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Resolving the taxonomic status of *Frankliniella schultzei* (Thysanoptera: Thripidae) colour forms in Kenya – a morphological-, biological-, molecular- and ecological-based approach

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Abstract. *Frankliniella schultzei* Trybom is a polyphagous pest and vector of tospoviruses worldwide. It occurs in dark and pale colour forms that are morphologically similar but differ in vector competency and geographic spread. In Kenya and other tropical regions, mixed populations of both colour forms are observed in similar habitats, so are considered as one species. To ascertain the taxonomic status of the two colour forms, they were characterized using morphological, molecular, biological and ecological approaches. Morphological characterization revealed differences between the colour forms on eight features and they separated into distinct clusters through principal component analysis. Restriction fragment length polymorphism of the internal transcribed spacer region (ITS-RFLP) analysis revealed differences between the two colour forms and was confirmed by differences in ITS2 sequences. Virgin pale females had female offspring (thelytoky), while virgin dark females had male offspring (arrhenotoky). Interbreeding of dark males with pale females resulted in pale females, indicating absence of interbreeding between the two colour forms. Laboratory colonies of pale forms lacked males and further analysis of *F. schultzei* males from *Ipomoea setosa* flowers in the field indicated the presence of dark males and the absence of pale males. Field surveys in Kenya indicated differences in distribution and host plant preferences among the colour forms. Lack of interbreeding, distinct host preferences and distribution, and morphological and molecular differences indicate that the two colour forms of *F. schultzei* could be different species. The results highlight the need for combining morphological, biological, molecular and ecological characteristics for resolving taxonomic status of closely related insects.

Key words: Common blossom thrips, colour forms, ITS-RFLP, ITS2, parthenogenesis, interbreeding, morphometry

Introduction

Frankliniella schultzei Trybom (Thysanoptera: Thripidae), known as the common blossom thrips (CBT), is a key crop pest causing worldwide economic loss (Kakkar *et al.*, 2014). The pest causes direct damage during feeding and oviposition, and indirect damage as a vector of tospoviruses (Sakimura, 1969). *Frankliniella schultzei* is one of the

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major insect pests affecting French bean production in Kenya (Nyasani *et al.*, 2010; 2012).

Frankliniella schultzei is distributed in two colour forms: dark and pale. The dark form is mainly distributed south of the Equator, while the pale form is mainly found north of the Equator (Sakimura, 1969). The two forms of CBT coexist in the same habitat with overlapping host ranges in India, the Philippines, New Guinea, Northern Australia and East Africa (Mound, 1968; Sakimura, 1969). Overlapping habitats, host ranges and similar morphological features apart from the differences in colour, suggest their grouping as one species. Hence, the pale form which was originally described as *Frankliniella sulphurea* (Schmutz, 1913) was synonymized with *F. schultzei* (Mound, 1968).

Most thrips species reproduce both sexually and parthenogenetically (Moritz, 1997). Although mixed colonies of both colour forms have been reported on some host plants (Mound, 1968), no official reports on the possibility of interbreeding between the two forms are available, and differences between the two forms are not well established at morphological and molecular levels.

The colour forms are known to differ in their vector competence with the dark form considered to be a good vector of at least four tospoviruses, whereas the pale form is a weak vector of only two (Sakimura, 1969; Cho *et al.*, 1988; Wijkamp *et al.*, 1995; Nagata and de Ávila, 2000). On the contrary, the pale forms are good vectors of *Maize chlorotic mottle virus* when compared to the dark forms (Nyasani *et al.*, 2015).

Therefore, the current study aimed to resolve the taxonomic status of the two colour forms of *F. schultzei* through combined assessment of morphological, molecular, biological and ecological differences as suggested by Sakimura (1969). Physical features were compared morphometrically, and molecular differences were assessed using ITS-RFLP and sequence analysis. Interbreeding experiments were conducted, and host and ecological preferences of the two colour forms determined. This study emphasizes the importance of 'integrative taxonomy' in accurate species identification and nomenclature (Dayrat, 2005), which is critical in quarantine pest diagnostic and management (Garza *et al.*, 2007).

Material and methods

Thrips rearing

The study was conducted at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. Isolated colonies of the two colour forms of *F. schultzei* were reared in *icipe*'s Animal Rearing and Containment Unit. The dark forms of *F. schultzei* originated from a population on *Malvaviscus grandiflorus* Kunth from Kasarani, Nairobi Province (GPS coordinates: −1.221, 36.897) and has been in culture for approximately 100 generations. The pale form of *F. schultzei* originated from a population on *Phaseolus vulgaris* (Linnaeus) in Kitui, Eastern Province (GPS coordinates: −1.298, 37.944) and has also been in culture for approximately 100 generations. Both colour forms were reared in ventilated plastic jars (1 litre) with thrips-proof nets covering the top. The two colour forms were reared on surface-sterilized French bean (*P. vulgaris* (L.) var. *samantha*) pods as described earlier by Niassy *et al.*, (2012).

Biological characterization – interbreeding

To generate virgin parents, larvae of the different colour forms were transferred individually into 90 mm Petri dishes lined with a paper towel and fed with a piece of French bean pod (about 7 cm long) until adult emergence. Emerged adults were sexed and utilized in interbreeding experiments. Twenty dark and 20 pale *F. schultzei* females were placed individually in 10 ml glass vials for mating with dark males in a female to male ratio of 1:4 for 3 days. After mating, all females were placed in separate Petri dishes containing sterile French bean pod for oviposition. Recently emerged larvae (F1) were transferred to separate Petri dishes and fed as described above until adult emergence. Concomitantly, control treatments of virgin dark or virgin pale females were conducted with 20 females of each colour form held in the absence of males. The experiment was replicated three times. We could not undertake the interbreeding of pale males with dark females as pale males were not observed in the colony. Twenty parental specimens of both forms (dark and pale) and their respective F1 offspring (virgin dark female, virgin pale female, dark male × dark female and dark male × pale female) were cleared, dehydrated and mounted on slides with Canada balsam using methods outlined by Moritz *et al.* (2013).

Morphological characterization

The identity of the specimens mounted on the slides was confirmed as *F. schultzei* using morphological features outlined in the Lucid key on 'Pest thrips of East Africa' (Moritz *et al.*, 2013). Eight morphological features were selected for further characterization of the specimens based on previous literature (Johansen, 2002; Hamodi and Abdul-Rassoul, 2004; Retana-salazar *et al.*, 2010; Wang *et al.*, 2010; Subramanian *et al.*, 2012; Moritz *et al.*, 2013).

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The features selected included the length of third ocellar setae (*Sl*), the distance of the third ocellar setae from the tangent of the anterior margin of the hind ocellar (*DfT*), the lengths of first ocellar setae (*Set1*), second ocellar setae (*Set2*), metanotal lateral setae (*Mls*), major setae on tergite X (*TX*), width of the forewings (*Fw*) and the appearance of posteromarginal comb of microtrichia on tergite VIII (*Comb*) (Fig. 1). Morphometric measurements were made on the specimens using a Leica DM LB® microscope fitted with Leica EC3® camera and Leica Application Suite Version 3.0.0 software (Leica Microsystems, Switzerland).

The length of third ocellar setae (*Sl*) and distance of the third ocellar setae from the tangent of the anterior margin of the hind ocellar (*DfT*), which are among the significantly different character states between the parents (Subramanian *et al*., 2012) were further used to characterize the F1 offspring from dark male × dark female and dark male × pale female crosses.

Molecular characterization

Individual specimens of *F. schultzei* stored in 95% ethanol were removed from 1 ml Eppendorf tubes and allowed to air dry for 2 min. Each specimen was then placed on a sterile microscope slide and their abdomen dissected using a sterile surgical blade. Total DNA was extracted from the dissected abdomen using ISOLATE II Genomic DNA Bioline Kit, following the manufacturer's instructions, with a slight modification of eluting the DNA in 30 µl elution buffer. The remaining body parts that consisted of head, thorax and a section of the abdomen were cleared and mounted on slides for morphological identification as described earlier.

The Internal Transcribed Spacer region (ITS2) was amplified from the DNA obtained using the primer pair ITS2 F 5′-CGAGTATCGATGAA-GAACGCAGC- 3′ (Despres *et al*., 1993) and ITS2 R 5′-AATGCTTAAATTTAGGGGGTA- 3′ (Campbell *et al*., 1994). Amplification was performed in 30 μl total reaction volumes, comprising 3 μl of genomic DNA solution, 10 pmol of each primer, 10mM dNTPs, 1.2 5mM $MgCl_2$, 5X Phusion HF buffer, 100% DMSO and 5 units of Phusion DNA Polymerase. The amplification was carried out in an Arktik Thermo Scientific® Thermocycler. The DNA was initially denatured at 98 °C for 1 min and 10 s followed by 35 cycles of denaturation for 20 s at 98 °C, annealing for 40 s at 57.2 °C and elongation for 45 s at 72 °C. The last cycle was followed by a 10-min incubation period at 72 °C to complete any partially synthesized strands. The amplification products were separated by electrophoresis in 1% agarose gel containing ethidium bromide. Amplified DNA products were purified using ISOLATE II PCR and the Gel Bioline Kit and samples were sent for sequencing.

ITS2 fragments obtained from amplification using ITS2F-ITS2R primer pair were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis using four restriction enzymes; AluI, HinfI, RsaI and HaeIII (Thermo Scientific, Inc). Each enzyme reaction was conducted separately. The reactions were done in 18 μl reaction volumes, comprising 10X Fast Digest green buffer, 2 units of restriction enzyme and 5 μl purified PCR products. The DNA was first digested at 37 °C for 14 h and then the reaction was stopped by denaturing the enzyme at 65 °C for 5 min. The restricted fragments were separated by electrophoresis in 2% agarose gel containing ethidium bromide at 70 V for 1.5 h and visualized under the UV imaging system.

Due to the absence of pale *F. schultzei* males in the colony, it was essential to confirm their presence in the wild. Male *F. schultzei* were collected from *Ipomoea setosa* Ker-Gawl flowers which hosted both pale and dark *F. schultzei*. All males were treated as tentative candidates. DNA was extracted from individual male *F. schultzei*, the ITS2 regions amplified and the fragments digested using *Alu1* restriction enzyme as described above. Since *Alu1* generated distinct markers among the parents (Fig. 2), it was selected for screening of the males from the wild population. Twenty of the males collected from *I. setosa* were also mounted on slides as described above and their morphometrics measured, based on the eight variables previously stated.

Ecological characterization

Host plant and ecological preferences of the two colour forms were assessed by analysing the geo-referenced database maintained in the Biosystematics Unit, *icipe*. A total of 1383 *F. schultzei* specimens were sampled between August 2008 and July 2009 from over 88 locations in Kenya. At each location, sampling was undertaken at random from 10 × 20 plants/location using the standard 'Plant tapping method' (Pearsall and Myers, 2000; Mfuti *et al*., 2016). The geo-referenced database includes information on the global positioning system (GPS) coordinates of each sampling location, host plant records, dates of collection and thrips species information.

Data analysis

Analysis of data obtained from the morphometrics study was performed using R version

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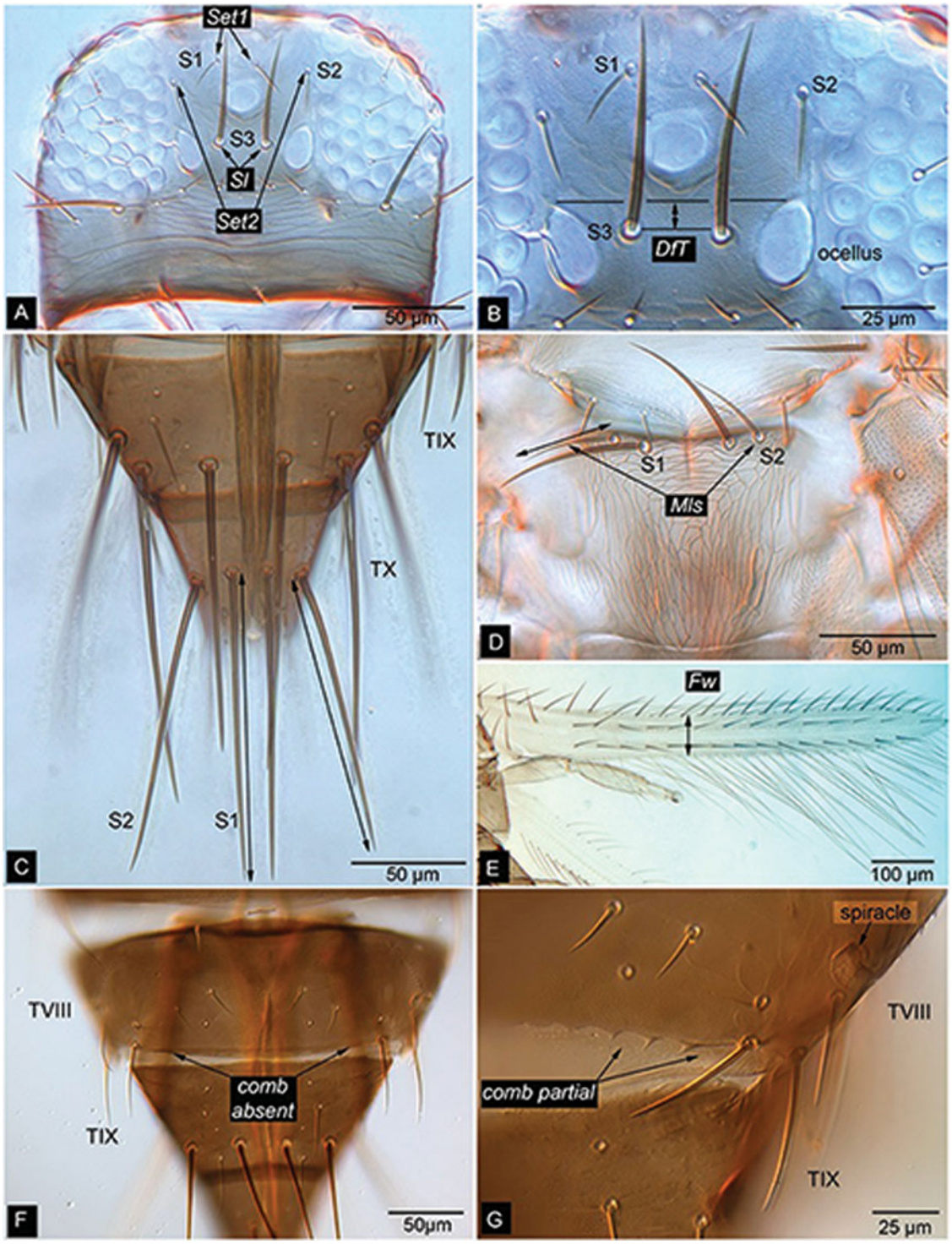

Fig. 1. Morphological features used in morphometric study. (A) *Set1, Set2* and *Sl*, first, second and third ocellar setae length, respectively; (B) *DfT*, distance of third ocellar setae from tangent of anterior margin of hind ocellar; (C) TIX and TX, Tergite 9 and 10, respectively; S1 and S2, TX major setae length (S=seta); (D) *Mls,* metanotal lateral setae length; (E) *Fw*, forewing width; (F–G) *Comb*, absence of comb and presence of partial comb, respectively; TVIII, tergite 8.

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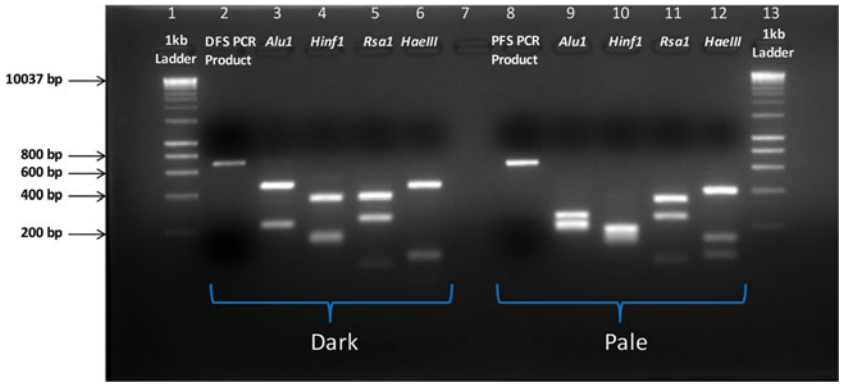

Fig. 2. ITS2-RFLP banding profile of dark and pale forms of *Frankliniella schultzei*. (Lanes 1 and 13) 1Kb ladder; (lanes 2 and 8) ITS2 PCR Product of dark and pale forms, respectively; (lanes 3–6 and 9–12) restriction of ITS2 region of dark and pale forms, respectively using restriction enzymes *Alu1*, *Hinf1*, *Rsa1* and *HaeIII*.

3.1.3 (R Core Team, 2015). Normality of data was checked using the Shapiro–Wilk test. Multiple comparisons of mean of the morphometric features between parents (dark and pale) and respective F1 off spring (dark male × dark female and dark male × pale female) were made using ANOVA in Microsoft® Excel 2007/XLSTAT©-Pro (Version 6.1.9, 2003, Addinsoft, Inc., Brooklyn, NY, USA). The level of significance was fixed at 95%. Principal component analysis (PCA) was then conducted using R 3.1.3 (R Core Team, 2015) to determine the relationship between dark and pale *F. schultzei* parental populations, as well as between dark *F. schultzei* males from the colony and *F. schultzei* males from *I. setosa*. This was determined, based on the clustering of the eight morphometric variables measured. ITS2 DNA sequences were analysed by first editing and aligning them using Geneious® version 8.0.2 (Kearse *et al.*, 2012). Consensus sequences were deposited in the GenBank and accession numbers were obtained. Homology searches of the ITS2 sequences against DNA databases were performed using nBLAST (http://www.ncbi.nlm.nih.gov/blast). Multiple sequence analysis of both dark and pale *F. schultzei* parental ITS2 sequences were conducted using Clustal W2 (Larkin *et al.*, 2007). Pairwise sequence divergence was calculated using the Kimura two parameter model (Kimura, 1980) in MEGA 6 (Tamura *et al.*, 2013). To visualize the patterns of divergence, a neighbour-joining tree (Saitou and Nei, 1987) was constructed from the evolutionary distances using a bootstrap of 1000 replicates and complete deletion option. The genetic distances were also used to generate a principal component plot using GenAlEx 6.41 (Peakall and Smouse, 2006). The descriptive statistical analysis on host plant and geographic preference were performed using Microsoft® Excel 2007/XLSTAT©-Pro (Version 6.1.9, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

Results

Biological characterization – interbreeding

Virgin dark *F. schultzei* produced only male F1 offspring. Virgin pale *F. schultzei* produced only female F1 offspring. The dark male × dark female produced dark F1 offspring, both male and female at a ratio of 1:1. Pale male × dark female produced only pale female F1 offspring (Table 1).

Morphological characterization

Multiple comparison of mean of morphometric features

Analysis of the parents revealed that there was significant difference between the two colour forms for *Set1* ($F_{1,38} = 32.29$, $P < 0.0001$); *Set2* ($F_{1,38} = 87.60$, $P < 0.0001$); *Sl* ($F_{1,38} = 187.38$, $P < 0.0001$); *Terg10* ($F_{1,38} = 51.53$, $P < 0.0001$); *DfT* ($F_{1,38} = 115.06$, $P < 0.0001$); *Mls* ($F_{1,38} = 29.31$, $P < 0.0001$) and *Fw* ($F_{1,38} = 21.30$, $P < 0.0001$). Post-hoc comparison using the Tukey HSD test indicated that the dark *F. schultzei* parents had significantly longer *Set1* (0.108 ± 0.004), *Set2* (0.123 ± 0.003), *Sl* (0.337 ± 0.005), *Terg10* (1.019 ± 0.012) as compared to the pale parents *Set1* (0.078 ± 0.005), *Set2* (0.086 ± 0.004), *Sl* (0.240 ± 0.007) and *Terg10* (0.900 ± 0.017). It was observed that the dark parents had significantly shorter *Mls* (0.130 ± 0.002), *Fw* (0.117 ± 0.002) and *DfT* (0.059 ± 0.003) as compared to the pale parents *Mls* (0.145 ± 0.003), *Fw* (0.130 ± 0.003) and *DfT* (0.103 ± 0.004) (Fig. 3).

Further analysis of parents compared to their F1 offspring revealed significant differences amongst the parents and F1 for *Sl* ($F_{3,91} = 74.85$, $P = < 0.0001$) and *DfT* ($F_{3,91} = 20.80$, $P = < 0.0001$). Post-hoc comparison using the Tukey HSD test indicated that *Sl* of dark parents (0.337 ± 0.005) was significantly longer than that of the F1 offspring of dark male × pale female cross (0.249 ± 0.012). Pale parents

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Table 1. Interbreeding of *Frankliniella schultzei* colour forms

Parents	Sex of F1 offspring	Sex ratio (female: male) of F1 offspring	Colour of F1 offspring	*Sl* and *DfT* of F1 offspring
Virgin dark female	Male	0:1	All dark	Similar to parents
Virgin pale female	Female	1: 0	All pale	Similar to parents
Dark male × dark female	Female and male	1:1	All dark	Similar to parents
Dark male × pale female	Female	1:0	All pale	Similar to pale mother

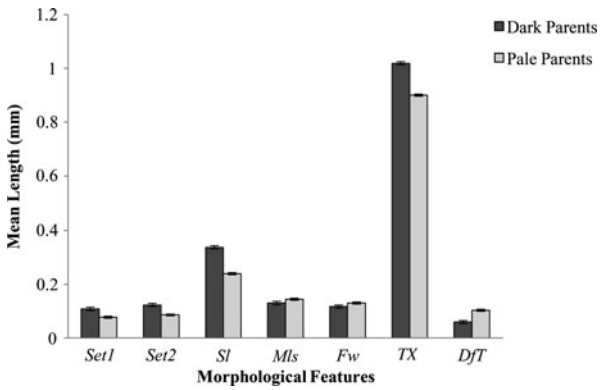

Fig. 3. Mean length of seven quantitative morphological features of parental populations of the two colour forms of *Frankliniella schultzei*.

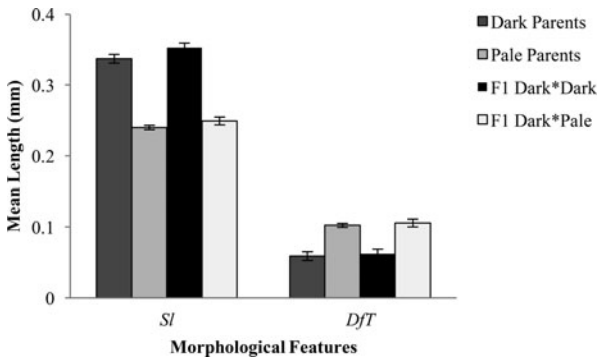

Fig. 4. Mean length of third ocellar setae (*Sl*) and distance of the third ocellar setae from the tangent of the anterior margin of the hind ocellar (*DfT*) of parental populations of the two colour forms of *Frankliniella schultzei* compared to their F1 offspring.

(0.240 ± 0.007) had significantly shorter *Sl* compared to F1 offspring of the dark male × dark female cross (0.353 ± 0.009). *Sl* was also significantly longer in the F1 offspring of the dark male × dark female cross (0.353 ± 0.009) than those from the dark male × pale female cross (0.249 ± 0.012). No significant differences were found in *Sl* between dark parents (0.337 ± 0.005) and dark male × dark female F1 offspring (0.353 ± 0.009) as well as between pale parents (0.240 ± 0.007) and dark male × pale female F1 offspring (0.249 ± 0.012) (Fig. 4). The *DfT* was significantly shorter in dark *F. schultzei* parents (0.059 ± 0.003) as compared to the F1 offspring from the dark male × pale female cross (0.106 ± 0.009). Pale parents (0.103 ± 0.004) had significantly longer *DfT* than the F1 offspring of the dark male × dark female cross (0.062 ± 0.007). Dark male × dark female offspring (0.062 ± 0.007) had significantly shorter *DfT* than dark male × pale female F1 offspring (0.106 ± 0.009). No significant difference in *DfT* was found between dark parents (0.059 ± 0.003) and F1 offspring of the dark male × dark female cross (0.062 ± 0.007) or between pale parents (0.103 ± 0.004) and F1 offspring of the dark male × pale female cross (0.106 ± 0.009) (Fig. 4).

Appearance of *Comb* in the dark form was either absent or incomplete with one or two pairs of marginal microtrichia while the pale form had an incomplete marginal *Comb* with three pairs of microtrichia (Fig. 1).

Principal component analysis of morphometric data

The first two principal components contributed to 94.1% of the total variance (PC1 = 86.9 and PC2 = 7.2). The scatter plot developed using the loadings generated by PC1 and PC2 showed significant differences between dark and pale *F. schultzei* as the two forms separated into two distinct clusters (Fig. 5). The four most different contributing features were the length of the major setae at tergite 10, length of the third ocellar setae, second ocellar setae length and distance of third ocellar setae from tangent of anterior margin of hind ocellar, each contributing 79, 52.5, 19.3 and 18% towards the distinct clustering of the colour forms, respectively.

Molecular characterization

ITS2-RFLP analysis

The ITS2 region of both dark and pale *F. schultzei* parents produced fragments of ~700 bp. Digestion of their respective amplicons using *Alu*1, *Hinf*1, *Rsa1* and *Hae*III restriction enzymes yielded differences in banding patterns and fragment sizes between the two colour forms (Fig. 2). Restriction by *Alu1* produced two bands in both colour forms, with a

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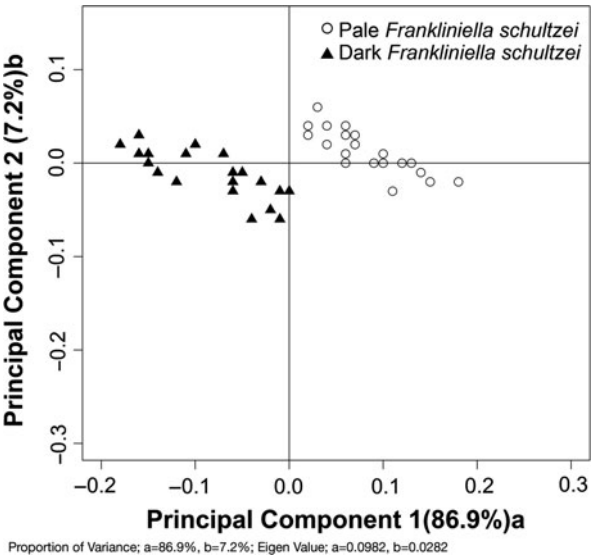

Fig. 5. Principal component analysis of morphometric character states of dark and pale forms of *Frankliniella schultzei.*

common ~200 bp band but the dark form had a second ~500 bp band, while the second band for the pale form was ~250 bp. There were also differences in the bands produced by *Hinf1* and *Rsa1* in both colour forms, with both colour forms having similar first bands of ~150 bp and ~250 bp in these two enzymes, respectively. However, restriction with *Hinf1* resulted in a second band of ~350 bp for the dark form, while the pale form had a second band of ~200 bp. Similarly, in *Rsa1,* the dark form had a second band of ~400 bp while the pale form had a second band of ~350 bp. Restriction with *HaeIII* resulted in a distinct band of ~150 bp for the pale form which was absent in the dark form.

Nucleotide sequence variation

The sequences obtained in this study were deposited in the GenBank; accession numbers KR105630–KR105633 for dark forms and accession numbers KR105634–KR105637 for pale forms. Multiple alignments of sequences from the dark and pale forms of *F. schultzei* in Clustal W2 showed consistent nucleotide differences between the two forms (Fig. 6). Distance estimates from the sequences obtained were used to generate a neighbour-joining tree which separated the dark and pale *F. schultzei* into two clades (Fig. 7). The genetic distance between the two colour forms ranged between 0.18 and 0.23. The average distance between the individuals within the same colour form was estimated between 0.00 and 0.01 among the dark forms and 0.00 and 0.04 among the pale forms, respectively (Table 2). The PCA of the genetic

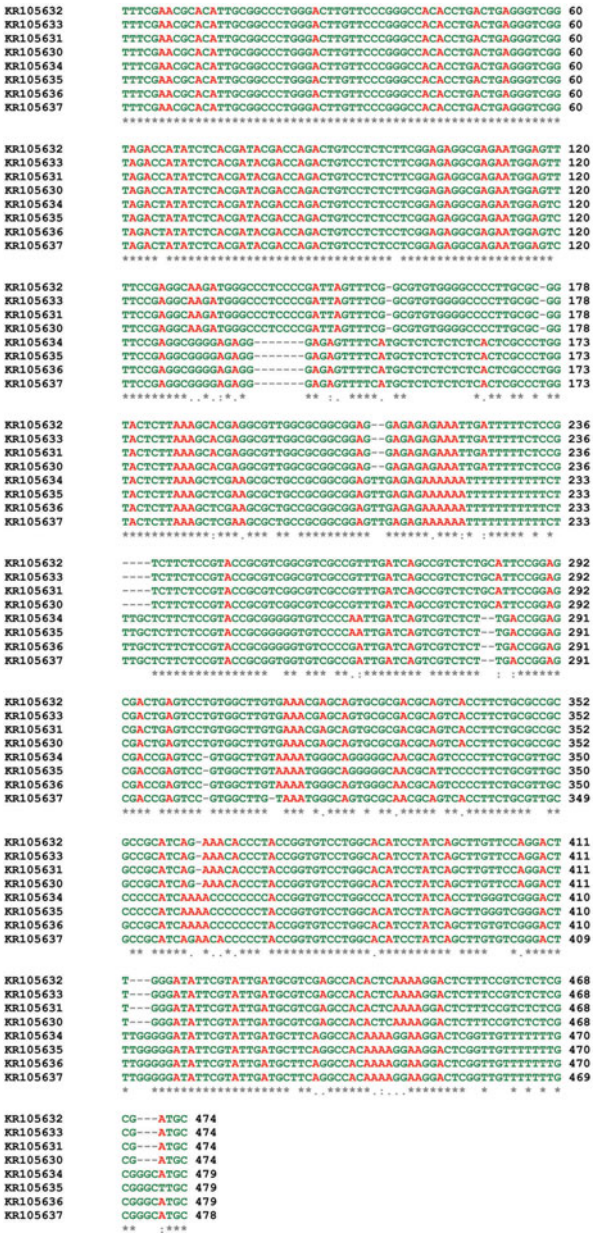

Fig. 6. Multiple sequence alignment of a 498 bp section from the ITS2 region. Dark (KR105630 – KR105633) and pale (KR105634 – KR105637) *Frankliniella schultzei* sequences.

distances resulted into two distinct clusters of the two colour forms (Fig. 8).

Confirmation of the absence of pale F. schultzei males in the environment

PCA conducted on specimens collected in the wild showed that there was no significant difference between males from the colony of dark *F. schultzei*

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Table 2. Genetic distances of *Frankliniella schultzei* ITS2 sequences. Dark (KR105630 – KR105633) and pale (KR105634 – KR105637)

	KR105630	KR105632	KR105633	KR105631	KR105634	KR105635	KR105636	KR105637
KR105630	0.00							
KR105632	0.00	0.00						
KR105633	0.00	0.01	0.00					
KR105631	0.01	0.00	0.01	0.00				
KR105634	0.23	0.23	0.23	0.23	0.00			
KR105635	0.22	0.22	0.22	0.22	0.02	0.00		
KR105636	0.20	0.20	0.20	0.20	0.03	0.02	0.00	
KR105637	0.19	0.19	0.19	0.18	0.04	0.04	0.02	0.00

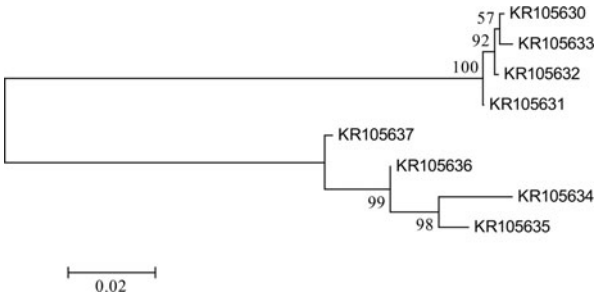

Fig. 7. Unrooted neighbour-joining tree of *Frankliniella schultzei* constructed from Kimura two-parameter distance model. Dark (KR105630 – KR105633) and pale (KR105634 – KR105637).

and those found in *I. setosa* flowers, the two populations clustered together (Fig. 9). These results were also supported by comparison of the ITS2-RFLP banding pattern produced by restriction of the ITS2 region of males collected from *I. setosa* flowers and males from the dark *F. schultzei* colony by restriction enzyme *Alu*1 (Thermo Scientific, Inc). All wild males had banding patterns identical to those of dark males in the laboratory colony (Fig. 10).

Ecological characterization

Host plant and geographical preference

Among the 1383 *F. schultzei* specimens observed from the thrips collections at *icipe*, the proportion of dark *F. schultzei* (68%) exceeded that of the pale *F. schultzei* form (32%). The dark colour form was predominant in Western and Nyanza provinces, which are dry to sub-humid mid-altitude regions in the Lake Victoria basin. The proportion of pale *F. schultzei* population increased gradually towards the coast with highest proportions in the low altitude hot and humid coastal province followed by the warm and dry medium altitude region in the Eastern Province (Fig. 11). The host plants with the highest proportion of dark *F. schultzei* were *Phaseolus* sp., *Cucurbita* sp. and *Ipomoea* sp. while those with the highest populations of pale forms were *Capsicum* sp., *Senna* sp. and *Ajuga remota* Benth (Labiatae). Host plants that had nearly equal proportions of both colour forms of *F. schultzei* were *Bidens pilosa* L. 1753, *Brassica oleracea* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai and *Solanum lycopersicum* L. (Fig. 12). The population of the dark colour form increased with altitude and is most prominent at altitudes of 700−1900 m. Above 1300 m, the population of the pale colour form decreased as altitude increased (Fig. 13).

Discussion

Thysanopterans are a highly diverse group of organisms with over 5200 recognized species (Moritz *et al.*, 2000). Within the group, subtle variations in the morphological character states delineate differences in species (Mound, 2002). Within thrips species, a high degree of variability in morphological character states have been reported in *Frankliniella occidentalis* (Pergande) (Chumak, 2014; Cluever *et al.*, 2015) and *Scirtothrips dorsalis* Hood (Rugman-Jones *et al.*, 2006; Hoddle *et al.*, 2008*a*; Dickey *et al.*, 2015). Similarly populations of some thrips species have been shown to differ in their modes of reproduction and host associations, as has been shown for *Thrips tabaci* (Jenser and Szénási, 2004; Jenser *et al.*, 2006; Westmore *et al.*, 2013; Nault *et al.*, 2014). The CBT, *F. schultzei* also exhibits a high degree of variability in terms of morphological characters (Johansen, 2002; Cavalleri and Mound, 2012) and populations have been grouped into pale and dark colour forms (Mound, 1968). However, the taxonomic status of the two colour forms of *F. schultzei* has been a subject of debate among thrips biologists. Some biologists, e.g., Bhatti *et al.* (2009), considered the pale form of *F. schultzei* as a separate species, *F. sulphurea* (Schmutz, 1913). Others consider the two colour forms as one species since they occur sympatrically with overlapping host plants (Mound, 1968; Hoddle *et al.*, 2008*b*). Apart from the differences in colour, analysis of eight morphometric character states undertaken in

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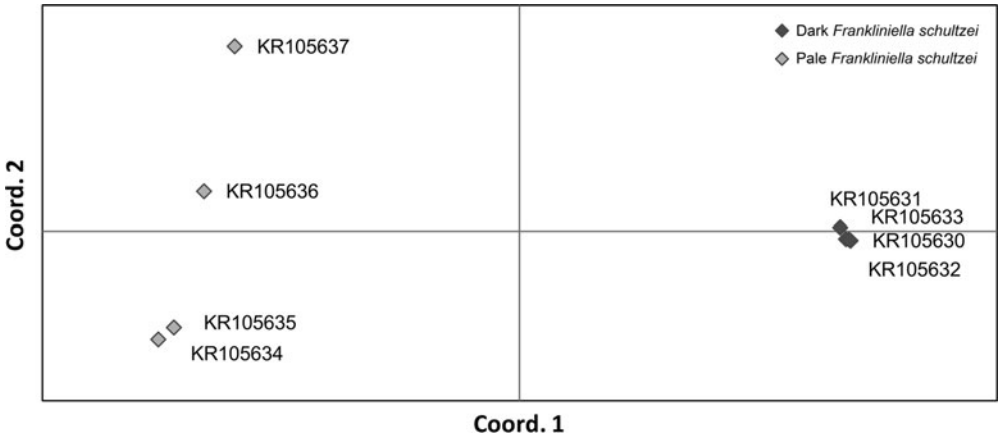

Fig. 8. Principal component analysis of genetic distances between dark and pale forms of *F. schultzei.* Dark (KR105630 – KR105633) and pale (KR105634 – KR105637) *Frankliniella schultzei.*

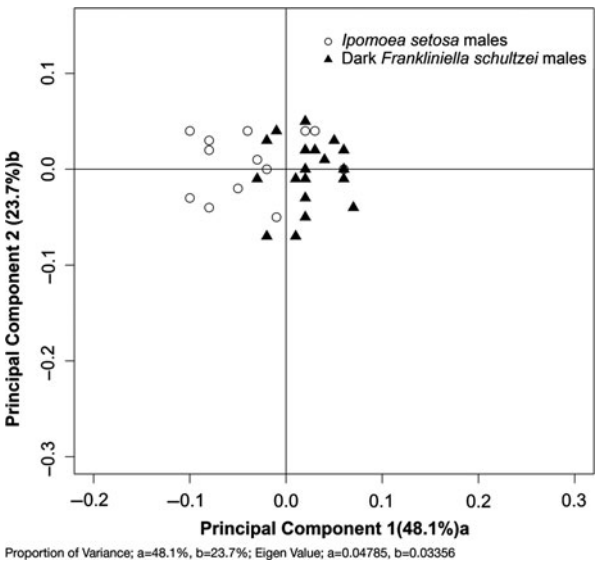

Fig. 9. Principal component analysis of morphometric characters of males from *Ipomoea setosa* and dark *Frankliniella schultzei* males from the laboratory colony.

this study revealed significant differences between the two colour forms. The length of the major setae at tergite 10, length of the third and second ocellar setae and distance of third ocellar setae from the tangent to the anterior margin of hind ocellar were key character states that differentiated the two colour forms.

In addition to differences for morphological character states, the two colour forms of *F. schultzei* exhibited significant variations at molecular level with genetic distances of up to 23% between them, grouping them into two distinct clades. Considering the fact that the ITS region has a high inter-specific variability and low intra-specific variability (Moritz *et al.*, 2001), the high level of molecular differences between the two colour forms of *F. schultzei* in the ITS regions argues strongly that they are two different species. The non-coding and highly evolving ITS2 region has been used to identify closely related thrips species (Toda and Komazaki, 2002; Rugman-Jones *et al.*, 2006; Farris *et al.*, 2010).

The pale and dark forms of *F. schultzei* have an allopatric distribution (Sakimura, 1969) with Kenya, Uganda, Sudan, India, New Guinea and Puerto Rico as regions where both colour forms are found (Mound, 1968; Kakkar *et al.*, 2014). In allopatric populations, genetic divergence usually leads to evolution of barriers (Gavrilets, 2004) which in turn could become a hindrance to interbreeding and reproductive isolation, therefore, contributing to speciation (Bush, 1975). Our study did not find evidence of pale *F. schultzei* males either in the colony or in the wild. The absence of males needs to be further confirmed in other host plants and regions. Circumstantial evidence for absence of males in *F. schultzei* is given by Wijkamp *et al.* (1995), where the population of pale *F. schultzei* used for transmission studies had only females, while the population of dark *F. schultzei* had both males and females. The absence of pale males and emergence of female progenies from virgin pale female indicates a thelytokous form of parthenogenetic reproduction in pale *F. schultzei*. On the other hand, emergence of male progenies from virgin dark females indicates an arrhenotokous form of parthenogenetic reproduction in dark *F. schultzei.* Endosymbionts such as *Wolbachia* spp. (Kumm and Moritz, 2008) are known to influence parthenogenetic reproduction. This needs to be further investigated with the two colour forms of *F. schultzei.* Emergence of female progenies similar to the female parent in dark male × pale female crosses indicates the existence of barriers to interbreeding between the colour

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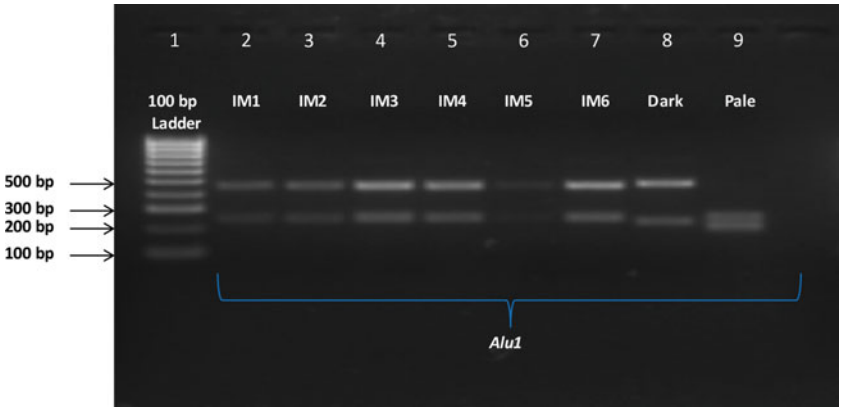

Fig. 10. ITS2-RFLP banding profile of males from *Ipomoea setosa*. (Lane 1) 100 bp ladder; (lanes 2–7) Restricted ITS2 region of males from *Ipomoea setosa* digested using restriction enzyme *Alu1*; (lane 8) dark *F.schultzei* male from colony; (lane 9) pale *F. schultzei* female from colony.

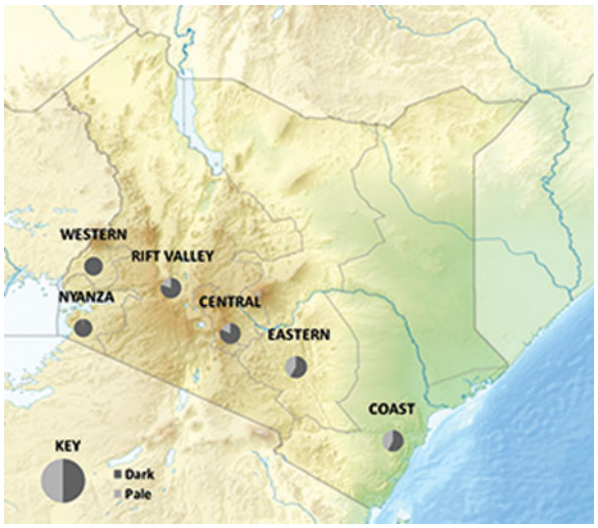

Fig. 11. Proportion of dark and pale *Frankliniella schultzei* in different provinces of Kenya.

forms and further strengthens the evidence for thelytokous reproduction in pale *F. schultzei.* The allopatric distribution of the two colour forms might have created differences in their mate-choosing response systems, creating a pre-mating barrier and eventually preventing interbreeding within the same ecosystem (Templeton, 1989; Grant and Grant, 2009) as observed in this study. In recent studies with *F. occidentalis*, Rugman-Jones *et al.*, (2010) found evidence of reproductive isolation in biologically and morphologically diverse sympatric populations of *F. occidentalis.* This was based on differences in nuclear as well as mitochondrial gene sequences, inferring that the diverse populations could be two cryptic species.

From an ecological perspective, the distribution of two colour forms appears to be influenced by the climate and host plant. Pale forms were more prevalent in the low altitude, hot and humid regions of the coast and warm and dry mid-altitudes of Eastern Province of Kenya, while the dark forms were prevalent in the high altitude, cool and wet regions of Central Province and in the warm and humid medium altitude regions of Western and Nyanza provinces of Kenya. The differences in agroecological preferences of the two colour forms could be related to their host plant preference. Host plants such as beans (*Phaseolus* sp.) and cucurbits (*Cucurbita* sp.) that are widely grown in high altitude regions with cool temperatures are predominantly infested by dark forms. Plants such as *Capsicum* sp., *Senna* sp. and *Ajuga remota* Benth, which thrive well in the low altitudes with warm and humid conditions were predominantly infested by the pale forms. Differences in prevalence of pale and dark *F. schultzei* might be related to variations in temperature in the different agro-ecologies. Temperature and colour of the insects influenced the body temperatures and thereby the insect physiology and their abundance. For instance, Murai and Toda (2001) reported that at lower rearing temperatures, *T. tabaci* adults were darker as compared to paler thrips when reared at higher temperatures. Similarly, in montane areas often characterized by cool temperatures, the dark forms of *F. occidentalis* were frequently observed (Hoddle *et al.*, 2008*b*). Dark forms of *F. occidentalis* were more abundant during the cooler months, while pale forms were abundant during the warmer months (Elimem *et al.*, 2011).

The morphometric, molecular, ecological and host plant differences between the dark and pale forms of *F. schultzei* (together with their inability to interbreed) lead us to conclude that the colour forms represent distinct species. Further investigation on the presence/absence of pale *F. schultzei* males in

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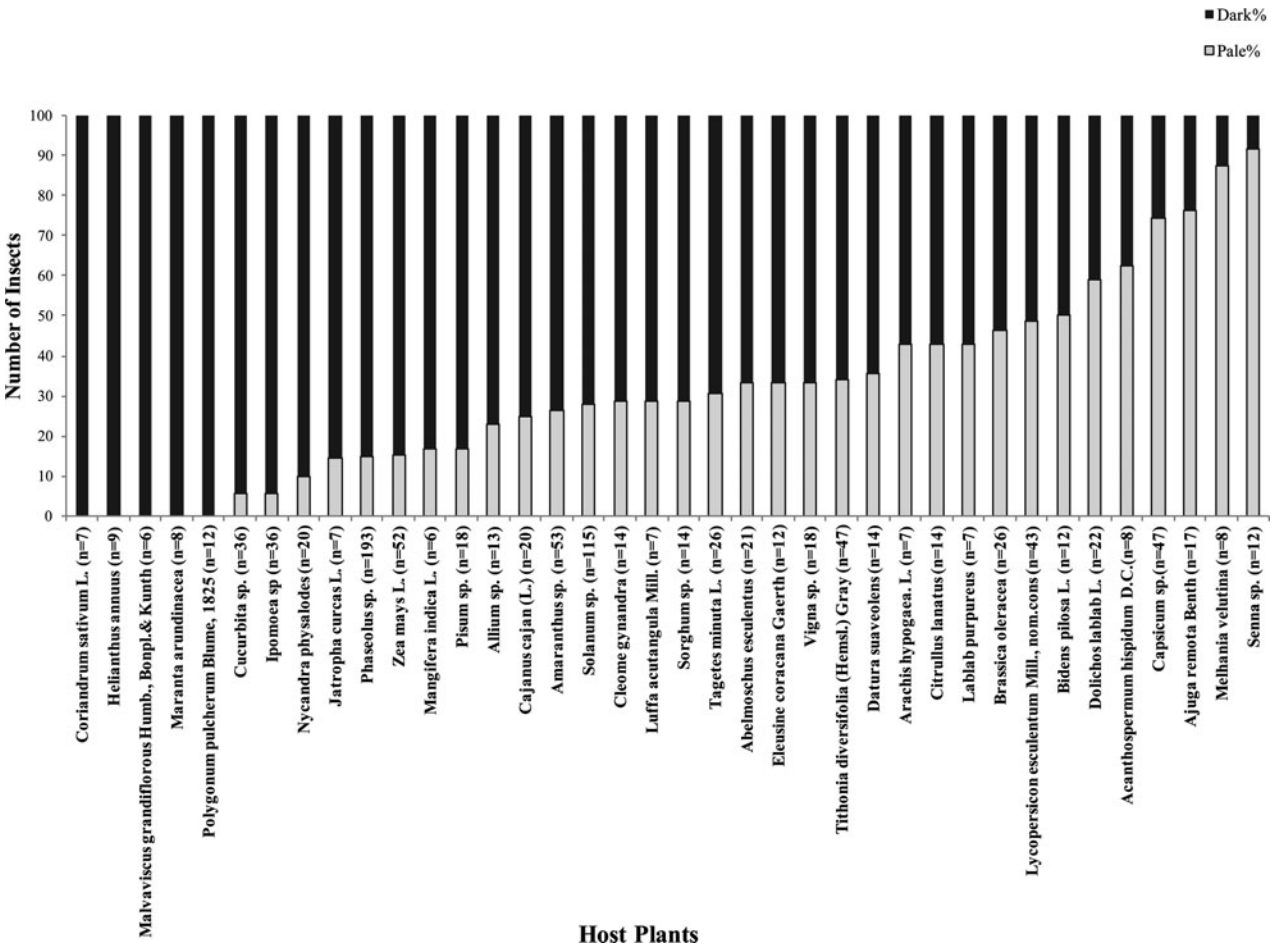

Fig. 12. Proportion of dark and pale forms of *Frankliniella schultzei* collected from different host plants in Kenya.

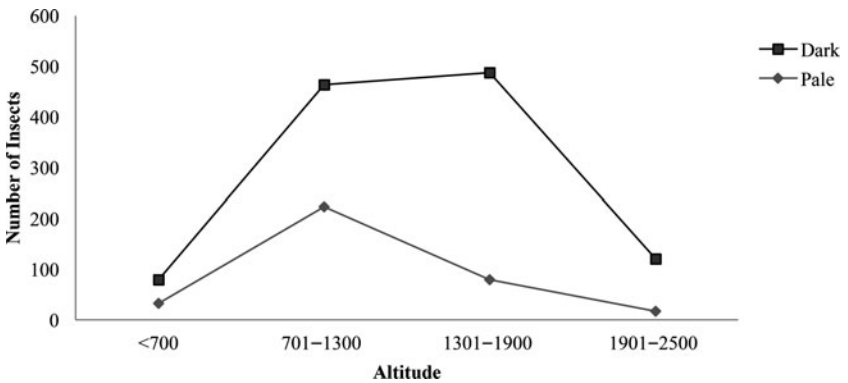

Fig. 13. Numbers of dark and pale forms of *Frankliniella schultzei* collected from different altitudinal classes in Kenya.

different regions and host plants, factors influencing the modes of parthenogenetic reproduction, differential response to climatic variables, and vector competence could provide valuable insights into the divergence of the two species. This study also highlights the importance of the integrative taxonomic approach for conclusively resolving the species status of closely related population of thrips. Effective management of insect pests begins with their unambiguous and accurate identification.

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References

Bhatti J., Alavi J., zur Strassen R. and Telmadarraiy Z. (2009) Thysanoptera in Iran 1938–2007. An overview. Part 1. *Thrips* 7–8, 1–373.

Bush G. L. (1975) Modes of animal speciation. *Annual Review of Ecology and Systematics* 6, 339–364.

Campbell B. C., Steffen-Campbell J. D. and Werren J. H. (1994) Phylogeny of the Nasonia species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Molecular Biology* 2, 225–237.

Cavalleri A. and Mound L. A. (2012) Toward the identification of *Frankliniella* species in Brazil (Thysanoptera, Thripidae). *Zootaxa* 30, 1–30.

Cho J. J., Mau R., Hamasaki R. and Gonsalves D. (1988) Detection of tomato spotted wilt virus in individual thrips by enzyme-linked immunosorbent assay. *Phytopathology* 78, 1348–1352.

Chumak P. Y. (2014) Biomorphological variability of *Frankliniella occidentalis* Pergande in relation to its invasion in greenhouses of Kyiv. *Russian Journal of Biological Invasions* 5, 56–60.

Cluever J. D., Smith H. A., Funderburk J. E. and Frantz G. (2015) Western flower thrips (*Frankliniella occidentalis*. *ENY 883(IN108900): Series of the Entomology and Nematology Department, UF/IFAS Extension*: 1–8. http://edis.ifas.ufl.edu/pdffiles/IN/IN108900.pdf.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051-07-0. http://www.R-project.org

Dayrat B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85, 407–415.

Despres L., Imbert-Establet D. and Monnerot M. (1993) Molecular characterization of mitochondrial DNA provides evidence for the recent introduction of *Schistosoma mansoni* into America. *Molecular and Biochemical Parasitology* 60, 221–230.

Dickey A. M., Kumar V., Hoddle M. S., Funderburk J. E., Morgan J. K., Jara-Cavieres A., Shatters R. G. J., Osborne L. S. and McKenzie C. L. (2015) *The Scirtothrips dorsalis* species complex: endemism and invasion in a global pest. *PLoS ONE* 10, e0123747. doi: 10.1371/journal.pone.0123747.

Elimem M., Harbi A. and Chermiti B. (2011) Evaluation of *Frankliniella occidentalis* different body colours and their development in a pepper crop greenhouse in the region of Moknine in Tunisia. *Bulletin of Insectology* 64, 9–13.

Farris R. E., Ruiz-Arce R., Ciomperlik M., Vasquez J. D. and Deleón R. (2010) Development of a ribosomal DNA ITS2 marker for the identification of the thrips, *Scirtothrips dorsalis*. *Journal of Insect Science* 10, 1–15.

Garza R., Ruiz R. and Ciomperlik M. A. (2007) Molecular identification for economically important thrips species, *Journal of Insect Science* 7 (28), 12. In *Proceedings of the VIII International symposium on Thysanoptera and Tospoviruses* September 11–15, 2005; Asilomar, Pacific Grove, California (ed Ullman D., Moyer J., Goldbach R. and Moritz G.).

Gavrilets S. (2004) *Fitness Landscapes and the Origin of Species*. Princeton University Press, Princeton, New Jersey. 476 pp.

Grant P. R. and Grant B. R. (2009) The secondary contact phase of allopatric speciation in Darwin's finches. *Proceedings of the National Academy of Sciences of the United States of America* 106, 20141–20148.

Hamodi A. A.-F. and Abdul-Rassoul M. S. (2004) Keys for identification of genera and species of thrips (Thysanoptera : Thripidae) from Middle of Iraq. *Bulletin of Iraq National History Museum* 10, 9–37.

Hoddle M. S., Heraty J. M., Rugman-Jones P. F., Mound L. A. and Stouthamer R. (2008a) Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. *Annals of the Entomological Society of America* 101, 491–500.

Hoddle M., Mound L. and Paris D. (2008b) Thrips of California. Cd-rom published by CBIT, Brisbane. http://keys.lucidcentral.org/keys/v3/thrips_of_california/Thrips_of_California.html

Jenser G., Lipcsei S., Szénási Á. and Hudák K. (2006) Host range of the arrhenotokous populations of *Thrips tabaci* (Thysanoptera: Thripidae). *Acta Phytopathologica et Entomologica Hungarica* 41, 297–303.

Jenser G. and Szénási Á. (2004) Review of the biology and vector capability of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Acta Phytopathologica et Entomologica Hungarica* 39, 137–155.

Johansen R. M. (2002) The Mexican *Frankliniella fusca* (Hinds), *F. pallida* (Uzel) and *F. schultzei* (Trybom) species assemblages, in the 'intonsa group' (Insecta, Thysanoptera: Thripidae). *Acta Zoológica Mexicana n.s.* 85, 51–82.

Kakkar G., Seal D. R., Jha V. K. and Bagnall F. (2014) Common blossom thrips, *Frankliniella schultzei* Trybom (Insecta : Thysanoptera : Thripidae). *EENY 477(IN860): Series of the Entomology and Nematology Department, UF/IFAS Extension*: 1–5. http://edis.ifas.ufl.edu/in860 [accessed on 23 November 2013]

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. and

Drummond A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28, 1647–1649.

Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–120.

Kumm S. and Moritz G. (2008) First detection of *Wolbachia* in arrhenotokous populations of thrips species (Thysanoptera: Thripidae and Phlaeothripidae) and its role in reproduction. *Environmental Entomology* 37, 1422–1428.

Larkin M. A., Blackshields G., Brown N. P., Chenna R., Mcgettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R., Thompson J. D., Gibson T. J. and Higgins D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948.

Mfuti D. K., Subramanian S., van Tol R. W., Wiegers G. L., de Kogel W. J., Niassy S., du Plessis H., Ekesi S. and Maniania N. K. (2016) Spatial separation of semiochemical Lurem-TR and entomopathogenic fungi to enhance their compatibility and infectivity in an autoinoculation system for thrips management. *Pest Management Science* 72, 131–139.

Moritz G. (1997) Structure, growth and development, pp.15–63. In *Thrips as Crop Pests* (edited by T. Lewis). CAB International, Cambridge, UK.

Moritz G., Brandt S., Triapitsyn S. and Subramanian S. (2013) Identification and information tools for Pest thrips in East Africa. QBIT, QAAFI, UQ. ISBN 978-1-74272-067-8. http://thripsnet.zoologie.uni-halle.de/key-server-neu/data/03030c05-030b-4107-880b-0a0a0702060d/media/Html/index.html

Moritz G., Delker C., Paulsen M., Mound L. A. and Burgermeister W. (2000) Modern methods in thrips identification and information (Insecta: Thysanoptera). *EPPO Bulletin* 30, 591–593.

Moritz G., Paulsen M., Delker C., Picl S. and Kumm S. (2001) Identification of thrips using ITS-RFLP analysis. In *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera* (eds Marullo R. and Mound L. A.), pp. 365–367.

Mound L. A. (1968) A review of R. S. Bagnall's Thysanoptera collections. *Bulletin of the British Museum (Natural History)* (Ent.) suppl. 11, pp. 1–181.

Mound L. A. (2002) Thysanoptera biodiversity in the Neotropics. *Revista de Biología Tropical* 50, 477–84.

Murai T. and Toda S. (2001) Variation of *Thrips tabaci* in colour and size, pp. 377–378. In *Proceedings of the Seventh International Symposium on Thysanoptera*. 2 July–7 July 2001, Reggio, Calabria, Italy, Commonwealth Scientific and Industrial Research Organisation.

Nagata T. and de Ávila A. C. (2000) Transmission of Chrysanthemum stem necrosis virus, a recently discovered tospovirus, by two thrips species. *Journal of Phytopathology* 148, 123–125.

Nault B. A., Kain W. C. and Wang P. (2014) Seasonal changes in *Thrips tabaci* population structure in two cultivated hosts. *PLoS ONE* 9, e101791. doi: 10.1371/journal.pone.0101791

Niassy S., Maniania N. K., Subramanian S., Gitonga L. M. and Ekesi S. (2012) Performance of a semiochemical-baited autoinoculation device treated with *Metarhizium anisopliae* for control of *Frankliniella occidentalis* on French bean in field cages. *Entomologia Experimentalis et Applicata* 142, 97–103.

Nyasani J. O., Kusia E. S. and Subramanian S. (2015) Thrips as pests and vectors of Maize chlorotic mottle virus, p. 49. In *Proceedings of the Tenth International Symposium on Thysanoptera and Tospoviruses*. 16 May–20 May 2015, Asilomar, California, Entomological Society of America.

Nyasani J. O., Meyhöfer R., Subramanian S. and Poehling H. M. (2012) Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. *Entomologia Experimentalis et Applicata* 142, 236–246.

Nyasani J. O., Meyhöfer R., Subramanian S. and Poehling H. M. (2010) Thrips species composition and abundance on French beans, associated crops and weed species in Kenya. *Journal of Insect Science* 10, 166. In *Proceedings of the Ninth International Symposium on Thysanoptera and Tospoviruses* 31 August–4 September 2009, Queensland, Australia.

Peakall R. and Smouse P. E. (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.

Pearsall I. A. and Myers J. H. (2000) Population dynamics of western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. *Journal of Economic Entomology* 93, 264–275.

Retana-salazar A. P., Cambero-campos O. J. and Sánchez A. (2010) Key to the Central American and Caribbean species of the *Frankliniella minuta* group (Thysanoptera: Thripidae) with the description of a new species. *Métodos en Ecología y Sistemática* 5, 27–35.

Rugman-Jones P. F., Hoddle M. S., Mound L. A. and Stouthamer R. (2006) Molecular identification key for pest species of Scirtothrips (Thysanoptera: Thripidae). *Journal of Economic Entomology* 99, 1813–1819.

Rugman-Jones P. F., Hoddle M. S. and Stouthamer R. (2010) Nuclear-mitochondrial barcoding exposes the global pest Western flower thrips (Thysanoptera: Thripidae) as two sympatric cryptic species in its native California. *Journal of Economic Entomology* 103, 877–886.

Saitou N. and Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406–425.

Sakimura K. (1969) A comment on the color forms of *Frankliniella schultzei* (Thysanoptera: Thripidae) in relation to transmission of the tomato spotted wilt virus. *Pacific Insects* 1, 761–762.

Schmutz K. (1913) Zur Kenntnis der Thysanopterenfauna von Ceylon. Sitzungsber. Kaiserl. Akad. Wiss., Math.-naturwiss. Kl. 72 (7), 991–1089.

Subramanian S., Kumm S., Nyasani J. O., Waiganjo M., Muchemi S. and Moritz G. (2012) Diversity, distribution and plant association of thrips belonging to *Frankliniella* genus in East Africa. Abstract No. O301TU12 in *Proceedings of the twenty fourth International Congress of Entomology*. 19 August−25 August 2012, Daegu, South Korea, Entomological Society of South Korea.

Tamura K., Stecher G., Peterson D., Filipski A. and Kumar S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.

Templeton A. R. (1989) The meaning of species and speciation: A genetic perspective, pp. 3−27. In *Speciation and its Consequences* (edited by D. Otte and J. A. Endler). Sinauer Associates Inc., Sunderland, Massachusetts.

Toda S. and Komazaki S. (2002) Identification of thrips species (Thysanoptera: Thripidae) on Japanese fruit trees by polymerase chain reaction and restriction fragment length polymorphism of the ribosomal ITS2 region. *Bulletin of Entomological Research* 92, 359–363.

Wang C. L., Lin F. C., Chiu Y. C. and Shih H. T. (2010) Species of *Frankliniella* Trybom (Thysanoptera: Thripidae) from the Asian-Pacific Area. *Zoological Studies* 49, 824–838.

Westmore G. C., Poke F. S., Allen G. R. and Wilson C. R. (2013) Genetic and host-associated differentiation within *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and its links to Tomato spotted wilt virus-vector competence. *Heredity* 111, 210–215.

Wijkamp I., Almarza N., Goldbach R. and Peters D. (1995) Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology* 85, 1069–1074.