Evidence from 12S Ribosomal RNA Sequences That Onychophorans Are Modified Arthropods


The evolutionary relationships of the onychophorans (velvet worms) and the monophyly of the arthropods have generated considerable debate. Cladistic analyses of 12S ribosomal RNA sequences indicate that arthropods are monophyletic and include the onychophorans. Maximum parsimony analyses and monophyly testing within arthropods indicate that myriapods (millipedes and centipedes) form a sister group to all other assemblages, whereas crustaceans (shrimps and lobsters) plus hexapods (insects and allied groups) form a well-supported monophyletic group. Parsimony analysis further suggests that onychophorans form a sister group to chelicerates (spiders and scorpions) and crustaceans plus hexapods, but this relationship is not well supported by monophyly testing. These relationships conflict with current hypotheses of evolutionary pathways within arthropods.

The question of whether arthropods, or jointed foot invertebrates, have a common ancestor has generated debate. Central to this discussion is the phylogenetic position of onychophorans, or velvet worms. These enigmatic invertebrates resemble slugs with legs (Fig. 1), and an early report described them as mollusks (chitons, limpets, and snails) (1). They have been described as the missing link between arthropods and annelids (segmented worms) because of physical similarities to both groups. Currently there are four major hypotheses of onychophoran evolutionary relationships (2–5) (Fig. 2). On the basis of morphological criteria, these hypotheses group myriapods (millipedes and centipedes) and hexapods (insects and allied groups) together to form the atelocerates. The morphological characteristics used by these models include the presence of anterior terantorial arms, the absence of preanal levator muscles, the absence of distinct appendages on the tritocerebral head segment (6), and appendage evolution (7).

Ultrastructural similarities between the sperm of onychophorans and eucrilliates (oligochaetes, branchiobdellids, and leeches) have led to the proposition that onychophorans are more closely related to certain annelids than to arthropods (2) (Fig. 2).
2A). Similarities in the embryonic development of onychophorans and atelocerates have been used as evidence for monophyly of these two groups to form the uniramians, although there is dissent over arthropod monophyly (Fig. 2, B and D). Investigators who discount the embryological evidence (4) have suggested that the closest extant relatives of atelocerates are the other mandibulate arthropods, the crustaceans (shrimps and lobsters) (Fig. 2C). The monophyly of this assemblage is further supported by the extraordinary similarity of the compound eye ommatidia of hexapods and crustaceans. The myriapod eyes are considered to be modified secondarily (8).

Of the existing numerical approaches to investigating phylogeny, maximum parsimony methods have been used most extensively (9). These methods minimize the amount of evolutionary change required to explain the available data. Maximum parsimony comparisons of partial cytoplasmic 18S ribosomal RNA (rRNA) sequences from chelicerates (spiders and scorpions), myriapods, crustaceans, and hexapods support the hypothesis that these arthropods are monophyletic relative to annelids and mollusks (10, 11). However, bootstrapping (12), a statistical method for obtaining an estimate of error, does not support this hypothesis (11). In contrast to maximum parsimony analyses, evolutionary parsimony analysis of a subset of these data does not suggest arthropod monophyly (13). Because there are relatively few 18S rRNA transversion positions (11), we sequenced a segment of the mitochondrial small ribosomal subunit, 12S rRNA (14), in order to investigate the phylogenetic position of onychophorans and the monophyly of arthropods. This region of 12S rRNA was chosen collapsed to yield a consensus. The series of parsimony analyses with T-PTP testing processes follows. Arthropods including onychophorans are monophyletic (T-PTP = 0.05, difference 4 steps), and a hypothetical arthropod ancestor is calculated using character state optimization based on the tree topology found within arthropods. This ancestor ancestor is then combined with the other outgroup taxa to test monophyly of annelids and mollusks. Annelids are monophyletic (T-PTP = 0.01, 18 steps), but a parallel test constrained mollusc monophyly gives a tree 3 steps longer than the converse. In an anterior analysis mollusks and annelids are monophyletic (T-PTP = 0.01, 12 steps). Onychophorans and arthropod monophyly is supported when annelids are the outgroup to all arthropods (T-PTP = 0.04, 5 steps, and T-PTP = 0.03, 3 steps, respectively). Monophyly of myriapods and the remaining arthropods is subsequently supported (T-PTP = 0.01, 4 steps, and T-PTP = 0.01, 3 steps, respectively). Chelicerae and crustacean monophyly is supported in parallel (T-PTP = 0.01, 3 steps, and T-PTP = 0.01, 9 steps). Crustaceans plus hexapods are monophyletic (T-PTP = 0.03, 1 step) when myriapods are the outgroup and the ingroup consists of C. longicaudata and M. tredicim and the onychophoran, dipteran, chelicerae, and crustacean ancestors. Tendipicercus longicaudata and M. tredicim are not significantly monophyletic in a parallel test (T-PTP = 0.11, 1 step). Subsequent parsimony analysis suggests chelicerates and crustaceans plus hexapods are monophyletic; however, T-PTP testing shows this is not significant (T-PTP = 0.31, 1 step). In a subsequent test of non-monophyletic, T-PTP testing could not reject the hypothesis that onychophorans and chelicerates are monophyletic (T-PTP = 0.50, 1 step). Hexapods are monophyletic (T-PTP = 0.01, 1 step) when onychophorans and chelicerates are the outgroup to crustaceans and hexapods. Parallel testing shows monophyly of C. longicaudata and M. tredicim is not well supported (T-PTP = 0.15, 2 steps). With the cranial ancestor as the outgroup and dipteran ancestor as the ingroup, monophyly of M. tredicim and C. longicaudata is again not significant (T-PTP = 0.69, 0 steps). Monophyly test of major groups within arthropods were properly defined as a priori tests (23). However, testing monophyly of chelicerates and crustaceans plus hexapods and non-monophyly of onychophorans and chelicerates was correctly a posteriori; the hypothesis of monophyly arose as a result of cladistic analysis. The simpler a priori test initially applied here presents a posteriori test, as T-PTP values will always be higher for the latter.
been pared study,ature proposal suggested that myriapods and chelicerates are the sister group to crustaceans plus hexapods. Although this tree was not well supported, no trees within 1% of the most parsimoniously recognized a monophyletic myriapod plus hexapo-

## Maximum parsimony analyses with T-PTP testing indicate that arthropods include onychophorans and are monophyletic relative to annelids and mollusks (Fig. 3A). This proposal is supported by neighbor-joining analysis with bootstrapping (Fig. 3B). Annelids were chosen as an appropriate outgroup to evaluate arthropod relationships in subsequent analyses with T-PTP testing (25). Parsimony analyses suggest that onychophorans form a sister group to chelicerae and crustaceans plus hexapods, but T-PTP testing shows the available 12S rRNA data cannot significantly resolve this trichotomy (Fig. 3A). In comparison to these results, neighbor-joining analysis suggests that onychophorans and chelicerates are sister taxa (Fig. 3B). These data imply that onychophorans are a highly specialized assemblage, neither a primitive "missing link" nor an appropriate outgroup for analyzing arthropod phylogenetic relationships. Parsimony analyses with T-PTP testing further indicate that myriapods represent the earliest arthropod branch and are the sister group to the remaining taxa including a monophyletic crustacean plus hexapod assemblage. This suggests that the diverse eye structures of myriapods are primitive and not derived (8) and that the morphological criteria used to unite the arthropod assemblage results from convergent evolution and are not shared derived characters (6). Phylogenetic analyses indicating that myriapods are basal to the remainder of the arthropoda suggest that the assemblage is older than previously thought. A myriapod-like fossil recently described from marine deposits in the Middle Cambrian superficially resembles _Portalia_ and _Redouibia_ from the Burgess Shale (26). The later fossils were originally described as holothurians (sea cucumbers) (27) but a more recent evaluation concluded their relationships are problematic (28).

Data obtained from 18S RNA (10, 11) support some of the evolutionary relationships proposed in our reconstructed parsimony analyses. Field and co-workers (10) reported that the myriapod occupied an unexpectedly deep position in their tree; however, they had difficulty placing it because of the long branch length. Maximum parsimony analyses with additional 18S rRNA sequences (11) suggested that myriapods and chelicerates are the sister group to crustaceans plus hexapods. Although this tree was not well supported, no trees within 1% of the most parsimonious recognized a monophyletic myriapod plus hexapo-

## Within the major assemblages, general congruence with independently derived phylogenies provides additional support for our reconstructed tree (Fig. 3A). Within onychophorans, _Plicataperipatus jamacaiensis_ was the sole member of the family _Peripatidae_ sequenced. This family is the sister group to the family _Peripatopsidae_. Within _Peripatopsidae_, the unnamed _Asterhion_ species is expected to be phylogenetically (29) and morphologically (30) distinct from _Euprostigmata_. Phylogenetic inferences generated from 18S rRNA analyses support monophyly of the chelicerates (11) and the crustaceans used in this study (31). There is also high congruence between 12S rRNA and morphologically derived trees within dipteran hexapods (32). Hexapod phylogenetic relationships are complicated by the accumulation of nucleotide substitutions in the 12S RNA of the thysanuran _Ceroepis monticulata_. The reconstructed tree clusters _C. longicauda_ and the hemiptera, _Maggieciadactas_. However, T-PTP tests do not support monophyly of this clade. Additional sequences will be required to resolve relationships between these hexapod orders.

These data demonstrate that 12S rRNA sequence data can resolve arthropod relationships over a broad taxonomic range. Some further corroboration of the significant monophyletic groups is found using 18S rRNA sequence data and electrophoretic and morphological characters within arthropod assemblages. We propose that arthropods include onychophorans and are monophyletic. Moreover, we cannot find support for monophyly of uniramians or arachnids and suggest phylogenetic relationships within arthropods should be reassessed. Our reconstructed tree represents a new framework for arthropod evolutionary pathways (33).
PREDATOR-INDUCED PHENOTYPICAL CHANGE IN BODY MORPHOLOGY IN CRUCIAN CARP

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In a field experiment where the presence or absence of piscivorous pike (Esox lucius) in ponds was manipulated, the morphology of crucian carp (Carassius carassius) diverged, such that individuals became deeper bodied in pond sections with pike. A laboratory experiment confirmed that the presence of this predator induced a change in body morphology in the carp. Estimation of prey vulnerability to predation by pike, a gape-limited predator, revealed that this increase in body depth resulted in crucian carp reaching a size that provided refuge from predation. However, this change in morphology incurs a cost through an increase in drag when the carp are swimming. Because crucian carp are limited by resources in the absence of piscivores and by the substantial cost of the defensive morph in their presence, phenotypic plasticity should be the optimal strategy for this species.

Various morphological structures in prey organisms function as efficient adaptations against predation (1), and these morphological defenses could be either constitutive or environmentally induced. The evolution and maintenance of inducible defenses is favored when the defense incurs a fitness cost, when predation intensity varies temporally or spatially, and when prey have reliable cues for predator detection (2, 3). Predator-induced morphological defenses occur in a number of invertebrates, mainly aquatic taxa (2). Waterborne cues from predators or chemicals released by injured conspecifics trigger the development of defenses that reduce predation rates (2, 4). However, induced defenses have been shown to incur a fitness cost through a reduction of growth or reproduction or both (2, 4, 5). Here, we report on a predator-induced change in body morphology in a vertebrate, the freshwater fish crucian carp (Carassius carassius).

Crucian carp are extremely vulnerable to predation (6, 7). In lakes with piscivores, especially pike Esox lucius, crucian carp populations consist of a small number of large individuals (6). However, without piscivores, crucian carp form dense populations of small individuals (6–8). The body morphology of monospecific pond populations and multispecies lake populations differ, with lake individuals much deeper bodied. The two morphs originally were considered as separate species (Cyprinus vulgaris and C. gibelo); however, in the early 1800s it was shown by transplant experiments that these two species were one (9). The presence of two morphs has previously been considered a result of differences in resource levels; however, we show that increased body depth can also be an inducible morphological defense that reduces the risk of predation.

For part of a study evaluating the effects of trophic structure on freshwater communities, we divided into halves two small, eutrophic ponds (Severin’s and Matz’s ponds, 0.1 ha each) with monospecific crucian carp populations and introduced pike into one half (10). After 12 weeks, crucian carp had diverged in body shape; in pond sections with pike, carp tended to have a deeper body (Fig. 1). Given this result, we hypothesized that the change in body morphology could be a result of several things: (i) selective predation, (ii) an increase in resource availability, or (iii) a predator-induced phenotypic modification of body shape. The small variance in body depth and the absence of overlap between treatments (Fig. 1) suggested no polymorphism with regard to this trait in the original population; thus, selective predation on genetically determined morphs could not account for the increase in body depth.

High resource availability may be responsible for the shift in morphology, as suggested by a study in Finland where crucian carp increased in body depth when introduced at a low density of 187 fish per hectare to a fishless pond (8). In our ponds, the reduction of the crucian carp density by pike permitted an increase in the density of large, cladoceran zooplankton (11). This increase in food availability in the pike section could account for the differences in the carp body depth. However, in another experiment we transplanted crucian carp from a pond with a...