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ACKNOWLEDGEMENTS. We thank the Director, National Institute of Ocean Technology (NIOT), Chennai and authorities of the Ministry of Earth Sciences, Government of India for providing necessary facility to conduct this study. We also thank Dilip Kumar Jha, NIOT, Port Blair for help in the preparation of the map of the study site.

Received 21 April 2011; revised accepted 22 August 2011

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Description and phylogenetic characterization of common hydra from India

Hydra, a freshwater polyp belonging to phylum Cnidaria and class Hydrozoa, is globally distributed except in the Antarctic region and Oceanic islands[1]. Although this organism has been extensively used as a model system in biology, there has been considerable uncertainty over its taxonomy, primarily due to lack of taxonomically distinct features. This created a doubt whether to put different hydra species under the genus *Hydra*, which was first reported by Carl Linné[2], or to follow Schulze's three genera classification[3]. Campbell[1] has classified the genus *Hydra* into four different groups: 'oligactis group' (stalked hydra), 'vulgaris group' (common hydra), 'braueri group' (gracile hydra) and 'viridissima group' (green hydra), based on morphological differences. A recent study based on molecular phylogenetic analysis has shown the reliability of Campbell's system of grouping different hydra species[4]. Hydra types were first studied in India by Annandale[5,6]. A detailed study based on morphological and physiological characters of Indian hydra types was conducted by Prasad and Mookerjee[7]. However, they have refrained from naming any species, except *Chlorohydra* (green hydra) collected from Hyderabad[7]. In India, a local species referred to as *Pelmatohydra oligactis*, is being used as a model system for studying regeneration, pattern formation and development[8–12], but has not been taxonomically described till date. It has so far been referred to as *P. oligactis* based on a personal communication between late L. H. Hyman and late Leela Mulherkar. However, a detailed taxonomic study of Pune hydra ecotype, especially in view of the prevailing principles of hydra taxonomy, has not been carried out. With increasing use of this organism as a model system, it is necessary to describe the taxonomic position and phylogenetic relationship of Indian hydra with other species of hydra.

Polyps collected from a local pond were cultured by standard method[13]. Live polyps were collected randomly from the culture and their body length was measured by placing a graph paper under the glass beaker containing the animals. Hydra at various stages of budding were randomly selected from a mass culture, relaxed by exposure to 2% urethane for 2 min and fixed in 4% paraformaldehyde overnight at 4°C (ref. 14). The pattern of emergence of tentacles was studied with an Olympus SZX16 stereomicroscope. Nematocysts were prepared for observation as described by David[15] and photographed with a Zeiss Axio ImagerZ1.

Total DNA was isolated from 50 polyps by the phenol/chloroform method[16]. Primers reported earlier[4] were used to amplify regions of mitochondrial 16S rRNA gene. PCR product was sequenced and a 379 bp sequence was submitted to GenBank (accession no. GU591886). Mitochondrial 16S rRNA sequences from other hydra species reported recently[17] were used for comparison with the sequence from Indian hydra. Sequence alignments were carried out using ClustalW[18] and cured manually.

Phylogenetic tree was constructed by neighbour joining (NJ) method based on *p*-distance using MEGA 4.0 software[19]. A separate analysis by maximum parsimony (MP) and maximum likelihood (ML) methods was carried out with PAUP* 4.0b10 (ref. 20). The MP analysis was performed with heuristic searches of 100 random additions with characters weighed equally and tree bisection and reconnection (TBR) branch swapping algorithm. The ML analysis was performed with general time reversible (GTR)

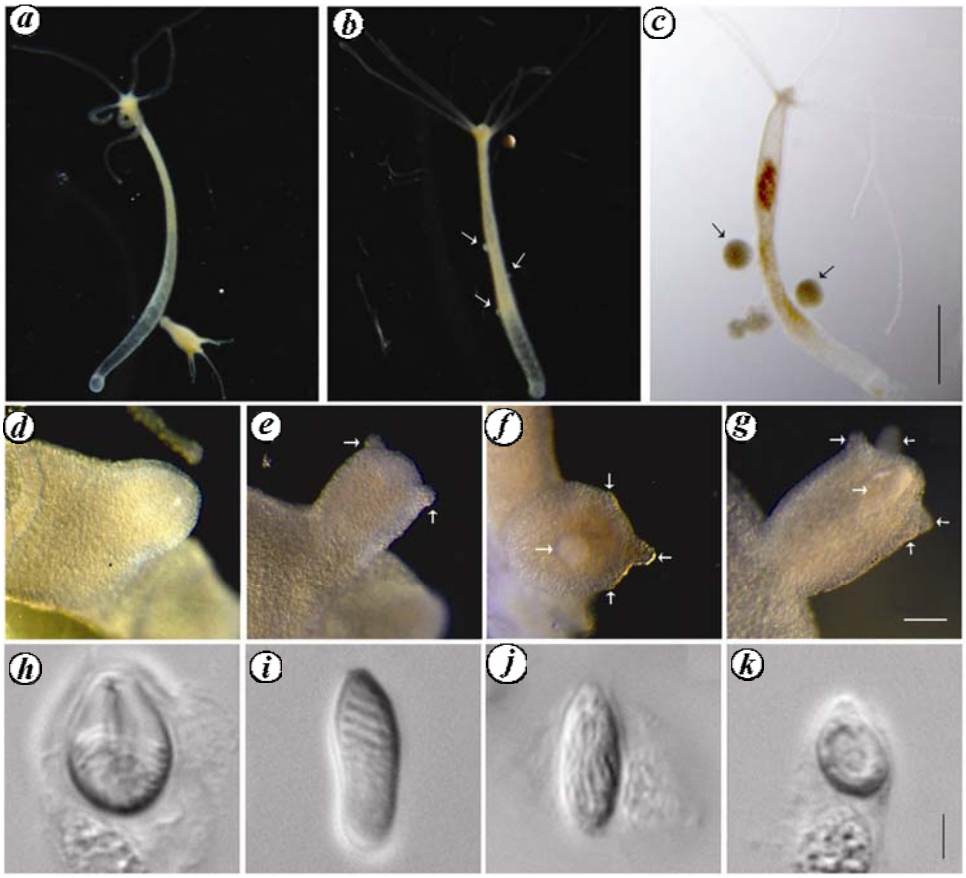

Figure 1. ***a–c***, *Hydra vulgaris* Ind-Pune. ***a***, Polyp with bud; ***b***, Polyp with testes (→) and ***c***, polyp with female gonads (→). ***d–g***, Asynchronous emergence of tentacles on bud: ***d***, No tentacles; ***e***, Two tentacle rudiments (→); ***f***, Both pairs of tentacle rudiments (→) and ***g***, Two big and three small tentacle rudiments (→). ***h–k***, Nematocysts: ***h***, Stenotele; ***i***, Holotrichous isorhiza; ***j***, Atrichous isorhiza and ***k***, Desmoneme. Scale bar: ***a–c*** = 2.5 mm, ***d–g*** = 200 μm and ***h–k*** = 5 μm.

model, selected by Akaike information criterion (AIC) implemented in DAMBE[21]. A discrete gamma distribution with four rate categories across the site heterogeneity was estimated in PAUP* 4.0b10 and used in the ML method. In both the methods gaps were treated as missing, initial tree was obtained by stepwise addition and TBR algorithm was used for branch-swapping. L5 strain of *Hydra oligactis* was used as outgroup. In all methods bootstrap test with 1000 replicates was conducted.

Taxonomy
Phylum: Cnidaria
Class: Hydrozoa
Family: Hydridae
Hydra vulgaris Ind-Pune (Figure 1 *a–c*)

Etymology
Ind-Pune: Ind refers to India and Pune is the type locality of the described strain.

Collection site
Samples were collected from a pond in University of Pune Campus, Pune, 18°31′N lat. and 73°51′E long., Maharashtra, India.

Description
General morphology: Polyps are without a distinct stalk and the number of tentacles per polyp is 5–6 (n = 30, where n is the sample size); rarely animals with seven tentacles have been observed. Tentacles are shorter than body column and stretched tentacles can extend a little longer than the body column. In all the 48 animals studied, tentacles emerged asynchronously on buds. In 56% of the buds, two tentacles emerged first, opposite each other, followed by two more perpendicular to the first pair, whereas the fifth one appeared randomly (n = 48; Figure 1 *d–g*).

Colour: Polyps are light brown in colour after feeding and appear pale on starvation (48 h). On prolonged starvation (> 72 h), they become white.

Measurements: Adult polyps (with bud) measure about 4–8 mm (n = 25) in the relaxed state and 0.6–4 mm (n = 27) when fixed. Polyps without buds can extend up to 12 mm (n = 102) in length.

Sexuality: Usual mode of reproduction is asexual (by budding). Sexual reproduction is rarely observed and the conditions under which gonads are induced are not clear. Polyps are dioecious in nature and many male gonads are found on the body column starting from just below the sub-hypostomal region to the budding zone. The testes are broadly triangular in shape with a slightly constricted apex, without a distinct nipple. They are alternately and spirally arranged on the body axis (Figure 1 *b*). More than one female gonad occur along the axis starting from the sub-hypostomal region to budding zone (Figure 1 *c*).

Nematocysts: Stenoteles are pyriform, 9.72 ± 1.37 μm × 7.73 ± 1.13 μm (n = 24; Figure 1 *h*), holotrichous isorhizae are paramecium-like and some cylindrical, 10 ± 0.43 μm × 4.18 ± 0.28 μm (n = 15; Figure 1 *i*), atrichous isorhizae are cylindrical, 8 ± 0.69 μm × 3.53 ± 0.29 μm (n = 20; Figure 1 *j*) and desmonemes are

small, pyriform, 4.28 ± 0.28 μm × 3.16 ± 0.28 μm (n = 12; Figure 1 *k*). The tubule is transversely coiled in holotrichous isorhiza (Figure 1 *i*).

Phylogenetic affinities: *H. vulgaris* Ind-Pune has characteristic morphological features of the *H. vulgaris* species, such as absence of a distinct stalk, the length of stretched tentacles equal to or slightly longer than the body axis and the transversely coiled pattern of tubule within the holotrichous isorhiza. The asynchronous emergence of tentacles on buds is the only feature it shares with the *H. oligactis* (*P. oligactis*). Molecular phylogenetic analysis based on mitochondrial 16S rRNA clearly shows its close affinity with members of common hydra, *H. vulgaris* (Figure 2). Pairwise distance (*p*-distance) of the Indian strain from *Hydra magnipapillata* 105 (105 strain of *H. vulgaris*) is 0.005 and from other members of Eurasia analysed here is 0.003.

A recent report based on molecular phylogeny shows the species relationships within the genus *Hydra*[22] found across the globe. Another report has shown further clear clades within the vulgaris group based on their geographical distribution (North America, South America, Oceania, South Africa and Eurasia)[17]. The hydra described here clusters with Eurasian members of *vulgaris* species (Figure 2). The genetic divergence among the Eurasian members of *vulgaris* species is low[17], but morphological features like total polyp size, colour, emergence pattern of tentacles on buds and consistency in gonad production vary. The Indian strain shows very little genetic divergence from other members of the clade. Morphological and cytological evidence also strongly suggests close affinity of this species with the vulgaris group and not with *P. oligactis*, as erroneously called earlier in non-taxonomic literature. Though it has peculiar characters like alternatively arranged male gonads and asynchronous emergence of tentacles, we cannot attribute the novel species status as these characters are variable within the vulgaris group[1]. The ecotype from Pune previously studied[7] has similar morphological features as *H. vulgaris* Ind-Pune, and appears to be the same species. This strain differs by > 40% (in terms of morphology and physiology described by Prasad and Mookerjee[7]) from the Calcutta ecotype, which was presumed to be *H. vulgaris* phase orientales, by Annandale (cited by Prasad and Mookerjee[7]).

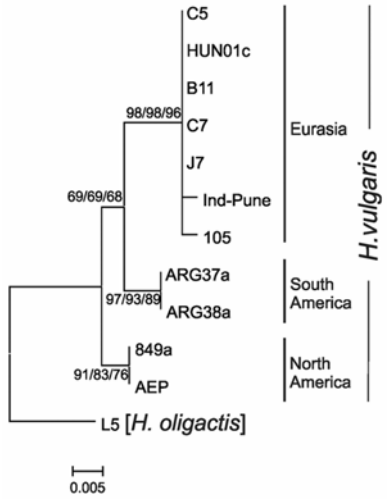

Figure 2. Phylogenetic tree based on 16S rRNA datasets using neighbour joining (NJ) method. Bootstrap values of NJ, maximum parsimony and maximum likelihood analysis are indicated above/below each node. Branch lengths are proportional to the scale bars given in substitutions per site. C5, HUN01C, B11, C7, J7, 105, ARG37a, ARG38a, 849a and AEP are strains of *H. vulgaris*[17]; L5 is a strain of *H. oligactis*[17] and Ind-Pune is the strain used in the present study.

On the basis of evidence presented here, this hydra can be classified only as a different strain of the *vulgaris* species, particular to the type locality mentioned, and for further referencing we call it as *H. vulgaris* Ind-Pune. Our data show that *H. vulgaris* Ind-Pune is closely related to *H. magnipapillata* 105, whose genome has been sequenced.

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ACKNOWLEDGEMENTS. We thank Prof. Richard Campbell, UCI, USA and Dr Hemant Ghate, Modern College, Pune, for valuable suggestions and comments on an earlier draft of this manuscript. P.C.R. and A.B. were recipients of SRF–NET from UGC and CSIR respectively. This work was funded by Department of Biotechnology, Government of India (Centre of Excellence in Epigenetics).

Received 20 December 2010; revised accepted 27 July 2011

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