Appendix S3: Morphological traits

Table A) List of 60 traits (T) used in morphometric analysis of surface and subterranean *Asellus aquaticus* ecomorphs from Slovenia and Romania. Measured regions or structures are abbreviated as: pl – plumose, prx – proximal, dst – distal, crd – robust simple setae along the margin, inf – inferior, sup – superior. Numerical counted traits (N), numerical metric traits (M) and ratios (written in italics) are denoted, the latter are briefly described: r – relative.

<table>
<thead>
<tr>
<th>Region, structure</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>Ratio [denominator]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Head</strong></td>
<td></td>
<td></td>
<td></td>
<td>r width [head L]</td>
</tr>
<tr>
<td>Body</td>
<td>BL</td>
<td>N</td>
<td>M</td>
<td>r width [BL]</td>
</tr>
<tr>
<td><strong>Antenna I</strong></td>
<td></td>
<td></td>
<td></td>
<td>r length [BL]</td>
</tr>
<tr>
<td>flagellum articles</td>
<td>A1</td>
<td>N</td>
<td>M</td>
<td>r length [antenna I r length]</td>
</tr>
<tr>
<td>aestethascs</td>
<td>A1A</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antenna II</strong></td>
<td>A2</td>
<td>N</td>
<td>M</td>
<td>r length [BL]</td>
</tr>
<tr>
<td>flagellum articles</td>
<td>A2N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pereopod I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 3</td>
<td>PE13</td>
<td>N</td>
<td>M</td>
<td>merus r width [merus length]</td>
</tr>
<tr>
<td>article 5</td>
<td>PE11</td>
<td>N</td>
<td>M</td>
<td>propodus r width [propodous length]</td>
</tr>
<tr>
<td>article 6, inf setae</td>
<td>PE1N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 6, inf seta</td>
<td>PE1S</td>
<td>N</td>
<td></td>
<td>dactylus longest inf seta r length [propodous length]</td>
</tr>
<tr>
<td><strong>Pereopod IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 4, dst sup setae</td>
<td>PE44D</td>
<td>N</td>
<td>M</td>
<td>carpus longest dst sup seta r length [carpus length]</td>
</tr>
<tr>
<td>article 5</td>
<td>PE45S</td>
<td>N</td>
<td>M</td>
<td>propodus r length [merus length]</td>
</tr>
<tr>
<td>article 5, crd inf setae</td>
<td>PE45SN</td>
<td>N</td>
<td>M</td>
<td>propodus longest crd inf seta r length [propodous length]</td>
</tr>
<tr>
<td>article 5, inf setae</td>
<td>PE45SU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup setae</td>
<td>PE45BU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup pl setae</td>
<td>PE45BN</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup setae</td>
<td>PE45SU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup pl setae</td>
<td>PE45BU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, dst sup setae</td>
<td>PE45D</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pereopod VII</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 2, sup setae</td>
<td>PE72N</td>
<td>N</td>
<td>M</td>
<td>merus longest dst sup seta r length [merus length]</td>
</tr>
<tr>
<td>article 3, dst sup seta</td>
<td>PE73S</td>
<td>N</td>
<td>M</td>
<td>propodus r length [merus length]</td>
</tr>
<tr>
<td>article 5</td>
<td>PE753</td>
<td>N</td>
<td>M</td>
<td>propodus longest crd inf seta r length [propodous length]</td>
</tr>
<tr>
<td>article 5, crd inf setae</td>
<td>PE75SN</td>
<td>N</td>
<td>M</td>
<td>propodus longest crd inf seta r length [propodous length]</td>
</tr>
<tr>
<td>article 5, inf setae</td>
<td>PE75BN</td>
<td>N</td>
<td>M</td>
<td>propodus longest crd inf seta r length [propodous length]</td>
</tr>
<tr>
<td>article 5, inf setae groups</td>
<td>PE75N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup setae</td>
<td>PE75SU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, sup pi setae</td>
<td>PE75BU</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>article 5, dst setae</td>
<td>PE75D</td>
<td>N</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>article 6, setae</td>
<td>PE76N</td>
<td>N</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>article 6, seta</td>
<td>PE76S</td>
<td>N</td>
<td>M</td>
<td>dactylus longest inf seta r length [dactylus length]</td>
</tr>
<tr>
<td><strong>Pleotelson</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marginal setae</td>
<td>PTS</td>
<td>N</td>
<td>M</td>
<td>r width [pleotelson length]</td>
</tr>
<tr>
<td>marginal setae</td>
<td>PTT</td>
<td>N</td>
<td>M</td>
<td>longest marginal seta r length [shortest marginal seta length]</td>
</tr>
<tr>
<td><strong>Pleopod I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protopodit</td>
<td>PL11</td>
<td>N</td>
<td>M</td>
<td>r width [protopodit length]</td>
</tr>
<tr>
<td>protopodit, retinaculum denticles</td>
<td>PL1R</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit</td>
<td>PL12</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit, pl setae</td>
<td>PL1F</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit, prx setae</td>
<td>PL1B</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit, concavity</td>
<td>Z</td>
<td>N</td>
<td>M</td>
<td>half concavity setae r number [whole concavity setae number]</td>
</tr>
<tr>
<td>exopodit, concavity symmetry</td>
<td>ZS12</td>
<td>N</td>
<td>M</td>
<td>half concavity r surface [whole concavity surface]</td>
</tr>
<tr>
<td><strong>Pleopod II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protopodit</td>
<td>PL21</td>
<td>N</td>
<td>M</td>
<td>r width [protopodit length]</td>
</tr>
<tr>
<td>endopodit, spur</td>
<td>O</td>
<td>N</td>
<td>M</td>
<td>r length [exopodit length]</td>
</tr>
<tr>
<td>exopodit</td>
<td>PL23</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit, setae</td>
<td>PL2B</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit, pl setae</td>
<td>PL2F</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td><strong>Pleopod V</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exopodit, area</td>
<td>PL5</td>
<td>N</td>
<td>M</td>
<td>r width [exopodit length]</td>
</tr>
<tr>
<td>exopodit</td>
<td>PL5AS</td>
<td>N</td>
<td>M</td>
<td>r surface [exopodit surface]</td>
</tr>
<tr>
<td><strong>Uropod</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protopodit</td>
<td>U</td>
<td>N</td>
<td>M</td>
<td>uropod L [BL]</td>
</tr>
<tr>
<td>endopodit, setae</td>
<td>US</td>
<td>N</td>
<td>M</td>
<td>longest seta r length [endopodit length]</td>
</tr>
<tr>
<td>endopodit, seta</td>
<td>UBN</td>
<td>N</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>exopodit</td>
<td>U23</td>
<td>N</td>
<td>M</td>
<td>r length [endopodit length]</td>
</tr>
</tbody>
</table>
Morphometric traits showing significant change in both the Romanian and the Slovenian surface/subterranean ecomorph pairs of *Asellus aquaticus*.

Significant increase (red) and decrease (blue) in size or number is given in % relative to the respective ancestral surface ecomorph. All traits except for the relative pleopod 5 size (PL5) and number of marginal setae on pleotelson (PTS) change in the same direction, indicating concordant response to selection pressure of the subterranean environment. All structures are drawn approximately to scale. Not indicated are complete eye loss and depigmentation in both subterranean ecomorphs. Trait codes are written next to each trait in the Slovenian subterranean ecomorph. Trait descriptions and further details are given in the table above. It should be noted that the figure does not summarize the evolution of all traits; for a comprehensive representation of trait evolution please see the heatmap (Fig. 3) in the main text.
Table B) Trait-by-trait comparison of Slovenian and Romanian ecomorph pairs of *Asellus aquaticus*. Summary of test results for homogeneity of variance (Levene’s test) and difference in means (t-test) for 62 morphological traits in surface and subterranean ecomorph pairs of *Asellus aquaticus* from Slovenia (PP/PR*) and Romania (MD/AW*). Arrow orientation indicates increase/decrease of the trait value in the subterranean (*) ecomorph relative to its ancestral surface form. Significance levels were adjusted to control the false discovery rate according to Benjamini and Hochberg (1995) at q = 0.001 for t-tests and q = 0.01 for Levene’s tests. P-values of t-tests significant at q = 0.01 are underlined. Traits with significant unidirectional or opposing change in both ecomorph pairs are shown on Figure 4.

<table>
<thead>
<tr>
<th>Traits</th>
<th>SLOVENIA: PP/PR* (N=24/24)</th>
<th>ROMANIA: MD/AW* (N=20/22)</th>
<th>Direction of change in cave ecomorph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Levene's test</td>
<td>t-test</td>
<td>Levene's test</td>
</tr>
<tr>
<td></td>
<td>p (q*=0.00019)</td>
<td>p (q*=0.00082)</td>
<td>p (q*=0.00008)</td>
</tr>
<tr>
<td>A2</td>
<td>0.049</td>
<td>0.000</td>
<td>0.485</td>
</tr>
<tr>
<td>A2N</td>
<td>0.058</td>
<td>0.000</td>
<td>0.314</td>
</tr>
<tr>
<td>PE44D</td>
<td>0.080</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>PE45SN</td>
<td>0.302</td>
<td>0.000</td>
<td>0.473</td>
</tr>
<tr>
<td>PE45SU</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PE45D</td>
<td>0.050</td>
<td>0.000</td>
<td>0.878</td>
</tr>
<tr>
<td>PE75BN</td>
<td>0.004</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>PE75N</td>
<td>0.000</td>
<td>0.000</td>
<td>0.032</td>
</tr>
<tr>
<td>PE75SU</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>USN</td>
<td>0.009</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>BP</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A1N</td>
<td>0.306</td>
<td>0.000</td>
<td>0.682</td>
</tr>
<tr>
<td>PE44S</td>
<td>0.000</td>
<td>0.000</td>
<td>0.021</td>
</tr>
<tr>
<td>PE73S</td>
<td>0.919</td>
<td>0.000</td>
<td>0.016</td>
</tr>
<tr>
<td>PE75S</td>
<td>0.288</td>
<td>0.000</td>
<td>0.034</td>
</tr>
<tr>
<td>Z</td>
<td>0.000</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>US</td>
<td>0.018</td>
<td>0.000</td>
<td>0.423</td>
</tr>
<tr>
<td>PTS</td>
<td>0.075</td>
<td>0.000</td>
<td>0.225</td>
</tr>
<tr>
<td>PL5</td>
<td>0.211</td>
<td>0.000</td>
<td>0.652</td>
</tr>
<tr>
<td>A1A</td>
<td>0.329</td>
<td>0.000</td>
<td>0.162</td>
</tr>
<tr>
<td>PE453</td>
<td>0.207</td>
<td>0.000</td>
<td>0.664</td>
</tr>
<tr>
<td>PE45BN</td>
<td>0.227</td>
<td>0.000</td>
<td>0.907</td>
</tr>
<tr>
<td>PE45BU</td>
<td>0.000</td>
<td>0.000</td>
<td>0.312</td>
</tr>
<tr>
<td>PE7</td>
<td>0.574</td>
<td>0.000</td>
<td>0.087</td>
</tr>
<tr>
<td>PE753</td>
<td>0.010</td>
<td>0.000</td>
<td>0.031</td>
</tr>
<tr>
<td>PE75BU</td>
<td>0.510</td>
<td>0.000</td>
<td>0.961</td>
</tr>
<tr>
<td>PE75D</td>
<td>0.000</td>
<td>0.000</td>
<td>0.079</td>
</tr>
<tr>
<td>U</td>
<td>0.015</td>
<td>0.000</td>
<td>0.049</td>
</tr>
<tr>
<td>UBN</td>
<td>0.394</td>
<td>0.000</td>
<td>0.021</td>
</tr>
<tr>
<td>PE13</td>
<td>0.016</td>
<td>0.000</td>
<td>0.168</td>
</tr>
<tr>
<td>PE11</td>
<td>0.470</td>
<td>0.000</td>
<td>0.035</td>
</tr>
<tr>
<td>PE1N</td>
<td>0.167</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PE1S</td>
<td>0.000</td>
<td>0.000</td>
<td>0.358</td>
</tr>
<tr>
<td>PE45S</td>
<td>0.430</td>
<td>0.000</td>
<td>0.303</td>
</tr>
<tr>
<td>PE76N</td>
<td>0.033</td>
<td>0.000</td>
<td>0.135</td>
</tr>
<tr>
<td>PE76S</td>
<td>0.754</td>
<td>0.000</td>
<td>0.027</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>PL11</td>
<td>0.002</td>
<td>0.000</td>
<td>0.988</td>
</tr>
<tr>
<td>PL1F</td>
<td>0.142</td>
<td>0.000</td>
<td>0.053</td>
</tr>
<tr>
<td>O</td>
<td>0.310</td>
<td>0.000</td>
<td>0.943</td>
</tr>
<tr>
<td>PL2B</td>
<td>0.000</td>
<td>0.000</td>
<td>0.548</td>
</tr>
<tr>
<td>PL2F</td>
<td>0.804</td>
<td>0.000</td>
<td>0.831</td>
</tr>
<tr>
<td>BWL</td>
<td>0.140</td>
<td>0.016</td>
<td>0.613</td>
</tr>
<tr>
<td>PE75SN</td>
<td>0.024</td>
<td>0.048</td>
<td>0.002</td>
</tr>
<tr>
<td>ZS12</td>
<td>0.019</td>
<td>0.417</td>
<td>0.083</td>
</tr>
<tr>
<td>U1U</td>
<td>0.326</td>
<td>0.386</td>
<td>0.185</td>
</tr>
<tr>
<td>A1</td>
<td>0.712</td>
<td>0.042</td>
<td>0.300</td>
</tr>
<tr>
<td>PTT</td>
<td>0.161</td>
<td>0.020</td>
<td>0.926</td>
</tr>
<tr>
<td>PL5AS</td>
<td>0.734</td>
<td>0.051</td>
<td>0.006</td>
</tr>
<tr>
<td>U23</td>
<td>0.289</td>
<td>0.002</td>
<td>0.264</td>
</tr>
<tr>
<td>PE72N</td>
<td>0.109</td>
<td>0.245</td>
<td>0.000</td>
</tr>
<tr>
<td>BL</td>
<td>0.462</td>
<td>0.021</td>
<td>0.036</td>
</tr>
<tr>
<td>H</td>
<td>0.011</td>
<td>0.024</td>
<td>0.116</td>
</tr>
<tr>
<td>PE4</td>
<td>0.219</td>
<td>0.224</td>
<td>0.101</td>
</tr>
<tr>
<td>PT</td>
<td>0.949</td>
<td>0.901</td>
<td>0.682</td>
</tr>
<tr>
<td>A1AN</td>
<td>1.000</td>
<td>1.000</td>
<td>0.222</td>
</tr>
<tr>
<td>PL1R</td>
<td>0.041</td>
<td>0.112</td>
<td>0.136</td>
</tr>
<tr>
<td>PL12</td>
<td>0.865</td>
<td>0.189</td>
<td>0.001</td>
</tr>
<tr>
<td>PL1B</td>
<td>0.466</td>
<td>0.002</td>
<td>0.876</td>
</tr>
<tr>
<td>ZB</td>
<td>0.247</td>
<td>0.003</td>
<td>0.446</td>
</tr>
<tr>
<td>PL21</td>
<td>0.098</td>
<td>0.018</td>
<td>0.254</td>
</tr>
<tr>
<td>PL23</td>
<td>0.380</td>
<td>0.132</td>
<td>0.126</td>
</tr>
</tbody>
</table>