A MOLECULAR PHYLOGENY FOR THE TRIBE DACINI (DIPTERA: TEPHRITIDAE): SYSTEMATIC AND BIOGEOGRAPHIC IMPLICATIONS

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Abstract

With well over 700 species, the Tribe Dacini is one of the most species-rich clades within the dipteran family Tephritidae, the true fruit flies. Nearly all Dacini belong to one of two very large genera, *Dacus* Fabricius and *Bactrocera* Macquart. The distribution of the genera overlap in or around the Indian subcontinent, but the greatest diversity of *Dacus* is in Africa and the greatest diversity of *Bactrocera* is in south-east Asia and the Pacific. The monophyly of these two genera has not been rigorously established, with previous phylogenies only including a small number of species and always heavily biased to one genus over the other. Moreover, the subgeneric taxonomy within both genera is complex and the monophyly of many subgenera has not been explicitly tested. Previous hypotheses about the biogeography of the Dacini based on morphological reviews and current distributions of taxa have invoked an out-of-India hypothesis; however this has not been tested in a phylogenetic framework. We attempted to resolve these issues with a dated, molecular phylogeny of 125 Dacini species generated using 16S, COI, COII and white eye genes. The phylogeny shows that *Bactrocera* is not monophyletic, but rather consists of two major clades: *Bactrocera* s.s. and the 'Zeugodacus group of subgenera' (a recognised, but informal taxonomic grouping of 15 *Bactrocera* subgenera). This 'Zeugodacus' clade is the sister group to *Dacus*, not *Bactrocera* and, based on current distributions, split from *Dacus* before that genus moved into Africa. We recommend that taxonomic consideration be given to raising Zeugodacus to genus level. Supportive of predictions following from the out-of-India hypothesis, the first common ancestor of the Dacini arose in the mid-Cretaceous approximately 80mya. Major divergence events occurred during the Indian rafting period and diversification of *Bactrocera* apparently did not begin until after India docked with Eurasia (50-35mya). In contrast, diversification in *Dacus*, at approximately 65mya, apparently began much earlier than predicted by the out-of-India hypothesis, suggesting that, if the Dacini arose on the Indian plate, then ancestral *Dacus* may have left the plate in the mid to late Cretaceous via the well documented India-Madagascar-Africa migration route. We conclude that the phylogeny does not disprove the predictions of an out-of-India hypothesis for the Dacini, although modification of the original hypothesis is required.

Keywords: *Bactrocera*, *Dacus*, Zeugodacus, phylogenetics, Gondwana

1.0 Introduction

The Tribe Dacini of the Tephritidae sub-family Dacinae contains approximately 770 described fruit fly species, as well as many undescribed species (Drew and Hancock, 2000). The Dacini is considered monophyletic (Han and Ro, 2009) consisting of four genera; two small genera, *Ichneumonopsis* Hardy (one sp) and *Monacrostichus* Bezzi (two spp), and two very large genera, *Dacus* Fabricius (245 spp) and *Bactrocera* Macquart (528spp) (Drew and Hancock, 2000). Both *Dacus* and *Bactrocera* are further subdivided into subgenera (*Bactrocera* – ~30, *Dacus* - ~7, depending on source) (Drew, 1972, 1989; Hancock and Drew, 2006; White, 2006), and sometimes within those subgenera into species groups (Drew, 1989). Nearly all *Dacus* and *Bactrocera* species have frugivorous larvae and, although most species are non-pest endemics of savannah areas (*Dacus*) or rainforests (*Bactrocera*) (Drew, 2004), a few species are horticultural pests of international importance (White and Elson-Harris, 1992).

The distribution of the Dacini extends from Africa, where *Dacus* dominates, across India and into Asia, Australia and the Pacific, where *Bactrocera* dominates (Drew, 2004). The greatest generic and species diversity of Dacini occurs in south-east Asia (inclusive of India), where all four genera are found (*Ichneumonopsis* and *Monacrostichus* are restricted to this region) (Drew and Hancock, 1994b; Drew et al., 1998; Drew and Raghu, 2002; Drew and Romig, 2007). The presence of Dacini in Africa, Australia and India suggests the group may have first evolved in Gondwana, but this is countered by the group's much higher diversity in Asia which suggests a Eurasian centre of origin.

Drew and Hancock (2000) and Drew (2004) have argued that the Dacini are of Gondwanan rather than Eurasian origin, with the ancestral flies having originated on the Indian plate after it rafted away from Gondwana. Once India docked with Eurasia around 50-35mya, ancestral *Dacus* moved westward and diversified in Africa, while ancestral *Bactrocera* moved eastward and diversified in the rainforests of south-east Asia and the Pacific. An important part of the argument (as summarised in Fig 19.1 of Drew and Hancock, 2000) is the subsequent movement of some *Dacus* species back into Asia and some *Bactrocera* species, particularly members of the Zeugodacus group of subgenera, back into Africa. The reasoning behind Drew and Hancock's (2000) hypothesis is based on the absence of Dacini

in South America (hence diversification only beginning after Gondwana started breaking up), the presence of basal genera (*Ichneumonopsis* and *Monacrostichus*) and subgenera (e.g., *B*. (*Tetradacus*)) in south western Asia, and the abundance of more derived *Dacus* in Africa and *Bactrocera* in south-east Asia, New Guinea and Australia.

Drew and Hancock's (2000) hypothesis for the evolution of the Dacini, while developed independently, is a 'text book' example of a general biogeographic pattern now known as the 'out-of-India' hypothesis. The out-of-India hypothesis has been used to explain why some clearly Gondwanan groups have high diversity in Asia and uses the same argument of Drew and Hancock: i.e., groups of Gondwanan origin rafted on the Indian plate away from Gondwana and then diversified following docking with Eurasia (Gower et al., 2002; Praveen Karanth, 2006; Conti et al., 2007; Datta-Roy and Karanth, 2009; Svenson and Whiting, 2009; Viseshakul et al., 2011).

While an out-of-India argument for the biogeography of the Dacini aligns with biogeographic patterns seen in other plant and animal taxa, it is not the only hypothesis to explain why a putative Gondwanan group may have high diversity in Asia. For other Gondwanan taxa with Asian elements, arguments have also been made for out-of-Africa (Masters et al., 2006; Surveswaran et al., 2010; Zhou et al., 2011) or out-of-Australia (Braby et al., 2005; Braby and Pierce, 2007; Goldblatt et al., 2008; Cruaud et al., 2011) scenarios. These hypotheses could also be applied to the Dacini, as could an out-of-Asia hypothesis (Kosuch et al. 2001; Köhler & Glaubrecht 2007) if the group is not Gondwanan. Drew and Hancock (2000) acknowledge that their Indian rafting hypothesis is dependent on there being congruence between the timing of species diversification events and geological events. What is also critical, but is unstated, is that their assumptions of Dacini phylogeny (especially with respect to early-branching versus late-branching genera, sub-genera and species) are correct. Independent molecular phylogenies, with estimated divergence times, are needed to address these issues.

Current molecular phylogenies pertinent to the Dacini are insufficient to address the problem posed. Most focus on the phylogeny of a specific genus (esp. *Bactrocera*, Muraji and Nakahara, 2001; Smith et al., 2003; Nakahara and Muraji, 2008; Zhang et al., 2010), or

where coverage includes both genera, the taxonomic coverage is insufficient for biogeographic analysis (Han and McPheron, 1997; Smith et al., 2002; Segura et al., 2006 ; Han and Ro, 2009). A major weakness for nearly all previous studies has been a lack of African *Dacus* material, but this has now been overcome (Virgilio et al., 2009). At a broader taxonomic scale, the Schizophora group of dipteran taxa, of which the Tephritidae form a part, are hypothesised to have originated around 60mya (Weigmann et al. 2003; Weigmann et al. 2011) and Schizophora first appear in the fossil record at around the same time (Winkler et al., 2010). By extension this implies that the Dacini could be much younger than would be expected based on Drew and Hancock's (2000) out-of-India scenario.

Using a combination of new data and sequences from GenBank, we present here a joint phylogenetic analysis of *Bactrocera* and *Dacus* based on the 16S, COI, COII and white eye genes. Specifically, within a dispersal biogeographical framework (sensu Cranston, 2005), we test the following arguments and predictions made by Drew and Hancock (2000) in support of their out-of-India hypothesis: (i) the earliest common ancestor of the Dacini should occur between 165mya (when India/Madagascar broke away from Africa) and 57-35 mya (progressive collision dates of India with Eurasia) (Ali and Aitchison, 2008); (ii) the original diversification of the Dacini into major clades should occur during the same period; (iii) *B*. (*Tetradacus*) should be an early-branching lineage within *Bactrocera*; (iv) major species level diversification of *Dacus* and *Bactrocera* should occur after 35mya, when final docking between India and Asia had been completed; and (v) the Australo-Pacific *Dacus* species should form at least two clades, one clade containing *D*. (*Callantra*) and a second clade that is sister to *Dacus* taxa from Africa. A further question which could be asked, that the African *B*. (*Zeugodacus)* species should be a monophyletic clade nested within the larger *B.* (*Zeugodacus*) clade, could not be addressed as we had no African *B*. (*Zeugodacus*) data available. We appreciate that such an approach necessarily cannot confirm an out-of-India origin for the Dacini, but it can help disprove it. Moreover, the phylogeny is also the most complete yet developed for the Dacini, covering approximately 20% of described species. We therefore use this phylogeny to address additional systematic issues related to subgeneric status, male lure response and level of polyphagy.

2.0 Methods

2.1 Taxon sampling

Sampling was directed towards obtaining and comparing a wide variety of subgenera within the major Dacini genera (*Bactrocera* and *Dacus*). We also attempted to incorporate taxa from across the geographical ranges of the two genera to provide better resolution of deeper evolutionary relationships. The primary new data used in this paper comes from a molecular dataset generated as part of the Ph.D. study of G.C.G. (Graham, 2006), supplemented by additional COI sequences for some species. Both data sets are comprised mostly of Australasian taxa from both genera that were predominantly identified in the laboratory of Prof. R.A.I. Drew (Griffith University, Brisbane, Australia). Single sequences for each gene were chosen to represent each species largely on the basis of completeness and geographical location (i.e., samples from a species' native range were preferred over invasive records). Seven dipteran outgroups were incorporated into the analysis: *Aedes aegypti* and *Anopheles gambiae* (S.O. Nematocera, Family Culicidae); *Tabanus rufofrater* (S.O. Brachycera, Family Tabanidae); *Drosophila melanogaster* (S.O. Brachycera, Family Drosophilidae); *Anastrepha ludens*, *Rhagoletis pomonella* and *Ceratitis capitata* (S.O. Brachycera, Family Tephritdae). Supplementary Table 1 includes the species names, subgeneric status, sequence origin, geographical range, male lure response and degree of polyphagy of the taxa included in the current analysis. Male lure response data was compiled from information in Drew (1989), Allwood et al. (1999), Clarke et al. (2005) and the online resource Pherobase (www.pherobase.com, accessed March-July 2011). Information concerning the degree of polyphagy exhibited by each species was retrieved from Drew (1989), Allwood et al. (1999) Hancock et al. (2000), Drew (2004) and Clarke et al. (2005).

2.2 Genetic procedures

2.2.1 Sequences supplied by G.C.G.: DNA was extracted from individual fly heads by boiling a tissue grindate in a suspension of Chelex resin, a modification of the method in Walsh et al. (1991). The remainder of the body was kept as a morphological voucher and all are currently held at the Australian National Insect Collection, Canberra. A 542 bp fragment of mitochondrial *16S* rRNA was amplified using the primers of Palumbi (1996) (forward: Mtd32 5' CCGGTCTGAACTCA GATCACGT 3'; reverse Mtd34 5' CGCCTGTTTAACAAAAACAT 3').

Additionally, a 690 bp fragment of mitochondrial cytochrome *c* oxidase subunit II (*COII*) was amplified using primers described by Simon *et al.* (1994) (forward: Atleu 5' ATGGCAGATTAGTGCAATGG 3'; reverse: Btlys 5' GTTTAAGAGACCAGTACTTG 3'). PCR reactions for both loci were performed in a total volume of 25 µL and contained 10 ng of DNA template, 0.2 µL of 5 U μL^{-1} *Taq* DNA polymerase (Qiagen, Hilden, Germany), 0.5 µL of 10 mM dNTP's (Qiagen), 0.5 µL of each primer and PCR buffer to a final concentration of 0.01 M Tris-HCl, 1.5 mM MgCl2, 0.05 M KCl, 0.1 mg mL^{-1} gelatine (pH 8.3) (Qiagen). The cycle protocol involved initial denaturation at 94°C 2 mins, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and a final cycle of 25°C for 2 min. PCR products were purified using a standard polyethylene glycol (PEG) precipitation procedure. Amplification of purified products was undertaken using an ABI *Taq* DyeDeoxy™ terminator sequencing protocol and products were cleaned using a standard ethanol precipitation protocol prior to sequencing at the Australian Genome Research Facility (University of Queensland, Brisbane, Australia). All sequences were deposited in GenBank (Accession Numbers XXXXX-XXXXX).

2.2.2 Sequences supplied by K.A.: Procedures for amplification and sequencing of additional mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences followed those outlined in Armstrong and Ball (2005). These sequences were deposited under GenBank Accession Numbers XXXXX-XXXXX.

2.3 GenBank data

GenBank searches were carried out during early 2011 for sequence data from members of both *Bactrocera* and *Dacus* for inclusion in the current study. We collated data for *16S* and *COII* that aligned to the regions sequenced by Graham (2006) and that possessed greater than 50% coverage of each region. Additional datasets were created by collating data for the entire *COI* gene (1535 bp) and a partial fragment of the nuclear gene *white-eye* (328 bp). Published data used here originated from the following studies: Beard et al. (1993), Lewis et al. (1995), Spanos et al. (2000), Gomulski et al. (2001), Smith et al. (2001), Mitchell et al. (2002), Morlais and Severson (2002), Smith et al. (2002), Jamnongluk et al. (2003), Mun et al. (2003), Smith et al. (2003), Armstrong and Ball (2005), Nakahara et al. (2005), Barr et al. (2006), Barr and McPheron (2006), Sota and Mogi (2006), Winterton et al. (2007), Elfekih et

al. (2009), Han and Ro (2009), Virgilio et al. (2009), Chua et al. (2010), Mazzon et al. (2010), Shearman et al. (2010), Van Houdt et al. (2010) and Zhang et al. (2010).

2.4 Alignment and Phylogenetic Analyses

Sequences were aligned by eye in BioEdit Version 7.0.5 (Hall, 1999) and coding regions were translated to amino acid sequences to check for the presence of internal stop codons. Alignment of *16S* sequences was performed initially by eye and was checked using MUSCLE Version 3.6 (Edgar, 2004). Five hypervariable regions were recognised among *16S* sequences and were excluded from all analyses. Tests for sequence saturation were conducted by calculating the mean ratio of transitions to transversions in Mega Version 4.0 (Kumar et al., 2008). Tajima's D tests of neutrality were estimated in DnaSP Version 5.0 (Librado and Rozas, 2009) using coalescent simulations to determine if sequences were evolving neutrally. Sequences were assessed for clock-like evolution using Tree-Puzzle Version 5.2 (Schmidt et al., 2002) under a Hasegawa-Kishino-Yano (HKY) model of sequence evolution. Partition-homogeneity tests were conducted in PAUP* Version 4.0b10 (Swofford, 2001) to provide support for the analysis of the four gene regions as a combined dataset.

The most appropriate substitution model for each locus was determined under the Akaike Information Criterion (AIC) using the online resource FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). Sequences were partitioned separately in combined analyses and the most appropriate model was applied to each partition individually where software options allowed. Phylogenies were reconstructed using mitochondrial and nuclear data both separately and combined. Bayesian phylogenetic inference was performed in MrBayes Version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) under the GTR model of sequence evolution, incorporating a gamma distribution of nucleotide frequencies and either variable (*COI*, *16S*) or equal (*COII*, *white-eye*) state frequencies according to the results of FindModel for each locus. Two simultaneous runs of 30 million generations were performed and convergence was maximised by ensuring the average standard deviation of split frequencies fell below 0.01, potential scale reduction factors approached 1.0. Maximum likelihood (1,000 bootstraps) reconstruction was performed using RAXML Version 7.0.3 (Stamatakis, 2006)

241 under the GTRMIX model of sequence evolution. Both MrBayes and RAXML runs were
242 carried out on the online CIPRES Science Gateway resource (Miller et al., 2010).
243
244 Tests of specific phylogenetic relationships were carried out using Shimodaira-Hasegawa
245 tests (SH test – (Shimodaira and Hasegawa, 1999) in PAUP*. Additional Bayesian
246 phylogenies were constrained to reflect expected patterns under particular evolutionary
247 scenarios and tested the resulting trees against the unconstrained topology. The specific
248 settings for constrained Bayesian runs were as described above. First, reciprocal monophyly
249 of *Bactrocera* and *Dacus* was constrained. Second, African *Dacus* were constrained as
250 monophyletic to the exclusion of Australasian *Dacus* taxa. Third, we constrained monophyly
251 of Australasian *Dacus* taxa. Statistical significance was obtained via 1000 random
252 resampling estimated log-likelihood (RELL) bootstrap replicates.
253
254 Times to most recent common ancestor (tmrca) were estimated for relevant nodes of the
255 partitioned dataset using BEAST Version 1.5.3 (Drummond and Rambaut, 2007). The
256 program r8s (Sanderson, 2003) was used to ultrametricise the Bayesian consensus topology
257 and the resulting tree used as a starting topology in BEAST to reduce computation time. An
258 exponential prior was set on the root height of the tree with hard lower and soft upper
259 bounds that encompassed the proposed timing of the Culicomorpha-Brachycera split
260 (238.5-295.4mya – Benton et al., 2009). Two lognormal priors were also set on internal
261 nodes: one on the node connecting all Brachyceran taxa that incorporated a zero offset of
262 60my, a mean of 70my and a standard deviation of 0.75; and a second prior on the node
263 that connected all Schizophoran taxa that used a zero offset of 15my, a mean of 30my and a
264 standard deviation of 0.75. The former calibration corresponded to the age estimate for the
265 oldest known stem group fossil schizophoran (Winkler et al., 2010), and the latter
266 corresponded to the age estimate for the oldest known stem group fossil tephritid, which
267 was recorded from Dominican amber of mid-Miocene to early Eocene age (Poinar Jnr.,
268 1992; Norrbom, 1994). We acknowledge the ongoing debate surrounding the appropriate
269 use of fossils in molecular phylogenetics (Heads, 2005; Yang and Rannala, 2006; Parham and
270 Irmis, 2008; Heads, 2010; Parham et al., 2011); however, we believe that lognormal prior
271 distributions, rather than hard maximum age constraints, make best use of stem group fossil
272 data (Ho and Phillips, 2009). Any differences in estimated divergence times between the

current study and existing, higher-order dipteran phylogenies likely stem from differences in the treatment of fossil data. The tree prior was set to 'Speciation: Yule Process', the HKY model of sequence evolution was used and a relaxed lognormal molecular clock prior was applied so that substitution rates were allowed to vary across branches. Two runs of 30 million generations were performed, sampling every 1000 generations. Three million generations were removed from each run as burnin prior to combining log files, producing a total run of 54 million generations, representing 54 000 samples.

3.0 Results

3.1 Analysis and tree topology

Sequence data for a total of 125 species was collated, comprising 84 *Bactrocera* and 41 *Dacus* taxa (see Supplementary Table 1 for gene fragments included per individual). Transition to transversion ratios were low for all loci (range 0.922-1.827), suggesting only limited homoplasy. Tajima's D tests of neutrality were non-significant for all loci ($P > 0.05$). A molecular clock hypothesis was rejected for all fragments, thus a relaxed clock was implemented for estimation of divergence times. Findmodel identified the GTR + γ model of substitution as the most appropriate for the *COI* dataset, the Symmetrical + γ model for *COII*, the unequal-frequency Kimura-3-parameter + γ model for *16S* and the equal-frequency transition model for the *white-eye* dataset. The partition homogeneity test was non-significant ($P = 1.000$), thereby supporting the combined analysis of the four gene regions.

Topologies were roughly concordant among methods of reconstruction (Figure 1), although nodal support for ML outputs typically was lower than for Bayesian analyses. Phylogenies inferred from separate mitochondrial and nuclear gene datasets appeared roughly concordant and produced good tip support but with lower support at deeper nodes (data not shown). The combined partitioned dataset produced topologies that were well supported across the tree. In contrast to the established taxonomy, the genera *Bactrocera* and *Dacus* were not reciprocally monophyletic: *Bactrocera* instead was paraphyletic. All but two members of the Zeugodacus group of subgenera (i.e., *B. (Zeugodacus), B. (Papuodacus), B*. (*Paradacus*), *B.* (*Paratridacus), B.* (*Austrodacus*) and *B.* (*Sinodacus*)) (sensu Drew and Hancock 2000, hereafter simply referred to as Zeugodacus) analysed here formed a clade

that was more closely related to *Dacus* than *Bactrocera*. The exceptions – *B. (Javadacus) unirufa* and *B. (Paratridacus) expandens* – were nested within the broader *Bactrocera* clade; a placement supported by recent morphological taxonomic study (R.A.I.Drew, pers. comm., 2011). The broader *Bactrocera* group was a grade and phylogenetic resolution for some backbone nodes within this group was often weak. *Dacus* was divided into two subclades of roughly equal numbers of taxa (*Dacus* clades A and B, Figure 1), within one of which (*Dacus* clade A) were nested two groups of species from the Australo-Pacific.

Below the subgenus group level, members of both genera predominantly did not cluster according to subgeneric taxonomic designation. Members of the subgenera *B. (Paradacus* and *Paratridacus)* were variously nested within the Zeugodacus clade. Taxa belonging to *D. (Dacus, Didacus* and *Leptoxyda)* were distributed throughout the *Dacus* clade. Individuals belonging to the subgenus group *Bactrocera (Bactrocera)* dominated the broader *Bactrocera* clade; however, the subgenus group *B. (Daculus)* was nested within this clade. Furthermore, members of the subgenus *B. (Afrodacus)* were paraphyletic within the broader *Bactrocera* clade. There were three exceptions to the pattern of widespread paraphyly below the genus level: the subgenera *D. (Callantra)*, which formed a sister group to a clade of mostly African *Dacus*; *B. (Tetradacus)*, a sister group to the remainder of the broader *Bactrocera* clade; and the subgenus *B. (Daculus)*, which was nested within a subclade of the broader *Bactrocera* group. Of those subgenera for which only a single representative could be acquired, *B. (Polistomimetes) murrayi*, *B. (Notodacus) xanthodes*, *B. (Gymnodacus) calophylli* and *B. (J.) unirufa* were nested within the broader *Bactrocera* clade; *D. (Lophodacus) umehi, D. (Metidacus) pergulariae* and *D. (Neodacus) quilicii* were nested within the *Dacus* clade; and *B. (Sinodacus) abdopallescens*, *B. (Austrodacus) cucumis* and *B. (Papuodacus) neopallescentis* were nested within the *B. (Zeugodacus)* clade. Most members of the *B (B.) dorsalis* species complex (except *B. (B.) caryeae* Kapoor, *B. (B.) endiandrae* Perkins and May and *B. (B.) kandiensis* Drew and Hancock) resolved as a strongly supported monophyletic group within the broader *Bactrocera* clade.

Taxa that respond to a particular male lure did not form reciprocally monophyletic groups and there was no distinct trend for the lure response of members of the major clades in the current phylogeny (Supplementary Figure 1). Likewise, phylogenetic placement of any given

337 species did not appear to be correlated with the degree of polyphagy it exhibits
338 (Supplementary Figure 2). No evidence was observed to support the notion that polyphagy
339 in dacine fruit flies is a derived trait.
340
341 *3.2 Biogeography*
342 In all major clades of the phylogeny, except one, representatives from a given geographical
343 region did not form predominantly monophyletic groups to the exclusion of taxa from other
344 regions. The one exception was one of two major clades within *Dacus*, in which all species
345 except one (*D. ciliatus*) were restricted to Africa. *Dacus ciliatus* is also an African species,
346 but has spread across the Middle East into Pakistan, northern India and Bangladesh, possibly
347 as the result of human assisted movement (Drew and Hancock, 2000). Both the Zeugodacus
348 clade and the broader *Bactrocera* clade show no relationship between phylogenetic
349 placement and geographical distribution (Figure 1). Even the *Dacus* clade, for which a valid
350 hypothesis for the reciprocal monophyly of African and Australo-Pacific members could be
351 established, shows that these groups are not reciprocally monophyletic. Instead, there
352 were two subclades within *Dacus*, both of which comprised some African taxa and one of
353 which possessed two separate groups of Australo-Pacific taxa.
354
355 SH tests of specific evolutionary relationships supported the current topology and provided
356 statistically significant outcomes for three tests. The paraphyly of *Bactrocera* was supported
357 ($P < 0.001$), rather than being reciprocally monophyletic to *Dacus*. Monophyly of African
358 *Dacus* was not supported ($P < 0.001$), instead forming two clades as described above.
359 Similarly, Australo-Pacific *Dacus* were supported as being paraphyletic ($P < 0.001$). Taken
360 with the paraphyly of African *Dacus*, this suggests that multiple independent migration
361 events from Africa or the Indian plate into the Asia-Pacific region may have occurred
362 following initial divergence of the genus.
363
364 Estimated divergence times for relevant nodes all possessed appropriate effective sample
365 sizes, indicating good support; although some 95% credibility intervals were broad (Figure 2
366 & Table 1). All sampled members of the Dacini were estimated to have last shared a
367 common ancestor during the late Cretaceous, approximately 80mya (95-65mya; node 'D'
368 Figure 2). Divergence of the broader *Bactrocera* clade apparently began around 70mya (84-

55mya; node 'F') with the branching off of the ancestor of the subgenus *B. (Tetradacus)*. This subgenus apparently then began to diverge around 61mya (74-48mya; node 'L'). *Dacus* and Zeugodacus last shared a common ancestor around 72mya (86-59mya; node 'E'). Divergence within the genus *Dacus* appears to have initiated around 66mya (79-54mya; node 'H'), with subsequent divergence of the ancestors of the Australo-Pacific subgenus *D. (Callantra)* occurring approximately 63mya (76-51mya; node 'I') and of a second group of Australo-Pacific *Dacus* around 46mya (55-34mya; node 'K'). The subgenus *D. (Callantra)* apparently began to diverge in the early Miocene 16mya (27-8mya; node 'N'). Members of the Zeugodacus subgenus group apparently shared a most recent common ancestor around 67mya (84-54mya; node 'G'). The well-resolved *B. dorsalis* species complex apparently has its origins in the Pliocene, with all members sharing a common ancestor around 6mya (8-4mya; node 'O').

4.0 Discussion

With 125 taxa included, the molecular phylogeny presented here represents the most comprehensive yet produced for the Dacini. The next largest molecular analysis of the Dacini included only 14 taxa (Smith et al., 2002), although studies of the individual genera (i.e., *Dacus* and *Bactrocera*) have included up to 32 species (Virgilio et al., 2009). Nevertheless, we acknowledge that this phylogeny is not complete: it is missing some subgenera from both *Bactrocera* and *Dacus,* along with African Zeugodacus and taxa from India and Madagascar, and it is also missing *Monochrostichus* and *Ichneumonopsis*. Yet despite these lacunae, we believe that the analysis undertaken allows us to make comment on the systematics and historical biogeography of the group.

4.1 Systematics

There are a number of issues raised by the phylogeny which are important for the systematics of the Dacini. Clearly the most important is the paraphyly of the *Bactrocera*, with the Zeugodacus group of *Bactrocera* subgenera, represented here by *B*. (*Zeugodacus*), *B*. (*Paratridacus*), *B*. (*Paradacus*), *B*. (*Sinodacus*), *B.* (*Austrodacus*) and *B*. (*Papuodacus*), sitting as an almost entirely monophyletic sister group to *Dacus*. The paraphyly of *Bactrocera* was also found using a weighted phylogenetic analysis of morphological traits by

White (2000), but he down-played its importance. Other molecular studies have recognised the monophly of the Zeugodacus group of subgenera (Muraji and Nakahara, 2001; Nakahara and Muraji, 2008), but not the paraphyly of *Bactrocera* (Smith et al., 2002; Smith et al., 2003). Only Segura et al. (2006) has previously hinted at this, with *B. (Z.) cucurbitae* (Coquillet) separated from other *Bactrocera* by *Dacus* species. Moreover, Segura et al (2006, and references therein) allude to similarities in plant host relationships and male lure response between members of the Zeugodacus subgenera group and *Dacus* taxa that support our proposed phylogenetic placement. In our study, two species in subgenera belonging to the Zeugodacus subgenera group, *B*. (*Javadacus*) *unirufa* (Drew) and *B*. (*Paratridacus*) *expandens* (Walker) do not sit in the Zeugodacus clade, both sitting with the other *Bactrocera*. This is supported by the indication that these species will be removed from Zeugodacus for morphological reasons in a new revision, when the definition of Zeugodacus will be tightened (R.A.I. Drew, pers. comm., 2011). We strongly suggest that Zeugodacus should, following revision of the Zeugodacus 'concept', be elevated to genus level. In the short term, it is important for applied workers to recognise that important pest taxa of the Zeugodacus group, such as *B*. *cucurbitae* (Coquillet), *B*. *cucumis* (French) and *B*. *tau* (Walker), are more closely related to African *Dacus* species, than they are to Asian pest species such as *B. dorsalis* (Hendel).

As was reported by Virgilio et al. (2009) in their revision of the African *Dacus*, we also found a number of the subgenera paraphyletic, including the very large subgenera *B*. (*Bactrocera*) and *B*. (*Zeugodacus*), and smaller groups such as *B*. (*Paradacus*) and *B*. (*Paratridacus*). We also have new data to support the finding of Virgilio et al. (2009) that *D*. (*Dacus*) and *D*. (*Didacus*) are not monophyletic. We are not the first to state that the subgeneric classification of the Dacini needs extensive revision (White, 2000; Smith et al., 2003; Graham, 2006; Virgilio et al., 2009). In contrast to the otherwise widespread paraphyly of previous taxonomic groupings, it is worth reporting that the addition of new data continues to support earlier analyses (Clarke et al., 2005) that the economically important *B*. (*Bactrocera*) *dorsalis* species complex (Drew and Hancock, 1994a) is a very recently derived, monophyletic clade.

No apparent correlation was observed between the current phylogenetic placement of a given species and that species' male lure response. This supports the assertion of White et al. (2003) that male lure response appears to be a highly changeable trait within the Dacini. The current data suggests that lure response has been important in the evolution of this group only at a very low taxonomic level, and the lability of this trait makes determining the ancestral character state difficult. The degree of polyphagy of each species is similarly paraphyletic; there is no apparent correlation between degree of polyphagy and phylogenetic placement. As with male lure response, this trait appears to be highly labile in the Dacini and the current pattern does not support the assertion based on a smaller dataset (Graham, 2006) that polyphagy is a derived trait.

4.2 Historical Biogeography

To test Drew and Hancock's (2000) original out-of-India hypothesis for the evolution of the Dacini in a time-scaled phylogenetic framework, we postulated in the Introduction a series of predictions. Overall, our results do not reject the original hypothesis, although with some modification (Figure 3), and we discuss our results here in the context of the original predictions. Specifically, (i) the earliest common ancestor of the Dacini, at approximately 80mya, fits the time-line needed for the prediction that the group arose on the rafting Indian plate; (ii) divergence of major clades is completed by approximately 70mya, well before Indian docking with Eurasia; (iii) *Bactrocera* (*Tetradacus*) is confirmed as an early-branching lineage, being the sister group to all other *Bactrocera* and, along with a small number of other *Bactrocera*, arose prior to docking; (iv) major species level diversification within *Bactrocera* apparently occurs only after Indian docking, possibly as the group moved into the south-east Asian rainforests (Drew, 2004); and, finally, (v) the Asian/Australian *Dacus* species do form two clades as predicted, one consisting of *D*. (*Callantra*), the other comprising members of predominantly African subgenera. In only one point, concerning the evolution of *Dacus*, do the proposed time-lines and phylogenetic relationships not match Drew and Hancock's (2000) evolutionary hypothesis for the group .

Drew and Hancock (2000) predicted the evolution of *Dacus* would follow a similar pattern to *Bactrocera,* with major diversification of the genus occurring in Africa, after India docked with Eurasia, and subsequent westward migration of the genus. The evolutionary time-lines

do not, however, match this prediction. Major diversification of *Dacus* was underway by around 65mya and almost complete (within our sample taxa) by 20mya: in contrast diversification of *Bactrocera* largely took place from roughly 40mya onward. This suggests that diversification of *Dacus* may have been initiated while the Indian plate was still rafting. However, dispersion of *Dacus* from the Indian plate into Africa post-docking does not appear probable, as this would have required large numbers of taxa, rather than a small number of ancestral taxa, to have migrated from India, across the Middle East and into Africa: this seems a less parsimonious scenario. An alternative scenario that fits the phylogeny and geological time-lines, may be that the ancestral *Dacus* lineage migrated from India to Africa via Madagascar during the late Cretaceous at about 60mya. In Africa, an early split apparently led to the evolution of two separate lineages, labelled here as *Dacus* clade A and B. These clades were also resolved by Virgilio et al. (2009), who recognised that *Dacus* clade A appeared to have specialised on the Apocynaceae and *Dacus* clade B on the Cucurbitaceae and Passifloraceae. Moreover, *Dacus* clade A comprises a mix of taxa from Africa and Australasia, while *Dacus* clade B is comprised exclusively of African taxa. Within *Dacus* clade A, Australasian taxa form two clusters. One very early off-shoot, representing the ancestor of *Dacus* (*Callantra*), may have rapidly moved back to India, most likely again via Madagascar, before migrating into the Asian region post-docking. A later off-shoot of *Dacus* clade A apparently left Africa at around 55mya, most likely migrating from the north-east of Africa through the current Middle East, becoming the Asian/Australian *Dacus*.

This explanation for the evolution of *Dacus* is congruent with a growing body of genetic and fossil evidence that suggests that many animal and plant groups migrated to and from India and Africa, via Madagascar (India and Madagascar were still largely joined or connected via land bridges), in the mid to late Cretaceous (Renner, 2004; Masters et al., 2006; Yoder and Nowak, 2006; Ali and Aitchison, 2008). This matches the time-line required not only for ancestral *Dacus* migration into Africa, but also the migration back of *D.* (*Callantra*). Fruit flies currently endemic to Madagascar, *D*. (*Tythocalama*) and *B*. (*Aglaodacus*), show morphological taxonomic character states linking them to Asian Dacini, leading to propositions such as "*An early offshoot from this Asian line* [Dacus (Callantra)] *appears to have reached Madagascar as the subgenus* Tythocalama" (Drew and Hancock, 2000). This supports a hypothesis of migration through Madagascar. A later migration out of north-east

Africa at approximately 45mya also matches known paleobiological migration events. Prior to the Eocene (55.8-33.9mya), the Tethys Sea formed a biogeographic barrier that separated the African plate from the European Plate. The sea shrank in size during the Paleocene (65-55.8mya), until it closed in the Eocene to become the Mediterranean Sea (Curray et al., 1982; Jolivet and Faccenna, 2000). This opened up new land bridges and facilitated migration events which are known to have impacted on many groups across all taxonomic levels (Brown and Lomolino, 1998; Hrbek and Meyer, 2003 ; Koufos et al., 2005), including Diptera (Cranston, 2005).

While most of the discussion above focuses on *Dacus*, it is also worth commenting on *Bactrocera* and the Zeugodacus group of subgenera. Both groups appear to have only begun significant speciation in the last 25-50 million years, suggesting that there may have been a consistent driver to diversification. While hard collision of India with Eurasia did not occur until around 35mya, the north-east corner of the Indian plate made 'glancing contact' with Sumatra and then Borneo as early as 57mya, potentially allowing the exchange of biota (Ali and Aitchison, 2008). Thus, if we accept an out-of-India hypothesis, the diversification of these groups may have occurred exclusively on the Indian plate or in this early India/Eurasia contact zone. Given that much of India during the Cretaceous was semi-arid to arid, with rainfall occurring in short, intense monsoons (Ghosh, 1997; Rogers et al., 2007) and subject to extensive volcanism (Hofmann et al., 2000), the latter scenario may be more plausible. Drew (2004) has argued that speciation of the Dacini in Asia has been largely driven by co-evolution with their rainforest host plants, but more recent literature on the evolution of biodiversity in the Sundaland biogeographic region suggests a more complex explanation, including repeated ecological and geological fragmentation and regularly changing environmental conditions (Taylor et al., 1999; Bird et al., 2005; Cannon et al., 2009; den Texa et al., 2010; Malohlava and Bocak, 2010).

Following the philosophy of Popperian science, we freely acknowledge that we cannot exclude alternative explanations for the history of this group based on the current dataset and analyses. However, we also find no evidence to disprove the out-of-India hypothesis proposed for this group by Drew and Hancock (2000) and believe the evolutionary scenario presented here reconciles of the dated phylogeny with known geological events, as well as

being supported by existing literature from other taxa. More complete sampling of the Tribe, including African Zeugodacus and taxa from Madagascar and India, may strengthen resolved evolutionary relationships and clarify those which are uncertain. Alternatively, addition of such taxa may change our understanding of the evolution of this group by revealing relationships that are currently unresolved. Such expansion may also allow the application of novel analytical techniques for investigating biogeographical hypotheses (e.g., Lagrange – Ree and Smith, 2008), which are inappropriate for the current study because of the non-geographically targeted nature of taxon sampling.

4.3 The fossil gap for Schizophora

Our divergence time estimates place the origin of the Dacini at around 80mya (95-65mya). The Dacini are a tribe nested within the Tephritidae, a family nested well inside the Tephritoidea, in turn nested well within the Schizophora (Wiegmann et al. 2011). Thus our divergence time estimates would place the origin of the Schizophora further back in time, probably into the early Cretaceous. This is congruent with Wiegmann et al.'s (2011) unconstrained age estimate for the Schizophora of 115mya (p. 2, Supporting Information). However, the earliest confirmed Schizophoran fossil is Paleogene, approximately 60mya (Winkler *et al.* 2010). These results suggest a 60 million year gap in the fossil record of the Schizophora. While divergence times estimated from molecular data should logically provide older dates than first fossil occurrence (Ho *et al.* 2005), a difference of this magnitude is surprising. Either there are many Cretaceous schizophoran fossils undiscovered, or some assumptions associated with the divergence time estimation have been violated, or a combination of these two effects (Ho and Larsen 2006; Ho *et al.* 2011). Indeed, schizophoran taxa recently discovered in Indian amber deposits of earliest Eocene age (~50mya – Rust et al., 2010) apparently represent relatively recent branches within the Schizophora (Weigmann et al., 2011), suggesting that our understanding of the tempo of schizophoran divergence may need revision.

4.4 Conclusions

Overall, our results represent the most comprehensive test of Drew and Hancock's (2000) hypothesis for the evolution of the Dacini fruit flies conducted to date. The pattern of phylogenetic relationships and divergence times inferred here largely conformed to

predictions and we could not reject an out-of-India hypothesis, though there were some subtle differences that have allowed us to modify our understanding of the biogeographic history of the group. We have revised Drew and Hancock's (2000) classic out-of-India dispersal hypothesis for the Dacini to also include an additional migration pathway between India and Africa via Madagascar. Moreover, the timing of major migrations of Dacini taxa between these landmasses appear to coincide well with documented continental movements. Additionally, our results support assertions that the subgeneric taxonomy of *Bactrocera* and *Dacus* requires significant revision. Taken together, these data represent valuable baseline information regarding the evolution of the group that will aid researchers in a wide variety of fields related to this economically important Tribe of fruit flies.

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6.0 References

Armstrong, K.F. & Ball S.L., 2005. DNA barcodes for biosecurity: invasive species identification. Philosophical Transactions of the Royal Society (Series B) 360, 1813-1823.

589 Ali, J., Aitchison, J., 2008. Gondwana to Asia: Plate tectonics, paleogeography and the
590 biological connectivity of the Indian sub-continent from the Middle Jurassic through
591 latest Eocene (166–35 Ma). Earth-Science Reviews 88, 145-166.
592 Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hamacek, E.L., Hancock, D.L., Hengsawad,
593 C., Jipanin, J.C., Jirasurat, M., Kong Krong, C., Kritsaneepaiboon, S., Leong, C.T.S.,
594 Vijaysegaran, S., 1999. Host plant records for fruit flies (Diptera: Tephritidae) in
595 south east Asia. The Raffles Bulletin of Zoology Supplement No. 7, 1-92.
596 Barr, N.B., Copeland, R.S., De Meyer, M., Masiga, D., Kibogo, H.G., Billah, M.K., Osir, E.,
597 Wharton, R.A., McPheron, B.A., 2006. Molecular diagnostics of economically
598 important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and
599 RFLP analyses. Bulletin of Entomological Research 96, 505-521.
600 Barr, N.B., McPheron, B.A., 2006. Molecular phylogenetics of the genus *Ceratitis* (Diptera:
601 Tephritidae). Molecular Phylogenetics and Evolution 38, 216-230.
602 Beard, C.B., Hamm, D.M., Collins, F.H., 1993. The mitochondrial genome of the mosquito
603 *Anopheles gambiae*: DNA sequence, genome organization, and comparisons with
604 mitochondrial sequences of other insects. Insect Molecular Biology 2, 103-124.
605 Benton, M.J., Donoghue, P.C.J., Asher, R.J., 2009. Calibrating and constraining molecular
606 clocks. In: Hedges, S.B., Kumar, S. (Eds.), The timetree of life. Oxford University Press,
607 Oxford, UK, pp. 35-86.
608 Bird, M.I., Taylor, D., Hunt, C., 2005. Palaeoenvironments of insular Southeast Asia during
609 the Last Glacial Period: a savanna corridor in Sundaland? Quaternary Science
610 Reviews 24, 2228-2242.
611 Braby, M.F., Trueman, J.W. H., Eastwood, R., 2005. When and where did troidine butterflies
612 (Lepidoptera: Papilionidae) evolve? Phylogenetic and biogeographic evidence
613 suggests an origin in remnant Gondwana in the Late Cretaceous. Invertebrate
614 Systematics 19, 113-143.
615 Braby, M.F., Pierce, N.E., 2007. Systematics, biogeography and diversification of the Indo-
616 Australian genus Delias Hübner (Lepidoptera: Pieridae): phylogenetic evidence
617 supports an 'out-of-Australia' origin. Systematic Entomology 32, 2-25.
618 Brown, J.H., Lomolino, M.V., 1998. Biogeography, 2nd Edition. Sinauer Associates,
619 Sunderland, MA.

620 Cannon, C.H., Morley, R.J., Bush, A.B.G., 2009. The current refugial rainforests of Sundaland
621 are unrepresentative of their biogeographic past and highly vulnerable to
622 disturbance. Proceedings of the National Academy of Sciences U.S.A. 106, 11188-
623 11193.
624 Chua, T.H., Chong, Y.V., Lim, S.H., 2010. Species determination of Malaysian *Bactrocera*
625 pests using PCR-RFLP analyses (Diptera: Tephritidae) Pest Management Science 66,
626 379–384
627 Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K., Yeates,
628 D.K., 2005. Invasive phytophagous pests arising through a recent tropical
629 evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. Annual Review
630 of Entomology 50, 293-319.
631 Conti, E., Eriksson, T., Schönenberger, J., Sytsma, K.J., Baum, D.A., 2007. Early tertiary out-of-
632 India dispersal of Crypteroniaceae: evidence from phylogency and molecular dating.
633 Evolution 56, 1931-1942.
634 Cranston, P.S., 2005. Biogeographical Patterns in the Evolution of Diptera. In: Yeates, D.K.,
635 Wiegmann, B.M. (Eds.), The Evolutionary Biology of Flies. Columbia University Press,
636 New York, pp. 274-311.
637 Cruaud, A., Jabbour-Zahab, R., Genson, G., Couloux, A., Peng, Y.-Q., Rong, Y.D., Ubaidillah,
638 R., Santinelo Pereira, R.A., Kjellberg, F., van Noort, S., Kerdelhue, C., Rasplus, J.-Y.,
639 2011. Out of Australia and back again: the world-wide historical biogeography of
640 non-pollinating fig wasps (Hymenoptera: Sycophaginae). Journal of Biogeography 38,
641 209-225.
642 Curray, J.R., Emmel, F.J., Moore, D.G., Raitt, R.W., 1982. Structure, tectonics and geological
643 history of the northeastern Indian Ocean. In: Nairn, A.E.M., Stehli, F.G. (Eds.), The
644 Ocean Basin and Margins, Vol. 6: The Indian Ocean. Plenum Press, New York, pp.
645 399-450.
646 Datta-Roy, A., Karanth, K.P., 2009. The Out-of-India hypothesis: What do molecules suggest?
647 Journal of Biosciences 34, 687-697.
648 den Texa, R.-J., Thorington, R., Maldonado, J.E., Leonard, J.A., 2010. Speciation dynamics in
649 the SE Asian tropics: Putting a time perspective on the phylogeny and biogeography
650 of Sundaland tree squirrels, *Sundasciurus.* Molecular Phylogenetics and Evolution 55,
651 711-720.

652 Drew, R.A.I., 1972. The generic and subgeneric classification of Dacini (Diptera: Tephritidae)
653 from the South Pacific area. Australian Journal of Entomology 11, 1-22.
654 Drew, R.A.I., 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian
655 and Oceanian regions. Memoirs of the Queensland Museum 26, 1-521.
656 Drew, R.A.I., 2004. Biogeography and speciation in the Dacini (Diptera: Tephritidae:
657 Dacinae). Bishop Museum Bulletin in Entomology 12, 165-178.
658 Drew, R.A.I., Hancock, D.L., 1994a. The *Bactrocera dorsalis* complex of fruit flies (Diptera:
659 Tephritidae: Dacinae) in Asia. Bulletin of Entomological Research Supplement Series
660 Supplement No 2, i-iii + 1-68.
661 Drew, R.A.I., Hancock, D.L., 1994b. Revision of the tropical fruit flies (Diptera: Tephritidae:
662 Dacinae) of south-east Asia. I. Ichneumonopsis Hardy and Monacrostichus Bezzi.
663 Invertebrate Taxonomy 8, 829-838.
664 Drew, R.A.I., Hancock, D.L., 2000. Phylogeny of the Tribe Dacini (Dacinae) based on
665 morphological, distributional, and biological data. In: Aluja, M., Norrbom, A.L. (Eds.),
666 Fruit flies (Tephritidae): Phylogeny and evolution of behavior. CRC Press, Boca Raton,
667 pp. 491-504.
668 Drew, R.A.I., Hancock, D.L., White, I.M., 1998. Revision of the tropical fruit flies (Diptera :
669 Tephritidae : Dacinae) of South-east Asia. II. *Dacus* Fabricius. Invertebrate Taxonomy
670 12 567 - 654.
671 Drew, R.A.I., Raghu, S., 2002. The fruit fly fauna (Diptera: Tephritidae: Dacinae) of the
672 rainforest habitat of the Western Ghats, India. The Raffles Bulletin of Zoology 50,
673 327-352.
674 Drew, R.A.I., Romig, M.C., 2007. Records of Dacine fruit flies and new species of *Dacus*
675 (Diptera: Tephritidae) in Bhutan. The Raffles Bulletin of Zoology 55, 1-21.
676 Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling
677 trees. BMC Evolutionary Biology 7, 214.
678 Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high
679 throughput. *Nucleic Acids Research,* **32(5)**, 1792-1797.
680 Elfekih, S., Makni, M., Haymer, D.S., 2009. Mitochondrial DNA markers in populations of
681 *Dacus punctatifrons* (Diptera: Tephritidae). Florida Entomologist 92, 518-520.
682 Ghosh, P., 1997. Geomorphology and palaeoclimatology of some Upper Cretaceous
683 palaeosols in central India. Sedimentary Geology 110, 25-49.

684 Goldblatt, P., Rodriguez, A., Powell, M.P., Davies, J.T., Manning, J.C., van der Bank, M.,
685 Savolainen, V., 2008 Iridaceae 'Out of Australasia'? Phylogeny, biogeography, and
686 divergence time based on plastid DNA sequences. Systematic Botany 33, 495-508.
687 Gomulski, L.M., Pitts, R.J., Costa, S., Saccone, G., Torti, C., Polito, L.C., Gasperi, G., Malacrida,
688 A.R., Kafatos, F.C., Zwiebel, L.J., 2001. Genomic organization and characterization of
689 the white locus of the Mediterranean fruitfly, *Ceratitis capitata*. Genetics 157, 1245-
690 1255.
691 Gower, D.J., Kupfer, A., Oommen, O.V., Himstedt, W., Nussbaum, R.A., Loader, S.P.,
692 Presswell, B., Müller, H., Krishna, S.B., Boistel, R., Wilkinson, M., 2002. A molecular
693 phylogeny of ichthyophiid caecilians (Amphibia: Gymnophiona: Ichthyophiidae): out
694 of India or out of South East Asia? Proceedings of the Royal Society (Series B) 269,
695 1563-1569.
696 Graham, G.C., 2006. Phylogenetics of the Australasian Dacinae. Unpublished PhD thesis,
697 School of Integrative Biology. The University of Queensland, Brisbane.
698 Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis
699 program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95-98.
700 Han, H.-Y., McPheron, B.A., 1997. Molecular phylogenetic study of Tephritidae (Insecta:
701 Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. Molecular
702 Phylogenetics and Evolution 7, 17-32.
703 Han, H.-Y., Ro, K.-E., 2009. Molecular phylogeny of the family Tephritidae (Insecta: Diptera):
704 New insight from combined analysis of the mitochondrial 12S, 16S, and COII genes.
705 Molecules and Cells 27, 55-66.
706 Hancock, D.L., Hamacek, E.L., Lloyd, A.C., Elson-Harris, M.M., 2000. The distribution and host
707 plants of fruit flies (Diptera: Tephritidae) in Australia. Brisbane, Department of
708 Primary Industries, Queensland.
709 Hancock, D.L., Drew, R.A.I., 2006. A revised classification of subgenera and species groups in
710 *Dacus* Fabricius (Diptera: Tephritidae). Instrumenta Biodiversitatis VII, 167-205.
711 Heads, M., 2005. Dating nodes on molecular phylogenies: a critique of molecular
712 biogeography. Cladistics 21, 62-78.
713 Heads, M., 2010. Evolution and biogeography of primates: a new model based on molecular
714 phylogenetics, vicariance and plate tectonics. Zoologica Scripta 39, 107-127.

715 Ho, S.Y.W., Phillips, M.J., Cooper A., Drummond, A.J., 2005. Time dependency of molecular
716 rate estimates and systematic overestimation of recent divergence times. Molecular
717 Biology and Evolution 22, 79-83.
718 Ho, S.Y.W., Larson G., 2006. Molecular clocks: when times are a-changin'. Trends in Genetics
719 22, 79-83.
720 Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic
721 estimation of evolutionary divergence times. Systematic Biology 58, 367-380.
722 Ho, S.Y.W, Lanfear, R., Bromham, L., Phillips, M.J., Soubrier, J., Rodrigo, A.G., Cooper, A.,
723 2011. Time-dependent rates of molecular evolution. Molecular Ecology 20, 3087-
724 3101.
725 Hofmann, C., Féraud, G., Courtillot, V., 2000. $^{40}Ar/^{39}Ar$ dating of mineral separates and
726 whole rocks from the Western Ghats lava pile: further constraints on duration and
727 age of the Deccan traps. Earth and Planetary Science Letters 180, 13-27.
728 Hrbek, T., Meyer, A., 2003. Closing of the Tethys Sea and the phylogeny of Eurasian
729 killifishes (Cyprinodontiformes: Cyprinodontidae). Journal of Evolutionary Biology 16,
730 17-36.
731 Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny.
732 Bioinformatics 17, 754-755.
733 Jamnongluk, W., Baimai, V., Kittayapong, P., 2003. Molecular evolution of tephritid fruit flies
734 in the genus *Bactrocera* based on the cytochrome oxidase I gene. Genetica 119, 19-
735 25.
736 Jolivet, L., Faccenna, C., 2000. Mediterranean extension and the Africa-Eurasia collision.
737 Tectonics 19, 1095-1106.
738 Lewis, D.L., Farr, C.L., Kaguni, L.S., 1995. *Drosophila melanogaster* mitochondrial DNA:
739 completion of the nucleotide sequence and evolutionary comparisons. Insect
740 Molecular Biology 4, 263-278.
741 Köhler, F., Glaubrecht M., 2007. Out of Asia and into India: on the molecular phylogeny and
742 biogeography of the endemic freshwater gastropod *Paracrostoma* Cossmann, 1900
743 (Caenogastropoda: Pachychilidae). Biological Journal of the Linnean Society 91, 627-
744 651.

745 Kosuch, J., Vences, M., Dubois, A., Ohler, A., Böhme, W., 2001. Out of Asia: mitochondrial
746 DNA evidence for an Oriental origin of Tiger frogs, genus *Hoplobatrachus.* Molecular
747 Phylogenetics and Evolution 21, 398-407.
748 Koufos, G.D., Kostopoulos, D.S., Vlachou, T.D., 2005. Neogene/Quaternary mammalian
749 migrations in Eastern Mediterranean. Belgium Journal of Zoology 135, 181-190.
750 Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: A biologist-centric software for
751 evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics 9,
752 299-306.
753 Librado, P., Rozas, J., 2009. DNAsp v5: a software for comprehensive analysis of DNA
754 polymorphism data. Bioinformatics 25, 1451-1452.
755 Malohlava, V., Bocak, L., 2010. Evidence of extreme habitat stability in a Southeast Asian
756 biodiversity hotspot based on the evolutionary analysis of neotenic net-winged
757 beetles. Molecular Ecology 19, 4800-4811.
758 Masters, J.C., de Wit, M.J., Asher, R.J., 2006. Reconciling the origins of Africa, India and
759 Madagascar with vertebrate dispersal scenarios. Folia Primatologica 77, 399-418.
760 Mazzon, L., Martinez-Sanudo, I., Simonato, M., Squartini, A., Savio, C., Girolami,V., 2010.
761 Phylogenetic relationships between flies of the Tephritinae subfamily (Diptera,
762 Tephritidae) and their symbiotic bacteria. Molecular Phylogenetics and Evolution 56,
763 312-326.
764 Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for
765 inference of large phylogenetic trees. Proceedings of the Gateway Computing
766 Environments Workshop (GCE), New Orleans, LA, pp. 1-8.
767 Mitchell, A., Sperling, F.A.H., Hickey, D.A., 2002. Higher level phylogeny of mosquitoes
768 (Diptera: Culicidae): mtDNA data support a derived placement for Toxorhynchites.
769 Insect Systematics and Evolution 33, 163-174.
770 Morlais, I., Severson, D.W., 2002. Complete mitochondrial DNA sequence and amino acid
771 analysis of the cytochrome C oxidase subunit I (COI) from *Aedes aegypti*. DNA
772 Sequence 13, 123-127.
773 Mun, J., Bohonak, A.J., Roderick, G.K., 2003. Population structure of the pumpkin fruit fly
774 *Bactrocera depressa* (Tephritidae) in Korea and Japan: Pliocene allopatry or recent
775 invasion? Molecular Ecology 12, 2941-2951.

776 Muraji, M., Nakahara, S., 2001. Phylogenetic relationships among fruit flies, *Bactrocera*
777 (Diptera, Tephritidae), based on the mitochondrial rDNA sequences. Insect
778 Molecular Biology 10, 549-559.
779 Nakahara, S., Ishida, T., Dohino, T., Mizuniwa, S., Kaneda, M., Muraji, M., 2005. Phylogenetic
780 relationships and discrimination among 12 *Bactrocera* species (Diptera: Tephritidae)
781 based on sequences of mitochondrial COII. Research Bulletin of the Plant Protection
782 Service Japan 41, 15-23.
783 Nakahara, S., Muraji, M., 2008. Phylogenetic analysis of *Bactrocera* fruit flies (Diptera:
784 Tephritidae) based on nucleotide sequences of the mitochondrial COI and COII
785 genes. Research Bulletin of Plant Protection Japan 44, 1-12.
786 Norrbom, A.L., 1994. New genera of Tephritidae (Diptera) from Brazil and Dominican amber,
787 with phylogenetic analysis of the tribe Ortalotrypetini. Insecta Mundi 8, 1-15.
788 Palumbi, S.R., 1996. Nucleic acids, II: the polymerase chain reaction. In: Hillis, D.M., Moritz,
789 C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer Associates, Sunderland,
790 Massachusetts, pp. 205-247.
791 Parham, J.F. and Irmis, R.B., 2008. Caveats on the use of fossil calibrations for molecular
792 dating: a comment on Near et al. The American Naturalist 171, 132-136.
793 Parham, J.F., Donoghue, P.C.J., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J.G.,
794 Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E., van
795 Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L.,
796 Makovicky, P.J., Müller, J., Smith, K.T., Theodor, J.M. & Warnock, R.C.M., 2011. Best
797 Practices for Justifying Fossil Calibrations. Systematic Biology
798 doi:10.1093/sysbio/syr107.
799 Poinar Jnr., G.O., 1992. Life in amber. Stanford University Press, Stanford.
800 Praveen Karanth, K., 2006. Out-of-India Gondwanan origin of some tropical Asian biota.
801 Current Science 90, 789-792.
802 Ree, R.H. & Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution
803 by dispersal, local extinction and cladogenesis. Systematic Biology 57, 4-14.
804 Renner, S.S., 2004. Multiple Miocene Melastomataceae dispersal between Madagascar,
805 Africa and India. Philosophical Transactions of the Royal Society of London (Series B)
806 359, 1485-1494.

807 Rogers, R.R., Krause, D.W., Curry-Rogers, K., Rasoamiaramanana, A.H., Rahantarisoa, L.,
808 2007. Paleoenvironment and paleoecology of *Majungasaurus crenatissimus*
809 (Theropoda: Abelisauridae) from the late Cretaceous of Madagascar. Journal of
810 Vertebrate Paleontology 27, 21-31.
811 Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under
812 mixed models. Bioinformatics 19, 1572-1574.
813 Rust, J., Singh, H., Rana, R.S., McCann, T., Singh, L., Anderson, K., Sarkar, N., Nascimbene,
814 P.C., Stebner, F., Thomas, J.C., Solorzano Kraemer, M., Williams, C.J., Engel, M.S.,
815 Sahni, A., Grimaldi, D.A., 2010. Biogeographic and evolutionary implications of a
816 diverse paleobiota in amber from the early Eocene of India. Proceedings of the
817 National Academy of Sciences, 107, 18360-18365.
818 Sanderson, M., 2003. r8s: inferring absolute rates of molecular evolution and divergence
819 times in the absence of a molecular clock. Bioinformatics 19, 301-302.
820 Schmidt, H.A., Strimmer, K., Vingron, M., von Haeseler, A., 2002. TREE-PUZZLE: maximum
821 likelihood phylogenetic analysis using quartets and parallel computing.
822 Bioinformatics 18, 502-504.
823 Segura, M.D., Callejas, C., Fernández, M.P., Ochando, M.D., 2006. New contributions
824 towards the understanding of the phylogenetic relationships among economically
825 important fruit flies (Diptera: Tephritidae). Bulletin of Entomological Research 96,
826 279-288.
827 Shearman, D.C.A., Frommer, M., Morrow, J. L., Raphael, K. A., Gilchrist, A. S., 2010.
828 Interspecific Hybridization as a Source of Novel Genetic Markers for the Sterile Insect
829 Technique in *Bactrocera tryoni* (Diptera: Tephritidae) Journal of Economic
830 Entomology 103, 1071-1079.
831 Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with
832 applications to phylogenetic inference. Molecular Biology and Evolution 16, 1114-
833 1116.
834 Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting,
835 and phylogenetic utility of mitochondrial gene sequences and a compilation of
836 conserved polymerase chain reaction primers. Annals of the Entomological Society of
837 America 87, 651-701.

838 Sota, T., Mogi, M., 2006. Origin of pitcher plant mosquitoes in *Aedes* (Stegomyia): a
839 molecular phylogenetic analysis using mitochondrial and nuclear gene sequences.
840 Journal of Medical Entomology 43, 795-800.
841 Smith-Caldas, M.R.B., McPheron, B.A., Silva, J.G., Zucchi, R.A., 2001. Phylogenetic
842 relationships among species of the fraterculus group (*Anastrepha*: Diptera:
843 Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase I.
844 Neotroprical Entomology 30, 565-573.
845 Smith, P.T., Kambhampati, S., Armstrong, K.A., 2003. Phylogenetic relationships among
846 *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA
847 sequences. Molecular Phylogenetics and Evolution 26, 8-17.
848 Smith, P.T., McPheron, B.A., Kambhampati, S., 2002. Phylogenetic analysis of mitochondrial
849 DNA supports the monophyly of Dacini fruit flies (Diptera: Tephritidae). Annals of the
850 Entomological Society of America 95, 658-664.
851 Spanos, L., Koutroumbas, G., Kotsyfakis, M., Louis, C., 2000. The mitochondrial genome of
852 the mediterranean fruit fly, *Ceratitis capitata*. Insect Molecular Biology 9, 139-144.
853 Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with
854 thousands of taxa and mixed models. Bioinformatics 22, 2688-2690.
855 Surveswaran, S., Wang, R.J., Su, Y.C.F., Saunders, R.M.K., 2010. Generic delimitation and
856 historical biogeography in the early-divergent 'ambavioid' lineage of Annonaceae:
857 Cananga, Cyathocalyx and Drepananthus. Taxon 59, 1721-1734.
858 Svenson, G.J., Whiting, M.F., 2009. Reconstructing the origins of praying mantises
859 (Dictyoptera, Mantodea): the roles of Gondwanan vicariance and morphological
860 convergence. Cladistics 25, 468-517.
861 Swofford, D.L., 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods),
862 Version 4. Sinauer Associates, Sunderland, MA, USA.
863 Taylor, D., Saksena, P., Sanderson, P.G., Kucera, K., 1999. Environmental change and rain
864 forests on the Sunda shelf of Southeast Asia: drought, fire and the biological cooling
865 of biodiversity hotspots. Biodiversity and Conservation 8, 1159-1177.
866 Van Houdt, J.K., Breman, F.C., Virgilio, M., De Meyer, M., 2010. Recovering full DNA
867 barcodes from natural history collections of Tephritid fruitflies (Tephritidae, Diptera)
868 using mini barcodes. Molecular Ecology Resources 10, 459-465.

869 Virgilio, M., De Meyer, M., White, I.M., Backeljau, T., 2009. African *Dacus* (Diptera:
870 Tephritidae): molecular data and host plant associations do not corroborate
871 morphology based classifications. Molecular Phylogenetics and Evolution 51, 531-
872 539.
873 Viseshakul, N., Charoennitikul, W., Kitamura, S., Kemp, A., Thong-Aree, S., Surapunpitak, Y.,
874 Poonswad, P. & Ponglikitmongkol, M. (2011) A phylogeny of frugivorous hornbills
875 linked to the evolution of Indian plants within Asian rainforests. *Journal of*
876 *Evolutionary Biology,* **24**, 1533-1545.
877 Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction
878 of DNA for PCR-based typing from forensic material. Biotechniques 10, 506-513.
879 White, I.M., 2000. Morphological features of the Tribe Dacini (Dacinae): Their signficance to
880 behavior and classification. In: Aluja, M., Norrbom, A.L. (Eds.), Fruit flies
881 (Tephritidae): Phylogeny and evolution of behavior. CRC Press, Boca Raton, pp. 505-
882 533.
883 White, I.M., 2006. Taxonomy of the Dacina (Diptera: Tephritidiae) of Africa and the Middle
884 East. African Entomology Memoir 2, 1-156.
885 White, I.M., Elson-Harris, M.M., 1992. Fruit flies of economic significance: Their
886 identification and bionomics. CAB International, Wallingford, UK.
887 Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.-W., Lambkin, C., Bertone,
888 M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J.,
889 Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N.,
890 Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T.,
891 Courtney, G.W., Freidrich, M., Meier, R., Yeates, D.K., 2011. Episodic radiations in the
892 fly tree of life. Proceedings of the National Academy of Sciences,
893 doi/10.1073/pnas.1012675108.
894 Winkler, I.S., Labandeira, C.C., Wappler, T., Wilf, P., 2010. Distinguishing Agromyzidae
895 (Diptera) leaf miners in the fossil record: new taxa from the paleogene of north
896 America and Germany and their evolutionary implications. Journal of Paleontology,
897 84, 935-954.
898 Winterton, S.L., Wiegmann, B.M., Schlinger, E.I., 2007. Phylogeny and Bayesian divergence
899 time estimations of small-headed flies (Diptera: Acroceridae) using multiple
900 molecular markers. Molecular Phylogenetics and Evolution 43, 808-832.

Yang, Z. and Rannala, B., 2006. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. Molecular Biology and Evolution 23, 212-226.

Yoder, A.D., Nowak, M.D., 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Annual Review of Ecology and Systematics 37, 405-431.

Zhang, B., Liu, Y.H., Wu, W.X., Wang, Z.L., 2010. Molecular phylogeny of *Bactrocera* species (Diptera: Tephritidae: Dacini) inferred from mitochondrial sequences of 16S rDNA and COI sequences. Florida Entomologist 93, 369-377.

Zhou, L., Su, Y.C.F., Thomas, D.C., Saunders, R.M.K., 2011. 'Out-of-Africa' dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). Journal of Biogeography, doi:10.111/j.1365-2699.2011.02598.x.

916 **Tables**

917 **Table 1.** Estimates of times to most recent common ancestor for nodes in the Dacini
918 phylogeny. Node labels refer to Figure 2.
919

Group/Clade	Node Label	Mean	95% Credibility Intervals	Effective Sample Size (ESS)
Brachycera	A	170.3	198.6-141.9	446.76
Schizophora	B	141.1	165.1-117.6	347.75
All Tephritids	C	110.9	131.4-91.2	298.38
All Dacinae	D	79.6	94.7-65.1	220.58
Dacus + Zeugodacus	E	72.2	86.3-59.3	206.6
Bactrocera excl. B. (Zeugodacus)	F	69	83.5-54.9	236.98
B. (Zeugodacus)	G	68.6	83.8-53.5	251.52
Dacus	H	65.8	78.5-53.5	206.47
Dacus clade A inc. D. (Callantra)	I	63.2	75.7-51.3	217.82
Bactrocera excl. B. (B. Tetradacus)	J	61.1	74.4-48.1	222.09
Austro-Pacific Dacus excl. D. (Callantra)	K	44.5	55.4-33.8	347.18
B. (Tetradacus)	L	21.4	30.9-12.4	287.55
B. (Daculus)	M	17.8	29-7.3	398.77
D. (Callantra)	N	16.4	27-7.8	697.69
B. (B. B.) dorsalis complex	O	6.2	8.4-4.3	391.45

920

921 **Supplementary Table 1.** Dacini species included in the current study. Geographical
922 distribution, male lure response and degree of polyphagy are given using the following
923 abbreviations: AUS = Australia, PAC = Pacific (inc. New Guinea), SEA = Southeast Asia, MA =
924 Mainland Asia (inc. Central Asia, Japan and Taiwan), SUB = Subcontinent (inc. Sri Lanka), AFR
925 = Africa, MED = Mediterranean; CL = Cue lure, W = Willison's lure, ME = Methyl eugenol, BA
926 = Benzyl acetate, VL = Vert-lure (Methyl p-hydroxybenzoate), U = Unknown, N = None; SS =
927 single species, G = multiple species within a genus, F = multiple genera within a family, P =
928 polyphagous (multiple families), U = unknown. References for sequences per locus per

929 species are given as GenBank Accession Numbers for existing data or author initials for new
930 data.

931

932 **Figure Legends**

933 **Figure 1.** Consensus Bayesian topology for the partitioned dataset of the Tribe Dacini. Node
934 values represent Bayesian posterior probabilities and ML bootstrap support: '-' denotes a
935 node unresolved by ML and '*' one that possesses posterior probabilities of 1.00 or
936 bootstrap support of 100. Branch lengths are in expected substitutions per site.
937 Geographical distribution is given for each species in the taxon label according to the
938 abbreviations in the legend.

939 **Figure 2.** Chronogram of Dacini divergence based on mean tmrca estimates from Table 1.
940 The scale bar is in units of millions of years. Lettered nodes are those for which tmrca was
941 estimated. A filled star denotes a node for which a prior calibration was used. Taxon labels
942 are removed for brevity and clade names follow those in Figure 1.

943 **Figure 3.** Schematic diagram of proposed model for dispersal biogeography of the Dacini.
944 Solid arrows show the movements of ancestral taxa and dashed arrows denote the
945 movement of continental blocks. (a) Dacini arise on the Indian plate after splitting from
946 Africa and major clades evolve ~80-65mya with ancestral *Dacus* migrating to Africa via
947 Madagascar ~65mya; (b) ancestor of *D*. (*Calllantra*) migrates back to India ~63mya; (c)
948 *Bactrocera*, *Zeugodacus* and *D*. (*Callantra*) migrate from India to Asia following docking ~57-
949 35mya; (d) a lineage of *Dacus* leaves north-east Africa for Asia ~45mya and ancestral *B.*
950 *(Daculus)* migrates into Africa from Asia ~18mya.

951 **Supplementary Figure 1.** Consensus Bayesian topology for the partitioned dataset of the
952 Tribe Dacini as per Figure 1. Here, male lure response is colour coded for each species
953 according to the abbreviations in the legend.

954 **Supplementary Figure 2.** Consensus Bayesian topology for the partitioned dataset of the
955 Tribe Dacini as per Figure 1. Here, degree of polyphagy is colour coded for each species
956 according to the abbreviations in the legend.