frontiers | Frontiers in Marine Science

REVIEW
published: 30 June 2022
doi: 10.3389/fmars.2022.799191

Challenges and Advances in the Taxonomy of Deep-Sea Peracarida: From Traditional to Modern Methods

Inmaculada Frutos[1*], *Stefanie Kaiser*[1†], *Łukasz Pułaski*[2,3], *Maciej Studzian*[2,3] *and Magdalena Błażewicz*[1]

[1] Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland, [2] Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland, [3] Laboratory of Transcriptional Regulation, Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland

OPEN ACCESS

Edited by:
Ana Colaço, Marine Research Institute (IMAR), Portugal

Reviewed by:
Oliver Simon Ashford, OceanMind, United Kingdom
Andres G. Morales-Nunez, University of Maryland Eastern Shore, United States

***Correspondence:**
Inmaculada Frutos
inmaculada.frutos@biol.uni.lodz.pl

†Present address:
Stefanie Kaiser, INES Integrated Environmental Solutions UG, c/o DZMB, Wilhelmshaven, Germany

Specialty section:
This article was submitted to Deep-Sea Environments and Ecology, a section of the journal Frontiers in Marine Science

Received: *21 October 2021*
Accepted: *05 May 2022*
Published: *30 June 2022*

Citation:
Frutos I, Kaiser S, Pułaski Ł, Studzian M and Błażewicz M (2022) Challenges and Advances in the Taxonomy of Deep-Sea Peracarida: From Traditional to Modern Methods. Front. Mar. Sci. 9:799191. doi: 10.3389/fmars.2022.799191

As one of the oldest branches of biology, taxonomy deals with the identification, classification and naming of living organisms, using a variety of tools to explore traits at the morphological and molecular level. In the deep sea, particular challenges are posed to the taxonomic differentiation of species. Relatively limited sampling effort coupled with apparent high diversity, compared to many other marine environments, means that many species sampled are undescribed, and few specimens are available for each putative species. The resulting scarce knowledge of intraspecific variation makes it difficult to recognize species boundaries and thus to assess the actual diversity and distribution of species. In this review article, we highlight some of these challenges in deep-sea taxonomy using the example of peracarid crustaceans. Specifically, we offer a detailed overview of traditional as well as modern methods that are used in the taxonomic analysis of deep-sea Peracarida. Furthermore, methods are presented that have not yet been used in peracarid taxonomy, but have potential for the analysis of internal and external structures in the future. The focus of this compilation is on morphological methods for the identification, delimitation and description of species, with references to molecular analysis included where relevant, as these methods are an indispensable part of an integrative taxonomic approach. The taxonomic impediment, i.e. the shortage of taxonomists in view of a high undescribed biodiversity, is discussed in the context of the existing large taxonomic knowledge gaps in connection with the increasing threat to deep-sea ecosystems. Whilst peracarid crustaceans are used here as an exemplary taxon, the methodology described has broad relevance to many other deep-sea taxa, and thus will support broader research into deep-sea biodiversity and ecology more widely.

Keywords: integrative taxonomy, morphology, microscopy, imaging, suprabenthos, Isopoda, Tanaidacea, Amphipoda

1 INTRODUCTION

The dichotomy in deep-sea biodiversity research consisting of a gap between the sheer scale of the deep sea and our incomplete knowledge of what actually lives there, is immense; areas away from the shelf edge making up the deep sea cover more than two-thirds of the Earth's global surface, but only a tiny portion of this has been examined by scientists (Ramirez-Llodra et al., 2010; Costello and Chaudhary, 2017). It is in part because of this limited knowledge that estimates of how many metazoan species to expect in the deep sea vary widely, ranging between 0.5 to more than 10 Mio. species (May, 1992; Grassle and Maciolek, 1992; Poore and Wilson, 1993; Lambshead and Boucher, 2003; Appeltans et al., 2012). There are currently > 26,000 named species catalogued in the World Register of Deep-Sea Species (WoRDSS; Glover et al., 2021), but certainly many more are to be discovered, especially among the inconspicuous, small-size and short-ranged fractions (Mora et al., 2011).

The discovery and description of the first species from the deep sea, the sea pen *Umbellula encrinus* (Linnaeus, 1758), heralded the beginning of the taxonomic study of deep-sea organisms. Remarkably, this coincided with the revision of the previous classification system and the birth of modern taxonomy as introduced by Linnaeus (1735) *Systema Naturae*. Our knowledge of deep-sea species has been thereby closely linked, on the one hand, with the ever-improving technology and logistics for taking samples from the deep sea and, on the other hand, with methodological advances to make external and internal parts of organisms visible. Here, the invention of the first compound microscopes towards the end of the 16th century had pushed taxonomic work forward considerably since it allowed to study the smaller size fractions and thus greatly increased the number of known species (Rosenthal, 2009; Manktelow, 2010). Regarded today as art, the detailed scientific illustrations of taxonomists at the earliest time such as Carl Linnaeus (1707–1778), Alexander von Humboldt (1769–1859), Ernst Haeckel (1834–1919), or Georg Ossian Sars (1837–1927) were indispensable in the absence of the photographic imaging techniques available today (**Figures 1A–G**). Isolated deep-sea samples had already been collected prior, but it was only 150 years ago that a global collection as part of the HMS *Challenge*r Expedition (1872–1876) could refute the thesis that the deep sea is devoid of life (Murray and Renard, 1891). Research into deep-sea biodiversity has gradually shifted from a more exploratory focus that involved a mere inventory of species to a more systematic approach that addresses issues such as how deep-sea diversity is structured. Likewise, taxonomy, as a legacy of Charles Darwin (1809–1882), Ernst Haeckel and more recently the German systematist Willi Hennig (1913–1976), has made a transition from classifying taxa based on their morphological appearance (phenetics) to using homologous characters to illuminate phylogenetic relationships (cladistics).

To date, referring to morphological features is still the means of choice when delimiting, identifying and describing deep-sea species. This is likely because it seems easy to apply, and others, such as the biological species concept *sensu* Mayr (1942; "Species are groups of interbred natural populations reproductively isolated from other such groups") cannot readily be applied due to the difficulty to obtain data on reproduction of deep-sea species (see also Brandt et al., 2012). With the advent of molecular approaches in taxonomy in general and deep-sea taxonomy in particular, however, many complications are associated with the phenotypic data, including evidence of sexually dimorphic or polymorphic species, convergence, and phenotypic plasticity (Raupach and Wägele, 2006; Vrijenhoek, 2009; Błażewicz-Paszkowycz et al., 2012; Brandt et al., 2012; Riehl et al., 2012; Błażewicz-Paszkowycz et al., 2014; Brandt et al., 2014; Mohrbeck et al., 2021). While molecular techniques have certainly helped expedite species identification and delimitation, phylogenetic relationships and biodiversity assessment, also on the background of intensifying anthropogenic impacts on deep-sea ecosystems, the description and naming of species remains pivotal to understanding their ecological function and evolution. Traditional taxonomy, however, in general cannot keep up with automated, high-throughput molecular methods that generate large amounts of data at a rapid pace, resulting in a large number of unnamed species on taxonomists' shelves, which remain unavailable for conservation purposes (Pante et al., 2015; Gellert et al., 2022). Moreover, for many (and not only) biologists, species identification also reduced to the pragmatic ability to distinguish between species remains far from a satisfactory solution. The simple curiosity to know and understand biodiversity in every detail at different levels of life organization, as well as the search for answers to *how* and *why*, goes beyond rapid and precise species identification (Will et al., 2005; Wheeler, 2018; Dupérré, 2020).

In that regard, morphological techniques used in deep-sea taxonomy did not stand still, but are constantly being further developed or have been introduced as new applications. For example, Confocal Laser Scanning Microscopy (CLSM) was originally developed in the 1950s to map the anatomy of the human nervous system and is now increasingly being used for the taxonomic analysis of microscopic invertebrates in the deep sea (Michels and Büntzow, 2010; Brandt et al., 2014; Meißner et al., 2017; Martínez Arbizu and Petrunina, 2018; Jennings et al., 2018; Kaiser et al., 2018; Błażewicz et al., 2019; Chim and Tong, 2020; Kaiser et al., 2021; Demidov et al., 2021). 3-D visualizations of internal structures are reconstructed from histological sections (Neusser et al., 2016; Bober et al., 2018; Gooday et al., 2018). Underwater Hyperspectral Imagery has been employed to aid identification of deep-sea megafaunal species owing to their specific spectral profiles alongside automated tools for the annotation of benthic fauna from video or still imagery (Langenkämper et al., 2017; Dumke et al., 2018; Kakui and Fujiwara, 2020; Singh and Mumbarekar, 2021).

The remit of this review article is to compile and evaluate available traditional and modern tools and techniques in morphology-based taxonomy with a focus on peracarid crustaceans. With more than 21,000 described species, the malacostracan superorder Peracarida is a highly diverse group containing about a third of the total richness of crustaceans (Appeltans et al., 2012; Wilson and Ahyong, 2015). Common to

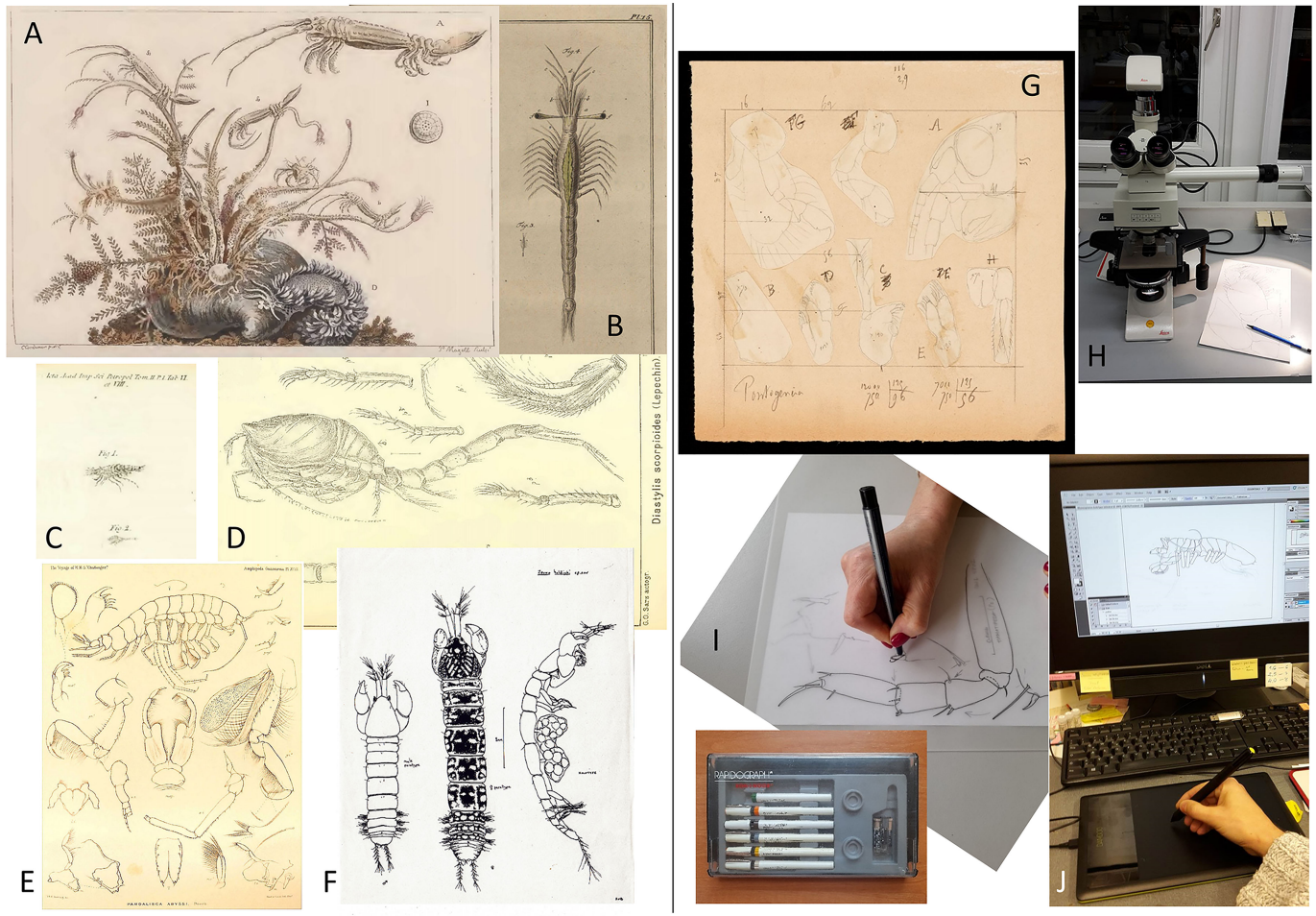

FIGURE 1 | Scientific illustrations of peracarids as complement of taxa description from past to present. **(A)** Isopod genus *Astacilla* Cordiner, 1793 illustrated by Cordiner (1793). **(B)** *Mesopodopsis slabberi* (van Beneden, 1861), the earliest illustrated mysid by Slabber (1778). **(C)** *Diastylis scorpioides* (Lepechin, 1780), the earliest published illustration of a cumacean as *Oniscus scorpioides* Lepechin, 1780 (see Holthuis, 1964). **(D)** *Diastylis scorpioides* (Lepechin, 1780), illustrated by G.O. Sars more than one century later (G.O. Sars, 1900). **(E)** The amphipod *Pardalisca abyssi* Boeck, 1871 illustrated after the voyage of H.M.S. *Challenger* during the years 1873–76 (Stebbing, 1888). **(F)** Original hand inked drawing made by Roger Bamber for the description of the tanaid *Zeuxo holdichi* Bamber, 1990. **(G)** Original plate outline with the drawings made by Édouard Chevreux for the amphipod description *Pontogeneia minuta* Chevreux, 1908 (Crustacean collection MNHN). **(H)** Compound microscope equipped with *camera lucida* to draw specimens for taxonomical purposes (photo I Frutos). **(I)** Preparing a plate by hand inking from previously made pencil drawings (photo I Frutos). **(J)** Electronical inking of drawings using a drawing tablet and computer (photo I Frutos).

all peracarids is brood care, whereby embryos are carried around in a ventral brood pouch formed by coxal oostegites until juveniles are released. Peracarids occur in all aquatic habitats, including caves, freshwater, stygobiont and marine environments, but only the oniscidean isopods contain truly terrestrial species. Besides extant species, they have occurrences in the fossil record, including deep-sea areas (Secrétan and Riou, 1986; Selden et al., 2016; San Vicente and Cartanyà, 2017; Luque and Gerken, 2019). Spanning different size classes, from meio- to megafauna, the highest diversity of peracarids is likely to be found within the macrofauna, where they represent one of the most diverse groups in the deep sea (Hessler and Jumars, 1974; Sanders et al., 1985; Frutos et al., 2017a; Brandt et al., 2019; Washburn et al., 2021). Peracarids are the main component of suprabenthos, which includes all swimming bottom-dependent animals performing, with varying amplitude, intensity, and regularity, seasonal or daily vertical migrations above the seafloor (Brunel et al., 1978; Frutos et al., 2017a; Ashford et al., 2018). Most species of deep-sea peracarids are benthic, with tanaidaceans and some isopod taxa living mostly infaunally, whilst many amphipods, isopods and cumaceans are known as good swimmers (Błażewicz-Paszkowycz et al., 2012; Poore and Bruce, 2012). Shrimp-like mysids and lophogastrids similarly have good swimming capacities, representing members of suprabenthic (mysids) and pelagic (lophogastrids) communities (San Vicente et al., 2014a). Although the variety of lifestyles, morphologies and functions of deep-sea peracarids is large, with some exceptions, a general suite of taxonomic working methods can be applied to their study (including the study of some fossil specimens).

This review is intended to describe the entire process required for the morphological examination of deep-sea peracarids, from deep-sea sampling to long-term storage in historical collections. The focus is on fixation and conservation for microscopy as well as the selection and application of imaging techniques. Although this compilation is dedicated to the morphological analysis, recommendations for sample preparation are also given with regard to genetic/omic studies as part of an integrative workflow. Given the great diversity of peracarids in the deep sea, we hope that this overview will find broad application and importance in exploring the cornerstone of any biological research there, the species.

2 METHODS FOR SAMPLE PREPARATION

Deep-sea science is indisputably expensive and logistically difficult. Study areas are usually far away from the coast, sampling itself takes long hours, and apart from vents or seeps, faunal densities are typically low. Moreover, the ship-time costs, the effort and number of people involved to get a sample, with all the physical difficulties to successfully work at great ocean depth, make deep-sea material very precious. While this is common sense, prior to sampling consideration should therefore be given to how best to sample, process and fix samples simultaneously for various purposes (e.g., morphological, molecular, ecological and biochemical) in order to get the most out of the material. At the same time, media and methods for long-term storage need to be evaluated so that the vouchers and slides are retained for future work. A full representation of the described workflow of sample collection and processing is shown in **Figure 2**.

2.1 Sampling and Sample Processing

Basically, two ways of collecting data are common: 1) still or video imagery *in situ,* and 2) direct sampling (Schiaparelli et al., 2016). Identification to the species level using images is difficult or even impossible for the megafauna (Hanafi-Portier et al., 2021; Horton et al., 2021), so that *ex-situ* examinations are required or even mandatory for the mostly much smaller Peracarida. The majority of deep-sea peracarids are sediment-bound, i.e. living in, on or just above the seabed (suprabenthic lifestyle). Depending on lifestyle and mobility of the target organisms, a variety of benthic sampling devices are used in deep-sea research. On soft bottoms, in general, coring devices, including box corer, multi- and megacorer, collect epi- and infaunal species; towed apparatus (trawls, sledges and dredges) is used for the epi- and supra-fauna; as well as baited and sediment traps, for the collections of more mobile and/or pelagic species. Manned submersibles or remotely operated vehicles (ROV) can help in the sample collection by means of push-corer, suction pump, small nets or picking up larger structures on hard substrata (for sampling specificities see Jamieson, 2016; Kaiser and Brenke, 2016; Kelley et al., 2016; Narayanaswamy et al., 2016; Frutos et al., 2017a). In water column studies, pelagic peracarid species are collected by means of mid-water trawls or plankton nets (Kürten et al., 2013; MacIsaac et al., 2014; Papiol et al., 2019); the latter are also suitable as collector of benthic peracarids if they are used as additional sampler attached to trawling devices such as otter or beam trawls (Nouvel and Lagardère, 1976; Lagardère, 1977). In addition, peracarids can also be sampled indirectly by examining the gut content of decapod or the fish stomach content, because they are their food source (Sorbe, 1981; Carrasón and Matallanas, 2001; Preciado et al., 2017). The advantages or disadvantages for the use of the aforementioned types of sampling devices are summarized in **Table 1**; however, an optimal choice is the combination of different equipment types to sample (Taylor et al., 2021; Ríos et al., 2022), which also provides complementary information on species behavior (Frutos and Sorbe, 2010; San Vicente et al., 2014b).

The choice of sampling devices depends on the target taxon (with regard to size class and lifestyle), seafloor topography, substrate type and depth, as well as data requirements (qualitative *vs*. quantitative). Benthic sledges are useful, for instance, to collect specimens with high swimming capacities (i.e. mysids and lophogastrids; Frutos, 2006), as well as relatively high specimen numbers, and thereby enable more coherent morphological and genetic assessment. Although sledges provide large numbers of peracarid fauna, additional equipment (such as opening/closing system of nets, flowmeters or pingers in the sledge frame) is required to better express abundances as densities (Brunel et al., 1978; Sorbe, 1983; Cartes et al., 1994; Dauvin et al, 1995; Frutos, 2006; Frutos et al., 2017a). Corers, by contrast, only provide low faunal densities, but offer

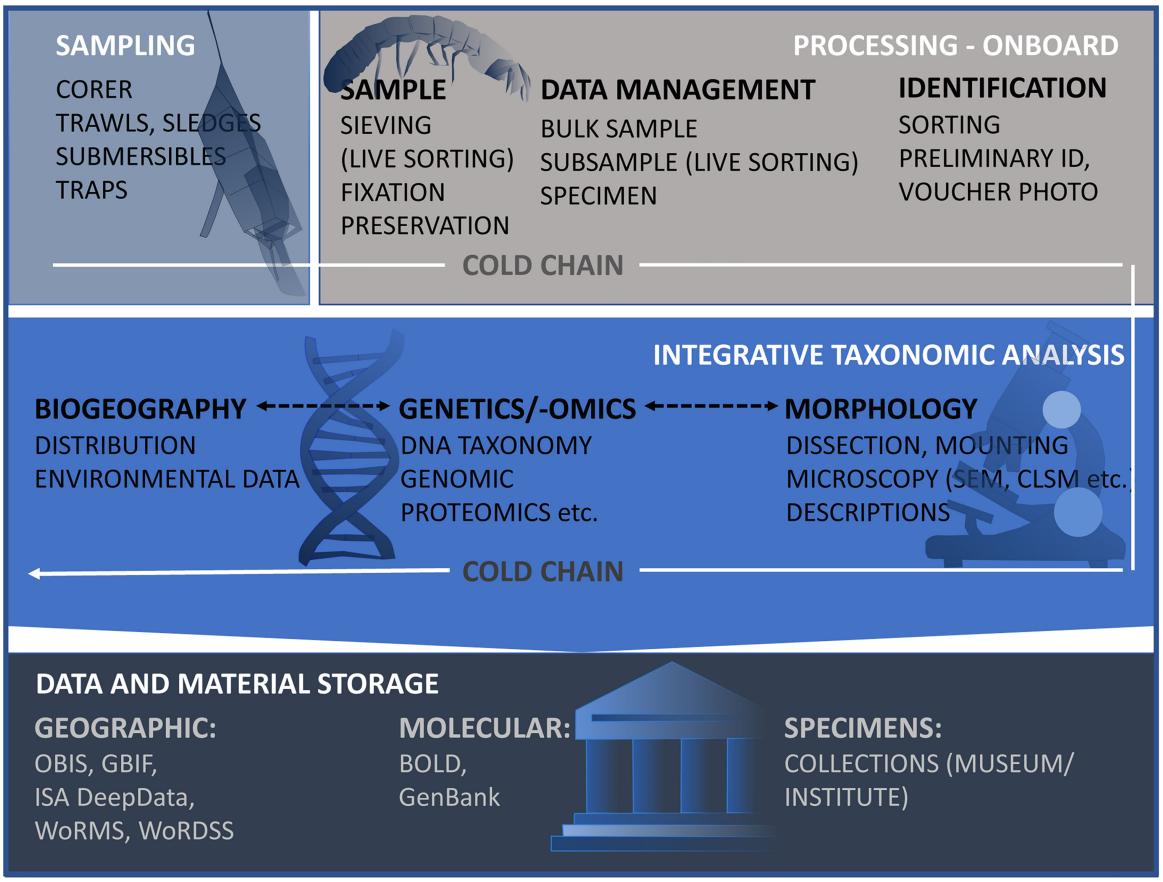

FIGURE 2 | Workflow to illustrate all steps that are required for the taxonomic investigation of the deep-sea peracarid fauna under the cold chain regime (Riehl et al., 2014) - from sampling, morphological taxonomic investigation, molecular and biogeographic analysis to the final storage of samples and data. Links to: OBIS, Ocean Biogeographic Information System[1]; GBIF, Global Biodiversity Information Facility[2]; DeepData, Deep Seabed and Ocean Database of the International Seabed Authority[3]; WoRMS, World Register of Marine Species[4]; WoRDSS, World Register of Deep-Sea Species[5]; BoLD, Barcode of Life Data System[6]; and Genbank[7]. ([1]https://obis.org/; [2]https://www.gbif.org/; [3]https://data.isa.org.jm/isa/map/; [4]http://www.marinespecies.org/; [5]http://www.marinespecies.org/deepsea/; [6]https://www.boldsystems.org/; [7]https://www.ncbi.nlm.nih.gov/genbank/).

quantitative insights when collecting undisturbed sediment surfaces (Jóźwiak et al., 2020; Lins and Brandt, 2020).

In all cases, minimizing mechanical damage to the specimens during sampling and processing to avoid loss of taxonomic information, and considering different preservation options for the same sample are important considerations. On the one hand, this includes careful handling during sampling and sample processing (washing and sieving), but also swift storage of the samples, especially if genetic or biochemical analyses are to be carried out. For example, precautions should be taken for trawled devices prior to sampling to avoid hard substrate entering the nets and grinding individuals (Kaiser and Brenke, 2016). Since sediment is part of the sample, it is important to remove it by sieving to maximize fixative concentration and thus improve sample preservation. As crustaceans can easily lose their legs and antennae, which is often essential for taxonomic identification, sediment samples should therefore be carefully sieved, if necessary with prior elutriation of the sediment samples in seawater.

Processing the samples for different purposes needs specimens to be removed from the sediment as soon as possible after the arrival of the sample on deck. Here, the maintenance in high ethanol content may arguably be even more crucial for genetic analysis (see *3.2.1 Light Microscopy*) than to maintain a cold chain protocol. The latter has been thought to be essential for molecular work on deep-sea isopods (Riehl et al., 2014). For sampling under tropical climatic conditions, however, it is strongly recommended that the samples are transferred to a cold environment as soon as possible. A disadvantage of fixing the entire sample in ethanol, however, is that the tegument/cuticle of the peracarids becomes hard and stiff and could impede further morphological examination (e.g. of subcuticular elements), while the setae required for morphological determination, become brittle and can break off. Furthermore, some morphological features can only be observed in live (unfixed) specimens. For example, in deep-sea amphipods, optical structures often can only be visualized in live animals: *Leucothoe cathalaa* is showing the

TABLE 1 | The most common types of sampling devices used for collecting peracarids.

Type of sampling	Type of sampler	Sampling Equipment	Advantages	Disadvantages	References
Direct (Biological)	Coring devices	Grab	Mainly infaunal species Quantitative samples	Small number of individuals Optimal at shallower depths	Esquete et al., 2014 Jakiel et al., 2018 Rodríguez et al., 2021
		Box-corer	Mainly infaunal species Does not disturb sediments Quantitative samples	Small number of individuals High-mobility species not represented	Chardy, 1979 Błażewicz-Paszkowycz et al., 2011 Wilson, 2017 Ashford et al., 2018
		Multi-corer	Meiobenthic species Undisturbed sediments Quantitative samples	Small-sized specimens small number of individuals	Schmidt and Martínez Arbizu, 2015; Rosli et al., 2016 Schmidt et al., 2018
	Towed devices	Dredge	Epibenthic species Hard-bottom sampling	only large specimens small number of individuals	Kensley, 1989 Bamber, 2007 Frutos et al., 2017b
		Beam trawl	Epibenthic species Large specimens	small number of individuals Accidental pelagic species	Moreira, 1973 Bruce, 2005 Serrano et al., 2017
		Sledge	High number of individuals Epi- and suprabenthic species	High-tech models are heavy and expensive	Hessler and Sanders, 1967 Buhl-Jensen, 1986 Almeida et al., 2017 Frutos et al., 2017a
		Otter trawl	Epi- and suprabenthic species Big-sized specimens Peracarids can be recovered from decapod/fish stomach contents	Small number of individuals From stomach contents, peracarids are partially digested	Sánchez et al., 2008 Serrano et al., 2011 Preciado et al., 2017
		Plankton net	Pelagic species Attached to trawls provides high numbers of benthic peracarids	Net can be damaged on rough bottoms	Nouvel and Lagardère, 1976 Zeidler, 1990; Shimomura and Ohtsuka, 2005 Kürten et al., 2013 Papiol et al., 2019
	Traps	Baited	Huge number of individuals	Only scavengers	Barnard and Ingram, 1990 Frutos and Sorbe, 2010 Horton et al., 2020
		Sediment	Specimens perfectly preserved Good-swimming peracarids	Accidental catches Unusable for genetics (formalin fixation)	Corbera, 2006 Guidi-Guilvard et al., 2007 Kraft et al., 2013
In situ observation	Underwater vehicles	ROV	Imaging species in their habitat Collecting peracarids from hard bottoms Species from vulnerable and extreme habitats Most of taxa are new to science	Species identification requires the specimen Small number of individuals	Tandberg et al., 2012 Corbari and Sorbe, 2018 Lörz and Horton, 2021
		Manned submersibles	Scientist is onboard to sample Collecting peracarids by means of push-corer & nets Species from vulnerable and extreme habitats Most of taxa are new to science	Species identification requires the specimen Small number of individuals	Shaw, 1989 Martin et al., 1993 Bellan-Santini and Thurston, 1996 Corbera et al., 2008

Sampling equipment is classified in general terms. Advantages/disadvantages are specified with regard to abundance or body size of collected individuals. For additional sampling equipment specificities see Jamieson, 2016; Kaiser and Brenke, 2016; Kelley et al., 2016; Narayanaswamy et al., 2016; Frutos et al., 2017a.

whitish pigmentation of the rounded eye before storage in preservative medium (**Figure 3E**, while its eyes are hardly visible in preserved specimens, even under light microscope (Frutos and Sorbe, 2013). Equally, samples that are to be frozen, e.g. for biochemistry studies, should be identified as accurate as possible and pictured before being preserved. Thus, live sorting should be considered, whenever possible, whereby the respective individuals are selected directly from the sample and individually identified, photographed and fixed (Brix et al., 2020; Ahyong et al., 2022).

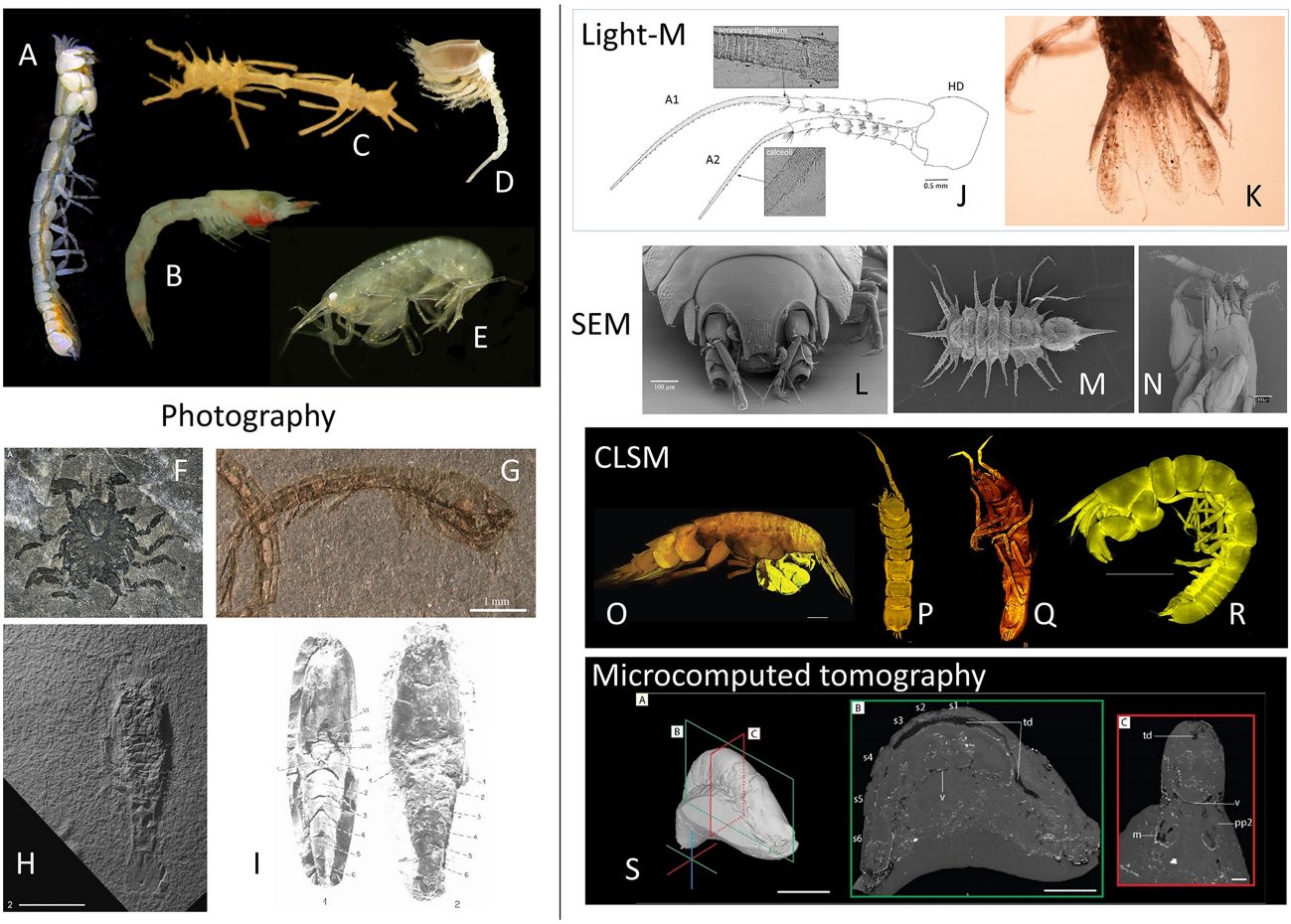

FIGURE 3 | Peracarida specimens visualized applying different modern imaging techniques to complement the taxonomical description of species. **(A–G)** Digital still camera on stereomicroscope. **(H, I)** Still camera. **(J, K)** dissected specimen under Light microscope. **(L–N)** Scanning Electron Microscope. **(O–R)** Confocal Laser Scanning Microscope. **(S)** Microcomputed tomograph. **(A)** The paranarthrurellid tanaidacean *Armatognathia swing* Błażewicz and Jóźwiak, 2019 from Błażewicz et al. (2019) under Creative Commons license. **(B)** The mysid *Paramblyops rostratus* (Holt and Tattersall, 1905) from Frutos (2017). **(C)** The ischnomesid isopod *Cornuamesus longiramus* (Kavanagh and Sorbe, 2006), and **(D)** the diastylid cumacean *Campylaspis vitrea* Calman, 1906 from Frutos et al. (2017a). **(E)** The leucothoid amphipod *Leucothoe cathalaa* Frutos and Sorbe, 2013 from Frutos and Sorbe (2013). **(F)** The first asellote isopod from the fossil record *Fornicaris calligarisi* Wilson and Selden, 2016 from Selden et al. (2016),© The Crustacean Society, reprinted with permission of Oxford University Press on benhalf of The Crustacean Society. **(G)** The oldest crown cumacean *Eobodotria muisca* Luque and Gerken, 2019 from Luque and Gerken (2019), reprinted with permission of Royal Society Publishing. **(H)** The oldest known fossil mysid *Aviamysis pinetellensis* San Vicente and Cartanyà, 2017 from San Vicente and Cartanyà (2017), reprinted with permission of Cambridge University Press. **(I)** Two fossil lophogastrids of family Lophogastridae from Secrétan and Riou (1986), reprinted with permission of Annales of Paléontologie. **(J)** The eusirid amphipod *Dorotea papuana* Corbari, Frutos and Sorbe, 2019 from Corbari et al. (2019). **(K)** The paranthurid isopod *Paranthura santiparrai* Frutos, Sorbe and Junoy, 2011 from Frutos et al. (2011). **(L)** The nannoniscid isopod *Austroniscus obscurus* Kaiser and Brandt, 2007 from Kaiser and Brandt (2007). **(M)** The paramunnid isopod *Pentaceration bifficlyro* Kaiser and Marner, 2012 from Kaiser and Marner (2012). **(N)** The paranthurid isopod *Paranthura santiparrai* Frutos, Sorbe and Junoy, 2011 from Frutos et al. (2010). **(O)** The oedicerotid amphipod *Oedicerina teresae* Jażdżewska, 2021 from Jażdżewska et al. (2022) under Creative Commons license. **(P)** The nannoniscid isopod *Thaumastosoma platycarpus* Hessler, 1970 from Kaiser et al. (2018). **(Q)** The nannoniscid isopod *Nannoniscus magdae* Kaiser, Brix and Jennings, 2021 from Kaiser et al. (2021). **(R)** The paranthrurellid tanaidacean *Paranarthrurella arctophylax* (Norman and Stebbing, 1886), from Błażewicz et al. (2019) under Creative Commons license. **(S)** Fossil lophogastrid specimen showing internal anatomy after microcomputed tomography, from Jauvion (2020).

2.2 Fixation

Fixation of specimens in taxonomic studies aims to prevent the spontaneous deterioration of taxonomically important features of the collected animals and thus its methods should be selected and applied with a thorough regard for the subsequently planned discovery pipeline of methods. The two main threats to morphological and genetic features of marine crustaceans that have to be prevented by fixation are dead cell/tissue autolysis by endogenous enzymes and destruction of biological material by microbial (bacterial/fungal) contaminants. An optimal fixative should aim to prevent both threats at the same time. Specimen fixation is of paramount importance if a significant time lapse occurs between collection and analysis, which is usually the case for marine samples, especially deep-sea ones, collected on board of research vessels and later analyzed in research institutions on dry land. In fact, the current average shelf life of new species

between discovery and description is about 21 years (Fontaine et al., 2012). Furthermore, good preservation is also extremely important for material of taxonomic significance, especially type material that has to be available for subsequent re-analysis in museum collections. While the term "preservation" is usually used for application of fixatives for prolonged storage of museum specimens, both underlying principles and specific compounds used are analogous to fixation for general purposes and will be discussed together here.

Fixation inevitably changes the physico-chemical properties of the specimen, so it has to be performed in a way that is compatible with downstream taxonomic techniques, both with regard to imaging morphology for identification purposes and to analyzing genetic and biochemical make-up of the specimen. Thus, selection of proper fixative is always a trade-off between efficiency and durability of preservation on one hand and lack of significant interference with taxonomically important features of the specimen (Eltoum et al., 2001). Among the properties that need to be considered are i.e.: crude shape changes which may result from physico-chemical processes (drying, osmotic swelling); delicate morphological elements that may be damaged during the fixation process itself; physical features that may deteriorate upon chemical reactions with the fixative, especially upon prolonged exposure (color, transparency, flexibility, malleability etc.); biochemical composition (e.g. lipid or carbohydrate content of specific tissues); integrity of nucleic acids and their accessibility to isolation; antigenic properties and/or enzymatic activity of proteins (Barbosa et al., 2014). With regard to deep-sea biological investigations, another consideration that has to be taken into account is the availability of fixative at the collection site: this includes questions of logistics (ease of transport, security), legal issues, shelf life of the fixative itself etc. Sometimes, a two-tier fixation protocol may be adopted, with simpler fixative applied on board the collection vessel for short-term preservation and subsequent exchange for museum-grade fixative during preparation for long-term storage in a biological collection. Of course, taxonomists are often confronted by the fact that the specimens to be examined have not been collected and preserved by themselves, so they no longer have a choice of fixation method, but some fixatives can be exchanged for others (e.g. ethanol can be replaced with formaldehyde and *vice versa*) prior to analysis if interference is expected (Pereira et al., 2019). As the published literature is contradictory about the compatibility of some fixation protocols with subsequent taxonomic analysis (especially by nucleic acid isolation, PCR and/or next generation sequencing) and anecdotal evidence for the suitability of individual protocols prevails, taxonomists are recommended to understand the physico-chemical principles of fixation and of genetic methods, so that an informed decision may be made. A classification of the fixatives most commonly used in the Peracarida taxonomic community and short description of their main advantages and disadvantages is included in **Table 2**.

In some cases, taxonomic studies are performed not on specimens from extant taxa collected while still alive, but on subfossil or fossil material which is already naturally "fixed" or transformed into a relatively permanent, physico-chemically stable form. Morphology of preserved tissues may be studied in such samples using the same imaging techniques as described below for extant material – optical microscopy, electron microscopy or microcomputed tomography (Sánchez-García et al., 2016; Nagler et al., 2017; Jauvion, 2020; Luque et al., 2021; Robin et al., 2021), but the physical preparation of the sample lacks the fixation step, instead involving mechanical preparation (slicing, milling, polishing). For some taxa of deep-sea Peracarida, morphological studies of fossils using recently available imaging techniques led to taxonomic corrections and reclassification of whole groups of specimens: a decapod tail described as amphipod (McMenamin et al., 2013; Starr et al., 2016); samples that upon close investigation contained not amphipods but previously unknown genera and species of tanaids (Vonk and Schram, 2007); a new mysid genus (Cartanyà, 1991; San Vicente and Cartanyà, 2017) or a new lophogastrid taxon (Secrétan and Riou, 1986; Jauvion, 2020).

2.2.1 Common Fixatives

The most common fixative types in aquatic zoology can be classified into two groups: those relying on quick dehydration and those relying on molecular cross-linking of biochemical components. Both aim to quickly and efficiently inhibit the activity of enzymes (endogenous or microbial ones) which could destroy the biological macromolecules that the specimen consists of: proteases for proteins, nucleases for nucleic acids or glycosidases for carbohydrates. Dehydration withdraws the main reaction substrate for hydrolytic reactions and inactivates enzymes by coagulation-mediated denaturation. Cross-linking prevents enzyme-substrate interactions by stopping diffusion as well as by preventing conformational changes of the enzyme molecule that are crucial for its activity. Some fixation methods aim also to inhibit major lytic enzyme groups by specific biochemical interactions with their co-substrates or active sites, or to target microbial life with antibiotic toxins (**Table 2**).

The most universal and frequently used fixatives based on the dehydration principle are aliphatic alcohols, especially ethanol. Ethanol works by quickly mixing with water, penetrating the specimen, and removing the solvation shells from proteins and other molecules. The most efficient and rapid-acting concentration of 95–96% is considered the optimal fixative both for field fixation and long-term storage when preservation of tissue structure, biochemical composition and DNA for genetic analysis are important (Palero et al., 2010; Wetzer, 2015; Martin, 2016; Beninde et al., 2020).

While 70% ethanol is also historically used for long-term storage in museum collections due to its superior anti-microbial activity, numerous studies have shown that the increased water content and insufficient lytic enzyme inhibition leads to detectable levels of DNA degradation, correlating with storage time and therefore making subsequent genetic studies on material stored in the manner more difficult – especially for taxonomically valuable material (e.g. type specimens) (Marquina et al., 2021); moreover, the high-water content and lowered pH of 70% ethanol may lead to cuticle decalcification upon long-term storage, which is important especially for those peracarids

TABLE 2 | The most common types of fixatives used by peracarid taxonomists with their advantages and disadvantages summarized.

Type of fixative	Active agent	Advantages	Disadvantages	References
Dehydrating	Ethanol	Efficient fixation, relatively non-toxic, allows posterior genetic studies	Tissue shrinkage and brittleness, fast evaporation, legal issues	Wetzer, 2015; Martin, 2016;
	Isopropanol	Stronger fixation than ethanol	Slower action than ethanol	Hughes and Kaji, 2016
	Hydrophobic solvent (Carnoy's)	Preservation of hard tissues	Damage to cellular components of the specimen, removal of pigments	Presnell and Schreibman, 1997
Cross-linking	Formaldehyde	Efficient fixation, low evaporation and shrinkage, high flexibility of exoskeleton	Damages nucleic acids and hampers their isolation, relatively toxic, needs buffering	Palero et al., 2010; Wetzer, 2015;
	Glutaraldehyde	More durable fixation and less toxic than formaldehyde	Difficult sample manipulation after fixation, irreversible damage to nucleic acids	Brooker et al., 2012b
Freezing	Phase transition	Cheap and easy, allows biochemical analysis	Effective in very short term, disrupts micromorphology	Martin, 2016; Turner et al., 2016;
Coagulant	Organic acids (Bouin's)	Quick fixation and good preservation of overall morphology	Dissolves calcium carbonate in exoskeleton, may disrupt delicate morphological features	Göpel and Wirkner, 2018
	Mercuric salts (Zenker's)	Fixative and anti-microbial action at the same time	Highly toxic, not efficient in preserving hard tissues	Fryer, 1968
	Osmium tetroxide	Good fixation for fat-rich tissues, serves as fixative and electron microscopy stain at the same time	Expensive, damages nucleic acids	Kaji et al., 2014
Anti-microbial	Antibiotic/antifungal agents	Long-term protection against microbial contamination	Must be combined with an actual fixative for preservation of specimen morphology	Stegner et al., 2015
Stabilising nucleic acids	Quaternary ammonium/ caesium ions (RNAlater)	Good DNA and RNA preservation	Very expensive, does not preserve morphology well	Wetzer, 2015; Porter, 2016
	Propylene glycol	Cheap, good DNA preservation	Distortion of some morphological features	Robinson et al., 2021
	EDTA/DMSO (DESS)	Good DNA preservation	Short-term storage	Boxshall et al., 2016; Lins et al., 2021;
	EDTA/SDS	Good DNA preservation	Destruction of protein-based morphological features	Pokluda et al., 2014

that have taxonomically important calcium carbonate deposits in different forms (amorphous, calcite, aragonite) in the exoskeleton, e.g. isopods. On the other hand, rapid and complete dehydration by concentrated ethanol has the disadvantage of making arthropod exoskeletons stiff and brittle, as their natural elasticity depends to a large extent on extracellular matrix proteins which lose their properties when denatured/coagulated by water loss, leading to mechanical damage in transport or during dissection (Costa et al., 2021). The fragility of tegument is especially problematic in the case of some deep-sea Peracarida where delicate appendages and armament are often essential for taxonomic identification – therefore, an addition of up to 5% glycerol (by volume) during fixation and preservation would be strongly recommended as it softens the exoskeleton and makes it less fragile. In some cases, the tegument may also become opaque due to coagulated protein precipitation, hampering internal observation (e.g., of musculature or gut content), and taxonomically important pigmentation may be partially or totally dissolved, e.g. making eyes difficult to notice visually (Frutos and Sorbe, 2013; Campean and Coleman, 2018). Therefore, while 95% ethanol remains the optimal concentration for on-site fixation and long-term storage, it may be preferably exchanged for 70% ethanol in sample transit and before laboratory manipulations. Absolute (~100%) ethanol is much more expensive than 95% ethanol and may sometimes introduce microscopic morphological artefacts due to its extreme hygroscopy.

Methanol, while used in histological fixation, is ineffective for long-term storage of specimens for taxonomic purposes and should be avoided since its dehydration power is relatively weak, leading to insufficient protein coagulation and residual lytic activities. Isopropanol is as efficient in protein coagulation as ethanol and does not stiffen carbohydrate structures (carapaces) as much, but this advantage is offset by its relatively high price and slow diffusion into larger biological structures, leading to potential loss of fine details or DNA contained in internal structures (King and Porter, 2004).

Despite prevailing misconceptions in literature about ethanol with additives that make it unsuitable for human consumption (so-called denatured alcohol), these additives (e.g. methanol, ether or acetone) have no discernible effect on the fixation process, long-term preservation and downstream applications (when nucleic acids are isolated for genetic analysis, these additives are removed together with ethanol itself, and they are present in far too low concentrations to impact downstream processes anyway). The same is true for traces of benzene or its derivatives present in absolute ethanol. The misplaced recommendations against using denatured alcohol for

specimen preservation for genetic analysis stem from faulty interpretation of several studies where "pure ethanol" at 95% was compared to "denatured alcohol" at 70% (as this is the concentration readily available commercially in many countries), and the above-mentioned inferior performance of the latter in DNA preservation was mistakenly ascribed to the denaturing additives (Wall et al., 2014). If denatured 95% ethanol is available, it may be used for fixing deep-sea Peracarida equally to pure 95% ethanol. The main advantages of ethanol as a fixative for taxonomy of deep-sea Peracarida include: low cost, fast action, potential for long-term storage, good preservation of DNA and proteins (including linear antigenic determinants). The main disadvantages include: high volatility (and therefore potential for evaporation from non-hermetic storage containers), flammability, legal issues (especially with transport to the collection site), need for time-consuming removal for some downstream applications (especially involving nucleic acid isolation), potential for morphological distortion by rapid water removal from small specimens with delicate exoskeletons, as well as fragility of dehydrated specimens.

The most frequently used cross-linking fixative is formaldehyde which reacts with proteins, nucleic acids as well as some lipids and carbohydrates to form a durable network of covalently linked macromolecules. For long-term storage, formaldehyde is usually used at concentration of 4% (or sometimes higher). The working solution is obtained by diluting so-called formalin (stabilized concentrated solution of ca. 36%) or by de-polymerizing the solid polymer paraformaldehyde. Formaldehyde penetrates tissues quickly and preserves structures efficiently, while not dehydrating the specimen at the molecular level, leading to full preservation of flexibility of appendages and tegument, making dissection easy. Since aquatic solutions of formaldehyde are acidic (due to hydrolysis and forming of geminal methanediol), it is crucial that this fixative is buffered to neutral or slightly basic pH (7.5–8.5) when used on marine crustaceans if biochemical integrity of the tegument is to be preserved, to prevent dissolution of calcium carbonate in their exoskeleton. The most frequently used buffering agents for this task are sodium borate (borax), sodium phosphate, sodium bicarbonate and hexamethylenetetramine (urotropin) (Presnell and Schreibman, 1997; Martin, 2016). On the other hand, decalcification in acidic formaldehyde solutions makes some tegument more transparent, allowing for easier microscopic observation of internal structures. For small aquatic animals with shells or carapaces, formaldehyde is sometimes combined with compounds that accelerate protein coagulation during the initial specimen soaking (picric and acetic acids) - this fixative is called Bouin's solution and may be recommended where careful preservation of deep tissue morphology is of importance. An alternative for formaldehyde is the higher molecular weight bifunctional molecule, glutaraldehyde, which forms more stable and durable crosslinks, but is much more expensive, makes tissues hard and difficult to dissect and prevents any subsequent molecular analysis. The advantages of formaldehyde for peracarid taxonomy, especially used in commercial and monitoring studies, include: low cost, fast action, capacity for long-term storage (low volatility). The main disadvantages are: high toxicity (which necessitates careful handling, especially during transport), strong biochemical changes which are sometimes irreversible (DNA and RNA may be isolated from formaldehyde-fixed specimens after de-crosslinking, but it is of significantly lower quality; while some proteins retain antigenic properties, some do not), deterioration of some physical features of the specimen (tissue hardening, "tanning" - generation of secondary pigments), deformation of microscopic features by spontaneously precipitating paraformaldehyde crystals. It has been demonstrated that formaldehyde-crosslinked nucleic acids are more labile to hydrolysis, which is why they yield worse quality sequencing data; de-crosslinking is most efficient at 70°C in dilute buffer at pH=8.0 (Evers et al., 2011).

2.2.2 Less Common Fixatives

A historically common preservation technique for short-term maintenance of collected specimens until the availability of more efficient fixative is refrigeration or freezing of sample in the seawater in which it was collected. Refrigeration does not stop degradation processes, it only slows them down, while freezing (e.g., flash-freezing in liquid nitrogen) strongly disrupts microscopic morphology owing to generation of ice crystals within tissues, so these methods are recommended only when the main purpose of material collection is biochemical analysis in the near future.

While ethanol works by dehydration at the molecular level, water may be removed from the specimen also physically by drying (spontaneous, heat-induced or using hygroscopic materials such as silica gel). While common as a preservation procedure in terrestrial arthropods, this method is of highly limited applicability for marine peracarids: morphology is strongly disturbed by the drying process itself and by marine water salts, dry specimens are extremely delicate with regard to mechanical damage, inhibition of lytic enzymes and microbial growth is inefficient, nucleic acid chains tend to break. The only exception is preparation of specimens for SEM where liquid needs to be removed while preserving micromorphology – freeze-drying (lyophilisation) or critical point drying in liquid carbon dioxide are the fixation methods of choice here.

Organic solvent-based dehydrating fixatives, which are commonly used in histology, are also sometimes applied for preservation of marine crustaceans, although this is mainly of historical significance and should be discouraged for modern taxonomic analysis. Specifically, acetone or Carnoy's solution (ethanol with chloroform and acetic acid) dissolve and wash out hydrophobic components of the specimen, including biological membranes and lipid pigments, much more strongly than ethanol, preserving only the crude external structures (e.g. the exoskeleton), which is not acceptable for museum-quality preservation.

A group of less frequently used fixatives are inorganic salt coagulants involving heavy metals that act on negatively charged groups in proteins and lipids. Osmium tetroxide is an efficient fixative for lipid-rich tissues, but its application for crustaceans is mostly limited to concurrent fixation and staining for electron microscopy (see below). Similarly, in some histological work on marine crustaceans, Zenker's fixative is used. This solution contains highly toxic mercuric chloride acting as coagulant and providing excellent tissue fixation for detailed histological

analysis. Its usage nowadays is limited, since it has to be handled with extreme care and produces hazardous waste that requires costly disposal.

Sometimes, antimicrobial additives (amphothericin, thimerosal, azide etc.) are used to prevent microbial contamination and degradation of the sample, but as they usually have a relatively narrow spectrum of action and do not influence the spontaneous degradation of dead tissue by endogenous enzymes, they can have an auxiliary function at best.

Several specialized fixatives have been developed for specimens destined for subsequent nucleic acid isolation and genetic analysis. While RNA is both inherently unstable and subject to degradation by ubiquitous and abundant RNAses, DNA (a more common object of genetic analysis for taxonomic purposes) is chemically very stable, degrading only under specific conditions, and its deterioration in unfixed specimens is mostly due to action of microbial digestive enzymes because tissues of marine invertebrates are very poor in endogenous nucleases. Thus, while commercial fixatives like RNAlater™ and other chaotropic salt-based protein denaturants aimed at rapid and efficient elimination of RNAse activity are crucial to any transcriptomic (RNA-based) analysis, they are very expensive and simpler fixatives (like ethanol) are just as efficient in DNAse inhibition if only DNA-based analysis is foreseen. Alternatives to ethanol as a fixative for DNA-based studies have been proposed (e.g. propylene glycol-containing antifreeze solution (Robinson et al., 2021) or solutions containing metal chelators that deprive DNAses of cofactors mixed with detergents (Pokluda et al., 2014) or polar solvents (Lins et al., 2021) and they facilitate subsequent DNA isolation, but they are not efficient in preserving morphology or in long-term prevention of microbial contamination, so they should be used only in targeted taxonomic studies (e.g. barcoding or metabarcoding). When selecting the fixative for a specimen that will (or may) be subjected to genetic analysis by DNA sequencing, it is important to take into account the specific technique to be used: some techniques (e.g. Illumina) sequence short fragments and thus may be efficiently used even on DNA of low quality, e.g. isolated from formaldehyde-fixed specimens; some techniques (e.g. nanopore) need long DNA molecules and thus should be applied only for material fixed with ethanol or DNA-specific fixatives. Importantly, both freeze-thaw cycles and drying-rehydration cycles contribute to DNA strand breakage and should be avoided if longer DNA is required.

2.3 Dissection for Morphological Examination

Body length of peracarids rarely exceeds several millimeters. For this reason, the morphological identification of the peracarids involves observation of the details of head/cephalothorax, thorax, and abdomen appendages as well as additional components such as labrum, labium or epignath. The dissection of microscopic size requires experience, "surgical" dexterity, and precise tools. The needles used for the preparation of larger crustaceans are much too large for working with small crustaceans, while thin entomological needles are too flexible for dissection of the crustaceans. Tungsten needles, with tips although extremely fine, remain rigid and inelastic, are an ideal solution for peracarid dissection. Nowadays there are many companies on the market that offer tungsten needles, but sharpening can also be done in the lab, using solution of KOH, as copper as cathode and a low electric voltage.

3. METHODS FOR MORPHOLOGICAL STUDIES

3.1 Preparing Drawings

Scientific drawings are the pillar of taxonomic research. Drawing practiced with the support of a *camera lucida* microscope enable future researchers to recognize named species (**Figure 1H**). In the early Linnean days of taxonomy, it was essential to prepare drawings to visualize features, but recently they are increasingly being replaced by other (e.g., photographic) techniques (Wilson, 2003; Anderson, 2014; d'Udekem d'Acoz and Verheye, 2017; Lörz and Horton, 2021), that are also being applied to fossilised specimens (Selden et al., 2016; Jauvion, 2020). There have been fierce debates over photographs or microscopic images to become substitutes for drawings or even types (cf. Zhang et al., 2017). Although changes to the International Code for Zoological Nomenclature now have a certain consistency with regard to the type problem (Zhang et al., 2017), the idea of describing species purely based on imagery or molecular taxonomic units (MOTUs) (Jörger and Schrödl, 2013; Sharkey et al., 2021) still remains the exception for peracarids.

Drawings provide an interpretation often in a rather schematic way. The traditional scientific drawing workflow is clearly a lengthy one, starting with pencil drawings, followed by inking, scanning, as well as editing and arranging plates (**Figures 1G–J**). Yet, pencil and ink drawings, on the one hand, aid in-depth examination of the morphology and, on the other hand, distracting details may be omitted if they are systematically uninformative. Besides, drawing habitus of poorly calcified specimens enables us to visualize the morphological characters which cannot be well pictured by camera because of low contrast. Images, on the other hand, ideally give a precise representation of the morphological structures (also with regard to coloration and patterns, see amphipod example above), even more so with the development of high-resolution imaging techniques (Kaiser et al., 2018; Błażewicz et al., 2019; Jażdżewska et al., 2022). In addition, photography is far less subjective than creating drawings, but despite these advantages has so far rarely found its way into peracarid taxonomy.

The preparation of drawings presenting details of morphological structure has been historically/traditionally carried out by means of a *camera lucida* attached to the microscope. This device is a simple system of mirrors (Wollaston, 1807) which makes it possible to reproduce an object (body habitus or appendages) on a sheet of paper placed next to the microscope (**Figure 1H**). Despite the simplicity of its design, the *camera* is a relatively expensive piece of optical microscope equipment: only few optical companies

manufacture them, and they are not usually exchangeable between different models of microscopes. In addition to the traditional use of *camera lucida*, focus-stacked microphotographs can be the baseline for drawings (Coleman, 2006) or even substitute for pencil drawings (d'Udeckem d'Acoz and Verheye, 2017; Wilson and Humphrey, 2020). Nevertheless, both *camera lucida* and stacked microphotographs techniques can also be applied together for producing drawings of fossils (Selden et al., 2016). The appropriate camera and acquisition software to equip the microscope are also expensive.

Microscopic images are useful to complement scientific drawings when studying rare (singleton or unique) species. While this is a general phenomenon in the description of species (Lim et al., 2012; Wells et al., 2019), it becomes particularly evident in the morphological analyses of deep-sea species including peracarids (Brandt et al., 2012; Higgs and Attrill, 2015). Drawings without dissecting parts of the specimen are often sought not to sacrifice the holotype, but it is thanks to the use of imaging techniques, chiefly non-destructive methods (such as CLSM, see below), it is possible to fill in missing gaps of morphological information. However, it is clear that not always taxonomist have access to all facilities to use such as useful techniques and methods.

So far, however, no efforts to refrain from drawings in peracarid taxonomy have been taken but, on the contrary to bring together as much information as possible (including molecular, ecological, and biogeographic) as part of an integrative process (Brix et al., 2015; Malyutina et al., 2018; Kaiser et al., 2018; Schnurr et al., 2018; Błażewicz et al., 2019; Jakiel et al., 2019; Riehl and De Smet, 2020; Kaiser et al., 2021). Above all, the use of digital drawing techniques and the corresponding software (something expensive as well) has made a significant contribution to reducing the time required for, and improving the quality of species illustrations (Coleman, 2003; Coleman, 2009; Bober and Riehl, 2014; Montesanto, 2015). However, much greater advances appear to have been achieved in the development of 3D reconstruction and imaging techniques.

3.2 Specialized Techniques of Specimen Imaging

Morphology (i.e., shape of the organisms and its parts) is still the most important taxonomic characteristic and thus methods of its recording and analysis – imaging methods – are crucial tools in the armory of a taxonomist of deep-sea Peracarida (**Figure 3**). Concentrating on imaging for taxonomic purposes, we need to differentiate the imaging of overall morphology (habitus) which may be performed without any previous zoological knowledge (**Figures 3A–I**), and imaging of specialized, taxonomically important features, the choice of which must be informed by accumulated knowledge and expertise. For deep-sea peracarids, where specimens are difficult to obtain (complicated logistics), available in limited numbers and thus are highly valuable, an important consideration is the distinction between imaging taxonomically important morphological features *in situ* (in intact specimens) *versus* imaging of prepared or isolated body parts (*ex situ*, after dissection and/or sectioning, **Figures 3J, K**), which may be sometimes necessary even for type specimens. When selecting imaging techniques, some thought must be also paid to the location of taxonomically distinctive features within the body of the crustacean – some techniques are exclusively suited to imaging external morphology (e.g. SEM, **Figures 3L–N**, or CLSM, **Figures 3O–R**), while others were developed specifically for imaging internal organs and hidden features (e.g. microCT, **Figure 3S**). Finally, a modern taxonomist must bear in mind that imaging can be used not only for purely morphological (shape-related) analysis, but specific contrast techniques are available to draw conclusions about biochemical composition of tissue elements as well as course of physiological processes which may be helpful as additional taxonomic characteristics and form an additional level of analysis (apart from morphological and genetic ones). **Table 3** includes recent examples of application of specific imaging techniques which will be reviewed below to Peracarida and other crustaceans.

3.2.1 Light Microscopy

3.2.1.1 Bright Field and Optical Contrast Microscopy

While bright field light microscopy is the original method in taxonomy of any small organisms, its applicability to deep-sea Peracarida is limited by the relative lack of inherent contrast in their bodies. Light microscopy relies mainly on absorption, refraction, and dispersion of incident rays in the specimen, and marine crustaceans tend to be colorless (low absorption) and with optical refringence that is uniform and similar to surrounding seawater. While habitus imaging may be performed on whole specimens by reflected light stereomicroscopy in air (Hegna, 2010), the resulting images are poor in details and thus of low usefulness in taxonomy.

Most commonly, zoological specimens are prepared in a procedure called mounting, where the animal is placed on a glass slide in a drop of liquid and covered with another flat piece of glass (the thickness of this cover glass is adapted to the working distance of the microscope objective to be applied). Mounting has two main purposes: to prevent the desiccation-related destruction of specimen, and to provide an environment with uniform refraction properties in order to minimize image blurring due to photon scattering on phase borders. Therefore, the mounting medium for marine crustaceans must mix well and rapidly with seawater, and its refractive index should be as close as possible to that of glass (1.52). While animals can be mounted in water itself for short-term observation (e.g. on board), it evaporates quickly and a different mounting medium is needed if the specimen is to be stored as microscope slide. The most important decision in the choice of mounting medium is related to the desired permanence of the slide: specimens in non-permanent (liquid- or gel-based) media may be manipulated, moved around, remounted, or even removed from the slide for other type of analysis; permanent (solidifying) medium preserves the slide permanently in the same attitude of the specimen. Sometimes, the mounting medium includes components that have additional functions with regard to the specimen itself: clearing (optical homogenization by removal of light-scattering inclusions) and/or maceration (chemical removal of unwanted tissue, e.g. muscles inside the tegument). These components are

TABLE 3 | Selected examples of literature references where different imaging techniques were used to study the taxonomy of peracarids and other crustaceans or were applied to visualize peracarids for non-taxonomic purposes.

Imaging method	Special technique	Staining/preparation for visualization	PERACARIDA		CRUSTACEA
			Taxonomy	Other purpose	taxonomy
Optical contrast light microscopy	Bright field	Alcian blue		Žnidaršič et al., 2018	
		Alizarin red	Haug et al., 2011a	Žnidaršič et al., 2018	
		Azure II		Wirkner and Richter, 2004; Mrak et al., 2012	
				Žnidaršič et al., 2018	
		Chlorazol black	Corbera and Martín, 2002		
		Hematoxylin	Hegna, 2010		
		Hematoxylin/eosin		Žnidaršič et al., 2018	
		Ink	Hegna, 2010		
		Lignin pink	Hadjab et al., 2020; Jażdżewska et al., 2022		
		Toluidine blue	Bober et al., 2018		
		None	Curatolo et al., 2013		
	Dark field	None			Haug et al., 2011b
Fluorescence microscopy	Widefield	Autofluorescence	Haug et al., 2011b	Giurginca et al., 2015	Eiler et al., 2016
			Haug et al., 2011a	Glenn et al., 2013	Marek, 2017
				Nagler and Haug, 2016	
		Alizarin red		Mrak et al., 2013	Haug et al., 2011b
		Hoechst		Kreissl et al., 2008	
		Chitin-binding probe		Žnidaršič et al., 2018	
		Immunofluorescence		Kenning and Harzsch, 2013	
				Kreissl et al., 2008	
	Laser scanning confocal	Autofluorescence	Hughes and Kaji, 2016	Bruce and Patel, 2020	Galassi et al., 1998
			Riehl and De Smet, 2020	Kakui, 2014	Michels, 2007
				Kenning and Harzsch, 2013	Lee et al., 2009
				Stegner et al., 2015	Valdecasas and Abad, 2011
		Acid fuchsin	Riehl and De Smet, 2020		Kottmann et al., 2013
		Congo red	Brökeland et al., 2010		Kihara and Martinez Arbizu, 2012
			Michels and Büntzow, 2010		Menzel, 2011
			Riehl and De Smet, 2020		Michels and Büntzow, 2010
		Congo red/acid fuchsin	Brandt et al., 2014		Kamanli et al., 2017
		Blankophor			Brooker et al., 2012a; Brooker et al., 2012b
		DiI		Stemme et al., 2014	
		Eosin Y			Lee et al., 2009
		Gomori			Brooker et al., 2012a; Brooker et al., 2012b
		Mercurochrome			Lee et al., 2009
		Phalloidin		Jirikowski et al., 2013	
				Jirikowski et al., 2015	
				Göpel and Wirkner, 2018	
		Rose bengal	Chim and Tong, 2020		
		Safranin			Lee et al., 2009
		Shirlastain A	Riehl and De Smet, 2020		
		Sytox Green		Wolff, 2009	
		Immunofluorescence		Kenning and Harzsch, 2013	
				Stegner et al., 2015	
				Stemme et al., 2014	

(Continued)

TABLE 3 | Continued

Imaging method	Special technique	Staining/preparation for visualization	PERACARIDA		CRUSTACEA
			Taxonomy	Other purpose	taxonomy
	Spinning disk confocal	Autofluorescence	Haug et al., 2011b		
Electron microscopy	TEM	Uranium/immunogold Uranium/lead Lectin-gold		Štrus et al., 2019 Geiselbrecht and Melzer, 2013a Žnidaršič et al., 2018	
	SEM	Gold		Geiselbrecht and Melzer, 2014 Kaji et al., 2016 Wirkner and Richter, 2004 Wolff, 2009	
		Gold/palladium	Haug et al., 2011a		
		Osmium		Kaji et al., 2014	
		Carbon	Bober et al., 2018 Brandt et al., 2014		
		Other	Hughes and Ahyong, 2016 Riehl and De Smet, 2020	Štrus et al., 2019	Kamanli et al., 2017 Haug et al., 2011b
	FIB-SEM	Gold/palladium	Haug et al., 2011a		
	SBF-SEM	Osmium/lead/gold		Kaji et al., 2016	
MicroCT	X-ray	Iodine None	Haug et al., 2011a	Štrus et al., 2019 Nagler and Haug, 2016 Wirkner and Richter, 2004 Göpel and Wirkner, 2018	Maeno et al., 2019 Landschoff et al., 2018 Haug et al., 2011b
	Synchrotron	None			Betz et al., 2007

usually acids (e.g. lactic acid) or bases (e.g. potassium hydroxide), and care must be taken not to exceed the necessary dosage and, if possible, to remove the agent before final mounting, as they may progressively destroy taxonomically important features or even the whole specimen during prolonged storage. **Table 4** lists the commonly used mounting media for microscopic imaging of Peracarida with their main advantages and disadvantages.

The most common components of non-permanent mounting media used for taxonomic imaging of small marine arthropods include: glycerol (higher refractive index than water and negligible evaporation; sometimes mixed with 10% saline to facilitate mixing during slide preparation), gelatin (less recommendable as it is prone to desiccation and cracking), polyvinyl alcohol (included in the popular commercial mounting medium Mowiol and in the complex self-made medium polyvinyl lactophenol), and chloral hydrate (included together with glycerol in popularly used Hoyer's medium, where it contributes to its high refractive index). They are often used in personally formulated mixtures based on experience and anecdotal evidence on performance – it is possible that some are more suitable for certain systematic groups of Peracarida than others, but systematic studies are lacking and it seems that subjective personal preference remains the main argument for mounting medium choice. Oil-based mounting media are also available, but rarely used for invertebrate taxonomy as they do not perform well with carbohydrate exoskeletons. Permanent (solidifying/hardening, either by physical curing or by chemical polymerisation) mounting media are also often used for museum specimen storage, but this practice prevents any further manipulation of the specimen (including potential new molecular discrimination techniques) and should be discouraged for rare type material where methodological developments in molecular studies may warrant the need for access to relatively unchanged biological material in distant future. However, permanent mounting may be recommended for long-term storage of dissected parts (e.g. appendages) which are of purely morphological value. While some resin-based solidifying media are marketed as reversible (they may be liquefied by heating with an excess of solvent), both morphological structure and biochemical composition is usually compromised by such treatment and all solidifying mounting media should be treated as permanent. The most common base ingredients of solidifying mounting media used in taxonomy of Peracarida include natural resins (Canada balsam, Euparal and others that solidify by gradual solvent evaporation and vitrification), synthetic resins (included in such preparations as DPX or Permount) and formaldehyde-based polymers (mainly dimethylhydantoin formaldehyde – DMHF – which is recognized as superior to resins due to much less cracking and bubbling artefacts; Bameul, 1990).

If the entire or dissected specimen is to be preserved in long-term storage in the form of microscope slide mounted in liquid medium, this slide must be also sealed using impermeant sealants that isolate the specimen from external moisture and oxygen (numerous commercial products are available, e.g. based on linseed oil, plant resins, paraffin or acrylic glue; even simple nail

TABLE 4 | Advantages and disadvantages of mounting media commonly used for light-microscopy studies of peracarids.

Type of mounting medium	Components/ media	Advantages	Disadvantages	References
Non-permanent liquid	Water	Easy application	Weak optical properties, strong evaporation, very low durability	Wittmann et al., 2016
	Glycerol	Good optical properties, easy application and removal for other techniques, very low evaporation	Need for complex sealing methods,	Maybury et al., 1991; Neuhaus et al., 2017
	Chloral hydrate (Hoyer's)	High refractive index, strong clearing action	Short-term storage, easy evaporation, difficult sealing	Kodama and Kawamura, 2019
Semi-permanent solidifying	Gelatin	Easy application	Easy cracking and microbial contamination in long-term storage	Jersabek, 2005; Neuhaus et al., 2017
	Polyvinyl alcohol (PVA)	May include clearing and macerating agents (lactic acid, phenol)	Possibility of microbial contamination, difficult remounting	Koomen and von Vaupel Klein, 1995; Neuhaus et al., 2017
Permanent resin-based	Euparal	Long-term preservation without dehydration	Time-consuming preparation, some dehydration	Coleman, 2006
	Canada balsam	Very durable (hundred-year permanence)	Impossible to remount, specimen no longer accessible for other methods, complex specimen preparation (dehydration)	Koomen and von Vaupel Klein, 1995; Neuhaus et al., 2017
	Dibuthyl phthalate (DPX)	Easy to apply, relatively durable	Toxic, generates some morphological distortion of delicate features	Geiselbrecht and Melzer, 2013b; Nagler and Haug, 2016;
Permanent polymer-based	Dimethylhydantoin formaldehyde (DMHF)	Easy to apply, concomitant maceration	Crystal formation during long-term storage, damage to nucleic acids	Steedman, 1958; Bameul, 1990; Bourque et al., 2020

varnish may be used for this purpose, but care must be taken that its components do not interfere with any staining that was applied) (Allington-Jones and Sherlock, 2007). When considering long-term storage in non-permanent mounting media, the question of microbial contamination potential must be also taken into account: glycerol-based media are most resistant to contamination, while microbes grow most easily in those containing gelatin. Since the function of the mounting medium requires the compounds involved to thoroughly permeate the specimen, it needs to be extensively washed if it is required at some later point to release it from the slide after microscopy for some other (e.g. genetic) analysis. Common liquid mounting media (e.g. glycerol-based) do not damage nucleic acids and can be removed by washing, but polymerizing permanent mounting makes isolating DNA from the sample impossible.

For transmitted light imaging, the standard procedure is to stain the specimen with light-absorbing dyes to create contrast. In current practice for taxonomic purposes, researchers aim to use non-selective stains to visualize most tissue types and structures (in crustaceans, the most important element being usually the exoskeleton and its outgrowths, especially on the appendages). The most commonly used dyes are hematoxylin (which stains nucleic acids – and thus living tissue – dark blue) (Hegna, 2010) and eosin (which stains most biological macromolecules, including those in the extracellular matrix and exoskeleton, pink), most often combining these two as counterstains (Žnidaršič et al., 2018). Other, more selective dyes can also be used to stain crustaceans, including azure II (stains polysaccharides, including cuticle components), alizarin red (stains calcium deposits in calcified carapace), chlorazol black (basic dye that stains anionic macromolecules, mainly nucleic acids), alcian blue (basic dye for acidic glycans in connective tissue), toluidine blue, lignin pink (both glycan-selective stains with differing affinity) or even the non-selective India ink that stains by physical interactions. Specimens stained using these techniques are usually mounted by immobilization on standard microscope slides, but sectioned or dissected samples may be also prepared after staining. Image is recorded by photographic cameras attached to standard light microscopes or even simply by drawing (see *Preparing Drawings*). If a specimen stained with a cationic dye is to be subsequently used for DNA isolation, an additional washing step may be included to remove the bound dye which might impact downstream reaction efficiency. Some fluorescent DNA-binding (intercalating) dyes (see below) are virtually impossible to remove from DNA during isolation, but there are few reports (from experiments on tissues of vertebrates) finding them interfering even in complex genetic procedures (e.g. next generation sequencing), so this should not be a critical issue in invertebrate taxonomy.

The indisputable advantage of bright field light microscopy imaging is the common availability of cheap instrumentation which requires little specialist training on the part of the researcher. Light microscopes are usually available, even on board research vessels, and can be used for imaging of freshly collected specimens before fixation. When combined with staining, this technique can provide convincing basis for quantitative measurements and rudimentary conclusions with regard to biochemical composition of some structures (e.g. carapace calcification). The central disadvantage is the

relatively poor contrast, both against the background and internally within the imaged specimen, leading to potential obfuscation of taxonomically important morphological differences and features. Standard light microscopy (both in reflected and transmitted light) is poor in rendering internal structures of the body and requires extensive dissection to image complex elements (like appendages). Efficiently imaging three-dimensional structures is not possible, even though they may be observed by stereomicroscopy (attempts have been made to construct and publish 3D images of Amphipoda to be viewed through red-cyan glasses, with limited success (Haug et al., 2011a). Nevertheless, taxonomical descriptions relying on bright field images of unstained or stained Peracarida continue to be routinely published, e.g. new amphipod species imaged after lignin pink staining (Hadjab et al., 2020) or new isopod species stained with chlorazol black (Pereira et al., 2019).

The contrast problem has led to the application of some specialized variants of optical contrast light microscopy (which all require technical add-on enhancements to the microscope itself which are relatively rare in zoological laboratories). One technique which has found use in taxonomically useful imaging of arthropods is dark field microscopy, where incident light is directed at the specimen in such a way that it does not pass into the objective unless deflected (reflected, refracted or scattered) by the specimen, leading to improved contrast against background and higher salience of delicate surface structures (Haug et al., 2011b). Another applicable method is polarization contrast that can underline differences in thickness and density of thicker homogenous structures formed by the cuticle (Fernández del Río et al., 2016; Melzer et al., 2021). Finally, interference contrast (also known as Nomarski contrast) is a powerful technique enabling the visualization of fine ultrastructural details. It has hitherto found application in deep-sea isopod and amphipod species taxonomy (Bruce, 1995; Bruce, 1997; Just, 2001; Tomikawa and Mawatari, 2006; Storey and Poore, 2009) but also in coastal and freshwater species (Shimomura and Mawatari, 1999; Shimomura and Mawatari, 2000; Tanaka, 2004; Jaume and Queinneck, 2007), demonstrating its power in imaging fine morphological structure of appendages (Maruzzo et al., 2007).

3.2.1.2 Fluorescence Microscopy

The most common solution to the contrast problem in biological microscopy is to make use of fluorescence, the physical phenomenon where some compounds (called fluorophores) absorb light of higher energy (lower wavelength) and subsequently emit light of lower energy (higher wavelength). This difference in wavelength, called Stokes shift, makes it possible to design microscopes which separate the incident (illumination) light from the light emanating from the sample, and thus obtain an image exclusively of the fluorescent elements within the sample. For most biological specimens, fluorescence microscopy requires staining with fluorescent dyes (fluorophore-containing compounds which bind to specific structures in the sample). Crustaceans (and arthropods in general), however, usually display relatively strong fluorescence of endogenous compounds (so-called autofluorescence) in intact specimens, allowing for easy fluorescence microscopy imaging and accounting for the widespread use of this technique in taxonomy. While biochemical studies of compounds responsible for autofluorescence in crustaceans are still too few and this field needs further intensive research, most parts of crustacean exoskeleton exhibit a broad-spectrum, near UV-excited autofluorescence that is a consequence of its highly cross-linked structure with glycan and protein components both contributing to the resulting fluorophores. Serendipitously, formaldehyde fixation tends to strengthen this broad-spectrum fluorescence component, making it even easier to image specimens fixed in this way (Hughes and Ahyong, 2016). Another source of autofluorescence is the elastomeric protein resilin, abundant in sites that are under strong mechanical stress such as tegument joints or mouthpart appendages, which contains dityrosine crosslinks that generate autofluorescence. Finally, some metabolic compounds (flavins, pterins, porphyrins, etc.) present in tissues also have fluorescent properties, enhancing the potential for fluorescent imaging of unstained specimens (Riehl and De Smet, 2020). Some arthropods have evolved dedicated autofluorescent compounds, probably important for ecological interactions, such as in some hoplocarid mantis shrimps with markings containing a yellow fluorescent fluorophore that are important in visual recognition or in shallow water copepods which contain dedicated fluorescent proteins similar to the more well-known ones from cnidarians. This ecologically motivated autofluorescence is even more common in terrestrial arthropods such as scorpions (which produce coumarin pigments) or millipedes (which rely on pterins). However, in crustaceans from the aphotic zone these dedicated fluorophores have not been detected yet and the observed autofluorescence seems to be a side effect of the biochemical structure of tissues and tegument (Glenn et al., 2013). Fluorescent properties may be used to enhance the visual signal generated by bioluminescence in deep-sea Peracarida that display this property, thus being ecologically important for visual communication within the species or between different species. Examples include, the lanceolid amphipod *Megalanceola stephenseni* (Chevreux, 1920) and amphipods from the families Pronoidae, Scinidae and Lysianassidae (Herring, 1981; Zeidler, 2009), mysids from family Mysidae (Herring, 1981) and the lophogastrid *Neognathophausia ingens* (Dohrn, 1870) (Frank et al., 1984), the fluorescence of which seems not to originate from the species itself, but rather to be dependent upon components of its food (Wittmann et al., 2014). This topic needs further studies on living specimens, preferably *in situ* (Macel et al., 2020). In any case, the presence of autofluorescence does not preclude the use of additional staining of specific structures in the crustacean body with fluorescent dyes for taxonomic purposes, but its continued presence needs to be taken into account for potential spectral overlap when selecting imaging channels.

When using fluorescence microscopy for taxonomic purposes, specimens are often stained with fluorescent dyes to further enhance contrast and facilitate the imaging of structures with defined biochemical composition. With regard to their mode of action, these dyes can be divided into four groups:

1) Broad specificity acidic dyes, which bind mainly to carbohydrates in the tegument. They are useful in detailed

imaging of appendages, exoskeleton protrusions etc., while staining virtually the whole body of the animal to a different extent. The most commonly used dyes from this group are acid fuchsin (Riehl and De Smet, 2020; Kaiser et al., 2021) and Congo red (Michels and Büntzow, 2010; Kihara and Martinez Arbizu, 2012). An interesting example is rose bengal, a halogenated fluorescein derivative which has the capacity to bind cellular components as well, but in the presence of abundant extracellular carbohydrate material binds mostly to it. In the taxonomy of deep-sea Peracarida, its main use is for transient staining of (usually formaldehyde-fixed) mixed material to aid in visual sorting (due to its strong color) (Hegna, 2010), but its fluorescent properties allow it also to be used in whole-body fluorescence microscopy (Chim and Tong, 2020).

2) Carbohydrate-specific dyes, mostly taken over from the textile industry. They are i.a. Blankophor/Calcofluor (Brooker et al., 2012b), Shirlastain or aniline blue, which bind mainly to chitin in the exoskeleton (Riehl and De Smet, 2020).
3) Calcium binding stains, that are useful to identify calcified parts of the skeleton, such as calcein or alizarin red (Haug et al., 2011a).
4) Cationic dyes, which mainly bind to nucleic acids and stain living tissues more or less uniformly. They are safranin, eosin, DAPI or Hoechst family dyes (Kakui and Hiruta, 2017).

More specialized fluorescent probes binding to cellular or subcellular elements with restricted distribution may also be used, e.g. cytoskeleton-specific binders such as phalloidin or fluorescent antibodies (this technique is called immunofluorescence), but this is of limited usefulness in taxonomy and more commonly found in physiological or embryological studies. Both autofluorescence and probe/dye fluorescence is subject to a phenomenon called photobleaching, where long-term illumination causes a chemical reaction that destroys fluorophore molecules, leading to decreased image brightness. This can be slowed down by including so-called anti-fade components in the mounting medium, but this is rarely necessary with the bright and stable fluorophores used for taxonomically relevant imaging of crustaceans.

With regard to instrumentation, the simplest application of the fluorescence microscopy principle is the widefield fluorescence microscope which uses the same optical principle as a bright field microscope, but separates optical paths of excitation and emission light using filters and dichroic mirrors. Images generated in a widefield microscope can be viewed directly through the eyepiece or recorded using photographic or motion cameras. They can also be overlaid in-microscope with bright field images, pinpointing the location of fluorescent structures within the whole body of the animal. Widefield image quality is restricted by the so-called out-of-focus blur, i.e. light emitted from above and below the focal plane which enters the objective and decreases the image sharpness. This can be strongly limiting in the imaging of small taxonomically important elements within a larger structure. Therefore, an increasing number of taxonomic studies make use of another fluorescence imaging modality, so-called confocal microscopy. A confocal microscope retains only the objective lens from a standard optical microscope setup and images only a single point within the sample (so-called confocal volume), using regulated apertures (here called pinholes) to cut off out-of-focus illumination from both excitation and emission light paths. Therefore, a confocal microscope does not generate an image, but measures the fluorescence intensity in a spatially defined point within the sample. An image is subsequently reconstructed digitally by dedicated computer software from data collected from various confocal volumes, as the illumination is scanned across the sample. The scan may be effected in two ways: either by using optically deflected laser beams (laser-scanning confocal microscopy, in zoology usually known under the less logical name confocal laser scanning microscopy or CLSM) or by using spinning discs (Nipkow discs) with multiple pinholes (spinning disc confocal microscopy). While spinning disc confocal microscopy generates images much faster and with higher inherent brightness, these advantages are mostly important in imaging live specimens, which is rare for deep-sea taxonomical purposes. The relative rarity and costliness of spinning disc microscopes combined with their lack of versatility make them a niche tool for crustacean taxonomy when compared to laser-scanning microscopes (Haug et al., 2011b).

A confocal image is not "recorded" in a way that a camera records a widefield image, but is reconstructed from individual pixels in silico, so the native form of this image is already digital and with no loss of quality upon digitization. Since the confocal volume can be moved across the sample in all directions, a confocal microscope can be used to record three-dimensional images of specimens, making it especially useful in crustacean taxonomy where many important features such as appendage structure are inherently three-dimensional (**Figures 3O–R**). Properties of light, however, restrict the image resolution in the Z axis (parallel to the long axis of the objective) to ca. 2–3 times less than lateral resolution, so confocal images are never truly 3D-isomorphic. If isomorphism is absolutely necessary for taxonomic purposes, several images with different specimen orientation must be recorded. Since laser scanning confocal microscopy involves moving a small confocal volume around a large specimen, it is notoriously slow, with a good resolution image of an average-sized deep-sea crustacean taking more than 10 hours to record. Moreover, because for good resolution it is necessary to use medium-magnification objectives which usually do not allow the whole animal to fit in a single field of view, sophisticated software must be used to reconstruct the whole image from several adjacent scans in a procedure called tiling – its success (the lack of visible artifacts on scan joints) depends largely on the quality of the objective (spherical aberration correction). When recording 3D confocal images and using them for taxonomy, the way that they will be presented and disseminated in the literature must be considered, because the original files are usually too large to include even as supplementary information in published articles. A number of 2D projections (most common being maximum intensity

projection and surface projection) have been developed to help present 3D data.

3.2.2 Electron Microscopy

Electron microscopy is a group of imaging techniques which use physical effects which happen when the sample is illuminated with a stream of high-energy electrons: usually, transmitted, scattered or secondary electrons are detected. The main advantage of electron microscopy in biological imaging is the potential to generate images of much higher inherent resolution than light microscopy, since the electron beam is equivalent to radiation with a very short wavelength compared to visible light. However, for purposes of taxonomy of macroscopic invertebrates, this aspect rarely comes into play, since subcellular features (and generally features of submicrometric size) are not often used as taxonomically defining. The variant of this technique that is most often used by taxonomists is scanning electron microscopy (SEM), where the sample is illuminated by a narrow electron beam which moves across its surface and secondary electrons emitted from every spot on the way (only from the surface since they have too low energies to escape from lower layers of material) are measured using an array of detectors, recreating in real time a spatial map of the surface relief. The main advantages of this method which make it so attractive for imaging for taxonomic purposes are: high sensitivity to small changes in surface geometry which makes it possible to efficiently image surface texture and generate high-resolution images of delicate and complex structures such as those abounding on crustacean exoskeletons and appendages; high depth of field which retains in focus structures that are far away from each other along the z axis, generating a realistic and sharp image of the whole macroscopic specimen while retaining sub-microscopic resolution; the ability to modify magnification in a wide range (from several-fold to tens of thousands-fold) in a contiguous, real-time manner while conducting observations; and the possibility to easily reconstruct three-dimensional measurements from images or generate true 3D images of the specimen by recording images from two different angles. However, the method has also significant disadvantages, mostly related to the onerous and highly invasive sample preparation required for imaging in a typical SEM instrument: since both the high-energy illumination electrons and the low-energy secondary electrons that are being imaged can be deflected by interactions with air molecules, low-pressure vacuum environment is needed around the sample, which means it cannot contain water (so, biological samples must be dehydrated before imaging); since atoms contained in organic compounds do not interact with high-energy electrons efficiently and do not generate many secondary electrons, it is often necessary to coat the specimen surface with a layer of higher atomic number atoms which will produce a brighter image; the absorption of electrons by the specimen generates a high static electrical charge which would quickly lead to scanning artifacts, discharges and specimen destruction if not removed, thus the specimen must be electrically conductive or coated with a material which conducts electricity. For these reasons, SEM is a destructive technique and specimens of Peracarida prepared for SEM imaging cannot usually be used subsequently for any further preparation or analysis using other methods. The sample preparation process for deep-sea crustaceans for SEM imaging has several important steps at which different approaches may be taken depending on specific needs of the researcher. Due to the high energies and harsh treatment involved, the specimen needs to be fixed in a strong fixating agent, usually glutaraldehyde or a mixture of glutaraldehyde and formaldehyde. Dehydration cannot be achieved by air drying as this would destroy delicate surface structures, so water is first replaced by an organic solvent (e.g. ethanol or acetone), and this solvent with higher vapor pressure may be either evaporated directly with less damage to the specimen or it may be replaced with liquid carbon dioxide which then evaporates in conditions around its phase transition critical point (where gas and liquid densities are equal, removing the damaging surface tension - so-called critical point drying). For imaging, the specimen may then be coated with a thin layer of metal (such as gold, platinum, palladium or their mixtures) which provides both better secondary electron emission and electrical conductance, or with a layer of powdered carbon (graphite) which only increases conductance. Another useful metal with unique properties is osmium – its tetroxide is an efficient fixative due to the ability to bind lipids (see the chapter on fixation), coating with osmium itself provides conductivity, and both treatments strongly increase contrast due to efficient secondary electron generation.

Apart from standard SEM, other electron microscopy techniques have been used to image aquatic crustaceans, including deep-sea Peracarida. Environmental SEM (ESEM) is a variation of SEM where differential pumping and pressure-limiting apertures allow the placement of the specimen in a gaseous environment. While this still requires a low-pressure environment, water vapor pressure may be kept at saturation levels, allowing the imaging of water-containing (non-dehydrated) specimens (Drumm, 2005). This is of high importance for potential taxonomic usage as imaging is thus non-destructive and the specimen may be re-used in studies using other methods (however, the pressures used in ESEM are usually low enough to cause the sample to freeze, and the freeze-thaw cycle may break up longer DNA molecules, so the sample may be no longer ideal for e.g. nanopore sequencing). The gaseous environment requires low electron beam energies and specialized detectors, which has both practical advantages (most importantly there is no need to coat the sample in conductive material as there is no static electricity build-up, confirming the non-destructive characteristics of this methodology) and disadvantages (the depth of field is severely limited, making low magnification imaging of large specimens difficult). While for terrestrial arthropods, this has allowed the imaging of even live individuals, the applicability of ESEM for aquatic animals is less apparent due to imaging artifacts from liquid droplets at fine structures, but the technological advances in recent years will probably remove this impediment. While SEM is usually used to image the specimen surface, it can be modified for three-dimensional imaging of deeper tissue layers, which is of special interest for crustacean taxonomists as it allows to recreate high-

resolution images of small appendages with complex structure (e.g. mouthparts). One such modification is serial block-face SEM (SBF-SEM) where the animal is stained with heavy metals (osmium, gold, uranium or lead), embedded in a block of epoxy resin and placed in the imaging chamber of a SEM microscope. The top layers of the block are subsequently serially removed with an ultramicrotome which is contained within the imaging chamber itself, and SEM images of the surface at each cutting depth are combined into a 3D image (Kaji et al., 2016). Alternatively, top layers of biological material (e.g. exoskeleton) may be removed by so-called ion beam milling (abrasion by bombardment with a focused stream of high-energy ions) in a technique called focused ion beam SEM (FIB-SEM) (Haug et al., 2011a). The advantage of FIB-SEM in comparison to SBF-SEM is that location of in-depth imaging is determined by the researcher, the 3D image resolution is uniform in all dimensions and the sample does not require embedding, while SBF-SEM is significantly faster (and having the resolution in the vertical dimension limited by the thickness of ultramicrotome slice is usually not a problem for taxonomically relevant features of crustacean bodies). Finally, traditional transmission electron microscopy (TEM), which involves preparing ultra-thin slices of the sample and treating them with heavy metal stains or probes, has also been applied in proof-of-concept studies to image the fine structure of tissue of some peracarids, but its applicability in taxonomy is not related to morphological studies, but limited to determination of differences in molecular composition of proteins, e.g. by immunogold staining, or carbohydrates, e.g. by lectin-gold staining (this potential has not yet been practically applied for taxonomic purposes in Peracarida).

3.2.3 X-Ray Microtomography

Computed tomography refers to any technique that allows three-dimensional imaging of internal structure by techniques that do not require physical dissection/slicing of the specimen (such as magnetic resonance imaging or positron emission tomography). However, in practical usage in zoology, this term (and the subordinate term microcomputed tomography, or μCT, when applied to microscopic objects) is understood exclusively as applying to imaging *via* X-ray illumination and multi-point detection of transmitted and scattered X-rays (more properly known as X-ray tomography). The principle is the three-dimensional analogue of standard medical X-ray imaging of tissue, with pixel size in the micrometric range. This allows the non-destructive imaging of internal structure of zoological specimens and has become one of mainstays of morphology studies of deep-sea crustaceans for taxonomic purposes (Gutiérrez et al., 2018) and specially for treatment of fossil records (Jauvion et al, 2016; Jauvion, 2020; see Figure 3S). Specimen preparation is simple: while the samples may be unfixed (e.g. flash-frozen), it is usual to use specimens fixed in the standard manner (since μCT allows for subsequent use of the same specimen in any other analysis or imaging protocol). Both ethanol and formaldehyde fixatives work fine, with some studies recommending the use of acidic coagulants (in the form of Bouin's fixative) to yield higher image contrast (this is, however, not necessary for crustacean taxonomy in most cases, as the inherent contrast between soft and hard tissue is sufficient anyway) (Wirkner and Richter, 2004). Image quality may be enhanced by stains (in this technique idiosyncratically called "contrast agents"), with the most common ones (providing superior X-ray scattering capabilities) containing atoms of iodine (e.g. Lugol's solution) or osmium (e.g. osmium tetroxide) (Grams and Richter, 2021). This staining helps especially to differentiate between soft tissues with different fat content, but it has not hitherto been shown to be important for crustacean taxonomy, with enough endogenous contrast present in virtually all cases. While the specimens do not need to be dry for imaging itself to be successful, the lengthy scanning process often leads to spontaneous evaporation (air-drying) and consequential morphological artifacts, which makes many researchers opt for specimen dehydration (usually by critical point drying) before μCT imaging. It must be reminded that this makes the sample unsuitable for some potential downstream analysis, including some optical microscopy methods (e.g. immunofluorescence) or nucleic acid isolation for long-chain sequencing. In some studies where precise discrimination between small internal features was necessary (e.g. in neuroanatomy of arthropods), higher energies of X-ray illumination (derived from a large device known as a synchrotron) have been used (Betz et al., 2007). However, for taxonomically important morphological features laboratory-scale μCT (which uses fully shielded bench-size X-ray sources) is fully sufficient. The main advantages of μCT for imaging morphological features of deep-sea Peracarida is the non-destructive character of imaging (thus, it can be used even for the most valuable type samples), the ease of sample preparation and the isomorphic resolution of three-dimensional images (allowing reliable measurement of spatial features). Disadvantages are limited to low access to relevant equipment in some academic centers (although this is currently changing with increasing affordability of μCT equipment) and lack of obvious links between physico-chemical composition of biological tissues and structures and contrast features of the image (which is, however, usually not important for taxonomists).

3.3 Species Descriptions

The naming of species according to defined standards serves to link new information with existing knowledge. The purpose of formal species descriptions is therefore to show how a species is characterized, how it differs from other known species, and ultimately to make the name available for biogeographical, conservational, or phylogenetic studies amongst others. In the past, species descriptions consisted only of the name and diagnosis of the most important segregating features, later detailed descriptions followed, which are extensive, time consuming and (arguably) not necessary (Riedel et al., 2013; Renner, 2016).

A detailed morphological description clearly contradicts ongoing efforts to accelerate taxonomic work. New methodologies and integrative approaches also do not contribute much to the goal of making taxonomy faster, on the contrary, they tend to increase complexity. This is also due to the

fact that with increasing use of molecular tools in taxonomy, often more (new) species are discovered than being described (Pante et al., 2015), a condition that is also observed in studies of deep-sea peracarids (Jennings et al., 2018; Brix et al., 2020; Kaiser et al., 2021; Mohrbeck et al., 2021). Reasons for this gap are manifold: for instance, definitive (morphological and molecular) evidence of a new species is absent, the authors lack taxonomic expertise or there is not enough time to describe all the species in the duration of a (post doc) project (Pante et al., 2015; Brix et al., 2020; Malyutina et al., 2020; Kaiser et al., 2021).

From a peracarid study point of view, there arguably has hardly been any progress in deviating from the so-called taxonomic impediment, i.e. the description of the many, especially small-sized taxa by declining number of taxonomists (Convention on Biological Diversity [CBD], 2010; Mora et al., 2011; Coleman, 2015; Engel et al., 2021). Over the past decades, novel tools have been introduced to put taxonomy into the fast lane, from automated species descriptions (e.g., using DELTA - DEscriptive Language for TAxonomy, Dallwitz et al., 2000), turbo- (Riedel et al., 2013) and cybertaxonomy (Zhang, 2008), to descriptions based exclusively on DNA sequences as diagnostic characters (Jörger and Schrödl, 2013). Turbotaxonomy, for example, describes the approach of linking molecular sequences, morphological descriptions, and high-resolution digital imaging to enable the rapid formal description of a relatively large number of new species (Riedel et al., 2013). While the appropriateness of some new approaches is certainly controversial (e.g., DNA sequences as diagnostic characters, Meier et al., 2022), so far only a few of the modern endeavors mentioned above have been translated into the description of new deep-sea peracarid species (e.g., Lowry and Myers, 2012; Sittrop et al., 2015).

The task of describing all peracarid species from the deep sea is enormous. Hundreds of species are already known within the Peracarida from there, especially within the Isopoda and Tanaidacea (Brandt et al., 2012; Błażewicz-Paszkowycz et al., 2012). Yet, the number of undescribed species is probably much larger, although robust estimates are scarce (Wilson, 2017). From the central abyssal Pacific, for example, 187 and 98 supposedly new species within Isopoda and Tanaidacea, respectively, could be identified from a single sample campaign (Błażewicz et al., 2019; Brix et al., 2020). Add to this, the need of taxonomic revisions and redescriptions of earlier works, which is crucial, but also leads to a step backwards in the description and assessment of deep-sea peracarid biodiversity (Brandt et al., 2012).

For the hypothetical case that around 10,000 deep-sea species within the Isopoda and Tanaidacea still have to be described, existing taxonomists would need around 1,000-2,500 years with a current average rate of 4–9 descriptions per year (**Figure 4**). However, this also requires that sufficient taxonomic expertise remains available and that its number do not decrease any further. Therefore, taxonomic intercalibration exercises in the form of the exchange of sketches and informal taxonomic information were encouraged in order to compare undescribed biodiversity between different regions (International Seabed Authority ISA, 2020; Lins et al., 2021; Washburn et al., 2021). Furthermore, lengthy morphometric investigations and descriptions of new species have already been replaced by proteomic profiles (Yeom et al., 2021). In addition, molecular methods such as e-DNA metabarcoding approaches are propagated, which record biodiversity in a certain area by circumventing formal species descriptions (Dell'Anno et al., 2015; Pawlowski et al., 2018).

Despite the urgency to describe deep-sea fauna in the wake of augmented human impacts, we believe that species should still be formally named and described. Furthermore, descriptions should adhere to common standards, such as according to the International Code of Zoological Nomenclature (ICZN). Species descriptions take time to be accurate and robust, but they could become standardized and more automated (e.g., using programs such as DELTA or MANTIS; Dallwitz et al., 2000; Naskrecki, 2008; Brown, 2013). In addition, experts for a specific group could agree on the lowest common denominator of diagnostic features necessary for the delineation and identification of species, while supplementary microscopic images (such as CLSM see above) provide further taxonomically important information, as well as biogeography, environmental parameters, or DNA barcoding. Overall, we agree with Glover et al. (2018) that only through a comprehensive study of deep-sea species can we gain a better understanding of their function and value for the for deep-sea ecosystems.

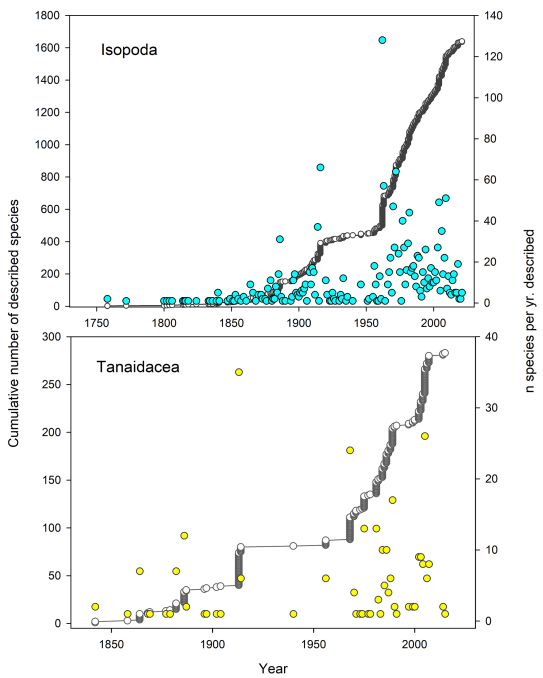

FIGURE 4 | Rate of deep-sea species descriptions within Isopoda (top) and Tanaidacea (below); the line indicates the cumulative number of species (corresponding left y-axis), and the scatter plot indicates the actual number of species described (right y-axis) per year. The average description rate in the last ten years was ~4 and 9 species for tanaidaceans and isopods respectively. Data retrieved from WoRDSS (Glover et al., 2021) and updated through WoRMS (WoRMS Editorial Board, 2021). According to WoRDSS (Glover et al., 2021) only species described from below 500 m are included.

4 DISCUSSION

Undoubtedly, peracarids are an integral part of deep-sea benthic ecosystems (Hessler and Wilson, 1983; Błażewicz-Paszkowycz et al., 2012; Frutos et al., 2017a). Within the particularly species-rich groups, isopods and tanaidaceans, so far around 2,000 species have been described (**Figure 4**), and that should be only a fraction of what is actually present. While well-established traditional methods are often still in use to describe and classify deep-sea Peracarida, new methodologies, notably molecular and microscopic imaging tools, have taken their taxonomic analysis to a new (integrative) level. Specifically, these methods have helped solve some common issues in peracarid taxonomy, including, but not limited to, the delineation of morphologically the same or similar species (Havermans et al., 2013; Brandt et al., 2014; Brix et al., 2015; Jakiel et al., 2020; Kaiser et al., 2021), those with strong sexual or ontogenetic dimorphism (Riehl et al., 2012; Błażewicz-Paszkowycz et al., 2014; Riehl and Kühn, 2020), polymorphism (Larsen, 2001) or incomplete, damaged specimens (Kaiser et al., 2018). The latter is more the rule than the exception. In particular, fragile peracarid crustaceans are damaged when taking samples from greater depths or during sample processing. In addition, fixatives, especially ethanol, although the latter being still first choice, also make the specimens brittle, so they tend to lose their legs or antennae (even if the latter may be mitigated by using small amounts of glycerol (Wilson and Humphrey, 2020). The ability to identify damaged specimens is therefore certainly an advantage of molecular methods over traditional morphological identification (Mohrbeck et al., 2015).

While the methodologies considered here are focused on deep-sea peracarids, they can be applied, in the same way, to the study of other benthic small-sized crustaceans, i.e. ostracods and copepods. With special requirements for efficient sampling (<300 μm mesh-size nets or multi-corer; see Narayanaswamy et al., 2016), the identification of specimens of meiofaunal harpacticoid copepods often demands the dissection of their smallest appendages (Kihara and Martinez Arbizu, 2012; Rossel and Martínez Arbizu, 2018). They are studied in a similar workflow using modern imaging tools under an integrative approach for species identification (Easton and Thistle, 2016; Khodami et al., 2020), however, special techniques adapted to their tiny size (i.e. mass spectrometry) are also suitable for their identification (Rossel and Martínez Arbizu, 2018; Rossel and Martinez Arbizu, 2019).

Overall, the introduction of new taxonomic methods for application to deep-sea specimens seems to be delayed compared to those in shallow waters or on land. 'Omic' approaches, for instance, are increasingly being utilized for classification and identification of species (Bourlat et al., 2013; Raupach et al., 2016; Rossel and Martinez Arbizu, 2019). Whole-genome data, that are already used to separate prokaryote strains, may also be applied to eukaryote taxonomy in the future (Raupach et al., 2016). Yet, in the marine realm and even more so in the deep sea, the application of genomics is still in its infancy. In recent years, genomes have been published for a number of marine species (Wilson et al., 2005; Ritchie et al., 2017; Li et al., 2019), here in particular for amphipods, but also genomes from a number of tanaidacean species have been now analyzed (Kakui and Kano, 2021). For deep-sea isopods and tanaidaceans this is still pending though. Another promising approach is proteomic fingerprinting, which has already been used successfully in the identification of deep-sea isopods (Paulus et al., 2021; Kürzel et al., 2022). The advantages are the faster and cheaper application of proteomics, for example compared to the molecular genetic approach. Yet, it requires a library of protein mass spectra and, overall, the technology is not yet mature enough to reliably delineate species from an unknown deep-sea sample from one another and thus needs further evaluation (Kürzel et al., 2022).

The 'hesitation' in testing new methods is probably partly due to the challenges of deep-sea sampling itself, as fauna densities are typically low especially at greater depths (Frutos and Sorbe, 2014; Wilson, 2017; Malyutina et al., 2018) and therefore the number of organisms usually needed for any kind of molecular analysis may not be achieved. In addition, most deep-sea peracarids, with the exception of a few giant isopods and amphipods, are small, and often only a few millimetres in size, which makes it difficult to extract DNA from these specimens while keeping a whole animal as a voucher. Finally, many of these methods come at a price, require special facilities and equipment as well as expertise (e.g. Pinu et al., 2019, but see Le et al., 2021). Yet, there is no question that now is the time to look more closely than ever before into describing deep-sea biodiversity, which also means to delve deeper into these new approaches, but also to critically evaluate those that have been applied so far (e.g. with respect to long-term preservation of samples and slides, **Table 4**). The deep-sea environment could be used to a greater extent for its resources in the future and is already affected by deep-sea fisheries (e.g. Clark et al., 2016), environmental pollution (Chiba et al., 2018) and climate change (Sweetman et al., 2017). So, time is of the essence to describe more species rather quickly in order to better understand these impacts and their consequences for deep-sea ecosystems.

Despite all the advances, taxonomy has probably never been as challenging as it is today. It starts with the fact that the importance of taxonomic research is not recognized and in turn not well promoted or funded (e.g. Wägele et al., 2011; Saunders, 2020; Britz et al., 2020). In part, this is because the quality of scientific progress is measured by the Impact Factor of journals, with taxonomic journals often falling behind (Wägele et al., 2011). Chairs with a purely taxonomic focus have become a rarity, and taxonomy has become often only a sub-area of otherwise molecular or ecological subjects (e.g., Lester et al., 2014). Since taxonomic research appears to have no future, only a few young scientists can get enthusiastic about the topic, and there is already a shortage of well-trained taxonomists evident today. This taxonomic impediment mentioned above, in which a decreasing number of taxonomists are faced with a high undescribed diversity, is also noticeable among (deep-sea) peracarid taxonomists (Błażewicz-Paszkowycz et al., 2012). Within the last ten years there have been seven and 16 active

taxonomists (only first authors counted), who have described deep-sea species within the Tanaidacea and Isopoda, respectively, but only few of them holding a permanent position (Glover et al., 2021). For amphipods, Coleman (2015) counted nine active taxonomists, although these include the entire diversity of this speciose group - from freshwater to marine. Yet, with regard to the methods and techniques presented in this review, we show how diverse and demanding the taxonomic work is, which not only includes the time-consuming work of describing new species, but often also dealing with unsolved phylogenetic histories including species' redescription and assessment of museum's type material. Among other things, this not only requires a taxonomist to have profound theoretical knowledge of species concepts and phylogenetic analytical methods but also methodical skills, for example in the application of various microscopy techniques or imaging processes as well as relevant molecular methods, while at the same time having to keep up with the pace of how the latter are developing.

It is not a new topic that taxonomic work is highly underrated, and at the same time it is not an individual problem that taxonomists do not get recognition for their work, but that is placed in a broader context and ultimately linked to how society values biodiversity and nature. In our opinion, this is exactly where we have to start, namely to convey taxonomic research and thus the diversity of life to other scientists, but also the wider public. New methods can play a special role here, because the application of the new imaging processes opens up a new world not only to taxonomists, but also to other scientists. SEM let us recognize surfaces that were previously invisible and provides information about the hardness of the tegument; CLSM or computed tomography help to recognize internal structures and thus contribute to the understanding of the functional morphology, embryology or even to the recognition of the material quality that defines the respective structures. All of this not only gives us the opportunity to learn what type of animal we are seeing, but also how it is constructed and how it functions. Thus, these new techniques (including imaging), which are primarily geared towards taxonomy, are an important link to other sciences thus making taxonomy a highly integrative field of science. For laypeople, of course, this only plays a subordinate role; instead, ethical and aesthetic reasons to value or reject something are often in the foreground (cf. Jamieson et al., 2021). Analogous to Haeckel's drawings, the art factor (microscopic images) could be used to reach the public and convince them of the beauty of deep-sea life, and thus also to raise their awareness of how biodiverse the deep sea is and that this diversity is threatened.

5 CONCLUSIONS

Learning more about the deep sea and its inhabitants is an urgent need, and taxonomy will play an important role in this endeavor. Therefore, changes must be addressed here too, in order to describe deep-sea species and thus biodiversity more quickly and at the same time to ensure high-quality taxonomic work. Although great advances have been made in microscopy and imaging tools, it has been shown that relying on morphology alone to describe species poses a number of pitfalls. Therefore, integrative taxonomy in describing deep-sea species is the way forward, as it provides multiple lines of evidence to reliably differentiate species from one another. So, whenever possible, both morphological and molecular (if fixation allows), as well as possibly a description of the environment among others should be sought when describing species. In this paper we have also discussed a number of methods that have not yet or only rarely been so far used in peracarid taxonomy, but that may become more important in the future. Here, particular mention should be made of (non-destructive) microscopic techniques such as CLSM, ESEM or μCT or 'omic' approaches including genomics and proteomics. Above all, however, taxonomic work is to be recognized as what it is, i.e. a multidisciplinary science that makes an essential part of research into deep-sea biodiversity and thus a significant contribution to its conservation.

AUTHOR CONTRIBUTIONS

IF and MB developed the idea; IF and SK produced the figures. All authors contributed to drafting and editing the text, read and approved the submitted version.

FUNDING

SK acknowledges a grant by the Polish National Agency for Academic Exchange (NAWA) under the ULAM program (PPN/ULM/2019/1/00169); IF acknowledges PPN/BFR/2019/1/00031/U/00001 PHC-Polonium; MB and IF acknowledge – NCN OPUS 2018/31/B/NZ8/03198 and NCN OPUS 2016/13/B/NZ8/02495.

ACKNOWLEDGMENTS

Preparation of this manuscript began during the Covid-19 pandemic, which did not allow co-authors to meet in person until restrictions were relaxed. The authors are therefore indebted to the online technology that made the advancement of this article possible. We would like to thank Laure Corbari and Paula Martín-Lefevre, Musée national d'Histoire Naturelle in Paris, who kindly provided the scan of the original plate outline of Chevreux; and two reviewers for their comments that substantially improved this manuscript.

REFERENCES

Ahyong, S. T., Chan, B. K. K., Chan, T.-Y., Corbari, L., Ďuriš, Z., Frutos, I, et al. (2022). "Crustacean Diversity and Discovery," in *The Marine Fauna and Flora of the Bismarck Sea. Patrimoines Naturels*. Eds. P. Bouchet, C. Payri, R. Sabroux and S Samadi. Paris:Muséum national d'Histoire Naturelle

Allington-Jones, L., and Sherlock, E. (2007). Choosing a Microscope Slide Sealant: A Review of Aging Characteristics and the Development of a New Test, Using Low Oxygen Environments. *NatSCA News* 12, 4–14.

Almeida, M., Frutos, I., Company, J. B., Martin, D., Romano, C., and Cunha, M. R. (2017). Biodiversity of Suprabenthic Peracarid Assemblages From the Blanes Canyon Region (NW Mediterranean Sea) in Relation to Natural Disturbance and Trawling Pressure. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 137, 390–403. doi: 10.1016/j.dsr2.2016.06.019

Anderson, G. (2014). Endangered: A Study of Morphological Drawing in Zoological Taxonomy. *Leonardo* 47 (3), 232–240. doi: 10.1162/LEON_a_00675

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., et al. (2012). The Magnitude of Global Marine Species Diversity. *Curr. Biol.* 22 (23), 2189–2202. doi: 10.1016/j.cub.2012.09.036

Ashford, O. S., Kenny, A. J., Barrio Frojan, C. R. S., Bonsall, M. B., Horton, T., Brandt, A., et al. (2018). Phylogenetic and Functional Evidence Suggests That Deep-Ocean Ecosystems are Highly Sensitive to Environmental Change and Direct Human Disturbance. *Proc. R. Soc B.* 285, 20180923. doi: 10.1098/rspb.2018.0923

Bamber, R. N. (2007). New Apseudomorph Tanaidaceans (Crustacea, Peracarida, Tanaidacea) From the Bathyal Slope Off New Caledonia. *Zoosystema* 29 (1), 51–81.

Bameul, F. (1990). Le DMHF: Un Excellent Milieu De Montage En Entomologie. *L'Entomologiste* 46 (5), 233–239.

Barbosa, P., Berry, D. L., and Kary, C. S. (2014). *Insect Histology: Practical Laboratory Techniques* (John Wiley and Sons, Ltd). doi: 10.1002/9781118876114

Barnard, J. L., and Ingram, C. L. (1990). Lysianassoid Amphipoda (Crustacea) From Deep-Sea Thermal Vents. *Smithson Contrib. Zool.* 449, 1–80. doi: 10.5479/si.00810282.499

Bellan-Santini, D., and Thurston, M. H. (1996). Amphipoda of the Hydrothermal Vents Along the Mid-Atlantic Ridge. *J. Nat. Hist.* 30, 685–702. doi: 10.1080/00222939600770381

Beninde, J., Möst, M., and Meyer, A. (2020). Optimized and Affordable High-Throughput Sequencing Workflow for Preserved and Nonpreserved Small Zooplankton Specimens. *Mol. Ecol. Resour.* 20, 1632–1646. doi: 10.1111/1755-0998.13228

Betz, O., Wegst, U., Weide, D., Heethoff, M., Helfen, L., Lee, W.-K., et al. (2007). Imaging Applications of Synchrotron X-Ray Phase-Contrast Microtomography in Biological Morphology and Biomaterials Science. I. General Aspects of the Technique and Its Advantages in the Analysis of Millimetre-Sized Arthropod Structure. *J. Microsc.* 227, 51–71. doi: 10.1111/j.1365-2818.2007.01785.x

Błażewicz, M., Jóźwiak, P., Jennings, R. M., Studzian, M., and Frutos, I. (2019). Integrative Systematics and Ecology of a New Deep-Sea Family of Tanaidacean Crustaceans. *Sci. Rep.* 9 (1), 1–70. doi: 10.1038/s41598-019-53446-1

Błażewicz-Paszkowycz, M., Bamber, R., and Anderson, G. (2012). Diversity of Tanaidacea (Crustacea: Peracarida) in the World's Oceans–How Far Have We Come? *PloS One* 7 (4), e33068. doi: 10.1371/journal.pone.0033068

Błażewicz-Paszkowycz, M., Bamber, R. N., and Cunha, M. R. (2011). New Tanaidomorph Tanaidacea (Crustacea: Peracarida) From Submarine Mud-Volcanoes in the Gulf of Cadiz (North-East Atlantic). *Zootaxa* 2769 (1), 1–53. doi: 10.11646/zootaxa.2769.1.1

Błażewicz-Paszkowycz, M., Jennings, R. M., Jeskulke, K., and Brix, S. (2014). Discovery of Swimming Males of Paratanaoidea (Tanaidacea). *Pol. Polar. Res.* 35 (2), 415–453. doi: 10.2478/popore-2014-0022

Bober, S., and Riehl, T. (2014). Adding Depth to Line Artwork by Digital Stippling – A Step-by-Step Guide to the Method. *Org. Divers. Evol.* 14 (3), 327–337. doi: 10.1007/s13127-014-0173-7

Bober, S., Riehl, T., and Brandt, A. (2018). An Organ of Equilibrium in Deep-Sea Isopods Revealed: The Statocyst of Macrostylidae (Crustacea, Peracarida, Janiroidea). *Zoomorph* 137 (1), 71–82. doi: 10.1007/s00435-017-0376-5

Bourlat, S. J., Borja, A., Gilbert, J., Taylor, M. I., Davies, N., Weisberg, S. B., et al. (2013). Genomics in Marine Monitoring: New Opportunities for Assessing Marine Health Status. *Mar. Poll. Bull.* 74 (1), 19–31. doi: 10.1016/j.marpolbul.2013.05.042

Bourque, D. A., Morey, K. C., Bradley, D. L., Fost, B., Daley, J. M., Jacobson, N., et al. (2020). Dimethyldimethyl Hydantoin: An Alternative Fluid for Morphological and Genetic Preservation. *Biopreserv. Biobank.* 18, 283–289. doi: 10.1089/bio.2020.0001

Boxshall, G. A., Kihara, T. C., and Huys, R. (2016). Collecting and Processing Non-Planktonic Copepods. *J. Crust. Biol.* 36, 576–583. doi: 10.1163/1937240X-00002438

Brandt, A., Alalykina, I., Brix, S., Brenke, N., Błażewicz, M., Golovan, O. A., et al. (2019). Depth Zonation of Northwest Pacific Deep-Sea Macrofauna. *Prog. Oceanogr.* 176, 102131. doi: 10.1016/j.pocean.2019.102131

Brandt, A., Błażewicz-Paszkowycz, M., Bamber, R. N., Mühlenhardt-Siegel, U., Malyutina, M. V., Kaiser, S., et al. (2012). Are There Widespread Peracarid Species in the Deep Sea (Crustacea: Malacostraca)? *Pol. Polar. Res.* 33 (2), 139–162. doi: 10.2478/v10183-012-0012-5

Brandt, A., Brix, S., Held, C., and Kihara, T. C. (2014). Molecular Differentiation in Sympatry Despite Morphological Stasis: Deep-Sea *Atlantoserolis* Wägele 1994 and Glabroserolis Menzies 1962 From the South-West Atlantic (Crustacea: Isopoda: Serolidae). *Zool. J. Linn. Soc* 172 (2), 318–359. doi: 10.1111/zoj.12178

Britz, R., Hundsdörfer, A., and Fritz, U. (2020). Funding, Training, Permits—the Three Big Challenges of Taxonomy. *Megataxa* 1 (1), 49–52. doi: 10.11646/megataxa.1.1.10

Brix, S., Leese, F., Riehl, T., and Kihara, T. C. (2015). A New Genus and New Species of Desmosomatidae Sars 1897 (Isopoda) From the Eastern South Atlantic Abyss Described by Means of Integrative Taxonomy. *Mar. Biodiv.* 45, 7–61. doi: 10.1007/s12526-014-0218-3

Brix, S., Osborn, K. J., Kaiser, S., Truskey, S. B., Schnurr, S. M., Brenke, N., et al. (2020). Adult Life Strategy Affects Distribution Patterns in Abyssal Isopods – Implications for Conservation in Pacific Nodule Areas. *Biogeosciences* 17, 6163–6184. doi: 10.5194/bg-17-6163-2020

Brökeland, W., Guðmundsson, G., and Svavarsson, J. (2010). Diet of Four Species of Deep-Sea Isopods (Crustacea: Malacostraca: Peracarida) in the South Atlantic and the Southern Ocean. *Mar. Biol.* 157, 177–187. doi: 10.1007/s00227-009-1308-9

Brooker, A., Bron, J., and Shinn, A. (2012a). Description of the Free-Swimming Juvenile Stages of *Lernaeocera Branchialis* (Pennellidae), Using Traditional Light and Confocal Microscopy Methods. *Aquat. Biol.* 14, 153–163. doi: 10.3354/ab00388

Brooker, A., Shinn, A., and Bron, J. (2012b). Use of Laser Scanning Confocal Microscopy for Morphological Taxonomy and the Potential for Digital Type Specimens (E-Types). *Aquat. Biol.* 14, 165–173. doi: 10.3354/ab00389

Brown, B. V. (2013). Automating the" Material Examined" Section of Taxonomic Papers to Speed Up Species Descriptions. *Zootaxa* 3683 (3), 297–299. doi: 10.11646/zootaxa.3683.3.8

Bruce, N. L. (1995). The Taxonomy and Phylogeny of Tube-Tailed Sphaeromatid Isopods (Crustacea) With Descriptions of New Species and a New Genus From Southern Australia. *Ophelia* 43 (2), 127–180. doi: 10.1080/00785326.1995.10429829

Bruce, N. L. (1997). A New Genus of Marine Isopod (Crustacea: Flabellifera: Sphaeromatidae) From Australia and the Indo- Pacific Region. *Mem. Mus. Vic.* 56, 145–234. doi: 10.24199/j.mmv.1997.56.08

Bruce, N. L. (2005). Two New Species of the Mesopelagic Isopod Genus *Syscenus* Harger 1880 (Crustacea: Isopoda: Aegidae) From the Southwestern Pacific. *Zootaxa* 1070, 31–42. doi: 10.11646/zootaxa.1070.1.2

Bruce, H. S., and Patel, N. H. (2020). Knockout of Crustacean Leg Patterning Genes Suggests That Insect Wings and Body Walls Evolved From Ancient Leg Segments. *Nat. Ecol. Evol.* 4, 1703–1712. doi: 10.1038/s41559-020-01349-0

Brunel, P., Besner, M., Messier, D., Poirier, L., Granger, D., and Weinstein, M. (1978). Le Traîneau Macer-GIROQ: Appareil Amélioré Pour L'échantillonnage Quantitatif De La Petite Faune Nageuse Au Voisinage Du Fond. *Int. Rev. Ges Hydrobiol.* 63 (6), 815–829. doi: 10.1002/iroh.19780630612

Buhl-Jensen, L. (1986). The Benthic Amphipod Fauna of the West-Norwegian Continental Shelf Compared With the Fauna of Five Adjacent Fjords. *Sarsia* 71, 193–208. doi: 10.1080/00364827.1986.10419690

Campean, A. J., and Coleman, C. O. (2018). A New Species of Sicafodia Just 2004 (Crustacea, Amphipoda, Sicafodiidae) From the North Atlantic. *Mar. Biodiver.* 48, 939–948. doi: 10.1007/s12526-017-0635-1

Carrasón, M., and Matallanas, J. (2001). Feeding Ecology of the Mediterranean Spiderfish, *Bathypterois Mediterraneus* (Pisces: Chlorophthalmidae), on the Western Mediterranean Slope. *Fish. Bull.* 99 (2), 266–274.

Cartanyà, J. (1991). Nous Crustacis Al Registre Fossil De La Conca De Barberà. *Reboll* 2, 27–30.

Cartes, J. E., Sorbe, J. C., and Sardà, F. (1994). Spatial Distribution of Deep-Sea Decapods and Euphausiids Near the Bottom in the Northwestern Mediterranean. *J. Exp. Mar. Biol. Ecol.* 179, 131–144. doi: 10.1016/0022-0981(94)90021-3

CBD, Secretariat of the Convention on Biological Diversity (2010). Guide to the Global Taxonomy Initiative. *CBD Tech. Ser.* 30 (1–195), i–viii.

Chardy, P. (1979). Structure of Deep Sea Asellota Assemblages in the Bay of Biscay; Relationships With the Abyssal Environment. *Ambio Special Rep.* 6, 79–82.

Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., et al. (2018). Human Footprint in the Abyss: 30 Year Records of Deep-Sea Plastic Debris. *Mar. Pol.* 96, 204–212. doi: 10.1016/j.marpol.2018.03.022

Chim, C. K., and Tong, S. J. (2020). Two New Species of Paratanaoid Tanaidaceans of the Family Incertae Sedis (Crustacea: Peracarida) From Polymetallic Nodule Fields in the Eastern Clarion-Clipperton Fracture Zone. *Zootaxa* 4758 (3), 461–485.

Clark, M. R., Althaus, F., Schlacher, T. A., Williams, A., Bowden, D. A., and Rowden, A. A. (2016). The Impacts of Deep-Sea Fisheries on Benthic Communities: A Review. *ICES J. Mar. Sci.* 73 (1), i51–i69. doi: 10.1093/icesjms/fsv123

Coleman, C. O. (2003). "Digital Inking": How to Make Perfect Line Drawings on Computers. *Org. Divers. Evol.* 14, 1–14. doi: 10.1078/1439-6092-00081

Coleman, C. O. (2006). Substituting Time-Consuming Pencil Drawings in Arthropod Taxonomy Using Stacks of Digital Photographs. *Zootaxa* 1360, 61–68. doi: 10.11646/zootaxa.1360.1.4

Coleman, C. O. (2009). Drawing Setae the Digital Way. *Zoosyst. Evol.* 85 (2), 305–310. doi: 10.1002/zoos.200900008

Coleman, C. O. (2015). Taxonomy in Times of the Taxonomic Impediment–Examples From the Community of Experts on Amphipod Crustaceans. *J. Crust. Biol.* 35 (6), 729–740. doi: 10.1163/1937240X-00002381

Corbari, L., Frutos, I., and Sorbe, J. C. (2019). *Dorotea* Gen. Nov., a New Bathyal Genus (Amphipoda, Eusiridae) From the Solomon Sea (Papua New Guinea). *Zootaxa* 4568, 69–80. doi: 10.11646/zootaxa.4568.1.4

Corbera, J. (2006). "Arthropoda Cumacea," in *Handbook of Deep-Sea Hydrothermal Vent Fauna. Denisia*, vol. 18. Eds. D. I. Desbruyères, M. Segonzac and M. Bright, 370–371.

Corbera, J., and Martín, D. (2002). Two New Cumacean Species (Crustacea: Peracarida) From Shallow Waters Off Thailand. *Sci. Mar.* 66, 407–415. doi: 10.3989/scimar.2002.66n4407

Corbera, J., Segonzac, M., and Cunha, M. R. (2008). A New Deep-Sea Genus of Nannastacidae (Crustacea, Cumacea) From the Lucky Strike Hydrothermal Vent Field (Azores Triple Junction, Mid-Atlantic Ridge). *Mar. Biol. Res.* 4 (3), 180–192. doi: 10.1080/17451000801898576

Cordiner, C. (1793). Remarkable ruins, and romantic prospects, of North Britain. With ancient monuments, and singular subjects of natural history. Peter Mazell, London. 96 plates with letterpress.

Costa, A. B., Silva, M.B.da, Fraga, R. E., Rocha, A.A.da, Nishiyama, P. B., Anjos, M.S.d., et al. (2021). Evaluation of an Alternative Technique for Preserving Crustaceans in Dry Conditions With Joint Mobility: A Proposal for Didactic Purposes. *Acta Sci. Biol. Sci.* 43, e53450. doi: 10.4025/actascibiolsci.v43i1.53450

Costello, M. J., and Chaudhary, C. (2017). Marine Biodiversity, Biogeography, Deep-Sea Gradients, and Conservation. *Curr. Biol.* 27 (11), R511–R527. doi: 10.1016/j.cub.2017.06.015

Curatolo, T., Calvaruso, C., Galil, B. S., and Lo Brutto, S. (2013). Geometric Morphometry Supports a Taxonomic Revision of the Mediterranean *Bathyporeia Guilliamsoniana* (Spence Bate 1857) (Amphipoda, Bathyporeiidae). *Crustaceana* 86, 820–828. doi: 10.1163/15685403-00003217

Dallwitz, M. J., Paine, T. A., and Zurcher, E. J. (2000) *Principles of Interactive Keys*. Available at: http://delta-intkey.com (Accessed 5 July 2012).

Dauvin, J. C., Sorbe, J. C., and Lorgeré, J. C. (1995). The Benthic Boundary Layer Macrofauna From the Upper Continental Slope and the Cap-Ferret Canyon (Bay of Biscay). *Oceanol. Acta* 18, 113–122.

Dell'Anno, A., Carugati, L., Corinaldesi, C., Riccioni, G., and Danovaro, R. (2015). Unveiling the Biodiversity of Deep-Sea Nematodes Through Metabarcoding: Are We Ready to Bypass the Classical Taxonomy? *PloS One* 10 (12), e0144928. doi: 10.1371/journal.pone.0144928

Demidov, O., Kihara, T. C., Martinez Arbizu, P., and Clark, P. F. (2021). The Megalopal Stage of the Hydrothermal Vent Crab *Austinograea Rodriguezensis* Tsuchida and Hashimoto 2002 (Decapoda: Bythograeidae): A Morphological Description Based on CLSM Images. *Zootaxa* 5040 (3), 365–387. doi: 10.11646/zootaxa.5040.3.3

Drumm, D. T. (2005). Comparative Morphology of the Mouthparts, Chelipeds and Foregut of Two Kalliapseudid Apseudomorphans (Crustacea: Tanaidacea). *P. Acad. Nat. Sci. Phil.* 154, 137–147. doi: 10.1635/0097-3157(2004)154[0137:CMOTMC]2.0.CO;2

d'Udekem d'Acoz, C., and Verheye, M. H. (2017). *Epimeria* of the Southern Ocean With Notes on Their Relatives (Crustacea, Amphipoda, Eusiroidea). *Eur. J. Taxon.* 359, 1–553. doi: 10.5852/ejt.2017.359

Dumke, I., Purser, A., Marcon, Y., Nornes, S. M., Johnsen, G., Ludvigsen, M., et al. (2018). Underwater Hyperspectral Imaging as an *in Situ* Taxonomic Tool for Deep-Sea Megafauna. *Sci. Rep.* 8 (1), 1–11. doi: 10.1038/s41598-018-31261-4

Dupérré, N. (2020). Old and New Challenges in Taxonomy: What Are Taxonomists Up Against? *Megataxa* 1, 59–62. doi: 10.11646/megataxa.1.1.12

Easton, E. E., and Thiestle, D. (2016). Do Some Deep-Sea, Sediment-Dwelling Species of Harpacicoid Copepods Have 1000-km-Scale Range Sizes? *Mol. Ecol.* 25, 4301–4318. doi: 10.1111/mec.13744

Eiler, S. M., Haug, C., and Haug, J. T. (2016). Detailed Description of a Giant Polychelidan Eryoneicus-Type Larva With Modern Imaging Techniques. *Spixiana* 39, 22–60.

Eltoum, I., Fredenburgh, J., Myers, R. B., and Grizzle, W. E. (2001). Introduction to the Theory and Practice of Fixation of Tissues. *J. Histotechnol.* 24, 173–190. doi: 10.1179/his.2001.24.3.173

Engel, M. S., Ceríaco, L. M. P., Daniel, G. M., Dellapé, P. M., Löbl, I., Marinov, M., et al. (2021). The Taxonomic Impediment: A Shortage of Taxonomists, Not the Lack of Technical Approaches. *Zool. J. Linn. Soc* 193, 381–387. doi: 10.1093/zoolinnean/zlab072

Esquete, P., Wilson, G. D. F., and Troncoso, J. S. (2014). Ecology and Systematics of a New Species of *Uromunna* (Crustacea: Isopoda) From Spanish Eelgrass Beds. *Helgol. Mar. Res.* 68 (2), 329–339. doi: 10.1007/s10152-014-0393-4

Evers, D. L., Fowler, C. B., Cunningham, B. R., Mason, J. T., and O'Leary, T. J. (2011). The Effect of Formaldehyde Fixation on RNA: Optimization of Formaldehyde Adduct Removal. *J. Mol. Diagn.* 13 (3), 282–288. doi: 10.1016/j.jmoldx.2011.01.010

Fernández del Río, L., Arwin, H., and Järrendahl, K. (2016). Polarizing Properties and Structure of the Cuticle of Scarab Beetles From the *Chrysina* Genus. *Phys. Rev. E* 94, 12409. doi: 10.1103/PhysRevE.94.012409

Fontaine, B., Perrard, A., and Bouchet, P. (2012). Twenty-One Years of Shelf Life Between Discovery and Description of New Species. *Curr. Biol.* 22, 943–944. doi: 10.1016/j.cub.2012.10.029

Frank, T. M., Widder, E. A., Latz, M. I., and Case, J. F. (1984). Dietary Maintenance of Bioluminescence in a Deep-Sea Mysid. *J. Exp. Biol.* 109, 385–389. doi: 10.1242/jeb.109.1.385

Frutos, I. (2006). *Estudio De Las Comunidades Suprabentónicas Submareales De La Ría De La Coruña Y Plataforma Continental Adyacente* (NW Península Ibérica: Universidad de Alcalá).

Frutos, I. (2017). "Mysida," in *Inventario De La Biodiversidad Marina De Galicia: Proyecto LEMGAL*(Santiago de Compostela:Consellería do Mar, Xunta de Galicia), 443–448 pp.

Frutos, I., Brandt, A., Sorbe, J. C., and Orejas Saco del Valle, C. (2017a). "Deep-Sea Suprabenthic Communities: The Forgotten Biodiversity," in *Marine Animal Forests. The Ecology of Benthos Biodiversity Hotspots*. Eds. S. Rossi, L. Bramanti and A. Gori (Springer), 475–503 pp. doi: 10.1007/978-3-319-21012-4_21

Frutos, I., Corbari, L., and Sorbe, J. C. (2017b). Diversity of Deep-Sea Amphipoda From Papua New Guinea (SW Pacific Ocean). *Biodivers. J.* 8 (2), 505–506.

Frutos, I., and Sorbe, J. C. (2010). *Politolana Sanchezi* Sp. Nov. (Crustacea: Isopoda: Cirolanidae), a New Benthic Bioturbating Scavenger From Bathyal Soft-Bottoms of the Southern Bay of Biscay Northeastern Atlantic Ocean.

Zootaxa (Alicante, Spain:XVI SIEBM Abstract book) 2640, 20–34. doi: 10.11646/zootaxa.2640.1.2

Frutos, I., and Sorbe, J. C. (2013). *Leucothoe Cathalaa* Sp. Nov. (Crustacea: Amphipoda: Leucothoidae) a New Bathyal Benthic Species From the Le Danois Bank ("El Cachucho" Spanish MPA), Southern Bay of Biscay. *J. Mar. Biol. Assoc. UK* 93 (3), 659–666.

Frutos, I., and Sorbe, J. C. (2014). Bathyal Suprabenthic Assemblages From the Southern Margin of the Capbreton Canyon ("Kostarrenkala" Area), SE Bay of Biscay. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 104, 291–309. doi: 10.1016/j.dsr2.2013.09.010

Frutos, I., Sorbe, J. C., and Junoy, J. (2010). "La Primera Especie Ciega Del Género *Paranthura* (Crustacea: Isopoda: Anthuridea) Habita En El Mar Cantábrico (N España)," in *XVI Simposio Ibérico de Estudios de Biología Marina*, Alicante (España, Septiembre 2010, Vol. 6–10.

Frutos, I., Sorbe, J. C., and Junoy, J. (2011). The First Blind *Paranthura* Species (Crustacea, Isopoda, Paranthuridae) From the "El Cachucho" Marine Protected Area (Le Danois Bank, Southern Bay of Biscay). *Zootaxa* 2971, 17–32. doi: 10.11646/zootaxa.2971.1.2

Fryer, G. (1968). Evolution and Adaptive Radiation in the Chydoridae (Crustacea: Cladocera): A Study in Comparative Functional Morphology and Ecology. *Phil. T. R. Soc London Ser. B.* 254, 221–382+384. doi: 10.1098/rstb.1968.0017

Galassi, D. M. P., De Laurentiis, P., and Giammatteo, M. (1998). Integumental Morphology in Copepods: Assessment by Confocal Laser Scanning Microscopy (CLSM). *Frag. Entomol.* 30, 79–92.

Geiselbrecht, H., and Melzer, R. R. (2013a). How do Mandibles Sense? – The Sensory Apparatus of Larval Mandibles in *Palaemon Elegans* Rathke 1837 (Decapoda, Palaemonidae). *Arthropod. Struct. Dev.* 42, 1–16. doi: 10.1016/j.asd.2012.09.001

Geiselbrecht, H., and Melzer, R. R. (2013b). Nervous Systems in 3D: A Comparison of Caridean, Anomuran, and Brachyuran Zoea-I (Decapoda). *J. Exp. Zool. B.: Mol. Dev. Evol.* 320, 511–524. doi: 10.1002/jez.b.22528

Geiselbrecht, H., and Melzer, R. R. (2014). Fine Structure and Ecdysis of Mandibular Sensilla Associated With the Lacinia Mobilis in *Neomysis Integer* (Leach 1814) (Crustacea, Malacostraca, Peracarida). *Arthropod. Struct. Dev.* 43, 221–230. doi: 10.1016/j.asd.2014.01.002

Gellert, M., Bird, G. J., Stępień, A., Studzian, M., and Błażewicz, M. (2022). A Hidden Diversity in the Atlantic and the SE Pacific: Hamatipedidae N. Fam. *Front. Mar. Sci.* 8, 773437. doi: 10.3389/fmars.2021.773437

Giurginca, A., Šustr, V., Tajovsky, K., Giurginca, M., and Matei, I. (2015). Spectroscopic Parameters of the Cuticle and Ethanol Extracts of the Fluorescent Cave Isopod *Mesoniscus Graniger* (Isopoda, Oniscidea). *ZooKeys* 515, 111–125. doi: 10.3897/zookeys.515.9395

Glenn, D., Caldwell, R. L., and Pakes, M. J. (2013). Fluorescence in Arthropoda Informs Ecological Studies in Anchialine Crustaceans, Remipedia, and Atyidae. *J. Crust. Biol.* 33, 620–626. doi: 10.1163/1937240X-00002170

Glover, A. G., Higgs, N., and Horton, T. (2021). World Register of Deep-Sea Species (WoRDSS). doi: 10.14284/352

Glover, A. G., Wiklund, H., Chen, C., and Dahlgren, T. G. (2018). Point of View: Managing a Sustainable Deep-Sea 'Blue Economy'requires Knowledge of What Actually Lives There. *Elife* 7, e41319. doi: 10.7554/eLife.41319.005

Gooday, A. J., Sykes, D., Góral, T., Zubkov, M. V., and Glover, A. G. (2018). Micro-CT 3D Imaging Reveals the Internal Structure of Three Abyssal Xenophyophore Species (Protista, Foraminifera) From the Eastern Equatorial Pacific Ocean. *Sci. Rep.* 8 (1), 1–12. doi: 10.1038/s41598-018-30186-2

Göpel, T., and Wirkner, C. S. (2018). Morphological Description, Character Conceptualization and the Reconstruction of Ancestral States Exemplified by the Evolution of Arthropod Hearts. *PloS One* 13, e0201702. doi: 10.1371/journal.pone.0201702

Grams, M., and Richter, S. (2021). Locomotion in *Anaspides* (Anaspidacea, Malacostraca) – Insights From a Morpho-Functional Study of Thoracopods With Some Observations on Swimming and Walking. *Zoology* 144, 125883. doi: 10.1016/j.zool.2020.125883

Grassle, J. F., and Maciolek, N. J. (1992). Deep-Sea Species Richness: Regional and Local Diversity Estimates From Quantitative Bottom Samples. *Am. Nat.* 139 (2), 313–341. doi: 10.1086/285329

Guidi-Guilvard, L. D., Thistle, D., and Khripounoff, A. (2007). Two-Year Temporal Variability of Small Hyperbenthos Collected 4 M Above the Bottom in the Deep, (2347 M) NW Mediterranean. *ICES* 05, 5.

Gutiérrez, Y., Ott, D., Töpperwien, M., Salditt, T., and Scherber, C. (2018). X-Ray Computed Tomography and its Potential in Ecological Research: A Review of Studies and Optimization of Specimen Preparation. *Ecol. Evol.* 8, 7717–7732. doi: 10.1002/ece3.4149

Hadjab, R., Ayati, K., and Piscart, C. (2020). A New Species of Freshwater Amphipods *Echinogammarus* (Amphipoda, Gammaridae) From Algeria. *Taxonomy* 1, 36–47. doi: 10.3390/taxonomy1010005

Hanafi-Portier, M., Corbari, L., Chan, T.-Y., Chen, W.-J., Chen, J.-N., Lee, M.-Y., et al. (2021). When Imagery and Physical Sampling Work Together: Towards an Integrative Methodology of Image-Based Megafauna Identification. *Front. Mar. Sci.* 8, 749078. doi: 10.3389/fmars.2021.749078

Haug, J. T., Haug, C., Kutschera, V., Mayer, G., Maas, A., Liebau, S., et al. (2011b). Autofluorescence Imaging, an Excellent Tool for Comparative Morphology: Autofluorescence Imaging. *J. Microsc. Oxford* 244, 259–272. doi: 10.1111/j.1365-2818.2011.03534.x

Haug, C., Mayer, G., Kutschera, V., Waloszek, D., Maas, A., and Haug, J. T. (2011a). Imaging and Documenting Gammarideans. *Int. J. Zool.* doi: 10.1155/2011/380829

Havermans, C., Sonet, G., d'Udekem d'Acoz, C., Nagy, Z. T., Martin, P., Brix, S., et al. (2013). Genetic and Morphological Divergences in the Cosmopolitan Deep-Sea Amphipod *Eurythenes Gryllus* Reveal a Diverse Abyss and a Bipolar Species. *PloS One* 8, e74218. doi: 10.1371/journal.pone.0074218

Hegna, T. A. (2010). Photography of Soft-Bodied Crustaceans *via* Drying, Whitening, and Splicing. *J. Crust. Biol.* 30, 351–356. doi: 10.1651/09-3253.1

Herring, P. J. (1981). Studies on Bioluminescent Marine Amphipods. *J. Mar. Biol. Assoc. U.K.* 61 (1), 161–176. doi: 10.1017/S0025315400045999

Hessler, R. R., and Jumars, P. A. (1974). Abyssal Community Analysis From Replicate Cores in the Central North Pacific. *Deep Sea Res. Oceanogr. Abstr.* 21 (3), 185–209. doi: 10.1016/0011-7471(74)90058-8

Hessler, R. R. , and Sanders, H. L. (1967). Faunal Diversity in the Deep-Sea. *Deep Sea Res. Part I Oceanogr. Res.* 14(1), 65–78. doi: 10.1016/0011-7471(67)90029

Hessler, R. R., and Wilson, G. D. F. (1983). "The Origin and Biogeography of Malacostracan Crustaceans in the Deep Sea," in *Evolution, Time, and Space: The Emergence of the Biosphere*, vol. 23 . Eds. R. W. Sims, J. H. Price and P. E. S. Whalley (Whalley: Systematics Association, Special Publication), 227–254.

Higgs, N. D., and Attrill, M. (2015). Biases in Biodiversity: Wide-Ranging Species Are Discovered First in the Deep Sea. *Front. Mar. Sci.* 2. doi: 10.3389/fmars.2015.00061

Holthuis, L. B. (1964). The Earliest Published Record of a Cumacean. *Crustaceana* 7, 317–318. doi: 10.1163/156854064X00533

Horton, T., Marsh, L., Bett, B. J., Gates, A. R., Jones, D. O. B., Benoist, N. M. A., et al. (2021). Recommendations for the Standardisation of Open Taxonomic Nomenclature for Image-Based Identifications. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.620702

Horton, T., Thurston, M. H., Vlierboom, R., Gutteridge, Z., Pebody, C. A., Gates, A. R., et al. (2020). Are Abyssal Scavenging Amphipod Assemblages Linked to Climate Cycles? *Prog. Oceanogr.* 184, 102318. doi: 10.1016/j.pocean.2020.102318

Hughes, L. E., and Ahyong, S. T. (2016). Collecting and Processing Amphipods. *J. Crust. Biol.* 36, 584–588. doi: 10.1163/1937240X-00002450

Hughes, L. E., and Kaji, T. (2016). Description of a New Species of Quadrivisio Stebbing 1907, From Songkhla Lake, Thailand (Crustacea: Peracarida: Amphipoda: Maeridae). *Raffles B. Zool.* 9 (64), 351–359.

ISA (2020). Workshop on Deep-Sea Taxonomic Standardization: Strategic Approaches for Collaboration. *ISA Workshop*, 15–16.

Jażdżewska, A. M., Brandt, A., Arbizu, P. M., and Vink, A. (2021). Exploring the Diversity of the Deep Sea — Four New Species of the Amphipod Genus *Oedicerina* Described Using Morphological and Molecular Methods. *Zool. J. Linn. Soc* 194(1), 181–225. doi: 10.1093/zoolinnean/zlab032 .

Jakiel, A., Palero, F., and Błażewicz, M. (2019). Deep Ocean Seascape and Pseudotanaidae (Crustacea: Tanaidacea) Diversity at the Clarion-Clipperton Fracture Zone. *Sci. Rep.* 9, 17305. doi: 10.1038/s41598-019-51434-z

Jakiel, A., Palero, F., and Błażewicz, M. (2020). Secrets From the Deep: Pseudotanaidae (Crustacea: Tanaidacea) Diversity From the Kuril-

Kamchatka Trench. *Prog. Oceanogr.* 183, 102288. doi: 10.1016/j.pocean.2020.102288

Jakiel, A., Stępień, A., and Błażewicz, M. (2018). A Tip of the Iceberg – Pseudotanaidae (Tanaidacea) Diversity in the North Atlantic. *Mar. Biodiv.* 48, 859–895. doi: 10.1007/s12526-018-0881-x

Jamieson, A. J. (2016). "Landers: Baited Cameras and Traps," in *Biological Sampling in the Deep Sea*. Eds. M. R. Clark, M. Consalvey and A. A. Rowden (John Wiley and Sons Ltd), 228–259 pp.

Jamieson, A. J., Singleman, G., Linley, T. D., and Casey, S. (2021). Fear and Loathing of the Deep Ocean: Why Don't People Care About the Deep Sea? *ICES J. Mar. Sci.* 78 (3), 797–809. doi: 10.1093/icesjms/fsaa234

Jaume, D., and Queinneck, E. (2007). A New Species of Freshwater Isopod (Sphaeromatidea: Sphaeromatidae) From an Inland Karstic Stream on Espíritu Santo Island, Vanuatu, Southwestern Pacific. *Zootaxa* 1653, 41–55. doi: 10.11646/zootaxa.1653.1.3

Jauvion, C. (2020). *De La Vie a La Pierre: Préservation Exceptionnelle D'arthropodes Marins Fossils* (MNHN Paris: Museum national d'histoire naturelle), 429 pp.

Jauvion, C., Audo, D., Charbonnier, S., and Vannier, J. (2016). Virtual Dissection and Lifestyle of a 165 - Million-Year-Old Female Polychelidan Lobster. *Arthropod. Struct. Dev.* 45, 122–132. doi: 10.1016/j.asd.2015.10.004

Jennings, R. M., Brix, S., Bober, S., Svavarsson, J., and Driskell, A. (2018). More Diverse Than Expected: Distributional Patterns of *Oecidiobranchus* Hessler 1970 (Isopoda, Asellota) on the Greenland-Iceland-Faeroe Ridge Based on Molecular Markers. *Mar. Biodiv.* 48 (2), 845–857. doi: 10.1007/s12526-018-0857-x

Jersabek, C. D. (2005). The 'Frank J. Myers Rotifera Collection' at the Academy of Natural Sciences of Philadelphia. *Hydrobiol* 546, 137–140. doi: 10.1007/s10750-005-4110-9

Jirikowski, G., Richter, S., and Wolff, C. (2013). Myogenesis of Malacostraca — the "Egg-Nauplius" Conceptrevisited. *Front. Zool.* 10, 76. doi: 10.1186/1742-9994-10-76

Jirikowski, G., Wolff, C., and Richter, S. (2015). Evolution of Eumalacostracan Development — New Insights Into Loss and Reacquisition of Larval Stages Revealed by Heterochrony Analysis. *Evo. Devo* 6, 4. doi: 10.1186/2041-9139-6-4

Jörger, K. M., and Schrödl, M. (2013). How to Describe a Cryptic Species? Practical Challenges of Molecular Taxonomy. *Front. Zool.* 10 (1), 1–27. doi: 10.1186/1742-9994-10-59

Jóźwiak, P., Pabis, K., Brandt, A., and Błażewicz, M. (2020). Epibenthic Sled Versus Giant Box Corer – Comparison of Sampling Gears for Tanaidacean Species Richness Assessment in the Abyssal Benthic Ecosystem. *Prog. Oceanogr.* 181. doi: 10.1016/j.pocean.2019.102255

Just, J. (2001). Bathyal Joeropsididae (Isopoda: Asellota) From South-Eastern Australia, With Description of Two New Genera. *Mem. Mus. Vic.* 58 (2), 297–333. doi: 10.24199/j.mmv.2001.58.16

Kaiser, S., and Brandt, A. (2007). Two New Species of the Genus *Austroniscus* Vanhoeffen 1914 (Isopoda: Asellota: Nannoniscidae) From the Antarctic Shelf. *Zootaxa* 1394, 47–68. doi: 10.11646/zootaxa.1394.1.3

Kaiser, S., and Brenke, N. (2016). "Epibenthic Sledges," in *Biological Sampling in the Deep Sea*. Eds. M. R. Clark, M. Consalvey and A. A. Rowden (John Wiley and Sons Ltd), 184–206 pp.

Kaiser, S., Brix, S., Kihara, T. C., Janssen, A., and Jennings, R. M. (2018). Integrative Species Delimitation in the Deep-Sea Genus *Thaumastosoma* Hessler 1970 (Isopoda, Asellota, Nannoniscidae) Reveals a New Genus and Species From the Atlantic and Central Pacific Abyss. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 148, 151–179. doi: 10.1016/j.dsr2.2017.05.006

Kaiser, S., Kihara, T. C., Brix, S., Mohrbeck, I., Janssen, A., and Jennings, R. M. (2021). Species Boundaries and Phylogeographic Patterns in New Species of *Nannoniscus* (Janiroidea: Nannoniscidae) From the Equatorial Pacific Nodule Province Inferred From mtDNA and Morphology. *Zool. J. Linn. Soc,* 193(3), 1020–1071. doi: 10.1093/zoolinnean/zlaa174

Kaiser, S., and Marner, M. (2012). A New Species of Pentaceration Just 2009 (Isopoda, Asellota, Paramunnidae) From the Challenger Plateau, New Zealand (Tasman Sea). *Zoosystem Evol.* 88, 171–184. doi: 10.1002/zoos.201200015

Kaji, T., Fritsch, M., Schwentner, M., Olesen, J., and Richter, S. (2014). Male Claspers in Clam Shrimps (Crustacea, Branchiopoda) in the Light of Evolution: A Case Study on Homology Versus Analogy: Male Claspers in Clam Shrimps. *J. Exp. Zool. (Mol. Dev. Evol.)* 322, 269–280. doi: 10.1002/jez.b.22574

Kaji, T., Kakui, K., Miyazaki, N., Murata, K., and Palmer, A. R. (2016). Mesoscale Morphology at Nanoscale Resolution: Serial Block-Face Scanning Electron Microscopy Reveals Fine 3D Detail of a Novel Silk Spinneret System in a Tube-Building Tanaid Crustacean. *Front. Zool.* 13, 14. doi: 10.1186/s12983-016-0146-0

Kakui, K. (2014). A Novel Transmission Pathway: First Report of a Larval Trematode in a Tanaidacean Crustacean. *Fauna Ryukyuana* 17, 13–22.

Kakui, K., and Fujiwara, Y. (2020). First *In Situ* Observations of Behavior in Deep-Sea Tanaidacean Crustaceans. *Zool. Sci.* 37, 1–4. doi: 10.2108/zs200028

Kakui, K., and Hiruta, C. (2017). Tube Construction by a Tanaidacean Crustacean Using a Novel Mucus Secretion System Involving the Anal Opening. *Zool. Lett.* 3, 20. doi: 10.1186/s40851-017-0082-7

Kakui, K., and Kano, Y. (2021). First Complete Mitochondrial Genome of a Tanaidacean Crustacean (*Arctotanais Alascensis*). *Zool. Sci.* 38 (3), 267–272. doi: 10.2108/zs200167

Kamanli, S. A., Kihara, T. C., Ball, A. D., Morritt, D., and Clark, P. F. (2017). A 3D Imaging and Visualization Workflow, Using Confocal Microscopy and Advanced Image Processing for Brachyuran Crab Larvae: 3D Imaging of Crab Larvae Using CLSM. *J. Microsc. Oxford* 266, 307–323. doi: 10.1111/jmi.12540

Kelley, C., Kerby, T., Sarradin, P.-M., Sarrazin, J., and Lindsay, D. J. (2016). "Submersibles and Remotely Operated Vehicles," in *Biological Sampling in the Deep Sea*. Eds. M. R. Clark, M. Consalvey and A. A. Rowden (John Wiley and Sons Ltd), 285–305 pp.

Kenning, M., and Harzsch, S. (2013). Brain Anatomy of the Marine Isopod *Saduria Entomon* Linnaeus 1758 (Valvifera, Isopoda) With Special Emphasis on the Olfactory Pathway. *Front. Neuroanat.* 7. doi: 10.3389/fnana.2013.00032

Kensley, B. (1989). Marine Isopod Crustaceans From the St. Paul and Amsterdam Islands, Southern Indian Océan. *Bull. Mus. Natn. Hist. Nat. 4° Sér.* 11 (1), 147–164.

Khodami, S., Mercado-Salas, N. F., and Martinez Arbizu, P. (2020). Genus Level Molecular Phylogeny of Aegisthidae Gisbrecht 1893 (Copepoda: Harpacticoida) Reveals Morphological Adaptations to Deep-Sea and Plagic Habitats. *BMC Evol. Biol.* 20, 36. doi: 10.1186/s12862-020-1594-x

Kihara, T. C., and Martinez Arbizu, P. (2012). Three New Species of Cerviniella Smirnov 1946 (Copepoda: Harpacticoida) From the Arctic. *Zootaxa* 3345, 1–33. doi: 10.11646/zootaxa.3345.1.1

King, J. R., and Porter, S. D. (2004). Recommendations on the Use of Alcohols for Preservation of Ant Specimens (Hymenoptera, Formicidae). *Insect. Soc.* 51, 197–202. doi: 10.1007/s00040-003-0709-x

Kodama, M., and Kawamura, T. (2019). A New Species of Bemlos Shoemaker 1925 (Amphipoda: Aoridae) From Deep Water Off Tanabe Bay, Japan, With a Review of the Deep-Sea Aorids and Their Adaptations to the Deep Sea. *J. Crust. Biol.* 39, 54–61. doi: 10.1093/jcbiol/ruy098

Koomen, P., and von Vaupel Klein, J. C. (1995). The Suitability of Various Mounting Media for Permanent Mounts of Small Chitinous Crustaceans, With Special Reference to the Observation of Integumental Organs. *Crustaceana* 68, 428–437. doi: 10.1163/156854095X01583

Kottmann, J., Kihara, T. C., Glatzel, T., and Veit-Köhler, G. (2013). A New Species of *Wellsopsyllus* (Copepoda, Harpacticoida, Paramesochridae) From the Deep Southern Ocean and Remarks on its Biogeography. *Helgol. Mar. Res.* 67, 33–48. doi: 10.1007/s10152-012-0302-7

Kraft, A., Bauerfeind, E., Nöthig, E.-M., Klages, M., Beszczynska-Möller, A., and Bathmann, U. V. (2013). Amphipods in Sediment Traps of the Eastern Fram Strait With Focus on the Life-History of the Lysianassoid Cyclocaris Guilelmi. *Deep Sea Res. Part I. Oceanogr. Res.* 73, 62–72. doi: 10.1016/j.dsr.2012.11.012

Kreissl, S., Uber, A., and Harzsch, S. (2008). Muscle Precursor Cells in the Developing Limbs of Two Isopods (Crustacea, Peracarida): An Immunohistochemical Study Using a Novel Monoclonal Antibody Against Myosin Heavy Chain. *Dev. Genes. Evol.* 218, 253–265. doi: 10.1007/s00427-008-0216-1

Kürten, B., Frutos, I., Struck, U., Painting, S. J., Polunin, N. V. C., and Middelburg, J. J. (2013). Trophodynamics and Functional Feeding Groups of North Sea Fauna: A Combined Stable Isotope and Fatty Acid Approach. *Biogeochemistry* 113, 189–212. doi: 10.1007/s10533-012-9701-8

Kürzel, K., Kaiser, S., Lörz, A. N., Rossel, S., Paulus, E., Peters, J., et al. (2022). Correct Species Identification and Its Implications for Conservation Using

Haploniscidae (Crustacea, Isopoda) in Icelandic Waters as a Surrogate. *Front. Mar. Sci.* 8, 795196. doi: 10.3389/fmars.2021.795196

Lagardère, J. P. (1977). Recherches Sur La Distribution Vertical Et Sur L'alimentation Des Crustacés Décapodes Benthiques De La Pente Continentale Du Golfe De Gascogne. *Bull. Cent. Etud. Rech. Sci. Biarritz* 11 (4), 367–440.

Lambshead, P. J. D., and Boucher, G. (2003). Marine Nematode Deep-Sea Biodiversity–Hyperdiverse or Hype? *J. Biogeogr.* 30 (4), 475–485. doi: 10.1046/j.1365-2699.2003.00843.x

Landschoff, J., Komai, T., du Plessis, A., Gouws, G., and Griffiths, C. L. (2018). MicroCT Imaging Applied to Description of a New Species of Pagurus Fabricius 1775 (Crustacea: Decapoda: Anomura: Paguridae), With Selection of Three-Dimensional Type Data. *PloS One* 13, e0203107. doi: 10.1371/journal.pone.0203107

Langenkämper, D., Zurowietz, M., Schoening, T., and Nattkemper, T. W. (2017). Biigle 2.0-Browsing and Annotating Large Marine Image Collections. *Front. Mar. Sci.* 4, 83. doi: 10.3389/fmars.2017.00083

Larsen, K. (2001). Morphological and Molecular Investigation of Polymorphism and Cryptic Species in Tanaid Crustaceans: Implications for Tanaid Systematics and Biodiversity Estimates. *Zool. J. Linn. Soc* 131, 353–379. doi: 10.1111/j.1096-3642.2001.tb02241.x

Lee, S., Brown, R. L., and Monroe, W. (2009). Use of Confocal Laser Scanning Microscopy in Systematics of Insects With a Comparison of Fluorescence From Different Stains. *Syst. Entomol.* 34, 10–14. doi: 10.1111/j.1365-3113.2008.00451.x

Le, J. T., Levin, L. A., Lejzerowicz, F., Cordier, T., Gooday, A. J., and Pawlowski, J. (2021). Scientific and Budgetary Tradeoffs Between Morphological and Molecular Methods for Deep-Sea Biodiversity Assessment. *Integr. Environ. Assess. Manage* 18(3), 665–663. doi: 10.1002/ieam.4466

Lepechin, I. (1780). Tres *Oniscorum* Species Descriptae. *Acta Acad. Sci. Imperialis Petropolitanae* 1778, 248–249.

Lester, P. J., Brown, S. D. J., Edwards, E. D., Holwell, G. I., Pawson, S. M., Ward, D. F., et al. (2014). Critical Issues Facing New Zealand Entomology. *N. Z. Entomol.* 37 (1), 1–13. doi: 10.1080/00779962.2014.861789

Lim, G. S., Balke, M., and Meier, R. (2012). Determining Species Boundaries in a World Full of Rarity: Singletons, Species Delimitation Methods. *Syst. Biol.* 61 (1), 165–169. doi: 10.1093/sysbio/syr030

Linnaeus, C. (1735). Systema Naturae; Sive, Regna Tria Naturae: Systematice Proposita Per Classes, Ordines, Genera and Species. *Haak*.

Lins, L., and Brandt, A. (2020). Comparability Between Box-Corer and Epibenthic-Sledge Data on Higher Taxon Level: A Case Study Based on Deep-Sea Samples From the NW Pacific. *Prog. Oceanogr.* 182, 102273. doi: 10.1016/j.pocean.2020.102273

Lins, L., Zeppilli, D., Menot, L., Michel, L. N., Bonifácio, P., Brandt, M., et al. (2021). Toward a Reliable Assessment of Potential Ecological Impacts of Deep-Sea Polymetallic Nodule Mining on Abyssal Infauna. *Limnol. Oceanogr. Meth.* 19, 626–650. doi: 10.1002/lom3.10448

Li, J. Y., Song, Z. L., Yan, G. Y., and He, L. S. (2019). The Complete Mitochondrial Genome of the Largest Amphipod, *Alicella Gigantea*: Insight Into Its Phylogenetic Relationships and Deep Sea Adaptive Characters. *Int. J. Biol. Macromol.* 141, 570–577. doi: 10.1016/j.ijbiomac.2019.09.050

Lörz, A.-N., and Horton, T. (2021). Investigation of the Amathillopsidae (Amphipoda, Crustacea), Including the Description of a New Species, Reveals a Clinging Lifestyle in the Deep Sea Worldwide. *ZooKeys* 1031, 19–39. doi: 10.3897/zookeys.1031.62391

Lowry, J. K., and Myers, A. A. (2012). Podosiridae, a New Family of North Atlantic Deep Sea Amphipod (Crustacea, Amphipoda). *Zootaxa* 3546 (1), 81–84. doi: 10.11646/zootaxa.3546.1.6

Luque, J., and Gerken, S. (2019). Exceptional Preservation of Comma Shrimp From a Mid-Cretaceous Lagerstätte of Colombia, and the Origins of Crown Cumacea. *Proc. R. Soc. B.* 286, 20191863. doi: 10.1098/rspb.2019.1863

Luque, J., Xing, L., Briggs, D. E. G., Clark, E. G., Duque, A., Hui, J., et al. (2021). Crab in Amber Reveals an Early Colonization of Nonmarine Environments During the Cretaceous. *Sci. Adv.* 7 (43), eabj5689. doi: 10.1126/sciadv.abj5689

Macel, M.-L., Ristoratore, F., Locascio, A., Spagnuolo, A., Sordino, P., and D'Aniello, S. (2020). Sea as a Color Palette: The Ecology and Evolution of Fluorescence. *Zool. Lett.* 6, 9. doi: 10.1186/s40851-020-00161-9

MacIsaac, K. G., Kenchington, T. J., Kenchington, E. L. R., and Best, M. (2014). The Summer Assemblage of Large Pelagic Crustacea in the Gully Submarine Canyon: Major Patterns. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 104, 51–66. doi: 10.1016/j.dsr2.2013.08.017

Maeno, A., Kohtsuka, H., Takatani, K., and Nakano, H. (2019). Microfocus X-Ray CT (microCT) Imaging of *Actinia Equina* (Cnidaria), *Harmothoe* Sp. (Annelida), and *Xenoturbella Japonica* (Xenacoelomorpha). *J. Vis. Exp.*, 150 e59161. doi: 10.3791/59161

Malyutina, M., Frutos, I., and Brandt, A. (2018). Diversity and Distribution of the Deep-Sea Atlantic *Acanthocope* (Crustacea, Isopoda, Munnopsidae), With Description of Two New Species. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 148, 130–150. doi: 10.1016/j.dsr2.2017.11.003

Malyutina, M. V., Kihara, T. C., and Brix, S. (2020). A New Genus of Munnopsidae Lilljeborg 1864 (Crustacea, Isopoda), With Descriptions of Two Abyssal New Species From the Clarion Clipperton Fracture Zone, North-Eastern Tropical Pacific. *Mar. Biodiv.* 50, 42. doi: 10.1007/s12526-020-01061-z

Manktelow, M. (2010). "History of Taxonomy," in *Lecture From Dept. Of Systematic Biology* (Uppsala University).

Marek, P. (2017). Ultraviolet-Induced Fluorescent Imaging for Millipede Taxonomy. *Res. Ideas Outcomes* 3, e14850. doi: 10.3897/rio.3.e14850

Marquina, D., Buczek, M., Ronquist, F., and Łukasik, P. (2021). The Effect of Ethanol Concentration on the Morphological and Molecular Preservation of Insects for Biodiversity Studies. *Peer J* 9, e10799 2020. doi: 10.7717/peerj.10799

Martin, J. W. (2016). Collecting and Processing Crustaceans: An Introduction. *J. Crust. Biol.* 36, 393–395. doi: 10.1163/1937240X-00002436

Martínez Arbizu, P., and Petrunina, (2018). Two New Species of Tantulocarida From the Atlantic Deep Sea With First CLSM Pictures of Tantulus Larva. *Mar. Biodiv.* 48 (1), 231–237. doi: 10.1007/s12526-016-0627-6

Martin, J. W., France, S. C., and Van Dover, C. L. (1993). *Halice Hesmonectes*, a New Species of Pardaliscid Amphipod (Crustacea, Peracarida) From Hydrothermal Vents in the Eastern Pacific. *Can. J. Zool.* 71, 1724–1732. doi: 10.1139/z93-244

Maruzzo, D., Minelli, A., Ronco, M., and Fusco, G. (2007). Growth and Regeneration of the Second Antennae of Asellus Aquaticus (Isopoda) in the Context of Arthropod Antennal Segmentation. *J. Crust. Biol.* 27, 184–196. doi: 10.1651/S-2756.1

May, R. (1992). Bottoms Up for the Oceans. *Nature* 357, 278–279. doi: 10.1038/357278a0

Maybury, C., Morrison, L., and Stewart, V. (1991). The Search for a Reliable Mounting Medium for Recent 'Live' Foraminifera. *J. Micropalaeontol.* 9, 172–172. doi: 10.1144/jm.9.2.172

Mayr, E. (1942). *Systematics and the Origin of Species* (New York: Columbia University Press), 315 pp.

McMenamin, M. A. S., Zapata, L. P., and Hussey, M. C. (2013). A Triassic Giant Amphipod From Nevada, USA. *J. Crust. Biol.* 33 (6), 751–759. doi: 10.1163/1937240X-00002192

Meißner, K., Bick, A., and Götting, M. (2017). Arctic *Pholoe* (Polychaeta: Pholoidae): When Integrative Taxonomy Helps to Sort Out Barcodes. *Zool. J. Linn. Soc* 179 (2), 237–262. doi: 10.1111/zoj.12468

Meier, R., Blaimerb, B. B., Buenaventura, E., Hartopb, E., vonRintelen, T., Srivathsana, A., et al. (2022). A Re-Analysis of the Data in Sharkey Et Al.'s, (2021) Minimalist Revision Reveals That BINs do Not Deserve Names, But BOLD Systems Needs a Stronger Commitment to Open Science. *Cladistics*, 38, 264–275. doi: 10.1111/cla.12489

Melzer, R. R., Spitzner, F., Šargač, Z., Hörnig, M. K., Krieger, J., Haug, C., et al. (2021). Methods to Study Organogenesis in Decapod Crustacean Larvae II: Analysing Cells and Tissues. *Helgol. Mar. Res.* 75, 2. doi: 10.1186/s10152-021-00547-y

Menzel, L. (2011). First Descriptions of Copepodid Stages, Sexual Dimorphism and Intraspecific Variability of Mesocletodes Sars 1909 (Copepoda, Harpacticoida, Argestidae), Including the Description of a New Species With Broad Abyssal Distribution. *ZooKeys* 96, 39–80. doi: 10.3897/zookeys.96.1496

Michels, J. (2007). Confocal Laser Scanning Microscopy: Using Cuticular Autofluorescence for High Resolution Morphological Imaging in Small Crustaceans. *J. Microsc. Oxford* 227, 1–7. doi: 10.1111/j.1365-2818.2007.01787.x

Michels, J., and Büntzow, M. (2010). Assessment of Congo Red as a Fluorescence Marker for the Exoskeleton of Small Crustaceans and the Cuticle of Polychaetes. *J. Microsc.* 238 (2), 95–101. doi: 10.1111/j.1365-2818.2009.03360.x

Mohrbeck, I., Horton, T., Jażdżewska, A. M., and Arbizu, P. M. (2021). DNA Barcoding and Cryptic Diversity of Deep-Sea Scavenging Amphipods in the Clarion-Clipperton Zone (Eastern Equatorial Pacific). *Mar. Biodiv.* 51 (2), 1–15. doi: 10.1007/s12526-021-01170-3

Mohrbeck, I., Raupach, M. J., Martínez Arbizu, P., Knebelsberger, T., and Laakmann, S. (2015). High-Throughput Sequencing—The Key to Rapid Biodiversity Assessment of Marine Metazoa? *PloS One* 10 (10), e0140342. doi: 10.1371/journal.pone.0140342

Montesanto, G. (2015). A Fast GNU Method to Draw Accurate Scientific Illustrations for Taxonomy. *ZooKeys* 515, 191–206. doi: 10.3897/zookeys.515.9459

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G., and Worm, B. (2011). How Many Species Are There on Earth and in the Ocean? *PloS Biol.* 9 (8), e1001127.

Moreira, P. S. (1973). Especies De Isopoda (Crustacea, Peracarida). *Programa Rio Grande Do Sul II*. Parte I, Publicaçao Especial do Instituto Oceanografico. 3(2), 213–229.

Mrak, P., Žnidaršič, N., and Štrus, J. (2013). Alizarin Red S Staining of the Crustacean Cuticle: Implementation in the Study of Porcellio Scaber Larvae. *ABS* 56, 51–62.

Mrak, P., Žnidaršič, N., Tušek-Žnidarič, M., Klepal, W., Gruber, D., and Strus, J. (2012). Egg Envelopes and Cuticle Renewal in *Porcellio* Embryos and Marsupial Mancas. *ZooKeys* 176, 55–72. doi: 10.3897/zookeys.176.2418

Murray, J., and Renard, A. F. (1891). *Report on Deep-Sea Deposits Based on the Specimens Collected During the Voyage of HMS Challenger in the Years 1872 to 1876* (HM Stationery Office).

Nagler, C., and Haug, J. T. (2016). Functional Morphology of Parasitic Isopods: Understanding Morphological Adaptations of Attachment and Feeding Structures in *Nerocila* as a Pre-Requisite for Reconstructing the Evolution of Cymothoidae. *PeerJ* 4, e2188. doi: 10.7717/peerj.2188

Nagler, C., Hyžný, M., and Haug, J. T. (2017). 168 Million Years Old "Marine Lice" and the Evolution of Parasitism Within Isopods. *BMC Evol. Biol.* 17, 76. doi: 10.1186/s12862-017-0915-1

Narayanaswamy, B. E., Bett, B. J., Lamont, P. A., Rowden, A. A., Bell, E. M., and Menot, L. (2016). "Corers and Grabs," in *Biological Sampling in the Deep Sea*. Eds. M. R. Clark, M. Consalvey and A. A. Rowden (John Wiley and Sons Ltd), 207–227 pp.

Naskrecki, P. (2008) *Mantis V. 2.0 - A Manager of Taxonomic Information and Specimens*. Available at: http://insects.oeb.harvard.edu/mantis (Accessed 30 July 2012).

Neuhaus, B., Schmid, T., and Riedel, J. (2017). Collection Management and Study of Microscope Slides: Storage, Profiling, Deterioration, Restoration Procedures, and General Recommendations. *Zootaxa* 4322, 1–173. doi: 10.11646/zootaxa.4322.1.1

Neusser, T. P., Jörger, K. M., Lodde-Bensch, E., Strong, E. E., and Schrödl, M. (2016). The Unique Deep Sea—Land Connection: Interactive 3D Visualization and Molecular Phylogeny of *Bathyhedyle Boucheti* N. Sp. (Bathyhedylidae N. Fam.)—The First Panpulmonate Slug From Bathyal Zones. *PeerJ* 4, e2738. doi: 10.7717/peerj.2738

Nouvel, H., and Lagardère, J. P. (1976). Les Mysidacés Du Talus Continental Du Golfe De Gascogne I. Tribu Des Erythropini (Genre *Erythrops* Excepté). *Bull. Mus. Natn. Hist. Nat.* 414 (291), 1243–1324.

Palero, F., Hall, S., Clark, P. F., Johnston, D., Mackenzie-Dodds, J., and Thatje, S. (2010). DNA Extraction From Formalin-Fixed Tissue: New Light From the Deep Sea. *Sci. Mar.* 74, 465–470. doi: 10.3989/scimar.2010.74n3465

Pante, E., Schoelinck, C., and Puillandre, N. (2015). From Integrative Taxonomy to Species Description: One Step Beyond. *Syst. Biol.* 64, 152–160. doi: 10.1093/sysbio/syu083

Papiol, V., Cartes, J. E., Vélez-Belchí, P., and Martín-Sosa, P. (2019). Near-Bottom Zooplankton Over Three Seamounts in the East Canary Islands: Influence of Environmental Variables on Distribution and Composition. *Deep Sea Res. Part I. Oceanogr. Res.* 149, 1030025. doi: 10.1016/j.dsr.2019.04.003

Paulus, E., Brix, S., Siebert, A., Arbizu, P. M., Rossel, S., Peters, J., et al. (2021). Recent Speciation and Hybridization In Icelandic Deep-Sea Isopods: An Integrative Approach Using Genomics and Proteomics. Authorea Preprints.

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A., et al. (2018). The Future of Biotic Indices in the Ecogenomic Era: Integrating (E) DNA Metabarcoding in Biological Assessment of Aquatic Ecosystems. *Sci. Tot. Environ.* 637, 1295–1310. doi: 10.1016/j.scitotenv.2018.05.002

Pereira, E., Roccatagliata, D., and Doti, B. L. (2019). *Xiphoarcturus* – a New Genus and Two New Species of the Family Antarcturidae (Isopoda: Valvifera) From the Mar Del Plata Submarine Canyon and its Phylogenetic Relationships. *Arthropod. Syst. Phyl.* 77, 303–323. doi: 10.26049/ASP77-2-2019-07

Pinu, F. R., Beale, D. J., Paten, A. M., Kouremenos, K., Swarup, S., Schirra, H. J., et al. (2019). Systems Biology and Multi-Omics Integration: Viewpoints From the Metabolomics Research Community. *Metabolites* 9 (4), 76. doi: 10.3390/metabo9040076

Pokluda, P., Čížek, L., Stříbrná, E., Drag, L., Lukeš, J., and Novotný, V. (2014). A Goodbye Letter to Alcohol: An Alternative Method for Field Preservation of Arthropod Specimens and DNA Suitable for Mass Collecting Methods. *Eur. J. Entomol.* 111, 175–179. doi: 10.14411/eje.2014.024

Poore, G. C. B., and Bruce, N. L. (2012). Global Diversity of Marine Isopods (Except Asellota and Crustacean Symbionts). *PloS One* 7, e43529. doi: 10.1371/journal.pone.0043529

Poore, G. C. B., and Wilson, G. D. (1993). Marine Species Richness. *Nature* 361 (6413), 597–598.

Porter, M. L. (2016). Collecting and Processing Mysids, Stygiomysids, and Lophogastrids. *J. Crust. Biol.* 36, 592–595. doi: 10.1163/1937240X-00002443

Preciado, I., Cartes, J. E., Punzón, A., Frutos, I., López-López, L., and Serrano, A. (2017). Food Web Functioning of the Benthopelagic Community in a Deep-Sea Seamount Based on Diet and Stable Isotope Analyses. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 137, 56–68. doi: 10.1016/j.dsr2.2016.07.013

Presnell, J. K., and Schreibman, M. P. (1997). *Humason's Animal Tissue Techniques. 5th ed* (Baltimore (Md: Johns Hopkins University press).

Ramirez-Llodra, E., Brandt, A., Danovaro, R., Mol, B. D., Escobar, E., German, C. R., et al. (2010). Deep, Diverse and Definitely Different: Unique Attributes of the World's Largest Ecosystem. *Biogeosciences* 7 (9), 2851–2899. doi: 10.5194/bg-7-2851-2010

Raupach, M. J., Amann, R., Wheeler, Q. R., and Roos, R. (2016). The Application of "–Omics" Technologies for the Classificationand Identification of Animals. *Org. Divers. Evol.* 16, 1–12. doi: 10.1007/s13127-015-0234-6

Raupach, M. J., and Wägele, J. W. (2006). Distinguishing Cryptic Species in Antarctic Asellota (Crustacea: Isopoda)-A Preliminary Study of Mitochondrial DNA in *Acanthaspidia Drygalskii*. *Antarc. Sci.* 18 (2), 191. doi: 10.1017/S0954102006000228

Renner, S. S. (2016). A Return to Linnaeus's Focus on Diagnosis, Not Description: The Use of DNA Characters in the Formal Naming of Species. *Syst. Biol.* 65 (6), 1085–1095. doi: 10.1093/sysbio/syw032

Riedel, A., Sagata, K., Suhardjono, Y. R., Tänzler, R., and Balke, M. (2013). Integrative Taxonomy on the Fast Track-Towards More Sustainability in Biodiversity Research. *Front. Zool.* 10 (1), 1–9. doi: 10.1186/1742-9994-10-15

Riehl, T., Brenke, N., Brix, S., Driskell, A., Kaiser, S., and Brandt, A. (2014). Field and Laboratory Methods for DNA Studies on Deep-Sea Isopod Crustaceans. *Pol. Polar. Res.*, 35(2) 203–224. doi: 10.2478/popore-2014-0018

Riehl, T., and De Smet, B. (2020). *Macrostylis Metallicola* Spec. Nov. — An Isopod With Geographically Clustered Genetic Variability From a Polymetallic-Nodule Area in the Clarion-Clipperton Fracture Zone. *PeerJ* 8, e8621. doi: 10.7717/peerj.8621

Riehl, T., and Kühn, M. A. L. (2020). Uniting What Belongs Together—Reevaluation of the Isopod Species *Macrostylis Grandis* and *M. Ovata* Using Ontogenetic, Morphological and Genetic Evidence. *Prog. Oceanogr.* 181, 102238. doi: 10.1016/j.pocean.2019.102238

Riehl, T., Wilson, G. D., and Hessler, R. R. (2012). New Macrostylidae Hansen 1916 (Crustacea: Isopoda) From the Gay Head-Bermuda Transect With Special Consideration of Sexual Dimorphism. *Zootaxa* 3277 (1), 1–26. doi: 10.11646/zootaxa.3277.1.1

Ríos, P., Cristobo, J., Altuna, A., Frutos, I., Manjón-Cabeza, E., García, Guillén, et al. (2022). Avilés Canyon System: Increasing the Benthic Biodiversity Knowledge. *Estuar. Coast. Shelf Sci.* 274, 107924. doi: 10.1016/j.ecss.2022.107924

Ritchie, H., Jamieson, A. J., and Piertney, S. B. (2017). Genome Size Variation in Deep-Sea Amphipods. *R. Soc Open Sci.* 4 (9), 170862. doi: 10.1098/rsos.170862

Robin, N., Gueriau, P., Luque, J., Jarvis, D., Daley, A. C., and Vonk, R. (2021). The Oldest Peracarid Crustacean Reveals a Late Devonian Freshwater Colonization by Isopod Relatives. *Biol. Lett.* 17, 20210226. doi: 10.1098/rsbl.2021.0226

Robinson, C. V., Porter, T. M., Wright, M. T. G., and Hajibabaei, M. (2021). Propylene Glycol-Based Antifreeze Is an Effective Preservative for DNA Metabarcoding of Benthic Arthropods. *Freshw. Sci.* 40, 77–87. doi: 10.1086/712232

Rodríguez, J. G., Garmendia, J. M., Muxika, I., Gómez-Ballesteros, M., Quincoces, I., Díez, I., et al. (2021). Macrofaunal Variability in the Continental Shelf and Canyons in the Southeastern Bay of Biscay. *Reg. Stud. Mar. Sci.* 48, 102012. doi: 10.1016/j.rsma.2021.102012

Rosenthal, C. K. (2009). The Beginning. *Nat. Cell Biol.* 11 (1), S6–S6. doi: 10.1038/ncb1938

Rosli, N., Leduc, D., Rowden, A. A., Clark, M. R., Probert, P. K., Berkenbusch, K., et al. (2016). Differences in Meiofauna Communities With Sediment Depth Are Greater Than Habitat Effects on the New Zealand Continental Margin: Implications for Vulnerability to Anthropogenic Disturbance. *PeerJ* 4, e2154. doi: 10.7717/peerj.2154

Rossel, S., and Martínez Arbizu, P. (2018). Automatic Specimen Identification of Harpacticoids (Crustacea : Copepoda) Using Random Forest and MALDI-TOF Mass Spectra, Including a *Post Hoc* Test for False Positive Discovery. *Methods Ecol. Evol.* 9, 1421–1434. doi: 10.1111/2041-210X.13000

Rossel, S., and Martinez Arbizu, P. (2019). Revealing Higher Than Expected Diversity of Harpacticoida (Crustacea: Copepoda) in the North Sea Using MALDI-TOF MS and Molecular Barcoding. *Sci. Rep.* 9 (1), 1–14. doi: 10.1038/s41598-019-45718-7

Sánchez-García, A., Peálver, E., Bird, G. J., Perrichot, V., and Delclòs, X. (2016). Palaeobiology of Tanaidaceans (Crustacea: Peracarida) From Cretaceous Ambers: Extending the Scarce Fossil Record of a Diverse Peracarid Group. *Zool. J. Linn. Soc.* 178, 492–522. doi: 10.1111/zoj.12427

Sánchez, F., Serrano, A., Parra, S., and Cartes, J. E. (2008). Habitat Characteristics as Determinant of the Structure and Spatial Distribution of Epibenthic and Demersal Communities of Le Danois Bank (Cantabrian Sea, N Spain). *J. Mar. Syst.* 72, 64–86. doi: 10.1016/j.jmarsys.2007.04.008

Sanders, H. L., Hessler, R. R., and Garner, S. P. (1985). *Hirsutia Bathyalis*, a New Unusual Deep-Sea Benthic Peracaridan Crustacean From the Tropical Atlantic. *J. Crust. Biol.* 5 (1), 30–57. doi: 10.2307/1548219

San Vicente, C., and Cartanyà, J. (2017). A New Mysid (Crustacea, Mysida) From the Ladinian Stage (Middle Triassic) of Conca De Barberà (Catalonia, NE Iberian Peninsula). *J. Paleontol.* 91 (5), 968–980. doi: 10.1017/jpa.2017.24

San Vicente, C., Frutos, I., and Cartes, J. E. (2014b). *Petalophthalmus Papilloculatus* Sp. Nov. (Crustacea: Mysida: Petalophthalmidae), A New Bathyal Suprabenthic Mysid From the Galicia Bank (NE Atlantic Ocean). *Zootaxa* 3765 (1), 77–91. doi: 10.11646/zootaxa.3765.1.5

San Vicente, C., Guerao, G., and Olensen, J. (2014a). "Lophogastrida and Mysida," in *Atlas of Crustacean Larvae*. Eds. J. W. Martin, J. Olensen and J. T. Høeg (Baltimore: Johns Hopkins University Press), 199–205.

Sars, G. O. (1900). An account of the Crustacea of Norway. Vol III. Cumacea. Part V and VI Diastylidae. Bergen. pp. 41–68, plates XXXIII–XLVIII.

Saunders, T. E. (2020). Taxonomy at a Crossroads: Communicating Value, Building Capability, and Seizing Opportunities for the Future. *Megataxa* 1 (1), 63–66. doi: 10.11646/megataxa.1.1.13

Schiaparelli, S., Schnabel, K. E., Richer de Forges, B., and Chan, T.-Y. (2016). "Sorting, Recording, Preservation and Storage of Biological Samples," in *Biological Sampling in the Deep Sea*. Eds. M. R. Clark, M. Consalvey and A. A. Rowden (John Wiley and Sons).

Schmidt, C., Escobar Wolf, K., Lins, L., Martínez Arbizu, P., and Brandt, A. (2018). Meiofauna Abundance and Community Patterns Along a Transatlantic Transect in the Vema Fracture Zone and in the Hadal Zone of the Puerto Rico Trench. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 148, 223–235. doi: 10.1016/j.dsr2.2017.12.021

Schmidt, C., and Martínez Arbizu, P. (2015). Unexpectedly Higher Metazoan Meiofauna Abundances in the Kuril-Kamchatka Trench Compared to the Adjacent Abyssal Plains. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 111, 60–75. doi: 10.1016/j.dsr2.2014.08.019

Schnurr, S., Osborn, K. J., Malyutina, M., Jennings, R., Brix, S., Driskell, A., et al. (2018). Hidden Diversity in Two Species Complexes of Munnopsid Isopods (Crustacea) at the Transition Between the Northernmost North Atlantic and the Nordic Seas. *Mar. Biodiv.* 48, 813–843. doi: 10.1007/s12526-018-0877-6

Secrétan, S., and Riou, B. (1986). Les Mysidacés (Crustacea, Peracarida) Du Callovien De La Voulte-Sur-Rhône. *Ann. Paleontol.* 72 (4), 295–323.

Selden, P., Wilson, G. D. F., Simonetto, L., and Dalla Vecchia, F. (2016). First Fossil Asellote (Isopoda: Asellota), From the Upper Triassic (Norian) of the Carnic Prealps (Friuli, Northeastern Italy). *J. Crust. Biol.* 36 (1), 68–86. doi: 10.1163/1937240X-00002387

Serrano, A., Cartes, J. E., Papiol, V., Punzón, A., García-Alegre, A., Arronte, J. C., et al. (2017). Epibenthic Communities of Sedimentary Habitats in a NE Atlantic Deep Seamount (Galicia Bank). *J. Sea Res.* 130, 154–165. doi: 10.1016/j.seares.2017.03.004

Serrano, A., Sánchez, F., Punzón, A., Velasco, F., and Olaso, I. (2011). Deep Sea Megafaunal Assemblages Off the Northern Iberian Slope Related to Environmental Factors. *Sci. Mar.* 75 (3), 425–437. doi: 10.3989/scimar.2011.75n3425

Sharkey, M. J., Janzen, D. H., Hallwachs, W., Chapman, E. G., Smith, M. A., Dapkey, T., et al. (2021). Minimalist Revision and Description of 403 New Species in 11 Subfamilies of Costa Rican Braconid Parasitoid Wasps, Including Host Records for 219 Species. *ZooKeys* 1013, 1–665. doi: 10.3897/zookeys.1013.55600

Shaw, P. (1989). New Amphipods From Geothermal Vent Sites Off the West Coast of Vancouver Island, British Columbia, With a Reappraisal of the Amphipod Family Sebidae. *Can. J. Zool.* 67 (8), 1882–1890. doi: 10.1139/z89-269

Shimomura, M., and Mawatari, S. F. (1999). *Paramunna Rhipis*, a New Species of Asellote Isopod (Paramunnidae) From Japan. *Crust. Res.* 28, 153–159. doi: 10.18353/crustacea.28.0_153

Shimomura, M., and Mawatari, S. F. (2000). *Santia Katoi* Sp. Nov., a New Isopod Crustacean From Shirahama, Japan. *Publ. Seto Mar. Bioi. Lab.* 39 (1), 29–34. doi: 10.5134/176292

Shimomura, M., and Ohtsuka, S. (2005). "Deep-Sea Asellote Isopods (Crustacea Peracarida) of Nansei Islands, Southwestern Japan," in *Deep-Sea Fauna and Pollutans in Nansei Islands*, vol. 29 . Eds. K. Hasegawa, G. Shinohara and M. Takeda (National Science Museum Monographs), 249–259.

Singh, R., and Mumbarekar, V. (2021). *Neural Network Model Approach for Automated Benthic Animal Identification* (ICT Express).

Sittrop, D. J., Serejo, C. S., Souza-Filho, J. F., and Senna, A. R. (2015). New Genera and Species of Urothoidae (Amphipoda) From the Brazilian Deep Sea, With the Re-Assignment of *Pseudurothoe* and *Urothopsis* to Phoxocephalopsidae. *J. Nat. Hist.* 49 (9–10), 527–552. doi: 10.1080/00222933.2014.953227

Slabber, M. (1769–1778a). Natuurkundige Verlustigingen 15, Tweede Waarneeming van een Steur-garnael met trompetswyze oogen, pp. 136–139, pls. 15, figs 3–4. Haarlem.

Sorbe, J. C. (1981). Rôle Du Benthos Dans Le Régime Alimentaire Des Poissons Démersaux Du Secteur Sud Gascogne. *Kieler Meeresforsch. Sonderh.* 5, 479–489.

Sorbe, J. C. (1983). Description D'un Traîneau Destiné a L'échantillonnage Quantitatif Etagé De La Faune Suprabenthique Néritique. *Ann. Inst. Océanogr.* 59 (2), 117–126.

Starr, H. W., Hegna, T. A., and McMenamin, M. A. S. (2016).). Epilogue to the Tale of the Triassic Amphipod: *Rosagammarus* McMenamin, Zapata and Hussey 2013 is a Decapod Tail (Luning Formation, Nevada, USA). *J. Crust. Biol.* 36 (4), 525–529. doi: 10.1163/1937240X-00002444

Stebbing, T. R. R. (1888). Report on the Amphipoda Collected by H.M.S. Challenger During the Years 1873–76. Report on the Scientific Results of the Voyage of H.M.S. Challenger During the Years 1873–1876. *Zoology* 29, xxiv + 1737.

Steedman, H. F. (1958). Dimethyl Hydantoin Formaldehyde: A New Water-Soluble Resin for Use as a Mounting Medium. *J. Cell Sci.* s3–99, 451–452. doi: 10.1242/jcs.s3-99.48.451

Stegner, M. E., Stemme, T., Iliffe, T. M., Richter, S., and Wirkner, C. S. (2015). The Brain in Three Crustaceans From Cavernous Darkness. *BMC Neurosci.* 16, 19. doi: 10.1186/s12868-015-0138-6

Stemme, T., Eickhoff, R., and Bicker, G. (2014). Olfactory Projection Neuron Pathways in Two Species of Marine Isopoda (Peracarida, Malacostraca, Crustacea). *Tissue Cell* 46, 260–263. doi: 10.1016/j.tice.2014.05.010

Storey, M., and Poore, G. C. B. (2009). New Species of *Brucerolis* (Crustacea: Isopoda: Serolidae) From Seas Around New Zealand and Australia. *Mem. Mus. Vic.* 66, 147–173. doi: 10.24199/j.mmv.2009.66.15

Štrus, J., Tušek-Žnidarič, M., Repnik, U., Blejec, A., and Summers, A. (2019). Microscopy of Crustacean Cuticle: Formation of a Flexible Extracellular Matrix in Moulting Sea Slaters *Ligia Pallasii*. *J. Mar. Biol. Ass.* 99, 857–865. doi: 10.1017/S0025315418001017

Sweetman, A. K., Thurber, A. R., Smith, C. R., Levin, L. A., Mora, C., Wei, C. L., et al. (2017). Major Impacts of Climate Change on Deep-Sea Benthic Ecosystems. *Elem. Sci. Anth.* 5(4).

Tanaka, K. (2004). A New Species of *Gnathia* (Isopoda: Cymothoida: Gnathiidae) From Ishigaki Island, the Ryukyus, Southwestern Japan. *Crustacean Res.* 33, 51–60. doi: 10.18353/crustacea.33.0_51

Tandberg, A. H., Tore Rapp, H., Schander, C., Vader, W., Sweetman, A. K., and Berge, J. (2012). *Exitomelita Sigynae* Gen. Et Sp. Nov.: A New Amphipod From the Arctic Loki Castle Vent Field With Potential Gill Ectosymbionts. *Polar. Biol.* 35, 705–716. doi: 10.1007/s00300-011-1115-x

Taylor, J., Devey, C., Le Saout, M., Petersen, S., Frutos, I., Linse, K., et al. (2021). The Discovery and Preliminary Geological and Faunal Descriptions of Three New Steinahóll Vent Sites, Reykjanes Ridge, Iceland. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.520713

Tomikawa, K., and Mawatari, S. F. (2006). A New Species of the Genus *Amathillopsis* (Crustacea: Amphipoda: Amathillopsidae) From Japan. *Species Div.* 11 (3), 199–207. doi: 10.12782/specdiv.11.199

Turner, L. M., Ricevuto, E., Massa Gallucci, A., Lorenti, M., Gambi, M.-C., and Calosi, P. (2016). Metabolic Responses to High Pco2 Conditions at a CO2 Vent Site in Juveniles of a Marine Isopod Species Assemblage. *Mar. Biol.* 163, 211. doi: 10.1007/s00227-016-2984-x

Valdecasas, A. G., and Abad, A. (2011). Morphological Confocal Microscopy in Arthropods and the Enhancement of Autofluorescence After Proteinase K Extraction. *Microsc. Microanal.* 17, 109–113. doi: 10.1017/S1431927610094213

Vonk, R., and Schram, F. (2007). Three New Tanaid Species (Crustacea, Peracarida, Tanaidacea) From the Lower Cretaceous Álava Amber in Northern Spain. *J. Paleont.* 81 (6), 1502–1509. doi: 10.1666/05-020.1

Vrijenhoek, R. C. (2009). Cryptic Species, Phenotypic Plasticity, and Complex Life Histories: Assessing Deep-Sea Faunal Diversity With Molecular Markers. *Deep Sea Res. Pt. II Top. Stud. Oceanogr.* 56 (19–20), 1713–1723. doi: 10.1016/j.dsr2.2009.05.016

Wägele, H., Klussmann-Kolb, A., Kuhlmann, M., Haszprunar, G., Lindberg, D., Koch, A., et al. (2011). The Taxonomist-an Endangered Race. A Practical Proposal for its Survival. *Front. Zool.* 8 (1), 1–7. doi: 10.1186/1742-9994-8-25

Wall, A., Campo, D., and Wetzer, R. (2014). Genetic Utility of Natural History Museum Specimens: Endangered Fairy Shrimp (Branchiopoda, Anostraca). *ZooKeys* 457, 1–14. doi: 10.3897/zookeys.457.6822

Washburn, T. W., Menot, L., Bonifácio, P., Pape, E., Błażewicz, M., Bribiesca-Contreras, G., et al. (2021). Patterns of Macrofaunal Biodiversity Across the Clarion-Clipperton Zone: An Area Targeted for Seabed Mining. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.626571

Wells, A., Johanson, K. A., and Dostine, P. (2019). Why are So Many Species Based on a Single Specimen? *Zoosymposia* 14 (1), 32–38. doi: 10.11646/zoosymposia.14.1.5

Wetzer, R. (2015). Collecting and Preserving Marine and Freshwater Isopoda (Crustacea: Peracarida). *Biodivers. Data J.* 3, e4912. doi: 10.3897/BDJ.3.e4912

Wheeler, Q. (2018). Blank Canvas: The Case for Descriptive Taxonomy. *Integr. Comp. Biol.* 58, 1118–1121. doi: 10.1093/icb/icy067

Will, K. W., Mishler, B. D., and Wheeler, Q. D. (2005). The Perils of DNA Barcoding and the Need for Integrative Taxonomy. *Syst. Biol.* 54, 844–851. doi: 10.1080/10635150500354878

Wilson, G. D. F. (2003). A New Genus of Tainisopidae Fam. Nov. (Crustacea: Isopoda) From the Pilbara, Western Australia. *Zootaxa* 245, 1–20. doi: 10.11646/zootaxa.245.1.1

Wilson, G. D. F. (2017). Macrofauna Abundance, Species Diversity and Turnover at Three Sites in the Clipperton-Clarion Fracture Zone. *Mar. Biodivers.* 47, 323–347. doi: 10.1007/s12526-016-0609-8

Wilson, G. D. F., and Ahyong, S. T. (2015). Lifestyles of the Species-Rich and Fabulous: The Deep-Sea Crustaceans. in *The Natural History of the Crustacea: Lifestyles and feeding Biology*. Eds.M. F. Thiel and L. Watling (Oxforf University Press), 279–298.

Wilson, G. D. F., and Humphrey, C. L. (2020). The Eophreatoicus Nicholls 1926 Species Flock From Kakadu and Arnhem Land, With a Description of a New Genus of Amphisopidae (Crustacea: Isopoda: Phreatoicidea). *Zootaxa* 4854, 1–303. doi: 10.11646/zootaxa.4854

Wilson, K., Thorndyke, M., Nilsen, F., Rogers, A., and Martinez, P. (2005). Marine Systems: Moving Into the Genomics Era. *Mar. Ecol.* 26 (1), 3–16. doi: 10.1111/j.1439-0485.2005.00041.x

Wirkner, C. S., and 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. doi: 10.1002/jemt.20076

Wittmann, K. J., Ariani, A. P., and Lagardère, J. P. (2014). "Orders Lophogastrida Boas 1883, Stygiomysida Tchindonova 1981, and Mysida Boas 1883 (Also Known Collectively as Mysidacea)," in *Treatise on Zoology – Anatomy, Taxonomy, Biology. The Crustacea*, vol. vol 4 Part B . Eds. J. C. von Vaupel Klein, M. Charmantier-Daures and F. R. Schram (Leiden: Koninklijke Brill NV), pp 189–pp 396.

Wittmann, K. J., Ariani, A. P., and Daneliya, M. (2016). The Mysidae (Crustacea: Peracarida: Mysida) in Fresh and Oligohaline Waters of the Mediterranean. Taxonomy, Biogeography, and Bioinvasion. *Zootaxa* 4142, 1–70. doi: 10.11646/zootaxa.4142.1.1

Wolff, C. (2009). The Embryonic Development of the Malacostracan Crustacean *Porcellio Scaber* (Isopoda, Oniscidea). *Dev. Genes Evol.* 219, 545–564. doi: 10.1007/s00427-010-0316-6

Wollaston, W. H. (1807). Description of the Camera Lucida. *J. Nat. Phil. Chem. Arts.* 17, 1–5.

WoRMS Editorial Board (2021) *World Register of Marine Species*. Available at: https://www.marinespecies.org.

Yeom, J., Park, N., Jeong, R., and Lee, W. (2021). Integrative Description of Cryptic *Tigriopus* Species From Korea Using MALDI-TOF MS and DNA Barcoding. *Front. Mar. Sci.* 8, 495. doi: 10.3389/fmars.2021.648197

Zeidler, W. (1990). Pelagic Amphipods, Infraorder Physosomata (Crustacea: Amphipoda. Hyperiidea) From the CSK International Zooplankton Collection (Western North Pacific) With the Description of Four New Species of *Scina*. *Publ. Seto Mar. Biol.* 34 (4/6), 167–200.

Zeidler, W. (2009). A Review of the Hyperiidean Amphipod Superfamily Lanceoloidea Bowman and Gruner 1973 (Crustacea: Amphipoda: Hyperiidea). *Zootaxa* 2000, 1–117. doi: 10.11646/zootaxa.2000.1.1

Zhang, Z. Q. (2008). Zoological Taxonomy at 250: Showcasing Species Descriptions in the Cyber Era. *Zootaxa* 1671 (1), 1–2. doi: 10.11646/zootaxa.1671.1.1

Zhang, R., Zhou, Y., Lu, B., and Wang, C. (2017). A New Species in the Genus *Styracaster* (Echinodermata: Asteroidea: Porcellanasteridae) From Hadal Depth of the Yap Trench in the Western Pacific. *Zootaxa* 4338, 153–162. doi: 10.11646/zootaxa.4338.1.8

Žnidaršič, N., Mrak, P., Rajh, E., Soderžnik, K.Ž., Čeh, M., and Štrus, J. (2018). Cuticle Matrix Imaging by Histochemistry, Fluorescence, and Electron Microscopy. *Resol. Discovery* 3, 5–12. doi: 10.1556/2051.2018.00052

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.