*Article*

# Morphological and Molecular Analysis of Australian Earwigs (Dermaptera) Points to Unique Species and Regional Endemism in the Anisolabididae Family

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## Supplementary Materials

**Table S1.** Location of field sites from which adult Dermaptera were collected.

| # | Site ID | State | Latitude | Longitude |
|---|---------|-------|----------|-----------|
| 1 | Muradup | WA | -33.8380 | 116.9522 |
| 2 | Frankland River | WA | -34.4123 | 117.0167 |
| 3 | Kojonup | WA | -33.8426 | 117.2405 |
| 4 | Mt. Barker | WA | -34.6348 | 117.5299 |
| 5 | Tenterden | WA | -34.3549 | 117.6573 |
| 6 | Amelup | WA | -34.3049 | 118.1039 |
| 7 | South Stirling | WA | -34.6245 | 118.2758 |
| 8 | Needilup | WA | -33.9553 | 118.7607 |
| 9 | Boxwood Hill | WA | -34.4059 | 118.8616 |
| 10 | Jacup | WA | -33.7822 | 119.1235 |
| 11 | Fitzgerald | WA | -33.6170 | 119.5841 |
| 12 | Wittenoom Hills | WA | -33.5208 | 122.2667 |
| 13 | Kapinnie | SA | -34.1155 | 135.5122 |
| 14 | Oleo Rd | SA | -34.3813 | 135.8071 |
| 15 | North Shield | SA | -34.6090 | 135.8298 |
| 16 | Yallunda Flat | SA | -34.3221 | 135.8397 |
| 17 | Warooka | SA | -34.9862 | 137.3193 |
| 18 | Petersville | SA | -34.3465 | 137.7843 |
| 19 | Manoora | SA | -33.9520 | 138.8513 |

| 20 | Milang | SA | -35.3834 | 138.9341 |
| 21 | Frayville | SA | -34.8256 | 139.2424 |
| 22 | Lake Hawdon South | SA | -37.2316 | 140.0259 |
| 23 | Bool Lagoon | SA | -37.2049 | 140.7111 |
| 24 | Inverleigh | VIC | -38.0862 | 143.9344 |
| 25 | Elmore | VIC | -36.4863 | 144.5513 |
| 26 | Finley | NSW | -35.6258 | 145.6040 |
| 27 | Devenish | VIC | -36.3327 | 145.8903 |
| 28 | Thoona | VIC | -36.3031 | 146.0989 |
| 29 | Traralgon | VIC | -38.2013 | 146.6533 |
| 30 | Grong Grong | NSW | -34.7250 | 146.7452 |
| 31 | Downside 1 | NSW | -34.9553 | 147.3101 |
| 32 | Downside 2 | NSW | -34.9574 | 147.3113 |
| 33 | Peak Hill | NSW | -32.6696 | 148.0390 |
| 34 | Wallendbeen | NSW | -34.5450 | 148.1110 |
| 35 | Canowindra | NSW | -33.4256 | 148.6678 |
| 36 | Tamworth | NSW | -31.1457 | 150.9742 |

Numbers refer to labels on Figure S1.

**Table S2.** Summary statistics for morphometric variables for the Anisolabididae morphospecies.

| Morphospecies | N | Paramere length | Paramere width | Maximum forceps length | Maximum forceps width | Forceps asymmetry index | Paramere length-width ratio | Basal width of forceps | Forceps length-width ratio |
|---|---|---|---|---|---|---|---|---|---|
| *Anisolabis* sp. 1 | 12 | 1.189 (0.092) | 0.396 (0.044) | 3.182 (0.501) | 2.476 (0.597) | 0.483 (0.196) | 3.027 (0.284) | 3.490 (0.627) | 1.320 (0.197) |
| | | 1.057 - 1.393 | 0.302 - 0.459 | 2.517 - 3.885 | 1.689 - 3.280 | 0.204 - 0.909 | 2.678 - 3.785 | 2.765 - 4.419 | 1.058 - 1.693 |
| *Anisolabis* sp. 2 | 18 | 1.087 (0.058) | 0.283 (0.027) | 3.073 (0.302) | 1.499 (0.150) | 0.148 (0.045) | 3.862 (0.354) | 2.392 (0.177) | 2.064 (0.241) |
| | | 0.974 - 1.198 | 0.229 - 0.325 | 2.468 - 3.633 | 1.190 - 1.754 | 0.067 - 0.219 | 3.361 - 4.633 | 1.945 - 2.624 | 1.750 - 2.744 |
| *Gonolabis forcipata* | 23 | 0.644 (0.034) | 0.415 (0.024) | 2.204 (0.260) | 1.471 (0.217) | 0.098 (0.040) | 1.558 (0.100) | 2.551 (0.280) | 1.513 (0.172) |
| | | 0.583 - 0.715 | 0.370 - 0.466 | 1.717 - 2.849 | 1.122 - 2.049 | 0.047 - 0.209 | 1.421 - 1.806 | 1.987 - 3.368 | 1.249 - 2.041 |
| *Gonolabis* nr. *gilesi* | 14 | 0.613 (0.043) | 0.353 (0.024) | 1.810 (0.318) | 1.402 (0.175) | 0.176 (0.147) | 1.744 (0.149) | 2.344 (0.237) | 1.302 (0.242) |
| | | 0.533 - 0.709 | 0.301 - 0.398 | 1.506 - 2.733 | 1.240 - 1.914 | 0.014 - 0.609 | 1.481 - 1.937 | 2.012 - 2.760 | 1.000 - 1.995 |
| *Gonolabis* sp. 1 | 32 | 0.529 (0.032) | 0.422 (0.026) | 3.434 (0.315) | 1.730 (0.272) | 0.051 (0.043) | 1.255 (0.074) | 2.927 (0.201) | 2.036 (0.394) |
| | | 0.472 - 0.590 | 0.369 - 0.484 | 2.859 - 4.116 | 1.276 - 2.257 | 0.001 - 0.174 | 1.129 - 1.463 | 2.579 - 3.320 | 1.498 - 2.868 |
| *Gonolabis* sp. 2 | 22 | 0.658 (0.039) | 0.452 (0.026) | 2.469 (0.206) | 1.489 (0.162) | 0.078 (0.036) | 1.459 (0.086) | 2.827 (0.230) | 1.674 (0.190) |
| | | 0.549 - 0.721 | 0.419 - 0.521 | 2.121 - 2.968 | 1.138 - 1.739 | 0.020 - 0.148 | 1.286 - 1.675 | 2.403 - 3.378 | 1.343 - 2.104 |

| | | 0.410 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Gonolabis* sp. 3 | 3 | 0.616 (0.084) | (0.031) | 2.390 (0.431) | 1.332 (0.341) | 0.072 (0.011) | 1.499 (0.121) | 2.450 (0.468) | 1.824 (0.169) |
| | | 0.520 - 0.677 | 0.381 - 0.442 | 1.893 - 2.655 | 0.938 - 1.531 | 0.059 - 0.079 | 1.365 - 1.601 | 1.910 - 2.724 | 1.719 - 2.018 |

"Forceps asymmetry" is an index calculated as the sum of the absolute differences between the heights and widths of a specimen's two forceps, "Paramere ratio" is the ratio of a specimen's right paramere's length to its width, and "Forceps ratio" is the ratio of the maximum forceps length to the maximum forceps width. These three measurements were dimensionless; all others were in mm. Individual cells show the mean, the standard deviation in brackets, and the minimum and maximum values below them. N refers to the number of individuals of each morphospecies measured. Note that only the three dimensionless variables plus the basal width of the forceps were used for the morphometric and classification analysis.

**Table S3.** Details of linear discriminant function analysis.

| Classification | Function | Variable | | | |
| | | Basal width of forceps | Forceps asymmetry index | Paramere length-width ratio | Forceps length-width ratio |
|---|---|---|---|---|---|
| Genus | 1 (NA) | -1.568 | -3.061 | -4.107 | -0.572 |
| *Anisolabis* | 1 (NA) | -0.593 | 0.216 | 2.267 | 3.865 |
| *Gonolabis* | 1 (0.966) | 1.427 | -1.653 | -8.550 | 1.534 |
| | 2 (0.024) | -3.802 | -0.224 | -1.078 | 2.272 |
| | 3 (0.009) | 0.910 | 14.878 | -1.398 | 0.568 |
| | 4 (0.001) | 1.012 | -2.706 | 5.547 | 2.375 |
| *Gonolabis* (*G*. sp. 3 removed) | 1 (0.975) | 1.245 | -1.720 | -8.600 | 1.588 |
| | 2 (0.018) | -3.639 | 4.728 | -2.290 | 2.055 |
| | 3 (0.007) | -2.266 | -13.008 | -0.513 | -0.477 |

"Classification" refers to the grouping variable used (i.e., "Genus" refers to the discrimination of the two genera, "*Anisolabis*" to that between *Anisolabis* spp., etc.). Function refers to the individual discriminant functions generated, of which there can be a maximum of k – 1, where k is the number of levels of the grouping variable (morphospecies or genera in this case). Functions are numbered in order of the proportion of variance explained by each, which is shown in brackets. "Forceps asymmetry" is an index calculated as the sum of the absolute differences between the heights and widths of a specimen's two forceps, "Paramere ratio" is the ratio of a specimen's right paramere's length to its width, and "Forceps ratio" is the ratio of the maximum forceps length to the maximum forceps width. These three measurements were dimensionless; the basal width of the forceps was in mm.

**Table S4.** Uncorrected p-distances within and between earwig species on the Barcode of Life Data Systems public repository's cytochrome oxidase c subunit 1 barcodes (507–680 bp).

| | N | *Chelidura guentheri* | *Forficula auricularia* A | *Forficula auricularia* B | *Labidura riparia* | *Nala lividipes* |
|---|---|---|---|---|---|---|
| *Chelidura guentheri* | 6 | 0.001 (0.001) | 0.020 | 0.020 | 0.019 | 0.020 |
| *Forficula auricularia* A | 558 | 0.231 | < 0.001 (< 0.001) | 0.015 | 0.020 | 0.020 |
| *Forficula auricularia* B | 130 | 0.220 | 0.102 | 0.003 (0.001) | 0.019 | 0.020 |
| *Labidura riparia* | 6 | 0.189 | 0.207 | 0.205 | 0.006 (0.002) | 0.017 |
| *Nala lividipes* | 5 | 0.199 | 0.197 | 0.196 | 0.147 | 0.007 (0.002) |

Only records with species names attached and with sequences > 500 bp were retrieved. Interspecific means and variances are shown on the left and right of the diagonal. Intraspecific mean shown on diagonal with variance in brackets. Variances were calculated using 1000 bootstraps. N refers to total individuals retrieved. Forficula auricularia A and B refers to cryptic sibling species [1].
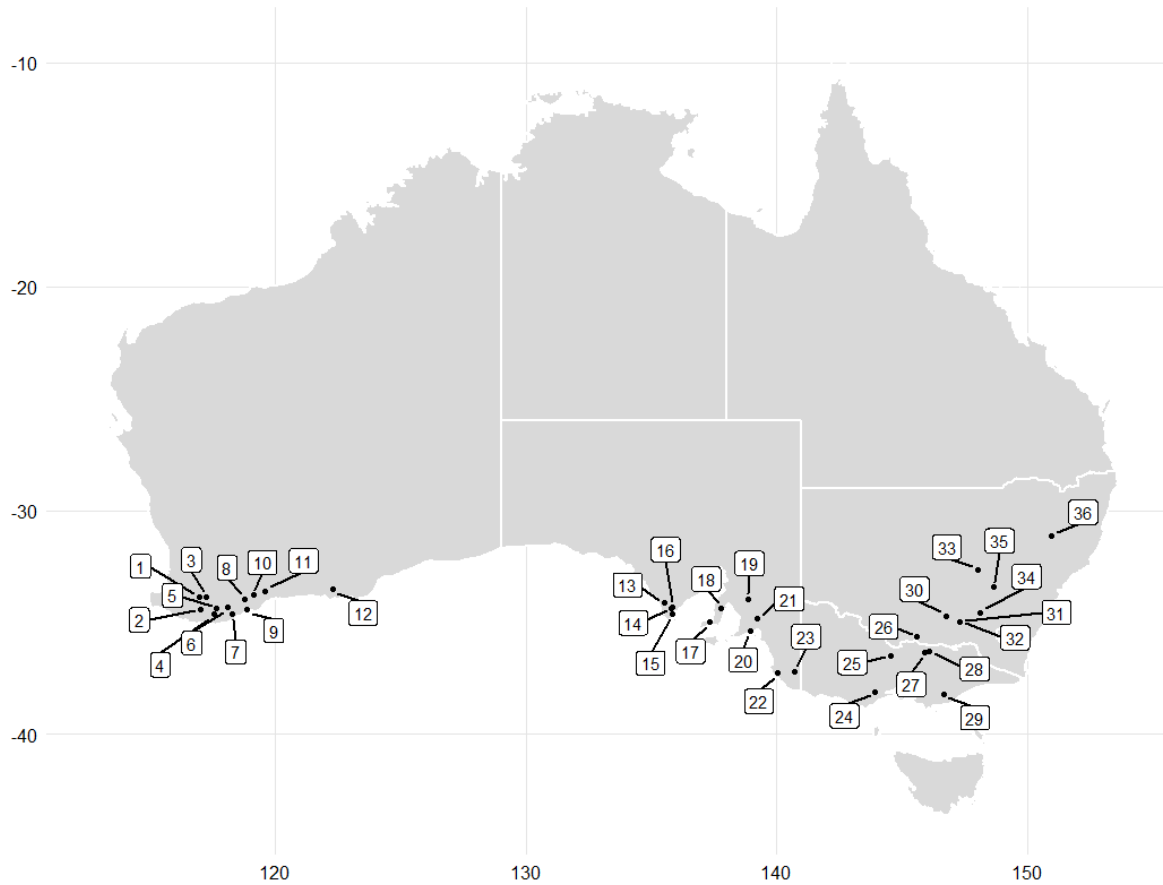
**Figure S1**. Map of field sites from which adult Dermaptera were collected. Numbers refer to sites listed in Table S1. Axes represent longitude and latitude.
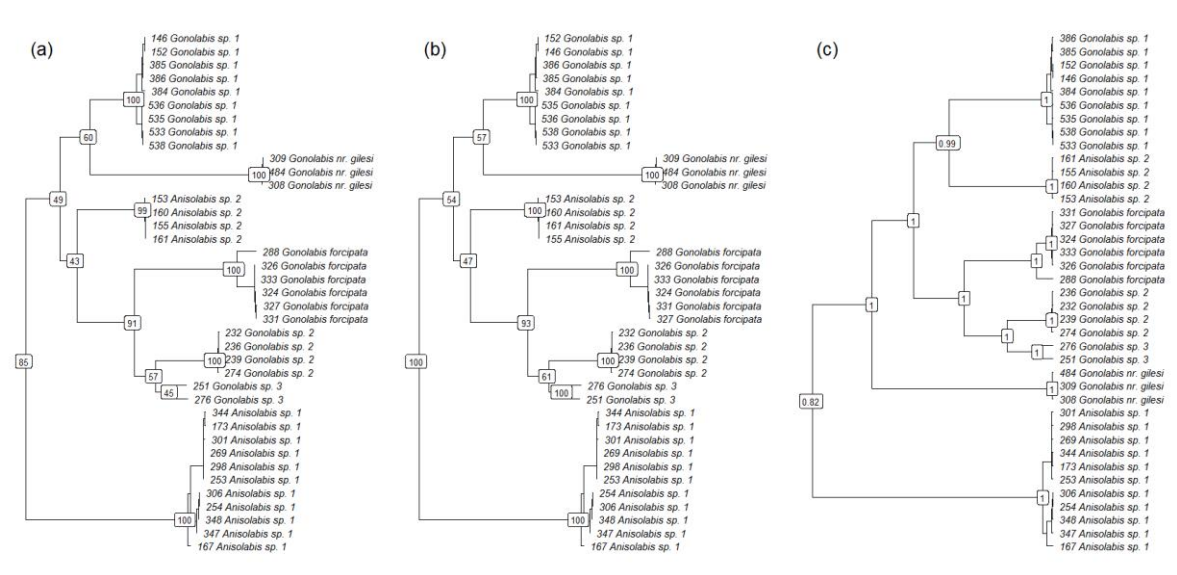


**Figure S2.** Gene trees inferred from alignment of mitochondrial sequence data (1016 bp) for Anisolabididae morphospecies (658 bp from cytochrome *c* oxidase subunit 1 and 358 bp from cytochrome *b*). Node labels represent bootstrap support from 1000 bootstraps (a and b) and posterior probability (c). (a) Best tree inferred under a maximum likelihood framework in RAxML using a general time reversible model of sequence evolution with a gamma-distributed rate parameter for site evolution estimated empirically; log-likelihood = −5689.987. (b) Tree inferred exactly as for (a) with a constraint tree representing the known morphospecies imposed on the search space; log-likelihood =

−5689.989. (c) Maximum clade credibility tree inferred using *BEAST, with models of site evolution estimated simultaneously to tree estimation using bModelTest and a birth-death process for the tree prior; 95% HPDI of tree log-likelihood = [−5276.756, −5254.856], mean log-likelihood = −5265.388.
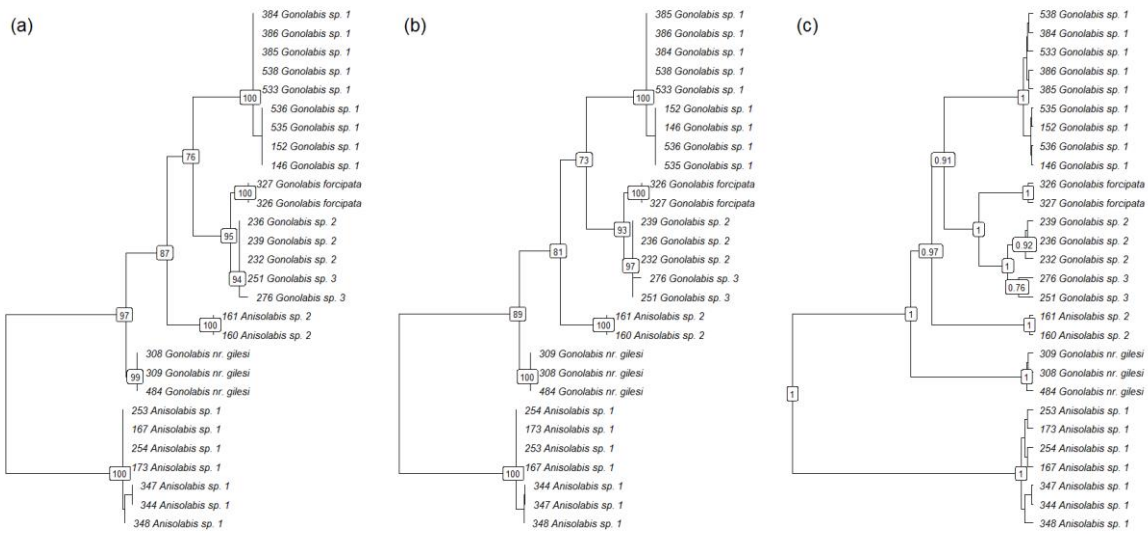


**Figure S3.** Gene trees inferred from alignment of a fragment of *28S* ribosomal DNA (442 bp) from Anisolabididae morphospecies. Node labels represent bootstrap support from 1000 bootstraps (a and b) and posterior probability (c). (a) Best tree inferred under a maximum likelihood framework in RAxML using a general time reversible model of sequence evolution with a gamma-distributed rate parameter for site evolution estimated empirically; log-likelihood = −1087.959. (b) Tree inferred exactly as for (a) with a constraint tree representing the known morphospecies imposed on the search space; log-likelihood = −1087.959. (c) Maximum clade credibility tree inferred using *BEAST, with models of site evolution estimated simultaneously to tree estimation using bModelTest and a birth-death process for the tree prior; 95% HPDI of tree log-likelihood = [−1123.086, −1102.800], mean log-likelihood = −1114.919.
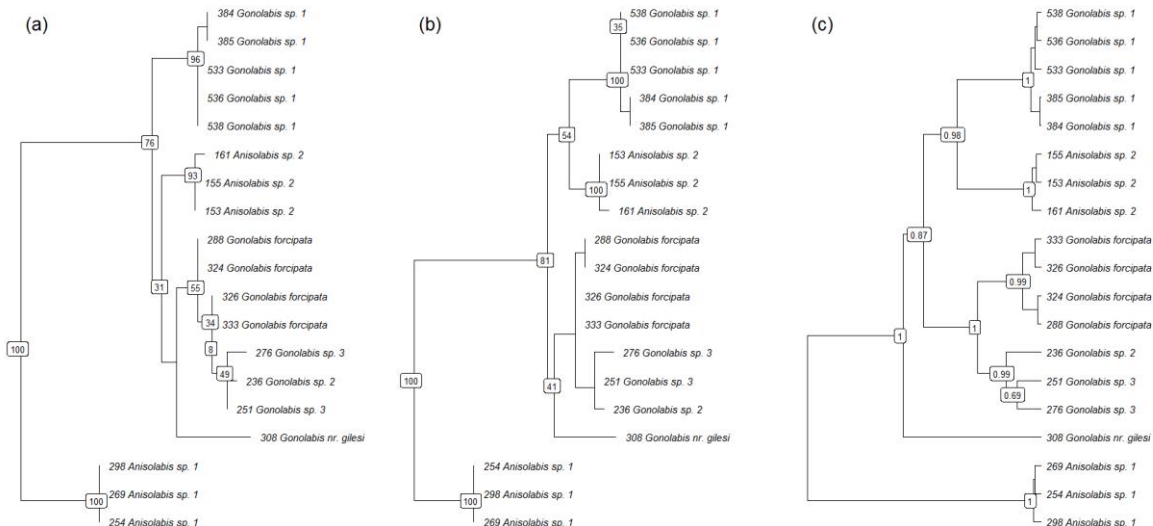


**Figure S4.** Gene trees inferred from alignment of a fragment of tubulin-alpha 1 (466 bp) from Anisolabididae morphospecies. Node labels represent bootstrap support from 1000 bootstraps (a and b) and posterior probability (c). (a) Best tree inferred under a maximum likelihood framework in RAxML using a general time reversible model of sequence evolution with a gamma-distributed rate parameter for site evolution estimated empirically; log-likelihood = −1183.262. (b) Tree inferred

exactly as for (a) with a constraint tree representing the known morphospecies imposed on the search space; log-likelihood = −1184.068. (c) Maximum clade credibility tree inferred using *BEAST, with models of site evolution estimated simultaneously to tree estimation using bModelTest and a birth-death process for the tree prior; 95% HPDI of tree log-likelihood = [−1086.380, −1054.702], mean log-likelihood = −1066.792.

**Reference**

1. Wirth, T.; Le Guellec, R.; Vancassel, M.; Veuille, M. Molecular and reproductive characterisation of sibling species in the European earwig (*Forficula auricularia*). *Evolution* **1998**, *52*, 260–265.