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

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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 Velddrift 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 Palaearctic 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 (Benjamini 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 Huddleston 1991; Askew and Shaw 1997; Shaw et al. 2009). Together these are the most effective parasitoids of *P. 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 *Piersis 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 Ichneumoidea 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...
with *P. brassicae* is confirmed. Apart from *C. glomerata*, the campoplegine ichneumonid, *Hypsopter ebinus* (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 rutipes* (Miller, 1759) and *Apecthis compunctor* (Linnaeus, 1758) are commonly reared from *P. brassicae*, with the gregarious *Blapsidus 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 [Epipamperca succincta (Meigen) is, however, a *Pieris* specialist], some with wide host ranges e.g. species of *Pales* Robineau-Desvoidy, *Compilura* Bouché, *Phryce* 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, *Pteromalus puparum* (Shaw 1982; Shaw et al. 2009), and confirm the identity of images are available on www.waspweb.org. *glomerata* in South Africa as well as DNA barcodes for *P. brassicae* provide a species determination for the microgastrine braconid (Claassens 1998; Picker and Griffiths 2011; Prinsloo and Uys 2015). 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.

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: AADD110) 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, MU1Z20-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,
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; Oceanian: Fiji and Hawaiian Islands and Palaearctic: Armenia, Azerbaijan, Azores, Belarus, Belgium, Bulgaria, Canary Islands, China (B), 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, the Czech Republic and France (Figure 5).


Biology. Gregarious endoparasitoid of Pieris brassicae larvae.

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

Nucleotide sequence

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

Nucleotide sequence

GGATATTAGGATTTTCTATAAGATTATTAATTTCGTT -
TAGAATTAGGAATACCTGGAAGATTAATTGGTAAT -
GATCAGATTTATAATAGAATTGTAACTTCTCATGCTTT -
TATTATAATTTTTTTATAGTTACCTGTAATAATTTG-
GAGGGGATCAATTCATTATCAAATTCATGCTGAGG

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)

Diagnosis. The diagnosis of Pteromalus puparum is here cited from Baur (2015): female femora infuscate; 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.

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 & Speith 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 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.

**ACKNOWLEDGEMENTS**

Simon van Noort was funded by South African NRF (National Research Foundation) grants: GUN 2068865, GUN 61497, GUN 79004, GUN 79211, GUN 81139, and GUN 98115. Cape Nature, the Eastern Cape Department of Environmental Affairs and the Northern Cape Department of Nature and Environmental Conservation provided collecting permits for South Africa.

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**REFERENCES**


Claassens AJM. 1995. Observations on the large white, *Pieris brassicae*