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8 Running head: Evolution of climbing performance

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11 **Allometric, phylogenetic and adaptive components of climbing performance**

12 **in seven species of didelphid marsupials**

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24 Abstract: Didelphid marsupials specialized in arboreal locomotion have morphological
25 features convergent with primates, hence used to test hypotheses about the evolution of
26 arboreality. To evaluate the extent of the convergence between didelphid marsupials and
27 prosimians, data on climbing behavior and performance are necessary. We analyzed the
28 relative contributions of allometric, phylogenetic, and adaptive aspects to the climbing
29 performance of seven species of didelphid marsupials of the Atlantic Forest of Brazil. These
30 species encompass a diversity of body sizes, use of the vertical strata, and lineages within
31 didelphid marsupials. Climbing performance was evaluated by velocity in climbing three
32 nylon ropes of 0.6, 0.9 and 1.25 cm of diameter. The cycle of maximum velocity was chosen
33 to measure relative stride length, frequency, and relative velocity. As expected, arboreal
34 species (*Gracilinanus microtarsus*, *Marmosops incanus*, *Micoureus paraguayanus*, and
35 *Caluromys philander*) had higher relative climbing velocities than more terrestrial species
36 (*Didelphis aurita*, *Philander frenatus*, and *Metachirus nudicaudatus*). Mostly stride frequency
37 was associated with the phylogeny and vertical use of the forest, but relative velocities
38 generally were determined by a combination of both stride length and frequency. Differences
39 in climbing performance originated early in the diversification of the group, but continued to
40 evolve because significant differences also were detected at the sub-family, tribe, and genus
41 levels, which seems to parallel the evolution of grasping abilities in primates. Increasing stride
42 frequency in climbing performance rather than stride length could be a more efficient strategy
43 to increase velocity, reducing metabolic costs and muscle fatigue.

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45 Key-words: Didelphimorphia, locomotion, Neotropical forest, performance, velocity, vertical
46 stratification.

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INTRODUCTION

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It has been proposed that locomotion on supports of small diameter was a determinant factor for the evolution of prehensile extremities in primates (Cartmill 1974). Grasping ability is particularly useful to climb fine branches and to move on supports of small diameter, but it is not restricted to primates. Lineages of didelphid marsupials are derived from an arboreal ancestor (Lemelin 1999), and extant species present a variety of habits and body sizes, with different degree of grasping abilities (Charles-Dominique 1983; Cunha and Vieira 2002; Szalay 1994; Vieira 2006). Consequently, didelphid marsupials have been used to test hypothesis of morphological and locomotory traits related to the evolution of arboreality (Argot 2001, 2002, 2003; Lemelin et al. 2003; Schmitt and Lemelin 2002). Didelphids more specialized in arboreal locomotion have morphological features convergent with primates (Lemelin 1999; Lemelin et al. 2003). For example, the foot of *Caluromys* resembles that of prosimians rather than *Monodelphis*, a terrestrial didelphid marsupial (Lemelin 1999; Lemelin et al. 2003). To evaluate the extent of the convergence between didelphid marsupials and prosimians, data on locomotory behavior and performance are necessary.

Arboreal locomotory behavior was studied in more detail for *Caluromys philander* (Cartmill et al. 2002; Lemelin et al. 2003; Schmitt and Lemelin 2002), and climbing behavior was compared between seven didelphid species of a variety of body sizes and habits (Antunes 2003). However, locomotory performance was studied only for arboreal walking performance (Delciellos and Vieira 2006, 2007). Delciellos and Vieira (2007) observed different combinations of stride length and frequency in the arboreal walking of didelphid marsupials. The species more restricted to the canopy, *C. philander*, had a relatively low velocity determined mostly by stride length, whereas the velocity of semi-terrestrial species, *Didelphis aurita* and *Philander frenatus*, was determined mostly by stride frequency. Arboreal didelphids of small size were the fastest, with velocities determined mostly by high stride

72 frequencies (Delciellos and Vieira 2007). Locomotory performance of didelphid marsupials
73 are associated with phylogeny, but its evolution does not seem to be concentrated at any level
74 (sub-family, tribe, or genus) (Delciellos and Vieira 2006).

75 A derived question is if the relative performances observed in arboreal walking are
76 also observed in other tasks related to the arboreal lifestyle, such as climbing ability. The
77 climbing behavior of didelphid marsupials does not vary as much as in primates, but species
78 can be ranked in their climbing ability. When climbing vertical supports, arboreal species (*C.*
79 *philander*, *Marmosops incanus*, *Micoureus paraguayanus*, and *Gracilinanus microtarsus*) and
80 *P. frenatus* kept their body always far from the support, *D. aurita* touched the support with its
81 chest and snout, and the whole body of the terrestrial *Metachirus nudicaudatus* was always
82 close or touching the support with its elbows close to the body (Antunes 2003). This variation
83 in climbing postural behaviour must have consequences for climbing performance, but were
84 not evaluated yet.

85 Maximum velocity is frequently used as a measure of performance in a variety of tasks
86 (Arnold 1983; Bennett 1989; Ricklefs and Miles 1994), being a compromise between stride
87 length and frequency (Hildebrand 1995). Climbing performance can also be evaluated by
88 maximum velocity (*e. g.* Isler 2004), even though other specific behaviors may also contribute
89 to performance. Velocity can be maximized by longer strides, more frequent strides, or both.
90 The exact combination favored by natural selection will depend on energetic costs of each
91 combination relative to the benefits acquired (Heglund and Taylor 1988). However, as in
92 other aspects of arboreal walking, climbing performance may not be necessarily adaptive, but
93 could also result from allometric and phylogenetic effects (Delciellos and Vieira 2006).

94 Allometric effects on climbing velocity have already been evaluated for mammals in
95 general. Cartmill (1974) reasons that animals that move cursorily on vertical supports, such as
96 squirrels and marmosets, must be small to cope with the high energetic costs compared to

97 terrestrial locomotion. Also, Gallardo-Santis et al. (2005) observed a significant relationship
98 between body size and mass-standardized climbing speed for species of marsupials and
99 rodents.

100 Here in, our main objective was to determine allometric, phylogenetic, and adaptive
101 aspects of the climbing performance of seven species of didelphid marsupials of the Atlantic
102 Forest of Brazil. Climbing performance was evaluated by maximum velocity. We also
103 determine how velocity changes with the support diameter.

104

105 MATERIALS AND METHODS

106 *Specimens of study.*—Animals were captured in remnants of Atlantic Forest near or in
107 the mountain range of Serra dos Órgãos (22°94'–22°32' S, 42°69'–43°06' W), State of Rio
108 de Janeiro, Brazil, in the municipalities of Guapimirim, Teresópolis, Cachoeiras de Macacu
109 and Sumidouro, from January 1999 to February 2004. Captures were part of the small
110 mammal surveys of the Project of Conservation and Use of Brazilian Biological Diversity
111 (PROBIO, MMA-GEF), and part of the Small Mammal Population Monitoring Program of
112 the Laboratório de Vertebrados, Universidade Federal do Rio de Janeiro.

113 The seven species studied were *Caluromys philander* (Linnaeus, 1758), *Didelphis*
114 *aurita* Wied-Neuwied, 1826, *Gracilinanus microtarsus* (Wagner, 1842), *Marmosops incanus*
115 (Lund, 1840), *Metachirus nudicaudatus* (Dermarest, 1817), *Micoureus paraguayanus*
116 (Moojen, 1943), and *Philander frenatus* (Olfers, 1818). These species differ in their use of the
117 vertical strata of the forest, and encompass most of the body size range of didelphid
118 marsupials (Table 1). Only individuals with four functional molars were considered adults and
119 tested (D'Andrea et al. 1994; Tyndale-Biscoe and Mackenzie 1976).

120 *Performance tests.*—The tests consisted of climbing three vertical nylon ropes of 0.6,
121 0.9, and 1.25 cm of diameter, simulating vines and lianas. One end of the rope was passed

122 through a pulley and left hanging free at one meter from the floor. The remaining rope on the
123 other side of the pulley was held by an observer. An animal was placed on the hanging end of
124 the rope, head up, and encouraged to climb by proximity of an observer, even touching the tail
125 of the animal or producing sounds with a key ring if necessary. Animals were kept climbing
126 but within the visual field of a camcorder (NTSC standard, 30 complete frames · s⁻¹, shutter
127 speed = 0.01 s) by releasing rope from the other side of the pulley. Animals that did not
128 respond to these stimuli or did not climb were excluded. Each test was repeated three times
129 for each individual, and in each trial had at least 10 stride cycles recorded, usually more than
130 that. Maximum velocity was reached quickly, after the first three or four strides. Animals
131 were tested during the afternoon in normal daylight conditions because no difference was
132 observed between diurnal and nocturnal tests (Vieira 1995). The floor was covered with five
133 centimeters thick foam to prevent any injury to the animal in case of fall.

134 *Image analyses.*—Images were digitized with ATI All-in-Wonder 128 video card and
135 software (ATI Technologies Inc.), and distances between hands and feet were measured with
136 SigmaScan Pro 5.0 (SPSS Inc.) on the digitized frames. The moment the right posterior foot
137 grabbed the rope was chosen as the starting and ending point of the stride cycle. Stride cycle
138 was the sequence of limb movements after the right posterior foot grabbed the rope. For each
139 individual and rope diameter, the stride cycle of maximum velocity was chosen to measure
140 stride length and frequency. Stride length was the distance covered by the right posterior foot
141 between the first and last frames of the chosen stride cycle. Because the rope was released to
142 keep the animal within the field vision of the camera, the starting and ending locations of the
143 cycle were not present in a single frame. Thus, stride length had to be measured in three
144 phases. First, step length was measured, the maximum distance between the right foot and
145 hand when both were grabbing the rope, which occurred in a video frame in the middle of the
146 stride cycle. Second, the minimum distance between right foot and hand when both were

147 grabbing the rope was measured. This minimum distance occurred after the step, when the
148 foot and posterior limb would leave its position and moved forward, towards the anterior
149 limb. When the foot grabbed the rope again it was close or touching the hand of the same side
150 of the body, which occurred in a frame at the end of the stride cycle. When this minimum
151 distance was zero, posterior and anterior limbs were grabbing almost the same point on the
152 rope at the start and end of the stride cycle. In this case, stride and step had the same length.
153 Frequently, however, the minimum distance was greater than zero, hence stride length –
154 measured by the initial and final position of the posterior foot - was shorter than step length.
155 Finally, stride length was obtained subtracting the minimum distance from step length. Stride
156 frequency was the inverse of stride duration, which was the number of frames for a stride
157 cycle multiplied by duration of a single frame (30^{-1} s). Velocity was stride length multiplied
158 by its frequency (Hildebrand 1988).

159 The effects of body mass, phylogeny, and vertical use of the forest were tested on
160 absolute stride length and frequency (see Statistical Analyses below). In simple comparisons
161 between species, relative velocity and relative stride length were used to emphasize
162 differences between species regardless of major differences in body size (Alexander and Jayes
163 1983; Alexander and Maloiy 1984; Van Damme and Van Dooren 1999). Biomechanical
164 comparisons of the terrestrial locomotion require dynamically similar conditions, which is
165 usually obtained for animals of different size using Froude numbers (Alexander and Jayes
166 1983). However, Froude numbers apply to terrestrial locomotion, and from an ecological
167 point of view, absolute or relative velocities seem more relevant (Van Damme and Van
168 Dooren 1999). Relative stride length was stride length of each individual divided by its head-
169 body length, and relative velocity was absolute stride frequency multiplied by relative stride
170 length. Therefore, relative velocity is in units of body lengths covered by unit of time (bl/s),
171 and relative stride length in units of body length (bl).

172 *Statistical analyses.*—The stability of mean values for each species in each support
173 diameter was evaluated by jackknifing the mean frequency, relative stride length, and relative
174 velocity (Sokal and Rohlf 1995). This procedure allowed to determine the sensitivity of the
175 results to the addition or removal of individuals, and to compare the performance of the same
176 species in different supports. For each species and support diameter, an individual was
177 removed from the sample and the average calculated. The individual was returned, a new
178 individual removed, and the mean recalculated. The process was repeated for all the
179 individuals of the sample (N), resulting in a total of N means. The grand mean, \bar{x} , and its
180 standard deviation were the estimate and standard error, se , of the jackknife. According to the
181 central limit theorem, a distribution of means should approach a normal distribution (Sokal
182 and Rohlf 1995), hence confidence intervals were calculated as $\bar{x} \pm 1.96 se$. Intraspecific
183 differences in performance between supports were considered significant when the 95%
184 confidence intervals did not overlap.

185 Relative velocity, relative stride length, and stride frequency were compared between
186 species using supports of diameters equivalent to their body sizes. Differences between
187 species were tested with one-way ANOVAs (Sokal and Rohlf 1995). Supports for comparison
188 were chosen based on the mean foot breadth of each species: 13 mm for *G. microtarsus*, 15.14
189 mm (± 0.4) for *M. incanus*, 19.0 mm (± 0.4) for *M. paraguayanus*, 23.0 mm (± 1.4) for *C.*
190 *philander*, 19.0 mm (± 1.4) for *P. frenatus*, 26.3 mm (± 4.2) for *M. nudicaudatus*, and 37.9
191 mm (± 4.2) for *D. aurita* (see Carvalho et al. 2000 for a description of the measure of foot
192 breadth). Accordingly, diameters of support equivalent to body size were 0.6 for *G.*
193 *microtarsus* and *M. incanus*, 0.9 cm for *C. philander*, *M. nudicaudatus*, *M. paraguayanus*,
194 and *P. frenatus*, and 1.25 cm for *D. aurita*.

195 Absolute stride length and frequency were log-transformed prior to the analyses to
196 increase homocedasticity and reduce non-linearity, particularly of frequency data, which

197 would follow a multinomial distribution (Sokal and Rohlf 1995). Normality was tested by
198 Shapiro Wilks test (Sokal and Rohlf 1995), and was not rejected in most of the supports and
199 species, either for stride length or stride frequency ($P > 0.05$ for 95.25% and 85.7% of the
200 tests respectively).

201 Log-transformed absolute stride length and frequency of the three supports were
202 combined and reduced by two Principal Component Analyses of the covariance matrix, one
203 for stride length and another for stride frequency. One or two components were selected in
204 each analysis based on the interpretability of the factor score coefficients, magnitude of the
205 eigenvalues, and variance explained. The scores of each individual were used in comparisons
206 between species, replacing the original variables with the selected components. The seven
207 species of marsupials were treated as single group in the Principal Component Analyses to
208 obtain a common size factor, which was tested by Pearson correlations between the logarithm
209 of body mass and the scores of each individual in the first principal component of stride
210 length, and frequency.

211 Phylogenetic relationships were incorporated in the comparisons between species in
212 each selected principal component by Nested Analysis of Covariance (review in Harvey and
213 Pagel 1991), using three nested levels of comparison, genus (within tribes), tribe (within sub-
214 families), and sub-family (within families). The levels were defined by the classification and
215 phylogenetic hypothesis of Kirsch et al. (1997), which used species as representative of their
216 genera (Fig. 1). We also tested the phylogenetic arrangement proposed by Jansa and Voss
217 (2000), which required an additional hierarchical level between tribe and sub-family.
218 However, the results were very similar, hence we report only the results based on Kirsh et al.
219 (1997). Thus, the lower rank of phylogenetic comparisons in ANCOVA was the genus.
220 Pairwise *a posteriori* comparisons between species were tested with Fischer LSD (Winer
221 1971). Sample size and degrees of freedom did not allow inclusion of the family level.

222 Vertical use of the forest was ranked from one to six based on the relative degree of use of the
 223 vertical strata (Table 1), and used as the covariate in ANCOVA.

224

225 RESULTS

226 Not surprisingly, arboreal species were relatively faster than terrestrial species in the
 227 climbing performance tests (Table 2). Among the arboreal species, the smaller *G.*
 228 *microtarsus*, *M. incanus*, and *M. paraguayanus* were faster than the larger *C. philander*,
 229 which is also the arboreal species more restricted to the canopy. Among terrestrial species, *P.*
 230 *frenatus* was faster than *D. aurita* and *M. nudicaudatus*. In all species there was a trend of
 231 increasing relative velocity with support diameter, reaching the highest velocities in the
 232 largest support (1.25 cm of diameter, Fig. 2). Relative velocities of most species were
 233 determined by a combination of both stride length and frequency (Fig. 3), but in *C. philander*
 234 and *M. paraguayanus* relative velocities were determined mainly by relative stride length
 235 (Fig. 3).

236 When compared between supports of diameters equivalent to body size, relative stride
 237 length, stride frequency, and relative velocity also differed significantly between species
 238 (relative stride length: $N=98$, $R^2=0.515$, $F=16.07$, $d.f.=6$, $P<0.001$; stride frequency: $N=98$,
 239 $R^2=0.737$, $F=42.45$, $d.f.=6$, $P<0.001$; relative velocity: $N=98$, $R^2=0.760$, $F=48.15$, $d.f.=6$,
 240 $P<0.001$). Again, arboreal species had higher values than terrestrial species in relative stride
 241 length, stride frequency, and relative velocity. *Gracilinanus microtarsus* had an exceptionally
 242 high relative velocity, stride length, and frequency. Most species pairs differed significantly in
 243 their climbing performance according to Fisher's post hoc LSD tests (Appendix). Exceptions
 244 were *C. philander*, *M. incanus* and *M. paraguayanus*, which did not differ between each other
 245 in any performance measure ($P>0.061$); *G. microtarsus* did not differ from *C. philander* and
 246 *M. paraguayanus* ($P>0.059$, but differed from *M. incanus* with $P=0.043$); *Didelphis aurita*

247 and *M. nudicaudatus* did not differ in stride frequency ($P=0.389$), and *D. aurita* and *P.*
248 *frenatus* did not differ in relative stride length ($P=0.093$).

249 For stride length, the first and second principal components (PC1sl e PC2sl) were
250 selected because they explained 88.4% of the original variation. PC1sl represents a factor
251 common to all supports because their factor scores on PC1sl were similar in sign and
252 magnitude (Table 3). However, PC1sl cannot be interpreted as a size factor because of its
253 weak correlation with body mass ($r= 0.338$, $P < 0.001$). Rather, it was interpreted as the
254 individual climbing ability, which in this case has a significant, but weak association with the
255 size of the individual. PC2sl was interpreted as the main effect of support diameter on stride
256 length. Positive values on PC2sl were associated with longer strides on the larger supports,
257 and shorter strides on the thinnest support.

258 Overall, PC1sl and PC2sl were not associated to the phylogenetic levels tested. The
259 only exception was a significant association of PC1sl with the tribe level (Table 4). In
260 pairwise comparisons, only the tribes of the arboreal genera differed significantly between
261 each other, all with $P= 0.038$ (Fisher LSD: Marmosopsini vs. Marmosini = 0.615,
262 Marmosopsini vs. *Caluromys* = 0.308, and Marmosini vs. *Caluromys* = -0.308). PC1sl and
263 PC2sl were not associated with the vertical use of the forest either (Table 4).

264 For stride frequency, only the first principal component was selected (PC1sf) because
265 it alone explained 90.9% of the original variation (Table 3). It was interpreted as the effect of
266 body size on stride frequency because all factor score coefficients had the same sign and
267 magnitude, and it was highly correlated with body mass ($r = -0.850$, $P < 0.001$). The size
268 effect on stride frequency (PC1sf) was affected significantly by all taxonomic levels and by
269 the vertical use of the forest (Table 4). In pairwise comparisons, all tribes differed
270 significantly between each other, with all $P < 0.001$ (Fisher LSD: Didelphini vs. Metachirini
271 = -9.479, Didelphini vs. Marmosopsini = -4.190, Didelphini vs. Marmosini = -5.289,

272 Didelphini vs. *Caluromys* = -4.739, Metachirini vs. Marmosopsini = 5.289, Metachirini vs.
273 Marmosini = 4.190, Metachirini vs. *Caluromys* = 4.739, Marmosopsini vs. Marmosini = -
274 1.099, Marmosopsini vs. *Caluromys* = -0.550, Marmosini vs. *Caluromys* = 0.550). All
275 pairwise comparisons between the three sub-families differed significantly with $P < 0.001$
276 (Fisher LSD: Didelphinae vs. [Thylamyinae + Marmosinae] = 3.365, Didelphinae vs.
277 Caluromyinae = 1.682, [Thylamyinae + Marmosinae] vs. Caluromyinae = -1.682).

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DISCUSSION

280 The general pattern of high relative velocities of arboreal species observed in climbing
281 performance was already observed in arboreal walking of didelphid marsupials (Delciellos
282 and Vieira 2006, 2007). Arboreal species have morphological specializations that could
283 explain their higher performance, such as reduced claws and longer digits, allowing a better
284 grasping ability (Lemelin 1999; Hamrick 2001). If these morphological features appeared
285 early in the divergence of didelphid lineages and indeed are responsible for their climbing
286 performance, differences between species in climbing performance also would have appeared
287 early in the evolution of these lineages. Indeed, in this study we detected significant
288 differences in climbing performance early in the evolution of the group, at the sub-family
289 level, but also at the tribe and genus levels, suggesting that there were further specializations
290 along the history of the group. Didelphid marsupials have been proposed as living ecological
291 models to understand the evolution of climbing ability in primates because of their similar use
292 of grasping abilities and fine branches (Lemelin, 1999). The early divergence in climbing
293 performance and continuous evolution along the lineages also may have parallel in the
294 evolutionary history of primates. Grasping may be an ancestral character for archontan
295 mammals - orders Scandentia, Primates, Dermoptera and Chiroptera (Szalay and Dagosto,
296 1988), and also suffered further evolution within each order.

297 In climbing performance of didelphid marsupials, most effects related to phylogeny
298 and vertical use of the forest were detected on stride frequency. It is possible that stride
299 frequency rather than stride length could be a more efficient strategy to increase climbing
300 velocity, reducing metabolic costs and muscle fatigue. The same would apply for other small
301 to medium size animals. The effect of gravity on body mass is probably most pronounced
302 when climbing a vertical support, when longer strides would require muscles to suspend a
303 force equivalent to the whole body mass of the animal. Longer strides imply a longer period
304 of time when only one limb of a pair (fore or posterior) supports the body mass. The other
305 limbs would be in a suspension phase, reaching an upper point on the support. Conversely, on
306 horizontal supports the limbs are not suspending the body, but supporting and projecting it
307 forwards. Increasing stride length becomes a viable strategy to increase velocity, which could
308 be determined by a combination of stride length and frequency, as observed in arboreal
309 walking (Delciellos and Vieira 2006, 2007). The hypothesis that more frequent strides are
310 more energetically efficient in climbing slender supports is speculative at the moment, and
311 would require biomechanical or physiological studies to be tested.

312 Although stride length was not associated with body size and vertical use of the forest,
313 relative velocity of most species was determined both by relative stride length and frequency.
314 Besides, there was a common factor affecting stride length similarly on the three supports,
315 indicating consistent individual variation, and this factor (PC1) differed significantly between
316 tribes. This idiosyncratic factor of stride length could result from the particular history of each
317 individual, or from its limb proportions. Further morphological and behavioral studies are
318 necessary to understand it, but regardless of the nature of this factor, climbing performance
319 cannot be evaluated and understood examining only stride frequency. Stride length also must
320 be considered.

321 In arboreal walking by the same didelphid marsupials there were two contrasting
322 strategies to increase velocity, increasing stride length and reducing frequency, or vice-versa
323 (Delciellos and Vieira 2007), but in climbing performance most species increased velocity by
324 a combination of both stride length and frequency. *Caluromys philander* and *M.*
325 *paraguayanus* were exceptions, increasing velocity predominantly by longer strides. In
326 arboreal walking, *C. philander* also increased velocity by longer and less frequent strides, but
327 *M. paraguayanus* had an opposite pattern, increasing velocity by more frequent but shorter
328 strides (Delciellos and Vieira 2007). Thus, *C. philander* was the only species that favored
329 longer strides in two aspects of arboreal performance, climbing and arboreal walking.

330 The climbing performance of two semi-terrestrial species, *P. frenatus* and *D. aurita*,
331 contrasted with their vertical use of the forest (Table 1). *Didelphis aurita* occasionally climbs
332 to the canopy, whereas *P. frenatus* rarely climbs higher than the understorey (Cunha and
333 Vieira 2002). Accordingly, the performance of *D. aurita* should be more similar to more
334 arboreal species than *P. frenatus*, but actually it was the opposite, the climbing performance
335 of *P. frenatus* was more similar to *C. philander*, an arboreal species of similar size. A similar
336 result for *P. frenatus* was already observed for arboreal walking performance (Delciellos and
337 Vieira 2006, 2007). A tentative hypothesis for these contrasting results may be the predatory
338 and foraging habits of species of *Philander* (Astúa de Moraes et al. 2003; Tuttle et al. 1981),
339 and the associated high encephalization coefficient, also more similar to arboreal than to
340 terrestrial species (Eisenberg and Wilson 1981). A predator has to capture, hold and subdue
341 prey, which require precise movements, perception of obstacles, and agility. These abilities
342 could secondarily enhance its climbing performance, which has the same requirements. Other
343 possibilities are that factors other than locomotory performance and behavior could be
344 restricting the use of the arboreal strata by *P. frenatus*, including the presence of *D. aurita*, a

345 potential competitor and predator (Cunha and Vieira 2002, 2005; Fonseca and Robinson
346 1990).

347 The worst performance of *M. nudicaudatus* was expected because of its cursorial
348 morphological specializations (Szalay 1994). Its long hindlimbs, elongated hindfeet, and
349 reduced grasping ability must restrict its ability to climb, similarly to observed in comparisons
350 between lizard species (Vanhooydonck et al. 2000).

351 The evolution of climbing performance in didelphid marsupials occurred early in
352 diversification of group, but continued along the evolution. It was mainly associated with
353 increasing stride frequency, in contrast with the evolution of arboreal walking, where stride
354 length and frequency were equally important (Delciellos and Vieira 2006). Stride frequency
355 also was the major determinant of performance associated with vertical use of the forest and
356 body size. Increasing stride frequency in climbing performance rather than stride length could
357 be a more efficient strategy to increase velocity, reducing metabolic costs and muscle fatigue.
358 This hypothesis, however, needs to be tested with other groups, such as primates, a natural
359 group for tests of adaptive hypothesis about the evolution of arboreality in mammals.

RESUMO

360
361 Marsupiais didelfídeos especializados na locomoção arborícola têm características
362 morfológicas convergentes com primatas, por isso utilizados para testar hipóteses sobre a
363 evolução da arborealidade. Para avaliar a extensão de convergências com primatas, dados
364 sobre comportamento e desempenho na escalada são necessários. Analisamos aqui as
365 contribuições relativas de aspectos alométricos, filogenéticos e adaptativos no desempenho
366 locomotor na escalada de sete espécies de marsupiais didelfídeos da Mata Atlântica do Brasil.
367 As espécies estudadas abrangem a amplitude de tamanho corporal, uso vertical da mata e
368 linhagens de marsupiais didelfídeos. O desempenho locomotor foi avaliado pela velocidade
369 na escalada de três cordas de nylon de 0,6, 0,9 e 1,25 cm de diâmetro. O ciclo de velocidade
370 máxima foi escolhido para a medir o comprimento de passada relativo, frequência e
371 velocidade relativa. Como esperado, as espécies arborícolas (*Gracilinanus microtarsus*,
372 *Marmosops incanus*, *Micoureus paraguayanus* e *Caluromys philander*) tiveram maiores
373 velocidades relativas na escalada do que as espécies mais terrestres (*Didelphis aurita*,
374 *Philander frenatus* e *Metachirus nudicaudatus*). De forma geral a frequência de passadas foi
375 associada com a filogenia e uso vertical da mata, mas a velocidade relativa da maioria das
376 espécies foi determinada por uma combinação de comprimento de passada e frequência.
377 Diferenças no desempenho locomotor na escalada apareceram no início da diversificação do
378 grupo, mas continuaram a evoluir porque diferenças significativas também foram detectadas
379 ao nível de subfamília, tribo e gênero, de forma semelhante à evolução da habilidade de dedos
380 opositores em primatas. O aumento da frequência em vez do comprimento de passada na
381 escalada parece ser uma estratégia mais eficiente para aumentar a velocidade, reduzindo os
382 custos metabólicos e a fadiga muscular.
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LITERATURE CITED

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LEGENDS FOR FIGURES

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501

502 **FIGURE 1.** Phylogenetic hypothesis for the genera of didelphid marsupials studied
503 based on Kirsch *et al.* (1997). Branch lengths are proportional to time of divergence.

504

505 **FIGURE 2.** Relative velocity of the species of didelphid marsupials on supports of
506 three diameters. Error bars are the jackknife confidence intervals.

507

508 **FIGURE 3.** Stride frequency (continuous lines) and relative length (discontinuous lines)
509 of the species of didelphid marsupials on supports of three diameters. Error bars are the
510 jackknife confidence intervals.

511 **TABLE 1.**— Mean ($\pm sd$) of morphological measurements based on the individuals studied, and use of the
 512 vertical strata of the species of didelphid marsupials. Relative tail length is tail length divided by body length. Sample
 513 size (N) is the total number of individuals tested, but not all individuals were tested in all supports. (¹Charles-
 514 Dominique, 1983; ²Emmons, 1990; ³Szalay, 1994; ⁴Cunha and Vieira, 2002; ⁵Schmitt and Lemelin, 2002).

Species	Head-body length (cm) ($\bar{x} \pm sd$)	Tail length (cm) ($\bar{x} \pm sd$)	Relative tail length	Body mass (g) ($\bar{x} \pm sd$)	N	Use of the vertical strata
<i>Gracilinanus microtarsus</i>	11.3 \pm 1.6	15.9 \pm 1.0	1.41	36.8 \pm 7.3	9	Arboreal of the canopy and understorey ^(1, 2)
<i>Marmosops incanus</i>	13.2 \pm 1.3	18.3 \pm 2.1	1.38	49.8 \pm 16.0	20	Arboreal of the understorey ⁽⁴⁾
<i>Micoureus paraguayanus</i> = (<i>M. demerarae</i>)	16.7 \pm 1.2	24.3 \pm 2.2	1.45	123.9 \pm 29.9	22	Arboreal of the canopy and understorey ^(1, 2)
<i>Caluromys philander</i>	20.9 \pm 2.06	28.5 \pm 1.2	1.36	230.8 \pm 70.3	6	Arboreal of the canopy ^(1, 2, 5)
<i>Metachirus nudicaudatus</i>	25.4 \pm 2.1	32.5 \pm 1.6	1.28	415.0 \pm 99.5	10	Specialized terrestrial ^(2, 3, 4)
<i>Philander frenatus</i>	25.7 \pm 2.5	29.5 \pm 2.8	1.15	382.3 \pm 137.1	19	Semi-terrestrial, using the understorey ⁽⁴⁾
<i>Didelphis aurita</i>	36.5 \pm 3.2	35.4 \pm 2.6	0.97	1226.3 \pm 321.6	17	Semi-terrestrial, using the canopy or the understorey ⁽⁴⁾

Support diameter (cm)	RELATIVE VELOCITY ($\text{cm} \cdot \text{s}^{-1}$)			FREQUENCY (strides $\cdot \text{s}^{-1}$)			RELATIVE STRIDE LENGTH (body length units)		
	0.6	0.9	1.25	0.6	0.9	1.25	0.6	0.9	1.25
<i>Caluromys philander</i>	1.17 ± 0.24	1.52 ± 0.79	1.96 ± 0.66	2.35 ± 0.44	2.30 ± 0.68	2.61 ± 0.58	0.50 ± 0.06	0.63 ± 0.18	0.74 ± 0.13
<i>Didelphis aurita</i>	0.24 ± 0.17	0.36 ± 0.20	0.42 ± 0.16	0.80 ± 0.10	0.93 ± 0.27	1.01 ± 0.19	0.29 ± 0.10	0.38 ± 0.12	0.42 ± 0.14
<i>Gracilinanus microtarsus</i>	3.99 ± 1.98	4.52 ± 1.57	6.03 ± 2.07	5.00 ± 1.28	5.56 ± 1.31	6.64 ± 1.14	0.77 ± 0.18	0.81 ± 0.17	0.89 ± 0.20
<i>Marmosops incanus</i>	1.81 ± 0.80	1.79 ± 0.71	2.56 ± 1.22	2.78 ± 0.63	2.69 ± 0.58	3.34 ± 1.22	0.63 ± 0.19	2.69 ± 0.58	0.74 ± 0.21
<i>Metachirus nudicaudatus</i>	0.14 ± 0.06	0.30 ± 0.20	0.39 ± 0.39	0.85 ± 0.33	0.97 ± 0.47	1.09 ± 0.64	0.18 ± 0.09	0.29 ± 0.09	0.32 ± 0.11
<i>Micoureus paraguayanus</i>	1.68 ± 0.92	2.01 ± 0.95	2.48 ± 0.85	2.69 ± 0.89	3.26 ± 1.80	2.99 ± 0.74	0.60 ± 0.18	0.64 ± 0.15	0.82 ± 0.12
<i>Philander frenatus</i>	0.53 ± 0.20	0.81 ± 0.30	1.23 ± 0.66	1.15 ± 0.25	1.65 ± 0.44	1.88 ± 0.61	0.45 ± 0.11	0.48 ± 0.09	0.62 ± 0.14

519 **TABLE 3.**— Principal components selected from the covariance matrices of
 520 stride length and frequency (log-transformed) on supports of three diameters. Values are
 521 the coefficients of the corresponding eigenvectors.

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Support Diameter (cm)	Stride length		Stride frequency
	PC1	PC2	PC1
0.6	0.370	-0.180	0.654
0.9	0.270	0.169	0.628
1.25	0.276	0.076	0.599
Eigenvalue	0.286	0.066	1.181
Proportion of variance explained	71.7	16.7	90.9

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524 **TABLE 4.**—Nested Analyses of Covariance (Nested ANCOVAs) for the three
 525 performance components selected. The nested levels were defined according to the
 526 classification and phylogenetic hypothesis of Kirsch *et al.* (1997), and the covariate was
 527 the vertical use of the forest strata (see Table 1).

	<i>F</i>	<i>d.f.</i>	<i>P</i>
Stride length PC1 (individual climbing ability) $R^2 = 0.491$			
Constant	0.21	1	0.652
Vertical use of the forest	0.53	1	0.467
Genus	1.65	2	0.198
Tribe	4.12	2	0.020
Sub-family	1.32	1	0.254
Stride length PC2 (support diameter) $R^2 = 0.189$			
Constant	0.01	1	0.936
Vertical use of the forest	0.05	1	0.829
Genus	0.41	2	0.668
Tribe	0.84	2	0.436
Sub-family	1.09	1	0.300
Stride frequency PC1 (size factor) $R^2 = 0.879$			
Constant	48.35	1	< 0.001
Vertical use of the forest	52.28	1	< 0.001
Genus	46.84	2	< 0.001
Tribe	30.40	2	< 0.001
Sub-family	53.46	1	< 0.001

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APPENDIX

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532 Pairwise comparisons between species (differences in adjusted least square means) in

533 supports of equivalent body-size diameters. Fisher's probabilities are in parentheses,

534 with significant probabilities in boldface.

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1. Relative stride frequency

	<i>C. philander</i>	<i>D. aurita</i>	<i>G. microtarsus</i>	<i>M. incanus</i>	<i>M. nudicaudatus</i>	<i>M. paraguayanus</i>	<i>P. frenatus</i>
<i>C. philander</i>	0.000 (1.000)						
<i>D. aurita</i>	-0.803 (<0.001)	0.000 (1.000)					
<i>G. microtarsus</i>	0.784 (<0.001)	1.587 (<0.001)	0.000 (1.000)				
<i>M. incanus</i>	0.201 (0.185)	1.003 (<0.001)	-0.583 (<0.001)	0.000 (1.000)			
<i>M. nudicaudatus</i>	-0.921 (<0.001)	-0.118 (0.389)	-1.705 (<0.001)	-1.121 (<0.001)	0.000 (1.000)		
<i>M. paraguayanus</i>	0.281 (0.061)	1.084 (<0.001)	-0.503 (<0.001)	0.081 (0.420)	1.202 (<0.001)	0.000 (1.000)	
<i>P. frenatus</i>	-0.335 (0.031)	0.468 (<0.001)	-1.119 (<0.001)	-0.536 (<0.001)	0.586 (<0.001)	-0.616 (<0.001)	0.000 (1.000)

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2. Relative stride length

	<i>C. philander</i>	<i>D. aurita</i>	<i>G. microtarsus</i>	<i>M. incanus</i>	<i>M. nudicaudatus</i>	<i>M. paraguayanus</i>	<i>P. frenatus</i>
<i>C. philander</i>	0.000 (1.000)						
<i>D. aurita</i>	-0.420 (0.002)	0.000 (1.000)					
<i>G. microtarsus</i>	0.214 (0.139)	0.633 (<0.001)	0.000 (1.000)				
<i>M. incanus</i>	-0.010 (0.938)	0.410 (<0.001)	-0.224 (0.043)	0.000 (1.000)			
<i>M. nudicaudatus</i>	-0.781 (<0.001)	-0.362 (0.002)	-0.995 (<0.001)	-0.772 (<0.001)	0.000 (1.000)		
<i>M. paraguayanus</i>	0.008 (0.950)	0.428 (<0.001)	-0.206 (0.059)	0.018 (0.833)	0.789 (<0.001)	0.000 (1.000)	
<i>P. frenatus</i>	-0.256 (0.050)	0.164 (0.093)	-0.470 (<0.001)	-0.246 (<0.001)	0.525 (<0.001)	-0.264 (0.003)	0.000 (1.000)

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3. Relative velocity

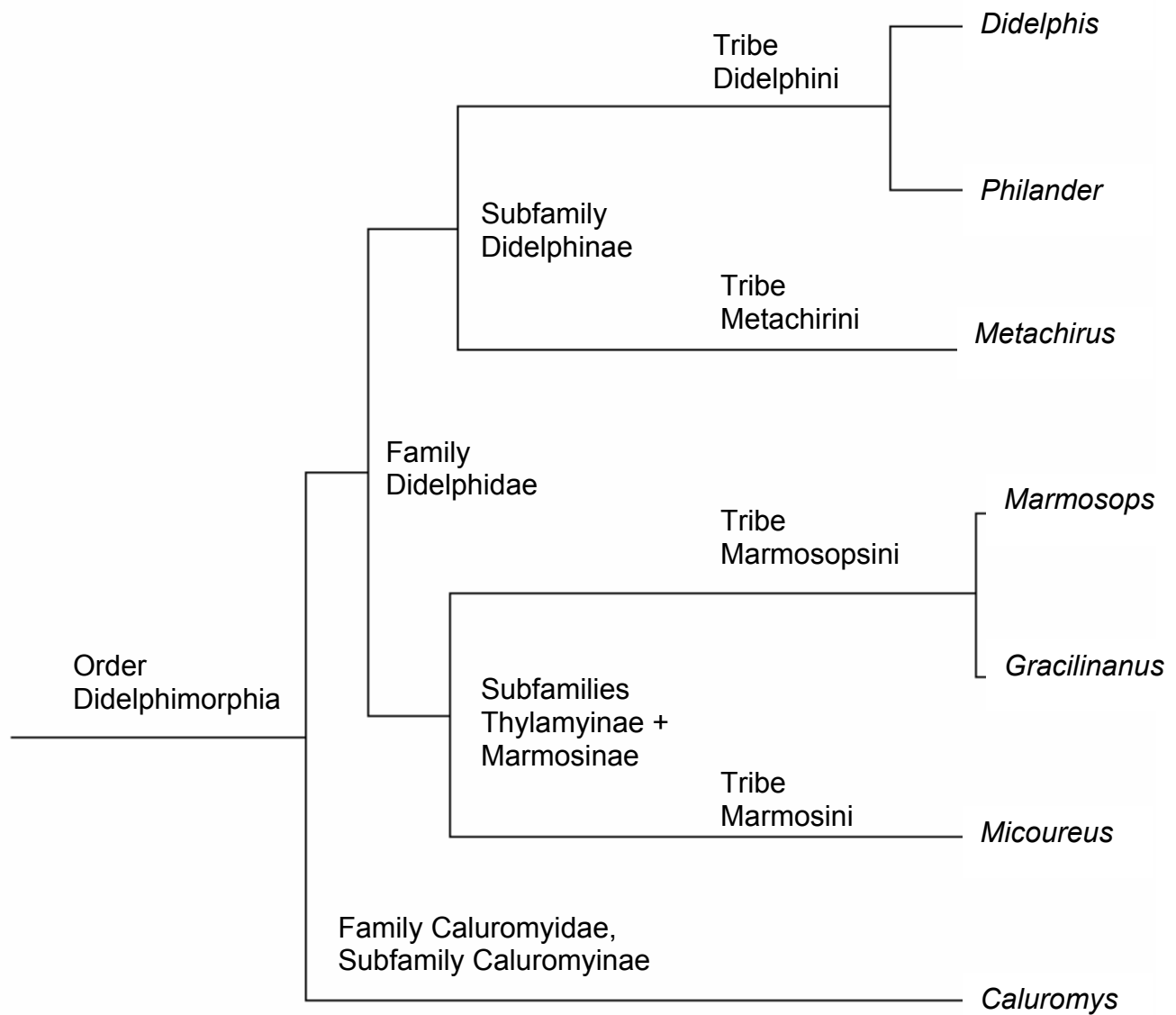
	<i>C. philander</i>	<i>D. aurita</i>	<i>G. microtarsus</i>	<i>M. incanus</i>	<i>M. nudicaudatus</i>	<i>M. paraguayanus</i>	<i>P. frenatus</i>
<i>C. philander</i>	0.000 (1.000)						
<i>D. aurita</i>	-1.223 (<0.001)	0.000 (1.000)					
<i>G. microtarsus</i>	0.998 (<0.001)	2.220 (<0.001)	0.000 (1.000)				
<i>M. incanus</i>	0.191 (0.370)	1.413 (<0.001)	-0.807 (<0.001)	0.000 (1.000)			
<i>M. nudicaudatus</i>	-1.702 (<0.001)	-0.480 (0.014)	-2.700 (<0.001)	-1.893 (<0.001)	0.000 (1.000)		
<i>M. paraguayanus</i>	0.289 (0.171)	1.512 (<0.001)	-0.708 (<0.001)	0.099 (0.485)	1.991 (<0.001)	0.000 (1.000)	
<i>P. frenatus</i>	-0.591 (0.007)	0.631 (<0.001)	-1.589 (<0.001)	-0.782 (<0.001)	1.111 (<0.001)	-0.880 (<0.001)	0.000 (1.000)

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557 Fig. 1.



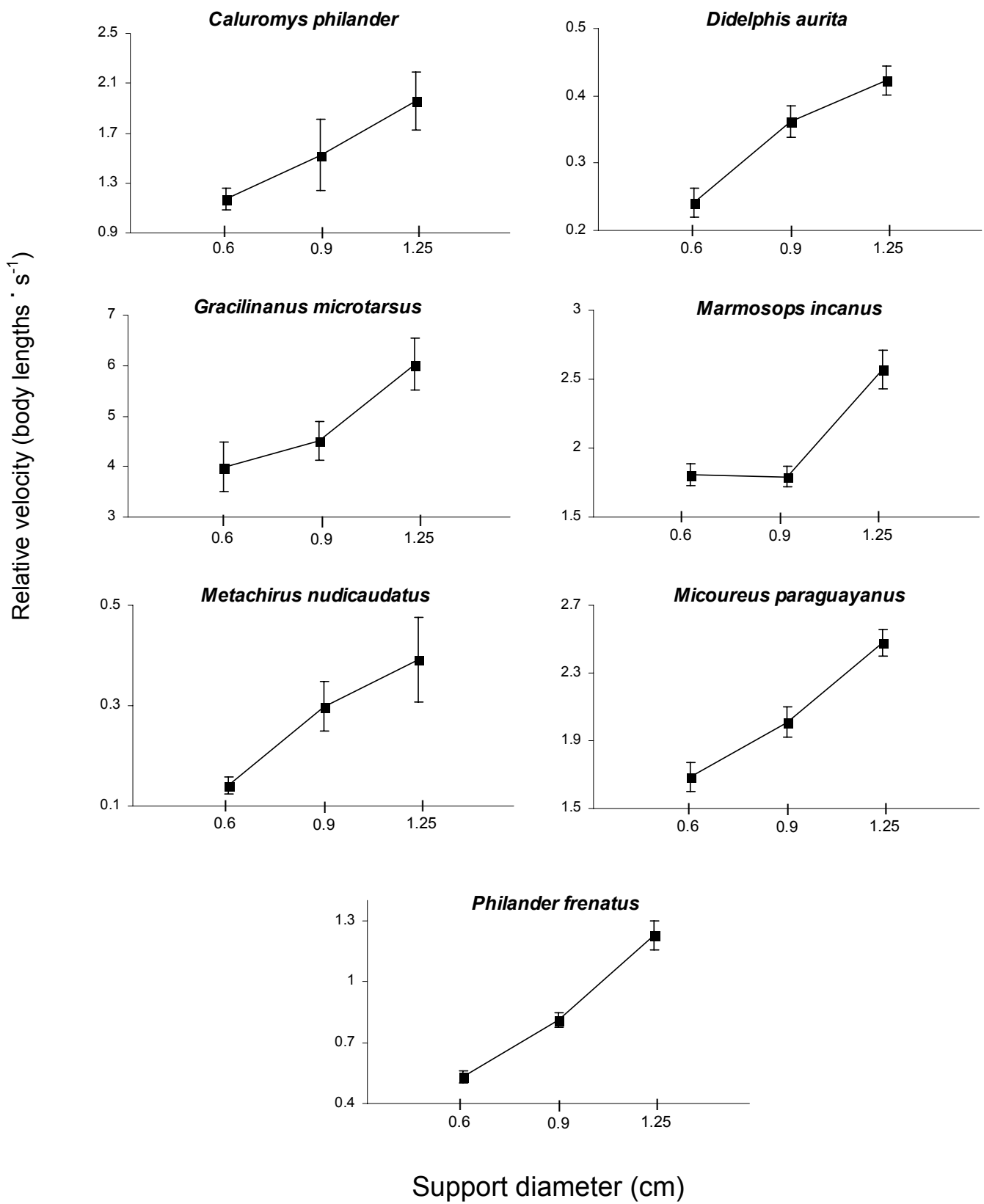
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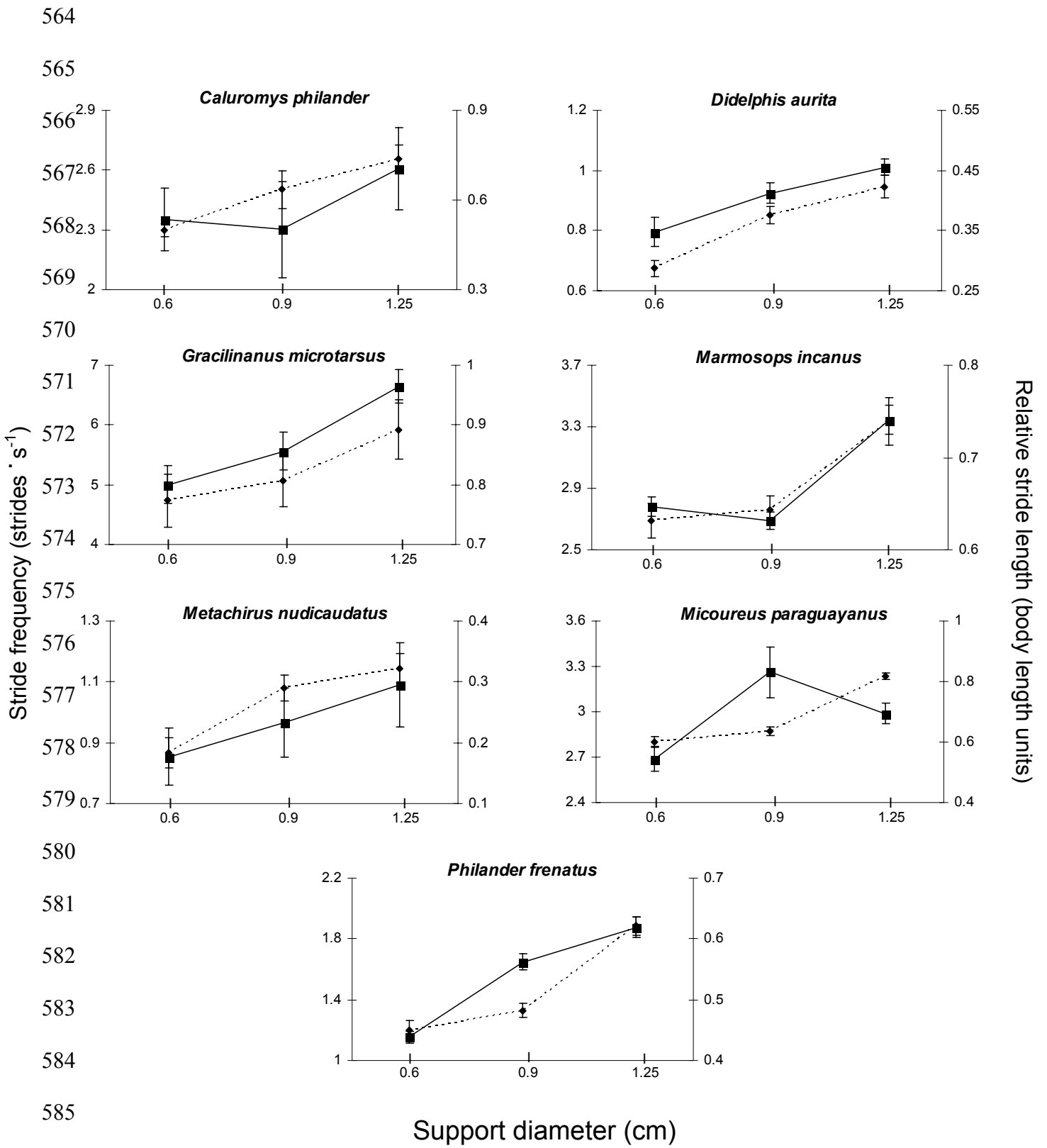
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562 Fig. 2.



563 Fig. 3.



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