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Australian parasitic *Ogyris* butterflies: east–west divergence of highly-specialized relicts

DANIEL J. SCHMIDT[1]*, ROGER GRUND[2], MATTHEW R. WILLIAMS[3] and JANE M. HUGHES[1]

[1]*Australian Rivers Institute, Griffith University, Nathan, QLD 4111, Australia*
[2]*9 Parkers Road, Torrens Park, SA 5062, Australia*
[3]*Department of Parks and Wildlife, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia*

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Not all butterflies are innocuous plant-feeders. A small number of taxa in the family Lycaenidae have graduated from mutualistic partnerships with ants to predatory or parasitic associations. These highly-specialized life histories, involving butterfly larvae living inside ant colonies, are often associated with rarity and vulnerability to extinction. In the present study, we examined the evolutionary relationships of a poorly-known group of seven taxa herein referred to as the *idmo*-group within the Australian lycaenid genus *Ogyris*. The *idmo*-group has a relictual distribution across southern Australia and includes taxa with highly-specialized phytophagous and myrmecophagous life histories. A phylogeny based on mitochondrial DNA (cytochrome oxidase I and cytochrome b] and the nuclear DNA locus elongation factor 1α (*EF1α*), generally agrees with current taxonomy and supports the recent elevation of endangered taxon *Ogyris halmaturia* to full species status. The transition to myrmecophagy was dated to the mid-Miocene (approximately 16 Mya), when southern Australia experienced a humid climate and extensive mesic biome. The arid Nullarbor Plain, a major biogeographical feature of central southern Australia, divides the remnants of this mesic biome into south-eastern and south-western isolates. Late-Miocene to Pliocene divergence estimates for polytypic *Ogyris* species across the Nullarbor were older than estimates made for similarly distributed birds, butterflies, mammals, and reptiles, which mostly date to the Pleistocene. The concept of highly-specialized life histories as evolutionary dead-end strategies is well exemplified by the *idmo*-group. Data compiled on the known extant subpopulations for *idmo*-group taxa show that all of these extraordinary butterflies are scarce and several face imminent threat of extinction. *Biological Journal of the Linnean Society*, 2013, ••, ••–••.

ADDITIONAL KEYWORDS: Bassian fauna – BEAST – *Camponotus* – Lycaenidae – molecular divergence – myrmecophagy – myrmecophily – Nullarbor – phylogeography.

INTRODUCTION

Many members of the cosmopolitan butterfly family Lycaenidae engage in mutualistic or commensal associations with ants ('myrmecophily'), and a relatively small fraction of these have secondarily evolved into parasites of their ant hosts (Pierce *et al*., 2002). A range of adaptations, including chemical and acoustic mimicry, facilitate the infiltration of these butterfly larvae into ant colonies where they complete growth and development by feeding on ant-brood ('myrmecophagy'; Pierce *et al*., 2002; Settele *et al*., 2011). Highly-specialized life histories are generally associated with reduced range size, patchy distributions, and increased extinction vulnerability (Filippi-Codaccioni *et al*., 2010; Garcia-Barros & Benito, 2010). Parasitic butterflies exemplify these patterns; the best-documented example being the large blues (*Maculinea* spp. Lycaenidae: Polyommatinae), which are flagship species for butterfly conservation in Europe (Thomas, Simcox & Clarke, 2009). The life cycle of *Maculinea arion* requires oviposition on a particular plant followed by adoption of larvae by a certain *Myrmica* ant that itself has microhabitat requirements favoured by

*Corresponding author. E-mail: d.schmidt@griffith.edu.au

particular land-use practices (Thomas *et al*., 2009). Great effort was required to understand the ecology and genetic structure of declining *Maculinea* populations before successful conservation strategies were adopted (Als *et al*., 2004; Thomas *et al*., 2009).

The Australasian genus *Ogyris*, commonly known as the 'azures', currently includes 15 species that all associate with ants to varying degrees (Braby, 2000; Braby & Douglas, 2008; Grund, 2010). Three of the four species within the morphologically and biogeographically distinct *Ogyris idmo* species-group (*sensu* Braby & Douglas, 2008) (i.e. *Ogyris idmo*, *Ogyris subterrestris*, and *Ogyris hamaturia*), hereafter referred to as the '*idmo*-group', have a myrmecophagous life history involving parasitism of large nocturnal *Camponotus terebrans* (Lowne) ants. The above three taxa are very similar to the extent that *O. subterrestris* was initially considered to be a dwarf form of *O. idmo*. The fourth species, *Ogyris otanes*, has larvae with a conventional phytophagous diet on root-parasitic plants *Choretrum* and *Leptomeria* (Santalaceae) and maintains an obligate myrmecophilous relationship with *C. terebrans* (Braby, 2000). Two additional *Ogyris* species (*genoveva* and *zosine*) have morphological similarities with the *idmo*-group. These species, which comprise the *genoveva*-group (*sensu* Braby & Douglas, 2008), have obligate myrmecophilous associations with large *Camponotus* spp. ants (but not *C. terebrans*) and are phytophagous on parasitic mistletoe (similar to *O. otanes*). However the *genoveva*-group is biogeographically distinct, with distributions covering much of eastern and northern Australia (Braby, 2000).

Similar to *Maculinea*, the *idmo*-group represents a challenge for butterfly conservation in Australia. All taxa in this group have highly-specialiszed life histories with small, patchy distributions and most are under threat in part or all of their range (Braby & Douglas, 2008; New, 2011). Currently, the functional ecology and genetic relationships within and among the members of this group await documentation.

The four species comprising the *idmo*-group incorporate seven recognized taxa (inclusive of subspecies). All are entirely restricted to coastal and semi-arid environments of southern Australia (Fig. 1). The group exhibits a disjunct biogeographical distribution pattern typical of the flora and fauna of southern Australia (Byrne *et al*., 2011), with each of the seven taxa restricted either to the south-east or south-west of the arid Nullarbor Plain (Fig. 1A, C, D). The Nullarbor Plain (the present surface of the Eucla basin) is the world's largest limestone karst, its surface largely exposed from approximately the end of the Middle Miocene (~14–12 mya) and covers almost one-third of southern Australia (Hou *et al*., 2008; Miller, James & Bone, 2012). The climate of the plain has evolved from temperate humid in the Miocene to increasingly arid through the Pliocene–Pleistocene (< 5 Mya) and, currently, is sparsely vegetated with shrubby steppe foliage (Miller *et al*., 2012). The aridity is compounded not by the lack of rainfall but, instead, because of the plain's lack of water retention near the surface as a result of its karst porosity. The plain isolates the southern mesic bioregions to its east and west (Fig. 1A) and its formation, along with true aridification of Australia's central deserts, induced ancient vicariance resulting in allopatric isolation and divergence of the unique biota of the south-west (Hopper & Gioia, 2004; Crisp & Cook, 2007; Byrne *et al*., 2011).

Recent interest in molecular dating of east–west divergence across the Nullarbor Plain suggests multiple episodes of isolation have occurred (Byrne *et al*., 2011). Relatively ancient interspecific divergences have been estimated for east–west sister lineages of plants and mesic zone-restricted animals, including freshwater fish, crayfish, frogs, and spiders (Crisp & Cook, 2007; Morgan, Roberts & Keogh, 2007; Schultz *et al*., 2009; Unmack *et al*., 2011; Rix & Harvey, 2012). These divergences vary from late-Oligocene to mid-Miocene in age, approximately 13–26 Mya. Intraspecific divergence for taxa with similar disjunct east–west distributions cluster towards more recent dates in the late-Pliocene to Pleistocene. Examples include reptiles, birds, butterflies, and mammals (Norgate *et al*., 2009; Dubey & Shine, 2010; Dolman & Joseph, 2012; Neaves *et al*., 2012). A detailed analysis of multiple bird taxa suggested that even within this recent time frame, east–west divergence might have occurred over at least three discrete periods ranging from approximately 50 kya to 1 Mya (Dolman & Joseph, 2012).

The taxonomic arrangement of the *idmo*-group indicates that east–west divergence may have been an important factor in its diversification. A former arrangement divided species *idmo* and *subterrestris* into pairs of east–west subspecies, and *otanes* into one eastern and two western subspecies (Field, 1999; Braby, 2000; Williams & Hay, 2001). A recent revision elevated the eastern subspecies of *idmo* to full species status (*Ogyris halmaturia*; Braby & Douglas, 2008; Grund, 2010). In the present study, we attempt to highlight the dire conservation prospects faced by this group resulting from the interplay between their highly-specialized life histories, an ancient association with a declining mesic biome (Byrne *et al*., 2011) and modern habitat destruction. The study aimed to: (1) present the first molecular phylogenetic analysis of the *idmo*-group with samples representing the current and former range of each taxon; (2) test the recent taxonomic elevation of *O. halmaturia*; (3) estimate the timescale of east–west divergence of taxa across the Nullarbor Plain; and (4) compile

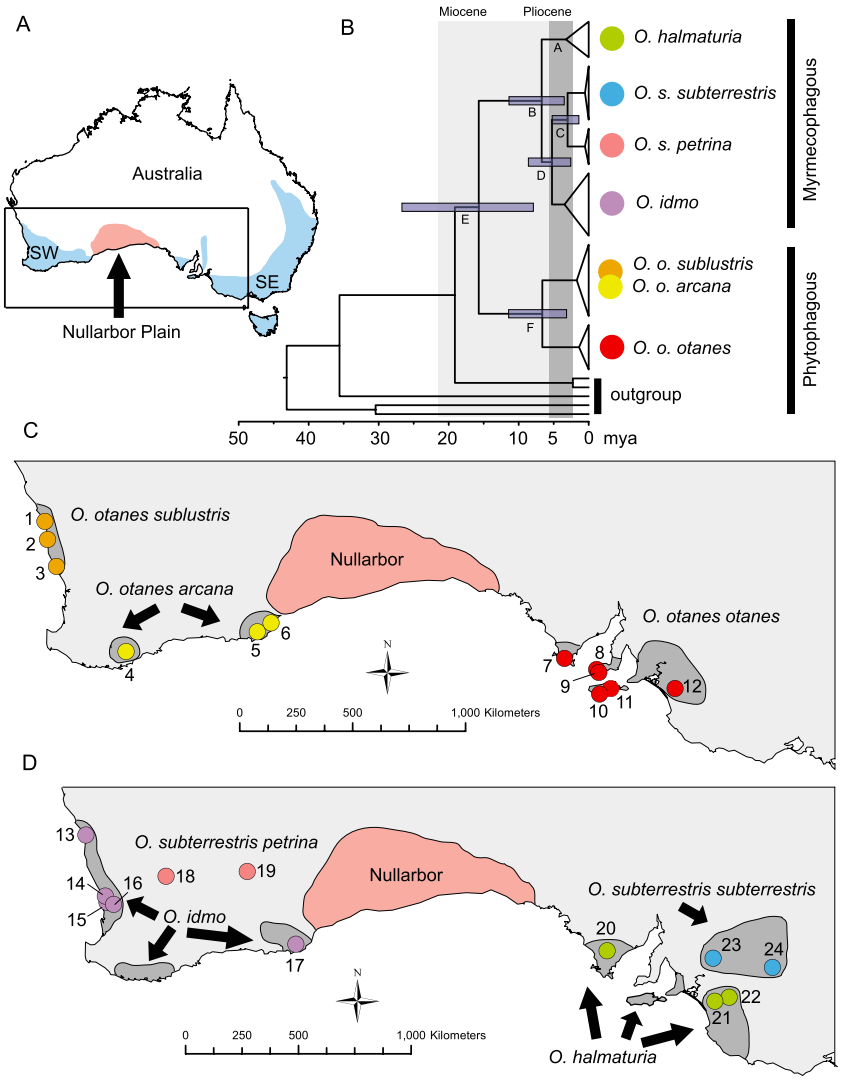

Figure 1. Distribution of *Ogyris idmo* species-group taxa, sample locations, and BEAST chronogram. A, Australian continent showing approximate extent of the southern mesic biome (blue) divided into south-western (SW) and south-eastern (SE) regions by the arid Nullarbor Plain (pink). Rectangle indicates study area enlarged in (C) and (D). B, BEAST maximum-clade credibility chronogram for seven *idmo*-group taxa based on the mitochondrial DNA dataset. Node bars represent 95% highest posterior density (HPD) intervals of node age and letters correspond to HPD values given in Table 2, C, geographical distribution of phytophagous taxon *Ogyris otanes*. Grey shaded areas delimit approximate historical distribution of subspecies *sensu* Braby (2000). Circles represent sample sites coloured by taxon; numbers correspond to sample sites listed in Table 1, D, geographical distribution and sample sites of myrmecophagous taxa *O. idmo*, *Ogyris halmaturia* and *Ogyris subterrestris*. Distributions in grey shading *sensu* Braby (2000). Subspecies *Ogyris subterrestris petrina* is known only from two sample sites, discovery of site 18 postdates Braby (2000).

information on the number of known extant subpopulations to highlight the conservation concern facing this extraordinary group of butterflies.

MATERIAL AND METHODS

SAMPLES AND MOLECULAR METHODS

Tissue samples were obtained from private and institutional collections (Table 1). Total genomic DNA was extracted from a single leg using a standard phenol–chloroform extraction method (Sambrook, Fritsch & Maniatis, 1989). Mitochondrial (mt)DNA regions cytochrome oxidase I (*COI*) and cytochrome b (*cytb*) and the nuclear DNA locus elongation factor 1α (*EF1α*) were amplified and sequenced *sensu* Schmidt & Hughes (2006). Primers were LCO1490 and C1-N-2191 for *COI* (Folmer *et al.*, 1994; Simon *et al.*, 1994); REVCB2H and REVCBJ for *cytb* (Simmons & Weller,

Table 1. Locality and collection data for sequenced specimens

Taxon	Specimen voucher code	Site code*	Location†	Latitude	Longitude	Collector	Date	Tissue Source§
Ogyris otanes otanes	RG226	12	Tintinara, Upper SE., SA	−35.883	140.057	R. Grund	19 February 1997	SAMA
	gen22	8	Innes, Yorke Pen., SA	−35.219	136.886	R. Fisher	25 March 1993	SAMA
	LH002	9	Marion Bay, Yorke Pen., SA	−35.24	136.979	L. Hunt	1995	SAMA
	RF003	11	Beyeria, Kangaroo I., SA	−35.787	137.595	L. Hunt	1995	SAMA
	RA459	10	Vivonne Bay, Kangaroo I., SA	−36.011	137.149	R. Grund	13 November 2003	RBG
	RA102d	7	South Eyre Pen., SA	−34.68	135.61	R. Grund	21 January 2002	RBG
Ogyris otanes sublustris	KB114	1	Port Denison, WA	−29.275	114.918	R. Grund	22 November 1997	SAMA
	KB117	1	Port Denison, WA	−29.275	114.918	R. Grund	1 December 1997	SAMA
	RF11	2	Leeman, WA	−29.948	114.978	R. Field	30 September 1993	SAMA
	RF18	2	Leeman, WA	−29.948	114.978	R. Field	2 October 1993	SAMA
	AAM-97-W057	3	2.6 km south of Lancelin, WA	−31.02	115.332	M. R. Williams	21 October 1997	MCZ
Ogyris otanes arcana	RF46	4	Stirling Range, WA	−34.415	118.154	R. Field	24 November 1993	SAMA
	AAM-97-U328	4	Stirling Range, WA	−34.415	118.154	A. A. E. Williams	4 November 1997	MCZ
	RF50	6	Wylie Scarp, WA	−33.272	123.919	R. Field	26 November 1993	SAMA
	RF203	5	Cape Arid NP, WA	−33.623	123.376	R. Field	6 November 1994	SAMA
Ogyris halmaturia	RA101a	20	Southern Eyre Pen., SA	−33.882	135.764	R. Grund	20 November 2001	RBG
	RA101b	20	Southern Eyre Pen., SA	−33.882	135.764	R. Grund	20 November 2001	RBG
	RG552	20	Southern Eyre Pen., SA	−33.882	135.764	R. Grund	7 November 1998	SAMA
	MBF-00-P653	22	Ngarkat Cons. Park, SA	−35.731	140.625	M. F. Braby & M. Moore	9 November 2006	MFB
	LH16	21	Tintinara, Upper SE., SA	−35.883	140.057	L. Hunt	15 November 1997	SAMA
Ogyris idmo	DL-01-Q561	13	Port Denison, WA	−29.275	114.918	D. Lohman	9 October 2000	MCZ
	KB90b	13	Port Denison, WA	−29.275	114.918	R. Grund	15 October 1997	RBG
	RF64	15	Perth, WA	−31.951	115.858	R. Field	10 December 1993	SAMA
	Y126	14	Carramar, Perth WA.	−31.699	115.785	M. & A. Williams	20 February 2002	DEC
	MW-97-Y097	16	Pickering Brook, WA	−32.036	116.122	M. R. Williams	13 November 1996	MCZ
	RF200	17	Cape Arid NP, WA	−33.623	123.376	R. Field	5 November 1994	SAMA
	RF201	17	Cape Arid NP, WA	−33.623	123.376	R. Field	6 November 1994	MV
	RE-02-A338	17	Cape Arid NP, WA	−33.623	123.376	S. S. Brown, C. E. Meyer & Weir, R. P.	11 September 2002	MCZ
Ogyris subterrestris subterrestris	RG119	23	Waikerie, SA	−34.182	139.983	R. Grund	9 October 1996	SAMA
	RF34	23	Waikerie, SA	−34.182	139.983	R. Field	October 1993	SAMA
	RG233	23	Waikerie, SA	−34.182	139.983	R. Grund	13 March 1997	SAMA
	SB001	24	5 km south of Coligan, VIC	−34.54	142.345	S. Brown	6 November 2006	SSB
	SB002	24	5 km south of Coligan, VIC	−34.54	142.345	S. Brown	5 November 2006	SSB
	SB003	24	5 km south of Coligan, VIC	−34.54	142.345	S. Brown	6 November 2006	SSB
Ogyris subterrestris petrina	RA463	19	Lake Douglas, WA	−30.749	121.465	R. Field	17 October 1991	SAMA
	RA465	19	Lake Douglas, WA	−30.749	121.465	A. Graham	14 December 1987	SAMA
	U243	18	near Merredin, WA‡	−31	118	A. A. E. Williams	20 October 2006	DEC
	U244	18	near Merredin, WA‡	−31	118	A. A. E. Williams	8 March 2007	DEC
	U245	18	near Merredin, WA‡	−31	118	A. A. E. Williams	22 October 2007	DEC
Ogyris genoveva (outgroup)	gAx1	_	Mt Alexina, VIC	−36.58	145.13	D. J. Schmidt	November 2004	GU
Ogyris zosine (outgroup)	zTw2	_	Tewantin, QLD	−26.25	152.59	D. J. Schmidt	24 August 2002	GU
Ogyris olane (outgroup)	ol2	_	Armidale, NSW	−30.31	151.41	D. J. Schmidt	2003	GU
Ogyris amaryllis (outgroup)	Wn8	_	Wandoan, QLD	−26.7	149.58	D. J. Schmidt	May 2003	GU
Ogyris abrota (outgroup)	aCn1	_	Crows Nest, QLD	−27.16	152.3	D. J. Schmidt	2002	GU

*Site code numbers correspond to Figure 1A, D.

†NP, National Park; NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

‡Precise details withheld for conservation purposes.

§Tissue source: DEC, Department of Environment and Conservation Western Australia; GU, Griffith University; MCZ, Museum of Comparative Zoology collection; MFB, Michael Braby collection; SAMA, South Australian Museum; RBG, Roger Grund collection; SSB, Steve Brown Collection.

2001); and *EF1α* was amplified in two overlapping fragments using primer pairs EF1-44F + EF1-M4R and EF1-51.9 + EF1-929 (Monteiro & Pierce, 2001; Kandul *et al*., 2004). Dried leg samples from some pinned specimens did not produce successful amplification of mtDNA or *EF1α*. To obtain at least some data for these samples (including the extinct type locality of *O. subterrestris petrina*), we resorted to amplification of three smaller overlapping fragments of *COI*, yielding a fragment of equivalent length to other samples. Additional primers and methods described in the Supporting information (Table S1). Sequences for one *O. idmo* sample and one *Ogyris amaryllis* sample were obtained from GenBank (accession numbers: DQ456524, DQ456605, EF116946, EF108424; Supporting Information, Table S2). New sequences were deposited in GenBank under accession numbers KF516683 – KF516805 (see Supporting Information, Table S2).

Phylogenetic analysis

Phylogenetic relationships of *idmo*-group butterflies were estimated using maximum likelihood (ML), Bayesian, and statistical parsimony methods. A ML analysis was performed on the mtDNA dataset using RaxML, version 7.6.3 via the CIPRES portal (Stamatakis, 2006; Miller, Pfeiffer & Schwartz, 2010). The best tree was found under the GTR+G substitution model with separate partitions for *COI* and *cytb*, and with 1000 bootstrap replicates to assess clade support (Stamatakis, Hoover & Rougemont, 2008). The Shimodaira–Hasegawa (SH) test was used to evaluate the recent taxonomic change involving elevation of *halmaturia* from subspecies under *idmo*, to full species status (Shimodaira & Hasegawa, 1999). A topological constraint was imposed on ML tree searches to unite *idmo* and *halmaturia* in a monophyletic clade reflecting their former conspecific classification. Deviation of log-likelihood values generated around the constrained topology relative to the unconstrained topology was assessed with the SH test using 1000 RELL bootstraps implemented in PAUP* (Swofford, 2000). The *EF1α* alignment contained relatively low variation and a haplotype network was used to represent genetic relationships among taxa at this locus. Haplotypes were resolved from diplotype sequences using PHASE, version 2.1 (Stephens, Smith & Donnelly, 2001), implemented in DNASP, version 5.10 (Librado & Rozas, 2009), and a haplotype network was created using statistical parsimony in TCS, version 1.21 (Clement, Posada & Crandall, 2000).

Molecular dating of divergences between south-eastern and south-western taxa was performed using a Bayesian approach in BEAST, version 1.7.5 through the CIPRES portal (Drummond & Rambaut, 2007; Miller *et al*., 2010). Divergence times were estimated under both strict and lognormal relaxed clock models; and under both coalescent constant size and Yule tree priors. We estimated divergence times using *COI* substitution rates commonly used in the literature for estimating interspecific divergence in butterflies. Two rates were used to describe a lognormal prior for the estimated clock rate, where 95% of the probability density was contained between 0.0039 and 0.0115 substitutions/site/lineage/million years, corresponding to the widely used rates of Zakharov, Caterino & Sperling (2004) and Brower (1994) respectively. Additionally, we estimated a species tree for the seven *idmo*-group taxa incorporating both mtDNA and *EF1α* using *BEAST, version 1.7.5 (Heled & Drummond, 2010). A Yule tree-model prior was used and tree models for mtDNA partitions (*COI*, *cytb*) were linked. Appropriate substitution models were selected for *COI* (TrN+G), *cytb* (TrN+I+G), and *EF1α* (TrNef+I+G) partitions using JMODELTEST, version 0.1 (Posada, 2008) and implemented separately for each partition in *BEAST. Convergence, mixing, and effective sample size of all model parameters (> 200) for BEAST and *BEAST runs were assessed using TRACER, version 1.5 (Drummond & Rambaut, 2007) after running analyses for 10^8 generations. Maximum clade credibility trees were produced using TREEANNOTATOR, version 1.7.4 and FIG TREE, version 1.3.1 (Drummond & Rambaut, 2007).

RESULTS

Phylogenetic relationships of the *Ogyris idmo* species-group

The *COI* alignment contained data from 45 individuals (including five congeneric outgroup taxa) was 628 bp in length, and included 167 variable positions (125 variable for the ingroup). The *cytb* alignment included 40 individuals, was 579 bp in length, and contained 171 variable positions (121 variable for the ingroup). The *EF1α* alignment included 42 individuals, was 1062 bp in length, and contained 76 variable positions (27 variable sites for the ingroup). Alignment of all partitions was unambiguous with no evidence of stop codons or frameshift mutations. Gene tree topologies from ML and Bayesian analyses of mtDNA data were in general agreement with current taxonomy for the seven *idmo*-group taxa. Five taxa were recovered as monophyletic clades with ML bootstrap support > 80% and posterior probabilities of 1.0 (i.e. *idmo*, *halmaturia*, *otanes*, *subterrestris*, and *petrina*); the two western subspecies of *otanes* (i.e. *arcana* and *sublustris*) were polyphyletic (Fig. 2A). The *EF1α* data contained less variation relative to mtDNA,

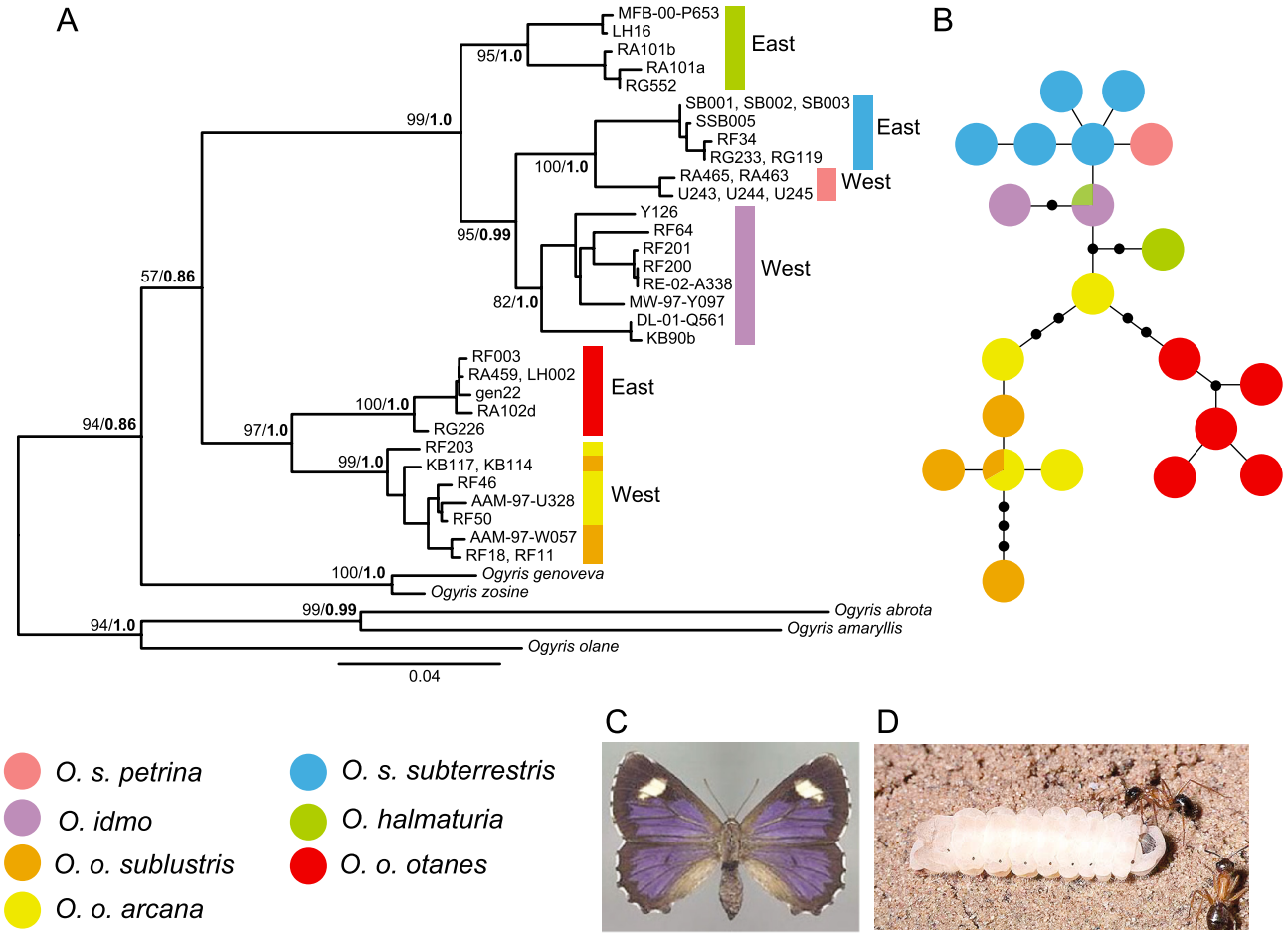

Figure 2. Gene trees of *Ogyris idmo* species-group taxa. A, maximum likelihood (ML) phylogram of mitochondrial DNA dataset (*COI*, *cytb*). Terminals labelled with specimen codes corresponding to Table 1; specimen codes separated by commas share identical haplotypes. Node labels are ML bootstrap support values followed by Bayesian posterior probabilities for nodes also found in the BEAST maximum clade credibility tree. B, statistical parsimony haplotype network of the phased elongation factor 1α (*EF1α*) dataset. Haplotype colour corresponds to the taxon label. Haplotypes shared between taxa are illustrated as frequency pies. C, *Ogyris halmaturia*, adult female upperside. D, *Ogyris subterrestris subterrestris*, larva with host ant, *Camponotus terebrans*. Images are reproduced with permission (http://www.sabutterflies.org.au/).

although a network of inferred haplotypes did resolve taxa into groups consistent with the mtDNA gene tree, differing primarily in haplotype sharing between species *idmo* and *hamaturia* (Fig. 2B). A topology test using mtDNA data supported the recent elevation of *halmaturia* to full species level. The difference in log-likelihood scores between an unconstrained ML tree and the best ML tree constrained to unite *idmo* and *halmaturia* in a monophyletic clade, showed a highly significant difference ($P < 0.001$; $\ln L_{\text{monophyly}} = -5278$; $\ln L_{\text{unconstrained}} = -5237$). Therefore, mtDNA supports the recent taxonomic elevation of *halmaturia*, whereas *EF1α* does not because most *idmo* and *halmaturia* samples share a common haplotype (Fig. 2B). The topology of the species tree produced by combining mtDNA and *EF1α* was identical to the mtDNA gene tree except for the arrangement of outgroup taxa, which was poorly supported (see Supporting information, Fig. S1). The species tree represents each taxon as a single terminal, and so the polyphyletic relationship found between subspecies *sublustris* and *arcana* (using mtDNA and *EF1α* separately) cannot be expressed in the species tree. All DNA matrices, along with the tree presented in Figure 2A, are available from TreeBase ID: 14796.

Timing of east–west divergence events

The Bayesian mtDNA tree (Fig. 1B) showed an identical topology to the ML tree (Fig. 2A) at supported

Table 2. Estimates of time to most recent common ancestor (TMRCA) for mitochondrial DNA nodes depicted in the maximum clade credibility tree (Fig. 1B) and comparison of TMRCA estimates under coalescent versus Yule tree prior using BEAST, version 1.7.5

	Coalescent tree prior			Yule tree prior		
Node*	Median value (millions of years)	95% highest posterior density (lower – upper)	ESS†	Median value (millions of years)	95% highest posterior density (lower – upper)	ESS
A	3.25	1.47–5.75	1430	3.04	1.54–5.31	1247
B	6.72	3.50–11.4	1242	6.14	3.11–10.36	1275
C	3.01	1.40–5.22	1373	2.83	1.30–4.83	1398
D	5.24	2.56–8.64	1302	4.82	2.45–8.15	1294
E	16.0	7.90–27.3	1213	13.45	6.38–22.36	1249
F	6.62	3.14–11.40	1362	5.91	2.91–10.20	1295

*Node corresponds to chronogram in Figure 1B.
†ESS, effective sample size.

nodes. Choice of tree prior did not affect TMRCA estimates made with BEAST, and a strict clock model could not be rejected after observing the marginal distribution of ucld.stdev included zero under a relaxed clock. Divergence between phytophagous and myrmecophagous clades within the *idmo*-group was estimated at approximately 16 Mya (node E, Fig. 1B; Table 2). Estimates of TMRCA for clades with disjunct distributions across the Nullarbor were Pliocene to late-Miocene in age; median estimates ranged from approximately 3.01 Mya for *O. substerrestris* (node C, Fig. 1B; Table 2) to approximately 6.62 mMya for *O. otanes* (node F, Fig. 1B; Table 2). Evidence for phylogeographical divergence was also found within taxa. An estimated divergence time of approximately 3.65 Mya separated populations of *O. halmaturia* on either side of the putative Eyrean biogeographical barrier that is marked by two large coastal inlets (Spencer and St Vincent Gulfs) along the southern coast, and aridity to the north (node A, Fig. 1B, D; Table 2).

Current conservation concerns

Available information on the number of extant subpopulations for the seven *idmo*-group taxa is compiled in Table 3. *Ogyris halmaturia* and both subspecies of *O. subterrestris* rank among the most threatened butterfly taxa on the Australian continent. Their presently known ranges are highly fragmented and consist of very few sites where subpopulations still persist. *Ogyris s. petrina* is known from only two disjunct localities in the semi-arid zone of south-west WA. The easternmost site depicted in Figure 1D (site 19) is the type locality where the butterfly became locally extinct in the 1990s soon after its discovery. Variation in mtDNA (*COI*) recovered from pinned specimens shows a very close relationship between individuals from the type locality (site 19) and those from a new site discovered in 2006 (site 18, Fig. 1D; compare samples U245 (site 18) and RA465 (site 19), Fig. 2A). Other taxa (*O. otanes otanes*, *O. subterrestris subterrestris*; Table 3) have become locally extinct over wide areas of their former distribution and the few remaining sites where these butterflies can be found are associated with areas of remnant mallee (dryland *Eucalyptus* woodland) habitat. The adult stage of *O. subterrestris* is sedentary, and so remaining subpopulations are demographically independent and require sufficient coverage and density of mallee habitat, as well as abundant *C. terebrans* ants to be self-sustaining (R. Grund, pers. observ.). The root-parasitic hosts of *O. otanes* are very susceptible to broad-acre agricultural clearing because regeneration appears to be linked to fire and/or digestion by birds including emus (R. Grund, pers. observ.). Larvae may denude individual host plants and so areas of habitat with high hostplant density are critical for survival of this species.

DISCUSSION

Phylogenetic relationships and evolution of ant-parasitic *Ogyris* butterflies

The evolutionary transition from a phytophagous to a myrmecophagous life history appears to have been relatively ancient in *Ogyris*, with divergence between *O. otanes* (phytophagous) and the remainder of the *idmo*-group (myrmecophagous) estimated at approximately 16 Mya. This timing coincides with the Miocene exposure of the Nullarbor Plain and a significant period of interspecific diversification between

Table 3. Conservation status of *Ogyris idmo* species-group taxa

Taxon	Life history	State conservation status	Known extant subpopulations*	Last confirmed or documented sighting	Source†
Ogyris subterrestris petrina Field	Myrmecophagous	Threatened (Critically Endangered), WA	South-west WA	Active, June 2013	MRW & AAEW
Ogyris subterrestris subterrestris Field	Myrmecophagous	Endangered, SA Vulnerable, VIC	1. Riverland, SA 2. Mildura area, VIC	1. ~2000 2. Active	RG & MM
Ogyris halmaturia (Tepper)	Myrmecophagous	Endangered, SA; Presumed extinct, VIC	1. Upper South-east, SA 2. Eyre Peninsula, SA	1. 2007 2. 2001	RG
Ogyris idmo (Hewitson)	Myrmecophagous	Not threatened, WA	> 10 subpopulations, south-west WA	Active, Oct 2012	MRW & AAEW
Ogyris otanes otanes (C & R Felder)	Obligate myrmecophilous; phytophagous	Threatened, Kangaroo I Endangered, SA Presumed extinct, VIC	1. Kangaroo I., SA 2. South-east, SA 3. Yorke Peninsula, SA 4. Eyre Peninsula, SA	1. Active 2. 1997 3. Active 4. 2005	RG
Ogyris otanes sublustris Williams & Hay	Obligate myrmecophilous; phytophagous	Not threatened, WA	~10 subpopulations, WA	Active, March 2013	MRW & AAEW
Ogyris otanes arcana Williams & Hay	Obligate myrmecophilous; phytophagous	Not threatened, WA	~10 subpopulations, WA	Active, October 2012	MRW, AAEW, GM, CM

*State abbreviation: SA, South Australia; VIC, Victoria; WA, Western Australia.
†Source of confirmed sighting by experienced lepidopterist: AAEW, Andrew Williams; CM, Cliff Meyer; GM, Grant Miller; MM, Mike Moore; MRW, Matthew Williams; RG, Roger Grund.

south-eastern and south-western components of the Australian flora and fauna (Crisp & Cook, 2007; Byrne *et al*., 2011; Unmack *et al*., 2011; Miller *et al*., 2012). Our results are consistent with the view that myrmecophagy is a derived trait within the (largely) myrmecophilous Lycaenidae (Pierce *et al*., 2002). The ancient transition to myrmecophagy coupled with limited species diversity in *Ogyris* is also consistent with phylogenetic analyses of other lycaenid genera containing myrmecophagous species (e.g. *Acrodipsas*, *Maculinea*; Eastwood & Hughes, 2003; Als *et al*., 2004). All *idmo*-group taxa depend on association with the widespread ant *Camponotus terebrans*. Patterns of genetic subdivision within *C. terebrans* based on allozymes suggest at least some diversification within the *idmo*-group may have occurred in parallel with their ant hosts. Eastern and western subspecies of *O. subterrestris* are associated with a northern (inland) form of the ant, which is genetically and morphologically distinct from a southern (coastal) form associated with *O. idmo* and *O. otanes* (McArthur, Adams & Shattuck, 1997).

Our moderately well-resolved mtDNA phylogeny of the *idmo*-group is largely in agreement with current taxonomy, including recent elevation of *O. halmaturia* (Braby & Douglas, 2008; Grund, 2010). *Ogyris halmaturia* is restricted to the south-east and sister to the remainder of the *idmo*-group, including monotypic *idmo* in the south-west and polytypic *subterrestris* with south-eastern and south-western subspecies (Fig. 1D). Variation in the *EF1*α dataset was insufficient to resolve *idmo* from *halmaturia*. *COI* and *EF1*α are the most common markers used for lower-level phylogenetics of Lepidoptera, and this difference in variation and phylogenetic signal is typically observed between these markers (Wilson, 2010). Lack of *EF1*α resolution does not necessarily diminish the status of *halmaturia*, which is strongly supported by mtDNA, morphological characters and the multilocus species tree (see Supporting information, Fig. S1).

The polyphyletic relationship between two western subspecies of *O. otanes* (*arcana* and *sublustris*) is not surprising given these taxa both occur in the south-west and probably share a more recent history than taxa divided by the Nullarbor. Similar structuring between western coastal and southern lineages within the south-west region has been recorded for frogs, lizards, and plants (Byrne *et al*., 2011). Polyphyly aside, these taxa are allopatric (Fig. 1C), exhibit consistent morphological differences (Williams & Hay, 2001), and do not share mtDNA haplotypes, indicating they may be at an intermediate state of genetic divergence. These features argue for maintaining the current classification according to a recent assessment of the subspecies concept (Braby,

Eastwood & Murray, 2012). Polyphyletic relationships between subspecies are commonly observed in molecular phylogenies, including that of the widespread congeneric species *O. amaryllis* (Schmidt & Hughes, 2006). These patterns are generally attributed to incomplete lineage sorting and/or historical gene flow (Schmidt & Hughes, 2006; McLean *et al*., 2012).

Phylogeography of southern Australia

Our molecular dating of intraspecific divergence across the Nullarbor fits well with the existing framework of southern Australian phylogeography. Estimated late-Miocene to Pliocene divergence events are slightly older than Plio-Pleistocene intraspecific divergences estimated for mesic-restricted birds, mammals, reptiles, and butterflies (Norgate *et al*., 2009; Salinas *et al*., 2009; Dubey & Shine, 2010; Dolman & Joseph, 2012). The most comprehensive analysis to date showed that the relatively older trans-Nullarbor divergence times among birds were associated with taxa restricted to more mesic habitats (Dolman & Joseph, 2012). The same pattern was observed in the present study. East–west divergence in the coastal species *O. otanes* was approximately twice the age of semi-arid inland species *O. subterrestris* (i.e. approximately 6.6 Mya compared to 3 Mya). A number of factors might explain relatively old east–west intraspecific divergence times found for *Ogyris* butterflies relative to other organisms. Demographic factors including small population size and limited dispersal ability would make these highly-specialized species susceptible to rapid isolation and divergence in response to sea-level rise and aridification during this period (Edwards & Beerli, 2000; Miller *et al*., 2012). Misspecification of models, priors, and clock rates can also have a significant influence on divergence time estimation using BEAST (Phillips *et al*., 2013). We conditioned our analysis on a range of clock rates and found no difference between results under alternative models. Accordingly, our results should be comparable with previous studies, most of which are based on mtDNA.

Conservation and the future prospects of highly-specialized *Ogyris* butterflies

The few remaining subpopulations of *O. halmaturia* and *O. subterrestris* are cause for international concern over the conservation status of these butterflies. Currently, none of these taxa are listed nationally as threatened fauna in Australia. Conservation assessment of two *idmo*-group taxa based on International Union for Conservation of Nature criteria suggest that *O. halmaturia* warrants listing as 'Endangered' (Braby & Douglas, 2008), and that *O. subterrestris petrina* is 'Critically Endangered'. It is likely that assessment of *O. s. subterrestris* and *O. o. otanes* will lead to similar conclusions based on the conservation status of these taxa at state level (Table 3).

Outlook

Ant-dependent life histories have persisted for millions of years in the *idmo*-group, yet it is likely that the highly-specialized nature of these associations have contributed to their recent precipitous decline in response to human-induced habitat change, including agriculture and urbanization. Populations that are naturally rare and localized because of specific habitat and/or host-ant requirements are especially susceptible to fragmentation of native vegetation as a result of their small size and weak demographic connectivity (Bruckmann, Krauss & Steffan-Dewenter, 2010; Krauss *et al*., 2010). *Ogyris idmo* species-group taxa have probably always been patchily distributed with widely separated subpopulations so that re-colonization of locally extinct colonies would become increasingly unlikely as fragmentation increases their isolation (New, 2011). In evolutionary terms, Pierce *et al*. (2002) noted that the scattered and species-poor phylogenetic distribution of myrmecophagous butterflies suggests they are extinction-prone dead-ends. Phylogenetic relationships and conservation concerns reported in the present study exemplify this pattern. The *idmo*-group is comprised of a limited number of relatively ancient taxa with relictual distributions either side of the Nullarbor Plain. More broadly, one might consider that ant–parasitic butterfly life histories face a similar evolutionary fate to other forms of parasitic life history that become locked into inescapable trade-offs (Lehtonen *et al*., 2013). In this case, parasitic butterflies pay for the short-term gain of enemy-free space and an unwitting food supply, with the long-term burden of highly complex life histories, leaving them vulnerable to changes in their specific habitat requirements.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Species tree of *Ogyris idmo* species-group taxa produced using *BEAST, version 1.7.5 and incorporating both mitochondrial DNA (*COI*, *cytb*) and elongation factor 1α (*EF1α*) partitions. Scale bar represents the time in million years. Branch support is indicated by posterior probabilities.
Table S1. Primers used for amplification of *COI*[a], *cytb*[b], and *EF1α*[c].
Table S2. GenBank accession numbers.