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	*R. shetterlyi*
Locke Claypans	LC	4	32 19'38"	134 57'15"	12-iii-01	3	1	1	*R. trichogena*
Locke Claypans	LC	5	32 18'29"	134 59'15"	13-iii-01	7	1	1	*R. trichogena*
Lake Acraman	AC	6	32 00'25"	135 18'59"	13-iii-01	6	1	1	*R. sp.nr. shetterlyi*
Kokatha 72.6 km South	KO	8	31 55'08"	135 13'24"	14-iii-01	2	1	1	*R. sp.nr. shetterlyi*
Kokatha 69.6 km South	KO	9	31 53'32"	135 13'15"	14-iii-01	6	1	1	*R. sp.nr. labyrintha*
Kokatha 43.9 km South	KO	10	31 39'39"	135 15'03"	14-iii-01	6	1	1	*R. sp.nr. shetterlyi*
Lake Harris	HS	12c	31 05'34"	135 19'54"	15-iii-01	2	1	1	*R. sp.nr. shetterlyi*
Lake Labyrinth	LA	13	30 42'32"	135 14'50"	15-iii-01	6	1	1	*R. labyrintha*
Lake Hart	HT	14b	31 13'44"	136 22'44"	16-iii-01	2	1	1	*R. shet.lutamatrix*
Island Lagoon	IL	106	31 23'53"	136 51'47"	iv-03	1	1	1	*R. shet. lutamatrix*
Island Lagoon	IL	15b	31 14'32"	136 28'27"	16-iii-01	3	1	1	*R. shet.lutamatrix*

Pimba 27.3 km South	PM	16b	31 26'07"	137 00'06"	16-iii-01	3	1	1	*R. shet.lutamatrix*
Yorkeys Crossing	YC	22 ind 2-4,6	32 24'12"	137 46'26"	18-iii-01	4	1	1	*R. cardinalba*
Glendambo 14.5 km North-West	GL	39	30 52'53"	135 38'18"	24-iii-01	5	1	1	*R. labyrintha*
Lake Yaninee	YA	48	32 58'05"	135 16'29"	28-iii-01	3	1	1	*R. trichogena*
Googs Lake	GO	49.3	31 34'19"	133 58'05"	29-iii-01	1	1	1	*R. trichogena*
Lake Macfarlane	MA	104	31 43'07"	136 36'18"	iv-03	1	1	1	*R. shet. lutamatrix*
Googs Lake	GO	49.1,2,4-6	31 34'19"	133 58'05"	29-iii-01	5	1	2	*R. trichogena*
Pt Wa 14.2 km South	PW	18	34 17'43"	138 14'22"	17-iii-01	6	1	3	*R. leucothrix*
Lake Bumbunga	BU	19	33 55'17"	138 09'54"	17-iii-01	6	1	3	*R. leucothrix*
Lochiel 1.0 km South	LO	20	33 56'44"	138 09'34"	18-iii-01	6	1	3	*R. leucothrix*
Lake Gilles	LL	47	33 01'54"	136 36'09"	28-iii-01	6	1	4	*R. leucothrix*
Lake Gilles	LL	1a	32 40'39"	136 54'21"	11-iii-01	6	2	5	*R. gillesensis*
Lake Harris	HS	12b	31 05'34"	135 19'54"	15-iii-01	5	3	6	*R. gagei*
Lake Harris	HS	209	31 09'43"	135 19'43"	19/iii/95	1	3	6	*R. gagei*
Lake Gairdner	GA	41	31 01'47"	135 23'58"	25-iii-01	4	4	7	*R. webbae*
Lake Gairdner	GA	3b	32 18'15"	135 51'18"	12-iii-01	2	4	7	*R. webbae*
Lake Harris	HS	12a	31 05'34"	135 19'54"	15-iii-01	6	5	8	*R. hudsoni*
Lake Gairdner	GA	42	31 04'00"	135 26'14"	25-iii-01	2	6	9	*R. gairdneri*
Yorkeys Crossing	YC	22.5, 7-10	32 24'12"	137 46'26"	18-iii-01	5	7	10	*R. cardinalba*
Lake Frome	FR	25b	30 37'36"	139 38'05"	19-iii-01	6	7	11	*R. cardinalba*
Lake Harry	HY	27	29 25'05"	138 16'43"	20-iii-01	3	7	11	*R. cardinalba*
Lake Eyre So.	EY	31	29 30'07"	137 14'17"	21-iii-01	6	7	11	*R. cardinalba*
Lake Eyre N.	EY	34	28 45'30"	136 52'37"	22-iii-01	6	7	11	*R. cardinalba*
Blanche Cup Spring	BC	32b	29 27'18"	136 52'06"	21-iii-01	6	7	11	*R. cardinalba*
Lake William	WI	36	28 59'33"	136 26'38"	22-iii-01	4	7	11	*R. cardinalba*
Lake Cadibarrawirra	CA	37	28 57'23"	135 35'41"	22-iii-01	6	7	11	*R. cardinalba*
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	*R. n.sp.*
Curtin Springs 31 km East	CS	112	25 13'23"	132 04'11"	iv-03	6	7	13	*R. n.sp*
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	*R. n.sp.*
Lake Hart	HT	14a	31 13'44"	136 22'44"	16-iii-01	6	8	17	*R. ozellae*
Island Lagoon	IL	15a	31 14'32"	136 28'27"	16-iii-01	2	8	17	*R. ozellae*
Pimba 27.3 km South	PM	16a	31 26'07"	137 00'06"	16-iii-01	1	9	18	*R. ozellae*
Yorkeys Crossing	YC	23	32 23'54"	137 45'55"	18-iii-01	5	9	18	*R. nudohumeralis*
Lake Frome	FR	25a	30 37'36"	139 38'05"	19-iii-01	6	9	18	*R. nudohumeralis*
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	*R. n.sp.*
vic. Well 24 - CSR	VW	120	23 05'19"	123 22'24"	iv-03	6	10	20	*R. n.sp.*
Lake Barlee	BR	76	28 54'29"	119 58'49"	08-iv-01	6	11	21	*R. n.sp.*
Lake Moore	MO	94b	29 25'15"	117 47'16"	14-iv-01	2	11	22	*R. n.sp.*
Lake Bennett	BE	114	22 46'10"	131 00'44"	iv-03	6	12	23	*R. n.sp.*
Lake Dora	DO	117	22 02'51"	123 06'56"	iv-03	6	13	24	*R. n.sp.*
Lake Austin	AU	90	27 39'23"	117 52'24"	13-iv-01	6	14	25	*R. browni*
Lake Noondie	NO	75	28 23'03"	119 42'06"	08-iv-01	4	14	26	*R. n.sp.*
Lake Deborah - West	DE	57.3	30 50'28"	119 03'33"	01-iv-01	1	14	27	*R. sp.nr blackburni*
Lake Deborah - East	DE	59a3,6	30 56'40"	119 29'26"	02-iv-01	2	14	27	*R. sp.nr blackburni*
Lake Marmion	MN	67b	29 54'36"	121 16'44"	04-iv-01	7	14	28	*R. praecipua*
Lake Marmion	MN	67a	29 54'36"	121 16'44"	04-iv-01	3	14	29	*R. sp.nr blackburni*
Lake Goongarrie	GG	204	29 59'65"	121 09'46'	7-iv-96	1	14	29	*R. aurifodina*
Lake Goongarrie	GG	68a	29 59'38"	121 09'33"	04-iv-01	9	14	29	*R. aurifodina*
Lake Goongarrie	GG	68b	29 59'38"	121 09'33"	04-iv-01	2	14	29	*R. sp.nr blackburni*
Lake Goongarrie	GG	68c	29 59'38"	121 09'33"	04-iv-01	3	14	29	*R. sp. interm.*
Lake Ballard	BA	201	29 31'46"	121 12'82"	8-iv-96	1	14	29	*R. trepida*
Lake Ballard	BA	69a	29 32'03"	121 11'49"	05-iv-01	1	14	29	*R. sp.nr blackburni*
Lake Ballard	BA	69b	29 32'03"	121 11'49"	05-iv-01	1	14	29	*R. sp.nr aurifodina*
Lake Barlee	BR	142	29 08' 43"	119 05'14"	iv-03	5	14	29	*R. n.sp.*
Lake Giles	GI	140	29 44' 29"	119 44'28"	iv-03	8	14	29	*R. n.sp.*
Lake Dundas	DU	52	32 23'10"	121 47'38"	31-iii-01	10	14	30	*R. sp.nr blackburni*
Lake Dundas	DU	205	32 23'22"	121 47'27"	13-iv-96	1	14	30	*R. n. sp.*
Lake Gilmore	GE	200	32 36'57"	121 33'61"	16-iv-96	4	14	30	*R. trepida*

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

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

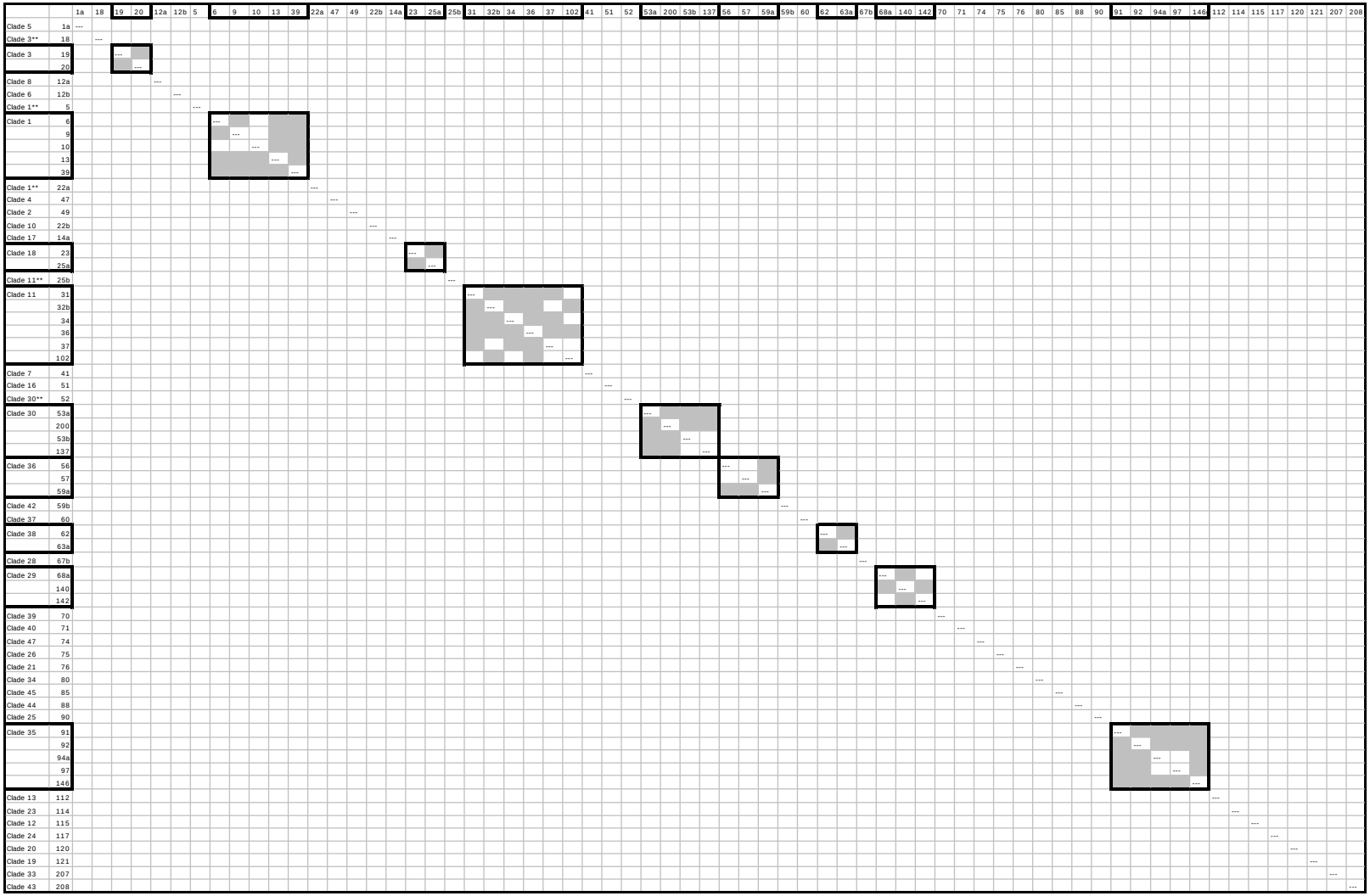
Table S2. Primers used in this study

Sequence: 5'-3'	**mtDNA Region**	**Direction**	**Reference**
CGCCTGTTTAACAAAAACAT	*rrnL+trnL2+nad1*	Forward	(13)
GCATCACAAAAAGGCTGAGG	*rrnL+trnL2+nad1*	Reverse	(13)
CTGCCAAAGTAAYAATATTCTTC	*rrnL*	Reverse	(14)
GAGGAGCAACTGTAATTACTAA	*cob*	Forward	(15)
AAAAGAAARTATCATTCAGGTTGAAT	*cob*	Reverse	(15)
CAACATTTATTTTGATTTTTTGG	*cox1*	Forward	(13)
TCCAATGCACTAATCTGCCATATTA	*cox1*	Reverse	(13)
GAAACATTTGGTTCATTAGG	*cox1*	Forward	Cardoso *et al.* unpublished
GAGTAGCTATGTTCAGC	*cox1*	Reverse	Cardoso *et al.* 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.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Clade 5 1a
Clade 3** 18
Clade 3 19
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Clade 37 60
Clade 38 62
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Clade 28 67b
Clade 29 68a
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Clade 39 70
Clade 40 71
Clade 47 74
Clade 26 75
Clade 21 76
Clade 34 80
Clade 45 85
Clade 44 88
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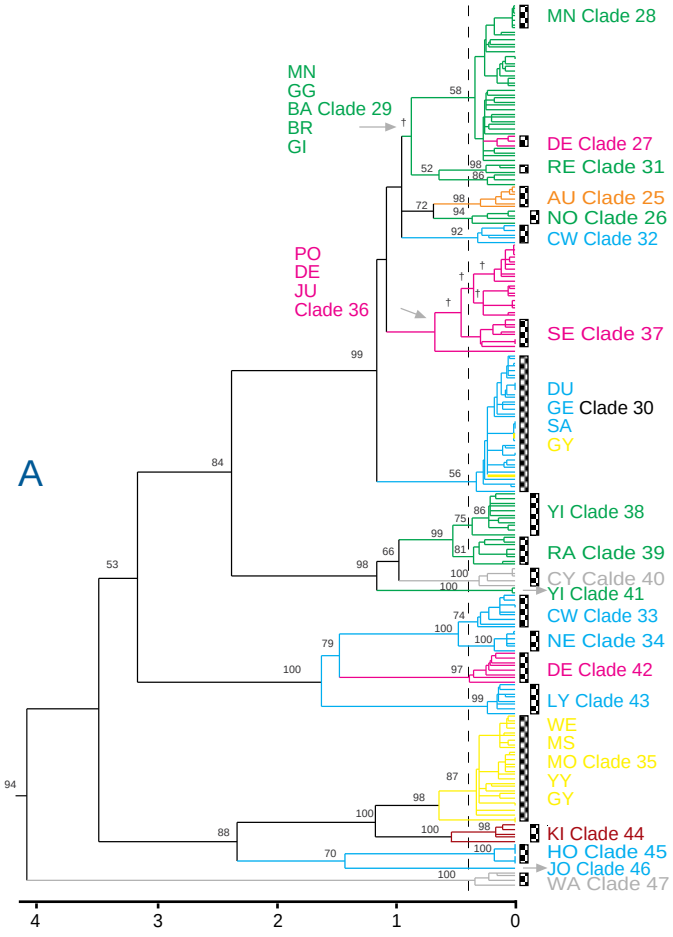
Clade 19 121
Clade 33 207
Clade 43 208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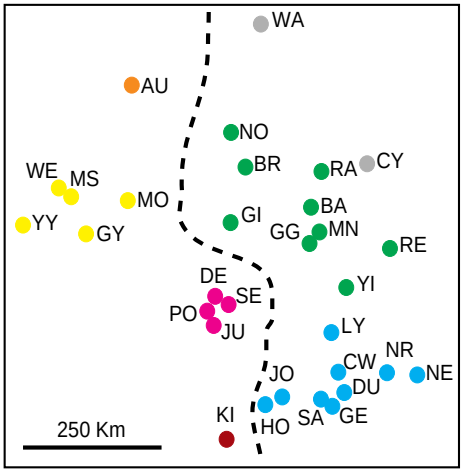
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