DNA barcodes, expanded distribution, and redescription of *Apanteles hemara* Nixon, 1965 (Hymenoptera, Braconidae, Microgastrinae), a potential biocontrol species against amaranth leaf-webbers in Africa

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Abstract

The microgastrine parasitoid wasp *Apanteles hemara* Nixon, 1965, is currently being considered as a potential biocontrol agent of amaranth leaf-webber pests in Africa. To facilitate future research and identification of the species, we characterize it from an integrative taxonomy perspective by providing a comprehensive morphological redescription, extensive illustrations (including the first images of the holotype), DNA barcodes, wasp biology, host data (Choreutidae and Crambidae caterpillars), and updated geographical distribution of the species (including eight new country records). Despite a wide distribution across four major biogeographical regions (mostly within the Old World tropics), the species seems to be relatively uniform from a molecular and morphological perspective, based on studied specimens from Africa and Asia.

Keywords

Microgastrinae, *Amaranthus*, biocontrol, DNA barcode, geographical distribution, Africa
Introduction

Apanteles hemara was described more than 50 years ago (Nixon 1965), and even at that time it was considered to have a wide distribution. The species has been recorded from many countries: Australia, Bulgaria, Canary Islands, Cape Verde Islands, China, Cyprus, France, Greece, India, Iran, Israel, Italy, Madeira Islands, Mauritius, mainland Portugal, Russia, Senegal, South Africa, mainland Spain, Turkey, Vietnam, and the former Yugoslavia (Austin 1992, Papp 1988, 2007, 2012, Long and Belokobylskij 2003, Shaw 2012, Kedar and Kumaranag 2013, Madl and van Achterberg 2014, Yu et al. 2016).

The host species attacked by A. hemara are also varied: it has been considered a regular solitary parasitoid of Tebenna micalis (Mann, 1857) (Choreutidae), wherever both species occur (Shaw 2012), but it is also recorded from several species of Crambidae: Cnaphalocrocis trapezalis (Guenée, 1854), Herpetogramma stultalis (Walker, 1859), Hydriris ornatalis (Duponchel, 1832), Omiodes indicata (Fabricius, 1775), Spoladea recurvalis (Fabricius, 1775) and Udea ferrugalis (Hübner, 1796) (Long and Belokobylskij 2004, Papp 2012, Madl and van Achterberg 2014, Yu et al. 2016). The wasp may play some role in the biological control of caterpillars of S. recurvalis attacking amaranth crops in India, although the parasitism rate is low (Peter and Balasubramanian 1984, Velmurugan et al. 2006, Arivudainambi et al. 2010).

Due to the wide geographical distribution and host associations, the species was described four times under different names, and it currently has three synonyms (see below for more details on that).

In order to better characterize the species, this paper provides the first molecular information for Apanteles hemara (DNA barcodes), expands the known distribution to an additional eight countries, and presents the first color pictures of the species, including the holotype.

Methods

We studied specimens from the California Academy of Sciences (CAS), Canadian National Collection of Insects, Ottawa (CNC), the International Centre of Insect Physiology and Ecology, Nairobi, Kenya (ICIPE), and Naturalis Biodiversity Center, Leiden, Netherlands (RMNH).

Specimens from several countries (Kenya, Madagascar, Republic of the Congo, United Arab Emirates, and Yemen) 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).

The holotype of *A. hemara* was photographed with a Leica DFC450 camera on a Leica M165 C microscope. Other specimens were photographed 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. For the Leica camera, the Zerene Stacker program (http://zerenesystems.com/cms/stacker) was used; software associated with the Keyence System produced focused images taken with that camera. Plates were prepared using Microsoft PowerPoint 2010.

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

**Results**

*Apanteles hemara* Nixon, 1965


**Holotype.** INDIA, Dehradun. Female (deposited in the Natural History Museum, London), examined.


**Distribution.** Afrotropical, Australian, Oriental and Palaearctic regions (Fig. 1). The species is widespread in the Old World tropics, especially Africa. We record here the presence of *Apanteles hemara* in eight additional countries. The new distribution records for Kenya, Madagascar, Republic of the Congo, United Arab Emirates, and Yemen are based on examined specimens from the CAS, CNC, ICIPE and RMNH collections. Additionally, three other countries (Egypt, Pakistan, and Saudi Arabia) are recorded based on BOLD records whose sequences match sequences of the species, although those specimens were not available to us for study. The new data expands the species distribution across mainland Africa (where it was already known from a few countries), to Madagascar and the Arabian Peninsula (where it had not been recorded before). Based on the specimens we examined, the species has been collected throughout the entire year.

**Diagnosis.** *Apanteles hemara* can be recognized by having antenna slightly shorter than body length, with flagellomere 14 length 1.3–1.6 × its width; vein R1 about four times as long as distance between ends of veins R1 and 3RS; hind legs with black coxa, yellow trochanter and trochantellus, brown metafemur, metatibia yellow on anterior 0.5–0.6 and brown on posterior 0.4–0.5, metatibial spurs white, and metatarsus brown; propodeum mostly smooth, but with entire areola entirely defined by strong carinae; tergites 1 and 2 with strong, longitudinal striaion; and ovipositor sheats shorter than metatibia (0.7–0.9 ×). *Apanteles hemara* belongs to the *ater* species group (sensu Nixon 1965), which unfortunately comprises “many aggregates of species that are not closely related but merge into one another through transitional forms” (Nixon 1965: 25). The world species were keyed out by Nixon (1965), but many more species have been described since, and thus that paper is now outdated. Updates are available for species of the *ater* group from Europe (Papp 1980), the former Soviet Union (Tobias 1986), China (Chen and Song 2004) and Mesoamerica (Fernandez-Triana et al. 2014) but unfortunately there is no updated key to world species.
Redescription. Body color mostly black, tergites 3+ dark brown, laterotergites and sternites 3+ light yellow-brown. Head black, except for dark orange-brown labrum, light yellow-white palpi and dark brown antennae. Front and middle legs yellow (except for coxae and mesofemur light yellow-brown to brown); hind leg with black coxa, yellow trochanter and trochantellus, brown metafemur, metatibia yellow on anterior 0.5–0.6 and brown on posterior 0.4–0.5, metatibial spurs white, and metatarsus brown. Wings with most veins transparent or white, except for brown veins R1, r, 2RS and M; pterostigma mostly brown, with a very small white spot at base. Anteromesoscutum and scutellar disc with relatively coarse and dense punctures (distance between punctures smaller than diameter of individual puncture). Propodeum mostly smooth, with areola entirely defined by strong carinae. Tergites 1 and 2 with strong, longitudinal striation, contrasting with remaining tergites which are smooth.

Body measurements (in mm) and ratios. Body length: 2.50–3.20, fore wing length: 2.50–3.00, ovipositor sheaths: 0.62–0.84, metafemur: 0.65–0.77, metatibia length: 0.87–0.95, tergite 1 0.36–0.49. Length of flagellomeres: 1st (0.18–0.22), 2nd (0.18–0.22), 3rd (0.17–0.21), 14th (0.08–0.10), 15th (0.08–0.10) and 16th (0.12). Length/width of flagellomere 2: 2.75–3.14; length/width of flagellomere 14: 1.28–1.60. Head height/width: 0.82–0.88; head slightly narrowing towards mandibles, width at clypeus base 0.88–0.88 × head width at antennal base. Malar line 1.12–1.50 × mandibular base. Ocular ocellar line 1.67–2.00 × posterior ocellus diameter; interocellar distance 1.71–2.17 × posterior ocellus diameter. Scutellar disc length 1.09–1.15 × width at base. Maximum height of mesoscutellum lunules 0.42–0.56 × maximum height of lateral face of mesoscutellum. Tergite 1 widening from anterior margin to two thirds of tergite length, then slightly narrowing towards posterior margin; tergite 1 length
1.64–2.09 × tergite width at posterior margin; tergite widths (at anterior margin/maximum width/posterior margin): 0.19–0.25/0.27–0.30/0.22–0.25. Tergite 2 width at posterior margin 2.31–2.64 × length medially. Tergite 2 length medially 0.48–0.64 × tergite 3 length medially. Metafemur length 3.25–3.67 × metafemur width. Pterostigma length 2.55–2.86 its width. Vein R1 length 1.15–1.27 × pterostigma length. Vein r length 1.82–2.20 × vein 2RS length.

Variation. Despite the widespread distribution of the species across four major biogeographical regions (mostly Old World tropics), the specimens we examined were very similar morphologically (Figs 4–8), with only minor variation in the color of the hind legs and tergites 3+ of the metasoma. However, we could not examine specimens from the Australian or the Palaeartic regions, which might be more variable than the specimens from Africa and Asia we studied.

Biology. Solitary parasitoid (Fig. 4E); over 41,280 parasitism cases were observed by us under laboratory condition at ICIPE in Kenya during a three years study. Hosts: Choreutidae, *Tebenna micalis*; Crambidae, *Cnaphalocrocis trapezalis, Herpetogramma stultalis, Hydriris ornatalis, Omiodes indicatae, Spoladea recurvalis, Udea ferrugalis*. For additional details see Comments below.

DNA barcodes. A total of 17 DNA barcodes were obtained from the specimens we studied. All sequences but one were over 600 base pairs (bp) long, with most representing full barcodes (658 bp). Additional sequences representing the species are found in BOLD, but we could not examine those specimens because they belong to projects that are not public yet. Overall, there are currently 32 sequences belonging to *A. hemara* in BOLD, 24 of them being public records and 19 being barcode compliant (Fig. 2). The species has been assigned the BIN number BOLD:AAB1927 (for the concepts of ‘BIN’ and ‘barcode compliant’ see Ratnasingham and Hebert 2007). The DNA barcode intraspecific variation (p-distance) for *A. hemara* averaged 0.61% (4 bp), with a maximum of 1.91% (12 bp), but more than half of the analyzed sequences differed by only 0.2–0.4 % (1–2 bp). There were six different haplotypes recognized among the barcode specimens. From a DNA barcode perspective, the nearest species in BOLD is *Apanteles xanthostigma* (Haliday, 1834), which differs from *A. hemara* by 6.93% (45 bp) (Figs 2, 3).

Comments. In Kenya, the parasitoid was collected during outbreaks of two amaranth leaf-webber species, *Spoladea recurvalis* and *Udea ferrugalis* on two species of amaranth, *Amaranthus cruentus* L. and *A. dubius* L. It was successfully reared under laboratory conditions on both amaranth leaf-webber species at ICIPE. However, it failed to attack the leafworm species *Spodoptera littoralis* (Boisduval, 1833) and *S. exigua* (Hübner, 1808) (Noctuidae). During population dynamics studies carried out under field conditions at high and mid altitude levels in Central Kenya, field parasitism rates on *S. recurvalis* as low as 3% were obtained during outbreak periods while parasitism rates as high as 25 to 75% were achieved outside outbreak periods. These observations prompted studies for potential augmentative biological control strategies for early interventions aiming at preventing or reducing outbreaks of the amaranth leaf-webbers in farmers’ fields. Under laboratory conditions (25 ± 2°C, 60 ± 10% RH and 12L:12D
photoperiod), *A. hemara* demonstrated high performance on *S. recurvalis* and *U. ferrugalis* both through high direct parasitism rates and significant non-reproductive mortalities caused to the hosts. The parasitized caterpillars can easily be distinguished from
non-parasitized ones within the first two days after the parasitoid’s oviposition in the larva, through a significant reduction in feeding, movement and the lack of windowing on the leaf epidermis. Subsequently their growth rate is reduced and within four days after the parasitoid’s oviposition, parasitized caterpillars are considerably smaller in size than their non-parasitized counterparts, turn creamish and will all die whether...
the parasitoid successfully emerged or not. The developmental times are 12 and 13 days for male and female parasitoids respectively. *Apanteles hemara* is currently being considered for a conservative and augmentative biological control program against the amaranth leaf-webbers in Africa.
Figure 5. *Apanteles hemara* female specimen from Kenya (Voucher code: CNC507541).
Figure 6. Apanteles hemara female specimen from Yemen (Voucher code: CNC661375).
Figure 7. *Apanteles hemara* female specimen from Madagascar (Voucher code: CNC661376).
Figure 8. *Apanteles hemara* female specimen from Oman (Voucher code: CNC661377).
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