

# Cell size versus body size in geophilomorph centipedes

Marco Moretto<sup>1</sup> · Alessandro Minelli<sup>1</sup> · Giuseppe Fusco<sup>1</sup>

Received: 22 December 2014 / Revised: 9 March 2015 / Accepted: 12 March 2015  
© Springer-Verlag Berlin Heidelberg 2015

**Abstract** Variation in animal body size is the result of a complex interplay between variation in cell number and cell size, but the latter has seldom been considered in wide-ranging comparative studies, although distinct patterns of variation have been described in the evolution of different lineages. We investigated the correlation between epidermal cell size and body size in a sample of 29 geophilomorph centipede species, representative of a wide range of body sizes, from 6 mm dwarf species to gigantic species more than 200 mm long, exploiting the marks of epidermal cells on the overlying cuticle in the form of micro-sculptures called scutes. We found conspicuous and significant variation in average scute area, both between suprageneric taxa and between genera, while the within-species range of variation is comparatively small. This supports the view that the average epidermal cell size is to some extent taxon specific. However, regression analyses show that neither body size nor the number of leg-bearing segments explain this variation, which suggests that cell size is not an usual target of change for body size evolution in this group of arthropods, although there is evidence of its correlation with other morphological variables, like cuticle thickness. Scute sizes of miniaturized geophilomorph species are well within the range of the lineage to which the species belong, suggesting recent evolutionary transitions to smaller body size.

**Keywords** Chilopoda · Cuticle sculpture · Epithelia · Evolution · Miniaturization

Communicated by: Sven Thatje

✉ Giuseppe Fusco  
giuseppe.fusco@unipd.it

<sup>1</sup> Department of Biology, University of Padova, Padova, Italy

## Introduction

Total body size and the relative dimensions of body parts are key attributes that shape most of a species' functions, behaviour, and ecological relationships. However, the developmental mechanisms that control size and shape are still unexplored or incompletely understood in most animal taxa (Nijhout and Callier 2015).

In the few animal species in which adequate studies have been performed, intraspecific variation in body size is the result of a complex interplay between variation in cell number and variation in cell size (e.g. Robertson 1959; Partridge et al. 1994; De Moed et al. 1997; Azevedo et al. 2002; Nijhout et al. 2014). Cell growth and cell proliferation are regulated by partly independent mechanisms that can respond separately to genetic and environmental variation (Schmelzle and Hall 2000; Nijhout 2003; Nijhout and Callier 2015).

Overall, within a species, differences in cell size are arguably more important, as a determinant of an individual's body size (e.g. Stevenson et al. 1995), but this is not necessarily true in interspecific comparisons (Arendt 2007). There are fundamental differences in the mechanisms by which distantly related groups, e.g. mammals and insects, control body size (Trumpp et al. 2001), despite the existence of an evolutionarily conserved signaling cascade that links external nutrient sources to cell size (Stocker and Hafen 2000). Thus, differences in body size between mice and elephants are mainly the result of differences in cell number (Conlon and Raff 1999), whereas cell size contributes significantly to body size variation among *Drosophila* species (Stevenson et al. 1995).

In comparative zoology, cell size is very seldom considered worth investigation, and its variation is seldom seen as a feature characterizing an evolutionary trend. The general view does not go beyond textbook generalizations, e.g. that the enormous variation in adult body size across the Metazoa

(more than 20 orders of magnitude in volume) is mainly realized through variation in cell number, rather than in cell size. But this is an incorrect and uninformative oversimplification because, despite the limited amount of available data, interesting patterns of cell size variation have been described.

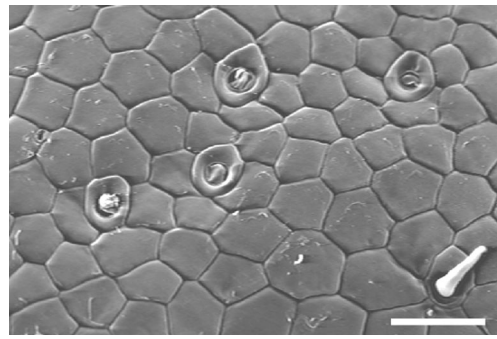
On one side there are descriptive data showing extreme variation in somatic cell size. For instance, very small cell size characterizes the loriciferans, minuscule marine invertebrates which measure at most 300  $\mu\text{m}$  as adults (less than several unicellular eukaryotes), but are made of more than 10,000 cells (Kristensen 1991), while in the appendicularians, another group of small marine invertebrates, the body (about 1 mm, e.g. in *Oikopleura dioica*, if we ignore the long but thin tail) is made of a very small number of cells, some of which are of enormous size (Brena et al. 2003).

On the other side, an opposite trend emerges from other studies on miniaturized metazoans. Miniaturization leads to considerable reorganization of structures in insects not only at the level of organs but also at the cellular level, with a marked decrease in the size of cells in the nervous system (Polilov 2015). However, low numbers of cells, corresponding to a larger average cell size, have been recorded for other miniaturized forms, e.g. the paedogenetic males of the two known species of the Cycliophora, *Symbion pandora* and *S. americanus* (30–40  $\mu\text{m}$  for about 60 cells, Neves et al. 2009).

Undoubtedly, the scattered available information on the control of cell size and the correlation between cell size and the animal's body size or complexity reveals a diversity of patterns, and the comparison between loriciferans and male cycliophorans shows that metazoan complexity is not strictly correlated to either body size or the overall number of cells of an individual (Neves et al. 2009).

In this paper, we present the result of a comparative investigations on epidermal cell size in a sample of 29 centipede species, all belonging to the order Geophilomorpha, spanning a wide range of size at full growth (body length 6–200 mm) and including species which, compared to the average size of their lineage's members, deserve to be considered miniaturized. This is the first study of this kind in any myriapod group and among the very few where the question of cell size is comparatively viewed in an evolutionary perspective, not strictly related to miniaturization. This was accomplished by exploiting the marks of epidermal cells on the overlying cuticle.

The cuticle of many arthropods more or less locally displays a common form of surface micro-sculpture, which takes the form of a polygonal pattern (Fig. 1). The individual convex polygonal fields forming this pattern are called *scutes* (Cals 1974) and are generally thought to correspond to the apical surface of as many cells in the underlying epidermis (Hinton 1970; Grassé 1975), the arthropod monolayer epithelium which secretes



**Fig. 1** Surface view of centipede cuticle (SEM microscopy). Polygonal pattern of scutes and some specialized structures (a sensillum and the pore openings of a few solitary epidermal glands) are visible. *Geoperingueyia* sp., penultimate sternite. Bar 10  $\mu\text{m}$ . Photo courtesy Leandro Drago

the cuticle (Rosenberg et al. 2011, Moussian 2013). Fusco et al. (2000) showed that in the head of the centipede *Lithobius* the polygonal surface pattern of the cuticle reproduces exactly the shape of the apical side of the epidermal cells at the stage of deposition of the very first layers of the cuticle, through a template mechanism.

In geophilomorph centipedes, a polygonal pattern is detectable in several cuticular districts, among which the cephalic shield is usually the place where scutes are most evident. Although not supported by direct evidence, it seems reasonable to suppose that the cuticular pattern of geophilomorphs represents a *cellular template pattern*, as in *Lithobius*. However, for the scope of the present study, it suffices to assume that the cuticle of geophilomorphs simply exhibits a *cellular derived pattern*, where the single scutes have unicellular origin, but their shape does not necessarily coincide with cell shape (Fusco et al. 2000). This is because we are not elaborating on scute shape but only on their average size, which simply derives from their number per unit area.

## Materials and methods

### Sample specimens

This analysis is based on 39 specimens representative of 29 species of all major clades of Geophilomorpha (Bonato et al. 2014a) and spanning the whole range of body size in the group, including two gigantic species and four species that can be considered miniaturized (Foddai et al. 2003). The 29 species are classified into nine suprageneric taxa, traditionally treated as families or subfamilies (Table 1).

Specimens belong to the Minelli-Bonato Chilopoda collection (Department of Biology, University of Padova, Italy), where they are preserved in 70 % ethanol.

**Table 1** Study dataset

Species	iCS	iBL	iLBS	iASA	sBL	sLBS	s.d. SA	CV SA	Sample (n)
<b>Mecistocephalidae</b>									
<i>Arrup kyushuensis</i> Uliana, Bonato & Minelli, 2007	0.76	12.1	41	90.7	12	41			
<i>Mecistocephalus karasawai</i> Uliana, Bonato & Minelli, 2007	1.39	33.0	47	71.9	33	49			
<i>Nannarrup hoffmanni</i> Foddai, Bonato, Pereira & Minelli, 2003	0.63	10.4	41	83.6	10	41	19.5	0.23	104
<i>Takashimaia ramungula</i> Miyosi, 1955	2.10	40.0	45	69.2	40	45			
<b>Oryidae</b>									
<i>Orya barbarica</i> (Gervais, 1835)	3.09	116.9	95	48.4	220	117			
<i>Orya barbarica</i> (Gervais, 1835)	3.22	123.0	97	45.8	220	117			
<b>Himantariidae</b>									
<i>Haplophilus souletinus</i> Brölemann, 1907	1.03	55.7	99	74.5	88	103	24.8	0.33	104
<i>Himantarium gabrielis</i> (Linnaeus, 1767)	1.83	57.8	105	42.2	195	139			
<b>Schendylinae</b>									
<i>Schendyla carniolensis</i> Verhoeff, 1902	0.82	20.6	43	89.5	45	45	21.5	0.24	104
<i>Dinogeophilus oligopodus</i> Pereira, 1984	0.32	6.0	29	104.2	6	31			
<i>Nannophilus eximius</i> (Meinert, 1870)	0.95	37.5	71	118.2	50	71	24.9	0.21	104
<i>Schendylops ramirezi</i> (Pereira, 2013)	0.35	7.0	29	109.9	7	29			
<i>Pectiniunguis geayi</i> (Brölemann & Ribaut, 1911)	0.85	27.1	51	106.2	25	51	24.3	0.23	104
<i>Pectiniunguis geayi</i> (Brölemann & Ribaut, 1911)	0.90	24.6	51	102.4	25	51	22.3	0.22	104
<i>Pectiniunguis geayi</i> (Brölemann & Ribaut, 1911)	0.60	17.5	47	102.9	25	51	23.6	0.23	104
<i>Pectiniunguis ducalis</i> Pereira, Minelli & Barbieri, 1995	1.20	33.2	67	99.8	52	69			
<b>Ballophilinae</b>									
<i>Ityphilus crabilli</i> Pereira, Minelli & Barbieri, 1994	0.78	27.4	47	64.1	21	53			
<i>Ityphilus donatellae</i> Pereira, 2012	0.34	11.0	43	48.8	11	43			
<b>Geophilinae</b>									
<i>Geophilus alpinus</i> Meinert, 1870	0.84	11.8	45	71.2	35	55	18.9	0.27	103
<i>Geophilus alpinus</i> Meinert, 1870	0.87	18.3	45	72.0	35	55	19.9	0.28	104
<i>Geophilus alpinus</i> Meinert, 1870	1.21	25.8	47	68.2	35	55	20.1	0.29	102
<i>Geophilus alpinus</i> Meinert, 1870	1.19	21.9	47	66.8	35	55	15.1	0.23	101
<i>Geophilus carpophagus</i> Leach, 1815	1.40	44.1	59	64.2	54	55	21.4	0.33	99
<i>Geophilus pygmaeus</i> Latzel, 1880	0.72	18.0	43	75.5	15	43	19.7	0.26	108
<i>Geophilus richardi</i> Brölemann, 1904	0.52	7.8	33	64.4	9	33	19.7	0.31	104
<i>Geophilus richardi</i> Brölemann, 1904	0.53	8.2	33	71.7	9	33	18.8	0.26	104
<i>Clinopodes flavidus</i> C. L. Koch, 1847	1.36	42.1	63	71.0	65	69	24.2	0.34	104
<i>Stenotaenia linearis</i> (C. L. Koch, 1835)	0.86	26.2	75	73.1	50	73	24.4	0.33	100
<b>Ribautiinae</b>									
<i>Pachymerium ferrugineum</i> (C. L. Koch, 1835)	1.11	27.8	51	90.4	45	49	23.2	0.26	100
<i>Pachymerium ferrugineum</i> (C. L. Koch, 1835)	1.02	22.9	51	86.2	45	49			
<i>Gnathoribautia bonensis</i> (Meinert, 1870)	1.47	52.9	71	84.1	55	75			
<i>Gnathoribautia bonensis</i> (Meinert, 1870)	1.02	22.3	71	80.2	55	75			
<i>Ribautia centralis</i> (Silvestri, 1907)	1.93	61.0	63	97.1	46	65	26.2	0.27	106
<i>Ribautia proxima</i> Pereira, Minelli & Barbieri, 1995	0.71	22.3	73	98.7	39	77	25.1	0.25	104
<i>Hyphidrophilus adisi</i> Pereira, Minelli & Barbieri, 1994	0.72	17.3	43	93.6	16	43			
<i>Hyphidrophilus adisi</i> Pereira, Minelli & Barbieri, 1994	0.77	18.1	43	101.1	16	43			
<b>Dignathodontidae</b>									
<i>Henia vesuviana</i> (Newport, 1845)	1.10	13.3	59	65.4	52	71			
<i>Dignathodon microcephalus</i> (Lucas, 1846)	0.68	52.0	45	63.0	60	79			
<b>Linotaeniidae</b>									
<i>Strigamia acuminata</i> (Leach, 1815)	1.22	35.4	39	41.4	34	41			

Seven species are represented by more than one individual. Single scute areas (SA) were measured in a subsample of 20 individuals

*iCS* individual cephalic size (compound measure, see text), *iBL* individual body length (mm), *iLBS* individual number of leg-bearing segments, *iASA* individual average scute area ( $\mu\text{m}^2$ ), *sBL* species adult body length (mm), *sLBS* species modal number of leg-bearing segments, *s.d. SA* standard deviation of individual scute area, *CV SA* coefficient of variation of individual scute area, *n* number of individually sampled scutes

## Sample cephalic area

A rectangular area 240  $\mu\text{m}$  wide and 180  $\mu\text{m}$  long (43,200  $\mu\text{m}^2$ ) was selected for recording scute pattern. This is an approximately flat cuticular area, localized in the antero-medial zone of the cephalic shield, where the scutes are generally well visible (Fig. 2). Depending on the size of the specimen, this area comprises about 1 to 30 % of the total dorsal area of the cephalic shield, and depending on the species, it includes from about 350 to about 1050 scutes. For the three very small species *Dinogeophilus oligopodus*, *Schendylops ramirezi*, and *Ityphilus donatellae*, whose whole cephalic shield width does not reach 240  $\mu\text{m}$ , a smaller area (measuring 5000, 10,000, and 10,000  $\mu\text{m}^2$ , respectively) was considered.

## Preparation

Specimens were placed in a lactophenol solution for 2 to 3 weeks, which makes tissues more transparent, before being processed. Most specimens were mounted undissected on a slide to be photographed with a digital camera Leica DMR under a light microscope. In few cases of very opaque heads, the cephalic shield was detached using entomological needles and eventually mounted on the microscope slide.

For *D. oligopodus* and *I. donatellae*, our counts were based on original drawings kindly provided by L. A. Pereira (National University of La Plata, Argentina) and for *S. ramirezi* on Figure 12 in Pereira (2013). These are camera lucida drawings, accurately reproducing the scute pattern on the centipedes' heads.

## Measurements

Depending on the specimen's overall size, body length of the individual (iBL) was measured under a dissecting microscope using a micrometric reticulum or with a calliper, and the individual's number of leg-bearing segments (iLBS) was counted under a light or dissecting microscope. Cephalic length and width and the number of scutes in the sample area were

measured on the digital image. A linear measure of individual cephalic size (iCS) was calculated as the square root of the sum of squares of cephalic length and width. Owing to animal contraction upon fixation, iBL can be affected by a larger relative measurement error than iCS; however, the latter could be a less significant measure in interspecific comparisons, due to evolutionary allometry. For these reasons in our analyses, we considered both measures.

Individual average scute area (iASA) was calculated dividing the surface of the sample area by the number of counted scutes. Scutes across the borders of the sample area were counted 1/2. For regression analysis, a linear measure of scute size (iSS) was obtained by the square rooting of iASA. Estimates of species scute size (sSS) were calculated by square rooting the average value of iASA of the individuals of the same species.

For 20 individuals of 14 species, using a digital pen on the digital image, the actual area of single scutes (SA) was measured in a subsample of about 100 scutes ( $n$  in Table 1) within the cephalic rectangular sample area (CASTI ImageNT version 2.6).

Species approximate body length (sBL) and species modal number of leg-bearing segments (sLBS) were assigned on the basis of several sources from the taxonomic literature.

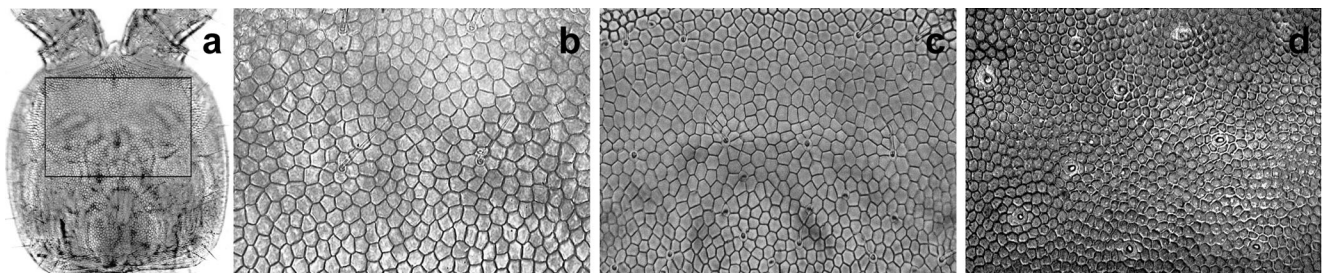
## Statistical analyses

Variation was scrutinized through one-way analysis of variance (ANOVA) and the Kruskal-Wallis non-parametric test. The relationship between morphometric variables was inspected through least-squares linear regression. All analyses were carried out with the package *Statgraphics Centurion*, ver. XVI.

## Results

### Scute area variation outline

Individual average scute area (iASA) varies from 41.4 to 118.2  $\mu\text{m}^2$  (Table 1, Fig. 2). There is a significant variation in iASA means and medians between the nine suprageneric



**Fig. 2** Geophilomorph scute patterns on the cephalic shield. **a** An example of the rectangular sampling area (here *Geophilus pygmaeus*). Depending on the cephalic size of the individual, this area can comprise different proportions of the whole cephalic shield dorsal area, although

approximately centred in the same relative position. **b–d** Examples of scute patterns in the sampled area (240 $\times$ 180  $\mu\text{m}$ ) in three species with different average scute area, from large to small, **b** *Pectiniunguis geayi*, **c** *Geophilus richardi*, **d** *Orya barbarica*



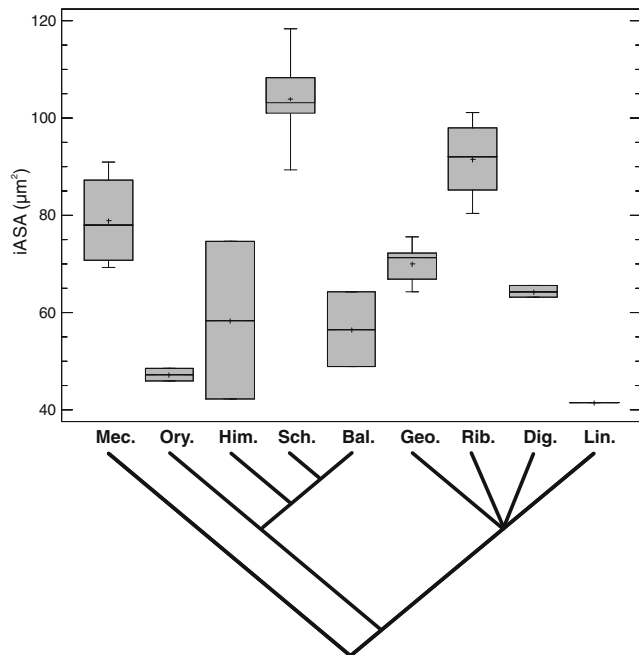
taxa (ANOVA,  $p < 0.0001$ , Kruskal-Wallis  $p = 0.0004$ ), with Linotaeniidae and Schendylinae at the two extremes (Fig. 3), and between genera (ANOVA,  $p < 0.0001$ , Kruskal-Wallis  $p = 0.0256$ ), with the smallest scutes in *Himantarium* and *Strigamia*, and the largest in *Nannophilus* (Fig. 4). The range of variation of aSA is generally modest within genera ( $< 8 \mu\text{m}^2$ ), only slightly larger in *Geophilus* ( $11.3 \mu\text{m}^2$ ) and *Ityphilus* ( $15.3 \mu\text{m}^2$ ).

**Scute area intraindividual variation**

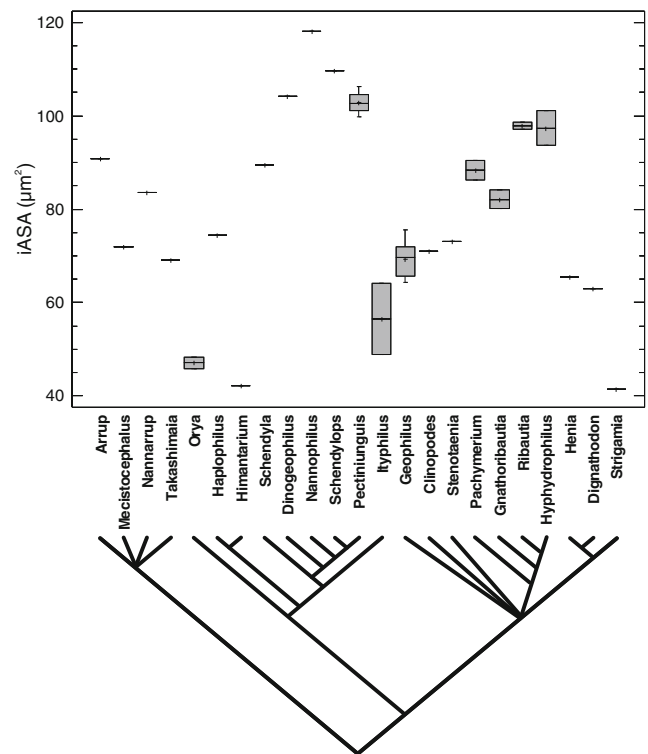
In the 20 individuals where single scute areas (SA) were measured, within-individual SA standard deviation ranges between  $15.1$  and  $26.2 \mu\text{m}^2$ , while the coefficient of variation ranges from 21 to 33 % (Table 1). Individual differences in SA standard deviation are however not significant (Cochran’s test,  $p > 0.06$ ).

**Scute area intraspecific variation**

Seven species are represented in the sample by two to four specimens. The within-species range of variation of aSA is small in all cases ( $< 8 \mu\text{m}^2$ ). For the three of these species where individual scute measures are available, between-individual variation resulted significant with respect to



**Fig. 3** Variation in individual average scute area (iASA) between geophilomorph main taxa. Phylogenetic relationships and taxonomic nomenclature after Bonato et al. (2014). Mec., Mecistocephalidae; Ory., Oryidae; Him., Himantariidae; Sch., Schendylinae; Bal., Ballophilinae; Geo., Geophilinae; Rib., Ribautiinae; Dig., Dignathodontidae; Lin. Linotaeniidae. Geophilinae and Schendylinae are probably not monophyletic taxa. In the boxplot, boxes represent the interquartile interval, with median (transverse line) and mean (small cross); vertical lines are ranges of variation



**Fig. 4** Variation in individual average scute area (iASA) between geophilomorph genera. Phylogenetic relationships and taxonomic nomenclature after Bonato et al. (2014). In the boxplot, boxes represent the interquartile interval, with median (transverse line) and mean (small cross); vertical lines are ranges of variation

within-individual variation in *Geophilus richardi* ( $n = 2$ , Student’s  $t$  test,  $p = 0.0067$ ) and non-significant in *Geophilus alpinus* ( $n = 4$ ) and *Pectiniunguis geayi* ( $n = 3$ ) (ANOVAs,  $p > 0.15$ ).

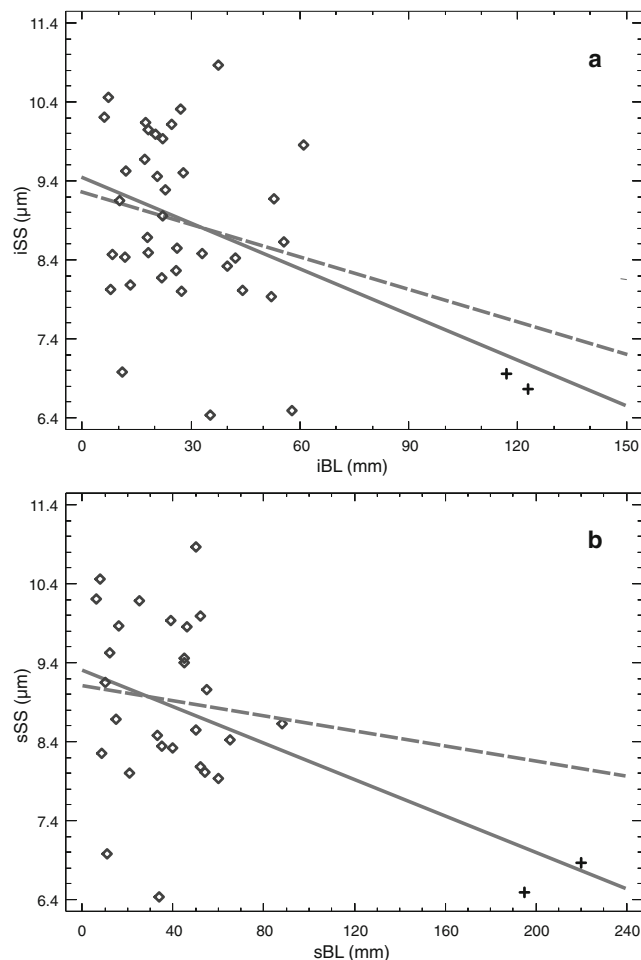
For *G. alpinus* (four individuals, body length  $11.8$ – $25.8$  mm) and *P. geayi* (three individuals, body length  $17.5$ – $27.1$  mm), linear regression coefficients of aSA vs. individual body length (iBL) are non-significant (both  $p > 0.92$ ). For the other five species represented by two individuals, only in two cases (*Pachymerium ferrugineum* and *Gnathoribautia bonensis*) one individual is sizably larger than the other. In both cases, the relative difference in aSA (for both species, 5 %) is concordant (same sign) with the difference in body size (19 and 82 %, respectively) but much smaller.

**Scute size versus body size**

Although there is no evidence of marked effects of individual size (an age correlate) on scute size, the subsequent interspecific analyses were conducted using both individual (iCS and iBL) and species (sBL) estimates of body size and individual (iSS) and species (sSS) estimates of scute size.

Regression analysis of scute size on either individual size (iBL and iCS) or species size (sBL) is strongly

influenced by points with a leverage several times larger than an average data point (Fig. 5). Hence, analysis was carried out both including and excluding those influential points. Regressions of iSS vs. iBL and iSS vs. iCS get similar results, as iBL and iCS are strongly correlated ( $r=0.90$ ), and in both regressions, the influential data points are the two individuals of *Orya barbarica*, which have a leverage between 5.6 and 7.1 times that of an average data point. With all data points in, regression coefficients are significant and negative ( $n=39$ ,  $p=0.0040$  for iBL and  $p=0.0030$  for iCS). Excluding the two influential points, no regression coefficient is significant. For sSS vs. sBL, there are two influential points, *O. barbarica* and *Himantarium gabrielis*, with a leverage of 6.9 and 5.2 times the average, respectively. With all data points, the regression coefficient is significant and negative ( $n=29$ ,  $p=0.0077$ ). Excluding the two influential points, the regression coefficient is not significant.



**Fig. 5** Regressions of individual scute size (iSS) vs. individual body length (iBL) (a), and species scute size (sSS) vs. species body length (sBL) (b). In both graphs, exclusion of two influential points (crosses) turns a significant regression coefficient (continuous line) into non-significant (dashed line). See text

Although regression analysis shows that there is no general correlation between species size and the average size of cephalic scutes, it is noteworthy that the two biggest species in our sample (*O. barbarica* and *H. gabrielis*) have among the smallest aSA ( $47.1$  and  $42.2 \mu\text{m}^2$ , respectively). On the contrary, the four smallest species (*G. richardi*, *D. oligopodus*, *S. ramirezi*, and *N. hoffmanni*) have scute sizes that span more than half of the whole aSA species range, from  $68.1 \mu\text{m}^2$  in *G. richardi* to  $109.9 \mu\text{m}^2$  in *S. ramirezi*.

### Scute size versus number of leg-bearing segments

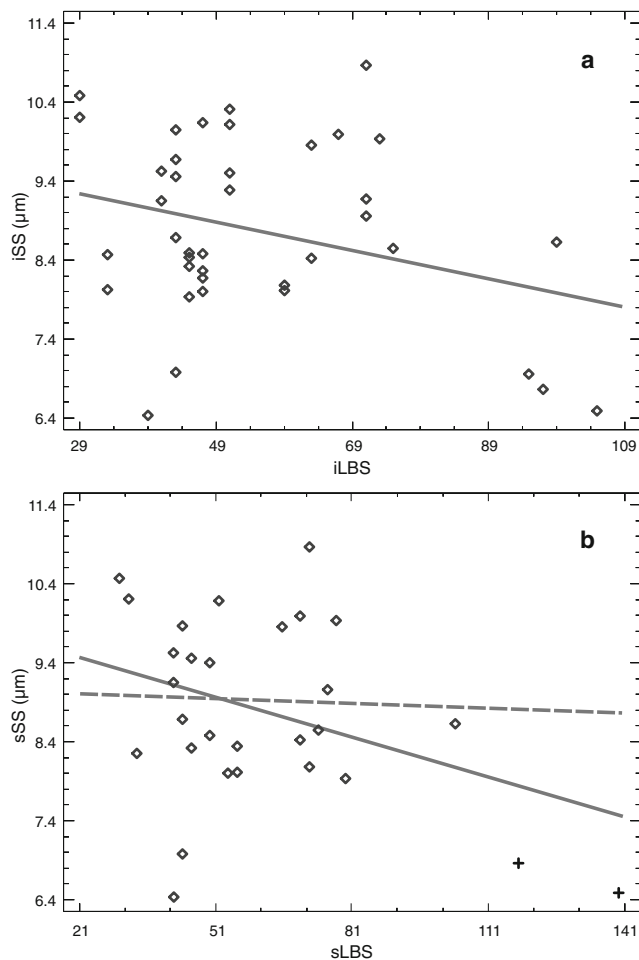
The conspicuous interspecific and intraspecific variation in the number of trunk segments in geophilomorph centipedes is traditionally expressed in terms of variation in the number of leg-bearing segments (Fusco 2005). The latter varies in the taxon between 27 and 191 and, as a nearly universal rule, only odd numbers occur (Leśniewska et al. 2009; Fusco and Minelli 2013). The number of leg-bearing segments (LBS) is considered an important phenotypic character in geophilomorph comparative studies, with a complex relationship to body size (Minelli et al. 2010).

Regression of iSS vs. iLBS is not significant ( $n=39$ ,  $p>0.071$ ) and regression of sSS vs. sLBS is barely significant ( $n=29$ ,  $p=0.0464$ ). However, excluding the two influential points represented by the polypodous species of the genera *Orya* and *Himantarium* (leverage of 3.0 and 5.4 times the average, respectively), the regression coefficient is not significant (Fig. 6).

Although the regression analysis shows that there is no general correlation between scute size and the number of leg-bearing segments, it is noteworthy that the two most polypodous species in our sample (*O. barbarica* and *H. gabrielis*) have among the smallest iASA ( $47.1$  and  $42.2 \mu\text{m}^2$ , respectively). However, another polypodous himantariid species (*Haplophilus souletinus*) has no particularly small scutes ( $74.5 \mu\text{m}^2$ ).

### Discussion

Epithelia can display highly dynamic behaviour, as cells continuously die or divide, and epithelial cells can change shape or intercalate as tissues change size and shape during morphogenesis (Guillot and Lecuit 2013). On the basis of live imaging of cell junction dynamics in *Drosophila*, Gibson et al. (2006) showed that a steady-state distribution of polygons formed by cell lateral surfaces is a fundamental property of epithelia with replicating cells. Studies in this area also show aspects of morphogenesis that are not under direct genetic control but are instead the result of the complex interactions



**Fig. 6** Regressions of individual scute size (iSS) vs. individual number of leg-bearing segments (iLBS) (a) and species scute size (sSS) vs. species modal number of leg-bearing segments (sLBS) (b). Regression coefficient is not significant in (a), and exclusion of two influential points (crosses) in (b) turns a barely significant regression coefficient (continuous line) into non-significant (dashed line). See text

between geometric and biomechanical properties of epithelial tissues (Gibson and Gibson 2009).

Our approach offers only a partial view of geophilomorph cell size, limited to the size of the cell’s apical side. Variation in cell apical surface could be directly correlated with cell height (small apical surfaces indicating smaller cells), inversely correlated with cell height (small apical surfaces indicating a thicker epithelium, with minor variation in cell volume), or not be correlated at all; intraspecific and interspecific variation in cell apical surface could be based on different relationships with epithelial thickness. Cell apical surface area and the corresponding scute area can thus result from an interplay of cell size and cell shape that cannot be disentangled in our measurements, but that represents an aspect of epithelia morphological variation which is relevant in determining arthropod body size through exoskeleton formation.

There is a conspicuous and significant variation in average scute area (iASA), both between the suprageneric taxa

traditionally treated as families or subfamilies and between genera, while the within-species range of variation is comparatively small. This supports the view that average scute area, and thus average epidermal cell density, is to some extent taxon specific. Although our dataset does not allow us to test this view rigorously, the phylogenetic pattern of iASA variation suggests some degree of phylogenetic inertia (Blomberg and Garland 2002). This is further supported by the limited amount of intrageneric variation in those genera (*Pectiniunguis*, *Geophilus*, *Ribautia*) where two or more species have been sampled, compared with variation between genera or suprageneric taxa.

However, in front of the considerable variation in average scute area, regression analyses show that neither body size nor the number of leg-bearing segments explain this variation. Nonetheless, a closer examination of our data invites some further considerations.

From the viewpoint of scute size, no pattern emerges for the taxonomic distribution of the species with the largest scutes (>90  $\mu\text{m}^2$ ), which are scattered among different taxa of different body size. On the contrary, a possible functional correlation emerges in the case of the geophilomorphs with the smallest scutes (<50  $\mu\text{m}^2$ ). These are *Orya barbarica*, *Himantarium gabrielis*, *Ityphilus donatellae*, and *Strigamia acuminata*, representatives of four distinct families. In these species, body size varies from large (*O. barbarica*, *H. gabrielis*) to intermediate (*S. acuminata*) to small (*I. donatellae*). These species seem to share the common trait of a relatively robust cephalic shield cuticle, as suggested by routine taxonomic practice under light microscopy. Cells with small apical surface, if not small also in volume, may express a higher potential of extracellular matrix (cuticle) production per unit area through ordinary secretory pathways (Moussian 2013).

From the perspective of adult body size, which in Geophilomorpha is broadly correlated with the number of body segments (Minelli et al. 2010), a premise is in order. Phylogenetic considerations (Bonato et al. 2003, 2014a) and palaeontological evidence (Bonato et al. 2014b) suggest that the range of variation in body size exhibited by living geophilomorphs is the result of diverging evolutionary trends toward both larger and smaller body sizes and both higher and lower number of segments, from an intermediate primitive condition. In fact, parsimony-based reconstruction of the state of these characters in the earliest representatives of Geophilomorpha suggests a body length in the interval of 1 to 3 cm and a number of leg-bearing trunk segments in the range 41–45.

Large species (BL > 30 mm) show no discernible patterns in average scute size, except for the noteworthy observation that the two largest species in our sample, *O. barbarica* and *H. gabrielis*, are among those with the smallest scutes. This observation can be hardly compared with similar situations in

other animal taxa, as evolution toward larger sizes has apparently not received the same attention than miniaturization.

Conversely, the study of the smallest species of a group has been the object of study in several taxa. A key question is how far different cellular organizations of taxa with different body size depend on some differential constraints on cell size or simply reflect a different story in miniaturization. Several studies have shown that a small body size associated to small cell size occurs more frequently when miniaturization is an old feature of the group (e.g. in hydroscaphid beetles (Beutel and Haas 1998) and in strepsipterans (Beutel et al. 2005)), as the possible outcome of an also long history of microanatomical adjustment. While at the same time, an association of small body size with a relatively large or unaltered cell size occurs in groups that evolved toward small sizes more recently (e.g. in plethodontid salamanders (Roth et al. 1994, 1995)).

In geophilomorphs, a clear boundary between “normal” and miniaturized species or lineages is difficult to trace. Morphological correlates of a strongly reduced body size can be apparent to very diverse degrees, and any generalizations are to some extent arbitrary. Here, by considering an operational threshold at 10 mm, we have four miniaturized species: *N. hoffmanni*, *D. oligopodus*, *S. ramirezi* and *G. richardi*. All of them have scute sizes which are well within the range for the non-miniaturized species in their family or subfamily; this is expected in the case of a relatively recent miniaturization in the presence of some phylogenetic inertia.

Summing up, we found no indications of an association between body size and cell size in geophilomorph centipedes. This, together with the wide range of variation in body size and the observed phylogenetic inertia in scute size, suggests that cell size is not a usual target of change for body size evolution in this group of arthropods. However, evidence suggesting less general patterns, such as the association of cell size with other morphological variables, may well deserve further investigation.

**Acknowledgments** We are indebted to Luis Pereira for access to his detailed drawings of *Dinogeophilus oligopodus* and *Ityphilus donatellae*, Lucio Bonato for suggesting a possible correlation between scute size and cuticle thickness, and Leandro Drago for kindly allowing us to use a SEM photograph of his as our Fig. 1. Wallace Arthur, Lucio Bonato, Carsten H. G. Müller, and two anonymous referees provided insightful comments on a previous version of the manuscript.

## References

- Arendt J (2007) Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. *Biol Rev* 82:241–256
- Azevedo RBR, French V, Partridge L (2002) Temperature modulates epidermal cell size in *Drosophila melanogaster*. *J Insect Physiol* 48:231–237
- Beutel RG, Haas A (1998) Larval head morphology of *Hydroscapha natans* LeConte, 1874 (Coleoptera, Myxophaga, Hydroscaphidae) with special reference to miniaturization. *Zoomorphology* 18:103–116
- Beutel RG, Pohl H, Hunefeld F (2005) Strepsipteran brains and effects of miniaturization (Insecta). *Arthropod Struct Dev* 34:301–313
- Blomberg SP, Garland T Jr (2002) Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *J Evol Biol* 10:899–910
- Bonato L, Foddai D, Minelli A (2003) Evolutionary trends and patterns in centipede segment number based on a cladistic analysis of Mecistocephalidae (Chilopoda: Geophilomorpha). *Syst Entomol* 28:539–579
- Bonato L, Drago L, Muriene J (2014a) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. *Cladistics* 30:485–507
- Bonato L, Edgecombe GD, Minelli A (2014b) Geophilomorph centipedes from the Cretaceous amber of Burma. *Paleontology* 57:97–110
- Brena C, Cima F, Burighel P (2003) Alimentary tract of Kowalevskiidae (Appendicularia, Tunicata) and evolutionary implications. *J Morph* 258:225–238
- Cals P (1974) Mise en évidence, par le microscope électronique à balayage, de champs morphogénétiques polarisés, exprimés par les cellules épidermiques normales dans l’appendice locomoteur des Arthropodes: *Tylos latreilli* (Audouin) (Crustacé, Isopode) et *Periplaneta americana* (L.) (Insecte Dictyoptère). *C R Acad Sci Paris* 279D:663–666
- Conlon I, Raff M (1999) Size control in animal development. *Cell* 96:235–244
- De Moed GH, De Jong G, Scharloo W (1997) Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genet Res* 70:35–43
- Foddai D, Bonato L, Pereira L, Minelli A (2003) Phylogeny and systematics of the Arrupinae (Chilopoda: Geophilomorpha: Mecistocephalidae) with the description of a new dwarfed species. *J Nat Hist* 37:1247–1267
- Fusco G (2005) Trunk segment numbers and sequential segmentation in myriapods. *Evol Dev* 7:608–617
- Fusco G, Minelli A (2013) Arthropod body segments and tagmata. In: Minelli A, Boxshall G, Fusco G (eds) *Arthropod biology and evolution. Molecules, development, morphology*. Springer, Berlin, pp 197–221
- Fusco G, Brena C, Minelli A (2000) Cellular processes in the growth of lithobiomorph centipedes (Chilopoda: Lithobiomorpha). A cuticular view. *Zool Anz* 239:91–102
- Gibson WT, Gibson MC (2009) Cell topology, geometry, and morphogenesis in proliferating epithelia. *Curr Top Dev Biol* 89:87–114
- Gibson MC, Patel AB, Nagpal R, Perrimon N (2006) The emergence of geometric order in proliferating metazoan epithelia. *Nature* 442:1038–1041
- Grassé PP (1975) La cuticule. In: Grassé PP (ed) *Traité de Zoologie, Tome VIII, Fascicule III, Insectes: Téguments, Système nerveux. Organes sensoriels*. Masson, Paris, pp 5–31
- Guillot C, Lecuit T (2013) Mechanics of epithelial tissue homeostasis and morphogenesis. *Science* 340:1185–1189
- Hinton HE (1970) Some little known surface structures. *Symp R Ent Soc Lond* 5:41–58
- Kristensen RM (1991) Loricifera. In: Harrison FW, Woollacott RM (eds) *Microscopic anatomy of invertebrates, Vol. 4, Aschelminthes*. Wiley-Liss, New York, pp 351–375
- Leśniewska M, Bonato L, Minelli A, Fusco G (2009) Trunk anomalies in the centipede *Stigmatogaster subterranea* provide insight into late-embryonic segmentation. *Arthropod Struct Dev* 38:417–426



- Minelli A, Maruzzo D, Fusco G (2010) Multi-scale relationships between numbers and size in the evolution of arthropod body features. *Arthropod Struct Dev* 39:468–477
- Moussian B (2013) The arthropod cuticle. In: Minelli A, Boxshall G, Fusco G (eds) *Arthropod biology and evolution. Molecules, development, morphology*. Springer, Berlin, pp 171–196
- Neves RC, Sørensen KJK, Kristensen RM, Wanninger A (2009) Cyclophoran dwarf males break the rule: high complexity with low cell numbers. *Biol Bull* 217:2–5
- Nijhout HF (2003) The control of body size in insects. *Dev Biol* 261:1–9
- Nijhout HF, Callier V (2015) Developmental mechanisms of body size and wing-body scaling in insects. *Annu Rev Entomol* 60:141–156
- Nijhout HF, Cinderella M, Grunert LW (2014) The development of wing shape in Lepidoptera: mitotic density, not orientation, is the primary determinant of shape. *Evol Dev* 16:68–77
- Partridge L, Barrie B, Fowler K, French V (1994) Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* 48:1269–1276
- Pereira LA (2013) Discovery of a second geophilomorph species (Myriapoda: Chilopoda) having twenty-seven leg-bearing segments, the lowest number recorded up to the present in the centipede order Geophilomorpha. *Pap Avulsos Zool (São Paulo)* 53:163–185
- Polilov AA (2015) Small is beautiful: features of the smallest insects and limits to miniaturization. *Annu Rev Entomol* 60:103–121
- Robertson FW (1959) Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. *Genetics* 44:869–896
- Rosenberg J, Müller CHG, Hilken G (2011) The Chilopoda—integument and associated organs—integument and cuticle. In: Minelli A (ed) *Treatise on zoology—the Myriapoda*, vol 1. Brill, Leiden, pp 67–70
- Roth G, Blanke J, Wake DB (1994) Cell size predicts morphological complexity in the brains of frogs and salamanders. *Proc Natl Acad Sci USA* 91:4796–4800
- Roth G, Rotluff B, Blanke J, Ohle M (1995) Brain size and morphology in miniaturized plethodontid salamanders. *Brain Behav Evol* 45:84–95
- Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. *Cell* 103:253–262
- Stevenson RD, Hill MF, Bryant PJ (1995) Organ and cell allometry in Hawaiian *Drosophila*: how to make a big fly. *Proc R Soc Lond B* 259:105–110
- Stocker H, Hafen E (2000) Genetic control of cell size. *Curr Opin Genet Dev* 10:529–535
- Trumpp A, Refaeli Y, Thordur Oskarsson T, Gasser S, Murphy M, Martin GR, Bishop JM (2001) c-Myc regulates mammalian body size by controlling cell number but not cell size. *Nature* 414:768–773