Primer

Crustaceans

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For many people, the term crustacean conjures up images of cooked lobsters, shrimp and crabs. While these particular animals embody many of the usual crustacean characteristics, they represent only a tiny fraction of this remarkable assemblage of animals (Figure 1). Crustaceans belong to the phylum Arthropoda, which is usually subdivided into four major groups: Crustaceans, insects, myriapods (centipedes and millipeds) and chelicerates (spiders, mites, scorpions, etc.). Like the other arthropods, crustaceans have a ventral nerve cord, jointed limbs, compound eyes, exoskeletons and body plans characterized by repeating segments that are often grouped into functional and morphological units called tagmata.

Even by arthropod standards, crustaceans are wildly successful. There are crustaceans in every type of marine and freshwater environment, and terrestrial forms, such as pillbugs, are found on all continents except Antarctica. The diversity of body forms and lifestyles is enormous. They are as common and widely distributed as the terrestrial wood louse and as rare and unfamiliar as the marine cave-dwelling remipedes. They range in size from microscopic copepods to 40-pound lobsters (Box 1).

As a general rule, the crustacean adult body plan is characterized by three primary tagmata — head, pereon (thorax) and pleon (abdomen) — plus a posterior telson (tail), but this body plan is not universal. For example, in some crustacean groups the head and anterior trunk segments are fused into a structure called the cephalothorax. Particularly notable variations on this body plan are seen in barnacles (described in more detail below) and the worm-like pentastomids. Most crustaceans are relatively soft-bodied with chitonous exoskeletons, but decapods, such as lobsters and crabs, have a hard calcium carbonate carapace. Both types of exoskeletons must be shed for growth. Crustaceans also display an exceptional diversity of appendage morphology, not only between species, but between different segments on the same animal. They have limbs specially adapted for walking, eating, grooming, swimming, mating, sensing the surrounding environment, brooding young, spearing prey, scraping surfaces and even snapping to make noise. Lobsters provide a good example of the limb diversity that can exist on even a single individual — the thoracic and abdominal appendages include maxillipeds (thoracic legs adapted for feeding), walking legs, swimming legs, genital appendages, and specialized grasping claws. One of the most highly specialized limbs among all the crustaceans is the raptorial appendage of the mantis shrimps (stomatopods), which can be deployed in as little as 2 milliseconds to club or spear prey. The force of the strike can even break the glass walls of an aquarium or the finger bones of a fisherman unlucky enough to have caught one of these animals.

As mentioned previously, some crustaceans, such as barnacles, depart so radically from the typical crustacean body plan that they are easily confused with animals of other phyla. Barnacles were particularly fascinating to Charles Darwin, and he spent many years of his life collecting and studying them. Most people, however, only encounter barnacles in their closed-up state in tide pools or on dock pilings, and usually only see the outer cone-shaped shell. Based partly on this shell structure, early zoologists first argued that barnacles were mollusks, thus putting them into the same group as clams. It was not until the early 1800s that they were recognized as crustaceans, a realization reached through the study of their development, as barnacle larvae closely resemble other crustacean larvae. An even more unusual body form is seen in the rhizocephalans, endoparasitic barnacles that exist as masses of reproductive cells within their crab host's body cavity, though their

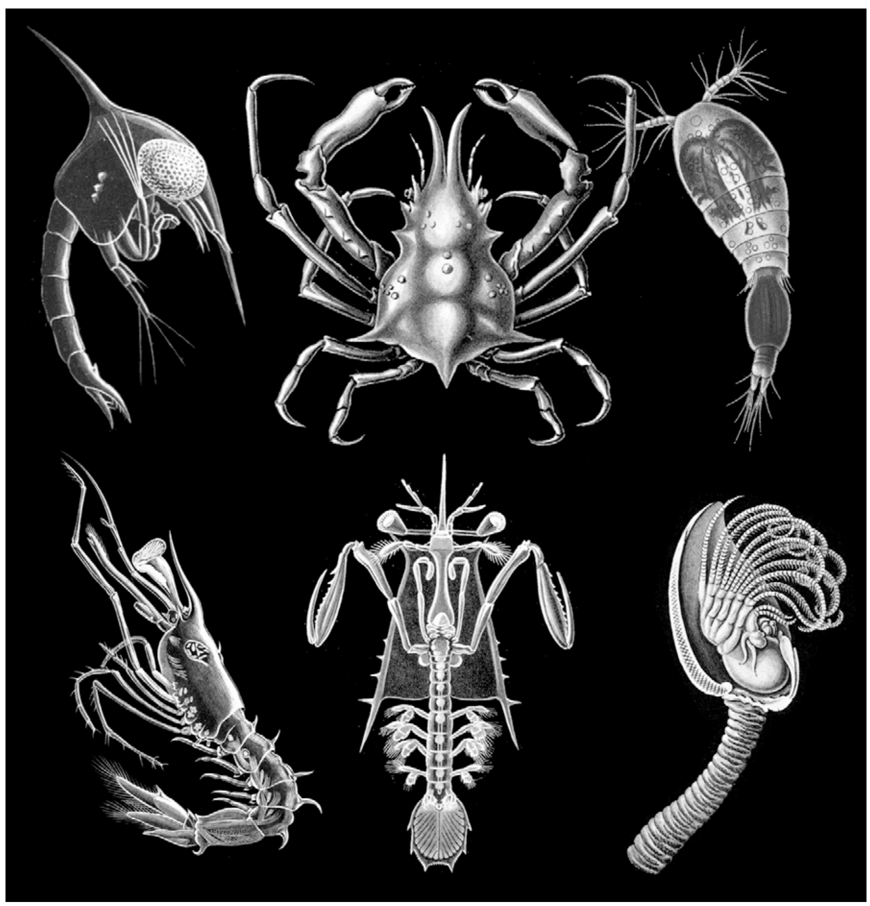

Figure 1. Some examples of crustacean diversity as illustrated by Ernst Haeckel.
Clockwise from the top left are a larval crab, adult crab, copepod, barnacle, juvenile stomatopod, and a shrimp. Reproduced from Ernst Haeckel's 'Kunstformen der Natur,' with permission from Ernst-Haeckel-Haus, Friedrich-Schiller-Universität Jena.

Box 1.

Major groups of crustaceans.

Branchiopods almost exclusively inhabit freshwater, but some, such as the brine shrimp *Artemia*, have re-adapted to salt water. Most species typically have a large number of identical trunk segments, the tadpole shrimp *Triops* has more than 50. Water fleas like *Daphnia* have a highly reduced number of segments and a derived body plan. Other groups have head shields that are shovel-shaped (*Triops*) or bilobed (clam shrimp).
Remipedes were discovered in the 1980s and inhabit marine caves that are difficult and dangerous to access. They resemble an aquatic centipede, having a very large number of homonomous limbed segments (up to 32). Very little is known about their lifecycle, behavior, or habits, and no young larvae or embryos have yet been described.
Cephalocarids are tiny saltwater sediment-dwellers with an anterior trunk of limbed homonomous segments and limbless posterior segments, roughly similar in morphology to brine shrimp with a head shield.
Copepods are generally tiny (less than 2 mm) and torpedo-shaped, and have a single median eye. They are found in fresh and salt water, may be planktonic or benthic, and make up an important part of marine plankton.
Ostracods, also known as seed shrimp, are generally tiny, but there are giant deep-ocean species. They are found in fresh and salt water, have a derived body plan in which segmentation is not readily apparent, and are encased in a bivalved hard carapace
Branchiurans are parasites of vertebrates. This group includes the fish lice and tongue worms (pentastomids). Pentastomids are elongate, limbless, and live in the sinuses of vertebrate hosts (including reptiles, birds and mammals). Fish lice have a flattened body covered by an oval carapace with mouthparts and antennae modified into suckers for sticking to and feeding on the host.
Tantulocarids are parasites that live on other deep-water crustaceans. Their hosts include copepods, isopods, and ostracods, so they are extremely tiny (less than 1/3 mm) and have body plans with reduced segmentation.
Thecostracans are the barnacles. In their adult forms, they are sessile filter-feeders with a reduced body plan and a calcerous shell, but larval stages are planktonic. One group, the parasitic rhizocephalans, is highly derived; they have typical barnacle larval forms, but the adult consists of little more than a mass of neurons and germ cells inside the body cavity of a crab host.
Mystacocarids are tiny (less than 1 mm) benthic animals that live in the spaces between sand grains. Their antennae are very long in relation to their bodies, and they resemble worms with heads and small branched limbs. Less than 20 species have been described.
Malacostracans are a hugely diverse group that includes all the things you probably think of when you think of crustaceans, including decapods (lobsters, crabs, and shrimp), peracarids (isopods, amphipods) and stomatopods (mantis shrimps).
Insects, according to the Pancrustacean hypothesis (Figure 2), are the most successful of the terrestrial crustaceans. Adults possess three thoracic segments with legs (and sometimes wings) and 8 to 11 limbless abdominal segments.

planktonic larvae clearly identify them as a barnacle.

Evolutionary history

Fossil crustaceans became relatively common after the diversification of hard-shelled decapods in the Permian a little less than 300 million years ago. Like most extant species, more ancient crustaceans had soft bodies that did not fossilize well, and indeed it turns out that crustaceans were present and quite widely diversified by the Cambrian period over 500 million years ago. The Burgess shale formation from the middle Cambrian is a particularly important repository of Cambrian arthropods because of the quantity and quality of the specimens and represents a very special time in crustacean evolution, when there were crustaceans that appear to belong to extant groups as well as animals such as *Branchiocaris* and *Marella* which cannot be assigned to any of the modern classes of crustaceans. The upper Cambrian Orsten fauna is a remarkable resource for illuminating crustacean evolution because it contains a vast number of larvae preserved in three dimensions. While the fossil record does not give a clear picture of the morphology of the ancestor of all crustaceans (the 'urcrustacean'), it has been argued that it probably would have had a fairly large number of identical or highly similar (homonomous) segments, each of which bore a pair of appendages. This inference of homonomous segmentation in the urcrustacean often leads to description of several extant groups, such as remipedes and branchiopods (brine shrimp, etc.) as 'primitive' — or more correctly, having retained ancestral bodyplan characteristics.

The relationships among the four major groups of arthropods are a matter of debate, but there is increasing evidence that crustaceans and insects are more closely related to one another than either is to the myriapods or chelicerates. A more factious phylogenetic debate concerns the relationships of the various groups of crustaceans to one another and to insects. According to one of the more recent phylogenies (Figure 2), the so-called Pancrustacean hypothesis, the insects actually arise from within the crustacean lineage, thus defining insects as the most successful group of terrestrial crustaceans. One theory is that crustaceans, which first evolved in the young Earth's seas, later colonized freshwater habitats as branchiopods, and finally invaded the land as insects. Consistent with this hypothesis, insects are most similar to branchiopods in terms of mitochondrial gene sequences and some aspects of embryogenesis, but there are other sequence analyses as well as anatomical and embryological evidence to unite insects with the malacostracans instead. The relationship between the major classes of crustaceans remains contentious, and for now it is probably best to consider them part of an unresolved phylogenetic tree that is likely to be hotly debated for some time to come.

Life history

Crustaceans are mostly aquatic, with some species adapted to terrestrial environments. Adaptation to life on land has occurred multiple times, and most terrestrial species such as coconut crabs and hermit crabs still require water for breeding; terrestrial isopods, such as pillbugs and woodlice, are the only exception to this rule. Even terrestrial species use gills for gas exchange and thus are susceptible to desiccation. Crustaceans are found in every type of aquatic ecosystem, and there are species adapted to extremes of temperature, pressure, salinity, and even anoxia. Intertidal and tidal pool species are especially adaptable, with individuals experiencing extensive temperature fluctuations and periodic drying on a daily basis. Besides living in virtually every aquatic habitat on earth, crustaceans also make their living in almost every conceivable way. Planktonic larvae are filter feeders, as are some crabs and the sessile barnacles. Other forms scavenge, graze, hunt prey or live as parasites.

Reproductive strategies vary considerably and include sexual and asexual lifestyles. The majority of crustacean species have separate male and female sexes, but some are hermaphroditic, and some even reproduce parthenogenetically, such as the Darwinulid ostracods. Adding to their oddity, barnacles have evolved the largest penises (relative to body size) in the animal kingdom, allowing sessile individual's to mate across the distance of several centimeters away. In most cases there is no maternal care, but the young of some species may live on the mother's body for a period of days after hatching. One group of crustaceans, the sponge dwelling pistol shrimp of the genus *Synalpheus*, exhibit an eusocial life-style otherwise seen only for bees, ants, wasps, termites, and mole rats: Within a colony of animals, there is only a single reproductive female ('queen') while the remaining of the individuals work to maintain the colony.

Early embryogenesis varies greatly throughout the crustaceans. Most are holoblastic showing complete cleavage of early cells, while some, such as isopods, are meroblastic, showing only partial cleavage during early development. After gastrulation, the anteroposterior body axis becomes apparent during formation of the germband. The head is established very early, and then the remaining body segments are added in an anterior to posterior sequence (Figure 3), reminiscent of the formation of segments in short-germ insects, such as grasshoppers and beetles.

The degree of development completed *in ovo* varies considerably. With the exception of changes associated with sexual maturation (sexually dimorphic limb morphology, gonad development), crustaceans such as isopods and amphipods emerge from the egg looking like miniature adults. Others, like brine shrimp, hatch with only head segments and a tail and form the rest of their body segments as they pass through several larval stages. There are also metamorphic developers such as decapods and krill. These groups pass through some bizarre-looking larval stages that may not resemble the adult form at all. Indeed, some larval forms differ so much from the adult that it has taken years for researchers to match adults with their corresponding larval forms; sometimes the only way to match them in the absence of DNA sequence information is to rear wild-caught larvae in the lab or persuade captive adults to breed.

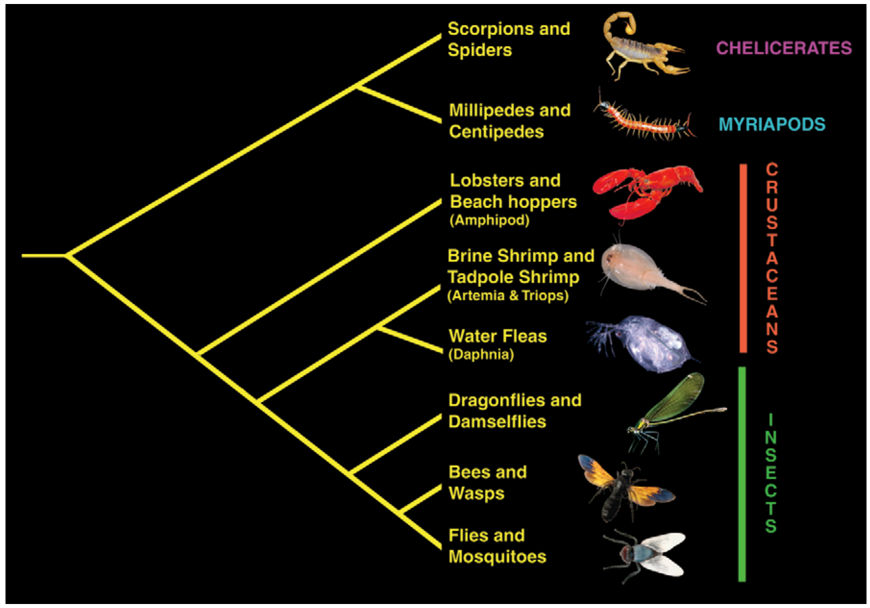

Figure 2. A proposed evolutionary relationship for some of the major arthropod groups.
In this scenario, chelicerates and myriapods are more closely related to each other than either is to insects or crustaceans. Insects are depicted as arising from within the crustacean lineage (the Pancrustacean hypothesis).

Current research

Identification of new species is still an important part of modern crustacean research. New species are being described at a rapid pace, especially from the deep sea and other previously inaccessible habitats. Living species of the class Remipedia were not discovered until the 1980s; prior to that, the group was represented only by a single fossilized specimen. Exploration and discovery continue to yield new information about species diversity, distribution, and ecological relationships.

Although crustacean phylogeny remains murky, advances in genomics and developmental biology are increasing our understanding of the evolutionary history of crustaceans as a group. There is also a long history of using crustaceans in physiology, neurobiology, and anatomy research, but it is becoming more common to see them used in developmental, cell and molecular biology, fields that, until recently, focused almost exclusively on a few model species. Non-model organisms are becoming increasingly popular research subjects in all fields as modern science regains its appreciation of comparative biology. As the use of non-model organisms becomes more widespread in molecular biology, there is an increasing need for genome information. One crustacean genome has been completed, that of the branchiopod *Daphnia pulex*, which was chosen for sequencing due to its relatively small genome size (250 Mb), its long history in ecological and evolutionary research, and its role as a sentinel species in aquatic ecosystems. EST sequencing from the porcelain crab, *Petrolisthes cinctipes*, is being used in research aimed at understanding how aquatic animals respond to thermal stress which may prove useful in predicting the outcome of global climate change. A genome sequencing project has also been approved for the amphipod *Jassa slatteryi*, and techniques for knocking down and misexpressing genes have been established in the amphipod *Parhyale hawaiensis*.

Humans and crustaceans

Crustaceans are important food sources all over the world and a few species, such as lobsters, crabs and shrimp, have become economically particularly important crustaceans, with some harvested from wild stocks and others being farmed. While some crustacean species are in decline, in at least one case human intervention has created

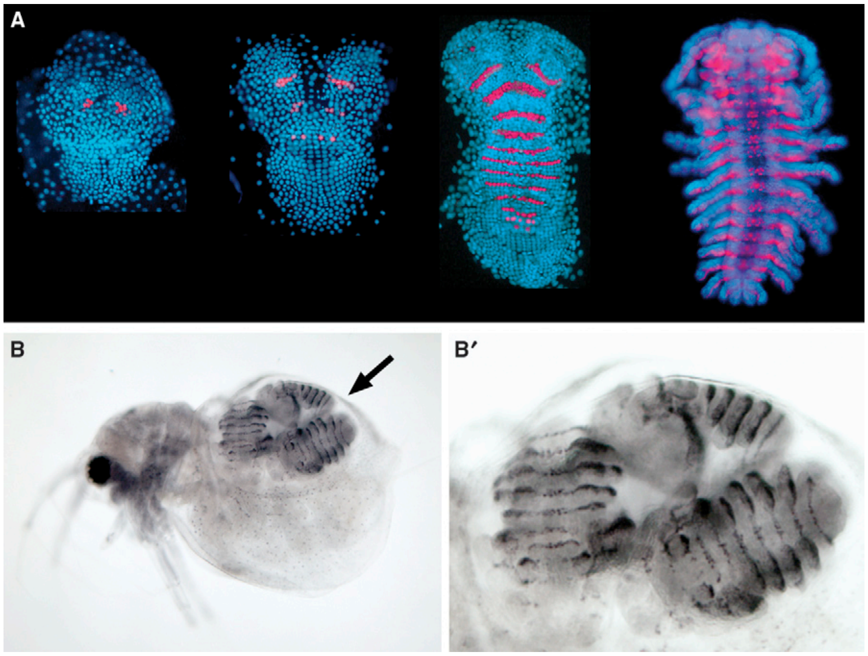

Figure 3. An example of sequential segmentation frequently observed in crustacean development.

(A) Series of sequentially older embryos of the amphipod *Parhyale hawaiensis* (anterior is oriented up). All the nuclei of the animal are shown in blue, while stripes of expression of the Engrailed protein (in red) mark the boundaries between segments. As the embryo develops, segments are added from the head to the tail. (B) Here the developing embryos (arrow) of the branchiopod *Moinodaphnia* are shown inside the brood pouch of the mother (Engrailed stripes are black). (B′) Higher magnification of the embryos within the brood pouch.

a boom in a wild population, although with some unintended consequences: As a way to feed the Soviet Union's growing northern population, king crabs native to the North Pacific were introduced into the Barents Sea in the 1960s. Now thriving, this population has been welcomed by the fishing industry, as these crabs are a profitable catch. Unfortunately, these crabs are also ravenous omnivores and have few natural predators in these waters. Their feeding activities strip bare huge swaths of seabed, depleting capelin and cod stocks by consumption of the fishes' eggs and competition for food. The king crabs are steadily making their way south towards European waters and despite short-term benefits damage to fisheries in the long term seems likely. Other crustacean species have also become invasive, including Chinese mitten crabs, which have spread through the ballast water of ships, and the crayfish *Procambarus clarkii*, a native of the US Gulf Coast, that has displaced many other crayfish from their habitats. The recent finding of a parthenogenetic crayfish strain in the aquarium trade also poses a potential threat if it makes its way into the wild.

Crustaceans are clearly a remarkable group of organisms with a long evolutionary history and remarkable adaptability. Given their primarily aquatic habitats, however, they are not as well studied as their terrestrial arthropod relatives, but our knowledge of their diversity, behavior, development, and physiology is growing rapidly.

Further reading

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Widespread recycling of processed cDNAs in dinoflagellates

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Dinoflagellates are ubiquitous algae with extraordinary nuclear genomes that are among the largest known [1–3]. Dinoflagellate nuclear genes are also exceptional in that an invariant 22 bp trans-spliced leader (SL) caps most or all mRNAs [4,5]. We re-examined cDNAs from diverse dinoflagellates, and show that about 25% of them include additional, sometimes multiple, relict SL sequences in tandem. All of these relict SLs are truncated after nucleotide 7 of the canonical SL, corresponding to an AG dinucleotide. Genomic sequences confirm that these additional SLs are genome-encoded, and that the relict SL provides the AG acceptor site for subsequent trans-splicing. Altogether, this shows that large numbers of genes in dinoflagellate genomes have been cycled through an mRNA intermediate at least once in recent evolutionary history, an event potentially encouraged by the conservation of splice acceptor sites within the SL. This dynamic cycling between the genome and mRNA predicts that introns will be rare in dinoflagellate genomes since they would be purged with each cycle, and those that do remain are most likely to have been inserted relatively recently.

An identical 22 bp leader is found on the 5′ end of most or all mRNAs in all known dinoflagellates, making the trans-splicing mechanism that adds this leader both ancient and pan-genomic. We aligned the 5′ end of approximately 500 full-length dinoflagellate cDNAs and found the conserved 22 bp SL sequence in all cases, but also found evidence for a second, relict SL immediately downstream of the first one in over 100 cDNAs (Figure 1A, and Figures S1 and S2 in Supplemental Data, published with this article online). A third SL was also present in six