One and the same: integrative taxonomic evidence that *Bactrocera invadens* (Diptera: Tephritidae) is the same species as the Oriental fruit fly *Bactrocera dorsalis*

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**Abstract.** The invasive fruit fly *Bactrocera invadens* Drew, Tsuruta & White, and the Oriental fruit fly *Bactrocera dorsalis* (Hendel) are highly destructive horticultural pests of global significance. *Bactrocera invadens* originates from the Indian subcontinent and has recently invaded all of sub-Saharan Africa, while *B. dorsalis* principally occurs from the Indian subcontinent towards southern China and South-east Asia. High morphological and genetic similarity has cast doubt over whether *B. invadens* is a distinct species from *B. dorsalis*. Addressing this issue within an integrative taxonomic framework, we sampled from across the geographic distribution of both taxa and: (i) analysed morphological variation, including those characters considered diagnostic (scutum colour, length of aedeagus, width of postsutural lateral vittae, wing size, and wing shape); (ii) sequenced four loci (ITS1, ITS2, cox1 and nad4) for phylogenetic inference; and (iii) generated a cox1 haplotype network to examine population structure. Molecular analyses included the closely related species, *Bactrocera kandiensis* Drew & Hancock. Scutum colour varies from red-brown to fully black for individuals from Africa and the Indian subcontinent. All individuals east of the Indian subcontinent are black except for a few red-brown individuals from China. The postsutural lateral vittae width of *B. invadens* is narrower than *B. dorsalis* from eastern Asia, but the variation is clinal, with subcontinent *B. dorsalis* populations intermediate in size. Aedeagus length, wing shape and wing size cannot discriminate between the two taxa. Phylogenetic analyses failed to resolve *B. invadens* from *B. dorsalis*, but did resolve *B. kandiensis*. *Bactrocera dorsalis* and *B. invadens* shared cox1 haplotypes, yet the haplotype network pattern does not reflect current taxonomy or patterns in thoracic colour. Some individuals of *B. dorsalis*/*B. invadens* possessed haplotypes more closely related to *B. kandiensis* than to conspecifics, suggestive of mitochondrial introgression between these species. The combined evidence fails to support the delimitation of *B. dorsalis* and *B. invadens* as separate biological species. Consequently, existing biological data for *B. dorsalis* may be applied to the invasive population in Africa. Our recommendation, in line with other recent publications, is that *B. invadens* be synonymized with *B. dorsalis*.

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Introduction

Fruit flies of the subfamily Dacinae (Diptera: Tephritidae) include some of the world’s most important horticultural pests (White & Elson-Harris, 1992). Within Dacinae, species of the genus Bactrocera Macquart (Drew & Hancock, 2000) have diversified prolifically in the South-east Asian and Pacific regions over the last 40 Ma (Drew & Hancock, 2000; Krosch et al., 2012). To differentiate diversity in this species-rich genus, it has been divided taxonomically into 22 subgenera and over 20 species complexes (informal species groups within subgenera) (Drew, 1989). The best known is the Oriental fruit fly, Bactrocera (Bactrocera) dorsalis (Hendel) complex, because it includes the most widely distributed and damaging pest species in the genus (Drew, 1989; Clarke et al., 2005).

The B. dorsalis species complex (hereafter the ‘dorsalis complex’) contains over 100 taxa that share a defined set of morphological characters, principally a mostly black scutum and abdominal terga III–V with a medial longitudinal dark band and variable dark patterns on the lateral margins (Drew & Hancock, 1994; Drew & Romig, 2013). While most members of the complex are readily identifiable and of little to no economic importance, the recently described B. invadens Drew, Tsuruta & White is morphologically very similar to B. dorsalis, and with similar economic pest status. This species was first detected in Africa in 2003 and has since become a destructive and highly invasive member of the complex, attacking over 40 fruit species and recorded from more than 30 African countries (Lux et al., 2003; Goergen et al., 2011; Khamis et al., 2012).

When first reported in Kenya, B. invadens was considered an ‘unusually variable’ invasive population of B. dorsalis (Lux et al., 2003: 358). These flies were initially identified as B. dorsalis because they were collected in methyl eugenol baited traps (few other African Bactrocera are known to respond to methyl eugenol) and they possessed morphological characters consistent with B. dorsalis (Lux et al., 2003). The African fly was considered a new species and named B. invadens following examination of specimens of the same B. dorsalis-like species from Sri Lanka, the purported native range (Drew et al., 2005, 2007). According to the formal taxonomic description by Drew et al. (2005) and a recent major revision of tropical fruit flies by Drew & Romig (2013), B. invadens is distinguished from B. dorsalis by: (i) a mostly dark orange-brown scutum with a dark fuscous to black lanceolate pattern; (ii) a longer aedeagus; (iii) a scutum with narrower postsutural vittae; (iv) a dark transverse band on the abdominal tergite III which broadly reaches tergite IV; and (v) a dark anterolateral marking on abdominal tergite V extended mesally. The abdominal characters are not referred to in Drew & Romig (2013), with scutum colour, aedeagus length, and postsutural lateral vittae the only diagnostic features provided. These morphological characters are, however, sufficiently variable to render some individuals of B. invadens virtually inseparable from B. dorsalis (Drew et al., 2005). The question therefore remains: how reliable are diagnostic characters of B. invadens in distinguishing it from B. dorsalis? And, if not, is B. invadens a distinct species?

Due to its economic impact, most studies on B. invadens have an applied focus on host use, seasonality and invasion dynamics (Mwatawala et al., 2006; Ekses et al., 2007; Rwomushana et al., 2008; Khamis et al., 2009; De Meyer et al., 2010), temporal occurrence and comparative demographic parameters (Vayssières et al., 2005; Salum et al., 2014), interactions with other fruit fly species and their parasitoids (Mohamed et al., 2008; Ekses et al., 2009; Rwomushana et al., 2009; Van Mele et al., 2009), and the development of market access protocols (Grout et al., 2011; Hallman et al., 2011). These considerable research efforts are based on the assumption that B. invadens is a biologically distinct species from B. dorsalis, a fundamental issue which is receiving increased attention. If B. invadens and B. dorsalis are the same species, the considerable existing regulatory arrangements and literature on B. dorsalis may be applied to the invasive population in Africa.

Of those studies investigating the biological relationship between B. invadens and B. dorsalis, results show that: (i) aedeagi of B. invadens from Sri Lanka are significantly longer than those of B. dorsalis from Taiwan (Drew et al., 2008); (ii) male pheromone constituents following methyl eugenol feeding between B. dorsalis and B. invadens are identical (Tan et al., 2011); (iii) there are extremely low wing-morphometric differences between these two species, together with the lowest estimate of evolutionary divergence between B. dorsalis and B. invadens following mitochondrial DNA analysis among multiple taxa (including Bactrocera kandiensis Drew & Hancock, another dorsalis-complex species) (Khamis et al., 2012); (iv) molecular analyses across a range of tephritid taxa have found no significant genetic differentiation between B. invadens and B. dorsalis (Frey et al., 2013; Leblanc et al., 2013; San Jose et al., 2013); and (v) B. invadens and B. dorsalis are fully sexually compatible as demonstrated by random mating and viable offspring to the second hybrid generation (Bo et al., 2014). Despite mounting evidence supporting their conspecificity, a recent major revision of South-east Asian fruit flies maintains B. invadens as a valid species which is no longer considered a member of the dorsalis complex (Drew & Romig, 2013).

Given that morphological characters based on a limited amount of material collected from Africa (Kenya, Benin, Cameroon and Uganda) and Sri Lanka (Asia) were the only features used to originally separate B. invadens from B. dorsalis (Drew et al., 2005), we re-examined purportedly diagnostic characters of both taxa from across a much wider geographic range to determine if variation was indeed discontinuous and supportive of two biologically distinct species or, in fact, continuous and supportive of a single morphologically variable species. We therefore focused on the characters previously used to differentiate the two putative species most recently by Drew & Romig (2013) (i.e. scutum colour, width of postsutural lateral vittae and aedeagus length). Additionally, we applied geometric morphometric wing shape analysis due to its demonstrated capacity in resolving fine-scale variation between real and putative cryptic insect taxa, as well as intraspecific population structure (Aytekin et al., 2007; Schutze et al., 2012b; Krosch et al., 2013).
We generated genetic datasets in addition to morphological and morphometric analyses, because morphologically identical populations may consist of multiple cryptic biological species (Bickford et al., 2007). Using two nuclear and two mitochondrial loci, which have proven discriminatory power for cryptic taxa within the dorsalis complex (Boykin et al., 2014), we carried out Bayesian and maximum likelihood phylogenetic analyses to test whether samples of B. invadens and B. dorsalis form distinct and well supported clades as predicted for two species, or whether individuals of B. invadens emerge unresolved within a larger B. dorsalis clade as predicted for one species. Moreover, we used one mitochondrial DNA locus (coxl) to construct a minimum spanning haplotype network; this form of analysis is well suited to inferring intraspecific relationships (Bandelt et al., 1999). Wherever possible, we used the same individuals as for morphological analysis to strengthen conclusions drawn within an integrative taxonomic framework (Schlick-Steiner et al., 2010; Yeates et al., 2011).

Samples of Bactrocera papayae Drew & Hancock from our previous work on the dorsalis complex from the Indo/Malay Archipelago (Schutze et al., 2012b; Krosch et al., 2013) were included in analyses due to considerable evidence that B. papayae is the same biological species as B. dorsalis (Perkins et al., 1990; Medina et al., 1998; Tan, 2003; Mahmood, 2004); hence inclusion of this material in a geographically wide-ranging study involving B. dorsalis is justified. We interpret our results in the context of the unified species concept (sensu de Queiroz, 2007), for which no single species character (e.g. mate compatibility, genetic divergence or morphological difference) is relied upon for their delimitation; rather, multiple lines of data are independently analysed to evaluate evidence for, or against, separately evolving metapopulation lineages. We discuss our findings within the context of the taxonomic history of B. dorsalis, particularly the relationship between these taxa and Dacus ferrugineus Fabricius, a species described in the late 18th century and a junior synonym of B. dorsalis.

### Materials and methods

#### Specimens

Twenty individuals from each of 13 locations were examined for morphological variation (n = 260). Of these, 200 were newly acquired for this study (Table 1) and combined with 60 specimens from Thailand, Taiwan, and Malaysia that were part of previous studies (Schutze et al., 2012b; Krosch et al., 2013). Molecular analyses included 94 newly acquired specimens (Table 1), which were combined with 312 individuals from Taiwan, Thailand, and Malaysia that were part of previous studies (Schutze et al., 2012b; Krosch et al., 2013). Molecular analyses included 94 newly acquired specimens (Table 1), which were combined with 312 individuals from Taiwan, Thailand, and Malaysia that were part of previous studies (Schutze et al., 2012b; Krosch et al., 2013).

#### Table 1.

<table>
<thead>
<tr>
<th>Country</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Date</th>
<th>COI</th>
<th>ND4-3</th>
<th>ITS1</th>
<th>ITS2</th>
<th>Aedeagus</th>
<th>Lateral</th>
<th>Scutum</th>
<th>Wing shape</th>
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<td>2.07</td>
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<td>5</td>
<td>5</td>
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<td>25.2</td>
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<td>4</td>
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<td>20</td>
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<td>n/a</td>
<td>Initiated March 2012</td>
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<td>33.96</td>
<td>August – October 2009</td>
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from Dahanu were used in molecular analyses because we were unable to amplify DNA from Bangalore material due to its age [1992; pinned loan material from the British Museum of Natural History (BMNH), London, U.K.]. Bangalore specimens were used for wing shape analysis, as the wings of specimens from Dahanu were too badly damaged. Remaining morphological analyses (aedeagus and lateral vitta morphometrics and scutum colour variation) were conducted on Dahanu material.

East Asian samples were from China, Thailand, Peninsular Malaysia, Taiwan, Indonesia and the Philippines. Specimens from Taiwan, Thailand and Malaysia were used in comparative morphological analyses; specimens from all locations were used in molecular analyses. Further, specimens from Malaysia, Thailand, Indonesia and the Philippines included individuals traditionally classified as *B. papayae* and *Bactrocera philippinensis* Drew & Hancock. However, as *B. philippinensis* has been synonymized with *B. papayae* (Drew & Romig, 2013) and there is now considerable evidence that these two species are synonymous with *B. dorsalis*, we deemed it appropriate to include them here as part of the wider study. All East Asian specimens were collected from the wild into methyl eugenol traps between 2009 and 2012.

We included other species from both within and outside the *B. dorsalis* complex as part of our molecular analysis. Those in the complex included *Bactrocera carambolae* Drew & Hancock (*n* = 61), *B. kandiensis* (*n* = 9), *Bactrocera apiifera* (Drew & Hardy) (*n* = 19), *Bactrocera cacuminata* (Hering) (*n* = 19) and *Bactrocera ocellipalis* (Bezzi) (*n* = 22); those from outside the complex were *Bactrocera musae* (Tryon) (*n* = 20) and *Bactrocera tryoni* (Froggatt) (*n* = 9). All sequences used for molecular analysis, except *B. kandiensis*, had been acquired in the earlier study of Boykin et al. (2014), with collection data reported therein. We analysed eight specimens of *B. kandiensis* which were collected into methyl eugenol traps in Sri Lanka (caught May 2007). Note that *B. kandiensis* is only included in the molecular analysis as too few samples were obtained for morphological analysis.

**Morphology and morphometrics**

Four morphological features were examined as part of this study: scutum colour variation, postsutural lateral vitta width, aedeagus length, and wing size and shape. While abdominal colour pattern is listed as differing between *B. dorsalis* and *B. invadens*, we did not include it as part of our study as it was too variable. Only specimens identified as *B. dorsalis* or *B. invadens* were included for analysis (i.e. no outgroups), of which we examined 20 individuals for each morphological feature from all new locations (except Myanmar and Yunnan, China) in addition to three locations included in a previous examination of *B. dorsalis*: Taiwan, Thailand and Malaysia. We excluded specimens from Myanmar and Yunnan because all individuals died as teneral adults and were unsuitable for morphological analysis.

**Scutum.** Scutum colour is a continuous variable and defining variants is largely arbitrary. However, in an attempt to document variation across the geographic range, we divided scutum colour into one of three types based on figure 4 in Drew et al. (2005): pale, intermediate or dark. Pale forms were entirely pale-brown or with negligible black colouration (<10% of the scutum with black markings; see the first two images of fig. 4 in Drew et al., 2005); intermediate forms possessed a weak to strong black lanceolate pattern on an otherwise pale-brown scutum (see images 3–6 in fig. 4 of Drew et al., 2005); and dark forms had entirely, or almost entirely, black scutums whereby the lanceolate pattern was no longer discernible (>80% of the scutum is black; see images 7–8 in fig. 4, Drew et al., 2005). This character was not subjected to statistical analysis due to the subjective nature of categorizing scutum colour. Instead, the three colour forms are simply presented graphically as frequency charts.

**Postsutural lateral vitta.** Measurements of postsutural vitta width were made at the widest point of the vitta using an eyepiece micrometer mounted into a Leica MZ6 stereo-microscope (Wetzlar, Germany). Analysis of variance (ANOVA) with post hoc Tukey test was used to assess for significant differences among sample sites.

**Aedeagus.** Abdomens were removed and immersed in 10% KOH solution overnight to soften the integument prior to dissection. Each aedeagus was excised from remaining genitalia structures, fully straightened out on a microscope slide and measured as for vittae. Aedeagus length was measured from the base of the aedeagus to the start of (and excluding) the distiphallus, following Krosch et al. (2013). ANOVA with post hoc Tukey test was used to assess for significant differences among sample sites.

**Wing shape.** One wing from each fly was removed for slide mounting, image capture and analysis. Usually the right wing was dissected; if damaged, the left was used (∼4% of specimens across the total dataset). Wings were mounted in Canada balsam and air-dried prior to image capture using an AnMo Dino-Eye microscope eyepiece camera (model no. AM423B; Taipei, Taiwan) mounted into a Leica MZ6 stereo-microscope. Fifteen wing landmarks were selected following Schutze et al. (2012a) and using the computer program TPSDIG2 v.2.16 (Rohlf, 2010). Landmark coordinate data were imported into the computer program MORPHO v.1.04a (Klingenberg, 2011) for shape analysis. Data were subjected to Procrustes superimposition to remove all but shape variation (Rohlf, 1999). Multivariate regression of the dependent wing shape variable against centroid size (independent variable; see below) was conducted to assess the effect of wing size on wing shape (i.e. allometry) (Drake & Klingenberg, 2008; Schutze et al., 2012a). The statistical significance of this regression was tested by permutation tests (10 000 replicates) against the null hypothesis of independence (MORPHO v.1.04a). Subsequent analyses were undertaken in MORPHO v.1.04a using the residual components as determined from the regression of shape on centroid size to correct for allometric effect.
The size of each wing (centroid size) was calculated in \textsc{morpho} v.1.04a. Centroid size is an isometric estimator of size calculated as the square root of the summed distances of each landmark from the centre of the landmark configuration (see fig. 1.10 in Zeldich et al., 2004). ANOVA with post hoc Tukey test was used to assess for significant differences among sample sites.

Canonical variate analysis (CVA) on wing shape data was undertaken on 13 a priori groups based on location collection. Significant differences were determined via permutation tests (1000 permutation rounds) for Mahalanobis distances among groups ($\alpha = 0.05$; Bonferroni-corrected). We also tested for isolation by distance (IBD; Wright, 1943), whereby we conducted a subset CVA using only individuals from the native range of \textit{B. dorsalis} and \textit{B. invadens} (i.e. all Asian and Indian subcontinent samples to the exclusion of invasive African samples) upon which we undertook regression analysis (\textsc{spss} v.21) on pairwise geographic distance (km) versus Mahalanobis distances calculated from CVA. We did this because \textit{B. dorsalis} has demonstrated a strong IBD effect with respect to wing shape variation within a biogeographical context in South-east Asia (Schütze et al., 2012b). African samples were excluded from this analysis because they are a recent invasive population (detected in 2003) and hence geographic distance would be artificially inflated with respect to any differences in wing shape.

\textit{Molecular analysis}

\textit{DNA extraction and PCR.} Total genomic DNA was extracted from 8 to 14 individuals from each of the sampled locations using the Bioline Isolate II extraction kit with minor modifications. The modifications involved a pre-crushing step where three legs from each individual were placed in lysis buffer and crushed using either a micro-pestle or 3 mm ball bearings using a Qiagen mixer mill (Venlo, The Netherlands). Four gene fragments were amplified for the molecular component of this study, which consisted of two nuclear (ITS1 and ITS2) and two mitochondrial loci (\textit{cox1} and \textit{nad4}). Primer sequences for ITS1 and ITS2 are as per Boykin et al. (2014), for \textit{cox1} are as per Folmer et al. (1994) and for \textit{nad4} are Teph\textsubscript{ND}4F2 (5’-WCC WAA RGC TCA TGT WGA AGC TCC-3’) and Teph\textsubscript{ND}4R2 (5’-WCC CCC TCT AAT AYA AAY WCC-3’). Note, the same \textit{nad4} region was amplified in the present study as in Boykin et al. (2014); however, the primers reported in Boykin et al. (2014) are incorrect. Each PCR reaction contained 2.5 uL of template DNA, 1xMyTAQ PCR buffer (Bioline, London, U.K.), 0.5 units of MyTAQ polymerase and 2.5 mM MgCl$_2$, in a total reaction volume of 25 uL. PCR cycling conditions consisted of an initial denaturation step for 3 min at 94°C, followed by 25–30 cycles of 94°C for 30 s, 47–52°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products for each gene fragment were purified using a Bioline Isolate PCR purification kit. Cycle sequencing of purified PCR products were conducted using ABI Big Dye$^\text{TM}$ Terminators v.3.1 chemistry. Following a standard isopropanol precipitation clean-up, fragments were sequenced on an ABI 3500 genetic analyser (Life Technologies, Carlsbad, CA, U.S.A.). Trace files were corrected and contigs formed using \textsc{sequencher} v. 5.0 (Gene Codes Corporation, 2004).

\textit{Phylogenetic methods.} Individual sequences for each of the four loci were aligned in \textsc{mega} 5.2.2 (Tamura et al., 2011). Alignments of \textit{cox1} and \textit{nad4} were trivial as no indels were found in this study; ITS1 and ITS2 were aligned by eye (alignments available from the authors upon request). Individual alignments were concatenated in \textsc{mega} and partitioned by codon position for protein-coding genes or loci for ribosomal RNA genes. Evolutionary models were inferred for each partition using \textsc{modeltest} version 3.6 (Posada & Crandall, 1998) (ITS1, HKY + G; ITS2, GTR + I + G; \textit{cox1}-1st, GTR-I; \textit{cox1}-2nd, F81; \textit{cox1}-3rd, GTR; \textit{nad4}-1st, GTR + G; \textit{nad4}-2nd, GTR; \textit{nad4}-3rd, HKY). Phylogenetic analyses were run using likelihood and Bayesian inference methods. Likelihood analyses used the \textsc{raxml} Blackbox webserver (http://phylobench.vital-it.ch/raxml-bb/index.php) (Stamatakis et al., 2008), with separate partitions, a gamma model of rate heterogeneity, estimated proportions of invariant sites, and branch lengths optimized on a per locus basis. Bayesian analyses used \textsc{mrbayes} version 3.2 (Ronquist et al., 2012) with unlinked partitions, two independent runs each with three hot chains and one cold chain, for 10 million generations. Convergence between runs was monitored within \textsc{mrbayes} (standard deviation of split frequencies <0.001) and in \textsc{tracer} v1.5.4 (Rambaut & Drummond, 2010). Two parallel datasets were analysed, one composed of all specimens for which at least two of the four loci had been sequenced (dataset no. 1, 406 specimens) and one in which all specimens had been sequenced for all four loci (dataset no. 2, 293 specimens). Our previous analyses of \textit{B. dorsalis} group flies (Boykin et al., 2014) showed that phylogenetic analyses are robust to such missing data for this gene set.

A haplotype network using \textit{cox1} data was constructed using the median-joining method followed by maximum parsimony postprocessing in \textsc{network} version 4.6.1.1 (Bandelt et al., 1999). This allows evolutionary relationships among individuals to be inferred under a statistical framework that does not force bifurcation and was thus compared with relationships resolved using phylogenetic methods.

\textit{Results}

\textit{Morphology and morphometrics}

\textit{Scutum colour variation.} All three colour variants were observed for flies collected from sites across Africa and the Indian subcontinent, albeit with varying relative proportions (Fig. 1). For instance, most individuals from Benin, India and Nepal had black scutums, whereas most Sri Lankan and Congolese flies had intermediate scutums (black lanceolate pattern). Pale forms were the least frequently observed form, except for
Fig. 1. Geographic distribution of scutum phenotypes for Bactrocera dorsalis and Bactrocera invadens from: (1) Benin, (2) Democratic Republic of Congo, (3) Kenya, (4) Mozambique, (5) Sudan, (6) Pakistan, (7) Nepal, (8) India, (9) Sri Lanka, (10) Thailand, (11) Taiwan, (12) Malaysia, and (13) China. Twenty specimens of either species were examined per location, with the relative proportion of pale, intermediate, or fully black scutums shown.

the Kenyan sample; however, note that these flies were taken from a laboratory colony.

All East Asian flies (with one exception) possessed predominantly black scutums with no pale or intermediate forms present. The one exception was the sample from Wuhan for which two flies (out of 20 screened) had a pale scutum. While not examined here, all specimens of B. dorsalis collected from further along the Indo-Malay Archipelago and into the Philippines previously examined by the authors possessed a fully black scutum.

Postsutural lateral vittae. Postsutural lateral vittae of African B. invadens specimens ranged from 0.13 to 0.21 mm; B. dorsalis and B. invadens from the Indian subcontinent ranged from 0.13 to 0.22 mm; and B. dorsalis from eastern Asia ranged from 0.15 to 0.23 mm. Postsutural lateral vittae width varied significantly across sample sites ($F_{12,247} = 10.76$, $P < 0.001$; Fig. 2A) with no significant differences among flies from Africa, Pakistan, India and Sri Lanka; flies from these locations had the narrowest vittae, with an average width ranging from 0.16 mm (Benin) to 0.17 mm (Pakistan). Nepalese flies had significantly wider lateral vittae (mean width = 0.18 mm) than flies from some African locations (Benin, D.R. Congo and Kenya) and Sri Lanka; however, they did not differ from Mozambican or Kenyan colony flies (2.71 and 2.76 mm, respectively). Furthermore, there was significant variation in aedeagus length among samples from eastern Asia: Malaysian aedeagi were significantly longer (> 2.8 mm) than those from other locations in the region (all < 2.8 mm). Only males from the Indian subcontinent possessed aedeagi of statistically similar lengths among all locations from within the region; there were varying levels of overlap in aedeagus length among populations from this region and those from Africa and eastern Asia.

Aedeagus. While similar to the vittae analysis, in that there was a significant difference among populations for aedeagus length ($F_{12,247} = 15.45$, $P < 0.001$), there was no west-to-east trend from Africa to eastern Asia for aedeagus length (Fig. 2B). Aedeagi from African B. invadens specimens ranged from 2.41 to 2.97 mm; B. dorsalis and B. invadens from the Indian subcontinent ranged from 2.38 to 2.91 mm; and B. dorsalis from eastern Asia ranged from 2.35 to 3.00 mm. Significant aedeagus length variation was observed within Africa; for example, Congolese males had significantly shorter aedeagi (average of 2.64 mm) than Mozambican or Kenyan colony flies (2.71 and 2.76 mm, respectively). Furthermore, there was significant variation in aedeagus length among samples from eastern Asia: Malaysian aedeagi were significantly longer (> 2.8 mm) than those from other locations in the region (all < 2.8 mm). Only males from the Indian subcontinent possessed aedeagi of statistically similar lengths among all locations from within the region; there were varying levels of overlap in aedeagus length among populations from this region and those from Africa and eastern Asia.

Wing shape. Wing centroid size significantly varied among sampled populations ($F_{12,247} = 5.013$, $P < 0.001$) and there was a significant allometric effect (4.09%; $P < 0.0001$). While there were differences in wing size among locations, there was no longitudinal trend from Africa to eastern Asia as observed.
Integrative taxonomy of B. invadens & B. dorsalis

A

B

C

Congolese wings were the smallest of all African locations (average centroid size of 6.06) and they were significantly different from Kenyan and Mozambican flies that possessed the largest wings (average centroid sizes of 6.49 and 6.61, respectively). Sudanese and Beninese flies had wings that were not significantly different from any other African location or from each other (average centroid sizes of 6.39 and 6.35, respectively). There was significant variation in the east-Asian samples, with the smallest wings belonging to Malaysian flies (average centroid size of 5.96; the smallest wings of the entire dataset) which were significantly different from Taiwanese and Chinese flies (average centroid sizes of 6.40 and 6.46, respectively). Contrary to African and East Asian samples, all wings sampled from the Indian subcontinent were not significantly different from each other with respect to size, ranging in average centroid size from 6.34 (Nepalese wings) to 6.48 (Indian wings).

Canonical variate analysis following correction for allometric effect (due to the significant result reported earlier) produced 12 canonical variates of which the first two explained 63% of the variation (Fig. 3). Group Mahalanobis distances were significantly different for all comparisons except among the following locations: (i) Sudan, Benin, and D.R. Congo; and (ii) Nepal and Pakistan. All African groups were closest neighbours except for Kenya, which separated in multidimensional space from other African samples relative to both Pakistan and Nepal (i.e. Pakistani and Nepalese wings were more similar in shape to Sudanese, Congolese, Beninese and Mozambican wings than was Kenya to any of the other African locations). The remaining Indian subcontinent groups (India and Sri Lanka) were relatively different from both African samples and those from the northern Indian subcontinent (i.e. Pakistan and Nepal). Further, despite their relative geographic proximity, wings from Indian flies were considerably different from Sri Lankan wings. Malaysian, Taiwanese and Thai wings were more similar in shape to wings from Pakistan and Nepal than those from India or Sri Lanka. Chinese wings were highly similar in shape to those from the southern Indian subcontinent, particularly Sri Lanka (Mahalanobis distance between China and Sri Lanka = 2.51).

Canonical variate analysis on Asian samples (i.e. excluding Africa) was conducted on eight a priori defined groups: Pakistan, Nepal, India, Sri Lanka, Thailand, Wuhan, Taiwan and Malaysia, resulting in seven canonical variates of which the first two explained 74% of the variation. There was no significant association between Mahalanobis distance and geographic distance ($r^2 = 0.001; P = 0.850$; Fig. 4).

Molecular analysis

Phylogenetics. New sequences were generated for up to four loci per specimen and combined with sequences from a previous study (Boykin et al., 2014) for phylogenetic analyses (GenBank accession numbers JX099580-JX099755, KC446030-KC447278 and KM 453245- KM453574; see Table S1). Bayesian and maximum likelihood analyses for both dataset no. 1 (two out of the four loci analysed, 406 individuals) and dataset no. 2 (all four loci, 293 individuals) yielded similar phylogenetic topologies, albeit with varying levels of nodal support with the highest values generally obtained for the

Fig. 3. Plot of first two variates following canonical variate analysis of geometric morphometric wing shape data for Bactrocera dorsalis and Bactrocera invadens collected from Africa (A), the Indian subcontinent (B) and East Asia (C). Twenty wings were analysed per location, with respective regions shaded in each of the three plots.

Bayesian analysis using dataset no. 1 (Fig. 5). All outgroups were well resolved, including B. carambolae recovered as sister to the larger B. dorsalis clade. The ingroup clade contained previously sequenced data for B. dorsalis, B. papayae and B. philippinensis from South-east Asia (collectively termed ‘B. dorsalis s.l.’) (Boykin et al., 2014), in addition to de novo data from individuals obtained from the expanded range of East Asia (B. dorsalis), the Indian subcontinent (B. dorsalis, B. invadens and B. kandiensis) and Africa (B. invadens). Specimens of B. dorsalis additional to the study of Boykin et al. (2014) were from Myanmar, China (Wuhan and Yunnan), India, Nepal and Pakistan. All newly included B. dorsalis and African specimens of B. invadens fell within the broader B. dorsalis clade with no evidence of statistically supported subclades. All African B. invadens specimens were either completely unresolved within the broader B. dorsalis clade or emerged as two weakly supported clades that included individuals from across all African countries sampled and representing the range of scutum colour variation (see Figures S1 and S2). The same was true for Sri Lankan B. invadens specimens, which were either fully unresolved within the B. dorsalis clade or which formed small, poorly supported groups nested within the larger clade. Scutum colour (i.e. red-brown, black or intermediate) did not align with the phylogeny in any consistent pattern (Fig. 5 inset).

Bactrocerakandiensis is the only subclade within B. dorsalis s.l. with significant nodal support (Fig. 5). This was driven by a unique indel pattern present in the ITS1 locus which, while diagnostic, is shorter than the indel that occurs in the same locus in B. carambolae (Boykin et al., 2014). Further, some specimens of B. dorsalis possessed cox1 haplotypes more closely related to B. kandiensis than other B. dorsalis haplotypes; these included five specimens from Sri Lanka (Bd1561–1564 and Bd1566), two from Myanmar (Bd1580 and Bd1582) and seven from India (Bd1691–1697). As these specimens did not possess the ‘B.
Integrative taxonomy of *B. invadens* & *B. dorsalis*

Fig. 5. Phylogenetic tree generated from Bayesian and maximum likelihood (ML) analysis for *Bactrocera dorsalis*, *Bactrocera invadens* and outgroups. East Asian specimens (*) include *Bactrocera papayae* and *Bactrocera philippinensis*. Nodal supports presented for each analytical approach and for both 2/4 and 4/4 loci analyses. Ingroup specimens from the Indian subcontinent, Africa and East Asia are highlighted in light grey, dark grey and black, respectively. Specimen identities have been removed for clarity (provided in Figure S1). Inset figure (lower left) shows scutum colour pattern mapped onto *Bactrocera dorsalis/invadens* clade. n.s., not significant.

*kandiensis* ITS1 indel, they resolved with the remainder of *B. dorsalis* in multi-locus analyses.

The median-joining network largely conformed to that presented in Schutze et al. (2012b), comprising East Asian *B. dorsalis* s.l. haplotypes, with new sequences from this paper placed throughout (Fig. 6). The central starburst-like pattern remained, with numerous singletons radiating from a common, widespread haplotype. Individuals of *B. kandiensis* formed a divergent and diverse cluster and were connected to the network by a very long branch, demonstrating that the position of this taxon in the multilocus phylogeny is not driven solely by ITS1 indel patterns. Within the *B. kandiensis* cluster there were several haplotypes from *B. dorsalis* flies from Sri Lanka, India and Myanmar, although, no haplotypes were shared between *B. dorsalis* and *B. kandiensis* flies. There was no apparent separation of haplotypes from flies identified morphologically as *B. invadens* or *B. dorsalis*; haplotypes of these two taxa were generally scattered throughout the network. Indeed, four *cox1* haplotypes were shared between the two taxa; one sampled from Nepal and Thailand populations, one from Sudan and Thailand, one from Taiwan, Malaysia, Nepal and Pakistan, and the common widespread haplotype sampled from all locations except Benin, Mozambique, Kenya and Sudan (Figure S3). Likewise, there was no clear geographical pattern in the network, although there were few haplotypes shared among individuals from broadly different regions. No clustering of haplotypes was apparent for individuals with different scutum colours (Figure S2). Five haplotypes were shared by individuals that differed at this trait (often the same haplotype was shared among sites and/or among *B. dorsalis* and *B. invadens* flies).

The interpretation of our combined results strongly suggests that *B. dorsalis* and *B. invadens* are one biological species. Genetic data, at both the multi-locus and haplotype levels, fail to show evidence of distinct clades and unique haplotypes, respectively, consistent with the presence of two species as reported in other studies (Khamis *et al.*, 2012; Frey *et al.*, 2013; San Jose *et al.*, 2013). Examination of morphology shows a great deal of variation among populations, some with apparent geographic structuring that relates to current taxonomy (especially scutum colour), but with other traits showing no such structure; in all cases the patterns in morphology do not align with any apparent genetic variation. To place our results within the broader context of *B. dorsalis* taxonomy and species delimitation over the centuries, a brief summary of the confusing taxonomic history of *B. dorsalis* is provided.

**Taxonomic history of Bactrocera dorsalis**

The species now known as *B. dorsalis* was first described by Fabricius in the late 18th century as a rust-red-coloured fly from ‘India Orientali’ under the name *Musca ferruginea* (Fabricius, 1794). Note that whilst India Orientali can be interpreted as the East Indies (Pont, 1995), the type specimen described by Fabricius is considered to be from East India (Drew & Romig, 2013). Further, treatments of other Fabrician collections, e.g. hymenopterans (van der Vecht, 1961), state that India Orientali usually refers to India, rather than other parts of South-east Asia (e.g. Indonesia). Fabricius (1805) subsequently transferred this species to the genus *Dacus* Fabricius, a combination that persisted until the 20th century. In the early part of the 1900s, however, the morphological variability of *D. ferrugineus* was noted by Froggatt (1910), specifically the scutum colour which ranged from black to rust-red. The black scutum variety was soon described by Hendel (1912) as a new species, *D. dorsalis* Hendel, following examination of specimens from Formosa (= Taiwan), and with a black scutum as the chief discriminatory character separating it from *D. ferrugineus* (which possessed a red-brown scutum).

Nevertheless, the view of the ‘Formosan type’ as a distinct species was not universally accepted, with studies over the next 50 years regarding *D. dorsalis* as either a species in its own right (Perkins, 1938) or simply a dark variety of *D. ferrugineus* (Bezzi, 1916; Miyake, 1919; Shiraki, 1933; Munro, 1939). Critically, Hendel himself accepted that *D. dorsalis* represented a black variety of *D. ferrugineus* and, after examining more specimens, explicitly stated that specimens from Taiwan corresponded with the Fabrician description (Hendel, 1915). Munro (1939) found north-west Indian specimens to exhibit a full range of thoracic colour forms (from pale, through intermediates, to dark) with no additional structural characters present to further distinguish any of these forms from each other, leading him to conclude they all belonged to the same species.

In the late 1960s, Hawaiian taxonomist D.E. Hardy undertook a revision of what was, by then, commonly known as the ‘Oriental fruit fly’ (Hardy, 1969). The key outcomes of this revision were the following: (i) the species name *ferrugineus* was invalid as it was preoccupied by another fly described by Scopoli (1763); (ii) the only valid name available for the Fabrician species was Hendel’s *D. dorsalis*; (iii) that *D. ferrugineus* must therefore become a junior synonym of *D. dorsalis*; (iv) that individuals of this species with a red-brown scutum colour were teneral adults yet to develop their final black-scutum colouration; (v) *D. dorsalis* was characterized as possessing only a black (or mostly black) scutum; and, finally, (vi) a number of closely related species existed which were to be placed in the newly formed, 16-member, *D. dorsalis* species complex. It was at this critical point that red-brown scutum forms were subsumed in subsequent treatments of *D. dorsalis*, including in major revisions towards the end of the 20th century by which time *D. dorsalis* had been reassigned to the genus...
Bactrocera and the complex expanded to more than 70 species with a black, or mostly black, scutum one of their defining characters (Drew, 1989; Drew & Hancock, 1994; Drew & Romig, 2013). Importantly, Hardy’s (1969) revision referred to previous work which detailed the range of colour forms, such as the Indian study by Munro (1939); however, Hardy specifically noted that Munro examined a limited sample range of 39 specimens, and that Hardy himself never observed such variability in the many thousands of specimens he examined from India and Pakistan (Hardy, 1969).

A morphologically variable fly closely allied to B. dorsalis was reported from Africa in 2003 (Lux et al., 2003). Although the newly detected species was considered highly variable, it showed morphological characters that were consistent with B. dorsalis (Lux et al., 2003). Our examination of specimens from Sri Lanka in comparison with African specimens has led to the conclusion that the invasion probably originated from the Indian subcontinent (NB: the same region from which B. invadens was reported from Africa in 2003 (Lux et al., 2003). Our examination of specimens from Sri Lanka in comparison with African specimens has led to the conclusion that the invasion probably originated from the Indian subcontinent (NB: the same region from which D. ferrugineus was probably first described by Fabricius) and that these morphologically variable flies were a new species, which was described by Drew et al. (2005) as B. invadens. An important side note for taxonomic consideration is that the type locality of B. invadens is Kenya (i.e. invasive range), not Asia (i.e. native range).

Morphological variation

Clearly there was confusion during the last century over the identity of B. dorsalis in relation to D. ferrugineus, particularly with respect to scutum colour variation. Earlier studies, such as that of Munro (1939), described specimens from the Indian subcontinent as exhibiting a range of thoracic colour forms, yet towards East Asia the darker, mostly black form, predominated. Our assessment of scutum colour variation of newly acquired specimens reflects this pattern, with a range of colour forms across the Indian subcontinent (Fig. 1) and entirely black forms occurring eastwards into the rest of Asia, with the exception of a small number of individuals from China. All flies in our study were collected from traps placed in the wild or sourced from colony material and were fully mature specimens, thereby conflicting with Hardy’s (1969) view that mature red-brown specimens of B. dorsalis do not exist. Indeed, the presence of a range of thoracic colour forms is similarly documented, either directly or through inference, in contemporary studies of material from the Indian subcontinent. Drew et al. (2007) recorded B. invadens in Bhutan, but this was considered as doubtful by Drew & Romig (2013); a recent illustrated key on Indian fruit flies states that while B. invadens does not occur in India, specimens keying out as B. dorsalis may more closely match descriptions given for B. invadens (David & Ramani, 2011). Further, a detailed survey of B. dorsalis from Bangladesh clearly demonstrated that individuals possess the range of thoracic and abdominal colour forms typical of African and Sri Lankan B. invadens (Leblanc et al., 2013). Given the evidence at hand, it is difficult to accept Hardy’s assertion that such colour variation does not exist in B. dorsalis.

Other presumably diagnostic morphological characters measured here, namely aedeagus length and width of post sutural lateral vittae, do not conform to variation expected under a two-species hypothesis. Bactrocera invadens is reported to possess longer aedeagi and narrower post sutural lateral vittae than B. dorsalis (Drew et al., 2005, 2008). Our results demonstrate that neither of these characters possesses diagnostic value as they either show no pattern at all (i.e. aedeagus length, Fig. 2B) or are continuously variable across a geographic cline (i.e. vittae width, Fig. 2A). Such results may therefore be indicative of population-level variation rather than species-level variation, similar to the latitudinal variation in aedeagus length documented for B. dorsalis in South-east Asia (Krosch et al., 2013).

Geometric morphometric shape analysis is a more sensitive tool for assessing morphological variation than simple linear measurements (Schutze et al., 2012b; Krosch et al., 2013). This study extends wing shape analysis from East Asia into the Indian subcontinent and Africa, revealing potentially insightful patterns of variation and points of origin. For example, while wing shape is highly similar among all African populations of B. invadens (as expected for a relatively newly established invasive population), there is greater difference in wing shape among populations of B. dorsalis throughout the native range of the Indian subcontinent and East Asia (Fig. 3). Furthermore, the wing shape of African flies is most similar to those from the northern range of the Indian subcontinent, namely Nepal and Pakistan (Fig. 3), while those from further south (i.e. India and Sri Lanka) have wings that are relatively different in shape from African and northern Indian subcontinent populations (Fig. 3). Wing shape can, under some circumstances, demonstrate a highly significant IBD signature, as found in the study of B. dorsalis s.l. in South-east Asia (Schutze et al., 2012b). In that study, wing shape was superior to population genetic data at resolving IBD signatures given a specific biogeographic hypothesis, demonstrating that, as geographic distance between populations increased, so did relative differences in wing shape. We therefore consider wing shape analysis to be a valuable tool for inferring the origin of an invasive species such as B. invadens, and that the African invasion may have come from the northern Indian subcontinent rather than Sri Lanka, as previously thought. This hypothesis could be tested further by a targeted population genetic study using markers of contemporary gene flow [e.g. microsatellites or restriction site associated DNA (RAD) tag].

A ‘one species’ hypothesis is supported by molecular data

While the significant morphological variation could be interpreted as the forms present in Africa and the Indian subcontinent representing different species (i.e. B. invadens in the west, B. dorsalis in the east), it is not supported by the molecular evidence. These data fail to resolve specimens from Africa and the Indian subcontinent as distinct from the broader B. dorsalis clade, a clade incorporating material from across the geographic range of B. dorsalis and B. invadens (Fig. 5). Moreover, mapping scutum colour onto individuals from the dorsalis clade reveals

little to no pattern in the distribution of colour forms (Fig. 5 inset). Previous molecular studies have found similar results. Neighbour-joining analysis of the cox1 barcoding gene, for instance, resolved specimens from a Hawaiian laboratory colony of B. dorsalis as a group within a larger clade of B. invadens from Africa and Sri Lanka, while splitting B. invadens into two clades, one with specimens from Africa and Sri Lanka (along with B. dorsalis), the other grouping Sri Lankan B. invadens with Sri Lankan B. kandiensis (Khamis et al., 2012). While Khamis et al. (2012) did not conclude that B. dorsalis and B. invadens were the same species, a more recent multi-locus phylogenetic study of a number of Bactrocera species found the following: (i) B. invadens was polyphyletic within the B. dorsalis s.l. clade; and (ii) B. invadens was genetically indistinguishable from many of the pest species within the group. The study did not, therefore, support the placement of B. invadens as an independent species outside B. dorsalis s.l. (San Jose et al., 2013). This conclusion was also reached by Frey et al. (2013), who explicitly stated that B. invadens should be synonymized with B. dorsalis as a result of their cox1 barcode study. These earlier molecular studies, while extensive in their own right, have incorporated a relatively limited sample range for either B. dorsalis or B. invadens, yet they nevertheless demonstrate that B. dorsalis and B. invadens are most probably a single species. This conclusion is reinforced in our phylogenetic study, which represents the most extensive molecular analysis of B. invadens and B. dorsalis from across much of their native and invasive geographic ranges. We also found haplotypes to be shared between individuals identified as either B. dorsalis or B. invadens in our analysis of the cox1 haplotype network (Fig. 6). Furthermore, the most common and widespread haplotype includes individuals that exhibit the full range of scutum colour forms from Thailand, Malaysia, India, Nepal, China, Pakistan, Sri Lanka and D.R. Congo (Figures S2 and S3).

The curious case of B. kandiensis – evidence of introgression?

Our analysis of the cox1 gene revealed an unexpected association among B. kandiensis, B. dorsalis and B. invadens, in that some Sri Lankan, Indian and Burmese specimens that were morphologically identified as either B. dorsalis or B. invadens possessed cox1 sequences more closely related to B. kandiensis haplotypes than conspecifics (Fig. 6). This was not reflected by nuclear data which, to the contrary, revealed an indel that consistently separated all B. kandiensis specimens from B. dorsalisinvadens. Our results reflect the barcode study of Khamis et al. (2012), who found a large proportion of Sri Lankan B. invadens specimens to be more closely related to B. kandiensis than to African B. invadens or Hawaiian B. dorsalis. Importantly, however, Khamis et al. (2012) examined a single gene, cox1, from only Sri Lankan specimens rather than those from other locations across the Indian subcontinent.

The presence of B. kandiensis haplotypes among B. invadens or B. dorsalis individuals raises the possibility of introgression – the permanent incorporation of genes from one population into another via hybridization (Dowling & Secor, 1997). Introgression in dacine fruit flies has been proposed for other taxa, such as the Australian species, B. tryoni, for which horizontal introgression of genetic material from its sister species, Bactrocera neoheralis (Hardy), was proposed as a potential adaptive mechanism allowing the expansion of B. tryoni into new climate regions (Lewontin & Birch, 1966). Under the current scenario, the presence of B. kandiensis mitochondrial DNA haplotypes in the genome of B. dorsalis, but not the reverse (i.e. B. dorsalis haplotypes in B. kandiensis), implies that such hybridization, if it has occurred, was unidirectional. Moreover, this pattern must have resulted from sex-biased couplings whereby B. dorsalis males mate with B. kandiensis females to produce offspring with B. dorsalis morphology but with B. kandiensis mitochondrial DNA. Preliminary field cage mating tests examining compatibility between B. kandiensis and B. dorsalis revealed no evidence of assortative mating between these two species (M.K. Schutze and W. Bo, unpublished data), reinforcing the potential for them to interbreed in sympathy. Bactrocera kandiensis is recorded only from Sri Lanka; however, it is suspected to exist also in southern India (Kapoor, 2005) and may occur sympatrically with B. dorsalis potentially as far east as Myanmar. This remains a hypothesis in need of further testing, with the incorporation of a wider sample range and continued research into mating compatibility between these taxa. Furthermore, we advocate additional studies including other species of the dorsalis complex, such as Bactrocera caryaeae (Kapoor). This economically important species is considered allopatric to B. kandiensis as it is recorded from India but not Sri Lanka, yet they emerge as sister taxa in phylogenetic analyses (Krosch et al., 2012) and are distinguished based on abdominal colour pattern (Drew & Romig, 2013).

Conclusions

Our integrative molecular and morphological study of B. invadens and B. dorsalis from across a wide geographic distribution supports the hypothesis that they represent a single biological species. These data, in accordance with mounting evidence from other studies (Tan et al., 2011; Khamis et al., 2012; Frey et al., 2013; San Jose, et al., 2013; Bo et al., 2014), highlight the need for formal synonymy between B. dorsalis and B. invadens and a subsequent revision of the current description of the Oriental fruit fly to encompass a wider range of morphological colour variants, particularly with respect to scutum colour. Further, given the taxonomic history of this species, we argue that the fly described as B. invadens is probably conspecific with that described by Fabricius (1794, 1805) as D. ferrugineus. The type specimen of ferrugineus designated by Fabricius and held by the Natural History Museum of Denmark is almost completely destroyed and so this proposition cannot be directly tested. However, what remains of this specimen (a thorax and partial abdomen) bears a strong resemblance to present-day B. invadens (Fig. 7), and a later-collected individual from Sri Lanka (collected 1899 and labelled by Hendel as Chaetodacus ferrugineus F.; specimen in the Natural History Museum, Vienna, Austria) has been confirmed as B. invadens.
Fig. 7. Holotype of Dacus ferrugineus Fabricius located in the Natural History Museum of Denmark. While nearly entirely destroyed, the taxonomically informative ‘red-brown’ colour of the thorax is still evident. Photo credit: Verner Michelsen.

(Drew & Romig, 2013). Synonymizing these species will have a profound impact on quarantine and trade access, especially for sub-Saharan Africa, which has been devastated by the rapid and destructive expansion of this species across the continent. However, we stress that although B. invadens is the same biological species as B. dorsalis, the potential for biological differences among populations remains, especially considering the broad geographic distributions and environmental conditions where these species are found. Finally, our data revealing the potential of hybridizing introgression between B. dorsalis/invadens and B. kandensis further exemplify the need for more research into the mechanisms of speciation and the evolution of the B. dorsalis species complex.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12114

Figure S1. Phylogenetic tree generated from Bayesian and Maximum Likelihood analysis for Bactrocera dorsalis, Bactrocera invadens, and outgroups. Nodal supports presented for each analytical approach and for both 2/4 and 4/4 loci analyses. Ingroup specimens from the Indian subcontinent, Africa, and eastern Asia are highlighted in light grey, dark grey, and black, respectively.

Figure S2. Median joining haplotype network generated from cox1 sequence data of Bactrocera dorsalis and Bactrocera invadens collected from Africa, the Indian subcontinent, and eastern Asia. Different colours represent different collection countries.

Table S1. Collection data and Genbank accession numbers for specimens of Bactrocera spp. used in Schutze et al.

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