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Appendix from N. J. Tatarnic and G. Cassis, "Surviving in Sympatry: Paragenital Divergence and Sexual Mimicry between a Pair of Traumatically Inseminating Plant Bugs"

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Field Collecting, French Polynesia

Over three trips we sampled insects from Tahiti Island. Specimens were collected predominantly by beating vegetation, with supplemental collection via light trapping. For a video tutorial on vegetation beating, visit the Planetary Biodiversity Plant Bug Inventory website (http://research.amnh.org/pbi/). Specimens examined for this study were collected from two localities:

Tahiti Island, Tahiti Iti, Taravao Plateau, 17.777°S, 149.255°W, 877 m asl. Specimens were collected off the flowers of *Metrosideros collina* (Myrtaceae; March 30–April, 7, 2012; February 1–6, 2012; May 15–25, 2011). During February 2012 specimens were also collected by light trap at the same location.

Tahiti Island, Rao Ape Aorai (base of "Belvedere Trail"), 17.551°S, 149.529°W, 331 m asl. Specimens collected off the flowers of *Schinus terebinthifolius* (Anacardiaceae; March 30–April 7, 2012).

Phylogenetic Analysis

Taxon Sampling

Taxa for the phylogenetic analyses were selected based on an existing phylogeny of the genus *Coridromius* (Tatarnic and Cassis 2010). These include *C. variegatus* (New Caledonia, Fiji), the three Australian endemics *C. chenopoderis*, *C. monotocopsis*, and *C. pilbarensis*, as well as a new, recently described species from Norfolk Island. Outgroup taxa include two Southeast Asian species: *C. chinensis* and *C. testaceous*. *Coridromius bicolor* from Vanuatu was unavailable for this study; however, based on our previous phylogenetic analysis, it is not closely related to either of the Tahitian species.

DNA was extracted using DNEasy Tissue Kits (www.Quiagen.com). Extractions and polymerase chain reaction (PCR) amplifications were conducted at the University of New South Wales (UNSW) and the Australian Museum (AM). Because of the bugs' minute size, whole specimens were used for DNA extraction. Voucher DNA is stored in the UNSW insect collection. Specimen voucher and collection data are supplied below.

Coridromius chenopoderis. Voucher ID C002: Australia: Western Australia: Burrup Conservation Area, near Karratha, 20.578°S, 116.795°E, 17 m asl, June 3, 2004, N. Tatarnic and S. Lassau (coll.), ex. *Atriplex amnicola* (Chenopodiaceae).

Coridromius monotocopsis. Voucher ID C004: Australia: New South Wales: Royal National Park, Wattamola Beach car park, 34.133°S, 151.113°E, 29 m asl, April 12, 2004, G. Cassis, P. Tinerella, N. Tatarnic, N. Velez, and K. McLachlan (coll.), ex. *Monotoca elliptica*.

Coridromius pilbarensis. Voucher ID C003. Australia: Western Australia: Pilbara Region, base of Mount Nameless, adjacent to gate. 22.727°S, 117.749°E, June 5, 2004, M. Bulbert, N. Tatarnic and S. Lassau (coll.), ex. *Acacia* sp. (Fabaceae).

Coridromius variegatus. Voucher ID C001: French Polynesia: New Caledonia: Province Sud: Les Bois du Sud campground, 22.172°S, 166.760°E, 200 m asl, April 23–25, 2005, G. Cassis, M. Wall, N. Tatarnic, and G. Monteith (coll.), ex. *Phyllanthus* sp. (Euphorbiaceae).

Coridromius norfolkensis. Voucher ID H308: Australia: Norfolk Island: Ball Bay, end of Marsh Road, 29.048°S, 167.980°E, 69 m asl, April 22, 2011, N. Tatarnic and A. Namyatova (coll.), ex. *Schinus terebinthifolius* (Anacardiaceae).

Coridromius tahitiensis. Voucher ID H205: French Polynesia: Society Islands: Tahiti Island: Tahiti Iti, Taravao Plateau, 17.777°S, 149.255°W, 877 m asl, May 19, 2011, G. Cassis and N. Tatarnic (coll.), *Metrosideros collina* (Myrtaceae).

Coridromius taravao. Voucher ID H305: French Polynesia: Society Islands: Tahiti Island: Tahiti Iti, Taravao Plateau, 17.777°S, 149.255°W, 877 m asl, May 19, 2011, G. Cassis and N. Tatarnic (coll.), ex. *Metrosideros collina* (Myrtaceae).

Coridromius chinensis. Voucher ID NT01: China: Yunnan Province: Xishuangbanna Tropical Botanical Garden, edge of gallery forest, 21.932°N, 101.245°E, 547 m asl, May 26, 2006, N. Tatarnic (coll.), ex *Macaranga* sp. (Euphorbiaceae).

Coridromius testaceous. Voucher ID NT12: China: Yunnan Province: Xishuangbanna Tropical Botanical Garden, edge of gallery forest, 21.932°N, 101.245°E, 547 m asl, May 26, 2006, N. Tatarnic (coll.), ex *Macaranga* sp. (Euphorbiaceae).

Analysis

A total of 892 aligned mitochondrial nucleotides were amplified from the 16S (450 bp) and CO1 (442 bp) regions, while 1,159 aligned nuclear nucleotides were amplified from two 28S subregions: D2 (637 bp) and D3–5 (522 bp). The primers used are shown in table A1:

PCR protocol was as follows: one initial denaturation at 94°C for 3 min; and then denaturation: 30 s at 94°C, annealing: one cycle each at 55°, 53°, 51°, 49°, and 47°C, and 35 cycles at 45°C for 30 s; extension: 72°C for 45 s; and a final extension of 72°C for 10 min (MJ Research PTC 100 Thermocycler). The PCR product was visualized on a 2% agarose gel to ensure single bands of the expected size and then sent to Macrogen (http://www.macrogen.com) for cleanup and sequencing.

Concatenation of forward and reverse sequences and multiple sequence alignments were performed in Geneious Pro 3.5.7 (http://www.geneious.com). Alignments were conducted using the Geneious Alignment method (default settings, five alignment iterations), followed by manual editing. Before analysis, PartitionFinder, version 1.0.1 (Lanfear et al. 2012), was used to select appropriate nucleotide substitution models for the different data set partitions (codon position and gene region). Data partitions were set for each gene region as well as each codon position for CO1. From this four best models were selected for our six subset partitions: HKY + G (16S); JC + I (CO1 position 2, D3–5); HKY + G (CO1 position 3); and K80 + I (CO1 position 1, D2). Phylogenetic analysis with the above partitions and nuclear substitution models was conducted using MrBayes 3.2 (Huelsenbeck and Ronquist 2001; four chains, 11,000,000 generations, burn-in 5,000). Genbank accession numbers are provided in table A2.

Table A1. Primers used for DNA amplification

Region	Primer	Primer sequence (5′–3′)	Source
16S	16SF	CGCCTGTTTATCAAAAACAT	Xiong and Kocher 1991
16S	16SR	CTCCGGTTTGAACTCAGATC	Xiong and Kocher 1991
CO1	C1-N-2609	GAAATACTGCTCCTATGGATA	Damgaard and Sperling 2001
CO1	C1-J-2183	CAACATTTATTTTGATTTTTTGG	Damgaard and Sperling 2001
28S	D2FA	CGGGTTGCTTGAGATGC	Unpublished, AMNH
28S	D2FR	CTCCTTGGTCCGTGTTTC	Unpublished, AMNH
28S	D3FA	TTGAAACACGGACCAAGGAG	Unpublished, AMNH
28S	D5FR	CGCCAGTTCTGCTTACCA	Unpublished, AMNH

Note: AMNH = American Museum of Natural History.

Table A2. Genbank accession numbers

Taxon (*Coridromius* spp.)	16S	CO1	28S, D2 subregion	28S, D3–5 subregion
C. chinensis	KF005578	...	KF036255	KF036264
C. testaceous	KF005579	KF036246	KF036254	KF036263
C. monotocopsis	KF005580	KF036249	KF036257	KF036268
C. chenopoderis	KF005581	KF036248	KF036256	KF036267
C. pilbarensis	KF005582	KF036252	KF036258	KF036269
C. tahitiensis	KF005583	KF036253	KF036261	KF036270
C. taravao	KF005584	KF036247	KF036262	KF036271
C. variegatus	KF005585	KF036250	KF036260	KF036265
C. norfolkensis	...	KF036251	KF036259	KF036266

Literature Cited Only in the Appendix

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Xiong, B., and T. D. Kocher. 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34:306–311.