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# First cytogenetic characterisation of the parasitoid wasp *Brachymeria (Pseudobrachymeria) vesparum* (Hymenoptera: Chalcididae)

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## Abstract

Cytogenetic analyses have been widely used to understand evolutionary processes and to resolve taxonomic uncertainties in insects. In particular, the mapping of specific chromosomal regions can provide insights about the genome organisation and interspecific relationships. Considering the importance of this approach and the scarcity of chromosomal data in some groups of Hymenoptera, this study provides the first cytogenetic characterisation of the parasitoid wasp, *Brachymeria (Pseudobrachymeria) vesparum* Bouček, 1992 (Hymenoptera: Chalcididae). This species was characterised by  $2n = 10$  metacentric chromosomes. The heterochromatin was located at pericentromeric and terminal regions, being particularly conspicuous due to occupying a large part of chromosomes from pair 2. In addition, guanine–cytosine-rich blocks ( $GC^+$ ) were detected in the interstitial region of two chromosomal pairs. The data obtained were found to be useful for inferring the chromosomal rearrangements involved in speciation within *Brachymeria*, in addition to providing cytotoxic markers for further comparative cytogenetic studies.

## Introduction

Cytogenetic studies are important for assessing the genetic variation and for inferring the karyoevolution of species (King 1993; Schubert 2007), being useful for cytotaxonomy and phylogenetics (Bitencourt *et al.* 2011; Miao *et al.* 2018; König *et al.* 2019). In addition, the methodological advances in cytogenetics have allowed the development of refined investigations into the microrearrangements in the karyotype of certain groups (Bitencourt *et al.* 2014; Gokhman *et al.* 2016). In particular, analyses about heterochromatin composition have revealed unique karyotypic traits at the species and population levels of many organisms, including insects (Lorite *et al.* 1999; Gokhman 2021).

A large number of cytogenetic reports in the order Hymenoptera are available. They range from chromosomal analyses to genome sequencing, but most are restricted to ants, social wasps, and bees (Pompolo and Takahashi 1990; Brito *et al.* 2005; Lorite and Palomeque 2010; Carvalho and Costa 2011; Menezes *et al.* 2013, 2014; Cristiano *et al.* 2014; Barros *et al.* 2016). For parasitoid wasps, cytogenetic information has been reported in about 500 species, which is considered a small number when compared to the remarkable richness of this group of insects (Gokhman 2009).

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Accordingly, the parasitoid wasps of the superfamily Chalcidoidea comprise nearly 23 000 species distributed into 22 families (Huber 2017). Amongst them, the Chalcididae represent a family characterised by wide variation in colour and size of species recognised as solitary endoparasitoids of several orders of holometabolous insects, mainly larvae of Diptera and pupae of Lepidoptera. Other species in this family have been recorded as parasitoids of Coleoptera, Hymenoptera, and Strepsiptera, and others yet are obligatory hyperparasitoids of Ichneumonoidea and Tachinidae (Tavares and Araújo 2007). In addition, gregarious and ectoparasitoid species are reported within Chalcididae (Universal Chalcidoidea Database Community 2023). Currently, this family is divided into 87 genera and 1464 valid species, including 28 genera and 439 taxa in Neotropical regions. Sixteen genera and 219 species are reported in Brazil (Universal Chalcidoidea Database Community 2023).

Considering the wide diversity of Chalcidoidea, this superfamily remains underrepresented in cytogenetic studies: karyotype information is available for only 1% (about 240) of valid species (Gokhman 2020). These data are even scarcer in the family Chalcididae, for which chromosomal studies are restricted to karyotyping based on traditional methods of five species from three genera (Hung 1986; Amalin *et al.* 1988; Johnson *et al.* 2001). *Brachymeria* is the most studied genus inasmuch as karyotypic data are available for *B. lasus* Walker ( $2n = 10$ ), *B. ovata* Say ( $2n = 10$ ), and *B. intermedia* Nees ( $2n = 6$ ) (Hymenoptera: Chalcididae) (Hung 1986), the latter being characterised by the lowest haploid number ( $n = 3$ ) ever recorded for Chalcidoidea (Gokhman 2020).

The goal of the present study is to provide the first chromosomal data in the parasitoid wasp, *Brachymeria* (*Pseudobrachymeria*) *vesparum* Bouček, 1992 (Hymenoptera: Chalcididae). We also report novel information about C-banding and base-specific fluorochrome staining for species in the Chalcididae family – information that can be used to infer the species' karyoevolutionary pathways.

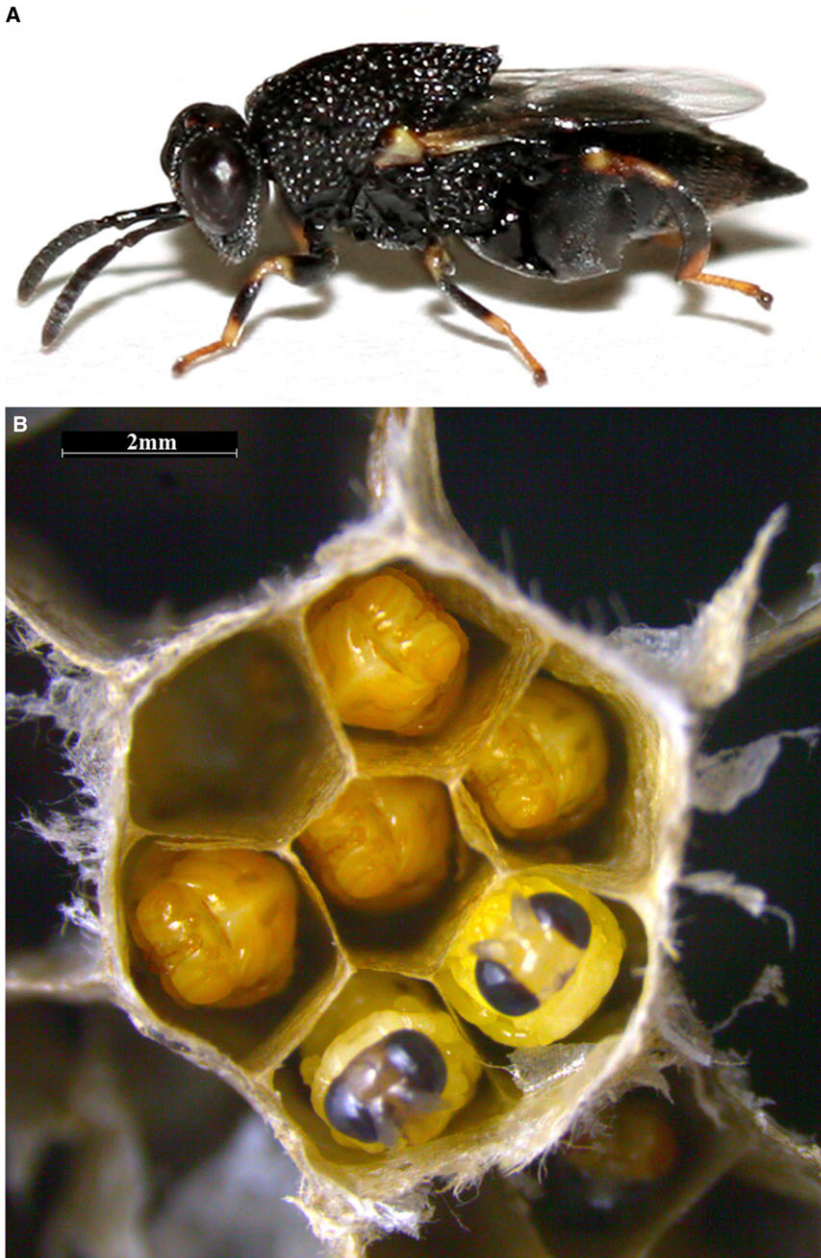
## Materials and methods

Adults and immatures of *B. vesparum* (Fig. 1A) were obtained from nests of the social wasp, *Polistes canadensis* Linnaeus (Hymenoptera: Vespidae), found as hyperparasites of immature forms of the parasitoid wasp, *Pachysomoides* sp. (Hymenoptera: Ichneumonidae) (Fig. 1B), at Universidade Estadual do Sudoeste da Bahia (UESB) – Campus II, Jequié, Bahia, Brazil ( $13^{\circ} 51' 4''$  S and  $40^{\circ} 4' 52''$  W). About 10 nests containing parasitised cells were collected. The adults that emerged were stored in 70% alcohol and were identified by taxonomist Dr. Marcelo Tavares, Universidade Federal do Espírito Santo (UFES; Vitória, Espírito Santo, Brazil).

The mitotic metaphases were obtained from the cerebral ganglia of larvae in the prepupal stage according to Imai *et al.*'s (1988) air-drying technique. Chromosomes were stained in 10% Giemsa solution (Merck KGaA, Darmstadt, Germany) in Sørensen buffer (0.06 M; pH 6.8, according to the protocol of Guerra and Souza 2002) for determining chromosomal number and morphology.

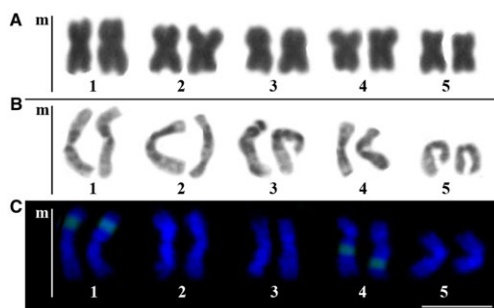
The pattern of heterochromatin distribution was determined by C-banding (Sumner 1972), with slight modifications (Siqueira *et al.* 2008). The regions in the pairs of bases rich in AT (adenine and thymine) and GC (guanine and cytosine) were mapped on chromosomes by base-specific fluorochrome staining, using chromomycin A<sub>3</sub> (CMA<sub>3</sub>), distamycin (DA), and 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich–Merck KGaA, Darmstadt, Germany), as reported by Schmid (1980).

The best metaphases were analysed and photographed using a Solaris-T microscope (BEL Engineering, Monza, Italy) fitted with a portable digital camera SCMOS mini USB2.0 SCMOS00350KPA model (MEKEY, Chongqing, China). The karyotypes were arranged by pairing chromosomes in decreasing order of size using Adobe Photoshop CS6 (Adobe, Inc., San Jose, California, United States of America), and the haploid number was determined from the



**Figure 1.** **A**, Representative specimen of *Brachymeria* (*Pseudobrachymeria*) *vesparum* obtained from nests of the social wasp *Polistes canadensis* (Hymenoptera: Vespidae) and **B**, hyperparasitizing immatures of the parasitoid wasp *Pachysomoides* sp. (Hymenoptera: Ichneumonidae). Approximate size, Fig. 1A: 1 mm.

modal frequencies. The chromosomal morphology was classified based on their arm ratio (Levan *et al.* 1964), using the software Image Pro Plus (<https://mediacy.com/image-pro/>). The ideograms representing the karyotype of *Brachymeria* species were established using the software EasyIdio, version 3.0 (Diniz and Xavier 2006), based on chromosomal measurements obtained in the present study and on data available in the literature for the genus (Hung 1986).



**Figure 2.** Metaphases of specimens of *Brachymeria (Pseudobrachymeria) vesparum*: **A**, after conventional Giemsa staining; **B**, C-banding; and **C**, base-specific fluorochrome staining. The scale bar corresponds to 10  $\mu\text{m}$ .

## Results

All specimens of *B. vesparum* showed  $2n = 10$  metacentric chromosomes (fundamental arm number or FN = 20) with a karyotype formula of  $2K = 10\ m$  (Fig. 2A). The C-banding revealed pericentromeric heterochromatic blocks at most chromosomes and at the terminal regions on pairs 3 and 4 and nearly the entire short arms of pair 2 (Fig. 2B). After base-specific fluorochrome staining (CMA<sub>3</sub>/DA/DAPI), most heterochromatic regions were AT-rich (AT<sup>+</sup>). However, GC-rich (GC<sup>+</sup>) sites were identified at interstitial regions on short and long arms of pairs 1 and 4, respectively (Fig. 2C). Heteromorphic GC<sup>+</sup> signals were also observed between homologous chromosomes from pair 4.

## Discussion

About 53 species of *Brachymeria* are described for the Neotropical region (Tavares and Araújo 2007), but to date, cytogenetic data are available for only three species. The diploid values reported in this genus range from  $2n = 6$  in *B. intermedia* (Hung 1986) to  $2n = 10$  in *B. lasus*, *B. ovata* (Hung 1986), and *B. vesparum* (present study).

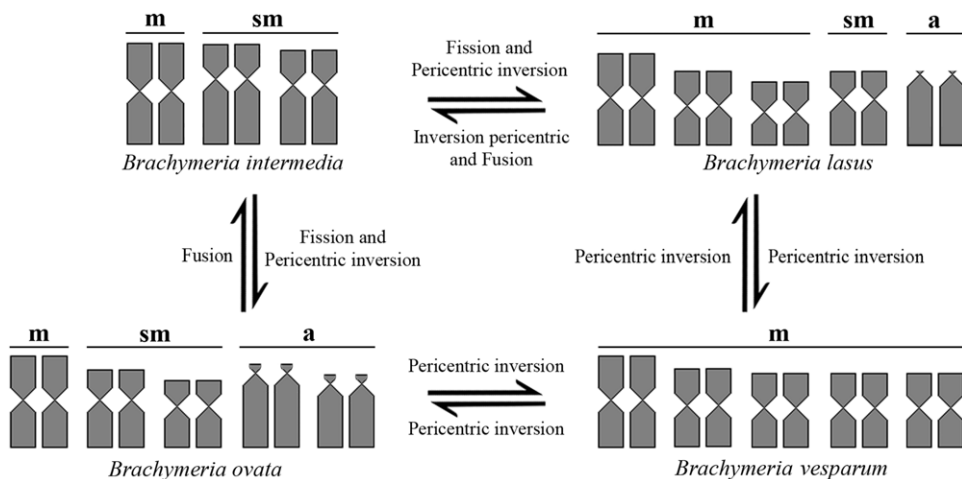
In general, the chromosomal information in parasitoid wasps of the superfamily Chalcidoidea, with few exceptions, indicates the species might be divided into two groups: (1) families characterised by low chromosomal numbers ( $n = 3-7$ ) and (2) families with higher chromosomal numbers ranging from  $n = 8$  to  $n = 11$  (Gokhman 2020). The putative ancestor haploid number of parasitoid hymenopterans ranges from 14 to 17, indicating multiple reduction events of chromosomal numbers within some lineages (Gokhman 2004), such as Pteromalidae and Eulophidae (Gokhman 2009, 2020). Conversely, the basal haploid values in Chalcidoidea vary from  $n = 3$  to  $n = 11$ , with a predominance of  $n = 5$  and  $n = 6$ . In this case, centric fusions are regarded as the main rearrangements involved in the karyoevolution of this group (Gokhman 2013, 2022).

The lowest chromosome number among Chalcidoidea,  $n = 3$ , has been found in *Brachymeria intermedia* and in certain members of the families Aphelinidae and Perilampidae (Hung 1986; Baldanza *et al.* 1999; Gokhman 2005). Despite the apparent conservatism of the diploid number in *Brachymeria*, variation in the karyotype formulae has been reported in this genus (Hung 1986). In general, congeneric species share karyotypes with a predominance of metacentric pairs, but submetacentric and acrocentric chromosomes have also been reported in the three species cytogenetically analysed (*B. intermedia*, *B. ovata*, and *B. lasus*; Hung 1986), except for *B. vesparum* from the present study (Table 1).

The unique karyotype formula herein reported for *B. vesparum*, when compared to the other congeneric species, suggests that structural rearrangements, such as pericentric inversions and centric fusions, played a key role in the karyotype evolution of these insects (Fig. 3). Even if the

**Table 1.** Cytogenetic data available in species of the family Chalcididae with their respective haploid (n) and diploid (2n) numbers, karyotype formulae, and origin of samples

Species	n	2n	Karyotypic formula	Country	References
<i>Brachymeria intermedia</i> (Ness, 1966)	3	6	2K = 2m + 4sm	Germany	Hung 1986
<i>Brachymeria lasus</i> (Walker, 1841)	5	10	2K = 6m + 2sm + 2a	Japan	Hung 1986
<i>Brachymeria ovata</i> (Say, 1824)	5	10	2K = 2m + 4sm + 4a	United States of America	Hung 1986
<i>Brachymeria (pseudobrachymeria) vesparum</i> (Boucek, 1992)		10	2K = 10m	Brazil	Present study
<i>Dirhinus himalayanus</i> (Westwood, 1836)	5	10	–	Philippines	Amalin <i>et al.</i> 1988
<i>Psilochalcis breviaalata</i> (Grissell and Johnson, 2001)	6	12	2K = 8m + 4sm	United States of America	Johnson <i>et al.</i> 2001

**Figure 3.** Ideograms representing the karyotypes of *Brachymeria* species (*B. intermedia*, *B. lasus*, and *B. ovata* by Hung (1986) and *B. (Pseudobrachymeria) vesparum*, from the present study), indicating the putative structural rearrangements that took place along the karyoevolution of the genus.

chromosome pairs are grouped into one-armed (acrocentric) and biarmed (meta/submetacentric) classes, the karyotype structure informs how the *Brachymeria* species have differentiated, given that *B. vesparum*, *B. lasus*, *B. ovata*, and *B. intermedia* present FN = 20, FN = 18, FN = 16, and FN = 12, respectively.

Genome architecture, including the chromosomal structure, is directly related to the transmission of genetic traits within and among populations and therefore is capable of promoting species diversification (Feder *et al.* 2011). Differences related to chromosomal rearrangements have been related directly to speciation events (Potter *et al.* 2017). For example, heterozygous inversions or translocations might affect gametogenesis, leading to infertility or affecting the survival of hybrid forms (Livingstone and Rieseberg 2003). In this way, the unique chromosomal features observed across *Brachymeria* species are species-specific markers for diagnosing congeners, thereby reinforcing their reproductive isolation.

Despite the major role of heterochromatin in the karyotype diversification of several animal groups (Bitencourt *et al.* 2011; Tavares *et al.* 2021), the analysis of heterochromatin distribution in the present study was poorly informative. C-bands at pericentromeric regions are commonly

reported in many species of the superfamily Chalcidoidea (Gokhman and Westendorff 2000; Gokhman 2022), and the presence of heterochromatic chromosomal arms has also been observed in some parasitoid wasps (Baldanza *et al.* 1999; Gokhman and Westendorff 2000). Such conspicuous C-bands most likely represent remnants of heterochromatin from chromosomes after fusion events, thereby supporting the role of chromosomal rearrangements in the evolution of this group of insects that previous studies (*e.g.*, Gokhman 2013) have proposed.

As for the composition of heterochromatin revealed by fluorochrome staining, the species examined in the present study revealed both AT- and GC-rich regions in two chromosome pairs, which Baldanza *et al.* (1999) reported in other parasitoids. Furthermore, the GC<sup>+</sup> sites on pair 4 were heteromorphic, possibly as a result of a heterozygous paracentric inversion, similar to what Tavares and Teixeira (2021) reported in *Pachodynerus nasidens* (Eumeninae). Gokhman *et al.* (2017, 2019) reported positive CMA<sub>3</sub> signals at the interstitial region in two parasitoid wasps, *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) and *Baryscapus silvestrii* Viggiani and Bernardo, 2007 (Hymenoptera: Eulophidae), as well as at the terminal regions of all chromosomes in the karyotype of *Trichospilus diatraeae* (Cherian and Margabandhu, 1942) (Hymenoptera: Eulophidae).

The mapping of nucleolus organiser regions (NORs) in karyotypic studies has also been carried out in cytogenetic studies of parasitoids (Baldanza *et al.* 1999; van Vugt *et al.* 2005), usually revealing single or double NORs in Chalcidoidea (Gokhman 2022). These regions can be identified by several methods, including base-specific fluorochrome staining because NORs are usually interspersed with GC-rich sites (CMA<sub>3</sub><sup>+</sup> signals; Schweizer 1980). However, it should be pointed out that additional fluorochrome signals unrelated to NORs might be present throughout the chromosomal DNA (Gokhman 2022). Accordingly, caution is recommended before concluding that the interstitial CMA<sub>3</sub><sup>+</sup> signals on pairs 1 and 4 of *B. vesparum* refer to NORs. For this reason, other techniques, such as silver nitrate staining and fluorescent *in situ* hybridisation with rDNA probes, should also be carried out to confirm the location of ribosomal cistrons.

Even though refined methods of chromosomal analyses have been performed on insects (van Vugt *et al.* 2005, 2009; Bolsheva *et al.* 2012; Gokhman *et al.* 2017), these reports remain scarce in parasitoid wasps (Gokhman 2010; Gebiola *et al.* 2012), and several taxonomic uncertainties in these insects have yet to be resolved. The karyotypic data described in the present study for *Brachymeria* advance the understanding of evolutionary mechanisms and cytogenetic patterns for the examined species. Moreover, the karyotype structure and the distribution of GC-rich heterochromatic sites provide useful diagnostic characters for cytotaxonomy. We therefore recommend that similar studies examine other members of the family Chalcididae to reveal and refine understanding of the evolutionary and systematic inferences in these insects, including the potential identification of cryptic forms or species complexes.

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**Competing interests.** The authors declare they have no competing interest.

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