

Research Paper

Evolution in the *Drosophila ananassae* species subgroup

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Supplementary Material

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SUPPLEMENT TABLES

Supplement Table 1. *Drosophila* strains, collection locations, and Genbank accession number for sequence used in this study.

Species	Strain	Exp.	Location	Country	Year	Collector/donor	Sequence accession numbers				
							<i>COI</i>	<i>Ddc</i>	<i>Gpdh</i>	<i>Pgi</i>	<i>kl2</i>
		*									
<i>D. ananassae</i>	AABBg1	2	Honolulu	Hawaii		Kyorin-fly					
<i>D. ananassae</i>	N81	4	Wau	Papua New Guinea	1981	Kyorin-fly					
<i>D. ananassae</i>	N138	1	Wau	Papua New guinea	1979	Kyorin-fly	FJ795551			FJ795615	
<i>D. ananassae</i>	WAU118	4	Wau	Papua New Guinea	1981	Kyorin-fly					
<i>D. ananassae</i>	O111	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. ananassae</i>	O156	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. ananassae</i>	PT2	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. ananassae</i>	POM468	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. ananassae</i>	POM486	1	Port Moresby	Papua New Guinea	1981	Kyorin-fly	FJ795551			FJ795616	
<i>D. ananassae</i>	D38	4	Coimbatore	India	1979	Kyorin-fly					
<i>D. ananassae</i>	PNP1	1	Phnom Penh	Cambodia	2003	Kyorin-fly	FJ795552	FJ795580	FJ795606	FJ795617	
<i>D. ananassae</i>	Kuching11	1	Kuching, Sarawak	Malaysia	2003	Ng	FJ795553			FJ795618	
<i>D. ananassae</i>	Klang10	1	Klang, Selangor	Malaysia	2003	Ng	FJ795554			FJ795619	
<i>D. ananassae</i>	Jakarta8	1	Jakarta	Indonesia	2004	Ng				FJ795620	
<i>D. ananassae</i>	BSB2	1	Bandar Seri Begawan	Brunei	2003	Kopp				FJ795621	
<i>D. ananassae</i>	KMJ1	1	Kumejima	Japan	2000	Kyorin-fly	FJ795555	FJ795593		FJ795622	
<i>D. ananassae</i>	HW	3,4	Honolulu	Hawaii		Kyorin-fly					
<i>D. ananassae</i>	HNL167	4	Honolulu	Hawaii	1981	Kyorin-fly					
<i>D. ananassae</i>	Tonga	3	Tongatapu	Tonga	1966	Kyorin-fly					
<i>D. ananassae</i>	TBU135	1	Tonbatapu	Tonga	1981	Kyorin-fly	FJ795556			FJ795623	

<i>D. pallidosa</i>	NAN4	3,4	Lautoka	Fiji	1981	Kyorin-fly				
<i>D. pallidosa</i>	NAN24	1	Kautoka	Fiji	1981	Kyorin-fly	FJ795557	FJ795594		FJ795624
<i>D. pallidosa</i>	NAN57	4	Lautoka	Fiji	1981	Kyorin-fly				
<i>D. pallidosa</i>	TBU155	1	Tongatapu	Tonga	1981	Kyorin-fly	FJ795558	FJ795595		FJ795625
<i>D. pallidosa</i>	TBU161	4	Tongatapu	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	TBU162	4	Tongatapu	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	VAV81	4	Vava'u	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	VAV92	1	Vava'u	Tonga	1981	Kyorin-fly	FJ795559			FJ795626
<i>D. pallidosa</i>	VAV103	4	Vava'u	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	VAV106	4	Vava'u	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	VAV107	4	Vava'u	Tonga	1981	Kyorin-fly				
<i>D. pallidosa</i>	PPG81	4	Pago Pago	Samoa	1981	Kyorin-fly				
<i>D. pallidosa</i>	PPG102	4	Pago Pago	Samoa	1981	Kyorin-fly				
<i>D. pallidosa</i>	PPG121	4	Pago Pago	Samoa	1981	Kyorin-fly				
<i>D. pallidosa</i>	NOU79	4	Noumea	New Caledonia	1981	Kyorin-fly				
<i>D. pallidosa</i>	NOU88	1,4	Noumea	New Caledonia	1981	Kyorin-fly	FJ795560	FJ795596		FJ795627
<i>D. pallidosa</i>	NOU90	4	Noumea	New Caledonia	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM447	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM454	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM437	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM446	1,4	Port Moresby	Papua New Guinea	1981	Kyorin-fly	FJ795561			FJ795628
<i>D. pallidosa-like</i>	POM455	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM462	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM473	1,3,4	Port Moresby	Papua New Guinea	1981	Kyorin-fly	FJ795562			FJ795629
<i>D. pallidosa-like</i>	POM474	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM456	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				
<i>D. pallidosa-like</i>	POM475	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly				

<i>D. pallidosa-like</i>	POM481	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	POM482	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	POM440	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	POM449	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	POM469	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	LAE339	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	LAE333	1,4	Lae	Papua New Guinea	1981	Kyorin-fly	FJ795563				FJ795630
<i>D. pallidosa-like</i>	LAE345	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	LAE346	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	LAE385	1,4	Lae	Papua New Guinea	1981	Kyorin-fly	FJ795564				FJ795631
<i>D. pallidosa-like</i>	LAE387	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. pallidosa-like</i>	PT18	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. pallidosa-like</i>	P122	4	Port Moresby	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	P132	4	Port Moresby	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	P71	4	Port Moresby	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	PT27	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. pallidosa-like</i>	PT140	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. pallidosa-like</i>	PT144	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. pallidosa-like</i>	O89	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O102	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O108	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O92	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O98	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O125	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O148	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i>	O157	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. pallidosa-like</i> <i>WAW</i>	WAW61	1,3,4	Wau	Papua New Guinea	1981	Kyorin-fly	FJ795565				FJ795632
<i>D. pallidosa-like</i> <i>WAW</i>	WAW92	1,4	Wau	Papua New Guinea	1981	Kyorin-fly					FJ795633

<i>D. pallidosa-like</i> WAU	Bulolo79-2	1	Bulolo	Papua New Guinea	1979	Kyorin-fly					FJ795634
<i>D. papuensis-like</i>	N116	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N121	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N123	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N125	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N128	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N131	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N133	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N134	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N89	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N113	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N135	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	N144	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	WAU142	1,3,4	Wau	Papua New Guinea	1981	Kyorin-fly	FJ795566				FJ795635
<i>D. papuensis-like</i>	WAU145	4	Wau	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	WAU148	4	Wau	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	WAU163	4	Wau	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	LAE308	1,4	Lae	Papua New Guinea	1981	Kyorin-fly	FJ795567	FJ795597			FJ795636
<i>D. papuensis-like</i>	LAE340	1,4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	LAE360	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	LAE376	4	Lae	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	PT125	4	Port Moresby	Papua New Guinea	1977	Kyorin-fly					
<i>D. papuensis-like</i>	P71	4	Port Moresby	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	P87	4	Port Moresby	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	S117	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	S167	4	Wau	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	POM442	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	POM484	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					
<i>D. papuensis-like</i>	POM459	4	Port Moresby	Papua New Guinea	1981	Kyorin-fly					

<i>D. papuensis-like</i>	O97	4	Lae	Papua New Guinea	1979	Kyorin-fly					
<i>D. papuensis-like</i>	AUS52	1	Cairns	Australia	1987	Kyorin-fly	FJ795568				FJ795637
<i>D. parapallidosa</i>	B43	4	Kota Kinabalu	Malaysia	1971	Kyorin-fly					
<i>D. parapallidosa</i>	B77	4	Kota Kinabalu	Malaysia	1971	Kyorin-fly					
<i>D. parapallidosa</i>	T184	3,4	Kota Kinabalu	Malaysia	1979	Kyorin-fly					
<i>D. parapallidosa</i>	T226	1,4	Kota Kinabalu	Malaysia	1979	Kyorin-fly			FJ795598		
<i>D. prapallidosa</i>	IRO21	1	Iriommote	Japan	1998	Kyorin-fly	FJ795569	FJ795581	FJ795599		FJ795638
<i>D. parapallidosa</i>	Lanyu3	1	Lanyu	Taiwan	2004	Ng	FJ795570				FJ795639
<i>D. parapallidosa</i>	Lanyu10	1	Lanyu	Taiwan	2004	Ng	FJ795571		FJ795600		FJ795640
<i>D. ochrogaster</i>	ochrogaster	1	Noumea	New Caledonia	1992	CNRS	FJ795572	FJ795582		FJ795607	FJ795641
<i>D. monieri</i>	monieri	1	Moorea	Society Islands	1987	CNRS	FJ795574	FJ795583		FJ795608	FJ795642
<i>D. phaeopleura</i>	434	1	Suva	Fiji	1966	TDSC	FJ795573	FJ795584		FJ795609	
<i>D. atripex</i>	B121	1	Chiang Mai	Thailand	1979	Y. Fuyama					
<i>D. atripex</i>	W192	1,2	Singapore	Singapore	1981	Kyorin-fly	FJ795575		FJ795601		FJ795643
<i>D. atripex</i>	Y226	1	Penang	Malaysia	1979	Kyorin-fly		FJ795585		FJ795610	
<i>D. bipectinata</i>	381.3	1	Chia-I	Taiwan	1967	TDSC	AJ844781	FJ795586	AJ844747		
<i>D. bipectinata</i>	CJB165	2	Coimbatore	India	1981	Kyorin-fly					
<i>D. parabipectinata</i>	401.0	1	Ari Ksatr	Cambodia	?	TDSC	AJ844799	FJ795587	AJ844752		
<i>D. m. malerkotlina</i>	391.0	1	Mysore	Mysore	1971	TDSC	AJ844758	FJ795588	AJ844732		FJ795644
<i>D. p. nigrens</i>	411.1	1	Samut, Songkham	Thailand	1971	TDSC	AJ844808		AJ844730		DQ239709
<i>D. p. nigrens</i>	L57	1	Kandy	Sri Lanka		Kyorin-fly		FJ795589			
<i>D. ercepeae</i>	ercepeae	1	La Reunion	La Reunion	1973	CNRS	FJ795576	FJ795590	FJ795602	FJ795611	FJ7956466

<i>D. merina</i>	merina	1	Mandraka	Madagascar	1987	CNRS	FJ795577	FJ795591	FJ795603	FJ795612	
<i>D. vallisimaia</i>	vallisimaia	1,2	Seychelles	Seychelles	1984	CNRS	FJ795578	FJ795592	FJ795604	FJ795613	FJ795647
<i>D. varians</i>	variens	1,2	Los Banos	Philippines		Kyorin-fly	FJ795579		FJ795605	FJ795614	

* 1 :Strains used for molecular phylogenetic reconstruction

2: Strains used for mitotic and meiotic chromosome preparation

3: Strains used for pre-mating isolation test

4: Strains used for F₁ males fertility test

** Kyorin-fly: Kyorin University *Drosophila* stock collection, Japan

CNRS : Laboratoire Populations, Genetique et Evolution, Centre National de la Recherche Scientifique, Gif sur Yvette, France

TDSC : Tucson *Drosophila* species Stock Center, University of Arizona, USA

Supplement Table 2. Primers used for PCR and sequencing.

Locus	Forward primer	Reverse primer	Approximate fragment length	Annealing temperature
<i>Gpdh</i>	TCAAGCTCGGCGACAACA	CCCATCAACACGGCGCAYGG	1000 bp	50°C
<i>Ddc</i>	AGTCCCAAGTTYCATGCCTACTTCCC	TGCCCTTCATCAGGTGGCGRTACTC	600 bp	55°C
<i>Pgi</i>	CCAAGCATTTTGTGGCCCTSTCGAC	AACGAGTTRATGTCCCAAATRATGCC	900 bp	55°C
<i>kl2</i>	CACAAGGAAGTCGTTGGCCTTT	TAAAATTGCTGCGCGTTCAGAG	500 bp	53-58°C
<i>COI</i>	CCAGCTGGAGGAGGAGATCC	CCAGTAAATAATGGGTATCAGTG	550 bp	55°C

Supplement Table 3. Maximum likelihood model parameters used in phylogenetic reconstruction.

Data set	Substitution Rate Matrix						Shape ^a	% inv ^b	Model
	AC	AG	AT	CG	CT	GT			
<i>COI</i>	0.0001	58.3675	86.5164	0.0001	448.302	1	0.1559	0	GTR+FG
<i>Ddc</i>	1	1.6337	0.6807	0.6807	4.1207	1	0.5996	0	TIM+FG
<i>Gpdh</i>	1	3.1748	1	1	7.84	1	0.6098	0.5565	TrN+I+FG
<i>kl2</i>	1.6193	3.4791	1.4849	0.3533	5.6662	1	0.2341	0	GTR+FG
<i>Pgi</i>	2.1168	4.4811	1.9334	1.1851	10.9492	1	1.1199	0.5719	GTR+I+FG
Combined	1.8191	3.8747	3.5083	1.8129	8.7016	1	0.5815	0.4703	GTR+I+FG

^a Shape parameter of the gamma distribution.

^b Percentage of invariable sites

APPENDIX TABLES

Appendix Table 1. Average number and the range of sex comb teeth in *D. parapallidosa* (T226), *D. pallidosa* (NAN24), and *D. ananassae* (HW)

Sex comb	Row	Species		
		<i>D. parapallidosa</i>	<i>D. pallidosa</i>	<i>D. ananassae</i>
metatarsus	1			1.0(0-3)
	2		1.4(0-2)	2.5(0-4)
	3	0.2(0-3)	4.0(1-6)	4.1(1-6)
	4	2.9(1-6)	5.1(3-7)	5.5(3-7)
	5	4.1(2-7)	4.9(4-7)	6.0(3-8)
2nd tarsus	1			
	2		0.1(0-1)	0.6(0-2)
	3	0.4(0-2)	2.2(1-3)	2.8(0-4)
	4	3.2(2-5)	3.5(3-5)	5.0(2-6)
	5	3.4(2-5)	4.0(3-5)	4.0(3-6)
No. of flies tested		140	160	50

Appendix Table 2. Average values and the range of taxonomic indexes of wings in *D. parapallidosa*, *D. pallidosa*, and *D. ananassae*.

Indexes	Species		
	<i>D. parapallidosa</i>	<i>D. pallidosa</i>	<i>D. ananassae</i>
C	1.3 (1.1-1.6)	1.4 (1.2-1.9)	1.4 (1.3-1.6)
4V	2.5 (2.0-3.0)	2.2 (1.7-2.8)	2.4 (2.0-3.1)
4C	2.0 (1.6-2.4)	1.7 (1.5-2.1)	1.8 (1.6-2.4)
5X	2.1 (1.5-3.0)	2.1 (1.4-2.7)	2.0 (1.5-2.6)
C3fg	0.54 (0.51-0.58)	0.52 (0.47-0.57)	0.51 (0.47-0.53)
No. of flies tested	60	62	75

Appendix Table 3. Major components of cuticular hydrocarbons in *D. parapallidosa*, *D. pallidosa*, and *D. ananassae*.

Species	Major cuticular hydrocarbon
<i>D. parapallidosa</i>	C31: (Z,Z)-5,25-hentriacontadiene [(Z,Z)-5-25-C _{31:2}]
<i>D. pallidosa</i> *	C33: (Z,Z)-5,27-tritriacontadiene [(Z,Z)-5,27,C _{33:2}]
<i>D. ananassae</i> **	C31: (Z,Z)-5,25-hentriacontadiene [(Z,Z)-5-25-C _{31:2}]

* Data from Oguma (1993) and Nemoto et. al. (1994)

** Data from Doi et. al. (1997)

Appendix A. *D. parapallidosa* Tobari, sp. nov.

D. parapallidosa (previously described as Taxon K by Tobari et al. (1993) and Tomimura et al. (1993)) shows consistent differences from the other taxa of the *ananassae* cluster with respect to reproductive isolation and Y-chromosomal haplotypes. Reproductive isolation between *D. parapallidosa* on the one hand, and *D. ananassae* and *D. pallidosa* on the other, is at least as strong as between the latter two species (Tables 3, 4). Based on this evidence, we propose to give this taxon the status of a new species.

Taxonomy

Drosophila (Sophophora) *parapallidosa* Tobari sp. n.

Diagnosis

External morphology of *D. parapallidosa* is indistinguishable from that of *D. pallidosa*.

Description

It is very hard to distinguish between *D. pallidosa* and *D. parapallidosa*, but some differences in several morphological characters are found as shown in Appendix Tables 1 ~3. All type specimens were deposited in National Science Museum, Tokyo, Japan (NSMT).

Holotype.

Male from the isofemale line T184, which was collected in August, 1979 at Kota Kinabalu, Malaysia by Y. Fuyama, F. Hihara, and T.K. Watanabe.

Paratypes.

Nine males and 10 females from the T184 line, and 10 males and 10 females from the T226 line that was collected in August, 1979 at Kota Kinabalu, Malaysia by Y. Fuyama, F. Hihara, and T.K. Watanabe.

Distribution.

KOTA KINABALU, Malaysia, LANYU, Taiwan, and OKINAWA, Japan.

Etymology.

The name indicates the phenotypic similarity to *D. pallidosa*.

Morphological characters

Sex combs

The number of sex comb teeth in *D. parapallidosa* is less than in *D. ananassae* and *D. pallidosa*. (Appendix Table 1)

Genitalia

Males of *D. parapallidosa* are difficult to distinguish from *D. pallidosa* males.

Wing index

Ranges of four traits: Costal index, 4V, 4C, and 5X are shown in Appendix Table 2. C3fg values overlap completely among species, ranging from 0.42 to 0.63; average values are 0.51, 0.52, and 0.54 in *D. ananassae*, *D. pallidosa*, and *D. parapallidosa*, respectively. Other indices overlap as well in all three species, though the mean values are slightly different.

Chromosome configurations

Mitotic chromosomes:

The Y and 4th chromosomes are slightly shorter in *D. parapallidosa* than in *D. pallidosa*. Both chromosomes are sub-telocentric in *D. parapallidosa*, while the Y chromosome is submetacentric in *D. ananassae* and metacentric in *D. pallidosa*.

Polytene chromosomes

D. parapallidosa has no Standard arrangement on 2L and 2R, but has *In(2L)B*, *In(2L)C*, and *In(2R)A*. *D. parapallidosa* also has the *In(3L)E* arrangement on the 3rd chromosome, which is not found in *D. ananassae* or *D. pallidosa*.

Cuticular Hydrocarbons

The main cuticular hydrocarbon of *D. parapallidosa* is the same as that of *D. ananassae*, but different from that of *D. pallidosa* as shown in Appendix Table 3.

Interspecific hybridization

Hybrid females are fertile in all interspecific crosses. F₁ males from crosses between *D. ananassae* or *D. pallidosa* females and *D. parapallidosa* males are sterile, while those from the reciprocal crosses are fertile. Mating success between species is shown in Table 3. There are large differences in the reciprocal crosses between *D. ananassae* and *D. pallidosa* (44-49% vs. 0-8%), but only a slight difference in the reciprocal crosses between *D. pallidosa* and *D. parapallidosa* (68% vs. 82%).