

The cavernicolous freshwater prawn in China, with description of two new species (Decapoda, Palaemonidae, *Macrobrachium*)

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Abstract

The karst area in southern China is recognized as a biodiversity hotspot for cave-dwelling organisms. However, the research of the cavernicolous species of the prawn genus *Macrobrachium* remains limited. This study aims to explore the species boundaries and diversity of this group and infer its phylogeny using an integrative approach. Molecular species delimitation analyses revealed five species excluding *M. elegantum*, for which molecular data were unavailable. Genetic gaps were observed among these species, with high interspecific distances (8.90%–27.43% for COI and 1.91%–9.63% for 16S) and low intraspecific distances (maximum 3.98% for COI and 0.47% for 16S). In contrast, morphological taxonomy identified three species and one species complex, which comprises three cryptic species. As a result, a total of six species were identified, including two new species, i.e. *Macrobrachium guizhouense* **sp. nov.** and *M. parvum* **sp. nov.** Among them, *M. tenuipes* and *M. parvum* **sp. nov.** are likely to be stygophiles, while the remaining species are likely to be stygobites. The phylogenetic trees based on (COI + 16S) revealed that these cave-dwelling species are polyphyletic, indicating the multiple independent cave invasions in the evolutionary history of this genus. Finally, these cavernicolous species exhibit opposite sexual dimorphism compared to epigean congeners, with females being larger than males. This may imply that they adopt a “pure search” mating mode. The findings enhance our understanding of the biodiversity and evolutionary history of subterranean *Macrobrachium* and provide fundamental data for the conservation of subterranean biodiversity.

Key Words

Phylogeny, species complex, species delimitation, stygobite, stygophiles, systematics

Introduction

Freshwater prawns of the genus *Macrobrachium* Spence Bate, 1868 constitute an essential component of freshwater ecosystems. To date, a total of 280 species of this genus have been recorded worldwide (DecaNet 2025), primarily concentrated in tropical and subtropical regions, including 41 species in China (Chen et al. 2021). The majority of these species are surface water dwellers.

In contrast, only 17 cavernicolous species are documented globally (Zhu et al. 2020) (note that *M. duanense* was omitted in the list in Zhu et al. (2020) and is included here). In China, current taxonomy on cavernicolous *Macrobrachium* has only depicted four species: *Macrobrachium lingyunense* (Li & Luo, 2001), *M. elegantum* Pan et al., 2010, *M. duanense* Lan et al., 2017 and *M. tenuipes* Zhu et al., 2020, all of which are restricted to Guangxi Zhuang Autonomous Region (hereafter Guangxi).

While the first three species are likely stygobites, *Macrobrachium tenuipes* is likely a stygophile or stygoxene due to its lack of obvious stygobiotic traits, such as pale body color and degenerated eyes (Zhu et al. 2020).

Macrobrachium linyunense (Li & Luo, 2001) is the first recorded cavernicolous species, with Shadong Cave, Linyun County as the type locality (Li and Luo 2001). However, the authors wrongly placed it in the genus *Typhlocaridina* of the family Atyidae, and the original description and illustrations were of poor quality. Subsequently, Li et al. (2006) described a species from the same cave as new and named it *Macrobrachium linyunense* Li et al., 2006 as well. Twelve years later, Cai and Ng (2018) finally synonymized the two names. *Macrobrachium elegantum* Pan et al., 2010 is the second recorded species from an unnamed cave in Jingxi County (Pan et al. 2010). Lan et al. (2017) described the third cavernicolous freshwater prawn, *Macrobrachium duanense* Lan et al., 2017 from a cave in Du'an County. However, the figures in the paper were vague and the species was only compared to the epigean and widespread species *M. nipponense* (De Haan, 1849) rather than other subterranean species. *Macrobrachium tenuipes* Zhu et al., 2020 is the fourth species collected from a karst cave in Mashan County (Zhu et al. 2020). The authors provided molecular data (COI and 18S) and constructed a phylogenetic tree, but none of the other three cave-dwelling species were included for comparison.

During recent investigations of stygofauna in southern China, we collected numerous specimens, covering all known species of subterranean freshwater prawns except for *Macrobrachium elegantum*. This provided an opportunity to conduct a taxonomical review of this group. The objectives of this study are: 1) to conduct species exploration, 2) to perform taxonomical delimitation to examine the validity of these species, and 3) to infer their phylogenetic relationships. As a result, two new species were discovered and described from five caves in Guizhou and Guangxi, *Macrobrachium duanense* was redescribed based on newly collected specimens, the detailed morphological differences between these cave-dwelling species were highlighted, and a well-supported phylogenetic tree was constructed. Additionally, we discussed the sexual dimorphism observed in these cavernicolous species. These findings significantly expand our understanding of the biodiversity of subterranean freshwater prawns in the region and provide valuable insights into their adaptation to subterranean environments and unique reproductive strategies.

Materials and methods

Specimen collection and preservation

After a series of expeditions, a total of eight caves in Guangxi and Guizhou were found to harbor several *Macrobrachium* species, including the type locality of

M. linyunense. Specimens were collected using cage nets that were set overnight. Live animals were first observed and photographed using a Sony A7R4A camera equipped with a Sony FE 90 mm macro lens. Subsequently, most of the specimens were preserved in 75% ethanol for morphological studies, while the remainder were preserved in absolute ethanol and stored at -40°C for molecular research. All specimens are deposited at the Institute of Biology, Guizhou Academy of Sciences, Guiyang, China (IBGAS).

Morphological study

Specimens were examined, photographed and measured using a Leica M205A stereomicroscope equipped with a Leica DFC450 camera and LAS X software ver. 5.1. The distribution map was generated with the R package GGMAPCN ver. 0.0.2 (see <https://github.com/Rimagination/ggmapcn>). All images were edited with PHOTOSHOP CC 2019 software ver. 20.0.0.

The following abbreviations are used in the text: **alt** (altitude), **cl** (carapace length, measured from the postorbital margin to the posterior margin of the carapace), **rl** (rostral length, measured from the rostral tip to the postorbital margin) and **tl** (total length, measured from the rostral tip to the posterior margin of the telson).

Molecular analyses

Species delimitation

Two to nine specimens from each cave (45 in total) were sampled for molecular analyses. Two mitochondrial genes (cytochrome c oxidase subunit I and 16S rDNA) were used to conduct the species delimitation analyses. Primer sequences for PCR amplification and Sanger sequencing are LCO1490 (GGTCAACAAAT-CATAAAGATATTGG)/ HCO2198 (TAAACTTCAGG-GTGACCAAAAAATCA) for COI (Folmer et al. 1994) and 16sA (ACTTGATATATAATTAAAGGGCCG)/ 16sB (CTGGCGCCGGTCTGAACTCAAATC) for 16S (Wowor et al. 2009). Finally, twenty-one COI sequences and twenty-two 16S sequences were successfully obtained. Two COI sequences of *M. tenuipes* from GenBank were also added in the analyses (Table 1). The COI sequences were aligned using CLUSTALW in MEGA ver. 7.0 (Kumar et al. 2016), based on amino acid translation. The more variable 16S sequences were aligned using the online version of MAFFT ver. 7.0 (Katoh et al. 2019) with the Q-INS-i algorithm. All other settings were left as default, and then trimmed using the program TRIMAL ver. 1.2 (Capella-Gutiérrez et al. 2009) set with the automated heuristic method. The COI and 16S sequences were aligned and trimmed to lengths of 632 bp and 427 bp, respectively. The pairwise Kimura 2-parameter distances were calculated with MEGA.

Table 1. Details of the specimens used for the molecular analyses. The GenBank accession numbers marked with an asterisk (*) indicate sequences obtained and uploaded to GenBank in this study.

Taxon	Voucher number	Collection data	GenBank number	
			COI	16S
<i>Macrobrachium guizhouense</i> sp. nov.	GBZD-646	Malai Cave, Libo County, Guizhou, China	PV400033*	PV405585*
	GBZD-647		-	PV405586*
	GBZD-648		PV400034*	PV405587*
	GBZD-649		PV400035*	PV405588*
	GBZD-650		PV400036*	PV405589*
<i>Macrobrachium parvum</i> sp. nov.	GBZD-827	Shuiyuandi Cave, Du'an County, Guangxi, China	PV400038*	-
	GBZD-828		PV400039*	-
	GBZD-829	Nonglitun Cave, Du'an County, Guangxi, China	PV400040*	PV405597*
	GBZD-830		PV400041*	PV405598*
	GBZD-831		PV400042*	PV405599*
	GBZD-832		PV400043*	PV405600*
	GBZD-833		PV400044*	PV405601*
	GBZD-834		PV400045*	PV405602*
<i>Macrobrachium duanense</i>	GBZD-651	Nongguangshang Cave, Du'an County, Guangxi, China	PV400032*	PV405582*
	GBZD-821	Nongshuitun Cave, Du'an County, Guangxi, China	-	PV405583*
	GBZD-822		-	PV405584*
<i>Macrobrachium lingyunense</i>	GBZD-751	Sha Cave, Lingyun County, Guangxi, China	PV400037*	PV405581*
<i>Macrobrachium tenuipes</i>	GBZD-835	Nongchitianchuang Cave, Du'an County, Guangxi, China	PV400046*	PV405590*
	GBZD-836		PV400047*	PV405591*
	GBZD-837		PV400048*	PV405592*
	GBZD-838		PV400049*	PV405593*
	GBZD-839		PV400050*	PV405594*
	GBZD-840		PV400051*	PV405595*
	GBZD-841		PV400052*	PV405596*
	A42	Mashan County, Guangxi, China	MK994931	-
	A49		MK994933	-
<i>Macrobrachium anhuiense</i>	-	Anhui, China	-	DQ194909
<i>Macrobrachium asperulum</i>	11213	Fujian, China	MN200397	DQ194908
<i>Macrobrachium bilineare</i>	A53	Jinxiu County, Guangxi, China	MN814447	-
<i>Macrobrachium edentatum</i>	-	Sichuan, China	AB250552	DQ194912
<i>Macrobrachium esculentum</i>	BIC-0255; MAS00110	Indonesia; Taiwan, China	MN526207	EU493145
<i>Macrobrachium fukienense</i>	MACR013	Fujian, China	FM958065	DQ194923
<i>Macrobrachium laevis</i>	A20; CUHK-LMT-CAR290-1	Gaoming, Guangdong;	MK412774	ON754348
<i>Macrobrachium latimanus</i>	-	Philippines	AB235276	DQ194937
<i>Macrobrachium maculatum</i>	A38	Gaoming, Guangdong, China; Anhui, China	MK412786	DQ194910
<i>Macrobrachium meridionale</i>	A27	Chancheng, Guangdong; Hainan, China	MK412779	DQ194948
<i>Macrobrachium nipponense</i>	GBZD-001	Guangzhao Reservoir, Qinglong, Guizhou, China	OR536638	OR537880
<i>Macrobrachium olfersii</i>	GUMB1115	-	-	EF588321
<i>Macrobrachium pentazona</i>	A46	Beijiang River, Qingyuan, Guangdong, China	MN814448	-
<i>Macrobrachium venustum</i>	CUHK-LMT-CAR304-1	Hong Kong, China	ON753715	ON754360
<i>Palaemon sinensis</i>	Hap_11	China	MT884029	LC582794
<i>Neocaridina palmata</i>	ZMB: Crustacea: 280401	China	KP168819	KP168779

Three independent species delimitation approaches were conducted, the Automatic Barcoding Gap Discovery (ABGD) (Puillandre et al. 2012), the multiple Poisson tree processes model (mPTP) (Kapli et al. 2017), and the Bayesian implementation of the Poisson tree processes model (bPTP) (Zhang et al. 2013), to identify the molecular operational taxonomic unit (MOTU). The ABGD analyses were conducted on the ABGD web interface (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>) under the default settings. The bPTP and mPTP analyses were conducted on the web servers (<http://species.h-its.org/ptp/> and <https://mptp.h-its.org/#/tree>), using the tree generated with RAXML ver. 8.2.10 (Stamatakis 2014) as the input data. All other settings were left as default.

Subsequently, we combined with the morphological evidence and the phylogram to test these MOTUs to obtain final species delimitations.

Phylogenetic analyses

After species delimitation analyses, these COI and 16S sequences were concatenated using MESQUITE ver. 3.6 (Maddison and Maddison 2019). Other sequences of sixteen epigeal species of *Macrobrachium* from adjacent areas and two non-*Macrobrachium* species, *Palaemon sinensis* (Palaemonidae) and *Neocaridina palmata* (Atyidae), as outgroup were derived from GenBank and added into the matrix (Table 1). Then, the matrix was imported into PHYLOSUITE ver. 1.2.1 (Zhang et al. 2020) for the following phylogenetic analyses: PARTITION-FINDER ver. 2.1.1 (Lanfear et al. 2017) plugin was used to determine the optimum partitioning scheme and the best-fitting model for each partition, using the corrected Akaike Information Criterion (AICc). We divided the matrix into four partitions, in which the COI gene

was split by codon positions. Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted to infer the phylogeny. ML were inferred using IQ-TREE ver. 1.6.8 (Nguyen et al. 2015) under Edge-linked partition model for 5000 ultrafast bootstraps. BI analysis was conducted using MRBAYES ver. 3.2.6 (Ronquist et al. 2012) with generated models in PARTITIONFINDER. Four independent Markov chain Monte Carlo (MCMC) were run simultaneously for forty million generations, and sampling was conducted every 1000 generations, with a burn-in of 25%. The process was terminated when the average standard deviation of splitting frequency falls below 0.01. Trees were visualized and edited using FIGTREE ver. 1.44 (Rambaut 2016).

Results

Species delimitation

The results of molecular species delimitation are summarized in Fig. 1. The ABGD, bPTP and mPTP analyses of COI clustered *Macrobrachium* spp. into four, six and five putative species, respectively. However, the bPTP result regarded GBZD-648 as an independent species, rather than clustering it with specimens from the same cave. The mPTP analysis of 16S identified all specimens as belonging to only one species. Therefore, this result was not considered further. The ABGD and bPTP analyses of 16S recognized four and five species, respectively.

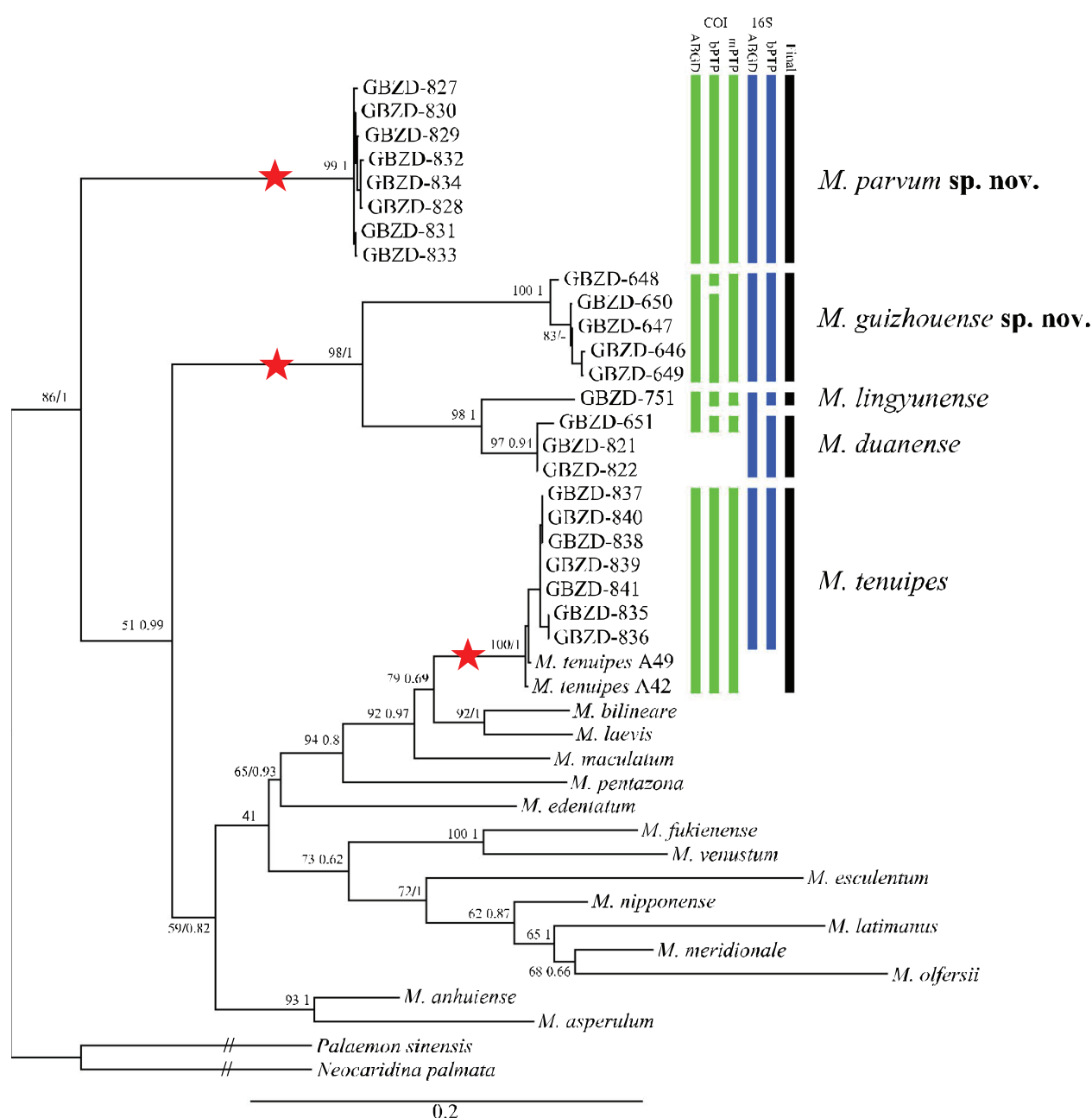


Figure 1. ML tree based on the concatenated dataset (COI + 16S) and results of the molecular species delimitation. Numbers at nodes are maximum likelihood percent bootstrap values (left) and Bayesian posterior probabilities (right). The red pentagrams represent cave colonization events.

In addition to GBZD-648, which was regarded as a species only by bPTP based on COI data, the differences among these results were reflected in whether *M. lingyunense* and *M. duanense* should be considered synonym or two different species. Both ABGD analyses supported the view that *M. lingyunense* and *M. duanense* should be considered the same species. However, given the large genetic divergences and the distant geographical distances, we regarded them as different species.

According to this scheme, the maximum intra-specific genetic distance of the COI gene was 3.98% (*M. guizhouense* sp. nov.), while the interspecific distances ranged from 8.90% to 27.43% (Table 2). The maximum intraspecific genetic distance of 16S was 0.47% (*M. parvum* sp. nov.), while the interspecific genetic distances ranged from 1.91% to 9.63% (Table 3). Both genes showed significant genetic gaps between intraspecific and interspecific distances.

Phylogenetic analyses

Topologies derived from the ML and BI analyses are similar, with both exhibiting generally high support values (Fig. 1). They differ only in the position of the clade comprising *M. anhuiense* and *M. asperulum*. In the ML analysis, it is a sister group to all epigeal species (bootstrap value = 59%). In the BI analysis, it is clustered with the clade including *M. tenuipes*, *M. bilineare*, *M. laevis*, *M. maculatum*, *M. pentazona*, and *M. edentatum* (posterior probability = 0.71; not shown in the figure). Given the low support for this clade on both trees, its phylogenetic location is uncertain.

The five subterranean species show the same topology in both trees and are found to be polyphyletic with strong supports. *M. parvum* sp. nov. is sister to all other species in the trees, with a bootstrap value of 86% and a posterior probability of 1. The monophyly of (*M. guizhouense* sp. nov. + (*M. lingyunense* + *M. duanense*)) is confirmed with high support (98% and 1). This clade is sister to the remaining species, with a bootstrap value of 51% and a posterior probability of 0.99. *Macrobrachium tenuipes* is clustered within the clade of all epigeal species and is sister to the clade comprising *M. bilineare* and *M. laevis* with relatively low support values (79% and 0.69).

Taxonomy

Family Palaemonidae Rafinesque, 1815

Genus *Macrobrachium* Spence Bate, 1868

Macrobrachium elegantum Pan et al., 2010

Macrobrachium elegantum Pan et al., 2010: 86, figs 2–4. Type locality: a cave in Xiaorui Village, Ludong Town, Jingxi County, Guangxi, China.

Material examined. None.

Diagnosis. Body semi-transparent. Carapace and abdomen smooth and glabrous. Rostrum straight, tip bifurcate and reaching beyond end of scaphocerite, 0.7 times of cl. Dorsal margin armed with 7 or 8 teeth, including 3 or 4 teeth behind orbit. Dorsal teeth placed more widely on anterior part. Ventral margin armed with 4 to 6 teeth. Eyes with cornea totally degenerated. Ocular peduncle small, elliptical and non-pigmented. Scaphocerite about 3.0 times longer than wide. Second pereopod slender, subequal in size and similar for both sexes. Ischium 0.9 times as long as merus; merus as long as carpus; carpus 1.5 times as long as palm; finger 1.7 times as long as palm, palm slightly inflated.

Distribution. Jingxi County, Guangxi, China.

Remarks. *Macrobrachium elegantum* is typical of a stygobitic organism, characterized by the complete absence of body color and the degeneration of eyes. In addition, the morphology of this species is distinctly different from that of other stygobitic species, thus supporting the validity of the species.

This species differs from all epigeal species as well as *M. parvum* sp. nov. and *M. tenuipes* by the completely degraded somatic pigmentation and eyes. It can be distinguished from *M. duanense*, *M. guizhouense* sp. nov. and *M. lingyunense* by the bifurcate tip of rostrum (unicuspidate in other three species), the different rostral formula (3–4 + 3–4 / 4–6 in *M. elegantum* vs. 2–3 + 6–7 / 2–4 in *M. duanense*), the slender scaphocerite (3.0 times longer than wide in *M. elegantum*, vs. 2.2 to 2.4 times in other three species) and the different ratios between the segments of second pereopods (Table 4).

Macrobrachium parvum Jiang & Zhou, sp. nov.

<https://zoobank.org/115A3B9A-8A21-4C6D-AD93-1A531D76B5A3>

Figs 2–4

Type materials. **Holotype:** • male (IBGAS-Dec-Pal-484-1) (tl 41 mm, cl 9.6 mm, rl 7.1 mm), China, Guangxi, Du'an County, Baoan Town, Nonglitun Cave, 24.0791°N, 107.8840°E, 10. IV. 2024, Zhou J.J. leg.

Paratypes: • 6 males (IBGAS-Dec-Pal-484-2–7) (tl 30.2–36.8 mm, cl 7.8–9.0 mm, rl 5.6–7.4 mm) and 15 females (IBGAS-Dec-Pal-484-8–22) (tl 31.7–41.4 mm, cl 7.5–11.4 mm, rl 6.0–7.6 mm), same data as holotype; • 2 males (IBGAS-Dec-Pal-485-1–2) (tl 39.6–44.0 mm, cl 9.4–10.2 mm, rl 6.6–8.5 mm) and 3 females (IBGAS-Dec-Pal-485-3–5) (tl 31.4–46.5 mm, cl 8.2–11.4 mm, rl 5.7–7.8 mm), Du'an County, Baoan Town, Nonglitun Cave, 11. IV. 2024, Zhou J.J. leg.; • 1 male (IBGAS-Dec-Pal-486-1) (tl 37.1 mm, cl 9.7 mm, rl 5.8 mm) and 12 females (IBGAS-Dec-Pal-486-2–13) (tl 26.5–48.3 mm, cl 6.8–12.7 mm, rl 4.1–7.0 mm), Guangxi, Du'an, Gaoling Town, Nongguangshang Cave, 24.0095°N, 108.0823°E, alt. 198 m, 16. IV. 2023, Zhou J.J. leg. 2 females (IBGAS-Dec-Pal-487-1–2) (tl 36.0–53.4 mm, cl 8.7–13.8 mm, rl 5.5–8.9 mm), Du'an County, Disu Town, Xiaodiao Village, Shuiyuandi Cave, 24.0061°N, 107.9840°E, 10. IV. 2024, Zhou J.J. leg.

Table 2. Pairwise Kimura 2-parameter distance (%) for COI sequences of cavernicolous *Macrobrachium* spp. from China.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. <i>M. duanense</i> _651																						
2. <i>M. guizhouense</i> _646	18.71																					
3. <i>M. guizhouense</i> _648	17.20	3.98																				
4. <i>M. guizhouense</i> _649	18.22	0.37	3.98																			
5. <i>M. guizhouense</i> _650	17.45	1.49	2.82	1.49																		
6. <i>M. linyunense</i> _751	8.90	18.43	17.22	17.95	17.67																	
7. <i>M. parvum</i> _827	26.59	24.99	24.84	24.46	25.34	24.86																
8. <i>M. parvum</i> _828	27.43	25.23	25.09	24.71	25.59	25.67	0.55															
9. <i>M. parvum</i> _829	27.13	24.95	24.81	24.43	25.30	25.39	0.37	0.18														
10. <i>M. parvum</i> _830	27.13	24.95	24.81	24.43	25.30	25.39	0.37	0.18	0.00													
11. <i>M. parvum</i> _831	26.88	24.71	24.56	24.18	25.05	25.14	0.18	0.37	0.18	0.18												
12. <i>M. parvum</i> _832	27.43	25.23	25.09	24.71	25.59	25.39	0.93	0.37	0.56	0.56	0.74											
13. <i>M. parvum</i> _833	26.88	24.71	24.56	24.18	25.05	25.14	0.18	0.37	0.18	0.18	0.00	0.74										
14. <i>M. parvum</i> _834	27.43	25.23	25.09	24.71	25.59	25.67	0.55	0.00	0.18	0.18	0.37	0.37	0.37									
15. <i>M. tenuipes</i> _835	19.35	19.96	18.31	19.47	18.73	17.78	23.76	24.00	24.28	24.28	24.03	24.00	24.03	24.00								
16. <i>M. tenuipes</i> _836	19.35	19.96	18.31	19.47	18.73	17.78	23.76	24.00	24.28	24.28	24.03	24.00	24.03	24.00	0.00							
17. <i>M. tenuipes</i> _837	19.84	19.93	18.29	19.45	18.71	18.25	23.72	23.97	24.25	24.25	24.00	23.97	24.00	23.97	0.55	0.55						
18. <i>M. tenuipes</i> _838	19.84	19.93	18.29	19.45	18.71	18.25	23.72	23.97	24.25	24.25	24.00	23.97	24.00	23.97	0.55	0.55	0.00					
19. <i>M. tenuipes</i> _839	19.58	19.68	18.04	19.20	18.46	18.01	23.45	23.69	23.97	23.97	23.72	23.69	23.72	23.69	0.37	0.37	0.18	0.18				
20. <i>M. tenuipes</i> _840	19.84	19.93	18.29	19.45	18.71	18.25	23.72	23.97	24.25	24.25	24.00	23.97	24.00	23.97	0.55	0.55	0.00	0.00	0.18			
21. <i>M. tenuipes</i> _841	19.58	19.68	18.04	19.20	18.46	18.01	23.45	23.69	23.97	23.97	23.72	23.69	23.72	23.69	0.37	0.37	0.18	0.18	0.00	0.18		
22. <i>M. tenuipes</i> _A42	19.30	20.17	18.52	19.68	18.94	17.74	22.39	22.63	22.90	22.90	22.66	23.17	22.66	22.63	1.30	1.30	1.30	1.30	1.12	1.30	1.12	
23. <i>M. tenuipes</i> _A49	19.32	19.93	18.29	19.45	18.71	17.76	22.63	22.87	23.14	23.14	22.90	23.42	22.90	22.87	1.12	1.12	1.12	1.12	0.93	1.12	0.93	0.55

Table 3. Pairwise Kimura 2-parameter distance (%) for 16S sequences of cavernicolous *Macrobrachium* spp. from China

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. <i>M. duanense_651</i>																					
2. <i>M. duanense_821</i>	0.24																				
3. <i>M. duanense_822</i>	0.24	0.00																			
4. <i>M. guizhouense_646</i>	4.13	3.88	3.88																		
5. <i>M. guizhouense_647</i>	4.13	3.88	3.88	0.00																	
6. <i>M. guizhouense_648</i>	4.13	3.88	3.88	0.00	0.00																
7. <i>M. guizhouense_649</i>	4.13	3.88	3.88	0.00	0.00	0.00															
8. <i>M. guizhouense_650</i>	4.13	3.88	3.88	0.00	0.00	0.00	0.00														
9. <i>M. linyunense_751</i>	2.15	1.91	1.91	4.62	4.62	4.62	4.62	4.62													
10. <i>M. parvum_829</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27												
11. <i>M. parvum_830</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27	0.00											
12. <i>M. parvum_831</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27	0.00	0.00										
13. <i>M. parvum_832</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27	0.00	0.00	0.00									
14. <i>M. parvum_833</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27	0.00	0.00	0.00	0.00								
15. <i>M. parvum_834</i>	8.84	8.57	8.57	9.63	9.63	9.63	9.63	9.63	8.27	0.00	0.00	0.00	0.00	0.00							
16. <i>M. tenuipes_835</i>	9.10	8.83	8.83	8.54	8.54	8.54	8.54	8.54	8.80	8.01	8.01	8.01	8.01	8.01	8.01						
17. <i>M. tenuipes_836</i>	9.10	8.83	8.83	8.54	8.54	8.54	8.54	8.54	8.80	8.01	8.01	8.01	8.01	8.01	8.01	0.00					
18. <i>M. tenuipes_837</i>	8.57	8.30	8.30	8.55	8.55	8.55	8.55	8.55	8.81	8.02	8.02	8.02	8.02	8.02	8.02	0.47	0.47				
19. <i>M. tenuipes_838</i>	8.57	8.30	8.30	8.55	8.55	8.55	8.55	8.55	8.81	8.02	8.02	8.02	8.02	8.02	8.02	0.47	0.47	0.00			
20. <i>M. tenuipes_839</i>	8.57	8.30	8.30	8.55	8.55	8.55	8.55	8.55	8.81	8.02	8.02	8.02	8.02	8.02	8.02	0.47	0.47	0.00	0.00		
21. <i>M. tenuipes_840</i>	8.57	8.30	8.30	8.55	8.55	8.55	8.55	8.55	8.81	8.02	8.02	8.02	8.02	8.02	8.02	0.47	0.47	0.00	0.00	0.00	
22. <i>M. tenuipes_841</i>	8.57	8.30	8.30	8.55	8.55	8.55	8.55	8.55	8.81	8.02	8.02	8.02	8.02	8.02	8.02	0.47	0.47	0.00	0.00	0.00	0.00

Table 4. Morphological comparison of cavernicolous *Macrobrachium* spp. from China.

Characters	Species					
	<i>M. elegantum</i>	<i>M. duanense</i>	<i>M. guizhouense</i> sp. nov.	<i>M. lingyunense</i>	<i>M. parvum</i> sp. nov.	<i>M. tenuipes</i>
Somatic pigmentation and eyes	completely degraded	completely degraded	completely degraded	completely degraded	reduced	normal
Rostral formula	3-4 + 3-4/4-6	2-3 + 6-7/2-4	3-4+5-7/3-4	2-4+5-7/3-4	2-4+3-6/2-5	3-4+8-9/3-4
Tip of rostrum	Bifurcate	unicuspidate	unicuspidate	unicuspidate	unicuspidate	unicuspidate
Ratio of RL/CL	0.7	0.41-0.53	0.46-0.79	0.54-0.56	0.55-0.83	0.79-0.99
CL	12.8-15.2	11.3-24.5	8.8-20.8	8.5-17.2	6.8-13.8	9.1-18.1
Scaphocerite length/width	3.0	2.4	2.2	2.4	3.0	4.1
Ratio of finger and palm of second pereopod	Finger longer than palm	Finger longer than palm	Finger longer than palm	Finger longer than palm	Finger longer than palm	Finger shorter than palm
Shape of palm of second pereopod	Inflated	Inflated	Inflated	Inflated	normal	normal
Ratios between segments of second pereopods (ischium: merus: carpus: palm: finger)	1:1.12:1.14:0.78:1.29	1:1.48:1.32:1.24:1.70	1:1.24:1.11:0.80:1.30	1:1.14:1.10:0.85:1.36	1:1.14:1.43:0.52:0.85	1:1.09:1.35:1.38:1.14
Moveable spine on uropodal diaeresis	-	Shorter than outer angle	Shorter than outer angle	Shorter than outer angle	Equal to outer angle	slightly longer than outer angle

Diagnosis. Body semi-transparent to yellowish with ochreous marks on surface of carapace and abdomen, all appendages semi-transparent. Carapace and abdomen smooth and glabrous. Rostrum slender, reaching end of scaphocerite, 0.5–0.8 times of cl, straight, or slightly upward. Dorsal margin with 5–10 teeth, including 2–4 teeth behind orbit, starting from about 1/3 of carapace length. Dorsal teeth equally spaced, anterior part of rostrum without or only with one tooth. Ventral margin with 2–5 teeth (mode 4). Eyes with cornea strongly degenerated, only small area on tip pigmented. Ocular peduncle small and elliptical. Scaphocerite about 3.0 times longer than wide. Second pereopod slender, subequal in size and similar for both sexes. Ischium 0.9 times as long as merus; merus 0.8 times as long as carpus; carpus as long as chela; finger 1.6 times as long as palm, palm not inflated. Uropodal diaeresis with inner movable spine subequal to outer angle.

Description. Body slender (Fig. 2). Rostrum slender, reaching end of scaphocerite, 0.5–0.8 times of cl, straight, or slightly upward distally. Dorsal margin with 5–10 teeth, including 2–4 teeth behind orbit, starting from about 1/3 of carapace length. Dorsal teeth equally spaced, anterior part of rostrum without or only with one tooth. Ventral margin with 2–5 teeth (mode 4) (Figs 2, 3A).

Eyes with cornea strongly degenerated, only small area on tip pigmented. Ocular peduncle small and elliptical (Figs 2, 3A).

Carapace smooth and glabrous. Antennal spine small, tip overreaching anterolateral margin of carapace. Hepatic spine small, lying behind and below antennal spine (Figs 2, 3A).

Abdomen smooth and glabrous. First to third pleurites broadly rounded, fourth and fifth pleurites slightly produced posteriorly. Sixth somite 1.4–1.9 times as long as fifth somite, with posteroventral angle slightly protruded in a sharp tip.

Telson 1.4–1.5 times length of sixth segment, 0.5–0.6 times of cl. Tapered posteriorly, with a sharp point. Dorsal surface with two pairs of spines. Posterior margin bearing two pairs of lateral spines. Inner spines obviously longer than outer spines, with plumose setae between inner spines (Fig. 4I).

Antennule with stout stylocerite, reaching about 1/4 length of basal segment of antennular peduncle. Basal segment broad, about 1.8 times as wide as second segment, as long as wide; distolateral spine of basal antennular segment small, reaching about 1/3 length of second segment. Second segment ca. 0.8 times as long as basal segment, ca. 0.9 times as long as distal segment. All segments except distal segment with submarginal plumose setae (Fig. 3B).

Scaphocerite about 3.0 times longer than wide. Inner margin somewhat convex; lateral margin strait, with sharp distolateral tooth, not reaching anterior margin (Fig. 3C).

Mandible typical of genus, with three-segmented palp of subequal length; incisor process with three sharp teeth; molar process robust, truncate distally (Fig. 3D).

Maxillular palp bilobed, upper lobe slender, digitiform, slightly longer than lower lobe, with few setae distally; lower lobe stout and small. Upper lacinia broadly elongated, distal margin with rows of strong spines, lower lacinia shorter than upper lacinia, tapering distally, densely setose (Fig. 3E).

Maxilla with simple palp; basal endite deeply bilobed, upper and lower lobes subequal and digitiform, with numerous simple setae distally; scaphognathite broad, about 3.7 times as long as wide (Fig. 3F).

First maxilliped with simple and small palp, basal and coxal endites distinct, tip of flagellum of exopod densely setose, epipod deeply bilobed (Fig. 3G).

Second maxilliped with 5-segmented endopod, flagellum with numerous plumose setae distally, epipod simple, with developed podobranch (Fig. 3H).

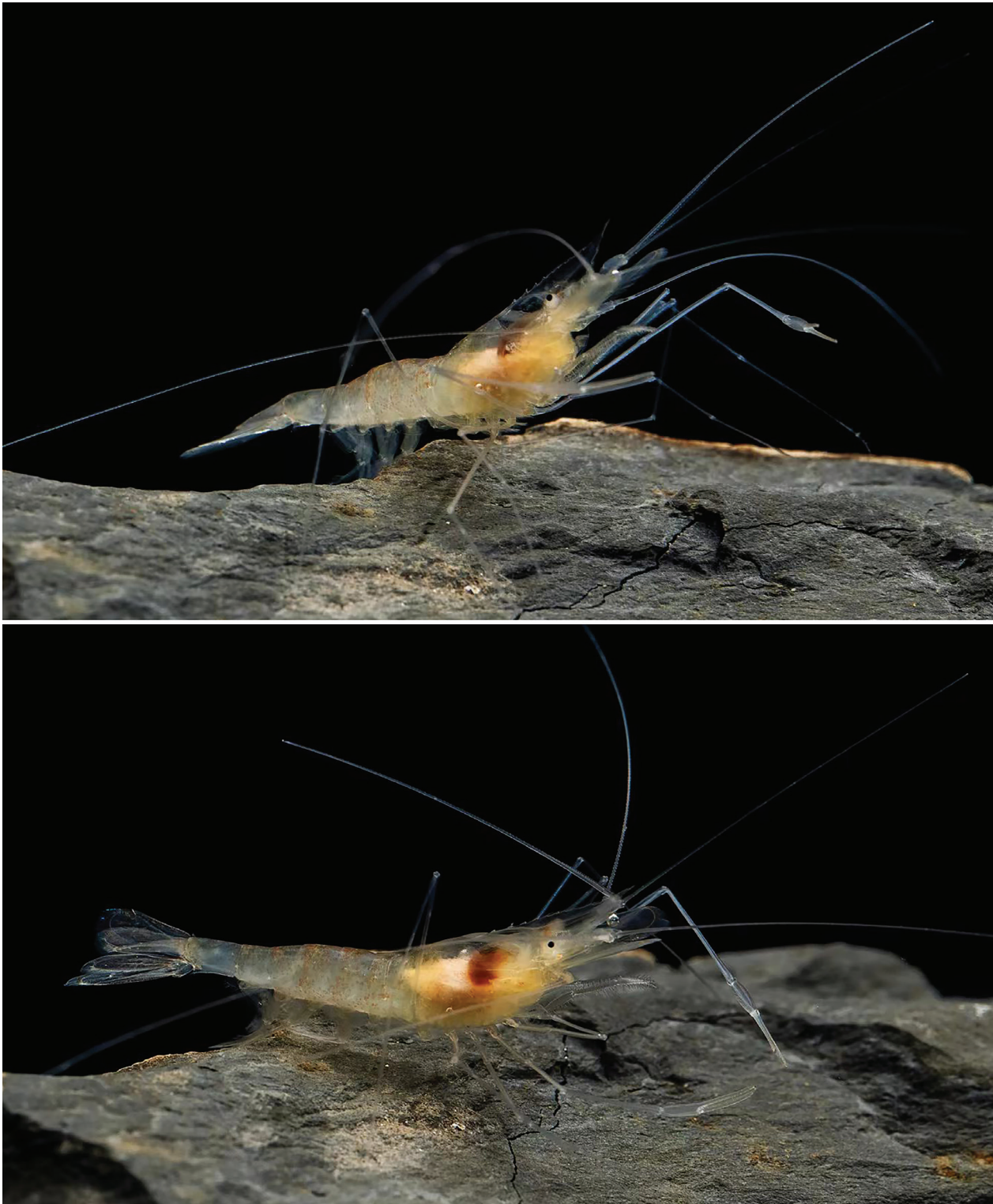


Figure 2. Live specimen of *Macrobrachium parvum* Jiang and Zhou, sp. nov. (female, IBGAS-Dec-Pal-484-8).

Third maxilliped with robust endopod; antepenultimate with row of simple setae on inner margin; penultimate 0.7 times length of antepenultimate, with rows of long, simple setae on inner margin; ultimate segment about 0.9 times penultimate segment, with rows of long, simple setae on inner and outer margins; exopod well-developed, reaching 0.7 times the length of antepenultimate, with plumose setae distally (Fig. 4A).

First pereiopod slender, reaching beyond end of scaphocerite. Ischium 0.6 times as long as merus; merus 0.8 times as long as carpus; carpus 2.7 times as long as chela; finger 1.2 times as long as palm (Fig. 4B, C).

Second pereiopod slender, subequal in size and similar for both sexes. Ischium 0.9 times as long as merus; merus 0.8 times as long as carpus; carpus as long as chela; finger 1.6 times as long as palm, palm not inflated (Fig. 4D, E).

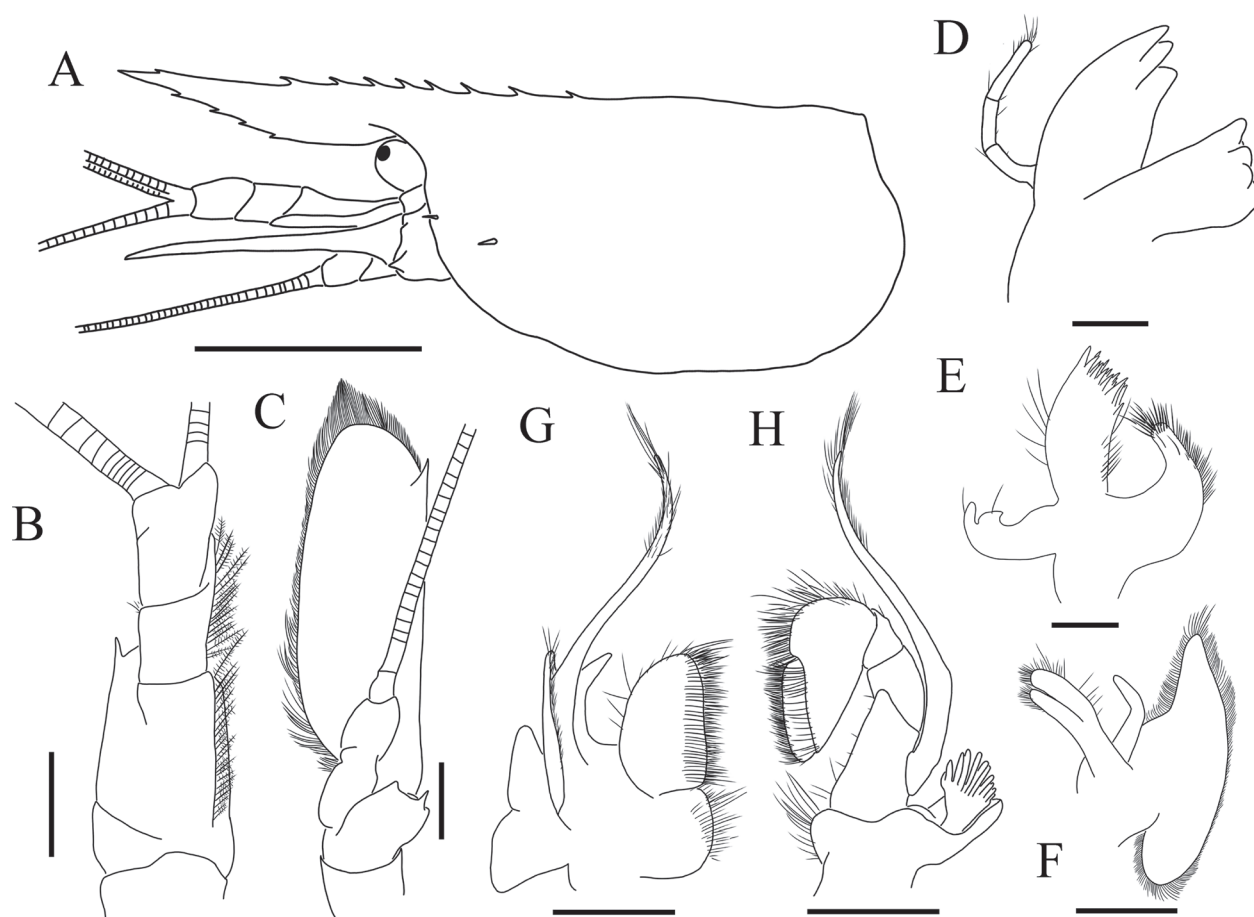


Figure 3. Holotype of *Macrobrachium parvum* Jiang and Zhou, sp. nov. (IBGAS-Dec-Pal-484-1). **A.** Cephalothorax and cephalic appendages, lateral view; **B.** Antennule; **C.** Antenna; **D.** Mandible; **E.** Maxillule; **F.** Maxilla; **G.** First maxilliped; **H.** Second maxilliped. Scale bars: 5 mm (**A**); 2.5 mm (**B**, **C**, **F–H**); 1 mm (**D**, **E**).

Third pereiopod slender, merus 1.4 times as long as carpus; carpus 0.7 times as long as propodus; propodus 5.3 times as long as dactylus (Fig. 4F).

Fourth pereiopod longer than third pereiopod, generally similar in form (Fig. 4G).

Fifth pereiopod slenderer and longer than third. Merus 1.4 times as long as carpus; carpus 0.7 times as long as propodus; propodus 7.0 times as long as dactylus; dactylus terminating in a small claw (Fig. 4H).

Male first pleopod with endopod about 1/3 length of exopod, inner margin concave, outer margin slightly convex.

Male second pleopod with well-developed appendix masculina bearing numerous spiniform setae. Appendix interna digitiform, reaching to 0.7 length of appendix masculina.

Uropodal diaeresis with inner movable spine subequal to outer angle.

Color. Body semi-transparent to yellowish with ochreous marks on surface of carapace and abdomen, all appendages semi-transparent (Fig. 2).

Etymology. The specific name is a Latin word meaning “little” referring to the relatively small body size of the species.

Distribution. Du’an County, Guangxi, China.

Habitat. The interior spaces of Nonglitun Cave and Shuiyuandi Cave are spacious, with broad pools located

approximately 50–100 meters from the entrances. The substrates of these pools consist of silt and rocks. Nongguangshang Cave is a section of an underground river, with numerous puddles about 50 meters from the entrance during the dry season. *Macrobrachium parvum* sp. nov. were discovered in these puddles. During the rainy season, the water levels in these three caves rise significantly, even overflowing the cave entrances to form small lakes.

Remarks. This species exhibits significant morphological and molecular divergence from other cave-dwelling congeners. Key diagnostic traits include a smaller body size, extremely slender appendages, and the presence of degenerated yet traceable body color and eyes. It can be distinguished from all epigeal species and *M. tenuipes* by the strongly reduced eyes with only small area in tip pigmented and the semi-transparent body color. This species differs from other four stygobiotic species in China by the pigmented eyes and body surface, the relatively smaller body size, the slender scaphocerite, and the extremely slender pereiopods, the shorter chela of second pereiopods and the moveable spine on uropodal diaeresis that are subequal to outer angle (Table 4).

Genetically, it demonstrates substantial interspecific divergence, with pairwise COI and 16S sequence differences exceeding 23% and 8%, respectively. Phylogenetic

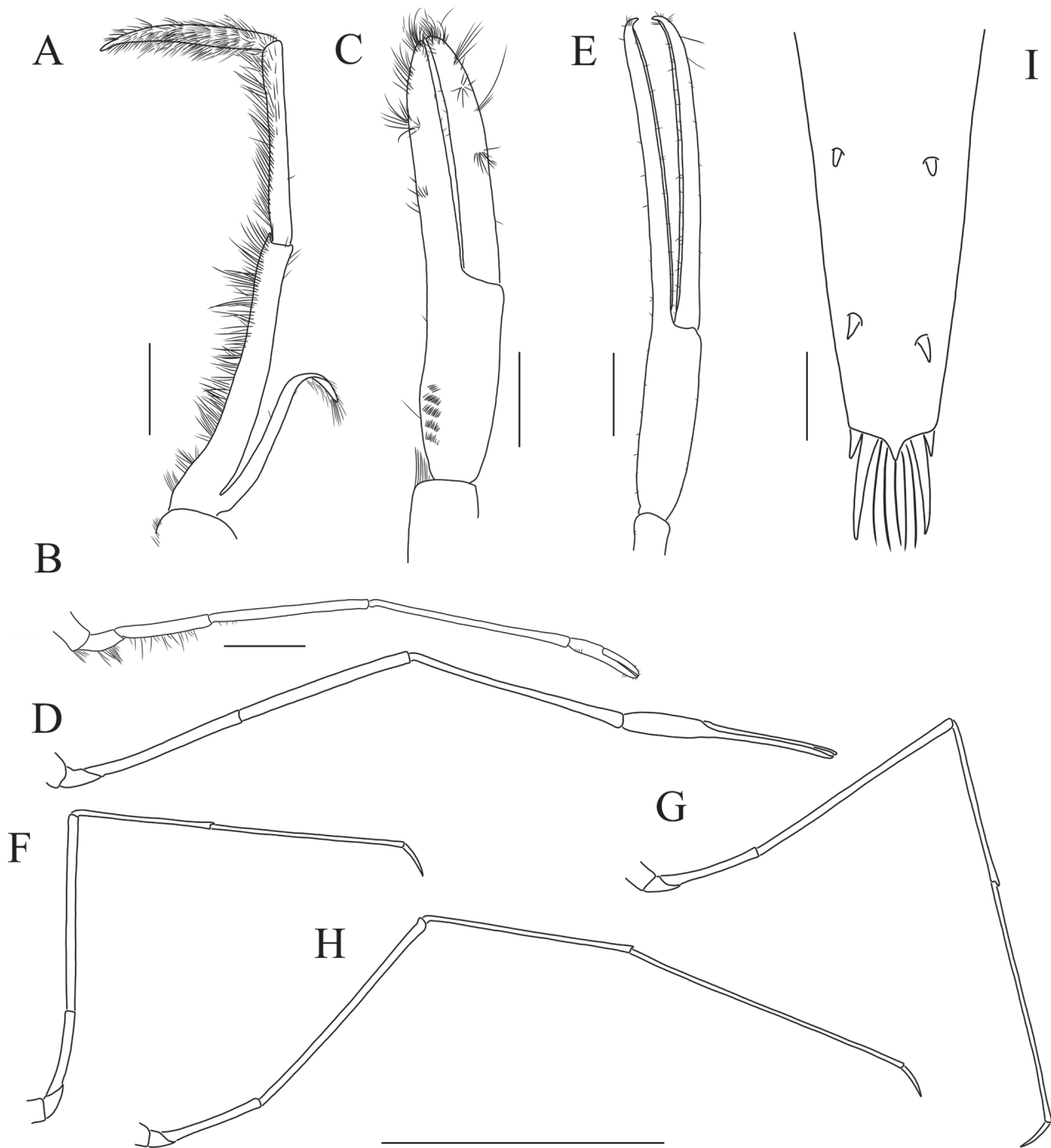


Figure 4. Holotype of *Macrobrachium parvum* Jiang and Zhou, sp. nov. (IBGAS-Dec-Pal-484-1). **A.** Third maxilliped; **B.** First pereopod; **C.** Chela of first pereopod; **D.** Second pereopod; **E.** Chela of second pereopod; **F.** Third pereopod; **G.** Fourth pereopod; **H.** Fifth pereopod; **I.** Telson. Scale bars: 2.5 mm (**A**, **B**, **E**); 2 mm (**C**, **I**); 10 mm (**D**, **F–H**) (same bar at the bottom).

analyses recover this taxon as a distinct evolutionary lineage. These combined morphological and molecular data robustly support its identity as a valid species.

The stygomorphic characteristics of this species indicate substantial adaptation to cave environments. However, the persistence of residual pigmentation and ocular structures suggest an incomplete transition to complete cave adaptation. We therefore classify it as a stygophile rather than a stygobite.

In Nongguangshang Cave and Shuiyuandi Cave, this species is inhabiting sympatrically with *M. duanense*.

***Macrobrachium tenuipes* Zhu et al., 2020**

Macrobrachium tenuipes Zhu et al., 2020: 512, figs 5–7, 8A, C. Type locality: a cave in Mashan County, Guangxi, China.

Material examined. • 2 males (IBGAS-Dec-Pal-488-1–2) (tl 38.1–39.8 mm, cl 7.9–9.0 mm, rl 6.7–7.8 mm) and 1 female (IBGAS-Dec-Pal-488-3) (tl 38.7 mm, cl 8.6 mm, rl 7.9 mm), Du'an County, Gaoling Town, Nongchitianchuan Cave, 24.0887°N, 108.0640°E, 10. IV. 2024, Zhou J.J. leg. • 1 male (IBGAS-Dec-Pal-489-1) (tl 47.0 mm,

cl 10.6 mm, rl 8.4 mm) and 3 females (IBGAS-Dec-Pal-489-2-4) (tl 47.0–77.2 mm, cl 10.9–19.6 mm, rl 9.2–13.6 mm), Guangxi, Du'an County, Gaoling Town, Nongchitianchuan Cave, 13. IV. 2024, Zhou JJ. leg.

Diagnosis. Body yellowish, all appendages generally translucent to faint yellow. Carapace and abdomen smooth and glabrous. Rostrum slender, slightly convex above orbital margin, 0.8–1.1 times of cl, overreaching scaphocerite. Dorsal margin with 11–12 teeth, including 3 or 4 teeth behind orbit. Dorsal teeth placed more widely on anterior part. Ventral margin with 3 or 4 teeth. Eyes well-developed. Scaphocerite about 4.1 times longer than wide. Second pereopod slender, subequal in size, similar in both sexes. Merus 1.1–1.2 times as long as ischium; carpus 1.2–1.3 times as long as merus, 1.1 times as long as palm; palm not inflated; finger 0.8–0.9 times as long as palm. Uropodal diaeresis with inner movable spine slightly longer than outer angle.

Distribution. Mashan County and Du'an County, Guangxi, China.

Habitat. The species was discovered in a karst window (a sinkhole in karst landscapes) of an underground river, which has an area of approximately 9,200 m² and a depth exceeding 60 m.

Remarks. The COI genetic distance of the collected specimens ranges from 0–1.30% compared to the type specimens (A42 and A49, see Table 2) and are morphologically consistent with the original description. This species can be distinguished from other cave-dwelling species by the obviously pigmented body and eyes (Table 4). As for its differences from epigean species, see Zhu et al. (2020).

The type locality of this species is in Mashan County (Zhu et al. 2020). With the discovery of this species in Du'an, its distribution range has been extended to the northwest by about 50 km. The genetic differentiation between populations of these two sites is insignificant, and they cluster with the epigean species in the phylogram. This may further suggest that this species is a stygophile, having recently migrated to caves. It probably has a wider distribution range in the surface environment, which enables gene exchange between different cave populations.

Macrobrachium lingyunense species complex

Diagnosis. Body semi-transparent to golden yellow, all appendages semi-transparent. Carapace and abdomen smooth and glabrous. Rostrum reaching end of scaphocerite, 0.4–0.8 times of cl, straight, or slightly upward. Dorsal margin with 8–11 teeth, including 2–4 teeth behind orbit, starting from about 1/3 of carapace length. Dorsal teeth equally space, or teeth more widely spaced on postorbital regions than on anterior. Ventral margin with 2–4 teeth. Eyes with cornea totally degenerated. Ocular peduncle small, elliptical and non-pigmented. Scaphocerite about 2.2–2.4 times longer than wide. Second pereopod moderately robust, subequal in size, similar in both sexes. Merus 1.1–1.5 times as long as the ischium;

carpus 0.9–1.0 times as long as merus, 1.1–1.4 times as long as palm; palm slightly inflated; finger 1.4–1.6 times as long as palm. Uropodal diaeresis with inner movable spine shorter than outer angle.

Remarks. This species complex consists of three cryptic species: *Macrobrachium duanense*, *M. guizhouense* sp. nov. and *M. lingyunense*. They all share the following diagnostic characters that distinguish them from other species: completely degraded somatic pigmentation and eyes, a relatively robust body, an unicuspidate rostral tip, a rostrum formula of (2–4 + 5–7/2–4), a broad scaphocerite, specific segment ratios in the second pereopods, and a slightly inflated palm of the second pereopod (Table 4). Phylogenetic analyses robustly support their monophyly, and molecular delimitation confirms their distinct species status. However, while morphologically distinguishable from other cave-dwelling congeners, these three species exhibit remarkably similar characteristics to one another, making morphological differentiation challenging.

Some subtle characters may be helpful in separating them. For instance, the palm of the second pereopod is longer than the ischium in *M. duanense*, but shorter in both *M. guizhouense* sp. nov. and *M. lingyunense*. The scaphocerite is relatively broader in *M. guizhouense* sp. nov. (2.2 times longer than wide), compared to 2.4 times in *M. lingyunense* and *M. duanense* (Table 4).

Macrobrachium duanense Lan et al., 2017

Figs 5–7

Macrobrachium duanensis Lan et al., 2017: 61, figs 1–3. Type locality: a cave in Nongchi village, Gaoling Town, Du'an County, Guangxi, China.

Material examined. • 2 males (IBGAS-Dec-Pal-490-1–2) (tl 53.7–78.2 mm, cl 15.3–20.5 mm, rl 7.2–9.3 mm) and 2 females (IBGAS-Dec-Pal-490-3–4) (tl 56.4–67.7 mm, cl 14.0–17.3 mm, rl 7.4–7.8 mm), Guangxi, Du'an Yao Autonomous County, Disu Town, Xiadiao Village, Shuiyuandi Cave, 24.0061°N, 107.9840°E, 10. IV. 2024, Zhou JJ. leg. • 4 females (IBGAS-Dec-Pal-491-1–4) (tl 52.7–86.9 mm, cl 13.0–24.5 mm, rl 6.2–11.2 mm), Guangxi, Du'an, Gaoling Town, Nongguangshang cave, 24.0095°N, 108.0824°E, alt. 198 m, 16. IV. 2023, Zhou JJ. leg. • 3 females (IBGAS-Dec-Pal-492-1–3) (tl 39.2–73.1 mm, cl 11.3–19.3 mm, rl 5.3–7.9 mm), Du'an, Nongshui Village, Nongshuitun Cave, 23.8413°N, 107.9981°E, 10. IV. 2024, Zhou JJ. leg.

Description. Body moderately robust (Fig. 5). Rostrum short and broad, reaching 3/4 to 4/5 of scaphocerite, 0.4–0.5 times of cl, slightly convex above orbital margin. Dorsal margin with 8–10 teeth (mode 9), including 2–3 teeth behind orbit, starting from about 1/4 of carapace length. Dorsal teeth equally spaced, except most posterior tooth more widely spaced than others. Ventral margin with 2–4 teeth (Fig. 6A).

Eyes with cornea totally degenerated. Ocular peduncle small, elliptical and non-pigmented (Figs 5, 6A).



Figure 5. Live specimen of *Macrobrachium duanense* Lan et al., 2017 (female, IBGAS-Dec-Pal-491-1).

Carapace (Figs 5, 6A) smooth and glabrous. Antennal spine small, tip reaching anterolateral margin of carapace. Hepatic spine small, lying behind and below antennal spine.

Abdomen (Fig. 5) smooth and glabrous. First to third pleurites broadly rounded, fourth and fifth pleurites slightly produced posteriorly. Sixth somite 1.2–1.5 times as long as fifth somite, with posteroventral angle slightly protruded.

Telson 1.5 times length of sixth segment, 0.4–0.5 times of cl. Tapered posteriorly, with a sharp point. Dorsal surface with two pairs of spines, occasionally with 1 or 3 teeth. Posterior margin bearing two pairs of lateral spines. Inner spines obviously longer than outer spines, with plumose setae between inner spines (Fig. 7I).

Antennule (Fig. 6B) with sharp stylocerite, reaching about half of basal segment of antennular peduncle. Basal segment broad, about 2 times as wide as second segment, as long as wide; distolateral spine of basal antennular segment slender, reaching beyond half of second segment. Second segment ca. 0.4 times as long as basal segment, ca. 0.5 times as long as distal segment. All segments except distal segment with submarginal plumose setae.

Scaphocerite about 2.4 times longer than wide. Inner margin somewhat convex, lateral margin strait, with stout distolateral tooth, not reaching anterior margin (Fig. 6C).

Mandible typical of genus, with three-segmented palp, distal segment slightly longer than the other two segments; incisor process with three sharp teeth; molar process robust, truncate distally (Fig. 6D).

Maxillular palp bilobed, upper lobe slender, slightly longer than lower lobe, with few setae distally; low-

er lobe stout and small, no setae. upper lacinia broadly elongated, distal margin with rows of strong spines, lower lacinia shorter than upper lacinia, tapering distally, densely setose (Fig. 6E).

Maxilla with simple palp; basal endite deeply bilobed, upper and lower lobes subequal and digitiform, with numerous simple setae distally; scaphognathite broad, about 3.8 times as long as wide (Fig. 6F).

First maxilliped with simple and small palp, basal and coxal endites distinct, tip of flagellum of exopod densely setose, epipod deeply bilobed (Fig. 6G).

Second maxilliped with 5-segmented endopod, flagellum with numerous plumose setae distally, epipod simple, with developed podobranch (Fig. 6H).

Third maxilliped with robust endopod; antepenultimate with row of simple setae on inner margin; penultimate 0.6 times length of antepenultimate, with rows of long, simple setae on inner margin; ultimate segment about 0.8 times penultimate segment, with rows of long, simple setae on inner and outer margins; exopod well developed, reaching 0.8 times the length of antepenultimate, with plumose setae distally (Fig. 7A).

First pereopod slender, reaching beyond end of scaphocerite. Ischium 0.6 times as long as merus; merus as long as carpus; carpus 1.7 times as long as chela; finger 1.2 times as long as palm (Fig. 7B, C).

Second pereopod moderately robust, subequal in size, similar in both sexes. Merus 1.5 times as long as the ischium; carpus 0.9 times as long as merus, 1.1 times as long as palm; palm slightly inflated; finger 1.4 times as long as palm, glabrous (Figs 7D, E).

Third pereopod slender, merus 1.8 times as long as carpus; carpus 0.5 times as long as propodus; propodus

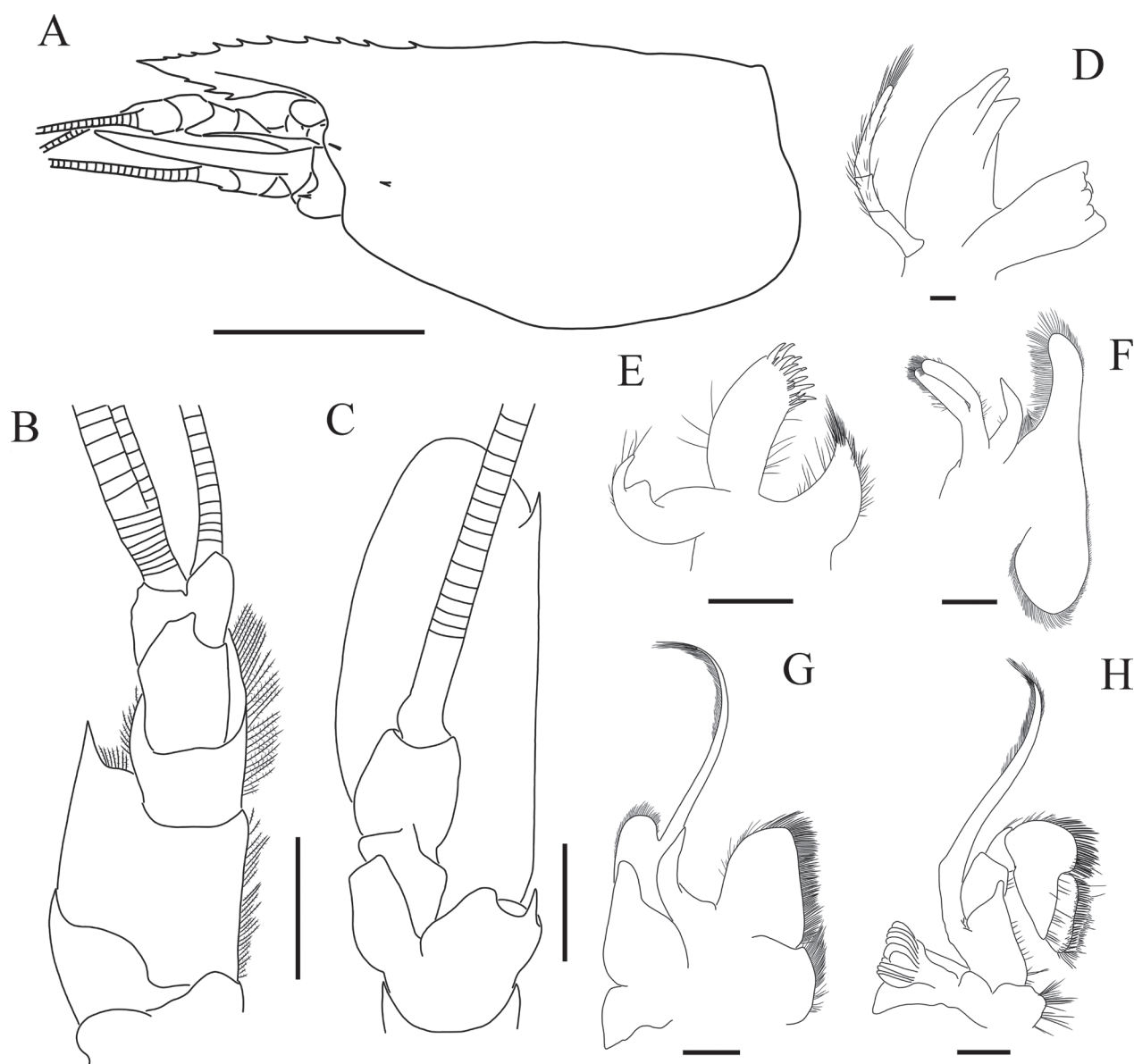


Figure 6. Male of *Macrobrachium duanense* Lan et al., 2017 (IBGAS-Dec-Pal-490-1). **A.** Cephalothorax and cephalic appendages, lateral view; **B.** Antennule; **C.** Antenna; **D.** Mandible; **E.** Maxillule; **F.** Maxilla; **G.** First maxilliped; **H.** Second maxilliped. Scale bars: 10 mm (**A**); 5 mm (**B**, **C**); 0.75 mm (**D**); 2.5 mm (**E–H**).

5.7 times as long as dactylus with several small spines on ventral margin (Fig. 7F).

Fourth pereopod longer than third pereopod, generally similar in form (Fig. 7G).

Fifth pereopod slenderer and longer than third. merus 1.4 times as long as carpus; carpus 0.6 times as long as propodus; propodus 8.9 times as long as dactylus, with several small spines on ventral margin; dactylus terminating in a small claw (Fig. 7H).

Male first pleopod with endopod shorter than half length of exopod, inner margin concave, outer margin slightly convex.

Male second pleopod with well-developed appendix masculina bearing numerous spiniform setae. Appendix interna digitiform, reaching to 0.6 length of appendix masculina.

Uropodal diaeresis with inner movable spine shorter than outer angle.

Color. Body semi-transparent to golden yellow, all appendages semi-transparent (Fig. 5).

Distribution. Du'an County, Guangxi, China.

Habitat. Nongshuitun Cave is similar to Nonglitun Cave and Shuiyuandi Cave, featuring broad pools in the dark zone 50–100 meters from the entrance, which may overflow out of the cave during the rainy season.

Remarks. As a stygobiotic species, this species differs from all epigeal species as well as *M. parvum* sp. nov. and *M. tenuipes* by the completely degraded somatic pigmentation and eyes. It can be diagnosed from *M. elegantum* by the unicuspidate tip of rostrum (bifurcate in *M. elegantum*), the broader scaphocerite (2.4 times longer than wide in *M. duanense* vs. 3.0 in *M. elegantum*), the different rostral formula ($2-3 + 6-7/2-4$ in *M. duanense* vs. $3-4 + 3-4/4-6$ in *M. elegantum*) and the different ratios between the segments of second

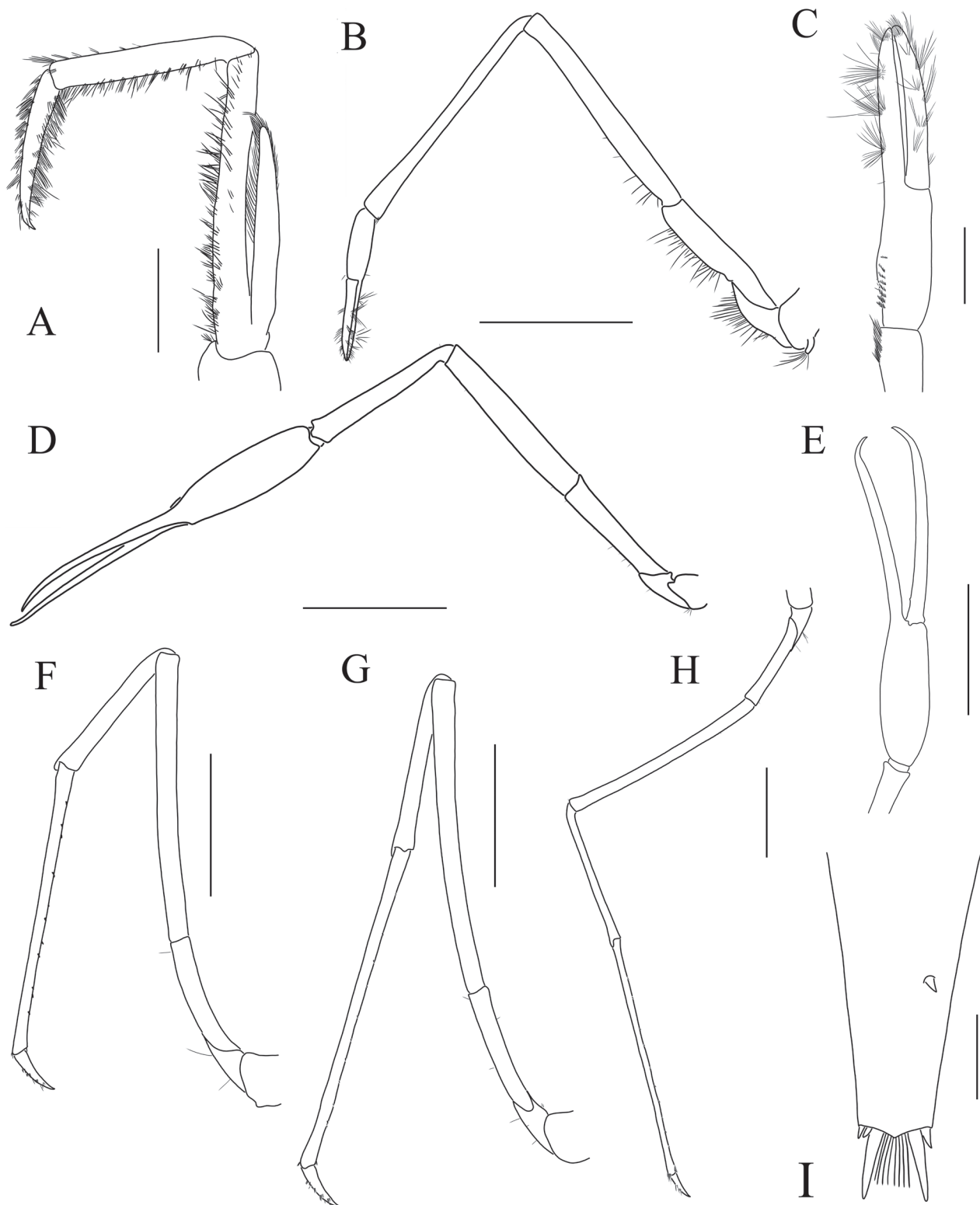


Figure 7. Male of *Macrobrachium duanense* Lan et al., 2017 (IBGAS-Dec-Pal-490-1). **A.** Third maxilliped; **B.** First pereopod; **C.** Chela of first pereopod; **D.** Second pereopod; **E.** Chela of second pereopod; **F.** Third pereopod; **G.** Fourth pereopod; **H.** Fifth pereopod; **I.** Telson. Scale bars: 5 mm (**A**); 10 mm (**B**, **D–H**); 2.5 mm (**C**, **I**).

pereopods. This species is very similar to its sister species, *M. guizhouense* sp. nov. and *M. lingyunense*, but it can be distinguished by the palm of second pereopods longer than ischium (shorter in *M. guizhouense* sp. nov. and *M. lingyunense*) (Table 4).

In the original description, Lan et al. (2017) used two spellings for the species name: ‘*Macrobrachium douanensis*’ in the Chinese description and ‘*M. duanensis*’ in the English title and abstract. Here, we adopt ‘*duan-*’ (the correct spelling of the county name) and adjust the suffix

to ‘-ense’ to match the neuter gender of the genus name (ICZN Articles 31.2 and 32.2, International Commission on Zoological Nomenclature, 1999).

The type locality of this species is a cave in Nongchi Village. Although we were unable to access the exact type locality, we collected specimens from three nearby caves, apart approximately 2 km (Nongguangshang Cave) to 25 km (Nongshuitun Cave) away. Given the extensive connectivity of the local cave system and the morphological congruence between our specimens and the original description, these specimens can be regarded as topotypes.

The original description and the figures of this species are poor. In addition, the authors only compared it to the epigeal and widespread species *M. nipponense*, rather than the stygobiotic species *M. lingyunense*. These hamper the correct identification of this species. Here, we redescribe this species and the results of the molecular delimitation analyses based on the topotypes of the two species, *M. duanense* and *M. lingyunense*, confirmed that they are two different species.

***Macrobrachium guizhouense* Jiang & Chen, sp. nov.**

<https://zoobank.org/DD88C826-FD38-4AF4-8B75-64FE156FC0F3>

Figs 8–10

Type materials. *Holotype*: • male (IBGAS-Dec-Pal-493-1) (tl 52.1 mm, cl 14.5 mm, rl 7.2 mm), China, Guizhou Province, Libo County, Jiaou Town, Malai Village, Malai Cave, 25.2722°N, 107.6638°E, alt. 790 m, 9. VI. 2023, Jiang X.K., Wu L. & Fan C. leg.

Paratypes: • 20 males (IBGAS-Dec-Pal-493-2–21) (tl 36.8–55.7 mm, cl 8.8–17.0 mm, rl 6.3–9.4 mm) and 44 females (IBGAS-Dec-Pal-493-22–65) (tl 31.5–61.7 mm, cl 7.8–20.8 mm, rl 5.3–9.8 mm), same data as holotype. • 1 male (IBGAS-Dec-Pal-494-1) (tl 50.1 mm, cl 11.6 mm, rl 9.2 mm) and 6 females (IBGAS-Dec-Pal-494-2–7) (tl 51.2–63.7 mm, cl 12.2–16.9 mm, rl 8.5–8.7 mm), Malai Cave, III. 2023, Luo T. et al. leg. • 6 males (IBGAS-Dec-Pal-495-1–6) (tl 52.1–54.4 mm, cl 12.1–13.1 mm, rl 7.7–9.2 mm) and 8 females (IBGAS-Dec-Pal-495-7–14) (tl 43.4–61.5 mm, cl 10.7–17.4 mm, rl 6.4–7.8 mm), Malai Cave, 10. XI. 2023, Liu Y.W. et al. leg. • 2 females (IBGAS-Dec-Pal-496-1–2) (tl 45.6–64.6 mm, cl 11.5–16.9 mm, rl 6.9–10.0 mm), Libo County, Jiaou Town, Gengzao Village, Gengzao Cave, 25.2735°N, 107.6777°E, alt. 775 m, 28. VI. 2023, Zhou J.J. et al. leg. • 1 male (IBGAS-Dec-Pal-497-1) (tl 63.1 mm, cl 15.0 mm, rl 9.4 mm) and 1 female (IBGAS-Dec-Pal-497-2) (tl 61.7 mm, cl 16.7 mm, rl 7.6 mm), Gengzao Cave, 8. XI. 2023, Jiang X.K. et al. leg.

Description. Body moderately robust (Fig. 8). Rostrum (Figs 8, 9A) reaching to end of scaphocerite, 0.4–0.7 times of cl, straight, or slightly upward distally. Dorsal margin with 8–11 teeth (mode 9), including 3–4 teeth behind orbit (mode 3), starting from about 1/3 of carapace length. Dorsal teeth equally space, or teeth more widely

spaced on postorbital regions than on anterior. Ventral margin with 2–4 teeth (mode 3).

Eyes with cornea totally degenerated. Ocular peduncle small, elliptical and non-pigmented (Figs 8, 9A).

Carapace smooth and glabrous. Antennal spine small, tip reaching anterolateral margin of carapace. Hepatic spine small, lying behind and below antennal spine (Figs 8, 9A).

Abdomen smooth and glabrous. First to third pleurites broadly rounded, fourth and fifth pleurites slightly produced posteriorly. Sixth somite 1.4–1.7 times as long as fifth somite, with posteroventral angle slightly protruded (Figs 8, 9A).

Telson 1.5 times length of sixth segment, 0.4–0.5 times of cl. Tapered posteriorly, with a sharp point. Dorsal surface with two pairs of small spines. Posterior margin bearing two pairs of lateral spines. Inner spines obviously longer than outer spines, with plumose setae between inner spines (Fig. 10G).

Antennule with sharp stylocerite, reaching about half of basal segment of antennular peduncle. Basal segment broad, about 1.5 times as wide as second segment, as long as wide; distolateral spine of basal antennular segment slender, reaching 0.4 times as long as second segment. Second segment as long as basal segment, ca. 1.2 times as long as distal segment. All segments except distal segment with submarginal plumose setae (Fig. 9B).

Scaphocerite about 2.2 times longer than wide. Inner margin somewhat convex; lateral margin strait, with stout distolateral tooth, not reaching anterior margin (Fig. 9C).

Mandible typical of genus, with three-segmented palp; three segments subequal in length; incisor process with three sharp teeth; molar process robust, truncate distally (Fig. 9D).

Maxillular palp deeply bilobed, upper lobe robust, longer than lower lobe, with few setae distally; lower lobe stout and small, devoid of setae with tip hook-like. Upper lacinia broadly elongated, distal margin with rows of strong spines, lower lacinia shorter than upper lacinia, tapering distally, densely setose (Fig. 9E).

Maxilla with simple palp; basal endite deeply bilobed, upper and lower lobes subequal and digitiform, with numerous simple setae distally; scaphognathite broad, about 4.7 times as long as wide (Fig. 9F).

First maxilliped with simple and small palp, basal and coxal endites distinct, tip of flagellum of exopod densely setose, epipod deeply bilobed (Fig. 9G).

Second maxilliped with 5-segmented endopod, flagellum with numerous plumose setae distally, epipod simple, with developed podobranch (Fig. 9H).

Third maxilliped with robust endopod; antepenultimate with rows of simple setae on inner margin; penultimate 0.6 times length of antepenultimate, with rows of long, simple setae on inner and lateral margins; ultimate segment about 0.8 times penultimate segment, with rows of long, simple setae; exopod well-developed, reaching

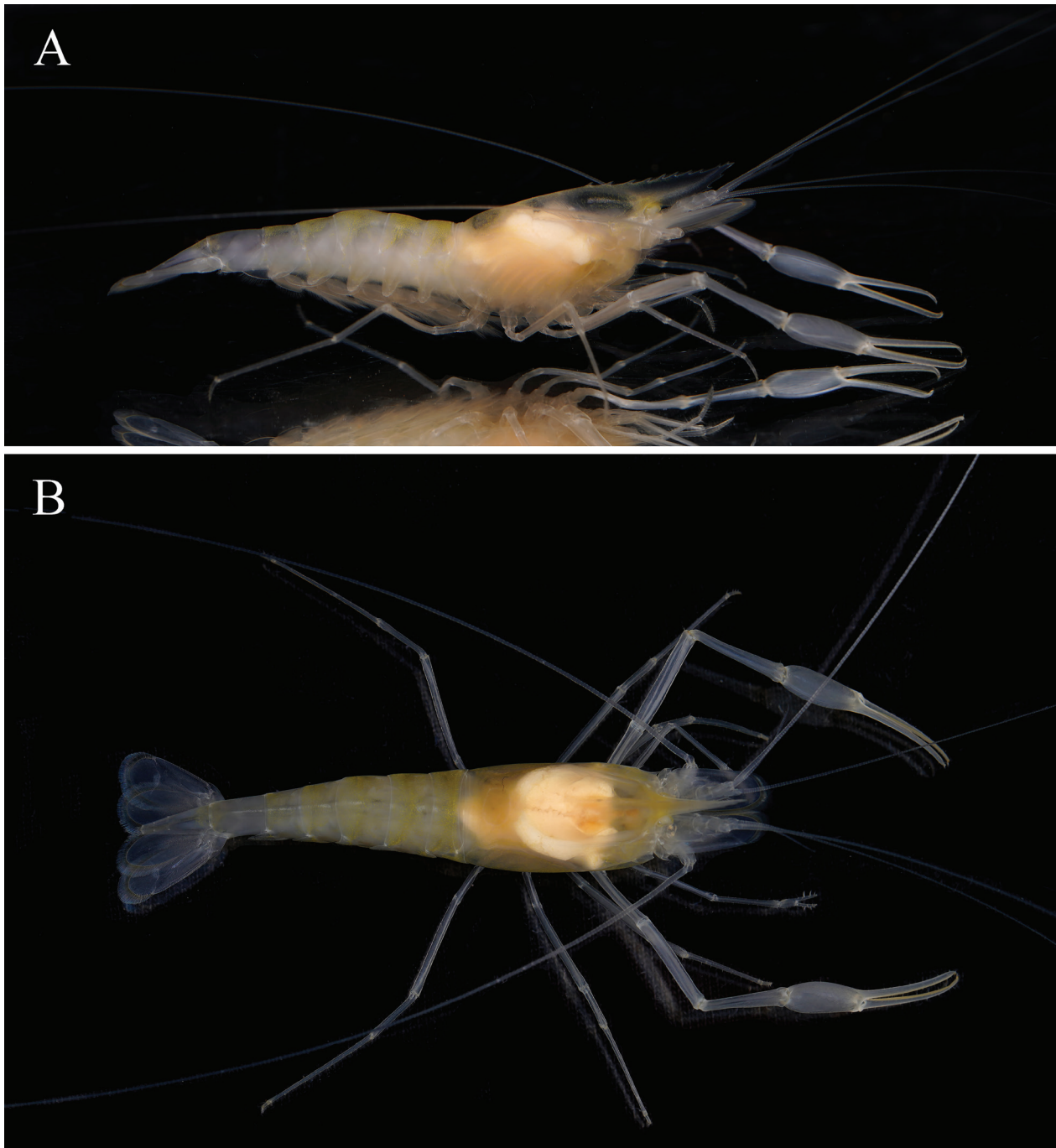


Figure 8. Live specimen of *Macrobrachium guizhouense* Jiang and Chen, sp. nov. (male, IBGAS-Dec-Pal-493-3). **A.** Lateral view; **B.** Dorsal view.

0.7 times the length of antepenultimate, with plumose setae distally (Fig. 10A).

First pereiopod slender, reaching beyond end of scaphocerite by carpus. Ischium 0.5 times as long as merus; merus 0.9 times as long as carpus; carpus 1.8 times as long as chela; finger 1.3 times as long as palm (Fig. 10B).

Second pereiopod moderately robust, subequal in size, similar in both sexes. Merus 1.2 times as long as the ischium; carpus 0.9 times as long as merus, 1.4 times as long as palm; palm slightly inflated; finger 1.6 times as long as palm (Fig. 10C, D).

Third pereiopod slender, merus 2.3 times as long as carpus; carpus 0.5 times as long as propodus; propodus 2.7 times as long as dactylus with several small spines on ventral margin (Fig. 10E).

Fourth pereiopod longer than third pereiopod, similar in form.

Fifth pereiopod slenderer and longer than third. merus 1.7 times as long as carpus; carpus 0.5 times as long as propodus; propodus 4.8 times as long as dactylus, with several small spines on ventral margin; dactylus terminating in a small claw (Fig. 10F).

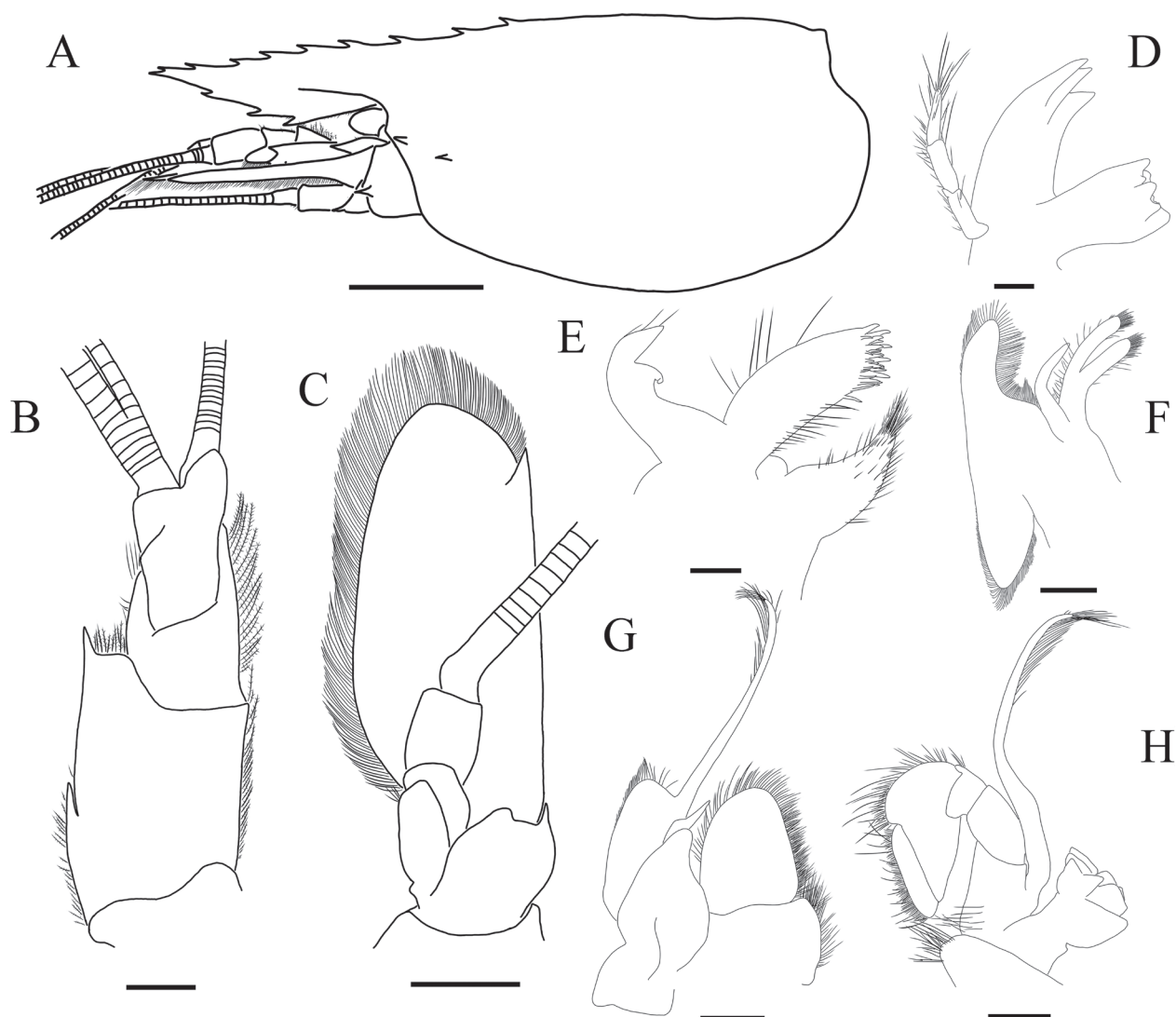


Figure 9. Holotype of *Macrobrachium guizhouense* Jiang and Chen, sp. nov. (IBGAS-Dec-Pal-493-1). **A.** Cephalothorax and cephalic appendages, lateral view; **B.** Antennule; **C.** Antenna; **D.** Mandible; **E.** Maxillule; **F.** Maxilla; **G.** First maxilliped; **H.** Second maxilliped. Scale bars: 5 mm (**A**); 2.5 mm (**B, D–H**); 5 mm (**C**).

Male first pleopod with endopod about half length of exopod, inner margin concave, outer margin slightly convex.

Male second pleopod with well-developed appendix masculina bearing numerous spiniform setae. Appendix interna digitiform, reaching to 0.6 length of appendix masculina.

Uropodal diaeresis with inner movable spine shorter than outer angle.

Color. Body semi-transparent to golden yellow, all appendages semi-transparent (Fig. 8).

Etymology. This species is named after the type locality, highlighting that this is the first stygobitic *Macrobrachium* species found in Guizhou Province.

Distribution. Libo County, Guizhou Province, China.

Habitat. Malai Cave and Gengzao Cave have similar environments and are both located within a village. Both caves slope gently downward. About 300 meters from their entrances; each cave contains a large pool. Due to the obstruction by rocks, the exact area and depths of the

pools are unclear. The substrates consist of silt and rocks. Local residents draw water from these pools for domestic use. *Macrobrachium guizhouense* sp. nov. were collected from these pools.

Remarks. This species differs from all epigean species as well as *M. parvum* sp. nov. and *M. tenuipes* by the completely degraded somatic pigmentation and eyes. It can be separated from *M. elegantum* by the unicuspidate tip of rostrum (bifurcate in *M. elegantum*), the broader scaphocerite (2.2 times longer than wide in *M. guizhouense* sp. nov. vs. 3.0 in *M. elegantum*), the different rostral formula ($3-4 + 5-7/3-4$ in *M. guizhouense* sp. nov. vs. $3-4 + 3-4/4-6$ in *M. elegantum*) and the different ratios between the segments of second pereopods. This species can be distinguished from *M. duanense* by the palm of second pereopods which is shorter than ischium; from *M. lingyunense* by the relatively broader scaphocerite (2.2 times longer than wide in *M. guizhouense* sp. nov. vs. 2.4 in *M. lingyunense*) (Table 4).

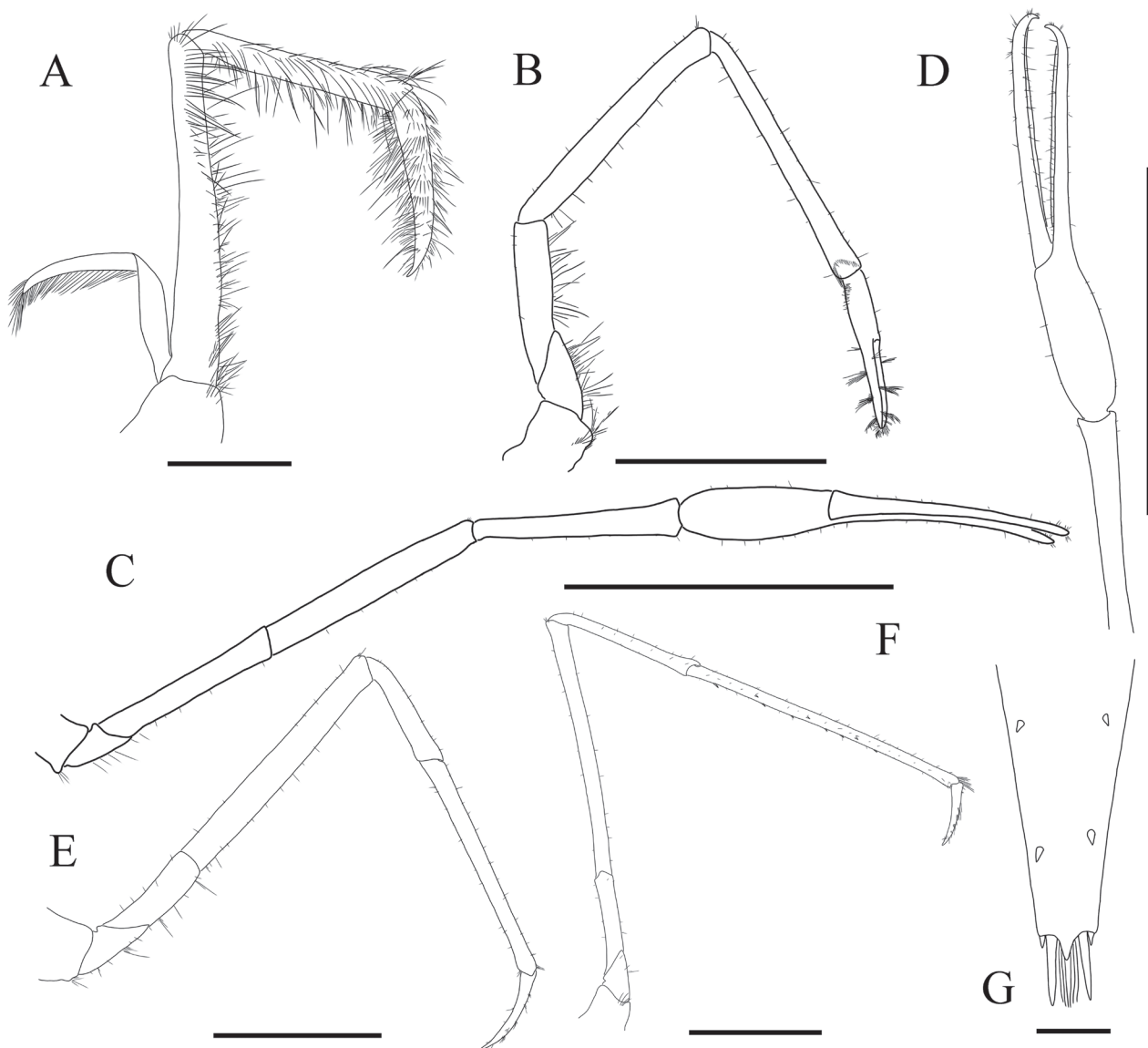


Figure 10. Holotype of *Macrobrachium guizhouense* Jiang and Chen, sp. nov. (IBGAS-Dec-Pal-493-1). **A.** Third maxilliped; **B.** First pereopod; **C.** Second pereopod; **D.** Chela of second pereopod; **E.** Third pereopod; **F.** Fifth pereopod; **G.** Telson. Scale bars: 2.5 mm (**A**, **B**, **G**); 10 mm (**C**, **D**); 5 mm (**E**, **F**).

Macrobrachium lingyunense (Li & Luo, 2001)

Fig. 11

Typhlocaridina lingyunensis Li & Luo, 2001: 72, fig. 1. Type locality: Sha Cave, Lingyun County, Guangxi, China.

Macrobrachium lingyunense Li et al., 2006: 277, figs 1–3; Cai and Ng 2018: 29.

Material examined. • 1 male (IBGAS-Dec-Pal-498-1) (tl 41.2 mm, cl 11.0 mm, rl 5.9 mm) and 2 females (IBGAS-Dec-Pal-498-2–3) (tl 37.6–41.9 mm, cl 10.3–11.1 mm, rl 5.8–6.2 mm), Guangxi, Lingyun County, Sha Cave, 24.4185°N, 106.6123°E, alt. 669 m, 25. XII.2023, Jiang X.K. & Liu Y.W. leg.

Distribution. Lingyun County, Guangxi, China.

Habitat. A small run-of-river hydropower station has been built at the entrance of Sha Cave, which generates electricity from May to December each year. The cave

consists of two layers. The upper layer features intermittent small pools. The lower layer is an underground river with a large water flow. The underground space of Sha Cave is extensive, with scattered large boulders from collapses and soil mounds. *Macrobrachium lingyunense* was discovered in the pools of the upper level, approximately 500 meters from the cave entrance, located within the dark zone.

Remarks. This species differs from all epigeal species as well as *M. parvum* sp. nov. and *M. tenuipes* by the completely degraded somatic pigmentation and eyes. It can be distinguished from *M. elegantum* by the tip of rostrum unicuspidate (bifurcate in *M. elegantum*), the broader scaphocerite (2.2 times longer than wide in *M. guizhouense* sp. nov. vs. 3.0 in *M. elegantum*), the different rostral formula (3–4 + 5–7/3–4 in *M. guizhouense* sp. nov. vs. 3–4 + 3–4/4–6 in *M. elegantum*) and the different ratios between the segments of second pereopods. This species can be distinguished from *M. duanense* by the palm of second pereopods shorter



Figure 11. Live specimen of *Macrobrachium lingyunense* (Li & Luo, 2001) (female, IBGAS-Dec-Pal-498-3).

than ischium; from *M. lingyunense* by the relatively slender scaphocerite (2.4 times longer than wide in *M. lingyunense* vs. 2.2 in *M. guizhouense* sp. nov.) (Table 4).

Discussion

Diversity, cave adaptation and evolution of Chinese cave *Macrobrachium*

As a hotspot for cave biodiversity, the karst areas in southern China harbor exceptional but poorly documented diversity of cave-adapted *Macrobrachium* prawns. Based on extensive field collections from its concentrated distribution areas, the karst cave clusters in southwestern China, and a thorough review of the literature, this study provides the first taxonomic delimitation and phylogenetic analyses of this group. Our systematic investigation revealed six species, including two new taxa *Macrobrachium guizhouense* sp. nov. and *M. parvum* sp. nov., representing a 50% increase over the previously documented diversity.

Biogeographically, cave *Macrobrachium* species from China concentrate in western Guangxi with Du'an County as a hotspot, hosting three species: *M. duanense*, *M. parvum* sp. nov., and *M. tenuipes* (Fig. 12). Outside this core area, high discovery potential exists in southern Guizhou, eastern Yunnan, and northern Indochina. These regions share similar climate and geological history (Huang et al. 2022), which are crucial for the formation of cave-dwelling species. Future explorations should prioritize these areas to assess the undiscovered diversity,

clarify evolutionary histories, and establish conservation priorities for these organisms.

These six species display a spectrum of stygomorphic adaptations. Four stygobites, *M. duanense*, *M. lingyunense*, *M. guizhouense* sp. nov., and *M. elegantum*, exhibit complete depigmentation, non-functional eyes (Figs 5, 8, 11), and are likely restricted to single cave networks. Notably, *M. parvum* sp. nov. demonstrates stronger cave adaptation than typical stygophiles, with nearly complete body depigmentation retaining only residual speckles and substantially reduced ocular structures (Fig. 2), suggesting it represents an advanced transitional form approaching stygobiotic status. In contrast, *M. tenuipes* retains morphological affinity with surface-dwelling congeners, displaying obvious pigmentation and functional eyes. These characteristics indicate its status as an early-stage cave colonizer.

Molecular analyses uncover three independent cave colonization events (Fig. 1). The basal position of *M. parvum* sp. nov. suggests an independent subterranean origin, while the monophyletic clade of the three stygobites, *M. duanense*, *M. lingyunense*, and *M. guizhouense* sp. nov. represents the second lineage of cave-adapted radiation. *M. tenuipes* clusters with surface-dwelling congeners (*M. bilineare* and *M. laevis*), indicating recent cave invasion, which is consistent with its morphological characters. The morphologically distinct *M. elegantum*, though lacking molecular data, may represent a fourth independent lineage. This hypothesis requires confirmation through future analyses. The multiple independent origins of cave-dwelling in this group

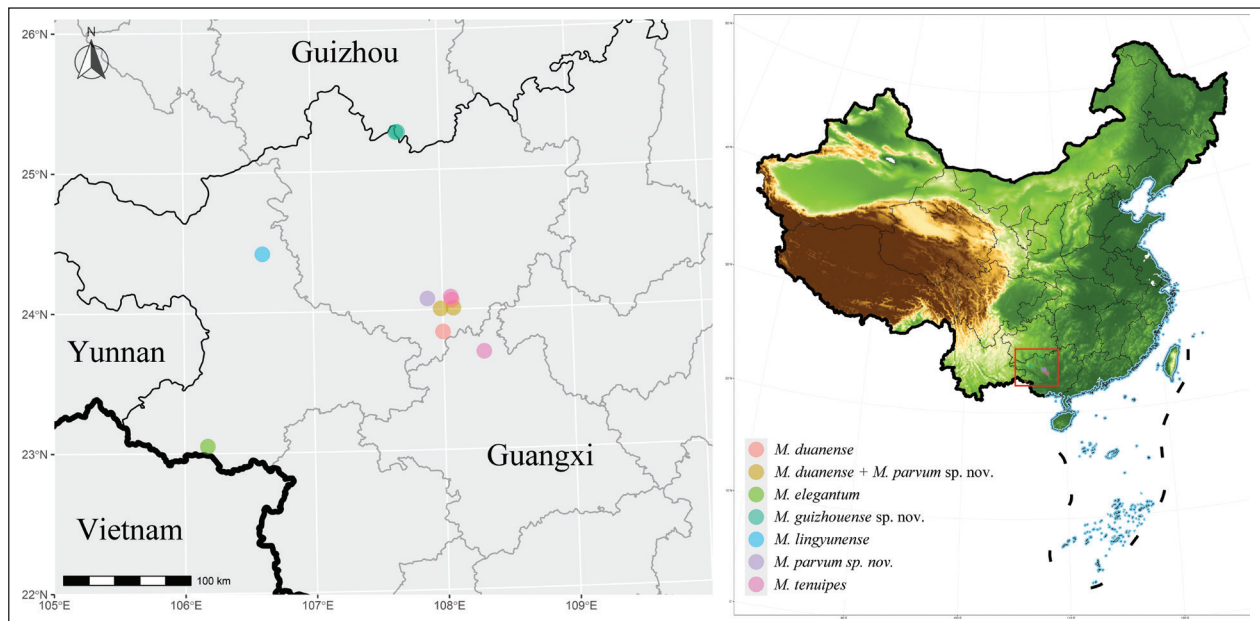


Figure 12. Distribution of cavernicolous *Macrobrachium* in China.

are remarkably similar to those in another crustacean family, Atyidae (von Rintelen et al. 2012).

The speciation mechanisms of cave organisms align with two non-exclusive hypotheses: 1) the Climatic Relict Hypothesis (Holsinger 1988; Peck and Finston 1993; Holsinger 2000; Juan et al. 2010), where ancestral populations sought cave refugia during climatic extremes, and 2) the Adaptive Shift Hypothesis (Howarth 1973, 1987; Holsinger 2000; Niemiller et al. 2008; Juan et al. 2010), involving active colonization driven by ecological opportunity. Recent studies on multiple biological taxa have shown that the formation of cave fauna in the subtropical East Asia is mainly affected by the rapid uplift of the Tibetan Plateau and the significant intensification of the East Asian monsoon climate, which is consistent with the climatic relict hypothesis (Li et al. 2022; Mao et al. 2022; Luo et al. 2025). However, there are no specific research reports on cave prawns from this area at present, and it remains to be confirmed whether these animals conform to the hypothesis.

It is noteworthy that some sympatric distributions have been observed so far, i.e. Nongguangshang cave and Shuiyuandi cave inhabited by both *M. duanense* and *M. parvum* sp. nov. This co-occurrence likely results from post-speciation dispersal mediated by karst drainage reorganization or seasonal flooding, rather than sympatric speciation. This reflects the complexity of the evolutionary history of Chinese cave prawn.

Owing to their close association with cave habitats and limited dispersal capabilities, cave-dwelling shrimps are particularly vulnerable to impacts from anthropogenic activities and habitat degradation. For example, in North America, two cave-dwelling species of the family Palaemonidae have been classified as Critically Endangered (Possibly Extinct) due to environmental pollution and invasive species (De Grave et al. 2015). To date, all six cave-dwelling species of *Macrobrachium* in China are

endemic, with distributions confined to single or adjacent caves. However, the scarcity of research data and the absence of comprehensive assessments have resulted in a significant knowledge gap regarding their conservation status and threats. Consequently, these species have not been included in either the China Species Red List or the IUCN Red List. To protect these rare and relict species, relevant conservation efforts are urgently needed.

Cryptic species in stygobiotic *Macrobrachium*

The intense convergent selective pressure in cave environments has led to the widespread existence of cryptic species among cave organisms (Juan et al. 2010), which have been discovered in various groups (Niemiller et al. 2012; Meleg et al. 2013; Zhang and Li 2014; Gladstone et al. 2019). However, among the 17 cave prawn species previously reported, no cryptic species have been identified or described. In this study, we have confirmed for the first time the existence of three cryptic species of stygobiotic prawn, *M. guizhouense* sp. nov., *M. duanense* and *M. lingyunense*, which collectively constitute a species complex. Morphologically, these three species exhibit minimal differences that make them morphologically difficult to separate from each other. Nevertheless, they display significant differences in the molecular data. Even though *M. lingyunense* and *M. duanense* have the smallest interspecific genetic distance, their COI and 16S genes exhibit clear gaps, indicating that their differentiation has reached the species level. Additionally, as they form a monophyletic group, this suggests that they were most likely descended from a common ancestor and have evolved independently within separate cave systems. This also implies that other cryptic species may potentially exist in numerous caves in the vicinity.

The sexual dimorphism in Chinese cave-dwelling *Macrobrachium*

Sexual differences in size and morphology are common in many animal taxa. Sexual dimorphism might evolve from sexual selection, intersexual food competition and reproductive role division (Hedrick and Temeles 1989). The typical sexual dimorphism in *Macrobrachium* is that the male has a larger body size and a pair of massive second pereopods from which the genus name was derived (Karplus and Barki 2018). According to the division of mating systems of caridean shrimp, *Macrobrachium* belongs to the ‘neighborhoods of dominance’ of the five mating types (Correa and Thiel 2003). It means that males need to defend receptive females and compete with other males to achieve reproductive success. Thus, male size and fighting structures are positively correlated with their mating success. Nevertheless, a few species in this genus show another type of mating system, namely ‘pure search’ (Nogueira et al. 2022). In this type, males need to search and then briefly fertilize receptive females rather than guarding them. Therefore, a small and agile body is better for an efficient search (Correa and Thiel 2003). Some species even evolved alternative mating tactics representing different ontogenetic developmental stages, e.g. *Macrobrachium rosenbergii*, which shows three morphotypes of adult males, including one type smaller than females and two types larger than females, representing the ‘pure search’ and ‘neighborhoods of dominance’ respectively (Karplus and Barki 2018).

The sexual dimorphism of epigean *Macrobrachium* spp. is typically large males and small females, but the cave-dwelling species collected in this research with considerable sample size, especially for the two new species, show opposite sexual dimorphism, that is, females are larger than males (total length of the largest individual in each species (mm): *M. duanense* male 78.2, female 86.9; *M. guizhouense* sp. nov. male 63.1, female 64.6; *M. linyunense* male 41.2, female 41.9; *M. parvum* sp. nov. male 44.0, female 53.4; *M. tenuipes* male 47.0, female 77.2), and the second pereopod shows no difference between sexes. This may be due to the lack of nutrients in the cave environment, which only sustains a low population density, resulting in less pressure for male intrasexual competition and more difficulty in encountering suitable female mates. This forces their mating strategy to shift from ‘neighborhoods of dominance’ to ‘pure search’. Females may also choose smaller males because larger size means more energy expenditure in this particular environment, or simply have no preference in size. Nevertheless, since little is known about the stygobiotic prawn behavior and ecology, e.g. no ovigerous female was collected for all subterranean species, our hypothesis may be refined with further evidence.

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