# Comparative morphological analysis of compound eye miniaturization in minute hymenoptera

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A B S T R A C T

Due to their small size, diminutive parasitic wasps are outstanding subjects for investigating aspects of body miniaturization. Information on minute compound eyes is still scarce, and we therefore investigated eye morphology in one of the smallest known hymenopteran species *Megaphragma mymaripenne* (body size 0.2 mm) relative to *Anaphes flavipes* (body size 0.45 mm) and compared the data with available information for *Trichogramma evanescens* (body size 0.4 mm). The eyes of all three species are of the apposition kind, and each ommatidium possesses the typical cellular organization of ommatidia found in larger hymenopterans. Compound eye miniaturization does not therefore involve a reduction in cell numbers or elimination of cell types. Six size-related adaptations were detected in the smallest eyes investigated, namely a) a decrease in the radius of curvature of the cornea compared with larger hymenopterans; b) the lack of extensions to the basal matrix from secondary pigment cells; c) the interlocking arrangement of the retinula cell nuclei in neighboring ommatidia; d) the distal positions of retinula cell nuclei in *M. mymaripenne*; e) the elongated shape of retinula cell pigment granules of both studied species; and f) an increase in rhabdom diameter in *M. mymaripenne* compared with *A. flavipes* and *T. evanescens*. The adaptations are discussed with respect to compound eye miniaturizations as well as their functional consequences based on optical calculations.

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## 1. Introduction

Structural peculiarities related to miniaturization can be traced in anatomy of many small insects (Polilov, 2015). The extremely small size affects most of the organ systems of tiny hymenoptera: the skeletal and the muscular system, as well as the reproductive (Polilov, 2007) and the nervous system (Makarova and Polilov, 2013). In the smallest insects modifications occur not only at the level of organs, but also at the cellular level (Polilov, 2012). Initial investigations on structural and functional changes in miniature compound eyes started about 10 years ago on eyes of small scarabaeid beetles (Meyer-Rochow and Gál, 2004), while more recent work dealing with lepidopteran compound eyes revealed morphologically intermediate eyes, in between apposition and superposition types (Honkanen and Meyer-Rochow, 2009; Fischer et al., 2011, 2012a,b, 2014). With respect to vision in the tiniest hymenopterans, to date only one study has been published

on the parasitoid wasp *Trichogramma evanescens* (Westwood 1833) with a body size of only 0.3—0.4 mm (Fischer et al., 2011). Work on the optics of this tiny species revealed size-related adaptational changes in eye morphology, and especially the very precise, alternating positions of the nuclei of regular retinula cells suggests that the described eye comes close to a fundamental limit with respect to the available space within the eye and its functional design (Fischer et al., 2011).

Nevertheless there exist species even smaller in size than *Trichogramma*, like *Megaphragma mymaripenne* (Timberlake 1924), with a body size around 0.2 mm. The question therefore arises how are organized and if special adaptations or even reductions in cell types are to be found. In order to study not only the minimal possible limits, but also questions regarding the priority with which different parameters have had an impact on eye design, a comparative investigation was initiated that included different species of parasitoid wasps of small body- and eye-sizes. We investigated the eye morphology of one of the smallest known hymenopteran species, *Megaphragma mymaripenne* (body size 0.2 mm), and of a larger species, *Anaphes flavipes* (body size

0.45 mm), and compared these with available information for *T. evanescens* (Fischer et al., 2011).

## 2. Material and methods

### 2.1. List of taxa examined

Adult females of two small parasitoid wasp species were studied. Specimens of the mymarid *A. flavipes* (Foerster 1841) (Mymaridae) were collected in the Moscow region (2009) and specimens of *Megaphragma mymaripenne* (Timberlake 1924) (Trichogrammatidae) in Blanes, Spain (2008) and Funchal, Madeira, Portugal (2009). *M. mymaripenne* were hatched from eggs of *Heliothrips haemorrhoidalis* (Bouché 1833), which were collected in July 2008 in Blanes and October 2009 in Madeira on *Viburnum tinus* L., 1753 (Adoxaceae). *A. flavipes* were collected with an insect net in places of possible habitat.

### 2.2. Light microscopy (LM)

Light-adapted specimens were fixed in formaldehyde–ethanol–acetic acid (FAE) and embedded in Araldite M. The samples were then sectioned at 1 μm with a Leica microtome (RM 2255) and stained with toluidine blue. The serial sections were photographed with a Tucsen digital camera on an Olympus BX43.

### 2.3. Transmission electron microscopy (TEM)

The sample material was fixed in 2% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2) for 12 h and post-fixed with 1% osmium tetroxide for 2 h in the same buffer. All specimens were fixed in the light-adapted state. The specimens, en-bloc stained with uranyl-acetate, were embedded in Epon 812 and sectioned with a Leica UC6 ultramicrotome at a thickness of 50 nm. After ultrathin sections were stained with lead citrate for 10–12 min, the samples were investigated with a Jeol JEM-1011 transmission electron microscope operated at 80 kV.

### 2.4. Scanning electron microscopy (SEM)

After ultrasonic cleaning, the specimens were critical point dried (Hitachi HCP-1) and coated with a layer of gold in a sputter coater (Hitachi IB-3). The samples were observed using a Jeol JSM-6380 SEM, operated at 20 kV.

### 2.5. Measurements

All measurements were made using software (Image J, Rasband, W.S, U.S. National Institutes of Health, Bethesda, MD). Eyes, 3–5 for each species, were used for measurements from the SEMs. For examinations involving LM and TEM, 6–10 eyes of either species were used. SEM was used to determine the dorso-ventral and antero-posterior extent of the eye, the total number of ommatidia per eye, their facet diameters, and the diameters of the ocelli. Longitudinal ultrathin sections for TEM were used to measure ommatidial lengths, the radii of curvature of eye and corneal facets, cone lengths, corneal thicknesses and to investigate the shapes and positions of the pigment granules within the retinula cells, as well as determine the interommatidial angles. Rhabdom diameters, shapes, and diameters of pigment granules of primary pigment cells (PPC) and secondary pigment cells (SPC) as well as retinula cells were measured from TEM cross sections. Measurements of rhabdom diameters were taken at the distal tip of the rhabdom.

### 2.6. Optical calculations

To compare the optical properties of the compound eyes, the anatomical measurements were used to calculate relevant parameters.

The focal lengths of the lenses were calculated on the assumption that the refractive indices in the crystalline cones and the cornea were homogenously distributed, using the thick lens formula (Jenkins and White, 1976):

$$P_l = P_1 + P_2 + P_3$$

with

$$P_1 = \frac{n_l - n}{r_1}, \quad P_2 = \frac{n' - n_l}{r_2}, \quad P_3 = -\frac{t}{n_l}P_1 P_2$$

The addition of the powers given by the front and back surfaces of the lens ($P_1$, $P_2$) provides the total lens power of the system. The indices n, $n_l$ and $n'$ describe the refractive indices of the air (n = 1), lens and image space; t is the thickness of the lens. The radii of curvature of the front and back surfaces of the lens are defined by $r_1$ and $r_2$, respectively. An investigation of the properties of the crystalline cones of these species appeared near to impossible because of their small size and the uncertainties of interference microscopy on such small structures (Kunze, 1979). Approximations were therefore taken from measurements on the compound eye of another hymenopteran, namely *Apis mellifera* (Varela and Wiitanen, 1970) for the lens ($n_l = 1.452$) and for the cone ($n' = 1.348$).

The focal length (f) and the image focal length (f') can then be calculated by:

$$f = \frac{n}{P_l} \quad \text{and} \quad f' = \frac{n'}{P_l}$$

The focal length (f) was calculated from the secondary nodal point N, the position of which can be determined by measuring the distance to the vertex of the back surface of the lens (Stavenga, 2003):

$$dn = \frac{n'(1 - tP_1/n_l)}{P_l} - f$$

The focal length allowed us to calculate the F-number of the compound eye, which serves as a value to compare the optical properties of different compound eyes (e.g. Warrant and McIntyre, 1993). The F-number is defined as the ratio of the facet diameter (A) to the focal length (A/f).

Furthermore the acceptance angle of the rhabdom $\Delta\rho_{rh}$ (diameter of the rhabdom/focal length) was calculated as well as the half-width of the Airy Disk ($\Delta\rho_l = \lambda/A$) with A = facet diameter and λ as the wavelength of light (Snyder, 1977, 1979). A wavelength of 0.5 μm (green light) was used in the calculation.

In order to determine the sensitivity of the ommatidia, the sensitivity formula was applied, modified for white light by Warrant and Nilsson (1998):

$$S_W = \left(\frac{\pi}{4}\right)^2 A^2 \left(\frac{d}{f}\right)^2 \left(\frac{kl}{2.3 + kl}\right)$$

where A = facet diameter, d = rhabdom diameter, f = focal length, k = absorption coefficient (0.0067 μm$^{-1}$ for invertebrates) and l = rhabdom length.