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BARCODING

DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae)

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Abstract

Recent DNA barcoding of generalist insect herbivores has revealed complexes of cryptic species within named species. We evaluated the species concept for a common generalist moth occurring in New Guinea and Australia, *Homona mermerodes*, in light of host plant records and mitochondrial cytochrome *c* oxidase I haplotype diversity. Genetic divergence among *H. mermerodes* moths feeding on different host tree species was much lower than among several *Homona* species. Genetic divergence between haplotypes from New Guinea and Australia was also less than interspecific divergence. Whereas molecular species identification methods may reveal cryptic species in some generalist herbivores, these same methods may confirm polyphagy when identical haplotypes are reared from multiple host plant families. A lectotype for the species is designated, and a summarized bibliography and illustrations including male genitalia are provided for the first time.

Keywords: barcoding, *Homona mermerodes*, host specificity, Lepidoptera, Tortricidae

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Introduction

Short sequences of cytochrome *c* oxidase I (COI), or animal DNA barcodes, can illuminate species boundaries when combined with other sources of information including taxonomic knowledge, morphology, and ecology (Rubinoff & Powell 2004; Schindel & Miller 2005; Dasmahapatra & Mallet 2006; DeSalle 2006; Rubinoff 2006; Miller 2007). Recent studies of mitochondrial DNA (mtDNA) variation in polyphagous insect species have revealed host races and cryptic species with ecological and/or morphological correlates of the DNA markers (Waring *et al.* 1990; Berkov 2002; Blair *et al.* 2005). Other molecular studies have favoured broader species concepts than those based on traditional morphological characters (Uechi *et al.* 2003; Vahtera & Muona 2006).

Hebert *et al.* (2004) used genetic distances to establish species limits in a morphologically and ecologically diverse hesperiid butterfly based on unique haplotypes corresponding to morphologically and ecologically distinct caterpillars. The correlation between molecular markers and ecology supported the delimitation of several new and relatively uniform species within a single hypervariable species. Re-analysis of these data by Brower (2006) using phylogenetic methods also indicated cryptic species although fewer in number than Hebert *et al.* (2004).

We took a similar approach to investigate whether the highly polyphagous and widespread *Homona mermerodes* Meyrick (Lepidoptera, Tortricidae) was one or more species. *H. mermerodes* was commonly reared in ecological studies of herbivorous insects in Papua New Guinea (Novotny *et al.* 2004) and was suspected of containing cryptic species because of its polyphagous habit, highly variable wing colouration, and because a closely related species, *Homona coffearia*, was found to be a species complex (Whittle *et al.* 1987). DNA barcodes provide an efficient molecular test of species concepts underpinning our previous ecological studies (e.g. Miller *et al.* 2003). *Homona* and related genera

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Table 1 Overview of *Homona* species known from New Guinea

H. aestivana (Walker) 1866	Male genitalia figured by Diakonoff (1968): Figure 303
H. mermerodes Meyrick (1910)	Caterpillar, adult and genitalia illustrated in Figures 1 and 2
H. phanaea Meyrick (1910)	An enigmatic species. Male genitalia were figured by Diakonoff (1968): Figure 35, but he later noted (Diakonoff 1983: 104) 'the identity of this species should be verified.'
Species near *salaconis* (Meyrick) (1912)	Similar to *Homona salaconis*, possibly an undescribed species. Male genitalia of typical *salaconis* from the Philippines figured by Diakonoff (1968): Figures 14 and 27
H. spargotis Meyrick (1910)	For many years lumped into *Homona coffearia*, which was recognized as a species complex by Whittle *et al.* (1987). Old misidentifications of *Homona nuenciana* Walker from New Guinea probably refer to this species.
H. trachyptera Diakonoff (1941)	Male genitalia figured by Diakonoff (1941): Plate 4 a Figure 1
Species Incertae sedis	Brown (2005) includes *Homona plumicornis* (Rothschild), 1916, from Admiralty Islands, originally described in Arctiidae: Lithosiinae. Miller has examined the holotype in BMNH, and it is obviously not a *Homona*, but its family placement remains unclear.

such as *Adoxophyes* are notoriously difficult to identify by wing pattern because of the extreme variability, which has driven the use of alternative characters such as pheromones and molecular genetics to diagnose species (Whittle *et al.* 1987; Lee *et al.* 2005).

Homona currently includes some 30 species distributed throughout Southeast Asia and Australia (Brown 2005) as the several African species should be placed in other genera (Razowski 2004: 174). *Homona* includes widespread polyphagous pest species, notably *H. coffearia* (Nietner) and *H. magnanima* Diakonoff (Dugdale *et al.* 2005). Diagnosis of many species in the genus is complicated by their sexual dimorphism and highly variable wing pattern. Six species are known from New Guinea (Table 1). Although many species were reviewed in a series of studies by Diakonoff (1948, 1953, 1968, 1983), the genus is in need of comprehensive revision to clarify its taxonomic status and the species involved.

Materials and methods

During our studies of caterpillar host specificity in Papua New Guinea rainforests (Madang, East Sepik, and West Sepik provinces), caterpillars of *Homona mermerodes* were collected, photographed, and reared to adults. Collecting was conducted throughout all months of the year at multiple sites throughout the northern lowlands of Papua New Guinea (PNG) during 1994–2006 (Novotny *et al.* 2002a; Miller *et al.* 2003). All individuals were collected by local assistants and brought to a field rearing station. Sampling effort was standardized across all hosts at all localities (Novotny *et al.* 2002a). Caterpillars were fed fresh foliage of the plant species on which they were collected and reared to adults whenever possible. Caterpillars and adults were first sorted to morphospecies by experienced parataxonomists and later matched to museum specimens at USNM (Smithsonian Institution) and BMNH (Natural History Museum, London). In addition, adults were light-trapped at four sites in northern Queensland.

DNA extraction and analysis

DNA sequences were obtained from 102 specimens of *Adoxophyes* sp. nr. *marmarygodes* Diakonoff, *Homona aestivana*, *H. mermerodes*, *Homona spargotis*, *Homona trachyptera*, and *Homona* sp. nr. *salaconis* from four localities in PNG and 24 specimens of *Homona mermerodes* and *Homona spargotis* collected by light trapping in Queensland, Australia. Food plants were recorded for PNG moths but were unavailable for Australian moths. PNG vouchers are deposited at USNM and the PNG National Agricultural Research Institute (NARI). Australian vouchers are deposited at USNM and Australian National Insect Collection (ANIC).

Sequences were produced at the University of Minnesota and at the University of Guelph. In Minnesota, DNA was extracted from legs of adult individuals and amplified using the QIAGEN DNeasy Tissue Kit. Primers LepF1 and *Lep*RI (Hebert *et al.* 2004) were used to obtain a 661-bp fragment of COI by cycle sequencing in both directions. Data were collected by polyacrylamide gel electrophoresis using an Applied Biosystems ABI 377 automated DNA sequencer. In Guelph, DNA was extracted from single legs and the same 661 bp fragment was amplified with a standard thermocycling regime (Hajibabaei *et al.* 2006). PCR products were subsequently sequenced on an ABI 3730 capillary sequencer. Sequences are available at the NCBI GenBank database (Accession nos EF070743–EF070863 and EF432740–EF432746) and at the Barcode of Life Database (see online Supplementary material).

Sequences were edited using SEQUENCHER version 4.2 and manually aligned. Haplotype diversity was examined prior to distance analysis with PAUP 4.0b10 (Swofford 2001). When identical haplotypes were obtained from different individuals, only a single individual was retained for phylogenetic analysis. Phenetic methods often used in studies of DNA barcoding are less explicit about distinguishing evolutionarily relevant information than character-based parsimony or likelihood-based methods

(Hillis & Huelsenbeck 1992; DeSalle & Siddall 2005; Cognato 2006). Therefore, species limits and relationships between *H. mermerodes* and similar species were evaluated using maximum parsimony. Parsimony analysis was conducted with PAUP 4.0b10 and heuristic searches of 1000 random addition sequence replicates with a maximum of 10 000 trees per replicate. To illustrate haplotype diversity, a neighbour-joining tree showing branch lengths was generated (Fig. 3) with a model of nucleotide substitution selected by MODELTEST 3.7 (Posada & Crandall 1998) according to the Akaike information criterion (AIC). The relative strength of clade support was assessed using nonparametric bootstrap resampling with 1000 replicates and 10 random addition sequence replicates per bootstrap replicate.

Adoxophyes sp. nr. *marmarygodes*, a morphospecies superficially similar to *Homona*, was used to root the phylogenetic trees. The morphospecies is near *Adoxophyes marmarygodes* Diakonoff, but lacks the female signum illustrated by Diaknoff (1952: 161), although Diaknoff's matching of sexes needs to be tested. Species limits within the complex of highland New Guinea *Adoxophyes* (*A. marmarygodes*, *A. tetraphracta* Meyrick (= *A. acropeta* Diakonoff), and *A. aniara* Diakonoff) are unclear and in need of revision.

Taxonomy

Homona mermerodes Meyrick (1910: 213) is known from the Solomon Islands, New Guinea, and Australia (Queensland). The Sumatra record by Diakonoff (1947: 342 and 1952: 404), was a misidentification, and was not repeated by Diakonoff (1983: 104). Museum collections and field experience indicate that it is widespread in the lowlands of New Guinea. It was not found in our study at 1700 m a.s.l. (Novotny *et al.* 2005), and mid-elevation specimens are uncommon in museum collections. The highest record known to us is 1900 m (Tapini, Loloipa River, Bome, PNG, in ANIC), and there are several records from PNG localities between 1500 m and 1200 m in ANIC, USNM, and BMNH.

The full bibliography of *H. mermerodes* is: Meyrick (1910): 213–214 [original description]; Meyrick (1912): 15 [checklist]; Meyrick (1913): 19 [checklist]; Meyrick (1938): 505 [distribution records]; Durrant (1916): 153 [distribution records]; Froggatt (1940): 13 [*Clerodendron* host record]; Diakonoff (1941): 38 [distribution record]; Diakonoff (1947): 342–343 [distribution records]; Diakonoff (1948): 509 [mentions possible relationships]; Diakonoff (1952): 404–405 [distribution records]; Diakonoff (1953): 18 [key]; Diakonoff (1983): 104 [checklist]; Simon Thomas (1958): 228; 1962: 89 [serious *Citrus* pest]; Basset *et al.* 1996: 180 [misidentification of *H. aestivana*]; Horak *et al.* (1996): 129 [checklist, including two new synonyms from Australia]; Novotny *et al.* 2003: 708 [*Piper* spp. host records]; Brown (2005): 388 [checklist]. Despite the fact that it is an occasionally important agricultural pest, the wings and genitalia of this species have never been illustrated and no diagnostic characters for identification have been published.

The wing shape and pattern are sexually dimorphic, and wing pattern is extremely variable (Fig. 1). *Homona mermerodes* is distinguished from all but one other species of *Homona* in New Guinea by its brown or orange brown hind wings (not orange or yellow). The other species with brown hindwings is *H. salaconis* (Meyrick) (Diakonoff 1983: 107) or a species close to it, which can be distinguished by the dark bar along the middle of the forewing costal edge in both sexes (longer than ever occurs in *H. mermerodes*) and the strongly sinuate costa in females. The male genitalia of *H. mermerodes* are characteristic: nearly the entire ventral half of the valva is sclerotized as a sacculus which culminates in a long, slender digit in the centre of the valva; the uncus is long and thin (Fig. 2). No variation in genitalia beyond species limits was found by extensive dissections of our reared specimens and of material in the ANIC, BMNH, Naturalis (Leiden), and USNM. General morphological variability excluding genitalia was also reviewed in the collection of Papua New Guinea National Agriculture Research Institute. General taxonomic methods are described in Holloway *et al.* (2001) and Miller *et al.* (2003).

Homona mermerodes was described (Meyrick 1910) from 26 specimens from Solomon Islands, New Guinea and Australia. Ian Common labelled a lectotype in 1956, but never published the designation. In order to be clear about the application of the name, we designate that female from Gizo, Solomon Islands, collected by A.S. Meek in 1905 in BMNH, with genitalia slide 3370, as lectotype. Three males from New Guinea in BMNH are labelled as paralectotypes, including genitalia slide 3498; we have not attempted to account for the remaining specimens.

Results

During a decade of sampling herbivorous insects in the northern lowlands of Papua New Guinea, we collected ~143 000 individual Lepidoptera (Novotny *et al.* 2002a, b; Weiblen *et al.* 2006). Among them, 1411 were identified as *Homona mermerodes*, of which 739 were reared to the adult stage. *H. mermerodes* was among the more widespread generalist species being successfully reared from about 40% of the host plant species we studied (44 out of 111 species) representing at least 19 plant families (Table 2). *H. mermerodes* was the 28th most common species in our sample (705 reared species, median species abundance 9 individuals). Species (and numbers of individuals) sequenced during the study included: *Adoxophyes* sp. nr. *marmarygodes* (5), *Homona aestivana* (7), *Homona mermerodes* (76), *Homona spargotis* (4), *Homona trachyptera* (22), and *Homona* sp. nr. *salaconis* (12). Sequences from these 126 moths yielded 34 unique haplotypes (Table 3). The best fitting

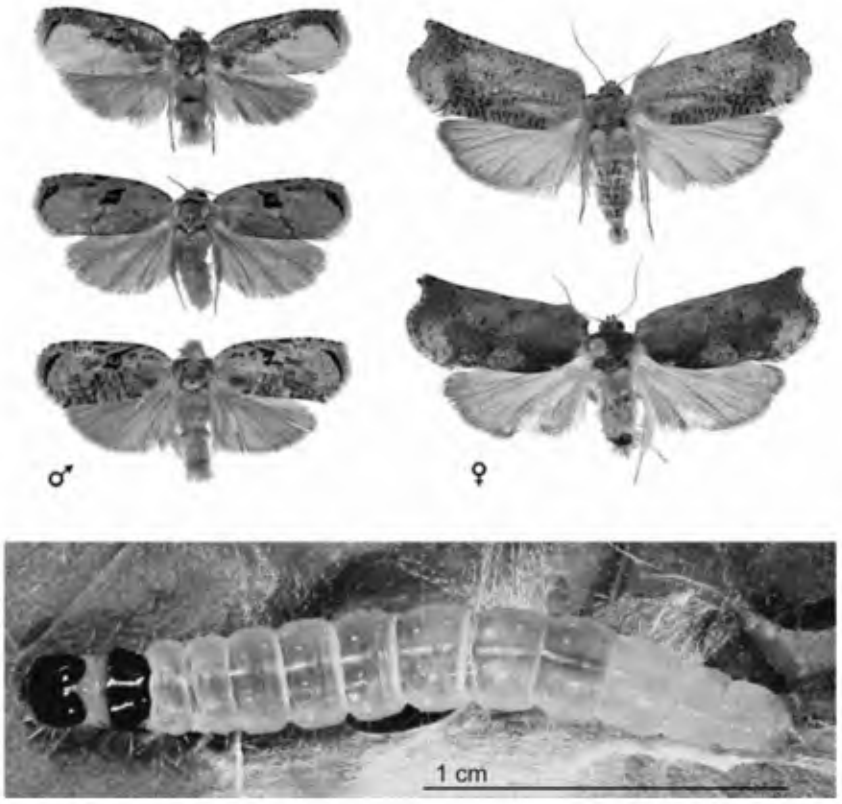

Fig. 1 *Homona mermerodes* adults and caterpillar.

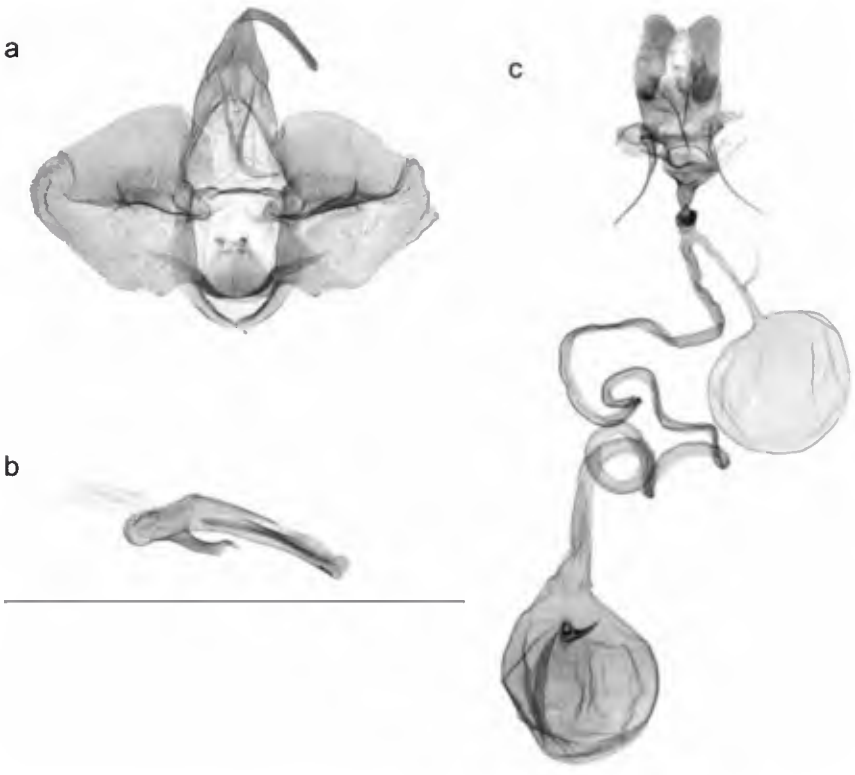

Fig. 2 (a) *Homona mermerodes* ventral view of the male genital capsule with aedeagus removed; (b) lateral view of aedeagus; (c) ventral view of female genitalia. [USNM slides 82 093 (male) and 125 226 (female)].

Table 2 Classification of host plants for *Homona* species in Papua New Guinea. Numbers of *Homona mermerodes* caterpillars feeding on each host plant species are listed. Plants not hosting *H. mermerodes* correspond to hosts for other *Homona* species listed in Table 1. Classification after Soltis *et al.* (2000)

Clade	Order	Host family	Host species	Code	Caterpillars
Euasterids I	Gentianales	Rubiaceae	*Amaracarpus calcicola* Merrill & Perry	AMA	0
Asterids	Ericales	Lecythidaceae	*Barringtonia* sp.	BAR	3
Eurosids I	Malpighiales	Phyllanthaceae	*Breynia cernua* (Poir.) Muell. Arg.	BRE	6
Monocots	Asparagales	Agavaceae	*Cordyline terminalis* P. Beauv.	COR	183
Euasterids I	Gentianales	Rubiaceae	*Dolicholobium oxylobum* K. Schum.	DOL	1
Monocots	Asparagales	Agavaceae	*Dracaena angustifolia* Roxb.	DRA	310
Magnoliids	Magnoliales	Eupomatiaceae	*Eupomatia laurina* R. Br.	EUP	180
Eurosids I	Rosales	Moraceae	*Ficus bernaysii* King	BER	1
Eurosids I	Rosales	Moraceae	*Ficus botryocarpa* Miq.	BOT	4
Eurosids I	Rosales	Moraceae	*Ficus copiosa* Steud.	COP	17
Eurosids I	Rosales	Moraceae	*Ficus dammaropsis* Diels	DAM	2
Eurosids I	Rosales	Moraceae	*Ficus hispidioides* S. Moore	HIS	2
Eurosids I	Rosales	Moraceae	*Ficus nodosa* Teysm. & Binn.	NOD	11
Eurosids I	Rosales	Moraceae	*Ficus phaeosyce* Laut. & K. Schum.	PHA	4
Eurosids I	Rosales	Moraceae	*Ficus pungens* Reinw. ex Blume	PUN	10
Eurosids I	Rosales	Moraceae	*Ficus septica* Burm.	SEP	2
Eurosids I	Rosales	Moraceae	*Ficus variegata* Blume	VAR	7
Eurosids I	Rosales	Moraceae	*Ficus wassa* Roxb.	WAS	2
Euasterids I	Lamiales	Verbenaceae	*Geunsia farinosa* Blume	GEU	0
	Gnetales	Gnetaceae	*Gnetum gnemon* L.	GNE	243
Eurosids I	Malpighiales	Euphorbiaceae	*Homalanthus novoguineensis* (Warb.) K. Schum.	HOM	0
Eurosids II	Malvales	Malvaceae	*Hibiscus tiliaceus* L.	HYB	1
Eurosids II	Malvales	Malvaceae	*Kleinhovia hospita* L.	KLE	29
Eurosids II	Sapindales	Rutaceae	*Lunasia amara* Blanco	LUN	63
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga aleuritoides* F. Muell.	MAA	8
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga clavata* Warb.	MAX	1
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga fallacina* Pax & Hoffm.	MAS	1
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga novoguineensis* J. J. Smith	MAU	2
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga quadriglandulosa* Warb.	MAQ	1
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga* sp.	GAB	2
Eurosids I	Malpighiales	Euphorbiaceae	*Macaranga* sp.	TOM	14
Euasterids I	Gentianales	Rubiaceae	*Mussaenda scratchleyi* Wernh.	MUS	5
Euasterids I	Gentianales	Rubiaceae	*Nauclea orientalis* (L.) L.	SAR	2
Euasterids I	Gentianales	Loganiaceae	*Neuburgia corynocarpa* (A. Gray) Leenh.	NEU	1
Euasterids II	Apiales	Araliaceae	*Osmoxylon sessiliflorum* (Lauterb.) W.R. Philipson	OSM	1
Eurosids I	Malpighiales	Euphorbiaceae	*Pimelodendron amboinicum* Hassk.	PIM	2
Magnoliids	Piperales	Piperaceae	*Piper aduncum* L.	PAD	32
Magnoliids	Piperales	Piperaceae	*Piper umbellatum* L.	PUB	30
Euasterids I	Lamiales	Verbenaceae	*Premna obtusifolia* R.Br.	PRE	2
Euasterids I	Gentianales	Rubiaceae	*Psychotria micrococca* (Laut. & Schum.) Val.	PSS	0
Euasterids I	Gentianales	Rubiaceae	*Psychotria ramuensis* Sohmer	PSL	2
Eurosids I	Fabales	Fabaceae	*Pterocarpus indicus* Willd.	PTE	59
Euasterids I	Lamiales	Bignoniaceae	*Spathodea campanulata* (L.) Kunth.	SPA	37
Rosids	Myrtales	Myrtaceae	*Syzygium malaccense* Merr. & Perry	SRS	27
Rosids	Myrtales	Myrtaceae	*Syzygium* sp.	SRB	40
Rosids	Myrtales	Myrtaceae	*Syzygium* sp.	SYZ	6
Eurosids II	Malvales	Malvaceae	*Sterculia schumanniana* (Lauterb.) Mildbr.	STR	0
Euasterids I	Gentianales	Apocynaceae	*Tabernaemontana aurantica* Gaud.	TAB	1
Euasterids I	Lamiales	Verbenaceae	*Teijsmanniodendron* sp.	TEI	3
Eurosids II	Malvales	Malvaceae	*Trichospermum pleiostigma* (F. Muell.) Kostermans	TRI	2

Table 3 *Homona* haplotypes and host plants in Australia and Papua New Guinea

Species	Haplotype	Moths sequenced	Host species (moths per host species)	Locality (moths per host locality)
Homona mermerodes	a	1	Unknown (light trapping)	Queensland, Australia
Homona mermerodes	b	8	Unknown (light trapping)	Queensland, Australia
Homona mermerodes	c	13	Unknown (light trapping)	Queensland, Australia
Homona mermerodes	d	1	Unknown (light trapping)	Queensland, Australia
Homona mermerodes	e	7	EUP (2), GNE (2), KLE (1), PAD (1), SRS (1)	Madang (Ohu, Baitabag, Mis), PNG
Homona mermerodes	f	1	LUN (1)	Madang (Mis), PNG
Homona mermerodes	g	42	COR (1), DRA (6), EUP (5), GNE (5), KLE (3), LUN (5), MAS (1), PAD (2), PTE (7), PUB (3), SPA (1), SRB (2), SRS (4), WAS (1)	Madang (Ohu, Baitabag, Mis), West Sepik (Utai, Yapsei; 4), PNG
Homona mermerodes	h	1	EUP (1)	Madang (Mis), PNG
Homona mermerodes	j	1	COR (1)	Madang (Ohu), PNG
Homona mermerodes	i	1	NOD (1)	Madang (Ohu), PNG
Adoxophyes sp. nr. *marmarygodes*	a	4	HOM (4)	Chimbu (Mu), PNG
Adoxophyes sp. nr. *marmarygodes*	b	1	HOM (1)	Chimbu (Mu), PNG
Homona aestivana	a	2	MAX (1), MUS (1)	Madang (Mis), West Sepik (Yapsei), PNG
Homona aestivana	b	2	GNE (1)	Madang (Baitabag, Ohu), PNG
Homona aestivana	c	1	MUS (1)	Madang (Mis), PNG
Homona aestivana	d	1	SPA (1)	Madang (Mis), PNG
Homona aestivana	e	1	GNE (1)	Madang (Ohu), PNG
Homona spargotis	a	1	PTE (1)	Madang (Mis), PNG
Homona spargotis	b	1	PUB (1)	Madang (Mis), PNG
Homona spargotis	c	1	PTE (1)	Madang (Mis), PNG
Homona spargotis	d	1	Unknown (light trapping)	Queensland, Australia
Homona trachyptera	a	5	HOM (5)	Madang (Baitabag), PNG Chimbu (Mu), PNG
Homona trachyptera	b	12	GEU (1), HOM (3) NOD (2), KLE (2), PTE (1), SRB (1), STR (1), TRI (1)	Madang (Baitabag, Mis, Ohu), PNG Chimbu (Mu), PNG Eastern Highlands (Wau), PNG
Homona trachyptera	c	2	GNE (1), KLE (1)	Madang (Mis, Baitabag) PNG
Homona trachyptera	d	2	HOM (1), WAS (1)	Madang (Baitabag), PNG Chimbu (Mu), PNG
Homona trachyptera	e	1	KLE (1)	Madang (Mis), PNG
Homona sp. nr. *salaconis*	a	1	SRB (1)	Madang (Mis), PNG
Homona sp. nr. *salaconis*	b	1	PAD (1)	Madang (Mis), PNG
Homona sp. nr. *salaconis*	c	1	SPA (1)	East Sepik (Wamangu), PNG
Homona sp. nr. *salaconis*	d	1	AMA (1)	Madang (Pau), PNG
Homona sp. nr. *salaconis*	e	1	SPA (1)	Madang (Ohu), PNG
Homona sp. nr. *salaconis*	f	1	PTE (1)	Madang (Pau), PNG
Homona sp. nr. *salaconis*	g	1	PAD (1)	Madang (Mis), PNG
Homona sp. nr. *salaconis*	h	4	MUS (1), PSS (1), PTE (1) SPA (1)	Madang (Baitabag, Mis, Ohu), PNG
Homona sp. nr. *salaconis*	i	1	NEU (1)	Madang, PNG

model of molecular evolution according to the Akaike information criterion (AIC) was general time reversible with estimated base frequencies, a parameter for the proportion of invariant sites and a shape parameter for the distribution of rate heterogeneity among sites (GTR + I + G). A neighbour-joining phylogram fitted with GTR + I + G model parameters is shown in Fig. 3. *Homona* phylogeny was rooted with two sequences from the *Adoxophyes* sp. nr. *marmarygodes*. There were 158 variable sites (23.9%) of which 113 were parsimony-informative (17.9%). The neighbour-joining tree was identical to one of > 10 000 most parsimonious trees. Although the strict consensus of these equally parsimonious trees did not conflict with the neighbour-joining tree, quite a number of branches collapsed. Monophyly of each *Homona* species was strongly supported (93–100% bootstrap value) whereas resolution and support for intraspecific clades was generally lacking (Fig. 3). There were only 11 parsimony-informative sites (1.7%) among *H. mermerodes* haplotypes and pairwise

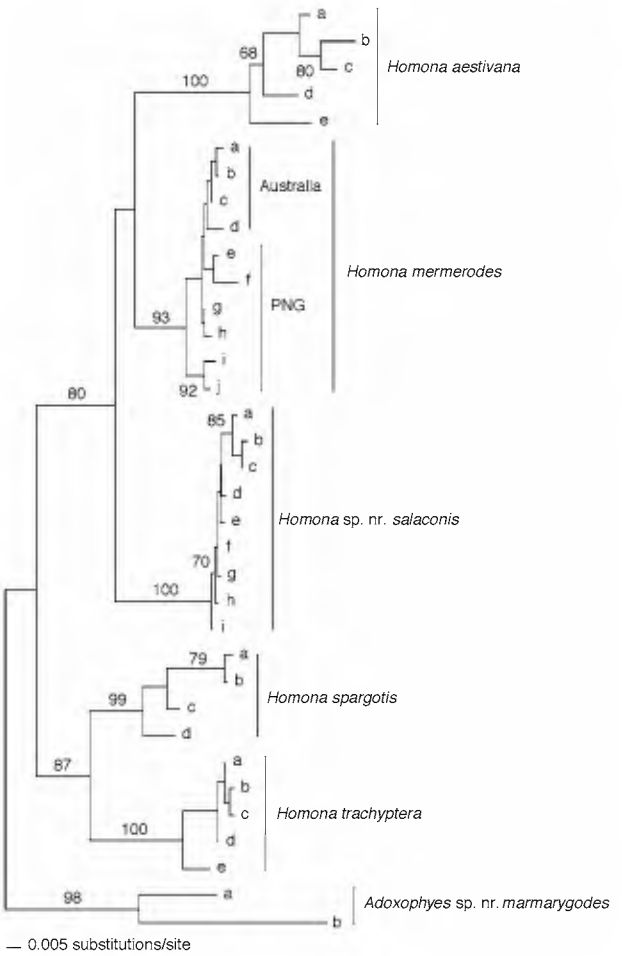

Fig. 3 Neighbour-joining phylogram for *Homona* moths rooted with two sequences from the *Adoxophyes* sp. nr. *marmarygodes*. The phylogram was obtained using maximum likelihood model parameters (GTR + I + G). Branch lengths are proportional to the number of substitutions per site and bootstrap percentages for clades supported by maximum parsimony analysis are listed above the branches.

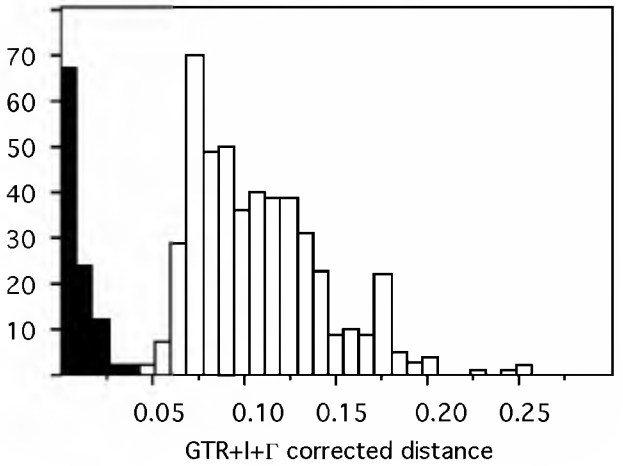

Fig. 4 Histogram of likelihood-corrected pairwise distances for 35 cytochrome *c* oxidase I haplotypes from six tortricid moth species in New Guinea and Australia. Intraspecific distances are indicated in black and interspecific differences in white.

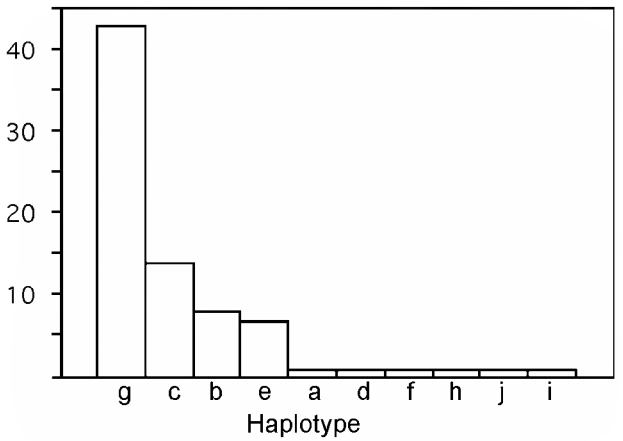

Fig. 5 Frequency of *Homona mermerodes* haplotypes from PNG and Australia.

distances averaged 0.010 ± 0.001 (X ± SD). Intraspecific distances were much lower on average than interspecific distances (0.107 ± 0.020) and were consistent with general patterns of variability of COI sequence in Lepidoptera (Hebert *et al.* 2003). Named species boundaries were confirmed by pairwise genetic distances forming a bimodal distribution with nearly discontinuous ranges of interspecific and intraspecific divergence (Fig. 4).

Homona mermerodes haplotype diversity was Poisson-distributed (Fig. 5) and the most common haplotype was encountered in 42 moths (Table 3). This haplotype was collected at multiple localities across northern New Guinea covering a distance of more than 500 km from Madang to West Sepik Province. A less common haplotype, distinguished by a C to T transition at position 277 out of 661, was encountered in seven instances. Four other New Guinea specimens exhibited unique mutations. Although sequences were double-checked for accuracy, it is still possible that some singleton haplotypes could be artefacts of DNA polymerase errors. Two out of four Australian haplotypes were encountered repeatedly. The paraphyly of New Guinea haplotypes with respect to the Australian material (Fig. 3) suggests a phylogeographical scenario in which Australia was colonized by *H. mermerodes* from New Guinea but clade support for this hypothesis is lacking.

Discussion

In contrast to a previous study identifying host-associated cryptic species in widespread generalist butterfly (Hebert *et al.* 2004), evaluation of species limits by DNA barcoding confirmed the polyphagous habit in *Homona* moths. The

most common *Homona mermerodes* haplotype, for example, was reared from 14 different plant species including 11 phylogenetically diverse families (Table 2). The second most-common haplotype was reared from five plant species including as many families. Although inferences about host specificity cannot be drawn from singleton haplotypes, the pattern of generalized feeding exhibited by the more common haplotypes and observations of *H. mermerodes* caterpillars feeding on 44 out of 111 host plant species sampled in New Guinea strongly support the polyphagous habit of this species (Weiblen *et al.* 2006). We found no evidence of host-associated mtDNA haplotype differentiation, which might be expected from the matrilineal inheritance of mtDNA and the fact that ovipositing females choose host plants. Intraspecific differentiation mediated by female choice and the tendency to respond to chemical cues similar to the larval host plants may sometimes lead to sympatric speciation (Corbet 1985; van Klinken 2000) but no host-associated differentiation was evident in *H. aestivana*, which fed on at least four different plant species in as many families, *H. trachyptera*, which fed on 10 plant species, or *Homona* sp. nr. *salaconis*, which fed on seven species.

Variation in wing colouration among the sampled individuals was substantial (Fig. 1), which is typical for *H. mermerodes* in museum collections. Variable wing patterns from the same mtDNA haplotype suggest a genetic polymorphism or phenotypic plasticity in this trait. On the contrary, genitalia of all dissected *H. mermerodes* showed little morphological variability, confirming that genital morphology is a legitimate source of data for evaluating species concepts in Lepidoptera (e.g. Miller 1994).

Our findings indicate that broad host range, morphological variability, and regionally distributed populations need not be associated with mtDNA divergence. DNA barcoding may lead to the discovery of cryptic species in some cases but in others the same technique may validate accepted species concepts and reinforce other information such as ecological data on diet breadth and specialization. In the case of *Homona mermerodes*, DNA barcoding confirms the polyphagous habit of a generalist moth.

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Supplementary material

The following supplementary material is available for this article:

Table S1 DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae)
Jiri Hulcr, Scott E. Miller, Gregory P. Setliff, Karolyn Darrow, Nathaniel Mueller, Paul Hebert and George D. Weiblen

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