

# Utility of DNA Sequences for Inferring Phylogenetic Relationships and Associating Morphologically Dissimilar Males and Females of the Bee-Killing Flies, Genus *Melaloncha* (Diptera: Phoridae)

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**ABSTRACT** The bee-killing flies, genus *Melaloncha*, are parasitoids of bees of the family Apidae, including stingless bees, bumble bees, and the western honey bee, *Apis mellifera* L. With >160 described species, *Melaloncha* is among the largest genera in the family Phoridae. Most species are known only from females as the males typically show too few differences to be useful in characterizing species. The monophyly of *Melaloncha* and its two subgenera (*Udamochiras* and *Melaloncha* s.s.) is strongly supported by morphological characters, but the phylogenetic relationships among the various species and species groups are not well understood. Here, we report on a preliminary molecular phylogenetic study of 30 exemplar *Melaloncha* species representing both subgenera and seven species groups by using a combination of nuclear (28S and CAD) and mitochondrial (12S, 16S, NDI, and COI) genes for a total of 3,306 bp. Maximum parsimony analysis suggested the following relationships: 1) *Melaloncha* is monophyletic, 2) the subgenus *M.* (*Udamochiras*) is monophyletic and is a sister-group to *M.* (*Melaloncha*), and 3) each of the species groups for which we had multiple species were monophyletic with the *M. furcata*-group being the sister-group to all other Group II taxa. These results support hypotheses of relationships based on morphological characters. The utility of the molecular data for associating morphologically dissimilar males and females is discussed.

**KEY WORDS** parasitoid, molecular systematics, Apidae, Neotropical

The genus *Melaloncha* Brues, commonly known as the bee-killing flies, is among the most biologically interesting lineages within the family Phoridae (Diptera). All species with known life histories are parasitoids of bees (Hymenoptera: Apidae), including stingless bees, bumble bees, and honey bees (Brown 2006). With the discovery of new collecting techniques (see Brown 2001) and because the introduced honey bee (*Apis mellifera* L.) is a suitable host for many *Melaloncha*, there has been increased interest and research activity on this group of flies that has resulted in the discovery of new species, an updated classification, and knowledge of new host records and behavioral traits (Brown 2004a, 2004b, 2005, 2006; Brown and Kung 2006; Gonzalez and Brown 2004).

Before 2004 there were only 32 described species of *Melaloncha* (Borgmeier 1968, 1971); however, recent taxonomic research (Brown 2004a, 2004b, 2005, 2006; Brown and Kung 2006; Gonzalez and Brown 2004) has resulted in the recognition of 163 species. The total number of species in the genus is estimated to be 200–300 (Brown 2006). In most cases, species are recognized based on structural characters of female

specimens as the males are often extremely different from females and have fewer distinguishing characters. Only in rare cases (e.g., when collected in copula) can *Melaloncha* males confidently be associated with their dissimilar female counterparts. Although earlier taxonomic treatments (e.g., Borgmeier 1934) described several male species of *Melaloncha*, it is a daunting task to associate any of these with their conspecific females. Molecular techniques have proven to be a useful tool for associating morphologically dissimilar males and females in the phorid genus *Phora* (Cook and Mostovski 2002). Therefore, one objective of the current study was to evaluate the utility of mitochondrial DNA sequences for associating two male *Melaloncha* species, one described by Borgmeier (1934) and the other undescribed, to their female counterparts. At a minimum, molecular methods should aid in the assignment of the two male species to specific species groups.

The taxonomy of *Melaloncha* has undergone extensive revision in the past 5 yr. Within *Melaloncha*, Brown (2004a) recognized two subgenera: *M.* (*Udamochiras*) Enderlein and *M.* (*Melaloncha*) Brues. The two subgenera can easily be distinguished based on structural characters, most notable is the presence [*M.* (*Udamochiras*)] or absence [*M.* (*Melaloncha*)] of large lateral setae on the abdominal tergites, especially the second tergite. Within *M.* (*Melaloncha*), Brown

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Table 1. List of taxa analyzed with collection data

Taxa	Species group	Locality
Subgenus <i>Udamochiras</i> Enderlein		
<i>Melaloncha colossia</i> (Enderlein)	<i>M. colossia</i>	Argentina: Uruguay
<i>Melaloncha horologia</i> Brown	<i>M. colossia</i>	Bolivia: 50 km N Caranavi
<i>Melaloncha spatula</i> Brown	<i>M. colossia</i>	Costa Rica: Rincon
<i>Melaloncha vargasi</i> Brown	<i>M. colossia</i>	Costa Rica: La Selva
Subgenus <i>Melaloncha</i> Brues		
Group I		
<i>Melaloncha immaculata</i> Brown	<i>M. digitalis</i>	Costa Rica: Las Cruces
<i>Melaloncha muricata</i> Brown	<i>M. punctifrons</i>	Costa Rica: Las Cruces
Group II		
<i>Melaloncha elviae</i> Brown	<i>M. cingulata</i>	Bolivia: Cumbre Alto Beni
<i>Melaloncha nudibasalis</i> Brown	<i>M. cingulata</i>	Costa Rica: Karen Morgensen
<i>Melaloncha variabilis</i> Brown	<i>M. cingulata</i>	Costa Rica: ZP El Rodeo
<i>Melaloncha chamaea</i> Brown	<i>M. furcata</i>	Peru: Tambopata Research Center
<i>Melaloncha inversa</i> Brown	<i>M. furcata</i>	Argentina: Iguazu
<i>Melaloncha kungae</i> Brown	<i>M. furcata</i>	Columbia: Amacayacu
<i>Melaloncha obscurella</i> Borgmeier	<i>M. furcata</i>	Costa Rica: ZP El Rodeo
<i>Melaloncha ovata</i> Brown	<i>M. furcata</i>	Costa Rica: Las Cruces
<i>Melaloncha pilula</i> Brown	<i>M. furcata</i>	Costa Rica: Las Cruces
<i>Melaloncha feleoeae</i> Brown	<i>M. stylata</i>	Bolivia: Hotel Don Quixote
<i>Melaloncha stylata</i> (Schiner)	<i>M. stylata</i>	Costa Rica: Rincon
<i>Melaloncha acoma</i> Brown & Kung	<i>M. unguilata</i>	Costa Rica: ZP El Rodeo
<i>Melaloncha borgmeieri</i> Brown & Kung	<i>M. unguilata</i>	Argentina: Uruguay
<i>Melaloncha candida</i> Brown & Kung	<i>M. unguilata</i>	Brazil: Caicara
<i>Melaloncha curtibrachia</i> Brown & Kung	<i>M. unguilata</i>	Costa Rica: Karen Morgensen
<i>Melaloncha fuscipalpis</i> Brown & Kung	<i>M. unguilata</i>	Peru: Tambopata Research Center
<i>Melaloncha nigrita</i> Borgmeier	<i>M. unguilata</i>	Argentina: Uruguay
<i>Melaloncha trita</i> Brown & Kung	<i>M. unguilata</i>	Costa Rica: ZP El Rodeo
<i>Melaloncha ustulata</i> Brown & Kung	<i>M. unguilata</i>	Costa Rica: Rincon
<i>Melaloncha clavata</i> Schmitz	Unplaced	Costa Rica: Las Cruces
<i>Melaloncha forficata</i> Brown	Unplaced	Bolivia: Arroyo Tuhiri
<i>Melaloncha hyalinipennis</i> Borgmeier	Unplaced	Peru: Tambopata Research Center
<i>Melaloncha maculata</i> Borgmeier	Unplaced	Costa Rica: ZP El Rodeo
<i>Melaloncha</i> cf. <i>striatula</i> Borgmeier	Unplaced	Peru: Tambopata Research Center
* <i>Melaloncha</i> cf. <i>genitalis</i> Borgmeier	<i>incertae sedis</i>	Bolivia: Coroico
* <i>Melaloncha</i> "yellow male"	<i>incertae sedis</i>	Peru: Tambopata Research Center
Outgroups	Subfamily	
<i>Phalacrotophora</i> sp.	Metopininae	Costa Rica: Rincon
<i>Phalacrotophora halictorum</i>	Metopininae	Costa Rica: Finca Montezuma
<i>Phalacrotophora punctiapex</i>	Metopininae	Brazil: Rio Branco
<i>Beckerina luteola</i>	Metopininae	USA: CA: Monrovia
<i>Dohrniphora gigantea</i>	Aenigmatiinae	Bolivia: Cumbre Alto Beni

Asterisk (\*) indicates the two male species for which sequence data was used to associate them to their morphologically dissimilar female counterparts.

(2006) identified two presumably monophyletic lineages (Groups I and II) based on the structure of the foretarsal claws. In Group I, the foretarsal claws are unmodified, whereas in Group II the foretarsal claws have a large basal lobe. Brown (2006) pointed out that the monophyletic status of Group I, which represents a small fraction of the total number of species in the subgenus, remains subject to further verification. Within Group II, several species groups have been recognized and treated: *M. cingulata*-group (Brown 2004), *M. furcata*-group (Brown 2005), *M. sinistra*-group (Brown 2006), *M. stylata*-group (Brown 2006) and *M. unguilata*-group (Brown and Kung 2006). Although there is strong support for the monophyly for each of these Group II lineages, the relationships of these groups to each other is not well understood.

Here, we report on a preliminary molecular systematic study of 30 *Melaloncha* species representing both subgenera and seven species groups, four of which are Group II subgenus *Melaloncha*. Specifically, we evaluate the utility of nuclear 28S and CAD (ru-

dimentary) and mitochondrial 12S, 16S, COI, and ND1 gene sequences for inferring phylogenetic relationships among selected *Melaloncha* species. The resulting molecular topology is discussed and compared with hypotheses of relationships based on structural characters. The molecular evidence, specifically 12S and ND1, is further used to place two distinctive but unassigned males (*M. genitalis* and *M. "yellow male"*) in their proper species.

## Materials and Methods

**Specimens.** A list of analyzed taxa with collection details is presented in Table 1. Brown (2004) identified the genera *Phalacrotophora* Enderlein and *Melitophora* Brues as being most closely related to *Melaloncha*. Outgroup taxa used in the current study included three species of *Phalacrotophora* (Phoridae: Metopininae), *Beckerina luteola* Malloch (Phoridae: Metopininae) and *Dohrniphora gigantea* (Enderlein) (Phoridae: Aenigmatiinae, sensu Brown 1992). The

**Table 2.** Oligonucleotide primers used in this study with GenBank accession numbers for sequences

Gene and primer code <sup>a</sup>	Sequence (5' end)	Reference	GenBank accession nos.
<b>Mitochondrial</b>			
16S-F (LR-N-13398)	CGCCTGTTTATCAAAAACAT	Simon et al. (1994)	EU068633-EU068666
16S-R (LR-J-12887)	CTCCGGTTTGAACCTCAGATCA		
12S-F (SR-J-14199)	TACTATGTTACCGACTTAT	Kambhampati and Smith (1995)	EU068601-EU068632
12S-R (SR-N-14594)	AAACTAGGATTAGATACCC		
COI-F (C1-J-2183)	CAACAYTTATTTTGATTYYTYGG	Simon et al. (1994)	EU068507-EU068539
COI-R (TL2-N-3014)	TCCATTGCACTAATCTGCCATATTA		
ND1-F (N1-J-11861)	ATCATAACGAAAYCGAGGTAA	Smith et al. (1999)	EU068473-EU068506
ND1-R (N1-N-12530)	CAACCTTTTWTGTATGC		
<b>Nuclear</b>			
28S D2-3-F	GCGAACAAGTACCCGTGAGGG	Belshaw and Quicke (1997)	EU068572-EU068600
28S D2-3-R	TAGTTCACCATCTTTCCGGGTC		
CAD (54F)	GTNGTNTTYCARACNNGNATGGT	Moulton and Wiegmann (2004)	EU068540-EU068571
CAD (405R)	GCNGTRTGYTCNNGRTGRAAYTC		
CAD (680R)	AANGCRTCNCGNACMACYTCRTAYTC		

<sup>a</sup> Mitochondrial primer nomenclature based on Simon et al. (1994); 3' nucleotide position based on sequence of *D. yakuba* (Clary and Wolstenholme 1985).

monotypic genus *Melittophora* is represented by the rarely collected *M. salti* Brues, a kleptoparasitoid of stingless bees, feeding on pollen in the brood cells (Salt 1929), and it is hypothesized to be the sister group to *Melaloncha* (Brown 2004). Unfortunately, specimens of *M. salti* were not available for molecular study. Voucher specimens, including dissected abdomens of sequenced specimens, are stored at the Natural History Museum of Los Angeles County (LACM), and surplus genomic extracts are stored at -70°C in the lab of P.T.S. Color images of male *Melaloncha* were deposited in Morphbank ([www.morphbank.com](http://www.morphbank.com)).

**DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Sequencing.** Genomic DNA was extracted using the DNAeasy tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Six different DNA fragments comprising portions of the nuclear 28S rRNA and CAD (rudimentary) and mitochondrial 12S rRNA, 16S rRNA, cytochrome oxidase I (COI) and NADH1 dehydrogenase (ND1) genes were amplified and sequenced using the oligonucleotide primers listed in Table 2. Seminested PCR was carried out on CAD by using the primer combination of 54F + 680R. One microliter of the resulting PCR product was used in a subsequent PCR reaction with the primer combination of 54F + 405R. All PCR amplifications were performed in 50- $\mu$ l volume by using annealing temperatures ranging from 40 to 55°C. Amplified products were purified on a QiaQuick PCR column (QIAGEN). DNA sequencing was performed using either the ABI d-Rhodamine Dye Terminator or Big Dye version 3 Cycle Sequencing Ready Reaction kits (Applied Biosystems, Foster City, CA) in a 5- $\mu$ l volume. Purified sequencing reactions were submitted to the University of Florida's DNA Sequencing Core Facility for sequencing of both strands on an ABI 377 DNA sequencer. Sequence electropherograms were read and edited using Sequence Navigator software (Applied Biosystems). Multiple sequence alignment was initially carried out using CLUSTALX (Thompson et al. 1997) with default parameters. The resulting alignment was further opti-

mized by excluding hypervariable regions that were present in the three rRNA genes. All sequences have been deposited in GenBank under the accession numbers listed in Table 2. The aligned data set (in Nexus format) is available at <http://www.phorid.net/phoridae.html> and <http://TreeBASE.org> under accession no. S2027.

**Phylogenetic Analysis.** Summary statistics for the DNA sequence data were calculated using PAUP\* 4.0b10 (Swofford 2003). Phylogenetic relationships were estimated by maximum parsimony (MP) in PAUP\* for the following data partition combinations: 1) all mitochondrial rRNA genes, 2) all mitochondrial protein-coding genes, 3) all mitochondrial genes, 4) all nuclear genes, 5) all rRNA genes, 6) all protein-coding genes, and 7) all genes. Unweighted MP analysis was carried out on all data partition combinations using the multiple equally parsimonious heuristic search option with tree bisection-reconnection and 1,000 random addition sequence replicates. Support for specific nodes on the MP trees was estimated by bootstrap analysis (Felsenstein 1985) (1,000 replications with 10 random addition sequence replicates). Bremer support indices (Bremer 1994) were calculated based on the strict consensus MP tree for all genes by using AUTODECAY version 4.0 (Eriksson 1998) and extracted onto the consensus tree using TREEVIEW (Page 1996).

## Results and Discussion

### Characterization of the Nucleotide Data

In total, 3,306 aligned bases (including gaps) of DNA sequence were obtained for a total of 35 taxa from portions of the nuclear 28S rRNA (263 bp), CAD (935 bp), and mitochondrial 12S rRNA (442 bp); 16S rRNA (499 bp); COI (641 bp); and ND1 (546 bp) genes. Of the 3,306 characters, 1,224 (37%) were variable, and 839 (25%) were parsimony informative. The base composition of the gene fragments was biased toward adenine and thymine, which together consti-

**Table 3.** Summary of tree and character statistics for seven data partitions

Data partition	Bases + gaps	PICs	TL	EPTs	CI	RI
Mitochondrial rRNA genes	921	137	560	5463	0.54	0.59
Mitochondrial protein-coding genes	1187	416	2143	3	0.36	0.48
All mitochondrial genes	2108	553	2723	2	0.39	0.50
All nuclear genes	1198	286	1048	160	0.63	0.67
All rRNA genes	1184	166	698	70	0.57	0.57
All protein-coding genes	2122	673	3109	1	0.43	0.53
All genes	3306	839	3824	2	0.45	0.53

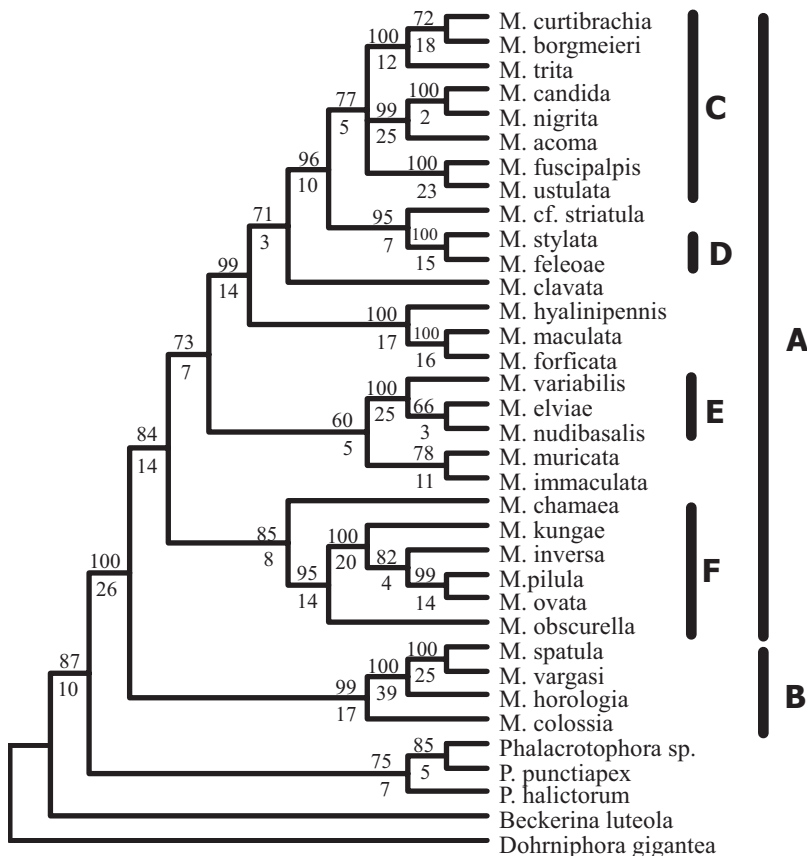
PICs, number of parsimony informative characters; TL, tree length; EPTs, number of equally parsimonious trees; CI, consistency index; and RI, retention index.

tuted an estimated 76% (mitochondrial) and 64% (nuclear) of the total.

### Phylogenetic Relationships

A summary of character statistics and results of unweighted parsimony analyses is presented in Table 3. Our discussion of phylogenetic relationships is based on the strict consensus of two equally parsimonious trees (EPTs) (TL = 3824) for all six genes analyzed simultaneously (Fig. 1). Other analyses

based on various permutations of the molecular data sets (Table 3) generally resulted in trees with less resolution and support and/or obscured relationships due to fewer included characters. The only difference between the two most parsimonious trees concerned the clade composed of species representing the *M. ungulata* group (Fig. 2). The eight exemplars of the *M. ungulata* group are classified into two subgroups: *M. ungulata* and *M. nigrita* (Brown and Kung 2006). In one of the trees both subgroups were recovered as monophyletic lineages, but in the other the *M. ungu-*



**Fig. 1.** Strict consensus of two equally parsimonious trees based on simultaneous analysis of portions of six genes (3,306 bp including gaps) for 30 *Melanochla* species. Tree length, 3,824; consistency index, 0.45; retention index, 0.53. Numbers above branches are bootstrap values (%), and those below branches are Bremer support indices. (A) Subgenus *Melanochla*. (B) Subgenus *Udamochiras*. (C) *M. ungulata* group. (D) *M. stylata* group. (E) *M. cingulata* group. (F) *M. furcata* group.

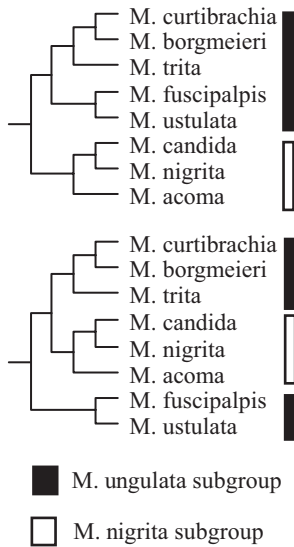


Fig. 2. Topological differences between the two equally parsimonious trees.

*lata* subgroup was rendered paraphyletic (Fig. 2). It is interesting to note that parsimony analysis of just the protein-coding genes (i.e., ND1, CO1, and CAD; see Table 3) resulted in a single tree that was identical to one of our most parsimonious trees based on the full complement of genes (i.e., the one that recovered the *M. ungulata* and *M. nigrita* subgroups as monophyletic lineages). Thus, the rRNA genes support a slightly different topology within the *M. ungulata* group than the protein-coding genes, but the character conflict is minimal with respect to the overall topology of the phylogeny. The topology of the strict consensus tree is largely congruent with hypotheses of relationships based on structural characters (Brown 2004). The following groups were recovered as monophyletic with strong statistical support (bootstrap %/Bremer support indices): subgenus *Udamochiras* (99/17), subgenus *Melaloncha* (84/14), *M. furcata* group (85/8), *M. cingulata* group (100/25), *M. stylata* group (100/15), and *M. ungulata* group (96/10) (Fig. 1.). Group I exemplars (*M. muricata* + *M. immaculata*) were recovered as sister taxa, but they were included in a clade composed of Group II taxa exclusive of the *M. furcata* group (73/7) (Fig. 1.).

The molecular data support the hypothesis of Brown (2004) that subgenus *Udamochiras* is a monophyletic sister-group to subgenus *Melaloncha* (Fig. 1). Members of subgenus *Udamochiras* possess lateral abdominal setae and anterodorsal setae on both the mid- and hind legs. These characters are largely absent among members of subgenus *Melaloncha*. In rare cases where members of subgenus *Melaloncha* possess lateral abdominal setation, tergite 2 is always bare. Within subgenus *Melaloncha*, several species groups are recognized: *M. cingulata*-group, *M. furcata*-group, *M. sinistra*-group, *M. stylata*-group, and *M. ungulata*-group. In addition to these groups there are also a

number of unassigned species. The relationships of the aforementioned species groups to one another, as well as the many unplaced members of subgenus *Melaloncha*, is not well understood. However, DNA sequence analysis provides some preliminary insight into the relationships of species in subgenus *Melaloncha*: (*M. furcata* group + (*M. cingulata* group + (*M. stylata* group + *M. ungulata* group))) (Fig. 1). The placement of the *M. furcata* group as a sister group to the clade composed of all other Group I + Group II taxa is noteworthy because all members of this group possess an  $R_{2+3}$  wing vein. This wing vein is primitively present in most phorids, but absent from most *Melaloncha*, including other members of subgenus *Melaloncha*. Brown (2005) considers the reappearance of this wing vein to be a synapomorphy within *Melaloncha*. It is also worth noting that included unplaced members of subgenus *Melaloncha* (represented by *M. hyalinipennis*, *M. maculata*, *M. forcicata*, *M. clavata*, and *M. cf. striatula*) do not form a monophyletic lineage. The phylogenetic relationships of species within subgenus *Melaloncha* outlined above are based on a small sampling of taxa and must remain subject to further verification. However, the combination of nuclear and mitochondrial genes used in this study seems to have some phylogenetic utility and would be appropriate to investigate the phylogeny of the entire genus with a more rigorous sampling of taxa (B.V.B. and P.T.S., unpublished data).

#### Associating Dissimilar Male and Female *Melaloncha*

Recent taxonomic research (Brown 2004a, 2004b, 2005, 2006; Brown and Kung 2006) has resulted in a five-fold increase in the number of recognized species in *Melaloncha*. In most cases, these newly described species are based almost exclusively on female specimens. Although the males of *Melaloncha* typically show too few differences to be useful in characterizing species, some species have been described from male specimens only (Borgmeier 1971). However, because male and female *Melaloncha* exhibit striking sexual dimorphism, it is not possible to associate any of the species described from male specimens to their female counterparts. A useful molecular tool for identifying unrecognized specimens is through direct sequence comparison with a database of sequences from known specimens. A variation of this technique was used by Cook and Mostovski (2002) to associate unknown females to known males in the phorid genus *Phora*. In the current study, a database of mitochondrial 12S and ND1 gene sequences (968 bp total) compiled for 87 *Melaloncha* species (data not shown; B.V.B. and P.T.S., unpublished data). Below, we report on the utility of this female-derived database for matching two male specimens: *M. genitalis* Borgmeier (hereafter referred to as *M. cf. genitalis*) and an undescribed, distinctly yellow male species to their putative female counterparts; and we discuss the taxonomic implications of the sequence comparisons.

**Specimens.** *M. genitalis* is known from a single male specimen from Espirito Santo, Brazil. Later,

**Table 4.** Average nucleotide differences for two *Melaloncha* male species against various *Melaloncha* taxonomic groupings for portions of the mitochondrial 12S and ND1 genes (968 bp)

	<i>M. (Melaloncha)</i>							
	<i>M. (Udamochiras)</i>	Group I	Group II	FurG	CinG	StyG	SinG	UngG
<i>M. genitalis</i>	104.3	74.8	52.1	78.7	58.8	19.5	86	46.5
<i>M. "yellow male"</i>	102.8	74.8	47.8	80.9	58.0	36.0	90	48.9

Group I ( $n = 4$ ), Group II (unplaced taxa,  $n = 17$ ), FurG = *M. fuscata* group ( $n = 12$ ); CinG = *M. cingulata* group ( $n = 5$ ); StyG = *M. stylata* group ( $n = 2$ ); SinG = *M. sinistra* group ( $n = 1$ ); UngG = *M. ungulata* group ( $n = 22$ ).

Borgmeier (1971) synonymized *M. ungulata* Borgmeier, a species known from both sexes (from Nova Teutonia, Santa Catarina, Brazil), with *M. genitalis*. This synonymy was based on the supposed inadequacy of the differences between males of the two species cited in his (Borgmeier 1959) key with this species. Brown and Kung (2004) compared the terminalia of *M. genitalis* and *M. ungulata* and found little structural similarity. Consequently, Brown and Kung (2006) removed *M. ungulata* from synonymy with *M. genitalis* and reinstated it as a valid species. Furthermore, they found no support for the inclusion *M. genitalis* in the *M. ungulata* species group. Among male specimens collected by Brown and his field assistants in Bolivia were two specimens with genitalia closely similar to those of the holotype of *M. genitalis*, representing either the same species or one closely related.

The "yellow male" species is distinctive because of its color: most *Melaloncha* males have a largely to completely dark brown to black thorax. In contrast, the yellow males are almost completely yellow, with the exception of two dark spots posterolaterally on the scutum.

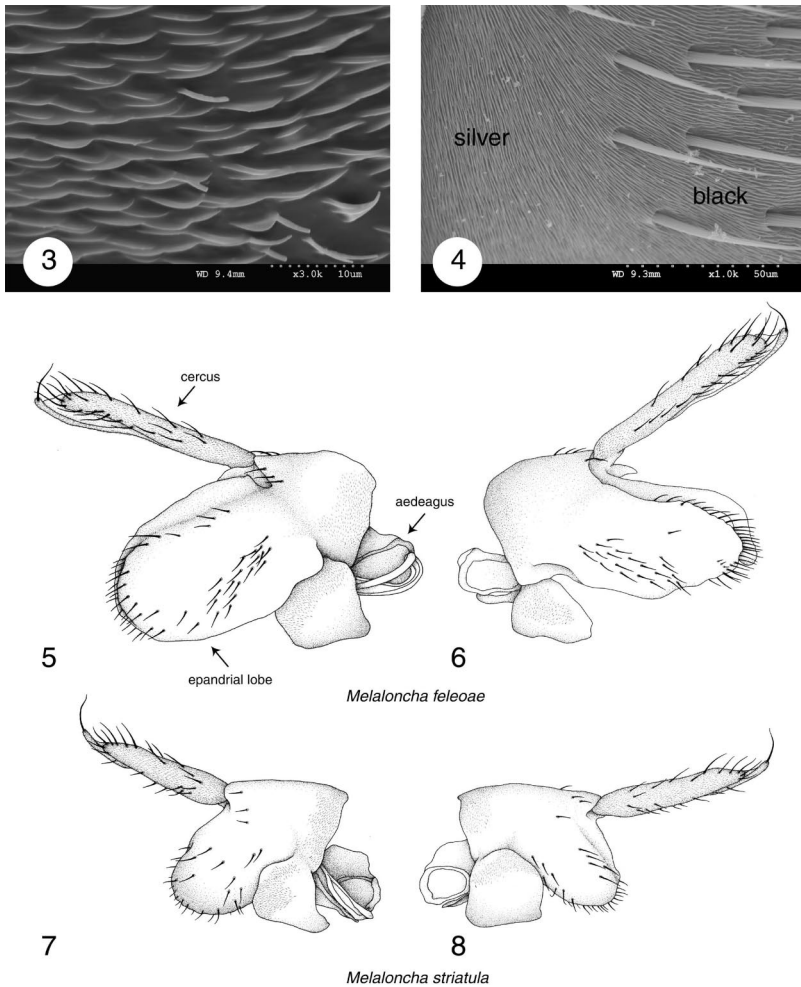
**Sequence Comparisons.** Table 4 shows the average pairwise nucleotide differences of *M. cf. genitalis* and *M. "yellow male"* against exemplar female *Melaloncha* species representing both subgenera, Groups I and II, and selected species groups within Group II. The data clearly indicate that both male species are subgenus *Melaloncha*, Group II taxa. We then compared the two

males against all Group II taxa for which we had sequence data from both the 12S and ND1 genes, a representative sample ( $n = 15$ ) is shown in Table 5; and the data suggest that *M. cf. genitalis* may be synonymous with the recently described female species *M. feleoeae* (Brown 2006). Direct sequence comparison of *M. cf. genitalis* and *M. feleoeae* resulted in only 2 bp differences of a total of 968 bases (0.2% divergence). Thus, the sequence data supports the removal by Brown and Kung (2004) of *M. genitalis* from synonymy with *M. ungulata* and at a minimum suggests placement of *M. genitalis* in the *M. stylata* group (which includes *M. feleoeae*).

Similarly, *M. "yellow male"* exhibited only 2 bp differences against *M. cf. striatula*. Originally described from Costa Rica, *Melaloncha striatula* Borgmeier is a widespread species that Brown (2006) found to be varied in ovipositor structure and grading into the closely similar *M. palpalis* Borgmeier. The specimen we designated "*M. cf. striatula*" was collected at Tambopata Research Center in Peru, and its corresponding females were placed in *M. striatula* by Brown (2006), who also noted more work was needed on the concept of this species. For the present, we consider the "yellow males" to belong to a species or species complex named *M. striatula*, but it is noteworthy that there were 16 bp differences (ND1 + 12S) between *M. cf. striatula* and *M. striatula* (Costa Rica: ZP El Rodeo), indicating that they might be separate species.

**Table 5.** Absolute pairwise distance matrix for 17 *Melaloncha* taxa including two male species for portions of the mitochondrial 12S and ND1 genes (968 bp)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>M. stylata</i>	—																
2 <i>M. feleoeae</i>	27	—															
3 <i>M. cf. striatula</i>	30	29	—														
4 <i>M. hyalinipennis</i>	54	40	45	—													
5 <i>M. acicula</i>	65	51	57	22	—												
6 <i>M. maculata</i>	59	43	46	42	48	—											
7 <i>M. striatula</i>	43	31	16	62	71	66	—										
8 <i>M. clavata</i>	39	30	28	41	56	58	40	—									
9 <i>M. gomezi</i>	39	32	30	48	61	62	44	21	—								
10 <i>M. gonzalezae</i>	43	36	34	53	66	65	49	29	26	—							
11 <i>M. gradata</i>	89	66	71	97	105	97	98	87	91	87	—						
12 <i>M. torquata</i>	46	29	18	60	70	61	22	43	41	50	88	—					
13 <i>M. forcifata</i>	69	49	50	43	51	40	69	58	62	64	96	67	—				
14 <i>M. dibitettii</i>	31	26	31	41	48	49	33	29	28	30	74	35	46	—			
15 <i>M. simoni</i>	59	46	48	58	67	67	65	54	52	60	92	65	73	52	—		
16 <i>M. genitalis</i>	37	2	31	55	67	59	46	38	36	46	86	43	66	30	59	—	
17 <i>M. "yellow male"</i>	41	31	2	58	70	61	19	40	38	47	93	18	67	35	60	41	—



Figs. 3–8. Structure of *Melaloncha* species. (3 and 4) *Melaloncha colossia* (Enderlein), male. (3) Microstructure of anepisternum, lateral. (4) Microstructure of abdominal tergite 2, lateral. (5–8) Male terminalia, left side, right side.

### Taxonomic Consequences

Based on the molecular data presented above, we place males of *Melaloncha* cf. *genitalis* in *M. feleuae* and the “yellow male” in *M. striatula*. Below, we describe these previously unknown males.

Few males of *Melaloncha* species have been described. The latest key to species for known males is that of Borgmeier (1971), with the only additions being those in Brown (2006) and Brown and Kung (2006). Male terminalia have been figured only for *M. digitalis* Borgmeier (1959: fig. 66). Our figures (Figs. 5–8) show the disposition of the aedeagus, which in *Melaloncha* extends much further anteriorly than in other phorids.

In previous works on this genus, Brown and co-authors have incorrectly referred to the silver sheen on the bodies of adult *Melaloncha* as “pollinosity.” Instead, this effect seems to be caused by two different phenomena on the body, one type on the pleuron and the other on the abdominal tergites. The faint silver sheen on the pleuron of virtually all species is caused

by microscopic parallel, curved ridges on the pleural plates (Fig. 3) that give a scalloped appearance. Some of these ridges have elongate processes, but the majority do not. On the abdomen, there is often a pattern of dark (or black) areas alternating with bright silver, reflective areas. Some species have less strongly contrasting colors, but the pattern remains. This silver color is apparently produced by extremely dense spinuli on the abdomen, as in the area marked “silver” in Fig. 4. In contrast, the black areas have much spinuli spaced much further apart, such that the underlying sclerite can be seen between them (area marked “black” in Fig. 4).

***Melaloncha feleuae* Brown. Male.** Body length 2.6 mm. Frons yellow, except ocellar triangle black; with scattered, sparse setulae close to eye margin and along ventral border. Flagellomere one yellow, except white basally; first two arisal segments yellow, apical segment black. Palpus white; palpal setae black, normally developed. Postocular and genal setae black. Scutum light brown, except posterior margin dark brown.

Scutellum dark brown. Pleuron mixed dark and light brown colors (see Morphbank accession no. 142664) with silver sheen. Legs dark brown, except apex of fore- and midfemur and all of fore- and midtibia lighter; forecoxa whitish yellow, mid- and hind coxae dark brown. Abdominal tergites black with silver areas. Venter of abdomen dark gray. Cercus yellow, except basal one fifth dark brown. Epandrial lobes greatly enlarged, especially on right side (Figs. 5 and 6).

In the latest key to *Melaloncha* males (Borgmeier 1971), *M. feleoae* keys to *M. genitalis* at couplet 10. In comparison with the holotype of *M. genitalis*, there are no obvious differences that we noted. As discussed above, we cannot rule out the possibility that these two names refer to the same species, but we are skeptical of this.

**Material Examined.** BOLIVIA: La Paz: Coroico, Hotel Don Quixote, 16.19° S, 67.72° W, 2♂ (one used for sequencing), 5-IV-2004, B. Brown, E. Zumbado, *Phoenix* palm flowers; 1,750 m (LACM collection).

***Melaloncha striatula* Borgmeier.** Male. Body length 2.2–2.4 mm. Frons yellow, except ocellar triangle black; with scattered, sparse setulae close to eye margin. Flagellomere one yellow, except white basally; first two arista segments yellow, apical segment dark brown. Palpus white; palpal setae black, normally developed. Postocular and genal setae black. Scutum light brown, except with posterolateral dark brown macula. Scutellum light brown with large antero-medial black macula. Pleuron yellowish-brown, except basalare dark brown (see Morphbank accession no. 142660), with faint silver sheen. Legs yellowish brown except fore- and hind coxae whitish yellow and apex of hind femur with dark brown macula. Abdominal tergites yellowish brown, black posteriorly and laterally, with faint silver sheen. Venter of abdomen yellow. Basal one half of cercus brown, apical one-half white. Male terminalia as in Figs. 7 and 8.

In the latest key to *Melaloncha* males (Borgmeier 1971), *M. striatula* keys to couplet 9, which give the choices of “Species of medium size (2.5 mm)” versus “smaller species (1.8–2.0 mm).” If the first lead is taken, *M. striatula* keys to *M. glabrifrons*, from which it differs by the smaller epandrial lobes and the uniform yellowish brown pleuron. If the second lead is taken, *M. striatula* males key to *M. clavata* Schmitz. The male specimen used by Borgmeier to represent *M. clavata*, however, is unlikely to be that species, and it could equally likely be many other *Melaloncha* (Brown, 2006, Brown and Kung, 2006).

Brown and Kung (2006) found that *M. nigrifrons* Borgmeier and *M. borgmeieri* Brown & Kung also keyed to *M. clavata* in Borgmeier’s key. The males of *M. nigrifrons* are easily recognized by their dark brown frons, whereas males of *M. borgmeieri* have a dark brown, sinuous line across the pleuron (see Morphbank accession no. 142917), and dark brown foretarsomeres. The male of *M. striatula* has yellowish brown legs and pleuron, and it has a yellow frons.

**Material Examined.** PERU: Madre de Dios: Tambopata Research Center, 13.14° S, 69.61° W, 3♂ (one used for sequencing), 21-24-VII-2001, B. Brown, G. Kung, honey-sprayed undergrowth (LACM).

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