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Sugarcane moth borers (Lepidoptera: Noctuidae and Pyraloidea): phylogenetics constructed using COII and 16S mitochondrial partial gene sequences

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Abstract

Sugarcane moth borers are a diverse group of species occurring in several genera, but predominately within the Noctuidae and Pyraloidea. They cause economic loss in sugarcane and other crops through damage to stems and stalks by larval boring. Partial sequence data from two mitochondrial genes, COII and 16S, were used to construct a molecular phylogeny based on 26 species from ten genera and six tribes. The Noctuidae were found to be monophyletic, providing molecular support for the taxonomy within this subfamily. However, the Pyraloidea are paraphyletic, with the noctuids splitting Galleriinae and Schoenobiinae from the Crambinae. This supports the separation of the Pyralidae and Crambinae, but does not support the concept of the incorporation of the Schoenobiinae in the Crambidae. Of the three crambine genera examined, *Diatraea* was monophyletic, *Chilo* paraphyletic, and *Eoreuma* was basal to the other two genera. Within the Noctuidae, *Sesamia* and *Bathytricha* were monophyletic, with *Busseola* basal to *Bathytricha*. Many species in this study (both noctuids and pyraloids) had different biotypes within collection localities and across their distribution; however the individual biotypes were not phylogenetically informative. These data highlight the need for taxonomic revisions at all taxon levels and provide a basis for the development of DNA-based diagnostics for rapidly identifying many species at any developmental stage. This ability is vital, as the species are an incursion threat to Australia and have the potential to cause significant losses to the sugar industry.

Introduction

Insect species feeding on sugarcane are diverse, numerous, and characteristically of limited geographical distribution (Box, 1953; Pemberton & Williams, 1969; FitzGibbon *et al.*, 1998). Few species are cosmopolitan; the majority are local species that have moved from feeding on grasses to feeding on introduced sugarcane (Strong *et al.*, 1976). Of particular importance are the moth borers, a group of diverse Lepidoptera, primarily noctuids and pyraloids, which are key pests in most of the world's sugar industries. The group includes species that have a long evolutionary association with *Saccharum* spp. (Poaceae) (e.g. *Sesamia grisescens* Warren (Noctuidae)), as well as species that have been spread by humans, (e.g. *Chilo sacchariphagus* Bojer (Crambidae)), and many species that have only recently adapted to feeding on cultivated sugarcane (e.g. *Diatraea* spp., *Eldana saccharina* Walker (Pyralidae), African *Sesamia* spp.) (Way & Turner, 1999; Bull, 2000).

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Sugarcane stalks at any stage of growth are prone to attack from moth borers that are loosely classified into four types (Metcalfe, 1969) according to the part of the stalk that they attack: shoot borers, top borers, rootstock borers, and internode, stalk or stem borers. However, a species is not necessarily restricted to one habit, e.g. *Chilo infuscatellus* Snellen (Crambidae) is found as a shoot, top and internode borer; the distinction between types is largely based on the stage of development of the stalk and is purely arbitrary (Metcalfe, 1969). Shoot borers kill the shoots, with the first noticeable sign of damage being the characteristic 'dead heart' following damage to the base of the spindle leaves. Top borers attack the youngest part of the plant top, and usually destroy the growing point. Young stalks die whereas older stalks often die or produce side shoots and sucrose content is usually adversely affected. Internode borers tunnel in, and sometimes through, the internodes. The stalks lose weight and subsequent fungal infection induces rotting and death of the whole stalk. Juice quality can also be affected. Rootstock borers enter at or below ground level; young stalks show 'dead hearts', whilst older ones are weakened or killed.

Despite their dominance in most sugar industries, moth borers are not significant pests of Australian sugarcane (Allsopp *et al.*, 2000), although species such as *Bathytricha truncata* Walker (Noctuidae) are minor pests. Genera such as *Chilo* Zincken, *Diatraea* Guilding, *Eldana* Walker, *Scirpophaga* Treitschke and *Sesamia* Guenée are either not present in Australia or are represented by species that do not feed on sugarcane (Nielsen *et al.*, 1996). Many of the shoot, stem and top borers found in Southeast Asia and Papua New Guinea have been identified as threats to the Australian industry (FitzGibbon *et al.*, 1999). For example, the Papua New Guinean noctuid *S. grisescens* could easily establish in northern Queensland (Allsopp & Sallam, 2001) and cause damage similar to that in Papua New Guinea, where it reduced annual sugar production during the early 1990s by 5–18%, or reduced sugar production in the late 1980s by up to US$8.4 million annually (Kuniata & Sweet, 1994). The detrimental impact on the Australian sugar industry from such pests could see sugar production significantly reduced, given that there are no existing control measures (Allsopp *et al.*, 2001).

Prompt identification of a species is critical in framing the correct response to any incursion, forming the basis for appropriate control and eradication measures. The Australian sugar industry has determined that the accurate and rapid identification of borer larvae is a biosecurity priority (Allsopp *et al.*, 2001). Given that larvae of many species are difficult to separate morphologically and laboratory rearing of larvae to adults for morphological identification can be laborious, DNA-based methods could provide a useful alternative. Phylogenetics is a tool frequently used for establishing inter- and intra-specific relationships between taxa and within populations. The mitochondrial large ribosomal RNA subunit (16S) and protein-coding cytochrome oxidase II (COII) genes have been used extensively to infer phylogenetic relationships in insect families such as Drosophilidae (Simon *et al.*, 1994), Tephritidae (Smith *et al.*, 2003) and Lepidoptera (Sperling & Hickey, 1994), and could be useful and appropriate for phylogenetic reconstruction of the moth borers of sugarcane. Only one study has used this technique on sugarcane moth borers; King *et al*. (2002) successfully used COI-COII sequence data to show that different biotypes of the pyralid *Eldana saccharina* exist in Africa.

Considerable debate exists in the taxonomic placement of the pyraloid subfamilies, with one approach placing them in the Pyralidae (Bleszynski, 1969, 1970; Fletcher & Nye, 1984; Holloway *et al.*, 1987, 2001; Common, 1990; Nielsen & Common, 1991; Zhang, 1994; Scoble, 1995; Schaffer *et al.*, 1996), and the other suggesting the subfamilies should be separated into the Pyralidae and Crambidae (Börner, 1925; Hasenfuss, 1960; Minet 1981, 1983, 1985; Shaffer, 1990; Solis, 1992; Maes, 1995, 1998a,b; Kristensen, 1998). In this study, molecular phylogenetics was used to provide a hypothesis of relationships between sugarcane moth borer species as well as the first stage in improving diagnostics. The study includes Australian endemic species and potential incursion threats to Australia.

Materials and methods

Sample sources and DNA extraction

Specimens were collected from Australia and sourced from overseas. A subset of some larval specimens collected from overseas and in Australia were reared through to adult and identified by morphology, or larvae were identified using current larval taxonomic keys. MNS or PGA identified Australian material; sugarcane entomologists in their respective country identified overseas material. Material was stored in 100% ethanol. Twenty-six taxa from ten genera were included (table 1). One species, *Cosmopterix* sp., is not a true 'moth borer', its larvae bore into the mid-ribs of sugarcane (Jarvis, 1927; Common, 1990); it was included as an outgroup, with *Opogona glycyphaga* Meyrick (Tineidae), for the phylogenetic analysis. Five individuals of each collection were used for analysis; where material was limited, fewer were used (table 1). DNA was extracted from hind proleg segments of individual larva or head and thorax of individual adults into a 96-well plate with the remaining insect tissue stored in 100% ethanol as laboratory voucher specimens. DNA was extracted as in Scott *et al*. (2003).

Cytochrome oxidase II amplification

Polymerase chain reaction (PCR) amplification of approximately 369 base pairs of the COII DNA fragment was carried out in 25 µl total reaction volumes containing 4 mM $MgCl_2$, 20 mM Tris-HCl, 100 mM KCl, 0.2 mM of each dNTP (Biotech, Perth, Western Australia, Australia), 0.2 µM of each primer A-298 (5′ATTGGACATCAATGATATTGA3′) and B-tLYS (5′GTTTAAGAGACCAGTACTTG3′) (Liu & Beckenbach, 1992; Simon *et al.*, 1994), 1U *Taq* polymerase (Qiagen, Clifton Hill, Victoria, Australia), and 20 ng DNA. Thermal cycling was performed in a PC960 Thermal Cycler (Corbett Research, Mortlake, New South Wales, Australia) using the cycling conditions of initial denaturation at 94°C for 1 min, then 35 cycles at: 94°C for 30 s; 50°C for 60 s; 72°C for 60 s, then a final extension at 72°C for 1 min and hold at 25°C.

16S amplification

Polymerase chain reaction amplification of approximately 378 base pairs of the 16S DNA fragment was carried out in 25 µl total reaction volumes containing 2.0 mM $MgCl_2$, 20 mM Tris-HCl, and 100 mM KCl, 0.2 mM each dNTP

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Table 1. Collection locations, numbers of specimens, and GenBank accession numbers for taxa included in the sugarcane borer phylogenetic analysis.

Family and subfamily	Species	Location	Stage	Number of individuals	GenBank accessions 16S	GenBank accessions COII
Cosmopterigidae, Cosmopteriginae	*Cosmopterix* sp.	Mackay, Australia	Larva	2	AY320442	AY320489
Crambidae, Crambinae	*Chilo auricilius* Dudgeon	India	Larva	3	AY320428	AY320475
	C. infuscatellus Snellen	India	Larva	3	AY320429	AY320476
		Thailand	Larva	5	AY320430	AY320477
	C. orichalcociliellus Strand	Kenya	Larva	5	AY320431	AY320478
	C. partellus Swinhoe	India	Larva	3	AY320432	AY320479
		Kenya	Larva	5	AY320433	AY320480
		Zimbabwe	Larva	3	AY320435	AY320482
		South Africa	Adult	5	AY320434	AY320481
	C. sacchariphagus Bojer	Thailand	Larva	4	AY320439	AY320486
		Mauritius	Larva	5	AY320437	AY320484
		Réunion	Larva/adult	5	AY320438	AY320485
	C. sacchariphagus indicus (Kapur)	India	Larva	3	AY320436	AY320483
	C. terrenellus Pagenstecher	Papua New Guinea	Larva	5	AY320440	AY320487
	C. tumidicostalis Hampson	Thailand	Adult	5	AY320441	AY320488
	Diatraea busckella Dyar & Heinrich	Venezuela	Larva	5	AY320443	AY320490
	D. centrella Möschler	El Rodeo, Venezuela	Larva	3	AY320445	AY320492
		Ecuador	Adult	4	AY320444	AY320491
	D. crambidoides Grote	Texas, USA	Adult	5	AY320446	AY320493
	D. grandiosella Dyar	Texas, USA	Larva	5	AY320447	AY320494
	D. rosa Heinrich	Yaritagua, Venezuela	Larva	3	AY320448	AY320495
	D. saccharalis Fabricius	Florida, USA	Adult	5	AY320450	AY320497
		Texas, USA	Adult	5	AY320453	AY320500
		Mexico	Larva/adult	5	AY320452	AY320499
		Jamaica	Adult	5	AY320451	AY320498
		Chivacoa, Venezuela	Larva	3	AY320454	AY320501
		Brazil	Adult	5	AY320449	AY320496
	Eoreuma loftini Dyar	Texas, USA	Larva/adult	5	AY320458	AY320505
Crambidae, Schoenobiinae	*Scirpophaga excerptalis* Walker	India	Larva	3	AY320460	AY320507
		Papua New Guinea	Larva	5	AY320461	AY320508
Noctuidae, Amphipyrinae	*Bathytricha truncata* Walker	Ayr, Australia	Larva	4	AY320424	AY320471
		Mackay, Australia	Larva	5	AY320426	AY320473
		Bundaberg, Australia	Larva	5	AY320425	AY320472
	Busseola fusca Fuller	South Africa	Larva	5	AY320427	AY320474
	Sesamia sp.	Ahvaz, Iran	Larva	3	AY320462	AY320509
	S. calamistis Hampson	Kenya	Larva	5	AY320463	AY320510
		Zimbabwe	Larva	5	AY320464	AY320511
	S. cretica Lederer	Ahvaz, Iran	Adult	3	AY320465	AY320512
	S. grisescens Warren	Papua New Guinea	Larva	5	AY320466	AY320513
	S. nonagrioides botanephaga Tams & Bowden	Ahvaz, Iran	Adult	3	AY320467	AY320514
Pyralidae, Galleriinae	*Eldana saccharina* Walker	Kenya	Larva	5	AY320455	AY320502
		Zimbabwe	Larva	5	AY320457	AY320504
		South Africa	Larva/adult	5	AY320456	AY320503
Tineidae, Hieroxestinae	*Opogona glycyphaga* Meyrick	Mackay, Australia	Larva	2	AY320459	AY320506

(Biotech, Perth, Western Australia, Australia), 0.2 μM of each primer 16ScbF (5′AAGATTTTAATGATCGAACAG) and 16ScbR (5′TGACTGTACAAAGGTAGCATA3′), 1U *Taq* polymerase (Qiagen, Clifton Hill, Victoria, Australia) and 20 ng DNA. Thermal cycling was performed in a PC960 Thermal Cycler (Corbett Research, Mortlake, New South Wales, Australia) using the cycling conditions of initial denaturation at 94°C for 2 min, then 40 cycles at: 92°C for 45 s; 50°C for 60 s; 72°C for 90 seconds, and with a final extension at 72°C for 2 min and hold at 25°C.

Visualization, purification and sequencing

Amplified PCR products were checked on 1.5% Tris-borate-EDTA agarose gel to confirm amplification success, before PCR purification using MultiScreen-PCR 96-well plates (Millipore, North Ryde, New South Wales, Australia). Sequencing was performed in the forward and reverse directions in a 12 μl total reaction volume containing 4 μl of AB V3.0 Big Dye Terminator chemistry (Applied Biosystems, Melbourne, Victoria, Australia), 3.2 pmol of primer, and 50 ng of purified PCR product in a PC960 Thermal Cycler (Corbett Research, Mortlake, New South Wales, Australia) using a cycling programme of 94°C for 5 min followed by 30 cycles at: 96°C for 10 s; 50°C for 5 s; 60°C for 4 min. Sequences were purified using Montage SEQ_{96} Sequencing Reaction Cleanup Kits (Millipore, North Ryde, New South Wales, Australia), and run on an AB 377 DNA sequencer (Applied Biosystems, Melbourne, Victoria, Australia) at the Australia Genome Research Facility (University of

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Queensland, Queensland, Australia). Sequence data for both the COII and 16S genes for all taxa are available at GenBank and accession numbers are given in table 1.

Alignment and phylogenetic analyses

Sequences were aligned with BioEdit (Hall, 1999). Consensus sequences were derived from aligned forward and reverse complemented sequences of multiple individuals from taxa collected from specific locations (table 1). Refined alignments were completed manually to improve positional homology assessments, under the assumption that gaps are rare and to preserve local positional homology in adjacent positions.

Phylogenetic analyses were performed using equal-weighted parsimony methods available in PAUP* (Swofford, 2002). The two mitochondrial genes sequenced are physically linked in the mitochondrial genome and were treated as one set of characters. Variation in characters between taxa was scored as polymorphic. Gaps positions were treated as a fifth base and missing sequence was coded as '?' and ambiguous characters coded as 'N'. Phylogenetic analysis of the data consisted of 1000 random stepwise additions searches, with tree bisection reconnection (TBR) branch swapping, multiple parsimony trees (MULPARS) and branches having maximum lengths of zero were collapsed to yield polytomies. Strict consensus of the most parsimonious trees (MPT) was computed by PAUP* (Swofford, 2002). Partition Bremer support (PBS) (Bremer, 1994) values were calculated with 20 heuristic searches of the data and PAUP* (Swofford, 2002) with 100 random-addition heuristic searches topographically constrained to find the most parsimonious trees without the nodes present in the combined analysis. Bootstrap analysis was undertaken to establish additional support values for nodes within the combined analysis. Support for all nodes was estimated by bootstrapping, which was conducted using 1000 replicates with 100 random additions heuristic searches of the combined data set.

Results

The nuclear genes, Internal Transcribe Spacer Unit 2 (ITS2) and Elongation factor-1α (EF1α), and the mitochrondrial genes, COII and 16S, were tested for usefulness in phylogenetic analysis. The nuclear genes proved to be either too variable or problematic in PCR amplification compared to the mitochondrial genes, so were avoided in this analysis. Sequenced COII and 16S fragments were submitted to GenBank, accession numbers are given in table 1. Multiple haplotypes occurred within specimens of a species from a single geographic location, although none of these were phylogenetically informative. A total of three most parsimonious trees were computed for the combined data, consisting of 298 phylogenetically informative characters and each tree gave a length of 1337 steps. A consensus tree was computed with a Consistency Index (CI) of 0.4031 and a Retention Index (RI) 0.6853.

Cosmopterix sp. (Gelechioidea) and *O. glycyphaga* (Tineoidea) are outgroups for the pyraloids and noctuids used in this phylogeny (fig. 1). The Amphipyradae are monophyletic, with each of the three genera being distinct. The pyraloids, however, are distinctly paraphyletic, splitting between the subfamilies Crambinae (*Chilo* and *Diatraea*) and the Schoenobiinae (*Scirpophaga*) and the Galleriinae *(Eldana*). Within the Crambinae, the genus *Diatraea* is monophyletic, but *Chilo* separates into two clades: *C. sacchariphagus* and *C. tumidicostalis* Hampson; *C. terrenellus* Pagenstecher, *C. orichalcociliellus* Strand, *C. infuscatellus*, *C. auricilius* Dudgeon and *C. partellus* Swinhoe. *Eoreuma loftini* Dyar is basal to the other crambines.

Genetic differences within species are also evident; phylogeographic separation is apparent between locations for a single species. *Scirpophaga excerptalis* Walker, *Sesamia calamistis* Hampson and *Diatraea centrella* Möschler show clear differences between the geographic locations examined. Other species show significant splits along geographic lines: *Eldana saccharina* Walker within Africa; *Bathytricha truncata* Walker within Australia; separation of Indian and African collections of *Chilo partellus*; separation of Asian and Mauritius–Réunion collections of *Chilo sacchariphagus*; separation of Mexican–South American and USA–Caribbean collections of *Diatraea saccharalis* Fabricius.

Discussion

The analysis covered all of the major genera of sugarcane moth borers and many of the major species that are incursion threats to the Australian sugar industry. The analysis contained 26 species from ten genera in six tribes. This study provides a DNA based phylogenetic analysis of this diverse group. It indicates that some groups are paraphyletic at family, subfamily and generic levels; other groups are monophyletic and accord well with current taxonomies.

Cosmopterix sp. (Gelechioidea) and *O. glycyphaga* (Tineoidea) were defined as outgroups, consistent with their general placement within lepidopteran classifications (e.g. Common, 1990; Nielsen & Common, 1991; Scoble, 1995; Nielsen *et al*., 1996; Holloway *et al*., 2001) and with more rigorous analysis of lepidopteran phylogenies (Nielsen, 1989).

The sugarcane noctuids, albeit only amphipyrines (sometimes amalgamated with the Acronictinae (Edwards, 1996), but probably not monophyletic (Scoble, 1995)), were monophyletic, suggesting a robust taxonomy within this subfamily. There is clear separation of *Busseola fusca* Fuller and *B. truncata* from *Sesamia* spp., suggesting that these three genera are valid. Tams & Bowden (1953) in their revision of African *Sesamia, Busseola* Thrau and related genera considered the first two distinct, although Holloway (1998) cast some doubt on the generic arrangement of the complex when he stated 'The whole complex needs further review and might even be treated as *Sesamia* sensu lato until the characters within it can be more completely assessed. Some sections of it might be considered plesiomorphic and therefore possibly paraphyletic.' However, he did maintain *Busseola* and *Sesamia* as valid genera, based on the shape of the costal process of the valve of the female genitalia.

Within *Sesamia,* our analysis clearly separates *S. cretica* (and an unidentified species from Iran) from *S. nonagrioides botanephaga* Tams & Bowden and *S. calamistis*. This is consistent with Tams & Bowden's (1953) separation of African *Sesamia* into two groups based on characters of the male antennae and of the male and female genitalia. Tams & Bowden (1953) speculated that the Oriental species of *Sesamia* were more closely related to the *cretica* group than to the *nonagrioides* group – our placement of the New Guinea

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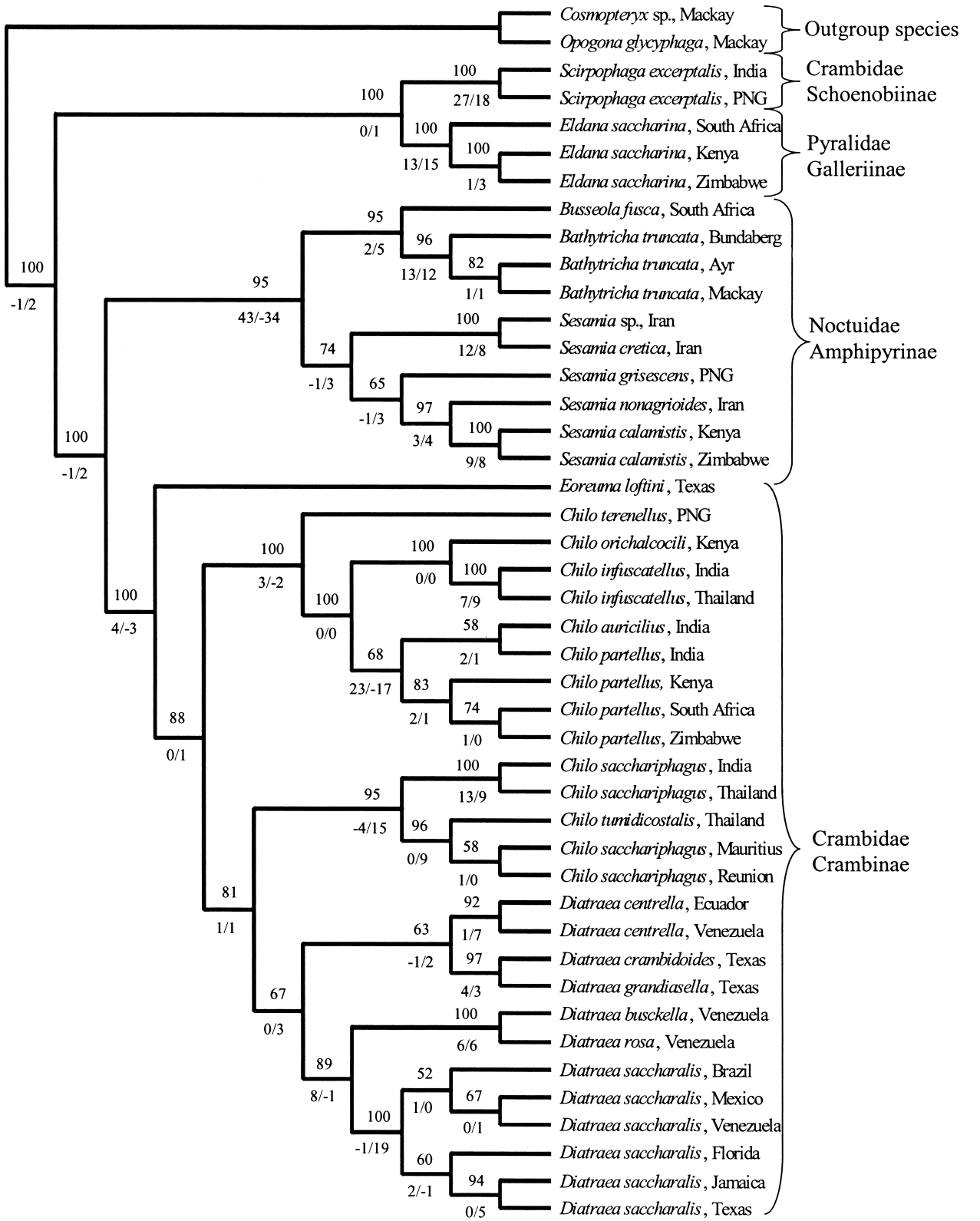

Fig. 1. Sugarcane moth stem and stalk borer phylogenetic tree with bootstrap values (%) above and partition Bremer support (PBS) values below the nodes (the left value indicating COII gene and the right value indicating 16S gene). The outgroup species are *Cosmopterix* sp. (Gelechioidea: Cosompterigidae) and *Opogona glycyphaga* (Tineoidea: Hieroxestinae). Collection localities are indicated after the species name.

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species *grisescens*, the most easterly occurring *Sesamia*, closer to the *nonagrioides* group does not support this hypothesis.

The sugarcane pyraloids are contained within two clades: the crambines (*Eoreuma loftini*, *Chilo* spp. and *Diatraea* spp.); and *Scirpophaga* (Schoenobiinae) and *Eldana* (Galleriinae). The separation of the pyralids from the crambines reflects one of the more contentious issues in lepidopteran phylogenetics. The more conservative view places all pyraloid subfamilies in the one family, the Pyralidae (e.g. Bleszynski, 1969, 1970; Fletcher & Nye, 1984; Holloway *et al.*, 1987, 2001; Common, 1990; Nielsen & Common, 1991; Zhang, 1994; Scoble, 1995; Schaffer *et al.*, 1996). However, a distinct division within this group was first noted by Börner (1925) and he split them into the Pyraliformes and Crambidiformes. This concept was refined further by Minet (1981, 1983, 1985), who placed the pyraloid subfamilies in either the Pyralidae or Crambidae depending on the presence or absence of a praecinctorium (a ventrally expanded medial flap anterior to the tympanal organs) and whether the tympanal organs are medially approximated or well separated. Systematic studies of pyraloid larvae by Hasenfuss (1960) provided further support for this division and this arrangement has met with some acceptance (e.g. Shaffer, 1990; Solis, 1992; Maes, 1995, 1998a,b; Kristensen, 1998). Both systems continue to be used, with 'arguments for and against rest[ing] not over phylogenetic structure … but on merits of tradition and ranking' (Holloway *et al.*, 2001). Our analysis partially supports the two-family concept; adult morphology places the Schoenobiinae with the Crambinae in the Crambidae. Our results suggest that the Crambidae *sensu lato* is paraphyletic.

Within the crambines, *Eoreuma* is clearly basal to *Chilo* and *Diatraea*, despite *E. loftini* being originally described in *Chilo*. According to Bleszynski (1969), *Diatraea* and *Chilo* form a compact monophyletic group, and are kept as distinct genera mainly for practical purposes. Our sequence data suggest that *Diatraea* is monophyletic, and that *Chilo* is paraphyletic, separating into two distinct clades. In our analysis, *Diatraea* resolves into two main groups: *centrella-crambidoides-grandiosella* and *busckella-rosa-saccharalis*. This differs from the implied phylogeny in Bleszynski's (1969) key, which groups the closely related *busckella-rosa* pair with *grandiosella*, and groups the closely related *crambidoides-saccharalis* pair with *centrella*. These two groups are differentiated on the patterns of dark spots on the wings, a character state that may not accurately reflect phylogeny.

Chilo was last revised by Bleszynski (1970), whose key separated *partellus* and *tumidicostalis* from the other species we examined on the basis of wing venation and *infuscatellus-sacchariphagus-terrenellus* from *auricilius-orichalcociliellus* on the basis of whether the forewings had metallic scales or not. Our arrangement is not consistent with this; we see two strong groups: (i) *auricilius*, *infuscatellus*, *orichalcociliellus*, *partellus* and *terrenellus*; (ii) *saccariphagus* and *tumidicostalis*. Indeed, the observed variation, and the closer relationship of the second group with *Diatraea* than with the first suggest that the two groups should be in separate genera. There are available names for groups of *Chilo* (Bleszynski, 1970), other than for the group containing the type species *phragmitella* Hübner – *Diphryx* Grote (type species *prolatella* Grote = *plejadellus* Zincken), *Proceras* Bojer (*sacchariphagus*), *Nephalia* (*crypsimetalla* Turner), *Hypiesta* (*argyrogramma* Hampson), *Silveria* Dyar (*hexhex* Dyer = *chiriquitensis* (Zeller)) and *Chilotraea* Kapur (*infuscatellus* Snellen). Obviously, only a thorough revision of the genus and consideration of related genera will resolve the situation.

There was minor variation among specimens of most species from one collection locality. However, this variation was not phylogenetically informative. In specimens collected at different localities, considerable variation was found that was phylogenetically informative. In species represented by only two collections (*Scirpophaga excerptalis*, *Sesamia calamistis*, *Chilo infuscatellus* and *Diatraea centrella*), that variation was enough to show the presence of distinct biotypes. *Bathytricha truncata* shows differentiation in its Australian distribution with distinct haplotypes in Bundaberg, Ayr and Mackay. Variation in *C. partellus* is evident, with phylogenetic differentiation of Kenyan, Zimbabwean, South African and Indian collections. This intraspecific variation is useful for developing molecular diagnostics, as a putative species may be molecularly identified to species level with possible indications of the country or population of origin. Further detailed investigation of these differentiations may reveal the presence of geographic isolation by distance, which may have an impact on potential biocontrol and eradication programmes.

Eldana saccharina shows phylogenetic differentiation between the Kenyan, Zimbabwean and South African collections. This species is almost certainly composed of different biotypes, with large phenotypical variation (Maes, 1998b), ecological differences (Conlong, 2001) and genetic differences among populations (King *et al.*, 2002). Phylogenetic similarities between these collection localities may be the result of host dispersal by humans.

In *Chilo sacchariphagus*, the two Asian populations are closely related, as are the populations from Mauritius and Réunion – the latter pair probably come from the same stock, being introduced from Asia by humans in the mid 1800s (Bleszynski, 1970; Williams, 1983). However, the closer relationship of the Mauritius–Réunion collections with *C. tumidicostalis* from Thailand than with the Indian–Thailand collections of *C. sacchariphagus* suggests that the species is polyphyletic. *Chilo sacchariphagus* is sometimes treated as three subspecies: *Chilo s. sacchariphagus*, *Chilo s. stramineellus* (Caradja) and *Chilo s. indicus* (Kapur). There are slight differences in the genitalia of the three subspecies, although the latter two are sometimes referred to simply as *C. sacchariphagus*. After examining several specimens, Bleszynski (1970) concluded that all populations belong either to one widely spread species, or to several phylogenetically very young species. He thought that geographical isolation of populations has resulted in slight variations in the genitalia, but that the differences can not be considered diagnostic. Further genetic studies of the complex may resolve this issue.

In *Diatraea saccharalis*, the six populations tested resolve into two groups: Mexico and South America, and the Caribbean and southern USA. The differences could reflect two dispersals (presumably human-assisted), one to the north and east and one to the south, from an original evolution on grasses, perhaps the wild ancestor of maize, in southern Mexico. Our study indicates that further investigation of this potential relationship may be warranted.

In this study, we have shown that molecular phylogenetics provides alternative hypotheses of relationships between sugarcane moth borers and validates some current hypotheses. Currently recognized genera and species are

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undoubtedly polyphyletic and there is strong evidence that the moth borers in the Pyraloidea need to be placed in at least two families. Future studies should concentrate on resolving these issues using a wider group of species. Our findings also impact on the potential development of DNA-based diagnostics – any system needs to be robust enough to account for the variation that we have seen but still be workable and produce results useful in managing incursions.

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