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ORIGINAL CONTRIBUTION

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Exploring levels of egg parasitism and variation in egg cuticular chemistry in different *Clavigralla* spp.

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Abstract

Clavigralla spp. (Hemiptera: Coreidae) are major pests of cowpea (*Vigna unguiculata* (L.) Walp, Fabacae), common bean (*Phaseolus vulgaris* L., Fabacae) and pigeon pea (*Cajanus cajan* L., Fabacae) in Africa. *Clavigralla* spp. egg parasitoids, *Gryon* spp. (Hymenoptera: Scelionidae), have previously been reported as potential biological control candidates. Little is known about the parasitism levels and their potential relationship with cuticular chemistry of *Clavigralla* spp. The aims of this study were to determine parasitism levels of *Clavigralla tomentosicollis* Stål (Hemiptera: Coreidae) and *C. elongata* Signoret (Hemiptera: Coreidae) eggs, and to explore the relationship between egg parasitism and egg cuticular chemistry. High parasitism levels were determined for *C. tomentosicollis* by collecting eggs from plants in mono-cropping and multi-cropping systems in farmers' fields in Bénin and Kenya between April and June 2016. Three species of *Clavigralla* were recorded: *C. tomentosicollis*, *C. shadabi* and *C. elongata*. *Clavigralla tomentosicollis* was the most common in both countries, while *C. shadabi* and *C. elongata* were only collected in Bénin and Kenya, respectively. An egg parasitoid (*Gryon* sp.) was recovered from egg batches collected from both countries. In parasitism assays using *Gryon* sp., the incidence of parasitism was higher in *C. tomentosicollis* eggs than that of *C. elongata*. Chemical analysis by coupled gas chromatography/mass spectrometry (GC/MS) of cuticular extracts obtained from *C. tomentosicollis* and *C. elongata* eggs identified fifteen compounds including ten alkanes of which the amounts varied between the two species. We speculate that *Clavigralla* spp. cuticular chemistry may serve as potential host location cues for *Gryon* sp.

KEYWORDS
egg parasitoid, occurrence, parasitism, pod sucking bug, semiochemical cues

1 | INTRODUCTION

The tribe Clavigrallini, which includes *Clavigralla* Spinola, is comprised of two genera and 44 species (Dolling, 1979). Commonly referred to as pod sucking bugs or brown spiny bugs, *Clavigralla tomentosicollis* Stål, *C. shadabi* Dolling and *C. elongata* Signoret (Hemiptera: Coreidae) all belong to the species complex that attack grain legume crops in Africa. These species occur widely in Nigeria, Burkina-Faso, Niger, Bénin, Tanzania and Kenya where they are the major pests of cowpea and French bean (Minja et al., 1999; Agunbiade et al., 2013; Chalam et al., 2016). *Clavigralla tomentosicollis* and *C. shadabi* were

The research was conducted at International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya.

previously recorded in some regions of Bénin (Dreyer, Baumgärtner, & Tamó, 1994; Shanower, Romeis, & Minja, 1999; Egho, 2010; Agboton et al., 2014). Gethi and Khaemba (1991) reported a high prevalence of *C. tomentosicollis* and *C. shadabi* on cowpea in maize intercropping systems in Mombasa, Kenya. *Clavigralla* spp. prefer to feed on legume pods and have been reported to cause yield losses of up to 90% and reduce seed viability of up to 85% (Dreyer et al., 1994; Abate & Ampofo, 1996; Koona et al., 2001; Oparaeke, 2006a, 2006b; Soyelu & Akingbohungbe, 2007; Dabire-Binso, Ba, Sanon, Drabo, & Bi, 2010; Dialoke et al., 2010).

Cultural control practices, pesticide applications and resistant crop varieties have in the past been used in the management of *Clavigralla* spp., but these were largely unsuccessful (Jackai & Adalla, 1997; Adipala, Nampala, Karungi, & Isubikalu, 2000; Koona, Osisanya, Jackai, Tamò, & Markham, 2002; Aliyu, Ladan, Ahmed, & Abdullahi, 2007). Several studies previously reported on the potential of egg parasitoids as biological control agents for pod sucking bugs eggs in Africa (Taylor, 1975; Asante, Jackai, & Tamò, 2000). For example, in northern Nigeria, *Gryon fulviventris* (Crawford) (Hymenoptera: Scelionidae), *Ooencyrtus utetheisae* (Risbec) (Hymenoptera: Encyrtidae) and *Anastatus* sp. (Hymenoptera: Eupelmidae) were reported to parasitize eggs of *C. tomentosicollis* (Asante et al., 2000). The same study reported that *C. tomentosicollis* eggs were the most parasitized by *G. fulviventris*, which suggests that the latter species could potentially be used as a biological control agent for *Clavigralla* spp. (Asante et al., 2000). *Gryon gnidus* (Nixon) (Hymenoptera: Scelionidae), another egg parasitoid of *C. tomentosicollis*, was reported in Nigeria by Taylor (1975) while Shanower, Anitha, Bhagwat, and Dreyer (1996) reported in Kenya and Tanzania that *Gryon clavigrallae* (Mineo) (Hymenoptera: Scelionidae) also parasitized eggs of *Clavigralla* spp.

Previous chemical ecology studies have documented that chemical cues are used by egg parasitoids in host location, foraging behaviour and parasitism. This has been demonstrated for *Nezara viridula* L. (Hemiptera: Pentatomidae) (Bin, Vinson, Strand, Colazza, & Jones Jr, 1993), *Earias vittella* Fab. (Lepidoptera: Noctuidae) and *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) (Maruthadurai, Gautam, & Mahesh, 2011) in their egg location by the egg parasitoid *Trichogramma brasiliensis* Ashmead (Hymenoptera: Trichogrammatidae). Likewise, *Trissolcus brochymenae* Ashmead (Hymenoptera: Platygastridae) is known to exploit *Murgantia histrionica* Hahn (Hemiptera: Pentatomidae) egg volatiles during host location and recognition (Conti, Salerno, Bin, Williams, & Vinson, 2003). Furthermore, volatiles from egg masses of *Orgyia postica* Walker (Lepidoptera: Lymantriidae) were found to attract the egg parasitoid *Telenomus euproctidis* Ratzeburg (Hymenoptera: Platygastridae) (Arakaki, Yamazawa, & Wakamura, 2011). These observations suggest that cuticular chemistry may play a role in parasitoid–pest interactions.

Despite the economic importance of *Clavigralla* spp. in Bénin and Kenya, little information exists on parasitoid–pest interactions. Furthermore, no studies have investigated the influence of *Clavigralla* spp. egg-derived chemicals in *Gryon* spp. foraging behaviour and parasitism. The aims of this study were threefold: to carry out morphological and genetic identification of the egg parasitoid recovered from *Clavigralla* spp. eggs; to determine levels of parasitism of different *Clavigralla* spp. eggs in the laboratory; and to determine whether egg cuticular chemistry differed between the different *Clavigralla* spp.

2 | MATERIALS AND METHODS

In Kenya, sampling was done during the long rains (April to May 2016) in the following six counties: Western Kenya (Kisumu), Rift Valley (Nakuru) and Eastern Kenya (Embu, Kitui, Machakos and Makueni). In Bénin, samples were collected during the long rainy season (May to June 2016) in ten villages from four regions: Southern Bénin (Abomey-Calavi), Eastern Bénin (Ketou, Pobe), Western Bénin (Klouekanme) and Central Bénin (Dassa-Zounme, Djidja). These counties and regions are considered as the main grain legume production areas in both countries (Minja et al., 1999; Gbaguidi, Dansi, Loko, Dansi, & Sanni, 2013; Ayenan, Ofori, Ahoton, & Danquah, 2017). The geographical coordinates were recorded at each site (Table 1).

2.1 | Sample collection

Collection of *Clavigralla* species and their eggs were carried out during the pod filling stage of the different crops which is the preferred phenological stage for attacking the crops (Koona et al., 2001). Eggs, nymphs and adults of *Clavigralla* spp. were collected in both mono-cropping and multi-cropping systems of French bean and pigeon pea that were in some cases associated with maize in Kenya. Collections in Bénin were done in mono-cropping systems of cowpea and pigeon pea. Each site was visited once in the morning (7:30–11:00) and in the afternoon (15:00–18:00). Visual inspection of plants was conducted to search for different life stages of these pests. At each site, two fields were visited. All specimens were placed individually into small cages (13.5 cm diameter and 12.0 cm height) that contained pods of plant hosts.

Ten egg batches collected from each site were incubated separately under laboratory conditions at 25 ± 1°C and 60%–70% RH, with a photoperiod of 12:12 hr (light: dark) until nymphs hatched or parasitoids emerged. The number of the emerged *Gryon* sp. per site was recorded. This parasitoid species that emerged from eggs were morphologically identified using the taxonomic keys presented by Masner (1975, 1976) and photographed by taxonomist Dr. Elijah Jacob Talamas, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL USA. Molecular techniques were used to confirm the morphological identity.

2.2 | DNA extraction, PCR and sequencing

The identity of the *Gryon* sp. samples collected was confirmed using PCR of the D2 region of 28S rDNA (28S) in the mitochondria. Genomic DNA from 95% ethanol-preserved specimens was extracted using

TABLE 1 Localities at which *Clavigralla tomentosicollis*, *Clavigralla shadabi* and *Clavigralla elongata* were recorded in Kenya and Bénin

		Clavigralla spp. collected			Coordinates of sampling		
Localities	**Villages**	***C. tom.***	***C. shad.***	***C. elong.***	**Latitude**	**Longitude**	**Elevation (m a.s.l.)**
Kenya							
Embu	Gatirari	+	–	–	S 00°40.532'	E 037°39.187'	1,060
	Jagawneth	+	–	+	S 00°44.847'	E 037°36.151'	1,049
Kisumu	Obino	+	–	+	S 00°05.066'	E 034°52.478	1,170
Kitui	Kithinzi	+	–	+	S 01°18.155'	E 038°02.019'	1,251
Machakos	Kitimani	+	-	-	S 01°10.060'	E 037°27.287'	1,228
Makueni	Kaiani	+	–	–	S 01°52.621'	E 037°42.793'	1,113
Nakuru	Kirobon	+	–	–	S 00°18.345'	E 035°59.224'	1,930
	Wata	+	-	+	S 00°16.413'	E 036°07.172'	1,883
	Karagita	+	–	+	S 00°48.170'	E 036°26.918'	2,001
Benin							
Abomey-Calavi	IITA Station	+	+	–	*N* 06°25.100'	E 002°19.925'	18
Dassa-zounme	Afossogbe	+	–	–	*N* 07°34.382'	E 002°11.195'	137
	Ganfon	+	-	-	*N* 07°49.371'	E002°08.399'	128
Djidja	Oumgbega	+	–	–	*N* 07°17.051'	E 002°02.420'	253
	Assantoun	+	–	–	*N* 07°17.704'	E 002°03.109'	259
	Dridji	+	+	–	*N* 07°23.801'	E 022°05.048'	167
Kétou	Camp	+	–	–	*N* 07°18.509'	E 002°37.424'	131
	Aguidi	+	+	–	*N* 07°18.543'	E 002°31.583'	68
Klouékanmey	Adja-hounmey	+	–	–	*N* 07°02.672'	E 001°47.592'	225
Pobè	Itchagba	+	–	–	*N* 07°06.705'	E 002°38.722'	34
	Occurrence level	**	*	*			

Note: (+) = present; (–) = absent; (*) = present; (**) = very present.
C. tom., *Clavigralla tomentosicollis*, *C. shad.*, *Clavigralla shadabi*, *C. elong.*, *Clavigralla elongate*.

high-quality DNA extraction for RT-PCR and Sequencing kit (Qiagen) as per manufacturer's instructions. The purity and concentration of the resultant extracted DNA were determined using Nanodrop 2000/2000c Spectrophotometer (Thermo Scientific). The PCR was carried out in a total reaction volume of 20 µl containing 5X My*Taq* Reaction Buffer (5 mMdNTPs, 15 mM $MgCl_2$, stabilizers and enhancers), 10 µmole of each primer (LepD2-Fw5'AGTCGTGTTGCTTGATAGTGCAG3' and LepD2 Rv5'TTGGTCCGTGTTTCAAGACGGG3' (Campbell, Steffen-Campbell, & Werren, 1994; Goolsby et al., 2006)), 0.5 mM $MgCl_2$, 0.25 µl My*Taq*DNA polymerase (Bioline, London, UK) and 15 ng/µl of DNA template. This reaction was set up in the Nexus Mastercycler gradient (Eppendorf). The following cycling conditions were used: initial denaturation for 2 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s annealing at 58.8°C and 1 min at 72°C, then a final elongation step of 10 min at 72°C. The target gene region was 700 base pairs. The amplified PCR products were resolved through a 1.2% agarose gel and bands analysed and documented using KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks). Successfully amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc. Europe Laboratory, the Netherlands, for bi-directional sequencing, and the platform used was Illumina. The successful sequences were assembled and edited using ChromasLite version 2.1.1 (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) and Geneious version 8 (http://www.geneious.com) (Kearse et al., 2012). The sequence identities were determined using Basic Local Alignment Search Tool (BLAST) hosted by the National Center for Biotechnology Information (NCBI).

2.3 | Laboratory rearing of egg parasitoid species and *Clavigralla* species

To aid laboratory assays, the parasitoid and the *Clavigralla* species *C. tomentosicollis* and *C. elongata* were reared in the insectary at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus, Nairobi, Kenya. Rearing was done at 25 ± 1°C and 60%–70% RH with a photoperiod of 12:12 hr (Light: Dark). Egg batches collected from different localities in Kenya were incubated separately in sterile clear plastic cages (9.0 cm diameter x 4.5 cm height) (Foodmate 0.5 L, Kenpoly Nairobi, Kenya), with ventilated lids. Emerged parasitoids were collected by means of an aspirator

and introduced into a cage containing *C. tomentosicollis* eggs that were less than 48 hr old. Parasitoids were fed on droplets of a 90% honey solution. In Bénin, field-collected eggs were incubated in the same way as described above, after which emerged parasitoids were put into vials containing 95% ethanol for molecular/morphological identification. All rearing was done following the same conditions described above.

Clavigralla tomentosicollis and *C. elongata* were reared on young healthy pods of French bean in cylindrical clear plastic cages (18 cm diameter x 6.5 cm high) (Foodmate 2 L, Kenpoly) with a ventilated lid. The cages were lined with paper towel to absorb excess moisture and bug excretions. Each cage contained five fresh pods of French bean. Thirty to forty adults were introduced into each cage using a fine brush and an aspirator. Batches of fifty eggs that were laid on the absorbent paper were transferred to new cages of the same dimensions every 48 hr. Any dead insects were removed from the containers.

2.4 | Parasitism bioassays

Parasitism levels of *C. tomentosicollis* and *C. elongata* eggs were studied under laboratory conditions as previously described by Asante et al. (2000). Each egg batch used contained 30 eggs. The experiment consisted of two treatments for each species: (a) one unparasitized egg batch of *C. tomentosicollis* or *C. elongata* eggs (control) and (b) one parasitized egg batch of *C. tomentosicollis* or *C. elongata* eggs (test). Each treatment was replicated five times with one egg batch per replicate. Thirty fresh unparasitized eggs (1 day old) of *C. tomentosicollis* or *C. elongata* were carefully introduced into sterile clear plastic cages (9 cm diameter and 4.5 cm height) (Foodmate 0.5 L, [Foodmate 0.5 L, Kenpoly]) with ventilated lids. Five newly emerged *Gryon* sp. females selected were collected by means of an aspirator and introduced into each test cage which contained fresh unparasitized *C. tomentosicollis* or *C. elongata* eggs for a period of 12 hr (6:00 to 18:00 hr). After 12 hr, the parasitoids were removed. All egg batches were incubated for 15 days which exceeds the date of expected parasitoid emergence by 2 days. The number of parasitoids that emerged from eggs was recorded daily from 10 to 15 days after exposure to the parasitoids.

2.5 | Extraction of egg cuticular components

To obtain parasitized eggs, *C. tomentosicollis* and *C. elongata* eggs were exposed to five females of *Gryon* sp. using the methods described above. Batches of parasitized and unparasitized eggs were then used to obtain cuticular extracts following the method described by Holman, Jørgensen, Nielsen, and d'Ettorre (2010). The parasitized eggs were extracted immediately after 12 hr exposure to the parasitoids. Both, the parasitized and unparasitized egg batches of each species were placed separately into two storage vials (2 ml) containing 100 µl of pentane (HPLC Grade, Sigma-Aldrich, purity ≥ 99%.) for 12 min. The extract was collected using a microsyringe and transferred into two storage vials for GC/MS analysis. All the experiments were conducted in the insectary of the International Centre of Insect Physiology and Ecology, Duduville campus, Nairobi at 25 ± 1°C and 60%–70% RH with a photoperiod of 12:12 hr (light: dark) cycle.

2.6 | Chemical analysis

Each cuticular extract (1 µl) was analysed by coupled gas chromatography/mass spectrometry (GC/MS) on an Agilent Technologies Series A 7,890 GC coupled to a 5975C MS (inert XL/ EI/CI MSD) triple axis mass detector, equipped with an HP5 MS low bleed capillary column (30 m × 0.250 mm i.d, 0.25 µm) (J&W) in the electron impact mode at 70 eV. The GC oven temperature was 35°C for 5 min with a rise of 10°C/min to 280°C for 10.5 min, then 5°C/min to 285°C and held at this temperature for 9 min. Identification of compounds was done by comparison of mass spectral data with library data Adams2, Chemecol and NIST11. The experiments were replicated three times. Quantification was based on calibration curves (peak area vs. concentration) generated from authentic standards of identified compounds.

2.7 | Chemicals

The following synthetic standards hexadecane, hexadecanoic acid, hexadec-9-enoic acid, octadecane, nonadecane, eicosane, tricosane, tetracosane and pentacosane, hexacosane, and heptacosane were all purchased from Sigma-Aldrich, Germany (purity ≥ 97%).

2.8 | Data analysis

All statistical analyses were performed in R Development Core Team (2012) software version 3.1.2 at 5% significance level. Percentage parasitism was calculated for each treatment. Data on egg parasitism levels of the two *Clavigralla* spp. were analysed using a generalized linear model affirming quasi-binomial distribution error. Principal component analyses (PCA) were performed to compare the chemical composition of parasitized and unparasitized *C. tomentosicollis* and *C. elongata* egg cuticles.

3 | RESULTS

3.1 | *Clavigralla* spp. and parasitoid emergence from field-collected eggs

Three species of *Clavigralla* including *C. tomentosicollis*, *C. shadabi* and *C. elongata* were recorded. *Clavigralla tomentosicollis* was present at all the study sites in both countries, and it was also more abundant than *C. shadabi* and *C. elongata*. *Clavigralla shadabi* was only collected in Bénin at the following sites: Djidja (Dridji), Ketou (Aguidi) and Abomey-Calavi (IITA station). In Kenya, *C. elongata* was recorded at Embu (Jagawneth farm), Kisumu (Obino), Kitui (Kithinzi) and Nakuru (Wata and Karagita) (Table 1, Figure 1). The only egg parasitoid which emerged from the collected eggs in both countries was morphologically identified as

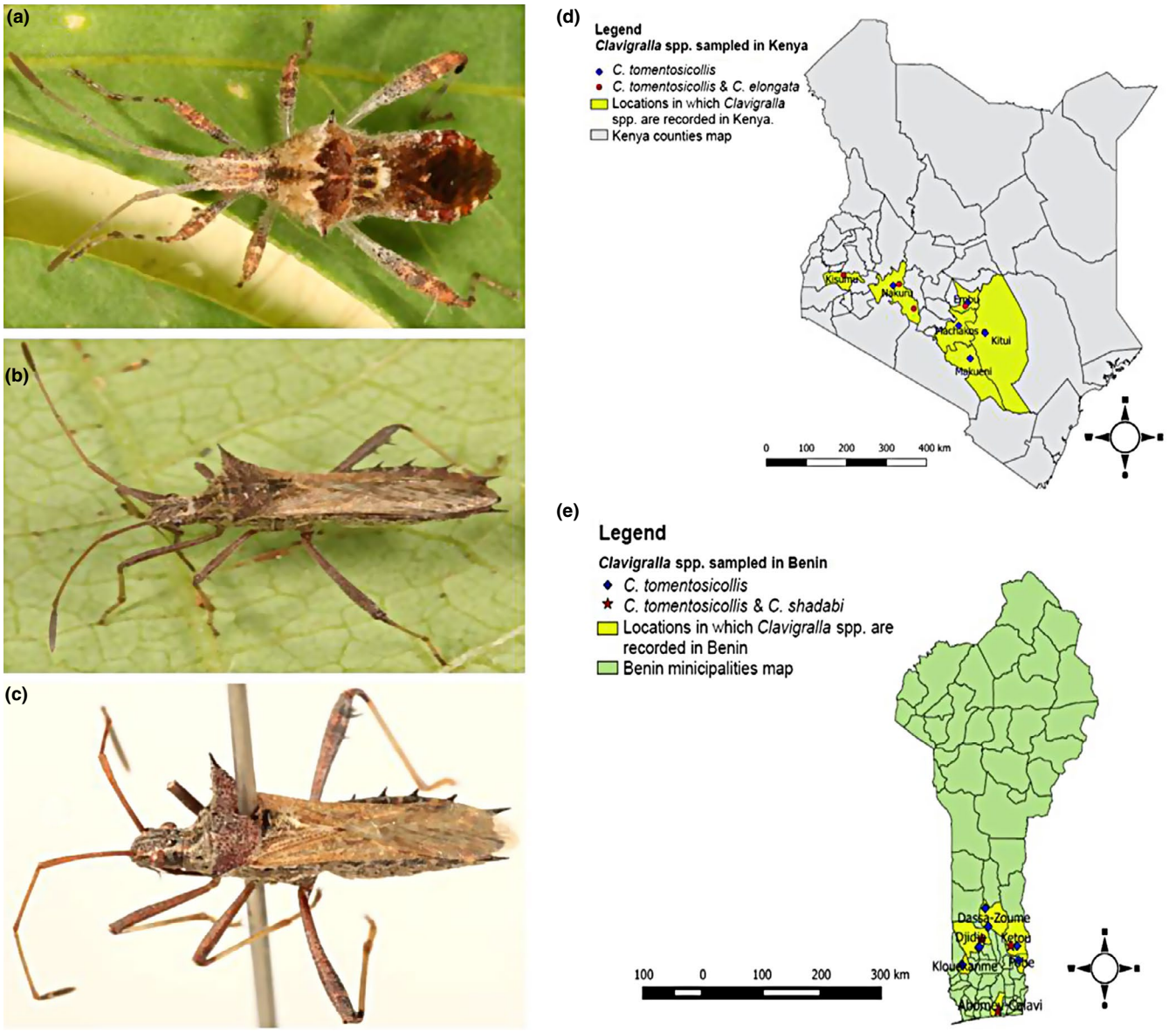

FIGURE 1 *Clavigralla* spp. and the localities they were collected from in Bénin and Kenya. (a) *Clavigralla tomentosicollis*; (b) *Clavigralla elongata*; (c) *Clavigralla shadabi*; (d and e) maps in yellow showing the different localities where sampling was done in Kenya (above) and in Bénin (below). The symbols "◆" and "★" indicate the species collected

Gryon sp. (Figure 2). In Bénin, parasitoids emerged from egg batches collected at the IITA station, Djidja (Dridji) and Ketou (Aguidi) were *Gryon* sp. Similarly, only *Gryon* sp. emerged from egg batches collected at the two sites (Machakos and Makueni) in Kenya. The numbers of emerged parasitoids recorded were, however, generally lower than those recorded in Bénin (Table 2). This preliminary data on field collection showed overall that the average number of emerged parasitoids per batch in Benin sites is two times greater than that of Kenya sites (Table 2).

3.2 | Identification of the parasitoid samples

BLAST query yielded 99% similarity of all the parasitoid sequences to *Gryon* sp. of accession number JX683193.1 (Table 3). This identity confirmed the morphological taxonomy, and which have been deposited in the GenBank with accession numbers (MK488003–MK488008). At present, the taxonomy of African *Gryon* lacks comprehensive and reliable identification tools, preventing us from attaching a species name to the species of *Gryon* reared in this study. The African species of *Gryon* are currently under revision by co-author (EJT), which includes the generation of a DNA barcode database for *Gryon* worldwide. Additional images of specimens from both the Bénin and Kenya colonies are available via the Hymenoptera Online Database (hol.osu.edu) and can be found by searching the specimen identification numbers (DPI_FSCA 00010233 and DPI_FSCA 00010182, respectively). As the revisionary work on *Gryon* progresses, identification of this species will be updated (Florida Department of Agriculture

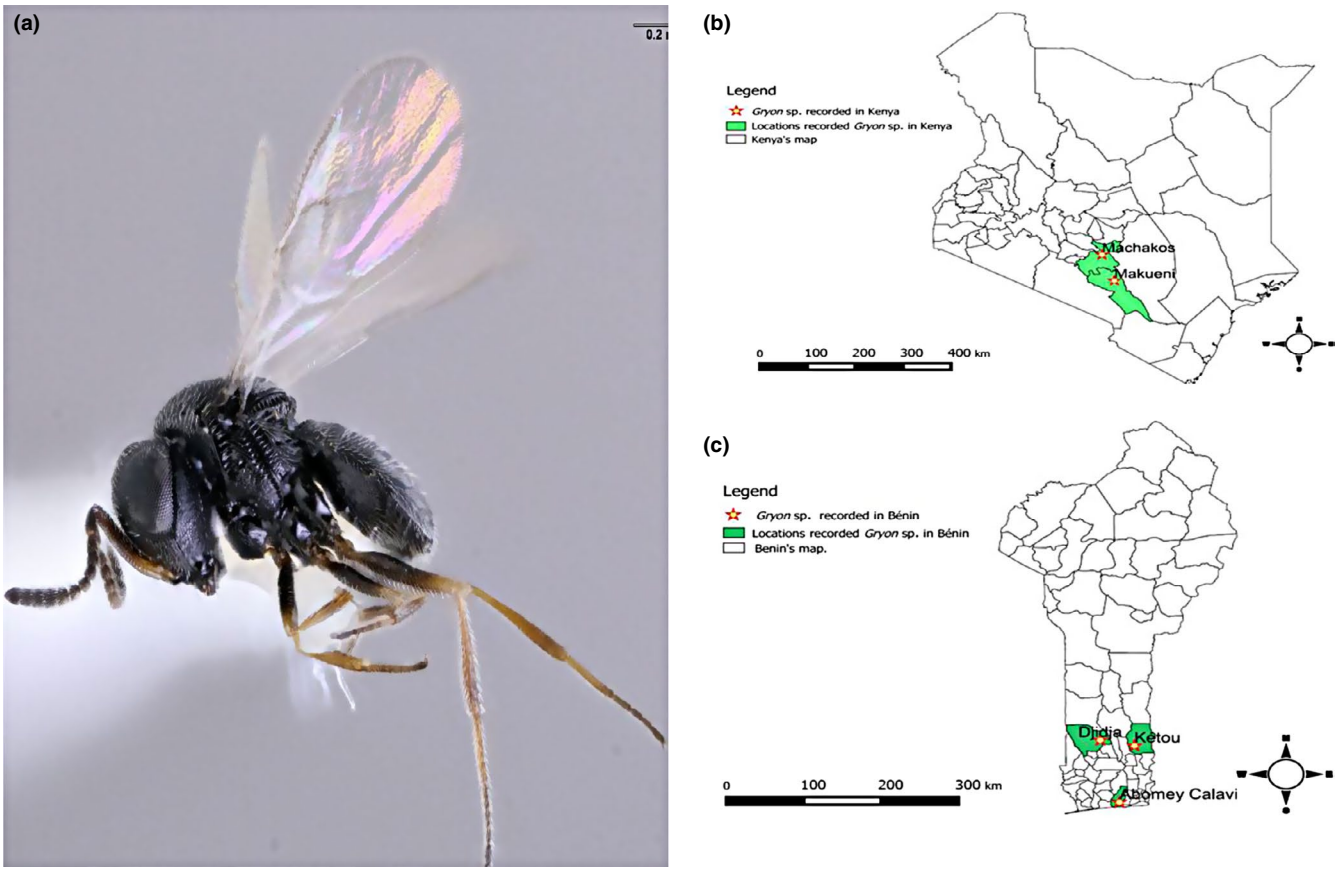

FIGURE 2 *Gryon* sp. recorded and locations they were collected from in Bénin and Kenya. (a) = *Gryon* sp. reared on *Clavigralla* spp. eggs, (b and c) = maps in green showing where *Gryon* sp. was collected in Kenya (above) and Bénin (below). Symbol "☆" indicates *Gryon* sp. recorded from *Clavigralla* spp. eggs

and Consumer Services, Division of Plant Industry, Gainesville, FL USA).

3.3 | Parasitism bioassays

There was a significant difference in the parasitism rate between *C. tomentosicollis* (mean percentage = 74.6 ± 7.2%) and *C. elongata* eggs (mean percentage = 35.3 ± 3.0%) ($F = 20.01$, $df = 8,1$; $p = 0.002$ (Figure 3).

3.4 | Chemical analysis

Overall, thirteen components were identified in the cuticular extracts of unparasitized and parasitized eggs of the two *Clavigralla* spp. These included two aliphatic acids, ten alkanes and one alkene whose identities were confirmed using authentic standards (Table 4; Figure 4). Four additional components were tentatively identified as phenols (two) an alkene and an alcohol based on mass spectral data only (Table 4). Differences between the cuticular chemistry of unparasitized and parasitized eggs were mainly quantitative rather than qualitative. Additionally, these differences varied within and between species and were, mainly in the dominant components, alkanes and their amounts (Table 4).

Principal component (PCA) analyses showed species separation of unparasitized and parasitized eggs (Figures 5 and 6) and were defined by seventeen cuticular components. The PCA biplot of the seventeen variables explained 77.1% (Dim 1 = 53.7% and Dim 2 = 23.4%) for unparasitized egg cuticular extract and 80.1% (Dim 1 = 57.1% and Dim 2 = 23%) for parasitized egg cuticular extract in the variance in the data set. Two groups were observed for each type of egg: group of specific cuticular extract components for the unparasitized *C. tomentosicollis* and *C. elongata* eggs and group of specific parasitized cuticular extract components for *C. tomentosicollis* and *C. elongata* eggs. Upon PCA examination, five cuticular hydrocarbons, two phenols and two aliphatic acids correlated with separation of *C. tomentosicollis* unparasitized eggs cluster from *C. elongata* unparasitized eggs (Figure 5a). The grouping of the parasitized specific cuticular extract components of *C. tomentosicollis* (heptacosane and eicosane) in the PCA separated this species from *C. elongata* (Figure 5b). Additionally, the heat map associated with the unparasitized and parasitized eggs cuticular extract components of the two species showed that the alkanes nonadecane, tricosane, tetracosane, hexacosane and the alcohols 2,4-bis(1-methyl-1-phenylethyl)- phenol, 2,4-bis(dimethylbenzyl)-6-t-butylphenol were present in high amounts in the cuticular extracts of unparasitized eggs of *C. tomentosicollis* but absent/trace from the

TABLE 2 Localities at which *Gryon* sp. were recorded from eggs of *Clavigralla tomentosicollis* in Bénin and Kenya and approximate numbers of *Gryon* sp. that emerged from field-collected eggs batches

Localities	Species collected	Coordinates		Elevation (m a.s.l.)	Number of *Gryon* sp. emerged from ten egg batches of *C. tomentosicollis* collected	Average of emerged parasitoids per batch
		Latitude	Longitude			
Kenya						
Machakos (Kitimani)	*Gryon* sp.	S 01°10.060'	E 037°27.287'	1,228	76	7.6
Makueni (Kaiani)	*Gryon* sp.	S 01°52.621'	E 037°42.793'	1,113	41	4.1
Bénin						
Abomey-Calavi	*Gryon* sp.	N 06°25.100'	E 002°19.925'	18	137	13.7
Djidja (Dridji)	*Gryon* sp.	N 07°23.801'	E 022°05.048'	167	119	11.9
Ketou (Aguidi)	*Gryon* sp.	N 07°18.543'	E 002°31.583'	68	63	6.3

cuticular extract of unparasitized eggs of *C. elongata*. Furthermore, eicosane and pentacosane were more concentrated in cuticular extract of unparasitized eggs of *C. tomentosicollis* than *C. elongata* unparasitized eggs, as well as hexadecanoic acid and octadecane which were present in high amounts in unparasitized cuticular egg extract of *C. tomentosicollis* and absent in the cuticular extract of unparasitized eggs of *C. elongata*. These compounds contributed to the separation of unparasitized eggs of the two species (Figure 6). The unidentified alkene, tricosane, tetracosane and squalene occurred in higher amounts in the cuticular extract of parasitized eggs of *C. elongata* than that of parasitized eggs of *C. tomentosicollis* and contributed largely to the separation of the two species. PCA analysis confirmed that most of the compounds were highly concentrated in the cuticular extract of unparasitized eggs than in cuticular extract of parasitized eggs of *C. tomentosicollis* but the contrary was observed for the cuticular egg extract of *C. elongata* (Figure 6).

4 | DISCUSSION

Field observations showed that *C. tomentosicollis* was the most common and abundant of the three *Clavigralla* spp. in Kenya and Bénin. *Clavigralla elongata* was recorded only in Kenya and *C. shadabi* at Djidja and Ketou in Bénin. The common and widespread occurrence of *C. tomentosicollis* in these different regions of Africa supports previous reports that these species occur in both east and west Africa (Dolling, 1979; Agunbiade et al., 2013; Chalam et al., 2016). Shanower et al. (1999) also reported *C. tomentosicollis* as a common species and *C. shadabi* as a pest of minor importance in Bénin and Nigeria. Our results support those by Dreyer and Baumgärtner (1996) which reported *C. tomentosicollis* and *C. shadabi* on cowpea in Bénin. In this study, *C. tomentosicollis* and *C. shadabi* were recorded throughout Bénin, confirming reports of its distribution across different agro-ecological zones (Dreyer et al., 1994; Agboton et al., 2014). Moreover, *C. shadabi* which was reported by Gethi and Khaemba (1991) in Mombasa, Kenya, was not recorded during this study, possibly due to geographical and seasonal differences, as well as the limited sampling that was done in Kenya. Nonetheless, our observation corroborates previous results which reported *C. tomentosicollis* and *C. elongata* on pigeon pea, hyacinth bean, gram and cowpea in Kenya (Khamala, 1978). Similar results were reported by Materu (1972) in Tanzania.

Gryon sp. was the only parasitoid species that emerged from *C. tomentosicollis* eggs in this study. We were not able to identify this egg parasitoid to species level because of the lack of reliable identification tools. Similar results were reported by Gariepy, Haye, and Zhang (2014) for some Scelionidae (*Telonomus* sp., *Trissolcus* sp.) and egg parasitoids of *Acrosternum hilare* Say (Hemiptera: Pentatomidae). Since that time, the generation of a DNA barcode database for *Trissolcus* Ashmead (Hymenoptera: Scelionidae) has enabled the development of molecular diagnostics that enable species to be identified based on the DNA barcode region.

TABLE 3 Results of *Gryon* sp. sequences data analyses

Sample name	ID from GenBank	Accession no.	Query %	E value	ID %
GRB–1-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99
GRB–3-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99
GRB–5-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99
GRK–8-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99
GRK–9-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99
GRK–10-D2	*Gryon* sp. CT–2012b voucher OSUC 266,775 28S ribosomal RNA gene, partial sequence	JX683193.1	76	0	99

Note: Abbreviations: GRB, *Gryon* from Bénin, GRK, *Gryon* from Kenya

This study showed that *Gryon* sp. seemed to be common in both Bénin and Kenya. This observation is consistent with previous studies, which showed that *G. fulviventris* was the most abundant egg parasitoid of *Clavigralla* spp. in northern Nigeria (Asante et al., 2000) compared to *O. utetheisae* and *Anastatus* sp. which were not recorded during this study. Previously, *G. gnidus* and *G. clavigrallae* have been reported as *Clavigralla* spp. egg parasitoids in Nigeria, Asia and India, respectively, and showed lower parasitism rates than that of *G. fulviventris* (Taylor, 1975; Shanower et al., 1996; Romeis, Shanower, & Madhuri, 2000). As reported from previous studies on *Gryon* spp., field parasitism rates can range from 69% to 74% (Taylor, 1975; Shanower et al., 1996; Asante et al., 2000). For example, the egg masses collected on cowpea in Nigeria showed that *G. fulviventris* parasitized up to 74% *C. tomentosicollis* eggs (Asante et al., 2000). Similar results have been reported for *G. gnidus* and *G. clavigrallae* showing that field parasitism rates of egg masses can reach 69% (Taylor, 1975; Shanower et al., 1996). Thus, although these observations suggest that *Gryon* sp. may be the principal and most common egg parasitoid of *Clavigralla* spp. in West Africa, future studies need to determine whether field parasitism rates of *Gryon* sp. in Benin and Kenya agree with this range (69%–74%).

Our results also showed that *Gryon* sp. parasitized higher numbers of *C. tomentosicollis* eggs than *C. elongata* eggs under laboratory conditions. This result confirms not only our field observations but also the parasitism rate of *G. fulviventris* reported in Nigeria on *C. tomentosicollis* eggs (Asante et al., 2000), and that of *G. clavigrallae* on *Clavigralla scutellaris* Spinola, and *C. gibbosa* (Westwood) (both Hemiptera: Coreidae) eggs in India (Romeis et al., 2000). This finding suggests that *C. tomentosicollis* eggs may contain contact chemical cues for parasitism by the parasitoid.

Chemical analysis showed significant quantitative differences in the profiles of unparasitized and parasitized eggs dominated by alkanes, which varied within and between the two species. Whereas these alkanes were upregulated in the cuticular profile of unparasitized eggs of *C. tomentosicollis*, they were downregulated in that of the eggs of *C. elongata*. The opposite pattern was observed in the parasitized eggs which may account for the higher parasitism of *Gryon* sp. of *C. tomentosicollis* eggs than those of *C. elongata*. Additional research will be needed across a wide range of samples collected from different localities and seasons in Kenya and Benin to confirm this suggestion and to investigate the possible contribution of background egg volatiles in parasitoid attraction. A previous study showed that the high concentration of hydrocarbons extracted from *E. vittella* larvae influenced the foraging behaviour and parasitism of *T. brasiliensis* (Maruthadurai

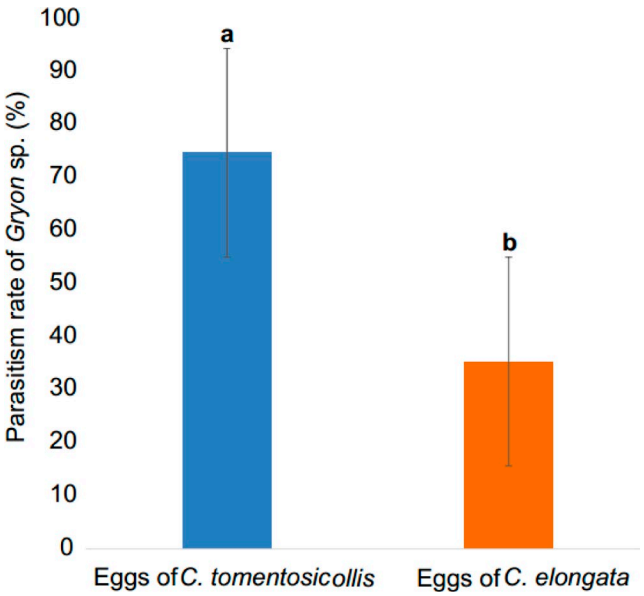

FIGURE 3 Comparison of parasitism rate of *Gryon* sp. ±SE (%) between *Clavigralla tomentosicollis* eggs and *Clavigralla elongata* eggs. Mean percentage was calculated for the parasitized eggs in five replications. Different letters "a" and "b" indicate significant differences

TABLE 4 Compounds identified in pentane extracts of parasitized and unparasitized eggs of *C. tomentosicollis* and *C. elongata*

Peak #	t_R(min)	Compound	Category	Retention Index	Mean amount detected (ng/egg/min ± *SE*)			
					C. elong. unparasitized eggs	*C. tom.* unparasitized eggs	*C. elong.* parasitized eggs	*C. tom.* parasitized eggs
1	22.82	Hexadec−9-enoic acid	Fatty acid	1848	tr	0.27 ± 0.09	-	-
2	22.99	Hexadecanoic acid	Fatty acid	1864	tr	0.28 ± 0.00	-	-
3	25.15	Octadecane	Alkane	2091	tr	0.06 ± 0.02	0.06 ± 0.02	tr
4	26.01	Nonadecane	Alkane	2185	tr	0.08 ± 0.00	-	-
5	26.05	Eicosane	Alkane	2189	0.06 ± 0.02	0.07 ± 0.03	tr	0.06 ± 0.00
6	27.47	Unidentified alkene[a]	Alkene	2357	-	-	0.08 ± 0.02	0.06 ± 0.00
7	27.49	Unidentified alcohol[a]	Alcohol	2359	0.27 ± 0.00	tr	-	-
8	27.63	Tricosane	Alkane	2376	tr	0.09 ± 0.03	0.07 ± 0.02	0.06 ± 0.00
9	27.79	2,4-bis(1-methyl−1-phenylethyl)- phenol[a]	Phenol	2395	tr	0.28 ± 0.00	-	-
10	27.93	2,4-bis(dimethylbenzyl)−6-t-butylphenol[a]	Phenol	2413	tr	0.29 ± 0.09	-	-
11	28.39	Tetracosane	Alkane	2470	tr	0.09 ± 0.03	0.07 ± 0.02	0.06 ± 0.02
12	29.18	Pentacosane	Alkane	2572	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
13	29.86	Hexacosane	Alkane	2659	tr	0.08 ± 0.00	0.07 ± 0.02	0.07 ± 0.00
14	30.14	Squalene	Alkene	2694	tr	0.06 ± 0.02	0.08 ± 0.01	0.07 ± 0.00
15	30.72	Heptacosane	Alkane	2860	0.06 ± 0.02	0.06 ± 0.02	tr	0.07 ± 0.02
16	32.82	Triacontane[a]	Alkane	2952	0.06 ± 0.02	tr	0.06 ± 0.02	0.06 ± 0.02
17	36.85	Hentriacontane[a]	Alkane	3832	0.06 ± 0.02	tr	0.07 ± 0.03	0.07 ± 0.02

Note: Identification based on comparison of retention time (RT) with mass spectral library data and retention index.
Quantification was based on calibration curves (peak area vs. concentration) generated from authentic standards of identified compounds;
(–), Not detected, tr, trace, *C. tom.*, *Clavigralla tomentosicollis*, *C. shad.*, *Clavigralla shadabi*, *C. elong.*, *Clavigralla elongata*
[a]compounds identified based on mass spectral data only.

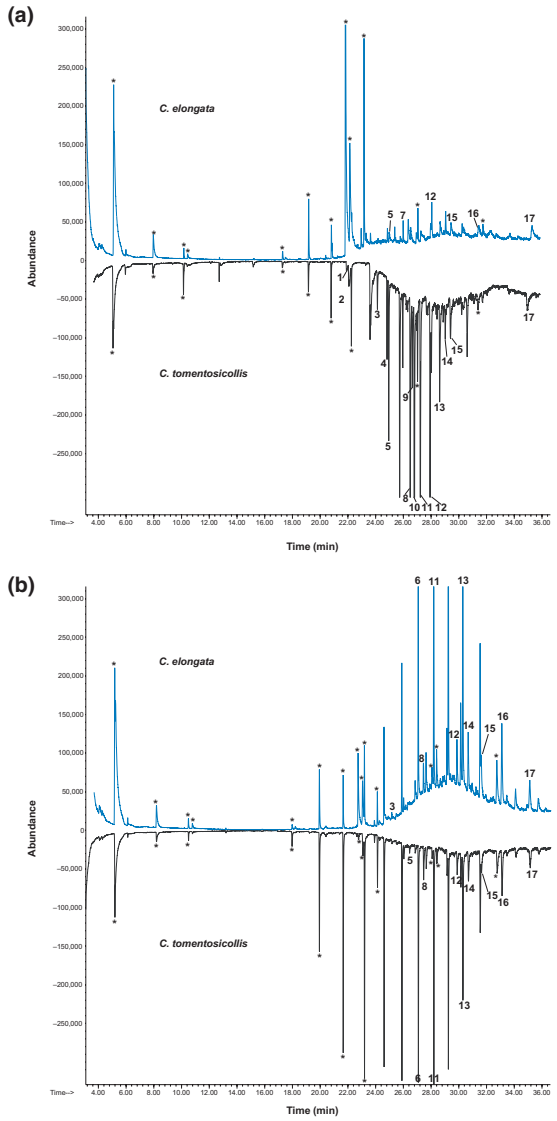

FIGURE 4 GC/MS profiles of cuticular extracts of unparasitized and parasitized eggs of *Clavigralla tomentosicollis* and *Clavigralla elongata*. (a) profiles of unparasitized eggs (above) and (b) profiles of parasitized eggs (below). Peak numbers indicate the identified compounds (Table 4); (*) means impurity

et al., 2011). Cuticular components extracted from pupa of *Lucilia sericata* Meigen (Diptera: Calliphoridae) and *Myrmecia gulosa* Fabricius (Hymenoptera: Formicidae) were found to be used for prey recognition (Dietemann, Peeters, Liebig, Thivet, & Hölldobler, 2003; Moore, Pechal, Benbow, & Drijfhout, 2017). Additionally, cuticular components have been implicated as important chemical cues used by trichogrammatids for location of their egg hosts (Paul, Srivastava, Dureja, & Singh, 2008). In the current study, hexadecenoic acid was identified as a specific compound in the cuticular extract of *C. tomentosicollis* unparasitized eggs. This compound was also reported by Michereff et al. (2016) to occur amongst the volatiles emitted by egg clusters of the stink bug *Euschistus heros* Fabricius (Heteroptera: Pentatomidae) as an attractant of the egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae). Our results suggest that cuticular chemistry of eggs of *Clavigralla* species may play a role in host location in parasitism of *Gryon sp*. Therefore, future study should explore the use of egg cuticular extracts to enhance *Gryon* sp. parasitism activity and management of *Clavigralla* spp.

Morphological and molecular tools were not able to identify this parasitoid (*Gryon* sp.) to species level. We identified cuticular chemicals compounds in the unparasitized eggs of *C. tomentosicollis*, which may play important roles in the host finding behaviour of *Gryon* spp. The importance of egg parasitoids in the management of *Clavigralla* species was previously highlighted in Nigeria and Tanzania using *Gryon gnidus*, *Ooencyrtus patriciae* Subba Rao (Hymenoptera: Encyrtidae) and *Ooencyrtus kuvanae* (How) (Hymenoptera: Encyrtidae) because of the high levels of combined egg parasitism (62%) observed (Matteson, 1981). Our results suggest that the parasitoid *Gryon* sp. could potentially be used as a biological control agent in the integrated management of *Clavigralla* spp. in Kenya and Bénin. It will also be important to evaluate the effect of the identified specific compounds associated with unparasitized eggs of *C. tomentosicollis* in the host location of *Gryon* sp. and to study the genetic diversity and identity of *C. tomentosicollis* from different regions of Africa.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

HK, JVB, FK, MT and BT conceived and designed research. HK conducted experiments in and analysed data. HK, JVB, FK, MT and BT

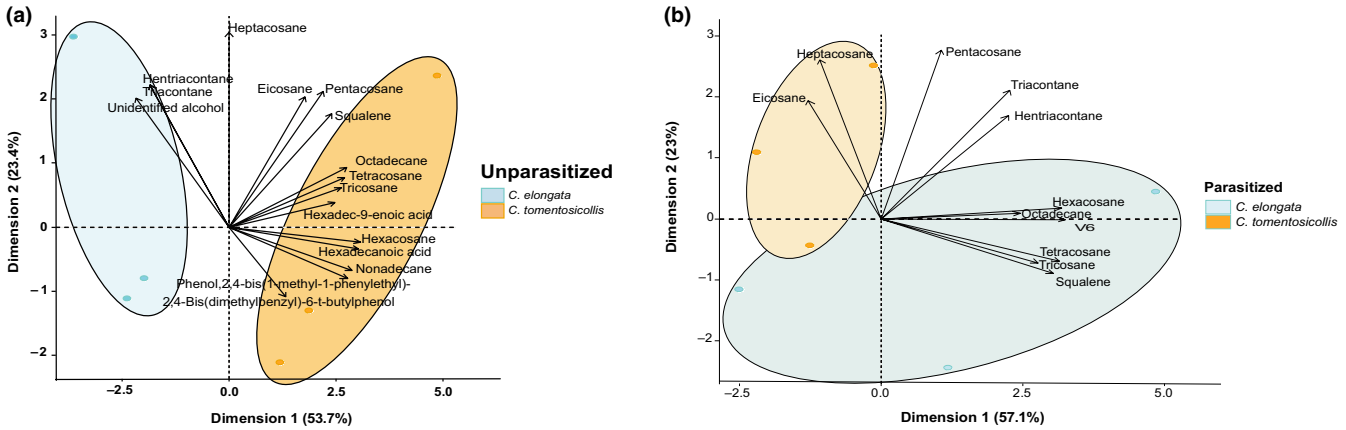

FIGURE 5 Plots of proportion of seventeen cuticular components of *Clavigralla tomentosicollis* and *Clavigralla elongata* eggs using principal component (PCA) analysis. Solid lines indicate specific component present in cuticular extract of unparasitized and parasitized eggs of *Clavigralla tomentosicollis* and *Clavigralla elongata*. (a) unparasitized eggs and (b) parasitized eggs

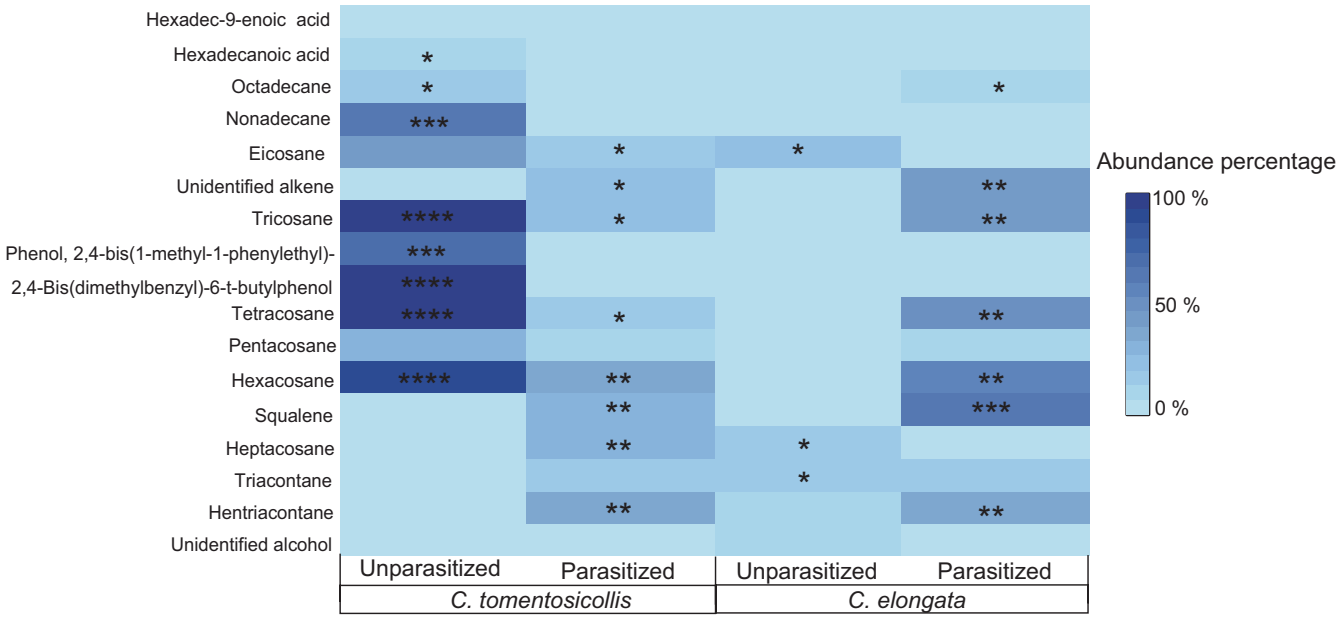

FIGURE 6 Heat map showing compounds detected in cuticular extracts of unparasitized and parasitized eggs of *Clavigralla tomentosicollis* and *Clavigralla elongata*. The number of asterisk (*) indicates the level of the compound content. The letters A, B, C and D indicate the different types of *Clavigralla tomentosicollis* and *Clavigralla elongata* eggs used

wrote the manuscript. EJT gave us support morphological identification of *Gryon* sp. All authors edited the manuscript and approved the final version.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA ACCESSIBILITY

Gryon sp. could be found in hol.osu.edu (deposit number: DPI_FSCA 00010233 and DPI_FSCA 00010182). Sequences data are available in GenBank with accession numbers (MK488003–MK488008). Raw chemical data are available in the Data S1 and S2.

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REFERENCES

Abate, T., & Ampofo, J. K. O. (1996). Insects pests of beans in Africa: Their ecology and management. *Annual Review of Entomology*, *41*(1), 45–73. https://doi.org/10.1146/annurev.en.41.010196.000401

Adipala, E., Nampala, P., Karungi, J., & Isubikalu, P. (2000). A review on options for management of cowpea pests: Experiences from Uganda. *Integrated Pest Managment Reviews*, *5*(3), 185–196. https://doi.org/10.1023/A:1011334312233

Agboton, C., Onzo, A., Ouessou, F. I., Goergen, G., Vidl, S., & Tamò, M. (2014). Insect fauna associated with *Anacardium occidentale* (Sapindales: Anacardiaceae) in Bénin, West Africa. *Journal of Insect Science*, *14*(1), 1–11. https://doi.org/10.1093/jisesa/ieu091

Agunbiade, T. A., Sun, W., Coates, B. S., Djouaka, R., Tamò, M., Malick, N., & Pittendrigh, B. R. (2013). Development of reference transcriptomes for the major field insect pests of cowpea: A toolbox for insect pest management approaches in west Africa. *PLoS ONE*, *8*(11), 1–15. https://doi.org/10.1371/journal.pone.0079929

Aliyu, M., Ladan, T., Ahmed, B. I., & Abdullahi, J. (2007). Studies on the efficacy of black soap and kerosene mixture on the control of Pod sucking bugs (*Clavigralla tomentosicollis* Stål) on Cowpea (*Vigna unguiculata* (L.) Walp. *Emirates Journal of Food and Agriculture*, *19*, 8–14. https://doi.org/10.9755/ejfa.v12i1.5171

Arakaki, N., Yamazawa, H., & Wakamura, S. (2011). The egg parasitoid *Telenomus euproctidis* (Hymenoptera: Scelionidae) uses sex pheromone released by immobile female tussock moth *Orgyia postica* (Lepidoptera: Lymantriidae) as kairomone. *Applied Entomology and Zoology*, *46*(2), 195–200. https://doi.org/10.1007/s13355-011-0031-4

Asante, S. K., Jackai, L. E. N., & Tamò, M. (2000). Efficiency of *Gryon fulviventris* (Hymenoptera: Scelionidae) as an egg parasitoid of *Clavigralla tomentosicollis* (Hemiptera: Coreidae) in Northern Nigeria. *Environmental Entomology*, *29*(4), 815–821. https://doi.org/10.1603/0046-225X-29.4.815

Ayenan, M. A. T., Ofori, K., Ahoton, L. E., & Danquah, A. (2017). Pigeonpea [(*Cajanus cajan* (L.) Millsp.)] production system, farmers' preferred traits and implications for variety development and introduction in Benin. *Agriculture and Food*. *Agriculture and Food Security*, *6*(1), 48.

Bin, F., Vinson, S. B., Strand, M. R., Colazza, S., & Jones, W. A. Jr (1993). Source of an egg kairomone for *Trissolcus basalis*, a parasitoid of *Nezara viridula*. *Physiological Entomology*, *18*(1), 7–15. https://doi.org/10.1111/j.1365-3032.1993.tb00443.x

Campbell, B. C., Steffen-Campbell, J. D., & 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*(4), 225–237. https://doi.org/10.1111/j.1365-2583.1994.tb00142.x

Chalam, V. C., Bhalla, S., Gupta, K., Singh, B., Khan, Z., & Dubey, S. C. (Eds). (2016). *Generic pest risk analysis: Import of transgenic soybean* (p. 146). New Delhi, India: ICAR-National Bureau of Plant Genetic Resources.

Conti, E., Salerno, G., Bin, F., Williams, H. J., & Vinson, S. B. (2003). Chemical cues from *Murgantia histrionica* eliciting host location and recognition in the egg parasitoid *Trissolcus brochymenae*. *Journal of Chemical Ecology*, *29*(1), 115–130.

Dabire-Binso, C. L., Ba, N. M., Sanon, A., Drabo, I., & Bi, K. F. (2010). Resistance mechanism to the pod-sucking bug *Clavigralla tomentosicollis* (Hemiptera: Coreidae) in the cowpea IT86D-716 variety. *International Journal of Tropical Insect Science*, *30*(4), 192–199. https://www.researchgate.net/publication/231922912

Dialoke, S. A., Agu, C. M., Ojiako, F. O., Onweremadu, E., Onyishi, G. O., Ozor, N., … Ugwoke, F. O. (2010). Survey of insect pests on pigeon pea in Nigeria. *Journal of SAT Agricultural Research*, *8*, 1–8.

Dietemann, V., Peeters, C., Liebig, J., Thivet, V., & Hölldobler, B. (2003). Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proceedings of the National Academy of Sciences*, *100*(18), 10341–10346.

Dreyer, H., Baumgärtner, J., & Tamó, M. (1994). Seed-damaging field pests of cowpea (*Vigna unguiculata* L. Walp) in Bénin: Occurrence and pest status. *International Journal of Pest Management*, *40*(3), 252–260. https://doi.org/10.1080/09670879409371893

Dreyer, H., & Baumgärtner, J. (1996). Temperature influence on cohort parameters and demographic characteristics of the two cowpea coreids *Clavigralla tomentosicollis* and *C. shadabi*. *Entomologia Experimentalis Et Applicata*, *78*(2), 201–213. https://doi.org/10.1111/j.1570-7458.1996.tb00783.x

Dolling, W. R. (1979). A revision of the African pod bugs of the tribe Clavigrallini (Hemiptera: Coreidae) with a checklist of the world species. *Bulletin of the British Museum (Natural History)*, *39*(1), 1–84.

Egho, E. (2010). Comparative studies on insect species of cowpea (*Vigna unguiculata* L. Walp.) in two agro-ecological zones during the early cropping season, in Delta State, Southern Nigeria. *Agriculture and Biology Journal of North America*, *1*(5), 946–949.

Gariepy, T. D., Haye, T., & Zhang, J. (2014). A molecular diagnostic tool for the preliminary assessment of host–parasitoid associations in biological control programmes for a new invasive pest. *Molecular Ecology*, *23*(15), 3912–3924. https://doi.org/10.1111/mec.12515

Gbaguidi, A. A., Dansi, A., Loko, L. Y., Dansi, M., & Sanni, A. (2013). Diversity and agronomic performances of the cowpea (*Vigna unguiculata* Walp.) landraces in Southern Benin. *International Research Journalof Agricultural Scienc E and Soil*, *3*(4), 121–133.

Gethi, M., & Khaemba, B. M. (1991). Damage by pod-sucking bugs on cowpea when intercropped with maize. *International Journal of Pest Management*, *37*(3), 236–239. https://doi.org/10.1080/09670879109371591

Goolsby, J. A., De Barro, P. J., Makinson, J. R., Pemberton, R. W., Hartley, D. M., & Frohlich, D. R. (2006). Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Molecular Ecology*, *15*(1), 287–297. https://doi.org/10.1111/j.1365-294X.2005.02788.x

Holman, L., Jørgensen, C. G., Nielsen, J., & d'Ettorre, P. (2010). Identification of an ant queen pheromone regulating worker sterility. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1701), 3793-3800. https://doi.org/10.1098/rspb.2010.0984

Jackai, L. E. N., & Adalla, C. B. (1997). Pest management practices in cowpea: a review. In: B. B. Singh, D. R. Mohan Raj, K. E. Dashiell, & L. E. N. Jackai (Eds), *Advances in cowpea research* (pp. 240–253). Co-publication of International Institute of Agriculture (IITA) and Japan International Center for Agricultural Sciences (JIRCAS). Ibadan, Nigeria: IITA, JIRCAS.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Khamala, C. P. M. (1978). Pests of grain legumes and their control in Kenya. In: R. Singh, H. F. VanEmolen, & T. A. Taylor (Eds). *Pests of Grain Legumes and their control in Kenya : ecology and control*. (pp. 127–134). London, UK: Academic Press Inc.

Koona, P., Osisanya, E. O., Jackai, L. E. N., Tamò, M., Tonye, J., & Ngeve, J. M. (2001). Interaction between pod age and position on damage to cowpea *Vigna unguiculata* by hemipteran pod-sucking bugs. *Bulletin of Entomological Research*, *91*(6), 453–459. https://doi.org/10.1079/BER2001125

Koona, P., Osisanya, E. O., Jackai, L. E. N., Tamò, M., & Markham, R. H. (2002). Resistance in accessions of cowpea to the coreid pod-bug *Clavigralla tomentosicollis* (Hemiptera: Coreidae). *Journal of Economic Entomology*, *95*(6), 1281–1288. https://doi.org/10.1603/0022-0493-95.6.1281

Maruthadurai, R., Gautam, R. D., & Mahesh, P. (2011). Kairomonal effect of host body washing on the egg parasitoid *Trichogramma brasiliensis* (Ashmead) (Hymenoptera: Trichogrammatidae). *Journal of Biological Control*, *25*(4), 298–304.

Masner, L. (1975). Two new sibling species of *Gryon haliday* (Hymenoptera, Scelionidae), egg parasites of blood-sucking

Reduviidae (Heteroptera). *Bulletin of Entomological Research*, *65*(2), 209–213. https://doi.org/10.1017/S0007485300005915

Masner, L. (1976). Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). *Memoirs of the Entomological Society of Canada*, *108*(S97), 1–87. https://doi.org/10.4039/entm10897fv

Materu, M. E. A. (1972). Morphology of adults and description of the young stages of *Acanthomia tomentosicollis* Stål. and *A. horrida* Germ. (Hemiptera, Coreidae). *Journal of Natural History*, *6*(4), 427–450. https://doi.org/10.1080/00222937200770401

Matteson, P. C. (1981). Egg parasitoids of hemipteran pests of cowpea in Nigeria and Tanzania, with special reference to *Ooencyrtus patriciae* Subba Rao (Hymenoptera: Encyrtidae) attacking *Clavigralla tomentosicollis* Stäl (Hemiptera: Coreidae). *Bulletin of Entomological Research*, *71*(4), 547–554. https://doi.org/10.1017/S0007485300010063

Michereff, M. F. F., Borges, M., Aquino, M. F. S., Laumann, R. A., Gomes, A. M., & Blassioli-Moraes, M. C. (2016). The influence of volatile semiochemicals from stink bug eggs and oviposition-damaged plants on the foraging behaviour of the egg parasitoid *Telenomus podisi*. *Bulletin of Entomological Research*, *106*(5), 663–671. https://doi.org/10.1017/S0007485316000419

Minja, E. M., Shanower, T. G., Songa, J. M., Ong'aro, J. M., Kawonga, W. T., Mviha, P. J., … Opiyo, C. (1999). Studies of pigeon pea insect pests and their management in farmers' fields in Kenya, Malawi, Tanzania and Uganda. *African Crop Science Journal*, *7*(1), 59–69. https://doi.org/10.4314/acsj.v7i1.2777

Moore, H. E., Pechal, J. L., Benbow, M. E., & Drijfhout, F. P. (2017). The potential use of cuticular hydrocarbons and multivariate analysis to age empty puparial cases of *Calliphora vicina* and *Lucilia sericata*. *Scientific Reports*, *7*(1), 1933. https://doi.org/10.1038/s41598-017-01667-7

Oparaeke, A. M. (2006a). The Potential for Controlling *Maruca vitrata* Fab. and *Clavigralla tomentosicollis* Stål. Using Different Concentrations and Spraying Schedules of Syzigium aromaucum (L.) Merr and Perr on Cowpea Plants. *Journal of Plant Sciences*, *1*(2), 132–137. https://doi.org/10.3923/jps.2006.132.137

Oparaeke, A. M. (2006b). Effect of Aqueous Extracts of Tropical Plants for Management *of Maruca vitrata* Fab. and *Clavigralla tomentosicollis* Stål. on Cowpea, *Vigna unguiculata* (L.) Walp Plants. *Journal of Entomology*, *3*(1), 70–75. https://doi.org/10.3923/je.2006.70.75

Paul, A. V. N., Srivastava, M., Dureja, P., & Singh, A. K. (2008). Semiochemicals produced by tomato varieties and their role in parasitism of *Corcyra cephalonica* (Lepidoptera: Pyralidae) by the egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *International Journal of Tropical Insect Science*, *28*(2), 108–116. https://doi.org/10.1017/S1742758408977493

R Development Core Team. (2012). *A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Romeis, J., Shanower, T. G., & Madhuri, K. (2000). Biology and field performance of *Gryon clavigrallae* (Hymenoptera: Scelionidae), an egg parasitoid of *Clavigralla* spp. (Hemiptera: Coreidae) in India. *Bulletin of Entomological Research*, *90*(3), 253–263. https://doi.org/10.1017/S0007485300000377

Shanower, T. G., Romeis, J., & Minja, E. M. (1999). Insect pests of pigeon pea and their management. *Annual Review of Entomology*, *44*(1), 77–96. https://doi.org/10.1146/annurev.ento.44.1.77

Shanower, T. G., Anitha, V., Bhagwat, V. R., & Dreyer, H. (1996). Parasitism of *Clavigralla* spp. (Hemiptera: Coreidae) eggs by *Gryon clavigrallae* Mineo (Hymenoptera: Scelionidae). *Journal of Biological Control*, *10*(1–2), 1–7. https://doi.org/10.1146/annurev.ento.44.1.77

Soyelu, O. L., & Akingbohungbe, A. E. (2007). Comparative assessment of feeding damage by pod-sucking bugs (Heteroptera: Coreidae) associated with cowpea *Vigna unguiculata* ssp. *unguiculata* in Nigeria. *Bulletin of Entomological Research*, *97*(1), 1–7. https://doi.org/10.1017/S0007485307004695

Taylor, T. A. (1975). *Gryon gnidus*, a scelionid egg-parasite of *Acanthomia tomentosicollis* (Hemiptera: Coreidae) in Nigeria. *Entomophaga*, *20*(2), 129–134. https://doi.org/10.1007%2FBF02371651

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*(24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876

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