Study finds surprising new branches on arthropod family tree

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Any way you look at it -- by sheer weight, species diversity or population -- the hard-shelled, joint-legged creepy crawlies called arthropods dominate planet Earth. Because of their success and importance, scientists have been trying for decades to figure out the family relationships that link lobsters to millipedes and cockroaches to tarantulas and find which might have come first.

In a scientific and technological tour de force that was nearly a decade in the making, a team of scientists from Duke University, the University of Maryland and the Natural History Museum of Los Angeles County have compared genetic sequences from 75 different species to draw a new family tree that includes every major arthropod lineage. Some of the relationships are so surprising that new names had to be coined for five newly-discovered groupings.

The work, which was supported by the National Science Foundation, appears early online Wednesday in the journal *Nature.*

A big surprise to tumble out of the new tree is that the closest living relatives of insects include a small and obscure group of creatures called remipedes that were only discovered in the late 1970s living in a watery cave in the Bahamas. With linear bodies like centipedes, simple legs and no eyes, it was thought that this small group -- now placed with cephalocarids in the newly-named Xenocarida or "strange shrimp" -- would be found at the base of the crustacean family tree.

Now, after analyzing 62 shared genetic sequences across all the arthropods, the researchers are putting the strange shrimp together with the six-legged insects, Hexapoda, to form a new group they dubbed Miracrustacea, or "surprising crustaceans." As a "sister clade" to hexapods, the Xenocarida likely represent the sort of creature that came onto land to start the spectacular flowering of the insect lineage, said Cliff Cunningham, a professor of biology at Duke who led the study.

Triops, a 2-inch crustacean that looks like a cross between a horseshoe crab and a mayfly, had also been thought of as an early crustacean, but it too was shown to have a relatively modern origin in the new analysis, Cunningham said.

"Taxonomists have been arguing about these things for decades, and people kept coming at this with one data set after another," Cunningham said. This latest study has created a fuller picture of the arthropod family tree by using more species and more genes, he said.

Beginning in 2001, Jeffrey Shultz, an associate professor of entomology at Maryland, led the efforts to figure out which species needed to be sequenced for a robust comparison, and then to round up suitable specimens of each. The study included nematodes, scorpions, dragonflies, barnacles, copepods and centipedes.

Remipedes, one of the two species of Xenocarida in the study, had to be fetched from partially submerged limestone caves in the Yucatan Peninsula and preserved just so. Bitty creatures called mystacocarids that live between grains of sand were captured by the Natural History Museum's Regina Wetzer, using a microscope on a Massachusetts beach.

Once assembled, the 75 species were then stripped down to their DNA for a painstaking search to find genetic sequences that would appear across all arthropods, enabling statistical comparisons.

The lab of Jerome Regier at Maryland's Center for Biosystems Research combed through 2,500 different combinations of PCR primers to find 62 protein-coding gene sequences that could be compared across all 75 species. Regier was an early proponent of using protein coding genes to sort out the arthropod tree, while most other researchers were using relatively less complex analyses from the DNA found in ribosomes and mitochondria.

The researchers ran four different statistical approaches, including two new ones invented at Maryland, "and they all came up with the same answer," Cunningham said. Earlier studies had not used as many genes or as many species, making this study about four times larger than anything done previously.

The spiders, ticks and scorpions of the subgroup Chelicerata are shown to have split from the line leading to insects and crustaceans even before the millipedes and centipedes of the subphylum Myriapoda. Most recent molecular studies had grouped these arachnids in Chelicerata together with millipedes and centipedes of the Myriapoda. But the new analysis puts millipedes and centipedes together with crustaceans and insects in a group taxonomists had long ago named Mandibulata.

"The only thing people thought they knew before molecular data was available was that the Myriapods were with the insects," Shultz said. But that turned out to be wrong. Even the grouping Crustacea is no longer correct, since it includes the six-legged insects.

Within the insect group Hexapoda, the good news for taxonomists who have grouped insects according to body shape and features is that they were pretty much on the mark, Shultz added.

There are still many holes that need to be filled in, Cunningham said, but at least the shape of the tree seems right. "Now the developmental

biologists can really piece things together."

Provided by Duke University