ON THE EVOLUTIONARY BIOLOGY OF *ELPIDIUM* OSTRACODS (LIMNOCYTHERIDAE, TIMIRIASEVIINAE): A PROPOSAL FOR PLURIDISCIPLINARY STUDIES

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Abstract. The present essay reviews the history of the research on Elpidium ostracods (Timiriaseviinae), a group exclusively known to live in micro-aquaria of phytotelmata from Neotropical bromeliaceans. A new species, *E. martensi* n. sp., is described and aspects dealing with functional morphology and taxonomy of the genus are presented. Related to these topics an evolutionary hypothesis and a programme of pluridisciplinary research are proposed. This should allow further improvement of our knowledge on the origin and evolution of the subfamily Timiriaseviinae, one of the most diverse cytheroid ostracod groups in inland waters since the beginning of the Mesozoic. Specifically, the following aspects are treated in-depth in the essay: (1) morphologic traits of the valves, useful for characterisation of Timiriaseviinae taxa; (2) the reversal of the valve overlap and hinge elements; (3) the diverse development of the posterior half of the female carapace, a quasi-independent morphological trait; (4) the morphological shapes of the male copulatory process; (5) the functional significance of the antero-ventral segment of the valve selvage for the life of *Elpidium* ostracods in the micro-aquaria of the bromeliaceans; (6) the necessary improvement of comparative descriptions of the limbs-chaetotaxy for Timiriaseviinae.

Key words. Non-marine Ostracoda, Timiriaseviinae, *Elpidium*, taxonomy, functional morphology, evolutionary hypothesis, prospects for pluridisciplinary research

INTRODUCTION

The ostracod genus *Elpidium* has a long history. The first species, *Elpidium bromeliarum*, was described by Müller (1880) using specimens from southern Brazil. During the last century, after 1940, several other species were described from Florida, Jamaica, Puerto Rico, Cuba and Honduras by Tressler (1941, 1956), Danielopol (1975) and supplemented in Colin and Danielopol (1980), Pinto and Jocqué (2013). Pinto and Purper (1970) offered a remarkably complete redescription for *E. bromeliarum* Müller, originally poorly characterised. Additional to taxonomical studies has been research on the possible phylogenetic relationships of *Elpidium* with other Timiriaseviinae. Colin and Danielopol (1980) showed the existence of morphological similarities between the Neotropical species of *Elpidium* with *Afrocythere rostrata* Klie, 1935 from western Africa. More recently, Pinto *et al.* (2008) in describing the first semi-terrestrial non-marine cytheroid *Intrepidocythere ibipora* Pinto, Rocha and Martens from Brazil pointed out affinities of this species with *Elpidium* and offered an hypothesis for its phylogenetic origin.

While many of the studies on Timiriaseviinae in the past dealt with morphologic and/or taxonomic aspects of this ostracod group, it is to the credit of Professor Paul D. N. Hebert and his students from the University of Guelph, to have started detailed genetic analysis in order to elucidate the taxonomy of *Elpidium* populations from Jamaica (Little and Hebert, 1996). Eight new species, left in open nomenclature, were proposed by these authors, based on genetic analysis. This latter study represents the first in-depth investigation on the comparative genetic structure of ostracod populations living in the microhabitats of phytotelmata. It clearly documented the strong genetic diversification of *Elpidium* populations leading to a high number of endemic ostracod species. These authors offered also hypotheses on different evolutionary biological processes which could lead to the high endemism, namely a combination of reduced geographic and ecological spread of populations with splitting of genetic pools of the founders due to bottleneck effects. The limited (geographically) spatial spread of individuals of *Elpidium* with colonisation of phytothelmata mentioned by Little and Hebert (1996) represents an apparent paradox when one observes the wide distribution of the "micro-aquaria" in the axillae of the bromeliaceans close to the terrestrial surface or located on the canopy of trees. It is now well documented, especially by the research of Lopez and co-workers (1999, 2005, 2009), that frogs represent effective phoretic vectors transporting on their sticky skin individuals of *Elpidium* or even transport them after ingestion.

Looking retrospectively at the research done on *Elpidium* and its related genera we note that this ostracod group was studied mainly by scientists with unidirectional research interests, mainly by taxonomists. However, *Elpidium* ostracods offer the possibility for study within a pluridisciplinary approach. This is especially the case if one is interested in evolutionary biological topics.

The present contribution is divided into three parts. We start with a special section on the taxonomic position of the genus *Elpidium* and the description of a new species *Elpidium martensi*. The second part deals with the analysis of six aspects related to the evolutionary morphology of *Elpidium* having practical implications for the systematics of the subfamily Timiriaseviinae. These aspects are:

1. Morphologic traits of the valves, useful for characterisation of Timiriaseviinae taxa which become visible when we change the scale of observation. We will deal with morphological traits of the peripheral margin of the valve, with details of the valve ornamentation and the body pigmentation, and finally with the hinge elements. All these aspects can be used for phylogenetic and taxonomic research when dealing with Timiriaseviinae.
2. The reversal of valve-overlap. This is the case of two *Elpidium* species, namely *E. purperae* Danielopol, 1980 and *E. martensi* n. sp. (this publication). This phenomenon is known to occur also in other ostracod groups and asks for interpretation, especially when used for taxonomic purposes, *eg* discussion in Triebel and Malz (1969), Bate (1970), Malz (1976, 1981), Colin and Danielopol (1980), Sames (2011).
3. The diverse development of the posterior half of the female carapace forming a brood pouch and where eggs and the first post-embryonic stages are stored. Here the problem is first to map the variety of carapace shapes and next to find a hypothesis for the origin of the disparity of this quasi-independent morphological trait (for this latter concept we followed Lewontin, 1978). We are interested to see, at least for *E. martensi* n. sp., how variable the posterior half of the carapace is in both sexes, as compared to the anterior half of the carapace. Additionally, we will try to see if there are privileged shapes, which could be selected for their adaptive value (*e.g.* advantages to store and respectively to protect a higher number of eggs and juveniles).
4. We will look to the diverse morphological shapes of the male copulatory process. We will digress on their possible origin using the existing evolutionary models discussed for Limnocytheridae by Horne *et al.* (1998) and Martens (2000).
5. The antero-ventral segment of the valve selvage has a sinuous shape. We will interpret its adaptive functional meaning for the life of *Elpidium* ostracods in the micro-aquaria of the bromeliaceans. By comparing this peculiar shape of the selvage with those existing in the semi-terestrial ostracod *Intrepidocythere ibipora* as described by Pinto *et al.* (2008) we hope to get a better insight into the adaptive function of the carapace to life in phytothelmata.
6. The necessary improvement of comparative descriptions of the limbs-chaetotaxy for Timiriaseviinae. The careful examination of the limbs of *Elpidium martensi* n. sp. compared with those of several Timiriaseviinae taxa showed us that it is necessary to refine descriptions of the limbs-chaetotaxy using complementary methods of study, for instance the same limb to be examined at lower magnification with transmitted light microscopy and details of the same limb but on other specimens have to be studied at high magnifications, *eg* using scanning electron microscopy. It is hoped that this approach will allow an improvement of the Timiriaseviinae systematics.

Finally, in the third part of our contribution we argue that the advancement of our knowledge on the evolutionary biology of *Elpidium*, and *ipso facto* viewed in a more general way this applies to the whole subfamily Timiriaseviinae, could be more profitable if we will work and communicate similar topics within pluridisciplinary groups. *Elpidium* ostracods should become model-organisms for evolutionary biological projects. In our view zoologists could cooperate and/or discuss with palaeontologists, with colleagues better trained in evolutionary ecology, with those experts in special topics of evolutionary genetics and/or molecular biology and certainly with animal physiologists and specialists in (palaeo)biogeography. Considering this latter cooperative research we will try to stimulate micropalaeontologists to re-examine various enigmatic podocopid ostracods, especially Mesozoic and Tertiary non-marine cypridoids and cytheroids from South America (*eg* from Brazil or Argentina) which could be related, as ancestral representatives of Timiriaseviinae, to the present-day *Elpidium* and *Intrepidocythere* species.

The apparently disparate presentation of this publication is due to the way this project was developed. Nicoletta Riedl worked on this project during 1992-1993 under the supervision of Dan L. Danielopol (DLD) at the Limnological Institute in Mondsee. Her work was supposed to represent a “Master thesis” to be submitted at the University of Salzburg, Institute of Zoology. She unfortunately left the project in 1993, depositing her data and material in the Benthos Laboratory of DLD at the Institute of Limnology, at that time belonging to the Austrian Academy of Sciences. With the retirement of DLD in 2007 from the Institute of Limnology, all the material was placed at the Universalmuseum Joanneum in Graz. By coincidence DLD met Ricardo Pinto in 2013 who was interested to continue investigations on *Elpidium*. We restarted the project reinvestigating especially *Elpidium martensi* n. sp. with the cooperation of Martin Gross and Julia da Silva Pereira. Within this new reformulation of the “*Elpidium* Project” we address here with special emphasis topics related to the evolutionary morphology and systematics of the Timiriaseviinae.

From the collection of *Elpidium* initially studied in 1992-1993 we will offer information on the new species *E. martensi* n. sp. and also on two other new species partly studied by N. Riedl (here named *Elpidium* sp. 1. and *Elpidium* sp. 2). These latter two species will be described in detail in a upcoming publication which will follow this one and which will honour Professor Paul D.N. Hebert and Professor Tom Little. Additionally, Ricardo L. Pinto (RLP) received from Professor Wilhelm Foissner (Univ. of Salzburg) the material of a new species (here named *Elpidium* sp. 3) from Jamaica, which will be described and published separately by them.

In this publication we insisted on illustrating our morphologic description of the valves and limbs using a high number of images taken with the scanning electron microscope (SEM).The figures presented are not redundant, they fully document a series of details generally overlooked and/or aspects of the valves and limbs which appear as stable information but which are not always possible to extract from a single individual. In this way we want to emphasize the importance of the morphologic description of ostracods using a series of individuals.

Julian Huxley (1973, p. 93) offered in his memoirs the following aphorism: *We see by expectation, habit and familiarity*. Transposed to our biological interests we should say that we expect to get better insight into the evolutionary biology of *Elpidium* mentioned above. We chose these questions because we already have the experience to look at ostracods as evolutionary subjects belonging to lineages that we follow within their historical development. Finally, we consider that present-day communication and by extension the cooperation between scientists specialised within various domains of research should become a familiar aspect of our scientific life.

MATERIAL AND METHODS OF STUDY

The *Elpidium* specimens used for this study were collected by Paul D. N. Hebert and his students during an excursion to Jamaica, February-March, 1991. The map in Figure 1 displays the sites 1 to 9 from were P.D.N. Hebert and his student group (HG) collected *Elpidium* material, from which specimens were studied in Mondsee during 1992-1993 and during 2013-2014 in Graz by DLD and MG, and in Brasilia by RLP and JdSP. Another collection was made at Hector’s River site in Jamaica by N. Riedl (NR) during her stay with the Hebert Group (HG), July 1993. The main interest of the HG was the molecular taxonomy of *Elpidium* (*cf.* Little and Hebert, 1996). The material was fixed in ethyl-alcohol. Here we used material from samples for which the site locations are visible in Figure 1. The material is stored at the Universal Museum Joanneum (UMJ), Department of Palaeontology, in Graz, Austria and at the Museu de Zoologia, University of São Paulo (MZUSP), São Paulo, Brazil. Additionally at UMJ are stored a collection of dissected male and female specimens in glycerine and limbs fixed on microscopic slides prepared by DLD and/or NR during work at the Limnological Institute in Mondsee, 1992–1993.

Elpidium martensi n. sp.

Hector’s River, Portland, Jamaica; (Fig 1b, site 7): sample 2/28/91, bromeliad plant Nr. 2, Coll. HG, 12 adult females (FF), 9 adult males (MM), 9 juveniles (JJ);, Coll. NR, about 130-140 specimens, FF, MM, JJ, sampled July 1993. From this latter sample in Graz, at UMJ are stored in alcohol in 2 vials the following material: 6 FF, 4MM in vial 1 and in a 2nd one about 50 specimens FF, MM, JJ, both labelled with the UMJ, Collection No. 211252. Additionally material used for SEM images and which illustrates this study is stored on micropalaeontological slides at UMJ, Collection No. 211248, 211249, 211250. Prepared limbs in glycerine, made by NR 1993, are stored on 7 slides at UMJ, Collection No. 211258. The rest of the NR-sample (about 80 specimens FF, MM, JJ) is stored at MZUSP. The type material originates from the sample collected by NR at Hector’s River during 1993, *i.e*. an adult male as holotype, adult female as allotype, and several adult individuals both males and females are stored at the MZUSP, on micropalaeontological slides and soft parts mounted on permanent slides or in 70% alcohol. These latter have the codes MZUSP 32812 (holotype), MZUSP 32813 (allotype), MZUSP 32814 and 32815 (paratypes).

Note: the type locality is mentioned on other maps also as Hectors River, hence we used here both names interchangeable.

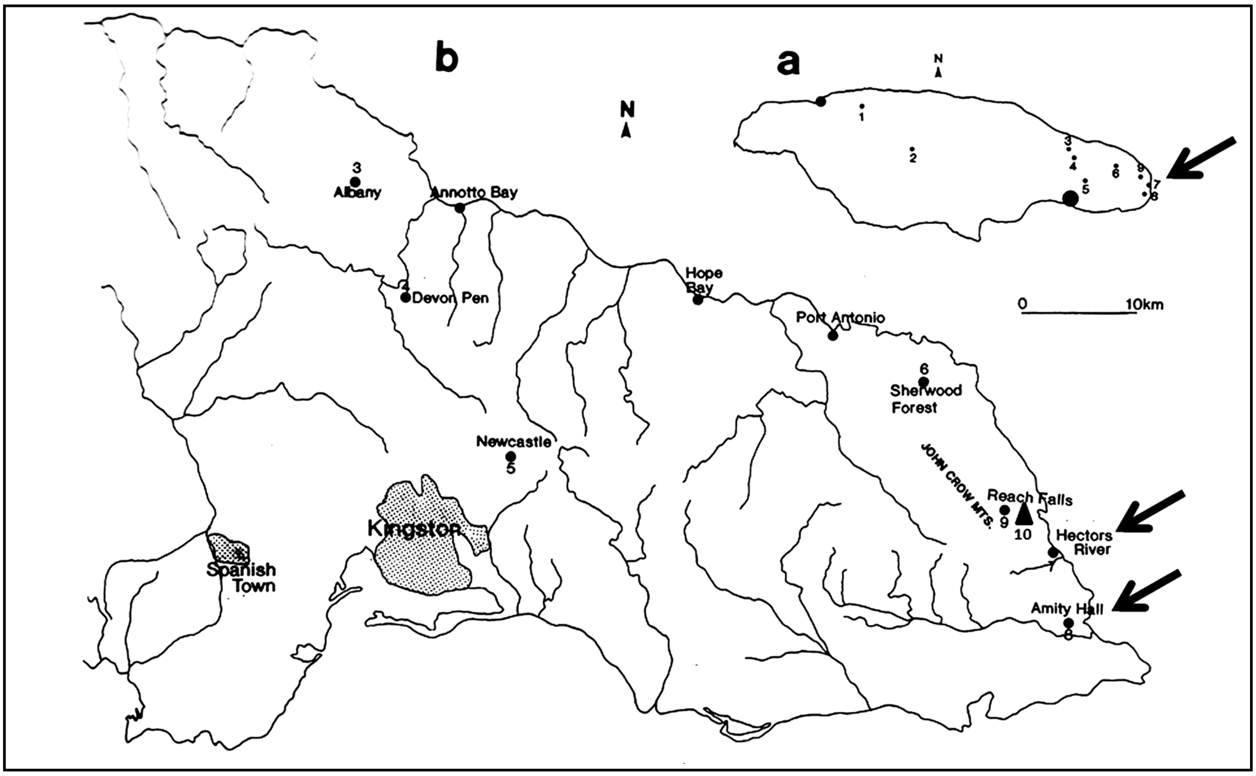


Figure 1 – Jamaica, with sites from where samples with *Elpidium* ostracods exist in our collection (samples from PDNH and his students collected February 1991 and from NR collected July 1993; **A** – general view with the location of sites (1 - Good Hope; 2 - Borobridge; 3 - Albany; 4 - Devon Pen; 5 - Hardwar Gap; 6- Sherwood Forest; 7 - Hector's River; 8 - Amity Hall; 9 – Reach Falls); **B** – details of the eastern part of the island with the positions of the sites 3-9: additionally site 10, Ecclesdown (triangle). The sites of Hector's River and Amity Hall indicated also by arrows (map produced by NR with information offered by PDNH and completed 2013 by DLD with information from W. Foissner).

Amity Hall, St. Thomas, south from Hector's River, Jamaica (Fig 1b, site 8): sample without date, Coll. HG, 29 FF, 23 MM, 24 JJ. At UMJ were deposited 4 MM, 2 FF fixed in alcohol and stored under the Collection No. 211253. Additional material, valves and carapaces used for the SEM, stored dry on micropalaeontological slides and used for the figures of our publication are registered at UMJ under the Collection No. 211251. Dry limbs, the mandibula and hemipenes used for the SEM at UMJ and illustrated in our publication (Figs 24 A-D) remained fixed on stub and have the same collection number as the valves, No. 211251. At the MZUSP, there is another male specimen (MZUSP 32816) dissected and dried for SEM illustration of appendages on a micropaleontological slide (limbs are mounted on a cover slip and kept inside the slide along with the valves). Also two males, two females and a juvenile are stored at the MZUSP in 70% alcohol (MZUSP 32817).

Elpidium sp. 1

Good Hope, south-east from Montego Bay (Fig 1a, site 1): sample 2/3/91, bromeliad plant Nr. 4, Coll. HG, 48 specimens, FF, MM, JJ. From this latter sample at UMJ in Graz are deposited 3 FF, 7 MM stored in alcohol and registered under the Collection No. 211254. Material dissected and limbs prepared by NR on 12 slides are stored at UMJ, Collection No. 21159. Rest of HG-sample stored at MZUSP.

Elpidium sp. 2

Sherwood Forest, Portland, south-east from Port Antonio, Jamaica (Fig 1b, site 6): sample 26/2/91, bromeliad plant Nr. 1, Coll. HG, 5 FF, 5 MM, bromeliad plant Nr. 2, 4 FF, ~ 15 MM, 3 JJ. At UMJ were deposited from this latter sample 10 MM, 2 FF, 2 JJ fixed in alcohol and stored under Collection No. 211255. Material dissected and limbs prepared by NR on 13 slides stored at UMJ, Collection No. 211260. Rest of HG-sample stored at MZUSP.

Elpidium sp. 3

Ecclesdown, Portland, east from Reach Falls, Jamaica (Fig 1b, site 10): 8FF, 3MM, Coll. W. Foissner, currently being described and will be stored at MZUSP.

For the study of the morphological traits of both the valves and the limbs we used both LM (light microscopy) and SEM techniques. Especially the latter, routinely used for valves, became an important help also for observation of minute details of the limbs-chaetotaxy, as well as on the exact hemipenis structure. The photographic documentation with SEM techniques was made at Universal Museum Joanneum in Graz by MG (Figs 7 - 10, 24 and 27) and at the Instituto de Geociências, Laboratório de Micropaleontologia in Brasilia by RLP (Figs 4-6, 21 and 23). We emphasize that in a first phase of study we used classic light microscopy techniques. For the complex structure of the hemipenis it was horizontally extended on a glass-slide covered by a glass-coverslip (Namiotko *et al.*, 2011). This latter preparation is important, especially for getting a standard extended position of the distal lateral lobe (DL). The SEM photos of the hemipenis either on its medial or lateral side helps to understand the shape. However, one has to note that because of the different preservation of the hemipenis during drying on the stub the DL shape changes in an uncontrolled way (see Figs 23 G and I, related to the description of *E. martensi* n. sp.).

For the ostracod shell, *i.e.* the carapace with its two valves, we follow the nomenclature used in Horne *et al.* (2002). For the carapace length we use the size classes proposed for Limnocytheridae by Gidó *et al.* (2007), namely small size (0.3 – 0.7 mm), large size (> 0.7 mm).

For the description of the limbs we used terms proposed by Broodbakker and Danielopol (1982) and Garm (2004). Special terms for the chaetotaxy are plumose setae (long setules cover the whole shaft), pappose setae (the shaft covered with short setules), serrate setae (setules are inserted on both sides of the length's axis of the seta), pectinate setae (setae with stiffed spikes comb-like shaped and ordered on one or two rows), pseudochaetae (series of setules secreted by the epicuticule). For the antennule (A1) we use the numbering of segments proposed by Smith and Tsukagoshi (2005) and discussed in Boxshall *et al.* (2010) within the framework of Crustacea development-patterns of this limb. For the coxal endite of the mandible (Horne, 2005) we introduce here a minor change in the labelling of the most internal tooth. This latter will be tooth No. 8 equivalent to the S1 used by Colin and Danielopol (1980, Fig 8 G). The labels of the maxillule's endites are numbered 1 to 3 starting from the outer lateral side (Colin and Danielopol. 1980, Fig 9 A).

We will distinguish for thoracopods, the basis (B) and the endopodite (E), this latter with endopodial segments numbered from E1 to E4 (Meisch, 2000 and here Figs 23 A-F). The posterior seta of the basis is a vestigial exopodite (Horne, 2005). We name it "exopodial seta". The E4 segment is fused to the distal claw, a trait typical for the Limnocytheridae (Danielopol *et al.*, 1989). In the present contribution we express the relative length of the endopodial seta of segment E1 and of the distal endopodial claw (for this latter the length of the 4th endopodial segment to which it is fused is also included) as a percentage ratio between their length to the referential length of 2nd endopodial segment, specific to each thoracopod (Lse/LE2 %). The length measurements were made following the procedure explained in Broodbakker and Danielopol (1982).

For the description of the hemipenis' position we use the convention proposed by Danielopol (1969), namely that there is a medial side internal to the body on which lies the copulatory complex and a lateral side on which there is a distal seta. The nomenclature of the various morphological traits of the hemipenis follows that proposed by Martens (1990). The numbering of the internal muscles within the peniferum (Fig 25) is proposed here for the first time.

The usage of the stereo-light microscope was especially useful for observing, through the transparency of the hard shell, either the colour of the epidermis or the eggs and juveniles stored in the posterior half of the shell-cavity.

Uni- and bivariate statistics were used for biometric analysis of the shell in its dorsal view. The shell's length (L) represents the standard (or reference) variable while the maximal height (H) and/or width (W) are dependent variables related to the growth degree of the shell, therefore we expressed their relative size as a percentage of the total shell's length (W/L %). The position of the maximal width along the length of the shell was expressed as the percentage of the anterior segment of the length intersecting the line of maximal width to the total length of the carapace, in percentage (Pa/L %). For the length and the maximal width of the shell we calculated the arithmetic mean with its standard error, the range of the variates as well as the percentage values of the relative width and of the relative position of the maximal width). The evaluation of the statistical differences between samples was done using the non-parametric test Mann-Whitney U-test. This latter calculates the average ranks for each sample and displays the statistical degree of sample separation (Sokal and Rohlf, 1995). The data were computed using the computer package BIOMstat, v. 3.30q (Rohlf and Slice, 1999) and the significance of the output data read on the Table U of the Statistical Tables (Rohlf and Sokal, 1995). One should note that the expression of the relative width of the valves in Pinto and Jocqué (2013) as L/W has a different meaning, namely it assumes that the width is the reference variable and the length of the shell is the dependent variable. This is biologically unrealistic because we recognise the enlargement of the shell depending on the increase of the shell's length (our reference for the diagnostic state of the valve development). The subtle increase of the maximal width (for obvious examples see the difference between the values of the Adult and A-1 shells in the Tables 1 and 2 of this publication) represents the accessory variable which is dependent on the length-variable.

For the biometric data we measured the maximal height and width of the shells from the photographs made either with the stereo-microscope (Fig 2 A) or with SEM. They were further processed with the computer facilities of the package ADOBE-PHOTOSHOP (Version CS3 Extended), following the procedure described by Stracke (2008). The outlined shells were further digitised using the Tps-dig software (Rohlf, 2003).

Geometric morphometric analysis of the outlines was performed using Linhart's B-spline algorithm (Neubauer and Linhart, 2008) and computed with the computer programme MORPHOMATICA v. 1.6 (Linhart *et al.*, 2006). We used for the first time all three variants of dissimilarity of shape as standardised for surface, namely for total outline-shape (examined in dorsal view), as well as the upper and the lower halves of

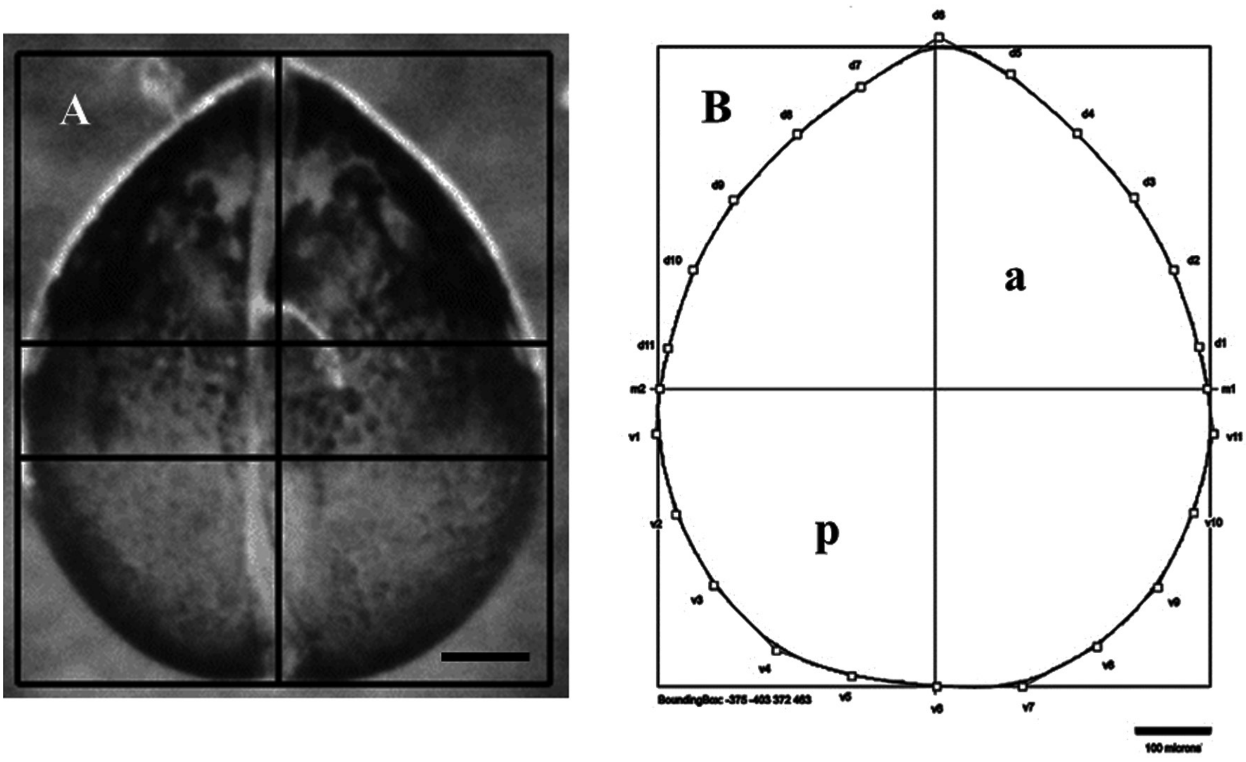


Figure 2 – *Elpidium martensi* n. sp., carapace in dorsalview. **A** – The major meristic axis: vertical line is the maximal length; the two horizontal lines represent where widths was measured, the upper line at the level of the adductor muscle scars, the lower line is the maximal width of the carapace. **B** – outline of the carapace with 24 control points and the two orthogonal axes of inertia (the intersection point of those axes represents the centroid of the outline); **a** – anterior half of the outline, **p** – posterior half, separated by the horizontal axis and the reference control points $m^1 - m^2$; the position of the other control points on the two half sections figured by squares, each being standardised by letters and numbers (on the anterior sector the control points are $d^1 \dots d^{11}$ and on the posterior one $v^1 \dots v^{11}$. Scale bar: 0.1 mm.

the outline of the carapace (Figs 2 A, B). This allowed us to approach the problem of the quasi-independent traits of anterior and posterior parts of the shell in dorsal view (*cf* next section, below). For the reconstruction of the shell-outline using B-splines we used 24 control points which produce an accurate geometric display (Fig 2 B). When photographing the *Elpidium* shells in dorsal view we orientated the image in ADOBE-PHOTOSHOP in such a way as to get the length axis passing as closely as possible through the line of separation of the two valves (Figs 3 A, C). MORPHOMATICA calculates the axis of inertia passing through the centre of gravity of the surface of the image (Neubauer and Linhart, 2008) hence the position of the length's axis (Figs 3 B, D) differs sometimes from those we observe in the microscope.

Disparity analysis of the shape of shells was done using multivariate statistics offered by the computer-package PRIMER v. 6 (Clarke and Gorley, 2006) and PERMANOVA+ for PRIMER v.6 (Anderson *et al.*, 2008). From PRIMER v. 6 we used the non-metric Multi-Dimensional Scaling program which constructs a 2-D map of the pairwise rank dissimilarities calculated on Euclidean distances. Additionally we used the ANOSIM test, which is a permutation statistic test reflecting the observed differences between and within the samples; the test is based on the rank similarities and calculates an index of discrimination between samples (R), which takes values between 1, when separation between two samples is absolute and 0, when samples are completely mixed. The test calculates also a global R value which indicates if within the collection of samples there is a trend toward a distinction between pairwise samples exists. Also the ANOSIM test offers probabilities value for the difference between samples. Clarke and Gorley (2006: p. 131) made the following remark: " R is largely not a function of the number of replicates (*i.e.* possible permutations) but an absolute measure of differences between two (or more) groups in the high-dimensional space of the data, whereas p (the probability value) …. gives an illusion of certitude which is not justified". However we mention in our data the probability values in order to strengthen especially the samples where p is higher than 5% and demonstrates that no difference exists. For the present study we propose a rule of thumb, namely when the R of the pairwise comparison has a value of 0.7 or higher is an indication for clear separation of samples.

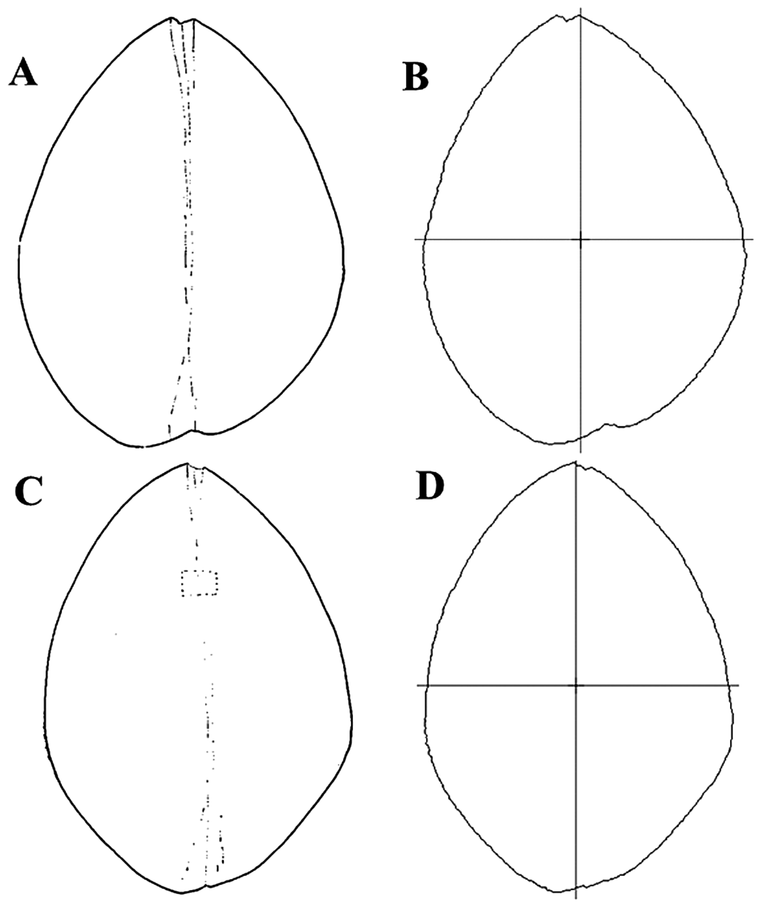


Figure 3 – Dorsal representation of an asymmetric carapace-shape (*Elpidium inaequivalvis*). **A, B** – female; **C, D** – male; **A, C** - drawn with *Camera lucida* from a stereo-microscope (*cf.* Danielopol, 1975, Figure 4, A, B); **B, D** – outlines of the previous carapace-figures reconstructed with the B-spline algorithm and the computer programme MORPHOMATICA.

From the PERMANOVA+ for PRIMER v.6 we used the ordination program PCO (Principal Coordinates Analysis). PCO places sample units into Euclidean axes using a matrix of inter-point dissimilarities calculated on Euclidean distances as original variables. The PCO allows us to represent within a multidimensional space the distribution of the outlines of the dorsal-shape of *Elpidium* shells. The resulting solution indicated the existence of three classes of shape represented by fairly well delineated morphotypes. In order to see how well the items were grouped within the classes of morphotypes we proposed, we performed a Canonical Analysis of Principal Coordinates (CAP) for the same data set using the routine existing in the computer-package PERMANOVA+ for PRIMER v.6.

The taxonomic system of the family Limnocytheridae adopted in this publication is that proposed by Martens *et al.* (1998) to which we added for the subfamily Timiriaseviinae Mandelstam, 1960 the nominotypical tribe Timiriaseviini Mandelstam, 1960. This is necessary in order to logically place the tribe Cytheridellini Danielopol and Martens, 1990 (in Danielopol *et al.*, 1990) within the Timiriaseviinae system. Originally Cytheridellini was considered to belong to Limnocytherinae (Colin and Danielopol, 1978). Martens (1995) in reanalysing its morphological characters relocated this taxon within the subfamily Timiriaseviinae without mentioning what should be done with the other genera already located within this subfamily. Therefore, this dilemma is solved here by inserting the nominatypical tribe Timiriaseviini for the genera recognised earlier in the subfamily Timiriaseviinae (Colin and Danielopol, 1980, Gidó *et al.*, 2007, Martens and Savatenalinton, 2011, Karanovic and Humphreys, 2014).

Finally, we have to note that in order to avoid repetitive descriptions for morphological traits of *Elpidium martensi* n. sp., selected aspects only appear in the primary description of this new species (*cf* first part of this contribution), as they will be presented in the second part of our essay within a comparative framework.

RESULTS AND DISCUSSION

A – Description of Elpidium martensi n. sp. and its systematic affinities to other Elpidium taxa

Class Ostracoda Latreille, 1802
Subclass Podocopa G.W. Müller, 1894
Order Podocopida G.O. Sars, 1866
Suborder Cytherocopina Baird, 1850
Superfamily Cytheroidea Baird, 1850
Family Limnocytheridae Klie, 1938
Subfamily Timiriaseviinae Mandelstam, 1960
Tribe Timiriaseviini Mandelstam, 1960
Genus *Elpidium* F. Müller, 1880

Type species (by original designation) - *Elpidium bromeliarum* F. Müller, 1880.

Species allocated to the genus - *Elpidium inaequivalvis* Danielopol, 1980 (in Colin and Danielopol, 1980, p. 18); *Elpidium laesslei* (Tressler, 1956); *Elpidium maricaoensis* (Tressler, 1941); *Elpidium merendonense* Pinto & Jocqué, 2013; *Elpidium pintoi* Danielopol, 1980 (in Colin and Danielopol, 1980, p. 18); *Elpidium purperae* Danielopol, 1980 (in Colin and Danielopol, 1980, p. 18, as *E. purperi*; the name is here corrected for gender, being named after Mrs. Ivone Purper); *Elpidium* sp. 1, *Elpidium* sp. 2, *Elpidium* sp. 3 (the last three *Elpidium* represent unnamed new species, mentioned for the first time in the present publication).

Diagnosis – Bisexual Timiriaseviinae with dimorphic carapace. Generally, females larger than the males and belonging to the large size class (> 0.7 mm). Carapace broad, generally larger in width than in height; ventral side flat. Males with greatest width at mid-length, females with posterior part expanded into a brood pouch carrying eggs and greatest width displaced more posteriorly than in the males. Epidermis below the shell, either uniform pale to dark coloured or covered with patches of colour. Strongly interlocking selvages along ventral margin of the carapace, leaving an antero-ventral gap between left and right valve margins. Hinge of protodont type (see section B 1 d below for this term). A1 with 5 functional articles; the first, bearing on the dorsal margin a subapical expansion with a tuft of tiny setae. A2 dimorphic. The distal endopodial segment of the male with a strongly pectinated claw (the homologue claw of the female slightly biserrate on the distal third of its posterior side). Distal endopodial segment of A2 with a tiny hyaline formation in both males and females. The X1 seta of the mandibular coxal endite, spoon-like shape. Eight mandibular teeth and only one internal S seta. Maxillular palp with two strongly plumose setae distally inserted. First and second maxillular endites bearing two spatulate claws each. Hemipenis with a short copulatory process which emerges on the medial side of the peniferum. The peniferal Lower Ramus present and shaped in a clasping process; the Upper Ramus absent. The distal lobe of the hemipenis is large and in some species with a ventral proximal digitiform expansion. On the lateral side of the hemipenis near the basis of the distal lobe a short seta present.

Elpidium martensi n. sp.

Type locality - Jamaica, Portland, Hector's River (Fig. 1b, site 7). Water accumulated among bromeliad leaf axils.

Additional locality - Jamaica, St. Thomas, Amity Hall (Fig 1b, site 8).

Type material

Holotype: a dissected male, with valves dried and coated for scanning electron microscopy stored on a micropaleontological slide and soft parts mounted on a permanent slide with CMC-9AF mounting medium (MZUSP 32812).

Allotype: an ovigerous female dissected and stored like the holotype (MZUSP 32813).

Paratypes: a dissected male (MZUSP 32814), with valves stored dry in a micropaleontological slide and soft parts mounted in a permanent slide with CMC-9AF mounting medium; two females (MZUSP 32815) kept whole in 70% ethanol.

Additional material: From Amity Hall a dissected male (MZUSP 32816) with limbs and carapace dried and coated for scanning electron microscopy; appendages are fixed on a glass coverslip and stored in a micropaleontological slide along with valves; two males, two females and 1 juvenile stored in alcohol (MZUSP 32817). Material investigated in Graz at UMJ and specifically used for description of the carapace and the limbs with the SEM is stored in micropalaeontological slides at UMJ, Collection No. 211248, 211249, 211250; additionally, material dissected in glycerine and limbs are stored on 7 slides at UMJ, Collection No. 211258. Specimens used for the documentation of valve reversal and the biometric traits of the carapace in the dorsal view (see next section) are now deposited in both collections, those of the UMJ and the MZUSP.

Derivation of name - The species is named in honour of Professor Koen Martens (Royal Belgian Institute of Natural Sciences, Brussels) in recognition of his remarkable contributions to the study of freshwater ostracods.

Description of male

Carapace (Figs 10 B, C, 11 A, B, Tables 1 and 2): Large-size carapace (mean length 0.78 – 0.82 mm, depending on site). In dorsal view the carapace is widely enlarged in its posterior half. The maximum width represents about 81-82 % of the carapace length and is placed at about 58 % from the anterior edge. Posterior part of the carapace, angular shaped. Valves approximately symmetric. Right valve overlaps the left one (section B 2). Ventral side flat (see next).

Right valve (Fig 4 A - I): In lateral view, moderately elongated (height/length ratio c. 59 % of the total length of the valve). Anterior margin narrowly rounded, dorsal margin evenly arched, posterior margin rounded, ventral margin flat (Fig 4 D). Position of the anterior margin advanced compared to the selvage (arrow in Fig 4 D). Selvage well developed with a hard rim on which lays a thin lamellar membrane (arrow in Fig 4 I). A funnel-shaped protrusion, visible on the antero-ventral third of the valve (section B 1 b). A hinge groove on the dorsal side fitting the hinge bar of the left valve is visible with enlargement at its anterior and posterior extremities (arrow in Fig 4 E and details in section B 1 d). Anterior and posterior vestibules of reduced size as compared to the total valve length; the former represents about 6 % starting from the anterior margin of the selvage, while the posterior vestibule is about one third smaller than the former one (Figs 4 A, B, H, I). On the external side of the valve, antero-ventrally, a small flange is visible. Surface of the valve, with minute foveolae (Fig 4 G). No sieve-pore plates visible. Epidermis below the carapace, coloured light brown (section B 1 e).

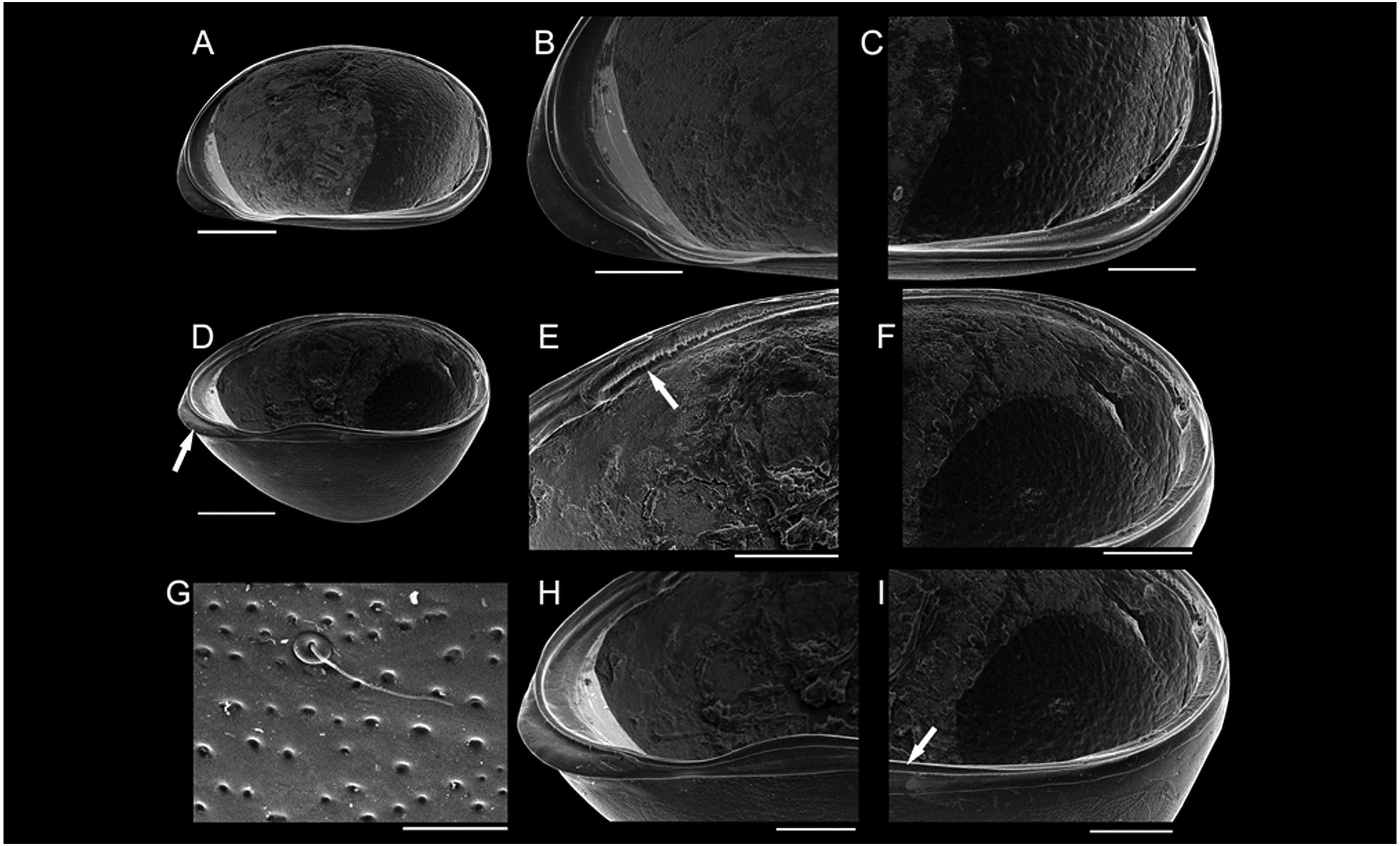


Figure 4 - *Elpidium martensi* n. sp., right valve, male, holotype (MZUSP 32812). **A** - Internal view, showing general aspect of valve. **B** - Internal view, showing detail of the antero-ventral part. C - Internal view, showing detail of the postero-ventral part. **D** - Oblique view, showing part of the ventral external surface – arrow indicates antero-ventral flange. **E** - Oblique view, showing internal detail of the antero-dorsal area – arrow indicates anteriormost part of the hinge groove. **F** - Oblique view, showing internal detail of postero-dorsal area, with posteriormost part of hinge groove. **G** - Oblique view, showing ventral detail of external surface with a seta emerging from a normal pore canal and alveolate (foveolae) ornamentation. **H** - Oblique view, showing detail of antero-ventral margin. **I** - Oblique view, showing detail of postero-ventral margin. Scale bars: A, D - 0.2 mm; B-C, E-F, H-I – 0.1 mm; G – 0.02 mm.

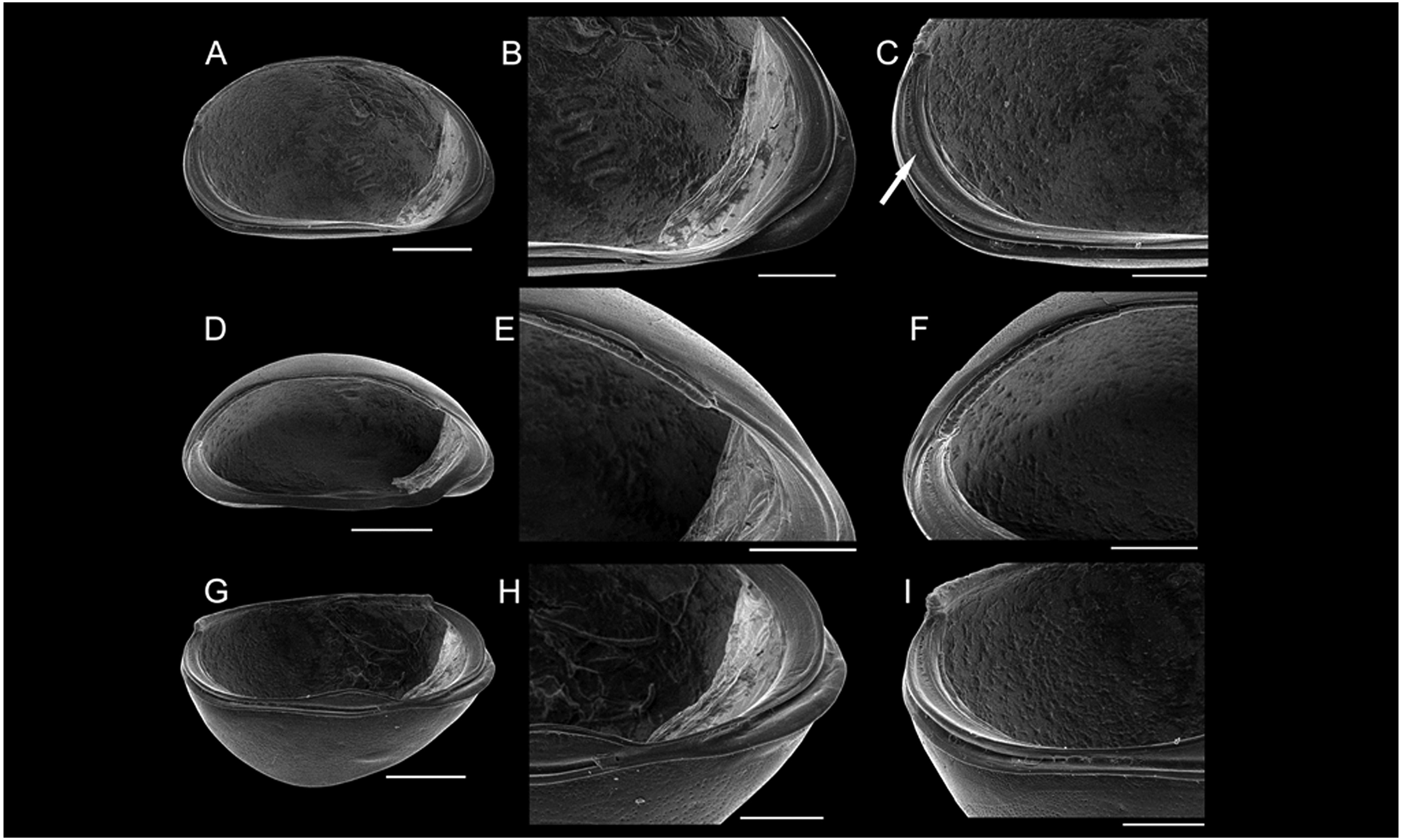


Figure 5 - *Elpidium martensi* n. sp., left valve, male, holotype (MZUSP 32812). **A** - Internal view, showing general aspect of valve. **B** - Internal view, showing detail of the antero-ventral part. **C** - Internal view, showing detail of the postero-ventral part – arrow indicates ciliate list on inner margin. **D** - Oblique view, showing part of the dorsal external surface, hinge bar and internal surface. **E** - Oblique view, showing detail of the anteriormost part of hinge bar. **F** - Oblique view, showing detail of posteriormost part of hinge bar. **G** - Oblique view, showing part of the ventral surface, internal surface and hinge bar. **H** - Oblique view, showing detail of antero-ventral margin. **I** - Oblique view, showing detail of postero-ventral margin. Scale bars: A, D, G - 0.2 mm; B-C, E-F, H-I - 0.1 mm.

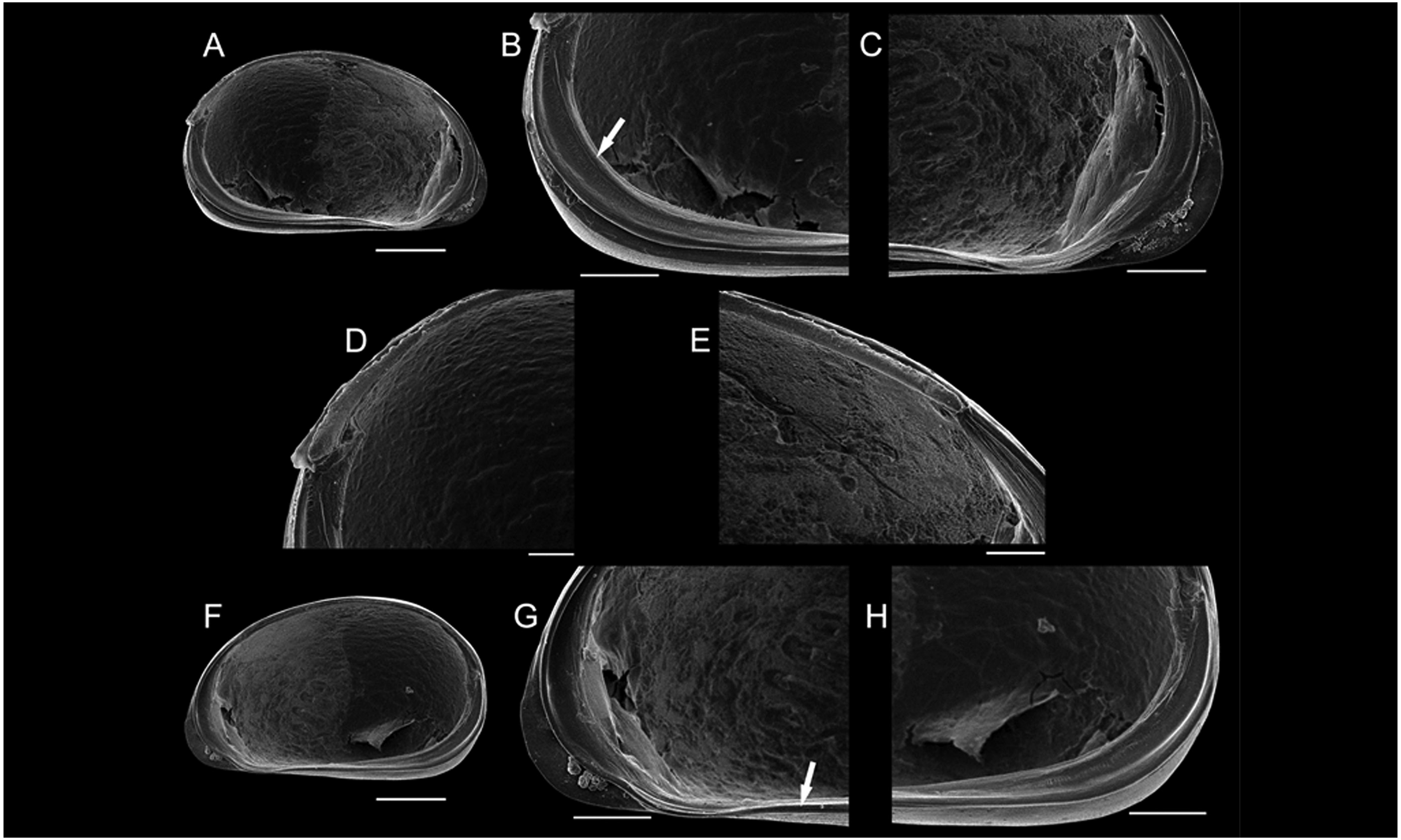


Figure 6 - *Elpidium martensi* n. sp., valves, female, allotype (MZUSP 32813). **A** - Internal view, showing general aspect of left valve. **B** - Internal view, showing detail of the postero-ventral part of left valve; arrow indicates the inner margin. **C** - Internal view, showing detail of the antero-ventral part of left valve. **D** - Internal view, showing detail of the postero-dorsal part of left valve with posteriormost part of hinge bar. **E** - Internal view, showing detail of the antero-dorsal part of left valve with anteriormost part of hinge bar. **F** - Internal view, showing general aspect of right valve. **G** - Internal view, showing detail of the antero-ventral part of right valve; arrow indicates the selvage. **H** - Internal view, showing detail of the postero-ventral part of right valve. Scale bars: A, F - 0.2 mm; B-C, G-H - 0.1 mm; D-E - 0.5 mm.

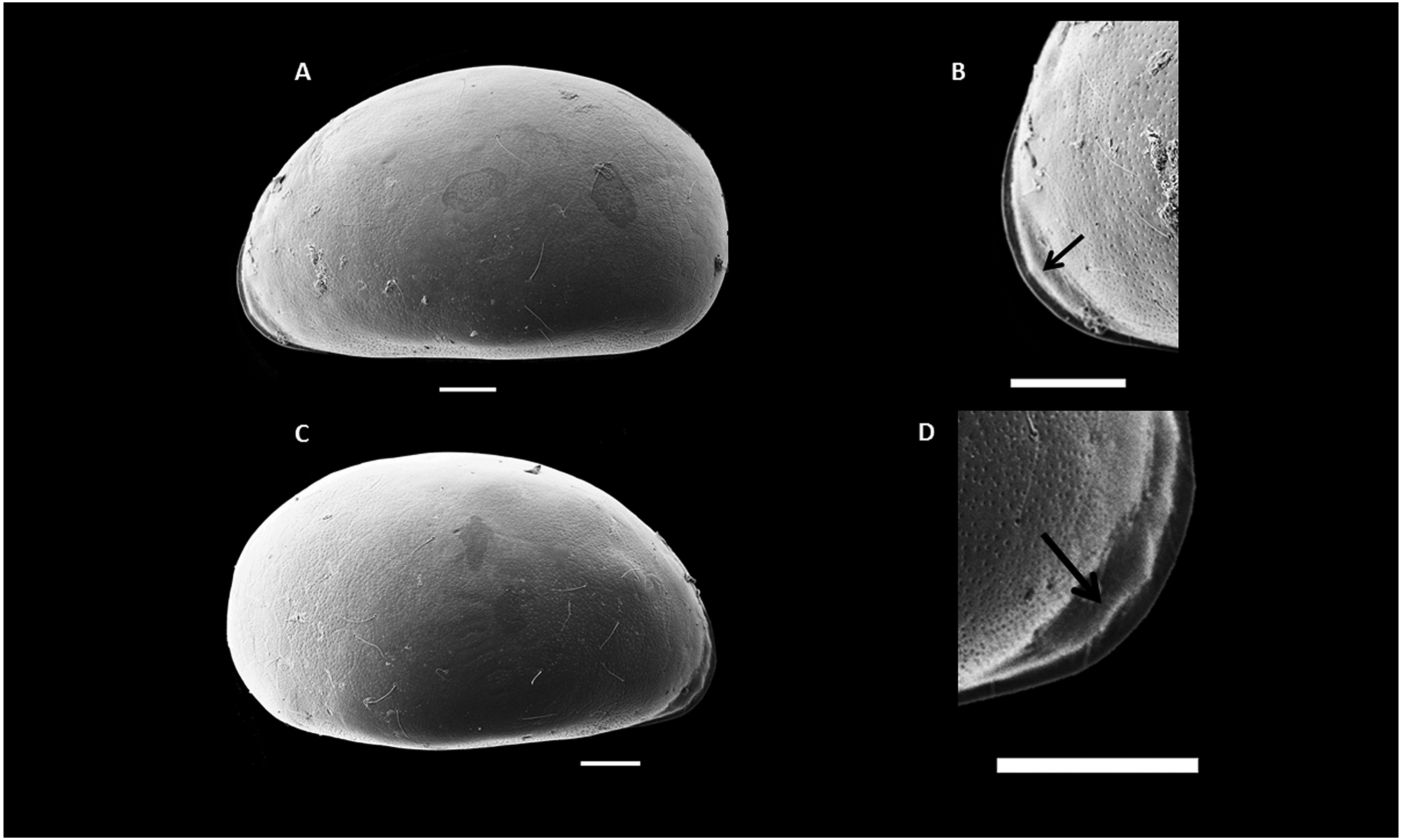


Figure 7 - *Elpidium martensi* n. sp., female, Hector's River, external view (valves stored at UMJ- Collection No. 211248); **A**, **B** – left valve; **A** - general view; **B** – detail of the anterior area with a slightly developed flange (*cf.* arrow); **C**, **D** – right valve; **C** - general view; **D** – detail of the anterior area with the slightly developed flange (*cf.* arrow). Scale bars: 0.1 mm.

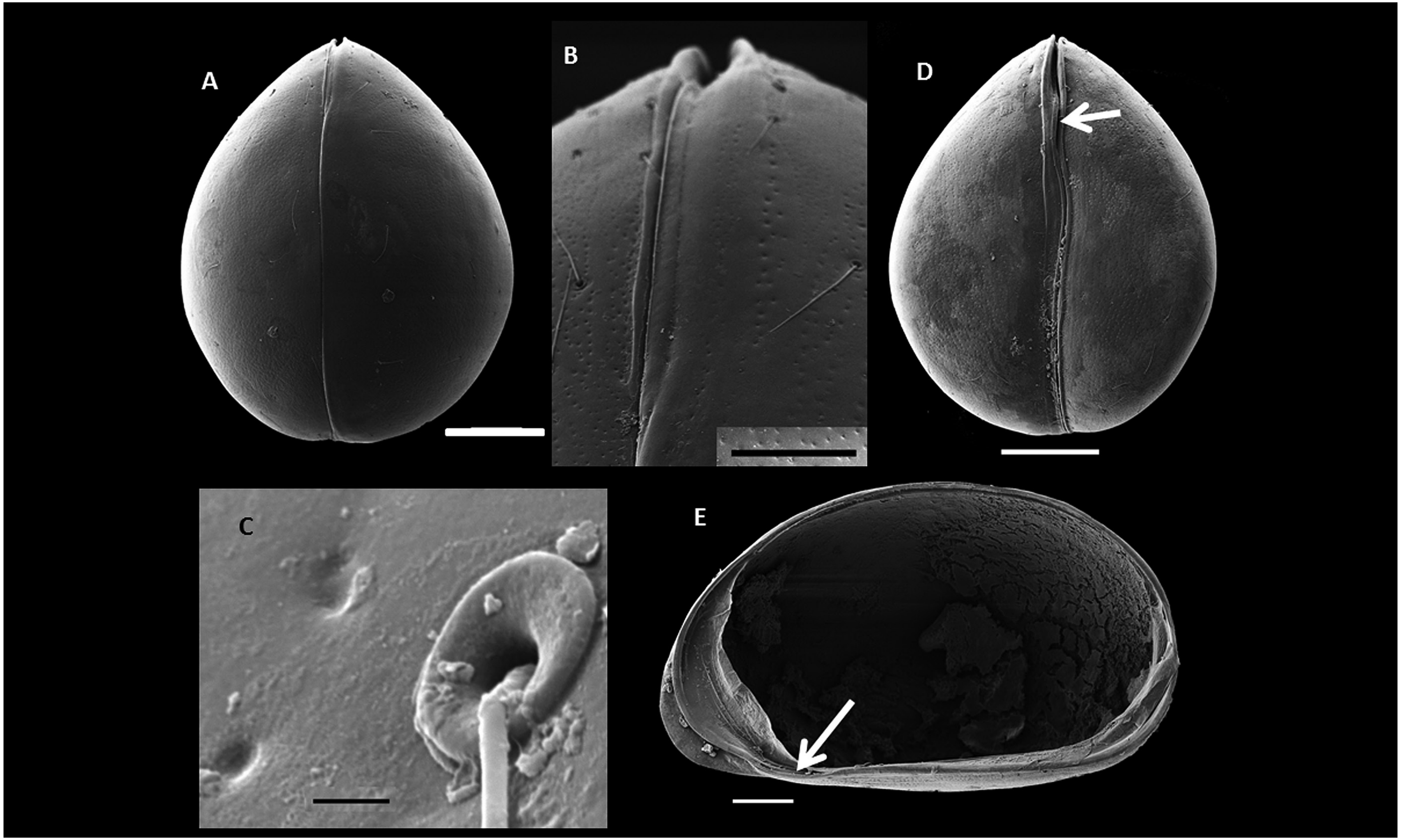


Figure 8 – *Elpidium martensi* n. sp., female, Hector's River (carapace and valves stored at UMJ- Collection No. 211249, 211250); **A** – **D**, aspects of the carapace morphology; **A** – dorsal view, scale bar, 0.2 mm ; **B** – detail of the anterior area, scale bar, 0.05 mm; **C** – detail of the valve's surface from Fig. 6 C, scale bar, 0.002 mm; **D** – ventral view, scale bar, 0.2 mm; **E** – right valve, inner view, scale bar, 0.1 mm; arrows point to the anterior bow funnel-shape of the selvage.

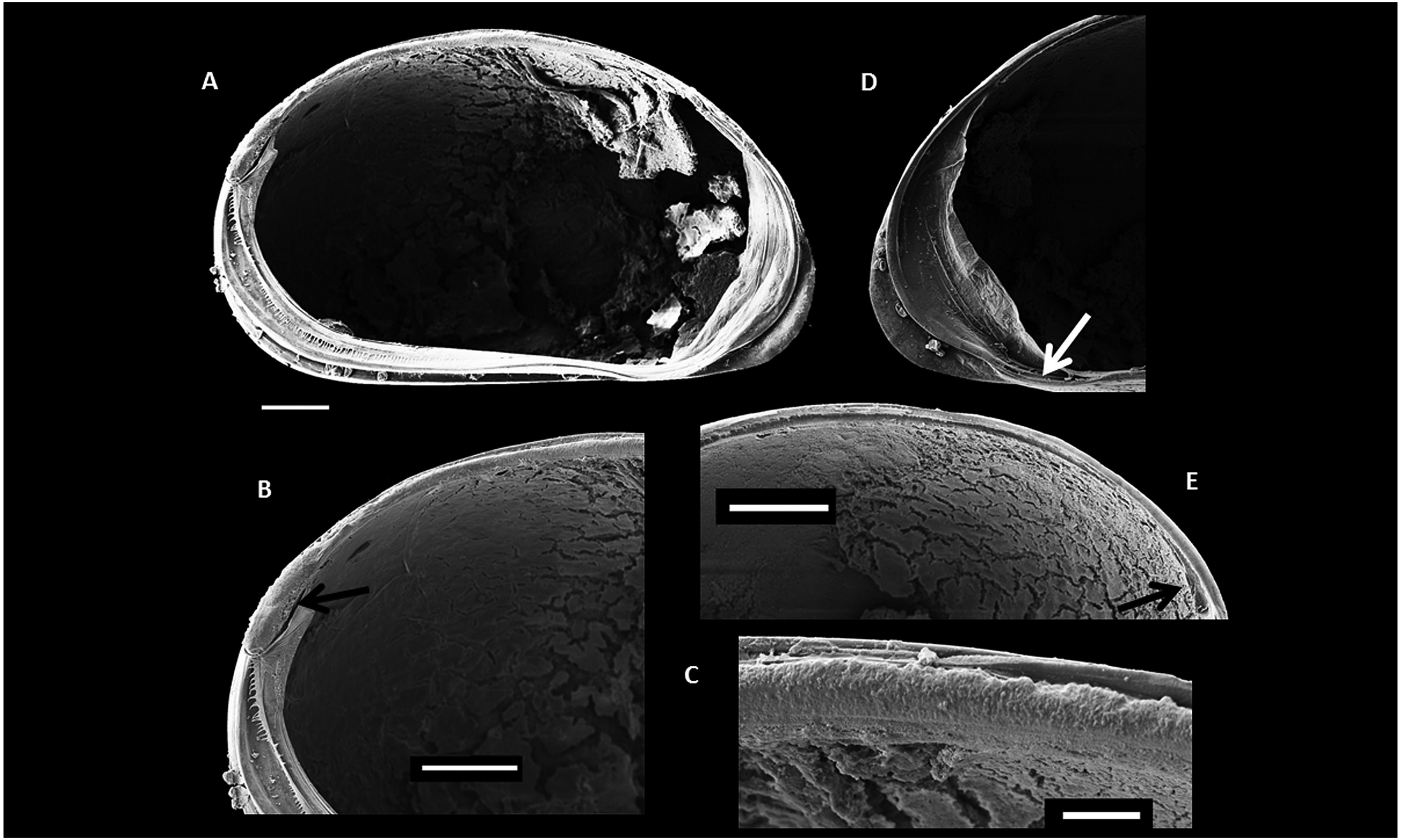


Figure 9 - *Elpidium martensi* n. sp., female, Hector's River (carapace and valves stored at UMJ- Collection No. 211250); **A** – **C**, left valve, inner view; **D** – **E**, right valve details; **A** – general view, scale bar 0.1 mm; **B** – detail of the postero-dorsal area with the posterior enlarged hinge bar (arrow), scale bar, 0.1 mm; **C** – detail of the anterior area of the hinge bar, scale bar 0.02 mm; **D**, **E** - details of the right valve, inner view, scale bar, 0.1 mm; **D** – anterior third with the prominent bow of the selvage (arrow); **E** - dorso-posterior area with the enlarged posterior hinge groove (arrow).

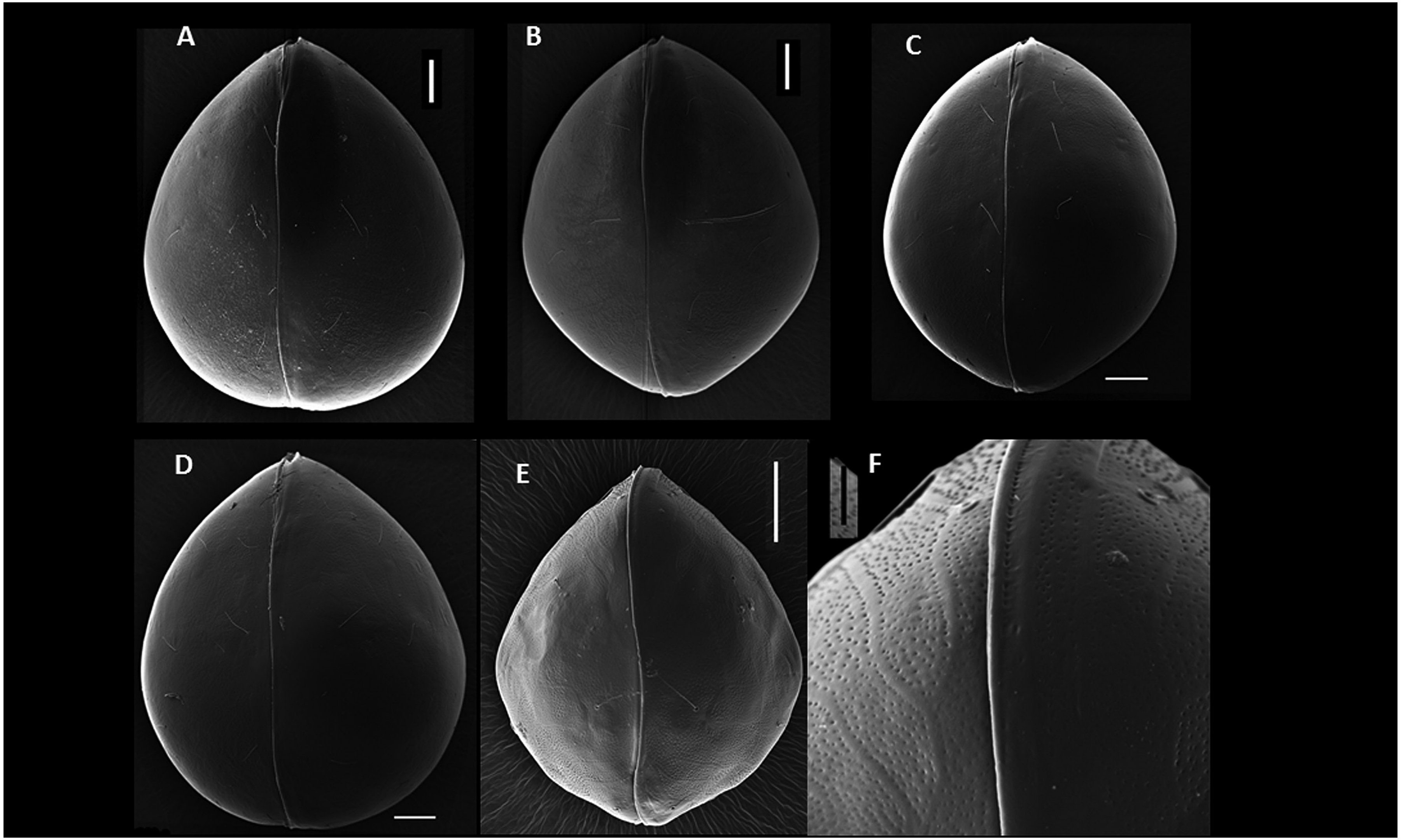


Figure 10 - *Elpidium martensi* n. sp., aspects of the carapace morphology, dorsal view; **A**, **B** – Hector's River (UMJ Collection No. 211250), **C** – **F** - Amity Hall (UMJ Collection No. 211251); **A**, **D** – female, **B**, **C** – male, **A** – **D** - scale bars, 0.1 mm; **E** – juvenile stage extracted from the inner space of the female's carapace, scale bar – 0.05 mm; **F** – detail from E, anterior area, scale bar – 0.01 mm.

Table 1 – *Elpidium martensi*, n. sp. Hector's River, details of the carapace dimensions (No. ind. – number of individuals examined; Mean ± SE – arithmetic mean and standard error, CV% - coefficient of variation).

Locality	Morph.Trait Dev. Stage & Sex	No. Ind.	Mean ± SE	Median	Range (Min-Max)	CV%
HectorsRiver (HR)						
	Length (L - mm)					
	Ad-F	15	0.86±0.01	0.85	0.78-0.92	5.12
	Ad-M	15	0.83±0.005	0.82	0.79-0.88	2.73
	A-1	10	0.68±0.004	0.68	0.66-0.70	1.83
	A-2	1	0.55	-	-	-
	A-3	1	0.43	-	-	-
	Max. Width (MW - mm)					
	Ad-F	13	0.74±0.012	0.75	0.67-0.79	5.96
	Ad-M	15	0.68±0.056	0.68	0.64-0.72	3.24
	A-1	10	0.54±0.006	0.545	0.51-0.57	3.37
	A-2	1	0.43	-	-	-
	A-3	1	0.31	-	-	-
	Rel. Width (MW/L - %)					
	Ad-F	13	85±0.47	86	83-89	2
	Ad-M	15	81.93±0.4	82	79-84	1.87
	A-1	10	78±0.5	79.5	76-81	2.02
	A-2	1	0.78	-	-	-
	A-3	1	73.47	-	-	-
	Rel. MW Position (%)					
	Ad-F	13	59.85±0.6	61	55-62	3.6
	Ad-M	15	58.07±0.61	58	54-65	4.09
	A-1	10	56.9±0.66	57	54-60	3.65
	A-2	1	57.62	-	-	-
	A-3	1	53	-	-	-

Table 2 – *Elpidium martensi*, n. sp. Amity Hall, details of the carapace dimensions (No. ind. – number of individuals examined; Mean ± SE – arithmetic mean and standard error, CV% - coefficient of variation).

Locality	Morph.Trait Dev. Stage & Sex	No. Ind.	Mean ± SE	Median	Range	CV%
Amity Hall (AH)						
	Length (L - mm)					
	Ad-F	9	0.83±0.007	0.84	0.80-0.86	2.62
	Ad-M	15	0.78±0.007	0.78	0.73-0.82	3.64
	A-1	6	0.61±0.02	0.615	0.55-0.66	7.04
	Max. Width (MW - mm)					
	Ad-F	9	0.71±0.009	0.71	0.66-0.74	3.66
	Ad-M	12	0.68±0.006	0.68	0.64-0.72	3.24
	A-1	6	0.46±0.02	0.46	0.40-0.51	11.3
	Rel. Width (MW/L - %)					
	Ad-F	9	84.22±0.27	84	83-85	0.99
	Ad-M	12	81.25±0.33	81	80-84	1.4
	A-1	6	75.66±1.2	76	71-79	3.89
	Rel. MW Position (%)					
	Ad-F	9	59.89±0.63	59	58-64	3.17
	Ad-M	12	59.33±0.84	58.5	56-64	4.94
	A-1	6	58.5±0.43	58	57-60	1.79

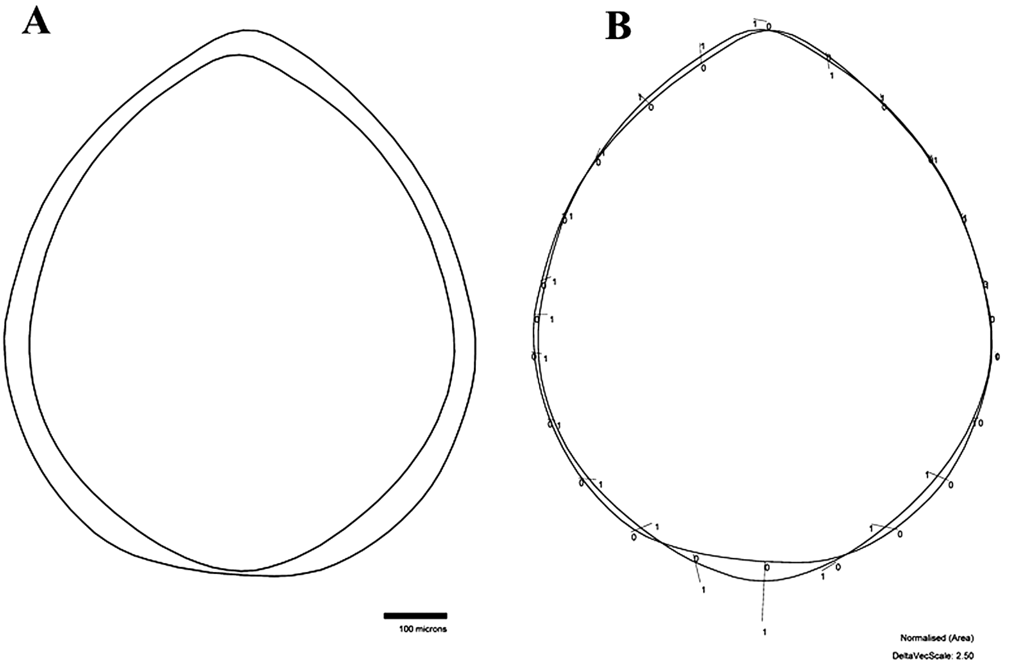


Figure 11 - *Elpidium martensi* n. sp., Hector's River, dorsal representation of the carapace as virtual mean outline of the female (n – 13) and the male (n – 15), calculated with the B-splines algorithm of the MORPHOMATICA programme; **A** – Superposition of outline in non-standardised for surface procedure, female outline, external to the male's one, scale bar, 0.1 mm; **B** – Same superposition but calculated after standardisation to an equal surface unit of 1 mm². The 24 control points and the vectors between outline sections are visible (vectors length increased 2.5 times of their original value); 0-outline, female and 1-outline, male (note that because of standardisation to equal surface the male's outline lays outside to the female one in their posterior part).

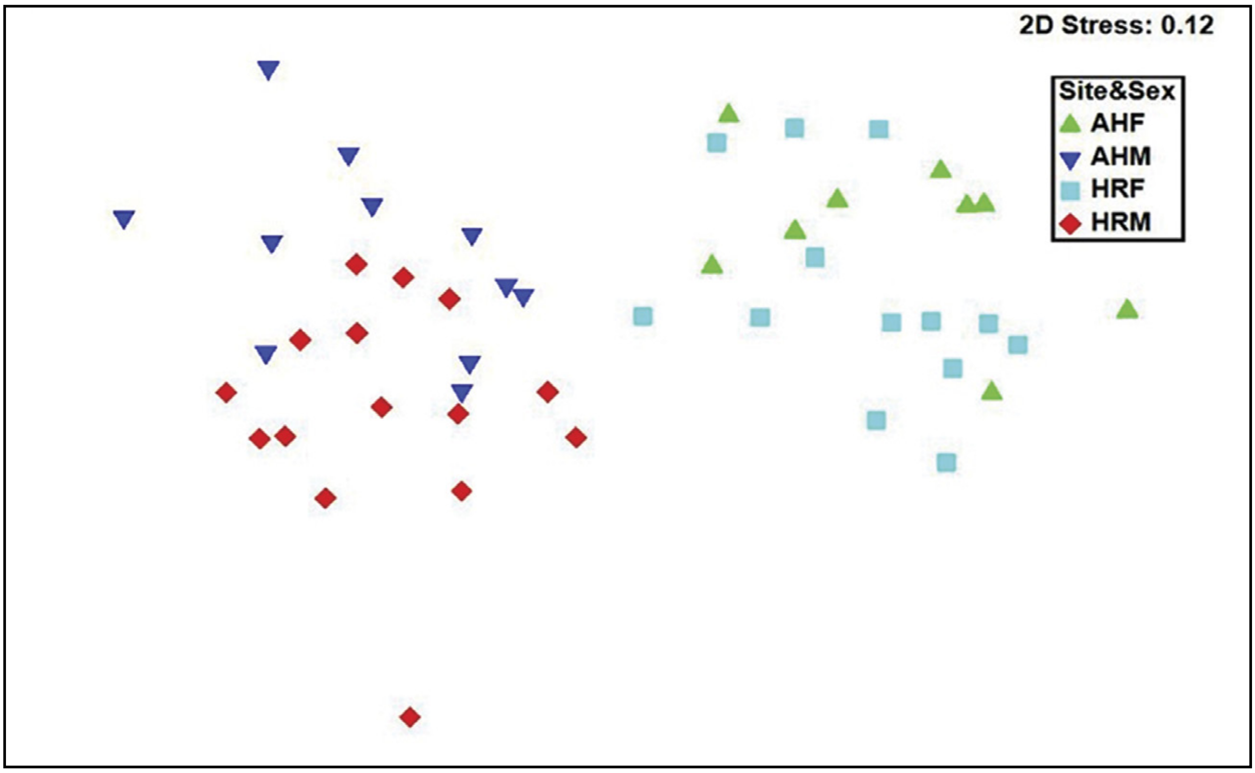


Figure 12 - *Elpidium martensi* n. sp., Distribution of the morphologic disparity of female (F) and male (M) outlines in dorsal view of the carapace for the samples of Amity Hall (AH) and Hector's River (HR), 2-D of a non-metric Multi Dimensional Scaling (n-MDS) plot, computed by the programme PRIMER-6. Note the lack of morphological separation for both females and males at the inter-site level as opposed to the clear separation of the two sexes (*cf* also the ANOSIM values, Table 3)

Table 3 – ANOSIM results for the carapace's shape in dorsal view calculated from standardised for equal surface outlines in various variants: for the whole outline, for the anterior and for the posterior halves of the outlines (AH - Amity Hall, HR - Hector's River, F – female, M- male, A-1 - juvenile stage); data computed with the statistic programme PRIMER v.6 (for additional details, see text).

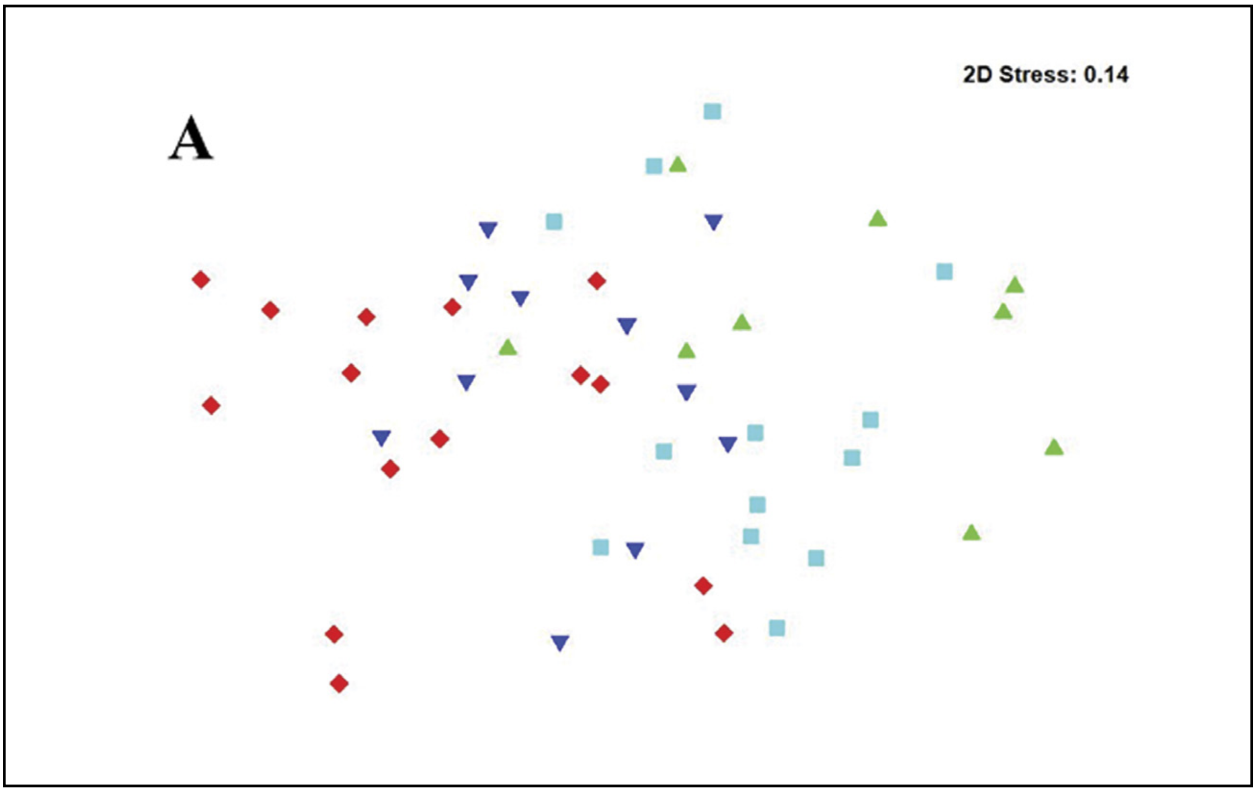
Total Carapace Shape (in dorsal view), Standardised for equal surface		
Pair-Samples	**R-Statistics**	**Significance level (P %)**
HR-F/HR-M	0.904	0.1
AH-F/AH-M	0.911	0.1
HR-F/AH-F	0.014	34.9
HR-M/AH-M	0.07	8.9
HR-A-1/HR-M	0.641	0.1
Anterior half of Carapace Shape, Standardised for equal surface		
HR-F/HR-M	0.407	0.1
AH-F/AH-M	0.315	0.1
HR-F/AH-F	0.102	10.3
HR-M/AH-M	0.13	2.5
HR-A-1/HR-M	0.252	0.7
Posterior half of Carapace Shape, Standardised for equal surface		
HR-F/HR-M	0.922	0.1
AH-F/AH-M	0.949	0.1
HR-F/AH-F	-0.018	51.7
HR-M/AH-M	0.036	22.8
HR-A-1/HR-M	0.742	0.1

Table 4 – Results of the Mann-Whitney U test for differences of the relative size of the carapace width (W/L%) within and between populations as well as between sexes and developmental stages.

Locality	Compared Pair	Pair No. Individuals	Pair Average Ranks	Critical Value U	Probability Significance
Hector's River (HR)	Female/Male	15:15	21.27:9.73	199	P > 0.001
HR	Male/A-1	15:10	17:17:6.75	137	P > 0.001
Amity Hall (AH)	Female/Male	9:15	19.39:8.37	129.5	P > 0.001
AH	Male/A-1	12:6	12.5:3.5	72	P > 0.001
HR/AH	Females	15:9	13.7:10.44	86	P = 0.13
HR/AH	Males	15:12	15.87:11.67	118	P = 0.08

Left valve (Fig 5): resembling the right valve but having on the dorsal side counterpart hinge elements. The H/L ratio slightly lower than those of the opposite valve, c. 58 %. A lamellar hinge bar is more strongly developed in its posterior side (Figs 5 D – F and details in section B 1 d). A ventro-posterior inner list with thin cilia is present (Fig 5 C and details in section B 1 c). Ventral side flat as in the right valve (Fig 5 G).

Antennula (Figs 20 A, 21 A): Five functional articles (I, II, III, IVa+IVb, V); article I expanded on the dorso-apical region with a group of tiny setae; segment II long, with a large ventral seta inserted baso-medially and reaching fused area of segments IVa+IVb; segment III with a small dorso-apical seta; segment IVa+IVb long, set medially with a ventral and two dorsal setae and apically with a ventral and three dorsal se-



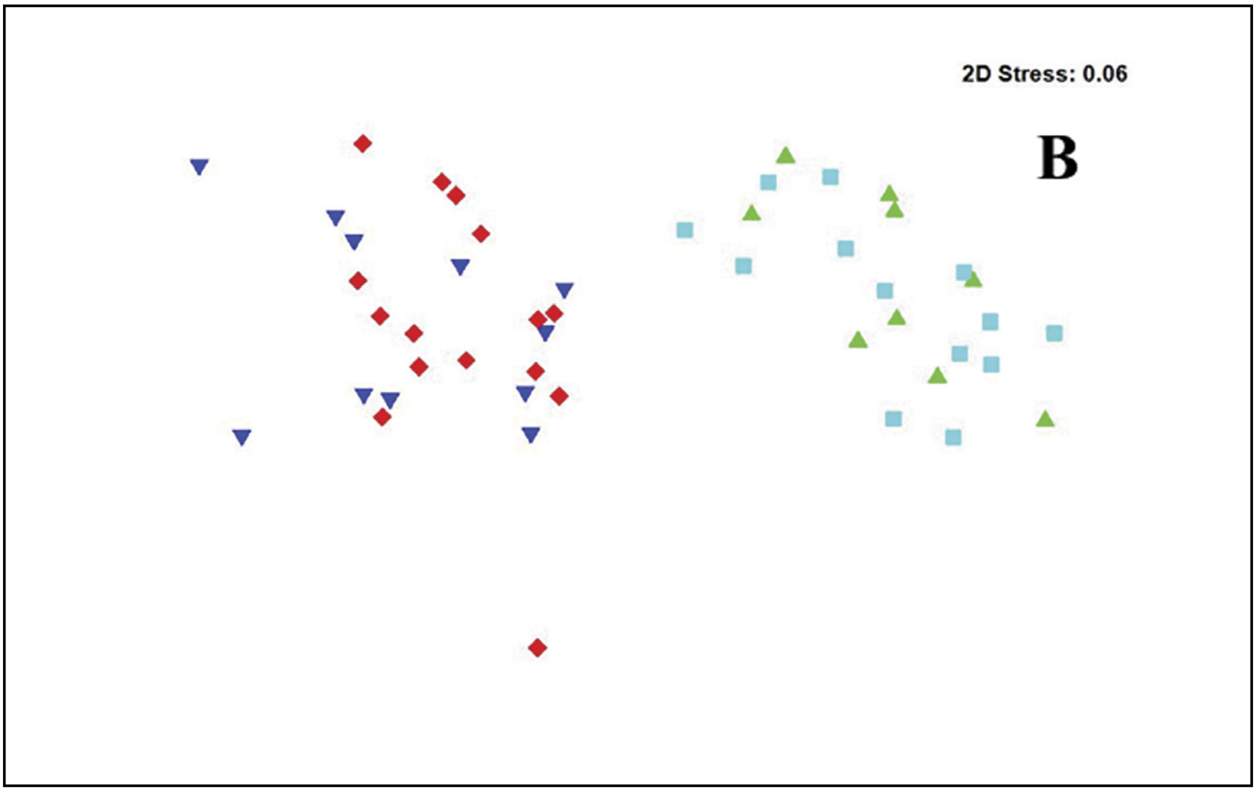


Figure 13 - *Elpidium martensi* n. sp., same data as in Figure 12 but: **A** – the quasi-independent anterior half of the outline (note the lack of morphological separation betweensites and the two sex), **B** - the quasi-independent posterior half of the outline (note the morphological separation between female and male outline-sections).

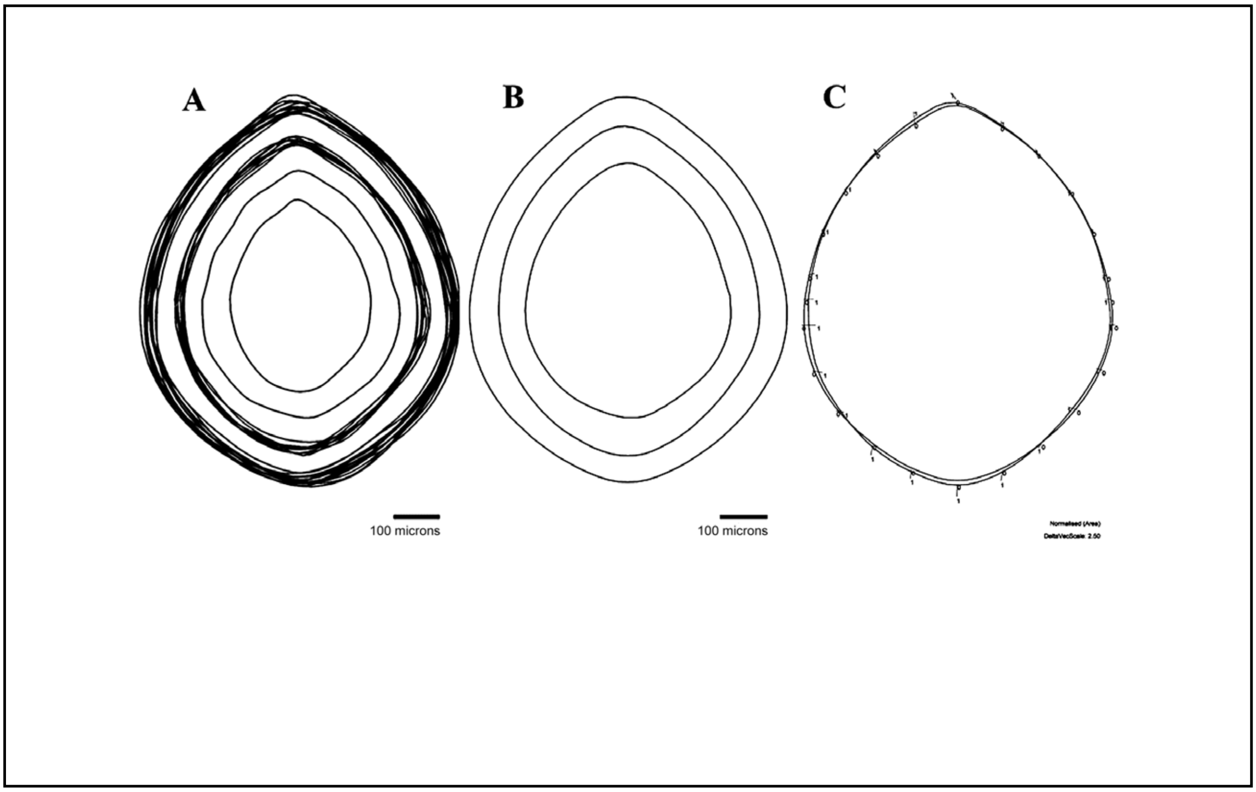


Figure 14 - *Elpidium martensi* n. sp., Hector's River, dorsal representation of the development trajectory of the carapace from A-3 to the adult male, computed with the B-splines algorithm of the Morphomatica programme; **A** – Superimposition of outlines in non-standardised for surface procedure, adult male outlines (n – 15), external to the A-1stage (n - 10), followed internally by A-2 (n - 1) and A-3 (n - 1); **B** – Superimposition of virtual mean outlines: adult male, A-1 and A-2. Scale bar: 0.1 mm; **C** - Same superposition for the mean outlines of the A-1 (1) on the adult male (0) calculated after standardisation to an equal surface unit of 1 mm^2. (vectors length increased 2.5 times of their original value).

tae; terminal article V with three setae and an aesthetasc Ya. Its distal chemosensorial section represents 14 % of the total length of this seta.

Antenna (Figs 20 B, 21 B - D): Protopodite two-segmented, basis, long and curved; endopodite three-segmented; 1st segment of intermediate size, with a long ventro-apical seta, which starts from a short expansion of the segment (Fig 21 B, arrow marks this strange structure). This antennal seta reaches the tip of the following segment. On the outer side of the 1st endopodial segment is visible an area of pseudochaetae, represented by long setules. 2nd segment (represented by the fusion of the 2nd and 3rd endopdial segments), long, ventro-medially with a seta and the Y aesthetasc. The chemosensorial section of this aesthetasc represents 11 % of the total length of the seta. On the dorsal side of the E2+E3 segment, there are two sub-apical setae; ventro-apically on the same segment, a strong biserrate seta and adjacent to it a minute seta. The 4th endopdial segment on its distal margin, two slender biserrate claws in dorsal position and ventrally a strong pectinate claw with one row of strong denticles. At the postero-distal corner of the E4, are inserted a minute seta and a hyaline ampulla; exopodite with a long spinneret tube and a tiny seta.

Mandible (Figs 20 C, 21 E, F, 24 D): Coxal endite with 8 teeth and a long serrate seta. The interdental X1 seta, spoon-shaped. The setae X2 and X3 slender and S-shaped. Additionally, in each interdental space exists a stiff spine. The 1st and the last tooth, bicuspidate, the other being tricuspidate. Mandibular palp with a large basis; internally on this latter are two medium-sized sub-equal setae and externally an exopodite with 3 long rays and a reflexed seta. Endopodite with three segments; first segment relatively small with two internal apical setae, one about half as long as the other; second segment with a ventro-apical seta, two dorso-apical setae and a centro-apical seta; terminal segment ventrally with a seta and externally with a claw.

Maxillula (Figs 20 D, 21 G): Maxillular basis with 3 endites. The inner one with 3 smooth setae; the next two endites, each with two spatulate bright setae and 2 visible (but probably 3) smooth setae. The endopodite short, with 2 strong plumose setae and a short spine. Exopodite (the respiratory plate) well developed, with 16 rays and one reflexed seta.

1st Thoracopod (Figs 22 A, 23 A, B): Basis with a pappose dorsal seta and 2 apical biserrate setae. Exopodial seta, long, plumose; 1st endopodial segment with one distal anterior biserrate seta, shorter than the length of the 2nd endopodial segment (E2). Distal endopodial segment fused with the dis-

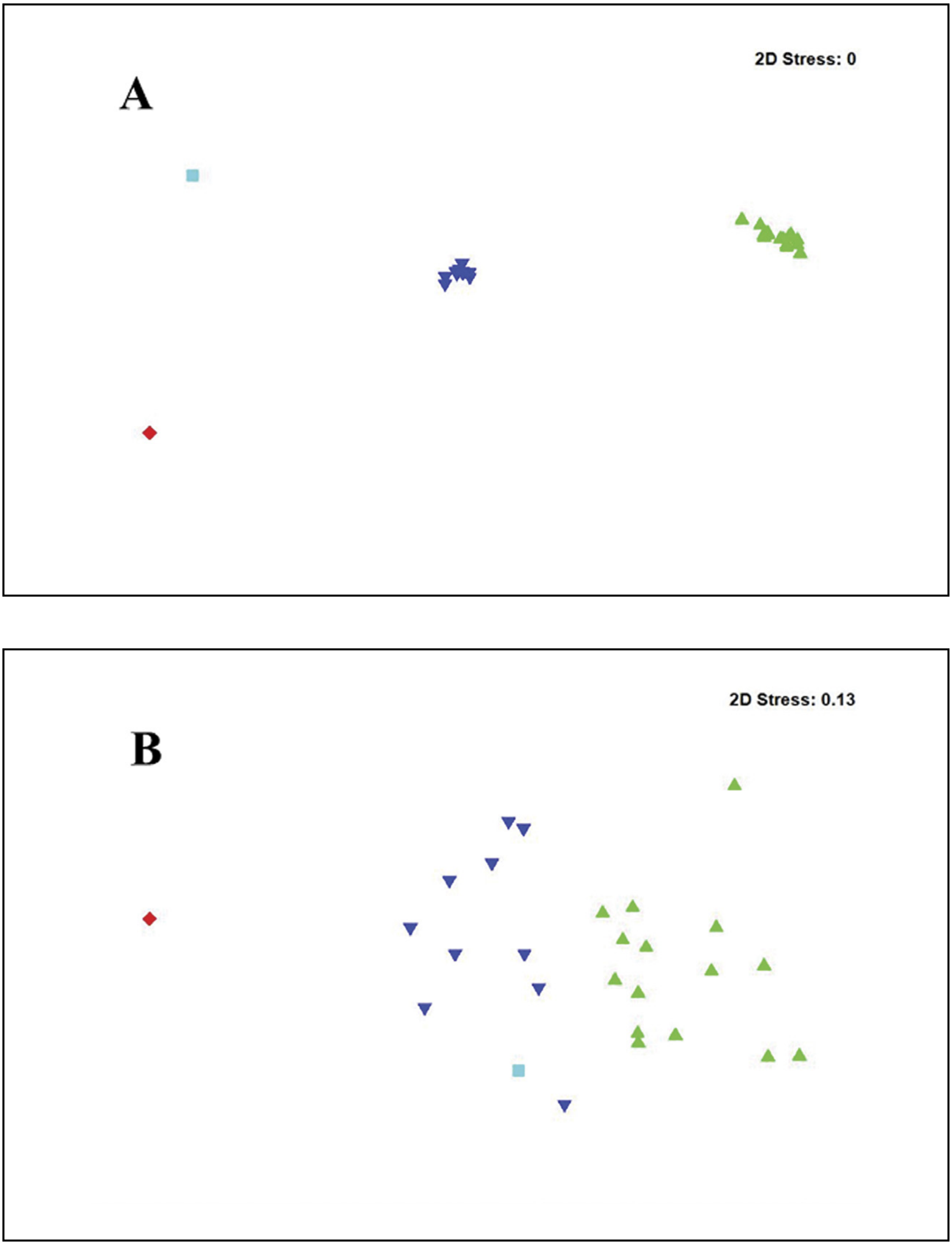
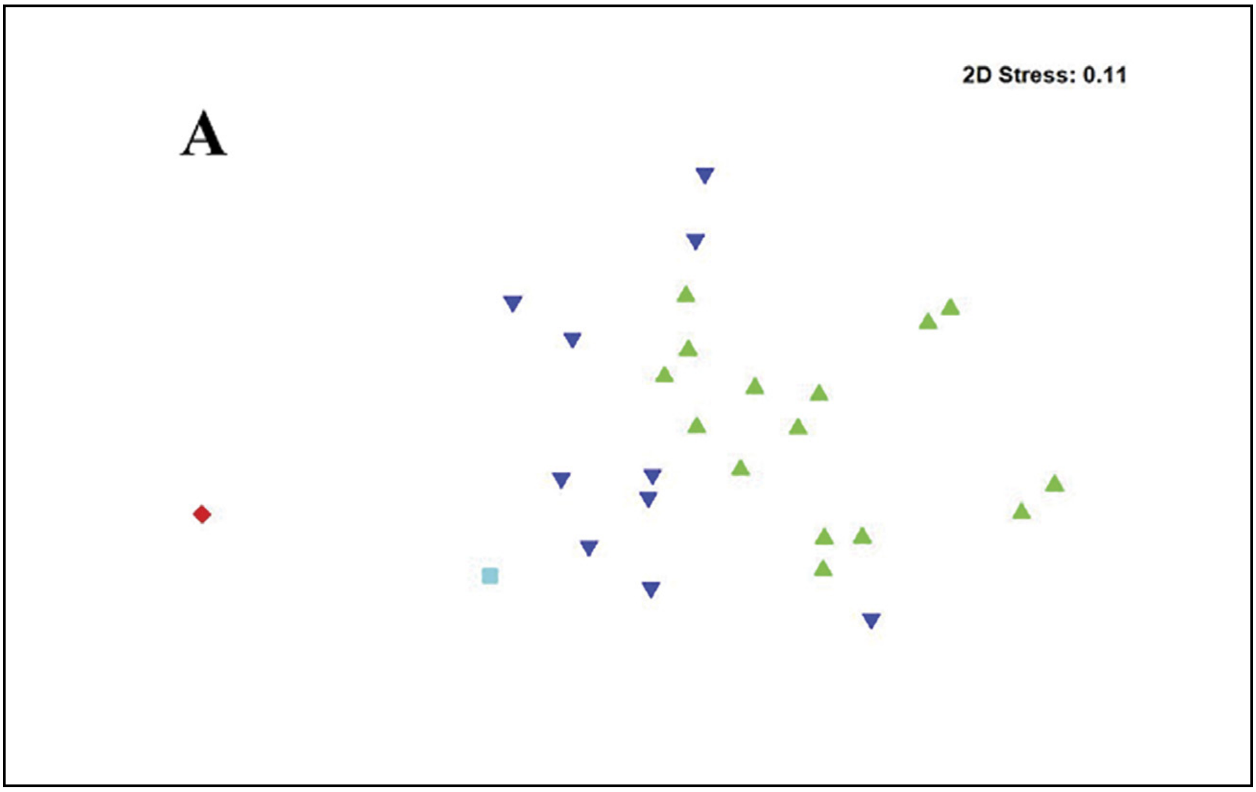


Figure 15 - *Elpidium martensi* n. sp., Hector's River, representation of the development trajectory of the carapace from A-3 to the adult male, in a 2-D plot of a non-metric Multi Dimensional Scaling (n-MDS), computed on a dissimilarity matrix of B–splines, by the programme PRIMER-6 (number of outlines as for the Figure 14); **A** – data of non-standardised outlines; **B** – same calculated for standardised data to equal surface unit.



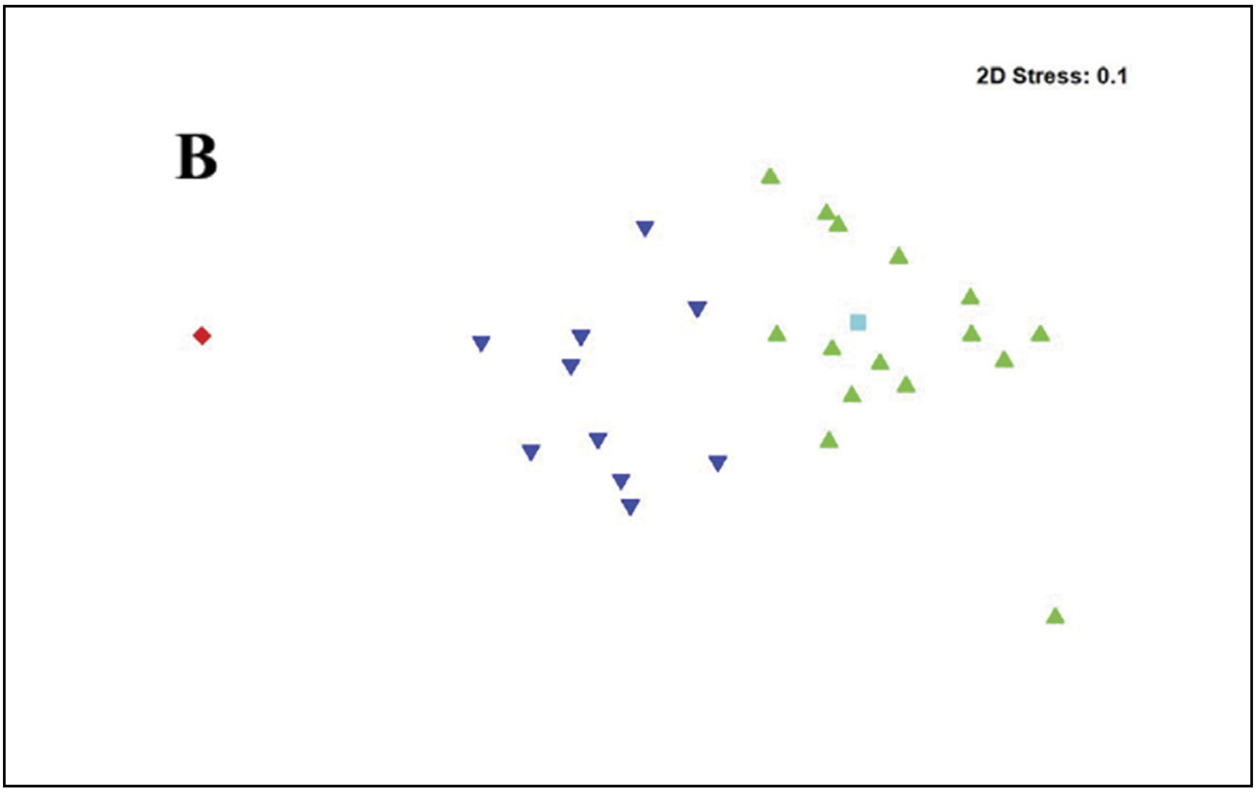


Figure 16 - *Elpidium martensi* n. sp., same representation as in Figure 15, **A** – the quasi-independent anterior half of the outline (note the lack of morphological separation between stages A-2, A-1 and the adult male); **B** - the quasi-independent posterior half of the outline (note the morphological separation between the A-1 and the adult male).

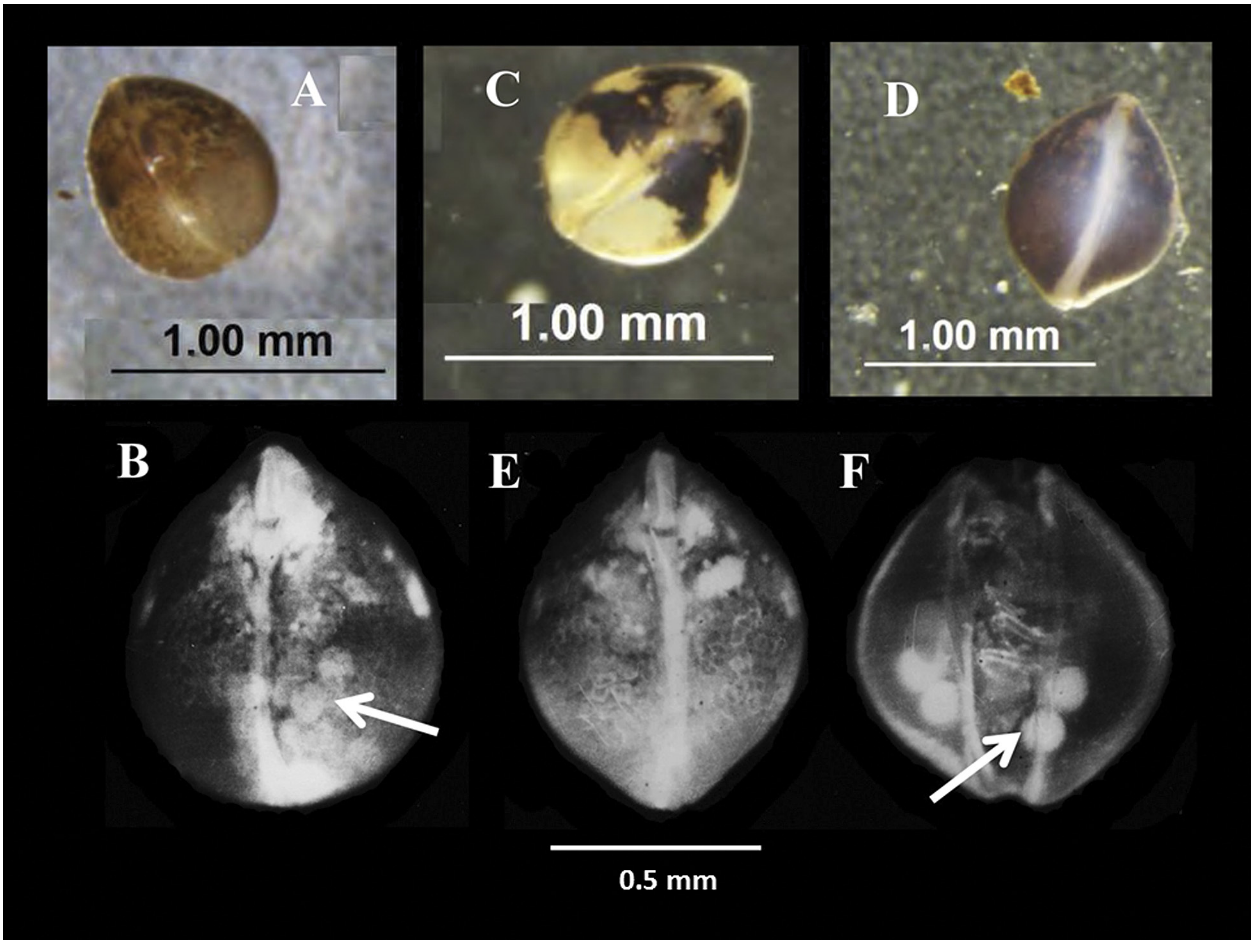


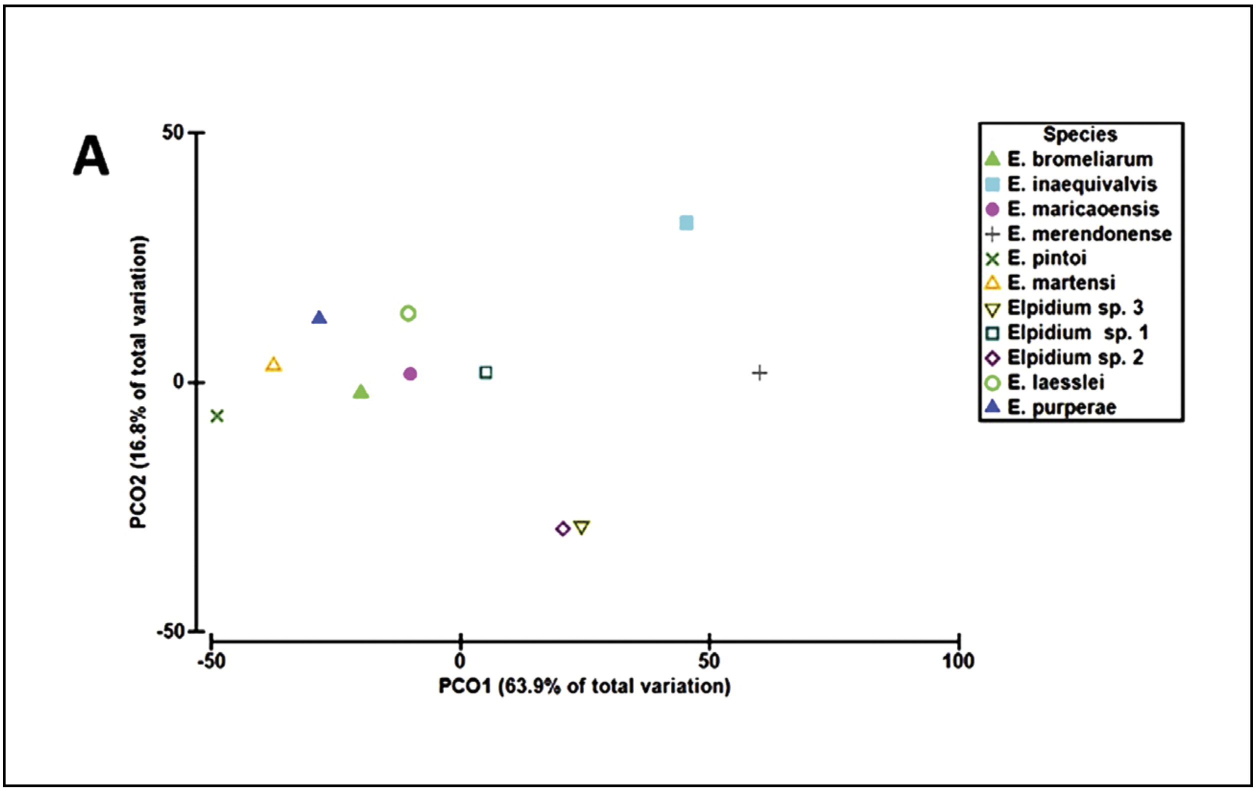
Figure 17 – View of various female carapaces of *Elpidium* with their peculiar pigmentation and the presence of eggs in the inner space of the carapace (see arrows); **A**, **B** – *Elpidium martensi* n. sp. Hector's River; **C** – *Elpidium* sp. 1, Good Hope; **D** - *Elpidium* sp. 2, Sherwood Forest; **E**, **F** dorsal and ventral views of *Elpidium* aff. sp. 2, Albany, site 3 (A, C, D, photos by D. L. Danielopol, 2014; B, E, F, photos by N. Riedl, 1993).

tal claw, 1.5 longer than the length of E2. Distal claw serrate on its anterior side.

2nd Thoracopod (Figs 22 B, 23 C): Similar to the preceding thoracic limb but slightly longer. Basis with only one apical seta which is pappose. The E1 segment longer than its homologue on the Th. 1. It is covered on the anterior side with filamentous pseudochaetae. The distal seta of E1 equal or slightly longer than the E2 segment. The distal endopodial segment fused to the distal claw and bearing a posterior minute seta, slightly longer than its homologue of Th. 1. Distal claw serrate on its anterior side, similar to those of Th. 1.

3rd Thoracopod (Figs 22 C, 23 D - F): This thoracopod is longer than those of the two preceding limbs, due to the elongation of the endopodite. Basis with 2 dorsal slender setae and a posterior plumose exopodial seta.This latter is c. 50 % shorter than its homologues of the Th. 1 and 2. The distal seta of E1 1.6 longer than the E2 segment and with 2 rows of longer setules. The distal endopodial segment fused to the distal claw, 3 times longer than the E2 segment; it is also biserrate as the precedent distal claws. Segment E1 with long filamentous pseudochaetae. E2 with anterior pseudochaetae and a transversal row of pectinate pseudochaetae on the posterior side. E3 with 3 distal rows of pseudochaetae on its anterior side (Fig 23 F).

Hemipenis (Figs 22 D, 23 G – I, 24 A – C, 25 A): Peniferum large and rounded containing muscles fixed on the inner wall or on special trabeculae (Fig 25 A). The peniferal muscles move the appendages of the copulatory complex, namely the copulatory process and the Lower Ramus, both placed on the medial side of the hemipenis (Fig 25 A and additional details in section B 4). On the apical margin of the peniferum a distal lobe without digital expansion, it is lancet-shaped and with a broad basis (this latter represents 50-60 % of its length). Copulatory process short, as a curved funnel and without distal glans. Lower Ramus L-shaped with a broader basis and a slightly slender concave distal segment. On the lateral side of the distal lobe a slender seta inserted on the apical margin of the peniferum.



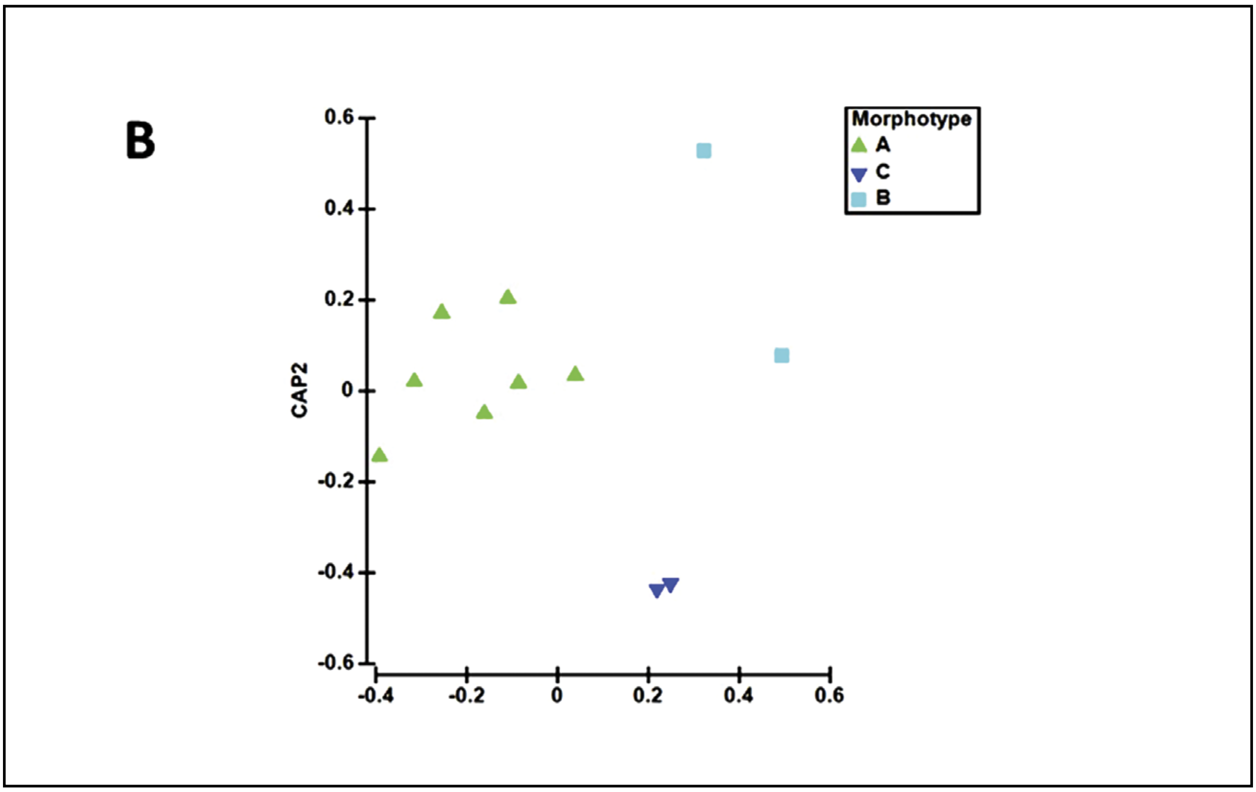


Figure 18 - Distribution of the morphologic disparity of outlines in dorsal view for female carapace of 11 *Elpidium* species. **A** - plot by Principal Coordinate Ordination (PCO), **B** – plot by Canonical Analysis of Principal Coordinates (CAP); **A** – **C** - the three species groups.

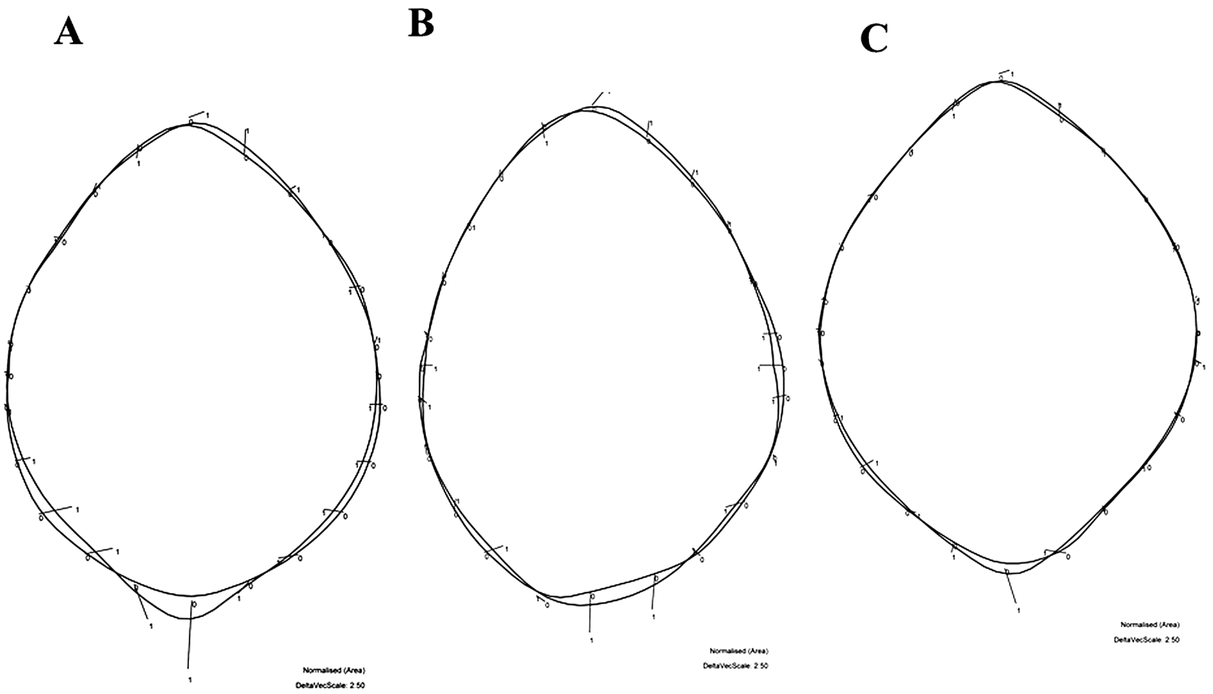


Figure 19 – Superimposition of female and male outlines of various *Elpidium* carapaces in standardised for surface procedure, female outline (0), male (1); **A** – *Elpidium* sp. 1 (Good Hope) virtual mean outlines of 3 female- and 4 male-outlines; **B** – *Elpidium inaequivalvis*, from Danielopol, 1975, figure 4, A, B); **C** - *Elpidium* sp. 2 (Sherwood Forest), virtual mean outlines of 3 female- and 4 male-outlines.

Additional description of female

Carapace (Figs 8 A – D, 10 A, D, 11 A, B, 17 A, B, 24 E, Tables 1 and 2): It differs from that of the male in the slightly larger size (mean length about 0.83 - 0.86 mm, depending on site). It is expanded in the posterior half forming a large brood pouch within which eggs and the first juvenile stages are stored (Fig 17, 24 E). This gives the carapace its cordiform shape in dorsal view. The maximum width represents about 85 % of the carapace length and is placed at about 60 % from the anterior edge. Posterior carapace-shape broader than those of the male but still only moderately rounded (Figs 8 A, D, 11. A, B, 17 A).

Left valve (Figs 6 A – E, 7 A, B, 9 A - C): In lateral view, slightly more arched than the male's homologue valve (height/length ratio c. 59 % of the total length, placed at 54 % from the anterior edge). A small flange visible too (Fig 7 B).

Right valve (Figs 6 F – H, 7 C, D, 8 E, 9 D, E, 27 A - D): Similar to the left valve but larger and higher, as it overlaps the latter. The H/L ratio is c. 61 % at 55 % from the anterior edge). Other details similar with those described for the male (*cf* illustration above).

Antenna (Fig 20 E): Female antenna differs from that of the male in the structure of the terminal segment, which bears three biserrate claws, similar to one another. Additionally a minute seta and a hyaline ampulla present.

Abdomen (Fig 22 E): Genital operculum rounded and rigid; internally with a trabecula. Caudal ramus consisting of a rounded lobe, with two closely placed setae and a third one displaced towards the external side. The posterior end of the body, rounded and with a stiff spine-like seta.

Differential Diagnosis – *Elpidium martensi* n. sp. with a reversal of the overlap of valves, *i.e.* the right valve covers the left one, is similar to *E. purperae* (*cf* description for this latter species, Danielopol, 1975). Females of *E. martensi* n. sp. display a carapace shape which places the species within the *Elpidium* species-group A (see details below, section B 3) differing from *E. inaequivalvis* and *E. merendonense* with asymmetric valves and *Elpidium* sp. 2 and *Elpidium* sp. 3 with poorly developed brood-pouch and weak carapace dimorphism. Hinge of inverse protodont type (details below, section B 1 d). From the other 10 species of *Elpidium* a "reversed-hinge" is known only for *E. purperae*. The new species *E. martensi* n. sp. with a mean length of the female carapace of 0.8 mm and the general cordiform shape (Tables 1 and 2) resembles *Elpidium bromeliarum* Müller, *E. pintoi* Danielopol, *E. purperae* Danielopol and *Elpidium* sp. 1. The distal lobe of the hemipenis, lancet-shaped and with a bright basis (this latter represents 50-60 % of its length) is peculiar to *E. martensi* n. sp. and *E. pintoi*. Most of the other *Elpidium* species have distal lobes provided with a

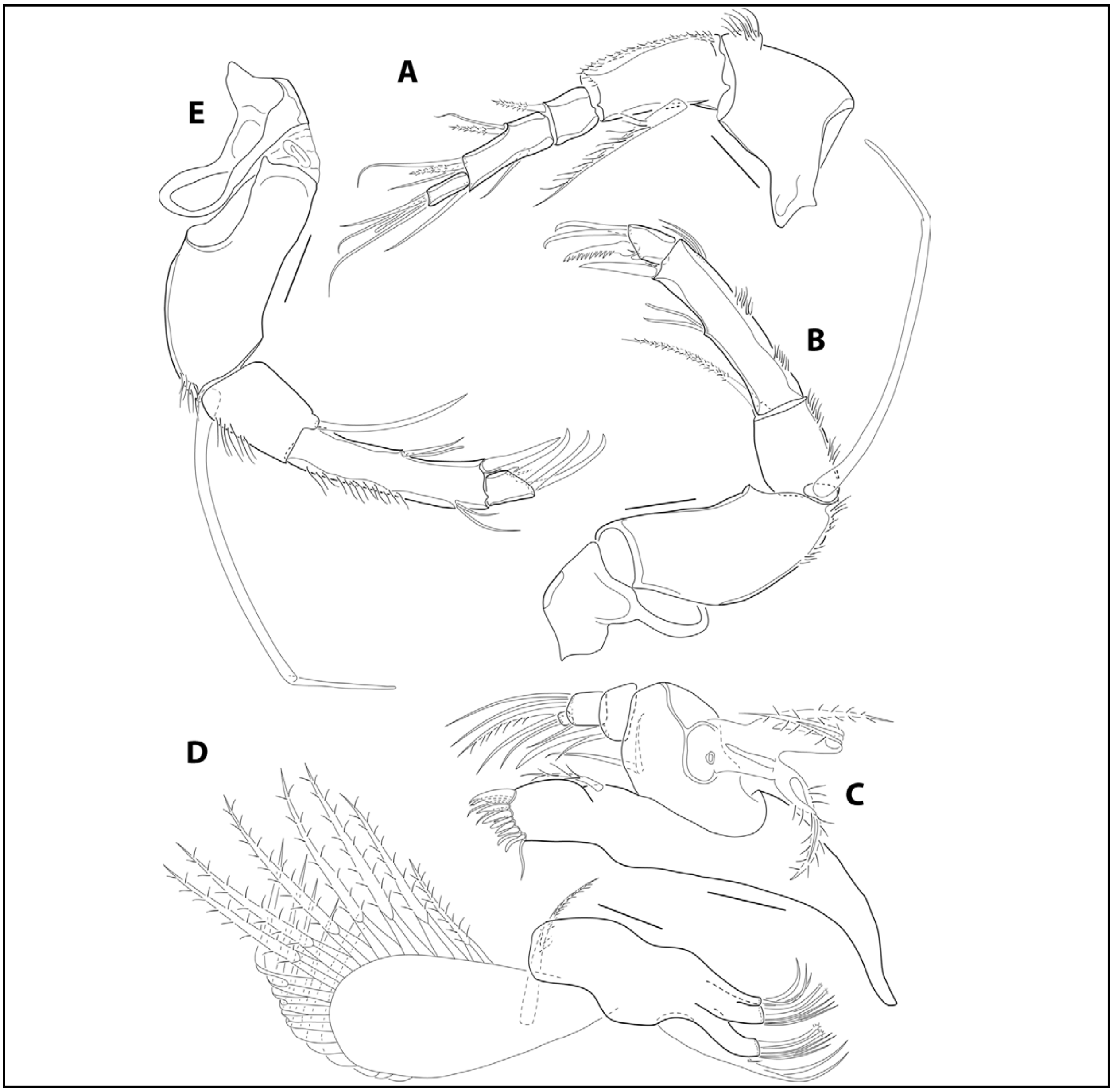


Figure 20 - *Elpidium martensi* n. sp., soft parts, line drawings. **A** - Antennula, male, paratype (MZUSP 32814); **B** - Antenna, male, paratype (MZUSP 32814); **C** - Mandible, male, holotype (MZUSP 32812); **D** - Maxillula, male, holotype (MZUSP 32812); **E** - Antenna, female, allotype (MZUSP 32813). Scale bars: 0.05 mm.

ventral digitiform projection. The exception to this group is *E. purperae* with an elongated distal lobe and without digitiform protrusion. The lobe's width of this latter species represents less than 50% of the length (Danielopol, 1975, Fig 8 D) differing in this way from our new species. Valves of *E. martensi* n. sp. display on their external side a minute flange and are covered with foveolae. For most *Elpidium* species no flange and ornamentation are described, with one exception, *E. laesslei*, for which Tressler (1956) figured a carapace with conspicuous fossae.

B - Analysis of morphologic traits relevant for the evolutionary morphology and systematics of Timiriaseviinae

1 - Morphology of the peripheral area and of the surface of Elpidium valves offers new directions for taxonomic diagnostic traits

We describe here five morphological structures of the valves which should be useful for a better differentiation of Timiriaseviinae taxa. These are: (a) the external marginal zone of the valves, (b) the position of the selvage on the anterior part of the valve, (c) the ciliated list on the inner lamella, (d)

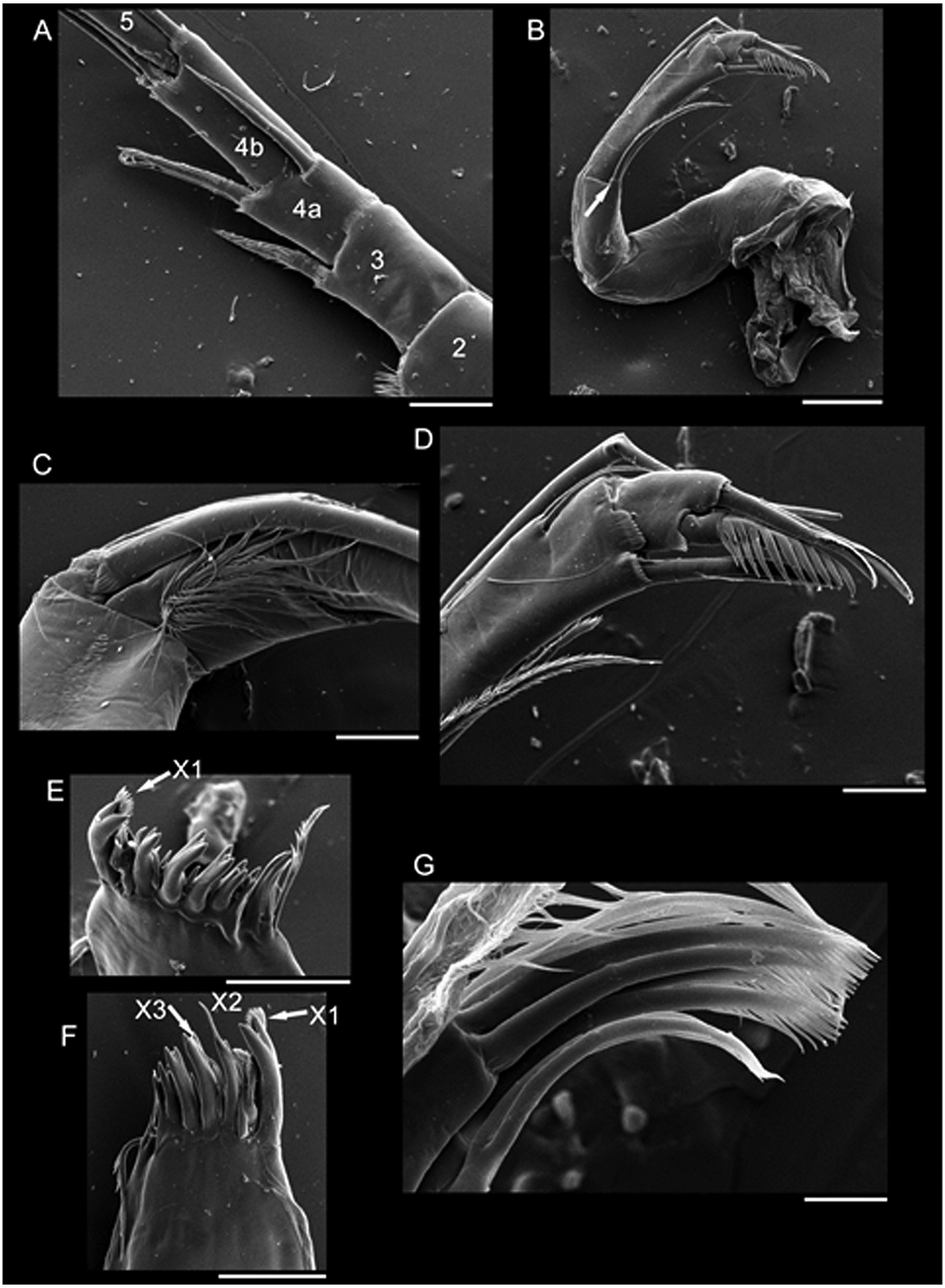


Figure 21 - *Elpidium martensi* n. sp., soft parts, scanning electron microscopy, male (MZUSP 32816). **A** – Antennula; **B** - Antenna; **C** - Antenna, detail of the basi-, exo- and endo-podite. **D** - Antenna, detail of the 3rd and 4th endopodial segments; **E**, **F** - Mandible, details of coxal endite; **G.** Maxillula, detail of internal claws and setae on endites (see the spatulate setae of the 1st and 2nd endite and the distal setae of the 3rd endite. Scale bars: A, C-F - 0.2 mm; B - 0.05 mm; G - 0.01 mm.

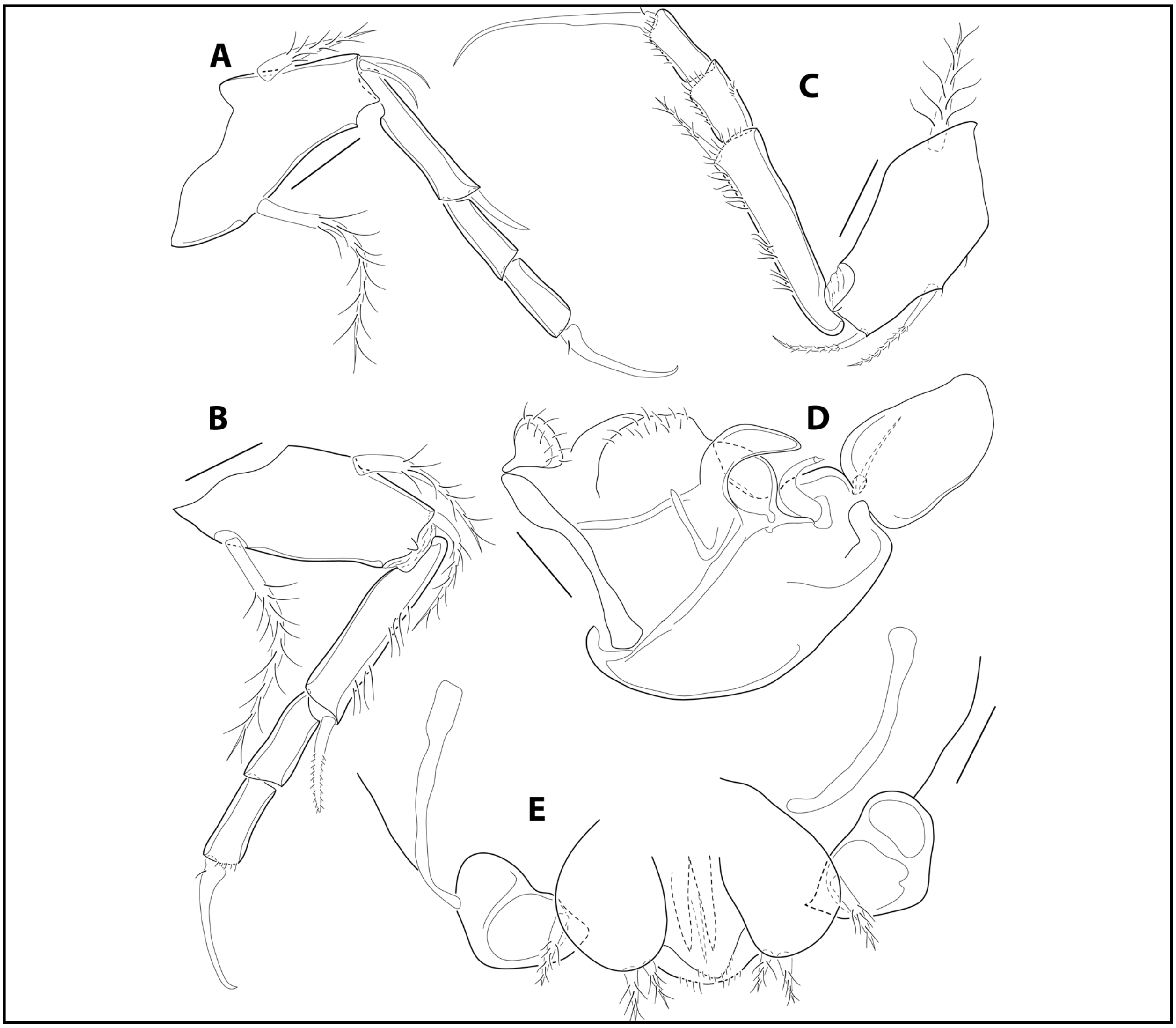


Figure 22 - *Elpidium martensi* n. sp., soft parts, line drawings. **A** – **D** male holotype (MZUSP 32812); **A** - First thoracic leg; **B** - Second thoracic leg; **C** - Third thoracic leg; **D** - Hemipenis, male; **E** - End of body, female, allotype (MZUSP 32813). Scale bars: 0.05 mm.

the hinge structure, and (e) the valve surface ornamentation including also the epidermis pigmentation.

Colin and Danielopol (1980) stressed that ostracods of the genus *Elpidium* display close morphological affinities with the genus *Afrocythere* from western Africa, and Pinto *et al.* (2008) documented close morphological similarities of their species *Intrepidocythere ibipora* from Brazil with species of *Elpidium*. Details on the five complexes of morphologic traits will help not only to improve the diagnoses of these genera based on carapace morphology but also to promote future phylogenetic studies where these traits can be illuminating.

a: The external marginal zone of the valves – *Elpidium martensi* n. sp. presents behind the anterior margin a slightly developed flange visible in lateral view on both valves (Figs 7 A – D). The antero-dorsal side of the left valve presents a short rim (Figs 8 A, B), which continues along the whole length of the ventral margin (Fig 8 D). The right valve displays also a marginal rim but only on the anterior third of the ventral marginal zone (Fig 8 D). Pinto and Jocqué (2013, Figs 1 H, I, 4 H, I) illustrated for their species *E. merendonense* a similar pattern of rims on the valves. For *Intrepidocythere ibipora*, Pinto *et al.* (2008, Fig 1 F) documented rims on the ventral side of the right and the left valves which run along 80% of the length of the valve-margin. Considering *Afrocythere rostrata*, Klie (1935) mentions on the anterior marginal area of both valves 12-14 protuberances, each bearing a seta. Neither *Elpidium* nor *Intrepidocythere* display such structures.

The observation of marginal rims was possible only with the SEM technique and at high magnification. Earlier descriptions of valves using LM and/or low SEM magnification did not allow their observation (Pinto and Purper, 1970; Danielopol, 1975). Colin and Danielopol (1980) illustrated with a SEM picture (*cf* their Plate 10, Fig. 9) the anterior rim on the

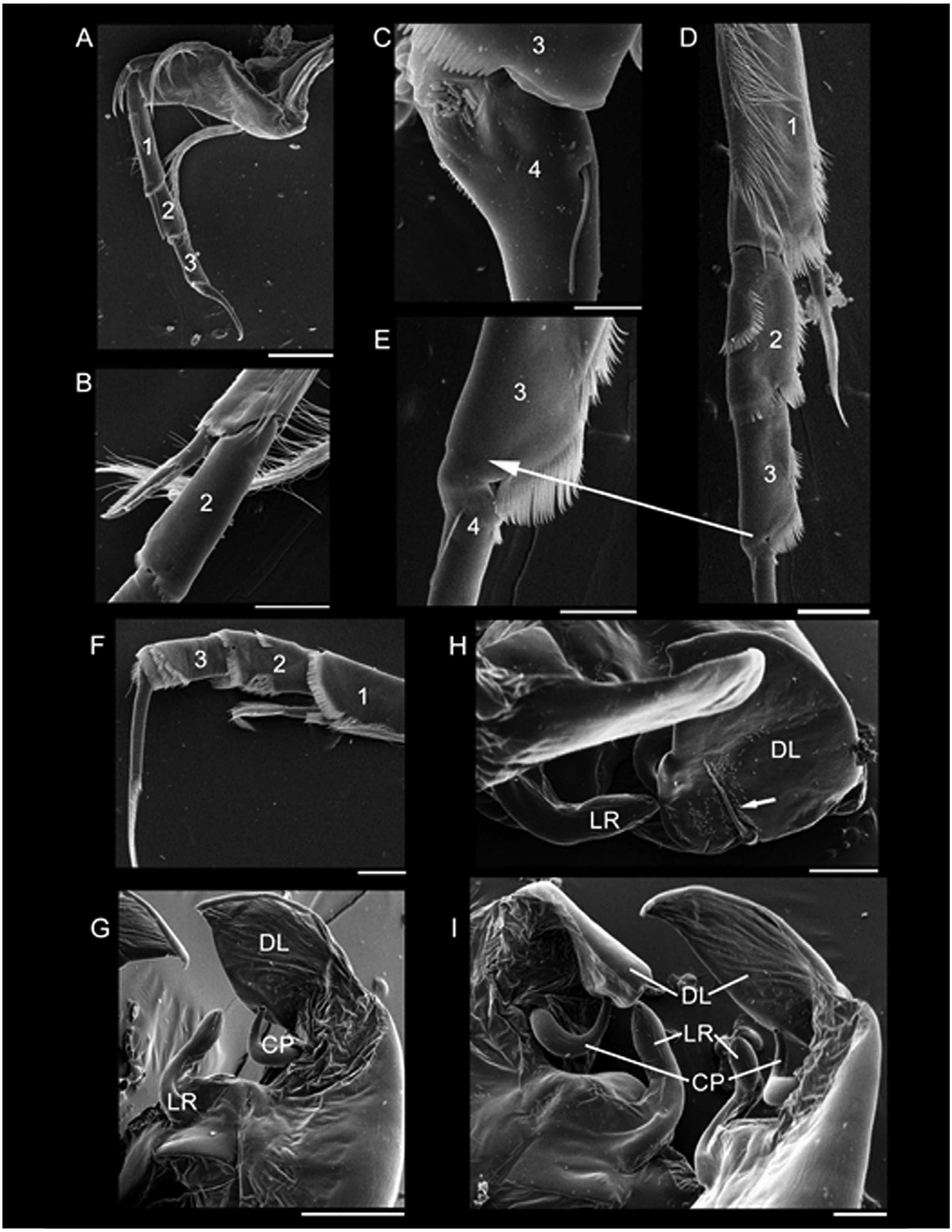


Figure 23 - *Elpidium martensi* n. sp., soft parts, scanning electron microscopy, male (MZUSP 32816). **A**. First thoracic leg. **B**. First thoracic leg, detail of the E1 segment with the endopodial seta and the E2segment. **C**. Second thoracic leg, detail of the E4 segment and the vestigial posterior seta. **D**. Third thoracic leg, detail of three endopodial segments. **E**. Third thoracic leg, detail of insertion of terminal claw. **F**. Third thoracic leg, detail of the endopodial segments with the endopodial seta E1 and the distal claw; **G** – left and right hemipenes, medio-lateral view with details of the DL and the LR; **H** - left and right hemipenes, apical view with details of the DL and LR (arrow points to the lateral seta; **I** – medial view of the hemipenes with the CP, DL and LR. Abbreviations: CP - copulatory process; DL - distal lobe; LR - lower ramus. Scale bars: A, G - 0.05 mm; B, D, F, H-I - 0.02 mm; C - 0.05 mm; E - 0.01 mm.

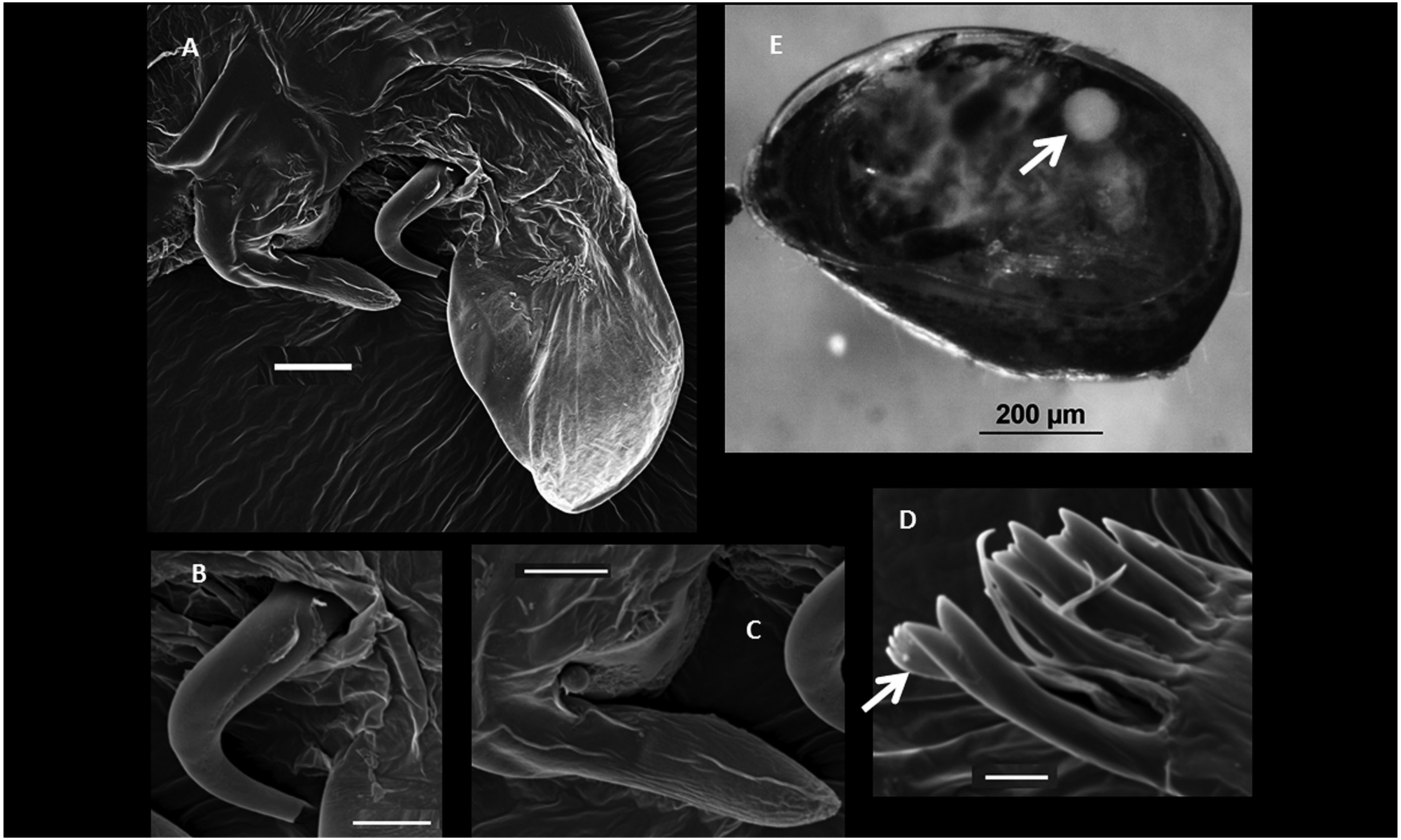


Figure 24 - *Elpidium martensi* n. sp., Amity Hall (UMJ Collection No. 211251), A – D male; A – C left hemipenis, medial position; **A** – general view; **B** – copulatory process; **C** – lower ramus; **D** – Mandible coxal endite (arrow points to the X1 spoon-shaped seta); **E** – female with a visible egg placed behind the central adductor muscles, within the inner space of the carapace.

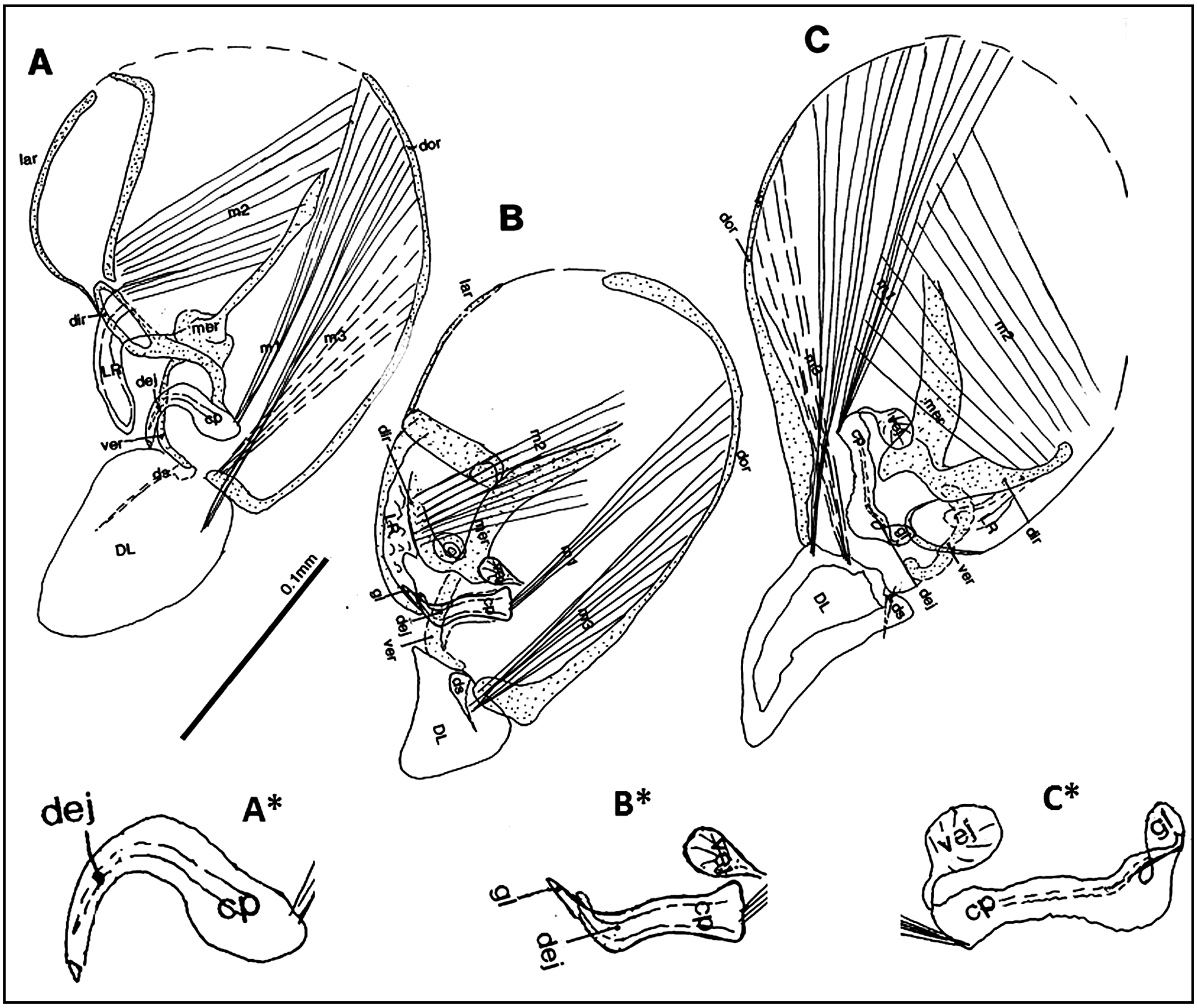


Figure 25 – Hemipenis of *Elpidium* species (items at UMJ, Collection Nos. 211258-211260) – **A** - *Elpidium martensi* n. sp., Hector's River, medial position; **B** - *Elpidium* sp. 1, Good Hope, lateral position; **C** - *Elpidium* sp. 2, Sherwood Forest, medial position; with asterisks, details of the copulatory process for each species. Symbols: DL – distal lobe, ds – distal seta, cp – copulatory process, dej – ductus ejaculatorius, gl – glans, vej – vesicula seminis, LR – Lower Ramus, m1 – m3, muscles (note that m1 moves the copulatory process; m2 contracts the penipherum; m3 is represented by a flexor and an extensor muscle, this latter not always visible; both muscles move the distal lobe DL); dor-dorsal ridge, lar – lateral ridge, mer – medial ridge, ver – ventral ridge.

dorsal side of *E. inaequivalvis*, without however understanding the interest of these structures for the systematics of the Timiriaseviinae. The present data should be useful for further comparative research on Timiriaseviinae valves, especially for fossil material where specialists have difficulties finding enough morphological traits for taxonomic and/or phylogenetic investigations.

b: The position of the selvage – Species of *Elpidium* are remarkable as compared to those belonging to the related genera *Afrocythere* and *Intrepidocythere* by the position of a displaced selvage interiorly to the anterior margin of both valves (*cf* for *E. martensi* n. sp., *inter alia* Figs 4 A, B, D, H, 5 A, B, D, G, H, 6 A, C, F, G). The anterior section of the selvage in the case of *I. ibipora* follows closely the outer margin of the valves (Pinto *et al.*, 2008). Following Klie (1935) the selvage of *A. rostrata* is not developed on the central part of the left valve as compared to the opposite valve. In both *Elpidium* and *Intrepidocythere* the selvage is well developed on the whole ventral side of both valves. The peculiarity of *Elpidium* species as compared to the species of the other two mentioned genera is the presence on the antero-ventral side of a funnel-shape inflexion (Figs 8 E, 9 A, D, 27 A, E) and a double bow on the recovering overlapping valve (in the case of *E. martensi* n. sp. on the right valve, Fig 8 D). Also remarkable is the ventral section of the selvage with a double-plication which starts near the bow-shaped formation (Figs 4 I, 8 E); the upper layer of this plication represents a chitineous lamella which is visible on the selvage of *Kovalevskiella phreaticola* Danielopol (Danielopol, 1969, Figs 1 and 2).

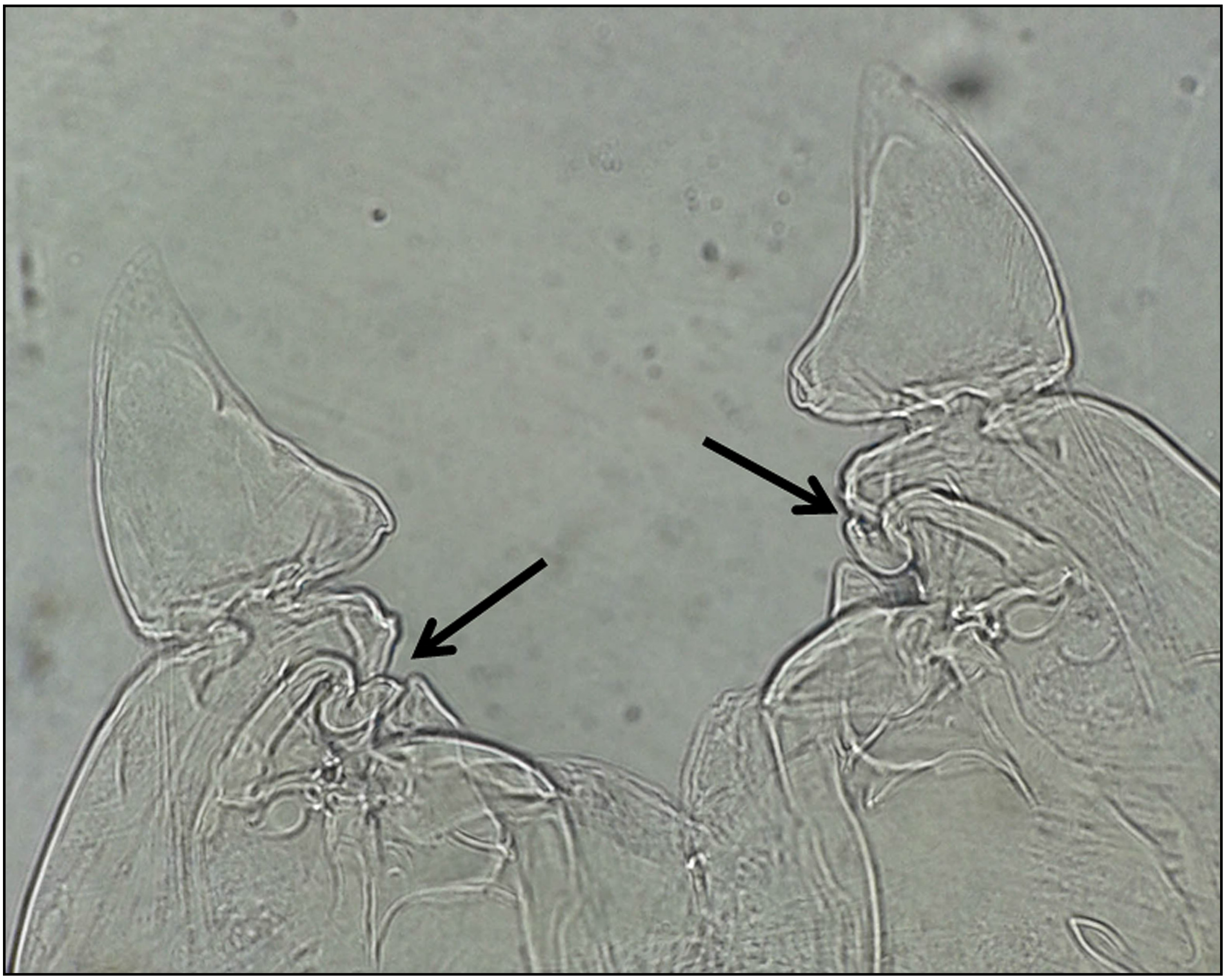


Figure 26 – Left and right hemipenes of *Elpidium* sp. 3, medial view (arrows point to the copulatory process); photo of a TLM-immage made by RP and JdSP.

c: The ciliated list on the inner lamella – this is a membrane located on the postero-ventral part of the inner lamella. It projects from behind the well-developed inner margin of the lamella. It is described for the first time in *E. martensi* n. sp. (Figs 5 C, 6 B, 9 A, B, 27 D) but we suspect that other *Elpidium* species display a similar trait. Details on the possible functional use of this peculiar morphological structure will be further presented in section 5 of the Results.

d: The hinge structure – Colin and Danielopol (1980), and more recently Karanovic and Humphreys (2014), mentioned that species of *Elpidium* display either adont or lophodont hinges. Present investigations show that *Elpidium* has no lophodont hinge and that the adont hinge is atypical and needs a better characterisation. Examination of *E. martensi* n. sp. using the SEM at high magnification shows the existence of a hinge bar with enlarged sections at the anterior and posterior extremities forming a kind of "proto-tooth" at each end (Figs 5 A, D - G, 6 A, D, E, 9 A, B). The posterior one (Figs 6 D, 9 B) is better developed than the anterior proto-tooth (Fig 6 E) which is more elongated and slender; the dorsal bar is dorsally slightly crenulated (Figs 9 A, C). The opposite (right) valve presents a hinge groove with an elongated anterior socket and an enlarged posterior one (Figs 4 A, E, F, 6 F, 8 E, 9 E, 27 A-C). Taking into consideration these peculiarities, the hinge of *Elpidium*, as compared to other Timiriaseviinae, represents an original type that we here name Protodont. It can be defined by the hinge bar on the smaller valve having enlarged sections at its extremities, these latter called proto-teeth. For *Afrocythere rostrata,* Klie (1935) mentioned the lack of cardinal teeth, so we consider it as an adont hinge. For *Intrepidocythere ibipora,* Pinto *et al.* (2008) described a typical lophodont hinge where the intercardinal bar is placed on the opposite valve to those bearing the hinge cardinal teeth, a situation existing also in the case of the other genera of Timiriaseviinae (Colin and Danielopol, 1980).

The information above and integrated within our knowledge of the diversity of hinge morphology of the Limnocytheridae (Martens, 1995) suggests that within the group of genera *Afrocythere*, *Elpidium* and *Intrepidocythere* that display closer morphological similarities, as compared to other gen-

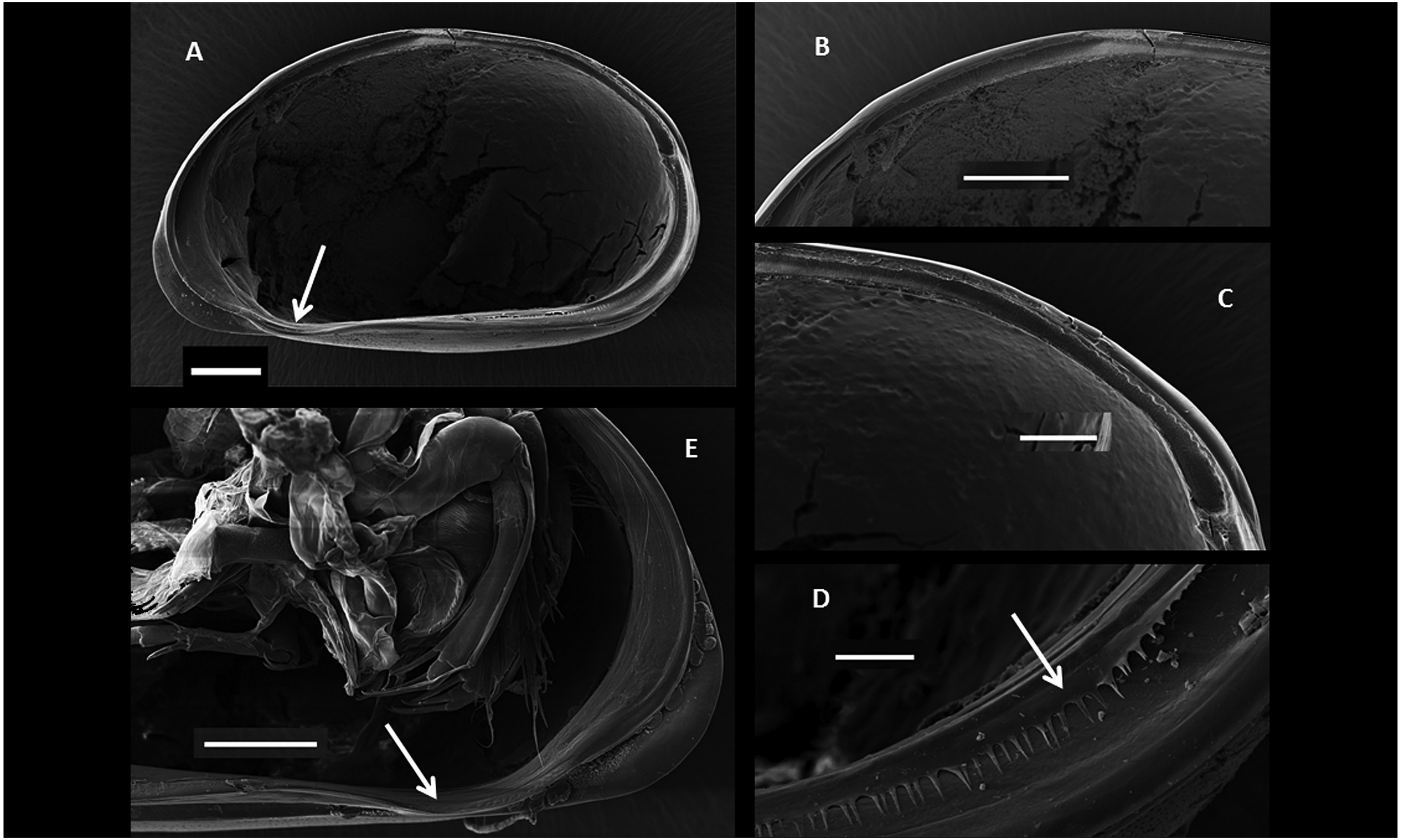


Figure 27 - *Elpidium martensi* n. sp., Amity Hall (UMJ Collection No. 211251); A – D – right female valve, inner view; **A** – general aspect (arrow points to the inflexed funnel shaped selvage); **B** – view of the enlarged anterior section of the hinge groove; **C** – same as in B but the enlarged posterior hinge groove; **D** – details of the posterior membraneous list of the inner lamella with cilia (arrow); **E** – detail of the female left valve with the position of the anterior limbs around the mouth (1st and 2nd antenna, mandible, labrum), arrow points to the antero-ventral funnel-shaped selvage.

era of Timiriaseviinae, the evolution of the hinge type proceeded from the simple adont one, as in *Afrocythere*, via an intermediary stage, the *Elpidium* protodont-type, and finally arrived to the lophodont *Intrepidocythere*-type. Therefore, within this group of three Timiriaseviinae genera, which possibly constitutes a phylogenetic lineage, the *Elpidium* hinge type displays a more primitive developmental stage as compared to the lophodont hinge of *Intrepidocythere*.

e: Valve surface ornamentation and the epidermis pigmentation - The ornamentation of the valves of *E. martensi* n. sp. with minute foveolae (Figs 4 G, 7 B, D, 8 C) is already visible on the early instar stored in the female brood-pouch (Fig 10 F). This trait is remarkable as only *E. laesslei* Tressler is known to have ornamented valves with large fossae (Tressler, 1956, Fig 1), otherwise *Elpidium* species have smooth valves (descriptions and/or illustrations in Pinto and Purper, 1970, Colin and Danielopol, 1980, Pinto and Jocqué, 2013). A question one should ask in the future is how the calcification of the valves proceeds in the case of *Elpidium* species. For *E. martensi* n. sp., the appearance of foveolae is visible from the early instars, and it is obvious that their formation depends on genetic cues. However, one should ask in the case of the large fossae on *E. laesslei* if they are due to an environmental cause such as special water chemistry in the micro-aquaria of bromeliaceans or if they are genetically cued.

At least in the case of *E. martensi* n. sp. we did not find sieve-pore plates similar to those known in representatives of the Limnocytherinae (Martens, 1990) and Timiriaseviinae Cytheridellini (Danielopol *et al.* 1989; Karanovic and Humphreys, 2014).

Tressler in his description of *E. maricaoensis* (Tressler, 1941, p. 268) noted for this species that the "color (is) gray with a much darker area on the anterior half of the valve which consists of a band of polygonal shaped dark brown areas across the two valves at the region of the eyes". For *E. bromeliarum*, Pinto and Purper (1970, p. 12) mentioned a variability of the epidermal colour from light to dark brownish. *Elpidium martensi* n. sp. (Fig 17 A) displays a light brown colour with a brighter transverse strip behind the ocular area which has a darker colour. *Elpidium* sp. 1 (Fig 17 C) presents a pattern resembling that described by Tressler (1941) for *E. maricaoensis,* namely in the anterior half around the ocular area there is a wide black area while the rest of the shell epidermis is white-yellow coloured. Finally, the epidermis below the valves in the case of *Elpidium* sp. 2 (Fig 17 D) displays a red-brown uniform colour. Casale and Danielopol (1990) discussed the diversity of colour pattern of several non-marine ostracods, including *Chlamydotheca unispinosa* (Baird), *Cypridopsis vidua* (O. F. Müller) and *Cypria ophtalmica* (Jurine*)*. In the case of the former two species it was noticed that the colour patterns were largely constant and could be mapped. This suggested to the authors that the colour pattern observed is genetically cued. For *Cypria ophtalmica,* Casale and Danielopol (1990) noted that the intensity of the coloration of the epidermal areas is environmentally cued. Individuals which came from the deeper part of lake Mondsee or from wells accessing groundwater displayed reduced pigmented areas, pale coloured, while the shallow-water individuals of this ostracod species from lake Mondsee were strongly pigmented. Hence, the same problem should in the future be investigated also for *Elpidium* species in order to see how much their shell ornamentation and epidermis pigmentation are due to genetic factors and how much to environmental cues.

In conclusion we are optimistic that the above discussion on the morphological traits belonging to the peripheral area and the surface of the valves will be useful for future research dealing with taxonomy and phylogenetic topics.

2 - The dextral-reversal of the valves as a taxonomic criterion for Elpidium martensi n. sp.

The dextral-reversal of valve traits used as a criterion for differentiation of taxa within groups is debatable. For *Miocyprideis janoscheki* Kollmann and for *Loxoconchissa foveolata* Triebel and Malz the reversal of valve traits was found variable. It was suggested that reversal of valve traits is due to local environmental cues and therefore has no value for taxonomic purposes (Kollmann, 1960; Triebel and Malz, 1969). In other cases, like the group of species now considered to belong to the genus *Hemicypris* Sars, the valve reversal represents the main generic criterion (Bate, 1970). Within the Timiriaseviinae, based on the reversal of the hinge elements, Colin and Danielopol (1980) suggested close phylogenetic relationships between three genera: *Rosacythere* Colin, *Kovalevskiella* Klein and *Frambocythere* Colin. Their supposition was strengthened by cladistic analysis of the fossil and Recent genera of the subfamily Timiriaseviinae (Gidó *et al.*, 2007; Karanovic and Humphreys, 2014, Fig 43).

Within the genus *Elpidium*, there are currently eight species taxonomically characterised (including *E. martensi* n. sp.) and three new species left in open nomenclature. From these 11 species only *E. purperae* Danielopol and *E. martensi* n. sp. present dextral-reversal of the valves, namely the right valve covers the left one at the periphery and the so-called positive elements of the hinge are also placed in the left valve (Danielopol, 1975, and description in previous sections). As mentioned above, *E. martensi* n. sp. differs from *E. purperae* by the shape of the distal lobe (DL) of the hemipenis, but it is surprisingly similar in morphology with those that of *E. pintoi* (Danielopol, 1975, Fig 8 A). Because of this latter situation a careful analysis of the argument that the dextral-reversal of the valves represents a valid taxonomic criterion for *Elpidium martensi* n. sp. becomes necessary.

We consider *a priori* for a morphologic fixed stability of the valve-reversal trait the following criteria: (1) the reversal should not display variability in samples with a significant number of specimens, in other words no specimens with opposite traits should occur in samples from the same locality and during different time periods; (2) the reversal should be visible in other populations located at different sites; (3) the reversal of the valves should be located into the developmental trajectory of the carapace (*i.e.* it should occur already in one of the post-embryonic stages; and (4) it should be congruent with other traits which define this new species.

We examined females, males and juveniles from the Hector's River site (18 specimens collected during February 1991 and 125 specimens during July 1993) and Amity Hall (75 specimens). All specimens displayed the trait of reversed valve overlap (Figs 8 A, B, D; 10 A-D). Selected specimens were verified for hinge reversal also showing the reverse position of the hinge elements, as described above. The areas around Hector's River and Amity Hall are separated by about 5.5 km distance. The reversal of the valves is evident on individuals extracted from the female brood pouch and representing the first or second post-embryonic stage (see Figs 10 E, F). From the same area Little and Hebert (1996) mentioned three populations belonging to a taxon they called "species A" which displays a similar distal lobe of the hemipenis with those of our *E. martensi* n. sp. and a genetic pattern totally unique as compared with other populations belonging to eight different species they mention in the above cited study. Therefore, we consider that our morphologic data are congruent with the genetic ones of Little and Hebert (1996).

We conclude that the reversal of valves observed in our *Elpidium* specimens is a valid taxonomic character. It is used for the characterisation of the new species *E. martensi* n. sp. in conjunction with the widely rounded posterior part of the carapace and quasi-symmetric valves as well as with the simple-shaped hemipenial distal lobe (*i.e.* without a ventral finger-shaped projection) and with the short copulatory process without glans.

It is also worth mentioning that our study is, quantitatively, one of the best documented cases for valve-reversal occurring in Recent ostracods. Another case using a similarly rich material is that of Malz (1981) for *Nipponocythere inversa* Malz. A long discussion on the general problem of the reversal of valve-traits is presented also by Schornikov (2011). We hope that our study for this *Elpidium* species will be complemented with additional comparative studies. It would be interesting to know if between *E. pintoi,* E. *purperae* and *E. martensi* n. sp. there are also ecological or biological differences. This type of question was addressed also by Gould *et al.* (1985) when discussing the sinistral coiling of gastropods. These authors postulated for the studied *Cerion* species that reversals of coiling are not anomalous cases and the difference between normal and reversal coiled individuals could be related to biological differences, such as a differential rate of mortality. Also as a general problem one could ask how the reversal phenomenon of valve traits becomes established within a population and leads to the occurrence of a new species.

3 - The importance of quasi-independent morphological traits for characterisation of Elpidium carapaces

A characteristic feature of the Timiriaseviinae ostracods is the development of a brood-care pouch in the posterior part of the female's carapace. Female and male carapaces within one species are dimorphic, with those of the latter being slightly smaller and laterally straighter than the shape of the opposite sex. Considering *E. martensi* n. sp. we tabulated in Tables 1 and 2 the meristic details for adults and the juveniles A-1- A-3 from Hector's River and Amity Hall. One can see the differences between the length and the maximal width of the carapaces existing between the adults and the juvenile developmental stages. Proceeding with the non-parametric Mann-Whitney U-test we obtained significant differences ($P > 0.001$) for the relative W/L% between the adult females and males and between these latter and the A-1 individuals but not between the adults of the two sites of Hector's River and Amity Hall (Table 4).

Danielopol (1975) when describing new species of *Elpidium* pointed out the presence of at least two types of shape for the females, one with a widely inflated posterior carapace and symmetric (or better quasi-symmetric) valves, as in the case of *E. bromeliarum*, and a second one with less posterior inflated carapace but with asymmetric valves (see here Fig 3 A, the example of *E. inaequivalvis*). Finally, a third type exists (here *Elpidium* sp. 2 and *Elpidium* sp. 3) where the female presents a more-or-less pointed posterior shape, very similar to the male (Figs 17 D-F). If you compare the shape of the carapaces in dorsal view of the present-day *Elpidium* species (11 species) you will immediately notice interspecific differences, some of them large, such as those between *E. bromeliarum* and *E. inaequivalvis*, and others of more subtle expression identifiable however by geometric morphometric methods.

Here we will show that it is possible to describe quantitatively the outline of the carapace, as viewed from the dorsal side, and further it is possible to delineate segments of the shell-outline as a quasi-independent morphological trait. For this latter point we follow the conceptual ideas of Lewontin (1978). This author observed that organisms have morphological traits which can locally change without compromising the architecture of the whole organismic module. This means that morphological characters are able to switch from one state to another in quasi-independence relative to the whole topological configuration of the structural or functional system within which they are inserted.

The carapace of *Elpidium* ostracods represents a structural module and its anterior and posterior parts are interpreted here as quasi-independent traits (Fig. 2, B the "a" - anterior and the "p"- posterior halves). Below, we describe for *E. martensi* n. sp. the morphological differences between the two traits using geometric morphometric techniques. Additionally, we will also demonstrate how with statistical criteria one can evaluate the degree of carapace shape separation between the post-embryonic stage A-1 and the adult male one. This latter point represents a persistent difficulty for ostracodologists working with cytheroids where just optical or meristic criteria are used. Finally, we will digress on the origin of the disparity of the carapace's shape within the genus *Elpidium*.

The differences between the female and male dorsal outlines can be visualised by superimposition and standardisation using geometric morphometric algorithms (*cf* section Material and Methods). Figure 11 displays the mean virtual shapes of *E. martensi* n. sp. taken from 13 adult females and 15 adult males of the Hector's River site. We superposed the virtual mean-outlines in two variants, namely using the size information (Fig 11 A) and standardised for an equal surface of one square millimetre (Fig 11 B). We note in both cases that the posterior part of the outlines diverge markedly. In the non-standardised for surface version the form of the female shape appears very inflated at its posterior part, while in the standardised version because the size of the male carapace shape is enlarged the posterior extremity and to a lesser extent the anterior lateral sides surpass the female's outline; this latter exceeds the male`s outline on the posterior lateral sides. The dissimilarity of the adults, female and male carapaces, in standardised for surface show a clear separation when plotted on a bi-dimensional morphospace with the non-metric Multi-Dimensional Scaling (n-MDS) algorithm. Figure 12 shows the n-MDS plot of the above mentioned data and we compared them with data for males and females from Amity Hall. We note that a clear separation of outlines is related to the sex but not for the sites. The differences or similarities of shape-type are confirmed through an analysis of similarities using ANOSIM in PRIMER v.6. Table 3 shows that the R statistic for the whole outline shape of female and male groups is close to 1 (0.904 and respectively 0.911). The opposite is the case for the inter-population dissimilarities of both sexes, namely the R statistic is very low (less than 0.1, hence without a notable shape separation).

We reanalysed the data using the same algorithms, as previously for the total carapace outline, but we now used the two halves of the carapace, the anterior and the posterior one, considered as quasi-independent traits (Fig 13). In Fig 13 A we do not see any separation of morphotype groups, for either sex or for sites. A better separation for sex is visible in Fig 13 B. The ANOSIM data (Table 3) for the R statistic display low values in all four cases (less than 0.7, a value which is by a rule-of-thumb the limit above which samples are well separated within the morphological space). The R values for the posterior half display higher values than those for the whole outline.

We analyse now for *E. martensi* n. sp. the juveniles. The shapes of the A-1 to A-3 stages resemble those of the adult male (Figs 14 A, B). When standardised-for surface, the shape differences shrink (Fig 14 C for the A-1 superposed on the adult male). However, still one notices obvious differences within the posterior half on both lateral and posterior extremity sites of the carapace.

We displayed the outline data for the male sample of Hector's River in a n-MDS, using the two algorithms, the non-standardised and the standardised one for equal surface (Fig.15 A, B). One can see in the former case that each stage is placed distantly one from the other and clearly reflects the image of Fig. 14 A. With the standardised algorithm (Fig 15. B), a slight separation between the A-1 and the adult male is visible but following the ANOSIM value (Table 3) the separation remains uncertain. The unique specimen A-2 is placed within the A-1 group, what means that it closely resembles specimens of this stage.

We repeated the analysis for the two quasi-independent traits, this time using the Hector's River set of data standardised for equal surface (Figs 13 A, B) and which was previously used for the whole outline. We note a lack of separation for morphotypes related to the developmental stages of the anterior half (Fig. 16 A) and a statistically significant separation is evident for the posterior half (Fig 16 B) of the carapace (The R value computed by ANOSIM is above 0.7 (0.742) in the latter case (Table 3). The difference of shape between the last juvenile stage and the adult male is due to the enlargement of the carapace (Fig 14 C) as a consequence of the full development of large hemipenes. This analysis of the 'male' juveniles compared to the adult male points again to the advantage of getting a better discrimination of the two outline sectors considered as quasi-independent traits. It is worth mentioning that all these data obtained with multivariate statistics on geometric morphometric data are congruent with the results obtained with the bivariate analysis and the Mann-Whitney U-test (Table 4). Therefore, for the future, one should use geometric morphometrics on quasi-independent traits and their analysis with multivariate statistical algorithms more intensively for study of developmental trajectories of the *Elpidium* carapaces. As we mentioned above there is a diversity of carapace shapes for *Elpidium* species, from species where the female has a widely inflated posterior carapace, as in *E. martensi* n. sp. showed above and *Elpidium* sp. 1 (Figs 17 A-C), to those with a conical shape which from the exterior does not indicate the existence of inside space for hatching eggs or juveniles, as in *Elpidium* sp. 2 (Figs 17 D-F). Horne *et al.* (1998) reviewed the information on the meaning of brooding within the inner carapace-space of ostracods. They endorsed the opinion of Van Harten (1990) related to *Cyprideis,* where brood care in the posterior part of the carapace has an adaptive value either for survival in stressful environments or for spatial dispersal by birds and/or with air currents. Prior to Van Harten (op. cit.), similar views were expressed by Sandberg (1964).

In the case of ostracods of the family Darwinulidae Pinto *et al.* (2005) mentioned that the brood quality could evolve through natural selection for increased fecundity of the females. Theoretically, one could approximate the effects of brood selection via the examination of the degree of the development of the posterior part of the female carapaces. Below we explore this idea in the case of *Elpidium* species. This is a question for which palaeontologists have much interest in order to apply such information to groups of ostracods represented exclusively by fossils.

The 11 species of *Elpidium* for which we have information on the female carapace morphology we display the disparity of the outlines in a 2D morphospace using Principal Coordinate Ordination (*cf* section Material and Methods). Figure 18 A shows three species clusters. The data analysed with the CAP routine of PERMANOVA+ for PRIMER shows that these groups are 100% well classified (Fig 18 B), namely group A, represented by seven species with widely developed posterior part and symmetric valves is separated from cluster B (two species) with valve-asymmetry and moderately enlarged carapace, and finally group C (two species) with conical posterior, resembling the male carapace. A difference in the sexual dimorphism of the shell outlines (when the outline of the male is superimposed on the female morphotype) is visible in Fig 19. One should note the differences of sexual dimorphism of the posterior part of the carapaces, the approximate symmetry differences for the group A (Fig 19 A) compared to the asymmetrical ones of the group B (Fig 19 B) and the minimal differences for the species-group C (Fig 19 C).

The data obtained through the Principal Coordinate Ordination (PCO) point clearly to the existence of 64% of species with a widely developed brood space inside the posterior part of the carapace (Fig. 18 A). This observation is in favour of natural selection acting for an increased brood. We emphasize that additional careful experiments are needed for an exact documentation of the way natural selection operates in the field for an increase of the posterior part of the carapace.

A problem which arises in the case of the females of *Elpidium* species with a minimal enlargement of the posterior half of the carapace, as for group C here, is the identification of their evolutionary state, namely do they at present display a primitive state or are they derived from ancestors with a well-developed brood pouch? A similar question was asked by one of us (RLP) when discussing the presence of eggs in the posterior part of *Microdarwinula inexpectata* Pinto, Rocha and Martens where eggs were found in the posterior part of the carapace but no special inner space exists for brood care (Pinto *et al.*, 2005). In this publication the hypothesis was advanced that the lack of brooding space is due to arrested development leading to a neothenic feature. Something similar was proposed by Van Harten (1990) for Neogene *Cyprideis* of the Paratethys, namely that some of the species could colonise new habitats in the deeper part of the lake where they could lose the capacity of brooding. However, Triebel (1956) described females of *Cyprideis stenopora* Triebel living in shallow water in the Galapagos with a minimal development of the posterior part of the carapace. Additionally, Sandberg (1964, p. 48) pointed out that females of *Cyprideis torosa* (Jones) display individual variability of the posterior segment of the shell, from clearly inflated to straight.

Considering the case of the *Elpidium* species group C, we need the examination of their developmental trajectory and this data should be compared with those of the *Elpidium* species of the group A. The example of the *Elpidium* species having females without enlargement of the posterior part of the carapace but still storing eggs within the domicilium (Fig 17 F) points to the need for observation of the fecundity of females related to the number of eggs and juveniles stored within the inner space of the carapace and the development of the posterior shell-outline of the different *Elpidium* species.

In conclusion, we hope to have convinced our readers that geometric morphometrics combined with classic observation on the limbs offer a new vista for evolutionary studies on the way *Elpidium* developed a variety of carapace types which all allow for broodcare. The degree of sexual dimorphism of the posterior part of the carapace could also be used for systematic purposes, namely as a diagnostic trait for species identification.

4 - The male copulatory process of Elpidium taxa - its possible usage for phylogenetic reconstructions

The copulatory process of the Limnocytheridae, as defined by Martens (1990), is a sclerotised tubular structure through which traverses the seminal ductus. It allows a direct transfer of the sperm into the female's vagina during the copulation process. The distal part of the copulatory process, the glans displays, in many cases, species-specific shapes which originate through sexual selection (Martens, 1990). This author showed that the diversity of glans-shape is viewed as an adaptive trait for direct species recognition during the mating process. In some species-groups of Limnocytherinae the copulatory process does not evolve diverse distal structures (Martens, op. cit.). This appears to be the case also for *Elpidium* where from 11 species where the copulatory process was figured, seven of them display a uniform shape like a curved short funnel, without a glans-like structure, similar to that of *E. martensi* n. sp. described above (Figs 22 D, 23 G, I, 24 A, B, 25 A). The three unnamed *Elpidium* species depart from this pattern. Copulatory process of *Elpidium* sp. 3 is tubular and long, with the distal part U-shaped (Fig 26). *Elpidium* sp. 1 and *Elpidium* sp. 2 display a bifid copulatory process. Closely located to the distal tip, where the seminal tube opens, one finds a lateral structure similar to a glans. This latter structure appears to be species specific (Fig 25 B, C). Summing up this information we propose the following hypothetical scenario: the funnel-shape type of copulatory process could represent the ground-pattern condition (a possible plesiomorphic state) for a large species group to which *E. bromeliarum, E. inaequivalvis, E. martensi* n. sp. and four other species belong. The copulatory process with a lateral glans of *Elpidium* sp. 1 and of *Elpidium* sp. 2, mentioned above, represents a more evolved state and apparently these latter species belong to a different species group. If this scenario is accepted then within the three classes of female carapace-shape we identified through morphometric analysis in the previous section, the symmetrical and inflated carapace shape of such species like *Elpidium martensi* n. sp. and *Elpidium* sp. 1 resulted through convergent and/or parallel evolution. Examples of convergent evolution of the posterior shape of the female carapace were discussed by Colin and Danielopol, (1980), namely the realisation of a brood pouch existing in distantly related genera of the Timiriaseviinae, *Cytheridella* Daday, belonging to the tribe Cytheridellini and *Metacypris* Brady and Robertson and *Elpidium* belonging to the nominatypical tribe Timiriaseviini.

The data and discussion presented in this section should interest palaeontologists who are confronted with extinct groups displaying carapaces which suggest that they belong to ostracod taxa with brood care (review in Horne *et al.*, 1998). Thus the three types of carapace-shape we describe here could be assigned by palaeontologists to different supra-specific taxa. Siveter *et al.* (2007) discuss for Palaeozoic ostracods a very similar case. The identification of limbs in a Silurian ostracod allowed these authors to classify their taxon within the extant group of Myodocopa even if the carapace alone showed similarities with the common Palaeozoic Palaeocopa for which soft parts are unknown. Siveter *et al.* (2007: 468) concluded their study as follows: "The discovery of the soft parts of this Palaeozoic ostracod urges caution in interpreting the affinities of other so-called *straight-hinged* ostracods based solely on shell morphology….The taxonomic assignment of many of the hundreds of such genera of Palaeozoic ostracods, which are based on shells alone, may be incorrect."

5 - The adaptive role of the antero-ventral funnel-shape of Elpidium's selvage

The antero-ventral section of the selvage of *Elpidium* valves isconcave shaped. It forms a kind of conical structure resembling a funnel turned with the tip downward. The position of the half of this "funnel" shape, seen from the lateral view of one of the valves is presented for *E. martensi* n. sp. in the figures 8 E, 9 D, 27 A, E. and in ventral view the projection is visible for the right valve (Fig 8 D). Cannon (1926) and later Adamczak (1969) described the structure and function of the ventral selvage section called 'bow-shaped projection', a morphologic peculiarity which exists in many podocopid ostracod groups. Both authors pointed out the adaptive function of these "ventral lips" for the suction of water and solid particles into the anterior part of the inner space of the ostracod carapace. The water once passed around the mouth-opening is directed laterally by the current made by the branchial plates of the maxillulae to the posterior part of the domicilium and is pushed outside. The water circulation through the inner space of organisms similar to those presented here for *Elpidium* is based on the Bernoulli principle who states that liquid or gases move in tubular structures because of different pressure within spaces with different diameter (Vogel, 1978). In thin tube sections the pressure is higher and in larger one lower, water flowing from high to low pressure. The funnel-shape structure of the antero-ventral part of the valve allows the suction of water which is then directed to the hypostome. The labrum of *Elpidium martensi* n. sp. has long cilia which most probably collect solid particles and direct them to the mouth and help the water circulation towards the lateral side between the inner side of the valve and the body; once here the branchial plate of the maxillula will move the water toward the posterior part of the domicilium; finally the water leaves the domicilium at the ventro-posterior side. We mentioned above the existence along the ventro-posterior side of the inner margin of the inner lamella a lamellar list provided with cilia (Fig 5 C, 6 B, 9 A, 27 D), which probably by its movement helps the water flow from the carapace's inner space. One should note that the semi-terrestrial *Intrepidocythere ibipora* does not display the antero-ventral funnel shape (Pinto *et al.*, 2008). This latter species probably does not need such a funnel-shaped selvage because of its mode of life within the thin layer of water which generally exists in semi-terrestrial habitats (Danielopol and Betch, 1980; Horne *et al.*, 2004). *Elpidium* species living in restricted habitats of phytotelmata with possible strong predation pressure and high accumulation of organic matter could be advantaged if they can get water inside the carapace without opening the valves widely. One should note that the present argument is based on morphological evidence and theoretical speculation. It needs future experimental validation with direct observation of living specimens and laboratory manipulation of artificial model as exemplified for other organisms by Vogel (1978).

6 - The necessary improvement of comparative descriptions of the limbs-chaetotaxy for Timiriaseviinae

Setae, claws, pseudochaetae, have specific structures and positions on the limbs. They can be defined also in relation to referential limb-segments (Broodbakker and Danielopol, 1982). The description of the new species *Elpidium martensi* n. sp. convinced us that many details of the chaetotaxy of the Timiriaseviinae limbs have been overlooked. Special attention should be directed to details of the secondary structure of setae and to the morphology and position of the pseudochaetae. They could in the future be helpful for defining taxa, especially at a supra – species level. Considering the chaetotaxy of the thoracopods of the Limnocytheridae, Martens (1990, 1992, 1996) documented a diversity of endopodial setae with definite shapes and/or lengths which could be used as diagnostic traits for species of *Limnocythere*, *Paralimnocythere* and/or *Korannacythere*. Colin and Danielopol (1978) noted that one of the diagnostic characters of the family Limnocytheridae is a vestigial seta of the 4th endopodial segment of the thoracopods. This latter endopodial segment is fused with the distal claw (here Figs 23 C, E). Danielopol (1970) presented comparative data on the antennal aesthetascs of *Metacypris cordata* Brady and Robertson, *Kovalevskiella phreaticola* (Danielopol) and *Afrocythere rostrata*. Additional data were described by Colin and Danielopol (1980) for these species and new information was offered for *Elpidium inaequivalvis* and *Cytheridella ilosvayi* Daday. Using transmitted light microscopy (TLM) the antennal aesthetascs, the interdental chaetotaxy of the coxal endite of the mandible and the thoracopod-setae were described in detail. For the present essay we discuss two aspects of limb chaetotaxy:

a: The improvement of information for the morphologic description of the chaetotaxy using SEM techniques – From the description of *E. martensi* n. sp. with TLM and a common 40X-objective one can see that some setae and claws are secondarily ornate, others appear smooth. This is the case for the endopodial seta of the 1st endopodial segment of the thoracopods T1 and T2 which in this magnification appear smooth (Figs 22 A, B). The same setae examined under high SEM magnification show that these setae are double serrate (Fig 23 B). The homologue seta of the T3, which in TLM appears plumose (Fig 22 C), in the SEM-picture it is visible as doubly serrate (Figs 23 D, F). The plumose exopodite of the thoracopods which appears as a hairy seta in TLM (Figs 22 A – C) could be clearly defined as a plumose seta due to the high magnificationof images obtained with the SEM (Figs 23 A, B). The exact interdental setae X1 of the coxal endite of the mandible which is generally either not recognised or not correctly drawn as spoon-shaped in low magnification with TLM, becomes clearly visible as such using the SEM (Figs 21 E, F, 24 D). Therefore we propose to follow the maxim "God is in the details" with the meaning that we need to revise most of the morphologic descriptions of setae if we want to use them for functional and structural aspects of Timiriaseviinae ostracods.

b: Possible usage of morphology of selected setae as diagnostic traits for Timiriaseviinae taxa – The spoon-like shape of the X1 seta of the coxal endite of the mandible is visible in representatives of *Elpidium* and *Kovalevskiella* Klein (Colin and Danielopol, 1980 and here the figures for *E. martensi* n. sp. cited above) as well as in *Metacypris digitiformis* Smith and Hiruta (Robin Smith unpublished image, communicated to DLD), *Dolekiella europaea* (Gidó *et al.*, 2007) and apparently exists also in *Thaicythere srisumonae* (Savatenalinton *et al.*, 2008, p. 423, Fig 8 A). Colin and Danielopol (1980) for *Cytheridella ilosvayi* figured an X1 seta without side enlargement, very similar with those of X2 seta (*cf* their Fig 1 A). *Cytheridella* is now the nominatypical genus defining the tribe Cytheridellini Danielopol and Martens (in Danielopol *et al.*, 1989) as revised by Martens (1995). The other genera mentioned above belong to what Colin and Danielopol (1978) included in the Timiriaseviinae Mandelstam, 1960. The shape of the X1 seta of the coxal endite of the mandible with morphological differences between *Cytheridella* and the other genera cited above can be used for a clear definition within the subfamily Timiriaseviinae of the two tribes, the Timiriaseviini and the Cytheridellini.

Within the Timiriaseviini two thoracopod setae appear to differ by their length when one compares species belonging to different genera. The length of the "exopodite seta" of the T1 and T2 are long and display an approximately similar length, while the homologous seta of the T3 is approximately 50% shorter in the case of *Elpidium merendonense* (Pinto and Jocqué, 2013, p. 53, Figs A-B) and here for *Elpidium martensi* n. sp. (Figs 22 A - C). A similar pattern is displayed also by *Intrepidocythere ibipora* (Pinto *et al.*, 2008, p. 36 Figs 3 B – D). In the case of *Kovalevskiella phreaticola* (Colin and Danielopol, 1980, Figs 4 B-D), *Metacypris digitiformis* (Smith and Hiruta, 2004, p. 41), *Dolekiella europaea* (Gidó *et al.*, 2007, p. 112, Figs 4 F-H), *Thaicythere srisumonae* (Savatenalinton *et al.*, 2008, p. 417 Fig 3 E, p. 419 Figs 4 A, B) and *Afrocythere rostrata* (Klie, 1935 p. 63 Fig 61) the "exopodite setae" of the T1 to T3 are of approximately equal length.

The seta of the endopodial segment E1 displays different lengths for the three thoracopods in the case of *E. martensi* n. sp. and *E. merendonense*. The T1 seta is shorter than the length of the E2 segment; it is about equal in length with the E2 in the case of the T2 and longer than the E2 in the T3 (Figs 22 A - C). Additionally, the endopodial seta of T3 is slender as compared to its homolog setae of the T1 and T2 which are more robust. For *E. martensi* n. sp. we saw in the SEM that these setae are doubly serrate but the T1 displays shorter setules as compared to its homologue seta on the T3(Figs 23 A, B, D, F). *Intrepidocythere ibipora* shows a pattern similar to those of the *Elpidium* endopodial setae mentioned here, namely there is an increase in their length when compared to the reference length of the E2 segment. In the figures of Pinto *et al.* (op. cit.) *Intrepidocythere* setae are longer compared to those of the two *Elpidium* species mentioned here. *Afrocythere rostrata,* from the figure of Klie (1935, p. 63, Fig 61), shows a different pattern with two short setae which do not reach to lower margin of the E2 on the T1 and T2 and a long seta on the T3 which ends close to the lower margin of the E3. Representatives of *Kovalevskiella*, *Metacypris* and *Thaicythere* display a short length for this endopodial seta on all three thoracopods, differing in this way from the above mentioned genera.

This cursory description of the mandibular and thoracic setae, when used comparatively, allows us to consider them as potential diagnostic traits for the various genera of the Timiriaseviini. They appear useful for a new expanded cladistical analysis of Timiriaseviinae, with the future potential to improve the systematics of this diverse cytheroid group. This intuitive view is based also on the long experience on the morphology and distribution of various groups of Timiriaseviinae that one of us (DLD) has accumulated. None of the present day phylogenetic scenarios proposed by Gidó *et al.* (2007), Savatenalinton *et al.* (2008), Karanovic (2009), Karanovic and Humphreys (2014) fully capture the complexity of the evolutionary pathways of Timiriaseviinae.

C: Final Discussion: Elpidium ostracods as potential model-organisms for evolutionary biological projects

Current information on *Elpidium* ostracods in the Neotropics, as derived from the observations of Lopez and coworkers (Lopez *et al.* 1999, 2005, 2009), suggests that these microcrustaceans have the propensity to develop rapidly in new habitats of phytotelmata, therefore it may be possible to culture them under laboratory conditions reproducing some of the ecological characteristics of the phytotelmata environment. The bromeliacean's micro-aquaria were metaphorically called by Little and Hebert (1996) 'ecological islands' and their study compared with those carried out on diverse endemic fauna living on true islands. If successful laboratory culturing can be achieved one should try to explain why representatives of this ostracod group live, as far as we know now, exclusively in the aquatic habitats of bromeliacean phytotelmata? Related to this basic biological question one should ask also what is the origin of these *Elpidium* ostracods, how old is this phylogenetic group? We already know that *Elpidium* is closely related either to the semiterrestrial ostracod *Intrepidocythere ibipora* (Pinto *et al.*, 2008) and/or to *Afrocythere rostrata* (Colin and Danielopol, 1980). To better understand phylogenetical relationships of these genera we need more information on the history of these groups, namely on their ecological specialisation and/or on their (palaeo)biogeographical distribution in time and space.

Communication with specialists who study other invertebrate groups displaying similar patterns, namely organisms widely spread through space but ecologically restricted to the bromeliacean phytotelmata, can be very helpful for developing new ideas for future research with *Elpidium*. We think of the research of Dunthorn *et al.* (2012) who suggested that the origin of microfauna (Protozoa) of bromeliacean phythotelmata could be derived from organisms living in semi-terrestrial swampy habitats. Here we see immediately a possible analogy for the ecological distribution of *Elpidium* and *Intrepidocythere*.

In the case of the *Elpidium* ostracods, one should try to find fossil ostracods which could be related to our living *Elpidium* taxa. We take as an example, even if not directly related to the present study, the fossil Timiriaseviinae species from the Late Cretaceous of Argentina, described by Uliana and Musacchio (1978) under the name "*Gomphocythere*" *payunensis* and/or those of Carignano and Varela (2011) identified as *Vecticypris* sp. from a Late Cretaceous non-marine deposit in Argentina. Such taxonomically interesting ostracods should be in the future more carefully examined for their morphology in order to try to find their possible relationships to the living *Elpidium* fauna.

Information offered by specialists on the origin, diversification and geographic spread of bromeliaceans could be used to fix the time-period within which ostracods belonging to taxa which evolved to the present day *Elpidium* could specialise with the phythotelmata. Givnish *et al.* (2007) show that bromeliaceans arose on moist terrestrial habitats on the Guyana shield about 70 Mya ago and spread centripetally in the New World. Molecular biological investigation, as in the case of some Coleoptera specialised to life in phytotelmata (Balke *et al.*, 2008), could also help to approximately define the time when ostracods specialised to bromeliads. In the case of the beetle Copelatinae (Dytiscidae), Balke *et al.* (2008) estimated that specialisation to the bromeliacean life occurred during the Tertiary.

In conclusion, we argue that a pluridisciplinary investigation of the *Elpidium* phylogenetic lineage should involve active communication and/or cooperation with various groups of specialists. This should become the rule if one expects to transform *Elpidium* ostracods as model-organisms for evolutionary biological projects. For progress in our comprehension of phylogenetic relationships between various groups of Timiriaseviinae and/or for the improvement of the taxonomic framework of this subfamily one should apply a pluridisciplinary approach using the expertise of specialists from various domains like crustacean morphology, molecular systematics, cladistics, geometric morphometrics, (palaeo)biogeography, ecology and physiology, etc. - all this could fulfill what Sluys (2013, p. 1103) called, "an integrative approach to taxonomy".

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