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

Tendai S. Mapondera[1], Treena Burgess[1,*], Mamoru Matsuki[1,3], Rolf G. Oberprieler[2]

[1] *Cooperative Research Centre for Forestry and Industry Pest Management Group (IPMG), School of Biological Sciences and Biotechnology, Murdoch University, South St, Murdoch, Western Australia 6150, Australia.*

[2] *CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, Australian Capital Territory 2601, Australia.*

[3] *present address: Centre of Excellence in Natural Resource Management, University of Western Australia and Department of Agriculture and Food Western Australia, 444 Albany Highway, Albany, Western Australia 6330, Australia.*

[*]Corresponding author, email: tburgess@murdoch.edu.au

Tel: +61 8 9360 7537, Fax: +61 8 9360 6303

Abstract The Eucalyptus Weevil, generally referred to as *Gonipterus scutellatus* Gyllenhal, is a significant pest of *Eucalyptus* species in Africa, America, Europe and New Zealand. It has recently also 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 *Gonipterus scutellatus* to comprise a complex of cryptic species, obscuring the identity of the various pest populations of the weevil in the world. We examined (i) whether the apparent cryptic species identifiable on genital differences have a genetic basis, (ii) the distribution of these species and (iii) the origin of the population in Western Australia. We studied specimens from across the range of Eucalyptus Weevil in Australia and obtained sequences of three genes from them: cytochrome oxydase I mtDNA, elongation-factor 1-α nuclear DNA and 18s rDNA. The cladogram of COI haplotypes resolved ten 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, *G. platensis* (Marelli), *G. pulverulentus* Lea and *G. scutellatus* Gyllenhal. 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). 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 *Gonipterus 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 programmes.

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

INTRODUCTION

Gonipterus scutellatus Gyllenhal, generally known as Eucalyptus Weevil or Eucalyptus Snout-Beetle, belongs to the Australo-Pacific weevil tribe Gonipterini (Coleoptera: Curculionidae). The genus *Gonipterus* currently contains about 20 described species, most of them occurring in eastern Australia, from Tasmania north into Queensland, and only a few in Western Australia. 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 plantations, 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 Western Australia (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 differences noted between specimens of Eucalyptus Weevil from WA and from south-eastern Australia suggested that uncertainty about the true identity of the weevil (Oberprieler, personal observation) 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* was 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* Gyllenhal (Mally 1924; Tooke 1955). Several other species names were later synonymised with it (Wibmer & O'Brien 1986; Zimmerman 1994), including that of *G. gibberus* Boisduval, 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). Taxonomic studies of the Gonipterini commenced in Australia in 2003 by one of us (RGO) 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 (Figs. 1c–d) (Oberprieler, unpublished 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 (Figs. 1b–c), not gradually attenuated as in the other species (Fig. 1a), and that thus far ten types of aedeagal sclerites can be distinguished in this group of species, most of which are currently impossible to distinguish on external characters. *Gonipterus scutellatus* was therefore indicated to comprise a complex of at least ten largely cryptic species (Newete *et al*. 2011). A taxonomic revision of this complex is in progress (Oberprieler, in preparation).

The purpose of this study is (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 determine the approximate distribution ranges of these entities in Australia and elsewhere and (3) the geographical origin of the population in WA. For this purpose we studied specimens collected from across the 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, Tasmania (TAS) and three regions in eastern Australia: south-eastern Queensland (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 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.

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 & 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 (Oberprieler, unpublished 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* as well of *G. exaratus* Fåhraeus, *G. gibberus* Boisduval and *G. notographus* Boisduval, whose names had been synonymised with that of *G. scutellatus* by Zimmerman (1994), and a syntype of *Dacnirotatus platensis* Marelli, whose name had been synonymised with *gibberus* by Marshall (1927) and with *scutellatus* by Wibmer & 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 100µg/ml Proteinase K and 100µg/ml 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

25mL reaction mixture contained 1 × PCR polymerization buffer (67 mM Tris–HCl, 16.6 mM ammonium sulphate, 0.45 % Triton X-100, 0.2 mg/ml, gelatine 0.2 mM of each dNTPs) (Fisher Biotech, Perth, Australia), 25 mM $MgCl_2$ (Fisher Biotech), 0.6 pmol of each primer (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 minutes at 95°C, followed by 40 cycles of denaturation for 30 seconds at 94°C, 30 seconds at the annealing temperature and two extensions for 2 and 7 minutes 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 State Agricultural and 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 & Hewitt (1995). A species from the closely related genus *Oxyops* (*O. pictipennis* Blackburn) 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.

Parsimony analysis was performed using PAUP* version 4.0b10 (Swofford 2003). 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*, and their sequences were duplicated in the phylogenetic analyses to stabilise the position of the terminal clades. The most parsimonious trees were obtained by performing heuristic searches, as described previously (Jung & Burgess 2009).

Bayesian analysis was conducted on the same aligned dataset. 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 4 chains started from random tree topology and

lasted for 10 000 000 generations. Trees were saved after each 1 000 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 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 9 950 sampled trees.

The 18S gene of rDNA did not vary among the specimens of *Gonipterus* sequenced (TreeBASE 11783), thus provided no phylogenetically useful information and was not analysed. Amplification of the EF1-α gene region was inconsistent, and the resultant dataset was incomplete (TreeBASE 11783). Although, this gene region separated *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, ten clearly different types of aedeagal sclerites were recognisable (Figs. 1a, 1e–l). The aedeagi of eight of them possessed a squarely protruding apex (Figs. 1b–c), thus representing species of the *G. scutellatus* complex, while the aedeagal apex of the other two was narrowly attenuated (Fig. 1a). Comparison of these ten 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, *G. platensis* (Marelli), *G. pulverulentus* Lea and *G. scutellatus* Gyllenhal, the four undescribed species here named *Gonipterus* sp. n. 1–4. Of the remaining two aedeagal types, one could be associated with *G. notographus* Boisduval, whose purported conspecificity with *G. scutellatus* (Zimmerman 1994) thus proved to be incorrect, while the other species was named *Gonipterus* sp. n. 5. Examination of the holotypes of *G. exaratus* and *G. gibberus* showed that these two species do not belong to the *G. scutellatus* complex and are therefore also not conspecific with *G. scutellatus*, and further that the species in South America regarded as *G. gibberus* (*e.g.*, by Rosado-Neto & Marques 1996) is in fact *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 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 (Oberprieler, in preparation).

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, 487 steps long (C.I. = 0.43, R.I. = 0.86, g1 = –0.35) (TreeBASE 11783). Due to the high level of homoplasy 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 11 strongly supported terminal clades, ten of which corresponded well with the ten species recognised on genital differences (the 11th representing the related genus *Oxyops*) (TreeBASE 11783). Within *Gonipterus*, the eight species of the *G. scutellatus* complex formed a well supported clade placed as sister-group of *G. notographus*, with *Gonipterus* sp. n. 5 forming the sister-taxon of the *G. scutellatus* complex plus *G. notographus* (though with only moderate support).

Eight strongly supported terminal clades (species) were resolved within the *G. scutellatus* complex, although in some clades there was considerable haplotype (intraspecific) variation, and those of haplotypes co44 and co65 (corresponding to *G. balteatus* and *G. scutellatus*) were based on duplicated sequences of single specimens. Four of the terminal clades corresponded to the described species *G. balteatus, G platensis, G. pulverulentus* and *G. scutellatus* and the other four to the undescribed *Gonipterus* sp. n. 1–4. *Gonipterus* sp. n. 4 was placed as sister-taxon of the other seven species, which together formed a strongly supported clade. Within the latter, *G. platensis* and *G. pulverulentus* formed a closely related species pair placed as sister-group of the remaining five species, which formed a moderately supported clade. In this clade, *G. scutellatus* was strongly supported as sister-taxon of a clade containing *Gonipterus* sp. n. 1–3, with *Gonipterus* sp. n. 2 and 3 forming a species pair though less strongly supported than suggested by the similarity of their genitalia (Figs. 1k–l).

All specimens sequenced of *G. pulverulentus*, *G. scutellatus* and *Gonipterus* sp. n. 1 were from Tasmania, while *G. platensis* specimens were from Tasmania, 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 Tasmania) and also showed high variation in COI haplotypes, ten 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 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): *Gonipterus 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 species were collected mostly on 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 *Gonipterus 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 & Marques (1996) in the two species of *Gonipterus* in South America and identified in other species in Australia (Oberprieler, personal observation) are (i) also consistently distinct in a larger set of specimens from a larger geographical range and (ii) congruent with well supported terminal clades of COI haplotypes. This indicates that the ten types of aedeagal sclerites identified in this set of specimens have a genetic basis and therefore represent ten distinct taxonomic (and evolutionary) entities, which, although largely indistinguishable externally, are nonetheless morphologically as well as genetically distinct species. As in the molecular-phylogenetic study of amorphocerine cycad weevils (Downie *et al*. 2008), the molecular data here also fully 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 under the same name in the literature. *Gonipterus "scutellatus"* in the traditional sense is therefore confirmed to constitute a complex of at least ten largely cryptic species (two not included in the COI analysis but identifiable on genitalia, and possibly others existing). Even though several species names have been associated with *G. scutellatus* in the past, only five of these ten species proved to be described.

The *Gonipterus* species in WA

Gonipterus platensis was first noticed 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 (Matsuki, personal observation). 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 a recent past. All other Australian specimens of *G. platensis* assessed in this study were 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 this island and that the population in WA is most likely to have been introduced from there. Also 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 accidentally introduced in New Zealand, southern South America (Argentina, Brazil, Chile), western North America (California, Hawaii) and Europe (Italy, Portugal, Spain) (Oberprieler, unpublished data).

In 2008, *Gonipterus* sp. n. 2 was also 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, a large number of this species was 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 and France (Newete *et al*. 2011; Oberprieler, unpublished data).

As currently known, three other species of *Gonipterus* occur in WA, all evidently native and probably endemic to the region. *Gonipterus citrophagus* Lea was described from the Swan River (Perth) feeding on citrus leaves (Lea 1894), but it probably naturally occurs on one or more WA species of *Eucalyptus*. It has recently been collected just north-west of the region with *E. globulus* plantations but has also been found in at least one plantation of *E. globulus*, the latter specimens mistakenly identified as *G. scutellatus* (Matsuki, personal observation). Available records indicate that it occurs 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 unavailable for sequencing, but none of them belongs to the *G. scutellatus* complex on genital characters.

Identification and distribution of the species

Details of the species of the *G. scutellatus* complex will be published in a pending taxonomic revision (Oberprieler, in preparation), 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 (Oberprieler, personal observation) 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 of 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 (Matsuki, personal observation). 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 is generally 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. There are indications that late-instar larvae differ between at least some of the species (Matsuki, personal observation), but such differences and also the association of different larvae with adults have not 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 only 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 as well on 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.

Gonipterus 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 did not yield another specimen (Matsuki, personal observation). No specimen from any location outside of Australia studied was found to represent this species, and it has evidently not been introduced anywhere in the world.

The species most often confused with *G. scutellatus*, *G. platensis*, is evidently also native and naturally endemic to TAS and again not very common there, all few records known to date emanating from the southern parts of the island and recent searches yielding only few specimens (C. Valente, Oberprieler, Matsuki, personal observation). 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, Italy) 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 only represented in this study from two locations in TAS and one in north-eastern NSW, *G. pulverulentus* is widespread in TAS (common along the east coast; Matsuki, personal observation) 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, Oberprieler, Matsuki, personal observation). *Gonipterus* species appear to prefer dry sclerophyll forests, as searches in wet sclerophyll forests in TAS have not yielded specimens so far (V. Patel and J. Elek, personal communication; Matsuki, personal observation).

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 also to St. Helena, Madagascar and Mauritius. It has also been introduced in southern France (Rabasse & Perrin 1978), its identity there confirmed by dissection of specimens both of the original introduction (at Menton) and of material recently collected in the same region (Oberprieler, unpublished data).

Gonipterus sp. n. 3 is closely related to *Gonipterus* sp. n. 2, both on genital and molecular characters, and externally indistinguishable 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 are thus far each only known 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, finally, 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, personal observation).

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 & 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 are generally compromised to misleading. For one, it is evident that none of them refer 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 can generally be attributed to the correct species, but in areas where more than one species are known or likely to occur, they must be treated with reservation. Thus, studies as conducted in WA (Loch 2006; Loch & Floyd 2001; 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*, while those in southern Africa (Mally 1924; Tooke 1953; Tribe 2003) largely apply to the undescribed *Gonipterus* sp. n. 2. However, the suspected presence of *G. platensis* in South Africa as well (Echeverri *et al*. 2007) makes the results of studies in colder 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* as preferred by *G. platensis*. The recent finding of *Gonipterus* sp. n. 2 on *E. smithii* near *E. globulus* plantations in WA (see above) similarly suggests that these two *Gonipterus* species may have quite different host preferences, although 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 be at least partly the result of a host-parasitoid mismatch. *Anaphes nitens* was originally collected in South Australia for importation to South Africa, despite the assumption that the *Gonipterus* species in South Africa had originated from Tasmania (Mally 1924; Tooke 1953; Tribe 2003). Once released, the wasp was so successful in controlling Eucalyptus Weevil in South Africa that even 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. This has generally been ascribed to a climatic effect, the wasps not being able to effectively control the weevils in spring when temperatures are low (Cordero Riviera *et al*. 1999; Sanches 2000). However, it

now appears that this failure of biocontrol is at least partly rooted in a mismatch between parasitoid and host, as the weevil in these areas, *G. platensis*, does not naturally occur in continental Australia but only in Tasmania. Two native Tasmanian species of *Anaphes*, *A. tasmaniae* Huber & Prinsloo and *A. inexpectatus* Huber & Prinsloo, 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. Both the molecular and the morphological data reveal that *Gonipterus* "*scutellatus*" comprises a monophyletic complex of at least eight species (two more identified on genital morphology 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. Their identities could thus be clarified, two named as *G. platensis* (Marelli) and *G. pulverulentus* Lea but the third undescribed. 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 and France is a natural host for the egg parasitoid *Anaphes nitens*, which is employed 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.

ACKNOWLEDGEMENTS

This study would not have been possible without the help of many colleagues and friends who collected specimens for us in Australia. For this, we thank R. Aigner (QLD DPI & F), M.

Anning (Timbercorp), A. Carnegie (NSW DPI), J. Elek (Forestry Tasmania), P. Entwistle (NSW DPI), R. Gillies (Timbercorp), L. Goodrem (Murdoch University), J. Holden (Timbercorp), A. Loch (NSW DPI), V. Patel (CRC Forestry), K. Pullen (CSIRO Ecosystem Sciences) and S. Read (Timbercorp). Additional specimens for sequencing were kindly submitted by M. Branco (Portugal), while C. Bellamy and L. Fox (California), M. de Meyer and P. Limbourg (Belgium), W. Haynes (Hawaii), S. Ide Mayorga (Chile), A. Lanteri (Argentina), J.-C. Malausa and H. Perrin (France), P. Mansilla (Spain), J. Moore, S. Newete and R. Stals (South Africa), C. Valente (Portugal), B. Viklund (Sweden) and R. Thompson (United Kingdom) provided specimens for morphological comparison and identification. We thank Diane White and Vera Andic (Murdoch University) for help with sequencing. Natalie Banks (CSIRO Ecosystem Sciences, Canberra) ably prepared the images of the genitalia, and Nicole Gunter (CSIRO Ecosystem Sciences) read and commented on the manuscript. Financial support for this study was provided by Cooperative Research Centre for Forestry and Industry Pest Management Group (Albany Plantation Forestry Company, ForestrySA, Great Southern Limited (now Gunns Forestry), Integrated Tree Cropping (now Elders Forestry), Hansol PI (now Great Southern Timber Holdings), Timbercorp (now Australian Bluegum Plantations), WA Forest Products Commission, and WA Plantation Resources).

REFERENCES

Burgess T & Wingfield MJ. 2002. Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review* **4**, 56–65.

Caterino MS & Sperling FAH. 1999. *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. *Molecular Phylogenetics and Evolution* **11**, 122–137.

Cho A, Mitchell A, Regier JC, Mitter C, Poole RW, Friedlander TP & Zhao S. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor 1a recovers morphology-based tree for heliothine moths. *Molecular Biology and Evolution* **12**, 650–656.

Clark AF. 1931. The parasite control of *Gonipterus scutellatus* Gyll. *New Zealand Journal of Science and Technology* **13**, 22–28.

Clarke AR, Paterson S & Pennington P. 1998. *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) oviposition on seven naturally co-occurring *Eucalyptus* species. *Forest Ecology and Management* **110**, 89–99.

Cordero Rivera A & Santolamazza Carbone S. 2000. The effect of three species of *Eucalyptus* on growth and fecundity of the Eucalyptus snout beetle (*Gonipterus scutellatus*). *Forestry* **73(1)**, 21–29.

579 Cordero Rivera A, Santolamazza Carbone S & Andres JA. 1999. Life cycle and biological control of
580 the Eucalyptus snout beetle (Coleoptera, Curculionidae) by *Anaphes nitens* (Hymenoptera,
581 Mymaridae) in north-west Spain. *Agricultural and Forest Entomology* **1(2)**, 103–109.
582 Cunningham SA, Floyd RB & Weir TA. 2005. Do *Eucalyptus* plantations host an insect community
583 similar to remnant *Eucalyptus* forests? *Austral Ecology* **30**, 103–117.
584 Downie DA, Donaldson JS & Oberprieler RG. 2008. Molecular systematics and evolution in an
585 African cycad-weevil interaction: Amorphocerini (Coleoptera: Curculionidae: Molytinae) weevils
586 on *Encephalartos*. *Molecular Phylogenetics and Evolution* **47**, 102–116.
587 Dungey HS & Potts BM. 2003. Eucalypt hybrid susceptibility to *Gonipterus scutellatus* (Coleotera:
588 Curculionidae). *Austral Ecology* **28**, 70–74.
589 Echeverri D, Slippers B, Hurley BP & Wingfield MJ. 2007. Population diversity and structure of the
590 *Eucalyptus* Snout-Beetle, *Gonipterus scutellatus* (Coleoptera, Curculionidae) in South Africa,
591 Spain, Chile and Uruguay. *Proceedings of the IUFRO 2007 Working Group 2.08.03 Meeting*
592 *"Eucalypts and Diversity: Balancing Productivity and Sustainability"; 22–26 October, 2007*;
593 Durban, South Africa.
594 Elliott HJ & de Little DW. 1984. *Insect Pests of Trees and Timber in Tasmania*. Forestry Commission
595 Tasmania, Hobart, Tasmania.
596 Felsenstein J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**,
597 783–791.
598 Graham GC, Meyers P & Henry RJ. 1994. A simplified method for preparation of fungal DNA for
599 PCR and RAPID analysis. *Biotechniques* **16**, 48–50.
600 Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for
601 Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.
602 Hanks LM, Millar JG, Paine TD & Campbell CD. 2000. Classical biological control of the Australian
603 weevil *Gonipterus scutellatus* (Coleoptera: Curculionidae) in California. *Environmental*
604 *Entomology* **29(2)**, 369–375.
605 Hillis DM & Huelsenbeck JP. 1992. Signal noise and reliability in molecular phylogenetic analysis.
606 *Journal of Heredity* **83**, 189–195.
607 Howland DE & Hewitt GM. 1995. Phylogeny of the Coleoptera based on mitochondrial cytochrome
608 oxidase I sequence data. *Insect Molecular Biology* **4**, 203–215.
609 Huerta-Fuentes A, Chiffelle-Gómez I, Serrano-Garón M, Vázquez-Silva T & Araya-Clericus J. 2008.
610 Susceptibility of eucalyptus species to *Gonipterus scutellatus* and electrophoretic profiles of adult
611 marker proteins. *Agrociencia* **42(3)**, 327–334.
612 Jung T & Burgess TI. 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody
613 hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov..
614 *Persoonia* **22**, 95–110.

615 Laffin RD, Dosdall LM & Sperling FAH. 2005a. Population structure and phylogenetic relationships
616 of *Ceutorhynchus neglectus* (Coleoptera: Curculionidae). *The Canadian Entomologist* **137**, 672–
617 684.
618 Laffin RD, Dosdall LM & Sperling FAH. 2005b. Population structure of the cabbage seedpod weevil,
619 *Ceutorhynchus obstrictus* (Marsham) (Coleoptera Curculionidae): Origins of North American
620 Introductions. *Environmental Entomology* **34(2)**, 504–510.
621 Lanfranco D & Dungey HS. 2001. Insect damage in Eucalyptus: a review of plantations in Chile.
622 *Austral Ecology* **26(5)**, 477–481.
623 Lea AM. 1897. Descriptions of new species of Australian Coleoptera. Part IV. *Proceedings of the*
624 *Linnean Society of New South Wales* **22**, 584–638.
625 Loch AD. 2005. Mortality and recovery of eucalypt beetle pest and beneficial arthropod populations
626 after commercial application of the insecticide alpha-cypermethrin. *Forest Ecology and*
627 *Management* **217**, 255–265.
628 Loch AD. 2006. Phenology of *Eucalyptus* weevil, *Gonipterus scutellatus Gyllenhal* (Coleoptera:
629 Curculionidae), and chrysomelid beetles in *Eucalyptus globulus* plantations in south-western
630 Australia. *Agricultural and Forest Entomology* **8**, 155–165.
631 Loch AD. 2008. Parasitism of the *Eucalyptus* weevil, *Gonipterus scutellatus* Gyllenhal, by the egg
632 parasitoid, *Anaphes nitens* Girault, in *Eucalyptus globulus* plantations in southwestern Australia.
633 *Biological Control* **47**, 1–7.
634 Loch, AD & Floyd RB. 2001. Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in
635 south-western Australia: History, current perspectives and future prospects. *Austral Ecology* **26**,
636 458–466.
637 Loch AD & Matsuki M. 2010. Effects of defoliation by *Eucalyptus* weevil, *Gonipterus scutellatus*,
638 and chrysomelid beetles on growth of *Eucalyptus globulus* in southwestern Australia. *Forest*
639 *Ecology and Management* **260**, 1324–1332.
640 Londt J. 1996. Milestone in biological control. *Antenna* **20(1)**, 24.
641 Mally CW. 1924. The Eucalyptus Snout-beetle (*Gonipterus scutellatus*, Gyll.). *Journal of the*
642 *Department of Agriculture, Union of South Africa*, **51**, 1–30.
643 Marshall GAK. 1927. New injurious Curculionidae (Col.). *Bulletin of Entomological Research* **17**,
644 199–218.
645 Nei M, Maruyama T & Chakraborty R. 1975. The bottleneck effect and genetic variability in
646 populations. *Evolution* **29**, 1–10.
647 Newete SW, Oberprieler RG & Byrne MJ. 2011. The host range of the Eucalyptus Weevil, *Gonipterus*
648 *"scutellatus"* Gyllenhal (Coleoptera: Curculionidae), in South Africa. *Annals of Forest Science*
649 **68(5)**, 1005–1013.
650 Nylander JAA. 2004. *MrModeltest. Version 2.* Program distributed by the author. Evolutionary
651 Biology Centre, Uppsala University.

652 Oberprieler RG. 2010. A reclassification of the weevil subfamily Cyclominae (Coleoptera:
653 Curculionidae). *Zootaxa* **2515**, 1–35.
654 Oberprieler RG, Marvaldi AE & Anderson RS. 2007. Weevils, weevils, weevils everywhere. *Zootaxa*
655 **1668**, 491–520.
656 Paine TD & Millar JG. 2002. Insect pests of eucalypts in California: implications of managing
657 invasive species. *Bulletin of Entomological Research* **92**, 147–151.
658 Phillips CL. 1996. *Insects, Diseases and Deficiencies associated with Eucalypts in South Australia.*
659 Primary Industries SA Forests, Adelaide, South Australia.
660 Pinet C. 1986. *Patasson nitens*, parasite specifique de *Gonipterus scutellatus* en France. *Bulletin*
661 *OEPP* **16(2)**, 285–287.
662 Rabasse JM & Perrin H. 1979. Introduction en France du charançon de l'Eucalyptus, *Gonipterus*
663 *scutellatus* Gyll. (Coleoptera Curculionidae). *Annales de Zoologie et Ecologie Animaux* **11(3)**,
664 337–345.
665 Ronquist F & Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed
666 models. *Bioinformatics* **19**, 1572–1574.
667 Rosado-Neto GH & Marques MI. 1996. Characteristics of adult, genitalia and immature forms of
668 *Gonipterus gibberus* Boisduval and *G. scutellatus* Gyllenhal (Coleoptera, Curculionidae). *Revista*
669 *Brasileira de Zoologia* **13**, 77–90.
670 Sanches MA. 2000. Parasitismo de ovos de *Gonipterus scutellatus* Gyllenhal, 1833 e *Gonipterus*
671 *gibberus* Boisduval, 1835 (Coleoptera, Curculionidae) por *Anaphes nitens* (Girault, 1928)
672 (Hymenoptera, Mymaridae) em Colombo (Parana, Brasil). *Arquivos do Instituto Biologico Sao*
673 *Paulo* **67(1)**, 77–82.
674 Sequeira AS, Normark BB & Farrell BD. 2000. Evolutionary assembly of the conifer fauna:
675 Distinguishing ancient from recent associations in bark beetles. *Proceedings of the Royal*
676 *Entomological Society of London* **267**, 2359–2366.
677 Simon C, Frati F, Beckenback B, Crespi H, Liu H & Flook P. 1994. Evolution, weighting, and
678 phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase
679 chain reaction primers. *Annals of the Entomological Society of America* **87**, 651–701.
680 Swofford DL. 2003. *Phylogenetic analysis using parsimony (*and other methods), version 4.* Sinauer
681 Associates, Sunderland, Massachusetts.
682 Tooke FGC. 1953. The eucalyptus snout-beetle, *Gonipterus scutellatus* Gyll. A study of its ecology
683 and control by biological means. *Entomology Memoirs, Department of Agriculture, Union of*
684 *South Africa*, **3**, 1–282.
685 Tribe GD. 2003. Biological control of defoliating and phloem- or wood feeding insects in commercial
686 forestry in southern Africa. In: *Biological Control in IPM Systems in Africa* (eds. P
687 Neuenschwander, C Borgemeister & J Langewald J) pp 113–129. International Institute of
688 Tropical Agriculture, Benin Station, Cotonou, Benin.

689 Valente C, Branco M & Oberprieler R. 2010. Biologicol control of *Gonipterus "scutellatus"*
690 (Coleoptera: Curculionidae) – how critical is the correct species identity? *IUFRO Conference*
691 *"Population Dynamics, Biological Control, and Integrated Management of Forest Insects", 12–*
692 *16 September 2010, Eberswalde, Germany. Book of Abstracts*, p 26.
693 (http://www.forestinsects.org/iufro/eberswalde/documents/IUFRO_2010_Eberswalde_Abstracts.p
694 df).
695 Vidal Sarmiento JA. 1955. Contribución a la aclaración definitiva del problema existente entre las
696 especies "*Gonipterus gibberus*" Boisd. y "*G. platensis*" Mar.. *Notas del Museo* **18**, 31–41.
697 Wibmer GJ & O'Brien CW. 1986. Annotated checklist of the weevils (Curculionidae *sensu lato*) of
698 South America (Coleoptera: Curculionoidea). *Memoirs of the American Entomological Institute*
699 **39**, 1–563.
700 Williams JR, Moutia LA & Hermelin PR. 1952. The biological control of *Gonipterus scutellatus* Gyll.
701 (Col. Curculionidae) in Mauritius. *Bulletin of Entomological Research* **42**, 23–28.
702 Zimmerman EC. 1994. *Australian Weevils (Coleoptera: Curculionoidea). Volume I – Orthoceri,*
703 *Anthribidae to Attelabidae*. The Primitive Weevils. CSIRO Publishing, Melbourne, Victoria.
704

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

Site no.	State	location	Host	Lat °S	Long °E	Individuals analysed #	Haplotypes §
1	WA	67km NW of Frankland	*E. globulus*	34° 04’	116° 32’	2 (42)	**co1, co68***
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)	**co67***
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	*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°0 4’	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**

Site no.	State	location	Host	Lat °S	Long °E	Individuals analysed #	Haplotypes §
29	TAS	Cradoc	*E.amigdalina*	43° 06’	147° 02’	1 (8)	co46
30	TAS	Dunrobbin Rd	*E. pulchella* *E. amygdalina* *E. ovata*	42° 31’	146° 09’	11 (52) 2 (11) 1 (5)	co10, co47, co48, co49, co50 (4), co51, co52, co53, co54, co61, co62
31	TAS	Eddys Rd	*E. nitens*	43° 03’	146° 47’	13 (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* *E. pulchella*	42° 54’	147° 20’	2 (8) 2 (6)	co10 (2), 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* *E. viminalis*	42° 14’	148° 01’	1 (10) 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 Australia	*Eucalyptus* spp.	28° 01’	152° 55’	9 (11)	co21 (2), co22, co23, co24, 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)	co.18, 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’	7 (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
56	NE-NSW	Mulcahy Plantation	*E. dunnii*	28° 37’	152° 28’	1 (18)	co38

Site no.	State	location	Host	Lat °S	Long °E	Individuals analysed #	Haplotypes §
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

Table 2 Primers used for amplification and sequencing

Primer name	Direction	Region	Location of 3' end[1]	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.*, 2005a)	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		This study	GGA GTA CTC GGG ATA ATT TAC G
GON-R	R	COI		This study	CCG ATT GAG GAA ATA GCG T
GON-MF	F	COI		This study	GAG GAT TAA CTG GTG TAG TAT TAG
GON-MR	R	COI		This study	GCT AAT ACT ACA CCA GTT AAT CC

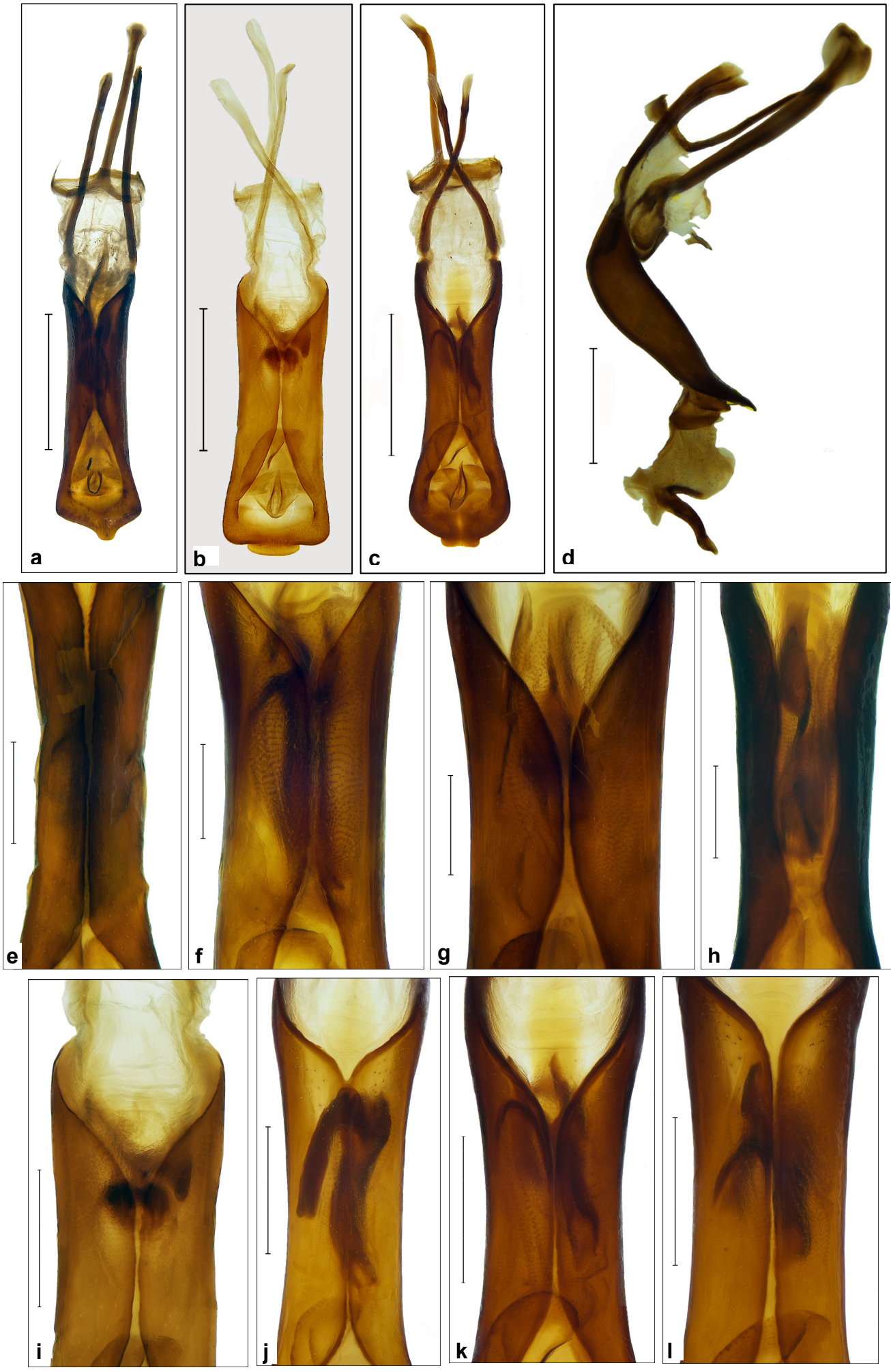
[1]Positions are relative to *Drosophila yakuba* for mtDNA (Simon *et al.*, 1994) and *Heliothodes diminutivus* (Cho *et al.*, 1995) for EF-1α and *Tenebrio molitor* sequence for 18S (GenBankX07810).
COI = Cytochrome oxidase 1, EF-1α = elongation factor-1-alpha and 18s rDNA = 18S ribosomal DNA.

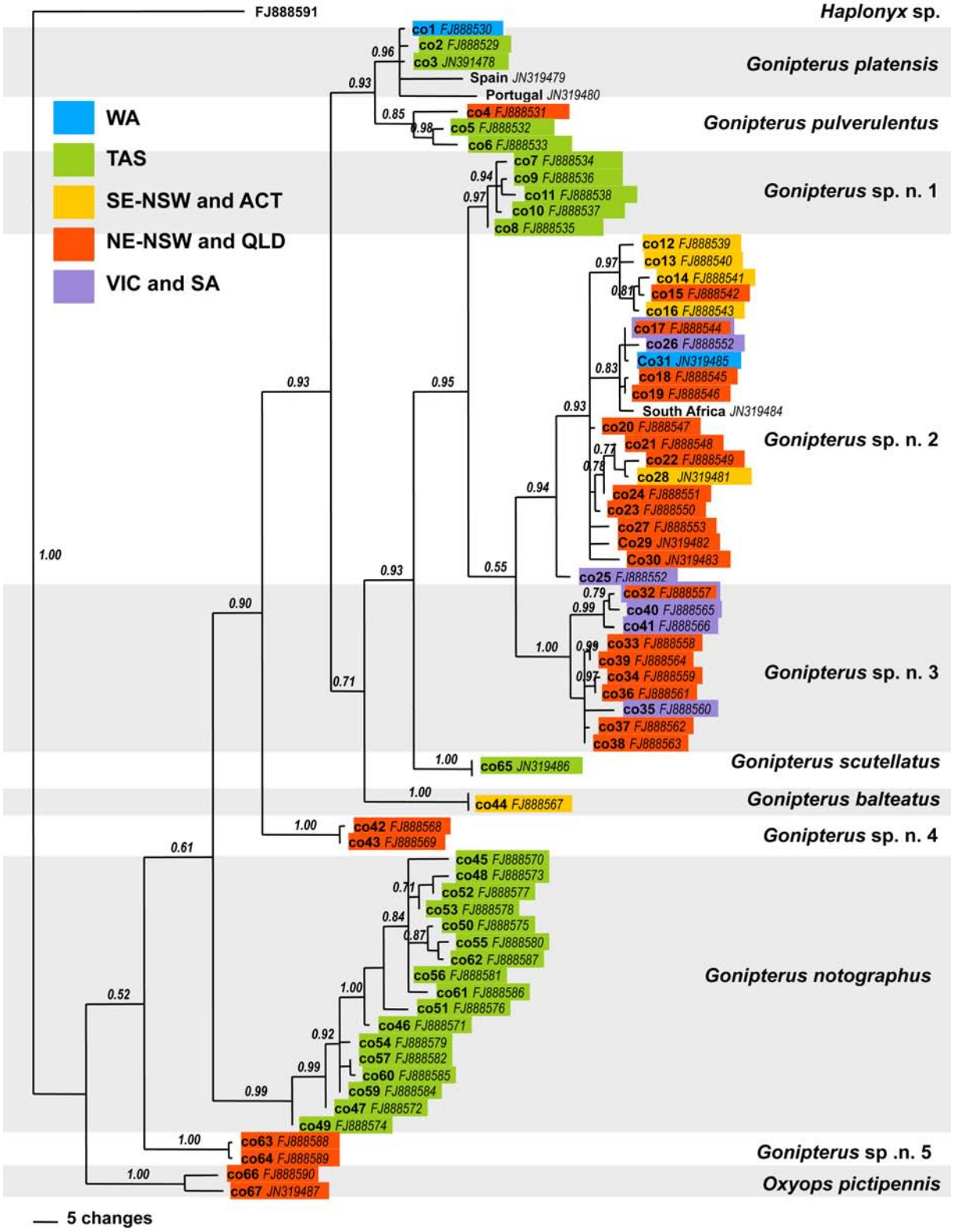
Figure captions

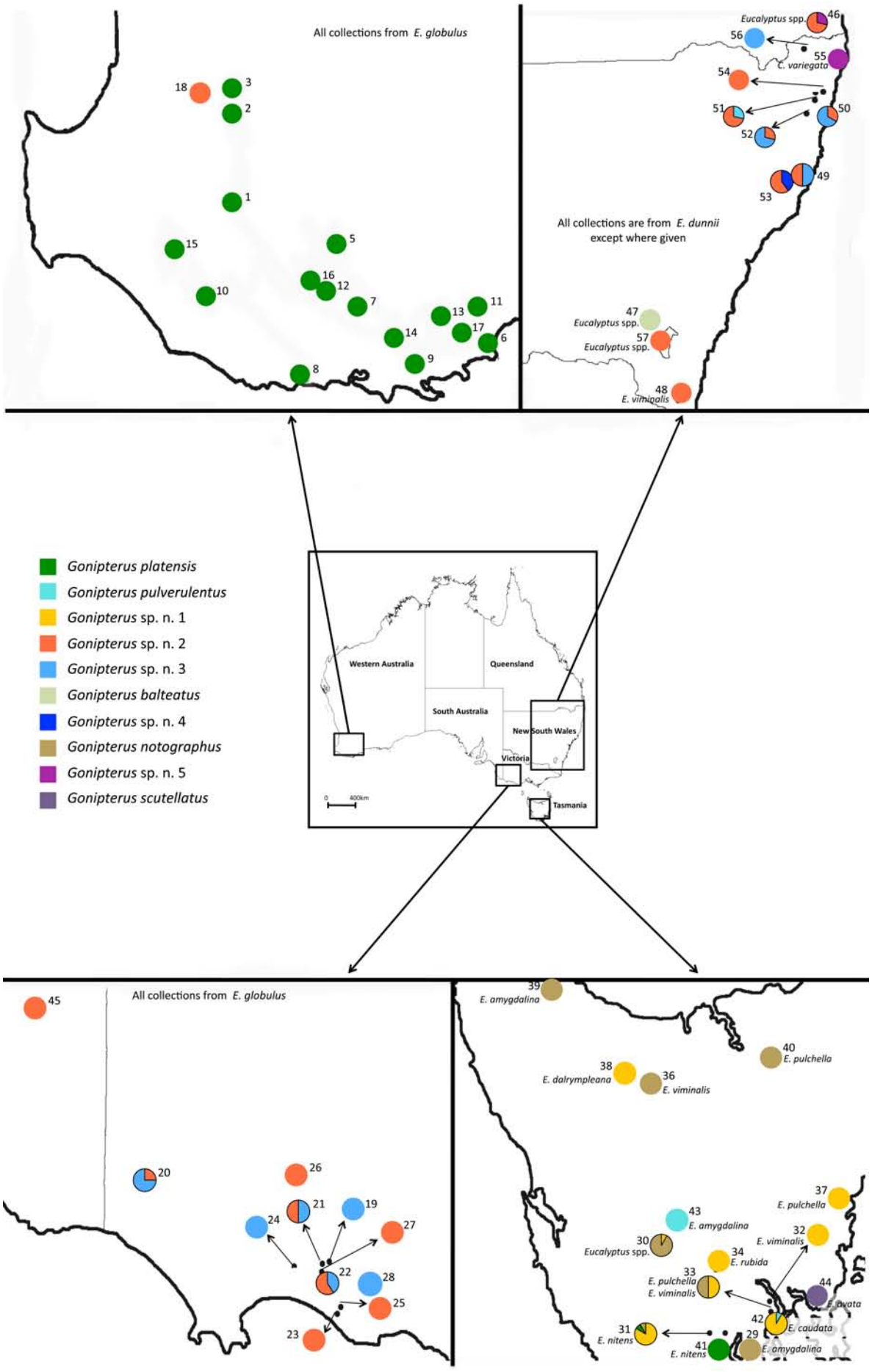
Fig. 1. Genital structures of *Gonipterus* species. (a)–(d) aedeagi; (e)–(l) mid-sections of aedeagi showing diagnostic internal sclerites, dorsal view. (a) *Gonipterus notographus* Boisduval, showing narrowly attenuated apex and long, composite internal sclerites protruding between anterior apodemes; dorsal view (Hobart, TAS); (b) *Gonipterus scutellatus* Gyllenhal, 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 (Tinderbox, TAS); (g) *Gonipterus platensis* (Marelli) (Albany, WA); (h) *Gonipterus balteatus* Pascoe (Adjumbgilly, NSW); (i) *Gonipterus scutellatus* Gyllenhal (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 1mm for Figs. (a)–(d), 0.5 mm for Figs. (e)–(l).

Fig. 2. Bayesian inference tree based on COI sequences showing phylogenetic relationships between species in the *Gonipterus scutellatus* complex. Numbers above branches represent posterior probability based on Bayesian analysis. COI haplotypes are colour-coded according to their region of origin in Australia; (i) WA, (ii) TAS, (iii) southern NSW and the ACT, (iv) northern NSW and southern QLD (v) southeast SA and southwest VIC (for specific locations see Table 1). The *Haplonyx* sp. was used as outgroup taxon.

Fig. 3. Distribution and frequency of *Gonipterus* species at each region within Australia; (a) WA, (b) QLD, NSW and ACT, (c) VIC, (d) TAS. Site numbers correspond with those in Table 1.


a
b
c
d
e
f
g
h
i
j
k
l


WA
TAS
SE-NSW and ACT
NE-NSW and QLD
VIC and SA
FJ888591
Haplonyx sp.
co1 FJ888530
co2 FJ888529
co3 JN391478
Spain JN319479
Portugal JN319480
Gonipterus platensis
co4 FJ888531
co5 FJ888532
co6 FJ888533
Gonipterus pulverulentus
co7 FJ888534
co9 FJ888536
co11 FJ888538
co10 FJ888537
co8 FJ888535
Gonipterus sp. n. 1
co12 FJ888539
co13 FJ888540
co14 FJ888541
co15 FJ888542
co16 FJ888543
co17 FJ888544
co26 FJ888552
Co31 JN319485
co18 FJ888545
co19 FJ888546
South Africa JN319484
co20 FJ888547
co21 FJ888548
co22 FJ888549
co28 JN319481
co24 FJ888551
co23 FJ888550
co27 FJ888553
Co29 JN319482
Co30 JN319483
co25 FJ888552
Gonipterus sp. n. 2
co32 FJ888557
co40 FJ888565
co41 FJ888566
co33 FJ888558
co39 FJ888564
co34 FJ888559
co36 FJ888561
co35 FJ888560
co37 FJ888562
co38 FJ888563
Gonipterus sp. n. 3
co65 JN319486
Gonipterus scutellatus
co44 FJ888567
Gonipterus balteatus
co42 FJ888568
co43 FJ888569
Gonipterus sp. n. 4
co45 FJ888570
co48 FJ888573
co52 FJ888577
co53 FJ888578
co50 FJ888575
co55 FJ888580
co62 FJ888587
co56 FJ888581
co61 FJ888586
co51 FJ888576
co46 FJ888571
co54 FJ888579
co57 FJ888582
co60 FJ888585
co59 FJ888584
co47 FJ888572
co49 FJ888574
Gonipterus notographus
co63 FJ888588
co64 FJ888589
Gonipterus sp .n. 5
co66 FJ888590
co67 JN319487
Oxyops pictipennis
5 changes


All collections from E. globulus
All collections are from E. dunnii except where given
Eucalyptus spp.
C. variegata
E. viminalis
Gonipterus platensis
Gonipterus pulverulentus
Gonipterus sp. n. 1
Gonipterus sp. n. 2
Gonipterus sp. n. 3
Gonipterus balteatus
Gonipterus sp. n. 4
Gonipterus notographus
Gonipterus sp. n. 5
Gonipterus scutellatus
Western Australia
Queensland
South Australia
New South Wales
Victoria
Tasmania
0 400km
All collections from E. globulus
E. amygdalina
E. pulchella
E. dalrympleana
E. viminalis
E. rubida
E. nitens
E. caudata
E. ovata