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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-

Table 1
List of species included in the analyses (taxonomic classification following Norrbom et al. (1999)). Vouchers sequenced at all five markers (COI, 16S, tRNApro, ND6, *period*) were included in dataset 1. Species sequenced at the mitochondrial COI and 16S gene fragments were assigned to dataset 2 (see text for explanations). The *Bactrocera* groups of subgenera are indicated according to Drew and Hancock (1999).

Subtribe	Genus	Species	Dataset	Subtribe	Genus	Group of subgenera	Subgenus	Species	Dataset
Gastrozonina	*Bistrispinaria*	*magniceps*	1 & 2	Dacina	*Bactrocera*	*Bactrocera*	*Afrodacus*	*jarvisi*	2
	Clinotaenia	*superba*	2					*minuta*	2
Ceratitidina	*Capparimyia*	*aenigma*	1 & 2				*Bactrocera*	*albistrigata*	1 & 2
		melanaspis	1 & 2					*aquilonis*	2
	Carpophthoromyia	*dimidiata*	1 & 2					*arecae*	2
		dividua	1 & 2					*bancroftii*	2
	Ceratitis	*anonae*	1 & 2					*breviaculeus*	1 & 2
		bremii	1 & 2					*bryoniae*	2
		caetrata	1 & 2					*cacuminata*	2
		capitata	2					*carambolae*	2
		catoirii	1 & 2					*caryeae*	2
		colae	1 & 2					*cognata*	2
		connexa	1 & 2					*correcta*	2
		cosyra	1 & 2					*curvipennis*	2
		discussa	1 & 2					*dorsalis*	1 & 2
		fasciventris	1 & 2					*endiandrae*	2
		flexuosa	1 & 2					*facialis*	2
		lentigera	1 & 2					*frauenfeldi*	2
		marriotti	2					*invadens*	1 & 2
		millicentae	1 & 2					*kandiensis*	2
		pedestris	1 & 2					*kirki*	2
		podocarpi	2					*kraussi*	1 & 2
		punctata	2					*laticaudus*	2
		quinaria	1 & 2					*latifrons*	1 & 2
		rosa	1 & 2					*makilingensis*	2
		rubivora	1 & 2					*manskii*	2
		silvestrii	1 & 2					*mayi*	2
		striatella	1 & 2					*melanotus*	2
	Neoceratitis	*cyanescens*	1 & 2					*melas*	1 & 2
	Perilampsis	*curta*	1 & 2					*musae*	2
		woodi	1 & 2					*nigrotibialis*	2
	Trirhithrum	*coffeae*	1 & 2					*occipitalis*	1 & 2
		demeyeri	1 & 2					*opiliae*	2
		nigerrimum	1 & 2					*papayae*	1 & 2
		quadrimaculatum	1 & 2					*philippinensis*	1 & 2
		teres	1 & 2					*psidii*	2
Dacina	*Dacus*	*aequalis*	2					*quadrisetosa*	2
		apostata	1 & 2					*trilineola*	2
		arcuatus	1 & 2					*trivialis*	2
		armatus	1 & 2					*tryoni*	2
		axanus	2					*umbrosa*	1 & 2
		bellulus	2					*unirufa*	2
		bivittatus	1 & 2					*zonata*	1 & 2
		chiwira	1 & 2				*Daculus*	*munroi*	1 & 2
		ciliatus	1 & 2					*oleae*	1 & 2
		demmerezi	1 & 2				*Gymnodacus*	*amplexa*	1 & 2
		diastatus	1 & 2					*mesomelas*	1 & 2
		durbanensis	1 & 2				*Notodacus*	*xanthodes*	2
		eclipsis	1 & 2				*Tetradacus*	*minax*	2
		famona	1 & 2			*Zeugodacus*	*Austrodacus*	*cucumis*	2
		fuscovittatus	1 & 2				*Zeugodacus*	*calumniata*	1 & 2
		humeralis	1 & 2					*caudata*	1 & 2
		hyalobasis	1 & 2					*chorista*	2
		kariba	1 & 2					*cucurbitae*	1 & 2
		langi	1 & 2					*synnephes*	1 & 2
		longicornis	1 & 2					*tau*	1 & 2
		longistylus	1 & 2						
		lounsburyii	1 & 2						
		masaicus	1 & 2						
		mediovittatus	1 & 2						
		pergulariae	1 & 2						
		phloginus	2						
		punctatifrons	1 & 2						
		quilicii	1 & 2						
		siliqualactis	1 & 2						
		sphaeristicus	1 & 2						
		telfairеae	1 & 2						
		tenebricus	2						
		theophrastus	1 & 2						
		transitorius	1 & 2						
		triater	1 & 2						
		umehi	1 & 2			(*outgroup*)	*Acanthiophilus*	*helianthi*	2
		vertebratus	1 & 2			(*outgroup*)	*Dectodesis*	*augur*	2

ma shape parameters, proportions of invariable sites and transition/transversion ratio estimated from the datasets and 100 bootstrap replicates to evaluate branch support. MP consensus trees (strict and majority rule consensus for dataset 1 and 2, respectively) were calculated in PAUP* (Swofford, 2002) through 1000 random addition replicates with tree-bisection-reconnection (TBR) branch swapping and rearrangement limit of 1,000,000 branch swaps. Characters were considered as "unord" with equal weights and gaps as missing data.

General differences in the tree topologies provided by the data partitions of dataset 1 were evaluated in a Bayesian framework through the Bayes factors method (Kass and Raftery, 1995; Nylander, 2004). The harmonic means of marginal likelihoods of models with fully linked tree topology (B_0) and with unlinked topologies for each gene fragment (B_1) were calculated in MrBayes 3.1. The support provided to the alternative model B_1 was evaluated using $2\log_e(B_0/B_1)$. Following Kass and Raftery (1995) a value of $2\log_e(B_0/B_1) > 10$ would strongly support the alternative concatenation model, while $2\log_e(B_0/B_1) < 2$ would not suggest a better success of the alternative partition strategy in predicting the data. Localised differences in clade support provided by the data partitions were quantified in a MP framework by calculating partitioned Bremer support (PBS) in TreeRot 3 (Sorenson and Franzosa, 2007). PBS quantifies the particular areas of a phylogeny with localised conflict between data partitions. The PBS for a particular clade in a given data partition is the extent (measured in number of steps) to which a data partition supports the most parsimonious tree, including a clade, over a constrained tree not including the clade in question. Positive PBS indices suggest support for that node in the given partition while negative suggest conflict with that node (Lambkin, 2004).

3. Results

The concatenation of data produced an alignment of 2338 bp for dataset 1 and of 1200 bp for dataset 2. The COI, 16S, tRNApro, ND6 and *period* gene fragments contributed for 658, 542, 75, 524 and 539 bp, respectively. Bayes factors (Kass and Raftery, 1995) do not suggest the occurrence of severe conflict in the phylogenetic signals provided by data partitions. In fact, the marginal likelihoods of different concatenation models were largely comparable (Table 2) with $2\log_e(B_0/B_1)$ always well below the threshold that would support alternative concatenation models (Brown and Lemmon, 2007; Kass and Raftery, 1995). This was considered enough to justify the full concatenation of data partitions and thus the total evidence approach adopted in this study. The Bayesian, ML and MP analyses of dataset 1, (Figs. 1, A2 and A3) yield two monophyletic clades that correspond to subtribes Ceratitidina (genera *Capparimyia*, *Carpophthoromyia*, *Ceratitis*, *Neoceratitis*, *Perilampsis* and *Trirhithrum*) and Dacina (*Dacus* and *Bactrocera*). Within Ceratitidina, the genera *Perilampsis* and *Capparimyia* are monophyletic in all tree reconstructions (Fig. 1), while *Trirhithrum* is monophyletic except for *T. demeyeri* which is placed in a clade including the two representatives of the genus *Carpophthoromyia* (*C. dimidiata* and *C. dividua*) and *Ceratitis connexa*. The other *Ceratitis* species do not form a monophyletic group, while the Bayesian and ML reconstructions place the only representative of the genus *Neoceratitis* (*N. cyanescens*) in a polytomy with *Perilampsis*, *Trirhithrum* (except *T. demeyeri*) and *Ceratitis* (except *C. connexa*) (Fig. 1, PP = 0.72). Dataset 1 divides the Dacina clade in three main groups corresponding to (a) the genus *Dacus*, (b) *B.* (*Zeugodacus*) and (c) all the other *Bactrocera* (Fig. 1). The relative position of these groups is hard to define. The Bayesian and ML trees reconstructions place the *Dacus*, *Zeugodacus* and *Bactrocera* clades in polytomy (due to the lack of support of the node including *Dacus* and *Bactrocera*), while the MP strict consensus tree places *Zeugodacus* as sister group of *Dacus* (Fig. A3).

The analysis of PBS (Figs. 1 and A3) shows that, within Dacina, the different data partitions of dataset 1 provide comparable support to the clades including (a) *Dacus*, (b) *Bactrocera* (*Zeugodacus*) and (c) the *Bactrocera* group of subgenera (Fig. 1). Similarly, most of the Ceratitidina internal nodes are comparably supported by the data partitions (e.g. see nodes corresponding to genera *Capparimyia* and *Perilampsis*). Yet, local conflict can be observed for the Ceratitidina clade that is mainly supported by the *period* (PBS = 31.0) and COI (PBS = 2.5) partitions but not by ND6 (PBS = −6.0) and 16S (PBS = −6.5). Similarly, localised conflict occurs for the main *Trirhithrum* clade (not supported by COI and ND6) as well as for the node including the genus *Carpophthoromyia* and the two *Ceratitis* and *Trirhithrum* outliers (not supported by COI, 16S and ND6). The analysis of the PBS decay indices also show that the placement of *Zeugodacus* as sister group of *Dacus* in the MP strict consensus tree (Fig. A2) is mainly determined by the mitochondrial partitions COI (PBS = 3.0), 16S (PBS = 3.2) and ND6 (PBS = 1.1), while the nuclear partition *period* (PBS = −4.4) provides a conflicting signal in this respect.

Dataset 2 (Fig. 2) shows the monophyly of subtribe Gastrozonina (here including two representatives of the subtribe that are recovered in a supported clade) and provides more extended taxon coverage (particularly for *Dacus* and *Bactrocera* that here include the COI and 16S sequences published in Krosch et al. (2012). Within Ceratitidina, results are largely consistent with those obtained for dataset 1, with *Perilampsis* and *Capparimyia* as the only monophyletic genera. Yet in the Bayesian analysis of dataset 2, *C. connexa* is sister species of *Capparimyia* while *Carpophthoromyia dimidiata* and *T. demeyeri* form a separate clade (Fig. 2). Within Dacina, the Bayesian tree reconstruction recovers the genus *Dacus* as monophyletic while *Bactrocera* is represented by (a) a main clade including all species from subgenus *Bactrocera* and the two *Afrodacus* representatives (*B. jarvisi*, *B. minuta*), (b) a

Table 2
Bayes factors evaluating the relative success of different partition strategies. A value of $2\log_e(B_0/B_1) < 2$ does not suggest a better success of the alternative model B_1 in predicting the data (Kass and Raftery, 1995).

Tree topology		Harmonic mean of marginal likelihoods	$2\log_e(B_0/B_1)$
B0:	Fully linked		
	COI + 16S + tRNApro + ND6 + *period*	−39121.77	
Alternative model			
B1:	COI unlinked	−39003.04	0.006
B1:	16S unlinked	−38880.28	0.012
B1:	tRNApro unlinked	−39095.79	0.001
B1:	ND6 unlinked	−38929.54	0.010
B1:	*period* unlinked	−38763.67	0.018
B1:	Fully unlinked	−38350.34	0.040

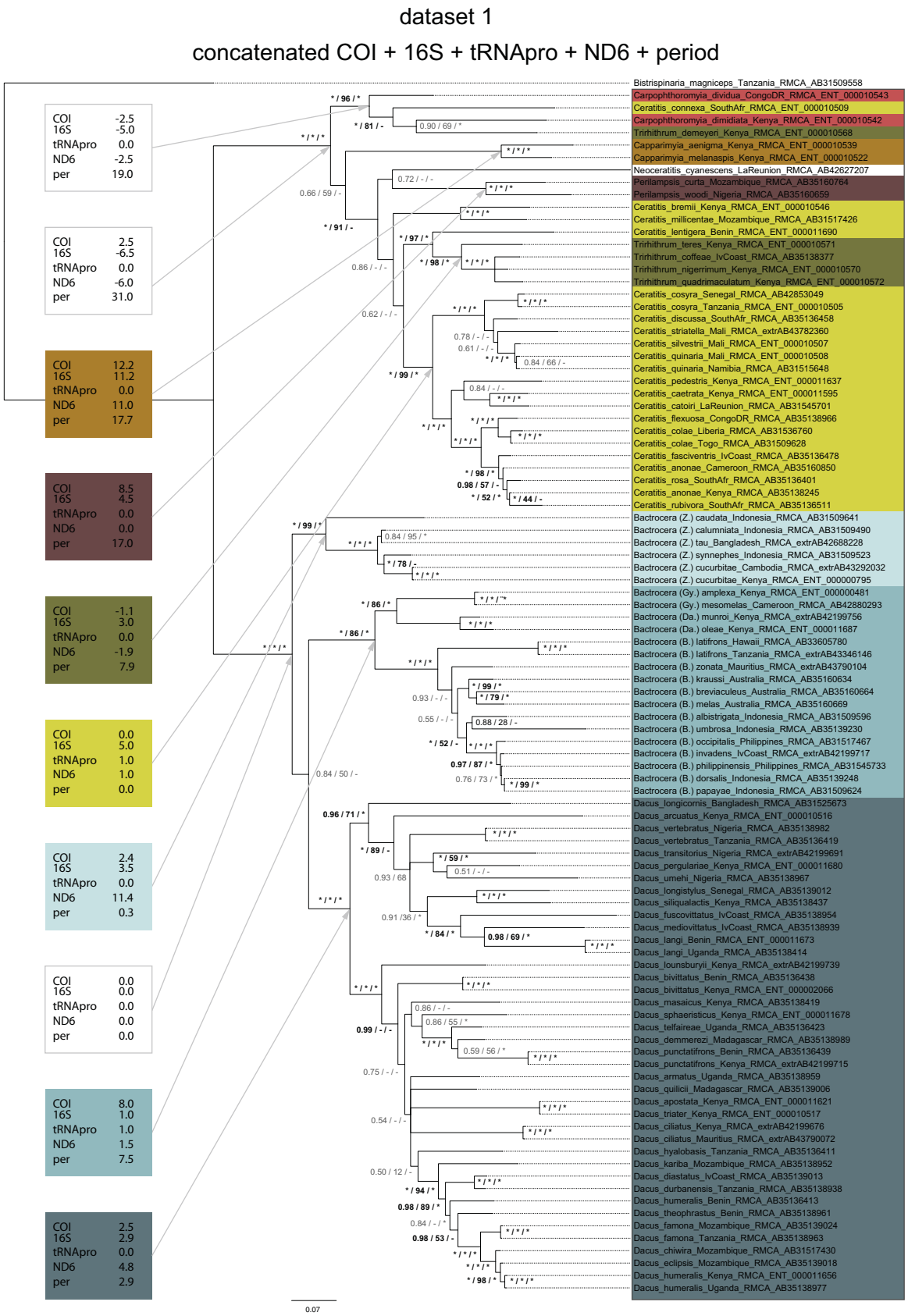

Fig. 1. Bayesian tree obtained from the analysis of 98 concatenated COI + 16S + tRNApro + ND6 + *period* sequences (2338 bp, dataset 1). For each node (separated by slashes): Bayesian PP (∗ = 1.00), ML bootstrap support (∗ = 100) and node presence (∗) or absence (–) in the MP strict consensus tree. Nodes with Bayesian PP ⩾ 0.95 are in bold. On the left: PBS indices of the most relevant clades (all PBS values in Fig. A3). Ceratitidina genera are highlighted in different colours. Within Dacina, (a) the genus *Dacus*, (b) *B.* (*Zeugodacus*) (Z.) and (c) the *Bactrocera* group of subgenera (B.: *B.* (*Bactrocera*), Da: *B.* (*Daculus*), Gy: *B.* (*Gymnodacus*)) are separately evidenced.

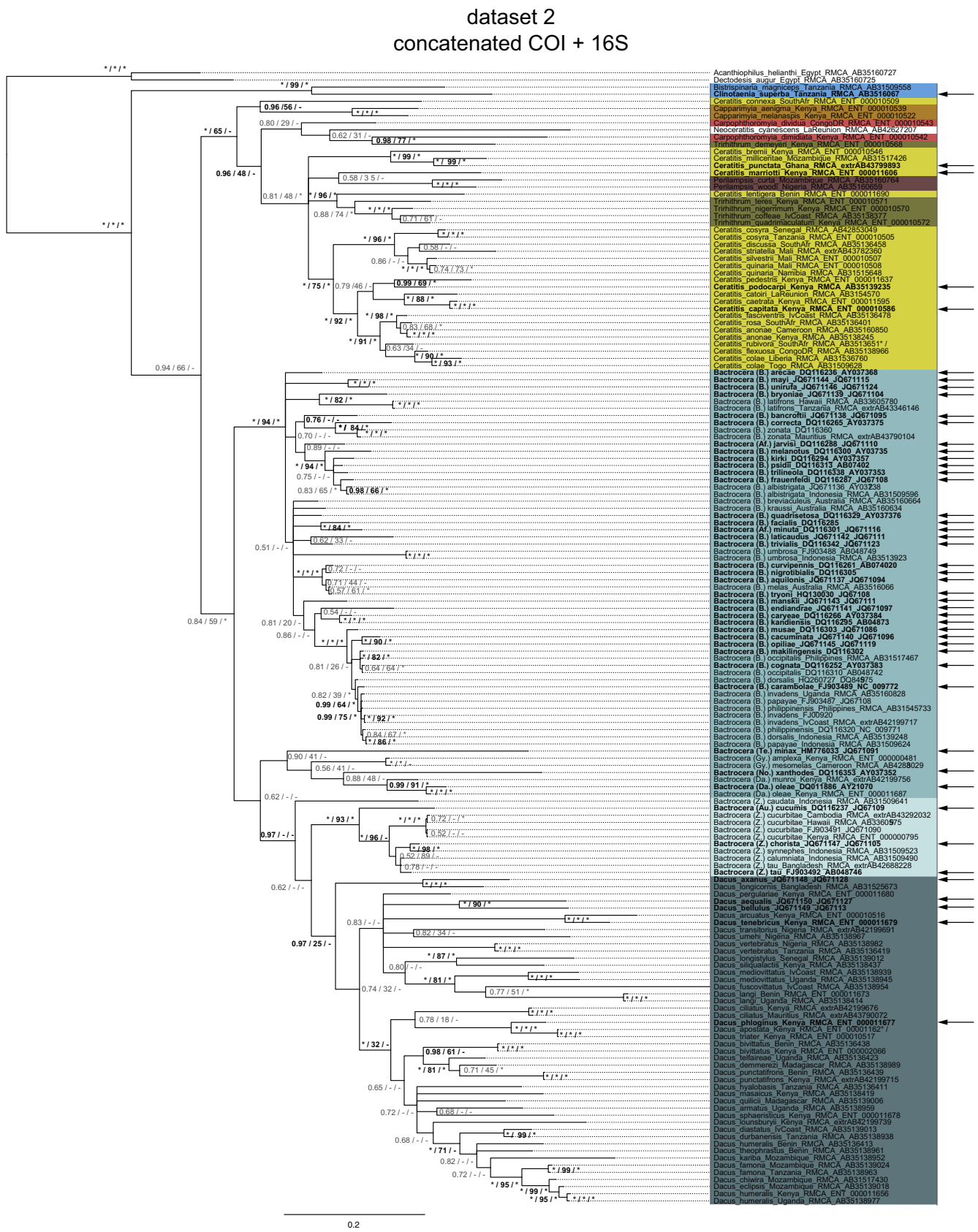

Fig. 2. Bayesian tree obtained from the analysis of 156 concatenated COI + 16S sequences (1200 bp, dataset 2). For each node (separated by slashes): Bayesian PP (∗ = 1.00), ML bootstrap support (∗ = 100) and node presence (∗) or absence (–) in the MP majority rule consensus tree. Nodes with Bayesian PP ⩾ 0.95 are in bold. Subtribe Gastrozonina and Ceratitidina genera are highlighted in different colours. Within Dacina, (a) the genus *Bactrocera*, (b) the *Zeugodacus* group of subgenera (Au: *B.* (*Austrodacus*), Z.: *B.* (*Zeugodacus*)) and (c) the *Bactrocera* group of subgenera (Af: B. (*Afrodacus*), B.: *B.* (*Bactrocera*), Da: *B.* (*Daculus*), Gy: *B.* (*Gymnodacus*), No: *B.* (*Notodacus*), Te: *B.* (*Tetradacus*)) are separately evidenced. Arrows indicate additional taxa compared to dataset 1 (mostly from Krosch et al., 2012).

not supported (hence paraphyletic) group including subgenera *Daculus* (*B. munroi*, *B. oleae*), *Gymnodacus* (*B. amplexa*, *B. mesomelas*), *Notodacus* (*B. xanthoides*), *Tetradacus* (*B. minax*) and (c) a clade corresponding to the *Zeugodacus* group of subgenera (here including *B.* (*Zeugodacus*) and *B.* (*Austrodacus*), see Table 1). Yet, analyses of dataset 2 did not recover *B. caudata* (type species of subgenus *Zeugodacus*) with the other representatives of the subgenus but either as a sister species of both the *Zeugodacus* group of subgenera and *Dacus* (Fig. 2) or in polytomy with *Dacus* and *Bactrocera* (Figs. A4 and A5).

4. Discussion

4.1. Subtribal classification and generic placement within subtribes

Phylogenetic relationships inferred from Bayesian, ML and MP analyses 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. The results of this study confirms the current subtribal subdivision of Dacini into three monophyletic groups, i.e. Ceratitidina, Dacina (as resulting from the analysis of dataset 1) and Gastrozonina (from dataset 2). Monophyly of the three clades was earlier suggested by Smith et al. (2002) but not by other studies (see Korneyev (1999) or Han and Ro (2009)). This study provides a more extensive coverage of Ceratitidina genera, with at least two species from five genera (*Ceratitis*, *Trirhithrum*, *Capparimyia*, *Carpophthoromyia*, *Perilampsis*), and one *Neoceratitis* representative. Similarly, the taxonomic coverage of Dacina was extended to include a combined set of both African and Asian *Dacus* and *Bactrocera*. The only other Dacina genera not covered in this study are *Ichneumonopsis* and *Monacrostichus*, very rare groups including only one and two species, respectively, and considered to be plesiomorphic sister groups of all other Dacina (see Drew and Hancock, 1999).

The monophyly of Gastrozonina could only be verified through a limited number of taxa (*B. magniceps*, *C. superba*) and could only be evaluated in dataset 2. The known biology of Gastrozonina is different from that of the other subtribes, in that Gastrozonina are known to feed on dead or living shoots of Poaceae, (especially bamboo species in Asia, see Dohm et al., 2014) while Ceratitidina and Dacina are known to be mainly fruit feeders (Aluja and Norrbom, 1999). A larger taxon sampling is thus needed to provide a more robust validation of Gastrozonina monophyly as well as more precise indications about its possible basal position compared to Ceratitidina and Dacina (Fig. 2).

Regardless the additional data provided here, the phylogenetic relationships within subtribes still remain uncertain. Only the small genera *Capparimyia*, and *Perilampsis* within Ceratitidina and the genus *Dacus* within Dacina were monophyletic, while all other genera considered (*Bactrocera*, *Ceratitis*, *Trirhithrum*, *Carpophthoromyia*) could still not be unambiguously resolved. Barr and McPheron (2006) investigated phylogenetic relationships within Ceratitidina and showed that *Ceratitis*, *Trirhithrum* and *Carpophthoromyia* were not monophyletic. In their study they also suggested that *Carpophthoromyia*, *Capparimyia*, and *Neoceratitis* might be sister taxa to *Ceratitis* (but these data were only partially presented in their paper). Our study does not provide support to this hypothesis as the *Ceratitis* group is split in a main clade including most species and other clades including other *Ceratitis*. While polytomies including species from different genera might in part be determined by low phylogenetic signal from the concatenated data, we should also consider re-evaluating the taxonomic position of a number of Ceratitidina species such as *T. demeyeri* (see also Barr and McPheron, 2006) or *C. connexa*, as they are consistently recovered in properly supported groups including other non-*Ceratitis* species.

Similarly, within Dacina, the phylogeny of *Bactrocera* shows incongruences with the currently accepted classification. Based upon morphological characters, Drew and Hancock (1999) divided the Asian and Pacific subgenera of *Bactrocera* into four groups, viz. the *Bactrocera*, *Melanodacus*, *Queenslandacus* and *Zeugodacus* group of subgenera. Recently, molecular analysis suggested that several representatives of different subgenera within the *Zeugodacus* group (i.e. subgenera *Austrodacus*, *Hemigymnodacus*, *Papuodacus*, *Paradacus*, *Paratridacus*, *Sinodacus*, and *Zeugodacus*), might be monophyletic and separate from representatives of the *Bactrocera* group (like *Afrodacus*, *Apodacus*, *Bactrocera*, *Gymnodacus*, *Javadacus*, *Daculus*, *Notodacus*, and *Tetradacus*) (Asokan et al., 2011; Krosch et al., 2012; Muraji and Nakahara, 2001; Smith et al., 2003). Feeding in Cucurbitaceae is strongly developed in the *Zeugodacus* group of subgenera (Drew and Hancock, 1999). Feeding preferences seem to be a main evolutionary factor in the closely related genus *Dacus*, whose representatives are oligophagous and form clades within the genus that include either Apocynaceae, Curcubitaceae, or Passifloraceae feeders (Virgilio et al., 2009). Krosch et al. (2012) have shown that the *Zeugodacus* group of subgenera is separated from the *Bactrocera* subgenera and phylogenetically closer to *Dacus*. In their study the *Zeugodacus* group of subgenera is divided between a sister clade to *Dacus*, including *B.* (*Zeugodacus*) *macrovittata* and *B.* (*Papuadacus*) *neopallescentis* (PP = 0.97), and a non-monophyletic group (PP = 0.82) with 18 other species from the *Zeugodacus* group of subgenera (with representatives of *B.* (*Austrodacus*), *B.* (*Paratridacus*), *B.* (*Paradacus*), *B.* (*Sinodacus*), and *B.* (*Zeugodacus*)). The Bayesian, ML and MP analyses of the longer concatenated DNA fragment (dataset 1) recovered *B.* (*Zeugodacus*) as a monophyletic group supported by both the mitochondrial (COI, 16S, tRNApro, ND6) and nuclear (*period*) partitions. Yet, even if there are indications that *B.* (*Zeugodacus*) is phylogenetically closer to *Dacus* than to the other *Bactrocera* subgenera (as suggested by the MP analysis of dataset 1 and by Krosch et al., 2012), the phylogenetic relationships between *Dacus*, *B.* (*Zeugodacus*) and the other *Bactrocera* subgenera still remain to be resolved. The fact that only the mitochondrial partitions of dataset 1 (and not the nuclear gene fragment) support *B.* (*Zeugodacus*) as a sister group of *Dacus* suggests a relatively recent evolutionary history for the differentiation between these groups.

4.2. Proposed revision of Bactrocera

The results of this work as well as of Krosch et al. (2012), Drew and Hancock (1999) and White (2006) reveal molecular, morphological and ecological differentiation between *Bactrocera* (*Zeugodacus*) and the other *Bactrocera* subgenera. The paraphyletic status of *B.* (*Zeugodacus*) *caudata*, resulting from the analysis of dataset 2 in this study, seems more related to the poorer phylogenetic signal provided by this dataset than to effective taxonomical issues (in fact, all *Zeugodacus* taxa are recovered in a supported monophyletic group when all molecular markers are included in the analysis of dataset 1). Additionally, *B.* (*Zeugodacus*) *caudata* unambiguously shows all morphological features of the higher taxon further supporting its subgeneric placement. Hence, we consider that the synopsis of the currently available data provides sufficient evidence to elevate to generic rank, *Zeugodacus* Hendel stat. nov. (as already suggested by Krosch et al., 2012), with type species *Dacus caudatus* Fabricius, 1805 as originally designated by Hendel (1927) for the subgenus.

Similarly, morphological differentiation can be observed between the *Bactrocera* and *Zeugodacus* groups of subgenera, as first evidenced by Drew and Hancock (1999) with respect to the structure of male sternite V and surstylus. White (2006) also evidenced that notopleural xanthine and medial postsutural vitta

support the grouping of the *Zeugodacus* group with *Dacus*. White (2006) also proposed a biological argument, i.e. the common use of Cucurbitaceae as host for species groups within *Zeugodacus* and *Dacus*. We, therefore, support the view of White (2006) with regard to morphological evidence for common ancestry of the *Zeugodacus* group and *Dacus* and thus the split of *Bactrocera* versus *Zeugodacus*. We also support the view of Krosch et al. (2012) with regard to molecular evidence for the latter split. We conclude that morphological, ecological and molecular evidence is sufficient to elevate the *Zeugodacus* group of subgenera to be placed in a separate genus, as it would more clearly reflect the interrelationships between *Bactrocera*, *Zeugodacus* and *Dacus*. Hence, the *Bactrocera* subgenera grouped under the *Zeugodacus* group (as well as all species currently comprised under these subgenera, see Norrbom et al. (1999) and Drew and Romig (2013) for current listing), should be presented under new generic combinations, i.e. *Zeugodacus* (*Asiadacus*) comb. nov., *Zeugodacus* (*Austrodacus*) comb. nov., *Zeugodacus* (*Diplodacus*) comb. nov., *Zeugodacus* (*Heminotodacus*) comb. nov., *Zeugodacus* (*Hemiparatridacus*) comb. nov., *Zeugodacus* (*Nesodacus*) comb. nov., *Zeugodacus* (*Niuginidacus*) comb. nov., *Zeugodacus* (*Papuodacus*) comb. nov., *Zeugodacus* (*Paradacus*) comb. nov., *Zeugodacus* (*Paratridacus*) comb. nov., *Zeugodacus* (*Parazeugodacus*) comb. nov., and *Zeugodacus* (*Sinodacus*) comb. nov. We also consider that the subgenus *Javadacus* should be excluded from inclusion in *Zeugodacus* since the only species included in the analysis by Krosch et al. (2012), i.e. *Bactrocera* (*Javadacus*) *unirufa*, is placed within the *Bactrocera* group. Exact placement requires further analysis of other species within this group. *Bactrocera* (*Paratridacus*) *expandens* is placed within the *Bactrocera* group (Krosch et al., 2012; Muraji and Nakahara, 2001). However, *B.* (*Paratridacus*) *decipiens* and *B.* (*Paratridacus*) *diversa* (considered to belong to *Hemigymnodacus* according to Drew and Romig (2013)) were placed in the *Zeugodacus* group. It is also suggested that for the time being all other subgeneric groups, i.e. the *Bactrocera*, *Melanodacus*, and *Queenslandacus* groups, remain placed under *Bactrocera*. No representatives of the latter group were included either in any of the recent studies or in our own analysis. African representatives of *Gymnodacus*, are considered different from *Gymnodacus* from the Oriental region and probably belong to the *Melanodacus* group, together with *Daculus* according to Drew and Hancock (1999). Our fully concatenated analysis (dataset 1) groups the African *Daculus* and *Gymnodacus* studied here in a strongly supported clade, placed as a sister group of *Bactrocera*. Representatives analyzed of *Afrodacus*, *Apodacus*, Oriental '*Gymnodacus*', *Notodacus*, *Paratridacus* and *Tetradacus* all group together with representatives of *Bactrocera* s.s. However, a more extensive sampling of representatives of all subgenera is required to further resolve the (sub)-generic classification of the taxa within *Bactrocera* s.l.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.01.007.

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