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First record of a gynandromorph of *Osmia submicans* MORAWITZ, 1870 (Hymenoptera, Megachilidae) – characterisation by morphological and morphometric parameters and critical note on gynander classification

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A b s t r a c t : The first record of a gynandromorph of *Osmia submicans* MORAWITZ, 1870 (Hymenoptera, Megachilidae) with a nearly complete bilateral asymmetry is presented. However, several alternating asymmetries of female and male traits occur additionally on the tagma level. The article presents an analysis of 25 morphological (non-meristic) characteristics (integument colour, pubescence, structural features) and 34 morphometric parameters of females and males. The morphometric differences were tested statistically. The left and right gynander sides were compared with those of females and males (using multivariate analysis for parameter measurements). The gynanders within the genus *Osmia* described so far in the literature were typified again and compared with the gynander presented here. The conventional type classification of gynanders is critically discussed. Criteria that may be relevant for an analysis of gynanders are presented.

K e y w o r d s : Anthophila, gynander, gynandromorphism, morphometric differentiation, principal component analysis.

Introduction

In a gynandromorph (gynander), female and male phenotypic traits occur in the same individual (AKRE et al. 1982). These features may be developed within different tagmata, such as head, mesosoma or metasoma (GORDH & HEADRICK 2001, MICHEZ et al. 2009, NARITA et al. 2010, HINOJOSA-DÍAZ et al. 2012). The first observation of a Hymenopteran gynander was made in the year 1801 by J.G. Lukas for a honeybee (*Apis mellifera*), and was published by LAUBENDER (1801). The specimen was characterised as 'Stacheldrohne' which means 'spiny drone' (DALLA TORRE & FRIESE 1899). According to a literature review by ALMEIDA et al. (2018), gynandromorphs have been reported among wild bees (Hymenoptera, Anthophila) in about 140 species within 35 genera. Specimens within the Andreninae, Apinae, Halictinae, Colletinae, Megachilinae and Melittinae predominate (ALMEIDA et al. 2018). A high proportion of gynandromorphs in the Megachilinae was indicated by WCISLO et al. (2004), with a particularly high number found in the genus *Megachile* (MITCHELL 1929, WCISLO et al. 2004, MICHEZ et al. 2009, HINOJOSA-DÍAZ et al. 2012, COELHO et al. 2016). Gynandromorphs also occur in high numbers within the genus *Xylocopa* (Xylocopinae) (LUCIA et al. 2009, 2010, 2012; LUCIA & GONZALEZ 2013; ZAMA & COELHO 2017).

Several possible causes for gynandromorphism in the systematic group of Anthophila have been discussed in the literature. These include chromosome elimination, polyspermy, double fertilisation of a binucleate egg, loss of a sex chromosome or up-/downregulation of sex-determining genes, mutations, and genetic incompatibilities (NESBITT & GARTLER 1971, NARITA et al. 2010). MICHEZ et al. (2009) hypothesise that mosaic gynanders may be the result of chromosomal aberrations and eliminations, or may be due to the expression of a single locus of complementary sex determination in different tissues during embryonic development. According to the authors, transverse gynanders and bilateral gynanders may be interpreted as the result of a solitary genetic defect. *Wollbachia* infections and stylopisation can also cause gynandromorphs (STOECKHERT 1924, SALT 1927, MITCHELL 1929, POPOV 1937).

In honeybees, gynandromorphs generally develop out of the combination of a diploid zygote and additional haploid male tissues derived from a second sperm (MORGAN 1905, ROTHENBUHLER et al. 1952, DRESCHER & ROTHENBUHLER 1963). In this case of polyspermy, more than one sperm penetrates the egg (BAER et al. 2016). This so-called 'polyspermy hypothesis' was primarily formulated by MORGAN (1905, 1916). Gynandromorphic bees can therefore carry several separate paternal genomes, which arise from the fact that additional sperms divide and, in addition to the diploid tissue of the zygote, also form androgenic, haploid, male tissues. Depending on the extent to which the maternal or paternal pronuclei have divided before the formation of the zygote, this can lead to different types of gynandromorphs. Haplodiploidy allows almost any combination of gametes present in an ovule (for example, the four maternal pronuclei and one or more sperm pronuclei) to fuse and to form a zygote, or not to fuse and to develop independently as a haploid tissue (AAMIDOR et al. 2018). In wild bees, the reason for gynandromorphy, which usually occurs in less than 0.001 per cent of solitary ones, is haplopolyploidy (JALLON & HOTTA 1979, AKRE et al. 1982, KINOMURA & YAMAUCHI 1994, LUCIA et al. 2009).

There are studies in which the behaviour of gynandromorphs is analysed in more detail (*Bombus*: MICHEZ et al. 2009, UGAJIN et al. 2016, MATSUO et al. 2018; *Chalicodoma*: BISCHOFF & ULRICH 1929; *Dasypoda hirtipes*: MICHEZ et al. 2009; *Euglossa*: GIANGARELLI & SOFIA 2011; *Megaloptera*: KRICHILSKY et al. 2020; *Osmia*: SAMPSON et al. 2010). It is generally assumed that gynandromorphic behaviour correlates with the phenotype of the abdomen rather than with the phenotype of the head or thorax (MAENO & TANAKA 2007, SAMPSON et al. 2010).

The rare occurrence of gynanders makes comprehensive studies in developmental biology or molecular genetics difficult, and histological analyses are also rare (BISCHOFF & ULRICH 1929). But all phenomena that occur in gynanders have led to a variety of hypotheses. This applies, for example, to questions of the regulation and coordination of phenotypic traits in a wide range of different body areas (HINOJOSA-DÍAZ et al. 2012, CAMARGO & GONÇALVES 2013). Studies on gynanders can also suggest explanations for mechanisms of sex determination and sex differentiation (NARITA et al. 2010).

In this publication, the first case of a gynander of *Osmia submicans* is presented. Besides a morphological (non-meristic) characterisation, further extensive morphometric as well as multivariate methods are included in the gynander analysis. The results are discussed in a broader framework that includes questions of typification and characterisation of gynanders in wild bees. Furthermore, a critical analysis of the type classification of gynanders is made, and further suggestions for typification and characterisation of gynanders are presented.

Review of gynandromorphs in *Osmia* described in the literature

Sixteen gynandromorphic individuals (belonging to eight species) within the genus *Osmia* have been reported so far. HINOJOSA-DÍAZ et al. (2012) mentioned only seven individuals. Gynanders were found most frequently in *O. caerulescens* (N = 5), followed by *O. rufa* (N = 3 each), *O. aurulenta* and *O. bicolor* (N = 2 each). In the species *O. fulviventris*, *O. obtusa*, *O. pentstemonis*, *O. ribifloris* gynanders have only been found once so far (Table 1). With the gynander of *O. submicans* presented here, the number will increase to 17 in the genus *Osmia* and nine species (Table 1).

Table 1: Gynanders described within the genus *Osmia*.

Gynander	Autor
O. aurulenta (PANZER, 1799)	WOLF (1991)
O. aurulenta (PANZER, 1799)	WOLF (2003b)
O. bicolor SCHRANK, 1781	BEAUMONT (1936)
O. bicolor SCHRANK, 1781	WOLF (1990)
O. caerulescens (LINNAEUS, 1758)	DALLA TORRE & FRIESE (1899)
O. caerulescens (LINNAEUS, 1758)	BENNO (1948)
O. caerulescens (LINNAEUS, 1758)	NIEUWENHUISEN (1995)
O. caerulescens (LINNAEUS, 1758)	TORRES & RAMOS (2000)
O. caerulescens (LINNAEUS, 1758)	TORRES & RAMOS (2000)
O. fulviventris (PANZER, 1798)	MORICE, F.D. (1903)
O. obtusa FRIESE, 1899	WOLF (2003a)
O. pentstemonis COCKERELL, 1906	SANDHOUSE (1923)
O. ribifloris biedermannii MICHENER, 1936	SAMPSON et al. (2010)
O. rufa (LINNAEUS, 1758)	NOSKIEWICZ (1923)
O. rufa (LINNAEUS, 1758)	BARENDRECHT (1943
O. rufa (LINNAEUS, 1758)	TUMŠS (1970) citation WCISLO (1999)
O. submicans MORAWITZ, 1870	KRATOCHWIL in this study

The descriptions of gynandromorphic *Osmia* specimens found in the literature (Table 1) were classified into five categories: (1) complete to partial bilateral asymmetry; (2) complete to partial bilateral but reciprocal asymmetry; (3) partial bilateral asymmetry with features of the frontal gynander type; (4) partial bilateral asymmetry with features of the frontal or transverse gynander type; (5) partial frontal asymmetry. Due to the different definitions of gynander types in the literature, the term ‘mixed type’ (mosaic gynander) is not used.

1. Complete to partial bilateral asymmetry

- *Osmia bicolor*: An almost complete gynander, which can be characterised as bilaterally asymmetrical, was described by WOLF (1990). Only the two antennae and the sternite 6 are similar to those of a female.

- *Osmia caerulescens*: The specimen characterised by BENNO (1948) shows an almost complete bilateral asymmetry. The exceptions are the antennae and the extremities, which are characterised as female.

- *Osmia obtusa*: A description of a complete bilaterally asymmetrical gynander of this species was shown by WOLF (2003a).

- *Osmia rufa*: The gynander described by BARENDRECHT (1943) is completely bilaterally asymmetrical.

2. Complete to partial bilateral but reciprocal asymmetry

- *Osmia caerulescens*: The gynander described in TORRES & RAMOS (2000) shows a perfect bilateral asymmetry in pubescence and in the colour of the integument of head, thorax and abdomen. This is also true for a second gynander, but the distribution of male and female characteristics of the abdomen is opposite to those of the head and thorax.

3. Partial bilateral asymmetry with features of the frontal gynander type

- *Osmia aurulenta*: In the gynander studied by WOLF (2003b), the head and metasoma are bilaterally asymmetrical, whereas the metasoma is characterised as purely female. The degree of bilateral asymmetry is less pronounced. The gynander, with its completely female abdomen, corresponds to a partially frontal gynander type.

- *Osmia caerulescens*: The gynander of NIEUWENHUISEN (1995) shows a partial bilateral asymmetry in the head area (antennae, mandible, partly also in the integument colour) and thorax (pubescence, integument colour, extremity). The abdomen is similar to that of a male. Thus, the specimen also corresponds to a partially frontal gynander type.

- *Osmia fulviventris*: A similar situation is documented in the gynander described by MORICE (1903). The head is bilaterally asymmetrical and the mesosoma partially bilaterally asymmetrical, whereas the metasoma is female in character.

- *Osmia pentstemonis*: The head and metasoma of the gynander described by SANDHOUSE (1923) are bilaterally asymmetrical and the metasoma corresponds to that of a female.

- *Osmia rufa*: In the gynander described by NOSKIEWICZ (1923), the head is bilaterally asymmetrical, while the rest of the body is characterised as female.

4. Partial bilateral asymmetry with features of the frontal or transverse gynander type

- *Osmia aurulenta*: The gynander characterised by WOLF (1991) shows simple bilateral asymmetries in the area of the head and the metasoma. The antennae and the extremities are typical for a female. The upper side of the abdomen is characterised as male, while the sternite, with ventral brush, is similar to that of a female. The abdomen thus corresponds to the transverse type.

- *Osmia bicolor*: The gynander presented by BEAUMONT (1936) is characterised as almost entirely female-like in the head region, while the thorax is bilaterally asymmetrical. The metasoma is quite complex in structure. All tergites are characterised as male. The first two sternites have bilateral asymmetry, sternite 3 is partially male and the others are all characteristically male.

- *Osmia ribifloris biedermannii*: This gynander described by SAMPSON et al. (2010) shows a bilaterally asymmetrical structure in the head region, whereas the mesosoma and metasoma (tergites 1 and 2) are only very partially characterised by bilaterally asymmetrical features.

5. Partial frontal asymmetry

- *Osmia caerulescens*: The specimen described by DALLA TORRE & FRIESE (1899) corresponds to the frontal gynander type (head characterised as female, thorax and abdomen characterised as male, but with partially female features). A bilateral asymmetry is only developed in the region of the clypeus.

The analysis of gynanders within the genus *Osmia* shows that bilateral asymmetries predominate, but these asymmetries show different degrees. They can comprise two tagmata, but also all three. There is also an example of a partially frontal gynander type (DALLA TORRE & FRIESE 1899).

Material and methods

1. Species characterisation

Osmia submicans was described by MORAWITZ (1870), and the lectotype and paralectotype were designated by ZANDEN (1991). The species is assigned to the Palaearctic subgenus *Pyrosmia* TKALCU, 1975, to which about 30 described species belong. All species are characterised by a metallic blue, green, violet or reddish integument and a pale pubescence. More rarely, the pubescence is reddish. The species often have hair bands at the tergite ends. The body sizes usually vary between 4 and 6 mm (MICHENER 2007). SCHEUCHL & WILLNER (2016) characterise the distribution range as follows: 'in the Mediterranean from the Canary Islands, on numerous Mediterranean islands to Egypt, in the Eurasian region from Portugal to Asia Minor and Central Asia, northwards to the German low mountain ranges and southern Russia'. The ssp. *hebraea* BENOIST, 1934 occurs in North Africa, Malta, Sicily, Cyprus, and in the Near East, while the ssp. *canaria* MAVROMOUSTAKIS, 1957 (La Gomera, Gran Canaria, Tenerife), the ssp. *columbina* ZANDEN, 1996 (La Palma), and the ssp. *lanzarotae* WARNCKE, 1992 (Lanzarote, Fuerteventura) occur in the Canary Islands. At the present time, the descriptions and comparative analysis of the subspecies are based on only a few morphological characteristics (ZANDEN 1996). The applied criteria for a differentiation have to be improved. The specimens from Crete are probably not identical to those of the nominate taxon. A revision of the taxa seems to be necessary. With regard to flower visitation, flowers of Fabaceae (for example, *Hippocrepis comosa* L.) are preferred (DUCKE 1900).

2. Origin of the gynander

Osmia submicans was collected in an area of weakly consolidated small dune fields between the coast and the 'Route du Sel' (Presq'île de Giens, Hyères, Département Var, Côte d'Azur, France, N43°3'33,4" E6°7'58,2", 6 m a.s.l) on 18.03.2008 (visual captures with net). The area was populated by large stands of flowering *Hippocrepis comosa* (Fig. 1). Forty-three males were found on *Hippocrepis comosa*, one male on *Sonchus arvensis* L., and one female and the gynander on *Hippocrepis comosa*. The ratio of females to males shows that the flight season of the species in the area had only just begun, and due to proterandry, almost only males were flight active at this time. The metasomal scopa of the female and the reduced metasomal scopa of the gynander did not carry pollen. The provisioning phase had not yet begun.

Fig. 1: *Osmia submicans* male, visiting flowers of *Hippocrepis comosa* (Presq'île de Giens, Hyères, France, 18.03.2008; same site where the gynander was detected); photo: A. Schwabe.

3. Preparation

The specimens were treated at low temperatures and set on a paper glue board and an insect pin. The genitalia were extracted from the metasoma by H. R. Schwenninger (Stuttgart, Germany) and dissected for further morphological analysis.

4. Morphological, morphometric and multivariate analysis

For the comparison of the female and male parts of the gynander, females and males were primarily analysed in more detail morphologically and tagma-specifically. The analysis was carried out on 25 morphological (non-meristic) parameters. The following parameters were taken into account[1]: the colouration of the integument (head, thorax including lateral propodeum, T1-T6); the length, density and colouration of the pubescence (vertex, frons, ocellus area, paraocular area, clypeus, scutum, scutellum, mesepisternum, T1-T6, S1-S6); the number of the flagellomeres; the structure of the anterior margin of clypeus, the mandible, the terminal tarsi, T7, S1-S6, the genitalia, the presence of a spine. The characterisation of the genitalia followed Akre et al. (1982), Michener (2007), and Packer (2003). In general, it should be noted that the designation left/right half of the body refers to the dorsal view. The terms 'head', 'mesosoma' and 'metasoma' are used for the individual tagmata. The term 'mesosoma' is retained, although the first abdominal segment is properly the propodeum (Michener 1944).

[1] T1, T2, etc. = first, second, etc., metasomal terga; S1, S2, etc. = first, second, etc., metasomal sterna.

Furthermore, 34 morphometric (meristic) parameters were analysed on seven females, seven males and the gynander (Table 2). The measured distances are shown in Fig. 2. Transversal sections were measured only halfway in females and males, so that a comparison with the left and right halves of the body of the gynander was possible. In no case could asymmetries in the females or in the males be detected. Longitudinal sections

Table 2: Abbreviations, character name, definition and magnification of the 34 parameters used for the morphometric analyses; definition of parameters and method of measurements according to MICHENER (2007). * = Since the antennal condyle (articulatory bulb) is mobile and the antennal fossa (antennal socket, antennal insertion) is often difficult to see, the lower annular sclerotised edge of the torylus, which is not covered by the antenna, is used for measurement. Abbreviations: fv = frontal view, dv = dorsal view, lv = lateral view, mam = measured along midline.

Abbreviation	Character name	Definition	Magnification
AOED	Antennocellar distance	Distance between the lower ocellus edge and the upper torylus edge of the antenna* (fv)	100x
AOUD	Antennocular distance	Distance between the antennal base and the eye (fv)	100x
BL	Body length	Maximal length of the body from the antennal base to the tip of the pygidium (dv)	16,25x
CAD	Clypeoantennal distance	Distance between the antennal base and the clypeus (fv)	100x
CL	Clypeus length	Maximal length of the clypeus (fv)	62,5x
EL	Eye length	Maximal length of the eye (lv)	62,5x
EW	Eye width	Maximal width of the eye (lv)	62,5x
HFL	Hind femur length	Maximal length of the hind femur (mam, lv)	62,5x
HL	Head length	Maximal length of the head from top of the vertex to the edge of the clypeus (fv)	40x
HML	Hind metatarsus length	Maximal length of the hind metatarsus (mam, lv)	100x
HMW	Hind metatarsus width	Maximal width of the hind metatarsus (mam, lv)	100x
HT2L	Hind tarsus 2 length	Maximal length of the second hind tarsus (mam, lv)	100x
HT2W	Hind tarsus 2 width	Maximal width of the second hind tarsus (mam, lv)	100x
HT3L	Hind tarsus 3 length	Maximal length of the third hind tarsus (mam, lv)	100x
HT3W	Hind tarsus 3 width	Maximal width of the third hind tarsus (mam, lv)	100x
HT4L	Hind tarsus 4 length	Maximal length of the fourth hind tarsus (mam, lv)	100x
HT4W	Hind tarsus 4 width	Maximal width of the fourth hind tarsus (mam, lv)	100x
HT5L	Hind tarsus 5 length	Maximal length of the fifth hind tarsus (mam, lv)	100x
HT5W	Hind tarsus 5 width	Maximal width of the fifth hind tarsus	100x
HTL	Hind tibia length	Maximal length of the hind tibia (mam, lv)	62.5x
HTW	Hind tibia width	Maximal width of the hind tibia (mam, lv)	100x
HW	Head half width	Maximal half width of the head from the outer ridge of the eye to the centre (fv)	40x
IAHD	Interantennal half distance	Half distance between the antennal bases (fv)	100x
IOHD	Interocellar half distance	Half distance between the upper ocelli (fv)	100x
LID	Lower interocular half distance	Shortest half distance of the lower distance between the eyes (fv)	62,5x
MCHW	Mesoscutum half width	Maximal width of the half of the mesoscutum from the margin to the centre, characterised by the medium line (dv)	40x
MOHW	Mesosoma half width	Half of the greatest width of the mesosoma (dv)	40x
MSL	Mesoscutum length	Maximal length of the mesoscutum along the median line from the posterior edge of the pronotum to the anterior edge of the scutellum (dv)	40x
OOD	Ocellular distance	Distance between the nearest ocellus and the eye (fv)	100x
PSL	Pterostigma length	Maximum length of the pterostigma; distance between the proximal base of the pterostigma and the position where the vein R1 has its typical width (MICHENER 2007)	100x
SHW	Scutellum half width	Maximal width from the margin of the scutellum to the centre of the scutellum (dv)	62,5x
SL	Scutellum length	Length of the scutellum along the median line from the posterior border of the the mesoscutum to the posterior border of the scutellum (dv)	62,5x
UID	Upper interocular distance	Shortest half distance of the upper distance between the eyes (fv)	62,5x
WL	Wing length	Maximal length of the forewing from the wing base to the wing tip	25x

using boxplots (boxplot function; R CORE TEAM 2016). The lower and upper parts of the box are the 25th and 75th percentiles (lower quartile and upper quartile), and the central marker indicates the median (the 50th percentile). The ends of the vertical lines indicate the minimum and maximum data values. The outliers were plotted individually with an 'o'. Data sets were compared using the Welch two sample t-test (R CORE TEAM 2016, RASCH et al. 2011). A principal component analysis (PCA) was used to show the morphometric differences between females and males compared to the gynander separately for the left and right side. The calculations were made with PAST 4.04 (HAMMER-MUNTZ et al. 2001). Morphological and morphometric analyses were carried out with a modular stereomicroscope Wild M3Z, Heerbrugg, Switzerland, with a 25x eyepiece (16.25x, 40x, 62.5x and 100x). INKSCAPE (2020) and EAZYDRAW (2020) programmes were used for the drawings.

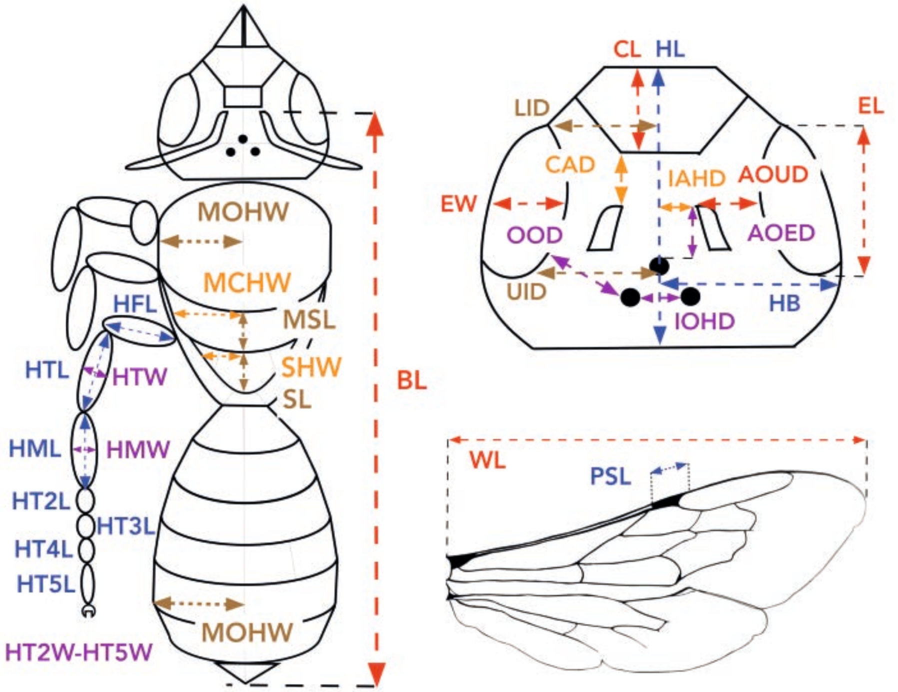

Fig. 2: Ranges of measurements of various morphometric parameters (abbreviations, character names, definitions; see Table 2).

5. Photo documentation

The macro-photos of the head and the upper and lower abdomen of the gynander were taken at the Upper Austrian State Museum Linz, Austria by L. Haitzinger. The photos of the gynander genitals were taken by J. Fricke (State Museum of Natural History, Karlsruhe, Germany).

Results

1. Qualitative morphological results

Table 3 shows the morphological differences of the two sexes and the distribution of female and male characteristics on the gynander. The analysis of the female and male features is shown schematically in Fig. 3 (left dorsal side; right ventral side). In the following, the results are presented tagma-specifically.

Table 3: Comparison of morphological characteristics of females, males and the gynander of *Osmia submicans*.

Colour Tagma	Area	Female	Male	Gynander
Prosoma	***Head***	Black to dark bluish or black to blue-green metallic coloured integument	Greenish-bronze to ore-coloured or blue-green to green metallic coloured integument	***Female character*:** left side black exactly to the centre with a slightly green metallic coloured integument near the inner upper side of the eye (also ore-coloured); the clypeus slightly ore-coloured. ***Male character*:** right side greenish-bronze or ore-coloured integument exactly to the centre of the head; in the area of the left ocellus a slightly blue-green metallic coloured integument
Mesosoma	***Mesoscutum***	Black to dark bluish metallic coloured integument	Greenish-bronze to ore-coloured or blue-green to green metallic coloured integument	***Female character*:** right side. ***Male character*:** left side
	Lateral propodeum	Black to dark bluish metallic coloured integument	Greenish-bronze to ore-coloured or blue-green to green metallic coloured integument	***Female character*:** right side. ***Male character*:** left side
	Tegulae	Black to dark bluish metallic coloured integument	Greenish-bronze to ore-coloured or blue-green to green metallic coloured integument	***Female character*:** left side. ***Male character*:** right side
Metasoma	***Tergites 1-6***	Black with slightly blue to blue-green metallic coloured (dark bluish) integument	Greenish-bronze to ore-coloured or blue-green to green metallic coloured integument	***Female character*:** right side of T1-T3; T4 only the left half of the right tergite side. ***Male character*:** left side of T1-T4 predominantly greenish-bronze to ore-coloured integument; T1 with dark bluish colour; T4 left side blue to blue-green metallic colour; right half of the right tergite side with greenish-bronze to ore-coloured integument; T5 and T6 predominantly greenish-bronze to ore colour

Table 3ff.

Pubescence				
Tagma	**Area**	**Female**	**Male**	**Gynander**
Prosoma	***Vertex***	Longer whitish-yellow hairs	Longer golden hairs	***Female character*:** left side. ***Male character*:** right side
	Frons	Single long grey-white hairs	Single long golden yellow hairs	***Female character*:** left side. ***Male character*:** right side
	Ocellus area	Only very slightly yellowish hairs	Dense long golden yellow hairs; the supraclypeal area with whitish-yellow hairs	***Female character*:** left side. ***Male character*:** right side
	Paraocular area	Longer denser white hairs	Upper half light golden yellow, lower half white hairs	**Female character:** left side. ***Male character*:** right side
	Clypeus	Single white-yellowish hairs on the clypeus area; anterior margin with short golden-yellow hairs	Dense long white hairs	***Male character*:** dense long white hairs, slightly yellowish at the base; on the left side of the clypeus shorter hairs
Mesosoma	***Scutum***	Few longer grey-white hairs, shorter at the edges	In the centre and laterally longer and denser golden yellow hairs	***Female character***
	Scutellum	Long grey-white hairs on the entire area	Longer and denser golden yellow hairs	***Female character***
	Mesepisternum	Callus (pronotal lobe) with long, dense, whitish-grey hairs; the mesopleures also with whitish-grey hairs	Callus long light golden hairs; the mesopleures above with light yellow, below with white hairs	***Female character***
Metasoma	***Tergite 1***	Long white lateral hairs (loose tufts)	Long light golden yellow lateral hairs (loose tufts)	***Female character*:** right side. ***Male character*:** left side
	Tergites 1-5	White hair bands; those of T1 and T2 interrupted	White hair bands; those of T1-T3 interrupted	***Female character*:** right side of T1 and T2 with interrupted white hair bands; T3 with continuous white hair band to the centre; T4 right half of the left side with a white hair band; T6 right side more densely covered with whitish hairs. ***Male character*:** left side of T1 and T2 with marginal golden yellow hairs; T3 and T4 with golden yellow, not interrupted loose hair bands; T4 right half of the right side with golden yellow hair band; T5 with continuous golden yellow hair band; T6 left side side with golden yellow hairs
	Sternites	Metasomal scopa with black hairs; the lateral hairs changing to brownish and whitish colour	No metasomal scopa; sternites very short and fine hairs	***Female character*:** left side. ***Male character*:** right side

Structure				
Tagma	**Area**	**Female**	**Male**	**Gynander**
Prosoma	***Antenna***	12 antennal segments	13 antennal segments	***Female character*:** left side. ***Male character*:** right side
	Clypeus anterior margin	Frontally two thickened arch-shaped structures with a small cavity	In the centre a small hump; on the left and on the right side two non-thickened arcuate thickenings	***Female character*:** the predominant left part. ***On the right side*:** abnormal structures with a marginal anteriorly exposed thorn-like outgrowth
	Mandible	Three sharply pointed inner teeth	Two teeths	***Female character*:** right side. ***Male character*:** left side
Mesosoma	***Distitarsus***	Simple claws	Cloven claws	***Female character*:** left side. ***Male character*:** right side
Metasoma	***Tergite 7***	T7 with uniform margin	T7 with three spines of equal length	***Female character***
	Sternites	Sternites more or less uniform and not ridged	S2 large, slightly rimmed inside, overlapping S3 in the centre; S3 arched rimmed, with dense yellowish hair fringe; S4 basal zone in the centre with dense fine pubescence.	***Female character*:** left side. ***Male character*:** right side
	Male genitalia		Present	***Male character*:** right side
	Female genitalia	Present		***Female character*:** left side
	Sting	Present		***Female character*:** left side

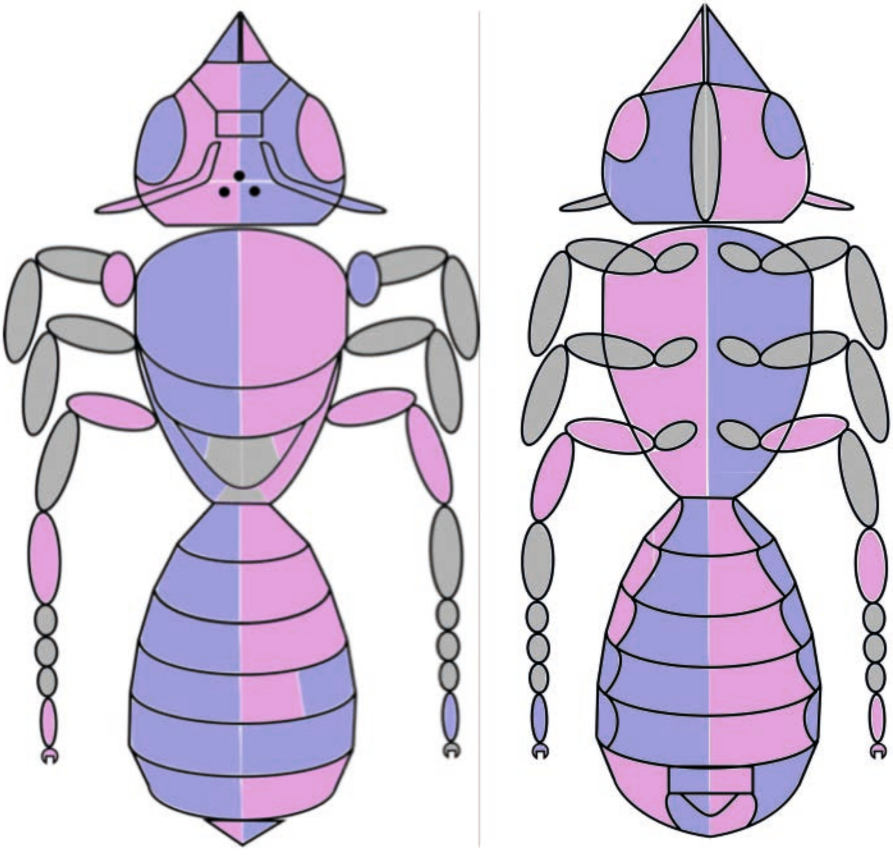

Fig. 3: Areas of the gynander with female (pink) and male (blue-violet) morphological features, schematised (left = dorsal side; right = ventral side). The results for femura, tibia, metatarsi and tarsi 5 were assigned on the basis of the morphometric results. In ventral view, the left side corresponds to the right half of the body, the right side to the left half of the body. No differences could be detected in areas marked in grey.

- Prosoma: The head of the gynander is shown in Fig. 4 (schema in Fig. 3). The mandible on the left side corresponds to a bidentate male mandible, and that on the right side to a tridentate female mandible. The remaining areas, with the exception of the clypeus and the eye, show female characteristics on the left side, and male characteristics on the right side. This applies to the colour of the integument and the colour of the pubescence in the vertex, frons, ocellus area and paraocular area. The antenna on the left side of the head has 12 antennal segments, that on the right side 13 segments. The clypeus also shows female characteristics in the integument colouration on the left side, and male characteristics on the right side. In pubescence, both sides of the clypeus correspond to that of a male (dense white hairs at the base of the clypeus, slightly tinged with yellow). On the left side of the clypeus, the pubescence is even more pronounced than on the right side. The anterior margin of the clypeus on the left side corresponds to a female (a small central hump, but no thickened, arcuate outgrowth). The right anterior margin has a straight outgrowth laterally, approximately analogous to the curved outgrowths of *O. cornuta* (LATREILLE, 1805) or *O. cerinthidis* MORAWITZ, 1876. However, such outgrowths are restricted only to females in these two species. The ventral genae show male characteristics in the integument colouration on the left half of the head (dorsal right side), but female characteristics on the right half of the head (dorsal left side). The left eye is shorter in length than the right eye (see morphometric results). The eye lengths of males and females are highly significantly different. Thus, the gynander has a male eye on the left side of the head, and a female eye on the right side (Fig. 4). The distribution of the ocelli is symmetrical.

Fig. 4: Head frontal. The greenish-bronze to ore-coloured integument on the left side of the head (right side of the body), the 13 antenna segments and the dense long golden-yellow pubescence of the ocellus area, frons and paraocular area show male characteristics. The black (dark bluish) integument, the 12 antenna segments and the patchy whitish pubescence of ocellus area, frons and paraocular area show female characteristics; photo: L. Haitzinger.

- Mesosoma: The mesothorax is characterised by male features on the left side and female features on the right side concerning the integument colour of the scutum, scutellum and mesepisternum (Fig. 3). Differences in pubescence are not visible in these areas. The lateral propodeum also shows a bilateral asymmetry of features, with male characteristics on the left side and female characteristics on the right side. The same applies to the lateral parts of the propodeum. In the centre, the propodeum is equally dark and dull-coloured in males and females. The left tegula is darker-coloured at the anterior margin, while the right side shows a metallic green colour. The tarsi of males and females are clearly different. The male terminal tarsus is divided (two-toothed), while the female is simply one-toothed. Thus, the left side is characterised as female and the right side as male.

- Metasoma: On the right side, T1-T3 and T6 show the integument colour and pubescence characteristic of a female (Fig. 3, Fig. 5), and on the left side those of a male. T5 shows male characteristics, T7 female ones. Remarkably, T4 shows half-female and half-male features. On the side where the tergites show male features, the underlying sternites are all characteristically female, and where the tergites show female features, the underlying sternites are male (Fig. 3, Fig. 6). The male sternites typically show a large S2, which is slightly edged and covers the S3 centrally. This has a dense yellowish hair band. T7 is purely female in character and does not bear the three characteristic spines of a male.

Fig. 5: The metasoma has a greenish-bronze to ore-coloured integument on the left dorsal side and a golden yellow pubescence on the tergite ends of T1-T3 and T6 (male characteristics). On the right side, it has a black (dark bluish) integument and a white pubescence (female characteristics). In T4, the left side has purely male characteristics, while the right side is female on one side and male on the other. T5 has male characteristics throughout; photo: L. Haitzinger.

Fig. 6: On the left side, the sternites with male characteristics are visible. They show the greenish ore-coloured integument which is also typical for males. The large S2 is characteristic for males. On the right side the smaller female sternites are formed, bearing the black metasomal scopa; photo: L. Haitzinger.

The genitalia are typical for a male on the right side and a female on the left (Fig. 7). They thus correspond to the sternites in position and sex-specificity. In the male genitalia, the gonocoxit 9 is detectable, with the gonostylus (gonoforceps) as well as the gonapophysis 9 (penis valve) with the volsella at the base. In the female genitalia, the gonocoxite 8 (valviver 1) from which the gonapophyses (valvula I) originate, and the gonocoxite 9 (valviver 2), with the valvulae II (anterior) and gonostyli (valvula III, sting sheath) can be recognised. The gonapophysis 9 (valvula II) is also named 'stylus'; the gonapophysis 8 (valvula I) is named 'lancet'.

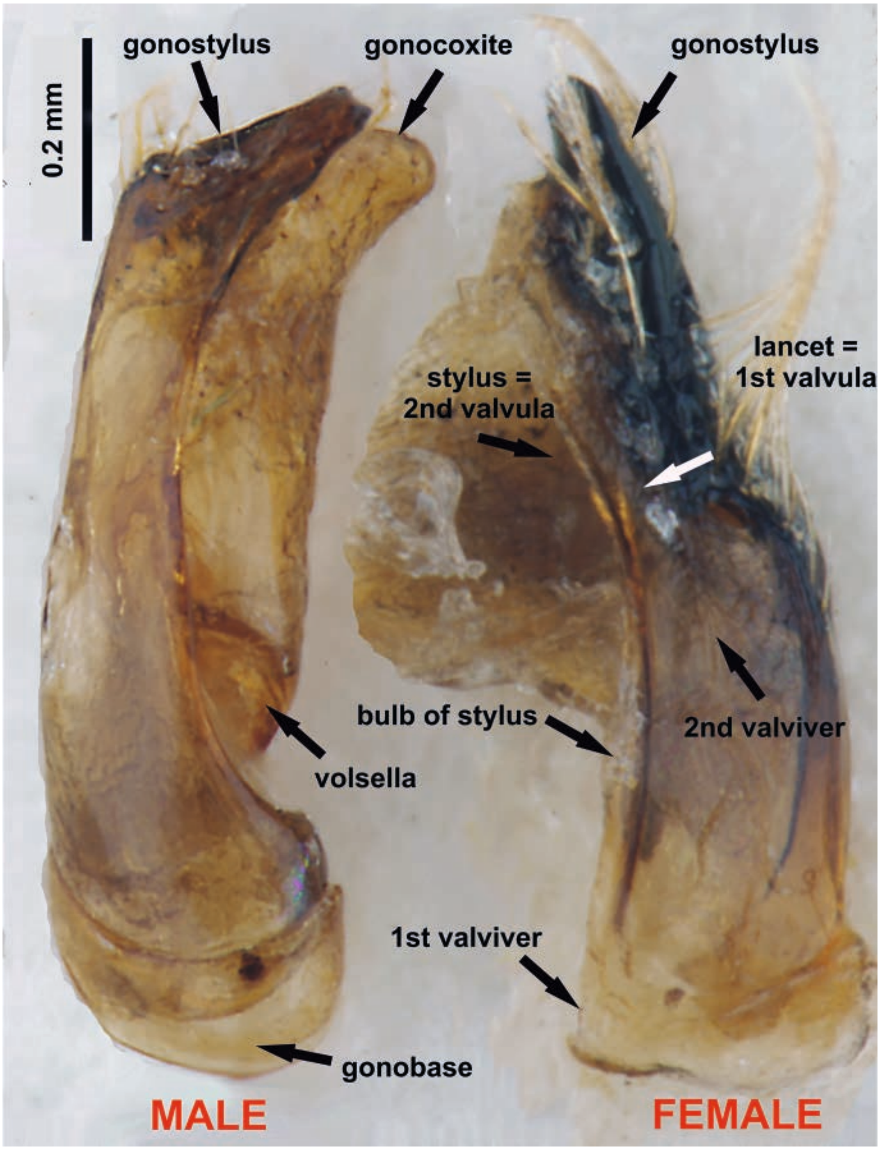

Fig. 7: Genitals of male and female characters in the gynandromorph specimen; photo: J. Fricke.

2. Morphometric results

The morphometric results of parameters are shown in alphabetical order in Table 4. In the following, the different parameters are discussed specifically and compared with the morphological results detected in the gynander. The morphometric results are additionally presented as boxplots in the case of significant differences (Fig. 8, Fig. 9).

Table 4: Morphometric results of the 34 parameters (Table 2) in females, males and the gynander, indicating the mean and standard deviation.

		AOED	AOUD	BL	CAD	CL	EL	EW	HFL	HL	HML	HMW	HT2L
Female	Mean	1.02	0.67	7.81	0.30	0.86	2.01	0.86	1.47	2.52	0.93	0.32	0.29
	SD	0.05	0.03	0.69	0.04	0.07	0.11	0.05	0.10	0.13	0.11	0.04	0.03
Male	Mean	0.91	0.50	6.99	0.30	0.73	1.85	0.90	1.33	2.22	0.79	0.20	0.29
	SD	0.02	0.02	0.40	0.00	0.03	0.04	0.04	0.05	0.08	0.04	0.02	0.01
	t	5.68	13.56	2.73	-0.25	4.63	3.44	-1.40	2.97	4.93	32.08	7.53	-0.26
	df	9	11	10	6	8	8	12	9	10	7	8	6
	P	0.000	0.000	0.022	0.808	0.002	0.009	0.188	0.015	0.000	0.000	0.000	0.801
		***	***	*	ns	**	**	ns	*	***	***	***	ns
Gynander	Left	0.9	0.6		0.3		1.8	0.9	1.4		0.9	0.3	0.3
	Right	1.0	0.6		0.2		2.0	0.9	1.4		0.8	0.3	0.3
	Total			7.9		0.8				2.3			

		HT2W	HT3L	HT3W	HT4L	HT4W	HT5L	HT5W	HTL	HTW	HW	IAHD	IOHD
Female	Mean	0.16	0.23	0.14	0.16	0.13	0.4	0.1	1.6	0.5	1.4	0.5	0.3
	SD	0.01	0.02	0.01	0.02	0.01	0.0	0.0	0.1	0.0	0.1	0.0	0.1
Male	Mean	0.15	0.23	0.13	0.16	0.13	0.4	0.1	1.4	0.4	1.3	0.4	0.3
	SD	0.01	0.01	0.01	0.01	0.01	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	t	1.49	0.46	1.55	-0.63	-0.28	-4.64	1.81	3.34	3.32	3.17	6.18	0.59
	df	12	10	12	11	10	10	8	11	12	10	9	9
	P	0.163	0.654	0.147	0.543	0.787	0.001	0.110	0.006	0.006	0.010	0.000	0.572
		ns	ns	ns	ns	ns	**	ns	**	**	*	***	ns
Gynander	Left	0.2	0.2	0.1	0.1	0.1	0.4	0.2	1.5	0.5	1.3	0.4	0.4
	Right	0.2	0.3	0.2	0.1	0.1	0.4	0.2	1.6	0.4	1.4	0.4	0.3
	Total												

		LID	MCHW	MOHW	MSL	OOD	PSL	SHW	SL	UID	WL
Female	Mean	0.8	1.1	1.3	1.7	0.7	0.5	0.6	0.7	0.9	5.7
	SD	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.2
Male	Mean	0.7	1.0	1.2	1.4	0.6	0.4	0.6	0.5	0.8	5.1
	SD	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.2
	t	8.51	5.13	2.01	4.44	8.42	2.00	6.88	5.53	5.03	4.99
	df	10	10	8	11	7	8	10	7	12	11
	P	0.000	0.000	0.079	0.001	0.000	0.080	0.000	0.000	0.000	0.000
		***	***	ns	**	***	ns	***	***	***	***
Gynander	Left	0.7	1.0	1.1		0.7	0.4	0.6		0.8	5.3
	Right	0.8	1.1	1.4		0.7	0.4	0.6		0.9	5.3
	Total				1.7				0.7		

There were significant differences between the female and male morphometric data for 22 parameters (Table 4). No differences occurred in 12 parameters (Table 4) which refer to the clypeoantennal distance (CAD), the eye width (EW), the interocellar half distance (IOHD), the mesosoma half width (MOHW), the tarsi 2 to 4 both in length and width (HT2L-HT4L, HT2W-HT4W), but for tarsus 5 only in its width (HT5W) and furthermore in the pterostigma length (PSL). In the following, those parameters that show significant sex-specific morphometric differences are compared within the gynander for female, male or intermediate sizes. The boxplot diagrams are shown in Fig. 8 and Fig. 9.

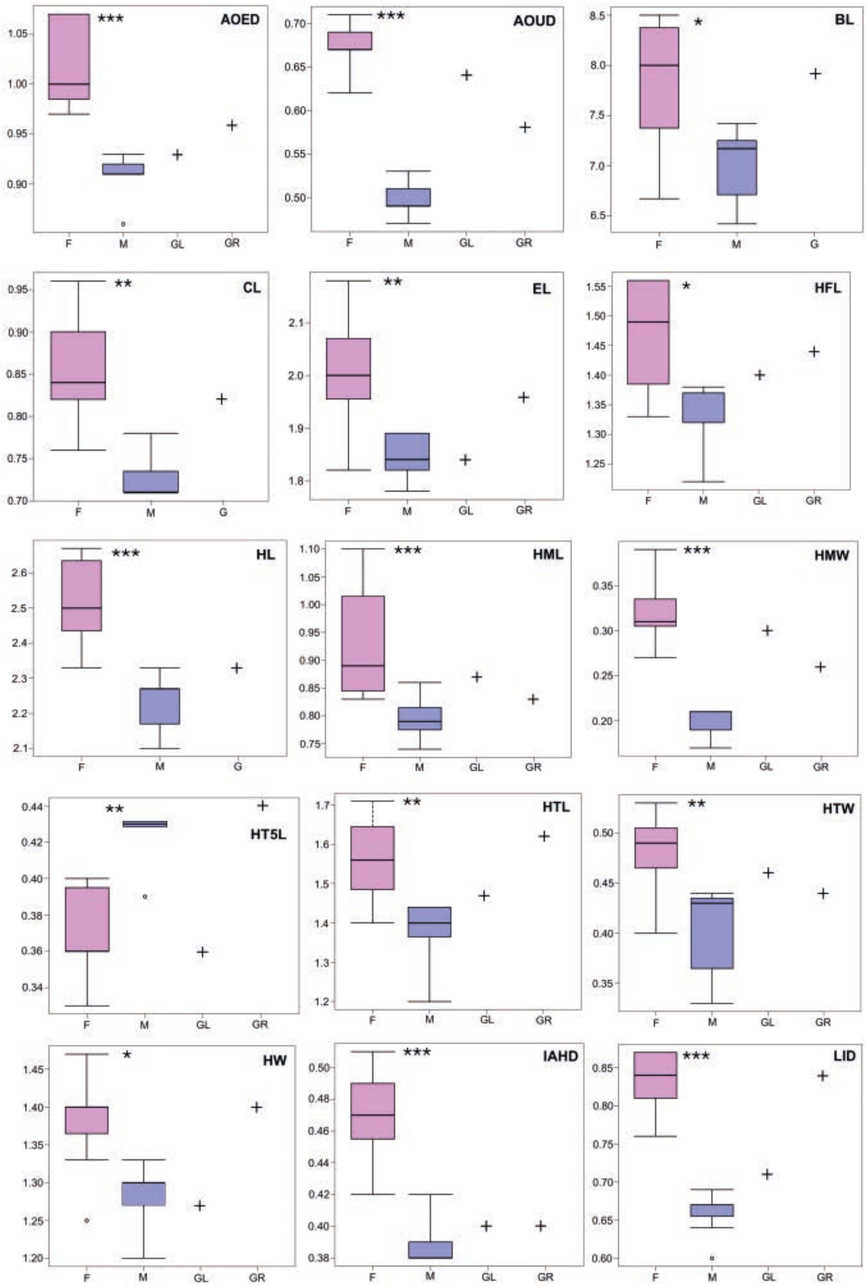

Fig. 8: Morphometric comparison between female, male, left and right side of the gynander for 15 parameters with significant sex-specific morphometric differences.

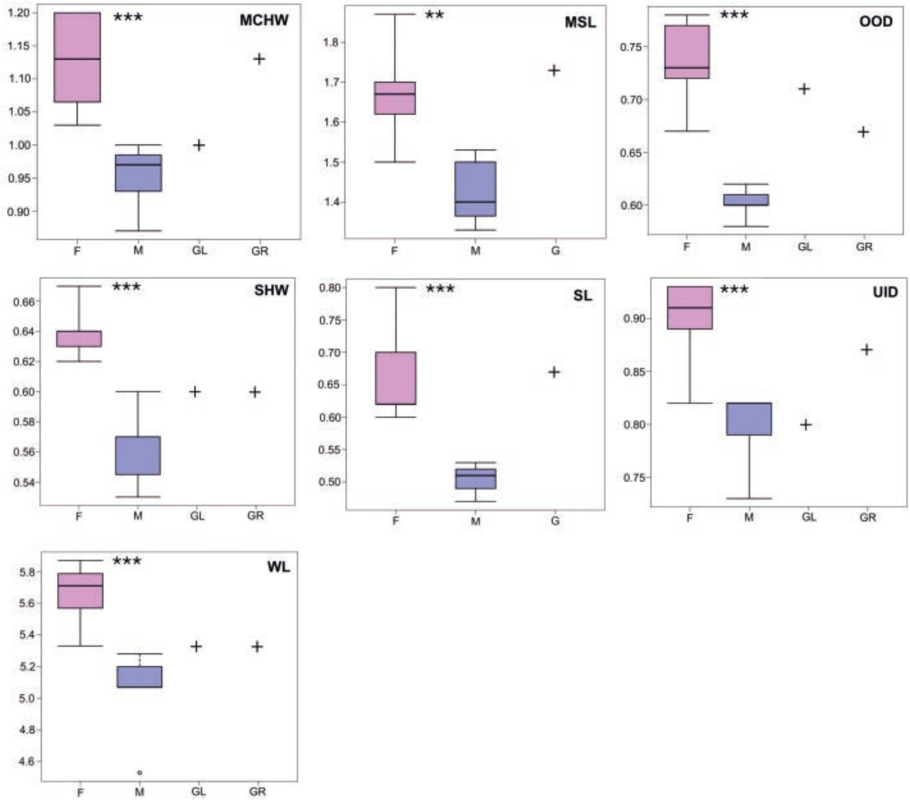

Fig. 9: Morphometric comparison between female, male, left and right side of the gynander for additional seven parameters with significant sex-specific morphometric differences.

- Antennocellar distance (AOED): Females and males differ highly significantly in the antennocellar distance, with the distance between the base of the antenna and the lower ocellus being larger in females than in males. Both the left and right sides of the head of the gynander show distances between those of females and males. The left side of the gynander's head, characterised as female by qualitative morphological features, has a smaller AOED distance than the right side of the head, and is therefore characterised as a male feature (Fig. 8).

- Antennocular distance (AOUD): The antennocular distance is highly significantly larger in females than in males. In the gynander, the distance on the left side is within the lower whisker range of the females, while the one on the right side shows an intermediate value (Fig. 8).

- Body length (BL): Females have a weakly significantly larger body length. The value of the gynander is close to the median value of the females; thus, the total gynander corresponds to the average size of a female (Fig. 8).

- Clypeus length (CL): Females have a significantly longer clypeus than males. The clypeus length of the gynander is close to the lower boxplot quartile of females (Fig. 8).

- Eye length (EL): The length of the compound eyes differs significantly between females and males. Females have longer and thus larger eyes than males. The eye length of the left side of the gynander is in the range of the median of the males, while that of the right side of the gynander is near the lower boxplot quartile of the females. Thus, the gynander has a male eye on the left side and a female eye on the right side (Fig. 8).

- Hind femur length (HFL): The hind femur is significantly longer in the female than in the male. In the gynander, both the left and the right hind femur show lengths corresponding to the female femur (Fig. 8).

- Head length (HL): Females have highly significantly longer heads than males. The length of the head of the gynander, on the other hand, has an intermediate value (Fig. 8).

- Hind metatarsus length (HML): The hind metatarsus of females is highly significantly longer than that of males. In the gynander, the length of the posterior metatarsus on the left side is between the median and the lower quartile of the female, the length of the posterior metatarsus on the right side is intermediate (Fig. 8).

- Hind metatarsus width (HMW): In addition to differing in the length of the hind metatarsus, females and males also differ highly significantly in the width of the hind metatarsus. Here, too, the value on the left side of the gynander lies between the median and the lower quartile of the female. On the right side, there is an intermediate value between the female and the male (Fig. 8).

- Hind tarsus 5 length (HT5L): The hind tarsus 5 is significantly longer in the male than in the female. The left side of the gynander corresponds to the tarsus 5 of the female, and the right side to the male (Fig. 8).

- Hind tibia length (HTL): The length of the hind tibia is significantly larger in the female than in the male. The left side of the gynander is intermediate, while the right side corresponds to that of the female (Fig. 8).

- Hind tibia width (HTW): The hind tibia is slightly significantly wider in the female than in the male. The widths of the left and right side of the gynander have intermediate values (Fig. 8).

- Head half width (HW): Females have significantly wider heads than males. The width of the left side of the head of the gynander corresponds to a female, and that of the right side to a male (Fig. 8).

- Interantennal half distance (IAHD): Females have a highly significantly larger interantennal distance than males. The distance on the left and right sides of the gynander corresponds to the values of a male (Fig. 8).

- Lower interocular half distance (LID): Females have a highly significantly larger distance between their lower compound eyes than males. Only on the right side of the gynander does this correspond to a female. On the left side, it is intermediate (Fig. 8).

- Mesoscutum half width (MCHW): Females have a highly significantly larger mesoscutum width than males. On the right side of the gynander, the mesoscutum half width corresponds to that of a female, while on the left side, it corresponds to that of a male (Fig. 9).

- Mesoscutum length (MSL): The mesoscutum of a female is significantly longer than that of a male. The value of the gynander is the same as that of the female (Fig. 9).

- Ocellular distance (OOD): Females have a highly significantly greater distance between the outer ocellus and the compound eye. On the left side of the gynander, this distance corresponds to that of a female, while on the right side, it has an intermediate value between a female and a male (Fig. 9).

- Scutellum half width (SHW): The scutellum is highly significantly wider in females than in males. The values of the left and right gynander sides are equal and lie between those of a female and a male (Fig. 9).

- Scutellum length (SL): The scutellum is highly significantly longer in females than in males. The values of the gynander side are the same as those for a female (Fig. 9).

- Upper interocular distance (UID): The distance between the lower ocellus and the eye is highly significantly different between males and females. On the left side of the gynander, the value corresponds to that of a male, while on the right side, it is within the lower whisker range of a female (Fig. 9).

- Wing length (WL): Females have highly significantly longer wings than males. The values of the left and right sides of the gynander are between those of a female and a male (Fig. 9).

Table 5 summarises the morphometric results of the different parameters of the gynander and characterises the values as female, male or intermediate. In 16 cases, assignments can be made to a female trait, and only in six cases to a male trait. In 15 cases, the values are intermediate and lie between those of the female and the male.

Table 5: Characterising the morphometric parameters of the gynander (GL = left gynander side, GR = right gynander side, G = total length of the parameter) as female (F), male (M) or intermediate trait (I). The abbreviations of the parameters are explained in Table 2.

Parameter	GL	GR	G
AOED	I	I	–
AOUD	F	I	–
BL	–	–	F
CL	–	–	F
EL	M	F	–
HFL	F	F	–
HL	–	–	I
HML	F	I	–
HMW	F	I	–
HT5L	F	M	–
HTL	I	F	–
HTW	I	I	–
HW	F	M	–
IAHD	M	M	–
LID	I	F	–
MSL	–	–	F
OOD	F	I	–
SHW	I	I	–
SL	–	–	F
UID	M	F	–
WL	I	I	–

With the exception of head length (HL), where an intermediate value exists, all length measurements, such as body length (BL), clypeus length (CL), mesoscutum length (MSL) and scutellum length (SL) were found to have values in the gynander that are typical of females. Hind femur length (HFL) shows female characteristics on both the left and right side of the body. Female characteristics on the left side of the gynander, combined with intermediate ones on the right, were found for antennocular distance (AOUD), hind metatarsus length (HML), hind metatarsus width (HMW) and ocellular distance (OOD). The reverse is true for the hind tibia length (HTL) and lower interocular half distance (LID), where intermediate values occur on the left side and female values on the right side.

Gynander sides with alternating female and male values occur in eye length (EL) and upper interocular distance (UID) (left male, right female) as well as in hind tarsus 5 length

(HT5L) (left female, right male). Antennocellar distance (AOED), hind tibia width (HTW), scutellum half width (SHW) and wing length (WL) consistently show intermediate values.

3. Characterisation of the gynander in morphology and morphometrics

The analysis of the gynander presented here shows that we are dealing with a type which cannot be typified with the definitions of DALLA TORRE & FRIESE (1899). Bilateral asymmetries clearly predominate, although several reciprocal asymmetries occur at the tagma level. Deviations occur in the posterior hind extremities and T4 and T5. Intermediate features between females and males are also detectable. A characterisation at tagma level shows the following result:

- **Prosoma:** The head of the gynander is bilaterally asymmetrical. According to integument colour and pubescence of vertex, frons, paraocular and ocellus area, the left side of the head is characterised female, the right side of the head male. The left antennocular distance (AOUD) and the left ocellular distance (OOD) also correspond to that of a female, but the value of the right side is intermediate. According to the mandibular structure, as well as the eye length (EL) and the upper interocular distance (UID), it is the opposite situation. The left side is characterised as male, the right as female. Differences in the dorsoventral plane are not detectable. The head thus corresponds to a double bilaterally asymmetrical type with isolated intermediate features.

- **Mesosoma:** The mesosoma also shows two bilateral asymmetries in the colouration of the integument. The scutum, scutellum and mesepisternum are characterised by male features on the left side and female features on the right side. In contrast, the left tegula is female in the colour of the integument, the right tegula is male, and the posterior tarsus and claw structure correspond to those of a female, while those of the right side correspond to those of a male. According to these characteristics, the metasoma can be classified as a double bilaterally asymmetrical type. The length of both femurs, however, is similar to that of a female. According to the pubescence, the whole mesosoma is characterised as female. Differences in the dorsoventral plane are not noticeable.

- **Metasoma:** The dorsal colouration of the mesosoma is also bilaterally asymmetrical (left side male, right side female). However, this does not affect all tergites. T5 has male characteristics throughout. In T4, the left side is characterised as male, and the right side as female on the one hand and as male on the other. Thus, the right T4 shows its own internal bilateral asymmetry. The sternites are also bilaterally asymmetrical, which affects the sternite structure. The female and male sides are characterised in the opposite way from the tergites (right side male, left side female). The genital structures also coincide with this result (right side male, left side female). According to these features, the mesasoma corresponds to a complex bilaterally asymmetrical type.

4. Multivariate morphometric analysis

Fig. 10 shows the morphometric parameters for females, males and the left and right gynander side in the context of a PCA. There is a characteristic separation into two clusters between the females and males. Both the left and right gynander sides occupy an intermediate position in the morphometric analysis.

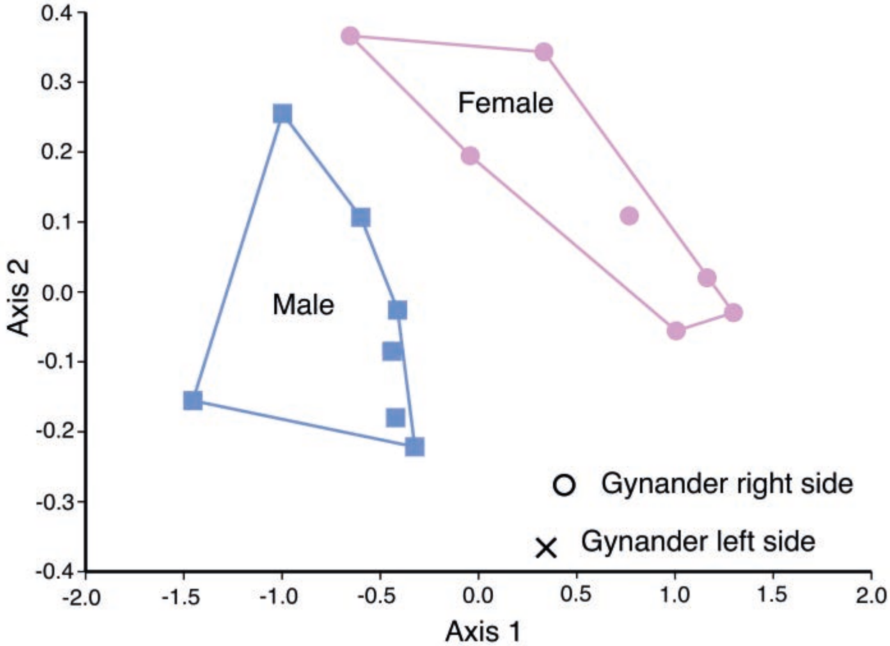

Fig. 10: PCA of 34 morphological parameters in females, males and the left and right side of the gynander (variance-covariance matrix). Pink dot = females; bluish square = males, cross = gynander left, circle = gynander right. Eigenvalue axis 1 = 0.6535, % variance = 84.67; eigenvalue axis 2 = 0.0580, % variance = 7.516.

Discussion

The gynander of *Osmia submicans* presented here shows partial bilateral but reciprocal asymmetry (group 2 in section 'Review of gynandromorphs in *Osmia* described in the literature'). It should be emphasised that several alternating asymmetries occur at the tagma level (male features on the left, female features on the right, reversed in another area of the tagma). However, the morphometric analysis also shows intermediate results.

A peculiarity of the gynander of *Osmia submicans* presented here is that the sagittal plane of the insect extends into a female and a male half extends to the genitalia. Among Anthophila, such examples have only been described in *Chalicodoma muraria* (BISCHOFF & ULRICH 1929), *Halictus eurygnathus* (POPOV 1937), *Ceratina rupestris* (LUCIA et al. 2012), and *Thyreus* cf. *redactulus* (ENGEL 2007).

The first to classify the gynandromorphs of Hymenoptera into types were DALLA TORRE & FRIESE (1899). This was the most differentiated classification of gynanders until now. The authors used 65 gynandromorph Hymenoptera species as database, whose descriptions (often of very different quality) originate from the literature, and which descriptions they also cited in detail. This type classification distinguishes the three main gynander types: lateral (different laterally); transversal (different above and below); frontal (different in front and behind); and mixed (composed of the first three types). In addition, the gynanders were differentiated according to female and male characteristics, and characterised according to different tagmata (head, thorax, abdomen) and the localisation there. This system created by DALLA TORRE & FRIESE (1899) does not take into account that mixed types can also occur within a tagma. Thus, these authors characterised a specimen of *Osmia aurulenta* as a frontal gynander with a female head and a male thorax and abdomen, although in the head region the clypeus on the left side showed a male colouration and pubescence. In such cases, DALLA TORRE & FRIESE (1899) made the decision according to the visually dominant feature. This was also applied in other cases analysed by the authors. DALLA TORRE & FRIESE (1899) referred to the problem that the terms are used inconsistently by different authors.

WCISLO et al. (2004) adopted the terms of DALLA TORRE & FRIESE (1899) in a modified form (bilateral asymmetric, anterior-posterior, dorso-ventral, mixed = mosaics or different combinations of the preceding three classes) and analysed 76 gynanders (64 species) from the literature, with the exception of a new description of the gynanders already evaluated by DALLA TORRE & FRIESE (1899). These gynanders were not included in the study by WCISLO et al. (2004). In contrast to DALLA TORRE & FRIESE (1899), WCISLO et al. (2004) also indicated intermediate formations and defined the term 'mixed' more broadly. However, the phenotypic characterisation of the specific gynanders, which is important for type formation, was extraordinarily brief. They also did not characterise the types in detail.

Without referring to DALLA TORRE & FRIESE (1899), MICHEZ et al. (2009) distinguished only three types: (1) the bilateral type, when an organism has female characteristics on one half of the body and male characteristics on the other half; (2) the transverse type, when there is a distribution on two asymmetrical planes; and (3) the mosaic type (mixed type), when the areas with male and female characteristics are mixed or distributed mosaically on the individual. Thus, MICHEZ et al. (2009) summarised the types transversal and frontal (anterior-posterior) of DALLA TORRE & FRIESE (1899). In MICHEZ et al. (2009), the concept of the mosaic type undergoes a considerable expansion, as it is no longer associated with the other gynander types and is only understood in the sense of a 'random distribution of the sexual characters through the body'. In this sense, LUCIA & GONZÁLEZ (2013) and ALMEIDA et al. (2018) also interpreted mixed or mosaic classes. In the more recent literature, this definition by MICHEZ et al (2009) has usually used (see, for example, GONZÁLEZ 2004, GIANGARELLI & SOFIA 2011, CAMARGO & GONÇALVES 2013, SPRING et al 2015, COELHO et al. 2016, ALVAREZ et al. 2019). Thus, it is not surprising that the mosaic type is considered the most common type among apoid Hymenoptera (WCISLO et al. 2004, LUCIA & GONZÁLEZ 2013).

Many of the gynander descriptions concerning representatives of Anthophila were not always detailed enough that typifications and assignments could be made accurately (see, for example, the characterisations of WOLF 1990, 1991, 2003a, 2003b). Many important parameters regarding the external morphological features often remained unconsidered, and only the particularly obvious and striking features were listed. In addition, a prior accurate comparative analysis of females and males was not always available, so that some sex-specific differences remained unrecognised. Eye size or end-tarsus structure, for example, were not always taken into account. A genital analysis (complete, incomplete or very rudimentary formations) is particularly important, but was also often omitted (ENGEL 2007). Morphometric analyses were usually hardly carried out, except for a few parameters such as body size, wing length and some others. The structures inside the insect body were often not taken into account, but these are difficult to dissect and analyse afterwards, especially in dead dried material (BISCHOFF & ULRICH 1929, RAMOS & RUZ 2013).

These are also the reasons why there are difficulties and deviations in the type classifications by different authors. A comparative analysis of the gynanders described so far in *Osmia* (Table 1) shows, among other aspects, that one and the same specimen was classified typologically completely differently by different authors. Thus, the gynander of *O. aurulenta* described by WOLF (1991) was assigned to the mosaic type according to MICHEZ et al. (2009), while according to WOLF (1991) it was a bilateral and mosaic gynander. The gynander of *O. bicolor* described by WOLF (1990) as an incomplete bilateral gynander was a mosaic gynander according to MICHEZ et al. (2009). The *Osmia rufa* gynander described by NOSKIEWICZ (1923) was described as a transverse gynander according to MICHEZ et al. (2009), but according to WCISLO et al. (2004), this gynander was characterised as predominantly female with bilateral asymmetry. It must be assumed that in such comparisons, the authors did not analyse the original specimens, but only used the descriptions in the literature as a basis. It is therefore not surprising that meta-analyses showing the frequency of the occurring types differ considerably (DALLA TORRE & FRIESE 1899, WCISLO et al. 2004, MICHEZ et al. 2009).

In DALLA TORRE & FRIESE 1899, the evaluation of 73 individuals of different wild bee species yielded the result that the lateral type was the most frequent (52%), with the proportions of females/males features equally distributed on the left or right. Second most common was the mixed type (25%), which would complement the lateral, transverse or frontal type in relatively equal proportions. In third place was the frontal type, at 22%. In more than half the cases, the head was similar to that of the female. A transverse type occurred in only one case. The authors also emphasised that among the gynanders, in addition to those with vestigial genitalia, there are also clear hermaphrodites (female and male sex). DALLA TORRE & FRIESE (1899) presented examples of this.

WCISLO et al. (2004) examined 64 wild bee species (76 cases, 6 families) with characteristics of gynandromporphy. According to WCISLO et al. (2004), 48% could be assigned to the mosaic type and about 35% to the lateral type. The anterior-posterior type occurred in about 15%, and the dorso-ventral transverse type in only about 3%. MICHEZ et al. (2009) analysed 109 cases from six bee families (106 according to a literature review) and, after their classification, concluded that transverse gynanders occurred most frequently (56%), followed by mosaic gynanders (33%) and bilateral types (9%). In the study by DALLA TORRE & FRIESE (1899), there is only one example of a transverse type, while in the publication by MICHEZ et al. (2009) this is the most frequently occurring type. Apart from the different degrees of accuracy in the gynander descriptions, the main reason for the divergent assignments to gynander types is clearly the inconsistent type characterisation, which does not always take into account the degree of complexity of gynander phenomena. In this respect, such meta-analyses do have deficiencies.

Concluding remarks: Proposals for further gynander classifications

The detailed analysis of the gynander of *Osmia submicans* presented here shows that due to the complexity of the characteristics, this gynander cannot be typified with the definitions of DALLA TORRE & FRIESE (1899). A characterisation should fulfil certain basic criteria, whereby before analysing a gynander, females and males must first be examined in detail and only afterwards an analysis of the gynander must be carried out. Many characteristics are not included in the usual species characterisations or in determination tables. In addition to female and male characteristics, attention must also be paid to intermediate characteristics. Single anomalies can also occur in gynanders, such as a horn formation on the right anterior clypeus side in the example of *Osmia submicans* presented here. Such abnormalities can also be caused by an ontogenetic incompatibility of female and male characteristics in the gynander individual.

The following criteria for a gynander analysis are suggested:

- Consideration of as many morphological and morphometric characteristics as possible
- Characterisation according to tagmata level
- Subdivision of the tagmata into symmetry planes (lateral, dorsoventral)
- Subdivision of tagmata according to substructures:
    - Prosoma: for example, mandible, proboscidian structures, vertex, frons, ocellus area, paraocular area, clypeus, antennae
    - Mesosoma: for example, pronotum, scutum, scutellum, postscutellum, propodeum mesopleuren, mesepisternum, tegulae, wings, femur, tibia, metatarsus, tarsi, claw
    - Metasoma: tergites T1-T7 and sternites S1-S8
- Differentiation of tagmata, including their substructures, according to integument colour; pubescence (for example, colour, length, density), and structures (for example, sculpturing and puncturing of the integument, antennal segments, mandible structure and shape, number of teeth of the terminal tarsus, anal structures)
- Recording of a sufficient number of morphometric data and a statistical comparison between the values of females, males and the gynander
- Analysis of the genitalia, taking into account sex specificity, structural completeness and anomalies
- Comprehensive photo documentation.

Morphological and morphometric characteristics and data should be checked for conformity. In addition to statistical analyses, multivariate methods are useful for the analysis of gynanders and the characterisation of female and male traits. Comparisons between gynanders and their typifications, which were made exclusively according to data in the literature, are not always possible due to the often very heterogeneous descriptions. Terms such as ‘incomplete lateral gynander’ are not helpful. The analysis of original gynander specimens from collections would be helpful in many cases. Unfortunately, the whereabouts of the gynanders described in the literature are not always known.

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Zusammenfassung

Es wird der erste Nachweis eines Gynanders von *Osmia submicans* MORAWITZ, 1870 mit einer fast vollständigen bilateralen Asymmetrie vorgestellt. Auf Tagma-Ebene konnten zusätzlich mehrere Asymmetrien weiblicher und männlicher Merkmale, die alternierend auftreten, festgestellt werden. Die Untersuchungen wurden unter Berücksichtigung von 25 qualitativen morphologischen Merkmalen (Integumentfarbe, Behaarung, Strukturmerkmale) und 34 morphometrischen Parametern durchgeführt. Weibchen und Männchen von *O. submicans* sind eingehend morphologisch und morphometrisch untersucht und die morphometrischen Unterschiede statistisch geprüft worden. Über eine multivariate morphometrische Analyse wurden die Messungen der linken und der rechten Gynanderseite mit den Werten von Weibchen und Männchen verglichen. Die bisher in der Literatur beschriebenen Gynander innerhalb der Gattung *Osmia* sind erneut typisiert und mit dem hier vorliegenden Gynander verglichen worden. Die herkömmliche Typisierung von Gynandern wird kritisch diskutiert. Kriterien, die für eine Analyse von Gynandern von Bedeutung sind, werden vorgestellt.

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