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ECOLOGY

Worldwide Observations of Remarkable Deep-Sea Squids

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The bathypelagic realm of the ocean encompasses >90% of the nonsubterranean biosphere, comprising the largest, yet least explored, ecosystem. Recent interest has focused on biodiversity in the deep ocean (*1*, *2*). Here, we report observations from submersibles of large unknown squids at bathypelagic depths in four ocean basins (Table 1). Sample frames from videotapes of some of the observations are presented in Fig. 1. Videotaped records of these encounters lasted up to 10 min.

Representative digitized video clips are available on *Science* Online (*3*). In most cases, the squids were first encountered within a few meters of the sea floor with their extremely large, terminal fins undulating slowly and with arms and tentacles typically held in a unique position: spread outward from the body axis, then abruptly bent anteriorly (Fig. 1, A and B). None of these squids were collected, but all share the following morphology: The arms cannot be distinguished from the tentacles (modified ventrolateral arms of decapod cephalopods), appearing as 10 identical brachial appendages, all of about equal thicknesses and lengths. The relative length of these appendages is far greater than the arm length in any known squid, although all appendages are highly contractile. The filamentous distal portions of the brachial appendages appeared lighter in color than the rest of the animal in some videos. During extreme close-ups of squids 7 and 8, suckers could be seen along the oral surfaces of the proximal arms but not on the filamentous segments distal to the elbowlike bends.

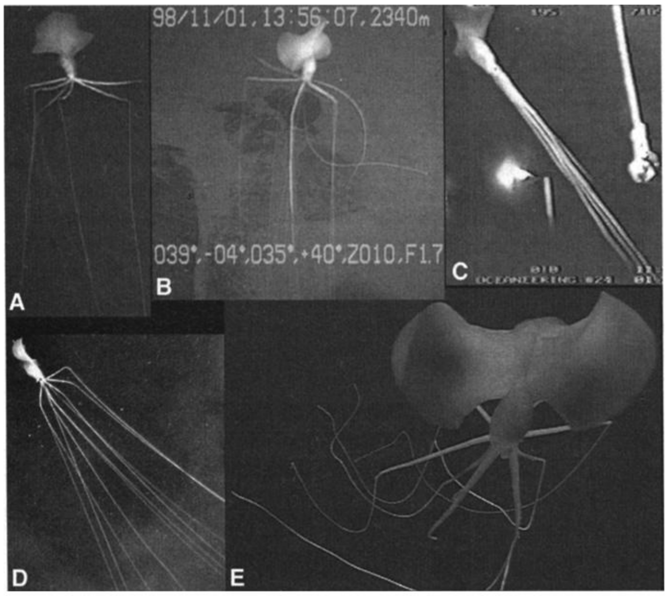

Fig. 1. Video frames of bathypelagic squids observed from submersibles. (**A**) Squid 2 (see Table 1); (**B**) squid 4; (**C**) squid 5; (**D**) squid 7; and (**E**) squid 8.

Most of the squids did not appear initially to react to the approach of the submersibles. In one case (squid 4), no escape response was noted even when the brachial appendages brushed the sample basket. After a few minutes, one animal (squid 7) moved slowly away, flapping its fins but with the arm crown still spread (Fig. 1D). However, when most squids either touched a submersible or encountered its turbulence, the escape reaction consisted of strongly flapping the fins and towing the retracted brachial crown (e.g., Fig. 1C). When squid 7 changed from hovering to rapid swimming, the first pulse included a contraction of the mantle, but no jetting was observed subsequently. One squid (squid 5) became entangled in the submersible and seemed to have difficulty releasing its arms and tentacles; during its attempts to swim away, multiple appendages stretched greatly.

We cannot be certain of the identity of these squids until specimens are captured; the morphology is very different from any known family. They are, however, most similar to the family Magnapinnidae. This family was recently described from two juveniles and a paralarva, all captured in epipelagic waters in the central and eastern Pacific Ocean (*4*). The Magnapinnidae is characterized by tentacles and arms with slender elongate tips and very large terminal fins. The squids reported here could be the unknown adults of this family. The sharp bend of the arms, which appears to coincide with a change in sucker distribution, could mark the junction between the arms and the terminal filaments seen on juvenile magnapinnids.

The inference from eight sightings within a few years of a completely unknown deep-sea animal at similar depths in the Gulf of Mexico and the Atlantic, Indian, and Pacific oceans is that these squids are comparatively common worldwide in the bathypelagic realm. That such a substantial animal is common in the world's largest ecosystem, yet has not previously been captured or observed, is an indication of how little is known about life in the deep ocean.

References and Notes

1. J. Asubel, *Oceanography* **12**, 4 (1999).
2. C. R. Smith, L. A. Levin, L. S. Mullineaux, *Deep Sea Res.* **45**, 1 (1998).
3. Videos and acknowledgements are available at www.sciencemag.org/cgi/content/full/294/5551/2505/DC1.
4. M. Vecchione, R. E. Young, *S. Afr. J. Mar. Sci.* **20**, 429 (1998)

Table 1. Summary of submersible observations on unusual bathypelagic squids.

Sighting	Fig.	Date	Submersible	Latitude	Longitude	Depth (m)	Comments
1		September 1988	*Nautile*	10°42.9′N	040°53.4′W	4735	Western Atlantic, off Brazil
2	1A	July 1992	*Nautile*	03°40′N	002°30′W	2950	Eastern Atlantic, off Africa
3		July 1992	*Nautile*	03°40′N	002°30′W	3010	60 m above bottom, possibly the same squid as squid 2
4	1B	November 1998	*Shinkai 6500*	32°45′S	057°13′E	2340	Indian Ocean, estimated length >1.5 m
5	1C	January 2000	Commercial ROV	28°37′N	088°00′W	2195	Gulf of Mexico, estimated length about 7 m
6		May 2000	*Nautile*	19°32′S	065°52′E	2576	Indian Ocean
7	1D	October 2000	*Alvin*	27°34.7′N	88°30.6′W	1940	Gulf of Mexico
8	1E	May 2001	*Tiburon*	21.9°N	158.2°W	3380	Central Pacific, estimated length 4 to 5 m

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GENETICS

Miniature Genome in the Marine Chordate *Oikopleura dioica*

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Oikopleura dioica is a small pelagic chordate belonging to the class of larvaceans (Fig. 1A). Chordates include vertebrates, cephalochordates, and urochordates, and urochordates are divided into ascidians, thaliaceans, and larvaceans. Larvaceans may be derived from the most basal branch of urochordates, and for this reason, they have been targeted for studies of chordate innovations, such as the tail, the notochord, or the dorsal nervous system (*1*, *2*). Found in all oceans, *Oikopleura dioica* can be permanently cultured in the laboratory. Its very short life cycle (4 days at 20°C, <2 days at 29°C) and high female fecundity (300 oocytes on average) offer incomparable perspectives for genetic analysis among nonvertebrate chordates. Here, we show that *Oikopleura dioica* is also attractive for genome-based studies, because of unusually high gene density and gene compaction.

Flow cytometry comparison of sperm DNA content between *Oikopleura* and the ascidian *Ciona intestinalis* yielded a value of 72 ± 13 Mb for the *Oikopleura* genome. In a large-scale shotgun sequencing, 128,386 short reads were assembled in 43,990 nonredundant contigs representing a total of 32.6 Mb. These data, treated with an existing model (*3*), suggest a minimal genome size of 51 Mb. The shotgun data set also accurately matched, on average, 50% of the length of 113 expressed sequence tags (ESTs), leading to a third estimation of 65 Mb. All these results concur to indicate that *Oikopleura* has the smallest genome ever found in a chordate.

We then compared the density of genes in *Oikopleura* and in *Ciona* through a BLASTX analysis of 407 1-kb-long genomic fragments from *Oikopleura* and four cosmid sequences from *Ciona* (*4*) divided into 1-kb fragments. The BLASTX alignments at a 10^{-09} e-value cutoff were twice as frequent with *Oikopleura* fragments (25% compared with 12%) and twice as long for *Oikopleura* (average 45 amino acids compared with 26 amino acids per kilobase, respectively). BLASTX analysis of 113 ESTs showed that this program failed to detect half of *Oikopleura* coding sequences. All BLASTX results suggest for *Oikopleura* an average gene density of one gene every 5 kb and about 15,000 genes (*4*).

To obtain a view of overall genome organization, we randomly cloned, sequenced, and annotated a few larger genomic fragments. One of them was a 145-kb-long bacterial artificial chromosome (BAC) insert for which we predicted 33 genes (Fig. 1B). The coding nature of 28 of them was confirmed by systematic cloning/sequencing of partial cDNAs. Another 18 genes were predicted in four distinct 12- to 14-kb plasmid inserts. In most genes, many small introns were found, with 62% of all introns smaller than 50 base pairs (bp) and 6% larger than 500 bp. Predicted intergenic regions were also very short, in several cases as small as 200 bp. Short repetitive sequences were detected in the largest intergenic regions as well as in some large introns. Four well-conserved genes of the BAC insert (stomatin, retinoblastoma-binding protein 7, protein phosphatase 2A PR65 subunit, and glycine transporter 1/2) were much shorter than their best candidate human orthologs (0.8, 1.6, 2.2, and 2.1 kb for *Oikopleura* genes compared with 15, 30, 35, and 12/50 kb in human, respectively). In these four conserved genes, only five intron positions out of 24 in *Oikopleura* and 41 to 43 in human were identical.

It is still premature to establish a link between the small genome and the short generation time of *Oikopleura*, even though a correlation between longevity and genome size has been found in birds (*5*). It is also unclear whether the high gene density uncovered in *Oikopleura* is an ancestral feature of chordates or, alternatively, if the genome has been markedly reduced in this lineage of urochordates. Regardless, important intron movements must have differentially affected the vertebrate and the *Oikopleura* lineages.

References and Notes

1. S. Bassham, J. Postlethwait, *Dev. Biol.* **220**, 322 (2000).
2. A. Nishino, N. Satoh, *Genesis* **29**, 36 (2001).
3. C. M. Fraser, R. D. Fleischmann, *Electrophoresis* **18**, 1207 (1997).
4. M. W. Simmen, S. Leitgeb, V. H. Clark, S. J. Jones, A. Bird, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4437 (1998).
5. P. Monaghan, N. B. Metcalfe, *Trends Genet.* **16**, 331 (2000).
6. Complete BAC insert, acknowledgements, and GenBank accession numbers are available at www.sciencemag.org/cgi/content/full/294/5551/2506/DC1.

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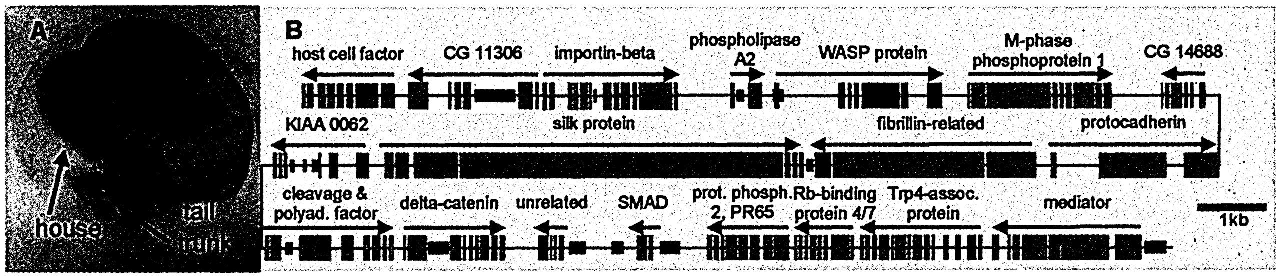

Fig. 1. (**A**) *Oikopleura dioica* in its "house," which allows seawater filtration. As an adult, the whole animal measures about 5 mm. (**B**) Organization of *Oikopleura dioica* genes in one BAC insert [see (*6*)], with exons detectable with BLAST (red), exons predicted by other tools (blue), regions of coding sequences confirmed by cDNA cloning (yellow), and repetitive sequences (green).