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The bee-killing flies, genus *Melaloncha* Brues (Diptera: Phoridae): a combined molecular and morphological phylogeny

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Abstract. The phylogeny of the bee-killing flies, genus *Melaloncha* Brues (Diptera: Phoridae) is analysed using six genes – *cytochrome oxidase I*, *16S* ribosomal DNA, *12S* ribosomal DNA, *NADH1 dehydrogenase*, *28S* ribosomal DNA and *CAD* – plus 47 morphological characters. A total of 91 specimens, including eight out-groups and 83 *Melaloncha* (representing 70 species) were included in the analyses. Parsimony analysis of the combined data set produced a single most parsimonious tree with varied Bremer and bootstrap support of interior nodes. Bayesian analysis of molecules only and of morphology + molecules produced trees largely in agreement with parsimony results, although with a few differences. Supported groups included subfamily Metopininae, genus *Melaloncha*, and subgenera *Melaloncha* s.s. and *Melaloncha* (*Udamochiras*) Enderlein. Within the subgenera, the previously recognized *Melaloncha furcata*, *Melaloncha cingulata*, *Melaloncha ungulata* and *Melaloncha stylata* groups were recovered, as well as some new groupings. The *M. furcata* group was placed as the sister group of other *Melaloncha* s.s., which is consistent with known host-attacking behaviour.

Introduction

Phoridae are small, poorly-known flies found worldwide, represented by about 4000 described species, but their actual number is probably closer to ten times this number. Revisions commonly uncover many undescribed species, especially in tropical regions (e.g. Brown, 2004a), but even in well-studied Europe (e.g. Bonet *et al*., 2006), many species remain to be described.

An outstanding characteristic of these flies is the variety of lifestyles they pursue (summarized by Disney 1994): larvae include generalized scavengers, specialized scavengers, herbivores, predators, parasitoids and true parasites. Most are terrestrial, although some are aquatic and possibly even marine. Many are associated with social insects, especially with termites, ants and bees.

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Among the most interesting phorids are the parasitoids. A great diversity of species are parasitoids of ants, including some that are being used to attempt biological control of invasive ant species (e.g. Porter, 1998, and many other recent papers). A much lesser known group are those that parasitize or are associated with bees. The largest group of bee-killing flies is the genus *Melaloncha* Brues, which has been revised recently (Brown, 2001, 2004b, c, 2005, 2006; Gonzalez & Brown, 2004; Brown & Kung, 2006; Kung, 2008). A total of 134 new species were described, bringing the total of the genus to 167 species. Further undescribed species have accumulated, and we estimate that the genus actually numbers between 200 and 300 species.

A phylogenetic framework for the group was proposed (Brown, 2004b), based on morphology. More recently, Smith & Brown (2008) evaluated Brown's (2004b) phylogenetic proposal using an independent set of molecular characters. In this study a molecular-based phylogeny of 30 species of *Melaloncha* representing both subgenera and selected species groups was inferred using a combination of nuclear and

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mitochondrial genes. Maximum parsimony analysis of the preliminary molecular data suggested that *Melaloncha* is monophyletic, and that *Melaloncha* (*Udamochiras*) Enderlein is a monophyletic sister group to *Melaloncha* (*Melaloncha*). The combination of nuclear and mitochondrial genes utilized in the study by Smith & Brown (2008) appear to be useful in phylogeny reconstruction, and are appropriate to investigate the entire genus with a more rigorous sampling of taxa, and the inclusion of morphological characters.

Through the revision process, much new fieldwork and collecting took place, and we accumulated specimens of *Melaloncha* for sequencing and molecular phylogenetic analysis. Here, we report on a combined molecular and morphological phylogenetic study of 83 *Melaloncha* representing both subgenera and 70 species. The resulting topology provides insights concerning the evolution of oviposition behaviour within this group of bee parasitoids.

Materials and methods

We agree with other authors (e.g. Nixon & Carpenter, 1996; Fitzhugh, 2006) that the best course of action with multiple data sources is to combine the data. We also present separate molecular analyses for comparative purposes.

A list of taxa analysed with collection details is presented in Appendix S1; numbers preceding *Melaloncha* names are sample numbers from our database. Out-group taxa included *Dohrniphora gigantea* (Enderlein) (Phoridae: Aenigmatiinae; classification sensu Brown, 1992) and two species of Hypocerinae: *Latiborophaga* sp. and *Stichillus* sp. In addition to *Melaloncha*, we included the following Metopininae as in-group taxa: three species of *Phalacrotophora* Enderlein, *Bekerina luteola* Malloch and *Apodicrania molinae* Borgmeier. 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. Colour images of specimens of *Melaloncha* species are deposited in Morphbank (http://www.morphbank.net). Terms for external structure are those of the Manual of Central American Diptera (Brown *et al.*, 2009).

Total genomic DNA was extracted from ethanol-preserved or dried flies using the DNAeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Six genes were targeted for amplification and sequencing, four of which are mitochondrial DNA (mtDNA): *cytochrome oxidase I* (*COI*), *16S* ribosomal DNA (*16S*), *12S* ribosomal DNA (*12S*) and *NADH1 dehydrogenase* (*ND1*), whereas two are nuclear DNA (nDNA): 28S ribosomal DNA (28S) and CAD (rudimentary). The following primer sets were used to isolate and amplify a fragment of each target gene using the polymerase chain reaction: C1-J-2183 and TL2-N-3014 for *COI* (Simon *et al.*, 1994), LR-N-13398 and LR-J-12887 for *16S* (Simon *et al.*, 1994; Kambhampati & Smith, 1995), SR-J-14199 and SR-N-14594 for *12S* (Kambhampati & Smith, 1995), N1-J-11861 and N1-N-12530 for *ND1* (Smith *et al.*, 1999; Smith & Brown, 2008), 28SD2-3-F and 28SD2-3-R for *28S* (Belshaw & Quicke, 1997) and 54F, 405R and 680R for *CAD* (Moulton & Wiegmann, 2004). Semi-nested PCR was carried out on *CAD* using the primer combination of 54F + 680R. A 1-μL portion of the resulting PCR product was used in a subsequent PCR reaction utilizing the primer combination of 54F + 405R.

The PCR cycling conditions for all reactions consisted of an initial denaturing period at 94°C for 1 min, followed by 35–40 cycles of 94°C for 30 s, 40–55°C for 40 s and 72°C for 1 min, followed by a final extension step at 72°C for 7 min. Prior to sequencing, product yield, specificity and potential contamination were checked by 1% agarose gel electrophoresis. Amplified products were purified on a QiaQuick PCR column (Qiagen, Valencia, CA). DNA sequencing was performed using either the ABI dRhodamine Dye Terminator or Big Dye v3 Cycle Sequencing Ready Reaction Kits (Perkin-Elmer, Foster City, CA) in a 5-μL 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 (.abi files) were read and edited using ABI's Sequence Navigator software. Multiple sequence alignment was carried out initially using ClustalX (Thompson *et al.*, 1997) using default parameters. The resulting alignment was optimized further by excluding hypervariable regions present in the three ribosomal genes; it was impossible to determine homology in these regions. All sequences have been deposited in GenBank under the following accession numbers: *COI* EU068507–EU068539, GU550334–GU550387; *16S* EU068633-EU068666, GU550180-GU550232; *12S* EU068601–EU068632, GU550124–GU550179; *ND1* EU068473–EU068506, GU550388–GU550440; *28S* EU068572–EU068600, GU550233–GU550279; and CAD EU068540–EU068571, GU550280–GU550333. The aligned data set (in Nexus format) is available at http://www.phorid.net/phoridae.html and http://TreeBASE.org under accession number S10272.

Morphological characters, selected from those discussed in Brown (2004b, 2005) and Brown & Kung (2006), are listed in Appendix S2.

Our data matrix included 91 taxa (83 *Melaloncha*, representing 70 species) and 3447 characters (47 of which were morphological), including gaps. In order to assess within-species divergence of taxa recognized by morphology, we sequenced more than one specimen of some species. Specifically, we sequenced two specimens of *Melaloncha simillima* Borgmeier (specimens 70 from Costa Rica and 152 from Argentina), *Melaloncha ronnai* Borgmeier (specimens 161 from Argentina and 250 from Costa Rica), *Melaloncha schiaffinoae* Gonzalez & Brown (specimens 147 and 148, both from Argentina), *Melaloncha gradata* Brown (specimens 99 from Bolivia and 193 from Peru), *Melaloncha gomezi* Brown (specimens 38 from Costa Rica and 160 from Argentina), *Melaloncha nigrifrons* Borgmeier (specimens 35 from Costa Rica and 186 from Bolivia), *Melaloncha acoma* Brown & Kung (specimens 31 and 267 from different sites in Costa

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Rica), *Melaloncha trita* Brown & Kung (specimens 19 from Costa Rica and 188 from Bolivia), four specimens of *Melaloncha adusta* Brown & Kung (specimens 122 and 289–291 from the same site in Costa Rica) and three specimens of *Melaloncha striatula* Borgmeier (specimens 1 from Peru, 26 from Costa Rica and 245 from a different site in Costa Rica).

Maximum parsimony analysis was performed using PAUP* 4.0b10 (Swofford, 2000). The combined matrix (molecular + morphology) was analysed using a heuristic tree–bisection–reconnection (TBR) search algorithm and 100 random-addition sequence replicates. Gaps were treated as missing data and all characters were weighted equally. Clade support values were estimated using non-parametric bootstrapping (Felsenstein, 1985) in PAUP*, with 1000 pseudoreplicates of a heuristic (TBR) search algorithm incorporating ten replicates of random stepwise addition of taxa. Bremer support indices (Bremer, 1994) were calculated using AUTODECAY 4.0 (Eriksson, 1998) in conjunction with PAUP*, which was also used to calculate summary statistics for the DNA sequence data.

Bayesian analysis was performed using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). Analysis of the molecular data alone used the settings nst = 6, rates = gamma (corresponding to the GTR + I + G model), as suggested by MRMODELTEST 2.3 (Nylander, 2004) for our data. This analysis was allowed to run for 7.65×10^6 generations, sampling frequency = 1000, until the standard deviation of split frequencies was less than 0.01. The first 1900 samples were discarded as the burn-in period. Analysis of the molecular plus morphological data used a partitioned approach, with two separate analyses. In the first set of data, the molecular characters were treated as a single partition and analysed as described above, but the morphological characters were analysed with the 'standard' discrete model (Lewis, 2001). In this analysis, 8.2×10^6 generations were needed to complete the analysis; the first 2050 samples were discarded as burn-in. In the second analysis, each gene was partitioned separately, and all partitions had parameters unlinked. Separate analysis of each gene with MRMODELTEST found that all required the same model (as above), and morphological characters were analysed again with the standard model. This analysis required 2.27×10^6 generations until the standard deviation of split frequencies was less than 0.01. The first 1000 samples were discarded as the burn-in period.

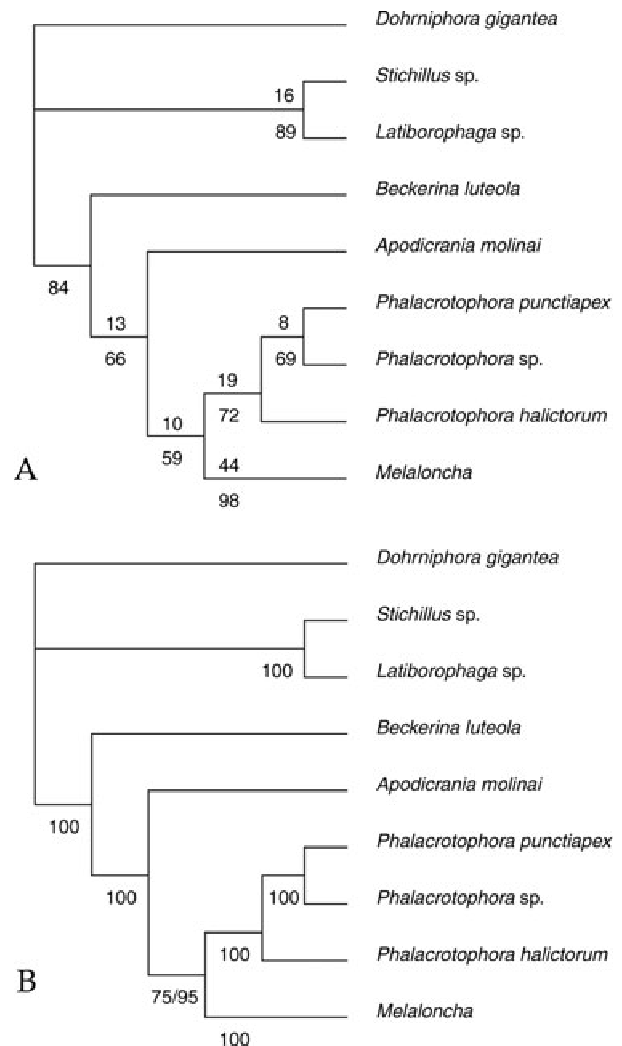

Fig. 1. Trees for out-groups and the genus *Melaloncha* only. (A) Single most parsimonious tree for combined molecular and morphological data. Bremer (above) and bootstrap (below) are shown for each branch for values above 0 or 50, respectively. (B) Bayesian analysis results. Clade credibility values (CCVs; Ronquist *et al*., 2005) given for each node: if differing between analyses, CCVs given as molecules/molecules + morphology.

Results

Of the 3447 characters, 1546 (45.8%) were variable and 1183 (34.3%) were parsimony informative. The base composition of the combined mitochondrial and combined nuclear data sets were each biased toward adenine and thymine (76% mitochondrial and 73% nuclear). A χ^2 test of homogeneity of base frequencies across taxa for the combined molecular data set yielded $P < 0.01$ ($\chi^2 = 473.40$, df = 270).

Unweighted parsimony analysis of the combined data resulted in a single tree (Figs 1a, 2, 4). This tree (length 8246 steps; consistency index, CI 0.30; retention index, RI 0.60) is nearly identical with the parsimony tree based on molecular data alone, except two further, basal branches in the *Melaloncha ungulata* group are resolved. Other analyses based on various permutations of the molecular datasets (see Table S1) resulted in an increase in the number of equally parsimonious trees and trees with less resolution and lower bootstrap support.

The monophyly of some expected, larger groups was supported with varying levels of bootstrap and Bremer support values. The two hypocerine taxa, *Stichillus* and *Latiborophaga*, grouped together (Brown, 1992), as did Metopininae (all taxa except *Dohrniphora* and the hypocerines) and the

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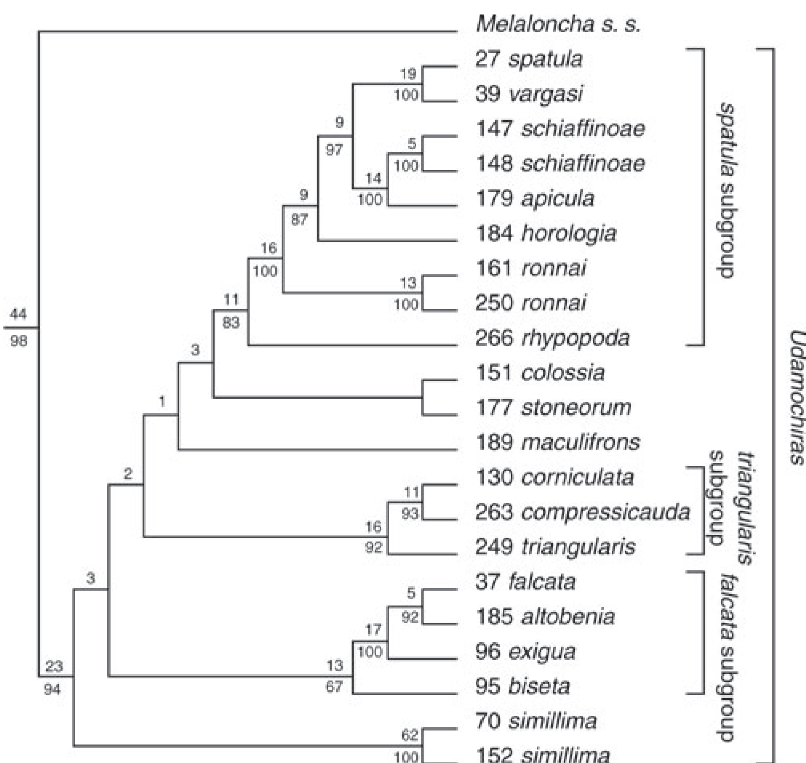

Fig. 2. Single most parsimonious tree for combined molecular and morphological data, subgenus *Melaloncha* (*Udamochiras*) only. Bremer (above) and bootstrap (below) are shown for each branch for values above 0 or 50, respectively.

genus *Melaloncha* itself (Fig. 1). Within *Melaloncha*, the two subgenera proposed by Brown (2004b), *Melaloncha* s.s. and *Udamochiras*, are both recovered (Figs 2, 4).

The relationships within *Udamochiras* are fully resolved (Fig. 2), but have low bootstrap and Bremer support values, except within some apical groups. Previously, the *Melaloncha spatula* subgroup consisted of *Melaloncha spatula* Brown, *Melaloncha vargasi* Brown and *Melaloncha horologica* Brown (Brown, 2004b), all of which are in our molecular data set; other taxa now placed in this group are *M. schiaffinoae* and *Melaloncha apicula* Brown. The species *M. ronnai* and *Melaloncha rhypopoda* Brown are strongly supported as successive sister taxa to the rest of the *M. spatula* subgroup. Two further well-supported groupings within *Udamochiras* are *Melaloncha triangularis* Brown + (*Melaloncha corniculata* Gonzalez & Brown + *Melaloncha compressicauda* Brown), herein termed the *M. triangularis* subgroup, and *Melaloncha biseta* Brown + (*Melaloncha exigua* Brown + (*M. altobenia* Gonzalez & Brown + *Melaloncha falcata* Brown)), hereafter the *M. falcata* subgroup; the relationships of all of these species were previously unknown.

Within subgenus *Melaloncha* s.s., we recovered seven sequential sister groups of species (Fig. 4). Three of these corresponded to species groups recognized on the basis of morphology: the *Melaloncha furcata* group (Brown, 2005), *Melaloncha cingulata* group (Brown, 2004c) and *M. ungulata* group (Brown & Kung, 2006). The relationships among these seven groups are not well supported, although there are robust groups within them (see Discussion).

The proportion of sites differing within species varied considerably (Table S2), but was usually much lower than the proportion of sites differing between them and closely related species. This relationship held for *M. simillima*, *M. ronnai*, *M. schiaffinoae*, *M. gradata*, *M. nigrifrons*, *M. acoma* and *M. adusta*.

In the case of *M. gomezi*, there were slightly more differences between the two specimens than between specimen 38 *M. gomezi* and *Melaloncha gonzalezae* Brown, and in the cladogram (Fig. 4), specimen 150 *M. gomezi* groups with *M. gonzalezi*. Similarly, specimen 19 *M. trita* had a slightly lower proportion of differences with specimen 8 *Melaloncha castanea* Brown & Kung than with specimen 188 *M. trita*. In the cladogram (Fig. 4), specimen 188 *M. trita* grouped with *M. castanea* and *Melaloncha strigosa* Brown & Kung.

The specimen of *Melaloncha torquata* Brown is apparently only slightly differentiated from the three specimens of *M. striatula*, although it was placed as the sister group to them in the cladogram (Fig. 4). These four specimens had a slightly higher proportion of differences from *Melaloncha stylata* (Schiner).

The results from the Bayesian analyses of molecules alone and of the two combined, partitioned data sets were largely similar to the parsimony results. The greatest difference was in subgenus *Udamochiras*, in which strongly supported relationships not found in the parsimony analysis were recovered (Fig. 3). As in the parsimony analysis, the three subgroups were well supported, but additional taxa were included: *M. simillima* was placed as the sister species to

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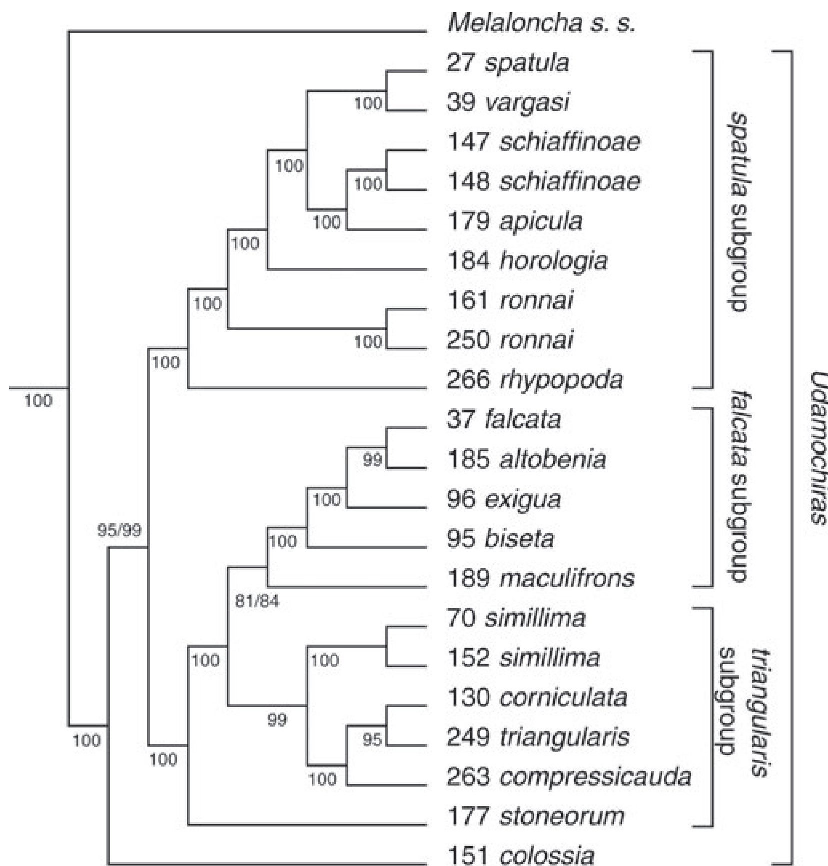

Fig. 3. Bayesian analysis results, subgenus *Melaloncha* (*Udamochiras*) only. Clade credibility values (CCVs) given for each node: if differing between analyses, CCVs given as molecules/molecules + morphology.

the rest of the *M. triangularis* subgroup, and *Melaloncha maculifrons* Brown was placed as the sister species to the rest of the *M. falcata* subgroup. Furthermore, the *M. triangularis* and *M. falcata* subgroups are supported strongly as sister groups, with *Melaloncha stoneorum* as a strongly supported out-group. Finally, *Melaloncha colossia* (Enderlein) is placed as the sister species to all other *Udamochiras*.

Within the subgenus *Melaloncha*, there was less variation among analyses (compare Fig. 4 with Fig. 5 and Figures S1, S2). The *M. cingulata* group and the *M. furcata* group are successive sister groups to the rest of the subgenus, and relationships within these two groups are unchanged. The *Melaloncha thompsonae* group becomes paraphyletic in the Bayesian analyses, but remains strongly supported as out-groups to the remaining species. The relationships of the *Melaloncha maculata* group, *Melaloncha clavata* Schmitz and the *M. gomezi* group are also unchanged. Some 'wild-card' taxa (Nixon & Wheeler, 1992), specifically *Melaloncha simoni* Brown, *Melaloncha berezovskiyi* Brown and *Melaloncha dibitettii* Brown, move among the more stable clades in various analyses. The *M. stylata* group is strongly supported, as is the *M. ungulata* group, although the precise make-up of these groups is affected by various wild-card taxa forming poorly-supported out-groups. Relationships within these two groups are stable, although within the *M. ungulata* group the species *M. acoma* and *M. adusta* group together, unlike in the parsimony analysis where *M. adusta* groups with *Melaloncha nigrita* Borgmeier + *Melaloncha candida* Brown; the Bayesian result is more concordant with the morphology, as *M. acoma* and *M. adusta* share a derived, bare oviscape structure (Brown, 2006).

The results of the second partitioned Bayesian analysis, with all parameters unlinked, was similar (Figure S2), except that *M. berezovskiyi*, *M. dibitettii*, and *M. simoni* are placed in a polytomy with the *M. ungulata* and *M. stylosa* groups.

Discussion

Phylogeny

Our largely molecular data set resolved the two subgenera, *Melaloncha* s.s. and *Udamochiras*, postulated by Brown (2004b). Of these two, *Udamochiras* is diagnosed by a greater number of morphological characters (Brown, 2004b) and has stronger support on the cladograms (Figs 2, 3).

Brown's (2004b: fig. 20) cladogram of relationships within *Udamochiras* featured a large group defined by character 6(1) (see Table S2), the presence of reduced dorsal interfrontal (DIF) setae. Most *Melaloncha*, the sister genus *Melittophora* and some *Phalacrotophora* lack such setae, whereas they are present and large in most other out-groups. The parsimony analysis of Brown (2004b: figs. 17, 20) therefore

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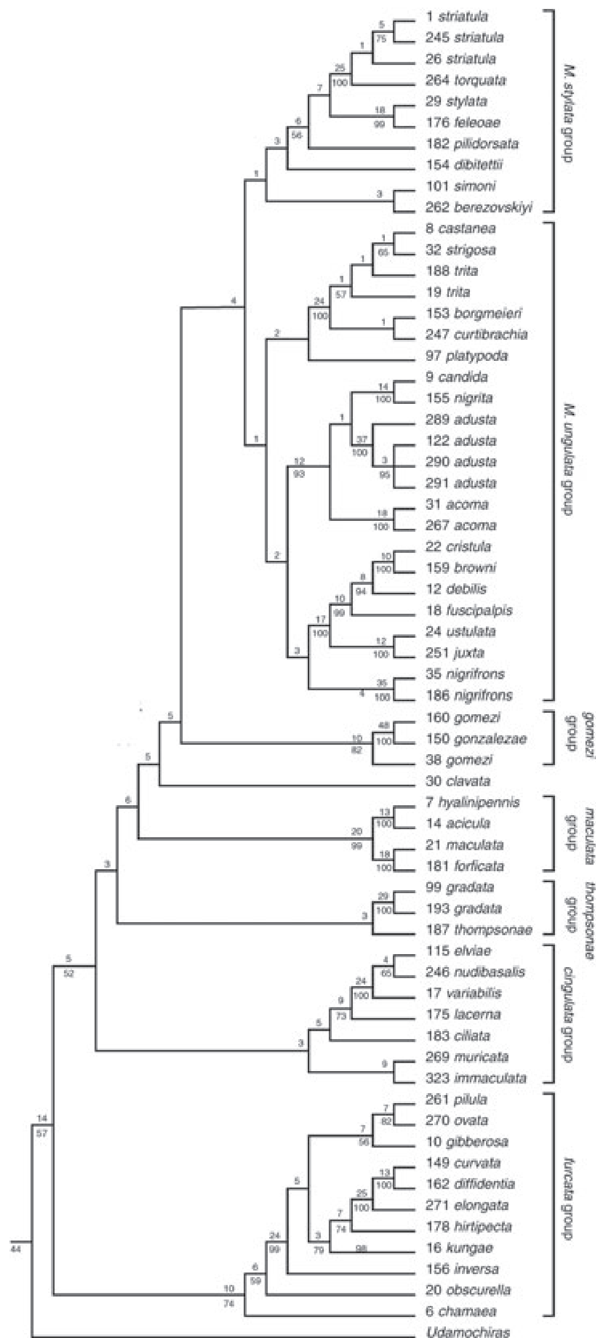

Fig. 4. Single most parsimonious tree for combined molecular and morphological data, subgenus *Melaloncha* only. Bremer (above) and bootstrap (below) are shown for each branch for values above 0 or 50, respectively.

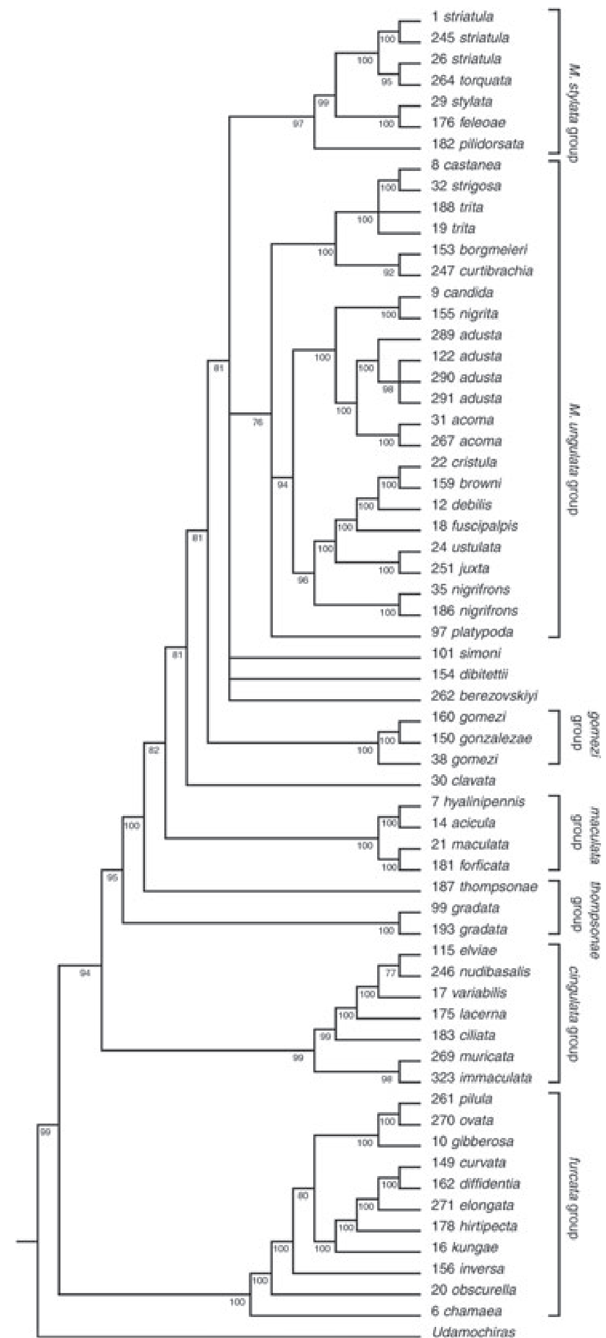

Fig. 5. Bayesian analysis results for combined molecular and morphological data, subgenus *Melaloncha* only. Clade credibility values (CCVs) given for each node.

treated state 6(1) as a synapomorphic character; however, DIF setae are absent in *M. colossia* (and other taxa of the *M. colossia* subgroup), which is embedded near the middle of the parsimony tree (Fig. 2). Bayesian analysis places *M. colossia* as the sister species of all other *Udamochiras* (Fig. 3). Unfortunately, *M. colossia* is the only species in our data set that lacks DIF setae. We need to sequence specimens of further *M. colossia* group species to determine if the complete loss of these setae has occurred one or more times.

Within *Melaloncha* s.s., Brown (2004b) recognized the *M. furcata*, *M. ungulata* and *M. cingulata* groups (all of which were recovered here), and two further, probably nonmonophyletic assemblages, designated group I and group

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II. Group-I species were characterized by simple foretarsal claws, as found in the out-group genera, and was considered to be probably nonmonophyletic. The rest of *Melaloncha* s.s. was hypothesized to be monophyletic, based on the presence of forked, modified foretarsal claws (character 31 in Brown, 2004b: fig. 18). According to our new analyses, however, species with simple foretarsal claws (*Melaloncha immaculata* Brown, *Melaloncha muricata* Brown, *M. berezovskiyi*, and *Melaloncha pilidorsata* Brown) are embedded within groups of species with modified foretarsal claws. Apparently, these species have reverted to a simple foretarsal claw from ancestors with modified claws.

According to our data, the *M. furcata* group is the sister group to all other *Melaloncha* s.s. Brown (2005) provided a preliminary cladogram, based on morphology, for this group, in which he recognized two monophyletic subgroups: the *M. furcata* subgroup (justified only by preliminary molecular characters) and the *Melaloncha obscurella* subgroup. In our current analyses, the *M. obscurella* subgroup, represented by *Melaloncha chamaea* Brown and *M. obscurella* Borgmeier, is not monophyletic, with the two species being successive sister groups to the *M. furcata* subgroup. There is at least one highly distinctive morphological character defining the *M. obscurella* subgroup, however, and we expect the inclusion of further species of this taxon in future analyses will provide more support for the subgroup. The *M. furcata* subgroup in our current analyses is consistent with, although much better resolved than, the tree proposed by Brown (2005), with the exception of the placement of *Melaloncha inversa* Brown. This species is clearly closely related to (and nearly identical with) *Melaloncha pilula* Brown, and its placement as the sister group of all other *M. furcata* subgroup species is probably wrong.

The *M. cingulata* group is placed as the sister group of the remaining groups in *Melaloncha* s.s., with low statistic support in the parsimony tree and high support in Bayesian trees. Relationships within the *M. cingulata* group were not proposed by Brown (2004c), but the group was hypothesized to include *M. thompsonae* Brown, which our current data does not support (it instead groups with or near *M. gradata*; Fig. 4). Brown (2006) proposed that *Melaloncha ciliata* Brown belonged in the *M. cingulata* group, which our new data supports (Fig. 4). The grouping of *Melaloncha lacerna* Brown, and thus the other nine species of the *Melaloncha sinistra* group (of Brown, 2006), in the *M. cingulata* group is a new result, but is supported by the presence of a punctate frons (in contrast to most *Melaloncha* and out-groups, which have a mostly smooth frons). Inclusion of *M. muricata* and *M. immaculata* in this group is not well supported in the parsimony analysis, but Bayesian analysis confirms this grouping.

Recognition of the *M. thompsonae* group is speculative, as it is not supported in the Bayesian analyses, and we have no supporting morphological characters. The species *Melaloncha lamellata* Borgmeier and *Melaloncha pertica* Brown were considered close relatives of *M. thompsonae* (within the *M. cingulata* group; Brown, 2004c), but we had no fresh specimens of these species to sequence.

The *M. maculata* group is an assemblage with extremely high statistical support. Although there are no distinctive morphological characters associated with this group, there are behavioral characters (see below) that further support its recognition.

Both *M. clavata* and the *M. gomezi* group are tentatively placed near the middle of the cladogram (Fig. 4). The specimen of *M. gomezi* from Argentina is more closely related to *M. gonzalezae* than to the other *M. gomezi* specimen (from Costa Rica), indicating that more taxonomic work on this group is necessary.

The remaining species are organized into the *M. ungulata* and *M. stylata* groups. The *M. ungulata* group was revised by Brown & Kung (2006), who proposed a phylogeny of the group that is largely consistent with our current data (although less well-resolved), with the exception that the *M. ungulata* series (in Fig. 4 this group runs from *M. castanea* down to *Melaloncha curtibrachia* Brown) does not arise within the group of *Melaloncha cristula* Brown through *M. nigrifrons*; instead it is the sister group of the other *M. ungulata*-group species. Additionally, the placement of *Melaloncha platypoda* Brown is novel.

Finally, the *M. stylata* group is a new assemblage, with strong support for at least *M. striatula*, *M. torquata*, *Melaloncha feleoae* Brown and *M. pilidorsata*. Inclusion of *M. dibitettii* and *M. berezovskiyi* is less certain. The grouping of *M. stylata* + *M. feleoae* is expected, as it was defined as an *M. stylata* group by Brown (2006), including three further species.

Behavioral concordance with phylogeny

A few host-attacking behaviours have been reported in the recent work on *Melaloncha*, and these are completely congruent with the cladograms presented herein. Females of *Udamochiras* and *M. furcata*-group species attack their hosts while in flight, darting down at stationary bees or even attacking bees in flight. This is in contrast to all other observed species, which all land on a substrate and curl their ovipositor under their bodies before attacking their hosts (see Brown & Kung, 2006: fig. 1). We therefore consider this latter behaviour a derived trait characteristic of *Melaloncha* s.s., exclusive of the *M. furcata* group (Fig. 6). Attacking in flight is thus the primitive mode of attack within the genus, but both behaviours are known for other genera of parasitic phorid flies.

The other behaviour we have observed is the 'piggybacking' attack of *M. maculata* Borgmeier and *Melaloncha hyalinipennis* Borgmeier (Brown, 2006). In these two species, females land (apparently undetected) on the back of their host, and stealthily attack the abdomen. Both of these species are placed in our well-supported *M. maculata* group (e.g. Fig. 4), and we predict that the other two species classified here, *Melaloncha acicula* Brown and *Melaloncha forficata* Brown, will be found to have the same behaviour.

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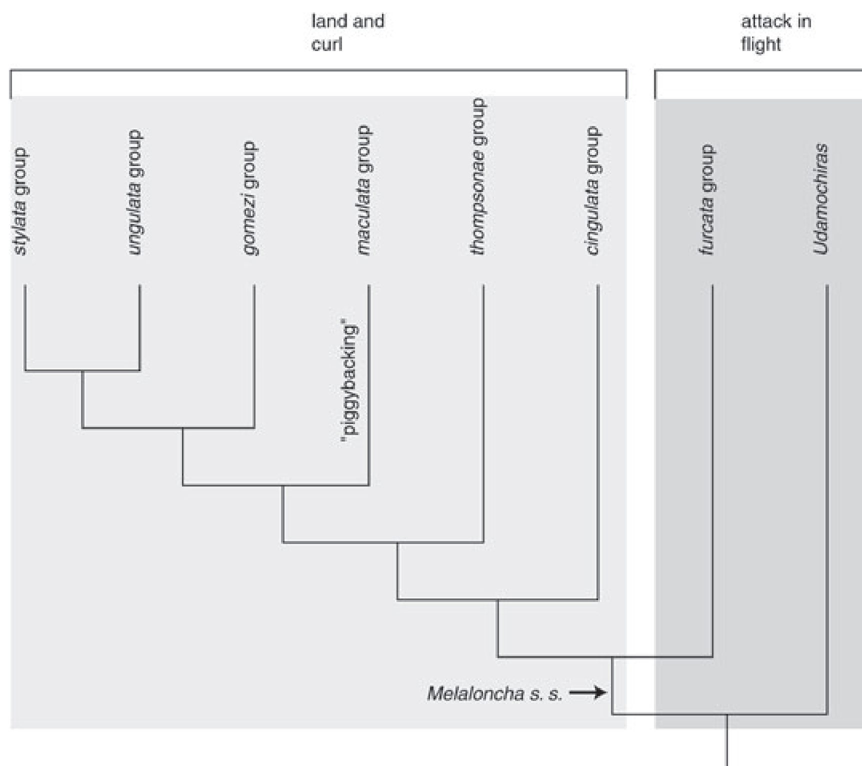

Fig. 6. Phylogenetic outline of host-attacking behaviour in *Melaloncha*.

Conclusions and further study

Our analyses have further resolved the phylogeny of *Melaloncha* bee-killing flies, largely confirming previous morphological analyses (Brown, 2004b, 2005; Brown & Kung, 2006), although disagreeing with them in some aspects. Greater resolution has been obtained at many levels, and some novel but morphologically justifiable new groupings have been obtained (for example, the placement of the *M. sinistra* group within the *M. cingulata* group), as well as a strongly supported new grouping, for which there are no known morphological characters. Some results point to taxonomic questions that need further attention (e.g. the status of *M. trita* and *M. gomezi*).

Future work on this genus should obviously include further taxa, and at least some additional genes, to seek greater support for relationships among species groups within *Melaloncha* s.s. Use of further nuclear genes, such as arginine kinase and *wingless*, which have been used with success in other groups of insects, will probably be necessary.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3113.2010.00540.x

Appendix S1. List of taxa included in the analysis, and the localities from which they were collected.

Appendix S2. Morphological characters for the *Melaloncha* data set.

Figure S1. Bayesian analysis results for molecular data, subgenus *Melaloncha* only.

Figure S2. Bayesian analysis results for combined molecular and morphological data, subgenus *Melaloncha* only, parameters unlinked.

Table S1. Summary statistics for various data set combinations.

Table S2. Proportion of sites differing within species and among their closest relatives in our sequence data.

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