- 651 Mm – Millimetre;
- 652 oss – Ossification islands on cornu branchiale II;
- 653 P – Parietale;
- 654 PG – Peak gape;
- 655 pro – Protraction;
- 656 pl – Processus lingualis;
- 657 ret – Retraction;
- 658 s – Second;
- 659 SO – Slow open phase lacking discrete SOI and SOII;
- 660 SO I – Slow Open I;
- 661 SO II – Slow Open II;
- 662 T – Transport;
- 663 TAG – Terrestrial aquatic gradient;
- 664 TCD – Total cycle duration;
- 665 THS – Total hyoid cycle;
- 666 TP – Tongue plateau;
- 667 TPG – Time to Peak Gape;
- 668 TPR – Tongue protraction;
- 669 TR – Tongue retraction;
- 670 TT – Tip of the tongue;
- 671 Tv – Tympanum ventral most point;
- 672 UJ – Upper jaw tip;
- 673 vent. – Ventral;
- 674

1

Figure 1

Phylogeny of turtle clades with a focus on Testudinoidea. Interrelationship following Shaffer and Thomson (2011). Those authors assign Platysternidae as sister taxon to Emydidae; here we show different hypothesis for the position of this species. 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 (*Geoemydida*, *Manouria*). B) Jaw prehension; the elongated tongue is protruded during prehension but does not contact the food and is only used for food transport (Emydidae). C) Prior to jaw prehension, the tongue contacts the food (advanced tortoises).



2

Figure 2

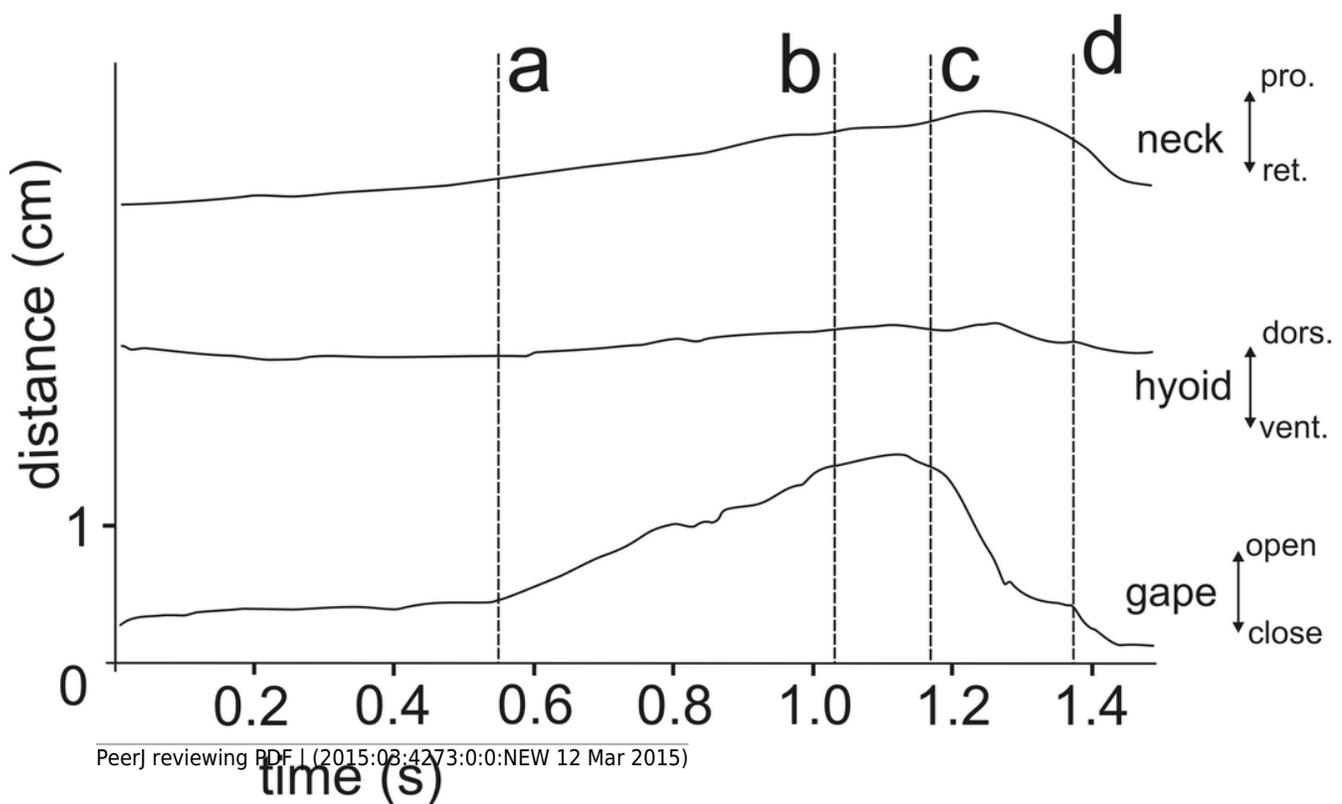
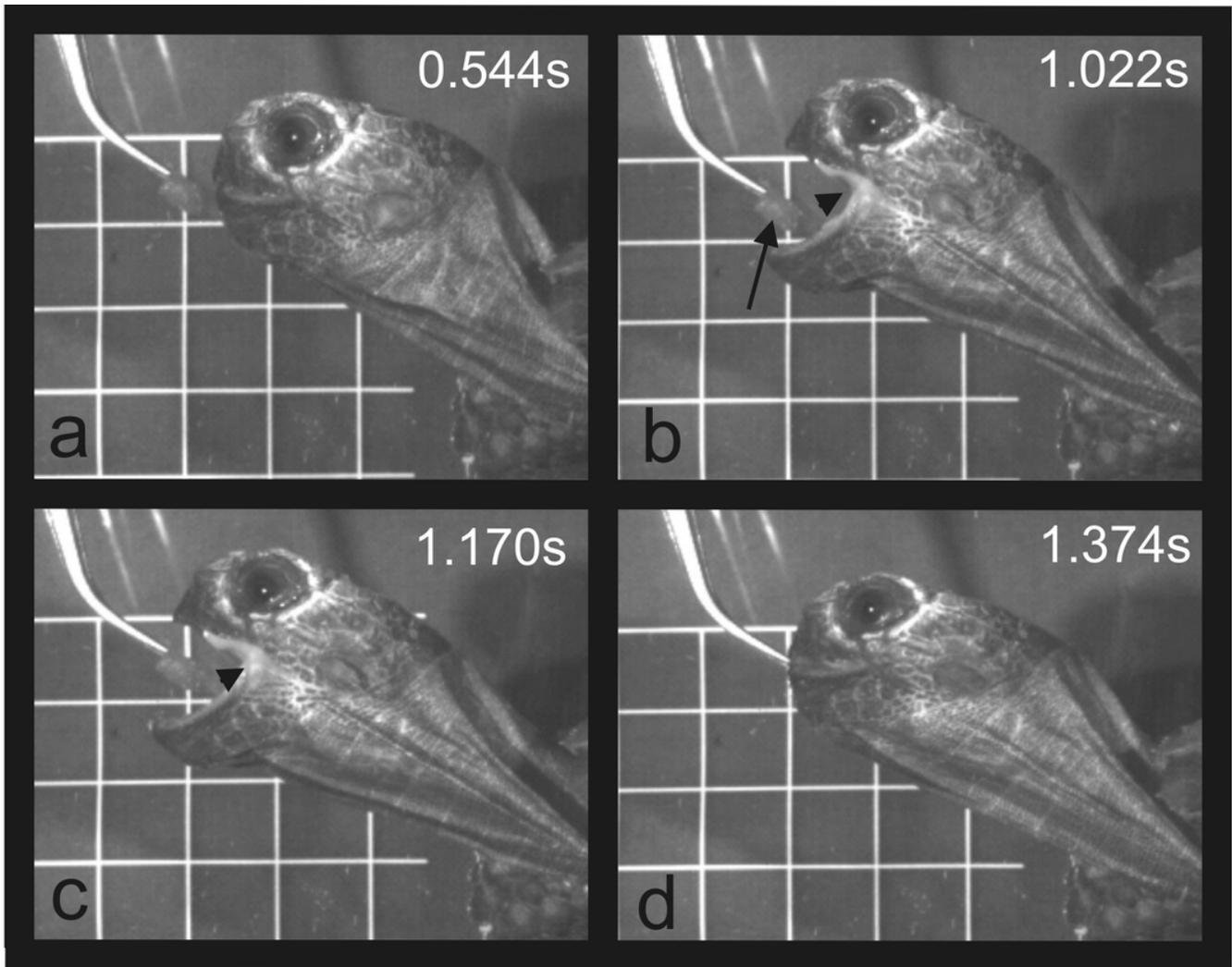
Selected frame from a high-speed video sequence (500 fr/s) of food transport in *Manouria emys*, showing the landmarks used for kinematic analyses: C, rostral tip of sagittal carapace, Hy, hyoid at the basis of ceratbranchial I; LJ, tip of the lower jaw; P, posteriormost point of crista supraoccipitale; 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 10x10 mm.



3

Figure 3

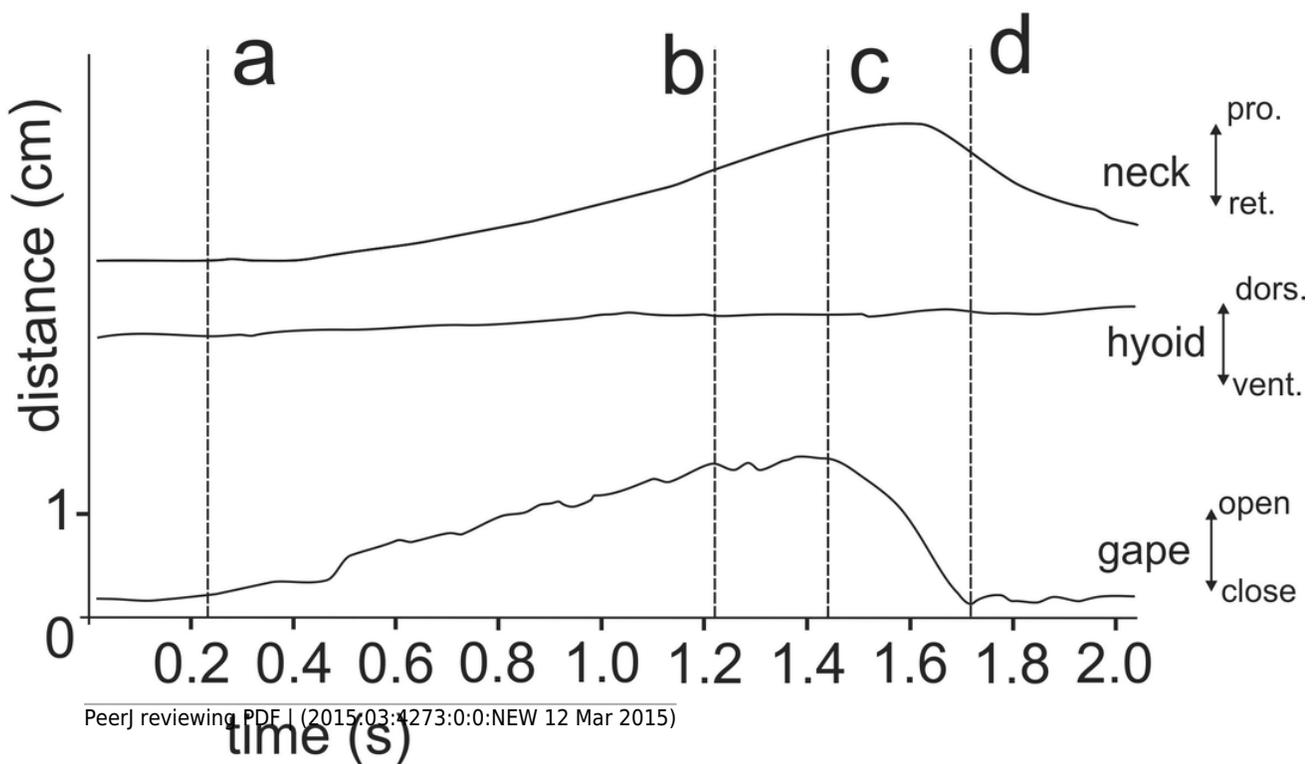
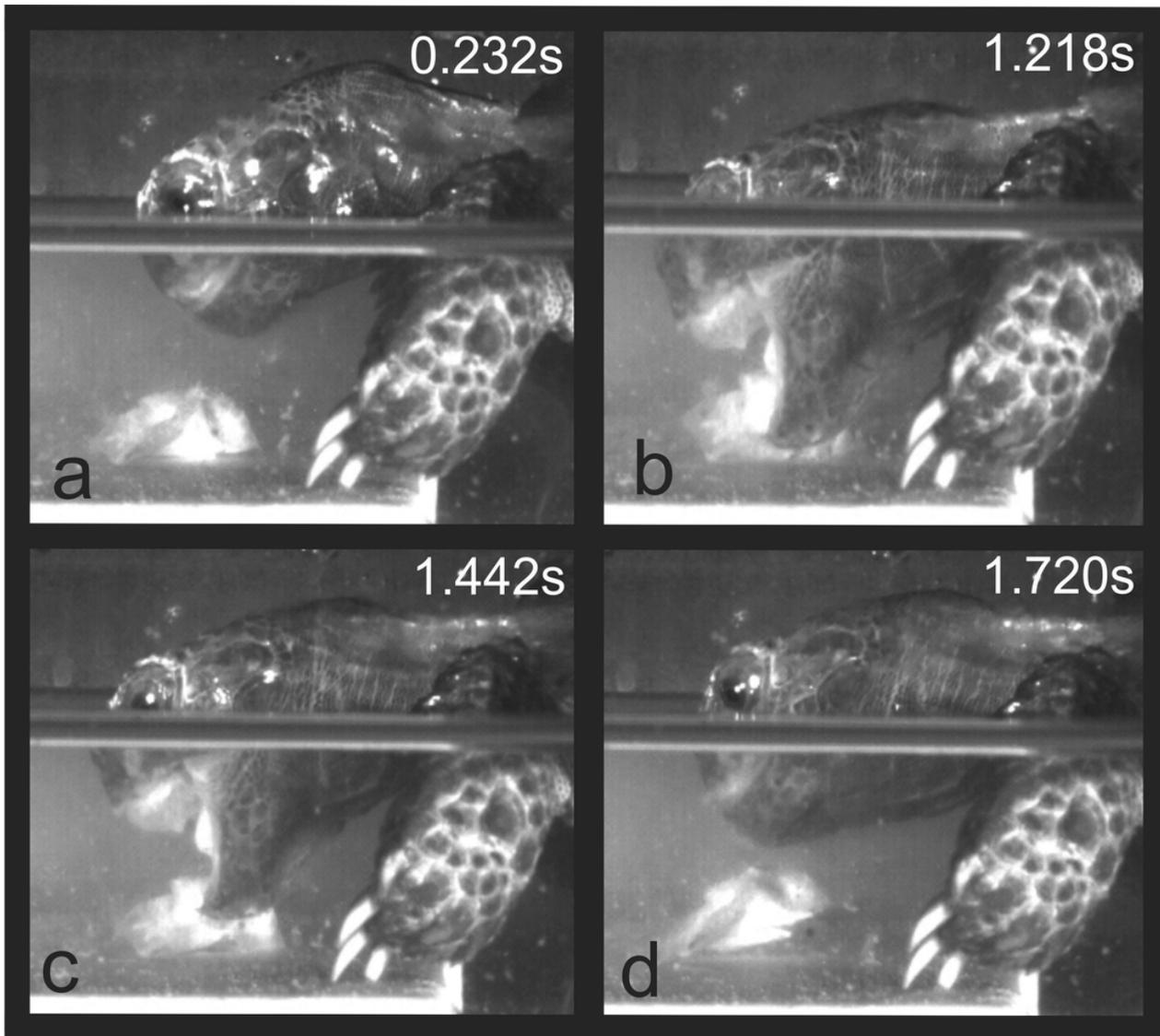
Selected frames and graphics (based on a high-speed video with 500 fr/s) represent the movements of jaws, hyoid and the head during terrestrial food uptake in *Manouria emys* when feeding on pieces of beef heart; a, slow open phase end (lacking discrete SOI and SOII); b, fast open end; c, fast close start; d, fast close end; arrow indicates the position of the food item; arrowheads represent the position of the tip of the tongue; grid 10x10 mm.



4

Figure 4

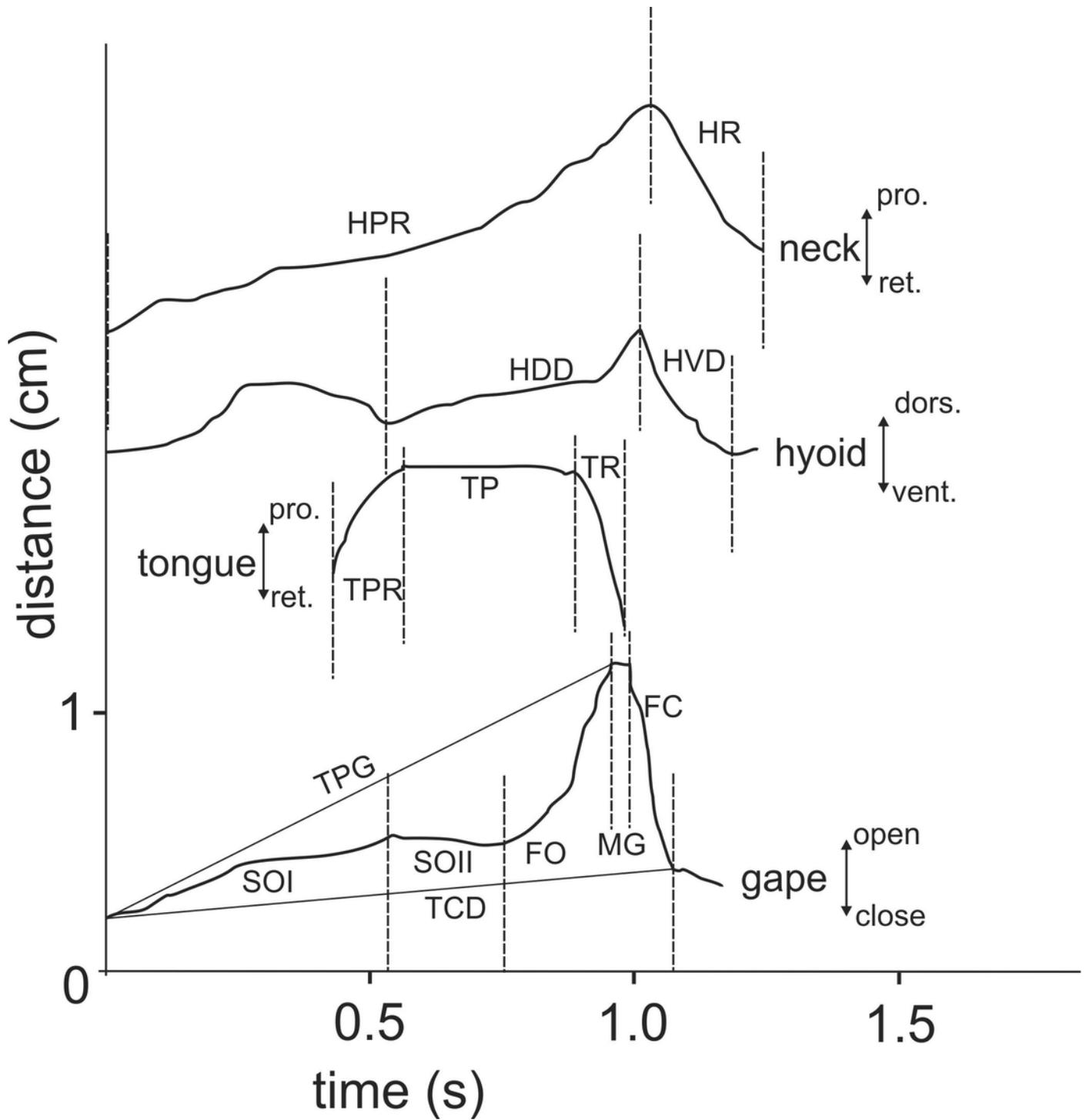
Selected frames and graphics (based on high-speed video with 250 fr/s showing the movements of jaws, hyoid, and head during attempts of aquatic food uptake in *Manouria emys*; a, start of the gape cycle; b, end of jaw opening; c, maximum gape end; d, fast closure end; note the lack of movement of the hyoid complex during the whole cycle; grid 10x10 mm. dors., - dorsal; vent. - ventral; pro - protraction; ret - retraction.



5

Figure 5

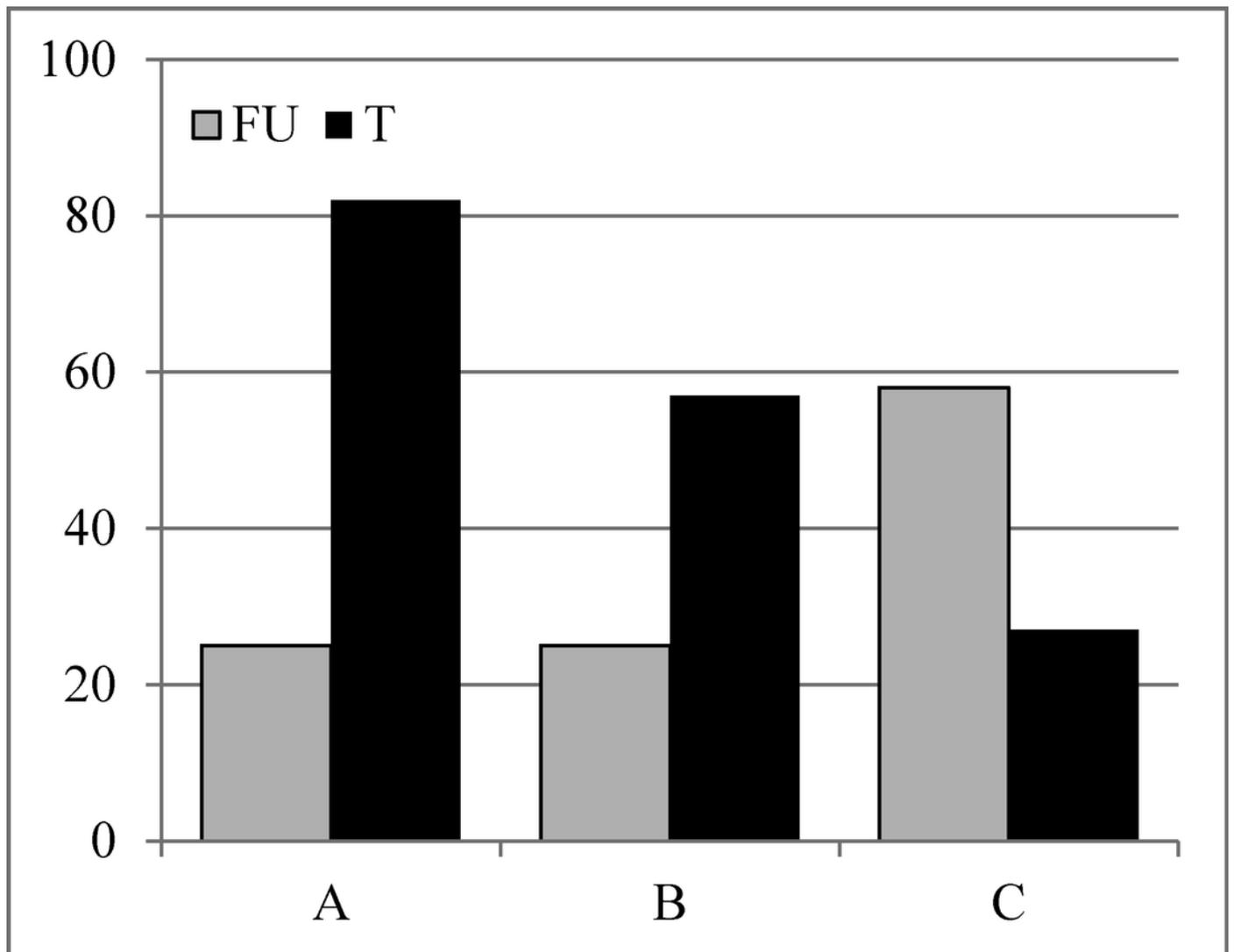
Selected graphics (based on a high-speed video with 500 fr/s) showing the movement patterns of jaws, hyoid, tongue and head during terrestrial food transport in *Manouria emys*; note the delay in hyoid ventral displacement relative to the start the retraction of the tongue tip, as well as the delay of both the tongue retraction and hyoid retraction relative to the start of the FO phase. Abbreviations: FC, fast closure; GCM, generalised cyclic model; HDD, hyoid dorsal displacement; HPR, head protraction; HR, neck retraction; HRV, hyoid retraction velocity; HVD, hyoid ventral displacement; MG, maximum gape; PG, peak gape; SO, slow open phase lacking discrete SOI and SOII; SO I, slow open phase I; SO II, slow open phase II; T, transport; TCD, total cycle duration; THC, total hyoid cycle; TP, tongue plateau; TPG, time to peak gape; TPR, tongue protraction; TR, tongue retraction.



6

Figure 6

Frequencies of occurrence of sequences with absence of selected variables in food uptake (FU) (light grey bars) and food transport (T) (black bars) phases, expressed as: percentages missing any possible split in slow and fast open gape phases - SO and FO (A); cycles in which SO phase was detectable, but the split of discrete slow gape phase in SOI and SOII is missing (B); lack of MG phase (C).



7

Figure 7

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

