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ORIGINAL ARTICLE

Modularity and developmental stability in segmented animals: variation in translational asymmetry in geophilomorph centipedes

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Abstract Does a modular body organization present a challenge for developmental control? We investigate the idea of a possible developmental cost of modularity by examining the relationship between modularity and developmental stability in a multi-segmented arthropod taxon: the geophilomorph centipedes. In a sample of eight species, we tested the correlation between developmental stability, estimated from measures of translational fluctuating asymmetry, and the number of trunk segments and some other morphological traits, both at the species and individual levels. We found sizeable differences in size and shape patterns of variation at the level of species. However, we did not find any clear evidence of correlation between fluctuating asymmetry and the number of trunk segments or the other morphological traits considered. Thus, our results provide no support to the idea of a possible trade-off between the cardinality of a modular system and the level of developmental precision in the phenotypic expression of its modules. The results of this exploratory study invite further investigations of patterns of translational fluctuating asymmetry in segmented animals and other modular organisms, as these have the potential to reveal features of developmental stability that cannot be captured by the study of bilateral asymmetry alone.

Keywords Canalization · Fluctuating asymmetry · Geometric morphometrics · Trade-offs

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Introduction

Modularity, here intended as the presence of serially homologous structures in the body of the same individual, is a common trait of the body architecture of many multicellular eukaryote taxa (Schlosser and Wagner 2004), and several studies have explored the processes of adaptation and taxonomic diversification in relation to the modular organization of particular body plans (Clune et al. 2013). In a macroevolutionary context, a modular organization of the phenotype is reputed highly evolvable through a mechanism of 'multiplication and differentiation' of the modules that allows acquisition of new functions while retaining the original ones (but see Fusco and Minelli 2013). However, comparably less attention has been paid to possible developmental costs associated with modularity.

Here, we focus on developmental noise, assessed as fluctuating asymmetry, as the phenotypic expression of such putative costs. There are different ways a growing organism can buffer developmental noise through ontogeny (Swaddle and Witter 1997), and some of them can be expected to be associated with modularity. For instance, it has been argued in a few case studies on bilateral asymmetries that developmental

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precision can be maintained through a mechanism of compensative growth among different body parts (Swaddle and Witter 1997; Piscart et al. 2005, but see Aparicio 1998), possibly as a result of a competition among the same parts for common resources (Klingenberg and Nijhout 1998). Under these conditions, where developmental precision is the result of mechanisms that operates throughout developmental feedback among body parts, it is possible that a high number of parts, at different distances from each other, as in the case of a long segmented body, can make more complex and less effective this form of control. On the contrary, if such control processes were inherent to each developing trait, then the number of body parts should have no effect on their stability and modularity would come at no cost. These conjectures are akin to those formulated in the general discussion on the developmental basis of phenotypic robustness, where the view of robustness being an intrinsic property of developing traits is opposed to that considering it being achieved through the action of separate, dedicated developmental processes (e.g. Debat and Peronnet 2013; Klingenberg 2015, p. 891; Felix and Barkoulas 2015).

Taking a developmental perspective on the evolution of modularity, we explore the association between modularity and developmental stability in a group of multi-segmented arthropods, the geophilomorph centipedes. Developmental stability is defined as the ability of an organism to buffer random perturbations of its developmental process (Nijhout and Davidowitz 2003; Fusco and Minelli 2010), and we investigate a possible trade-off between the number of body segments and the level of developmental stability, as revealed by the precision of segment phenotypic expression.

Geophilomorph centipedes (Chilopoda, Geophilomorpha) are terrestrial arthropods with a highly polymerous and rather homonomous (i.e. morphologically scarcely differentiated) segmental body organization. A survey through the entire primary taxonomic and faunistic literature of the group (Leśniewska et al. 2009) showed that most of the published records of putatively congenital defects in the segmental pattern are from taxa characterized by high numbers of segments. Also, in the geophilomorph *Haplophilus subterraneus*, a species with a high incidence of naturally occurring individuals with morphological anomalies, Fusco et al. (2015) found a positive correlation between the number of body segments and the presence of these anomalies. These observations suggest that the production of high numbers of modules might be associated with a reduction in the developmental precision of their expression, i.e. it may exist as a trade-off between the cardinality of a modular system and its developmental stability.

Here, this hypothesis is tested for a broader taxonomic sample encompassing several species, and with a method applicable to any type of modular organism. This phenotype-based approach quantifies developmental stability by measuring within-individual deviations from the expected body symmetry which are manifested as random non-heritable differences among repeated homologous structures known as fluctuating asymmetry (FA) (e.g. Debat and David 2001; Graham et al. 2010; Klingenberg 2015).

In organisms with bilateral symmetry, random deviations from left-right symmetry, or bilateral FA (Palmer and Strobeck 1986), are commonly employed to investigate levels and patterns of developmental stability (Polak 2003). However, for organisms with a modular body organization (e.g. segmented or radial organisms), other types of body symmetry, more specific of their body architecture, can be used to study developmental stability through the quantification of FA (Savriama and Klingenberg 2011). Segmented animals, like annelids, arthropods, vertebrates, and many other taxa (Minelli and Fusco 2004), present a form of translational symmetry along their main body axis that can be effectively exploited through the analysis of translational FA for the study of developmental stability (Astaurov 1930; Freeman et al. 1993; Fusco and Minelli 2000a; Savriama and Klingenberg 2011; Raz et al. 2012; Savriama et al. 2012; 2015).

In a sample of eight species, representative of two geophilomorph genera which display sizeable inter-specific variation in the number of trunk segments, we studied the association between developmental stability, inferred from fluctuating translational asymmetry, and the number of trunk segments and other morphological traits at the level of species and individuals.

Materials and methods

Sample species

The trunk of geophilomorphs comprises one anterior segment bearing a pair of venomous maxillipedes, a variable number of segments bearing one pair of legs each, and a terminal apodous ano-genital region of uncertain segmental composition (possibly, three segments, see Fusco 2005; Fusco and Minelli 2013) (Fig. 1). Within this group, the length of the series of leg-bearing segments, which does not change during post-embryonic development, varies conspicuously across species, sexes, and individuals. The variation in the number of trunk segments is thus traditionally expressed in terms of variation in the number of leg-bearing segments (LBS).

We selected eight species within the geophilomorph centipede genera *Stenotaenia* and *Stigmatogaster*, which are not closely related (estimated divergence time 321 MY, Murienne et al. 2010) but have in common a high inter-specific heterogeneity in the number of trunk segments and adult body size. In *Stenotaenia*, the number of leg-bearing segments varies between 43 and 115, and the body length at full growth ranges from 1 to 8 cm. In *Stigmatogaster* (here inclusive of the

Fig. 1 Translational symmetry in geophilomorph centipedes. **a** Habitus of a geophilomorph centipede in dorsal view. The *thick line* indicates the section of the trunk analysed. **b** Ventral view of a portion of the trunk of an exemplar of *Stigmatogaster subterranea* showing the series of metasternites. The *borders* of one metasternite are highlighted by a *white contour*
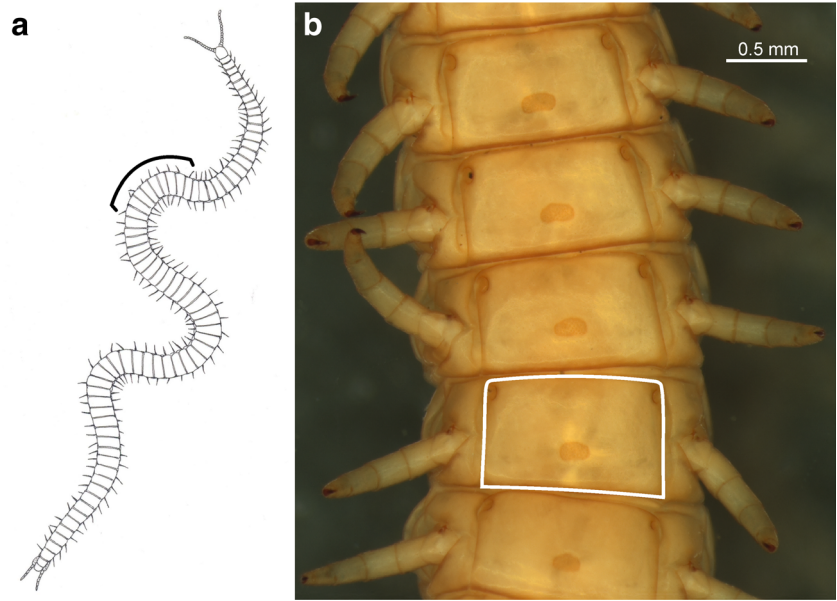
species that some authors ascribe to the genus *Haplophilus*), the number of leg-bearing segments varies between 69 and 165, with a body length in the range 5–18 cm (Bonato et al. 2011). The four species within each genus were selected to cover as much as possible these wide ranges of variation. For *Stenotaenia*, the species are *S. linearis*, *S. naxia*, *S. romana*, and *S. sorrentina*, and for *Stigmatogaster*, they are *S. dimidiata*, *S. gracilis*, *S. souletina*, and *S. subterranea*.

We analysed ten specimens sampled from a single population for each species, except for *S. dimidiata* for which only eight specimens were available. Specimens of *S. souletina* and *S. subterranea* are from M. Leśniewska's collection (Department of General Zoology, Mickiewicz University, Poznań, Poland), and all the remaining specimens are from the Minelli-Bonato Chilopoda collection (Department of Biology, University of Padova, Italy). All specimens are stored in 70 % ethanol.

Segment sampling

Because of the intra- and inter-specific variation encountered in the number of trunk segments, the question of the homology of segments has no simple and univocal solution (Fusco and Minelli 2013). However, previous studies showed that, for a given geophilomorph species, the relative position of the segments along the trunk, rather than their absolute (ordinal) position, appears to be the major determinant of their size and shape (Berto et al. 1997; Fusco and Minelli 2000b).

Accordingly, for each specimen in all species, we analysed a series of nine contiguous segments centred at an homologous relative position along the trunk, namely at one third of the entire series of leg-bearing segments

(Fig. 1). For example, we analysed segments 11 to 19, for a specimen of *S. romana* with 45 LBS ($45/3 = 15 \pm 4$), and segments 28 to 36 for a specimen of *S. gracilis* with 95 LBS ($95/3 \cong 32 \pm 4$). These sets of segments correspond to a region of the body characterized by identical embryonic profile of Hox gene expression (Brena 2015) and by modest segment heteronomy (Astaurov 1930; Fusco and Minelli 2000a). For each segment, we considered a single, non-articulated exoskeletal structure, the largest ventral sclerite (metasternite). This is a cuticular plate which is approximately flat for most of its span and is therefore suitable for two-dimensional (2D) morphometric analyses (see below).

Image acquisition

Nine photographs for each specimen (one for each selected metasternite) were taken through confocal laser scanning microscopy (CLSM). This technique takes advantage of the autofluorescence of the arthropod cuticle, allowing imaging of detailed structures without dissection or elaborate (and often non reversible) sample preparations (Klaus and Schawaroch 2006).

Specimens were transferred from ethanol to glycerol 100 % through gradual transitions in solutions of increasing glycerol concentration until complete glycerol replacement. For CLSM sessions, specimens were whole-mounted on slides, ventral side up, in glycerol. For each metasternite, a stack of 2D images ('optical slices') was acquired with a Leica DMI 6000B microscope equipped with a Leica TCS SP5 unit, using 543 nm helium/neon laser and a 550–680-nm band-pass emission filter.

From the stack of a metasternite, a single photograph (with scale bar) was produced creating a projection of the slices using the function "Z-Project" in the software ImageJ (ver. 1.47u). With the same software, with the function "Volume Viewer", the inclination of the metasternite with respect to the optical plane of the microscope was quantified for subsequent positioning correction (see below). On average, these angles resulted in the order of 1–2 sexagesimal degrees.

Landmark choice and data acquisition

For each segment, ten landmarks on the metasternite (five per side) have been chosen (Fig. 2). The landmarks are the positions of as many *idionymic* sensory setae (or epidermal sensilla), i.e. setae that are serially homologous across trunk segments within an individual (Minelli and Fusco 2013), and homologous across individuals within species (Fusco and Minelli 2000a). Although in general it is not possible to confidently homologise idionymic setae across different species, the homology of the selected setae among the eight species considered here is highly probable given the geometric similarity of their configurations. In any case, as the main target of this study is not the shape of setal configurations, but rather their segmental fluctuations, possible non-homology among the certain setae in different species are not expected to sensibly

affect our correlation analyses. Note that the spatial localization of the setae should not be considered indicative of the size and shape of the sclerite bearing them but rather of the topology of the underlying peripheral nervous system (sensory system).

To assess measurement error due to digitalization, landmarks were digitized twice by the same operator (MV) in two independent working sessions 2 days apart using TPSDig 2 (ver. 2.17, Rohlf 2015). The program tpsUTIL (ver. 1.60, Rohlf 2015) was used to build. TPS files from the images and combine them into a single dataset. Another potential source of measurement error is the orientation of the samples during the acquisition of images. Traditionally, the method used to handle this problem is to replicate both positioning and data acquisition. In our study, because of the relative stiffness of the geophilomorph trunk, independent orientations of the sclerites under the microscope in different sessions are not always attainable, thus potentially introducing a systematic measurement bias. For this reason, we adopted a different protocol, which takes advantage of CLSM. We did not replicate positioning and to compensate for possible non-perfect co-planarity of the plane of the metasternite (specific of each segment and specimen) with respect to the optical plane of the microscope, we transformed the raw coordinates through a rigid
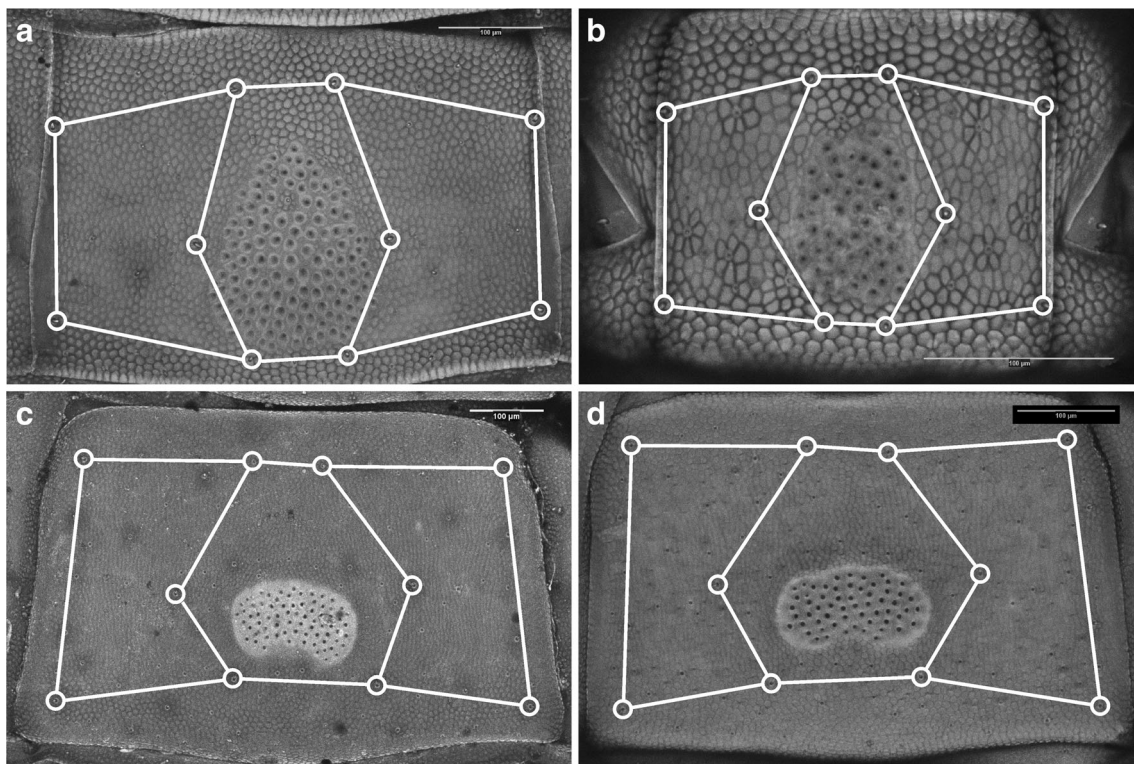

Fig. 2 Examples of the CSLM metasternite photographs and landmark digitalization. The ten landmarks are positioned at the basis of as many idionymic setae (see text). **a** *Stenotaenia linearis*. **b** *Stenotaenia romana*. **c** *Stigmatogaster gracilis*. **d** *Stigmatogaster subterranea*

geometric rotation contrary to the specific angles of inclination of the metasternite along their X and Y axes. These angles were obtained by the vertical stack (Z-axis) of images of each metasternite. Thus, for each landmark, new transformed (X_t, Y_t) coordinates were calculated as ($X/\cos(\alpha_X)$, $Y/\cos(\alpha_Y)$), where X and Y are the original raw coordinates and α_X and α_Y are the angles of inclination of the sclerite (in radians) with respect to the two axes. These are the raw coordinates used in subsequent morphometric analyses.

Translational fluctuating asymmetry

In centipedes, each segment exhibits bilateral symmetry and the suite of segments is arranged according to translational symmetry. The whole organism therefore displays a complex combination of bilateral and translational symmetries. Here, we followed the general approach of Savriama and Klingenberg (2011), specifically designed for the size and shape analysis of complex symmetric structures, to characterize the patterns of translational asymmetry. Each individual segment was analyzed with the method of object symmetry prior to further analyses in order to extract the symmetric component of shape variation of segments (Mardia et al. 2000; Kent and Mardia 2001; Klingenberg et al. 2002). From these symmetric averages of original and reflected (appropriately relabeled) configurations, we then used the method of matching symmetry—generalized for the study of any type of symmetry—to analyse the variation in translational symmetry of segments along the body axis of the centipedes. The effects of segment heteronomy were corrected by regression of size (centroid size) and shape (Procrustes residuals) data on segment ordinal position along the antero-posterior axis. This is a key step to separate constitutive size and shape variation of metasternites along the trunk (segment heteronomy) from FA along the same body axis. The relatively smooth transitions in segment size and shape exhibited by geophilomorph centipedes along their main body axis (Fusco and Minelli 2000b) and the relatively short series of segment investigated (nine segments) allow one to satisfactorily approximate the effect of the constitutive variation in the segmental series with linear regression models.

For size measures (centroid size), we also divided individual deviations by the individual mean segment size in order to correct for the heterogeneity in size among individuals and species.

The analyses of asymmetry were based on the conventional decomposition of sums of squares in a two-way analysis of variance (ANOVA) traditionally used in studies of FA to separate the components of symmetric and asymmetric variation into biologically meaningful units (Leamy 1984; Palmer and Strobeck 1986, 2003; Klingenberg and McIntyre 1998; Klingenberg et al. 2002; Savriama and Klingenberg 2011).

In our design for the study of translational asymmetry, the 'individual' main effect stands for the variation among individuals, the 'segment' main effect stands for directional asymmetry (the average deviation from one segment to the segments mean), the interaction term 'individual-by-segment' accounts for translational matching fluctuating asymmetry (i.e. the variation, among individuals, in the heterogeneity among segments), and measurement error (ME) represents the variation between digitizing sessions (Palmer and Strobeck 2003; Klingenberg and McIntyre 1998). The FA10 index is computed as the mean square of the interaction term minus the mean square for ME adjusted for their appropriate numbers of degrees of freedom (Palmer and Strobeck 2003). Along with this species-level estimate of developmental instability, we computed an individual-level estimate of translational FA as the within individual, inter-segment variance, standardized by the mean segment size.

Correlation analyses

We investigated the relationship between FA and a small set of candidate predictor morphometric variables, both for species and individuals. Species body length at adulthood (sBL) and species modal number of leg-bearing segments (sLBS) were assigned on the basis of several sources from the taxonomic literature (see Bonato and Minelli 2014 and references therein). Species epithelial resolution (sER) was estimated on a few metasternite photographs for each species. This is calculated as the square root of the approximate number of epidermal cells comprised by the configuration of landmarks. A square root transformation converts this surface measure into a one-dimensional morphometric variable. The number of epidermal cells was estimated by counting the convex polygonal fields (scutes) on the surface of the metasternite cuticle, which correspond to the apical surface of as many epidermal cells (Fusco et al. 2000; Moretto et al. 2015). This number varies from 500 in *S. romana* to 10,000 in *S. subterranea* (Fig. 2). The idea is that the position of the setae, which are somehow embedded in the epidermis, is to some extent 'discretized' by the mosaic of epidermal cells, possibly with a negative effect on the precision of their position that is inversely proportional to the epithelial resolution. In other words, the lower the number of cells, the lower the precision of the position of the setae. The only morphometric variable collected on the individuals is the number of leg-bearing segments of the specimens (LBS).

To account for possible effects of the phylogenetic relationships, we assembled a phylogenetic hypothesis for the eight species based on Bonato et al. (2014) and Del Latte et al. (2015). We first tested whether there was a significant phylogenetic signal in size and shape FA as

well as sBL, sLBS, and sER, by computing Blomberg's
K-statistics, as performed in the R library *picante*
(Kembel et al. 2010). We then tested the correlation be-
tween size and shape FA and between these and the co-
variates, using phylogenetic generalized linear models
(pgls) implemented in the R library *caper* (Orme et al.
2013). As the number of species is low, we implemented
these tests both on the raw variables and using their
ranked values to get a non-parametric version of the anal-
yses (E. Rezende, personal communication).

Finally, we also investigated whether the patterns of shape
translational FA were influenced by the phylogeny by com-
puting the association (RV coefficient) between the matrix of
phylogenetic distances and the matrix of angles between pairs
of vectors depicting the dominant direction of shape FA for
each species (PC1 of variance-covariance matrix of shape
FA).

All morphometric and statistical analyses were pro-
grammed and carried out in R (R core team 2015).

Results

Phylogenetic signal

Neither size nor shape FA exhibits a significant phylogenetic
signal (respectively, $K=0.45$, $P=0.65$ and $K=0.62$, $P=0.28$).
In contrast, sBL, sLBS, and sER all exhibit a significant phy-
logenetic signal (sBL $K=1.37$, $P=0.014$; sLBS $K=1.23$,
$P=0.009$; sER $K=1.66$, $P=0.015$).

Association of translational FA with other morphometric variables

When using a parametric pgls, we found a significant correla-
tion between size and shape estimates of FA, both at the level
of species (pgls $r=0.807$, $P=0.015$, $n=8$) and individuals
($r=0.54$, $P=0.001$, $n=78$, pooled individuals corrected for
species differences). The correlation turns to non-significant
at the level of species when using the ranked values ('non-
parametric pgls' $r=0.31$, $P=0.44$).

At the level of species (Fig. 3), we found no significant
correlation between size FA and any of the other variables,
regardless the method used (parametric pgls: sLBS $r=0.59$
$P=0.11$; sBL $r=0.58$, $P=0.12$; sER $r=0.32$, $P=0.4$; non-
parametric pgls: sLBS $r=0.16$, $P=0.69$; sBL $r=0.13$,
$P=0.74$; sER $r=0.05$, $P=0.89$). For shape FA, in turn, sig-
nificant correlations were detected in the parametric analysis
with sLBS ($r=0.68$, $P=0.046$) and sBL ($r=0.69$, $P=0.044$)
but not with sER ($r=0.49$, $P=0.22$). When using the non-
parametric version of the test, these correlations are no longer
significant (sLBs $r=0.51$, $P=0.19$; sBL $r=0.65$, $P=0.075$),
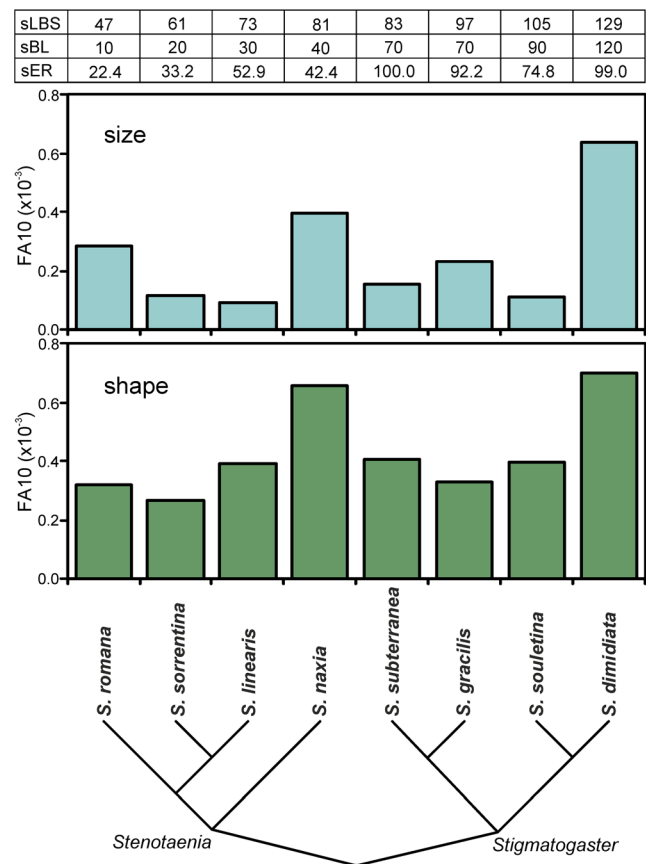whereas the correlation with sER turns significant ($r=0.72$,

sLBS	47	61	73	81	83	97	105	129
sBL	10	20	30	40	70	70	90	120
sER	22.4	33.2	52.9	42.4	100.0	92.2	74.8	99.0

Fig. 3 Developmental instability, estimated as fluctuating translational
asymmetry (FA10) for size (*upper panel*) and shape (*lower panel*), in
eight species of geophilomorph centipedes, mapped on a cladogram
obtained by the collation of the cladograms of the two genera.
Stenotaenia cladogram is from Del Latte et al. (2015); *Stigmatogaster*
cladogram is from Bonato et al. (2014). The table on the top shows three
species covariates: modal number of leg-bearing segments (*sLBS*), adult
body length (*sBL*), and epithelial resolution (*sER*) (see text)

$P=0.044$). The significant correlations with shape FA, either
parametric or non-parametric, are nevertheless only marginal-
ly significant and highly dependent on a single, influential
value, suggesting an overall weak effect.

At the individual level, correlations between size and shape
FA and LBS are non-significant within species (all $P>0.1$,
$n=10$, $n=8$ for *S. dimidiata*), except for *S. souletina*, where
the correlation between shape FA and LBS is significant
($r=-0.788$, $P=0.006$, $n=10$). Here, however, the significant
result again depends on a single influential point with high
leverage. When the influential point is excluded from the anal-
ysis, this correlation also turns out to be non-significant. To
compensate for the low statistical power resulting from the
low sample size within each species, we also tested for the
association between shape and size FA and LBS for pooled
individuals after correction for species differences in FAs and
LBS. The results remain unchanged, and no significant corre-
lation is detected for either shape FA ($r=-0.12$, $P=0.31$) or
size FA ($r=-0.13$, $P=0.26$).

Patterns of shape variation for translational FA

In order to investigate and visualize the patterns of translational FA, we used principal component analysis (PCA) of the FA covariance matrices. Figure 4 displays together the average shapes of the configurations of the setae for each species and the vectors depicting the main directions of shape FA. The four species that belong to the genus *Stenotaenia* show similar average setal configuration shapes, vaguely lenticular, with mostly variation in longitudinal direction. The four *Stigmatogaster* species exhibit more angular and more diverse average shapes. While *S. subterranea* and *S. gracilis* show a posterior convex profile in the setal configuration (landmarks 3, 4, 8, and 9), *S. souletina* and *S. dimidiata* display a 'bow-tie' morphology, with convex profiles of both anterior and posterior setal rows (landmarks 1, 2, 6, and 7 and landmarks 3, 4, 8, and 9).

Inspecting the patterns of shape FA depicted from the first PC, a clear phylogenetic structure emerges. Within the genus *Stenotaenia*, the main features of variation in translational FA are comparable among the species. They mostly affect landmarks 4, 5, 9, and 10 that characterize the more internal part of the setal configuration. By contrast, the main aspects of variation in asymmetry are not as similar as for the species belonging to the genus *Stigmatogaster*. They concern landmarks 5 and 10 in *S. subterranea*, 1 and 6 for both *S. souletina* and *S. dimdiatus*, and are spread more equally over all landmarks for *S. gracilis*. To sum up, the closely related *S. sorrentina* and *Stenotaenia linearis* exhibit fairly similar patterns of shape

FA, which are somewhat also close to that of the other two *Stenotaenia* species. Differently, within the *Stigmatogaster* group, while *S. souletina* and *S. dimidiata* show very similar patterns of FA, the other two *Stigmatogaster* species are more divergent from both the first two and from each other, *S. subterranea* FA pattern somehow resembling that of the *Stenotaenia* species.

This phylogenetic structure of shape translational FA patterns is not supported by the RV statistics, which compares the matrices of phylogenetic distances and of angles between shape FA PC1 vectors ($RV = 0.39$, $P = 0.26$). This test should be considered with caution though, as the power to detect a phylogenetic signal is poor due to the low number of species.

Discussion

In our analysis of the association between the number of body segments and the precision of their phenotypic expression in geophilomorph centipedes, size and shape translational FA appear to capture different aspects of phenotypic precision, both at the levels of species and individuals. These are likely reflecting some constitutive features of the mechanisms underlying developmental stability in these modular animals.

Our results provide no support to the idea of a possible trade-off between the cardinality of a modular system and its developmental stability. Despite some weak signs of a correlation with shape FA, we did not find any compelling
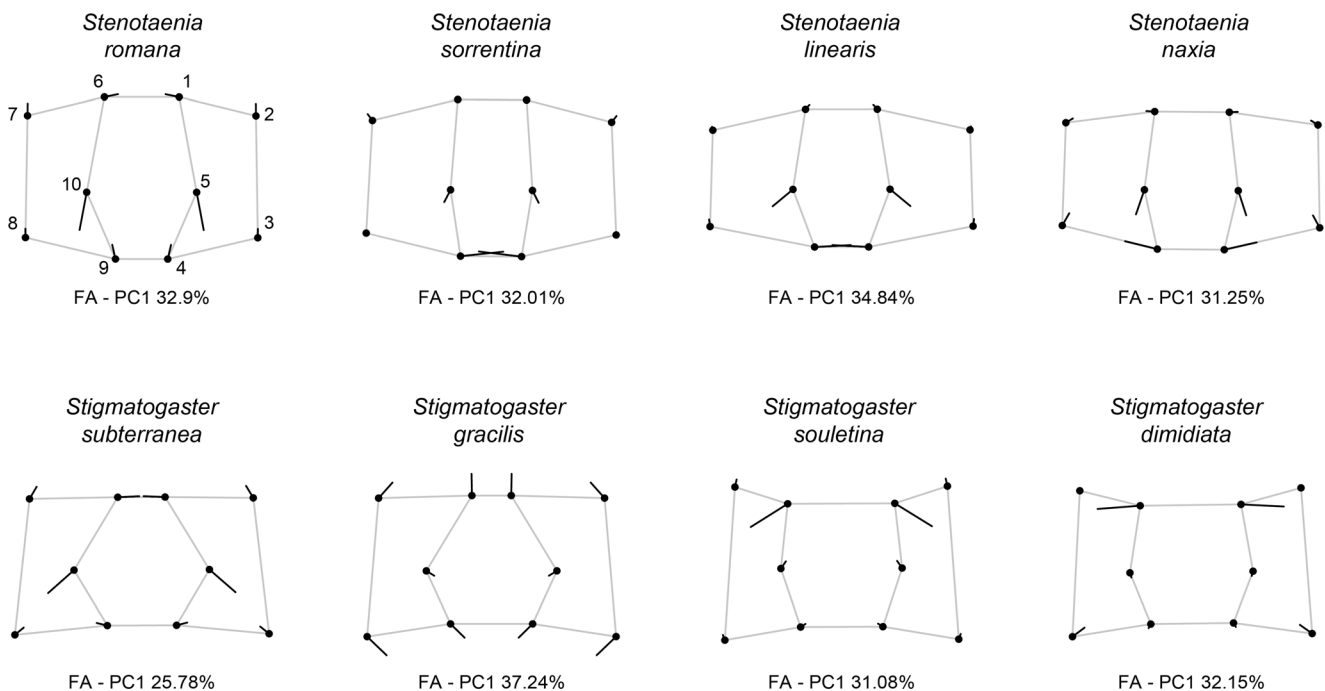

Fig. 4 Shape component of within species translational fluctuating asymmetry, shown as lollipop graphs. *Dots* represent the mean shape and *black segments* indicate magnitude and direction of the first principal component in shape FA (percentage of variation accounted by PC1 is reported under each graph). The labelling of landmarks, indicated for *Stenotaenia romana*, is the same for all species

evidence of a correlation between the number of trunk segments and developmental precision at the level of either species or individuals. Likewise, we found no significant correlation between FA and other covariates, like body length and the epithelial resolution at the level of either species or individuals. Overall, the number of trunk segments does not seem to be an influential factor for the observed variation in translational FA, and there is no indication that the latter is influenced by any of the other morphological traits considered here.

Despite the fact that phylogenetic signal in the pattern of shape FA is not supported by the RV test, there are sizeable differences between the two genera and within the genus *Stigmatogaster* in the pattern of shape variation for translational FA (Fig. 4). For the more internal setae (landmarks 1, 4, 5, 6, 9, and 10), these differences could be possibly related to some kind of steric interference with the *ventral pore-field*, an area of clustered pores present on the metasternites (Fig. 1). Indeed, this area, which has species-specific location and extension on the sclerite, and segmental occurrence along the leg-bearing trunk (Turcato et al. 1995), is relatively more conspicuous in *Stenotaenia* than in *Stigmatogaster*. Another possible driving factor could be the ontogenetic order in which the setae are formed. Horneland and Meidell (2009) have studied the post-embryonic development of the metasternite setal pattern in the geophilomorph model species *Strigamia maritima*. They observed that the setae appear progressively on metasternites, in a stereotypic succession such that the level of completion of the pattern can be used to identify developmental stages. In this species, the size and recognizability of the setae is proportional to the number of stages that have elapsed since their appearance. Our landmark choice was guided by the same morphological principle, but we cannot exclude that in different species, and even in case of setal homology, these can have a different ontogenetic history.

Beyond the specific hypothesis tested here, geophilomorphs stand as a valuable model system for exploring several aspects of developmental stability in modular organisms. For instance, it would be interesting to investigate, with the same morphometric framework used here, the relationship between bilateral and translational fluctuating asymmetries in these animals. In principle, at least at the level of species, it is possible to have segmental patterns with high translational FA and low bilateral FA (for instance, with translational irregularity affecting concordantly right and left sides) or, vice versa, low translational FA and high bilateral FA (for instance, with signed bilateral asymmetry equal in all segments of an individual but differing among individuals). With an exploratory study based on distance measures, Fusco and Minelli (2000a) found a significant correlation between translational and bilateral FA at the level of the individual in the geophilomorph species *Pleurogeophilus mediterraneus* and *Strigamia acuminata*.

High correlation should not be necessarily expected between asymmetry values of different characters in the same organism, because of the diversity of stress levels affecting the traits, the possible diversity in developmental buffering among traits, and/or their distinct times of sensitivity to developmental noise. This subject has received much attention and the existence of an individual asymmetry parameter, although often discussed, remains an open question (e.g., Leamy 1993; Clarke 1998; Debat et al. 2011). In contrast, the correlation between different types of FA for the same morphological trait still remains mostly unexplored so far (Savriama and Klingenberg 2011). Translational FA might be particularly relevant to assess developmental stability: bilateral FA studies have long been known to be affected by a power issue as they aim at assessing developmental stability with only two points (i.e. right and left, e.g. Whitlock 1996). Translational FA should perform better in that respect, and its correlation with bilateral FA would thus be of interest. It would be also very interesting to explore in different species the ontogeny of translational FA across successive post-embryonic developmental stages. As sensory setae are mostly formed during early post-embryonic development (Brena 2014, 2015), ontogenetic changes in FA patterns could provide new insight into the mechanisms of developmental buffering in segmental patterning (Aparicio 1998; Klingenberg 2003).

This exploratory study demonstrates that the powerful morphometric and statistical toolkit devised to analyse developmental stability in bilaterally symmetric organisms can be successfully extended to more complex instances of symmetry. This encourages further investigations of the patterns of translational FA in segmented animals, and in modular organism in general. However, as our treatment of constitutive segment heteronomy (by linear regression) rests on specific segmental features of the species under study and on a suitable choice of the examined segmental series, further methodological development will be necessary to devise a more general solution to this problem.

Beyond technical aspects, investigating different types of organismal symmetry and understanding their relations with traditional bilateral FA has the potential to reveal features of developmental canalization and homeostasis that cannot be captured by the study of bilaterally asymmetries alone.

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