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Higher phylogeny of frugivorous flies (Diptera, Tephritidae, Dacini): Localised partition conflicts and a novel generic classification

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ABSTRACT

The phylogenetic relationships within and among subtribes of the fruit fly tribe Dacini (Ceratitidina, Dacina, Gastrozonina) were investigated by sequencing four mitochondrial and one nuclear gene fragment. Bayesian, maximum likelihood and maximum parsimony analyses were implemented on two datasets. The first, aiming at obtaining the strongest phylogenetic signal (yet, having lower taxon coverage), consisted of 98 vouchers and 2338 concatenated base pairs (bp). The second, aiming at obtaining the largest taxonomic coverage (yet, providing lower resolution), included 159 vouchers and 1200 concatenated bp. Phylogenetic relationships inferred by different tree reconstruction methods were largely congruent and showed a general agreement between concatenated tree topologies. Yet, local conflicts in phylogenetic signals evidenced a number of critical sectors in the phylogeny of Dacini fruit flies. All three Dacini subtribes were recovered as monophyletic. Yet, within the subtribe Ceratitidina only *Perilampsis* and *Capparimyia* formed well-resolved monophyletic groups while *Ceratitis* and *Trirhithrum* did not. *Carpophthoromyia* was paraphyletic because it included *Trirhithrum demeyeri* and *Ceratitis connexa*. Complex phylogenetic relationships and localised conflict in phylogenetic signals were observed within subtribe Dacina with (a) *Dacus*, (b) *Bactrocera* (*Zeugodacus*) and (c) all other *Bactrocera* species forming separate clades. The subgenus *Bactrocera* (*Zeugodacus*) is therefore raised to generic rank (*Zeugodacus* Hendel stat. nov.). Additionally, *Bactrocera* subgenera grouped under the *Zeugodacus* group should be considered under new generic combinations. Although there are indications that *Zeugodacus* and *Dacus* are sister groups, the exact relationship between *Zeugodacus* stat. nov., *Dacus* and *Bactrocera* still needs to be properly resolved.

1. Introduction

Fruit flies (Diptera: Tephritidae) are considered as one of the most important groups of agricultural pests (White and Elson-Harris, 1994). With more than 4600 species, classified in more than 500 genera, it is also one of the most speciose groups of Diptera (Norrbom et al., 1999; Pape et al., 2009). Representatives of the family have been the subject of fundamental research in different fields and served as a model for studies on, among others, speciation events (Clarke et al., 2005; Schwarz et al., 2005), invasion history and strategy (Bonizzoni et al., 2004; Duyck et al., 2007; Khamis et al., 2009) or mutual associations between organisms (Aluja and Mangan, 2008; Mazzon et al., 2010).

Largely phytophagous (some rare exceptions being found among Phytalmiini and Acanthonevrini), most pest species are found in groups whose larvae develop in fruits while others develop in, or are associated with, flowers of Asteraceae and other plant families. Interestingly, feeding strategies and host range are largely correlated with higher taxonomic classification (Han and McPheron, 1997), with fruit infesting tephritids, largely restricted to the trypetine tribes of Carpomyini (i.e. *Rhagoletis*), Dacini (i.e. *Bactrocera*, *Ceratitis*, *Dacus* and *Trirhithrum*) and Toxotrypanini (i.e. *Anastrepha* and *Toxotrypana*). Drew (2004) emphasized the fact that in the genus *Bactrocera*, there are close co-evolutionary relationships between host plants and fly speciation. Similar associations were also found for representatives of the African *Ceratitis* and *Dacus* species (De Meyer, 2005; Erbout et al., 2011;

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Virgilio et al., 2009) while other generic groups show particular host ranges (like *Perilampsis* on Loranthaceae, *Capparimyia* on Capparaceae, *Neoceratitis* on Solanaceae, predominantly of the genus *Lycium*) (De Meyer, 2009; De Meyer and Freidberg, 2005).

In the Old World tropics, the Dacini constitutes the economically most important lineage. In particular the genera *Bactrocera*, *Ceratitis* and *Dacus* include notorious pests, some of which have resulted in adventive populations of invasive alien species throughout the world. Despite their economic relevance, the higher classification and phylogenetic relationships within these groups are still debated. In the widely accepted classification presented by the world catalogue of Norrbom et al. (1999), the tribe Dacini comprises the subtribes Ceratitidina, Dacina and Gastrozonina. Yet, authors like Korneyev (1999) and Drew and Hancock (1999), elevate the Dacini to subfamily level, and the corresponding subtribes to tribal level. Korneyev (1999) provided a morphological framework for the phylogenetic relationships among higher groups of Tephritidae and concluded that the relationships among subfamilies and tribes have not yet been satisfactorily defined. Drew and Hancock (1999) divided the Asian and Pacific *Bactrocera* into four groups of subgenera: *Bactrocera* group, *Melanodacus* group, *Queenslandacus* group and *Zeugodacus* group. The taxonomic placement of both the *Zeugodacus* subgenus and group is particularly intriguing. White (2006) first suggested that the subgenus *Zeugodacus* might be in fact sister group to *Dacus* and Krosch et al. (2012) confirmed the occurrence of strong phylogenetic affinities between the whole *Zeugodacus* group of subgenera and the genus *Dacus*.

In recent years molecular phylogenetic studies (Han and McPheron, 1997; Han and Ro, 2009; Smith et al., 2002) have provided new insights but could not fully resolve the interrelationships within the family. Earlier studies were limited to either verifying the monophyly of the Dacini as a whole (Smith et al., 2002) or dealt with subgeneric relationships, especially with regard to *Bactrocera* (Jamnongluk et al., 2003; Muraji and Nakahara, 2001; Smith et al., 2003; Zhang et al., 2010). Most of these studies had a restricted taxon sampling with focus on the Asian fauna. Only the more recent work by Krosch et al. (2012) refers to the relationship between *Dacus* and *Bactrocera*, but does not take other dacine genera into account. Except for the relationships within the genus *Ceratitis* (see Barr and McPheron, 2006; Erbout et al., 2011) and African *Dacus* (see Virgilio et al., 2009) the monophyly, and phylogenetic position of African (sub-) genera is still largely unknown, particularly with respect to Ceratidina, whose representatives are predominantly found in Africa.

A better understanding of the suprageneric relationships within the tribe Dacini could provide a more stable framework for studies on host plant specificity, climatic thresholds, and attractiveness to lures. The objective of this work is to provide a more comprehensive phylogenetic analysis addressing points, at tribal, generic and subgeneric level, that were not considered in earlier studies or for which the information was inconclusive or limited in terms of taxonomic coverage. In particular, the following questions were put forward: (a) can the three dacine subtribes (i.e. Ceratitidina, Dacina, Gastrozonina) be recognized as monophyletic groups and what is their interrelationship? (b) what is the status of those genera currently taxonomically classified under Ceratitidina? and (c) what is the position of *Zeugodacus* versus other Dacina groups?

2. Material and methods

We sampled 157 vouchers belonging to 129 species and 10 genera, from (a) six of the 12 Ceratitidina genera, (*Capparimyia*, *Carpophthoromyia*, *Ceratitis*, *Neoceratitis*, *Perilampsis* and *Trirhithrum*), (b) two of the four Dacina genera, (*Bactrocera* and *Dacus*) and (c) two out of the 27 Gastrozonina genera (*Bistrispinaria* and *Clinotaenia*), with strong emphasis on African representatives (Table 1 and A.1). Two tephritines were also included as outgroups for tree reconstructions (see below).

DNA was extracted from both pinned and ethanol preserved specimens using the DNeasy Blood and Tissue Kit (Qiagen) and following the manufacturer's protocol. PCR products were purified by means of GFX purification columns (GE Healthcare), subjected to sequencing reactions using the Big-Dye cycle sequencing kit (Applied Biosystems) and finally sequenced in both directions with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Four mitochondrial gene fragments, COI, 16S, tRNApro, ND6, and part of the nuclear locus *period* were sequenced using primers and laboratory procedures described in Barr and McPheron (2006), and Virgilio et al. (2009). Nucleotide sequences were aligned using the muscle routine implemented by SeaView 4 (Gouy et al., 2010). Before analyses, coding regions were translated into amino acids to verify the possible presence of internal stop codons.

Two datasets were analysed. The first aimed at obtaining the strongest phylogenetic signal (longer concatenation but with lower taxon coverage), the second at obtaining the largest taxonomic coverage (but had a lower resolution, due to the shorter concatenated DNA fragment). The first (dataset 1) was composed by 98 concatenated COI + 16S + tRNApro + ND6 + *period* sequences, the second (dataset 2) 159 concatenated COI + 16S gene fragments (Table 1). To complement the predominantly African sampling of Dacina, dataset 2 also included sequences from forty-nine of the *Dacus* and *Bactrocera* vouchers from Krosch et al. (2012). These additional COI and 16S sequences (highlighted in grey in Table A1) allowed extending the taxon coverage of Asian *Dacus* and *Bactrocera* by including 46 of the 71 *Bactrocera* specimens and three of the 42 *Dacus* specimens considered in dataset 2. *Bistripinaria magniceps* (Gastrozonina) was used as a root for the tree reconstructions of dataset 1, while *Acanthiophilus helianthi* and *Dectodesis augur* (tribe Tephritini) were used as outgroups for dataset 2.

Phylogenetic relationships were mainly inferred through Bayesian tree reconstructions as implemented in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) on the Mobyle SNAP Workbench portal (Monacell and Carbone, 2014). Evolutionary models were selected for each gene fragment according to the Akaike information criterion of jModelTest 2 (Darriba et al., 2012). The general time reversible model (Tavaré, 1986) either with invariant positions and gamma distributed rates (GTR + I + G), or with gamma distributed rates (GTR + G), was used for the mitochondrial gene fragments (COI, 16S, tRNApro, ND6), whereas GTR + G was used for the nuclear partition (*period*). All MrBayes analyses employed a cold chain and three incrementally heated chains. Starting trees for each chain were random and the default values of MrBayes were chosen for all settings (including prior distributions). MrBayes metropolis coupled Markov Chains Monte Carlo (MCMC) were run for 15–40 million generations (until the average standard deviation of split frequencies fell below 0.01) with heating temperatures from 0.001 to 0.2 (Huelsenbeck and Ronquist, 2001). Trees were sampled every 1000 generations with 50% of trees discarded as burn-in. Only nodes with Bayesian posterior probabilities (PP) $\geqslant 0.95$ were considered as supported, all other not supported nodes as polytomies.

In order to evaluate if the molecular phylogeny obtained from the Bayesian approach was robust to different reconstruction methods, we also performed maximum likelihood (ML) and maximum parsimony (MP) tree reconstructions. ML analyses were performed in PhyML 3.0 (Guindon et al., 2010) using the GTR model of substitution (Tavaré, 1986), four substitution rate categories, gam-