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# 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 behaviour in *Manouria emys***

6

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18

19 **ABSTRACT**

20

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

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38

39 **Key words:** turtle, food uptake, feeding kinematics, evolution, Tetrapoda, terrestrial transition

## 41 INTRODUCTION

42 Comprising more than 180 species, the cryptodiran taxon Testudinoidea represents the most diverse  
43 group of extant turtles (e.g., Fritz and Havaš, 2007; Thomson and Shaffer, 2010; Turtle Taxonomy  
44 Working Group 2014). Traditionally, it contains three major extant groups, including the emydid  
45 turtles, the geoemydid turtles, and the testudinid turtles (tortoises) (Fig. 1). All molecular phylogenetic  
46 studies confirm a sister group relationship of Testudinidae and Geoemydidae (Iverson et al., 2007;  
47 Shaffer, 2009; Thomson and Shaffer, 2010; Barley et al., 2010) and Emydidae evolved independently  
48 from the former groups (Fig. 1). The relationship of Platysternidae to other turtle groups remains  
49 unresolved (Fig. 1; reviewed by Parham et al. 2006), but most molecular studies support a closer  
50 relationship to emydids (e.g., Shaffer and Thomson, 2010, Crawford et al., 2015). Palaeontological  
51 studies have shown that all testudinoids share a common aquatic ancestor, from which terrestrial  
52 species evolved (Danilov, 1999; Sukhanov, 2000; Joyce and Gauthier, 2004).

53 The majority of the Triassic stem turtles were terrestrial as indicated by the design and proportions  
54 of the limbs, which were adapted for terrestrial locomotion (for a comprehensive discussion see Joyce,  
55 in press). With the emergence of modern turtles (Testudines) during the Jurassic period (e.g., Danilov  
56 and Parham, 2006; Sterli, 2010; Sterli and de la Fuente, 2011), a general transition of turtles into an  
57 aquatic environment occurred (Willis et al., 2013). The invasion of aquatic environments induced  
58 diversification into several subgroups (see Joyce, 2007; Thomson and Shaffer, 2010). Due to the  
59 different physical properties (drag, viscosity, etc.) of air and water, the new environment required  
60 morphological and functional adaptations of the locomotion- and feeding system to enable efficient  
61 swimming behaviour and aquatic food uptake (i.e. suction feeding) (Schumacher, 1973; Lemell et al.,  
62 2002).

63 Among modern turtles, a transition from an aquatic to a semi-terrestrial or fully terrestrial lifestyles  
64 and the capacity to exploit terrestrial food sources has evolved independently within all three major

65 testudinoid lineages (for overview see Summers et al., 1998; Natchev et al., 2009). At least eight  
66 emydid species are able to feed on land as well as under water (see Bels et al., 1997, 2008; Summers et  
67 al., 1998; Stayton, 2011). During terrestrial feeding, such amphibious emydids use their jaws to grasp  
68 food items (jaw prehension). Similarly, all amphibious geoemydids studied to date also use jaw  
69 prehension in terrestrial food uptake (see Heiss et al., 2008; Natchev et al., 2009). In contrast, all  
70 testudinids studied so far use the tongue to touch the food items, a behaviour referred to as “lingual  
71 prehension” (see Wochesländer et al., 1999; Bels et al., 2008). According to Bels et al. (2008), lingual  
72 prehension is obligatory for all tortoises.

73 The tortoises show a clear tendency towards herbivory and emancipation from water as living and  
74 feeding medium (see Pritchard, 1979; Ernst and Barbour, 1989; Bonin, Devaux and Dupre, 2006). In  
75 fact, testudinids seem to have lost their ancestral ability to feed under water and exclusively rely on  
76 terrestrial trophic ecologies. Some predominantly terrestrial geoemydids are able to complete the whole  
77 feeding process on land and under water (Natchev et al., 2010). Similarly, testudinids with tendencies  
78 towards an amphibious lifestyle might have retained the ancestral feature to feed underwater. Hence,  
79 information on bimodal feeding mechanisms in tortoises is of great importance to understand the  
80 evolution of terrestrial feeding mechanisms and subsequent evolution of the predominantly terrestrial  
81 lifestyle in tortoises.

82 The genus *Manouria*, being of the most ‘basal’ extant tortoises, with a strong association to aquatic  
83 environments (Høybye-Mortensen, 2004; Stanford et al., 2015) constitutes a suitable model to study  
84 the feeding mechanisms in testudinids. Its partial aquatic feeding habit purported to be associated with  
85 the observed morphological extension of the palatines onto the triturating surface of the upper jaw  
86 (character 30 sensu Gerlach, 2001), a diagnostic feature common to geoemydid (=batagurid) turtles.  
87 Another geoemydid-like feature is the unique existence of class II mental glands (Winokur and Legler,  
88 1975).

89 The present study was conceived to provide a detailed analysis of the feeding behaviour in a  
90 species of the genus *Manouria*. *Manouria emys* is found in close association with water. Hence, we  
91 designed experiments to reveal whether this species is able to complete the entire feeding process  
92 under both aquatic and terrestrial conditions as some geoemydids do.

93 Wochesländer et al. (1999, 2000) and Bels et al. (2008) stated that the feeding kinematics in all  
94 testudinids involve two common features: an obligatory lingual prehension and the split of the gape  
95 cycle in four main phases [slow open phase I (SOI); slow open phase II (SOII); fast open phase (FO);  
96 fast close phase (FC)]. Accordingly, the second main goal of this study is to test whether these  
97 elements are also typical for the feeding behaviour in the ‘basal’ modern tortoises, i.e. *Manouria*.  
98 Based on the results of our experiments, we formulate hypotheses concerning the evolution of the food  
99 prehension mode within different turtle groups. Additionally, we test and adjust the classical feeding  
100 models in lower tetrapods and discuss their general validity.

## 102 MATERIALS AND METHODS

### 104 Ecological background

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

111 *M. emys* inhabits tropical evergreen monsoon forests and exhibits high tolerance for soil moisture.  
112 It is commonly found reposing in wet areas, buried in mud or under the leaf litter where it may spend

113 long periods of time. It is active even during rainy weather and direct sun exposure and basking are not  
114 required. Furthermore, this species has a mostly crepuscular and nocturnal lifestyle (Ernst, Altenburg,  
115 and Barbour, 2000; Vetter and Daubner, 2000, Stanford et al., 2015).

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

119

#### 120 Experimental setting

121 Animal husbandry and experiments were in strict accordance with the Austrian Protection of  
122 Animals Act. The animals used in the present study were obtained commercially and kept at 12 h  
123 dark/light cycles in a large terrarium (150x100 cm ground area) with a permanently filtered water basin  
124 and spacious terrestrial area. The turtles were fed different fruits, vegetables, commercially obtained  
125 tortoise pellets, dead mice, as well as pieces of cattle heart and liver, offered on the terrestrial part of  
126 the terrarium. Carapace lengths in the three subadult experimental animals ranged between 109-135  
127 mm with body masses between 234-236 g. For filming terrestrial feeding, the specimens were put in a  
128 dry glass cuvette (24x60x30 cm). When the food was offered on the floor of the cuvette, the tortoises  
129 permanently twisted their necks and rotated their heads in attempt to grasp the food item. The side  
130 movements made the filming of the animals in strict lateral view not possible and the landmarks were  
131 not clearly visible during the sequence. By the use of forceps for food display we completely  
132 eliminated these problems and were able to shoot perfect lateral plans of the feeding turtles. The food  
133 in the feeding experiments was offered at a position which was similar to the position on which we  
134 offered the food in the terrarium where the tortoises were housed. The animals did not extend their  
135 necks extraordinary to reach the food items (see Supplements). The position of the offered food was

136 completely “natural” for the tortoises and they needed to stretch their necks forwards rather than  
137 downwards, which did not impacted other kinematic patterns of the feeding cycles.

138 As food items we used small pieces of cattle heart measuring approximately 5x5x5 mm. The  
139 turtles were filmed from lateral aspect (with a reference grid 1x1 cm in the background) via the digital  
140 high-speed camera system Photron Fastcam-X 1024 PCI (Photron limited; Tokyo, Japan) at 500 fps  
141 with a highly light-sensitive objective AF Zoom - Nikkor 24-85 mm (f/2,8-4D IF). Two “Dedocool  
142 Coolh” tungsten light heads with 2 x 250 W (ELC), supplied by a “Dedocool COOLT3” transformer  
143 control unit (Dedo Weigert Film GmbH; München, Germany) were used for illumination. We filmed  
144 and analysed the food uptake and the food transport cycles in eight feeding sequences for each  
145 specimen.

146 The setting for filming aquatic feeding of submerged food comprised the experimental aquarium  
147 filled with water to a level of 3 cm and presentation of food items in front of the turtle’s snout. In order  
148 to reduce the light intensity and for optimisation of the digitising process, the frame rate was reduced to  
149 250 fps. As the tortoises were unable to grasp the food item in a total of 36 trials, the kinematics of the  
150 feeding apparatus were analysed (see below) in nine selected representative feeding trials.

151 For both terrestrial and “aquatic feeding” sequences, horizontal (X-axis) and vertical (Y-axis)  
152 coordinates of relevant landmarks (see Fig. 2) were digitised frame by frame using “SIMI-MatchiX”  
153 (SIMI Reality Motion Systems; Unterschleißheim, Germany). Based on the displacement of the  
154 markers, we were able to calculate the gape amplitude (distance between the tip of the upper and lower  
155 beak), head movement (distance between the anterior tip of the carapace and the point “P” on Fig. 2),  
156 tongue movements (distance between the most ventral point on tympanum and the tip of the tongue  
157 when visible), and hyoid movements (distance between the point “P” on Fig. 2 and the basis of the  
158 posterior ceratobranchial). To compare the kinematic feeding pattern of *M. emys* to those of other  
159 studied turtles and to understand the coordination between the elements of the feeding apparatus, these

160 data were used for calculation of the following kinematical variables: duration of Slow open phase  
161 (SO); duration of Slow open phases I and II (SOI and SOII) when present; duration of fast open (FO);  
162 duration of maximum gape phase (MG); duration of fast close (FC); time to peak gape (TPG); total  
163 cycle duration (TCD); duration of hyoid protraction (HyDD); duration of hyoid retraction (HyVD);  
164 duration of the total hyoid cycle (THC); hyoid retraction velocity (HyRV); duration of head protraction  
165 (HP); duration of head retraction (HR); duration of tongue protraction (TP); tongue retraction velocity;  
166 delay of the start of hyoid retraction relative to the tongue retraction start; delay of reaching peak gape  
167 relative to start of the hyoid retraction; delay of reaching peak gape relative to tongue retraction start  
168 (see Table 1).

#### 169 Statistics

170 We tested whether there are any differences among the frequency of occurrences of defined  
171 patterns both in food uptake (FU) and food transport (T), i.e. sequences with: missing split of the jaw  
172 opening in SO and FO; without detectable split of discrete SOI and SOII slow gape phase; lacking MG  
173 phase. In order to provide the comparisons, Chi-square test with Yates' correction was performed.  
174 Then we tested for possible existence of differentiation in kinematical variables in both feeding stages.  
175 All variables were tested with the Shapiro-Wilk test for normal distribution. When the p-value was less  
176 than the chosen alpha level ( $p < 0.05$ ), the null hypothesis was rejected and data were excluded from  
177 further analyses. In addition, all variables included in Table 1 were tested with Levene's and Brown-  
178 Forsythe tests and then processed with Welch's ANOVA for heteroscedastic data. Tukey's honest  
179 significant difference test (HSD) was performed for post-hoc analyses when applicable.  
180 Furthermore, in order to express the degree of individual differentiation among the studied specimens,  
181 a Canonical discriminant analysis (CDA) was performed. Standard descriptive statistics including  
182 mean, range, standard deviation [(SD) and confidential interval at 95% CI] were presented.  
183

184

## 185 RESULTS

186

187 When feeding on land, the Asian forest tortoises always grasped food by the jaws. After food  
188 uptake, one to four transport cycles followed prior to oesophageal packing (see Schwenk, 2000). The  
189 tip of the tongue was barely visible during food uptake (see Fig. 3b-c) indicating that the tongue was  
190 not protracted. By contrast, during transport cycles, the cyclic movements of the tongue were well  
191 visible as it was rhythmically pro- and retracted to transport the food item towards the oesophagus (Fig.  
192 5).

193 When trying to feed under water (Fig. 4), *M. emys* submerged its head under the water level, and  
194 by protruding the head, the animals were trying to position the gaping mouth around the food item. The  
195 gape cycle was newer split in slow and fast jaw open phases. The tongue tip was not visible from the  
196 lateral aspect and the hyolingual complex did not protract prior reaching peak gape. No retraction of  
197 the hyoid complex was detected prior jaw closure. The gape cycle duration exceeded for one and a half  
198 seconds and was  $1.94 \pm 0.36$  s (mean  $\pm$  SD). Despite the unsuccessful attempts, the turtles repeatedly  
199 tried to catch the submerged food. In several events, we were able to detect that the food item was  
200 carried away by the bow wave induced by jaw closing.

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

207 When testing the variables of the feeding kinematics (Table 1), nine variables, which describe the  
208 food uptake process, were detected to show significant differences between individuals. In transport  
209 cycles, six out of 18 variables differed significantly amongst individuals (see Table 1). When testing  
210 for differences between grasping and transport cycles, seven out of nine variables differed  
211 significantly: fast jaw open duration (FO;  $F_{\text{Welch}}(1,43)=15.17$ ,  $p=0.011$ ); maximum gape duration (MG;  
212  $F_{\text{Welch}}(1,26)=15.89$ ,  $p=0.001$ ); fast closing (FC;  $F_{\text{Welch}}(1,26)=7.86$ ,  $p=0.010$ ); time to peak gape (TPG;  
213  $F_{\text{Welch}}(1,72)=46.78$ ,  $p<0.001$ ); total gape cycle duration (TCD;  $F_{\text{Welch}}(1,72)=52.50$ ,  $p<0.001$ ); head  
214 protraction duration (HP;  $F_{\text{Welch}}(1,67)=52.23$ ,  $p<0.001$ ); and head retraction duration (HR;  $F_{\text{Welch}}$   
215  $(1,47)=12.57$ ,  $p=0.002$ ).

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

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

226

## 227 **DISCUSSION**

228

229 *M. emys* repetitively tried to feed on dispersed food items under water, which was an unexpected  
230 and hitherto unknown behaviour among tortoises. However, *M. emys* always failed to consume the

231 submerged food. On land, *M. emys* grasped food with the jaws, just like all known aquatic or  
232 semiterrestrial turtles do, but not with the tongue as formerly predicted for all tortoises. On the basis of  
233 our results we discuss several important evolutionary, behavioural, and functional aspects.

234

### 235 **Evolution of food uptake among turtles**

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

246 Among extant turtles, the ability to complete the whole feeding process (including food uptake,  
247 food manipulation and transport, esophageal packing, and swallowing) on land has been tested and  
248 documented for only six species so far. All of them were members of Testudinoidea (Fig. 1; see also  
249 Summers et al., 1998; Bels et al., 2008; Natchev et al., 2009). The terrestrial mode of food uptake  
250 differs dramatically among and within the three testudinoid subgroups (see Bels et al., 1997, 2008;  
251 Summers et al., 1998; Wochesländer et al., 1999, 2000; Natchev et al., 2009, present study).  
252 Correspondingly, it appears as if terrestrial feeding re-evolved several times independently amongst  
253 turtles. Unfortunately, only limited experimental data are available on feeding mechanisms in emydids

254 and geoemydids. Further functional and palaeontological investigations may help to sort out the issues  
255 on the evolution of the feeding behaviour and the morphology of the feeding apparatus in testudinoids.

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

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

274 Based on experimental data, Natchev et al. (2009) described and summarised three categories of  
275 terrestrial food uptake modes among Testudinoidea: (A) Jaw prehension with retracted hyolingual  
276 complex, as observed in the geoemydid genus *Cuora* (Natchev et al., 2009); (B) Jaw prehension with  
277 slightly protracted hyolingual complex, as observed in emydids (Bels et al., 1997; Stayton, 2011); (C)

278 Lingual prehension: The tongue touches the food item prior to food uptake and possibly carries the  
279 food item into the mouth, as documented in all tortoises studied so far (Wochesländer et al. 1999, Bels  
280 et al. 2008). The food uptake mode of *M. emys*, however, differs substantially from that of all  
281 remaining tortoises (category C). In fact, the hyolingual complex in *M. emys* remained fully retracted  
282 during the food prehension on land, and the first contact with the food item was by the jaws.  
283 Accordingly, the feeding mechanism of *M. emys* should be assigned to category A, along with that of  
284 semi-aquatic geoemydids.

285 We now aim to construct a theoretical scenario on the evolution of terrestrial feeding mechanisms  
286 in turtles. Given the aquatic origin of all living turtles, the functional transition from aquatic to  
287 terrestrial feeding mechanisms could hypothetically have involved four stages, beginning with an  
288 exclusively aquatic feeding ancestor. In different lineages and stages, turtles may have left their aquatic  
289 environments for various reasons e.g. for exploiting new food niches. The species that retained  
290 predominantly aquatic life styles may grasp food by the jaws on land, but have to drag it into the water  
291 for further intraoral (hydrodynamic based) transport. In recent turtles, such behaviour was documented  
292 in the kinosternids *C. angustatus* (Weisgram, 1985a,b) and *Sternotherus odoratus* (Heiss et al., 2010;  
293 Natchev et al., 2011), as well as in the emydid *Trachemys scripta* (Weisgram, 1985b; Weisgram et al.,  
294 1989) and other emydids (see Stayton, 2011). Turtles of the second hypothetical evolutionary stage  
295 grasped food by the jaws and the tongue was used for intraoral food transport on land. Such species  
296 would still have retained their underwater feeding ability by using hydrodynamic mechanisms. When  
297 grasping food on land, the tongue remained retracted or was protracted without however touching the  
298 food item. Among extant turtles, such a feeding mode is found in the geoemydid genus *Cuora* (Heiss et  
299 al., 2008; Natchev et al., 2009, Natchev et al., 2010) and in some emydids (Bels et al., 1997; Summers  
300 et al., 1998, Stayton, 2011). In the next theoretical evolutionary step (stage three), behavioural and  
301 morphological adaptations for terrestrial feeding were further advanced, increasing the efficiency of

302 terrestrial food transport at the expense of the ability to use effective hydrodynamic mechanisms in  
303 water. Such species still grasped food items with their jaws on land (as typical for aquatic or  
304 semiaquatic turtles), but were no longer able to take up dispersed food if submerged, which features  
305 prominently in the present case of *M. emys*. Most importantly, stage three could have marked the  
306 “point of no return” in the evolution of terrestrial feeding in extant tortoises. Finally, in a fourth stage,  
307 turtles became fully terrestrial and their tongue was obligatorily involved in food uptake as  
308 documented in the tortoises *Testudo (Eurotestudo) hermanni boettgeri* (Weisgram, 1985b;  
309 Wochesländer et al., 1999; 2000), *Kinixys belliana*, *Geochelone elephantopus* and *G. radiata* (Bels et  
310 al., 2008).

311 According to Bels et al. (2008), tortoises exhibit very stereotypical kinematical patterns in food  
312 ingestion and transport. These authors predicted: obligatory lingual prehension for initial food uptake:  
313 split of the gape cycle in slow open (SO) and fast open (FO) phases: start of the tongue retraction at the  
314 beginning of the fast jaw open phase. Specifically, the authors demonstrated these elements of the  
315 feeding behaviour in the tortoises *G. radiata*, *G. elephantopus*, and *K. belliana*, and Wochesländer et  
316 al. (1999, 2000) in *T. hermanni boettgeri*.

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

322 We propose that hyolingual protrusion evolved in the lineage forming to advanced tortoises (Fig.  
323 1). *Manouria emys* has a large tongue with massive intrinsic and extrinsic musculature (see Heiss et al.,  
324 2011). The advanced and complex lingual musculo-skeletal architecture permits the turtle to protrude  
325 the tongue outside the margins of the rhamphothecae (see Fig. 2). However, *M. emys* does not use

326 lingual food prehension as typical for all other tortoises studied so far. In fact, it seems that the  
327 *Manouria* [and perhaps *Gopherus* (N.N. personal observations)] “linage” has retained the jaw  
328 prehension mechanism inherited from earlier aquatic ancestors. It seems that the tortoises in general  
329 evolved fleshy tongues which improve the food transport performance and advanced tortoises only  
330 refined the behaviour of food uptake on land via lingual food contact prior to jaw closure (see  
331 Wochesländer et al., 1999; Bels et al., 2008).

332

### 333 **Function of the protruded tongue in the testudinid’s food uptake**

334 What would the potential advantage of the obligatory lingual protrusion, found in the more derived  
335 tortoises, be? One possible explanation is that the tongue is used as a prehensile organ for food  
336 ingestion analogous to that found in other tetrapod groups (for overview see Schwenk, 2000; Schwenk  
337 and Wagner, 2001). However, for tortoises such an analogous explanation might be put into question. By  
338 examining all published data available (Wochesländer et al., 1999, Bels et al., 2008), we were not able  
339 to find any convincing evidence that tortoises collect food with their tongues - they just touch it. In all  
340 published feeding sequences, the contact between the food and the tongue is clearly demonstrated - yet,  
341 in all cases, after the initial contact of the tongue with the food, the head moves forward and the food  
342 item is not dragged into the mouth by tongue retraction, but is grasped by the jaws during the fast jaw  
343 closing (FC gape phase). Hence, in extant tortoises, the tongue may not be the main organ that is  
344 catching (collecting) the food, but these are in fact the jaws. Initial food ingestion in tortoises might not  
345 be considered “lingual prehension” (see Schwenk, 2000; Bels et al., 2008) in the strict sense, but  
346 should be regarded as “jaw prehension following lingual contact”. This is imperative for differentiating  
347 and prompts the question: why is an obligatory contact of the tongue to the food present in extant  
348 tortoises (except *Manouria* and also belike *Gopherus*) during food uptake? In other words: why do  
349 tortoises apply a more complex and presumably more energetically expensive food uptake mechanism

350 by including movements of the hyolingual complex in addition to the movements of the neck and jaws  
351 alone?

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

366

### 367 **Intraoral food transport on land**

368 The execution of the transport cycles demand complicated coordination of the activities of the  
369 contractile elements such as the jaw opening and closing muscles, head protracting and retracting  
370 muscles, intrinsic and extrinsic lingual muscles, as well as muscles that protract and retract the  
371 hyolingual complex as a whole unit (Jones et al., 2012; Werneburg, 2011; 2013). In contrast to this  
372 complicated choreography, the mode of food prehension in *M. emys* suggests less complex  
373 neuromotoric coordination between neck and jaw movements. Yet, the execution of the transport

374 cycles is often more than twice shorter in duration (see Table 1). A possible explanation for the longer  
375 duration of food uptake cycles relative to transport cycles might be that during transport the  
376 coordination centres of the muscle activities execution are permanently supplied with information  
377 concerning the position of the food item within the oropharynx and the proper movements can be  
378 executed precisely in a shorter time.

379 Wochesländer et al. (1999, 2000) and Bels et al. (2008) hypothesised that the feeding kinematical  
380 patterns in tortoises adhere exactly to those of the generalized cycle model (GCM) proposed by  
381 Bramble and Wake (1985). In his work on the feeding mechanisms in domestic animals, Bels (2006)  
382 established that the pre-programmed GCM is universally valid among different groups of tetrapods,  
383 including anamniotes, sauropsids, and mammals. The feeding kinematics of the 'basal' extant Asian  
384 forest tortoise differs in some aspects from those proposed by the GCM. The kinematics of the feeding  
385 system in *M. emys* seems to be pre-programmed, but under permanent feed-back control. The values of  
386 the gape and hyoid/hyolingual cycle patterns in the three specimens studied here show high degrees of  
387 variation, both concerning food uptake and food transport (see Table 1 and Figures 5-7). The slow  
388 open phases (SO) are not obligatory. The gape cycle often includes a phase of retaining maximum gape  
389 (see Table 1, Figure 3, 5). In turtles, the maximum gape phase (MG) was described for the gape cycle  
390 in *Cuora sp.* (Natchev et al., 2009, 2010) and was confirmed for other turtles (Natchev et al., 2011).  
391 The presence of a prolonged maximum gape phase (MG) can be easily overlooked when the frame rate  
392 of the film sequence is not high enough (i.e., step between successive frames over 10 milliseconds).

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

398

399 **Relations between the habitat preferences and the diet in turtles**

400 In the evolution of the testudinids there is a clear shift not only in the habitat preferences (from  
401 aquatic to terrestrial), but corresponding shifts are also seen in dietary preferences. In the particular  
402 taxon it manifests in a tendency toward herbivory. Most of the recent tortoises rely on diets mainly  
403 composed of plant material (for overview see Pritchard, 1987; Ernst et al., 2000; Bonin et al., 2006).  
404 The partly carnivorous lifestyle of *Manouria sp.* (Bonin et al., 2006) may be a relic of the carnivorous  
405 diet of the ancestor of the tortoises and supports the transitional status of the genus.

406 Apparently the feeding media (air vs. water) and the dietary shift had a large influence on the  
407 overall feeding behaviour of the testudinids (see Bels et al., 2008). The suction mechanism was lost  
408 and replaced by a jaw food prehension system (this study) or by “lingual prehension” (Wochesländer  
409 et al., 1999; Bels et al., 2008). Probably, the switch to herbivory determined the reorganisations in the  
410 morphology of the jaw muscle system and the proportions of the skull in tortoises (see Werneburg  
411 2011, 2012, 2013). By feeding predominantly on plants (immobile items), the tortoises may prolong  
412 the duration of the food uptake cycles and have more time to adjust their prehension kinematics to  
413 every single feeding situation.

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

421 Jurassic (as Testudines). There may be several reasons for the secondary terrestriality in tortoises and  
422 presumably one of the main was the inter- and intraspecific concurrence for food resources.

423

## 424 **CONCLUSIONS**

425

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

438

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440

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449

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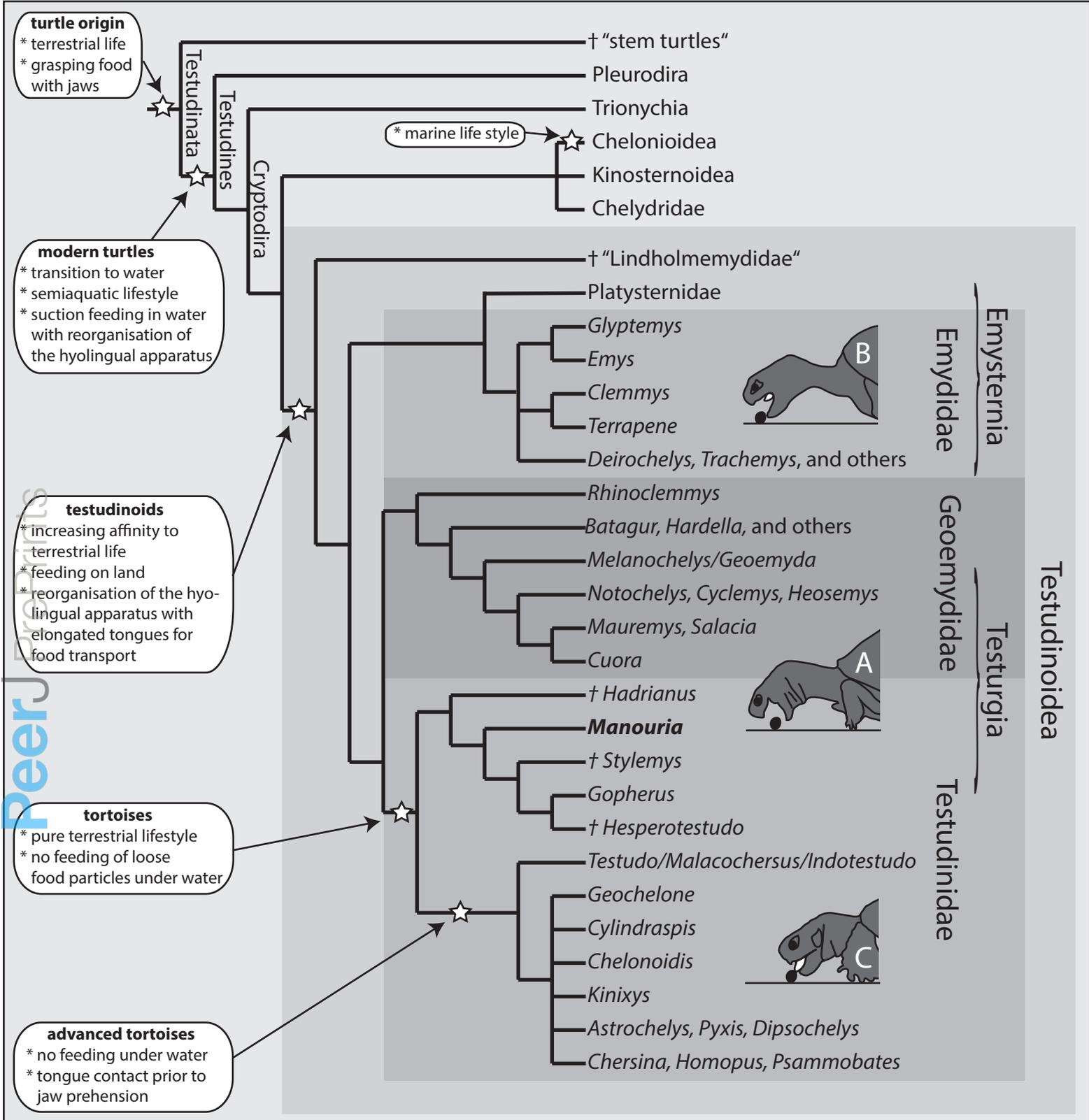
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596

## Figure 1 (on next page)

### Figure 1

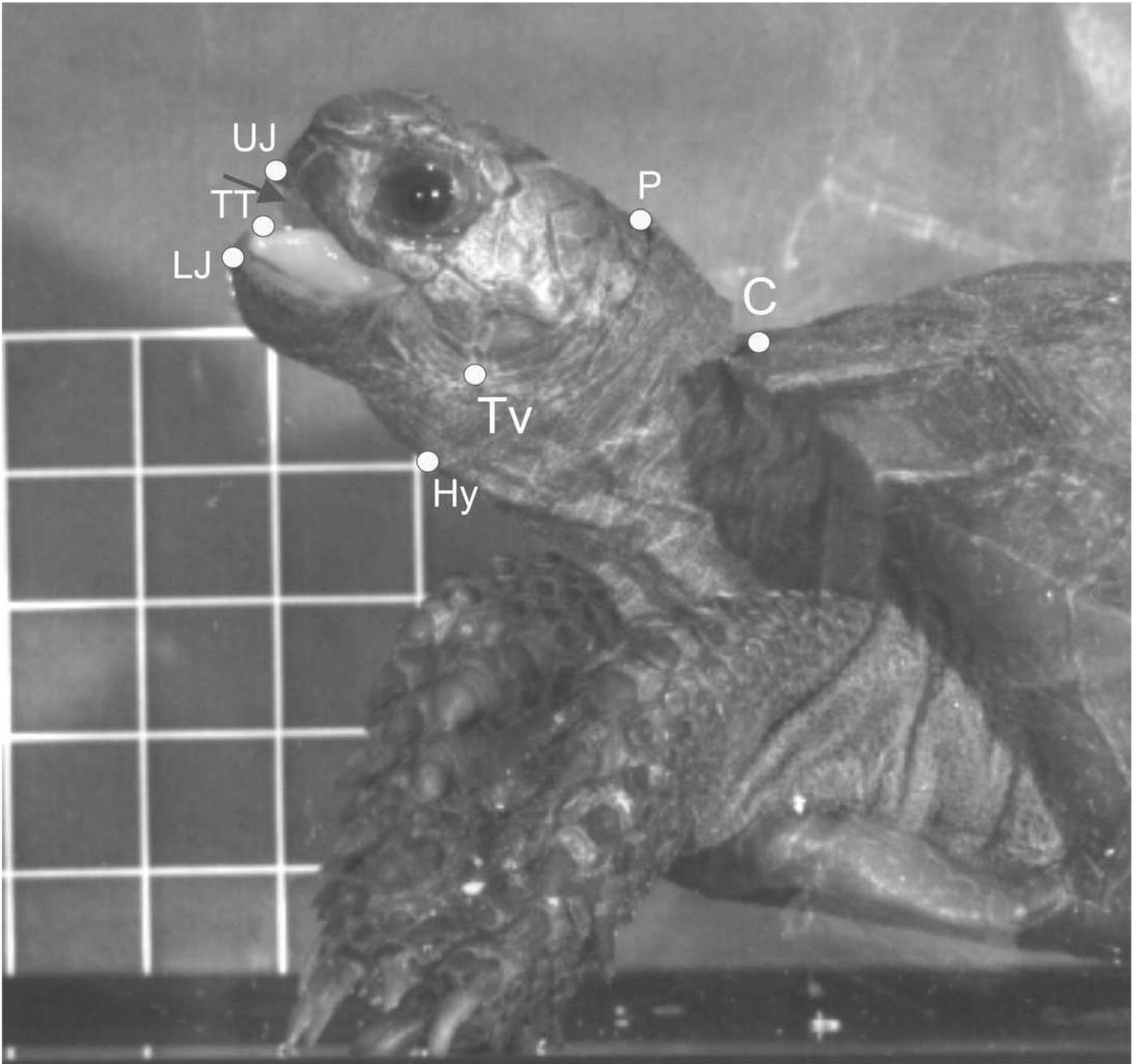
Phylogeny of turtle clades with a focus on Testudinoidea. Interrelationship following Shaffer and Thomson (2011). Those authors assign Platysternidae as sister taxon to Emydidae; here we show different hypothesis for the position of this species. Major evolutionary changes are listed; for details see 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

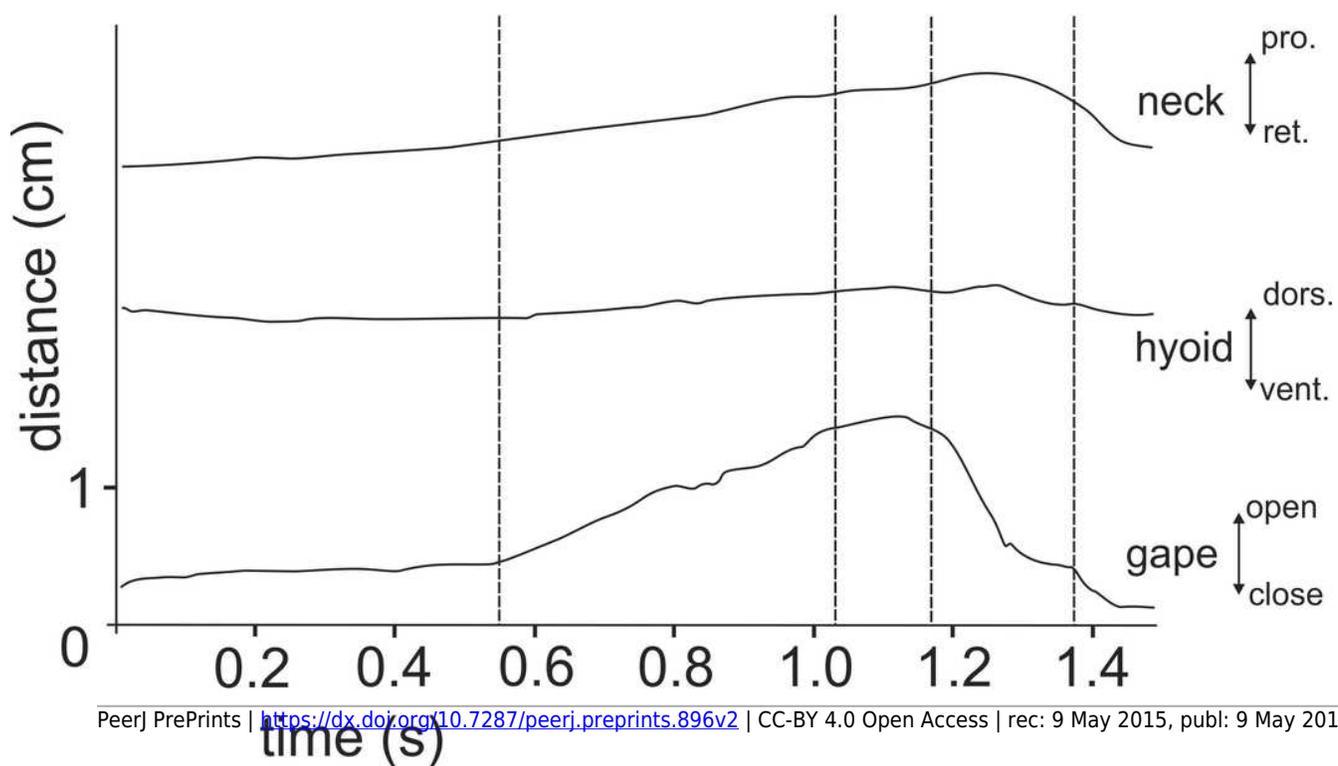
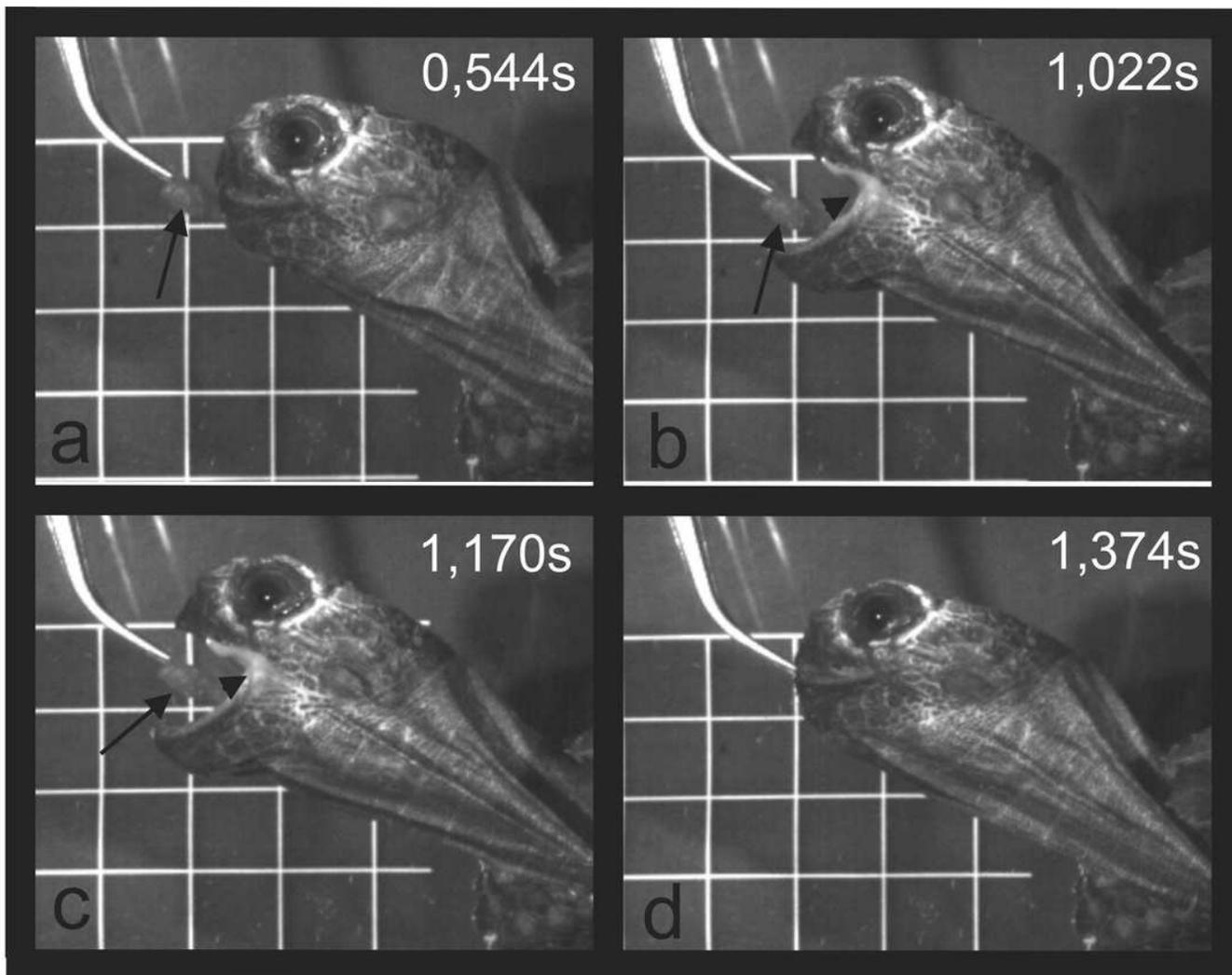
Selected frame from a high-speed video sequence (500 fr/s) of food transport in *Manouria emys*, showing the landmarks used for kinematic analyses: C, rostral tip of sagittal carapace, Hy, hyoid at the basis of ceratbranchial I; LJ, tip of the lower jaw; P, posteriormost point of crista supraoccipitale;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

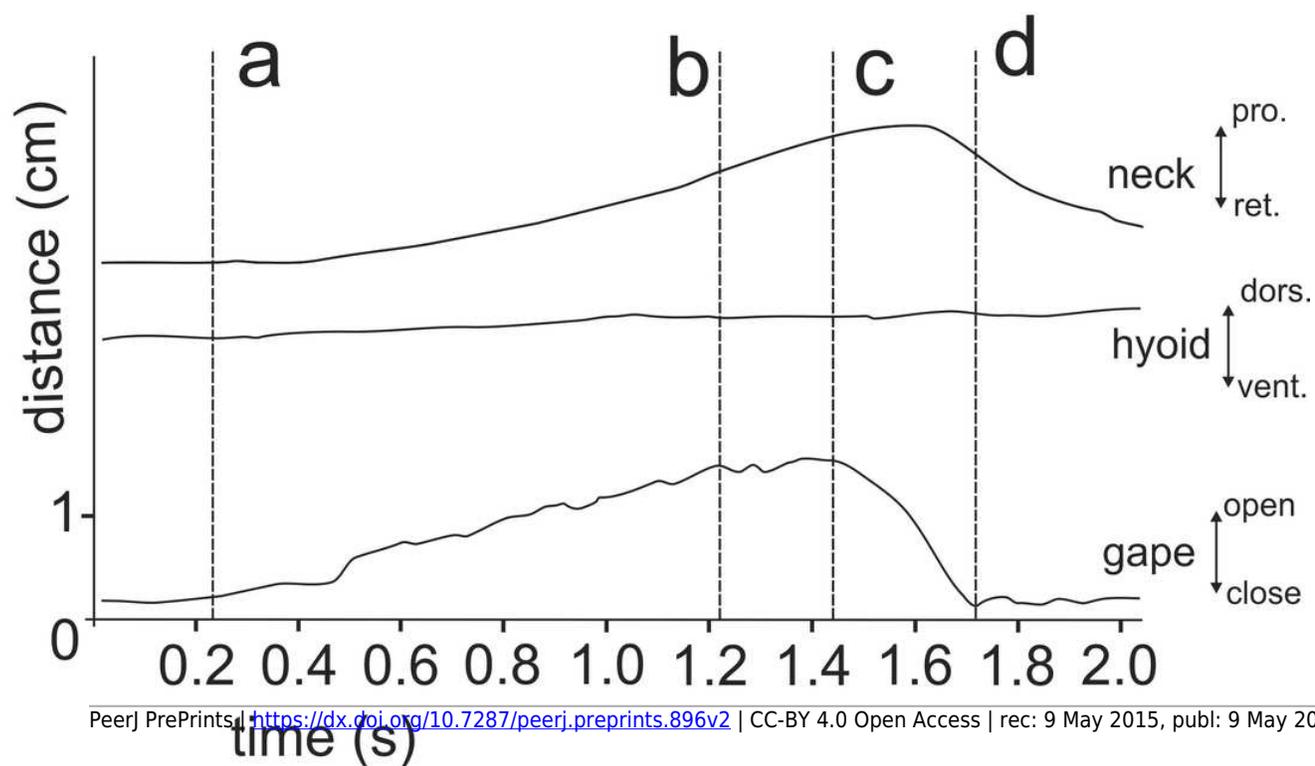
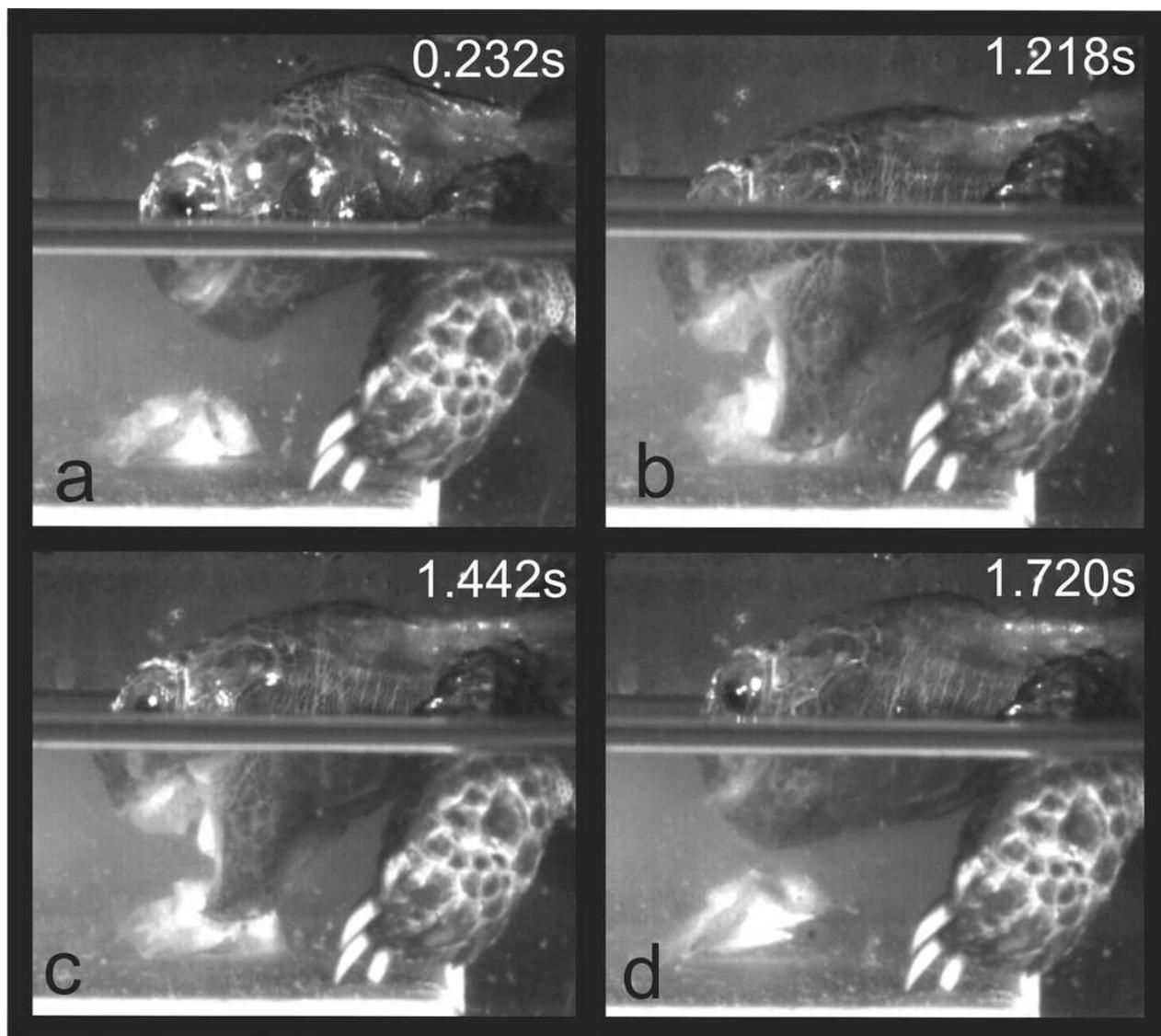
Selected frames and graphics (based on a high-speed video with 500 fr/s) represent the movements of jaws, hyoid and t head during terrestrial food uptake in *Manouria emys* when feeding on pieces of beef heart; a, slow open phase end (lacking discrete SOI and SOII); b, fast open end; c, fast close start;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



# 4

## Figure 4

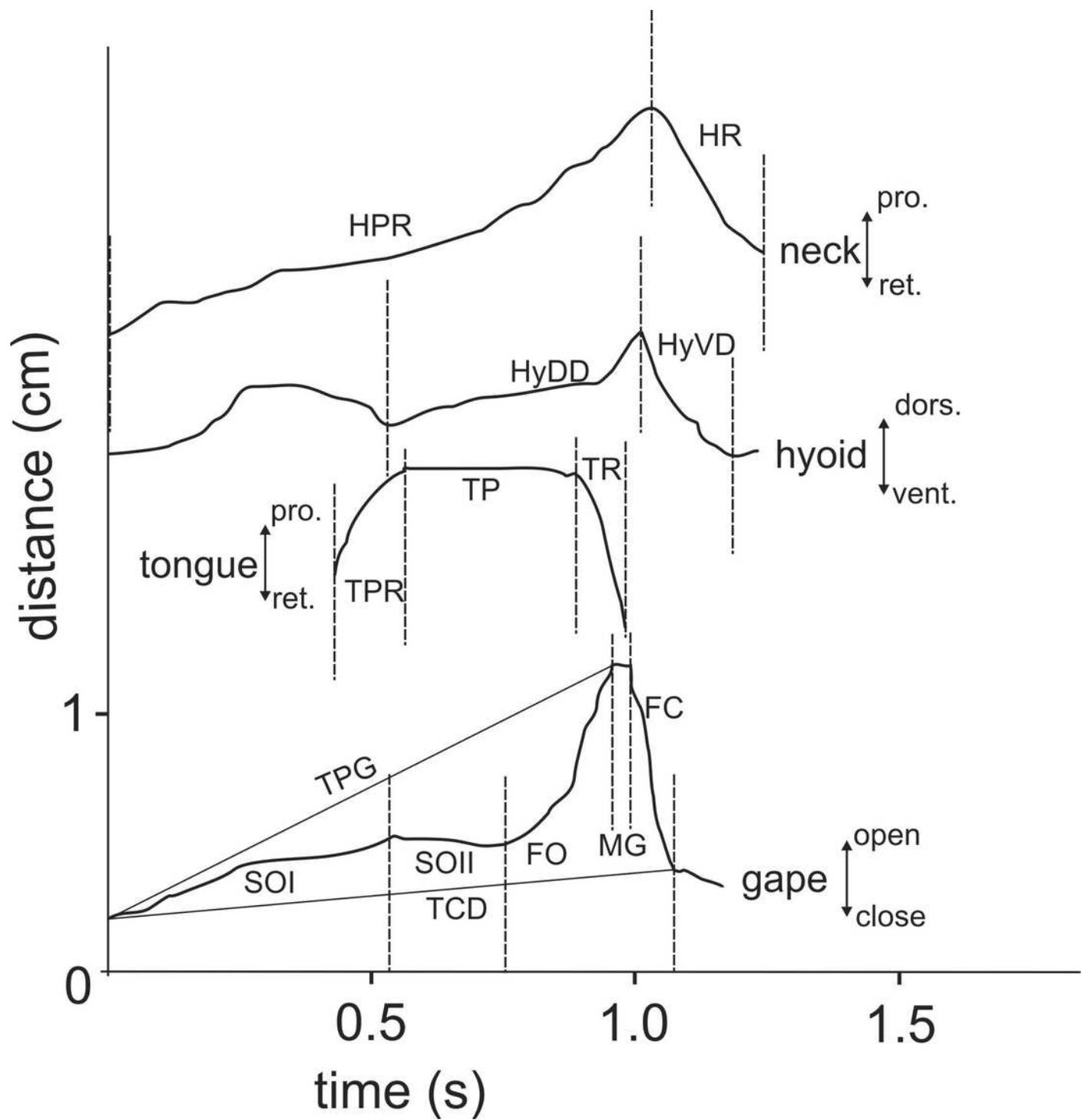
Selected frames and graphics (based on high-speed video with 250 fr/s showing the movements of jaws, hyoid, and head during attempts of aquatic food uptake in *Manouria emys*; a, start of the gape cycle; b, end of jaw opening; c, maximum gape end; d, fast closure end; note the lack of movement of the 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).

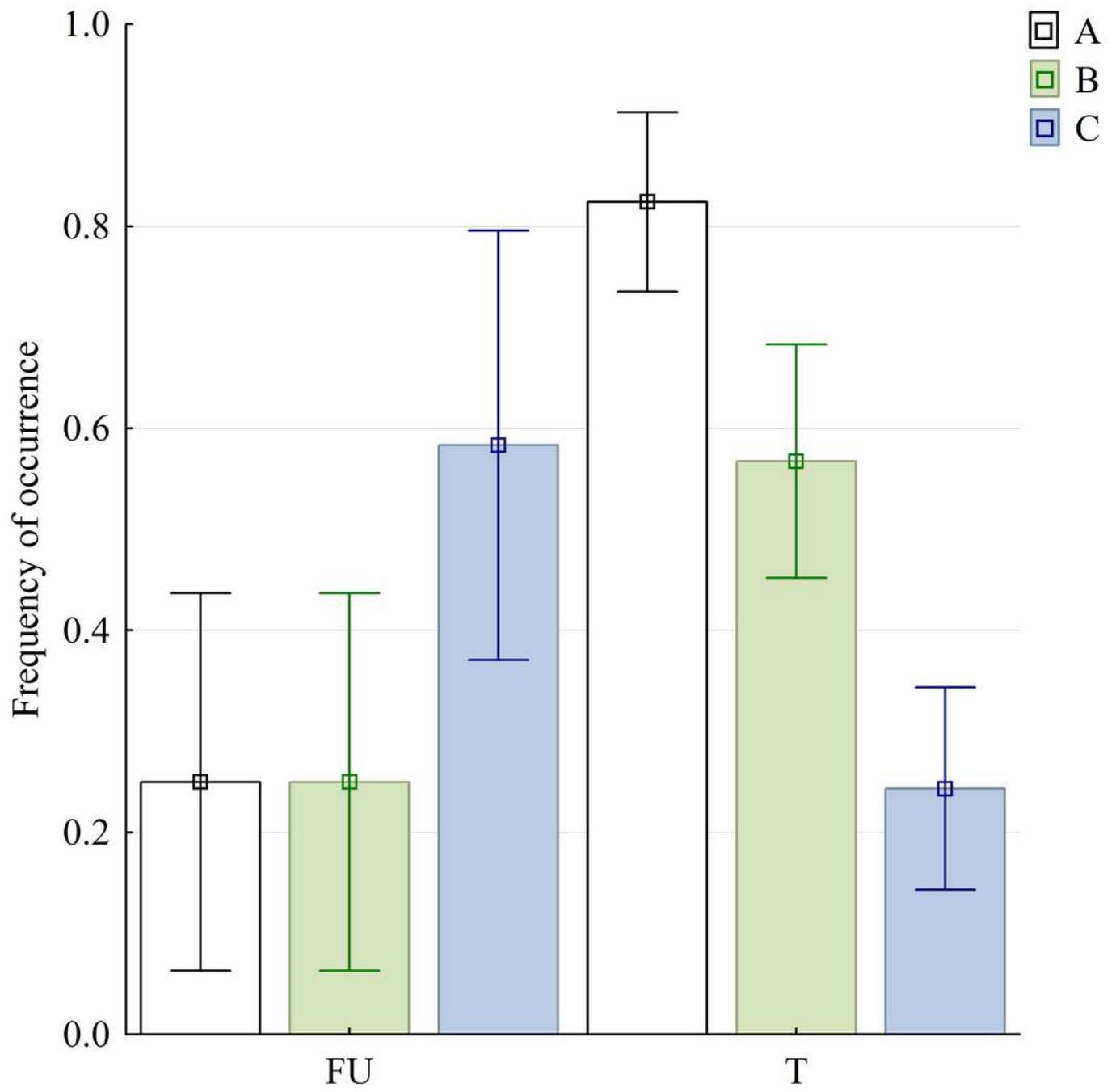
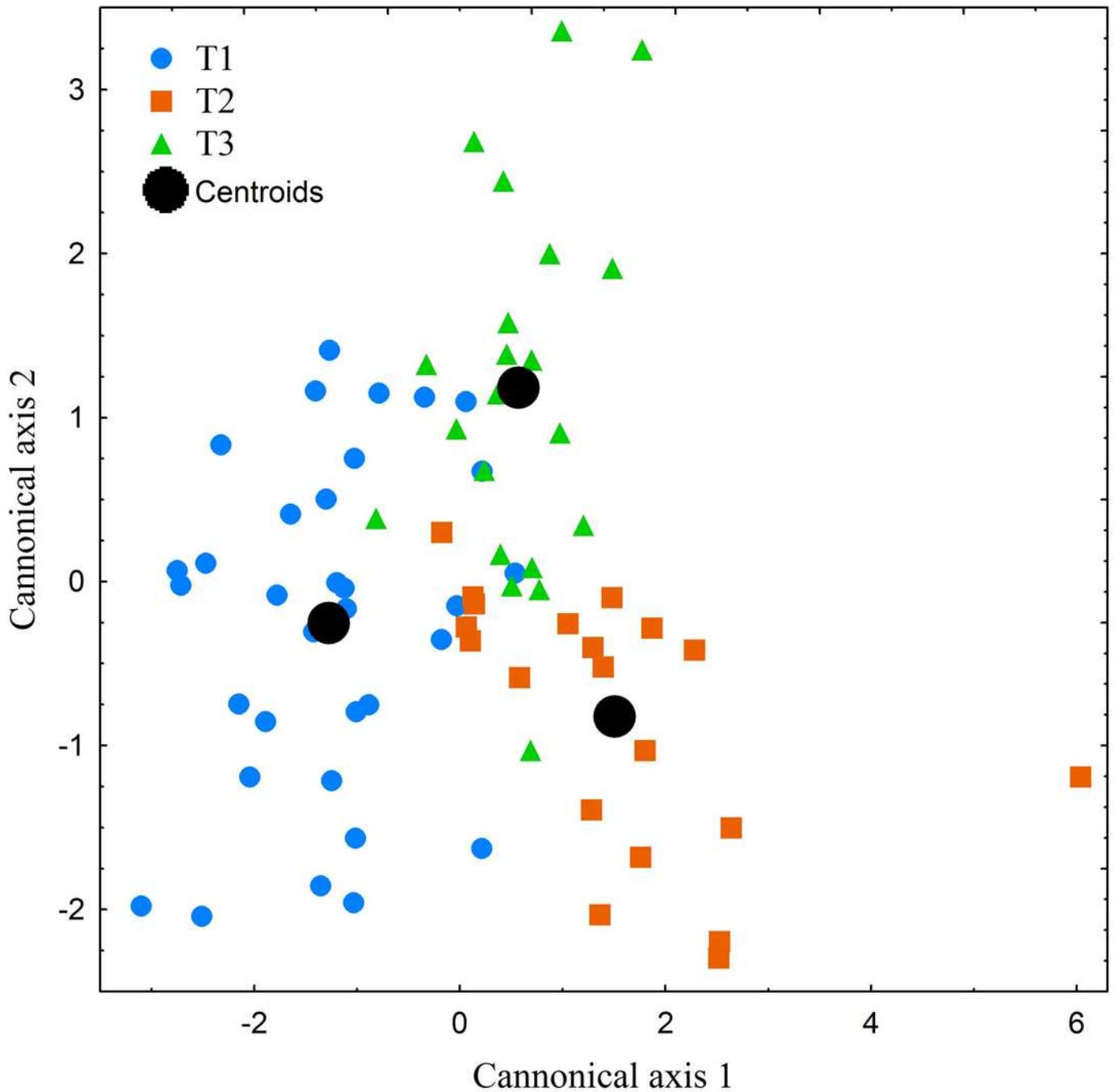


Figure 7

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



**Table 1** (on next page)

Table 1

## 2 Table and table legend

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

6

Variable	Food uptake (FU)			p1	Transport (T)			p2	I vs. T	
	individual 1 (n=8)	individual 2 (n=8)	individual 3 (n=8)		individual 1 (n=33)	individual 2 (n=20)	individual 3 (n=21)		p3	
SOI duration [s]	0.168 $\pm$ 0.060 n=2	0.618 $\pm$ 0.231 n=3	0,562 n=1	n.c.	0.146 $\pm$ 0.016 n=18	0.126 $\pm$ 0.014 n=14	0.115 $\pm$ 0.015 n=11	0,378	0,068	
SOII duration [s]	0.738 $\pm$ 0.508 n=2	0.453 $\pm$ 0.294 n=3	1,024 n=1	n.c.	0.147 $\pm$ 0.014 n=18	0.187 $\pm$ 0.027 n=13	0.190 $\pm$ 0.021 n=11	0,187	0,072	
FO duration [s]	0.450 $\pm$ 0.060 n=2	0.379 $\pm$ 0.150 n=3	0,694 n=1	n.c.	0.122 $\pm$ 0.009 n=25	0.126 $\pm$ 0.012 n=15	0.102 $\pm$ 0.006 n=19	0,111	0.011*	
MG duration [s]	0.079 $\pm$ 0.017 n=4	0.095 $\pm$ 0.031 n=4	0.166 $\pm$ 0.044 n=6	0,271	0.025 $\pm$ 0.003 n=6	0.033 $\pm$ 0.004 n=10	0.042 $\pm$ 0.019 n=4	0,318	0.001*	
FC duration [s]	0.157 $\pm$ 0.079 n=8	0.105 $\pm$ 0.036 n=8	0.158 $\pm$ 0.030 n=8	0.024*	0.089 $\pm$ 0.020 n=33	0.186 $\pm$ 0.040 n=20	0.119 $\pm$ 0.088 n=21	0,155	0.010*	
TPG [s]	0.943 $\pm$ 0.144 n=8	0.989 $\pm$ 0.177 n=8	1.784 $\pm$ 0.137 n=8	0.002*	0.408 $\pm$ 0.021 n=33	0.439 $\pm$ 0.038 n=20	0.403 $\pm$ 0.028 n=21	0,187	<0.001*	
TCD duration [s]	1.139 $\pm$ 0.148 n=8	1.128 $\pm$ 0.169 n=8	2.073 $\pm$ 0.144 n=8	0.001*	0.499 $\pm$ 0.020 n=33	0.655 $\pm$ 0.098 n=20	0.510 $\pm$ 0.030 n=21	0,311	<0.001*	
HDD duration [s]					0.281 $\pm$ 0.025 n=30	0.216 $\pm$ 0.035 n=14	0.169 $\pm$ 0.019 n=21	0.005*		
HVD duration [s]					0.176 $\pm$ 0.011 n=31	0.167 $\pm$ 0.014 n=17	0.149 $\pm$ 0.009 n=21	0,162		
THC duration [s]					0.456 $\pm$ 0.028 n=30	0.384 $\pm$ 0.041 n=14	0.317 $\pm$ 0.022 n=21	0.002*		
HRV velocity [cm/s]					0.718 $\pm$ 0.059 n=31	0.938 $\pm$ 0.107 n=17	0.551 $\pm$ 0.071 n=21	0.016*		
HP duration [s]	1.345 $\pm$ 0.159 n=8	1.204 $\pm$ 0.246 n=8	2.494 $\pm$ 0.177 n=8	0.001*	0.220 $\pm$ 0.049 n=32	0.864 $\pm$ 0.132 n=14	0.464 $\pm$ 0.089 n=10	0.001*	<0.001*	
HR duration [s]	0.296 $\pm$ 0.041 n=8	0.487 $\pm$ 0.086 n=7	0.704 $\pm$ 0.174 n=8	0,052	0.236 $\pm$ 0.027 n=14	0.211 $\pm$ 0.025 n=13	0.316 $\pm$ 0.065 n=10	0,333	0.002*	
TP duration [s]					0.165 $\pm$ 0.008 n=30	0.160 $\pm$ 0.017 n=19	0.133 $\pm$ 0.025 n=13	0,483		
TR velocity [cm/s]					7.459 $\pm$ 0.550 n=31	5.798 $\pm$ 0.547 n=20	6.562 $\pm$ 0.595 n=13	0,121		
delay of HVD start relative to TR start [s]					-0.2011 $\pm$ 0.026 n=30	-0.039 $\pm$ 0.231 n=15	-0.082 $\pm$ 0.025 n=14	0.005*		
delay of TPG relative to HVD start [s]					-0.007 $\pm$ 0.007 n=31	-0.016 $\pm$ 0.008 n=17	-0.032 $\pm$ 0.005 n=21	0.014*		
delay of TPG relative to TR start [s]					-0.062 $\pm$ 0.007 n=31	-0.045 $\pm$ 0.013 n=20	-0.055 $\pm$ 0.008 n=14	0,521		

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