Assessments of phenotypic variations and variability as a tool for understanding evolutionary processes in echinoids

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Preface

This doctoral thesis consists of following published manuscripts:

Schlüter, N., Wiese, F. and Reich, M. (2015) Systematic assessment of the Atelostomata (Spatangoida and Holasteroida; irregular echinoids) based on spine microstructure. *Zoological Journal of the Linnean Society*. 175: 510–524. doi:10.1111/zoj.12291

Schlüter, N., Wiese, F., Kutscher, M. (2016) Heterochronic evolution in the Late Cretaceous echinoid *Gauthieria* (Echinoidea, Phymosomatidae). *Cretaceous Research*. 57: 294–305. doi:10.1016/j.cretres.2015.09.005

Schlüter, N. (2016) Ecophenotypic variation and developmental instability in the Late Cretaceous echinoid *Micraster brevis* (Irregularia; Spatangoida). *PLoS ONE*. 11 (2): e0148341. doi:10.1371/journal.pone.0148341

Contents

Chapter 1

Introduction

Evidence of evolutionary relationships among organisms are the inheritance of information and development of characters (either genetic, or morphologic), which should be similar due to a common ancestor. Darwin (1869) identified necessities for evolution by natural selection; variation within populations is needed. Accordingly, evolution can be studied by assessing variations in homologous characters of related taxa. The term homoplasy, generally describes a similarity between traits, but not necessarily of the same developmental origin, which is known as convergence. Parallelism and reversals as a subject of homoplasy, however, have similar or even the same developmental pathways, and, hence, are sometimes considered as homology (Hall, 2002).

The concept of homologous characters is essential for phylogenetic analyses, by applying the principle of parsimony to the origins of homologies, and it is a useful tool to reconstruct systematic relationships under an evolutionary subtext (see chapter 2; Schlüter et al., 2015). Nevertheless, homologies are evidence for evolution, but provide no information on evolutionary mechanisms (Gilbert & Bolker, 2001). Both evolution, as well as phenotypic variations can be studied on a hierarchy-level. Evolution can be investigated at the scale of microevolution (changes within a species-level), macroevolution (above the species level) and megaevolution (at the levels of families, orders or higher) (Arthur, 2003). Similarly, studies of variations can be applied to either comparisons of species, or higher systematic orders, or going into detail by exploring variations within species, within populations or even within-individuals.

Evolution is traditionally defined as a change in allele frequencies as a consequence of mutation (Dobzhansky, 1937). However, this definition does not necessarily explain phenotypic adaptation or changes in form, as recently genotype-phenotype mapping revealed much more complex interactions (gene-gene and gene-environment interaction) than the gene-centric view of “one gene, one protein” postulates (compare Pigliucci, 2007). This, in turn, leads to the distinction of variation and variability. Variation determines the observable differences within a population or an investigated entity (e.g. species or population), whereas variability describes a predisposition to vary (Wagner & Altenberg, 1996; Willmore et al., 2007).

The developmental basis underlying phenotypic variability is manifold in its origins, which is governed by genetic and environmental interactions (West-Eberhard, 2003). Some important mechanisms, which can be assessed by studies of the phenotype, are briefly summarised in the following chapters.

1.1. Evolutionary development

King and Wilson's influential hypothesis (1975) suggested that the discrepancy between the evident phenotypical differences and the large degree in genetic analogies between humans and chimpanzees are best explained by changes in the regulation of the genes, rather than by mutations in the coding gene sequences. Later, Jacob (1977) coined the term "tinkering" for reshuffling pieces of existing genes as the major process of adaptation in evolution. It also needs to be mentioned that the famous Britten-Davidson model for gene expression in eukaryotic organisms (Britten & Davidson, 1969) anticipated the current ideas and knowledge of the gene regulatory network (as being composed of regulatory and structural genes) by empirical data. However, a lot of time elapsed until these ideas were widely accepted. Analytical improvements to detect the expression of the gene products in recent decades allowed the comparison of gene expression levels among organisms and, thus, to approve the ideas of King and Wilson and others, resulting in the establishment of the concept of "evolutionary development" ["evo-devo"; see Carroll (2008) for a more concise synopsis].

Changes in regulatory genes predict the general form and trait diversity in and among organisms more adequately than previously assumed, that mutations in structural genes account largely for this diversity of life (Davidson, 2006; Hoekstra & Coyne, 2007). "Evo-devo" addresses mechanisms in anatomical modifications by comparing and analysing developmental trajectories. Four mechanisms are traditionally defined as dominant mechanisms for macroevolutionary changes and mechanisms of developmental reprogramming (Arthur, 2000):

modifications in the regulatory genes

a) heterochrony	changes in time of gene expression
b) heterometry	changes in amount of gene expression
c) heterotopy	changes in location of gene expression

modifications in the encoding genes

d) heterotypy	changes in sequence of the gene being expressed

Heterochrony was framed by Gould (1977) and brought back to evolutionary studies by referring to Haeckel`s famous biogenetic law ("ontogeny recapitulates phylogeny") (Haeckel, 1866), which included the ideas of King and Wilson (1975). The concept of heterochrony considers changes in the timing and the rate of gene expressions (Raff & Wray, 1989). It is studied by comparing the developmental timing (onset and offset) or developmental rate of trajectories between ancestor and descendant taxa (e.g. Alberch, 1980). Heterochrony has become a classic approach to study evolutionary development and has attracted considerable attention in palaeontological studies (see

chapter 3; Schlüter et al., 2016 and e.g. McNamara, 1987, 1989; McKinney & McNamara, 1991, and references therein).

Heterotopy: A spatial displacement of a trait within an organism by activation, or inactivation, of a particular gene expression (Arthur, 2000).

Heterometry refers to changes in the amount of gene expression. It can alter a distinct trait such that its value will increase or decrease in size. Heterometry can be assessed by investigating either size changes among phylogenetic lines or related groups by comparing the relative size of a distinct trait, or a trait which increases in size in relation other traits of an individual (Arthur, 2000; 2011).

Heterotypy is distinguished to previous mechanisms by the change in the expressed protein itself due to a mutation in the encoding gene (structural gene). The effect of previous mechanisms is based on changes in the regulatory mechanism of the gene regulatory network (Arthur, 2000).

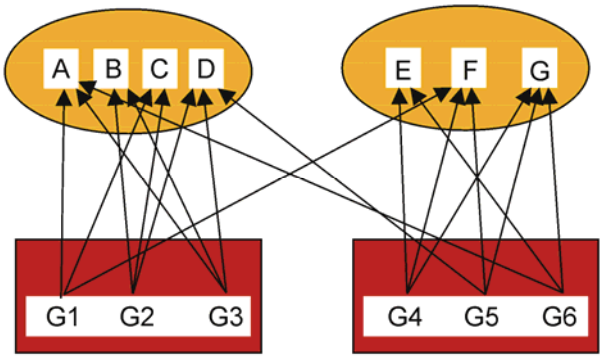

Figure 1. Pleiotropy. Two modular trait complexes, composes of the traits (A-D) and (E-G), arrows indicate the effects of the genes (G1-G6) on particular traits. The pleiotropic effects within each modular trait are larger than among the traits (modified after Wagner, 1996).

Modularity is associated with the mechanisms described above and the concept of morphological homology. It refers to an autonomous development of distinct traits or the expression of subsets in a trait by affecting subsets of the related gene regulatory circuit (Fig. 1) (Wagner et al., 2007). Modularity is enhanced by regulatory genes, *cis*-regulatory elements, which are found upstream to an encoding gene sequence (Davidson, 2001, 2006). Transcription factors and other molecules attach to the binding sites of the *cis*-regulatory sequences and activate the transcription of the downstream gene sequence (Carroll et al., 2001). While changes in protein-coding genes (e.g. encoding for diffusible transcription factors) can have pleiotropic effects (Wittkopp & Kalay, 2012), affecting gene regulation in multiple, often independent traits (Carroll, 2008), mutations in the non-coding regulatory genes can lead to increased modularity. Accordingly, pleiotropy can affect integration among developmental modules or traits. Additionally, covariation between modules can also arise by the same

environmental or functional stimuli (Klingenberg, 2008a). Following is that modularity has positive effects on phenotypical diversification and contributes to evolvability. Less integrated traits are able to react in different manners to selection pressures (Cheverud, 1984; Wagner & Altenberg, 1996; Klingenberg, 2005).

1.2. Robustness: Canalization and developmental stability

Whereas the crucial base of any modification is found in mutations, these modifications are not necessarily translated into the phenotype. Variability is constrained by robust development. The concept of robustness is of major importance for the phenotypic modification and variability. Robustness is traditionally referred to the concepts of canalization and developmental instability. However, there is still little consensus about the definitions of both concepts (see Dworkin, 2005). The term canalization was defined by Waddington (1942) as a property of a developmental process to be resistant against minor variations, either being of environmental or genetic origin. Schmalhausen (1949) developed a similar concept, but independently from Waddington (1942).

These variations are able to perturb the development of a genotype and subsequently change the normal developmental pathway (e.g. Gibson & Wagner, 2000; Gibson & Dworkin, 2004). Accordingly, even if standing genetic variation is available, the phenotypic value will remain invariant, if a developmental pathway is canalised (Fig. 2). The available cryptic genetic variation within a genotype will be exposed and likely affects the developmental pathway in such a way that the expressed phenotype will deviate from the "target" phenotype (see chapters 2 & 4; Schlüter et al., 2015; Schlüter, 2016).

Waddington (1942, 1953, 1956) demonstrated that, if organisms are subjected to new stimuli, pathways during development can change and later be manifested (genetic assimilation), illustrated by his famous metaphor "epigenetic landscape" (Waddington, 1957) (Fig. 3).

In addition, canalization allows for accumulation of further cryptic genetic variation (Rutherford,

2000; Gibson & Dworkin, 2004; Paaby & Rockmann, 2014), which is the logic consequence of the suppression of variation. Mutations cannot be selected against, if these are not phenotypically expressed. A possible mechanism for canalization is found in chaperone proteins (e.g. heat shock protein family [HSP]) (Rutherford & Lindquist, 1998; Rutherford, 2003; Sangster et al., 2008). If HSPs are impaired, for instance by environmental stressors, or a mutation which affects the HSP encoding gene, previous cryptic genetic variants can be released. Likewise, other feasible mechanisms contribute to canalization. For instance, through gene-gene interactions (epistasis) the genetic background can buffer against mutations. Perturbations in the development caused by knockout mutations ("loss of function") can be compensated by redundancies in the gene regulatory network,

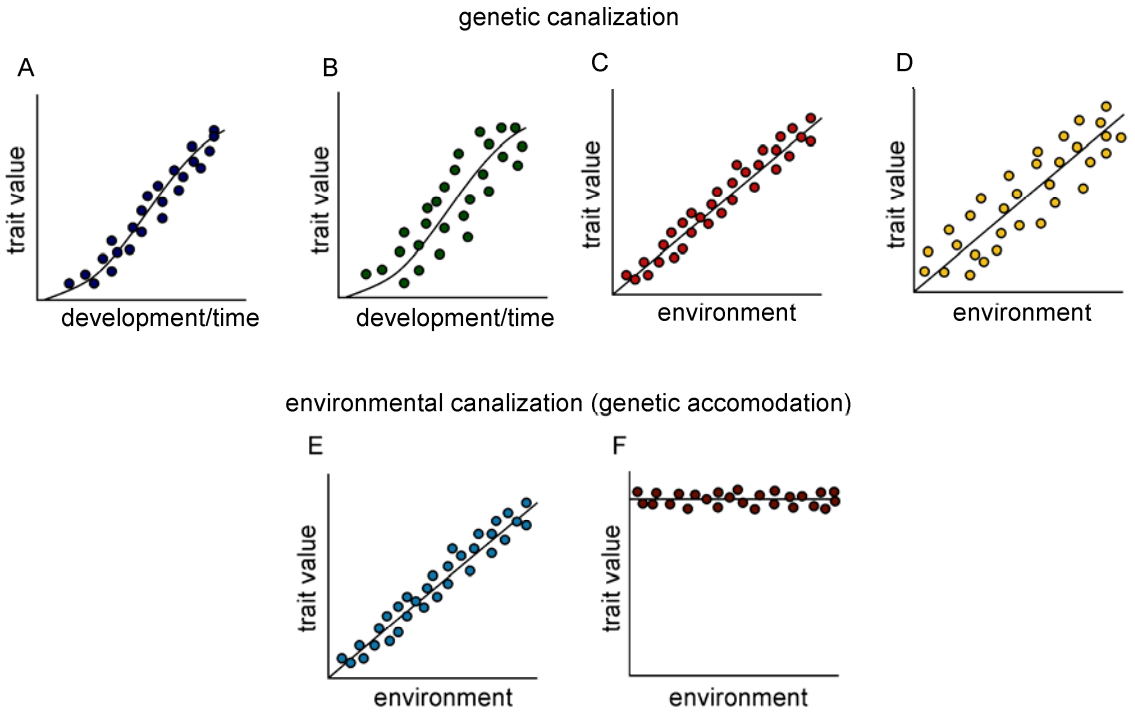

Figure 2. Canalization. Genetic canalization, variations in the degree of genetic canalization of populations along a developmental trajectory, the bold line represents the mean phenotype of the respective trajectory: A, B: In A the individuals are closer to the mean phenotype, than compared to B and thus reveal a better canalised development. C, D: Variations in response to a distinct, continuously varying environmental variable (reaction norm). While in C the individuals are in their phenotypical response closer to the target phenotype, the individuals in D show a higher phenotypic variance, which is a case for a lesser degree of canalization than compared to C. E, F: Genetic accommodation of a previously plastic trait. E: phenotypical response to an environmental variable. F: In the descendent population the previous plastic phenotype has been fixed by experiencing genetic accommodation at distinct position of the trait value.

e.g. by gene duplications (Siegal & Bergman, 2002; Ledón-Rettig et al., 2014). Canalization can only be measured by comparing the degree of variation, or variance, among populations. Therefore, it is not meaningful to draw conclusions about the degree of canalization based on a single population (Dworkin, 2005).

Developmental stability is defined as the propensity of a genotype to pursue a distinct developmental trajectory, if subjected to the same condition (Hallgrímsson et al., 2002). Developmental instability is usually assessed by within-individual variation. Phenodeviants such as normally bilateral individuals, which reveal a high degree of asymmetry between both sides (fluctuating asymmetry, Van Valen, 1962; see chapter 4; Schlüter, 2016), are indicative and are frequently used as an estimator for developmental instability (e.g. Palmer & Strobeck, 1986; 2003, Klingenberg & McIntyre, 1998). Stochastic gene expression is generally expected to be of particular importance for the generation of developmental noise (Klingenberg, 2003; Willmore & Hallgrímsson,

2005). Stochastic gene expression refers to randomised amounts and frequencies in transcription and translation of a gene product (McAdams & Arkin, 1997; Kaern et al., 2005).

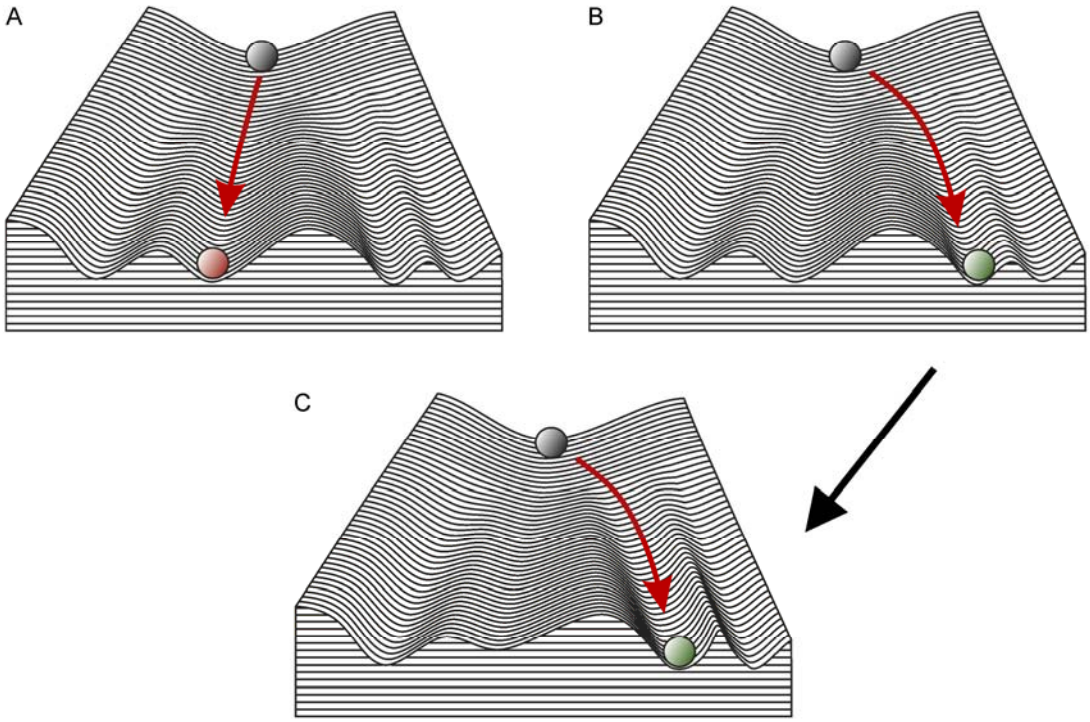

**Figure 3. Waddington`s epigenetic landscape.** A modification of Waddington`s epigenetic landscape (Waddington, 1957), showing potential differentiation of pluripotent cells (stem cells, represented by greyish marbles), within in a single genotype. A: normal development. B: modified development outcome, due to (environmental, genetical) perturbations, cues, respectively. C: fixed modified developmental pathway in response to mutations, note that this particular pathway is surrounded by steeper hills due to canalization.

Fluctuations in gene expression will result in randomly available gene products, which can be crucial for the activation of subsequent developmental cascades in the gene regulatory network (Willmore & Hallgrímsson, 2005). Such stochastic gene expressions can have a negative impact on the formation of particular traits, for instance, if the gene expression level does not exceed a distinct threshold necessary for the formation or activation of a trait (Klingenberg, 2003), the phenotypic outcome will deviate from normal gene expression. Such circumstances indicate phenotypic differentiation without a change in the genetic background. A possible factor influencing fluctuations in gene expression can be explained by mutations, which lead either to a reduced or to a loss in function of an allele (Cook et al., 1998, Klingenberg, 2004). Developmental instability also depends on the behaviour of developmental systems (Klingenberg, 2004; Leamy & Klingenberg, 2005; Willmore & Hallgrímsson, 2005). For example, in nonlinear developing systems, if alleles (corresponding to a developmental pathway) contribute to their activity (e.g. in gene expression) in a non-additive fashion, the developmental mapping function (genotype-phenotype relation) reacts in a nonlinear fashion (see Fig.

4), a curvatic surface of the phenotypic value (Klingenberg, 2004). Developmental stability in such cases would be dependent on the position of the mapping, for instance, developmental perturbations would have more drastic morphological effects at a location with a steep slope (e.g. a genotype with a mutant allele), but at a position with a level mapping function, the effects would be only small (see Fig. 4) (Klingenberg, 2004).

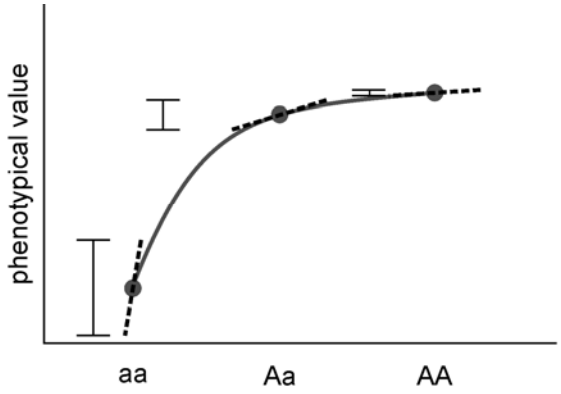

Figure 4. Relationship of developmental instability and nonlinear development. Slopes of the developmental mapping function at the locations corresponding to the three genotypes. Each genotype shows differences in the sensitivity of the phenotype to perturbations during development. The dashed lines and additionally the solid ranged lines give the possible phenotypic outcome due to developmental instability, which are different for each genotypes because of their local slopes within the nonlinear developmental mapping function (allele A is dominant over the a allele) (modified from Klingenberg, 2004).

Such consequences for developmental instability can also be applied for nonlinear developmental pathways (Willmore & Hallgrímsson, 2005)

Other factors, like environmental stress (e.g. adverse temperatures, or pollution), can have similar effects on stochastic gene expression. Developmental stability, the converse of developmental instability, buffers against such perturbations. In logical consequence, developmental stability and canalization are related. However, studies on the relation of canalization and developmental stability revealed controversial results. In several cases, no such relation was found (e.g. Debat et al., 2000).

For instance, studies testing the influence of malfunction of HSPs in fluctuating asymmetry, as an indicator of developmental instability, revealed no effect on variation in fluctuating asymmetry (Debat et al., 2006). However, complexity in gene regulatory networks is generally assumed to be crucial for robustness in developmental pathways in both canalization and developmental stability (Klingenberg, 2004; Wagner, 2005; Garfield et al., 2013).

Canalization and developmental instability are similarly affected by stress (Parsons, 1990; Badyaev, 2005). Selection can have a tremendous impact on canalization and developmental stability. Stressful environmental conditions can disturb “normal” development and induce novel developmental pathways. This implies that, in turn, non-stabilizing selection e.g. directive selection

and disruptive selection, are promoting less robust development (Pélabon et al., 2010). Less canalised, or less developmental stable traits, on the other hand, are under the conditions of stabilizing selection costly, hence traits which are more robust in development are favoured (Siegal & Bergman, 2002). Furthermore, it is assumed that canalization is a by-product (Gibson & Wagner, 2000) of, or evolves under stabilising selection (Siegal & Bergman, 2002).

In general, it is expected that phenotypical diversification and innovation is associated with adverse environment, by the fact that stress can reveal cryptic genetic variation and affect variability, as well by fostering mutation rates (Parsons, 1988; Hoffmann & Parsons, 1997). Stress plays an essential role in evolution. For example, stem members of radiating lineages or periods in the natural history of rapid diversification and the emergence of phenotypic innovations are assumed to be linked to periods of decreased canalization and/or increased developmental instability (Wilkins, 2003). Evidence from natural history to demonstrate such relationships is few and far between (e.g. Williamson, 1981; Webster, 2007). Assumptions on early bursts in diversity are mainly based on estimation of evolutionary rates based on phylogenetic approaches, with different outcomes (e.g. Foote, 1991; Hughes et al., 2013; Hopkins & Smith, 2015). These models, however, do not refer in detail to the interplay of development (canalization, developmental instability) and environment, rather on disparity estimates between clades.

1.3. Phenotypic plasticity

Phenotypic plasticity is a well-known phenomenon, has gained more attraction in evolutionary biology during the last decades (Rollo, 1995; Pigliucci, 1998; West-Eberhard, 2003). Phenotypic plasticity can be defined as the environmental sensitivity of a genotype to produce alternative phenotypes (Fusco & Minelli, 2010). Woltereck (1909) coined the term “reaction norm” for the production of a single phenotype as a response to an environmental variable. More dramatic transformations are caused by polyphenism, another case of phenotypic plasticity. Polyphenism causes discrete phenotypic variations by a single genotype, induced by environmental triggers (DeWitt & Scheiner, 2004).

Often, phenotypic plasticity is misunderstood as the converse of canalization (e.g. Zelditch, et al., 2012), if interpreted as the insensitivity to environmental perturbations or the reliability to produce a target phenotype. However, a reaction norm can imply a continuous range of target phenotypes as well, and organisms can differ in the variances of their plastic response. According to this, it still makes sense to speak of a canalised plastic development (see Pigliucci, 2010).

The role of phenotypic plasticity in evolution has been controversially debated; it would either promote (Waddington, 1942; West-Eberhard, 2003, 2005; Wund, 2012) or retard and constrain evolutionary changes. An argument for retarding evolution is that phenotypic plasticity potentially

shields genetic variability from selection by buffering the environmental influence (Schlichting & Smith, 2002). Plastic traits can contribute to evolution by promoting diversification (Waddington, 1942; West-Eberhard, 2003, 2005; Wund, 2012). For instance, it can promote diversification among populations inhabiting different environments, by accommodation of alternative phenotypes. The processes of genetic assimilation (Waddington, 1942, 1953, 1956) and genetic accommodation (West-Eberhard, 2003) are able to contribute to fixation of environmentally introduced alternative phenotypes (West-Eberhard, 2005; Suzuki & Nijhout. 2006; Braendle & Flatt, 2006; Schlichting & Wund, 2014). Accordingly, these topics are integral elements of the controversial debate of whether or not genes are followers or leaders in phenotypic diversification and adaptation. Genes can appear as leaders through mutational variation, or as genetic manifestations following an environmental modification of the phenotype (e.g. Palmer, 2004; Schwander & Leimar, 2011).

In addition, phenotypic plasticity plays a major role in evolution, simply by the fact that it allows organisms to migrate, settle and survive in novel environments. In addition, these novel stimuli are able to uncover previously hidden genetic variation by evoking plastic reactions to new environmental triggers, and simultaneously, it allows for accumulation of cryptic genetic variation by concealing potential phenotypic variation to selection regimes (Schlichting & Smith, 2002). In conclusion, phenotypic plasticity can be of major importance in facilitating phenotypic diversification. For instance, the flexible stem hypothesis (West-Eberhard, 2003) predicts that adaptive radiations can arise by divergence from ancestral populations as a consequence of phenotypic plasticity. Descendent species are enabled by the inherited plastic developmental pathways to encounter variable environments and ecological niches. Through natural selection, these prior plastic developmental trajectories would experience a loss of phenotypic plasticity by genetic accommodation in the derived species. Such a scenario would lead to evolution without a large genetic variation. Studies on cases of adaptive radiation were able to confirm this model (Wund et al., 2008; Tebbich et al., 2010; Muschick et al., 2011). In conclusion, phenotypic plasticity potentially enhances variability and evolvability.

As phenotypic plasticity is a property of the genotype, it is difficult to infer phenotypic plasticity in the fossil record, hampered by the fact that any knowledge about the genotype is lost. Accordingly, any ecophenotypic variation in fossil populations could also have been the result of genetic variation. Some authors have made statements on phenotypic plasticity on fossil taxa in the recent past. However, these conclusions are often only very speculative and based only on the fact that in different environments different proportions of phenotypic alternatives/variations occur (e.g. Wilmsen & Mosavinia, 2010). Other possible developmental effects such as variations in the degree of canalization among populations were frequently ignored.

To provide more reliable inferences about plasticity in the fossil record, it was suggested to evaluate if *a)* particular modified traits would not be unique or exclusive in a population, *b)* the whole population would react similarly, *c)* other related taxa reveal similar morphological reactions, *d)*

phenotypic variation are recurrent within a lineage under the condition of similar experienced environmental stimuli (McNamara & McKinney, 1991; Chauffe & Nichols, 1995; West-Eberhard, 2003). Accordingly, phenotypic plasticity is difficult, but not impossible to assess in the fossil record (see chapter 4; Schlüter, 2016).

1.4. The study object: the natural history of echinoids

On account of the high-magnesium-calcite skeleton, the preservation potential of echinoids is comparably good, and accordingly, the fossil record is good (Kier, 1974; Smith, 1984). Irregular echinoids especially occur in the fossil record often stratigraphically continuously. Moreover, the skeletons of echinoids are very complex and provide a diverse array of characters to study (compare Kroh & Smith, 2010).

In general, echinoids are ideal subjects for studies in patterns of phenotypic variation and evolution. Indeed, there are numerous studies on their evolution, either based on solely morphological patterns (e.g. Rowe, 1899; McNamara, 1987, 1989; David & Laurin, 1996; Villier et al., 2004; Kroh & Smith, 2010), or a combination of molecular and morphological analysis (Littlewood & Smith, 1995; Jeffery et al., 2003, Egea et al., 2016). So far, however, systematic approaches and evolutionary analysis predominantly rely on the architecture of the rigid tests. Other appendages, like teeth and pedicellariae, were less widely studied and applied for such purposes (e.g. Mortensen, 1950, 1951; Coppard et al., 2012; teeth of regular echinoids, Reich & Smith, 2009; Kroh & Smith, 2010, Ziegler et al., 2012).

Echinoids developed during the Palaeozoic (first occurrence date: Middle Ordovician, Lefebvre et al, 2013), reaching a peak in the Lower Carboniferous, declining from the Upper Carboniferous towards the Triassic (Smith, 1984, Kroh, 2011). Due to their low preservation potential, based on their only imbricated test, palaeozoic echinoids are characterised by a generally poor fossil record (Smith & Kroh, 2013). They have been restricted to rather quiet, offshore habitats (Smith, 1984). From the archaeocidarids crown group, echinoids emerged during the middle Permian, divided into cidaroids and euechinoids (Thompson et al., 2015). After a recovery from the Permian/Triassic extinction event, which only two lineages surpassed (cidaroids and euechinoids), a further diversification started again in the Upper Triassic (Smith, 1984). This was the initiation of a radiation, which continued during the Mesozoic. Morphological innovations occurred within this period, and the euechinoids clade diverged into the Echinothurioida, Micropygoida, Diadematoida,

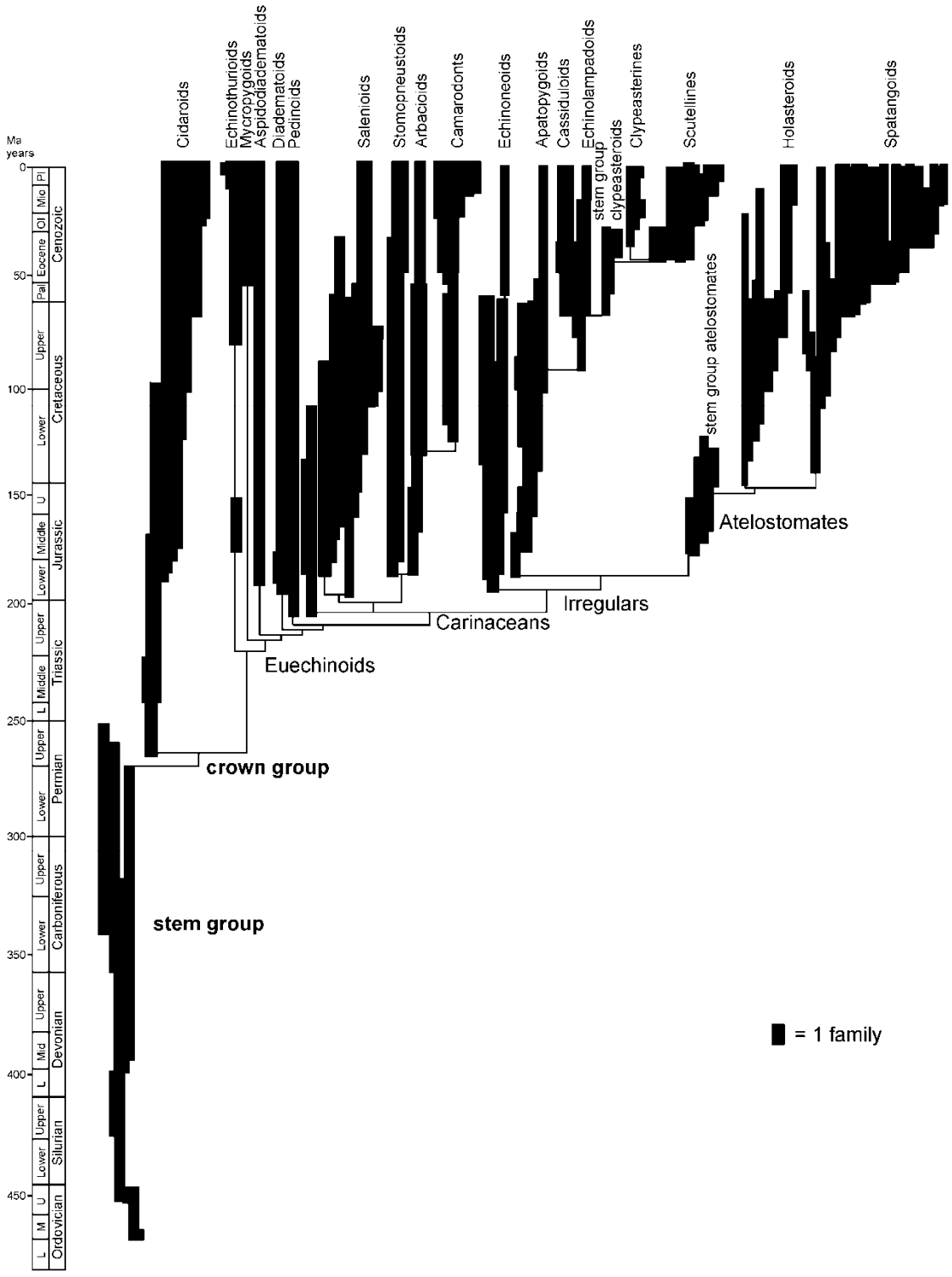

Figure 5. Stratigraphic distribution of the echinoid families. One family is represented by one bar (modified from (Smith & Kroh, 2013). Note that the results on the origin of the Cidaroida of Thompson et al. (2015) are not considered here.

Aspidodiadematoida, Pedinoida, Calycina, Echinacea and Irregularia (Smith & Kroh, 2013). Irregularia established a highly divergent morphology, from pentaradial in the "regular" echinoids to a secondary bilateral symmetry along the anterior-posterior axis, which is associated with a transformation of the apical disc due to a progressive migration of the periproct from the apex to the posterior margin of the test (Saucède et. al., 2007; Saucède et. al., 2015). Irregular echinoids comprise, besides primitive or basal groups (e.g. Holectypoida, Echinoneoida) the diverse groups of Neognathostomata (Cassiduloida, Echinolampadoid, Clypeasteroida) and Atelostomata (Spatangoida, Holasteroida) (Smith & Kroh, 2013).

Novel morphologies enabled the group of irregular echinoids to explore new habitats, shifting from an epifaunal to an often infaunal living mode (Kier, 1974; Smith, 1984). Whereas neognathostomates prefer shallower habitats with coarser sediments, and, the atelostomates exploited and adapted to finer grained sediment in deeper water (Telford & Mooi, 1996; Barras, 2008; Smith & Kroh, 2013).

During the latest Jurassic to early Cretaceous, atelostomate echinoids evolved and split into the Spatangoida and Holasteroida. However, little is known of this era of divergence of groups, due to a comparably low sedimentary record of this time interval and sometimes unfavourable preservation conditions (Kroh et al., 2014). After the Cretaceous, holasteroids declined in their abundance and diversity and are today restricted to the deep sea (Smith, 2004). Another important event in the Cenozoic was the advent of the Clypeasteroida.

From the Mesozoic on, irregular echinoids developed a high morphological diversity with high evolutionary rates, sometimes resulting in bizarre-looking and highly specialised shapes, such as *Hagenowia* (Ernst et al., 1971; Gale & Smith, 1982) and *Pourtalesia* (Saucède et al., 2004). In contrast, regular echinoids, evolved slowly with a lower morphological diversification due to assumable developmental constraints (Hopkins & Smith, 2015)). Likewise, from the Mesozoic onwards echinoids exploited a wide range of different habitats from the shallow shelfs to the deep-sea. Echinoids were in the past and are today, an important part of the marine benthos fauna, having important ecological functions, such as serving as predators (Baumiller et al., 2010) or grazers on coral reefs (Hawkins & Lewis, 1982).

1.5. Methods

The methods applied reflect a progress in analytical techniques. Beginning with classic linear measurements and predominantly descriptive comparisons in the second chapter, descriptive methods and mainly linear measurements are applied in the third chapter. Finally, in the fourth chapter, descriptive methods are complemented by predominantly contemporary geometric morphometric approaches with 3D reconstructed images (photogrammetry). Variations in shape and covariation

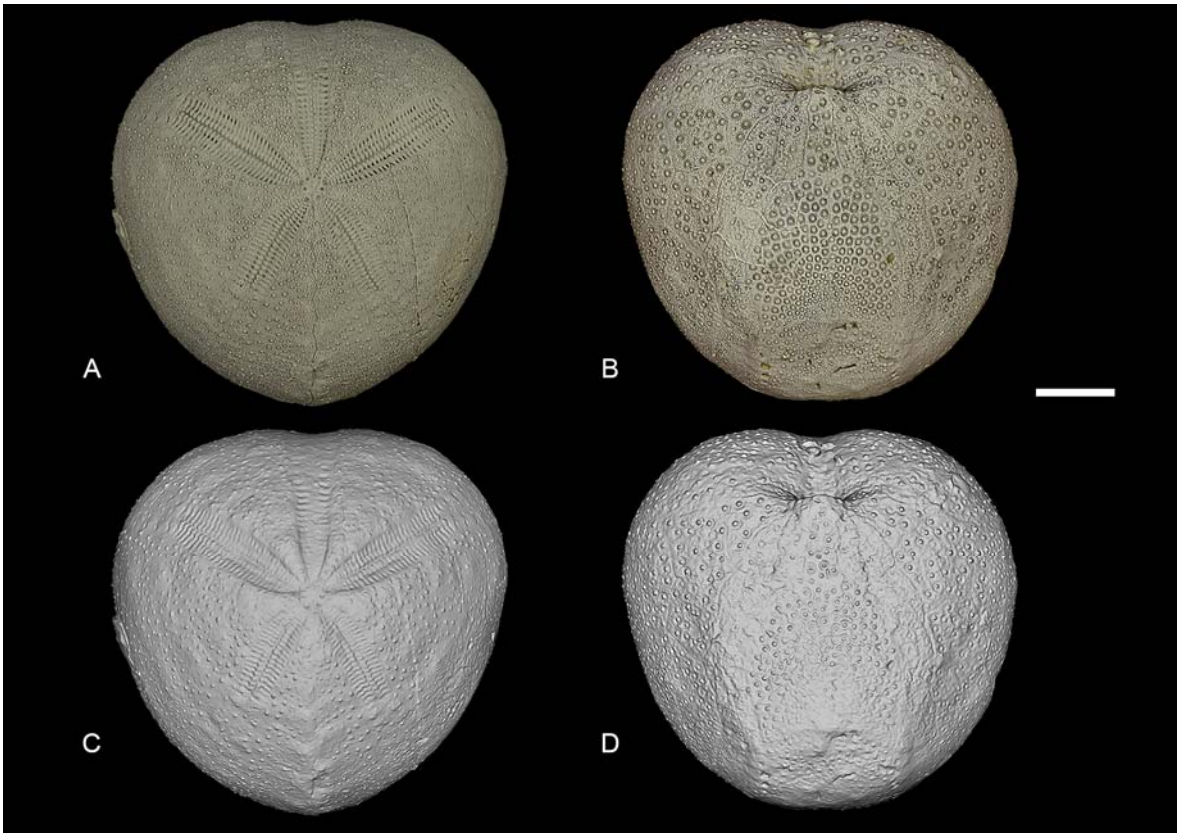

Figure 6. 3D models of *Micraster brevis* (GSUB E3867, Erwitte area, Westphalia, Germany) in apical (A, C) and oral (B, D) view. A-B: given as an OBJ file showing the texture surface, which enables to see the photographic details. C-D: polygon mesh models of the same specimen. Scale bar equals 1 cm.

between subsets within shape configurations can be tested by a more objective characterization with the help of geometric morphometrics (Bookstein, 1991; Klingenberg, 2010) than by subjective descriptions. The advantage of landmark based geometric morphometric analyses over simple linear measurements is that the shape of an entity can be measured rigorously and quantified very precisely. Additionally, it enables comparisons and analysis of organisms as a whole, and thus, not comparing measurements of specific traits as stand-alone (in combinations with other dimensions, like length, or height), which are detached from their morphological context and hence, only capture little information. Accordingly, linear measurements contain information on shape variations, which are given by the ratios, in which one measurement of interest is dependent on the size of another dimension (Zelditch, et al., 2012). Consequently, it is difficult to extract or combine information on shape variations of a specific entity homologous in their position in all studied specimens (Bookstein, 1991). This, however, results in a drawback due to the fact that novel innovations are excluded from the comparison, if they do not originate from pre-existing traits by duplications (for details see Klingenberg, 2008b). Shape variations can be presented and studied more easily with geometric morphometric approaches, so such an approach was chosen in the fourth chapter.

Geometric morphometric analyses rely either on 2D or 3D images. The choice of dimension also depends on the object of interest. Analyses of three dimensional objects, studied by 2D images, may be less accurate (Cardini, 2014). However, generation of 3D images of objects is often laborious, and requires expensive technical devices. On the other hand, 3D models based on photogrammetry provide a very useful alternative, though rarely applied, tool in morphometric studies to for example, laser scanning. Photogrammetric software reconstructs 3D coordinates in a series of overlapping 2D images, recorded along small angles across the object. The resulting 3D models are useful for geometric morphometric purposes, similar to products of other devices. Generally, geometric morphometric methods are a powerful tool in evolutionary biology, for instance investigating disparities (Drake & Klingenberg, 2010), or the degree of canalization among populations or species (Willmore et al, 2005; Willmore & Zelditch 2006) by comparing the total shape variances. They enable the analysis of developmental instability within and among populations (Klingenberg & McIntyre, 1998) and phylogenetic analysis based on shape variation can be conducted.

1.6. Aims and scope

The following three chapters can be regarded as thematic complexes of studies on phenotypic variations and variability in order to elucidate their origins on a systematic hierarchy-level in echinoids.

Chapter 2 seeks systematically valuable characters in the microstructure of recent atelostomate echinoids (Spatangoida, Holasteroida). Within the last 145 Myr years, the atelostomate group of echinoids has become an important component of the marine benthos. Systematic studies within this echinoid group are predominantly based on test morphology (Kroh & Smith, 2010). An important feature of echinoids is their spines. Attempts have been made to use the variation in their spines for systematic purposes but without any meaningful results, leading to them being regarded as a poor variable and thus, insignificant morphological character. In this study, 973 spines of 74 atelostomate taxa have been investigated in detail with respect to their microstructures. The results revealed several homologies shared among the particular orders of holasteroids and spatangoids. However, a single character (pattern of perforation of the inner cylinder) was found to be reliable to distinguish between spines of spatangoids and holasteroids. Interestingly, a single outlier in this respect was found within the holasteroids [*Corystus relictus* (de Meijere, 1903)], which was similar to the pattern found in spines of spatangoids. An anomaly in a single spine of this taxa, combining both patterns of perforation, suggests that the deviant holasteroid taxa bears the genetic potential to develop both character states.

Chapter 3 considers a group of fossil echinoids (regular echinoids; Phymosomatidae), which has traditionally been neglected in palaeolontigical studies of evolution. This may be due to their less rigid skeleton architecture and, accordingly, their relatively poor fossil record (Kier, 1977). Moreover, regular echinoids reveal a low phenotypical variation in comparison with irregular echinoids, which hampers traditional approaches in phylogenetic studies. Thus, only little is known about their evolutionary relationship, and virtually no knowledge exists about evolutionary processes leading to their diversity. In this study, ontogenetic trajectories among three species of the genus *Gauthieria* are recognised: *G. radiata* (Sorignet, 1850), *G. spatulifera* (Forbes in Dixon, 1850), and *G. princeps* (von Hagenow, 1840). These trajectories enable comparisons among the three species and thus reveal heterochronic processes within their evolution. As mentioned above, detailed phylogenetic results which are crucial for drawing conclusions about heterochronic development are completely missing from this group. Nevertheless, this work shows that comparisons of ontogenetic variations are useful tools to uncover evolutionary links between otherwise only low variable taxa.

Chapter 4 studies variations within a species of the Late Cretaceous irregular echinoid *Micraster*. In this work, populations from different habitats from the early Coniacian are investigated, two populations from the Münsterland Cretaceous Basin (Germany) and one population from the North Cantabrian Basin (Spain). Variations on different levels are investigated, from between populations and habitats to variations within-individuals. The aim of this work was to discuss and trace back the mechanisms of the respective variations, in terms of being largely genetically influenced, or either as being a sign of phenotypic plasticity, and to test for the presence of stochastic variations as a result of developmental instability.

Micraster is regarded as a well-known example of a progressive modifying lineage. The influence of the environment on variations in this phenotype were neglected by previous studies. However, this study demonstrates the possibility to distinguish between largely genetic influenced variations and variations due to phenotypic plasticity, and to further explore developmental instabilities in this fossil taxa.

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Chapter 2

Systematic assessment of the Atelostomata (Spatangoida and Holasteroida; irregular echinoids) based on spine microstructures

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Abstract

Spines of irregular echinoids occur in very high abundance in each specimen and display distinct architectures due to the specialised functions of the spines. However, studies on the spine-microstructures of atelostomate echinoids have rarely been carried out so far. Accordingly, only little is known about their specific morphology. This work aims to elaborate differences in spine morphologies of selected Atelostomata (Spatangoida and Holasteroida) in detail and discussing spine microstructure for its potential systematic value. Based on 82 atelostomate species (56 spatangoids, 26 holasteroids) we show that the perforation pattern in the internal cylinder of the spine (helicoidal versus horizontal pattern) provides a safe distinction between the Spatangoida and Holasteroida. According to this character we discuss the geological history of atelostomate echinoids, in particular their migration into the deep sea, based on well preserved records of fossil spines.

Keywords: classification – morphology – sea urchins – Echinoidea – Atelostomata – Spatangoida – Holasteroida

2.1. Introduction

Holasteroid and spatangoid echinoids (the only extant atelostomate irregular echinoids) evolved around 145 Mya (Eble, 2000; Kroh & Smith, 2010), and became an important component of the Cretaceous shelf benthos (Kier, 1974; Smith, 1984; Eble, 2000); however, systematic approaches to these atelostomates predominantly rely on test architecture. The appendages of echinoids in general have been studied in detail elsewhere (pedicellariae, including atelostomate taxa, Mortensen, 1950, 1951; Coppard et al., 2012; teeth of regular echinoids, Ziegler et al., 2012); however, there is little knowledge on the morphology and microstructure of atelostomate spines. Agassiz (1872–1874: 651) gave detailed descriptions on spine microstructure in the major extant echinoid groups, also including

Superorder Atelostomata von Zittel, 1879		
Order Spatangoida L. Agassiz, 1840		
	Family Hemiasteridae H. L. Clark, 1917	*Holanthus expergitus* (Lovén, 1874)
Suborder	Family Micrasteridae Lambert, 1920	*Isopatagus obovatus* Mortensen, 1948
Micrasterina Fischer, 1966	Family Aeropsidae Lambert, 1896	*Aeropsis fulva* (A. Agassiz, 1898)
Suborder	Family Loveniidae Lambert, 1905	*Breynia australasiae* (Leach, 1815)
Brissidina Stockley, Smith		*Echinocardium cordatum* (Pennant, 1777)
Littlewood & MacKenzie-Dodds, 2005		*Echinocardium mediteraneum* (Forbes, 1844)
		Lovenia elongata (Gray, 1845)
		Lovenia subcarinata Gray, 1851
	Family Spatangidae Gray, 1825	*Spatangus capensis* Döderlein, 1905
		Spatangus purpureus Müller, 1776
		Spatangus raschi Lovén, 1870
	Family Maretiidae Lambert, 1905	*Granobrissoides hirsutus* (Mortensen, 1950)
		Gymnopatagus magnus Agassiz & Clark, 1907
		Homolampas sp.
		Maretia planulata (Lamarck, 1816)
		Nacospatangus laevis (H.L. Clark, 1917)
		Nacospatangus tylota (H.L. Clark, 1917)
		Spatagobrissus mirabilis H.L. Clark, 1923
	Family Palaeotropidae Lambert, 1896	*Paleotrema loveni* (A. Agassiz, 1879)
	Family Eurypatagidae Kroh, 2007	*Eurypatagus ovalis* Mortensen, 1948
		Eurypatagus parvituberculatus (H.L. Clark, 1924)
		Linopneustes fragilis (de Meijere, 1903)
		Linopneustes longispinus (A. Agassiz, 1878)
		Linopneustes murrayi (A. Agassiz, 1879)
		Paramaretia multituberculata Mortensen, 1950
	Family Brissidae Gray, 1855	*Anametalia regularis* (H.L. Clark, 1925)
		Brissopsis lyrifera (Forbes, 1841)
		Brissus agassizii Döderlein, 1885
		Brissus latecarinatus (Leske, 1778)
		Brissus obesus Verrill, 1867
		Meoma ventricosa grandis Gray, 1851
		Meoma ventricosa ventricosa (Lamarck, 1816)
		Metalia nobilis Verrill, 1867
		Plagiobrissus grandis (Gmelin, 1791)
		Rhynobrissus hemiasteroides A. Agassiz, 1879
		Rhynobrissus pyramidalis A. Agassiz, 1872

Table 1. List of taxa investigated.

sections (Agassiz, 1872–1874: plates XXXV, XXXVI, XXXVII). Mooi & David (1996) documented some miliary spines from selected Holasteroida, and Stephenson (1963; on *Echinocorys scutata* Leske, 1778) and Saucède et al. (2009; on *Calymne relicta* Thomson, 1877) presented the spine morphology of a single species in great detail. Other studies treated spines cursorily (Agassiz, 1881; Hesse, 1900; Mortensen, 1950, 1951; Stephenson, 1963; Kroh, 2002) and, because of their apparently variable and thus insignificant morphological characters, atelostomate spines were not seriously considered to be taxonomically significant (but see Kroh & Smith, 2010). In order to gauge the possible systematic value of atelostomate spines, we studied the morphology and microstructure of 973 spines of 74 extant atelostomate taxa (for details, see Tables A1-15), following the systematic

Order Spatangoida L. Agassiz, 1840		
Suborder Paleopneustina Markov & Solovjev, 2001	Family Schizasteridae Lambert, 1905	*Abatus cavernosus* (Philippi, 1845)
		Abatus cordatus (Verrill, 1876)
		Aceste bellidifera Thomson, 1877
		Brisaster capensis (Studer, 1880)
		Brisaster fragilis (Düben & Koren, 1846)
		Moira atropos (Lamarck, 1816)
		Protenaster australis (Gray, 1851)
		Schizaster compactus (Koehler, 1914)
		Schizaster edwardsi Cotteau, 1889
		Tripylaster philippii (Gray, 1851)
	Family Prenasteridae Lambert, 1905	*Agassizia scrobiculata* Valenciennes, 1846
		Tripylus excavatus Philippi, 1845
	Paleopneustine	*Amphipneustes lorioli* Koehler, 1901
		Amphipneustes marsupialis (Koehler, 1926)
	unnamed clade	*Heterobrissus hystrix* (A. Agassiz, 1880)
	Family Paleopneustidea A. Agassiz, 1904	*Paleopneustes cristatus* A. Agassiz, 1873
		Plesiozonus diomedeae Mortensen, 1948
	Family Pericosmidae Lambert, 1905	*Faorina chinensis* Gray, 1851
		Pericosmus akabanus Mortensen, 1939
		Pericosmus macronesius Koehler, 1914

Table 2. List of taxa investigated (continuation of Table 1).

Order Holasteroida Durham & Melville, 1957		
Infraorder Urechinina Duncan, 1889	Family Plexechinidae Mooi & David, 1996	*Plexechinus spectabilis* Mortensen, 1948
	Family Corystusidae Foster & Philip, 1978	*Corystus relictus* (de Meijere, 1903)
	Family Pourtalesiidae A. Agassiz, 1881	*Ceratophysa ceratopyga valvaecristata* Mironov, 1976
		Ceratophysa rosea (A. Agassiz, 1879)
		Cystocrepis setigera (A. Agassiz, 1898)
		Echinocrepis rostrata Mironov, 1973
		Echinosigra (*Echinogutta*) *amphora* Mironov, 1974
		Echinosigra (*Echinosigra*) *phiale* (Thomson, 1873)
		Echinosigra (*Echinosigra*) *vityazi* Mironov 1997
		Pourtalesia heptneri Mironov, 1978
		Pourtalesia jeffreysi Thomson, 1873
		Pourtalesia laguncula A. Agassiz, 1879
		Pourtalesia thomsoni Mironov, 1976
	Family Urechinidae Duncan, 1889	*Cystechinus loveni* A. Agassiz, 1898
		Pilematechinus vesica (A. Agassiz, 1879)
		Urechinus naresianus A. Agassiz, 1879
	Family Carnarechinidae Mironov, 1993	*Carnarechinus clypeatus* (A. Agassiz, 1879)
	Family Calymnidae Mortensen, 1907	*Sternopatagus sibogae* de Meijere, 1903

Table 3. List of taxa investigated (continuation of Table 2).

classification of Kroh & Smith (2010): 56 Spatangoida, with members of the Hemiasteridae, Micrasteridae, Loveniidae, Spatangidae, Maretiidae, Palaeotropidae, Eurypatagidae, Brissidae, Loveniidae, Schizasteridae, Prenasteridae, Palaeopneustine unnamed clade, Paleopneustidea, and Pericosmidae; 18 Holasteroida, with species of the Plexechinidae, Corystusidae,

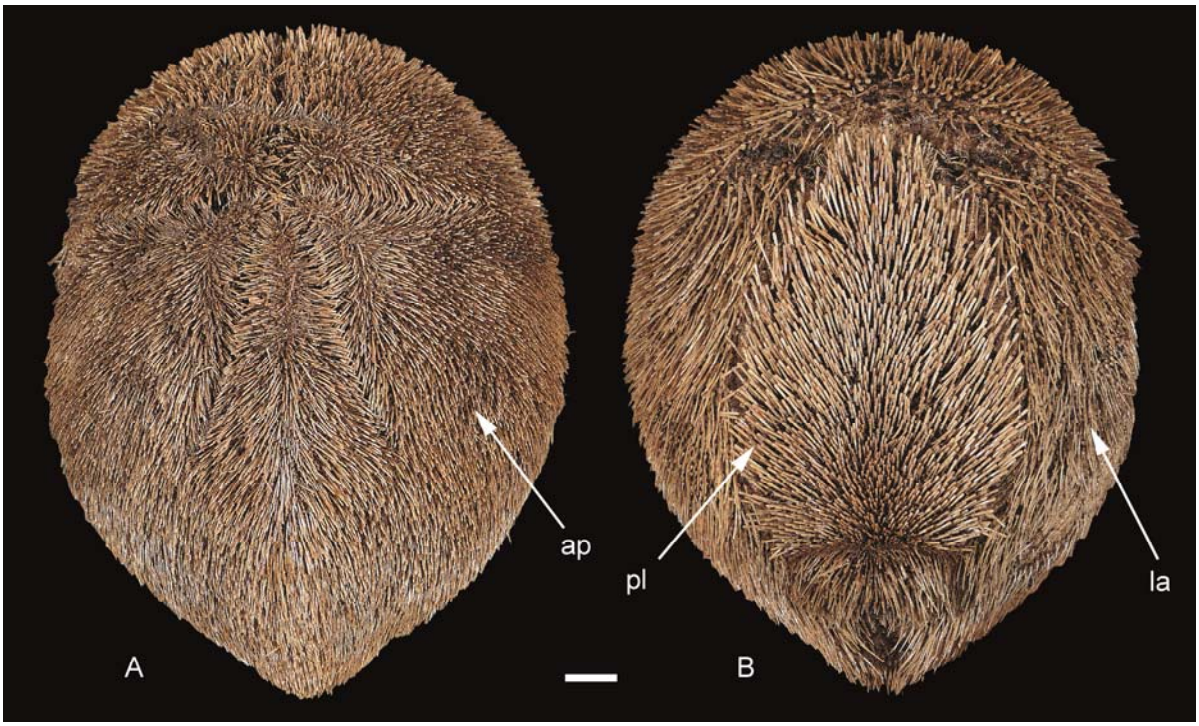

Figure 1. Test with spines (*Brissus latecarinatus*, ZMUC-ECH-602): apical side (A), oral side (B). Arrows indicate approximately from where spines have generally been collected (ap: apical, pl: plastronal, la: lateral). Scale bar equals 1 cm.

Pourtalesiidae, Urechinidae, Carnarechinidae, and Calymnidae (Tables 1–3). In addition, published drawings (A. Agassiz, 1881) from eight holasteroid taxa were studied for the perforation of the internal cylinder (see Table 4).

2.2. Material

Most of the material comes from the Theodor Mortensen collection (Natural History Museum Copenhagen), which is one of the largest collections of recent echinoids worldwide. Further taxa come from the Natural History Museum of Berlin and the Geoscience Museum of the University of Göttingen.

There is a mismatch between the number of Holasteroida and the number of Spatangoida in the collections: today, holasteroids are restricted to the deep-sea, and given the fragile nature of their tests and spines, specimens often lack the complete spine canopy, or spine tips are broken off as a result of the collecting technique (e.g. dredging).

2. 2. 1. Institutional abbreviations

GZG, Geowissenschaftliches Zentrum der Georg-August-Universität Göttingen, Göttingen, Germany; ZMB, Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der

Humboldt-Universität zu Berlin, Berlin, Germany; ZMUC, Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark.

2.3. General morphology of spines in atelostomate echinoids

Irregular echinoids are armed with a dense coat of often small spines (Fig. 1). These play a very important role in the differing lifestyles of the echinoids. With the distinct functions of the spines (e.g. locomotion, protection, and transport of food particles), the architecture of the spines is highly adapted to the function. Smith (1980) gave a detailed description on the overall shape and function of spines (for characters mentioned in the text, see Fig. 1). The proximal part of the spine, the acetabulum, is articulated to the mamelon of the tubercle. The base is connected via muscles to the areole, which surrounds the mamelon. The shape of the base varies because of the function of the spine. If the movement of the spine is preferentially unidirectional, the area of muscle attachment is enlarged in the corresponding direction, both in the areole and in the base. The widened area at the top of the base is called the milled ring, which also serves for muscle attachment. The shaft of a spine is generally slender, with either a pointed or a spatulate tip towards the distal ends. The spatulate tip is often found in spines specialised for burrowing/locomotion, and is localised on the oral and possibly on the lateral side also. The spines show a distinct longitudinal striation, which is produced by longitudinal wedges running over the whole length of the shaft. The wedges are generally wedge- or club-shaped in cross section (Fig. 2B). The hollow centre of the spines (lumen or axial cavity, Fig. 2B) is encompassed by a cylinder ('Axialscheide' of Hesse, 1900), which is perforated (Fig. 2C). The bladelike wedges are connected to the cylinder via bridges (Fig. 2A, B).

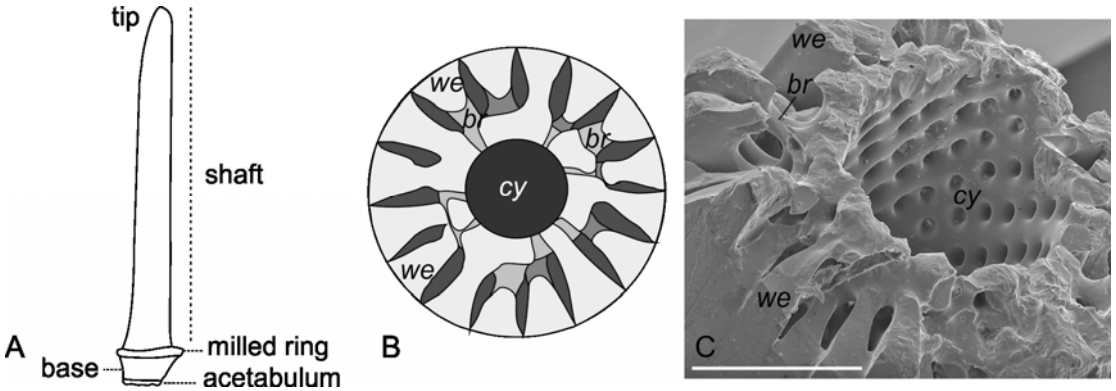

Figure 2. Spine morphology: general (A), spatangoid spine in section (B), and internal structure in a broken spine of *Spatangus raschi* (C). Abbreviations: *we* = wedge, *br* = bridges, *cy* = cylinder.

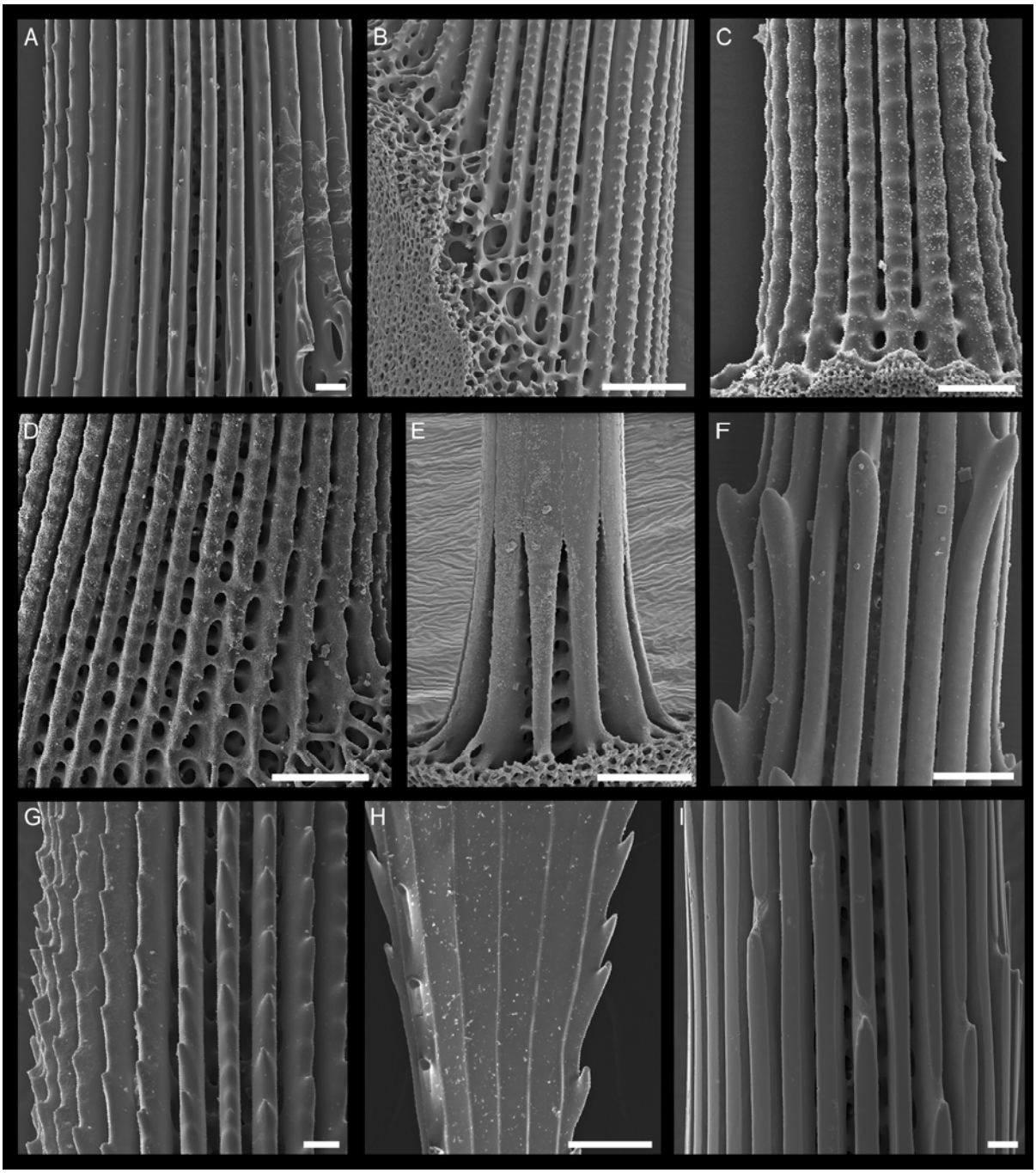

Figure 3. Ornamentation of spines: (A) *Abatus cordatus* (ZMB.Ech 2230_5), (B) *Breynia australisae* (ZMUC-ECH-610), (C) *Tripylaster philippii* (ZMUC-ECH-612), (D) *Moira atropos* (ZMUC-ECH-613), (E) *Pourtalesia heptneri* (ZMUC-ECH-655), (F) *Paleopneustes cristatus* (ZMUC-ECH-113), (G) *Amphipneustes lorioli* (ZMUC-ECH-666), (H) *Echinosigra phiale* (ZMB.Ech 5436_2), (I) *Rhynobrissus pyramidalis* (GZG.INV.78903). Scale bars equal 20 μm (G, I), 30 μm (A), 100 μm (B, C, D, E, F, H).

2.4. Methods

The spines were extracted from the oral side (plastronal area), lateral side and apical side of the tests (see Fig. 1), if spines were available in these areas. These areas could not always be sampled in all

specimens because of incomplete spine preservation. The spines were macerated and cleared of organic remains with hypochlorous acid (3%), and afterwards were washed in distilled water. For longitudinal sections, to assess the perforation of the cylinder, spines were glued on stubs and opened with a nail file. Prior to SEM investigation, samples were sputtered with gold, and analyses and photographic documentation were performed at the Section of Palaeontology, Freie Universität Berlin, with a Zeiss Supra 40VP scanning electron microscope. All measurements were made with ImageJ, three measurements were made and averaged, and the correlation analysis was performed in R v. 3.0.1 (R Development Core Team, 2013).

2.5. Systematic assessment

We tested 7 spine characters (1 internal and 6 external) for a systematic assessment.

i) Ornamentation of the wedges close to the base: Four states can be discriminated: a serrated-like appearance of the wedges (Fig. 3A), a distinct, horizontal, or scattered running pustulation in the wedges (Fig. 3B), a beaded ornamentation (Figs. 3B-D), or naked wedges throughout (Fig. 3E). It appears that some states occur together in a single spine (e.g. Fig. 3B).

ii) Absence/presence of thorns: One internal and six external thorns were generally treated as being absent or present. We did not distinguish between distinct shapes of the thorns (see Figs. 3F–I).

iii) The presence of a beaded ornamentation: We distinguished between spines with a beaded structure (see Fig. 4A–C, H–J) and spines without any ornamentation. Furthermore, the position and extension of the beaded structure on the spine was considered: (1) spines with a beaded base only; (2) the beaded structure extended at least to half the length of the spine; (3) the base of the shaft is smooth and the beaded structure starts higher; (4) naked spines.

iv) The distances between the wedges: The distance between the wedges was related to the width of the wedges. Wedges were measured at the widest point of each wedge, and in between them. To gain a descriptive parameter for statistical analysis, the distance between the wedges was divided by the width of the wedges. The smaller the distance between the septa, the smaller the result: a result of 0 means no distance between the wedges, and a result of 1 means the distance between the wedges and the width of the wedges are equal.

v) The shape of the wedges: The shape of the wedges was suggested by Hesse (1900) as a systematic character. He distinguished the following groups: (1) *Echinocardium* group, based on cuneiform wedges (flatter outer surface and triangular shape; Fig. 5A); (2) *Brissus* group, based on a fan-shaped appearance of the proximal parts of the wedges (after a thinner bridge, connecting cylinder and

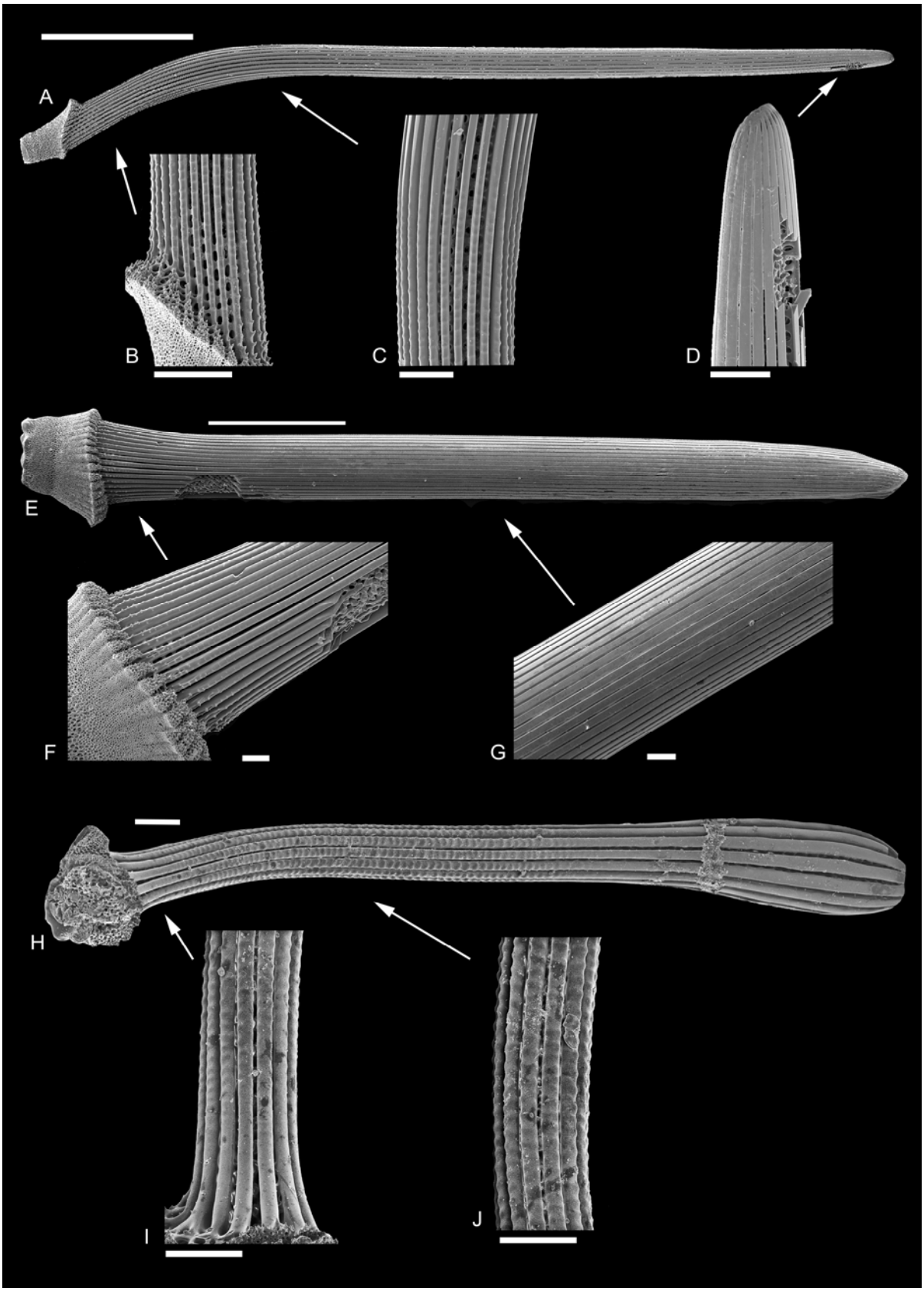

Figure 4. Ornamentation of spines: **(**A-D) *Echinocardium cordatum* (GZG.INV.78890); (E-G) *Brissus agassizii* (GZG.INV.78900); (H-J) *Holanthus expergitus* (ZMUC-ECH-651). Scale bars equal 40 μm (B-D), 100 μm (F-J), 200 μm (A), 1 mm (E).

wedges, the width of the wedges increases rapidly towards the periphery), the surface of the wedges is more flatter (Fig. 5B, C); (3) *Prenaster* group, based on club-shaped wedges (rounded to wellrounded outer surface; Fig. 5D–F).

vi) Number of the wedges: The diameter of the spine was measured at three different sites of the spine: close to the base of the shaft, the middle part, and at the top. These measurements were averaged and then correlated with the number of wedges. The correlation of these data was performed for all species grouped together.

vii) The perforation of the cylinder: The arrangement of the pores was differentiated between pores running horizontally (Fig. 6A) and helicoidally (Fig. 6B).

2.6. Results

A detailed compilation of the results of the analyses can be obtained from the table provided in Tables A1-13. A generalised overview of the results for each family is given in a simplified phylogenetic tree of the Spatangoida and the Holasteroida (Fig. 7).

i) Ornamentation of the wedges close to the base: It appears that the development of a pustulation, or serration, is a shared apomorphy among several spatangoid taxa, as these features could not be observed in holasteroid spines. An occurrence of distinct ornamentation states in spatangoids, which follows a systematic grouping at the family level, could not be detected. The development of ornamentation is possibly more stable at the genus level than at the family level: both species of *Abatus* share the same state (serrated ornamentation), the species of *Nacospatangus* (naked and pustule-like surfaces) and *Linopneustes* (naked throughout). By contrast, species of *Echinocardium* differ from one another: whereas *Echinocardium cordatum* (Pennant, 1777) has spines with a pustulated surface, *Echinocardium mediterraneum* (Forbes, 1844) has naked wedges at the base. Moreover, individuals occur with both types of spines.

ii) Absence/presence of thorns: Thorns occur in holasteroids as well as in spatangoids. Spines with thorns occur scattered among several families. The simple presence or absence of thorns does not reveal a systematic pattern in this study.

iii) Presence of a beaded ornamentation: Holasteroid echinoids never display such ornamentation, which is why we believe that beaded ornamentation is in part apomorphic to the Spatangoida; however, this state seems to occur randomly in spatangoids. Regarding simple availability, it does not strictly follow any systematic grouping: taxa with beaded ornamentation present or absent are found in species regardless of their natural grouping. Furthermore, this feature is variable even in a single echinoid, which can possess beaded as well as naked spines. On the other hand, the degree of

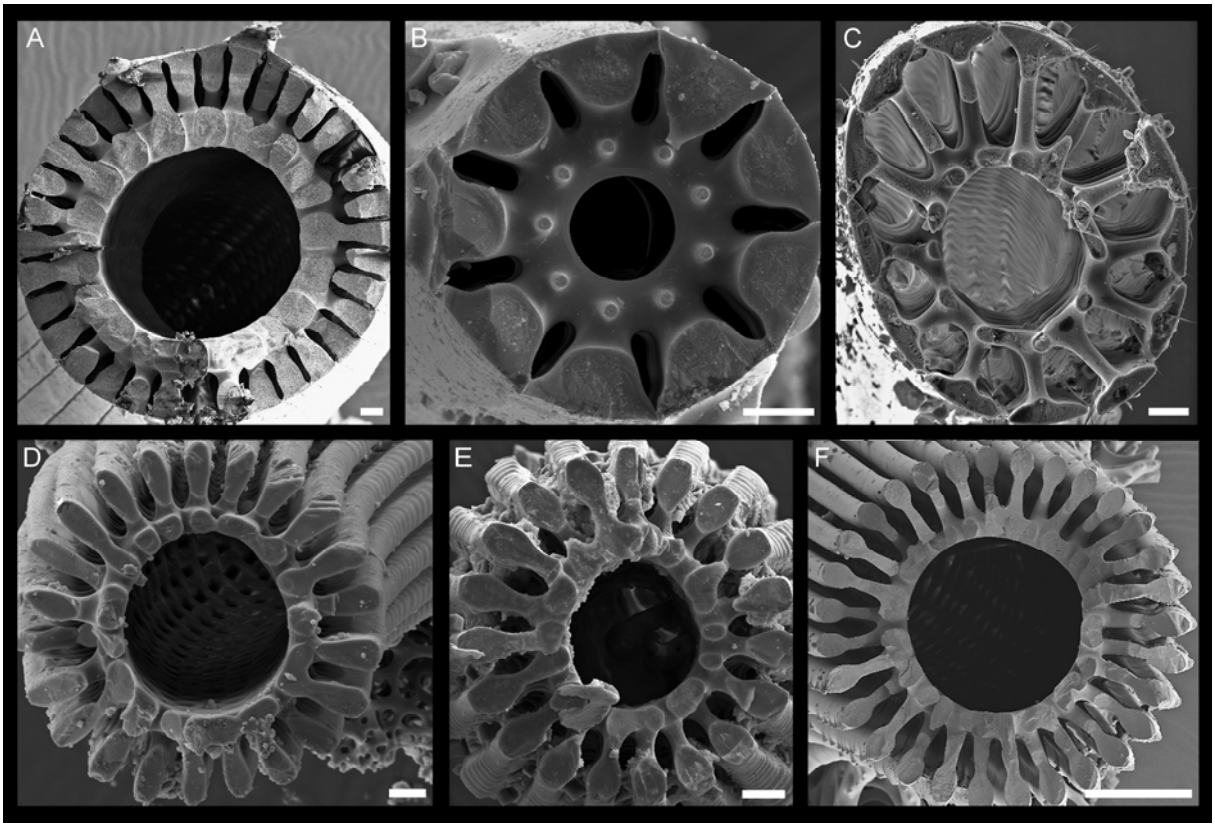

Figure 5. Wedges shape of *Echinocardium* group: (A) *Linopneustes fragilis* (ZMUC-ECH-643); *Brissus* group: (B) *Sternopatagus sibogae* (ZMB.Ech-7426); (C) *Echinocardium mediteraneum* (ZMUC-ECH-622); and *Prenaster* group: (D) *Tripylus excavatus* (ZMUC-ECH-637); (E) *Plesiozonus diomedeae* (ZMUC-ECH-135); (F) *Amphipneustes marsupialis* (ZMUC-ECH-640). Scale bars equal 10 μm (C), 20 μm (A, B, D, E), 100 μm (F).

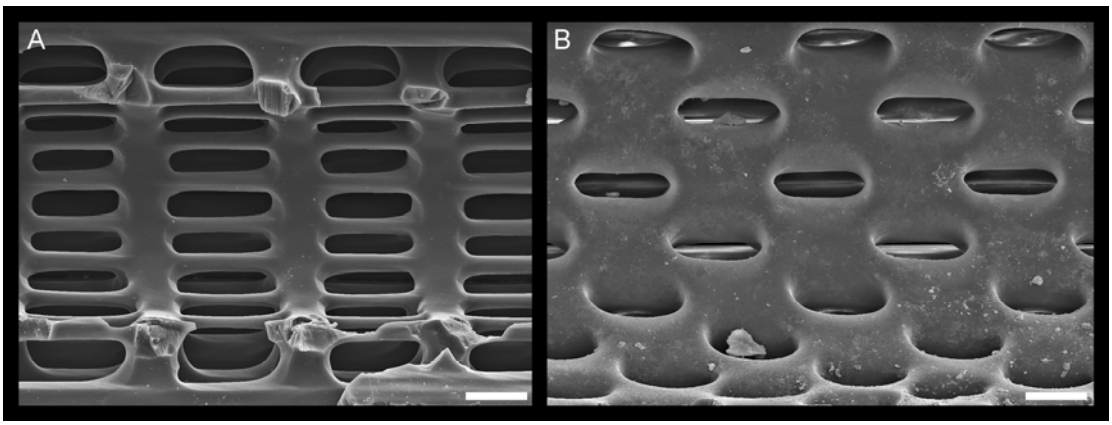

Figure 6. Perforation of the internal cylinder: (A) horizontal arrangement in *Ceratophysa rosea* (ZMB.Ech-7419) and (B) helicoidal arrangement in *Gymnopatagus magnus* (ZMUC-ECH-641). Scale bars equal 20 μm.

expansion of the beaded structure might bear some limited systematic value at the family level. Taxa of the suborders Micrasterina, Brissidina, and the hemiasterid *Holanthus expergitus* (Lovén, 1871) have beaded ornamentation on the lower part of the shaft only, whereas there are several species in the

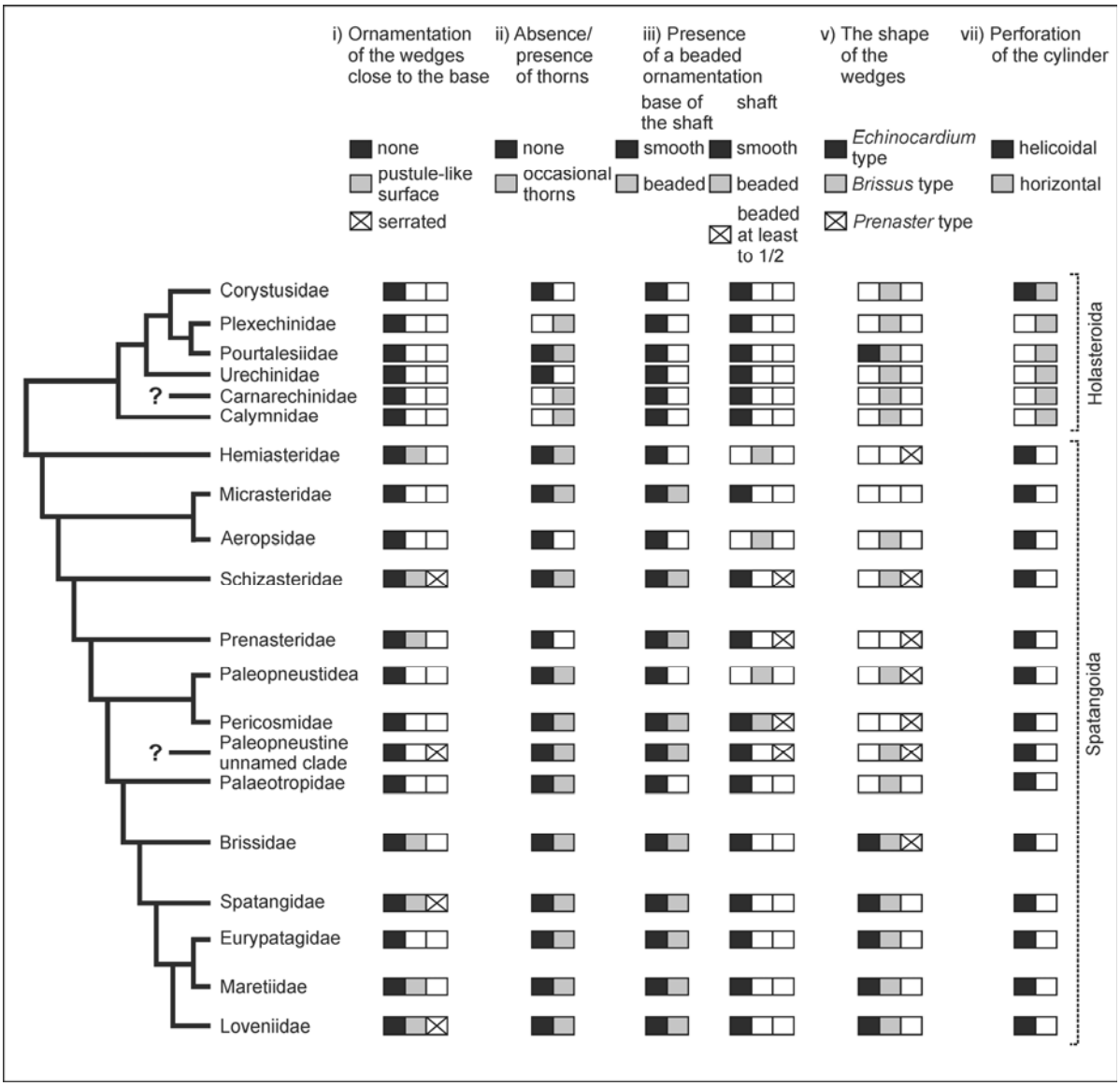

Figure 7. Simplified phylogenetic tree showing the different observed characters for each family (after Kroh & Smith, 2010; for a more detailed phylogeny of the Holasteroida: after Smith, 2004 and Mironov *et al.*, 2013).

suborder Palaeopneustina [*Abatus cavernosus* (Philippi, 1845), *Abatus cordatus* (Verrill, 1876), *Amphipneustes lorioli* Koehler, 1901, *Brisaster capensis* (Studer, 1880), *Brisaster fragilis* (Düben & Koren, 1844), *Faorina chinensis* Gray, 1851, *Moira atropos* (Lamarck, 1816), *Pericosmus macronesius* Koehler, 1914, *Protenaster australis* (Gray, 1851), *Schizaster edwardsi* Cotteau, 1889, *Schizaster compactus* (Koehler, 1914), and *Tripylus excavatus* Philippi, 1845] in which the beaded ornamentation continues beyond.

iv) The distances between the wedges: This character potentially bears some limited value for systematics in atelostomate echinoids. The members of the families Spatangidae, Maretiidae, and Eurypatagidae studied here have spines in which the wedges are more fused to each other, similar to the families Palaeotropidae and Micrasteridae, although these are represented by a single taxon only,

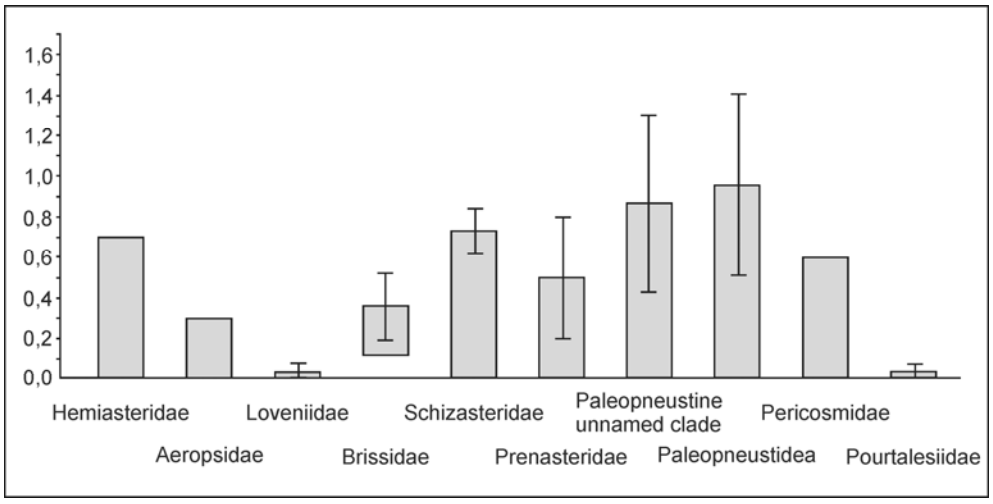

Figure 8. Boxplot showing the mean values and ranges of distance between wedges for the studied atelostomate families (the numbers on the vertical axis give the rleation of distance between the divided by the width of the wedges); families which show no distances between the wedges are not presented (Micrasteridae, Spatangidae, Maretiidae, Palaeotropidae, Eurypatagidae, Plexechinidae, Corystusidae, Urechinidae, Carnarechinidae, Calymnidae).

and are thus not significant. Pericosmids and schizasterids have mostly distanced wedges (compare Fig. 8), and *Aceste bellidifera* Thomson, 1877 is the only schizasterid species with spines that are largely fused wedges. Admittedly, this conclusion is putative and needs to be evaluated for its systematic value with larger data sets.

v) The shape of the wedges: It appears that all types of wedge shapes can occur together in different spines of the same species (*Rhynobrissus hemiasteroides*, A. Agassiz, 1879). Furthermore, the variability of shape types within families can be relatively large, where all types of shapes are present (e.g. Loveniidae and Brissidae). By contrast, schizasterids have the *Brissomorpha* (= *Prenaster*) type only, except for *Aceste bellidifera* and comparable monotonous holasteroids, which have only the *Brissus* type, except for *Pourtalesia jeffreysi* Thomson, 1873. Hesse (1900) grouped 15 atelostomate taxa (fossil and recent) based on the shape of the wedges in section into three groups: (1) *Echinocardium* group with *Echinocardium cordatum*, *Spatangus* sp., *Hemipatagus hoffmanni* (Goldfuss, 1829), *Spatangus purpureus* Leske, 1778, *Schizaster canaliferus* (Lamarck, 1816), *Maretia planulata* (Lamarck, 1816), *Stegaster facki* Stolley, 1892 [probably misidentified, possibly a junior synonym of *Plesiocorys* (*Sternotaxis*) *heberti* (Cotteau in Cotteau & Triger, 1860) or a similar species]; (2) *Brissus* group with *Brissus* sp. and *Brissus carinatus* (Lamarck, 1816) [= *Brissus latecarinatus* (Leske, 1778)]; (3) *Prenaster* group with *Prenaster fuchsi* (Laube, 1871), *Micraster* sp., *Schizaster* sp., *Echinocorys ovata* (Leske, 1778), *Hemipneustes striatoradiatus* (Leske, 1778), and *Metalia maculosa* (Gmelin, 1791) (= *Metalia spatagus* Linnaeus, 1758). This clustering does not

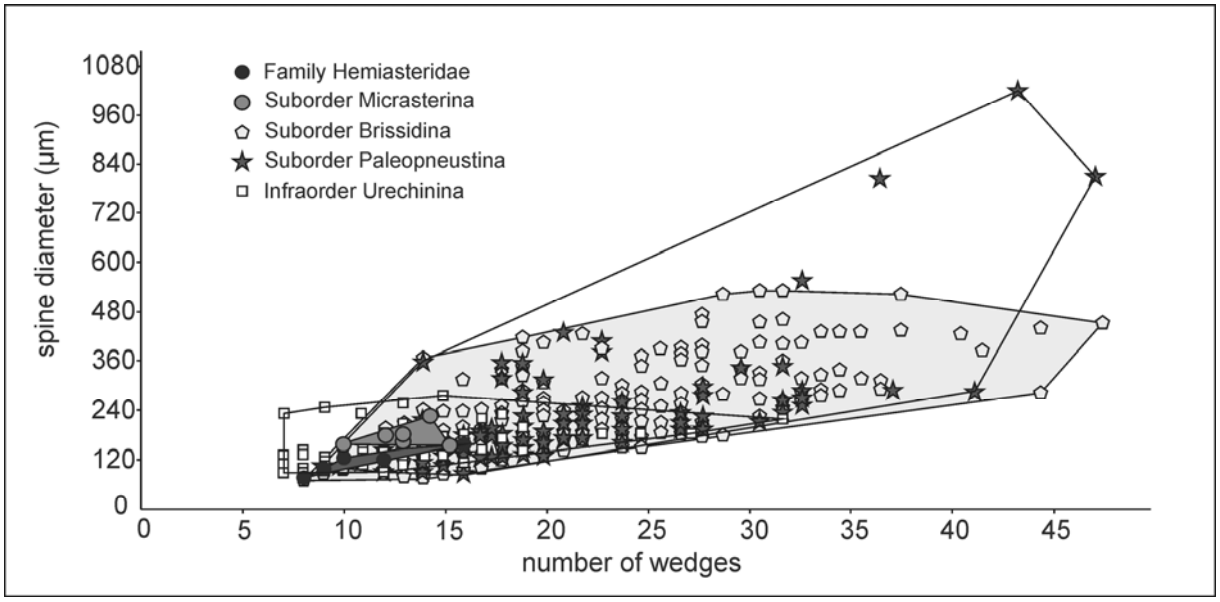

Figure 9. Scatter plot that shows the relationship between number of wedges and spine diameter, including convex hulls for each group; for a better overview only higher systematic levels are distinguished, if possible.

Cystechinus wyvillii A. Agassiz, 1879	pl. XL, figs. 59-60
Calymne relicta Thomson, 1877	pl. XL, figs. 64, 65
Cystechinus wyvillii A. Agassiz, 1879	pl. XLI, figs. 24-27
Echinocrepis cuneata A. Agassiz, 1879	pl. XLI, fig. 31
Spatagocystis challengeri A. Agassiz, 1879	pl. XLI, fig. 40
Ceratophysa ceratopyga (A. Agassiz, 1879)	pl. XLI, figs, 44-46
Pourtalesia hispida A. Agassiz, 1879	pl. XLI, figs. 47, 48
Helgocystis carinata (A. Agassiz, 1879)	pl. XLI, figs. 50-52

Table 4. Holasteroid species, which reveal a horizontal arrangement in pores in the internal cylinder (A. Agassiz, 1881).

reflect the natural systematic grouping, and our data support that this character is of no value for a systematic assessment.

vi) Number of the wedges: The number of wedges and diameter of the spine are strongly positively correlated (Fig. 9, Pearson's correlation coefficient = 0.74; Tables A5-13). This suggests that the number of wedges is simply related to a growth factor, and hence is not relevant for systematic purposes.

vii) The perforation of the cylinder: Spatangoid spines reveal cylinders with a helicoidal pore arrangement in the cylinder throughout. In contrast in holasteroid spines the pores are exclusively arranged horizontally. Additionally, the drawings of holasteroid spines from eight taxa in Agassiz (1881) reveal a

horizontal pore pattern in the cylinder also (Table 4). The only outlier in this group is *Corystus relictus* (de Meijere, 1903), which, in contrast, has helicoidally arranged pores. Both patterns were found in a single spine, however (Fig. 10). Given that the spiral pattern is the target phenotype in spines of *C. relictus*, this phenomenon can be interpreted as a phenodeviant, sporadically occurring

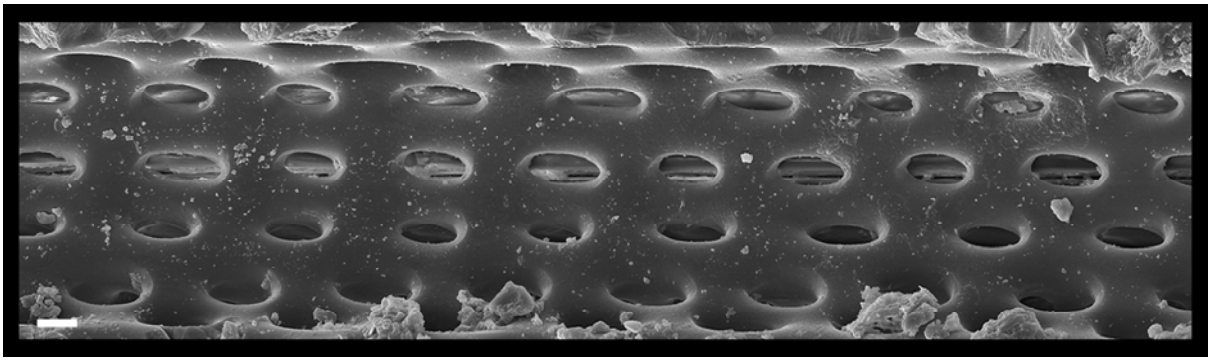

Figure 10. View on the inner side of the cylinder of a spine of *Corystus relictus* (ZMUC-ECH-605); the pores are arranged in a horizontal pattern (left half), from approximately half of the image it changes to a helicoidal pattern. Scale bar equals 20 μm.

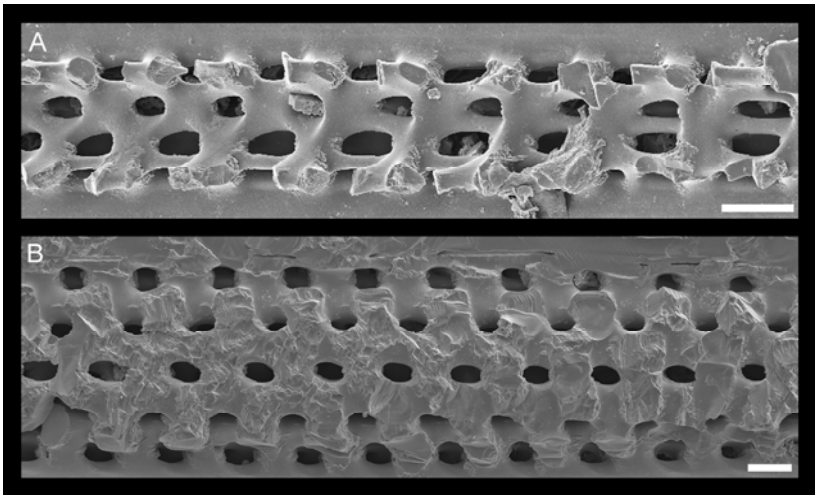

Figure 11. View on the the cylinder of spines of atelostomate echinoids of early/middle Albian age (Falkland plateau, A: GZG.INV.94999, B: GZG.INV.95000): view on the inner side (A), the pores are arranged in a horizontal pattern (right half), from approximately half of the image it changes to a helicoidal pattern; view on the outer side (B), the pores are arranged in a helicoidal pattern throughout. Scale bars equal 20 μm.

abnormal morphology (Rasmuson, 1960). Phenodeviants can be the result of developmental instability, caused by genetic and/or environmental perturbations (Polak, 2003). Those perturbations have an effect on the gene regulatory cascade, and thus potentially reveal cryptic genetic variation (Gibson & Dworkin, 2004). We postulate a scenario that could explain this aberrancy: genetic information from both pore arrangement patterns is available in this specimen, and the developmental pathway for horizontal pores has been reactivated, or switched, as a result of perturbations. This might also hold true for the species *C. relictus*. Even rare abnormalities can give important clues to evolutionary development (West-Eberhard, 2003). We propose that the arrangement of the holes perforating the cylinder turns out to be a reliable character to delineate between holasteroid and spatangoid spines, at least for the majority of the taxa investigated here.

2.7. Conclusions

From the seven characters investigated, only the perforation of the cylinder provides a feature of unequivocal systematic value, enabling a discrimination of the Holasteroida (horizontal pore orientation in the internal cylinder) and Spatangoida (helicoidal pore orientation in the cylinder) (Fig. 7). Finally, a beaded surface and other ornaments like pustules or serrations are exclusively found in the Spatangoida, but never occur in the Holasteroida, which bears some potential for systematic assessments. Our work suggests that spine morphology can serve in parts as a supplementary source for phylogenetic analysis in atelostomate echinoids. The results also bear implications for the evaluation of Atelostomata occurrences in the geological record: from the early–middle Albian (Lower Cretaceous, 110 Myr old), we found Atelostomata spines in deep-sea sediments of Deep Sea Drilling Project (DSDP) Site 327 (eastern Falkland Plateau). These spines exhibit both helicoidal and horizontal pore arrangements, as indicated by astonishingly well-preserved microstructures of the spines (Fig. 11). Interestingly, the horizontal state co-occurs in a single spine with the helicoidal pattern, similar to the deviant spine of *Corystus*. As disasteroid echinoids (stem-group members of the Atelostomata) still occur in this age (Smith & Crame, 2012), we cannot exclude the possibility that these spines were belonging to other atelostomates than holasteroids and spatangoids. A postmortem down-slope transport of the spines from shallower areas is unlikely. This area, as the name suggests, is a plateau since the early/middle Albian, surrounded by deeper basins (Barker et al., 1977). Nevertheless, these finds indicate that the colonization of the deep-sea by the Atelostomata happened earlier than has previously been thought (Smith, 2004). These data are in good accordance with the results of Thuy et al. (2012), who showed that the origin of some modern deep-sea echinoderm faunas (especially ophiuroids and holothuroids) dates back at least to the early Cretaceous (Aptian, c. 120 Mya). In addition, it is the spines of the Atelostomata and not the echinoids test, which are preserved in Integrated Ocean Drilling Program (IODP) deep-sea samples in large numbers (Wiese et al., 2015). Thus, our results potentially provide a new tool to assess this as yet unexplored source of information in order to reconstruct the distribution and dispersal of the Atelostomata in the deep-sea through time.

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2.10. Appendix. Supplementary data

Superorder Atelostomata von Zittel, 1879			*i) Ornamentation of the wedges close to the base*	*ii) Absence/presence of thorns*
Order Spatangoida L. Agassiz, 1840				
	Family Hemiasteridae H. L. Clark, 1917	*Holanthus expergitus* (Lovén, 1874)	none & pustule-like surface	none & occasional thorns
Suborder Micrasterina Fischer, 1966	Family Micrasteridae Lambert, 1920	*Isopatagus obovatus* Mortensen, 1948	none	none & occasional thorns
	Family Aeropsidae Lambert, 1896	*Aeropsis fulva* (A. Agassiz, 1898)	none	none
Suborder Brissidina Stockley, Smith, Littlewood & MacKenzie-Dodds, 2005	Family Loveniidae Lambert, 1905	*Breynia australasiae* (Leach, 1815)	pustule-like surface	none & occasional thorns
		Echinocardium cordatum (Pennant, 1777)	serrated	none
		Echinocardium mediteraneum (Forbes, 1844)	none	none
		Lovenia elongata (Gray, 1845)	none	none & occasional thorns
		Lovenia subcarinata Gray, 1851	none	none & occasional thorns
	Family Spatangidae Gray, 1825	*Spatangus capensis* Döderlein, 1905	none & pustule-like surface	none
		Spatangus purpureus Müller, 1776	none	none & occasional thorns
		Spatangus raschi Lovén, 1870	serration of vertical thorns	none & occasional thorns
	Family Maretiidae Lambert, 1905	*Granobrissoides hirsutus* (Mortensen, 1950)	none	none & occasional thorns
		Gymnopatagus magnus A. Agassiz & H.L. Clark, 1907	none	occasional thorns
		Homolampas sp.	none	none & occasional thorns
		Maretia planulata (Lamarck, 1816)	none	none & occasional thorns
		Nacospatangus laevis (H.L. Clark, 1917)	none & pustule-like surface	none & occasional thorns
		Nacospatangus tylota (H.L. Clark, 1917)	none & pustule-like surface	none
		Spatagobrissus mirabilis H.L. Clark, 1923	pustule-like surface	none
	Family Palaeotropidae Lambert, 1896	*Paleotrema loveni* (A. Agassiz, 1879)	none	none & occasional thorns
	Family Eurypatagidae Kroh, 2007	*Eurypatagus ovalis* Mortensen, 1948	none	none & occasional thorns
		Eurypatagus parvituberculatus (H.L. Clark, 1924)	none	none
		Linopneustes fragilis (de Meijere, 1903)	none	none & occasional thorns
		Linopneustes longispinus (A. Agassiz, 1878)	none	none & occasional thorns
		Linopneustes murrayi (A. Agassiz, 1879)	none	occasional thorns
		Paramaretia multituberculata Mortensen, 1950	none	none
	Family Brissidae Gray, 1855	*Anametalia regularis* (H.L. Clark, 1925)	none	none
		Brissopsis lyrifera (Forbes, 1841)	none	none
		Brissus agassizii Döderlein, 1885	pustule-like surface	none
		Brissus latecarinatus (Leske, 1778)	none & pustule-like surface	none
		Brissus obesus Verrill, 1867	none & pustule-like surface	none
		Meoma ventricosa grandis Gray, 1851	none	none
		Meoma ventricosa ventricosa (Lamarck, 1816)	none	none
		Metalia nobilis Verrill, 1867	none & pustule-like surface	none
		Plagiobrissus grandis (Gmelin, 1791)	none & pustule-like surface	none & occasional thorns
		Rhynobrissus hemiasteroides A. Agassiz, 1879	pustule-like surface	none
		Rhynobrissus pyramidalis A. Agassiz, 1872	none	occasional thorns
Suborder Paleopneustina Markov & Solovjev, 2001	Family Schizasteridae Lambert, 1905	*Abatus cavernosus* (Philippi, 1845)	none & serration of vertical thorns	none
		Abatus cordatus (Verrill, 1876)	none & serration of vertical thorns	none & occasional thorns

Table A 1. List of investigated taxa and summary of the results.

Superorder Atelostomata von Zittel, 1879			*i) Ornamentation of the wedges*	
Order Spatangoida L. Agassiz, 1840			*close to the base*	*ii) Absence/presence of thorns*
		Brisaster capensis (Studer, 1880)	none & pustule-like surface	none
		Brisaster fragilis (Düben & Koren, 1846)	none	none
		Moira atropos (Lamarck, 1816)	none	none
		Protenaster australis (Gray, 1851)	pustule-like surface	none & occasional thorns
		Schizaster compactus (Koehler, 1914)	none & pustule-like surface	none
		Schizaster edwardsi Cotteau, 1889	none	none
		Tripylaster philippii (Gray, 1851)	none & serration of vertical thorns	none
	Family Prenasteridae Lambert, 1905	*Agassizia scrobiculata* Valenciennes, 1846	none & pustule-like surface	none
		Tripylus excavatus Philippi, 1845	none	none
	Paleopneustine	*Amphipneustes lorioli Koehler, 1901*	none & serration of vertical thorns	none & occasional thorns
		Amphipneustes marsupialis (Koehler, 1926)	none & serration of vertical thorns	none
	unnamed clade	*Heterobrissus hystrix* (A. Agassiz, 1880)	none	none
	Superfamily Paleopneustidea A. Agassiz, 1904			
	Family Paleopneustidea A. Agassiz, 1904	*Paleopneustes cristatus* A. Agassiz, 1873	none	occasional thorns
		Plesiozonus diomedeae Mortensen, 1948	none	none
	Family Pericosmidae Lambert, 1905	*Faorina chinensis* Gray, 1851	none	none
		Pericosmus akabanus Mortensen, 1939	none	none
		Pericosmus macronesius Koehler, 1914	none	none & occasional thorns
Order Holasteroida Durham & Melville, 1957				
Infraorder Urechinina Duncan, 1889	Family Plexechinidae Mooi & David, 1996	*Plexechinus spectabilis* Mortensen, 1948	none	occasional thorns
	Family Corystusidae Foster & Philip, 1978	*Corystus relictus* (de Meijere, 1903)	none	none
	Family Pourtalesiidae A. Agassiz, 1881	*Ceratophysa ceratopyga valvaecristata* Mironov, 1976	none	none
		Ceratophysa rosea (A. Agassiz, 1879)	none	none
		Cystocrepis setigera (A. Agassiz, 1898)	none	none & occasional thorns
		Echinocrepis rostrata Mironov, 1973	none	none & occasional thorns
		Echinosigra (*Echinogutta*) *amphora* Mironov, 1974	none	none & occasional thorns
		Echinosigra (*Echinosigra*) *phiale* (Thomson, 1873)	none	none & occasional thorns
		Echinosigra (*Echinosigra*) *vityazi* Mironov 1997	none	none
		Pourtalesia heptneri Mironov, 1978	none	none
		Pourtalesia jeffreysi Thomson, 1873	none	none
		Pourtalesia laguncula A. Agassiz, 1879	none	none & occasional thorns
		Pourtalesia thomsoni Mironov, 1976	none	none & occasional thorns
	Family Urechinidae Duncan, 1889	*Cystechinus loveni* A. Agassiz, 1898	none	none
		Pilematechinus vesica (A. Agassiz, 1879)	none	none
		Urechinus naresianus A. Agassiz, 1879	none	none
	Family Carnarechinidae Mironov, 1993	*Carnarechinus clypeatus* (A. Agassiz, 1879)	none	occasional thorns
	Family Calymnidae Mortensen, 1907	*Sternopatagus sibogae* de Meijere, 1903	none	occasional thorns

Table A 2. List of investigated taxa and summary of the results (continued).

Superorder Atelostomata von Zittel, 1879	*iii) Presence of a beaded ornamentation*		*iv) Distances between* the wedges	*v) The shape of the wedges*	*vii) Perforation of the cylinder*
Order Spatangoida L. Agassiz, 1840	*base of the shaft*	*shaft*			
Holanthus expergitus (Lovén, 1874)	smooth	beaded	0.7	*Prenaster* type	helicoidal
Isopatagus obovatus Mortensen, 1948	smooth & beaded	smooth	0	*Brissus* type	helicoidal
Aeropsis fulva (A. Agassiz, 1898)	smooth	beaded	0.3	*Prenaster* type	helicoidal
Breynia australasiae (Leach, 1815)	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Echinocardium cordatum (Pennant, 1777)	beaded	smooth	0.2	*Echinocardium*type	helicoidal
Echinocardium mediteraneum (Forbes, 1844)	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Lovenia elongata (Gray, 1845)	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Lovenia subcarinata Gray, 1851	beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Spatangus capensis Döderlein, 1905	beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Spatangus purpureus Müller, 1776	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Spatangus raschi Lovén, 1870	smooth & beaded	smooth	0	*Brissus* type	helicoidal
Granobrissoides hirsutus (Mortensen, 1950)	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Gymnopatagus magnus A. Agassiz & H.L. Clark, 1907	smooth	smooth	0	*Brissus* type	helicoidal
Homolampas sp.	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Maretia planulata (Lamarck, 1816)	smooth	smooth	0	*Brissus* type	helicoidal
Nacospatangus laevis (H.L. Clark, 1917)	beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Nacospatangus tylota (H.L. Clark, 1917)	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Spatagobrissus mirabilis H.L. Clark, 1923	beaded	smooth	0	*Echinocardium* type	helicoidal
Paleotrema loveni (A. Agassiz, 1879)	smooth	smooth	0	*Brissus* type	helicoidal
Eurypatagus ovalis Mortensen, 1948	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Eurypatagus parvituberculatus (H.L. Clark, 1924)	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Linopneustes fragilis (de Meijere, 1903)	smooth	smooth	0	*Echinocardium*type	helicoidal
Linopneustes longispinus (A. Agassiz, 1878)	smooth	smooth	0	*Echinocardium*type	helicoidal
Linopneustes murrayi (A. Agassiz, 1879)	smooth	smooth	0	*Echinocardium*type	helicoidal
Paramaretia multituberculata Mortensen, 1950	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Anametalia regularis (H.L. Clark, 1925)	beaded	smooth	0	*Brissus* type	helicoidal
Brissopsis lyrifera (Forbes, 1841)	beaded	smooth	1.5	*Echinocardium* & *Prenaster* type	helicoidal
Brissus agassizii Döderlein, 1885	smooth	smooth	0.4	*Prenaster* type	helicoidal
Brissus latecarinatus (Leske, 1778)	smooth & beaded	smooth	0.3	*Brissus*, *Prenaster* type	helicoidal
Brissus obesus Verrill, 1867	smooth & beaded	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Meoma ventricosa grandis Gray, 1851	smooth & beaded	smooth	0	*Prenaster* type	helicoidal
Meoma ventricosa ventricosa (Lamarck, 1816)	smooth & beaded	smooth	1.3	*Prenaster* type	helicoidal
Metalia nobilis Verrill, 1867	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Plagiobrissus grandis (Gmelin, 1791)	smooth	smooth	0	*Echinocardium* & *Brissus* type	helicoidal
Rhynobrissus hemiasteroides A. Agassiz, 1879	smooth & beaded	smooth	0 & 0.4	*Echinocardium, Brissus & Prenaster type*	helicoidal
Rhynobrissus pyramidalis A. Agassiz, 1872	smooth	smooth	0	*Brissus* type	helicoidal
Abatus cavernosus (Philippi, 1845)	beaded	beaded at least to 1/2	0.9	*Prenaster* type	helicoidal
Abatus cordatus (Verrill, 1876)	beaded	smooth & beaded at least to 1/2	1.2	*Prenaster* type	helicoidal
Aceste bellidifera Thomson, 1877	smooth	smooth	0	*Brissus* type	helicoidal
Brisaster capensis (Studer, 1880)	beaded	smooth & beaded at least to 1/2	0.9	*Prenaster* type	helicoidal
Brisaster fragilis (Düben & Koren, 1846)	beaded	beaded at least to 1/2	0.6	*Prenaster* type	helicoidal
Moira atropos (Lamarck, 1816)	beaded	beaded at least to 1/2	0.7	*Prenaster* type	helicoidal
Protenaster australis (Gray, 1851)	smooth & beaded	smooth & beaded at least to 1/2	0.5	*Prenaster* type	helicoidal
Schizaster compactus (Koehler, 1914)	smooth & beaded	smooth & beaded at least to 1/2	0.6	*Prenaster* type	helicoidal

Table A 3. List of investigated taxa and summary of the results (continued).

Superorder Atelostomata von Zittel, 1879	*iii) Presence of a beaded ornamentation*		*iv) Distances between* the wedges	*v) The shape of the wedges*	*vii) Perforation*
Order Spatangoida L. Agassiz, 1840	*base of the shaft*	*shaft*			*of the cylinder*
Schizaster edwardsi Cotteau, 1889	beaded	beaded at least to 1/2	0.7	*Prenaster* type	helicoidal
Tripylaster philippii (Gray, 1851)	beaded	beaded at least to 1/2	1.2	*Prenaster* type	helicoidal
Agassizia scrobiculata Valenciennes, 1846	smooth	smooth	0.2	*Prenaster* type	helicoidal
Tripylus excavatus Philippi, 1845	beaded	beaded at least to 1/2	0.8	*Prenaster* type	helicoidal
Amphipneustes lorioli Koehler, 1901	beaded	beaded at least to 1/2	1.1	*Prenaster* type	helicoidal
Amphipneustes marsupialis (Koehler, 1926)	smooth	smooth	1.5	*Prenaster* type	helicoidal
Heterobrissus hystrix (A. Agassiz, 1880)	smooth	smooth	0	*Brissus* type	helicoidal
Paleopneustes cristatus A. Agassiz, 1873	smooth	beaded	0.5	*Brissus & Prenaster type*	helicoidal
Plesiozonus diomedeae Mortensen, 1948	smooth	beaded	1.4	*Prenaster* type	helicoidal
Faorina chinensis Gray, 1851	beaded	beaded at least to 1/2	0.6	*Prenaster* type	helicoidal
Pericosmus akabanus Mortensen, 1939	smooth & beaded	beaded	0.6	*Prenaster* type	helicoidal
Pericosmus macronesius Koehler, 1914	smooth & beaded	smooth & beaded at least to 1/2	0.6	*Prenaster* type	helicoidal
Order Holasteroida Durham & Melville, 1957					
Plexechinus spectabilis Mortensen, 1948	smooth	smooth	0	*Brissus* type	horizontal
Corystus relictus (de Meijere, 1903)	smooth	smooth	0	*Brissus* type	helicoidal & horizontal
Ceratophysa ceratopyga valvaecristata Mironov, 1976	smooth	smooth	0	*Brissus* type	horizontal
Ceratophysa rosea (A. Agassiz, 1879)	smooth	smooth	0.4	*Brissus* type	horizontal
Cystocrepis setigera (A. Agassiz, 1898)	smooth	smooth	0	*Brissus* type	horizontal
Echinocrepis rostrata Mironov, 1973	smooth	smooth	0	*Brissus* type	horizontal
Echinosigra (*Echinogutta*) *amphora* Mironov, 1974	smooth	smooth	0	*Brissus* type	horizontal
Echinosigra (*Echinosigra*) *phiale* (Thomson, 1873)	smooth	smooth	0	*Brissus* type	horizontal
Echinosigra (*Echinosigra*) *vityazi* Mironov 1997	smooth	smooth	0	*Brissus* type	horizontal
Pourtalesia heptneri Mironov, 1978	smooth	smooth	0	*Brissus* type	horizontal
Pourtalesia jeffreysi Thomson, 1873	smooth	smooth	0	*Echinocardium & Brissus* type	horizontal
Pourtalesia laguncula A. Agassiz, 1879	smooth	smooth	0	*Brissus* type	horizontal
Pourtalesia thomsoni Mironov, 1976	smooth	smooth	0	*Brissus* type	horizontal
Cystechinus loveni A. Agassiz, 1898	smooth	smooth	0	*Brissus* type	horizontal
Pilematechinus vesica (A. Agassiz, 1879)	smooth	smooth	0	*Brissus* type	horizontal
Urechinus naresianus A. Agassiz, 1879	smooth	smooth	0	*Brissus* type	horizontal
Carnarechinus clypeatus (A. Agassiz, 1879)	smooth	smooth	0	*Brissus* type	horizontal
Sternopatagus sibogae de Meijere, 1903	smooth	smooth	0	*Brissus* type	horizontal

Table A 4. List of investigated taxa and summary of the results (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
	Family Hemiasteridae H. L. Clark, 1917	*Holanthus expergitus* (Lovén, 1874)	8	71.2
			8	84.5
			9	100.3
			10	122.5
			12	116.4
			13	144.9
			16	155
Suborder Micrasterina Fischer, 1966	Family Micrasteridae Lambert, 1920	*Isopatagus obovatus* Mortensen, 1948	9	157.5
			11	178.8
			14	156.3
	Family Aeropsidae Lambe, 1896	*Aeropsis fulva* (A. Agassiz, 1898)	12	138.5
			12	157.5
			13	223
Suborder Brissidina Stockley, Smith, Littlewood & MacKenzie-Dodds, 2005	Family Loveniidae Lambert, 1905	*Breynia australasiae* (Leach, 1815)	20	213.9
			21	186.1
			23	253
			27	253.7
			27	392.5
			28	386.4
		Echinocardium cordatum (Pennant, 1777)	32	241.6
			34	278.5
			45	283
		Echinocardium mediteraneum (Forbes, 1844)	13	76.8
			15	80.8
			28	174.9
		Lovenia elongata (Gray, 1845)	20	172.9
		Lovenia subcarinata Gray, 1851	21	135.1
			25	211.9
			27	234.2
	Family Spatangidae Gray, 1825	*Spatangus capensis* Döderlein, 1905	21	185.1
			22	195.5
			27	403.5
			31	317.1
			33	320
			34	325.8
			36	320
		Spatangus purpureus Müller, 1776	10	91.9
			12	106
			12	119.5
			12	193.4
			13	212.6
			14	169.3
			14	236.8
			15	117
			15	137.3
			15	154.2
			15	234

Table A 5. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (μm)
Order Spatangoida L. Agassiz, 1840				
			16	163.3
			16	234.6
			16	317.7
			17	144.1
			17	172.2
			17	243.7
			19	177.4
			19	384.9
			19	418.7
			20	219.3
			20	305.6
			20	406.8
			21	240.3
			22	235.8
			23	208.8
			23	274.6
			23	395.9
			24	281.9
			24	293.8
			25	241.9
			25	252.1
			25	260.7
			25	346.9
			26	306.4
			27	264.3
			28	273
			29	279.6
			34	290.5
		Spatangus raschi Lovén, 1870	8	73.1
	Family Maretiidae Lambert, 1905	*Gymnopatagus magnus* A. Agassiz & H.L. Clark, 1907	14	182
			15	166.9
			15	187.2
			16	190.9
			22	432.7
			31	536.2
		Granobrissoides hirsutus (Mortensen, 1950)	16	162.6
			16	167.1
			28	347.5
			28	411.7
			32	404.3
		Homolampas sp.	12	110.1
			12	110.4
			13	138.3
			13	149.2
		Maretia planulata (Lamarck, 1816)	15	162.6
			17	173.9
			18	230.3
			19	176

Table A 6. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (μm)
Order Spatangoida L. Agassiz, 1840				
			20	239.7
		Nacospatangus laevis (H.L. Clark, 1917)	12	99.8
			13	93
			14	128.5
			18	142.6
			19	158.6
			20	121.4
			21	156.6
		Nacospatangus tylota (H.L. Clark, 1917)	13	66.1
			14	74.9
			22	237.4
			22	248.1
			24	183
		Spatagobrissus mirabilis H.L. Clark, 1923	31	457.2
			32	542.3
			36	431.3
			38	438.7
			45	445.1
	Family Palaeotropidae Lambert, 1896	*Paleotrema loveni* (A. Agassiz, 1879)	9	93.7
	Family Eurypatagidae Kroh, 2007	*Eurypatagus ovalis* Mortensen, 1948	19	290.6
			20	265
			22	274.7
			23	321.2
		Eurypatagus parvituberculatus (H.L. Clark, 1924)	16	133.7
			18	248.7
			19	258.9
			19	323.8
			21	149.6
			23	319.3
		Linopneustes fragilis (de Meijere, 1903)	18	213.1
			23	199.4
			24	268
			25	282.7
			35	286.9
		Linopneustes longispinus (A. Agassiz, 1878)	28	474.2
			28	457.6
		Linopneustes murrayi (A. Agassiz, 1879)	19	182.9
			23	236
			30	312.1
			30	385.9
			31	265.6
			37	309.5
		Paramaretia multituberculata Mortensen, 1950	31	320.6
			32	463.4
	Family Brissidae Gray, 1855	*Anametalia regularis* (H.L. Clark, 1925)	13	76.5
			15	128.6

Table A 7. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
			17	145.4
			22	236.1
			25	177.5
			26	212.5
			26	395.3
		Brissus agassizii Döderlein, 1885	34	433.9
			37	290.3
			42	389.9
			48	451.9
		Brissus latecarinatus (Leske, 1778)	19	173.9
			25	371.4
		Brissus obesus Verrill, 1867	15	124.1
			21	220.7
			21	230.8
		Meoma ventricosa grandis Gray, 1851	23	205.2
			24	146.4
			24	269.7
			26	245.2
			29	179.8
			33	410.7
		Meoma ventricosa ventricosa (Lamarck, 1816)	21	228.5
			23	209.6
			27	277.2
			27	364.5
			30	325
			31	333.6
			35	340.1
			35	430.3
			38	527.2
			41	424.8
		Metalia nobilis Verrill, 1867	12	108.3
			13	112.3
			15	175.3
			21	198.5
			23	188.4
			23	224.1
			24	300.7
		Plagiobrissus grandis (Gmelin, 1791)	22	219.8
			26	251.4
			27	282.8
			27	379.1
			31	406.3
			33	314.6
		Rhynobrissus hemiasteroides A. Agassiz, 1879	15	151.6
			17	97.1
			21	175.1
			26	189
			28	201.7
			31	267.9

Table A 8. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
		Rhynobrissus pyramidalis A. Agassiz, 1872	33	251.8
			24	169.5
			25	147
			27	171.4
			29	171.3
Suborder Paleopneustina Markov & Solovjev, 2001	Family Schizasteridae Lambert, 1905	*Abatus cavernosus* (Philippi, 1845)	19	341.6
			27	233.3
			37	287.2
		Abatus cordatus (Verrill, 1876)	22	202.5
			24	220
			28	213.6
			28	302.6
		Aceste bellidifera Thomson, 1877	12	140
			16	187.1
			22	216.9
			22	224.2
			24	223.9
		Brisaster capensis (Studer, 1880)	12	99.2
			18	117.9
			18	125.6
		Brisaster fragilis (Düben & Koren, 1846)	16	92.3
			22	164.2
			22	203.1
		Moira atropos (Lamarck, 1816)	15	109
			15	121.5
			17	117.5
			20	126.4
			20	149.9
			20	174.1
		Protenaster australis (Gray, 1851)	14	99.4
			14	369.3
			24	198.9
		Schizaster compactus (Koehler, 1914)	18	117.1
			24	165.5
			31	224.6
		Schizaster edwardsi Cotteau, 1889	15	175.5
			17	182.2
			17	195.3
			17	196.2
			20	182.6
		Tripylaster philippii (Gray, 1851)	19	283.2
			20	128.5
			22	238.2
			24	167
			27	213.5
			28	194.9
	Family Prenasteridae Lambert, 1905	*Agassizia scrobiculata* Valenciennes, 1846	10	98.1
			12	98.9

Table A 9. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
			14	129
			15	164.3
		Tripylus excavatus Philippi, 1845	10	101
			10	101.3
			18	144.7
			19	177.8
			22	199
			22	199.9
	Paleopneustine unnamed clade	*Amphipneustes lorioli* Koehler, 1901	20	163.3
			22	190.7
			22	191
			27	192.7
			32	264.1
			32	250.9
			33	252.4
			33	282
			41	282.8
		*Amphipneustes marsupialis (*Koehler, 1926)	23	385
			24	258.5
			28	279
			30	342.8
			32	358.2
		Heterobrissus hystrix (A. Agassiz, 1880)	18	317.1
			18	342.1
			33	567.2
			29	524.4
			43	1025.6
			37	796.2
			47	815.4
	Superfamily Paleopneustidea A. Agassiz, 1904			
	Family Paleopneustidae A. Agassiz, 1904	*Paleopneustes cristatus* A. Agassiz, 1873	18	318.1
			19	359.2
			20	313.4
			21	429.7
			23	408
		Plesiozonus diomedeae Mortensen, 1948	11	111.9
			13	122.6
			14	132.5
			14	141.3
			14	151.9
			19	132
			19	153.6
	Family Pericosmidae Lambert. 1905	*Faorina chinensis* Gray, 1851	15	158.1
			16	139.5
			16	144.5
			16	146.9
			16	154.5

Table A10. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
			19	188.1
			21	169.4
			21	199.7
			22	241.9
		Pericosmus macronesius Koehler, 1914	13	100.2
			18	168.4
			18	169.5
			18	174.3
			19	222.7
Order Holasteroida Durham & Melville, 1957				
Infraorder Urechinina Duncan, 1889	Family Plexechinidae Mooi & David, 1996	*Plexechinus spectabilis* Mortensen, 1948	11	123.9
			13	159.1
			13	203.9
			15	175.3
	Family Corystusidae Foster & Philip, 1978	*Corystus relictus* (de Meijere, 1903)	9	124.5
	Family Pourtalesiidae A. Agassiz, 1881	*Ceratophysa ceratopyga valvaecristata* Mironov, 1976	11	140.1
			12	176.6
			15	176.2
			19	136.3
			16	163.7
			23	183.1
		Ceratophysa rosea (A. Agassiz. 1879)	14	146.1
		Cystocrepis setigera (A. Agassiz. 1898)	8	124.7
			9	246
		Echinocrepis rostrata Mironov, 1973	7	87.7
			8	88.2
			8	92.7
			8	93.5
			16	109.2
			25	178.6
			32	216.3
		Echinosigra (Echinogutta) amphora Mironov, 1974	15	155.9
			17	221.3
			18	173.9
			18	232
		Echinosigra (Echinosigra) phiale (Thomson, 1873)	9	80.2
			12	86.5
		Echinosigra (Echinosigra) vityazi Mironov, 1997	8	91.1
			9	91.6
			10	108.2
		Pourtalesia heptneri Mironov, 1978	9	105.3
			10	102.1
			10	109.8
			10	117.1

Table A11. List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879			number of wedges	diameter of the spines (µm)
Order Spatangoida L. Agassiz, 1840				
			11	125
			12	121.7
			12	125.1
			12	149.6
			19	188.1
			21	169.4
			21	199.7
			22	241.9
		Pericosmus macronesius Koehler, 1914	13	100.2
			18	168.4
			18	169.5
			18	174.3
			19	222.7
Order Holasteroida Durham & Melville, 1957				
Infraorder Urechinina Duncan, 1889	Family Plexechinidae Mooi & David, 1996	*Plexechinus spectabilis* Mortensen, 1948	11	123.9
			13	159.1
			13	203.9
			15	175.3
	Family Corystusidae Foster & Philip, 1978	*Corystus relictus* (de Meijere, 1903)	9	124.5
	Family Pourtalesiidae A. Agassiz, 1881	*Ceratophysa ceratopyga valvaecristata* Mironov, 1976	11	140.1
			12	176.6
			15	176.2
			19	136.3
			16	163.7
			23	183.1
		Ceratophysa rosea (A. Agassiz. 1879)	14	146.1
		Cystocrepis setigera (A. Agassiz. 1898)	8	124.7
			9	246
		Echinocrepis rostrata Mironov, 1973	7	87.7
			8	88.2
			8	92.7
			8	93.5
			16	109.2
			25	178.6
			32	216.3
		Echinosigra (*Echinogutta*) *amphora* Mironov, 1974	15	155.9
			17	221.3
			18	173.9
			18	232
		Echinosigra (*Echinosigra*) *phiale* (Thomson, 1873)	9	80.2
			12	86.5
		Echinosigra (*Echinosigra*) *vityazi* Mironov, 1997	8	91.1
			9	91.6
			10	108.2
		Pourtalesia heptneri Mironov, 1978	9	105.3
			10	102.1

Table A12 List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Superorder Atelostomata von Zittel, 1879		number of wedges	diameter of the spines (μm)
Order Holasteroida Durham & Melville, 1957			
		10	109.8
		10	117.1
		11	125
		11	125
		12	121.7
		12	125.1
		12	149.6

Table A13 List of investigated taxa, shown are number of wedges and diameter for each anaylsed spine (continued).

Holanthus expergitus (Lovén, 1874)	ZMUC-ECH-651, ZMUC-ECH-652	[n=12 (external: n=11, internal: n=1)]	Ingolf station 37, Davis Strait, North Atlantic
Isopatagus obovatus Mortensen, 1948	ZMUC-ECH-253 (Paratype)	[n=7 (external: n=4, internal: n=3)]	Sulu Sea, 09°38`N, 121°11` E
Aeropsis fulva (A. Agassiz, 1898)	ZMUC-ECH-649	[n=15 (external: n=3, internal: n=2)]	waters between Acapulco & Panama, 09°23`N, 89°32` W
Breynia australasiae (Leach, 1815)	ZMUC-ECH-610, ZMUC-ECH-611	[n=27 (external: n=19, internal: n=9)]	Bowen, Queensland
Echinocardium cordatum (Pennant, 1777)	GZG.INV.78890-97 (from a single specimen)	[n=14 (external: n=7, internal: n=7)]	North Atlantic Ocean, Bretagne, France
Echinocardium mediteraneum (Forbes, 1844)	ZMUC-ECH-622	[n=11 (external: n=7, internal: n=4)]	Arcachon, France
Lovenia elongata (Gray, 1845)	ZMUC-ECH-645, ZMUC-ECH-646	[n=29 (external: n=16, internal: n=13)]	Bowen, Queensland
Lovenia subcarinata Gray, 1851	ZMUC-ECH-647, ZMUC-ECH-648	[n=23 (external: n=13, internal: n=10)]	Koh Kood, Siam
Spatangus capensis Döderlein, 1905	ZMUC-ECH-642	[n=15 (external: n=10, internal: n=5)]	False Bay, South Africa
Spatangus purpureus Müller, 1776	ZMUC-ECH-623 - 625; ZMUC-ECH-657 - 660	[n=64 (external: n=55, internal: n=19)]	Kattegat, North Sea / Plymouth Great Britain
Spatangus raschi Lovén, 1870	ZMB.Ech 4806_1 - 4806_10 (from a single specimen)	[n=8 (external: n=5, internal: n=3)]	locality unknown
Granobrissoides hirsutus (Mortensen, 1950)	ZMUC-ECH-182 (Holotype)	[n=10 (external: n=7, internal: n=3)] [inside: n=3 (la=1, pl=2)]	SE of Lakes (+) Entrance,Victoria
Gymnopatagus magnus A. Agassiz & H.L. Clark, 1907	ZMUC-ECH-641	[n=10 (external: n=6, internal: n=4)]	Philippines, 13°36`N, 123°40`E
Homolampas sp.	ZMUC-ECH-629	[n=7 (external: n=4, internal: n=3)] [inside: n=3 (ap=3)]	Kei Islands (Station 46), Indonesia
Maretia planulata (Lamarck, 1816)	ZMUC-ECH-614	[n=11 (external: n=6, internal: n=5)]	Koh Lan, Siam
Nacospatangus laevis (H.L. Clark, 1917)	ZMUC-ECH-631	[n=14 (external: n=10, internal: n=4)]	Cortez Bank, California, USA
Nacospatangus tylota (H.L. Clark, 1917)	ZMUC-ECH-632	[n=11 (external: n=7, internal: n=4)]	Kei Islands (Station 32), Indonesia
Spatagobrissus mirabilis H.L. Clark, 1923	ZMB.Ech-7417	[n=11 (external: n=6, internal: n=5)]	Cape Town, South Africa
Paleotrema loveni (A. Agassiz, 1879)	ZMUC-ECH-630	[n=12 (external: n=8, internal: n=4)]	Philippines
Eurypatagus ovalis Mortensen, 1948	ZMUC-ECH-540 (Paratype)	[n=8 (external: n=6, internal: n=2)]	Philippines, 13°20`N, 123°14`E
Eurypatagus parvituberculatus (H.L. Clark, 1924)	ZMUC-ECH-633	[n=10 (external: n=7, internal: n=3)]	Durban, South Africa
Linopneustes fragilis (de Meijere, 1903)	ZMUC-ECH-643	[n=11 (external: n=9, internal: n=2)]	Philippines
Linopneustes longispinus (A. Agassiz, 1878)	ZMB.Ech 3281_1 - 3 (from a single specimen)	[n=4 (external: n=3, internal: n=1)]	western Atlantic Ocean, Bahamas Islands
Linopneustes murrayi (A. Agassiz, 1879)	ZMUC-ECH-668	[n=13 (external: n=9, internal: n=4)]	Sagami Sea
Paramaretia multituberculata Mortensen, 1950	ZMUC-ECH-41 (Lot of Coptype)	[n=12 (external: n=7, internal: n=5)]	Eastern Australia, 38°10`S, 149°25`E
Anametalia regularis (H.L. Clark, 1925)	ZMUC-ECH-644	[n=8 (external: n=4, internal: n=4)]	Java Sea
Brissopsis lyrifera (Forbes, 1841)	ZMUC-ECH-606, ZMU-ECH-607	[n=20 (external: n=11, internal: n=9)]	Skagen, Denmark
Brissus agassizii Döderlein, 1885	GZG.INV.78898 – 78903 (from a single specimen)	[n=9 (external: n=6, internal: n=3)]	Tasman Sea, Sydney, Australia
Brissus latecarinatus (Leske, 1778)	ZMUC-ECH-602	[n=12 (external: n=7, internal: n=5)]	Hilo, Hawaii, USA
Brissus obesus Verrill, 1867	ZMUC-ECH-626, ZMUC-ECH-656	[n=30 (external: n=21, internal: n=9)]	Taboguilla, Panama
Meoma ventricosa grandis Gray, 1851	ZMUC-ECH- 600	[n=15 (external: n=10, internal: n=5)]	Torolita, Panama
Meoma ventricosa ventricosa (Lamarck, 1816)	ZMUC-ECH-619	[n=13 (external: n=9, internal: n=4)]	Loango Key, Carribean Sea

Table A14. List of taxa, collection numbers and ammount of investigated spines, separated in spines for external analyses and internal and provenance (with coordinates, if available) of the specimens.

Metalia nobilis Verrill, 1867	ZMUC-ECH-620, ZMUC-ECH-664	[n=26 (external: n=15, internal: n=11)]	Taboguilla, Panama
Plagiobrissus grandis (Gmelin, 1791)	ZMUC-ECH-621	[n=13 (external: n=8, internal: n=5)]	Bahama Islands
Rhynobrissus hemiasteroides A. Agassiz, 1879	ZMUC-ECH-628	[n=13 (external: n=9, internal: n=4)]	Bowen, Queensland
Rhynobrissus pyramidalis A. Agassiz, 1872	GZG.INV.78903 - 78906 (from a single specimen)	[n=8 (external: n=4, internal: n=4)]	West Pacific, Taiwan
Abatus cavernosus (Philippi, 1845)	ZMB.Ech 7215_1 - 7215_4 (from a single specimen)	[n=5 (external: n=4, internal: n=1)]	southern Indian Ocean, Kerguelen Islands
Abatus cordatus (Verrill, 1876)	ZMB.Ech 2230_1 - 2230_8 (from a single specimen)	[n=10 (external: n=8, internal: n=2)]	southern Indian Ocean, Kerguelen Islands
Aceste bellidifera Thomson, 1877	ZMUC-ECH-653	[n=10 (external: n=8, internal: n=2)]	Albatross St. 2043, NE Coast of the USA,
Brisaster capensis (Studer, 1880)	ZMUC-ECH-650, ZMUC-ECH-667	[n=16 (external: n=13, internal: n=3)]	Cape Peninsula, South Africa, 34°17`S, 17°58`E
Brisaster fragilis (Düben & Koren, 1846)	ZMB.Ech 2766_1 - 2766_6 (from a single specimen)	[n=9 (external: n=6, internal: n=3)]	North Atlantic, NW of Tromsø, Norway
Moira atropos (Lamarck, 1816)	ZMUC-ECH-613, ZMUC-ECH-636	[n=27 (external: n=16, internal: n=11)]	Saint Thomas, Carribean Sea
Protenaster australis (Gray, 1851)	ZMUC-ECH-639	[n=14 (external: n=7, internal: n=7)]	Ellenbrook Beach, Western Australia
Schizaster compactus (Koehler, 1914)	ZMUC-ECH-638	[n=14 (external: n=8, internal: n=6)]	Thirumalai Vasal, Madras, India
Schizaster edwardsi Cotteau, 1889	ZMUC-ECH-635	[n=13 (external: n=8, internal: n=5)]	waters around Guinea, 06°17`N, 3°24`E
Tripylaster philippii (Gray, 1851)	ZMUC-ECH-612	[n=14 (external: n=8, internal: n=6)]	Patagonia, Argentina, 42°42`S, 64°48`W
Agassizia scrobiculata Valenciennes, 1846	ZMUC-ECH-634	[n=10 (external: n=6, internal: n=4)]	Taboguilla, Panama
Tripylus excavatus Philippi, 1845	ZMUC-ECH-637	[n=16 (external: n=11, internal: n=5)]	Falkland Islands, South Atlantic Ocean
Amphipneustes lorioli Koehler, 1901	ZMUC-ECH-666	[n=12 (external: n=11, internal: n=1)]	Discovery St. 1652, 75°56`S, 178°35`W
Amphipneustes marsupialis (Koehler, 1926)	ZMUC-ECH-640	[n=8 (external: n=6, internal: n=2)]	Antarctic
Heterobrissus hystrix (A. Agassiz, 1880)	ZMUC-ECH-627	[n=14 (external: n=9, internal: n=5)]	Frederiksted, Saint Crox, Carribean Sea
Paleopneustes cristatus A. Agassiz, 1873	ZMUC-ECH-113 (Syntype)	[n=12 (external: n=8, internal: n=4)]	Barbados
Plesiozonus diomedeae Mortensen, 1948	ZMUC-ECH-135 (Paratype)	[n=15 (external: n=10, internal: n=5)]	Philippines, 06°52`N, 126°14`E
Faorina chinensis Gray, 1851	ZMUC-ECH-608	[n=14 (external: n=9, internal: n=5)]	Nha Trang, Vietnam
Pericosmus akabanus Mortensen, 1939	ZMUC-ECH-60 (Syntype)	[n=12 (external: n=10, internal: n=2)]	Gulf of Aqaba, Red Sea
Pericosmus macronesius Koehler, 1914	ZMUC-ECH-615	[n=10 (external: n=6, internal: n=4)]	Port Louis, Mauritius
Plexechinus spectabilis Mortensen, 1948	ZMUC-ECH-617 (Syntype)	[n=9 (external: n=7, internal: n=2)]	Sulu Sea, western North Pacific, 08°13`N, 120°37`E
Corystus relictus (de Meijere, 1903)	ZMUC-ECH-604, ZMUC-ECH-605	[n=25 (external: n=16, internal: n=9)]	Kiushiu Japan
Ceratophysa ceratopyga valvaecristata Mironov, 1976	ZMB.Ech-7418	[n=12 (external: n=6, internal: n=6)]	voyage of the RV Sonne - 223, St. 7-11, 43°2,66` - 43°1,75`N, 152°59,46` - 52°58,59`W.
Ceratophysa rosea (A. Agassiz, 1879)	ZMB.Ech-7419	[n=2 (external: n=1, internal: n=1)]	voyage of the RV Dmitry Mendeleyev, St. 20, 19°37` - 19°36`N, 62°20` - 62°21`W
Cystocrepis setigera (A. Agassiz, 1898)	ZMB.Ech-7420	[n=4 (external: n=2, internal: n=2)]	voyage of the RV Dmitry Mendeleyev, St. 1648, 10°46,2`S, 79°00,8`W
Echinocrepis rostrata Mironov, 1973	ZMB.Ech-7422	[n=13 (external: n=11, internal: n=2)]	voyage of the RV Vityaz, St. 5605, 46°10`N, 153°07`E
Echinosigra (Echinogutta) amphora Mironov, 1974	ZMB.Ech-7423	[n=11 (external: n=7, internal: n=4)]	voyage of the RV Vityaz, St. 3166, 44°43`N, 153°49`E

Table A15. List of taxa, collection numbers and ammount of investigated spines, separated in spines for external analyses and internal and provenance (with coordinates, if available) of the specimens (continued).

Cystocrepis setigera (A. Agassiz, 1898)	ZMB.Ech-7420	[n=4 (external: n=2, internal: n=2)]	voyage of the RV Dmitry Mendeleyev, St. 1648, 10°46,2`S, 79°00,8`W

Echinocrepis rostrata Mironov, 1973	ZMB.Ech-7422	[n=13 (external: n=11, internal: n=2)]	voyage of the RV Vityaz, St. 5605, 46°10`N, 153°07`E
Echinosigra (Echinogutta) amphora Mironov, 1974	ZMB.Ech-7423	[n=11 (external: n=7, internal: n=4)]	voyage of the RV Vityaz, St. 3166, 44°43`N, 153°49`E
Echinosigra (Echinosigra) phiale (Thomson, 1873)	ZMB.Ech 5436_1 - 5436_3 (from a single specimen), ZMUC-ECH-665	[n=7 (external: n=5, internal: n=2)]	Antarctic Ocean, Kaiser Wilhelm II Land, eastern Antarctica / Tasman Sea, Sydney, Australia, 45°57`S, 164°32`E
Echinosigra (Echinosigra) vityazi Mironov, 1997	ZMB.Ech-7421	[n=12 (external: n=4, internal: n=8)]	voyage of the RV Vityaz, St. 4954, 09°34,9`N, 90°50,4`E
Pourtalesia heptneri Mironov, 1978	ZMUC-ECH-655 Paratype	[n=19 (external: n=9, internal: n=10)]	voyage of the RV Vityaz, St. 7271, 05°34,6` - 05°39,9`S, 131°06,2` - 131°06,5`E / voyage of the RV Vityaz, St. 7271, 05°37`S, 131°07,5`E
Pourtalesia jeffreysi Thomson, 1873	ZMB.Ech 4462_1 - 4462_2 (from a single specimen), ZMUC-ECH-609	[n=10 (external: n=6, internal: n=4)]	SE of Jan Mayen, 70°5'N 8°26'W
Pourtalesia laguncula A. Agassiz, 1879	ZMUC-ECH-618	[n=6 (external: n=3, internal: n=3)]	Sagami Sea, Japan
Pourtalesia thomsoni Mironov, 1976	ZMUC-ECH-654	[n=12 (external: n=9, internal: n=3)]	voyage of the RV Akademik Mst. Keldysh, St. 2309, 55°13` - 55°12`N, 67°29` - 67°26`E
Cystechinus loveni A. Agassiz, 1898	ZMB.Ech-7427	[n=13 (external: n=8, internal: n=5)]	voyage of the RV Vityaz, St. 3364, 48°21`N, 168°54`E
Pilematechinus vesica (A. Agassiz, 1879)	ZMB.Ech-7428	[n=5 (external: n=4, internal: n=1)]	voyage of the RV Akademik Mst. Keldysh, St. 2306, 54°57,11` - 57°07`N, 165°49,9` - 165°51`W
Urechinus naresianus A. Agassiz, 1879	ZMB.Ech 5432_1 - 5432_4 (from a single specimen), ZMUC-ECH-616	[n=6 (external: n=5, internal: n=1)]	Antarctic Ocean, Kaiser Wilhelm II Land, eastern Antarctica / Ingolf station 37, Davis Strait, North Atlantic
Carnarechinus clypeatus (A. Agassiz, 1879)	ZMB.Ech-7429	[n=8 (external: n=5, internal: n=3)]	voyage of the RV Dmitry Mendeleyev, St. 1365, 34°25`S, 128°12`E
Sternopatagus sibogae de Meijere, 1904	ZMB.Ech-7426	[n=8 (external: n=5, internal: n=3)]	voyage of the RV Vityaz, St. 7325, 01°51,5`S, 144°40,8`E

Table A15. List of taxa, collection numbers and ammount of investigated spines, separated in spines for external analyses and internal and provenance (with coordinates, if available) of the specimens (continued).

Chapter 3

Heterochronic evolution in the Late Cretaceous echinoid *Gauthieria* (Echinoidea, Phymosomatidae)

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Abstract

Based on representatives of the Late Cretaceous genus *Gauthieria* (*Gauthieria radiata* - *Gauthieria spatulifera* - *Gauthieria princeps*), ontogenetic trajectories within the family Phymosomatidae are described for the first time. Due to shared similarities in their ontogenetic development, an intimate evolutionary relationship must be assumed. This interpretation is most supported by analyses of the development in the ambulacral plating pattern (alternation of simple plates and compound plates), which is not commonly found among the Phymosomatoidae. This pattern, however, is present among all three species during development. The developmental trajectories of 8 further characters were included in this study (arrangement of the adapical pore pairs, number of pore pairs, pore pair numbers in ambital ambulacral plates, number of interambulacral plates, peristomal opening, apical opening diameter, test height, radial ornament of the areoles). The evolution in this lineage is characterised by several different heterochronic processes, which suggest a dissociated heterochronic evolution, indicating a developmental modularity. Additionally, the systematic treatment of *G. princeps* is discussed on account of the presented results.

Keywords: Echinoidea – Phymosomatidae – ontogeny – evolution – heterochrony – Late Cretaceous

3.1. Introduction

Studies on the developmental processes of related fossil species can provide important insights into their evolutionary development and relationship. The concept of heterochrony is a classic approach in evolutionary studies (Gould, 1977; Alberch et al.,1979; Raff & Wray, 1989). It refers to a linkage between ontogeny and evolution, in other words, heterochrony refers to alternations in rates and/or timing of developmental events between ancestors and their descendants. In particular, by comparing ontogenetic trajectories of ancestors and descendants, changes in onsets, or offsets, respectively, as well as the rate of development (increase or decrease) of particular traits can be observed. These can be discriminated as either paedomorphosis (the retention of juvenile features of an ancestor in adults of the descendant, which can occur by neotony, postdisplacement or progenesis), or as peramorphosis (addition of developmental stages in the descendent compared to the ancestor, which can occur by

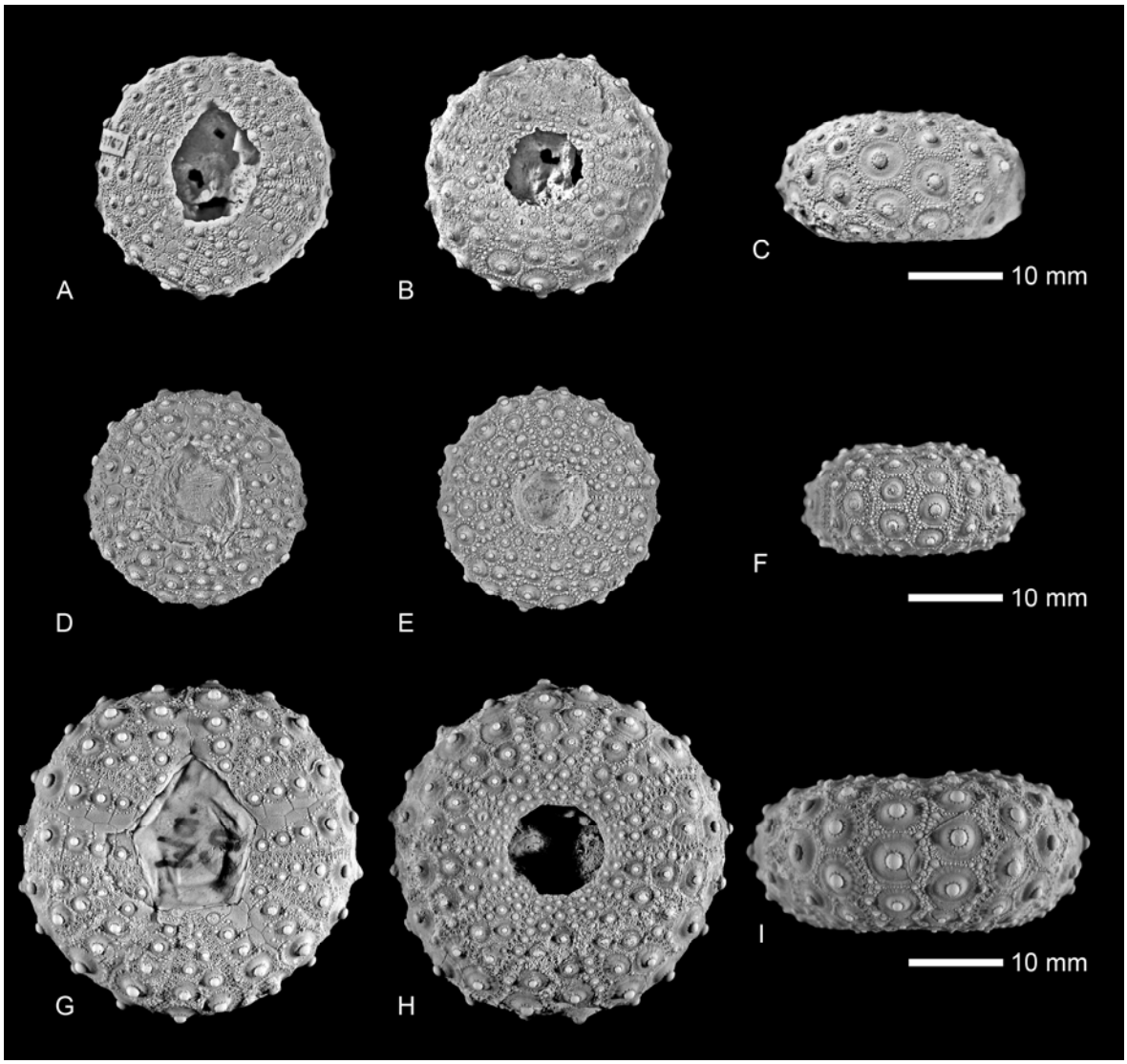


Figure 1. Large sized specimens. (A-C) *Gauthieria radiata*, *Mytiloides labiatus* Zone; early Turonian; Dover, Kent, Great Britain, BMNH E9767 (A: aboral, B: oral, C: lateral view); (D-F) *Gauthieria spatulifera*, Santonian, Kent, Great Britain, MB.E.8443 (D: aboral, E: oral, F: lateral view); (G-I) *Gauthieria princeps*, early Maastrichtian, quarry at the Waldmeisterstraße, Sassnitz, Rügen, GZG.INV.18624 (G: aboral, H: oral, I: lateral view). All specimens coated with ammonium chloride prior to photography.

acceleration, predisplacement or hypermorphosis), for a more recent review of heterochrony see Klingenberg (1998). Heterochronic evolution was described for several fossil (e.g. McNamara, 1982; Korn, 1992), including irregular echinoids (McKinney, 1984; McNamara, 1989; Ciampaglio & D'orazio, 2007). For fossil irregular echinoids numerous evolutionary lineages had been identified (e.g. Rowe, 1899; Ernst, 1972; Gale & Smith, 1982; David & Fouray, 1984; Villier et al., 2004). Phylogenetic studies on fossil regular echinoids on a species level are only very scattered (e.g. Jeffery & Emlet, 2003). This case is even worse for the family Phymosomatoidae Pomel, 1883, for which evolutionary studies are not available. Although phymosomatoid echinoids were common and widespread in Europe and western Asia in the Late Cretaceous, their value for evolutionary biology

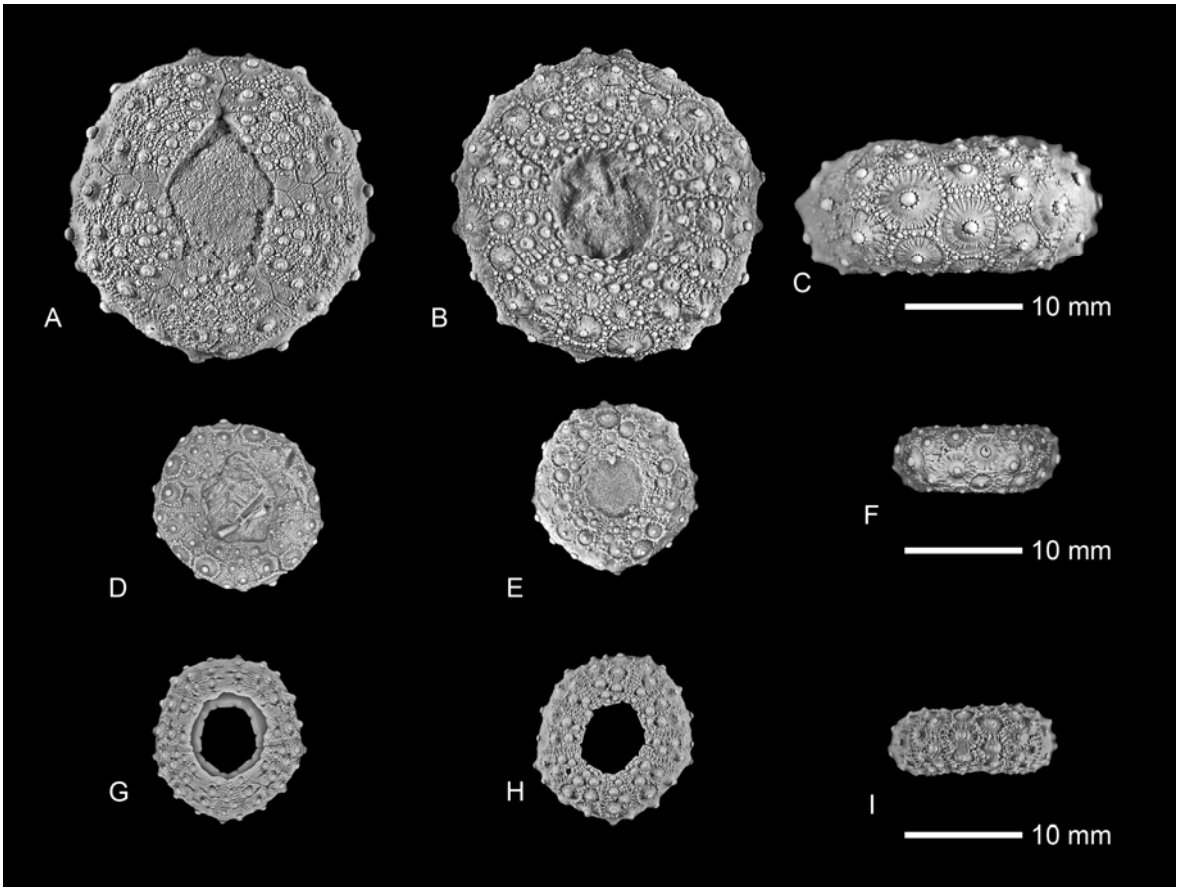


Figure 2. Small sized specimens. (A-C) *Gauthieria radiata*, late Turonian, Strehlen, Germany, MB.E.8440.5 (A: aboral, B: oral, C: lateral view); (D-E) *Gauthieria spatulifera*, *Echinocorys conica*/*Galeola papillosa* Zone, early Campanian, Germania IV quarry, Misburg, Hannover, Germany, MB.E 8565 (D: aboral, E: oral, F: lateral view); (G-I) *Gauthieria princeps*, Maastrichtian, Hemmoor quarry, Germany, GPIH04838 (G: aboral, H: oral, I: lateral view). All specimens coated with ammonium chloride prior to photography.

has not yet been tested. This might be due to their rather poor preservation potential, as for regular echinoids in general (Kier, 1977). Further problems arise because of the often rather invariate morphology in regular echinoids and their comparatively low degrees of morphological diversification (Hopkins & Smith, 2015). Additionally, their use in evolutionary studies is hampered by a number of taxonomic problems, related to the identity of certain species (e.g. Schlüter et al., 2012a; Schlüter et al., 2012b).

One of these taxonomic problems can be exemplified by small-sized representatives of *Gauthieria* Lambert, 1888 from the Late Cretaceous (Santonian to Maastrichtian) of northern Europe (compare Smith &Wright, 1996). Based on the large morphological similarity, particularly in the ambulacral plating pattern (intercalated simple elements between compound plates) and sometimes strongly radially striated peri-areole area in primary tubercles, specimens were often referred to *Gauthieria radiata* (Sorignet, 1850) (Figs. 1A-C and 2A-C) (e.g. Nestler, 1966; Geys, 1980; Kutscher, 1985c, 2003), which is in fact, common in the Turonian-Coniacian, or regarded as being very similar

to it, hence referred to *Gauthieria pseudoradiata* (Schlüter, 1899) (e.g. Ravn, 1928; Kutscher, 1985c; Smith & Wright, 1996; Jeffery, 1997; Jagt, 2000 (pars); Smith & Jeffery, 2000; see Schlüter et al., 2012b for a detailed discussion on the state of the latter species). However, it was only rarely considered that these small-sized specimens represent simply a juvenile stage of another species (Smith & Wright, 1996), and we infer that these small-sized specimens are juveniles of *Gauthieria spatulifera* (Forbes, in Dixon, 1850) (Figs. 1D-F and 2D-F) and *Gauthieria princeps* (von Hagenow, 1840) (Figs. 1G-I and 2G-I). Our assumption is based on the fact that these small-sized specimens co-occur spatially and temporally with *G. spatulifera* (Santonian-lower Campanian) and *G. princeps* (upper Campanian- upper Maastrichtian). Other species of *Gauthieria*, co-occurring with the two previous taxa, are excluded from this assumption. *Gauthieria wetherelli* (Woodward, 1856), being the only known other Santonian species from Northern Europe, is similar to *G. spatulifera*. It deviates, however, by having pore pairs, which are not inclined towards the peristome. In addition, the ambulacral plates next to the peristome show no intercalations of simple elements, unlike in *G. spatulifera* (see Smith & Wright, 1996). The very rare species *Gauthieria middletoni* (Woodward, 1856) (late Campanian; England, Norfolk) is closely similar to *Gauthieria princeps*. The only difference is the presence of enlarged secondary tubercles in the adapical interambulacral plates in G. middletoni. Due to its paucity and to the fact that *G. middletoni* is mostly not found in the same localities as *G. princeps* we do not attribute the studied small-sized specimens to *G. middletoni*. Specimens of *G. radiata* can be distinguished from *G. spatulifera* and *G. princeps* in the radial striation of the peri-areole area, which is not found in larger sized specimens of the latter species. Further they differ by the appearance of adapically biserial arranged pore pairs: pore pairs of *G. radiata* are strictly uniserial arranged but are biserial in *G. spatulifera* and *G. princeps*. Furthermore, the test size is similar in *G. radiata* and *G. spatulifera* (c. 26 mm, c. 29 mm respectively) while *G. princeps* reaches a diameters of c. 58 mm. An additional criterion to distinguish *G. spatulifera* from *G. princeps* is the diameter the peristome, which is larger and more invaginated (at comparable sizes) in *G. princeps* than in *G. spatulifera*. Essential for heterochronic analyses are phylogenetic data (McKinney & McNamara, 1991). These, however, do not exist for the Phymosomatoida, but we can assume an ancestor-descendant relationship from *G. radiata* - *G. spatulifera* - *G. princeps*, inferred by their successive stratigraphic occurrence. This study displays the first description of an ontogenetic development in species of the Phymosomatoidae and closer evolutionary relationships and development in phymosomatoid echinoids by invoking the concept of heterochrony. The comparison of the ontogenetic trajectories observed in the three species considered enables us to evaluate whether or not they are really closely related phylogenetically. If there is a phylogenetic relation among these species, there should exist shared similarities in their development.We considered nine characters (see below) and tested them for heterochronic modifications.

3.2. Material and methods

The assumption of an evolutionary relation between *G. radiata*, *G. spatulifera* and *G. princeps* was tested based on 117 specimens (*G. radiata*: 43, *G. spatulifera*: 38, *G. princeps*: 36). Most of the material originates from southern England. Additional specimens from northern Germany and northern France were included (Fig. 3, see appendix, including specimens and provenance). From deformed specimens, longest and shortest axis in diameter were taken. The averaged values of these were included in the analyses (raw data are provided as online supplementary information). Measurements were taken to the nearest 0.01 mm (digital calliper). The following traits were analysed: ambulacral plating pattern, arrangement of the adapical pore pairs, number of pore pairs, pore pair numbers in ambital ambulacral plates, number of interambulacral plates, peristomal opening, apical opening diameter, test height, radiation in the areoles.

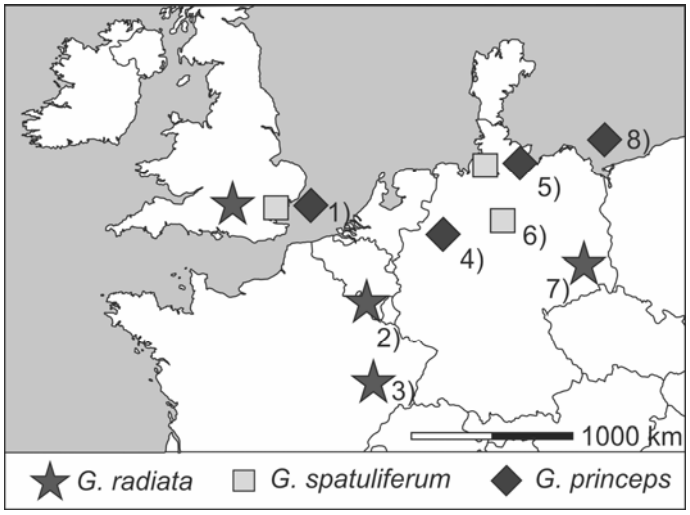


Figure 3. Geographic map, indicating the orgin of the studied material; 1) = England (refer to online appendix for detailed informations); 2) = Berzieux, Étigny France; 3) = Saint-Julien, France; 4) = Coesfeld, Germany; 5) = Hannover, Germany; 6) = Hemmoor, Lägerdorf, Germany; 7) = Strehlen, Germany; 8) = Rügen, Germany.

3.2.1. Regression analysis

Linear regressions were carried out using the software PAST (Hammer et al., 2001) to compare interspecific growth trajectories, in which morphological variables were logarithmically transformed. To give an estimate of growth trajectories for individual species and for interspecies comparisons, we used the reduced major axis regression (RMA) (McKinney & McNamara, 1991) and compared at the statistical significance level of 0.05. RMA treats size and trait variables more equally, compared to least square linear regression (McKinney & McNamara, 1991). In the latter case, the size would be treated as an "independent" variable, but size takes part in the same developmental system as the traits. Analysis of covariance (ANCOVA) was applied, calculated from least square regressions, to test for significant differences of slopes and y-intercepts between species. To study heterochronic processes, we used size (diameter) as

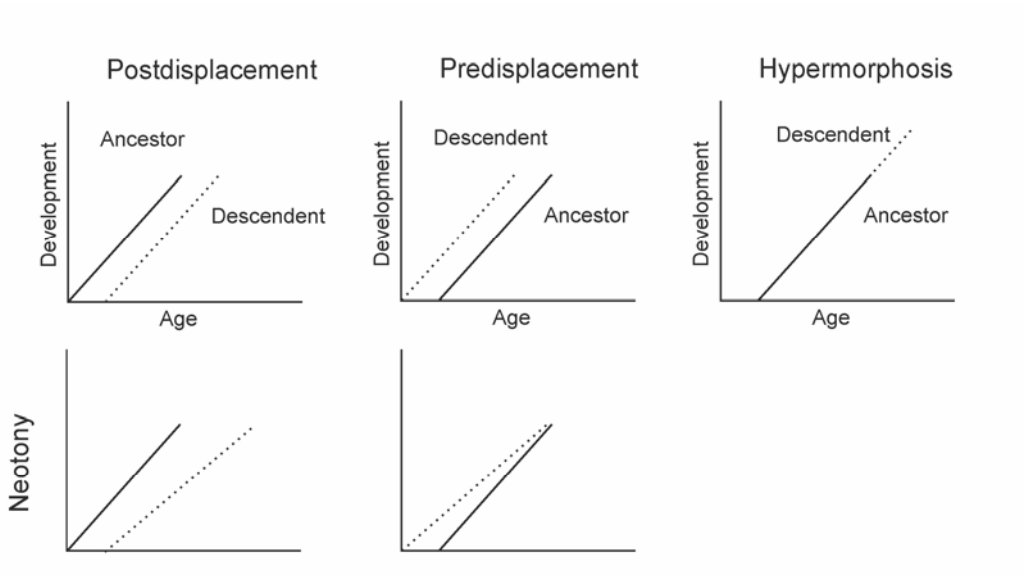


Figure 4. Illustration of heterochronic processes and the effect of pairwise combination of heterochronic processes, depicted are only the processes occurring among the here studied species, refer to Klingenberg (1998) for more details (modified after previously mentioned references).

a proxy for age, or developmental timing, which results in "allometric heterochrony" (McKinney & McNamara, 1991). In the terminology for heterochrony we have followed the concept of Alberch et al. (1979). Heterochronic processes can be combined (Fig. 3), as long as those combinations are not affecting the same trait in opposite directions (Alberch et al., 1979; Raff & Wray, 1989; Reilly et al., 1997; Klingenberg, 1998). These combinations are reasoned by the fact that more than one of the parameters of the growth function can change simultaneously, i.e. a growth trajectory can be predisplaced and accelerated (see Fig. 4). The slope of the regression lines characterises the developmental rate, while the y-intercept (value of the y-axis, where regression line crosses) indicates the displacement, as either pre- or postdisplaced, of the trajectory in relation to the particular ancestor.

3.2.2. Abbreviations

BNHM The Natural History Museum, Department of Palaeontology, London, Great Britain; MB.E. Museum für Naturkunde, Leibniz-Institut für Evolutions und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Berlin, Germany; GZG Geowissenschaftliches Zentrum der Georg-August-Universität Göttingen, Göttingen, Germany; GPIH Geologisch-Paläaontologisches Institut und Museum, Universität Hamburg, Hamburg, Germany; MNHN-F Muséum national d'Histoire naturelle, Paris, France.

3.3. Results

Ambulacral plating pattern (Figs. 5 and 6): In phymosomatoid echinoids an alternation of simple and compound plates is found generally only in the proximity of the apex. These simple elements fuse later in later stages of ontogeny to the compounded plates. In *G. radiata*, however, an alternation of simple and compound plates occurs consistently in the entire ambulacral zone throughout its ontogeny.

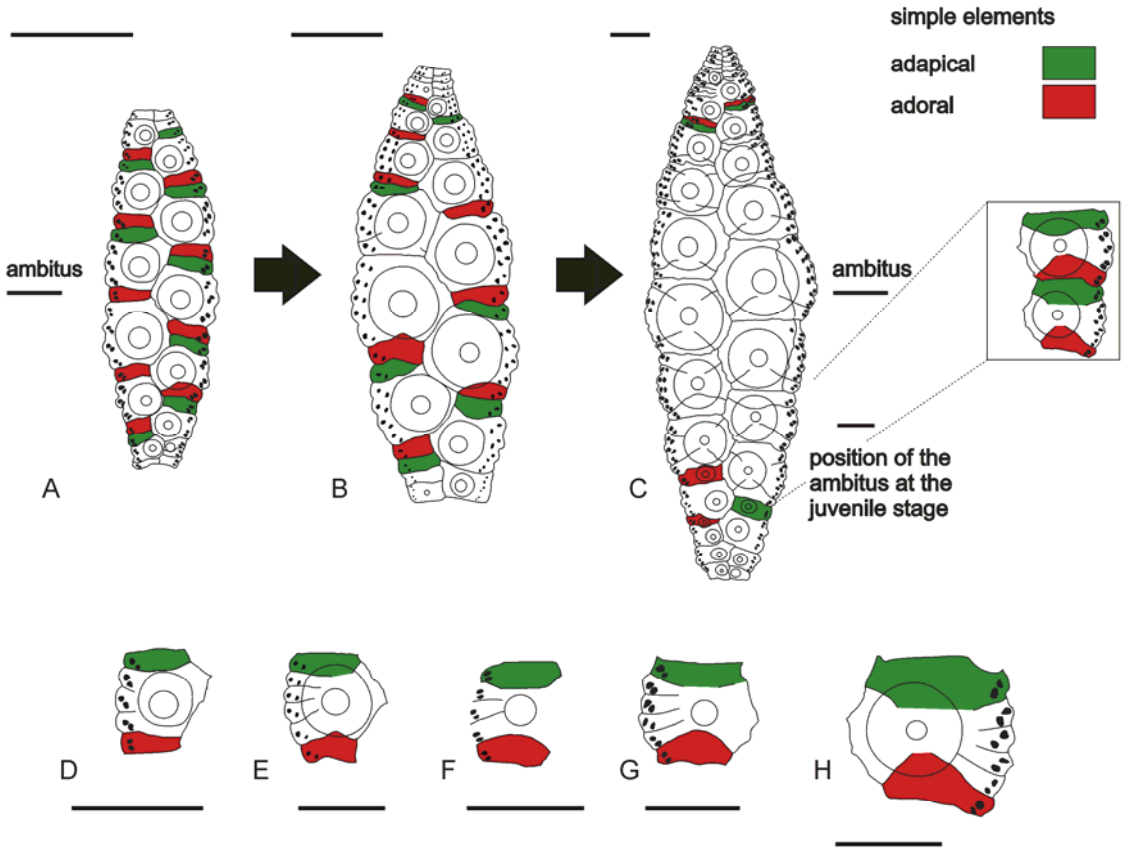


Figure 5. (A-C) Plating style drawings of the ambulacral zones of different growth stages of *G. princeps*, simple elements (bearing only one pore pair) are coloured, granules and smaller tubercles were omitted; (D-H) shape change by fusion to compound plates of simple elements (A, D = GZG.INV.18602, B, E = GZG.INV.18605, C, H = GZG.INV.18623, F = MB.E.8567, G = GZG.INV.18630; all from the early Maastrichtian of Rügen). Scale bars equal 2 mm (A-C), 1 mm respectively (D-H). Note that differences in pore pair number per plate represent intraspecific variation observed in the material.

One simple element is regularly available in each direction (adoral and adapical) of each compound plate. In *G. spatulifera* and *G. princeps*, however, this pattern is found in smaller-sized tests only (see below). With an increase in size, the simple elements above the ambitus fuse

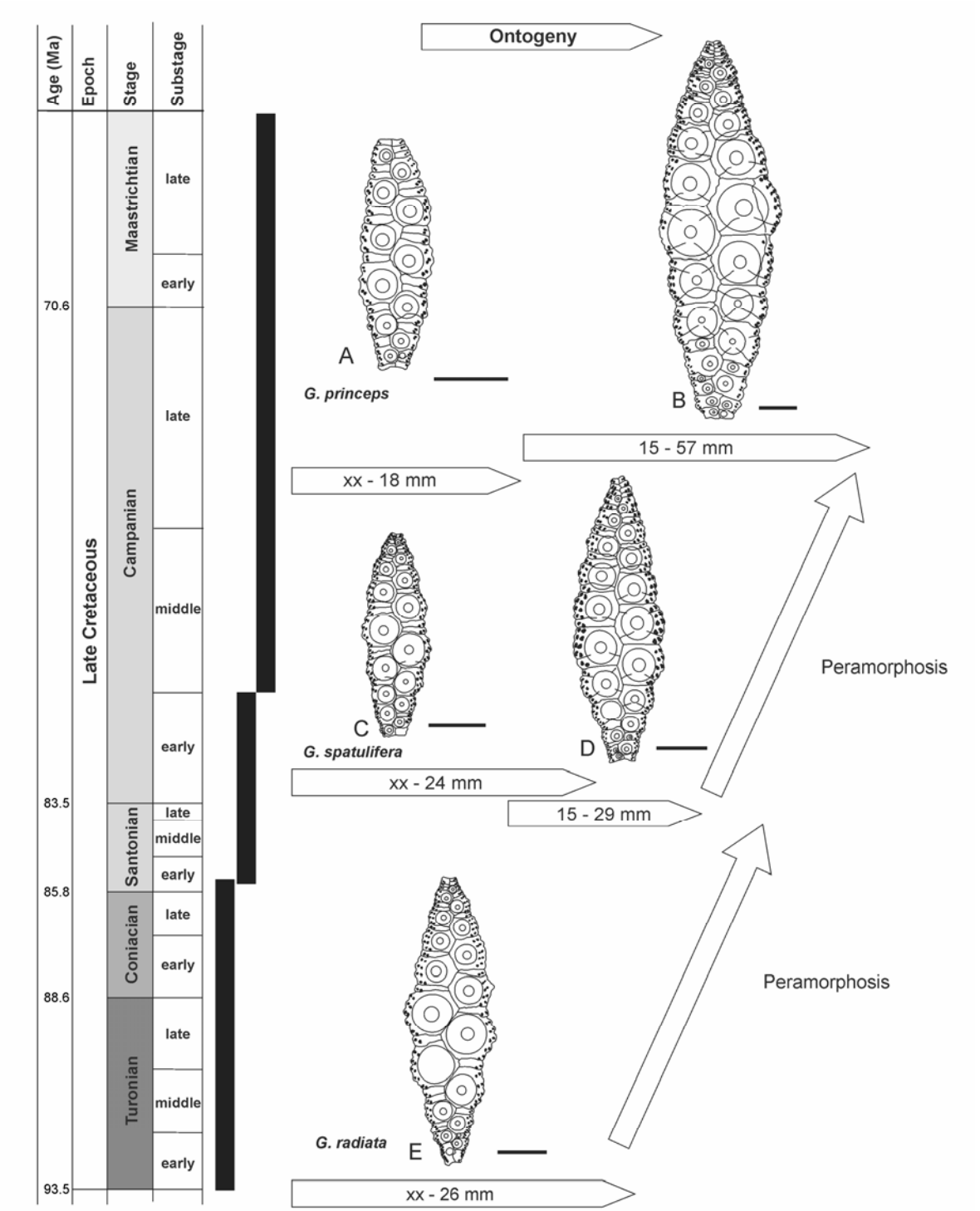


Figure 6. Ambulacral plating pattern in *G. princeps*, *G. spatulifera* and *G. radiata*; indicating different patterns at distinct developmental stages for *G. princeps* and *G. spatulifera* (A, C showing alternations of simple plates and compound plates ambitally); (A) early Maastrichtian, Jasmund peninsula; Rügen, Germany, GZG.INV.18602; (B) early Maastrichtian, cliff complex XI; Jasmund peninsula; Rügen; Germany, GZG.INV.18623; (C) *Micraster coranguinum* Zone; early - middle Santonian; Quidhampton; Wiltshire; Great Britain, BMNH E35778; (D) *Micraster coranguinum* Zone; early - middle Santonian, Tolhurst's Pit, Kent. Great Britain, BMNH E17149; (E) *Mytiloides labiatus* Zone; early Turonian; Dover, Kent, Great Britain, BMNH E9767. Scale bars equal 2 mm.

successively into to the compound plates (Fig. 5B). This is accompanied by a change of shape of these elements, as they migrate towards the centre of the adjacent larger compound plate (Fig. 5D-H). The simple elements have adapically and adorally a shape with almost horizontal sutures in the beginning. By growth of the adoral element into adapical direction, and, vice versa, they form progressively a bell shape (adoral elements), and a U-shape (adapical elements) respectively. In larger specimens, simple elements, which are not fused to the compounded plates, occur only at the apical opening and in the proximity of the peristome (Fig. 5C). Accordingly, the involved heterochronic process can easily be regarded as a predisplacement (peramorphosis) in *G. spatulifera* and *G. princeps* in comparison to *G. radiata*. In *G. spatulifera* and *G. princeps* test diameters, at which simple plates at the ambitus are absent for the first time, are similar (c. 15 mm). However, the size range, in which these simple elements at the ambitus are still present, is larger in *G. spatulifera* (15-24 mm) compared to *G. princeps*. In the latter species, such simple elements are already fused to the compounded plates after test diameters of 18 mm. Therefore, the development in *G. princeps* is predisplaced in several specimens, relative also to the previous species.

Arrangement of the adapical pore pairs (Figs. 7 and 8): In *G. radiata*, the adapical pore pairs are strictly uniserial. In *G. spatulifera*, a biserial pore arrangement can be found in test diameters larger than 16 mm, arguing for a predisplacement development compared to *G. radiata*. In *G. princeps*, however, the pore pairs tend to become biserially arranged only after diameters of 21 mm. Sometimes, a biserial arrangement is completely missing in large-sized specimens of *G. princeps* (Fig. 8A). However, significant differences between *G. spatulifera* and *G. princeps* in the regression slopes and y-intercepts were not found in both analyses, neither in the non-log transformed regression (Fig. 8A), nor in the log transformed regression (Fig. 8B). Accordingly, the only heterochronic process by which *G. princeps* is characterised, compared to *G. spatulifera*, is a hypermorphosis.

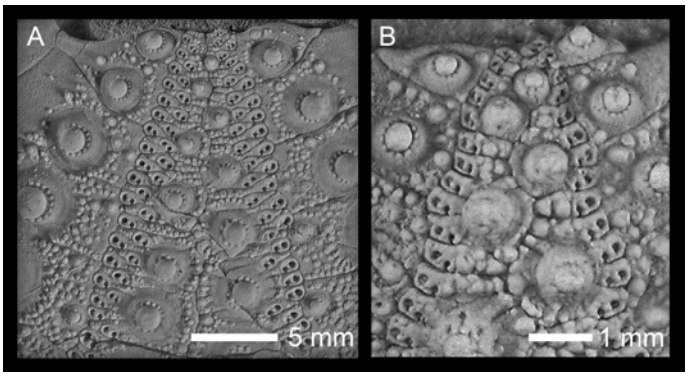
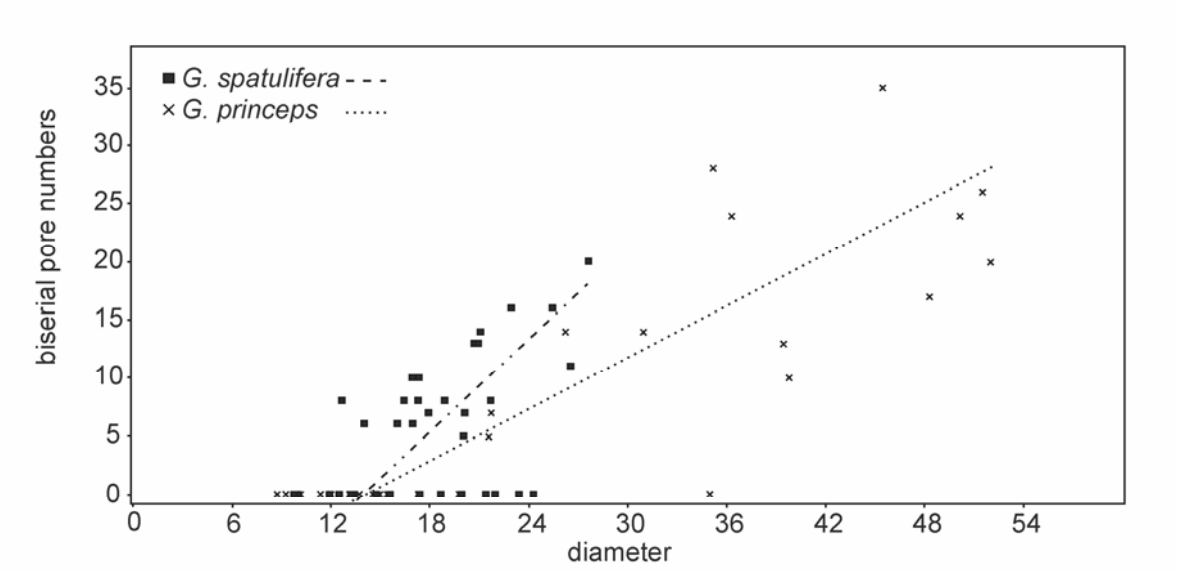


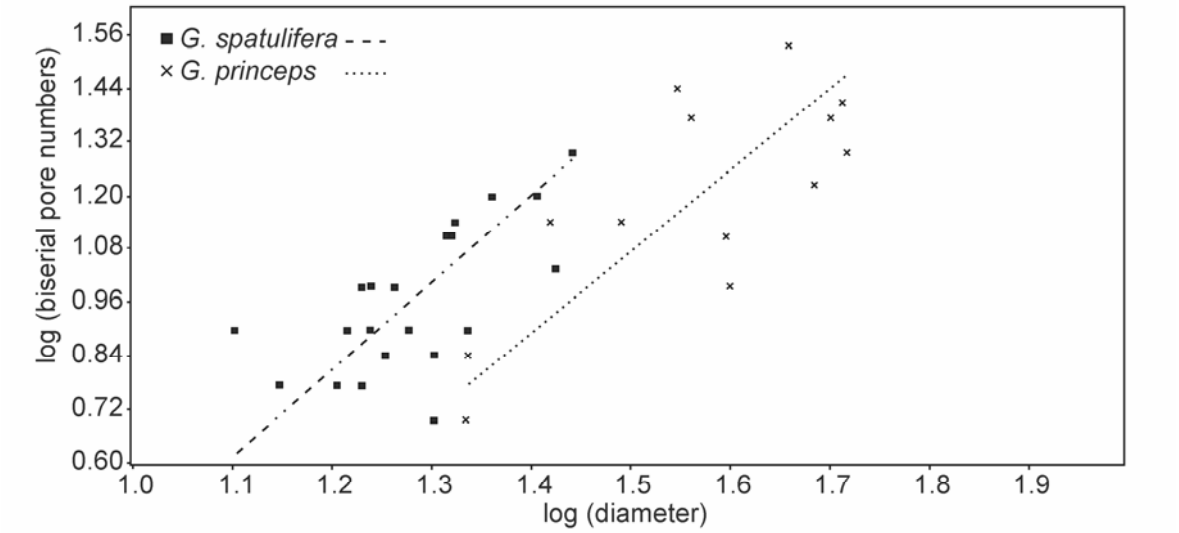
Figure 7. Arrangement of adapical ambulacral pore pairs. (A) Biserial arranged pore pairs; (B) uniserial arranged pore pairs. (A) *G. princeps*, early Maastrichtian, Wittenfelde quarry, Rügen, Germany, GZG.INV.18627; (B) *G. radiata*, late Turonian, Strehlen, Germany, MB.E.8440. All specimens coated with ammonium chloride prior to photography.

Number of pore pairs (Fig. 9): *G. spatulifera* is characterised by an earlier onset in growth trajectory (predisplaced) compared to *G. radiata*, the y-intercepts are significantly different (P = 0.001966). *G. princeps* has a lower y-intercept than *G. spatulifera* (P = 0.001718), indicative for a



A

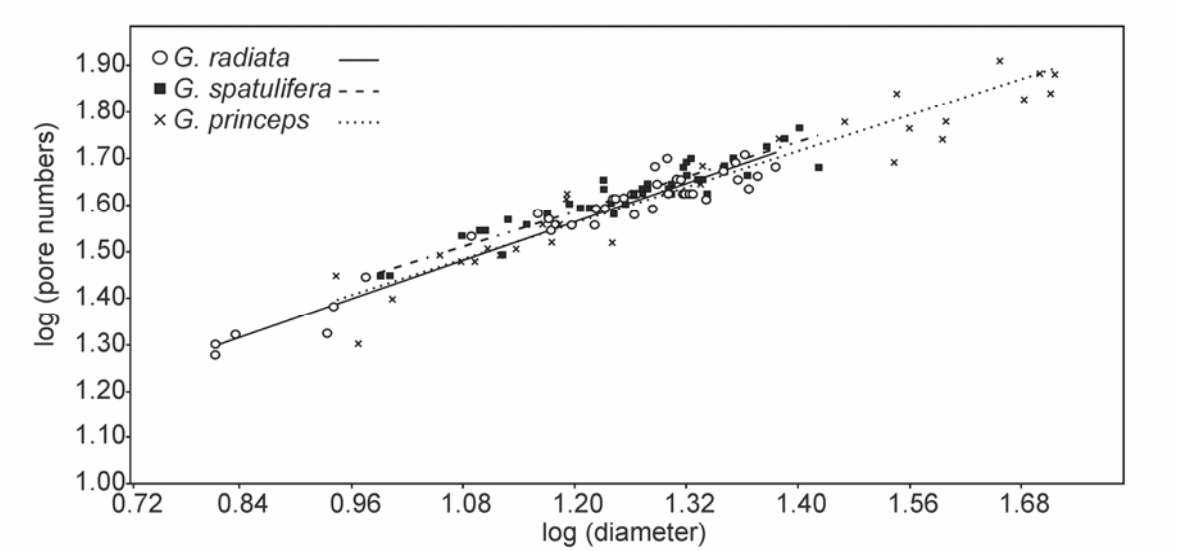
species	regression slope	y-intercept	correlation
G. spatulifera	1.33 ± 0.19	-18.75 ± 13.26	r^2 = 0.28
G. princeps	0.74 ± 0.07	-10.69 ± 4.68	r^2 = 0.73



B

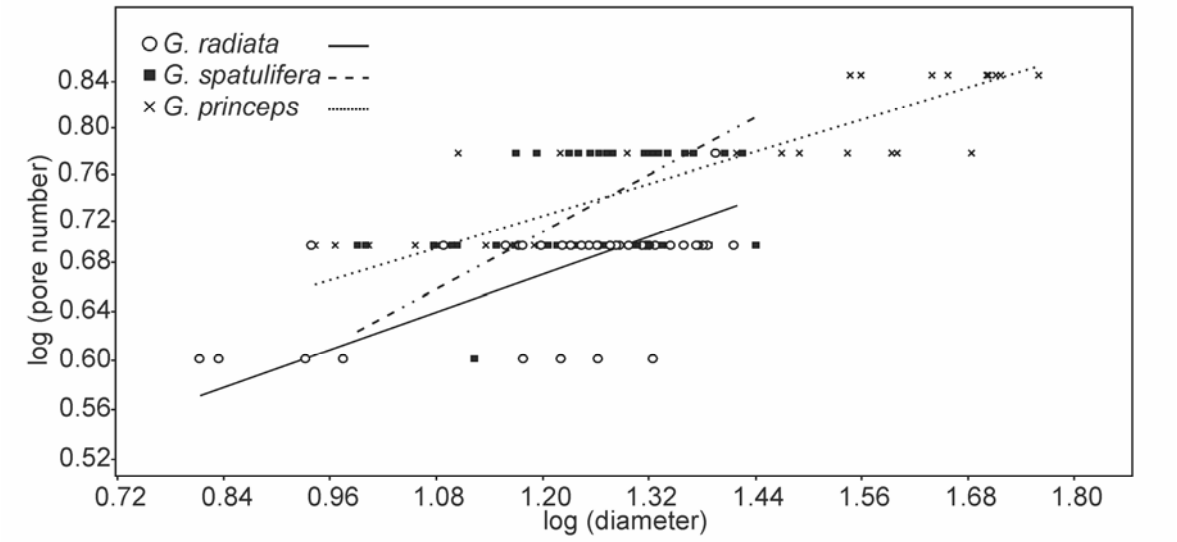
species	regression slope	y-intercept	correlation
G. spatulifera	1.94 ± 0.32	-1.52 ± 0.17	r^2 = 0.44
G. princeps	1.8225 ± 0.36	-1.6489 ± 0.32	r^2 = 0.55

Figure 8. A: Bivariate scatter plot of the numbers of pore pairs, which are biserial arranged, against test diameter. B: Bivariate scatter plot showing the log transformed values of biserial arranged pore pair numbers against test diameter. Including regression line slopes, y-intercepts and correlations for each species.



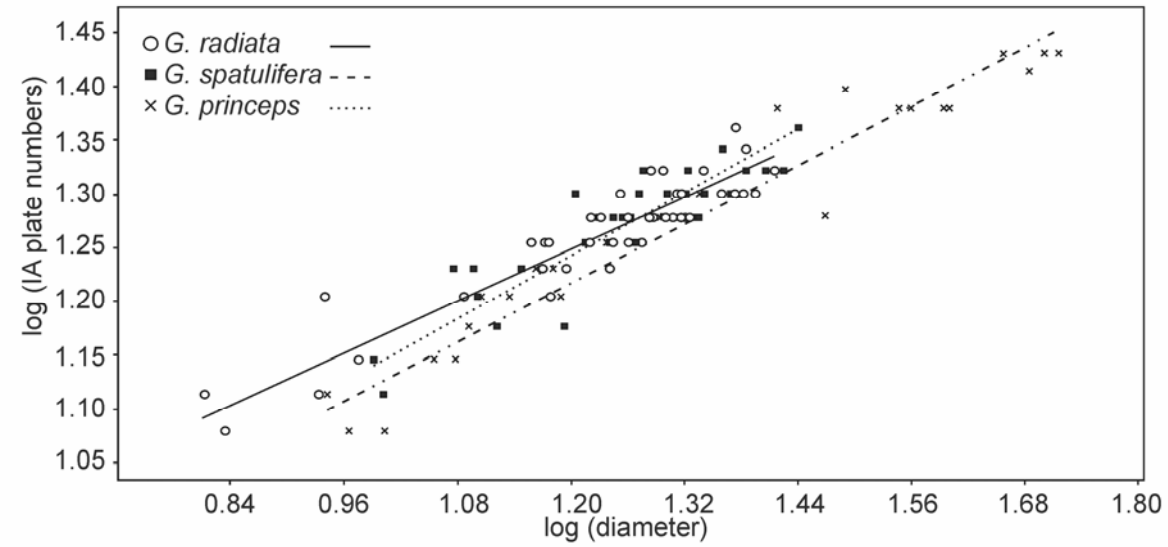
species	regression slope	y-intercept	correlation
G. radiata	0.68 ± 0.02	0.73 ± 0.001	r^2 = 0.92
G. spatulifera	0.62 ± 0.03	0.83 ± 0.002	r^2 = 0.87
G. princeps	0.65 ± 0.03	0.78 ± 0.002	r^2 = 0.92

Figure 9. Bivariate scatter plot showing the log transformed values of pore pair numbers against test diameter, including the values for regression line slopes, y-intercepts and correlations for each species.



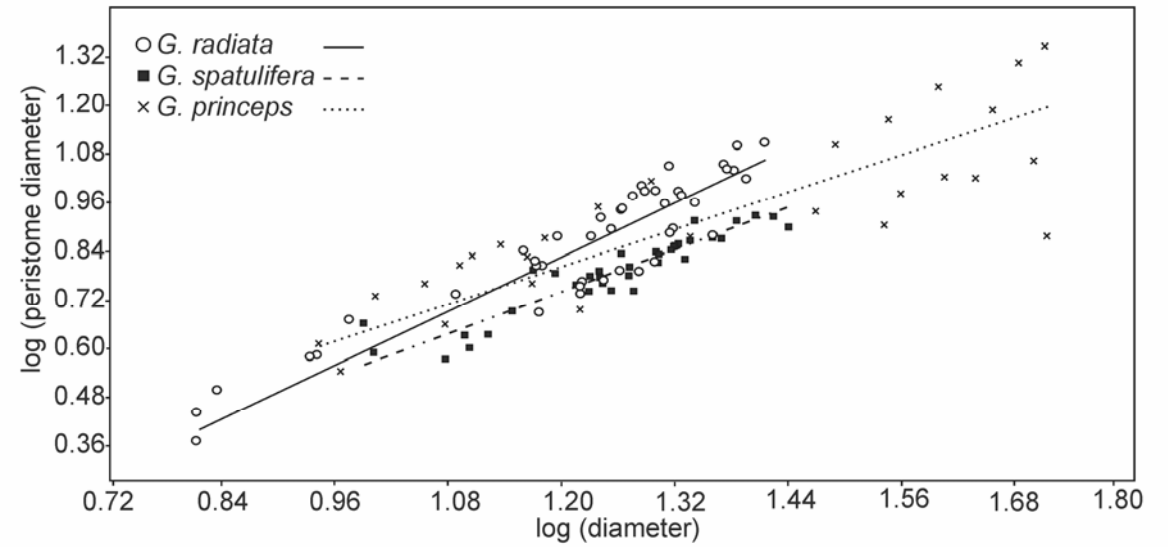
species	regression slope	y-intercept	correlation
G. radiata	0.27 ± 0.03	0.35 ± 0.001	r^2 = 0.38
G. spatulifera	0.41 ± 0.06	0.21 ± 0.006	r^2 = 0.16
G. princeps	0.22 ± 0.02	0.44± 0.0008	r^2 = 0.76

Figure 10. Bivariate scatter plot showing the log transformed values of pore pair numbers per ambital plate against test diameter, including the values for regression line slopes, yintercepts and correlations for each



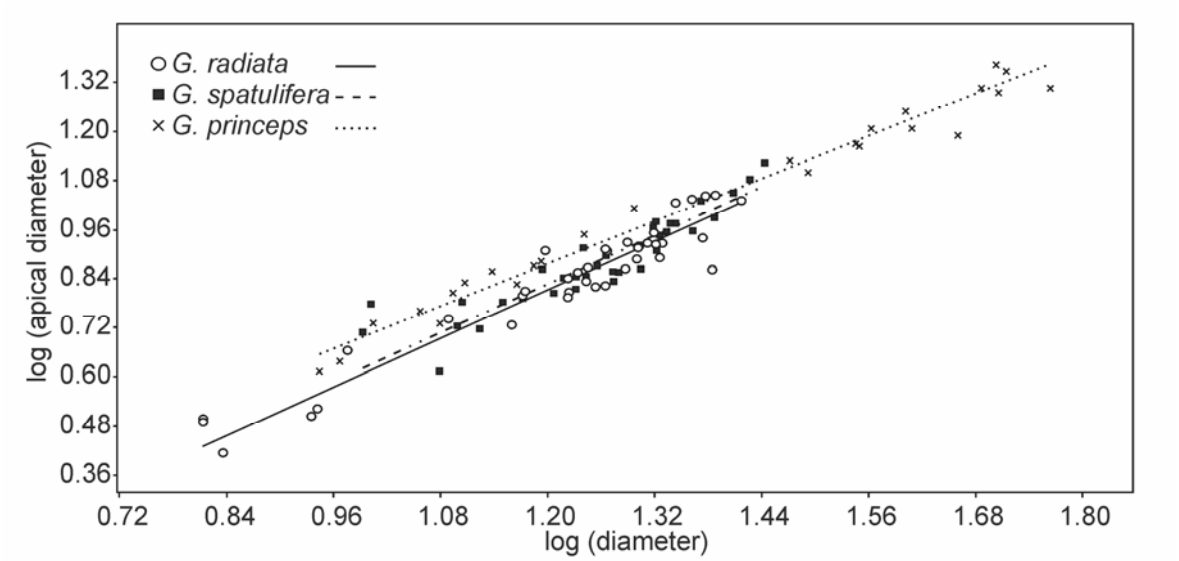
species	regression slope	y-intercept	correlation
G. radiata	0.40 ± 0.02	0.76 ± 0.0008	r^2 = 0.87
G. spatulifera	0.49 ± 0.03	0.65 ± 0.002	r^2 = 0.79
G. princeps	0.45 ± 0.02	0.66 ± 0.0009	r^2 = 0.94

Figure 11. Bivariate scatter plot showing the log transformed values of inertambulacral plate numbers against test diameter, including the values for regression line slopes, y-intercepts and correlations for each species.



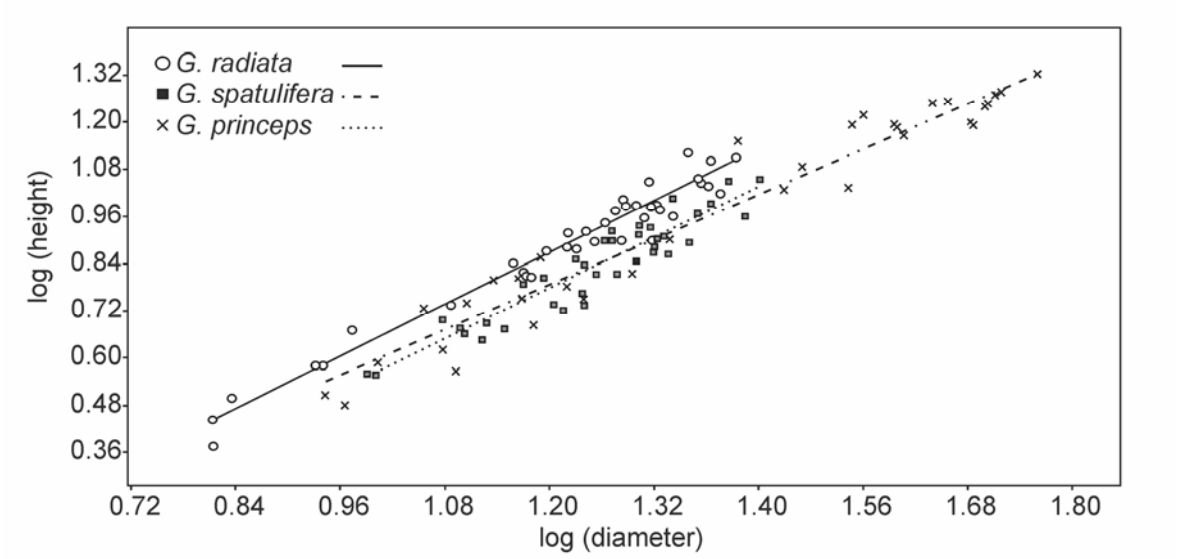
species	regression slope	y-intercept	correlation
G. radiata	1.10 ± 0.06	-0.56 ± 0.006	r^2 = 0.86
G. spatulifera	0.87 ± 0.05	-0.31 ± 0.004	r^2 = 0.88
G. princeps	0.78 ± 0.08	-0.13 ± 0.01	r^2 = 0.70

Figure 12. Bivariate scatter plot showing the log transformed values of peristome diameters against test diameter, including the values for regression line slopes, y-intercepts and correlations for each species.



species	regression slope	y-intercept	correlation
G. radiata	0.99 ± 0.05	-0.37 ± 0.004	r^2 = 0.92
G. spatulifera	0.99 ± 0.07	-0.36 ± 0.007	r^2 = 0.81
G. princeps	0.86 ± 0.02	-0.16 ± 0.001	r^2 = 0.98

Figure 13. Bivariate scatter plot showing the log transformed values of the diameter of the apical openings against test diameter, including the values for regression line slopes, yintercepts and correlations for each species.



species	regression slope	y-intercept	correlation
G. radiata	1.11 ± 0.03	-0.47 ± 0.002	r^2 = 0.95
G. spatulifera	1.15 ± 0.06	-0.61 ± 0.007	r^2 = 0.87
G. princeps	1.01 ± 0.04	-0.44 ± 0.003	r^2 = 0.93

Figure 14. Bivariate scatter plot showing the log transformed values of the test height against test diameter, including the values for regression line slopes, y-intercepts and correlations for each species.

postdisplacement. No significant differences in the regression slopes between the three species, hence, in their developmental rates, could be found.

Pores at the ambitus (Fig. 10): Higher numbers of ambital ambulacral pores are found earlier in development in *G. spatulifera* and *G. princeps* than in *G. radiata*, which is due to changes in their development. The growth trajectories of *G. spatulifera* and *G. princeps* are governed by postdisplacement, predisplacement respectively, in relation to *G. radiata*. Compared to *G. radiata*, both have significantly different y-intercepts ($P < 0.0001$). However, the growth trajectories of *G. spatulifera* and *G. princeps* are not significantly different to each other, which might be a consequence of insufficient statistical power in this case. The significant differences in the y-intercepts of both species in relation to *G. radiata* reveal antithetic onsets in each growth trajectory. Accordingly, we tentatively argue for a predisplaced development in *G. princeps* in comparison to *G. spatulifera*, as given by their y-intercepts.

Number of interambulacral plates (Fig. 11): *G. radiata* and *G. spatulifera* are comparable in the number of interambulacral plates. Their trajectories reveal no significant differences *G. princeps* has lower numbers in IA plates than *G. spatulifera*; the differences in their y-intercepts are only weak, but significant ($P = 0.0007478$), arguing for a slightly earlier onset (predisplacement) in *G. princeps*. Significant differences in the regression slopes of the species were not found.

Peristomal opening (Fig. 12): The diameter of the peristome is largest in *G. radiata*, through neotenic predisplacement (slopes: $P < 0.001215$, y-intercepts: $P < 0.0001$) the diameter decreases towards *G. spatulifera*. The recurrent increase in the peristome diameter in *G. princeps* is explained by a predisplaced onset in development compared to *G. spatulifera* ($P = 0.001115$) and neoteny in the developmental rate in relation to *G. radiata* ($P = 0.001215$).

Apical opening diameter (Fig. 13): *G. spatulifera* and *G. radiata* are very similar to each other in the dimension of the apical opening (measured by its longest axis) during their entire development. Only the y-intercept is somewhat higher in *G. spatulifera* than in *G. radiata*, but not significantly different. In *G. princeps* differences to previous species are more obvious, revealing larger size differences during the earlier development through a significant predisplaced trajectory (compared to *G. radiata*: $P < 0.0001$, in comparison with *G. spatulifera*: $P = 0.0001829$). Differences in developmental rate between all species are not significant.

Test height (Fig. 14): The test height in *G. spatulifera* and *G. princeps* is decreased in comparison to *G. radiata*. The growth trajectory of *G. spatulifera* and *G. princeps* changed in

comparison with *G. radiata* by a postdisplacement, predisplacement respectively ($P < 0.001$, in each case). The slopes and y-intercepts of *G. spatulifera* and *G. princeps* are not significantly different. As the changes in the particular growth trajectories of *G. princeps* and *G. spatulifera* in comparison to *G. radiata* are in contrast to each other, we, however, argue for a predisplacement in the growth trajectory of *G. princeps* in comparison to *G. spatulifera*, inferred by their y-intercepts. The absence of significant differences in the y-intercepts might be due to a lack of statistical power.

Radiation in the areoles (Fig. 15): A distinct radial striation in the areoles of the tubercles can be found in any sizes of *G. radiata* (Fig. 15E, F). In *G. spatulifera* (Fig. 15C, D) and *G. princeps* (Fig. 15A, B), however, is strongest in small sized specimens (< 15 mm). In the latter two species, this radiation successively becomes less pronounced during development. Accordingly, a predisplacement in *G. spatulifera* and *G. princeps*, relative to *G. radiata*, is found. A difference between *G. spatulifera* and *G. princeps* in the onset of the vanishment this striation was not be found in the present study.

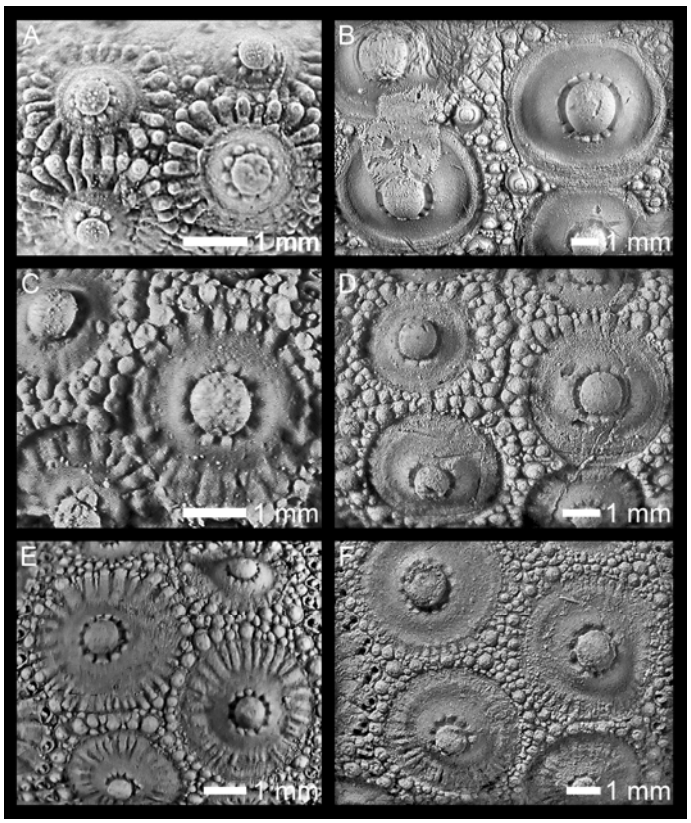


Figure 15. Radiation in interambulacral tubercles at different size stages. A-B: *Gauthieria princeps* (A: early Maastrichtian, cliff complex XVIII; Jasmund peninsula; Rügen; Germany, GZG.INV.18602; B: early Maastrichtian, Rügen, Germany, MB.E.6877); C-D: *Gauthieria spatulifera* (C: early Campanian, Schinkel quarry, Lägerdorf, Germany, GPIH04841; D: Chalk Group, Upper Chalk Formation, Kent; Great Britain, BMNH E12570); E-F: *Gauthieria radiata* (E: late Turonian, Strehlen, Germany, MB.E.8440.5; F: *Mytiloides labiatus* Zone; early Turonian; Dover, Kent, Great Britain, BMNH E9767). All specimens coated with ammonium chloride prior to photography.

3.4. Discussion

The evolution in this lineage is characterised by several changes in the developmental rate or "timing" of developmental events in the herein studied traits (Table 1). Only a single trait (peristome diameter) in *G. spatulifera* shows a combination of changes in the onset and in the rate of development. *G.*

princeps is characterised by a tremendous size increase (up to *c*. 58 mm), reaching double the test diameter of its ancestors (*G. radiata*: *c*. 26 mm, *G. spatulifera*: *c*. 29 mm). Peramorphic processes, like neotony and hypermorphosis, are often responsible for size increase in general (McKinney & McNamara, 1991). Hypermorphosis could be detected in the biserial pore arrangement, neoteny could not be found in the development of *G. princeps* in this study. Detections of dissociated heterochronies (McKinney & McNamara, 1991) or mosaic heterochrony (David, 1989, 1990), in the studied developmental changes are suggestive of a modularity in the development of distinct traits, more or less autonomous developing traits (Wagner & Altenberg, 1996; Klingenberg, 2014). Modularity can facilitate evolutionary diversification (Klingenberg, 2005). Addressing the question of the comparatively low diversification in regular echinoids (see above) by comparing degrees of modularity and integration in traits during development could be interesting and promising for future studies. In some other species of *Gauthieria*, e.g. *Gauthieria mosae* Geys, 1980 (late Campanian; Netherlands), *Gauthieria meandrina* (Schlüter, 1883) (late Maastrichtian; Netherlands), *Gauthieria alterna* Kutscher, 1985a (early - late Maastrichtian; northern Germany, Denmark), similar plating patterns in the ambulacral zones (alternating simple and compound plates) occur, comparable to the species studied in this paper. This suggests a close evolutionary relationship to the species tested. Furthermore, in previously mentioned species a retention of this plating pattern is found throughout their development (paedomorphic), similar to *G. radiata* or small specimens of *G. spatulifera* and *G. princeps*. It further emphasises that the described lineage is probably more differentiated than illustrated in our study, at least during their late stratigraphic occurrence. However, we did not attempt to access material in collections for this study, as specimens of these species are only very rare (Geys, 1980; Kutscher, 1985a; Jagt et al., 1998; Schlüter et al., 2012b).

3.5. Conclusion

On account of the morphological intermediate specimens between mature specimens of *G. spatulifera*, *G. princeps* respectively, and the small-sized forms from the Santonian - Maastrichtian ("*G. radiata*"), a very likely ontogenetic development is herein established. The prevailing morphological similarities in all species, revealed by their ontogenetic development, are found in the ambulacral plating pattern, consisting of alternating simple and compound plates. This development is probably the most convincing result of an intimate evolutionary relationship between the species.

3.6. Systematic remarks

Gauthiosoma Kutscher, 1985b (type species: *Cidarites princeps* von Hagenow, 1840) was established on account of biserial arranged pore pairs towards the apex, which would be different to *Gauthieria*.

This designation was followed by others (e.g. Jagt, 2000; Smith & Jeffery, 2000; Smith & Kroh, 2011). Smith & Wright (1996), however, were of the opinion that the adapical arrangement of the pore pairs would not be sufficient to distinguish between *Gauthiosoma* and *Gauthieria*; where development of biserial arranged pore pairs adapically would rather be a question of size. This study, however, shows that this view is erroneous as *G. spatulifera* and *G. princeps* develop a biseriality at sizes where the pore arrangement in *G. radiata* is uniserial. Nevertheless, the opinion of Smith and Wright (1996) on the state of the genus *Gauthiosoma* is agreed here. The studied species examined in this study represent the most probable members of an evolutionary lineage, hence, being not in a sister-group relationship. Consequently, *Gauthieria* would become paraphyletic by the exclusion of *Cidarites princeps*. A further retainment of the genus *Gauthiosoma* would thus rather represent an artificial construction in a systematic treatment. Smith & Kroh (2011), however, regard *Gauthiosoma* as a junior synonym of *Cosmocyphus* Pomel, 1883. *Cosmocyphus* (type species: *Cyphosoma saemanni* Coquand, 1860) is in its morphology close to *Gauthieria*; both share a sunken peristome and biserial pore arrangement adapically. Smith & Kroh (2011) stated that *Cosmocyphus* differs from *Gauthieria* Lambert, 1888 only in the development of biserial arranged pore pairs adapically (uniserial in *Gauthieria*), which was not confirmed in our study. The type species *C. saemanni*, however, differs to *Gauthieria* and *Gauthiosoma*, in having no simple elements intercalated between larger, compound plates close to the oral opening, further the pore pairs in oral direction are lesser inclined than in *Gauthieria* and *Gauthiosoma*, in which they are running strongly oblique. For these reasons, the here studied species are incompatible with the genus *Cosmocyphus* and *Gauthiosoma* is treated as a synonym of *Gauthieria* rather than *Cosmocyphus* here.

Trait	Heterochronic process	
	G. spatulifera vs. *radiata*	*G. princeps* vs. *spatulifera*
Ambulacral plate pattern	Predisplacement	Predisplacement
Biserial pore arrangement	Predisplacement	Hypermorphosis
Number of pore pairs	Predisplacement	Predisplacement
Pores at the ambitus	Postdisplacement	Predisplacement
Number of IA plates	No changes in development	Predisplacement
Peristome diameter	Neotenic predisplacement	Predisplacement
Apical opening diameter	no changes in development	Predisplacement
Test height	Postdisplacement	Predisplacement
Radiation in the areoles	Predisplacement	No changes in development

Table 1. Summary of the results; involved heterochronic processes are in relation to the particular ancestral species.

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3.9. Appendix. Supplementary data

species	collection-nr.	stratigraphy	locality	test diameter	test height	peristome diameter
Gauthieria spatulifera						
	BMNH EE7914	3 m below Whittaker's 3-inch Band; *Micraster coranguinum* Zone; early Santonian; Late Cretaceous	Cooper's Pit; Kent; England	18.33	7.91	6.82
	BMNH EE7915	3 m below Whittaker's 3-inch Band; *Micraster coranguinum* Zone; early Santonian; Late Cretaceous	Cooper's Pit; Kent; England	20.06	8.21	6.5
	BMNH EE7921	lower *Uintacrinus socialis* Zone; late Santonian, Late Cretaceous	Kingsgate; Kent; England; Great Britain	21.07	7.99	7.2
	BMNH EE7922	lower *Uintacrinus socialis* Zone; late Santonian, Late Cretaceous	Kingsgate; Kent; England; Great Britain	12.66	4.58	4
	BMNH E12568	Santonian; Late Cretaceous	Kent, England; Great Britain	16.96	7.11	5.52
	BMNH E12569	Chalk Group; Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	20.9	7.63	7.11
	BMNH E12570	Chalk Group; Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	26.56	9.14	8.39
	BMNH E12572	Chalk Group; Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	21.94	10.12	8.21
	BMNH E12573	Chalk Group; Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	18.7	8.41	6.32
	BMNH E12574	Chalk Group; Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	21.42	8.08	6.6
	BMNH E17148	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Northfleet, Kent; England; Great Britain	20.85	7.37	7.1
	BMNH E17149	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Tolhurst's Pit; Kent; England; Great Britain	24.28	9.86	8.21
	BMNH E17150	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Cliffe, Kent, England; Great Britain	18.68	7.89	6.02
	BMNH E17155	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Foots Cray, Kent, England; Great Britain	19.95	7.02	6.91
	BMNH E34086	Chalk Group, Upper Chalk Formation; Late Cretaceous	Kent; England; Great Britain	22.93	7.81	7.46
	BMNH E34107	Coniacian; Santonian, Late Cretaceous	Gravesend, Kent, England; Great Britain	25.44	11.26	8.46
	BMNH E35775	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Quidhampton; Wiltshire; England; Great Britain	23.42	9.27	7.41
	BMNH E35776	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Quidhampton; Wiltshire; England; Great Britain	13.25	4.41	4.3
	BMNH E35778	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Quidhampton; Wiltshire; England; Great Britain	11.94	4.98	3.75
	BMNH E35791	Coniacian - Maastrichtian; Late Cretaceous	Wiltshire, Hampshire; England; Great Britain	17.4	6.87	5.96
	BMNH E35835	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Micheldever; Hampshire; England; Great Britain	17.93	6.5	5.54
	BMNH E35836	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Micheldever; Hampshire; England; Great Britain	18.94	6.5	5.52
	BMNH E35838	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Micheldever; Hampshire; England; Great Britain	20.08	8.67	6.8
	BMNH E35849	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Micheldever; Hampshire; England; Great Britain	16.03	5.43	5.89
	BMNH E35854	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Micheldever; Hampshire; England; Great Britain	20.69	8.58	6.97
	BMNH E40577	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Thanet Coast; Kent; England; Great Britain	21.69	7.3	7.37
	BMNH E40578	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Thanet Coast; Kent; England; Great Britain	16.43	5.28	5.73
	BMNH E40592	*Uintacrinus* band; *Marsupites testudinarius* Zone; late Santonian; Late Cretaceous	Thanet Coast; Kent; England; Great Britain	14.79	6.13	6.24
	BMNH E40593	*Uintacrinus* band; *Marsupites testudinarius* Zone; late Santonian; Late Cretaceous	Thanet Coast; Kent; England; Great Britain	17.39	5.41	6.2
	BMNH E40594	*Uintacrinus* band; *Marsupites testudinarius* Zone; late Santonian	Thanet Coast; Kent; England; Great Britain	10.02	3.6	3.9
	BMNH E40595	*Uintacrinus* band; *Marsupites testudinarius* Zone; late Santonian	Thanet Coast; Kent; England; Great Britain	9.79	3.63	4.6

Table A1. List of taxa investigated, collection numbers, provenance and measurement and qualitative data.

species	collection-nr.	stratigraphy	locality	test diameter	test height	peristome diameter
	BMNH E4716a	Upper Chalk; Late Cretaceous	Sussex; England; Great Britain	15.61	6.35	6.1
	BMNH E40601	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Thanet; Kent; England; Great Britain	12.51	4.73	4.28
	BMNH E40602	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Thanet; Kent; England; Great Britain	14.05	4.7	4.93
	BMNH E46766	Chalk Group; Upper Chalk Formation; Late Cretaceous	Bromley; Kent; England; Great Britain	16.99	7.09	6.02
	BMNH E57533	Chalk Group; Upper Chalk Formation; Late Cretaceous	Bromley; Kent; England; Great Britain	27.6	11.34	7.91
	GPIH04840	Flintlayer F31; *Offaster senonensis* Zone; early Campanian; Late Cretaceous	Alsen quarry, Lägerdorf, Germany	13.42	4.91	na
	GPIH04841	early Campanian; Late Cretaceous	Schinkel quarry, Lägerdorf, Germany	17.3	5.85	6.11
Gauthieria radiata						
	BMNH E1932	Upper Chalk; Late Cretaceous	no location data; Great Britain	23.68	11.11	8.11
	BMNH E4705	*Holaster planus* Zone; late Turonian; Late Cretaceous	Sussex; England; Great Britain	14.42	6.95	4.39
	BMNH E4697	*Holaster planus* Zone; late Turonian; Late Cretaceous	Sussex; England; Great Britain	18.85	9.45	6.98
	BMNH E9767	*Mytiloides labiatus Zone; early Turonian; Late Cretaceous*	Dover; Kent; England; Great Britain	26.01	12.84	7.62
	BMNH E10278	Grit Bed; *Mytiloides labiatus Zone*; early Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	22.88	13.27	6.47
	BMNH E10266	*Mytiloides labiatus Zone; early Turonian; Late Cretaceous*	West of Dover, England; Great Britain	20.37	9.05	6.08
	BMNH E10267	*Mytiloides labiatus* Zone; early Turonian; Late Cretaceous	West of Dover, England; Great Britain	19.26	10.04	5.87
	BMNH E10270	*Mytiloides labiatus* Zone; early Turonian; Late Cretaceous	West of Dover, England; Great Britain	18.31	8.77	5.55
	BMNH E10271	*Mytiloides labiatus* Zone; early Turonian; Late Cretaceous	West of Dover, England; Great Britain	17.45	8.37	5.3
	BMNH E10264	*Mytiloides labiatus* Zone; early Turonian; Late Cretaceous	West of Dover, England; Great Britain	24.32	12.61	7615
	BMNH E17168	*Micraster cortestudinarium* Zone; early Coniacian; Late Cretaceous	Borstal Manor Pit; Rochester, Kent; England; Great Britain	14.91	6.44	5.48
	BMNH E17172	*Holaster planus* Zone; late Turonian; Late Cretaceous	Cuxton; Kent; England; Great Britain	19.39	9.76	6.57
	BMNH E17182	*Holaster planus* Zone; late Turonian; Late Cretaceous	Blue Bell Hill; Kent; England; Great Britain	24.14	10.93	9.03
	BMNH E1837-1	Turonian; Late Cretaceous	Southern England; Great Britain	23.53	11.32	7.48
	BMNH E39714	*Terebratulina lata* Zone; middle Turonian; Late Cretaceous	Coast; Devon; England; Great Britain	6.84	3.14	2.49
	BMNH E39723	*Holaster planus* Zone; late Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	21.07	9.73	6.84
	BMNH E39724	*Holaster planus* Zone; late Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	21.2	9.52	6.88
	BMNH E39725	*Holaster planus* Zone; late Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	19.95	9.73	6.96
	BMNH E39726	*Holaster planus* Zone; late Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	20.82	7.88	7.13
	BMNH E39727	*Holaster planus* Zone; late Turonian; Late Cretaceous	Dover; Kent; England; Great Britain	18.37	8.81	5.9
	BMNH E39734	*Micraster cortestudinarium* Zone; early Coniacian; Late Cretaceous	Chatham; Kent; England; Great Britain	20.58	11.2	7.09
	BMNH E40751	*Holaster planus* Zone; late Turonian; Late Cretaceous	Boswell Farm Pit, Louth, (Pit No. 8 of A. Rowe), Lincolnshire, Great Britain	21.93	9.1	7.09
	BMNH E40752	*Holaster planus* Zone; late Turonian; Late Cretaceous	Boswell Farm Pit, Louth, (Pit No. 8 of A. Rowe), Lincolnshire, Great Britain	15.69	7.47	5.66
	BMNH E40891	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Dover; Kent; England; Great Britain	14.82	6.55	4.61
	BMNH E40892	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Dover; Kent; England; Great Britain	9.43	4.7	3.37

Table A2. List of taxa investigated, collection numbers, provenance and measurement and qualitative data.

species	collection-nr.	stratigraphy	locality	test diameter	test height	peristome diameter
	BMNH E40893	*Micraster coranguinum* Zone; early - middle Santonian; Late Cretaceous	Dover; Kent; England; Great Britain	12.24	5.43	4.67
	BMNH E41016	*Holaster planus* Zone; late Turonian; Late Cretaceous	Small Pit in upper part of Level Crossing Lane, Barnes Close (Pit No. 8 of A. Rowe), Great Britain	17.03	7.54	6.23
	MB.E.8441.1	late Turonian; Late Cretaceous	Strehlen;Germany	20.72	9.8	7.7
	MB.E.8441.2	late Turonian; Late Cretaceous	Strehlen;Germany	19.16	7.98	6.17
	MB.E.8441.3	late Turonian; Late Cretaceous	Strehlen;Germany	18.28	8.15	6.19
	MB.E.8441.4	late Turonian; Late Cretaceous	Strehlen;Germany	16.66	8.4	5.82
	MB.E.8442.1	late Turonian; Late Cretaceous	Strehlen;Germany	17.52	6.98	5.92
	MB.E.8442.2	late Turonian; Late Cretaceous	Strehlen;Germany	16.59	7.01	5685
	MB.E.8444	Late Turonian; Late Cretaceous	Strehlen;Germany	16.61	7.73	5.44
	MB.E.8440.1	late Turonian; Late Cretaceous	Strehlen;Germany	19.87	10.05	6.54
	MB.E.8440.5	late Turonian; Late Cretaceous	Strehlen;Germany	15.04	7	4.9
	MNHN-F-A20066	late Turonian; Late Cretaceous	Etigny; Yonne; France	24.87	10.44	7.53
	MNHN-F-A20068-1	late Turonian; Late Cretaceous	Berzieux; Marne; France	6.5	2.77	3.1
	MNHN-F-A20068-2	late Turonian; Late Cretaceous	Berzieux; Marne; France	8.58	3.81	3.53
	MNHN-F-A20068-3	late Turonian; Late Cretaceous	Berzieux; Marne; France	8.72	3.83	3.58
	MNHN-F-A20068-4	late Turonian; Late Cretaceous	Berzieux; Marne; France	6.5	2.36	2.99
	MNHN-F-A20060-1	late Turonian; Late Cretaceous	Saint-Julien; France	17.86	7.84	5.78
	MNHN-F-A20060-2	late Turonian; Late Cretaceous	Saint-Julien; France	15.1	6.4	4.99
Gauthieria princeps						
	BMNH EE4815	late Campanian, Late Cretaceous	Coesfeld, Germany	45.42	17.86	12.79
	BMNH EE5540	*Belemnitella mucronata* Zone; late Campanian; Late Cretaceous	Attoe's Pit; Norfolk; England; Great Britain	14.59	6.36	5.63
	BMNH E10546	Chalk Group; Upper Chalk Formation; Late Cretaceous	Trimmingham; Norfolk; England; Great Britain	26.2	14.16	9.69
	BMNH E10547	Chalk Group; Upper Chalk Formation; Late Cretaceous	Trimmingham; Norfolk; England; Great Britain	39.37	15.53	11.57
	BMNH E35751	*Belemnitella mucronata* Zone; late Campanian; Late Cretaceous	Clarendon; Wiltshire; England; Great Britain	11.36	5.3	4.97
	BMNH E39802	*Belemnitella mucronata* Zone; late Campanian; Late Cretaceous	Earlham Lime Works; Norfolk; England; Great Britain	35.17	15.59	10.82
	BMNH E40600	*Belemnitella mucronata* Zone; late Campanian; Late Cretaceous	Studland Bay; Dorset; England; Great Britain	8.76	3.2	3.3
	GPIH04835	late Maastrichtian; Late Cretaceous	Hemmoor quarry; Germany	17.34	5.62	6.04
	GPIH04836	late Maastrichtian; Late Cretaceous	Hemmoor quarry; Germany	19.73	6.48	7.4
	GPIH04837	Maastrichtian; Late Cretaceous	Hemmoor quarry; Germany	12.75	5.48	4815
	GPIH04838	Maastrichtian; Late Cretaceous	Hemmoor quarry; Germany	12.37	3.7	4695
	GPIH04839	late Maastrichtian; Late Cretaceous	Hemmoor quarry; Germany	15.21	4.82	5.45
	GZG.INV.18530	early Maastrichtian; Late Cretaceous	Jasmund peninsula; Rügen; Germany	40.38	14.64	10.53
	GZG.INV.18564	early Maastrichtian; Late Cretaceous	Kieler Bach; Jasmund peninsula; Rügen; Germany	50.14	17.47	11.57
	GZG.INV.18567	early Maastrichtian; Late Cretaceous	Wittenfelde quarry; Rügen; Germany	13.15	na	na
	GZG.INV.18593	early Maastrichtian; Late Cretaceous	cliff complex VIII; Jasmund peninsula; Rügen; Germany	14.76	5.64	na

Table A3. List of taxa investigated, collection numbers, provenance and measurement and qualitative data.

species	collection-nr.	stratigraphy	locality	test diameter	test height	peristome diameter
	GZG.INV. 18597	early Maastrichtian; Late Cretaceous	Promoisel quarry; Rügen; Germany	21.71	7.99	7.5
	GZG.INV. 18599	early Maastrichtian; Late Cretaceous	Jasmund peninsula; Rügen; Germany	21.57	na	na
	GZG.INV. 18600	early Maastrichtian; Late Cretaceous	Jasmund peninsula; Rügen; Germany	14.95	na	na
	GZG.INV. 18601	early Maastrichtian; Late Cretaceous	cliff complex V; Jasmund peninsula; Rügen; Germany	29.5	10.62	8.66
	GZG.INV. 18602	early Maastrichtian; Late Cretaceous	cliff complex XVIII; Jasmund peninsula; Rügen; Germany	11.95	4.17	4.56
	GZG.INV. 18605	early Maastrichtian; Late Cretaceous	cliff complex X; Jasmund peninsula; Rügen; Germany	15.5	7.2	na
	GZG.INV. 18609	early Maastrichtian; Late Cretaceous	cliff complex IV; Jasmund peninsula; Rügen; Germany	9.25	3.03	3.5
	GZG.INV. 18622	early Maastrichtian; Late Cretaceous	Promoisel quarry; Rügen; Germany	34.92	10.72	8.02
	GZG.INV. 18624	early Maastrichtian; Late Cretaceous	quarry at the Waldmeisterstraße; Sassnitz; Rügen ; Germany	36.3	16.44	9.62
	GZG.INV. 18625	early Maastrichtian; Late Cretaceous	Wittenfelde quarry; Rügen; Germany	48.28	15.8	11.4
	GZG.INV. 18626	early Maastrichtian; Late Cretaceous	Promoisel quarry; Rügen; Germany	16.59	6.03	4.96
	GZG.INV. 18627	early Maastrichtian; Late Cretaceous	Wittenfelde quarry; Rügen; Germany	57.63	21	na
	GZG.INV. 18628	early Maastrichtian; Late Cretaceous	Wittenfelde quarry; Rügen; Germany	51.48	18.5	na
	GZG.INV. 95001	early Maastrichtian; Late Cretaceous	Kieler Bach; Jasmund peninsula; Rügen; Germany	13.67	6.28	5.11
	GZG.INV. 95002	early Maastrichtian; Late Cretaceous	Kieler Bach; Jasmund peninsula; Rügen; Germany	10.07	3.89	3.97
	GZG.INV. 95003	early Maastrichtian; Late Cretaceous	Wittenfelde quarry; Rügen; Germany	50.37	17.78	na
	GZG.INV. 95004	early Maastrichtian; Late Cretaceous	Promoisel quarry; Rügen; Germany	43.56	17.67	na
	MB.E.687 7	early Maastrichtian; Late Cretaceous	Rügen; Germany	5.154	19.13	11
	GZG.INV. 78438	late Campanian, Late Cretaceous	Ahlten, Germany	30.93	12.24	8.78
	GZG.INV. 78432	late Campanian, Late Cretaceous	Ahlten, Germany	39.76	15.44	10.87

Table A4. List of taxa investigated, collection numbers, provenance and measurement and qualitative data.

species	collection-nr.	peristome diameter	longest axis of the apical opening	number of pore pairs	number of biserial pore pairs	number of IA plates	presence of simple ambulacral plates ambital	pores ambital	presence of radiation in the areoles
Gauthieria spatulifera									
	BMNH EE7914	6.82	7.88	42	10	19	no	6	no
	BMNH EE7915	6.5	8.35	42	5	20	no	5	no
	BMNH EE7921	7.2	8.82	50	14	21	no	6	no
	BMNH EE7922	4	6.07	35	8	16	yes	5	yes
	BMNH E12568	5.52	6.52	45	10	19	no	6	no
	BMNH E12569	7.11	8.13	46	13	20	no	6	no
	BMNH E12570	8.39	12.12	55	11	21	no	6	no
	BMNH E12572	8.21	9.44	42	0	20	no	6	no
	BMNH E12573	6.32	7.2	42	0	18	no	6	no
	BMNH E12574	6.6	9.02	45	0	19	yes	6	no
	BMNH E17148	7.1	9.56	49	13	20	no	5	no
	BMNH E17149	8.21	9.77	46	0	21	no	5	no
	BMNH E17150	6.02	6.81	43	0	20	yes	5	no
	BMNH E17155	6.91	na	43	0	19	yes	5	no
	BMNH E34086	7.46	9.06	48	16	22	no	6	no
	BMNH E34107	8.46	11.17	53	16	21	no	6	no
	BMNH E35775	7.41	10.73	50	0	20	yes	6	no
	BMNH E35776	4.3	5.2	31	0	15	yes	4	yes
	BMNH E35778	3.75	4.12	34	0	17	yes	5	yes
	BMNH E35791	5.96	6.99	38	0	18	no	6	yes
	BMNH E35835	5.54	7.44	40	7	19	no	6	yes
	BMNH E35836	5.52	7.19	44	8	21	no	6	no
	BMNH E35838	6.8	7.3	44	7	20	no	5	no
	BMNH E35849	5.89	6.38	39	6	20	yes	5	yes
	BMNH E35854	6.97	9.23	48	13	19	no	6	no
	BMNH E40577	7.37	9.46	45	8	19	no	5	no
	BMNH E40578	5.73	6.95	39	8	18	yes	5	no
	BMNH E40592	6.24	6.2	38	0	17	no	6	yes
	BMNH E40593	6.2	na	38	10	na	no	6	no
	BMNH E40594	3.9	6.01	28	0	13	yes	5	yes
	BMNH E40595	4.6	5.1	28	0	14	yes	5	yes
	BMNH E4716a	6.1	7.31	40	0	15	yes	6	yes

Table A5. List of taxa investigated, collection numbers, provenance and measurement and qualitative data.

species	collection-nr.	peristome diameter	longest axis of the apical opening	number of pore pairs	number of biserial pore pairs	number of IA plates	presence of simple ambulacral plates ambital	pores ambital	presence of radiation in the areoles
Gauthieria spatulifera									
	BMNH E40601	4.28	5.29	35	0	17	yes	5	yes
	BMNH E40602	4.93	6.08	36	6	17	yes	5	yes
	BMNH E46766	6.02	6.99	43	6	19	no	5	no
	BMNH E57533	7.91	13.28	58	20	23	no	5	no
	GPIH0480	na	na	37	0	15	no	na	yes
	GPIH0481	6.11	8.22	40	8	15	no	5	yes
Gauthieria radiata									
	BMNH E1932	8.11	11.05	45	0	23	yes	5	yes
	BMNH E4705	4.39	5.32	38	0	18	yes	5	yes
	BMNH E4697	6.98	na	40	0	18	yes	5	yes
	BMNH E9767	7.62	10.76	48	0	21	yes	5	yes
	BMNH E10278	6.47	10.83	47	0	20	yes	5	yes
	BMNH E10266	6.08	8.45	45	0	19	yes	5	yes
	BMNH E10267	5.87	7.3	48	0	21	yes	5	yes
	BMNH E10270	5.55	6.66	38	0	18	yes	5	yes
	BMNH E10271	5.3	6.84	41	0	17	yes	5	yes
	BMNH E10264	7615	11.1	43	0	22	yes	5	yes
	BMNH E17168	5.48	6.45	35	0	18	yes	5	yes
	BMNH E17172	6.57	8.5	44	0	19	yes	5	yes
	BMNH E17182	9.03	7.26	51	0	20	yes	5	yes
	BMNH E1837-1	7.48	8.74	49	0	20	yes	5	yes
	BMNH E39714	2.49	2.59	21	0	12	yes	4	yes
	BMNH E39723	6.84	7.8	42	0	19	yes	4	yes
	BMNH E39724	6.88	8.44	42	0	19	yes	5	yes
	BMNH E39725	6.96	8.31	42	0	19	yes	5	yes
	BMNH E39726	7.13	8.4	42	0	20	yes	5	yes
	BMNH E39727	5.9	8.08	42	0	18	yes	5	yes
	BMNH E39734	7.09	na	45	0	20	yes	5	yes
	BMNH E40751	7.09	10.6	41	0	21	yes	5	yes
	BMNH E40752	5.66	8.11	36	0	17	yes	5	yes
	BMNH E40891	4.61	6.31	37	0	17	yes	5	yes
	BMNH E40892	3.37	4.61	28	0	14	yes	4	yes
	BMNH E40893	4.67	5.53	34	0	16	yes	5	yes

Table A6. List of taxa investigated, collection numbers, provenance and measurement and qualitative data (continued).

species	collection-nr.	peristome diameter	longest axis of the apical opening	number of pore pairs	number of biserial pore pairs	number of IA plates	presence of simple ambulacral plates ambital	pores ambital	presence of radiation in the areoles
Gauthieria radiata									
	BMNH E41016	6.23	7.13	39	0	19	yes	5	yes
	MB.E.8441.1	7.7	9.08	42	0	19	yes	5	yes
	MB.E.8441.2	6.17	na	39	0	19	yes	5	yes
	MB.E.8441.3	6.19	8.2	42	0	19	yes	4	yes
	MB.E.8441.4	5.82	6.4	39	0	19	yes	5	yes
	MB.E.8442.1	5.92	7.4	41	0	18	yes	5	yes
	MB.E.8442.2	5685	6.95	36	0	18	yes	4	yes
	MB.E.8444	5.44	6.28	36	0	18	yes	5	yes
	MB.E.8440.1	6.54	7.77	50	0	21	yes	5	yes
	MB.E.8440.5	4.9	na	na	0	18	yes	4	yes
	MNHN-F-A20066	7.53	na	46	0	20	yes	6	yes
	MNHN-F-A20068-1	3.1	3.08	20	0	13	yes	4	yes
	MNHN-F-A20068-2	3.53	3.19	21	0	13	yes	4	yes
	MNHN-F-A20068-3	3.58	3.34	24	0	16	yes	5	yes
	MNHN-F-A20068-4	2.99	3.15	19	0	13	yes	4	yes
	MNHN-F-A20060-1	5.78	6.61	41	0	20	yes	5	yes
	MNHN-F-A20060-2	4.99	na	36	0	16	yes	5	yes
Gauthieria princeps									
	BMNH EE4815	12.79	15.45	81	35	27	no	7	no
	BMNH EE5540	5.63	6.7	36	0	17	yes	5	yes
	BMNH E10546	9.69	na	55	14	24	no	6	no
	BMNH E10547	11.57	na	55	13	24	no	6	no
	BMNH E35751	4.97	5.76	31	0	14	yes	5	yes
	BMNH E39802	10.82	14.64	69	28	24	no	7	no
	BMNH E40600	3.3	4.1	28	0	13	yes	5	yes
	GPIH04835	6.04	8.89	33	0	18	yes	5	no
	GPIH04836	7.4	10.3	42	0	19	no	6	yes
	GPIH04837	4815	6.75	32	0	16	yes	6	yes
	GPIH04838	4695	6.37	30	0	15	yes	5	yes
	GPIH04839	5.45	7.43	36	0	17	no	na	yes
	GZG.INV.18530	10.53	16.03	na	na	na	no	na	no
	GZG.INV.18564	11.57	23.07	76	24	27	no	7	no

Table A7. List of taxa investigated, collection numbers, provenance and measurement and qualitative data (continued).

species	collection-nr.	peristome diameter	longest axis of the apical opening	number of pore pairs	number of biserial pore pairs	number of IA plates	presence of simple ambulacral plates ambital	pores ambital	presence of radiation in the areoles
Gauthieria princeps									
	GZG.INV.18593	na	na	na	na	na	yes	5	yes
	GZG.INV.18597	7.5	9.35	48	7	20	no	5	no
	GZG.INV.18599	na	na	44	5	na	no	na	no
	GZG.INV.18600	na	na	33	0	na	na	na	yes
	GZG.INV.18601	8.66	13.44	na	na	19	no	6	no
	GZG.INV.18602	4.56	5.37	30	0	14	yes	5	no
	GZG.INV.18605	na	7.62	42	0	16	yes	5	yes
	GZG.INV.18609	3.5	4.35	20	0	12	yes	5	yes
	GZG.INV.18622	8.02	14.78	49	0	na	no	6	no
	GZG.INV.18624	9.62	16.06	58	24	24	no	7	no
	GZG.INV.18625	11.4	20.19	67	17	26	no	6	no
	GZG.INV.18626	4.96	na	na	na	na	yes	6	no
	GZG.INV.18627	na	20.28	na	0	na	no	7	no
	GZG.INV.18628	na	22.21	69	26		no	7	no
	GZG.INV.95001	5.11	7.2	32	0	16	yes	5	yes
	GZG.INV.95002	3.97	5.37	25	0	12	yes	5	yes
	GZG.INV.95003	na	19.7	na	na	na	no	7	no
	GZG.INV.95004	na	na	na	na	na	no	7	no
	MB.E.6877	11	na	76	20	27	no	7	no
	GZG.INV.78438	8.78	12.6	60	14	25	no	6	no
	GZG.INV.78432	10.87	17.64	60	10	24	no	6	no

Table A8. List of taxa investigated, collection numbers, provenance and measurement and qualitative data (continued).

Chapter 4

Ecophenotypic variation and developmental instability in the Late Cretaceous echinoid *Micraster brevis* (Irregularia; Spatangoida)

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Abstract

The Late Cretaceous echinoid genus *Micraster* (irregular echinoids, Spatangoida) is one of the most famous examples of a continuous evolutionary lineage in invertebrate palaeontology. The influence of the environment on the phenotype, however, was not tested so far. This study analyses differences in phenotypical variations within three populations of *Micraster* (*Gibbaster*) *brevis* from the early Coniacian, two from the Münsterland Cretaceous Basin (Germany) and one from the North Cantabrian Basin (Spain). The environments of the Spanish and the German sites differed by the sedimentary characteristics, which are generally a crucial factor for morphological adaptations in echinoids. Most of the phenotypical variations (position of the ambitus, periproct and development of the subanal fasciole) among the populations can be linked to differences in their host sediments. These phenotypic variations are presumed to be an expression of phenotpic plasticiy, which has not been considered in *Micraster* in previous studies. Two populations (Erwitte area, Germany; Liencres area, Spain) were tested for stochastic variation (fluctuating asymmetry) due to developmental instability, which was present in all studied traits. However, differences in the amount of fluctuating asymmetry between both populations were recognised only in one trait (amount of pore pairs in the anterior paired petals). The results strengthen previous assumptions on ecophenotypic variations in *Micraster*.

Keywords: echinoid – palaeontology – fossil – phenotype – variation – phenotypic plasticity –fluctuating asymmetry

4.1. Introduction

The environment plays a principal role in adaptive evolution by shaping through natural selection the means of population phenotypes and by evoking phenotypic variation without altering the genetic background, e.g. through uncovering cryptic genetic variation (decanalization), or phenotypic plasticity (Pigliucci, 1998; Gibson & Wagner, 2000; West-Eberhard, 2003; Gibson & Dworkin, 2000). Phenotypic plasticity, however, is a property of a genotype and, thus, its norm of reaction is influenced by genetic variation as well (DeWitt & Scheiner, 2004).

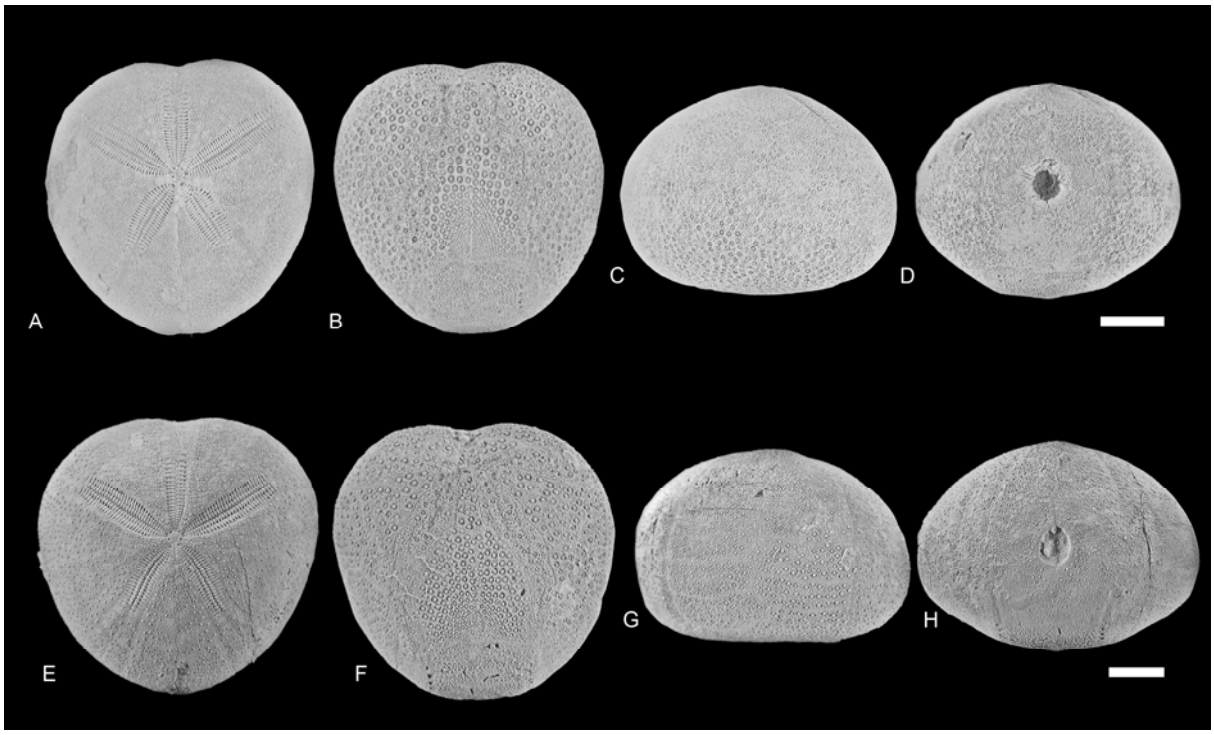

Figure 1. ***Micraster brevis*.** (A-D) Specimen from the Liencres area, Spain (MB.E.8251) in apical, oral, lateral and posterior views. (E-H) Specimen from the Erwitte area, Germany (GSUB E3867) in apical, oral, lateral and posterior views. Scale bar equals 1 cm.

The developmental origins of observed phenotypic variation are often open to debate in palaeontological studies, reasoned in the complex interplay of genes and environment. Typically, the fossil record provides, to some extent, only the factor environment in this equation. However, to decipher patterns in development, evolution and speciation in deep time, it is meaningful to address these mechanisms in studies for fossil taxa as well (Jablonski, 1998). However, another source of phenotypic variation — developmental instability — is easy to explore, but received limited attention by palaeontologists. As a consequence of developmental instability, environmental or genetic stressors perturb developmental pathways (within populations of similar genotypes and stable environments) and lead to an increase in phenotypic variation (Palmer, 1994; Willmore et al., 2007). Developmental instability can be measured by fluctuating asymmetry, random (subtle) deviations from perfect symmetry, as the genes on both sides of a symmetric organism are identical, barring somatic mutations (Van Valen, 1962). Developmental stability, on the other hand, is the ability to buffer the development against those perturbations. Developmental instability has ample origins and the processes leading to it are still not well understood (reviewed in Klingenberg, 2003; Willmore & Hallgrímsson, 2005). Stochastic gene expression contributes to developmental noise (McAdams & Arkin, 1997; Kaern et al., 2005), caused, for example, by environmental stressors, such as pollution, or mutations that reduce the activity of a gene.

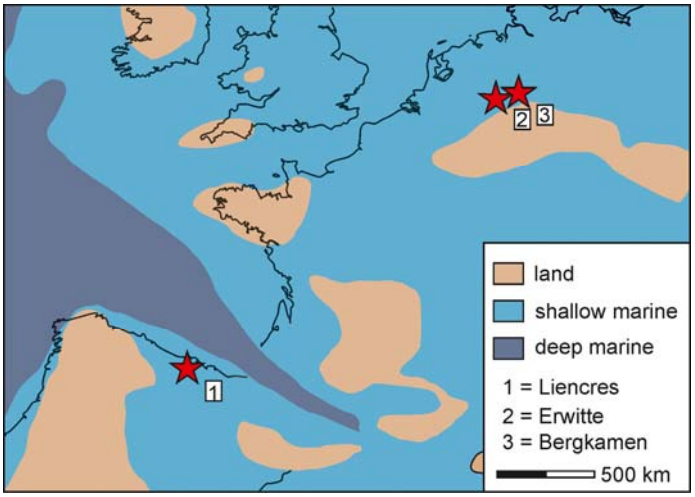

Figure 2. Palaeogeographic map. Simplified palaeogeographic map of western Europe during the Late Cretaceous (modified after Ziegler, 1988).

Phenotypic variations also cause confusion, especially for the species concept in palaeontology, exemplified by the Late Cretaceous fossil echinoid *Micraster*. According to their high variability, extensive taxonomic works resulted in vast names of species (compare Lambert & Thiéry, 1909–1925). *Micraster* was geographically widespread (western Asia, Europe, northern Africa: Stokes, 1975) and inhabited a wide spectrum of environments. Moreover, *Micraster* provides some of the most studied fossil examples in speciation (e.g. Rowe, 1899; Ernst, 1970, 1972; David & Fouray, 1984; Smith & Wright, 2012), due to their well-traceable shifts of phenotypic variations in time. The influence of the environment on the phenotypic variation in *Micraster*, however, was only vaguely assumed (Ernst, 1970, 1972; Drummond, 1983), and Ernst and Seibertz (1977) suggested that species of *Micraster* could have developed local ecophenotypism. However, it was assumed that the evolution in *Micraster* would reflect a step-wise increase of adaptation towards a stable environment (Smith, 1984). These ideas, however, have never been tested.

To analyse and discuss the influence of the environment on the phenotype of *Micraster*, three populations of *Micraster* (*Gibbaster*) *brevis* (Fig. 1, following: ***M. brevis***) were compared, which was widespread during the early Coniacian in Europe (compare Olszewska-Nejbert, 2007). Villier et al. (2001) demonstrated that well-elongated pores in the frontal ambulacrum (associated to tube feet for gaseous exchange) in the Lower Cretaceous spatangoid *Heteraster* indicate an adaptation to warm shallow waters, which was also likely the case for ***M. brevis***.

Three populations were compared. Two samples come from the Münsterland Cretaceous Basin (MCB: Grimberg (IV) mine shaft, Erwitte area, Germany), and one comes from the North Cantabrian Basin (NCB: Liencres area, northern Spain) (Fig. 2).

Palaeoenvironments can be deduced from the composition of the rocks. While the MCB is characterised by wackestones with fine siliciclastic and calcareous ooze and a low content of dispersed bioclasts (calcispheres and foraminifera) (Fig. A1 A–C), the facies of the NCB contains silty packstones with abundant and coarser-grained, reworked silici- and bioclastics (e.g. bivalves,

foraminifera, hexactinellid sponges, echinoderms) (Fig. A1 D–E). More details on the lithology can be found in Wiese (1997) for the NCB and (Seibertz, 1979; Kaplan & Skupin, 1998) for the MCB. Additionally, stronger sea currents and a shallower sea level can be inferred by the sediment nature (coarse grains, angular silt) for Liencres (Wiese, 1997) in comparison to the Grimberg (IV) mine shaft and the Erwitte area (fine-grained matrix). Accordingly, a somewhat more proximal position is inferred for Liencres than for the Erwitte area, which had a more basinal position in the innershelf basin (Münsterland Cretaceous Basin) (Kaplan & Skupin, 1998). Furthermore, a somewhat higher palaeotemperature for the Liencres area can be assumed, as a consequence of its lower palaeolatitude (for the Cenomanian age compare Voigt et al., 2003).

In order to assess the impact of the environment on *M. brevis*, shape variations between the populations were analysed by a geometric morphometric approach, and additional comparative semi-quantitative analyses of four further traits were conducted (ornamentation of the periplastronal ambulacrals and the interradial area of the paired petals, projection of the labrum, development of the subanal fasciole) (see Table 1). Characters which had been previously assumed to be influenced by the sedimentary environment in spatangoid taxa were included in the analyses (e.g. position of the periproct, development of the subanal fasciole, shape of the plastron, inflation of the test) (Higgins, 1974; Zaghbib-Turki, 1990; Kanazawa, 1992; François & David, 2006; Saitoh & Kanazawa, 2012), as well as traits, in which modifications are traditionally regarded as being the result of a continuous process in the evolution of *Micraster* (e.g. ornamentation of the periplastronal ambulacrals and theinterradial area of the paired petals, projection of the labrum, shape of the test) (Rowe, 1899; Nichols, 1959; Ernst, 1970, 1972; Fouray, 1981) (Table 1).

In a second part, the populations were tested for the presence and differences in the level of fluctuating asymmetry (FA) to assess if both populations deviated in their degree of developmental stability. For FA analyses, morphometric (periplastronal ambulacrals, paired petals) and meristic traits (the amount of pore pairs in the anterior and posterior paired petals) were considered.

4.2. Material and Methods

4.2.1. Material

In total, 126 specimens were studied (File A1), which originate from the coastline of Liencres (northern Cantabria, Spain, 49 specimens), from the abandoned Grimberg (IV) mine shaft (27 specimens), close to Bergkamen (Westfalia, Germany), and from the vicinity of Erwitte (Westphalia, Germany, 50 specimens). The material from the Erwitte area was collected by Ekbert Seibertz (Wolfsburg, Germany) during the construction of the highway A44 in the 1970s. The specimens from the Liencres and the Erwitte areas originate from a quasi-isochronous short-term interval of 2 m

morphometric traits	**landmarks**	**morphological function**	**evolutionary significance**
position of the apical shield	1	growth dependent in several taxa, possible related to burrowing strategy (Nichols, 1959; Smith, 1984)	shift in position from anterior to posterior (Rowe, 1899; Ernst, 1970, 1972; Stokes, 1975; Fouray, 1981; David & Fouray, 1984; Smith & Wright; 2012)
shape of the paired petals	2-9	associated tube feet are related to gaseous exchange (Smith, 1980)	increase of length (Rowe, 1899; Stokes, 1975; Smith & Wright; 2012)
shape of the plastron (sternal plates)	10, 12, 14-19, 21-22	related to locomotion behaviour, nature of the substrate respectively (Kanazawa, 1992; Saitoh & Kanazawa, 2012)	not considered so far
position of the periplastronal ambulcral plates	11-15, 19-23	not known	possible shift into the anterior direction, and elongation of the perilabral plate, compare (Smith & Wright; 2012)
depth of the anterior notch	24-26	related to feeding strategies (Nichols, 1959)	a trend towards a deepening of the notch (Nichols, 1959)
position of the ambitus	24-28	related to burrowing depth (Kanazawa, 1992)	lowered in the *Gibbaster* branch (Ernst, 1970, 1972)
position of the widest point of the test	27-28	not known, possible related to burrowing strategy (Smith, 1984)	shift towards the posterior direction (Rowe, 1899; Ernst, 1970, 1972; Stokes, 1975; Fouray, 1981; David & Fouray, 1984; Smith & Wright; 2012)
position of the periproct	30	related to burrowing depth (Kanazawa, 1992)	lowered in the *Gibbaster* branch (Ernst, 1970, 1972)
non-morphometric traits		**morphological function**	**evolutionary significance**
pore numbers in the paired petals		associated tube feet are related to gaseous exchange (Smith, 1980)	increase in numbers (Rowe, 1899; Stokes, 1975; Smith & Wright; 2012)
structure in the interradial area in the paired petals		not known	accentuation of the interradial structure and ornamentation (Rowe, 1899; Ernst, 1970, 1972; Stokes, 1975; Fouray, 1981; David & Fouray, 1984; Smith & Wright; 2012)
structure in the periplastronal ambulcral plates		not known	accentuation of the granulation (Rowe, 1899; Ernst, 1970, 1972; Stokes, 1975; Fouray, 1981; David & Fouray, 1984; Smith & Wright; 2012)
projection of the labrum		not known, assumed to be related to a change in feeding strategy (Nichols, 1959)	increase of the projection of the labrum, accordingly the peristomal opening is completely covered (Rowe, 1899; Ernst, 1970, 1972; Stokes, 1975; Fouray, 1981; David & Fouray, 1984; Smith & Wright; 2012)

Table 1. Investigated traits, their function and evolutionary significance.

thickness (equivalent to approximately 30,000 years) from the basal *Cremnoceramus crassus crassus/Cremnoceramus deformis deformis* Zone, early Coniacian (compare (Kaplan & Kennedy, 1994; Wiese, 1997). The Grimberg material is from the lower *Cr. crassus crassus/Cr. deformis deformis* Zone, collected between sinking depths of 286 and 282 m (Tröger, 1974), corresponding to

an interval of approximately 60,000 years. Although *M. brevis* is very abundant in each of the studied localities, specimens are often distorted or incomplete, which limited the sample size in this study. For the comparison of shape variations, 86 specimens in total were analysed: 14 from the Grimberg VI mine shaft, 37 from the surroundings of Erwitte, and 35 from the Liencres area. To explore the shape variability of all populations, a Principal Component Analysis (PCA) was conducted.

For FA analysis, specimens that were not adequately preserved (slight deformations) were excluded, resulting in 33 specimens from Erwitte and 35 from the Liencres area. The material from the Grimberg mine shaft was insufficient in numbers to give reliable results, and omitted for this study.

Semiquantitative analyses were conducted by inclusion of 121 specimens in total (46 Liencres area, 49 Erwitte area, 26 Grimberg area) to examine the development of the subanal fasciole, the variation of the interradial structure of the paired petals, and the granulation of the periplastronal area.

For analysis of the projection of the labrum, data from 96 specimens were obtained (33 Liencres area, 46 Erwitte area, 17 Grimberg area). Due to the fragile nature of the labrum tip, the extension of the labrum was not considered in the morphometric analyses, as it would have reduced the available material.

To investigate for differences in variation of the average count and variation in FA of pore pair numbers, 35 specimens were included for the Erwitte population and 36 specimens for the Liencres population. The same material was used to test for FA in pore pair numbers in the anterior and the posterior paired petals.

4.2.2. Institutional abbreviations

To denote the repositories of specimens illustrated and/or referred to in the text, the following abbreviations are used: (MB.E.) Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Berlin, Germany; (BGR) Bundesanstalt für Geowissenschaften und Rohstoffe Berlin, Berlin, Germany; (GSUB) Geowissenschaftliche Sammlung der Universität Bremen, Bremen, Germany.

4.2.3. Methods – geometric morphometrically based analyses

In order to realise 3D models of the material, specimens were mounted on a stick and positioned on a turntable, and photos were obtained from all available perspectives of the echinoids by rotating the turntable across small angles. Overview photographs were supplemented by close-ups for capturing highly detailed 3D models. For photogrammetric reconstructions resulting in digital three-dimensional models, the images were elaborated with Autodesk® 123D® Catch. Each 3D model was reconstructed by implementing and aligning 70 2D images. This process results in a mesh, which can be exported as

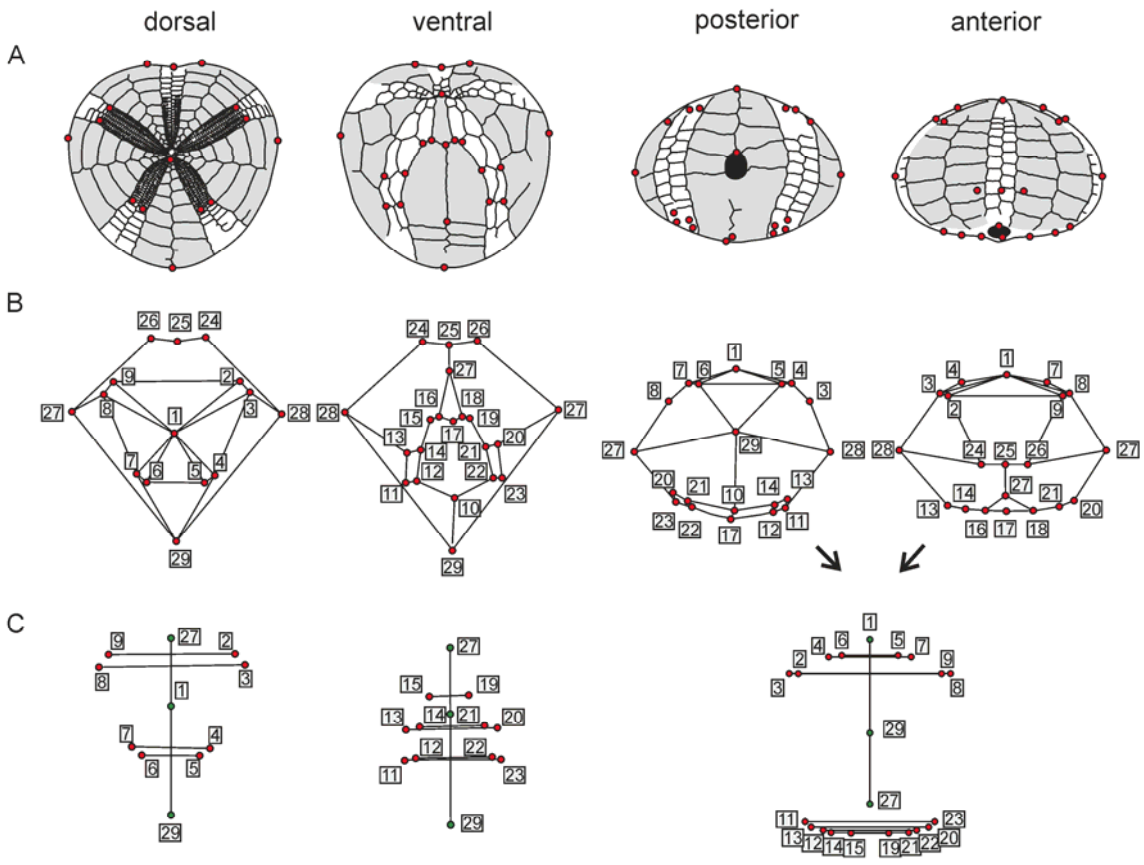

Figure 3. Landmark configurations. (A) Plating drawings (after specimen GSUB E3867) depicting the landmark configurations for the specific analyses. (B) Global shape variation, shown as wireframe graph. (C) Fluctuating asymmetry analysis. For FA analysis: red circles represent paired landmarks, green circles represent median landmarks.

a textured object file that features the photographic detailed surfaces of the specimen. These files were applied for further data collection for geometric morphometric purposes. For a more detailed and practical guide for photogrammetry, it is referred to the works of Falkingham (2012) and Malison and Wings (2014).

Landmarks were digitised in the freeware MeshLab (Visual Computing Lab - ISTI - CNR; http://meshlab.sourceforge.net/) from the textured 3D models. The Cartesian coordinates were analysed with the morphometric software package MorphoJ (Klingenberg, 2010). Prior to each analysis a Procrustes fit was performed, in which the shape of each specimen was rescaled to unit centroid size, which removes information on size. Centroid size is the square root of the sum of squared distances from a configuration of landmark to the centre of the shape configuration (centroid) (Bookstein, 1991). The rescaled shapes are then translated to the same position, and rotated to the best fitting orientation of the landmark configurations. Shape variations within the whole sample were investigated through a PCA by assessing a set of 30 landmarks (Fig. 3).

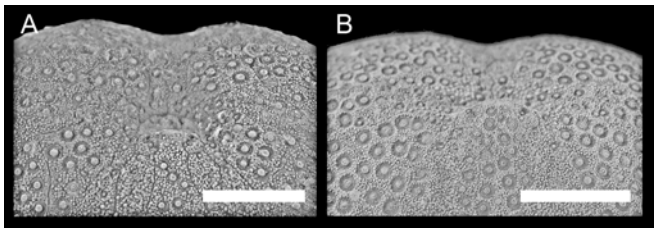

Figure 4. Variation in the projection of the labral plate. (A) Weakly projecting, not covering the peristomal opening (GSUB E3847, Erwitte area, Germany), (B) strongly projecting, covering completely the peristomal opening (MB.E.8251, Liencres area, Spain). Scale bars equal 1 cm.

A Procrustes ANOVA was performed to assess for the possible presence of FA and to quantify the relative amounts of shape variation in asymmetry and measurement error (Klingenberg, 1998). Because measurement error can inflate FA, it is preferred to test for its significance. The Procrustes ANOVA contains the factor sides (fixed) and individuals (random). Directional asymmetry (DA) is tested by the side factor, while FA is estimated by the individual-by-side interaction term. Occurrence of antisymmetry was estimated by examining the scatter plots of shape asymmetry for bimodality. Other asymmetric variations, e.g. DA, and antisymmetry are not suitable for measuring developmental instability as they have a genetic component and should be therefore avoided in such studies (Palmer, 1994). As the studied traits here have object symmetry (objects which are symmetric by themselves), tests for size dependency of FA are precluded (Klingenberg, 2002). Accordingly, only shape FA can be considered here. A set of replicated models of each specimen was used to quantify the measurement error. For the analysis of FA, 21 landmarks, 18 paired and 3 median landmarks (Fig. 3), were digitised. Only landmarks of type 1 (Bookstein, 1991) were chosen, which were precisely defined by intersections with other plate sutures, or very well locatable. The landmark configuration included the shape of the periplastronal ambulacrals and paired petals. The Procrustes ANOVA was independently applied for the Erwitte and the Liencres populations. In both samples a Procrustes ANOVA was computed for the global shape and for each trait separately (periplastronal ambulacral plates, paired petals). Levene's test was performed to test for differences in FA among populations, which is more robust to departures from normality (Palmer, 1994). To visualise and investigate shape variations, a PCA was conducted for each population, which took symmetric and asymmetric components into account.

4.2.4. Methods – variation in non-morphometric character

4.2.4.1. Subanal fasciole, projection of the labrum, interradial structure of the paired petals, and granulation of the periplastronal area

To define the degree of coverage by the labrum and the projection of the labrum, three descriptive states were used: *i)* open peristome (Fig. 4 A), *ii)* peristome is completely covered (labrum reachs the frontal margin of the peristome), and *iii)* the labrum exceeds significantly the margin of the peristome (Fig. 4 B). In terminology for the development of the subanal fasciole, Néraudeau and colleagues (1998) was followed. According to the terminology and classification of Rowe (1899), Fouray (1981) and Olszewska-Nejbert (2007), the populations were studied to compare the development of the interradial structure of the paired petals and the granulation of the periplastronal area.

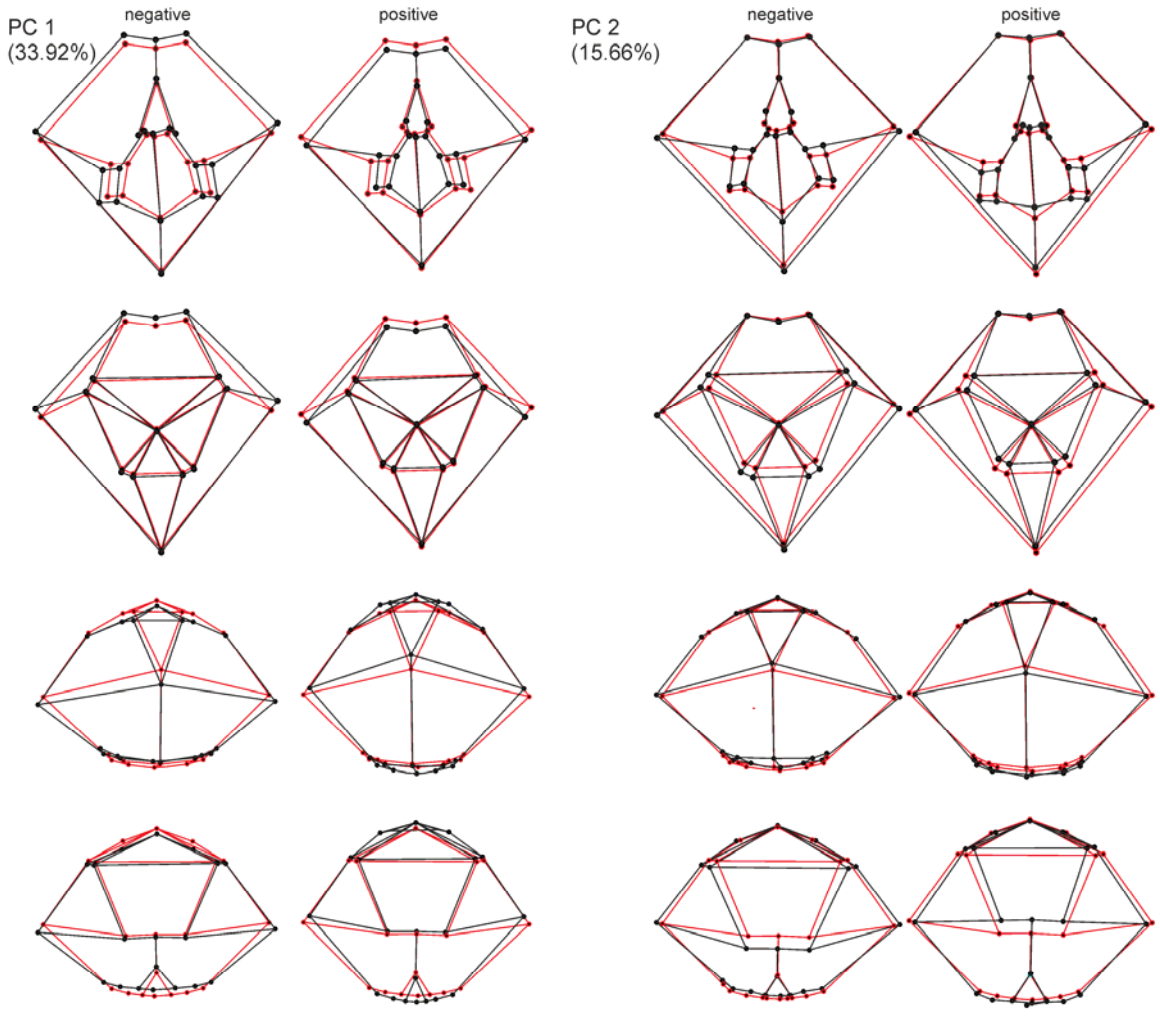

Figure 5. Global shape variation. Wireframe graphs show the shape changes from the mean shape (red) to shape changes associated within PC1 and PC2 with a negative and positive direction.

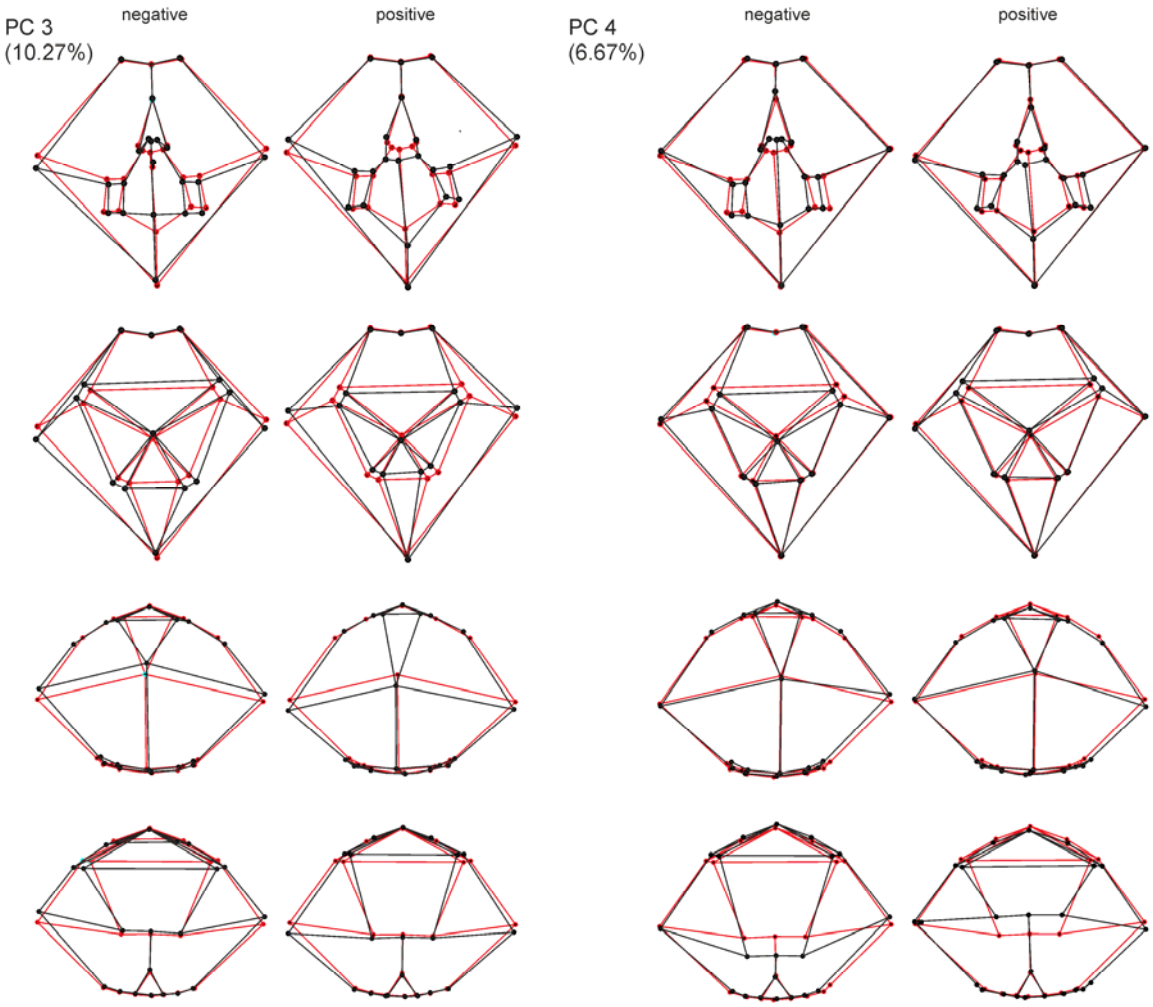

Figure 6. Global shape variation. Wireframe graphs show the shape changes from the mean shape (red) to shape changes associated within PC3 and PC4 with a negative and positive direction.

4.2.4.2. Pore pair numbers versus test length

The number of pore pairs from the Erwitte and the Liencres populations were counted with the help of the image analysis software ImageJ (Schneider et al., 2012). The pore number averages of the anterior and the posterior paired petals of each specimen [(R+L)/2] were plotted against the test length. An ANCOVA was computed to assess for significance of differences between slopes among the populations.

4.2.4.3. FA analysis of pore pair numbers in the anterior and the posterior paired petals

The counts of the pore pairs were not repeated, as they can be confidently performed without any measurement error. Grubb's test was applied to check for outliers, as outliers otherwise could artificially inflate the results of FA. Prior to FA analyses, a size dependency of FA was tested by

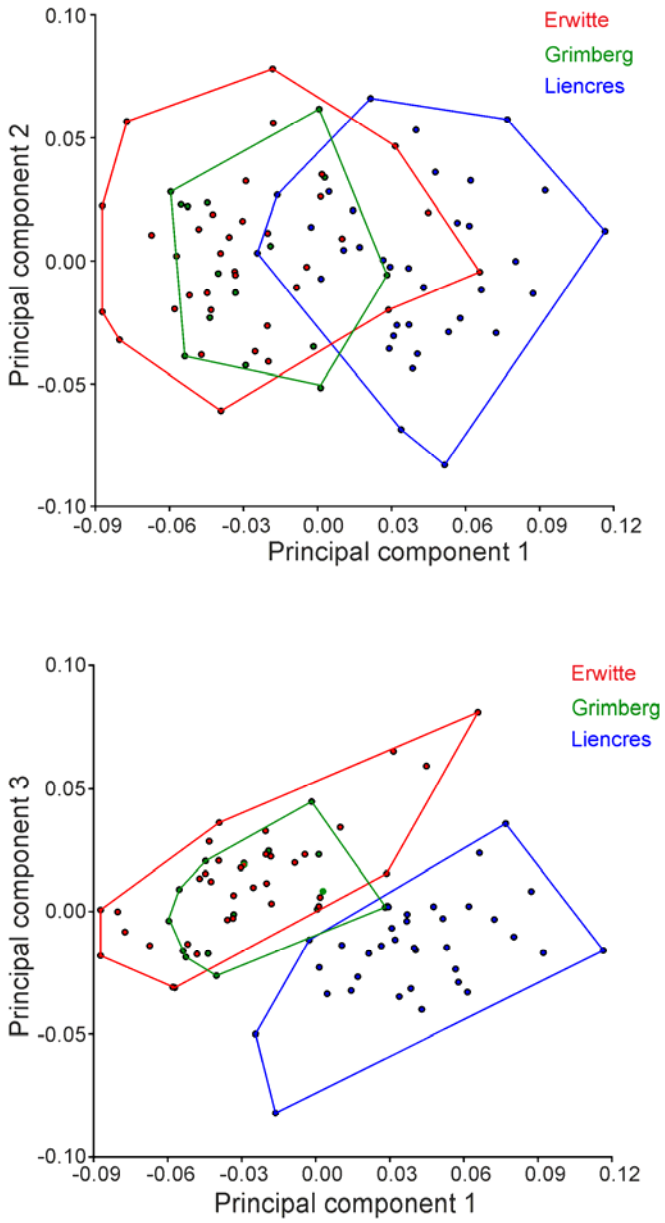

Figure 7. Principal component analysis scatter plots. (A) PC1 versus PC2. (B) PC1 versus PC3.

calculating a Spearman rank correlation of unsigned values and averaged trait size. FA, which varies with trait size, could otherwise obscure the analysis (Palmer & Strobeck, 1986). Normal distribution was assessed with a Shapiro–Wilk test, to test for ideal FA, since the property of FA is a normal distribution of right side–left side differences with a mean of zero, and hence to exclude the possible occurrence of antisymmetry (Palmer & Strobeck, 1986). A one-sample t-test was performed to test if the data sets deviate from a mean of zero, in which case DA would be present.

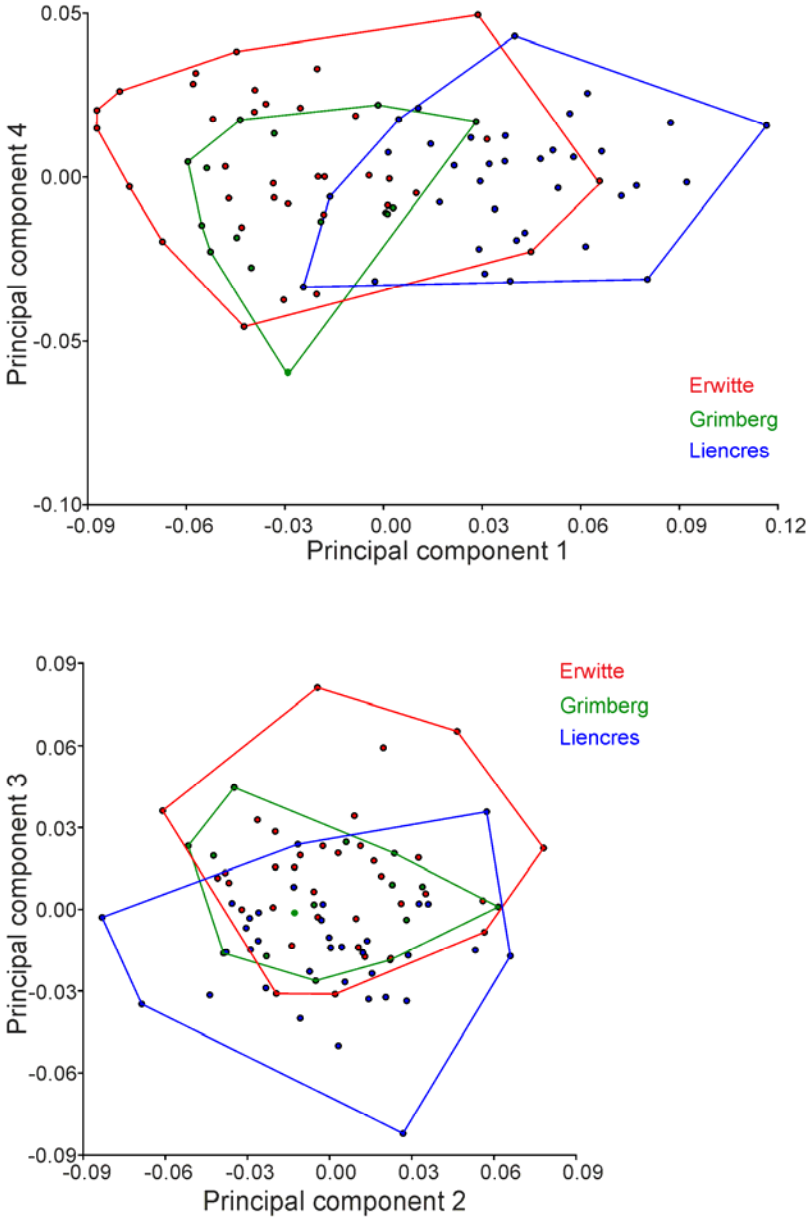

Figure 8. Principal component analysis scatter plots. (A) PC1 versus PC3. (B) PC2 versus PC3.

Following Palmer and Strobeck (1986; 2003), two indices were computed to estimate developmental instability. FA1 displays the mean of unsigned differences between the right and left sides [mean |R-L|], and FA4a assesses the variance within a given trait [0.798√var (R–L)].

The FA4a index is a modified version of the FA4 index, which has the advantage (compared to FA1) of not being biased by the presence of DA (Palmer, 1994). If DA is present, the influence of DA on the asymmetry values could be assessed by comparing DA, as the mean (R–L), with the value of FA4a. In the case of DA not exceeding FA4a, the variation in the trait is mainly due to

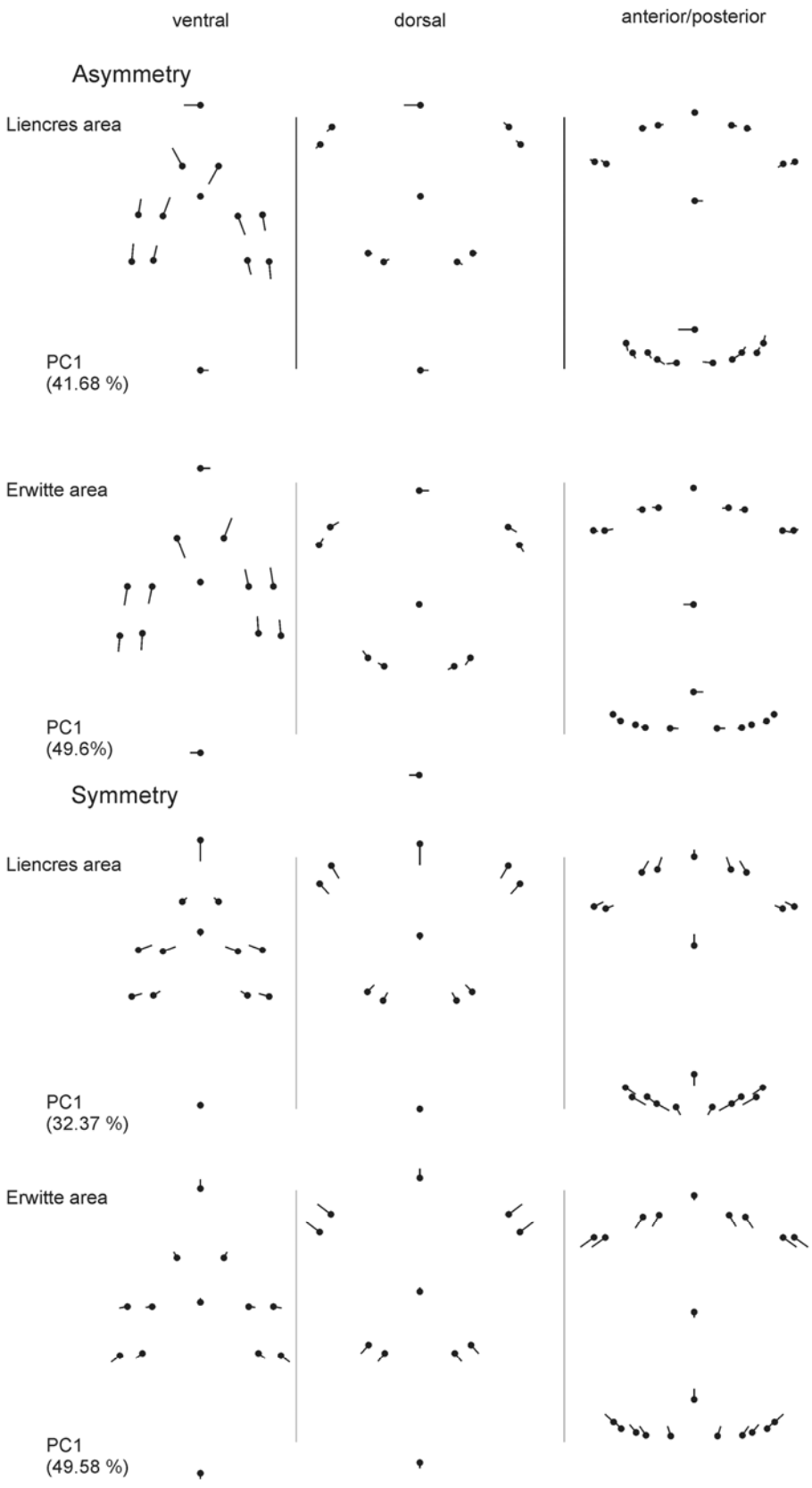

Figure 9. Shape variation of the asymmetric and symmetric component. Shape variation on PC1 for the population from the Liencres and the Erwitte area.

developmental instability (Palmer and Strobeck, 2003). Levene's test was conducted by using the unsigned values to evaluate differences of FA among populations.

4.3. Results

4.3.1. Morphometric variation

4.3.1.1. Shape analysis

The first four PCs account for 66.53% of the total shape variance (Figs. 5 and 6). PC 1 reveals the best separation between the German populations and the Spanish population (Figs. 7 and 8); they reveal, however, some overlap, but a larger dispersion of the Spanish population is found along the positive direction of PC 1 — both German populations disperse rather in a negative direction. The latter both have in any of the describing principal components large overlaps.

PC 1 (33.92% of total variance) is linked in a positive direction with a higher positioned periproct and ambitus, and the widest point of the test is set in the posterior position. The sternal plates are generally slimmer and shorter, the periplastronal ambulacral plates are set more in the anterior direction, and the plastronal area is more inflated. Furthermore, the shape of the test is more tumid, less elongated, and taller with a strongly positive PC 1.

PC 2 (15.66% of total variance) is associated in a positive direction with a posterior shift of the periplastronal ambulacral plates, combined with shorter sternal plates. The paired petals are less extended. PC 3 (10.27% of total variance) pertains to shape variations with a change in a positive direction in a lower-levelled ambitus and periproct. Furthermore, the widest point is more anteriorly situated, and the more asymmetric sternal plates are found in a rather anterior position and have a broader contact to the labrum. The periplastronal ambulacral plates moved in the anterior direction, and the paired petals are shorter by a concurrent shift of the apical shield closer to the anterior margin than in relation to a negative direction.

In the positive direction of PC 4 (6.67% of total variance) a change in shape is illustrated mainly by a lower test with a higher position of the deepest impression in the notch, and the maximum width is found in the posterior direction. The apical shield is closer to the anterior notch, combined with longer anterior paired petals. The sternal plates are more asymmetric than in a change in the negative direction, and are in a broader contact to the labral plates; the periplastronal ambulacral plates are positioned in the anterior direction.

4.3.1.2. Fluctuating asymmetry analysis

Directional asymmetry is statistically significant in the Erwitte population for the global shape variation and for the separately analysed periplastronal ambulacrals and paired petals; in the Liencres population, DA is significant in the paired petals, in the global shape variations, and in the periplastronal ambulacrals it is significant only at a *P*-value level of 0.05. FA is significant for all analysed shape variations in the Erwitte and in the Liencres populations (Table 2). The measurement error is compared to the interaction term (individual-by-side) is only minor throughout and is therefore negligible. In both populations, FA is most conspicuous in the oral ambulacral plates and shape changes refer mainly to shifts of the periplastronal ambulacral plates along the posterior–anterior axis (Fig. 9, see Figs. A2, A3, A4 for PC2, PC3, PC4 respectively), whereas variations in the symmetric component are largely associated with the shape of the plastron. Differences in the amount of FA among both populations (see Table A1-3 for individual FA scores) were for all shape variations not significant, as revealed by Levene's test (global shape: $P = 0.49$; periplastronal ambulacrals: $P = 0.87$, paired petals: $P = 0.37$).

4.3.2. Variation in non-morphometric characters

4.3.2.1. Subanal fasciole, projection of the labrum, interradial structure of the paired petals, and granulation of the periplastronal area

An obvious trend is seen in the development of the subanal fasciole, which is most pronounced in the Liencres population (Fig. 10 A, Table A4), where a trace of a subanal fasciole is always present. In a large proportion of the Grimberg and Erwitte populations, on the other hand, no subanal fasciole could be detected (46% and 41% respectively, Figs. 10 A and 11 A). Likewise, more complete types of fasciole development are found in specimens from the Liencres area (parafasciole: 41%, Fig. 11 C; orthofasciole: 7%, Fig. 11 D, Table A4). In both populations, specimens having a parafasciole were only found in the Grimberg material; orthofascioles are completely missing.

A similar tendency is found for the projection of the labrum. A minor part of the Liencres material has an uncovered peristome. Predominantly, the labrum is covering and/or even exceeding the margin of the peristome. This is in contradiction to the observations made in the German populations, in which the majority of the specimens have only weakly projecting labral plates; the peristomes are, to a large extent, uncovered. No significant differences between all populations were observed in the development interradial structure of the paired petals (Fig. 12) and the granulation of the periplastronal area (results are not shown).

Population	Trait	Effect	SS	MS	df	F	*P* (param.)
Erwitte area							
	general shape	Individual	0.26086047	0.00054346	480	6.76	<0.0001
		Side	0.0053173	0.00037981	14	4.72	<0.0001
		Individual x Side	0.03602375	8.041E-05	448	38.12	<0.0001
		Measurement error	0.00201864	2.1093E-06	957		
	periplastronal ambulacralia	individual	0.28937101	0.00100476	288	3.86	<0.0001
		side	0.01070259	0.00133782	8	5.14	<0.0001
		Individual x Side	0.06665542	0.00026037	256	68.21	<0.0001
		Measurement error	0.00214141	3.8171E-06	561		
	paired petals	Individual	0.18856263	0.0009821	192	10.21	<0.0001
		Side	0.0028151	0.00056302	5	5.85	<0.0001
		Individual x Side	0.01538751	9.6172E-05	160	16.46	<0.0001
		Measurement error	0.00212048	5.8416E-06	363		
Liencres area							
	general shape	Individual	0.21060018	0.00041294	510	5.68	<0.0001
		Side	0.00283036	0.00020217	14	2.78	0.0005
		Individual x Side	0.03462216	7.2736E-05	476	36.89	<0.0001
		Measurement error	0.00200114	1.9716E-06	1015		
	periplastronal ambulacralia	Individual	0.26783721	0.00087529	306	3.52	<0.0001
		Side	0.00539528	0.00067441	8	2.71	0.0069
		Individual x Side	0.06765032	0.00024871	272	60.98	<0.0001
		Measurement error	0.00242686	4.0788E-06	595	-0.01	
	paired petals	Individual	0.1466068	0.00071866	204	7.31	<0.0001
		Side	0.00283686	0.00056737	5	5.77	<0.0001
		Individual x Side	0.01671819	9.8342E-05	170	17.41	<0.0001
		Measurement error	0.00217429	5.6475E-06	385		

Table 2 Procrustes ANOVA results for the populations from Erwitte and Liencres area.

4.3.2.2. Pore numbers versus test length

The slope for the anterior paired petals of the Liencres population differs somewhat from the Erwitte population (0.93 and 0.90 respectively, Fig. 13). The ANCOVA, however, revealed no significant differences among both populations ($P = 0.57$). Similar results are found for the posterior paired petals, and the slope in the Liencres population is larger (0.93) than in the Erwitte population (0.82), but insignificantly different ($P = 0.45$).

4.3.2.3. Fluctuating asymmetry analysis for the pore numbers in the paired petals

The values of each FA index and mean asymmetries are given in Table 3 (see Table A6 for individual FA values). One outlier from the set of posterior paired petals was excluded from the following analyses according to Grubb's test. All studied traits reveal a normal distribution ($P > 0.05$); accordingly, antisymmetry is not present in the studied traits. A correlation of FA with the trait size

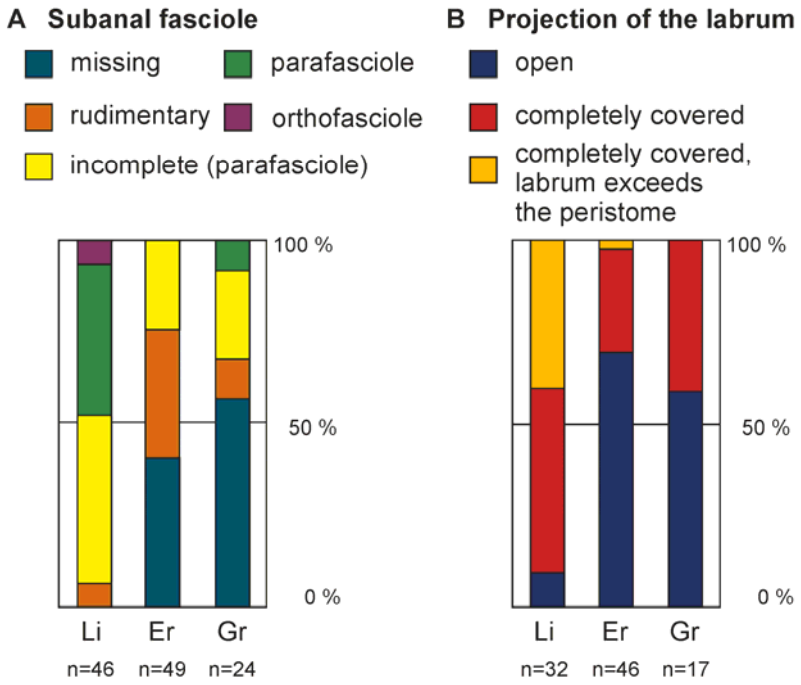

Figure 10. Variation in development of the subanal fasciole. Bar charts indicating the percentage for the particular populations in (A) the development of the subanal fascioles, (B) the development of the projection of the labrum (Li = Liencres area; Er = Erwitte area; Gr = Grimberg).

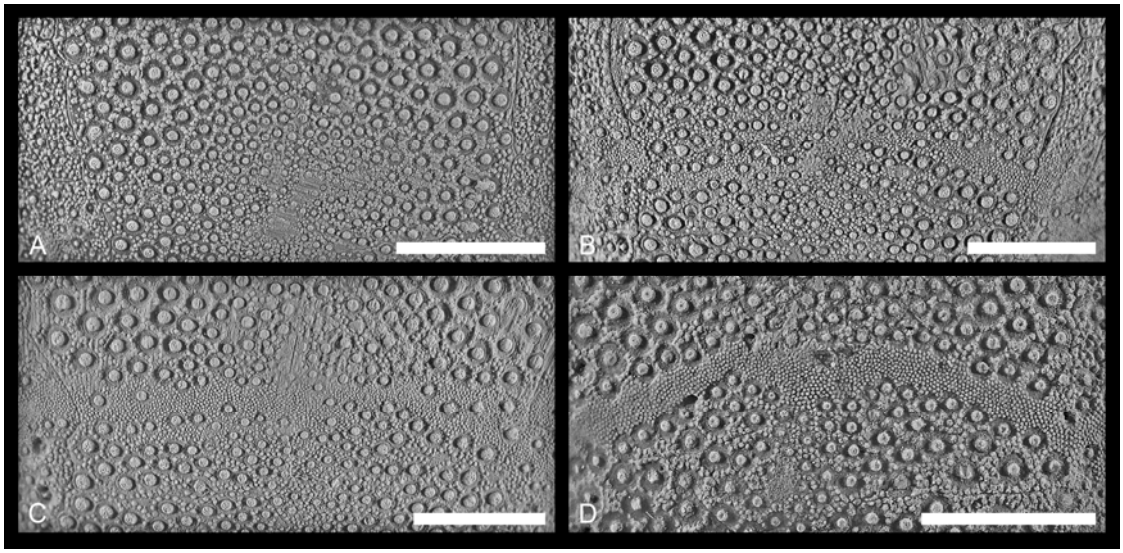

Figure 11. Photographs illustrating variation in development of the subanal fasciole. (A) Not present (GSUB E3847, Erwitte area, Germany). (B) Incomplete (GSUB E3850, Erwitte area, Germany). (C) Protofasciole (BGR X 06195, Grimberg IV shaft, Germany). (D) Orthofasciole (MB.E.3873, Liencres area, Spain). Scale bars equal 0.5 cm.

could neither be detected in the anterior nor in the posterior paired petals. The differences of FA between the Erwitte and Liencres populations in the posterior petals are not significant, as concluded by Levene's test ($P = 0.84$); DA could not be detected. The presence of DA in the anterior petals was

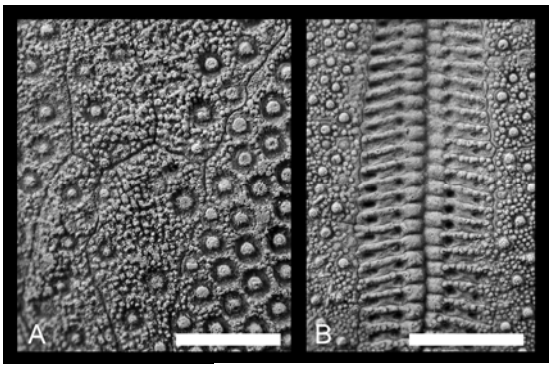

Figure 12. Development in the structure of periplastronal area and the interporiferous area of the paired petals. (A) Granular periplastronal area (MB.E.8196, Liencres, Spain). (B) Subdivided interporiferous area of the paired petals (GSUB E3867, Erwitte area, Germany). Scale bars equal 0.5 cm.

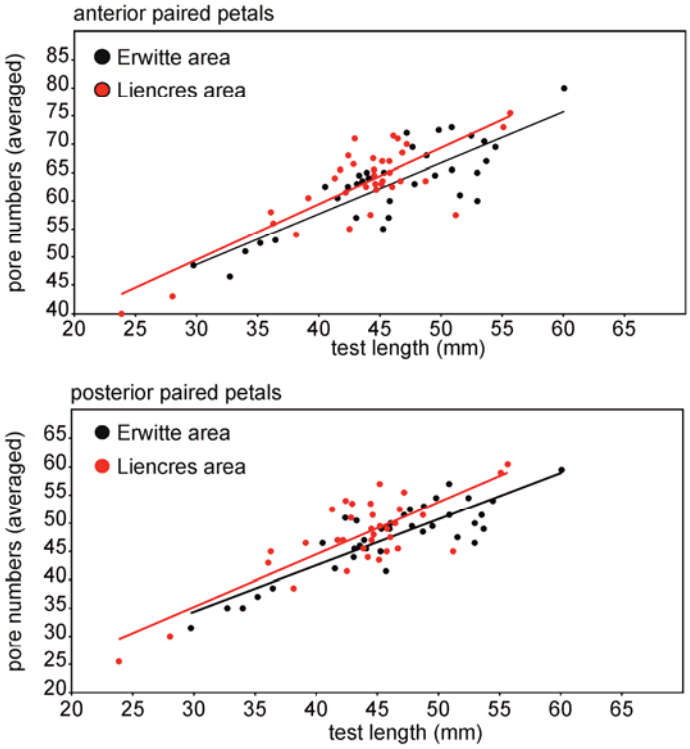

Figure 13. Bivariate scatter plot of the numbers of pore pairs against test length.

confirmed by a t-test in both samples. Estimated by a comparison of the mean (R–L) and FA4a for each sample, the values of the FA4a are larger than the mean (R–L) in both cases; accordingly, the asymmetry variation accounts largely for FA. The FA scores (FA1 & FA4a) for the anterior petals in the Liencres population are significantly higher ($P < 0.001$) than in the Erwitte population (Table 3 and Fig. 14). The range in between-sides differences in this trait is sufficient for avoiding biased estimates of FA, as suggested by Swain (1987) for the reliability of meristic traits to assess developmental instability.

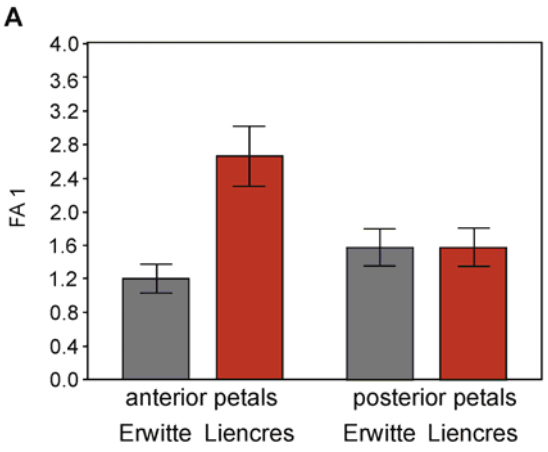

Figure 14. FA1 scores of the pore pairs numbers. Bar charts of the FA scores (FA1 index) for pore counts of the anterior and the posterior paired petals of the Liencres and the Erwitte population.

4.4. Discussion

4.4.1. Shape variation

The Spanish and the German populations of *M. brevis* are distinguished by the displacement of the periproct and the ambitus, which are generally in a higher position in the Spanish specimens. Furthermore, the shape of the plastron is slimmer, shorter and more inflated in the Liencres population than in the German populations. The latter populations show a large overlap in their morphospace and are virtually indistinguishable. The morphological differences between the German and the Liencres populations argue for an adaption to the grain size of their host sediments. With regard to the burrowing behaviour, the Spanish and the German populations must have been different. An inflated plastron, like in the Liencres populations, is found in deeper burrowing spatangoids (Saitoh & Kanazawa, 2012). The position of the periproct is related to the burrowing depth of the animal, and deeper burrowing species have a higher positioned periproct than shallow or epifaunal burrowers (Kanazawa, 1992; Olszewska-Nejbert, 2007). The generally higher situated periproct in the Liencres sample supports the interpretation of a deeper burrowing behaviour.

A similar pattern for the variation in the plastron shape in relation to the sediment was found in the extant spatangoid *Echinocardium cordatum* among populations from the waters of Great Britain and New Zealand (Higgins, 1974). Curiously, however, the relation to the grain size is in contradiction to the pattern which can be observed in *M. brevis*.

In *E. cordatum*, however, broader shapes are related to mud and clay, and narrow shapes to sand and gravel. Only the degree in inflation of the plastronal area is in both species linked to coarser substrates. These contradictory outcomes are probably related to differences in the way of locomotion and burrowing mechanisms among both species, which demonstrates interspecific variations (Kanazawa, 1992; Walker & Gagnon, 2014). *Echinocardium cordatum*, for example, is a deeply burrowing species (Kanazawa, 1992), which is in contrast with the assumed shallow burrowing depth

of *M. brevis*. These different patterns in plastron shape are, however, worth to be studied in more detail elsewhere. Regardless of these deviations, both studies show a strong linkage between the substrate and plastron shape. Egea and colleagues (2016) mention that phenotypic plasticity has an influence on the morphology of *E. cordatum*, unfortunately without giving specific examples. Another example of how the morphology in spatangoid echinoids changed in relation to the sediment is given by the fossil *Toxaster granosus kiliani* (Lower Cretaceous) (François & David, 2006). It responded to an increase in the abundance of clasts in the sediment by an inflation of the test and by a higher positioned periproct. This pattern was also observed in *E. cordatum* from northern France (François & David, 2006). These observations correspond to the results found in *M. brevis*.

The phenotypical differences within *M. brevis* either relied on genetic differentiation or were reasoned in phenotypic plasticity. Although phenotypic plasticity is ubiquitous in organisms, it is challenging to draw conclusions about phenotypic plasticity in the fossil record (discussed for the case in ammonoids: De Baets et al., 2015). McNamara and McKinney (1991), Chauffe and Nichols (1995), and West-Eberhard (2003), however, proposed criteria to evaluate phenotypic plasticity in the fossil record. It was argued that phenotypic plasticity can be assumed, for instance, if the modified trait is not occurring uniquely; all forms of (isogenic) taxa would modify in the same way, and other related taxa would modify in a similar way (if being exposed to the same stimuli). This evaluation can be hampered by the fact that phenotypic plasticity, however, is a property of a genotype. Accordingly, the reaction norm (the range of phenotypic expression of a genotype across an environmental variable) can vary within or between populations (Scheiner, 1994). In addition, initially, plastic traits can change by genetic accommodation, hence being fixed (West-Eberhard, 2003; Braendle & Flatt, 2006; Suzuki & Nijhout, 2006). Nevertheless, the relationship between the position of the ambitus, the periproct respectively, and the grain size in fossils *T. granosus kiliani*, *M. brevis* and the extant *E. cordatum*, as mentioned above, suggests an influence of phenotypic plasticity. This is possibly true for the development of the plastron shape as well, but further confirmation is needed.

The populations of *E. cordatum* from New Zealand and Great Britain, analysed by Higgins (1974) with respect to the plastron shape, are yet genetically divergent (Egea et al., 2016), but whether these genetic differentiations contributed to the shape variations is unclear.

The high and rapid dispersal potential in *Micraster*, due to their planktotrophic larvae (Cunningham & Jeffery Abt, 2009), would have enabled a gene flow between the Spanish and German populations. However, gene flow between both areas may have been asymmetric; several examples suggest that a north to south directed migration of taxa existed during the Late Cretaceous (Wiese & Voigt, 2002). In the case of the cryptic species complex *E. cordatum* (Chenuil & Féral, 2001; Egea et al., 2016), planktotroph larvae allowed for widespread settlement of genetically homogeneous clades. However, the possibility of genetic variation in *M. brevis* cannot be totally excluded.

Other shape variations are shared by all three populations and cannot be referred to distinct habitats. Thus, insofar as it is not possible at present to associate these variations with an environmental gradient, their development may be attributed to genetically influenced variation.

4.4.2. Fluctuating asymmetry

Environmental stressors, like assumable temperature clines, potentially could have contributed only to a negligible amount of FA. Moreover, shared inheritable factors (e.g. effects of non-additive gene–gene interactions, see Klingenberg & Nijhout, 1999) could have contributed to similar patterns and degrees of FA in the periplastronal ambulacrals found in both populations. This is, of course, very speculative and needs to be discussed further by inclusion of more populations of different habitats. Additionally, assessments on phenotypic variance can be biased by time averaging, which potentially inflates phenotypic variance within larger time intervals (Hunt, 2004). However, it was tried to limit this bias by restricting the time interval under study as much as possible.

Remarkably, variation within individuals can exhibit conspicuous variability in the periplastronal ambulacral plates, in which one plate can be very shortened, while the opposing plate can show a very extended shape (Fig. 15). These within-individual variations reflect intra-populational variation in the development of these plates, as described by the first four principal component factors for global shape variations. The pattern of the symmetric shape and the asymmetric shape variation, however, are not congruent. This disagreement is possibly explained by the fact that a large amount of variation is attributed to the shape variation in the plastron, which is associated with the position of the periplastronal ambulacral plates. These stochastic variations might give some clues to the developmental process of this trait. Inferred from the probability that these extreme asymmetries are a result of stochastic gene expression, i.e. fluctuations in the amount of a gene product (Chalancon et al., 2012), it suggests that the (symmetric) variation in these plates was a result of homometry (changes in the amount of gene products, see Arthur, 2000). McNamara (1987), however, suggested that displacement in the periplastronal ambulacral plates of spatangoid echinoids is reasoned in allometry. Studies on allometric variation in this trait of *M. brevis* are needed to give better ideas on their development.

A study on FA of two populations of *Echinocardium flavescens* (Saucède et al., 2006) has shown that variations in the periplastronal ambulacrals are most intense along the main growth (longitudinal) axis and are, to some extent, constrained to this direction. This suggests that shape variations of FA in the periplastronal ambulacrals are, to some degree, under common developmental constraints. The amount of FA in *E. flavescens*, however, generally increased along the anterior–posterior axis, unlike in *M. brevis*, where the anterior landmarks are more influenced by FA. This assumes that regulatory mechanisms between *M. brevis* and *E. flavescens* are partially different. DA is

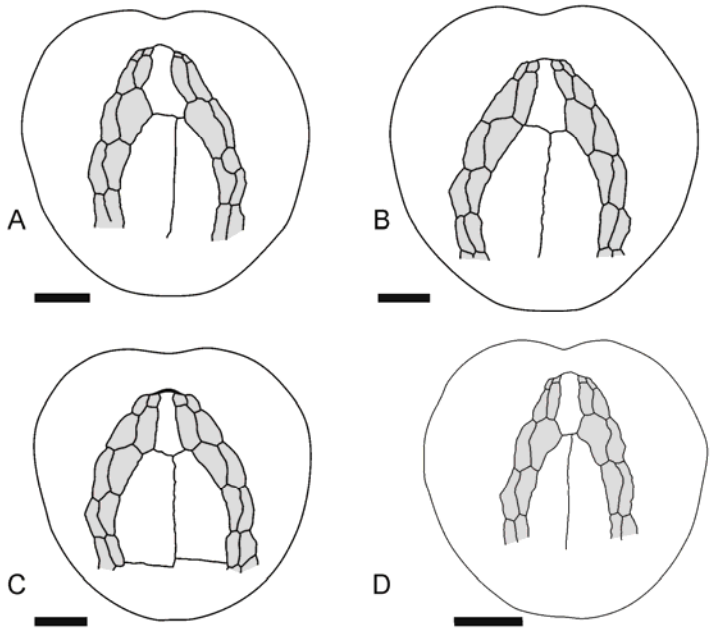

Figure 15. Sketches illustrating the variation in the periplastronal ambulacral plates. (A-B) Conspicuous asymmetric development. (C-D) Symmetric development, with longer ambulacral plates (C) and shorter plates (D) next to the labrum (A: MB.E.8196, Liencres area, Spain; B: GSUB E3847, Erwitte area, Germany; C: GSUB E3867, Erwitte area, Germany; D: MB.E.8257, Liencres area, Spain). Scale bars equal 1 cm.

thought to be ubiquitous in the skeleton of irregular echinoids, which is assumed to be related to the asymmetry of the digestive system (Lawrence et al., 1998, ;Stige et al., 2006). Accordingly, the occurrence of DA in at least one sample (Erwitte population) is not surprising.

4.4.3. Variation in non-morphometric characters

4.4.3.1. Subanal fasciole and peristome coverage by the labrum

The subanal fasciole is better developed in the Liencres specimens, while it is often totally absent in the German populations. The task of the subanal fasciole is to provide a water current, by movement of the ciliated spines, and thus to sweep the faeces away from the body (Smith, 1984). A sustainment of such a feature is more reasonable at a deeper burrowing depth. The development of the fasciole had been linked to the nature of the sediment in several taxa of fossil spatangoids by previous authors (Higgins, 1974; Néraudeau et al., 1998), which is in agreement with the present observations on *M. brevis*. Néraudeau (2001) already stated that the occurrence and development of fascioles in general are influenced by phenotypic plasticity. Fascioles (e.g. subanal, or lateral fascioles) in fossil spatangoids are better developed if these inhabited finer grained sediments, whereas in coarse grained sediments fascioles tend to be lesser developed, or even become lost. As this pattern is in an inverse relation to the here recognised variation, it has to be considered that the need for fascioles is influenced by the burrowing behaviour (Smith, 1984), fascioles are generally rather found in deeper burrowing taxa. Accordingly, a need for fascioles corresponds to permeability of the sediments (governed by e.g. grain size) and burrowing depth. According to the tendency for better-developed

subanal fascioles in the Liencres population, it is probably related to the foregoing burrowing depth, due to coarser-grained sediment.

It is unclear, however, to which function the projection of the labrum is related (De Ridder & Lawrence, 1982). Nichols (1959), on the other hand, interpreted this development as being associated with a change in feeding habits, from a food supply from underneath the peristome towards a transport of particles from the surface to the peristome via the frontal notch. However, so far there is no clear evidence in support of this idea.

A stronger projection of the labrum can be observed within the evolution from *M. leskei* (late Turonian) to *M. coranguinum* (late Coniacian), e.g. (Smith, 1984). *Micraster brevis* is assumed to be a side branch of this lineage (Ernst, 1970, 1972); thus, it is remarkable that a change of the labrum projection apparently took place independently in different species of *Micraster*. Accordingly, the development of the labrum in *Micraster* would then be homoplastic, as a result of either parallelism or convergence. An increase in the projection of the labrum also appeared in other spatangoid lineages, e.g. McNamara & Philip (1980), which supports the idea of homoplastic development in this trait. Moreover, the here-observed differences in the degree of projection in the labrum between the populations argue for a large influence of the environment on the development of the labrum. To what degree this development was mediated through phenotypic plasticity or genetically determined must remain unclear without comparable case studies.

Interestingly, the interradial structure of the paired petals and the granulation of the periplastronal area are the most invariant traits considered here. This finding could indicate low genetic variation, and/or low environmental influences, or high developmental stability, or canalization, which buffers against any perturbations.

4.4.3.2.Pore numbers versus test length

Elongated pores such as those in the paired petals of *Micraster* are linked to ambulacral tube feet, which are specialised for gaseous exchange (Smith, 1980). Accordingly, as suggested by the works of Stokes (1983) and Zaghbib-Turki (1990), an adaptation towards higher temperatures in southern palaeolatitudes, by an increase in the petaloid pore pair numbers, could have been expected in the population from the Liencres area. However, no significant differences between the populations could be found. This could argue either for insufficient statistical power or for similar temperatures in both realms. Unfortunately, reconstructions of palaeotemperatures of the early Coniacian of these areas are not available.

4.4.3.3. Fluctuating asymmetry analysis for the pore pair numbers in the paired petals

The pore numbers in the anterior paired petals show a higher amount of FA in the Liencres population, which indicates a higher level of developmental instability, caused by either environmental factors or genetic stressors. For instance, possible higher sea temperatures could have posed an environmental stressor in this case, as water temperatures and oxygen content are intimately linked, and must have served as a selective force for this trait. It is suggested (Møller & Swaddle, 1997; Karvonen et al., 2003) that functionally important traits are more stable in development and, thus, reveal only low levels of FA, as they would be subjected more to stabilising selection. As the pore pairs in the paired petals have a highly important function, however, it could therefore be assumed that the Liencres population was exposed to non-stabilising selection.

Metric FA analysis of the ambulacral plates, including the paired petals, revealed no significant differences among both populations. This is in contradiction to the results of the meristic analysis, which suggests either insufficient sample sizes to detect significant differences in FA of shape variation, or that different processes in development of the shape and the pore pair numbers were involved. The latter idea is supported by comparisons of individual metric FA scores (computed only for the anterior paired petals) with the meristic unsigned asymmetry values of the paired petals, in which values of either analysis are only weakly to moderately related (see Fig. A5). However, comparisons of FA analysis considering the position and the numerical values of the same trait had different outcomes, in which positional was more sensitive to developmental instability (Polak, 1997), which is inconsistent with findings of the current study.

4.5. Conclusions

This study confirms the assumption that *Micraster* developed local ecophenotypism (Ernst & Seibertz, 1977). It is most likely that especially, the nature of the sediment (e.g. grain size) had a large influence on the morphology of *M. brevis*. The phenotypic variations suggest different burrowing behaviours of the populations in their respective environment. The morphological features in the Liencres population indicate a greater burrowing depth than in the German populations, which likely was attributed to the coarser sediment in Liencres.

Morphological variations in the position of the ambitus, the periproct and the subanal fasciole were most likely influenced by phenotypic plasticity, and potentially also the shape of the plastron. The projection of the labrum was achieved independently in different species of *Micraster*. The findings of more pronounced projecting labral plates in the Spanish sample, however, raise the question to which degree the environment played a shaping force and what kind of factors were involved. Further comparisons to distinct *Micraster* populations/species are required to gain more insights into the dependence between environmental factors and the development of this trait.

Variations due to developmental instability exist in the Liencres and in the Erwitte populations. Differences in the amount of FA, however, were only significant for the numbers of pore pairs in the anterior paired petals. Differences in environmental factors, which could have provoked these higher FA values, are unclear. Temperature gradients, for instance, to which this trait would have most reasonably responded, could not be detected, since data on palaeotemperatures are not available. Similarities among the populations in FA levels of the periplastronal ambulacral plates could have resulted from common perturbations in their developmental regulatory mechanisms.

Other shape modifications (asymmetry in the sternal plates, contact of the labrum and the sternal plates, position in the periplastronal ambulacral plates, position of the apical shield, and the widest point of the test), which were traditionally regarded as being involved in a continuous process of evolution in *Micraster*, revealed no distinct relation to specific populations and, hence, to environmental differences. It is noteworthy that two of the here-studied traits (interradial structure of the paired petals, granulation of the periplastronal) were very robust in their development, as they reveal apparently no variation. The influence of environmental variation, however, was able to create an increase in morphological diversity, which is worth studying in the evolutionary context of the *Micraster* lineage.

Concepts and mechanisms of variability, such as phenotypic plasticity and developmental robustness, are important topics and are of great interest for evolutionary development. The majority of works addressing these concepts, however, relied on extant organisms. Data from the fossil record, however, are invaluable to our understanding of evolution in nature. Accordingly, works on these topics are worth to be extended to the fossil record and have the potential to provide important insights into trends and patterns in evolutionary history, which can be incorporated into ideas of great interest in evolutionary biology.

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4.8. Appendix. Supplementary data

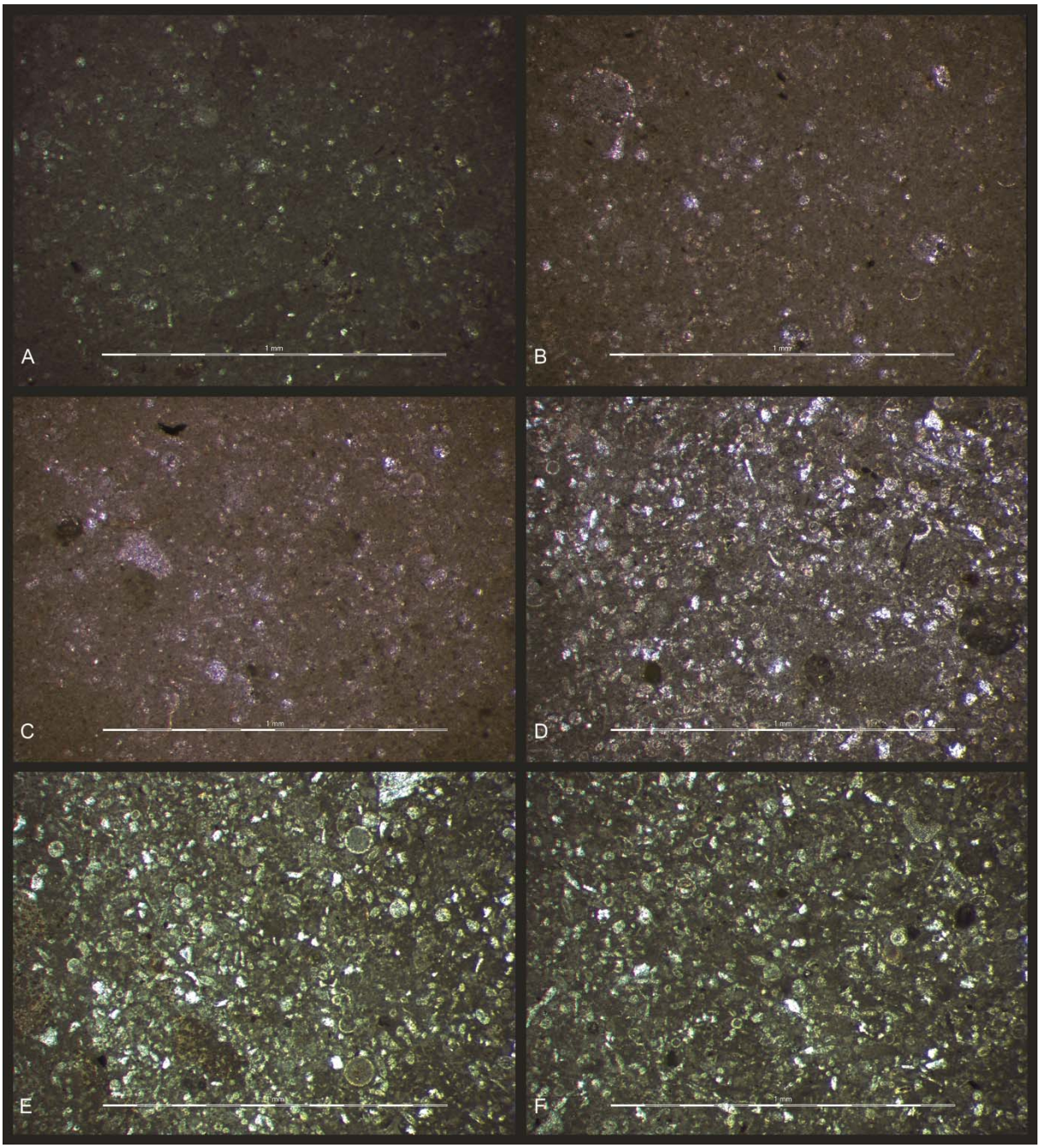

Figure A1. Thin sections**. (**A) Grimberg. (B, C) Erwitte. (D-F) Liencres. (A-C) Wackestones contain clay and silt, with relatively low content of bioclasts. (D-F) Silty packstones with abundant bioclasts and siliciclasts.

A) Morphometric variation

Shape analysis

Grimberg IV shaft: Kr 47601 – 06, Kr 4768 – 13, Kr 476015, X 06195
Erwitte area: GSUB E3812 – 20, E3822, E3825 – 28, E3839 – 41, E3843 – 61
Liencres area: EE4485, MB.E.3870, 3872, 8008, 8190, 8194, 8196 – 97, 8200, 8231, 8234, 8240, 8245, 8247 – 48, 8251, 8254, 8258 – 59, 8261, 8264, 8266, 8270, 8274 – 75, 8304, 8309, 8310, 8320, 8326 – 27, 8328, 8399, 8400, 8458

Fluctuating asymmetry analysis

Erwitte area: GSUB E3812 - 15, E3817 - 19, E3821 - 22, E3825 - 27 E3839 - 61
Liencres area: EE4485, MB.E.3870, 3872 – 73, 8008, 8190, 8191, 8194, 8196 – 97, 8200, 8231, 8234, 8240, 8245, 8247 – 48, 8251, 8254, 8258 – 59, 8261, 8265 – 66, 8270, 8274 – 75, 8304, 8308, 8309, 8310, 8320, 8326 – 28, 8399, 8400, 8458

B) Non-morphometric variation

Variation in the subanal fasciole

Grimberg IV shaft: Kr 47601 – 17, 47619 – 26, X 06195
Erwitte area: GSUB E3812 –61
Liencres area: MB.E.3870, 3873, 8008, 8190, 8191, 8194, 8196 – 97, 8199, 8200, 8202, 8228, 8231, 8234, 8237, 8240, 8241, 8245, 8247 – 48, 8251, 8254 – 55, 8257 – 59, 8261, 8264, 8265 – 66, 8279, 8274 – 75, 8304, 8308, 8309, 8310, 8316, 8320, 8322, 8326 – 27, 8328, 8399, 8400, 8458

Projection of the labrum,

Grimberg IV shaft: Kr 47601, Kr 47603 – 06, Kr 47611, Kr 47613 , Kr 47616 – 18, Kr 47620 - 26
Erwitte area: GSUB E3812 – 13, E3815 – 16, E3818 – 22, E3824 - 41, E3843 - 61
Liencres area: MB.E.3873, 8008, 8190, 8191, 8194, 8196, 8199, 8200, 8202, 8228, 8231, 8234, 8237, 8238, 8240, 8245, 8247 – 48, 8251, 8254 – 55, 8257 – 59, 8261, 8264, 8266, 8279, 8308, 8327, 8399, 8400, 8458

Interradial structure of the paired petals and granulation of the periplastronal area

Grimberg IV shaft: Kr 47601 – 47626
Erwitte area: GSUB E3812 –61
Liencres area: EE4485, MB.E.3870, 3872 – 73, 8008, 8190, 8191, 8194, 8196 – 97, 8199, 8200, 8202, 8228, 8231, 8234, 8237, 8238, 8240, 8241, 8245, 8247 – 48, 8251, 8254 – 55, 8257 – 59, 8261, 8264, 8265 – 66, 8279, 8274 – 75, 8304, 8308, 8309, 8310, 8316, 8320, 8322, 8326 – 27, 8328, 8399, 8400, 8458

Variations in pore pair numbers of the paired petals / FA analyses in pore pair numbers of the paired petals

Erwitte area: GSUB E3812 - 14, E3816 - 21, E3823 - 26. E3828, E3831, E3839, E3841 - 45, E3847 - 52, E3854 - 57, E3859, 63, E3866 - 67
Liencres area: EE4485, MB.E.3873, 3875, 8008 - 09, 8191, 8194, 8202, 8228, 8234, 8237, 8238, 8241, 8247 – 48, 8251, 8254, 8258 – 60, 8265, 8270, 8273, 8275 – 76, 8304, 8309 - 10, 8316, 8318, 8320, 8324, 8326, 8329, 8400, 8458

File A1. Material included for particular analysis.

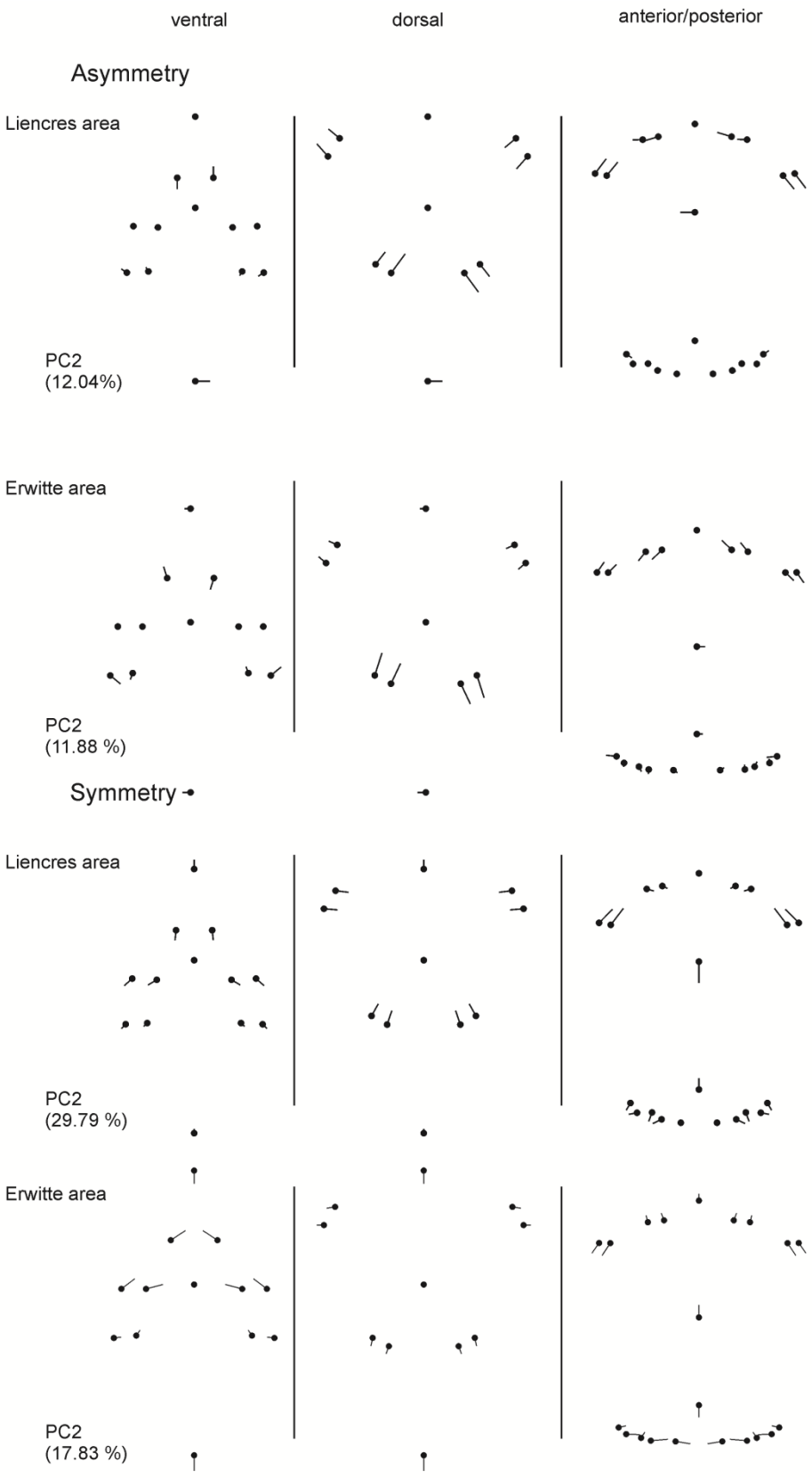

Figure A2. Shape variation of the asymmetric and symmetric component (PC2) for the population from the Liencres and the Erwitte area.

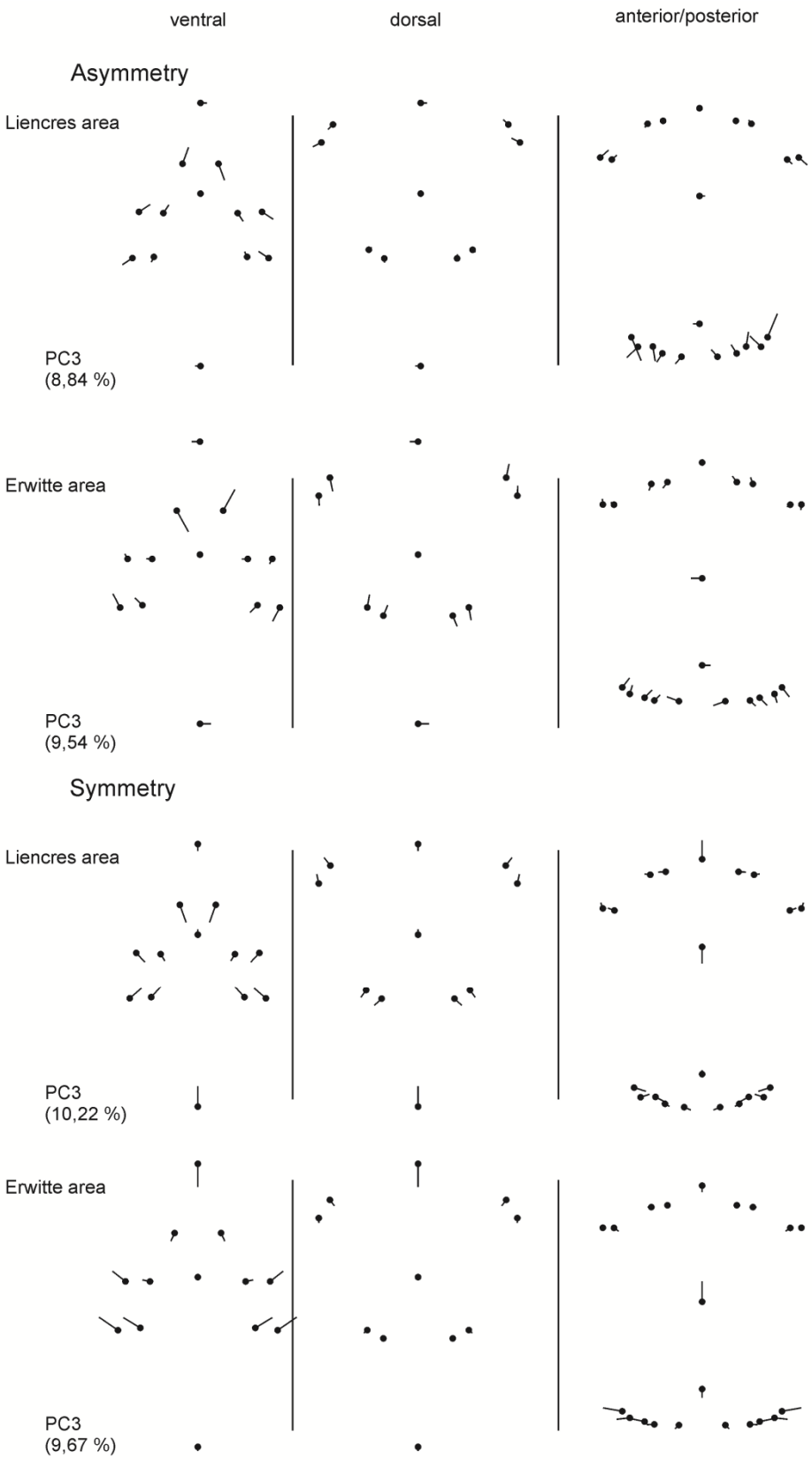

Figure A3. Shape variation of the asymmetric and symmetric component (PC3) for the population from the Liencres and the Erwitte area.

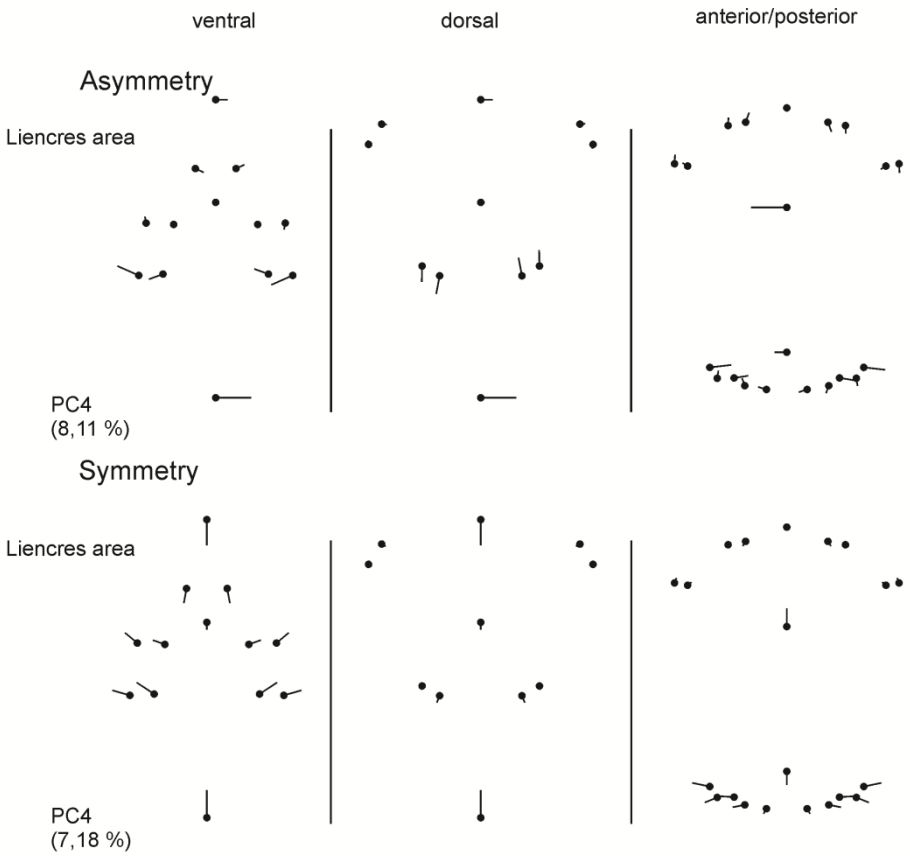

Figure A4. Shape variation of the asymmetric and symmetric component (PC4) for the population from the Liencres area and the Erwitte area.

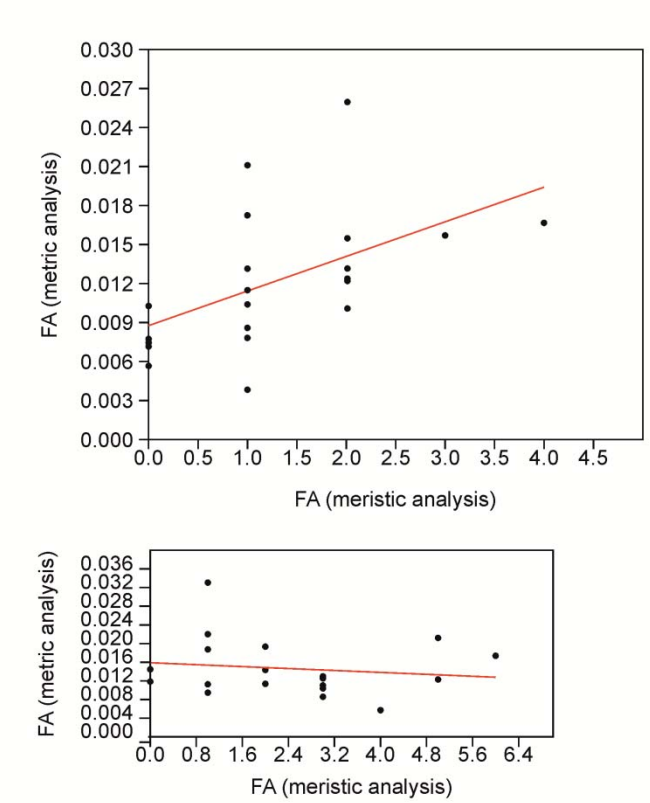

Figure A5. Comparison of the individual metric and meristic FA values for the specimens from Liencres area (A) and from the Erwitte area (B).

Global shape variation			
Liencres area		**Erwitte area**	
Id	**Procrustes FA score**	**Id**	**Procrustes FA score**
EE4485	0.024197804	GSUB E3822	0.014414974
MB.E.3870	0.021592186	GSUB E3821	0.020744664
MB.E.3872	0.011619225	GSUB E3825	0.017739304
MB.E.8008	0.017339887	GSUB E3826	0.012964364
MB.E.8190	0.016246431	GSUB E3846	0.02310569
MB.E.8194	0.020621467	GSUB E3812	0.017351757
MB.E.8196	0.038857947	GSUB E3814	0.021995606
MB.E.8197	0.027017598	GSUB E3815	0.020213289
MB.E.8200	0.014101571	GSUB E3817	0.011468436
MB.E.8231	0.014495551	GSUB E3818	0.026923907
MB.E.8234	0.013218722	GSUB E3819	0.022568137
MB.E.8240	0.020330872	GSUB E3827	0.040500493
MB.E.8245	0.026203968	GSUB E3813	0.016216099
MB.E.8247	0.019878017	GSUB E3839	0.010794428
MB.E.8248	0.022421795	GSUB E3840	0.017793129
MB.E.8251	0.017903009	GSUB E3841	0.013952166
MB.E.8254	0.024235894	GSUB E3843	0.017874458
MB.E.8258	0.029932506	GSUB E3844	0.023652146
MB.E.8259	0.01445233	GSUB E3845	0.013181751
MB.E.8261	0.014627679	GSUB E3847	0.050753241
MB.E.8265	0.019978334	GSUB E3848	0.031481291
MB.E.8266	0.019982252	GSUB E3849	0.019340242
MB.E.8270	0.020940247	GSUB E3850	0.012894059
MB.E.8274	0.019854784	GSUB E3851	0.019243162
MB.E.8275	0.011931275	GSUB E3852	0.018636248
MB.E.8304	0.009635107	GSUB E3853	0.023998995
MB.E.8309	0.017119431	GSUB E3855	0.011124726
MB.E.8310	0.018819016	GSUB E3856	0.01365553
MB.E.8320	0.029088181	GSUB E3857	0.022753474
MB.E.8326	0.014057138	GSUB E3858	0.014717851
MB.E.8327	0.026902019	GSUB E3859	0.035063817
MB.E.8328	0.012085249	GSUB E3860	0.025918802
MB.E.8400	0.040332042	GSUB E3861	0.019142975
MB.E.8401	0.023738627		
MB.E.8458	0.019126663		

Table A1. Individual FA scores for the metric analysis.

Shape variation in the paired petals			
Liencres area		**Erwitte area**	
Id	**Procrustes FA score**	**Id**	**Procrustes FA score**
EE4485	0.021654908007412384	GSUB E3822	0.00730889909322648
MB.E.3870	0.016021175861774963	GSUB E3821	0.011465464140793418
MB.E.3872	0.006893673684905784	GSUB E3825	0.018786149135176115
MB.E.8008	0.011441204618299769	GSUB E3826	0.00946086239499326
MB.E.8190	0.01774103176949572	GSUB E3846	0.00996064630145697
MB.E.8194	0.013273522153130586	GSUB E3812	0.015991792009577857
MB.E.8196	0.008306403127295955	GSUB E3814	0.026068779290039746
MB.E.8197	0.014513140913279333	GSUB E3815	0.013486106033296745
MB.E.8200	0.008758989084451487	GSUB E3817	0.010391559937066653
MB.E.8231	0.0098589048717602	GSUB E3818	0.004739262957925292
MB.E.8234	0.007209319275715448	GSUB E3819	0.013842575556037478
MB.E.8240	0.009870066771175506	GSUB E3827	0.011148627539289128
MB.E.8245	0.021638953955820397	GSUB E3813	0.015669350903917353
MB.E.8247	0.0169214647863942	GSUB E3839	0.012175705889061394
MB.E.8248	0.018858766175367952	GSUB E3840	0.017988010252214897
MB.E.8251	0.01288036519988653	GSUB E3841	0.012603246244404223
MB.E.8254	0.03184956589899418	GSUB E3843	0.014036424382333363
MB.E.8258	0.018579265318594478	GSUB E3844	0.018851704628779782
MB.E.8259	0.013627129296673141	GSUB E3845	0.011789100273371569
MB.E.8261	0.014100502941038155	GSUB E3847	0.025433731909985234
MB.E.8265	0.011662532230877319	GSUB E3848	0.009335683216833323
MB.E.8266	0.016317350740556447	GSUB E3849	0.0051919598404860335
MB.E.8270	0.014906732381379203	GSUB E3850	0.004793513235690507
MB.E.8274	0.012172646397329418	GSUB E3851	0.021768046245615808
MB.E.8275	0.010627415375773996	GSUB E3852	0.016532075471416035
MB.E.8304	0.00891769747256565	GSUB E3853	0.013791719427944524
MB.E.8309	0.016451905692517216	GSUB E3855	0.013903262514819442
MB.E.8310	0.01967688992127521	GSUB E3856	0.00890566362819675
MB.E.8320	0.018844644493376302	GSUB E3857	0.030852644787158844
MB.E.8326	0.011145856743506127	GSUB E3858	0.006897111484787562
MB.E.8327	0.01958438240189527	GSUB E3859	0.012453842845108845
MB.E.8328	0.007457199976403906	GSUB E3860	0.019760321070112594
MB.E.8400	0.03016277848039887	GSUB E3861	0.01915764973136021
MB.E.8401	0.022881942529209776		
MB.E.8458	0.015352664262518308		

Table A2. Individual FA scores for the metric analysis (continued).

Shape variation in the periplastronal ambulacrals			
Liencres area		**Erwitte area**	
Id	**Procrustes FA score**	**Id**	**Procrustes FA score**
EE4485	0.029639211552944723	GSUB E3822	0.017521097129795448
MB.E.3870	0.02543795546623998	GSUB E3821	0.025582657843059995
MB.E.3872	0.015534881414152399	GSUB E3825	0.014951724060907778
MB.E.8008	0.0196530236216953	GSUB E3826	0.016062354042323177
MB.E.8190	0.012374188403293953	GSUB E3846	0.029501630818466143
MB.E.8194	0.02539683406775433	GSUB E3812	0.020562259590462373
MB.E.8196	0.05288300222103713	GSUB E3814	0.01702336484102698
MB.E.8197	0.03588819082930029	GSUB E3815	0.025448419375970165
MB.E.8200	0.018493335859824628	GSUB E3817	0.011267543698266925
MB.E.8231	0.01755769089627426	GSUB E3818	0.037151446822954834
MB.E.8234	0.017290109142699357	GSUB E3819	0.028161570350135483
MB.E.8240	0.025723428985228503	GSUB E3827	0.054501249868748596
MB.E.8245	0.029607454872609976	GSUB E3813	0.014774645091152538
MB.E.8247	0.023123633695105845	GSUB E3839	0.008989592446445323
MB.E.8248	0.029602715700301166	GSUB E3840	0.01611690569731028
MB.E.8251	0.017989906240298996	GSUB E3841	0.01460335059945433
MB.E.8254	0.016874674482176593	GSUB E3843	0.0187658014973484
MB.E.8258	0.03667114835571218	GSUB E3844	0.025722320121645485
MB.E.8259	0.012087639810631455	GSUB E3845	0.013619152227829745
MB.E.8261	0.01232786652349558	GSUB E3847	0.06374789585438526
MB.E.8265	0.02518865727240762	GSUB E3848	0.03947350658787092
MB.E.8266	0.025729531546970198	GSUB E3849	0.02533163292775681
MB.E.8270	0.02074564343734494	GSUB E3850	0.017502545166418096
MB.E.8274	0.024743428210465674	GSUB E3851	0.014359693889741797
MB.E.8275	0.011954583613674725	GSUB E3852	0.01928484157126892
MB.E.8304	0.007974078877838879	GSUB E3853	0.029157454223063088
MB.E.8309	0.017138350092105264	GSUB E3855	0.006359296904189897
MB.E.8310	0.02017444339518608	GSUB E3856	0.018142075156736177
MB.E.8320	0.03570384495750921	GSUB E3857	0.012215263810978911
MB.E.8326	0.01631142460957764	GSUB E3858	0.01987768546902729
MB.E.8327	0.03108724479044859	GSUB E3859	0.045377307543826684
MB.E.8328	0.012635045024827008	GSUB E3860	0.02957538531550744
MB.E.8400	0.05329779527974889	GSUB E3861	0.019404814114930502
MB.E.8401	0.025652398986632872		
MB.E.8458	0.02024208992817502		

Table A3. Individual FA scores for the metric analysis (continued).

	peristome not covered	peristome completely covered	peristome completely covered, labrum exceeds the peristome margin
Grimberg IV	59.00%	41.00%	0.00%
Erwitte area	70.00%	28.00%	2.00%
Liencres area	9.00%	51.00%	39.00%

Table A4. Variation in the projection of the labrum for each population.

	subanal fasciole				
	none	rudimentary	incomplete	parafasciole	orthofasciole
Grimberg IV	46.00%	21.00%	25.00%	8.00%	0.00%
Erwitte area	41.00%	35.00%	24.00%	0.00%	0.00%
Liencres area	0.00%	7.00%	45.00%	41.00%	7.00%

Table A5. Variation in the development of the subanal fasciole for each population.

	ID	length	R anterior	L anterior	R posterior	L posterior	Ra-La	Rp-Lp	
Liencres area									
	EE 4585	42.2	62	61	47	47	1	0	
	MB.E. 3873	42.94	73	69	53	54	4	-1	
	MB.E. 3875	51.2	58	57	46	44	1	2	
	MB.E. 8008	55.1	73	73	60	58	0	2	
	MB.E. 8009	28.04	47	39	30	30	8	0	
	MB.E. 8191	42.41	68	68	54	54	0	0	
	MB.E. 8194	45.14	63	63	43	44	0	-1	
	MB.E. 8202	36.28	56	56	46	44	0	2	
	MB.E. 8228	46.83	67	70	52	53	-3	-1	
	MB.E. 8234	39.14	60	61	46	47	-1	-1	
	MB.E. 8237	42.51	58	52	40	43	6	-3	
	MB.E. 8238	41.31	63	65	52	53	-2	-1	
	MB.E. 8241	46.46	73	69	50	50	4	0	
	MB.E. 8247	55.66	75	76	60	61	-1	-1	
	MB.E. 8248	45.19	70	64	56	58	6	-2	
	MB.E. 8251	46.68	65	62	46	45	3	1	
	MB.E. 8254	41.75	68	63	46	48	5	-2	
	MB.E. 8258	44.53	66	63	50	44	3	6	
	MB.E. 8259	44.62	65	61	52	51	4	1	
	MB.E. 8260	46.09	75	68	50	50	7	0	
	MB.E. 8265	42.84	68	65	50	52	3	-2	
	MB.E. 8270	46.06	64	61	48	47	3	1	
	MB.E. 8273	44.51	65	66	50	48	-1	2	
	MB.E. 8275	43.86	65	60	46	45	5	1	* outlier
	MB.E. 8276	44.7	62	62	52	44	0	8	
	MB.E. 8304	36.08	59	57	42	44	2	-2	
	MB.E. 8309	44.45	67	68	52	55	-1	-3	
	MB.E. 8310	46	63	62	48	50	1	-2	
	MB.E. 8316	45.77	69	65	46	44	4	2	
	MB.E. 8318	23.86	39	41	25	26	-2	-1	
	MB.E. 8320	38.15	55	53	38	39	2	-1	
	MB.E. 8324	45.8	64	66	49	49	-2	0	
	MB.E. 8326	48.73	65	62	51	52	3	-1	
	MB.E. 8329	44.22	60	55	43	45	5	-2	
	MB.E. 8458	47.19	71	69	53	58	2	-5	
	MB.E. 8400	45.21	63	64	51	48	-1	3	

Table A6. Individual values for the pore pair numbers, including asymmetry values.

ID	length	R anterior	L anterior	R posterior	L posterior	Ra-La	Rp-Lp
Erwitte area							
GSUB E3812	49.81	72	73	55	54	-1	1
GSUB E3814	60.07	82	78	57	62	4	-5
GSUB E3816	47.19	73	71	51	52	2	-1
GSUB E3817	45.26	56	54	44	46	2	-2
GSUB E3818	41.53	61	60	42	42	1	0
GSUB E3819	43.92	66	64	47	47	2	0
GSUB E3820	47.98	67	65	51	52	2	-1
GSUB E3821	44.08	65	63	45	46	2	-1
GSUB E3823	43.12	63	63	45	46	0	-1
GSUB E3824	47.67	70	69	52	53	1	-1
GSUB E3825	42.38	63	62	50	52	1	-2
GSUB E3826	43.3	64	65	49	52	-1	-3
GSUB E3828	32.73	47	46	35	35	1	0
GSUB E3831	29.75	48	49	32	31	-1	1
GSUB E3839	40.52	63	62	47	47	1	0
GSUB E3842	48.81	68	68	52	54	0	-2
GSUB E3843	53.7	67	67	47	51	0	-4
GSUB E3844	53.53	70	71	53	50	-1	3
GSUB E3845	48.73	64	63	49	48	1	1
GSUB E3847	45.71	58	56	41	42	2	-1
GSUB E3848	43.57	63	64	45	47	-1	-2
GSUB E3849	50.87	73	73	58	56	0	2
GSUB E3850	47.83	63	63	49	50	0	-1
GSUB E3851	49.51	65	64	52	47	1	5
GSUB E3852	50.89	66	65	51	52	1	-1
GSUB E3854	52.46	71	72	55	54	-1	1
GSUB E3855	43.06	56	58	45	43	-2	2
GSUB E3856	52.97	68	68	50	50	0	0
GSUB E3857	45.8	60	60	50	49	0	1
GSUB E3859	35.21	54	51	36	38	3	-2
GSUB E3860	33.99	52	50	35	35	2	0
GSUB E3862	54.45	68	71	52	56	-3	-4
GSUB E3863	52.96	65	65	47	46	0	1
GSUB E3866	51.55	61	61	47	48	0	-1
GSUB E3867	36.45	54	52	39	38	2	1

Table A7. Individual values for the pore pair numbers, including asymmetry values (continued).

Multimedia A1. A 3D model of GSUB E3840 (Erwitte area) shows the landmark configuration. This file (.obj file)can be opened (import mesh) in the open-source software MeshLab (Visual Computing Lab—ISTI—CNR), available at: http://meshlab.sourceforge.net/. The landmark coordinates (GSUB E3840_picked_points.pp) can be loaded via the PickPoints function. (ZIP)
(Multimedia A1 available at: http://journals.plos.org/plosone/article/asset?unique&id=info:doi/10.1371/journal.pone.0148341.s007)

5. Conclusions and perspectives

The results of the main chapters show that the study of variation and variability in the fossil record is a fruitful area to illustrate and investigate topics in modern evolutionary theory. However, it is not possible to decipher any distinct genetic change. Nonetheless, as recent works have demonstrated, it is not the gene *per se* that causes phenotypic evolution, rather it is the variation in gene expression that influences evolutionary changes. Furthermore, it is possible to easily draw conclusions on the nature of variations in gene expression, either genetic, or non-genetic (stochastic). Phenodeviants, where alternative phenotypical states co-occur within a single individual (in other words as a result of stochastic gene expression, or developmental errors), can be of greater value as indirect evidence that changes in gene expression are involved, rather than variation in the genetic background. Moreover, the results imply that changes on the microevolutionary and macroevolutionary scale can be driven by related factors, simply by influences on variations in gene expression. In conclusion, studies on the variability in the fossil record are able to provide examples for the idea of "evo-devo" and the extended version of the evolutionary synthesis, which addresses the importance of environmental influences on variations in gene expression.

The results of each chapter have practical implications for further specific investigations. In general, studies of variation, addressing interspecific, intraspecific variation and within-individual variation are of great importance for addressing questions of major importance in evolutionary (palaeo-) biology. In future studies on the current topics, it might be possible to achieve a more detailed picture of evolutionary patterns of the respective echinoid taxa. Moreover, the ideas are applicable to all kinds of fossil groups and accordingly, would improve the understanding of phenotypic variation during natural history in general.

Such comparisons of ontogenetic variation among species (**Chapter 3**) offers the possibility for echinoid taxa providing only a minor potential of characters, e.g. due to homoplastic variations, to analyse evolutionary relationships. Further, an extension of more detailed studies of modular development and integration of traits could have implications for addressing the question of the divergence in diversification rates among regular echinoids in relation to irregular echinoids. Such studies could reveal potential developmental constraints of evolvability respectively in particular clades.

Besides the fact that spine morphology can serve in part as a supplementary source for phylogenetic analysis in the atelostomate echinoid (**Chapter 2**), and allows the estimation of the origin of deep sea atelostomate echinoids by a careful re-assessment of the IODP/ODP/DSDP material, spines of atelostomate echinoids can give a valuable insight into the natural history of this clade, i.e. the divergence within the atelostomates. Further, additional data about the divergence within the atelostomates could be gained. However, further information about the microarchitecture of

stem members of atelostomate echinoids would be required. So far, the results of such quality are solely given for the derived atelostomates (Spatangoida, Holasteroida).

Investigations on intraspecific and within-individual variation of spine material (i.e. by phenodeviant specimens) among different habitats, e.g. onshore versus offshore habitats, can be helpful to estimate and test if offshore environments, like the deep-sea for instance, were more resilient than shelf habitats and provided less environmental stress, which could have been a trigger for evolutionary evolvability.

Similarly, studies on the Late Cretaceous echinoid genus *Micraster* (**Chapter 4**) can contribute to the question of the reasons of divergence and evolution in natural history. Previously, such questions were addressed predominantly on estimates of taxonomic and morphological disparity, with very few exceptions, which investigated intraspecific variation, as a key property of developmental canalization. Analyses addressing the role of canalization and developmental instability during the *Micraster* evolution could contribute to the major tasks of interest in evolutionary biology. Are radiating lineages lesser canalised and more instable in their development during their initial phase of diversification and if so, are these phenomenon related to more stressful habitats? Investigations including habitats with a more homogenous and habitats with a rather heterogenous, fluctuating palaeoenvironment would help to address the hypotheses if evolutionary radiation or divergence is linked to free ecospaces, or is it governed by a lesser entrenchment of developmental attributes, or due to an interaction of both scenarios. Accordingly, phenotypic plasticity played what role in the rise and fall of the echinoid *Micraster*, canalization or developmental