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# Utilizing integrative taxonomy uncovers a new stygobiotic *Caridina* species (Decapoda: Caridea: Atyidae) from Guizhou Province, China

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**Integrative taxonomy uncovers a new stygobiotic *Caridina* species (Decapoda: Caridea: Atyidae) from Guizhou Province, China**

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**Abstract**

Collecting much-needed information on the taxonomy, distribution, and ecology of cave-dwelling shrimp is vital for addressing the urgent challenges in conservation biodiversity in fragile cave ecosystems. *Caridina unicolor* **sp. nov.**, a new atyid shrimp from an underground stream of Yaoshui Cave, Daqikong scenic area, Libo County, Guizhou Province, southwestern China is described based on morphology and DNA analysis (mitochondrial COI). *Caridina unicolor* **sp. nov.** differs from epigean congeners by its smaller eyes which ranges from reduced to completely blind; colorless body and appendages; long stylocerite and sixth abdominal segment; and relatively large eggs. In comparison to other cave species, *Caridina unicolor* **sp. nov.** presents a long rostrum and stylocerite; slender sixth abdominal segment; and unique shape of the appendix masculina. Data on the habitat, ecology, and levels of threat are provided and suggests that it should be categorized as Critically Endangered (CR) under the

current IUCN criteria.

## **Keywords**

*Caridina*, COI, freshwater biodiversity, southwestern China, speleology, taxonomy, conservation biodiversity

## **Introduction**

China is rich in subterranean environments, with more than 500,000 documented caves, most of which are located in the southwest karst region, such as Guangxi, Guizhou, and Yunnan Province (Chen 2006; Ran & Yang, 2015). This underground setting has a unique ecological habitat characterized by permanent darkness, relatively constant air and water temperature, and scarcity of food supply (Rétaux & Casane 2013; Culver & Pipan 2019; Mammola et al. 2019). They may have served as faunal refuges and indeed are known to harbor an impressive array of shrimp species (Holthuis 1974, 1977; Hobbs et al. 1977; Hart & Manning 1981; Liang & Yan 1981; Mejía-Ortíz & Hartnoll 2006; Li 2007; Cai & Ng 2009; 2018; Pan et al. 2010; Zhu et al. 2020). Though the stygobiont shrimps are prone to geographic and genetic isolation, they exhibit a suite of convergent characteristics under natural selection, including reduction or loss of eyes, loss of pigment, elongated antennae and ambulatory appendages. Non-visual sensory structures are enhanced with the loss of vision, which ultimately leads to speciation (Barr 1968; Peck 1986; Holthuis 1986; Christiansen 2005; Mejía-Ortíz et al. 2006). They serve an important underground ecological role and have a position near the base of the food chain. By consuming organic matter such as leaves and twigs that get flushed into caves, they are the primary decomposers and make nutrients available to other organisms in the ecosystem, such as fish and crabs (Botosaneanu, 1985).

In China, most cave systems have not been adequately surveyed because of the difficulty in sample collection. To date, 24 described species of 4 genera of atyids are presently known as inhabitants of the subterranean aquatic realm, some of which are completely adapted to subterranean life, and the majority from the genus *Caridina* (Liang & Yan 1981; Guo et al. 1992,1996; Liang & Zhou 1993; Cai 1995; Cai & Li 1997; Cai & Liang 1999; Cai & Ng 1999,2018; Liang et al.1999,2005; Li & Li 2010; Xu et al.2020).

Guizhou Province is the central area of karst landforms in southwest China, with 73% of the land covered with carbonate rocks (He & Li 2016; Zhou et al. 2017). The karstic nature of this area promotes the formation of submerged cave systems, with more than 700 large cave systems documented (Zhang & Zhu 2012). Although Guizhou presents great biospeological potential, most submerged cave systems have not been adequately surveyed or studied. The research concerning the cave-dwelling atyids in Guizhou started with a study by Cai & Li (1997), who described *Caridina demenica* with pigmented reduced eyes from Libo County. Liang et al. (2005), subsequently revealed another three new atyids: *Caridina caverna* which is blind and depigmented; *C. acuta* which has slightly reduced eyes and pigmentation; and *Neocaridina brevidactyla* which apparently does not show any adaptation to subterranean life. Cai & Ng (2018) reported a new cave-dwelling atyid *Caridina jiangkou* from Jiangkou County, which is also of the typical epigean form. More recently, Xu et al. (2020) reported *Caridina sinanensis* from Sinan County, which is unpigmented, and the cornea is vestigial with only a small pigment spot.

During our biospeological surveys in Guizhou Province, stygobiont atyid shrimps belonging to the genus *Caridina* Edwards, 1837, were collected from Yaoshui Cave, Daqikong scenic

area, Libo County. The specimens collected could not be assigned to any known species of this genus based on a combination of morphological and molecular features (COI). *C. unicolor* **sp. nov.** is rare and has a restricted distribution; its taxonomic uniqueness also suggests that it may be relictual. The impact of anthropogenic activities on the new species are also noted and suggests it is in need of urgent conservation intervention.

## **Materials and methods**

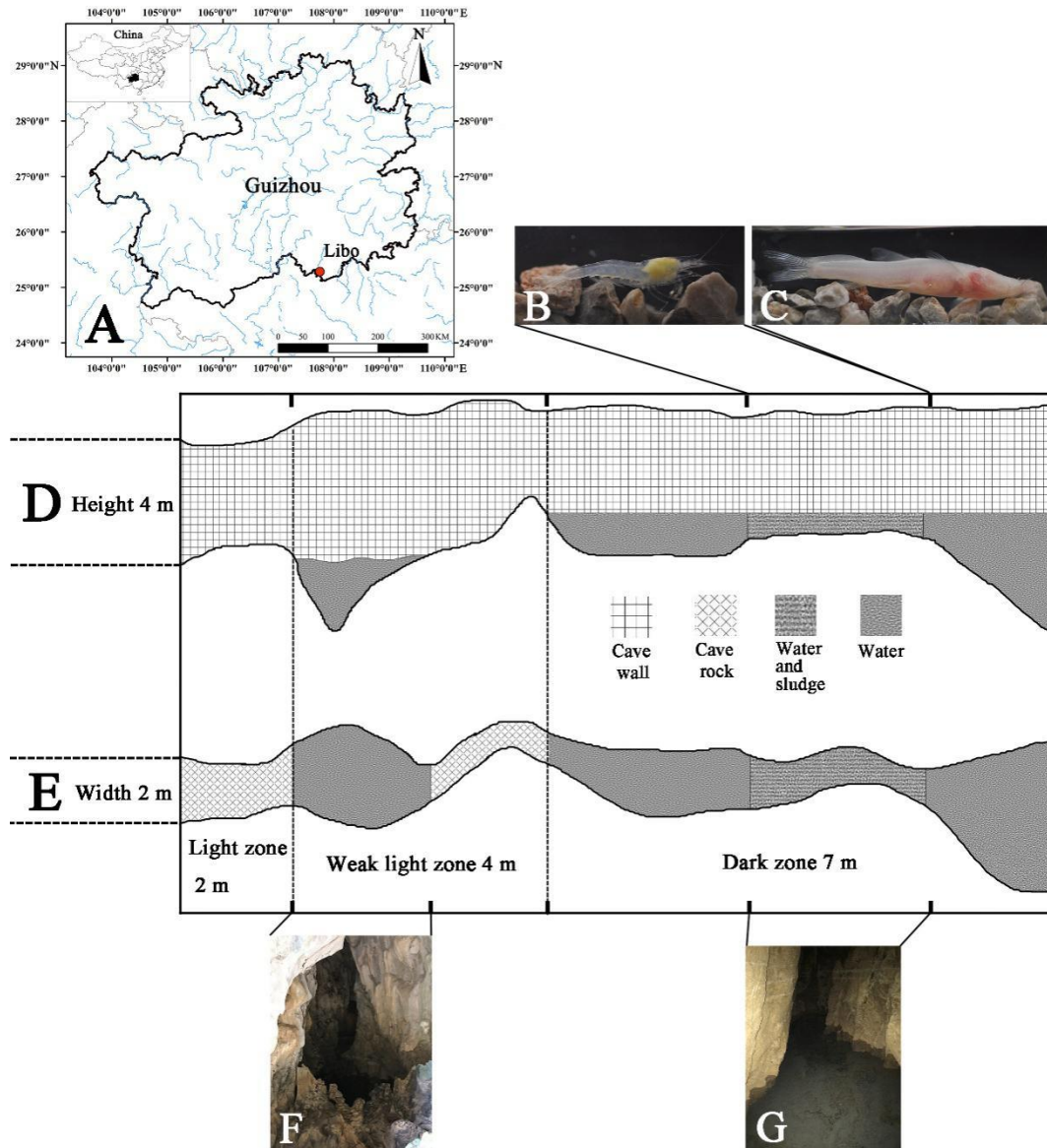
### **Study cave and ecological data**

Daqikong scenic area is named after a seven span bridge on the Dagou River near Mengtang Village, Wangmeng Township. It is situated about 25 km southwest of Libo County, at the border region of Guizhou and Guangxi in southwestern China. Yaoshui Cave is near the Mengtang scenic spot of Daqikong scenic area, at 25° 17' 1" N, 107° 45' 8" E and an altitude of 520 m. The entrance is about 120 m away from the tour plank road, located halfway up a limestone hill. The opening of the cave is arched and 2 m wide and 4 m high. There is naked shale above the entrance, with some ferns, bryophytes and vines present in the surrounding areas (Fig. 5A). Beyond the entrance is a rocky horizontal passage which is 2 m wide. Shrimps were observed in a pool (2 m<sup>2</sup>, 0.8 m deep, and in the weak light zone) situated 2 m from the entrance. The weak light zone channel is rugged and can only be entered along the tunnel wall. Another 4 m in is the underground river (in total darkness). In some areas, it is shallow (0.1-0.5 m) with a muddy bottom. However, in the deeper parts (1.3-2.5 m), the bottom becomes rocky and slopes precipitously to unknown depths. A number of shrimps were taken in the shallows. This cave was inhabited by bats and a thick layer of bat guano was also found on the ground.

During the November collection trip, the water was clear and the water parameters of the river were: temperature 21 °C, pH 7.5, and dissolved oxygen 8.8 mg l<sup>-1</sup>.

Water levels of Yaoshui Cave fluctuate dramatically throughout the year. During spring and summer, heavy precipitation inundates the passage and blocks access to the entrance. Also, an outflowing stream snakes down into Dagou River (Fig. 5B). During autumn and winter, due to lower water levels, some pools may become dry, but the underground river does not dry up. The outline of the Yaoshui Cave is shown in Figure 1.

The cave was visited by Deng, et al. in 2011. Twenty-six cavefish specimens were collected and a new species, *Oreonectes daqikongensis* Deng, Wen, Xiao & Zhou, 2016 was described. They mention that only the blind fish was found in the underground river, and no other fish, shrimp, or aquatic animal were found (Deng, et al. 2016). This blind fish was collected in the deeper parts of the river during this survey (Fig.1C).



**Figure 1** Type locality and schematic diagram of the cave of *Caridina unicolor* sp. nov. **A** Yaoshui Cave is located near Libo County and is marked by the red dot. **B** the sampling point of shrimp. **C** the sampling point of fish. **D** left view of the cave. **E** top view of the cave. **F** a pool in a weak light zone. **G** river in the dark zone.

### Sample collection

On August 3, the cave gallery was inundated due to the rain. We managed to wade in, but only a juvenile female was collected. On November 27, the water level was lower, we successfully entered the cave. Under the light of our headlights, a school of translucent white shrimps were

observed swimming or clinging to the cave wall of the river. 42 individuals were collected in the shallow muddy areas. Samples were collected with a hand net (mesh size 0.6 mm). The sampling scene was recorded with photographs and video-recordings. Specimens were placed in oxygenated polythene bags, anaesthetized with ice, then transported back to the hotel. The shrimps were fixed in 95% ethanol after they were photographed. The ethanol was changed after 24 h with fresh 75% ethanol. The blind fish (*Oreonectes daqikongensis*) was also collected.

Basic hydrological and physicochemical parameters of the cave were measured by the following instruments: Bosch GLM-30 Laser rangefinder, eTrex Venture GPS locator, JWSA2-2 temperature and hygrometer, BDO820 Portable dissolved oxygen determining meter, and BPH-220 pH measuring apparatus.

### **Morphological analysis**

Specimens were examined using a dissecting microscope (Olympus SZX7). Morphometric measurements on selected characters and illustrations were made using a digital camera (DP22) mounted on a stereomicroscope (Olympus SZX7) with Olympus CellSens Entry v1.18 software. The measuring method of morphometric characters follows that of von Rintelen & Cai (2009). The following abbreviations are used throughout the text: alt (altitude), cl (carapace length), rl (rostral length), and tl (total length). All measurements are in millimeters. Specimens were deposited in the Department of Animal Science, School of Life Science and Engineering, Foshan University (FU).

### **Molecular data and analysis**

Genomic DNA was extracted from abdominal muscle tissue using the Universal Genomic DNA



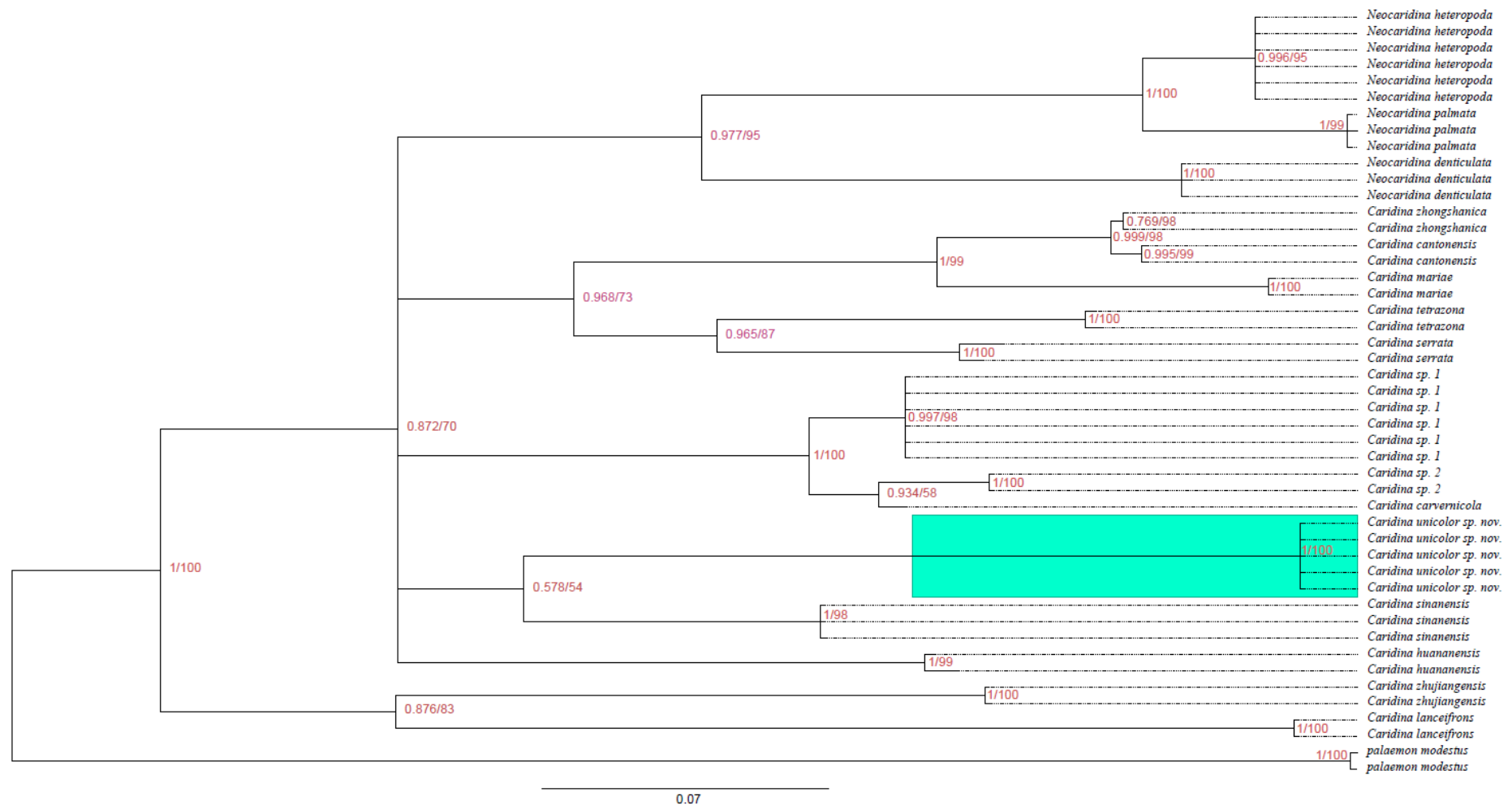
Kit (Beijing, China), following manufacturer instructions. Segments of the mitochondrial cytochrome oxidase I (COI) were amplified by using the primers LCO1490 and HCO2198 (Folmer et al.1994). PCRs were run in 50 $\mu$ l volume reactions. COI cycling conditions were: 94°C for 3 min followed by 35 $\times$  (94°C for 30s/46 $\pm$ 1°C for 60s/72°C for 60s) , with a final elongation step of 5 min at 72°C. PCR products were forwardly sequenced using primers with an Applied Biosystems 3730 Analyzer (Applied Biosystems, Foster City, CA, USA).

For the phylogenetic analyses, we included species similar in morphology to the new species and also other *Caridina* species that are known to occur in the neighboring areas. A total of 47 nucleotide sequences of 13 *Caridina* and 3 *Neocaridina* species and an outgroup (Table 1) were incorporated and analyzed with MAFFT v7.313 (Katoh & Standley 2013). All the COI sequences were obtained by PCR. The new sequencing results are corrected for 617~bp (COI) for subsequent analysis. All the sequences were aligned with Mega 7.0 (Kumar et al. 2016) software using the muscle alignment module. Genetic distances were calculated using Maximum Composite Likelihood. The best partitioning strategy was selected according to MrBayes v.3.2.6 (Ronquist et al. 2012) information criterion (BIC) and default parameters. Bayesian phylogeny was performed using PhyloSuite v1.2.2 (Zhang et al. 2020). Maximum likelihood (ML) was performed using IQ-TREE 1.6.12 (Nguyen et al. 2015). According to the Bayes information criterion (ModelFinder (Kalyaanamoorthy et al. 2017) default recommendation), the best model for Bayesian inference (BI) and maximum likelihood method (ML) are GTR+I+G and TIM2+G+I, respectively. Markov chain Monte Carlo (MCMC) analysis was performed with two simultaneous runs starting with random trees to approximate the posterior probabilities of trees. Each run consisted of four chains, with default heating

parameters and ran for  $4 \times 10^6$  generations, discarding the first 25% as burnin.

**Table 1.** Species used in the molecular analysis, with details on sampling locations and GenBank accession numbers (COI).

Species	Samplling locality	GenBank numbers
<i>Caridina unicolor</i> <b>sp.nov.</b>	Libo, Guizhou	MW237749—MW237753
<i>Caridina sinanensis</i>	Sinan, Guizhou	MT433962—MT433964
<i>Caridina zhongshanica</i>	Zhongshan, Guangdong	MN701597—MN701598
<i>Palaemon modestus</i>	Dongting lake, Hunan	MK412768—MK412769
<i>Neocaridina denticulata</i>	Hulun lake, Inner Mongolia	MW222157—MW222159
<i>Neocaridina palmata</i>	Hong Kong, China	MW226891—MW226893
<i>Caridina mariae</i>	Nankun Mountarin, Huizhou	MN701601—MN701602
<i>Caridina serrata</i>	Dong'ao Island, Zhuhai	MN701599—MN701600
<i>Caridina tetrazona</i>	Zhuhai, Guangdong	MN701593—MN701594
<i>Caridina cantonensis</i>	Qingyuan, China	MN701589—MN701590
<i>Caridina</i> sp. 2	Huanjiang, Guangxi	MW237763—MW237764
<i>Caridina huananensis</i>	Yingde, Qingyuan	MN701607—MN701608
<i>Caridina lanceifrons</i>	Dongfang, Hainan	MN701605—MN701606
<i>Caridina zhujiangensis</i>	Dong'ao Island, Zhuhai	MN701603—MN701604
<i>Caridina carvernicola</i>	Mashan, Guangxi	MW237867
<i>Neocaridina heteropoda</i>	Guilin, Guangxi	MW221964—MW221966 MW222154—MW222156
<i>Caridina</i> sp. 1	Mashan, Guangxi	MW237861—MW237866



**Figure 2.** Bayesian inference (BI) tree and maximum likelihood method (ML) tree of 16 atyids and outgroups (*Palaemon modestus*) based on the COI gene. Support values at the nodes represent posterior probability.

**Table 2.** Pairwise genetic distance among 17 species based on COI (above diagonal) gene.

Speices	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>Caridina unicolor</i> <b>sp. nov.</b>																	
2 <i>Caridina sinanensis</i>	0.13																
3 <i>Caridina zhongshanica</i>	0.17	0.14															
4 <i>Palaemon modestus</i>	0.24	0.20	0.19														
5 <i>Neocaridina denticulata</i>	0.18	0.14	0.15	0.20													
6 <i>Neocaridina palmata</i>	0.18	0.14	0.16	0.21	0.05												
7 <i>Caridina mariae</i>	0.17	0.15	0.08	0.21	0.16	0.17											
8 <i>Caridina serrata</i>	0.16	0.12	0.11	0.21	0.14	0.16	0.12										
9 <i>Caridina tetrazona</i>	0.17	0.14	0.12	0.21	0.17	0.17	0.13	0.09									
10 <i>Caridina cantonensis</i>	0.16	0.14	0.01	0.19	0.15	0.16	0.08	0.11	0.12								
11 <i>Caridina</i> sp. 2	0.15	0.11	0.14	0.21	0.15	0.15	0.15	0.13	0.14	0.15							
12 <i>Caridina huananensis</i>	0.16	0.12	0.14	0.21	0.15	0.17	0.14	0.13	0.14	0.14	0.14						
13 <i>Caridina lanceifrons</i>	0.19	0.18	0.18	0.22	0.20	0.18	0.19	0.18	0.18	0.18	0.19	0.19					
14 <i>Caridina zhujiangensis</i>	0.18	0.16	0.17	0.20	0.18	0.18	0.19	0.18	0.19	0.17	0.16	0.18	0.17				
15 <i>Caridina carvernica</i>	0.16	0.12	0.14	0.21	0.14	0.15	0.14	0.13	0.13	0.14	0.02	0.13	0.20	0.15			
16 <i>Neocaridina heteropoda</i>	0.18	0.14	0.16	0.20	0.13	0.15	0.16	0.15	0.15	0.16	0.16	0.16	0.18	0.20	0.15		
17 <i>Caridina</i> sp. 1	0.16	0.11	0.14	0.21	0.15	0.15	0.14	0.14	0.14	0.14	0.05	0.14	0.19	0.15	0.03	0.15	

## Results

### Taxonomy

Systematic account

Family Atyidae De Haan, 1849

Subfamily Atyinae De Haan, 1849

Genus *Caridina* H. Milne Edwards, 1837

#### ***Caridina unicolor* sp. nov.**

(Figs.3-5)

**Material examined. Holotype:** Adult male (FU, 2018-11-27-01), tl 21.8 mm, cl 5.1 mm, rl 3.0 mm; Yaoshui Cave, Mengtang Village, Wangmeng Township, Daqikong scenic area, Libo County, Guizhou Province, China (25° 17' 1" N, 107° 45' 8" E, alt. 520.0 m), November 27, 2018.

**Paratypes:** 1 male (FU, 2018-11-27-02) tl 25.9 mm, cl 6.1 mm, rl 3.1 mm; 16 males (FU, 2018-11-27-03) tl 17.4-25.9 mm, cl 4.2-6.0 mm, rl 2.4-3.4 mm; 10 females (FU, 2018-11-27-04), tl 17.8-25.2 mm, cl 4.5-6.1 mm, rl 2.4-3.4 mm; cl 4.9-6.6 mm, same data as for holotype. **Paratypes:** 6 males (FU, 2018-11-27-05) tl 18.4-25.1 mm, cl 4.4-5.9 mm, rl 2.5-3.0 mm; 9 females (3 ovigerous) (FU, 2018-11-27-06), tl 17.5-25.0 mm, cl 4.5-6.0 mm, rl 2.4-3.1 mm; cl 5.0-6.3 mm, same data as for holotype.

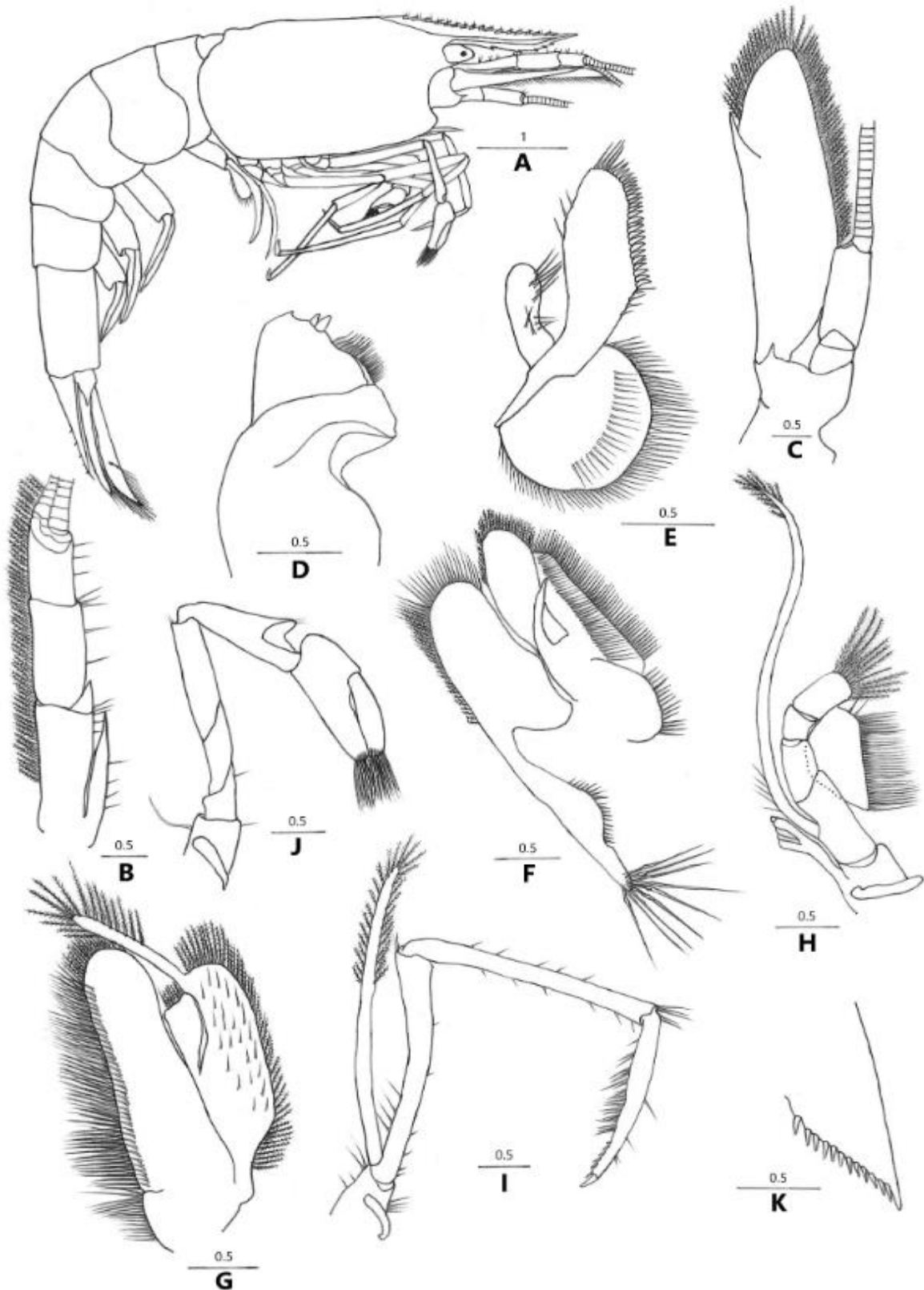
#### **Comparative material examined:**

*Caridina sinanensis* Xu, Li, Zhang & Guo 2020. Holotype: Adult male (FU, 2019-01-25-01), tl 16.7 mm, cl 4.8 mm, rl 1.5 mm; a cave river at Pengjiaao, Tangtou Town, Sinan County,

Guizhou Province, southwestern, China (27° 44' 10" N, 108° 11' 58" E, alt. 294.7m), January 25, 2019. Paratypes: 1 male (FU, 2019-01-25-02) cl 5.4 mm; 1 male (FU, 2019-01-25-03) cl 6.8 mm; 1 male (FU, 2019-01-25-04) cl 4.8 mm; 2 males (FU, 2019-01-25-05), cl 4.2-6.2 mm; 20 females (9 ovigerous) (FU, 2019-01-25-05), cl 4.9-6.6 mm.

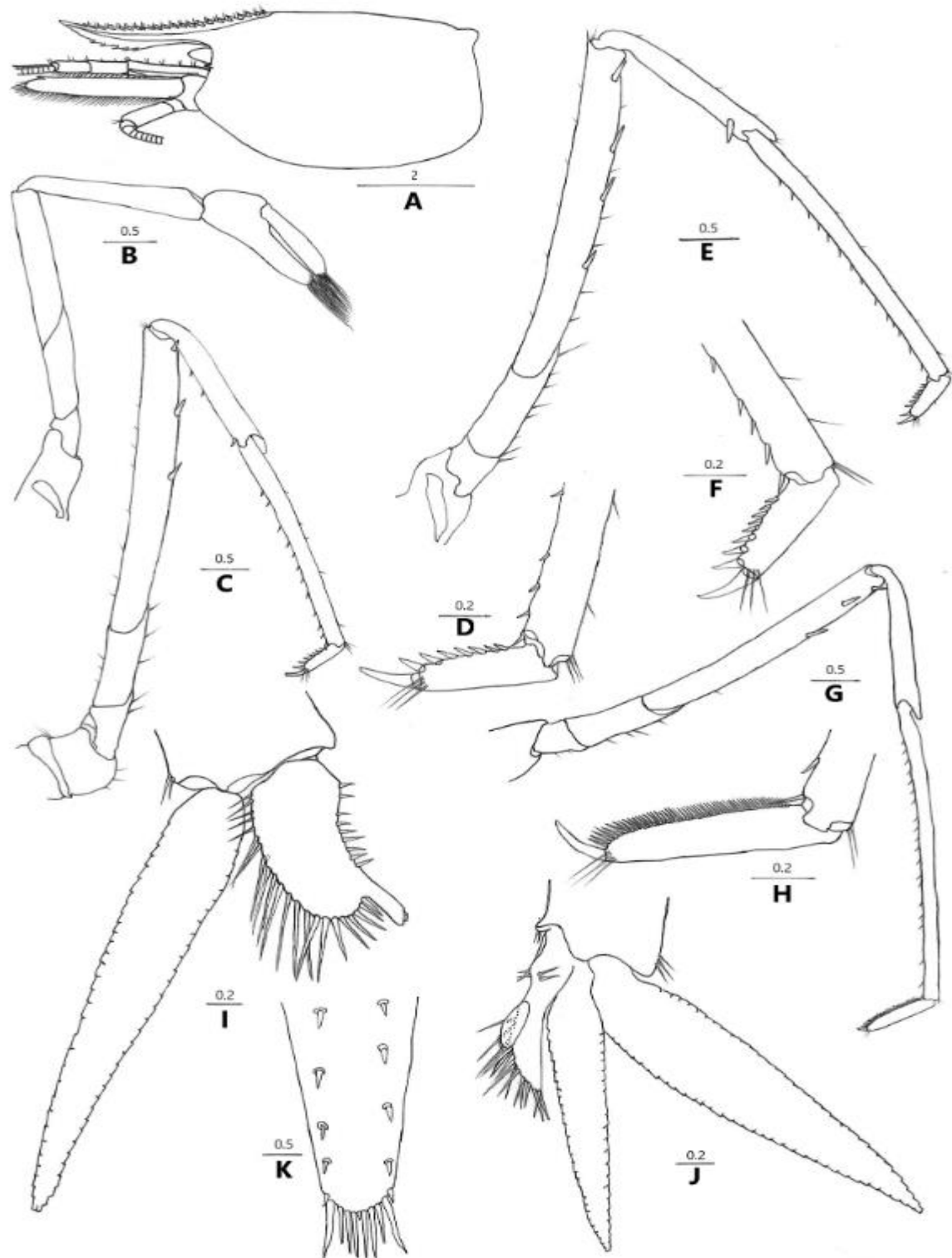
**Diagnosis:** Body and appendages without coloration, translucent. Rostrum slender, slightly elevated at base, reaching to base of 3<sup>rd</sup> segment of antennular peduncle to end of scaphocerite; straight, slightly sloping downwards, sometimes with tip turned upwards; rostral formula 6-10+11-27/4-15. First pereopod carpus 0.83-0.91 × as long as chela, 2.3-2.7 × as long as high; chela 2.2-2.5 × as long as broad; fingers 1.1-1.4 × as long as palm. Second pereopod carpus 1.3-1.4 × as long as chela, 5.4-5.6 × as long as high; chela 2.4-2.6 × as long as broad; fingers 1.6-1.8 × as long as palm. Third pereopod propodus 3.8–4.0× as long as dactylus, 13.6–14.4× breadth, with 8-11 thin spines on the posterior and lateral margins. Fifth pereopod propodus 4.0-4.7 × as long as dactylus, 17.6–20.5× breadth, with 17-20 thin spines on the posterior and lateral margins, dactylus terminating in one claw, with 50-55 spinules on flexor margin. Endopod of male subrectangular, slightly wider proximally, length 0.39–0.46 × exopod length, 2.0-2.2 × proximal breadth, ending broadly rounded; inner margin slightly concave, bearing spine-like setae, outer margin slightly convex, proximally 1/3 bearing nearly equal length short spine-like setae, distally 2/3 bearing nearly equal length long spine-like setae, and top bearing nearly equal length stout spine-like setae; appendix interna well developed, arising from distal 1/3 of endopod, beyond the end of endopod, distally with cincinulli. Appendix masculina rod-shaped and gradually tapering into a triangular tip, reaching about 0.48-0.51× length of endopod, with numerous long spined setae

on proximal and distal regions; endopod reaching about  $0.76-0.79 \times$  length of exopod; appendix interna well developed, reaching about  $0.58-0.78 \times$  length of appendix masculine, with cincinulli distally. Uropodal diaeresis with 10-12 movable spinules. Females carry 10-15 eggs, size of undeveloped eggs (without eyespots)  $0.83-0.88 \times 1.18-1.26$  mm.



**Figure 3.** *Caridina unicolor* sp. nov. **A** entire animal, lateral view. **B** antennule. **C** antenna. **D** mandible. **E** maxillula. **F** maxilla. **G** first maxilliped. **H** second maxilliped. **I** third maxilliped. **J** first pereopod. **K** diaeresis of uropodal exopod. **A** holotype. **B-K** paratype.





**Figure 4.** *Caridina unicolor* sp. nov. **A** carapace and cephalic appendages, lateral view. **B** second pereopod. **C** third pereopod. **D** dactylus of third pereopod. **E** fourth pereopod. **F** dactylus of fourth pereopod. **G** fifth pereopod. **H** dactylus of fifth pereopod. **I** first male pleopod. **J** second male pleopod. **K** distal margin of telson. **A-K** paratype.

**Description:** Body (Figs.3A,5C-F): Slender, smooth, colorless, translucent, sub-cylindrical, and medium-sized, males up to 25.9 mm tl, females up to 25.2 mm tl.

Rostrum (Figs.3A, 4A, 5C-F): 0.47–0.77 of cl, slender, slightly elevated at base, reaching to base of 3<sup>rd</sup> segment of antennular peduncle to end of scaphocerite; straight, slightly sloping downwards (55.8%, N=43), tip sometimes turned upward (44.2%); armed dorsally with 17–27 teeth, including 6–10 (usually 5–8) on the carapace, with 4–15 (usually 5–8) ventral teeth; lateral carina dividing rostrum into two unequal parts, continuing posteriorly to the orbital margin.

Eyes (Figs.3A, 4A, 5C-F): Small, bullet-like, reduced, with a short stalk, lacking facets, cornea pigmentation has great variability in the eye phenotype, with the smaller pigmented cornea (79%, N=43), or totally pigmentless and blind (21%).

Carapace (Figs.3A, 4A): Smooth, swollen; antennal spine acute, fused with inferior orbital angle; pterygostomial angle angular, produced forward; pterygostomial spine absent.

Antennule (Fig. 3B): Reaching slightly short of scaphocerite; stylocerite long, reaching end of the basal segment, basal segment robust, anterolateral angle with broadly produced sharp projection, reaching 0.25 length of 2<sup>nd</sup> segment; about 0.72-0.76 × of combined length of 2<sup>nd</sup> and 3<sup>rd</sup> segments, 2<sup>nd</sup> segment as long as 0.76-0.82× of basal segment, 1.2-1.3× of 3<sup>rd</sup> segment; all segments with sub-marginal plumose setae.

Antenna (Fig. 3C): Peduncle about 0.44-0.58 times as long as scaphocerite; scaphocerite 3.1-3.8× as long as wide, outer margin straight, aetose, ending in a strong sub-apical spine, inner and anterior margins with long plumose setae.

Mouthparts characteristic of the genus. Mandible with well-developed incisor and molar

processes; left incisor process with a single short sharp outer tooth, two long stout inner teeth, 7 curving setae followed by a patch of long setae; molar process stout and with triturative surface (Fig.3D). Maxillula with broadly rounded lower lacinia, with several rows of marginal and submarginal plumose setae; upper lacinia elongate, medial edge straight, with 25-35 strong spinules and simple setae; palp simple, longer than wide, slightly expanded distally, with 4 simple setae at basal part and 6 at distal part (Fig. 3E). Maxilla with well-developed scaphognathite, tapers posteriorly, with a regular row of long plumose setae and short marginal plumose setae continuing down the proximal triangular process distally, furnished with numerous long plumose setae; upper and middle endite with marginal, simple, denticulate setae, with plumose setae distally; lower endite with long simple marginal setae; palp shorter than the cleft of upper endite, wider than distal setose proximally (Fig. 3F). First maxilliped palp broad, with terminal plumose setae; caridean lobe broad, with marginal plumose setae; exopodal flagellum well developed, with marginal plumose setae distally; ultimate and penultimate segments of endopod indistinctly divided; medial and distal margins of an ultimate segment with marginal and sub-marginal rows of simple, denticulate and plumose setae; penultimate segments with marginal long plumose setae (Fig.3G). The second maxilliped with endopodite ultimate and penultimate antennomeres fused, slightly concave, reflected against basal antennomeres, inner margin of ultimate, penultimate, and basal segments with long setae of various types; exopod flagellum long, slender with marginal plumose setae distally. Podobranchium comb-like (Fig. 3H). Third maxilliped with three-segmented endopod, reaching slightly beyond scaphocerite; penultimate segment 0.89–0.96× of the basal segment; distal segment 0.75–0.91× of the penultimate segment, ending in a large claw-like spine

surrounded by simple setae, preceded by about 7–9 spines on the distal third of the posterior margin, with a clump of long and short simple, serrate setae proximally; exopod flagellum well developed, about  $0.25\text{--}0.34\times$  of the penultimate segment of endopod, distal margin with long plumose setae (Fig.3I).

First pereopod (Fig. 3J): Short, reaches the end of eyes; chela length  $2.2\text{--}2.5\times$  breadth,  $1.1\text{--}1.2\times$  length of the carpus; movable finger length  $3.1\text{--}3.4\times$  breadth,  $1.1\text{--}1.4\times$  length of the palm, setal brushes well developed; carpus excavated disto-dorsally, length  $2.3\text{--}2.7\times$  breadth, about the same length of merus.

Second pereopod (Fig. 4B): Reaches about the end of 3<sup>rd</sup> antennular peduncle segment, slenderer and longer than the first pereopod; chela length  $2.4\text{--}2.6\times$  breadth,  $0.73\text{--}0.77\times$  length of the carpus; movable finger length  $4.5\text{--}4.8\times$  breadth, and  $1.6\text{--}1.8\times$  length of the palm, setal brushes well developed; carpus length  $5.4\text{--}5.6\times$  breadth, slightly excavated distally,  $1.0\text{--}1.1\times$  length of merus.

Third pereopod (Fig. 4C, D): Reaches beyond the end of scaphocerite; dactylus length  $4.4\text{--}4.7\times$  breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears 6–7 spines; propodus length  $3.8\text{--}4.0\times$  of the dactylus, bearing 8–11 spinules on posterior margin,  $13.6\text{--}14.4\times$  breadth; carpus length  $0.65\text{--}0.72\times$  of the propodus; merus length  $1.9\text{--}2.1\times$  of the carpus, with about 3 strong spines on the posterior margin.

Fourth pereopod (Fig. 4E, F): Reaches end of 3<sup>rd</sup> segment of antennular peduncle; dactylus length  $4.2\text{--}5.0\times$  breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears 7–8 spines; propodus length  $3.8\text{--}4.6\times$  of the dactylus, bearing 12–15 spinules on posterior margin,  $14.6\text{--}17.4\times$  breadth; carpus length  $0.58\text{--}0.73\times$  of the

propodus; merus length  $1.9\times$  of the carpus, with about 3 strong spines on the posterior margin.

Fifth pereopod (Fig. 4G, H): Reaches end of 3<sup>rd</sup> segment of antennular peduncle; dactylus length  $4.2\text{--}5.6 \times$  breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears comb-like row 50–55 spines; propodus length  $4.0\text{--}4.7 \times$  of the dactylus, bearing 17–20 spinules on posterior margin,  $17.6\text{--}20.5\times$  breadth; carpus length  $0.55\text{--}0.60 \times$  of the propodus; merus length  $1.5\text{--}1.6 \times$  of the carpus, with about 3 strong spines on the posterior margin.

First four pereopods with epipod. Branchial formula typical for the genus.

First pleopod (Fig. 4I): Endopod of male subrectangular, slightly wider proximally, length  $0.39\text{--}0.46 \times$  exopod length,  $2.0\text{--}2.2 \times$  proximal breadth, ending broadly rounded; inner margin slightly concave, bearing spine-like setae, outer margin slightly convex, proximally  $1/3$  bearing nearly equal length short spine-like setae, distally  $2/3$  bearing nearly equal length long spine-like setae, and top bearing nearly equal length stout spine-like setae; appendix interna well developed, arising from distal  $1/3$  of endopod, reaching beyond the end of endopod, with cincinulli distally.

Second pleopod (Fig. 4J): Appendix masculina rod-shaped and gradually tapering into a triangular tip, reaching about  $0.48\text{--}0.51\times$  length of endopod, with numerous long spine setae on proximal and distal margins; endopod reaching about  $0.76\text{--}0.79\times$  length of exopod; appendix interna well developed, reaching about  $0.58\text{--}0.78 \times$  length of appendix masculine, with cincinulli distally.

Telson (Fig. 4K):  $0.40\text{--}0.55 \times$  of cl, shorter than sixth abdominal segment,  $0.67\text{--}0.96 \times$  length of the sixth abdominal segment, posteriorly tapering, with median projection, dorsal

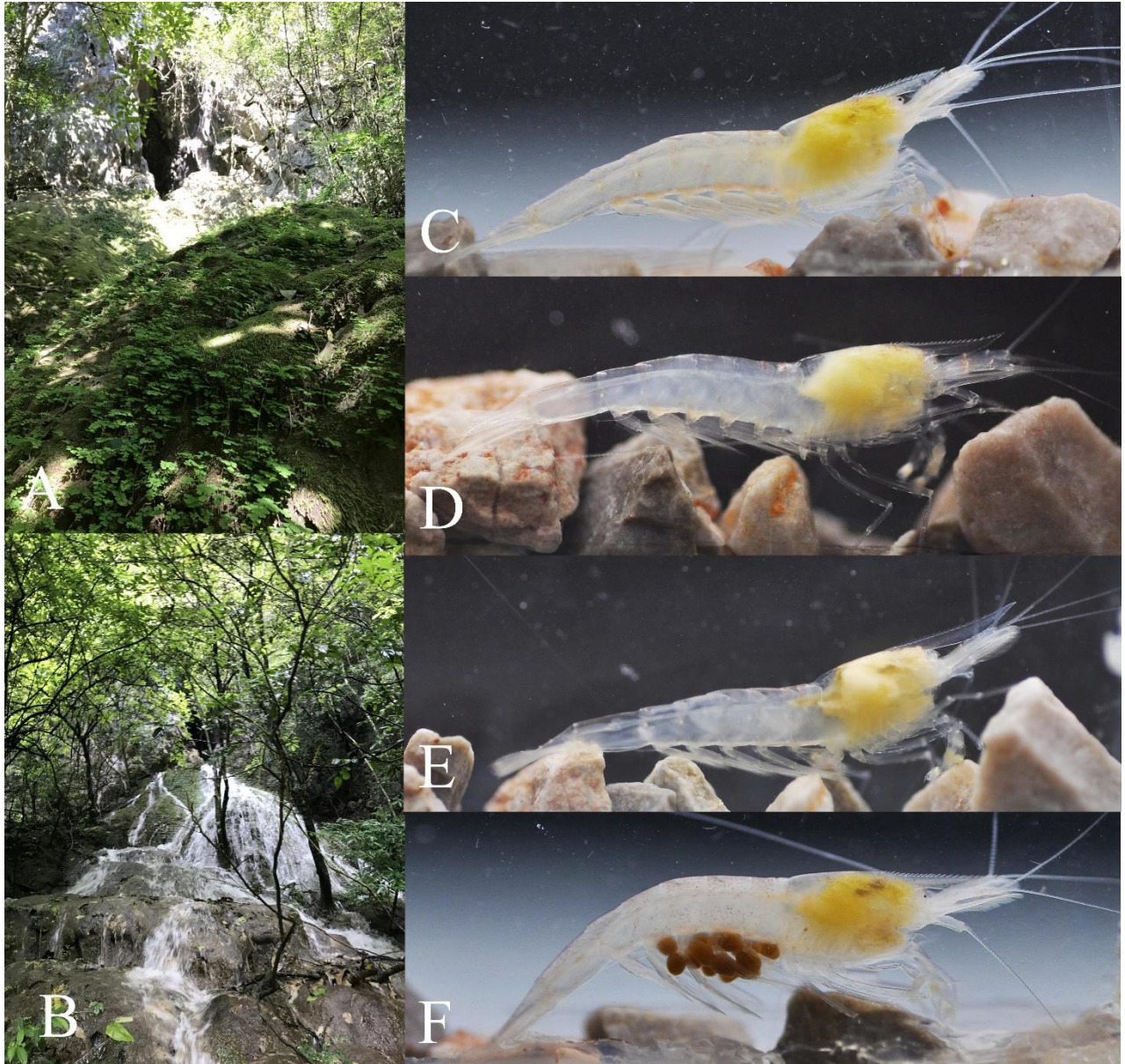
surface with 5 pairs of stout movable spine setae including the pair at posterolateral angles; posterior margin with 4 pairs of intermedial strong spiniform setae, sublateral pair shorter than lateral and inner pairs. Exopodite of the uropod bears a series of 10–12 movable spinules along the diaresis, the last one shorter than the lateral process (Fig. 3K).

Females carry 10-15 eggs, size of undeveloped eggs (without eyespots)  $0.83\text{--}0.88 \times 1.18\text{--}1.26$  mm.

Coloration (Fig. 5C-F): Body and appendages are colorless and translucent; vestigial pigment present at the center of the cornea or without pigment; internal organs (gonads and hepatopancreas) are yellow; eggs in females brown.

Etymology: *Caridina unicolor* **sp. nov.** is named after the colorless and transparent body color.





**Figure 5.** Habitats, variable features, and live coloration of *Caridina unicolor* sp. nov. **A** surrounding environment of Yaoshui Cave during the dry season. **B** same site during the rainy season. **C-E** variable rostrum and eye size in life. **F** ovigerous in life.

### Remarks

*Caridina unicolor* sp. nov. might be more closely related to the epigean species than to its supposed cave congeners. It is morphologically similar to *C. guiyangensis* Liang 2002, from Guiyang, Guizhou Province in the long rostrum and indentation, the shape of endopod of the

male first pleopod and appendix masculina. Although no molecular comparison with *C. guiyangensis* could be accomplished, *C. unicolor* **sp. nov.** can easily be distinguished from the latter by the reduced eyes, colorless body and appendages (versus developed eyes and pigmentation in *C. guiyangensis*); the long stylocerite, reaching to the end of the antennule basal segment (versus reaching 0.85 x of basal segment in *C. guiyangensis*); the long penultimate segment of the 3<sup>rd</sup> maxilliped, which is distinctly longer than the basal segment and distal segment (versus penultimate segment as long as basal segment and distinctly shorter than distal segment in *C. guiyangensis*); absence of a projection on the base of the inner margin of male first pleopod endopod (versus with projection in *C. guiyangensis*); and relatively large eggs, size of undeveloped eggs 0.83–0.88 × 1.18–1.26 mm (versus 0.63–0.75 × 1.05–1.13 in *C. guiyangensis*). In comparison to other cave species within *Caridina*, *C. sinanensis* Xu, Li, Zhang & Guo, 2020, is most similar in sharing the long 6<sup>th</sup> abdominal segment, and the variably pigmented cornea. However, the new species differs from the latter by possessing a relatively long and slender rostrum which reaches beyond the end of the 3<sup>rd</sup> antennular peduncle segment (versus stouter, reaching to the end of the 2<sup>nd</sup> segment in *C. sinanensis*); the long stylocerite, reaching to the end of the basal segment of the antennule (versus reaching 0.75–0.88 x of basal segment in *C. sinanensis*); and completely different shape of the endopod of the 1<sup>st</sup> pleopod and appendix masculina of the 2<sup>nd</sup> pleopod in males (Fig.4I, J, versus Fig.4H, J, K in Xu et al., 2020). There are another six atyids, *Caridina acuta*, *C. caverna*, *C. demenica*, *C. jiangkou*, *C. sinanensis*, and *Neocaridina brevidactyla*, that have been reported from nearby caves from Guizhou Province. *Caridina unicolor* **sp. nov.** differs from *N. brevidactyla* in the completely different shape of the endopod of 1<sup>st</sup> pleopod and appendix masculina of the 2<sup>nd</sup> pleopod in



males; lack of a hook-like projection on the posterior part of coxa of the 2<sup>nd</sup> pereopod and a pterygostomian spine. *C. unicolor* **sp. nov.** differs from all other cave species in having a long rostrum and stylocerite (with the longest rostrum and stylocerite amongst all known seven cave atyids); slender sixth abdominal segment which is distinctly longer than the telson (only *C. sinanensis* has a slender sixth abdominal segment that is slightly longer than the telson, other cave species have a stout sixth abdominal segment which is distinctly shorter than the telson); and an appendix masculina that is unique in shape and gradually tapers into a triangular tip. These taxa can be separated from each other by morphological differences as discussed in Xu et al., 2020.

### **Molecular phylogenetic results**

We analyzed 47 COI sequences in all. Five specimens of *Caridina unicolor* **sp. nov.** were used in the molecular phylogenetic analysis shown in Figure 2. Specimens assigned to *Caridina unicolor* **sp. nov.** formed a clade distinct from other species. *Caridina unicolor* **sp. nov.** is well distinguished from the other 16 atyids with a sequence divergence of 13.7% - 24.5% (COI). The sequence divergence between *Caridina unicolor* **sp. nov.** and *C. sinanensis* is the closest. The topology of the Bayesian (BI) trees and the ML tree are basically similar. Phylogenetic trees revealed the relationship between *Caridina unicolor* **sp. nov.** and 16 other species of atyids, with the posterior probability and bootstrap values from the BI and ML analysis shown in Figures 2. According to Hebert, Ratnasingham & de Waard, 2003, the genetic distances supports *Caridina unicolor* **sp. nov.** as a new species.

### **Conservation**

Threats to cave shrimp are of concern due to the uniqueness of the habitat and increasing

anthropogenic activities. Based on the information available, the Yaoshui Cave and its faunas are potentially at risk from excessive levels of external disturbance. Daqikong scenic area is famous for its marvelous primeval forests, steep canyons, spectacular caves, and underground rivers. Over the years, tourism to the region has improved the welfare of local residents and has become a major industry in this area. Moreover, new recreational trails and amusement facilities have been built in the scenic area. It is almost inevitable that these new projects will put great pressure and impact on caves and their faunas. In addition, land development and agriculture lead to habitat degradation and groundwater pollution, which also has a negative impact on the survival of this species.

So far, no freshwater shrimps are protected by the national legislation. The Announcement of the Ministry of Agriculture and Rural Areas of China has failed to categorize the strictly endemic cave species as Endangered (CITES Appendix aquatic wild species of China, no. 69, 2018). *Caridina unicolor* is new to science and the conservation status remains unassessed. Using the criteria provided by the IUCN (2019) Red List Categories and Criteria (version 14) (IUCN,2019), the new species should be considered as a critically endangered (CR) species on account for its exceptional rarity, restricted distribution, and exposure to serious anthropogenic impacts.

The Yaoshui Cave, which is home to two unique and range-restricted species (atyid shrimp, *Caridina unicolor*, and loach fish, *Oreonectes daqikongensis*) is biologically significant without question. These strictly-adapted cave species must be considered as important units for conservation, and devising an effective conservation strategy is clearly an urgent priority. It has also become obvious that there is a need to collect more baseline data, such as the exact

population size, structure, natality, and mortality rates. Regular monitoring may be necessary to ensure populations are sustained in the face of further anthropogenic disturbances. Equally important, a captive breeding program of these cave species should be developed. In addition, we propose that non-invasive and non-destructive projects, such as eco-tourism, should be promoted. Last but not least, we also appeal to local farmers to lower the usage of agricultural pesticides, herbicides, and fertilizers to help reduce the amount of hazardous chemicals that are leached into the groundwater.

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