

Identity of wasp parasitoids (Hymenoptera) attacking *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae) in South Africa

Simon van Noort^{1,2} , Jose Fernandez-Triana³ , Hannes Baur^{4,5}  and Mark R. Shaw⁶ 

¹Research and Exhibitions Department, South African Museum, Iziko Museums of South Africa, Cape Town, South Africa

²Department of Biological Sciences, University of Cape Town, South Africa

³Canadian National Collection of Insects, Ottawa, Canada

⁴Department of Invertebrates, Natural History Museum Bern, Switzerland

⁵Institute of Ecology and Evolution, University of Bern, Switzerland

⁶National Museums of Scotland, Edinburgh, UK

The alien invasive large cabbage white, *Pieris brassicae* (Linnaeus, 1758) (Pieridae), although introduced into South Africa more than 27 years ago, has not dramatically expanded its distribution, possibly because it is effectively attacked by two parasitoid wasp species. Even though there is a cohort of parasitoid species associated with *P. brassicae* in the Northern Hemisphere, these are the only two recorded parasitoids in South Africa. We determine these parasitoid wasps as *Cotesia glomerata* (Linnaeus, 1758) (Braconidae: Microgastrinae) and *Pteromalus puparum* (Linnaeus, 1758) (Pteromalidae), and provide high resolution images and DNA barcodes to facilitate their recognition. This is the first record of *C. glomerata* from the Afrotropical region.

INTRODUCTION

The large cabbage white, *Pieris brassicae* (Linnaeus, 1758) (Pieridae), was accidentally introduced into South Africa sometime prior to its first detection in July 1994 in Sea Point, Cape Town (Claassens 1995, 1996, 1998, 2014; Gardiner 1995; Geertsema 1996; Picker and Griffiths 2011, 2017; van Noort 1996). The species is extremely common on the Cape Peninsula (Claassens 1995, 1996, 1998, 2014; Steele 1998), and the adults are present for most of the year (Prinsloo & Uys 2015), although population levels can fluctuate dramatically from year to year (Janion-Scheepers & Griffiths 2020). The species is a registered invasive species (Robinson et al. 2020), but has not spread much beyond a core south-western Cape distributional range, extending north as far as Velddrif on the Western Cape coast, and east to Keurboomstrand on the southern Cape coast, with two outlying isolated records from Walvis Bay in Namibia (iNaturalist 2021, https://www.inaturalist.org/observations?taxon_id=55401).

Although *P. brassicae* appears to have had limited impact on cruciferous vegetable production in South Africa, even though it has the potential to do so (Geertsema 1996), there is evidence that it is having an impact as an effective pollinator on invasive ornamental plants in South Africa, such as devil's beard (*Centranthus ruber* L.) and purple loosestrife (*Lythrum salicaria* L.) (Geerts et al. 2017; Le Roux et al. 2020). Besides cabbage, the caterpillars commonly feed on nasturtiums (*Tropaeolum majus* L.) and sweet alyssum [*Lobularia maritima* (L.) Desv.] in gardens and parks in South Africa, as well as on a common weed, *Rapistrum rugosum* (L.) (Wild Mustard or Turnip Weed) (Claassens 1995, 2014; Prinsloo & Uys 2017; van Noort 1996).

This butterfly, indigenous to the Palearctic region in the northern hemisphere, is an invasive species in other areas of the southern hemisphere besides South Africa: Chile where it has been established for over 36 years (Benyamini 1996; Gardiner 1974; Neira et al. 1989), and New Zealand where it has purportedly subsequently been eradicated (Brown et al. 2019). In Europe and Chile (and formerly in New Zealand), *P. brassicae* larvae are commonly parasitized by the microgastrine *Cotesia glomerata* (Linnaeus, 1758) (Braconidae), and pupae by *Pteromalus puparum* (Linnaeus, 1758) (Pteromalidae) (Karnavar 1983; Peters 1991; Shaw and Fitton 1989; Shaw and Huddleston 1991; Askew and Shaw 1997; Shaw et al. 2009). Together these are the most effective parasitoids of *Pieris brassicae* and parasitism levels may exceptionally be as high as 100% (Peters 1991), but are usually much lower than this (Shaw et al. 2009). Both parasitoid species were originally released in New Zealand in the early 1930s as biological control agents for *P. rapae* (Cameron et al. 1989; Phillips et al. 2014), a misguided attempt as *C. glomerata* is better adapted to *P. brassicae*, and *P. puparum* has a wide host range (Shaw et al. 2009). The records of two other microgastrine braconids from *P. brassicae* clearly stem from host misidentifications: *Cotesia rubecula* (Marshall, 1885), which almost exclusively attacks *Pieris rapae* in nature, and *Cotesia ruficrus* (Haliday, 1834), which has a wide host repertoire, but does not attack *P. brassicae* (Shaw et al. 2009). Other Microgastrinae species mentioned in some references (e.g. Greathead & Greathead 1992; Harvey et al. 1999, see also compilation of references in Yu et al. 2016) are almost certainly incorrect and should not be considered further. On a global level *P. brassicae* is attacked by several other wasp species as well as fly parasitoids. Again many of the records of Ichneumonoidea cited in Yu et al. (2016), as well as in the primary literature (Greathead & Greathead 1992; Neira et al. 1989; Razmi et al. 2011; Shahram & Debjani 2013) need to be treated with great suspicion unless their identity and positive association

CORRESPONDENCE

Simon van Noort

EMAIL

svannoort@iziko.org.za

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with *P. brassicae* is confirmed. Apart from *C. glomerata*, the campoplegine ichneumonid, *Hyposoter ebeninus* (Gravenhorst, 1829), is another koinobiont larval parasitoid attacking *P. brassicae* (Shaw et al. 2016), and a *Brachymeria* species (Chalcididae) is also an occasional primary parasitoid of the pupa (Shaw et al. 2009). Idiobiont pupal parasitoids are usually less specialised (Shaw 1994), and in Europe *Pimpla rufipes* (Miller, 1759) and *Apechthis compunctor* (Linnaeus, 1758) are commonly reared from *P. brassicae*, with the gregarious *Blapsidotes vicinus* (Gravenhorst, 1829) being less commonly recorded from this host (Shaw 1982; Shaw et al. 2009). A number of non-host specific tachinid fly species develop as larval and larval-pupal parasitoids [*Epicamponera succincta* (Meigen) is, however, a *Pieris* specialist], some with wide host ranges e.g. species of *Pales* Robineau-Desvoidy, *Compsilura* Bouché, *Phryxe* Robineau-Desvoidy and *Exorista* Meigen regularly attack *P. brassicae*, and at least in the case of *E. succincta* outcompete *C. glomerata* (Shaw 1982; Shaw et al. 2009). Polyphagous *Trichogramma* Westwood (Trichogrammatidae) egg parasitoids commonly attack *Pieris* Schrank hosts in Europe (Shaw 1982). Incidence and levels of hyperparasitism in this system are currently unknown.

In South Africa, two species of Tachinidae in the genera *Winthemia* Robineau-Desvoidy and *Exorista*, and a species of *Pteromalus* (Pteromalidae: Chalcidoidea) (Claassens 1995, 1996), as well as a species of *Cotesia* (Claassens 1998) – cited as a species of *Apanteles* in Prinsloo & Uys (2015) and Janion-Scheepers & Griffiths (2020) – have been reared from *P. brassicae*. The pteromalid wasp associated with *P. brassicae* in South Africa was subsequently recorded as the cosmopolitan *P. puparum* (Claassens 1998; Picker and Griffiths 2011; Prinsloo and Uys 2015), which is a gregarious pupal endoparasitoid (Askew and Shaw 1997; Shaw 1982; Shaw 2002; Shaw et al. 2009). We here provide a species determination for the microgastrine braconid wasp as *C. glomerata*, which is a gregarious larval endoparasitoid (Shaw 1982; Shaw et al. 2009), and confirm the identity of *Pteromalus puparum*. To facilitate their future recognition we provide high resolution images of these two species reared from *P. brassicae* in South Africa as well as DNA barcodes for *C. glomerata*. All images presented here as well as supplementary images are available on www.waspweb.org.

MATERIALS and METHODS

A leg from each of three selected specimens of *Cotesia* reared from *P. brassicae* in South Africa was submitted to Barcode of Life Data System (BOLD) for barcoding. DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova et al. 2006). Total genomic DNA was re-suspended in 30 µl of dH₂O, a 658 base pairs (bp) region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (<http://v4.boldsystems.org/index.php>), and a composite sequence was generated for all successful amplifications. All information for the sequences associated with each individual specimen barcoded can be retrieved from the BOLD system (Ratnasingham and Hebert 2007). We use the Barcode Index Number (BIN) system to discuss species limits, following the BIN concept detailed in Ratnasingham and Hebert (2013).

Images were acquired at SAMC with a Leica LAS 4.9 imaging system, comprising a Leica® Z16 microscope (using either a 2× or 5× objective) with a Leica DFC450 Camera and 0.63× video objective attached. The imaging process, using an automated Z-stepper, was managed using the Leica Application Suite V 4.9 software installed on a desktop computer. Diffused lighting was achieved using a Leica LED5000 HDI dome. All images presented in this paper, as well as supplementary images, are available at www.waspweb.org.

Depository of specimens

SAMC: Iziko South African Museum, Cape Town (curator Simon van Noort)

RESULTS

Barcoding

Of the three *Cotesia* samples submitted to BOLD, two rendered full DNA barcodes (658 base pairs), the third (38754_B02_SAM-HYM-P020703; FSA1906-21), which was a dry specimen mounted in 1998 shortly after collection, returned only a 286 bp length that was determined to represent a potential contamination. The ethanol preserved specimen (38754_B03_SAM-HYM-P020703; FSA1907-21) from this same collecting event had been stored in 96% ethanol until extraction on 27 November 2020 for submission to BOLD. The third specimen (38754_B11_SAM-HYM-P095102; FSA1915-21) was freshly collected on emergence on 1 December 2020 and killed in 96% ethanol shortly prior to submission. The *Pteromalus* extraction failed to produce a sufficient sequence length for barcoding (i.e. 281 base pairs).

The South African *Cotesia* specimens match perfectly with several sequences of specimens of *Cotesia glomerata* from Chile, the Czech Republic and France (Figure 5). They also match fairly closely (99.5–99.6% of base pairs shared) with other sequences of *C. glomerata* in BOLD from Australia, Canada, the Czech Republic, Hungary, Paraguay, Spain, Switzerland and United States. Other specimens of *C. glomerata* in BOLD from India, Pakistan and Turkey are slightly less related (98.7% of similar base pairs), but still within the limits of what is considered as a single BIN (BOLD: AAD1110) in BOLD, which includes all specimens of *C. glomerata* with sequences deposited there.

Braconidae, Microgastrinae

Cotesia glomerata (Linnaeus, 1758) (Figures 1–4)

Material examined. South Africa, Western Cape Province. 6 FM: Cape Town, Sea Point, 33.917°S 18.383°E, 8 m, 22 September 1998, A.J.M. Claassens, ex *Pieris brassicae*, 2 pins with caterpillar skin & cocoon cases, *Cotesia* sp. det. S. van Noort, 1998, SAM-HYM-P020669 (SAMC); 6 FM: Cape Town, Sea Point, 33.917°S, 18.383°E, 8 m, 28 September 1998, A.J.M. Claassens, ex *Pieris brassicae*, (additional specimens in ethanol), *Cotesia* sp. det. S. van Noort, 1998, SAM-HYM-P020671 (SAMC); 7 FM: Stellenbosch, 29 September 1998, V.B. Whitehead & V. Booth, ex *Pieris brassicae*, 1 pin with caterpillar case & cocoon - see material in EtOH, *Cotesia* sp. det. S. van Noort, 1998, SAM-HYM-P020670 (SAMC); 31 FM: Cape Town, Kirstenhof, 34.069°S 18.452°E, 1 November 1998, S. van Noort, ex *Pieris brassicae*, emerged 9 November 1998, *Cotesia* sp. det. S. van Noort, 1998, SAM-HYM-P020703 (SAMC); 2 M: Cape Town, Devil's Peak, October 2016, N. Larsen, ex *Pieris brassicae* larva, SAM-HYM-P095176 (SAMC); ditto SAM-HYM-P095177 (SAMC); Muizenberg, Clifton Road, 34.101°S 18.475°E, caterpillar collected 23 November 2020, wasp larvae emerged from host caterpillar and spun cocoon on 25 November 2020; adult wasps emerged 1 December 2020, S. van Noort, MUIZ20-COT231120, *Cotesia* sp. det. S. van Noort, 2020, SAM-HYM-P095102 (SAMC); ditto SAM-HYM-P095103 (SAMC); ditto SAM-HYM-P095104 (SAMC); ditto SAM-HYM-P095105 (SAMC); ditto SAM-HYM-P095106 (SAMC); ditto SAM-HYM-P095107 (SAMC); ditto SAM-HYM-P095108 (SAMC); ditto SAM-HYM-P095109 (SAMC); ditto SAM-HYM-P095110 (SAMC); ditto SAM-HYM-P095111 (SAMC); ditto SAM-HYM-P095112 (SAMC).

Diagnosis. The sequences available in BOLD support the assignment of the two South African barcodes to *C. glomerata*, as they cluster with sequences of that species originating from Chile,

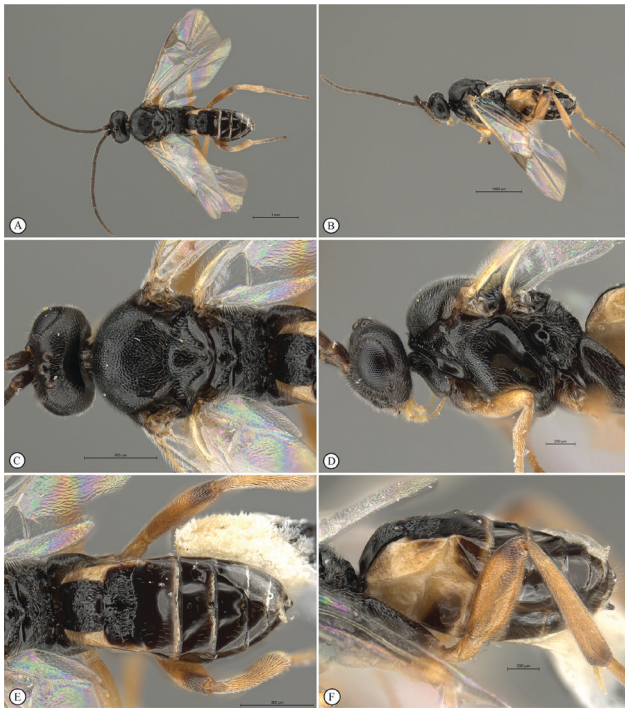


Figure 1. *Cotesia glomerata*, female SAM-HYM-P020703 (SAMC). A, habitus, dorsal view. B, habitus, lateral view. C, head, mesosoma, dorsal view. D, head, mesosoma, lateral view. E, metasoma, dorsal view. F, metasoma, lateral view

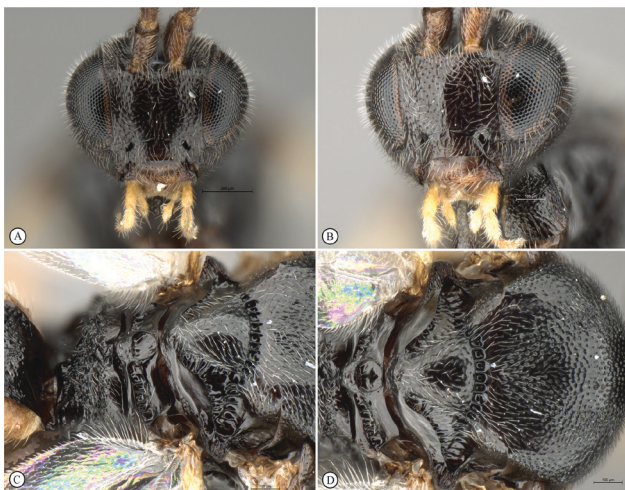


Figure 2. *Cotesia glomerata*, female SAM-HYM-P020703 (SAMC). A, head, anterior view. B, head antero-lateral view. C, scutellum, propodeum, dorso-lateral view. D, scutellum, propodeum, dorsal view

the Czech Republic and France (Figure 5).

Distribution. *Australasian:* Australia (ACT, NSW, QLD), New Zealand; *Nearctic:* Canada (BC, NB, ON, QC) and USA (CA, CO, CT, DC, FL, IL, IA, LA, MD, MA, MI, MN, NH, NJ, NY, OR, PA, SC, VT, VA, WA, WI); *Neotropical:* Barbados, Brazil (SP), Chile and Uruguay; *Indomalayan (Oriental):* China (GZ, HN, JS, SH, SN, TW, ZJ), India, Pakistan and Vietnam; *Oceania:* Fiji and Hawaiian Islands and *Palearctic:* Armenia, Azerbaijan, Azores, Belarus, Belgium, Bulgaria, Canary Islands, China (BJ, HE, HA, JL, LN, NM, NX, SN, XJ), Croatia, Cyprus, Czech Republic, Denmark, Egypt, Estonia, Finland, France, Georgia, Germany, Hungary, Iran, Ireland, Israel, Italy, Japan, Jordan, Kazakhstan, Korea, Latvia, Lithuania, Macedonia, Malta, Moldova, Mongolia, Morocco, Netherlands, Poland, Portugal, Romania, Russia (AD, AST, BU, KGD, KAM, KHA, KIR, KDA, KRS, MOS, PRI, ROS, SAK, SPE, SAR, TAM, VGG, VLG, YAR), Serbia, Slovakia, Spain,



Figure 3. *Cotesia glomerata*, female SAM-HYM-P020703 (SAMC). A, first and second tergites, dorso-lateral view. B, scutellum, propodeum, dorso-posterior view. C, first and second tergites, dorso-anterior view. D, wings, dorsal view (inset: data labels)

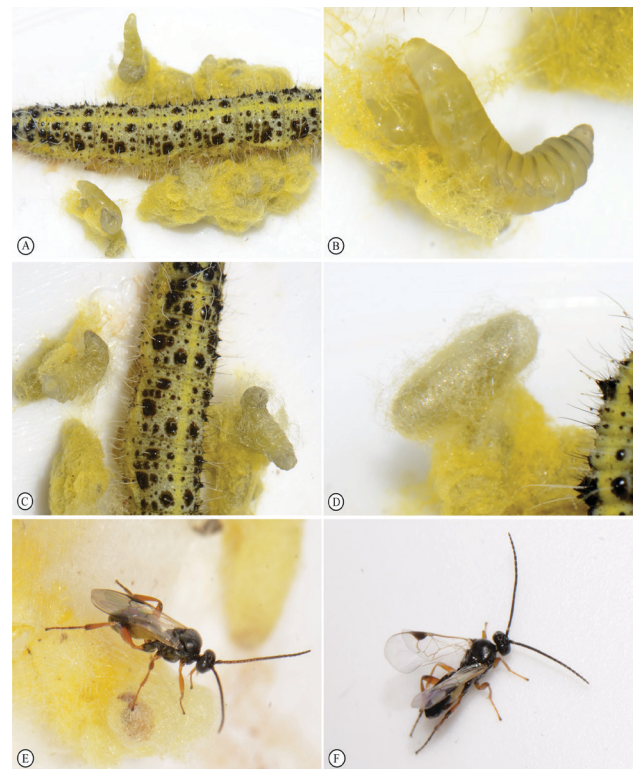


Figure 4. *Cotesia glomerata*, Muizenberg collecting event: MUIZ20-COT231120 (SAMC). A, wasp larvae shortly after emergence from host *Pieris brassicae* caterpillar. B, cocoon spinning commencement. C, cocoon spinning. D, cocoon completion. E and F, recently emerged adult wasp.

Sweden, Switzerland, Syria, Turkey, Ukraine, United Kingdom and Uzbekistan (Fernandez-Triana et al. 2020).

Biology. Gregarious endoparasitoid of *Pieris brassicae* larvae.

Barcode sequences for specimen: 38754_B03_SAM-HYM-P020703 (sequence code in BOLD: FSA1907-21)

Nucleotide sequence

TTTCTATAAGATTATTAATTCGTTT TAGAATTAG-
GAATACCTGGAAGATTAATTGGTAATGATCAGATT-
TATAATAGAATTGTAACCTTCTCATGCTTTTATTATA-
ATTTTTTTTATAGTTATAACCTGTAATAATTGGCG-
GTTTTGGAAATTGATTAATTCCTTTGATGTTAG-
GATCTCCAGATATATCTTTCTCGAATAATAATATA-
AGTTTTTGATTATTGATCCCTTCTTTAATATTATTAAT-

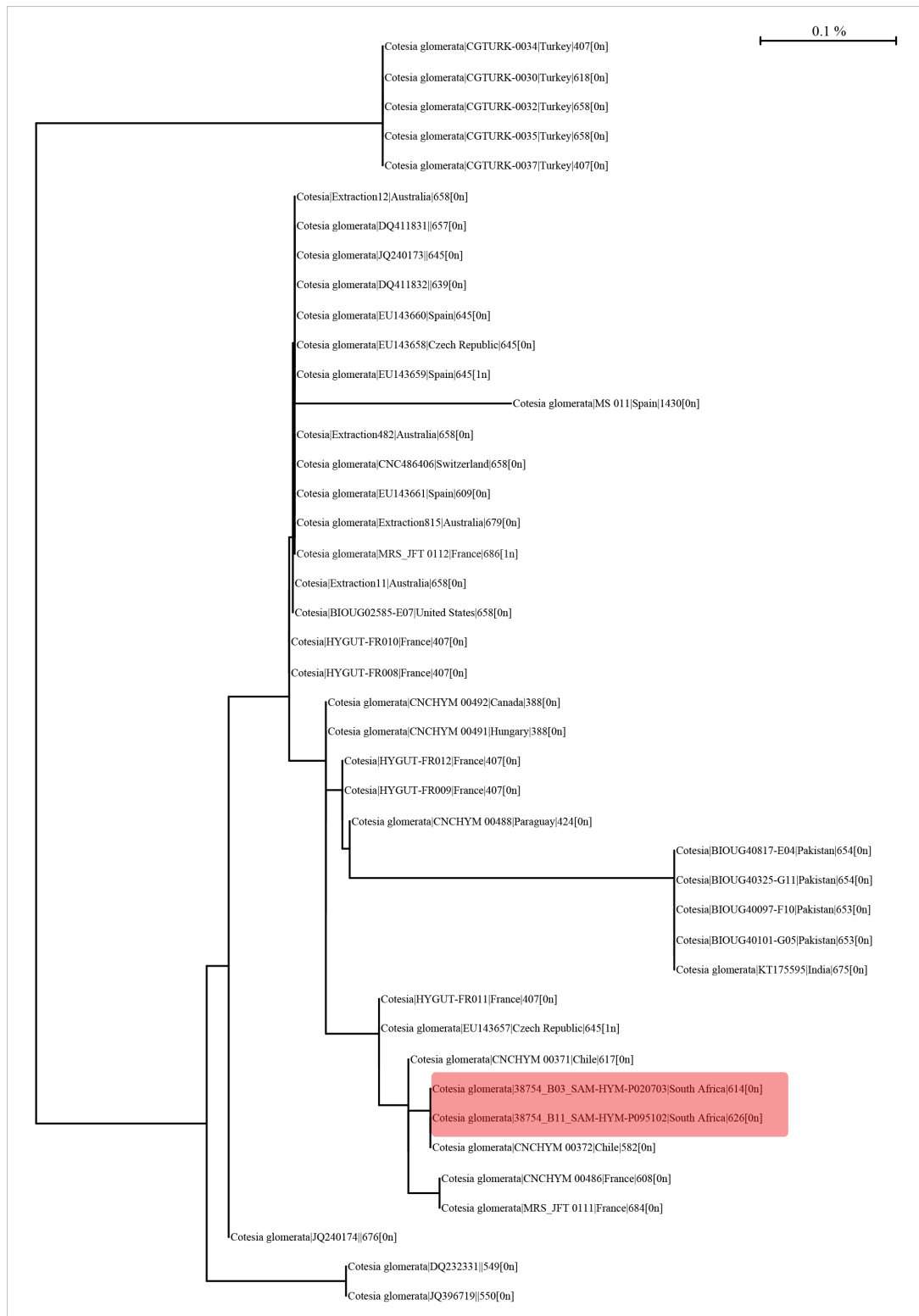


Figure 5. Neighbour joining tree (Kimura 2 parameter) of all *Cotesia glomerata* sequences available in the Barcode of Life Data system, with BIN (BOLD: AAD1110)

TATAAGAAGATTTATTAATGTAGGAGTTGGAACGTG-
GTTGAACTGTTTATCCTCCTTTATCATTAATTTTAGGT-
CATGGGGGAATATCAGTTGATTTAGGAATTTTTCTT-
TACATTTAGCTGGTGCATCTTCAATTATAGGTGCTGTA-
AATTTTATTACTACTATTATAAATATACGTTCAAAT-
TATTTAATATAGATAAAATATCTTTATTTTCTTGATCT-
GTATTTATTACTGCAATTTTATTATATTATCTTTACCT-
GTTTTAGCAGGTGCTATTACTATATTATTAAGTATC-
GAAATATAAATACAAGATTTTTTGATCCATCAGGTG-

GAGGGGATCCAATTCTTTATCAACATTTATTT

Barcode sequences for specimen: 38754_B11_SAM-HYM-P095102 (sequence code in BOLD: FSA1915-21)

Nucleotide sequence

GGATATTAGGATTTTCTATAAGATTATTAATTCGTT-
TAGAATTAGGAATACCTGGAAGATTAATTGGTAAT-
GATCAGATTTATAATAGAATTGTAACCTCTCATGCTTT-
TATTATAATTTTTTTTATAGTTATACCTGTAATAATTG-

GCGGTTTTGGAAATTGATTAATTCCTTTGATGTTAG-GATCTCCAGATATATCTTTTCCTCGAATAAATAATATA-AGTTTTTGATTATTGATCCCTTCTTTAATATTATTAAT-TATAAGAAGATTTATTAATGTAGGAGTTGGAACGTG-GTTGAACTGTTTATCCTCCTTTATCATTAAATTTAGGT-CATGGGGGAATATCAGTTGATTTAGGAATTTTTCTT-TACATTTAGCTGGTGCATCTTCAATTATAGGTGCTGTA-AATTTTATTACTACTATTATAAAATATACGTTCAAATTTATTAAATATAGATAAAATATCTTTATTTCTTGATCT-GTATTTATTACTGCAATTTTATTATTATTATCTTTACCT-GTTTTAGCAGGTGCTATTACTATATTATTAACGTATC-GAAATATAAATACAAGATTTTTTGATCCATCAGGTG-GAGGGGATCCAATCTTTATCAACATTTATTT

Pteromalidae, Pteromalinae

***Pteromalus puparum* (Linnaeus, 1758) (Figures 6–8)**

Material examined. South Africa, Western Cape Province. 9 FM: Cape Town, Pinelands, 19 October 1995, J.B. Ball, ex *Pieris brassicae* pupa, *Pteromalus puparum* det. S. van Noort, 1998, SAM-HYM-P011872 (SAMC); 10 FM: Cape Town, Edgemoed, 29 April 1997, I.G.T. Taylor, ex *Pieris brassicae* pupa, *Pteromalus puparum* det. S. van Noort, 1998, SAM-HYM-P019377 (SAMC); 4 FM: Stellenbosch, pupa collected July 1997, emerged 22 August 1997, V.B. Whitehead & V. Booth, ex *Pieris brassicae* pupa, *Pteromalus puparum* det. S. van Noort, 1998, (photos: SAM EN#C001448–1450), SAM-HYM-P019409 (SAMC); 4 FM: Stellenbosch, pupa collected July 1997, emerged 23 September 1997, V.B. Whitehead & V. Booth, ex *Pieris brassicae* pupa, *Pteromalus puparum* det. S. van Noort, 1998, (photos: SAM EN#C001448–1450), SAM-HYM-P021093 (SAMC); 10 FM: Stellenbosch, pupa collected July 1997, emerged 3–6 September 1997, V.B. Whitehead & V. Booth, ex *Pieris brassicae* pupa (2 pupae out of 9 parasitized), *Pteromalus puparum* det. S. van Noort, 1998, (photos: Photo SAM EN#C001448 – 1450), SAM-HYM-P019410 (SAMC); 11 FM: Cape Town, Sea Point, 33.917°S 18.383°E, 8 m, 1 November 1998, A.J.M. Claassens, ex *Pieris brassicae* pupa, *Pteromalus puparum* det. S. van Noort,

1998, SAM-HYM-P021092 (SAMC); 20F 1M: Kleinmond, M. Brink, MAGRIETB-160929-3, ex pupa on curtain collected 29 September 2016. Wasps emerged 7 October 2016. SAM-HYM-P088315 (SAMC).

Diagnosis. The diagnosis of *Pteromalus puparum* is here cited from Baur (2015): female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL slightly greater than OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster obtusely pointed, rarely more than 1.6 times as long as broad (for abbreviations and terminology, see Baur 2015).

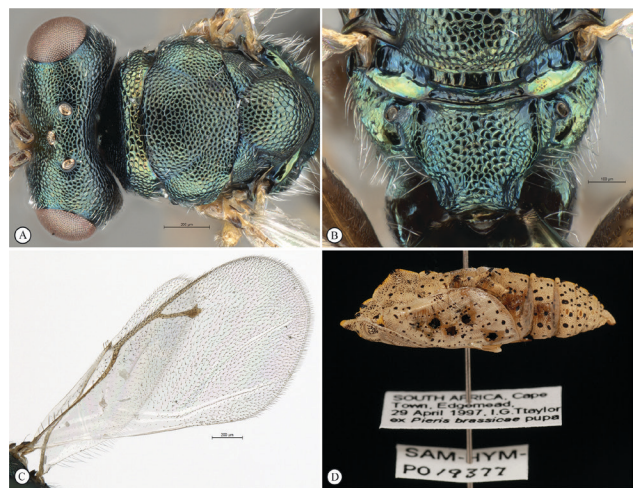


Figure 7. *Pteromalus puparum*, female SAM-HYM-P019277A (SAMC). A, head, metasoma, dorsal view. B, propodeum, dorsal view. C, wings. D, host *Pieris brassicae* pupa showing four *P. puparum* exit holes through wing case.

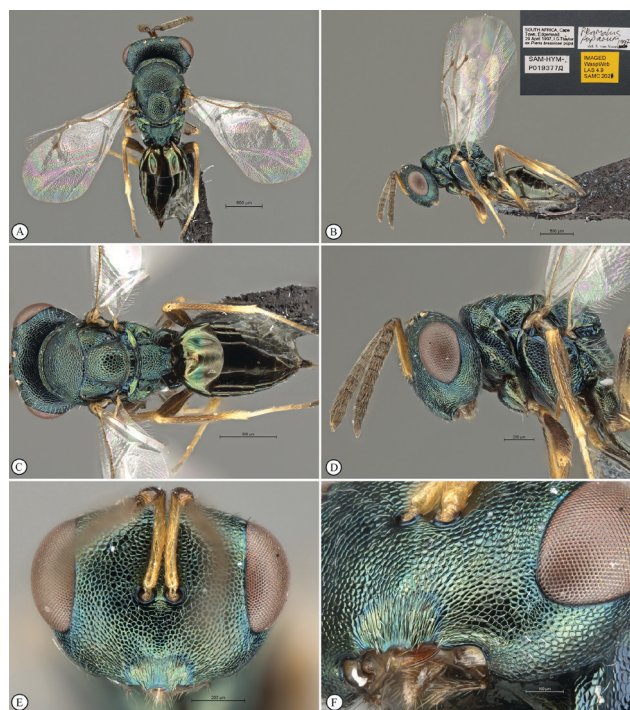


Figure 6. *Pteromalus puparum*, female SAM-HYM-P019277A (SAMC). A, habitus, dorsal view. B, habitus, lateral view (inset: data labels). C, head, mesosoma, metasoma, dorso-posterior view. D, head, metasoma, lateral view. E, head, anterior view. F, clypeus and gena, anterio-lateral view.

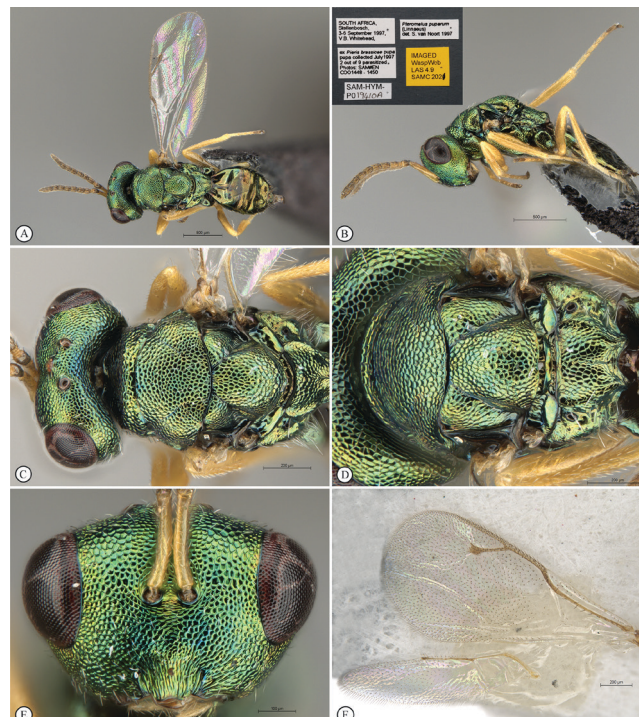


Figure 8. *Pteromalus puparum*, male SAM-HYM-P019410A (SAMC). A, habitus, dorsal view. B, habitus, lateral view (inset: data labels). C, head, mesosoma, dorsal view. D, metasoma, dorsal view. E, head, anterior view. F, wings.

Graham's (1969) key to *Pteromalus* as well as the key published in Klimmek and Baur (2018) allow recognition of the species.

Distribution. The species is currently considered to be cosmopolitan (Noyes 2019). However, there are many (older) records that need reassessment and confirmation of their validity.

Biology. Gregarious pupal endoparasitoid of *Pieris brassicae*. The host caterpillar, in its final instar (sometimes even before it has finished feeding), or as a prepupa, is usually mounted by the female parasitoid, which often goes unnoticed. Oviposition only takes place when the soft, fresh pupal cuticle is exposed as the prepupa moults (Shaw 2002). Also commonly recorded as a parasitoid of other butterflies, in particular many species of Nymphalidae, Papilionidae and Pieridae (Graham 1969). Further supposed hosts cited in Noyes (2019), especially those involving Coleoptera, Hemiptera or Diptera, must be considered with suspicion. *Pteromalus puparum* has always been one of the best known species of the genus (e.g. Graham 1969). For this reason, many rearings were often attributed (often in error) to this species.

Barcode sequences. Failed.

DISCUSSION

Unfortunately the barcode extraction for *P. puparum* failed, but the identity of this species was confirmed by one of the authors (viz. Hannes Baur). Two of the three *C. glomerata* specimens submitted to BOLD produced full DNA barcodes. The specimen that failed was a dry specimen mounted in 1998 shortly after collection, but a specimen from the same collecting event that had been stored in 96% ethanol was successfully sequenced, supporting the value of different modes of specimen preservation. The haplotype variation present within the global population of *C. glomerata* (based on the available sequences in BOLD from specimens worldwide, depicted in Fig. 5), might indicate a variable species, or perhaps a complex of related species, but solving that will require additional studies of the world fauna and more specimens to be sequenced, which is beyond the scope of the present work. Our results confidently place the South African specimens within the present concept of *C. glomerata*.

Although the hot and dry Western Cape summer was hypothesised to curtail population expansion of the large cabbage white, *P. brassicae*, evidence that the species can enter diapause during unfavourable summer conditions in southern Europe (Held & Spieth 1999) and Chile (Benyamini 1996) has probably enabled adaptation of the South African alien population to persist in the Western Cape. The impact of this alien butterfly on local ecosystems and agriculture in South Africa is little understood, and is begging for assessment, as is the efficiency of parasitoids present in South Africa in controlling this pest butterfly species. The mode and date of introduction of the two exotic parasitoid species, *C. glomerata* and *P. puparum* is unknown, but given the latter's global distribution and multiple host species association, its presence in South Africa possibly preceded the introduction of *P. brassicae* circa 1994. Given that *Cotesia glomerata* is host-specific on only a few genera of Pieridae (*Aporia*, *Pieris*, *Pontia*, and more rarely *Anthocharis* and *Euchloe*) in its (presumed) Palaearctic area of origin, it is likely to have colonised South Africa subsequent to the introduction of *P. brassicae*, although both parasitoids could feasibly have come into South Africa together with *P. brassicae*, being present and developing at the time of introduction inside immature life stages of the host butterfly. This mode of parasitoid introduction has occurred elsewhere, such as in the case of the 1991 accidental introduction of the Glanville fritillary butterfly (*Melitaea cinxia* Linnaeus, Nymphalidae), which included the parasitoid

wasp, *Hyposoter horticola* (Gravenhorst) (Ichneumonidae: Campopleginae) and its associated hyperparasitoid *Mesochorus* cf. *stigmaticus* (Ichneumonidae: Mesochorinae), from the main Åland Islands in Finland to an isolated island in the archipelago (Duplouy et al. 2021). To date no indigenous African wasp parasitoids have been reared from *P. brassicae*, and given the length of presence of the butterfly in South Africa it appears that they may not be able to adapt to this alien species, or that the conditions for host recruitment have not yet arisen. It is perhaps surprising that no indigenous ichneumonid pipeline pupal parasitoids of butterflies, which often have wide host ranges (Shaw 1994), have been reared from *P. brassicae*. Conversely, it is not a surprise that local tachinid fly parasitoids with wide host ranges have recruited this exotic butterfly. Given the current globalisation phenomenon it is possible that further exotic parasitoids of *P. brassicae* may also establish in South Africa, although the only real contender that is a regular parasitoid of *P. brassicae* larvae is *Hyposoter ebeninus* (Shaw et al. 2016). There may actually be a need for introduction of this species as a further potential biocontrol agent of the large cabbage white, but this is contingent on future studies assessing its potential host repertoire as well as the parasitism rate of the two currently present species.

The results presented in this paper, confirming the identity of the two prevalent parasitoids attacking the large cabbage white in South Africa, provide a first step towards enabling further investigation of the impact, biology and potential expansion of this alien invasive butterfly species in the region. Further research, especially regarding parasitism of the egg and pupal stages, is expected to reveal more parasitoid species in South Africa.

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ORCID IDs

Simon van Noort – <https://orcid.org/0000-0001-6930-9741>
Jose Fernandez-Triana – <https://orcid.org/0000-0003-0425-0309>
Hannes Baur – <https://orcid.org/0000-0003-1360-3487>
Mark R Shaw – <https://orcid.org/0000-0002-6651-8801>

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