Molecular phylogeny of *Cotesia* species (Hymenoptera: Braconidae) inferred from a 16S gene

The genus *Cotesia* (Hymenoptera: Braconidae) was erected by Cameron in 19th century. *Apanteles* Foerster was listed as a synonym until the generic reclassification of the Microgastrinae: Braconidae by Mason[1]. *Cotesia* is a large group of primary parasitoids of Lepidoptera, with about 1500–2000 species worldwide[1]. Many *Cotesia* species are important natural enemies of agricultural and forestry pests, and several species have been used as biocontrol agents of lepidopteran pests in temperate regions of the world. The biology of the *Cotesia* species is diverse and taxonomy is controversial with relatively recent usage of the generic name *Cotesia*[1]. The present study is aimed to identify the collected *Cotesia* species by using a 16S gene, to measure the homology between different *Cotesia* populations and to assess the usefulness of this genetic region for phylogenetic studies.

Cotesia plutellae, *C. flavipes*, *C. sesamiae*, *C. glomerata* and *C. rubecula* were procured from nine different geographic origins, viz. Australia, Benin, India, Kenya, Malaysia, South Africa, Taiwan, Thailand and United Kingdom (Table 1). The specimens used in the present study were identified by the supplier. Genomic DNA was extracted from individual *Cotesia* specimens following a modified cetylmethyl ammonium bromide (CTAB) protocol with an additional polyethylene glycol precipitation[2]. The 16S gene region of the mitochondrial genome was amplified using the primer pairs[3,4], 16Saf (5′-CACCTGTTTATCAAAAACAT-3′) and 16Sar (5′-CTTATTCAACATCGAGGTC-3′). Negative control lacking template DNA was included in all experiments. The PCR mixture (25 ml) contained 2.5 µl of 10x reaction buffer, 15 mM $MgCl_2$ (Biotherm™ Gene Graft, Germany), 20 pmol of each primer, 2 mM of dNTPs (MBI Fermentas) and one unit of thermostable *taq* DNA polymerase (Biotherm™– Gene Graft, Germany). Amplification conditions were the initial hot start at 94°C for 3 min followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min and extension at 72°C for 1 min, and final extension of 72°C for 7 min. To confirm successful amplification, 5 µl of the product was electrophoresed on a 1.2% agarose gel and the gel stained with ethidium bromide. The amplified PCR products were purified using QIAquick spin column (Qiagene®) and products

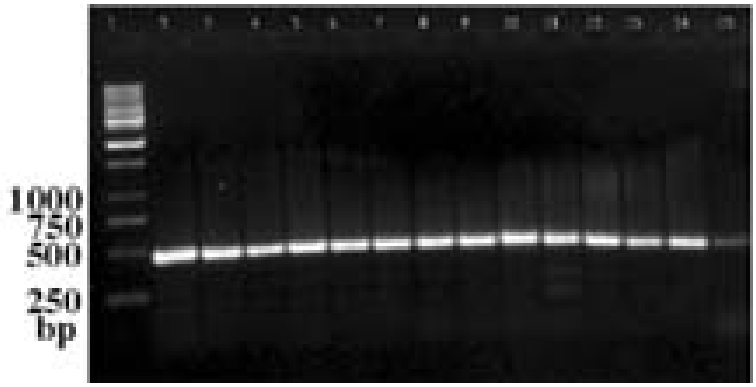

Figure 1. Amplified PCR product of a 16S gene. Lane 1. 1 kb DNA ladder, *Cotesia plutellae* from (lane 2, Malaysia; lane 3, Benin (Africa); lane 4, South Africa; lane 5, Taiwan/Kenya; lane 6, United Kingdom; lane 7, Kenya; lane 8, Thailand; lane 9, India; lane 10, *C. glomerata* from India; lane 11, *C. rubecula* from Australia; lane 12, *C. plutellae* from Taiwan; lane 13, *C. flavipes* from Kenya; lane 14, *C. flavipes* from Thailand; lane 15, *C. sesamiae* from Kenya.

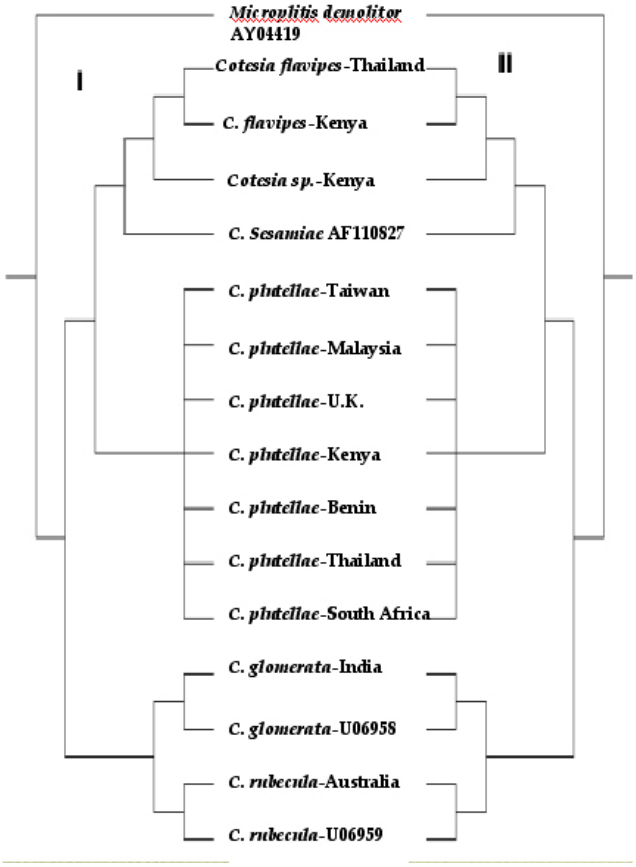

Figure 2. Strict consensus tree from 16S gene analysis. I. Maximum parsimony tree (length = 83, CI = 0.680, RI = 0.814). II. Maximum likelihood tree.

Table 1. Geographical origins of the *Cotesia* populations

Species	Host	Origin	Source
Cotesia plutellae	*Plutella xylostella*	Malaysia	MARDI-Malaysia
C. plutellae	*P. xylostella*	Benin	CIRAD-France
C. plutellae	*P. xylostella*	South Africa	Dr Rami Kafir, Rhodes University
C. plutellae	*P. xylostella*	Taiwan	AVRDC-Taiwan
*C. plutellae**	*P. xylostella*	Taiwan-Kenya	ICIPE-Nairobi
C. plutellae	*P. xylostella*	United Kingdom	Dr Tanja Schuler, Rothamsted Station
C. plutellae	*P. xylostella*	Kenya	ICIPE-Nairobi
Cotesia sp.	*P. xylostella*	Kenya	ICIPE-Nairobi
C. plutellae	*P. xylostella*	Thailand	Chatuchak-Bangkok
*C. plutellae**	*P. xylostella*	India	UHF-Solan
C. glomerata	*Pieris brassicae*	India	UHF-Solan
C. rubecula	*P. rapae*	Australia	Dr Sassan Asgari, Adelaide University
C. flavipes	*Chilo partellus*	Kenya	ICIPE-Nairobi
C. flavipes	*C. partellus*	Thailand	Chatuchak-Bangkok
*C. sesamiae**	*Sesamia* spp., *C. partellus, C. orichalcociliellus* and *Busseola fusca*	Kenya	ICIPE-Nairobi
C. glomerata-U06958	*Pieris brassicae*	USA	NCBI Databank
C. rubecula-U06959	*Pieris rapae*	USA	NCBI Databank
C. sesamiae-AF110827	*Sesamia* spp., C. *partellus, C. orichalcociliellus* and *B. fusca*	USA	NCBI Databank

*Sequencing reaction failed.

electrophoresed on 1.2% agarose gel stained with ethidium bromide to determine the success of purification. The purified PCR product from different *Cotesia* species was sequenced using 16Sar and 16Saf primers at MWG-Ebersberg, Germany. The sequences were aligned with MEGALIGN® (DNAStar Inc.). Maximum parsimony and maximum likelihood analysis were performed with PAUP™ 4.0b10[5], under the branch-and-bound search option, with gaps treated as missing data. *Microplitis demolitor (*AY04419) was used as an outgroup.

Primers amplified an absolute length of approximately 500 bp of16S gene for all the species of *Cotesia* used in this study (Figure 1). Sequences showed a high degree of homology within species (> 98%) and thus, the samples could be confidently assigned to a species, regardless of their geographic origin. From this we conclude that the 16S gene is a reliable marker for identifying *Cotesia* spp. Maximum parsimony and maximum likelihood analysis resulted in a single tree for each approach (Figure 2). *C. plutellae*, *C. flavipes*, *C. sesamiae* and *Cotesia* sp. formed one clade, while *C. glomerata* and *C. rubecula* were rendered as sister species. Both groups were consistently separated from the outgroup. With exception of *C. plutellae*, our results are in agreement with those of Michel-Salzat and Whitfield[6], who placed *C. plutellae* together with *C. glomerata,* based on the analysis of combined data from four genes. We conclude that a combined analysis of several molecular characters of different levels of conservation may represent a better approach for phylogenetic studies. Further molecular work on Cotesia species combined with taxonomic characters may help to understand the evolution of the species. The sequences for the 16S gene were submitted to GenBank with accession numbers: AY936211 to AY936222.

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ACKNOWLEDGEMENTS. We thank scientist(s) for providing specimens used in the study. R.S.R. thanks DAAD, Bonn, Germany for a fellowship.

Received 30 September 2005; revised accepted 26 August 2006

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