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Cotesia icipe sp. n., a new Microgastrinae wasp (Hymenoptera, Braconidae) of importance in the biological control of Lepidopteran pests in Africa

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Abstract

A new species of Microgastrinae, *Cotesia icipe* Fernández-Triana & Fiaboe, **sp. n.**, is described from eastern Africa. It was reared in Kenya as a solitary parasitoid from two major amaranth pests, *Spodoptera littoralis* (Boisduval, 1833) and *S. exigua* (Hübner, 1808); study of specimens in collections also revealed its presence in four other countries in the Afrotropical region (Madagascar, Saudi Arabia, South Africa, and Yemen). Morphological, molecular and biological characters are used to describe the new species and to distinguish it from all 12 previously described species of Afrotropical *Cotesia*. *Cotesia icipe* shows potential in the biological control of key Lepidopteran pests in small scale farming conditions in Africa.

Keywords

Microgastrinae, *Cotesia*, *Spodoptera*, biological control, Africa

Introduction

The subfamily Microgastrinae (Hymenoptera, Braconidae) is the single most important group of parasitoid wasps attacking caterpillars, with many species used or being considered as biocontrol agents against Lepidopteran pests in agriculture and forestry

(Whitfield 1997). It comprises more than 2,700 described species (Yu et al. 2016) with many thousands of additional, undescribed ones (Rodriguez et al. 2013). The genus *Cotesia* is currently the second largest, with almost 300 described species worldwide (Yu et al. 2016) but many hundreds awaiting description.

There are 12 species of *Cotesia* recorded from the entire Afrotropics (Rousse and Gupta 2013, Yu et al. 2016, Kaiser et al. 2017). We do not include in that total *Cotesia plutellae* (Kurdjumov, 1912), which was recently removed from synonymy with *Cotesia vestalis* (Haliday, 1834) and considered as a valid species by Rousse and Gupta (2013). Rousse and Gupta based their decision on a paper from Rincon et al. (2006) that found that "*Cotesia plutellae*" seemed to comprise two partially incompatible population aggregates. While we agree that specimens previously identified as "*Cotesia plutellae*" might comprise a complex of morphologically cryptic species –as is the case with many other species of Microgastrinae (e.g., Fernández-Triana 2010, Fernández-Triana et al. 2014, Kaiser et al. 2017), without further study, especially of the type specimens involved, there is no real basis for raising that name from synonymy. It is clear from both papers (Rincon et al. 2006, Rousse and Gupta 2013) that those authors never studied the pertinent material, and at present there is no sound evidence for a conclusion as to which type specimens (if any) are associated with their segregates. Therefore, we consider here the name *Cotesia plutellae* (Kurdjumov, 1912) to be just a synonym of *Cotesia vestalis* (Haliday, 1834).

This paper describes a new species of *Cotesia* from Kenya (also present in other Afrotropical countries), which has been found to be important in the biological control of two major amaranth pests, *Spodoptera littoralis* and *S. exigua* (Lepidoptera, Noctuidae). The parasitoid was found during various field studies carried out by the International Centre of Insect Physiology and Ecology (*icipe*), in the scope of developing an IPM programme against amaranth pests in East Africa. In Kenya, specimens of the new species were collected from different locations where the project activities are carried out and there is a potentially much wider distribution. The new species is compared to the other 12 species of *Cotesia* previously recorded from the Afrotropical region, and diagnostic characters to recognize it are provided.

Methods

Specimens of the new species were collected from different locations in Central Kenya: Yatta, (01.23044°S; 37.45789°E) and Mwea (0.6309°S; 37.35117°E); Kitengela (1.6°S; 36.85°E) and Thika (1.00269°S; 37.07858°E). The material has been deposited in the International Centre of Insect Physiology and Ecology, Nairobi, Kenya (*icipe*), the Canadian National Collection of Insects, Ottawa (CNC) and the National Museums of Kenya (NMK).

Morphological terms and measurements of structures follow those used by Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernández-Triana et al. (2014). The abbreviations T1, T2, and T3 refer to meta-

somal mediotergites 1, 2, 3; and F1-F16 refer to antennal flagellomeres 1 to 16. The description of the new species contains ratios commonly used in taxonomic studies of Microgastrinae (e.g., ovipositor sheaths length/metatibia length); we also provide the raw measurements of morphological structures (in mm) as they allow for additional ratios to be explored in the future, if needed.

Seven specimens (including the holotype and paratypes, with voucher codes CNC507547–CNC507553) were sampled for DNA barcodes (the 5' region of the cytochrome c oxidase I (CO1) gene, Hebert et al. 2003). DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova et al. 2006). Total genomic DNA was re-suspended in 30 µl of dH2O, a 658-bp region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (http://v4.boldsystems.org/index.php), and a composite sequence was generated for all successful amplifications. All information for the sequences associated with each individual specimen can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007).

Photos were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a range of 10–130 ×. Multiple images were taken of a structure through the focal plane and then combined to produce a single in-focus image using the software associated with the Keyence System. Plates were prepared using Microsoft PowerPoint 2010.

A map with the distribution of the species was generated using SimpleMappr (Shorthouse 2010).

Original descriptions and/or authenticated specimens of the 12 *Cotesia* species previously recorded for the Afrotropical region were checked. In the Detailed diagnosis section below, the new species is compared individually with all other species previously described from the Afrotropics, and diagnostic characters to separate it from all of them are presented. Morphological comparisons are based mostly on female specimens, as male Microgastrinae often are difficult to identify (e.g., Whitfield 1997, Fernández-Triana et al. 2014).

A neighbor-joining tree (K2P) of all specimens in the Barcoding of Life Data System (BOLD) currently associated with the new species (BIN BOLD:ABZ7318) was generated using the capabilities available in BOLD (for an explanation of the BIN concept see Ratnasingham and Hebert 2007). A second neighbor-joining tree (K2P) was generated for all Afrotropical species of *Cotesia* with available sequences over 500 base pairs in BOLD (the data was accessed on September 2017).

Results

Cotesia icipe **Fernández-Triana & Fiaboe, sp. n.**
http://zoobank.org/E697611F-08A4-4CA7-B300-E35B50675BD9

Holotype. Female, Kenya, NMK. Holotype locality: Yatta. Holotype labels: KENYA, Yatta, 1°13'49.59"S 37°27'28.41"E, 1184m, coll. ICIPE. Voucher code: CNC507547.

Paratypes. 3 females (CNC507548, CNC507549, CNC507553), 3 males (CNC50750, CNC50751, CNC50752); same locality of holotype. Paratypes deposited in the CNC, ICIPE and NMK.

Other specimens considered as this species. There are 10 DNA barcodes in BOLD from Madagascar, Saudi Arabia, South Africa and Yemen (Fig. 4) whose sequences match those of the Yemen holotype and paratypes and thus we consider them all as conspecific. However we only include in the type series those specimens from Kenya that we were able to examine.

Diagnosis. Metasoma with T3 dark brown to black centrally, yellow laterally; hind legs mostly yellow, except for metacoxa (mostly black, with small yellow spot on apical 0.1), brown spots on apical 0.1 of metafemur (dorsally), apical 0.1 of metatibia dark brown, and metatarsus entirely dark brown; tegula and humeral complex yellow; fore wing with most veins brown. Besides coloration, *C. icipe* has scuto-scutellar sulcus with eight carinae; T1 almost parallel-sided, very slightly widening towards posterior margin; T2 relatively small, quadrate and not covering the entire surface of the tergum, and T3 1.3x as long as T2 length. The above combination of characters is sufficient to separate the species from all other described species of Afrotropical *Cotesia*, but see the Detailed diagnosis section below for one-to-one comparisons of *C. icipe* versus every other species. Beyond morphological characters, from a molecular perspective there is also sufficient information to recognize the new species. Eight out of the 12 previously described Afrotropical species of *Cotesia* have DNA barcodes available in BOLD (Fig. 5), and they are all clearly different from *C. icipe*.

Description. Female (Fig. 1). Metasoma brown dorsally, except for T3 (which is centrally brown, laterally yellow); most of laterotergites and sternites yellow, hypopygium brown; F2 length 1.78–2.00 × F14 length; metafemur length 3.60–3.80 × metafemur width; metatibia inner spur length 1.17 × metatibia outer spur length; metatibia inner spur length 0.52 × first segment of metatarsus length; T1 entirely sculptured with coarse punctures and a polished knob centrally on posterior margin; T1 very slightly widening towards posterior margin (width at posterior margin 1.1–1.2 × its width at anterior margin); T1 length centrally 1.5–1.7 × its width at posterior margin; T2 entirely sculptured, with coarse punctures along all margins and longitudinal striation centrally; T2 more or less rectangular, width at posterior margin 2.0 × its length centrally; T3 smooth, with rows of setae that are more dense on posterior half of tergite; T3 length centrally 1.3 × T2 length centrally; ovipositor sheaths length 0.16–0.19 × metatibia length. ***Body measurements*** (all in mm). Body length: 2.20–2.50; fore wing length: 2.20–2.50; ovipositor sheaths: 0.12–0.15; metafemur length 0.65; metafemur width: 0.17–0.18; metatibia length: 0.76–0.80; metatibia inner spur: 0.21; metatibia outer spur: 0.18; first segment of metatarsus: 0.40. T1 length centrally: 0.35–0.37; T1 width at anterior margin: 0.19; T1 maximum width: 0.25; T1 width at posterior margin: 0.22–0.24; T2 width at posterior margin: 0.32; T2 length centrally: 0.16; T3 length centrally: 0.21; length of F1: 0.17–0.19; length of F2: 0.16–0.18; length of F3: 0.16–0.17; length of F14: 0.09; length of F 15: 0.08; length of F16: 0.10.

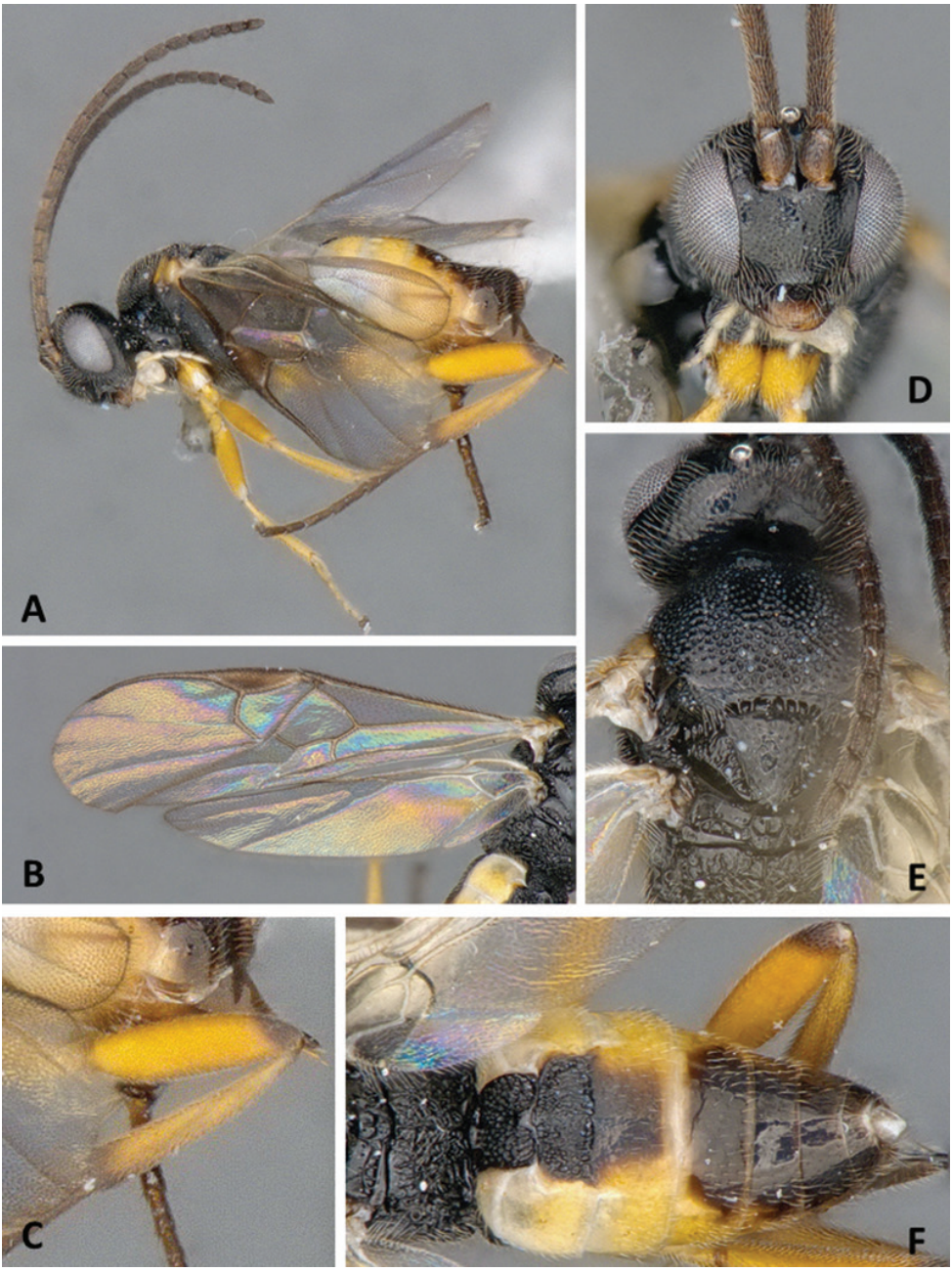

Figure 1. *Cotesia icipe*, female holotype. **A** Habitus lateral **B** Wings **C** Hind leg, hypopygium and ovipositor **D** Head frontal **E** Head and mesosoma dorsal **F** Propodeum and metasoma dorsal.

Male (Fig. 2). As female but with darker metasoma dorsally, sometimes with T3 entirely dark brown to black.

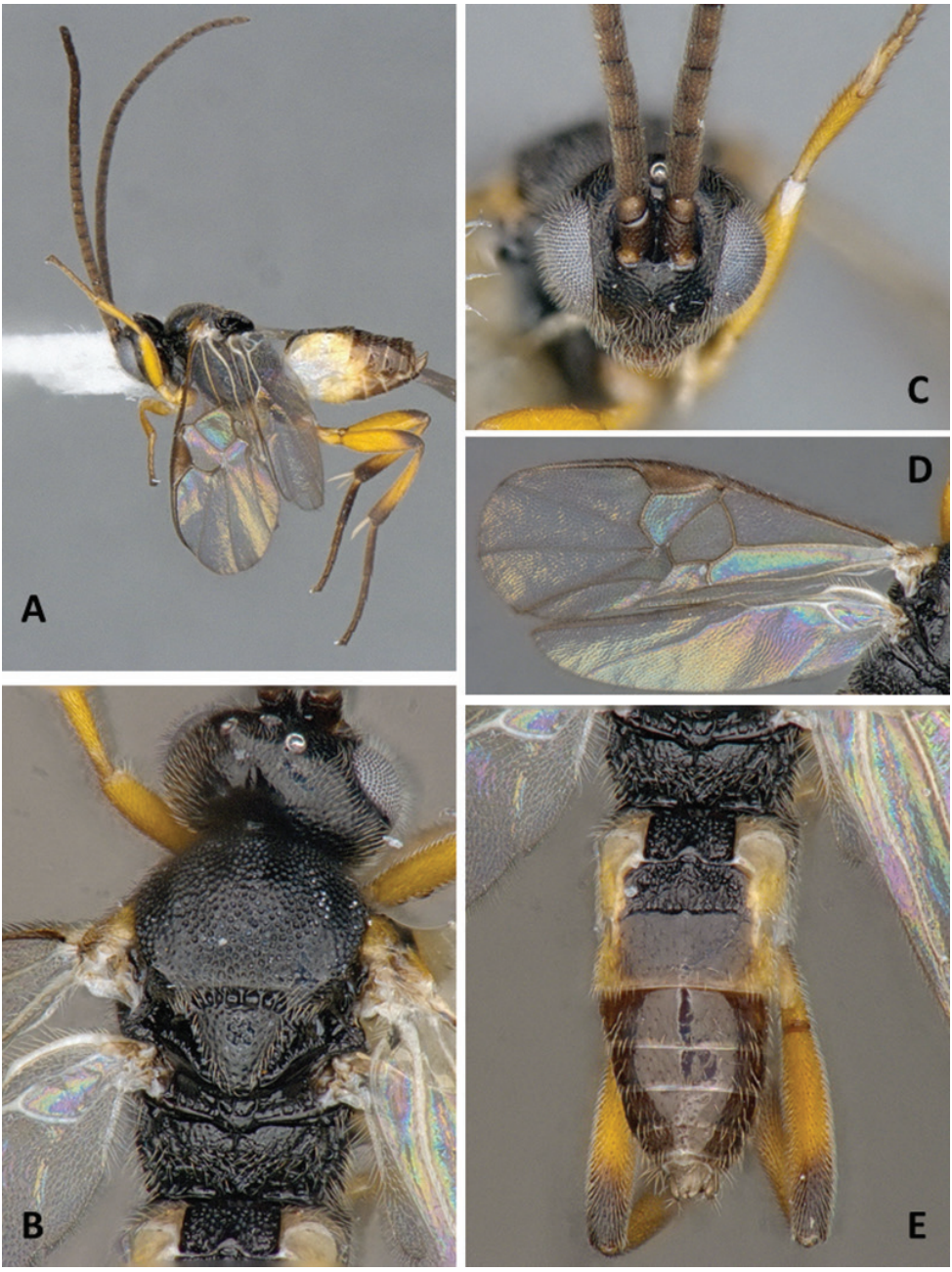

Figure 2. *Cotesia icipe*, male paratype. **A** Habitus lateral **B** Head and mesosoma dorsal **C** Head frontal **D** Wings **E** Propodeum and metasoma dorsal.

Biology. Based on 4,000+ parasitism cases observed under laboratory condition at *icipe* in Kenya, *C. icipe* is a solitary larval endoparasitoid of *Spodoptera littoralis* (Boisduval, 1833) and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera, Noctuidae). It was

Figure 3. Known distribution of *Cotesia icipe* in the Afrotropical region. Blue dots show distribution based on specimens of the type series (Kenya), red dots show distribution based on specimens with DNA barcodes matching those of the Kenya series.

successfully reported to attack those two noctuid species tested on amaranth. However, it failed to parasitize three Crambidae species tested on the same host plant: *Spoladea recurvalis* (Fabricius, 1775), *Udea ferrugalis* (Hübner, 1796) and *Herpetogramma bipunctalis* (Fabricius, 1794). Further studies of host range are warranted to explore the full potential of the new species in the biological control of key pests in small scale farming conditions in Africa. The female prefers ovipositing on second instar host larvae. The development time from egg to adult is two weeks and ovipositing females fed with honey can live more than two weeks at 25°C (our unpublished data).

Distribution. Afrotropical: Kenya, Madagascar, Saudi Arabia, South Africa, Yemen (Fig. 3).

DNA barcodes. Currently (as of September 2017) there are in BOLD 19 available sequences from five Afrotropical countries: Kenya (9 sequences), Madagascar (1), Saudi Arabia (3), South Africa (1) and Yemen (5). Although we could not examine the specimens from Saudi Arabia or South Africa, their sequences are identical to the rest, and thus we consider them all to represent the species *Cotesia icipe*. They belong to BIN BOLD:ABZ7318; however, that BIN is likely to contain more than one species, as the sequences currently assigned to it are grouped in different clusters (Fig. 4). The Afrotropical sequences are in a single cluster, separated by more than 1% different

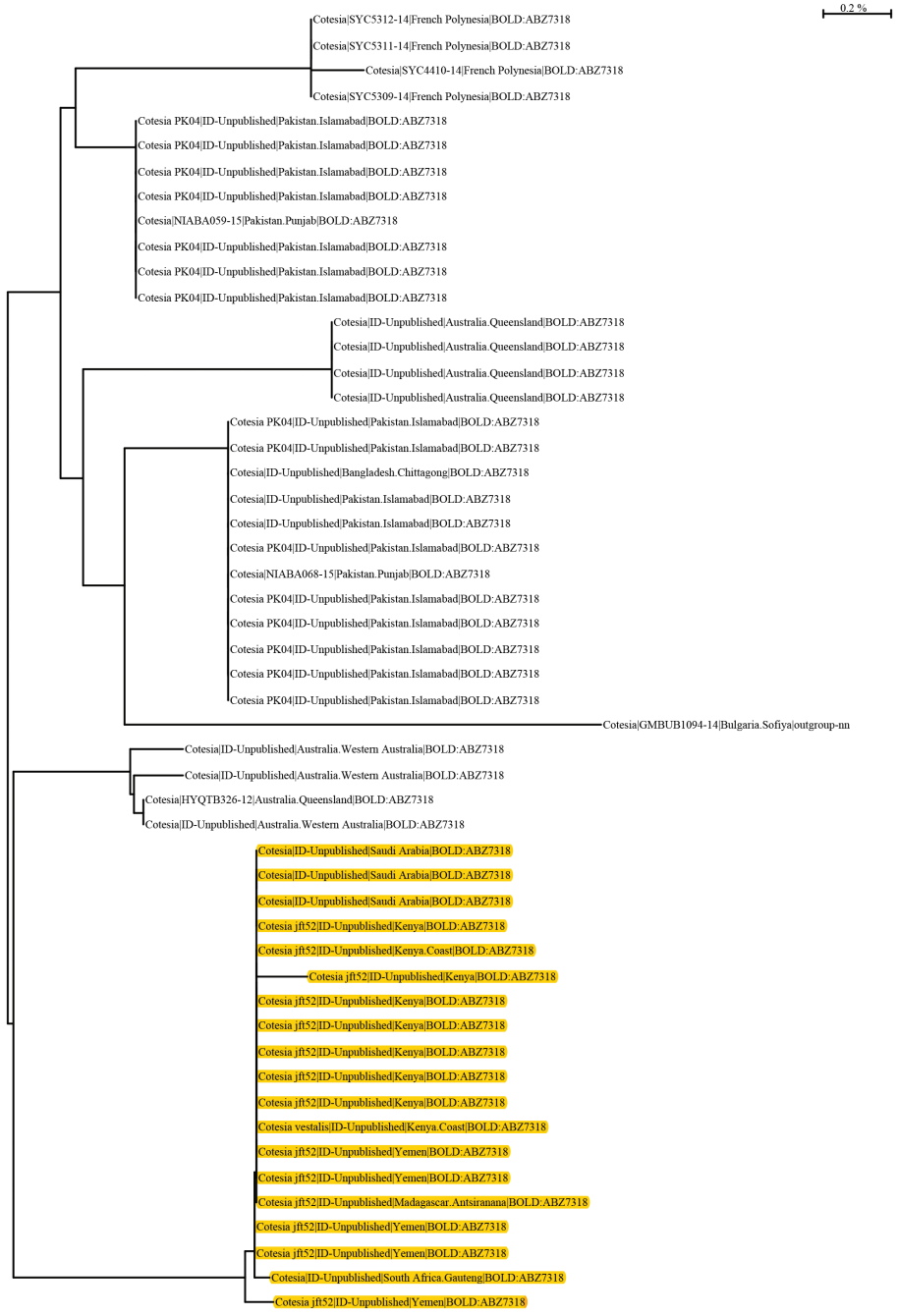

Figure 4. Neighbor-joining tree (K2P) of all specimens in the Barcoding of Life Data System (BOLD) currently associated with BIN BOLD:ABZ7318 (for explanation on the BIN concept see Methods of this paper). The cluster highlighted in Yellow in the tree shows the specimens considered in the present paper as *Cotesia icipe*.

Figure 5. Neighbor-joining tree (K2P) of Afrotropical species of *Cotesia* in the Barcoding of Life Data System (BOLD) with over 500 base pairs; data accessed on September 2017. Specimens named as "*Cotesia* jft52" in BOLD actually belong to the species *Cotesia icipe* (cluster highlighted in Yellow), and specimens named as "*Cotesia* jft68" in BOLD actually belong to the species *Cotesia chrysippi* (Viereck, 1911).

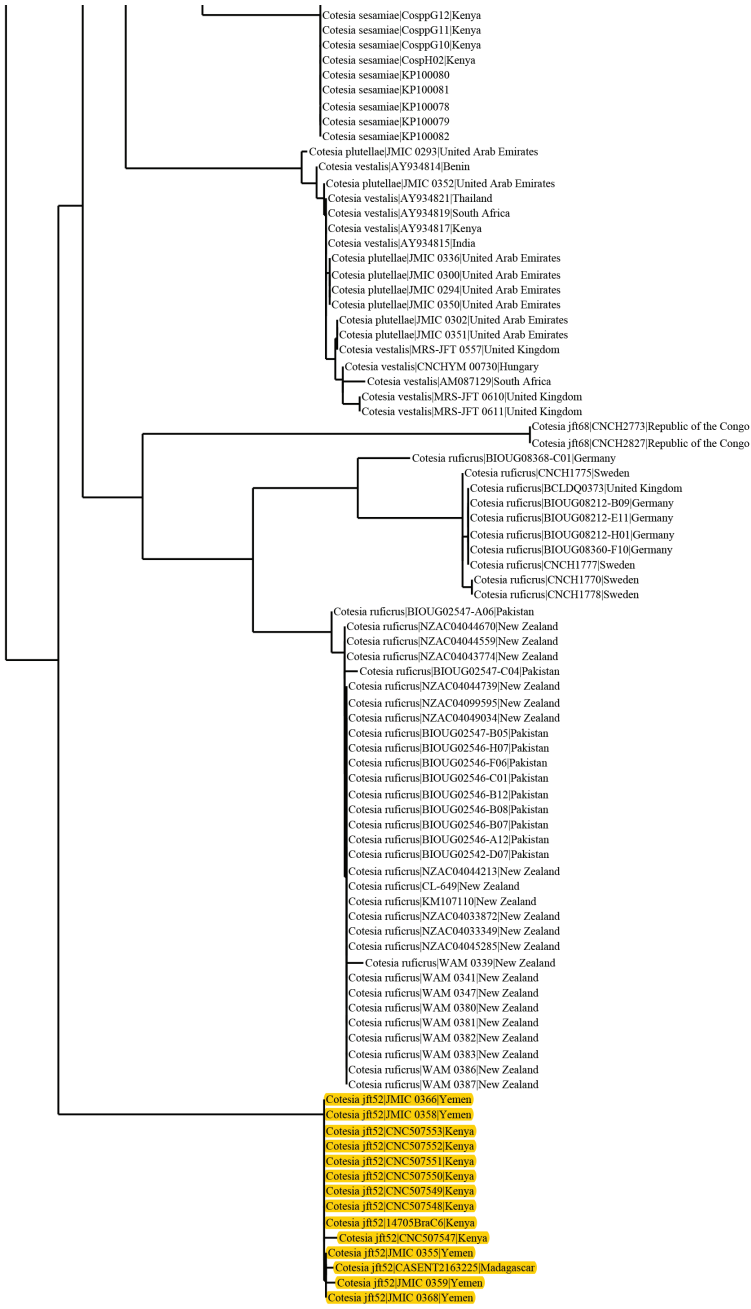

Figure 5. Continued. Neighbor-joining tree (K2P) of Afrotropical species of *Cotesia* in the Barcoding of Life Data System (BOLD) with over 500 base pairs; data accessed on September 2017. Specimens named as "*Cotesia* jft52" in BOLD actually belong to the species *Cotesia icipe* (cluster highlighted in Yellow), and specimens named as "*Cotesia* jft68" in BOLD actually belong to the species *Cotesia chrysippi* (Viereck, 1911).

base pairs from the other sequences in that BIN (from Australia, French Polynesia and Pakistan, all in separate clusters). Solving the species limits for this BIN is beyond the scope of this paper, but for the time being we consider that only the Afrotropical sequences represent *Cotesia icipe*.

Etymology. We dedicate this species to the "International Centre of Insect Physiology and Ecology (*icipe*)" for its long-term promotion of Integrated Pest Management and for building the capacity of thousands of African young scientists over the years in insect sciences.

Detailed diagnosis. Additionally, and to facilitate future work on the group, we detail below how each of the other 12 species of *Cotesia* previously described from the Afrotropics individually differ from *C. icipe*.

Cotesia bignellii (Marshall, 1885) has metasoma (entirely) and hind legs (mostly) dark brown, very different from the body color of *C. icipe* as detailed above. *C. bignellii* also parasitizes a different family of Lepidoptera hosts (Nymphalidae) and it is mostly distributed in Europe (Yu et al. 2016).

Cotesia chrysippi (Viereck, 1911) parasitizes a different family of Lepidoptera hosts (Nymphalidae) (Yu et al. 2016) and it has a very different color, with a reddish-brown metasoma dorsally, dark brown tegula and humeral complex and fore wing with most veins white.

Cotesia decaryi (Granger, 1949) is only known from Madagascar and probably represents a local endemic from that country. It parasitizes a different family of Lepidoptera hosts (Lasiocampidae) (Yu et al. 2016). It has metacoxa mostly smooth (rugose-punctate in *C. icipe*), T1 clearly widens towards posterior margin, T2 covers most of the tergum dorsally, and T3 is about the same length than T2 (T3 1.3 × as long as T2 length in *C. icipe*).

Cotesia flagellator (Wilkinson, 1930) is only known from Uganda (Yu et al. 2016); it has scuto-scutellar sulcus with 4–5 (very rarely 6) major carinae (*C. icipe* has 8–9, rarely 7 major carinae); metacoxa mostly smooth, with only indefinite coarse punctures anterodorsally (metacoxa mostly with shallow punctures in *C. icipe*); T2 2.2 × as long as wide at posterior margin (2.0 × in *C. icipe*); T3 1.5 × as long as T2 length (1.3 × in *C. icipe*).

Three related species, ***Cotesia flavipes*** Cameron 1891**,** ***Cotesia sesamiae*** (Cameron, 1906) and ***Cotesia typhae*** Fernández-Triana, 2017, have all been recently revised in Kaiser et al. (2017), including extensive illustrations. They are all part of the *flavipes* complex and can be distinguished by relatively short antennae (much shorter than body length, usually not surpassing the length of head and mesosoma; whereas the antenna of *C. icipe* is comparatively much longer, about as long as body), body relatively depressed, and metasoma extensively to slightly pale in coloration (yellow, orange or light brown) but clearly much lighter in color than *C. icipe*. Beyond the substantial morphological differences these three species parasitize different host species within the families Crambidae and Noctuidae (Yu et al. 2016, Kaiser et al. 2017).

Cotesia pistrinariae (Wilkinson, 1929) has a strongly narrowed T1 centrally, which is unique among all known species of *Cotesia*; extensive illustrations of *pistrinariae* can be found in Gupta et al. (2016). It also parasitizes a different family of Lepidoptera hosts (Pieridae) (Yu et al. 2016).

Cotesia vestalis (Haliday, 1834) has T1 clearly widening towards posterior margin and T2 covering most of the tergum dorsally, both tergites are also much more coarsely sculptured than in *C. icipe*; the coxae (especially pro- and mesocoxae) are also darker colored as compared to *icipe*. There are many host records attributed to this species (Yu et al. 2016), some of them dubious; they include mostly Erebidae, Nymphalidae and Plutellidae, with a couple of Noctuidae species different from those parasitized by *C. icipe*.

Cotesia vanessae (Reinhard, 1880) is mostly a Palearctic species, recently found to have spread into the Nearctic (Hervet et al. 2014), and only marginally distributed in Africa (Yu et al. 2016). It has tegula and humeral complex dark brown to black, metasoma entirely black dorsally, and legs mostly dark brown. Numerous host records, some of them certainly inaccurate, are attributed to this species (Yu et al. 2016).

Of all described Afrotropical species ***Cotesia ruficrus*** (Haliday, 1834) looks most similar to *C. icipe* from a morphological perspective; however, it has T1 clearly wider at posterior margin as compared to anterior margin (almost the same width in *C. icipe*); T2 covers most of the tergum surface; and vein R1 in fore wing is comparatively much shorter, about the same length of pterostigma, and with the distance between end of vein R1 and the end of vein 3RSb being more than 0.4 × the length of vein R1 (in *C. icipe* the vein R1 in fore wing is comparatively much longer, clearly longer than pterostigma length, and with the distance between end of vein R1 and the end of vein 3RSb being less than 0.2 × the length of vein R1); also, the overall coloration is lighter and more reddish than in *C. icipe*. Furthermore, from a biological point of view, *C. ruficrus* is a gregarious parasitoid while *C. icipe* is a solitary parasitoid. Numerous host records, some of them certainly inaccurate, are attributed to *C. ruficrus* (Yu et al. 2016).

Cotesia rugosa (Szépligeti, 1914) presents the most difficult case to assess, as it is only known from the male holotype, collected in 1912 at an altitude of 2,000 m, on the western side and near of Mount Kenya (Szépligeti 1914, Papp 2008). *C. icipe* has been collected not too far from that locality, at close to 1,200 m. However, and according to the original description, *C. rugosa* has different colored legs, including many red areas (legs mostly yellow in *C. icipe*, with a few areas brown); tegula reddish-brown (yellow in *C. icipe*); T2 as long as T3 (0.7–0.8 × in *C. icipe*); T1 is described as 'almost transversal' (Szépligeti 1914: 184) which, within the context of the Microgastrinae species described in that paper, seems to indicate that T1 is strongly widened towards posterior margin, i.e., T1 width at posterior margin is about as long as T1 length medially (whereas T1 is 1.5 × as long as wide at posterior margin in *C. icipe*).

Discussion

The finding of the new species *C. icipe* is of particular interest because it successfully attacks Noctuids of the genus *Spodoptera* (Agbodzavu et al. in press), which includes notorious pest species. The tropical and subtropical genus *Spodoptera* includes several economically important species in Africa (Brown and Dewhurst 1975). The most destructive ones reported in the Afrotropical regions include *S. littoralis*, *S. exigua*, *S. exempta* (Brown and Dewhurst 1975) and *S. frugiperda* (Goergen et al. 2016). The first two have been confirmed as suitable hosts for *C. icipe*, which therefore represents a new component in the environmental friendly management of both pests in small scale farming systems (Agbodzavu et al. in press). *Spodoptera littoralis* and *S. exigua* are herbivores with over 90 known host plants including cotton, tobacco, soybean, cabbage, amaranth, coffee, potato and wheat, among others (Abdullah et al. 2000, Agbodzavu et al. in press, Azidah and Sofian-Azirun 2006, Clarke-Harris et al. 2004, Saeed et al. 2017, Santiago-Alvarez and Ortiz-Garcia 1992). Young larvae of both pests feed on terminal leaf clusters, seedlings, and stems of host crops, leading to skeletonized plants (Bohmfalk et al. 1999, East et al. 1989, McDougall et al. 2013). Further studies are also warranted to assess the performance of *C. icipe* on *S. exempta* and *S. frugiperda*. The African armyworm *S. exempta* is a serious pest of maize and rice, the most important staple crops across Africa (Grzywacz et al. 2008, Okello-Ekochu and Wilkins 1996, Sithole 1989) while the fall armyworm *S. frugiperda* is a newly reported invasive pest on the continent, causing currently the highest damage on maize, rice, sorghum and millet (Cock et al. 2017, Goergen et al. 2016). *Cotesia icipe* represents therefore a potential candidate for natural control of pests of these staple crops.

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Supplementary material 1

Tree for Barcode Index Number – BOLD:ABZ7318

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Data type: molecular data

Link: https://doi.org/10.3897/jhr.61.21015.suppl1

Supplementary material 2

Tree Result – Search: Sample IDs; Seq Length(500 bp); Include public records (170 records returned) (170 records selected)

Authors: Komi K.M. Fiaboe, Jose Fernández-Triana, Faith W. Nyamu, Komi M. Agbodzavu

Data type: moleculara data

Link: https://doi.org/10.3897/jhr.61.21015.suppl2