# Genetic structure of Octopus minor around Chinese waters as indicated by nuclear DNA variations (Mollusca, Cephalopoda) 

Faiz Muhammad ${ }^{1,2}$, Zhen-ming Lü', Liqin Liu', Li Gong', Xun Du', Muhammad Shafi ${ }^{3}$, Hubdar Ali Kaleri ${ }^{4}$<br>I National Engineering Research Center of Marine Facilities Aquaculture, College of Marine Sciences and Technology, Zhejiang Ocean University 2 Center of Excellence in Marine Biology, University of Karachi $\mathbf{3}$ Lasbella University of Agriculture, Water and Marine Sciences 4 Department of Animal Science and Aquaculture, Dalhousie University, Canada<br>Corresponding author: Zhen-ming Lü (nblzmnb@163.com)

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#### Abstract

Octopus minor is an economically important resource commonly found in Chinese coastal waters. The nuclear gene (RD and ODH) approach of investigation has not reported in this species. Rhodopsin (RD) and octopine dehydrogenase (ODH) genes were used to elaborate the genetic structure collected from eight localities ranging from the northern to the southern coast of China. In total, 118 individuals for the RD gene and 108 for the ODH were sequenced. Overall (RD and ODH) genes resulted in high ( $0.741 \pm 0.032 ; 0.805 \pm 0.038$ ) haplotype and low nucleotide ( $0.01261 \pm 0.00165 ; 0.00747 \pm 0.00086$ ) diversity. Molecular variance displayed higher values among the populations and lower values within the population where the fixation index $\mathrm{F}_{\mathrm{ST}}$ denoted 0.880 and 0.584 in RD and ODH genes respectively. The Dongshan population clustered separately in a phylogenetic tree as in the haplotype networking assessment. The current data suggests that the Dongshan population needs separate management.


## Keywords

Cephalopoda, China Coast, Nuclear DNA, Octopuses

## Introduction

The class Cephalopoda embraces animals which are exclusively marine inhabitants. They have immense commercial and ecological significance, including a profound contribution as a source of protein for humans. The cephalopod has lobed and folded brain like that of vertebrates and are ingenious, migrant and largest of all molluscs (Mather and Kuba 2013; Cheng et al. 2013; Larson et al. 2015) and it is sensitive to environmental factors (Emery et al. 2016; Wang and Zheng 2017

Among cephalopods, the octopuses contribute $33 \%$ to the existing cephalopod assembly. Three hundred species of octopus are thought to exist along the coastal waters of Korea, China, and Japan (Roper et al. 1984; Norman and Sweeney 1997; Kang et al. 2012). One hundred thirty-four species are reported only in Chinese waters including the profit-making species like Octopus minor, Amphioctopus fangsiao, and Cistopus chinensis (Lu et al. 2012).

Previously, mtDNA molecular markers were popular because of their high mutation rate, maternal inheritance, and non-recombination (Vaseeharan et al. 2013). In the last decade, the nuclear DNA markers have been widely used for various investigations including identification, population genetics, comparisons between wild and captive populations, demographic evaluations, and rehabilitation projects (Chauhan and Rajiv 2010).

Carlini et al. (2000) was first who used nuclear DNA (Actin) for phylogenetic analysis of coleoid species subsequently other authors continued nuclear approach of investigation such as, (Warnke et al. 2003; Lindgren et al. 2004; Strugnell et al. 2005). The nuclear gene provides more information than mtDNA genes (Graybeal 1994). The rhodopsin and octopine dehydrogenase genes were used for phylogenetic and phylogeographic analysis of octopuses (Strugnell and Nishiguchi 2007; Toussaint et al. 2012). However, meagre information is available on population genetics of octopuses using RD and ODH genes. The RD and ODH genes are less complex and can provide better results than 18 S rDNA. Our present study aims to focus on collecting fundamental information about the population structure of this species using nuclear genes.

## Materials and methods

Samples were collected from eight locations (Fig. 1). Thereafter, they were preserved in $95 \%$ ethanol and transported to the laboratory. The total genomic DNA was isolated from muscle tissues using a standard protocol. Target genes were amplified by PCR using primers (Table 1). The total $25 \mu \mathrm{l}$ PCR mixture included (DNA template $1.25 \mu$ l, each primer $1.25 \mu$ l, ES Taq $12.5 \mu \mathrm{l}$, and $8.75 \mu \mathrm{l}$ water). Thermocycler conditions were as follows: denaturation at $94^{\circ} \mathrm{C}$ for $5 \mathrm{~min}, 35$ cycles $94^{\circ} \mathrm{C}$ for 30 s , annealing at $55^{\circ} \mathrm{C}$ for 30 s , extension at $72{ }^{\circ} \mathrm{C}$ for 30 s , and the final extension at $72^{\circ} \mathrm{C}$ for 7 min . Electrophoresis was performed on a $1.2 \%$ agarose gel and was sequenced using the same oligonucleotide primers. Sequences were aligned using MEGA 6 software (Tamura et al. 2013). Analysis of genetic


Figure I. Map showing the collection locations.

Table I. Oligonucleotide primers used for the amplification of nuclear rhodopsin and octopine dehydrogenase genes in Octopus minor.

| Gene | Primer and sequence (5'-3') | Tm (C) | size (bp) | Reference |
| :--- | :---: | :---: | :---: | :---: |
| Rhodopsin | RDF 5'-GCTTTCCTCATGATTATC-3' | 50 | 653 | Toussaint et al. 2012 |
|  | RDR 5'-TTCTCCATCATTGCCATC-3' |  |  |  |
| Octopine <br> dehydrogenase | ODHF 5'-AAATCCCGACCAAACATG-3' | 50 | 618 | Toussaint et al. 2012 |
|  | ODHR 5'-GTTAAGTTTGTACCAGTC-3' |  |  |  |

differentiation, AMOVA, molecular diversity indices, genetic differentiation values, and $\mathrm{F}_{\text {ST }}$ values were determined with the ARLEQUIN software (Excoffier and Lischer 2010). Calculations of gene flow $\left(\mathrm{N}_{\mathrm{m}}\right)$ were performed using formula $N_{m}=\left(1-\mathrm{F}_{\mathrm{ST}}\right) / 2 \mathrm{~F}_{\mathrm{ST}}$ Haplotype and nucleotide diversity were estimated using DnaSP (Librado and Rozas 2009). The neighbor joining tree was constructed to check the genetic relationship between populations using MEGA 6 (Tamura et al. 2013). The haplotype networking was created using NETWORK software version 5.0.0.1 (Bandelt et al. 1999).

## Results

Rhodopsin Gene (RD): A fragment of 637 bp of RD was sequenced from 118 individuals. RD gene showed 33 polymorphic sites and 13 haplotypes. Haplotype diversity (HD) ranged from 0.090 to 0.833 , nucleotide diversity $(\pi)$ remained in the lower range (0.00027-0.001), where the average number of differences $(\mathrm{k})$ ranged from 0.166 to 1.388 (Table 2). Molecular variance revealed a higher percentage among the populations ( $88.04 \%$ ) while lower values were retrieved within (11.96\%) populations; fixation index $\mathrm{F}_{\mathrm{ST}}$ was 0.880 (Table 3). The pairwise $\mathrm{F}_{\mathrm{ST}}$ values ranged from 0.014 to 0.989. The lowest gene flow was observed in Dongshan population (Table 4). The values of Tajima's D tests generally showed negative values with exception to Xiamen population. The Wenzhou and Dongshan populations were statistically significant

Table 2. Genetic diversity parameters for RD and ODH sequences in Octopus minor.

| Gene | Population | Number of segregating sites | Number of ha2lotypes | Haplotype diversity (Hd) | Nucleotide diversity ( $\pi$ ) | Average number of differences (k) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Rhodopsin (RD) | Dalian | 3 | 4 | 0.676 | 0.001 | 0.819 |
|  | Dongshan | 4 | 2 | 0.090 | 0.001 | 0.363 |
|  | Nantang | 6 | 6 | 0.760 | 0.001 | 1.150 |
|  | Qingdao | 2 | 2 | 0.404 | 0.001 | 0.426 |
|  | Shanghai | 1 | 2 | 0.250 | 0.00040 | 0.250 |
|  | Wenzhou | 1 | 2 | 0.166 | 0.00027 | 0.166 |
|  | Xiamen | 4 | 5 | 0.833 | 0.002 | 1.388 |
|  | Zhoushan | 2 | 3 | 0.292 | 0.0004 | 0.304 |
| Octopine dehydrogenease (ODH) | Dalian | 7 | 7 | 0.758 | 0.003 | 2.164 |
|  | Dongshan | 4 | 5 | 0.775 | 0.002 | 1.341 |
|  | Nantang | 8 | 8 | 0.828 | 0.003 | 2.076 |
|  | Qingdao | 6 | 6 | 0.647 | 0.003 | 1.800 |
|  | Shanghai | 6 | 6 | 1.000 | 0.004 | 2.733 |
|  | Wenzhou | 2 | 2 | 0.222 | 0.001 | 0.444 |
|  | Xiamen | 5 | 6 | 0.717 | 0.001 | 0.897 |
|  | Zhoushan | 7 | 7 | 0.794 | 0.003 | 2.025 |

Table 3. Analysis of molecular variance of Octopus minor using RDand ODH.

| Gene | Source of variation | df | Sum of squares | variance component | Percentage (\%) |
| :--- | :--- | :--- | :--- | :--- | :--- |
| RD | Among population | 8 | 573.404 | 5.57703 Va | 88.04 |
|  | Within population | 110 | 83.334 | 0.75758 Vb | 11.96 |
|  | Total | 118 | 656.737 | 6.33461 |  |
| ODH | Fixation Index FST: | $\mathbf{0 . 8 8 0}$ |  |  |  |
|  | Among population | 8 | 174.799 | 1.79694 Va | 58.44 |
|  | Within population | 99 | 126.490 | 1.27768 Vb | 41.56 |
|  | Total | 107 | 301.290 | 3.07462 |  |
|  | Fixation Index FST: | $\mathbf{0 . 5 8 4}$ |  |  |  |

( $\mathrm{P}<0.05$ ). Fu's Fs values were positive and statistically non-significant in all populations (Table 6). The phylogenetic analysis of eight populations separated Dongshan population with $99 \%$ bootstrap support (Fig. 2). The networking analysis revealed that haplotype three (Hap 3) was shared by six populations (Dalian, Nantong, Zhoushan, Qingdao, Shanghai, and Xiamen), Hap 4 was contributed by five populations (Dalain, Nantong, Qingdao, Xiamen, Zhoushan), Hap 2 shared between Dalian, Zhoushan, and Nantong, while Hap 7 appeared in two populations (Qingdao, Nantong), Hap 11 and Hap 12 were present in the Wenzhou and Xiamen populations, and Hap 5 and Hap 6 were independently represented in the Dongshan population (Fig. 4). Octopine dehydrogenase $(O D H)$ : A fragment of 597 bp was sequenced from 108 individuals, revealed 27 polymorphic sites and 29 haplotypes. Haplotype diversity remained higher ( $0.222-1.000$ ) than nucleotide diversity $(\pi)(0.001-0.004)$. The average number of differences $(\mathrm{k})$ ranged from 0.444 to 2.733 (Table 2). Molecular variance was revealed to be higher among the populations (58.44\%) while lower within a population $(41.56 \%)$. Fixation index $\left(\mathrm{F}_{\text {ST }}\right)$ was determined as 0.584 (Table 3). The pairwise $\mathrm{F}_{\text {ST }}$ values ranged from 0.018 to 0.925 . The lowest gene flow was observed in Dongshan population whereas highest $\mathrm{N}_{\mathrm{m}}$ values were shown between Wenzhou and Xiamen populations (Table 5). Tajima’s D showed negative values except in Dongshan, Shanghai, and Zhoushan populations whereas only Xiamen population was statistically significant ( $\mathrm{P}<0.05$ ). Fu's Fs presented negative values except Dongshan, Qingdao, and Wenzhou populations while only Shanghai and Xiamen populations were statistically

Table 4. Pairwise $\mathrm{F}_{\mathrm{ST}}$ below diagonal and gene flow $\left(\mathrm{N}_{\mathrm{m}}\right)$ values above diagonal RD gene.

|  | Dalian | Dongshan | Nantong | Qingdao | Shanghai | Wenzhou | Xiamen | Zhoushan |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dalian | - | 0.012 | 15.125 | 35.214 | 4.654 | 0.071 | 7.436 | $\infty$ |
| Dongshan | 0.975 | - | 0.016 | 0.008 | 0.006 | 0.005 | 0.018 | 0.007 |
| Nantong | 0.032 | 0.968 | - | 1.112 | 0.2 | 0.298 | $\infty$ | 2.996 |
| Qingdao | 0.014 | 0.983 | 0.043 | - | 7.696 | 0.149 | 7.564 | 19.5 |
| Shanghai | 0.097 | 0.987 | 0.200 | 0.061 | - | 0.103 | 2.104 | 23.309 |
| Wenzhou | 0.875 | 0.989 | 0.626 | 0.770 | 0.828 | - | 0.575 | 0.118 |
| Xiamen | 0.063 | 0.964 | $\infty$ | 0.062 | 0.192 | 0.465 | - | 0.002 |
| Zhoushan | $\infty$ | 0.986 | 0.143 | 0.025 | 0.021 | 0.809 | 0.154 | - |

Table 5. Pairwise $\mathrm{F}_{\mathrm{ST}}$ below diagonal and gene flow $\left(\mathrm{N}_{\mathrm{m}}\right)$ values above diagonal ODH gene.

|  | Dalian | Dongshan | Nantong | Qingdao | Shanghai | Wenzhou | Xiamen | Zhoushan |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dalian | - | 0.086 | 0.884 | 14.2 | $\infty$ | 2.530 | 3.632 | $\infty$ |
| Dongshan | 0.853 | - | 0.086 | 0.075 | 0.107 | 0.040 | 0.050 | 0.082 |
| Nantong | 0.018 | 0.852 | - | $\infty$ | $\infty$ | 1.836 | 2.441 | 17.357 |
| Qingdao | 0.034 | 0.869 | $\infty$ | - | 9.303 | 3.885 | 4.261 | 23.309 |
| Shanghai | $\infty$ | 0.823 | $\infty$ | 0.051 | - | 1.702 | 2.406 | $\infty$ |
| Wenzhou | 0.165 | 0.925 | 0.214 | 0.114 | 0.227 | - | 25.815 | 6.852 |
| Xiamen | 0.121 | 0.908 | 0.170 | 0.105 | 0.172 | 0.019 | - | 12.32 |
| Zhoushan | $\infty$ | 0.859 | 0.028 | 0.021 | $\infty$ | 0.068 | 0.039 | - |



Figure 2. Neighbour-joining phylogenetic tree constructed based on RD gene sequences. Key: $\mathrm{DL}=\mathrm{Dalian}$, $\mathrm{N}=$ Nantong, $\mathrm{Q}=$ Qingdao, $\mathrm{S}=$ Shanghai, $\mathrm{W}=$ Wenzhou, $\mathrm{X}=$ Xiamen, $\mathrm{Z}=$ Zhoushan.

Table 6. Tajima's D and Fu's FS tests, corresponding $p$ value for eight population of $O$. minor.

| Gene | Population | Tajima's D |  | Fu's Fs |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\mathbf{D}$ | $\boldsymbol{p}$ | $\boldsymbol{F}$ | $\boldsymbol{p}$ |
| RD | Dalian | -0.260 | 0.449 | 1.447 | 0.805 |
|  | Dongshan | -2.139 | 0.001 | 3.781 | 0.939 |
|  | Nantang | -0.390 | 0.385 | 0.017 | 0.513 |
|  | Qingdao | -0.416 | 0.405 | 1.360 | 0.789 |
|  | Shanghai | -1.054 | 0.213 | 1.414 | 0.688 |
|  | Wenzhou | -1.629 | 0.017 | 1.558 | 0.752 |
|  | Xiamen | 0.061 | 0.533 | 0.087 | 0.518 |
|  | Zhoushan | -1.125 | 0.167 | 0.934 | 0.666 |
|  | Dalian | -0.455 | 0.335 | -0.66426 | 0.345 |
|  | Dongshan | 0.353 | 0.694 | 0.45027 | 0.612 |
|  | Nantang | -0.220 | 0.445 | -0.77911 | 0.367 |
|  | Qingdao | -0.031 | 0.526 | 0.00070 | 0.532 |
|  | Shanghai | 0.665 | 0.741 | -2.73435 | 0.021 |
|  | Wenzhou | -1.512 | 0.057 | 2.30182 | 0.843 |
|  | Xiamen | -1.579 | 0.027 | -2.26025 | 0.022 |
|  | Zhoushan | 0.579 | 0.300 | -0.95837 | 0.265 |



Figure 3. Neighbour-joining tree constructed based on the ODH gene. Key: DL = Dalian, $\mathrm{N}=$ Nantong, $\mathrm{Q}=$ Qingdao, $\mathrm{S}=$ Shanghai, $\mathrm{W}=$ Wenzhou, $\mathrm{X}=$ Xiamen, $\mathrm{Z}=\mathrm{Zhoushan}$.
significant (Table 6). The neighbour-joining phylogenetic tree described two distinct clades where Dongshan population clustered separately with $100 \%$ bootstrap support (Fig. 3). The median joining network analysis described that Hap1 shared by seven populations followed by Hap2, which appeared in four populations, namely Dalian, Nantong, Qingdao, and Zhoushan. Hap 13 contributed by three populations (Dalian, Shanghai, and Zhoushan) similarly Hap 6 appeared in Dalian, Shanghai and Xiamen populations, Hap 17 shared by Nantong, Zhoushan, and Xiamen populations, Hap 16 shared by Nantong and Xiamen, Hap 19 appeared in Qingdao and Shanghai populations while Hap, 25 shared between Zhoushan and Wenzhou populations. Hap 8-12 were independently representing Dongshan population (Fig. 5).

## Discussion

Octopus minor is a bottom-dwelling inhabitant and its migration is limited. The dispersal capacity has immense influence on population genetics. Tag- recapture investigations of $O$. vulgaris reported to be restricted within one km from the point of release (Melis


Figure 4. Median-joining networking drawn based on RD gene haplotypes. Colours represent the corresponding population frequencies. Key: Dalian; Dongshan; Nantong; Qingdao; Shanghai; Wenzhou; Xiamen; Zhoushan.
et al. 2018 and references therein). Conversely, dispersal range of $O$. minor juveniles and adults is not precisely known. The basic information of genetic variation and population structure is valuable for stocking, fisheries management, and conservation (Feng et al. 2017). Several divergent forces cause genetic differentiation, including geographic isolation, current and life history characteristics (Gao et al. 2016). In fact, oceanic processes are complex and a single reason cannot be claimed as source of divergent force. There are many islands and gulfs in China's sea, which can contribute to the gene flow complications of the populations (Gao et al. 2016). Earlier several studies were under taken including complete mitochondrial genome of this species (Cheng et al. 2012). Previous investigations on population genetics of $O$. minor present subtle to significant differences (Gao et al. 2016; Yang et al. 2015; Lü et al. 2013; Kang et al. 2012; Xu et al. 2011; Sun et al. 2010; Li et al. 2010). Our present analysis of two nuclear DNA gene sequences in $O$. minor collected from eight locations imparted variation. The haplotype diversity of RD gene ranged ( $0.090-0.833$ ), the higher haplotype diversity was noted in Xiamen population and lowest haplotype diversity showed in Dongshan population. The ODH gene haplotype diversity was higher in Shanghai population (1.000) and was lowest in Wenzhou population (0.222). Comparative studies of Kang et al. (2012) between Korean and Chinese populations showed less haplotype diversity in Korean population than Chinese populations. However, only three Chinese populations were


Figure 5. Median-joining networking drawn based on ODH gene haplotypes. Colours represent the corresponding population frequencies. Key: Dalian; Dongshan; Nantong; Qingdao; Shanghai; Wenzhou; Xiamen; Zhoushan.
sampled (Dalian, Tianjin, and Rongcheng) from Bohai and Yellow seas, which showed 0.43 to 0.64 values of haplotype diversity. This result is in line with present study. Chang et al. (2010) reported higher haplotype diversity in Lianyungang (0.934) and lowest ( 0.342 ) in Xiamen population but RD and ODH gene showed higher values ( $0.833 ; 0.717$ ) in Xiamen population. Yang et al. (2015) included five populations and reported that Qingdao has high diversity and more diverse than others but present research described high diversity of all northern populations (Table 2). The AMOVA results denoted higher values among populations and lower values within populations, where the fixation index $\mathrm{F}_{\text {ST }}$ was much higher. Yang et al. (2015) reported lower AMOVA values ( $10.88 \%$ ) between populations and higher values ( $89.12 \%$ ) within population, which is discordant. Higher $\mathrm{F}_{\mathrm{ST}}$ values indicate a lower level of gene flow $\left(\mathrm{N}_{\mathrm{m}}\right)$ and higher genetic differentiation among populations (Hedrick and Goodnight 2005; Ye et al. 2015). The lowest gene flow between Dongshan with other counterpart populations was observed. $\mathrm{F}_{\mathrm{ST}}$ value of 0.05 is considered to indicate negligible genetic differentiation, while a value greater than 0.25 demonstrate high genetic differentiation within the analyzed population (Weir and Cockerham 1996). Based on this standard, the results obtained in this study showed high differentiation between Dongshan and other counterpart populations (Tables 4, 5).

The Tajima's D analysis of the RD gene showed negative values except for the Xiamen population, unlike to the results of the CO1 studies (Chang et al. 2010). The ODH gene revealed negative values for Tajima's D except for three populations, Shanghai, Zhoushan, and Dongshan. Results are similar to mtDNA COI investigations
(Chang et al. 2010) where the Zhoushan population had positive values; Shanghai and Nantong populations were not included in COI studies. The RD gene revealed 13 haplotypes; among them Hap 3 was common, shared by 6 populations. None of the haplotypes of the Dongshan population was shared by other counterpart populations with similar COII and Cytb investigations (Lü et al. 2013; Li et al. 2013). Nevertheless, it differs with COII and Cytb (Lü et al. 2013; Li et al, 2013) where Wenzhou, Zhoushan, and Wenzhou, Xiamen populations remained isolated populations respectively. The ODH gene described 29 haplotypes among which Hap1 remained common, which was shared by seven populations. It is consistent with Lü et al. (2013) and Li et al. (2013) with respect to Dongshan population whereas differ with reference to Wenzhou and Xiamen populations. Studies of Kang et al. (2012) described sharing of one haplotype between Chinese and Korean populations. The neighbour-joining phylogenetic tree of both the genes distinctly clustered Dongshan population separately as in Cyt b, and COII studies (Li et al. 2013; Lü et al. 2013). However, COII unveiled Wenzhou and Zhoushan populations as separate clade (Lü et al. 2013); similarly, 16S rRNA showed the Xiamen population as a separate clade (Li et al. 2010). Chang et al. (2010) reported Wenzhou and Xiamen populations as separate clade. Kang et al. (2012) investigated three clades where only one Chinese population (Dalian) parted as sub-clade along with Korean populations. Yang et al. (2015) using AFLP and Sun et al. (2010) using CO1 reported two clades, which is inconsistent with present study. The discrepancy of data is consequences of various unknown oceanic process, genetic markers used and range of sampling locations (Lü et al. 2013). To understand the data variation of $O$. minor along the Chinese coast, it is imperative to study the larval and adult dispersal range along with seasonal oceanic process during ontogenesis. Present data does not support the isolation by distance (IBD) because the geographic coastal distance between Dongshan and Xiamen is only 157 KM while the gene flow between them is meagre as shown by RD and $\mathrm{ODH}(0.018 ; 0.050)$, whereas the coastal distance between Dalian and Xiamen is approximately 3700 Km but the gene flow is higher (7.436; 3.632). Lü et al. (2013) was also noted gene flow discordance but the concrete reason is unknown. Melis et al. (2018) emphasized the causes of meagre larval dispersal in $O$. vulgaris including high mortality, philopatric behaviour of larvae and potential cryptic berries. Nevertheless, the above-mentioned factors are difficult to be disentangled, furthermore, oceanic fronts (temperature, salinity, density, turbidity, nutrients, velocity), upwelling and current systems can also influence the larval dispersal (Melis et al. 2018). It has long been entrenched that genetic structure of populations affected by glacier activities where sea level encountered climatic fluctuations during the Pleistocene period and caused gene flow restrictions in marine organisms (Imbrie et al. 1992). However, relatively high haplotype diversity reported in freshwater fish inhabiting nonglaciated regions or temperate regions (Bernatchez and Wilson 1998; Tan et al. 2015). Most of the studies related to $O$. minor gave high haplotype diversity in this region, including the present study.

Our present investigation has significant implications for conservation and favourable management of $O$. minor along the Chinese coast and it also shed light on the
need for separate management of the Dongshan population: once an evolutionary lineage is lost, there is no possibility to be recover it (Mortiz 2002). We also recommend infield ontogenetic studies and larval migration ranges observations along with physical oceanographic parameters in future studies to understand the population genetics data inconsistency in $O$. minor reported along Chinese coastal waters.

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# Philippiphonte aspidosoma gen. et sp. n., a radically divergent member of the Laophontidae from shell gravel in the East Sea, South Korea, including a review of Folioquinpes Fiers \& Rutledge, 1990 (Copepoda, Harpacticoida) 

Rony Huys', Jimin Lee ${ }^{2}$<br>I Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK 2 Marine Ecosystem and Biological Research Center, Korea Institute of Ocean Science \& Technology, 385 Haeyangro, Yeongdo-gu, Busan, 49111, South Korea

Corresponding author: Rony Huys (r.huys@nhm.ac.uk)

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#### Abstract

The primarily marine subtidal family Laophontidae not only contains more valid genera than any other family in the Harpacticoida, it is also one of the most speciose ones in the order, currently accommodating 327 species and subspecies. Based on published records, 25 laophontid species in 12 genera have so far been reported from Korean waters. Here both sexes of a new genus and species of Laophontidae are described, collected from shell gravel off Dokdo Island in the East Sea. Philippiphonte aspidosoma gen. et sp. n. displays a radically divergent morphology, including an extreme dorsoventrally depressed body shape which is reminiscent of members of the family Porcellidiidae. The convergent evolution of dorsoventrally flattened body plans in the Harpacticoida is briefly discussed.

The distribution and habitat preference of laophontid species recorded from the Korean peninsula are summarised. The authenticity of the Korean record of Folioquinpes mangalis Fiers \& Rutledge, 1990 from washings of invertebrates and intertidal stones from Jeju Island is reassessed in the light of a discussion of the genus. Folioquinpes pseudomangalis sp. n. and $F$. indicus sp. n. are proposed as new species for Folioquinpes mangalis Fiers \& Rutledge, 1990 sensu Kim (2013) and Laophonte chathamensis Sars, 1905 sensu Sewell (1924), respectively. A key to species of Folioquinpes Fiers \& Rutledge, 1990 is provided.


## Keywords

Copepoda, Dokdo island, Folioquinpes indicus sp. n., F. pseudomangalis sp. n., key to species, Laophontidae, shell gravel

## Introduction

The Laophontidae is one of the most speciose families in the Harpacticoida, currently accommodating 327 species and subspecies and containing more valid genera (74!) than any other family in the order. Members of the family can be found in tropical to polar waters and typically occur subtidally in fine to coarse-grained sandy sediments at shallow depths. Laophontids have also radiated into a wide range of other habitats, including saltmarshes and intertidal mudflats (Barnett 1968; Bodin 1976), the fronds and holdfasts of macroalgae (Hicks 1977a-b), the interstitial environment of sandy beaches (Cottarelli et al. 1986, 2008; Fiers 1990, 1991), brackish lagoons (Hamond 1972; Heip 1969; Lee and Chang 2008), anchihaline and coastal marine caves (Chappuis 1938; Huys and Lee 2000) and the deep sea (Huys and Lee 2000; Lee and Huys 1999). Some species have entered into symbiotic associations with sea anemones, bryozoans, holothurians, chitons, and particularly crustaceans (Huys 2016; Yeom et al. 2018) while a few have colonised freshwater lacustrine habitats (Defaye and Dussart 2011; Lee and Chang 2005).

Based on published records, 25 laophontid species in 12 genera have so far been reported from Korean waters. The species listed in an unpublished PhD dissertation (Kim 2002) are not considered here. Microchelonia koreensis (Kim, 1991) was recorded from two species of holothurian kept in aquaria at fish markets, including Holothuria (Mertensiothuria) hilla Lesson, 1830 (family Holothuriidae) in Busan (Korea Strait), and Apostichopus japonicus (Selenka, 1867) (family Stichopodidae) in Gangneung (East Sea coast) and Mokpo (Yellow Sea coast) (Kim 1991). The same species was subsequently collected in washings of the latter host obtained at 15 m depth in Uljin (East Sea coast) (Kim 2013). Song and Chang (1995) collected Onychocamptus bengalensis (Sewell, 1934) from crab burrows on an intertidal mud flat on Chindo (Jindo) Island, southwestern Korea. Lee and Chang (2005) added a second record from Sokcho, northern East Sea coast and reported another two Onychocamptus species [O. mohammed (Blanchard \& Richard, 1791) and O. vitiospinulosa (Shen \& Tai, 1963)] from streams, freshwater lakes and oligohaline reservoirs. Additional records from estuaries, lakes and saltmarshes were listed for both species by Chang (2009, 2010). Quinquelaophonte koreana Lee, 2003 was described from a sandy beach in Taean on the west coast of the Korean peninsula (Lee 2003) while Song et al. (2010) reported Laophonte cornuta Philippi, 1840, Paralaophonte lacerdai Jakobi, 1953, P. obscura Vervoort, 1962, Heterolaophonte discophora Willey, 1929 and H. hamata Jakobi, 1954 from phytal communities on Ulva pertusa Kjellman in Pohang, eastern Korea. Lee et al. (2012) cited Paralaophonte congenera (Sars, 1908) as one of the most common laophontid species in Korea. Kim (2013) listed eight (sub)species in the genus Laophonte Philippi, 1840 (L. cornuta; L. thoracica Boeck, 1865; L. inopinata T. Scott,

1892; L. denticornis, T. Scott, 1894; L. inornata A. Scott, 1902; L. dinocerata Monard, 1926; L. elongata barbata Lang, 1934; L. longistylata Willey, 1935), three species in the genus Paralaophonte Lang, 1948 [P. macera (Sars, 1908), P. lacerdai, P. obscura], in addition to Heterolaophonte discophora, Harrietella simulans T. Scott, 1906, Echinolaophonte mirabilis (Gurney, 1927), Folioquinpes mangalis Fiers \& Rutledge, 1990, Robustunguis minor Fiers, 1992 and Psammoplatypus proprius (Lang, 1965). Several of these species were found in submerged wood infested by teredinid shipworms and limnoriid isopods or in washings of invertebrates such as sponges, barnacles, soft corals, and oysters. However, the great majority of these records should be considered as accidental associations (Huys 2016). Finally, Jejulaophonte hyeopjaeensis Back \& Lee, 2014 was recently described from a sandy beach on Jeju Island (Back and Lee 2014).

Both sexes of a new species were collected from shell gravel off Dokdo Island in the East Sea. The new species displays a radically divergent morphology and cannot be accommodated in any of the currently recognised genera. It is here fixed as the type species of a new genus, Philippiphonte gen. n., and described in detail. The authenticity of the Korean record of Folioquinpes mangalis from washings of invertebrates and intertidal stones from Jeju Island is reassessed in the light of a review of the genus Folioquinpes Fiers \& Rutledge, 1990.

## Materials and methods

Samples were collected by SCUBA diving by scooping the upper $\sim 5 \mathrm{~cm}$ of sublittoral sediments around Dokdo Island, East Sea (Sea of Japan), South Korea (Figure 1) during April and June 2015, and August 2016, and transferred to 1 -litre plastic bottles to which $7 \% \mathrm{MgCl}_{2}$ solution was added; after 5-10 min the samples were fixed in $10 \%$ formalin. In the laboratory, specimens were extracted from the sediments by flotationcentrifugation using the Ludox HS-40 colloidal silica polymer (Burgess 2001), and rinsed and filtered through a $63 \mu \mathrm{~m}$ mesh-size sieve with tap water. Copepods were sorted under a Leica M165C stereomicroscope, transferred to glycerine, and then dissected in lactic acid. Whole specimens and appendages were drawn using a camera lucida mounted on a Leica DM2500 microscope equipped with differential interference contrast. After examination, the dissected parts were mounted in lactophenol mounting medium and sealed.

The descriptive terminology is adopted from Huys and Boxshall (1991) and Huys et al. (1996). Abbreviations used in the text are: ae, aesthetasc; P1-P6, for swimming legs 1-6; exp, enp and benp for exopod, endopod and baseoendopod, respectively; exp (enp) $-1(-2,-3)$ to denote the proximal (middle, distal) segments of a ramus; apo for apophysis. The term 'acrothek' denotes the trifid setal structure found primitively on the apical margin of the distal antennulary segment (Huys and Iliffe 1998).

Type specimens were deposited in the National Biological Resources Center (NIBR), Incheon, Republic of Korea. Additional material was stored in the Korea Institute of Ocean Science and Technology (KIOST), Busan, Korea.


Figure I. Localities in Dokdo island, Korea where Philippiphonte aspidosoma gen. et sp. n. was collected: I Gajaebawi (type locality) $\mathbf{2}$ Mulgol $\mathbf{3}$ Old harbour.

## Systematics

Order Harpacticoida Sars, 1903
Family Laophontidae T. Scott, 1905
Subfamily Laophontinae T. Scott, 1905 sensu Huys \& Lee (2000)

## Genus Philippiphonte gen. n.

http://zoobank.org/F82FCADE-CF6B-4AAF-A465-F9DC8E73F9CD

Diagnosis. Laophontidae. Body extremely dorsoventrally flattened, porcellidiid-like. Distinct sexual dimorphism in size, urosomal segmentation, antennule, P3 endopod, P5, and P6. Rostrum large, inverted trapezoid; anterior margin slightly convex in $q$, virtually straight in $\widehat{J}^{\top}$. Cephalothoracic shield broadly bell-shaped; lateral margins fringed with closely set spinules. Pedigerous somites bearing legs $2-4$ with strongly developed pleurotergites, those of leg 4-bearing somite backwardly produced and embracing leg 5-bearing somite and anterior half of genital double-somite; each provided with strong spinules along lateral margins. Leg 5-bearing somite reduced, without marked pleurotergites. Genital double-somite completely fused. Second and third abdominal somites with lobate pleurotergites, those of penultimate somite embracing anal somite and anterior half of caudal rami. Anal somite without expanded pleurotergites; operculum naked. Caudal rami flattened, longer than wide, with straight outer and markedly convex inner margin; with medially directed spinules along inner margin and finer spinules along outer margin; with seven setae, all of which located near posterior margin of ramus; setae IV-V with fracture planes and fused at base.

Antennule slender, 5 -segmented and with aesthetasc on segment 3 in $q$, subchirocerate, 8 -segmented and with aesthetasc on segment 5 in $\sigma^{\lambda}$; without spinous pro-
cesses on segments $1-2$; segments $1-3$ with setules along anterior margin; segment 3 elongate in $q$. Antenna with allobasis bearing unipinnate seta along abexopodal margin. Exopod 1-segmented, with four elements. Mandible with slender gnathobase; palp small, comprising unisetose basis with incorporated endopod and discrete exopod, armed with three and one seta(e), respectively. Maxillule without defined rami; armature of palp represented by one lateral and three distal setae. Maxilla with two coxal endites; endopod with two setae. Maxilliped elongate and slender; syncoxa with one seta; endopod represented by acutely recurved claw with minute accessory seta at its base.

Legs 1-4 with very wide and narrow intercoxal sclerites. Leg 1 with sparsely plumose inner and outer seta on basis; exopod 3-segmented with long outer spine on exp-1, extending beyond distal margin of exp-3 and bearing stiff spinules along its outer margin; exp-2 with outer unipinnate spine; exp-3 with two unipinnate spines and two geniculate setae; endopod 2 -segmented, prehensile, enp-1 unarmed, enp-2 with short claw but accessory seta not discernible. Legs 2-4 with transversally elongate bases, with long (P2-P3) or short (P4) outer seta; with 3-segmented exopods and 2-segmented endopods (except for P3 endopod 3-segmented in $\delta^{\top}$ ); outer exopodal spines typically unipinnate in distal half only, inner setae very long and plumose; outer margin of $\mathrm{P} 2-\mathrm{P} 4$ enp-2 with double row of flimsy setular extensions. Leg $3 \widehat{\sigma}$ with outwardly recurved, spinous apophysis on enp-2; enp-3 with one inner and two apical setae. Armature formulae:

|  | Exopod | Endopod |
| :---: | :---: | :---: |
| P2 | 0.1 .123 | 0.120 |
| P3 | 0.1 .223 | $0.121\left[0 . \mathrm{apo} .120 \mathrm{in} \mathrm{o}^{\top}\right]$ |
| P4 | 0.1 .223 | 0.121 |

Leg 5 biramous; baseoendopod very elongate, backwardly recurved, with outer basal seta arising from short dorsal setophore; endopodal armature represented by three setae in $q$ and one seta in $\delta^{\lambda}$; exopod with four elements in $q$ and three elements in $\delta^{\lambda}$.

Genital field $q$ located near border with leg 5-bearing somite. P6 forming well developed operculum with two small setae in $\mathcal{+}$; asymmetrical in $\delta^{\lambda}$ (with dextral or sinistral configuration), with outer distal corner bearing one minute seta.

Type species. Philippiphonte aspidosoma gen. et sp. n. (by original designation).
Etymology. The genus is dedicated to Rudolph Amandus Philippi (14 September 1808-23 July 1904), author of the type genus Laophonte Philippi, 1840 and of the first publication to adopt the term "copepod" in its title (Philippi 1843). Many of Philippi's $(1840,1843)$ generic names such as Aenippe, Euryte, Idomene, Idya (= Tisbe), Metis, Oncaea, Psamathe (= Scutellidium) and Thyone (= Porcellidium) were named after figures of Ancient Greek mythology and so was also Laophonte, named after a daughter of Pleuron, and the wife of Thestius, by whom she had Althaea and Leda.

## Philippiphonte aspidosoma sp. n.

http://zoobank.org/7C22C6B6-91CD-42FC-934E-4FCCEAA5979B
Figs 2-7
Type locality. South Korea, East Sea (Sea of Japan), Gajaebawi, Dokdo island (Liancourt Rocks), $37^{\circ} 14^{\prime} 49.37^{\prime \prime} \mathrm{N}, 131^{\circ} 51^{\prime} 48.24^{\prime \prime} \mathrm{E}$, shell gravel, 22 m depth (Figure 1).

Type material. Holotype $q$ dissected on 11 slides (reg. no NIBRIV0000816435), allotype $\widehat{ }$ dissected on 11 slides (reg. no NIBRIV0000816434), remaining paratypes ( 9 우, $1 \delta^{\text {² }}$ ) preserved in formalin (reg. no NIBRIV0000816433). All type specimens were collected on 23 April 2015 from the type locality and are deposited in the National Biological Resources Center (NIBR), Incheon.

Additional material examined. $1 \delta^{\wedge}$ from Mulgol, Dokdo island, $37^{\circ} 14^{\prime} 35.16{ }^{\prime \prime} \mathrm{N}$, $131^{\circ} 51^{\prime} 51.37^{\prime \prime} \mathrm{E}, 15 \mathrm{~m}$ depth, 27 June 2015 (reg. no MInRB-Hr15-L001); $1 \delta^{\lambda}$ from the old harbour of Dokdo island, $37^{\circ} 14^{\prime} 27.31^{\prime \prime} \mathrm{N}, 131^{\circ} 52^{\prime} 16.69^{\prime \prime} \mathrm{E}, 12 \mathrm{~m}$ depth, 27 June 2015 (reg. no MInRB-Hr15-L002); 2 우, $4 \boldsymbol{O}^{\lambda} \delta^{3}, 24$ August 2016 from type locality (Figure 1) (reg. no MInRB-Hr15-L003). All specimens are deposited in the collections of the Korea Institute of Ocean Science and Technology (KIOST), Busan.

Description of female. Body length from anterior margin of rostrum to posterior margin of caudal rami 536-612 $\mu \mathrm{m}$ (mean $=574 \mu \mathrm{~m} ; n=12$; holotype $=552 \mu \mathrm{~m}$ ); maximum width measured at level of leg 3 -bearing somite: $338 \mu \mathrm{~m}$ (in holotype). Body (Figure 2A) extremely dorsoventrally flattened, porcellidiid-like; except for digestive tract and ovaries completely transparent; dorsal surface of all somites covered with minute setules and denticles (not illustrated); ventral surface of urosomites without surface ornamentation (except for spinule rows around posterior margin). Rostrum large, prominent in dorsal aspect, inverted trapezoid; anterior margin slightly convex, anterolateral corners each with sensillum. Cephalothoracic shield broadly bell-shaped, about 1.5 times wider than long; lateral margins fringed with closely set spinules; dorsal surface with symmetrical pattern of sensilla; posterior margin with setules and spinules. Pedigerous somites bearing legs 2-4 with strongly developed pleurotergites, those of leg 4-bearing somite backwardly produced and embracing leg 5 -bearing somite and anterior half of genital double-somite; each provided with strong spinules along lateral margins and shorter spinules along posterior margin; sensillar pattern as illustrated. Leg 5-bearing somite reduced, without marked pleurotergites; posterior margin with spinules dorsally and setules dorsolaterally. Genital double-somite completely fused; original segmentation marked by sensillar pattern, faint dorsal suture and paired arrangement of backwardly directed pleurotergites, each fringed with long spinules laterally and shorter spinules or setules posteriorly; anterior pair larger than posterior pair; ventral posterior margin with paired rows of tiny spinules (Figure 3A). Second and third abdominal somites with lobate pleurotergites, those of penultimate somite embracing anal somite and anterior half of caudal rami; dorsal and lateral ornamentation as in previous somites; ventral posterior margin with paired rows of tiny spinules (Figure 3A). Anal somite without expanded pleurotergites (Figs 2A; 4A-B); dorsal surface

with paired tube-pores and sensilla flanking rounded, naked anal operculum; ventral surface with two pairs of tube-pores and tiny spinules near bases of caudal rami; anal frill triradiate, well developed, provided with long setular extensions.

Caudal rami (Figure 4A-B) flattened, about 2.2 times longer than maximum width, with straight outer and markedly convex inner margin; with elaborate ornamentation consisting of strong, medially directed spinules along inner margin, finer spinules along outer margin, and two spinule rows in anterior half of ventral surface. Armature consisting of seven setae, all of which located near posterior margin of ramus; seta I minute, positioned dorsally near naked seta II; seta III located at outer distal corner, naked; setae IV-V with fracture planes and fused at base; seta IV sparsely pinnate, about $18 \%$ of body length; seta V very long, about 3.5 times length of seta IV (Figure 2A), with minute spinules in middle third and sparse setules in distal quarter; seta VI located at inner distal corner; seta VII located near posterior margin of ramus, triarticulate at base and sparsely plumose in distal third.

Antennule (Figure 2B) 5-segmented, slender; without spinous processes on segments 1-2; segment 1 with setules along anterior and ventral distal margin, those on the latter being particularly long; anterior margin of segments 2 and 3 (proximal half only) with short setules; segment 3 longest, about 2.4 times as long as segment 1 (measured along anterior margin), with aesthetasc ( $114 \mu \mathrm{~m}$ ) arising from socle and fused at base to long naked seta. Armature formula 1-[1 plumose], 2-[4 +4 plumose], $3-[5+2$ plumose $+(1+\mathrm{ae})], 4-[1], 5-[8+$ acrothek $]$; apical acrothek consisting of two basally fused setae, aesthetasc not observed.

Antenna (Figure 2C) with allobasis, bearing two spinule rows and slender unipinnate seta along abexopodal margin. Exopod 1-segmented, with two lateral and two apical bipinnate setae (outer one slightly spiniform). Free endopod with two spines and one seta laterally, and distal armature consisting of two geniculate setae, one long (fused at base to vestigial seta) and two short pinnate spines.

Mandible (Figure 3B) with slender gnathobase bearing several multicuspidate teeth and one unipinnate seta. Palp small, comprising basis with incorporated rami; armature of basis represented by one plumose seta originating from small articulating socle; endopod represented by one short and two long plumose setae; exopod represented by one sparsely pinnate seta.

Maxillule (Figure 3C) with well-developed syncoxal arthrite bearing two spinule rows on posterior surface and total of eight elements along distal margin. Coxal endite with one naked seta and one unipinnate spine. Basis without defined rami; armature represented by one lateral and three distal setae (innermost of which spiniform and unipinnate).

Maxilla (Figure 3D). Syncoxa with spinules along distal outer margin and two coxal endites; proximal endite with naked seta and basally fused unipinnate spine, distal endite with two setae of which innermost one fused at base. Allobasis produced into distally unipinnate claw, with accessory armature consisting of small naked seta and unipinnate spiniform element. Endopod represented by a minute segment with two basally fused setae.


Figure 3. Philippiphonte aspidosoma gen. et sp. n. (q): A urosome and right leg 5, ventral B mandible [inset showing gnathobase from different angle] $\mathbf{C}$ maxillule, anterior [inset showing small unipinnate element arising from posterior surface] $\mathbf{D}$ maxilla $\mathbf{E}$ maxilliped $\mathbf{F}$ leg 1, anterior [exp-2 and -3 disarticulated].

Maxilliped (Figure 3E) elongate and slender. Syncoxa with one sparsely plumose seta and tuft of long setules near distal inner corner and additional inner setules around base. Basis without ornamentation except for few spinules near outer distal corner. Endopod represented by acutely recurved claw with minute accessory seta at its base.

Leg 1 (Figure 3F) with very wide and narrow intercoxal sclerite. Basis with sparsely plumose inner (anterior) and outer seta. Exopod 3-segmented, all segments of about equal size; exp- 1 with long outer spine, extending beyond distal margin of exp-3 and bearing stiff spinules (gradually increasing in size distally) along its outer margin; exp-2 and -3 wider than long, with tuft of setules along inner margin; exp-2 with outer spine being unipinnate in its distal half; exp-3 with two unipinnate spines and two geniculate setae (pinnules restricted to apical parts of elements). Endopod 2-segmented, prehensile; enp-1 elongate, about five times as long as wide, unarmed, with long spinules along proximal half of inner margin; enp-2 with short, acutely recurved claw, outer distal corner with few spinules but accessory seta not discernible.

Legs 2-4 (P2-P4) (Figs 4C-D; 5A) with widely separated members connected by narrow intercoxal sclerites. Praecoxae represented by small U-shaped sclerite. Coxae with spinular ornamentation on anterior surface as figured. Bases transversally elongate, becoming progressively longer from P2 to P4; outer margin with setules (P2) or multiple rows of spinules (P3-P4); with long (P2-P3) or short (P4) outer seta, bipinnate in P2 only; anterior surface with tube-pore. Exopods 3-segmented; exp-1 without inner seta; inner margin of exp-1 and -2 with few long setules; outer margin of all segments with spinular ornamentation as figured; P3 exp-3 with tube-pore on anterior surface; outer exopodal spines typically unipinnate in distal half only (except for outer spine of exp-1 and proximal outer spine on exp-3 of P 4 being bipinnate); inner setae very long and plumose. Endopods 2 -segmented; enp-1 unarmed, shorter than enp-2, with setules along both inner and outer margins; outer margin of enp-2 with double row of flimsy setular extensions; outer distal spine of P3 enp-2 bipinnate. Spine and setal formulae of swimming legs as for genus.

Leg 5 (Figure 3A) consisting of baseoendopod and 1 -segmented exopod. Baseoendopod subcylindrical and elongate (about 8.5 times as long as average width), backwardly recurved and fused at base to pleural wall of somite; bearing outer basal seta arising from short setophore (located dorsally); endopodal armature consisting of long seta located at about two-thirds the segment length, and two closely set, minute setae originating near boundary with exopod; all setae naked; proximal third with tube-pore on ventral surface. Exopod about one third the size of baseoendopod; inner margin with one bipinnate and one unipinnate seta, distal margin with long plumose and short naked seta.

Genital field (Figure 3A) located in anterior third of genital double-somite, near border with leg 5-bearing somite. Genital apertures closed off by opercula derived from vestigial sixth legs, each bearing two minute, naked setae. Copulatory pore median, of moderate size. Egg-sac not observed.

Description of male. Slightly smaller than female; body length from anterior margin of rostrum to posterior margin of caudal rami $461-527 \mu \mathrm{~m}$ (mean $=489 \mu \mathrm{~m}$; $n$


Figure 4. Philippiphonte aspidosoma gen. et sp. n. ( $q$ ): A anal somite and left caudal ramus, dorsal B anal somite and left caudal ramus, ventral $\mathbf{C} \operatorname{leg} 2$, anterior $\mathbf{D}$ leg 3, anterior.


Figure 5. Philippiphonte aspidosoma gen. et sp. n.: A leg 4 , anterior Burosome đ (excluding leg 5 -bearing somite), ventral $\mathbf{C}$ anal somite and right caudal ramus $\hat{\delta}^{\hat{}}$, dorsal $\mathbf{D}$ anal somite and right caudal ramus $\hat{\delta}$, ventral $\mathbf{E} \operatorname{leg} 5 \hat{\delta}$, ventral.


Figure 6. Philippiphonte aspidosoma gen. et sp. n. ( $\left(^{\top}\right)$ : A habitus, dorsal $\mathbf{B}$ antennule, ventral [segments $1-4$ disarticulated; insert showing apical armature of segment 8 in dorsal aspect] $\mathbf{C l e g} 3$ endopod, anterior.
$=8$; allotype $=523 \mu \mathrm{~m}$ ); maximum width measured near posterior margin of cephalothorax: $315 \mu \mathrm{~m}$ (in allotype). Body (Figure 6A) of similar shape, transparency and with virtually identical ornamentation as in female. Rostrum comparatively narrower than in female and with virtually straight anterior margin. Genital and first abdominal somites completely free; posterior margin of former with continuous row of short spinules or setules posteriorly; lobate pleurotergites of genital somite more slender than in female. Anal somite and caudal rami (Figure 5B-D) as in female.

Antennule (Figure 6B) 8-segmented, subchirocerate, with geniculation between segments 5 and 6; without spinous processes on segments $1-2$. Segment 1 as in female; anterior margin of segments 2 and 3 (proximal half only) with setules; segment 4 represented by an incomplete U-shaped sclerite; segment 5 swollen, with large aesthetasc $(125 \mu \mathrm{~m})$ arising from socle and fused at base to long naked seta; segments 5 and 6 with setae modified into basally fused spinous processes. Armature formula 1-[1 plumose], 2-[ $4+5$ plumose], $3-[7+1$ plumose], $4-[2], 5-[8+2$ spinulose +2 spinous processes $+(1+\mathrm{ae})], 6-[2$ spinous processes], $7-[1], 8-[7+$ acrothek $]$; apical acrothek consisting of two basally fused setae, aesthetasc not observed.

Leg 3 (Figure 6C) with 3-segmented endopod. Enp-1 shortest, with few setules on both outer and inner margins. Enp-2 forming slender, outwardly recurved, spinous apophysis (homologue of outer distal spine of enp-2 in female) provided with barb along inner margin and minute projections near apex; setules present on both outer and inner margins. Enp-3 with one inner and two apical plumose setae.

Leg 5 (Figure 5E) consisting of baseoendopod and 1 -segmented exopod. Baseoendopod elongate, backwardly recurved and fused to pleural wall of somite; bearing outer basal seta arising from short setophore (located dorsally); endopodal armature consisting of two closely set, vestigial setae near boundary with exopod; proximal third with tube-pore on ventral surface. Exopod about one third the size of baseoendopod; inner margin with one strong, bipinnate seta, distal margin with one long and one short naked seta.

Sixth legs (P6) (Figure 5B) asymmetrical with functional right member articulating at base and closing off genital aperture and left member fused at base to genital somite; each vestigial sixth leg with minute naked seta. Spermatophore oval, relatively small (65 $\mu \mathrm{m}$ ).

Etymology. The specific epithet is derived from the Greek ó $\sigma \pi i ́ s$, meaning shield, and $\sigma \tilde{\omega} \mu \alpha$, meaning body, and alludes to the dorsoventrally flattened shield-shaped body form.

## Discussion

## Taxonomic position of Philippiphonte aspidosoma gen. et sp. n.

The new genus can readily be identified as a member of the family Laophontidae because of the morphology of leg 1, including the presence of a pedestal on the basis used
for the insertion of the endopod, the displacement of the inner spine onto the anterior surface of the basis, and the modification of the outer (= anterior) distal element of enp-2 into a large non-geniculate claw (Huys 1990a). The inner (= posterior) distal element on enp-2 which is typically reduced to the size of a setule in laophontids appears to be absent in P. aspidosoma. The new genus is placed in the subfamily Laophontinae based on the following synapomorphies as defined by Huys and Lee (2000): (a) male antennule with up to three segments distal to geniculation, (b) mandible without discrete exopod, (c) maxilliped with maximum two setae on syncoxa, (d) P1 enp-1 without inner seta, (e) P2 enp-2 without outer spine, (f) proximal outer setae of female P5 exopod with distinctly separated insertion sites, and (g) absence of cup-shaped transformed pores on legs, somites or caudal rami.

Philippiphonte aspidosoma is morphologically radically divergent from other members of the family, justifying its assignment to a new genus. Unique autapomorphies that define the genus Philippiphonte include (a) the extremely dorsoventrally flattened, porcellidiid-like body shape in both sexes (Figs 2A, 6A), (b) the inverted trapezoid shape of the rostrum (Figs 2A, 6A), (c) the flattened caudal rami with elaborate spinular ornamentation along inner and outer margins (Figs 4A-B, 5C-D), (d) the remarkably slender antennules in the $q$, characterised by a very elongate third segment (Figure 2B), (e) basal seta of mandibular palp originating from small articulating socle (Figure 3B), (f) P1 exp-1 with long outer spine, extending beyond distal margin of exp-3 and bearing stiff spinules (Figure 3F), (g) legs 2-4 with widely separated members connected by narrow intercoxal sclerites and with transversally elongate bases, becoming progressively longer from P2 to P4 (Figs 4C-D, 5A), and (h) P5 baseoendopod subcylindrical, elongate, backwardly recurved and fused at base to pleural wall of supporting somite in both sexes (Figs 3A, 5E). Another character of interest is the presence of only one seta on the male sixth legs (Figure 5B), the plesiomorphic 2-setae condition being typical for members of the Laophontidae. The only other reported exception is found in the esolinid Applanola hirsuta (Thompson \& Scott, 1903) which has unarmed sixth legs (Huys and Lee 2000: fig. 19D).

The interrelationships of the Laophontidae are poorly resolved despite decades of morphological studies with the least confidently resolved part of the tree being the relative positions of the 65 genera in the subfamily Laophontinae. The significance of patterns of swimming leg sexual dimorphism in unravelling relationships among certain laophontid lineages has been demonstrated repeatedly in a number of studies (e.g., Lee and Huys 1999; Gómez and Boyko 2006; McCormack 2006; Huys and Lee 2009). However, except for the apophysis on the male P3 endopod (Figure 6C) no other sexual dimorphism is expressed on legs 2-4 of P. aspidosoma. The presence of such an apophysis is phylogenetically uninformative at generic level since it is a convincing synapomorphy uniting the families of the Laophontoidea (Huys 1990a; Huys and Lee 1998, 1999). Given the many autapomorphic character traits expressed in its body plan, the identity of the closest relative of $P$. aspidosoma will probably remain elusive until the arrival of molecular data. The swimming legs of P. aspidosoma are characterised by the presence of a double row of setular extensions along the weakly chi-
tinised outer margin of P2-P4 enp-2 (Figs 4C-D,5A). Similar parallel rows of flimsy extensions, possibly surrounding a glandular opening, have previously been reported in Marbefia carthyi (Hamond, 1968) (Huys and Lee 2009: Figs 5B, 6A-C). Although the latter represent positional homologues to the structures observed in P. aspidosoma there is no additional morphological evidence suggesting a close relationship between Philippiphonte and Marbefia Huys \& Lee, 2009. The same applies to the laophontid genera Asellopsis Brady \& Robertson, 1873, Platylaophonte Bodin, 1968, Applanola Huys \& Lee, 2000 and Peltidiphonte Gheerardyn \& Fiers in Gheerardyn et al., 2006, all of which have a more or less dorsoventrally depressed body shape, but display no other apomorphic similarities in support of a direct relationship with Philippiphonte. As previously suggested by Gheerardyn et al. (2006) the somewhat similar body shape in these genera is more than likely the result of convergent evolution.

All members of the families Porcellidiidae and Peltidiidae are exclusively dorsoventrally depressed. However, flattened body shapes have also evolved in many other harpacticoid lineages (Figure 7). In some harpacticoid genera the dorsoventral flattening of the body is an adaptation to a mode of life associated with smooth, flat surfaces such as macroalgae (Noodt 1971; Hicks 1980) or the inside surface of gastropod shells used by anomuran decapods (Huys 2016). Since such substrates are commonly exposed to strong water currents, a low-profile body shape evidently helps the copepod maintaining its position on the surface, designed to disturb the water flow as little as possible. In other cases, dorsoventral flattening has been assumed to be an adaptation to life on coral fragments in an environment with strong currents (Gheerardyn et al. 2006) or with invertebrate hosts (Huys 1990b). In its least modified form flattening involves only the prosome with no (e.g., Scutellidium spp.; Figure 7P) or very moderate (e.g., Xouthous spp.; Donsiella spp.; Peltobradya spp.; Figure 7I-J, L, O) dorsoventral depression of the urosome. However, in most low-profile body shapes the latter tagma displays a similar modification as the prosome, often blurring the boundary between both. A common way by which urosomal flattening is achieved is by enlargement of the genital double-somite resulting from transversal expansion (Figure $7 \mathrm{G}-\mathrm{H}, \mathrm{K}, \mathrm{M}$ ) and/or the formation of pleural wings (Figure 7A-D, F, N). The genital double-somite can become very large in relation to the rest of the body (e.g., Paramenophia spp.; Figure 7 H ) and can incorporate additional somites posterior to it to form a genital complex which embraces the anal somite and caudal rami. This condition is found in some genera of the Peltidiidae (Neopeltopsis Hick, 1976; Figure 7C, F) and all members of the Porcellidiidae (Figure 7B).

The shape of the rostrum, the general ovoid, shield-shaped habitus and the degree of dorsoventral compression in Philippiphonte are somewhat reminiscent of the condition in the Porcellidiidae (compare Figure 7A, B). However, the morphology of the rest of the body shows important differences. In all Porcellidiidae the dorsal cephalic shield and epimeral plates of the free pedigerous somites are typically provided with a marginal hyaline membrane. Hence, during attachment the body is sealed around most of its perimeter by a membranous extension applied to the surface of the substratum, offering optimal suction efficiency. Attachment is achieved with the aid of a ventral


Figure 7. Harpacticoid copepods exhibiting dorsoventrally flattened body shapes (females only, dorsal view; genital double-somite shaded). A Philippiphonte aspidosoma (Laophontidae) B Porcellidium viride (Philippi, 1840) (Porcellidiidae) C Neopeltopsis pectinipes Hicks, 1976 (Peltidiidae) D Hamondia superba Huys, 1990 (Hamondiidae) E Alteutha oblonga (Goodsir, 1845) (Peltidiidae) F Peltidium purpureum Philippi, 1839 (Peltidiidae) G Zaus abbreviatus Sars, 1904 (Harpacticidae) H Paramenophia platysoma (Thompson \& Scott, 1903) (Thalestridae) I Xouthous purpurocinctus (Norman \& Scott, 1905) (Pseudotachidiidae) J Xouthous parasimulans (Médioni \& Soyer, 1968) (Pseudotachidiidae) K Mucropedia kirstenae Bouck, Thistle \& Huys, 1999 (Harpacticidae) L Donsiella phycolimnoriae Hicks, 1990 (Pseudotachidiidae) M Alteuthoides kootare Hicks, 1986 (Peltidiidae) N Peltidiphonte rostrata Gheerardyn \& Fiers in Gheerardyn, Fiers, Vincx \& De Troch, 2006 (Laophontidae) O Peltobradya bryozoophila Médioni \& Soyer, 1968 (Ectinosomatidae) P Scutellidium arthuri Poppe, 1884 (Tisbidae).
sucker formed by the modified mandibular palps and first pair of legs (Tiemann 1986). In $P$. aspidosoma the lateral margins of the cephalothorax and pleurotergites of the free pedigerous somites are fringed with closely set spinules and no specialised suction device is present. In $P$. aspidosoma the somites bearing legs $2-4$ are transversally expanded forming large pleurotergites while in porcellidiids only those bearing legs 2-3 are modified in a similar way, the leg 4-bearing somite being markedly smaller (note that in members of the laophontid genus Peltidiphonte such well-developed pleurotergites are present on the somites bearing legs $2-5$ : Figure $7 N$ ). The genital complex in the Porcellidiidae forms a single pair of backwardly produced extensions which typically embrace the anal somite and caudal rami. In P. aspidosoma the genital double-somite has two sets of strongly developed pleurotergites and the second and third abdominal somites each one pair, the last one surrounding the anal somite and part of the caudal rami (Figure 2A).

The transition from an epibenthic to a mesopsammic life style has evolved independently and successfully many times in various lineages of the Harpacticoida. Adaptation to the three-dimensional labyrinth of the interstitial system of sand grains and shell gravel is primarily achieved by miniaturisation and/or the adoption of vermiformicity or a cylindrical body shape, thus enhancing flexibility and wriggling ability. Dwarfism often leads to a simplification in body morphology, most commonly resulting in the loss of swimming leg segments and rami or even entire limbs. Within the Laophontidae such regressive evolution linked to an interstitial mode of life can be observed in at least 14 genera characterised by a cylindrical body form, including Laophontina Norman \& Scott, 1905, Klieonychocamptoides Noodt, 1958, Afrolaophonte Chappuis, 1960, Stygolaophonte Lang, 1965, Mexicolaophonte Cottarelli, 1977, Galapalaophonte Mielke, 1981, Novolaophonte Cottarelli, Saporito \& Puccetti, 1983, Indolaophonte Cottarelli, Saporito \& Puccetti, 1986, Amerolaophontina Fiers, 1991, Wellsiphontina Fiers, 1991, Spiniferaphonte Gheerardyn \& Fiers, 2007, Raowellsia Özdikmen, 2008, Aequinoctiella Cottarelli, Bruno \& Berera, 2008, and Fiersiphontina Bruno \& Cottarelli, 2011 (Noodt 1958; Lang 1965; Cottarelli 1977; Mielke 1981; Cottarelli et al. 1983, 1986, 2008; Wells and Rao 1987; Fiers 1990, 1991; Gheerardyn et al. 2007; Bruno and Cottarelli 2011). An alternative - less common - adaptation to the interstitial environment is flattening of the body but this appears to occur only in copepods that inhabit substrata with larger crevices such as shell gravel. Within the Laophontidae adoption of a dorsoventrally depressed body form as an adaptation to the interstitial habitat has evolved convergently at least twice, i.e. in Peltidiphonte and Philippiphonte.

## A review of the genus Folioquinpes Fiers \& Rutledge, 1990

Fiers and Rutledge (1990) proposed the genus Folioquinpes to accommodate Laophonte chathamensis Sars, 1905 and a new species F. mangalis. Sars's (1905) description did not include a discussion on possible relationships but Sewell (1924) believed that there was a close affinity with Laophonte mohammed Blanchard \& Richard, 1891, to the ex-
tent that both species may well turn out to be synonymous. Nicholls (1941) placed $L$. chathamensis, together with L. mohammed and L. bengalensis Sewell, 1934, in the mo-hammed-group of the genus Laophonte. This group of fresh and brackish water forms is effectively equivalent to the mohammed-group delimited by Lang (1948) within the genus Onychocamptus Daday, 1903. Lang $(1944,1948)$ resurrected the latter genus after it had previously been synonymised with Laophonte by Zykoff (1904). Lang did not expound on the new placement of $L$. chathamensis but it is conceivable that the short antennule in the female, the presence of only three setae on the female P5 endopodal lobe and the shape of the P1 have influenced his generic assignment.

Mielke (1981) found two ovigerous females in the Galápagos which he provisionally identified as Onychocamptus spec. He suspected possible conspecificity with $O$. chathamensis which was subsequently confirmed by Fiers \& Rutledge (1990). The latter authors highlighted the absence of distinct swimming leg sexual dimorphism in $O$. chathamensis, a character standing in marked contrast with the modified 3-segmented P3 endopod and strongly built P2-P4 exopods exhibited by males of other Onychocamptus species. Sars (1905) stated that the exopods of P3-P4 were somewhat more strongly developed in the male but Fiers and Rutledge (1990) failed to find any sexual dimorphism upon re-examination of material from Papua New Guinea, Guadeloupe and the Philippines (Fiers, unpubl. data). In F. mangalis, they did, however, note that the outer spines on P2-P4 were stronger in the male. The foliaceous P5 exopod showing a reduced armature in the female and the absence of strongly modified $\mathrm{P} 2-\mathrm{P} 4$ exopods and sexual dimorphism on the P3 endopod in the male, were regarded as the primary diagnostic features of Folioquinpes (Fiers and Rutledge 1990).

Schizas and Shirley (1994), who were unaware of the publication of Folioquinpes, recognised two lineages within Onychocamptus, based on the shape of the P5 endopodal lobe in the female: the mohammed-group and the chathamensis-group [including $O$. chathamensis and Onychocamptus spec. sensu Mielke (1981)].

Folioquinpes chathamensis and $F$. mangalis assume a bizarre geographical distribution, including widely separated records from the Atlantic and Western Pacific oceanic basins. Remarkably, both species have been recorded from the northern coast of Papua New Guinea and exist in relative proximity in the Caribbean. Unless both species are widely distributed throughout the Indo-Pacific (for which there is no compelling evidence at present) this may indicate the existence of a complex of sibling species, each with a more restricted distribution. In this context, Mielke (1981) did not rule out the possibility that his Galápagos material of $F$. chathamensis represents a distinct "subspecies".

Sewell's (1924) specimens of $F$. chathamensis from Chilika (= Chilka) Lake, India differ from Sars' (1905) type description in (a) the female P5, showing three outer setae on the exopod and a very short, blunt spine apically, (b) the more slender P1 endopod, and (c) the shorter P4 enp-2. Since no variability in these characters has been recorded by either Sars (1905) or Fiers and Rutledge (1990), both of whom examined ample material, the differences recorded in the Chilika Lake population are considered sufficient to warrant separate specific status; Sewell's (1924) material is consequently renamed here as
F. indicus sp. n. Similarly, Rühe's (1914) record of F. chathamensis from South Africa requires confirmation. His illustrations show distinctly longer caudal rami, reduced pleural extensions on the abdominal somites, and longer setae on the female P5 baseoendopod. Rühe's concise description does not enable us to reach a final verdict on the specificity of his specimens. Pending the re-examination of new material, Laophonte chathamensis sensu Rühe (1914) is here considered species inquirenda in Folioquinpes. The recent record of F. mangalis from South Korea (Kim 2013) is based on a misidentification and attributed below to a new species, F. pseudomangalis sp. n. Differentiating characters between the four species of Folioquinpes and those of related genera are summarised in Table 1.

Diagnosis. Laophontidae. Body moderately to strongly dorsoventrally depressed. Integument of cephalothorax and body somites with dense pattern of long spinules; dorsal posterior margins of somites with sensillate tubercles. Rostrum partially delimited at base; prominent and bell-shaped, with (F. mangalis, F. pseudomangalis sp. n.) or without (F. chathamensis, $F$. indicus sp. n.) spinules between apical sensilla. Genital double-somite $q$ bilaterally incised, with dorsal and lateral transverse chitinous ribs marking original segmentation. Pleural extensions of $q$ abdominal somites moderately to strongly (conical) developed. Caudal ramus elongate, cylindrical, with spinules along inner and (often) outer margin; with seven setae; seta V well developed, with fracture plane, fused to short seta IV; seta VI reduced, setiform; ramus slightly sexually dimorphic in $F$. mangalis (inner margin less convex in $\delta^{\top}$ ). Anal operculum spinulose.

Sexual dimorphism in antennule, P5, P6 and in genital segmentation. Slight dimorphism in exopods of $\mathrm{P} 3-\mathrm{P} 4$, abdominal ornamentation and caudal ramus shape.

Antennule short and 4 - or 5 -segmented in $q$, all segments densely spinulose; 8 -segmented and subchirocer with three segments distal to geniculation in 0 ; segment 1 with strong spinules along anterior margin; segment 2 sometimes with small blunt process near posterior margin; with aesthetasc on segment $3(q)$ or $5\left(\delta^{\top}\right)$ and probably as part of acrothek on apical segment; segment $6 \sigma^{\lambda}$ with three hyaline extensions. Antenna with four setae on exopod; allobasis with abexopodal seta. Mandibular palp elongate, 1-segmented; with one basal, one exopodal and three endopodal setae. Maxillule with defined exopod bearing two setae. Maxilla with three endites on syncoxa; endopod represented by two setae. Maxilliped moderately robust; syncoxa with one seta; basis with spinules along both margins; endopodal claw curved, with accessory seta at base.

P1 with 2-segmented exopod, with long pinnate outer spine on exp-1, and three spines and two geniculate setae on exp-2; endopod moderately stout, enp-1 without inner seta, enp-2 with minute seta and short, strong claw. Swimming legs with 3 -segmented exopods and 2 -segmented endopods in both sexes; segments and/or outer spines of P3-P4 exopods somewhat stronger in $\widehat{o}^{\lambda}$. Armature formula as follows:

|  | Exopod | Endopod |
| :---: | :---: | :---: |
| P2 | 0.1 .123 | 0.220 |
| P3 | $0 .(0-1)^{*} .123$ | $0 .(2-3) 21$ |
| P4 | *: variability in $F$ mangalis | 0.111 |

Table I. Number of segments in female antennule (A1), number of setae on antennary exopod (A2) and armature of P2-P4 (female) and P5 (both sexes) of members of Onychocamptus Daday, 1903 and allied genera.

|  | A1 | A2 | P2 |  | P3 |  | P4 |  | P5 + |  | $\begin{gathered} \hline \mathbf{P 5} \delta^{\top} \\ \hline \exp \\ \hline \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | $\exp$ | enp | $\exp$ | enp | $\exp$ | enp | exp | benp |  |
| Onychocamptus |  |  |  |  |  |  |  |  |  |  |  |
| mohammed (Blanchard \& Richard, 1891) | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 3 | 2 |
| bengalensis (Sewell, 1934) | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 3 | 2 |
| besnardi Jakobi, 1954 | 5 | 4 | 0.1 .123 | 0.220 | 0.1 .123 | $0.321^{\text {a }}$ | 0.1.022 | 0.111 | 3 | 3 | 2 |
| vitiospinulosa (Shen \& Tai, 1963) | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 2 | 2 |
| anomalus (Ranga Reddy, 1984) | 5 | 1 | 0.1.123 | 0.220 | 0.1 .123 | 0.321 | 0.1.122 | 0.111 | 4 | 3 | 3 |
| taifensis Kikuchi, Dai \& Itô, 1993 | 5 | 4 | 0.1.123 | 0.120 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 3 | 2 |
| krusensterni Schizas \& Shirley, 1994 | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | $0.1 .122^{\text {b }}$ | 0.111 | 3 | 3 | 2 |
| Folioquinpes |  |  |  |  |  |  |  |  |  |  |  |
| chathamensis (Sars, 1905) | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 3 | $2^{\text {c }}$ |
| indicus sp. $\mathbf{n}$. | 5 | 4 | 0.1 .123 | 0.220 | 0.1 .123 | 0.321 | 0.1.123 | 0.111 | 4 | 3 | ? |
| mangalis Fiers \& Rutledge, 1990 | 4 | 4 | 0.1.123 | 0.220 | $0.0-1^{\text {d }} .123$ | 0.221 | 0.0.123 | 0.111 | 4 | 2 | 2 |
| pseudomangalis sp. $\mathbf{n}$. | 4 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.221 | 0.0.123 | 0.111 | 4 | 3 | 2 |
| Onychoquinpes |  |  |  |  |  |  |  |  |  |  |  |
| permixtionis Gómez \& Morales-Serna, 2013 | 5 | 4 | 0.1.123 | 0.220 | 0.1.123 | 0.321 | 0.1.123 | 0.111 | 3 | 3 | 2 |
| Psammolaophonte ${ }^{\text {e }}$ |  |  |  |  |  |  |  |  |  |  |  |
| spinicauda Wells, 1967 | 5 | 2 | 0.0.022 | 0.020 | 0.0.022 | 0.021 | 0.0.022 | 0.011 | 4 | 3 | 3 |

 mented $q$ antennule, sensillar tubercles (socles) on the body somites, a strongly developed seta V on the caudal ramus and a trisetose $q$ P5 baseoendopod in $P$. spinicauda (cf. Wells 1967) indicates that this genus is closely affiliated to the Onychocamptus-Folioquinpes-Onychoquinpes-group of genera.

P5 $q$ large, with separate, densely hirsute rami; exopod elongate-oval, arising from pedestal, with $2-3$ setae laterally and one short dilated spine apically; baseoendopod with triangular or rectangular endopodal lobe bearing one apical and $1-3$ lateral setae. P5 ot incorporated into supporting somite; endopodal lobe completely absent (no armature); exopod typically not defined at base, small, with two setae.

P6 $q$ with two minute setae; P6 $\delta^{\pi}$ asymmetrical, membranous flaps with two setae.
Copepodids IV-V without modified P4 in $P$ (cf. Fiers 1998).
Euryhaline but primarily brackish or freshwater, free-living.
Type species. Folioquinpes mangalis Fiers \& Rutledge, 1990 (by original designation).

Other species. F. chathamensis (Sars, 1905), F. indicus sp. n., F. pseudomangalis sp. n. Species inquirenda. Folioquinpes chathamensis (Sars, 1905) sensu Rühe (1914)

## Key to species

1 Anterior margin of rostrum with spinules between sensilla; antennule $q$ 4-segmented; P3 enp-2 with two inner setae; P3-P4 exp-2 without inner seta2

- Anterior margin of rostrum without spinules between sensilla; antennule $q$ 5-segmented; P3 enp-2 with three inner setae; P3-P4 exp-2 with inner seta ... 3
2 Cephalothorax bilaterally incised; P5 $q$ endopodal lobe with two setae .......................................................... F. mangalis Fiers \& Rutledge, 1990
- Cephalothorax not bilaterally incised; P5 $q$ endopodal lobe with three setae.... F. pseudomangalis sp. n.

3. P4 enp-2 longer than enp-1; P5 exopod $q$ with two outer setae F. chathamensis (Sars, 1905)

- $\quad \mathrm{P} 4$ enp-2 shorter than enp-1; P5 exopod $q$ with three outer setae .F. indicus sp. n.


## Folioquinpes chathamensis (Sars, 1905)

Laophonte chathamensis Sars, 1905
Folioquinpes chathamensis (Sars, 1905) Fiers and Rutledge (1990)
Onychocamptus spec. sensu Mielke (1981): Fiers and Rutledge (1990)

Original description. Sars (1905): 391-393; Plate 17 (figs 103-118).
Additional description. Mielke (1981 as Onychocamptus spec.): 52; Abb. 28.
Type locality. New Zealand, Chatham Islands, Wharekauri (= Chatham Island), Te Whanga Lagoon; shallow brackish water.

Body length. $480 \mu \mathrm{~m}$ (q), slightly smaller ( (đ) [Sars 1905]; 430-450 $\mu \mathrm{m}$ (q) [Mielke 1981].

Remarks. Fiers and Rutledge (1990) stated that armature and shape of the male P5 differed between $F$. chathamensis and $F$. mangalis. Sars's (1905) text description is not informative with regard to the number and position of armature elements. His figure (figure 118) suggests that the P5 is distinctly bilobate, having one endopodal and three exopodal setae. However, the accompanying figure legend states that the left member is illustrated, implying that Sars had figured it in dorsal aspect. The "endopodal" seta is therefore the outer basal arising from a setophore (and not an endopodal lobe). Comparison with $F$. mangalis also suggests that there are only two exopodal elements, the third one representing the sensilla originating from a lateral tubercle. Based on this reinterpretation there is probably no difference in male P5 morphology between both species. The absence of the typical baseoendopodal incision in the female P5, separating the endopodal lobe and the pedestal bearing the exopod, is also attributable to an observational error by Sars (1905: Taf. 17, fig. 116).

Folioquinpes chathamensis resembles $F$. indicus sp. n. in the absence of spinules along the anterior margin of the rostrum, the 5 -segmented condition of the female antennule, the presence of three inner setae on the distal endopodal segment of leg 3, and of the inner seta on the middle exopodal segment of legs 3-4. The alternative states, including the 4 -segmented female antennule, are displayed in the other two species of the genus (Table 1).

Hamond (in Hicks 1977a: 457) collected F. chathamensis near Sydney and Melbourne while Newton and Mitchell (1999) obtained it in mud samples from the Hopkins River estuary in south-western Victoria. It remains unclear whether Lewis's (1984) single record from an estuarine lagoon in New Zealand is new or refers to Sars's (1905) type locality. Fiers (1995) recorded the species from the 'aufwuchs' covering submerged mangrove pneumatophores in the Celestún Lagoon, northwest of the Yucatán Peninsula (Mexico). Gómez and Morales-Serna (2013) erroneously cited Suárez-Morales et al. (2009) as the source for the Gulf of Mexico record but their checklist only refers to Fiers and Rutledge's (1990) record of $F$. mangalis from Louisiana. The latter authors also examined material from Guadeloupe, Papua New Guinea and Taal (Bombón) Lake, a freshwater lake on the island of Luzon in the Philippines (Fiers, unpubl. data). Mielke $(1981,2003)$ found the species in a sandy beach in Bahía Academy (Santa Cruz), Galápagos. A single African outlier has been reported from the brackish coastal Ebrié Lagoon in Ivory Coast (Dumont and Maas 1988). The records by Rühe (1914) and Sewell (1924) refer to other species (see below).

Newton and Mitchell (1999) observed during estuarine mud incubation experiments that F. chathamensis developed to egg-bearing female stage in only six days at $20^{\circ} \mathrm{C}$, suggesting that dormancy occurred at an advanced copepodid stage rather than the egg.

## Folioquinpes mangalis Fiers \& Rutledge, 1990

Original description. Fiers and Rutledge (1990): 122-124; fig. 9.
Type locality. Papua New Guinea, Capital District, Motupore Island; mangrove along northern shore; algae on pneumatophores.

Body length. $600 \mu \mathrm{~m}(q), 400 \mu \mathrm{~m}$ ( $\mathrm{\delta}^{\top}$ ) [Fiers and Rutledge 1990].
Remarks. Folioquinpes mangalis differs from its congeners in the bilaterally incised cephalothorax and the more strongly developed P5 $q$ endopodal lobe which bears only two setae. The dense spinular ornamentation on the anterior surface of leg 5 has not been documented in other species of the genus. The species is similar to P. pseudomangalis sp. n . in the strongly depressed body, the distinct pleural extensions on the urosomites, the 4 -segmented female antennule, the lack of the inner seta on P4 exp-2 (and P3 exp-2 but see below) and the presence of only two inner setae on P3 enp-2.

Fiers and Rutledge (1990) found two specimens with an inner seta on P3 exp-2; the absence of this seta appears to represent the normal condition. They also figured only two outer spines on P1 exp-2 (their figure 9 g ) but mentioned three in the text, which is here regarded as the correct condition.

Folioquinpes mangalis has been found on pneumatophores of mangrove trees along the southern (type locality) and northern coast (Sepik River delta) of Papua New Guinea and on Spartina alterniflora stems from marshes in Cocodrie, Louisiana (Fiers and Rutledge 1990; Rutledge and Fleeger 1993). It was subsequently found in samples of decaying leaves and sediment, from a Rhizophora apiculata-dominated mangrove forest bordering the Sungai Merbok estuary in north-western peninsular Malaysia (Gee and Somerfield 1997; Somerfield et al. 1998). Kim (2013) recently identified two specimens from Jeju Island, Korea as $F$. mangalis but this material is believed to represent a different species (see below).

## Folioquinpes indicus sp. n.

http://zoobank.org/DB1E5425-B537-4C6D-8F79-DD695B7C7B75
Laophonte chathamensis Sars, 1905 sensu Sewell (1924)

Original description. Sewell (1924 as Laophonte chathamensis): 830-832; Plate LVII, fig. 2 ( $q$ only).

Type material. The original material collected by R.B. Seymour Sewell is no longer available for re-examination. In accordance with ICZN (1999) Arts 16.4 and 72.5.6 the female specimen illustrated by Sewell (1924) in his plate LVII (fig. 2) is here fixed as the holotype of $F$ indicus sp. n.

Type locality. India, Odisha State, Chilika (Chilka) Lake; anchorage at Barkul due east; tow-nettings of brackish water plankton.

Body length. $400 \mu \mathrm{~m}(q)$ [Sewell 1924].
Remarks. Females of $F$. indicus differ from those of $F$. chathamensis primarily in the morphology of the P5 exopod which is more oval, has three outer setae (instead of two) and a very short, blunt spine apically (Sewell may have missed the flagellate tip). Additional differences include the more slender P1 endopod (enp-1:enp-2 ratio 5.3 vs 4.6) and the shorter P4 enp-2 (enp-1:enp-2 ratio 1.1 vs 0.8 ).

The authenticity of other records from the Indian peninsula is unclear since none was accompanied by illustrations. Chappuis (1941) recorded Onychocamptus chathamensis from the River Sina and the River Bhima (near Pandharpur) in Maharashtra State, approximately 250 km inland from the Indian west coast. In a later report Chappuis (1954) added records from Mhaisgaon (River Sina) and Dabhol (Vashishti River), both in Maharashtra State, and from coastal lagoons in two districts of the Union Territory of Puducherry, i.e. Mayyazhi (Mahé) and Karaikal, along the southwestern and southeastern coasts of the Indian peninsula, respectively. Folioquinpes chathamensis has recently been recorded from the middle and/or lower reaches of the River Godavari and River Krishna in Andhra Pradesh (Jayaram 1995; Ranga Reddy 2001, 2014; Ranga Reddy and Schminke 2009a-b; Ranga Reddy and Totakura 2010; Totakura et al. 2016). These hyporheic freshwater records, all from the east coast of India, most likely refer to F. indicus. Ranga Reddy (2002) reported "F. chathamensis" from a bore well on the Nagarjuna University campus, near Guntur town (Andhra Pradesh). The species is also known from Port Canning near Kolkata, West Bengal (Forró and Dussart 1985).

## Folioquinpes pseudomangalis sp. n. <br> http://zoobank.org/37483F03-31C2-4710-9180-DD05779A6440

Folioquinpes mangalis Fiers \& Rutledge, 1990 sensu Kim (2013)

Original description. $\operatorname{Kim}$ (2013 - as Folioquinpes mangalis): 38-43; figs 13-16.
Type locality. Korea, Jeju Island, Aewol; washings of invertebrates and intertidal stones.

Type material. In accordance with ICZN (1999) Arts 16.4 and 72.5 .6 the female specimen illustrated by $\operatorname{Kim}(2013)$ in his fig. 13A is here fixed as the holotype of $F$. pseudomangalis sp. n .

Body length. $600 \mu \mathrm{~m}(\uparrow), 400 \mu \mathrm{~m}\left(\circlearrowleft^{\lambda}\right)$ [to be confirmed - see below].
Remarks. Kim (2013) copied Fiers and Rutledge's (1990) text description virtually verbatim (with the exception of the mouthparts which were not described in the original account). This explains the discrepancies between Kim's (2013) text and some of his illustrations and also casts doubt on the accuracy of the body length given for both sexes of the Korean specimens which is allegedly identical to that of $F$. mangalis.

Kim's (2013) specimens are most similar to $F$. mangalis but differ from Fiers and Rutledge's description in a number of characteristics, justifying their assignment to a distinct species: (a) cephalothorax not bilaterally incised, (b) caudal rami relatively shorter, (c) second antennulary segment $q$ without blunt process, (d) both exopod and endopod of P4 markedly less elongate, (e) $q$ P5 endopodal lobe with three setae and markedly shorter while exopod relatively more slender, and (f) $\uparrow$ P5 rami without dense spinular ornamentation on anterior surface.

## Folioquinpes chathamensis (Sars, 1905) sensu Rühe (1914)

Laophonte chathamensis Sars, 1905 sensu Rühe (1914)

Original description. Rühe (1914): 33; fig. 11 ( $q$ only).
Type locality. South Africa, Western Cape Province, Cape Town, Muizenberg, Sandvlei; freshwater lake.

Body length. 470-670 $\mu \mathrm{m}$ (q) [Rühe 1914].
Remarks. Rühe's (1914) illustrations are limited to the P5 and the abdomen in dorsal aspect. Differences with $F$. chathamensis include the distinctly longer caudal rami, the reduced pleural extensions on the abdominal somites, and the setae on the P5 baseoendopod being distinctly longer. Rühe (1914) suspected that Sars (1905) had misinterpreted the apical blunt spine on the P5 exopod as a single element rather than two adjacent ones. Mielke's (1981) illustration, which confirms Sars's observation, indicates that the space between the two apical spines in Rühe's fig. 11b is in reality the inner core of the basally dilated spine. We suspect that Rühe has misinterpreted as a real phenomenon what he has seen only in optical section. Pending the discovery of fresh material the Western Cape population attributed to Folioquinpes chathamensis is here regarded as a species inquirenda in the genus.

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# A new remarkable species of Alloscorpiops Vachon, 1980 from Myanmar (Burma) (Scorpiones, Scorpiopidae) 

Wilson R. Lourenço', Ondřej Košulič2 ${ }^{2}$<br>I Muséum national d'Histoire naturelle, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (ISYEB), UMR7205-CNRS, MNHN, UPMC, EPHE, CP 53, 57 rue Cuvier, 75005 Paris, France 2 Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemèdëllská 3, Brno, Czech Republic

Corresponding author: Wilson R. Lourenço (wilson.lourenco@mnhn.fr)
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#### Abstract

Among the genera of the family Scorpiopidae Kraepelin, 1905 Alloscorpiops remains yet rather discrete. New species were added to this genus only recently, increasing its number from two to six. Therefore, species of Alloscorpiops can be considered rare and uncommonly collected. One particular new species, Alloscorpiops viktoriae sp. n., is described based on two females and one pre-adult male collected from the northern part of central Myanmar (Burma). The new species presents most features exhibited by scorpions of the genus Alloscorpiops, but it is characterised by a moderate to small size, very strongly marked granulation, and a particular trichobothrial pattern. Aspects of the ecology and distribution of the new species are discussed and compared with those of other species of genus Alloscorpiops.


## Keywords

Alloscorpiops, biodiversity, Burma, new species, Scorpion, Scorpiopidae, southeast Asia

## Introduction

As already outlined by Lourenço and Pham (2015a, b) in a revision of the genus Scorpiops (Lourenço and Pham 2015a, b), Vachon (1980) described three new subgenera, Alloscorpiops, Euscorpiops, and Neoscorpiops, in addition to the nominotypical subgenus Scorpiops. Alloscorpiops was defined based on an important 'majorante' neobothriotaxy with 10-12 ventral trichobothria on the surface of pedipalp chela-hand, whereas the other subgenera presented only four trichobothria. Vachon (1980) assigned two species to this subgenus: Scorpiops (Alloscorpiops) anthracinus Simon, 1887 (as type species of the subgenus) and Scorpiops (Alloscorpiops) lindstroemii Thorell, 1889.

Stockwell (1989), in an unpublished thesis dissertation, proposed raising all the subgenera within the family Scorpiopidae to the rank of genera; however, his proposition could not be validated since his dissertation was never published. Finally, Lourenço (1998) confirmed this decision. The four subgenera were elevated to generic rank and the monotypic genera Parascorpiops Banks, 1928 and Dasyscorpiops Vachon, 1974 were added, thus bringing the total number of genera to six.

In the present note, one additional new species belonging to the genus Alloscorpiops is described from the region of Magway in the northern part of central Myanmar. Specimens were collected in an open sandy riverbed, which is not a common habitat type for this group of scorpions, as all known species are usually found in dry or humid tropical forest ecosystems (e.g., Kovařík 2013; Kovařík et al. 2013; Lourenço 2017). Moreover, currently all Alloscorpiops species occupy an area around central to southern Thailand or the Mekong region in eastern Indochina. Therefore, the new Alloscorpiops species extends its area of distribution, with central Myanmar now forming its northernmost point. It may represent yet another endemic element in the fauna of this country.

## Materials and methods

Illustrations and measurements were produced using a Wild M5 stereo-microscope with a drawing tube and an ocular micrometre. Measurements follow Stahnke (1970) and are given in mm. Trichobothrial notations follow Vachon (1974, 1980), morphological terminology mostly follows Vachon (1952) and Hjelle (1990), and chelicerae dentition follows Vachon (1963). Locality data were recorded using portable GPS units (Garmin Oregon 450). The map background was downloaded from Free Vector Maps platform system and modified in Adobe Illustrator CS3 and Photoshop CS4.

## Present composition of the genus Alloscorpiops Vachon, 1980

Alloscorpiops anthracinus (Simon, 1887), Myanmar
Alloscorpiops lindstroemii (Thorell, 1889), Myanmar
Alloscorpiops calmonti Lourenço, 2013, Laos
Alloscorpiops citadelle Kovařík, 2013, Thailand

Alloscorpiops wongpromi Kovařík, Soleglad \& Košulič, 2013, Laos, Thailand
Alloscorpiops troglodytes Lourenço \& Pham, 2015, Vietnam
Alloscorpiops viktoriae sp. n., central Myanmar (this study)

## Taxonomic treatment

## Family Scorpiopidae Kraepelin, 1905 <br> Genus Alloscorpiops Vachon, 1980

## Alloscorpiops (Alloscorpiops) viktoriae sp. n.

http://zoobank.org/FF6038DF-4F70-4B05-B5BA-92D443AA5445
Figs 1-12

Diagnosis. The new species shows several of the characteristics already defined for the genus Alloscorpiops (Vachon, 1980). General colouration reddish brown to dark brown. Global size moderate to small in relation to other species of the genus; adult female with 50.9 mm in total length and a very strong overall granulation. The new species is also characterised by the trichobothrial patterns of some 'territories' or series. Femur with three trichobothria, $\boldsymbol{d}, \boldsymbol{i}$ and $\boldsymbol{e}$. Patella with the trichobothria $\boldsymbol{d} \mathbf{1}$ and $\boldsymbol{d} \mathbf{2}$ on the dorsal surface; $\boldsymbol{i}$ on the internal surface, 15-17 $V$ on the ventral surface and only 22 trichobothria on the external surface ( $6 \boldsymbol{e t}, 7 \boldsymbol{e s t}, 2 \boldsymbol{e m}, 2 \boldsymbol{e s b}, 5 \boldsymbol{e b}$ ). Chela-hand with an unusual trichobothrial number on the $\boldsymbol{V}$ series of 8-9 on the ventral surface, $\boldsymbol{D} \boldsymbol{t}$ on the dorsal surface, $\boldsymbol{D} \boldsymbol{b}$ on the external surface, $\boldsymbol{i b}$ and $\boldsymbol{i t}$ on the internal surface, five $\boldsymbol{E}, \boldsymbol{E s t}, \boldsymbol{E} \boldsymbol{s} \boldsymbol{b}$ and three trichobothria in the $\boldsymbol{E} \boldsymbol{b}$ series on the external surface. The annular ring is strongly marked. Pectines with 8-9 teeth in females and 8-8 in the only known male; fulcra reduced.

Material. Myanmar (Burma), Magway region, Kyakhtu District, River Stream, Sandy habitat, GPS $21^{\circ} 27^{\prime} 36^{\prime \prime} \mathrm{N}$; $94^{\circ} 16^{\prime} 24^{\prime \prime} \mathrm{E}, 398 \mathrm{~m}$ a.s.l., 29/I/2016 (O. Košulič). Female holotype (RS-9122) and male-juvenile paratype (RS-9123) deposited in the Muséum national d'Histoire naturelle, Paris. Female paratype (VS-55342) deposited in the Mendel University of Brno, Czech Republic.

Etymology. The new species is named after a young lady, Viktorie Košuličová, the daughter of O. Košulič. Coincidently, the new species was also found in the region close to the most impressive peak of Central Myanmar, Mt. Victoria.

Description. The general coloration is reddish brown to dark brown. Carapace and tergites reddish brown. Metasomal segments brown to dark brown; telson reddish brown; base of aculeus yellow and tip slightly reddish. Chelicerae yellow with intense variegated brownish spots; teeth reddish. Pedipalps dark brown; granulations on chela fingers almost reddish. Legs reddish brown. Venter reddish to reddish yellow; genital operculum and pectines yellow.

Morphology. Carapace strongly granular, furrows moderately to very deep. Median eyes anterior to the centre of carapace; three pairs of lateral eyes, the third pair only slightly smaller than the first two. Sternum pentagonal, longer than wide. Genital operculum formed by two semi-oval plates in female. Tergites strongly granulated; VII


Figures I-2. Alloscorpiops viktoriae sp. n. Female holotype. Habitus. Dorsal and ventral aspects.


Figures 3-5. Alloscorpiops viktoriae sp. n. Female holotype. 3 Chelicera, dorsal aspect 4 Metasomal segments VI-V and telson, lateral aspect 5 Cutting edge of movable finger with rows of granules. Scale bars 2 mm .


Figures 6-I 2. Alloscorpiops viktoriae sp. n. Female holotype. Trichobothrial pattern. 6-8 Chela, dorsoexternal, ventral and internal aspects 9 Femur, dorsal aspect 10-12 Patella, dorsal, external and ventral aspects. Scale bars 2 mm .
with four moderately marked carinae. Pectinal tooth count 8-8 (9-9) in females, 8-8 in male; fulcra reduced. Sternites smooth and shiny; VII with four weak carinae and some granulations. Metasomal segments I and II wider than long; segments III to V longer than wide; 10-8-8-8-7 carinae present on segments I-V, strongly marked; dorsal carinae on segments I-IV, with strongly marked posterior spinoid granules on segments III-IV; metasomal tegument moderately to strongly granulated; ventral carina on segment V with weak spinoid granules. Telson vesicle almost smooth, with some isolated granulations. Pedipalps: femur with dorsal internal, dorsal external, ventral internal and ventral external carinae moderately to strongly marked; tegument moderately granular. Patella with dorsal internal, ventral internal, dorsal external, ventral external and external carinae strongly marked; several spinoid granules present on internal aspect, two of which are very conspicuous; the interno-ventral being larger than the interno-dorsal granule; tegument moderately granular. Chela with dorsal marginal, external secondary, ventral internal and ventral carinae moderately to strongly marked; other carinae moderately marked; tegument granulated dorsally and ventrally. Chelal fingers with two longitudinal series of granules, almost fused, and a few inner and outer accessory granules. Chelicerae dentition as illustrated in Figure 3; four/five teeth on ventro-internal face of movable finger. Trichobothriotaxy type $\mathbf{C}$, as presented in Figs 6-12.

Relationships. Although geographically closer to the others species of Alloscorpiops described from Myanmar (Burma) the new species shows some affinities with Alloscorpiops troglodytes from Vietnam, in particular by the reduced number of trichobothria in some territories. Both species can, however, be readily distinguished by the structure of their tegument, which is weakly granular, almost smooth in A. troglodytes and strongly granular in $A$. viktoriae sp. n. Besides this, they differ in their overall size, pattern of pigmentation, and general morphology (see also the following key).

Morphometric values (in mm ) of female holotype. Total length (including telson) 50.9. Carapace: length 8.0; anterior width 4.1; posterior width 7.9. Mesosoma length 17.8. Metasomal segment I: length 2.2 , width 3.1 ; II: length 2.4 , width 2.7 ; III: length 3.2 , width 2.6 ; IV: length 3.6 , width 2.4 ; V: length 6.3 , width 2.2 , depth 2.2 . Telson length 7.4. Vesicle: width 2.2, depth 2.1. Pedipalp: femur length 7.9 , width 3.0 ; patella length 7.2 , width 3.3 ; chela length 15.9 , width 4.4 , depth 4.3 ; movable finger length 7.7.

## Simplified key to the species of Alloscorpiops

1 Chela of pedipalp with 3 trichobothria on the Eb series.............................. 2

- Chela of pedipalp with 5 trichobothria on the Eb series................................

Alloscorpiops (Laoscorpiops) calmonti
2 Chela of pedipalp with $10-13$ ventral trichobothria; patella with $15-22$ ventral trichobothria .4

- Chela of pedipalp with 8-9 ventral trichobothria; patella with 14-17 ventral trichobothria.

3 Tegument of carapace and tergites almost smooth.... Alloscorpiops troglodytes

- Tegument of carapace and tergites strongly granulated $\qquad$ Alloscorpiops viktoriae sp. n.
4 Patella of pedipalp with 15-16 ventral and 23-25 external trichobothria....5
- Patella of pedipalp with 19-21 ventral and 29-37 external trichobothria.... 6

5 Patella of pedipalp with 16 ventral and 23 external trichobothria
Alloscorpiops anthracinus

- Patella of pedipalp with 15 ventral and 25 external trichobothria

Alloscorpiops lindstroemii
6 Patella of pedipalp with 19-21 ventral and 29-34 external trichobothria.
Alloscorpiops citadelle

- Patella of pedipalp with 21-22 ventral and 33-37 external trichobothria

Alloscorpiops wongpromi

## Type locality and habitat of Alloscorpiops viktoriae sp. n.

The new species $A$. viktoriae sp . n . was found in Magway region in the northern part of central Myanmar along the border with Chin State. This region is situated on the eastern slopes of the Chin Hills of Arakan Mountains, which includes a large area of tropical and subtropical broadleaf forests (Leimgruber et al. 2005). The Arakan Mountains act as a barrier to the southwestern monsoon and thus shield central Myanmar, making their eastern slopes much drier (Wu et al. 2004). The hilly landscape area of the type locality was located at a rather lower elevation, approximately $300-400 \mathrm{~m}$ a.s.l. The area is significantly disturbed by anthropogenic influence and covered mainly by fragmented dry dipterocarp and bamboo forests, agricultural fields, and uncultivated rocky-sandy habitats with scattered woody vegetation. The specimens were found hiding under stones in sandy riparian habitats along a small water stream (Figure 13). The stream was surrounded by dry dipterocarp and bamboo forests with moderately open canopy coverage in a very dry condition (Figure 13). Several specimens of Lychas mucronatus (Fabricius, 1798) were also sympatrically found. No other species of scorpions were observed during the field trip to this area. We suggest that specimens of $A$. viktoriae sp. n. moved from very dry conditions of dipterocarp and bamboo forests to the stream in search of higher humidity during the peak of the dry season. Such a pattern can also be found in other arthropod groups during long-term dry conditions in tropical forests (Murphy and Lugo 1986).

## Distribution and ecological affinities of species belonging to the genus Alloscorpiops

Until now, all species from the genus Alloscorpiops were distributed between $15^{\circ}$ and $8^{\circ}$ of geographical latitude and $98^{\circ}$ to $107^{\circ}$ of geographical longitude. This area is located from central Thailand to the eastern region of Indochina along southern Laos


Figure I3. Type locality of Alloscorpiops viktoriae sp. n. in Magway region of Central Myanmar. A Overall view on natural habitat of the new species B Detail view on the same habitat. All specimens were found under stones located directly in humid riverbed. Photographs by Ondřej Košulič (A); Šárka Mašová (B).


Figure 14. Map of southeast Asia showing the known distribution of the species belonging to the genus
Alloscorpiops: Alloscorpiops anthracinus (I), Alloscorpiops lindstroemii (2), Alloscorpiops calmonti (3), Alloscorpiops citadelle (4), Alloscorpiops wongpromi (5), Alloscorpiops troglodytes (6) and Alloscorpiops viktoriae sp. n. (7).
and central Vietnam to southern Thailand where the southernmost distribution of this genus is (Figure 14). Therefore, our finding of $A$. viktoriae sp. n. indicates the northernmost location of this genus and significantly extends the region of distribution of Alloscorpiops further to the central of Myanmar (Figure 14). We suppose that more findings may be discovered in suitable locations in southeast Asia; however, it also seems that these scorpions are very localised and due to their life strategy and good hiding abilities they may be easily overlooked in tropical ecosystems.

With the exception of $A$. troglodytes, which was found in a cave habitat in a limestone area of central Vietnam (Lourenço 2015), all species were usually discovered in lowland dry dipterocarps or bamboo forests (Kovařík et al. 2013; Lourenço 2013) or dry evergreen tropical forests (Simon 1887; Thorell 1889). One questionable situation has been observed for $A$. citadelle Kovařík, 2013. This species was described from a type locality in Khlong Phanom in southern Thailand. The region is characterised by the presence of humid tropical rain forests influenced from the south by the Malesia bioregion. Furthermore, Kovařík (2013) also described this species based on one juvenile paratype from Sai Yok in central Thailand. However, this area is influenced by different biogeographical regions (mainly from Indochina) with the presence of forest types (e.g., dry evergreen and deciduous forests) and ecological characteristics quite distinct from those in southern Thailand (Knight and Holloway 1990). Therefore, we suggest that Kovařík (2013) probably misidentified this juvenile paratype as A. citadelle. The specimen from central Thailand could in fact be associated with another species of Alloscorpiops such as $A$. anthracinus or $A$. lindstroemii, which naturally occur in forest ecosystems of this region or with some other possibly new species from this genus.

In general, most Alloscorpiops species have been collected and observed from similar microhabitat conditions sharing ecological strategies similar to other groups from the family Scorpiopidae (e.g., Qi et al. 2005, Kovařík et al. 2015). Specimens of Alloscorpiops can be found and observed in the already mentioned habitats during the evening or at night in a sit-and-wait position resting inverted on overhanging surfaces of rock or soil walls. Some of them may occupy more protected places in fissures of cracked rock walls. When disturbed, the scorpions usually escape and hide deeper in the rock fissures or soil burrows (Kovařík et al. 2013). They can also be found under dead wood (Lourenço 2013), in crevices in caves (Lourenço 2015) or under stones such as A. viktoriae sp. n.

In conclusion, it can be suggested that $A$. viktoriae sp. n. represents a remarkable species of scorpion for Myanmar and its finding significantly extends the distribution range of this southeast-Asian endemic genus further to the north from its original area. Presumably more species from this group will be found in suitable habitats across southeast Asia.

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# Two new species of the genus Methocha from Laos (Hymenoptera, Tiphiidae) 

Keisuke Narita', Toshiharu Mita²<br>I Entomological Laboratory, Graduate School of Bioresourse and Bioenvironmental Sciences, Kyushu University, Motooka 744, Fukuoka, Japan 2 Entomological Laboratory, Faculty of Agriculture, Kyushu University, Motooka 744, Fukuoka, Japan<br>Corresponding author: Toshiharu Mita (t3mita@agr.kyushu-u.ac.jp)

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Abstract<br>Two new species of Methocha, M. cariniventris and M. granulosa are described from Laos.

## Keywords

Cicindelinae, new record, parasitoid, taxonomy

## Introduction

The genus Methocha Latreille contains 86 species throughout the world except for the Australian region (Krombein 1982, Tsuneki 1986, Agnoli 2011, Kimsey 2011, Terayama and Mita 2015). This genus shows considerable sexual dimorphism, with winged males and wingless, ant-like females. Although the life history of most species is still unknown, they are considered to be parasitoids of Cicindelinae beetle larva (Coleoptera, Carabidae) (Adlerz 1903, 1906, Bouwman 1909, Champion and Champion 1914, Williams 1919, Pagden 1926, Main 1927, Yasumatsu 1931, Iwata 1936, Krombein 1982).

The tiphiid subfamily Methochinae includes two genera, Methocha and Karlissa Krombein (Kimsey 1991, Brothers and Carpenter 1993). Some molecular phylogenetic studies suggested that Methochinae and Tiphiinae were paraphyletic, and Methochinae were moved to Thynnidae (Pilgrim et al. 2008, Debevec et al. 2012). However, their close relationship was rather strongly supported by recent studies based on transcriptomes (Peters et al. 2017) and UCEs (Branstetter et al. 2017). Although family concept remains disputable, here we follow the classification proposed by Aguir et al. (2013).

Methocha are most diverse in the Oriental region. Up to now, 52 species have been recorded (Krombein 1982, Tsuneki 1986, Agnoli 2011, Terayama and Mita 2015), and 15 species are known from Southeast Asia, including Malaysia, Philippines, and Indonesia. However, none of them have been recorded from Laos. We had an opportunity to examine some specimens of Methocha from Laos collected by biotic surveys on 2009 and 2011, and found several interesting specimens. These specimens were classified into two new species by the following characters; shape of the clypeus, sculpturing of the pronotum, the mesopleuron, and the propodeum, and infuscation of the wings.

## Materials and methods

Specimens examined are deposited in the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan (ELKU). The terminology follows those of Richards (1977) and Krombein (1982). The following abbreviations are used in descriptions:

| BL | body length; | FWL | fore wing length; |
| :--- | :--- | :--- | :--- |
| HL | head length; | ML | mesosoma length; |
| HW | head width; | MW | mesosoma width; |
| WF | width of frons; | T | metasomal tergite; |
| POL | length between posterior ocelli; | S | metasomal sternite; |
| AOL | length between anterior ocellus | AMW | anterior width ofT1; |
|  | and posterior ocellus; | MTL | T1 length; |
| DAO | diameter of anterior ocellus; | PMW | posterior width of T1. |
| EL | eye length; |  |  |

Observations were made on an Olympus SZX-1212 stereomicroscope. Photo images were taken by a Canon EOS-60D with a Canon MP-E $65 \mathrm{~mm} 1-5 \times$ a Macro lens and processed by image stacking software, Combine ZM (Hadley 2010).

## Results and discussion

## Methocha cariniventris sp. n.

http://zoobank.org/EDCC6019-5CB6-4F50-B81D-3E645062DD7A
Figs 1-12
Material examined. Holotype, $\widehat{ }$ §, LAOS: Mt. Phou Pan Gnai, Houa Phan Prov., VI. 2009, H. Kojima leg. (ELKU).

Diagnosis. This species is characterized by combination of the following characters: the distally wide mandibles; the translucent distal margin of the clypeus; the areolate propodeum; infuscate near apical $1 / 2$ of the wings. This species is similar to M. foveiventris Lin, 1966 and M. punctata Williams, 1919 in the body size, but it can be distinguished by the striate propodeum (reticulate rugose in $M$. foveiventris) and the translucent distal margin of the clypeus (opaque in M. punctata).

Description. Male. Head (Figs 5, 6). Height 0.7 times as long as wide in frontal view; temple weakly rounded; ocelli forming regular triangle, POL: AOL: $\mathrm{DAO}=1.0$ : 1.0: 0.6; vertex punctate: punctures smaller and sparser than those on frons, $1.0-5.0$ puncture diameters apart, with interspaces smooth; frons densely punctate: punctures $0-1.0$ puncture diameters apart, deeply excavated above antennal lobes; antennal lobes developed; gena punctate as vertex; clypeus (Figure 4) densely covered with small punctures, distinctly convex, semicircular laterally, apical $1 / 5$ translucent, subtruncate; mandible not narrowed distally, lower tooth longer than upper tooth; maxillary palpus shorter than length of pronotum, length (width) of segments II-VI showing following ratio: 1.0 (0.3): 2.0 ( 0.3 ): 3.0 ( 0.3 ): $2.4(0.3): 2.4(0.3)$; flagellum weakly flattened, length (width) of flagellomeres I-III showing following ratio: 1.7 (1.0): 2.3 (1.0): 2.4 (1.0).

Mesosoma (Figure 3). Pronotal disc punctate: punctures 1.0-3.0 puncture diameters apart, with interspaces smooth, anterior transverse carina absent, lateral surface punctate as dorsal surface, transversely faintly rugulose medially; mesoscutum punctate: punctures smaller and denser anteriorly, with interspaces smooth, sometimes coriaceous, larger and sparser medially; notauli indicated by transversely striate wide depression, reaching posterior $1 / 3$, posterior margin effaced; mesoscutellum elevated, dorsal surface sparsely, lateral slope finely punctate, with interspaces smooth, lateral lower surface transversely carinate; mesepisternum convex, coarsely punctate: punctures 1.0 puncture diameter apart, with interspaces smooth, narrow surface above episternal sulcus densely punctate: punctures $0-0.5$ puncture diameters apart, episternal sulcus deep, precoxal sulcus obscure, faintly depressed; metanotum elevated, smooth, laterally striate, anterior margin with deep crescentic depression; metapleural region antero-dorsally smooth, posteriorly rugose; dorsal surface of propodeum (Figure 7) irregularly longitudinally areolate, with interspaces smooth, lateral surface coarsely puncto-reticulate, posterior surface transversely rugose.


Figures I-3. Methocha cariniventris sp. n., male, holotype. I Dorsal habitus $\mathbf{2}$ lateral habitus $\mathbf{3}$ mesosoma, lateral view.


Figure 4. Frontal head of Methocha cariniventris sp. n. male, holotype. Arrow a indicating translucent distal apex of clypeus; arrow $b$ indicating labrum.


Figures 5-I 2. Methocha cariniventris sp. n., male, holotype. $\mathbf{5}$ Head, frontal view $\mathbf{6}$ head, lateral view $\mathbf{7}$ propodeum $\mathbf{8}$ hind claw 9 T 1 , dorsal view $\mathbf{1 0}$ right paramere, lateral view (right side is dorsal) II aedeagus, dorsal view, arrow indicating apical membranous lobe $\mathbf{1 2}$ aedeagus, lateral view (right side is dorsal). Scale bars are given in mm.

Legs. Hind coxa dorsally carinate, parabolically elevated in lateral view; claws (Figure 8 ) curved at apical $1 / 2$, tridentate, subapical tooth stout, truncate, basal tooth minute, less than $1 / 5$ as long as subapical tooth.

Metasoma. Tergites smooth with sparsely located punctures and setae; AMW: MTL: PMW = 1.0: 2.9: 2.5; T1 (Figure 9) with pair of strong carinae present before spiracle, rugose between carinae, medial furrow present around anterior $4 / 5$, shallower and wider posteriorly; anterior transverse depression of T2 costate, T3-T7 without carinae, smooth; posterior margin of S2-S6 with row of brown stout setae; punctures on S 7 smaller than those on $\mathrm{S} 1-\mathrm{S} 6,1.0-2.0$ punctures diameters apart, posterior margin of S 7 not cleft; ventral surface of hypopygium longitudinally striate, hairy; paramere (Figure 10) narrow, apical $1 / 4$ slender; aedeagus (Figs 11, 12) with apical membranous lobe, apical hook faintly curved ventrad, aedeagal apodeme moderately curved ventrad except distal apex.

Color. Black; scape brown; distal margin of clypeus translucent yellow; maxillary palpus dark yellow; apical $1 / 3$ of mandible dark brown; tegula dark brown; wings weakly tinged with brown, with apical $1 / 2$ strongly infuscate; legs dark brown.

Measurements. BL 11.0 mm , HL 1.5 mm , HW 2.1 mm , WF 1.0 mm , EL 1.1 mm , ML 3.9 mm , MW 1.7 mm , FWL 4.7 mm .

Etymology. The name is derived from the propodeal sculpture.
Distribution. Laos: Houa Phan Province.
Remarks. The female is unknown.

## Methocha granulosa sp. n.

http://zoobank.org/D38A72B4-9143-4AD6-94CD-A7876FC283FE
Figs 13-23

Materials examined. Holotype, $\widehat{o}^{\lambda}$, LAOS: $19^{\circ} 33^{\prime} \mathrm{N}, 103^{\circ} 41^{\prime} \mathrm{E}$, 570 m alt., Hot spring near Ban Ban, Houa Phan Prov., 4. IV. 2011, T. Mita leg. (ELKU); Paratypes, 3§, with same data as holotype (ELKU).

Diagnosis. This species is characterized by combination of the following characters: the distally wide mandibles; the translucent distal margin of the clypeus; the distinct pleuro-propodeal suture; the striate and granulate propodeum; the faintly infuscate wings. This species can be easily distinguished from other Asian species by the above-mentioned characters.

Description. Male. Head (Figs 16, 17). Height 0.7-0.9 times as long as wide in frontal view; temple weakly rounded; ocelli forming regular triangle, POL: AOL: $\mathrm{DAO}=1.0: 1.0: 0.6-0.8$; vertex punctate: punctures smaller and sparser than those on frons, $1.0-4.0$ puncture diameters apart, with interspaces smooth; frons densely punctate: punctures $0.5-1.0$ puncture diameters apart, deeply excavated above antennal lobes; antennal lobes developed; gena punctate as vertex; clypeus covered with small punctures, distinctly convex, apical $1 / 5$ translucent, with apex subtruncate; mandible not narrowed distally, lower tooth longer than upper tooth; maxillary palpus slightly shorter than length of pronotum, length (width) of segments I-VI showing following ratio: 4.2 (1.2): 3.3-5.0 (1.2): 5.0-6.7 (1.2): 6.7-8.3 (1.2): 5.8-6.7 (1.2): 5.0-8.3 (1.2); length (width) of flagellomeres I-III showing following ratio: 2.0-2.4 (1.6): 2.6-2.9 (1.6): 2.7-3.0 (1.6).

Mesosoma (Figure 15). Pronotal disc more or less covered with shallow punctures, with interspaces smooth, anterior transverse carina absent, lateral surface smooth; mesoscutum punctate: punctures denser and smaller around anterior $1 / 3$, larger and sparser at posterior $2 / 3$, punctures sometimes entirely sparser; notauli indicated by transversely striate wide depression, reaching posterior $1 / 2$ to $2 / 3$, posterior margin effaced; mesoscutellum elevated, dorsal surface sparsely, lateral slope finely punctate, sometimes almost entirely impunctate, lateral lower surface transversely rugose by weak carinae, sometimes almost smooth; mesepsternum convex, smooth, with several small punctures, episternal sulcus deep, precoxal sulcus obscure, faintly depressed; metanotum elevated, smooth, laterally striate, anterior margin with deep crescentic


Figures I3-I5. Methocha granulosa sp. n., male, paratype. I3 Dorsal habitus $\mathbf{1 4}$ lateral habitus $\mathbf{1 5}$ mesosoma, lateral view.
depression; metapleural region smooth, postero-dorally longitudinally rugose, with pleuro-propodeal suture distinct, arising from lower pit to propodeal spiracle; dorsal surface of propodeum (Figure 18) more or less longitudinally rugose on anterior $1 / 3$, with interspaces granulate, medial $1 / 3$ transversally rugose, with interspaces granulate, lateral surface sparsely punctate: punctures $2.0-4.0$ punctures diameters apart, with interspaces smooth, around pleural-propodeal suture rugose.

Legs. Hind coxa dorsally carinate, parabolically elevated in lateral view; claw (Figure 19) strongly curved at apical $1 / 3$, tridentate, subapical tooth stout, truncate, basal tooth minute, less than $1 / 5$ as long as subapical tooth.

Metasoma. Tergites smooth with sparsely located punctures and setae with fine punctures; ATL: MTL: PTL = 1.0: 2.9-3.3: 2.4-3.0; T1 (Figure 20) with pair of strong carinae present before spiracle, rugose and weakly excavated medially between carinae, medial furrow present on anterior $4 / 5$, shallower posteriorly; anterior transverse depression of T2-T4 costate, T5-T7 without carinae, smooth; posterior margin of S2-S6 with row of brown setae; $S 7$ with large punctures: punctures 1.0 puncture diameter apart, apical margin semicircularly cleft; ventral surface of hypopygium rugose, hairy; paramere (Figure 21) narrow, apical $1 / 3$ slender and nearly straight; aedeagus (Figs 22, 23) with apical membranous lobe, apical hook strongly curved ventrad; aedeagal apodeme almost straight except distal apex laterally.

Color. Black; antenna dark brown; distal margin of clypeus translucent yellow; maxillary palpus dark testaceous; apical $1 / 4$ of mandible dark brown; tegula brown; wings faintly infuscate; legs brown.

Measurements. BL 7.00-7.5 mm; HL 0.9-1.3 mm, HW 1.2-1.4 mm, WF 0.6-0.7 mm , EL $0.7-0.8 \mathrm{~mm}$, ML $2.3-2.7 \mathrm{~mm}$, MW $1.0-1.3 \mathrm{~mm}$, FWL $4.5-6.0 \mathrm{~mm}$.

Etymology. The name is derived from the propodeal sculpture.
Distribution. Laos: Houa Phan Province.
Remarks. The female is unknown.


Figures I6-23. Methocha granulosa sp. n., male, holotype. 16 Head, frontal view 17 head, lateral view 18 propodeum 19 hind claw 20 T 1 , dorsal view 21 right paramere (right side is dorsal), lateral view $\mathbf{2 2}$ aedeagus, dorsal view, arrow indicating apical membranous lobe $\mathbf{2 3}$ aedeagus, lateral view (right side is dorsal). Scale bars are given in mm .

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# Description of Triatoma mopan sp. n. from a cave in Belize (Hemiptera, Reduviidae, Triatominae) 

Patricia L. Dorn', Silvia A. Justi², ${ }^{2,6}$, Carolina Dale ${ }^{3}$, Lori Stevens ${ }^{2}$, Cleber Galvão ${ }^{4}$, Raquel Lima-Cordón ${ }^{2}$, Carlota Monroy ${ }^{5}$<br>I Department of Biological Sciences, Loyola University New Orleans, New Orleans, LA, USA 2 Department of Biology, University of Vermont, Burlington, VT, USA $\mathbf{3}$ Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil 4 Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomineos, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil 5 LENAP, University of San Carlos, Guatemala City, Guatemala 6 The Walter Reed Biosystematics Unit, Smithsonian Institution Museum Support Center, 4210 Silver Hill Rd, Suitland, MD 20746-2863, USA

Corresponding author: Silvia A. Justi (silvinhajusti@gmail.com) Academic editor: G. Zhang | Received 27 November 2017 | Accepted 4 June 2018 | Published 17 July 2018
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#### Abstract

In this paper, Triatoma mopan sp. n. is described based on five males and six females collected in the Rio Frio cave, Cayo District, Belize. This species is similar to Triatoma dimidiata (Latreille), but can be distinguished by characters found on the pronotum, legs, and abdomen. Geometric morphometry and phylogenetic comparisons are also provided. Presently, the species is known only from the type locality and is a potential Chagas vector.


## Keywords

Belize, Chagas disease, new species, Triatoma dimidiata, Triatoma mopan

## Introduction

Species belonging to Triatominae Jeannel, 1919 (Insecta, Hemiptera, Reduviidae) are important as vectors of Chagas disease. Presently, more than 150 species within 15 genera (Justi and Galváo 2017) are recognized as valid in this subfamily. The most speciose genus is Triatoma Laporte, 1832, which includes Triatoma dimidiata (Latreille, 1811) and Triatoma infestans (Klug, 1834), historically, two of the most relevant Chagas disease vectors in Central and South America, respectively.

Because of the substantial morphological variation of T. dimidiata across its large geographic distribution (southern Mexico to Peru), this species has been split and synonymized many times since its original description (reviewed in Dorn et al. 2007). This includes morphotypes (previously considered species or subspecies) at extreme ends of the geographic distribution. In the north (Mexico, described as T. maculipennis Stål, 1859), specimens of T. dimidiata have smaller bodies, shorter heads, and larger eyes compared with specimens from the south (Colombia, once called T. capitata Usinger 1941).

Lent and Wygodzinsky (1979) examined 160 T. dimidiata specimens, including the entire distribution, and concluded that, in general, there is a clinal variation with size increasing southwards, and synonymized T. maculipennis and T. capitata with T. dimidiata; these authors also state that there are many exceptions to that rule, and commented specifically on cave specimens. Upon comparison of one "T. dimidiata" specimen from the Rio Frio cave, in Belize, with five specimens from the Lanquin cave in Guatemala, the authors conclude that they appear identical. The morphological distinctions between the cave specimens and the other specimens of T. dimidiata were regarded simply as cave adaptations.

Following the synonymizing of the species (Lent and Wygodzinsky 1979), many qualitative and quantitative phenotypic, biochemical, and molecular studies have sought to clarify the systematics of T. dimidiata. Phylogenetic studies of T. dimidiata based on DNA sequence analyses of nuclear and mitochondrial genes (Bargues et al. 2008, Monteiro et al 2003, Dorn et al. 2016) and genome-wide data (Justi et al. 2018) have shown that T. dimidiata is composed of at least three phylogenetic species (Mishler and Brandon 1987), referred to as : T. dimidiata sensu strictu (or groups 1 and 2), T. sp. affinnis dimidiata (group 3) and T. sp. aff. dimidiata - cave (group 4); the latter comprises only specimens from the Rio Frio cave, Cayo District, Belize. Interestingly, Dorn et al. (2016) included in their study specimens from both caves mentioned in Lent and Wygodzinsky (1979) and found that specimens from Lanquin are recovered within T. dimidiata s.s., whereas the ones from Rio Frio compose a distinct clade.

In this manuscript, we describe $T$. sp. aff. dimidiata - cave, the lineage from the Rio Frio cave, as Triatoma mopan sp. n. (Hemiptera, Reduviidae, Triatominae), a new species of the genus Triatoma.

## Materials and methods

## Specimen collection

We conducted field work on June 15, 2016 in the Rio Frio cave, Cayo District, Belize [(coordinates: 16.956939/-88.979675) under permits covering the research (\#IA/H/1/16 (03), Institute of Archaeology), collecting (\#WL/1/1/16 (33), Forest Department) and export (\#WL/1/7/16 (29), Forest Department) of specimens from Belize. The sole purpose of this field work was to collect enough specimens from the Rio Frio cave Triatoma population to reliably compare this population with T. dimidiata from other localities. We collected specimens from the Rio Frio cave, Cayo, Belize because of previous results of phylogenetic studies that showed this population to be an independent lineage distinct from all other populations included under the umbrella of T. dimidiata, and to be the only phylogenetic species found in this particular cave. We collected 15 adult males and 13 adult females and more than 70 nymphs of various lifecycle stages. For this study, we focus on the adult morphology.

## Morphological identification and description

Adults collected in the Rio Frio cave could not be taxonomically identified using the key for Triatoma species (Lent and Wygodzinsky 1979). Because of the morphological similarities mentioned by Lent and Wygodzinsky (1979) between T. dimidiata and the single specimen they examined from the Rio Frio cave, six T. mopan males and seven T. mopan females were compared with specimens from the Lanquin cave, Guatemala, as well as representatives of the entire distribution of T. dimidiata, including photographs of the type specimens from the extreme ends of the range, T. dimidiata capitata (from Colombia), and T. dimidiata maculipennis (from Mexico). It was not possible to directly compare with the T. dimidiata holotype, since this specimen has been lost; thus, T. dimidiata specimens used in this study were identified following the key and description in Lent and Wygodzinsky (1979). The specimens examined here were from the Triatominae collection of the Oswaldo Cruz Institute (CTIOC) in Rio de Janeiro, Brazil (Table 1) and the type specimens were from Zoologisches Museum, Berlin (T. dimidiata maculipennis) and the California Academy of Sciences, USA (T. dimidiata capitata).

## Morphological study

Character observation and measurements were made with a stereoscopic Zeiss Stemi SV11 microscope, using a graduated eyepiece micrometer, and photos were taken using a Nikon Coolpix 990 digital camera. The following characters were measured:

| TL | total length of the body |
| :--- | :--- |
| LOP and WOP | length and width of pronotum |
| AOR and POR | length of the ante- and post-ocular region |
| SYN | length of the inter-ocular region or synthlipsis |
| HL and WOH | length and width of the head <br> width of the eye |
| WE | total length of scutellum <br> TS |
| length of process of scutellum |  |
| POS | length of antennal segments <br> A1-A4 |
| R1-R3 | labial segments (Figure 1, Table 2). |

The terminology and measurements used for the description were based on Lent and Wygodzinsky (1979). For comparison, these characters were also measured for specimens of T. dimidiata. For each sex separately, the two species were compared for each of the characters with a t -test using JMP ${ }^{\circ}$ ver 13 (SAS Institute, Inc., Cary NC, USA). Significance was adjusted for multiple comparisons with the method of Benjamini and Hochberg (1995). Boxplots comparing the characters measured were also created using R (The R Development Core Team 2008) and the code and plots are provided as Suppl. material 1.

## Morphometric study

Morphometric comparison of the dorsal portion of the heads was made based on eight landmarks (Figure 2). Thirteen T. mopan specimens (eleven from the field and two from a Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos - LNIRTT colony, previously regarded as T. dimidiata but found to be T. mopan based on DNA sequence (T. sp. aff. dimidiata - cave; Justi et al. 2018) were compared with all known morphotypes of T. dimidiata s.l. (T. dimidiata maculipennis: one specimen from Mexico; T. dimidiata capitata: four specimens including the holotype; Triatoma dimidiata s.1.: one each from Mexico and Peru, three from Costa Rica, two from Ecuador, six from Guatemala, including two from the Lanquin cave; T. sp. aff. dimidiata: two from Guatemala); and T. gerstaeckeri: five from the southern United States (Table 1). Triatoma gerstaeckeri was included because this species was recovered as a sister taxon to T. mopan (then identified as T. dimidiata) in a recent phylogenetic study (Justi et al. 2016). Size variation observed in colony specimens (Jaramillo et al. 2002) did not interfere with the analyses since for geometric morphometric analysis size information is removed. Additionally, colony specimens were not used for character (morphology) measurements.

In order to compare the head shape variation between T. mopan and closely related taxa, we used eight landmarks (clear, visible, homologous intersections between structures; Jaramillo 2011), taken for each specimen using StereoMorph (Olsen and Westneat 2015). Triatoma dimidiata specimens were grouped according to morpho-

Table I. Specimens used for morphological and morphometric comparison. CTIOC: Triatominae Collection of the Oswaldo Cruz Institute.

| Species | Specimen origin | ID/voucher number | Geographic Origin | Sex | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: |
| H゙ | field | A10800 | Huehuetenango, Guatemala | F |  |
|  | field | A10727 | Huehuetenango, Guatemala | M |  |
|  | CTIOC | 2838 | Colombia | M | capitata morphotype |
|  | Colony LNIRTT* | N/A | Colombia | F | capitata morphotype |
|  | CTIOC | N/A | Santa Boyaca, Colombia | F | capitata morphotype |
|  | CTIOC | 2463 | Costa Rica | F |  |
|  | CTIOC | 2592 | Costa Rica | F |  |
|  | CTIOC | 2587 | San Jose, Costa Rica | F |  |
|  | Colony LNIRTT* | N/A | Equador | F |  |
|  | Colony LNIRTT* | N/A | Equador - genitalia | M |  |
|  | CTIOC | 3385 | Candelaria Caves, Alta Verapaz, Guatemala | M |  |
|  | CTIOC | A6160 | Lanquin Caves, Guatemala | M |  |
|  | CTIOC | 3388 | Lanquin Caves, Guatemala | M |  |
|  | CTIOC | 3377 | Peten | M |  |
|  | CTIOC | 3379 | Peten | F |  |
|  | CTIOC | A9703 | Peten | F |  |
|  | CTIOC | 8937 | Mexico | M | maculipennis morphotype |
|  | CTIOC | N/A | Peru | M |  |
|  | CTIOC | 2769 | N/A | M |  |
|  | California Academy of Sciences | N/A | Boyacá, Colombia | M | capitata holotype |
|  | CTIOC | 6242 | Mexico | F |  |
|  | CTIOC | N/A | San Marcos, Texas, US | M |  |
|  | CTIOC | N/A | Texas | M |  |
|  | CTIOC | 6239 | N/A | F |  |
|  | CTIOC | 6241 | N/A | F |  |
|  | Colony 16 LNIRTT | N/A | Belize | F | colony started in 12/05/2006 |
|  | Colony 147 LNIRTT | N/A | Belize | F | colony started in 12/05/2006 |
|  | Field | 2016BZ001 | Cayo District, Rio Frio Cave, | F |  |
|  | Field | 2016BZ002 | Cayo District, Rio Frio Cave, Belize | F |  |
|  | Field | 2016BZ003 | Cayo District, Rio Frio Cave, Belize | F |  |
|  | Field | 2016BZ004 | Cayo District, Rio Frio Cave, Belize | F |  |
|  | Field | 2016BZ005 | Cayo District, Rio Frio Cave, Belize | F |  |
|  | Field | 2016BZ006 | Cayo District, Rio Frio Cave, Belize | F |  |
|  | Field | 2016BZ007 | Cayo District, Rio Frio Cave, Belize | M |  |
|  | Field | 2016BZ008 | Cayo District, Rio Frio Cave, Belize | M |  |
|  | Field | 2016BZ009 | Cayo District, Rio Frio Cave, Belize | M |  |
|  | Field | 2016BZ011 | Cayo District, Rio Frio Cave, Belize | M |  |
|  | Field | 2016BZ013 | Cayo District, Rio Frio Cave, Belize | M |  |



Figure I. Scheme of the characters measured on the head.

Table 2. Sequence information and specimens used for the phylogenetic reconstruction and calculation of genetic distances.

| Taxon | Sequence ID | Locality | ITS-2 | Cyt b |
| :---: | :---: | :---: | :---: | :---: |
| T. dimidiata | 1 | Sta. Theresa, Toledo | DQ871354 | FJ197155 |
|  | 10 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 11 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 12 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 13 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286695 | JN585881 |
|  | 14 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | KT874438 | JN585881 |
|  | 15 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585893 |
|  | 16 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585894 |
|  | 17 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585894 |
|  | 18 | Angeles, San Rafael, Heredia, Costa Rica | KF192843 | JN585894 |
|  | 19 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | KT874433 | JN585895 |
|  | 2 | Mérida, Yucatán, Mexico | FJ197146 | FJ197157 |
|  | 20 | Colombia | AM286703 | KT998309 |
|  | 21 | Colombia | AM286703 | KT998309 |
|  | 22 | Colombia | AM286704 | KT998309 |
|  | 23 | Colombia | KF192845 | KT998310 |
|  | 24 | Lanquin, Alta Verapaz, Guatemala | AM286702 | KT998313 |
|  | 25 | Lanquin, Alta Verapaz, Guatemala | AM286702 | KT998314 |
|  | 26 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286694 | KT998315 |
|  | 27 | El Masical, San Antonio, Copán, Honduras | AM286694 | KT998316 |
|  | 28 | El Masical, San Antonio, Copán, Honduras | AM286695 | KT998316 |
|  | 29 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | KT998317 |
|  | 3 | Lanquin, Alta Verapaz, Guatemala | AM286694 | JN585861 |
|  | 30 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286696 | KT998318 |


| Taxon | Sequence ID | Locality | ITS-2 | Cyt b |
| :---: | :---: | :---: | :---: | :---: |
| T. dimidiata | 31 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286695 | KT998319 |
|  | 32 | El Masical, San Antonio, Copán, Honduras | AM286694 | KT998320 |
|  | 33 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286693 | KT998321 |
|  | 34 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | KT874435 | KT998321 |
|  | 35 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | KT874437 | KT998321 |
|  | 36 | El Masical, San Antonio, Copán, Honduras | AM286693 | KT998322 |
|  | 37 | El Masical, San Antonio, Copán, Honduras | KT874436 | KT998322 |
|  | 38 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286693 | KT998325 |
|  | 39 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286694 | KT998325 |
|  | 4 | Lanquin, Alta Verapaz, Guatemala | AM286702 | JN585861 |
|  | 40 | El Lodo Negro, San Marcos Sierra, Intibuca, Honduras | AM286695 | KT998325 |
|  | 41 | El Masical, San Antonio, Copán, Honduras | KT874434 | KT998325 |
|  | 42 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | KT998327 |
|  | 43 | Angeles, San Rafael, Heredia, Costa Rica | AM286693 | KT998328 |
|  | 44 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | KT874432 | KT998330 |
|  | 45 | Angeles, San Rafael, Heredia, Costa Rica | KF192844 | KT998331 |
|  | 46 | San Pedro Columbia, Toledo district, Belize | FJ197153 | FJ197154 |
|  | 5 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 6 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 7 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 8 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
|  | 9 | Caserío la Bendición, Monte Largo, Santa Ana, El Salvador | AM286693 | JN585881 |
| T. sp. aff dimidiata | 1 | Calla Creek, Cayo District, Belize | FJ197152 | FJ197156 |
|  | 2 | Mérida, Yucatán, Mexico | FJ197150 | FJ197158 |
|  | 3 | Mérida, Yucatán, Mexico | FJ197147 | FJ197159 |
|  | 4 | Teya, Yucatán, Mexico | KT874439 | KT998296 |
|  | 5 | Huehuetenango, Guatemala |  |  |
|  | 5 | Huehuetenango, Guatemala |  |  |
| T. mopan | 1 | Río Frio Cave, Cayo District, Belize | KF192846 | JN585883 |
|  | 2 | Río Frio Cave, Cayo District, Belize | KF192847 | JN585884 |
|  | Col16 |  |  |  |
| T. infestans |  |  | AJ576054 | JN006799 |
| T. gerstaeckeri |  | Chihuahua | JQ282707 | JQ282723 |
| T. brailovskyi |  | Jalisco | JQ282704 | JQ282720 |
| T. hegneri |  |  | AM286727 | JN585830 |

type, and all comparisons were done amongst groups. Generalized Procrustes analysis was performed so shapes were directly comparable, without the effect of size and this was followed by Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA). ANOVA statistics were used to compare variance between the group means for Centroid size (ANOVA) and shape (Procrustes ANOVA), with the null hypothesis that the means are not different among groups. All analyses were performed using the package MorphoJ (Klingenberg lab 2014).


Figure 2. Landmarks selected for the morphometric comparison. Photograph Raquel Lima.

## Phylogeny and genetic distance

Portions of the nuclear Internal transcribed spacer 2 (ITS-2) and mitochondrial cytochrome b (cytb) gene sequences from all nominal species belonging to the dimidiata species subcomplex and species affinis (T. dimidiata, T. sp. aff. dimidiata, T. gerstaeckeri, T. brailovskyi, T. hegneri, and T. mopan; Table 2; (Justi and Galvão 2017) were used for phylogenetic reconstruction and comparison of genetic distances, with T. infestans as outgroup. Triatoma mopan specimens used for this purpose were the same as described from Rio Frio cave, Cayo, Belize in Monteiro et al. (2013), Dorn et al. (2016), Justi et al. (2018), including two specimens from the LNIRTT colony. PCR and sequencing of these genes was performed as previously described (Dorn et al. 2016).

Sequences were aligned using MAFFT version 7 (Katoh and Standley 2013) for ITS-2, with the algorithm Q-INS-I and ClustalW (Larkin et al. 2007) implemented on MEGA v. 6 (Tamura et al. 2013) for cytb. JModeltest (Darriba et al. 2015) was used to assess the best fit model of evolution under AIC criterion. Maximum likelihood phylogenies were reconstructed independently for each marker using PhyML v.3.1 (Guindon and Gascuel 2003) with 100 bootstrap replicates. The best-fit model for ITS-2 was HKY+G and for cytb, TPM2uf+G.

In order to evaluate previously reported genetic distances (Monteiro et al. 2003, Monteiro et al. 2004) and assess comparable intra- and interspecific distances with previously reported data (K2-p cytb distances < 2\% for interspecific comparisons), we used the package ape (Paradis et al. 2004), in R (The R Development Core Team 2008); the code is provided as Supp. material 1 (S2).

## Taxonomy

Family Reduviidae Latreille, 1807
Subfamily Triatominae Jeannel, 1919
Genus Triatoma Laporte, 1832
Triatoma mopan Dorn, Justi \& Dale, 2018, sp. n.
http://zoobank.org/94C6EBF5-D78A-4294-97EC-AAD58AAE2769
Figure 6
Material. Holotype Male. BELIZE: Cayo: Rio Frio Cave, coordinates: 16.956939/$88.979675,15$ June 2016, Dorn, Justi, Stevens, Monroy, CTIOC, FIOCRUZ. Paratypes. Five males and six females, Cayo: Rio Frio Cave, coordinates: $16.956939 /-$ 88.979675, 15 June 2016, Dorn, Justi, Stevens, Monroy (CTIOC; FIOCRUZ [four males and five females]; National Museum of Natural History, Smithsonian Institution [one male and one female])

Diagnosis. Triatoma mopan has an overall vestiture similar to T. dimidiata, generally with a more slender body. It is believed to have been wrongly identified as $T$. dimidiata by Lent and Wygodzinsky (1979). Upon close examination, T. mopan can be easily distinguished from T. dimidiata specimens by the consistently pale-yellow hemelytra, and uniformly dark brown to black head and pronotum with scarce pale setae, absent in T. dimidiata. Triatoma mopan has the pronotum with a straight latitudinal depression dividing it in half and the anterior lobe of the pronotum rugose without any distinguishable tubercles (all the examined morphotypes of T. dimidiata present such tubercles) (Figure 3). In addition, the fore-femora with $1+1$ apical, small denticles, $2+1$ subapical denticles in both males and females; and mid-femora with $1+1$ apical, small denticles, $2+2$ asymmetrical subapical larger denticles on males and $2+2$ larger, asymmetrical subapical denticles on females. In contrast, T. dimidiata presents $1+1$ small subapical denticles on both fore- and mid-femora in both sexes (Figure 4). Abdominal spiracles are close or adjacent to the connexival suture and surrounded by spot slightly darker then the tegument in T. mopan, but never adjacent and always surrounded by a well-defined dark spot in T. dimidiata (Figure 5).

Description. Length of male 26.6-30.1 mm., of female 32.1-38 mm.; width of pronotum of male $6.2-6.3 \mathrm{~mm}$., of female $6-7.4 \mathrm{~mm}$ (Table 4). Overall color dark brown or black, with connexivum and corium pale yellow. Pilosity pale yellow, short and scarce (Figure 6).

Head distinctively rugose dorsally (Figure 7), averaging twice as long as width across the eyes ( $1: 0.39-0.46$ ) and slightly longer than the pronotum (1:1.09-1.23). Short, scarce, pale yellow pilosity present in both dorsal and ventral portions (Figure 7). Anteocular region from 2.8 to 3.2 times as long as post ocular (1:0.31-0.35), post ocular region with sides almost straight, subparallel and not rounded. Eyes in lateral view surpassing the level of the ventral surface of the head. Ratio of the width of eye to synthlip-


Figure 3. Drawings of the pronotum of T. mopan and distinct T. dimidiata morphotypes. Note that for T. dimidiata the anterior lobe shows discal and lateral tubercles, that are absent in T. mopan, which also presents a shorter and rounder anterolateral angle. Drawings by Carolina Dale.
sis: $1: 1.81-2.46$. Ocelli very small, with diameter averaging about half the distance from their anterior border to the posterior margin of the eye. Antenniferous tubercles subcylindrical, situated slightly posterior of middle of anteocular region of head; first antennal segment attaining to the level of or surpassing the apex of clypeus; second segment with many strong setae. Antennal segments presenting a darker to lighter coloration from the first to the fourth segment, going from dark brown (first segment), reddish brown (second segment) to pale yellow (third and fourth segments). Ratio of antennal segments: 1:2.71-3.4:2.5-2.6:1.15. Labium slender; first visible segment extending almost to the base of the antenniferous tubercle in males and to the level of apex of antenniferous tubercle in females; second visible segment extending to the anterior portion of the thorax, almost reaching the stridulatory sulcus in males, and attaining level of posterior border of head on females; third visible segment attaining almost to the posterior portion of the stridulatory sulcus in males, and to the anterior half of the stridulatory sulcus on females (Figure 7). Ratio of visible labium segments: 1:1.79-2.15:0.44-0.69. Color dark brown (first visible segment) to light reddish brown (third visible segment). Setae pale yellow, with length and density increasing from the first visible through the third visible segment, being the longest and denser on the third segment (Figure 7).


Figure 4. Comparison between the fore and mid femora of T. mopan and T. dimidiata from Jutiapa, Guatemala. Drawings: Carolina Dale. Photographs Silvia Justi.

## Triatoma mopan sp.n Triatoma dimidiata



Figure 5. Comparison between the position and color of the abdominal spiracles from T. mopan and T. dimidiata from Jutiapa, Guatemala. Drawings by Carolina Dale. Photographs Silvia Justi and Raquel Lima.


Figure 6. Overall vestiture of T. mopan male and female. Scalebar 10 mm . Photographs Raquel Lima.


Figure 7. Comparison between the dorsal and ventral portions of the heads from T. mopan and T. dimidiata from Jutiapa, Guatemala males and females. Photographs: Silvia Justi and Raquel Lima.

## Triatoma mopan $\mathrm{sp} . \mathrm{n}$. Triatoma dimidiata



Figure 8. Comparison between the pronotum morphology of T. mopan and T. dimidiata from Jutiapa, Guatemala males and females. Photographs: Silvia Justi.

Neck dark, with $1+1$ lateral light brown to yellowish spots. Pronotum uniformly dark brown to black, with a distinctive depression forming a straight latitudinal line from the neck to the posterior portion of the pronotum (Figure 8). Anterior lobe rugose, without distinctive tubercles (Figures 3 and 8). Anterolateral angles, short, rounded, in some specimens subconical. Scutellum shallowly rugose, with central median depression heart shaped, apical process about as long as body of scutellum, subcylindrical, slightly downwardly bent at apex. Hemelytra usually surpassing the apex of abdomen but leaving female genital segments exposed. Basal portion of clavus dark brown, apex pale yellow. Corium pale yellow, with the extreme apex black, and with a dark central spot of variable size. Membrane almost as pale as the corium. Legs uniformly dark. Forelegs with $1+1$ apical small denticles $2+1$ subapical denticles on both males and females. Middle legs with $1+1$ apical small denticles $2+2$ asymmetrical subapical denticles on males and $2+2$ asymmetrical subapical denticles on females (Figure 4).

Abdomen ventrally convex, minutely striate transversally, with scarce yellow pilosity. Mostly dark brown, with yellow spots extending from the connexival suture (Figure 9). Spiracles close or adjacent to connexival suture, usually surrounded by a spot slightly darker than tegument (Figure 9). Connexival segments piceous or black on anterior third to half across entire width, almost always as an extension of the piceous portion of the abdomen, pale posteriorly.

## Triatoma mopan $\mathrm{sp} . \mathrm{n}$. Triatoma dimidiata



Figure 9. Comparison between ventral views of from T. mopan and T. dimidiata from Jutiapa, Guatemala males and females. Photographs: Silvia Justi and Raquel Lima.

External genitalia dark brown to black, almost round, with setae darker than the rest of the tegument in males; triangular, with long reddish setae on females (Figure 9).

Distribution. To date, the species is only known from the type locality.
Type-locality. Rio Frio cave, Cayo District, Belize, coordinates 16.956939/88.979675.

Etymology. The specific epithet mopan was chosen to honor the indigenous Mopan people, one of the Mayan groups historically and presently in this area of Belize and Guatemala, and comprises the lineage previously referred to as $T$. sp. aff. dimidiata (Group 4 - cave; Monteiro et al. 2013, Stevens et al. 2014, Dorn et al. 2016, Justi et al. 2018).

Host-parasite data. Specimens of T. mopan sp. n. collected prior to its description, in the same Rio Frio cave, were found to be infected with Trypanosoma cruzi (Monroy, unpublished data). The type series was not investigated for parasite infection in order to preserve the integrity of the samples.

Earlier studies completed by our research group, identifying blood sources on specimens of the then undescribed T. mopan collected in the same Rio Frio cave indicate that this species feeds on human, pig, goat or sheep, rat, mouse, duck, bat, opossum, and synanthropic and wild vertebrates (Stevens et al. 2014).

Bionomics. Found in caves, in cracks in rocks near water, in humid environment.

## Results

## Morphological and morphometric study

Character measures used for the description were compared separately for males and females between T. mopan and the morphotypes of T. dimidiata (Table 3). Barplots showing the mean and standard deviation for each of the significantly different characters are shown on Figure 10, and the bar plots for all the characters are shown in the Suppl. material 1. After the correction for multiple comparisons five of the 18 characters varied between species for females and six of 17 for males (because of missing third and fourth antennae segments, not all 19 characters were measured for T. dimidiata (see Table 3). Four characters were significantly different between species for both females and males (HL, POR, R3 and SYN. For females, the two species also differed for the R1 and R2. For males, A1 and AOR differed between species (Table 3).

Head shape comparison between T. mopan specimens and T. dimidiata and T. gerstaeckeri revealed a unique separate cluster comprising T. mopan for both PCA and CVA (Figure 11). ANOVA results led to the rejection of the null hypothesis ( $\mathrm{p}<0.0001$ ), that is, our results indicate that the variance between the means of the groups are different. PCA results show that most of the variation is from landmarks corresponding to the antennal tubercle and the anterior portion of the eye (Figure 12).

## Phylogenetic reconstruction and genetic distances

Both ML phylogenies recovered T. mopan as an independently evolving lineage, with the highest bootstrap support recovered for each phylogeny. Triatoma mopan was recovered as a sister taxon to T. dimidiata upon ITS-2 phylogenetic reconstruction and as sister to T. gerstaeckeri when cytb was used for the reconstruction (Figure 13).

Pairwise Kimura 2-parameter genetic distances revealed T. mopan to diverge at least $2 \%$ from the other lineages when distances were calculated for ITS-2; while for cytb the minimum pairwise distance between T. mopan and the closest examined species increases to about 10\% (Figure 14).

## Discussion

Phylogenetic studies of the diverse T. dimidiata have long shown the taxa to be composed of at least three independently evolving lineages (Dorn et al. 2007, 2016, Bargues et al. 2008, Herrera-Aguilar et al. 2009, Monteiro et al. 2013, Justi et al. 2018). One such lineage, the Triatoma from the Rio Frio cave in Belize, was first observed to be a separate phylogenetic species by Monteiro et al. 2013, and referred to as T. dimidiata group 4. Later, more comprehensive studies confirmed the specific phylogenetic status of the lineage referred to as $T$. sp. aff. dimidiata - cave, pending the formal description of the new species (Dorn et al. 2016, Justi et al. 2018). In this study, we describe the
Table 3. Character measurements ( mm ) of T. mopan and T. dimidiata specimens and significance of t -test results for the comparison between the sexes of both species. Key: asterisk ( ${ }^{*}$ ) significant value based on Benjamini-Hochberg multiple comparison False Discovery Rate, $\mathrm{FDR}=0.05$. n.s. non-significant value, N/A - not available.

| Character | T. mopan females |  |  | T. dimidiata females |  |  | P -value | T. mopan males |  |  | T. dimidiata males |  |  | P-value |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | N | Mean (mm) | $\begin{gathered} \text { Min-Max } \\ (\mathrm{mm}) \\ \hline \end{gathered}$ | N | Mean (mm) | Range (mm) |  | N | Mean (mm) | Range (mm) | N | Mean (mm) | Range (mm) |  |
| A1 | 6 | 1.63 | 1.406-1.781 | 6 | 1.492 | 1.200-1.750 | n.s. | 5 | 1.538 | 1.406-1.625 | 6 | 1.223 | 1.000-1.563 | $<0.02^{*}$ |
| A2 | 6 | 4.719 | 4.500-5.281 | 5 | 4.2 | $3.400-4.800$ | n.s. | 5 | 4.769 | 4.125-5.313 | 4 | 4.255 | 4.000-4.719 | n.s. |
| A3 | 3 | 4.365 | 4.219-4.531 | 3 | 3.567 | 3.100-3.800 | n.s. | 5 | 4.194 | 3.688-4.469 | 0 | N/A | N/A | N/A |
| A4 | 1 | 1.844 | 1.844-1.844 | 3 | 2.617 | 1.750-3.100 | N/A | 5 | 2.725 | 0.000-4.188 | 0 | N/A | N/A | N/A |
| AOR | 6 | 3.781 | 3.688-4.063 | 3 | 2.567 | 1.800-3.250 | n.s. | 5 | 3.513 | 3.313-3.813 | 7 | 2.734 | 2.250-3.500 | <0.002* |
| BOS | 6 | 1.823 | 1.563-2.063 | 3 | 1.717 | 1.550-1.950 | n.s. | 5 | 1.525 | 1.188-2.000 | 7 | 1.857 | 1.500-2.063 | n.s. |
| HL | 6 | 6.192 | 5.923-6.538 | 6 | 5.383 | 4.650-5.900 | < 0.005* | 5 | 5.569 | 5.308-5.846 | 7 | 4.758 | 4.308-5.615 | <0.005* |
| LOP | 6 | 5.295 | 5.000-6.000 | 6 | 4.767 | 3.950-5.400 | n.s. | 5 | 4.723 | 4.308-5.077 | 7 | 4.805 | 3.950-7.000 | n.s. |
| POR | 6 | 1.281 | 1.188-1.375 | 6 | 0.942 | 0.800-1.050 | <0.0001* | 5 | 1.15 | 1.125-1.188 | 7 | 0.929 | 0.750-1.188 | <0.005* |
| POS | 6 | 1.458 | 1.000-1.688 | 3 | 1.55 | 1.200-1.750 | n.s. | 5 | 1.425 | 0.813-1.625 | 7 | 1.307 | 1.063-1.625 | n.s. |
| R1 | 6 | 2.133 | 2.000-2.250 | 6 | 1.808 | 1.650-2.000 | < 0.002 | 5 | 1.87 | 1.750-2.050 | 6 | 1.8 | 1.650-2.150 | n.s. |
| R2 | 6 | 4.133 | 3.750-4.450 | 6 | 3.417 | 2.900-3.850 | <0.002 | 5 | 3.83 | 3.750-3.950 | 6 | 3.292 | 2.950-4.000 | n.s. |
| R3 | 6 | 1.202 | 1.000-1.610 | 6 | 0.967 | 0.850-1.050 | $<0.05$ n.s. | 5 | 1.2 | 1.150-1.250 | 6 | 0.933 | 0.700-1.150 | <0.01* |
| S | 6 | 1.308 | 1.231-1.538 | 6 | 1.033 | 0.900-1.250 | <0.005* | 5 | 1.169 | 1.077-1.231 | 7 | 0.95 | 0.850-1.231 | <0.005* |
| TL | 6 | 34.58 | $\begin{gathered} 32.170- \\ 38.000 \\ \hline \end{gathered}$ | 6 | 33.12 | $\begin{gathered} 28.830- \\ 34.200 \\ \hline \end{gathered}$ | n.s. | 5 | 28.73 | $\begin{gathered} 26.670- \\ 30.170 \\ \hline \end{gathered}$ | 7 | 29.98 | $\begin{gathered} 23.830- \\ 35.500 \\ \hline \end{gathered}$ | n.s. |
| TS | 6 | 3.281 | 2.750-3.500 | 3 | 3.15 | 2.500-3.650 | n.s. | 5 | 2.95 | 2.750-3.188 | 7 | 3.164 | 2.750-3.688 | n.s. |
| WAE | 6 | 2.487 | 2.385-2.692 | 6 | 2.492 | 2.200-2.800 | n.s. | 5 | 2.323 | 2.231-2.462 | 7 | 2.415 | 2.154-2.900 | n.s. |
| WE | 6 | 0.599 | 0.500-0.625 | 6 | 0.733 | 0.600-0.900 | n. | 5 | 0.594 | 0.563-0.625 | 7 | 0.654 | 0.500-0.800 | n.s. |
| WOP | 6 | 6.526 | 6.077-7.385 | 6 | 6.633 | $2.800-8.000$ | n.s. | 5 | 6.308 | 6.231-6.385 | 7 | 5.352 | 3.100-7.385 | n.s. |



Figure IO. Barplot of the character measures that were significantly different for at least one comparison between male and females of T. mopan and T. dimidiata (see Table 3).

Table 4. Triatoma mopan character measures for males, females and the holotype.

|  | Males (mm) |  |  |  |  | Females (mm) |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Min. | Mean | Max. | sd | Min. | Mean | Max. | $\boldsymbol{s d}$ | (mm) |
| A1 | 1.41 | 1.538 | 1.63 | 0.086 | 1.41 | 1.63 | 1.78 | 0.138 | 1.59 |
| A2 | 4.13 | 4.77 | 5.31 | 0.483 | 4.5 | 4.718 | 5.28 | 0.293 | 5.19 |
| A3 | 3.69 | 4.194 | 4.47 | 0.303 | 4.22 | 4.363 | 4.53 | NA | 4.31 |
| A4 | 2.75 | 2.952 | 3.19 | 0.197 | 2.75 | 3.282 | 3.5 | 0.279 | 3.13 |
| AOR | 3.31 | 3.512 | 3.81 | 0.189 | 3.69 | 3.782 | 4.06 | 0.145 | 3.56 |
| BOS | 1.19 | 1.526 | 2 | -0.332 | 1.56 | 1.823 | 2.06 | 0.191 | 1.5 |
| HL | 5.31 | 5.57 | 5.85 | 0.261 | 5.92 | 6.193 | 6.54 | 0.217 | 5.85 |
| LOP | 4.31 | 4.724 | 5.08 | 0.305 | 5 | 5.295 | 6 | 0.371 | 4.77 |
| POR | 1.13 | 1.154 | 1.19 | 0.033 | 1.19 | 1.282 | 1.38 | 0.076 | 1.19 |
| POS | 0.81 | 1.426 | 1.63 | -0.349 | 1 | 1.46 | 1.69 | 0.28 | 1.63 |
| R1 | 1.75 | 1.87 | 2.05 | 0.130 | 2 | 2.133 | 2.25 | 0.103 | 2.05 |
| R2 | 3.75 | 3.83 | 3.95 | 0.084 | 3.75 | 4.133 | 4.45 | 0.225 | 3.85 |
| R3 | 1.15 | 1.2 | 1.25 | 0.05 | 1 | 1.202 | 1.61 | 0.211 | 1.25 |
| S | 1.08 | 1.168 | 1.23 | 0.063 | 1.23 | 1.308 | 1.54 | 0.120 | 1.15 |
| TL | 26.67 | 28.74 | 30.17 | 1.677 | 32.17 | 34.58 | 38 | 2.056 | 29.67 |
| TS | 2.84 | 3.408 | 4.19 | NA | 1.84 | 1.84 | 1.84 | NA | 3.13 |
| WAE | 2.23 | 2.324 | 2.46 | 0.084 | 2.38 | 2.485 | 2.69 | 0.117 | 2.31 |
| WE | 0.56 | 0.592 | 0.63 | 0.025 | 0.5 | 0.602 | 0.63 | 0.052 | 0.59 |
| WOP | 6.23 | 6.306 | 6.38 | 0.075 | 6.08 | 6.525 | 7.38 | 0.458 | 6.38 |

## PCA

CVA


Figure II. PCA and CVA of the Morphometric comparison of the heads of T. mopan, T. gerstaeckeri, Triatoma from Lanquin and T. dimidiata different morphotypes. Note that both T. mopan and Triatoma from Lanquin form clusters separated from the other species.


## PC1

Figure I2. PCA thin plate showing that landmarks 1 and 2 are the most variable within the compared specimens.


Figure 13. ML phylogenies reconstructed using ITS-2 (right) and cytb (left) sequences. Numbers above branches represent bootstrap support > 50. T. mopan clade is highlighted in purple.

K2p cytb pairwise distances


K2p ITS-2 pairwise distances


Figure 14. Plot of the K2-p distances calculated pairwise between T. mopan and all the other specimens used for the phylogenetic reconstruction. Dotted line indicates $2 \%$ divergence.

Triatoma lineage from the Rio Frio cave, in Belize and and name it Triatoma mopan, after the Mopan people of that area.

We have compared T. mopan with T. dimidiata using different systematic approaches: classic morphology, geometric morphometric and molecular phylogeny, and the results agree in that these are separate species. Diagnostic characters were observed on the pronotum, legs and abdomen and PCA and CVA results place both in separate clusters (Figures 3, 4, 5, 11).

The morphological comparison of T. mopan with the description of T. dimidiata (Lent and Wygodzinsky 1979) and photos of the holotypes of T. dimidiata capitata and T. dimidiata maculipennis (Figure 15), along with the molecular phylogenetic results published previously (Dorn et al. 2007, 2009, 2016, Bargues et al. 2008, Monteiro et al. 2013, Justi et al. 2018) show the uniqueness of the Rio Frio species. All diagnostic characters and the description of the species, allowed us to observe a combination of characters unique to T. mopan.

Lent and Wygodzinsky (1979) observed 160 specimens of T. dimidiata and concluded that the morphological variation observed within the taxa displays a clinal pattern with size increasing southwards. Amongst those, they describe five specimens from the Lanquin cave, in Guatemala, highlighting differences in the length and ratio of the antennae, and mentioning characteristics related to the cave environment, such as diminished pigmentation and eye and ocelli size when compared with non-cave populations of T. dimidiata. The authors also stated that the single specimen examined from the Rio Frio cave "is identical phenotypically with the specimens from Lanquin cave". In light of this statement, we compared T. mopan with the Lent and Wygodzinsky (1979) description and to two specimens from the Lanquin cave population and noticed distinctive characteristics in the head (Table 5), wing, and abdomen coloration pattern (Figure 16) that clearly separate T. mopan and the Lanquin population of Triatoma. Likely convergent evolution due to the cave environment is observed in the diminished pigmentation and relative smaller eye and ocelli sizes and the absence of tubercles on the anterior lobe of the pronotum. Even though Lent and Wygodzinsky stated that these are T. dimidiata populations from caves, it is not possible to taxonomically place these Lanquin and Rio Frio cave specimens using their key to the species of Triatoma (combination of characters described on dychotomy 39 does not correspond to either population).

The geometric morphometric comparison of the head shape (PCA and CVA) also placed the Lanquin specimens closer to T. dimidiata (but separate from) than to T. mopan, which forms a unique separate cluster (Figure 11). Similar results were previously found, placing the Lanquin population in a separate cluster from the T. dimidiata populations (Bustamante et al. 2004). Additionally, previous molecular phylogeny results have shown that the Triatoma specimens collected in the Lanquin cave fall within T. dimidiata s.s (Monteiro et al. 2013, Dorn et al. 2016), while the ones collected in the Rio Frio cave comprise an independently evolving lineage. These results were confirmed by the broader phylogenetic recontruction and comparison of genetic distances performed for this study (Figures 13, 14). The widely distinct morphology of

Table 5. Character comparison between T. mopan, T. dimidiata, and Triatoma from Lanquin cave, based on the description of Lent and Wygodzinsky (1979) for the latter.

|  | T. mopan | Triatoma Lanquin | T. dimidiata |
| :--- | :---: | :---: | :---: |
| Antenna | first antennal segment <br> attaining to the level of or <br> surpassing the apex of clypeus | first antennal segment <br> surpassing the apex of clypeus | first antennal segment <br> attaining to the level of the <br> apex of clypeus |
| POR/AOR | $2.8-3.2$ | 4 | $2.5-3$ |
| Ratio antennae <br> segments | $1: 2.7-3.4: 2.5-2.6: 1.15$ | $1: 2.5: 2.2: 2.3$ | $1: 3.2-3.8: 2.552 .2$. |
| Eyes | surpassing the level of the <br> ventral surface of the head | not surpassing the level of the <br> ventral surface of the head | attaining to the level of the <br> ventral surface of the head |



Figure 15. Triatoma dimidiata capitata (Photo: Rachel Diaz-Bastin, California Academy of Sciences), T. mopan (Photo: Raquel Lima-Cordón), and T. dimidiata maculipennis (Photo: © Schurian / MfN.berlin) holotypes. Photographs are not to scale.
the Lanquin cave specimens, combined with these phylogenetic results show the need for a deeper study of this population to better understand its evolution and taxonomy.

The comparison of the morphology of T. mopan with T. dimidiata shows a clear trend in cave adaptation evolution. Besides the diminished overal pigmentation of the specimens, T. mopan exhibits much denser sensillae on the visible labial segments


Figure 16. Comparison between Triatoma from Lanquin and T. mopan. Photographs to scale. Photo: Carolina Dale.
(Figure 7), which are significantly longer than in T. dimidiata, probably to compensate for the reduced visual cues in such an environment.

In combination, the morphological characters with molecular phylogeney and geometric morphometry of the head show that T. mopan is an independently evolving lineage, separate from T. dimidiata. The comparison with the types of T. dimidiata maculipennis, T. dimidiata capitata and the description given by Lent and Wygodzinsky (1979) for T. dimidiata, in the absence of the type, make it clear that T. mopan is a separate species, not previously formally described as any morphotype or subspecies of T. dimidiata.

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## Supplementary material I

## T. mopan $\times$ T. dimidiata - boxplots comparing the characters.

Authors: Patricia L. Dorn, Silvia A. Justi, Carolina Dale, Lori Stevens, Cleber Galváo, Raquel Lima-Cordón, Carlota Monroy
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## Supplementary material 2

## T. mopan description - R codes.

Authors: Patricia L. Dorn, Silvia A. Justi, Carolina Dale, Lori Stevens, Cleber Galvão, Raquel Lima-Cordón, Carlota Monroy
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# Micropeplus liweiae sp. n ., a new species from Sichuan, China (Coleoptera, Staphylinidae, Micropeplinae) 

Cheng-Bin Wang', Ri-Xin Jiang², Jiang Zhu ${ }^{3}$<br>I Innovation College, Mianyang Normal University, 166 Mianxing West Road, Mianyang 621000, Sichuan Province, PR China 2 Department of Biology, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, PR China 3 College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, 388 Guangxin Road, Guangzhou 510550, Guangdong Province, PR China<br>Corresponding author: Jiang Zhu (jiangzhu_gz@163.com)

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#### Abstract

A new species of micropepline beetle is described from Sichuan, China, Micropeplus liweiae sp. n. (Coleoptera, Staphylinidae). Important morphological characters of the new species are illustrated by colour plates.


## Keywords

China, Micropeplinae, Micropeplus, new species, Staphylinidae, taxonomy

## Introduction

Micropeplus Latreille, 1809 is the most speciose genus of Micropeplinae (Coleoptera, Staphylinidae), with members distributed in Ethiopian, Nearctic, Neotropical, Oriental and Palaearctic Regions. Campbell (1968, 1992, 1995) established eight speciesgroups to classify the species of the genus.

In the fauna of China, 22 species had been recorded before this study (Herman 2001; Schülke and Smetana 2015; Grebennikov and Smetana 2015; Zheng et al. 2016). Grebennikov and Smetana (2015) revealed the amazing hyper diversity of Micropeplinae in Southwest China. In this paper, Micropeplus liweiae sp. n., a new species belonging to the staphylinoides species-group is described and illustrated from Sichuan Province, China. The type specimens were collected under a rock in an alpine meadow.

## Materials and methods

Specimens were relaxed and softened in a hot saturated solution of potassium hydroxide for 4 minutes（for dry mounted specimens）or 8 minutes（for alcohol－preserved specimens）， and then transferred to distilled water to rinse the residual potassium hydroxide off and stop any further bleaching．The softened specimens were moved into glycerin and dissected there to observe morphological details．After examination，the body parts were mounted on a glass slip with Euparal Mounting Medium for future studies．Habitus photographs were taken using a Canon MP－E $65 \mathrm{~mm} \mathrm{f} / 2.8$ 1－5X macro lens on a Canon 7D camera， and a Canon MT－24EX macro twin light flash was used as light source．Observations，pho－ tographs，and measurements of morphological details were performed using an Olympus CX31 microscope with a Canon G9 camera．The final deep focus images were created with Zerene Stacker 1.04 stacking software．Adobe Photoshop CS6 was used for post processing．

The material examined for this study is deposited in the following collections（with names of curators in parentheses）：SNUC－Insect Collection of Shanghai Normal University，Shanghai，China（L．Tang）；SYSB－Museum of Biology，Sun Yat－sen Uni－ versity，Guangzhou，China（F．－L．Jia）．

Measurement criteria in millimetres（ mm ）are used as follows：

Body length length between the anterior apex of clypeus and the abdominal apex along the midline．
Elytral length

Elytral width Fore body

Head length length between the anterior apex of clypeus and the posterior mar－ gin of occiput along the midline．
Head width widest part of head（including compound eyes）．
Pronotal length length of the pronotum along the midline．
Pronotal width widest part of pronotum．

## Results

Genus Micropeplus Latreille， 1809
Vernacular name：铠甲属

## Micropeplus liweiae sp．n．

http：／／zoobank．org／A6D7C159－AA58－4903－8E47－E85A0F2E5CA5
Figs 1A，B；2A－H
Vernacular name：力伟铠甲

Type material．Holotype：${ }^{\top}$ ，CHINA，Sichuan：Xiaojin County［ 小金县］，near Siguniang Shan［接近四姑娘山］， $30.921623^{\circ} \mathrm{N}, 102.889709^{\circ} \mathrm{E}$ ，under rock，

4258m，14．VII．2017，Jiang Zhu leg．（SYSB）．Paratype： 1 \＆，same data as holotype （SNUC）．

Diagnosis．This new species is very similar to Micropeplus songi Zheng，Li \＆Yan， 2014 from Mt．Wahui［瓦灰山］，Sichuan，but it is easy to distinguish it from the latter by a combination of the following characteristics：head with microreticulate surface； elytral punctures moderate－sized，distinctly smaller but more numerous than that of M．songi；elytral interspace I with two to three rows，II with three to four rows，III with four rows，IV with one row and $V$ with three rows；metathoracic wings fully developed； aedeagal parameres with two long setae at apex．

Description．Male．Body small， 2.81 mm long．Length（mm）of different body parts：head（ 0.31 ），pronotum（ 0.55 ），elytra（1．05），fore body（1．91），aedeagus（ 0.58 ）． Width（mm）：head（0．64），pronotum（1．16），elytra（1．22）．（Head width）／（pronotal width $)=0.55,($ pronotal length $) /($ elytral length $)=0.52$ ．

Habitus（Figure 1A）elliptical，generally convex and sublustrous．Head，disc of pronotum，elytra and abdomen blackish brown to black；basal eight antennomeres and apical half of ultimate antennomere，maxillary and labial palpi，legs，and sides of pronotum yellowish brown to brown．

Head（Figure 2A）transverse，widest across eyes，width／length $=2.06$ ．Clypeus with anterior margin broadly subrounded．Vertex with a longitudinal carina along midline in basal half；area on both sides of carina weakly impressed；one fine transverse carina and two oblique carinae at middle of inner side of each eye；spaces between carinae mi－ croreticulate．Eyes distinctly prominent．Antennae have 9 antennomeres with single－ segmented clubs；antennomere I more robust than other antennomeres except IX，and about equal to length of II＋III combined；II narrowed apically，shorter and slightly narrower than I；III－VI longer than wide，narrower than II，III－V subequal and longer than VI；VII and VIII transverse；IX largest and oval，covered with dense pubescence．

Pronotum subtrapezoidal，widest just before hind angles，width／length $=2.11$ ． Sides almost obliquely linear，gradually narrowing from posterior to anterior，with ca． 4 small teeth；anterior margin broadly and distinctly emarginate and slightly arched in middle；posterior margin bisinuate；anterior angles distinctly projected forwards；pos－ terior angles nearly rectangular；surface finely granulate；lateral areas broadly explanate； median area elevated dorsally，with fourteen cells enclosed by costae（including two small ones after anterior margin）．

Scutellum shield－like with finely granulate surface．
Elytra subquadrate，width／length $=1.16$ ，widest at about apical $2 / 5$ ．Each elytron with 6 costae，one sutural，two discal，one humeral，one pseudepipleural（straight，dis－ tinctly separated anteriorly and posteriorly from epipleural costa）and one epipleural； interspaces between costae with irregular rows of moderate－sized punctures，interspace I with two to three rows，II with three to four rows，III with four rows，IV with one row and $V$ with three rows．Metathoracic wings fully developed．

Metastemum with elongate median impression，wide and deep at base，weaken－ ing anteriorly，about half as long as metasternum．Abdomen gradually narrowed to－ wards segment VI and abruptly narrowed from VII to apical end；tergites III－VII each strongly and transversely depressed in basal half，III with one short median longitudi－


Figure I. Habitus of Micropeplus liweiae sp. n. (dorsal view): A holotype $\widehat{\boldsymbol{B}}$ B paratype $q$. Scale bars: 0.5 mm .
nal carina, IV to VI each with three longitudinal carinae almost throughout length of tergite, VII with three abbreviated carinae in about basal $1 / 3$, VIII (Figure 2G) with apical edge almost straight; sternite VIII (Figure 2H) with apical edge distinctly and subroundly emarginated, and semilunarly depressed before emargination.

Protibia without tooth on medial margin; meso- (Figure 2B) and metatibiae (Figure 2C) each armed with a large subtriangular tooth at about apical $1 / 3$ of medial margins.

Aedeagus (Figure 2D-F) stout; median lobe weakly bent ventrad in apical part; parameres fused with median lobe, each with two long setae at apex.

Female. Similar to male in general appearance (Figure 1B), including anterior margin of clypeus which lacking sexual dimorphism, but distinct in the following characters: meso- and metatibiae without tooth on medial margins, and sternite VIII without emargination at apical edge.

Habitat. Specimens were found under a rock in an alpine meadow (Figure 3), with high altitude over 4000 m .

Distribution. China (Sichuan).


Figure 2. Micropeplus liweiae sp. n., holotype $\widehat{\delta}^{-}$: $\mathbf{A}$ head $\mathbf{B}$ mesotibia $\mathbf{C}$ metatibia $\mathbf{D - F}$ aedeagus $\mathbf{G}$ abdominal tergite VIII $\mathbf{H}$ abdominal ventrite VIII. Scale bars: $0.5 \mathrm{~mm}(\mathbf{A}) ; 0.1 \mathrm{~mm}(\mathbf{B}-\mathbf{H}) .(\mathbf{A}-\mathbf{D}, \mathbf{G}$ dorsal view $\mathbf{E}$ lateral view $\mathbf{F}, \mathbf{H}$ ventral view).


Figure 3. Habitat of Micropeplus liweiae sp. n. The new species was collected under a rock (red arrow) in an alpine meadow of Xiaojin, Sichuan.

Etymology. The specific epithet is dedicated to Ms. Li-Wei Liu, the mother of Jiang-Zhu (the collector and corresponding author), for her care and constant support to him.

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# Two new species of the Aenictus wroughtonii species group (Hymenoptera, Formicidae, Dorylinae) from Thailand 

Weeyawat Jaitrong', Jiraporn Ruangsittichai ${ }^{2}$<br>I Natural History Museum, National Science Museum, Technopolis, Khlong 5, Khlong Luang, Pathum Thani, 12120 Thailand 2 Department of Medical Entomology, Faculty of Tropical Medicine, Mabidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok, 10400 Thailand<br>Corresponding author: Jiraporn Ruangsittichai (jiraporn.rua@mahidol.ac.th)<br>Academic editor: B. Fisher | Received 23 May 2018 | Accepted 9 July 2018 | Published 19 July 2018<br>http://zoobank.org/8480FFF6-6476-40E7-A08C-47DDC92ED854<br>Citation: Jaitrong W, Ruangsittichai J (2018) Two new species of the Aenictus wroughtonii species group (Hymenoptera, Formicidae, Dorylinae) from Thailand. ZooKeys 775: 103-115. https://doi.org/10.3897/zookeys.775.26893


#### Abstract

The Aenictus wroughtonii species group is widely distributed in Asia. The members of this group are characterised by a slender body, long legs, anterior clypeal margin with 5-10 denticles and a weakly-developed subpetiolar process. Twelve worker-based species of this group have been recorded from Asia. Herein, two new species from Thailand (Aenictus nuchiti sp. n. and Aenictus samungi sp. n.) are added to this group. A key to the Asian species of this group is provided.


## Keywords

Aenictus wroughtonii species group, Aenictus minutulus species group, army ant, distribution, new species, taxonomy, Thailand

## Introduction

The Aenictus wroughtonii species group was established by Wilson (1964) based on the external morphology of the worker caste. Subsequently, Jaitrong et al. (2010) and Jaitrong and Yamane (2011) redefined the species group and listed seven worker-based species from the Oriental region. Recently, Sharaf et al. (2012) and Staab (2014) described new species of the group from Saudi Arabia and Southeast China, respectively.

To date, 10 species have been recorded in the $A$. wroughtonii group from Greece, Turkey, Iran, Israel, India, Sri Lanka, Southeast China and Taiwan to Southeast Asia (Aktaç et al. 2004, Jaitrong et al. 2010, Jaitrong and Yamane 2011, Sharaf et al. 2012, Staab 2014).

During our survey of the Asian Aenictus, two unidentified species belonging to the A. wroughtonii group were found from Thailand. After carefully examining specimens of these two species under a stereomicroscope and comparing them with the type material of closely related species, it was concluded that both species are new to science. In the present study, the two new species are described and a key for the Asian species based on the worker caste is provided.

## Materials and methods

The holotypes and paratypes of Aenictus nuchiti sp. n. and Aenictus samungi sp. n. were pin-mounted dry specimens. The holotypes, paratypes and syntypes of seven species (A. artipus Wilson, 1964; A. biroi Forel, 1907; A. camposi Wheeler WM \& Chapman, 1925; A. sagei Forel, 1901; A. stenocephalus Jaitrong \& Yamane, 2010; A. vieti Jaitrong \& Yamane, 2010; A. wroughtonii Forel, 1890) in the A. wroughtonii species group were examined. High-resolution images of the holotype of $A$. gutianshanensis Staab, 2014 available in Antweb (2018) were also examined. Most morphological observations were made with a ZEISS Discovery V12 stereoscope.

Multi-focused montage images were produced using NIS-Elements-D-[Sequence6*-Focused] from a series of source images taken by a Nikon Digital SightRil camera attached to a Nikon AZ100M stereoscope. Type specimens of each species were measured for the following parts using a micrometer (accurate to 0.01 mm ).

The abbreviations used for the measurements and indices are as follows:

HL Maximum head length in full-face view, measured from the anterior clypeal margin to the midpoint of a line drawn across the posterior margin of the head.
HW Maximum head width in full-face view.
ML Mesosomal length measured from the point at which the pronotum meets the cervical shield to the posterior margin of the metapleuron in profile.
PH Petiolar node height, measured in profile, the maximum vertical height of the petiole from summit to lower most part of subpetiolar process.
PL Petiole length measured from the anterior margin of the peduncle to the posteriormost point of the tergite in profile.
SL Scape length excluding the basal constriction and condylar bulb.
TL Total length, axial length of body, summed HL (including mandibles) + ML + $\mathrm{PL}+$ postpetiole length + gaster length.
CI Cephalic index, HW/HL $\times 100$.
PI Petiolar index, PH/PL $\times 100$.
SI Scape index, SL/HW $\times 100$.

Abbreviations of the type depositories are as follows:

| MHNG | Muséum d'histoire naturelle, Geneva, Switzerland |
| :--- | :--- |
| SKYC | Seiki Yamane Collection, Japan |
| THNHM | Natural History Museum of the National Science Museum, Thailand |
| USNM | National Museum of Natural History, Smithsonian Institution, Washington |
|  | DC, USA |

The general terminology for the worker caste of the ants follows Hölldobler and Wilson (1990) and Bolton (1994). The studies of Jaitrong et al. (2010) and Jaitrong and Yamane (2011) have been referred to for the important characteristics of the genus Aenictus.

## Results

## Taxonomy

## Aenictus wroughtonii species group

Figs 1A, 3A
Diagnosis. (modified from Jaitrong and Yamane 2011). Head narrow, oval or elliptical; occipital margin lacking collar (distinct carina). Antenna short or long, comprising 10 segments; scape short, attaining mid-length of head or longer attaining or extending beyond the posterolateral corner of head. Anterior clypeal margin roundly convex with 5-10 denticles. Mandible triangular, with masticatory margin bearing $8-12 \mathrm{~min}$ ute inconspicuous denticles in addition to a large apical tooth with a sharp apex; basal margin of mandible lacking denticles. Frontal carina short; parafrontal ridge feeble and incomplete. Mesosoma narrow and elongate. Legs very slender. Propodeal junction in profile angulate (Fig. 1A) or rounded (Fig. 3A). Subpetiolar process weakly developed or almost absent. Head and gaster entirely smooth and shiny. Body yellow, yellowish brown to dark brown; typhlatta spot absent.

Distribution. Greece, Iran, Israel, Turkey, Saudi Arabia, India, Sri Lanka, Southeast China, Taiwan, Vietnam, Thailand, Malay Peninsula (West Malaysia), Sumatra, Borneo (Sabah, Sarawak, and Brunei) and Philippines (Negros and Luzon) (Aktaç et al. 2004, Jaitrong et al. 2010, Jaitrong and Yamane 2011, Sharaf et al. 2012, Staab 2014).

Currently valid names. Aenictus arabicus Sharaf \& Aldawood, 2012, A. artipus Wilson, 1964; A. biroi Forel, 1907; A. camposi Wheeler WM \& Chapman, 1925; A. gutianshanensis Staab, 2014, A. nuchiti sp. n., A. rhodiensis Menozzi, 1936, A. sagei Forel, 1901; A. samungi sp. n., A. stenocephalus Jaitrong \& Yamane, 2010; A. vieti Jaitrong \& Yamane, 2010; A. wroughtonii Forel, 1890.


Figure I. Aenictus nuchiti sp. n. (holotype, worker, THNHM-I-02612). A Body in profile B Body in dorsal view $\mathbf{C}$ Head in full-face view.

## Descriptions of new species

## Aenictus nuchiti sp. n.

http://zoobank.org/C0440537-2B93-4EA6-8A29-0D7CACEB3ABB
Figs 1-2, 4

Types. Holotype (THNHM-I-02612, THNHM), 55 paratype workers (THNHM-I-02614, MHNG, SKYC, THNHM, USNM) and queen (THNHM-I-02613, THNHM), N Thailand, Chiang Mai Province, Omkoi District, Omkoi Forest, DDF (dry
dipterocarp forest), $17.89583333^{\circ} \mathrm{N}, 98.40750000^{\circ} \mathrm{E}$, ca 1000 m a.s.l., 16.VII.2016, W. Jaitrong leg., Colony no. TH16-WJT-859.

Non-type material examined. 28 workers, Thailand, Chiang Mai Province, San Sai District, San Sai Luang Sub-district, near Maejo University Campus, mixed deciduous forest, collected from leaf litter, $18.92777778^{\circ} \mathrm{N}, 99.05083333^{\circ} \mathrm{E}$, ca 350 m a.s.l., 15.X.2015, N. Likhitrakarn leg., Colony no. NL151015-1 (THNHM).

Worker measurements. Holotype: HL 0.53; HW 0.40; ML 0.69; PH 0.17; PL 0.18; SL 0.41; TL 2.28; CI 75; PI 91; SI 104. Paratype workers ( $\mathrm{n}=10$ ): HL $0.50-$ 0.53; HW 0.38-0.43; ML 0.66-0.73; PH 0.13-0.17; PL 0.17-0.18; SL $0.40-0.43$; TL 2.24-2.41; CI 77-83; PI 90-91; SI 94-104.

Queen measurements. (paratype). HL 0.92; HW 01.06; ML 1.55; PH 0.53; PL 0.53; SL 0.64; TL 5.31; CI 114; PI 100; SI 61.

Description of Worker. (Holotype and paratypes; Fig. 1). Head in full-face view elliptical, clearly longer than broad with slightly convex sides and almost straight posterior margin. Antennal scape short, extending beyond the mid-length of the head but not reaching the posterolateral corner of the head; antennal segment II slightly longer than III-VI; the last (X) almost as long as VIII and IX combined and as long as II and III combined. Frontal carina thin and short, not extending beyond level of posterior margin of torulus. Clypeus short, with its anterior margin roundly convex, bearing 7 denticles. Mandible with an apical tooth large and curved, followed by a medium-sized subapical tooth and a series of 10-12 minute teeth on masticatory margin. Mesosoma in profile with pronotum strongly convex dorsally, demarcated from mesonotum by a shallow transverse groove; mesonotum convex, sloping gradually to metanotal groove; mesopleuron demarcated from metapleuron by a shallow groove. Propodeum in profile lower than promesonotum, with a weakly convex dorsal outline; propodeal junction angulate; declivity of propodeum widely and shallowly concave, encircled by a thin rim. Petiole in profile slightly longer than high, with a dorsal outline convex; seen from above relatively narrow with sides almost parallel; subpetiolar process present, its ventral outline convex, without angle or tooth; postpetiole slightly shorter than petiole but seen from above slightly broader than petiole; its node short, clearly shorter than high.

Head, antennal scapes, pronotum, petiole, postpetiole, gaster, femora and tibiae of legs entirely or extensively smooth and shiny. Antennal flagellum densely punctate; mesothorax and propodeum with dense punctures; metapleuron partly or extensively smooth.

Body with relatively sparse standing hairs mixed with sparse short hairs over surface; longest pronotal hair $0.10-0.13 \mathrm{~mm}$ long. Head, mesonotum, propodeum and gaster dark brown; pronotum, waist, antennae and legs reddish brown.

Description of Queen. (Paratype, Fig. 2). Head in full-face view subrectangular, posteriorly narrow and gradually widening anteriorly, slightly shorter than broad, with sides weakly convex and posterior margin concave; upper frons weakly concave. Antennal scape flat, relatively short, about half as long as head, basally narrow, widening considerably apicad; flagellum of antenna missing (for this specimen). Frontal carina indistinct. Parafrontal ridge absent. Anterior clypeal margin concave, without denti-


Figure 2. Aenictus nuchiti sp. n. (paratype, queen, THNHM-I-02613). A Body in profile B Body in dorsal view $\mathbf{C}$ Head in full-face view.
cles. Mandible half as long as head length, with a slender, inner margin that is convex while lateral margin weakly concave; masticatory margin without denticles. Mesosoma elongate; in profile, pronotum convex dorsally; mesonotum weakly concave; propodeal dorsum almost straight; seen from above pronotum and propodeum broader than mesonotum; propodeal junction low, roundly convex; propodeal declivity weakly convex, not encircled by a rim. Petiole longer than high, with its dorsal outline slightly elevated posteriorly, with petiole in profile posterodorsal corner bluntly angulate; seen from above petiole with a distinct longitudinal furrow running from anterior face to posterior face; subpetiolar process large, subtriangular, with its apex pointed downwards. Gaster large and elongate; first tergite narrower and shorter than second, its
anterior slope weakly concave; second tergite largest; third as long as first; tip of gaster missing in this specimen. Legs relatively long and slender; femora and tibiae clavate.

Entire body smooth and shiny, with relatively dense standing hairs; hairs slightly shorter on pronotum than on head, mandible and antennal scape; longest pronotal hair 0.08 mm long. Head dark brown; lateral and ventral faces of head and mandible reddish brown; scapes and legs yellowish brown. Mesosoma with ground colour reddish brown; lateral faces of pronotum and mesonotum, entire mesopleura and propodeal declivity dark brown. Petiole with ground colour reddish brown; lower portion of petiole, posterior slope of petiole and subpetiolar process dark brown; gaster with ground colour dark brown; lateral faces of second tergite reddish brown.

Etymology. The species is named after Mr Supachai Nuchit (Royal Forest Department, Thailand) who kindly helped us with ant collecting at Pa Omkoi National Forest, Chiang Mai Province.

Distribution. Northern Thailand (Chiang Mai Province).
Comparative diagnosis. Aenictus nuchiti sp. n. is most similar to A. biroi, A. camposi, A. gutianshanensis and $A$. vieti in having dense punctures on the mesosoma and an angulate propodeal junction. However, $A$. nuchiti is much smaller than the latter four (TL 2.24-2.41 mm, HW $0.38-0.43 \mathrm{~mm}$ in A. nuchiti; TL $>2.6 \mathrm{~mm}$, HW $>0.43 \mathrm{~mm}$ in the latter four). It has a short antennal scape that reaches only two-thirds the head length (in contrast, reaching or extending beyond the posterolateral corners of the head in the latter four). This species can be distinguished from $A$. gutianshanensis and A. vieti by the configuration of the subpetiolar process (ventral outline roundly convex and without anterior angle in $A$. nuchiti; ventral outline with anterior angle in $A$. biroi, A. gutianshanensis and A. vieti). Aenictus nuchiti is similar to $A$. biroi and $A$. camposi in the unarmed subpetiolar process. In $A$. nuchiti, however, the body size is much smaller than that of $A$. biroi and the head is clearly longer than broad in A. nuchiti (almost as long as broad in $A$. biroi). The body colour is dark brown in $A$. nuchiti, whereas it is entirely yellow in $A$. camposi. The propodeal declivity is broader and widely rounded above in $A$. nuchiti but is narrow and tapers distinctly above in $A$. camposi.

## Aenictus samungi sp. n.

http://zoobank.org/758F945A-D992-438B-ACFC-BD8E55283AB6
Figs 3, 5
Types. Holotype (THNHM-I-02615, THNHM) and 15 paratype workers (THNHM-I-02616, MHNG, SKYC, THNHM, USNM), Thailand, Tak Province, Um Phang District, Thung Yai Wildlife Sanctuary, Yuyi Junction, DEF (dry evergreen forest), $15.44861111^{\circ} \mathrm{N}$, $99.04694444^{\circ} \mathrm{E}$, ca 1100 m a.s.l., 25.IX.2016, W. Jaitrong leg., TH16-WJT-1069.

Non-type material examined. One worker, Thailand, Tak Province, Near Myanmar border, Tung Yai [Thung Yai] W.S., 23.V.1999, W. Jaitrong leg. (THNHM).

Measurements. Holotype: HL 0.41; HW 0.31; ML 0.53; PH 0.17; PL 0.12; SL 0.20 ; TL 1.75; CI 76; PI 143; SI 63. Paratypes ( $\mathrm{n}=11$ ): HL $0.40-0.43$; HW $0.31-$


Figure 3. Aenictus samungi sp. n. (holotype, worker, THNHM-I-02615). A Body in profile B Body in dorsal view $\mathbf{C}$ Head in full-face view.
0.33; ML 0.51-0.53; PH 0.17-0.18; PL 0.12-0.13; SL 0.20-0.23; TL1.72-1.78; CI 76-80; PI 138-143; SI 63-70.

Description of Worker. (holotype and paratypes; Fig. 3). Head in full-face view clearly longer than broad, with its sides weakly convex and posterior margin almost straight or feebly concave. Antennal scape short, only slightly extending mid-length of head; antennal segment II (pedicel) clearly longer than each of III-VI; X longer than VII, VIII and IX combined. Frontal carinae fused at level of anterior margin of torulus, extending beyond level of posterior margin of torulus. Clypeus short, with its anterior


Figure 4. Type locality of Aenictus nuchiti sp. n., dry dipterocarp forest in Chiang Mai Province, northern Thailand.


Figure 5. Type locality of $A$. samungi sp. n., dry evergreen forest in Tak Province, western Thailand.
margin bearing 7 denticles. Mandible subtriangular, with apical tooth large and curved, followed by a medium-sized subapical tooth, 4-5 minute teeth and a medium-sized basal tooth on masticatory margin. Mesosoma in profile almost flat dorsally; metanotal groove distinct. Propodeum in profile with a nearly straight dorsal outline; propodeal junction rounded; propodeal declivity weakly convex, not encircled by a rim. Petiole including subpetiolar process shorter than high, with its dorsal outline convex; subpetiolar process rather developed, with its ventral outline roundly convex, without angle or tooth; postpetiole shorter than petiole and shorter than high, in profile slightly elevated posteriorly.

Head, antennal scape, promesonotum, propodeal dorsum, petiole, postpetiole, gaster, femora and tibiae of legs entirely or extensively smooth and shiny; mesopleuron, metapleuron and lateral face of propodeum superficially reticulate. Antennal flagellum densely finely punctate.

Body with relatively sparse standing hairs mixed with sparse short hairs over surface; longest pronotal hair $0.07-0.08 \mathrm{~mm}$ long. Head, mesosoma, waist, gaster, antennae and legs yellowish brown; mandible dark brown.

Etymology. The species is named after Mr Yuthana Samung (Faculty of Tropical Medicine, Mahidol University, Thailand) who kindly helped us in taking pictures of Thai ants, including the two new species discovered in the present study.

Distribution. Western Thailand (Tak Province).
Comparative diagnosis. Aenictus samungi sp. n. can be easily distinguished from the other members of the $A$. wroughtonii species group by the following characteristics: smallest species of the group (HW $0.31-0.33 \mathrm{~mm}$ in $A$. samungi; HW $>3.7 \mathrm{~mm}$ in other members); petiole shorter than high (as long as or longer than high in other members); promesonotum with almost flat or straight dorsal outline (convex and sloping gradually to metanotal groove in other members); antennal scape short only just reaching mid-length of the head (at least two-thirds of the head length or beyond the posterolateral corner of the head in other members). Both Aenictus nuchiti sp. n. and $A$. samungi sp . n . have small bodies and short antennae, but can be easily separated from each other by the different conditions of the propodeum (Figs 1A vs. 3A).

Key to Asian species of the Aenictus wroughtonii species group based on worker caste
1 Antennal scapes short, when laid back only attaining mid-length or twothirds of the head length

2

- Antennal scape long, when laid back attaining or extending beyond the posterolateral corner of the head ....................................................................... 4
2 HW 0.31-0.33 mm; antennal scape attaining only mid-length of the head; petiole excluding subpetiolar process shorter than high (Thailand)
A. samungi sp. n.
- $\quad \mathrm{HW}>0.37 \mathrm{~mm}$; antennal scape attaining two-thirds of the head length; petiole excluding subpetiolar process longer than high
- Propodeal junction angulate

5 Subpetiolar process almost absent, anteroventrally not angulate (India)
A. wroughtonii

- Subpetiolar process weakly developed; its anteroventral corner angulate...... 6

6 Entire pronotum and petiole sculptured (punctate or reticulate) (China)...... A. gutianshanensis

Pronotum and petiole largely smooth and shiny 7
7 HW 1.00-1.04 mm (Aktaç et al. 2004, measurements of syntypes); posterior margin of head concave (Greece, Iran, Israel, Turkey)
A. rhodiensis

- $\quad \mathrm{HW}<0.60 \mathrm{~mm}$; posterior margin of head almost straight or convex.......... 8

8 Scape short; SI 100 or less; body hairy; the longest pronotal hair 0.23-0.25 mm ; subpetiolar process very low, with ventral outline almost straight (India, Nepal, Afghanistan) A. sagei Scape long; SI 130-140; body with sparse hairs; the longest pronotal hair approximately $0.15-0.18 \mathrm{~mm}$; subpetiolar process with ventral outline slightly convex (China, Vietnam, Thailand) A. artipus Ventral outline of subpetiolar process convex, anteroventrally not angulate ... $\mathbf{1 0}$
Ventral outline of subpetiolar process convex or almost straight; its anteroventral corner angulate. 11
10 Declivity of propodeum narrower, seen from back strongly tapering above; petiole longer than high (PI 84-86); body smaller with TL $2.6-2.7 \mathrm{~mm}$; antenna longer with SI 122-135 (Thailand, Indonesia, Malaysia, Philippines) . A. camposi Declivity of propodeum broader and more rounded above; petiole almost as long as high (PI 95-100); body larger with TL 3.1-3.2 mm; antenna shorter with SI 114-118 (Sri Lanka)
.A. biroi
Ventral outline of subpetiolar process strongly convex in anterior half; mesonotum partly and propodeum almost entirely densely sculptured; pronotum clearly demarcated from mesonotum by a shallow transverse groove (Taiwan, Vietnam and Thailand) A. vieti

- Ventral outline of subpetiolar process almost straight; entire mesonotum and propodeum smooth and shiny, with at most superficial sculpture; pronotum only weakly demarcated from mesonotum (Thailand).
.A. stenocephalus


## Discussion

These two new species are also similar to members of the Aenictus minutulus species group (A. changmaianus Terayama \& Kubota, 1993, A. minutulus Terayama \& Yamane, 1989, A. minimus Jaitrong \& Hashimoto, 2012) in general appearance and by having a short petiole, short antennal scapes (reaching only to mid-length of the head) and subtriangular mandibles (masticatory margin with a large apical tooth, medium-sized subapical and basal teeth and 2-6 denticles between them) (Jaitrong and Hashimoto 2012). However, herein, both species were treated as members of the $A$. wroughtonii group because they have a serrate anterior clypeal margin, the most important characteristic that separates the $A$. wroughtonii group from the $A$. minutulus group (anterior clypeal margin without denticles in the $A$. minutulus group) (see the key for the species groups of Aenictus in Jaitrong and Yamane (2011)).

Aenictus nuchiti sp. n. is a rare species. The type series was collected from a bivouac under a large rotting $\log$ in a dry dipterocarp forest (Fig. 4), ca 1000 m a.s.l., during the wet season. Numerous immature specimens (all were pupae) were found in the bivouac, and no worker activity was seen around the log. Another colony (NL1510151) was collected from a leaf litter in a dry dipterocarp forest, ca 350 m a.s.l. This new species is sympatric with $A$. artipus belonging to the same species group in at least the Chiang Mai Province (Wilson 1964; Jaitrong et al. 2010; Jaitrong and Yamane 2011).

Aenictus samungi sp. n . is also a rare species and is known only from the type locality (ca 1100 m a.s.l.). The type series was collected from a foraging column on a forest path in a dry evergreen forest (Fig. 5), western Thailand near the Myanmar border; no immature and prey were seen along the column. Workers were fast-running.

## Acknowledgements

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# A primitive honey bee from the Middle Miocene deposits of southeastern Yunnan, China (Hymenoptera, Apidae) 


#### Abstract

Michael S. Engel ${ }^{1,2}$, Bo Wang ${ }^{3,4}$, Abdulaziz S. Alqarni ${ }^{5}$, Lin-Bo Jia ${ }^{6}$, Tao $\mathrm{Su}^{7}$, Zhe-kun Zhou ${ }^{6,7}$, Torsten Wappler ${ }^{8}$

I Division of Entomology, Natural History Museum, and Department of Ecology \& Evolutionary Biology, 1501 Crestline Drive - Suite 140, University of Kansas, Lawrence, Kansas 66045-4415, USA 2 Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79h Street, New York, New York 10024-5192, USA 3 State Key Laboratory of Palaeobiology and Stratigraphy, Center for Excellence in Life and Palaeoenvironment, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China 4 Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Science, Beijing 100101, China 5 Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia 6 Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China 7 Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China 8 Natural History Department, Hessisches Landesmuseum Darmstadt, Friedensplatz 1, D-64283 Darmstadt, Germany


Corresponding author: Torsten Wappler (torsten.wappler@hlmd.de)

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#### Abstract

While fossils of honey bees (Apini: Apis Linnaeus) are comparatively abundant in European Oligocene and Miocene deposits, the available material from Asia is scant and represented by only a handful of localities. It is therefore significant to report a new deposit with a fossil honey bee from southern China. Apis (Synapis) dalica Engel \& Wappler, sp. n., is described and figured from Middle Miocene sediments of Maguan County, southeastern Yunnan Province, China. This is the first fossil bee from the Cenozoic of southern China, and is distinguished from its close congeners present at the slightly older locality of Shanwang, Shandong in northeastern China. The species can be distinguished on the basis of wing venation differences from other Miocene Apis.


## Keywords

Aculeata, Apinae, Apis, Apoidea, Miocene, taxonomy

## Introduction

Honey bees (genus Apis Linnaeus) are iconic insects. The domesticated Western honey bee, Apis mellifera Linnaeus, is one of the most intensely studied animals (Winston 1991). Although most work focuses on $A$. mellifera for obvious apicultural and agricultural purposes, A. cerana Fabricius is also intensively managed and the remaining species are similarly exploited for their wax and honey. Honey bees comprise seven extant species of the corbiculate apine tribe Apini (Engel 1999a; Radloff et al. 2011), all of which are highly eusocial, with fixed queen and worker castes. This eusocial organization is shared with the related tribe Meliponini (stingless bees), while bumble bees (Bombini) occupy the primitively eusocial behavioral grade (Michener 1974, 2007). The putatively basalmost tribe of corbiculate bees, the Euglossini (orchid bees), are solitary or communal, with a few examples of primitive eusocial behavior in some species (Boff et al. 2015; Andrade et al. 2016). Relationships among these tribes have been controversial, although most evidence converges on a Darwinian null-hypothesis supporting a single origin of eusociality in the common ancestor of Bombini + Meliponini + Apini, and a single origin of the highly eusocial grade in the common ancestor of Meliponini + Apini (Michener 1990; Schultz et al. 1999, 2001; Engel 2001a; Noll 2002; Cardinal and Packer 2007; Canevazzi and Noll 2015; Porto et al. 2016, in press). Alternatively, some molecular evidence has placed meliponines as sister to bombines (e.g., Cameron and Mardulyn 2001; Kawakita et al. 2008; RodriguezSerrano et al. 2012), although in the most recent such analysis data from Euglossini were excluded (Kwong et al. 2017), and the potential impact of excluding one of the four surviving corbiculate tribes for driving spurious results has not been explored.

As is the case for most bees, the fossil record of corbiculate Apinae is comparatively sparse and largely confined to the Cenozoic, with a heavy bias toward material of Eocene through Miocene ages (Zeuner and Manning 1976; Engel 2001b, 2005; Ohl and Engel 2007; Michez et al. 2012). Euglossini have a meagre record, confined to the Early Miocene (Burdigalian) and younger deposits (Engel 1999b, 2014; HinojosaDíaz and Engel 2007), although an enigmatic and difficult to interpret compression from the latest Eocene of North America could represent a stem-group euglossine (Dehon et al. 2014). Bombini have a slightly stronger record (Rasnitsyn and Michener 1991; Michez et al. 2012; Wappler et al. 2012; Prokop et al. 2017), which is in need of revision but demonstrates the persistence of the crown group since at least the Eocene. Perhaps owing to the fact that all species are highly eusocial, often with large numbers of individuals within perennial colonies, fossils of Meliponini and Apini are the most abundant. In fact, in sheer numbers meliponine fossils outpace those of all other bees combined, although this is entirely due to a preponderance of material of workers from
one species, Proplebeia dominicana (Wille \& Chandler), from the Early Miocene of the Dominican Republic (Camargo et al. 2000). All other fossil stingless bee species are rare, but span from the end of the Cretaceous (Maastrichtian) to Pleistocene copals (Michener 1982; Michener and Grimaldi 1988; Engel 2001b; Greco et al. 2011; Engel and Michener 2013a, 2013b). Honey bees, again largely based on fossils of the worker caste, are known from a sparse number of deposits (Zeuner and Manning 1976; Nel et al. 1999), but at some they can be found in large numbers (e.g., Armbruster 1938; Kotthoff et al. 2011). These fossils span a range of ages from the earliest Oligocene through to the Pleistocene (Engel 1998a, 1999a, 2006; Engel et al. 2009; Kotthoff et al. 2011), although the taxonomic status of several putative species remains to be evaluated. Aside from these tribes, three other corbiculate tribes were once present - Electrobombini, Electrapini, and Melikertini (Engel 1998b, 2001b; Wappler and Engel 2003; Patiny et al. 2007; Engel et al. 2013, 2014). These extinct tribes were all eusocial, with the latter two belonging to the highly eusocial clade (Engel 2000b, 2001a, 2001b), and for at least one there is relatively detailed information on pollen collection for populations from the Lutetian of Germany (Wappler et al. 2015; Grímsson et al. 2017). More extensive work is needed regarding the refinement of relationships, but it is possible that one group of electrapines, genus Thaumastobombus Engel, was more closely related to honey bees owing to the presence of a barbed sting (Engel 2001).

Among the fossil Apini, there is apparently a gradation of taxa leading from the earliest Oligocene to the Miocene appearance of the first species of the clade comprising the surviving subgenera Micrapis Ashmead, Megapis Ashmead, and Apis s. str. (Engel 1998a, 1999, 2006). The extant clades form a monophyletic group relative to earlier species, the subgenera Priorapis Engel, Synapis Cockerell, and Cascapis Engel composing a basal grade (Engel 1998a, 1999, 2006). While most of the fossil species are found across Eurasia, well within the bounds of the modern, native distribution of Apis in Europe, Africa, and Asia, at least one species occurred within western North America during the Middle Miocene (Engel et al. 2009; Kotthoff et al. 2013). Within Asia there are few localities with sufficiently preserved material of honey bees (e.g., Stauffer 1979; Hong 1983; Zhang 1989, 1990; Engel 2006), most specimens deriving from the Upper Miocene deposits of Shanwang in northeastern China (Hong 1983; Zhang 1989, 1990). Herein we report the finding of a new fossil honey bee species from the Middle Miocene deposits of southern China. The species belongs to Synapis, expanding not only the paleogeographic distribution of this group but extending their temporal presence slightly later into the Miocene, approximately $1-2$ million years younger than those records from the Northeast.

## Materials and methods

Insect fossils were collected from the northwestern Maguan Basin, southeastern Yunnan, southwestern China ( $23^{\circ} 01^{\prime} \mathrm{N}, 104^{\circ} 23^{\prime} \mathrm{E}, 1320 \mathrm{~m}$ a.s.l.) (Figure 1). The Cenozoic sediments in Maguan are composed of the Paleogene Yanshan Group, Neogene Huazhige Formation, and Quaternary deposits (Zhang 1976; Bureau of Geology and


Figures I-3. Fossil locality in Maguan County, southeastern Yunnan Province, China. I Outcrop overview, green arrow showing layers bearing the present fossil 2 Example of preservation, Acer cf. coriaceifolia H. Lév. (Sapindaceae) preserved together with a nematoceran fly (position indicated by white arrow) 3 Schematic cross section of the studied area.

Mineral Resources 1990; Zhang et al. 2015b). The basal Paleogene Yanshan Group is characterized by coarse breccias and lacks fossils (Zhang 1976; Zheng et al. 1999). Sitting unconformably on the Paleogene deposits, the Huazhige Formation is a fluviolacustrine deposit, composed of light-gray or light-yellow pelitic laminated siltstone and mudstone, and bears abundant animal and plant fossils (Figure 2) (Zhang 1976; Zhang et al. 2015b). The Quaternary deposits overly unconformably on the Huazhige Formation (Zhang 1976; Zhang et al. 2015b).

The sediments bearing the present insect fossils are characterized by cyclic deposits of light-yellow or light-grey pelitic laminated mudstone and siltstone (Figure 3). They belong to the Huazhige Formation according to stratigraphic correlations (Zhang 1976; Zhang et al. 2015b). The Huazhige Formation is also well developed in the Wenshan Basin approximately 50 km to the north of the Maguan Basin, and the two basins are inferred to be the same age (Bureau of Geology and Mineral Resources

1990; Lebreton-Anberrée et al. 2016). The age of the Huazhige Formation in the Wenshan Basin was assigned to $16.5-15.2 \mathrm{Ma}$ based on a recent palaeomagnetic study (Lebreton-Anberrée et al. 2016). Therefore, the age of the Huazhige Formation in the Maguan Basin should also be the Middle Miocene.

Besides insect fossils, the sediments bear abundant fossils of fishes, birds, as well as plants in excellent preservation (Figure 2). A preliminary study of plant fossils from the outcrop shows that the plant flora was dominated by Fagaceae and Fabaceae, accompanied by other elements such as Calocedrus Kurz (Zhang et al. 2015a), Sequioa Endl. (Cupressaceae) (Zhang et al. 2015b), Bauhinia L. (Fabaceae), Burretiodendron Rehder (Malvaceae) (Lebreton-Anberrée et al. 2015), Cedrelospermum Saporta (Ulmaceae) (Jia et al. 2015), and Ailanthus Desf. (Simaroubaceae), indicating a subtropical evergreen forest with warm and wet environment.

For the description, morphological terminology is adapted from Engel (2001b) and Michener (2007), with formats following previous studies on fossil honey bees (e.g., Engel 2006; Engel et al. 2009) and presented in the context of furthering refinements of species-level diagnoses for bees (e.g., Engel 2011; Gonzalez et al. 2013). The fossil is carbonized and so the integumental coloration or even patterning of lighter versus darker areas is not preserved. Photographs were taken using a Zeiss Stereo Discovery V16 microscope system at the State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences.

## Systematic paleontology

## Tribe Apini Latreille, 1802

Genus Apis Linnaeus, 1758
Subgenus Synapis Cockerell, 1907

## Apis (Synapis) dalica Engel \& Wappler, sp. n.

http://zoobank.org/865F24F0-8027-4C7B-9B52-68116485FBDA
Figs 4-9

Holotype. Worker (Figure 4), NIGP154200; Middle Miocene, approximately 16.515.2 Ma (around the Tortonian-Serravallian boundary); northeastern suburb of Maguan, Maguan County, Wenshan Zhuang \& Miao Autonomous Prefecture, Yunnan Province, China. The holotype is deposited in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, China.

Diagnosis. The new species is most similar to those Miocene honey bees described from Shandong Province, China. Apis dalica differs from them in the gently arched basal vein (comparatively straight in the specimens from Shandong), which is also closer to $1 \mathrm{cu}-\mathrm{a}$ (separated by about a vein width versus several vein widths and even up to $0.5-0.75$ times crossvein length in material from Shandong: refer to figures presented by Zhang 1989, and Zhang et al. 1994). In addition, in A. longitibia Zhang


Figures 4-7. Holotype worker of Apis (Synapis) dalica Engel and Wappler, sp. n., from Maguan County, southeastern Yunnan Province, China. 4 Entire holotype (NIGP154200) as preserved 5 Reconstruction of wing venation; forewing above, hind wing below $\mathbf{6}$ Detail of foreleg. 7 Detail of apical sterna. Abbreviations: $\mathrm{ppl}=$ propleuron, $\mathrm{mcx}=$ mesocoxa, $\mathrm{tr}=$ trochanter, $\mathrm{fm}=$ femur, $\mathrm{tb}=$ tibia.
and $A$. miocenica Hong $2 \mathrm{rs}-\mathrm{m}$ is comparatively straight (Zhang 1989; Zhang et al. 1994), rather than the distinctly arcuate form of $A$. dalica. In A. shandongica Zhang and $A$. miocenica $1 \mathrm{~m}-\mathrm{cu}$ is not so prominently arched and only so at its anterior end rather than strongly so and at midlength in A. dalica. Lastly, in all of the material from Shandong (Zhang 1989; Zhang et al. 1994), 1Rs originates in a strongly proximal position relative to the base of the pterostigma, rather than near the base of the pterostigma in $A$. dalica. The pterostigma of $A$. dalica is more distinctly developed than in modern species and most other fossil species of Apis.

Description. Worker. Total length (as preserved) 17.06 mm ; preserved in ventral orientation, with head thrust forward, wings extended obliquely away from body, and legs largely tucked underneath the body with most podites not preserved or indiscernible; coloration not preserved (appearing uniformly charcoal black). Head apparently slightly longer than wide as interpreted in ventral position; malar space elongate, longer


Figures 8-9. Wings of Apis (Synapis) dalica Engel and Wappler, sp. n., from Maguan County, southeastern Yunnan Province, China. 8 Details of right forewing 9 Details of left forewing.
than basal mandibular width; head narrower than mesosoma. Leg podites incompletely preserved. Metasoma typical for worker honey bee, length (as preserved) 9.03 mm , maximum width 4.36 mm ; apical margins of sterna somewhat concave, those more basal sterna relatively straight, apical most sterna more strongly concave; sting not extended but slightly evident extending along midline of apical sterna (Figure 7).

Forewing with venation typical of Apis and subgenus Synapis (Figs 4, 5, 8, 9), length 8.54 mm , maximum width 2.18 mm ; basal vein ( 1 M ) slightly distad 1cu-a, separated from 1cu-a by distance scarcely greater than vein width, gently arched before meeting 1 Rs; 1 Rs about as long as $1 \mathrm{Rs}+\mathrm{M}$ and not in line with 1 M ; first submarginal cell smallest, with 2Rs sinuate (rather than relatively straight); r -rs about as long as anterior margin of second submarginal cell; second submarginal cell trapezoidal, with 1 rs -m relatively straight and strongly slanted apically such that posterior border of cell is slightly more than three times length of anterior border; 1 m -cu meeting posterior border of second submarginal cell at basal third of cell length, with distinct abscissal stub present at about angle of midlength, stub projecting into proximal border of second medial cell; third submarginal cell relatively broad anteriorly, with 2 rs -m arcuate, anterior border of third submarginal cell distinctly longer than anterior border of second submarginal cell; $\mathrm{aRs}_{2}$ absent (sensu Tan et al. 2008); 2 m -cu meeting posterior border of third submarginal cell near apical quarter of cell length, crossvein relatively straight. Hind wing with typical Apis venation, length 6.37 mm , maximum width 1.39 mm ; linear series of distal hamuli
present along anterior wing margin (precise number not discernible); distal abscissa M ('indica' vein) present, about as long as rs-m (Figure 5).

Etymology. The specific epithet refers to the Medieval Dali Kingdom which occupied the area of Yunnan from its founding in 937 AD at the close of the Nanzhao Kingdom and until its termination by Kublai Khan (1215-1294) and the Mongol invasion in 1253 AD.

## Discussion

Fossil honey bees are comparatively uncommon in Asia relative to the wealth of material available from a variety of European deposits of Oligocene and Miocene ages (e.g., Nel et al. 1999; Kotthoff et al. 2011, 2013). In fact, most fossil honey bees in Asia have been found at a single locality in Shandong Province (Zhang 1989; Zhang et al. 1994). Unfortunately, the descriptions and available photographs of the material from Shandong are incomplete and there is reason to believe that some of the species from these deposits are synonyms of each other (Engel 1998, 1999), particularly in light of the fact that species of Apis can be notoriously variable in many features (e.g., Ruttner 1988; Radloff et al. 2010; Kotthoff et al. 2011, 2013). Thus, the present dearth of abundant specimens from which to work hampers a more comprehensive understanding of apine diversity in Asia during the Neogene, a period of time in which considerable diversification was apparently underway among honey bees such that by the present day the greatest number of species of Apis may be found across the Indomalayan region (e.g., Engel 1999, 2012; Michener 2007; Radloff et al. 2011).

The discovery of $A$. dalica expands the known localities with fossil honey bees southward in China and the presence of highly eusocial bees and critical pollinators within the Miocene of fauna of Yunnan. It is hoped that further exploration will recover larger numbers of workers from which the general morphometrics of the species can be determined and more precisely circumscribe the taxon among other Apini, as well as refine phylogenetic relationships among early honey bees. Phylogenetic studies on the modern species have demonstrated that open-nesting is ancestral for the genus (Engel and Schultz 1997). Given that most of the known fossil Apis fall basal to the clade of modern subgenera (e.g., Engel 2006; Kotthoff et al. 2013), and that $A$. dalica's wing venation places it among species of the extinct subgenus Synapis, it is presumed that $A$. dalica would have constructed their nests in exposed localities, perhaps attached to the branches of trees or sturdy bushes. Such perennial colonies would have been more impacted by temperature changes over the course of the year, implying that the local paleoclimate was comparatively steady.

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# New records of the endemic Sicilian land snail species Marmorana (Murella) muralis (O. F. Müller, I774) from the north of Tunisia (Pulmonata, Gastropoda) 

Issaad Kawther Ezzine ${ }^{1}$, Najet Dimassi ${ }^{1}$, Beat Pfarrer ${ }^{2,3}$, Khaled Said ${ }^{1}$, Eike Neubert ${ }^{2,3}$<br>I Laboratoire de génétique, biodiversité et valorisation des bio-ressources, Institut Supérieur de Biotechnologie de Monastir, Avenue Taher Hadded (B.P 74) Monastir 5000, Tunisia 2 Natural History Museum Bern, Bernastr. 15, CH-3005 Bern, Switzerland 3 Institute of Ecology and Evolution, University of Bern, CH-3012 Bern, Switzerland<br>Corresponding author: Issaad Kawther Ezzine (issaadkawther.ezzine@gmail.com)

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#### Abstract

Marmorana (Murella) muralis is known as an endemic species of Sicily Island, which is introduced in many European countries. Here, M. (M.) muralis is recorded from the north of Tunisia. In order to confirm the identification of samples collected from several localities, shell morphology, details of genital organs and two mitochondrial markers (COI and 16S) were investigated. The results of the molecular study, as well as the morphological and anatomical studies confirm the identification of all Tunisian samples as $M$. (M.) muralis. The analysis of mitochondrial markers shows a low divergence between Sicilian and Tunisian samples suggesting a recent introduction of $M$. (M.) muralis to the North of Tunisia. The comparison of morphological characters of $M$. (M.) muralis with shell characters of Murella nicollei described by Pallary (1926) confirms that the latter should be considered as synonym of $M$. (M.) muralis.


## Keywords

16S, COI, anatomy, Marmorana (Murella) muralis morphology, Murella nicollei, Sicily, Tunisia

[^1]
#### Abstract

Résumé Marmorana (Murella) muralis est connue comme une espèce endémique de lîle de Sicile qui est actuellement introduite dans plusieurs pays européens. Dans la présente étude, nous enregistrons, la présence de $M$. (M.) muralis dans différentes localités du Nord de la Tunisie. Dans le but de valider l'authenticité des spécimens collectés de différentes localités, la morphologie de la coquille, l'anatomie de l'appareil génital ainsi que deux marqueurs mitochondriaux (COI et $16 S$ ) ont été analysés. Les résultats de l'étude moléculaire ainsi que ceux de l'étude morphologique et anatomique confirment qu'il s'agit bien de l'espèce Sicilienne M. (M.) muralis. L'analyse des deux marqueurs mitochondriaux montre une faible divergence entre les populations Sicilienne et Tunisienne suggérant ainsi une récente introduction de cette espèce en Tunisie. La comparaison des critères morphologiques de l'espèce $M$. (M.) muralis avec les critères de la coquille de l'espèce Murella nicollei décrite par Pallary (1926) confirme que cette dernière doit être considérée comme synonyme de l'espèce $M$. (M.) muralis.


## Mots clés

16S, COI, anatomie, Marmorana (Murella) muralis, morphologie, Murella nicollei, Sicile, Tunisie

## Introduction

Land snails compose a group of invertebrates which are characterized by low mobility and dispersal capacity. The evolution of morphological characters within land snail species is widely influenced by the environmental and ecological conditions. Marmorana (Murella) muralis is an endemic helicid species from Sicily Island, which is characterized by an extremely high variability of shell morphology as well as molecular characters (Fiorentino et al. 2013). It was demonstrated that paleogeographical factors and environmental changes affected the shell morphology of $M$. (M.) muralis in Sicily (Fiorentino et al. 2013). This species was introduced by humans to many other European areas such as Tuscany in Italy, Sardinia, the Baleares, Portugal and Bouches-du-Rhône in France. Tunisia is a quite well sampled area as evidenced by Letourneux and Bourguignat (1887), who documented land snails from a plethora of localities. Interestingly, they never recorded the presence of Marmorana (Murella) Pfeiffer, 1877 in the area. It was Pallary in 1926, who was the first to describe a Murella, Murella nicollei, from Tabarka in northwest Tunisia.

Recent sampling efforts by the senior author revealed the presence of a Marmorana (Murella) taxon at several localities in the north of Tunisia. The present study aims to 1) identify the samples collected from Tunisia based on morphological and molecular characters, 2) determine the possible origin of each Tunisian population known and 3) clarify the status of Murella nicollei Pallary, 1926.

## Materials and methods

Living specimens were collected by hand at several localities in Tunisia during two periods: spring 2014, and winter 2015/2016. Geographic coordinates were recorded using a GPS device. For subsequent molecular analyses, specimens were preserved and stored in $80 \%$ ethanol until dissection and DNA extraction.

## Morphological and anatomical studies

First assessments of the shell morphological characters were done by using simple magnifying glasses. Preserved animals were dissected under a LEICA M212 stereo microscope using thin tweezers. The genital organs of the specimens were removed from the body, and the outer morphology of the complete hermaphroditic genital organ (situs) and further morphological details were investigated. After that, shells, genital situs, and details of the genital organs were photographed with a LEICA DFC 425 camera combined with a LEICA M205 C stereo microscope. The multifocal images were processed by using Imagic IMS software (Imagic, Switzerland).

## Molecular study

Ten specimens of $M$. (M.) muralis collected from northern Tunisia were used in this study. We also included sequences of Italian $M$. (M.) muralis specimens (Fiorentino et al. 2008; Fiorentino et al. 2010; Fiorentino et al. 2013; Neiber and Hausdorf 2015), M. serpentina (Férussac, 1821) (Fiorentino et al. 2008, Fiorentino et al. 2010), and M. cf. globularis (Philippi, 1836) (Fiorentino et al. 2008) for comparison with our specimens. Almost all cytochrome c oxidase subunit I (COI) haplotypes published by Fiorentino et al. (2013) were included in the study to estimate the divergence between Tunisian and Italian populations. Macularia sylvatica (Draparnaud, 1801) and Macularia niciensis (Férussac, 1821) were selected as outgroup (Neiber and Hausdorf 2015). All specimens used are listed in Table 1. Sequenced specimens are housed in the voucher collection of the NMBE (Naturhistorisches Museum der Burgergemeinde Bern).

## DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from foot muscle tissue of each specimen using a standard phenol chloroform method (Estoup et al. 1996). Two mitochondrial gene fragments were chosen for analyses in the present study: cytochrome c oxidase subunit I (COI) of 711 base pairs (bp) length and the gene of the 16 S ribosomal RNA subunit (16S rRNA) for an approximately 470-480 bp fragment. Polymerase chain reactions (PCR) were performed in a reaction mixture containing 15 ng of DNA template, 1X reaction buffer $(1.5 \mathrm{mM}), 0.1 \mathrm{mM}$ of each primer pair, 0.2 mM dNTPs, Taq polymerase $(1.25 \mathrm{U})$ and adjusted till a total volume of $25 \mu \mathrm{l}$ with DNAase free water/sterilized water (UNIMED) $\left(\mathrm{H}_{2} \mathrm{O}\right)$. PCR reactions were run under the following conditions: 3 $\min$ at $95^{\circ} \mathrm{C}$, followed by 35 cycles of 1 min at $95^{\circ} \mathrm{C}, 1 \mathrm{~min}$ at $40^{\circ} \mathrm{C}$ and 1 min at 72 ${ }^{\circ} \mathrm{C}$ and finally, 5 min at $72{ }^{\circ} \mathrm{C}$ for COI . For 16 S the amplification conditions were: 3 $\min$ at $95^{\circ} \mathrm{C}$, followed by 35 cycles of 1 min at $95^{\circ} \mathrm{C}, 1 \mathrm{~min}$ at $50^{\circ} \mathrm{C}$ and 1 min at $72{ }^{\circ} \mathrm{C}$, and finally, 5 min at $72{ }^{\circ} \mathrm{C}$. PCR products were sequenced using automated and standardised ABI 3730 XL sequencing run with a read length up to 1.100 bp (PHRED20 quality) and using the same primers as for the PCR (Table 2).

Table I. Taxa examined in this study: species, localities, voucher, and GenBank accession numbers for COI, and 16 S fragments.

| Species | Voucher number | Localities | Latitude | Longitude | GenBank accession numbers |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | COI | 16S |
| M. (M.) muralis | NMBE 551462 | Manzel Abderrahmen, Bizerte, Tunisia | $37.232494^{\circ}$ | $9.868065^{\circ}$ | MG780362 | MG774439 |
| M. (M.) muralis | NMBE 551463 | Manzel Abderrahmen, Bizerte, Tunisia | $37.232494^{\circ}$ | $9.868065^{\circ}$ | MG780363 | MG774440 |
| M. (M.) muralis | NMBE 551464 | Manzel Abderrahmen, Bizerte, Tunisia | $37.232494^{\circ}$ | $9.868065^{\circ}$ | MG780364 | MG774441 |
| M. (M.) muralis | NMBE 551454 | Manzel Jemil, Bizerte, Tunisia | $37.249964^{\circ}$ | $9.914793^{\circ}$ | MG780365 | MG774442 |
| M. (M.) muralis | NMBE 551460 | Manzel Jemil, Bizerte, Tunisia | $37.249964^{\circ}$ | $9.914793^{\circ}$ | MG780366 | MG774443 |
| M. (M.) muralis | NMBE 551461 | Manzel Jemil, Bizerte, Tunisia | $37.249964^{\circ}$ | $9.914793^{\circ}$ | MG780367 | MG774444 |
| M. (M.) muralis | NMBE 551465 | Haouaria, Nabeul, Tunisia | $37.052299^{\circ}$ | $11.010219^{\circ}$ | MG780368 | MG774445 |
| M. (M.) muralis | NMBE 551457 | Kelibiya, Nabeul, Tunisia | $36.838017^{\circ}$ | $11.115843^{\circ}$ | MG780369 | MG774446 |
| M. (M.) muralis | NMBE 551458 | Kelibiya, Nabeul, Tunisia | $36.838017^{\circ}$ | $11.115843^{\circ}$ | - | MG774447 |
| M. (M.) muralis | NMBE 551459 | Kelibiya, Nabeul, Tunisia | $36.838017^{\circ}$ | $11.115843^{\circ}$ | MG780370 | MG774448 |
| M. (M.) muralis [Fiorentino et al. 2010] | FGC 35940 | Joppolo, Italy | - | - | EU189905 | EU189872 |
| M. (M.) muralis [Fiorentino et al. 2010] | FGC 35948 | Marsala, Sicily, Italy | - | - | EU189904 | EU189871 |
| M. (M.) muralis [Fiorentino et al. 2010] | FGC 35922 | Selinunte, Italy | - | - | EU189907 | EU189874 |
| M. (M.) muralis [Fiorentino et al. 2010] | FGC 36598 | Fiumedinisi, Sicily, Italy | - | - | GU391370 | GU391399 |
| $\begin{aligned} & \hline \text { M. (M.) muralis } \\ & \text { [Neiber and } \\ & \text { Hausdorf 2015] } \end{aligned}$ | MN 503 | Lazio, Italy | $41.885278^{\circ}$ | $12.480833^{\circ}$ | KR705023 | KR704983 |
| M. cf. globularis [Fiorentino et al.2008] | FGC 35918 | Caltabellotta, Sicily, Italy | - | - | EU189919 | EU189886 |
| M. (M.) muralis [Fiorentino et al. 2013] | H1 | Erice, Sicily, Italy | - | - | JX827102 | - |
|  | H2 | Erice, Sicily, Italy | - | - | JX827103 | - |
|  | H3 | Erice, Sicily, Italy | - | - | JX827104 | - |
|  | H4 | Erice, Sicily, Italy | - | - | JX827105 | - |
|  | H5 | Erice, Sicily, Italy | - | - | JX827106 | - |
|  | H6 | Monte Cofano, Sicily, Italy | - | - | JX827107 | - |
|  | H8 | Erice, Sicily, Italy | - | - | JX827108 | - |
|  | H9 | Monte Monaco, \|Sicily, Italy | - | - | JX827109 | - |
|  | H10 | Erice, Sicily, Italy | - | - | JX827110 | - |


| Species | Voucher number | Localities | Latitude | Longitude | GenBank accession numbers |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | COI | 16S |
| M. (M.) muralis [Fiorentino et al. 2013] | H11 | Monte Monaco, Sicily, Italy | - | - | JX827111 | - |
|  | H12 | Erice, Sicily, Italy | - | - | JX827112 | - |
|  | H13 | Erice, Sicily, Italy | - | - | JX827113 | - |
|  | H14 | Monte Monaco, Sicily, Italy | - | - | JX827114 | - |
|  | H15 | Monte Cofano, Sicily, Italy | - | - | JX827115 |  |
|  | H16 | Monte Monaco, Sicily, Italy | - | - | JX827116 | - |
|  | H17 | Monte Monaco, Sicily, Italy | - | - | JX827117 | - |
|  | H18 | Monte Monaco, Sicily, Italy | - | - | JX827118 | - |
|  | H19 | Monte Monaco, Sicily, Italy | - | - | JX827119 | - |
|  | H20 | Monte Monaco, Sicily, Italy | - | - | JX827120 | - |
|  | H22 | Monte Monaco, Sicily, Italy | - | - | JX827122 | - |
|  | H23 | Monte Monaco, Sicily, Italy | - | - | JX827123 | - |
|  | H24 | Monte Monaco, Sicily. Italy | - | - | JX827124 | - |
|  | H25 | Monte Cofano, Sicily. Italy | - | - | JX827125 | - |
|  | H26 | Monte Cofano, Sicily, Italy | - | - | JX827126 | - |
|  | H27 | Monte Cofano, Sicily, Italy | - | - | JX827127 | - |
|  | H28 | Monte Monaco, Sicily, Italy | - | - | JX827128 | - |
|  | H29 | Monte Monaco, Sicily, Italy | - | - | JX827129 | - |
|  | H30 | Monte Monaco, Sicily, Italy | - | - | JX827130 | - |
|  | H31 | Monte Cofano, Sicily, Italy | - | - | JX827131 | - |
|  | H32 | Monte Cofano, Sicily, Italy | - | - | JX827132 | - |
|  | H33 | Monte Monaco, Sicily, Italy | - | - | JX827133 | - |
|  | H34 | Monte Monaco, Sicily, Italy | - | - | JX827134 | - |
|  | H35 | Monte Monaco, Sicily, Italy | - | - | JX827135 | - |
|  | H36 | Monte Monaco, Sicily, Italy | - | - | JX827136 | - |


| Species | Voucher number | Localities | Latitude | Longitude | GenBank accession numbers |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | COI | 16S |
| M. (M.) muralis [Fiorentino et al. 2013] | H37 | Monte Monaco, Sicily, Italy | - | - | JX827137 | - |
|  | H38 | Monte Monaco, Sicily, Italy | - | - | JX827138 | - |
|  | H39 | Monte Monaco, Sicily, Italy | - | - | JX827139 | - |
|  | H40 | Monte Monaco, Sicily, Italy | - | - | JX827140 | - |
|  | H41 | Monte Sparagio, Sicily, Italy | - | - | JX827141 | - |
|  | H42 | Monte Sparagio, Sicily, Italy | - | - | JX827142 | - |
|  | H43 | Monte Sparagio, Sicily, Italy | - | - | JX827143 | - |
|  | H44 | Monte Sparagio, Sicily, Italy | - | - | JX827144 | - |
|  | H45 | Monte Sparagio, Sicily, Italy | - | - | JX827145 | - |
|  | H46 | Erice, Sicily, Italy | - | - | JX827146 | - |
|  | H47 | Erice, Sicily, Italy | - | - | JX827147 | - |
|  | H48 | Monte Monaco, Sicily, Italy | - | - | JX827148 | - |
|  | H50 | Erice, Sicily, Italy | - | - | JX827149 | - |
|  | H51 | Erice, Sicily, Italy | - | - | JX827150 | - |
|  | H37 | $\begin{aligned} & \text { Monte Monaco, } \\ & \text { Sicily, Italy } \end{aligned}$ | - | - | JX827137 | - |
|  | H38 | Monte Monaco, Sicily, Italy | - | - | JX827138 | - |
|  | H39 | Monte Monaco, Sicily, Italy | - | - | JX827139 | - |
|  | H40 | Monte Monaco, Sicily, Italy | - | - | JX827140 | - |
|  | H41 | Monte Sparagio Sicily, Italy | - | - | JX827141 | - |
|  | H42 | Monte Sparagio, Sicily, Italy | - | - | JX827142 | - |
|  | H43 | Monte Sparagio, Sicily, Italy | - | - | JX827143 | - |
|  | H44 | Monte Sparagio, Sicily, Italy | - | - | JX827144 | - |
|  | H45 | Monte Sparagio, Sicily, Italy | - | - | JX827145 | - |
|  | H46 | Erice, Sicily, Italy | - | - | JX827146 | - |
|  | H47 | Erice, Sicily, Italy | - | - | JX827147 | - |
|  | H48 | Monte Monaco, Sicily, Italy | - | - | JX827148 | - |
|  | H50 | Erice, Sicily, Italy | - | - | JX827149 | - |


| Species | Voucher number | Localities | Latitude | Longitude | GenBank accession numbers |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | COI | 16S |
| M. (M.) muralis [Fiorentino et al. 2013] | H51 | Erice, Sicily, Italy | - | - | JX827150 | - |
|  | H52 | Monte Monaco, Sicily, Italy | - | - | JX827151 | - |
|  | H53 | Monte Monaco, Sicily, Italy | - | - | JX827152 | - |
|  | H54 | Monte Monaco, Sicily, Italy | - | - | JX827153 | - |
|  | H55 | Monte Monaco, Sicily, Italy | - | - | JX827154 |  |
| M. serpentina [Fiorentino et al. 2008] | FGC 35931 | Siena, Italy | - | - | EU189932 | EU189899 |
| M. serpentina [Fiorentino et al. 2010] | FGC 32381 | Sardinia: <br> Casa Cantoniera, Italy | - | - | GU391369 | GU391397 |
| Macularia sylvatica [Neiber and Hausdorf 2015] | UB-ZMH- <br> DNA-2843 | Schaffhausen Switzerland | $47.676389^{\circ}$ | $8.614722^{\circ}$ | KR705039 | KR705002 |
| Macularia niciensis [Neiber and Hausdorf 2015] | $\begin{array}{\|c} \text { MN } 2370 \text {-Hel- } \\ 218 \end{array}$ | Provence-Alpes-Côte d'Azur_France | $43.700000^{\circ}$ | $7.241667^{\circ}$ | KR705037 | KR705000 |

Table 2. List of primers used for PCR and sequencing.

| Gene | Name | Sequences | Reference |
| :--- | :---: | :---: | :---: |
| COI | COIF <br> COIR | 5'-ACTCAACGAATCATAAAGATATTGG-3' <br> 5'-TATACTTCAGGATGACCAAAAAATCA-3' | Folmer et al. 1994 |
| 16 S | 16 Sar <br> 16Sbr | 5'-CGCCTGTTTATCAAAAACAT-3' <br> 5'-CCGGTCTGAACTCTGATCAT-3' | Simon et al. 1994 |

## Sequence analyses

Forward and reverse sequences were assembled, checked for ambiguities and aligned using the default settings of the ClustalW multiple alignment algorithm as implemented in Bioedit V 7.2.5 (Hall 1999) and trimmed for 655 bp and 414 bp respectively for COI and 16S. Obtained sequences were deposited in GenBank under the accession numbers MG780362-MG780370 and MG774439-MG774448 (Table 1).

Aligned Tunisian and Sicilian sequences were analysed using DnaSP v5.10.01 (Librado and Rozas 2009) to estimate the number of informative sites and nucleotide diversity for each marker. The K2P values were estimated using Mega v. 6 (Tamura et al. 2013). The relationships of inferred haplotypes of Tunisian and Italian $M$. (M.) muralis were estimated using the Minimum Spanning Network (MSN) method (Bandelt et al. 1999) im-
plemented PopART v1.7 (Leigh and Bryant 2015). Because of lack of sequences available on GenBank, we produced the haplotype network separately for COI and 16 S markers.

## Phylogenetic analysis

Concatenated sequences of the two mitochondrial markers were analysed by Bayesian inference of phylogeny. The sequence data was initially partitioned into four partitions: three partitions corresponding to the codon positions of COI and one partition for 16 S . Based on the Akaike Information Criterion (AIC), the substitution models F81, K81uf+G, $\mathrm{Tr} \mathrm{N}+\mathrm{I}$ and $\mathrm{HKY}+\mathrm{G}$ were chosen as best models, respectively, for the first, second and third codon positions of COI and for 16S by PartitionFinder v 1.1.1 (Lanfear et al. 2012). For the Bayesian Inference, we used Mr Bayes v3.2.2 (Ronquist and Huelsenbeck 2003) using the partition scheme and substitutions models suggested by PartitionFinder. Four independent runs were conducted for $10^{6}$ generations, sampling every 1000 . The first $25 \%$ trees were discarded as default burn-in and a majority-rule consensus tree was calculated from the remaining trees. Convergence between runs was assessed by comparing the traces using Tracer v1.5 (Rambaut and Drummond 2007). The topology obtained, and the posterior probabilities for each node were displayed with Figtree V1.4.0 (Rambaut 2012).

## Results morphology and anatomy

## Marmorana (Murella) muralis (O. F. Müller, 1774)

Description. Shell medium-sized, depressed globular, thick, solid basic colour beige; large protoconch, clear, smooth, consisting of $1 \frac{1}{2}$ whorls; teleoconch consisting of $31 / 2$ slightly flattened whorls, distinctly ribbed; last whorl slightly keeled, larger than the rest whorls, descending towards aperture; aperture sub-spherical; peristome thick, white; suture moderately deep; underside with single interrupted spiral band; moderately ribbed, umbilicus completely covered by the reflected columellar margin (Fig 1).

Male genital anatomy. Penis club-shaped, thick; epiphallus as long as penis; retractor muscle inserting into the distal part of the epiphallus; flagellum twice the length of epiphallus; penial papilla elongated, with a slit-like pore on one side.

Female genital anatomy. Dart sac simple, well developed, two glandulae mucosae, non-ramified, inserting into the middle part of the vagina near the base of the dart sac; bursa copulatrix and diverticulum inserting into the proximal part of the vagina (Fig. 2).

## Haplotype network and genetic diversity

Among nine Tunisian and 58 Italian partial COI sequences of $M$. (M.) muralis (Fig. 3), 46 distinct haplotypes were found, suggesting an extremely high haplotype diversity ( $\mathrm{Hd}=0.9815$ ) (Fig. 4A). With 45 haplotypes detected, Italian sequences are


Figure I. Marmorana (Murella) muralis (O. F. Müller, 1774) and Murella nicollei Pallary, 1926. A Menzel Jemil, Bizerte, 17.ii.2015, NMBE 534231, leg. Ezzine, D = 16.79 mm B Kelibiya, 10.i.2016, NMBE 551457, leg. Ezzine, $\mathrm{D}=16.98 \mathrm{~mm}$. Photographs Bochud \& Ezzine C Murella nicollei, Tabarka. Scale bar: D 15.5 mm (copy of the original publication).


Figure 2. Anatomy of genital organs of Marmorana (Murella) muralis (Müller, 1774). A Situs B Details of dart sac C Details of epiphallus D Penial papilla. Abbreviations: A. atrium, Bc. Bursa copulatrix, D. Diverticulum, Ds. Dart sac, E. Epiphallus, F. Flagellum, Gm. Glandulae mucosae, mrp. Penial retractor muscle, P. Penis, Vd. Vas deferens.


Figure 3. Localisation of Tunisian and Italian specimens used in the study.
highly diverse $(\mathrm{Hd}=0.9903)$, while only 3 haplotypes were found in Tunisia ( $\mathrm{Hd}=$ 0.5596 ). The haplotype network therefore suggests a relatively low genetic variability of COI sequences from Tunisian specimens compared to sequences from Italian specimens. Tunisian and Italian specimens share two haplotypes: the first is represented by the COI sequences of the samples collected from Manzel Jemil, Manzel Abderrahmen and the sequence from Selinunte. The second haplotype is represented by the sequence of the sample collected at Haouaria and the sequences H1, H4, and H50 from Erice (Fiorentino et al. 2013). The sequences of the samples collected at Kelibiya represent a unique haplotype that is neither shared with the other specimens from Tunisia nor with any of the specimens from Italy. Within the Tunisian sequences, the highest K2P value (0.078) was recorded between the haplotype of the sequence from Haouaria and the sequences from Kelibiya however; the lowest value ( 0.01 ) was reported between the sequences from Kelibiya and those from Manzel Jemil and Manzel Abderrahmen. Between Tunisian and Italian populations, the highest K2P value ( 0.081 ) was registered between the sequences from Kelibiya and the


Figure 4. Haplotype network showing the relationships among Italian and Tunisian specimens of M. (M.) muralis. A Haplotype network based on partial COI sequences B Haplotype network based on partial 16 s sequences $\mathbf{C}$ Haplotype network based on concatenated partial COI and $16 S$ sequences.
sequences from Erice (H5, H10) and Monte Monaco (H22, H28, H30, H38, H52). The lowest was recorded between the sequence from Haouaria and the sequences H1, H4 and H50 (Fiorentino et al. 2013) on the one hand and the sequences from Manzel Jemil- Manzel Abderrahmen and the sequences from Selinunte on the other hand (Fiorentino et al. 2010). The nucleotide divergence within Tunisian population reached a value of 0.0178 but the divergence was slightly higher ( 0.0316 ) within Italian population. Moreover, the divergence between Tunisian and Italian populations reached a value of 0.0353 .

The analysis of ten Tunisian and six Italian 16 S partial fragments shows five haplotypes suggesting a low haplotype diversity ( 0.450 ) (Fig. 4B). Sequences of Italian specimens represent four haplotypes ( $\mathrm{Hd}=0.80$ ), while sequences from Tunisian specimens represent only two haplotypes ( $\mathrm{Hd}=0.20$ ). Tunisian and Italian samples share one haplotype, which was represented by sequences of Tunisian specimens from Kelibiya, Manzel Jemil, Manzel Abderrahmen and sequences of Italian specimens from Lazio, Selinunte and Marsala. The sequences of specimens from Haouaria, Fiumedi-
nisi, Caltabellotta and Joppolo represented four different haplotypes. The maximum value of K2P distance (0.02), within Tunisian sequences, is recorded between the sequence from Haouaria and the rest. While the maximum value recorded between Tunisian and Italian populations is 0.044 between the sequence from Haouaria and the sequences from Fiumedinisi and Caltabellotta. The nucleotide divergence of the 16 S partial fragment is remarkably low within Tunisian population (0.00409), as well as, between Tunisian and Italian populations (0.00828).

The analysis of nine Tunisian and six Italian concatenated sequences (COI, 16S) recovered seven different haplotypes among them (Fig. 4C). One haplotype is shared by Tunisian and Italian populations. The rest is divided into two Tunisian and four Italian haplotypes.

## Phylogeny

The topology, obtained by Bayesian inference based on the concatenated COI and 16 S data set was rooted with Macularia sylvatica and Macularia niciensis as outgroups (Fig. 5). The Marmorana species form two opposite clades well supported ( $\mathrm{PP}=1$ ): The first one is formed by the samples of $M$. (M.) serpentina and the second is formed by both Tunisian and Italian M. (M.) muralis. Within the $M$. (M.) muralis clade, M. cf. globularis and the M. (M.) muralis of Fiumedinisi are situated at the base of the clade with a high value of posterior probability ( $1-0.93$ ). The rest sequences form three well supported clades. The first is composed by the sequences of Marsala and Lazio, the second is formed by the sequences of Kelibiya, Joppolo, and Selinunte (0.92) and the third clade is formed by the sequences of Manzel Abderrahmen, Manzel Jemil, Haouaria and Erice (0.82).


Figure 5. Bayesian 50\% majority-rule consensus tree based on the analysis of concatenated partial COI and 16S sequences showing the relationships among Tunisian and Italian Marmorana (Murella) muralis samples.

## Discussion

## Morphology and anatomy

Marmorana (Murella) muralis is known as an endemic species of Sicily but it was introduced to several localities in southern Europe (Fiorentino et al. 2008). The morphological and anatomical characters of the Tunisian samples show the same morphological and genital anatomical traits presented by Fiorentino et al. (2010). Thus, these specimens are here considered to represent $M$. (M.) muralis. In Tunisia, this taxon was first recorded by Pallary (1926: 49, pl. VIII, fig. 9) under the name Murella nicollei from Tabarka. The photo of Murella nicollei (Fig. 1C) confirms the same shell morphological traits characterizing M. (M.) muralis. Thus, we consider Murella nicollei Pallary, 1926 to represent synonym of $M$. (M.) muralis. The species was probably introduced by Italian people, who lived in Tabarka for a long time. In fact, Italian people colonised Tabarka since the middle of the XVI century (Valérian 2012). The maximum number of Sicilian people settling in Tunisia was reached in 1891 (De Montety 1937). Since its description, there is no record of this species from Tabarka known to the authors. During the last decade, Tabarka was visited several times by Abbes and Ezzine, but neither empty shells nor living specimens of $M$. (M.) muralis could be found. The extinction of $M$. (M.) muralis in the area could be the result of 1) a negative ecological selection caused by the climatic conditions in Tabarka, or 2) the fragmentation and urbanisation of its habitat by human activities, which easily could reduce the population. Being an alien species to Tabarka it is quite possible that it could not well disperse in the area. As a result, the population is gone extinct. However, despite its extinction in Tabarka, it does well in the other Tunisian localities recorded here. Fiorentino et al. (2013) demonstrated that the shell morphology is highly affected by environmental changes in Sicily Island; the Tunisian populations seem not yet to be influenced by the new environment so far. The absence of any environmental effects on the shell supports the hypothesis that the species was quite recently introduced to the country.

## Network haplotype and genetic diversity

The nucleotide divergence of the COI sequences reaches a maximum value of 0.0316 ( $3.16 \%$ ) between Tunisian and Italian populations. This value does not exceed the threshold of intraspecific divergence of land snails (4\%) as suggested by Davison et al. (2009), and is comparable to the threshold of $3 \%$ suggested by Hebert et al. (2003) to characterize animal species in general. Furthermore, this value is smaller than the intraspecific divergence of the Tunisian Xerocrassa latastei reported by Ezzine et al. (2017). The comparison of the nucleotide divergence, the haplotype diversity, and the K2P value between Tunisian and Italian COI sequences shows a high diversity of this marker. The divergence between Tunisian and Italian populations might be the result of the isolation caused by the Mediterranean Sea, which can be considered a geographical barrier causing the restriction of passive gene flow between the two populations.

The analysis of the results obtained by the 16 S sequences shows low values of nucleotide divergence, haplotype diversity, and K2P distance between Italian and Tunisian sequences, suggesting a weak diversity of this marker. The comparison of the parameters of the COI and 16 S and the haplotype network show that COI is more polymorphic than 16 S . COI seems to be suitable to estimate the divergence not only on species but also on population level. The haplotype network of the concatenated data confirms the results obtained by COI and 16 S separately and shows that Italian populations are more diversified than the Tunisian ones. This supports the hypothesis of a recent introduction to Tunisia.

The haplotype network of COI sequences shows that the haplotype from Manzel Jemil and Manzel Abderrahmen is similar to the haplotypes from Selinunte and Joppolo, the haplotype from Haouaria is similar to the sequences from Erice, which can be interpreted as a hint to the origin of these particular populations. Interestingly, the haplotype from Kelibiya is unique and not shared with Italian populations. The divergence of the haplotype of Kelibiya may have two reasons: 1) these snails have been introduced from a genetically unknown population on Sicily, or 2) or it could be the result of the isolation of the population inside the castle. In fact, we visited Kelibiya several times, the population seems to be isolated but well adapted to the environment within the castle. The species does not live outside the castle. Geographical isolation is widely accepted to represent the main cause of genetic divergence within a species (Graybeal 1995; Baum and Shaw 1995; Olmstead and Reeves 1995). However, this is a process that requires many generations and might lead to changes in shell morphology as seen in Sicily. This is not the case here, so we assume that the first hypothesis has a higher probability.

## Phylogeny

The analysis of the topology obtained by the Bayesian Inference method shows that Tunisian specimens form one well supported clade ( $\mathrm{PP}=1$ ) together with the Italian samples of $M$. (M.) muralis (Fig. 5) and thus proves that the Tunisian samples are conspecific with this species. The topology obtained did not divide the samples used into separate Sicilian and Tunisian clades, and the presence of a Tunisian or Sicilian ancestral clade could not be shown. Additionally, the shell morphology seems not to be affected by the environmental difference between Sicily and Tunisia, as might have been expected in case of a longer presence of the species in Tunisia.

To better understand the population dynamics of this species, more studies including more samples from Tunisia and from Italy will be needed.

## Conclusions

Based on morphological, anatomical, and mitochondrial markers, the present study confirms that the recently collected Tunisian samples of a Marmorana species belong
to $M$. (M.) muralis. The absence of this species in the collection of Letourneux and Bourguignat (1887) leads to the hypothesis that the species may have recently been introduced to Tunisia, i.e. earliest after 1887. The first record for the species comes from Tabarka (Pallary 1926), but the species has gone extinct there. The recent populations from Tunisia share some Sicilian haplotypes indicating an origin from Selinunte, Erice and other well-known populations on Sicily; the population from Kelibiya is more isolated and does not relate to any genetically known population on Sicily. The haplotype networks of the COI, 16S and concatenated fragments prove that Italian populations are more diversified than the Tunisian. The shell morphology of the Tunisian populations is rather homogenous. We therefore conclude that the present distribution pattern is result of a recent anthropogenic introduction of the species in the north of Tunisia, which occurred sometime in the last 90 years. The species has to be considered a neozoon for the Tunisian malacofauna. It has to be emphasized that the development of the hitherto known four populations and their future dispersal in the country need to be observed. The impact of this alien species on the endemic land snail fauna of Tunisia needs serious future monitoring.

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