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A New Species of Green Hydra (Hydrozoa: Hydrida) from China

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A new species of green freshwater hydra (Cnidaria, Hydrozoa: Hydrida), *Hydra sinensis*, is described from Guangdong Province, China. The chief distinction between *H. sinensis* sp. nov. and three other green hydras (*H. hadleyi, H. viridissima*, and *H. plagiodesmica*) is in the holotrichous isorhizae. *Hydra sinensis* sp. nov. differs from *H. plagiodesmica* in the shape of the holotrichous isorhizae, and from *H. viridissima* and *H. hadleyi* in the tubule of the capsule of the holotrichous isorhizae. The capsule tubule coils two times in 86% and three times in 14% of holotrichous isorhizae (n=50) in *H. sinensis* sp. nov.; we observed no tubules coiling four times. In contrast, the capsule tubule coils three or four times in *H. viridissima* and *H. hadleyi*, and no tubules coiling two times have been reported. In addition, holotrichous isorhizae, which are mainly located around the hypostome, are sparse in the tentacles of *H. sinensis* sp. nov., whereas the majority of holotrichous isorhizae is located on the tentacles in most other hydras. A molecular phylogenetic analysis using the nuclear small subunit (18S) ribosomal RNA gene indicated a close relationship between *H. sinensis* and *H. viridissima*. *Hydra viridissima* did not group within a clade of four individuals of *H. sinensis*, indicating a possible sister-species relationship between the two species. Morphological characters in combination with the molecular phylogenetic evidence support *Hydra sinensis* as a new species.

Key words: Hydridae, taxonomy, new species, China

INTRODUCTION

Hydras are abundant on all continents except Antarctica (Campbell, 1987). Freshwater polyps of the genus *Hydra* (Cnidaria, Hydrozoa) have long been of general interest because various species of *Hydra* have revealed fundamental principles underlying development, differentiation, regeneration, and symbiosis (Hemmrich et al., 2007). Five new species and one new record have been reported from China: *Hydra mohensis* Fan and Shi, 1999; *H. daqingensis* Fan, 2000; *H. beijingensis* Fan, 2003; *H. harbinensis* Fan and Shi, 2003; *H. robusta* (Shi et al., 1987), and *Hydra polymorphus* Chen and Wang, 2008. *Hydra polymorphus* is from the wetlands of Zhaoqing, Guangdong Province, whereas the other species are distributed in the northern Yangtze River region. The diversity of *Hydra* species has not yet been well studied in most areas of China. Here we describe a new species of green freshwater hydra collected from Guangdong Province, southern China.

MATERIALS AND METHODS

Morphological examination

Green hydras were collected from Guangdong Province, China. Specimens were deposited in the Morphological Research Laboratory (MRL) of the School of Life Science, Shenzhen University, Guangdong, China. Some polyps were brought alive into the laboratory and studied under culture. They were fed fresh nauplii of *Artemia salina*. The morphological characteristics of the hydras were examined after 24 h of starvation. Images of live polyps were captured with a Leica DC 300 digital camera (Leica Microsystems, Switzerland). The lengths of the body column and tentacles, and embryotheca size, were measured with the software of the Leica DC 300. Nematocyst images were captured with an Olympus DP70 digital camera (Olympus, NY, USA) and nematocyst sizes were measured with this camera's software.

DNA preparation, PCR, sequencing, and phylogenetic analysis

Genomic DNA from four starved individual polyps preserved in 80% ethanol was isolated with the NP-40 method described by John et al. (1991). The target small-subunit (18S) ribosomal RNA gene was amplified by PCR using primers h18sf (5'-tggttgatcctgccagt-3') and h18sr (5'-atccttcngcaggttcacc-3'). The resulting PCR fragments were cloned into pGEMT vector (Promega, Madison, Wisconsin) and transformed into DH5α *Escherichia coli* cells. Plasmid inserts were sequenced by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). All sequences have been submitted to GenBank (accession nos. FJ265731–FJ265734). *Nematostella vectensis* (Cnidaria, Actiniaria, Edwardsiidae) was chosen as the outgroup (Genebank AF254382). The 18S sequences we obtained were aligned with those obtained from GenBank for other species, including *H. circumcincta* (AF358080), *H. carnea* (EF059947), *H. robusta* (EF059946), *H. oligactis* (EF059945), *H. magnipapillata* (EF059942), *H. littoralis* (AF358082), *H. vulgaris strain AEP* (EF059943), and *H. viridissima* (EF059949). All sequences were trimmed to the length of the shortest sequence obtained, resulting in aligned sequences 1053 bp long. Sequences alignment was carried out with the program Clustal W (Thompson et al., 1994). To infer phylogenetic relationships among the taxa, we conducted maximum parsimony (MP) and maximum likelihood (ML) analyses implemented in PHYML (Guindon

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and Gascuel, 2003) and Mega 4.0 (Kumar et al., 1994), respectively. Bootstrap support values were determined from full heuristic searches of 1000 replicates. The ML and MP trees were drawn by using TreeView 1.6.6 (Page, 1996) and Mega 4.0, respectively.

RESULTS

Taxonomic description

Hydra sinensis sp. nov.

(Figs. 1–3)

Type material. Holoype: CNI0001, a sexual individual. Paratypes: CNI0002–CNI0011, ten sexual individuals; CNI0012–CNI0021, ten asexual individuals. The holoype and paratype specimens were collected at Dongjiang River, Huizhou City, Guangdong Province, China (23°06'N, 114°24'E) on 4 September 2007 by A. T. Wang. The type specimens were deposited in the National Zoological Museum of China.

Etymology. The species name refers to China.

Description. Polyps have 5–8 tentacles, which are half to two times the column length (Fig. 1A–E). The frequencies of 5, 6, 7, and 8 tentacles were 3%, 31%, 54%, and 12%, respectively (n=106 individuals). Most polyps were 11 to 14 mm in column length when relaxed, but in a few individuals the column length reached 18 mm. The tentacles form sequentially in the order shown in Fig. 2M and N. The tentacle number in budding polyps is usually identical to that in the parent, and additional tentacles form after the budding polyps break free. *Hydra sinensis* sp. nov. is a species of green hydra; unicellular *Chlorella* algae were observed in the endodermal epithelial cells (Fig. 3). A positive relationship was found between light intensity and the density of *Chlorella* algae in the endoderm.

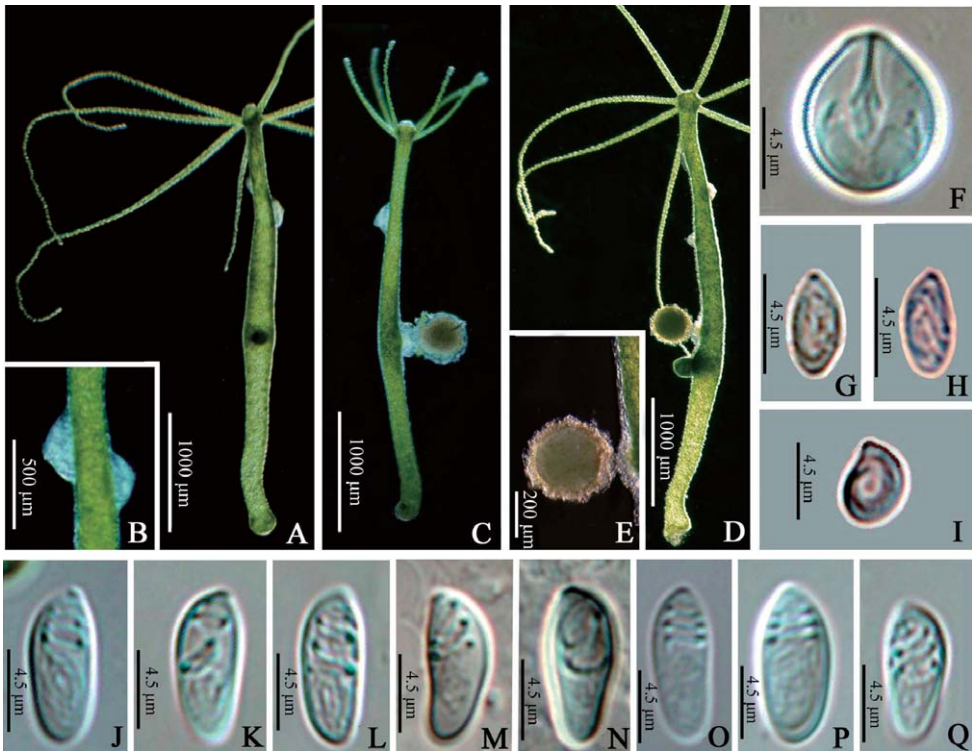

Fig. 1. Photographs of *Hydra sinensis* sp. nov. **(A, C, D)** Hermaphroditic polyps. **(B)** Testis. **(E)** Embryotheca. **(F)** Stenotele. **(G, H)** Atrichous isorhizae. **(I)** Desmoneme. **(J–Q)** Holotrichous isorhizae.

Nematocysts have the following forms: 1) stenotele, eudipleural pyriform shape, 8.93±0.41 μm×7.24±0.36 μm (n=50) (Figs. 1F, 2A); (2) holotrichous isorhiza, short claviform, paramecium-like, banana-like, and elliptoid shapes, 8.97±0.51 μm×3.64±0.28 μm (n=57) (Figs. 1J–Q, 2B–F); (3) atrichous isorhiza, long melon seed-like shape, 4.65±0.32 μm×2.45±0.18 μm (n=23) (Figs. 1G, H, 2G); and (4) desmoneme, pyriform shape, 4.61±0.22 μm×3.30±0.20 μm (n=41) (Figs. 1I, 2H). The

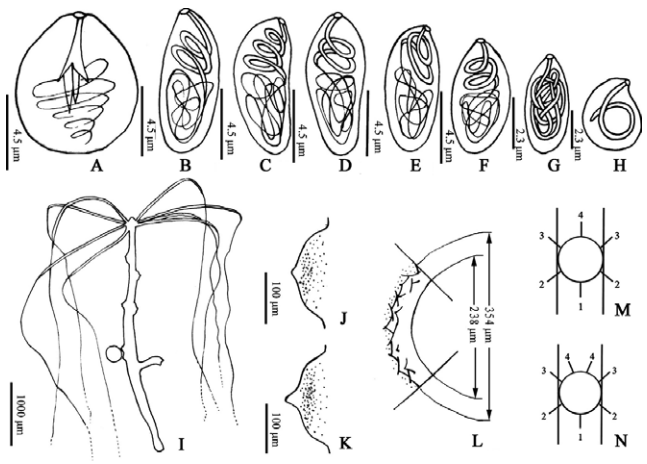

Fig. 2. Structure of *Hydra sinensis* sp. nov. **(A)** Stenotele. **(B–F)** Holotrichous isorhizae. **(G)** Atrichous isorhiza. **(H)** Desmoneme. **(I)** Hermaphroditic polyp. **(J, K)** Testes. **(L)** Embryotheca (with scale). **(M, N)** Order of tentacle formation.

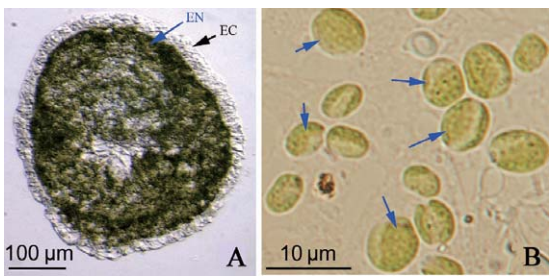

Fig. 3. Cross sections of the body column of *Hydra sinensis* sp. nov. EC, ectoderm; EN, endoderm. In panel B, arrows indicate the *Chlorella* algae in the endodermal epithelial cells.

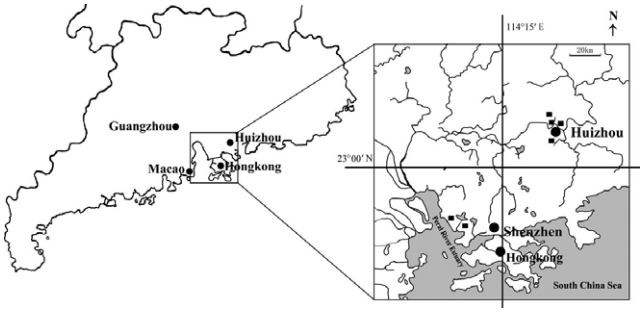

Fig. 4. Map of southern China showing collecting localities for *Hydra sinensis sp. nov.* in the present study. Solid squares (■) indicate sampling sites.

tubule of the capsule in the holotrichous isorhizae coils two (86%) or three times (14%) (n=50); no tubules were observed coiling four or more times. Interestingly, holotrichous isorhizae, which are locate mainly around the hypostome, are sparse in the tentacles of *H. sinensis* sp. nov., whereas the majority of holotrichous isorhizae is located on the tentacles in most other hydras.

Reproducing individuals of *H. sinensis* sp.nov. are hermaphroditic (Figs. 1A–E, 2I). Sexual reproduction can be induced by unfavorable conditions such as dense culture or food shortage. Three days without food induced sexual reproduction in *H. sinensis*. One to five coniform testes formed beneath the tentacles (Fig. 2J, K). Subsequently, an ovary with a stem formed over the budding zone (Figs. 1C, 2I). Ninety percent of individuals developed only one ovum, 354±23 μm in diameter (n=10). No change in body shape was observed in polyps during the transition from asexual to sexual reproduction. The embryotheca is brown, covering a layer of transparent, membrane-like material.

Habitat and collection sites. Localities where *H. sinensis* sp. nov. was found are shown in Fig. 4. The first specimens were collected in shallow water at the edge of the Dongjiang River, Huizhou City, Guangdong Province, China (23°06'N, 114°24'E; altitude 30 m). The Dongjiang River, a branch of the Pear River, lies in the Tropical Monsoon Region and is the most important source of freshwater for Shenzhen City and Hongkong. No input of industrial pollutants into the river was observed. Water where *H. sinensis* sp. nov. was found was clear, slow-moving, with a pH of 7.5 and a temperature of around 21.7°C. Several kinds of plankton were found at sites where *H. sinensis* sp. nov. was collected, including planarians (e.g., *Dugesia japonica*) and *Rotaria*. More recently, *H. sinensis* sp. nov. was also collected in shallow ponds in Shenzhen, Guangdong Province, China (Fig. 4).

Molecular phylogenetic analysis

The MP and ML trees based on analyses of the 18S rRNA sequences are closely similar (Figs. 5, 6). *Hydra sinensis* sp. nov. is closely related to *H. viridissima*, and *H. viridissima* and four individuals of *H. sinensis* sp. nov. comprise a sister group to the other *Hydra* species, with 98% bootstrap support. *Hydra viridissima*, however, does not cluster within the clade of four individuals of *H. sinensis* sp. nov.

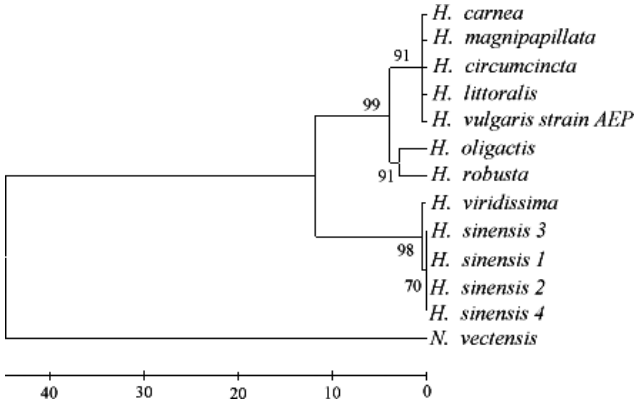

Fig. 5. Maximum-parsimony tree resulting from analysis of partial 18S rRNA gene sequences from species of *Hydra*. Numbers near nodes are bootstrap values in percent. The scale indicates the number of substitutional steps.

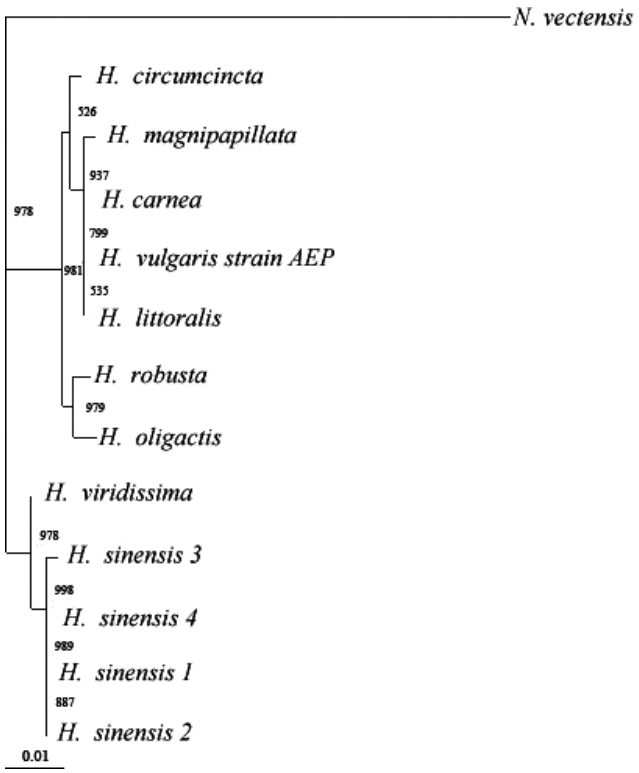

Fig. 6. Maximum-likelihood tree resulting from analysis of partial 18S rRNA gene sequences from species of *Hydra*. The scale bar indicates branch length in substitutions per site.

DISCUSSION

Taxonomic comparisions

Hydra sinensis sp. nov. is hermaphroditic. To date, over 20 species of hermaphroditic hydras have been decribed, including *H. mariana* Cox and Young, 1973; *H. plagiodesmica* Dioni, 1968; *H. intaba* Ewer, 1948; *H. viridissima* Pallas, 1766 (Semal-van Gansen, 1954); *H. hadleyi* Forrest, 1959; *H. hymanae* Hadley and Forrest, 1949; *H. parva* Ito, 1947; *H. graysoni* Maxwell, 1972; *H.*

circumcincta Schulze, 1914 (Tardent et al., 1968); *H. vulgaris* Pallas, 1766 (Boecker, 1915); *H. utahensis* Hyman, 1931a; *H. minima* Forrest, 1963; *H. lirosoma* Campbell, 1987; *H. mohensis* Fan and Shi, 1999; *H. harbinersis* Fan, 2003; *H. madagascarensis* Campbell, 1999; *H. umfula* Ewer, 1948; *H. carnea* L. Agassiz, 1850 (Hyman, 1931b); *H. salmacidis* Silveira et al., 1997; and *H. oxycnidoides* Schulze, 1927 (Stepanjants et al., 2006).

Among these hermaphroditic hydras, there are three green hydra species, *H. hadleyi*, *H. viridissima*, and *H. plagiodesmica*, known to form permanent and stable symbiotic associations with photosynthetic unicellular *Chlorella* algae. *Hydra hadleyi* and *H. plagiodesmica* occur in North America (Forrest, 1959) and South America (Dioni, 1968), respectively. *Hydra viridissima* is found in Europe and Madagascar Africa (Semal-van Gansen, 1954; Wolle, 1978; Holstein, 1995; Campbell, 1999). *Hydra hadleyi* and *H. viridissima* are the most similar morphologically; the major distinction between the two is in the structure of the embroytheca. Although both *H. viridissima* and *H. hadleyi* were described as having two chambers in the embryotheca, a larger secondary chamber is a diagnostic character for *H. hadleyi* (Forrest, 1959). *Hydra plagiodesmica* differs from *H. viridissima* and *H. hadleyi* chiefly in the stubbiness of the holotrichous isorhiza (Dioni, 1968).

The chief distinction between *H. sinensis* sp. nov. and the three other green hydras is in the holotrichous isorhizae. *Hydra sinensis* sp. nov. differs from *H. plagiodesmica* in the shape of the holotrichous isorhizae. *Hydra sinensis* sp. nov. has several shapes of holotrichous isorhizae, including short claviform, paramecium-like, banana-like, and elliptoid, while the holotrichous isorhizae of *H. plagiodesmica* are stubby. *Hydra sinensis* sp. nov. differs from *H. viridissima* and *H. hadleyi* in the tubule of the capsule of the holotrichous isorhizae. The tubule coils two (14%) or three (86%) times in *H. sinensis* sp. nov. (n=50), and no tubules were observed to coil four times. In contrast, the tubule coils three (Wolle, 1978; Holstein, 1995; Silveira, 1997) or four times (Campbell, 1999) in *H. viridissima*, and no tubules coiling two times have been reported. In addition, holotrichous isorhizae are located mainly around the hypostome and are sparse on the tentacles of *H. sinensis* sp. nov., whereas most other hydras have the majority of holotrichous isorhizae on the tentacles.

Hemmrich et al. (2007) analyzed the phylogenetic relationships among eight scientifically important members of the genus *Hydra* with sequence data from two nuclear rRNA genes (18S and 28S). *Hydra viridissima*, the only species of green hydra that Hemmrich et al. (2007) analyzed, was basal in phylogenetic trees and emerged as the sister group to all the other species of *Hydra*. *Hydra sinensis* sp. nov. was closely related to *H. viridissima* in our trees (Fig. 3). As *H. viridissima* is widely distributed, it is essential to know the sampling locality of the *H. viridissima* from which the DNA sequence came. The 18S sequence we used for *H. viridissima* (EF059949) was taken from Hemmrich et al. (2007), who provided no indication of geographical origin of their *H. viridissima* sample. Since the research of Hemmrich et al. (2007) was conducted in the Zoological Institute, Christian Albrechts University, Germany, by German scientists, we suppose that the *H. viridissima* sample they used was collected somewhere in Europe.

Not surprisingly, the four samples of *H. sinensis* sp. nov. formd a clade, and this clade comprised the sister group to *H. viridissima*. Although the fact that *H. sinensis* sp. nov. and *H. viridissima* are sister groups does not necessary indicate whether or not they are the same species, that *H. viridissima* did not cluster within the clade of four individuals of *Hydra sinensis* sp. nov. suggested the possibility that *Hydra sinensis* sp. nov. is a new species. Morphological characters in combination with the molecular phylogenetic evidence support *Hydra sinensis* as a new species. To our knowledge, this is the first species of green hydra reported in Asia.

The taxonomy within the Hydridae (hydroids) is controversial and not well resolved (Holstein, 1995; Stepanjants et al., 2000; Anokhin, 2004; Hemmrich et al., 2007). Carl Linné described the first *Hydra* species taxonomically in 1758, as *Hydra polypus* (Linné, 1767). Several additional species were subsequently identified and described in a single genus, *Hydra* (Pallas, 1766; Linné, 1767). Schulze (1914) proposed three genera, *Hydra*, *Pelmatohydra*, and *Chlorohydra*, according to general differences in body plan (body shape, stalk, symbiotic algae), different modes of tentacle formation during budding, and differences in specific types of nematocytes. Thereafter, some researchers agreed with the three-genus taxonomy (Hyman, 1940; Stepanjants et al., 2000; Anokhin, 2004), whereas other still supported Linné's one-genus taxonomy (Campbell, 1987; Holstein, 1995). We are exploring evidence to confirm whether or not the green hydras should be excluded from *Hydra*. Preliminary data (not shown) may indicate that *Chlorohydra* is an independent genus, in which case *H. sinensis* sp. nov. would belong in *Chlorohydra*.

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