

A peer-reviewed version of this preprint was published in PeerJ on 11 August 2015.

[View the peer-reviewed version](https://peerj.com/articles/1172) (peerj.com/articles/1172), which is the preferred citable publication unless you specifically need to cite this preprint.

Natchev N, Tzankov N, Werneburg I, Heiss E. 2015. Feeding behaviour in a 'basal' tortoise provides insights on the transitional feeding mode at the dawn of modern land turtle evolution. PeerJ 3:e1172
<https://doi.org/10.7717/peerj.1172>

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

Nikolay Natchev, Nikolay Tzankov, Ingmar Werneburg, Egon Heiss

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

7 Nikolay Natchev^{1,2}, Nikolay Tzankov³, Ingmar Werneburg⁴, Egon Heiss⁵

8

9 ¹ Department of Integrative Zoology, Vienna University, Althanstrasse 14, 1090 Vienna, Austria

10 ² Faculty of Natural Science, Shumen University, Universitetska 115, 9700 Shumen, Bulgaria

11 ³ Section Vertebrates, National Museum of Natural History, Bulgarian Academy of Sciences, Tzar
12 Osvoboditel 1, 1000 Sofia, Bulgaria

13 ⁴ Museum für Naturkunde, Leibniz-Institut für Evolutions- & Biodiversitätsforschung an der
14 Humboldt-Universität zu Berlin, Invalidenstraße 43, 10115 Berlin, Germany

15 ⁵ Institute of Systematic Zoology and Evolutionary Biology, Friedrich-Schiller-University Jena,
16 Erbertstr. 1, 07743 Jena, Germany

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.

37

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 ± 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_{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 apprehensive organ in modern
437 tortoises and the food uptake mode represents a derived jaw prehension system.

438

439 **ACKNOWLEDGEMENTS**

440

441 We would like to thank Josef Weisgram, Andreas Wanninger, Patrick Lemell, Christian Beisser
442 and Thomas Schwaha (Department for Integrative Zoology, University of Vienna) for providing
443 material and suggestions for the executions of our experiments. Alexander Westerström contributed
444 sorely to the revision of our manuscript. Stefan Kummer, Katherina Singer, Monika Lintner and

445 Marion Hüffel are acknowledged for the careful housing of the animals. The reviewers provided
446 helpful comments and suggestions to improve our paper. The study was funded by the FWF Austrian
447 Science Fund, Project no. P20094-B17 on which E.H. and N.N. were employed and the Advanced
448 Postdoc Mobility fund P300P3_158526 of the Swiss National Science Foundation granted to I.W.
449

451

452 **REFERENCES**

453

454 Aerts P, Van Damme J, Herrel A. 2001. Intrinsic mechanics and control of fast cranio-cervical
455 movements in aquatic feeding turtles. *American Zoologist* 41:1299-1310.

456 Anderson NJ. 2009. Biomechanics of feeding and neck motion in the softshell turtle, *Apalone*
457 *spinifera*. Rafinesque. D. A. USA: Idaho State University. P. 89.

458 Barley A J, Spinks R C, Thomson, Shaffer HB. 2010. Fourteen nuclear genes provide phylogenetic
459 resolution for difficult nodes in the turtle tree of life. *Molecular Phylogenetics and Evolution*
460 55:1189-1194.

461 von Bayern L F. 1884. *Zur Anatomie der Zunge - eine vergleichend-anatomische Studie*. München,
462 Literarisch-Artistische Anstalt (Theodor Riedel).

463 Bels VL, Davenport J, Delheusy V. 1997. Kinematic analysis of the feeding behaviour in the box turtle
464 *Terrapene carolina* (L.), (Reptilia: Emydidae). *Journal of Experimental Zoology* 277:198-212.

465 Bels VL. 2006. *Feeding in domestic vertebrates - from structure to behaviour*. UK: CABI Publishing

466 Bels VL, Baussart S, Davenport J, Shorten M, O'Riordan RM, Renous S, Davenport J. 2008.
467 Functional Evolution of feeding behaviour in turtles. In: Wyneken J, Godfrey MH, Bels V, eds.
468 *Biology of turtles*. USA: CRC Press Taylor & Francis Group. p. 189-212.

469 Bonin F, Devaux B, Dupre A. 2006. *Turtles of the world*. Translated by Pritchard PCH. UK, Baltimore:
470 Johns Hopkins University Press.

471 Bramble DM. 1973. Media dependent feeding in turtles. *American Zoologist* 13:1342.

- 472 Bramble DM, Wake DB. 1985. Feeding Mechanisms of Lower Tetrapods. In: Hildebrand M, Bramble
473 DM, Liem KF, Wake DB, eds. *Functional Vertebrate Morphology 13*. Massachusetts and London,
474 England: Harvard University Press Cambridge. p. 230-261.
- 475 Crawford NG, Parham JF, Sellas AB, Faircloth BC, Glenn TC, Papenfuss TJ, Henderson JB, Hansen
476 MH, Simison WB. 2015. A phylogenomic analysis of turtles. *Molecular Phylogenetics and*
477 *Evolution* 83:250–257
- 478 Danilov I. 1999. A new lindholmemydid genus (Testudines: Lindholmemydidae) from the mid-
479 Cretaceous of Uzbekistan. *Russian Journal of Herpetology* 6(1):63-71.
- 480 Danilov I, Parham JF. 2006. A redescription of ‘Plesiochelys’ tatsuensis from the Late Jurassic of
481 China, with comments on the antiquity of the crown clade Cryptodira. *Journal of Vertebrate*
482 *Paleontology*. 26(3):573-580.
- 483 Das I. 1995. *Turtles and tortoise of India*. Bombay, India: Oxford University Press. p. 179.
- 484 Ernst CH, Altenburg RGM, Barbour RW. 2000. *Turtles of the World*. World Biodiversity Database,
485 CD-ROM Series, Windows, Version 1.2. Amsterdam: Biodiversity Center of ETI.
- 486 Fritz U, Havaš P. 2007. Checklist of chelonians of the world. *Vertebrate Zoology* 57(2):149-368.
- 487 Gerlach J. 2001. Tortoise phylogeny and the ‘Geochelone’ problem. *Phelsuma*, suppl. A. 9:1–24.
- 488 Iverson J, Brown BRM, Akre TS, Near TJ, Le M, Thomson RC, Starkey DE. 2007). In search of the
489 tree of life for turtles. *Chelonian Research Monographs* 4:85-106.
- 490 Heiss E, Plenk H, Weisgram J. 2008. Microanatomy of the Palatal Mucosa of the Semiaquatic Malayan
491 Box Turtle, *Cuora amboinensis*, and Functional Implications. *Anatomical records* 291(7):876-885.

- 492 Heiss E, Natchev N, Schwaha T, Salaberger D, Lemell P, Beisser C, Weisgram J. 2011. Oropharyngeal
493 Morphology in the Basal Tortoise *Manouria emys emys* With Comments on Form and Function of
494 the Testudinid Tongue. *Journal of Morphology* 272:1217-1229.
- 495 Høybye-Mortensen K, 2004, The tortoise *Manouria emys emys*: behaviour and habitat in the wild. M.
496 Sc. Thesis, University of South Denmark. p 100.
- 497 Jones MEH, Werneburg I, Curtis N, Penrose R, O'Higgins P, Fagan MJ, Evans SE. 2012. The head
498 and neck anatomy of sea turtles (Cryptodira: Chelonioidea) and skull shape in Testudines. *PLOS*
499 *ONE* 7(11):e47852.
- 500 Joyce WG. 2007. Phylogenetic relationships of Mesozoic turtles. *Bulletin of the Peabody Museum for*
501 *Natural History* 48(1):3-102.
- 502 Joyce WG. 2015 (in press). The origin of turtles: a paleontological perspective. *Journal of*
503 *Experimental Zoology, Part B, Molecular and Developmental Evolution*
- 504 Joyce WG, Gauthier JA .2004. Palaeoecology of Triassic stem turtles sheds new light on turtle origins.
505 *Proceedings of the Royal Society London Series B - Biological Sciences* 271:1-5.
- 506 King G. 1996. Reptiles and herbivory. Chapman & Hall. London
- 507 Lambert FR, Howes JR. 1994. Ranging, breeding behaviour and food of Asian brown
508 tortoise *Manouria emys* in Borneo. *Malayan Nature Journal* 48:125-131.
- 509 Lauder GV, Prendergast T. 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra*
510 *serpentina*. *Journal of Experimental Biology* 164:55-78.
- 511 Lemell P, Beisser CJ, Weisgram J. 2000. Morphology and function of the feeding apparatus of *Pelusios*
512 *castaneus* (Chelonia; Pleurodira). *Journal of Morphology* 244:127-135.

- 513 Lemell P, Lemell C, Snelderwaard P, Gumpenberger M, Wochesländer R, Weisgram J. 2002. Feeding
514 patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). *Journal of Experimental Biology* 205:1495-
515 1506.
- 516 Lourenço JM, Claude J, Galtier N, Chiari Y. 2012. Dating cryptodiran nodes: Origin and
517 diversification of the turtle superfamily Testudinoidea. *Molecular Phylogeny and Evolution* 62:
518 496–507
- 519 Natchev N, Heiss E, Lemell P, Weisgram J. 2008. Kinematic analysis of prey capture, prey transport
520 and swallowing in the Common Musk Turtle *Sternotherus odoratus* (Chelonia, Kinosternidae).
521 *Comparative Biochemistry and Physiology Part A* vol. 150:95.
- 522 Natchev N, Heiss E, Lemell P, Stratev D, Weisgram J. 2009. Analysis of prey capture and food
523 transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata*
524 (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. *Zoology* 112:113-127
- 525 Natchev N, Lemell P, Heiss E, Beisser C, Weisgram J. 2010. Aquatic feeding in a terrestrial turtle: a
526 functional-morphological study of the feeding apparatus in the Indochinese box turtle *Cuora*
527 *galbinifrons* (Testudines, Geoemydidae). *Zoomorphology* 129:111–119.
- 528 Natchev N, Heiss E, Singer K, Kummer S, Salaberger D, Weisgram J. 2011. Structure and Function of
529 the Feeding Apparatus in the Common Musk Turtle *Sternotherus odoratus* (Chelonia,
530 Kinosternidae). *Contributions to Zoology* 80:143-156.
- 531 Nutphand W. 1979. *The Turtles of Thailand*. Thailand: Siamfarm Zoology Garden p. 222.
- 532 Parham JF, Feldman CR, Boore JL. 2006. The complete mitochondrial genome of the enigmatic
533 bigheaded turtle (Platysternon): description of unusual genomic features and the reconciliation of

534 phylogenetic hypotheses based on mitochondrial and nuclear DNA. *BMC Evolutionary Biology*
535 6:1-11.

536 Pritchard PCH 1979. *Encyclopaedia of Turtles*. Neptune NJ : TFH Publications, Inc.

537 Rasmussen ARJ, Murphy C, Ompi M, Gibbons JW, Uetz P. 2011. Marine Reptiles. *PLOS ONE*
538 6(11):e27373.

539 Schumacher GH. 1973. The head muscles and hyolaryngeal skeleton of turtles and crocodylians. In:
540 Gans C, Parsons TS eds. *Biology of Reptilia*. USA:, New York: Academic Press. p. 101-199.

541 Schwenk K. 2000. *Feeding: Form, Function and Evolution in tetrapod Vertebrates*. San Diego-San
542 Francisco-New York-Boston-London-Sydney-Tokyo: Academic Press.

543 Schwenk K, Wagner G. 2001. Function and the Evolution of Phenotypic Stability: Connecting Pattern
544 to Process. *American Zoologist* 41:552–563.

545 Shaffer HB. 2009. Turtles (Testudines). Hedges SB, Kumar S eds. *The Time Tree of Life*. New York,
546 USA: Oxford University Press. p. 398-401.

547 Stanford CB, Wanchai P, Schaffer C, Schaffer R, Thirakhupt K. 2015. *Manouria emys* (Schlegel and
548 Müller 1840) – Asian Giant Tortoise, Giant Asian Forest Tortoise. In: Rhodin AGJ, Pritchard PCH,
549 van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB, Mittermeier RA, eds. *Conservation Biology*
550 *of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and*
551 *Freshwater Turtle Specialist Group*. Chelonian Research Monographs (ISSN 1088-7105) No. 5,
552 doi:10.3854/crm.5.086.emys.v1.2015

553 Stayton CT. 2011. Terrestrial feeding in aquatic turtles: environmentdependent feeding behavior,
554 modulation and the evolution of terrestrial feeding in Emydidae. *Journal of Experimental Biology*
555 214:4083–4091.

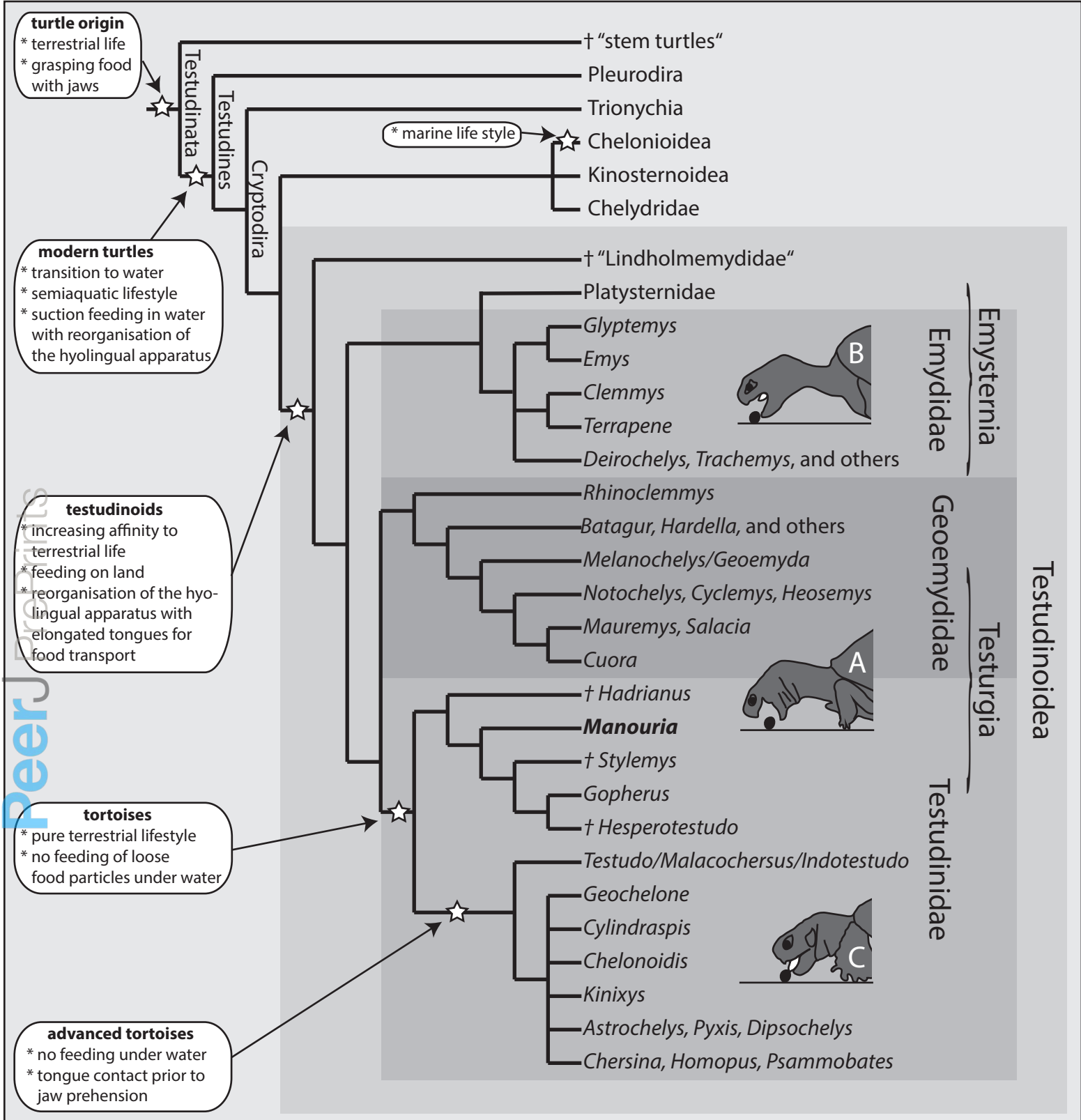
- 556 Sterli J. 2010. Phylogenetic relationships among extinct and extant turtles: the position of Pleurodira
557 and the effects of the fossils on rooting crown-group turtles. *Contributions to Zoology* 79:93-106.
558
- 559 Sterli J, de la Fuente MS. (2011). Re-description and evolutionary remarks on the Patagonian horned
560 turtle *Niolamia argentina* Ameghino, 1899 (Testudinata, Meiolaniidae). *Journal of Vertebrate*
561 *Paleontology* 31(6):1210-1229.
- 562 Sukhanov VB. 2000. Mesozoic Turtles of Middle and Central Asia. In: Benton MJ, Shishkin MA,
563 Unwin DM, Kurochkin EN, eds. *The Age of Dinosaurs in Russia and Mongolia*. Cambridge, UK:
564 University Press. P. 309-367.
- 565 Summers AP, Darouian KF, Richmond AM, Brainerd EL. 1998. Kinematics of Aquatic and Terrestrial
566 Prey Capture in *Terrapene carolina*, With Implications for the Evolution of Feeding in Cryptodire
567 Turtles. *Journal of Experimental Zoology* 281:280-287
- 568 Thomson R C, Shaffer HB. 2010. Sparse supermatrices for phylogenetic inference: taxonomy,
569 alignment, rogue taxa, and the phylogeny of living turtles. *Systematic Biology* 59(1):42-58.
- 570 Van Damme J, Aerts P. 1997. Kinematics and functional morphology of aquatic feeding in Australian
571 snake-necked turtles (Pleurodira; Chelodina). *Journal of Morphology* 233:113-125.
- 572 Vetter H, Daubner M. 2000. Das Schildkrötenlexikon auf CD. Bergheim: L. Staackmann Verlag KG.
- 573 Weisgram J. 1985a. Feeding mechanics of *Claudius angustatus* Cope 1865. In: Duncker HR, Fleischer
574 G, eds. *Fortschritte der Zoologie*. Stuttgart, Germany: Gustav Fischer Verlag. p. 257-260.
- 575 Weisgram J. 1985b. Zum Mechanismus der Nahrungsaufnahme bei Schildkröten. Eine vergleichend-
576 funktionsanatomische Studie an *Claudius angustatus* COPE 1865, *Pseudemys scripta elegans*

- 577 WIED 1839 und *Testudo hermanni hermanni* GMELIN 1789. D. Phil. Thesis Austria, Vienna:
578 University of Vienna. 130 p.
- 579 Werneburg I. 2011. The cranial musculature in turtles. *Palaeontologia Electronica* 14(2):15a:99 pages.
- 580 Werneburg I. 2012. Temporal bone arrangements in turtles: an overview. *Journal of Experimental*
581 *Zoology Part B: Molecular and Developmental Evolution* 318: 235-249.
- 582 Werneburg I. 2013. Jaw musculature during the dawn of turtle evolution. *Organismal Diversity and*
583 *Evolution* 13:225-254.
- 584 Werneburg I. 2014. Konvergente Evolution herbivorer Landwirbeltiere [ein Review]. In: Maier
585 W, Werneburg I (Eds.) "Schlüsselereignisse der organismischen Makroevolution". *Scidinge Hall*
586 *Verlag* Zürich, p. 295-331.
- 587 Willis KL, Christensen-Dalsgaard J, Ketten DR, Carr CE. 2013. Middle ear cavity morphology is
588 consistent with an aquatic origin for Testudines. *PLOS ONE* 8(1):e54086.
- 589 Winokur RM, Legler JM. 1975. Chelonian mental glands. *Journal of Morphology* 147:275-291.
- 590 Winokur BM. 1988. The buccopharyngeal mucosa of the turtles (Testudines). *J Morphol.* 196:33-52.
- 591 Wochesländer R, Hilgers H, Weisgram J. 1999. Feeding Mechanism of *Testudo hermanni boettgeri* (
592 Chelonia, Cryptodira). *Netherland Journal of Zoology* 49:1-13.
- 593 Wochesländer R, Gumpfenberger M, Weisgram J. 2000. Intraoral food transport in *Testudo hermanni*
594 (Chelonia, Cryptodira) – a radiographic video analysis. *Netherland Journal of Zoology* 50:445-454.
595
596

Figure 1 (on next page)

Figure 1

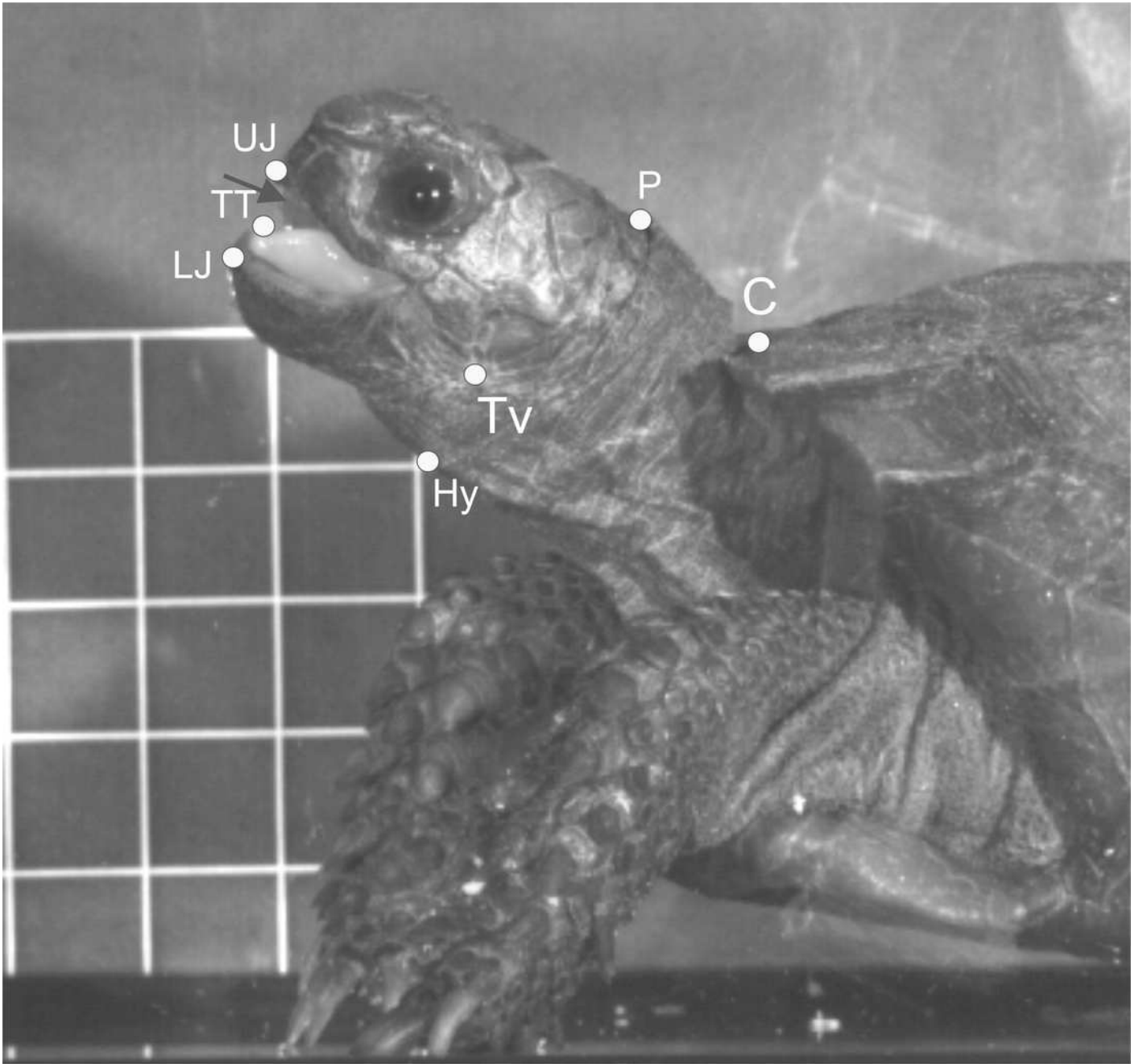
Phylogeny of turtle clades with a focus on Testudinoidea. Interrelationship following Shaffer and Thomson (2011). Those authors assign Platysternidae as sister taxon to Emydidae; here we show different hypothesis for the position of this species. Major evolutionary changes are listed; for details see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

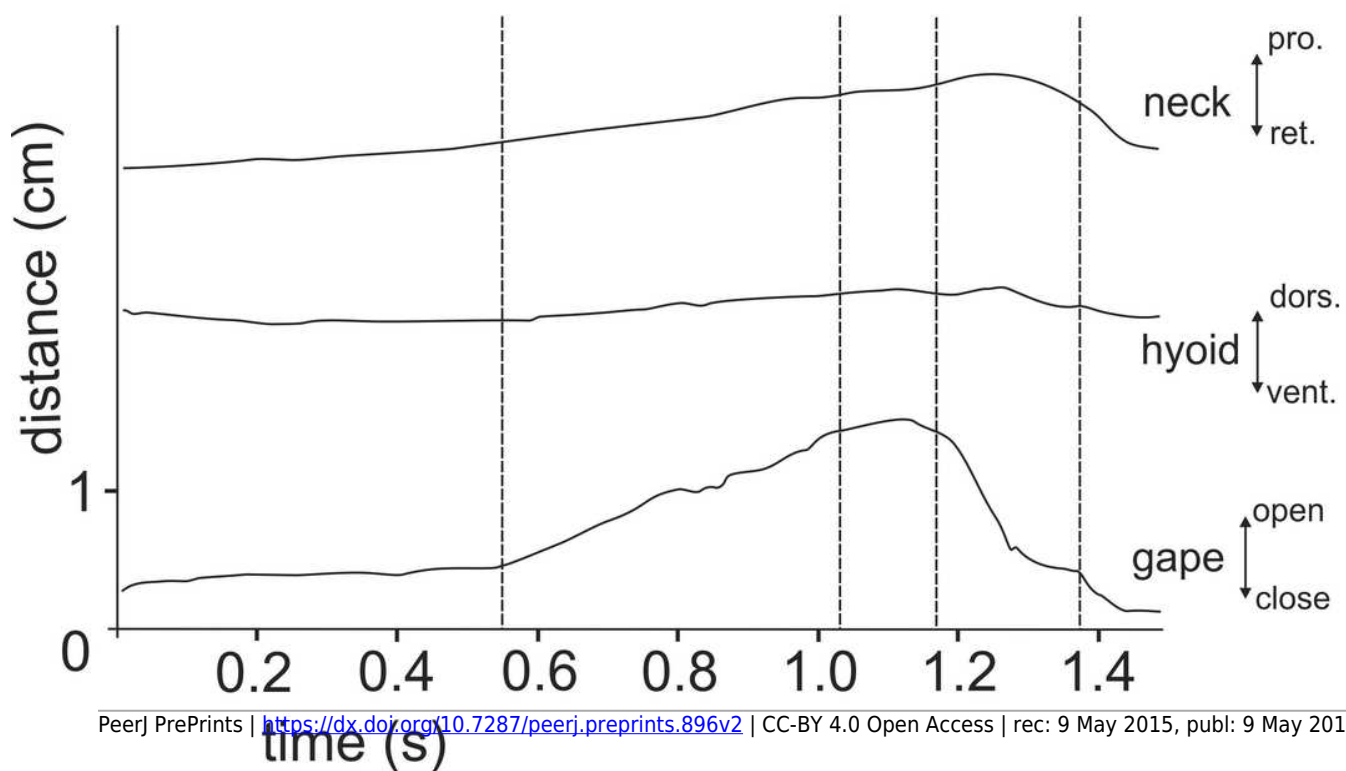
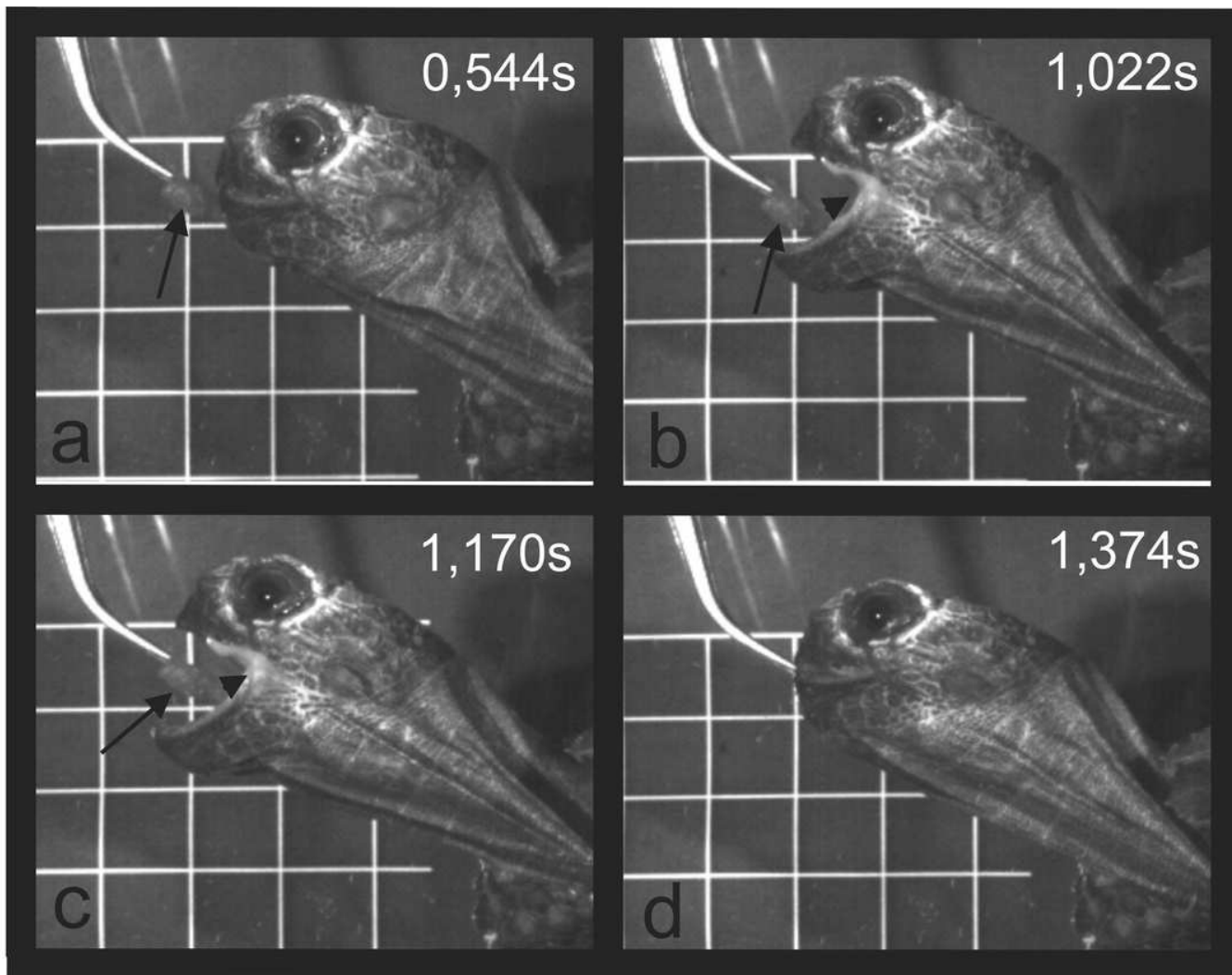
Selected frame from a high-speed video sequence (500 fr/s) of food transport in *Manouria emys*, showing the landmarks used for kinematic analyses: C, rostral tip of sagittal carapace, Hy, hyoid at the basis of ceratbranchial I; LJ, tip of the lower jaw; P, posteriormost point of crista supraoccipitale;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

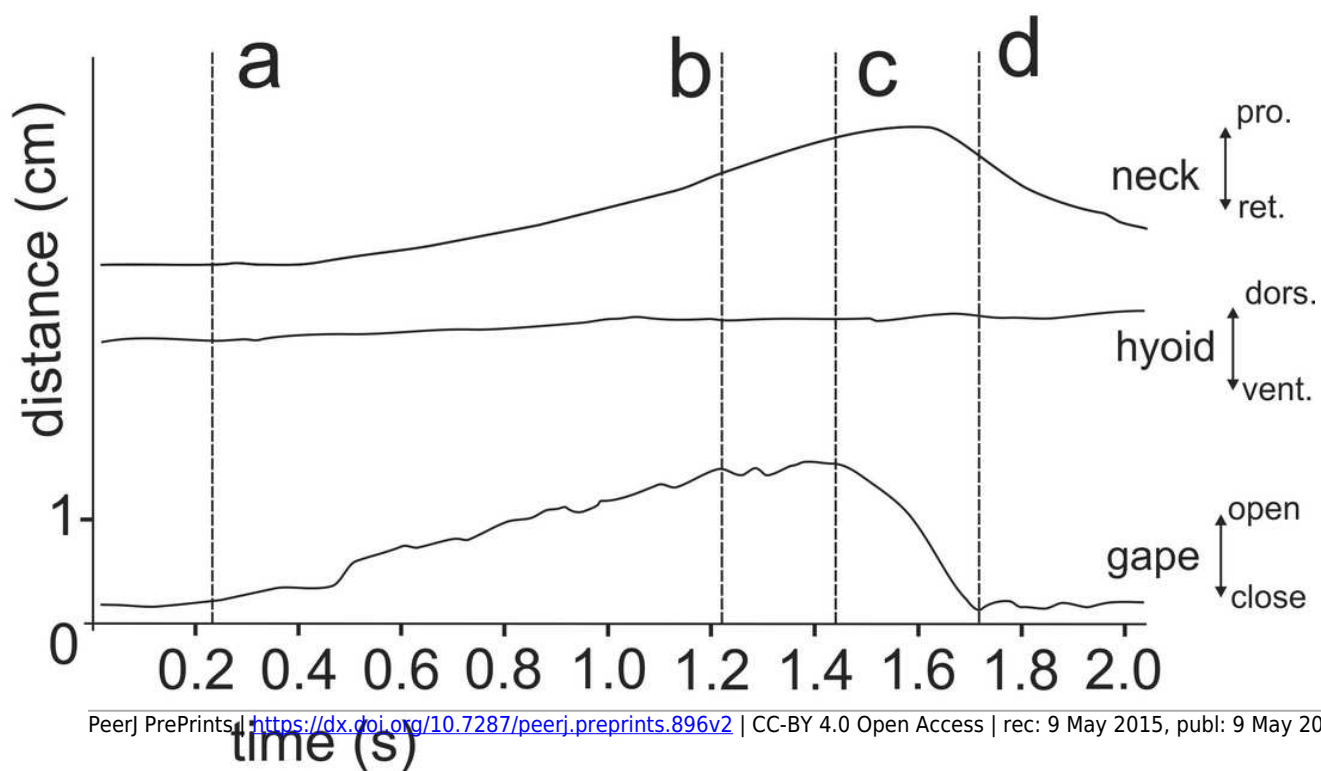
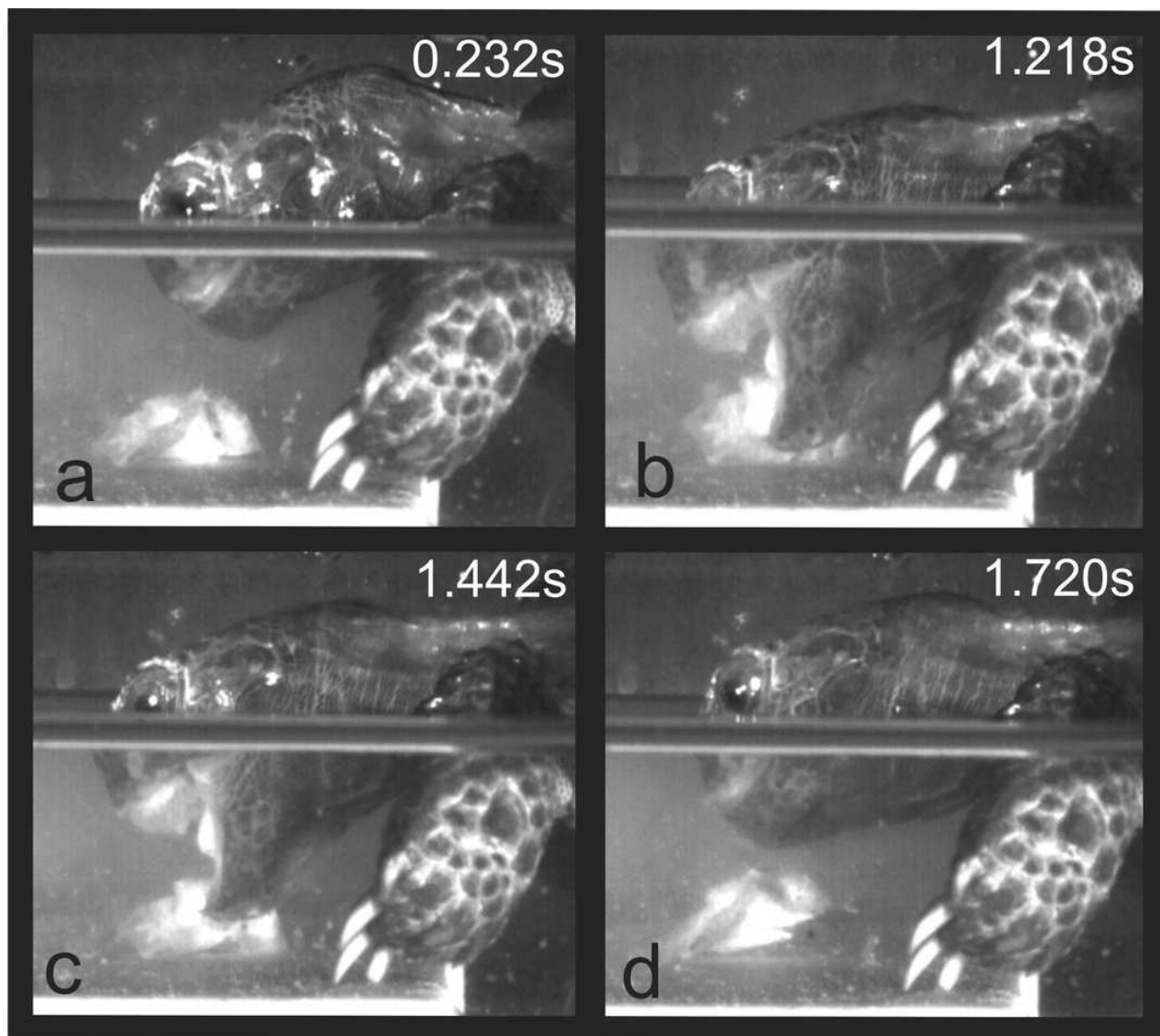
Selected frames and graphics (based on a high-speed video with 500 fr/s) represent the movements of jaws, hyoid and t head during terrestrial food uptake in *Manouria emys* when feeding on pieces of beef heart; a, slow open phase end (lacking discrete SOI and SOII); b, fast open end; c, fast close start;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

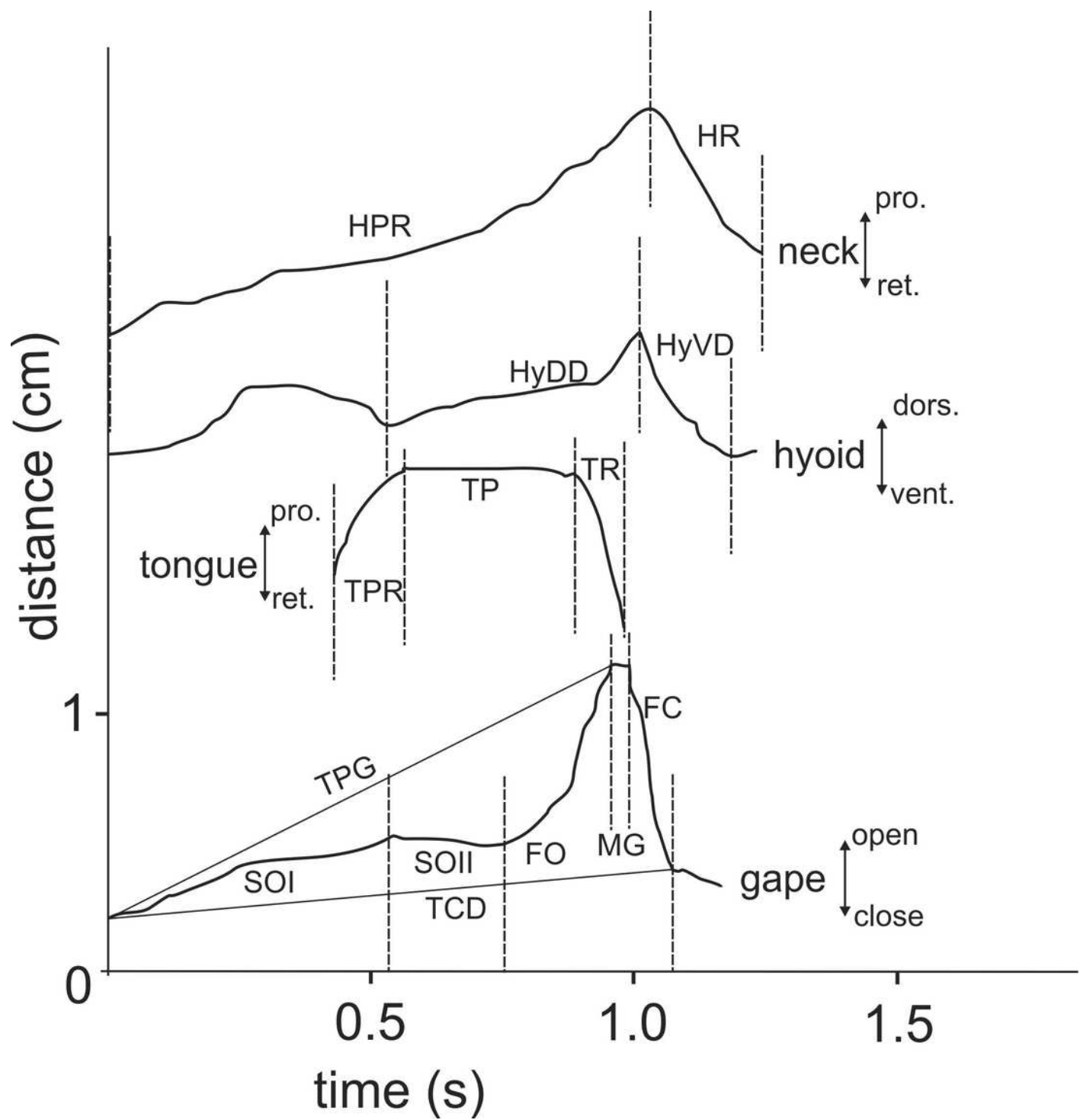
Selected frames and graphics (based on high-speed video with 250 fr/s showing the movements of jaws, hyoid, and head during attempts of aquatic food uptake in *Manouria emys*; a, start of the gape cycle; b, end of jaw opening; c, maximum gape end; d, fast closure end; note the lack of movement of the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

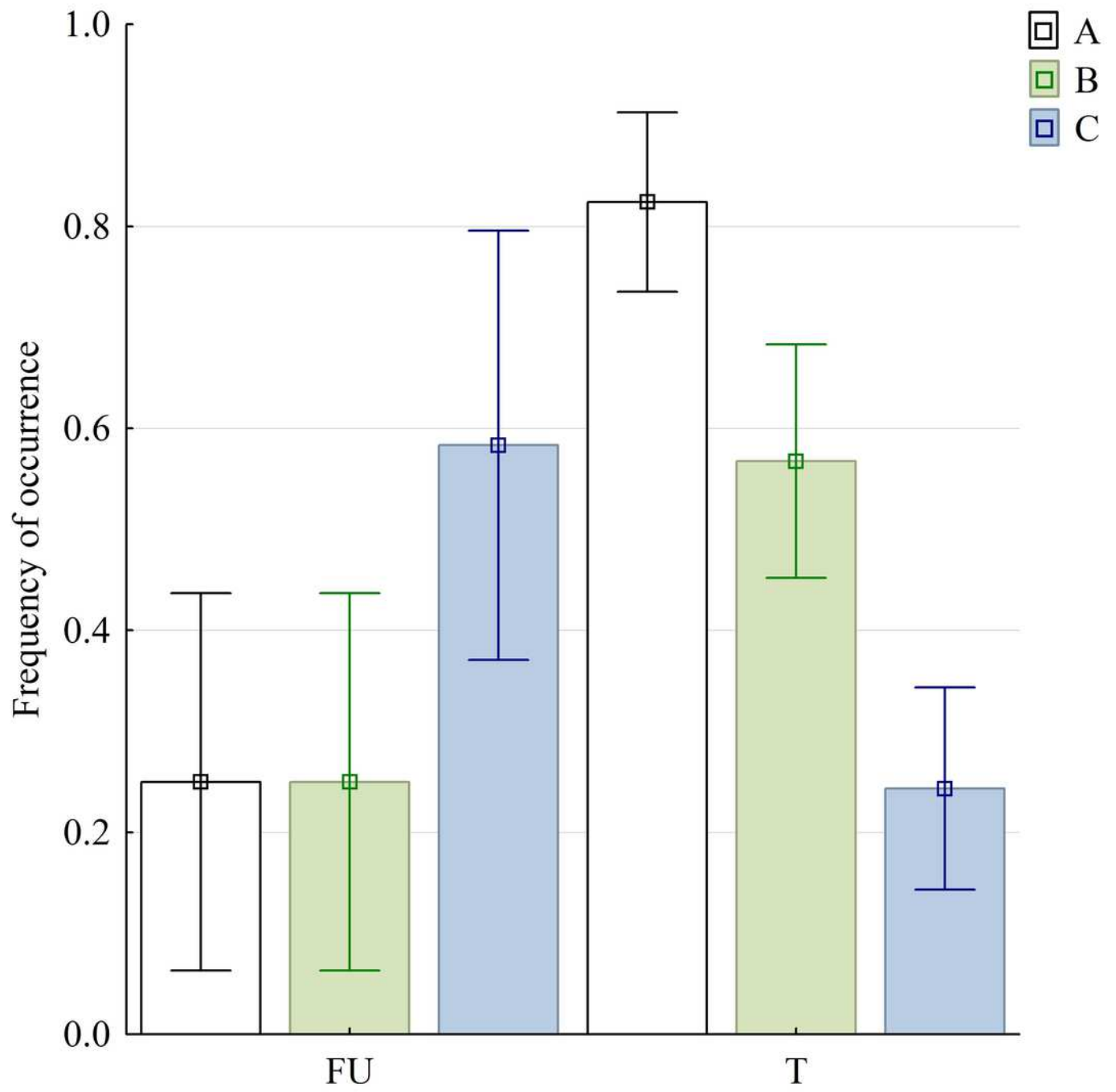


Figure 7

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

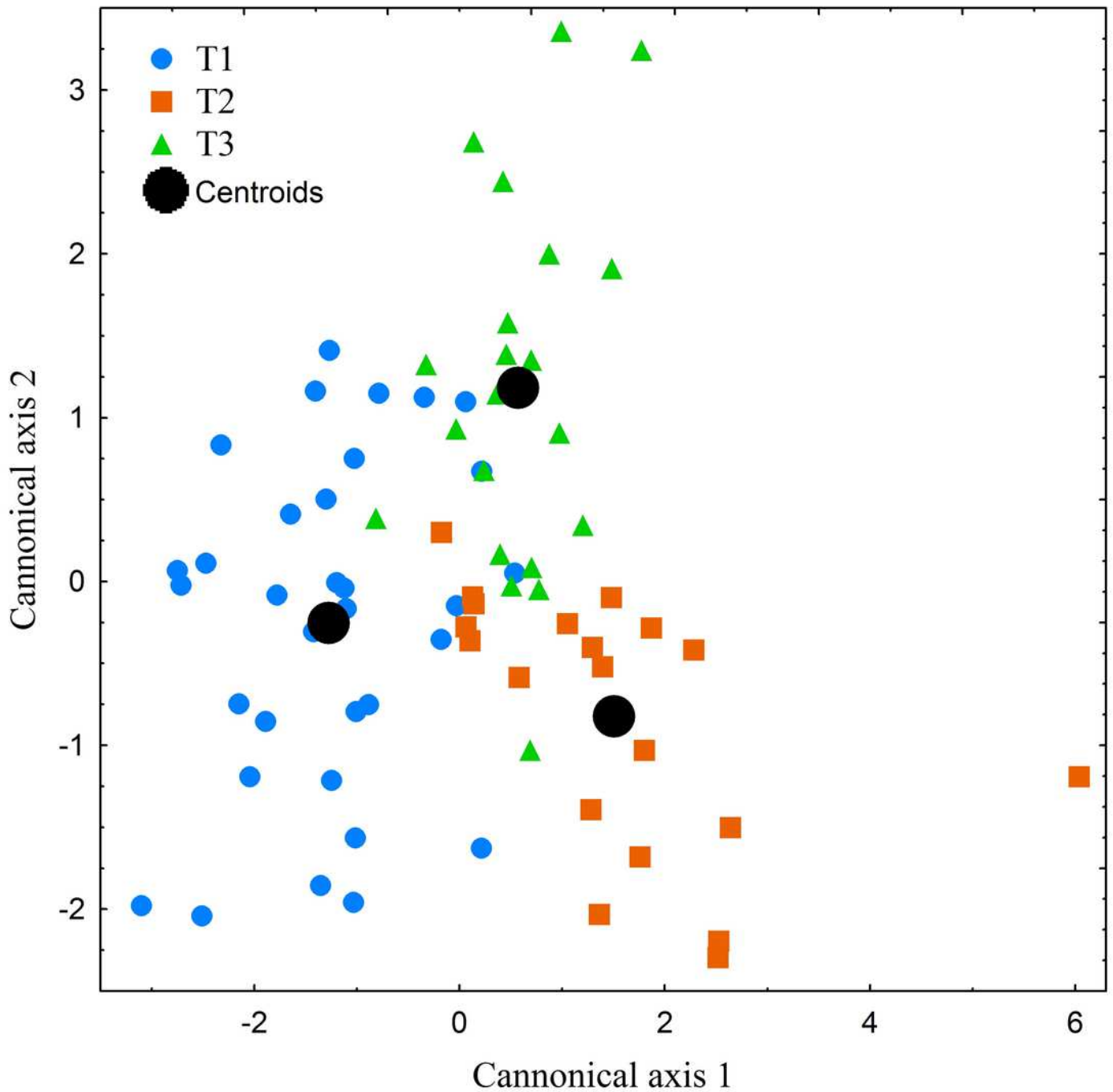


Table 1 (on next page)

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		

7
8