RESEARCH ARTICLE



# Spatial structure and morphometric relationships of the deep-sea shrimp Solenocera acuminata (Decapoda, Solenoceridae) in the Colombian Caribbean

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#### Abstract

Given the potential interest in targeting *Solenocera acuminata* in a new deep-sea fishery in the Colombian Caribbean, biological information is needed to support the management of this species. The objective of this study is to provide biological information about size structure, size at sexual maturity, morphometric relationships, abundances and spatial and bathymetric distribution of *S. acuminata* in the Colombian Caribbean. Specimens of *S. acuminata* were collected during four deep-sea prospecting surveys in the Colombian Caribbean Sea, which were conducted between Punta Gallinas and the Gulf of Uraba. A total of 87 exploratory fishing trawls were made between 100 and 550 m depth. Sexual dimorphism was evident, with males being smaller than females. The size at sexual maturity of the females was 95.2 mm total length (23.82 mm CL). Relatively high biomass values were found in the northern zone of the Colombian Caribbean, between Santa Marta and Riohacha. In the southern zone, higher at night (mean 1.82 kg/km<sup>2</sup>) than during daylight (mean 0.15 kg/km<sup>2</sup>). This species was distributed between 150 and 400 m depth and the highest biomass was associated with depths between 330 and 380 m. Before starting a new fishery, more research is needed to understand the life cycle parameters of deep-sea resources, such as growth, reproduction, recruitment, mortality, spawning areas and times, nursery areas and associated biodiversity.

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#### **Keywords**

Biomass, Caribbean, decapods, deep-sea crustacean, distribution, orange shrimp

# Introduction

Amongst decapod crustaceans, some species of the family Solenoceridae, which inhabit mostly tropical and subtropical zones, have been recognised worldwide for their importance in the development of many deep-sea fisheries (Holthuis 1980; Alves-Júnior et al. 2017; Purushothaman et al. 2017). Additionally, some shrimp of the genus *Solenocera* represent a high percentage of catches in these fisheries (Demestre and Abelló 1993; Despalatovic et al. 2006; Puentes et al. 2007; Villalobos-Rojas and Wehrtmann 2018).

*Solenocera acuminata* (Pérez-Farfante and Bullis 1973), also called "orange shrimp", is distributed in the Caribbean Sea, including the region from the Bahamas to French Guiana, at depths between 31 and 662 m (Pérez and Kensley 1997). Although some aspects of distribution and abundance of this species have been described previously (Maynou et al. 1996; Guéguen 1997, 1998a, 2000; Charbonnier et al. 2010), detailed information about ecology and fishery-biology of this resource is lacking in other countries where the species is caught. This shrimp is endobenthic during daytime and benthic at night (Guéguen 1998a) and is only caught during the night (Charbonnier et al. 2010). This species generally inhabits the upper part of slopes (Maynou et al. 1996; Guéguen 1997, 1998a) and dense aggregations are found on muddy sediments. This species is found along the continental slope of French Guiana (western tropical Atlantic) within a very narrow bathymetric distribution (between 200 and 300 m), where *S. acuminata* is clearly dominant, reaching a maximum abundance, with average yields of 10 kg/hour by trawl (Guéguen 1997, 1998a, 2000).

Given the potential interest in S. acuminata for a new deep-sea fishery in the Colombian Caribbean, biological fisheries information, such as spatial distribution, growth, size structure, morphometric relationships and size at sexual maturity, is needed for an efficient fisheries management (Hilborn and Walters 1992; Haedrich and Barnes 1997; Shin et al. 2005). This allows the design and implementation of management measurements, such as protected breeding areas and fishing ban, that support sustainable use, as well as monitoring and conservation strategies (Crocos and van der Velde 1995; Ramírez-Llodra 2002; Aragón-Noriega and García-Juárez 2007). The occurrence of S. acuminata has been reported in the Colombian Caribbean Sea in areas, such as Magdalena, Tayrona, Palomino and La Guajira (Campos et al. 2005), with high values of occurrence frequency (41.3%), representing 2.1% in biomass and 2.1% in abundance in scientific surveys (Pérez et al. 2019). Recently, studies on the diversity of continental slope decapods and the biology of deep-sea species with potential commercial importance have been developed in the central and southern western Atlantic (Wehrtmann et al. 2012; Pérez et al. 2019). However, knowledge of some species is still quite limited. Currently, information about the biology and ecology of *S. acuminata* in the Colombian Caribbean is scarce. Most studies have reported only taxonomic records and biological information has been limited to qualitative aspects, with little information on the distribution and abundance of this species (Campos et al. 2005). The lack of knowledge on the life cycle of most deep-sea species with potential commercial interest limits the development and implementation of appropriate management measures (Villalobos-Rojas and Wehrtmann 2011). Therefore and because there are currently no management plans in place in Colombia, it is necessary to broaden our knowledge about deep-sea species and their role in the ecosystem to support their conservation and sustainable use. The objective of this study is to provide biological information about the spatial and bathymetric distribution, abundance, size structure, size at sexual maturity and morphometric relationships of *S. acuminata* in the Colombian Caribbean.

## Materials and methods

Specimens of *S. acuminata* (Fig. 1) were collected during four deep-sea prospecting surveys in the Colombian Caribbean Sea, which were conducted between Punta Gallinas and the Gulf of Urabá (Fig. 2), in August and December 2009 and March and May 2010. Sampling was carried out onboard the commercial shrimp trawler "Tee Claude". A Furuno FCV-1150 echo sounder with a transducer with a frequency of 28 kHz was used to identify the trawlable soft sea bottoms. A total of 87 exploratory fishing trawls were carried out in depths ranging from 100 to 550 m using a shrimp trawl with an opening of 11.6 m at the footrope and a cod-end mesh size of 44.5 mm from knot to knot. The hauls lasted 30 minutes and were conducted at an average speed of 2.5 knots, which was estimated using a Garmin Map 76CSx GPS. The swept area, which was used to



Figure 1. A specimen of Solenocera acuminata captured in the Colombian Caribbean.

calculate the catch per unit area (CPUA; kg/km<sup>2</sup>), was estimated from the spread of the net (11.6 m) using the vulnerability correction factor for shrimp trawl nets (0.7) (Sparre and Venema 1995) and the speed of the vessel (average of 2.5 knots) (King 2007).

In the laboratory, the total wet weight (W) of the S. acuminata individuals was measured using a digital balance with an accuracy of 0.01 g. Afterwards, the samples were measured using a caliper with an accuracy of 0.01 mm, recording twelve morphometric measurements, based on previous studies developed by Tzeng et al. (2001) and Tzeng and Yeh (2002). The recorded measurements were (1) total length (TL; from the posterior margin of the ocular margin indent to the telson), (2) antennal spine width (ASW), (3) hepatic spine width (HSW), (4) cephalothorax length (CL; from the posterior margin of the ocular indent to the posterior margin of the carapace, excluding the rostrum), (5) diagonal cephalothorax length (DCL), (6) first abdominal segment length (FSL), (7) first abdominal segment width (FSW), (8) first abdominal segment height (FSH), (9) second abdominal segment length (SSL), (10) sixth abdominal segment height (SISH), (11) abdomen length (AbL; this measurement, which is used since the head is removed in fishing activities, extends from the end of the thorax to the telson) and (12) head length (HL; from the rostrum to the posterior margin of the carapace) (Fig. 3). The individuals were sexed, with males being identified by the presence of a petasma and females by the presence of a thelycum. The macroscopic maturity stage was determined for females using, as a reference, the five-stage scale (immature, early maturing, late maturing, mature and spent-recover) proposed for Solenocera choprai (Dineshbabu and Manissery 2008).

#### Data analysis

Size structure was analysed by means of a frequency distribution, grouping the data in 5 mm intervals, for both females and males. Differences in the size frequency distribution between females and males were tested using a non-parametric Kruskal-Wallis Test (Gotelli and Ellison 2004).

The length-weight relationship was determined using the potential equation (W = *a* TL<sup>*b*</sup>), the parameters of which were obtained from least squares fitting, having previously performed a linearisation of the function by logarithmic transformation: ln W = ln a + b ln TL, where *W* is the total weight in g, *TL* is the total length in mm, *a* is the intercept (condition factor or initial growth coefficient) and *b* is the growth coefficient (Ferreira et al. 2008). As a measure of goodness of fit, the coefficient of determination (r<sup>2</sup>) was used. A 95% confidence interval was also estimated for the parameters and a Student's t-test was conducted to determine if the sample presented isometric growth (H0:  $\beta$  = 3,  $\alpha$  = 0.05). On the other hand, morphometric relationships were identified by a linear model (Y = a + bX) using least squares estimation, where (Y = TL), X = each independent variable (ASW, HSW, CL, DCL, FSL, FSW, FSH, SSL, SISH, AbL and HL) and *a* and *b* are the parameters of the equation. To evaluate the existence of possible differences between the slopes of the sexes, an analysis of covariance was performed (ANCOVA) (Zar 2009).

The analysis of the size at sexual maturity was performed by the logistic function:

$$P = \frac{1}{\left(1 + e^{(a+b^*X)}\right)}$$

where *P* is the proportion of mature females, *a* and *b* are the parameters and *X* corresponds to total length (TL) or cephalothorax length (CL). The size at sexual maturity is obtained by  $TL_{50\%} = (-a/b)$  and  $CL_{50\%} = (-a/b)$  (King 2007), fitting the logistics curve using a generalised linear model (GLM) with a binomial distribution and logistics link (Dobson 2002; Wheeler et al. 2009) using the GLM function in R software. The estimated  $TL_{50\%}$  and  $CL_{50\%}$  confidence intervals were calculated using a bootstrap procedure that randomly re-sampled the data 10,000 times, resulting in corresponding numbers for the generalised and estimated linear models of  $TL_{50\%}$  and  $CL_{50\%}$ . The 2.5 and 97.5 percentiles of the  $TL_{50\%}$  and  $CL_{50\%}$  estimates were used as the confidence intervals (CI) (Haddon 2001).

The total and cephalothorax lengths were determined to be the primary measurements for the break point analysis in females and males, since these measurements are the most frequent recorded values in crustaceans (Özcan and Katağan 2011). The TL and CL were linked to the different morphometric measurements recorded for each individual (Queirós et al. 2013) to be compared with the size at sexual maturity calculated with the logistic function. A segmented regression model from the segment package in R software was used for estimating the break point; this model is based on the relationships between two explanatory variables that are represented by two straight lines connected by a break point (Muggeo 2003, 2008). The fitting was made by minimising the gap parameter, which measures the space between the two regression lines on each side of the break point. When the algorithm converges, the gap parameter approaches zero, minimising the standard error of the break point. The break point was considered an indicator of the size at the beginning of maturity for females and males, as long as the t value associated with the gap parameter was less than two (Muggeo 2008). In addition, the Davies Test was used to test for significant differences in slopes between fitted segments (Davies 1987; Muggeo 2008; Queirós et al. 2013; Williner et al. 2014).

The spatial distribution of the biomass (kg/km<sup>2</sup>) and size (CL, mm) of *S. acuminata* was determined by a geostatistical method (Rivoirard et al. 2000; Paramo and Roa 2003), in which the populations are seen as spatially stochastic processes, with the variable of interest varying randomly at any given location (Paramo and Roa 2003). First, spatial distribution was modelled using an average model for the entire sampling region of interest, based on the computed structural tool. This structural tool and model are the experimental and model variograms, respectively (Paramo and Roa 2003). Several variogram models (e.g. spherical, exponential and Gaussian models) were fitted to the experimental variogram according to the weighted least square minimisation criterion (Cressie 1993). Then, ordinary point kriging was used to interpolate the data for the not-sampled stations inside



**Figure 2.** Study area in the Colombian Caribbean Sea. The white circles indicate the sampling stations and the black circles indicate the sampling station where *Solenocera acuminata* was observed.

the spatial distribution area (Isaaks and Srivastava 1989). Finally, an intrinsic geostatistical method was applied to estimate the variance in the mean biomass (Rivoirard et al. 2000; see Paramo and Roa 2003 for more explanation). The spatial analysis was performed by R software (geoR library) (Ribeiro and Diggle 2001). A non-parametric Kruskal-Wallis Test was used to test for possible differences in biomass (kg/km<sup>2</sup>) during the day and night (Gotelli and Ellison 2004). A cumulative frequency distribution (CFD) (Perry and Smith 1994) was applied to evaluate the relationship between *S. acuminata* biomass and depth. The maximum absolute vertical distance between the curves was calculated to determine the statistical significance (P) of the difference between curves. The hypothesis of a random relationship between both CFDs was evaluated with 2000 randomisations by Monte Carlo re-sampling for the CPUA and depth (Perry and Smith 1994; see Paramo et al. 2003).

# Results

A total of 147 individuals were captured in 26 stations (Fig. 2), of which 59.9% (88 individuals) were female, 37.4% (55 individuals) were male and 2.7% (4 individuals) were indeterminate. The sizes of the females and males ranged from 56.18 to 146.70 mm TL (mean 105.95  $\pm$  18.10 mm) and from 71.18 and 113.22 mm TL (mean 91.46  $\pm$  11.30 mm), respectively. The females' CL varied from 12.92 to 38.17 mm (mean



**Figure 3.** Diagram of a shrimp showing the body segments measured (Tzeng et al. 2001): total length (TL), antennal spine width (ASW), hepatic spine width (HSW), cephalothorax length (CL), diagonal cephalothorax length (DCL), first abdominal segment length (FSL), first abdominal segment width (FSW), first abdominal segment height (FSH), second abdominal segment length (SSL), sixth abdominal segment height (SISH), abdomen length (AbL) and head length (HL).

**Table 1.** Parameters of the relation between size and weight in female (F) and male (M) *Solenocera acuminata* from the Colombian Caribbean; *a*: intercept, *b*: the allometry coefficient, CI: confidence intervals.

Sex	Ν	a	a (CI 95%)	b	b (CI 95%)	r2	t - test	F	P-value
						-	(b)	(ANCOVA)	(ANCOVA)
F	88	0.00002	0.00001 to 0.00004	2.868	2.734 to 3.002	0.955	0.054	30.17	< 0.05*
М	55	0.00002	0.00001 to 0.00004	2.848	2.681 to 3.016	0.956	0.075		

\* Significant (*P* < 0.05).

 $27.03 \pm 4.98$  mm) and that of the males between 17.33 and 27.97 mm (mean 22.17  $\pm$  2.6 mm). The weights of the females and males fluctuated between 3.50 and 38.40 g (14.91  $\pm$  7.63 g) and 4.30 and 15.30 g (8.44  $\pm$  3.01 g), respectively. Statistically significant differences in sizes and weight were found between the sexes (*P* < 0.05) and sexual dimorphism was evident, with males being smaller than females (Fig. 4).

The analysis of the length-weight relationship indicated allometric growth ( $b \neq 3$ ) in females, while males showed isometric growth (b = 3) (Table 1). The results of the ANCOVA revealed significant differences between the slopes of females and males in the weight-length relationship (Fig. 5a, Table 1). The morphometric relationships between TL vs. ASW, HSW, CL, DCL, FSL, FSW, FSH, SSL, SISH, AbL and HL showed high coefficients of determination ( $r^2 > 0.81$ ) indicating a high correlation between sizes. The ANCOVA showed statistically significant differences between



**Figure 4.** Frequency distributions of **a** total length (TL) and **b** cephalothorax length (CL) for females (F) and males (M) of *Solenocera acuminata*.

parameter b of females and males in linear relationships (TL vs. CL, HL, FSL and FSW). In contrast, there was no significant difference in parameter b (TL vs. AbL, ASW, HSW, FSH, DCL, SSL and SISH) (Fig. 5b–l, Table 2).

The size at sexual maturity was calculated with a total of 68 females (34% immature and 66% mature). The size at sexual maturity ( $TL_{50\%}$ ) of females was 95.2 mm TL (95% CI = 94.22–96.77) and 23.82 mm CL (95% CI = 23.6–24.2) (Fig. 6). The parameters of the logistic model of  $TL_{50\%}$  were as follows: a = 13.25 and b = 0.14; for the logistic model of  $CL_{50\%}$ , a = 11.59 and b = 0.49; for both cases,  $r^2 = 0.99$ .

A total of 68 females and 42 males were analysed separately in the break point analyses. The values shown in Table 3 correspond to those estimates that showed significant differences between the slopes (Davies' Test, P < 0.05) and high coefficients of determination ( $r^2 > 0.817$ ), indicating a high correlation between sizes. For females, the slopes of the linear regression of the first segment were always greater than those of the second segment, while for the males, the slopes of the linear regression with CL showed statistical significance only in females, with a break point of 23.80 ± 1.83 mm for FSL vs. CL. On the other hand, the segmented regressions, performed with TL as the main measure, were significant only for the FSL vs. TL and HL vs. TL relationships in females, showing break point values of 88.87 ± 4.92 mm and 99.85 ± 5.17 mm, respectively. For the males, a break point of 96.07 ± 33.3 mm was evident in the SISH vs. TL relationship (Table 3, Fig. 7a–d).

**Table 2.** Parameters and confidence intervals (95%) of morphometric relationships in females and males of *Solenocera acuminata*: Total length (TL), cephalothorax length (CL), head length (HL), abdomen length (AbL), antennal spine width (ASW), hepatic spine width (HSW), first abdominal segment height (FSH), diagonal cephalothorax length (DCL), first abdominal segment length (FSL), first abdominal segment width (FSW), second abdominal segment length (SSL) and sixth abdominal segment height (SISH). Degrees of freedom for all relationships = 139.

Morphometric	Sex	Ν	a	a (C.I. 95%)	b	b (C.I. 95%)	r <sup>2</sup>	F	P-value
relationship								(ANCOVA)	(ANCOVA)
TL = a+b*CL	F	88	10.613	5.503 to 15.723	3.526	3.341 to 3.713	0.943	5.066	0.026*
	М	55	1.353	-8.029 to 10.735	4.064	3.644 to 4.484	0.876		
TL = a+b*HL	F	88	5.711	3.018 to 8.403	2.755	2.682 to 2.828	0.985	5.245	0.024*
	М	55	3.231	-1.961 to 8.423	2.971	2.797 to 3.144	0.957		
$TL = a+b^*AbL$	F	88	7.488	3.496 to 11.479	1.452	1.394 to 1.509	0.967	0.441	0.508
	М	55	5.352	-0.914 to 11.618	1.416	1.313 to 1.518	0.936		
TL = a+b*ASW	F	88	22.119	16.852 to 27.385	8.674	8.141 to 9.208	0.924	3.35	0.069
	М	55	29.376	22.262 to 36.489	7.774	6.896 to 8.653	0.856		
TL = a+b*HSW	F	88	18.917	14.598 to 23.237	7.26	6.907 to 7.613	0.951	3.888	0.051
	М	55	29.622	21.993 to 37.252	6.501	5.71 to 7.293	0.837		
TL = a+b*FSH	F	88	12.828	8.784 to 16.871	6.921	6.626 to 7.217	0.962	2.095	0.15
	М	55	6.758	-1.529 to 15.046	7.517	6.787 to 8.247	0.889		
TL = a+b*DCL	F	88	24.507	20.143 to 28.871	2.413	2.286 to 2.539	0.94	1.287	0.259
	М	55	17.759	8.854 to 26.663	2.625	2.302 to 2.928	0.84		
TL = a+b*FSL	F	88	-39.069	-50.748 to -27.391	64.398	59.248 to 9.547	0.88	219.94	< 0.05*
	М	55	41.748	35.046 to 48.449	21.641	18.782 to 24.5	0.81		
TL = a+b*FSW	F	88	19.288	15.077 to 23.499	6.783	6.459 to 7.106	0.953	8.958	0.003*
	М	55	6.453	-0.612 to 13.517	8.029	7.368 to 8.692	0.918		
TL = a+b*SSL	F	88	2.839	-3.214 to 8.892	18.833	17.743 to 19.923	0.932	2.012	0.158
	М	55	5.067	-4.155 to 14.289	17.353	15.514 to 19.192	0.871		
TL = a+b*SISH	F	88	10.115	4.845 to 15.385	10.923	10.333 to 11.514	0.94	3.856	0.052
	М	55	1.694	-6.316 to 9.704	12.362	11.266 to 13.457	0.906		

\* Significant (*P* < 0.05).

**Table 3.** The break point estimated by segmented regression for morphometric relationships of *Solenocera acuminata*: first abdominal segment length (FSL) vs. cephalothorax length (CL), FSL vs. total length (TL) and head length (HL) vs. TL for females; sixth abdominal segment height (SISH) vs. TL for males. The intercept and slope are presented for each segment.

Sex	n	Relationship	Break point (mm)	±SE	Segment	Intercept	Slopes	$\mathbf{r}^2$	Davies' test P-value
F	68	FSL vs. CL	23.80	1.83	First	0.470	0.069	0.817	0.039*
					Second	1.078	0.044		
		FSL vs. TL	88.87	4.92	First	0.187	0.021	0.88	0.002*
					Second	0.911	0.013		
		HL vs. TL	99.85	5.17	First	-4.154	0.387	0.986	0.003*
					Second	0.295	0.343		
М	42	SISH vs. TL	96.07	3.33	First	1.914	0.057	0.906	0.018*
					Second	-2.832	0.106		

\* Significant (*P* < 0.05).

The spatial structure of the biomass  $(kg/km^2)$  of *S. acuminata* was modelled using an omnidirectional variogram, which is represented by a spherical model. The variogram showed a 59.33% nugget as a percentage of the sill (nugget = 0.39; sill = 0.27;



**Figure 5.** Morphometric relationships of the females (grey circles) and the males (black circles) of *Solenocera acuminata* in the Colombian Caribbean: **a** total weight (W) vs. total length (TL) **b** TL vs. cephalothorax length (CL) **c** TL vs. head length (HL) **d** TL vs. abdomen length (AbL) **e** TL vs. antennal spine width (ASW) **f** TL vs. hepatic spine width (HSW) **g** TL vs. first abdominal segment height (FSH) **h** TL vs. diagonal cephalothorax length (DCL) **i** TL vs. first abdominal segment length (FSL) **j** TL vs. first abdominal segment width (FSW) **k** TL vs. second abdominal segment length (SSL) and **I** TL vs. sixth abdominal segment height (SISH).



**Figure 6. a** Size at sexual maturity and total length ( $TL_{50\%}$ ) and **b** cephalothorax length ( $CL_{50\%}$ ) of *Solenocera acuminata* females in the Colombian Caribbean.



**Figure 7.** Break points estimated for morphometric relationships in females and males of *Solenocera acuminata*: Female: **a** first abdominal segment length (FSL) vs. cephalothorax length (CL) **b** FSL vs. total length (TL) **c** head length (HL) vs. TL; male: **d** sixth abdominal segment height (SISH) vs. TL.



**Figure 8.** Spatial distribution of the **a** biomass (kg/km<sup>2</sup>) and **b** cephalothorax length (CL) (mm) of females *Solenocera acuminata* in the Colombian Caribbean.

range = 8.81 km). The spatial structure of the CL for females was also modelled by a spherical model. The variogram showed 0.00% of the nugget as a percentage of the sill (nugget = 0.00, sill = 10.72, range = 13.64 km). Relatively high biomass values were found in the northern zone of the Colombian Caribbean, between Santa Marta and Riohacha, where the mean biomass was 0.94 kg/km<sup>2</sup> (coefficient of variation, CV = 39.97). In the southern zone, higher biomass was found between Cartagena and the Morrosquillo Gulf and the mean biomass in this zone was 0.89 kg/km<sup>2</sup> (CV = 17.55) (Fig. 8). The spatial distribution of CL in females showed that the largest shrimp were found off the coast in the north and northwest of Riohacha and to the west of Punta Gallinas. The smaller individuals (~ 21–22 cm CL) in this study were found closer to the coast (~ 10 nautical miles, at 150 m of depth) to the northeast of Santa Marta.



**Figure 9.** Box plot of the biomass (kg/km<sup>2</sup>) of *Solenocera acuminata* in the Colombian Caribbean differentiated by time of day.



**Figure 10.** Cumulative density functions (CFDs) of the depth (f(t)) and the weighted biomass  $(kg/km^2)$  of *Solenocera acuminata*. f(t) is shown by the thick black line, g(t) is shown by the thin grey line and the dotted line (d) is the absolute difference between g(t) and f(t). The depth preferences are shown as the grey and black straight lines.

However, in the southern area, two aggregations of small individuals (~ 21–22 cm CL) were found off the coast between Cartagena and the Gulf of Morrosquillo (Fig. 8).

The biomass of *S. acuminata* showed significant differences (P = 0.002) with the diel pattern, with higher values at night (mean  $1.82 \pm 3.81 \text{ kg/km}^2$ ) than during day-time (mean  $0.15 \pm 0.37 \text{ kg/km}^2$ ) (Fig. 9).

The relationship between the biomass of *S. acuminata* and depth (m) showed significant associations (P < 0.01). This species was distributed between 150 and 400 m and the highest biomass was associated with depths ranging from 330.00 to 380.90 m (Fig. 10).

# Discussion

Information about the reproductive biology of a species is one of the most important aspects in the assessment of strategies for managing exploited populations (Li et al. 2012). The study of the reproductive biology in penaeid shrimp can facilitate our understanding of the adaptive strategies and reproductive potential of a species related to its environment (Gillett 2008). Although S. acuminata is of commercial interest, there are few studies about the biology of some species of the genus Solenocera and their roles in the ecosystems in which they are found (Demestre and Abelló 1993; Ohtomi and Irieda 1997; Ohtomi et al. 1998; Dineshbabu and Manissery 2008; Villalobos-Rojas and Wehrtmann 2011). The size at sexual maturity for females of S. acuminata (95.2 mm TL; 23.82 mm CL) is the first report for the species. The maximum TL in this study (females: 146.70 mm; males: 113.22 mm) are within the range of sizes recorded for females of a species of the same genus Solenocera agassizii in the Colombian Pacific (Rodríguez et al. 2012) and both sexes of this species in the Pacific Ocean in Costa Rica (Villalobos-Rojas and Wehrtmann 2011, 2018). Furthermore, the maximum CL values in this study (females: 38.17 mm; males: 27.97 mm) are similar to those of the same species in French Guiana (Guéguen 1997, 1998b) and slightly lower than those reported in the Colombian Caribbean Sea (Campos et al. 2005). However, they are within the range reported for the Western Atlantic (Pérez-Farfante and Bullis 1973) and French Guiana (Guéguen 1997, 1998b). For males, the maximum CL (27.97 mm) was within the ranges reported by previous authors (Pérez-Farfante and Bullis 1973; Guéguen 1997; Campos et al. 2005).

The mean size differences found between females and males are common amongst solenocerid shrimp (Dineshbabu and Manissery 2008; Li et al. 2012; Villalobos-Rojas and Wehrtmann 2018). The higher number of females than males in the larger size classes observed in *S. acuminata* (Fig. 4) has also been observed in other solenocerid species (Ohtomi and Irieda 1997; Dineshbabu and Manissery 2008; Li et al. 2012). The main factors that affect these variations in the sizes of females and males are differential longevity, mortality, migration behaviour and growth rates (Villalobos-Rojas and Wehrtmann 2018). Differences in the sizes of females and males may be due to differential mortality, nutrition restriction, greater activity of one sex, migration of one of the sexes at a given period and the use of different habitats by sex (Charnov and Hannah 2002; Chiba et al. 2006; Lizárraga-Cubedo et al. 2008; Baeza and Piantoni 2010; Grabowsky et al. 2014).

The highest biomass of *S. acuminata* was found in the northern zone of the Colombian Caribbean. The northeast trade winds drive the surface currents to the west and southwest, almost parallel to the coast, leading to Ekman transport away from the coast, which is responsible for upwelling in the northern zone of the study area and increased productivity along the Guajira coast (Andrade et al. 2003; Paramo et al. 2003, 2009; Correa-Ramírez et al. 2020). In fact, in the Guajira Region, high biomasses have been found for other deep-sea crustaceans, such as *Aristaeomorpha foliacea*, *Pleoticus robustus* (see Paramo and Saint-Paul 2012a), *Penaeopsis serrata* (see Paramo and Saint-Paul 2012b), *Metanephrops binghami* (see Paramo and Saint-Paul 2012c), *Glyphocrangon longleyi*, *Glyphocrangon neglecta* (see Pacheco et al. 2018) and *Agononida longipes* (see Espitia et al. 2019).

Morphometric relationships are an important factor for biological studies of fishery resources and stock assessment. In addition, for the management, it is very important to know the size structure, body growth and size at sexual maturity of this species (Hilborn and Walters 1992), all of which influence the structure and function of marine ecosystems (Haedrich and Barnes 1997; Shin et al. 2005). However, sometimes for practical reasons or due to body damage, only data from some body parts can be recorded (Zetina et al. 1996). Therefore, morphometric relationships have been established to calculate sizes and weight. The morphometric relationships analysed in this study can be useful for population studies of the same species in different geographic locations. The size structure, growth type and morphometric relationships are important parameters of life history and of great utility for the management of a new deep-sea fishery in the Colombian Caribbean. The most frequent dimensions used amongst the wide variety of morphometric measurements in penaeid shrimp are carapace length, total length, body width and wet weight (Özcan and Katağan 2011). Currently, studies on morphometric relationships in deep-water shrimp are scarce. However, these analyses indicate whether there are morphometric variations between several body measurements for the same species in a period of time (Kapiris 2005). These variations may be due to reproductive factors, since filled gonads can influence morphometry.

Morphometric analyses performed by Rudolph (1997, 1999, 2002) on other decapods indicated that, in Samastacus spinifrons and Parastacus pugnax, females have longer and wider abdomens than males. However, in this study, the first abdominal segment was shorter (Fig. 5i), but wider (Fig. 5j) in females than in males. In some male decapods, gonopods and abdominal segments do not increase in size faster than the carapace or the total length (Daniels 2001). However, females have a marked increase in size and changes in the shape of abdominal segments, especially the first abdominal segment, as well as pleopods and other parts of the body, which increases the area available for the fixation of eggs on the pleopods, acting as an incubation chamber for developing eggs (Daniels 2001). Therefore, it is necessary to study whether these growth patterns are related to different types of habitats, ecology, migration and reproduction. All the morphological variations, observed between the sexes, could be associated with differences in the growth pattern of females (e.g. larger maximum size and higher growth rate) compared to those of males (Kapiris 2005). Morphometric variations can be caused by evolutionary and environmental factors and genetic analysis should be used to confirm that these variations are associated with changes in reproductive morphology, rather than with environmental differentiation (Tzeng et al. 2001).

Penaeid shrimp usually show an allometric coefficient (b) close to 3. Female S. acuminata in the Colombian Caribbean followed an allometric growth pattern, which is consistent with previous studies regarding other decapods (Guéguen 1997; Josileen 2011; Özcan and Katağan 2011; Li et al. 2012). Males of S. acuminata followed an isometric growth pattern, as reported by Josileen (2011) and Segura and Delgado

(2012) for other decapods. The length-weight relationship slope values of females in this study were similar to those reported in Kagoshima Bay, southern Japan, for Solenocera melantho (see Ohtomi and Irieda 1997) and higher than those reported for Solenocera membranacea in the coastal Aegean Sea of Turkey (Özcan and Katağan 2011) and S. melantho in the East China Sea (Li et al. 2012). Boschi (2016) reported allometric growth of other juvenile decapods in Argentina, such as *Pleoticus muelleri* and Artemesia longinaris, due to fluctuations in the relationship between different body parts. Several studies have shown similar results on sexual dimorphism in decapod crustaceans (Romero-Sedano et al. 2004; Faye et al. 2015; Ramírez et al. 2020), with females being larger than males and this sexual dimorphism is thought to be related to differences in the functions of male gonopods and female pleopods (Daniels 2001). In fact, after the complete development of gonopods, an isometric growth pattern or even a negative allometric pattern is reported (Fadlaoui et al. 2019). The growth pattern of some specific body parts, such as abdominal segment, gonopods and pleopods, shows variations in the degree of allometry during the course of the development, which may coincide with gonad maturation, providing an important estimate for the size at which these animals are ready for spawning (Marochi et al. 2016; Fadlaoui et al. 2019). However, most studies on the functional significance of isometric and allometric growth have been superficial and more care needs to be taken in explaining these biological phenomena (Daniels 2001). On the other hand, allometric analysis can also provide valuable information about evolutionary modifications in the growth of species (Tzeng and Yeh 2002).

Knowledge of the reproductive season and the average size at sexual maturity of a species with potential applications in fisheries is fundamental to designing and establishing monitoring and control strategies for its conservation. The spatial size structure of S. acuminata in the Colombian Caribbean was determined for the first time, which is interesting because it indicates possible breeding areas. However, for the Colombian Caribbean, there is currently no reproductive information available for the orange shrimp (S. acuminata). The analysis performed by Guéguen (1998a) on the continental slope of French Guiana revealed two seasons of sexual maturation of the gonads in females of S. acuminata (between May and June and between November and December). A very similar pattern arises in the species S. agassizii, which showed two spawning peaks per year from 2005 to 2011, one from May to July and another from December to January (Villalobos-Rojas and Wehrtmann 2018). However, these authors mentioned that this peak of high reproductive intensity fluctuates and is mainly associated with changes in water temperature and salinity, as well as food availability. The size at sexual maturity for females of S. acuminata (95.2 mm TL and 23.82 mm CL) is the first report for the species; these values serve as a reference point for this species in the Colombian Caribbean. In addition, an important factor should be considered in relation to the assignment of ovarian maturity stages performed in this work, wherein Stage II (early maturing) is classified as an immature female. Nevertheless, due to the sampling period, it is possible that females classified as being in Stage II had previously extruded a clutch of eggs and that their ovaries were in regeneration, which may cause

confusion during classification. Incorrect classifications in our study may have affected the estimation of the maturity ogive. Moreover, it is recommended that, to validate these results, the future studies should use a histology analysis to obtain a more accurate ogive estimation (Flores et al. 2020). Therefore, it is recommended to carry out a monthly study to determine the reproductive characteristics of *S. acuminata*, such as its reproductive season and maximum reproductive potential; in addition, histological studies should be used to verify the stages of gonadal maturity and the activity of moults during the year to develop management measures, such as a temporal and/or spatial closure.

The discontinuities in the growth rates of some parts of the body in crustaceans may indicate variations in the morphological size of individuals at the onset of sexual maturity (Hall et al. 2006; Claverie and Smith 2009; Josileen 2011; Queirós et al. 2013; Severino-Rodrigues et al. 2016). The estimated maturity ogive in this study was associated with the results obtained in the break point analysis. The analysis of the first abdominal segment length (FSL) versus CL in the females in this study had relatively similar results as the analysis of the size at sexual maturity: FSL vs. CL = 23.80 mm CL (Table 3) and  $CL_{50\%} = 23.82 \text{ mm}$  (Fig. 6). These findings indicate that the variation in the length of the first abdominal segment, according to CL and TL, can be related to the morphological size at the onset of sexual maturity. Therefore, in this species, the growth of the first abdominal segment may be delayed until individuals reach CLs of 23.8 mm and TLs of 88.87 mm (Fig. 7a, b). This is the approximate time at which the onset of sexual maturity occurs (following a moult in puberty) and an abdominal morphology consistent with reproduction (i.e. a wider abdomen) and with the objective of incubating eggs is observed as occurring in other crustaceans (Rudolph 2002; Cusba and Paramo 2017). However, this change may be also associated with the postmaturing phase, when adult females with optimal abdominal sizes require energy for egg production, reducing the rate of abdominal growth (Claverie and Smith 2009). Therefore, evident changes in the size of the body area in the abdomen may be a good indicator of the beginning of morphological sexual maturity in *S. acuminata* females. However, the morphological size of the maturity (23.8 mm CL and 88.87 mm TL) is uncertain due to the inconsistencies that exist in the estimation of the maturity ogive presented in this study. For HL and SISH, several changes occur in decapods, particularly in the increase in the SISH (Boschi 2016). This increase could also be related to individuals that are close to mating, during which a thoracic-abdominal junction occurs from the male to the female, with the male facing the ventral regions of the female (Boschi 2016).

Guéguen (1997, 1998a) and Charbonnier et al. (2010) reported that *S. acuminata* is probably nocturnal-feeding, since it is captured only at night and burrowing during the day. This behaviour may be a crucial factor in the catch yields recorded during the present cruise, where daytime hauls showed significantly lower catches than night-time hauls. These animals rest when light increases and feed in darkness (Aguzzi and Company 2010). These patterns of diel periodicity agree with our results for *S. acuminata*, which also indicated a nocturnal feeding behaviour, as the largest catches were

recorded during nocturnal trawls and burrowing behaviour during daylight. This behaviour should be considered in the sampling design of future surveys aimed at evaluating the population of *S. acuminata* in the Colombian Caribbean.

Size structure, size at sexual maturity, growth type and morphometric relationships presented in this work are initial reference parameters for fisheries managers. This important information could be useful and strengthened in future research in order to establish and implement management and conservation strategies for *S. acuminata*. Before starting a new fishery, more research is needed to understand the life cycle parameters of this deep-sea resource, such as its growth, reproduction, recruitment, mortality, spawning areas and times, nursery areas and associated biodiversity.

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RESEARCH ARTICLE



# First record of the genus *Pseudamblyopus* (Coleoptera, Erotylidae) in China, with description of a new species

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#### Abstract

The genus *Pseudamblyopus* Araki, 1941 is reported from China for the first time. *Pseudamblyopus sinicus* Liu & Li, **sp. nov.** is described and illustrated, and one species previously placed in *Scelidopetalon* Del-keskamp, 1957 is transferred to *Pseudamblyopus* resulting in the following new combination: *Pseudamblyopus varicolor* (Arrow, 1925). A key to all known species of *Pseudamblyopus* is given.

#### Keywords

Key, new combination, new record genus, taxonomy

# Introduction

The family Erotylidae currently contains approximately 3500 species in 258 genera worldwide, with the highest diversity in South America, Africa, and Asia (Leschen et al. 2010). The tribe Tritomini Curtis, 1834 is the largest tribe in the subfamily Erotylinae sensu stricto, one of six subfamilies in the family Erotylidae, with 92 genera and approximately 1200 species (Leschen et al. 2010; Skelley and Powell 2018). The genus *Pseudamblyopus* was erected by Araki in 1941 with *Amblyopus palmipes* Lewis, 1889 as its type species. Until now, only two species, *Pseudamblyopus similis* (Lewis, 1887) in Japan and Russia and *P. palmipes* (Lewis, 1889) in Japan, have been reported (Chûjô and Chûjô 1990; Wegrzynowicz 2007). In the Fauna Japonica, Chûjô (1969)

described these species in detail. The genus *Pseudamblyopus* is similar to the genera *Amblyopus* Lacordaire, 1842 and *Scelidopetalon* Delkeskamp, 1957. Both *Pseudamblyopus* and *Scelidopetalon* can be distinguished from *Amblyopus* by their small and finely facetted eyes (Araki 1941; Dai and Zhao 2013). While most species of *Scelidopetalon* differ from those of *Pseudamblyopus* by having the antennomere XI much wider than long, in *Scelidopetalon varicolor* (Arrow, 1925) and other species of *Pseudamblyopus* antennomere XI is almost as long as wide or only slightly wider (Dai and Zhao 2013). The ratio of width to length of the antennomere XI of all species in *Pseudamblyopus* is 1.2–1.67:1, while *Scelidopetalon* is 2.1–2.5:1 except *S. varicolor*. The antennomere XI of *S. varicolor* is 1.5× as wide as long, which is the same as that in other representatives of *Pseudamblyopus varicolor* (Arrow, 1925), comb. nov.]. *Pseudamblyopus sinicus* sp. nov. from Guangdong Province, China, is described and illustrated. Before this study, there was no record of *Pseudamblyopus* in China.

#### Materials and methods

The abdominal segments and the genitalia were detached from the body after softening in hot water. Male and female genitalia were placed in 5% NaOH boiling solution for 5 min and then cleaned with distilled water. Morphological characters were illustrated using a Nikon SMZ800N stereomicroscope and modified with Adobe Photoshop CS6.0. Habitus photographs were taken with an Olympus E-M5II camera. Terminology for major structures follow Lawrence et al. (2010, 2011). We have examined all specimens of *Scelidopetalon* and *Pseudamblyopus* deposited in the Museum of Hebei University (**MHBU**), Natural History Museum (**NHML**), and Department of Biology, Shanghai Normal University (**SHNU**).

#### Species of Scelidopetalon and Pseudamblyopus examined in the current studies

Scelidopetalon instabilis (Gorham, 1896) [Petaloscelis] (Burma, Vietnam) from the NHML.

Scelidopetalon similis (Arrow, 1925) [Petaloscelis] (Assam Valley) from the NHML.

Scelidopetalon solidus (Arrow, 1925) [Petaloscelis] (India) from the NHML.

*Scelidopetalon fasciatus* (Arrow, 1926) [*Petaloscelis*] (Sumatra, N. Borneo) from the **NHML**. *Scelidopetalon arrowi* Delkeskamp, 1957 (Singapore) from the **NHML**.

*Scelidopetalon monommoides* (Arrow, 1917) [*Petaloscelis*] (Cameroon) from the **NHML**. *Scelidopetalon biwenxuani* Dai & Zhao, 2013 (China) from the **SHNU**.

*Pseudamblyopus varicolor* (Arrow, 1925), comb. nov. [*Petaloscelis*] (India) from the **NHML**. *Pseudamblyopus palmipes* (Lewis, 1889) [*Amblyopus*] (Japan) from the **NHML**.

Pseudamblyopus similis (Lewis, 1887) [Amblyopus] (Russia, Japan) from the NHML.

#### Taxonomy

#### Genus Pseudamblyopus Araki, 1941

#### Type species. Amblyopus palmipes Lewis, 1889.

**Diagnosis.** Body small to medium-sized, oval to elongate oval, distinctly convex dorsally. *Head* with a pair of stridulatory files on the occipital region; lacinia without teeth at apex; terminal maxillary palpomere nearly triangular to semicircular; mentum much longer than wide, sharply and triangularly ridged on its surface; terminal labial palpomere elongate but not dilated terminally. Compound eye small and finely facetted; antennae rather short, antennomere III nearly equal in length to antennomere IV and V combined; antennal club compactly articulated, antennomere XI irregularly rounded, almost as long as wide, and much narrower than preceding segment. *Pronotum* approximately twice as wide at the base as long. The base of pronotum narrower than the base of elytra. *Elytra* convex, with eight regular rows of fine punctures on each elytron located in bottom of longitudinal furrows (striae). *Prosternum* rather short, prosternal process wide, widened posteriorly, markedly emarginate at its posterior border. *Prosternum* with prosternal lines, metaventrite with postmesocoxal lines and basal abdominal ventrite with postmetacoxal lines.

Legs rather short and robust; tibiae markedly expanded terminally.

*Sexual dimorphism*: male with legs more robust than in female, with extended and more dilated protarsi.

**Distribution.** Japan (Hokkaido, Honshu, Shikoku, Kyushu), China (Guang-dong), India (Nilgiri Hills), Russia (Far East).

#### Key to species of the genus Pseudamblyopus

Partly based on Arrow (1925) and Dai and Zhao (2013).

1	Elytra with indistinct reddish patch at base. Body length: 4.0-5.0 mm. Dis-
	tribution: India (Nilgiri Hills)Pseudamblyopus varicolor
_	Elytra without reddish patch at base
2	Pronotum with two colors, black semicircular spot at the basal border of pro-
	notum. Body length: 4.6–5.1 mm. Distribution: China (Guangdong)
_	Pronotum uniformly reddish brown
3	Leg reddish-brown. Body length: 3.5-5.5 mm. Distribution: Japan (Hok-
	kaido. Honshu, Shikoku) and Russia (Far East) Pseudamblyopus similis
_	Leg black. Body length: 4.5–6.5 mm. Distribution: Japan (Honshu, Shikoku,
	Kyushu)Pseudamblyopus palmipes

#### Pseudamblyopus sinicus Liu & Li, sp. nov.

http://zoobank.org/212740A3-1C5B-46EE-B903-071389B9342B Figures 1–14

**Material examined.** *Holotype* (MHBU), ♂: China; Guangdong Province, Nankun Mountain; 23°09'1.47"N, 113°20'42.70"E; VII/30/2010; Hao Yu Liu, leg. *Paratype* (MHBU), (1 ♀). 1 ♀: same data as holotype.

**Diagnosis.** Body oval, distinctly convex dorsally, smooth and glossy; general color reddish brown. Pronotum with basal border of pronotum slightly infuscate and one black semicircular spot at basal middle, scutellar shield, elytra, basal border of prosternum, mesoventrite, metaventrite and abdominal ventrites black. Antennomere III slightly longer than antennomeres IV and V combined. Terminal maxillary palpomere triangular, about 1.8× as wide as long. Pronotum nearly trapezoidal, convex dorsally; 1.6× as wide as long, finely and closely punctured. Tibiae strongly expanding at apex.

**Comparison.** This species is similar to *Pseudamblyopus similis* due to the shape and color of the body. The new species can be identified using the above key to species. It is distinguished by its pronotum having the black semicircular spot at the basal border; scutellar shield subangulate posteriorly; basal border of prosternum, mesoventrite, metaventrite, and abdominal ventrites black. In contrast to the new species, *P. similis* has its pronotum without black spot; scutellar shield rounded posteriorly; basal border of prosternum, mesoventrite, metaventrite, and abdominal ventrite, and abdominal ventrites set to the new species.

**Description.** Body length: 4.6–5.1, width: 3.0–3.2mm. Body oval, distinctly convex dorsally, smooth and glossy, general color reddish brown, basal border of pronotum slightly infuscated, with one black semicircular spot at basal middle, scutellar shield, elytra and basal border of prosternum, mesoventrite, metaventrite and abdominal ventrites black (Fig. 1).

*Head* (Fig. 2) large, with and dense punctures. Labrum semicircular, with golden pubescence at anterior border; mandibles protruding; clypeus with narrow border and lateral continuations nearly reaching eyes, feebly emarginate in middle, with one fovea on each side of base; frontoclypeal suture incomplete. Compound eye large, moderately prominent, finely faceted; interocular distance 0.62× width of head. Antennae (Fig. 3) short, extending to basal half of pronotum, with golden setae; antennomere I (scape) rather large; antennomere II subtriangular; antennomere III slightly longer than antennomere IV and V combined; antennomeres VI–VIII nearly equal; antennomere VII and VIII somewhat expanding; antennomeres IX and X bowl-shaped and much wider than long, antennomere XI (width: length = 1.67:1) irregularly rounded; relative lengths of antennomere (Fig. 4) triangular, sides rounded, nearly 1.8× as wide as long. Labial terminal palpomere (Fig. 5) subcylindrical. Mentum (Fig. 6) with subtriangular plate, both sides with marginal border, middle area depressed; submentum (Fig. 6) finely and sparsely punctured, with few setae.

**Pronotum** (Fig. 7) nearly trapezoidal, widest at basal, convex dorsally; finely and densely punctured. Anterior margin shallowly bisinuate, with narrow and complete marginal border; lateral margins broadly rounded, with expressed border; basal margin



Figure 1. Dorsal habitus of Pseudamblyopus sinicus sp. nov. Scale bar: 1.00 mm.

weakly bisinuate, with border at both sides, but not along median antescullar part. Anterior and posterior angles rounded, each with one pore. **Prosternum** (Fig. 8) almost impunctate laterally, with fine and sparse punctures medially, with golden setae; anterior border produced to short point in middle, with narrow and complete marginal border; prosternal process with subtriangular depression at apical emargination, surface with golden pubescence; prosternal lines extending anterior margin of procoxal cavities. **Scutellar shield** large, subpentagonal, transverse, with fine and dense punctures, subangulate posteriorly. **Elytra** with eight striae bearing distinct and rather coarse punctures.

*Mesoventrite* (Fig. 9) wide, each side with one shallow depression. *Metaventrite* coarsely punctured at sides and finely punctured in middle; postmesocoxal lines long, extenging to basal 3/5 of metaventrite. *Abdomen* with coarse and dense punctures laterally and slightly finer ones medially; postmetacoxal lines extending to basal 3/4 of ventrite I.

Legs short, femora dilated medially; tibiae (Fig. 10) subtriangular.



Figures 2–14. *Pseudamblyopus sinicus* sp. nov. 2 head 3 antenna 4 maxillary palpus 5 labial palpus 6 mentum and submentum 7 pronotum 8 prosternum 9 mesoventrite 10 protibia and protarsus 11 aedeagus, lateral view 12, 13 ovipositor, ventral, and dorsal views 14 female spermatheca. Scale bars: 0.25 mm (a), 0.5 mm (b).

*Male genitalia* (Fig. 11): median lobe weakly curved, gradually narrow from base to apex; median strut long, with apex dilated and about as long as median lobe. Flagel-lum present and without sclerite at base.

*Female genitalia* (Figs 12, 13): ovipositor with narrow styli at apex of coxite, covered with long setae; spermatheca (Fig. 14) nearly oval.

Distribution. China (Guangdong Province).

Etymology. The species epithet means Chinese.

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RESEARCH ARTICLE



# Two new crane fly species of the subgenus Vestiplex Bezzi, 1924 (Diptera, Tipulidae, Tipula) from Yunnan and Sichuan, China, with a key to species in the *immota* species group

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#### Abstract

Two new crane fly species, *Tipula (Vestiplex) gongdangensis* **sp. nov.** and *T. (V.) dechangensis* **sp. nov.** are described and illustrated based on materials collected in the Yunnan and Sichuan provinces, China. A key is provided to distinguish males of the new species from those of other species in the *T. (V.) immota* Alexander, 1935 species group which is proposed here for the first time.

#### Keywords

Hypopygium, ovipositor, taxonomy, Tipulinae

# Introduction

The World fauna of the subgenus *Vestiplex* Bezzi, 1924 currently amounts to 176 described species, distributed throughout the Holarctic and Oriental regions (Oosterbroek 2021). The Chinese fauna of *Vestiplex* is richly represented, with 69 species and one subspecies (Oosterbroek 2021).

The subgenus *Vestiplex* can be recognized by females having a powerfully constructed and heavily sclerotized cercus, usually with a serrated ventral margin, although margins may be smooth in some of the Asiatic species (Alexander 1935, 1965; Alexander and Byers 1981). The hypogynial valve is short to rudimentary, in the shape of blades or plates, or filamentous (Starkevich et al. 2019a, 2020).

Some species of the subgenus *Vestiplex* have tergite 9 of the male hypopygium forming a shallowly concave and sclerotized saucer (Alexander 1935; Alexander and Byers 1981; Starkevich et al. 2020), while other species have tergite 9 divided by a pale membrane at the midline, with the posterior margin of the dorsal portion simple or bearing additional lobes, ventrally membranous or terminating in a pair of plates, sclerotized processes, armatures or flattened lobes (Alexander 1935; Alexander and Byers 1981; Men et al. 2017; Pilipenko et al. 2019; Starkevich et al. 2019a, b).

Two new species were detected while sorting and identifying specimens of *Vestiplex* from the Sichuan and Yunnan provinces, China. In the present paper, the new species are described and illustrated. The *Tipula* (*V*.) *immota* Alexander, 1935 species group is proposed here for the first time. A key to separate all known species in this species group is given.

#### Materials and methods

Adult crane flies were collected at ultraviolet light and with an insect net, and preserved in 96% ethanol. Specimens were studied with a Nikon SMZ800 (Nikon, Japan) and an Olympus SZ61 (Olympus, Japan) stereomicroscopes. Images were taken with a Canon EOS 80D (Canon, Japan) mounted on an Olympus SZX10 (Olympus, Japan) stereomicroscope and with a KUY NICE (KUY, China) mounted on an Olympus SZ61 stereomicroscope. The genitalia were studied after boiling in a 10% NaOH solution for 5–10 minutes.

Descriptive terminology follows that of Ribeiro (2006) and Cumming and Wood (2017). The term "appendage of sternite 9" (A9S) is adopted from Mannheims (1963), and the terms "ventral lobe" and "dorsal lobe" of A9S were adopted from Gelhaus (2005).

Abbreviations for institutional collections used herein: **AQNU** = Anqing Normal University, Anqing, China; **NRC** = Nature Research Centre, Vilnius, Lithuania; **USNM** = United States National Museum of Natural History, Washington, D.C., USA; other abbreviation: **PS** = slide by Pavel Starkevich.

Abbreviations for terms of the terminalia: **adm**, adminiculum; **AIA**, anterior immovable apodeme; **ap**, anal plate; **A9S**, appendage of sternite 9, **bu**, bursa; **c**, cercus; **CG**, clasper of gonostylus; **dl**, dorsal lobe of appendage of sternite 9; **dp**, dorsal portion of tergite 9; **EA**, ejaculatory apodeme; **gcx**, gonocoxite; **h**, hypogynial valve; **LG**, lobe of gonostylus; **PIA**, posterior immovable apodeme; **s**, sternite; **sp**, spermatheca; **sp** d, spermathecal duct; **t**, tergite; **vl**, ventral lobe of appendage of sternite 9; **vp**, ventral portion of tergite 9.

#### Taxonomy

#### Tipula (Vestiplex) immota Alexander, 1935 species group

The *immota* group can be distinguished from other *T.* (*Vestiplex*) species by tergite 9 having 1) a pair of brown, inconspicuously protruded rounded lobes dorsally, located on either side of the midline, 2) posterior margin terminating into a pair of short, heavily blackened processes and 3) membranous, brown ventral portion with additional microscopically darkened dots.

Males of the *immota* group can also be recognized by the following features: gonocoxite dorsally produced into a horn or spine; clasper of gonostylus terminating into an extended upper beak, dorsal crest nearly rectangular or angular, with a blackened ridge originating from the dorsal corner and a suffused black rim along dorsal surface; lower beak absent; sternite 9 with dorsal lobe of A9S short, fused basally with ventral lobe; adminiculum flattened basally and dilated, with median portion distinctly protruded. The female is known only for *T. (V.) dechangensis* sp. nov. and is characterized by a smooth cercus and a short, blackened, plate-like hypogynial valve.

The *immota* group is close to the *bicornigera* species group (Starkevich et al 2019a). Both groups are characterized by an armed gonocoxite, tergite 9 with additional extensions on posterior margin, and dorsal lobe of A9S reduced. The *bicornigera* group can also be separated by the hypertrophic ventral portion of tergite 9, the absence of protruded lobes on the dorsal surface, and posteromedian lobes having the same level of sclerotizasion as the rest of the tergal surface, not heavily blackened as in the *immota* species group.

#### Tipula (Vestiplex) dechangensis sp. nov.

http://zoobank.org/59772C00-8AC4-4499-A956-AF7B340E2010 Figs 1–21, 36

**Type material.** *Holotype*: male, CHINA, Sichuan, road Dechang/Miyi, 27°05.34'N, 102°01.40'E, alt. 2100 m, 10 May 2018, R. Butvila & A. Saldaitis (NRC); preserved in ethanol.

**Paratypes:** 3 males, 2 females, male genitalia slide No. PS0421m, wing slide No. PS0440m, female genitalia slide No. PS0422f, same data as holotype (NRC); preserved in ethanol.

**Comparative material examined.** *Tipula* (*V*.) *bicornuta* Alexander, 1920: Holotype, male, **CHINA**, Taiwan, Funkiko, 21 April 1917, pinned, T. Shiraki (USNM); antenna, wing and genitalia on slide (USNM). *Tipula* (*V*.) *immota* Alexander, 1935: Holotype, male, **CHINA**, Sichuan, Kwanhsien, alt. 2000–4000 ft, 15–31 May 1933, Graham (USNM); pinned; paratype, male, topotypic (USNM), antenna, leg, wing and genitalia on slide (USNM).

**Diagnosis.** *Tipula* (*V*.) *dechangensis* sp. nov. can be recognized by the following combination of characters: body yellow, abdomen with basal segments yellow, tergites



Figures 1, 2. Tipula (Vestiplex) dechangensis sp. nov. I holotype male, lateral view 2 wing. Scale bar: 2.0 mm.

trivittate, distal segments, including hypopygium, dark brown; male antenna reaching base of abdomen if bent backward. Hypopygium with gonocoxite apically with a black spine that is curved at tip; tergite 9 divided at midline by a pale membrane, posterior margin with U-shaped notch, dorsal surface with pair of brown, inconspicuously protruded, rounded lobes, posterior margin terminating with a pair of black and short, wedge-shaped processes; adminiculum with a rounded preapical incision in lateral view. Female with cercus nearly straight, outer margin smooth, without visible serration, hypogynial valve in the shape of short brown plates, obtuse at apex.

**Description** (Figs 1–20). Adult male (Fig. 1) (n = 4). Lengths: body 11.3–13.2 mm, wing 14.3–15.5 mm, antenna 5.1–6.6 mm.

*Head.* Yellowish, vertex and occiput yellowish with dark brown median line. Rostrum yellowish, with short nasus. Antenna 13-segmented, elongate, if bent backward reaching base of abdomen. Scape, pedicel and first flagellomere yellow; following flagellomeres basally brown, apically light brown. Each flagellomere except first slightly enlarged at base (Fig. 1). Apical flagellomere small, reduced. Verticils shorter than their corresponding segments. Palpus brownish-yellow.


**Figures 3–9.** Male terminalia of *Tipula (Vestiplex) dechangensis* sp. nov., holotype **3** hypopygium, lateral view **4** tergite 9, caudal view **5** tergite 9, dorsal view **6** hypopygium, caudal view **7** lobe of gonostylus (left) **8** clasper of gonostylus (left), lateral view **9** hypopygium, caudal view. Scale bars: 0.5 mm (**10–13**); 1.0 mm (**14, 15**).

**Thorax.** Pronotum light brown, with darker median line. Prescutum and presutural scutum brownish, with four brown longitudinal stripes. Interspaces grey, median pair fused anteriorly, with anterior margins inconspicuously bordered by darker brown. Scutum grey, scutal lobes each with two brown spots. Scutellum and mediotergite grey, with dark brown median line. Pleura yellowish, thinly grey-dusted (Fig. 1). Leg with coxa and trochanter yellow; femur yellow, with darkened tip; tibia and tarsal segments dark brown; tarsal claw with tooth (Fig. 1). Wing light brown (Fig. 2), cell sc



Figures 10–15. Male terminalia of *Tipula (Vestiplex) dechangensis* sp. nov., holotype 10 right gonocoxite, lateral view 11 lobes of appendage of sternite 9 12 adminiculum, lateral view 13 adminiculum, ventral view 14 sperm pump, dorsal view 15 sperm pump, lateral view.

not darker than ground colour, stigma dark brown, variegated by light areas including apical area of cell c and medial area of first cell of cell  $r_1$ , a light band across apical area of cell  $r_1$ , base of cells  $r_3$ ,  $r_4$  and  $r_5$ , and median area of discal cell; remaining light area including base and apical areas of bm. Wing venation:  $R_1$  complete,  $R_{2+3+4}$  subequal in length to  $R_3$ ,  $R_4$  as long as Rs,  $R_5$  curved in apical half, r-m as long as base of  $R_5$ , discal cell narrow, at least 3 times as long as petiole of cell  $m_1$ , cell  $m_1$  more than 4× longer than its petiole. Halter pale yellow, knob brown basally, pale yellow apically.

**Abdomen.** Abdominal segments 1–6 yellow, tergites trivittate, rest of segments, including hypopygium, brown (Fig. 1).

*Hypopygium* (Figs 3–15). Tergite 9 and sternite 9 totally separated. Tergite 9 completely divided at midline by a pale membrane (Fig. 5). Dorsal portion of tergite 9 laterally pale yellow, medially with a pair of brown, inconspicuously protruded, rounded lobes located on either side of midline; posterior margin covered with setae, medially with a U-shaped notch and a pair of black and short, wedge-shaped processes; posterolateral margin rounded (Figs 4, 5). Ventral portion membranous, brown, each half triangular at margin (Fig. 5). Gonocoxite not fused with sternite 9, posterior part produced into a dorsally-directed spine (Figs 3, 6, 10). Lobe of gonostylus narrowed,



**Figures 16–20.** Female terminalia of *Tipula (Vestiplex) dechangensis* sp. nov. (paratype) **16** ovipositor, left lateral view **17** eighth sternite with hypogynial valves, ventral view **18** sternite 9 with bursa **19** furca **20** spermatheca with spermathecal duct. Scale bars: 1.0 mm (**16, 17**); 0.5 mm (**18–20**).

slightly curved, finger-shaped (Fig. 7). Clasper of gonostylus yellow, terminating in an extended upper beak; dorsal crest nearly rectangular with short, blackened ridge generated from the dorsal corner; a suffused black rim along dorsal surface; lower beak absent; base with short lobe and covered with setae (Figs 3, 6, 8). Sternite 9 with ventral lobe of A9S dark brown, nearly triangular, covered with setae (Fig. 11). Dorsal lobe of A9S yellow, densely covered with setae, short, finger-shaped, fused with ventral lobe at base (Fig. 11). Adminiculum triangular in ventral view, basally flattened and dilated (Fig. 12); median portion, before apex, distinctly protruded with margin raised at base; with a rounded preapical incision in lateral view, apex narrowed (Fig. 13). Sperm pump with ejaculatory apodeme fan-shaped, with a small V-shaped notch medially (Fig. 14). Posterior immovable apodeme of same length as ejaculatory apodeme, with paired arms curved dorsally, terminating in an acute apex in lateral view (Fig. 15). Anterior immovable apodeme broad, flattened, obtuse in dorsal view. Aedeagus more than 3.5 times as long as sperm pump (Fig. 15).

**Female.** Adult (n = 2). Lengths: body 18.4–20.1 mm, wing 15.1–16.8 mm, antenna 2.9–3.1 mm.



Figure 21. Type locality of *Tipula (Vestiplex) dechangensis* sp. nov. China, Sichuan, road Dechang/Miyi, alt. 2100 m.

Generally similar to male. Antenna yellow, if bent backward reaching presutural scutum. Flagellar segments, except first, slightly enlarged at base. Abdomen yellow, tergites trivittate, with distinct dorsal stripe.

**Ovipositor** (Figs 16–20). Tergite 10 shiny yellow. Cercus yellow, nearly straight, with tip obtuse and outer margin smooth (Fig. 16). Sternite 8 yellow, with hypogynial valve brown (Figs 16, 17).

Hypogynial valve in the shape of short plate, brown (Fig. 17). Posterior margin of hypogynial valve medially incised, terminating in an obtuse apex in ventral view. Posterior part of sternite 9 covered with short filaments, shovel-shaped, medially with a groove, posterior margin rounded (Fig. 18). Anterior part of sternite 9 narrow, nearly straight (Fig. 18). Furca long, posteriorly flattened, anteriorly narrow (Fig. 19). Three spermathecae, spherical (Fig. 20).

**Biology and distribution.** The new species is known from the Dechang, located at the eastern edge of the Tibetan plateau in Sichuan Province, China (Fig. 36). Two males and three females were collected at ultraviolet light at the beginning of May, at an altitude of around 2100 m. The new species was collected in the dry valley of a small river, with slopes covered by mixed forest dominated by various broad-leaved trees such as oaks (*Quercus dentata* Thunberg, *Q. glauca* Thunberg), poplars (*Populus cathayana* Rehder, *P. simonii* Carrière), elm (*Ulmus parvifolia* Jacquin),

rhododendrons (*Rhododendron brachycarpum* G. Don, *R. dauricum* Linnaeus), and bamboos (*Phyllostachys* ssp., *Borinda* ssp., *Fargesia* spp.) (Fig. 21).

**Etymology.** The new species name is derived from the type locality, Dechang, in Sichuan, China.

**Disscussion.** *Tipula* (V) *dechangensis* sp. nov. is most similar to T. (V) gongdangensis sp. nov. in body and antenna colouration, and in the shape of the gonocoxite, the clasper of the gonostylus and the dorsal lobe of A9S. These two species can be separated by following details of tergite 9: posterior margin with U-shaped notch, black processes es wedge-shaped, and posterolateral corner without extension in T. (V.) *dechangensis* sp. nov.; posterior margin broadly emarginated, without U-shaped notch, black processes nearly triangular, and posterolateral corner extended in T. (V.) gongdangensis sp. nov. Both species can be also separated by differences in the adminiculum, which has a preapical incision in T. (V.) *dechangensis* sp. nov. that is absent in T. (V.) gongdangensis sp. nov., and by the ventral lobe of A9S, which is well developed in T. (V.) *dechangensis* sp. nov. and indistinct in T. (V.) gongdangensis sp. nov.

#### Tipula (Vestiplex) gongdangensis sp. nov.

http://zoobank.org/D6CD9F93-8220-4241-8979-3C7453D7C492 Figs 22–36

**Type material.** *Holotype*: male, **CHINA**, Yunnan, Gongshan County, Bingzhongluo, Gongdangshenshan, 27.97°N, 98.66°E, 12 June 2019, leg. Men QL & Lv L (AQNU), preserved in ethanol.

Paratypes: 23 males, topotypic (AQNU), preserved in ethanol.

**Diagnosis.** *Tipula (V) gongdangensis* sp. nov. can be recognized by the following combination of characters: body yellow, abdomen with basal segments yellow, tergites trivittate, distal segments, including hypopygium, dark brown; male antenna reaching base of abdomen if bent backward. Hypopygium with gonocoxite armed with a black spine; tergite 9 divided at midline by a pale membrane, broadly emarginated at posterior margin, dorsal surface with a pair of brown, inconspicuously protruded, rounded lobes, posterior margin terminating with a pair of short, black, triangular processes. Adminiculum with median portion before apex distinctly protruded, apex curved, acute in lateral view.

**Description** (Figs 22–34). Adult male (Fig. 22) (n = 24). Lengths: body 10.5–11.2 mm, wing 14.0–14.2 mm, antenna 5.6–5.9 mm.

*Head.* Yellowish, vertex and occiput yellowish, medially with narrow dark brown line. Rostrum yellowish, with short nasus densely covered with black setae. Antenna 13-segmented, elongate, if bent backward reaching base of abdomen. Scape, pedicel and first flagellomere yellow; following flagellomeres basally brown, apically light brown, producing an indistinct bicoloured appearance (Fig. 22). Each flagellomere, except first, slightly enlarged at base, segments progressively shortened and narrowed. Apical flagellomere small, reduced. Verticils shorter than their corresponding segments. Palpus brownish-yellow.



Figure 22, 23. *Tipula (Vestiplex) gongdangensis* sp. nov. 22 holotype male, lateral view of holotype 23 wing. Scale bar: 2.0 mm.

**Thorax.** Pronotum light brown, with darker median area. Prescutum and presutural scutum brownish, with four brown longitudinal stripes. Interspaces between median and lateral stripes grey, with light and short setae. Median pair with anterior margins and inner margins on apical 1/4 suffused with black. Scutum grey, scutal lobes greypruinose, each with two brown spots. Scutellum and mediotergite grey-pruinose, with dark brown median line. Pleura yellowish, thinly dusted with brown (Fig. 22). Leg with coxa and trochanter yellow; femur yellow with tip dark brown; tibia and tarsal segments dark brown; tarsal claw with a tooth. Wing light brown (Fig. 23), cell sc darker than ground colour, stigma dark brown with a light area at base, variegated by light areas, including apical area of cell c and median area of 1<sup>st</sup> cell of cell r<sub>1</sub>, a light band across apical area of cell r<sub>1</sub>, base of cells r<sub>3</sub>, r<sub>4</sub> and r<sub>5</sub>, and median area of discal cell; remaining area light, including base and apical areas of cell bm, and median and apical areas of



Figures 24–34. Male terminalia of *Tipula (Vestiplex) gongdangensis* sp. nov., holotype 24 hypopygium, dorsal view 25 tergite 9, caudal view 26 hypopygium, lateral view 27 hypopygium, ventral view 28 lobe of gonostylus (left) 29 clasper of gonostylus (left), lateral view 30 lobes of appendage of sternite 9 31 adminiculum, lateral view 32 ejaculatory apodeme 33 sperm pump, dorsal view 34 sperm pump, lateral view. Scale bars: 0.5 mm (23–30); 0.8 mm (31–33).



**Figure 35.** Type locality of *Tipula (Vestiplex) gongdangensis* sp. nov. China, Yunnan, Gongshan County, Bingzhongluo, Gongdangshenshan.

cells cua and cup. Wing venation:  $R_1$  atrophied in basal half,  $R_{2+3+4}$  slightly shorter than  $R_3$ ,  $R_4$  distinctly shorter than  $R_5$ , curved in apical half, r-m distinctly longer than base of  $R_5$ , discal cell narrow, 3× as long as petiole of cell  $m_1$ , cell  $m_1$  more than 4× as long as its petiole. Halter with stem yellow, knob brown, with apical part lighter.

*Abdomen.* Abdominal segments 1–6 yellow, with brown lateral and median stripes; remaining segments, including hypopygium, brown (Fig. 22).

**Hypopygium.** (Fig. 24–34). Tergite 9 and sternite 9 totally separated (Fig. 24). Tergite 9 completely divided at midline by a pale membrane (Fig. 24). Dorsal portion of tergite 9 medially with a pair of brown, inconspicuously protruded, rounded lobes, located on either side of midline; posterior margin emarginated, with a pair of black, short, nearly triangular processes, posterolateral margin extended into rounded lobes that are covered with setae. Ventral portion membranous, brown, with darker areas provided with brown microscopic dots; anal plates narrowed, attached to rounded margins of ventral portion (Fig. 25). Gonocoxite entirely cut off from sternite 9, ba-



Figure 36. Collecting sites of *Tipula (Vestiplex)* in China: Sichuan, *Tipula (V.) dechangensis* sp. nov. (tri-angle); Yunnan, *Tipula (V.) gongdangensis* sp. nov. (circle).



Figures 37, 38. Genitalia slides of C. P. Alexander (USNM) 37 *Tipula (Vestiplex) bicornuta*, holotype, gonocoxite, lobe of gonostylus and clasper of gonostylus 38 *Tipula (V) immota*, paratype, gonocoxite, lobe of gonostylus and clasper of gonostylus. Scale bar: 0.05 mm (37, 38).

sally covered with setae, dorsally produced into a slightly curved spine with an acute tip (Figs 26, 27). Lobe of gonostylus slightly flattened in middle (Fig. 28). Clasper of gonostylus yellow, upper beak extended, dorsal crest angular, with a black ridge originating from the dorsal corner; with a suffused black rim along dorsal surface; lower beak absent; base with triangular lobe and covered with setae (Figs 24, 26, 29). Sternite 9 with ventral lobe of A9S dark brown, nearly triangular, covered with setae (Figs 27, 30). Dorsal lobe of A9S yellow, densely covered with setae, short, narrowed towards apex, fused with ventral lobe at base (Fig. 30). Adminiculum triangular in ventral view, basally flattened and dilated (Fig. 27); median portion, before apex, distinctly protruded; apex curved, acute at tip (Fig. 31). Sperm pump (Figs 32–34) with ejacula-

tory apodeme V-shaped, each arm expanded and rounded apically (Fig. 32). Posterior immovable apodeme with strongly curved paired arms, terminating in an acute apex in lateral view (Fig. 34). Anterior immovable apodeme broad, flattened, round in dorsal view (Fig. 33). Aedeagus more than 4× as long as sperm pump (Fig. 34).

Female. Unknown.

**Biology and distribution.** A total of 24 males were collected with insect nets around the middle of June, 2019 on Gongdang Mountain, located in the south of Bingzhongluo town of Nujiang Lisu Autonomous Prefecture, Yunnan Province (Fig. 36). The new species was collected at altitudes of approximately 2000–2400 m, in mixed mountain forest dominated by various deciduous trees and bushes (Fig. 35).

**Etymology.** The specific epithet refers to the type locality, Gongdang Mountain, Yunnan, China.

**Discussion.** *Tipula* (V.) *gongdangensis* sp. nov. and *T*. (V.) *dechangensis* sp. nov. are closely related to *T*. (V.) *dashahensis* (Yang et al. 2005: p. 381, fig. 1A–C) based on the shape of the clasper of the gonostylus and of the dorsal lobe of A9S, but they differ by the shape of gonocoxite, which is stout, horn-shaped in *T*. (V.) *dashahensis* and is slender, spine-shaped in *T*. (V.) *dechangensis* sp. nov. and *T*. (V.) *gongdangensis* sp. nov. The two new species can also be separated by the intermediate stripes on the prescutum and presutural scutum, which are fused in *T*. (V.) *dashahensis* and separated, except at the anterior margin, in both of the newly described species.

## Key to species (male) of the *immota* species group

1	Gonocoxite apically stout, horn-shaped, broad at base, gradually narrowing
	toward apex (Figs 37, 38; Yang et al. 2005: p. 381, fig. 1A)2
_	Gonocoxite apically with a slender spine (Figs 3, 26)
2	Prescutum and presutural scutum with fused intermediate stripes (Yang et al.
	2005)
_	Prescutum and presutural scutum with intermediate stripes separated, con-
	fluent only at anterior margin
3	Flagellum bicolorous. Femora brownish-yellow, blackened at tip, preceded by
	a slightly brighter ring. Clasper of gonostylus slightly curved, beak broadened,
	rounded at tip; dorsal margin extended into indistinct teeth (Fig. 37)
_	Flagellar segments black basally and dark brown distally. Femora brown, tip
	broadly dark brown. Clasper of gonostylus bent almost at a right angle, with
	beak narrowing toward apex; dorsal margin extended into two distinct teeth
	(Fig. 38)
4	Gonocoxite with curved, spine-shaped tip (Fig. 3). Tergite 9, at posterior
	margin, with median U-shaped notch; posterolateral margin of tergite 9 not
	extended (Fig. 5)
_	Gonocoxite with tip not curved (Fig. 26). Tergite 9 broadly emarginated at
	posterior margin, without median U-shaped notch; posterolateral margin of
	tergite 9 extended into short lobes (Fig. 24) T. (V.) gongdangensis sp. nov.

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RESEARCH ARTICLE



# A new Antipathozoanthus species (Cnidaria, Hexacorallia, Zoantharia) from the northwest Pacific Ocean

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#### Abstract

A new species of zoantharian within the genus *Antipathozoanthus* is described based on specimens collected from the coast of mainland Japan, northwest Pacific Ocean. *Antipathozoanthus tubus* **sp. nov.** is characterized by its substrate (epibiotic on polychaete tube) and habitat (exposed rock). As well, the results of molecular phylogenetic analyses using concatenated multiple genetic markers also support the distinction between *A. tubus* **sp. nov.** and its congenerics. *Antipathozoanthus tubus* **sp. nov.** is the first species of *Antipathozoanthus* species reported to be epibiotic on polychaete tubes, and is the second species in the genus that is not associated with antipatharians.

## Keywords

molecular phylogeny, polychaete, Sagami Bay, symbiosis, zoantharians

# Introduction

The order Zoantharia Rafinesque, 1815 (Cnidaria: Anthozoa) consists of primarily colonial hexacorallians that are commonly found in most marine environments, including extreme environments such as intertidal zones and methane cold seeps in

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the deep sea (Reimer et al. 2007; Sinniger et al. 2010). The number of studies in Japanese waters on these species have increased in recent decades and have played key roles in the systematic re-appraisal and revision of zoantharians around the globe (Reimer and Fujii 2017). Moreover, the number of overall diversity records of zoantharian species from Japan has increased since 2006 by the addition of more than 20 formally described species (see Reimer and Fujii 2017). In particular, zoantharians within the suborder Macrocnemina Haddon & Shackleton, 1891 have been well studied in Japan as most newly described species belong to this suborder, and the total number of macrocnemic zoantharian species continues to rise by reports of many possibly undescribed species in Japanese waters (e.g., Sinniger et al. 2010; Reimer et al. 2010, 2019).

Antipathozoanthus Sinniger, Reimer & Pawlowski, 2010 within the family Parazoanthidae Delage & Hérouard, 1901 is a genus that has been the subject of recent research in Japanese waters (Sinniger et al. 2010; Kise et al. 2017). This genus currently contains five species (Reimer and Sinniger 2021), with records from Madagascar (Sinniger et al. 2010), the Red Sea, Palau, Maldives, Japan (Reimer et al. 2014; Kise et al. 2017; Reimer et al. 2019) in the Indo-West Pacific, and Ecuador (Reimer and Fujii 2010; Bo et al. 2012; Jaramillo et al. 2018) in the eastern Pacific Ocean, as well as from St. Helena (Santos et al. 2019), Cape Verde, Principe Islands (Ocaña and Brito 2003; Ocaña et al. 2007; Sinniger et al. 2010), and Curaçao (Montenegro et al. 2020) in the Atlantic Ocean and the Caribbean. As the generic name indicates, Antipathozoanthus species generally utilize antipatharians (Hexacorallia: Antipatharia) as their obligate substrate (Sinniger et al. 2010). However, A. obscurus Kise et al., 2017 described from Okinawa, Japan, and the Red Sea, is not associated with any antipatharians and instead is found in cracks and caves on coral-reef substrates (Kise et al. 2017). Thus, substrate specificity to antipatharians within the genus Antipathozoanthus is not all-inclusive, unlike as originally theorized (Sinniger et al. 2010).

Recently, we collected two specimens in Japanese waters of an undescribed species belonging to the genus *Antipathozoanthus*, which were unexpectedly found as epibionts on an empty polychaete tube. Here, we formally describe this new species, *Antipathozoanthus tubus* sp. nov., utilizing morphological and phylogenetic data. With this addition, the entire Japanese zoantharian fauna now comprises 37 recorded species, representing 16 of the 28 currently-recognized genera across nine families (see also Reimer and Fujii 2017; Kise et al. 2017, 2018, 2019; Reimer et al. 2019).

## Materials and methods

#### Specimen collection

The examined specimens were collected in shallow waters of Sagami Bay, Kanagawa, Japan on 2019 and 2020, by SCUBA (Table 1). Specimen images were taken in situ for gross external morphological observation.

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I. Information of sp	liy Species		thidae Antipathozoanthus	tubus sp. nov.							A. hickmani				A. cavernus			A. remengesaui		A. obscurus		

## DNA extraction, PCR amplification, and sequencing

We extracted genomic DNA from tissue of the holotype specimen preserved in 99.5% EtOH using a spin-column DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. PCR amplification using Hot Star Taq Plus Master Mix Kit (Qiagen, Hilden, Germany) was performed for each of COI (mitochondrial cytochrome oxidase subunit I), mt 12S-rDNA (mitochondrial 12S ribosomal DNA), mt 16S-rDNA (mitochondrial 16S ribosomal DNA), 18S-rDNA (nuclear 18S ribosomal DNA), ITS-rDNA (nuclear internal transcribed spacer region of ribosomal DNA), and 28S-rDNA (nuclear 28S ribosomal DNA) using published primers (Medlin et al. 1988; Folmer et al. 1994; Apakupakul 1999; Chen et al. 2002; Sinniger et al. 2005; Swain 2009, 2010; Fujii and Reimer 2011). All PCR products were purified with 1 U of shrimp alkaline phosphatase (SAP) and 5 U of Exonuclease I (Takara Bio Inc., Shiga, Japan) at 37 °C for 40 min followed by 80 °C for 20 min. Cleaned PCR products were sequenced in both directions on an ABI 3730Xl Genetic Analyzer (Applied Biosystems, Thermofisher) at the Fasmac Co., Ltd., Kanagawa, Japan. Obtained sequences in this study were deposited in GenBank under accession numbers MW652759-MW652774.

## Molecular phylogenetic analyses

Forward and reverse sequences were assembled and edited in Geneious v10.2.3 (Kearse et al. 2012). Multiple alignments for each marker were performed with previously published Parazoanthidae sequences obtained from GenBank (Suppl. material 1: Table S1) using MAFFT ver. 7.110 (Katoh and Standley 2013) with the auto algorithm under default parameters for all genetic markers. In this study, sequences of two selected taxa within the zoantharian genus *Epizoanthus* were used as outgroups. We obtained a dataset of 549 bp for 34 sequences of COI, 757 bp for 22 sequences of mt 12S-rDNA, 772 bp for 40 sequences of mt 16S-rDNA, 1756 bp for 23 sequences of 28S-rDNA. These alignments were subsequently concatenated to obtain a final dataset of 5672 bp for 40 OTUs. All aligned datasets are available from the first author and at treebase.org (ID: S27965).

Phylogenetic analyses were performed on the concatenated dataset using Maximum likelihood (ML) and Bayesian inference (BI). ModelTest-NG v0.1.6 (Darriba et al. 2019) under the Akaike information criterion was used to select the best fitting model for each molecular marker, independently for ML and BI. The best selected models for ML and BI analyses were HKY+G for COI, GTR+I+G for mt 12S-rD-NA, SYM+I+G for mt 16S-rDNA, HKY+I+G for 18S-rDNA, TPM3uf+I+G (BI: HKY+I+G) for ITS-rDNA, and GTR+I+G for 28S-rDNA. Independent phylogenetic analyses were performed using model partition per each region in RAxML-NG v0.9.0 (Kozlov et al. 2019) for ML, and MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003) for BI. RAxML-NG was configured to use 12345 initial seeds, search for the best tree among 100 preliminary parsimony trees, branch length was scaled and automatically optimized per partition, and model parameters were also optimized. MrBayes was configured following the models and parameters as indicated by ModelTest-NG, 4 MCMC heated chains were run for 5,000,000 generations with a temperature for the heated chain of 0.2. Chains were sampled every 200 generations. Burn-in was set to 1,250,000 generations at which point the average standard deviation of split frequency (ASDOSF) was steadily below 0.01.

## Morphological observations

Morphological data were collected from whole, dissected, and serial sections of the preserved specimens. Histological sections of 10–15 mm thickness were made using a RM-2125 RTS microtome (Leica, Germany) and were stained with hematoxylin and eosin after decalcification with a Morse solution for 48 h (1:1 vol; 20% citric acid: 50% formic acid). Classification of marginal muscle shapes followed Swain et al. (2015). Cnidae analyses were conducted using undischarged nematocysts from tentacles, column, actinopharynx, and mesenterial filaments of two polyps of holotype specimen under a Nikon Eclipse80i stereomicroscope (Nikon, Tokyo). Cnidae sizes were measured using ImageJ ver. 1.45 (Rasband, 2012). Cnidae classification generally followed England (1991) and Ryland and Lancaster (2004).

## Abbreviations

NSMT National Science Museum, Tsukuba, Ibaraki, Japan;CMNH Coastal Branch of Natural History Museum and Institute, Chiba, Japan.

## Results

Taxonomic description

## Order Zoantharia Rafinesque, 1815 Suborder Macrocnemina Haddon & Shackleton, 1891 Family Parazoanthidae Delage & Hérouard, 1901

## Genus Antipathozoanthus Sinniger, Reimer & Pawlowski, 2010

**Diagnosis** (revised from Sinniger et al. 2010; Swain and Swain 2014, 2015; Kise et al. 2017; additions in **bold**). Macrocnemic zoantharians with cteniform endodermal muscle or endo-meso transitional sphincter muscle. Encrustations of the column to the outer mesoglea. No mesogleal canals or encircling sinus. Tentacles at least 26 in number. Substrate consists of antipatharians, **external surfaces of parchment-like tubes of polychaetes**, or calcium carbonate (coral reef).

**Type species.** *Gerardia macaronesicus* Ocaña & Brito, 2003, by original designation. **Remarks.** We herein modify the diagnosis of *Antipathozoanthus*, as *A. tubus* sp.

nov. is clearly located within the clade of *Antipathozoanthus* with very high support in our molecular phylogenetic analyses. Skeletal secretion as has been reported in *A. macaronesicus* (Ocaña & Brito, 2003) was not found in any other *Antipathozoanthus* species, including *A. tubus* sp. nov.

#### Antipathozoanthus tubus sp. nov.

http://zoobank.org/70CBDCBE-87C2-4A84-AF9D-D4841A082CEC Figures 1–3

**Material examined.** *Holotype.* NSMT-Co 1742, collected from Iwa Beach, Sagami Bay, Kanagawa, Japan (35°09'36"N, 139°08'36"E) at a depth of 14 m by M. Obuchi, 26 July 2019, divided in two pieces, one portion fixed in 99.5% EtOH and the other in 5–10% saltwater formalin. *Paratype.* NSMT-Co 1743, collected from Kotogahama, Sagami Bay, Kanagawa, Japan (35°08'48"N, 139°09'05"E) at a depth of 14 m by M. Obuchi, 6 June 2020, divided in two pieces, one portion fixed in 99.5% EtOH and the other in 70% EtOH.

Material examined for comparison. Antipathozoanthus obscurus NSMT-Co1602 (holotype), collected from Cape Bise, Motobu, Okinawa-jima Island, Japan, by J.D. Reimer, 14 August 2014. Antipathozoanthus remengesaui NSMT-Co1603 (holotype), collected from Blue Hole, Palau, by J.D. Reimer, 15 September 2014. Antipathozoanthus cavernus NSMT-Co1604 (holotype), collected from Sakurajima, Kagoshima, Japan, by J.D. Reimer, 20 September 2015. Antipathozoanthus hickmani CMNH-ZG-05883 (paratype), collected from Roca Onan, Pinzon Island, Galapagos, Ecuador, by A. Chiriboga, 14 March 2007.

Type locality. Iwa Beach, Sagami Bay, Kanagawa, Japan

**Description.** *External morphology.* Colonial zoantharian, with cylindrical polyps connected by well-developed dark red colored coenenchyme (Fig. 1a). External branched tube of dead polychaete mostly covered by coenenchyme. Scapus of column dark red in situ, dark brown in preserved specimens. Capitulum of column orange in situ, dark violet in preserved specimens. Column and coenenchyme heavily encrusted with visible sand and silica particles in ectodermal tissue to outer mesoglea (Fig. 1c, d). Preserved, contracted polyps 2.0–6.0 mm in height, 1.0–3.0 mm in diameter. In situ, opened polyps approximately < 8.0 mm in height, < 10 mm in diameter. Oral disk 5.0–8.0 mm in diameter, orange to light orange in coloration. Number of oral furrows the same as the number of tentacles, and cream white circular protrusion in central oral disk bears slit-like mouth aligned with directives. Tentacles arranged in two rows (15–17 inner endocoelic tentacles and 15–17 outer exocoelic tentacles), as long as the expanded oral disk diameter. Number of tentacles 30–34, transparent in coloration. 15–17 marginal teeth present under inner endocoelic tentacles (Fig. 1b). Tips of tentacles usually cream in coloration. Capitular ridges indiscernible.



**Figure 1.** Images of external morphology of *Antipathozoanthus tubus* sp. nov. (holotype: NSMT-Co 1742) **a** colony on branched polychaete tubes in situ **b** close-up image of polyps in situ **c** colony on branched polychaete tubes in preserved condition **d** close-up image of closed polyp. Abbreviations: MT: marginal teeth, T: tube of polychaete. Scale bars: 10 mm (**a**, **c**), 5.0 mm (**b**), 0.5 mm (**d**).

**Internal morphology.** Azooxanthellete. Mesentery number 30–34, complete 15–17, incomplete 15–17. Mesenteries in macrocnemic arrangement (Fig. 2c). Mesoglea thickness 0.01–0.10 mm, and thicker than ectoderm. Developed siphonoglyph distinct and U-shaped. Mesenterial filaments present (Fig. 2a). Endodermal marginal muscle, short comb-like mesogleal pleats supporting the entire length of the marginal muscle (cteniform endodermal marginal muscle: Fig. 2b). Basal canals of mesenteries absent (Fig. 2d). Additionally, possible gametes observed in several longitudinal sections.

**Cnidae.** Basitrichs and microbasic *b*-mastigophores, microbasic *p*-mastigophores, holotrichs, and spirocysts (Fig. 3, Table 2).

**Habitat and distribution.** Northwestern Pacific Ocean: Sagami Bay, Kanagawa, Japan at depths < 14 m.

**Associated host.** We could not identify host polychaete species as there were no polychaetes in the tubes. However, the tubes that *Antipathozoanthus tubus* sp. nov. was attached to may belong to species within the genus *Eunice*, as polychaete species that build parchment-like branched tubes have been reported from this genus (e.g., Díaz-Díaz et al. 2020).



**Figure 2.** Image of internal morphology of *Antipathozoanthus tubus* sp. nov. (holotype: NSMT-Co 1742) **a** longitudinal section of polyp **b** closed-up image of cteniform endodermal marginal muscle **c**, **d** crosssection of polyp. Abbreviations: A: actinopharynx, MF: mesenterial filament, CEMM: cteniform endodermal marginal muscle, DD: dorsal directives, VD: ventral directives, S: siphonoglyph, 5<sup>th</sup>: 5<sup>th</sup> mesentery from dorsal directives, M: mesoglea, CM: complete mesentery, IM: incomplete mesentery. Scale bars: 0.5 mm (**a**, **c**), 0.1 mm (**b**, **d**).

**Molecular phylogeny.** Both ML and BI phylogenetic analyses showed similar topologies as indicated in Fig. 4. The genus *Antipathozoanthus* appeared as a monophyletic clade located within the family Parazoanthidae with strong nodal support (ML=100%, BI=1) and was close to a Parazoanthidae clade containing species associated with stalked hexactinellid sponges. Within *Antipathozoanthus*, two subclades were formed; one subclade consisted of the antipatharian-associated species *A. macaronesicus, A. hickmani, A. remengesaui,* and *A. cavernus* (ML = 100%, BI = 0.97), and the other subclade consisted of *A. tubus* sp. nov. and *A. obscurus* (ML = 82%, BI = 0.92). Genetic distances in COI, 16S-rDNA, and ITS-rDNA sequences between *A. tubus* sp. nov. and other *Antipathozoanthus* species were 0.000 to 0.009, 0.002 to 0.010, and



Figure 3. Cnidae in the tentacles, column, actinopharynx, and mesenterial filaments of holotype of Antipathozoanthus tubus sp. nov. Abbreviations: HL: holotrich large, HM: holotrich medium, B: basitrichs, BM: microbasic b-mastigophores, SBM: special microbasic b-mastigophores, PM: microbasic p-mastigophores, S: spriocysts.

Tissue	Type of cnidae	Antipathozoanthus tubus sp. nov.							
		Length	Width	Mean±SD	Frequency	n			
		(min-max)	(min-max)	(Length × Width)					
Tentacles	Spirocysts	8.0-19.0	1.0-4.0	15.6±2.0 × 2.1±0.5	Numerous	325			
	Bastrichs	7.0-16.0	1.0 - 4.0	$10.4 \pm 1.5 \times 2.0 \pm 0.7$	Numerous	37			
	Holotrichs medium	12.0-19.0	7.0-8.0	17.8±2.6 × 7.6±0.5	Occasional	6			
	Holotrichs large	20.0-22.0	8.0-11.0	$20.7\pm0.6 \times 9.4\pm0.8$	Occasional	10			
Column	Special microbasic b-mastigophores	12.0	6.0	-	Rare	1			
Actinopharynx	Spirocysts	10.0-16.0	1.0-3.0	$12.7\pm1.6 \times 2.4\pm0.7$	Occasional	9			
	Bastrichs	11.0-15.0	2.0-3.0	$12.5 \pm 1.0 \times 2.3 \pm 0.4$	Numerous	37			
	Microbasic b-mastigophores	8.0-15.0	2.0-3.0	$10.0\pm1.9 \times 2.6\pm0.5$	Rare	5			
	Microbasic p-mastigophores	9.0-11.0	3.0	$10.0\pm0.8 \times 3\pm0$	Rare	3			
	Holotrichs medium	16.0-19.0	5.0-8.0	$18.3 \pm 0.1 \times 6.8 \pm 0.1$	Rare	4			
	Holotrichs large	20.0-22.0	8.0-10.0	$20.7\pm0.9 \times 9.0\pm0.7$	Occasional	15			
Mesenterial filaments	Microbasic b-mastigophores	10.0 - 14.0	2.0-3.0	$12.2\pm1.8 \times 2.5\pm0.5$	Rare	4			
	Microbasic p-mastigophores	8.0-16.0	2.0-4.0	$10.1\pm0.2 \times 3.2\pm0.6$	Numerous	60			
	Holotrichs medium	12.0-19.0	5.0-10.0	$17.8 \pm 1.9 \times 9.3 \pm 1.2$	Common	23			
	Holotrichs large	20.0-25.0	10.0-12.0	$21.1\pm1.2 \times 10.7\pm0.5$	Numerous	36			

**Table 2.** Cnidae types and sizes observed in *Antipathozoanthus tubus* sp. nov. Frequency: relative abundance of cnidae type in decreasing order; numerous, common, occasional, rare (n = number of cnidae).

0.010 to 0.128, respectively. As well, *A. tubus* sp. nov. and other *Antipathozoanthus* species shared unique insertion/deletion patterns in 16S-rDNA sequences.

Remarks. Antipathozoanthus tubus sp. nov. can be easily distinguished from A. remengesaui Kise et al., 2017, A. macaronesicus (Ocaña & Brito, 2003), and A. hickmani Reimer & Fujii, 2010 by the number of tentacles as well as different coloration; Antipathozoanthus remengesaui, A. macaronesicus, and A. hickmani have up to 42 tentacles (Ocaña and Brito 2003; Reimer and Fujii 2010; Kise et al. 2017), while A. tubus sp. nov. has fewer tentacles (30-34). The dark red colored polyps and coenenchyme of A. tubus sp. nov. are not found in these other three Antipathozoanthus species. In addition, A. tubus sp. nov. differs from A. cavernus Kise et al., 2017 with regards to polyp coloration (A. cavernus has orange or light orange polyps: Kise et al. 2017). Although A. tubus sp. nov. and A. obscurus Kise et al., 2017 are phylogenetically close, their COI, 16SrDNA, and ITS-rDNA sequences are all unique (genetic distances in COI, 16S-rDNA and ITS-rDNA sequences between A. tubus sp. nov. and A. obscurus were 0.009, 0.03, and 0.12, respectively). As well, these two species can be separated by coloniality; polyps of A. obscurus are connected by a stolon forming a mesh network (Kise et al. 2017), while polyps of A. tubus sp. nov. are connected by a well-developed coenenchyme. Furthermore, A. macaronesicus, A. remengesaui, A. cavernus, A. hickmani, and A. obscurus have holotrichs in their column (Ocaña and Brito 2003; Reimer and Fujii 2010; Kise et al. 2017), while holotrichs were not observed in the column of A. tubus sp. nov.

Antipathozoanthus is a circumglobally distributed genus, as species have reported from the Indian, Pacific, and Atlantic Oceans (Ocaña and Brito 2003; Sinniger et al. 2010; Reimer and Fujii 2010; Bo et al. 2012; Reimer et al. 2014; Kise et al. 2017), with members living from shallow waters (*A. obscurus* at 3 m depth; Kise et al. 2017) to mesophotic depths (153–169 m for *Antipathozoanthus* sp. sensu Reimer et al. 2019). The most distinctive attributes of *A. tubus* sp. nov. are its substrate and habitat.



**Figure 4.** Maximum likelihood tree based on combined dataset of COI, mt 12S-rDNA, mt 16S-rDNA, 18S-rDNA, ITS-rDNA, and 28S-rDNA sequences. Number at nodes represent ML bootstrap values (> 50% are shown). White circles on nodes indicate high support of Bayesian posterior probabilities (>0.95).

Antipathozoanthus macaronesicus, A. hickmani, A. remengesaui, and A. cavernus are found on antipatharians within the families Antipathidae and Myriopathidae (Ocaña and Brito 2003; Reimer and Fujii 2010; Sinniger et al. 2010; Bo et al. 2012; Kise et al. 2017), while A. obscurus is directly attached to coral reef carbonate (Kise et al. 2017). On the other hand, A. tubus sp. nov. is the only species of the genus found to date on tubes of polychaetes. Four Antipathozoanthus species are known from low light environments; A. macaronesicus, A. remengesaui, and A. cavernus have been found in cave entrances, and A. obscurus is found in crevasses and/or coral reef caves (Ocaña and Brito 2003; Kise et al. 2017). On the other hand, the habitat of A. tubus sp. nov. is not a low-light environment, but the specimens were instead found on a polychaete tube attached to exposed rock.

Within Parazoanthidae, until now, *Isozoanthus altisulcatus* Carlgren, 1939 is the only species described as living on the tubes of polychaetes. However, several morphological differences exist between *A. tubus* sp. nov. and *I. altisulcatus*. Capitular ridges are developed and conspicuous in *I. altisulcatus*, whereas they are indiscernible in *A. tubus* sp. nov. The marginal teeth on the capitulum found in *A. tubus* sp. nov. were not observed in *I. altisulcatus*. Although Carlgren (1939) did not describe the numbers of tentacles of *I. altisulcatus*, the numbers of mesenteries are 34–42 (Carlgren 1939).

As numbers of tentacles are known to be equal to the number of mesenteries (Bourne 1900), the number of tentacles of *I. altisulcatus* is likely to be 34–42, which is greater than the number of tentacles of *A. tubus* sp. nov. (30–34).

Genetic distances of COI sequence between *A. tubus* sp. nov. and other *Antipathozoanthus* species can be considered as intra-generic differences based on previous comparisons of genetic distances (Sinniger et al. 2010). As well, *A. tubus* sp. nov. shared unique insertion/deletion patterns in 16S-rDNA sequences with other *Antipathozoanthus* species. Thus, we consider that *A. tubus* sp. nov. should belong to the genus *Antipathozoanthus* and does not warrant the erection of a new parazoanthid genus.

**Etymology.** *Antipathozoanthus tubus* sp. nov. is named from the Latin *tuba*, as this species is found on polychaete tubes. The Japanese name is 'Iwa-tsuno-sunaginchaku'.

## Discussion

Japanese waters are composed of a wide variety of physical, geographical, and topographical environments due to the latitudinal extension of Japan spanning from the near-tropics of Okinawa to the near-subarctic Hokkaido, and also to the dynamic geology of the region, and thus, Japanese waters have high marine species diversity levels (Fujikura et al. 2010). At the same time, it is estimated that more than 70% of the marine taxa in this region remain undescribed (Fujikura et al. 2010). The order Zoantharia is one such taxon for which much work remains to be done. Although many zoantharian studies have been conducted in Japan, taxonomic studies are still biased by region; southern Japan including Kochi and the Ryukyu Archipelago have been focused on in comparison to other regions (Reimer and Fujii 2017). As a result, 16 species have been described based on type specimens collected from southern Japan (mainly from the Ryukyu Archipelago) (e.g., Irei et al. 2015). In other regions, historical taxonomic works have been conducted in Sagami Bay by Lwowsky (1913), Tischbierek (1929), and Carlgren (1934), with the description of three macrocnemic species; Hydrozoanthus gracilis Lwowsky, 1913, Epizoanthus cnidosus Tischbierek, 1929 (junior synonym of Hydrozoanthus gracilis), and Epizoanthus ramosus, Carlgren 1934. As well, Hertwig (1882) reported the carcinoecium-forming Epizoanthus parasiticus (Verill, 1864) based on the specimens collected from the Sea of Enshu during the Challenger expedition. However, few taxonomic studies have been conducted in these regions since these past historical works, and for many other regions, almost no literature exists (e.g. the Sea of Japan). Thus, in order to understand species richness and the distribution patterns of zoantharians in Japan, further diversity studies with sampling efforts focused on understudied regions are required.

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## Supplementary material I

## Table S1

Authors: Hiroki Kise, Masami Obuchi, James Davis Reimer

Data type: GenBank accession numbers

- Explanation note: GenBank accession numbers used for phylogenetic analyses in this study. Newly obtained sequences indicated in bold.
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RESEARCH ARTICLE



# Revision of the Palaearctic species of Lysitermus Foerster (Hymenoptera, Braconidae, Hormiinae)

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#### Abstract

The three Palaearctic species of *Lysitermus* Foerster, 1863 (Braconidae, Hormiinae, Lysitermini) are revised. The type species is described for the first time together with both of the other species. *Lysitermus suecicus* (Hedqvist, 1957) is a new synonym of *L. tritoma* (Bouček, 1956), and *L. longiventris* (Tobias, 1976) of *L. talitzkii* (Tobias, 1971), **stat. nov.** 

## Keywords

Bulgaria, *Diplodoma laichartingella*, Finland, France, key, Lysitermini, Malta, new record, Portugal, Psychidae, Romania, Sweden

# Introduction

The little-known Palaearctic and Afrotropical genus *Lysitermus* Foerster, 1863 (Braconidae, Hormiinae, Lysitermini) was described by Foerster in 1863 without a description of its type species, *L. pallidus* Foerster, 1863. The identity of the genus was unclear, which resulted in three new generic names for this taxon (*Rogadinaspis* Bouček, 1956; *Paracedria* Hedqvist, 1957; *Prolysitermus* Tobias, 1971). Hedqvist (1963) recognised his earlier mistake and synonymised the first two with *Lysitermus* Foerster. He also synonymised their type species (*Rogadinaspis tritoma* Bouček, 1956 and *Paracedria suecicus* Hedqvist, 1957) with *L. pallidus*. The type series of *Lysitermus* was examined by the first author in 1979, and it proved to be also congeneric with *Prolysitermus* Tobias (van Achterberg 1982) and *Lysitermus* sensu Tobias (1971, 1976) was renamed in *Tritermus* van Achterberg, 1982. In the generic revision of Afrotropical and West Palaearctic genera of Rogadinae (van Achterberg 1991) the author attempted to construct the first key to the European species based mainly on the type series and their original descriptions (but the latter was absent for *L. pallidus*). Four species were recognised by using the shape of the second metasomal suture (sinuate in *L. tritoma*; Figs 24, 25), subparallel-sided metasoma (of *L. longiventris* (Tobias, 1976)) and the relative length of the median carina of the propodeum (for *L. pallidus* and *L. suecicus*). *Lysitermus talitzkii* (Tobias, 1971) was synonymised with *L. pallidus* by Belokobylskij and Tobias (1986).

The second author reared a series of *L. pallidus* from *Diplodoma laichartingella* (Goeze, 1783) (Lepidoptera, Psychidae). After studying the reared material, plus small reared series in the National Museums of Scotland and Naturalis Biodiversity Center, it turned out that there are only two species present in the material from western Europe, with most likely a third one in south-eastern Europe, and that the characters used in the previous key (van Achterberg 1991) were too variable to be useful. In this paper, a new key is presented and *L. pallidus* is described and illustrated for the first time, together with a redescription of *L. tritoma* and *L. talitzkii*.

Although developmental details are hardly known, *Lysitermus* species have been reared as solitary or weakly gregarious parasitoids of case-bearing lepidopterous larvae of Psychidae (*Luffia* and *Diplodoma* spp.) and Tineidae (*Eudarcia derrai* (Gaedike, 1983)) (Gupta and Quicke 2018; Mifsud et al. 2019; this paper). Recently, one species has been found in a mass-rearing from *Inonotus radiatus* bracket fungus on common alder (*Alnus glutinosa* (L.)) in Sweden (Jonsell et al. 2016), probably from Psychidae or Tineidae hiding or feeding in the fungus.

## Material and methods

The specimens studied were (rarely) collected in Malaise traps and directly conserved in 70% alcohol or reared from their hosts and preserved dry. For identification of the subfamily Lysiterminae, see van Achterberg (1993), and for identification of the genus *Lysitermus*, see van Achterberg (1991).

Morphological terminology follows van Achterberg (1988, 1993), including abbreviations for wing venation. Measurements are taken as indicated by van Achterberg (1988): for the length and the width of a body part the maximum length and width is taken, unless otherwise indicated. The length of the mesosoma is measured from the anterior border of the mesoscutum to the posterior border of the propodeum and of the first tergite from the posterior border of the adductor to the medio-posterior margin of the tergite. An asterisk indicates a new record for the country.

Observations and descriptions were made with an Olympus SZ40 stereomicroscope with  $2\times$  objective lens and fluorescent lamp. Photographic images were made with Sony A7RIII 42.4MP camera combined with Canon MPE 65 mm/1–5× macro lens at f2.8 and a Youngnuo YN14EX ring flash. For photo stacking Helicon Focus 7 software (method C pyramid) was used. Additional photos of the Finnish specimen were made with a Nikon DS-Ri2 camera mounted on Nikon SMZ25 stereomicroscope and combined with Zerene Stacker focus stacking software. **BZL** stands for Oberösterreichisches Landesmuseum, Biologiezentrum, Linz; **CSV** for Simo Väänänen Collection; **FMNH** for Finnish Museum of Natural History, Helsinki; **MTMA** for Hungarian Natural History Museum, Budapest; **NMS** for National Museums of Scotland, Edinburgh; **NRS** for Swedish Natural History Museum, Stockholm; **RMNH** for Naturalis Biodiversity Center, Leiden; **ZJUH** for Zhejiang University, Hangzhou; **ZISP** for Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia and **ZMB** for Zoologisches Museum, Humboldt Universität, Berlin.

## Taxonomy

Lysitermus Foerster, 1863

Figs 1-47

- *Lysitermus* Foerster, 1863: 236 [not Tobias 1971: 205, 1976: 49]; Hedqvist 1963: 35; Shenefelt 1975: 1154–1155; van Achterberg 1982: 125; Belokobylskij and Tobias 1986: 63–64; van Achterberg 1991: 19, 1995: 93; Jonsell et al. 2016: 12, 18; Forshage et al. 2016: 12. Type species (by monotypy): *Lysitermus pallidus* Foerster, 1863 [examined].
- *Rogadinaspis* Bouček, 1956: 441; Hedqvist 1963: 35 (as synonym of *Lysitermus* Foerster, 1863); Shenefelt 1975: 1155; van Achterberg 1991: 19, 1995: 93. Type species (by monotypy): *Rogadinaspis tritoma* Bouček, 1956 [examined].
- *Paracedria* Hedqvist, 1957: 219; Hedqvist 1963: 35 (as synonym of *Lysitermus* Foerster, 1863); Shenefelt 1975: 1155; van Achterberg 1991: 19, 1995: 93. Type species (by monotypy): *Paracedria suecicus* Hedqvist, 1957 [examined].
- Prolysitermus Tobias, 1971: 205–206; Shenefelt 1975: 1155; Tobias 1976: 49; van Achterberg 1982: 125 (as synonym of *Lysitermus* Foerster, 1863), 1991: 19, 1995: 93. Type species (by monotypy): Prolysitermus talitzkii Tobias, 1971 [examined].

**Diagnosis.** See van Achterberg (1991: 19). The type species of *Lysitermus* Foerster was not described in 1863; the species name is valid because it was the only species included in the new genus. Indirectly, the type species is characterised by the two features mentioned in the key to the genera (vein 2-SR of fore wing absent and only three metasomal segments visible) but its unequivocal recognition has been problematical. Therefore, we illustrate here recently reared specimens from Sweden, because the cotypes of *L. pallidus* in ZMB are less suitable for redescription and in any case not currently available.

**Biology.** Facultative gregarious parasitoids of case-bearing lepidopterous larvae of Psychidae and Tineidae; they are almost certainly idiobiont ectoparasitoids (Gupta

and Quicke 2018; Mifsud et al. 2019; this paper). There is no mummification of the host larva, as in Rogadinae, and the host remains in the host case are compatible with ectoparasitoism (M.R. Shaw pers. comm.). The records of Scolytini (*Pityophthorus micrographus* (Linnaeus, 1758), *Polygraphus poligraphus* (Linnaeus, 1758)) are based on a mass rearing from a dead *Picea abies* tree infested by both species (Hedqvist 1957). As *Lysitermus* has been reared from case-bearing larvae so far and there is no direct rearing known from Curculionidae (Scolytini), we consider both records very doubtful.

Notes. Lysitermus Foerster and the widespread Old World genus Acanthormius Ashmead, 1906, are very similar and should be synonymised in future if molecular data show that *Acanthormius* and *Lysitermus* are paraphyletic. Up to now, with only few species sampled, the Old World Lysitermus species sampled clusters with Afrotritermus Belokobylskij, 1995 and Atritermus Belokobylskij, Zaldivar-Riverón & Quicke, 2007 and not with the Acanthormius clade (Jasso-Martínez et al. 2021). Therefore, we refrain from synonymising both genera, despite that both have vein CU1a of the fore wing at the level of vein 2-CU1 or above (Fig. 2) and lack a parastigma (but the parastigma is also rarely absent in Aulosaphoides van Achterberg, 1995). However, in Acanthormius vein 2-SR of the fore wing is complete and the third tergite, excluding lamella, usually protruding latero-apically. In Lysitermus vein 2-SR is often largely or entirely absent, if present then nearly always its posterior third unpigmented and only the lamella of the third tergite is protruding. The development of vein 2-SR is very variable; the specimen in Figure 2 has the vein in one wing only pigmented, as figured, but in the other wing entirely sclerotised. In Aulosaphoides vein CU1a of the fore wing is situated distinctly below the level of vein 2-CU1 (at the same level or above in *Lysitermus*) and vein r is emitted distinctly before the middle of the pterostigma (submedially in *Lysitermus*). Trissarthrum Ashmead, 1900, is traditionally included in Lysitermus (Wharton 1993; van Achterberg 1995; Yu et al. 2016), but its Neotropical type species, T. maculipennis Ashmead, 1900, from St. Vincent has a complete vein 2-SR of the fore wing and the propodeal areola comparatively narrow. Therefore, it may be a different Neotropical genus near Acanthormius lacking the apico-lateral protruding part of the third tergite and having vein M+CU1 of the fore wing non-tubular and vein 1-M of hind wing much longer than vein M+CU (van Achterberg 1995). Both characters are also present in the only other described species from the New World, L. woolleyi Wharton, 1993 from Mexico. The Australian Lysitermus sp. 1 listed by Jasso-Martínez et al. (2021) clusters with the Afrotropical genera Afrotritermus Belokobylskij, 1995 and Atritermus Belokobylskij, Zaldívar-Riverón & Quicke, 2007. The listed Neotropical Lysitermus sp. 2 (= Trissarthrum) clusters with the Acanthormius clade (Jasso-Martínez et al. 2021). Lysitermus without Trissarthrum has an Old World distribution, known from the Afrotropical (Papp and van Achterberg 1999; van Achterberg 2000), Australian (Jasso-Martínez et al. 2021), and Palaearctic regions (Yu et al. 2016).

The position of the tribe Lysitermini is uncertain, but there is increasing evidence for a subordinate position in the Hormiinae. Recently, Lysitermini are either included as a tribe in the Rogadinae *sensu lato* (Chen and van Achterberg 2019) or the Hormiinae (Jasso-Martínez et al. 2021), or treated as a separate subfamily (Quicke et al. 2020).

## Key to Palaearctic species of the genus Lysitermus Foerster

1 Area behind stemmaticum finely granulate and more or less rugulose anteriorly (Fig. 8); scutellum finely striate antero-laterally and granulate medially (Fig. 3); apical lamella of third metasomal tergite in  $\mathcal{Q}$  distinctly protruding laterally (Fig. 1), with approx. 25 carinae and wide in dorsal view (Figs 4, 5), its border distinctly serrate in lateral view (also in  $\Im$  comparatively wide, but in lateral view less serrate, without smooth apical rim; Figs 9-11); third tergite of  $\mathcal{Q}$  mainly yellowish brown, less contrasting with second tergite (Figs 1, 5, 26; more or less darkened in 3: Fig. 9) ...... *L. pallidus* Foerster, 1863 Area behind stemmaticum smooth or largely so (Figs 18, 32, 41); scutellum smooth, without lateral striae (Figs 14, 28, 32, 36, 41), but rarely granulate medially; apical lamella of third tergite less protruding laterally (Figs 12, 19, 23, 29, 30, 39), with 8-16 carinae and medium-sized in dorsal view (Figs 16, 21, 24, 25, 27, 31), usually straight to slightly serrate in lateral view (Figs 12, 29, 30, 39); third tergite dark brown or brown, darker than yellowish brown second tergite (Figs 15, 20, 22, 31), but in melanistic specimens second tergite more or less dark brown (Figs 27-29, 39, 43) ......2 Apical lamella of third metasomal tergite concave medio-posteriorly (Figs 16, 2 21, 24), but sometimes intermediate (Figs 25, 27) and with a distinct and smooth rim in front of it (Figs 15, 20, 23, 27); hind tibia (except apically) parallel-sided or nearly so (Figs 12, 29); third antennal segment yellow or yellowish brown (Figs 12, 17, 19, 22, 29), rarely mainly dark brown; vein 3-SR of fore wing 1.3–2.0× longer than vein r (Figs 13, 22) ..... Apical lamella of third metasomal tergite straight medio-posteriorly or nearly so (Figs 31, 37, 39, 44, 47) and rim in front of it less developed and more or less sculptured (Figs 30, 31, 33, 37, 46, 47); hind tibia gradually widened (Figs 30, 45); third antennal segment dark brown or largely so (Figs 33, 45); vein 3-SR of fore wing usually 2.3-3.0× longer than vein r (Figs 30, 33, 34), but sometimes approx. 1.4× (Figs 43, 45) ..... ...... L. talitzkii (Tobias, 1971), stat. nov.

## Lysitermus pallidus Foerster, 1863

Figs 1–11, 26

*Lysitermus pallidus* Foerster, 1863: 236; Shenefelt 1975: 1155; Belokobylskij and Tobias 1986: 64; van Achterberg 1991: 20; Gupta and Quicke 2018: 429.

**Material.**  $5 \ 9 \ + 1 \ 0 \ (= type series of$ *L. pallidus*; ZMB), "[**Germany**], Aachen", "Frst", "*pallidus* $Furst", "Fam. 3 Hecaboloidae, n. gen. (rest illegible)"; <math>4 \ 9 \ + 1 \ 0 \ (RMNH)$ , "**Sweden**: Skåne, Fjälkestad, Balsberget, ex cases of *Diplodoma laichartingella* (Goeze)



Figure 1. Lysitermus pallidus Foerster, ♀, Sweden, habitus lateral. Photograph: R. Soethof.

(Psychidae), coll. 25.vi.2019, em. (indoors) 18.ii.2020, F. Skeppstedt"; 1  $\bigcirc$  (NMS), "**Finland**: Lemland, Flaka, Björkö, 59.98°N, 20.19°E AI, 3.viii.2004, ex ?Psychid [= *Diplodoma laichartingella*] case, N. R. Fritzen", " $\bigcirc$  Ant 17, *Lysitermus pallidus* Foerster, det. M. R. Shaw 2015".

**Redescription.** Figured and reared  $\bigcirc$  from Sweden (RMNH), length of body 2.2 mm, and of fore wing 1.6 mm.

*Head.* Antenna 1.2× as long as fore wing, with 16 segments, slightly widened apically (Fig. 1), scapus oblique apically, length of third segment 1.3× fourth segment; third, fourth and penultimate segments 5.0, 4.0, and 2.4× their width, respectively; face largely smooth, with long erect setae and 1.7× wider than high; clypeus smooth, upper half distinctly convex and ventral half depressed; head in dorsal view 1.8× broader than long medially, eye 1.3× longer than temple dorsally, strongly and roundly narrowed posteriorly (Fig. 7); vertex finely granulate and superficially rugulose near stemmaticum (Fig. 8); occipital carina strong and in front of it crenulate; POL: diameter of posterior ocellus: OOL = 4:2:6; eye in lateral view 1.6× as high as wide, temple distinctly broadening ventrally and width of eye 1.2× minimum width of temple (Fig. 1); malar space 0.7× as long as height of eye and 1.3× as long as basal width of mandible; frons smooth, except for some fine rugulae.

**Mesosoma.** Mesosoma  $1.4 \times$  as long as high in lateral view; mesoscutum granulate and with rather long, narrow, medio-posterior groove (Fig. 3); notauli shallow, complete, largely smooth and anteriorly connected to lateral irregular carina; scutellar sulcus with 3 carinae; scutellum finely striate antero-laterally and superficially granu-



**Figures 2–8.** *Lysitermus pallidus* Foerster,  $\bigcirc$ , Sweden **2** wings **3** mesosoma dorsal **4** metasoma dorsal **5** second and third metasomal tergites dorsal **6** head anterior **7** head dorsal **8** detail vertex dorsal. Photographs: R. Soethof.

late medially (Fig. 3); propodeum granulate and with large areola, its median carina much shorter than anterior side of areola (Fig. 3); side of pronotum rugose-crenulate and secondary granulate sculpture; mesopleuron largely smooth and convex (Fig. 1); precoxal sulcus rather deep and only anterior half present, with few crenulae; metapleuron densely rugulose and with long setae; mesosternal sulcus deep, narrow and smooth. *Wings.* Fore wing: pterostigma elongate triangular and 4× longer than wide

(Fig. 2), r issued near its middle and 1.2× longer than width of pterostigma; 2-SR complete and sclerotized left wing) or only basal two-thirds pigmented (right wing, Fig. 2); 3-SR 1.6× longer than r; SR1 straight and reaching tip of wing; m-cu distinctly postfurcal, rather short (Fig. 2); subdiscal cell distally closed, CU1b close to m-cu and CU1a at same level as 2-CU1 (Figs 1, 2). *Legs.* Hind coxa largely smooth; length of femur, tibia and basitarsus of hind leg 5.5, 11.4, and 10.2× their width, respectively; hind tarsus compressed.

**Metasoma.** Length of first tergite  $0.6 \times$  its apical width, its surface longitudinally striate and with additional granulate sculpture between striae, dorsal surface evenly convex, its dorsal carinae lamelliform and medially interconnected; medial length of second tergite  $0.9 \times$  its basal width, and  $1.4 \times$  as long as third tergite; second and third tergites longitudinally striate (but on middle of third tergite weakly developed) and secondary granulate sculpture; second transverse suture coarsely crenulate and nearly straight (Fig. 4); third tergite antero-laterally without minute, tooth-shaped protuberance and distinctly narrowed posteriorly (Figs 4, 5), with narrow, rugulose, and rather dull apical rim medially (Fig. 5) and distinctly serrate latero-apically (Fig. 4); apical lamella of third metasomal tergite distinctly protruding laterally (Fig. 1), concave and wide in dorsal view, with approx. 25 carinae (Figs 4, 5); setose part of ovipositor sheath  $0.23 \times$  as long as fore wing and  $0.5 \times$  as long as hind tibia, nearly parallel-sided (Fig. 1).

**Colour.** Yellowish brown; third tergite mainly yellowish brown but laterally darkened, not contrasting with similarly coloured second tergite (Figs 1, 5, 26); mesosoma brown, but mesoscutum and scutellum largely dark brown; antenna (basal segments yellowish), pterostigma (but basal quarter yellow) and veins M+CU1 apically, 1-CU1, 1-SR, 1-M, r, and veins of apical half of fore wing dark brown (Fig. 2); palpi and tarsi pale yellowish; remainder of legs yellowish brown; wing membrane infuscate, but with subhyaline band below base of pterostigma (Fig. 1).

*Variations.* Antenna with 16–17 segments; length of body 2.1–2.3 mm, and of fore wing 1.6–1.7 mm; length of ovipositor sheath  $0.21-0.23 \times$  as long as fore wing; vein 2-SR of fore wing varies in reared series from nearly complete to entirely absent (Fig. 2), most frequently only basal half present as pigmented but unsclerotised vein; notauli complete to posterior third obsolescent; second metasomal tergite  $1.3-1.5 \times$  longer than second tergite; lamella of third tergite more or less serrate (Figs 1, 5).

**Male.** Very similar to female, but metasoma slenderer (Fig. 11); head dark brown dorsally; third tergite more or less darkened in  $\Diamond$  (Fig. 9), striate and/or granulate and its lamella serrate and with approx. 20 carinae (Figs 9–11); pterostigma dark brown basally.

**Distribution.** Finland, Germany, Moldova, \*Sweden.

**Biology.** Five specimens of *Lysitermus pallidus* hatched from ten final instar larval cases of *Diplodoma laichartingella* (Goeze, 1783) (Lepidoptera, Psychidae) collected in Sweden by the second author. Dissection of the final instar larval cases showed that only three had been parasitized and five specimens had hatched from them. It clearly indicates that *L. pallidus* is a gregarious larval ectoparasitoid of this host, but probably a facultative one perhaps depending on the size (first- or second-year stage?) of the


**Figures 9–11.** *Lysitermus pallidus* Foerster, ♂, Sweden **9** habitus lateral **10** third metasomal tergite dorsal **11** propodeum and metasoma dorsal. Photographs: R. Soethof.

host. In northern Europe *D. laichartingella* has a two-year life cycle which raises the question of whether *L. pallidus* is a parasitoid only of fully grown larva and, therefore, has a two-year lifecycle like its host (Kunz 1989) or whether it might be able to complete its lifecycle in a juvenile larval case as well. The *D. laichartingella* cases are usually found in woodland areas where the caterpillar feeds on algae/mosses as well as dead insects on tree trunks, especially, of *Fagus sylvatica* L. and *Quercus robur* L. The specimen from Finland has also been reared from *D. laichartingella* (det. M. Mutanen) but was referred by Gupta and Quicke (2018) as reared from an unidentified psychid host.

#### Lysitermus tritoma (Bouček, 1956)

Figs 12-29

Rogadinaspis tritoma Bouček, 1956: 441.

*Lysitermus tritoma*; Hedqvist, 1963: 35; Shenefelt 1975: 1155; van Achterberg 1991: 20; Gupta and Quicke 2018: 429; Mifsud et al. 2019: 54 [examined].

Paracedria suecicus Hedqvist, 1957: 219.

*Lysitermus suecicus*; Hedqvist, 1963: 35 (as synonym of *L. pallidus*); Shenefelt 1975: 1155; van Achterberg 1991: 20; Gupta and Quicke 2018: 429; Mifsud et al. 2019: 54 [examined]. Syn. nov.

**Material.** 1  $\bigcirc$  (FMNH), "Finland: Ab, Parainen, Pexor, 60.26°N, 22.25°E, Malaise trap 1a, 25.vi.–6.vii.2020, Juho Paukkunen"; 1 ♀ (CSV), same label data, but 6–19. vii.2020, Juho Paukkunen & Jonathan Scotson;  $1 \Leftrightarrow 1 \Leftrightarrow (RMNH)$ , "S. Sweden: Uppland, Edsbro, Kristineholm, S15 or S20, coll. 28.i.2009, ex Inonotus radiatus on Alnus glutinosa, C. Gonzales Alonso, RMNH'11"; 1 3 (RMNH), "Netherlands: UT, UTM FF 6560, Amerongen, unmanaged Quercus robur [forest], ex dead stem [in] cage 9(h), 29.v.-12.vi.2001, L. Moraal, RMNH'02"; 1 ♀ (NMS), "France: Lot-et-Garonne, Bernac, 28.vii.[19]90, M.R. Shaw"; 1 ♀ + 2 ♂ (RMNH), "**Spain**, Llansa, 1986, ex *Luffia lapidella*, H. Hendrickx, RMNH'96"; 1 Q (RMNH), "**Portugal**, Oeiras, 18–22. viii.1979, A. van Harten"; 9 9 + 4 8 (RMNH), "Portugal, Cascais fort, ex Luffia lapi*della*, coll. 27.vii.1994, H. Hendrickx"; 5 ♀ + 3 ♂ (RMNH), idem, but coll. 4.v.1995, ex Luffia sp.; 1  $\bigcirc$  (RMNH), idem, but coll. 1.iii.1995; 10  $\bigcirc$  + 2  $\bigcirc$  (RMNH, ZJUH), idem, but Cascais (and mislabelled as from Azores), ex Luffia ferchaultella (Psych.), coll. 27.vii.1994, em. 21.viii.1994; 2 ♀ (RMNH, MTMA) "**Italy**: Sardinia centr., Bruncu Istiddi, 900 m, ex Luffia sp. n.?, viii.1975, E. Hartig & Gozmany";  $1 \stackrel{\frown}{\downarrow} + 1 \stackrel{\frown}{\bigcirc} (NMS)$ , "Malta, Buscett, 22.xi.2006, ex larva on Luffia lapidella (Goeze, 1783) [Psychidae], M. Zerafa"; 2  $\bigcirc$  + 1  $\bigcirc$  (NMS), "Malta: Mosta valley, larva on *Eudarcia derrai* (Gaedike, 1983) [Tineidae], coll. 22.ii.2010, em. v.2010, M. Zerafa";  $3 \ 9 \ + 5 \ 0 \ (BZL, NMS)$ , "Romania: Siebenbürgen, Munt Apuseni (Trascaului), Umg. Posaga 2 km oberhalb, 600 m, ex Dahlica rakosy oder Apt. helicoidella [Psychidae], em. Ende April 2005, M. Weidlich";  $2 \ 9 + 1 \ 6$  (BZL, NMS), "**Bulgaria**: Macedonia, Pirin planina – Süd, Umg. Jane Sandanski, 1220 m, el. 13.v.2000, M. Weidlich, ex Dahlica sp. [Psychidae]".



Figure 12. Lysitermus tritoma (Bouček), ♀, France, habitus lateral. Photograph: R. Soethof.

**Redescription.** Figured  $\bigcirc$  from France (NMS), length of body 1.6 mm and of fore wing 1.4 mm.

**Head.** Antenna 1.1× as long as fore wing, with 15 segments, rather widened apically (Fig. 12), scapus oblique apically, length of third segment 1.1× fourth segment; third, fourth and penultimate segments 5.0, 4.5, and 3.1× their width, respectively; face largely smooth, with long erect setae and 1.7× wider than high; clypeus smooth, upper half distinctly convex and ventral half depressed; head in dorsal view 1.8× broader than long medially, eye 1.4× longer than temple dorsally, strongly and roundly narrowed posteriorly (Fig. 18); vertex smooth and shiny, including area near stemmaticum (Fig. 18); occipital carina moderately strong and in front of it indistinctly micro-crenulate; POL: diameter of posterior ocellus: OOL = 2:2:3; eye in lateral view 1.6× as high as wide, temple distinctly broadening ventrally, smooth and width of eye 1.6× minimum width of temple laterally (Fig. 12); malar space 0.4× as long as height of eye and 1.5× as long as basal width of mandible; frons smooth and shiny.



Figures 13–18. Lysitermus tritoma (Bouček),  $\bigcirc$ , France 13 wings 14 mesosoma dorsal 15 propodeum and metasoma dorsal 16 second and third metasomal tergites dorsal 17 head anterior 18 head posterodorsal. Photographs: R. Soethof.

*Mesosoma*. Mesosoma 1.5× as long as high in lateral view; mesoscutum granulate and with 2 rather short carinulae and no medio-posterior groove (Fig. 14); notauli shallow, distinctly developed anteriorly and posterior half obsolescent, largely smooth and anteriorly connected to lateral irregular carina; scutellar sulcus with 1 carina; scutellum smooth and shiny, convex (Fig. 14); propodeum shiny, largely smooth between carinae and with large areola, its median carina approx. as long as anterior side of areola (Fig. 14):



**Figures 19–21.** *Lysitermus tritoma* (Bouček),  $3^{\circ}$ , Malta **19** habitus dorso-lateral **20** propodeum and metasoma dorsal **21** second and third metasomal tergites dorsal. Photographs: R. Soethof.



**Figures 22–25.** *Lysitermus tritoma* (Bouček),  $\bigcirc$ , 22 holotype *L. suecicus*, **23–25** types of *L. tritoma* **22** habitus dorsal **23** metasoma lateral **24, 25** detail third tergite dorsal. **22** from Forshage et al. (2016) and **23–25** from Bouček (1956).

14); side of pronotum rugose-crenulate and with some granulate sculpture; mesopleuron largely smooth and convex (Fig. 12); precoxal sulcus rather deep and only in anterior half present, with few crenulae anteriorly; metapleuron largely smooth and with long setae; mesosternal sulcus deep, narrow and smooth. *Wings.* Fore wing: pterostigma elongate triangular and 3.5× longer than wide (Fig. 13), r issued from its middle and slightly longer than width of pterostigma; 2-SR completely absent (Fig. 13); 3-SR 1.5× longer than r; SR1 straight and reaching tip of wing; m-cu rather short (Fig. 13); subdiscal cell distally closed, CU1b close to m-cu and CU1a at same level as 2-CU1 (Fig. 13). *Legs.* Hind coxa largely smooth; length of femur, tibia and basitarsus of hind leg 5.0, 9.2, and 5.2× their width, respectively; hind tarsus hardly compressed.

*Metasoma.* Length of first tergite 0.6× its apical width, its dorsal surface evenly convex, surface longitudinally striate and with additional granulate sculpture between striae, its dorsal carinae lamelliform and medially interconnected; medial length of second tergite 0.9× its basal width, and 1.4× as long as third tergite; second and third tergites longitudinally striate and with distinct secondary granulate sculpture; second transverse suture coarsely crenulate and nearly straight (Fig. 15); third tergite anterolaterally without minute tooth-shaped protuberance and distinctly narrowed posteriorly (Figs 15, 16), with distinct smooth and shiny apical rim (Fig. 15); apical lamella of third metasomal tergite moderately protruding laterally (Fig. 12), concave and wide in dorsal view, with approx. 8 carinae (Fig. 16); setose part of ovipositor sheath 0.21× as long as fore wing and 0.6× as long as hind tibia, slightly widened apically (Fig. 12).

**Colour.** Dark brown; third tergite dark brown, contrasting with largely yellowish brown second tergite (Figs 15, 16); first tergite yellowish brown; antenna (basal segments yellowish), pterostigma (but basal fifth yellow) and veins M+CU1 apically, 1-CU1, 1-M, and veins of apical half of fore wing dark brown (Fig. 13); palpi and tarsi pale yellowish; remainder of legs yellowish brown; wing membrane infuscate, but band below base of pterostigma and marginal cell partly, subhyaline (Fig. 13).

*Variations.* Antenna with 14–17 segments; length of body 1.5–1.9 mm, and of fore wing 1.3–1.5 mm; length of ovipositor sheath 0.21–0.26× as long as fore wing; vein 2-SR of fore wing varies from completely absent (Figs 13, 19), complete (Fig. 29) to small unsclerotised trace (Fig. 45); notauli complete to posterior third obsolescent; scutellar sulcus with 1–3 carinae; precoxal sulcus smooth or with few crenulae; metapleuron largely smooth or with some rugulae medially; median carina of propodeum approx. as long as anterior side of areola or much shorter; second metasomal tergite 1.3–1.5× longer than second tergite; lamella of third tergite hardly serrate (Fig. 12), with 8–16 carinae; colour of body is very variable: southern specimens are brownish yellow with only third tergite dark brown and northern specimens are largely dark brown (except first and second tergites, but in both Finnish specimens also darkened; Figs 27–29), sometimes hind coxa, femur and tibia largely brown or dark brown (Fig. 29).

**Male.** Very similar to female, but metasoma slenderer (Fig. 20); antenna with 16 or 17 segments; length of body 1.4–1.6 mm, and of fore wing 1.3 mm; head dark brown dorsally; linear medio-posterior depression of mesoscutum absent or slightly



**Figures 26–27. 26** *Lysitermus pallidus* Foerster,  $\mathcal{Q}$ , Sweden (freshly emerged specimen), habitus lateral **27** *L. tritoma* (Bouček), dark  $\mathcal{Q}$ , Finland, metasoma dorsal. Photographs: F. Skeppstedt (**26**) and P. Malinen (**27**).

impressed; third tergite dark brown and contrasting with yellowish second tergite (Fig. 20), striate and/or granulate and its lamella hardly serrate (Figs 19–21, 45); pterostigma dark brown basally.

Biology. Reared from lepidopterous case-bearing larvae belonging to Psychidae (*Luffia ferchaultella* (Stephens, 1850); *L. lapidella* (Goeze, 1783); *L. sp.; Dahlica sp.*)



**Figures 28–29.** *Lysitermus tritoma* (Bouček), dark  $\mathcal{D}$ , Finland **28** body dorsal **29** habitus lateral. Photograph: P. Malinen.

and Tineidae (*Eudarcia derrai* (Gaedike, 1983); Mifsud et al. 2019). It has been reared from *Inonotus radiatus* bracket fungi on *Alnus glutinosa* (L.) (Jonsell et al. 2016), most likely from host cases hiding in the fungi.

**Distribution.** \*Bulgaria, Czech Rep., \*Finland, \*France, Italy (Sardinia), Malta, \*Netherlands, Poland, \*Portugal (mainland), \*Romania, \*Spain (mainland), Sweden.

**Notes.** The medio-longitudinal carina of the propodeum is very variable in length, from about as long as oblique anterior side of propodeal areola to much shorter and the second metasomal suture varies from distinctly sinuate (typical *L. tri-toma*; Figs 24, 25) to straight (typical *L. suecicus*; Figs 15, 20, 22). After more reared specimens became available, intermediates of both characters have been found, and there are no grounds to separate any longer the two species, as has been proposed by van Achterberg (1991).

The holotype of *L. suecicus* (NRS) is incorrectly figured in the original description. For instance, the second tergite is not twice as long as the third tergite but 1.5× (Fig. 22), the ovipositor sheath is not widened but subparallel, and the pterostigma is not robust but rather slender (Fig. 22).

#### Lysitermus talitzkii (Tobias, 1976) stat. nov.

Figs 30-47

Prolysitermus talitzkii Tobias, 1971: 205; Shenefelt 1975: 1155.

*Lysitermus talitzkii*; Belokobylskij and Tobias 1986: 64 (as synonym of *Lysitermus pallidus*); van Achterberg 1991: 20 [examined].

Prolysitermus longiventris Tobias, 1976: 50, 253.

*Lysitermus longiventris*; Belokobylskij and Tobias 1986: 64; van Achterberg 1991: 20. Syn. nov.

**Material.** Holotype of *Prolysitermus talitzkii*,  $\mathcal{O}$  (ZISP), "MCCP [= **Moldova**], Kishinev, radiolu, 2.vi.[19]62, 65, Talitzkij", "Holotypus *Praelysitermus* [sic!] *talitzkii* Tobias, [19]71"; 1  $\mathcal{O}$  paratype of *P. talitzkii* (ZISP) from Moldova, Străşeni, 21.vii.1961. Holotype of *Prolysitermus longiventris*,  $\mathcal{O}$  (ZISP), "[**Russia:**] Sochi-Lazarevskoe, 3–4.v.[1]973, V. Tobias", "*Prolysitermus longiventris* sp. n., Tobias, det. 1973", "Holotypus *Prolysitermus longiventris* Tobias, 1976"; 1  $\mathcal{Q}$  paratype (ZISP), "28. iv.[19]75", "[Russia:] Krasnodarskiy kray, Lazarevskoe, les na terrasirovannych sklonach, 28.iv.1975, V. Tobias", "Paratypus *Prolysitermus longiventris* Tobias, 1976"; 1  $\mathcal{O}$  paratype (ZISP), "11.v.[19]75", and same label data as  $\mathcal{Q}$  paratype.

**Redescription.** Paratype  $\bigcirc$  of *L. longiventris*, length of fore wing 1.4 mm, and of body 1.6 mm.

*Head.* Antenna  $1.1 \times$  as long as fore wing, with 15 segments, rather widened apically (Fig. 30), scapus oblique apically, length of third segment  $1.1 \times$  fourth segment; third, fourth, and penultimate segments 6.6, 5.8, and 2.9 $\times$  their width, respectively; face largely smooth, with long erect setae and 2.2 $\times$  wider than high; clypeus smooth



Figures 30–32. *Lysitermus talitzkii* (Tobias), ♀, paratype of *L. longiventris* 30 habitus lateral 31 metasoma dorsal 32 head and mesoscutum dorsal. Photographs: K. Samartsev.

and distinctly convex; head in dorsal view  $1.6\times$  broader than long medially, eye  $1.4\times$  longer than temple dorsally, strongly and roundly narrowed posteriorly (Fig. 32); vertex smooth and shiny, including area near stemmaticum (Fig. 32); occipital carina strong medio-dorsally and in front of it micro-crenulate; POL:diameter of posterior ocellus: OOL = 20:12:35; eye in lateral view  $1.5\times$  as high as wide, temple distinctly broadening ventrally, smooth, and width of eye  $1.3\times$  minimum width of temple laterally; malar space  $0.5\times$  as long as height of eye and  $1.7\times$  as long as basal width of mandible; frons smooth and shiny.

*Mesosoma*. Mesosoma 1.5× as long as high in lateral view; mesoscutum granulate and shiny, medio-posteriorly with indistinct groove (Fig. 32); notauli only anteriorly distinct, largely smooth, but anteriorly crenulated; scutellar sulcus with one median carina and 6 weak crenulae; scutellum largely smooth and shiny, convex (Fig. 32); propodeum shiny, largely smooth between carinae and with large areola, its median carina shorter than anterior side of areola; side of pronotum and mesopleuron largely smooth; precoxal sulcus rather shallow and only its anterior half present, largely smooth; metapleuron largely smooth and with long setae. *Wings*. Fore wing: pterostigma elongate triangular and 5.0× longer than wide (Fig. 30), r issued from its middle and 0.7× shorter than width of pterostigma; 2-SR absent except for some pigmentation (Fig. 30); 3-SR 3.1× longer than r; SR1 straight and reaching tip of wing; m-cu rather short and largely unpigmented; subdiscal cell distally closed, CU1b far from



**Figure 33.** *Lysitermus talitzkii* (Tobias), *A*, holotype of *L. longiventris*, habitus lateral. Photograph: K. Samartsev.

m-cu and CU1a above level of 2-CU1 (Fig. 30). *Legs.* Hind coxa largely smooth; length of femur, tibia and basitarsus of hind leg 4.5, 11.1, and 8.4× their width, respectively; hind tarsus compressed.

**Metasoma.** Length of first tergite  $0.6 \times$  its apical width, its surface longitudinally striate and with additional granulate sculpture between striae, dorsal surface evenly convex, its dorsal carinae lamelliform and medially interconnected anteriorly; medial length of second tergite  $0.9 \times$  its basal width, and  $1.4 \times$  as long as third tergite; second and third tergites longitudinally striate and with distinct additional granulate sculpture between striae; second transverse suture coarsely crenulate and nearly straight (Fig. 31); third tergite antero-laterally with minute tooth-shaped protuberance and gradually narrowed posteriorly (Fig. 31), with distinct and sculptured posterior rim; apical lamella of third metasomal tergite moderately protruding laterally (Fig. 31), straight and narrow medially in dorsal view, with approx. 14 carinae; setose part of ovipositor sheath  $0.4 \times$  as long as hind tibia.

**Colour.** Mainly dark brown; third tergite dark brown, contrasting with largely yellowish brown second tergite (Figs 30, 31); first tergite yellowish brown; scapus infuscated, pedicellus, third and fourth segments yellow; remainder of antenna, ptero-



**Figures 34–42.** *Lysitermus talitzkii* (Tobias),  $\mathcal{E}$ , holotype of *L. longiventris* **34** fore wing **35** head and mesosoma lateral **36** propodeum and first and second metasomal tergites dorsal **37** metasoma dorso-lateral **38** metasoma lateral **39** metasoma dorso-lateral **40** head anterior **41** head and mesonotum dorsal **42** hind coxa and femur lateral. Photographs: K. Samartsev.

stigma, and veins M+CU1 apically, 1-CU1, 1-M, and veins of apical half of fore wing brown; palpi and tarsi pale yellowish; remainder of legs yellowish brown; wing membrane subhyaline.



**Figures 43–44.** *Lysitermus talitzkii* (Tobias), *C*, paratype of *L. longiventris* **43** habitus lateral **44** metasoma dorsal. Photographs: K. Samartsev.

**Male.** Colour very variable: body entirely dark brown (Fig. 43) to partly yellowish brown (Fig. 45); metasoma slender, darkened and antero-lateral small tooth-like protuberance of third tergite either absent (holotypes, but area slightly convex; Figs 36, 47) or present (Fig. 44); first tergite weakly narrowed posteriorly (Figs 36, 44) or parallel-sided (Fig. 47); vein 3-SR of fore wing 1.4–3.0× longer than vein r (Figs 34, 43, 45); length of body1.4–1.9 mm and fore wing 1.4–1.5 mm.

Biology. Unknown.

Distribution. Moldova, Poland (Huflejt 1997), Russia.

**Notes.** The male holotype of *L. talitzkii* (Tobias, 1971) was considered to be a synonym of *L. pallidus* Foerster by Belokobylskij and Tobias (1986) and van Achterberg (1991), but after examination of reared series consisting of both sexes it is obvious that the holotype male with its gradually widened hind tibia (Fig. 45) fits better with *L. longiventris*, as defined above, and the latter is synonymised with it. The holotype has the notauli shallowly impressed posteriorly (van Achterberg 1995) although normally the posterior half of the notauli are absent, but specimens with vaguely to distinctly indicated notauli have been examined among specimens of the closely related *L. tritoma* from Sweden (holotype of *L. suecicus*), Italy, and Portugal, and, therefore, we do not consider the more developed notauli as a valid reason to maintain *L. longiventris* as a species different from *L. talitzkii*.

Lysitermus longiventris (Tobias) was described from N. Caucasus and is very similar to L. tritoma (Bouček) but differs by having the third metasomal tergite of-



**Figures 45–47.** *Lysitermus talitzkii* (Tobias), ♂ holotype, but 47 of ♂ paratype **45** habitus lateral **46,47** metasoma dorsal. Photographs: K. Samartsev.

ten with a minute tooth-like protuberance antero-laterally and its posterior lamella straight medially or nearly so, the metasoma slightly slenderer, the third tergite slightly less narrowed posteriorly, and vein 3-SR of the fore wing usually more than twice as long as vein r. The reduction of the longitudinal rugae on the third tergite is considered less distinctive because reduction of sculpture is common in *Lysitermus* males and to a lesser degree in females. Other differences given by Belokobylskij and Tobias (1986), such as the first tergite as long as wide at apex and the second tergite longer than its apical width, disagree with the only figure in the very short original description and the figures included here (Figs 31, 36, 44). Characters such as the third tergite less sculptured than second tergite and without distinct lamella apically, the face twice as wide as high, and the width of the hypoclypeal depression equal to distance from eye to depression are more or less also found in males of *L. tritoma*.

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RESEARCH ARTICLE



# Taxonomic reassessment and phylogenetic placement of Cyrtodactylus phuketensis (Reptilia, Gekkonidae) based on morphological and molecular evidence

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#### Abstract

The taxonomy and phylogeny of the *Cyrtodactylus pulchellus* complex along the Thai-Malay Peninsular region has been the focus of many recent studies and has resulted in the recognition of 17 species. However, the majority of these studies were focused on Peninsular and insular Malaysia where there were specimens and genetic vouchers. The taxonomic status and phylogenetic relationships of the Thai species in this complex remain unresolved, due to the lack of genetic material of some species, especially *C. phuketensis* and *C. macrotuberculatus* from Thai populations. In this study, we investigated the phylogenetic relationship between *C. phuketensis* and its closely related species *C. macrotuberculatus*, using both morphometric and molecular data. Phylogenetic analyses of mitochondrial NADH dehydrogenase subunit 2 (ND2) gene revealed that *C. phuketensis* is embedded within a *C. macrotuberculatus* clade with 1.45–4.20% (mean 2.63%) uncorrected pairwise sequence divergences. Morphological comparisons showed nearly identical measurements of *C. phuketensis* and *C. macrotuberculatus*, thus rendering *C. phuketensis* a junior synonym of *C. macrotuberculatus*, thus rendering *C. phuketensis* a junior synonym of *C. macrotuberculatus*.

#### Keywords

*Cyrtodactylus macrotuberculatus*, Malaysia, morphology, phylogeny, taxonomic status, Thailand, Thai-Malay Peninsula

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## Introduction

*Cyrtodactylus* is a genus of the bent-toed geckos which is widely distributed across South Asia to Melanesia (Wood et al. 2012; Grismer et al. 2020, 2021a). This genus is the most speciose group of gekkotans, with 306 species currently recognized (Uetz et al. 2020). Due to discoveries of hidden taxa within species complexes over decades, species diversity of *Cyrtodactylus* has remarkably increased, especially in Southeast Asia (e.g., Grismer et al. 2012, 2018; Riyanto et al. 2017; Pauwels et al. 2018; Murdoch et al. 2019; Quah et al. 2019). In the last decade, the integrative approach of molecular and morphological data has been applied to study species boundaries, evaluate taxonomic status, and the adaptive evolution in habitat preference in *Cyrtodactylus* (Grismer et al. 2015, 2020, 2021a, b; Nielsen and Oliver 2017).

The Cyrtodactylus pulchellus group (Grismer et al. 2021a) is widely distributed along the Thai-Malay Peninsular region and extends from lowland to over 1,500 meters above sea level (Grismer 2011). It was recognized as a single species until morphological differences from an insular population (Pulau Langkawi, Kedah, Malaysia) were noticed and a new species, C. macrotuberculatus Grismer and Ahmad, 2008 was recognized. Several populations of C. pulchellus were considered to be part of a species complex that may reveal hidden diversity and unnamed species (Grismer 2011; Grismer et al. 2012). To date, taxonomic revisions of the C. pulchellus group have recovered 17 named species based on molecular and morphological data, including C. astrum Grismer et al., 2012, C. australotitiwangsaensis Grismer et al., 2012, C. bintangrendah Grismer et al., 2012, C. bintangtinggi Grismer et al., 2012, C. dayangbuntingensis Quah et al., 2019, C. evanquahi Wood et al., 2020, C. hidupselamanya Grismer et al., 2016, C. jelawangensis Grismer et al., 2014, C. langkawiensis Grismer et al., 2012, C. lekaguli Grismer et al., 2012, C. lenggongensis Grismer et al., 2016, C. macrotubercu*latus, C. pulchellus* Gray, 1827, *C. phuketensis* Sumontha et al., 2012 (only morphological data provided), C. sharkari Grismer et al., 2014, C. timur Grismer et al., 2014, and C. trilatofasciatus Grismer et al., 2012. Among this complex group, C. macrotuberculatus and C. phuketensis showed minor morphological differences based on the original description (Sumontha et al. 2012) but no genetic data were provided for elucidating their phylogenetic placement within the *C. pulchellus* group.

*Cyrtodactylus phuketensis* was described as a new species from Ban Bangrong, Thalang District, Phuket Province by Sumontha et al. (2012). It was similar to *C. macrotuberculatus* in having tuberculation on ventral surface of the forelimbs, gular region and ventrolateral folds, and relatively larger ventral scales (compared to other species in *C. pulchellus* complex). In the original description, *C. phuketensis* was separated from *C. macrotuberculatus* by having three dark bands between limb insertions, 19 subdigital lamellae on the 4<sup>th</sup> toe, the presence of a precloacal groove in both sexes, and eight dark caudal bands on an original tail.

During our field surveys, nine specimens of *C. phuketensis* were collected from the type locality and nearby areas and we found variation in the number of body bands and overlap in the ranges of putatively diagnostic meristic characters when compared

to *C. macrotuberculatus*. Therefore, this study aims to reassess the taxonomic status of *C. macrotuberculatus* and *C. phuketensis* using morphological and genetic data from the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and flanking tRNAs. The analyses were performed on newly collected specimens from southern Thailand and from the type specimens of both species.

## Materials and methods

#### Specimen sampling

During October 2017 and June 2019, field surveys were conducted at five localities in southern Thailand, including the type locality of *C. phuketensis* (Fig. 1; Table 1). Specimens were investigated and captured by hand during the night (1900–2300). Liver or muscle tissues were individually preserved in 95% ethyl alcohol and stored at -20 °C for molecular analysis. Specimens were fixed in 10% formalin and later transferred to 70% ethyl alcohol. Preserved specimens were deposited in the herpetological collections of the Zoological Museum, Kasetsart University, Thailand (**ZMKU**). Additional specimens were also examined from the Princess Maha Chakri Sirindhorn Natural History Museum (**PSU**), Prince of Songkhla University, Thailand; Thailand Natural History Museum (**THNHM**), Thailand; La Sierra University Herpetological Collection (**LSUHC**), La Sierra University, Riverside, California, USA; and the Zoological Reference Collection (**ZRC**) of Lee Kong Chian Natural History Museum at National University of Singapore.

## Molecular analyses

Total genomic DNA was extracted from 95% ethanol-preserved muscle or liver tissue using a NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Germany). Mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and flanking tRNAs were amplified via double-stand Polymerase Chain Reaction (PCR) using primers L4437a (tRNAmet: 5' AAGCTTTCGGGGCCCATACC 3') and H5934 (COI: 5' AGRGT-GCCAATGTCTTTGTGRTT 3') (Macey et al. 1997). PCR amplification occurred with an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 48-52 °C for 30 sec, extension at 72 °C for 1 min 30 sec, and final extension at 72 °C for 7 min. Amplification products were purified using NucleoSpin Gel and PCR Clean-Up kit (Macherey-Nagel GmbH & Co. KG, Germany) and visualized on 1.0% agarose gel electrophoresis. Purified PCR products were sequenced in both directions using amplifying primers on an ABI 3730XL DNA Sequencer (Applied Biosystems, CA, USA). Sequences were manually edited and aligned in Geneious R11 (Biomatters, Ltd, Auckland, New Zealand). The ND2 nucleotide sequences were translated to amino acid for the protein-coding region and to ensure the lack of stop codons. All sequences were deposited in GenBank under the accession numbers MW809294 to MW809309 (Table 1).



**Figure 1.** Map illustrating the known geographic distribution of *Cyrtodactylus macrotuberculatus* and *C. phuketensis*. Yellow star: the type locality of *C. macrotuberculatus* at Gunung Raya, Pulau Langkawi, Kedah, Malaysia. Green star: the type locality of *C. phuketensis* at Thalang District, Phuket Island, Phuket Province. Yellow circles: *C. macrotuberculatus* samples used in this study. Green circle: *C. phuketensis* samples used in this study. Yellow squares: the distribution of *C. macrotuberculatus* taken from Grismer et al. (2012), and Quah et al. (2019). The samples used correspond to those in Table 1.

Phylogenetic relationships were inferred using two model based approaches, Bayesian Inference (BI) and Maximum Likelihood (ML). Outgroup species used to root the tree were Hemidactylus frenatus, Agamura persica, Tropiocolotes steudneri, C. elok, C. intermedius, C. interdigitalis, and Cyrtodactylus sp. based on Wood et al. (2012). The best-fit nucleotide substitution model for each of the three codon partitions and tRNAs was selected under the Bayesian Information Criterion (BIC) in PartitionFinder2 on XSEDE (Lanfear et al. 2016) using CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010). BI analysis was executed in MrBayes 3.2.6 on XSEDE (Ronquist et al. 2012) using CIPRES with the TRN+I+G for the 1st and 2nd codon position, and TIM+I+G for the 3<sup>rd</sup> codon position and tRNAs. Four chains (three hot and one cold) were run for 10,000,000 generations and sampled every 1,000 generations using Markov chain Monte Carlo (MCMC). To build a consensus tree, we discarded the first 25% of each run as burn-in and assessed stationarity by plotting log-likelihood score in Tracer ver. 1.7.1. (Rambaut et al. 2018). The ML analysis was performed on the web server W-IQ-TREE (Trifinopoulos et al. 2016) with 1,000 bootstrap replicates using ultrafast bootstrap approximation (Minh et al. 2013). Nodes having Bayesian posterior probabilities (BPP) of  $\ge 0.95$  and ultrafast bootstrap support (UFB) of  $\ge 95$  were considered to be strongly supported (Huelsenbeck and Ronquist 2001; Wilcox et al. 2002; Minh et al. 2013). Uncorrected pairwise sequence divergence was calculated to assess within and among species differences using the default settings in MEGA X 10.0.5 (Kumar et al. 2018).

# Morphological measurements

Morphological and meristic characters were modified from the previous studies of Grismer and Ahmad (2008) and Grismer et al. (2012). Measurements were taken with digital calipers to the nearest 0.1 mm for the following sixteen characters:

- SVL snout-vent length, taken from the tip of snout to the vent;
- **TW** tail width, taken at the base of the tail immediately posterior to the postcloacal swelling;
- TL tail length, taken from vent to the tip of the tail, original or regenerated;
- **FL** forearm length, taken from the posterior margin of the elbow while flexed 90° to the inflection of the flexed wrist;
- **TBL** tibia length, taken from the posterior surface of the knee while flexed 90° to the base of the heel;
- **AG** axilla to groin length, taken from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body;
- **HL** head length, the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout;
- **HW** head width, measured at the angle of the jaws;
- HD head depth, the maximum height of head from the occiput to the throat;
- ED eye diameter, the greatest horizontal diameter of the eye-ball;
- **EE** eye to ear distance, measured from the anterior edge of the ear opening to the posterior edge of the eye-ball;
- **ES** eye to snout distance, measured from anterior most margin of the eye-ball to the tip of snout;
- **EN** eye to nostril distance, measured from the anterior margin of the eye-ball to the posterior margin of the external nares;
- **IO** inter orbital distance, measured between the anterior edges of the orbit;
- EL ear length, the greatest vertical distance of the ear opening;
- IN internarial distance, measured between the nares across the rostrum.

Additional scale counts and non-meristic characters evaluated were the number of supralabial and infralabial scales counted from the largest scale immediately posterior to the dorsal inflection of the posterior portion of the upper jaw to the rostral and mental scales, respectively; the number of paravertebral tubercles between limb insertions counted in a straight line immediately left of the vertebral column; the number of longitudinal rows of body tubercles counted transversely across the center of the dorsum from one ventrolateral fold to the other; the number of longitudinal rows of ventral scales counted transversely across the center of the dorsum from one ventrolateral fold to the other; the number of longitudinal rows of ventral scales the other; the presence or absence of tubercles on the ventral surface of the forearm; the presence or absence of tubercles in the gular region, throat, and lateral margins of the abdomen; the number of subdigital lamellae beneath the fourth toe counted from the base of the first phalanx to the claw; the total number of precloacal and femoral pores (i.e., the contiguous rows of femoral and precloacal scales bearing pores combined as a single meristic referred to as the femoroprecloacal pores); the presence or absence of a precloacal depression or groove; the degree of body tuberculation, weak tuberculation referring to dorsal body tubercles that are low and rounded whereas prominent tuberculation refers to tubercles that are raised and keeled; the width of the dark body bands relative to the width of the interspace between the bands; number of dark caudal bands on the original tail; the white caudal bands of adults immaculate or infused with dark pigment; and whether or not the posterior portion of the original tail in hatchlings and juveniles less than 50 mm SVL was white or whitish and faintly banded or boldly banded.

#### Morphological analyses

All statistical analyses were performed using the base statistical software in RStudio v. 1.2.1335 (RStudio Team 2018). To remove potential effects of allometry, mensural characters were scaled to SVL using the following allometric equation:  $X_{adj} = X-\beta(SVL-SVL_{mean})$ , where  $X_{adj} =$  adjusted value; X = measured value;  $\beta$  = unstandardized regression coefficient for each OTU; SVL = measured snout-vent length; SVL<sub>mean</sub> = overall average SVL of each OTU (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000). Male and female measurements were analyzed separately to remove potential effects of sexual dimorphism. For morphological analyses, TL (tail length) was excluded due to their different conditions (e.g., original, broken, and regenerated). Importantly, the following type material and topotypic specimens were included in the analysis: *C. macrotuberculatus* (holotype and three paratypes) and *C. phuketensis* (holotype, paratype and three topotypes). Prior to the morphological analyses, individuals were assigned on the basis of molecular data except *C. phuketensis* based on its distribution into three groups (= species): *C. macrotuberculatus*, *C. phuketensis*, and *C. pulchellus*.

Principal component analysis (PCA) was implemented in the R package Facto-MineR (Lê et al. 2008) to discover or reduce dimensionality of the original character variables in order to recover characters bearing the highest degree of variation among groups. Fifteen scaled morphometric and seven meristic characters (scalations) were concatenated and used for the PCA analyses separately by sex. For females, femoroprecloacal pore counts were excluded from the PCA due to their presence in only males.

For univariate analyses, all transformed mensural characters were tested for normality using the Shapiro-Wilk Test. Equality of variances was tested using F-tests. Morphological differences of both males and females between *C. macrotuberculatus* and *C. phuketensis* were examined using a *t*-test (for normally distributed and equal variance data), Welch's *t*-test (for unequal variance data) and Mann-Whitney U test (for non-normally distributed data) at a significant level of 95%.

## Results

#### Phylogenetic relationships

The aligned matrix contained 1,453 bp of ND2 gene and its flanking tRNAs for 101 samples of the *C. pulchellus* complex including outgroups (Table 1). The standard deviation of split frequencies among the two Bayesian runs was 0.003263, and the Estimated Sample Size (ESS) of all parameters were  $\geq 200$ . The BI and ML analyses generated similar topologies and strong nodal support for most clades, and only the ML tree is shown (Fig. 2). According to phylogenetic analyses, *C. phuketensis* is nested within *C. macrotuberculatus* with strong support (1.00 BPP, 100 UFB), thus rendering *C. macrotuberculatus* paraphyletic. *Cyrtodactylus macrotuberculatus* (including *C. phuketensis*) was recovered as sister lineage to a clade containing *C. pulchellus* and *C. evanquahi*. Uncorrected pairwise sequence divergence (*p*-distance) between *C. phuketensis* and this sister lineage was higher than 8.45% and within the *C. phuketensis* and *C. macrotuberculatus* clade, it ranged from 1.45–4.20% (mean 2.63%; Table 2). The *p*-distance within species ranged from 0.00–0.36% (mean 0.14%) for *C. phuketensis* and 0.00–4.38% (mean 2.48%) for *C. macrotuberculatus*.

## Morphology

A total of 45 preserved specimens from three species groups (*C. macrotuberculatus* = 29, *C. phuketensis* = 10, and *C. pulchellus* = 6) were used for principal component analysis (Table 3). The PCA of males showed complete overlap between *C. macrotuberculatus* and *C. phuketensis*, and they were completely separated from *C. pulchellus* along the first two principal components (Fig. 3A). The first three principal component (PC1) accounted for 53.17% of the variation. The first principal component (PC1) accounted for 25.78% of the variation and was most heavily loaded on  $HL_{adj}$ ,  $ES_{adj}$ ,  $EN_{adj}$  and ventral scales; the PC2 accounted for 17.56% of the variation and was most heavily loaded on  $TBL_{adj}$ ,  $IO_{adj}$ , supralabial and infralabial scales; and the PC3 accounted for 9.83% of the variation and was loaded most heavily on longitudinal tubercles (Table 3).

Along the first two PC plots, the PCA of females revealed complete overlap between *C. macrotuberculatus* and *C. phuketensis*, which were distinctly separated from *C. pulchellus* (Fig. 3B). The first three principal components of females accounted for 52.99% of the variation. The first principal component (PC1) accounted for 23.14% of the variation and was most heavily loaded on TW<sub>adj</sub>, AG<sub>adj</sub>, ES<sub>adj</sub>, EN<sub>adj</sub>, ventral scales and number of the 4<sup>th</sup> toe lamellae; the PC2 accounted for 16.59% of the variation and was most heavily loaded on HL<sub>adj</sub>, supralabial and infralabial scales; the PC3 accounted for 13.26% of the variation and was loaded most heavily on TBL<sub>adj</sub> and EL<sub>adj</sub> (Table 3).

Summary univariate statistics of morphological characters of adult males and females are shown in Table 4. In adult males, *C. macrotuberculatus* (N = 18) and *C. phuketensis* (N = 6) were not significantly different in most morphological characters (*t*-tests

Table	I. Specimens	of Cyrtoda	<i>ictylus</i> use	d in (A	A) mo	lecular	and/or	(B)	morphol	ogical	analyse	es in	this
study.	WM = West M	alaysia; TH	H = Thaila	nd.									

Species	Locality	Museum No.	GenBank Accession No	Type of	Reference
Hemidactylus frenatus	Unknow	NC 00155	IX519468	A	Grismer et al. (2012)
Agamura persica	Pakistan, Baluchistan Province, Makran	FMNH 247474	JX440515	A	Grismer et al. (2012) Grismer et al. (2012)
$T \rightarrow 1 + 1 + 1 + 1$	District, Gwadar	ID 20	IV//0520		C
Tropiocolotes steuaneri	WAAD LEE CONTRACT CONTRACT	JD 28	JA440520	A	Grismer et al. (2012)
Cyrtoaactylus elok	TLL CL L .: D .: KL KL: L L	LSUHC 04/1	JQ889180	A	Grismer et al. $(2012)$
C. intermedius	I H, Chantaburi Province, Khao Khitchakut District	LSUHC 9513	JX519469	А	Grismer et al. (2012)
	TH, Chantaburi Province, Khao Khitchakut District	LSUHC 9514	JX519470	А	Grismer et al. (2012)
	Laos, Khammouan Province, Nakai District	FMNH 255454	JQ889181	А	Grismer et al. (2012)
Cyrtodactylus sp.	TH, Loei, Phu Rua	FMNH 265806	JX519471	А	Grismer et al. (2012)
C. astrum	WM, Perlis, Gua Kelam	LSUHC 8806	JX519481	А	Grismer et al. (2012)
	WM, Perlis, Gua Kelam	LSUHC 8808	JX519479	А	Grismer et al. (2012)
	WM, Perlis, Kuala Perlis	LSUHC 8815	JX519482	А	Grismer et al. (2012)
	WM, Perlis, Kuala Perlis	LSUHC 8816 (paratype)	JX519483	А	Grismer et al. (2012)
C. australotitiwangsaensis	WM, Pahang, Genting Highlands	LSUHC 6637 (holotype)	JX519484	А	Grismer et al. (2012)
0	WM, Pahang, Fraser's Hill	LSUHC 8086	IX519486	А	Grismer et al. (2012)
	WM, Pahang, Fraser's Hill	LSUHC 8087	IX519485	А	Grismer et al. (2012)
C. hintangrendah	WM. Kedah. Bukit Palang	LSUHC 9984	IX519487	A	Grismer et al. (2012)
Ci Unitangi Citatan	WM Kedah Bukit Mertaiam	LSUHC 10331 (paratype)	MN125076	A	Quah et al. (2012)
	WM Kedah Bukit Mertajam	I SUHC 10519	MN125077	A	Qualitet al. (2019) Qualitet al. (2019)
	WM Kedah Bukit Mertajam	I SUHC 10520 (paratype)	MN125078	A	Quali et al. (2019) Quali et al. (2019)
C hintanatinari	WM Daral: Bulit Larut		IV510/02	Δ	Criemer et al. (2012)
C. binungiinggi	W/M Dearly Parlain Lanut	LSUIIC 8802	JX510404	л л	Grismer et al. (2012)
C. Januar Januaria	WM Vadah Davana Bundan Jaland	LSOTIC 9000 (paratype)	JAJ19494	л л	Ouch $at al (2012)$
C. aayangbunungensis	WM, Kedan, Dayang Bunung Island	LSURC 14333	MIN123090	A	Qualitiet al. $(2019)$
	WM, Kedan, Dayang bunting Island	LSUHC 14554	MIN125091	A	Quan et al. $(2019)$
C 1:	WM, Kedah, Dayang Bunting Island	LSUHC 14355	MIN125092	A	Quah et al. (2019)
C. evanquam	WM, Kedah, Gunung Baling	BYU 53435 (holotype)	MIN586889	A	Wood et al. (2020)
	WM, Kedah, Gunung Baling	BYU 53436 (paratype)	MN586890	A	Wood et al. (2020)
	WM, Kedah, Gunung Baling	BYU 53437 (paratype)	MN586891	A	Wood et al. (2020)
C. hidupselamanya	WM, Kelantan, Felda Chiku 7	LSUHC 12158 (paratype)	KX011412	A	Grismer et al. (2016)
	WM, Kelantan, Felda Chiku 7	LSUHC 12160 (paratype)	KX011414	A	Grismer et al. (2016)
	WM, Kelantan, Felda Chiku 7	LSUHC 12161 (paratype)	KX011415	А	Grismer et al. (2016)
	WM, Kelantan, Felda Chiku 7	LSUHC 12162 (paratype)	KX011416	А	Grismer et al. (2016)
	WM, Kelantan, Felda Chiku 7	LSUHC 12163 (holotype)	KX011417	А	Grismer et al. (2016)
	WM, Kelantan, Felda Chiku 7	LSUHC 12173 (paratype)	KX011420	А	Grismer et al. (2016)
C. jelawangensis	WM, Gunung Stong, Kelantan	LSUHC 11060 (paratype)	KJ659850	А	Grismer et al. (2014)
	WM, Kelantan, Gunung Stong	LSUHC 11061 (paratype)	KJ659851	А	Grismer et al. (2014)
	WM, Gunung Stong, Kelantan	LSUHC 11062 (holotype)	KJ659852	А	Grismer et al. (2014)
C. langkawiensis	WM, Kedah, Pulau Langkawi, Wat Wanaram	LSUHC 9123 (paratype)	JX519500	А	Grismer et al. (2012)
	WM, Kedah, Pulau Langkawi, Wat Wanaram	LSUHC 9124 (paratype)	JX519499	А	Grismer et al. (2012)
	WM, Kedah, Pulau Langkawi, Wat Wanaram	LSUHC 9125	JX519496	А	Grismer et al. (2012)
	WM, Kedah, Pulau Langkawi, Wat Wanaram	LSUHC 9435	JX519495	А	Grismer et al. (2012)
C. lekaguli	TH, Phang-nga Province, Takua Pa District	ZMKU 00720	KX011425	А	Grismer et al. (2016)
	TH, Phang-nga Province, Takua Pa District	ZMKU 00721	KX011426	А	Grismer et al. (2016)
	TH, Phang-nga Province, Takua Pa District	ZMKU 00722	KX011427	А	Grismer et al. (2016)
	TH, Phang-nga Province, Takua Pa District	ZMKU 00723	KX011428	А	Grismer et al. (2016)
C. lenggongensis	WM, Perak, Lenggong Valley	LSUHC 9974 (holotype)	IX519490	А	Grismer et al. (2012)
00 0	WM, Perak, Lenggong Valley	LSUHC 9975 (paratype)	IX519488	А	Grismer et al. (2012)
	WM, Perak, Lenggong Valley	LSUHC 9976 (paratype)	IX519489	А	Grismer et al. (2012)
	WM. Perak, Lenggong Valley	LSUHC 9977 (paratype)	IX519491	A	Grismer et al. (2012)
C. macrotuberculatus	WM, Kedah, Kuala Nerano	BYU 51869	MN125085	A	Ouah et al. (2019)
	WM Kedah Kuala Nerang	BYU 51870	MN125086	A	Quah et al. (2019)
	WM Kedah Gunung Jerai	LSUHC 5939	IX519513	A	Grismer et al. (2012)
	WM Kedah Cupupg Jerai	LSUHC 5999	IX519512	Δ	Grismer et al. (2012)
	WM Kedah Cumung Jorai	LSUIIC 5555	IV51051/	A	Crismer et al. $(2012)$
	WM Kedah Pulay Landrawi Lubul	I SUHC 6920	IV510505	A	Grismer et al. $(2012)$
	Sembilang		JAJ19303	A	Grisinei et al. (2012)
	WM, Kedah, Pulau Langkawi, Gunung Machinchang	LSUHC 7560	JX519503	А	Grismer et al. (2012)

Species	Locality	Museum No.	GenBank Accession No.	Type of analysis	Reference
C. macrotuberculatus	WM, Kedah, Pulau Langkawi, Gunung Raya	LSUHC 9428	JX519506	А, В	Grismer et al. (2012), This study
	WM, Kedah, Pulau Langkawi, Gunung Rava	LSUHC 9429	_	В	This study
	WM, Kedah, Pulau Langkawi, Gunung Rava	LSUHC 9432	_	В	This study
	WM, Kedah, Pulau Langkawi, Gunung Machinchang	LSUHC 9448	JX519507	А	Grismer et al. (2012)
	WM, Kedah, Pulau Langkawi, Gunung Machinchang	LSUHC 9449	JX519509	А	Grismer et al. (2012)
	WM. Kedah. Hutan Lipur Sungai Tupah	LSUHC 9671	IX519510	А	Grismer et al. (2012)
	WM, Kedah, Hutan Lipur Sungai Tupah	LSUHC 9672	IX519511	А	Grismer et al. (2012)
	WM Kedah Hutan Lipur Sungai Tupah	LSUHC 9693	IX519517	A	Grismer et al. (2012)
	WM Perlis Perlis State Park	LSUHC 9980	IX519515	A	Grismer et al. (2012)
	W/M Dealie Dealie State Faik	LSUIIC 0081	JX510516	A D	Crimer et al. (2012)
	Why relis, relis state raik	1.51110 10027	JX510510	л, D	(2012), This study
	WIM, Perlis, Bukit Chabang	LSUHC 1003/	JA519519	A	Grismer et al. (2012)
	WM, Perlis, Bukit Chabang	LSUHC 10038	JX519518	A	Grismer et al. (2012)
	WM, Perlis, Perlis State Park	LSUHC 1006/	-	В	This study
	WM, Kedah, Bukit Wang	LSUHC 10329	MN125088	A	Quah et al. (2019)
	WM, Kedah, Bukit Wang	LSUHC 10330	MN125087	А	Quah et al. (2019)
	WM, Perlis, Perlis State Park	ZRC 2.4869	-	В	This study
	WM, Kedah, Pulau Langkawi, Gunung Raya	ZRC 2.6754 (holotype)	-	В	This study
	WM, Kedah, Pulau Langkawi, Gunung Raya	ZRC 2.6755 (paratype)	-	В	This study
	WM, Kedah, Pulau Langkawi, Gunung Raya	ZRC 2.6756 (paratype)	-	В	This study
	WM, Kedah, Pulau Langkawi, Telaga Tujuh	ZRC 2.6757/ LSUHC 7173 (paratype)	JX519508	А	Grismer et al. (2012)
	WM, Kedah, Pulau Langkawi, Lubuk Semilang	ZRC 2.6758 (paratype)	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00871	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00872	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00873	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00874	MW809294	А, В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00875	MW809295	А, В	This study
	TH, Songkhla Province, Hat Yai District, Thung Tam Sao	ZMKU R 00876	MW809296	А, В	This study
	TH, Songkhla Province, Hat Yai District, Thung Tam Sao	ZMKU R 00877	MW809297	А, В	This study
	TH, Songkhla Province, Hat Yai District, Thung Tam Sao	ZMKU R 00878	MW809298	А, В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00879	_	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00880	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00881	-	В	This study
	TH, Satun Province, Mueang Satun District, Adang Island	ZMKU R 00882	-	В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00883	MW809299	А, В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00884	-	В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00885	-	В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00886	—	В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00887	MW809300	А, В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00888	_	В	This study
	TH, Satun Province, Mueang Satun District, Rawi Island	ZMKU R 00889	_	В	This study

Species	Locality	Museum No.	GenBank	Type of	Reference
			Accession No.	analysis	
C. macrotuberculatus (as C. phuketensis)	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00890	MW809301	А, В	This study
	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00891	MW809302	А, В	This study
	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00892	MW809303	А, В	This study
	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00893	MW809304	А, В	This study
	TH, Phuket Province, Thalang District, Thep Krasatti	ZMKU R 00894	MW809305	А, В	This study
	TH, Phuket Province, Thalang District, Thep Krasatti	ZMKU R 00895	MW809306	А, В	This study
	TH, Phuket Province, Thalang District, Thep Krasatti	ZMKU R 00896	MW809307	А, В	This study
	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00897	MW809308	А, В	This study
	TH, Phuket Province, Kathu District, Kathu Waterfall	ZMKU R 00898	MW809309	А	This study
	TH, Phuket Province, Thalang District, Thep Krasatti	PSUZC-RT 2010.58	-	В	This study
	TH, Phuket Province, Thalang District, Thep Krasatti	THNHM 15378	-	В	This study
C. pulchellus	WM, Penang, Pulau Pinang, Empangan Air Itam	LSUHC 6668	JX519523	А	Grismer et al. (2012)
	WM, Penang, Pulau Pinang, Moongate Trail	LSUHC 6726	JX519527	А	Grismer et al. (2012)
	WM, Penang, Pulau Pinang, Moongate Trail	LSUHC 6727	JX519526	А, В	Grismer et al. (2012), This study
	WM, Penang, Pulau Pinang, Moongate Trail	LSUHC 6728	JX519525	А, В	Grismer et al. (2012), This study
	WM, Penang, Pulau Pinang, Moongate Trail	LSUHC 6729	JX519528	А, В	Grismer et al. (2012), This study
	WM, Penang, Pulau Pinang, Moongate Trail	LSUHC 6785	JX519524	А	Grismer et al. (2012)
	WM, Penang, Pulau Pinang, Air Terjun Titi Kerawang	LSUHC 9667	JX519520	А	Grismer et al. (2012)
	WM, Penang, Pulau Pinang, Air Terjun Titi Kerawang	LSUHC 9668	JX519521	А	Grismer et al. (2012)
	WM, Penang, Pulau Pinang, Air Terjun Titi Kerawang	LSUHC 10022	JX519522	А, В	Grismer et al. (2012), This study
	WM, Penang, Pulau Pinang	ZRC 2.4854	-	В	This study
	WM, Penang, Pulau Pinang	ZRC 2.4857	-	В	This study
C. sharkari	WM, Pahang, Merapoh, Gua Gunting	LSUHC 11022 (holotype)	KJ659853	А	Grismer et al. (2014)
C. timur	WM, Gunung Tebu, Terengganu	LSUHC 10886	KJ659854	А	Grismer et al. (2014)
	WM, Gunung Tebu, Terengganu	LSUHC 11183 (paratype)	KJ659855	А	Grismer et al. (2014)
	WM, Gunung Tebu, Terengganu	LSUHC 11184 (paratype)	KJ659856	А	Grismer et al. (2014)
	WM, Gunung Tebu, Terengganu	LSUHC 11185 (paratype)	KJ659857	A	Grismer et al. (2014)
C. trilatofasciatus	WM, Pahang, Cameron Highlands	LSUHC 10064	JX519529	A	Grismer et al. (2012)
	WM, Pahang, Cameron Highlands	LSUHC 10065	JX519530	A	Grismer et al. (2012)
	WIM, Pahang, Cameron Highlands	LSUHC 10066	JX519531	A	Grismer et al. (2012)

**Table 2.** Percentage uncorrected pairwise sequence divergence (*p*-distance) for *Cyrtodactylus macrotuberculatus, C. phuketensis*, and closely related species calculated from 1,453 base pairs of the mitochondrial gene ND2 and the flanking tRNAs. Numbers in bold represent the mean and the range of within species *p*-distances.

	Species	N	1	2	3	4
1	C. pulchellus	9	1.02 (0.14-2.20)			
2	C. evanquahi	3	7.42 (6.64-8.38)	0.24 (0.14-0.36)		
3	C. macrotuberculatus	27	8.93 (7.47-10.48)	8.08 (6.64-8.38)	2.48 (0.00-4.38)	
4	C. phuketensis	9	9.41 (8.71-10.29)	8.79 (8.45-8.95)	2.63 (1.45-4.20)	0.14 (0.00-0.36)



**Figure 2.** Reconstructed phylogenetic relationships of the *Cyrtodactylus pulchellus* complex based on 1,453 bp of ND2 and flanking tRNAs. The phylogenetic tree is from the Maximum Likelihood analysis with Bayesian posterior probabilities (BPP) and ultrafast bootstrap support values (UFB), respectively. Black circles represent nodes supported by BPP and UFB of 1.0 and 100. Samples in bold are new sequence from this study.

Characters		Males			Females	
-	PC1	PC2	PC3	PC1	PC2	PC3
SVL <sub>adi</sub>	0.067	-0.193	-0.291	0.080	-0.043	-0.047
TW <sub>adi</sub>	0.558	0.589	-0.029	-0.823	0.036	0.055
FL <sub>adi</sub>	0.586	-0.425	0.023	-0.040	0.507	-0.474
TBL	0.467	-0.690	0.027	-0.055	0.452	-0.752
AG <sub>adi</sub>	-0.185	-0.442	0.415	0.685	-0.284	-0.283
HL <sub>adj</sub>	0.784	0.141	-0.033	0.384	0.730	0.436
HW <sub>adj</sub>	0.439	0.569	-0.144	0.232	0.405	-0.163
HD <sub>adi</sub>	0.577	0.539	-0.221	0.516	-0.070	0.040
ED <sub>adi</sub>	0.585	-0.173	0.374	-0.364	0.777	0.337
EE <sub>adi</sub>	0.484	0.314	-0.580	0.581	0.338	-0.056
ES <sub>adi</sub>	0.858	-0.053	-0.017	0.669	0.260	0.455
EN <sub>adi</sub>	0.774	-0.289	0.004	0.726	-0.087	0.133
IO <sub>adj</sub>	0.147	0.662	0.003	0.602	0.117	0.174
EL <sub>adj</sub>	0.351	-0.097	-0.360	0.141	0.190	0.714
IN <sub>adj</sub>	0.527	0.224	-0.184	-0.216	0.150	-0.473
Supralabials	-0.217	0.622	0.270	-0.070	-0.745	0.307
Infralabials	0.218	0.627	0.402	-0.258	-0.690	0.340
Paravertebral tubercles	-0.157	0.472	0.409	-0.287	-0.196	-0.168
Longitudinal tubercles	0.363	0.215	0.642	-0.133	0.215	0.464
Ventral scales	0.761	-0.297	0.338	-0.665	0.497	0.230
4 <sup>th</sup> toe lamellae	0.439	-0.324	-0.217	-0.864	0.095	0.283
Femoroprecloacal pores	0.512	-0.196	0.468	/	/	/
Eigenvalue	5.671	3.864	2.164	4.859	3.484	2.784
Percentage of variance	25.776	17.562	9.834	23.136	16.592	13.258
Cumulative proportion	25.776	43.338	53.172	23.136	39.728	52.986

**Table 3.** Summary statistics and factor loadings of the principal component analysis from morphological characters for males and females *Cyrtodactylus macrotuberculatus, C. phuketensis*, and *C. pulchellus*. Morphological character abbreviations are defined in the Materials and methods. / = data unavailable.

and Mann-Whitney *U* tests, p = 0.1765-0.9523) except only IO<sub>adj</sub> (*t*-test, p = 0.0256). In adult females, *C. macrotuberculatus* (N = 11) and *C. phuketensis* (N = 4) were not significantly different in twelve morphological characters (*t*-tests and Welch's *t*-test, p = 0.2325-0.9626) whereas only three characters were significantly different which are TBL<sub>adj</sub> (*t*-test, p = 0.0495), AG<sub>adj</sub> (Mann-Whitney U tests, p = 0.0176) and IN<sub>adj</sub> (Welch's *t*-test, p = 0.0129).

Sumontha et al. (2012) distinguished *C. phuketensis* from *C. macrotuberculatus* by using the number of subdigital lamellae on the fourth toe, number of dark body bands, and the presence of a precloacal groove in females. Based on examination of the type material and newly collected specimens of *C. phuketensis* from Phuket Island, these diagnostic characters overlap with those characters of *C. macrotuberculatus*. In this study, *C. phuketensis* has 19–21 total subdigital lamellae on the fourth toe (vs. 19–23 in *C. macrotuberculatus*); three or four dark body bands (vs. three or four in *C. macrotuberculatus*), and no precloacal groove in females (also absent in *C. macrotuberculatus*; Table 5).



**Figure 3.** Plots for the first two principal components of morphological characters from **A** males, and **B** females resulting from the principal component analyses of *Cyrtodactylus macrotuberculatus* (yellow circles), *C. phuketensis* (green squares) and *C. pulchellus* (red diamonds). The letters in the scatter plots refer to holotype (= H), paratype (= P) and topotype (= T).

Characters	cters Males Females							
	C. macrotuberculatus	C. phuketensis	t-test	р	C. macrotuberculatus	C. phuketensis	t-test	р
	N = 18	N=6			N = 11	N=4		
SVL	$105.8 \pm 8.9$	$105.3 \pm 10.0$	0.112	0.9122	$103.7 \pm 10.1$	$104.4 \pm 16.0$	-0.098	0.9237
	(88.9-117.9)	(93.2-115.3)			(84.1-115.7)	(84.8-117.6)		
TW	$9.5 \pm 0.9$	$9.6 \pm 1.2$	-0.061	0.9523	$8.1 \pm 1.4$	$8.2 \pm 2.1$	-0.352	0.7307
	(8.0-11.6)	(7.9-10.9)			(5.9–10.3)	(6.2–10.5)		
FL	$17.2 \pm 1.5$	$17.2 \pm 2.0$	-0.098	0.9229	$16.8 \pm 1.6$	$16.8\pm2.7$	0.048	0.9626
	(14.2-18.9)	(14.4-19.0)			(13.6-18.3)	(13.3-19.3)		
TBL	$20.3 \pm 1.6$	$20.6\pm2.6$	-0.631	0.5348	$19.9 \pm 1.8$	$20.4 \pm 3.2$	-2.166	0.0495*
	(17.3-22.8)	(17.4–19.0)			(16.3-21.9)	(16.5-23.2)		
AG	$50.4 \pm 4.6$	$51.5 \pm 5.9$	-1.034	0.3125	$50.7 \pm 4.5$	$53.4 \pm 7.9$	4 <sup>b</sup>	0.0176*
	(41.3-58.6)	(44.7-57.4)			(42.0-56.0)	(43.4-60.2)		
HL	$29.4 \pm 2.2$	$29.2\pm2.7$	0.590	0.5613	$28.5 \pm 3.1$	$28.4\pm4.4$	0.575ª	0.5758
	(24.6-33.3)	(25.6-31.7)			(22.8-32.3)	(23.1-32.1)		
HW	$20.0 \pm 1.8$	$19.8\pm2.7$	0.416	0.6816	$19.0 \pm 1.9$	$18.7\pm2.6$	0.815	0.4297
	(16.7-22.9)	(16.3-22.5)			(15.6-21.0)	(15.8-21.4)		
HD	$12.0 \pm 1.3$	$12.2\pm1.9$	-0.840	0.4100	$11.3 \pm 1.3$	$11.5 \pm 2.0$	-1.054	0.3109
	(9.7-14.1)	(9.9-14.2)			(9.0-13.4)	(9.1–13.5)		
ED	$6.9 \pm 0.6$	$6.7\pm0.5$	1.397	0.1765	$6.7 \pm 0.7$	$6.5 \pm 1.1$	1.253	0.2325
	(5.8–7.9)	(5.6-7.0)			(5.6–7.6)	(7.4–9.6)		
EE	$8.6 \pm 1.0$	$8.6 \pm 1.0$	62 <sup>b</sup>	0.6261	$8.5 \pm 0.8$	$8.6 \pm 1.1$	-0.106	0.9171
	(6.5–9.8)	(7.1 - 9.4)			(7.0-9.4)	(7.4–9.6)		
ES	$11.7 \pm 1.0$	$11.7 \pm 1.1$	56 <sup>b</sup>	0.9225	$11.5 \pm 1.2$	$11.4\pm1.8$	0.271ª	0.7912
	(10.0-13.6)	(10.3-12.9)			(9.3-13.1)	(9.2-12.9)		
EN	$8.7 \pm 0.7$	$8.7 \pm 0.7$	0.090	0.9288	$8.6 \pm 0.9$	$8.5 \pm 1.3$	0.610	0.5521
	(7.3–9.8)	(7.6–9.5)			(6.8–9.8)	(6.8–9.6)		
IO	$5.1 \pm 0.6$	$4.8\pm0.6$	2.394	0.0256*	$4.8 \pm 0.8$	$4.6 \pm 0.9$	0.835	0.4186
	(4.0-6.3)	(4.1-5.5)			(3.4–5.7)	(3.6-5.5)		
EL	$2.3\pm0.3$	$2.2\pm0.4$	0.504	0.6192	$2.4 \pm 0.4$	$2.2\pm0.5$	1.125	0.281
	(1.7-2.8)	(1.4-2.6)			(1.6-3.0)	(1.5-2.5)		
IN	$2.2\pm0.3$	$2.3\pm0.3$	-0.352	0.7282	$2.0 \pm 0.5$	$2.3\pm0.3$	-1.785ª	0.0129*
	(1.7 - 2.7)	(1.7 - 2.5)			(1.3 - 2.9)	(1.9 - 2.6)		

**Table 4.** Comparisons of fifteen morphological characters between *Cyrtodactylus macrotuberculatus* and *C. phuketensis*. Data are given as mean and standard deviation, followed by range in parentheses. Morphological character abbreviations are defined in the Materials and methods. Key: <sup>a</sup> tested by Welch's *t*-test, <sup>b</sup> tested by Mann-Whitney U test, \* significance level at p < 0.05.

# **Systematics**

The phylogenetic analyses recovered *C. phuketensis* as being nested within the *C. macrotuberculatus* and bearing a low genetic divergence (mean 2.63%) which was similar to that within *C. macrotuberculatus* populations (mean 2.48%). In concordance, the statistical analyses of meristic and mensural characters of *C. phuketensis* widely overlap with those of *C. macrotuberculatus*. Based on these data, we propose that *C. phuketensis* from Phuket Island, Phuket Province is a junior synonym of *C. macrotuberculatus* which can be recognized as follows.

# Taxonomy

## *Cyrtodactylus macrotuberculatus* Grismer and Ahmad, 2008 Figure 4

*Cyrtodactylus macrotuberculatus* Grismer & Ahmad, 2008: 55; Grismer 2011: 406; Grismer et al. 2012: 45.

Cyrtodactylus phuketensis Sumontha et al., 2012: 62.

	C. macrotuberculatus	C. phuketensis	C. macrotuberculatus	C. phuketensis
	(Grismer and Ahmad, 2008)	(Sumontha et al., 2012)	This study	This study
Supralabials	10-12	12-13	9-12	9–13
Infralabials	8-11	9-10	7-11	7-10
Tuberculation	Prominent	Prominent	Prominent	Prominent
Tubercles on ventral surface of forelimbs	Yes	Yes	Yes	Yes
Tubercles in gular region	Yes	Yes	Yes	Yes
Ventrolateral fold tuberculate	Yes	Yes	Yes	Yes
Paravertebral tubercles	40-47	40-43	34-49	39-45
Longitudinal rows of tubercles	22-26	23-24	19-27	20-24
Ventral scales	19-22	22-24	17-28	20-24
4 <sup>th</sup> toe lamellae	21-23	19	19-23	19-21
Femoroprecloacal pores	35-37	33-36	28-42	31-33
Precloacal groove present in females	No	Yes	No	No
Precloacal depression in males	No	No	Deep	Deep
No. of body bands	4	3 (3.5 one individual)	3–4	3–4 (3+1 incomplete band)
Body band/interspace ratio	/	/	0.95-1.74	1.02-1.50
Dorsum bearing scattered pattern of white tubercles	No	No	No	No
Hatchlings/juveniles with white tail tip	No	No	No	No
Dark caudal bands on original tail	/	8	7-10	7-8
White caudal bands in adults immaculate	/	/	No	No
Maximum SVL	120.0	114.7	117.87	117.61
Sample size	5	3	29	10

**Table 5.** Summarized diagnostic characters of *Cyrtodactylus macrotuberculatus* and *C. phuketensis* taken from original descriptions (Grismer and Ahmad 2008; Sumontha et al. 2012) and this study based on type materials and newly additional specimens. / = data unavailable.

**Type specimens.** *Holotype* (adult male, ZRC 2.6754) from Malaysia, Kedah, Pulau Langkawi, Gunung Raya; *Paratypes*: Malaysia, Kedah, Pulau Langkawi, Gunung Raya: ZRC 2.6755–2.6756, Telaga Tujuh: ZRC 2.6757, Lubuk Semilang: ZRC 2.6758.

Additional specimens examined (including types of *C. phuketensis*). MALAYSIA – Kedah, Pulau Langkawi, Gunung Raya: LSUHC 09428–09429, LSUHC 09432; Perlis, Perlis State Park: LSUHC 09981, LSUHC 10097, ZRC 2.4869. THAILAND – Satun Province, Mueang Satun District, Adang Island: ZMKU R 00871–00875, ZMKU R 00879–00882, Rawi Island: ZMKU R 00883–00889; Songkhla Province, Hat Yai District, Chalung Sub-district: ZMKU R 00876–00878; Phuket Province, Thalang District: PSUZC-RT 2010.58, THNHM 15378, ZMKU R 00894–00896, Kathu District: ZMKU R 00890–00893, ZMKU R 00897–00898 (Table 1).

**Expanded diagnosis.** Cyrtodactylus macrotuberculatus can be separated from all other species of *C. pulchellus* complex by having the following combination of characters (Table 6): (1) maximum SVL 117.9 mm (mean 105.0  $\pm$  SD 9.8, N = 39); (2) 9–13 supralabial and 7–11 infralabial scales; (3) prominent tuberculation on body; (4) tubercles on ventral surface of forelimbs, gular region, in ventrolateral body folds, and anterior one-third of tail; (5) 34–49 paravertebral tubercles; (6) 19–27 longitudinal tubercle rows; (7) 17–28 ventral scales; (8) 19–23 subdigital lamellae on the fourth



**Figure 4.** Male holotype of *Cyrtodactylus macrotuberculatus* from Pulau Langkawi, Kedah, Peninsular Malaysia (ZRC 2.6754) in preservative **A** dorsal view **B** ventral view **C** tuberculate gular region and throat, and **D** enlarge femoroprecloacal scale row and pores.

toe; (9) 28–42 femoroprecloacal pores in males; (10) deep precloacal groove in males; (11) three or four dark dorsal body bands; (12) body band wider than interspace; (13) 7–10 (N = 12) ringed dark caudal bands on original tail; (14) white caudal bands

**Table 6.** Morphological measurement (mm), meristic and non-meristic data from males and females of *Cyrtodactylus macrotuberculatus*. Morphological character abbreviations are defined in the Materials and methods.

Characters	Adult males $(N = 24)$		Adult fema	ales (N = 15)	<b>All</b> (N = 39)		
	Mean $\pm$ SD	(Min-Max)	Mean $\pm$ SD	(Min-Max)	Mean $\pm$ SD	(Min-Max)	
SVL	105.7 ± 9.0	(88.9–117.9)	$103.9 \pm 11.3$	(84.1-117.6)	$105.0 \pm 9.8$	(84.1-117.9)	
TW	$9.5 \pm 1.0$	(7.9–11.6)	$8.2 \pm 1.5$	(5.9-10.5)	$9.0 \pm 1.4$	(5.9-11.6)	
FL	$17.2 \pm 1.6$	(14.2–19.0)	$16.8\pm1.8$	(13.3–19.3)	$17.1 \pm 1.7$	(13.3-19.3)	
TBL	$20.4\pm1.9$	(17.3–23.5)	$20.0\pm2.1$	(16.3–19.3)	$20.2\pm2.0$	(16.3-23.5)	
AG	$50.7 \pm 4.9$	(41.3-58.6)	$51.4 \pm 5.4$	(42.0-60.2)	$51.0 \pm 5.0$	(41.3-60.2)	
HL	$29.4\pm2.3$	(24.6-33.3)	$28.5\pm3.3$	(22.8-32.3)	$29.0\pm2.7$	(22.8-33.3)	
HW	$20.0\pm2.0$	(16.3-22.9)	$18.9\pm2.0$	(15.6–21.4)	$19.6\pm2.0$	(15.6-22.9)	
HD	$12.1\pm1.4$	(9.7–14.2)	$11.3\pm1.4$	(9.0-13.5)	$11.8\pm1.4$	(9.0-14.2)	
ED	$6.8\pm0.6$	(5.6–7.9)	$6.7\pm0.8$	(5.2–7.5)	$6.8 \pm 0.7$	(5.2–7.9)	
EE	$8.6\pm1.0$	(6.5–9.8)	$8.5\pm0.9$	(7.0–9.6)	$8.6\pm0.7$	(5.2–7.9)	
ES	$11.7\pm1.0$	(10.0–13.6)	$11.5\pm1.3$	(9.2-13.1)	$11.6\pm1.1$	(9.2–13.6)	
EN	$8.7 \pm 0.7$	(7.3–9.8)	$8.6 \pm 1.0$	(6.8–9.8)	$8.6 \pm 0.8$	(6.8-9.8)	
IO	$5.0 \pm 0.6$	(4.0-6.3)	$4.8\pm0.8$	(3.4–5.7)	$4.8\pm0.7$	(3.4-6.3)	
EL	$2.3 \pm 0.3$	(1.4-2.8)	$2.3 \pm 0.4$	(1.5-3.0)	$2.3 \pm 0.4$	(1.4-3.0)	
IN	$2.2\pm0.5$	(1.7-2.7)	$2.1\pm0.5$	(1.3-2.9)	$2.2\pm0.4$	(1.3-2.9)	
HL/SVL	$0.28\pm0.01$	(0.27-0.30)	$0.27\pm0.01$	(0.26-0.29)	$0.28\pm0.01$	(0.26-0.30)	
HW/HL	$0.68\pm0.03$	(0.62-0.74)	$0.67\pm0.02$	(0.62-0.70)	$0.67\pm0.03$	(0.62 - 0.74)	
HD/HL	$0.41\pm0.02$	(0.37 - 0.45)	$0.40\pm0.01$	(0.38 - 0.42)	$0.41\pm0.02$	(0.37 - 0.45)	
ES/HL	$0.40\pm0.01$	(0.37-0.41)	$0.40\pm0.00$	(0.39-0.41)	$0.40\pm0.01$	(0.37 - 0.41)	
ED/HL	$0.23\pm0.01$	(0.21 - 0.27)	$0.23\pm0.01$	(0.22-0.25)	$0.23\pm0.01$	(0.21-0.27)	
EL/HL	$0.08\pm0.01$	(0.05 - 0.10)	$0.08\pm0.01$	(0.05 - 0.10)	$0.08\pm0.01$	(0.05 - 0.10)	
AG/SVL	$0.48\pm0.02$	(0.43-0.51)	$0.50\pm0.01$	(0.47-0.52)	$0.49\pm0.02$	(0.43-0.52)	
FL/SVL	$0.16\pm0.00$	(0.15-0.17)	$0.16\pm0.00$	(0.16-0.17)	$0.16\pm0.00$	(0.15-0.17)	
TBL/SVL	$0.19\pm0.01$	(0.18-0.21)	$0.19\pm0.00$	(0.18 - 0.20)	$0.19\pm0.01$	(0.18 - 0.21)	
TL/SVL	$1.29\pm0.04$	(1.23–1.35)	$1.27\pm0.04$	(1.24–1.34)	$1.28\pm0.04$	(1.23–1.35)	
Supralabials	9.	-13	9.	-12	9-	-13	
Infralabials	7-	-11	7-	-11	7-	-11	
Tuberculation	Pror	ninent	Pror	ninent	Pron	ninent	
Tubercles on ventral surface of forelimbs		Yes		Yes	Y	les	
Tubercles in gular region		Yes	2	Yes	Y	les	
Ventrolateral fold tuberculate		Yes	2	Yes	Y	les	
Paravertebral tubercles	37	-49	34	-47	34	-49	
Longitudinal rows of tubercles	19	-27	19	-26	19	-27	
Ventral scales	17	-28	19	-26	17	-28	
4 <sup>th</sup> toe lamellae	19	-23	19	-23	19–23		
Femoroprecloacal pores	28	3-42	1	No	28	-42	
Precloacal depression		Yes	1	No	Only	in males	
No. of body bands	3	or 4	3	or 4	3	or 4	
Body band/interspace ratio	0.95	-1.75	1.03	-1.62	0.95	-1.74	
Dark caudal bands on original tail	7	/_9	7-	-10	7-10		

infused with dark pigmentation in adults; (15) posterior portion of tail in hatchlings and juveniles bands not white.

**Description of adult males.** SVL of adult males range from 88.9-117.9 mm (mean 105.7, N = 24); head moderate in length (HL/SVL 0.27–0.30), width (HW/HL 0.62–0.74), somewhat flattened (HD/HL 0.37–0.45), distinct from neck, triangular in dorsal profile; lores concave; frontal and prefrontal regions deeply concave; canthus

rostralis sharply rounded; snout elongate (ES/HL 0.37–0.41), rounded in dorsal profile, laterally constricted; eye large (ED/HL 0.21–0.27); ear opening elliptical, moderate in size (EL/HL 0.05-0.10) obliquely oriented; eve to ear distance greater than diameter of eye; rostral rectangular, divided dorsally by an inverted Y or I-shaped furrow, bordered posteriorly by large left and right supranasals and small internasal, bordered laterally by external nares and first supralabials; external nares bordered anteriorly by rostral, dorsally by one large anterior supranasal, posteriorly by two postnasals, ventrally by first supralabial; 9–13 rectangular supralabials extending to just beyond upturn of labial margin, tapering abruptly below midpoint of eye; 7–11 infralabials not tapering in size posteriorly; scales of rostrum and lores slightly raised, larger than granular scales on top of head and occiput, those on posterior portion of canthus rostralis slightly larger; scales on top of head and occiput intermixed with enlarged tubercles; large, boney frontal ridges bordering orbit confluent with boney, transverse, parietal ridge; dorsal superciliaries elongate, smooth, largest anteriorly; mental triangular, bordered laterally by first infralabials and posteriorly by left and right trapezoidal postmentals that contact medially for 40-50% of their length posterior to mental; single row of slightly enlarged, elongate sublabials extending posteriorly to 5th-7th infralabial; small, granular, gular scales intermixed with numerous large, conical tubercles grading posteriorly into larger, conical tubercles on throat which abruptly transition into large, flat, smooth, imbricate, pectoral and ventral scales.

Body relatively short (AG/SVL 0.43–0.51) with well-defined, tuberculate, ventrolateral folds; dorsal scales small, granular, interspersed with large, trihedral, regularly arranged, keeled tubercles separated by no more than three granules at their base; tubercles extend from top of head onto approximately one-half of tail but not onto regenerated tail; tubercles on occiput and nape relatively small, those on body largest; approximately 19–27 longitudinal rows of dorsal tubercles at the mid body; approximately 37–49 paravertebral tubercles; 17–28 flat, imbricate, ventral scales and much larger than dorsal scales; precloacal scales large, smooth; deep precloacal groove (= depression).

Forelimbs moderate in stature, relatively short (FL/SVL 0.15–0.17); virtually no granular scales on dorsal surface of forelimbs, only large, trihedral, keeled tubercles; palmar scales slightly rounded; digits well-developed, inflected at basal, interphalangeal joints; subdigital lamellae nearly square proximal to joint inflection, only slightly expanded distal to inflection; digits more narrow distal to joints; claws well-developed, sheathed by dorsal and ventral scale; hind limbs more robust than forelimbs, moderate in length (TBL/SVL 0.18–0.21), virtually no granular scales on dorsal surfaces of hind limbs, only large, trihedral, keeled tubercles; ventral scales of thigh flat, smooth, imbricate; ventral, tibial scales flat, imbricate, slightly keeled; two rows of enlarged, flat, imbricate, femoroprecloacal scales extend from knee to knee through precloacal region where they are continuous with enlarged, pore-bearing precloacal scales; 28–42 contiguous, pore-bearing femoroprecloacal scales forming an inverted T bearing a deep, precloacal groove; eight to eleven pores bordering groove; postfemoral scales immediately posterior to the pore-bearing scale row conical, forming an abrupt union
on posteroventral margin of thigh; plantar scales low, slightly rounded; digits welldeveloped, inflected at basal, interphalangeal joints; subdigital lamellae proximal to joint inflection nearly square, only slightly expanded distal to inflection; digits more narrow distal to joints; claws well-developed, sheathed by a dorsal and ventral scale; 19–23 subdigital lamellae on the 4<sup>th</sup> toe.

Original tail (TL/SVL) moderate in proportions, 123-135% of SVL (mean 128, N = 12), 7.9–11.6 mm in width at base, tapering to a point; dorsal scales at base of tail square, smooth, flat, subimbricate, lacking tubercle on regenerated tail; median row of transversely enlarged, subcaudal scales; shallow caudal furrow; two to five small, postcloacal tubercles at base of tail on hemipenial swellings; all postcloacal scales flat, large, imbricate.

**Coloration of adult male ZMKU R 00871 in life (Fig. 5)**. Ground color of head, body, limbs, and dorsum light-brown to yellowish brown; wide, dark-brown nuchal band edged anteriorly and posteriorly by thin, creamy-white lines bearing tubercles extends from posterior margin of one eye to posterior margin of other eye; four similar body bands between nuchal loop and hind limb insertions edged anteriorly and posteriorly by thin, creamy-white lines bearing tubercles, first band terminates at shoulders, second and third bands terminate just dorsal to ventrolateral folds, the fourth band terminates at femurs; dark body bands slightly larger than light-colored interspaces; one additional dark-brown band posterior to hind limbs; original portion of tail bearing eight ringed, dark-colored bands separated by seven, narrower, off-white bands infused with dark pigmentation; ventral surfaces of head smudged with brown; abdomen and limbs beige, slightly darker, lateral regions.

**Coloration in preservative (Fig. 6).** Color pattern of head, body, limbs, and tail similar to that in life with some fading. Ground color of head, body, limbs, and dorsum tan; dark body and dark caudal bands lighter than in life.

**Variation.** *Cyrtodactylus macrotuberculatus* usually varies in coloration and banding pattern (Figs 7–8). In females, a precloacal groove and pores are absent (Fig. 9). PSUZC-RT 2010.58 and THNHM 15378 have a shallow precloacal groove. Three dark body bands occur in PSU 2010.58, THNHM 15378, ZMKU R 00889–00894 and ZMKU R 00897. In ZMKU R 00887, the second dorsal band bifurcates just dorsal to the ventrolateral fold. ZMKU R 00895 has four bands and the third band is incomplete. The third body band in ZMKU R 00896 is broken on the left of the midline and contacts the fourth body band bilaterally. Nuchal loop and body bands of ZMKU R 00883, ZMKU R 00895, and ZMKU R 00898 edged anteriorly and posteriorly by thin, light-yellow lines and tubercles; and dorsal superciliaries are light-yellow (Fig. 8). Variation in morphometric and meristic data are shown in Table 6.

**Distribution.** *Cyrtodactylus macrotuberculatus* is distributed on the mainland and only known from one island in Peninsular Malaysia and southern Thailand (Fig. 1). This species is known from Pulau Langkawi (Gunung Raya, Telaga Tujuh, Gunung Machinchang, and Lubuk Semilang), Kedah, Peninsular Malaysia (Grismer and Ahmad 2008). Other populations are found from Peninsular Malaysia; Kedah (Bukit Wang, Gunung Jerai, Hutan Lipur Sungai Tupah, Kuala Nerang, and Ulu Muda) and



**Figure 5.** Adult male *Cyrtodactylus macrotuberculatus* from Adang Island, Satun Province, Thailand (ZMKU R 00871) in life.

Perlis (Bukit Chabang, Chuping and Perlis State Park; [Grismer 2011; Grismer et al. 2012; Quah et al. 2019]). In Thailand, *C. macrotuberculatus* was recorded from Phatthalung Province (Grismer et al. 2012); Phuket Province, Kathu District (Kathu Waterfall) and Thalang District (Thep Krasatti Sub-district, previously type locality of *C. phuketensis*); Satun Province, La-ngu District, and Mueang Satun District (Adang and Rawi Islands); Songkhla Province, Rattaphum District (Grismer et al. 2012) and Hat Yai District (Ton Nga Chang Waterfall).

**Natural history.** Based on specimens in Thailand, all individuals were found in similar habitat type, lowland forest habitat along granitic rock streams and surrounding areas (elevation 7–186 m asl) during a night survey (1900–2200; Fig. 10). The geckos were found mostly on rock boulders, vegetation (trunk of tree, buttress root, rotting wood and vines), and sometimes on the ground with leaf litter and high humidity (26.3–30.8 °C in temperature, 73.8–100% in relative humidity). Gravid female (ZMKU R 00876) contained four eggs during December. One juvenile (ZMKU R 00898, 56.50 mm in SVL) was found on a tree trunk in January. The varied microhabitats within which this species occurs, are consistent with its characterization as a habitat generalist (Grismer et al. 2020, 2021b) and may account for its wide peninsular and insular distribution relative to other species of the *pulchellus* group whose distributions are much less extensive or site-specific (Grismer et al. 2012, 2014, 2016; Quah et al. 2019; Wood et al. 2020).

In Thailand, *C. macrotuberculatus* were found sympatric with other gecko species, *Cnemaspis adangrawi* Ampai et al., 2019 on Adang and Rawi Islands, Satun Province



**Figure 6.** Adult male *Cyrtodactylus macrotuberculatus* from Adang Island, Satun Province, Thailand (ZMKU R 00871) in preservative **A** dorsal and **B** ventral views.

(Ampai et al. 2019); *Cnemaspis phuketensis* Das and Leong, 2004, *Cyrtodactylus oldhami* Theobald, 1876, and *Gekko (Ptychozoon) tokehos* Grismer et al., 2019 at Kathu and Thalang District, Phuket Province; *G. (P.) tokehos, Cnemaspis kumpoli* Taylor, 1963, and *Gehyra mutilata* (Weigmann, 1834) at Hat Yai District, Songkhla Province.

**Comparison.** *Cyrtodactylus macrotuberculatus* is distinguished from all other 15 species in the *C. pulchellus* complex by a combination of morphological characters (Table 7). It differs from all other species by having prominent tuberculation on the body; tubercles on ventral surface of forelimbs, gular region, and in ventrolateral body folds; 34–49 paravertebral tubercles; 19–27 longitudinal tubercle rows; 17–28 ventral scales;



**Figure 7.** Variation in dorsal body band pattern of *Cyrtodactylus macrotuberculatus* from Thailand. From left to right, upper: ZMKU R 00878, ZMKU R 00873 from Adang Island, Satun Province; and ZMKU R 00887 from Rawi Island, Satun Province. Lower: ZMKU R 00889 from Rawi Island, Satun Province; ZMKU R 00896 and ZMKU R 00895 from Phuket Province.

19–23 subdigital lamellae on the fourth toe; 28–42 femorprecloacal pores in males; deep precloacal groove in males; no scattered white spots on dorsum; 7–10 dark-ringed caudal bands on original tail; white caudal bands on original tail infused with dark pigmentation in adults. Additional comparisons between *C. macrotuberculatus* and other species in *C. pulchellus* complex are in Table 7.



**Figure 8.** Color in life of *Cyrtodactylus macrotuberculatus* from Thailand **A** adult male ZMKU R 00883 from Rawi Island, Satun Province **B** subadult female ZMKU R 00895 from Thalang District, Phuket Province, and **C** juvenile ZMKU R 00898 from Kathu District, Phuket Province.



**Figure 9.** Precloacal region in female specimens of *Cyrtodactylus macrotuberculatus* **A** paratype ZRC 2.6758, from Telaga Tujuh, Pulau Langkawi, Malaysia, and **B** ZMKU R 00896 from Thalang District, Phuket Province, Thailand.

Based on molecular data, *C. macrotuberculatus* is the sister lineage to a clade composed of *C. pulchellus* and *C. evanquahi*. It can be separated from those two species by having tubercles on ventral surface of forelimbs, gular region, and in ventrolateral body folds (*vs.* absent in *C. evanquahi* and *C. pulchellus*); 17–28 ventral scales (*vs.* 29–33 in *C. evanquahi* and 29–34 in *C. pulchellus*); deep precloacal groove in males (*vs.* a shallow in *C. evanquahi*); three or four dark dorsal bands (*vs.* six or seven bands in *C. evanquahi* and only four bands in *C. pulchellus*); white posterior caudal region absent (*vs.* present in *C. evanquahi*); hatchlings and juveniles without white tail tip (*vs.* present in *C. evanquahi*).

#### Discussion

*Cyrtodactylus macrotuberculatus* and *C. phuketensis* are considered to be conspecific with the latter restricted to Phuket Island whereas *C. macrotuberculatus* is found on the Thai-Malay Peninsula and adjacent islands. The distinct characteristics between these two species were based solely on morphological comparisons by Sumontha et al. (2012). Our study provided additional morphology and molecular evidence to reassess the taxonomic status of *C. macrotuberculatus* and *C. phuketensis* from Thai populations and determine that these closely related populations are conspecific. Phylogenetic

**Table 7.** Diagnostic characters of *Cyrtodactylus macrotuberculatus* and related species within *C. pulchellus* complex. W = weak; P = prominent; / = data unavailable. Some information was collected from the following literature (Grismer et al. 2012, 2014, 2016; Quah et al. 2019; Wood et al. 2020).

	australotiti- wangsaensis	bin- tangtinggi	bintan- grendah	evan- quahi	lenggon- gensis	pulchellus	sharkari	trilato- fasciatus	<i>macrotu-</i> <i>berculatus</i> (this study)	astrum	dayangbun- tingensis	hidupse- lamanya	lang- kawiensis	lekaguli	jelawa- ngensis	timur
Supralabials	9-12	9–13	8-12	9 or 10	10 or 11	9–11	11	10-12	9–13	10-12	12–14	9–12	9–12	10-12	9–12	10-12
Infralabials	9–13	8-11	8 or 10	9 or 10	8-10	8-10	10	8-11	7-11	9–12	10-11	8-11	8-10	9–11	9-11	8-10
Tuberculation	Ъ	Р	Ъ	Ъ	M	Ъ	M	Ъ	Ρ	M	M	M	M	M	Ъ	M
Tubercles on ventral surface of forelimbs	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes	No
Tubercles in gular region	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
Ventrolateral fold tuberculate	No	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	°N	No
Paravertebral tubercles	37-45	31-42	36-44	31–34	36-41	33-43	31	34–38	34-49	40-57	35–36	39-48	34-44	30-50	36-42	38-43
Longitudinal rows of tubercles	22–30	21–26	22–25	18–23	22-25	22–26	24	23-27	19–27	20–29	20-22	19–24	21–25	20-24	23–25	21–24
Ventral scales	32-40	36-40	31–39	29–33	32 or 33	29–34	41	33–36	17–28	31-46	36–39	26–33	38-43	31-43	31–36	31-40
4 <sup>th</sup> toe lamellae	21–25	21-24	21-24	22-23	20-23	21–26	24	22-27	19–23	20-24	21–23	19–24	19–21	20-25	21–24	21–25
Femoroprecloacal pores	39-45	37-41	41-46	32–36	39-41	33–39	46	41-46	28-42	31–38	26-29	17-22	30	30–36	36	21 or 22
Precloacal groove in males	Deep	Deep	Deep	Shallow	Deep	Deep	Shallow	Deep	Deep	Deep	Deep	Deep	Deep	Deep	Deep	Deep
No. of body bands	3(1) or 4	3(1)  or  4	4	6 or 7	4 or 5	4	4	3	3-4	4	4	4	4 or 5	4 or 5	4	4
Body band/interspace ratio	1.00-2.00	1.00-1.25	1.00- 1.25	0.82 - 1.10	0.50– 1.25	0.75-1.25	1.75	2.00– 2.75	0.95–1.74	1.00– 2.00	0.75	1.00- 1.25	0.75–1.00	1.00– 2.00	1.00- 1.50	1.00- 1.25
Dorsum bearing scattered pattern of white tubercles	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No	No
Hatchlings/juveniles with white tail tip	No	No	No	Yes	~	No	-	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Adult posterior caudal region white	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	No	No.	No
Dark caudal bands on original tail	7–8	8-10	8 or 9	9-11	14	8-10	~	6-7	7–10	13 or 14	۲<	8-10	11–16	12-14	10	8-10
White caudal bands in adults immaculate	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No	Yes	No	No	No.	Yes
Maximum SVL	120.10	111.1	114.40	96.00	103.1	114.1	100.1	122.2	117.9	108.3	99.00	102.7	99.8	103.5	119.8	120.5
Sample size	12	14	9	3	4	13	-	9	39	=	3	14	10	13	4	5



**Figure 10.** Habitats of *Cyrtodactylus macrotuberculatus* in Thailand **A** Adang Island, Satun Province **B** Kathu District, Phuket Province, and **C** Hat Yai District, Songkhla Province.

analyses from this study are concordant with the phylogenetic studies of Grismer et al. (2012, 2014, 2016), Quah et al. (2019), and Wood et al. (2020). Based on the dataset of ND2 gene and its flanking tRNA, the phylogenetic analyses recovered a clade of *C. macrotuberculatus* – including *C. phuketensis* – as a strongly supported monophyletic group consisting of multiple insular populations. Some substructuring occurs within the *C. macrotuberculatus* which could be the result of limited gene flow among isolated populations (Hurston et al. 2009; Jang et al. 2011) or local adaptation to different selection pressures in widely distributed habitat generalist.

Sumontha et al. (2012) diagnosed *C. phuketensis* by the number of bands between the limb insertions and the presence of a precloacal groove in the female paratype. We re-examined the type series of both species (except female paratype of *C. phuketensis*, QSMI 1170) and newly collected specimens. Variation in the number of bands was found in both species, similar to several species of the *C. pulchellus* group such as *C. bintangrendah*, *C. australotitiwangsaensis* and *C. lenggongensis* (Grismer et al. 2012, 2016).

Within the *C. pulchellus* group, a continuous series of enlarged femoroprecloacal scales forming an inverted T in the precloacal region is present in both sexes; however, the precloacal groove was found only in males. In the present study, the newly collected female specimens from the type locality of *C. phuketensis* had a continuous series of enlarged femoroprecloacal scales but lacked a precloacal groove (or depression) (Fig. 9). Therefore, this character is the same as in *C. macrotuberculatus* and all other species in the *C. pulchellus* group. The presence of a precloacal groove in the female specimen

of *C. phuketensis* examined in Sumontha et al. (2012) was an erroneous observation (fig. 4 in Sumontha et al. 2012). The absence of a precloacal depression was used as a diagnostic character separating *C. macrotuberculatus* from *C. phuketensis* (see Grismer and Ahmad 2008; Sumontha et al. 2012). Based on the terminology of the precloacal depression in Mecke et al. (2016), the described specimens were re-examined and the presence of a precloacal depression (as precloacal groove) was observed in both *C. macrotuberculatus* (deep depression) and *C. phuketensis* (shallow depression). The PSUZC-RT 2010.58 and THNHM 15378 specimens are two males of *C. phuketensis*, in which the precloacal grooves are shallow (all others are deep) and could result from their poor state of preservation; thus, the character of this specimen was not included in the present diagnostic characters of *C. macrotuberculatus*.

Evidence from both overlapping ranges of morphology and relatively low sequence divergence indicate that *C. phuketensis* is an inconsistent pattern variation of *C. macro-tuberculatus*. We concluded that *C. phuketensis* should be treated as a junior synonym of *C. macrotuberculatus* based on the priority of names designated by International Code of Zoological Nomenclature (ICZN). Additional surveys should be conducted to determine their geographic distribution and the degree of variation and patterns of gene flow within this species.

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# Overview of Indian and Nepali representatives of the Cincticostella nigra (Uéno, 1928) complex (Ephemeroptera, Ephemerellidae), with discussion about Cincticostella Allen, 1971 species complexes

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#### Abstract

The concept of the *Cincticostella nigra* (Uéno, 1928) (Ephemeroptera: Ephemerellidae) complex is clarified and Indian and Nepali representatives of the complex are reviewed. Four new species are described viz. *Cincticostella changfai* Martynov & Palatov, **sp. nov.**, *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth, **sp. nov.**, *Cincticostella shinichii* Martynov & Palatov, **sp. nov.** and *Cincticostella wangi* Selvakumar, Martynov & Subramanian, **sp. nov.** The larva of *C. corpulenta* (Braasch, 1981) is re-described, based on the holotype and paratypes. *Cincticostella gosei* (Allen, 1975) is recorded from India for the first time. Morphological differences of the species complexes of *Cincticostella* Allen, 1971 are analysed. A new species complex, *Cincticostella gosei* complex, is proposed. Summaries of distribution data and habitat preferences, as well as new larval diagnoses, are presented for all species of the *Cincticostella nigra* complex.

#### **Keywords**

China, Ephemerellinae, Ephemerelloidea, Indomalayan Region, new records, new species, Pannota

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# Introduction

This article is a further contribution to a series of papers (Martynov et al. 2019, 2021) about spiny crawler mayflies (Ephemeroptera: Ephemerellidae) of the Indian subregion, in this case devoted to Indian and Nepali representatives of the genus *Cincticostella* Allen, 1971 (Ephemerellinae: Ephemerellini) that were not previously covered as part of the *C. insolta* (Allen, 1971) complex.

Due to uncertain relationships of species in the genus Cincticostella and possible polyphyly, we use the term "complex" to indicate distinctly different groups of species - the C. insolta complex (see Xie et al. 2009; Martynov et al. 2019; Zheng and Zhou 2021) and what we clarify here as the C. nigra (Uéno, 1928) complex, which corresponds to the group of nine species described by Xie et al. (2009) as having "the basic body pattern" for the genus. Up to now, the C. nigra complex in this sense has been comprised of the following nine species (Jacobus and McCafferty 2008; Xie et al. 2009): C. colossa Kang & Yang, 1995, C. corpulenta (Braasch, 1981), C. elongatula (McLachlan, 1875), C. fusca Kang & Yang, 1995, C. gosei (Allen, 1975), C. levanidovae (Tshernova, 1952), C. nigra (Uéno, 1928), C. orientalis (Tshernova, 1952) and C. szechuanensis Xie, Jia, Chen, Jacobus & Zhou, 2009. Amongst these, Xie et al. (2009) noted some differences from "the basic body pattern" of the larva, in that C. levanidovae and C. orientalis have reduced maxillary palps and C. gosei lacks maxillary palps. Zhang et al. (2020) subsequently described the male adult of C. fusca and remarked about its "novel ephemerellid form and possibly...new evolutionary trend in the group", but they did not recognise a new species group or complex for it.

The *C. nigra* complex, as well as the family Ephemerellidae in general, has been relatively poorly investigated in India and Nepal. Only one species of the *C. nigra* complex has been reported from these countries: *C. corpulenta* described from Nepal by Braasch (1981), based on the larval stage. Another species, *C. indica* (Kapur & Kripalani, 1961) is known only from India, based on the original material of the female adult stage. Given that few adults of this genus are known and that female alates generally cannot be identified with confidence, we do not assign *C. indica* to a species complex; however, we treat it below.

# Material and methods

Materials from India and Nepal were used in this research. New larval material was collected by kick-net sampling and hand-picking. All of this material is stored in 80–95% ethanol. Some specimens were mounted on slides with Canada balsam or Hoyer's medium.

The holotype of *C. corpulenta* from the Stuttgart State Museum of Natural History [**SSMNH**] (Stuttgart, Germany) was also examined, along with two paratypes from the Purdue University Entomological Research Collection [**PERC**] (West Lafayette, Indiana, USA). The type material of *C. changfai* sp. nov. (holotype and 31 paratypes – *IN Nepa5Cinsp1/1; Nepa5Cinsp1/2–4*, slide 640; *Indi2Cinsp*, slides 630, 657, 658),

*C. shinichii* sp. nov. (holotype and 1 paratype – *IN Nepa7Cinsp*, slides 647, 649) and specimens of *Cincticostella* sp. A (two larvae – *IN Nepa1Cinsp*, slide 634) are now housed in the National Museum of Natural History of the National Academy of Sciences of Ukraine [**NMNH NASU**], Kyiv, Ukraine. Type material of *C. funki* sp. nov. is deposited in the Zoological Survey of India [**ZSI**], Chennai, India (holotype and one paratype – *IN ZSI/SRC-I/E-512, ZSI/SRC-I/E-513*) and in NMNH NASU (one paratype – *IN Indi1Cinsp*, slide 632); *C. wangi* sp. nov. (holotype and eight paratypes – *IN 5575/H13*) and *C. gosei* (17 larvae) are deposited deposited in ZSI, Kolkata.

Photographs of specimens and their body parts were taken using a Leica M205A microscope, Zeiss Stemi 2000 binocular with Canon Power Shot A 640 and Ulab XY-B2T microscope with Canon Power Shot A 630. Some figures were subsequently improved with Adobe Photoshop CS5 and Helicon Focus 6.

Our hypotheses of species are based on morphological species concepts. Two middle instar larvae from a single morphotype were collected in Nepal in 2014. These larvae belong to the *C. nigra* complex and slightly differ from all known species. However, the poor material and absence of late larval instars do not allow us to describe a new species adequately and with confidence. Therefore, we give a provisional species designation only, along with diagnostic features.

# **Results and discussion**

The analysis of original material, historical collections and literature data showed that there are seven species belonging to *C. nigra* and *C. gosei* complexes (see details below) within India and Nepal: *C. corpulenta* (Braasch, 1981), *C. gosei* (Allen, 1975), four new species (*C. changfai* Martynov & Palatov, sp. nov., *C. shinichii* Martynov & Palatov, sp. nov., *C. wangi* Selvakumar, Martynov & Subramanian, sp. nov. and *C. funki* Martynov, Selvakumar, Palatov & Vasanth, sp. nov.) and one provisional species that we designate as *Cincticostella* sp. A. The taxonomic status of *C. indica* (Kapur & Kripalani, 1961) is discussed below. In addition, we report the first record of *C. gosei* for India.

Diagnoses of species provide characters that distinguish them within their corresponding complexes.

The following nominal species are treated in alphabetical order, with the provisional species listed at the end.

# *Cincticostella changfai* Martynov & Palatov, sp. nov. http://zoobank.org/C04D239A-E4B2-4E72-B238-3F42C3616861

Figs 1-4

**Description. Larva.** Late instars: body length 10.7–14.0 mm; caudal filaments length 6.0–9.1 mm. Body robust, yellowish-brown to brown (Fig. 1A).

**Head:** Without tubercles and ridges. Genae moderately developed, rounded (Fig. 1B). *Mouthparts* (Fig. 2A–G): Labrum (Fig. 2C) wide, angles rounded; anteromedian emargination wide and shallow (labrum height in emargination/maximum labrum height ratio – 0.84–0.87); surface densely covered with long, stout hair-like setae, very short rounded scales with feathered margin and empty scale sockets; anterior margin and angles with thin and stout hair-like setae; central part of anterior margin also with feathered setae. Mandibles (Fig. 2A, B) with empty scale sockets, very short scales and long hair-like setae, the greatest number of the hair-like setae near outer margin; prostheca consisting of protuberance with tuft of middle-sized hair-like setae. Right (planate) mandible with row of long thick hair-like setae (5–8)



**Figure 1.** Larva of *Cincticostella changfai* Martynov & Palatov, sp. nov., paratype **A** habitus, dorsal view, scale bar 1 mm **B** head **C** anterolateral projections of pro- and mesonotum **D** thorax.



Figure 2. Larva of *Cincticostella changfai* Martynov & Palatov, sp. nov., paratype A planate mandibleB angulate mandible C labrum D hypopharynx E maxillary palp F maxilla G labium.

under mola and tuft of short or middle-sized hair-like setae above; outer and inner incisors (kinetodontium) with three teeth. Left (angulate) mandible with few short hair-like setae at end of mola; outer incisor apex with four teeth; inner incisor with four teeth, the two central ones distinctly larger. Rounded apices of superlinguae with long stout and thin hair-like setae; surfaces of lingua covered with thin hairlike setae (Fig. 2D). Irregular rows of short, pointed, stout setae (up to 9) present on lingua surface near base; these rows mainly subtransverse relative to longitudinal axis of body. Maxilla (Fig. 2F) with two dentisetae, their inner margins serrate. Apex and apical part of maxilla surfaces with numerous long, stout hair-like setae; inner margin of galea-lacinia covered with dense row of stout hair-like setae; surface of galea-lacinia near base with group of 13–22 different-sized stout hair-like setae. Maxillary palp long, 3-segmented, with distinct articulations (Fig. 2E, F). Segments I and II with long, hair-like setae; most stout setae located on segment I; segment III short and rounded apically, with several fine setae. Labium with rounded glossae. Whole ventral surface of labium, dorsal surface of glossae and apices of paraglossae densely covered with long, stout, hair-like setae (Fig. 2G). Labial palp 3-segmented; segment I and segment II flattened and subequal in length, ventral side covered with long stout and thin hair-like setae; central part of segment I dorsal surface densely covered with empty scale sockets and very short rounded scales with feathered margins. Segment III elongated (length/width ratio = 2.66-3.5, average – 2.9), rounded apically, covered with numerous short fine setae.

**Thorax:** Dorsal surface of thorax covered with few scattered short, hair-like setae. Pronotum expanded laterally, with broad, rounded, anterolateral projections (Fig. 1D). Anterolateral projections of mesothorax well-developed, not notched, broad, somewhat rounded, with outer margins not subparallel to lateral aspect of body (Fig. 1C). Thoracic surface without distinct ridges and tubercles. In mature larvae, paired posterior projections between fore wing pads moderately developed, triangulate, cleft between them wide; apical parts of outer margins of projections not pressed against wing pads.

In late instars, femora of all legs slightly flattened (length/width ratio = fore femur 2.17–2.38; middle femur 2.56–2.86; hind femur 2.63–2.86), each one with longitudinal ridge, especially visible on middle and hind femora (Fig. 3A–C). Average length ratio of leg's parts (femur : tibia : tarsus): foreleg 1.90 : 1.63 : 1.00; middle leg 2.40 : 2.27 : 1.00; hind leg 2.59 : 2.77 : 1.00. Outer margins of all femora without apical projection.

Whole dorsal surface of fore femur with scattered middle-sized, hair-like setae; basal part of surface with group of spine-like setae, bifurcated stout setae and stout hair-like setae. Distal part of dorsal surface with narrow, transverse band consisting of irregular, sparse row of mainly long and middle-sized, pointed, bifurcated and only sometimes rounded apically, stout setae (some of them situated on chalazae) (Fig. 3A, D, E); also several short, rounded and bluntly pointed apically, stout setae situated in same band. Outer margin of fore femur and dorsal surfaces along it covered by different-sized stout setae with pointed, rounded or bifurcated apices and few hair-like setae; most hair-like setae in basal part. Inner margin of fore femur and adjacent part of dorsal surface densely covered with spine-like setae and stout hair-like setae; dorsal surface along inner margin with similar setae, but more scattered. Inner margins of fore tibia and tarsus and dorsal surfaces along those margins densely covered with spine-like setae of fore tibia also with row of long, pointed and bifurcated, stout setae and scattered hair-like setae. Outer margin of fore tibia and tarsus with few hair-like setae only (solitary and in tufts).

Dorsal surfaces of middle and hind femora covered with few hair-like setae and numerous short and several middle-sized rounded or bluntly pointed (sometimes



**Figure 3.** Larva of *Cincticostella changfai* Martynov & Palatov, sp. nov., paratype. **A** fore femur **B** middle femur **C** hind femur **D**, **E** stout setae of dorsal surface of fore femur **F** stout setae of outer margin of hind femur **G**, **H** tarsal claws.

bifurcated) apically stout setae (Fig. 3B, C, F). Inner margins of middle and hind femora without rows of stout setae, with several hair-like setae. Outer margins of middle and hind femora with irregular rows of different-sized (mainly long), pointed and

bifurcated, stout setae (Fig. 3F); also with few solitary and in tufts hair-like setae; longest hair-like setae in basal parts of margins.

Inner margins of middle and hind tibiae and tarsi densely covered with spine-like setae and stout, hair-like setae; inner margins of middle and hind tibiae also with long, pointed and bifurcated, stout setae. Outer margins of middle and hind tibiae with rows of long, pointed and bifurcated, stout setae. Outer margins of middle and hind tarsi with hair-like setae (solitary and in tufts) only.

Ventral surfaces of all tibiae and tarsi with hair-like setae (solitary and in tufts) and long, thin, pointed, stout setae; stout setae situated in apical parts of tibiae and tarsi and along their inner margins.

Tarsal claws of all legs hooked, usually with two subequal denticles (seldom with one denticle) (Fig. 3G, H) and several subapical setae (setae often broken, as in our figures, but sockets still visible).

**Abdomen:** Central parts of dorsal surfaces of terga II–IX with two medial fields of middle-sized and small stout setae (Fig. 4A, C) with rounded, bluntly pointed or bifurcated apices. Surfaces of all terga covered with few hair-like setae, very short scales with feathery margins and empty scale sockets (most number of two last situated on lateral areas of tergal surfaces). Whole sternal surfaces covered with middle-sized hair-like setae, very short scales with feathery margins and empty scale sockets.

Posterior margins of terga I–II each with row of long hair-like setae; of tergum III with few long thin bluntly pointed or bifurcated apically stout setae and hair-like setae; of terga IV–VII with several rounded and pointed (sometimes bifurcated) stout setae near paired projections; of terga VIII–X with rows of short and middle-sized, mainly rounded apically, stout setae (these rows extending from paired projections to lateral margins). Lateral margins of terga IV–VIII covered with numerous short stout setae.

Pairs of pointed, not bifurcated projections present on abdominal terga II–X, with those on terga II–IV and X smaller than others; those on terga V–IX strongest (Fig. 4A–C). Projections on terga IX elongated and pointed. Surfaces of paired projections on terga V–X covered with short stout setae with mainly rounded apices. Posterolateral projections of abdominal segments IV–IX present.

Dorsal surfaces of abdominal gills covered with hair-like setae and scale sockets; shapes of gills as in Fig. 4D–H; gill III without medial, transverse band of weakened membrane; ventral lamella of gills III–V bifurcated; ventral lamella of gills VI without medial cleft.

Caudal filaments subequal in length, with elongated, bluntly pointed or rounded (sometimes bifurcated) apically stout setae and hair-like setae at articulations (Fig. 4I, J).

## Adults. Unknown.

**Etymology.** The new species is named in honour of Dr. Chang-Fa Zhou (Nanjing Normal University, China), who contributed significantly to the study of the genus *Cincticostella*.

**Diagnosis.** The new species can be easily distinguished from other representatives of the *C. nigra* complex by the following combination of characters: (i) genae moderately developed, rounded (Fig. 1B); (ii) labrum with shallow anteromedian emargination



**Figure 4.** Larva of *Cincticostella changfai* Martynov & Palatov, sp. nov., paratype **A** abdomen, dorsal view **B** paired submedian projections of terga IX and X **C** paired submedian tubercles of tergum VIII **D–H** gills III–VII **I** caudal filaments, proximal part **J** caudal filaments, middle part.

(Fig. 2C); (iii) maxillary palp well-developed (Fig. 2E, F); (iv) segment III of maxillary palp small, rounded apically (Fig. 2E); (v) prothoracic anterolateral projections small, broad, rounded (Fig. 1C, D); (vi) mesothoracic anterolateral projections well-developed,

not notched, somewhat rounded, with outer margins not subparallel to lateral aspect of body (Fig. 1C, D); (vii) surface of thorax covered with a few scattered common, short, hair-like setae; (viii) fore femur with narrow, transverse band consisting of irregular, sparse row of mainly long and middle-sized, pointed, bifurcated and sometimes rounded apically, stout setae (Fig. 3A, D, E); also several short, rounded and bluntly pointed apically, stout setae situated in the band; (ix) tarsal claw with two subequal denticles mainly (Fig. 3G, H); (x) pairs of pointed, not bifurcated projections present on abdominal terga II–X, with those on terga II–IV and X smaller than others; those on terga V–IX strongest; projections on terga IX elongated and pointed (Fig. 4A–C).

Distribution. Known only from Nepal and northern India.

**Habitat.** Larvae of this species were collected in middle-sized rivers (wide 4–15 m) in deep valleys, at an altitude of about 1000 m a.s.l. on the South slope of the Great Himalaya Range (India, Uttarakhand State and Bagmati Zone, Central Nepal) (Fig. 24A). These rivers had low water temperature (12–13 °C), relatively high current velocity (about 0.7–0.8 m/s) and mainly stony bottom. Larvae were collected from stones at sections with current velocity 0.3–0.5 m/s together with *Baetis* (s.str.) sp., different Heptageniidae (*Electrogena*?), *Euthraulus* sp., Glossosomatidae and Hydropsychidae. The studied valleys of the rivers are densely populated by humans; therefore, the rivers are under the significant anthropogenic pressure. Investigated rivers can be classified as alpha- or beta-mezosaprobic waterbodies.

**Type material.** *Holotype*: NEPAL: larva, Bagmati zone, Shivapuri Nagarjun National Park, Gohare Khola River (near Mahankal village), 27.885842°N, 85.531386°E, h ~ 1050 m a.s.l., 4.iii.2007, Chertoprud M.V. leg. – *IN Nepa5Cinsp1/1* [NMNH NASU]. *Paratypes*: NEPAL: 22 larvae (one larva on slide 640), same data as holotype. – *IN Nepa5Cinsp1/2*–4 [NMNH NASU]; INDIA: 9 larvae (3 larvae on slides 630, 657, 658), Uttarakhand, Almora District, Ramganga River (300 m above Patangoari Village), 29.941569°N, 79.414394°E, h ~ 1050 m a.s.l., 3.ii.2011, Palatov D.M., Chertoprud M.V. leg. – *IN Indi2Cinsp* [NMNH NASU]. 6 larvae, Uttarakhand, Chamoli District, Pindar River (2 km above of the Karnaprayag Town), 30.251625, 79.229203, h ~ 780 m a.s.l., 4.ii.2011, Palatov D.M. leg. – *IN Indi4Cinsp1/1–3* [NMNH NASU].

#### Cincticostella corpulenta (Braasch, 1981)

Figs 5-8

*Ephemerella (Drunella) corpulenta* Braasch, 1981 *Cincticostella corpulenta* (Braasch, 1981) in Jacobus and McCafferty 2008

**Remarks.** A supplemental narrative description is provided, based primarily on the study of a slide of the holotype (larva, male) and two paratypes in ethanol. Some characters are given simply as in the original description (Braasch 1981) due to subsequent damage, fading or distortion of the aging specimens.



**Figure 5.** Larva of *Cincticostella corpulenta* (Braasch, 1981), holotype **A** head **B** anterolateral projection of mesonotum **C** thorax **D**, **E** anterolateral projections of pronotum.

**Description. Larva (male).** Body brown according to Braasch (1981), body length 10 mm; caudal filaments length 6 mm. *Head*: Without tubercles and ridges. Braasch (1981) described head as elongated [original text in German: *Kopf lang-gestreckt*], but in holotype genae, these are poorly developed, therefore head appears oval dorsally (Fig. 5A).

Dorsal surface of head covered with numerous very short scales, empty scale sockets and short, hair-like setae. *Mouthparts* (Fig. 6A–H): Labrum wide, angles rounded; anteromedian emargination deep (labrum height in emargination/maximum labrum height ratio -0.73) (Fig. 6C). Anterior margin of labrum mainly with long, stout and thin, hair-like setae; dorsal surface densely covered mainly with long, stout hair-like setae (mainly in apical half), very short scales, empty scale sockets and short hair-like setae.

Mandibles (Fig. 6A, B) covered with empty scale sockets, very short scales and long hair-like setae (stout and thin), hair-like setae most numerous near outer margins and central parts of surfaces; both prosthecae consisting of protuberance with tuft of middle-sized hair-like setae. Planate mandible with row of six long, stout hair-like setae under mola and tuft of short hair-like setae above; outer incisor with three teeth; inner incisor (kinetodontium) bifurcated. Outer incisor of angulate mandible apex with four teeth; inner incisor with three teeth (two central ones distinctly larger). Superlinguae



**Figure 6.** Larva of *Cincticostella corpulenta* (Braasch, 1981), holotype **A** angulate mandible **B** planate mandible **C** labrum **D** superlingua **E** maxillary palp **F** maxilla **G** labial palp **H** labium.

with rounded apices covered with long, stout hair-like setae (Fig. 6D); lingua with shorter setae. Lingua surface near base with transverse row of six short, pointed, stout setae. Maxilla (Fig. 6F) with two dentisetae with inner margins serrate. Apex and apical part of maxilla surfaces with numerous long, stout, hair-like setae; galea-lacinia with numerous, long, stout, hair-like setae on inner margin. Group (26, 29) of different-sized, stout, hair-like setae (some very long), situated on surface of galea-lacinia near

base. Maxillary palp long, 3-segmented; joins between segments distinct (Fig. 6E). Segments I and II with long, hair-like setae, most strong setae situated on segment I; segment III slightly elongated and rounded apically. Glossae rounded; dorsal surfaces covered with short and middle-sized, stout, hair-like setae. Apices of paraglossae densely covered with mostly long and middle-sized, stout, hair-like setae; ventral surface of labium densely covered with mostly long, stout, hair-like setae (Fig. 6H). Labial palp 3-segmented (Fig. 6G); segment I and II distinctly flattened and subequal in length; ventral sides densely covered with long, hair-like setae (stout and thin); dorsal surfaces with long, stout, hair-like setae along outer and inner margins; central part of segment I dorsal surface also densely covered with scale sockets and very short rounded scales with feathered margins in some sockets. Segment III distinctly elongated (length/width ratio = 3.40–4.15), rounded apically, with short, fine setae mainly at apex. Submentum with scattered long, stout, hair-like setae, very short rounded scales with feathered margins in some sockets.

**Thorax:** Dorsal surface of thorax covered with scattered short, waved and hooked, stout hair-like setae, thin and stout, hair-like setae and scale sockets (Fig. 23B); most distinct waved and hooked setae on veins of fore wing pads. Pronotum with moderately convex, rounded and broad anterolateral angles (Fig. 5C–E). Anterolateral projections of mesothorax poorly developed, rounded and not notched, with margins not subparallel to lateral aspect of body (Fig. 5B, C). Thoracic surface without distinct ridges and tubercles. Two blunt posterior projections present between fore wing pads; cleft between projections wide; apical parts of outer margins of projections pressed against wing pads.

Legs slightly flattened (length/width ratio = fore femur 2.08; hind femur 2.56), each one with longitudinal ridge (Fig. 7A, B). Average length ratio of leg's parts (femora : tibia : tarsi): foreleg 1.95 : 1.68 : 1; hind leg 2.59 : 2.38 : 1. Outer margins and proximal parts of dorsal surfaces of femora covered with irregular row of differentsized, stout setae with mostly rounded or bifurcated apices (Fig. 7A, B, E). Basal half of inner margin of fore femur and adjacent part of dorsal surface densely covered with spine-like setae and long, stout, hair-like setae. Apical half of dorsal surface of fore femur with transverse, sparse, band of mainly middle-sized and long, stout setae with bifurcated or rounded apices (some setae situated on chalazae) (Fig. 7A, C); several elongated, pointed or bifurcated apically, stout setae with feathered margins situated on dorsal surface near basal margin. Additionally, whole dorsal surface of fore femur covered with very short, rounded scales with feathered margins, empty scale sockets and different-sized hair-like setae. Inner margin and adjacent part of dorsal surface of fore tibia with numerous long, stout, hair-like setae and few, thin, hair-like setae (solitary and in tufts); another part of dorsal surface of fore tibia with row of long, bifurcated apically, stout setae and scattered hair-like setae (solitary and in tufts). Inner parts of ventral and dorsal surfaces of fore tibia and tarsus with numerous long, stout, hair-like setae (some with feathered margins in apical part); additionally, whole ventral and dorsal surfaces of fore tibia and tarsus with scattered hair-like setae (solitary and in tufts). Outer margins of fore tibia and tarsus with only long, hair-like setae. Outer margin of hind femur without apical projection; whole margin and part of dorsal



**Figure 7.** Larva of *Cincticostella corpulenta* (Braasch, 1981), holotype **A** fore femur **B** hind femur **C** stout setae of dorsal surface of fore femur **D** stout setae of hind tibia **E** stout setae of outer margin of hind femur **F** tarsal claw.

surface covered with different-sized, stout setae with rounded and bifurcated apices. Whole dorsal surface covered with numerous short, rounded and bifurcated, stout setae, scale sockets, short scales with feathered margins in some sockets and scattered short, sometimes waved, stout, hair-like setae. Inner margin of hind femora with only solitary thin, hair-like setae. Outer margin of hind tibia with regular row of long, stout setae with pointed and bifurcated apices and scattered hair-like setae (Fig. 7B, E). Dorsal surface of hind tibia with irregular row of long, stout setae with bifurcated apices, situated along inner margin (Fig. 7D); whole surface covered with scattered long hair-like setae (solitary and in tufts) and short, stout, hair-like setae (some apparently waved or hooked). Inner margins and ventral surfaces of hind tibia and tarsus as in fore leg.

Tarsal claws of all legs hooked, with one large denticle and several subapical setae (Fig. 7F).

*Abdomen:* Central part of dorsal surface of terga II–IX with two medial fields of mainly short stout setae with rounded or bluntly pointed, sometimes bifurcated, apices (Fig. 8A–C). Surfaces of all terga covered with not numerous hair-like setae (mainly stout, waved of hooked), empty scale sockets and very short rounded scales with feathered margins (most scales and sockets situated laterally). Whole sternal surfaces covered with scattered empty scale sockets, scales and hair-like setae. Posterior margins of tergum I with row of mostly long, hair-like setae; posterior margins of terga II–III (especially tergum II) with row of elongated, mostly bluntly pointed and bifurcated apically, stout setae and stout, hair-like setae; posterior margins of terga IV–VII with several elongated mostly rounded and bluntly pointed apically, stout setae, extending from paired projections to lateral margins; posterior margins of terga VIII–X with rows of elongated stout setae with mostly rounded apices (rows extending from paired projections to lateral margins) (Fig. 8B–E). Lateral margins of terga IV–VIII covered with rounded apically stout setae most numerous.

Pairs of not bifurcated projections present on abdominal terga II–IX, relatively weakly developed (Fig. 8A–E). Projections on terga VI–VIII strongest; those on terga II and III smallest and rounded; those on tergum IV elongated and bluntly pointed; those on terga V–VIII pointed; those on tergum IX short and bluntly pointed. Posterior margin of tergum X smooth, without any projections (Fig. 8E). Surfaces of paired projections on terga II–IX with several mostly short, stout setae with rounded apices.

Abdominal gills dorsal surfaces covered with hair-like setae and scale sockets; gill III oval, with somewhat extended posteromedial angles without medial, transverse band of weakened membrane. Due to slide-mounting of holotype, gills deformed and not separated from abdomen.

Caudal filaments subequal in length; middle parts with elongated stout setae with bluntly pointed or bifurcated apices (Fig. 8F) at articulations and hair-like setae (solitary and in tufts) on surfaces of segments.

**Diagnosis.** This species can be distinguished from all other species of the *C. nigra* complex by the following combination of characters: (i) genae poorly developed, head oval dorsally (Fig. 5A); (ii) labrum with deep anteromedian emargination (Fig. 6C); (iii) maxillary palp well-developed (Fig. 6E); (iv) segment III of maxillary palp small, rounded apically (Fig. 6E); (v) prothoracic anterolateral projections rounded and broad (Fig. 5C–E); (vi) mesothoracic anterolateral projections poorly developed, rounded, not notched, with margins not subparallel to lateral aspect of body (Fig. 5B); (vii) short, waved and hooked, stout hair-like setae presented on dorsal surface of thorax (Fig. 23B); (viii) dorsal surface of fore femur with transverse, sparse, band of mainly middle-sized and long, stout setae with bifurcated or rounded apices (Fig. 7A and C); (ix) tarsal claw with one large denticle (Fig. 7F); (x) pairs of pointed, relatively weakly developed, projections present on abdominal terga II–IX; those on terga II and III smallest and rounded; those on terga VI–VIII strongest; those on terga V–VIII pointed; those on tergum IX short and bluntly pointed; posterior margin of tergum X smooth, without any projections (Fig. 8A–E).



**Figure 8.** Larva of *Cincticostella corpulenta* (Braasch, 1981), holotype **A** abdomen on slide, dorsal view **B** submedian projection of tergum VII **C** paired submedian projections of tergum VIII **D** paired submedian projections of tergum IX **E** tergum X **F** caudal filament.

Distribution. Nepal (Braasch 1981).

**Remarks.** Adult stages unknown. The holotype (on slide) has the middle legs and one hind leg missing.

**Habitat.** No data, but assumed to be cold water rivers and streams, based on what we know about the Trisuli River near Dhunche.

**Type material examined.** *Holotype*: NEPAL: larva on slide, Himalaya, Trisuli Khola vor Dhunche, 1950 m a.s.l. NN, 30.04.1978, Leg. I. Sivec [SSMNH]. *Paratypes*: two larvae, same data as holotype [PERC]. *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth, sp. nov. http://zoobank.org/D1E54A80-A4A5-421E-9612-C1E8C6077BA2 Figs 9–12

**Description. Larva**. Late instars: body length 10.5–11 mm; caudal filaments length 6–7 mm. Body robust, yellowish-brown, abdomen darker (Fig. 9A). Head, thorax and abdomen with longitudinal medial white line; on abdomen line is most broad (Figs 9A–C, 12A, C). Anterolateral projections of mesothorax and lateral areas of pronotum are yellowish-whitish. Pronotum and mesonotum with indistinct whitish – yellowish spots.

Head: Without tubercles and ridges. Genae rounded, moderately developed (Fig. 9B). Mouthparts (Fig. 10A-F): Anteromedian emargination of labrum shallow and narrow (Fig. 10C). Anterolateral angles of labrum rounded. Dorsal surface of the labrum covered mainly with long, thin and stout hair-like setae; setae decreasing in length towards central notch. In addition, setae with feathered margins, small hairlike setae and solitary empty scale sockets presented on labrum surface. Largest hairlike setae and their greatest number on mandibles situated in the centre of their outer margin and adjacent areas (Fig. 10A and B). Additionally, mandibles bearing few empty scale sockets. Prostheca consisting of protuberance with tuft of middle-sized, hair-like setae. Planate mandible with row of long thick hair-like setae (7-8) under mola and tuft of short or middle-sized hair-like setae above; outer and inner incisors (kinetodontium) with three teeth. Angulate mandible with few short hair-like setae at the end of mola; outer incisor apex with four teeth; inner incisor with four teeth, two central teeth distinctly larger. Apices of superlinguae rounded, bearing long, thin and stout, hair-like setae; surfaces of lingua covered with thin hair-like setae (Fig. 10D). Lingua with shallow medial concave on anterior margin. Surface of lingua with irregular, subtransverse to longitudinal axis of the body, rows of short, pointed, stout setae (8-9). Maxilla with two dentisetae; their inner margins serrated. Apical part of maxilla densely covered with long, stout hair-like setae (Fig. 10E). Inner margin of galea-lacilia with a dense row of stout hair-like setae, longest setae situated proximal. In addition, a group of 16–24 long, stout setae situated on surface of galea-lacilia base. Maxillary palp long, 3-segmented, with distinct articulation (Fig. 10E). Segment III elongated with several fine setae, bluntly pointed apically. Segments I and II subequal in a length, covered mainly with a long, stout hair-like setae. Labium robust, glossae rounded; labial palp 3-segmented (Fig. 10F). Segments I and II flattened, subequal in length; on dorsal surfaces of segments stout setae (long, hair-like) present mainly near margins; ventral surfaces, outer and inner margins of segments densely covered with long, stout, hair-like setae; central part of dorsal surface near inner margin on segment I with scale sockets with feathery setae. Segment III narrow, elongated (length/width ratio = 2.5-2.7), with rounded apex, covered with short fine setae. Whole ventral surface of labium covered mainly with long, stout, hair-like setae; dorsal surface of glossae and apices of paraglossae covered mainly with similar kind of setae.



**Figure 9.** Larva of *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov., holotype (**A**) and paratype (**B–D**) **A** habitus, dorsal view, scale bar 2 mm **B** head **C** thorax **D** anterolateral projection of mesonotum.

**Thoras:** Dorsal surface of thorax covered with scattered mainly short, waved and hooked, stout hair-like setae (Fig. 23B). Pronotum expended laterally; anterolateral projections broad, small and rounded (Fig. 9C). Anterolateral projections of mesothorax well-developed, with rounded posterior angles, outer margins not notched and not sub-parallel to lateral aspect of body (on slide with immature larva – subparallel) (Fig. 9C, D).



**Figure 10.** Larva of *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov., paratype **A** angulate mandible **B** planate mandible **C** labrum **D** hypopharynx **E** maxilla **F** labium.

Thoracic surface without any distinct ridges and tubercles. In mature larvae, paired posterior projections between fore wing pads small, rounded with narrow cleft between them; apical parts of outer margins of projections not pressed against wing pads (Fig. 9C).

All femora slightly flattened (length/width ratio = fore femur 2.0–2.1; middle femur 2.0–2.2; hind femur 2.0–2.2) (Fig. 11A–C), each one with longitudinal ridge, especially



Figure 11. Larva of *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov., paratype A fore femur B middle femur C hind femur D stout setae of dorsal surface of fore femur E tarsal claw.

visible on middle and hind femora. Average length ratio of leg's parts (femora: tibia : tarsi): foreleg 1.48: 1.43 : 1.00; middle leg 1.61 : 1.72 : 1.00; hind leg 2.10 : 2.32 : 1.00. All femora outer margins without apical projections, any distinct serration also absent.

Whole dorsal surface of fore femur covered with scattered middle-sized, hair-like setae and scale sockets with small scales in some of them; basal part of surface with group of spine-like setae, bifurcated, stout setae and stout, hair-like setae. Additionally, numerous long, bifurcated apically, stout setae covering dorsal surface of fore femora along basal half of its inner margin. Distal part of surface with relatively wide, transverse, band consisting of irregular, sparse rows of different-sized (mainly long), rounded apically (only sometimes bluntly pointed with shallow bifurcation of apex), stout setae; some of setae situated on chalazae (Fig. 11D). Outer margin of fore femur and dorsal surfaces along it covered with different-sized stout setae with long rounded and bifurcated apices and numerous middle-sized hair-like setae. Inner margins of fore tibia and tarsus and dorsal surfaces along those margins densely covered with spine-like setae and stout, hair-like setae and scattered hair-like setae. Outer margin of fore tibia and tarsus with few hair-like setae only (solitary and in tufts).

Dorsal surface of middle and hind femora covered with few scattered hair-like setae and scale sockets with small scales in some of them; also, surface covered with numerous short, rounded apically, stout setae (Fig. 11B, C). Inner margins of middle and hind femora without stout setae, only solitary hair-like setae present. Outer margins of middle and hind tibiae with moderately dense irregular rows of mainly long and pointed, stout setae and few hair-like setae amongst them.

Inner margins of middle and hind tibiae and tarsi densely covered with spine-like setae and stout, hair-like setae; inner margins of middle and hind tibiae also with long, pointed and stout setae. Outer margin of middle and hind tibiae with irregular row of long, pointed and bifurcated, stout setae. Outer margin of middle and hind tarsi with hair-like setae (solitary and in tufts) only.

Ventral surfaces of all tibiae and tarsi with hair-like setae (solitary and in tufts) and long, thin, pointed, stout setae; stout setae situated in apical parts of tibiae and tarsi and along their inner margins.

Tarsal claw of all legs hooked, with two (rarely three) denticles distanced from each other; basal denticle (rarely two denticles) distinctly larger; distal denticle directed angled forward (Fig. 11E); claw also bears several subapical setae.

**Abdomen:** Posterior margins of tergum I with thin and stout, hair-like setae only; several similar setae present on surface of tergum I. Submedian areas of terga II–IX surfaces, posterior margins of terga VIII–X (excluding central area between submedian projections) and all paired submedian projections covered with small and middle-sized, oval or, sometimes, with slightly divergent margins, stout setae with rounded apices. Additionally, all terga surfaces covered with scattered, small, stout hair-like setae, short, thin, hair-like setae, scale sockets and short scales in some of them. Sterna covered with scattered thin and stout, hair-like setae and scale sockets.

Pairs of pointed, not bifurcated, projections present on abdominal terga II–IX; those on terga V–VIII strongest; those on terga II–IV distinctly smaller than others (Fig. 12A–C). Posterior margin of tergum X smooth, without projections. Abdominal segments IV–IX with posterolateral projections; on segment IX, they are most developed and directed backwards and laterally. Posterior margins of terga VIII–X with rows of short, stout setae with rounded apices (these setae almost absent in central areas of the margins). Lateral margins of terga IV–VIII covered with small stout setae with apices rounded.

Dorsal surface of abdominal gills covered with scattered hair-like setae and scale sockets; shape of gills as in Fig. 12D–G; medial, transverse band of weakened membrane absent on gill III.

Caudal filaments subequal in length, with mainly elongated, rounded apically, stout setae and hair-like setae at articulations.

## Adults. Unknown.

**Etymology.** The new species is named in honour of Dr. David Funk (Stroud Water Research Center, USA), who contributed significantly to the study of Ephemerellidae.

**Diagnosis.** The species is morphologically close to *C. shinichii* sp. nov. (see below), but can be distinguished from this species and other representatives of the complex by the following combination of the characters: (i) genae rounded and moderately



**Figure 12.** Larva of *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov., holotype (**A–C**) and paratype (**D–G**). **A** abdomen, dorsal view **B** paired submedian projections of terga VI–VIII, lateral view **C** paired submedian projections of terga V–IX, dorsal view **D–G** gills III–VI.

developed (Fig. 9B); (ii) anteromedian emargination of labrum shallow and narrow (Fig. 10C); (iii) maxillary palp well-developed (Fig. 10E); (iv) segment III of maxillary palp elongated, rounded apically (Fig. 10E); (v) anterolateral projections of prothorax small, broad and rounded (Fig. 9C); (vi) mesothoracic anterolateral projections well-developed, with rounded posterior angles, outer margins not notched and not subparallel to lateral aspect of body (on slide with immature larva – subparallel) (Fig. 9C, D); (vii) surface of thorax covered with scattered mainly short, waved and hooked, stout hair-like setae (Fig. 23B); (viii) fore femur with relatively wide, transverse band consisting of irregular, sparse rows of different-sized (mainly long), rounded apically (only sometimes bluntly pointed with shallow bifurcation of apex), stout setae (Fig. 11A, D);
(ix) tarsal claw with two denticles distanced from each other; basal denticle distinctly larger; distal one directed angled forward (Fig. 11E); (x) pairs of pointed projections present on abdominal terga II–IX; those on terga II–IV distinctly smaller than others; projections on tergum IX elongated and distinctly pointed; posterior margin of tergum X without paired projections (Fig. 12A–C).

**Distribution.** Northern India and the India-China border region (Uttarakhand State and Arunachal Pradesh).

**Habitat.** In Arunachal Pradesh, larvae of *C. funki* sp. nov. were collected from the Rike River (type locality) (2–5 m wide) at intermediate high mountain areas, on the Eastern Himalayan Range (Fig. 24B). Apparently, they prefer lotic water bodies with relatively low water temperature (9 °C–10 °C in sampling period), average current velocity (0.4–0.7 m/s), the bottom of river containing pebbles, cobbles and low level of silt particles and there is a median degree of anthropogenic pressure. Rivers inhabited by the species can be characterised as oligosaprobic. Larvae were collected from silted gravel and cobbles. Larvae of the mayfly *Baetis* sp., stoneflies Nemouridae and Peltoperlidae and caddisflies *Himalopsyche* sp. and *Rhyacophila* sp., were registered in the microhabitats along with *C. funki* sp. nov.

In Uttarakhand State, larvae of *C. funki* sp. nov. were collected from a small river (2–4 m wide) in medium high mountains, on the southern slope of the Great Himalayan Range (Fig. 24C). Apparently, they prefer small lotic waterbodies with relatively low water temperature (12 °C in sampling period), average current velocity (0.3– 0.6 m/s), mosaic bottom and low degree of anthropogenic pressure. Rivers inhabited by the species can be characterised as oligosaprobic. Larvae were collected from silted gravel of the mayfly *Caenis* sp., stoneflies *Mesonemoura* sp. and *Kamimuria* sp. and the caddisfly *Lepidostoma* sp.

**Type material.** *Holotype:* INDIA: larva, Arunachal Pradesh, Papumpare District, vicinity of Parang Village, Rike River, 27.32797°N, 93.50308°E, h ~1285 m a.s.l., 14.xii.2018, Coll. Bikramjit Sinha – IN *ZSI/SRC-I/E-512* [ZSI]. *Paratypes:* INDIA: one larva, same data as holotype – *IN ZSI/SRC-I/E-513* [ZSI]. One larva (on slide 632), Uttarakhand, Almora District, 2<sup>nd</sup> order left tributary of Ramganga River (in Dwarahat forest, 10.1 km north-eastwards of Chaukhutia Town), 29.925608 N, 79.445983 E, h ~ 1200 m a.s.l., 2.ii.2011, Palatov D.M. leg. – *IN Indi1Cinsp* [NMNH NASU].

# Cincticostella gosei (Allen, 1975)

Figs 13-15

*Ephemerella (Cincticostella) gosei* Allen, 1975 *Serratella thailandensis* Allen, 1980 (junior objective synonym, Edmunds and Murvosh 1995)

**Diagnosis.** This species can be distinguished from other *Cincticostella* species by the following combination of characters: (i) head brown, with three white to yellow spots near



**Figure 13.** Larva of *Cincticostella gosei* (Allen, 1975) **A** habitus, dorsal view, scale bar 2 mm **B** head **C** thorax **D** anterolateral projections of pro- and mesonotum **E** dorsal surface of hind femur central area **F** abdomen, dorsal view.



**Figure 14.** Larva of *Cincticostella gosei* (Allen, 1975) **A** angulate mandible **B** planate mandible **C** labrum **D** hypopharynx **E** maxilla **F** labial palp **G** labium.

ocelli (spots near lateral ocelli the largest) (Fig. 13A, B); (ii) body covered with numerous large scale sockets and small scales in some of them (Figs 13B–E, 14A–C, 15A–C); (iii) head without paired protuberances (Fig. 13B); (iv) genae moderately developed (Fig. 13B); (v) labrum with moderate anteromedian emargination (Fig. 14C); (vi) maxillary palp absent (Fig. 14E); (vii) glossae short, inner margins of paraglossae subparallel to longitudinal axis of body, held tightly against glossae (Fig. 14G); (viii) labial palp segments I and II not flattened, elongated (Fig. 14F, G); (ix) prothoracic anterolateral



Figure 15. Larva of *Cincticostella gosei* (Allen, 1975) **A** fore femur **B** middle femur **C** hind femur **D** tarsal claw **E–H** gills III–VI.

projections well-developed, bluntly pointed, directed forward (Fig. 13C, D); (x) mesothoracic anterolateral projections poorly developed, not notched, rounded (Fig. 13C, D); (xi) setal transverse band on dorsal surface of fore femur consisting of only several middle-sized stout setae; sometimes stout setae absent absolutely; (xii) inner margin of fore femur only with scattered, thin, hair-like setae (Fig. 15A); (xiii) middle and hind femora moderately widened (Fig. 15B, C); (xiv) dorsal surfaces of middle and hind femora without stout setae (Figs 13E, 15B, C); (xv) outer margins of middle and hind femora without expressed projections (Fig. 15B, C); (xvi) tarsal claw with 5–6 denticles and several subapical setae (Fig. 15D); (xvii) small paired submedial projections present on terga II–IX; projections on tergum IX broad and rounded (Fig. 13F).

**Distribution.** Thailand (Allen 1975, 1980) and the India-China border region (new data).

**Remarks.** The larva of this species was properly described from Thailand by Allen (1975, 1980). We report this species for the first time from India. Adult stages are unknown. Main distinguishing characters of species are shown on Figs 13–15. The characters typical for representatives of *C. insolta* and *C. nigra* complexes are analysed because of the separate position of this species (see below).

**Habitat.** Cold fast-flowing river with cobbles and gravel. The Ranga River habitat is shown in Martynov et al. (2019: fig. 152).

**Material examined. INDIA:** 17 larvae, Arunachal Pradesh, Lower Subansiri District, Ranga River, 27.396404°N, 93.757378°E, h ~ 625 m a.s.l., 06.xi.2015, Coll. Bikramjit Sinha – *IN 5346/H13* [ZSI].

# Cincticostella indica (Kapur & Kripalani, 1961), nomen dubium, new status

*Ephemerella indica* Kapur & Kripalani, 1961

Cincticostella indica (Kapur & Kripalani, 1961) in Jacobus and McCafferty 2008

**Remarks.** *Cincticostella indica* was described only from the female adult stage (Kapur and Kripalani 1961) and it was included in *Cincticostella* by Jacobus and McCafferty (2008), based on the colouration of the abdomen of the female adult, which is similar to that of several other *Cincticostella* species. Its status was regarded as questionable by Xie et al. (2009). Recently, we tried to examine the holotype of *C. indica*, but the type specimen could not be located from the Zoological Survey of India, Kolkata (Hubbard and Srivastava 1984). Hence, *C. indica* cannot be identified and, given that the type material is apparently lost, we hereby regard it as a *nomen dubium*.

# Cincticostella shinichii Martynov & Palatov, sp. nov.

http://zoobank.org/04CA8710-E234-49FC-8822-AE53DEFD6F08 Figs 16–19

**Description. Larva.** Middle and late instars: body length of mature larva 12.0 mm, caudal filaments length 7.8 mm. Body yellowish-brown to brown, robust, covered with scale sockets and small scales in some of them.

*Head:* Without tubercles and ridges. Genae moderately developed, rounded (Fig. 16A). Dorsal surface of head moderately covered with very short, elongated, rounded apically stout setae and few short hair-like setae. *Mouthparts* (Fig. 17A–G):



**Figure 16.** Larva of *Cincticostella shinichii* Martynov & Palatov, sp. nov., holotype (**A**, **B**, **D**, **E**) and paratype (**C**) **A** head **B** anterolateral projection of mesonotum **C** thorax **D**, **E** anterolateral projections of pronotum.

Labrum wide, angles rounded; anteromedian emargination relatively deep and wide (labrum height in emargination/maximum labrum height ratio - 0.67-0.71) (Fig. 17C); anterior margin covered mainly with different-sized hair-like setae and several short, feathered setae in emargination. Surface of labrum densely covered with long hair-like setae, very short rounded scales with feathery margin and empty scale sockets. Mandibles covered with empty scale sockets, very short rounded scales with feathery margin and long hair-like setae, the greatest number of the hair-like setae on and near outer margin (Fig. 17A, B). Prostheca consisting of protuberance with tuft of middle-sized hair-like setae. Planate mandible with row of long thick hair-like setae (7–8) under the mola and tuft of middle-sized or short hair-like setae above; outer incisor with three teeth, inner incisor bifurcated. Angulate mandible with few short hairlike setae at the end of mola; outer incisor apex with four teeth; inner incisor with two distinct central teeth and one-two small lateral teeth. Hypopharynx with denser and longer setae on superlinguae apical part, lingua with short, hair-like setae (Fig. 17D); also, two subtransverse, submedian rows of short, pointed, stout setae (7-9) situated on lingua surface near base. Superlinguae with rounded apices. Maxilla (Fig. 17F) with two dentisetae with inner margins serrate. Apex and apical part of maxilla surfaces with numerous long, stout, hair-like setae; galea-lacinia with numerous long, stout and thin, hair-like setae on inner margin; also, in mature larvae galea-lacinia with group of 22-24 different-sized, stout, hair-like setae on surface near base. Maxillary palp long,



**Figure 17.** Larva of *Cincticostella shinichii* Martynov & Palatov, sp. nov., paratype (**A**, **B**, **G**) and holotype (**C**-**F**) **A** angulate mandible **B** planate mandible **C** labrum **D** hypopharynx **E** maxillary palp **F** apical half of maxilla **G** labium.

3-segmented (Fig. 17E). Segments I and II with long hair-like setae; most strong setae situated on segment I; segment III elongated and bluntly pointed apically, with several short fine setae. Joints of maxillary palp segments distinct. Labium (Fig. 17G) with rounded glossae; dorsal surface of glossae and apices of paraglossae covered mainly with long, stout, hair-like setae; whole ventral surface of labium covered mainly with long, stout, hair-like setae. Labial palp 3-segmented; segment I and segment II flattened and subequal in length; their ventral surfaces, outer and inner margins densely covered with long, stout, hair-like setae; central areas of dorsal surfaces of the segments almost without setae, only near margins long, stout, hair-like setae present; central part

of dorsal surface of segment I with scale sockets and very short rounded scales with feathery margin in some of them. Segment III long (length/width ratio = 3.27-3.85, average – 3.53), rounded apically, covered with numerous short fine setae.

**Thorax:** Dorsal surface of thorax covered with numerous small stout setae with divergent margins, rounded or bifurcated apices and less numerous short, strait, thin and stout, hair-like setae and scale sockets (Fig. 23A). Pronotum expanded laterally, with small, broad and rounded apically anterolateral projections (Fig. 16C–E). Anterolateral projections of mesothorax well-developed, with rounded posterior angles; outer margins not subparallel to lateral aspect of body, not notched, with very shallow sag only (Fig. 16B, C). Thoracic surface with small, indistinct ridges and tubercles. In mature larvae, paired posterior projections between fore wing pads small, rounded, but with deep and narrow cleft between them; apical parts of outer margins of projections not pressed against wing pads (Fig. 16C).

In late instars, femora of all legs slightly flattened (length/width ratio = fore femur 2.00–2.17; middle femur 2.17–2.33; hind femur 2.33–2.44) and bearing longitudinal ridge, especially visible on middle and hind femora (Fig. 18A–C). Average length ratio of leg's parts (femur : tibia : tarsus): foreleg 1.95 : 1.68 : 1.00; middle leg 2.40 : 2.15 : 1.00; hind leg 2.70 : 2.84 : 1.00. Outer margins of all femora without apical projections.

Dorsal surface of fore femur with several elongated, pointed and bifurcated, stout setae with feathered margins near basal margin; also apical half of dorsal surface of fore femur with transverse, relatively wide and dense band of mainly middle-sized and short, bifurcated, bluntly pointed or rounded apically, stout setae (some situated on chalazae) (Fig. 18A, D, E). Basal half of inner margin of fore femur and adjacent part of dorsal surface with irregular row of spine-like setae and stout, hair-like setae. Outer margin of fore femur with numerous, mainly middle-sized and long, bifurcated apically, stout setae. Fore tibia and tarsus: outer margins of these with few hair-like setae (solitary and in tufts); inner margins with irregular, dense row of stout, hair-like setae. Dorsal surfaces of fore tibia with scattered hair-like setae and irregular, longitudinal row of long, stout setae with pointed and bifurcated apices; dorsal surface of fore tarsus with hair-like setae (solitary and in tufts) and long, stout hair-like setae.

Dorsal surface of middle and hind femora covered with numerous mainly middlesized and short, rounded or bifurcated apically, stout setae (Fig. 18B, C). Except for the setae mentioned above, dorsal surface of all femora covered with scattered thin, hairlike setae and scale sockets with small scales in some of them. Inner margins of middle and hind femora with solitary hair-like setae only. Outer margins of middle and hind femora with irregular rows of mainly long, pointed and bifurcated, stout setae and few hair-like setae (Fig. 18B, C, F).

Outer margins of middle and hind tibiae with regular rows of long, pointed and bifurcated, stout setae and few hair-like setae (solitary and in tufts) amongst them (Fig. 18G). Dorsal surfaces of middle and hind tibiae and tarsi with hair-like setae (solitary and in tufts) and waved and hooked, stout, hair-like setae mainly. Dorsal surface of middle tibia also with row of long, pointed and bifurcated, stout setae near



**Figure 18.** Larva of *Cincticostella shinichii* Martynov & Palatov, sp. nov., paratype (**A–C, E, F, H**) and holotype (**D, G**) **A** fore femur **B** middle femur **C** hind femur **D, E** stout setae of dorsal surface of feemur **F** stout setae of outer margin of hind femur **G** stout setae of outer margin of middle tibia **H** tarsal claw.

inner margin. Inner margin of middle and hind tibiae densely covered with long, stout hair-like setae and long, pointed or bifurcated, stout setae amongst them. Middle and hind tarsi: outer margins with hair-like setae (solitary and in tufts) only; inner margins densely covered with long, stout hair-like setae. Ventral surfaces of all tibiae and tarsi with numerous hair-like setae (solitary and in tufts) and long, thin, pointed, stout setae with feathered margins; stout setae situated in apical parts of tibiae and tarsi and along their inner margins.

Tarsal claws of all legs hooked, with one large denticle and several subapical setae (Fig. 18H).

**Abdomen:** Central part of dorsal surface of terga II–X with two medial fields of middle-sized and small, stout setae with bifurcated, bluntly pointed or rounded apices (Fig. 19A). Surfaces of all terga also covered with scattered short, waved, stout hair-like setae, short, thin, hair-like setae, scale sockets and very short scales with feathered margins in some of them (greatest number of two last situated on lateral areas of tergal surfaces).

Posterior margins of tergum I with row of long, hair-like setae; of terga II–III (especially tergum II) with row of long, thin, bluntly pointed or rounded apically, stout setae and hair-like setae; of terga IV–VII with several elongated rounded or bifurcated apically stout setae near paired projections; of terga VIII–X with rows of elongated or short, stout setae with rounded or bifurcated apices (these rows extending from paired projections to lateral margins). Lateral margins of terga IV–VII covered with numerous rounded or bifurcated apically stout setae. Posterolateral projections of abdominal segments IV–IX present (Fig. 19A).

Pairs of pointed, not bifurcated projections present on abdominal terga II–IX (Fig. 19A, B); projections on terga II–IV smaller than others; those on terga V–VIII strongest; projections on tergum IX elongated and distinctly pointed; posterior margin of tergum X smooth, without any projections. Surfaces of paired projections on terga V–IX covered with short stout setae with mainly rounded apices.

Dorsal surfaces of abdominal gills covered with hair-like setae (mainly in apical part) and scale sockets; shape of gills as in Fig. 19C–G; gill III without medial, transverse band of weakened membrane (Fig. 19C).

Caudal filaments subequal in length, with elongated stout setae with bluntly pointed, bifurcated or pointed apices and hair-like setae at articulations (Fig. 19H, I).

Adults. Unknown.

**Etymology.** The new species is named in honour of Dr. Shin-ichi Ishiwata (Kanagawa Environmental Research Center, Japan), who contributed significantly to the study of *Cincticostella* species.

**Diagnosis.** This new species is close to *C. corpulenta*, but can be differentiated from this and other species of the complex by the following combination of characters: (i) genae moderately developed, rounded (Fig. 16A); (ii) labrum with deep anteromedian emargination (Fig. 17C); (iii) maxillary palp well-developed (Fig. 17E, F); (iv) segment III of maxillary palp small, bluntly pointed apically (Fig. 17E); (v) prothoracic anterolateral projections broad and rounded (Fig. 16C–E); (vi) mesothoracic anterolateral projections well-developed, with rounded posterior angles; outer margins not subparallel to lateral aspect of body, not notched, with very shallow sag only (Fig. 16B, C); (vii) small, stout setae with divergent margins, rounded or bifurcated apices present on



**Figure 19.** Larva of *Cincticostella shinichii* Martynov & Palatov, sp. nov., holotype (**A** and **I**) and paratype (**B–H**) **A** abdomen, dorsal view **B** tergum X and posterior margin of tergum IX (black arrow shows posterior margin of tergum X lack of paired submedian projections) **C–G** gills III–VII **H** caudal filament, middle part **I** caudal filament, proximal part.

dorsal surface of thorax (Fig. 23A); (viii) dorsal surface of fore femur with transverse, relatively wide and dense band of mainly middle-sized and short, bifurcated, bluntly pointed or rounded apically, stout setae (Fig. 18A, D, E); (ix) outer margins middle and hind femora with dense irregular rows of mainly middle-sized and long, stout setae

(Fig. 18 B, C, F); (x) tarsal claw with one large denticle (Fig. 18H); (xi) pairs of pointed projections present on abdominal terga II–IX; projections on tergum IX elongated and distinctly pointed; tergum X without paired projections (Fig. 19A, B).

Distribution. Known only from Nepal.

**Habitat.** Larvae of *C. shinichii* sp. nov. were collected from stones with algal fouling in sections with current velocity of about 0.3–0.8 m/s of a large river (10–17 m wide) within low mountains on the southern slope of the Great Himalayan Range (Bagmati Zone, Central Nepal). Investigated rivers are under the significant anthropogenic pressure and can be classified as alpha- or beta-mezosaprobic waterbodies. Apparently, larvae inhabit rhithral zones of waterbodies with relatively high average current velocity and the predominance of large stones at the bottom. Larvae of *Stenopsyche*, Glossosomatidae, Hydropsychidae, *Baetis* (s.str.) sp. and representatives of other Ephemerellidae genera were collected along with the new species.

**Type material.** *Holotype*: NEPAL: larva (on slide 647), Bagmati Zone, Shivapuri Nagarjun National Park, Melamchi River (1 km below Talamarang village), 27.844497°N, 85.557433°E, h ~ 900 m a.s.l., 4.iii.2007, Chertoprud M.V. leg. – *IN Nepa7Cinsp* [NMNH NASU]. *Paratype*: NEPAL: larva (on slide 649), same data as holotype. – *IN Nepa7Cinsp* [NMNH NASU].

*Cincticostella wangi* Selvakumar, Martynov & Subramanian, sp. nov. http://zoobank.org/C2DA1FE7-465B-4F1E-8F99-891714EF1B36 Figs 20–21

**Description. Larva.** Body length 6.0–7.7 mm. Caudal filaments length 5.0–5.5 mm. Body colour yellowish-brown (Fig. 20A).

**Head:** Ocelli, clypeus dorsal surface and vertex without tubercles and ridges (Fig. 20B). Genae moderately developed. Anteromedian emargination of labrum deep and wide (labrum height in emargination/maximum labrum height ratio -0.79) (Fig. 21C), anterior margin covered with long, thin and stout hair-like setae decreasing in length towards central notch. Dorsal surface of labrum densely covered with different hair-like setae, very short rounded scales and empty scale sockets.

Mandibles (Fig. 21A, B) with numerous long, stout hair-like setae in central part on dorsal surfaces and outer margins. Basal half of mandibles surfaces also covered with very short scales and empty scale sockets. Planate mandible: outer incisor with three teeth, inner incisor bifurcated; row of setae under mola consists of 8–9 long, stout hair-like setae. Angulate mandible with outer incisor consisting of four teeth, second one longer than others, inner incisor with three–four teeth, central ones distinctly larger. Superlinguae of hypopharynx with numerous long, stout hair-like setae on apices; lingua with very sparse and tiny setae on dorsal and ventral surfaces, anterior margin with shallow medial concave (Fig. 21D). Lingua surface near base bears irregular rows of short, pointed, stout setae (7–9); these rows subtransverse relative to the longitudinal axis of body. Maxilla (Fig. 21E) with two dentisetae; apex of maxilla



**Figure 20.** Larva of *Cincticostella wangi* Selvakumar, Martynov & Subramanian, sp. nov., holotype (**A**) and paratypes (**B** and **C**) **A** habitus, dorsal view, scale bar: 2 mm **B** head **C** thorax.

with bushy group of long stout and thin hair-like setae; inner margin of galea-lacinia with dense row of long, stout hair-like setae; surface galea-lacinia base with few, mainly long and middle-sized, stout hair-like setae near inner margin. Maxillary palp well-developed, 3-segmented, with segments I and II subequal in length; segment I slightly broader than segment II, segment III short and pointed. Joints between maxillary palp segments distinct (Fig. 21E). Segments I and II with numerous long, stout hair-like setae. Labium (Fig. 21F): ventral surface densely covered with long, stout and thin hair-like setae; dorsal surface of glossae and apices of paraglossae covered by similar setae. Labial palp 3-segmented; segments I and II flattened and subequal in length; covered with long, stout and thin, hair-like setae on ventral surfaces; segment I with group of scale sockets and scales in some of them in central part of dorsal surface. Segment III rounded apically, elongated (length/width ratio = 3.0-3.5); apex covered with few short, fine setae.



Figure 21. Larva of *Cincticostella wangi* Selvakumar, Martynov & Subramanian, sp. nov., paratypesA planate mandible B angulate mandible C labrum D hypopharynx E apical half of maxilla F labium.

**Thorax:** Pronotum expended laterally, with anterolateral angles small and projecting forward (Fig. 20C); mesonotum projections moderately developed, rounded, their outer margins not notched. Dorsal surface of thorax with scattered short, relatively strait, stout hair-like setae (Fig. 23C); thoracic nota without ridges or projections. Paired posterior projections between fore wing pads, moderately developed, rounded, cleft between them shallow and smooth; apical parts of outer margins of projections not pressed against wing pads.

Femora of all legs flattened (length/width ratio = fore femur 2.0–2.1; middle femur 2.0–2.1; hind femur 2.0–2.2), with longitudinal ridges (Fig. 22A–C). Femora of all legs longer than tibiae and tibiae longer than tarsi. Outer margins of all femora without apical projections.

Fore femur with distinct transverse band of numerous, mainly extremely long to middle-sized, stout setae with deeply bifurcated apices (some situated on chalazae) (Fig. 22A, D); another part of dorsal surface with scattered hair-like setae and several pointed, stout setae in basal area and along outer margin. Outer margin of fore femur with few stout, bifurcated and pointed, stout setae; main number of the setae situated in transverse row area. Basal half of inner margin and adjacent area of dorsal surface densely covered with spine-like setae and stout hair-like setae.

Dorsal surfaces of middle and hind femora almost completely covered with different-sized, bifurcated, stout setae, some very long (Fig. 22B, C); only longitudinal ridges and distal part of surfaces from ridges to outer margins without these stout setae. In addition, scattered hair-like setae, very small scales and empty scale sockets cover all dorsal surfaces of middle and hind femora. Outer margins of middle and hind femora covered with long, bifurcated stout setae decreasing in length towards base of femora; basal half of outer margins also with long, stout hair-like setae. Inner margins of middle and hind femora without stout setae, only with scattered hair-like setae.

Dorsal surface of all tibiae with longitudinal rows of long, bifurcated stout setae; in middle and hind legs, these rows situated closer to inner margins of tibiae. Outer margin of fore tibia with thin hair-like setae only; in middle and hind tibiae also with regular rows of long, bifurcated, stout setae.

Claws of all legs with one denticle each and several subapical setae (Fig. 22E).

**Abdomen:** Paired, pointed projections present on abdominal terga II–VIII; posterior margin of tergum VIII almost straight, its protuberances very small and smooth; posterior margin of terga IX and X without projections (Fig. 22F, G). Dorsal surfaces of terga above projections and surfaces of projections covered with short stout setae. Posterior margins of terga VIII–IX in submedian areas covered with rows of elongated, rounded apically, stout setae; posterior margin of tergum X with a discontinuous row of stout setae.

Gill III without medial transverse band of weakened membrane, dorsal lobe of gills III–V similar in shape (Fig. 22F), gill VI diminished in size compared to gills III–V, gill VII very small and wholly covered by gill VI.

Caudal filaments subequal in length, with pointed, stout setae and hair-like setae on posterior edge of each segment, setae shorter than corresponding segment.

Adults. Unknown.

**Etymology.** The new species is named in honour of Dr. T.-Q. Wang (formerly Purdue University, USA), who contributed significantly to the study of Ephemerelloidea.

**Diagnosis.** This species can be distinguished from other representatives of *C. nigra* complex by the following combination of characters: (i) genae moderately developed,



**Figure 22.** Larva of *Cincticostella wangi* Selvakumar, Martynov & Subramanian, sp. nov., paratypes **A** fore femur **B** middle femur **C** hind femur **D** stout setae of dorsal surface of fore femur **E** tarsal claw and tarsus **F** abdomen, dorsal view **G** terga VI–X.



**Figure 23.** Setae on dorsal surface of thorax of some representatives of *Cincticostella nigra* (Uéno, 1928) complex **A** dominant kind of stout setae – numerous small stout setae with divergent margins, bifurcated apices **B** dominant kind of stout setae – short, waved and hooked, stout hair-like setae **C** dominant kind of stout setae – scattered short, relatively strait, stout hair-like setae.

rounded; (ii) labrum with deep and wide anteromedian emargination (Fig. 21C); (iii) maxillary palp well-developed (Fig. 21E); (iv) segment III of maxillary palp small, pointed; (v) prothoracic anterolateral projections small projecting forward (Fig. 20C); (vi) mesonotum projections mesonotum projections moderately developed, rounded, their outer margins not notched (Fig. 20C); (vii) dorsal surface of thorax with scattered short, relatively strait, stout hair-like setae (Fig. 23C); (viii) fore femur with distinct transverse band of numerous, mainly extremely long to middle-sized, stout setae with deeply bifurcated apices (Fig. 22A, D); (ix) tarsal claw with one denticle (Fig. 22E); (x) paired, pointed projections present on abdominal terga II–VIII; posterior margin of tergum VIII with very small and smooth protuberances; posterior margin of terga IX and X without projections (Fig. 22F, G); (xi) posterior margins of terga VIII–IX in submedian areas covered with rows of elongated, rounded, stout setae (Fig. 22F, G).

Distribution. India-China border region.

**Habitat.** Cold fast-flowing stream with cobbles and gravel. The type locality of *C. wangi* sp. nov. is shown in Martynov et al. (2019: fig. 151).

**Type material.** *Holotype*: INDIA: larva, Arunachal Pradesh, Lower Subansiri District, Tale Valley, unnamed stream, 27.537201°N, 93.959883°E, h ~ 2370 m a.s.l., 14.iv.2015, Coll. K.A. Subramanian – *IN 5575/H13* [ZSI]. *Paratypes*: INDIA: 8 larvae, same data as holotype – *IN 5575/H13* [ZSI].

## Cincticostella sp. A

**Diagnosis.** Immature larva: (i) genae moderately developed, rounded; (ii) anteromedian emargination of labrum shallow (labrum height in emargination/maximum labrum height ratio -0.85); (iii) maxillary palp well-developed; (iv) segment III of maxillary palp, thin, elongated, rounded apically; (v) group of 13–16 setae different-sized, stout, hair-like situated on surface of galea-lacinia near base; (vi) prothoracic anterolateral projections



**Figure 24.** Habitats of *Cincticostella changfai* Martynov & Palatov, sp. nov. (**A**) and *Cincticostella funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov. (**B** and **C**). **A** Ramganga River (300 m above Patangoari Village), Uttarakhand State, Almora District, India **B** Rike River (vicinity of Parang Village), Arunachal Pradesh, Papumpare District, India **C** second order left tributary of the Ramganga River (10.1 km northeastwards of Chaukhutia Town), Uttarakhand State, Almora District, India.

small; (vii) mesothoracic anterolateral projections well-developed, subparallel to lateral aspect of body, not notched; (viii) surface of thorax covered with scattered short, thin, hair-like setae and few scale sockets and very short rounded scales with feathered margins in some of them; few waved and hooked setae cover mainly wing pads; (ix) dorsal surface of fore femur with sparse, transverse row of mainly long, stout setae with slightly convergent margins and bifurcated apices; (x) tarsal claw with one large denticle and several subapical setae; (xi) pairs of pointed projections present on abdominal terga II–IX; those on terga V–VIII strongest; those on tergum IX moderately developed, rounded apically.

## Distribution. Nepal.

**Remarks.** Two middle larval instars (body length 5.5–6.5 mm; caudal filaments length 5.2–5.3 mm) of this operational taxonomic unit (OTU) were collected in Nepal in 2014. However, the poor material and absence of late larval instars do not allow us to describe a new species adequately. We hope that this account will facilitate discovery of additional material of this OTU.

**Habitat.** Larvae of *Cincticostella* sp. A inhabit cold, moderate flowing springs with stones, gravel and clumps of moss. Larvae were collected from such a spring (up 1.3 m wide) in medium high mountains, on the southern slope of the Great Himalayan

Range. This was a small, lotic waterbody with relatively low water temperature (10  $^{\circ}$ C in sampling period), average current velocity (0.2–0.6 m/s), mosaic bottom and a low degree of anthropogenic pressure.

**Material examined.** NEPAL: 2 larvae (one on slide 634), Gandaki Zone, Kaski District, stream at the Tolka-Lambruk Road, 28.365000°N, 83.831667°E, h ~ 2000 m a.s.l., 27.i.2014, Chertoprud M.V., Marinskiy V.V. leg. – *IN Nepa1Cinsp* [NMNH NASU].

## Cincticostella of India and southern limit of the genus distribution

To date, nine species of the genus *Cincticostella* are known from India: *C. bifurcata* Xie, Jia, Chen, Jacobus & Zhou, 2009; *C. braaschi* Jacobus & McCafferty, 2008; *C. changfai* Martynov & Palatov, sp. nov.; *C. funki* Martynov, Selvakumar, Palatov & Vasanth sp. nov.; *C. gosei* (Allen, 1975); *C. insolta* (Allen, 1971); *C. ranga* Selvakumar & Subramanian, 2019; *C. richardi* Martynov & Palatov, 2019 and *C. wangi* Selvakumar, Martynov & Subramanian, sp. nov. (Martynov et al. 2019, new data). Distribution of the species within India falls within the Himalayan Region. In all known cases, the species inhabit coldwater streams and rivers with significant current velocity and coarse substrate.

Overall, only three of all known species of *Cincticostella* occur at latitudes that are more southern than the Himalayan Region, namely *C. gosei*, *C. insolta* and *C. femorata*. The southernmost records of *C. insolta* are from Thailand, where the species inhabits coldwater flows in northern upland region of the country (Martynov et al. 2019). *Cincticostella femorata* inhabits similar waterbodies in the Region (Martynov et al. 2019; Zheng and Zhou 2021). However, this species and *C. gosei* penetrate southwards in Thailand, where they would seem to prefer warmer biotopes (e.g. Gose 1969). These records form the southern border of the genus distribution at about 12.6°N latitude.

# Species complexes in Cincticostella

*Cincticostella* comprises at least 21 valid species (Table 2) (Jacobus and McCafferty 2008; Xie et al. 2009; Martynov et al. 2019; Auychinda et al. 2020; new data), but it remains poorly investigated. For the vast majority of species, only the larval stage is known – as is the case for all representatives of the *C. insolta* complex, except for *C. femorata* (Zhang et al. 2020; Zheng and Zhou 2021).

Allen (1975) divided all representatives of the genus into two species groups, based on larval characters: the *nigra*-group and the *insolta*-group. According to Allen (1975), representatives of the *nigra*-group lack head tubercles and their middle and hind pairs of femora are narrow, not enlarged and the margins are without serration; the *insolta*-group was characterised by possessing suboccipital head tubercles and the middle and hind pairs of femora being enlarged with serrated margins and/or protuberances. Later, the *insolta*-group was designated as the separate subgenus *Rhionella* Allen, 1980; all other species, including *C. gosei*, were placed in *Cincticostella* s.str. (Allen 1980). In the revision of Ephemerellidae genera, *Rhionella* was strictly synonymised with *Cincticostella* (Jacobus and McCafferty 2008), based on the position of its type species within the tree of other *Cincticostella* species (fig. 94 in Jacobus and McCafferty 2008).

	Characters	C. insolta complex	C. nigra complex	C. gosei complex
1	Two pairs of suboccipital tubercles	present	absent	absent
2	Serration of margins of middle and hind femora	present	absent	absent*
3	Presence of numerous large, rounded, scale sockets on body surface	present	absent	present
4	Rate of anterolateral emargination of labrum	shallow	from shallow to deep	moderate
5	Maxillary palp	reduced, articulations of segments not distinct, especially between segments I and II	mainly well-developed, articulations of all segments distinct**	absent
6	Segments I and II of labial palp	wide	wide	relatively narrow, elongated
7	Stout setae on outer margin of fore femur	several stout setae only	numerous stout setae	several stout setae only
8	Stout setae on dorsal surface of middle and hind femora	absent or up to several stout setae in basal area	surface with numerous stout setae	absent
9	Shape of hind femur	strongly or moderately widened	moderately widened	moderately widened
10	Stout setae on dorsal surface of abdominal terga and paired projections	absent	present	absent

**Table 1.** Distinguishing larval characters of *Cincticostella* species complexes.

Notes: \*Gose's (1969: figs 50 and 51) text and illustrations, which became the basis for the *C. gosei* description (Allen 1975), indicate that the outer margins of middle and hind femora have distinct serration; however, all specimens investigated by us (Fig. 15B, C) lacked distinct serrations on the femora.

\*\*According to Ishiwata (2003), specimens of *C. orientalis* from Japan have the maxillary palp reduced, but with distinct articulations of segments; specimens studied by us from Russia (Primorsky Krai) had the maxillary palp reduced, without distinct articulations.

C. insolta complex	C. gosei complex	C. nigra complex
C. bifurcata Xie, Jia, Chen, Jacobus & Zhou, 2009	C. gosei (Allen,	C. changfai Martynov & Palatov, sp. nov.
C. braaschi Jacobus & McCafferty, 2008	1975)	C. colossa Kang & Yang, 1995
C. femorata (Tshernova, 1972)		C. corpulenta (Braasch, 1981)
C. insolta (Allen, 1971)		C. elongatula (McLachlan, 1875)
C. ranga Selvakumar & Subramanian, 2019		C. funki Martynov, Selvakumar, Palatov & Vasanth sp. nov.
C. richardi Martynov & Palatov, 2019		C. fusca Kang & Yang, 1995
C. sivaramakrishnani Martynov & Palatov, 2019		C. levanidovae (Tshernova, 1952)
C. tornata Auychinda & Gattolliat, 2020		C. nigra (Uéno, 1928)
		C. orientalis (Tshernova, 1952)
		C. shinichii Martynov & Palatov, sp. nov.
		C. szechuanensis Xie, Jia, Chen, Jacobus & Zhou, 2009
		C. wangi Selvakumar, Martynov & Subramanian, sp. nov.
		Cincticostella sp. A

Table 2. Systematic structure of Cincticostella.

In light of the several new species described since Jacobus and McCafferty (2008) revised the generic classification of the group and considering possible polyphyly, we re-evaluated the larval morphology of *Cincticostella* species for understanding the difference of complexes and expediency of their designation; available material and literature data were used for this analysis (Tshernova 1952; Allen 1975; Braasch 1981; Kang and Yang 1995; Ishiwata 2003; Jacobus and McCafferty 2008; Xie et al. 2009; Martynov et al. 2019; Auychinda et al. 2020; Zhang et al. 2020; Zheng and Zhou 2021).

Based on our research, we conclude that the establishment of the discussed complexes was for expediency. In fact, the *C. insolta* and *C. nigra* complexes are more distanced morphologically from each other than previously thought (see Allen 1975), based on our new distinguishing characters (see characters 3–10 in Table 1, below). All but one species of *Cincticostella* clearly fit into the *C. insolta* or *C. nigra* complexes as here defined; only *C. gosei* has a combination of characters that does not completely fit into any of these two complexes. Therefore, the monotypic *C. gosei* complex is proposed herein (Tables 1 and 2). We acknowledge possible paraphyly of the other complexes.

The following morphological features place *C. gosei* closer to the *C. insolta* complex than to the *C. nigra* complex: presence of numerous large, rounded, scale sockets on body surface, absence of maxillary palp (this character state may prove to be related to significant reduction of the maxillary palp, including reduction of segments' articulations), number of stout setae on the outer margin of the fore femur and absence of numerous stout setae on dorsal surfaces of the middle and hind femora, complete absence of stout setae on dorsal surfaces of abdominal terga and paired projections.

Ogden et al. (2009) included only two species from this genus in their combined evidence for phylogenetic analyses of Ephemerellidae. Further investigation of imaginal characters after new stage associations (e.g. Zhang et al. 2020; Zheng and Zhou 2021) and molecular investigation (Martynov, in progress) will help to better understand the structure and relationships of species in the genus *Cincticostella*.

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RESEARCH ARTICLE



# Two new species of the millipede genus *Tylopus* Jeekel, 1968 from Shan State, Myanmar (Diplopoda, Polydesmida, Paradoxosomatidae)

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## Abstract

The predominantly Indochinese to southern Chinese millipede genus *Tylopus* presently comprises 76 described species, including two new, *T. monticola* **sp. nov.** and *T. sutchariti* **sp. nov.**, both described and illustrated based on material from a limestone mountain in Taunggyi District, Shan State, Myanmar. Both new species have been found to occur syntopically near limestone caves and are assumed to be narrowly endemic to the Taunggyi Mountains, southwestern Shan State, Myanmar. A key to all six *Tylopus* species known to occur in Myanmar is provided, and their distributions are also mapped.

## Keywords

Key, map, Paradoxosomatinae, Sulciferini, taxonomy, Tylopus monticola sp. nov., Tylopus sutchariti sp. nov.

# Introduction

The predominantly Indochinese to southern Chinese millipede genus *Tylopus* Jeekel, 1968, has long been recognized as one of the most speciose and widespread not only within the family Paradoxosomatidae, but also in the entire class Diplopoda (Likhi-trakarn et al. 2010, 2016; Golovatch 2019). This genus, formerly known as *Agnesia* Attems, 1953, has been reviewed and rediagnosed several times (Jeekel 1965, 1968; Golovatch and Enghoff 1993; Likhitrakarn et al. 2010), but most taxonomic works have focused on adding new species descriptions, presenting a key and a distribution map to reveal the high diversity of the genus (Nguyen 2012; Liu and Luo 2013, Golovatch 2013, 2014, 2018, 2019, 2020; Likhitrakarn et al. 2014, 2016). At the moment, 74 species of *Tylopus* are known from Indochina and the adjacent parts of southern China and Myanmar (formerly Burma). Most of the known species diversity of *Tylopus* is encountered in Thailand and Vietnam.

Myanmar forms part of the Indo-Burma biodiversity hotspot (Myers et al. 2000; Sodhi et al. 2004). It supports extremely high biodiversity and abundant natural resources, including millipedes (Diplopoda). At present, Myanmar's known millipede diversity has gradually been revealed to amount to 96 species from 36 genera, 13 families and eight orders, containing 74 endemic and only five widespread synanthropic species (Likhitrakarn et al. 2017, 2018; Pimvichai et al. 2018; Srisonchai et al. 2018a, b). Furthermore, there are 527 millipede records from Burmese amber (Burmite; Cretaceous, ca 100 Mya), representing 13 of the 16 extant orders. Only the orders Sphaerotheriida, Julida and Siphonocryptida have not yet been reported from Burmite. Against this background, no fossil of the family Paradoxosomatidae, one of the largest and most diverse in the entire class Diplopoda globally, has previously been recorded from Myanmar (Wesener and Moritz 2018).

Four *Tylopus* species, all endemic, have been found in Myanmar. The first two species of *Tylopus* to be revealed from that country were *Tylopus doriae* (Pocock, 1895) and *T. silvestris* (Pocock, 1895), both described by Pocock (1895). It was 120+ years later that two further species were added: *T. brehieri* Golovatch, VandenSpiegel & Semenyuk, 2016 and *T. punctus* Likhitrakarn, Golovatch & Panha, 2016 (Golovatch et al. 2016; Likhitrakarn et al. 2016). Myanmar's climate, geology, topography and, partly, its biota are very similar to those of the neighbouring Thailand; consequently the *Tylopus* species. This is undoubtedly due to many areas of Myanmar still being difficult to access, remaining poorly collected and often even dangerous, coupled with local natural history research being rudimentary and secluded. Hardly surprisingly, the arthropod fauna of Myanmar is poorly known and understudied. Studies on the millipede diversity of Myanmar have recently resumed since the British colonial times, chiefly due to the activities of the Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, headed by one of us (SP).

The present paper puts on record two new species of *Tylopus* collected from a limestone mountain in the Taunggyi District, southwestern Shan State, Myanmar. A key to and updated distributions of all six species of *Tylopus* currently known to occur in Myanmar are also provided.

# Materials and methods

New material was collected in Myanmar, especially in limestone mountain areas, with the support of Fauna & Flora International (**FFI**) in 2015–2017, collaborating with the Animal Systematics Research Unit (**ASRU**), Chulalongkorn University. The collecting activities took place under the limestone conservation projects which aim to protect biodiversity in limestone habitats (Grismer et al. 2018a, b, c; Fauna & Flora International 2021).

Live animals were photographed in the laboratory using a Nikon 700D digital camera with a Nikon AF-S VR 105 mm macro lens. Specimens were preserved in 75% ethanol, and morphological observations were carried out in the laboratory using an Olympus stereo microscope. Scanning electron micrographs (SEM) of gonopods coated with gold were taken using a JEOL, JSM–5410 LV microscope, returned to alcohol after SEM examination. Digital images of the specimens were taken in the laboratory and assembled using the "Cell<sup>D</sup>" automontage software of the Olympus Soft Imaging Solution GmbH package. In addition, line drawings of gonopod characters were also prepared. Both holotypes, as well as most of the paratypes are housed in the Museum of Zoology, Chulalongkorn University (CUMZ), Bangkok, Thailand; some paratypes are donated to the collection of the Zoological Museum, State University of Moscow (ZMUM), Russia, as indicated in the text.

Collecting sites were located by GPS using the WGS84 datum. The distribution maps of all *Tylopus* species recorded from Myanmar were executed using QGIS 3.18.0 (QGIS Development Team 2021). Google satellite maps were downloaded via the QuickMapServices plugin. The images were enhanced and arranged in plates with Adobe Photoshop CS6 software.

In the synonymy sections, D stands for the original description and/or subsequent descriptive notes, K for the appearance in a key, L for the appearance in a species list, and M for a mention.

Terminology concerning gonopodal and somatic structures mostly follows Golovatch and Enghoff (1993) and Likhitrakarn et al. (2010, 2016). Abbreviations of certain gonopodal structures in the figures are explained both in the text and figure captions.

# Taxonomy

Family Paradoxosomatidae Daday, 1889 Subfamily Paradoxosomatinae Daday, 1889 Tribe Sulciferini Attems, 1898

# Genus Tylopus Jeekel, 1968

# Tylopus brehieri Golovatch, VandenSpiegel & Semenyuk, 2016

Tylopus brehieri Golovatch, VandenSpiegel & Semenyuk, 2016: 335 (D).

**Record from Myanmar.** Shan State, Kyauk Khaung (= Stone Cave) (Golovatch et al. 2016).

## Tylopus doriae (Pocock, 1895)

Orthomorpha doriae Pocock, 1895: 823 (D).
Orthomorpha Doriae (sic!) – Attems, 1898: 339 (L, K).
Orthomorpha (Kalorthomorpha) doriae – Attems, 1936: 204 (L).
Orthomorpha (Orthomorpha) doriae – Attems, 1937: 80 (D, K).
Orthomorpha doriae – Weidner, 1960: 85 (L).
Agnesia doriae – Jeekel, 1965: 100 (D, K).
Tylopus doriae – Jeekel, 1968: 60 (M); Golovatch and Enghoff 1993: 103 (D, K); Enghoff 2005: 99 (R); Likhitrakarn et al. 2010: 25 (L, K); 2014: 65 (L, K); 2016: 35 (L, K); Nguyen and Sierwald 2013: 1298 (L).

**Records from Myanmar.** Yado, 1000–1400 m; Bia-Po, 1000–1200 m, Meteleo, 900–1200; Puepoli, 900–1200 m (Pocock 1895).

**Remark.** Also found in Doi Suthep National Park (1400–1500 m), Chiang Mai Province, Thailand (Enghoff 2005).

## Tylopus punctus Likhitrakarn, Golovatch & Panha, 2016

Tylopus punctus Likhitrakarn, Golovatch & Panha, 2016: 29 (D).

**Record from Myanmar.** Mintaingbin Forest Camp, ca 35 km north of Aungban, Chan State, 20°55'20"N, 96°33'60"E, ca 1300 m a.s.l. (Likhitrakarn et al. 2016).

## *Tylopus silvestris* (Pocock, 1895)

*Orthomorpha silvestris* Pocock, 1895: 824 (D). *Orthomorpha silvestris* – Attems, 1914: 238 (L); 1936: 205 (L); 1937: 94 (L). *Agnesia silvestris* – Jeekel, 1965: 104 (D, K). *Tylopus silvestris* – Jeekel 1968: 60 (M); Golovatch and Enghoff 1993: 90 (M, K); Likhitrakarn et al. 2010: 26 (L, K); 2016: 38 (L, K); Nguyen and Sierwald 2013: 1300 (L).

Record from Myanmar. Village of Thao (Carin Ghecu, 1200–1400 m) (Pocock 1895).

## Tylopus monticola sp. nov.

http://zoobank.org/FD497FBD-67B7-4171-9905-ED6D2011DF51 Figs 1A, 2–4

**Material examined.** *Holotype:* MYANMAR – **Shan State** • 3; Taunggyi District, near Montawa Cave; elev. 1204 m; 20°45'15.9"N, 97°01'03.4"E; 21 Sep. 2016; J. Sutcharit, R. Srisonchai leg.; CUMZ. *Paratypes:* MYANMAR – **Shan State** • 3 9; same collection data as holotype; CUMZ • 1 9; same collection data as holotype; ZMUM • 3 9; near Aye Say Tee Cave; elev. 1583 m; 20°47'29.5"N, 97°03'01.6"E; 21 Sep. 2016; J. Sutcharit, R. Srisonchai leg.; CUMZ • 1 3; Parpant area, outside the cave; elev. 1159 m; 20°15'03.7"N, 97°14'23.9"E; 23 Sep. 2016; J. Sutcharit, R. Srisonchai leg.; CUMZ • 1 3; Same collection data as previous; ZMUM.

**Diagnosis.** Using the latest key to *Tylopus* species (Likhitrakarn et al. 2016), as well as the information concerning all 12 congeners described since (Golovatch et al. 2016; Golovatch 2018, 2019, 2020; Golovatch and Semenyuk 2018), *T. monticola* sp. nov. keys out to *T. rugosus* Golovatch & Enghoff, 1993 on account of the particularly strong similarities in the gonopodal structure (Fig. 8). Thus, even though they both share most of the somatic and gonopodal features, the new species differs in the large and long process z with a serrate edge along the dorsal margin, which protrudes beyond the apicolateral lobe (I) (Figs 3B–D, 4A, B) (vs. smaller and not protruding beyond I) (Fig. 8B, C), all  $\mathcal{S}$  legs with the prefemora swollen laterally except for leg 1 (vs. except for legs 1 and 2), coupled with the pleurosternal carinae complete crests with an evident, sharp, caudal denticle produced past the rear tergal margin on segments 4–7, gradually decreasing in size until segments 15(16) ( $\mathcal{S}$ ) or 13(12) ( $\mathcal{P}$ ) (Fig. 2B, D, E) (vs. same, but gradually decreasing in size until segment 18).

**Description.** Length of holotype ca 30 mm; adult paratypes 29–31 ( $\Diamond$ ) or 32–35 mm ( $\wp$ ), width of midbody pro- and metazonae of holotype, 2.4 and 3.6 mm; adult paratypes 2.4–2.5 and 3.4–3.7 mm ( $\Diamond$ ) or 2.9–3.5 and 3.7–4.5 mm ( $\wp$ ), respectively.

Colouration of live animals dark brown (Fig. 1A); calluses of paraterga, venter and legs lighter brown; colouration of alcohol material after two years of preservation faded to dark brown; head, antennae and tip of epiproct light brown, calluses of paraterga yellowish brown to pallid, venter and legs light brown to light yellowish (Fig. 2).

Clypeolabral region and vertex sparsely setose, epicranial suture distinct. Antennae short (Figs 1A, 2A, B), reaching body segment 3 ( $\Diamond$ ) or 2 ( $\bigcirc$ ) when stretched dorsally.



**Figure 1.** Habitus, live colouration **A** *Tylopus monticola* sp. nov.,  $\Diamond$  paratype (CUMZ) **B** *Tylopus sutchariti* sp. nov.,  $\Diamond$  holotype (CUMZ). Scale bars: 1 cm.

In width, head < segment 3 < 4 < 5 < 6 < collum < segment 2 < 7-16 ( $\mathcal{E}$ ,  $\mathcal{Q}$ ); thereafter body gently and gradually tapering. Collum with three transverse rows of strong setae: 3+3 anterior, 2+2 intermediate, and 3+3 posterior; a small lateral incision near midway; caudal corner of paraterga rounded, slightly declined ventrad, not produced past rear tergal margin (Fig. 2A, B).

Tegument rather smooth and shining, prozonae very finely shagreened, metaterga mainly smooth, but often rugulose; surface below paraterga finely microgranulate (Fig. 2A–F). Postcollum metaterga with two transverse rows of rather long setae: 2+2in anterior and 3+3 in posterior row, the latter often abraded, but then readily traceable as insertion points. Tergal setae long, strong, slender, about 1/3 metatergal length. Axial line visible both on pro- and metazonae.

Paraterga strongly developed (Fig. 2A–F), especially well so in  $3^{\circ}$ , subhorizontal, slightly upturned posteriorly, always lying high, at upper 1/3 of midbody height, but remaining below dorsum; anterior edge well-developed, mostly regularly rounded and narrowly bordered, fused to callus; caudal corner narrowly rounded, extending increasingly past rear tergal margin, especially strongly so on segments 15–19; in segments 16–19, tips strongly curved mesad, posterior edge slightly oblique (Fig. 2A, C, F); paraterga very thin blunt blades in lateral view, a little thicker only on pore-bearing segments (Fig. 2D). Calluses on paraterga delimited by a sulcus only dorsally. Paraterga 2 broad, lateral edge with three evident incisions: one in anterior 1/3, one at midway, and



**Figure 2.** *Tylopus monticola* sp. nov.,  $\delta$  holotype (CUMZ) **A**, **B** anterior part of body, dorsal and lateral views, respectively **C**, **D** segments 10 and 11, dorsal and lateral views, respectively **E–G** posterior part of body, lateral, subdorsal and subventral views, respectively **H**, **I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

one at posterior 1/3; anterior incision particularly evident. Paraterga 3 and 4 with two small incisions at lateral edge (Fig. 2A), one in anterior 1/3, the other at posterior 1/3; anterior incision also particularly evident. Lateral edge of paraterga of following segments with two small incisions, one in anterior 1/3, the other at midway, caudal incision being smaller in pore-bearing segments (Fig. 2C). Ozopores evident, lateral, lying in an ovoid groove at about 1/3 metatergal length in front of posterior edge of metaterga (Fig. 2D). Transverse sulcus usually distinct (Fig. 2A, C, F), slightly incomplete on segment 18, complete and clearly visible on metaterga 5–17, deep, reaching the bases of paraterga, arcuate, faintly beaded at bottom. Stricture between pro- and metazonae narrow, shallow, beaded at bottom down to base of paraterga (Fig. 2A, C, F). Pleurosternal carinae complete crests on segment 2–3(4) (Fig. 2B), with an evident and sharp denticle caudally on segments 4(5)-7 ( $\mathcal{J}$ ,  $\mathcal{Q}$ ), thereafter increasingly well reduced and remaining only a small sharp caudal tooth until segment 15(16) ( $\mathcal{J}$ ) or 13(12) ( $\mathcal{Q}$ ), thereafter missing (Fig. 2B, D, E). Epiproct (Fig. 2E–G) conical, flattened dorsoventrally, subtruncate, with two evident apical papillae directed caudally, both pointed at



**Figure 3.** *Tylopus monticola* sp. nov., ∂ holotype (CUMZ), left gonopod **A**, **B** mesal and lateral views, respectively **C–F** distal part, submesal, lateral, suboral and subcaudal views, respectively. Scale bars: 0.2 mm.

tip; pre-apical papillae evident, lying close to tip. Hypoproct subtrapeziform (Fig. 2G), small setiferous knobs at caudal edge well-separated and evident.

Sterna densely setose, without modifications (Fig. 2G); cross-impressions shallow; a deeply notched sternal lobe between  $\bigcirc$  coxae 4 (Fig. 2H, I). Legs long and slender, midbody ones ca 1.4–1.5 ( $\bigcirc$ ) or 0.9–1.0 ( $\bigcirc$ ) as long as body height; all  $\bigcirc$  legs except leg 1 with prefemora swollen laterally; femora and tibiae with particularly dense setae and ventral microgranulations; legs on segments 7–18 with an evident adenostyle (tubercle) medially on each postfemur and tibia (Fig. 4C); tarsal brushes absent.

Gonopods (Figs 3, 4A, B) simple; coxite slightly curved caudad, sparsely setose distoventrally. Prefemorite densely setose, about 1/3 as long as femorite + "postfemoral" part. Femorite rather stout, expanded distad, suberect, showing a distinct mesal groove/hollow (**g**); apicolateral lobe (**l**) simple; process **z** large and long, serrate along



**Figure 4.** *Tylopus monticola* sp. nov.,  $\mathcal{S}$  holotype (CUMZ) **A**, **B** left gonopod, mesal and lateral views, respectively **C** leg of segment 10. Abbreviations: **g** mesal groove/hollow of femorite, **h** short and stout process of femorite, **l** apicolateral lobe of femorite, **z** serrate process of femorite. Scale bars: 0.5 mm.

dorsal margin and protruding beyond apicolateral lobe (**l**); process **h** short and stout, suberect, with a narrowly rounded tip; solenophore long and slender, typically coiled, tip subtruncate.

**Name.** To emphasize the habitats where this new species was discovered; "*monti-cola*" meaning a mountain-dweller or a highlander; noun in apposition.

**Remark.** The species was found quite far away (about 120 air-km) from the type locality of the most similar species, *T. rugosus* Golovatch & Enghoff, 1993 (Fig. 9). Both new species described here have been found to occur syntopically.

## Tylopus sutchariti sp. nov.

http://zoobank.org/5385A3A0-C129-41F3-9A8C-C3910EF9178C Figs 1B, 5–7

**Material examined.** *Holotype*: MYANMAR – **Shan State** • ♂; Taunggyi District, near Montawa Cave; elev. 1204 m; 20°45'15.9"N, 97°01'03.4"E; 21 Sep. 2016; R. Srison-chai leg.; CUMZ. *Paratype*: MYANMAR – **Shan State** • 1 ♀; same collection data as holotype; CUMZ.

**Diagnosis.** This new species comes to a dead end in couplet 5 in the latest key to *Ty-lopus* species (Likhitrakarn et al. 2016), but it seems to be particularly similar to the later



**Figure 5.** *Tylopus sutchariti* sp. nov.,  $\mathcal{J}$  holotype (CUMZ) **A**, **B** anterior part of body, dorsal and lateral views, respectively **C**, **D** segments 10 and 11, dorsal and lateral views, respectively **E–G** posterior part of body, lateral, subdorsal and subventral views, respectively **H**, **I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

described and grossly sympatric *T. brehieri* Golovatch, VandenSpiegel & Semenyuk, 2016, especially in its gonopod conformation. Both species compared come from Shan State, Myanmar (Golovatch et al. 2016), but *T. sutchariti* sp. nov. differs in the presence of a small and triangular gonopod process **h** (vs. absent), and the large and subtrapeziform apicolateral lobe (**l**) with a smooth apical margin (Figs 6B, D, 7B) (vs. a subtriangular **l** with an apically rugose and denticulate margin), as well as the pleurosternal carinae being complete crests with a caudal tooth clearly visible until segments 16 ( $\eth$ ) or 13 ( $\updownarrow$ ), thereafter missing (Fig. 5B, D, E) (vs. visible until segment 10), while the sternal lobe between  $\eth$  coxae 4 is deeply notched (Fig. 5H, I) (vs. prominent and subquadrate).

**Description.** Length 24.1 ( $\mathcal{C}$ ) or 22.5 mm ( $\mathcal{Q}$ ), width of midbody pro- and metazona 1.8 and 2.7 mm ( $\mathcal{C}$ ) or 2.2 and 2.6 mm ( $\mathcal{Q}$ ), respectively.

Colouration of live animals dark brown (Fig. 1B); venter and legs brown; colouration of alcohol material after two years of preservation blackish, calluses of paraterga yellowish brown, head and antennae dark brown, venter and legs light yellowish, increasingly darker brown distally (Fig. 5).



**Figure 6.** *Tylopus sutchariti* sp. nov.,  $\mathcal{E}$  holotype (CUMZ), left gonopod **A**, **B** mesal and lateral views, respectively **C–F** distal part, submesal, sublateral, suboral and subcaudal views, respectively. Scale bars: 0.2 mm.

Clypeolabral region and vertex sparsely setose, epicranial suture distinct. Antennae short (Figs 1B, 5B), reaching body segment 3 ( $\mathcal{F}$ ) or surpassing body segment 2 ( $\mathcal{Q}$ ) when stretched dorsally. In width, head < segment 3 < 4 < 5 < collum < segment 2 < 6–17 ( $\mathcal{F}$ ,  $\mathcal{Q}$ ); thereafter body gently and gradually tapering. Collum with three transverse rows of strong setae: 3+3 anterior, 1+1 intermediate, and 3+3 posterior; a small lateral incision near midway; caudal corner of paraterga rounded, slightly declined ventrad, not surpassing rear tergal margin (Fig. 5B).

Tegument rather smooth and shining, prozonae very finely shagreened, metaterga smooth and finely rugulose; surface below paraterga finely microgranulate (Fig. 5A–F). Postcollum metaterga with two transverse rows of rather long setae: 2+2 in anterior and 2(3)+2(3) in posterior row, the latter often abraded, but then readily traceable as



**Figure 7.** *Tylopus sutchariti* sp. nov.,  $\Diamond$  holotype (CUMZ) **A, B** left gonopod, mesal and lateral views, respectively **C** leg of segment 10. Abbreviations: **g** mesal groove/hollow of femorite, **h** short and triangular process of femorite, **l** apicolateral lobe of femorite, **m** dorsoapical lobe of femorite, **sl** tip of solenomere, **sph** solenophore, **z** short and simple process of femorite. Scale bars: 0.5 mm.

insertion points. Tergal setae long, strong, slender, about 1/3 metatergal length. Axial line visible only on metazonae.

Paraterga strongly developed (Fig. 5A–F), especially well so in  $3^{\circ}$ , set high, at upper 1/3 of midbody height, slightly upturned, but remaining below dorsum; anterior edge well-developed, mostly regularly rounded and narrowly bordered, continuous with callus; caudal corner narrowly rounded to fully pointed, extending increasingly past rear tergal margin, especially well so on segments 15–19; on segments 16–19, tips strongly curved mesad, posterior edge slightly oblique (Fig. 5A, C, F); paraterga very thin blunt blades in lateral view, a little thicker only on pore-bearing segments (Fig. 5D). Calluses on paraterga delimited by a sulcus both dorsally and ventrally. Paraterga 2 broad, horizontal, anterior edge angular, lateral edge with three evident incisions, one in anterior 1/3, middle one at midway, caudal incision near tip; anterior incision particularly evident. Paraterga 3 and 4 with two small incisions at lateral edge (Fig. 5A), one in anterior 1/3, the other at midway, anterior one also particularly evident. Following segments each with lateral edge showing an evident incision near front 1/3 (Fig. 5C). Ozopores evident, lateral, lying in an ovoid groove at about 1/3 metatergal length in front of posterior edge of metaterga (Fig. 5D). Transverse sulcus usually distinct



**Figure 8.** Gonopod structure of *Tylopus rugosus* Golovatch & Enghoff, 1993, ♂ holotype, left gonopod **A–C** mesal, lateral and dorsal views, respectively. Abbreviations: **h** strong hook-shaped process of femorite, **l** apicolateral lobe of femorite, **z** serrate process of femorite. Scale bar: 0.5 mm (after Golovatch and Enghoff 1993).

(Fig. 5A, C, F), complete and visible on metaterga 5–18, deep, narrow, reaching bases of paraterga, line-shaped, clearly beaded at bottom. Stricture between pro- and metazonae wide, deep, clearly ribbed at bottom down to base of paraterga (Fig. 5A, C, F). Pleurosternal carinae complete crests on segment 2–4 (Fig. 5B), with anteriorly bulged crests and a sharp denticle caudally on segments 5–8 ( $\mathcal{F}$ ,  $\mathcal{Q}$ ), thereafter increasingly reduced and broken, remaining only a small sharp caudal tooth until segment 16 ( $\mathcal{F}$ ) or 13 ( $\mathcal{Q}$ ), thereafter missing (Fig. 5B, D, E). Epiproct (Fig. 5E–G) conical, flattened dorsoventrally, subtruncate, with two evident apical papillae directed caudally, both



**Figure 9.** Distributions of *Tylopus* species recorded from Myanmar. Pink circle: *T. brehieri* Golovatch, VandenSpiegel & Semenyuk, 2016; Red Asterisk: *T. punctus* Likhitrakarn, Golovatch & Panha, 2016; Yellow inverted triangle: *T. monticola* sp. nov.; Blue square: *T. sutchariti* sp. nov. and *T. monticola* sp. nov.; Orange diamond: *T. silvestris* (Pocock, 1895); Green triangle: *T. doriae* (Pocock, 1895).

pointed at tip; pre-apical papillae evident, lying close to tip. Hypoproct roundly sub-trapeziform (Fig. 5G), small setiferous knobs at caudal edge well-separated and evident.

Sterna densely setose, without modifications (Fig. 5G); cross-impressions shallow; a deeply notched sternal lobe between  $\Im$  coxae 4 (Fig. 5H, I). Legs long and slender, midbody ones ca 1.4–1.5 ( $\Im$ ) or 1.1–1.2 ( $\Im$ ) as long as body height;  $\Im$  legs of segments 4–17 with prefemora distinctly swollen laterally;  $\Im$  legs of segments 2–16 each with femur, postfemur, tibia and tarsus with particularly dense setae and carrying ventral microgranulations (Fig. 7C), tarsal brushes absent.

Gonopods (Figs 6, 7A, B) complex; coxa slightly curved caudad, sparsely setose distoventrally. Prefemorite as usual, densely setose, about 1/2 as long as femorite + "postfemoral" part. Femorite rather stout, suberect, expanded distad, showing a distinct mesal groove/hollow (**g**) and a prominent, rounded, dorso-apical lobe (**m**), apicolateral
lobe (**l**) large, subtrapeziform, with a smooth apical margin, mostly delimited at base by a transverse sulcus, with process **h** short and triangular; process (**z**) rather short and simple, narrowly rounded at tip. Solenophore (**sph**) typically coiled, lamellar, expanded apically into a subtruncate lobe, almost fully sheathing a similarly long, flagelliform solenomere, with only its tip (**sl**) being exposed.

**Name.** To honour Dr. Chirasak Sutcharit, Professor at the Department of Biology of the Chulalongkorn University, Bangkok, who participated in collecting the type series.

**Remark.** Both new species described here have been found to occur syntopically (Fig. 9).

# Key to species of Tylopus currently known to occur in Myanmar, chiefly based on ${\ensuremath{\varDelta}}$ characters

1	All $\Diamond$ prefemora normal, not bulged laterally2
_	Most d prefemora clearly swollen laterally (Figs 4C, 7C)
2	Body smaller: width up to 2.1–2.5 mm. Midbody paratergal corner very nar-
	rowly rounded and not protruding caudad past rear margin. Gonopod pro-
	cess <b>h</b> small and pointed
_	Body larger: width 3.0 mm. Midbody paratergal corner nearly pointed and
	protruding caudad past rear margin. Gonopod process <b>h</b> absent
	<i>T. silvestris</i> (Pocock, 1895)
3	Paratergal calluses with only one incision. Gonopod postfemoral lobe 1
	much longer than broad; area basal to I delimited by a distinct cingulum
	(Figs 6B, D, 7B)
_	Paratergal calluses with two incisions. Gonopod postfemoral lobe l either as
	long as broad or longer; no cingulum basal to l (Figs 3B, D, 4B)5
4	Sternal lobe between $\eth$ coxae 4 deeply notched (Fig. 5H, I). Gonopod pro-
	cess h small and triangular, while apicolateral lobe (1) large and subtrapezi-
	form with a smooth apical margin (Figs 6B, D, 7B) T. sutchariti sp. nov.
-	Sternal lobe between $\eth$ coxae 4 prominent and subquadrate. Gonopod pro-
	cess $\mathbf{h}$ absent, while apicolateral lobe (1) subtriangular with an apically rugose
	and denticulate margin
	T. brehieri Golovatch, VandenSpiegel & Semenyuk, 2016
5	Gonopod process $z$ large and long, protruding beyond apicolateral lobe (I)
	(Figs 3B–D, 4B). All $\Diamond$ legs with prefemora swollen laterally except for leg 1.
	Pleurosternal carinae present before segment 16 T. monticola sp. nov.
_	Gonopod process $z$ smaller and not protruding beyond apicolateral lobe (I)
	(Fig. 8B, C). All $\circlearrowleft$ legs with prefemora swollen laterally except for legs 1 and
	2. Pleurosternal carinae present until segment 18
	T. rugosus Golovatch & Enghoff, 1993

## Discussion

Of a total of 76 species of *Tylopus* presently known globally, including two new described above, most of the diversity (31 species, or >41%) comes from Thailand, followed by Vietnam (21 species), Laos (12 species), southern China (8 species) and Myanmar (6 species). Almost all *Tylopus* species appear to be confined to montane woodlands exceeding 500 m in elevation (Likhitrakarn et al. 2016). Furthermore, most of them (92%) are short-range endemics or confined to a small area (< 4000 km<sup>2</sup>). Many species occur sympatrically, some even syntopically, but then they tend to differ in the timing of sexual maturity or mating season. For instance, the Doi Inthanon and Doi Suthep mountains, both in northern Thailand, support at least 10 congeners each (Likhitrakarn et al. 2014). Unfortunately, most of the known species (75%) have only been collected once and from a single locality.

The genus *Tylopus* seems to be particularly similar to two genera of the large and mostly Asian tribe Sulciferini, viz. *Oxidus* Cook, 1911 and *Hedinomorpha* Verhoeff, 1934. All three share the presence of a unique gonopodal apicolateral lobe (I) separated from the femorite by a more or less distinct, basal, (sub)transverse sulcus. Golovatch (2021) has recently discussed the morphological differences between these three genera, *Tylopus* being distinct primarily in the particularly elaborate gonopodal telopodite. The distinction of *Tylopus* from *Oxidus* has also been confirmed by molecular evidence (Nguyen et al. 2017).

In addition to putting on record two new, presumably narrowly endemic species of *Tylopus* from the Taunggyi Mountains, southwestern Shan State, Myanmar, and thus bringing the number of *Tylopus* spp. of Myanmar to a total of six, we map their distributions (Fig. 9). Only *T. doriae* has been recorded from two countries, Myanmar and Thailand, whereas the other five seem to be more strongly localized. Given that Myanmar remains one of the largest, but least-surveyed countries in the East Indies, and considering the large limestone montane areas it harbours, there can hardly be any doubt that more species of *Tylopus* will be found there in the future.

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## New or little-known Boreoheptagyia (Diptera, Chironomidae) in China inferred from morphology and DNA barcodes

**RESEARCH ARTICLE** 

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## Abstract

The male adult of *Boreoheptagyia zhengi* Lin & Liu, **sp. nov.** is described and illustrated based on material collected in China. Associated morphological characteristics and reference to its DNA barcode are provided. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) is newly recorded from China based on both a male and female, with additional associated data on the DNA barcode of the male adult. A neighbor-joining tree based on available *Boreoheptagyia* DNA barcodes and a key to the adults of *Boreoheptagyia* from China are given.

## Keywords

COI, Diamesinae, integrative taxonomy, new species, non-biting midges

## Introduction

Brundin (1966) erected the genus *Boreoheptagyia* with *Heptagyia rugosa* Saunders, 1930 as type species by original designation. Larval populations of this genus live in cool, fast-flowing streams and other harsh environments (Thienemann 1954; Oliver 1989).

At present, the genus includes 24 valid species worldwide (Ashe and O'Connor 2009). Among them, 17 are restricted to the Palaearctic Region including the two recently described species *B. joeli* Makarchenko, 2020 and *B. ortladamellica* Rossaro, 2017 (Rossaro 2017; Makarchenko et al. 2020), seven to the Oriental Region and one to the Nearctic Region. Only *B. rotunda* Serra-Tosio, 1983 occurs in both Palaearctic and Oriental regions (Ashe and O'Connor 2009; Rossaro 2017). Seven species are currently reported from China: *B. alulasetosa* Makarchenko, Wu & Wang, 2008, *B. ambigua* Makarchenko, Wu & Wang, 2008, *B. brevitarsis* (Tokunaga, 1936), *B. similis* (Chaudhuri & Ghosh, 1981), *B. tibetica* Makarchenko, Wang & Willassen, 1996, *B. xinglongiensis* Makarchenko, Wu & Wang, 2008; and *B. joeli* Makarchenko, 2020 (Makarchenko et al. 1996; Wang 2000; Makarchenko et al. 2008; Makarchenko et al. 2020).

The DNA barcode corresponding to the 658-bp fragment of the mitochondrial gene cytochrome *c* oxidase I (COI) has been identified as the core of a global bio-identification system at the species level (Hebert et al. 2003a, b) and has proved to be useful in the delimitation of non-biting midge species and has provided important evidence to confirm new species (Anderson et al. 2013; Lin et al. 2015; Giłka et al. 2018; Lin et al. 2018; Song et al. 2018; Lin et al. 2019; Liu et al. 2021).

In the present study, morphology and the DNA barcode of *B. zhengi* Lin & Liu, sp. nov. are provided based on material collected in Yunnan Province, China. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) is newly recorded from China based on a male and female, the latter was associated with the male by standard DNA barcodes. DNA barcode analysis including the partial COI sequences of species of genus *Boreoheptagyia* is conducted. A key to the known adults of *Boreoheptagyia* from China is also given.

## Materials and methods

The examined adults were preserved in 85% ethanol and stored in the dark at 4 °C before morphological and molecular analyses. Genomic DNA was extracted from the thorax and head using a Qiagen DNA Blood and Tissue Kit at Nankai University, Tianjin, China (**NKU**), following the standard protocol (Lin et al. 2018) except for the final elution volume of 100  $\mu$ l. After DNA extraction, the exoskeleton of each specimen was mounted in Euparal on a microscope slide together with the corresponding wings, legs, antennae and abdomen, following the procedures outlined by Sæther (1969). Morphological terminology follows Sæther (1980).

Digital photographs of the mounted specimens were taken at 300-dpi resolution using a Nikon Digital Sight DS-Fil camera mounted on Nikon Eclipse 80i compound microscope using the software NIS-Elements F v.4.60.00. at the College of Life Sciences, Nankai University, Tianjin, China (NKU).

The universal primers LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify the standard 658-bp mitochondrial COI barcode region. Polymerase chain reaction (PCR) amplifications followed Song et al. (2018) and were conducted in a 25  $\mu$ l volume including 12.5  $\mu$ l 2× Es Taq MasterMix (CoWin Biotech Co., Beijing, China), 0.625  $\mu$ l of each primer, 2  $\mu$ l of template DNA and 9.25  $\mu$ l of deionized H<sub>2</sub>O. PCR products were electrophoresed in 1.0% agarose gel, and purified and sequenced in both directions at Beijing Genomics Institute Co., Ltd., Beijing, China.

Raw sequences were assembled and edited in Geneious Prime 2020 (Biomatters Ltd., Auckland, New Zealand). Alignment of the sequences was carried out using the MUSCLE algorithm (Edgar 2004) on amino acids in MEGA X (Kumar et al. 2018). The pairwise distances using the Kimura 2-Parameter (K2P) substitution model of ten species within the genus *Boreoheptagyia* were calculated in MEGA. The neighbor-joining tree was constructed using the K2P substitution model, 1000 bootstrap replicates and the "pairwise deletion" option for missing data in MEGA. Novel sequence, trace-files, and metadata of the new species are uploaded to the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2013). GenBank accessions of the Chinese specimens are list in Table 1. The holotype of the new species and other examined specimens are deposited in the collection of the College of Life Sciences, Nankai University, Tianjin, China.

## Results

Taxonomic description

*Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) Figs 1–4

Diamesa kurobebrevis Sasa & Okazawa, 1992: 58. Toyamadiamesa kurobebrevis Sasa & Kikuchi, 1995: 205. Boreoheptagyia kurobebrevis Endo, 2002: 12; Makarchenko et al. 2008: 8; Ashe and O'Connor 2009: 265.

**Material examined.** Male (NKU & BOLD sample ID: LGS63), 1 male, China, Guizhou Province, Qiandongnan Miao and Dong Autonomous Prefecture, Leishan, Leigongshan Natural Reserve, Fangxiang, Getoucun, 26.396014°N, 108.260933°E, 1070 m a.s.l., Malaise trap, 10–30. i. 2020, leg. H.-J. Yu. 1 female, same as above.

**Diagnostic characters.** *B. kurobebrevis* can be distinguished from other related species in having: antenna with five flagellomeres; wing membrane covered with microtrichia on entire surface. Costal extension 175  $\mu$ m long. R with 31 setae, R<sub>1</sub> with 34 setae, R<sub>4+5</sub> with 31 setae. Superior volsella rounded; inferior volsella finger-like and well-sclerotized; gonostylus with 2 small megasetae.

Adult male (N = 1). Total length 2.95 mm. Wing length 2.60 mm. Total length/ wing length 1.13. Wing length/length of profemur 2.17.

**Coloration** (Fig. 1). Head, thorax and abdomen mostly dark brown. Ground color of scutum yellow, stripes and postnotum dark brown, scutellum brown, abdominal tergites almost uniformly yellow, hypopygium dark brown, femur yellow in basal three-

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Species										airwise	e genetic	distanc	sec									GenBank accessions
Boreoheptagyia zhengi																						MZ128909
Boreoheptagyia brevitarsis	15.1																					MZ128906
Boreoheptagyia kurobebrevis	14.9	14.1																				MZ128908
1	14.9	14.1	0.0																			MZ128907
Boreoheptagyia joeli	13.7	11.2	12.9	12.9																		MT240752
1	14.3	11.9	13.5	13.5	0.0																	MT240753
1	14.1	11.9	13.3	13.3	0.8	1.1																MT240754
<u> </u>	13.9	11.8	13.1	13.1	0.8	0.5	0.9															MT240755
Boreoheptagyia sarymsactyensis	13.9	13.6	15.9	15.9	11.0	10.8	11.1	10.6														MT240756
1	14.1	13.8	16.1	16.1	11.2	11.0	11.3	10.8	0.2													MT240757
	14.3	13.4	16.1	16.1	10.8	10.6	11.0	10.4	0.8 (	).6												MT240758
Boreoheptagyia brevitarsis	12.1	11.8	12.9	12.9	9.2	9.9	9.9	9.7 1	1.7 1	1.9 1	12.2											MT240774
Boreoheptagyia sp. EAM-2017	13.2	12.5	13.3	13.3	10.3	10.1	10.4	6.6	7.0	7.2	7.5 11	0.										KY640386
Boreoheptagyia alulasetosa	17.1	15.7	16.6	16.6	12.7	13.3	13.1	12.9 1	4.2 1	4.4 1	14.4 14	1 6.3	5.1									MZ128904
Boreoheptagyia iranica	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7 1	6.1 1	6.3 1	16.3 12	3.8 14	4.0 15	Ŀ.								MT240768
	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7 1	6.1 1	6.3 1	16.3 15	3.8 14	4.0 15	.7 0.								MT240769
1	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7 1	6.1 1	6.3 1	16.3 15	3.8 14	4.0 15	.7 0.	0.0							MT240770
	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7 1	6.1 1	6.3 1	16.3 12	3.8 14	4.0 15	.7 0.	0.0	0.0						MT240771
Boreoheptagyia brevitarsis	12.5	12.7	13.9	13.9	10.4	11.1	11.1	11.0 1	2.4 1	2.6 1	1 12.9	.2 11	1.7 14	.7 14	2 14.2	2 14.2	14.2					MT240772
	11.6	12.3	13.7	13.7	9.6	10.1	10.2	9.9	1.5 1	1.7 1	12.0 0	.8 11	1.0 14	.9 14	0 14.0	14.0	14.(	1.5				MT240773
	14.7	0.5	13.9	13.9	11.0	11.7	11.9	11.5 1	3.3 1	3.5 1	[3.1 1]	.7 12	2.6 15	.7 15	0 15.0	15.0	15.0	12.6	12.2			MZ128905
	11.8	11.9	13.1	13.1	9.6	10.1	10.2	9.9	1.8 1	2.0 1	2.4 0	.5 1(	9.8 15	.3 13	6 13.6	5 13.6	13.6	5 1.4	0.8	11.9		MT240775
	11.7	12.3	13.5	13.5	9.9	10.2	10.2	10.1	1.7 1	1.8 1	2.2 0	.8	1.1 14	.9 14	2 14.2	2 14.2	14.2	1.9	0.9	12.2	1.1	MT240775



Figure 1. Boreoheptagyia kurobebrevis (Sasa & Okazawa, 1992), male adult in ethanol.



**Figure 2.** Boreoheptagyia kurobebrevis (Sasa & Okazawa, 1992), male adult A wing B thorax C hypopygium. Scale bars: 200  $\mu$ m.



Figure 3. Boreoheptagyia kurobebrevis (Sasa & Okazawa, 1992), female adult.



**Figure 4.** *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992), female adult **A** genitalia with gonapophysis VIII and seminal capsules, ventral **B** cercus. Scale bars: 50 µm.

**Table 2.** Lengths (in  $\mu$ m) and proportions of legs of *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) in China, male (N = 1).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
P <sub>1</sub>	1200	1380	920	500	270	80	130	0.67	3.57	2.80	2.64
P <sub>2</sub>	1300	1220	750	480	240	70	120	0.61	3.59	3.36	3.50
P <sub>3</sub>	1260	1450	900	500	250	70	90	0.62	3.97	3.01	3.89

quarter with brown ring apically, tibia pale medially with brown rings in basal one-fifth and distal one-third.

*Head.* Antenna with five flagellomeres. AR 0.82. Temporal setae 8, not separable into inner and outer verticals. Clypeus with 20 setae. Tentorium 75  $\mu$ m long; 25  $\mu$ m wide. Lengths of palpomere 1–5 (in  $\mu$ m): 38, 50, 105, 158, 250. Length ratio of palpomeres 5/3: 2.38.

*Thorax* (Fig. 2B). Antepronotum with 9 anterolateral setae; acrostichals 28; dorso-centrals 17 in two rows; prealars 10. Scutellum with 46 setae.

**Wing** (Fig. 2A). Brachiolum with 5 setae; wing membrane with macrotrichia on entire surface; costal extension 175  $\mu$ m long. Distribution of setae on veins: R, 31; R<sub>1</sub>, 34; R<sub>4.5</sub> 3. Anal lobe well-developed; squama with 16 setae. VR 1.08.

*Legs.* Length (in  $\mu$ m) of spurs of: P<sub>1</sub>, 40; P<sub>2</sub>, 55 and 55; P<sub>3</sub>, 80 and 50. Width (in  $\mu$ m) of tibial apex of: P<sub>1</sub>, 60; P<sub>2</sub>, 70  $\mu$ m; P<sub>3</sub>, 100. Comb on hind tibia with Comb of hind tibia with 12 setae. Lengths (in  $\mu$ m) and proportions of legs as in Table 2.

Hypopygium (Fig. 2C). Tergites IX without anal point. Tergites IX with 30 setae.

**Gonocoxite** 300  $\mu$ m long. Superior volsella rounded, 60  $\mu$ m long. Inferior volsella well-sclerotized, finger-like, bearing 17 setae. Gonostylus 140  $\mu$ m long, with two small megasetae. HR 2.14; HV 2.11.

**Genitalia of female** (N = 1) (Figs 3, 4). *Gonocoxite* IX 100 µm long, 45 µm wide, with 7 setae. Cercus 88 µm long, 75 µm wide, with 47 setae and covered with macro-trichia. Seminal capsule 158 µm long, 75 µm wide, sclerotized part 98 µm long.

## Boreoheptagyia zhengi Lin & Liu, sp. nov.

http://zoobank.org/9B5BB346-3355-469A-9F0E-125C350041E7 Figs 5, 6

**Type material.** *Holotype*: male (NKU & BOLD sample ID: XL3519), Yunnan Province, Baoshan City, Mangkuan County, Gaoligongshan National Nature Reserve, Baihualing, near a remote wild hot spring and a stream, 25.3105556°N, 98.795000°E, 1475 m a.s.l., light trap, 22.v.2018, leg. X.-L. Lin.

**Diagnostic characters.** According to the morphological characters of the adult male, the new species keys to the genus *Boreoheptagyia*. The new species is distinguished from its other congeners by the following combination of characters: antenna with seven flagel-lomeres; wing membrane covered with microtrichiae on almost entire surface except a bare



Figure 5. Boreoheptagyia zhengi Lin & Liu, sp. nov., male adult, holotype.

area near anal lobe; femora and tibiae of all legs pale in basal half, other portions brown; superior volsella tongue shape with small projection; gonostylus with one megaseta.

Adult male (N = 1). Total length 2.10 mm. Wing length 1.42 mm. Total length/ wing length 1.48. Wing length/length of profemur 1.63.

**Coloration** (Fig. 5). Head, thorax and abdomen yellow-brown. Wing membrane with microtrichiae on almost entire surface except a bare area near anal lobe. Femur and tibia of all legs pale in basal half, other portions brown.

*Head* (Fig. 6A, B). Antenna with seven flagellomeres; ultimate flagellomere 55  $\mu$ m long; AR 0.31. Eyes bare without dorsomedial extension. Temporal setae 14, not separable into inner and outer verticals. Clypeus with nine setae. Lengths of palpomere 1–5 (in  $\mu$ m): 20, 30, 60, 80, 150. Palpomere ratio (5<sup>th</sup>/3<sup>rd</sup>): 2.50.

*Thorax* (Fig. 6C). Antepronotum with five setae; acrostichals 27; dorsocentrals 14 in two rows; prealars five. Scutellum with 30 setae.

*Wing* (Fig. 6D). VR 1.04. Brachiolum with three setae. Costa without extension. Squama with eight setae. R with 11 setae,  $R_1$  with four setae,  $R_{4+5}$  with five setae. Anal lobe developed.

*Legs.* Spur of front tibia 35  $\mu$ m long, of mid tibia 38  $\mu$ m long; of hind tibia 55 and 33  $\mu$ m long. Width of front tibia apex 50  $\mu$ m, of mid tibia apex 50  $\mu$ m, of hind tibia apex 63  $\mu$ m. Comb of hind tibia with 15 setae. Lengths (in  $\mu$ m) and proportions of legs as in Table 3.



**Figure 6.** *Boreoheptagyia zhengi* Lin & Liu, sp. nov., male adult, holotype **A** hea **B** antenna **C** thorax **D** wing **E** hypopygium, dorsal view **F** hypopygium, ventral view. Scale bars: 100 μm.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
P <sub>1</sub>	870	810	440	200	120	50	80	0.54	4.71	3.82	2.57
$P_2$	860	850	480	220	120	50	100	0.56	4.47	3.56	3.91
P <sub>3</sub>	920	890	510	250	120	50	100	0.57	4.46	3.55	4.23

**Table 3.** Lengths (in  $\mu$ m) and proportions of legs of *Boreoheptagyia zhengi* Lin & Liu, sp. nov., male holotype (N = 1).

*Hypopygium* (Fig. 6E, F). Tergites IX without anal point. Tergites IX with 23 setae. Gonocoxite 200  $\mu$ m long. Superior volsella tongue shape with small projection, 50  $\mu$ m long. Inferior volsella sclerotized, finger-like, 30  $\mu$ m long. Gonostylus 75  $\mu$ m long, with one megaseta, 10  $\mu$ m long. HR 2.67; HV 2.80.

Female and immature stages unknown.

**Etymology.** The species is named '*zhengi*' after Prof. Le-Yi Zheng, for his outstanding contribution to the knowledge of insect taxonomy in China; noun in nominative case.

Key to the known adult males of Boreoheptagyia Brundin from China

1	Antenna with 13 flagellomeres2
_	Antenna less than 9 flagellomeres
2	Alula with 3–4 setae. Gonostylus short and inflated, with very short, narrow
	apical partB. alulasetosa Makarchenko, Wu & Wang
_	Alula without setae. Shape of gonostylus different
3	Dorsocentrals only in single anterior group on scutum4
_	Dorsocentrals in anterior and posterior groups on scutum
4	Prealars 12. Inferior volsella with some distal teeth
	<i>B. ambigua</i> Makarchenko, Wu & Wang
_	Prealars 1-4. Inferior volsella without teeth
	B. xinglongiensis Makarchenko, Wu & Wang
5	Prealars 17–28B. brevitarsis (Tokunaga)
_	Prealars 10 B. similis (Chaudhuri & Ghosh)
6	Antenna with 8–9 flagellomeres
_	Antenna with 6–7 flagellomeres7
7	Wing developed, antenna with 7 flagellomeres <i>B. zhengi</i> Lin & Liu, sp. nov.
_	Wing reduced, antenna with 6 flagellomeres
	B. tibetica Makarchenko, Wang & Willassen

## Discussion

Morphological characters of *B. kurobebrevis* from China fit well with the original description by Sasa and Okazawa (1992) and Makarchenko et al. (2008), but there are a few differences in numeric measurements: total length (2.95 mm), AR 0.82 and scutellum with 46 setae in Chinese specimen, as compared with total length 3.34 mm, AR 0.64 and scutellum with 50 setae in Japanese specimen (Makarchenko et al. 2008).





**Figure 7.** Neighbor-joining tree for six species of the genus *Boreoheptagyia* based on K2P distance in DNA barcodes. Numbers on branches represent bootstrap support (>70%) based on 1000 replicates; scale equals K2P genetic distance.

The new species can be easily separated from other related members of the genus by the following combination of morphological characters found in the male adult: antenna with seven flagellomeres; wing membrane covered with macrotrichia on almost the entire surface except a bare spot near the anal lobe. *Boreoheptagyia zhengi* sp. nov. keys out close to *B. tibetica* from which it can be separated in having: 1) antenna with seven flagellomeres in *B. zhengi* Lin & Liu, sp. nov., whereas the latter has six flagellomeres; 2) well-developed anal lobe in the new species and the wing membrane with microtrichiae on almost the entire surface except for a bare area near the anal lobe, whereas *B. tibetica* has a reduced anal lobe and wing membrane with macrotrichia on the entire surface; 3) differing number of chaetae on thorax: (acrostichals 27, dorso-centrals 14 in two rows, prealars 5 in the new species) compared with (acrostichals 14, dorsocentrals 6, prealars 15–16 in *B. tibetica*).

The neighbor-joining tree based on COI DNA barcodes of *Boreoheptagyia* revealed nine distinct genetic clades (Fig. 7). The new species *B. zhengi* sp. nov. separates from *B. brevitarsis* by more than 11% divergence in COI barcodes (Fig. 7; Table 1). In addition, there are two genetic clades of *Boreoheptagyia brevitarsis* (Fig. 7), indicating that a potential cryptic species or misidentification. A further integrative taxonomic study on *Boreoheptagyia brevitarsis* is needed when the more public vouchers are available to access.

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