Testing the stages model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika

Moritz Muschick$^{1,2}$, Patrik Nosil$^{1}$, Marius Roesti$^{2}$, Marie Theres Dittmann$^{3,4}$, Luke Harmon$^{5}$ & Walter Salzburger$^{2}$

Electronic Supplementary Material

Index:

8 Supplementary Methods

9 Supplementary Tables

10 Supplementary Figures
Supplementary Methods

Sampling

Sampling was performed between 2008 and 2012 in the southern basin of Lake Tanganyika and under the permission of the Lake Tanganyika Research Unit, Department of Fisheries, Republic of Zambia. Specimens were caught with gillnets set by snorkelling and scuba diving, by harpooning, by angling, or, in a few cases, were obtained from local fishermen. For sample preparation, we followed our standard operating procedure [described in 21]. In short, all specimens were photographed, measured and weighted; a fin-clip or a piece of muscle-tissue preserved in ethanol was taken as DNA and stable isotope sample. In total, we collected trait data for 51 LT cichlid species, which is about one quarter of the endemic species of this lake and covers 36 of the 53 genera and 10 of the 16 tribes. The choice of species was restricted to those occurring in the Southern part of the lake, where sampling took place. We used a number of sampling techniques to avoid methodical bias. Species were chosen from a larger data set solely on grounds of data completeness, i.e. data for all traits had been obtained for a given species. Efforts had been made to complete the data set also for less abundant species, in order to reduce representation bias towards more abundant species.

Trait data new to this study

The gill raker apparatus was investigated for four morphological traits: we separately counted the number of gill rakers on the dorsal and ventral bone of the first gill arch. Gill raker length was determined as the average length of rakers two, three and four on the ventral gill arch, counted from the joint with the dorsal arch bone onwards. We further measured the ventral gill arch length, as proxy for the spacing between gill rakers along the gill arch [32, 34]. These traits were measured to the nearest 0.01 mm and counted on one side within each specimen using a Leica MZ7.5 stereomicroscope. All gill raker trait assessments essentially followed previous investigations in threespine stickleback [32].

Brain tissue was removed from the neurocranium in the field and stored in either Ethanol or RNAlater (Sigma-Aldrich, Saint Louis, USA). In the laboratory, preserved fish brains were drained and dried at 60°C overnight in an incubator and subsequently weighed to the nearest milligram. A systematic bias between the two preserving liquids was apparent, with salt residues from RNAlater increasing the brain weight relative to fish body weight. We therefore adjusted the RNAlater sample measurements to fit the linear model of brain weight and body
weight for ethanol-preserved samples. To investigate gut length, we removed the entire alimentary canal (‘gut’) from the anus to the posterior end of the stomach from freshly caught specimens. Each gut was unwound, stretched out, and measured to the nearest millimetre.

In order to evaluate overall body colouration, we adopted and modified an existing colour-scoring scheme developed for Lake Tanganyika cichlids [41] using representative photographs. We used the 12 landmarks described in Salzburger et al. [41] plus information on lips (light/dark/yellow/blue), facial pattern (uniform colouration/stripes/dots), caudal fin pattern (uniform/stripes/blotches), caudal fin colouration (light/dark/yellow/orange/blue/brown/red), as well as sexual dimorphism (yes/no) and polychromatism (yes/no). From these 101 binary-coded colouration traits we retained the 83 that showed variation across species and had each character state found in at least two species. As no information on within-species variance was available, colour was not included in analyses requiring such variation.

Data re-used from a previous study

Phylogenetic relationships were derived from the enforced molecular-clock phylogeny of Muschick et al. [21] by pruning it to the 51 species included in this study. This phylogeny is based on one mitochondrial marker (ND2, alignment length: 1047 bp), and two nuclear markers (ednrbl and phpt, with alignment lengths of 542 bp and 424 bp, respectively). Body shape was assessed by using Cartesian coordinates of landmarks derived from lateral, standardized photographs using TPSDig [42]. Seventeen homologous landmarks covered the whole body and captured ecologically important shape features such as fin insertion points, body depth and length and relative size of head and trunk. Landmark coordinates were procrustes aligned in MORPHOJ [43] and, together with centroid size, analysed in R [44]. LPJ shape information was obtained similar to body shape. Twenty-eight landmark positions were recorded on scans of excised and cleaned lower pharyngeal jawbones in occlusal perspective. Twenty of these landmarks were semi-landmarks and subjected to a sliding process in TPSRELW [45] to more accurately capture the curved shapes of LPJ outlines. Of this set, 8 landmarks and 6 semi-landmarks were used for analysis. Procrustes alignment was performed in MORPHOJ and the symmetric component of shape variation and the centroid size exported for analysis in R.

Stable isotope data were used as proxies for habitat preference and trophic niche ($\delta^{13}$C and
δ₁³N, respectively). Stable isotope ratios of carbon (δ₁³C) are related to the source of carbon in the diet and thereby reveal approximate habitat preference on a limnetic-benthic axis [46]. The heavier stable isotope of nitrogen accumulates with trophic level making δ₁⁵N values can a good proxy for the trophic level of a species. Muschick et al. [21] used mass spectrometry on dried and pulverized white muscle tissue to reveal the isotopic composition. It is expressed in the conventional δ notation as permil (‰) deviation versus atmospheric N₂ and Pee Dee Belemnite.

Data transformation prior to statistical analyses

Prior to statistical analyses we log transformed all trait values, apart from landmark procrustes coordinates, gill raker counts, and colouration scores. We then regressed all trait values (excluding colouration traits) onto log transformed body weight using regression coefficients from the phyl.resid function of the R package PHYTOOLS version 0.2-40 [47]. Retained residuals of species means or individual trait values were used for subsequent analyses. Brain weight and gut length data were univariate, and hence treated accordingly. Data collected for the other traits were multivariate. We therefore reduced dimensionality within each trait complex using scaling, centring and principal components analysis (PCA). We calculated eigenvectors of species means of multivariate residuals using phylogenetic Principal Component Analysis (pPCA) as implemented in PHYTOOLS and retained principal components (PC) that each explained at least 10% of the total variance. Eigenvectors derived from the pPCA on species means were used to calculate PC scores of individuals. Residuals of univariate traits were scaled and centred only. As the colouration matrix consisted of binary coded data, colouration scores were reduced in dimensionality using detrended correspondence analysis. We kept the first three detrended correspondence axes (DFA) for further analyses.

Ecological specialization and overlap between species

We used plots of linear discriminants (LD) to illustrate each species’ position in morphospace for the multivariate data (body and lower pharyngeal jaw shape, gill rakers), and boxplots to illustrate the univariate data (gut length, brain weight). In the first case, we plotted LD₁ against LD₂, representing each species as polygons defined by the most extreme individuals,
to illustrate the subdivision of the morphospace among species and to indicate niche overlap between species. The boxplots show the log transformed, regressed, scaled, and centred trait values for each species, sorted by median.

Next, we calculated the between-species distances for each trait. Traits for which within-species variance information was available were used to calculate the Mahalanobis distance from the within-species covariance matrix. Since Mahalanobis distances are scaled by the within-group variance, we used them as a generalized measure of trait divergence, which can be compared among traits. Conceptually, this approach is similar to measuring evolutionary rate in haldanes by using the pooled standard variation to standardize character change over time [S1].

Since no such data were available for colour, we used the absolute (Manhattan) distance in the ordinated colour space derived from the presence/absence colour trait matrix. Distance matrices from different traits were then tested for correlations between each other using three-way partial Mantel tests, correcting for phylogenetic distance. The phylogenetic distance matrix was calculated using the cophenetic function in R on the molecular phylogeny. Significance of the Mantel statistic was tested with 9999 permutations.

Supplementary Reference:
<table>
<thead>
<tr>
<th>species</th>
<th>abbreviation</th>
<th>body shape</th>
<th>LPI shape</th>
<th>gut length</th>
<th>gill raker</th>
<th>brain weight</th>
<th>stable isotopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allolampelus calicus</td>
<td>alc</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Allolampelus compressiceps</td>
<td>alc</td>
<td>23</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Allolampelus fasciatus</td>
<td>alc</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Asplenothalassia agrestis</td>
<td>asag</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Astaciulus bulbus</td>
<td>abbul</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Autonoeusus alabrinus</td>
<td>aaul</td>
<td>36</td>
<td>19</td>
<td>9</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Beaufortiammus tiligulatus</td>
<td>btil</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Bouleniarmus mclurensis</td>
<td>bmm</td>
<td>17</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Callostoelasma macrospices</td>
<td>calmac</td>
<td>16</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Chalinomurus brachyuris</td>
<td>cbbr</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Cyphothelasma gibberosa</td>
<td>cphgb</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Ctenochromis horei</td>
<td>cthe</td>
<td>31</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Cyphothelasma funeifer</td>
<td>cpyfu</td>
<td>45</td>
<td>34</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Cyprichelasma leptomosoma</td>
<td>cplep</td>
<td>16</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Enantiopus melanogenys</td>
<td>emel</td>
<td>20</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Eriothalassia cyanostictus</td>
<td>erccy</td>
<td>15</td>
<td>15</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Gnaithochromis pefferi</td>
<td>gnapf</td>
<td>13</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Grinnemastia lamellata</td>
<td>gila</td>
<td>20</td>
<td>15</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Haplotelasma microlepis</td>
<td>hapmic</td>
<td>15</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Haplotelasma trifasciatus</td>
<td>hapri</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>11</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Interoboscois loocik</td>
<td>intlo</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Juxtaoctopus ornatus</td>
<td>juborn</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Lamprologus calathicus</td>
<td>lamcal</td>
<td>21</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Lamprologus lemoonii</td>
<td>lamle</td>
<td>13</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Leptodiscus calathicus</td>
<td>lepca</td>
<td>26</td>
<td>18</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Leptodiscus elongatus</td>
<td>lepel</td>
<td>34</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Leptodiscus profundicola</td>
<td>lepfo</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Limnotelasma dardenni</td>
<td>limdar</td>
<td>29</td>
<td>22</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Lobochilotes labatius</td>
<td>loblab</td>
<td>31</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Neolamprologus funeifer</td>
<td>nefun</td>
<td>13</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Neolamprologus modestus</td>
<td>nemo</td>
<td>25</td>
<td>17</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Neolamprologus pulcher</td>
<td>nespul</td>
<td>21</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Neolamprologus savanyi</td>
<td>nessav</td>
<td>22</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Neolamprologus sexfasciatus</td>
<td>nessex</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Neolamprologus teteapotanus</td>
<td>nettep</td>
<td>10</td>
<td>23</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Ophthalassia ventralis</td>
<td>opver</td>
<td>17</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Paracyprichromis brienii</td>
<td>pcbyr</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Perissodus microlepis</td>
<td>permic</td>
<td>16</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Petrochilaspis euphrates</td>
<td>peteph</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Petrochilaspis fimbria</td>
<td>petfim</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Petrochilaspis macrognathus</td>
<td>petmac</td>
<td>18</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Petrochilaspis palaunoton</td>
<td>petpol</td>
<td>10</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Plecopterus straeni</td>
<td>plstr</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Pseudolampridoon eurystomus</td>
<td>pseu</td>
<td>13</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Simochromis diagramma</td>
<td>simdia</td>
<td>27</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Telmatichromis temporalis</td>
<td>teltemp</td>
<td>11</td>
<td>16</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Trichoglossus mori</td>
<td>trmori</td>
<td>28</td>
<td>16</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Tylochilaspis polylepis</td>
<td>typlpol</td>
<td>11</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Variabilichromis mori</td>
<td>varmori</td>
<td>23</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Xenotroplus flavipinnus</td>
<td>xenfina</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Xenotroplus aplopterus</td>
<td>xenap</td>
<td>32</td>
<td>19</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

| sum                          | 51           | 886        | 649        | 401        | 419        | 334         | 577            |
| mean                         | -            | 17.4       | 12.7       | 7.9        | 8.2        | 6.5         | 11.3           |
| median                       | -            | 15         | 11         | 8          | 10         | 7           | 10             |
**Table S2. Statistical analyses of species’ separation in morphospace.** MANOVA and ANOVA results for multivariate traits and univariate traits, respectively.

<table>
<thead>
<tr>
<th>Trait</th>
<th>approx. F</th>
<th>Df1</th>
<th>Df2</th>
<th>Pr (&gt;F)</th>
<th>Wilks’ λ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>body shape</td>
<td>65.35</td>
<td>50</td>
<td>835</td>
<td>0</td>
<td>0.0084</td>
</tr>
<tr>
<td>lpf shape</td>
<td>53.95</td>
<td>50</td>
<td>598</td>
<td>0</td>
<td>0.0059</td>
</tr>
<tr>
<td>gill raker</td>
<td>59.51</td>
<td>50</td>
<td>368</td>
<td>0</td>
<td>0.0121</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gut length</td>
<td>38.29</td>
<td>50</td>
<td>350</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>brain weight</td>
<td>21.58</td>
<td>50</td>
<td>283</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>


Table S3. Macroevolutionary models fitted to a molecular phylogeny and trait data of *Tanganyikan cichlid fishes*. Parameter estimates and quality of model fit for macroevolutionary models, and estimation and significance of Blomberg’s K. Sample-size corrected Akaike Information Criterion (AICc) indicates model fit, with a smaller score being a better fit. The best fitting model for each trait among the evolutionary process and rate variation models is indicated by italicized AICc score.
<table>
<thead>
<tr>
<th>Evolutionary process</th>
<th>body shape</th>
<th>lpi shape</th>
<th>gill raker</th>
<th>brain</th>
<th>gut</th>
<th>colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pPC 1</td>
<td>pPC 2</td>
<td>pPC 3</td>
<td>pPC 1</td>
<td>pPC 2</td>
<td>pPC 3</td>
</tr>
<tr>
<td>Brownian motion (σ²)</td>
<td>252.44</td>
<td>103.95</td>
<td>82.21</td>
<td>175.05</td>
<td>136.19</td>
<td>86.05</td>
</tr>
<tr>
<td>AIC_c</td>
<td>269.91</td>
<td>231.76</td>
<td>222.02</td>
<td>248.05</td>
<td>242.35</td>
<td>210.69</td>
</tr>
<tr>
<td>Ornstein-Uhlenbeck (α)</td>
<td>16.39</td>
<td>0</td>
<td>35.5</td>
<td>15.98</td>
<td>32.22</td>
<td>21.25</td>
</tr>
<tr>
<td>AIC_c</td>
<td>270.85</td>
<td>230.76</td>
<td>218.85</td>
<td>248.93</td>
<td>240.20</td>
<td>208.79</td>
</tr>
<tr>
<td>white noise (σ²)</td>
<td>11.76</td>
<td>5.53</td>
<td>3.97</td>
<td>10.12</td>
<td>6.69</td>
<td>4.42</td>
</tr>
<tr>
<td>AIC_c</td>
<td>276.94</td>
<td>238.48</td>
<td>221.52</td>
<td>269.31</td>
<td>248.21</td>
<td>227.1</td>
</tr>
<tr>
<td>Rate variation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pagel's δ</td>
<td>6.35</td>
<td>3.83</td>
<td>5.20</td>
<td>3.02</td>
<td>4.89</td>
<td>3.36</td>
</tr>
<tr>
<td>AIC_c</td>
<td>275.92</td>
<td>230.89</td>
<td>218.55</td>
<td>248.52</td>
<td>240.5</td>
<td>208.65</td>
</tr>
<tr>
<td>Early Burst (a)</td>
<td>32.82</td>
<td>50.55</td>
<td>70.99</td>
<td>31.97</td>
<td>64.44</td>
<td>42.5</td>
</tr>
<tr>
<td>AIC_c</td>
<td>270.85</td>
<td>231.61</td>
<td>218.85</td>
<td>248.93</td>
<td>240.20</td>
<td>208.79</td>
</tr>
<tr>
<td>Phylogenetic signal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pagel's λ</td>
<td>0.88</td>
<td>0.88</td>
<td>0.44</td>
<td>0.94</td>
<td>0.83</td>
<td>0.95</td>
</tr>
<tr>
<td>AIC_c</td>
<td>268.45</td>
<td>233.47</td>
<td>223.07</td>
<td>250.42</td>
<td>242.64</td>
<td>213.06</td>
</tr>
<tr>
<td>Blomberg's K</td>
<td>0.41</td>
<td>0.34</td>
<td>0.44</td>
<td>0.67</td>
<td>0.48</td>
<td>0.63</td>
</tr>
<tr>
<td>P value</td>
<td>0.011</td>
<td>0.06</td>
<td>0.003</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>
**Figure S1.** Gill raker structures on a ventral gill arch of *Simochromis diagramma* (tribe Tropheini).
Figure S2. Phenotype-environment correlations. Species means for PC 1 (or DCA 1, in the case of colour) of traits was plotted against species means of $\delta_{13}C$, $\delta_{13}N$, and the first principal component of both. $\delta_{13}C$ is considered to be an indicator of macrohabitat choice, while $\delta_{15}N$ is a proxy for trophic level.
Figure S3. Breakpoint regressions of trait distances on phylogenetic distance. Between-
species Mahalanobis distances for multivariate traits, and Manhattan distances scaled by
within-species variance plotted against phylogenetic distance derived from the ultrametric
phylogeny of [21]. Breakpoint regression models were fitted for each trait, partitioning the
correlation of distances into two phases of trait-divergence build-up. A linear model without
breakpoint was the best fit for brain weight distances. The lack of correlation in the second
phase indicates little change in average between-species trait distances over evolutionary time
for all traits. Attained average trait distances can be compared across traits as they are
standardized by within-species variance.