



Review article

No limits: Breaking constraints in insect miniaturization

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ABSTRACT

Small arthropods are not simply scaled-down versions of their larger closest relatives, as changes in morphology and functional characters are largely governed by scaling laws. These same scaling laws set strict limits to size change toward smaller sizes. The evolution of extreme miniaturized forms involves the breaking of these constraints, by means of design innovations that allow evolutionary change to evade the limits posed by scaling laws. Here we review several cases studies in insects and other arthropods that illustrate this evolutionary path. We examine morphologies commonly recurring in miniaturized forms but not exclusive to them, morphologies exclusive to miniaturized forms and novel functional solutions supported by unconventional morphologies. We also discuss miniaturization and its evolvability taking into consideration arthropod postembryonic development and modular body organization. The modification of features commonly supposed not to change appears as a recurring pattern in arthropod miniaturization.

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1. Introduction

In organismal biology, scaling relationships describe in quantitative terms how measurable traits of animals, plants, fungi and microorganisms scale with body size. The theory of biological scaling provides a suitable context to consider the phenomenon of miniaturization in an evolutionary perspective.

Throughout the whole range of living forms, many features tend to scale with size in a surprisingly straightforward way. Size-scaling typically follows a simple power law behaviour, where the explanatory variable is the mass of the organism (McMahon and Bonner, 1983; Calder, 1984; Schmidt-Nielsen, 1984). Universality and simplicity of these relationships have suggested that some general principles underlay much of the coarse-grained structure and organisation of living systems, also in consideration of the fact that the exponent of these power laws usually takes a limited set of values, which are typically multiples of 1/4 (West and Brown, 2005). Revisiting this topic in terms of fractal geometry, West et al. (1997) proposed that the quarter-power allometric scaling laws and other features of the dynamical behaviour of biological systems reflect constraints inherent in the generic properties of life-sustaining hierarchically branching networks such as animal

circulatory systems and seed plant vascular systems. These were assumed to have invariant terminal units, to be space-filling and somehow optimised by natural selection. However, moving from the more fundamental features of organism design to the details of their morphology, where the relationship between size of different body parts and overall body size simply reflects the organism's change in shape with size (Klingenberg, 2016), the exponent of the power law is evidently not constrained to quarter powers.

A major distinction in scaling relationships is between *isometric scaling*, where the proportion of a given trait's size with respect to body size remains constant across the size range, and *allometric scaling*, where the variation in a specific feature like the size of a given body part (e.g., brain size) or a functional parameter (e.g., metabolic rate) is not proportional to the variation in body size (Huxley, 1932). The biological significance of scaling depends on the context in which size variation is studied (Shingleton et al., 2007). *Static allometry* refers to scaling relationship among the individuals of a species at the same developmental stage, whereas *ontogenetic allometry* refers to the relationship between the same organism's conditions at different developmental stages. Both static and ontogenetic allometry are relations at intraspecific level, whereas a third category, *evolutionary allometry*, considers scaling relationships among individuals of different species at the same (or at least comparable) developmental stages (for a review on allometry in arthropods see Minelli and Fusco, 2013; a recent review on insects is Polilov and Makarova, 2017).

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A corollary of these scaling laws is that there are limits to size change, toward both larger and smaller sizes. These limits may have different specific causes, especially in relation of whether intra-specific or interspecific size variation is under consideration. However, a common ground for these limitations are the constraints posed by the allometric relationship (so-called *allometric constraints*; e.g., Voje et al., 2014), that impair the functionality of the system when the size of the latter is pushed beyond some given upper or lower thresholds. This happens because of the physical properties of biological matter, or the physicochemical dynamics of some biological processes (e.g., metabolite transport). These limits are real, unless an *innovation* occurs. Larger or smaller organisms beyond the limits set by the scaling laws can evolve only provided that innovations occur that either change the material composition of the system or its structural design, or both (West, 2017).

The focus of this article is on the possibility for arthropods, with a special emphasis on insects, to escape the constraint to size changes imposed by the current properties of the system. Evolutionary innovations continuously allow evolutionary change to evade the physical, metabolic, or design constraints posed by current ‘technology’, through the ‘invention’ of new technologies. Miniaturization of insects and other arthropods (reviewed in Polilov, 2015; 2016a) provides beautiful examples of these distinctive evolutionary pathways, which open vistas into evolutionary innovations and their evolvability.

2. Conservation and change in the morphology of miniaturized arthropods

The study of miniaturized arthropods provides abundant examples of the morphological and functional consequences of the disruption of structural patterns otherwise conserved over a wide range of non-extreme size values. Deviations from the latter differ both in respect to uniqueness, recurrence and functional consequences. Uniqueness means that corresponding morphologies are not found in arthropods other than miniaturized taxa, while recurrence means the degree to which these morphologies have evolved in parallel in different miniaturized lineages. As for the functional consequences of these changes, we will only briefly mention them, in so far as these are likely to contribute an explanation for the lower limits of the miniaturized forms (as a whole, or in particular lineages). We will focus instead on the emergence of novel functional arrangements from which the animal finds a viable solution to a key functional problem despite (and following) a major deviation from conventional structure.

We will thus articulate the following summary of comparative data on morphological and functional aspects of miniaturization in insects and other arthropods into three sections: (1) unconventional morphologies commonly recurring in miniaturized forms but not exclusive to them; (2) unconventional morphologies exclusive to miniaturized forms; (3) novel functional solutions supported by unconventional morphologies in miniaturized forms. For a list and taxonomical overview of the arthropod species discussed in this article, see Appendix 1.

2.1. Unconventional morphologies commonly recurring in miniaturized forms but not exclusive to them

2.1.1. Cell number and cell size

Throughout the animal kingdom, reduction in size is mainly associated with a reduction in the number of cells forming the individual organs and eventually the whole body, but a reduction in average cell size is also frequently involved, especially when body size reduction is pushed towards extreme values. In some clades, size reduction is mainly due to only one of the two factors, as in the

Loricifera, with more than 10,000 tiny cells in a body with a length of 300 μm or less (Kristensen, 1991), strongly contrasting the very few (ca. 500), but unusually large cells forming the body (0.5–1.0 mm in length) of the appendicularian *Oikopleura dioica* (Brena et al., 2003). Only very few systems have been studied in adequate detail, nevertheless these suggest that the two reduction trends (cell number and cell size) can be causally associated. For example, in the small-size *Drosophila* mutant *chico*, reduction in cell number accounts for 68% of the reduction in wing area, compared to wild type, while the remaining 32% is due to reduction in average cell size (Böhni et al., 1999).

In the trade-off between reduction in cell number and reduction in cell size, the trend accompanying miniaturization in insects is mainly a reduction in cell number, except for neurons, where a moderate reduction in cell number is accompanied by a remarkable and sometimes extreme reduction in cell size (Polilov, 2015). Nevertheless, holometabolans microinsects have fewer neurons than larger members of related taxa (Polilov, 2008; Makarova and Polilov, 2013a,b), the minimum number recorded being around 10,000 cells in the adult brain (Polilov, 2015). This generalization seems to apply also to the smallest beetles, among which *Nanosella* has about 11,000 cells in the cerebrum, and *Mikado* about 12,000 cells (Makarova and Polilov, 2013a). The figure of 23,000 cells in the cerebrum of *Primorskiella* (Polilov, 2008) is probably an overestimation (Polilov, pers. comm.).

A reduction in the number of neurons, compared with larger relatives, is more obvious in hemimetabolans: there are only 8800 cells in the brain of the miniaturized *Liposcelis bostrychophila*, a psocopteran only 0.89–0.96 mm long, whereas in a psocopteran of quite large size such as *Copostigma* sp. (adult body length 7 mm), the brain cells are about 37,000 (Makarova and Polilov, 2017). In the thysanopteran *Heliothrips haemorrhoidalis* (Makarova and Polilov, 2017b) the diameter of brain cells varies between 2.18 and 4.94 μm in the first instar nymphs, and between 2.22 and 4.85 μm in the adult. The number of these cells increases from about 4400 units in the first instar nymph to 14,000 in the adult, whereas the relative volume of the brain decreases from 7.92% in the I instar nymph (body length 0.45 mm) to 1.68% in the adult (body length 1.4 mm).

Miniaturization can affect distinct types of brain cells differently, as shown by the detailed study of van der Woude and Smid (2017) on *Trichogramma evanescens* (adult body length 0.3 mm). While the number of serotonergic neurons appears to be little affected by miniaturization, many dopaminergic and some octopaminergic neuron clusters contain fewer neurons than in larger insects.

The number of ommatidia is dramatically reduced in miniaturized insects (Fischer et al., 2011, 2012a; 2012b), but the number and arrangement of cells in each ommatidium is largely conserved, except for a few taxa in which the typical arrangement is somehow disrupted (discussed below). The overall size of the ommatidium is reduced, but at most by a factor of 2: the smallest ommatidia (6 μm in diameter) are those of *T. evanescens* (Fischer et al., 2011), to be compared to those of the other Chalcidoidea, the diameters of which range between 7 and 13 μm .

A strong reduction in neuron size has been documented in miniaturized insects belonging to different orders, among both the hemimetabolans (Psocoptera: *Liposcelis bostrychophila*, Makarova and Polilov, 2017a) and the holometabolans, including Coleoptera (*Hydroscapha natans*, Beutel and Haas, 1998; *Mikado* sp., Polilov and Beutel, 2009; *Acrotrichis grandicollis*, *Nanosella* sp. and *Ptinella tenella*, Makarova and Polilov, 2013a; *Meru phyllisae*, Beutel et al., 2005), Strepsiptera (the primary larva of *Mengenilla chobauti*, Beutel et al., 2005) and Hymenoptera (*Anaphes flavipes* and *T. evanescens*, Polilov, 2007; Makarova and Polilov, 2013b). In most

instances, a cell size of ca. 2 μm seems to be the lower limit (Beutel and Haas, 1998; Grebennikov and Beutel, 2002; Beutel et al., 2005; Grebennikov, 2008), but neurons ranging between 1.3 and 4.3 μm have been found in the psocopteran *L. bostrichophila* (compared to 3.1–10.3 μm in the larger relative *Copostigma* sp.; Makarova and Polilov, 2017a).

In the smallest neurons of miniaturized insects, the volume of the nucleus can be up to 90% of the total cell volume (Grebennikov, 2008), but, to the contrary, the nucleus eventually disappears in some extremely miniaturized hymenopterans (see below). A critical factor in determining the lower size limit of nervous cells is perhaps the distribution of mitochondria, that have been reported to be lacking in axons of less than 98 nm in diameter in two miniaturized hymenopterans, *Encarsia formosa* (Hustert, 2012) and *Trichogramma brassicae* (Fischer et al., 2018).

2.1.2. Modularity and meristic changes

A strong reduction in cell number is probably responsible for the lack of functional boundaries between modular structures that are found in arthropods of larger size. In strongly miniaturized forms, there may not be cell rows in sufficient number to allow the production of many pattern units, e.g. in periodic patterns. This includes body segments, antennal and tarsal articles, and articulated exoskeletal sclerites in general, as noted by many authors (e.g., Sörensson, 1997; Beutel and Haas, 1998; Pohl, 2000; Grebennikov and Beutel, 2002; Polilov, 2005, 2007, 2008; Polilov and Beutel, 2009, 2010; Yavorskaya et al., 2014). Loss or the especially rapid evolution of numerous gene clusters conserved in other Hymenoptera, including genes coding for key transcription factors involved in embryo segmentation (e.g. *knirps*) (Lindsey et al., 2018) is observed in the genome of the miniature trichogrammatid wasp *Trichogramma pretiosum*.

Extensive 'segment fusion' involving most of both the thorax and the abdomen is common in two clades of Collembola: the Symphypleona, whose smallest representatives are 120 μm long (Bellinger et al., 1996–2018), with several species of *Sphaeridia* in the range 150–200 μm (e.g., Bretfeld and Schulz, 2012) and the Neelipleona, with the genus *Megalothorax*, whose representatives range in length between 0.2 and 0.7 mm (Schneider and D'Haese, 2013). In the smallest insect known thus far, the male of the mymarid *Dicopomorpha echmepterygis* (body length 139 μm) only two abdominal segments are discernible (Mockford, 1997) and in *Megaphragma mymaripenne* (body length 200 μm) the sternites are indistinct (Polilov, 2017a). These are really extreme conditions, as a full number of segments is found even in very small Ptiliidae (Polilov and Beutel, 2009) and Thripidae (Moritz, 1982).

Lack of disjunction of usually distinct sclerites is common among miniaturized insects, in both larvae and adults. Larval examples include mymarid hymenopterans (Polilov, 2007), the first instar larva of *M. chobauti* (Osswald et al., 2010) and beetles, such as *Sericoderus lateralis* (Polilov and Beutel, 2010). In the larva of *Mikado* sp., no well-defined sclerites are found (Polilov and Beutel, 2009). Fused sclerites are also common in the adults, e.g. of Coleoptera (Myxophaga, Beutel, 1999; Ptiliidae, Polilov, 2005, 2008; Polilov and Beutel, 2009; Corylophidae, Polilov and Beutel, 2010). Remarkable is the absence of sutures in the head capsule of adult and larval Ptiliidae and Corylophidae (Grebennikov, 2008; Polilov and Beutel, 2009, 2010). On the head capsule of *H. haemorrhoidalis* only a single distinct suture is visible (Polilov and Shmakov, 2016).

This trend affects also the endoskeleton of the thorax, individual elements of which are sometimes lacking in miniaturized taxa (Polilov, 2015, 2016a). For example, the metafurca, i.e. the common stem of the metendosternite, is missing in the hymenopteran *Megaphragma* (Polilov, 2017a). In the first instar larva of the

strepsipteran *Mengenilla chobauti*, the whole thoracic endoskeleton is absent (Osswald et al., 2010).

A reduction in the number of distal articles of the antennae, tarsi and, sometimes, palps is common in miniaturized insects, but far from universal. In the Myxophaga, a basally branching clade of beetles, all the members of which can be considered miniaturized, a full number of 11 antennomeres is retained only in the Sphaeriusidae (Yavorskaya et al., 2018), whereas smaller numbers are present in the adults of the remaining families: 9 in Torridincolidae, either 5 or 8 in Hydroscephalidae and only 4 in Lepiceridae (Reichardt, 1973; Anton and Beutel, 2006). However, compared with the number of antennomeres found in other beetle families (Minelli, 2005), only the smaller numbers (4 and 5) can be regarded as correlated with diminutive body size. Extreme is instead the reduction of segmentation in the antenna of the males of *D. echmepterygis*, which have only one antennomere (Mockford, 1997). In this case, not even the distinction between the proximal 'true' articles (scapus, pedicel) and the distal flagellum is recognizable.

A trend towards the reduction in the number of tarsomeres is also widespread, but rarely pushed to the extreme. For example, compared to a plesiomorphic number of 5 tarsal articles in the whole of the Staphyliniformia, Ptiliidae have only 2 or 3 (Hall, 1999). However, in the extremely miniaturized male of *D. echmepterygis* the tarsi are reduced to one article only, usually fused to the tibia (Mockford, 1997), while they are usually of 4 or 5 segments in the other mymarids. This can be compared with the condition in the aphid *Atarsos grindeliae* (Gillette, 1911), which lacks tarsi entirely.

In the insect's internal anatomy, this trend in reducing segmentation is paralleled by the oligomerization of the nervous system, i.e. by the concentration of ganglia, described in all groups of miniaturized insects (Polilov, 2005, 2007, 2008, 2015, 2016b; Polilov and Beutel, 2009, 2010). Fading articulation into segmental units affects also the brain of very small insects (Grebennikov, 2008); functional data would be interesting, to be compared to the condition in copepod nauplii, which also lack clear brain segmentation, but have distinct functional units that receive visual inputs (Lacalli, 2009).

Modular, although non segmental structures of the insect body are the veins of the wings. The wing apparatus of the majority of microinsects is characterized by a reduced number of veins (Polilov, 2007; Polilov and Beutel, 2009). The wings of all flying microinsects are narrow and contain only three veins at most, often very weakly developed; sometimes the number of veins is reduced to one.

2.1.3. Organ symmetry challenged

Miniaturization can affect body symmetry. With strong body size reduction, there may not be enough space for both elements of what is usually a pair of organs. Reduction to one organ is quite widespread in the case of gonads. In male Ptiliidae, the right testis is completely missing, as is one of the two ovaries in the females of some species of the same family (Polilov, 2005, 2008; Polilov and Beutel, 2009). Female with a single ovary are recorded for many other insect (and non-insect) groups with extremely small species, like eriophyoid mites (Nuzzaci and Alberti, 1996). However, the same morphology occurs also in a number of large size animals (only one ovary is developed in most birds and in many insects, e.g., viviparous leaf beetles, aphids, dipterans, scarabaeine beetles; Minelli, 2003).

Much more remarkable is the case of the nervous system, in terms of organ position and, more important, of organ size. We have no functional or behavioural evidence as to the possible consequences of nervous system asymmetry, but this is clearly compatible with the animal's vital functions. The brain of first

instar larvae of corylophid beetles is strongly asymmetrical, with the left half considerably larger than the right half (Polilov and Beutel, 2009, 2010). Likewise, in the diminutive ptiliid beetles of tribe Nanosellini, the left distal process of the protocerebrum is larger than the right one (Polilov, 2008). Similarly, the protocerebrum of the I instar nymph of the psocopteran *L. bostrychophila* has asymmetrical outgrowths (in this case, the right one being the larger) that extend into the prothorax (Polilov, 2016b). In the adult females of the mymarid wasps, the abdominal ganglion shifts into the right half of the abdomen (Polilov, 2007).

A different consequence of a strong size reduction along the transversal axis is the loss of laterality (Minelli et al., 2010) we observe in the ‘cyclopic’ organization of the visual apparatus of cladocerans, where one median compound eye replaces the usual pair of laterally located eyes; less conspicuous, but of functional importance, is the partial symphysis of the chelicerae of the tiny symphytognathid spiders: these appendages are fused medially, at the base at least and often along the entire length (Moritz, 1993).

2.2. Unconventional morphologies exclusive to miniaturized forms

Miniaturized insects offer a number of ‘natural experiments’ that can help getting a better understanding of the evolvability of basic traits of animal structure that are very seldom challenged, even in the manipulations hitherto performed by developmental geneticists.

Of the two kinds of phenomena briefly discussed in this section, one points to the occurrence of a large set of (arguably functional) anucleate cells in the nervous system of some of the smallest insects – an important addition to the very small set of instances of anucleate cells in metazoans, the best known of which are the red blood cells of mammals.

The other phenomenon is the extension of brain structures into ‘segmental domains’ other than those to which the neuromeres belong. This means displacing part of the brain into a tagma other than the head, more often the thorax, but occasionally even up to the anterior abdomen.

2.2.1. Anucleate cells

The examples presented in the previous paragraphs document the effect of miniaturization in breaking the conventional rules of insect body architecture, but have no consequences for cell structure: only cell size and/or number are commonly affected, as summarized above. For example, despite a decrease in cell size and in the volume of the cytoplasm, the neurons of *L. bostrychophila* preserve the full set of organelles usually found in a nerve cell (Makarova and Polilov, 2017a). However, a remarkable reduction of the cytoplasm is common. In the neurons of the Ptiliidae, the nucleus occupies 80–90% of the cell volume (Polilov, 2005). In no miniaturized insect is the nuclear volume remarkably reduced; this has been interpreted by Polilov (2005) as an indication that a limit to miniaturization is probably due to the fact that genome size cannot be reduced beyond a viable minimum.

However, among the tiniest of miniaturized insects even the presence of a nucleus, the most characteristic trait of the eukaryotic cell, is eventually challenged. In *M. mymaripenne*, as described by Polilov (2012), the central nervous system (CNS) undergoes dramatic changes at metamorphosis. The CNS of the pupa, whose structure does not deviate from the organization typical of insects, includes ca. 7400 nucleated cells, whose total volume is nearly one fifth of the whole body volume. The number of neurons provided with a nucleus is much smaller in the adult; these nucleated neurons are accompanied by a small number of cell bodies in different stages of lysis and by some 7000 anucleate cells. Anucleate neurons

have been also found in *M. amalphanum* (Polilov, 2017a) and in *M. caribea* (Polilov, 2017b).

2.2.2. Tagmosis challenged

In a number of miniaturized insects, either at the larval or the adult stage, or both, part of the brain extends beyond the posterior boundary of the head, as defined by exoskeletal and internal articulation structures.

This condition is widespread among the smallest coleopteran larvae. Examples are found among the representatives of the basal suborders Archostemata, with *Micromalthus debilis* (Beutel and Hörnschemeyer, 2002), and Myxophaga, with *H. natans* (Beutel and Haas, 1998) and *Sphaerius acaroides* (Yavorskaya et al., 2018), but also in the polyphagan families Corylophidae (*S. lateralis*; Polilov and Beutel, 2010; Yavorskaya and Polilov, 2016) and Ptiliidae. Among the latter, in *Ptinella* the brain extends posteriorly to the mesothorax (Grebennikov and Beutel, 2002), while in *Mikado* sp. the brain is completely shifted to the thoracic segments and in the first instar larva it extends to the second abdominal segment (Polilov and Beutel, 2009).

Moving to adult beetles, in *Sphaerius* a considerable part of the posteriorly inclined protocerebrum reaches into the prothorax (Yavorskaya et al., 2018). The brain of adults of the majority of ptiliid beetles is located in the head (Makarova and Polilov, 2013a), but in the smallest representatives of the family, e.g. *Mikado* sp., part of the cerebrum and the subesophageal ganglion are displaced to the prothorax (Polilov and Beutel, 2009).

Similar shifts have not been reported for adults of any other group of insects (e.g., Mymaridae; Polilov, 2007), but examples are known for early post-embryonic stages, of very small size, of strepsipterans and thysanopterans. In the first instar larva of the strepsipteran *M. chobauti*, brain and subesophageal complex are shifted to the thorax and anterior abdomen (Osswald et al., 2010), while in the first instar larvae of the thysanopteran *H. haemorrhoidalis* the subesophageal ganglion and the thoracic ganglia have asymmetrical processes protruding into the leg coxae (Polilov, 2015). A shift of part of the subesophageal ganglion into the coxa has also been described in the nymphs of small spiders (Quesada et al., 2011).

Two coarse-grained variable traits of arthropod body architecture are *segmental* and *tagmatic pervasivity*. These refer to how much of the anatomy of a given species (or instar) presents a segmental or tagmatic organization, respectively (Fusco and Minelli, 2013). The loss in pervasivity in both segmentation and tagmosis, generally associated to the evolution of parasitic forms, is evidently a recurrent structural trait in miniaturization as well. This provides support to the idea that segments and tagmata are to some extent epiphenomenal features of body organization, emerging when particular conditions for segmentation (e.g., occurrence of several serial structures in register) and regional specialization are met (Minelli and Fusco, 2004; Fusco, 2008; Fusco and Minelli, 2013), but these structural conditions can easily break apart in different circumstances, as in the case of extremely small body sizes.

2.3. Novel functional solutions supported by unconventional morphologies in miniaturized forms

In arthropods there are two main kinds of compound eyes, indicated as *apposition* and *superposition* eyes, structurally and functionally very different from each other (Meyer-Rochow, 2015). As a basic operating principle, in the apposition eye each lens with its associated photoreceptors behaves as an independent visual unit (the *ommatidium*), contributing to a mosaic-like image of the outside world, whereas in the superposition eye the optical elements contribute together to produce a single image. A key

anatomical features of superposition eyes is the existence of a wide transparent *clear zone* beneath the optical elements, which allows the light rays collected by many lenses to be focused on the deep-lying retinal layer. The apposition eye produces an image of higher resolution than the superposition eye, but requires brighter light, thus crepuscular and nocturnal insects, e.g. moths, as well as many mid-water and deep-sea crustaceans (Frank, 2017) generally rely on more sensitive superposition compound eyes.

In a classic study on miniature compound eyes in arthropods, Meyer-Rochow and Gál (2004), through mathematical modelling of the optics of superimposition eyes of different sizes, showed that a minimal size exists, below which effective superposition can no longer occur. According to these authors, their results would provide an explanation as to why apposition eyes exist in very small scarabaeid beetles (members of a taxon characterized by the common occurrence of superposition eyes) and may also account for why juveniles of species in which the adults possess superposition eyes, frequently bear eyes that resemble apposition eyes (e.g., in malacostracans; Richter, 1999). Successive ultrastructural studies in small-size lepidopterans – e.g. *Ectoedemia argyropeza* (Honkanen and Meyer-Rochow, 2009), *Camreraria ohridella* (Fischer et al., 2012a) and *Stigmella microtheriella* (Fischer et al., 2012b) – but also hymenopterans (Fischer et al., 2011; Makarova et al., 2015) and coleopterans (Jia and Liang, 2014) revealed the occurrence of morphologically *intermediate eyes*, with features in-between apposition and superposition eyes (review in Meyer-Rochow, 2015). Size reduction in the compound eyes of small species belonging to clades in which superposition eyes are the rule, seems to be strictly correlated with structural modifications of eye design (Fischer et al., 2012a, 2012b). Makarova et al. (2015) list six adaptive alterations in the eye design which are related to miniaturization in *M. mymaripenne*. The evolution of intermediate eyes from superposition precursors may have occurred several times independently in various taxa of miniaturized insects (Fischer et al., 2014).

Sensitivity and resolution cannot be increased simultaneously in an eye of a given size (Warrant and McIntyre, 1993) as, once eye size is fixed, facet diameters are inversely proportional to their number. It is observed that both facet number and diameter decrease with smaller body size, and as the latter is accompanied by an increasing level of diffraction, it seems that the maintenance of a minimal number of facets is needed to fulfil the individual optical requirements of the species, and that this option is apparently favoured over a smaller number of larger facets, although with a smaller diffraction index (Fischer et al., 2014). Meyer-Rochow (2015: 468) concludes “To what extent general rules set the size limitations and what the contributions of optical, physical, and chemical factors are in this regard, needs to be examined in more detail.”

3. Miniaturization in context

3.1. Miniaturized juveniles

Research on miniaturized animals has long been biased by a strict focus on miniaturized adults. The diminutive and often extremely much smaller size of juveniles has only been seen as a necessary adjustment of the ontogenetic schedule culminating in an unusually small adult. However, as forcibly explained by Eberhard and Wcislo (2011: 163), “most arthropods must function independently just after emerging from the egg, when they are much smaller than an adult”.

The smallest arthropod larvae are somehow smaller than 100 μm . The first nauplius of the marine copepod *Dactylopusia tisboides* is 80 μm long (Lacalli, 2009); those of the parasitic tantulocarids *Tantaulacus dieteri* (Mohrbeck et al., 2010) and *Stygotantulus stocki* (Boxhall and Huys, 1989) ca. 85 μm and 94 μm , respectively.

Among the miniaturized insects, first instar larvae of comparable size are only known among the parasitoids. The smallest primary larvae of the Strepsiptera are just 80 μm long (Pohl, 2000); the size of the primary larva of the smallest mymarid wasps is not known, but will be hardly larger, at least in the case of species whose adult length is less than 200 μm . Again, it is among the parasitoids (more precisely, among egg parasitoids) that all the insects with the smallest adult size (300 μm or less) are found (Polilov, 2015). As first remarked by Ivanova-Kazas (1961), the yolk content in the eggs of these diminutive hymenopterans is very poor or even virtually zero. Metabolically, this is compensated for by developing in the nutritional medium provided by the host's egg; morphologically, this circumstance allows extreme size reduction not only of the parasitoid's egg, but also of its post-embryonic stages, the adult included. In terms of comparative developmental biology, their condition has been described as a strong ‘desembryonization’ (Ivanova-Kazas, 1961; Polilov, 2015); we would alternatively say that in these insects the usual divide between embryonic and post-embryonic development is obviously challenged. This interpretation allows to see another possible path through which evolutionary change can evade constraints, of developmental origin in this case. The requirement of a closed system for the morphogenetic processes of early embryonic development is fulfilled by a substitutive, aptly sheltered environment, where the same processes (or their equivalents) can progress during post-embryonic (post-hatching) development.

In free-living insects, the size of the first larva cannot be pushed much below the adult size, especially because of the resistance of the nervous system to a drastic reduction in size (Polilov and Beutel, 2009, 2010). For example, the adult of *S. lateralis* is 950–1200 μm long, and the first larva is more than half that size (540–660 μm) (Polilov and Beutel, 2010). More conspicuously, in the smallest miniaturized beetles the body length of the first larva is approximately the same as the adult length. For example, in *Mikado* sp., the first instar larva is 390–450 μm long, the adult 390–455 μm (Polilov and Beutel, 2009).

The first post-embryonic instars of miniaturized hemimetabolans are of essentially the same size as those of the smallest beetles: a length of 300–350 μm has been recorded for the psocopteran *L. bostrychophila* (Polilov, 2016b; Makarova and Polilov, 2017a), while the first instar larva of the thysanopteran *H. haemorrhoidalis* is 430–480 μm (Polilov and Shmakov, 2016).

3.2. Miniaturization throughout metamorphosis

In holometabolous insects, internal resources are recycled, to a variable extent, during the pupal stage or immediately thereafter. This may reduce the need for the adult to feed, and thus to possess functional mouthparts. Among the smallest of insects, the extremely miniaturized male of *D. echmepterygis* has rudimentary mouthparts (Mockford, 1997). The mouthparts are described as normal in the miniscule phorid fly *Euryplatea nanaknihali*, only 400 μm long (Brown, 2012), but the proboscis is greatly reduced in another phorid, *Megapropodiphora arnoldi*, only 395 μm long (Brown, 2018). Valuable resources to be spent in reproductive maturation are likely obtained by *S. lateralis* by the dismantling of musculature during postpupal development (Polilov, 2011).

In hemimetabolans, generally, there is no major structural change along the post-embryonic development. According to Polilov (2015, 2016b) this may explain why the smallest (adult) hemimetabolans, with a length of ca. 0.5 mm, are quite larger than the smallest holometabolans. To some extent, such a difference exists even if we do not take into consideration miniaturization in the parasitoid hymenopterans, for which desembryonization plays an additional role, as mentioned above. But this is not the whole

story. Important structural changes, to some extent comparable to those in holometabolans, occur in thysanopterans and especially in some clades of sternorrhynchous homopterans, in scale insects especially. To date, homopterans have never been considered in studies on insect miniaturization, as they would deserve. Let's just mention that the first instar nymphs of some scale insects are among the smallest free-living, mobile terrestrial arthropods: those of *Mycetaspis personata* are only 85 μm long (Beshr et al., 2009).

More generally, compared to holometabolans, hemimetabolous insects have lesser chances of 'breaking the rules', just because they have no opportunity for a post-embryonic reorganization of the whole body structure. We agree with Polilov (2015) that in hemimetabolans the first post-embryonic stage is likely the critical stage that fixes limits to miniaturization. Its size must be large enough to accommodate a nervous system with functional efficiency and thus of a size (cell number) and complexity comparable to the size and complexity it will have in the adult: as remarked by Polilov (2015; see also Polilov and Shmakov, 2016). The relative volume of the CNS is therefore much larger in the first post-embryonic instar than in the adult (four or five times in *H. haemorrhoidalis*, 3.8 times in *Liposcelis* sp.).

There is possibly another reason for why insect metamorphosis appears as a 'preadaptation' for miniaturization. After metamorphosis, adult insects do not grow. Adult structures are subject to the functional constraints of a self-sustaining biological system, but are dispensed from extensive involvement in development. Also, in many cases, the organs in the adult have to function only for a short while, corresponding to the animal's short life span. Some of the innovations in adult design we have seen in the previous paragraphs (e.g., anucleate cells) can certainly be viewed in this perspective.

3.3. Unbroken developmental constraints

Quite long ago, Rensch (1948) floated the idea that in animals miniaturization is limited by the size of the egg. This seems to apply to arthropods too, unless a major constraint is broken: filling the egg with an adequate amount of yolk upon which the embryo may survive and develop until a juvenile is produced, able to feed itself. As mentioned above, this constraint is broken in parasitoid wasps.

However, in the other insect clades with a trend towards miniaturization, this constraint remains. Egg size affects adult size in two ways: on the one side, because egg size sets the starting point upon which the adult will be formed, through an ontogenetic schedule along which size will grow, fueled by the intake of external food resources; on the other, and more important, because egg size constrains the size of the ovary, or ovaries, and thus the size of the female adult. As in the smallest representatives of other animal groups, in miniaturized insects the relative volume of the reproductive system is larger than in their bigger relatives (Polilov, 2015).

The length of an egg of ptiliid beetles is up to half the female's body length; of course, only one egg at the time can be brought to maturity (Dybas, 1966; Polilov, 2008; Grebennikov, 2008) and the reproductive apparatus, as mentioned before, is frequently reduced to one ovary only, as in Nanosellini (Polilov and Beutel, 2009).

Evolutionary trends are much less consistent in the case of the male gametes: within one family (Ptiliidae), some genera possess very short spermatozoa (De Marzo, 1992; Polilov, 2005), while in others the spermatozoa are longer than the body (De Marzo, 1992; Dybas and Dybas, 1987).

4. Prospects and concluding remarks

Miniaturized arthropods are not simply scaled-down versions of their larger closest relatives. Size reduction is generally accompanied by specific, non-proportional size changes in

different body structures, as captured by wide-ranging allometric relationships. Although since Galileo (*Discourses and Mathematical Demonstrations Relating to Two New Sciences*, as mentioned in this context by West, 2017: 39) much more attention has been paid to the problems associated with getting bigger, rather than to those associated with getting smaller, changes of proportions of body organs associated with the latter trend have received increasing attention in recent years (e.g., Hanken and Wake, 1993; Polilov and Makarova, 2017).

However, beyond these changes that in some way follow some wide-ranging rules dictated by developmental and functional constraints at different sizes, something more drastic happens when size reduction reaches the domain of extreme miniaturization. A number of works have documented the profound reorganization of structures and tissues associated with miniaturization (review in Polilov, 2016a). Among these profound changes, in principle one would like to distinguish between (adaptive) features imposed by the need to overcome an extant allometric constraint (e.g., disposal of the cell nucleus in some tissues) and (neutral) features deriving from the 'loss of resolution' determined by the fixed, or variable but not indefinitely compressible, size of patterning units (Hanken and Wake, 1993), e.g., with effects on wing venation, sclerite articulation or setal patterns.

In his influential book *The Structure of Scientific Revolutions* Kuhn (1962), Thomas Kuhn contrasted the activity of *normal science*, which he described as scientific work done within a prevailing framework (or paradigm) to *paradigm shifts*, which characterize scientific revolutions. Analogously, in principle at least, we could distinguish 'normal' evolutionary change along an allometric line in a morphospace or in a morpho-functional space, from 'paradigm-shift' innovations, where former allometric constraints are broken and new scaling relationships based on a new innovative organismal design emerge. This evolutionary change by 'paradigm-shift' would qualify as a kind of 'saltatory process', although this should not be intended as a sudden transition with no intermediates, or a qualitative rather than quantitative change, but simply as a 'jump' from a region of constrained change to another region of the morphospace.

In introducing the wide scope of scaling laws in organism body architecture, Schmidt-Nielsen (1984:32) warned that "Allometric equations are useful for showing how a variable quantity is related to body size, all other things being equal (which most certainly they are not)." Arthropod miniaturization seems largely to be a story of modification of features commonly supposed not to change.

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Appendix 1. Miniaturized insects and other arthropods cited in the article.

Collembola

Megalothorax Willem, 1900 (Neelidae)
Sphaeridia Linnaniemi, 1912 (Sminthuridae)

Psocoptera

Liposcelis bostrychophila Badonnel, 1931 (Liposcelidae)

Homoptera

Atarsos grindeliae Gillette, 1911 (Aphididae)
Mycetaspis personata (Comstock, 1883) (Diaspididae)

Thysanoptera

Heliethrips haemorrhoidalis (Bouché, 1833) (Thripidae)

Coleoptera

Micromalthus debilis LeConte, 1878 (Micromalthidae)

Hydroscapha natans LeConte, 1874 (Hydroscaphidae)

Sphaerius acaroides Waltl, 1838 (Sphaeriusidae)

Lepiceridae

Torrincolidae

Meru phyllisae Spangler and Steiner, 2005 (Meruidae)

Acrotichis grandicollis (Mannerheim, 1844) (Ptiliidae)

Mikado Matthews, 1889 (Ptiliidae)

Nanosella Motschoulsky, 1869 (Ptiliidae)

Primorskiella Polilov, 2008 (Ptiliidae)

Ptinella tenella (Erichson, 1845) (Ptiliidae)

Sericoderus lateralis (Gyllenhal, 1827) (Corylophidae)

Strepsiptera

Mengenilla chobauti Hofeneder, 1910 (Mengenillidae)

Hymenoptera

Encarsia formosa Gahan, 1924 (Aphelinidae)

Megaphragma amalphanum Viggiani, 1997

(Trichogrammatidae)

Megaphragma caribea Delvare, 1993 (Trichogrammatidae)

Megaphragma mymaripenne Timberlake, 1924

(Trichogrammatidae)

Trichogramma brassicae Bezdenko, 1968 (Trichogrammatidae)

Trichogramma evanescens Westwood, 1833

(Trichogrammatidae)

Trichogramma pretiosum Riley, 1879 (Trichogrammatidae)

Anaphes flavipes (Förster, 1841) (Mymaridae)

Dicopomorpha echmepterygis Mockford, 1997 (Mymaridae)

Lepidoptera

Stigmella microtheriella (Stainton, 1854) (Nepticulidae)

Ectoedemia argyropeza (Zeller, 1839) (Nepticulidae)

Cameraria ohridella Deschka and Dimic, 1986 (Gracillariidae)

Diptera

Euryplatea nanaknihali Brown, 2012 (Phoridae)

Megapropodiphora arnoldi Brown, 2018 (Phoridae)

Copepoda

Dactylopusia tisburyoides (Claus, 1863) (Harpacticoida)

Dactylopusiidae)

Tantulocarida

Stygotantulus stocki Boxhall and Huys, 1989 (Basipodellidae)

Tantaulacus dieteri Mohrbeck, Arbizu and Glatzel, 2010

(Deoterthridae)

Araneae

Symphytognathidae

Acari

Eriophyoidea

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