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Identification and molecular phylogenetics of the cryptic species of the *Gonipterus scutellatus* complex (Coleoptera: Curculionidae: Gonipterini)

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Abstract The Eucalyptus Weevil, generally referred to as *Gonipterus scutellatus* Gyllenhal, 1833 is a significant pest of *Eucalyptus* species in Africa, America, Europe and New Zealand. It has recently become a pest of *Eucalyptus globulus* plantations in Western Australia, despite the presence there of the mymarid egg-parasitoid *Anaphes nitens* (Girault). Recent taxonomic study has indicated *G. scutellatus* to comprise a complex of cryptic species, obscuring the identity of the various pest populations of the weevil in the world. We examined (1) whether the apparent cryptic species identifiable on genital differences have a genetic basis; (2) the distribution of these species; and (3) the origin of the population in Western Australia. We studied specimens from across the distribution range of Eucalyptus Weevil in Australia and obtained sequences of three genes from them: cytochrome oxidase I mtDNA, elongation-factor 1-α nuclear DNA and 18s rDNA. The cladogram of COI haplotypes resolved 10 well supported clades fully corresponding with genital-morphologically distinct species, eight of them constituting a monophyletic *G. scutellatus* complex. Only four of these species proved to be described, as *G. balteatus* Lea, 1897 *G. platensis* (Marelli, 1926), *G. pulverulentus* Lea, 1897 and *G. scutellatus* Gyllenhal, 1833. The pest species in the world were found to be *G. platensis* (New Zealand, America, western Europe), *G. pulverulentus* (eastern South America) and an undescribed species (Africa, France, Italy). The population of *G. platensis* in Western Australia showed little genetic variation and is indicated to be a recent introduction from Tasmania. The discrimination of the cryptic species of the *G. scutellatus* complex enables improvements in the management of the pest species in terms of biological control and plantation practices. Our study highlights the critical importance of proper taxonomic studies underpinning biocontrol programs.

Key words cytochrome oxidase I (COI), Eucalyptus Weevil, genital structure, mtDNA, plantation forestry.

INTRODUCTION

Gonipterus scutellatus Gyllenhal, 1833 generally known as Eucalyptus Weevil or Eucalyptus Snout-beetle, belongs to the Australo-Pacific weevil tribe Gonipterini (Coleoptera: Curculionidae). The genus *Gonipterus* Schoenherr. currently contains about 20 described species, most of them occurring in eastern Australia, from Tasmania (TAS) north into Queensland (QLD), and only a few in Western Australia (WA). Eucalyptus Weevils, variously referred to as *G. scutellatus* in the literature, have been accidentally introduced in New Zealand (1890), Africa (1916), South America (1925), Europe (1975) and North America (1994), where they spread rapidly and from where they also apparently colonised islands in the Atlantic, Indian and Pacific Oceans. In all these areas outside of their native range, they cause severe damage to *Eucalyptus* trees (Myrtaceae), both adults and larvae feeding on leaves (Tooke 1953). Within their native distribution range, however, their numbers are thought to be controlled effectively by *Anaphes nitens* (Girault) (Hymenoptera: Mymaridae), a tiny wasp that parasitises their eggs (Tooke 1953). *Anaphes nitens* has therefore been introduced for biological control of Eucalyptus Weevil in parts of the world where the weevils have become serious defoliators of eucalypt trees, with generally good but not always complete success (e.g. Clark 1931; Williams *et al*. 1952; Tooke 1953; Pinet 1986; Cordero Rivera *et al*. 1999; Hanks *et al*. 2000; Sanches 2000; Lanfranco & Dungey 2001).

In the 1990s, Eucalyptus Weevil was found to cause severe and extensive damage in plantations of Tasmanian Blue Gum (*Eucalyptus globulus*) in WA (Loch & Floyd 2001). Although *A. nitens* has been reared from its eggs in WA, the parasitoid is not as effective in controlling the weevil there as it is in the eastern states of Australia. Loch (2008) explored the possible reasons for this breakdown in biological control in WA and suggested that a seasonal mismatch of the life cycles of host and parasitoid was the most likely factor, but genital

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Fig. 1. Genital structures of *Gonipterus* species. (a)–(d) aedeagi; (e)–(l) mid-sections of aedeagi showing diagnostic internal sclerites, dorsal view. (a) *Gonipterus notographus* Boisduval, 1835 showing narrowly attenuated apex and long, composite internal sclerites protruding between anterior apodemes; dorsal view (Hobart, TAS); (b) *Gonipterus scutellatus* Gyllenhal, 1833 showing very broad, squarely truncate apex and small, composite internal sclerites at base of aedeagus; dorsal view (Steppes, TAS); (c) *Gonipterus* sp. n. 3, showing narrower but also squarely truncate apex and larger, sinuate internal sclerite; dorsal view (Tidbinbilla, ACT); (d) *Gonipterus* sp. n. 3, endophallus with sinuate internal sclerite extruded as during copulation; lateral view (Tidbinbilla, ACT); (e) *Gonipterus* sp. n. 4 (Rocks River Crossing, NSW); (f) *Gonipterus pulverulentus* Lea, 1897 (Tinderbox, TAS); (g) *Gonipterus platensis* (Marelli, 1926) (Albany, WA); (h) *Gonipterus balteatus* Pascoe, 1870 (Adjumbgilly, NSW); (i) *Gonipterus scutellatus* Gyllenhal, 1833 (Steppes, TAS); (j) *Gonipterus* sp. n. 1 (Blackwood Creek, TAS); (k) *Gonipterus* sp. n. 2 (Josephville, QLD); (l) *Gonipterus* sp. n. 3 (Bessiebelle, VIC). Scale bars 1 mm for Figs. (a)–(d), 0.5 mm for Figs. (e)–(l).

differences noted between specimens of Eucalyptus Weevil from WA and from south-eastern Australia suggested that uncertainty about the true identity of the weevil (R. Oberprieler pers. obs. 2007) was likely to confound the situation (Loch 2008). The origin and arrival of Eucalyptus Weevil in WA is unclear. The absence of old authentic records in museum collections in WA and elsewhere indicates that it is not native to WA but has been introduced there, yet no direct evidence is available of when and from where this may have occurred. Its sudden noticeable appearance and rapid expansion in the region suggested that it had been introduced in WA a short time prior to the early 1990s (Cunningham *et al*. 2005), but it may have been present in small numbers in native forests in WA for a longer time and increased dramatically only after *E. globulus* had been widely established in plantations there (Loch & Floyd 2001).

These issues raised serious questions about the precise identity of Eucalyptus Weevil in WA. The identification of Eucalyptus Weevil had been problematical from its first appearance in South Africa in 1916, where, after numerous different opinions by various experts of the time, its identity was finally settled as being *G. scutellatus* (Mally 1924; Tooke 1953). Several other species names were later synonymised with it (Wibmer & O'Brien 1986; Zimmerman 1994), including that of *G. gibberus* Boisduval, 1835 which had always been treated as a distinct species in South America, specifically so on differences in the genitalia (Vidal Sarmiento 1955; Rosado-Neto & Marques 1996). One of the authors of the present study (RGO) commenced taxonomic studies of the Gonipterini in Australia in 2003, which confirmed that differences in certain features of the male genitalia are indeed species-diagnostic in *Gonipterus*, specifically the structure of the complex sclerite(s) situated inside the aedeagus in repose and extruded during copulation (Fig. 1c,d) (R. Oberprieler unpubl. data). Study of the male genitalia of all described species of *Gonipterus* and of numerous other specimens revealed that *G. scutellatus* and a number of closely similar species can be distinguished from all others by having the apex of the aedeagus abruptly and squarely extended (Fig. 1b,c), not gradually attenuated as in the other species (Fig. 1a). Thus far 10 types of aedeagal sclerites can be distinguished in this group of species, most of which are currently impossible to distinguish on external characters. *G. scutellatus* was therefore indicated to comprise a complex of at least 10 largely cryptic species (Newete *et al*. 2011). A taxonomic revision of this complex is in preparation by one of the authors of the present study (RGO).

The purposes of this study are: (1) to examine whether these morphological differences have a genetic basis and whether the entities as identifiable on genital characters can be corroborated by molecular differences, i.e. whether *G. scutellatus* is a genetically homogeneous species with variable genital structure or a complex of genetically as well as morphologically distinct though externally cryptic species; (2) to establish the approximate distribution ranges of these entities in Australia and elsewhere; and (3) to determine the geographical origin of the population in WA. For this purpose we studied specimens collected from across the distribution range of the Eucalyptus Weevil in Australia and obtained sequences of three genes from them for phylogenetic analysis. We then studied the genitalia, specifically the internal sclerites of the aedeagus, of at least one sequenced male specimen from almost all sites.

MATERIALS AND METHODS

Study area and specimen sources

Specimens were collected from south-western WA, TAS and three regions in eastern Australia: south-eastern QLD/north-eastern New South Wales (NSW), south-eastern NSW/Australian Capital Territory (ACT) and south-western Victoria (VIC)/south-eastern South Australia (SA) (Table 1). Specimens were collected in plantations of *Eucalyptus globulus* (WA, VIC and SA), *E. nitens* (TAS), *E. dunnii* and *Corymbia variegata* (north-eastern NSW), *E. viminalis* (south-eastern NSW) and unidentified *Eucalyptus* spp. (QLD and south-eastern NSW), as well as on *Eucalyptus* spp. in native forests (TAS and ACT). A few *Gonipterus* specimens from South Africa, Spain and Portugal were also included in the analysis. All specimens were preserved in absolute ethanol. Their legs were used for the molecular analysis, and their bodies were retained in ethanol for morphological assessment. Additional dried specimens in museum collections, mainly the Australian National Insect Collection (ANIC) at CSIRO Ecosystem Sciences in Canberra, ACT, were studied to evaluate the genital differences against the genitalia of type and other authentically identified specimens of all described *Gonipterus* species. Critical type specimens were borrowed from the Naturhistoriska Riksmuseet in Stockholm, Sweden (NHRS), the Institut Royal des Sciences Naturelles de Belgique in Brussels, Belgium

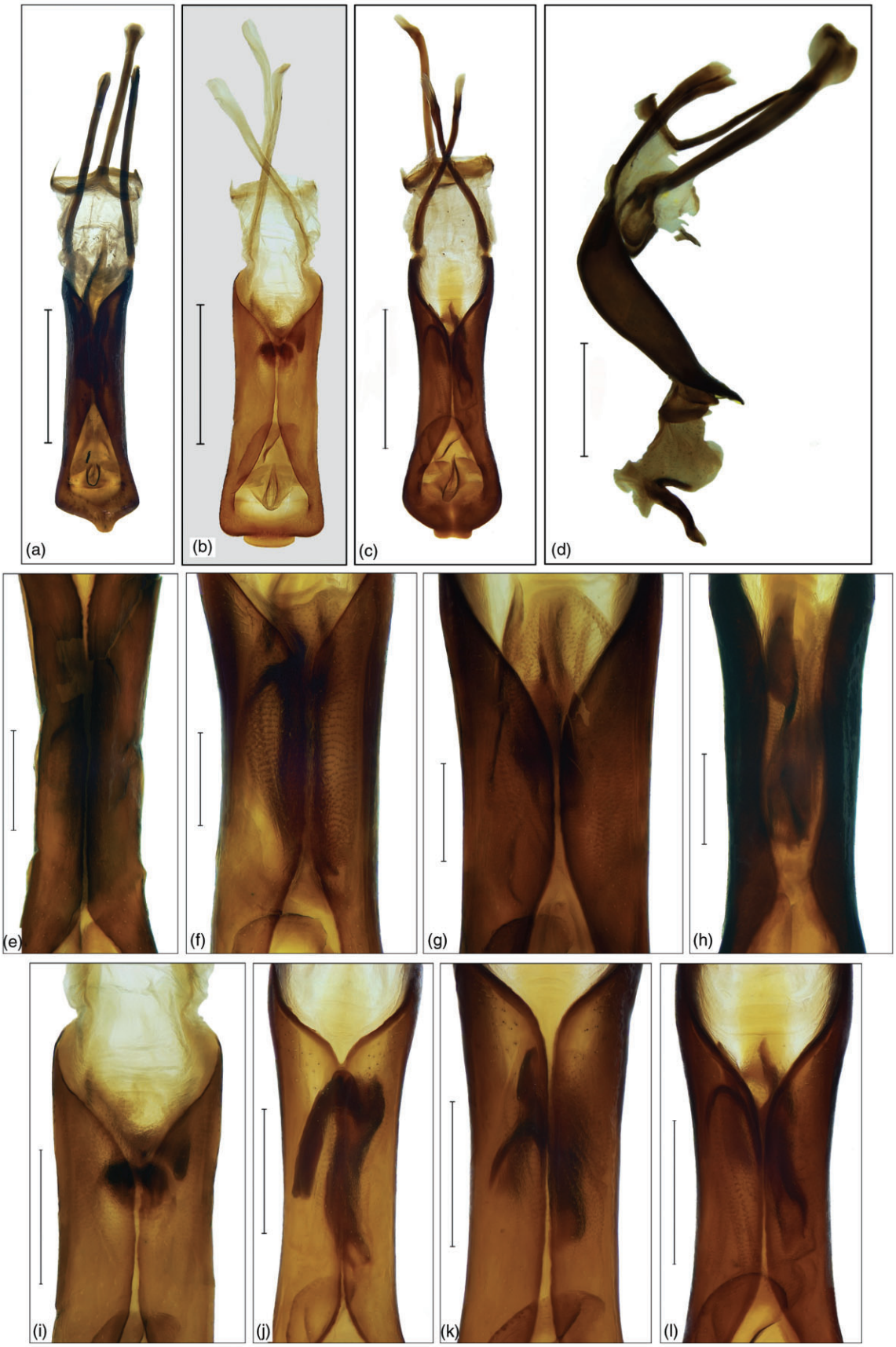

Table 1 Collection localities of *Gonipterus* specimens. Site numbers correspond to those in Figure 3 and haplotype numbers to those in Figure 2

Site no.	State	Location	Host	Lat °S	Long °E	Individuals analysed†	Haplotypes‡
1	WA	67 km NW of Frankland	*E. globulus*	34° 04′	116° 32′	2 (42)	**co1, co67§**
2	WA	Avery plantation	*E. globulus*	33° 33′	116° 32′	1 (16)	**co1**
3	WA	Barbour plantation	*E. globulus*	33° 32′	116° 25′	6 (63)	**co1**
4	WA	Black plantation	*E. globulus*	34° 51′	118° 05′	4 (17)	**co66§**
5	WA	Brickhouse Jones plantation	*E. globulus*	34° 20′	117° 16′	1 (40)	**co1**
6	WA	Cheyne plantation	*E. globulus*	34° 51′	118° 21′	22 (50+)	**co1**
7	WA	Forest Hill plantation	*E. globulus*	34° 37′	117° 25′	1 (6)	**co1**
8	WA	Guthrie plantation	*E. globulus*	35° 05′	117° 01′	4 (4)	**co1**
9	WA	ITC seed orchard	*E. globulus*	34° 56′	117° 48′	3 (56)	**co1**
10	WA	Karri Downs plantation	*E. globulus*	34° 34′	116° 20′	1 (2)	**co1**
11	WA	Kingscliff plantation	*E. globulus*	34° 39′	118° 16′	1 (45)	**co1**
12	WA	McIntosh plantation	*E. globulus*	34° 32′	117° 10′	1 (36)	**co1**
13	WA	Millinup plantation	*E. globulus*	34° 41′	117° 58′	2 (38)	**co1**
14	WA	Moltoni plantation	*E. globulus*	34° 18′	116° 04′	1 (40)	**co1**
15	WA	Moir plantation	*E. globulus*	34° 47′	117° 41′	1 (38)	**co1**
16	WA	Rocky Gully plantation	*E. globulus*	34° 31′	117° 04′	2 (49)	**co1**
17	WA	South Sister plantation	*E. globulus*	34° 48′	118° 09′	2 (9)	**co1**
18	WA	Sherwood Springs plantation	*E. globulus*	33° 30′	116° 06′	3 (5)	**co31**
19	VIC	Basil plantation	*E. globulus*	38° 09′	141° 59′	1 (12)	**co41**
20	VIC	Cleves plantation	*E. globulus*	37° 55′	141° 08′	4 (4)	**co17, co32(3)**
21	VIC	Dyson plantation	*E. globulus*	38° 09′	141° 59′	2 (8)	**co17, co32**
22	VIC	Freckelton plantation	*E. globulus*	38° 12′	142° 00′	44 (44)	**co17(24), co25, co26, co32(13), co35(5)**
23	VIC	Leaura plantation	*E. globulus*	38° 18′	142° 04′	1 (3)	**co17**
24	VIC	Linsay plantation	*E. globulus*	38° 10′	141° 51′	2 (3)	**co40**
25	VIC	Riordan plantation	*E. globulus*	38° 18′	142° 04′	2 (8)	**co17**
26	VIC	Stephens plantation	*E. globulus*	37° 54′	141° 51′	2 (2)	**co17**
27	VIC	The Gums plantation	*E. globulus*	38° 10′	141° 59′	2 (3)	**co17**
28	VIC	Torrone plantation	*E. globulus*	38° 14′	142° 12′	2 (3)	**co32**
29	TAS	Cradoc	*E.amygdalina*	43° 06′	147° 02′	1 (8)	**co46**
30	TAS	Dunrobbin Rd	*E. pulchella*	42° 31′	146° 09′	11 (52)	**co47-49, co50(4), co51-54**
			E. amygdalina			2 (7)	**co61, co62**
			E. ovata			1 (5)	**co10**
31	TAS	Eddys Rd	*E. nitens*	43° 03′	146° 47′	12 (25)	**co2, co9(2), co10(8), co55**
32	TAS	Hobart Domain	*E. viminalis*	42° 51′	147° 19′	1 (2)	**co8**
33	TAS	Hobart Sandy Bay	*E. viminalis*	42° 54′	147° 20′	2 (8)	**co10(2)**
			E. pulchella			2 (6)	**co56, co57**
34	TAS	Karanja	*E. rubida*	42° 40′	146° 50′	1 (2)	**co7**
36	TAS	Liena	*E. viminalis*	41° 33′	146° 14′	1 (1)	**co59**
37	TAS	Mayfield	*E. pulchella*	42° 14′	148° 01′	1 (10)	**co11**
			E. viminalis			1 (10)	**co11**
38	TAS	Moina	*E. dalrympleana*	41° 29′	146° 04′	1 (2)	**co11**
39	TAS	New Haven Rd	*E. amygdalina*	40° 58′	145° 27′	1 (1)	**co60**
40	TAS	Nunamarra	*E. pulchella*	41° 23′	147° 18′	1 (14)	**co45**
41	TAS	Oigles Rd	*E. nitens*	43° 10′	146° 52′	2 (2)	**co2, co3**
42	TAS	Tinderbox	*E. caudata*	43° 02′	147° 20′	16 (67)	**co6, co9, co10(13), co11**
43	TAS	Wayatinah	*E. amygdalina*	42° 23′	146° 31′	1 (2)	**co5**
44	TAS	near Kerevie	*E. ovata*	42° 46′	147° 48′	1 (1)	**co65**
45	SA	Kymhooper plantation	*E. globulus*	37° 23′	140° 37′	2 (4)	**co17**
46	QLD	Gelita plantation	*Eucalyptus* spp.	28° 01′	152° 55′	9 (11)	**co21(2), co22-24, co30**
47	SE-NSW	Buccleuch SF	*Eucalyptus* sp.	35° 09′	148° 41′	1 (5)	**co44**
48	SE-NSW	Coolangubra SF	*E. viminalis*	36° 53′	149° 24′	4 (18)	**co13(2), co14, co16**
49	NE-NSW	Coombes plantation	*E. dunnii*	31° 39′	152° 25′	2 (3)	**co18, co32**
50	NE-NSW	Crabtree plantation	*E. dunnii*	30° 08′	153° 06′	3 (32)	**co19, co33, co34**
51	NE-NSW	Dyraaba Station plantation	*E. dunnii*	29° 48′	152° 50′	7 (31)	**co4, co17, co20(3), co21, co27**
52	NE-NSW	Frost plantation	*E. dunnii*	30° 07′	152° 37′	13 (26)	**co19(4), co36(2), co37(3), co38, co39(3)**
53	NE-NSW	Gibson plantation	*E. dunnii*	31° 44′	152° 03′	6 (16)	**co18(3), co29, co42, co43**
54	NE-NSW	Grafton Ag station	*E. dunnii*	29° 37′	152° 57′	1 (1)	**co15**
55	NE-NSW	Morrow plantation	*C. variegata*	28° 44′	153° 26′	3 (37)	**co63, co64(2)**
56	NE-NSW	Mulcahy plantation	*E. dunnii*	28° 37′	152° 28′	1 (18)	**co38**
57	ACT	Tidbinbilla	*Eucalyptus* sp.	35° 28′	148° 54′	4 (4)	**co12, co13(2), co28**

†Number of specimens collected in parentheses. ‡Number of specimens in parentheses when more than one haplotype sequenced from a site. §*Oxyops* samples.

(IRSNB) and the Royal Museum for Central Africa in Tervuren, Belgium (RMCA).

Morphological study and species identification

For morphological discrimination of species and identification of specimens, sequenced and other specimens were dissected and their genitalia cleared for study. Rosado-Neto and Marques (1996) described and illustrated a number of differences in male and female genitalia between the two *Gonipterus* species recorded from South America, but examination of long series of all described *Gonipterus* species (R. Oberprieler unpubl. data) revealed that only the structure of the internal sclerite(s) of the aedeagus in the males varies distinctively and consistently between the species, whereas differences in the female genitalia are too subtle and variable to permit discrimination of the species. Therefore, and because reliable association of the sexes on external features is mostly impossible in the *G. scutellatus* complex, only males were used for morphological assessment of the samples analysed in this study. More than 100 male specimens were dissected from the samples collected at the 56 sites listed in Table 1, in many cases several specimens per sample. A few samples included only females and could therefore not be used for morphological assessment of the specimens.

Genitalia were prepared for study in the standard manner, by macerating the entire abdomen of the specimen in a warm 10% solution of potassium hydroxide, extracting and rinsing the aedeagus in 80% ethanol and studying and photographing it in temporary storage in glycerine. Photographs of the aedeagi were compiled using a Leica M205C stereo microscope, a Leica DFC500 digital camera and the Leica Application Software that montages images taken at different focus levels.

For identification of the species, authentically identified male specimens of all described species of *Gonipterus*, as housed in the ANIC, and of critical type specimens held in other collections were examined and, where necessary, dissected. Holotypes were studied of *G. scutellatus* (NHRS) as well as of *G. exaratus* Fåhraeus, 1840; *G. gibberus* Boisduval, 1835 and *G. notographus* Boisduval (IRSNB), whose names had been synonymised with that of *G. scutellatus* by Zimmerman (1994), and a syntype of *Dacnirotatus platensis* Marelli, 1926 (RMCA), whose name had been synonymised with *gibberus* by Marshall (1927) and with *scutellatus* by Wibmer and O'Brien (1986).

DNA extraction, PCR amplification and sequencing

Of each specimen, legs were cut off, frozen in liquid nitrogen and ground to a fine powder. DNA was extracted in hexadecyl trimethyl ammonium bromide (CTAB) according to the protocol of Graham *et al*. (1994), modified by the addition of 100 $\mu g/mL^{-1}$ proteinase K and 100 $\mu g/mL^{-1}$ RNAse A to the extraction buffer. Extracted DNA was stored at –20°C.

Genes sequenced consisted of a 1.2 kbp fragment of the 18S gene of rDNA, a 530 bp fragment of the cytochrome oxidase I (COI) gene of mtDNA and a 541 bp fragment of the elongation factor-1α (EF-1α) gene of nuclear DNA. Primers used for amplification of these regions are listed in Table 2. Polymerase chain reaction (PCR) was performed using GeneAmp PCR System 2700 Thermal Sequencer (Applied Biosystems, Australia). Each 25 mL reaction mixture contained 1 × PCR polymerisation buffer (67 mM Tris–HCl, 16.6 mM ammonium sulphate, 0.45% Triton X-100, 0.2 mg/mL^{-1}, gelatine 0.2 mM of each dNTPs) (Fisher Biotech, Perth, Australia), 25 mM $MgCl_2$ (Fisher Biotech), 0.6 pmol of each primer

Table 2 Primers used for amplification and sequencing

Primer name	Direction	Region	Location of 3′ end†	Reference	Sequence (5′ – 3′)
Starsky	F	EF-1α	0	(Cho *et al*. 1995)	CAC ATY AAC ATT GTC GTS ATY GG
Luke	R	EF-1α	541	(Cho *et al*. 1995)	CAT RTT GTC KCC GTG CCA KCC
F420	F	18S rDNA	420	(Sequeira *et al*. 2000)	GGC GAC GCA TCT TTC AAA TGT CTG
R1626	R	18S rDNA	1626	(Sequeira *et al*. 2000)	GGC ATC ACA GAC CTG TTA TTG CTC AAT CTC
C1-J-2183 (Jerry) (CJ)	F	COI	2183	(Simon *et al*. 1994)	CAA CAT TTA TTT TGA TTT TTT GG
C1-N-2659c (CN)	R	COI	2659	(Laffin *et al*. 2005)	ACT AAT CCT GTG AAT AAA GG
TL2-N-3014 (PAT)	R	COI	3014	(Simon *et al*. 1994)	TCC AAT GCA CTA ATC TGC CAT ATT A
Ron	F	COI	1751	(Simon *et al*. 1994)	GGA TCA CCT GAT ATA GCA TTC CC
Mila	R	COI	2659	(Simon *et al*. 1994)	GCT AAT CCA GTG AAT AAT GG
K698	F	COI	1460	(Simon *et al*. 1994)	TAC AAT TTA TCG CCT AAA CTT CAG CC
K741 1999	R	COI	2578	(Caterino & Sperling 1999)	TGG AAA TGT GCA ACT ACA TAA TA
GON-F	F	COI	2215	This study	GGA GTA CTC GGG ATA ATT TAC G
GON-R	R	COI	2194	This study	CCG ATT GAG GAA ATA GCG T
GON-MF	F	COI	2468	This study	GAG GAT TAA CTG GTG TAG TAT TAG
GON-MR	R	COI	2447	This study	GCT AAT ACT ACA CCA GTT AAT CC

†Positions are relative to *Drosophila yakuba* for mtDNA (Simon *et al*. 1994) and *Heliothodes diminutivus* (Cho *et al*. 1995) for EF-1α and *Tenebrio molitor* sequence for 18S (GenBankX07810).

COI, cytochrome oxidase 1; EF-1α, elongation factor-1-alpha; 18s rDNA, 18S ribosomal DNA.

(GeneWorks, Adelaide, Australia), approximately 5 ng DNA and 1 unit Taq DNA polymerase (Fisher Biotech). The PCR thermal cycling program was as follows: initial denaturation for 2 min at 95°C, followed by 40 cycles of denaturation for 30 s at 94°C, 30 s at the annealing temperature and two extensions for 2 and 7 min at 72°C.

Products obtained from PCR amplification were visualised on agarose gels to verify fragment sizes and purified with Ultrabind®DNA purification kit (MO BIO Laboratories, Solana Beach, California, USA). Amplicons were sequenced at the WA State Agricultural Biotechnology Centre at Murdoch University using an ABI Prism 377 DNA sequencer or by Macrogen Inc. (http://www.macrogen.com/eng/macrogen).

Phylogenetic analysis

The COI alignment did not include any gaps or indels. Non-informative characters were removed prior to analysis, and characters were unweighted and unordered. The COI data set was trimmed from 530 bp to 417 bp so that it commenced with the first codon of the COI fragment, as set out by Howland and Hewitt (1995). A species from the closely related genus *Oxyops* Schoenherr, 1826 (*O. pictipennis* Blackburn, 1894) was included in the analysis, and a species of the cryptopline genus *Haplonyx* was used as outgroup taxon. The sister-group of the Gonipterini is as yet unclear, but the tribe is currently classified in the subfamily Curculioninae (Oberprieler *et al*. 2007; Oberprieler 2010), which also contains the tribe Cryptoplini.

All sequence data were included in the initial analysis. Haplotypes were identified and coded (resulting in haplotypes numbered co1–co67). A single representative of each haplotype was utilised in the subsequent analyses. Only single specimens were available for *G. scutellatus* and *G. balteatus*. Parsimony analysis was performed using Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b10 (Swofford 2003). The most parsimonious trees were obtained using heuristic searches with random stepwise addition in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-descent option off. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis & Huelsenbeck 1992). Branch and branch node support (BS) was determined using 1000 bootstrap replicates (Felsenstein 1985).

Bayesian analysis was conducted on the same aligned data set. MrModeltest v2.2 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v3.1 (Ronquist & Huelsenbeck 2003). The Markov Chain Monte Carlo (MCMC) analysis of four chains started from random tree topology and lasted for 10 000 000 generations. Trees were saved after each 1000 generations, resulting in 10 000 saved trees. Burn-in was set at 500 000 generations, after which the likelihood values were stationary, leaving 9950 trees, and posterior probabilities (PP) were then calculated. PAUP* 4.0b10 was used to reconstruct the consensus tree, and maximum posterior probability was assigned to branches after a 50% majority rule consensus tree was constructed from the 9950 sampled trees.

The 18S gene of rDNA did not vary among the specimens of *Gonipterus* sequenced (TreeBASE 11783); it thus provided no phylogenetically useful information and was not analysed. Amplification of the EF1-α gene region was inconsistent, and the resultant data set was incomplete (TreeBASE 11783). Although this gene region distinguished *Oxyops* from *Gonipterus*, it did not resolve known species of *Gonipterus*, and was therefore also excluded from further analysis.

RESULTS

Morphological assessment and species identification

Among the genitalia of the set of *Gonipterus* males as dissected from the samples in this study, 10 clearly different types of aedeagal sclerites were recognisable (Fig. 1a,e–l). The aedeagi of eight of them possessed a squarely protruding apex (Fig. 1b,c), thus representing species of the *G. scutellatus* complex, whereas the aedeagal apex of the other two was narrowly attenuated (Fig. 1a). Comparison of these 10 aedeagal types with the aedeagi of all described species of *Gonipterus*, including critical type specimens as detailed above, revealed that five of them could be associated with described species, while the other five represented undescribed species. Four of the eight species of the *G. scutellatus* complex proved to be described, as *G. balteatus* Pascoe, 1870; *G. platensis* (Marelli, 1926), *G. pulverulentus* Lea, 1897 and *G. scutellatus* Gyllenhal, 1833 the four undescribed species here are coded as *Gonipterus* sp. n. 1–4. Of the remaining two aedeagal types, one could be associated with *G. notographus* Boisduval, 1835 whose purported conspecificity with *G. scutellatus* (Zimmerman 1994) thus proved to be incorrect, while the other species was named *Gonipterus* sp. n. 5. On the basis of the aedeagal features of the holotypes of *G. exaratus* and *G. gibberus*, these two species do not belong to the *G. scutellatus* complex either and are thus also not conspecific with *G. scutellatus*; the species regarded as *G. gibberus* in South America (e.g. by Rosado-Neto & Marques 1996) proved to be *G. pulverulentus*. The two remaining types of aedeagi with a square apex found thus far were not represented in the material examined in this study; one of them represents *G. geminatus* Lea, 1897 and the other another undescribed species. Details of the taxonomic and nomenclatural changes resulting from this study will be published in a pending revision of the *G. scutellatus* complex.

Phylogenetic analysis

COI amplification was successful for 237 specimens and yielded 67 unique haplotypes. The aligned data set consisted of 417 characters, 138 of which were parsimony-informative. Initial heuristic searches of unweighted characters in PAUP resulted in >1000 most parsimonious trees, 472 steps long

(CI = 0.43, RI = 0.86, g1 = –0.35) (TreeBASE 11783). Due to the high level of homoplasy (0.57) in the data set, a Bayesian analysis based on a substitution model was deemed to be a more suitable method. Four models returned equivalent likelihoods: the HKY substitution model, HKY with the proportion of invariable site (I) parameter, the general time reversible (GTR) substitution model with gamma (G) parameter, and finally GTR+G+I. Each substitution model produced trees with consistent topology, and only the tree resulting from the GTR+G analysis is presented here (Fig. 2).

The analysis resolved nine strongly supported terminal clades, with two additional lineages represented by single specimens but still clearly distinct (Fig. 2). Of these 11 lineages, 10 corresponded well to the 10 species recognised on genital differences (the 11th representing the related genus *Oxyops*) (TreeBASE 11783). Three of the terminal clades corresponded to the described species *G. platensis*, *G. pulverulentus* and *G. notographus* and the other four to the undescribed *Gonipterus* sp. n. 1–5, while the two lineages based on single specimens corresponded to *G. balteatus* and *G. scutellatus*. The eight species of the *G. scutellatus* complex formed a well supported clade (PP = 0.95, BS = 0.77), placed as sister-group of *G. notographus*. Six strongly supported terminal clades (species) were resolved within the *G. scutellatus* complex, with some clades showing considerable haplotype (intraspecific) variation. *Gonipterus* sp. n. 4 was placed as sister taxon of the other seven species, which together formed a strongly supported clade (PP = 0.97, BS = 0.77). Within the latter, *G. platensis* and *G. pulverulentus* formed a closely related species pair (PP = 0.90, BS = 0.80) placed as sister-group of the remaining five species, which formed a moderately supported clade (PP = 0.68, BS = 0.85). In this, *G. scutellatus* placed as sister taxon of a clade containing *Gonipterus* sp. n. 1–3 (PP = 1.00, BS = 0.85), with *Gonipterus* sp. n. 2 and 3 forming a species pair though less strongly supported (PP = 0.55, BS = 0.64) than suggested by the similarity of their genitalia (Fig. 1k,l).

All specimens sequenced of *G. pulverulentus, G. scutellatus* and *Gonipterus* sp. n. 1 were from TAS, while *G. platensis* specimens were from TAS, WA, Spain and Portugal. In contrast, those of *Gonipterus* sp. n. 2 and sp. n. 3 were from large areas in mainland south-eastern Australia (excluding TAS) and also showed high variation in COI haplotypes, 10 haplotypes recorded from 43 specimens in *Gonipterus* sp. n. 3 and 19 from 61 specimens in *Gonipterus* sp. n. 2. Two additional haplotypes of *Gonipterus* sp. n. 2 were found in WA and South Africa.

Relationship between COI haplotypes and geographical location (Fig. 3)

South-western WA (Fig. 3a)

Gonipterus platensis was widely distributed within *E. globulus* plantations throughout WA. All specimens share the same haplotype (co1). *Gonipterus* sp. n. 2 was collected from one of the more northerly *E. globulus* plantations.

South-eastern QLD/north-eastern NSW (Fig. 3b: top half)

In the plantations in this region, *G. pulverulentus*, *Gonipterus* sp. n. 2, *Gonipterus* sp. n. 3, *Gonipterus* sp. n. 4 and *Gonipterus* sp. n. 5 were collected, the first four on *Eucalyptus dunnii* in plantations in NSW and the last on *Corymbia variegata* in a plantation in north-eastern NSW. *Gonipterus* sp. n. 2 was also collected on unidentified *Eucalyptus* species in plantations in QLD.

South-eastern NSW/ACT (Fig. 3b, bottom half)

Gonipterus balteatus and *Gonipterus* sp. n. 2 were found in this region, on unidentified species of *Eucalyptus* in plantations as well as in native forest and on *E. viminalis* in a plantation. *Gonipterus* sp. n. 3 is also known from the region, but no specimens were included in the molecular analysis.

South-western VIC/south-eastern SA (the Green Triangle) (Fig. 3c)

All specimens were collected on *E. globulus* in plantations and were *Gonipterus* sp. n. 2 and *G*. sp. n. 3. The former was found in eight of the eleven plantations sampled in this region and the latter in six, while both species were found together in three plantations.

TAS (Fig. 3d)

G. scutellatus, *G. pulverulentus*, *G. platensis*, *G. notographus* and *Gonipterus* sp. n. 1 were collected in TAS. Specimens of *G. notographus* were collected mostly on *E. amygdalina* and *E. pulchella* (of the subgenus *Eucalyptus*) in native forests, with two records on *E. nitens* in plantations. In contrast, the other *Gonipterus* species were collected mostly on *Eucalyptus* species of the subgenus *Symphyomyrtus* (*E. nitens* in plantations and *E. caudata*, *E. dalrympleana*, *E. ovata*, *E. viminalis* and *E. rubida* in native forests), with the exception of one record of *G. pulverulentus* on *E. amygdalina*. Seventeen COI haplotypes from 21 specimens were found in *G. notographus*, and five COI haplotypes from 32 specimens in *Gonipterus* sp. n. 1.

DISCUSSION

The *G. scutellatus* species complex

Analysis of the mitochondrial COI gene and the male genitalia of this set of *Gonipterus* specimens confirmed that differences in the aedeagal sclerites as detected by Vidal Sarmiento (1955) and Rosado-Neto and Marques (1996) in the two species of *Gonipterus* in South America and identified in other species in Australia (R. Oberprieler pers. obs. 2007–2011) are: (1) consistently distinct in a larger set of specimens from a larger geographical range; and (2) congruent with well supported terminal clades of COI haplotypes. Thus, the 10 types of aedeagal sclerites identified in this set of specimens have a genetic basis and therefore represent 10 distinct taxonomic (and evolutionary) entities, which, although largely

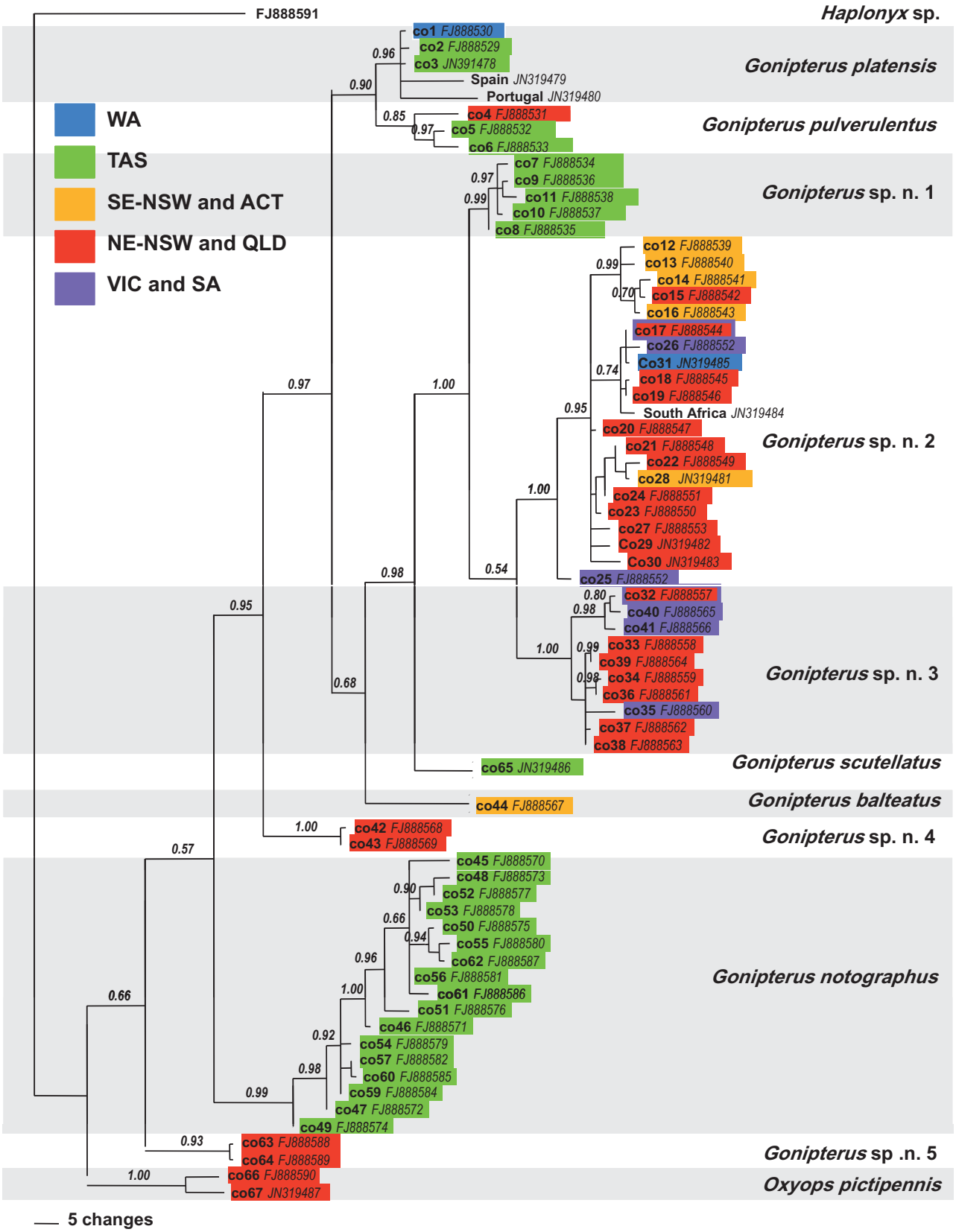

Fig. 2. Bayesian inference tree of COI sequences showing hypothesised phylogenetic relationships between species in the *Gonipterus scutellatus* complex and two related species *G. notographus* and *Gonipterus* sp. n. 5. Numbers above branches represent Bayesian posterior probability. COI haplotypes are colour-coded according to their region of origin in Australia (for specific locations see Table 1). The *Haplonyx* sp. was used as outgroup taxon.

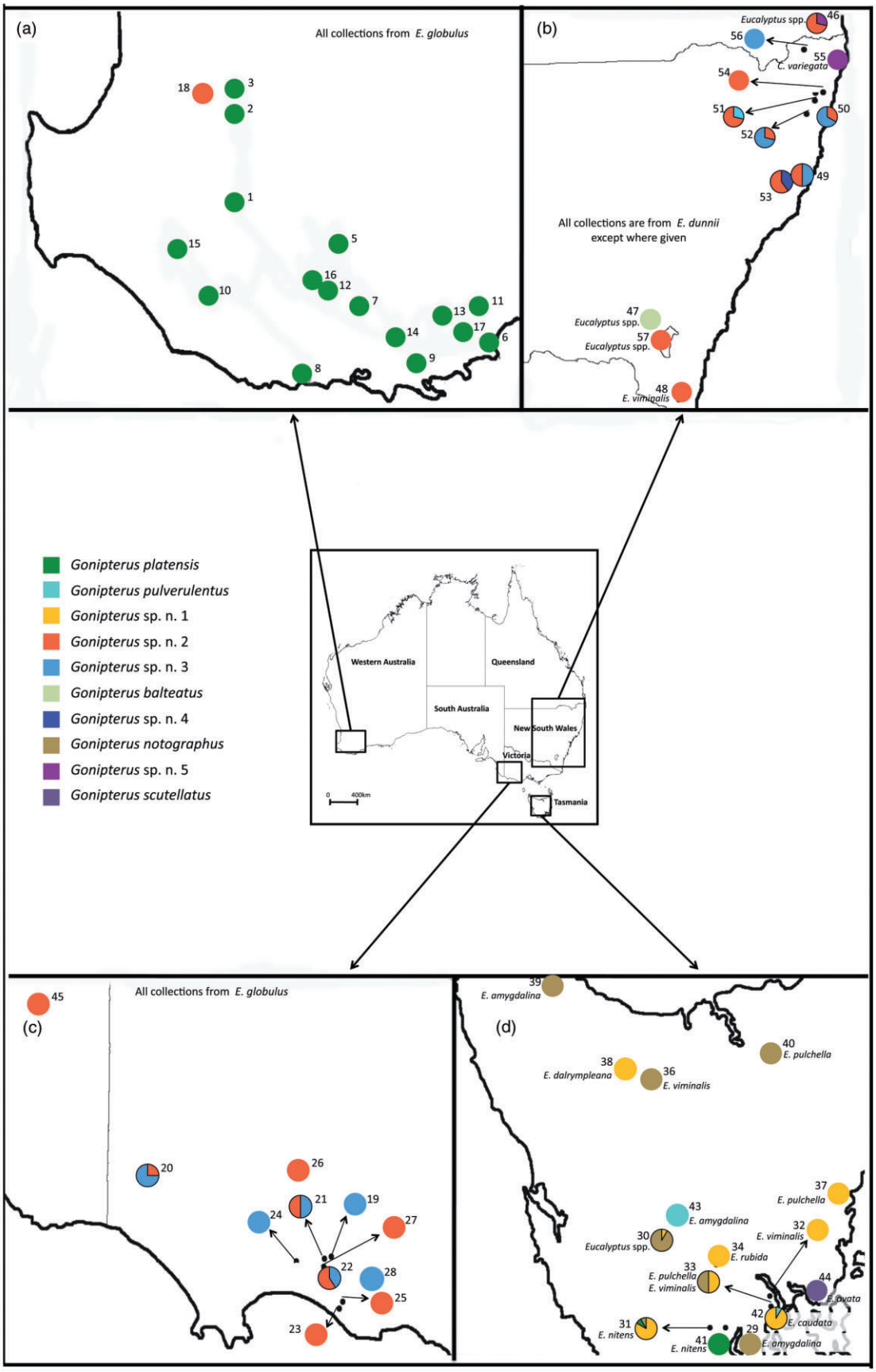

Fig. 3. Frequency of sequenced specimens of *Gonipterus* species at each collection site within Australia; (a) WA (b) QLD, NSW and ACT (c) VIC and SA (the Green Triangle) (d) TAS. Site numbers correspond to those in Table 1.

indistinguishable externally, nonetheless are distinct morphologically as well as in COI sequences. As in the molecular-phylogenetic study of amorphocerine cycad weevils (Downie *et al*. 2008), the molecular data here support the validity of species recognised on morphological differences, albeit subtle ones manifested largely in the male genitalia. A group of eight of these *Gonipterus* species, sharing a similar aedeagus and forming a well-supported clade on their COI haplotypes, includes *G. scutellatus* and several others treated previously under the same name in the literature. Thus, *Gonipterus 'scutellatus'* in the traditional sense constitutes a complex of at least 10 largely cryptic species (two not included in the COI analysis but identifiable on genitalia, and possibly others existing). Although several species names have been associated with *G. scutellatus* in the past, only five of these 10 species proved to have been described.

The *Gonipterus* species in WA

Gonipterus platensis was noticed first in large numbers in plantations of *Eucalyptus globulus* in WA in the early 1990s (Loch & Floyd 2001). By 2005, it was found throughout the geographical extent of *E. globulus* plantations in south-western WA (M. Matsuki pers. obs. 2005). We collected specimens throughout this extent of plantations and found only one COI haplotype among 51 specimens sequenced from 16 sites in WA (Table 1, Fig. 2). This lack of haplotype diversity in *G. platensis* in WA is in strong contrast with other *Gonipterus* species in south-eastern Australia, where multiple COI haplotypes were found in specimens of *Gonipterus* sp. n. 1, *Gonipterus* sp. n. 2 and *Gonipterus* sp. n. 3 at single locations.

The observed lack of diversity of COI haplotypes in *G. platensis* in WA can be the result of a founder effect or a bottleneck (Nei *et al*. 1975). Of these two possibilities, the founder effect due to the introduction of *G. platensis* to WA is more likely than a bottleneck in the recent past. All other Australian specimens of *G. platensis* assessed in this study are from TAS, but unfortunately the COI haplotype occurring in WA was not found among them, and therefore the origin of *G. platensis* in WA cannot be determined with certainty at this stage. However, all additional Australian specimens of *G. platensis* in the ANIC as studied are also only from TAS, and it therefore appears that this species is naturally endemic to TAS and that the population in WA is most likely to have been introduced from there. Additionally, its common host in WA, *Eucalyptus globulus*, is endemic to TAS and southern VIC but has been introduced in many parts of the world, often with associated pests and diseases (Burgess & Wingfield 2002). Similarly, *G. platensis* has been introduced accidentally into New Zealand, southern South America (Argentina, Brazil, Chile), western North America (California, Hawaii) and western Europe (Portugal, Spain) (R. Oberprieler unpubl. data).

In 2008, *Gonipterus* sp. n. 2 was found in a plantation of *E. globulus* in south-western WA. Three individuals sequenced from this population all had the same COI haplotype (Table 1, Fig. 2). In 2010, many individuals of this species were found in plantations of *E. smithii* near the plantation of first discovery. Again, we did not find the haplotype of this population in any other specimen of *Gonipterus* sp. n. 2 as sequenced, but the haplotypes clustering together with it (Fig. 2) are mostly from VIC, suggesting that its origin lies in the Green Triangle. Like *G. platensis*, *Gonipterus* sp. n. 2 has been introduced in other countries, but in contrast to *G. platensis* only in Africa, France and Italy (Newete *et al*. 2011; R. Oberprieler unpubl. data).

As currently known, three other species of *Gonipterus* occur in WA, all evidently native and probably endemic to the region. *Gonipterus citrophagus* Lea, 1897 was described from the Swan River (Perth) feeding on citrus leaves (Lea 1897), but probably it occurs naturally on one or more WA species of *Eucalyptus*. It has been collected recently just north-west of the region with *E. globulus* plantations and has been found also in at least one plantation of *E. globulus*, the latter specimens mistakenly identified as *G. scutellatus* (M. Matsuki pers. obs. 2008). Available records suggest a natural distribution in the south of WA, from Perth across to the SA border. The other two species are undescribed and occur in the Geraldton-Kalbarri region further north, but little is known about them. These three species were not collected during this study and thus were unavailable for sequencing, but on the basis of genital characters none belongs to the *G. scutellatus* complex.

Identification and distribution of the species

Details of the species of the *G. scutellatus* complex will be published in the pending taxonomic revision, but we here present some further information on the species dealt with in this study so as to assist their recognition and treatment in other parts of the world. Identification of the *Gonipterus* species covered in this study on external characters is difficult at best. No reliable external morphological characters for distinguishing the species have been identified so far (R. Oberprieler pers. obs. 2007–2011) and, even if eventually found from careful study of long series of specimens, will probably be very subtle and difficult to use for routine identification for most of the species. However, live fresh specimens of at least *G. balteatus*, *G. platensis*, *G. pulverulentus*, *Gonipterus* sp. n. 1, *Gonipterus* sp. n. 2/3 and also *G. notographus* may be identified to species with reasonable certainty based on the pattern formed by the white scales and waxy covering on their thorax and elytra (M. Matsuki pers. obs. 2008). Unfortunately, the process of killing and preservation (pinned or in ethanol) tends to dissolve the wax and/or dislodge the scales, thus to obscure the colour pattern, so that this feature generally is not useful for pinned and otherwise preserved specimens. Old specimens in collections additionally tend to accumulate grease and dirt and are even more difficult to identify. Morphological identification of all species should therefore ultimately always include dissection and study of the male genitalia. Late-instar larvae may differ between at least some of the species (M. Matsuki pers. obs. 2008); however, neither such differences nor the association of different larvae with adults has been investigated in Australia.

From this study and that of numerous other specimens in collections (mainly the ANIC), a general distribution pattern

of the various species may be concluded. The collection records compiled in this study obviously present an incomplete picture of the distribution range of any of the species. In particular, the lack of records from eastern VIC and the mid-coast of NSW is due to a lack of sampling rather than representing discontinuous distributions. Due to the confused identities and cryptic nature of the species of the *G. scutellatus* complex, distribution and also host records in the literature and of specimens identified in collections are totally unreliable. Most species are quite common in collections, but in nearly all cases study of the male genitalia is necessary for accurate species identification and evaluation of given locality and/or host records.

G. scutellatus appears to be endemic to TAS and uncommon to rare, with only one recent (2008) collection record and a small number of older ones available thus far. Intensive search for this species at and around the recent collection site yielded no specimen (M. Matsuki pers obs. 2008). No specimen from any location outside of Australia studied was found to represent this species, and it evidently has not been introduced anywhere in the world.

Gonipterus platensis is the species confused most often with *G. scutellatus*, and, while evidently also native and naturally endemic to TAS, it is not very common there, all of the few records known to date emanating from the southern parts of TAS and recent targeted searches yielded few specimens (C. Valente, M. Matsuki, R. Oberprieler pers. obs. 2008). Outside of Australia this is, however, the most widely distributed species, occurring widely in New Zealand, eastern and western South America, south-western North America (California) and Western Europe (Portugal, western Spain) as well as on the Canary Islands and Hawaii. On recent evidence (Echeverri *et al*. 2007) it also appears to be present in South Africa.

Although represented in this study only from two locations in TAS and one in north-eastern NSW, *G. pulverulentus* is widespread in TAS (common along the east coast; Matsuki pers. obs.) as well as on the eastern Australian mainland from SA to southern QLD. It has been introduced only in eastern South America, where it occurs in Argentina, Brazil and Uruguay and is generally referred to as *G. gibberus* (which, however, is a different species not belonging to the *G. scutellatus* complex and not introduced in South America).

Gonipterus balteatus, represented in our study from only one site in south-eastern NSW, occurs from SA through VIC and NSW into southern QLD and has not been introduced elsewhere in the world.

Of the four undescribed species of the *G. scutellatus* complex, *Gonipterus* sp. n. 1 is found throughout the drier parts of south-eastern TAS and fairly common on *E. globulus* and *E. viminalis* (C. Valente, M. Matsuki, R. Oberprieler pers. obs. 2008). *Gonipterus* species appear to prefer dry sclerophyll forests, as searches in wet sclerophyll forests in TAS have not yielded specimens so far (V Patel & J Elek, pers. comm. 2008; M. Matsuki pers. obs. 2008).

Gonipterus sp. n. 2 was the most widely sampled species in our study, and it occurs from SA through VIC and NSW into southern QLD but evidently not in TAS. This is the species introduced almost a century ago in South Africa, from where it spread northwards along the eastern side of Africa and to St. Helena, Madagascar and Mauritius. It has also been introduced in Italy (Arzone 1976; Maltzeff & Colonnelli 1994) and southern France (Rabasse & Perrin 1979), its identity there confirmed by dissection of specimens both of original introductions (Menton in France) and of material collected recently in these countries (R. Oberprieler pers. obs. 2007–2011).

Gonipterus sp. n. 3 is closely related to *Gonipterus* sp. n. 2, both on genital and molecular characters, and is indistinguishable externally from it. It is indicated to occur from western VIC to northern NSW and to overlap with *Gonipterus* sp. n. 2 in its distribution range. No specimens from outside of Australia examined so far are referable to it, and it thus appears not to have been introduced in other parts of the world.

Gonipterus sp. n. 4 and sp. n. 5 thus far each are known only from a few specimens collected at single localities in northern NSW, the latter (not in the *scutellatus* complex) being the only one in our study not found on *Eucalyptus* but on the related genus *Corymbia*.

Gonipterus notographus is rather common and widespread in TAS and also occurs in higher-altitude regions of VIC and NSW. Its egg capsule is slightly smaller, on average, than that of other *Gonipterus* species in TAS (V Patel pers. obs. 2007–2008).

Implications for management and control of Eucalyptus Weevil

The results or our study allow correction of at least some of the identifications of the *Gonipterus* species subjected to recent studies in Australia. All studies of *G. 'scutellatus'* in WA (Loch & Floyd 2001; Cunningham *et al*. 2005; Loch 2005, 2006, 2008; Loch & Matsuki 2010) refer to *G. platensis*, while in TAS the main species in the oviposition studies of Clarke *et al*. (1998) is *G. notographus* (based on voucher specimens in the ANIC and on host preference), and also *G. 'scutellatus'* in the study of Dungey and Potts (2003) appears to be *G. notographus*. *Gonipterus 'scutellatus'* in Elliott and de Little (1984) probably encompasses all five *Gonipterus* species known from TAS; the photo of the adult in this publication is of *G. pulverulentus*. On the basis of the distribution range and a photo of adult, the *G. 'scutellatus'* in SA in Phillips (1996) is *Gonipterus* sp. n. 2.

Because *Gonipterus* '*scutellatus*' as treated in the literature comprises a complex of species and different species are introduced in various parts of the world, studies on host and climate preferences of Eucalyptus Weevil and on susceptibility of different eucalypt species to its attack as reported in the literature generally are compromised to outright misleading. For one, evidently none refers to the real *G. scutellatus*. In regions outside of Australia where, as far as known, only one species of *Gonipterus* has been introduced, such biological and ecological results generally can be attributed to the correct species, but in areas where more than one species are known or likely to occur, records must be treated with reservation. Thus, studies as conducted in WA (Loch & Floyd 2001; Loch 2006; Loch & Matsuki 2010), New Zealand (Clark 1931), Spain

(Cordero Rivera & Santolamazza Carbone 2000), Chile (Lanfranco & Dungey 2001; Huerta-Fuentes *et al*. 2008) and California (Paine & Millar 2002) all pertain to *G. platensis*, whereas those in southern Africa (Mally 1924; Tooke 1953; Tribe 2003) apply largely to the undescribed *Gonipterus* sp. n. 2. However, the suspected additional presence of *G. platensis* in South Africa (Echeverri *et al*. 2007) makes the results of studies in cooler regions such as Lesotho (Richardson & Meakins 1986) much more doubtful. A recent field and laboratory study of feeding and oviposition preferences of authentic *Gonipterus* sp. n. 2 in South Africa (Newete *et al*. 2011) showed the preferred host of this species to be *Eucalyptus smithii* rather than *E. globulus*, the preferred host species of *G. platensis*. The recent finding of *Gonipterus* sp. n. 2 on *E. smithii* near *E. globulus* plantations in WA (see discussion above) supports this apparent difference in host preference. However, in our study *Gonipterus* sp. n. 2 was also collected on *E. globulus* in parts of the Green Triangle and on *E. dunnii* in northern NSW and an unidentified *Eucalyptus* species in south-eastern QLD, where *E. smithii* does not occur. Studies of *Gonipterus* host preferences and of eucalypt susceptibility and resistance to attack by *Gonipterus* therefore have to ascertain the correct identity of the weevil species.

Our results have similar implications for the biological control of Eucalyptus Weevil. As Loch (2008) suspected, the failure of the egg-parasitoid *Anaphes nitens* to properly control the numbers of *G. platensis* in WA is indicated to result, at least partly, from a host-parasitoid mismatch. *Anaphes nitens* was collected originally in SA for importation to South Africa, despite the assumption that the *Gonipterus* species in South Africa had originated from TAS (Mally 1924; Tooke 1953; Tribe 2003). Once released, the wasp was so successful in controlling Eucalyptus Weevil in South Africa that a memorial was erected for it (Londt 1996). As it turns out, however, the success of this biological control effort is purely due to chance as the host weevil, *Gonipterus* sp. n. 2, is in fact native in the same region (south-eastern continental Australia) as the parasitoid. In other parts of the world where Eucalyptus Weevil had become a pest in eucalypt plantations, the importation of *A. nitens* from South Africa proved less successful. Generally this failure has been ascribed to a climatic effect, the wasps being unable to control the weevils effectively in spring when temperatures are low (Cordero Rivera *et al*. 1999; Sanches 2000). However, on the basis of the findings of the current study, it now appears that this failure of biocontrol is rooted at least partly in a mismatch between parasitoid and host, as the weevil in these areas, *G. platensis*, does not occur naturally in continental Australia. Two native Tasmanian species of *Anaphes*, *A. tasmaniae* Huber & Prinsloo and *A. inexpectatus* Huber & Prinsloo 1990, are now under trial in Portugal and show a similar cold tolerance as *G. platensis* and hence much greater potential of controlling it than *A. nitens* (Valente *et al*. 2010).

CONCLUSIONS

Our study provides an example of successful resolution of the confused and controversial composition of a group of economically important but taxonomically difficult (cryptic) insect species by a combination of morphological and molecular data. While genetic data allow crucial testing of morphological species concepts, they cannot resolve such situations on their own, without correlation with taxonomic and nomenclatural concepts (such as holotypes) that carry the names of species. On the basis of both molecular and morphological data, *Gonipterus* '*scutellatus*' comprises a monophyletic complex of at least eight species (two more identified on genital structure but not included in the molecular analysis) that differ diagnostically only in the aedeagal sclerite of the male genitalia, while external features (such as scale patterns) are of limited use in distinguishing some of the species. Only half of these species proved to be described, and three species (but not the real *G. scutellatus*) have become invasive in eucalypt plantations outside of Australia. These invasive species have now been identified as *G. platensis* (Marelli, 1926), *G. pulverulentus* Lea, 1897 and a third, undescribed species. The proper discrimination and identification of these various *Gonipterus* species has important implications both for forest management in Australia and for the biological control of the three introduced species in other countries, indicating in particular that only the undescribed species in Africa, Italy and France is a natural host for the egg parasitoid *Anaphes nitens*, which is used to control all of them. This century-old case of 'blind' biocontrol illustrates the need to base biocontrol programs on much more careful identification and, where necessary, taxonomic study of both target species and biocontrol agents.

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