# Sexual dimorphism in shells of Cochlostoma septemspirale (Caenogastropoda, Cyclophoroidea, Diplommatinidae, Cochlostomatinae) 

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#### Abstract

Sexual dimorphisms in shell-bearing snails expressed by characteristic traits of their respective shells would offer the possibility for a lot of studies about gender distribution in populations, species, etc. In this study, the seven main shell characters of the snail Cochlostoma septemspirale were measured in both sexes: (1) height and (2) width of the shell, (3) height and (4) width of the aperture, (5) width of the last whorl, (6) rib density on the last whorl, and (7) intensity of the reddish or brown pigments forming three bands over the shell. The variation of size and shape was explored with statistical methods adapted to principal components analysis (PCA) and linear discriminant analysis (LDA). In particular, we applied some multivariate morphometric tools for the analysis of ratios that have been developed only recently, that is, the PCA ratio spectrum, allometry ratio spectrum, and LDA ratio extractor. The overall separation of the two sexes was tested with LDA cross validation.

The results show that there is a sexual dimorphism in the size and shape of shells. Females are more slender than males and are characterised by larger size, a slightly reduced aperture height but larger shell height and whorl width. Therefore they have a considerable larger shell volume (about one fifth) in the part above the aperture. Furthermore, the last whorl of females is slightly less strongly pigmented and mean rib density slightly higher. All characters overlap quite considerably between sexes. However, by using cross validation based on the 5 continuous shell characters more than $90 \%$ of the shells can be correctly assigned to each sex.


## Keywords

Sexual dimorphism, shell features, C. septemspirale, PCA ratio spectrum, allometry ratio spectrum, LDA ratio extractor

## Introduction

Molluscs can be hermaphrodites or gonochoristic organisms with separate sexes throughout their life span, with the latter group sometimes being subject to differing phenotypic traits. Sexual dimophism has been recorded for unionoid mussels as well as for some marine, freshwater and terrestrial snails (for examples see: Raven 1990; Kenchington and Glass 1998; Gofas 2001; Minton and Wang 2011). In gastropods there are two large groups, the hermaphroditic pulmonate snails, and the remaining groups (formerly called prosobranch molluscs) with separate sexes, bearing an operculum to close the shell. The latter group mainly inhabits marine habitats, while the former group forms the majority of terrestrial species. However, there are several "prosobranch" families, which successfully adapted to terrestrial life. As in all other bisexual organisms, sexual dimorphism may occur, and thus there is the question whether this phenomenon can be found here as well.

Sex determination without harming the living individuals often is a problem. Usually, the animals hide inside the shell, what makes it impossible to determine its sex. The solution for this problem could be a method that helps to distinguish between the sexes based on sex-related shell features. This could also help to analyse the sex ratio within given populations even when only shells are available, as often is the case in malacology and particularly in museum's collections.

Quite recently, De Mattia et al. (2011) intensively discussed the Cochlostoma species from Sabotino Hill (NE Italy), where three species of this genus occur. Next to anatomical and conchological character states, the authors also used landmark based, geometric morphometry to obtain reliable shell discriminating characters. To avoid a bias caused by sexual dimorphism, De Mattia et al. (2011) used three male and female specimens of each species, but the contribution of each sex to the average shell form was not addressed by them.

In their papers on the systematics of Pyrenean and Cantabrian cochlostomatid species, Raven (1990: 21 Fig. 1) as well as Gofas (2001) already mentioned that there is a sexual shell dimorphism which is reflected in the shells of males being smaller and stouter than females. According to Maassen (2007), this phenomenon is not uncommon among operculated families. In contrast, AnimalBase without referring the source states that there is no significant sexual dimorphism in C. septemspirale (last visit July 2011). As the shell is one of the most useful characters for species recognition in Cochlostoma (Gofas 2001) it might also be useful for identification of the sex. As far as known, sex dimorphism in this species is not expressed by differing behaviour between the sexes. However, increasing fecundity with increasing body size in females could be a good reason for sex dimorphism (Shine 1989). There are some differences in the reproductive system that might lead to sexual dimorphisms in shells. Females have to produce eggs, so it would be logic that they need a larger body, resulting in a higher volume of the shell which should be measurable in some shell metric Gofas (2001) mentioned a difference in pigmentation of the shell be-
tween the sexes in some Cochlostoma species, but gives no explanation for this difference. A last feature that often plays an important role in taxonomic studies is the rib density (ribs/mm) on the whorls of the shell (Welter-Schultes 2010). It is known that this feature is usually quite homogeneous within a single population, but it also can show an important geographical variation (Gofas 2001). For this reason, a comparison of this feature between the sexes is interesting as well.

In this study, Cochlostoma septemspirale (Razoumowsky, 1789) is used to find such sex-related shell features. The distribution of C. septemspirale extends from the Pyrenees to southern Germany and the central Balkans (Wagner 1897). In Switzerland, C. septemspirale is especially common throughout the Jura, from where it has been described by Razoumowsky in 1789 . Although C. septemspirale is one of the most widely distributed Cochlostoma species, there is not much known about ecology, physiology, and reproduction biology of this species to date.

## Material and methods

For analysing differences between males and females, only mature individuals have been tested. These can be recognised by a thickened outer lip (Gofas 2001).

The aim of this study was to analyse different shell characteristics of 5 populations with 30-50 individuals per population. The seven main characters were (1) height and (2) width of the shell, (3) height and (4) width of the aperture, (5) width of the last whorl, (6) rib density on the last whorl, and (7) intensity of the reddish or brown pigments forming three bands over the shell (for definition of measurements, see Fig. 1).

## Field procedures

The collection of the 220 samples of C. septemspirale was performed during two days in the Jura. Here, 6 sites had been sampled; the collection time on each site was about an hour. All collected specimens were preserved in plastic bags and later processed in the lab.

## Localities

Snails have been collected on the $5^{\text {th }}$ and on the $19^{\text {th }}$ of April. The first site was situated above Le Landeron (NE) (6 individuals); the second site was above La Neuveville (BE) (42 individuals), and the third site near Orvin (BE) (35 individuals) On the $19^{\text {th }}$ of April, the first two sites were situated near Nods (BE) with 35 and 47 individuals respectively; the last site was on the Twannberg (BE) (55 individuals). The habitat structure of these sites was similar: south-facing slopes, mainly beech forest, with some interspersed little limestone boulders; usually with low shrubs and


Figure I. The 5 distances measured on the shell of Cochlostoma septemspirale: shell height and width, aperture height and width, and whorl width.
a herb layer and also a moderately thick layer of foliage. For more information to the localities refer to Appendix 1.

## Separation of the sexes

To sex the collected specimens, it is necessary to check the genital organs of each specimen. This cannot be done in the contracted state, when the animal is retracted in the shell with the operculum closing the aperture. To relax the animals, they were stored in bottles filled with water over night. In a next step, the relaxed specimens were poured with boiling water to fix them in the expanded state. After this procedure, males and females could easily be separated with the naked eye even. Only in three cases, the stereo-microscope was needed. All individuals were stored in alcohol ( $85 \%$ ) until the next step.

## Measurements and conservation

Each specimen was photographed in a standardized orientation in up to 10 subsequent steps using a microscope camera (Leica DFC425). Afterwards, these multifocus images where combined to a single image by using ImageAccess Standard software. The measuring module of this software provided then measurements of height and width of shell, height and width of aperture and the width of the last whorl. The numbers of ribs on the last whorl and the pigmentation were also examined on the photos. Each specimen was stored in a glass tube with an individual number and preserved in $85 \%$ alcohol. Each lot received a catalogue number and was stored in the collection of the NMBE providing individual access to each specimen. All photos are stored on the NMBE Server and are available for subsequent research.

## Data analysis

Continuous characters (measurements): 5 continuous variables (Table 1) were treated with a multivariate analysis in order to explore the most significant variation in size and shape of the shell. According to Jolicoeur and Mosimann (1960), shape in general tends to provide more reliable information than size on the morphology of organisms. Size is often considered as a nuisance because it is strongly dependent on ecological factors (e.g., McCoy et al. 2006). Separation of size and shape in multivariate studies of morphological data is problematic (e.g., Claude 2008). One way to address this problem is by using principal component analysis (PCA), where the first principal component of PCA is usually considered as a general size axis, while the remaining principal components represent the shape space. However, the first principal component also includes size related shape information (Jolicoeur and Mosimann 1960) and has been identified by Jolicoeur (1963) heuristically as a multivariate allometric size axis (see Baur and Leuenberger 2011 for a model based statistical derivation). The mixture of size and size related shape information in the first component makes the interpretation of the other components of a PCA rather difficult. Baur and Leuenberger (2011) therefore have developed new methods that allow the interpretation of principal components in terms of ratios. With their tools it is possible to strictly separated size and shape. In a first step, an isometric size axis (below called "isosize") is defined as the geometric mean of the original measurements and thus comprises only differences in scaling (for the exact definition isosize, see Baur and Leuenberger (2011: 816)). Isometry free shape variables are obtained by projecting the measurements orthogonal to isosize. A PCA calculated on the covariance matrix of these shape variables then accounts solely for differences in proportions. Baur and Leuenberger (2011) developed a graphical tool for the interpretation of principal components in shape space that they called the "PCA ratio spectrum". To assess the amount of allometry in the data, Baur and Leuenberger (2011) sug-

Table I. Range, interquartile range, median, and number of specimens of each sex for the 5 distance measurements. Significance levels of t test: '***' $\mathrm{p}<0.001$, '**' $\mathrm{p}<0.01$, '*' $\mathrm{p}<0.05$, ' '' $\mathrm{p}<0.1$, '’ $\mathrm{p}<1$. All measurements in $\mu \mathrm{m}$.

| shell height ${ }^{* * *}$ |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| sex | min. | 1st quartile | median | 3rd quartile | max. | N |
| females | 6915 | 7428 | 7572 | 7780 | 8261 | 104 |
| males | 6549 | 6956 | 7120 | 7271 | 7873 | 116 |
| shell width |  |  |  |  |  |  |
| sex | min. | 1 st quartile | median | 3 rd quartile | max. | N |
| females | 3615 | 3822 | 3878 | 3954 | 4207 | 104 |
| males | 3573 | 3837 | 3929 | 4003 | 4332 | 116 |
| aperture height ${ }^{* * *}$ |  |  |  |  |  |  |
| sex | min. | 1 st quarte | median | 3rd quartile | max. | N |
| females | 2249 | 2535 | 2596 | 2681 | 2953 | 104 |
| males | 2189 | 2587 | 2689 | 2772 | 2962 | 116 |
| aperture width |  |  |  |  |  |  |
| sex | min. | 1 st quartile | median | 3 3rd quartile | max. | N |
| females | 2166 | 2504 | 2564 | 2652 | 2902 | 104 |
| males | 2161 | 2456 | 2564 | 2650 | 2893 | 116 |
| whorl width $* * *$ |  |  |  |  |  |  |
| sex | min. | 1 st quartile | median | 3 3rd quartile | max. | N |
| females | 3069 | 3202 | 3258 | 3325 | 3531 | 104 |
| males | 2994 | 3117 | 3175 | 3228 | 3383 | 116 |

gested to plot the isosize against each significant shape component and introduced another graphical tool, the "allometry ratio spectrum".

To examine how well the sexes are separated, the data were subjected to a linear discriminant analysis (LDA). The performance of the LDA was assessed by means of cross validation (Rencher 2002: 310), where one specimen is omitted from the analysis and classified according to the discriminant function found for the remaining specimens in the data set. The number of correctly classified cases is a measure of how well the result will generalize for an independent data set. Finally, the "LDA ratio extractor" (see Baur and Leuenberger 2011) was applied for finding the best ratios for separating the sexes. The measure $\delta$, also introduced by Baur and Leuenberger (2011: 818, formula 14), was calculated to see how much of the total differences are due to size and how much are due to shape.

## Other characters

The rib density (ribs $/ \mathrm{mm}$ ) on the last whorl was determined on the same photographs that were used for taking measurements. For counting the ribs, the last whorl was magnified on the screen. The total number of ribs on the last whorl was divided by whorl width in mm .

Pigmentation has been recorded at the nominal level. There was no intention to include it in the PCA, because PCA has only been used for a size and shape analysis. To determine the intensity of the reddish or brown pigments, that form three bands over the shell, we also used the photographs of the specimens. The pigmentation was determined on the last whorl of the shell. The intensity of the bands was difficult to classify, so first the senior author worked out an appropriate method for classifying the intensity of pigmentation. Finally, a 3-level classification scheme $(0=$ missing band, $1=$ interrupted band, and $2=$ continuous band) was adopted and the shells were classified in a first run by the senior author. To avoid any bias, that easily might be introduced by a person involved in the study, an independent person repeated the classification of specimens in a second run.

All statistical and graphical analyses were performed with R , version 2.11.1 ( R Development Core Team 2010). For the calculation of the multivariate methods of Baur and Leuenberger (2011) slightly modified versions of the R-scripts provided by the authors were employed (see section Supplementary material of their paper). In order to decide how many components to retain we plotted the scree graph (Rencher 2002: 398-399). Fisher's exact test was applied for contingency tables (frequencies of pigmentation categories) and therefore the function "fisher.test()" was used. For testing differences in the mean we applied the $t$-test by using the function "t.test()" or the non-parametric Wilcoxon rank sum test and the function "wilcox.test()", in case the distribution of the data points deviated from normality. The fit to a normal distribution was checked with Quantile-Quantile plots, i.e. by plotting the respective variables with functions "qqnorm()" and "qqline()".

## Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at doi: $10.5061 /$ dryad.ns7v7.

## Results

## Size and shape analysis of distance measurements:

To get an overview, the range, interquartile range and median of the 5 continuous shell characters are given in Table 1. The shells of females are higher and the last whorl is wider compared to males, whereas the aperture is higher in males ( t -tests, $\mathrm{p}<0.001$ ). For the two remaining characters, no significant differences between sexes were found.

The multivariate analysis offered a closer look concerning the variation in size and shape of the shells of C. septemspirale. As mentioned in the Material and methods section, we calculated isosize (i.e., the geometric mean of all variables) as a general size measure. Although the range was more or less overlapping, mean isosize was significantly higher for females compared to males (Wilcoxon test, $\mathrm{p}<0.01$ ).

A principal component analysis (PCA) performed in isometry free shape space allowed us to explore variation in shape. The scree graph (not shown) suggested the first two principal components (PC) which explained $80.4 \%$ of the total variance in the data. In Figure 2, the scatterplot of shape PC 1 against shape PC 2 is presented. In the graph, a distinct shift in the shape of females and males is evident, as the two cluster of points are only moderately overlapping. The shift is rather pronounced along shape PC1, but only weakly discernible along shape PC2. For the interpretation of the two components we used the PCA ratio spectrum (Fig. 3) as detailed by Baur and Leuenberger (2011). Following their description, ratios calculated from characters lying far apart in the spectrum explain a large portion of the variance of a shape PC; on the other hand, ratios from close characters contribute very little. According to the PCA ratio spectrum in Fig. 3a, shape PC1 was dominated by the ratio shell height:aperture height, while the PCA ratio spectrum of shape PC2 (Fig. 3b) suggested the ratio shell width:aperture width.

The first ratio, shell height:aperture height, showed a large mean difference between sexes (Wilcoxon test, $\mathrm{p}<0.001$; Fig. 4a). Females have a larger shell height as well as a smaller aperture height than males (Table 1). Because the width of the last whorl is also larger in females (Table 1), their shells should have considerably more volume in the part above the aperture. Indeed, mean volume is 1.19 times larger for females.

The mean of the other ratio, shell width:aperture width, differed slightly between sexes (Fig. 4b), although the differences in the means of shell width and aperture width are themselves not significant (Table 1). Mean shell width is nevertheless slightly larger in males, which is why the differences in the ratio became slightly significant (Wilcoxon test, $\mathrm{p}=0.044$ ). Because shell width and aperture width do not differ between sexes, the greater whorl width should therefore cause a less pronounced lateral protrusion of the female aperture. Indeed, aperture protrusion - calculated by subtracting whorl width from shell width - has distinctly lower values for females than for males (Wilcoxon test, p < 0.001; Fig. 4c).

To assess the amount of allometry in the data we plotted isosize against each of the shape PCs, in order to see how strongly shape correlates with size. Judging from the respective graphs (Figs 5a and 5b) only a very moderate correlation of shape PC2 with isosize is discernible, shape PC1 is clearly uncorrelated. Therefore allometric variation was of marginal importance concerning our data set. This observation is further supported by the allometry ratio spectrum. The latter is used in a very similar manner as a PCA ratio spectrum, except that the ratios calculated from distant characters are those showing the most distinctive allometric behaviour. Inspection of the allometry ratio spectrum in Figure 5c reveals aperture width:whorl width as the ratio with the strongest allometric variation. Obviously, it is not among the most dominant ratios of the PCA ratio spectrum of shape PC1 or shape PC2 (Fig. 2).

Often, a researcher might simply wish to identify the sex of a shell. We therefore performed a linear discriminant analysis (LDA) that reveals the best separation of two groups by using the total information in the data. The performance of the LDA was then assessed with cross validation (Table 2). In our analysis, it was possible to correctly classify $92.3 \%$ of the females and $88.7 \%$ of the males.


Figure 2. Scatterplot of first against second principal component in shape space; blue dots females, red triangles males.

For practical reasons, a few characters that would allow quick and easy identification of most specimens might sometimes be useful, for instance in field work. A discriminant function is too awkward for such purpose, because its application is complicated and it lacks an intuitive element. One or two ratios would be preferable, as these are easily calculated and differences in proportions can sometimes even be estimated by eye. We therefore applied the LDA ratio extractor (see Baur and Leuenberger 2011:816-817) for finding the optimal ratios for separating females and males. The two best ratios were shell height:width and aperture height:width (Fig. 6). Unfortunately, the sexes overlapped quite considerably in their range which is why ratios are apparently of limited use for identification. Baur and Leuenberger (2011) also introduced the measure $\delta$ that indicates how well shape discriminates in relation to size. A value of delta close to unity means that separation is mainly due to size, whereas for a value close to zero mostly shape is important. In our case, $\delta$ is 0.139 for the first ratio vector and thus the separation mainly stems from variation in shape.

## Anaylsis of other characters

The rib density, determined on the last whorl, was largely overlapping between the sexes, although the mean was slightly higher for females (Wilcoxon test, $\mathrm{p}<0.05$; Fig. 7).

Pigmentation of the last whorl revealed a significant difference in the lower band, while pigmentation of the median and upper band was the same for males and females (Fig. 8). The figure is based on the classification made by the person that was not further involved in the study. However, classification by the senior author gave a very similar result.


Figure 3. PCA ratio spectrum of first $(\mathbf{a})$ and second principal component $(\mathbf{b})$ in shape space.


Figure 4. Boxplots of the ratios shell height:aperture height (a) and shell width:aperture width (b), and aperture protrusion (c). Significance levels of Wilcoxon test: ${ }^{(* * * '} \mathrm{p}<0.001,^{\prime * * \prime} \mathrm{p}<0.01,{ }^{(*)} \mathrm{p}<0.05$, ' $\mathrm{p}<0.1$, ' ' $\mathrm{p}<1$.

## Sex ratio in the investigated six populations

The numbers of males and females within the investigated populations are presented in Table 3. Population no. $1(\mathrm{n}=6)$ is excluded here, the sample size is considered to be too small. For the remaining five populations, the observed average of females $/ \mathrm{males}$ approaches a value of nearly 1 , i.e. there is an equal number of females and males present. Two populations show a value > 1 (= more females), two show a value < 1 (= less females), and one population is balanced.
a
Isometric size axis versus first PC in shape space

b
Isometric size axis versus second PC in shape space


C
Allometry Ratio Spectrum

| aperture.w |  |
| :---: | :---: |
|  |  |
| shell.h |  |

Figure 5. Scatterplots of isosize against first (a) and isosize against second principal component (b) in shape space; blue dots females, red triangles males. Allometry ratio spectrum (c).

## Discussion

As expected, there is a sexual dimorphism in shells of $C$. septemspirale. Females are larger than males, as revealed by larger mean isosize, a result already indicated by Gofas (2001). However, the main differences are due to a considerable variation in shell shape. Aperture height is reduced in females, while shell height and whorl width are both larger. Females therefore are not only larger, they also have about one fifth more volume in the central part of the teleoconch above the aperture.

These differences in size and shape of the sexes may be explained by the differing morphology of the reproductive systems between females and males. Females have


Figure 6. Scatterplot of best (shell height:shell width) against second best ratio (aperture height:aperture width) for separating females from males; blue dots females, red triangles males.

Table 2. Identification of specimens based on cross validation.

| $\boldsymbol{s e x}$ | identification as |  | correctly identified |
| :--- | ---: | ---: | ---: |
|  | female | male |  |
| females | 96 | 8 | $92.3 \%$ |
| males | 13 | 103 | $88.8 \%$ |

Table 3. Sex ratio in five populations of Cochlostoma septemspirale.

| population | $q$ | $\sigma^{\pi}$ | No. of specimens | sex ratio $Q / \delta^{\pi}$ |
| :---: | :---: | :---: | :---: | :---: |
| 2 | 21 | 21 | 42 | 1.00 |
| 3 | 13 | 22 | 35 | 0.59 |
| 4 | 19 | 16 | 35 | 1.19 |
| 5 | 26 | 21 | 47 | 1.24 |
| 6 | 23 | 32 | 55 | 0.72 |
| Sums | 102 | 112 | 214 |  |
| mean |  |  |  | 0.95 |

a seminal receptacle and a bursa copulatrix well separated from the oviduct (Prince 1967; Varga 1984; De Mattia et al. 2011). The ovarium, which is situated in whorls 3 and 4 contains up to 40 eggs, which increase in size during growth and are transferred via the oviduct to the uterus in the last whorl. Finally, clutch size in C. septemspirale reaches up to 10 eggs, with a diameter of $1.0-1.1 \mathrm{~mm}$ (Prince 1967). In contrast, males have only a germinative gland with a simple duct that ends in the penis on the right side of the head.

Mean rib density is slightly higher in females, but the ranges are more or less overlapping (Fig. 7).


Figure 7. Boxplots of rip density. Significance levels of Wilcoxon test: '***' $\mathrm{p}<0.001$, '**' $\mathrm{p}<0.01$, '*' $\mathrm{p}<0.05$, $\because \mathrm{p}<0.1$, ' $\mathrm{p}<1$.


Figure 8. Bar diagrams showing the extent of pigmentation on the lower whorl: classification (missing, interrupted, continuous) of lower (a), median (b), and upper band (c); blue bars females, red bars males. Significance levels of Fisher's exact test: '***' $\mathrm{p}<0.001$, '**' $\mathrm{p}<0.01$, '*' $\mathrm{p}<0.05$, ' ${ }^{\prime} \mathrm{p}<0.1$, ' $\mathrm{p}<1$.

In the character state "banding of the last whorl", males are more strongly pigmented on the lower band than females (Fig. 8). Currently there is no explanation for this result, as males and females live in mixed populations under the same environmental conditions. It also cannot play a role in sex recognition, because the optical abilities of snails are limited to recognition of darkness or brightness (Chase 2001).

From Figure 2 and 6 it is evident that continuous character variation is quite strongly overlapping between sexes. However, the result of the cross validation indicated that about $90 \%$ of the shells can be correctly assigned (Table 2).

From the results of the calculation of the sex ratios it can be concluded that the ratio between males and females is approximately balanced. However, the number of investigated populations is much too small to come to a generalized conclusion. The observed ratio variability can also be due to a too small population sample.

As we have shown above, multivariate morphometric methods could be successfully applied to populations of C. septemspirale from the Jura. In particular, it was possible to uncover subtle, but nevertheless important morphological differences between sexes, that otherwise might have gone unnoticed. Future investigations will show whether our results apply for other populations from the distribution area of C. septemspirale or even other species of Cochlostoma. In practice, the methods are easily applicable in a wide variety of ecological and systematic studies and offer the additional benefit to exploit museum's material with their mainly dry shell collections.

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## Appendix I

## Localities

NMBE 30979
Le Landeron, $47.06760435^{\circ} \mathrm{N}, 7.06510484^{\circ} \mathrm{E}$ (DD), $596 \mathrm{~m}, 5.4 .2011$, leg. Reichenbach et al.

NMBE 30980
Chasseral, at the crossroads Diesse / Nods Chasseral, $47.06996106^{\circ} \mathrm{N}, 7.09448308^{\circ} \mathrm{E}$ (DD), $620 \mathrm{~m}, 5.4 .2011$, leg. Reichenbach et al.

NMBE 30981
Orvin, $47.15439027^{\circ} \mathrm{N}, 7.19399571^{\circ} \mathrm{E}$ (DD), $833 \mathrm{~m}, 5.4 .2011$, leg. Reichenbach et al.

NMBE 30982
Route de Chasseral, Combes de Nods, $47.11286416^{\circ} \mathrm{N}, 7.04331994^{\circ} \mathrm{E}$ (DD), 1154 $\mathrm{m}, 19.4 .2011$, leg. Reichenbach et al.

NMBE 30983
Route de Chasseral, 1 km towards Nods, $47.12018349^{\circ} \mathrm{N}, 7.07096815^{\circ} \mathrm{E}$ (DD), 1054 m, 19.4.2011, leg. Reichenbach et al.

NMBE 30984
Gaicht in direction to Twannberg, $47.10991059^{\circ} \mathrm{N}, 7.15971977^{\circ} \mathrm{E}$ (DD), 794 m , 19.4.2011, leg. Reichenbach et al.

# A new species and new records of Laelaspis Berlese (Acari, Laelapidae) from Iran 

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#### Abstract

This paper reports on three species of mites of the genus Laelaspis in Iran - Laelaspis calidus Berlese from Pheidole pallidula, L. humeratus (Berlese) from Tetramorium caespitum and L. dariusi Joharchi \& Jalaeian, sp. $\mathbf{n}$. from soil. The new species is described and illustrations provided.


## Keywords

Laelapidae, taxonomy, Formicidae, Iran, myrmecophiles

## Introduction

The Laelapidae is one of the largest families of free-living Mesostigmata, but it has not yet achieved a stable classification (Tenorio 1982, Joharchi et al. in press). Hypoaspis Canestrini and related genera have had an especially complicated and confusing history, including Laelaspis Berlese, 1903, which has often been treated as a subgenus
of Hypoaspis Canestrini, 1884 (Hunter 1961, Hunter and Glover 1968, Karg 1982, 1993, Faraji et al. 2008). Joharchi et al. (2011) treated Laelaspis as a separate genus, and gave a diagnosis and comparison of diagnostic characters for the closely related genera Gymnolaelaps and Pseudoparasitus. That concept of Laelapsis is followed here.

Joharchi et al. previously reported on five species of mites of the genus Laelaspis and on several genera associated with ants in Iran (Joharchi et al. in press, Joharchi et al. 2011). Joharchi et al. have previously provided a key to species of Laelaspis occurring in the Western Palaearctic Region with a summary of their host associations and biology (Joharchi et al. in press). We now expand the study to include further species in the genus Laelaspis Berlese, 1903, mainly associated with ants and soil.

The cosmopolitan genus Laelaspis includes 17 species in the Western Palaearctic Region and most species are associated with ants or their nests. However, a few were collected with small mammals or in soil, and most species have only been collected on few occasions, so it is difficult to draw any firm conclusions about their host specificity (Joharchi et al. in press). Six species of Laelaspis have been reported previously from Iran (Joharchi et al. in press). Unidentified species of Laelaspis were also reported from Iran by Kamali et al. (2001) and Nemati and Babaeian (2010). The purpose of this paper is to describe another species of Laelaspis and increase our knowledge of the Iranian fauna of Laelapidae.

## Materials and methods

Mites associated with ants and soil were collected in Alborz, Khorasan, Kerman and Yazd Provinces over a period of two years (2010-2012). Mites were removed from ants' nests by individual hand picking and by extraction from ant nest and soil material using Tullgren funnels. Mites were cleared in Nesbitt's solution and mounted in Hoyer's medium. The nomenclature used for the dorsal idiosomal chaetotaxy is that of Lindquist and Evans (1965), the leg chaetotaxy is that of Evans (1963a) the palp chaetotaxy that of Evans (1963b), and names of other anatomical structures mostly follow Evans and Till (1979). We use the term "lyrifissures" to refer to slit-shaped sensilli, and "pore" for circular or oval-shaped cuticular openings of unspecified function. The holotype and paratypes of the new species are deposited in the Acarological collection, Department of Plant Protection, Yazd Branch, Islamic Azad University (YIAU); paratypes are also deposited in the Jalal Afshar Zoological Museum, College of Agriculture, University of Tehran, Iran (JAZM) and in the Australian National Insect Collection, CSIRO Ecosystem Sciences, Canberra, Australia (ANIC). All measurements in the descriptions are given in micrometres ( $\mu \mathrm{m}$ ).

## Results

Genus Laelaspis Berlese

http://species-id.net/wiki/Laelaspis
Laelaps (Laelaspis) Berlese, 1903: 13.

Type species. Laelaps astronomicus Koch, 1839, by original designation.
Diagnosis. See Joharchi et al. (2011).
Notes on the genus. Laelaspis belongs to a group of genera of Laelapidae in which the genital shield of the female is greatly expanded, so that its posterior margin abuts the anal shield and its lateral margins extend outward behind coxae IV. The expanded genito-ventral shield in these genera captures at least two pairs of ventral setae in addition to the genital setae on the the extreme edges of the shield. Laelaspis is distinguished from Gymnolaelaps by its two-tined palp tarsal claw, the absence of pre-sternal shields, and the presence of two distinct $\Lambda$-shaped lines on the genital shield. Laelaspis differs from Pseudoparasitus because Pseudoparasitus has at least two pairs of setae on the surface of the genital shield, well separated from the edges of the shield, while all the genital setae of Laelaspis and Gymnolaelaps are on the extreme edges of the shield.

## Laelaspis calidus Berlese

http://species-id.net/wiki/Laelaspis_calidus
Laelaspis calidus Berlese, 1924: 255; Hunter 1961: 676.
Hypoaspis (Laelaspis) calidus.- Aswegen and Loots 1970: 27.
Hypoaspis (Laelaspis) calida.- Karg 1982: 250; 1989: 120.

Specimens examined. Six females, Anar, Kerman, $53^{\circ} 30^{\prime} \mathrm{N}, 18^{\circ} 55^{\prime} \mathrm{E}$, alt. 1152 m 10 November 2011, O. Joharchi coll., in nest of Pheidole pallidula.

Notes. Laelaspis calidus was described from east Africa (Berlese 1924), also has been recorded at Kilimanjaro near Marangu from moss and litter (Aswegen and Loots 1970) and has not been reported since. It is easily recognised by the bidentate movable digit and the seven-toothed fixed digit, the serrated postanal seta and seta Z 5 two to three times as long as J 5 . This species has been found from moss and litter, but has not been reported from the nests of ants. It is now recorded in Iran for the first time from the ant nests.

## Laelaspis dariusi Joharchi \& Jalaeian sp. n.

urn:lsid:zoobank.org:act:BB2DA9B6-4ACF-4F3B-A516-3E33EA3AD1F0
http://species-id.net/wiki/Laelaspis_dariusi
Figures 1-8

Specimens examined. Holotype, female, Iran, Khorasan Razavi Province, Kalate Naderi (Laeen), $37^{\circ} 07^{\prime} \mathrm{N}, 59^{\circ} 29^{\prime} \mathrm{E}$, alt. $858 \mathrm{~m}, 26$ Mar 2010, S. Paktinat-saeej coll., in soil of apple orchard. Paratypes, seven females, same data as holotype (in YIAU, JAZM and ANIC).

Description of the female. Figures 1-8. Dorsal idiosoma. Dorsal shield length 524-534, width 406-426 ( $\mathrm{n}=8$ ) (Fig. 1). Shield oval shaped, with reticulation, more distinct in opisthonotal region; with 39 pairs of long setae, 22 podonotal, 17 opisthonotal, including two pairs of Zx setae between J and Z setae, almost all setae slightly swollen at base, with pointed tip (Fig. 4), podonotal setae very long, reaching well past base of next posterior setae, setae of central area of dorsal shield decreasing in length from anterior to posterior ( $\mathrm{j} 3, \mathrm{z} 274-82$, j4 69-74, j6, J1, J3 54-57), lateral setae thicker than central setae, almost all marginal setae including Z5 slightly serrated (Fig. 3), length 89-99, almost double length of J 5 , length $45-50$; opisthonotal region with three unpaired supernumerary seta Jx in each specimen. Shield with three pairs of large circular to oval-shaped pores, other pores inconspicuous.

Ventral idiosoma (Fig. 2). Tritosternum with columnar base (15-17 long $\times 9-10$ wide), paired pilose laciniae, length $67-69$ (Fig. 7), pre-sternal shields absent, presternal area with some weak transverse lines. Sternal shield length 111-116, narrowest between coxae II (87-89) widest between coxae II \& III (151-153), with slightly concave posterior margin and undulating anterior margin, with three pairs of long and smooth sternal setae, st $142-47$, st2 59-62, st3 67-69, reaching well past base of next posterior setae, one pair of lyrifissures adjacent to setae $s t 1$, a pair of larger lyrifissures between $s t 2$ and $s t 3$; antero-lateral surface of sternal shield with lineate ornamentation, central area smooth. Metasternal platelets absent, metasternal setae st4 (27-32) and metasternal pores located in soft skin; endopodal plates II/III fused to sternal shield, endopodal plates III/IV elongate, narrow, curved. Genital shield broad, length 277-285, maximum width 248-260, posterior margin rounded, abutting anal shield, surface with characteristic ornamentation including distinct $\Lambda$-shaped lines and polygonal ornamentation, bearing the long genital setae st 5 (87-89) and two pairs of long setae (89-99) on its lateral edges. Paragenital pores located on soft skin lateral to shield behind coxae IV. Anal shield subtriangular, length 104-109, width 126-131; its anterior half with lineate ornamentation and a pair of lateral pores; post-anal seta $42-45 \mu \mathrm{~m}$, longer and thicker than para-anal setae, 22-25. Opisthogastric skin with long, narrow and oval metapodal plates (62-64 long $\times 8-10$ wide) very close to genital shield, and 15 pairs of slightly serrate setae, each arising on small sclerotised platelet, and seven pairs of pores. Exopodal plates forming subtriangular extensions behind coxae IV, narrow elongate exopodal plates II/III not fused to peritrematal shield. Peritreme extending from coxa IV to anterior


Figures I-8. Laelaspis dariusi Joharchi and Jalaeian sp. n., female. I Dorsal shield 2 Ventral idiosoma $\mathbf{3}$ Seta Z5 enlarged $\mathbf{4}$ Dorsal shield seta s3 $\mathbf{5}$ Hypostome $\mathbf{6}$ Epistome $\mathbf{7}$ Tritosternum $\mathbf{8}$ Chelicera.
of coxa I, peritrematal shield narrow, post-stigmatal section conspicuous, with two pairs of pores.

Gnathosoma. Epistome triangular, smooth (Fig. 6). Hypostomal groove with six rows of denticles each bearing $8-10$ small teeth, and smooth anterior transverse line. Hypostome with four pairs of setae, internal posterior hypostomal setae h3 longest (Fig. 5). Corniculi robust and horn-like, reaching mid-level of palp femur. Palp chaetotaxy: trochanter 2 (v1 thick), femur 5, genu 6, tibia 12, tarsus 15 ; all setae smooth and needle-like, palp tarsal claw two-tined. Fixed digit of chelicera with six blunt teeth (Fig. 8); pilus dentilis short and robust; dorsal seta short, prostrate; movable digit with two teeth; arthrodial membrane with a rounded flap and short filaments.

Legs. Legs II and III short (302-312, 282-288), I and IV longer (430-446, 372392). Leg I: coxa $00 / 10 / 10$, trochanter $11 / 10 / 21$ (ad thick), femur $23 / 22 / 22$ (ad2, ad3, al1, pl1 and pl2 thick), genu 2 3/2 3/1 2 (all dorsal thick), tibia 2 3/2 3/1 2. Leg II: coxa $00 / 10 / 10$ (all setae thick), trochanter $10 / 11 / 21$, femur $23 / 12 / 2$ 1 (ad1, pd2 and pv1 thick), genu $23 / 12 / 12$ (all ventral thick), tibia 2 2/1 2/12 (all ventral thick). Leg III: coxa $00 / 10 / 10$ (all setae thick), trochanter $10 / 10 / 21$ (al and $a v$ thick), femur $12 / 11 / 01$ ( $a d 1$ and $a d 2$ thick), genu $2 / 12 / 11$ (ventral setae thick), tibia: $21 / 12 / 11$ (ventral setae thick). Leg IV: coxa $00 / 10 / 00$, trochanter 1 0/1 0/2 1 (av thick), femur 1 2/1 1/0 1 (al long, $a d 1$ and $a d 2$ thick), genu 2 2/1 3/0 1 (ventral thick), tibia $21 / 13 / 12$; all setae fine and needle-like unless otherwise noted. Tarsi I-IV with 18 setae $33 / 23 / 23+m v$, $m d$. All pre-tarsi with a pair of claws and a long thin membranous ambulacrum.

Insemination structures not seen, apparently unsclerotised.
Etymology. The species is named in memory of Darius I (Old Persian: Dārayava(h)us'), also known as Darius the Great, was the third king of the Achaemenid Empire, who proved to be a strong and wise ruler and he was tolerant toward other religions and cultures, promoted learning, agriculture, forestation, and the construction of highways. He also built the great palace cities of Susa and Persepolis.

Notes. Laelaspis dariusi differs from all other species in the genus by its dorsal shield setae in central area decreasing in length from anterior to posterior, seta Z5 much longer than J5; seta v 1 on the palp trochanter thick, sternal setae long and smooth, long enough to well past base of next posterior setae, movable digit of chelicera with two teeth and fixed digit of chelicera with six blunt teeth.

## Laelaspis humeratus (Berlese)

http://species-id.net/wiki/Laelaspis_humeratus
Laelaps (Laelaspis) humeratus Berlese 1904: 425.
Hypoaspis humerata.— Evans and Till 1966: 212; Lapina 1976: 43.
Laelaspis humeratus.- Hull 1925: 210; Willmann 1951:113; Hunter 1961: 675;
Salmane 2001a: 131; Salmane 2001b: 34; Salmane and Brumelis 2010: 390.
Hypoaspis (Laelaspis) humerata.— Karg 1979: 102; 1982: 250; 1989: 120.

Laelaspis humerata.- Bregetova 1977: 545.
Specimens examined. One female, Alborz, Karaj, $35^{\circ} 56^{\prime} \mathrm{N}, 51^{\circ} 22^{\prime} \mathrm{E}$, alt. $2000 \mathrm{~m}, 11$ July 2011, O. Joharchi coll., in nest of Tetramorium caespitum.

Notes. Laelaspis humeratus was described from Luxemburg (Berlese 1904), and has been recorded from Latvia (Lapina 1976; Salmane 2001a, 2001b), Russia and Austria (Bregetova 1977), and England (Hull 1925; Evans and Till 1966). This species was found associated with at least two genera of ants (Lasius and Tetramorium), freeliving in soil, litter and meadows, and from the nests of mammals. This species is easily recognised by the large number of long, thick and wavy opisthonotal setae, the bidentate movable digit and the tridentate fixed digit. Haddad Irani-Nejad et al. (2003) recorded an unidentified species as Laelaspis near humerata (Berlese, 1904), but the identity of that species cannot be confirmed because the specimens have been lost, so this is the first record of $L$. humeratus from Iran.

## Discussion

Before the start of this study, six species of Laelaspis had been reported from Iran. We have added new information on $L$. calidus and $L$. bumeratus.

Joharchi et al. have previously discussed the distinction between Laelaspis and Gymnolaelaps and Pseudoparasitus (Joharchi et al. 2011). The biology of most species of Laelaspis has not been studied, but the limited information that is available shows that they are predatory (Hunter 1964). Laelaspis appears to be a genus of predators that feed on other small invertebrates in their hosts' nests, but are not harmful to the ants. High populations of acarids may be harmful to ants, so the presence of predators such as Laelaspis may be beneficial, forming a symbiotic relationship with its ant hosts. The ecological role of Laelaspis in mammal nests is also unknown, but it appears likely that they are predators, feeding on other nest inhabitants such as acarid mites (Rasmy et al. 1987).

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# Surprising longhorned beetle (Coleoptera, Cerambycidae) richness along an Italian alpine valley 

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#### Abstract

In this paper we report about 88 longhorned beetles (Cerambycidae) species found in 6929 hectares and distributed along an altitudinal gradient of 1500 m of an Italian alpine valley (Val Genova, central-eastern Italian Alps). The species richness, result merging data from sixty years (1947-2007) of entomological surveys, corresponds to the $32 \%$ of the Italian cerambycid fauna confirming the high richness/surface ratio, probably unique in the Alps. The effect of thirteen environmental variables was tested on the species richness, but only the elevation resulted able to affect it. The species richness decrease with altitude not gradually, but experience a strong step above 1700 m a.s.l.. The highest species richness (average values of 42 species) was recorded at the lowest and mid elevations (between 800 and 1600 m a.s.l.). The species turnover along the altitudinal gradient is low suggesting moderate habitat turnover along the valley.

One of the eighty-eight observed species, Tragosoma depsarium, is classified near threatened by the IUCN. Our data suggest that the wilderness of the valley close to the suitable management of grasslands and forests, help to support high level of cerambycids diversity. This biodiversity is good indicators of health of the wood saproxylic assemblages, as well an important food source for many vertebrate predators.


## Keywords

Cerambycids, saproxylic, species richness, protected areas, Val Genova, Alps

## Introduction

The longhorned beetles (Coleoptera: Cerambycidae) can be considered one of the richest families of animals with about 35,000 described species (Hurka 2006). The European species richness amounts to a total of 677 species (Sama 2009). This family of beetles is widespread in all Europe from the Mediterranean basin to the highest latitude due to the ability to colonize from temperate to hot regions. Cerambycids are strictly phytophagous occurring herbaceous, shrubby and arboreal vegetation (Sama 2006).

From the ecological point of view, longhorned beetles might potentially be excellent indicator species of the health of the wood saproxylic assemblages (Dajoz 2000) because of their habitat specificities, and because they are relatively easy to identify (Speight 1989, Sama 2006). The larvae of most longhorned beetles develop within either living or dead wood and feed by mining galleries in the wood. There is a great range in the breadth of host tree species that may used by the larvae of different species (Hanks 1999). This range of habitat specificity within the family extends from monophagous species specialized on a single host tree species to generalist species that can make use of a large variety of trees genera. Many species, at the adult stages, have an important role as pollinators and mainly the larvae, but also the adult, are an abundant component in the diet of forest birds like the woodpeckers. Thanks to these functional roles in the ecosystems, the cerambycids can be considered good indicators on the state of conservation of biodiversity, but also sensible to human ecosystem management (Sama 2006). In Italy the taxonomical and chorological knowledge is satisfactory. At today, for the Italian peninsula are known 274 species belonging to 119 genera representing one of the nations with the highest species richness in Europe (Sama 2009). This richness can be justified by the longitudinal extension of the mainland, the central position within the Mediterranean area and the presence of highly diversified habitats, both from geomorphologic and climatic point of view (Sama 2006).

In the central-eastern Italian Alps there is a valley belonging to the Adamello-Brenta Natural Park (Trentino - Alto Adige Province), named Val Genova which is characterized by the total absence of urbanized areas, and that is characterized by a mosaic of different natural and human-managed habitats which have not suffered modifications for at least one century. The human activities are limited to the hay meadows, pastures and to the cut of some forested areas. This valley had attracted the attention of many entomologists since the middle of the last century. In the last sixty years many surveys was done by different entomologists to catch cerambycids, therefore we merged presence/absence historical data with those collected by us. The obtained database was used to test the following hypothesis: i) the species richness decreases gradually along the altitudinal gradient, ii) the presence of a mosaic of natural and human-managed habitats supports high values of species richness.

## Methods

## Study area

The study area is an alpine valley named Val Genova ( $46^{\circ} 09^{\prime} \mathrm{N}, 10^{\circ} 40^{\prime} \mathrm{E}$ ). It is located in the central-eastern Italian Alps, in the Trentino - Alto Adige Region, and belongs to the Adamello-Brenta Natural Park. The valley is about 20 Km long, and the area considered for the cerambycids catchment it is distributed along an altitudinal gradient of about 1500 metres ( $800-2200 \mathrm{~m}$ a.s.l.), and inside an area of 6929 hectares. The average precipitation is over 1000 mm , and the most rainy periods are during May and October with precipitation over 100 mm . Different climatic factors determine the vegetation gradient along the valley. At the lowest elevation (800-1000 m a.s.l.) there is dominance of broadleaf woods with Fagus sylvatica, Alnus incana, Acer pseudoplatanus, Corylus avellana, Fraxinus excelsior, Carpinus betulus, Laburnum anagyroides, Betula pendula, Salix alba, Robinia pseudoacacia. At the mid-elevations (1100-1800 m a.s.l.) the broadleaf forests (mainly Betula) are mixed with conifers characterized by the presence of Abies excelsa, Abies alba and Pinus sylvestris. The timberline is around 1900 m a.s.l., while the treeline (with Larix decidua) is around 2100 m a.s.l.. Above 2100 m shrubs of Alnus viridis, Rhododendron ferrugineum and rare Pinus mugo appear. Along this altitudinal and vegetation gradient there are many grasslands (e.g. pastures, meadows) human-managed for at least one century. In particular the hay meadows are located at the low-mid elevation ( $<1500 \mathrm{~m}$ a.s.l.), whereas the pastures are from the mid to the highest elevation (1300-2200 m a.s.l.). Studies performed in the AdamelloBrenta Natural Park evidenced just an increase of $10 \%$ of the forest coverage since the Second World War up today due to the increase of neglected areas, in particular at the highest elevations (Bronzini 2005).

## Database creation

The database has been created by merging data collected during the field surveys performed by Moscardini (1956), Contarini (1988), Sama (1988), Martinelli (1995), and Pedroni (1998, 2000), with our presence/absence data recorded in 2007.

Our survey of longhorned beetles was carried out from May to September 2007, two-three times a week along the bottom of the valley and at the same elevation chosen by Moscardini in 1947 (Moscardini 1956). Other areas located on the slope of the valley were analyzed. To increase the probability of success, the survey of adults was organized on sunny days within the hottest hours (10:00 a.m. - 18:00 p.m.) netting on the herbaceous, shrubby and arboreal vegetation with sweep net and entomological umbrella. Once a week nocturnal collections were made using the Wood's UV lamp (Southwood 1978).

The surveys performed during these sixty years are not comparable with the aim to describe the species richness trend along the time because the sites visited by each entomologist have been not always the same, and the sampling effort was different.

Longhorned beetles were identified using Pesarini and Sabbadini (1994) and Rastelli et al. (2001). Some specimens were compared with those preserved in the collections of the Museo delle Scienze of Trento (Italy). The updated species nomenclature is based on Sama (2009).

## Statistical analyses

The Incidence-based Coverage Estimator of species richness (ICE) has been used to estimate the gamma-biodiversity (Hortal et al. 2006) of longhorned beetles living in the valley. The species accumulation curve has been drawn using the Mao Tau index to observe how species richness increases with the number of sampling performed by each entomologist (Colwell et al. 2005). The correlation between thirteen different environmental variables (elevation; aspect; distance from: houses, rivers, lakes, main roads, secondary roads; habitats diversity, percentage of: open areas, forests, glades; slope and land cover type) recorded within a buffer of 200 metres around the sampling point, and the species richness $(S)$ has been computed by Spearman's correlation analyses. This correlation was preferred respect to the Pearson correlation due to the few sampling points. The Linear Regression Analyses has been performed to test the effect of the environmental variables on the species richness. Each environmental variable was calculated by GIS-approach. The ANOSIM test, based on Jaccard's index of similarity, has been performed to evaluate the presence of similarities in the assemblages' composition along the elevational gradient. A dendrogram of similarity has been drown to show the similarities along the elevation gradient.

Analyses have been computed using SPSS 13.0 (SPSS, Inc., Chicago IL), PAST 2.0 (http://folk.uio.no/ohammer/past) and Estimate S (http://viceroy.eeb.uconn. edu/EstimateS).

## Results

The database realized merging data collected in sixty years (1947-2007) by different entomologists produced a checklist of 88 species (Tab. 1) observed along the altitudinal gradient comprise between 800 and 2200 m a.s.l. A new species, never recorded before, has been observed during the last sampling season performed in 2007; the species is Phytoecia cilindrica (Linnaeus, 1758). The ICE index estimated for the valley a total of 93 species indicating that about the $91 \%$ of the species has been sampled; the species accumulation curve is not tending to the asymptote (Fig. 1), but it is gradually increasing confirming that more species could be cached, yet.
Table I. Cerambycids observed along the altitudinal gradient of the Val Genova (* presence) The species are ordered on the base of their frequency at each elevational sampling point. (IUCN abbreviations: $\mathrm{LC}=$ least concern; $\mathrm{NT}=$ near threatened; EU endem $=$ European endemism)

| Species / Elevation (m) | 800 | 900 | 1060 | 1100 | 1250 | 1430 | 1500 | 1640 | 1790 | 2000 | 2200 | IUCN Red List |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Pachytodes cerambyciformis (Schrank, 1781) | * | * | * | * | * | * | * | * | * | * |  |  |
| Tetropium castaneum (Linné, 1758) | * | * | * | * | * | * | * | * | * | * |  |  |
| Monochamus sutor (Linné, 1758) | * | * | * |  | * | * | * | * | * | * |  | LC |
| Alosterna tabacicolor (De Geer, 1775) | * | * | * | * | * | * | * | * |  |  |  |  |
| Anastrangalia dubia (Scopoli, 1763) | * | * | * | * | * | * | * | * |  |  |  |  |
| Anastrangalia sanguinolenta (Linné, 1758) | * | * | * | * | * | * | * | * |  |  |  |  |
| Evodinus clathratus (Fabricius, 1792) |  |  | * | * | * | * | * | * | * | * |  |  |
| Leptura quadrifasciata (Linné, 1758) | * | * | * | * | * | * | * | * |  |  |  |  |
| Monochamus sartor (Fabricius, 1787) | * | * |  | * | * | * | * | * | * | * |  | LC - European endemism |
| Rhagium inquisitor (Linné, 1775) | * | * | * | * | * | * | * | * |  |  |  |  |
| Ruptela maculata (Poda, 1761) | * | * | * | * | * | * | * | * |  |  |  |  |
| Saperda scalaris (Linné, 1758) |  |  | * | * | * | * | * | * | * | * |  | LC |
| Stenostola dubia (Laichrting, 1784) |  | * | * | * | * | * | * | * | * | * |  |  |
| Stenurella melanura (Linné, 1758) | * | * | * | * | * | * | * | * |  |  |  |  |
| Acmaeops pratensis (Laicharting, 1784) | * |  | * | * | * | * | * | * |  |  |  |  |
| Aromia moschata moschata (Linné, 1758) |  | * | * | * | * | * | * | * |  |  |  | LC |
| Callidium violaceum (Linnaeus, 1758) |  |  |  | * | * | * | * | * | * | * |  | LC |
| Gaurotes virginea (Linné, 1758) | * | * | * | * | * | * |  | * |  |  |  |  |
| Hylotrupes bajulus (Linné, 1758) | * | * | * | * | * | * | * |  |  |  |  | LC |
| Lepturobosca virens (Linné, 1758) |  |  | * | * | * | * | * | * | * | * |  |  |
| Oxymirus cursor (Linné, 1758) |  |  | * | * | * | * | * | * | * |  | * |  |
| Agapanthia villosoviridescens (De Geer, 1775) |  |  | * | * | * | * | * | * |  |  |  |  |
| Judolia sexmaculata (Linné, 1758) |  |  | * | * | * | * | * | * |  |  | * |  |
| Molorchus minor (Linné, 1758) |  |  | * | * | * | * | * | * |  |  |  | LC |
| Paracorymbia hybrida (Rey, 1885) |  |  | * | * | * | * | * | * |  |  |  |  |
| Paracorymbia maculicornis (De Geer, 1775) |  |  | * | * | * | * | * | * |  |  |  |  |


| Species / Elevation (m) | 800 | 900 | 1060 | 1100 | 1250 | 1430 | 1500 | 1640 | 1790 | 2000 | 2200 | IUCN Red List |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Pidonia lurida (Fabricius, 1776) |  |  | * | * | * | * | * | * |  |  |  |  |
| Rhagium bifasciatum (Fabricius, 1775) |  |  | * | * | * | * | * | * |  |  |  |  |
| Stenocorus meridianus (Linné, 1758) | * | * | * | * | * | * |  |  |  |  |  |  |
| Tetropium fuscum (Fabricius, 1787) |  |  | * |  | * | * | * | * | * | * |  |  |
| Achanthoderes clavipes (Schrack, 1781) |  | * | * | * | * | * |  |  |  |  |  |  |
| Anastrangalia reyi (Heyden, 1889) |  |  |  | * | * | * | * | * |  |  |  |  |
| Clytus arietis (Linné, 1758) | * |  | * | * | * | * |  |  |  |  |  | LC |
| Pachyta quadrimaculata (Linné, 1758) | * | * |  | * | * | * |  |  |  |  |  |  |
| Stenurella bifasciata (Muller, 1776) | * | * | * | * | * |  |  |  |  |  |  |  |
| Tetropium gabrieli (Weise, 1905) |  |  |  | * | * | * | * | * |  |  |  |  |
| Cortodera femorata (Fabricius, 1787) |  | * |  |  | * | * |  | * |  |  |  |  |
| Pseudalosterna livida (Fabricius, 1776) |  |  | * | * | * | * |  |  |  |  |  |  |
| Rhagium mordax (De Geer, 1775) |  |  | * | * | * | * |  |  |  |  |  |  |
| Stictoleptura rubra (Linné, 1758) | * | * |  | * | * |  |  |  |  |  |  |  |
| Anaglyptus mysticus (Muller, 1766) |  | * |  |  | * | * |  |  |  |  |  | LC |
| Asemum striatum (Linné, 1758) |  |  |  |  | * | * |  | * |  |  |  |  |
| Dinoptera collaris (Linné, 1758) | * | * | * |  |  |  |  |  |  |  |  |  |
| Lamia textor (Linné, 1758) | * | * | * |  |  |  |  |  |  |  |  |  |
| Obrium brunneum (Fabricius, 1792) | * |  | * |  | * |  |  |  |  |  |  | LC |
| Pachyta lamed (Linnè, 1758) |  |  |  |  |  | * | * | * |  |  |  |  |
| Pogonocherus hispidulus (Piller \& Mitterpacher, 1783) | * | * | * |  |  |  |  |  |  |  |  |  |
| Prionus coriarius (Linné, 1758) | * | * | * |  |  |  |  |  |  |  |  | LC |
| Saphanus piceus (Licharting, 1784) |  |  | * | * | * |  |  |  |  |  |  |  |
| Spondylis buprestoides (Linné, 1758) | * | * | * |  |  |  |  |  |  |  |  |  |
| Strangalia attenuata (Linné, 1758) | * | * | * |  |  |  |  |  |  |  |  |  |
| Tragosoma depsarium (Linné, 1767) |  |  |  |  |  | * | * | * |  |  |  | NT |
| Cerambyx scopolii Fuesslins, 1775 | * |  |  |  |  | * |  |  |  |  |  | LC |


| Species / Elevation (m) | 800 | 900 | 1060 | 1100 | 1250 | 1430 | 1500 | 1640 | 1790 | 2000 | 2200 | IUCN Red List |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Cholorophorus figuratus (Scopoli, 1763) | * |  |  |  |  | * |  |  |  |  |  | LC |
| Clytus lama (Mulsant, 1847) | * |  |  |  |  | * |  |  |  |  |  | LC - European endemism |
| Exocentrus punctipennis Mulsant \& Guillebeau, 1856 | * | * |  |  |  |  |  |  |  |  |  |  |
| Leiopus nebulosus (Linné, 1758) |  |  |  |  | * | * |  |  |  |  |  |  |
| Mesosa nebulosa (Fabricius, 1781) | * | * |  |  |  |  |  |  |  |  |  |  |
| Oberea pupillata (Gyllenhal, 1817) |  |  |  |  |  | * |  | * |  |  |  |  |
| Paracorymbia fulva (De Geer, 1775) | * | * |  |  |  |  |  |  |  |  |  |  |
| Parmena unifasciata (Rossi, 1790) | * | * |  |  |  |  |  |  |  |  |  |  |
| Phytoecia cylindrica (Linnaeus, 1758) | * | * |  |  |  |  |  |  |  |  |  |  |
| Phytoecia nigricornis (Fabricius, 1781) | * | * |  |  |  |  |  |  |  |  |  |  |
| Pogonocherus fasciculatus (De Geer, 1775) |  |  | * |  |  |  |  | * |  |  |  |  |
| Saperda carcharias (Linné, 1758) | * | * |  |  |  |  |  |  |  |  |  |  |
| Stenopterus rufus (Linné, 1767) | * | * |  |  |  |  |  |  |  |  |  | LC |
| Stenostola ferrea (Schrank, 1776) | * |  |  |  |  | * |  |  |  |  |  |  |
| Acmaeops septentrionis (Thompson, 1666) |  |  |  |  | * |  |  |  |  |  |  |  |
| Aegomorphus clavipes (Schrank, 1781) | * |  |  |  |  |  |  |  |  |  |  |  |
| Anoplodera rufipes (Schaller, 1783) |  |  | * |  |  |  |  |  |  |  |  |  |
| Anoplodera sexguttata (Fabricius, 1775) |  | * |  |  |  |  |  |  |  |  |  |  |
| Arhopalus ferus (Mulsant, 1839) |  | * |  |  |  |  |  |  |  |  |  |  |
| Arhopalus rusticus (Linné, 1758) |  |  | * |  |  |  |  |  |  |  |  |  |
| Brachyta interrogationis (Linné, 1758) |  |  |  |  |  |  |  |  | * | * |  |  |
| Callidium aeneum (De Geer, 1775) |  |  |  |  | * |  |  |  |  |  |  | LC |
| Cholorophorus sartor (Muller, 1766) |  | * |  |  |  |  |  |  |  |  |  | LC |
| Corymbia scutellata scutellata (Fabricius, 1781) |  |  | * |  |  |  |  |  |  |  |  |  |
| Exocentrus lusitanus (Linné, 1767) | * |  |  |  |  |  |  |  |  |  |  |  |
| Glaphyra umbellatarum (Schreber, 1759) |  |  |  |  |  | * |  |  |  |  |  | LC |


| Species / Elevation (m) | $\mathbf{8 0 0}$ | $\mathbf{9 0 0}$ | $\mathbf{1 0 6 0}$ | $\mathbf{1 1 0 0}$ | $\mathbf{1 2 5 0}$ | $\mathbf{1 4 3 0}$ | $\mathbf{1 5 0 0}$ | $\mathbf{1 6 4 0}$ | $\mathbf{1 7 9 0}$ | $\mathbf{2 0 0 0}$ | $\mathbf{2 2 0 0}$ | IUCN Red List |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Grammoptera ruficornis (Fabricius, 1781) |  | $*$ |  |  |  |  |  |  |  |  |  |  |
| Mesosa curculionoides (Linné, 1758) | $*$ |  |  |  |  |  |  |  |  |  |  |  |
| Oberea oculata (Linné, 1758) | $*$ |  |  |  |  |  |  |  |  |  |  |  |
| Oplosia cinerea Mulsant, 1839 (=fennica <br> Paykull, 1800) |  |  |  |  | $*$ |  |  |  |  |  |  |  |
| Saperda octopunctata (Scopoli, 1772) |  |  | $*$ |  |  |  |  |  |  |  |  |  |
| Saperda populnea (Linnè; 1758) |  |  |  |  |  |  |  | $*$ |  |  |  | LC |
| Stenopterus ater (Linné, 1767) |  | $*$ |  |  |  |  |  |  |  |  |  |  |
| Stenurella nigra (Linnè, 1758) | $*$ |  |  |  |  |  |  |  |  |  |  | LC |
| alpha diversity | 43 | 41 | 46 | 38 | 48 | 49 | 32 | 37 | 12 | 11 | 2 |  |



Figure I. Accumulation curve on the number of species observed during the surveys.

The Spearman's correlation analysis highlighted that species richness is correlated to two variables: the elevation (rho $=-0,74 ; \mathrm{P}=0,01$ ), and of the distance from the secondary roads (rho $=-0,65 ; \mathrm{P}=0,03$ ), but elevation and the distance from the secondary roads are positively auto-correlated (rho $=0,73 ; \mathrm{P}=0,01$ ). The Linear Regression Analyses performed to test the effect of elevation and distance from the secondary roads on the species richness show that elevation is the only variable able to affect the negatively the specie richness (ANOVA test: $\mathrm{F}_{2,10}=10,27 ; \mathrm{P}=0,006$; elevation: $\mathrm{t}=$ $-2,58 ; \mathrm{P}=0,033$; distance from the secondary roads: $\mathrm{P}=0,95$ ). In particular, species richness decreased with increasing elevation, but this trend is not gradual; the highest values of $S$ are between 800 and 1600 m a.s.l. $\left(\mathrm{S}_{\text {mean }}=41,14\right)$. Within this elevational gradient, any significant trend in the species richness resulted ( $\mathrm{P}=0,45$ ) (Fig. 2).The species richness decrease strongly above 1600 metres, and between 1700 and 2200 metres; the average species richness is 8 .

ANOSIM test demonstrated that species composition along the valley varied with a significantly low turnover (ANOSIM, $\mathrm{r}=0.21 ; \mathrm{P}<0.01$ ). The dendrogram built on the base of the Jaccard similarity showed the cluster of three main groups (Fig. 3): the first one is between 800 and 900 m asl, the second one is between 1060 and 1640 m , and the third is above 1790 m a.s.l..

## Discussion

The high species richness $(S=88)$ found in Val Genova valley reflect the high wilderness of this area due to the cerambycids being excellent indicators of the health of the wood decomposer community because of their habitat specificities (Nilsson et al.


Figure 2. Relationship between the species richness $(\mathrm{S})$ and the altitudinal gradient.


Figure 3. Dendrogram drawn on the base of the Jaccard similarities between the sites.
2001). This surprising species richness, found in $69,3 \mathrm{~km}^{2}$, corresponds to the $60 \%$ of the Trentino - Alto Adige Region ( $13607 \mathrm{~km}^{2}$ ) cerambicid fauna, to the $32 \%$ of the Italian and the $13 \%$ of that European one (Fauna Europaea 2011). Probably the agrosilvopastural management conduced during the decades has been not extensive like as happened in similar areas of the Alps. The agrosilvopastural management adopted in the studied valley is able to prevent, or at least to minimize, the forest colonization in the neglected grasslands and aimed to maintain into the forest patches with necromass stocks able to attract the cerambycid decomposers community.

The effects of the environmental variables on the species richness showed that only the elevational gradient determines a decrease in the number of species, and a low spatial assemblage's turnover. This result is in agreement with Baselga (2008) who highlighted that species richness gradient is a phenomenon more environmentally deterministic than turnover that is independent of the richness gradient. The species richness trend was not gradual, as expected, but affected by a step around 1700-1800 metres. This step coincides with the timberline. So, under the timberline, the species richness maintains high values due to the presence of a spatially structure vegetation, and by the mosaic of agrosilvopastural activities.

Twenty-five percent of the species observed in the Val Genova have been evaluated in the IUCN redlist of saproxylic beetles (Nieto and Alexander 2010) and have been considered species of "least concern" (LC), but within these species Tragosoma depsarium is considered "near threatened" (NT), and two (Monochamus sartor and Clytus lama) are endemic of Europe (Tab. 1). Besides, within these species of European conservation interest, there are some of local monitoring interests because they have not been observed anymore since at least twenty years, therefore reported for the last time by Contarini (1988), and Sama (1988). These species are eight, and specifically: Parmena unifasciata, Grammoptera ruficornis, Oberea oculata, Anoplodera sexguttata, Arhopalus ferus, A. rusticus, Paracorymbia fulva, and Phytoecia nigricornis.

The forest management is known to negatively affect saproxylic beetles (Paillet et al. 2010). This finding suggests that the forests on the Val Genova valley are not extensively exploited by humans (mainly the thinning and the clearing) favouring the amount of dead and stressed trees. Raje et al. (2011) reports that the forest productivity is negatively correlated with cerambycid richness. A decrease in the productivity favour availability of dead wood, fallen limbs and more exposed inner wood offering favourable conditions for the development of the cerambycids. These beetles represent an important food source for many vertebrate and invertebrate predators, such that the benefits could propagate through the food web.

In conclusion, the biodiversity of longhorned beetles observed in the Val Genova valley can be considered surprising due to the high number of species living into an area with the following features: small size and distributed along a wide elevational gradient.

The possibility to observe so many cerambycids in a limited space is to our knowledge, unique in the Alps. It suggest that other taxonomic groups should be considered with the purpose to increase the entomological knowledge of this valley that probably is the only one not urbanized, at least in the Italian Alps.

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# Two new species of Olecryptotendipes Zorina, 2007 from China (Diptera, Chironomidae) 

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#### Abstract

Two new species, Olecryptotendipes exilis sp. n. and $O$. melasmus sp. n. are described and illustrated as males and Chinese males of $O$. lenzi are re-examined. A key to all known males of Olecryptotendipes is provided.


## Keywords

Chironomidae, Olecryptotendipes, new species, key, China

## Introduction

Based on the morphology of the males in the Russian Far East, Cryptotendipes lenzi Zorina, 2001 and C. secundus Zorina, 2003 were described by Zorina (2001, 2003). Zorina (2007) collected the larvae and pupae of $C$. lenzi, and showed the two species should be placed in a new genus, named Olecryptotendipes Zorina, 2007. The type species is Cryptotendipes lenzi Zorina, 2001. To date, the genus only contains the two aforementioned species. Yan et al. (2005) recorded Cryptotendipes lenzi from Xinjiang Autonomous Region in China.

The males of Olecryptotendipes are characterized by Y-shaped anal tergite bands; posterior part of tergite IX elongated with setae; superior volsella with sclerotized part and membranous ridge, with dorsal and ventral setae, microtrichia absent or present ventrally and weak inferior volsella (Zorina 2007). For the diagnosis of pupa and larva, refer to Zorina (2007).

In the present paper, two new species are recorded. Prof. Ole Sæther and Dr. M. Spies have checked the specimens. The two new species don't belong to the genera Cryptotendipes Lenz, 1941 and Chernovskiia Sæther, 1977 because of the presence of sclerotized superior volsella and lobate inferior volsella. In addition, the species of the genus Cryptotendipes lack an inferior volsella and the margin of the gonostylus is usually with an expansion. Species of the genus Chernovskiia are also without lobate inferior volsella, but with foot-shaped superior volsella, which also present in species of genera Paracladopelma Harnisch, 1923 and Beckidia Kieffer, 1913. The Y-shaped anal tergite bands (Figs 2, 8), the shoulder-like margin of tergite IX (Figs 2, 8), the sclerotized superior volsella (Figs 4, 10) and lobate inferior volsella (Figs 3, 9) show them to belong to the genus Olecryptotendipes (O. Sæther and M. Spies, pers. comm.). However, we have no specimens of larvae and pupae which are important to place the species properly.

The larvae of Olecryptotendipes inhabit sandy substrate in rivers (Zorina 2007). The Chinese specimens were collected from temperate zones and subtropical mountain areas in Palaearctic and Oriental China (Map 1).

Two new species, $O$. exilis sp. n. and $O$. melasmus sp. n., are described and illustrated based on material from China and a key to the males of Olecryptotendipes is provided.

## Material and methods

The terminology follows Sæther (1980) with the additions and corrections given by Sæther (1990). The material examined was mounted on slides in Canada balsam following the procedure outlined by Sæther (1969).

The type material and other material studied are housed in the College of Life Science, Department of Biology, Nankai University, Tianjin, China (BDN).

## Taxonomy

## Amended generic diagnosis

Based on Zorina (2007) and the Chinese specimens, we amend the generic diagnosis. The following combination of characters will separate the genus Olecryptotendipes from other members of the Harnischia complex: Total length 2.7-3.6 mm; AR 1.83-2.24; frontal tubercles absent; anal tergite bands of Y-shaped; posterior margin of tergite IX


Map I. Distribution in China for the genus Olecryptotendipes. A $O$. exilis sp. n. B O. lenzi Zorina C $O$. melasmus sp. n.
with caudolateral shoulders; anal point parallel-sided or widest at about apical $1 / 3$; superior volsella consisting of sclerotized part, dorsal and ventral setae present; inferior volsella with a weak blunt caudal projection, covered with microtrichia; gonostylus parallel-sided or slender to apex, inner margin without expansion.

## Olecryptotendipes exilis sp. n.

urn:lsid:zoobank.org:act:843E24C2-A4C2-4BDF-B681-582F6CBF1692
http://species-id.net/wiki/Olecryptotendipes_exilis
Figs 1-4

Diagnostic characters. The species is separated by the slender posterolateral projection of the superior volsella and the lobate inferior volsella, the posterolateral weak lobes of the anal tergite, and the parallel-sided anal point.

Description. Male imago ( $\mathrm{n}=2$ ). Total length 2.70-2.94 mm. Wing length $1.38-1.50 \mathrm{~mm}$. Total length / wing length 1.96. Wing length / length of profemur 1.92-2.08.

Coloration. Thorax and legs dark brown. Abdomen with tergite I-V yellowish brown and tergite VI-VIII and hypopygium dark brown.

Head. AR 1.97-2.02. Ultimate flagellomere 590-600 mm long. Frontal tubercles absent. Temporal 13-15 setae, including 4 inner verticals, 5-6 outer verticals and 4-5


Figures I-4. Olecryptotendipes exilis sp. n., male. I wing. $\mathbf{2}$ hypopygium (dorsal view) $\mathbf{3}$ hypopygium (ventral view) $\mathbf{4}$ superior volsella.
postorbitals. Clypeus with 13 setae. Tentorium 103-110 mm long, 28-30 mm wide. Palpomere lengths (in mm): 36-38, 38-40, 118-120, 140-145, 203-210. Palp segment $5^{\text {th }} / 3^{\text {rd }} 1.72-1.75$.

Thorax. Antepronotals with 6-7 setae, dorsocentrals 8-10, acrostichals 8, prealars 3. Scutellum with $12-13$ setae.

Wing (Fig. 1). VR $1.19-120$, R with $16-18$ setae, $\mathrm{R}_{1}$ with $8-10$ setae, $\mathrm{R}_{4+5}$ with $10-11$ setae. Brachiolum 2 setae. Squama with $2-3$ setae.

Legs. Front tibia with 3 subapical setae, 113-120, 138-144 and 141-150 $\mu \mathrm{m}$ long, spurs of mid tibia 30-35 and 37-46 $\mu \mathrm{m}$ long excluding comb, comb with 32-36 teeth, $10 \mu \mathrm{~m}$ long; spurs of hind tibia $28-30$ and $38-47 \mu \mathrm{~m}$ long excluding comb, comb with 42-48 teeth, $10-12 \mu \mathrm{~m}$ long. $\mathrm{Ta}_{1}$ of mid legs with only 1 sensilla chaetica, sensilla chaetica absent in hind legs. Lengths (in $\mu \mathrm{m}$ ) and proportions of legs as in Table 1.

Hypopygium (Figs 2-4). Tergite IX with weak lobes bearing 3-4 setae at each side of base of anal point. Laterosternite IX with 3 setae. Anal point $45-50 \mathrm{~mm}$ long, $5-6 \mathrm{~mm}$ wide, originating from caudal margin of anal tergite, completely parallel-side. Anal tergite bands Y-shaped. Phallapodeme $75-82 \mathrm{~mm}$ long. Transverse sternapodeme $56-60 \mathrm{~mm}$ long. Superior volsella (Fig. 4) slightly curved, with apical, partially sclerotized beak-like protrusion and slender spur-like posterolateral projection, bearing two long setae beside the beak-like protrusion, and covered with microtrichia in inner parts. Inferior volsella with a moderately blunt caudal projection, covered with microtrichia, and not reaching beyond margin of anal tergite. Gonocoxite $98-104 \mathrm{~mm}$ long, with 4 strong inner marginal setae. Gonostylus 168-175 mm long, slightly swollen at base, curved medially, moderately slender to apex, bearing 17-20 setae along inner margin. HR 0.58-0.59; HV 1.61-1.68.

Type material. Holotype $\circlearrowleft^{\lambda}$ (BDN No. 1291). CHINA: Hainan Province, Ledong Li Nationality Autonomous County, Jianfengling Nature Conservation area, $18^{\circ} 14^{\prime} 45.96^{\prime \prime N}, 109^{\circ} 30^{\prime} 42.69^{\prime \prime} \mathrm{E}, 21 . i v .1985$, X. Wang. Paratype $1 \sigma^{\Uparrow}$ (BDN No. 03578), same data as holotype.

Etymology. From Latin exilis, slender, in reference to the slender posterolateral projections of superior volsella.

Distribution. The species was collected in a subtropical mountain area in Hainan province in Oriental China.

Table I. Lengths ( $\mu \mathrm{m}$ ) and proportion of legs of Olecryptotendipes exilis sp. n., male ( $\mathrm{n}=2$ ).

|  | $\mathbf{F e}$ | $\mathbf{t i}$ | $\mathbf{t a}_{\mathbf{1}}$ | $\mathbf{t a}_{2}$ | $\mathbf{t a}_{\mathbf{3}}$ | $\mathbf{t a}_{\mathbf{4}}$ | $\mathbf{t a}_{\mathbf{5}}$ | $\mathbf{L R}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{p}_{1}$ | $680-720$ | $500-520$ | $920-1080$ | $490-515$ | $380-410$ | $310-320$ | $150-160$ | $1.84-1.08$ |
| $\mathrm{p}_{2}$ | $630-650$ | $530-550$ | $340-365$ | $190-220$ | $120-135$ | $70-80$ | $60-70$ | $0.64-0.66$ |
| $\mathrm{p}_{3}$ | $700-720$ | $710-725$ | $480-495$ | $250-260$ | $220-230$ | $110-115$ | $90-95$ | 0.68 |

## Olecryptotendipes lenzi (Zorina)

http://species-id.net/wiki/Olecryptotendipes_lenzi
Cryptotendipes lenzi Zorina, 2001: 31; Zorina, 2007: 350; Yan et al. 2005: 4
Specimens examined. China, Xinjiang Autonomous Region: 13 すへ, Kaba Altay, Baihualin Nature Conservation area, $48^{\circ} 03^{\prime} 39.05^{\prime \prime N}, 86^{\circ} 25^{\prime} 7.04^{\prime \prime} \mathrm{E}, 15-16 . v i i .2002$, Tang HQ, sweep net.

Diagnostic characters. Based on Zorina (2001) and Yan et al. (2005), the species is easily distinguished from the other species of the genus by having shoulder-like posterior margin of tergite IX and digitiform superior volsella with a longitudinal keel.

Distribution. This species is distributed in the Russian Far East and northwestern China.

## Olecryptotendipes melasmus sp. n.

urn:lsid:zoobank.org:act:A177BD2D-75F5-43F5-A269-E0FA3D9B22DC http://species-id.net/wiki/Olecryptotendipes_melasmus
Figs 5-10

Diagnosis. The species can be separated by the blackish brown spots on thorax and legs, the distally swollen anal point and the gonostylus with basal weak expansion.

Description. Male imago ( $\mathrm{n}=2$ ). Total length 3.13-3.60 mm. Wing length $1.50-1.88$ mm . Total length / wing length 1.91-2.09. Wing length / length of profemur 2.05-2.14.

Coloration. Thorax (Fig. 5) yellowish brown, with median black brown vittae. Femur of front leg yellowish green with distal parts dark brown, tibia dark brown except for median parts yellowish green, tarsi dark brown with basal $3 / 4$ of ta ${ }_{1}$ yellowish green; femora and tibia of mid and hind legs yellowish green with distal parts of femora and basal parts of tibia dark brown, $\mathrm{ta}_{1}$ to $\mathrm{ta}_{5}$ lightly brown (Fig. 6). Abdomen yellowish green to brown, with tergite I-V yellowish green, tergite VI-VIII and hypopygium yellowish brown.

Head. AR 1.94-2.24. Ultimate flagellomere 620-760 mm long. Frontal tubercles absent. Temporal 13-15 setae, including 4-5 inner verticals, 4-6 outer verticals and $4-5$ postorbitals. Clypeus with 13-21 setae. Tentorium 113-120 mm long, 27-37 mm wide. Palpomere lengths (in mm): 37-40, 39-45, 133-172, 133-158, 193-223. Palp segment $5^{\text {th }} / 3^{\text {rd }} 1.30-1.45$.

Thorax. Antepronotum with 2 setae, dorsocentrals 8-10, acrostichals 4-7, prealars $4-5$. Scutellum with $12-15$ setae.

Wing (Fig. 7). VR $1.15-1.20$, R with $12-16$ setae, $\mathrm{R}_{1}$ with $11-16$ setae, $\mathrm{R}_{4+5}$ with 18-25 setae. Brachiolum 2 setae. Squama with 3-4 setae.

Legs. Front tibia with 3 subapical setae, 113-135, 133-150 and $145-170 \mu \mathrm{~m}$ long, spurs of middle tibia $28-30$ and $35-37 \mu \mathrm{~m}$ long excluding comb, comb with 22-34 teeth, $10-11 \mu \mathrm{~m}$ long; spurs of hind tibia 30-32 and 42-43 $\mu \mathrm{m}$ long excluding comb, comb with 34-45 teeth, 10-12 $\mu \mathrm{m}$ long. Sensilla chaetica of mid and hind legs absent. Lengths (in $\mu \mathrm{m}$ ) and proportions of legs as in Table 2.

Table 2. Lengths ( $\mu \mathrm{m}$ ) and proportion of legs of Olecryptotendipes melasmus sp. n., male ( $\mathrm{n}=2$ ).

|  | $\mathbf{F e}$ | $\mathbf{T i}$ | $\mathbf{t a}_{\mathbf{1}}$ | $\mathbf{t a}_{\mathbf{2}}$ | $\mathbf{t a}_{\mathbf{3}}$ | $\mathbf{t a}_{4}$ | $\mathbf{T a}_{\mathbf{5}}$ | $\mathbf{L R}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{P}_{1}$ | $730-880$ | $500-650$ | $1100(1)$ | $580(1)$ | $430(1)$ | $350(1)$ | $150(1)$ | $1.69(1)$ |
| $\mathrm{P}_{2}$ | $640-800$ | $530-700$ | $330-410$ | $180-220$ | $120-150$ | $70-85$ | 60 | $0.59-0.62$ |
| $\mathrm{P}_{3}$ | $760-910$ | $720-940$ | $470-580$ | $280-340$ | $220-270$ | $120-140$ | $70-80$ | $0.62-0.65$ |



Figures 5-10. Olecryptotendipes melasmus sp. n., male. $\mathbf{5}$ thorax $\mathbf{6}$ leg $\mathbf{7}$ wing. $\mathbf{8}$ hypopygium (dorsal view) $\mathbf{9}$ hypopygium (ventral view) 10 superior volsella.

Hypopygium (Figs 8-10). Tergite IX with weak shoulder-like corners, bearing 1320 setae at base of anal point. Laterosternite IX with $4-7$ setae. Anal point originating from anterior of caudal margin of anal tergite in dorsal view, constricted basally, swollen apically, $43-50 \mathrm{~mm}$ long, $8-10 \mathrm{~mm}$ wide at base, $9-12 \mathrm{~mm}$ wide at apex. Anal tergite bands Y-shaped. Phallapodeme $68-80 \mathrm{~mm}$ long. Transverse sternapodeme $44-70 \mathrm{~mm}$ long. Superior volsella (Fig. 10) curved basally, straight distally, with large posterolateral projection, which is constricted medially forming a sharp angle, longitudinal membranous ridge present, bearing two long setae in distinct pits; covered with microtrichia in inner parts of superior volsella. Inferior volsella with reduced lobate caudal projection. Gonocoxite $85-100 \mathrm{~mm}$ long, with 4 strong inner marginal setae. Gonostylus $150-190 \mathrm{~mm}$ long, slightly swollen at base, concave medially, with rounded apex, bearing $8-20$ setae along basal inner margin, and $10-14$ setae along distal inner margin. HR 0.53-0.57; HV 1.89-2.09.

Type material. Holotype $\widehat{\delta}$ (BDN No. 04250). CHINA: Shaanxi Province, Baoji City, Feng County, Qinling, Dongyu, $33^{\circ} 54^{\prime} 42.03^{\prime \prime N}, 106^{\circ} 31^{\prime} 21.10^{\prime \prime} \mathrm{E}, 30$. vii. 1994, sweep net, W. Bu. Paratype (BDN No. 20598). CHINA: $1 \delta^{\lambda}$, Fujian Province, Jianning County, $26^{\circ} 49^{\prime} 51.25^{\prime \prime} \mathrm{N}, 116^{\circ} 50^{\prime} 45.90^{\prime \prime} \mathrm{E}, 25 . \mathrm{ix} .2002$, light trap, Z. Liu.

Etymology. From Greek, melasma, spot, in reference to the dark brown spots on the thorax and legs.

Distribution. The species is known from Palaearctic and Oriental China (Shaanxi Province; Fujian Province).

Systematic remarks. Male diagnosis: Based on the variation in Chinese material (O. exilis sp. n., O. melasmus sp. n.), the generic description given by Zorina (2007) should be emended as follows: "Antennal ratio 1.83-2.06" should be changed to "Antennal ratio $1.83-2.24$ ". "Total length $3.0-3.5 \mathrm{~mm}$ " should be emended to "Total length 2.7-3.6 mm."

Based on the description and figures of Zorina (2003), posterior margin of tergite IX of $O$. secundus not elongate but with caudolateral shoulders bearing 4 setae, which seem to $O$. exilis sp. n. The gonostylus of $O$. lenzi and $O$. secundus nearly parallelsided, but which is slender to apex of the two new species. The species $O$. secundus only with sclerotized superior volsella and without membranous ridges, it seems to $O$. exilis sp. n. with sclerotized beak-like protrusion. From the above, the generic characters of Olecryptotendipes should be emended as follows: "Posterior margin of tergite IX with caudolateral shoulders; gonostylus parallel-sided or slender to apex; superior volsella consisting of sclerotized part, dorsal and ventral setae present."

## Key to males of the genus Olecryptotendipes in the world

1 Acrostichals absent; R and $\mathrm{R}_{1}$ without setae; superior volsella lacking microtrichia ventrally

- Acrostichals present; R and $\mathrm{R}_{1}$ with setae; superior volsella with microtrichia ventrally

2 Anal point swollen distally; thorax with dark brown spots ... O. melasmus sp. n.

- Anal point parallel-sided, thorax without dark brown spots ......................... 3

3 Inferior volsella absent, gonostylus parallel-sided .............. O. lenzi (Zorina)

- Inferior volsella lobe-like, gonostylus swollen at base, moderately slender to apex O. exilis sp. n.


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# Two new species of Bryophaenocladius Thienemann, 1934 (Diptera, Chironomidae) from China 

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#### Abstract

Two new species of Bryophaenocladius Thienemann, 1934, B. mucronatus sp. n. and B. parictericus sp. n. are described and illustrated as males. A key to male imagines of the genus from China is presented.


## Keywords

Chironomidae, Bryophaenocladius, new species, key, China

## Introduction

The genus Bryophaenocladius was erected by Thienemann in 1934 with Orthocladius muscicola Kieffer, 1906 as type species. To date, more than 100 species have been recorded all over the world (Andersen and Schnell 2000, Ashe and Cranston 1990, Chaudhuri et al. 2001, Du and Wang 2010, Du et al. 2011, Freeman and Cranston 1980, Liu and Wang 2005, Makarchenko and Makarchenko 2006, 2009, 2011, Sæther 1973, Sæther et al. 2000, Sasa and Kikuchi 1995, Spies and Reiss 1996, Strenzke 1957, Wang 2000, Wang et al. 2001, 2004, 2006, Yamamoto 2004). So far 7 species of the genus were recorded in China, namely B. cuneiformis Armitage, 1987, B. parimberbus Du and Wang, 2010,
B. propinquus (Brundin, 1947), B. scanicus (Brundin, 1947), B. vernalis (Goetghebuer, 1921), B. wufengensis Du and Wang, 2010, and B. xinglongensis Du and Wang, 2010.

The adult males of most Bryophaenocladius species can be recognized by strong and decumbent acrostichals beginning close to antepronotum; wing membrane without setae, but with coarse punctation visible at 40 x magnification, squama with one to several setae; tibial spurs strongly developed, with well developed, but not divergent lateral denticles; hind tibial comb well developed; sensilla chaetica absent; tergite IX distinctive, with strongly pigmented, semi-circular band running around posterior margin; anal point projecting from setose area, large, semicircular to triangular; virga consisting of simple spines; gonostylus often distinctly broadened, strong megaseta (Cranston et al. 1989). However, there are exceptions to nearly all of these diagnostic characters. $B$. psilacrus Sæther is lacking acrostichals (Sæther 1982). Several species with bare squama (Andersen and Schnell 2000). The tibial spurs may be essentially without lateral denticles as in most Afrotropical species (Wang et al. 2001) and thus differ from the typical condition with lateral denticles separated but not as much as in Chaetocladius Kieffer. Tergite IX and the anal point may deviate from the typical form and it is the association of those species which are most in doubt such as B. productus (Freeman, 1953) (Sæther 1973).

After examinzing the type specimen of B. bicolor Wang, Sæther \& Andersen, 2001 and the specimens of $B$. ictericus (Meigen, 1830) collected from Canada, China and Sweden, two new species from oriental China are described. A key to male imagines of Bryophaenocladius from China and a distribution map of genus Bryophaenocladius in China is presented (Fig. 1).


Figure I. Distribution in China for the genus Bryophaenocladius A B. cuneiformis Armitage, 1987 B B. mucronatus sp. n. C B. parictericus sp. n. D B. parimberbus Du \& Wang, 2010 E B. propinquus (Brundin, 1947) F B. scanicus (Brundin, 1947). G B. vernalis (Goetghebuer, 1921) H B. wufengensis Du \& Wang, 2010 I B. xinglongensis Du \& Wang, 2010.

## Materials and methods

The morphological nomenclature follows Sæther (1980) and the abbreviations of parts measured follow Qi et al. (2012). The material examined was mounted on slides, following the procedure outlined by Sæther (1969). Measurements are given as ranges followed by the mean, when three or more specimens are measured, followed by the number of specimens measured ( n ) in parentheses. Examined specimens in this study are deposited in the College of Life Science, Nankai University, China and College of Life Science, Taizhou University, China.

## Key to male imagines of Bryophaenocladius from China

1 Third palpomere with apical projection ..... 2

- Third palpomere without apical projection ..... 4
2 Squama with setae; AR>1.0 ..... 3
- $\quad$ Squama bare; $\mathrm{AR}<1.0$ B. parictericus sp. n.
3 Inferior volsella unobvious B. xinglongensis Du \& Wang, 2010
- Inferior volsella obvious B. cuneiformis Armitage, 1987
4 Squama bare ..... 5
Squama setose ..... 6
5 Crista dorsalis absent; inferior volsella obvious
B. vernalis (Goetghebuer, 1921)
Crista dorsalis present; inferior volsella unobvious
B. parimberbus Du \& Wang, 2010
6 Anal point broad. ..... 7
- Anal point slender ..... 8
7 Inferior volsella finger-shaped B. propinquus (Brundin, 1947)
Inferior volsella almost rectangular B. scanicus (Brundin, 1947)
8 Pseudospurs present on $\mathrm{ta}_{1}, \mathrm{ta}_{2}$ of mid and hind legs ... B. mucronatus sp. n.- Pseudospurs absentB. wufengensis Du \& Wang, 2010


## Taxonomy

## Bryophaenocladius mucronatus sp. n.

urn:lsid:zoobank.org:act:09174531-D113-4061-B288-6627445DFCAF
http://species-id.net/wiki/Bryophaenocladius_mucronatus
Figures 2-4

Diagnosis. The male imago can be distinguished from known species of the genus by the following combination of characters: third palpomere without apical digitiform projection; squama with $1-7,4$ setae; pseudospurs present on $\operatorname{ta}_{1}$ and $t a_{2}$ of mid and


Figures 2-4. B. mucronatus sp. n. $\mathbf{2}$ wing $\mathbf{3}$ hypopygium (dorsal view) $\mathbf{4}$ hypopygium (ventral view).
hind legs; anal point hyaline, slender with pointed apex; tergite IX columnar; inferior volsella thumb-shaped, with $0-5,3$ setae.

Description. Male imago ( $\mathrm{n}=29$ ). Total length $2.20-3.00,2.51 \mathrm{~mm}$. Wing length 1.33-1.76, 1.55 mm . Total length/wing length $1.43-1.90,1.65$. Wing length/ length of profemur 2.50-3.34, 2.75.

Coloration. Dark brown.
Head. AR 1.13-1.43, 1.26. Ultimate flagellomere 415-455, $430 \mu \mathrm{~m}$ long. Temporal setae $7-11,9$ including 2-4, 3 inner verticals; 4-6, 5 outer verticals and $1-2,2$ postorbitals. Clypeus with 2-5, 3 setae. Tentorium 105-150, $130 \mu \mathrm{~m}$ long, 18-25, 20 $\mu \mathrm{m}$ wide. Stipes $105-110,108 \mu \mathrm{~m}$ long, $7-10,8 \mu \mathrm{~m}$ wide. Palpomere lengths (in $\mu \mathrm{m}$ ): $20-50,35 ; 30-95,47 ; 55-110,80 ; 60-100,80 ; 100-125,113$. L: $5^{\text {th }} / 3^{\text {rd }} 1.40-1.82$, 1.56. Third palpomere without apical digitiform projection.

Wing (Fig. 2). Anal lobe developed. Coarse punctation easily visible at 40x magnification. VR 1.16-1.33, 1.26. Costa extension 40-63, $48 \mu \mathrm{~m}$ long. Brachiolum with $1-3,2$ setae. R with 3-6, 4 setae; $\mathrm{R}_{4+5}$ with $0-1,0$ seta. Remaining veins bare. Squama with 1-7, 4 setae.

Thorax. Antepronotum with 3-8, 4 lateral setae. Dorsocentrals 5-13, 9; acrostichals 3-10, 7; prealars 2-5, 3. Scutellum with 2-8, 4 setae.

Legs. Spur of fore tibia 16-65, $45 \mu \mathrm{~m}$ long; spurs of mid tibia $20-40,33 \mu \mathrm{~m}$ and 12-27, $20 \mu \mathrm{~m}$ long; spurs of hind tibia 42-58, $50 \mu \mathrm{~m}$ and $11-40,23 \mu \mathrm{~m}$ long. Lateral denticles appressed to main shaft. Hind tibial comb with 6-16, 13 spines. Pseudospurs
present on $\mathrm{ta}_{1}$ and $\mathrm{ta}_{2}$ of mid and hind legs, $18-23,20 \mu \mathrm{~m}$ long. Width at apex of fore tibia 23-38, 30 mm , of mid tibia 25-35, 27 mm , of hind tibia $30-40,35 \mathrm{~mm}$. Lengths (in $\mu \mathrm{m}$ ) and proportions of legs as in Table 1.

Table I. Lengths (in $\mu \mathrm{m}$ ) and proportions of legs of B. mucronatus sp. n .

|  | $\mathbf{P}_{1}$ | $\mathbf{P}_{2}$ | $\mathbf{P}_{3}$ |
| :---: | :---: | :---: | :---: |
| fe | $500-594,553$ | $588-650,622$ | $580-690,643$ |
| ti | $620-783,723$ | $570-704,661$ | $648-810,765$ |
| $\mathrm{ta}_{1}$ | $370-450,415$ | $240-324,288$ | $300-450,415$ |
| $\mathrm{ta}_{2}$ | $220-270,240$ | $140-190,170$ | $160-250,220$ |
| $\mathrm{ta}_{3}$ | $160-200,180$ | $105-135,123$ | $135-200,173$ |
| $\mathrm{ta}_{4}$ | $105-130,118$ | $60-90,75$ | $80-110,95$ |
| $\mathrm{ta}_{5}$ | $80-100,86$ | $60-95,80$ | $75-100,88$ |
| LR | $0.52-0.63,0.57$ | $0.42-0.48,0.45$ | $0.46-0.59,0.55$ |
| BV | $2.59-2.62,2.61$ | $3.37-3.57,3.47$ | $3.13-3.28,3.19$ |
| SV | $2.96-3.10,3.03$ | $4.32-4.58,4.47$ | $3.16-3.51,3.31$ |
| BR | $2.17-2.86,2.41$ | $2.22-3.00,2.47$ | $3.33-4.35,3.81$ |

Hypopygium (Figs 3-4). Anal point hyaline, slender, with pointed apex, 45-90, $70 \mu \mathrm{~m}$ long, 25-35, $30 \mu \mathrm{~m}$ wide. Anal point length/width: 2.14-2.71, 2.45. Tergite IX columnar, with 10-22, 15 setae, laterosernite IX with 4-8, 6 setae. Phallapodeme $45-85,70 \mu \mathrm{~m}$ long. Transverse sternapodeme arcuate with developed oral projection, $68-100,88 \mu \mathrm{~m}$ long. Gonocoxite $175-212,190 \mu \mathrm{~m}$ long. Gonostylus 68-100, 87 $\mu \mathrm{m}$ long with $1-2,1$ megaseta, $8-13,10 \mu \mathrm{~m}$ long. Crista dorsalis low. Inferior volsella thumb-shaped, 23-35, $27 \mu \mathrm{~m}$ long, with $0-5$, 3 setae. Virga 10-25, $16 \mu \mathrm{~m}$ long, composed of $1-9,5$ spines. HR 1.95-2.36, 2.12. HV 2.59-3.00, 2.71.

Type materials. Holotype: $\widehat{o}^{\star}$ (BDN. I4B20), China, Zhejiang Province: Quzhou City, Kaihua County, Gutian Mountain, $29^{\circ} 14^{\prime} 35^{\prime \prime} \mathrm{N}, 118^{\circ} 06^{\prime} 41^{\prime \prime} \mathrm{E}, 18 . i v .2011$,
 ince, Lishui City, Qinyuan County, $27^{\circ} 45^{\prime} 08^{\prime \prime} \mathrm{N}, 119^{\circ} 12^{\prime} 26^{\prime \prime} \mathrm{E}, 15 . \mathrm{iv} .1994, \mathrm{Wu}$ H, sweeping net; Fujian Province: $11 \delta^{\top} \delta^{\lambda}$, Wuyi Mountain, $27^{\circ} 38^{\prime} 22^{\prime \prime} \mathrm{N}, 117^{\circ} 56^{\prime} 56^{\prime \prime} \mathrm{E}$, 26.iv.1993, Wang XH, sweeping net; Sichuan Province: $7 \circlearrowleft^{\top} 0^{\lambda}$, Wenchuan County,
 Nature Reserve, $30^{\circ} 45^{\prime} 23^{\prime \prime} \mathrm{N}, 103^{\circ} 13^{\prime} 55^{\prime \prime} \mathrm{E}, 27$. vii. 1987 , Li XZ, sweeping net.

Etymology. The species name is from Latin mucronatus, pointed, referring to the shape of apex of anal point.

Remarks. The present new species resembles to B. bicolor Wang, Sæther \& Andersen, 2001 in the shape of anal point, but it can be separated from B. bicolor in the following combination of characters in Table 2.

Female and immature stages unknown.
Distribution. The species was found in Fujian, Sichuan and Zhejiang Provinces (Oriental China).

Table 2. Differences between B. mucronatus sp.n. and B. bicolor Wang, Sæther \& Andersen, 2001.

|  | B. mucronatus sp. n. |  <br> Andersen, 2001 |
| :--- | :--- | :--- |
| Finger-shaped extension <br> on third palpomere | absent | present |
| Seta on $\mathrm{R}_{1}$ | bare | $4-5$ setae |
| $\mathrm{LR}_{1}$ | $0.52-0.63,0.57$ | $0.76-0.82,0.80$ |
| Pseudospurs $_{\text {Crista dorsalis }}$ | present on $\mathrm{ta}_{1}$, ta of mid and hind legs | absent |
|  | present | reduced |

## Bryophaenocladius parictericus sp. n.

urn:lsid:zoobank.org:act:125AC1CD-0CD6-46B4-AACF-D43C1084EFA6
http://species-id.net/wiki/Bryophaenocladius_parictericus
Figs 5-9

Diagnosis. The male imago can be distinguished from known species of the genus by the following combination of characters: AR $0.52-0.55$; third palpomere with apical digitiform projection; Costa extension 115-143, $122 \mu \mathrm{~m}$ long; squama bare; mid tibia comb with 3-7, 5 spines; anal point hyaline, slender with blunt apex; crista dorsalis absent; inferior volsella bubble-shaped, with 8-12, 9 setae.

Description. Male imago $(\mathrm{n}=6)$. Total length $2.65-3.082 .76 \mathrm{~mm}$. Wing length 1.63-2.48, 2.22 mm . Total length/wing length $1.10-1.46,1.26$. Wing length/length of profemur 2.78-3.19, 3.03.

Coloration. Dark brown.
Head (Fig. 5). AR 0.52-0.55 ( $\mathrm{n}=2$ ). Ultimate flagellomere 230-245 ( $\mathrm{n}=2$ ) $\mu \mathrm{m}$ long. Temporal setae 3-9, 7 including 2-7, 4 inner verticals; $0-4$, 2 outer verticals and $0-2,1$ postorbital. Clypeus with 4-7, 5 setae. Tentorium 109-148, $129 \mu \mathrm{~m}$ long, $15-$ $25,20 \mu \mathrm{~m}$ wide. Stipes $80-100,90 \mu \mathrm{~m}$ long, $5-8,6 \mu \mathrm{~m}$ wide. Palpomere lengths (in $\mu \mathrm{m}): 16-25,20 ; 35-52,41 ; 90-143,114 ; 42-65,57 ; 60-80,71$. L: $5^{\text {th }} / 3^{\text {rd }} 0.76-0.80$, 0.78 . Third palpomere with apical digitiform projection.

Wing (Fig. 6). Anal lobe not developed. Coarse punctation easily visible at 40x magnification. VR 1.02-1.23, 1.17. Costa extension 115-143, $122 \mu \mathrm{~m}$ long. Brachiolum with 1 seate. R with 5-9, 7 setae. Remaining veins bare. Squama bare.

Thorax. Antepronotum with 2-5, 3 lateral setae. Dorsocentrals 8-10, 9; acrostichals 6-7, 7; prealars 2-4, 3. Scutellum with 3-7, 6 setae.

Legs (Fig. 7). Spur of fore tibia 40-58, $48 \mu \mathrm{~m}$ long; spurs of mid tibia 30-42, 38 $\mu \mathrm{m}$ and $21-32,25 \mu \mathrm{~m}$ long; spurs of hind tibia $40-63,52 \mu \mathrm{~m}$ and $21-32,28 \mu \mathrm{~m}$ long. Lateral denticles appressed to main shaft. Mid tibial comb with 3-7, 5 spines; hind tibial comb with $9-14,12$ spines. Mid and hind legs without tarsal pseudospurs. Width at apex of fore tibia 35-45, 40 mm , of mid tibia 33-38, 36 mm , of hind tibia 40-48, 45 mm . Lengths (in $\mu \mathrm{m}$ ) and proportions of legs in Table 3.

Hypopygium (Figs 8-9). Anal hyaline, slender with blunt apex, 40-55, $48 \mu \mathrm{~m}$ long, $15-20,18 \mu \mathrm{~m}$ in width. Anal point length/width: 2.22-2.75, 2.51. Tergite IX


Figures 5-9. B. parictericus sp. n. $\mathbf{5}$ third palpomere $\mathbf{6}$ wing $\mathbf{7}$ mid tibia $\mathbf{8}$ hypopygium (dorsal view) $\mathbf{9}$ hypopygium (ventral view).

Table 3. Lengths (in $\mu \mathrm{m}$ ) and proportions of legs of Bryophaenocladius parictericus sp. n.

|  | $\mathbf{P}_{1}$ | $\mathbf{P}_{\mathbf{2}}$ | $\mathbf{P}_{\mathbf{3}}$ |
| :---: | :---: | :---: | :---: |
| fe | $510-893,718$ | $600-914,798$ | $620-977,735$ |
| ti | $710-1134,916$ | $660-987,873$ | $770-1260,994$ |
| $\mathrm{ta}_{1}$ | $360-670,558$ | $320-504,427$ | $400-683,611$ |
| $\mathrm{ta}_{2}$ | $240-389,322$ | $170-263,232$ | $220-315,282$ |
| $\mathrm{ta}_{3}$ | $180-273,235$ | $140-189,176$ | $180-284,233$ |
| $\mathrm{ta}_{4}$ | $100-147,132$ | $70-105,98$ | $80-126,115$ |
| $\mathrm{ta}_{5}$ | $70-108,96$ | $70-95,87$ | $70-105,95$ |
| LR | $0.51-0.64,0.58$ | $0.45-0.51,0.48$ | $0.52-0.58,0.54$ |
| BV | $2.68-2.92,2.84$ | $3.22-3.68-3.47$ | $3.25-3.35,3.30$ |
| SV | $3.24-3.39,3.31$ | $3.94-4.11,4.01$ | $3.31-3.48,3.39$ |
| BR | $2.14-2.67,2.33$ | $2.00-2.14,2.09$ | $1.50-2.11,2.01$ |

with 6-13, 9 setae, laterosernite IX with 3-5, 4 setae. Phallapodeme 48-91, $77 \mu \mathrm{~m}$ long. Oral projection of transverse sternapodeme vestigial, 75-96, $85 \mu \mathrm{~m}$ long. Gonocoxite 170-221, $194 \mu \mathrm{~m}$ long. Gonostylus slightly curved, 80-101, $92 \mu \mathrm{~m}$ long. Megaseta 13-21, $18 \mu \mathrm{~m}$ long. Crista dorsalis absent. Inferior volsella bubble-shaped, 18-27, 22 $\mu \mathrm{m}$ long, with 8-12, 9 setae. Virga absent. HR 1.88-2.50, 2.08. HV 2.62-3.48, 3.02.

Type materials. Holotype: ${ }^{\top}$ (BDN. K7A22), China, Zhejiang Province: Taizhou City, Xianju County, Shenxianju Scenic Area, $28^{\circ} 42^{\prime} 14^{\prime \prime} \mathrm{N}, 120^{\circ} 36^{\prime} 25^{\prime \prime} \mathrm{E}, 14.1 \mathrm{iv} .2011$,


Yajiang County, $30^{\circ} 01^{\prime} 52^{\prime \prime} \mathrm{N}, 101^{\circ} 00^{\prime} 52^{\prime \prime} \mathrm{E}, 10 . v i .1996,3050$ meters above sea level, Wang XH, sweeping net.

Etymology. Named in closing to the species B. ictericus (Meigen, 1830).
Remarks. The present new species resembles to B. ictericus (Meigen, 1830) in the shape of inferior volsella, but it can be separated by following combination of characters in Table 4.

Female and immature stages unknown.
Distribution. The species was found in Sichuan and Zhejiang Provinces (Oriental China).

Table 4. Differences between B. parictericus sp. n. and B. ictericus (Meigen, 1830)

|  | B. parictericus sp. n. | B. ictericus (Meigen, 1830) |
| :--- | :--- | :--- |
| Antennal ratio (AR) | $0.52-0.55$ | $1.19-1.73,1.56$ |
| Finger-shaped extension on third palpomere | present | absent |
| Length of Costal extension | $115-143,122 \mu \mathrm{~m}$ | $64-105,98 \mu \mathrm{~m}$ |
| Length of megaseta | $13-21,18 \mu \mathrm{~m}$ | $7-14,11 \mu \mathrm{~m}$ |
| Gonostylus | bended | straight |
| Virga | absent | present |

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# Mariapanteles (Hymenoptera, Braconidae), a new genus of Neotropical microgastrine parasitoid wasp discovered through biodiversity inventory 

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#### Abstract

A new genus of microgastrine parasitoid wasps, Mariapanteles Whitfield \& Fernández-Triana, gen. n., is described from rain forests of the Neotropics. The new genus is related to the common and speciose genus Pseudapanteles, but can be distinguished from the latter by having a complete transverse carina on the propodeum which forks around the spiracles. A molecular analysis based on data from COI from specimens of the proposed new genus plus possibly related genera confirms its generic distinctness. A key to two known species, M. felipei Whitfield, sp. n. (Costa Rica) and M. dapkeyae Fernández-Triana, sp. n. (Brazil) is provided. Evidence from collections suggests that there are other undescribed Neotropical congenerics. Specimens of Mariapanteles were likely confused in the past with the genus Beyarslania (referred to as Xenogaster until recently) but present information suggests that Beyarslania is restricted to the Afrotropical region while the Neotropical species clearly belong to a different genus, which we propose as new.


## Keywords

Microgastrinae, new genus, Area de Conservación Guanacaste, Costa Rica, Brazil, Neotropics, parasitoid wasp, rain forest

## Introduction

In the past few decades, biodiversity inventories in the neotropics have begun to incorporate intensive rearing programs for caterpillars, their food plants, and their parasitoids. The most extensive of these programs is in the Area de Conservación de Guanacaste (ACG) in northwestern Costa Rica. ACG contains 130,000 terrestrial hectares of contiguous conserved dry forest, cloud forest, and rain forest, extending from the Pacific Ocean to $2,000 \mathrm{~m}$ elevation and then down into the Caribbean lowlands (Janzen 2000, Janzen and Hallwachs 2011). The caterpillar/plant/parasitoid inventory is one of the main components of ACG conservation (e.g. Janzen and Hallwachs 2011, 2012; Janzen et al. 2009; Smith et al. 2006, 2007, 2008; Sharkey et al. 2011; Burns et al. 2007, 2008; Schauff et al. 2001; Gauld and Janzen 2004). Microgastrinae have been one of the most intensively studied groups of ACG parasitoids (e.g., Valerio et al. 2009; Smith et al. 2008; Grinter et al. 2009; Deans et al. 2003, Whitfield et al. 2011), but many hundreds of species of these tiny wasps still remain to be discovered and described. Microgastrines are probably the single most speciose higher taxon of parasitoids of Lepidoptera (Whitfield 1995, 1997), and it is estimated that 800+ species occur in ACG alone (Janzen and Hallwachs, inventory in progress).

The ACG inventory has encountered a rain forest species of Microgastrinae that superficially resembles the rare Old World genus Beyarslania - widely known as Xenogaster until a recent nomenclatural change (Kocak and Kemal 2009). This genus is only known from a single described species from South Africa, but undescribed species have been thought to exist in the Neotropics (Whitfield 1997; Campos and Diego 2001). As we studied the ACG specimens and other similar Neotropical specimens, we realized that these undescribed species are more informatively placed in a new genus, which we erect and describe here along with descriptions of two species contained within it. A molecular phylogenetic analysis based on data from the COI ("barcoding") gene is included to provide a further test of the monophyly of this putative new genus.

## Methods

The specimens for this study came from two main sources: the ACG inventory (Janzen and Hallwachs 2012, Janzen et al. 2009) and unsorted Neotropical wasps from the Canadian National Collection of Insects (CNC) in Ottawa. The genotype for the new genus has been deposited in the Smithsonian Institution (NMNH) and the remaining specimens have been deposited in the CNC, Illinois Natural History Survey (INHS) and the Natural History Museum, London (BMNH).

The morphological terms and morphological measurements follow mostly Wharton and Sharkey (1997) and Valerio and Whitfield (2009).

Photos were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a range of $13-130 \times$. Plates for the illustrations were prepared using Adobe Photoshop, but images were not digitally enhanced.

DNA barcodes for these and all other ACG inventory Microgastrinae were obtained using DNA extracts prepared from single legs using a glass fibre protocol (Ivanova et al. 2006). Extracts were re-suspended in $30 \mu \mathrm{l}$ of dH 2 O , and a 658-bp region near the 5' terminus of the COI gene was amplified using standard primers (LepF1-LepR1) following established protocols (Smith et al. 2006, 2007, 2008). If the initial 658 bp amplification was not successful composite sequences were generated using internal primers. Primer information for individuals sequences can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) using the accessions detailed in the online supplementary table, but primers are as detailed in Smith et al. (2008). Full details of methodology are as in (Smith et al. 2006, 2007, 2008). All sequence data are available on BOLD (www.barcodinglife. org) in the public Dataset, "Mariapanteles (Hymenoptera: Braconidae), a new genus of Neotropical microgastrine parasitoid wasp discovered through biodiversity inventory". All collection information, BOLD, and GenBank accessions for all sequences are listed in the Table in online supplementary materials.

All available DNA barcodes for Pseudapanteles (32 New World species, most of which are undescribed) and Mariapanteles ( 2 species) that were at least 300 bp long were downloaded from BOLD (see the online supplementary table). DNA barcodes for selected species of Prasmodon, Diolcogaster, Microplitis, Cotesia, Apanteles, and Neoclarkinella were also downloaded to be used as outgroups in the phylogenetic analyses. Genbank accession numbers of all sequences used are given in Table 1. Prior to analysis, identical Pseudapanteles sequences were removed from the dataset so that each unique sequence was only represented once. Sequences were aligned in Geneious Pro 5.5.6 (Drummond et al. 2011) using default settings for MUSCLE alignment. The first character was deleted from the aligned matrix because most sequences were missing this character. Bayesian phylogenetic analysis was performed in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) through the CIPRES Science Gateway V.3.1 (Miller et al. 2010). Model selection was based on Bayesian Information Criteria as implemented in JModelTest v.0.1.1 (Posada 2008). Two independent analyses with 4 chains each were run in parallel for 10 million generations under a GTR $+\mathrm{I}+\mathrm{G}$ model. The parameter trace files of each run were observed in Tracer v.1.5 (Rambaut and Drummond 2009) to verify that the runs had converged on the same stationary distribution, and to decide on the appropriate number of generations to discard as burn-in. A maximum clade credibility tree was constructed from these 18 million post-burn-in generations in TreeAnnotator v1.7.0 (Rambaut and Drummond 2012). The above protocol was followed for two additional analyses in which all $3^{\text {rd }}$ codon positions were removed from the dataset to correct for potential problems stemming from saturation in $3^{\text {rd }}$ codon positions of COI.

Table I. Specimens included in the COI molecular analyses and their GenBank Accession numbers. Sample IDs are DHJPAR numbers (assigned to ACG specimens submitted to BOLD) or other IDs submitted to BOLD. More complete data on all specimens are included in Supplementary File 1.

| Taxon | Sample ID | Genbank Accession Number |
| :---: | :---: | :---: |
| Apanteles Rodriguez03 | DHJPAR0012802 | JQ847281 |
| Apanteles Rodriguez05 | DHJPAR0012285 | EU396474 |
| Apanteles Rodriguez169 | DHJPAR0038032 | JQ848826 |
| Cotesia Whitfield03 | DHJPAR0013374 | JQ848576 |
| Diolcogaster Choi04 | DHJPAR0004153 | HQ549146 |
| Mariapanteles dapkeyae F.-T. | CNCHYM 03387 | JQ849377 |
| Mariapanteles felipei Whitfield | DHJPAR0025453 | HQ549955 |
| Mariapanteles felipei Whitfield | DHJPAR0025443 | JN282317 |
| Microplitis Whitfield19 | DHJPAR0031685 | HQ548870 |
| Neoclarkinella sp.jft 10 | WAM 0011 | JQ852287 |
| Neoclarkinella sp.jft 1 | GOU 0608 | JQ849914 |
| Prasmodon Whitfield02 | DHJPAR0038222 | HQ548705 |
| Prasmodon Whitfield05 | DHJPAR0012956 | HQ548880 |
| Pseudapanteles gouleti F.-T. | CAM 0874 | JQ848150 |
| Pseudapanteles sp. jft8 | CNCHYM 03312 | JQ849707 |
| Pseudapanteles sp. jft16 | CNCHYM 03343 | JQ575645 |
| Pseudapanteles sp. jft19 | CNCHYM 03355 | JQ850586 |
| Pseudapanteles sp. jft23 | CNCHYM 03372 | JQ850275 |
| Pseudapanteles sp. jft23 | CNCHYM 03372 | JQ850275 |
| Pseudapanteles sp. jft23 | CNCHYM 03369 | JQ854346 |
| Pseudapanteles sp. jft25 | CNCHYM 03377 | JQ853286 |
| Pseudapanteles sp. jft25 | CNCHYM 03377 | JQ853286 |
| Pseudapanteles sp.jft29 | 10BBHYM-1279 | JQ852886 |
| Pseudapanteles sp.jft29 | Micro0094 | JQ852261 |
| Pseudapanteles sp.jft29 | Micro0269 | JQ850735 |
| Pseudapanteles Whitfield01 | DHJPAR0004755 | JQ849938 |
| Pseudapanteles Whitfield02 | DHJPAR0025345 | JQ850204 |
| Pseudapanteles Whitfield05 | DHJPAR0031341 | JQ852695 |
| Pseudapanteles Whitfield05 | DHJPAR0026205 | HQ549736 |
| Pseudapanteles Whitfield05 | DHJPAR0033906 | JQ576585 |
| Pseudapanteles Whitfield06 | DHJPAR0031347 | HQ930249 |
| Pseudapanteles Whitfield06 | DHJPAR0031191 | JQ849350 |
| Pseudapanteles Whitfield06 | DHJPAR0034081 | JQ853689 |
| Pseudapanteles Whitfield07 | DHJPAR0013217 | JQ852406 |
| Pseudapanteles Whitfield08 | DHJPAR0026281 | JQ849770 |
| Pseudapanteles Whitfield09 | DHJPAR0027627 | JN281617 |
| Pseudapanteles Whitfield09 | DHJPAR0027692 | HM430666 |
| Pseudapanteles Whitfield09 | DHJPAR0027392 | JQ849832 |
| Pseudapanteles Whitfield09 | DHJPAR0026088 | JQ854468 |
| Pseudapanteles Whitfield09 | DHJPAR0026012 | JQ853711 |
| Pseudapanteles Whitfield09 | DHJPAR0026026 | HQ549676 |
| Pseudapanteles Whitfield09 | DHJPAR0027661 | JQ576067 |
| Pseudapanteles Whitfield09 | DHJPAR0033842 | HQ550374 |
| Pseudapanteles Whitfield09 | DHJPAR0031297 | JQ855464 |
| Pseudapanteles Whitfield10 | DHJPAR0012880 | JQ854478 |


| Taxon | Sample ID | Genbank Accession Number |
| :---: | :---: | :---: |
| Pseudapanteles Whitfield11 | DHJPAR0031742 | JQ847036 |
| Pseudapanteles Whitfield12 | DHJPAR0025380 | HQ550092 |
| Pseudapanteles Whitfield14 | DHJPAR0025854 | HQ549888 |
| Pseudapanteles Whitfield15 | DHJPAR0025751 | JQ849472 |
| Pseudapanteles Whitfield17 | DHJPAR0026060 | JN281784 |
| Pseudapanteles Whitfield18 | DHJPAR0027669 | JQ853458 |
| Pseudapanteles Whitfield19 | DHJPAR0027440 | JQ848147 |
| Pseudapanteles Whitfield19 | DHJPAR0026033 | JQ847890 |
| Pseudapanteles Whitfield19 | DHJPAR0026268 | JQ852987 |
| Pseudapanteles Whitfield19 | DHJPAR0027440 | JQ848147 |
| Pseudapanteles Whitfield19 | DHJPAR0026033 | JQ847890 |
| Pseudapanteles Whitfield19 | DHJPAR0026268 | JQ852987 |
| Pseudapanteles Whitfield19 | DHJPAR0025022 | HQ926353 |
| Pseudapanteles Whitfield19 | DHJPAR0026008 | JQ850045 |
| Pseudapanteles Whitfield19 | DHJPAR0025959 | JQ849085 |
| Pseudapanteles Whitfield19 | DHJPAR0025866 | JQ852050 |
| Pseudapanteles Whitfield19 | DHJPAR0026534 | HQ550096 |
| Pseudapanteles Whitfield19 | DHJPAR0027150 | HQ548886 |
| Pseudapanteles Whitfield19 | DHJPAR0025831 | JQ574919 |
| Pseudapanteles Whitfield20 | DHJPAR0040493 | JQ849256 |
| Pseudapanteles Whitfield20 | DHJPAR0027329 | JQ848647 |
| Pseudapanteles Whitfield20 | DHJPAR0039680 | JQ848927 |
| Pseudapanteles Whitfield20 | DHJPAR0041914 | JQ847496 |
| Pseudapanteles Whitfield21 | DHJPAR0038402 | EU396445 |
| Pseudapanteles Whitfield21 | DHJPAR0027221 | JN282176 |
| Pseudapanteles Whitfield23 | DHJPAR0031749 | JQ852705 |
| Pseudapanteles Whitfield27 | DHJPAR0043058 | JQ850352 |

## Results

## Molecular confirmation of Mariapanteles monophyly

Evidence (beyond the morphologcial data presented below) for the separation of these two genera comes from DNA barcode data (Figs 13, 14). When comparing the two species of Mariapanteles to 32 species of Pseudapanteles (most of them undescribed) for which there are CO1 sequences available in BOLD (www.boldsystems.com), the two genera are recovered as separate groups.

Bayesian analysis of the COI sequences, with and without $3^{\text {rd }}$ codon positions included, clearly supports Mariapanteles as being a separate distinct group from Pseudapanteles (Figs 13, 14). Pseudapanteles was recovered as being monophyletic with posterior probability (PP) of 1 when $3^{\text {rd }}$ codon positions were included and PP of 0.8 when $3^{\text {rd }}$ codon positions were removed. Monophyly of Mariapanteles was recovered with PP of 1 in both analyses. Furthermore, Mariapanteles was not recovered as the sister group to Pseudapanteles in either analysis. Several other genera included as potentially disrupting Mariapanteles monophyly also failed to do so in the analyses, so we have concluded that Mariapanteles is indeed a distinct genus.

## Mariapanteles Whitfield \& Fernández-Triana, gen. n.

urn:lsid:zoobank.org:act:917FB3C2-D102-4884-9FC4-787CC81AD5E9
http://species-id.net/wiki/Mariapanteles
Figs 1-12

Type species. Mariapanteles felipei Whitfield sp. n., by present designation.
Genus diagnosis. Propodeum with a complete transverse carina that forks around spiracles and reaches the lateral margin of propodeum, where it intersects a raised lateral carina. Fore wing without areolet (veins r-m and 3RS absent). First mediotergite with a sharp median groove on the basal half. The only other genera of Neotropical microgastrines with a complete transverse carina on the propodeum, Clarkinella and Prasmodon, both lack a medial groove on the first mediotergite and have an areolet in the forewing (a small areolet in Clarkinella, a large and quadrangular one in Prasmodon). Mariapanteles resembles Pseudapanteles in fore wing venation, shape of mediotergites 1 and 2, and general appearance of the body. However, Pseudapanteles has an elongate, bifurcate glossa, lacks a complete transverse carina on the propodeum, and the hypopygium has a large translucent fold with many pleats; the glossa of Mariapanteles is not bifurcate and the hypopygium has a median translucent fold with no or only a few pleats visible.

Description. Body length $2.4-2.6 \mathrm{~mm}$, fore wing length $2.6-2.9 \mathrm{~mm}$, antenna about the same length as body. Pronotum with two lateral grooves present, the lower one excavated. Mesoscutum more or less uniformly sculptured by impressed punctures. Mesoscutum 1.3-1.4× wider than long. Mesoscutum and scutellum uniformly covered by dense, pale yellowish pilosity. Scutellum length/width at base 1.0-1.1X. Scutellar suture broad, with 4-8 costulae. Posterior band of scutellum polished. Scutellar lateral face with the polished area thin ( $15-25 \%$ the face height) and about half the face width. Mesopleuron mostly smooth and glabrous, except for punctures on the anterior margin and setae on all margins. Metapleuron mostly smooth, with some punctures and setae in the apical half; metapleuron with a crenulate, longitudinal sulcus running from lower margin near metacoxa through spiracle. Metapleural carina raised, with a short lamella. Propodeum mostly smooth; median carina well defined and raised its entire length, and with a clearly complete transverse carina that reaches the spiracles and forks around them (there may also be additional, shorter transverse carinae, some of them radiating from the median carina but not reaching the spiracles). Transverse carina on propodeum delimiting two areas, the anterior, basal one being more or less horizontal; the posterior, apical one is declivous. Mediotergite 1 mostly smooth and with a deep medial groove on its basal half; slightly widening for the first quarter of its length, then narrowing towards apex. Mediotergite 2 mostly smooth, transverse, subtriangular to trapezoidal in shape. Mediotergite 3 and following, unsculptured, polished and with sparse setae. Hypopygium mostly inflexible but with a medial, translucent fold ventrally where none or few (1-2) pleats are distinguishable. Ovipositor sheaths fully setose, $0.7 \times$ as long as metatibia length. Metacoxa long, surpassing the length of the third metasomal tergite. Metatibial inner spur longer than outer spur, and
about half the length of metatarsomere 1 . Metafemur more than $3.0 \times$ as long as wide. Fore wing without an areolet, vein R1a longer than stigma length, and vein $r$ and $2 R S$ evenly curved to very slightly arched (with no clear limits between the two veins). Hind wing with edge of vannal lobe medially straight to slightly concave and with uniformly distributed setae that are shorter than those at base and apex of the lobe.

Distribution. The genus occurs in Central and South American rain forests. We describe two new species, one from Costa Rica (ACG, from rain forest at 400 m ) and one from Brazil (Mato Grosso and Goiás; the localities are presumed to have been rain forests at the time the specimens were collected). The CNC collection contains two additional specimens from other areas of Brazil that may represent additional species, but because they are singletons we do not describe them here. It is likely that more species of this new genus will be found in Neotropical rain forests.

Biology. Unknown. All specimens have been collected with Townes-type Malaise traps.

Etymology. Mariapanteles is dedicated to María Marta Chavarría Díaz of ACG and San Jose, Costa Rica, in recognition of her 30+ years of dedication to Costa Rican conservation, biodiversity systematics, and biodiversity development throughout Costa Rica, and very specifically within Area de Conservación de Guanacaste.

Comments. Mariapanteles is closely related to Pseudapanteles, and future revisions of the phylogeny of Microgastrinae might find that its erection renders Pseudapanteles paraphyletic. For example, and according to Mason (1981), some species of Pseudapanteles could have a multiple or indefinite transverse carina, in which case the complete transverse carina in Mariapanteles might be seen as the extreme in a continuum from having no transverse carina to having the complete transverse carina of Mariapanteles. However, we consider that the presence of a complete transverse carina on the propodeum, forking around the propodeal spiracles, may be a strong autapomorphy that defines Mariapanteles. There are only four other genera of Microgastrinae with a similar, complete transverse carina on the propodeum: Beyarslania, Clarkinella, Neoclarkinella, and Prasmodon. However, they all appear to be only distantly related to Mariapanteles because they all lack a sharp median groove on mediotergite 1 and/or have an areolet in the fore wing.

The described species of Pseudapanteles never have a complete transverse carina. Most of the specimens in collections just have a simple median carina, with only few species having irregular transverse striations arising along the length of the median carina (but even in those cases they never reach the spiracle and never form a fork around them). One example can be seen in the original description and pictures of the species Pseudapanteles gouleti Fernández-Triana, from Canada (Figure 18, page 24, in Fernández-Triana 2010). However, the carination pattern is not comparable to a complete transverse carina - as displayed by Mariapanteles.

The presence of a bifurcate glossa is a strong autapomorphy for Pseudapanteles (it is only present in three other distantly related Microgastrinae genera: Napamus, Promicrogaster and Sendaphne). Furthermore, the differences between Mariapanteles and Pseudapanteles with respect to the pleated area of the hypopygium are also consistent in the separation of these two genera.

Currently Pseudapanteles has nine described species (Yu et al. 2009), and a wide distribution within the New World, ranging from Canada to South America (one of the species has also been introduced to Hawaii (Coulson 1992)). The actual number of species is much higher, and we have seen in collections several dozen undescribed species, mostly from the Neotropics. The vast majority of those specimens are remarkably invariant in having a bifurcate glossa and in lacking a transverse carina on the propodeum.

For all of the above reasons along with the molecular results, we have decided that Mariapanteles is a distinct, separate genus that may be closely related to Pseudapanteles.

As for the former records of Neotropical species of the genus Beyarslania (at that time called Xenogaster) (Whitfield 1997; Campos 2001), these are based on confusion with specimens of what we have described here as Mariapanteles. Based on the available evidence, we now consider Beyarslania to be restricted to the African tropics. The Neotropical specimens thought to belong to that genus should be identified as Mariapanteles.

## Key to Mariapanteles species described here

1 Body colour mostly yellow in females and males -at most, males with tergite 3+ brown (Fig. 8); scutellum mostly smooth and scutellar suture with 4-6 costulae (Fig. 12); propodeum with a complete transverse carina that is clearly independent of other smaller transverse carinae (Figs 9, 12) [Mato Grosso and Goiás, Brazil] Mariapanteles dapkeyae sp. n.

- Body colour in females mostly orange-yellowish but with some areas brown or reddish brown (apical edge of scutellum, metascutellum, some carina on propodeum, central area on mediotergites 3+) (Fig. 1), male with much darker coloration, especially in the interocellar area, mesosoma and metasoma (Fig. 2); scutellum with impressed punctures and scutellar suture with 6-8 costulae (Fig. 6); propodeum with a completely transverse carinae not always clearly delimited from other smaller transverse carina (Fig. 6) [ACG, northwestern Costa Rica]

Mariapanteles felipei sp. n.

## Species descriptions

## Mariapanteles felipei Whitfield, sp. n.

urn:lsid:zoobank.org:act:2A5E1320-5C93-45FE-AEB0-AF9382929A78
http://species-id.net/wiki/Mariapanteles_felipei
Figs 1-6

Holotype. Female (NMNH). COSTA RICA: Alajuela Province, Sector Rincon Rain Forest of ACG, Caribe, Rio Francia, 400 m, latitude 10.90093, longitude -85.28915; 11-17.vii.2007, Malaise Trap. Voucher code: DHJPAR0025453.

Paratype. Male (NMNH). Same data as for holotype, except for collecting date: 22-28.viii.2007. Voucher code: DHJPAR0025443.

Description. Female. Antenna about the same length as body; body length 2.6 mm ; forewing 2.9 mm . Head: face with shallow and sparse punctures and sparse, uniformly distributed setae; face width at antennal base/face width at clypeus edge: 1.1×; intertentorial pit distance/face width at clypeus edge: $0.5 \times$; compound eye height/head height: $0.8 \times$; head height/width: $0.8 \times$; face width at antennal base/head maximum width: $0.6 x$; malar space/basal width of mandible $1.3 x$; clypeus width/height: 3.1x. Length/width of flagellomeres: 2nd (2.3x), 8th (2.5x), 14th (1.3x). Length of flagellomere $2^{\text {nd }} /$ length of flagellomere $14^{\text {h. }}: 2.2 x$. Ocello-ocular distance/posterior ocelli diameter: $2.3 \times$; distance between posterior ocelli/ocelli diameter: $1.4 \times$.

Mesosoma. Pronotum with two lateral grooves, the lower one excavated. Mesoscutum more or less uniformly sculptured by impressed punctures (distance between punctures about the same as their diameter). Mesoscutum $1.4 \times$ wider than long. Mesoscutum and scutellum uniformly covered by dense, pale-coloured pilosity. Scutellum similarly sculptured to mesoscutum. Scutellum length/width at base $1.0 \times$. Scutellar suture broad, with $6-8$ costulae. Posterior band of scutellum polished. Scutellar lateral face with polished area less than $30 \%$ the face height and about half the face width. Mesopleuron mostly smooth and glabrous, except for punctures on the anterior margin and setae on the all margins; separated from metapleuron by a crenulated sulcus. Metapleuron mostly smooth, with some punctures and setae in the apical half; metapleuron with a crenulate, longitudinal sulcus running from lower margin near metacoxa through spiracle. Metapleural carina raised with a short lamella. Propodeum mostly smooth, with a median carina well defined and raised its entire length; and with a clearly complete transverse carina that reaches the spiracles and forks around them (also with additional, shorter transverse carinae, some of them radiating from the median carina but not reaching the spiracles). Transverse carina on propodeum delimiting two areas, the anterior, basal one being more or less horizontal, while the posterior, apical one is declivous.

Metasoma. Mediotergite 1 mostly smooth and with a deep medial groove over its basal half; slightly widening for the first quarter of its length, then narrowing towards apex; basal width/apical width $2.1 \times$; length/apical width $4.8 \times$. Mediotergite 2 mostly smooth, transverse, subtriangular to trapezoidal in shape; basal width/apical width $0.4 \times$; length/apical width $0.4 \times$. Mediotergite $31.5 \times$ the length of mediotergite 2. Mediotergite 3 and following unsculptured, polished and with sparse setae. Hypopygium mostly inflexible but with a median, translucid fold ventrally where no pleats are distinguishable. Ovipositor sheaths fully setose, $0.7 \times$ as long as metatibia length.

Legs. Metacoxa long, surpassing the length of the third metasomal tergum. Metatibial inner spur $1.6 \times$ the length of outer spur, and $0.6 \times$ the length of metatarsomere 1 . Metafemur $3.2 \times$ as long as wide.

Wings. Vein R1a $1.3 \times$ as long as stigma length. Stigma $3.1 \times$ as long as wide. Length of R1a about $12 \times$ as long as the distance between its end and the end of 3 RSb. Vein $r$ and 2RS evenly curved to very slightly arched, with no clear limits between the two veins. Vein 2 M about the same length of vein (RS +M )b. Edge of vannal lobe of hind wing medially straight to slightly concave and with uniformly distribute setae which are shorter than those at base and apex of the lobe.


Figures I-6. Mariapanteles felipei Whitfield. I Dorsal habitus, female 2 Dorsal habitus, male $\mathbf{3}$ forewing, female $\mathbf{4}$ head and mesoscutum, dorsal view, female $\mathbf{5}$ hypopygium and ovipositor, lateral view 6 metanotum and propodeum, female, dorsal view.

Colour: Mostly an orange-yellowish species. Antennal flagellomere and dorsal part of scape brown. Apical edge of scutellum, metascutellum and some carina on propodeum, reddish-brown. Central area on mediotergites 3 and following dark brown. Forewing stigma and most of the wing veins dark brown.

Male. Like the female except for darker coloration as follows: interocellar area, propodeum, metascutellum, apical edge of scutellum, most of the lateral face of scutellum, and most of mediotergites $2+$, dark brown to black.

Distribution. The known specimens were captured in July-August 2007 (full rainy season) by the same Malaise trap placed in old growth rain forest understory on the banks of Rio Francia, where it crosses the access road through Sector Rincon Rain Forest of ACG, at 400 m .

Molecular data. The two known specimens bear the same DNA barcode. The nucleotide sequence in fasta format is:
>Mariapanteles felipei
ATTTTATATTTTTTATTTGGAATATGATCTGGAATATTAG-GATTTTCATTAAGAATAATTATCCGATTAGAGTTAGGCACACCAGGAA-GATTAATTAGAAATGATCAAATCTATAATAGAATTGTTACATCACAT-GCTTTTATCATAATTTTTTTTATAGTTATACCAATTATAATTGGAG-GATTTGGTAATTATTTAATTCCATTAATATTAGCAACTCCTGATATAT-CATTCCCACGAATAAATAATATGAGATTTTGATTACTAATTCCTTCAT-TATTTTTATTAATTTTTAGAAGATTTATTAATACAGGAGTAGGTACAG-GTTGAACAGTTTATCCACCTTTATCATCAAATTTAGGACATAGAGG-TATATCAGTTGATTTAGGAATCTTTTCTCTACATTTAGCAGGAGCCT-CATCAATTATAGGAGCAATTAATTTTATTACAACAATTAAAAATATAC-GAGTTAAATTATTAAAAATAGATAAAATTTCTTTATTTACTTGATCAGTTT-TAATTACAGCAATTTTATTATTATTATCTTTACCAGTTTTAGCAGGAG-CAATTACTATACTTTTAACAGACCGAAATTTAAATACATCATTTTTTGATCCTTCAGGAGGTGGGGATCCAATTTTATACCAACATTTATTT

Etymology. Mariapanteles felipei is dedicated to Luis Felipe Chavarría Díaz of ACG and San Jose, Costa Rica, in recognition of his 30+ years of dedication to Costa Rican conservation, biodiversity systematics, and biodiversity development throughout Costa Rica, and very specifically within Area de Conservación de Guanacaste.

Comments. The biology of this species, collected with Malaise traps, is unknown. Since its inception in 1978, the ACG caterpillar and parasitoid inventory (Janzen et al. 2009) has achieved Microgastrinae rearings from 9,000+ wild-caught caterpillars and has Malaisetrapped 5,000+ individual Microgastrinae in dry forest, cloud forest and rain forest (Janzen and Hallwachs 2012, Smith et al. 2008); this intense effort has yielded only two conspecific individuals of Mariapanteles, both from the same Malaise trap a few weeks apart. While this may suggest that the species is "rare", it has been the experience of the ACG inventory that when the wasp is finally reared and therefore its host caterpillar known, or the Malaise trap is placed in the "right" place, it may well be found to be common.

## Mariapanteles dapkeyae Fernández-Triana, sp. n.

 urn:lsid:zoobank.org:act:EF2F8657-FEBB-4AEE-8501-06B104771B86http://species-id.net/wiki/Mariapanteles_dapkeyae
Figs 7-12

Holotype. Female (CNC). BRAZIL: Mato Grosso, Sinop; x-xi.1975, Malaise Trap; M. Alvarenga col.

Paratype. 5 Females and 4 Males (CNC, with 1 female each deposited in INHS and BMNH). Same data as for holotype, except for collecting date (x. 1974 for all specimens but two males with collecting date: x.1975). Two males deposited in the CNC have DNA Voucher codes: CNCHYM 03387 and CNCHYM 07145. 1 Female (CNC). BRAZIL: Goiás, Jatai; xi.1972; F. M. Oliveira col.

Description. Female. Antenna about the same length as body; body length 2.4 mm ; forewing 2.6 mm . Head. Face with shallow and sparse punctures and sparse, uniformly distributed setae. Face width at antennal base/face width at clypeus edge: $1.1 \times$; intertentorial pit distance/face width at clypeus edge: $0.4 \times$; compound eye height/head height: $0.8 \times$; head height/width: $0.8 \times$; face width at antennal base/head maximum width: $0.5 \times$; malar space/basal width of mandible $1.4 \times$; clypeus width/height: $3.5 \times$. Length/width of flagellomeres: 2nd (2.4×), 8th (2.5x), 14th (1.3x). Length of flagellomere $2^{\text {nd }} / l$ length of flagellomere $14^{\text {th }}: 2.2 \times$. Ocello-ocular distance/posterior ocelli diameter: $2.2 \times$; distance between posterior ocelli/ocelli diameter: $1.3 \times$.

Mesosoma. Pronotum with two lateral grooves present, the lower one excavated. Mesoscutum more or less uniformly sculptured by shallowly impressed punctures (distance between punctures about the same as their diameter). Mesoscutum $1.3 \times$ wider than long. Mesoscutum and scutellum uniformly covered by dense, pale yellow pilosity. Scutellum mostly smooth, with very shallow and sparse punctures. Scutellum length/width at base $1.1 \times$. Scutellar suture broad, with 4-6 costulae. Posterior band of scutellum polished. Scutellar lateral face with polished area less than $20 \%$ the face height and less than half the face width. Mesopleuron mostly smooth and glabrous, except for punctures on the anterior margin and setae on all margins; separated from metapleuron by crenulate sulcus. Metapleuron mostly smooth, with some punctures and setae in the apical half; metapleuron with a crenulated, longitudinal sulcus running from lower margin near metacoxa through spiracle. Metapleural carina raised with a short lamella. Propodeum mostly smooth; with a median carina well defined and raised its entire length; and with a clearly complete transverse carina that reaches the spiracles and forks around them (there are also additional, shorter transverse carinae). Transverse carina on propodeum delimiting two areas, the anterior, basal one that is more or less horizontal, and the posterior, apical one is declivous.

Metasoma. Mediotergite 1 mostly smooth and with a deep medial groove over its basal half; slightly widening for the first quarter of its length, then narrowing to-


Figures 7-I 2. Mariapanteles dapkeyae Fernandez-Triana. $\mathbf{7}$ lateral habitus, female 8 dorsal habitus, female 9 fore wing, female $\mathbf{I 0}$ head and mesoscutum, dorsal view, female II hypopygium and ovipositor, lateral view $\mathbf{1 2}$ metanotum, propodeum and anterior metasomal tergites, female, dorsal view.
wards apex; basal width/apical width $1.5 \times$; length/apical width $3.3 \times$. Mediotergite 2 mostly smooth, transverse, subtriangular to trapezoidal in shape; basal width/apical width $0.3 \times$; length/apical width $0.4 \times$. Mediotergite $31.5 \times$ the length of mediotergite 2. Mediotergite 3 and following unsculptured, polished and with sparse setae. Hypopygium mostly inflexible but with a median, translucid fold ventrally where 1-2 weak pleats are sometimes distinguishable. Ovipositor sheaths fully setose, $0.7 \times$ as long as metatibia length.

Legs. Metacoxa long, surpassing the length of the third metasomal tergum. Metatibial inner spur $1.4 \times$ the length of outer spur, and 0.5 X the length of metatarsomere 1 . Metafemur $3.2 \times$ as long as wide.

Wings. Vein R1a $1.3 \times$ as long as stigma length. Stigma $3.3 \times$ as long as wide. Length of R1a about $14 \times$ as long as the distance between its end and the end of 3 RSb . Vein $r$ and 2RS evenly curved to very slightly arched, with no clear limits between the two veins. Vein 2 M about the same length of vein (RS+M)b. Edge of vannal lobe of hind wing medially straight to slightly concave and with uniformly distribute setae which are shorter than those at base and apex of the lobe.

Colour: Mostly yellow, with antennal flagellomere, forewing stigma and most of the wing veins, light brown.

Male. Mostly like females, but some specimens with darker interocellar area and mediotergites 3+. We associate these males with these females because of their morphological similarity.

Distribution. The specimens were collected with Malaise traps in two Brazilian localities (less than 1000 km apart) which are presumed to have been rain forests at the time of collecting.

Molecular data. Two paratype male specimens rendered partial barcodes (361 bp for the one with DNA voucher code CNCHYM 03387, and 164 bp for the one with DNA voucher code CNCHYM 07145). The nucleotide sequence shown below corresponds to the longer sequence in the barcode region of COI :
>Mariapanteles dapkeyae
TAAGATTTTGATTATTAATTCCATCTTTATTTATATTAATTTTT AGAAGATTTATTAATACAGGAGTAGGTACAGGTTGAACAGTATACCCACCATTATCATCAAATTTAAGACATAGGGGCATATCAGTCGATT TAAGAATTTTTTCTTTACATTTAGCAGGAACTTCATCAATTATAG-GAGCAATTAATTTTATTACAACAATTAAAAATATACGAGTTAAAT-TATTTAAAATAAATAAAATTTCTTTATTTAATTGATCAGTTTTAATTA-CAGCAATTTTATTATTATTATCATTACCAGTATTAGCAGGTGCTATTACTATACTTTTAACAGATCGAAATTTAAATACATCATTTTT

Etymology. This species is dedicated to Tanya Heckmann Dapkey of Philadelphia, Pennsylvania, USA, in recognition of her seven years of diligent and highly accurate sorting, processing, databasing, and de-legging ACG microgastrine wasps for DNA barcoding.

Comments. The biology of this species, collected with Malaise traps, is unknown. In the CNC collection there are two additional specimens of Mariapanteles from Bra-


Figure 13. Maximum clade credibility tree for Pseudapanteles and Mariapanteles based on Bayesian analysis of COI sequences, with $3^{\text {rd }}$ codon positions included (see text for details). Values at nodes are posterior probabilities.
zil: one female from Piedra Azul, Minas Gerais; and one male from Rio Javari, Estirar do Equador, Amazonas. Both specimens differ morphologically from M. dapkeyae. Additionally, the male specimen (with DNA voucher code CNCHYM 03380) rendered a partial DNA barcode (164bp) which has 7 base pairs different ( $4.3 \%$ ) from


Figure 14. Maximum clade credibility tree for Pseudapanteles and Mariapanteles based on Bayesian analysis of COI sequences, with $3^{\text {rd }}$ codon positions excluded (see text for details). Values at nodes are posterior probabilities.
the barcoded specimens of $M$. dapkeyae. We believe those two specimens may represent additional species, but because they are singletons we have not described them as new species here.

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## Appendix I

Detailed information about collection, BOLD, and GenBank number of species. (doi: 10.3897/zookeys.208.3326.app) File format: Excel spreadsheet (xls).

Explanation note: Appendix I contains all available DNA barcodes for Pseudapanteles ( 32 New World species, most of which are undescribed) and Mariapanteles (2 species) that were at least 300 bp long were downloaded from BOLD.

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[^0]:    Citation: Whitfield JB, Fernández-Triana JL, Janzen DH, Hallwachs W, Smith MA, Cardinal S (2012) Mariapanteles (Hymenoptera, Braconidae), a new genus of Neotropical microgastrine parasitoid wasp discovered through biodiversity inventory. ZooKeys 208: 61-80. doi: 10.3897/zookeys.208.3326.app

