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THE PANCRUSTACEAN CONUNDRUM: A CONFLICTED PHYLOGENY WITH EMPHASIS ON CRUSTACEA

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Abstract

Pancrustacea represents a diverse clade of arthropods and includes the crustaceans and insects. The morphological and ecological diversity is among the most impressive of all invertebrates, with representatives including barnacles, shrimps, crabs, copepods, ostracods, brine shrimp, dragonflies, grasshoppers, true bugs, butterflies, and beetles, among others. For decades, morphological and molecular studies have attempted to reconstruct the evolutionary relationships between the major lineages, but a consensus on pancrustacean phylogeny is still under debate. Much of the uncertainty surrounding the evolutionary history of this ancient group stems from uneven and unrepresentative sampling, conflicting data, and different analytical approaches. First, we will introduce the major lineages of Pancrustacea and discuss the evidence for monophyly within these groups. Next, we will review the pioneering morphological and molecular studies that have contributed to current understanding of higher-level (class/subclass) relationships within Pancrustacea, with an emphasis on Crustacea. Within these sections, we will start with a series of early studies and describe how the development of methods and technology allowed for the advancement of morphological and molecular phylogenetics. A summary of current knowledge of pancrustacean phylogeny will be followed by a brief discussion of new emergent methods in the field that can be applied to future phylogenetic and phylogenomic studies.

INTRODUCTION

Pancrustaceans are a morphologically diverse clade of arthropods with a rich fossil record dating back approximately 514 million years into the Cambrian (Zhang et al. 2007, Wolfe et al. 2016). With the recent inclusion of Hexapoda, the group represents a monophyletic taxon with over 1,000,000 described species, accounting for more than half of the described metazoan species (Brusca

et al. 2016). These include a variety of familiar and unfamiliar forms, including the seed shrimp, fish lice, and tongue worms of Oligostraca; the crabs, lobsters, shrimps, copepods, and barnacles of Multicrustacea; and the insects, remipedes, and cephalocarids of Allotriocarida (Fig. 4.1). Morphological disparity across pancrustaceans is the most astonishing of all invertebrates, with body sizes ranging from the order of micrometers to meters. The ecological and associated physiological diversity is equally impressive, with representatives found from the open ocean to a single isolated anchialine cave, from abyssal oceanic plains to coral reefs, and from terrestrial to aquatic ecosystems. Many species are vital for ecosystem health and survival through their role in pollination, trophic food webs, and community structure, while others play a fundamental role in the economy through commercial fisheries, aquaculture, and the aquarium and terrarium trade. Many pose a threat because they represent invasive species or vectors for disease, while others are targets of significant conservation efforts as they are threatened by anthropogenic encroachment, habitat loss, and climate change. Pancrustaceans are often used as model species for studies in evolution, adaptation, biodiversity, developmental biology, physiology, and ecosystem health and resilience. Work on pancrustaceans has contributed to a critical understanding of fundamental ecological and evolutionary processes and patterns across the planet.

Due to the biological, economic, and ecological importance of Pancrustacea, the need for a resolved phylogeny is pressing. An understanding of evolutionary relationships is the foundation for future studies in ecology, biogeography, diversification, speciation, and conservation biology, among other disciplines. With the inclusion of Hexapoda, monophyly of the Pancrustacea is well established. This is supported by numerous phylogenetic analyses, including nuclear protein-coding genes (Regier et al. 2005, 2010), transcriptomes (Meusemann et al. 2010, von Reumont et al. 2012), morphology (Schram and Koenemann 2004, Strausfeld and Andrew 2011, Legg et al. 2013), and combined morphological and molecular data (Oakley et al. 2013). However, internal relationships among the 90 orders remain only partially resolved and, for the most part, unknown. Only ~45 of the 90 orders within Pancrustacea have been included in past molecular analyses (most studies contain far fewer), thus, ~50% of ordinal diversity has never been sampled. Likewise, the most comprehensive morphological phylogeny to date included 35 orders (753 characters), omitting 60% of ordinal diversity (Legg et al. 2013). Unsurprisingly, many underrepresented groups have been excluded due to the difficulties in collection methods, rarity in nature, or, in the case of molecular work, the inability to obtain high-quality molecular grade material. For these reasons, some of the most functionally diverse and taxonomically challenging groups have remained a phylogenetic mystery with respect to their position in the Tree of Life.

Even in the face of sampling challenges, substantial advancements have been made in pancrustacean phylogenetics. Herein, we focus on the higher-level relationships (class or subclass and above) within Pancrustacea, with an emphasis on the major lineages within Crustacea. First, we briefly introduce the major lineages within Crustacea and succinctly discuss the evidence for the monophyly or polyphyly of these groups. Next, we review the morphological and molecular phylogenetic studies that have contributed to our current understanding of higher-level pancrustacean relationships. We conclude with a synthesis of these findings and the future of phylogenetics and phylogenomics within the group.

INTRODUCTION TO THE MAJOR GROUPS

The pancrustacean clade is comprised of three major groups: Oligostraca (Ostracoda, Branchiura, Pentastomida, and Mystacocarida), Multicrustacea (Malacostraca, Copepoda, Thecostraca, and Tantulocarida), and Allotriocarida (Hexapoda, Branchiopoda, Cephalocarida, and Remipedia) (Oakley et. al 2013). While this clade has also been called Tetraconata (introduced by Dohle
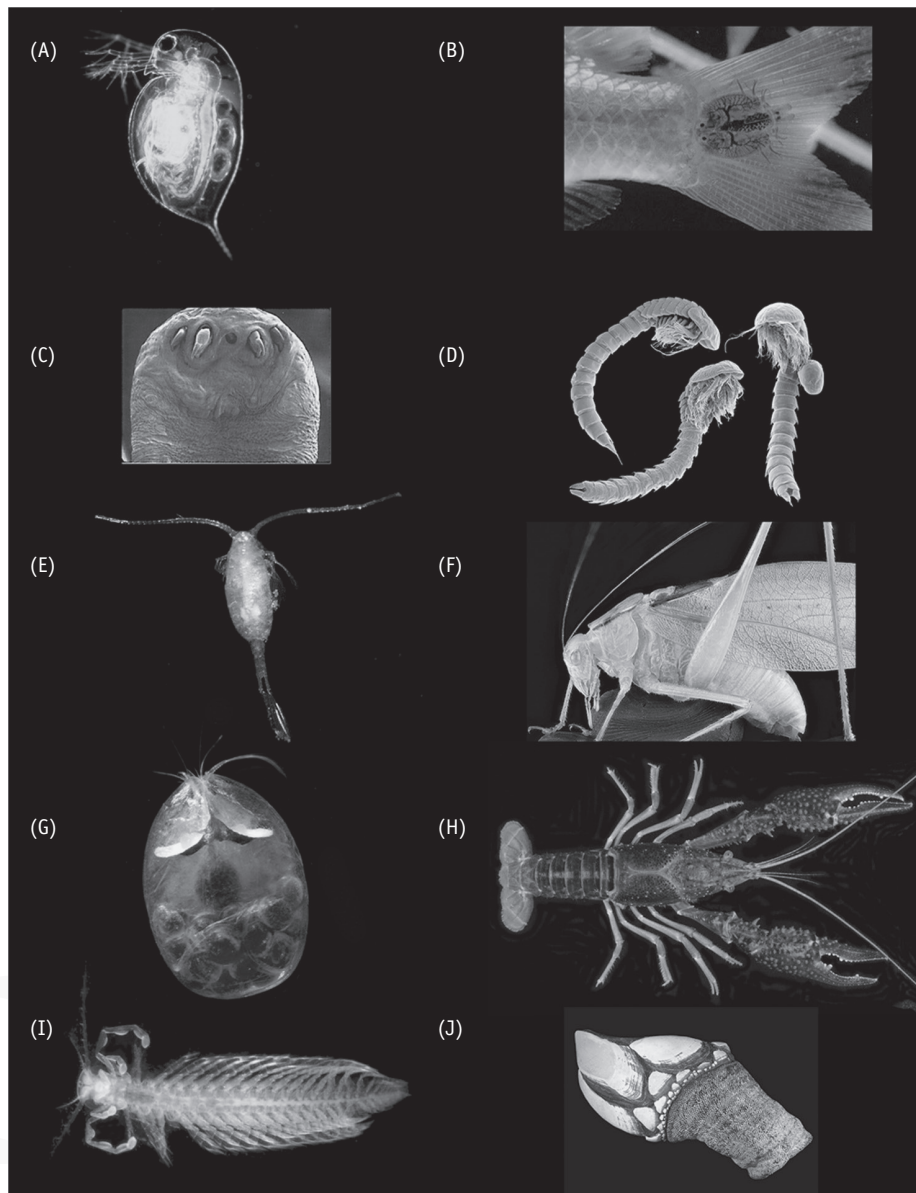
Fig. 4.1.
Examples demonstrating the morphological diversity across Pancrustacea. (A) *Daphnia pulex*, Branchiopoda (from Gewin 2005, under License CC-BY); (B) *Argulus foliaceus*, Branchiura (© G. Boxshall, under License CC-BY-NC-SA); (C) *Porocephalus crotali*, Pentastomida (from Abele et al. 1989, with permission from Oxford University Press); (D) *Lightiella monniotae,* Cephalocarida (© D. Waloszek and J. Olesen); (E) *Ridgewayia* sp., Copepoda (© T. Iliffe); (F) *Amblycorypha oblongifolia*, Hexapoda (from USGS Bee Inventory and Monitoring Lab); (G) *Gigantocypris* sp., Ostracoda (© D. Fenolio/ DEEPEND); (H) *Procambarus clarkii*, Malacostraca (© D. Felder); (I) *Cryptocorynetes* sp., Remipedia (© T. Iliffe); (J) *Pollicipes cornucopia*, Thecostraca (© H. Hillewaert, under License CC-BY-SA). See color version of this figure in centerfold.

2001) based on the shared ommatidia in the compound eyes, the name Pancrustacea (introduced by Zrzavỳ and Stys 1997) will be used herein. Across Pancrustacea there are 2 subphyla, 7 classes, 16 subclasses, and 90 orders, of which 59 orders belong to Crustacea (Table 4.1). Here we will introduce the major lineages (class or subclass) across Pancrustacea and briefly summarize studies that have provided evidence for these groupings.

Branchiopoda

This class of crustaceans includes the fairy/brine shrimp, tadpole shrimp, clam shrimp, and water fleas (Fig. 4.1A). The monophyly of Branchiopoda has been widely established based on phylogenetic analysis of molecules (Koenemann et al. 2010, Regier et al. 2010, von Reumont et al. 2012), morphology (Legg et al. 2013), and combined molecular and morphological data (Oakley et al. 2013). This group contains the only fully annotated non-insect crustacean genome to date, *Daphnia pulex,* as of 2016 (see GIGA Community of Scientists 2014 for list of genomic resources).

Branchiura and Pentastomida

These two subclasses include some of the most infamous parasitic crustaceans. Branchiurans, also known as fish lice, are obligate ectoparasites of fish and amphibians (Fig. 4.1B). They are economically important, as they can decimate fish aquacultures. Pentastomids are a small group of vermiform animals commonly known as tongue worms, living as obligate endoparasites of vertebrate respiratory tracts (though larvae infect intermediate host species; Fig. 4.1C). The unusual morphology of pentastomids (adults are so degenerated that they lack any recognizable arthropod characters) have led to uncertain placement within the metazoan Tree of Life with allies such as Arthropoda, Tardigrada, Annelida, Platyhelminthes, and Nematoda (Riley et al. 1978, de Oliveira Almeida and Christoffersen 1999). However, placement within the arthropods and, more specifically, Pancrustacea, is now widely accepted (see section "Where Are We Now?"). Branchiura include one order, Arguloida, whereas Pentastomida include two orders, Cephalobaenida and Porocephalida.

Cephalocarida

This small group of crustaceans (Fig. 4.1D) was discovered in 1955 in benthic marine sediments, living as part of the interstitial meiofauna. They can be found up to depths of 1,500 m and feed on detrital material within the sediment. Cephalocarids are certainly monophyletic but have been included in few molecular and morphological trees, with uncertain phylogenetic placement.

Copepoda

Copepods are a well-known, diverse group of tiny crustaceans found in nearly all aquatic environments from fresh to hypersaline waters, submerged caves, and terrestrial ponds (Fig. 4.1E). They comprise up to 91% of the ocean's zooplankton biomass (Kosobokova and Hopcraft 2010) and are thus an integral part of the marine food chain. Past studies have recovered copepods as monophyletic (Huys et al. 2007, Oakley et al. 2013), and, historically, they have been divided into 10 orders, namely Calanoida, Cyclopoida, Gelyelloida, Harpacticoida (benthic copepods), Misophrioida, Monstrilloida, Mormonilloida, Platycopioida, Poecilostomatoida, and Siphonostomatoida (fish parasites). However, a very recent study based on molecular data provided an updated position of copepods within Pancrustacea, tested the major subdivisions of Copepoda, and resolved deep internal relationships (Khodami et al. 2017). This paper found 7 of the 10 orders to be monophyletic (Platycopioida, Calanoida, Misophrioida, Monstrilloida, Siphonostomatoida, Gelyelloida, and Mormonilloida), redefined two orders (Cyclopoida and Harpacticoida), and proposed a new

Table 4.1. Current Classification of Extant Pancrustacea[*]

Subphylum Crustacea	Clade	Class	Subclass	Order
	Oligostraca			
			Mystacocarida	
				Mystacocaridida
		Ichthyostraca		
			Branchiura	
				Arguloida
			Pentastomida	
				Cephalobaenida
				Porocephalida
		Ostracoda		
			Myodocopa	
				Halocyprida
				Myodocopida
			Palaeocopa	
			Podocopa	
				Podocopida
			Platycopa	
				Platycopina
	Multicrustacea			
		Hexanauplia		
			Copepoda	
				Calanoida
				Canuelloida
				Cyclopoida
				Gelyelloida
				Harpacticoida
				Misophrioida
				Monstrilloida
				Mormonilloida
				Platycopioida
				Siphonostomatoida
			Tantulocarida	
				Tantulocarida
			Thecostraca	
				Akentrogonida
				Cryptophialida
				Cyprilepadiformes
				Dendrogastrida
				Facetotecta (Infraclass)
				Ibliformes
				Kentrogonida
				Laurida
				Lepadiformes

Table 4.1. *(Continued)*

Subphylum	Clade	Class	Subclass	Order
				Lithoglyptida
				Scalpelliformes
				Sessilia
		Malacostraca		
			Eumalacostraca	
				Amphipoda
				Anaspidacea
				Bathynellacea
				Bochusacea
				Cumacea
				Decapoda
				Euphausiacea
				Isopoda
				Lophogastrida
				Mictacea
				Mysida
				Spelaeogriphacea
				Stygiomysida
				Tanaidacea
				Thermosbaenacea
			Hoplocarida	
				Stomatopoda
			Phyllocarida	
				Leptostraca
	Allotriocarida			
		Branchiopoda		
			Calmanostraca	
				Notostraca
			Diplostraca	
				Anomopoda
				Ctenopoda
				Cyclestherida
				Haplopoda
				Laevicaudata
				Onychopoda
				Spinicaudata
			Sarsostraca	
				Anostraca
		Cephalocarida		
				Brachypoda
		Remipedia		
				Nectiopoda
Hexapoda				~31 orders

*Following Recent Studies and World Register of Marine Species (crustacean taxa) and Oakley et al. (2013).

order, Canuelloida (Table 4.1). Even with these advancements, shallow-level relationships are still in dire need of phylogenetic research.

Hexapoda

Tracing the phylogenetic relationships of insects, with their tremendous diversity, remains a challenge in evolutionary biology (Fig. 4.1F). Currently, Hexapoda is divided into three lineages including the insects (Insecta), coneheads (Protura), and springtails (Collembola; Misof et al. 2014). The monophyly of hexapods is well established (Regier et al. 2010, Sasaki et al. 2013, Dell'Ampio et al. 2014, Misof et al. 2014), and recent phylogenomic methods have been applied to resolve the evolutionary relationships and origins among major lineages (Misof et al. 2014). As with any group with such extensive diversity, familial, generic, and species relationships within the 31 orders continue to be targets for phylogenetic research, and many questions still remain. Extensive discussion on hexapod relationships, origins, and phylogenetic placement within Pancrustacea can be found in Chapter 5.

Ostracoda

This diverse class of aquatic crustaceans is commonly referred to as seed shrimp (Fig. 4.1G). They can be easily recognized due to their bivalved, calcified carapace, with the limbs often fully enclosed. These carapaces are prone to fossilization; hence ostracods have an excellent carapace-only fossil record dating over 478 mya (Wolfe et al. 2016). Ostracod monophyly is supported by phylogenetic analyses using nuclear protein-coding genes, transcriptomes, and/or morphology (Legg et al. 2013, Oakley et al. 2013, Wolfe and Hegna 2014). However, relationships within the two major lineages, Myodocopa and Podocopa, remain unresolved.

Malacostraca

This hyperdiverse class of crustaceans includes approximately 17 orders (with the recent inclusion of Amphionidacea into Decapoda; De Grave et al. 2015) and ~20,000 species. This group contains well-known representatives such as amphipods, isopods, crabs, lobsters, shrimps, stomatopods, as well as less familiar forms (Fig. 4.1H). Malacostraca is a well-supported clade within Pancrustacea, with consistent evidence from analyses of morphology (Wills et al. 1998, Legg et al. 2013), molecules (Regier et al. 2005, 2010, von Reumont et al. 2012), and total evidence (Oakley et al. 2013). Although several nested groups within Malacostraca have also been recovered consistently as monophyletic (i.e., Eumalacostraca, Eucarida, Decapoda), many taxonomic and phylogenetic questions still exist within and across major lineages. For example, peracarids, including isopods, amphipods, and mysids, among others, have been neglected in phylogenetic studies in spite of their tremendous biodiversity. Although some studies have recognized peracarids as a monophyletic group (Richter and Scholtz 2001, Poore 2005, Wills et al. 2009, Wirkner and Richter 2010), lack of sampling has left internal relationships unresolved (Jenner et al. 2009). To date, no higher-level molecular studies are available for peracarids. The monophyly and placement of the Superorder Syncarida within Crustacea remains questionable, with previous studies finding Syncarida as para- or polyphyletic (Camacho 2003, Camacho et al. 2002, Jenner et al. 2009, Wills et al. 2009).

Mystacocarida

The Mystacocarida is a subclass of minute (<1 mm), elongate, and cylindrical crustaceans originally described as a new order by Pennak and Zinn (1943). All extant species (~13) are found living in between sand grains as part of the meiofauna, feeding upon microalgae and bacteria that grow in

these environments. Although inconspicuous, mystacocarids have been included in higher-level molecular and morphological phylogenies.

Remipedia

The remipedes are a fascinating and enigmatic group of blind crustaceans with a global distribution restricted to anchialine caves and oceanic blue holes (Fig. 4.1I). The class is composed of one extant order, Nectiopoda, containing 8 families, 12 genera, and 29 species (Olesen et al. 2017, Hoenemann et al. 2013). Although remipedes may appear morphologically primitive (Yager 1994), they possess a highly specialized nervous system (Stemme et al. 2012, 2013) and feeding mouthparts and are voracious predators (von Reumont et al. 2014). The monophyly of Remipedia has been established based on molecular (Neiber et al. 2011) and robust morphological analyses (Koenemann et al. 2007), although internal relationships remain contentious.

Thecostraca

This charismatic group includes sessile and parasitic barnacles (Fig. 4.1J). Twelve or thirteen (if tanulocarids are included) orders make up the thecostracans, and these include bizarre representatives such as the Facetotecta (y-larvae), with no known adult form, and rhizocephalans that parasitize decapods, causing a series of behavioral, reproductive, and morphological changes in the host. Superficially, adults do not seem to resemble pancrustaceans or even arthropods (though dissection of a barnacle reveals that they use a series of biramous thoracic limbs for feeding). Their affinities were discovered by early 20th-century observation of their larvae, which share many characters with other pancrustaceans. Thecostraca is considered to be monophyletic based on total evidence (Pérez-Losada et al. 2014); however, this hypothesis has not been formally addressed in a robust evolutionary framework. In a recent study the parasitic group Tantulocarida was suggested to be part of Thecostraca based on one ribosomal gene (Petrunina et al. 2013), but more evidence is needed to resolve this issue. Although some studies have been done at the ordinal level (e.g., Sessilia, Pedunculata; Pérez-Losada et al. 2004), many basic phylogenetic and taxonomic questions remain across and within Thecostraca.

THE HISTORY OF PANCRUSTACEAN PHYLOGENETICS WITH AN EMPHASIS ON HIGHER-LEVEL RELATIONSHIPS

The higher-level phylogenetic relationships within Pancrustacea have been fraught with controversy. Several phylogenetic hypotheses, both morphological and molecular, have been proposed with little consensus in sight (Figs. 4.2 and 4.3). For the purpose of this review, we will introduce some of the monumental studies that have contributed to our current understanding of pancrustacean relationships. Due to the high volume of morphological and molecular studies that have emerged over the past few decades, we will not have the space to discuss them all. For this reason, we will limit our discussion to some of the most profound contributions in pancrustacean phylogenetics, with emphasis on subclass and class-level crustacean relationships (for detailed discussion on Hexapoda-Crustacea relationships, see Chapter 5).

Morphological Insights: from Observation to Rigorous Analysis

The earliest hypotheses of crustacean relationships were, of course, based broadly on morphology (Fig. 4.2). Linnaeus classified six genera containing 87 species we now recognize as crustaceans (as
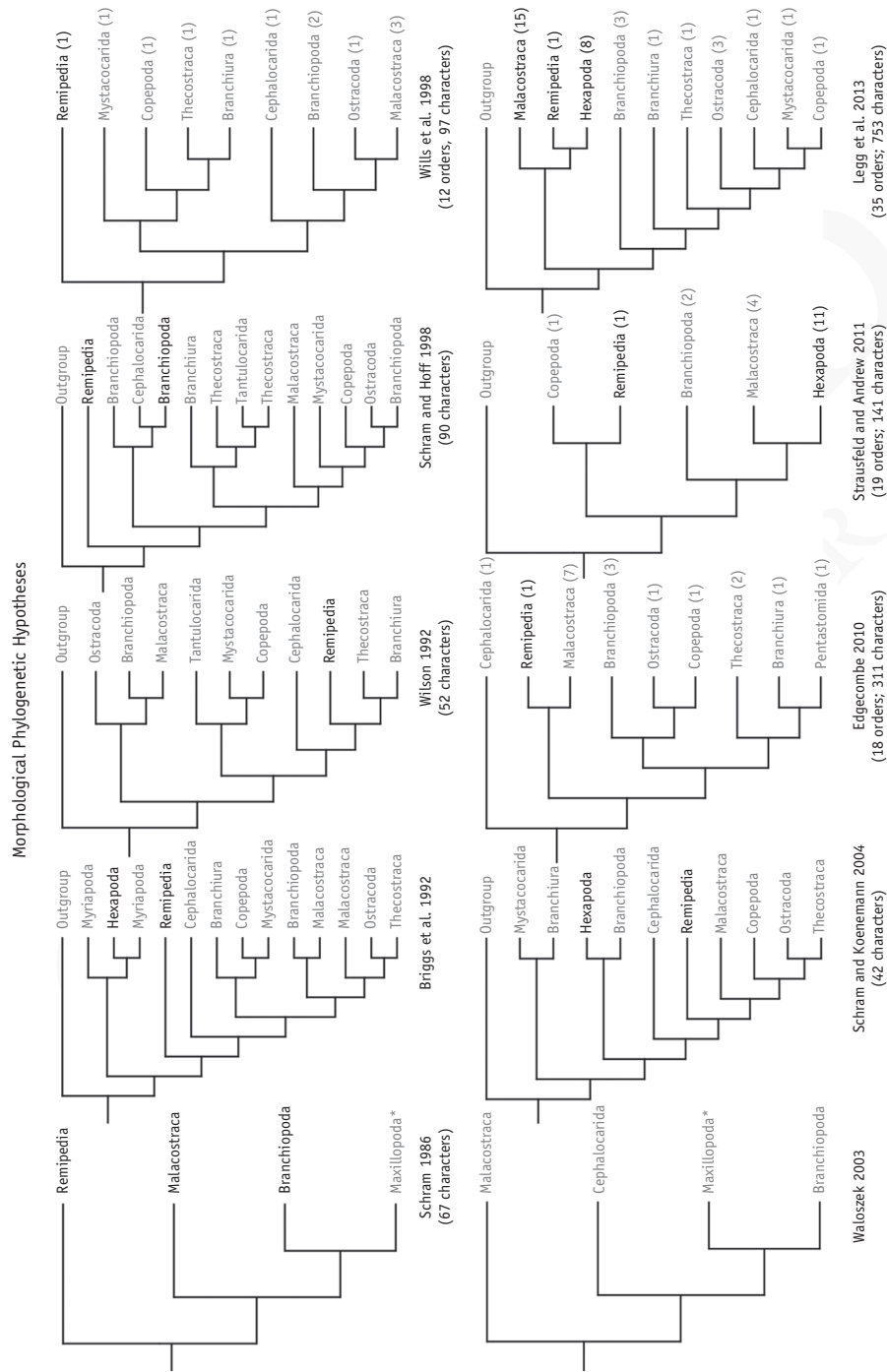
Morphological Phylogenetic Hypotheses

Fig. 4.2.

Alternative hypotheses of the Pancrustacean phylogeny based on morphological data. Numbers adjacent to taxon names in the tree represent how many orders for that taxon were included in the analyses (when available). Caption below citation indicates the total number of orders and characters used in the analyses. Fossil taxa excluded from phylogram for clarity. Class Maxillopoda, denoted by an asterisk, no longer constitutes a valid taxon. However, the source literature did not specify the maxillopodan taxa employed in said analyses. See color version of this figure in centerfold.
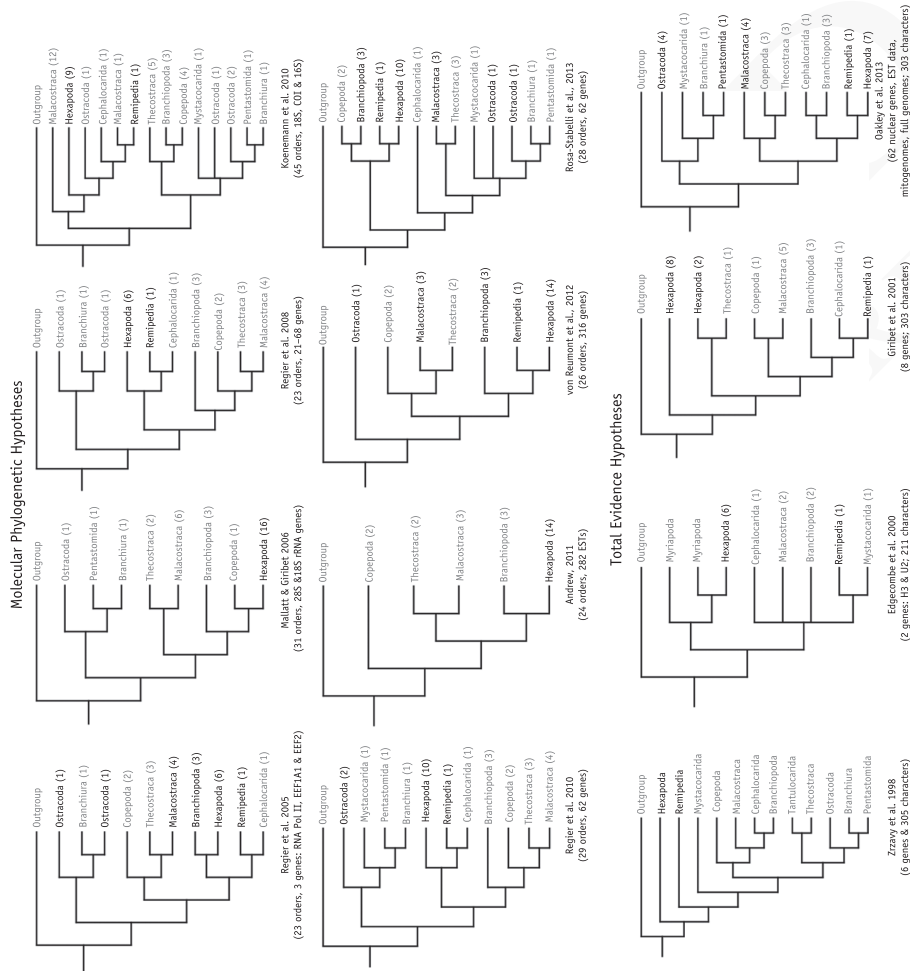
Molecular Phylogenetic Hypotheses

Regier et al. 2005
(23 orders, 3 genes: RNA Pol II, EEF1A1 & EEF2)

Outgroup
Ostracoda (1)
Branchiura (1)
Ostracoda (1)
Copepoda (2)
Thecostraca (3)
Malacostraca (4)
Branchiopoda (3)
Hexapoda (6)
Remipedia (1)
Cephalocarida (1)

Mallatt & Giribet 2006
(31 orders, 28S &18S rRNA genes)

Outgroup
Ostracoda (1)
Pentastomida (1)
Branchiura (1)
Thecostraca (2)
Malacostraca (6)
Branchiopoda (3)
Copepoda (1)
Hexapoda (16)

Regier et al. 2008
(23 orders, 21–68 genes)

Outgroup
Ostracoda (1)
Branchiura (1)
Ostracoda (1)
Hexapoda (6)
Remipedia (1)
Cephalocarida (1)
Branchiopoda (3)
Copepoda (2)
Thecostraca (3)
Malacostraca (4)

Koenemann et al. 2010
(45 orders, 18S, COI & 16S)

Outgroup
Malacostraca (12)
Hexapoda (9)
Ostracoda (1)
Cephalocarida (1)
Malacostraca (1)
Remipedia (1)
Thecostraca (5)
Branchiopoda (3)
Copepoda (4)
Mystacocarida (1)
Ostracoda (2)
Ostracoda (1)
Pentastomida (1)
Branchiura (1)

Regier et al. 2010
(29 orders, 62 genes)

Outgroup
Ostracoda (2)
Mystacocarida (1)
Pentastomida (1)
Branchiura (1)
Hexapoda (10)
Remipedia (1)
Cephalocarida (1)
Branchiopoda (3)
Copepoda (2)
Thecostraca (3)
Malacostraca (4)

Andrew, 2011
(24 orders, 282 ESTs)

Outgroup
Copepoda (2)
Thecostraca (2)
Malacostraca (3)
Branchiopoda (3)
Hexapoda (14)

von Reumont et al., 2012
(26 orders, 316 genes)

Outgroup
Ostracoda (1)
Copepoda (2)
Malacostraca (3)
Thecostraca (2)
Branchiopoda (3)
Remipedia (1)
Hexapoda (14)

Rosa-Stabelli et al. 2013
(28 orders, 62 genes)

Outgroup
Copepoda (2)
Branchiopoda (3)
Remipedia (1)
Hexapoda (10)
Cephalocarida (1)
Malacostraca (3)
Thecostraca (3)
Mystacocarida (1)
Ostracoda (1)
Ostracoda (1)
Branchiura (1)
Pentastomida (1)

Total Evidence Hypotheses

Zrzavy et al. 1998
(6 genes & 305 characters)

Outgroup
Hexapoda
Remipedia
Mystacocarida
Copepoda
Malacostraca
Cephalocarida
Branchiopoda
Tantulocarida
Thecostraca
Ostracoda
Branchiura
Pentastomida

Edgecombe et al. 2000
(2 genes: H3 & U2; 211 characters)

Outgroup
Myriapoda
Myriapoda
Hexapoda (6)
Cephalocarida (1)
Malacostraca (2)
Branchiopoda (2)
Remipedia (1)
Mystacocarida (1)

Giribet et al. 2001
(8 genes; 303 characters)

Outgroup
Hexapoda (8)
Hexapoda (2)
Thecostraca (1)
Copepoda (1)
Malacostraca (5)
Branchiopoda (3)
Cephalocarida (1)
Remipedia (1)

Oakley et al. 2013
(62 nuclear genes, EST data,
mitogenomes, full genomes; 303 characters)

Outgroup
Ostracoda (4)
Mystacocarida (1)
Branchiura (1)
Pentastomida (1)
Malacostraca (4)
Copepoda (3)
Thecostraca (3)
Cephalocarida (1)
Branchiopoda (3)
Remipedia (1)
Hexapoda (7)

Fig. 4-3.

Alternative hypotheses of the Pancrustacean phylogeny based on genetic data (rows 1 and 2) and combined morphological and genetic data (row 3). Numbers adjacent to taxon names in the tree represent how many orders for that taxon were included in the analyses (when available). Caption below citation indicates the total number of orders, genes, and/or characters used in the analyses. Fossil taxa excluded from phylogram for clarity. Abbreviations: RNA Pol II, RNA polymerase II; EEF1A1, elongation factor alpha subunit 1, EEF2, elongation factor 2; 28S, 28S ribosomal mitochondrial gene; 18S, 18S ribosomal mitochondrial gene; COI, cytochrome oxidase I; 16S, 16S ribosomal mitochondrial gene; EST, expressed sequence tags; U2, small nuclear RNA. See color version of this figure in centerfold.

well as a xiphosuran and several molluscs; Boxshall 2007). For over 200 years, hexapod relationships with crustaceans were rarely assessed, as they were assumed to be more closely related to myriapods (Snodgrass 1938, Tiegs and Manton 1958, Manton 1977; also see Chapter 5). Early research on crustacean systematics focused on reconstructing characteristics of the hypothetical crustacean ancestor. For this endeavor, gross morphology of the exoskeleton and differentiation of the limbs and body regions were emphasized, followed by internal anatomy and functional morphology (Manton 1977). Crustaceans were assumed to plesiomorphically share a five-segmented head bearing two pairs of antennae, gnathobasic mandibles, and early development characterized as a nauplius (either free-living larva or egg-nauplius; Tiegs and Manton 1958, Cisne 1982). Walossek's (1999) concept of the crustacean "groundplan" added several possible synapomorphies for the group: the presence of a proximal endite on post-antennular limbs, multi-annulated exopods on post-antennular limbs, and post-antennular limbs with no more than five podomeres on the endopod.

Work in the later 20th century frequently compared crustaceans to trilobites, with an assumption that the newly discovered Cephalocarida were "primitive" (Hessler and Newman 1975, Cisne 1982). The notostracan and anostracan Branchiopoda also received special attention, as they shared with Cephalocarida a long series of undifferentiated thoracic limbs and the flattened, leaf-like limb morphology (Fig. 4.4A). Thus, Branchiopoda and Cephalocarida were united in the literature as a clade called Phyllopoda; however, the same name has been used to refer to other clade compositions, sometimes including Ostracoda, excluding Cephalocarida and Leptostraca, or referring to non-anostracan Branchiopoda; thus the name Phyllopoda is now seldom used (Martin and Christiansen 1995, Martin and Davis 2001). Once discovered, remipedes were also viewed as a model for ancestral crustaceans, on the basis that their uniramous thoracic limbs must be plesiomorphic (e.g., Emerson and Schram 1990). Assumptions of remipede plesiomorphy have colored polarization of morphological characters in several early analyses (see below), where they were by default assumed to be the outgroup to other crustaceans.

Within Crustacea, one of the earliest described taxonomic divisions was that between Malacostraca and Entomostraca. The Entomostraca comprised most other crustaceans (Branchiopoda, Branchiura, Copepoda, Mystacocarida, Ostracoda, Thecostraca, and later Cephalocarida), based on the shared lack of a mandibular palp in the adult, presence of a maxillule with four median endites, and abdomen of at least four limb-less somites (Walossek 1999, Boxshall 2007). In Walossek's studies of developmental sequences, Cephalocarida are viewed as secondarily specialized and unrelated to Branchiopoda (Walossek 1999).

Taxonomy was revolutionized by the paradigm of phylogenetic systematics (Hennig 1966) and swiftly thereafter by the development of software to calculate the most parsimonious or most likely topology. The first cladistic analyses of any crustacean clades used matrices of morphological characters, scored as shared (homologous) between taxa at the familial or ordinal level, and analyzed using maximum parsimony (Tanaidacea: Sieg 1983; Eumalacostraca: Schram 1984). Shortly thereafter, the first comprehensive cladistic analysis of crustacean morphology was conducted by Schram (1986; Fig. 4.2). The morphological matrix comprised 23 extant taxa, coded for 67 characters (Schram 1986). Hexapoda were not included. The most parsimonious topology supported Remipedia as the earliest branching crustaceans, with Malacostraca as sister to a clade of Phyllopoda + Maxillopoda (Schram 1986). Note that in this case, Phyllopoda refers specifically to the clade containing Branchiopoda, Cephalocarida, and Leptostraca, united by the presence of multiramous, leaflike thoracic limbs (Schram 1986). Monophyly of Maxillopoda (Branchiura, Copepoda, Mystacocarida, Ostracoda, and Thecostraca) was supported by the presence of 11 trunk segments, fewer than six thoracic segments, a short bulbous heart, and a naupliar eye (Schram 1986). The clade of Maxillopoda and Phyllopoda was characterized by lack of a mandibular palp in the adult (Fig. 4.4B), a character that has recently been deconstructed on developmental grounds (Fig. 4.4C; Wolfe and Hegna 2014).
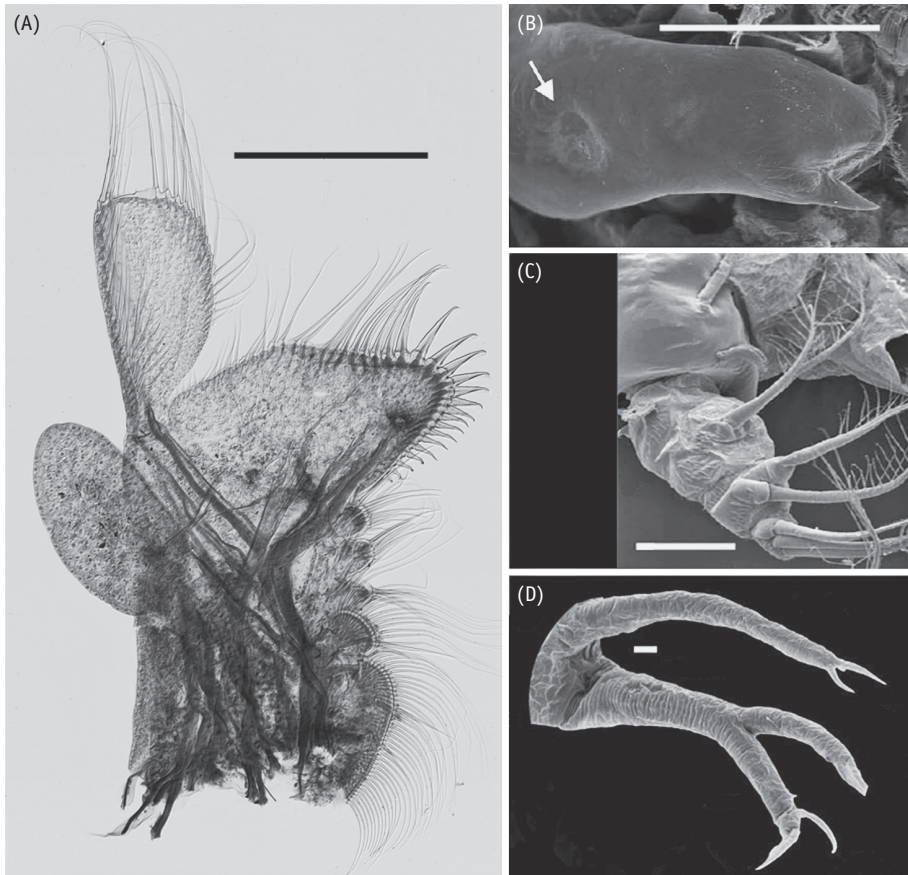
Fig. 4.4.
Morphological characters from limbs and body organization. (A) The phyllopodous eighth trunk limb of an anostracan branchiopod, *Branchinencta brushi*, with large epipodite visibly lacking intrinsic musculature (YPM IZ 024277, photo courtesy of E. Lazo-Wasem). Scale bar = 500 μm. (B) Adult mandible of the anostracan *Branchinectus raptor*, with a scar (arrow) indicating the position where the mandibular palp was lost during ontogeny (modified from Olesen 2009, with permission from Museum für Tierkunde Dresden, Senckenberg Naturhistorische Sammlungen Dresden). Scale bar = 1 mm. (C) Well-developed larval mandibular palp in an anostracan, *Eubranchipus grubii*, a character shared across most crustaceans (modified from Olesen 2009). Scale bar = 50 μm. (D) The deutocerebral antennule and tritocerebral antenna of the remipede *Pleomothra apletocheles* represent a traditional synapomorphy for all non-hexapod crustaceans (modified from Olesen et al. 2014, with permission from Johns Hopkins University Press). The antennule is always uniramous (above, with a distal secondary branch, or flagellum), while many taxa have truly biramous post-antennular limbs (below) (Boxshall 2004). Scale bar = 50 μm.

Exceptionally preserved fossils (such as those from the Cambrian Burgess Shale and Orsten deposits, and the Devonian Rhynie Chert) and their ability to polarize character evolution did not escape notice. In the 1990s, a series of morphological matrices that included fossils were constructed and analyzed. An early analysis (17 fossil and extant taxa coded for 59 characters) divided crustaceans into a clade comprising Branchiopoda + Malacostraca and another clade rendering Maxillopoda as polyphyletic (i.e., containing Cephalocarida and Remipedia; Fig. 4.2; Wilson 1992). The same year, 46 fossil and extant taxa (across all arthropods) coded for 134 characters were

analyzed using principal component analysis and maximum parsimony (Briggs et al. 1992; Fig. 4.2). Remipedia was the earliest branching lineage of crustaceans, with Cephalocarida branching next. Maxillopoda was polyphyletic, with a relationship supported only between Mystacocarida + Branchiura + Copepoda (Briggs et al. 1992). Malacostraca was also polyphyletic, based on inclusion of two representative taxa. Decapoda was sister to Thecostraca + Ostracoda, while Leptostraca was sister to Branchiopoda and several fossils (Briggs et al. 1992). At the time, only one of six most parsimonious trees was presented (Briggs et al. 1992), implying there may have been conflict (homoplasy) unreported in this matrix.

An updated matrix of 64 fossil and extant taxa coded for 90 characters was analyzed under several parameters, removing fossils and different categories of morphological data (Schram and Hof 1998; Fig. 4.2). All results supported Remipedia as the earliest branching lineage within the crown group. In the most complete analysis, Remipedia was sister to Phyllopoda (excluding Leptostraca) + all other crustaceans. Maxillopoda was a paraphyletic grade (separate clades of Branchiura + Thecostraca and Mystacocarida + Copepoda + Ostracoda) with respect to a monophyletic Malacostraca (Schram and Hof 1998). Meanwhile, in the same book, a different matrix of 64 fossil and extant taxa (across all arthropods) coded for 97 characters (Wills et al. 1998) produced dramatically different results (Fig. 4.2). The main similarity was the basal position of Remipedia. Maxillopoda was largely monophyletic (with the exception of Ostracoda; Wills et al. 1998). Phyllopoda was paraphyletic with respect to a monophyletic Malacostraca (Wills et al. 1998). Note that codings in these analyses were still based on "groundplans," i.e., the terminals were not scored based on characters observed from particular species or specimens but from expert knowledge of features common to orders or families.

Total evidence analyses (simultaneously optimizing data from morphological matrices and DNA sequences) also emerged in the 1990s and early 2000s, at the same time as the first crustacean phylogenies incorporating fossils. However, the first total evidence analyses combined morphology and molecular data from extant species only. The initial analysis was presented by Wheeler et al. (1993) but only included two non-hexapod crustacean species. More comprehensive sampling came several years later, sampling 36 extant taxa (across arthropods) for 211 morphological characters and 2 genes, H3 and U2 (Edgecombe et al. 2000; Fig. 4.3). Crustaceans were resolved as monophyletic, with little internal resolution. Interestingly, the presence of a second antenna (Fig. 4.4D) and nauplius were symplesiomorphic, although in this early analysis, morphological signal is strongly observed in the topology (Edgecombe et al. 2000). Cephalocarida or Cephalocarida + Branchiopoda (i.e., partial Phyllocarida) was the most basal clade, with Remipedia + Mystacocarida as sister to monophyletic Malacostraca (Edgecombe et al. 2000).

The next and most seminal total evidence analysis sequenced 8 genes in addition to coding 303 morphological characters for 54 extant taxa across arthropods (Giribet et al. 2001; Fig. 4.3). An important advance was the adoption of species-based morphological coding, where the matrix was constructed of character states relevant to the same species (rather than an assumed groundplan) that were represented by molecular sequences. Although crustacean paraphyly was recovered with respect to hexapods in the optimal topology, a thecostracan barnacle was nested within a clade also containing *Drosophila* and a dipluran (apterygote hexapod). Other analyses exploring this issue found that *Drosophila* had an aberrant gene sequence; thus the significance of this result was restricted to hexapods as the sister group of crustaceans, to the exclusion of myriapods (Giribet et al. 2001). Within non-hexapod crustaceans, Copepoda + Malacostraca comprised the sister group to (Cephalocarida + Remipedia) + Branchiopoda (Giribet et al. 2001). In hindsight, the relationship between Cephalocarida and Remipedia is strikingly similar to an early molecular analysis with extensive taxon sampling, discussed below (Regier et al. 2010).

A new morphological analysis incorporated 31 fossil and extant taxa coded for 42 characters, but based on groundplan codings (Schram and Koenemann 2004; Fig. 4.2). The earliest branching crustaceans were Branchiura + Mystacocarida, resulting in polyphyly of Maxillopoda. Another
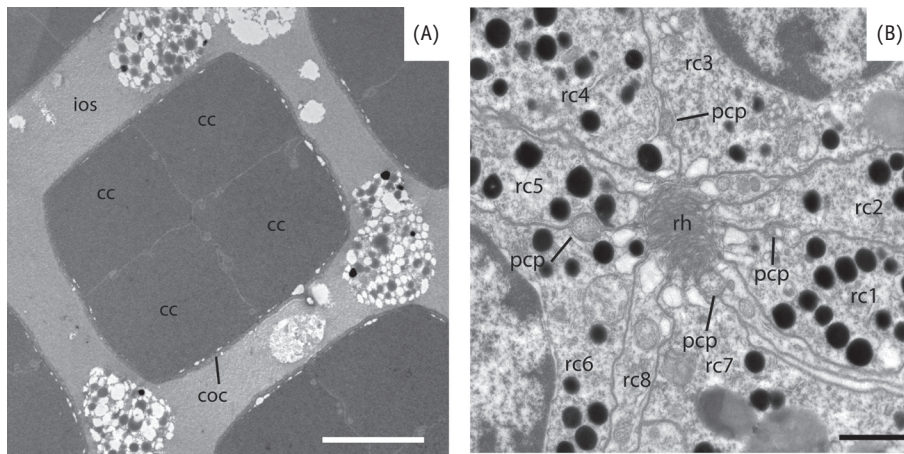
Fig. 4.5.
Morphological characters from the visual system, defining the alternate clade name of Tetraconata (shared by malacostracans and hexapods). (A) Cross section through the square crystalline cone of an ommatidium in the decapod *Palaemon elegans*, clearly showing the tetracone wrapped in a sleeve of the two corneagenous cells. Scale bar = 5 μm. (B) Cross section of the distal part of the retinula of the decapod *Albunea carabus*, containing eight retinula cells surrounding the rhabdom in the center. The irregular, unpigmented eighth retinula cell is a tetralobed apparatus in the interspace of the seven regularly pigmented retinula cells. Between the retinula cells are four proximal processes of the cone cells. Scale bar = 1 μm. Abbreviations: cc, crystalline cone cell or Semper cell; coc, corneagenous cell (circumconical sheath); ios, interommatidial space; pcp, proximal process of cone cell; rc, retinula cell; rh, rhabdom. Photos courtesy of C. Müller.

supported clade comprised Cephalocarida, Remipedia, monophyletic Malacostraca, and the remainder of Maxillopoda (Schram and Koenemann 2004). This paper was notable as it was the first significant morphological cladistic result depicting crustacean paraphyly with respect to Hexapoda (i.e., with Branchiopoda as the hexapod sister group); however, it did not present a substantial discussion of character support apart from some possible similarities from the then-emerging field of developmental genetics (Schram and Koenemann 2004).

Meanwhile, many conserved morphological characters supported crustacean paraphyly with respect to Hexapoda but had been overlooked by systematists. Shared architecture of the nervous system was indeed recognized since the late 19th century, without direct phylogenetic context (reviewed by Strausfeld 2012). The presence of four crystalline cone cells in the eye (Fig. 4.5) is eponymous for the 'Tetraconata' (Dohle 2001, Richter 2002). Neural morphology supported a sister relationship not only between crustaceans and hexapods but also between specific crustacean groups (i.e., paraphyly of crustaceans). Neurophylogeny was then developed, which identified morphological characters from the nervous system to argue in support of a sister relationship between Malacostraca and Hexapoda (Harzsch 2006). These comparisons, however, were merely mapped onto an assumed tree topology. Meanwhile, similar results were also discovered in a series of neural cladistic analyses, where neural characters were used as the basis of a morphological matrix (the most recent of which is Strausfeld and Andrew 2011; Fig. 4.2). This matrix included 42 extant taxa (across arthropods) coded for 141 characters only from the nervous system. Only one species each of Maxillopoda and Remipedia were included; they formed the most basal clade within paraphyletic Pancrustacea (Strausfeld and Andrew 2011). Branchiopoda was sister to the clade of Malacostraca + Hexapoda, a result that suggests the secondary simplification of the branchiopod brain (Strausfeld

and Andrew 2011, Strausfeld 2012). Neural characters continue to increase in importance, and data are even available from select fossils (Ma et al. 2015).

Seminal studies by Edgecombe (2010) and Legg et al. (2013) demonstrated congruence between molecular and morphological phylogenies of arthropods when fossils are included in the analysis (Fig. 4.2). Each of these papers built paired morphological phylogenies of their given data matrix, with and without fossils. The inclusion of 391 characters coded for 16 fossil and 68 extant taxa added fossil character combinations that conflict with Pentastomida falling outside Euarthropoda, instead supporting their grouping within the Crustacea (Zrzavỳ et al. 1998, Edgecombe 2010). A much more extensive analysis of 753 morphological characters for 215 fossil and 96 extant arthropod taxa further increased congruence between molecular and morphological phylogenies (Legg et al. 2013). The most significant result was recovery of paraphyletic Crustacea with respect to Hexapoda, found only in analyses including fossils. Analyses excluding fossils produced monophyletic sister groups of Crustacea and Hexapoda, likely driven by convergence in terrestrial characters between hexapods and myriapods (Legg et al. 2013). Futhermore, the extant sister group of Hexapoda was Remipedia in the fossil analysis, a relationship first proposed from transcriptome data (von Reumont et al. 2012, Legg et al. 2013, Oakley et al. 2013). Note, however, that with or without fossils, this matrix recovered monophyletic Maxillopoda, including Ostracoda (Legg et al. 2013).

Oakley et al. (2013) conducted the most extensive total evidence analysis of pancrustacean phylogeny, with a morphological data partition coding 16 fossil and 93 extant taxa for 183 characters. A massive molecular partition was added, with 1,001 genes represented, the results of which are discussed below (see section "Molecular Insights"). Briefly, monophyly of Ostracoda was supported within Oligostraca (including support for Pentastomida with Branchiura, i.e., Ichthyostraca). There was weak support for a new clade, Hexanauplia, comprising Copepoda and Thecostraca (also supported by larva-based analysis by Wolfe and Hegna 2014). Remipedia was again supported as the sister group of Hexapoda (Oakley et al. 2013). Simultaneous analysis of fossil and extant morphology with extant molecular data promises to improve divergence time estimates by adding uncertainty in fossil ages directly to the model ("tip dating"; e.g., O'Reilly et al. 2015), an approach that has not yet been applied to non-hexapod pancrustaceans.

Most recent morphological studies continue to support crustacean paraphyly, recently showing Remipedia as the sister group to Hexapoda, congruent with phylogenomic work (von Reumont et al. 2012, Legg et al. 2013, Oakley et al. 2013). Mounting morphological evidence for crustacean paraphyly casts doubt on the previously designated synapomorphies of Crustacea (none of which have truly persisted in the modern molecular era). Sperm morphology and subsequent phylogenies support a position for Pentastomida within crustaceans, usually grouped with Branchiura as the clade Ichthyostraca (Zrzavỳ et al. 1998, Edgecombe 2010, Oakley et al. 2013). There is potential support for the Hexanauplia clade (Oakley et al. 2013), but widespread support for Maxillopoda still persists in morphology-only studies and is a significant challenge to reconcile with molecular data (see section "Molecular Insights"). None of the higher-level clades continually found in recent molecular analyses (Oligostraca, Multicrustacea, Allotriocarida) have ever been supported by morphology alone, driving the search for new morphological characters and fossil data to make sense of possible character transformations upon robust molecular branches.

Molecular Insights: from Single Gene Trees to the Genomics Revolution

The monophyly of Crustacea has long been questioned from both a morphological and a molecular perspective. Few apomorphies have been proposed to unite the group (Lauterbach 1983, Richter 2002, Muller et al. 2003), and all recent molecular studies show hexapods nested within paraphyletic Crustacea (see below). Although this relationship is now largely accepted, the internal relationships including the

sister group to Hexapoda and internal relationships among Crustacea are still widely debated. Below, we will review some of the pioneering studies that investigated pancrustacean relationships using molecular data over the past two decades. It is important to note that several monumental phylogenetic studies that focus on hexapod-crustacean relationships or arthropod phylogeny have been omitted due to limited sampling across Pancrustacea (see Chapter 5; see also section "Morphological Insights").

Prior to the 21st century, molecular investigations of crustacean phylogeny included limited taxon sampling and markers. Some of the earliest studies to investigate crustacean relationships used 18S, a nuclear small-subunit ribosomal DNA gene, to generate single gene trees (Abele et al. 1989, Abele et al. 1992, Spears and Abele 1999). One of these studies included 10 crustacean species and 3 other arthropod outgroups (Spears and Abele 1999). Findings of this study rejected the hypothesis that crustaceans possessing foliaceous limbs (branchiopods, cephalocarids, phyllocarids) form a monophyletic clade. They recovered three major groupings, some of which are still supported today (i.e., Malacostraca), but scarce sampling of taxa and markers did not allow for robust testing of Pancrustacea relationships (for a discussion on the use of single markers in phylogenetic reconstruction, see Timm and Bracken-Grissom 2015).

In the early 2000s, the ability to generate larger amounts of sequence data allowed for the investigation of evolutionary relationships with more robust datasets. One outstanding question in the early 21st century was the uncertain phylogenetic position of Pentastomida in relation to other arthropod groups. Using complete mtDNA sequences and gene arrangements, Lavrov et al. (2004) concluded that pentastomids are crustaceans with a close affinity to branchiurans. Although this work was done over a decade ago, the monophyly of Branchiura + Pentastomida is still commonly accepted. This relationship is supported based on sperm morphology (Wingstrand 1972, Storch and Jamieson 1992, Giribet and Ribera 2000), morphology + protein coding genes (Zrzavý et al. 1998), transcriptomes (Oakley et al. 2013), and molecules only (Møller et al. 2008, Regier et al. 2010).

Around the same time, researchers began to substantially increase taxon sampling to investigate phylogenetic relationship across a much broader representation of crustaceans. Although molecular studies had provided support for Pancrustacea, the internal relationships within Crustacea were contentious. In addition to increased sampling, the use of nuclear protein-coding genes allowed Regier et al. (2005) to test the monophyly of Crustacea across 62 arthropod lineages and 2 outgroups. Using three nuclear markers, i.e., elongation factor-1α, RNA polymerase II, and elongation factor-2, Regier et al. (2005) reconfirmed previous studies that united hexapods and crustaceans, but with uncertain interrelationships (Fig. 4.3). In addition to recovering a pancrustacean clade, Regier et al. (2005) recovered four major groups within Crustacea. First, Branchiopoda were recovered as a monophyletic group sister to Hexapoda, but with low support. Second, Cephalocarida + Remipedia were recovered as a monophyletic group. Third was the recovery of Malacostraca + Cirripedia (Thecostraca includes Cirripedia) + Copepoda, with Malacostraca + Cirripedia strongly supported across all analyses. The final major grouping was Ostracoda + Branchiura, abeit with low bootstrap support values. Findings from this study rendered the traditional Maxillopoda (Thecostraca, Copepoda, Mystacocarida, Branchiura, Ostracoda) para- or polyphyletic. The non-monophyly of Maxillopoda had long been suspected based on rampant morphological variation across the group (Martin and Davis 2001) and is supported by subsequent phylogenetic analyses (Mallatt and Giribet 2006, Regier et al. 2008, Regier et al. 2010). Other deep relationships were recovered with uncertain or conflicting support (i.e., high posterior probabilities but low bootstrap).

The following year, Mallatt and Giribet (2006) used complete ribosomal RNA sequences, 28S and 18S, to investigate the relationships across Ecdysozoa (i.e., arthropods, nematodes, tardigrades, onychophorans, nematomorphs, priapulans, kinorhynchs, and loriciferans; Fig. 4.3). They included 20 crustaceans and 25 hexapods for a total of 45 pancrustaceans and included

newly generated and published sequences. Many overarching findings concurred with those of Regier et al. (2005) but differed slightly due to the taxa and molecular markers included in the study. Branchiura + Pentastomida were recovered with high support. Ostracoda were included as sister to this clade, but with no support. Similar to Regier et al. (2005), Malacostraca and Cirripedia formed a monophyletic group, however the current study found Copepoda to be sister to Hexapoda. This sister relationship between copepods and hexapods was later found to be an analytical artifact based on a sensitivity analysis (Mallatt and Giribet 2006). As in Regier at al. (2005), Branchiopoda were ultimately deemed to be the sister group to Hexapoda, and Maxillopoda were recovered as non-monophyletic. Several deeper relationships were left unanswered, and the authors noted that the phylogenetic positions of ostracods and copepods were extremely unstable.

A non-trivial issue to consider in any morphological or molecular study is the selection of analysis parameters in phylogenetic reconstruction. An important study by Koenemann et al. (2010) examined the conflict in arthropod phylogenetic relationships under different analysis parameters. This study was based on two mitochondrial markers, 16S rDNA and COI, and the nuclear ribosomal marker, 18S. In conclusion, several different phylogenetic reconstructions were recovered, highlighting the confounding effects of secondary structure on tree building. The major findings from this study recovered several monophyletic groups within Pancrustacea, namely Malacostraca and Insecta. As seen in previous studies (Regier et al. 2005, Mallatt and Giribet 2006) Maxillopoda was recovered as paraphyletic (Fig. 4.3). More surprisingly, Hexapoda emerged as a paraphyletic group. Resolving arthropod relationships was not a primary goal of this research, rather this study brought into light the careful consideration that needs to be given to alignment methods, nucleotide coding, and model selection during phylogenetic reconstruction (also see Timm and Bracken-Grissom 2015).

For the past decade, most molecular phylogenies relied on a limited number of nuclear genes or mitochondrial sequences to infer pancrustacean relationships. However, in two consecutive monumental studies, Regier et al. (2008, 2010) investigated arthropod relationships again based on the largest dataset to date: 62 single-copy nuclear protein-coding genes representing over 41kb of sequence data. Here, we will focus on the later study, Regier et al. (2010), which included greater taxon sampling across Pancrustacea (see Fig. 4.3). In this study, Regier et al. included 75 arthropod species and 5 outgroups, with 25 and 19 species represented by crustaceans and hexapods, respectively. For the first time, Remipedia were included in a molecular phylogeny. This resulted in a monophyletic clade including Remepedia + Cephalocarida (=Xenocarida, as seen in previous studies; Giribet et al. 2001) and sister to Hexapoda (=Miracrustacea). As seen in previous studies, Thecostraca + Malacostraca + Copepoda were recovered with high bootstrap support and herein referred to as Multicrustacea. Branchiopoda were recovered as sister to Multicrustacea, a relationship that had not been recovered in previous studies (Regier et al. 2005, Mallatt and Giribet 2006). Pentastomida and Branchiura (=Ichthyostraca) formed a strongly supported clade, as seen in other molecular phylogenies that included both groups (Lavrov 2004, Mallatt and Giribet 2006). For the first time, Mystacocarida were included in a comprehensive molecular analysis and showed an affinity to Ichthyostraca, but with no support. Ostracoda formed a statistically significant clade with Ichthyostraca + Mystacocarida (=Oligostraca). Importantly, this was the first phylogenomic study that recovered high support for several deep relationships across Pancrustacea and supported the notion that increased taxon and gene sampling was needed to improve resolution across the arthropod Tree of Life.

A year later, Andrew (2011) used expressed sequence tags (ESTs) mined from GenBank to infer insect–crustacean relationships. ESTs are short fragments of mRNA sequenced from cDNA libraries that can be used in phylogenetic inference. This phylogenomic approach allowed Andrew (2011) to identify ~282 orthologs (=67,726 amino acids) across 156 taxa (23 species of Crustacea and 63 of Hexapoda for a total of 86 pancrustaceans). The major finding from this study confirmed the

Tetraconata (=Pancrustacea) hypothesis with strong support (Fig. 4.3). As seen in other studies (Regier et al. 2005, 2008, 2010, Mallatt and Giribet 2006) Malacostraca were recovered as a monophyletic group; however, many orders within Malacostraca were excluded in the tree (only Decapoda, Amphipoda, and Euphausicea included). Thecostraca (represented as Cirripedia) were recovered as sister to Malacostraca, and Copepoda were recovered as sister to Thecostraca + Malacostraca (=Multicrustacea). With the recovery of Multicrustacea, Maxillopoda were found to be paraphyletic with respect to Malacostraca. Findings from the EST datasets were in accordance with overarching phylogenetic patterns recovered with protein-coding (Regier et al. 2005, 2008, 2010), ribosomal and/or mitochondrial DNA datasets (Mallatt and Giribet 2006, Koenemann et al. 2010); however, the lack of sampling by Andrew (2011) prohibited a comprehensive evaluation of pancrustacean phylogeny.

Several consistent, yet general patterns of Pancrustacea phylogeny were emerging. However, the limited sampling across the group hindered robust testing of fundamental questions such as "What is the sister group to Hexapoda?" A group of researchers set out to answer this question using EST data in a series of analyses to evaluate pancrustacean relationships (von Reumont et al. 2012; Fig. 4.3). Although the number of pancrustacean species differed across analyses, Ostracoda, Malacostraca, Cirripedia, Copepoda, Branchiopoda, Remipedia, and Hexapoda were included in all. Von Reumont et al. (2012) consistently found Remipedia to be the sister to Hexapoda, similar to that of Regier et al. (2010), which found Remipedia-Cephalocarida (=Xenocarida) as sister to Hexapoda. These findings challenged previous thoughts that suspected remipedes were an early-branching lineage on the crustacean Tree of Life (Schram 1986); however, in recent years, mounting evidence based on morphological, developmental, molecular, and neuroanatomical data has suggested a more derived placement (see section "Remipedia"). Other findings included the monophyly of Pancrustacea, Malacostraca, Multicrustacea, and Branchiopoda. As seen in most other studies, the phylogenetic relationships of deep lineages remain uncertain.

Further studies continued to test the effects of analytical approaches (Rota-Stabelli et al. 2013), similar to those performed a few years earlier (Koenemann et al. 2010). In a set of varied data analyses and parameters, Rota-Stabelli et al. (2013) reused the Regier et al. (2010) dataset to investigate the effects of nucleotide or amino acid alignments in deep phylogenomic reconstruction. More specifically, they performed a series of analyses to test if the same relationships would emerge using different models and different starting alignments (nucleotides vs. amino acids). Rota-Stabelli et al. (2013) found that the two different alignments resulted in strikingly different pancrustacean relationships (Fig. 4.3). The authors concluded that the nucleotide dataset that recovered a Branchiopoda + Malacostraca + Thecostraca + Copepoda clade was inaccurate based on codon (serine) biases in the alignment. Alternatively, the amino acid dataset recovered a Branchiopoda + Copepoda + Remipedia + Hexapoda clade, and sometimes (depending on the model used) Cephalocarida. They also recovered Malacostraca + Oligostraca + Thecostraca. The authors argued that the relationships recovered using amino acids resulted in relationships more similar to EST datasets (Meusemann et al. 2010, von Reumont et al. 2012) and that taxa with similar codon composition tended to group together artificially, implying that amino acid–based studies may be more robust.

The same year, Oakley et al. (2013) investigated the phylogenetic position of Ostracoda, a group often neglected in many higher-level phylogenetic studies. In addition to studying the position of ostracods across the pancrustacean Tree of Life, this study sought to answer several outstanding questions concerning higher-level relationships across Pancrustacea. Using a robust total evidence dataset comprising morphology, EST, mitochondrial genome, nuclear genome, and rDNA data, Oakley et al. (2013) found support for several pancrustacean clades. This study found Ostracoda to be a monophyletic group (but see Oakley et al. 2013 for a detailed discussion) closely related to a clade comprising Mystacocarida, Branchiura, and Pentastomida (= Oligostraca). Oligostraca were recovered to be the sister group to the remaining Pancrustacea. As

seen in several other studies, Multicrustacea were recovered with high support (Regier et al. 2010); however, internal relationships between Malacostraca, Cirripedia, and Copepoda remained debatable. As recovered by von Reumont et al. (2012), Remipedia were found as sister to Hexapoda, albeit with low support. A new clade, Allotriocarida, was recovered that included Cephalocarida, Branchiopoda, Remipedia, and Hexapoda, which contradicted the findings of Regier et al. (2010; i.e., with respect to position of Branchiopoda). It was noted that the recovery of Allotriocarida was most probably attributed to the data selection (proteomic and transcriptomic datasets). It is also important to note that data from the remipede and cephalocarid were recycled from the Regier et al. (2010) dataset, possibly perpetuating relationships recovered from previous studies (for a discussion on data recycling, see Timm and Bracken-Grissom 2015). Even so, this was the most comprehensive molecular dataset to date, recovering several well-supported clades with Pancrustacea.

WHERE ARE WE NOW? A SYNTHESIS OF HIGHER-LEVEL SUPPORT AND OUTSTANDING QUESTIONS

Although several outstanding questions still remain across Pancrustacea phylogeny, many higher-level relationships are consistently recovered in molecular studies. The three major clades that include all pancrustacean lineages include Multicrustacea, Oligostraca, and, based on recent evidence, Allotriocarida. It is important to note that none of these higher-level clades (Oligostraca, Multicrustacea, Allotriocarida) are supported by morphology alone; however, all recent total evidence approaches concur with the relationships discussed below.

Multicrustacea are consistently recovered as a monophyletic group with high support and include Malacostraca, Copepoda, and Thecostraca. Monophyly was first demonstrated by nuclear protein-coding genes (Regier et al. 2010) and later supported by EST sequences (von Reumont et al. 2012) and combined analysis of molecular and morphological data (Lee et al. 2013, Oakley et al. 2013). This clade has, however, not been recovered in one recent molecular-only study (Rota-Stabelli et al. 2013) nor any morphology-only phylogenetic analyses, presumably owing to widespread support for Malacostraca as sister to the rest of Pancrustacea (the Entomostraca hypothesis; see Walossek 1999, and Wolfe and Hegna 2014 for a morphological deconstruction of Entomostraca). The internal relationships of Multicrustacea are less well resolved. Although Malacostraca continue to be seen as monophyletic, many rare or understudied groups (e.g., Anaspidacea, Bochusacea, Mictacea, Spelaeogriphacea) have not yet been available for molecular phylogenetic analyses. Additionally, the phylogenetic position of Malacostraca relative to Copepoda and Thecostraca is unresolved, with competing hypotheses suggesting (Malacostraca, (Copepoda+Thecostraca)) (Oakley et al. 2013) or ((Malacostraca + Thecostraca), Copepoda) (Regier et al. 2010, von Reumont et al. 2012).

The superclass Oligostraca also continues to be supported in many molecular phylogenetic studies. The clade, composed of Mystacocarida, Branchiura, Pentastomida, and Ostracoda, has been recovered with nuclear protein coding genes (Regier et al. 2010, Zwick et al. 2012) and total evidence approaches that combine morphology and molecules (Zrzavý et al. 1998, Lee et al. 2013, Oakley et al. 2013). Some internal relationships are well accepted, while others need much attention. Currently, the monophyly of Branchiura + Pentastomida (=Ichthyostraca) is commonly accepted. This relationship is supported by sperm morphology (Wingstrand 1972, Storch and Jamieson 1992, Giribet and Ribera 2000). Additional lines of evidence based on total evidence (Zrzavý et al. 1998, Oakley et al. 2013) and molecules only (Møller et al. 2008, Regier et al. 2010) also support this group. Due to recent total evidence approaches (Oakley et al. 2013), the Ostracoda are monophyletic and fall as a sister clade to Ichthyostraca + Mystacocarida.

Increased taxon sampling from across ostracods and mystacocarids would help test these findings.

The last and perhaps most contentious clade, Allotriocarida, includes Branchiopoda, Cephalocarida, Remipedia, and Hexapoda. Monophyly of this clade was proposed by a combined phylogenetic analysis of transcriptomes, nuclear protein-coding genes, and morphology (Oakley et al. 2013). Internal relationships are in complete flux. The sister lineage to Hexapoda is hotly debated and has been argued to be Remipedia (von Reumont et al. 2012, Oakley et al. 2013), Remipedia + Cephalocarida (=Xenocarida; Regier et al. 2010), or possibly Branchiopoda (Regier et al. 2005, Mallatt and Giribet 2006). Future phylogenetic studies need to target lineages within this group to resolve these important outstanding questions.

Finally, there is growing evidence for Altocrustacea, a group that unites Multicrustacea and Allotriocarida. Monophyly has been supported by phylogenetic analysis of nuclear protein-coding genes (Regier et al. 2010, Zwick et al. 2012), EST data (von Reumont et al. 2012), and combined analysis of morphology and nuclear protein-coding genes (Lee et al. 2013) or morphology and transcriptomes (Oakley et al. 2013). However, this clade has been challenged as paraphyletic (containing Oligostraca) by Rota-Stabelli et al. (2013) and has not been supported by morphological data alone.

THE FUTURE OF PANCRUSTACEAN PHYLOGENY AND CONCLUDING REMARKS

The field of phylogenetics has undergone an exciting revolution in recent years, and emergent methods have the ability to transform morphological and molecular pancrustacean phylogenetics. New technologies for morphological description, such as micro-computed and synchrotron radiation X-ray tomography, autofluorescence imaging, whitening, immunolabeling, spatiotemporal gene expression, and geochemical and taphonomic analysis of fossils, continue to reveal insights into new characters (e.g., Hegna 2010, Haug et al. 2011, Eriksson et al. 2015, Ma et al. 2015, Stegner et al. 2015). These techniques are critical for expanding morphological sampling to taxa that are only available as fixed museum specimens (i.e., difficult-to-collect material which may be unavailable for nucleic acid extraction) and fossils. Besides morphological methods, recent advancements in sequencing techniques have yet to be applied widely across pancrustaceans. Such advancements include anchored hybrid enrichment (AHE) methods for high-throughput sequencing (Lemmon et al. 2012, Lemmon and Lemmon 2013). AHE is capable of targeting hundreds of loci, allowing for resolution from deep to shallow phylogenetic scales (Lemmon et al. 2012, Lemmon and Lemmon 2012). The starting material for this method is genomic DNA, allowing the use of ethanol-preserved specimens. This approach can therefore leverage the thousands of ethanol-preserved tissues housed in museum collections, eliminating challenges associated with tissue collection, preservation, and handling of RNA-preserved or "fresh" specimens. This presents an advantage over alternative "phylotranscriptomic" approaches. A recent study has demonstrated the potential of AHE methods, successfully recovering ~410 loci and ~86 kb (kilobases) of genomic data across the order Decapoda (Wolfe et al. 2019). With heterogeneous sequencing methods and even genome sizes, 100% coverage of all genes for all species may be intractable; optimizing sampling in computer analyses for phylogenetically informative genes may prove fruitful (Fernández et al. 2016). As new morphological and molecular characters are added to taxon sampling, pancrustacean systematists must be mindful of other developments in large-scale phylogenetic analysis, such as methods to mitigate conflict where the topologies of individual gene trees differ from the summary "species tree" when many genes are included (Edwards 2009).

The future of pancrustacean phylogeny is promising, and many lessons have been learned from a conflicted past. It is imperative that concerted efforts be made to include rare and understudied groups to validate or refute assumptions of monophyly within and among major pancrustacean lineages. Increased taxon sampling in combination with new molecular and morphological characters need to be incorporated into future studies to increase resolution at deep splits and avoid the perpetuation of artifacts being included in the same data across multiple studies (i.e., taxon recycling; see Timm and Bracken-Grissom 2015). As learned from previous studies (Koenemann et al. 2010, Rota-Stabelli et al. 2013), future datasets must undergo a series of robust analyses with different parameters to account for potential biases in models, alignments (nucleotides vs. amino acids), and data (i.e., codon usage, gene tree conflict). Emergent technologies in morphological and molecular phylogenetics have the ability to overcome many of these obstacles. Past studies have contributed greatly to our understanding of pancrustacean relationships, and future endeavors must build upon this knowledge to resolve the pancrustacean Tree of Life.

REFERENCES

Abele, L.G., W. Kim, and B.E. Felgenhauer. 1989. Molecular evidence for inclusion of the phylum Pentastomida in the Crustacea. Molecular Biology and Evolution 6:685–691.

Abele, L.G., T. Spears, W. Kim, and M. Applegate. 1992. Phylogeny of selected maxillopodan and other crustacean taxa based on 18S ribosomal nucleotide sequences: a preliminary analysis. Acta Zoologica 73:373–382.

Andrew, D.R. 2011. A new view of insect-crustacean relationships. II. Inferences from expressed sequence tags and comparisons with neural cladistics. Arthropod Structure and Development 40:289–302.

Boxshall, G.A. 2004. The evolution of arthropod limbs. Biological Reviews 79:253–300.

Boxshall, G.A. 2007. Crustacean classification: on-going controversies and unresolved problems. Zootaxa 1668:313–325.

Briggs, D.E., R.A. Fortey, and M.A. Wills. 1992. Morphological disparity in the Cambrian. Science 256:1670–1673.

Brusca, R., W. Moore, and S. Shuster. 2016. Invertebrates. Sinauer Associates, Sunderland, MA.

Camacho, A.I. 2003. Historical biogeography of Hexabathynella, a cosmopolitan genus of groundwater Syncarida (Crustacea, Bathynellacea, Parabathynellidae). Biological Journal of the Linnean Society 78:457–466.

Camacho, A.I., I. Rey, B.A. Dorda, A. Machordom, and A.G. Valdecasas. 2002. A note on the systematic position of the Bathynellacea (Crustacea, Malacostraca) using molecular evidence. Contributions to Zoology 71:123–130.

Cisne, J.L. 1982. Origin of the Crustacea. Pages 65–93 in L.G. Abele, editor. The Biology of Crustacea. Vol 1. Systematics, the Fossil Record, and Biogeography. Academic Press, New York.

De Grave, S., T.Y. Chan, K.H. Chu, and J.M. Landeira. 2015. Phylogenetics reveals the crustacean order Amphionidacea to be larval shrimps (Decapoda: Caridea). Scientific Reports 5:17464.

De Oliveira, W., and M.L. Christoffersen. 1999. A cladistic approach to relationships in Pentastomida. Journal of Parasitology 85:695–704.

Dell'Ampio, E., K. Meusemann, N.U. Szucsich, R.S. Peters, B. Meyer, J. Borner, M. Petersen, A.J. Aberer, A. Stamatakis, M.G. Walzl, B.Q. Minh, A. von Haeseler, I. Edesberger, G. Pass, and B. Misof. 2014. Decisive data sets in phylogenomics: lessons from studies on the phylogenetic relationships of primarily wingless insects. Molecular Biology and Evolution 31:239–249.

Dohle, W. 2001. Are the insects terrestrial crustaceans? A discussion of some new facts and arguments and the proposal of the proper name 'Tetraconata' for the monophyletic unit Crustacea+Hexapoda. Annales De La Societe Entomologique De France 37:85–103.

Edgecombe, G.D. 2010. Palaeomorphology: fossils and the inference of cladistic relationships. Acta Zoologica 91:72–80.

Edgecombe, G.D., G.D.F. Wilson, D.J. Colgan, M.R. Gray, and G. Cassis. 2000. Arthropod cladistics: combined analysis of histone H3 and U2 snRNA sequences and morphology. Cladistics 16:155–203.

Edwards, S.V. 2009. Is a new and general theory of molecular systematics emerging? Evolution 63:1–19.

Emerson, M.J., and F.R. Schram. 1990. The origin of crustacean biramous appendages and the evolution of arthropoda. Science 250:667–669.

Eriksson, M.E., F. Terfelt, R. Elofsson, A. Maas, F. Marone, A. Lindskog, D. Waloszek, B. Schmitz, and M. Stampanoni. 2015. Baring it all: undressing Cambrian 'Orsten' phosphatocopine crustaceans using synchrotron radiation X-ray tomographic microscopy. Lethaia 49:312–326.

Fernández, R., G.D. Edgecombe, and G. Giribet. 2016. Exploring phylogenomic relationships within Myriapoda and the effects of matrix composition and occupancy on phylogenomic reconstruction. Systematic Biology 65:871–889.

Gewin, V. 2005. Functional genomics thickens the biological plot. PLoS Biology 3:e219.

GIGA Community of Scientists. 2014. The Global Invertebrate Genomics Alliance (GIGA): developing community resources to study diverse invertebrate genomes. Journal of Heredity 105:1–18.

Giribet, G., and C. Ribera. 2000. A review of arthropod phylogeny: new data based on ribosomal DNA sequences and direct character optimization. Cladistics 16:204–231.

Giribet, G., G.D. Edgecombe, and W.C. Wheeler. 2001. Arthropod phylogeny based on eight molecular loci and morphology. Nature 413:157–160.

Harzsch, S. 2006. Neurophylogeny: architecture of the nervous system and a fresh view on arthropod phyology. Integrative and Comparative Biology 46:162–194.

Haug, J.T., C. Haug, V. Kutschera, G. Mayer, A. Maas, S. Liebau, C. Castellani, U. Wolfram, E.N. Clarkson, and D. Waloszek. 2011. Autofluorescence imaging, an excellent tool for comparative morphology. Journal of Microscopy 244:259–272.

Hegna, T.A. 2010. Photography of soft-bodied crustaceans via drying, whitening, and splicing. Journal of Crustacea Biology 30:351–356.

Hennig, W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

Hessler, R.R., and W.A. Newman. 1975. A trilobitomorph origin for the Crustacea. Fossils and Strata 4:437–459.

Hoenemann, M., M.T. Neiber, W.F. Humphreys, T.M. Iliffe, L. Difei,, F.R. Shram, and S. Koenemann. 2013. Phylogenetic analysis and systematic revision of Remipedia (Nectiopoda) from Bayesian analysis of molecular data. Journal of Crustacean Biology 33(5):603–619.

Huys, R., J. Llewellyn-Hughes, S. Conroy-Dalton, P.D. Olson, J.N. Spinks, and D.A. Johnston. 2007. Extraordinary host switching in siphonostomatoid copepods and the demise of the Monstrilloida: integrating molecular data, ontogeny and antennulary morphology. Molecular Phylogenetetics and Evolution 43:368–378.

Jenner, R., C. Dhubhghaill, M. Ferla, and M. Wills. 2009. Eumalacostracan phylogeny and total evidence: limitations of the usual suspects. BMC Evolutionary Biology 9:21.

Khodami, S., J.V. McArthur, L. Blanco-Bercial and P.M. Arbizu. 2017. Molecular phylogeny and revision of copepod orders (Crustacea: Copepoda). Scientific Reports 7:9164.

Koenemann, S., F.R. Schram, M. Honemann, and T.M. Iliffe. 2007. Phylogenetic analysis of Remipedia (Crustacea). Organisms Diversity and Evolution 7:33–51.

Koenemann, S., R.A. Jenner, M. Hoenemann, T. Stemme, and B.M. von Reumont. 2010. Arthropod phylogeny revisited, with a focus on crustacean relationships. Arthropod Structure and Development 39:88–110.

Kosobokova, K.N., and R.R. Hopcraft. 2010. Diversity and vertical distribution of mesozooplankton in the Arctic's Canada Basin. Deep Sea Research II 57:96–110.

Lauterbach, K.E. 1983. Zum Problem der Monophylie der Crustacea. Abhanglungen und Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg 26:293–320.

Lavrov, D.V., W.M. Brown, and J.L. Boore. 2004. Phylogenetic position of the Pentastomida and (pan) crustacean relationships. Proceedings of the Royal Society B: Biological Sciences 271:537–544.

Lee, M.S.Y., J. Soubrier, and G.D. Edgecombe. 2013. Rates of phenotypic and genomic evolution during the Cambrian explosion. Current Biology 23:1889–1895.

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Legg, D.A., M.D. Sutton, and G.D. Edgecombe. 2013. Arthropod fossil data increase congruence of morphological and molecular phylogenies. Nature Communications 4:2485.

Lemmon, E.M., and A.R. Lemmon. 2013. High-throughput genomic data in systematics and phylogenetics. Annual Review of Ecology, Evolution, and Systematics 44:99–121.

Lemmon, A.R., S.A. Emme, and E.M. Lemmon. 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. Systematic Biology 61:727–744.

Ma, X., G.D. Edgecombe, X. Hou, T. Goral, and N.J. Strausfeld. 2015. Preservational pathways of corresponding brains of a Cambrian euarthropod. Current Biology 25:2969–2975.

Mallatt, J., and G. Giribet. 2006. Further use of nearly complete, 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. Molecular Phylogenetics and Evolution 40:772–794.

Manton, S.M. 1977. The Arthropoda: Habits, Functional Morphology, and Evolution. Clarendon Press, Oxford, UK.

Martin, J.W., and J.C. Christiansen. 1995. A morphological comparison of the phyllopodous thoracic limbs of a leptostracan (*Nebalia* sp.) and a spinicaudate conchostracan (*Leptestheria* sp.), with comments on the use of Phyllopoda as a taxonomic categor. Canadian Journal of Zoology 73:2283–2291.

Martin, J.W., and G.E. Davis. 2001. An updated classification of the Recent Crustacea. Natural History Museum of Los Angeles County: Science Series 39:1–124.

Meusemann, K., B.M. von Reumont, S. Simon, F. Roeding, S. Strauss, P. Kuck, I. Ebersberger, M. Walzl, G. Pass, S. Breuers, V. Achter, A. von Haeseler, T. Burmester, H. Hadrys, J.W. Wägele, and B. Misof. 2010. A phylogenomic approach to resolve the arthropod Tree of Life. Molecular Biology and Evolution 27:2451–2464.

Misof, B., S. Liu, K. Meusemann, et al. 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346:763–767.

Møller, O.S., J. Olesen, A. Avenant-Oldewage, P.F. Thomsen, and H. Glenner. 2008. First maxillae suction discs in Branchiura (Crustacea): development and evolution in light of the first molecular phylogeny of Branchiura, Pentastomida, and other "Maxillopoda." Arthropod Structure and Development 37:333–346.

Muller, C.H.G., J.L. Rosenberg, S. Richter, and V.B. Meyer-Rochow. 2003. The compound eye of *Scutigera coleoptera* (Linneaus, 1758) (Chilopoda: Notostigmophora): an ultrastrutural reinvestigation that adds support to the Mandiulata concept. Zoomorphology 122:191–209.

Neiber, M.T., T.R. Hartke, T. Stemme, A. Bergmann, J. Rust, T.M. Iliffe, and S. Koenemann. 2011. Global biodiversity and phylogenetic evaluation of Remipedia (Crustacea). PLoS ONE 6:e19627.

O'Reilly, J.E., M. dos Reis, and P.C. Donoghue. 2015. Dating tips for divergence-time estimation. Trends in Genetics 31:637–650.

Oakley, T.H., J.M. Wolfe, A.R. Lindgren, and A.K. Zaharoff. 2013. Phylotranscriptomics to bring the understudied into the fold: monophyletic ostracoda, fossil placement, and pancrustacean phylogeny. Molecular Biology and Evolution 30:215–233.

Olesen, J. 2009. Phylogeny of Branchiopoda (Crustacea)—character evolution and contribution of uniquely preserved fossils. Arthropod Systematics and Phylogeny 67:3–39.

Olesen, J., S.V. Martinsen, T.M. Iliffe, and S. Koenemann. 2014. Remipedia. Pages 84–89 *in* J.W. Martin, J. Olesen, and J.T. Hoeg, editors. Atlas of Crustacean Larvae. Johns Hopkins University Press, Baltimore.

Olesen, J., K. Meland, H. Glenner, and T.M. Iliffe. 2017. *Xibalbanus cozumelensis*, a new species of Remipedia (Crustacea) from Cozumel, Mexico, and a molecular phylogeny of *Xibalbanus* on the Yucatan Peninsula. European Journal of Taxonomy 316:1–27.

Pennak, R.W., and D.J. Zinn. 1943. Mystacocarida, a new order of Crustacea from intertidal beaches in Massachusetts and Connecticut. Smithsonian Miscellaneous Collections 103:1–11.

Pérez-Losada, M., J.T. Hoeg, and K.A. Crandall. 2004. Unraveling the evolutionary radiation of the thoracian barnacles using molecular and morphological evidence: A comparison of several divergence time estimation approaches. Systematic Biology 53:244–264.

Pérez-Losada, M., J.T. Hoeg, and K.A. Crandall. 2009. Remarkable convergent evolution in specialized parasitic Thecostraca (Crustacea). BMC Biology 7:15.

Petrunina, A.S., T.V. Neretina, N.S. Mugue, and G.A. Kolbasov. 2013. Tantulocarida versus Thecostraca: inside or outside? First attempts to resolve phylogenetic position of Tantulocarida using gene sequences. Journal of Zoological Systematics and Evolutionary Research 52:100–108.

Poore, G.C.B. 2005. Peracarida: monophyly, relationships, and evolutionary success. Nauplius 13:1–27.

Regier, J.C., J.W. Shultz, and R.E. Kambic. 2005. Pancrustacean phylogeny: hexapods are terrestrial crustaceans and maxillopods are not monophyletic. Proceedings of the Royal Society B: Biologial Sciences 272:395–401.

Regier, J.C., J.W. Shultz, A.R.D. Ganley, A. Hussey, D. Shi, B. Ball, A. Zwick, J.E. Stajich, M.P. Cummings, J.W. Martin, and C.W. Cunningham. 2008. Resolving arthropod phylogeny: exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. Systematic Biology 57:920–938.

Regier, J.C., J.W. Shultz, A. Zwick, A. Hussey, B. Ball, R. Wetzer, J.W. Martin, and C.W. Cunningham. 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. Nature 463:1079–1083.

Richter, S. 2002. The Tetraconata concept: hexapod-crustacean relationships and the phylogeny of Crustacea. Organisms, Diversity and Evolution 2:217–237.

Richter, S., and G. Scholtz. 2001. Phylogenetic analysis of the Malacostraca (Crustacea). Journal of Zoological Systematics and Evolutionary Research 39:113–136.

Riley, J., A.A. Banja, and J.L. James. 1978. The phylogenetic relationships of the Pentastomida: the case for their inclusion within the Crustacea. International Journal of Parasitology 8:245–254.

Rota-Stabelli, O., N. Lartillot, H. Philippe, and D. Pisani. 2013. Serine codon-usage bias in deep phylogenomics: pancrustacean relationships as a case study. Systematic Biology 62:121–133.

Sasaki, G., K. Ishiwata, R. Machida, T. Miyata, and Z.H. Su. 2013. Molecular phylogenetic analyses support the monophyly of Hexapoda and suggest the paraphyly of Entognatha. BMC Evolutionary Biology 13:236–236.

Schram, F.R. 1984. Relationships within eumalacostracan Crustacea. Transactions of the San Diego Society of Natural History 20:301–312.

Schram, F.R. 1986. Crustacea. Oxford University Press, New York.

Schram, F.R., and C.H.J. Hof. 1998. Fossils and the interrelationships of major crustacean groups. Pages 223–302 in G.D. Edgecombe, editor. Arthropod Fossils and Phylogeny. Columbia University Press, New York.

Schram, F.R., and S. Koenemann. 2004. Are the crustaceans monophyletic? Pages 319–329 in J. Cracraft and M.J. Donoghue, editors. Assembling the Tree of Life: Where We Stand at the Beginning of the 21st Century. Oxford University Press, New York.

Sieg, J. 1983. Evolution of Tanaidacea. Pages 229–256 in Crustacean Phylogeny. Crustacean Issues, Vol. 1. A.A. Balkema, Rotterdam, Netherlands.

Snodgrass, R.E. 1938. Evolution of the Annelida, Onychophora and Arthropoda. Smithsonian Miscellaneous Collections 97:1–159.

Spears, T., and L.G. Abele. 1999. Phylogenetic relationships of crustaceans with foliaceous limbs: an 18S rDNA study of Branchiopoda, Cephalocarida, and Phyllocarida. Journal of Crustacean Biology 19:825–843.

Stegner, M.E., T. Stemme, T.M. Iliffe, S. Richter, and C.S. Wirkner. 2015. The brain in three crustaceans from cavernous darkness. BMC Neuroscience 16:19–19.

Stemme, T., T.M. Iliffe, G. Bicker, S. Harzsch, and S. Koenemann. 2012. Serotonin immunoreactive interneurons in the brain of the Remipedia: new insights into the phylogenetic affinities of an enigmatic crustacean taxon. BMC Evolutionary Biology 12:168–168.

Stemme, T., T.M. Iliffe, B.M. von Reumont, S. Koenemann, S. Harzsch, and G. Bicker. 2013. Serotonin-immunoreactive neurons in the ventral nerve cord of Remipedia (Crustacea): support for a sister group relationship of Remipedia and Hexapoda? BMC Evolutionary Biology 13:119–119.

Storch, V., and B.G.M. Jamieson. 1992. Further spermatological evidence for including the Pentastomida (tongue worms) in the Crustacea. International Journal for Parasitology 22:95–108.

Strausfeld, N.J. 2012. Arthropod Brains: Evolution, Functional Elegance, and Historical Significance. Belknap Press, Cambridge, MA.

Strausfeld, N.J., and D.R. Andrew. 2011. A new view of insect-crustacean relationships. I. Inferences from neural cladistics and comparative neuroanatomy. Arthropod Structure and Development 40:276–288.

Tiegs, O.W., and S.M. Manton. 1958. The evolution of the Arthropoda. Biological Reviews 33:255–333.

Timm, L., and H.D. Bracken-Grissom. 2015. The forest for the trees: evaluating molecular phylogenies with an emphasis on higher-level Decapoda. Journal of Crustacean Biology 35:577–592.

von Reumont, B.M., R.A. Jenner, M.A. Wills, E. Dell'Ampio, G. Pass, I. Ebersberger, B. Meyer, S. Koenemann, T.M. Iliffe, A. Stamatakis, O. Niehuis, K. Meusemann, and B. Misof. 2012. Pancrustacean phylogeny in the light of new phylogenomic data: support for Remipedia as the possible sister group of Hexapoda. Molecular Biology and Evolution 29:1031–1045.

von Reumont, B.M., A. Blanke, S. Richter, F. Alvarez, C. Bleidorn, and R.A. Jenner. 2014. The first venomous crustacean revealed by transcriptomics and functional morphology: remipede venom glands express a unique toxin cocktail dominated by enzymes and a neurotoxin. Molecular Biology and Evolution 31:48–58.

Walossek, D. 1999. On the Cambrian diversity of Crustacea. Pages 3–27 *in* F.R. Schram and J.C. von Vaupel Klein, editors. Crustaceans and the Biodiversity Crisis: Proceedings of the Fourth International Crustacean Congress, Amsterdam. Brill Academic Publishers, Leiden, The Netherlands.

Wheeler, W.C., P. Cartwright, and C.Y. Hayashi. 1993. Arthropod phylogeny: a combined approach. Cladistics 9:1–39.

Wills, M.A., D.E.G. Briggs, R.A. Fortey, M. Wilkinson, and P.H. Sneath. 1998. An arthropod phylogeny based on fossil and Recent taxa. Pages 33–105 *in* G.D. Edgecombe, editor. Arthropod Fossils and Phylogeny. Columbia University Press, New York.

Wills, M.A., R.A. Jenner, and C.N. Dhubhghaill. 2009. Eumalacostracan evolution: conflict between three sources of data. Arthropod Systematics and Phylogeny 67:71–90.

Wilson, G.D.F. 1992. Computerized analysis of crustacean relationships. Acta Zoologica 73:383–389.

Wingstrand, K.G. 1972. Comparative spermatology of a pentastomid, *Raillietiella hemidactyli*, and a branchiuran crustacean, *Argulus foliaceus*, with a discussion of pentastomid relationships. Det Kongelige Danske Videnskabernes Selskab Biologiske Skrifter 19:1–72.

Wirkner, C.S., and S. Richter. 2010. Evolutionary morphology of the circulatory system in Peracarida (Malacostraca; Crustacea). Cladistics 26:143–167.

Wolfe, J.M., and T.A. Hegna. 2014. Testing the phylogenetic position of Cambrian pancrustacean larval fossils by coding ontogenetic stages. Cladistics 30:366–390.

Wolfe, J.M., A.C. Daley, D.A. Legg, and G.D. Edgecombe. 2016. Fossil calibrations for the arthropod Tree of Life. Earth Science Reviews 160:43–110.

Wolfe, J.M., J.W. Breinholt, K.A. Crandall, A.R. Lemmon, E.M. Lemmon, L.E. Timm, M.E. Siddall, and H.D. Bracken-Grissom. 2019. A phylogenomic framework, evolutionary timeline, and genomic resources for comparative studies of decapod crustaceans. Proceedings of the Royal Society B 286:20190079.

Yager, J. 1994. Speleonectes gironensis, new species of remipede (Crustacea: Remipedia: Speleonectidae) from anchialine caves in Cuba, with remarks on biogeography and ecology. Journal of Crustacean Biology 14:752–762.

Zhang, X.G., D.J. Siveter, D. Waloszek, and A. Maas. 2007. An epipodite-bearing crown-group crustacean from the Lower Cambrian. Nature 449:595–598.

Zrzavý, J., and P. Stys. 1997. The basic body plan of arthropods: insights from evolutionary morphology and developmental biology. Journal of Evolutionary Biology 10:353–367.

Zrzavý, J., V. Hypsa, and M. Vlaskova. 1998. Arthropod phylogeny: taxonomic congruence, total evidence and conditional combination approaches to morphological and molecular data sets. Pages 97–107 *in* R.A. Fortey and R.H. Thomas, editors. Arthropod Relationships. Chapman and Hall, London.

Zwick, A., J.C. Regier, and D.J. Zwickl. 2012. Resolving discrepancy between nucleotides and amino acids in deep-level arthropod phylogenomics: differentiating serine codons in 21-amino-acid models. PLoS ONE 7:e47450.

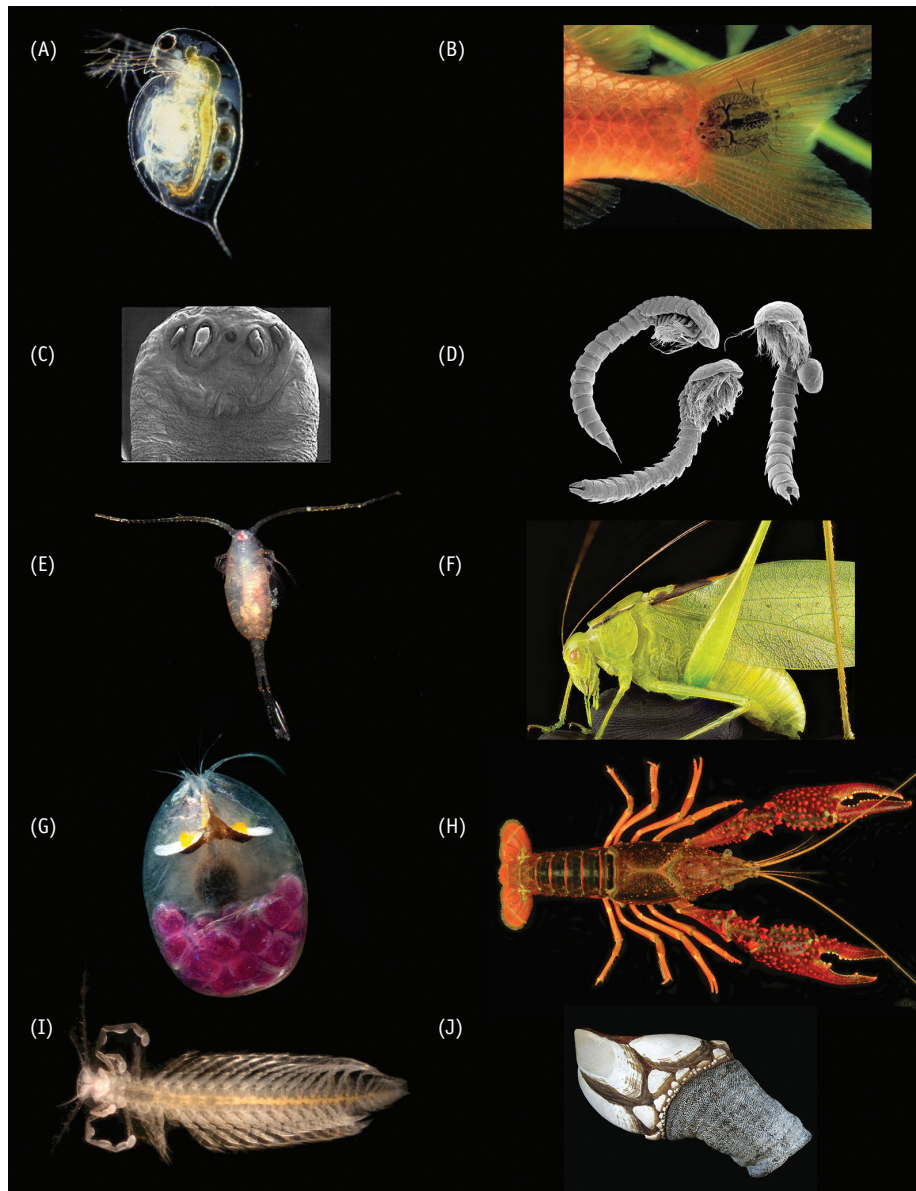**Fig. 4.1.**
Examples demonstrating the morphological diversity across Pancrustacea. (A) *Daphnia pulex*, Branchiopoda (from Gewin 2005, under License CC-BY); (B) *Argulus foliaceus*, Branchiura (© G. Boxshall, under License CC-BY-NC-SA); (C) *Porocephalus crotali*, Pentastomida (from Abele et al. 1989, with permission from Oxford University Press); (D) *Lightiella monniotae*, Cephalocarida (© D. Waloszek and J. Olesen); (E) *Ridgewayia* sp., Copepoda (© T. Iliffe); (F) *Amblycorypha oblongifolia*, Hexapoda (from USGS Bee Inventory and Monitoring Lab); (G) *Gigantocypris* sp., Ostracoda (© D. Fenolio/ DEEPEND); (H) *Procambarus clarkii*, Malacostraca (© D. Felder); (I) *Cryptocorynetes* sp., Remipedia (© T. Iliffe); (J) *Pollicipes cornucopia*, Thecostraca (© H. Hillewaert, under License CC-BY-SA).
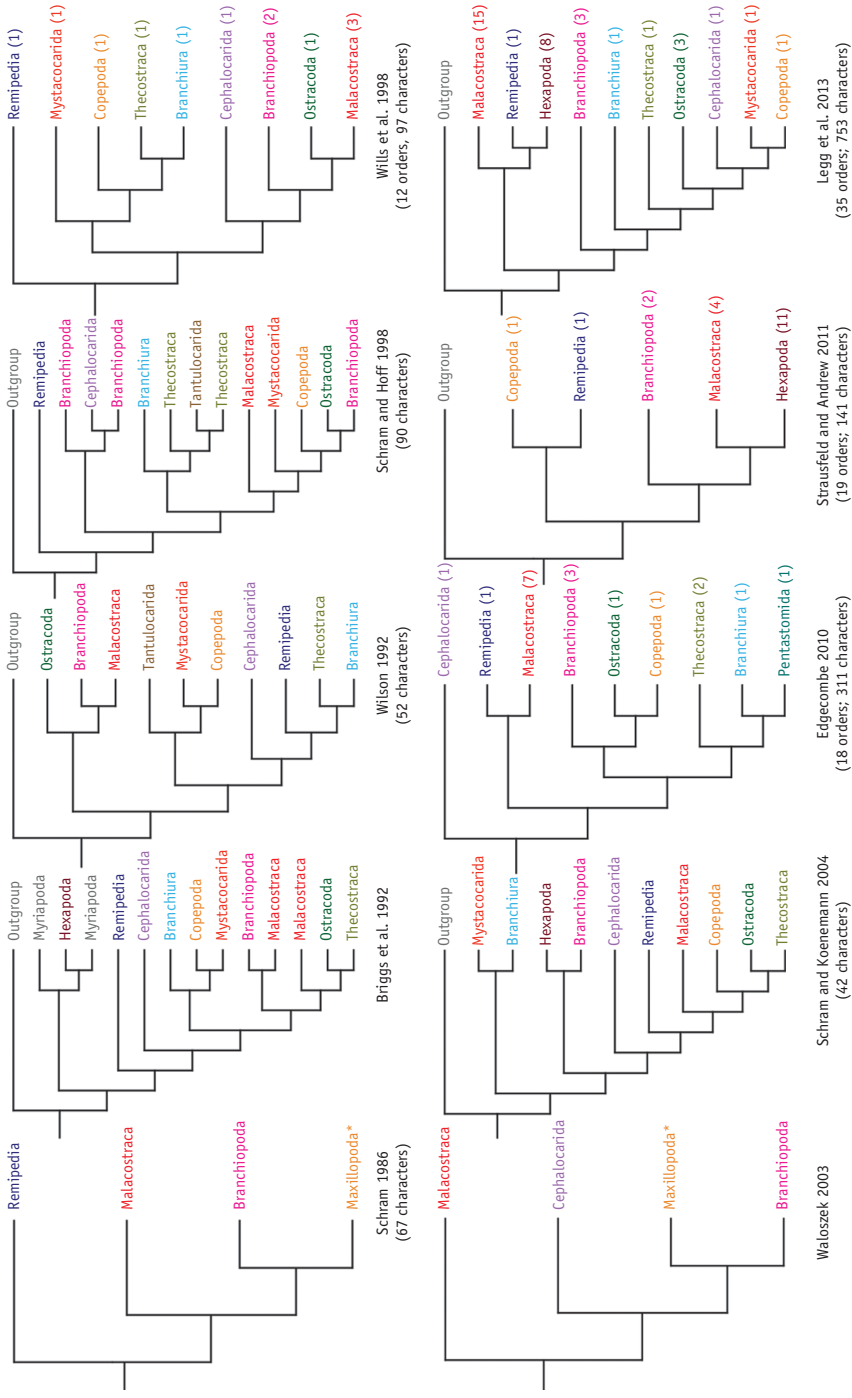
Morphological Phylogenetic Hypotheses

Schram 1986
(67 characters)

Briggs et al. 1992

Wilson 1992
(52 characters)

Schram and Hoff 1998
(90 characters)

Wills et al. 1998
(12 orders, 97 characters)

Waloszek 2003

Schram and Koenemann 2004
(42 characters)

Edgecombe 2010
(18 orders; 311 characters)

Strausfeld and Andrew 2011
(19 orders; 141 characters)

Legg et al. 2013
(35 orders; 753 characters)

Fig. 4.2.
Alternative hypotheses of the Pancrustacean phylogeny based on morphological data. Numbers adjacent to taxon names in the tree represent how many orders for that taxon were included in the analyses (when available). Caption below citation indicates the total number of orders and characters used in the analyses. Fossil taxa excluded from phylogram for clarity. Class Maxillopoda, denoted by an asterisk, no longer constitutes a valid taxon. However, the source literature did not specify the maxillopodan taxa employed in said analyses.
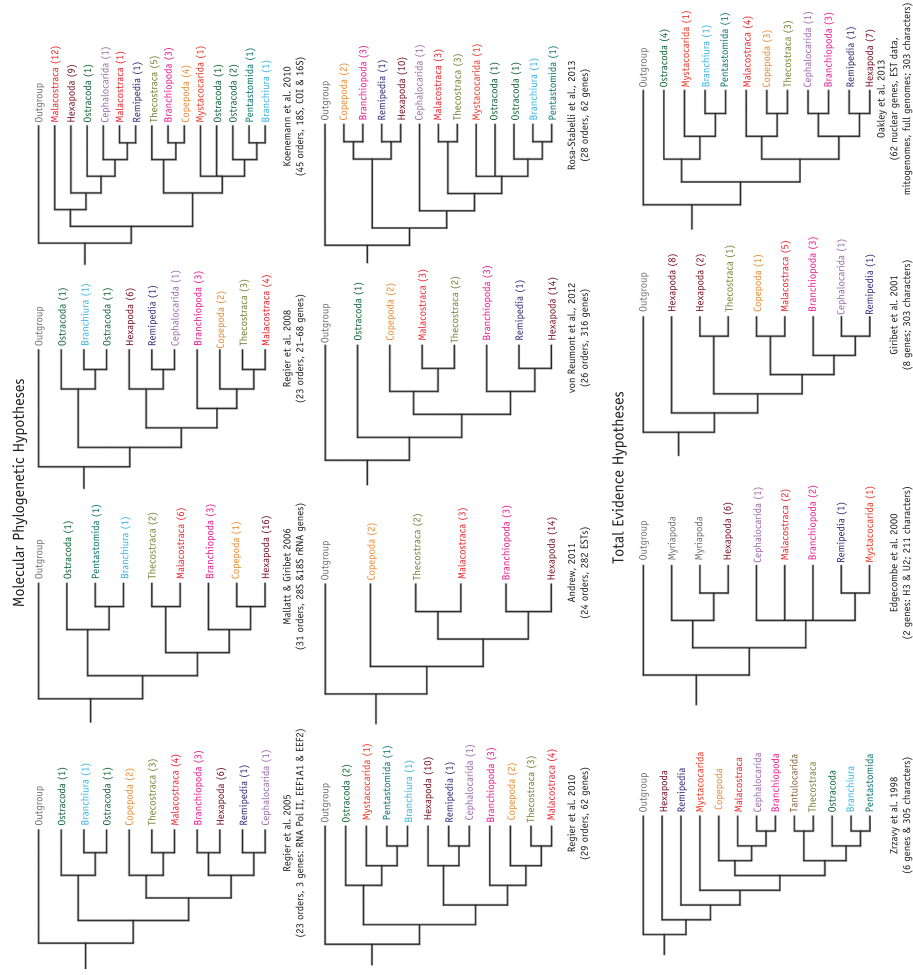
Fig. 4-3.
Alternative hypotheses of the Pancrustacean phylogeny based on genetic data (rows 1 and 2) and combined morphological and genetic data (row 3). Numbers adjacent to taxon names in the tree represent how many orders for that taxon were included in the analyses (when available). Caption below citation indicates the total number of orders, genes, and/or characters used in the analyses. Fossil taxa excluded from phylogram for clarity. Abbreviations: RNA Pol II, RNA polymerase II; EEF1A1, elongation factor alpha subunit 1; EEF2, elongation factor 2; 28S ribosomal mitochondrial gene; 18S, 18S ribosomal mitochondrial gene; COI, cytochrome oxidase I; 16S, 16S ribosomal mitochondrial gene; EST, expressed sequence tags; U2, small nuclear RNA.