WILEY-BLACKWELL

Cladistics 26 (2010) 143–167

Cladistics

10.1111/j.1096-0031.2009.00278.x

Evolutionary morphology of the circulatory system in Peracarida (Malacostraca; Crustacea)

Christian S. Wirkner* and Stefan Richter

Institut für Biowissenschaften, Universität Rostock, Allgemeine & Spezielle Zoologie, Universitätsplatz 2, 18055 Rostock, Germany

Accepted 22 June 2009

Abstract

We demonstrate that by formulating guidelines for evolutionary morphology the transparency, reproducibility, and intersubject testability of evolutionary hypotheses based on morphological data can be enhanced. The five main steps in our concept of evolutionary morphology are (i) taxon sampling, (ii) structural analysis, (iii) character conceptualization, (iv) phylogenetic analysis, and (v) evolutionary interpretation. We illustrate this concept on the example of the morphology of the circulatory organs in peracarid Malacostraca. The analysis is based on recently published accounts in which detailed structural analyses were carried out, and on the older literature. Detailed conceptualizations of 22 characters of the circulatory system are given for 28 terminals. In a further step these characters are included in a recently revised matrix, resulting in 110 characters. The resulting parsimony analysis yielded a single most parsimonious tree with a length of 309 steps. The most significant results are that Peracarida is monophyletic, Amphipoda is the sister taxon to the Mancoida *sensu stricto*, the relict cave-dwelling taxa Thermosbaenacea, Spelaeogriphacea, and Mictocarididae form a monophylum and Tanaidacea is the sister group to a monophylum comprising Cumacea and Isopoda. The evolutionary analysis shows that the ground pattern features of the circulatory organs in Peracarida are a tubular heart extending through the whole thorax, a posterior aorta with lateral arteries, and a ventral vessel system. Important features within the Peracarida are the backward shift of the anterior border of the heart, the reduction of the ventral vessel system, and two patterns of cardiac arteries, one common to the amphipod and tanaidacean terminals, and one to the cumacean and isopod terminals.

Introduction

A plea for morphological characters in a cladistic framework

With regard to the question of whether morphological data should still be used in a cladistic context even when molecular data are available or should not be used in a cladistic context, previous authors' views have ranged from dismissing morphological data completely in cladistic analyses (Hillis and Wiens, 2000; Scotland et al., 2003) to expressing strong support for it (Jenner, 2004; Sudhaus, 2007). Arguments for the use of morphological data have been listed in great detail by Sudhaus (2007), so we limit ourselves here to stressing only those that seem to be of importance in the framework of 'evolutionary morphology' outlined below. First, although the problem of combining morphological and molecular data has not yet been satisfactorily solved, from a theoretical point of view the 'total evidence approach' is seen as the most promising (Kluge, 1989). This approach is understood here as the concept that the degree of corroboration of a certain hypothesis increases with the severity with which this hypothesis has been tested. The severity of the test increases with the quantity and quality of data. Quantity includes the diversity of character complexes and molecular loci. Quality increases with the testability of the characters themselves. Therefore, excluding morphology from phylogenetics entails testing hypotheses less severely (Richter, 2005).

*Corresponding author:
E-mail address: christian.wirkner@web.de

With regard to the dismissal of morphological data and the claim that they should be mapped primarily onto molecular trees, morphological characters would still have to be conceptualized. In this context, the only step not taken would be the establishment of the branching pattern, i.e. the cladistic analysis of morphological data. None of the other steps, however, i.e. structural analysis, homology research, and character conceptualization, can be replaced by molecular data. This line of argument makes clear that with reference to the 'total evidence approach' mentioned above, as morphological data matrices are now to hand, they might just as well be analysed cladistically alongside the molecular data. Furthermore, if only the data gathered from model organisms were to be mapped onto the 'tree' it would lead to a vast underestimation of organismic diversity and morphological disparity of life. What would be the sense in a molecular tree consisting of a large number of terminals of which only a small number were represented morphologically? To understand fully the complex process of organismic evolution in combination with fields such as ecology, biogeography and physiology, morphology is essential. However, some of the criticism directed against morphology can be put to good use in increasing the transparency and intersubjective testability of morphological methodology.

Methodology of evolutionary morphology

We define here five major steps that need to be taken between collecting morphological data and producing an evolutionary hypothesis: (i) taxon sampling, (ii) structural analysis, (iii) character conceptualization, (iv) phylogenetic analysis, and (v) evolutionary interpretation.

(i) Taxon sampling. Taxon sampling is crucial in any comparative study. The choice of objects of study from the pool of possible groupings of individuals depends on the scientific questions to be answered. In the context of evolutionary morphology comparison is generally between representatives of species, but extensive comparative anatomical or developmental studies using sophisticated techniques can only feature a limited number of species. As a result, the a priori choice of terminals is even more crucial for research on internal morphology than it is in the study of external morphology or in molecular systematics. Ideally, all major lineages of the group of interest should be represented and data from different organ systems should be available for the same species, to permit the consistent employment of an exemplar approach (Yeates, 1995; Prendini, 2001) using only species as terminals. This would give 'total evidence' the maximum of explanatory power. Most anatomical and developmental studies from the nineteenth century, however, were performed on European species, resulting in an uneven distribution of knowledge across a given pool of species. At least some kind of combination of terminals (on the closest level of relationship possible) has to be accepted, then, if these important pieces of information are to be taken into account.

(ii) Structural analysis. The techniques used in structural analyses have improved greatly in the last decade thanks to technical progress in areas such as confocal laser scanning microscopy (cLSM), light microscopy (LM) and micro-computer-tomography (μCT) in particular (Wirkner and Richter, 2004; Betz et al., 2007). Perhaps an equally important development, however, is the improvement of visualization methods. For the first 200 years of morphological investigation, structures had to be reduced to a two-dimensional (2D) drawing right from the structural analysis stage. Through techniques such as cLSM or μCT it is now possible to analyse structures non-destructively in three dimensions. Furthermore, virtual-reality methods, e.g. computer-aided 3D reconstruction, and the possibility to embed 3D images into PDF files (Ruthensteiner and Heß, 2008) remove the need to revert to two dimensions at any point in the process, from the first analysis to the presentation of the results. Four-dimensional microscopy even allows a time-scale to be included in comparative analyses of developing organisms (Hejnol et al., 2006; Wolff and Scholtz, 2006). Morphological databases, e.g. MorphDBase, http://www.morphdbase.de, are another effective tool in enhancing the reproducibility of morphological data.

(iii) Character conceptualization. Structural analyses, however, do not automatically lead to characters usable in cladistic analyses. In this regard, the demarcation of features is crucial and cannot be substituted by computational methods but necessitates certain decisions and the formulation of hypotheses by the scientist performing the work (Pleijel, 1995; Wilkinson, 1995; Fitzhugh, 2006). Characters can be seen as the units of evolutionary transformation (*sensu* Hennig, 1966) with each character state being derived from one other state (Grant and Kluge, 2004). Furthermore, because both individuals and organ systems are complex and integrated entities, any division into character states is the result of a specific, more or less artificial concept. In most cases the demarcations of the characters and character states are not known to us because we are often not aware of the coherence (interdependence) between different features. A comparison between different taxa that involves the establishment of hypotheses of homology (e.g. see Richter, 2005; Szucsich and Wirkner, 2007), however, provides evidence of such coherence. Providing the rationale for defining characters and character states (character conceptualization), therefore, is a crucial step in evolutionary morphology.

An important subject related to steps (ii) and (iii) is morphological terminology. This discourse has been revived recently (Ramirez et al., 2007; Edgecombe, 2008; Vogt, 2008, 2009; but see also Kluge, 1993; Lee and Scanlon, 2002). The overriding goal, with which we agree completely, is better transparency, reproducibility, communicability, and inter-subject consensus regarding empirical data (following Vogt, 2008). This is exactly what we are trying to achieve with our attempt to formulate guidelines for evolutionary morphology. There is, however, no agreement on a coherent system on which terms should be based. It has been argued that morphological terms should either be completely independent of any homology statements (Vogt, 2008) or depend on (primary) homology statements (Edgecombe, 2008). In our view, the two points of view cannot be separated. On the one hand, we agree with Vogt (2008) that in the "purely" descriptive part of any morphological work, assumptions regarding homology should be avoided, although we doubt that after 150 years of study of evolutionary morphology (not taking into account the previous non-evolutionary approaches to homology) it is possible to find any term which has not previously been discussed in the framework of evolution. On the other hand, when it comes to the conceptualization of characters, which in itself is nothing other than the establishment of homology hypotheses under a specific concept of homology, the terminology used needs to take homology assumptions into consideration. In most cases identical terminology will be used: heart, ostia, cardiac arteries, descending artery, and aorta posterior are used in the first step as descriptive terms and can be defined without the notion of homology, but in the second step, the character conceptualization, homology, is implied. One example might illustrate this in more detail. In cumaceans, a paired "posterior aorta" has been described (Oelze, 1931; Siewing, 1952). We decided to call this paired structure the "last pair of cardiac arteries" because for us, part of the notion of a "posterior aorta" is that it is unpaired (Wirkner and Richter, 2008). At the same time, calling this structure the "last pair of cardiac arteries" implies that the cumacean vessel is not homologous to other types of "posterior aortae" but rather to cardiac arteries. Inventing a new, purely descriptive term for the situation in cumaceans just to avoid any homology-related implications would be meaningless unless carried out consistently for all kinds of posterior aorta and all kinds of cardiac arteries in the various taxa.

(iv) Cladistic analysis. A cladistic analysis has to be the basis of any discussion of the evolutionary changes of any organ system. It enables homology hypotheses at least of character states to be corroborated or rejected on the basis of strict cladistic principles. As mentioned above, there is little sense in analysing a single character complex on its own and then discussing potential evolutionary transformations, which is why a 'total evidence' approach is seen as more promising (Kluge, 1989).

(v) Evolutionary interpretation. We regard the main aim of evolutionary morphology to be the reconstruction of the evolution of organ systems and even whole organisms. Pursuit of this goal involves reconstructing the sequential order of character states, i.e. transformations from one character state to the next, in every single character to reveal the evolution of characters as transformation series (*sensu* Hennig, 1966). Evolutionary analyses do not necessarily have to be the final step, as causal explanations, i.e. mechanisms, of the evolutionary transformations might also feature in evolutionary morphology. From a technical point of view these transformations are easily visualized with software such as MacClade (Maddison and Maddison, 1992) or Mesquite (Maddison and Maddison, 2008). We are fully aware that it is possible to carry out character tracing in a probabilistic framework (Pagel, 1999), but for consistency reasons we believe nonetheless that the use of parsimony analyses necessitates the use of parsimonious character mapping (see also Wirkner, 2009).

Herein, we substantiate the five-step approach of evolutionary morphology with a case study of the peracarid circulatory system. Taxon sampling and the technical aspects of the cladistic analysis and character tracing are described in the Materials and methods section, whereas conceptualization of circulatory system characters as major step is described together with the results of the cladistic analysis and the evolutionary interpretation under the Results.

The discourse on peracarid circulatory systems and phylogeny

The first studies on the circulatory system in Peracarida (and other Malacostraca) date back to the early nineteenth century (e.g. Audoin and Milne-Edwards, 1827). Later in the century the first comparative studies were carried out (Delage, 1881, 1883; Claus, 1879, 1884a,b, 1888). After this classical period of comparative morphology came a phase of mostly monographical descriptive papers dealing with one or two representatives (Oelze, 1931; Klövekorn, 1934). It was not until the middle of the twentieth century that the first phylogenetic–evolutionary comparison of the circulatory system in Malacostraca was carried out by Rolf Siewing (e.g. Siewing, 1952, 1953, 1954, 1956). His studies were part of a comparative survey of the morphology of all major organ systems in Malacostraca with the explicit goal of extracting homologous characters (*Spezialhomologien*) for the development of phylogenetic hypotheses (Siewing, 1956, 1960, 1963a).

In the last few years we have applied a combination of modern and classical techniques such as corrosion casting, 3D reconstruction based on histology and μCT in a broad survey of the circulatory system of various peracarids (Wirkner and Richter, 2003, 2004, 2007a,b,c, 2008, 2009) partly to revisit Siewing's ideas, but even more in an attempt to establish new phylogenetic hypotheses to act as the foundation for a substantial character-based discussion of the evolutionary changes of this major organ system. These two aspects will be outlined in the present study.

Siewing's (1956, 1963a) hypothesis influenced many later researchers and the discourse on peracarid phylogeny that followed is summarized here under its four major aspects: first, the debate on the monophyly of Peracarida; secondly, and closely related to that, the discussion on the phylogenetic position (and monophyly status) of Mysidacea (= Mysida and Lophogastrida); thirdly, the question of the inclusion or exclusion of Thermosbaenacea in Peracarida; and finally, the discussion on the position of Isopoda, either as part of Mancoida together with Cumacea and Tanaidacea or as a sister taxon to Amphipoda, thus forming Edriophthalma.

With regard to the first two points, Watling (1981, 1983, 1999) and Watling et al. (2000) favoured a polyphyletic Peracarida as he regarded Mysidacea to be more closely related to the Eucarida. He did not believe in a monophyletic Mysidacea, but saw them as being a paraphyletic assemblage. It is particularly interesting to note that the similarities between the circulatory system of Mysidacea and that in Euphausiacea and Decapoda and its differences to the remaining Peracarida was one of his major arguments in favour of peracarid polyphyly. Molecular data do not support the monophyly of Peracarida either, as they all exclude Mysida from Peracarida and corroborate a closer relationship with either Euphausiacea (Jarman et al., 2000; based on 28S rRNA data), Euphausiacea and Stomatopoda (Meland and Willassen, 2007; based on 18S rRNA but Mysida except Stygiomysidae), Eucarida (Spears et al., 2005; based on 18S rRNA data), or even outside the Eumalacostraca (Jenner et al., 2009; molecular data set). On the contrary, the sister-group relationship between Mysida and Lophogastrida is strongly supported by morphological evidence (Hessler, 1982; De Jong-Moreau and Casanova, 2001; Wirkner and Richter, 2007a) and the most comprehensive morphological cladistic analyses of Malacostraca by Richter and Scholtz (2001), Poore (2005), and Jenner et al. (2009); morphological data set) also support monophyletic Mysidacea as the sister group to the remaining Peracarida.

According to Siewing (1956, 1963a), Thermosbaenacea is the sister group to Peracarida. This was also postulated by Pires (1987) who undertook one of the first cladistic analyses of the Peracarida. Wagner (1994), in his revision of Thermosbaenacea, included a cladistic analysis of the Peracarida. His consensus tree showed the three relict cave- or deep sea-dwelling taxa Spelaeogriphacea, Mictacea, and Thermosbaenacea on one branch nested within the Peracarida. Most later studies (e.g. Schram and Hof, 1998; Wills, 1998; Watling, 1999) located the Thermosbaenacea within the peracarid groups, with the exception of Richter and Scholtz (2001). In their analysis the position of Thermosbaenacea is not stable, but on the basis of evidence from developmental data they still hypothesize a sister-group relationship between Thermosbaenacea and Peracarida and thus reinforce Siewing's hypothesis. The relationships between Spelaeogriphacea and Mictacea are complicated by the suggestion that Mictocarididae are closely related to Spelaeogriphacea (forming Cosinzeneacea) and separated from the Hirsutiidae (Bochusacea) (Gutu, 1998; Gutu and Iliffe, 1998).

Over the years various phylogenetic studies have supported a sister-group relationship between Amphipoda and Isopoda (e.g. Schram, 1986; Wagner, 1994; Wills, 1998; Poore, 2005), largely on the basis of the presence of sessile eyes, the reduction of a carapace and the reduction of exopods on the thoracopods. Contrarily, Richter and Scholtz (2001) found support for Siewing's clade of Isopoda, Tanaidacea, and Cumacea (mancoids *sensu* Hessler, 1982), a clade also supported by recent molecular studies (Spears et al., 2005; Meland and Willassen, 2007).

Materials and methods

In the present study, two major aspects were of importance in the selection of terminals: first, the putative phylogenetic position within a certain taxon, or where no satisfying phylogenetic information was available a broad representation of morphological disparity, and secondly, the availability of data. The latter depends mainly on the availability of specimens because most of the information on the circulatory system comes from our previous publications.

In Lophogastrida, *Lophogaster typicus*, *Neognathophausia ingens*, and *Eucopia unguiculata* represent the three families. Within Mysida, Boreomysinae (represented by *Boreomysis arctica*) is probably basally offbranching within Mysidae (Meland and Willassen, 2007). In addition, *Neomysis integer* was chosen. No information is available for representatives of the other families in Mysida. In Isopoda, the three basal offbranching taxa are Phreatoicidea, Asellota, and Oniscidea (according to Wägele, 1989; Brusca and Wilson, 1991). Representatives of each of these taxa were considered. The status of phylogenetic resolution in

Amphipoda is less than satisfactory, and no well-corroborated hypothesis exists to date. However, most authors favour three or four main lineages within Amphipoda: Ingolfiellidea, Caprellidea, Hyperiidea, and Gammaridea (Kim and Kim, 1993; Gruner, 1993). In the present study, two species of gammarideans, one species of caprellids, one species of hyperiids, and one species of ingolfielids are considered. In Cumacea, phylogenetic analyses are scarce and only superficial to date (Haye et al., 2004). Therefore, three species from three families were chosen, representing at least the two lineages with and without a free telson, respectively. In Tanaidacea, three widely accepted, major recent lineages can be distinguished (Sieg, 1983; Larsen and Wilson, 2002): Apseudomorpha, Neotanaidomorpha, and Tanaidomorpha. This study considers *Apseudes bermudeus*, *Neotanais* sp., and *Tanais dulongii*—one representative of each group. As representatives of the relict and cave-dwelling taxa, *Tethysbaena argentarii* (Thermosbaenacea), *Spelaeogriphus lepidops* (Spelaeogriphacea), and *Mictocaris halope* (Mictacea, Mictocarididae) were obtained. Hirsutiidae were excluded from the analysis because no material could be obtained on which to investigate the circulatory system. Outgroup taxa are represented by only one representative each (two in the case of Decapoda) (see Table 1).

Cladistic analyses

Because the major goal of the present work is to provide new data from the circulatory system and to test their influence on resulting phylogenetic hypotheses no entirely new matrix has been created. Instead the matrix of Richter and Scholtz (2001) was revised by including new data that have become available for some of the terminals (see Supporting information), and all the terminals were transformed into exemplars (on the species level). As the relationships within Peracarida are the focus of the present analysis, Bathynellacea, Amphionidacea, Stenopodidea, and Reptantia were not considered. The original characters relevant to the circulatory system were taken out of the matrix (characters 61–66 of Richter and Scholtz, 2001) and 22 new circulatory organ characters (see below) were added to the remaining characters. The remaining characters where checked against the literature, resulting in a matrix for the Peracarida consisting of 110 characters and 28 terminals. All characters were treated as unordered.

Morphological data were analysed with parsimony in NONA v. 2.0 (Goloboff, 1998) using Winclada v. 1.00.08 (Nixon, 1999–2002) as a shell. One thousand random addition sequence replicates (RAS) were undertaken followed by tree bisection and reconnection

Table 1
Data sources

Taxon	Species	Source
Stomatopoda	*Squilla oratoria* (de Haan, 1844)	Komai and Tung (1931)
Leptostraca	*Nebalia bipes* (O. Fabricius, 1780)	Siewing (1956)
Decapoda	*Melicertus kerathurus* (Forskål, 1775)	Mayrat (1958)
	Palaemonetes vulgaris (Say, 1818)	Brody and Perkins (1930)
Anaspidacea	*Anaspides tasmaniae* Thomson, 1892	Siewing (1954, 1956))
Euphausiacea	*Euphausia superba* Dana, 1852	Zimmer (1913)
Thermosbaenacea	*Tethysbaena argentarii* (Stella, 1951)	Wirkner and Richter (2009)
Lophogastrida	*Lophogaster typicus* M. Sars, 1857	Wirkner and Richter (2007a)
	Eucopia unguiculata (Willemoes-Suhm, 1875)	Siewing (1956)
	Neognathophausia ingens (Dohrn, 1870)	Belman and Childress (1976)
Mysida	*Boreomysis arctica* (Krøyer, 1861)	Wirkner and Richter (2007a)
	Neomysis integer (Leach, 1815)	Wirkner and Richter (2007a)
Amphipoda	*Orchestia cavimana* Heller, 1865	Wirkner and Richter (2007b)
	Hyalella azteca Saussure, 1858	Wirkner and Richter (2007b)
	Hyperia galba (Montagu, 1813)	Wirkner and Richter (2007b)
	Caprella mutica Schurin, 1935	Wirkner and Richter (2007b)
	Trogloleleupia leleupi Ruffo, 1951	Siewing (1963b)
Spelaeogriphacea	*Spelaeogriphus lepidops* Gordon, 1957	Wirkner and Richter (2007c)
Mictocarididae	*Mictocaris halope* Bowman and Iliffe, 1985	Wirkner and Richter (2007c)
Cumacea	*Diastylis rathkei* (Krøyer, 1841)	Oelze (1931); Wirkner and Richter (2008)
	Hemilamprops rosea (Norman, 1863)	Wirkner and Richter (2008)
	Leucon nasica (Krøyer, 1841)	Wirkner and Richter (2008)
Tanaidacea	*Tanais dulongii* (Audouin, 1826)	Wirkner and Richter (2008)
	Apseudes bermudeus Bacescu, 1980	Wirkner and Richter (2008)
	Neotanais sp.	Wirkner and Richter (2008)
Isopoda	*Paramphisopus palustris* (Glauert, 1924)	Wirkner and Richter (2003)
	Porcellio scaber Latreille, 1804	Wirkner and Richter (2003)
	Asellus aquaticus (Linnaeus, 1758)	Silen (1954)

(TBR) branch swapping. In order to avoid spending too much time searching tree space in suboptimal islands, the number of trees held per replicate was limited to 1000. Nodal support was determined using bootstrapping and jackknifing, where proportions were calculated from 1000 replicates using 100 RAS + TBR in Winclada/Nona.

Tracing of character evolution

To evaluate the evolution of selected characters, characters states were mapped onto the resulting cladogram using the software Mesquite 2.5 (build77) by Maddison and Maddison (2008). The 'ancestral state reconstruction method' with the parsimony model 'unordered' was chosen as the most conservative option for optimizing ancestral states.

Results

As in other Euarthropoda, the circulation system in Malacostraca is made up of the hemolymph and the structures through which the hemolymph is circulated, which are collectively termed the hemolymph circulation system (HCS). This latter system consists of two major components: the hemolymph vascular system (HVS) and the hemolymph lacunar system (HLS). The former is made up of a central circulating pump in combination with various vessels that lead off to different regions of the body. The central pumping structure is the tubular heart which extends (in most malacostracans) through a substantial part of the body underneath the dorsal cuticle. Off this central vessel, a varying number of mostly segmentally arranged and paired vessels branch which supply the different regions of the body with hemolymph and are therefore termed arteries. True veins, i.e. vessels that are directly connected to the heart proper and bring hemolymph to the heart, are absent in Euarthropoda. The hemolymph re-enters the heart through slit-like openings known as ostia. All arteries have open endings through which the hemolymph enters the HLS. The HLS is made up of structures through which hemolymph is channelled but which are not directly (structurally) connected to the HVS. Those structures that are primarily involved in circulation are termed "sinuses". All the spaces between the organs in which hemolymph flows but which are not primarily involved in circulation are termed "lacunae". The most prominent sinus is the dorsal or pericardial sinus. It is confined by the dorsal diaphragm (or pericardial membrane) and the dorsal cuticle. Further examples of sinuses are the podo-pericardial sinuses that lead hemolymph out of the legs (and gills) into the pericardial sinus. These sinuses are confined by a connective tissue membrane that is attached to the lateral cuticle.

In the following, the rationale for the conceptualization of the circulatory organ characters is given. A list of the sources of all data on the general morphology is given in Table 1; sources of ultrastructure data are given directly in the text. A list of all characters and character states of the circulatory organs is given in Table 2.

Characters concerning the structure and extension of the heart

As in most euarthropods, the central circulatory structure in Malacostraca is the heart. Nevertheless, some features of this organ vary greatly throughout the different taxa, in particular its length and position and the histological composition and ultrastructure of the heart wall. In Peracarida, the mostly tubular heart is made up of a single layered myocardium that is surrounded by a connective tissue sheath. In decapods, however, more than one layer is reported (Baccetti and Bigliardi, 1969; Howse et al., 1971). In most peracarid species the heart is attached on the dorsal side by elastic tissue strands to the cuticle and on the ventral side to the dorsal diaphragm (but see character "attachment of heart to dorsal diaphragm"). It generally extends through the greater part of the thorax, except in *Tethisbaena argentarii* (Thermosbaenacea) and *Epipenaeon japonica* (Isopoda; Hiraiwa, 1933), for example, where only a short heart occurs. In Euphausiacea and Decapoda, too, the heart is a short globular structure located in the posterior part of the cephalothorax.

Myocardial ultrastructure. The myocardial ultrastructure of Malacostraca (and other Arthropoda) was studied in great detail during the 1980s by the "Comparative Cellular Cardiology Research Group" at the University of Bergen. The comparative outcome of over 20 papers on different species was summarized in three main papers (Nylund et al., 1987; Tjønneland et al., 1987; Nylund and Tjønneland, 1989); the following information is mainly taken from Nylund et al. (1987). Peracaridan taxa that were not studied are Thermosbaenacea, Spelaeogriphacea, Mictacea, and Lophogastrida. Because no data are available for our mysid exemplars, the data for *Praunus flexuosus* (Nylund, 1981) are scored for *N. integer*, as *P. flexuosus* also belongs to Mysidae (cf. Meland, 2003). This procedure is suggested by, for example, Giribet et al. (2001) as a way of avoiding the loss of information in an exemplar background. The same applies to data on *Gammarus pulex* (Larsen, 1983), here scored for *O. cavimana*. Information on *Asellus aquaticus* was taken from Wägele (1992).

The myofibres of crustacean hearts are always striated. The membrane system of these myofibres is well developed. Transverse tubules (T tubules) of the sarcolemma run into the myofibres. These are termed Tz

Table 2
Table of characters conceptualized for the circulatory system in malacstracan terminals.

		1		2		3		4		5		6		7	8	
		Location of T tubules		Location of couplings		Presence of M line		Anterior border of heart		Posterior border of heart		Attach. heart/dors. diaphragm		Ostia patterns	Number of pairs of ostia	
1	*Nebalia bipes*	?	?	?	?	?	?	Thl	0	PI IV	6	?	?	0	7 pairs	1
2	*Squilla oratoria*	at Z level	0	at H level	1	?	?	Thl	0	PI V	7	?	?	0	13 pairs	0
3	*Melicertus kerathurus*	at Z level	0	at H level	1	diffuse	2	?	?	Th8	0	directly	0	1	5 pairs	2
4	*Palaemonetes vulgaris*	?	?	?	?	?	?	?	?	Th8	0	directly	0	1	?	?
5	*Anaspides tasmaniae*	at Z level	0	at H level	1	distinct	1	Thl	0	PI IV	6	?	?	9	1 pair	5
6	*Euphausia superba*	at Z level	0	at H level	1	distinct	1	?	?	Th8	0	?	?	?	2 pairs	4
7	*Tethysbaena argentarii*	?	?	?	?	?	?	Thl	0	Th4	4	via susp. strands	1	8	1 pair	5
8	*Lophogaster typicus*	?	?	?	?	?	?	Thl	0	PI 1	5	directly	0	2	3 pairs	3
9	*Eucopia unguiculata*	?	?	?	?	?	?	Th2	1	Th8	0	directly	0	?	3 pairs	3
10	*Neognathophausia ingens*	?	?	?	?	?	?	?	?	Th8	0	directly	0	?	?	?
11	*Boreomysis arctica*	?	?	?	?	?	?	Thl	0	Th8	0	directly	0	2	2 pairs	4
12	*Neomysis integer*	at Z + H level	1	at H level	1	distinct	1	Thl	0	Th8	0	directly	0	2	2 pairs	4
13	*Orchestia cavimana*	at Z + H level	1	at H level	1	distinct	1	Th2	1	Th7	1	directly	0	5	3 pairs	3
14	*Hyalella azteca*	?	?	?	?	?	?	Th2	1	Th7	1	directly	0	5	3 pairs	3
15	*Hyperia galba*	?	?	?	?	?	?	Th2	1	Th7	1	directly	0	5	3 pairs	3
16	*Caprella mutica*	?	?	?	?	?	?	Th2	1	Th6	2	directly	0	5	3 pairs	3
17	*Troglololeupia leleupi*	?	?	?	?	?	?	Th3	2	Th5	3	directly	0	5	3 pairs	3
18	*Spelaeogriphus lepidops*	?	?	?	?	?	?	Th2	1	Th8	0	via susp. strands	1	4	2 pairs	4
19	*Mictocaris halope*	?	?	?	?	?	?	Th2	1	Th5	3	via susp. strands	1	7	1 pair	5
20	*Hemilamprops rosea*	at Z + H level	1	at H level	1	distinct	1	Th3	2	Th7	1	directly	0	3	1 pair	5
21	*Diastylis rathkei*	?	?	?	?	?	?	Th3	2	Th7	1	directly	0	3	1 pair	5
22	*Leucon nasica*	at Z + H level	1	at H level	1	distinct	1	Th3	2	Th6	2	directly	0	3	1 pair	5
23	*Tanais dulongii*	at Z level	0	at H level	1	absent	0	Th3	2	Th7	1	directly	0	?	1 pair	5
24	*Apseudes bermudeus*	?	?	?	?	?	?	Th3	2	Th8	0	directly	0	5	2 pairs	4
25	*Neotanais* sp.	?	?	?	?	?	?	Th3	2	Th8	0	directly	0	5	2 pairs	4
26	*Paramphisopus palustris*	at Z + AI level	2	?	?	?	?	Th3	2	Pl IV	6	directly	0	6	2 pairs	4
27	*Porcellio scaber*	at Z + AI level	2	at AI level	0	distinct	1	Th6	3	Pl V	7	directly	0	?	?	?
28	*Asellus aquaticus*	at Z + AI level	2	at AI level	0	distinct	1	Th6	3	?	?	?	?	6	2 pairs	4
		0 = at Z level 1 = at Z + H level 2 = at Z + Al level		0 = at AI level 1 = at H level		0 = absent 1 = distinct 2 = diffuse		0 = Thl 1 = Th2 2 = Th3 3 = Th6		0 = Th8 1 = Th7 2 = Th6 3 = Th5 4 = Th4 5 = Pl I 6 = Pl IV 7 = Pl V		0 = directly 1 = via suspending strands		legend see below	0 = 13 pairs 1 = 7 pairs 2 = 5 pairs 3 = 3 pairs 4 = 2 pairs 5 = 1 pairs	

Legend for character 7 (Ostia pattern): 0, segmental; 1, two pairs dorsally, two pairs laterally and one pair latero-ventrally; 2, two pairs in close vicinity, separated by membrane; 3, one distinct, large pair; 4, one pair at transition Th5 to Th6, one pair at transition Th6 to Th7; 5, one pair in Th4, one pair in Th5; 6, two pairs, arranged asymmetrically; 7, one pair in Th2; 8, one pair in Thl; 9, one pair in Th3

Table 2
(*Continued*)

		9	10		11		12		13		14		15	16	
		Cardiac arteries pattern	Presence of ALAs		Destination of ALAs		Destination of cardiac arteries		Descending artery		Splitting of descending artery		Ventral vessel	Posterior aorta	
1	*Nebalia bipes*	0	absent	1	-	-	thp/plp	0	absent	0	-	-	-	present	0
2	*Squilla oratorio*	0	present	0	md	2	thp/plp	0	absent	0	-	-	0	present	0
3	*Melicertus kerathurus*	1	present	0	md, Al, A2	3	visceral	1	present	1	absent	0	1	present	0
4	*Palaemonetes vulgaris*	1	present	0	md, Al, A2	3	visceral	1	present	1	absent	0	1	present	0
5	*Anaspides tasmaniae*	5	present	0	md	2	thp/plp	0	present	1	present	1	1	present	0
6	*Euphausia superba*	?	present	0	A2	1	visceral	1	present	1	present	1	1	absent	1
7	*Tethysbaena argentarii*	-	-	-	-	-	-	-	-	-	-	-	-	absent	1
8	*Lophogaster typicus*	0	present	0	Al, A2	0	visceral	1	present	1	present	1	1	present	0
9	*Eucopia unguiculata*	0	present	0	?	?	visceral	1	present	1	present	1	1	present	0
10	*Neognathophausia ingens*	0	present	0	Al, A2	0	visceral	1	present	1	?	?	1	present	0
11	*Boreomysis arctica*	2	absent	1	-	-	visceral	1	present	1	present	1	1	present	0
12	*Neomysis integer*	2	absent	1	-	-	visceral	1	present	1	present	1	1	present	0
13	*Orchestia cavimana*	3	present	0	md	2	visceral	1	absent	0	-	-	-	present	0
14	*Hyalella azteca*	3	present	0	md	2	visceral	1	absent	0	-	-	-	present	0
15	*Hyperia galba*	3	absent	1	-	-	visceral	1	absent	0	-	-	-	present	0
16	*Caprella mutica*	-	-	-	-	-	-	-	-	-	-	-	-	present	0
17	*Trogloleleupia leleupi*	3	?	?	?	?	visceral	1	absent	0	-	-	-	absent	1
18	*Spelaeogriphus lepidops*	-	-	-	-	-	-	-	-	-	-	-	-	absent	1
19	*Mictocaris halope*	-	-	-	-	-	-	-	-	-	-	-	-	absent	1
20	*Hemilamprops rosea*	4	absent	1	-	-	thp/plp	0	absent	0	-	-	-	absent	1
21	*Diastylis rathkei*	4	absent	1	-	-	thp/plp	0	absent	0	-	-	-	absent	1
22	*Leucon nasica*	4	absent	1	-	-	thp/plp	0	absent	0	-	-	-	absent	1
23	*Tanais dulongii*	3	present	0	md	2	visceral	1	absent	0	-	-	-	absent	1
24	*Apseudes bermudeus*	3	absent	1	-	-	visceral	1	absent	0	-	-	-	absent	1
25	*Neotanais* sp.	3	absent	1	-	-	visceral	1	absent	0	-	-	-	absent	1
26	*Paramphisopus palustris*	4	present	0	md	2	thp/plp	0	absent	0	-	-	-	absent	1
27	*Porcellio scaber*	?	present	0	md	2	thp/plp	0	absent	0	-	-	-	absent	1
28	*Asellus aquaticus*	4	present	0	md	2	thp/plp	0	absent	0	-	-	2	absent	1
		Legend see below	0 = present 1 = absent		0 = Al, A2 1 = A2 2 = md 3 = md, Al, A2		0 = thp/plp 1 = visceral		0 = absent 1 = present		0 = below ventral nerve cord 1 = above ventral nerve cord		legend see below	0 = present 1 = absent	

Legend for character 9 (Cardiac artery pattern): 0, segmental; 1, two pairs off anterior part of heart, descending artery; 2, one pair, three unpaired, descending artery; 3, three pairs in adjacent thoracic segments; 4, one pair supplying anterior thoracopods, three pairs supplying one pair of thoracopods each, one pair supplying pleon; 5, no lateral cardiac arteries.

Legend for character 15 (Ventral vessel): 0, sending branches into ventral nerve cord, connected to dorsal vessel through medial branches off some lateral cardiac arteries; 1, sending branches into thoracopods, connected to dorsal vessel through descending artery.

Table 2
(*Continued*)

		17		18		19		20		21		22	
		Aortic dilation + mobp		Aortic dilation + ml03/ml04		Lateral stomach arteries		Optical arteries		Pericerebral ring		Podo-pericardial sinuses	
1	*Nebalia bipes*	absent	1	?	?	absent	0	pair off aorta	1	present	0	?	?
2	*Squilla oratorio*	present	0	absent	0	absent	0	Common trunk off aorta	0	absent	1	?	?
3	*Melicertus kerathurus*	?	?	?	?	absent	0	Common trunk off aorta	0	absent	1	?	?
4	*Palaemonetes vulgaris*	present	0	absent	0	absent	0	Common trunk off aorta	0	absent	1	?	?
5	*Anaspides tasmaniae*	?	?	absent	0	absent	0	Common trunk off aorta	0	present	0	?	?
6	*Euphausia superba*	?	?	?	?	absent	0	Common trunk off aorta	0	absent	1	?	?
7	*Tethysbaena argentarii*	absent	1	present	1	present	1	-	-	present	0	?	?
8	*Lophogaster typicus*	present	0	present	1	absent	0	Common trunk off aorta	0	absent	1	in thorax	0
9	*Eucopia unguiculata*	present	0	?	?	absent	0	?	?	absent	1	?	?
10	*Neognathophausia ingens*	present	0	?	?	absent	0	?	?	?	?	in thorax	0
11	*Boreomysis arctica*	absent	1	present	1	absent	0	Common trunk off aorta	0	absent	1	in thorax	0
12	*Neomysis integer*	absent	1	present	1	absent	0	Common trunk off aorta	0	absent	1	in thorax	0
13	*Orchestia cavimana*	absent	1	present	1	absent	0	pair off aorta	1	present	0	in thorax	0
14	*Hyalella azteca*	absent	1	present	1	absent	0	pair off aorta	1	present	0	in thorax	0
15	*Hyperia galba*	absent	1	present	1	absent	0	pair off aorta	1	present	0	in thorax	0
16	*Caprella mutica*	absent	1	present	1	absent	0	-	-	present	0	in thorax	0
17	*Trogloleleupia leleupi*	absent	1	?	?	?	?	-	-	?	?	?	?
18	*Spelaeogriphus lepidops*	absent	1	present	1	present	1	pair off aorta	1	absent	1	in thorax	0
19	*Mictocaris halope*	absent	1	present	1	absent	0	absent	2	present	0	in thorax	0
20	*Hemilamprops rosea*	absent	1	present	1	absent	0	-	-	absent	1	in thorax	0
21	*Diastylis rathkei*	absent	1	present	1	absent	0	-	-	absent	1	in thorax	0
22	*Leucon nasica*	absent	1	present	1	absent	0	-	-	absent	1	in thorax	0
23	*Tana is dulongii*	absent	1	present	1	present	1	Common trunk off aorta	0	present	0	in thorax	0
24	*Apseudes bermudeus*	absent	1	present	1	present	1	-	-	present	0	in thorax	0
25	*Neotanais* sp.	absent	1	present	1	present	1	-	-	present	0	in thorax	0
26	*Paramphisopus palustris*	absent	1	present	1	absent	0	Common trunk off aorta	0	absent	1	in pleon	1
27	*Porcellio scaber*	absent	1	present	1	present	1	Common trunk off aorta	0	absent	1	in pleon	1
28	*Asellus aquaticus*	absent	1	present	1	absent	0	Common trunk off aorta	0	absent	1	in pleon	1
		0 = present 1 = absent		0 = absent 1 = present		0 = absent 1 = present		0 = Common trunk/split aorta 1 = pair off aorta 2 = absent		0 = present 1 = absent		0 = in thorax 1 = in pleon	

tubules when they branch off at the level of the Z bands, but T tubules also occur at the H level (*G. pulex*, Amphipoda; *P. flexuosus*, Mysida; *H. rosea* and *L. nasica*, Cumacea) and the AI level (isopod terminals) (character 1). Longitudinal tubules (LT tubules) branch off the T tubules, thus completing a second network around the myofibres. In addition, a fenestrated sheath is formed around each sarcomere by the sarcoplasmatic reticulum. The two different forms of invaginations form couplings with the sarcoplasmatic reticulum on different levels of the sarcomeres (AI level or H level; character 2). Within Malacostraca, they are only found at AI level in *P. scaber* and *A. aquaticus* (Isopoda). With regard to the striation of the sarcomeres the Z material is always arranged in thin Z lines and the M line is distinctly present in all studied species except *Tanais dulongii* (= *Tanais cavolinii* of Nylund, 1986) and diffuse in Dendrobranchiata (character 3).

Character 1: Location of T tubules in relation to sarcomere striation. 0 = at Z level; 1 = at Z and H level; 2 = at Z and AI level.

Character 2: Location of couplings in relation to sarcomere striation. 0 = at AI level; 1 = at H level.

Character 3: M line. 0 = absent; 1 = distinct; 2 = diffuse.

Anterior border of the heart. The anterior border of the heart is marked by the transition of the heart into the anterior aorta. In all species studied, a valve made up of two vertical flaps is found at this transition. A further characteristic of the anterior border is that the myocardium ends at this transition so that the wall of the anterior aorta is mainly made up of connective tissue. The position of the anterior border varies greatly between and within the different taxa.

Two major problems occur in evaluating its position exactly. First, in some taxa (*L. typicus*, Fig. 1E; *B. arctica*, Fig. 1D; *T. argentarii*, Fig. 1B; *L. nasica*, Fig. 1G) it lies in the cephalothorax. This renders an exact segmental allocation difficult. Nonetheless, for *N. bipes, S. oratoria, T. argentarii*, and the lophogastrid and mysid terminals the 1st thoracic segment is scored, as in these taxa the anterior border lies in the anterior part of the cephalothorax. For the cumacean terminals the 3rd thoracic segment is scored, as the heart begins in a more posterior position in the cephalothorax. Secondly, the anterior border of the heart can lie on the border of two adjacent segments. In these cases, the posterior adjacent segment is scored.

In some taxa the transition of the heart into the anterior aorta is situated on the border of the cephalothorax and the first free thoracic segment. However, this position is not homologous in the different taxa as the number of segments included in the cephalothorax varies.

Within Isopoda, the anterior border of the heart varies between the different taxa (for further details see Wirkner and Richter, 2003). In the species used for this analysis it lies in either the 3rd or the 6th thoracic segment (see Table 2).

Character 4: Anterior border of heart: 0 = in thoracic segment 1 (Th1); 1 = Th2; 2 = Th3; 3 = Th6.

Posterior border of the heart. The posterior border of the heart is marked either by the blind end of the heart (Fig. 1A,B,C,F), its transition into the posteriormost pair of cardiac arteries (Fig. 1G,I) or its transition into the posterior aorta (Fig. 1D,E,H). The transition of the heart into either the posteriormost pair of cardiac arteries or the posterior aorta is marked by valvular structures and the myocardium is not continued. The position is also variable between and within the malacostracan taxa and therefore difficult to pinpoint in some cases (see the problems discussed with regard to locating the anterior border of the heart). The anteriormost position is found in *T. argentarii* (Fig. 1B) in the 2nd thoracic segment, while the posteriormost position is found in some isopod species on the border of the Vth and VIth pleonal segments.

Character 5: Posterior border of heart: 0 = Th8; 1 = Th7; 2 = Th6; 3 = Th5; 4 = Th4; 5 = Th2; 6 = Pl I; 7 = Pl IV; 8 = Pl V.

Attachment of the heart to the dorsal diaphragm. The way the heart is attached to the dorsal diaphragm can vary to some extent, at least in Peracarida. In most taxa, a broad area of the heart is directly attached to the dorsal diaphragm (e.g. amphipod terminals; see figs 2A, 3C, 5B in Wirkner and Richter, 2007b). In contrast, in *T. argentarii*, *M. halope*, and *S. lepidops* the heart is connected to the dorsal diaphragm via connective tissue strands and membranes (see fig. 2G in Wirkner and Richter, 2007c).

Character 6: Attachment heart – dorsal diaphragm: 0 = directly; 1 = via suspending tissue.

Characters concerning the ostial openings

Ostia are slit-like openings in the heart wall that allow hemolymph to enter the heart lumen from the pericardial sinus. They are equipped with lips that close the openings during systole to prevent hemolymph from leaving the heart lumen.

Number and position of pairs of ostia. Within Peracarida, paired ostia that lie laterally opposite each other occur in most species. In some taxa the openings are shifted along the longitudinal axis. A further difference concerns the number of pairs of ostia, which varies from a segmental arrangement of 13 pairs to one pair. However, numbers can also change within taxa.

Pinpointing the exact location of the ostia (i.e. allocating them to a specific segment) is hampered in three main cases, first in taxa where ostia lie in the cephalothorax, e.g. in *L. typicus* and the mysid species (Fig. 1D,E), secondly where ostia are situated on the border of two adjacent segments (e.g. some Cumacea), and thirdly where pairs of ostia are asymmetrically distributed along the heart so that one ostium lies in one segment and the other in a different segment, e.g. in *P. palustris*, Isopoda (Fig. 1F). This complicates the scoring of these characters. As a result, distinct distribution patterns along the heart (character 7) and the total number of pairs of ostia (character 8) are scored separately. Within Malacostraca, nine different patterns are distinguished. A segmental arrangement as seen in *N. bipes* and *S. oratoria* is interpreted to be the plesiomorphic state. A further pattern can be observed in Decapoda, where the ostia are arranged dorsally, laterally, and ventrally at the globular heart. As the two lateral pairs of ostia situated in the globular heart of *E. superba* cannot be satisfactorily homologized to those in the decapod species, they are scored as "?". *L. typicus* and the mysid terminals have at least two pairs of ostia assembled closely together and tilted slightly obliquely above each other (Fig. 1D,E; 7 : 2, inlet). *L. typicus* and *E. unguiculata* (Lophogastrida) have three pairs of ostia. The large, uniquely shaped pair of ostia found in cumacean species is coded as a separate state (Fig. 1G; 7 : 3). *A. bermudeus*, *Neotanais* sp., and the amphipod terminals share a common state with at least two pairs of ostia lying in the 4th and 5th thoracic segments, respectively (*T. dulongii,* which only has one pair, is coded as inapplicable rather than defining an additional state). The isopod terminals show highly asymmetrically arranged ostia, also entered as a separate state. The two pairs of ostia in *S. lepidops* which lie at the borders of the 5th–6th and 7th–8th thoracic segments are deemed a separate state. Problematic in terms of conceptualization are the taxa with only one pair of ostia (*T. argentarii*, *M. halope*, cumacean terminals, *A. tasmaniae*, and *T. dulongii*). They are scored as the same state in character 9. But as the position of the single ostia pair varies in the different taxa, each taxon with only one pair of ostia is scored separately in character 8 (ostia pattern).

Character 7: Ostia pattern: 0 = segmental; 1 = two pairs dorsally, two pairs laterally and one pair lateroventrally; 2 = two pairs in close vicinity, separated by connective tissue membrane (see Wirkner and Richter, 2007a, fig. 5E); 3 = one distinct, large pair; 4 = one pair at transition Th5 to Th6, one pair at transition Th6 to Th7; 5 = one pair in Th4, one pair in Th5; 6 = two pairs, arranged asymmetrically; 7 = one pair in Th2; 8 = one pair in Th1; 9 = one pair in Th3.

Character 8: Number of pairs of ostia: 0 = 13; 1 = 7; 2 = 5; 3 = 3; 4 = 2; 5 = 1.

Characters concerning the cardiac arteries

Within Crustacea, lateral cardiac arteries are only described in Malacostraca. Cardiac arteries are made up of connective tissue walls and lead to different points in the body cavity such as long body appendages, lacunae, and distinct tissues.

Number and position of cardiac arteries. Within Peracarida, all cardiac arteries emanating from the heart are paired, except in Mysida, where next to the first pair of cardiac arteries four (descending artery included) unpaired arteries lead off the heart ventrally (scored as a separate state; Fig. 1D, 9 : 2). Within Peracarida, only the lophogastrid terminals show a segmental arrangement of these arteries (ten pairs in *L. typicus*; Fig. 1E, 9 : 0). In other peracarid taxa the number is lower, right down to their complete absence in *S. lepidops*, *M. halope*, and *T. argentarii* (Fig. 1A–C). As with the ostia, locating them precisely is complicated by their position either in the cephalothorax or on the border of two adjacent segments. Here again, distribution patterns of cardiac arteries are scored. In accordance with Siewing (1956) the stomatopod, leptostracan, and lophogastrid terminals are scored as showing a segmental pattern. *A. tasmaniae* is scored a state of its own due to the fact that cardiac arteries are only described in the pleon (Siewing, 1954, 1956). As with the ostia (see above), cardiac arteries in *E. superba* are difficult to homologize with those in the decapod terminals and therefore *E. superba* is scored as "?". In the cumacean terminals (Fig. 1G, 9 : 4), the five pairs of cardiac arteries show a strikingly similar distribution pattern in comparison to the posterior five pairs of arteries in the isopod terminals (Fig. 1F, 9 : 4). As a result, the first pair of cardiac arteries in the cumaceans is homologized with the second in the isopods and the general pattern as one state (for details see Wirkner and Richter, 2008). In both, the amphipod (Fig. 1H, 9 : 3) and tanaidacean terminals (Fig. 1I, 9 : 3), three pairs of cardiac arteries branch off the heart (in addition to the anterior lateral arteries) in thoracic segments 4, 5 and 6 (except in *C. linearis*, where no cardiac arteries occur).

Character 9: Cardiac arteries pattern: 0 = segmental; 1 = two pairs off anterior part of heart, descending artery; 2 = one pair, three unpaired, descending artery; 3 = three pairs in adjacent thoracic segments; 4 = one pair supplying anterior thoracopods, three pairs supplying one pair of thoracopods each, one pair supplying pleon; 5 = one pair in Th1, one pair in Th8, five pairs in pleon.

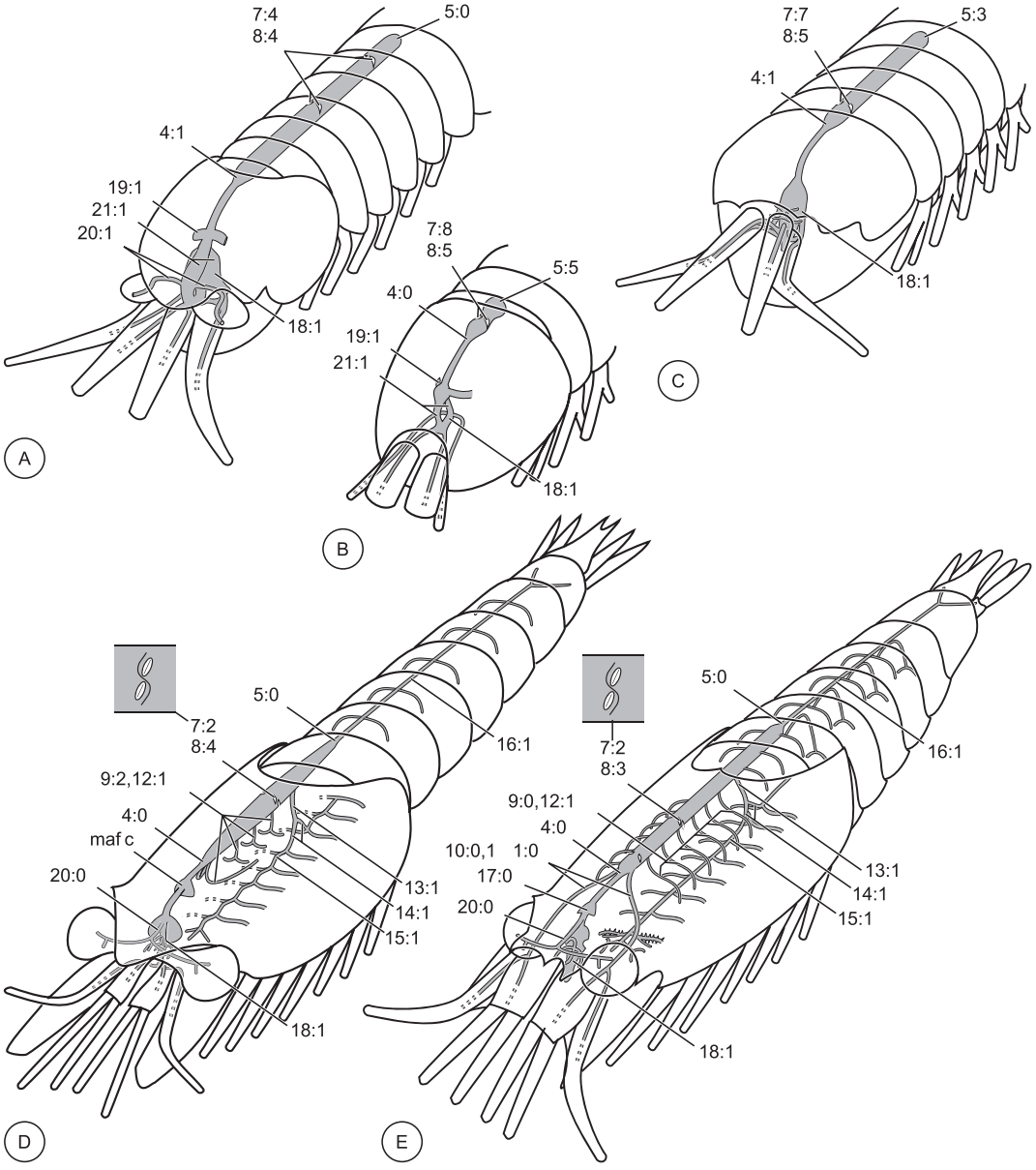
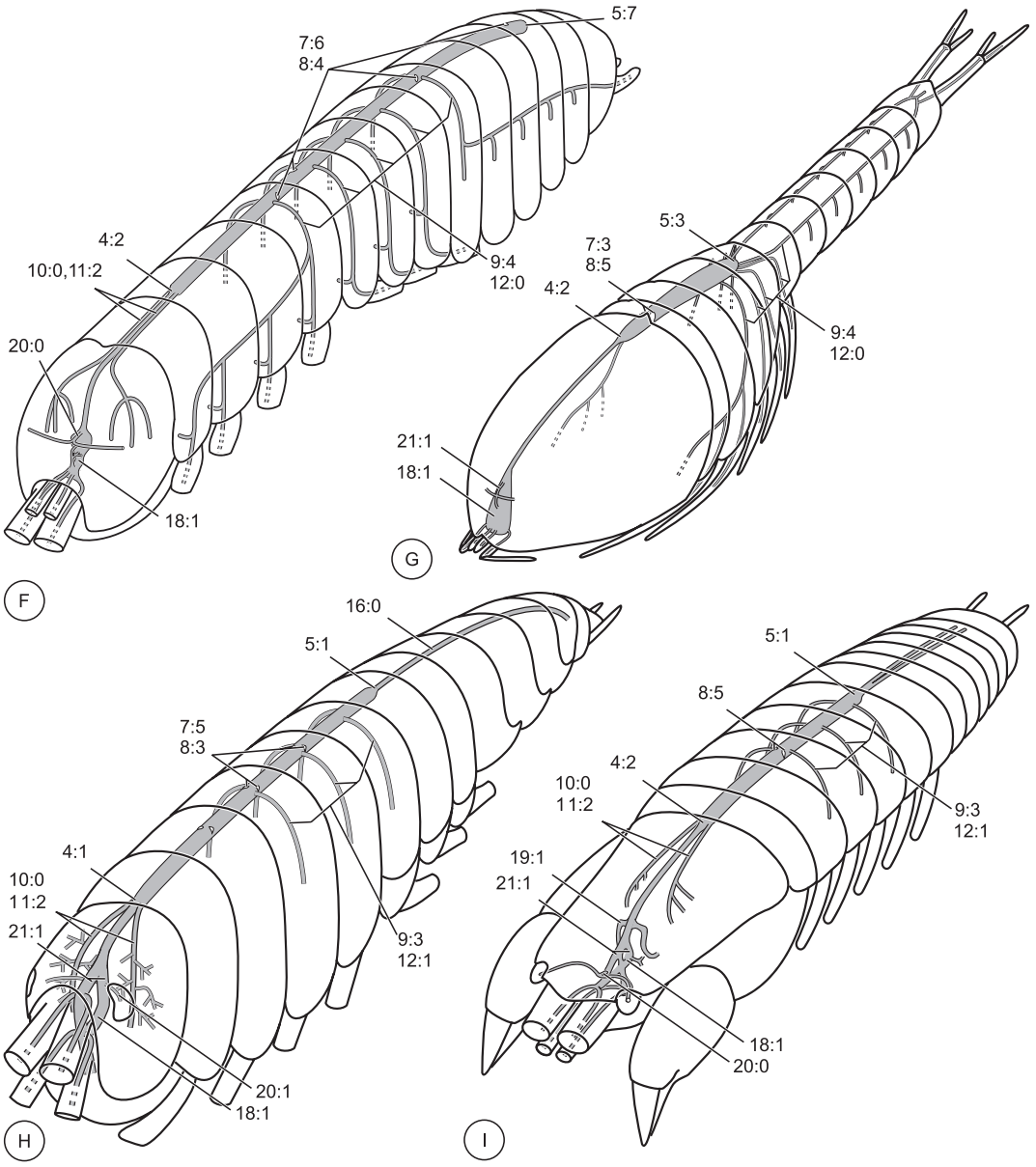


Fig. 1. Distribution of character states of the circulatory system in Peracarida mapped onto schematic representations. Bold numbers represent character numbers, other numbers refer to character states (see also Table 2). For further explanations see text. (A) ***Spelaeogriphus lepidops*** (after Wirkner and Richter, 2007c). **4**, anterior border of heart: 1, in thoracic segment 2; **5**, posterior border of heart: 0, in thoracic segment 8; **7**, ostia pattern: 4, one pair at transition Th5 to Th6, one pair at transition Th6 to Th7; **8**, number of ostia pairs: 4, two; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **19**, lateral stomach arteries: 1, present; **20**, optical arteries: 1, pair off aorta; **21**, pericerebral ring: 1, present. (B) ***Tethysbaena argentarii*** (after Wirkner and Richter, 2009). **4**, anterior border of heart: 0, in thoracic segment 1; **5**, posterior border of heart: 5, in thoracic segment 2; **7**, ostia pattern: 8, one pair in Th1; **8**, number of ostia pairs: 5, one; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **19**, lateral stomach arteries: 1, present; **21**, pericerebral ring: 1, present. (C) ***Mictocaris halope*** (after Wirkner and Richter, 2007c). **4**, anterior border of heart: 1, in thoracic segment 2; **5**, posterior border of heart: 3, in thoracic segment 5; **7**, ostia pattern: 7, one pair in Th2; **8**, number of ostia pairs: 5, one; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; (D) ***Boreomysis arctica*** (after Wirkner and Richter, 2007a). **4**, anterior border of heart: 0, in thoracic segment 1; **5**, posterior border of heart: 0, in thoracic segment 8; **7**, ostia pattern: 2, two pairs in close vicinity, separated by membrane (inlet showing arrangement); **8**, number of ostia pairs: 4, two; **9**, cardiac arteries pattern: 2, one pair, three unpaired, descending artery; **12**, destination of cardiac arteries: 1, visceral; **13**, descending artery: 1, present; **14**, splitting of descending artery: 1, present; **15**, type of ventral vessel: 1, sending branches into thoracopods, connected to dorsal vessel through descending artery (see text for explanation); **16**, posterior aorta: 0, present; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **20**, optical arteries: 0, pair with common trunk off aorta. (E) ***Lophogaster typicus*** (after Wirkner and Richter, 2007a). **4**, anterior border of heart: 0, in thoracic segment 1; **5**, posterior border of heart: 6, in pleonal segment 1; **7**, ostia pattern: 2, two pairs in close vicinity, separated by membrane (inlet showing arrangement); **8**, number of ostia pairs: 3, three; **9**, cardiac arteries pattern: 0, segmental; **10**, presence of ALAs: 0, present; **11**, destination of ALAs: 0, A1 and A2; **12**, destination of cardiac arteries: 1, visceral; **13**, descending artery: 1, present; **14**, splitting of descending artery: 1, present;



15, type of ventral vessel: 1, sending branches into thoracopods, connected to dorsal vessel through descending artery (for explanation see text); **16**, posterior aorta: 0, present; **17**, aortic dilation and mobp: 0, present; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **20**, optical arteries: 0, pair with common trunk off aorta. (F) ***Paramphisopus palustris*** (after Wirkner and Richter, 2003). **4**, anterior border of heart: 2, in thoracic segment 3; **5**, posterior border of heart: 7, in pleonal segment 4; **7**, ostia pattern: 6, isopod; **8**, number of ostia pairs: 4, two; **9**, cardiac arteries pattern: 4, one pair supplying anterior thoracopods, three pairs supplying one pair of thoracopods each, one pair supplying pleon; **10**, presence of ALAs: 0, present; **11**, destination of ALAs: 2, mandibles; **12**, destination of cardiac arteries: 0, thoracopods; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **20**, optical arteries: 0, pair with common trunk off aorta. (G) ***Leucon nasica*** (after Wirkner and Richter, 2008). **4**, anterior border of heart: 2, in thoracic segment 3; **5**, posterior border of heart: 3, in thoracic segment 5; **7**, ostia pattern: 3, one distinct, large pair; **8**, number of ostia pairs: 5, one; **9**, cardiac arteries pattern: 4, one pair supplying anterior thoracopods, three pairs supplying one pair of thoracopods each, one pair supplying pleon; **12**, destination of cardiac arteries: 0, thoracopods; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **21**, pericerebral ring: 1, present. (H) ***Hylalella azteca*** (after Wirkner and Richter, 2007b). **4**, anterior border of heart: 1, in thoracic segment 2; **5**, posterior border of heart: 1, in thoracic segment 7; **7**, ostia pattern: 5, one pair in Th4, one pair in Th5; **8**, number of ostia pairs: 3, three; **9**, cardiac arteries pattern: 3, three pairs in adjacent thoracic segments; **10**, presence of ALAs: 0, present; **11**, destination of ALAs: 2, mandibles; **12**, destination of cardiac arteries: 1, visceral; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **20**, optical arteries: 1, pair off aorta; **21**, pericerebral ring: 1, present. (I) ***Apseudes bermudeus*** (after Wirkner and Richter 2008). **4**, anterior border of heart: 2, in thoracic segment 3; **5**, posterior border of heart: 1, in thoracic segment 7; **7**, ostia pattern: 5, one pair in Th4, one pair in Th5; **8**, number of ostia pairs: 3, three; **9**, cardiac arteries pattern: 3, three pairs in adjacent thoracic segments; **10**, presence of ALAs: 0, present; **11**, destination of ALAs: 2, mandibles; **12**, destination of cardiac arteries: 1, visceral; **18**, aortic dilation with internalized oesophageal muscles m103 and m104: 1, present; **20**, optical arteries: 1, pair off aorta; **21**, pericerebral ring: 1, present.

Presence of the anterior lateral arteries (ALA). In close vicinity to the anterior aorta a pair of cardiac arteries branch off the heart in *L. typicus* (Fig. 1E, 10 : 0), mysid terminals (Fig. 1D, 10 : 0), some Amphipoda (*H. azteca*; Fig. 1H, 10 : 0), isopod terminals (Fig. 1F, 10 : 0), and some Tanaidacea (*T. dulongii*; Fig. 1I, 10 : 0). They also occur in Euphausiacea, Anaspidacea, Decapoda, and Stomatopoda.

Character 10: Presence of ALA: 0 = present; 1 = absent.

Destination of the ALA. The anterior lateral arteries lead to four different destinations. In *S. oratoria*, *T. dulongii* (Fig. 1I, 11 : 2), the isopod terminals (Fig. 1F, 11 : 2), and the two amphipods *H. azteca* (Fig. 1H, 11 : 2) and *O. cavimana* they run to the mandibular adductor muscles, in *L. typicus* (Fig. 1E, 11 : 0) they supply both the first and the second antennae, in *E. superba* the second antennae only, and in the decapod terminals the mandibular adductor muscles and the first and second antennae. In the mysid terminals (Fig. 1D) a pair of arteries branches off near the anterior end of the heart, but they bend ventrally posterior to the stomach and then run in a posterior direction laterally of the midgut to supply the midgut glands so are not homologized with ALA but with the hepatic arteries occurring in Decapoda, Euphausiacea and Anaspidacea.

Character 11: Destination of ALA: 0 = A1 + A2; 1 = A2; 2 = md; 3 = md, A1 + A2.

Destination of the other cardiac arteries. Two major destinations are distinguishable in the case of the cardiac arteries, which in the lophogastrid (Fig. 1E, 12 : 1), the mysid (Fig. 1D, 12 : 1), the amphipod (Fig. 1H, 12 : 1), and the tanaid terminals (Fig. 1I, 12 : 1) supply visceral organs such as the gut and the gonads, or in the isopod terminals (Fig. 1F, 12 : 0), the cumacean terminals (Fig. 1G, 12 : 0) and *S. oratoria* via the main stems the thoracopods. In this latter case, side branches also supply the viscera. A special case is described in *A. tasmaniae* where the cardiac arteries supply the pleopods. This is considered here as serially homologous to the supply of the thoracopods and therefore the character state is formulated as supplying either thoracopods or pleopods.

Character 12: Destination of cardiac arteries: thp or plp = 0; visceral = 1.

Arterial valves. Arteries leaving the heart are equipped with a valve that has two flaps. These valves most probably prevent hemolymph that has already entered the arteries from being sucked back into the lumen of the heart during systole. As this character occurs in all studied taxa it is phylogenetically uninformative at the level of Malacostraca.

Presence of descending artery. A descending artery is described as an artery that emanates from the heart and connects to the ventral vessel. It is normally greater in diameter than the other cardiac arteries. Within Peracarida, a descending artery is only present in Lophogastrida (Fig. 1E, 13 : 1) and Mysida (Fig. 1D, 13 : 1). A similar structure also occurs in Decapoda, Euphausiacea, and Anaspidacea. However, four details are prone to variation (intra- and/or interspecific). First, the descending artery can be unpaired (Mysida and some Decapoda) or paired, whereby the term paired also has to be defined. In *N. ingens* (Belman and Childress, 1976) and some decapod specimens (intraspecific variation; see Defretin, 1934; Wilkens et al., 1997; Vogt et al., 2009) two arteries connect the dorsal and ventral vessels, while in the other studied species only one branch of a pair of arteries makes the connection (the other branch being a visceral artery which is also smaller in diameter). The single descending artery can either be the left (*Eucopia* sp.; Siewing, 1956) or the right branch of the pair (e.g. *L. typicus*), or vary within a taxon (Defretin, 1934; Wilkens et al., 1997; Vogt et al., 2009). Further variation is seen in the segmental affiliation of the descending artery, which can lie either in the 6th thoracic segment (reported for some Brachyura at least; Mayrat et al., 2006), the 7th segment (most taxa, e.g. Lophogastrida, Mysida, and Euphausiacea) or even the 8th segment (some Decapoda; Mayrat et al., 2006). The homology of the descending artery has been a matter of detailed discussion (Siewing, 1956; Richter, 1994; Mayrat et al., 2006). Both the metameric origin and the thoracic connective through which the branches of the descending artery run were considered as criteria for homologization. However, neither criterion can serve as a robust homology hypothesis as they vary within and between the different taxa (see above). Nonetheless we still believe that as a structure connecting the dorsal with the ventral vessel the descending artery is homologous. The fact that this connection is made in most cases through an unpaired artery (one stem of a pair of arteries) in the posterior part of the thorax makes it, in combination with the criteria listed above, unlikely to be a product of convergent evolution.

Character 13: Descending artery: 0 = absent; 1 = present.

Splitting pattern of the descending artery. Dorsally to the nerve cord the descending artery splits into two or three branches that run down between the connectives in *A. tasmaniae*, *E. superba*, the lophogastrid (Fig. 1E, 14 : 1), and mysid terminals (Fig. 1D, 14 : 1). However, intraspecific variation in this feature complicates its

phylogenetic evaluation (see Wirkner and Richter, 2007a). The smallest common correspondence is that the descending artery splits above the nerve cord and the anterior branch (often referred to as the sternal artery) supplies at least the fifth to the first thoracopods, while the posterior branch (the caudal artery) supplies at least thoracopods 8. Therefore, only the presence of a splitting of the descending artery above the nerve cord is scored. In Decapoda, the descending artery pierces the ventral nerve cord undivided and connects to the ventral vessel.

Character 14: Splitting of descending artery: 0 = below ventral nerve cord; 1 = above ventral nerve cord.

Characters concerning the remaining HVS

Ventral vessel. A ventral vessel is described in several arthropod taxa. Two major states are discernible: the vessel runs either along the dorsal side of the nerve cord (in which case it is termed a supraneural vessel) or ventrally of the nerve cord (making it a subneural vessel). Within Malacostraca, both forms are present. A supraneural artery is described in Anaspidacea while in Stomatopoda, Decapoda, Euphausiacea, Lophogastrida, Mysida, and some Isopoda a subneural artery occurs. These vessels have been homologized in the literature in various ways, yet the positional criterion alone is not sufficient for homologization.

The stomatopod ventral vessel runs through the whole body and is connected to the dorsal vessel through a number of shunts (rami communicantes) branching off from cardiac arteries. The function of the ventral vessel in Stomatopoda is to supply the ventral nerve cord (Siewing, 1956). As this makes homology with other ventral vessels doubtful, a conservative scoring system is applied in which *S. oratoria* has its own state. The isopod ventral vessel resembles in some points that in stomatopods (Silen, 1954) but is most probably a structure acquired within Isopoda (see Wirkner and Richter, 2003). In the remaining taxa [Lophogastrida (Fig. 1E, 15 : 1), Mysida (Fig. 1D, 15 : 1), Euphausiacea, and Anaspidacea], however, the ventral vessel is connected to the dorsal vessel via a descending artery (see above) and supplies the thoracopods (and mouthparts). However, in Anaspidacea the ventral vessel is described as a supraneural vessel, or to be more exact, the sternal artery runs above the nerve cord while the caudal artery pierces the ventral nerve cord. This led Siewing (1956) to interpret the ventral vessel in Anaspidacea as having evolved independently. We, however, believe that it is homologous with the sternal arteries in Lophogastrida, Mysida, and Euphausiacea as it also supplies the thoracopods (via arteries that run down between the connectives) and is connected to the descending artery (see also Discussion).

Character 15: Presence of ventral vessel: 0 = sending branches into ventral nerve cord, connected to dorsal vessel through medial branches off some lateral cardiac arteries; 1 = sending branches into thoracopods, connected to dorsal vessel through descending artery.

Posterior aorta. The definition of a posterior aorta is narrowed down here to an unpaired artery elongating the heart posteriorly. The paired arteries emanating from the posterior end of the heart in *E. superba*, cumacean (Fig. 1G), and tanaidacean terminals (Fig. 1I) are, therefore, not posterior aortae (Wirkner and Richter, 2008) as was believed by earlier authors (e.g. Oelze, 1931; Siewing, 1953). In Cumacea, in particular, this last pair of cardiac arteries can be homologized with the last pair of cardiac arteries in Isopoda (see character 10 state 4). Consequently, within Peracarida, a posterior aorta is only scored as present in the lophogastrid (Fig. 1E, 16 : 0), mysid (Fig. 1D, 16 : 0), and amphipod terminals (Fig. 1H, 16 : 0). In Decapoda, Mysida, and Lophogastrida, the posterior aorta is equipped with lateral branches that mostly supply musculature in the pleon.

Character 16: Posterior aorta: 0 = present; 1 = absent.

Anterior aorta. An unpaired artery which continues the heart in an anterior direction and is separated from it by a valve is called the anterior aorta. An artery of this nature occurs in all studied arthropod species and can be homologized throughout all Euarthropoda (see Pass, 1991; Wirkner and Pass, 2002; Wirkner and Prendini, 2007). It is therefore phylogenetically uninformative at the level of Malacostraca.

Myoarterial formations. Myoarterial formations are described in a number of malacostracan species as dilated parts of the anterior aorta into which different muscles or pairs of muscles are internalized. These units are interpreted functionally as accessory pumping structures, as the intrinsic muscles might act as pumping motors (see also Discussion below). However, the only thorough functional–morphological studies to have been carried out concern Decapoda (Baumann, 1917; Powar, 1973; Steinacker, 1978) and Isopoda (Huber, 1992). And as pointed out by Huber (1992), the morphological disparity that exists in this regard is reflected in similar linguistic diversity: cor frontale, auxiliary heart, accessory heart, and frontal heart are all names given to structures that are thought to compensate for a reduced hemolymph pressure in the anterior cephalothorax, thereby mainly supplying the brain and associated

nervous structures (such as the optical lobes). Three major types of myoarterial formations are described within Malacostraca: the first form consists of aortic dilations that are associated with the oesophageal dilator muscles m103 and m104 (myoarterial formation a; see Wirkner and Richter, 2007a; and Discussion below). These myoarterial formations are only described in Peracarida (including Lophogastrida and Mysida; Fig. 1A–I, 18 : 1) and no reports of similar structures in other taxa were found in the literature. A second form, first described in Decapoda, is made up of muscles (musculi occuli basales posteriores, mobp: Baumann, 1917; Powar, 1973) that run from the dorsal cuticle of the anterior cephalothorax to a cuticular bar between the eyes, and an aortic dilation situated just above the brain (myoarterial formation b). Within Peracarida only *L. typicus* possesses this form (Wirkner and Richter, 2007a; Fig. 1E, 17 : 0). The third form occurs in Mysida: stomach dilators can be found inside an aortic dilation that is attached to the posterior stomach wall (myoarterial formation c; often confused with the second form in the literature; Fig. 1D, maf c). This formation is not scored here as it only occurs in Mysida.

Character 17: Myoarterial formation b: 0 = mobp internalized into anterior aorta (present); 1 = mobp not internalized into anterior aorta (absent).

Character 18: Myoarterial formation a: 0 = oesophageal dilator muscles m103 and m104 internalized into anterior aorta (present); 1 = oesophageal dilator muscles m103 and m104 not internalized into anterior aorta (absent).

Lateral stomach arteries (la off aorta). In *T. argentarii* (Fig. 1B, 19 : 1), *S. lepidops* (Fig. 1A, 19 : 1), and the tanaidacean terminals (Fig. 1I, 19 : 1) a pair of arteries emanates from the aorta laterally on the level of the anteriormost point of the stomach chamber. These arteries run backwards and downwards along the lateral stomach chamber wall, and for this reason and because of their position can be deemed to be homologous.

Character 19: Lateral stomach arteries: 0 = absent; 1 = present.

Optical arteries. The optic lobes and the eyes are supplied either by optical arteries stemming from a common trunk that splits off the anterior aorta (Fig. 1E,D,F, 20 : 0), or by a pair of arteries that branch off the anterior loop of the pericerebral vessel (Fig. 1A, H, 20: 1). The distally splitting anterior aorta described in Stomatopoda, Anaspidacea, and Decapoda is interpreted as being homologous to the arteries with a common trunk. The cumacean terminals are scored as "?" as supply to the eyes could not be satisfactorily clarified by Wirkner and Richter (2008), although in *D. rathkei*, Oelze (1931) described an unpaired artery branching off the aorta that splits to supply the eyes.

Character 20: Optical arteries: 0 = common trunk/split aorta; 1 = pair of arteries

Pericerebral ring. In *S. lepidops* (Fig. 1A, 21 : 1), *T. argentarii* (Fig. 1B, 21 : 1), and the cumacean (Fig. 1G, 21 : 1), amphipod (Fig. 1H, 21 : 1), and tanaidacean terminals (Fig. 1I, 21 : 1) the aorta splits into a dorsal and a ventral stem with the dorsal stem running dorsally around the brain while the ventral stem runs along the anterior oesophagus wall. The two stems reunite ventrally of the brain, thus completing a vessel ring around the brain. Various arteries branch off this vessel ring. An equivalent structure is missing in all other studied terminals.

Character 21: Pericerebral ring: 0 = absent; 1 = present.

Characters concerning the HLS

Podo-pericardial sinuses. In all studied peracaridan species, podo-pericardial sinuses (e.g. Fig. 2C, F in Wirkner and Richter, 2007b) extend the dorsal diaphragm into the legs to channel hemolymph into the pericardial sinus. This is thought to be plesiomorphic for the Malacostraca, as the epipodial gills (which insert at the thoracopods) are the plesiomorphic condition of respiration (Gruner, 1993). In Isopoda, however, parts of the pleopods function as gills (Wägele, 1989, 1992) and the posterior border of the heart is shifted into the pleon (see above), which means that the dorsal diaphragm can also be found in the pleon. Podo-pericardial sinuses, however, are only found in the pleon. In Amphipoda, the dorsal diaphragm also extends into the pleon but podo-pericardial sinuses are only present in the thorax.

Character 22: Podo-pericardial sinuses: 0 = in thorax; 1 = in pleon.

Respiratory carapace. In some Malacostraca the carapace can function along with various types of gills as an additional respiratory and therefore well-irrigated structure. The carapace is thought to be less effective than the gills. However, this type of respiration plays a major role during development as it is functional before the gills develop. Here, character 7 of Richter and Scholtz (2001) (character 29 in the present matrix) is changed to reflect the subsequently discovered elaborate capillary network in the carapace in *L. typicus* (see Wirkner and Richter, 2007a).

Character 29: Respiratory carapace: 0 = absent; 1 = present.

Cladistic analysis

After revision and addition of the 22 new circulatory organ system characters the matrix could be extended to 110 characters (Nexus file available as Supporting information). The cladistic analysis resulted in one single most parsimonious tree with a length of 309 steps (see Fig. 2). After "hard collapsing unsupported nodes" three nodes within Amphipoda and Cumacea collapsed. As relationships within peracarid taxa do not form the focus of this study these are not dealt with further.

The phylogenetic relationships that emerge from our cladistic analysis agree in large part with the analysis by Richter and Scholtz (2001), which was not unexpected in light of the significant overlap in the characters scored. All high-rank peracarid taxa are monophyletic and therefore in the following only their names are used. In contrast to Watling (1981, 1983, 1999) and Watling et al. (2000) and recent molecular systematic studies (Jarman et al., 2000; Spears et al., 2005; Meland and Willassen, 2007; Jenner et al., 2009, molecular data set) Peracarida is found here to be monophyletic and to include Mysida and Lophogastrida, which are sister groups. Like Richter and Scholtz (2001), we found no support for an amphipod–isopod clade (in contrast to Poore, 2005; and others) but instead for a mancoid clade comprising Isopoda, Cumacea, and Tanaidacea. However, unlike Richter and Scholtz (2001), we found Cumacea to be the sister group to Isopoda rather than to Tanaidacea, which is also the result to emerge from the 18S rRNA-based studies of Spears et al. (2005) and Meland and Willassen (2007). Thermosbaenacea is resolved as the sister group to Spelaeogriphacea and Mictacea nested within Peracarida (see also Wagner, 1994).

Evolution of the circulatory system in Peracarida (tracing of character evolution)

The circulatory systems in Peracarida are highly diverse and seem to have gone through major evolutionary changes, but some evolutionary trends are discernible nonetheless. Internal relationships within the peracaridan high-rank taxa (which cannot be satisfyingly reconstructed with the taxon sampling presented here) are not considered in the evolutionary analysis, and relationships outside the Peracarida are not the focus of this study.

The heart. As mentioned in the section on character conceptualization, pinpointing the exact location of the anterior and posterior borders of the heart is complicated by factors such as their position in the cephalothorax or on the border of two segments. Nonetheless, within Malacostraca, the heart seems to have its anterior extension in the 1st thoracic segment or even the head. Within Peracarida, this state is still observable within Lophogastrida and Mysida. Thermosbaenacea might have regained this state secondarily (Fig. 3A). But despite this, the significant reduction in the length of their heart surely casts them in a different light (see Wirkner and Richter, 2009). Within Peracarida, the anterior border of the heart shifted backwards in two steps (Fig. 3A), first in the lineage leading to Amphipoda etc. and second in the lineage leading to Mancoida *s.str*. This stepwise shift can be interpreted as predisposition for the further backward shift within Isopoda (see Wirkner and Richter, 2003).

No such clear evolutionary pattern is visible in the posterior border of the heart (Fig. 3B). In the plesiomorphic state within Malacostraca, the heart might have occupied the whole body (also feasible by comparison with Branchiopoda, e.g. Vehstedt, 1941) as its posterior border lies in the 4th or 5th pleonal segment in Stomatopoda and Leptostraca. The situation in Decapoda is derived, with a short globular heart, which also shows a unique histological design in adaptation to high circulatory demands in connection with body size etc. (e.g. McMahon and Burnett, 1990). Within Peracarida, the posterior border is restricted to the posterior segments of the thorax, i.e. the 5th to 8th segments. The posterior position of the border in the pleon in Isopoda has to be interpreted as a secondary process (see also Siewing, 1956; Wägele, 1992).

Ostia. One problem involved in obtaining evolutionary information on the position and/or number of ostia lies in the huge variability of these factors throughout the different taxa. Nevertheless, by considering ostial distribution patterns in combination with the numbers of ostia some trends do become evident.

The particular arrangement of two pairs of ostia in Lophogastrida and Mysida (see character 8 and Delage, 1883) might be an apomorphy for Mysidacea, as a certain degree of complexity is present: the two pairs lie in close proximity, both tilted slightly backwards above each other. Furthermore, a connective tissue membrane divides the two ostia from each other on each side (see also Wirkner and Richter, 2007a). On the other hand, the three pairs of ostia in *L. typicus* and *E. unguiculata* (character 9) are not homologous to those in Amphipoda (Fig. 3D). In Tanaidacea, two pairs of ostia are described in thoracomeres 3 and 4 (with the exception of *T. dulongii*, see Results). Together with the three pairs of cardiac arteries in the 4th, 5th, and 6th segments (*T. dulongii*, *A. bermudeus*, *Neotanais* sp.) this very

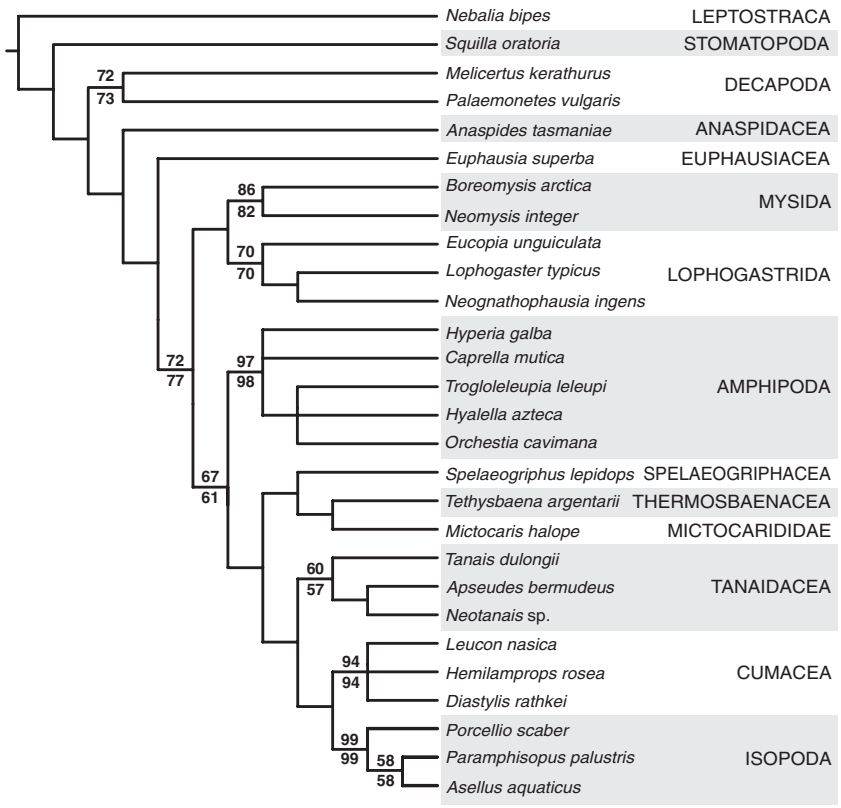
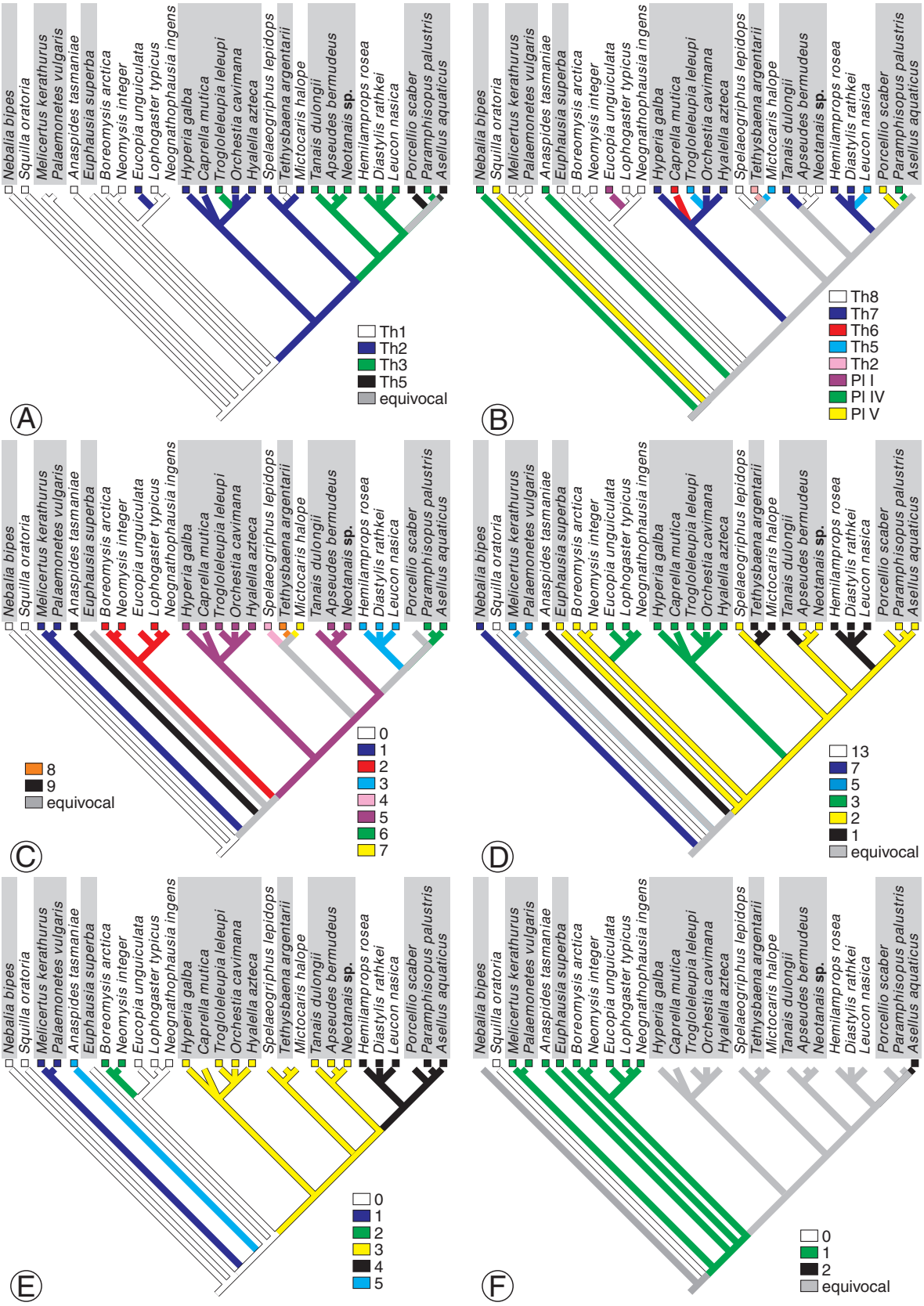


Fig. 2. Most parsimonious tree based on 110 morphological characters (see Table 2 and Materials and methods for details). Bootstrap values above branches, jackknife values below branches (both based on 1000 replicates; for details see also Materials and methods).

closely resembles the situation in Gammaridea at least (i.e. *O. cavimana*, *H. azteca*). The same ostial pattern is therefore assigned to Tanaidacea and Amphipoda and it becomes plausible that this pattern evolved only once (Fig. 3C; lilac; see also Wirkner and Richter, 2007b, 2008). The uniquely shaped pair of ostia found in the cumacean species (see Wirkner and Richter, 2008; Oelze, 1931) can also be interpreted as an apomorphy of Cumacea. A particular problem is posed by the single ostia pairs occurring in Anaspidacea, Thermosbaenacea, Cumacea, and Mictacea. While an independent reduction to one pair of ostia is feasible in Anaspidacea, *T. dulongii*, and Cumacea (Fig. 3D), it would be more parsimonious to interpret the presence of only one pair of ostia in *T. argentarii* and *M. halope* as homologous.

Cardiac arteries. The cardiac arterial system mainly serves the function of supplying organs and long body appendages with oxygenated hemolymph. Although the general body design and the position of organs did not change drastically during the evolution of Malacostraca, the distribution and destinations of the various arteries branching off the heart in various malacostracans did change to some extent. However, within high-rank taxa some uniformity is detectable. All the tanaidacean

Fig. 3. Tracing of character evolution on most parsimonious tree (see Fig. 2). Shaded boxes indicate monophyletic high-rank taxa (see Fig. 2). (A) Character 4: anterior border of heart. Th1, in first thoracic segment; Th2, in second thoracic segment; Th3, in third thoracic segment; Th5 in fifth thoracic segment. (B) Character 5: posterior border of heart. Th8, in eighth thoracic segment; Th7, in sixth thoracic segment; Th5, in fifth thoracic segment; Th2, in second thoracic segment; Pl I, in first pleonal segment; Pl IV, in fourth pleonal segment; Pl V, in fifth pleonal segment. (C) Character 7: ostia pattern. 0 = segmental; 1 = two pairs dorsally, two pairs laterally and one pair latero-ventrally; 2 = two pairs in close vicinity, separated by membrane; 3 = one distinct, large pair; 4 = one pair at transition Th5 to Th6, one pair at transition Th6 to Th7; 5 = one pair in Th4, one pair in Th5; 6 = two pairs, arranged asymmetrically; 7 = one pair in Th2; 8 = one pair in Th1; 9 = one pair in Th3. (D) Character 8: pairs of ostia. (E) Character 9: pattern of cardiac arteries. 0 = segmental; 1 = two pairs off anterior part of heart, descending artery; 2 = one pair, three unpaired, descending artery; 3 = three pairs in adjacent thoracic segments; 4 = one pair supplying anterior thoracopods, three pairs supplying one pair of thoracopods each, one pair supplying pleon; 5 = one pair in Th1, one pair in Th8, five pairs in pleon. (F) Character 15: type of ventral vessel. 0 = sending branches into ventral nerve cord, connected to dorsal vessel through medial branches off some lateral cardiac arteries; 1 = sending branches into thoracopods, connected to dorsal vessel through descending artery.


Nebalia bipes
Squilla oratoria
Melicertus kerathurus
Palaemonetes vulgaris
Anaspides tasmaniae
Euphausia superba
Boreomysis arctica
Neomysis integer
Eucopia unguiculata
Lophogaster typicus
Neognathophausia ingens
Hyperia galba
Caprella mutica
Troglolleupia leleupi
Orchestia cavimana
Hyalella azteca
Spelaeogriphus lepidops
Tethysbaena argentarii
Mictocaris halope
Tanais dulongii
Apseudes bermudeus
Neotanais sp.
Hemilamprops rosea
Diastylis rathkei
Leucon nasica
Porcellio scaber
Paramphisopus palustris
Asellus aquaticus
A
Th1
Th2
Th3
Th5
equivocal
B
Th8
Th7
Th6
Th5
Th2
Pl I
Pl IV
Pl V
C
8
9
equivocal
0
1
2
3
4
5
6
7
D
13
7
5
3
2
1
equivocal
E
0
1
2
3
4
5
F
0
1
2
equivocal

species so far described share at least three cardiac artery pairs in the 4th, 5th, and 6th thoracic segments. In most Amphipoda, too, three pairs of cardiac arteries are described as lying in the 4th–6th segments. In the studied caprellids (Wirkner and Richter, 2007b) and some Hyperiidea (not studied here; Claus, 1879), no cardiac arteries are present. This pattern can be interpreted as homologous for Amphipoda and Tanaidacea (Fig. 3E). Isopoda and Cumacea also share a common cardiac artery pattern with a pair of arteries supplying the fifth and anterior thoracopods (at least thoracopods 3; see Results), the next pairs each supplying one pair of thoracopods (i.e. thoracopods 6–8) and the last pair of cardiac arteries running into the pleon, where lateral arteries branch off in each segment. These patterns can be considered homologous (see also Wirkner and Richter, 2008). The reasons for changes in the distribution and destinations of cardiac arteries are not obvious. One aspect is particularly interesting: in Stomatopoda and Leptostraca thoracopods are supplied by cardiac arteries. This is taken over in Decapoda, Anaspidacea, and Euphausiacea, and within the Peracarida in Lophogastrida and Mysida by the ventral arterial system that supplies these appendages. However, Amphipoda and Tanaidacea do not have a ventral vessel, and nor do arteries run into the thoracopods. Hemolymph exchange is solely accomplished by the podo-pericardial sinuses. However, as these sinuses also occur in other taxa with an arterial supply to the thoracopods, it does not answer the question of how the lack of arterial supply is compensated for. Miniaturization might have played a role in the evolution of the small cave- or deep sea-dwelling taxa Thermosbaenacea, Spelaeogriphacea, and Mictacea. Our results suggest a single reduction of their cardiac arteries.

The ventral vessel system. As reported above, a ventral vessel system occurs in Stomatopoda, Decapoda, Euphausiacea, Anaspidacea, Lophogastrida, Mysida, and Isopoda, yet some problems arise with the homologization and evolution of these vessels. For the ventral vessel in Isopoda at least, a convergent evolution is strongly supported (Fig. 3F). With regard to a ventral vessel that is connected to the dorsal vessel via a descending artery, a further indication of the homology of the sternal artery in Anaspidacea and the other taxa might be the development of the ventral vessel system. In Crustacea (as in most other arthropods), the dorsal vessel develops during embryogenesis as a result of the fusion of latero-dorsal mesodermal cell clusters. These mesodermal cells meet in the dorsal midline of the body leaving a cavity in the middle that later in development becomes the lumen of the dorsal vessel (Siewing, 1969). The descending artery and the ventral vessel, on the other hand, develop in Decapoda in a secondary process during embryogenesis, laterally growing out from the dorsal vessel (Weygoldt, 1961). Early in development, the descending artery pierces the ganglia anlagen and continues as the ventral vessel. However, the difference in position of the sternal artery in Anaspidacea might be due to its delayed development. In *Anaspides tasmaniae*, the development of these vessels seems to take place after the hatching of the embryo as it is not observable during embryogenesis (Hickman, 1936). The present phylogenetic analysis corroborates that a ventral vessel, connected via a descending artery, evolved once in the stem lineage leading to Caridoida, i.e. Decapoda, Anaspidacea, Euphausiacea, Lophogastrida, and Mysida (Fig. 3F; green), and was lost within Peracarida in the lineage leading to Amphipoda and Mancoida *s.l.* Nonetheless, this contradicts the polyphyletic origin of Mysidacea favoured, for example, by Spears et al. (2005) and Meland and Willassen (2007). In this case the ventral vessel system would have to have evolved convergently at least twice.

The reason for the reduction of a ventral vessel system within Peracarida can only be speculated on. One common reason for the reduction of arterial systems is assumed to be the evolution of small body sizes, e.g. in Hexapoda (Pass, 2000), but there is no evidence to suggest that Amphipoda and Mancoida *s.l.* had especially small ancestors. With regard to the ventral vessel in Stomatopoda, a homologization to the ventral vessel in the other taxa is possible (Fig. 3F). Nonetheless, a re-investigation of the stomatopod ventral vessel is needed to corroborate this hypothesis.

Myoarterial formations or cor frontale. One of the most enigmatic structures of the circulatory system in Crustacea is the so-called "cor frontale". There are many accounts of it in the literature and several textbooks show aortic dilations termed cor frontale (Gruner, 1993; Brusca and Brusca, 2003), but detailed information (morphological or functional) is scarce and a comprehensive comparative analysis remains to be carried out. Due to the lack of experimental studies and therefore lack of information on the function of the structures at issue, these structures are termed myoarterial formations here (following Mayrat et al., 2006), as they are associations of different parts of the anterior aorta with different internalized muscles, located in the cephalothorax.

From our comparative morphological studies in combination with the literature, three different forms of myoarterial formation can be distinguished:

Myoarterial formation a: an aorta dilation into which the oesophageal dilator muscles m103 and m104 are internalized.

Myoarterial formation b: an aortic dilation through which the musculi oculi basales posteriores run, which represents the cor frontale most frequently described in Decapoda (Baumann, 1917; Powar, 1973). The structures resemble each other in Decapoda and *L. typicus* in

that the same pair of muscles are internalized in a dilation of the anterior aorta which lies a short distance dorsal to the brain.

Myoarterial formation c: A voluminous dilation of the anterior aorta posterior to the stomach chamber with internalized stomach musculature only found in Mysida.

As a basis for comparison of the muscles associated with the stomach, the detailed comparative–morphological and functional description of the stomach in *Asellus aquaticus* by Scheloske (1976) was chosen. The paired oesophageal muscles m103 and m104 run from the anterior cuticle to the anterior wall of the oesophagus. Similar muscles are found in all studied peracarid species and are therefore homologized with those described in *A. aquaticus* and termed m103 and m104. Functional aspects of the myoarterial formation a are discussed in Wirkner and Richter (2007c).

Posterior aorta. A posterior aorta has been described in a number of malacostracan species. It mostly constitutes an unpaired artery emanating from the posterior end of the heart. Confusingly, the last pair of cardiac arteries leading into the pleon in Euphausiacea and Cumacea was also termed posterior aortae (Zimmer and Gruner, 1956; Oelze, 1931). This has led to the interpretation that the last pair of arteries in Isopoda constitutes posterior aortae, despite the fact that the heart continues in a posterior direction after the branching of these arteries (Siewing, 1956). For the sake of precision, only an unpaired artery leading off the posterior end of the heart should be termed a posterior aorta. If this definition is applied a clear evolutionary scenario becomes evident (not shown). A posterior aorta is plesiomorphic for Malacostraca and has been reduced twice: once in Euphausiacea and once in the stem lineage leading to Mancoida *s.l.* This lack has been compensated for by the last pair of cardiac arteries that now supply the pleon in Mancoida *s.str.* In Thermosbaenacea, Spelaeogriphacea, and Mictacea, no cardiac arteries occur.

Discussion

Other hypotheses on the evolution of the circulatory system

The evolutionary phylogenetic analysis of the circulatory system in Malacostraca carried out by Siewing (1956) has been the most detailed to date. Although in some cases only single species were studied, and taxa such as Thermosbaenacea, Spelaeogriphacea, and Mictacea were not available, his statements about the evolution of this organ system are far reaching and can still be considered relatively accurate from a modern point of view. Although Siewing's main focus was on the high rank evolution of Malacostraca, we focus here on some points of his study that deal with the evolution in Peracarida.

First, Siewing stresses the close resemblance between the HVS in Mysidacea and that in Euphausiacea and Decapoda and concludes that Eucarida and Peracarida must be more closely related. However, from a cladistic point of view, these correspondences have to be considered as plesiomorphies (see above). Secondly, his views of the HVS within Peracarida converge with those presented here. He sees strong similarities between the HVS in Amphipoda and Tanaidacea on the one hand and that in Isopoda and Cumacea on the other. Nevertheless, these correspondences do not lead him to argue for a closer relationship between the latter two taxa.

Watling (1981, 1983, 1999) and Watling et al. (2000) presented alternative viewpoints on malacostracan phylogeny. In his 1983 paper, Watling tried to explain the morphological disparity of peracaridans by assigning individual structures, such as the mandibles and carapace (but also developmental patterns), to different types. On the basis of data from the literature, he also classified the circulatory organs in this manner, distinguishing four major types of circulatory system within Malacostraca. The first type (Type I) is described as having an elongated dorsal blood vessel, posterior ventrally directed arteries, and a descending artery. Anaspidacea, Lophogastrida, Mysida, and Euphausiacea are all assigned to Type I. Cumacea, Tanaidacea, Thermosbaenacea, and "probably" the Spelaeogriphacea are interpreted to belong to Type II, while the amphipod CS is considered to be Type III. As isopods differ from all the others in having "paired anterior aortae, a subneural artery running the length of the body and lateral arteries leading directly into the pereopods" (Watling, 1983), they are characterized as Type IV.

With regard to Type I, a characterization of the arterial assemblage of the descending artery and connected ventral vessel does seem appropriate as it is rather uniform throughout these taxa (see characters 5 + 6). However, heart length, numbers of ostia, and the HVS in the cephalothorax vary greatly between Anaspidacea, Lophogastrida, Mysida, and Euphausiacea. In Anaspidacea, the tubular heart runs from the 1st thoracic segment to the IVth pleonal segment, while it is a short globular organ in Euphausiacea. Numbers of cardiac arteries vary from five to ten, making it difficult to establish an overall pattern. Type II also seems to be inadequate, for the following reasons. First, the HVS in Cumacea does not appear to be reduced as reported by earlier authors (e.g. Siewing, 1956). On the contrary, a pattern of arteries is discernible similar to that described in Isopoda (see Wirkner and Richter, 2008). Secondly, the HVS in Thermosbaenacea and Spelaeogriphacea differs greatly from that in Cumacea and Amphipoda in that the

former taxa only have an anterior aorta branching off the heart. Last but not least, the patterns of ostia and cardiac arteries even differ between Cumacea and Amphipoda to some extent. In the former, these arteries run into the thoracopods, while in Amphipoda they supply the viscera. Furthermore, it is not clear why Watling assigned Amphipoda to Type III, because as discussed in the character analysis, Amphipoda share more likely patterns of ostia and cardiac artery distribution with the tanaids (see also Wirkner and Richter, 2008). With respect to Isopoda, Watling used the common scheme of circulatory organs presented by Siewing (1960), but as this resembles a derived state within Isopoda (for details see Wirkner and Richter, 2003) it is of little value in a phylogenetic or evolutionary scenario of high-rank taxa. On the other hand, characters associated with the descending artery and the ventral vessel support some of the nodes of the phylogeny presented by Watling et al. (2000). These characters, however, can be interpreted as plesiomorphies in Mysidacea when they are regarded as Peracarida, while peracaridan characters such as the oostegites or the lacinia mobilis must have evolved convergently if a eucarid descent applies.

Résumé

By including 22 newly conceptualized characters of the circulatory system in Malacostraca into the revised data matrix by Richter and Scholtz (2001), we were able to obtain a comprehensive morphological data matrix on Malacostraca. The evolution of the circulatory system in Peracarida as based on the cladistic analysis of the 110 characters over 28 taxa can be summarized as follows:

Circulatory system characters in the ground pattern of Peracarida are a tubular heart extending through the entire thorax, a posterior aorta with lateral arteries, and a ventral vessel system made up of a descending artery (that splits above the ventral nerve cord into a sternal and a caudal artery) and a subneural vessel (that supplies the thoracopods and most probably also the two maxillae). With regard to the number of pairs of ostia and cardiac arteries and the supply of the antennae and the mouthparts no clear statements can be made, although two pairs of ostia are suggested as the state at the base of the Peracarida.

Character changes in the stem lineage leading to Amphipoda + Mancoida *s.l.* are the reduction of a ventral vessel system, the shift of the anterior border of the heart backwards into the 2nd thoracic segment and a pattern of three segmentally arranged pairs of ostia. While a posterior aorta prevails, lateral arteries are absent. Cardiac arteries supply the viscera only and the antennae are supplied by arteries branching off the aorta.

The three taxa Spelaeogriphacea, Mictacea, and Thermosbaenacea share a common ancestry in which the HVS was greatly reduced to a heart and an anterior aorta.

In the stem lineage to Mancoida *s.str.* a further backward shift of the anterior border of the heart into thoracic segment 3 occurred and the reduced posterior aorta was replaced by the last pair of cardiac arteries. As Tanaidacea share the same pattern of cardiac arteries as Amphipoda this can also be accounted to the ground pattern of Mancoida *s.str.*

Cumacea and Isopoda are sister groups and share a common pattern of cardiac arteries. The cardiac arteries mainly supply the thoracopods and the pleon.

Contradicting the claims made in many textbooks, the Isopoda display a peracarid circulatory system and are deeply nested within Peracarida. The convergent evolution of their heart and a sister-group relationship to Amphipoda are excluded.

Acknowledgements

We thank Drs George D. F. Wilson, Gregory D. Edgecombe, and Frederick R. Schram for helpful comments and Lucy Cathrow for improving the English text. Our research was supported by the German Science Foundation (DFG) WI 3334/1-2 (to C.S.W.) and RI 837/6-1 (to S.R.).

References

Audoin, V., Milne-Edwards, H., 1827. Recherches anatomique et physiologiques sur la circulation dans les Crustacés. Annales des sciences naturelles Sér I. 11, 283–314, 352–393.

Baccetti, B., Bigliardi, E., 1969. Studies on the fine structure of the dorsal vessel of arthropods. II. The "heart" of a crustacean. Z. Zellforsch. Mikrosk. Anat. 99, 25–36.

Baumann, H., 1917. Das Cor frontale bei decapoden Krebsen. Zool. Anz. 49, 137–144.

Belman, B.W., Childress, J.J., 1976. Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biol. Bull. 150, 15–37.

Betz, O., Wegst, U., Weide, D., Heethoff, M., Helfen, L., Lee, W.K., Cloetens, P., 2007. Imaging applications of synchrotron X-ray phase-contrast microtomography in biological morphology and biomaterials science. 1. General aspects of the technique and its advantages in the analysis of millimetre-sized arthropod structure. J. Microsc. 227, 51–71.

Brody, M.S., Perkins, E.B., 1930. The arterial system of *Palaemonetes*. J. Morphol. 50, 127–142.

Brusca, R.C., Brusca, G.J., 2003. Invertebrates. Sinauer Associates, Sunderland, MA.

Brusca, R.C., Wilson, G.D.F., 1991. A phylogenetic analysis of the Isopoda with some classificatory recommendations. Mem. Queensland Mus. 31, 143–204.

Claus, C., 1879. Der Organismus der Phronimiden. Arb. Zool. Inst. Univ. Wien 2, 59–146.

Claus, C., 1884a. Über *Apseudes latreillii* Edw. und die Tanaiden. Arb. Zool. Inst. Univ. Wien 5, 319–332.

Claus, C., 1884b. Zur Kenntnis der Kreislauforgane der Schizopoden und Decapoden. Arb. Zool. Inst. Univ. Wien 5, 271–318.
Claus, C., 1888. Über *Apseudes latreillii* Edw. und die Tanaiden. Arb. Zool. Inst. Univ. Wien 7, 139–220.
De Jong-Moreau, L., Casanova, J.-P., 2001. The foreguts of the primitive families of the Mysida (Crustacea, Peracarida): a transitional link between those of the Lophogastrida (Crustacea, Mysidacea) and the most evolved Mysida. Acta Zool. 82, 137–147.
Defretin, R., 1934. Sur la Présence d'une Artère sternale double chez l'Écrevisse. Valeur morphologique de ce Vaisseau. B. Soc. Zool. Fr. 59, 231–236.
Delage, Y., 1881. Contribution a l'étude de l'appareil circulatoire des Crustacés Édriophthalmes marins. Arch. Zool. exp. gen. 9, 1–173.
Delage, Y., 1883. Circulation et respiration chez les Crustacés Schizopodes (*Mysis* Latr.). Arch. Zool. exp. gen. 1, 105–130.
Edgecombe, G.D., 2008. Anatomical nomenclature: homology, standardization and datasets. Zootaxa 1950, 87–95.
Fitzhugh, K., 2006. The philosophical basis of character coding for the inference of phylogenetic hypotheses. Zool. Scripta 35, 261–286.
Giribet, G., Edgecombe, G.D., Wheeler, W.C., 2001. Arthropod phylogeny based on eight molecular loci and morphology. Nature 413, 157–161.
Goloboff, P.A., 1998. Nona. Ver. 1.9. American Museum of Natural History, New York.
Grant, T., Kluge, A.G., 2004. Transformation series as an ideographic character concept. Cladistics 20, 23–31.
Gruner, H.-E., (Ed.) 1993. Arthropoda. Lehrbuch der Speziellen Zoologie. Gustav Fischer Verlag, Jena, Stuttgart, New York.
Gutu, G., 1998. Spelaeogriphacea and Mictacea (partim) suborders of a new order, Cosinzeneacea (Crustacea, Peracrida). Trav. Mus. Hist. Nat. 'Grigore Antipa' 40, 121–129.
Gutu, G., Iliffe, T.M., 1998. Description of a new hirsutiid (n.g., n.sp.) and reassignment of this family from order Mictacea to the new order, Bochusacea (Crustacea, Peracrida). Trav. Mus. Hist. Nat. 'Grigore Antipa' 40, 93–120.
Haye, P.A., Kornfield, I., Watling, L., 2004. Molecular insights into cumacean family relationships (Crustacea, Cumacea). Mol. Phylogenet. Evol. 30, 798–809.
Hejnol, A., Schnabel, R., Scholtz, G., 2006. A 4D-microscopic analysis of the germ band in *Porcellio scaber* (Malacostraca, Peracarida) – developmental and phylogenetic implications. Dev. Genes. Evol. 216, 755–767.
Hennig, W., 1966. Phylogenetic Systematics. University of Illinois Press, Chicago, IL.
Hessler, R.R., 1982. The structural morphology of walking mechanisms in eumalacostracan crustaceans. Philos. Trans. R. Soc. Lond. B Biol. Sci. 296, 245–298.
Hickman, V., 1936. The embryology of the syncarid crustacean *Anaspides tasmaniae*. Pap. Proc. Roy. Soc. Tasmania 1936, 1–35.
Hillis, D.M., Wiens, J.J., 2000. Molecules versus morphology in systematics: Conflicts, artifacts, and misconceptions. In: Wiens, J.J. (Ed.), Phylogenetic Analysis of Morphological Data. Smithsonian Institution Press, Washington D.C, pp. 1–19.
Hiraiwa, Y.K., 1933. Studies on a bopyrid, *Epipenaeon japonica* Thielemann. I. Morphological studies in both sexes. J. Sci. Hiroshima Univ. B 2, 49–70.
Howse, H.D., Ferrans, V.J., Hibbs, R.G., 1971. A light and electron microscopic study of the heart of a crayfish, *Procambarus clarkii* (Giraud). II. Fine structure. J. Morphol. 133, 353–374.
Huber, B.A., 1992. Frontal heart and arterial system in the head of Isopoda. Crustaceana 63, 57–69.
Jarman, S.N., Nicol, S., Elliott, N.G., McMinn, A., 2000. 28S rDNA evolution in the Eumalacostraca and the phylogenetic position of krill. Mol. Phylogenet. Evol. 17, 26–36.
Jenner, R.A., 2004. Accepting partnership by submission? morphological phylogenetics in a molecular millennium. Systemat. Biol. 53, 333–342.
Jenner, R.A., Ní Dhubhghaill, C., Ferla, M.P., Wills, M.A., 2009. Eumalacostracan phylogeny and total evidence: limitations of the usual suspects. BMC Evol. Biol. 9, 21.
Kim, C.B., Kim, W., 1993. Phylogenetic relationships among gammaridean families and amphipod suborders. J. Nat. Hist. 27, 933–946.
Klövekorn, J., 1934. Das Organsystem der Blutbewegung bei *Gammarus pulex* L. Z. wiss. Zool. 146, 153–192.
Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of the relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38, 7–25.
Kluge, A., 1993. *Aspidites* and the phylogeny of pythonine snakes. Rec. Aust. Mus., Suppl. 19, 1–77.
Komai, T., Tung, Y.M., 1931. On some points of the internal structure of *Squilla oratoria*. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B 6, 2–16.
Larsen, L.N., 1983. The heart ultrastructure of *Gammarus lacustris* G.O. Sars and Gammarus pulex (L.) (Crustacea, Amphipoda). Zool. Anz. 210, 289–295.
Larsen, K., Wilson, G.D.F., 2002. Tanaidacean phylogeny, the first step: the superfamily Paratanaidoidea. J. Zool. Syst. Evol. Res. 40, 205–222.
Lee, M.S.Y., Scanlon, J.D., 2002. Snake phylogeny based on osteology, soft anatomy and ecology. Biol. Rev. 77, 333–401.
Maddison, W.P., Maddison, D.R., 1992. MacClade: Analysis of Phylogeny and Character Evolution. Version 3.0. Sinauer Associates, Sunderland, MA.
Maddison, W.P., Maddison, D.R., 2008. Mesquite: a modular system for evolutionary analysis. Version 2.5. http://mesquite project.org.
Mayrat, A., 1958. Le Système atériel des Pénéides. Comparaison avec les autres Décapodes et les Mysidacés (Recherches sur l'Appareil circulatoire des Crustacés, II). Arch. Zool. exp. gen. 95, 69–78.
Mayrat, A., McMahon, B.R., Tanaka, K., 2006. The circulatory system. In: Forest, J., von Vaupel Klein, J.C., Schram, F.R. (Eds.), Treatise on Zoology – Anatomy, Taxonomy, Biology. The Crustacea Revised and Updated from the Traité de Zoologie, 2. Koninklijke Brill Academic Publishers, Leiden pp. 3–84.
McMahon, B.R., Burnett, L.E., 1990. The crustacean open circulatory system: a reexamination. Physiol. Zool. 63, 35–71.
Meland, K., 2003. Phylogeny, evolution and biogeography of the mysid genus *Pseudomma* (Crustacea, Peracarida, Mysidacea), including a phylogenetic analysis of the order Mysidacea. Ph.D. thesis.
Meland, K., Willassen, E., 2007. The disunity of "Mysidacea" (Crustacea). Mol. Phylogenet. Evol. 44, 1083–1104.
Nixon, K.C., 1999–2002. Winclada, Ver. 10. Published by the author, NY.
Nylund, A., 1981. The heart ultrastructure of *Praunus flexuosus* (Müller) and *Mysis relicta* (Lovén) (Crustacea, Mysidacea). J. Crust. Biol. 1, 558–566.
Nylund, A., 1986. Myocardial ultrastructure of *Tanais cavolinii* (Crustacea: Tanaidacea) and some phylogenetic considerations. J. Crust. Biol. 6, 199–206.
Nylund, A., Tjønneland, A., 1989. Crustacean heart ultrastructure and its phylogenetic implications, with special reference to the position of the isopods within the eumalacostracan phylogeny. Mon. Zool. Italiano 4, 29–42.
Nylund, A., Økland, S., Tjønneland, A., 1987. The crustacean heart ultrastructure and its bearing upon the position of the isopods in the eumalacostracan phylogeny. Zool. Scripta 16, 235–241.
Oelze, A., 1931. Beiträge zur Anatomie von *Diastylis rathkei* Kr. Zool. Jahrb., Abt. Anat. Ontog. Tiere 54, 235–294.

Pagel, M., 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48, 612–622.
Pass, G., 1991. Antennal circulatory organs in Onychophora, Myriapoda and Hexapoda: Functional morphology and evolutionary implications. Zoomorphology 110, 145–164.
Pass, G., 2000. Accessory pulsatile organs: Evolutionary innovations in insects. Ann. Rev. Entomol. 45, 495–518.
Pires, A.M.S., 1987. *Potiicoara brasiliensis*: a new genus and species of Spelaeogriphacea (Crustacea: Peracarida) from Brazil, with a phylogenetic analysis of the Peracarida. J. Nat. Hist. 21, 225–238.
Pleijel, F., 1995. On character coding for phylogeny reconstruction. Cladistics 11, 309–315.
Poore, G.C.B., 2005. Peracarida: monophyly, relationships and evolutionary success. Nauplius 13, 1–27.
Powar, C.B., 1973. The accessory heart of the crab *Paratelphusa guerinii* (Milne-Edwards). Zool. Anz. 190, 77–85.
Prendini, L., 2001. Species or supraspecific taxa as terminals in cladistic analysis? groundplans versus exemplars revisited. Syst. Biol. 50, 290–300.
Ramirez, M.J., Coddington, J.A., Maddison, W.P., Midford, P.E., Prendini, L., Miller, J., Griswold, C.E., Hormiga, G., Sierwald, P., Scharff, N., Benjamin, S.P., Wheeler, W.C., 2007. Linking digital images to phylogenetic data matrices using a morphological ontology. Systemat. Biol. 56, 283–294.
Richter, S., 1994. Aspekte der phylogenetischen Systematik der Malacostraca (Crustacea) unter besonderer Berücksichtigung der Caridoida auf der Grundlage vergleichend-morphologischer Untersuchungen der Komplexaugen und anderer Merkmale. PhD Thesis, Freie Universität Berlin, pp. 1–171.
Richter, S., 2005. Homologies in phylogenetic analyses – concept and tests. Theory Biosci. 124, 105–120.
Richter, S., Scholtz, G., 2001. Phylogenetic analysis of the Malacostraca (Crustacea). J. Zool. Syst. Evol. Res. 39, 113–136.
Ruthensteiner, B., Heß, M., 2008. Embedding 3D models of biological specimens in PDF publications. Microsc. Tech. Res. 71, 778–786.
Scheloske, H.-W., 1976. Vergleichend-morphologische und funktionelle Untersuchungen am Magen von *Asellus aquaticus* (L.) (Asellidae, Isopoda). Zool. Jahrb., Abt. Anat. Ontog. Tiere 95, 519–573.
Schram, F.R., 1986. Crustacea. Oxford Universtiy Press, New York, Oxford.
Schram, F.R., Hof, C.H.J., 1998. Fossils and the interrelationships of major crustacean groups. In: Edgecombe, G.D. (Ed.), Arthropod Fossils and Phylogeny. Columbia University Press, New York. pp. 233–302.
Scotland, R.W., Olmstead, R.G., Bennett, J.R., 2003. Phylogeny reconstruction: the role of morphology. Systemat. Biol. 52, 539–548.
Sieg, J., 1983. Evolution of Tanaidacea. In: Schram, F.R. (Ed.), Crustacean Phylogeny. A.A. Balkema, Rotterdam. pp. 229–256.
Siewing, R., 1952. Morphologische Untersuchungen an Cumaceen (*Cumopsis goodsiri* v. Beneden). Zool. Jahrb., Abt. Anat. Ontog. Tiere 72, 522–559.
Siewing, R., 1953. Morphologische Untersuchungen an Tanaidaceen und Lophogastriden. Z. wiss. Zool. 157, 333–426.
Siewing, R., 1954. Über die Verwandtschaftsbeziehungen der Anaspidaceen. Verh. Dtsch. Zool. Ges. 16, 240–252.
Siewing, R., 1956. Untersuchungen zur Morphologie der Malacostraca (Crustacea). Zool. Jahrb., Abt. Anat. Ontog. Tiere 75, 39–176.
Siewing, R., 1960. Neuere Ergebnisse der Verwandtschaftsforschung bei den Crustaceen. Wissenschaftl. Zeitschr. Universität Rostock 9, 343–358.
Siewing, R., 1963a. Studies in malacostracan morphology: results and problems. In: Whittington, H.B., Rolfe, W.D.I. (Eds.), Phylogeny and Evolution of Crustacea. Museum of Comparative Zoology, Special Publication, Cambridge, Mass. pp. 85–103.
Siewing, R., 1963b. Zur Morphologie der aberranten Amphipodengruppe Ingolfiellidae und zur Bedeutung extremer Kleinformen für die Phylogenie. Zool. Anz. 171, 75–91.
Siewing, R., 1969. Lehrbuch der vergleichenden Entwicklungsgeschichte der Tiere. Parey Verlag, Hamburg.
Silen, L., 1954. On the circulatory system of the Isopoda Oniscoidea. Acta Zool. 35, 11–70.
Spears, T., DeBry, R.W., Abele, L.G., Chodyla, K., 2005. Peracarid monophyly and interordinal phylogeny inferred from small-subunit ribosomal DNA sequences (Crustacea: Malacostraca: Peracarida). Proc. Biol. Soc. Wash. 118, 117–157.
Steinacker, A., 1978. The anatomy of the decapod crustacean auxiliary heart. Biol. Bull. 154, 497–507.
Sudhaus, W., 2007. Die Notwendigkeit morphologischer Analysen zur Rekonstruktion der Stammesgeschichte. Spec. Phyl. Evol. 1, 17–32.
Szucsich, N.U., Wirkner, C.S., 2007. Homology: a synthetic concept of evolutionary robustness of patterns. Zool. Scripta 36, 281–289.
Tjønneland, A., Økland, S., Nylund, A., 1987. Evolutionary aspects of the Arthropod heart. Zool. Scripta 16, 157–175.
Vehstedt, R., 1941. Über Bau, Tätigkeit und Entwicklung des Rückengefäßes und des lacunären Systems von *Artemia salina*, var. *arieta*. Z. wiss. Zool. 154, 1–39.
Vogt, L., 2008. Learning from Linnaeus: towards developing the foundations for a general structure concept for morphology. Zootaxa 1950, 123–152.
Vogt, L., 2009. The future role of bio-ontologies for developing a general data standard in biology: chance and challenge for zoomorphology. Zoomorphology, 128, 201–217.
Vogt, G., Wirkner, C.S., Richter, S., 2009. Symmetry variation in the heart-descending artery system of the parthenogenetic Marbled Crayfish. J. Morphol. 207, 221–226.
Wägele, J.W., 1989. Evolution und phylogenetisches System der Isopoda. Zoologica 140, 1–262.
Wägele, J.W., 1992. Isopoda. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic Anatomy of Invertebrates. Wiley-Liss, New York, Chichester, etc. pp. 529–617.
Wagner, H.P., 1994. A monographic review of the Thermosbaenacea (Crustacea: Peracarida) – A study on their morphology, taxonomy, phylogeny and biogeography. Zool. Verhand. 291, 3–338.
Watling, L., 1981. An alternative phylogeny of the peracarid crustaceans. J. Crust. Biol. 1, 201–210.
Watling, L., 1983. Peracaridan disunity and its bearing on eumalacostracan phylogeny with a redefinition of eumalacostracan superorders. Crust. Iss. 1, 213–228.
Watling, L., 1999. Towards understanding the relationship of the peracaridan orders: the necessity of determing exact homologies. In: Schram, F.R., von Vaupel Klein, J.C. (Eds.), Crustaceans and the Biodiversity Crisis. Proceedings of the Fourth International Crustacean Congress. Brill NV, Leiden, The Netherlands. pp. 73–89.
Watling, L., Hof, C.H.J., Schram, F.R., 2000. The place of the Hoplocarida in the malacostracan pantheon. J. Crust. Biol. 20, 1–11.
Weygoldt, P., 1961. Beitrag zur Kenntnis der Ontogenie der Dekapoden: Embryologische Untersuchungen an *Palaemonetes varians* (Leach). Zool. Jahrb., Abt. Anat. Ontog. Tiere 79, 223–270.
Wilkens, J.L., Yazawa, T., Cavey, M.J., 1997. Evolutionary derivation of the American lobster cardiovascular system: a hypothesis based on morphological and physiological evidence. Invert. Biol. 116, 30–38.
Wilkinson, M., 1995. A comparison of two methods of character construction. Cladistics 11, 297–308.
Wills, M.A., 1998. Crustacean disparity through the Phanerozoic: comparing morphological and stratigraphic data. Biol. J. Linn. Soc. 65, 455–500.

Wirkner, C.S., 2009. The circulatory system in Malacostraca – evaluating character evolution on different phylogenetic hypotheses. Arthrop. Syst. Phyl. 67, 57–70.
Wirkner, C.S., Pass, G., 2002. The circulatory system in Chilopoda: evolutionary and phylogenetic aspects. Acta Zool. 83, 197–202.
Wirkner, C.S., Prendini, L., 2007. Comparative morphology of the hemolymph vascular system in scorpions – A survey using corrosion casting, MicroCT, and 3D-reconstruction. J. Morphol. 268, 401–413.
Wirkner, C.S., Richter, S., 2003. The circulatory system in Phreatoicidea: implication for the isopod ground pattern and peracarid phylogeny. Arth. Str. Dev. 32, 337–347.
Wirkner, C.S., Richter, S., 2004. Improvement of microanatomical research by combining corrosion casts with MicroCT and 3D reconstruction, exemplified in the circulatory organs of the woodlouse. Microsc. Res. Tech. 64, 250–254.
Wirkner, C.S., Richter, S., 2007a. The circulatory system in Mysidacea – Implications for the phylogenetic position of Lophogastrida and Mysida (Malacostraca, Crustacea). J. Morphol. 268, 311–328.
Wirkner, C.S., Richter, S., 2007b. Comparative analysis of the circulatory system in Amphipoda (Malacostraca, Crustacea). Acta Zool. 88, 159–171.
Wirkner, C.S., Richter, S., 2007c. The circulatory system and its spatial relations to other major organ systems in Spelaeogriphacea and Mictacea (Malacostraca, Crustacea) – a three-dimensional analysis. Zool. J. Linn. Soc. 149, 629–642.
Wirkner, C.S., Richter, S., 2008. Morphology of the haemolymph vascular system in Tanaidacea and Cumacea: – Implications for the relationships of "core group" Peracarida (Malacostraca; Crustacea). Arth. Str. Dev. 37, 141–154.
Wirkner, C.S., Richter, S., 2009. The hemolymph vascular system in *Tethysbaena argentarii* (Thermosbaenacea, Monodellidae) as revealed by 3D reconstruction of semi-thin sections. J. Crust. Biol. 29, 13–17.
Wolff, C., Scholtz, G., 2006. Cell lineage analysis of the mandibular segment of the amphipod *Orchestia cavimana* reveals that the crustacean paragnaths are sternal outgrowths and not limbs. Front. Zool. 3, 19.
Yeates, D.K., 1995. Groundplans and exemplars: paths to the tree of live. Cladistics 11, 343–357.
Zimmer, C., 1913. Untersuchungen über den inneren Bau von *Euphausia superba* Dana. Zoologica 67, 65–127.
Zimmer, C., Gruner, H.-E., 1956. 6. Buch, III. Teil Euphausiacea. In: Gruner, H.-E. (Ed.), 5. Band, 1. Abteilung Custacea. Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig, pp. 1–185.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Nexus file of matrix containing 28 malcostracan terminals and 110 characters.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.