

# Evolutionary species delineation for the DNA taxonomy of unidentified insects

## Supplementary Online Material

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**Table S1.** Collecting sites and locality codes, sample number, collecting coordinates and dates, the number of individuals sampled, and their membership in nests in the statistical parsimony analysis and in the clades (species) of the Wiens-Penkrot (WP) analysis based on the combined data of the three mtDNA regions. Also given is the species name initially assigned during field collecting (which was not further assessed at a later stage; N/a, no species assignment made by collector). The list includes 115 entries, of which 108 groups were separated in the field based on different collecting sites or dates, or morphological differences of specimens if from the same site and time.

| Lake / site           | Code | Sample | Latitude  | Longitude  | date      | Individuals sequenced | Nest no. | WP Clade | Tentative taxon identification |
|-----------------------|------|--------|-----------|------------|-----------|-----------------------|----------|----------|--------------------------------|
| Lake Gairdner         | GA   | 3a     | 32 18'15" | 135 51'18" | 12-iii-01 | 2                     | 1        | 1        | <i>R. shetterlyi</i>           |
| Locke Claypans        | LC   | 4      | 32 19'38" | 134 57'15" | 12-iii-01 | 3                     | 1        | 1        | <i>R. trichogena</i>           |
| Locke Claypans        | LC   | 5      | 32 18'29" | 134 59'15" | 13-iii-01 | 7                     | 1        | 1        | <i>R. trichogena</i>           |
| Lake Acraman          | AC   | 6      | 32 00'25" | 135 18'59" | 13-iii-01 | 6                     | 1        | 1        | <i>R. sp.nr. shetterlyi</i>    |
| Kokatha 72.6 km South | KO   | 8      | 31 55'08" | 135 13'24" | 14-iii-01 | 2                     | 1        | 1        | <i>R. sp.nr. shetterlyi</i>    |
| Kokatha 69.6 km South | KO   | 9      | 31 53'32" | 135 13'15" | 14-iii-01 | 6                     | 1        | 1        | <i>R. sp.nr. labyrinth</i>     |
| Kokatha 43.9 km South | KO   | 10     | 31 39'39" | 135 15'03" | 14-iii-01 | 6                     | 1        | 1        | <i>R. sp.nr. shetterlyi</i>    |
| Lake Harris           | HS   | 12c    | 31 05'34" | 135 19'54" | 15-iii-01 | 2                     | 1        | 1        | <i>R. sp.nr. shetterlyi</i>    |
| Lake Labyrinth        | LA   | 13     | 30 42'32" | 135 14'50" | 15-iii-01 | 6                     | 1        | 1        | <i>R. labyrinth</i>            |
| Lake Hart             | HT   | 14b    | 31 13'44" | 136 22'44" | 16-iii-01 | 2                     | 1        | 1        | <i>R. shet.lutamatrix</i>      |
| Island Lagoon         | IL   | 106    | 31 23'53" | 136 51'47" | iv-03     | 1                     | 1        | 1        | <i>R. shet. lutamatrix</i>     |
| Island Lagoon         | IL   | 15b    | 31 14'32" | 136 28'27" | 16-iii-01 | 3                     | 1        | 1        | <i>R. shet.lutamatrix</i>      |

|  |    |              |           |            |           |   |     |    |                            |
|--|----|--------------|-----------|------------|-----------|---|-----|----|----------------------------|
| Pimba 27.3 km South                      | PM | 16b          | 31 26'07" | 137 00'06" | 16-iii-01 | 3 | 1   | 1  | <i>R. shet.lutamatrix</i>  |
| Yorkeys Crossing                         | YC | 22 ind 2-4,6 | 32 24'12" | 137 46'26" | 18-iii-01 | 4 | 1   | 1  | <i>R. cardinalba</i>       |
| Glendambo 14.5 km North-West             | GL | 39           | 30 52'53" | 135 38'18" | 24-iii-01 | 5 | 1   | 1  | <i>R. labyrintha</i>       |
| Lake Yaninee                             | YA | 48           | 32 58'05" | 135 16'29" | 28-iii-01 | 3 | 1   | 1  | <i>R. trichogena</i>       |
| Googs Lake                               | GO | 49.3         | 31 34'19" | 133 58'05" | 29-iii-01 | 1 | 1   | 1  | <i>R. trichogena</i>       |
| Lake Macfarlane                          | MA | 104          | 31 43'07" | 136 36'18" | iv-03     | 1 | 1   | 1  | <i>R. shet. lutamatrix</i> |
| Googs Lake                               | GO | 49.1,2,4-6   | 31 34'19" | 133 58'05" | 29-iii-01 | 5 | 1   | 2  | <i>R. trichogena</i>       |
| Pt Wa 14.2 km South                      | PW | 18           | 34 17'43" | 138 14'22" | 17-iii-01 | 6 | 1   | 3  | <i>R. leucothrix</i>       |
| Lake Bumbunga                            | BU | 19           | 33 55'17" | 138 09'54" | 17-iii-01 | 6 | 1   | 3  | <i>R. leucothrix</i>       |
| Lochiel 1.0 km South                     | LO | 20           | 33 56'44" | 138 09'34" | 18-iii-01 | 6 | 1   | 3  | <i>R. leucothrix</i>       |
| Lake Gilles                              | LL | 47           | 33 01'54" | 136 36'09" | 28-iii-01 | 6 | 1   | 4  | <i>R. leucothrix</i>       |
| Lake Gilles                              | LL | 1a           | 32 40'39" | 136 54'21" | 11-iii-01 | 6 | 2   | 5  | <i>R. gillesensis</i>      |
| Lake Harris                              | HS | 12b          | 31 05'34" | 135 19'54" | 15-iii-01 | 5 | 3   | 6  | <i>R. gagei</i>            |
| Lake Harris                              | HS | 209          | 31 09'43" | 135 19'43" | 19/iii/95 | 1 | 3   | 6  | <i>R. gagei</i>            |
| Lake Gairdner                            | GA | 41           | 31 01'47" | 135 23'58" | 25-iii-01 | 4 | 4   | 7  | <i>R. webbae</i>           |
| Lake Gairdner                            | GA | 3b           | 32 18'15" | 135 51'18" | 12-iii-01 | 2 | 4   | 7  | <i>R. webbae</i>           |
| Lake Harris                              | HS | 12a          | 31 05'34" | 135 19'54" | 15-iii-01 | 6 | 5   | 8  | <i>R. hudsoni</i>          |
| Lake Gairdner                            | GA | 42           | 31 04'00" | 135 26'14" | 25-iii-01 | 2 | 6   | 9  | <i>R. gairdneri</i>        |
| Yorkeys Crossing                         | YC | 22.5, 7-10   | 32 24'12" | 137 46'26" | 18-iii-01 | 5 | 7   | 10 | <i>R. cardinalba</i>       |
| Lake Frome                               | FR | 25b          | 30 37'36" | 139 38'05" | 19-iii-01 | 6 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake Harry                               | HY | 27           | 29 25'05" | 138 16'43" | 20-iii-01 | 3 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake Eyre So.                            | EY | 31           | 29 30'07" | 137 14'17" | 21-iii-01 | 6 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake Eyre N.                             | EY | 34           | 28 45'30" | 136 52'37" | 22-iii-01 | 6 | 7   | 11 | <i>R. cardinalba</i>       |
| Blanche Cup Spring                       | BC | 32b          | 29 27'18" | 136 52'06" | 21-iii-01 | 6 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake William                             | WI | 36           | 28 59'33" | 136 26'38" | 22-iii-01 | 4 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake Cadibarrawirra                      | CA | 37           | 28 57'23" | 135 35'41" | 22-iii-01 | 6 | 7   | 11 | <i>R. cardinalba</i>       |
| Lake Torrens                             | TO | 102.2-5      | 31 33'39" | 137 41'09" | iv-03     | 4 | 7   | 11 | N/a                        |
| Lake Lewis                               | LS | 115          | 22 57'32" | 132 32'01" | iv-03     | 6 | 7   | 12 | <i>R. n.sp.</i>            |
| Curtin Springs 31 km East                | CS | 112          | 25 13'23" | 132 04'11" | iv-03     | 6 | 7   | 13 | <i>R. n.sp</i>             |
| Salt Creek Coorong                       | SC | 291          | 36 02'00" | 139 32'57" | 2000      | 1 | 7   | 14 | N/a                        |
| Edithburgh (Yorke Peninsula) 9.4 Km West | ED | 292          | 35 06'00" | 137 41'59" | 2000      | 1 | (7) | 14 | N/a                        |
| Edithburgh 15.4 Km                       | ED | 293          | 35 06'00" | 137 41'59" | 2000      | 1 | (7) | 14 | N/a                        |

|                      |    |        |            |            |           |    |    |    |                            |
|----------------------|----|--------|------------|------------|-----------|----|----|----|----------------------------|
| West                 |    |        |            |            |           |    |    |    |                            |
| Tailem Bend          | TB | 288    | 35 08'00"  | 139 16'07" | 2000      | 1  | 7  | 15 | N/a                        |
| Tailem Bend          | TB | 289    | 35 08'00"  | 139 16'07" | 2000      | 1  | 7  | 15 | N/a                        |
| Norse 83.4 km East   | NR | 51     | 32 04'30"  | 122 35'29" | 30-iii-01 | 7  | 7  | 16 | <i>R. n.sp.</i>            |
| Lake Hart            | HT | 14a    | 31 13'44"  | 136 22'44" | 16-iii-01 | 6  | 8  | 17 | <i>R. ozellae</i>          |
| Island Lagoon        | IL | 15a    | 31 14'32"  | 136 28'27" | 16-iii-01 | 2  | 8  | 17 | <i>R. ozellae</i>          |
| Pimba 27.3 km South  | PM | 16a    | 31 26'07"  | 137 00'06" | 16-iii-01 | 1  | 9  | 18 | <i>R. ozellae</i>          |
| Yorkeys Crossing     | YC | 23     | 32 23'54"  | 137 45'55" | 18-iii-01 | 5  | 9  | 18 | <i>R. nudohumeralis</i>    |
| Lake Frome           | FR | 25a    | 30 37'36"  | 139 38'05" | 19-iii-01 | 6  | 9  | 18 | <i>R. nudohumeralis</i>    |
| Lake Torrens         | TO | 102.1  | 31 33' 39" | 137 41'09" | iv-03     | 1  | 9  | 18 | N/a                        |
| Lake Dissappointment | DI | 121    | 23 14'10"  | 122 42'13" | iv-03     | 6  | 10 | 19 | <i>R. n.sp.</i>            |
| vic. Well 24 - CSR   | VW | 120    | 23 05'19"  | 123 22'24" | iv-03     | 6  | 10 | 20 | <i>R. n.sp.</i>            |
| Lake Barlee          | BR | 76     | 28 54'29"  | 119 58'49" | 08-iv-01  | 6  | 11 | 21 | <i>R. n.sp.</i>            |
| Lake Moore           | MO | 94b    | 29 25'15"  | 117 47'16" | 14-iv-01  | 2  | 11 | 22 | <i>R. n.sp.</i>            |
| Lake Bennett         | BE | 114    | 22 46'10"  | 131 00'44" | iv-03     | 6  | 12 | 23 | <i>R. n.sp.</i>            |
| Lake Dora            | DO | 117    | 22 02'51"  | 123 06'56" | iv-03     | 6  | 13 | 24 | <i>R. n.sp.</i>            |
| Lake Austin          | AU | 90     | 27 39'23"  | 117 52'24" | 13-iv-01  | 6  | 14 | 25 | <i>R. browni</i>           |
| Lake Noondie         | NO | 75     | 28 23'03"  | 119 42'06" | 08-iv-01  | 4  | 14 | 26 | <i>R. n.sp.</i>            |
| Lake Deborah - West  | DE | 57.3   | 30 50'28"  | 119 03'33" | 01-iv-01  | 1  | 14 | 27 | <i>R. sp.nr blackburni</i> |
| Lake Deborah - East  | DE | 59a3,6 | 30 56'40"  | 119 29'26" | 02-iv-01  | 2  | 14 | 27 | <i>R. sp.nr blackburni</i> |
| Lake Marmion         | MN | 67b    | 29 54'36"  | 121 16'44" | 04-iv-01  | 7  | 14 | 28 | <i>R. praecipua</i>        |
| Lake Marmion         | MN | 67a    | 29 54'36"  | 121 16'44" | 04-iv-01  | 3  | 14 | 29 | <i>R. sp.nr blackburni</i> |
| Lake Goongarrie      | GG | 204    | 29 59'65"  | 121 09'46' | 7-iv-96   | 1  | 14 | 29 | <i>R. aurifodina</i>       |
| Lake Goongarrie      | GG | 68a    | 29 59'38"  | 121 09'33" | 04-iv-01  | 9  | 14 | 29 | <i>R. aurifodina</i>       |
| Lake Goongarrie      | GG | 68b    | 29 59'38"  | 121 09'33" | 04-iv-01  | 2  | 14 | 29 | <i>R. sp.nr blackburni</i> |
| Lake Goongarrie      | GG | 68c    | 29 59'38"  | 121 09'33" | 04-iv-01  | 3  | 14 | 29 | <i>R. sp. interm.</i>      |
| Lake Ballard         | BA | 201    | 29 31'46"  | 121 12'82" | 8-iv-96   | 1  | 14 | 29 | <i>R. trepida</i>          |
| Lake Ballard         | BA | 69a    | 29 32'03"  | 121 11'49" | 05-iv-01  | 1  | 14 | 29 | <i>R. sp.nr blackburni</i> |
| Lake Ballard         | BA | 69b    | 29 32'03"  | 121 11'49" | 05-iv-01  | 1  | 14 | 29 | <i>R. sp.nr aurifodina</i> |
| Lake Barlee          | BR | 142    | 29 08' 43" | 119 05'14" | iv-03     | 5  | 14 | 29 | <i>R. n.sp.</i>            |
| Lake Giles           | GI | 140    | 29 44' 29" | 119 44'28" | iv-03     | 8  | 14 | 29 | <i>R. n.sp.</i>            |
| Lake Dundas          | DU | 52     | 32 23'10"  | 121 47'38" | 31-iii-01 | 10 | 14 | 30 | <i>R. sp.nr blackburni</i> |
| Lake Dundas          | DU | 205    | 32 23'22"  | 121 47'27" | 13-iv-96  | 1  | 14 | 30 | <i>R. n. sp.</i>           |
| Lake Gilmore         | GE | 200    | 32 36'57"  | 121 33'61" | 16-iv-96  | 4  | 14 | 30 | <i>R. trepida</i>          |

|                              |    |                 |           |             |             |    |      |    |                              |
|------------------------------|----|-----------------|-----------|-------------|-------------|----|------|----|------------------------------|
| Lake Gilmore                 | GE | 53a             | 32 36'34" | 121 33'44"  | 31-iii-01   | 4  | 14   | 30 | <i>R. sp.nr blackburni</i>   |
| Lake Gilmore                 | GE | 53b             | 32 36'34" | 121 33'44"  | 31-iii-01   | 11 | 14   | 30 | <i>R. eburneola</i>          |
| Salmon Gums 42 km North-West | SA | 137             | 32 38'24" | 121 30' 22" | iv-03       | 4  | 14   | 30 | <i>R. trepida</i>            |
| Lake Goorly                  | GY | 146.1,2         | 29 57'42" | 117 01'10"  | iv-03       | 2  | 14   | 30 | <i>R. n.sp.</i>              |
| Lake Rebecca                 | RE | 65              | 30 09'57" | 122 39'05"  | 04-iv-01    | 3  | 14   | 31 | <i>R. sp.nr blackburni</i>   |
| Lake Cowan                   | CW | 207.7,9         | 32 03'07" | 121 40'97"  | 12/15-iv-96 | 2  | 14   | 32 | <i>R. pseudotrepida</i>      |
| Lake Cowan                   | CW | 54b             | 32 03'34" | 121 41'03"  | 31-iii-01   | 3  | 14   | 32 | <i>R. pseudotrepida</i>      |
| Lake Cowan                   | CW | 207.1-6, 8      | 32 03'07" | 121 40'97"  | 12/15-iv-96 | 7  | 15   | 33 | <i>R. blackburni</i>         |
| Lake Cowan                   | CW | 54a             | 32 03'34" | 121 41'03"  | 31-iii-01   | 2  | 15   | 33 | <i>R. blackburni</i>         |
| Newman Rocks L               | NE | 80              | 32 07'05" | 123 11'08"  | 10-iv-01    | 6  | 15   | 34 | <i>R. sp.nr blackburni</i>   |
| Lake Weelhamby               | WE | 91              | 29 11'29" | 116 27'51"  | 14-iv-01    | 5  | 16   | 35 | <i>R. n.sp.</i>              |
| Mongers Lake                 | MS | 92              | 29 21'41" | 116 41'09"  | 14-iv-01    | 5  | 16   | 35 | <i>R. n.sp.</i>              |
| Lake Moore                   | MO | 94a             | 29 25'15" | 117 47'16"  | 14-iv-01    | 6  | 16   | 35 | <i>R. n.sp.</i>              |
| Yarra Yarra Lakes            | YY | 97              | 29 46'53" | 115 50'29"  | 18-iv-01    | 4  | 16   | 35 | <i>R. n.sp.</i>              |
| Lake Goorly                  | GY | 146.3-10        | 29 57'42" | 117 01'10"  | iv-03       | 8  | 16   | 35 | <i>R. n.sp.</i>              |
| Lake Polaris                 | PO | 56              | 31 12'42" | 119 19'05"  | 01-iv-01    | 5  | 17   | 36 | <i>R. sp.nr blackburni</i>   |
| Lake Polaris                 | PO | 202             | 31 12'75" | 119 19'24"  | 4-iv-96     | 1  | 17   | 36 | <i>R. trepida</i>            |
| Lake Deborah - West          | DE | 57 ind 1,2,4-6  | 30 50'28" | 119 03'33"  | 01-iv-01    | 5  | 17   | 36 | <i>R. sp.nr blackburni</i>   |
| Lake Deborah - East          | DE | 59a.1,2,4,5,7,8 | 30 56'40" | 119 29'26"  | 02-iv-01    | 6  | 17   | 36 | <i>R. sp.nr blackburni</i>   |
| Lake Julia                   | JU | 58              | 31 08'10" | 119 22'01"  | 02-iv-01    | 3  | 17   | 36 | <i>R. sp.nr blackburni</i>   |
| Lake Seabrook                | SE | 60              | 31 00'06" | 119 37'53"  | 02-iv-01    | 8  | 17   | 37 | <i>R. sp.nr blackburni</i>   |
| Lake Yindarlgooda            | YI | 62              | 30 45'16" | 121 50'29"  | 03-iv-01    | 5  | 18   | 38 | <i>R. sp.nr aurifodina</i>   |
| Lake Yindarlgooda            | YI | 63a             | 30 36'40" | 121 57'55"  | 03-iv-01    | 6  | 18   | 38 | <i>R. sp.nr aurifodina</i>   |
| Lake Raeside                 | RA | 70              | 28 58'59" | 121 22'34"  | 05-iv-01    | 7  | 18   | 39 | <i>R. sp.nr aurifodina</i>   |
| Lake Raeside                 | RA | 203             | 28 58'48" | 121 22'48"  | 8-iv-96     | 1  | (18) | 39 | <i>R. trepida</i>            |
| Lake Carey                   | CY | 71              | 28 50'47" | 122 12'18"  | 05-iv-01    | 5  | 19   | 40 | <i>R. n.sp.nr aurifodina</i> |
| Lake Yindarlgooda            | YI | 133             | 30 36'40" | 121 57'55"  | iv-03       | 1  | (20) | 41 | <i>R. n.sp.</i>              |
| Lake Yindarlgooda            | YI | 63b             | 30 36'40" | 121 57'55"  | 03-iv-01    | 1  | 20   | 41 | <i>R. n.sp. unkown grp.</i>  |
| Lake Deborah                 | DE | 211             | ---       | ---         | 16-vii-99   | 1  | 21   | 42 | <i>R. n.sp. ("earina")</i>   |
| Lake Deborah - East          | DE | 212             | ---       | ---         | 24-vii-99   | 1  | (21) | 42 | <i>R. n.sp. ("earina")</i>   |
| Lake Deborah - East          | DE | 59b             | 30 56'40" | 119 29'26"  | 02-iv-01    | 6  | 21   | 42 | <i>R. n.sp. ("earina")</i>   |
| Lake Lefroy                  | LY | 55              | 31 26'32" | 121 34'13"  | 01-iv-01    | 1  | 22   | 43 | <i>R. salicursoria</i>       |
| Lake Lefroy                  | LY | 206             | 31 26'30" | 121 33'57"  | 2-iv-95     | 1  | (22) | 43 | <i>R. salicursoria</i>       |

|               |    |     |           |            |            |   |    |    |                          |
|---------------|----|-----|-----------|------------|------------|---|----|----|--------------------------|
| Lake Lefroy   | LY | 208 | 31 26'57" | 121 34'12" | 9/12-iv-96 | 6 | 22 | 43 | <i>R. salicursoria</i>   |
| Lake King     | KI | 88  | 33 05'27" | 119 37'02" | 12-iv-01   | 5 | 23 | 44 | <i>R. igneicolloides</i> |
| Lake Hope     | HO | 85  | 32 33'58" | 120 20'26" | 11-iv-01   | 6 | 24 | 45 | <i>R. sp.nr avita</i>    |
| Lake Johnston | JO | 210 | 32 26'02" | 120 38'41" | 3-iii-95   | 1 | 25 | 46 | <i>R. avita</i>          |
| Lake Way      | WA | 74  | 26 43'52" | 120 15'16" | 07-iv-01   | 4 | 26 | 47 | <i>R. n.sp.</i>          |

**Table S2. Primers used in this study**

| Sequence: 5'-3'             | mtDNA Region           | Direction | Reference                            |
|-----------------------------|------------------------|-----------|--------------------------------------|
|                             |                        |           |                                      |
| CGCCTGTTTAACAAAAACAT        | <i>rrnL+trnL2+nad1</i> | Forward   | (13)                                 |
| GCATCACAAAAAGGCTGAGG        | <i>rrnL+trnL2+nad1</i> | Reverse   | (13)                                 |
| CTGCCAAAGTAAAYAATATTCTTC    | <i>rrnL</i>            | Reverse   | (14)                                 |
|                             |                        |           |                                      |
| GAGGAGCAACTGTAATTACTAA      | <i>cob</i>             | Forward   | (15)                                 |
| AAAAGAAARTATCATTTCAGGTTGAAT | <i>cob</i>             | Reverse   | (15)                                 |
|                             |                        |           |                                      |
| CAACATTTATTTTGATTTTTTGG     | <i>cox1</i>            | Forward   | (13)                                 |
| TCCAATGCACTAATCTGCCATATTA   | <i>cox1</i>            | Reverse   | (13)                                 |
| GAAACATTTGGTTCATTAGG        | <i>cox1</i>            | Forward   | Cardoso <i>et al.</i><br>unpublished |
| GAGTAGCTATGTTTCAGC          | <i>cox1</i>            | Reverse   | Cardoso <i>et al.</i><br>unpublished |

## Supplementary Figure Legends

**Fig. S1.** Pairwise  $F_{st}$  analysis among populations. The figure shows the result of  $F_{st}$  analyses in pairwise comparisons between all populations of four or more individuals. Grey shading =  $F_{st}$  not significant; all others are significant. Where the phylogenetic analysis revealed that a local sample consisted of distantly related groups likely to represent different species not recognised in the field, these were either split or some specimens were omitted from the analysis. This affected population 22 which was split into sample 22a and 22b, and excluded specimens 146.1, 146.2, 207.7 and 207.9. If populations with non-significant pairwise  $F_{st}$  values were aggregated into larger entities, groupings were identical to those obtained with the WP method, plus the separation of two populations from Clade 1 and one population each from Clade 3, 11, and 30 (see Fig. 2 of main text).

**Fig. S2** Distribution of genotypes in paleo-drainages. Colors indicate different paleo-river according to Fig. 5 in: W. J. E. van de Graaff, R. W. A. Crowe, J. A. Bunting, M. J. Jackson, *Zeitschrift fuer Geomorphology* 21, 379-400 (1977). The dashed line marks a major division between current drainage systems. Note that genotypes up to approximately 0.7 myrs of divergence are confined to a single paleo-river.



