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Suspended mummies in *Aleiodes* species (Hymenoptera: Braconidae: Rogadinae) with descriptions of six new species from western Uganda based largely on DNA sequence data

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Abstract

A group of species of the rogadine braconid genus *Aleiodes* are shown to produce a distinctive mummy, which is ''J''-shaped and is formed after the host larva, in all cases an ennomine geometrid moth, has dropped from a plant suspended in midair by a silk thread. The group includes one described species, *A. buzurae* He & Chen from China, and a species complex from tropical Africa (W. Uganda). All the African specimens reared from suspended mummies looked morphologically virtually indistinguishable, though there was considerable colour variation that segregated the specimens into five groups. Three gene fragments (nuclear 28S D2-3 rDNA, the nuclear ITS2 region and part of the mitochondrial cytochrome oxidase 1 gene (CO1)) were sequenced to assess if these specimens represented a single variable species or a complex of morphologically cryptic species. Results show variation in all three gene fragments, with strong signal in the CO1 gene, parsimony analysis of which revealed six well supported groups corresponding to the colour variants, except that two specimens with nearly identical colour differed considerably in their CO1 sequences. Large, and difficult to align, variation was found in the ITS2 fragments, which by eye also supported the same six groupings. Limited variation was found in the 28S fragment, but one position supported monophyly of the two specimens belonging to one of the species circumscribed by the other genes. These groups are considered to correspond to separate species, which are described as new: *A. barnardae* Quicke & Shaw, *A. basutai* Quicke & Shaw, *A. kanyawarensis* Quicke & Shaw, *A. kasenenei* Quicke & Shaw, *A. mubfsi* Quicke & Shaw and *A. trevelyanae* Quicke & Shaw. The possible function of the specialised mummification behaviour is discussed and some observations on rates of hyperparasitism are presented.

Keywords: *Parasitoid, insect behaviour, cryptic species, internal transcribed spacer, barcoding*

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Introduction

Aleiodes is a cosmopolitan and species-rich genus of the braconid wasp subfamily Rogadinae. In common with all other members of the Rogadinae *sensu stricto*, the species are koinobiont endoparasitoids of Lepidoptera larvae (Shaw 2003), which they eventually kill and mummify. The parasitoid pupates within the host mummy and eventually emerges from it as an adult. Most *Aleiodes* species, for which rearings have been recorded, are solitary (a very few are gregarious, forming moderately large broods) and, typically, the host is killed in its penultimate larval instar (Shaw 1983; Shaw and Huddleston 1991). Strong and consistent modification of host behaviour just before the mummification process has been seen to be caused by many W. Palaearctic *Aleiodes* species (Shaw 1994 and unpublished data). In these cases, the host generally either undertakes some activity that is not habitual before settling to await its death (such as climbing high from the normal feeding, resting or pupation site, as seen in the arctiid, lymantrid and lasiocampid hosts of *Aleiodes alternator* (Nees)), or that anticipates an activity that unparasitised hosts would perform at a later time (such as precocious entry into leaf litter or soil, as seen in the penultimate instar *Orthosia* (Noctuidae) hosts of *Aleiodes dissector* (Nees), or preparation of an aerial site akin to that in which pupation occurs, such as the frail, roomy spinnings made by penultimate instar *Leucoma salicis* (L.) (Lymantriidae) parasitised by *Aleiodes pallidator* (Thunberg)).

Attack by parasitoids on *Aleiodes* mummies (''pseudohyperparasitism'') is generally very high and the various behaviours noted above presumably arose, at least partly, in response to this persistent pressure, as mummies are always formed in sites of relatively low predictability, or given greater physical protection, as a result. Here, we report an apparently hitherto unrecorded modification of host behaviour prior to mummification seen in a compact species-group of *Aleiodes*, occurring in western Uganda, attacking Geometridae feeding on understory plants and saplings. There is evidence that a related species from China has a similar habit.

During August of 2002 and 2003, in the undisturbed and lightly logged wet montane forest of Kibale National Park, western Uganda (Struhsaker 1997), one of us (DLJQ) observed and collected a number of mummified geometrid larvae, which were suspended by a silk thread 8–50 cm long from leaves of a range of understory plants and tree saplings. These mummified larvae, belonging to one or more species of Ennominae (Lepidoptera: Geometridae), were shaped like a letter ''J'', in that the head and thorax were bent ventrally under the rest of the body (which contained the *Aleiodes* pupa) and, as the silk strand emanated from the mouthparts, the resulting suspended mummy was balanced so as to be virtually horizontal (with the dorsum of the caterpillar skin lying below; Figure 1). Most of these mummies were collected suspended from the herb *Marantochloa leucantha* (K.Schum.) Milne-Redh. (Marantaceae), but some were also from *Piper capense* L. f. (Piperaceae) and *Vangueria* Comm. ex Juss. (Rubiaceae), *Lovoa* Harms (Meliaceae) and *Urera* Gaudich (Urticaceae) species. The 10 emerging *Aleiodes* from all of these were remarkably similar morphologically, but varied greatly in colour, which prompted us to employ molecular markers to try to determine whether they represented a single variable species or a group of closely related ones. For this purpose, we sequenced the D2-D3 region of the nuclear 28S rDNA gene, part of the mitochondrial CO-I gene, and the smaller internal transcribed spacer (ITS2) of the nuclear ribosomal gene complex.

Chen and He (1997; Figure 177) illustrate a mummy collected in China, which is essentially indistinguishable from ours from western Uganda (Figure 1), ascribed to *Aleiodes buzurae* He & Chen, a Chinese species reared from the ennomine geometrid *Buzura*

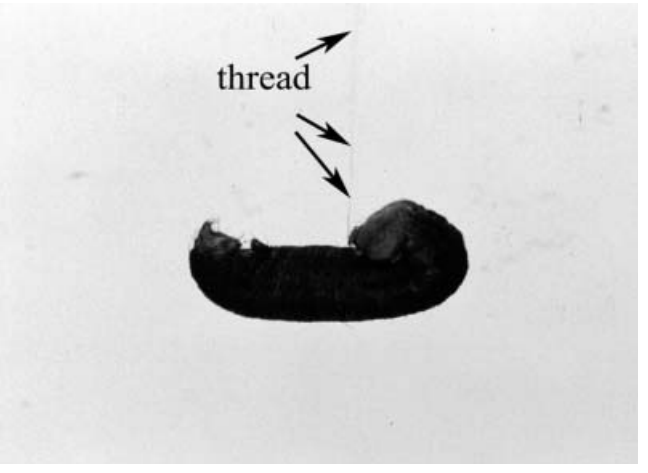


Figure 1. Photograph of a suspended mummy showing J-shaped configuration with silk thread (arrowed) arising near larval mouth.

suppressaria (Guenée) (=*Biston*, according to Scoble (1999)) that clearly has close affinity with the species found in western Uganda. Neither in the original description (He and Chen 1990) nor in the revision (Chen and He 1997) is mention made of the mummy being suspended from a thread but, in view of the date of collection (1954) of the original material by another person, it seems likely that this feature had not been appreciated by these authors rather than that it was not so. Indeed, the configuration of the host mummy illustrated by Chen and He (1997) really leaves little other possibility.

Molecular methods

DNA was extracted from single legs preserved in absolute ethanol using an ethanol precipitation method with final elution into 30 µl of water. PCR was carried out in 20 µl reactions containing 1.0 µl of DNA extract, 10 pmol of primers (Table I), 10 nmol of dNTPs (Amersham Pharmacia Biotech: APB), 1.0 U of Taq polymerase (Bioline) and 2 µl of 10 × reaction buffer (2.0 mM $MgCl_2$). PCR conditions were 94°C for 30 s, 50°C for 30 s and 72°C for 60 s (35 cycles with an initial denaturation for 2 min and a final extension for 7 min). PCR products were purified using GFX gel band purification kit (APB) and sequenced directly using BigDye terminators.

Primer sequences are given in Table I. The COI primers LCO/HCO (also called Folmer primers, after Folmer et al. (1994)) were COI forward=LCO 1490 and COI

Table I. Primer sequences.

Gene fragment	Direction	Primer sequence (5′→3′).
28S	Forward	GCG AAC AAG TAC CGT GAG GG
	Reverse	TAG TTC ACC ATC TTT CGG GTC
COI	Forward	GGT CAA CAA ATC ATA AAG ATA TTG G
	Reverse	TAA ACT TCA GGG TGA CCA AAA AAT CA
ITS2	Forward	TGT GAA CTG CAG GAC ACA TG
	Reverse	ATG CTT AAA TTT AGG GGG T

reverse=HCO 2198. The forward ITS2 primer was designed based on the 5.8S rDNA sequence of *Trichogramma minutum* Riley (GenBank accession numbers U36235 and U36236) anchoring between the 63rd and 81st positions. The reverse primer, which anchors at the beginning of the 28S rDNA sequence, was modified (terminal base removed) from that of Porter and Collins (1991).

Materials

Aleiodes apiculatus (Fahringer) and *A. testaceus* (Telenga) were included as outgroups because, in a larger study of the phylogeny of *Aleiodes* species based on analysis of the 28S D2-D3 rDNA gene region (Mori et al. in preparation), these appeared in groups on either side of the clade, including the suspended mummy taxa. One additional unidentified Afrotropical individual, AL0468, from the east shore of Lake Naivasha, Kenya, was included because it appears, on the basis of DNA sequence data and morphology, also to belong to this group.

For comparison of variation in the CO1 gene fragment of the African specimens with that within known and well-supported European species, sequences were obtained from seven specimens of *A. pictus* (Herrich-Schäffer), five specimens each of *A. coxalis* (Spinola) and *A. ruficornis* (Herrich-Schäffer), and from four of *A. dissector* (Nees), collected from a wide range of localities in the UK and Europe.

Four additional species, representing *A. compressor* (Herrich-Schäffer), *A. unipunctator* (Thunberg), an unidentified species from Las Cuevas, Belize (AL0005) and one from the Amani Hills, Tanzania (AL0044), were also included to provide broad representation of the genus.

DNA sequences are deposited in the EMBL/GenBank database; accessions numbers, provenances and voucher numbers are given in Appendix A.

Cladistic methods

Sequence data were analysed using maximum parsimony with PAUP* (Swofford 1999). Bootstrapping on the 15 taxon data set used 500 bootstrap replicates, each search of the pseudoreplicate using branch-and-bound searching. Maximum parsimony analysis of the 38 taxon data set used 1000 random additions, tree bisection–reconnection branch swapping, with only one tree saved each time: trees of the most parsimonious length obtained were found in more than 90% of random additions.

Results

Comparison with Aleiodes buzurae

The western Ugandan material is similar to *A. buzurae* in having a distinctive and strong rugulose–reticulate sculpture of the first four metasomal tergites and mid-dorsal and lateral sinuate emargination of the posterior of the fourth metasomal tergite. Although obviously belonging to the same compact species group, *A. buzurae* differs from all of the Ugandan material examined in its more sharply-defined and deeper postero-lateral emargination on the 4th metasomal tergite, its pattern of metasomal markings (Figure 6), and its somewhat slenderer legs in females (Figure 175 in Chen and He 1997).

Molecular results

Analysis of the 28S D2+D3 sequence data for the suspended mummy specimens revealed no phylogenetic structure, a strict consensus of the >1 million equally parsmonious trees being completely unresolved. However, a small number of substitutions were apparent. Both specimens of *A. trevelyanae* sp. n. differed from all others by a single substitution in the D3 region, the single specimen of *A. mubfsi* sp. n. differed from all others by two bases in the D2 region (corresponding to positions 107 and 199 of the alignment presented by Belshaw et al. (1998; Figure 1 loc. cit.), and *A. kasenenei* sp. n. differed from all others at one base in the D3 region.

Although intraspecific variation can be found in both CO1 and ITS sequences (Alvarez and Hoy 2002), the variation observed in the CO1 sequences was greatly in excess of that observed between multiple conspecific individuals (even from widely different localities) for a number of other *Aleiodes* species. For example, Figure 2 shows a phylogram, derived from maximum parsimony analysis of CO1 sequence data, for the *A. buzurae* complex specimens and multiple individuals of four well-supported European species of *Aleiodes*, and within these the total CO1 variation on the tree corresponds to at most 10 base changes among the *A. pictus* individuals from six widely separated localities, seven base changes in each of *A. coxalis* and *A. ruficornis* and six in *A. dissector*. The variation between the individuals of *A. barnardae* sp. n. (six bases) and *A. trevelyanae* sp. n. (one base) is, therefore, equivalent to that found within the European species, given that they are all from the same small region of forest. Of the Ugandan species we recognise, the closest (on the basis of their COI

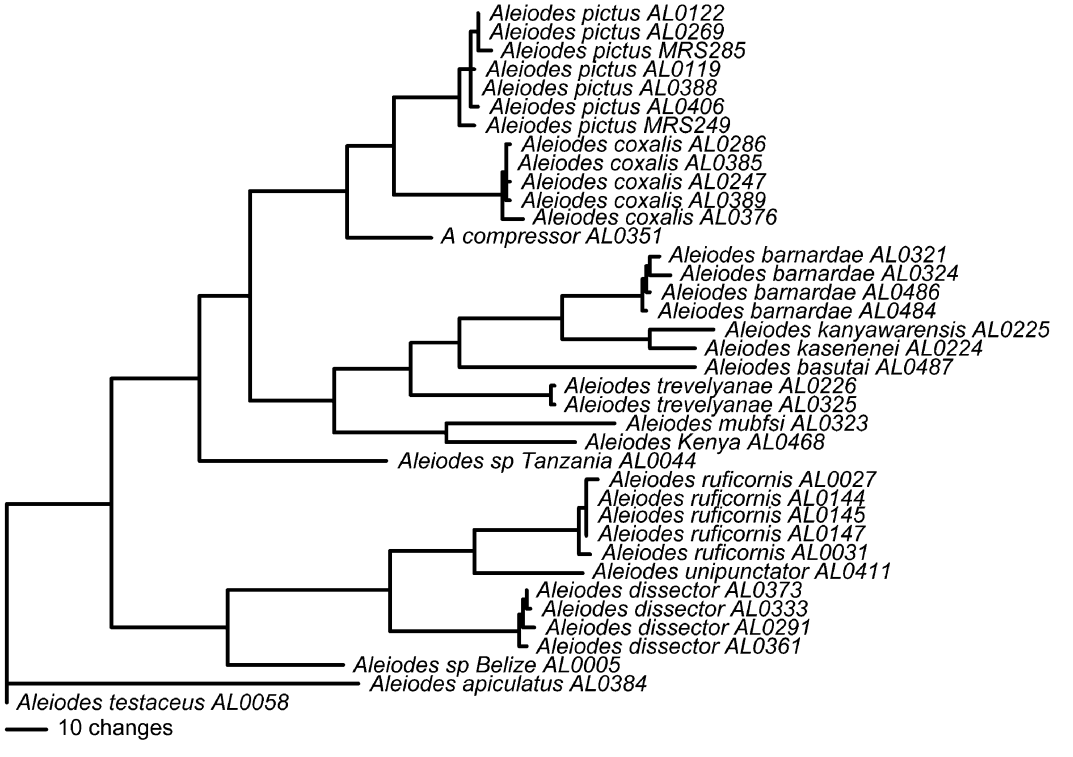


Figure 2. Phylogram from analysis of CO1 DNA sequence data for individuals of the *A. buzurae*-group and related species, and also multiple representatives of four European species for comparison.

sequences) are *A. kanyawarensis* sp. n. (one female) and *A. kasenenei* sp. n. (one male) (see Figures 2 and 3), which have widely differing colour patterns. The COI sequences of these two specimens, differing at 27 base positions, were still more than twice as different from one another as are conspecific members of any of the other species (Figure 4), and their ITS2 sequences have markedly different inserts (Figure 5). In contrast, the ITS2 regions of the four individuals of *A. barnardae* n.sp. were identical, as were those of both of the *A. trevelyanae* sp. n. specimens. We, therefore, conclude that the specimens reared in Kibale represent a complex of morphologically practically identical, but genetically isolated distinct species.

Monophylies of both *A. trevelyanae* sp. n. and of *A. barnardae* sp. n. are indicated by 100% bootstrap support in the analysis of their COI sequence data (Figure 3).

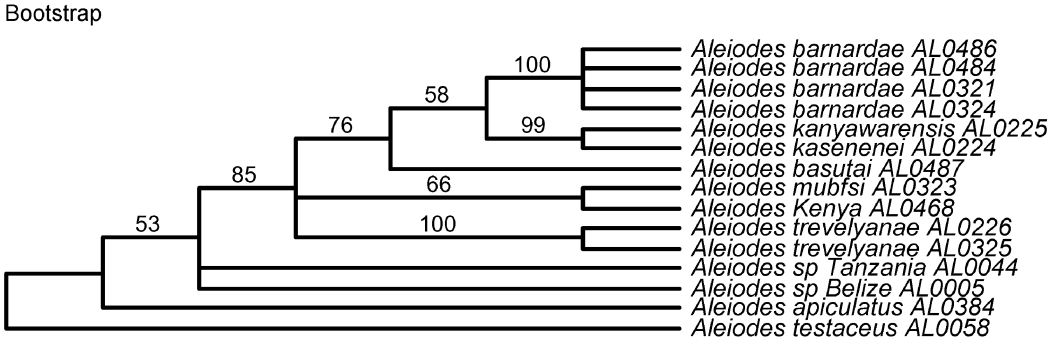


Figure 3. Bootstrap consensus tree from analysis of CO1 sequence data for *Aleiodes* reared from suspended mummies in Uganda plus another member of the *A. buzurae*-group and outgroups.

Taxon	Contiguous part of CO1 sequence
Fragment 1	$ *! * * * * *** ! * * *
barnardae n.sp.	GGGAGAATTTTAAAAAATGATCAAATTTATAATGGAATAGTAACATTACATGCTTTTATTATAATTTTTTTTAT
basutui n.sp.	GGGAGAATTTTAAAAAATGACCAAATTTATAATGGAATAGTAACCTTACATGCTTTCATTATAATTTTTTTTAT
kanyawarensis n.sp.	GGGAGAATTTTAAAAAATGACCAAATTTATAATGGAATAGTGACATTACATGCTTTCATTATAATTTTTTTTAT
kasenenei n.sp.	GGAAGAATTTTAAAAAATGACCAAATTTATAATGGAATAGTGACATTACATGCTTTCATTATAATTTTTTTTAT
mubfsi n.sp.	GGTAGTATTTTAAAAAATGATCAAATTTATAATGGAATAGTAACTTTACATGCTTTTATTATAATTTTTTTTAT
trevelyanae n.sp.	GGAAGAATTTTAAAAAATGACCAAATTTATAATGGTATGGTAACATTACATGCTTTCATTATAATTTTTTTTAT
A. sp. Kenya	GGAAAAATTTTAAGAAACGATCAAATTTACAATAATATAGTAACTTTACATGCTTTTATTATAATTTTTTTTAT
A. sp. Tanzania	GGAAGAATTTTAAAAAATGATCAAATTTATAATGGAATAGTAACTTTACATGCTTTTATTATAATTTTTTTTAT
Fragment 2	! ! * ! ! * $ * ! * ! ! *! !! $ $ * !** * !
barnardae n.sp.	AGTTATGCCTATTATAATTGGGGGATTCGGAAATTGATTAATCCCATTAATATTAGGAGCCCCAGATATAGCTT
basutui n.sp.	AGTTATACCAATCATAATTGGAGGGTTTGGAAATTGATTAATTCCATTAATATTAGGAGCTCCTGATATAGCTT
kanyawarensis n.sp.	AGTTATACCAATTATAATTGGGGGATTCGGAAATTGATTAATTCCTTTAATATTAGGCGCGCCAGATTTTGCTT
kasenenei n.sp.	AGTCATACCAATTATAATTGGAGGGTTTGGGAATTGATTAATTCCTTTAATATGGGGCGCGCCAGTTTTTGCTT
mubfsi n.sp.	AGTGATACCTATTATAATCGGGGGATTTGGTAACTGACTAATTCCTTTAATATTAGGGGCCCCTGATATAGCTT
trevelyanae n.sp.	AGTTATACCAATTATAATTGGGGGCTTTGGAAATTGATTAATTCCACTAATATTAGGAGCACCTGACATAGCTT
A. sp. Kenya	AGTTATACCAATTATAATTGGAGGATTTGGAAATTGATTAATTCCATTAATATTAGGAGCCCCAGACATAGCTT
A. sp. Tanzania	AGTTATACCTATTATAATTGGAGGATTTGGAAACTGATTAATTCCTTTAATATTAGGAGCACCAGATATAGCAT

Figure 4. Selected contiguous fragment of the CO1 gene in individuals of the *Aleiodes buzurae*-group from Uganda and Kenya with bold characters indicating substitutions. Asterisks show phylogenetically informative substitutions at species level; exclamation marks show apomorphies for individual species in the *buzarae* group and $ sites that have both unique and informative variation.

Taxon	ITS2 fragments
Fragment 1	* * ———————————— ** **** *
barnardae **n.sp.**	CATTTGATTTTTTTT----------CAATTGT**A**CAAGCGAAATAAA
basutui **n.sp.**	**T**ATTTGATTTTTTTT----------C**TT**T**AAA**TCAAGCGAAATAAA
kanyawarensis **n.sp.**	CATTTGATTTTTTTTCTTTTTAATT--------AAATCAATTGTCCAAGCGAAATAAA
kasenenei **n.sp.**	CATTTGATTTTTTTT----------CAATTGT**A**CAAGCGAAATAAA
mubfsi **n.sp.**	CATTTG**T**TTTTTTTTTTTTTTXXXXXXXCAAAAAAAXTCATTTGTCCAAGCGAAAT**G**AA
trevelyanae **n.sp.**	CATTTGATTTTTTTTTATTTA----------AAATCAATTGT**A**CAAGCGAAATAAA
Fragment 2	* * * ————————————*— ——
barnardae **n.sp.**	TTTGCRTGTTAARACA**T**AYACGTGTKGTTATATAATTTATTATATATATA-**G**AGGCATATAAAA--TT
basutui **n.sp.**	TTTGCATGTTAAGACACATACGTGTTGTATAATAATTTATTATATA-----AAGGCATNNAAAA--TT
kanyawarensis **n.sp.**	TTTGCATGTTAAGACACATACGTGTT**A**TTA--TAATTTATTATATA-----AAGGCATATAAAA--TT
kasenenei **n.sp.**	TTTGCATGTTAAGACA**T**ATACGTGTTGTTATATAATTTATTATATATATAG**G**AGGCATATAAAA--TT
mubfsi **n.sp.**	TTTGCATGTTAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXTTATATTAA----AGGCATATAAAAAATT
trevelyanae **n.sp.**	TTTGCATGTTAAGACACATA**T**GTGTTGTTA----------GGCATATAAAA--TT

Figure 5. Two fragments of the ITS2 region aligned by eye showing marked differences between species in the *Aleiodes buzurae*-group. Asterisks and bold font indicate substitutions in length-conserved regions and lines show regions of length variation. Xs indicate uncertainty about number and identity of bases.

Furthermore, a sister group relationship between *A. kanyawarensis* sp. n. and *A. kasenenei* sp. n. obtained 99% bootstrap support. Other relationships between the *buzurae* group species were equivocal.

Systematics

Superficial key to species

1. Metasomal tergites entirely pale (whitish to pale yellow-brown) 2
– At least metasomal tergite 3 and 4 with dark brown markings 4

2. Pterostigma largely to entirely black (Figure 7) . . . . . *barnardae* sp. n.
– Pterostigma largely pale yellowish, only the margin beyond origin of vein r grey (Figure 14) 3

3. Metasomal tergite 4 largely pale yellow . . . . . *mubfsi* sp. n.
– Metasomal tergite 4 largely yellow-white blending to pale yellow posteriorly . . . . . *kasenenei* sp. n.

4. Except for narrow yellowish lateral flush, metasomal tergites 3 and 4 largely dark brown to their posterior margins (Figure 19); occiput smokey-brown contrasting with frons; propodeum broadly medially brown-black . . *kanyawarensis* sp. n.
– Metasomal tergite 3 with posterior margin pale and with a pale central band for its full length; tergites 1 and 2 extensively pale yellow, the extent of the brown markings less than that of the yellow areas; vertex yellowish, not darker than mesonotum; propodeum variable 5

5. Pterostigma entirely dark brown (Figure 10); propodeum yellow (same colour as mesonotum) (Figure 10); metasomal tergite 2 whitish with small brown sublateral marks in apical half (Figure 12); metasomal tergite 3 with pair of brown marks wider posteriorly than anteriorly (Figure 12) . . . . . *basutai* sp. n.

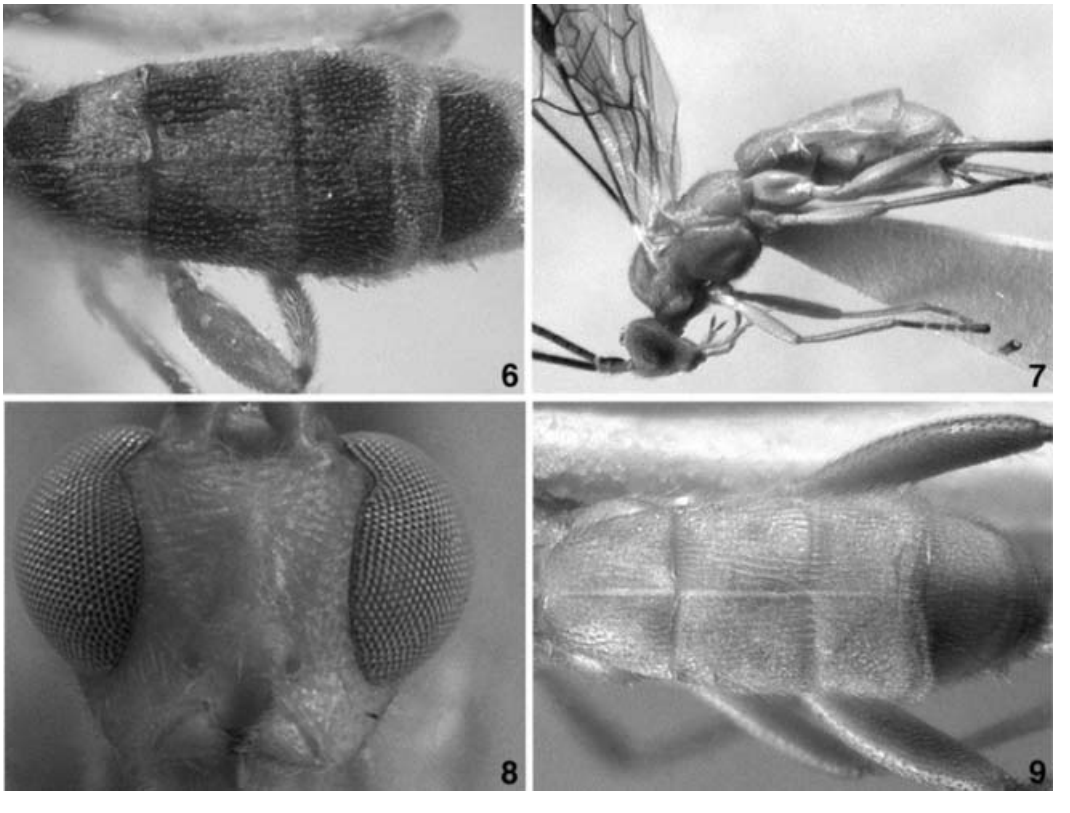
– Pterostigma distinctly pale cream coloured on basal 0.15 (Figure 24); propodeum at least with lateral brown-black marks (Figure 20); brown-black markings on metasoma more extensive and marks on tergite 3 oval to round, not widening posteriorly (Figures 22, 25) . . . . . *trevelyanae* sp. n.

None of the species described here is known from more than one sex. The two species known only from males, *A. kasenenei* n.sp. and *A. kanyawarensis* n.sp. share a largely pale pterostigma (Figure 14) and a more rectangular second submarginal cell of the fore wing. These might be secondary sexual features because the molecular phylogenetic analyses (see Figures 2 and 3) indicate that these are not particularly closely related despite their similar pterostigmal colour pattern.

Aleiodes barnardae **Quicke & Shaw**, sp. n.
(Figures 7–9)

Material examined

Holotype: Female, Uganda, Kibale Forest National Park, Kanyawara, August 2002, reared from suspended mummy of an ennomine geometrid (NMS).



Figures 6–9. Automontage photographs of features of the *Aleiodes buzurae*-group. (6) *A. buzurae*, metasoma. (7)–(9) *A. barnardae* sp. n., habitus, face and metasoma, respectively.

Paratypes: Three females, same locality as holotype, one collected and reared August 2002, two in August 2003.

Description

Females. Length of body 4.2–4.8 mm; length of fore wing 4.0–4.2mm; length of tergite 2+3 1.1mm.

Antenna with 40–42 segments (41 in holotype), 1.35 times longer than fore wing. Terminal flagellomere strongly acuminate, 3.4 times longer than wide. Median flagellomeres 2.25 times longer than wide. Sculptured parts of 1st and 2nd flagellomeres equally long. Third segment of maxillary palp 1.3 and 1.6 times longer than the 4th and 5th segments, respectively. Inter-tentorial distance 1.46 times tentorio-ocular distance. Width of clypeus:width of face=1.0:2.3. Width of head:width of face:height of eye=3.1:1.0:1.8. Face with small elongate median bulge, lateral to this with distinctly transverse rugose striae. Frons depressed and with distinct carina bordering anterior two-thirds of depression laterally, close to but separate from margin of eye. Stemmaticum coarsely rugose. Occipital carina broadly effaced medially.

Mesosoma 1.63 times longer than deep, coriaceous; mid-posterior part of mesoscutum more coarsely sculptured.

Fore wing: Lengths of veins r:3-SR:SR1=1.0:2.5:5.6. Lengths of veins 2-SR:3-SR:r-m=1.42:2.6:1.0. Vein 2-CU1 1.4 times longer than vein 1-CU1. Hind wing: Vein M+CU 1.4 times 1-M. Base of wing evenly setose.

Length of fore femur (excluding trochantellus):tibia=1.0:1.15. Length of hind femur (excluding trochantellus):tibia:basitarsus=2.0:2.7:1.0. Apex of hind tibia without a comb (setal fringe). Claws simple.

Metasomal tergites 2 and 3 with complete mid-longitudinal carina. Second tergite 1.38 times wider posteriorly than medially long. Third tergite 1.7 times wider posteriorly than medially long. Second suture rather weak, slightly anteriorly pointed medially. Posterior margin of 5th metasomal tergite with well-defined posterolateral emargination.. Ovipositor sheath 0.75 times length of hind basitarsus.

Yellow except the following: palps, malar region of face below anterior tentorial pits, fore and mid coxa and trochantellus, posterior half of 1st metasomal tergite, all of metasomal tergites 2–4 white; pronotum, metapleuron, middle of propodeum and hind trochanter whitish; tip of mandible, flagellum, scapus and pedicellus laterally and medially black; wing venation except basal 0.03 of fore wing vein C+SC+R, fore and mid tarsus, apical 0.05 hind tibia and hind tarsus (except paler telotarsus) dark greyish. Ovipositor sheath black with basal 0.2 whitish.

Molecular features

The ITS2 sequence of *A. barnardae* n. sp. is virtually identical to that of *A. kasenenei* n. sp (Figure 5), but these two species differ in their CO1 sequences at many 3rd codon positions (see Figure 4).

Etymology

Named after Sue Barnard for her friendship and help during the 2002 Kibale field trip.

***Aleiodes basutai* Quicke & Shaw**, sp. n.
(Figures 10–13)

Material examined

Holotype: Female, Uganda, Kibale Forest National Park, Kanyawara, viii-2003, reared from mummified, suspended geometrid larva (NMS)

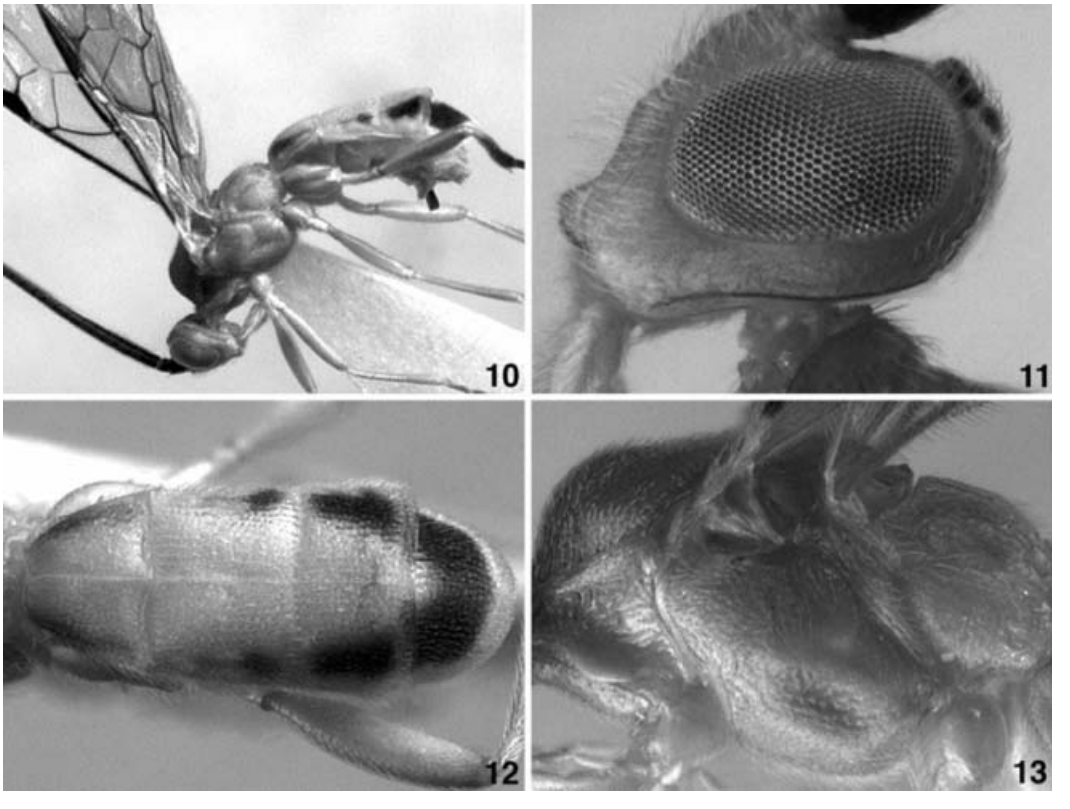
Description

Length of body 4.2 mm, of fore wing 4.0 mm. Antenna with 41 segments. Width of clypeus:width of face=1.0:2.4.

Morphologically like *A. barnardae* sp. n. (q.v.) except for colour (see key).

Molecular features

Displays nine unique base substitutions in the CO1 gene fragment, of which one is shown in Figure 4. The ITS2 fragment shows five unique substitutions in the length-conserved part (Figure 5, upper panel) and an indel of unique length (Figure 5, lower panel).

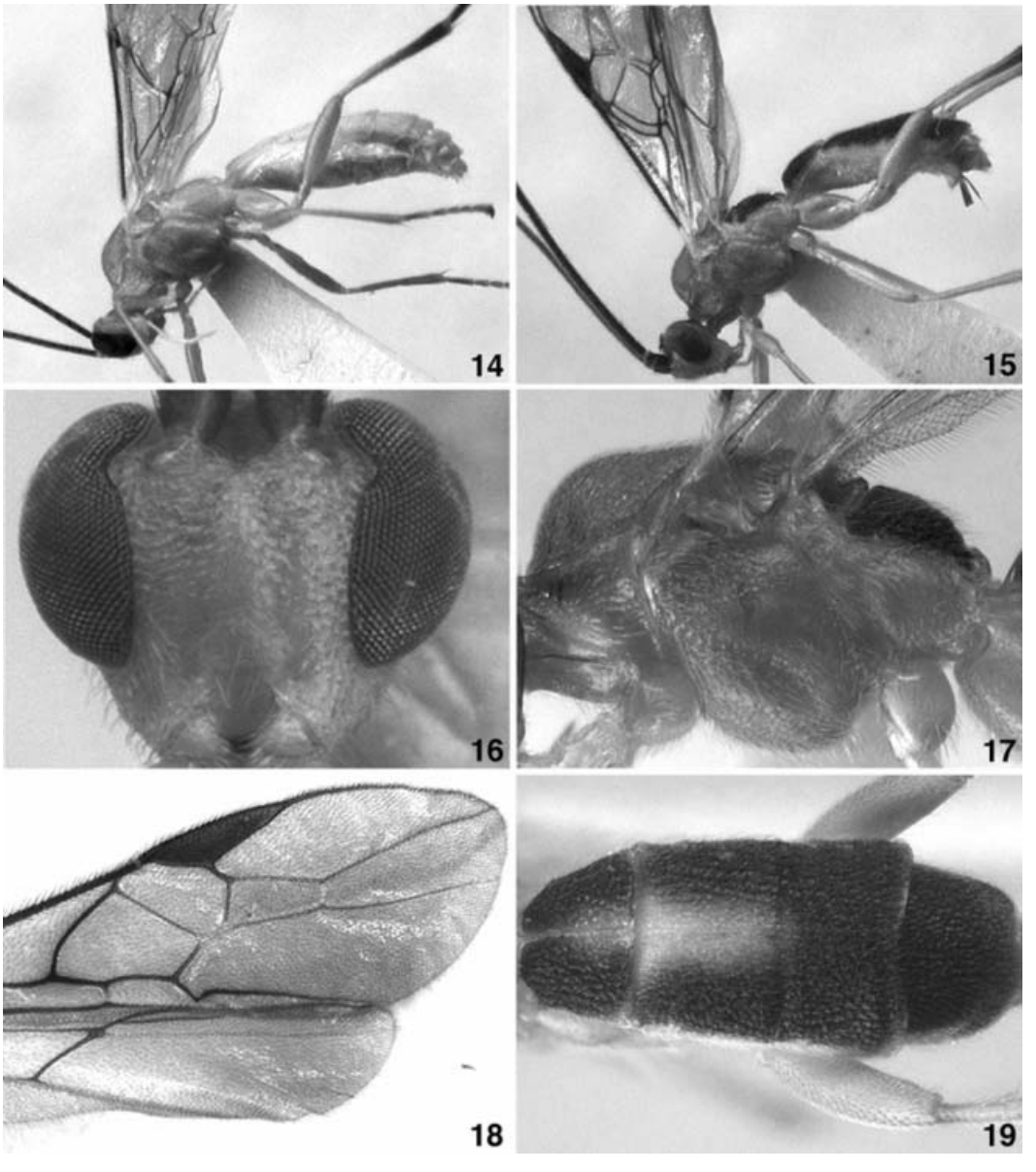


Figures 10–13. Automontage photographs of features of *Aleiodes busutai* sp. n., habitus, head lateral aspect, metasoma and mesosoma, respectively.

Etymology

Named after Dr Gill Basuta of Makerere Biological Field Station, for his help and great knowledge and enthusiasm at Kibale.

***Aleiodes kanyawarensis* Quicke & Shaw**, sp. n.
(Figures 15–19)



Figures 14–19. Automontage photographs of features of the *Aleiodes buzurae*-group. (14) *Aleiodes kasenenei* sp. n., habitus; (15)–(19) *Aleiodes kanyawarensis* sp. n., habitus, face, mesosoma, wings and metasoma, respectively.

Material examined

Holotype: Female, Uganda, Kibale Forest National Park, Kanyawara, viii-2002, reared from mummified, suspended geometrid larva (NMS)

Description

Length of body 4.4 mm, of fore wing 4.0 mm. Antenna with 41 segments. Width of clypeus:width of face=1.0:2.05.

As for *A. barnardae* sp. n. except for colour. Largely pale honey-yellow, stemmaticum black, antennae except small ventral mark on scape, occiput, propodeum except narrowly laterally and posteriorly, first metasomal tergite except anterior semicircular area and narrowly medio-posteriorly, second metasomal tergite except broadly medially and narrowly laterally, third and fourth tergites except narrowly laterally, apex of hind tibia and hind tarsus brown or brown-black; malar region paler yellow; fore and mid coxae and trochanters yellow-white; wings clear with dark brown venation and entirely black pterostigma.

Molecular features

Displays two unique sequences in the indel regions of the ITS2 gene (Figure 5).

Etymology

Named after the type locality.

***Aleiodes kasenenei* Quicke & Shaw**, sp. n.
(Figure 14)

Material examined

Holotype: Male, Uganda, Kibale Forest National Park, Kanyawara, viii-2002, reared from mummified, suspended geometrid larva (NMS)

Description

Length of body 4.5 mm, of fore wing 4.1mm. Antenna with 40 segments.

As for *A. barnardae* sp. n. except for colour. Pterostigma largely pale buff with borders and apical quarter grey. Metasomal tergites 1–4 largely pale yellow-white, narrowly more ochreous-yellow laterally.

Molecular features

Differs from all other species in the group by a single base substitution in the D3 region of the 28S gene. In terms of the ITS2 region, it has similar inserts and deletions to *A. barnardae* sp. n. (Figure 5).

Etymology

Named after Dr John Kasenene of Makerere University Biological Field Station, for his knowledge of Kibale and support for the Tropical Biology Association.

Aleiodes mubfsi **Quicke & Shaw**, sp. n.

Material examined

Holotype: Female, Uganda, Kibale Forest National Park, Kanyawara, viii-2002, reared from mummified, suspended geometrid larva (NMS)

Description

Length of body 4.5 mm, of fore wing 3.5mm. Antenna with 38 segments.

As for *A. barnardae* sp. n. except for colour. Pterostigma largely pale. Metasomal tergites largely pale ochreous yellow, tergite 1 postero-medially, tergite 2 broadly medially and tergite 3 with a small anteromedial area rather more yellow-white.

Molecular features

Displays two unique substitutions in the 28S D2 region (corresponding to positions 107 and 199 of the alignment presented by Belshaw et al. (1998; Figure 1, loc. cit.). The indel regions of the ITS2 gene were hard to read in the only known specimen, possibly due to intragenomic polymorphism; however, the sequence displays a unique base substitution (see Figure 5, upper panel).

Etymology

Named after the adapted acronym of the Makerere University Biological Field Station (MUBFS) at Kanyawara.

Aleiodes trevelyanae **Quicke & Shaw**, sp. n.

(Figures 20–25)

Material examined

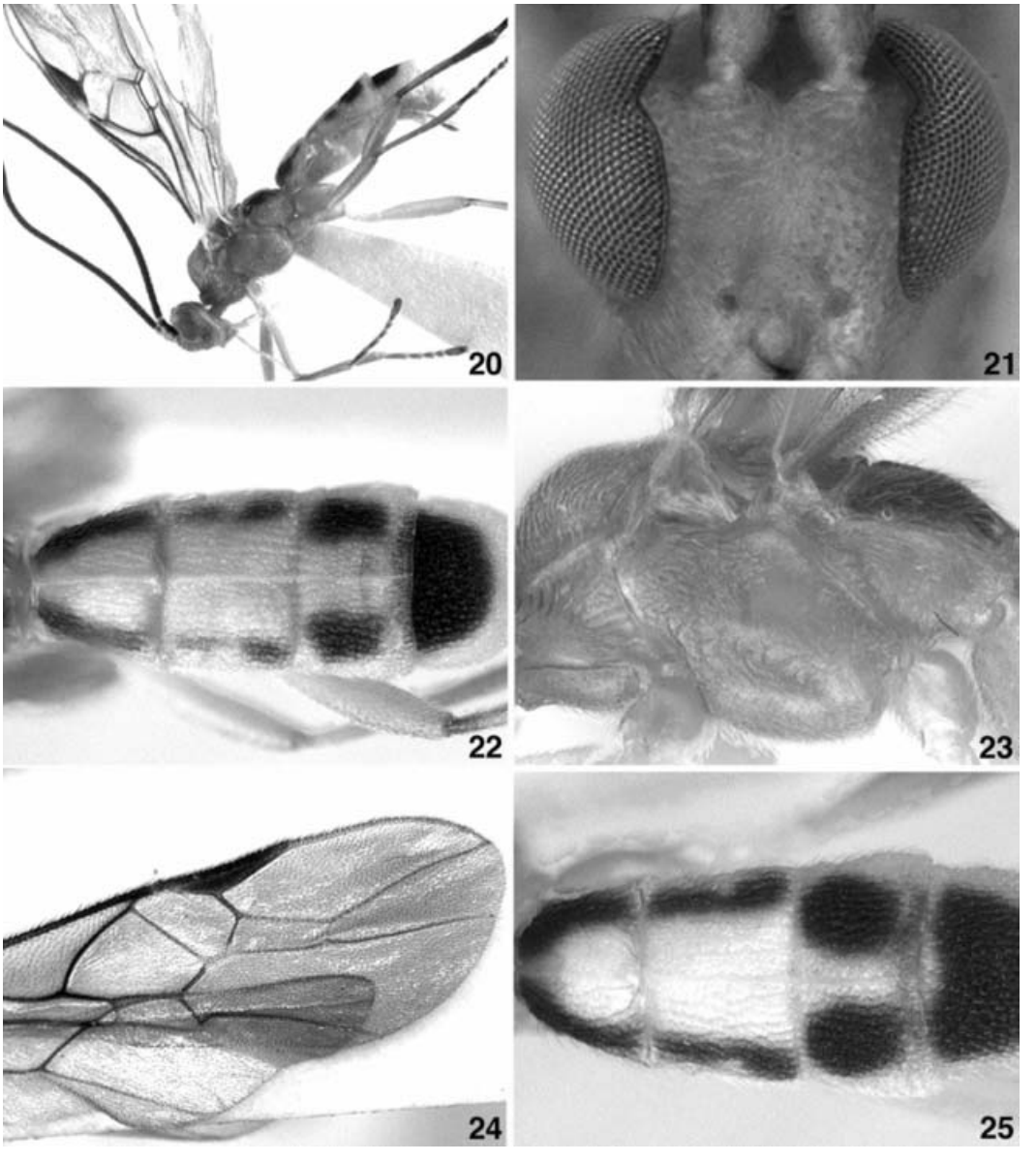
Holotype: Female, Uganda, Kibale Forest National Park, Kanyawara, August 2002, reared from suspended mummy (NMS)

Paratype: One female, same locality as holotype, reared August 2002

Description

Length of body 4.1 mm, of fore wing 3.5mm. Antenna with 40 segments.

Otherwise as for *Aleiodes barnardae* sp. n. except colour. Head, mesosoma and legs largely pale yellow except antenna and dorsal half of scape and pedicellus, stemmaticum, pair of longitudinal submedial stripes on propodeum (holotype) or propodeum largely medially (paratype, Figure 23) brown-black; middle part of propodeum yellow-white (holotype); mid tibia and tarsus grey-brown, hind tibia except dorsally on basal third, hind tarsus brown-black, fore and mid coxae and trochanters white-yellow; metasoma white to yellow-white with sublateral dark brown marks on tergites 1–3 and broadly medially on tergite 4. Wing venation largely black-brown, paler on distal veins, pterostigma yellow-white on basal 0.25 remainder brown-black (Figures 20 and 24). Width of clypeus:width of face=1.0:2.3.



Figures 20–25. Automontage photographs of features of *Aleiodes trevelyanae* sp. n. (20)–(24) Habitus, face, metasoma, mesosoma and wings of holotype; (25) metasoma of paratype.

Molecular features

Both sequenced individuals possessed a unique substitution in the D3 region of the 28S gene. The CO1 gene fragment sequenced was identical for both individuals and displays 15 unique substitutions, four of which are shown in Figure 4. Both length variable parts of the ITS2 sequences had indels of unique length and sequence.

Etymology

Named after Dr Rosie Trevelyan, the 'chief mzungu female' of the Tropical Biology Association.

Discussion

Use of DNA in tropical insect identification

Despite our extensive study of the specimens reared from the suspended mummies at Kibale, we have been unable to discern any morphological differences among them, though there is clear discontinuous variation in colour pattern. Without molecular evidence, we would simply have considered this as a variable or colour-polymorphic species. However, the high level of support for multiple clusters based on analysis of COI sequence data (Figures 2 and 3), and the congruence between these and the colour pattern and visually-recognised clusters of ITS2 sequences (Figure 5), indicates that these clusters are reproductively isolated even though sympatric and, therefore, we consider them to represent discrete species. Importantly, COI is mitochondrial and ITS2 is nuclear and, therefore, in sympatric, sexually reproducing species congruence in haplotypes of these two markers provides strong evidence that these are reproductively isolated species.

These results not only illustrate the use of both ITS2 and CO1 genes for discriminating species, something which has attracted a lot of attention recently (Porter and Collins 1991; Paskewitz et al. 1993; Hebert et al. 2003a,b; van Veen et al. 2003), but also indicates that estimates of species diversity and global species richness, based purely on morphological assessment, might be considerable underestimates. Furthermore, although most of the *A. buzurae*-group species recognised here are distinguishable on the basis of colour, two are virtually identical so, even if colour had been used as an indicator, at least one cryptic species pair would have been missed. Apart from the academic interest in knowing what proportion of morphologically defined species are actually complexes of biologically and genetically delimited cryptic species, it will also be important to the understanding of food webs, especially in the tropics, where this approach is being used to try to understand why species diversity is generally so tropico-centric but where the taxonomy is least well known.

The data presented here indicate that in the genus *Aleiodes* there is some variation in the CO1 sequence among conspecific individuals, typically five or six base changes (within the approximately 650 base pair fragment amplified) separating individuals on a most parsimonious tree, whereas more than 15 changes distinguished even the two most closely related of the species described as new in this paper (*A. kanyawarensis* sp. n. and *A. kasenenei* sp. n.). In addition, little intraspecific variation was found in the ITS2 fragment but different species showed moderate to large differences in the length-variable zones (Figure 5).

In many *Aleiodes* species, including some of the *A. buzurae* complex studied here, reading the ITS2 sequences after direct sequencing was very hard, because most individuals had some intragenomic variation and, more particularly, intragenomic length variation. The sequences presented here (Figure 5; Appendix A) concentrate on the obviously dominant signal (probably representing the variant that was commonest among the multiple genomic copies of the region), but even with experienced human pherogram reading it was not possible accurately to determine all bases in this variant in the variable regions (see Xs in Figure 5 sequences). Thus, whereas CO1 sequencing might be reliably automated, there will sometimes be arbitrary decisions about the level of variation that is assumed to

represent interspecific variation. In contrast, ITS2 (or ITS1) sequences might be more reliable indicators of species boundaries (different nuclear gene pools), but they may also be less practicable as intragenomic variants can compromise automated sequencing in some cases.

Mummification strategy

Many geometrid larvae escape from danger by dropping from their food plant on a silken thread, through which they regain access to the feeding site once danger is perceived to have passed. As *Aleiodes* species often exploit the latent behaviours of their hosts in order to pupate in greater safety, it is perhaps not surprising to find species that exploit this danger-avoiding reflex of certain geometrid larvae by causing the host to drop on a thread before being mummified. However, the host behaviour noted in the *Aleiodes* species-group sampled by us in western Uganda is not effective at completely preventing attack by pseudohyperparasitoids. In 2002, we collected a total of 19 suspended mummies, of which seven subsequently produced *Aleiodes*, eight produced hyperparasitoids, and four failed to emerge. Three of the hyperparasitoids belonged to groups known to behave as true hyperparasitoids, i.e. attacking the primary parasitoid while the latter is still feeding. These were two specimens of one species of *Mesochorus* (Ichneumonidae) and an *Afroperilampus* sp. (Perilampidae), and both of these made emergence holes like those of *Aleiodes*. The remaining five mummies produced three species of Eulophidae (some gregarious) that all belonged to groups likely to behave only as pseudohyperparasitoids in the context (one species each of the genera *Pediobius* and *Tetrastichus*, and a further unplaced species of Tetrastichinae). Because pseudohyperparasitism is generally an on-going process affecting the primary parasitoid throughout its cocooned period, and because some of the mummies were collected before this period was over, only a minimum level of hyperparasitism (about 50% overall) can be estimated from the above small collection (and even then that would presume that the overall level did not vary at other dates). In particular, it appears that mummification at the end of the thread was not preventing at least three Chalcidoidea species from exploiting the mummies as strongly presumed pseudohyperparasitoids (i.e. ~33% of the mummies collected).

Acknowledgements

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Appendix A: Specimen data, associated codes (attached to specimens all of which are deposited in the National Museums of Scotland, Edinburgh) and EMBL/GenBank sequence accessions numbers.

			EMBL/GenBank accessions numbers		
Taxon	Provenance	Voucher code	CO-1	28S D2-D3	ITS2
A. barnardae sp. n.	Kibale, Uganda	AL0320	–	EF115429	EF115480
A. barnardae sp. n.	Kibale, Uganda	AL0321	EF115445	EF115430	EF115483
A. barnardae sp. n.	Kibale, Uganda	AL0324	EF115446	EF115431	EF115484
A. barnardae sp. n.	Kibale, Uganda	AL0484	EF115444	–	EF115482
A. barnardae sp. n.	Kibale, Uganda	AL0486	EF115443	–	EF115481
A. basutai sp. n.	Kibale, Uganda	AL0487	EF115442	–	EF115485
A. kanyawarensis sp. n.	Kibale, Uganda	AL0225	EF115450	EF115435	EF115489
A. kasenenei sp. n.	Kibale, Uganda	AL0224	EF115451	EF115436	EF115490
A. mubifsi sp. n.	Kibale, Uganda	AL0323	EF115447	EF115432	EF115486
A. trevelyanae sp. n.	Kibale, Uganda	AL0325	EF115449	EF115434	EF115488
A. trevelyanae sp. n.	Kibale, Uganda	AL0226	EF115448	EF115433	EF115487
A. apiculatus	Berkshire, England	AL0273	–	EF115440	–
A. apiculatus	Berkshire, England	AL0384	EF115455	–	EF115494
A. compressor	Cumbria, England	AL0351	EF115458	–	–
A. coxalis	Berkshire, England	AL0247	EF115459	–	–
A. coxalis	Berkshire, England	AL0286	EF115460	–	–
A. coxalis	Dordogne, France	AL0376	EF115461	–	–
A. coxalis	Berkshire, England	AL0385	EF115462	–	–
A. coxalis	Orseg, Hungary	AL0389	EF115463	–	–
A. dissector	Perthshire, Scotland[1]	AL0291	EF115471	–	–
A. dissector	Perthshire, Scotland[1]	AL0333	EF115474	–	–
A. dissector	Beynam, Turkey[1]	AL0361	EF115472	–	–
A. dissector	Beynam, Turkey	AL0373	EF115473	–	–
A. pictus	Berkshire, England	AL0119	EF115464	–	–
A. pictus	Berkshire, England	AL0122	EF115465	–	–
A. pictus	Norfolk, England[2]	AL0269	EF115466	–	–
A. pictus	Midlothian, Scotland	AL0388	EF115467	–	–
A. pictus	Norfolk, England	AL0406	EF115468	–	–
A. pictus	Dordogne, France	MRS249	EF115469	–	–
A. pictus	Gloucester, England	MRS285	EF115470	–	–
A. ruficornis	Berkshire, England	AL0027	EF115476	–	–
A. ruficornis	no data	AL0031	EF115475	–	–
A. ruficornis	Berkshire, England	AL0144	EF115477	–	–
A. ruficornis	Berkshire, England	AL0145	EF115478	–	–
A. ruficornis	Berkshire, England	AL0147	EF115479	–	–
A. testaceus	Berkshire, England	AL0058	EF115454	–	EF115493
A. testaceus	Berkshire, England	AL0285	–	EF115439	–
A. unipunctator	Angus, Scotland	AL0411	EF115456	–	–
A. sp.	Naivasha, Kenya	AL0468	EF115452	EF115437	EF115491
A. sp.	Tanzania	AL0044	EF115453	EF115438	EF115492
A. sp.	Belize	AL0005	EF115457	EF115441	–

[1]ex *Orthosia incerta* (Hufnagel); [2]ex *Diarsia rubi* (Vieweg); [3]ex indet green noctuid larva on low herbs.