Integrative taxonomy of Antarctic and Subantarctic Sea Spiders (Pycnogonida) using morphological and molecular methods

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

Vorgelegt von

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List of publications

Weis A, Friedrich S, Melzer RR (2011) Antarctic Pycnogonida housed at the Bavarian State Collection of Zoology. *Zoosystematics and Evolution* 87 (2), 297-317.

Weis A, Melzer RR (2012) Chilean and Subantarctic Pycnogonida collected by the 'Huinay Fjordos' Expeditions 2005-2011. *Zoosystematics and Evolution* 88 (2), 187-205.

Weis A, Melzer RR (2012) How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity* 10(3), 361-374.

Weis A, Meyer R, Dietz L, Dömel J, Leese F, Melzer RR (2013) *Pallenopsis patagonica* (Hoek, 1881) – a species complex revealed by morphology and DNA barcoding with description of a new species of *Pallenopsis* Wilson, 1881. *Zoological Journal of the Linnean Society*. Accepted.

Disclaimer

I herewith declare that the nomenclaturally relevant acts in this thesis have to be regarded as unpublished according to article 8 of the International Code of Zoological Nomenclature (ICZN 1999), and will only become availabe by the referring publications.

Declaration of author's contribution

In this dissertation, I present the results from my doctoral research conducted from May 2010 until August 2013 in four chapters, carried out under the supervision of Prof. Dr. Roland R. Melzer at the Ludwig-Maximilians-University of Munich.

For all publications listed above Andrea Weis and PhD supervisor Prof. Dr. Roland R. Melzer designed the projects.

Contribution to Article I:

Weis A, Friedrich S, Melzer RR (2011) Antarctic Pycnogonida housed at the Bavarian State Collection of Zoology. *Zoosystematics and Evolution* 87 (2), 297-317.

Andrea Weis accomplished the data collection, performed morphological analyses (using light- and scanning electron microscope) and designed all figures. Stefan Friedrich was responsible for the collection management at the Bavarian State Collection of Zoology. Andrea Weis led the manuscript writing under the guidance of Prof. Dr. Roland R. Melzer. 10 of the 28 Antarctic species have already been discussed in the Diploma Thesis of Andrea Weis 2009.

Contribution to Article II:

Weis A, Melzer RR (2012) Chilean and Subantarctic Pycnogonida collected by the 'Huinay Fjordos' Expeditions 2005-2011. *Zoosystematics and Evolution* 88 (2), 187-205.

Andrea Weis accomplished the data collection, performed morphological analyses (using light- and scanning electron microscope) and designed all figures. Manuscript concept and writing was done by Andrea Weis under the guidance of Prof. Dr. Roland R. Melzer.

Contribution to Article III:

Weis A, Melzer RR (2012) How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity* 10(3), 361-374.

Andrea Weis carried out morphological and molecular analyses and designed all figures and tables. Manuscript concept and writing was done by Andrea Weis under the guidance of Prof. Dr. Roland R. Melzer.

Contibution to Article IV:

Weis A, Meyer R, Dietz L, Dömel J, Leese F, Melzer RR (2013) *Pallenopsis patagonica* (Hoek, 1881) – a species complex revealed by morphology and DNA barcoding with description of a new species of *Pallenopsis* Wilson, 1881. *Zoological Journal of the Linnean Society*. Accepted.

Andrea Weis coordinated and conducted the sampling of pycnognid material. R. Meyer, F. Leese, J. Dömel and L. Dietz sampled and provided specimens for the study. J. Dömel generated part of the molecular sequences. Andrea Weis carried out all morphological and phylogenetic analyses except the NeighborNet analyses which was performed by F. Leese. Design and preparation of figures and diagrams including also the drawings of the new species *Pallenopsis yepayekae* Weis nov. spec., in Weis et al. accepted was done by Andrea Weis. Manuscript writing was done by Andrea Weis under guidance of Prof. Dr. Roland R. Melzer. F. Leese supported manuscript writing.

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Ort, Datum   Dipl.-Biol. Andrea Weis

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Ort, Datum   Prof. Dr. Roland R. Melzer

Zusammenfassung

Pycnogonida oder Meeresspinnen sind ausschließlich marine Arthropoden, welche in allen Weltmeeren zu finden sind: von den Polen zu den Tropen und von Gezeitenbereichen bis in die Tiefsee. Viele Studien über Pycnogoniden konzentrieren sich auf antarktische Regionen, da Pycnogonida dort eine erstaunliche Artenvielfalt aufweisen. Ziel der vorliegenden Arbeit ist es das Studiengebiet auf bis dato noch relativ unerforschte subantarktische Gebiete mit Fokus auf die chilenische Küste und die dazugehörigen Fjorden auszubreiten. Insgesamt sind 40 Arten aus 9 der 11 Familien der Pycnogonida vertreten, und zwar: Ammotheidae, Colossendeidae, Callipallenidae, Nymphonidae, Pallenopsidae, Phoxichilidiidae, Pycnogonidae, Rhynchothoracidae und Austrodecidae. Die Tiere wurden detailliert mit dem Licht-, sowie dem Rasterelektronenmikroskop untersucht. Zur kompletten und aktualisierten Veranschaulichung der diagnostischen Merkmale der verschiedenen Arten werden zwei Bildatlanten (einer über antarktische und einer über chilenische/subantarktische) dargestellt und mit Ergebnissen früherer Literatur verglichen. Darüberhinaus sind die Angaben zu der geographischen Verbreitung der einzelnen Arten aktualisiert und zusammengefasst.

Ein weiteres Ziel der vorliegenden Arbeit sind die molekularen Analysen eines Teils des mitochondrialen proteincodierenden Genes COI (cytochrome oxidase subunit 1) von 76 chilenischen oder subantarktischen Pycnogonida, sowie die Gegenüberstellung molekularer und morphologischer Ergebnisse innerhalb eines integrativ-taxonomischen Ansatzes. Der phylogenetische Consensus-Baum zeigt 10 deutlich gut unterstützte Äste. Neben der Berechnung von intra- und interspezifischer Varianz wurden zur weiteren Überprüfung der Artgrenzen auch ein statistisches Parsimonie-Netzwerk sowie eine GMYC-Analyse herangezogen. Besonderer Fokus liegt hierbei auf 16 Tieren der Art *Achelia assimilis* (Haswell, 1885), welche sich in vier deutliche Unteräste untergliedern, die genau mit den verschiedenen geographischen Regionen korrespondieren. Da die morphologischen Unterschiede innerhalb der Variabilität liegen, wie sie in der Literatur für diese kosmopolitische Art beschrieben sind, repräsentieren die vier Unteräste von *A. assimilis* eher geographisch begrenzte Unterarten, bedingt durch die drastische Vereisung Patagoniens während des Känozoikums.

Ein weiteres interessantes Beispiel, auf welches die integrative Taxonomie angewendet wird, stellt *Pallenopsis patagonica* (Hoek, 1881) dar. Diese Art gehört zu den taxonomisch problematischsten und variabelsten Pycnogonida-Arten der am südlichsten gelegenen Küsten Südamerikas sowie der Subantarktis und Antarktis. Neben detaillierten morphologischen Studien, welche auch wichtiges Typenmaterial von Hoek (1881) beinhalteten, wurde eine

phylogenetische Analyse von mitochondrialen COI-Sequenzen von 47 *Pallenopsis*-Exemplaren durchgeführt. Dabei können zwei größere Linien unterschieden werden, nämlich: die „Falkland"-Linie, zu welchem der ursprüngliche *P. patagonica* gehört, und die „Chile"-Linie, welcher als eine neue Art *P. yepayekae* Weis nov. spec., in Weis et al. accepted beschrieben wird. Zudem geben weitere Linien innerhalb des *Pallenopsis*-Komplexes einen Hinweis auf das mögliche Vorhandensein weiterer kryptischer Arten.

Summary

Pycnogonids or sea spiders are exclusively marine arthropods found all over the ocean, from the poles to the tropics, and from littoral zones to abyssal depths. Many pycnogonid studies focus on the Antarctic area, since there they appear with remarkable species richness. The present study aims to extend the spectrum to hitherto relatively unexplored Subantarctic regions with special focus on the Chilean coast and inner fjords. Altogether 40 species from 9 of the 11 pycnogonid families are represented, namely: Ammotheidae, Colossendeidae, Callipallenidae, Nymphonidae, Pallenopsidae, Phoxichilidiidae, Pycnogonidae, Rhynchothoracidae and Austrodecidae. Specimens were studied in detail with light and scanning electron microscopy (SEM). To depict complete and updated sets of the species' diagnostic features two pictorial catalogues (one Antarctic and one Chilean/Subantarctic) are illustrated and compared to results from previous literature. Furthermore data on the species' geographic distribution are updated and summarized.

Further aim of the present thesis was the molecular analyses of the mitochondrial protein-coding gene COI (cytochrome c oxidase subunit 1) from 76 Chilean/Subantarctic pycnogonids and to combine the results in an integrative taxonomic approach with the morphological results. In the phylogenetic consensus tree 10 distinct, well-supported branches are displayed. Beneath the calculation of intra- and interspecific distances, a statistical parsimony network as well as a GMYC analysis were used to check for species boundaries. Special focus lies hereby on the 16 specimens of *Achelia assimilis* (Haswell, 1885), that represented four distinct subbranches corresponding to the different geographic regions. Since the morphological differences among the specimens lie well within the variation described in the literature for this cosmopolitan species, the four branches of *A. assimilis* might rather represent geographically limited subspecies as a result of drastic glaciation during the Cenozoic.

Another interesting candidate for applying integrative taxonomy displays *Pallenopsis patagonica* (Hoek, 1881) one of the taxonomically most problematic and variable pycnogonid species from the southern South American coast and Subantarctic/Antarctic area. Besides detailed morphological studies including also important type material used by Hoek (1881), a phylogenetic analysis of mitochondrial COI sequences of 47 *Pallenopsis* specimens was conducted. Two major clades could be identified, namely the "Falkland" clade, to which the nominal *P. patagonica* is assigned, and the "Chilean" clade, which is described here as a new species *P. yepayekae* Weis nov. spec., in Weis et al. accepted. In addition further clades of the *Pallenopsis* complex give a hint of even more putative cryptic species.

Table of contents

Weis A, Friedrich S & Melzer RR (2011) Antarctic Pycnogonida housed at the Bavarian State Collection of Zoology. *Zoosystematics and Evolution* 87 (2), 297-317.

Weis A & Melzer RR (2012a) Chilean and Subantarctic Pycnogonida collected by the 'Huinay Fjordos' Expeditions 2005-2011. *Zoosystematics and Evolution* 88 (2), 187-205.

1. General Introduction

1.1. Introduction to Pycnogonids

Pycnogonids or sea spiders are almost perfectly hidden in the benthic organisms they feed on (mainly hydrozoans but also anemones or small polychaetes (Bain, 1991; Arango & Brodie, 2003)), furthermore moving so slowly that they are very hard to detect.
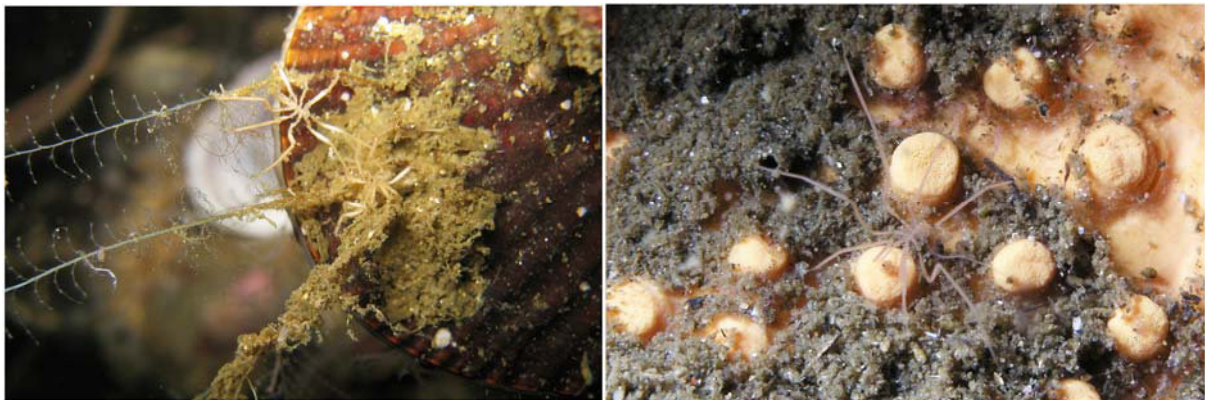
Fig. 1. *Anoplodactylus californicus* (left) and *Callipallene margarita* (right); Photos by Michael Schrödl and Roland Melzer (left) and Roland Meyer (right).

Viewing a pycnogonid gives the impression that the whole animal exists only of legs and appendages, lacking any kind of body. Already Stebbing (1902) therefore introduced the term "nobodies" as a popular name for this bizarre exclusively marine group of arthropods. Also the name Pantopoda implies its unique derived character of numerous multi-articulate ambulatory legs (Arango, 2003). While the term Pycnogonida compasses the valid name for the class including also fossils from the lower Silurian, lower Devonian and Jurassic (about 425 MYa, 400 MYa and 150 MYa respectively) the order Pantopoda denominates exclusively extant forms (Bamber & El Nagar 2013). Whereas most pycnogonids are octopodous, possessing four pair of walking legs, there are also found some decapodous (five pairs of legs) or dodecapodous (six pairs of legs) species in the Antarctica (Schram & Hedgpeth, 1978). Body sizes of pycnogonids range from less than 2 mm to about 6 cm reaching therefore a maximum leg span of about 70-75 cm (Westheide & Rieger, 2007; Child, 1995). Although their body appendages and segmentation patterns are highly specialized and modified in different taxa and sexes (Arnaud & Bamber 1987), the basic pycnogonid body plan consists of a body divided into three sections: cephalon, thorax or trunk and abdomen (Fig. 2).
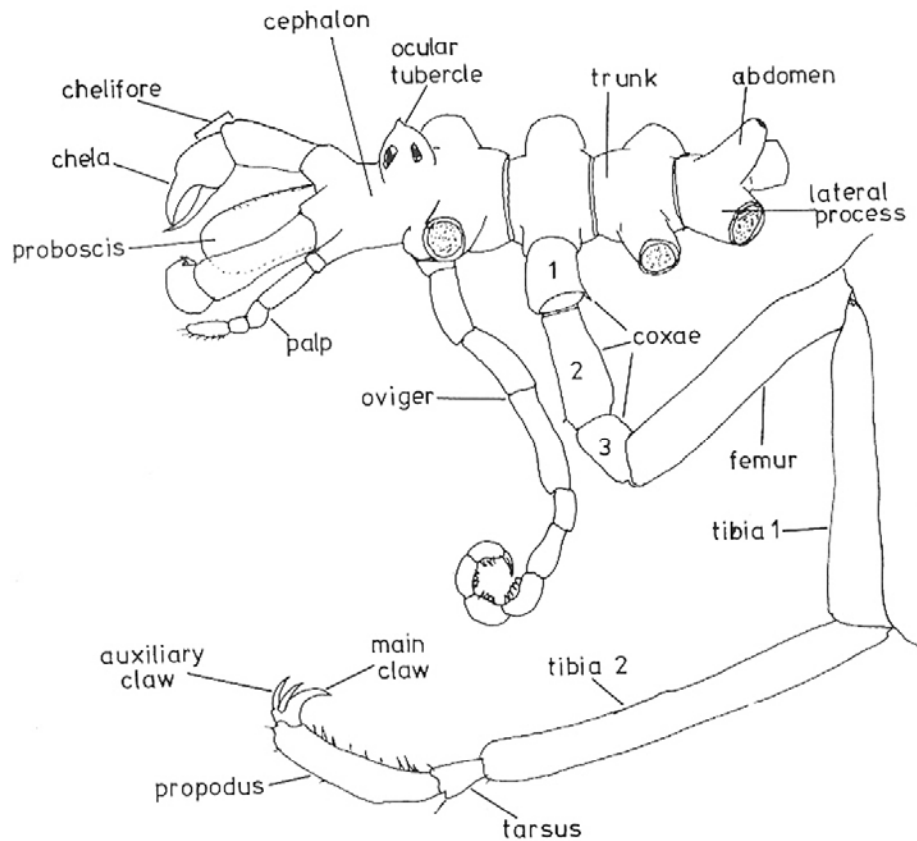
Fig. 2. Lateral view of an "idealized" pycnogonid (after Child, 1979).

Unfortunately there are many ways describing and naming the segmentation pattern of pycnogonids. Depending on the different literature the body of a pycnogonid is either termed thorax or trunk. Furthermore following the segmentation pattern of Chelicerata where pycnogonids belong to, they are sometimes divided in cephalothorax or prosoma and abdomen or opisthosoma. To avoid any misunderstanding a better alternative would be: cephalosoma (since it already bears the first pair of walking leg; Winter 1980), leg segments 2-4 and abdomen. The homology of the segmentation pattern of pycnogonids with the remaining arthropods is supported by gene expression and neuroanatomy studies (Jager et al. 2006; Manuel et al. 2006; Brenneis et al. 2008) (see also below).

The cephalon bears the ocular tubercle (dorsally), the proboscis (pycnogonid autapomorphy) and the first four sets of appendages: the cheliphores, palps, ovigers and the first pair of walking legs. As mentioned above depending on species or sex, some animals may lack one or more sets of cephalic appendages, making them crucial to the confident identification of pycnogonids (Stock, 1965; Arnaud & Bamber, 1987). Although the cheliphores are present in all pycnogonid larvae they show varying degrees of reductions in adults across genera (King, 1973; Maxmen et al. 2005). Cheliphores help to manipulate food whilst feeding and are also

2

suitable for holding onto the surrounding habitat (Arnaud & Bamber 1987). The palps of pycnogonids are composed of between one and twenty articles, although most vary between five and ten segments (Helfer & Schlottke, 1935), representing further important classification tools (Hedgpeth, 1955). Palps are used whilst feeding, cleaning or as sensory organs (King, 1973). Most ovigers show ten articles (varies with genera and sex) with the last four articles forming a strigilis (sickle-shaped claw) with denticulate spines for most species (Child, 1998). Depending on the genus the number of denticulate spines varies enormously or are even lacking completely. These appendages are used for grooming and by the males for egg carrying until hatching or longer (King, 1973).

The thorax consists of typically three segments, each bearing one pair of walking legs. The legs are composed of nine articles and connected with the thorax by lateral processes: coxa 1, coxa 2, coxa 3, femur, tibia 1, tibia 2, tarsus, propodus and claw (with or without auxiliary claws). The abdomen is mostly very small, which is also a distinct pycnogonid autapomorphy, situated at the posterior end of the trunk and terminating in the anus (Arnaud & Bamber, 1987).

The integument of pycnogonids is composed of a chitinous cuticle, but unlike to crustaceans, the cuticle of pycnogonids is never stiffened by calcium deposits (Arnaud & Bamber, 1987) and consists of three layers: the epi-, ecto-, and endocuticles (King, 1973).

1.2. Phylogeny

Both the presence of an exoskeleton and the segmentation of body parts clearly place pycnogonids within the monophyletic phylum Arthropoda (Weygoldt, 1986; Hickman et al. 2000; Dunlop & Arango 2004). However, their exact phylogenetic position is often controversial and still under debate (Dunlop & Arango, 2004). The apparent resemblance of pycnogonid's body shape to spiders (see also their nomination as sea spiders) and crabs gave rise to different interpretations of the phylogenetic classifications, either to the arachnids or to the crustaceans, which are both within the phylum Arthropoda. The hypotheses concerning their relationship to crustaceans, which was based on similarities in their larval morphology and development (Thompson, 1904) was recently refused by fossil descriptions (Hedgpeth, 1978; Siveter et al. 2004) and even molecular data (Giribet et al. 2001; Arango & Wheeler 2007; Arabi et al. 2010; Masta et al. 2010; Regier et al. 2010).

King (1973) already stated that pycnogonids should have the status of a subphylum within the Arthropoda. This hypothesis is strongly supported by the distinct autapomorphies that are the

pycnogonid oviger, their prominent proboscis and highly reduced abdomen (Hedgpeth, 1955). Nowadays pycnogonids are considered as a basally branching lineage within the arthropod tree (Weygoldt & Paulus 1979; Schram 1978; Zrzavy et al. 1997; Edgecombe et al. 2000; Giribet & Ribera 2000; Giribet et al. 2001; Arango & Wheeler 2007; Regier et al. 2010). Pycnogonida are placed as an own class within the Chelicerata as a sister group to Xiphosura and Arachnida (Westheide & Rieger, 2007). In addition fossil records from the Cambrian and Silurian age provide information that pycnogonids are among the oldest clades of arthropods (Arango, 2003). Further studies concerning the brain development of pycnogonids and their protocerebral innervation of the cheliphores compared to other extant arthropods display further strong support for the placement of pycnogonids as the most basally deriving group of extant arthropods (Budd & Telford 2005; Maxmen et al. 2005). However, more recent studies based on developmental expression patterns and neuroanatomy suggest pycnogonid cheliphores to be chelicerae homologues (Jager et al. 2006; Manuel et al. 2006; Brenneis et al. 2008). This evidence for a deutocerebral affiliation of the pycnogonid cheliphores (Brenneis et al. 2008) would be consistent with the placement of sea spiders as sister group to other chelicerates (Masta et al. 2010).

1.3. Biodiversity and Natural habitat of pycnogonids

Currently pycnogonids are counting more than 1300 species worldwide (Arango & Wheeler 2007; Munilla and Soler-Membrives 2008;-Bamber & El Nagar 2013) classified into 11 families, namely: Ammotheidae, Ascorhynchidae, Austrodeciade, Callipallenidae, Colossendeidae, Endeidae, Nymphonidae, Pallenopsidae, Phoxichilidiidae, Pycnogonidae and Rhynchothoracidae (Bamber 2007; Bamber & El Nagar 2013). Pycnogonids are exclusively marine arthropods, present in all oceans from the poles to the tropics, and from the littoral zone to abyssal depths (Bamber 2007; Park et al. 2007). In the Antarctic area pycnogonids appear with remarkable species richness (Stiboy-Risch 1993). While Hodgson (1927) described the seas around the Antarctic continent as the centre of speciation for pycnogonids ("Hauptquartier der Pantopoden"), later the Antarctic Ocean was also considered as a centre for geographic dispersion and evolutionary radiation (King 1973; Fry & Hedgpeth 1969; Hedgpeth 1947).

The bigger part of recent pycnogonid studies concentrates on the Antarctic area (see for example Pushkin 1993, Child 1994, Child 1995, Munilla and Soler-Membrives 2008, Nielsen et al. 2009, Krabbe et al. 2010, Arango et al. 2011, Dietz et al. 2011). Over the last 35 million

years the Antarctic has been affected by many glaciation events (Wilson et al. 2009), with the last maximum about 15.000 years ago (Huybrechts 2002). Thereby many species that could not adapt to such vast climate change got extinct, and following postglacial recolonization events offered the chance for evolution of new species. The often changing environmental conditions force the benthos communities to adapt faster to different living conditions and therefore pushing divergence to higher levels. Thatje et al. (2005) already mentioned: „Enhanced habitat structuring through local disturbance has been frequently used to explain Antarctic species richness and community structure (Gutt & Piepenburg 2003)." Benthic organisms could have survived the last glacial period by either migration to the deep sea or occupation of shallow water niches (Thatje et al. 2005), explaining the high percentage of cryptic species found in the region (Held 2003; Held & Wägele 2005; Wilson et al. 2007; Hunter & Halanych 2008; Mahon et al. 2008). Furthermore the process of repeated glacial and interglacial cycles has been termed the "Antarctic diversity pump" (Clarke & Crame 1989, 1992). Similar glacial events are also known for the relative unexplored Subantarctic region, offering another interesting study area.

The Antarctic Peninsula extends far north not far away from the southern tip of South America connected beyond that by the Humboldt Current (Brattström & Johanssen 1983). Therefore the about 90.000 km long southern Chilean coastline (including the southernmost tip of South America) offers by its impressive fjord regions (fig. 3) a unique possibility for studying speciation processes of pycnogonids.

Fig. 3. Overview of part of the Comau fjord at Huinay (Chile); Photo by Roland Meyer.

About 25.000-15.000 years ago the entire Chilean coastline including fjords and channels was covered by the Patagonian ice shield (Clapperton 1993, McCulloch et al. 2000). After this Last Glacial Maximum the Chilean fjord regions were subsequently re-colonized by benthic communities (Försterra 2009) including also pycnogonids (Melzer et al. 2006). As already Thatje and his colleagues (2005) hypothesized, survival of benthic communities during such glacial periods, was possible only in the deep sea or in shelters on the continental shelf. But along the Chilean coast the Pacific Ocean shows very steep slopes without any stepping stones which would be essential for survival of benthos communities. Thus postglacial recolonization occurred mainly from glacial refugia in the North and/or South. Furthermore Thatje et al. (2005) suggest that as a result of glacial isolation, taxa with poor dispersal abilities might form cryptic species. This is the case for pycnogonids, which exhibit a holobenthic life cycle (King 1973; Arnaud & Bamber 1987) lacking a pelagic larval stage, thus showing relatively limited dispersal abilities compared to pelagobenthic animals like for example crustaceans. All this makes the Chilean fjord region to a unique exceedingly interesting study area for studying speciation processes, cryptic species or even species that are new to science.

A further region that has to be mentioned are the Falkland Islands, that are connected to the South American shelf, located about 500 km to the east of Patagonia (Leese et al. 2008). Beyond that major ocean current systems facilitate dispersal of specimens (Leese et al. 2008): on the one hand the Falkland current arriving from Tierra del Fuego and passing the Falklands on the Westside and on the other hand the Humboldt Current arriving from the Antarctic and passing the Chilean coast (Brattström & Johanssen 1983). Furthermore evidence from marine species supports the fact that migration of species between the continental South America and the Falkland Island is occurring repeatedly (Leese et al. 2008). Biogeographically the Falkland Islands are either seen as a distinct region (Powell 1965) or as part of the Magellanic region. The latter can be supported by the Falkland current and resembles also my point of view.

Whereas the Chilean coast lacks any stepping stones which would be essential for survival of benthos communities, the Falkland Islands, lying between 51°S and 52° 30' S and 57° 45' W and 61° 30' W in the South Atlantic (Wakeham-Dawson et al. 2009), display a different case. The area around the Falkland Islands and Burdwood bank provided potential glacial refugia for benthos communities including pycnogonids (Clapperton 1993).

1.4. Sourcing of pycnogonid material

1.4.1. Huinay Scientific Field station (Chile) and "Huinay Fjordos" expeditions

Getting access to the isolated and highly nested Chilean fjord channels for collecting pycnogonid material became possible by collaboration with the Huinay Scientific Field station (fig. 4).

Fig. 4. Huinay Scientific Field Station (left), laboratory (right) and starting point for expeditions to the Chilean fjords (Upper left and lower photo by Roland Meyer; Upper right photo by Roland Melzer).

The private and non-profit institution "Fundacion San Ignacio del Huinay" is located (like the name implies) in Huinay in the Comau and Leptepu fjord, Palena Province, Region X (de los Lagos), Chile and founded by Endesa Chile and the Pontificia Universidad Católica de Valparaíso. The Huinay Scientific Center (inaugurated in December 2001) seeks to defend and preserve the bio-geographical patrimony of the Huinay area and of the surrounding Patagonian fjord region of Chile. Between 2005 and 2013 the Huinay Foundation carried out 16 adventurous and successful expeditions organized by Dr. Verena Häussermann and Günter Försterra along the Chilean coast and inner fjords and collected hundreds of pycnogonids by SCUBA diving. Amongst others for example the Yepayek a small ranger boat of the CONAF (Corporación Nacional Forestal) under captain German Coronado Vasquez and the crew members Victor Munoz Aguero and Guillermo Igor Almonacid carried the scientists to the different places in the Chilean fjords. Our aim was to collect pycnogonids from a broad geographic area of the Chilean coast from the north to the south as well as from outer fjord channels to more inner situated/sheltered fjords like for example the Reñihué fjord. A detailed overview of the different collecting sites is shown in paper II, figure 1.

An overview of marine benthic fauna of Chilean Patagonia is provided in "Marine Benthic Fauna of Chilean Patagonia" (Häussermann & Försterra 2009). Furthermore the molecular data of the Chilean pycnogonids are part of the barcoding project of the Canadian Centre for Barcoding at the Department of Zoology, University of Guelph, Ontario (project code: CFAP, project name: Chilean Fjord Pycnogonids). COI sequences of the Chilean pycnogonids of the present thesis are available from BOLD (http://www.boldsystems.org/).

1.4.2. Additional material

We received additional Chilean pycnogonid material including our northernmost sample site (30° 22.893' S and 71° 57.759' W) from Javier Sellanes López (Universidad Católica del Norte, Facultad De Ciencias Del Mar, Coquimbo, Chile). Furthermore Dr. Vladimir Laptikhovsky from the Falkland Islands Fisheries Department forwarded us supplementary material from the Westside of the Falkland Islands.

Antarctic pycnogonid material was received by Enrico Schwabe (Zoologische Staatssammlung München) during different expedition journeys of the FS Polarstern (ANDEEP-SYSTCO), which were mainly located in the Weddell Sea, Amundsen Sea and other ocean areas around the Antarctic Peninsula (overview of Antarctic collecting sites are shown in paper I, figure 1). Furthermore we received material from the ICEFISH 2004

expedition of the FS Nathaniel B. Palmer and one further sample of the FS Polarstern (by Florian Leese, Ruhr University Bochum).

Another important point concerning Chilean pycnogonid material are the collections from older expedition journeys like the H.M.S. Challenger Expedition (1872-1876) (Hoek 1881), the Antarctic Swedish Expedition (1901-1903) (Loman 1923a, b) and the Lund University Chile Expedition dating back to the 1950s (Hedgpeth 1961). Therefore additional specimens from the Swedish Museum of Natural History including the Loman collection as well as one specimen from the Lund Chile Expedition, determined by Hedgpeth were loaned for morphological analyses. Furthermore I examined Hoeks type material of *P. patagonica* (Hoek, 1881) from the Challenger expedition, which we loaned from the Natural History Museum London (Miranda Lowe).

1.5. Identifying species boundaries

According to Mayden (1997) there are existing more than 20 species definitions lacking any standardized operational criteria to delimit them (Sites & Marshall 2004). Hence the answer of the supposed simple question "what is a species?" is often subjective depending on the species concept one applies. The traditional biological species concept characterizes species as "…groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." (Mayr 1942).

For the present case, this definition is not practicable. Therefore morphospecies, and more recently DNA-based species delimitation are required. In the present thesis all species (Antarctic and Subantarctic) are firstly defined by various morphological traits including SEM (see paper I and paper II). For the Chilean/Subantarctic pycnogonids additional molecular COI data were consulted (see paper III and paper IV) and corroborated with the morphological results using integrative taxonomy (Dayrat 2005; Padial et al. 2010; Schlick-Steiner et al. 2010).

1.5.1. Morphological studies

Species that are exclusively established on morphology are also called "morphospecies" by Cain (1954). Furthermore Dayrat (2005) introduced the term "morphodiversity" and stated that: "…morphospecies are hypotheses that should be tested via different approaches and with different kinds of data." This suggests that defining species only by morphology (as any other

9

species concept) has some limits. For example defining and interpreting character states adheres always a subjective component (Padial et al. 2010). Beyond that morphology is also described as a complex and non-neutral marker which could lead to under- or over-estimation of biodiversity (Lefebure 2006). Another problem is the existence of so-called cryptic species that are amongst others found in the study area of the present Thesis. During the glacial maxima gene flow was inhibited, leading to genetic separation without morphological change resulting in high levels of cryptic species (Grant & Linse 2009). Hence further tools for species delineation are needed, for example molecular approaches such as recent methodological developments like DNA barcoding.

1.5.2. Molecular analyses

Many molecular studies concern common marine invertebrate groups like molluscs (Wilson et al. 2009, Jörger et al. 2010), echinoderms (Hunter & Halanych 2008) or crustaceans (Lefebure et al. 2006, Raupach & Wägele 2006, Oliveira-Biener et al. 2010, Meyer et al. 2013). Also pycnogonids are gaining more and more interest (see Mahon et al. 2008, Nielsen et al. 2009, Arabi et al. 2010, Krabbe et al. 2010, Masta et al. 2010, Arango et al. 2011, Dietz et al. 2011).

Ten years ago Paul Hebert and his team introduced a segment of the mitochondrial cytochrome oxidase I gene "as the core of a global bioidentification system for animals" the so-called DNA barcodes (Hebert et al. 2003a, 2003b). This approach also enables to document and study cryptic species that are difficult or impossible to identify by morphological traits solely. Besides the fact that barcoding works for all life stages, it can also be applied where no morphological information is available for example degraded specimens, fragments of organisms or even pieces of tissues (Birky 2007, Palumbi and Cipriano 1998).

The proposal by Hebert et al. (2003a,b) to use COI-barcoding as a general method for specimen identification and discovery of new species led to the formation of international Consortium for the Barcode of Life (CBOL) (Birky 2007). CBOL provides a public database for barcodes, BOLD (www.boldsystems.org), where COI-sequences are linked to voucher specimens deposited in museums or other institutions (for further information see also www.barcoding.si.edu/ or www.barcodeoflife.org/).

In the recent years the combination of morphological and molecular data in phylogenetic studies was hotly debated (Goldstein & DeSalle 2010). Combined approaches such as integrative taxonomy are gaining more and more interest and acceptance (Dayrat 2005; Gibbs

2009; Heethoff et al. 2011; Padial et al. 2010; Roe & Sperling 2007; Schlick-Steiner et al. 2010; Schwentner et al. 2011; Will et al. 2005). The present thesis uses for the first time both traditional (morphology) and modern (molecular) techniques in a combined approached called integrative taxonomy for analysing pycnogonids from the Chilean fjords.

The need to assess the current state of biodiversity poses a significant time challenge, because under the current conditions species may become extinct before they have even been described (Wilson et al. 2007).

2. Aims of the Thesis

2.1. Morphological analyses of Antarctic and Subantarctic/Chilean pycnogonids using modern techniques

An important basis for defining species boundaries using integrative taxonomy requires detailed knowledge of morphology. Since nowadays more modern techniques are available (like the scanning electron microscope) we have the opportunity to deepen our morphological knowledge compared to previous traditional species delimitations.

Hence one aim of the present study is to contribute to a detailed light and scanning electron microscopy (SEM) atlas of the Antarctic and Subantarctic pycnogonid fauna including also their biogeographic data. Due to the possibility of using more modern techniques like the SEM the morphological knowledge/understanding could be deepened. Altogether 40 species (28 Antarctic plus 12 Chilean and Subantarctic species with *Colossendeis megalonyx* Hoek, 1881 occuring in both areas) are illustrated and discussed concerning the species' diagnostic features and compared to results from previous literature. The complete pycnogonid material has been collected on Antarctic and Subantarctic/Chilean expedition journeys during the last two decades and is housed at the Bavarian State Collection of Zoology (SNSB-ZSM). Surveys of Antarctic pycnogonids stored in natural history collections can provide a useful basis for future studies (Dunlop et al. 2007). Since species descriptions of pycnogonids are in most instances very old and sampled specimens might show some discrepancies to already described species, our aim is to facilitate future species determinations by providing detailed and extended depth of field pictures using more modern techniques like the powerful SEM. The present thesis displays the first light and scanning electron microscopy atlas of the Antarctic and Subantarctic with focus on the Chilean fjords and surrounding area.

2.2. Documentation of COI sequences and species delimitations of pycnogonids from the Chilean fjords and surrounding area using integrative taxonomy

A further aim of the present thesis is the documentation of COI sequences of pycnogonids from the Chilean fjords and surrounding areas for the first time. So far molecular studies on pycnogonids have mostly be done for the Antarctic area (see for example Mahon et al. 2008, Nielsen et al. 2009, Krabbe et al. 2010, Masta et al. 2010, Arango et al. 2011, Dietz et al. 2011), but never touched the South American Magellan region, Tierra del Fuego or the Chilean fjord region. Pycnogonid research in the latter regions included only morphological analyses (see Loman 1923a,b , Hedgpeth 1961, Sielfeld 2003, Melzer et al. 2006, Melzer 2009). In the present study molecular analyses of 80 pycnogonids from the Chilean and Subantarctic region (including also three Antarctic and one Australian specimens) are discussed.

2.2.1. *Achelia assimilis* (Haswell, 1885)

Special focus lies on the species *Achelia assimilis* being one of the most abundant species in this region. While the morphological differences of the studied specimens lie well within the variation described in the literature, the molecular results show four distinct COI branches. Therefore *A. assimilis* is an excellent example for studying cryptic species. The morphological variation of *A. assimilis* is illustrated in detailed light and scanning electron microscopy pictures. Different haplotypes of the species are discussed concerning their different geographic locations. Furthermore the extraordinary distribution pattern gives hints concerning the speciation processes after the last glaciation. Possible evolutionary scenarios for the origin of the different branches concerning the species *A. assimilis* are considered and discussed.

2.2.2. *Pallenopsis patagonica* (Hoek, 1881)

The second aim on the molecular level is to shed more light on the *Pallenopsis patagonica* species complex using morphological and molecular data. Beyond detailed morphological studies (using light and scanning electron microscopy) a phylogenetic analyses of 47 mitochondrial COI sequences was conducted. From these 47 specimens 39 have been primarily identified under the umbrella of *Pallenopsis patagonica*. Consulting morphological

and molecular data we show that *P. patagonica* constitutes a species-rich complex which needs a thorough taxonomic revision. With the present thesis I achieved the first step to unscramble the complex taxonomy of *P. patagonica* and beyond that describe a *Pallenopsis* species that is new to science, namely: *Pallenopsis yepayekae* Weis nov. spec., in Weis et al. accepted.

3. Paper I

Weis A, Friedrich S & Melzer RR (2011) Antarctic Pycnogonida housed at the Bavarian State Collection of Zoology. *Zoosystematics and Evolution* 87 (2), 297-317.

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Antarctic Pycnogonida housed at the Bavarian State Collection of Zoology

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Abstract

The Antarctic pycnogonid material housed at the Bavarian State Collection of Zoology is reviewed. It represents 28 species from 8 of the 11 pycnogonid families, namely: Ammotheidae, Austrodecidae, Callipallenidae, Colossendeidae, Nymphonidae, Pallenopsidae, Pycnogonidae and Rhynchothoracidae. The animals were studied in detail with a scanning electron microscope (SEM). Series of light microscopic pictures were also taken in order to depict complete sets of the species' diagnostic features. The latter are discussed and compared to data from previous literature.

Key Words

Introduction

The Pycnogonida or sea spiders are an extraordinary group of exclusively marine arthropods present in all oceans. Their phylogenetic position is still under debate. They have either been considered as a basally branching lineage within the arthropod tree (Zrzavy et al. 1997; Edgecombe et al. 2000; Giribet & Ribera 2000; Giribet et al. 2001; Arango & Wheeler 2007; Regier et al. 2010) or seen as derived arachnids and placed as a sister group to the Acari (e.g. Masta et al. 2010). Their holobenthic lifecycle (eggs are carried by the males on their ovigera, and larvae deposited on adequate foraging grounds; Arnaud & Bamber 1987; Heß & Melzer 2003; Bain & Govedich 2004a, b) indicates that their dispersal capacity is limited. Nevertheless, pycnogonids are found from the poles to the tropics, and from littoral zones to abyssal depths (Bamber 2007; Park et al. 2007).

In the Antarctic area, they appear with remarkable species richness (Stiboy-Risch 1993). Hodgson (1927) already considered this continent as the centre of speciation for pycnogonids ("Hauptquartier der Pantopoden"). Beyond that, the Southern Ocean has been described as a centre of pycnogonid geographic dispersal and evolutionary radiation (Hedgpeth 1947; Fry &

Hedgpeth 1969). So far, 264 austral pycnogonid species have been recorded, representing 19.6 % of the 1344 species described worldwide (Munilla & Soler-Membrives 2008). From these 264 species 108 are endemic to Antarctic waters, 62 to the sub-Antarctic, and 63 are common in both regions (Munilla & Soler-Membrives 2008). According to the latter authors, genera with most of their species in austral waters are *Ammothea* Leach, 1814; *Austrodecus* Hodgson, 1907; *Colossendeis* Jarzinsky, 1870; *Nymphon* Fabricius, 1794 and *Pallenopsis* Wilson, 1881.

A likely cause for this large species record is the last ice age, since during the Cenozoic glacial periods community survival in the Antarctic was only possible by migration to the deep sea or through occupation of shallow water niches (Thatje et al. 2005). The following recolonisation from different refuges could have favoured speciation processes, including in various cryptic species complexes found around the Antarctic in many taxa discovered in the last years using molecular taxonomy (e.g., Held 2003; Held & Wägele 2005). Examples for two well studied cryptic species complexes among pycnogonids are *Nymphon australe* (Arango et al. 2009) and *Colossendeis megalonyx* (Krabbe et al. 2010). Clearly, pycnogonids make a highly interesting taxon for the study of speciation processes in the Antarctic Ocean.

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All this explains why there is a rapidly growing body of molecular studies on Antarctic pycnogonids (see Arango 2002, 2003; Arango & Wheeler 2007; Mahon et al. 2008; Arango 2009; Nielsen et al. 2009; Krabbe et al. 2010; Masta et al. 2010).

However, in addition to the molecular data detailed morphological analyses are needed, and surveys of Antarctic pycnogonids stored in natural history collections can provide a useful basis for future studies (see also Dunlop et al. 2007).

Since collection specimens sometimes show discrepancies to published species descriptions, especially if the latter are relatively old, one aim of our study is to avoid such problems and facilitate future species determinations and morphological reinvestigations, especially concerning cryptic species.

Representatives of some of the major genera of Antarctic pycnogonids are housed at the Bavarian State Collection of Zoology, distributed among 28 species. We here contribute a catalogue of this material, which has been collected on Antarctic expedition journeys in the last 16 years. Furthermore, we present the classification given in PycnoBase (Bamber & Nagar 2011) as a taxonomic backbone, and illustrate a general overview of the most prominent characteristics of the collected species.

Material and methods

The collecting sites and species distribution of the Antarctic pycnogonids studied are displayed in Figure 1. Sampling sites are mainly located in the Weddell Sea, Amundsen Sea and other ocean areas around the Antarctic Peninsula; details are given in the species chapters. Most of the material was collected using an Agassiz trawl or a Rauschert dredge, and fixed in either 75 % or 96 % ethanol.

Species determinations were performed with a variety of literature suitable for Antarctic pycnogonids. In addition to the works of Hoek (1881), Hodgson (1907), Bouvier (1913) and Stock (1957), the discovery reports by Gordon (1932, 1938, 1944) as well as the descriptions and keys given by Fry & Hedgpeth (1969) proved helpful, since they provide many drawings. Determinations were checked further using the more recent works of Child (1994, 1995) and Pushkin (1993). Synonyms were looked up in PycnoBase (Bamber and Nagar 2011) and Müller's (1993) "World Catalogue and Bibliography of the recent Pycnogonida".

Specimens were documented using an Olympus SZX stereo microscope equipped with a Jenoptic Prog-Res C12 digital camera (2580 × 14; 1944 px; 96 dpi; colour depth 24 bit). For each specimen, several shots focused at different levels along the z-axis were taken, then edited and combined to a single respective image with greater depth of field using the computer software CombineZ and/or Syncroscopy Auto Montage. SEM preparations were made according to methods described in Montoya Bravo et al. (2009). Specimens were examined in a LEO 1430VP at 15 kV.

## Annotated catalogue

General remarks

A total of 28 Antarctic pycnogonid species could be identified. In all cases, the major morphological char-

acteristics correspond well with the respective descriptions published earlier.

The species most frequently recorded in the literature (Munilla & Soler-Membrives 2008), *Nymphon australe*, is one of the most abundant species in our Antarctic pycnogonid collection as well (16 specimens). However, the most common species in our collection is *Colossendeis megalonyx* (17 specimens). To our knowledge, *Ammothea magniceps*, *Cilunculus cactoides*, *Nymphon compactum*, *N. eltaninae*, *N. longicoxa* and *N. proceroides* are recorded for the first time from the Weddell Sea, the first of these species even from the Antarctic (Fig. 1). In addition, the depth ranges of some species could be expanded; for example, the record of *Colossendeis longirostris* from 3800 m is the deepest ever for this species.

Classification

Order **Pantopoda** Gerstäcker, 1863
Suborder **Eupantopodida** Fry, 1978
Superfamily **Ascorhynchoidea** Pocock 1904
Family **Ammotheidae** Dohrn, 1881
   Achelia communis (Bouvier, 1906)
   Achelia spicata (Hodgson, 1915)
   Ammothea magniceps Thompson, 1884
   Ammothea longispina Gordon, 1932
   Cilunculus cactoides Fry & Hedgpeth, 1969
Superfamily **Colossendoidea** Hoek, 1881
Family **Colossendeidae** Hoek, 1881
   Colossendeis australis Hodgson, 1907
   Colossendeis longirostris Gordon, 1938
   Colossendeis megalonyx Hoek, 1881
   Colossendeis tortipalpis Gordon, 1932
Superfamily **Nymphonoidea** Pocock, 1904
Family **Nymphonidae** Wilson, 1878
   Nymphon australe Hodgson, 1902
   Nymphon biarticulatum (Hodgson, 1907)
   Nymphon charcoti Bouvier, 1911
   Nymphon compactum Hoek, 1881
   Nymphon eltaninae Child, 1995
   Nymphon longicollum Hoek, 1881
   Nymphon longicoxa Hoek, 1881
   Nymphon mendosum Hodgson, 1907
   Nymphon proceroides Bouvier, 1913
   Nymphon proximum Calman, 1915
   Nymphon villosum Hodgson, 1907
   Pentanymphon antarcticum Hodgson, 1904
Family **Callipallenidae** Hilton, 1942
   Austropallene cornigera Möbius, 1902
   Austropallene gracilipes Gordon, 1944
Family **Pallenopsidae** Fry, 1978
   Bathypallenopsis macronyx (Bouvier, 1911)
   Pallenopsis hodgsoni Gordon, 1938
Superfamily **Pycnogonoidea** Pocock, 1904
Family **Pycnogonidae** Wilson, 1878
   Pycnogonum gaini Bouvier, 1910
Superfamily **Rhynchothoracoidea** Fry, 1978
Family **Rhynchothoracidae** Thompson, 1909
   Rhynchothorax australis Hodgson, 1907
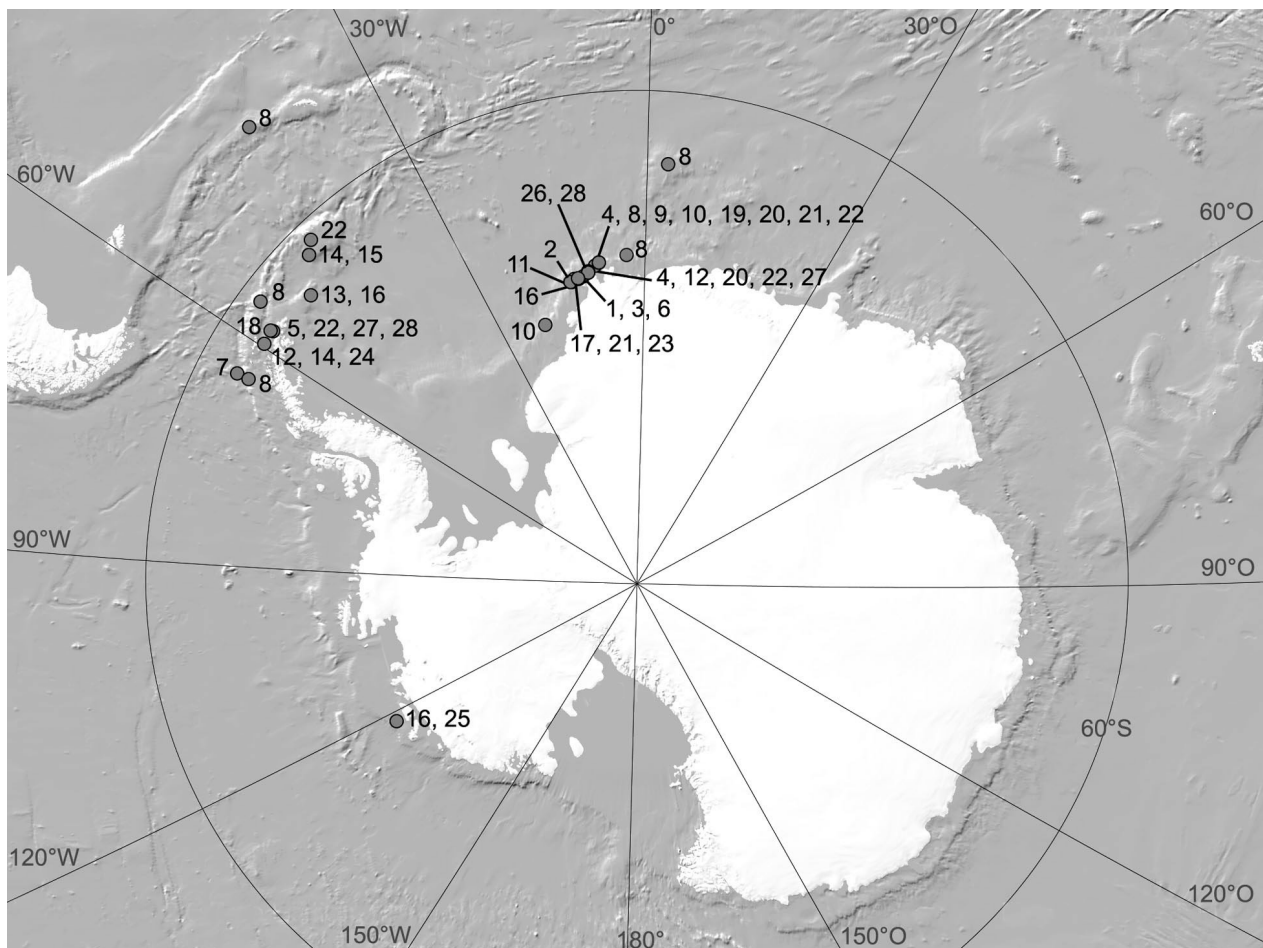
Figure 1. Overview of collecting sites of Antarctic pycnogonids deposited at the Bavarian State Collection of Zoology. Species are numbered as follows: **1** – *Achelia communis*, **2** – *Achelia spicata*, **3** – *Ammothea magniceps*, **4** – *Ammothea longispina*, **5** – *Cilunculus cactoides*, **6** – *Colossendeis australis*, **7** – *Colossendeis longirostris*, **8** – *Colossendeis megalonyx*, **9** – *Colossendeis tortipalpis*, **10** – *Nymphon australe*, **11** – *Nymphon biarticulatum*, **12** – *Nymphon charcoti*, **13** – *Nymphon compactum*, **14** – *Nymphon eltaninae*, **15** – *Nymphon longicollum*, **16** – *Nymphon longicoxa*, **17** – *Nymphon mendosum*, **18** – *Nymphon proceroides*, **19** – *Nymphon proximum*, **20** – *Nymphon villosum*, **21** – *Pentanymphon antarcticum*, **22** – *Austropallene cornigera*, **23** – *Austropallene gracilipes*, **24** – *Pallenopsis macronyx*, **25** – *Pallenopsis hodgsoni*, **26** – *Pycnogonum gaini*, **27** – *Rhynchothorax australis*, **28** – *Austrodecus glaciale*.

Suborder **Stiripasterida** Fry, 1978
Family **Austrodecidae** Stock, 1954
   Austrodecus glaciale Hodgson, 1907

Ammotheidae
Achelia Hodge, 1864

Achelia communis **(Bouvier, 1906)**
Figures 2a–d

Ammothea communis Bouvier, 1906: 44–50, figs 23–32

Synonyms
Ammothea affinis Bouvier, 1907
Achelia brucei Calman, 1915
Ammothea hoeki Loman, 1923

Material examined. ZSMA20100168: 1 specimen; Antarctica, Weddell Sea; 16.12.2003; 06:38–06:54; FS Polarstern; Cruise: PS 65; Station 173-1; Exp.: ANT XXI/2; 70°56.82′ S, 010°31.76′ W – 70°56.77′ S, 010°31.17′ W; AGT; 279.0–296 m.

Remarks. This species is very similar to *Achelia spicata*, from which it can be distinguished by the presence of anterior cephalic spurs (Fig. 2b). Furthermore, in *A. communis* the propodal spines are much straighter, more slender and further apart (Fig. 2d); see also Fry & Hedgpeth (1969) and the remarks on *A. spicata* below.

Achelia spicata **(Hodgson, 1915)**
Figures 2e–f

Austrothea spicata Hodgson, 1915: 147
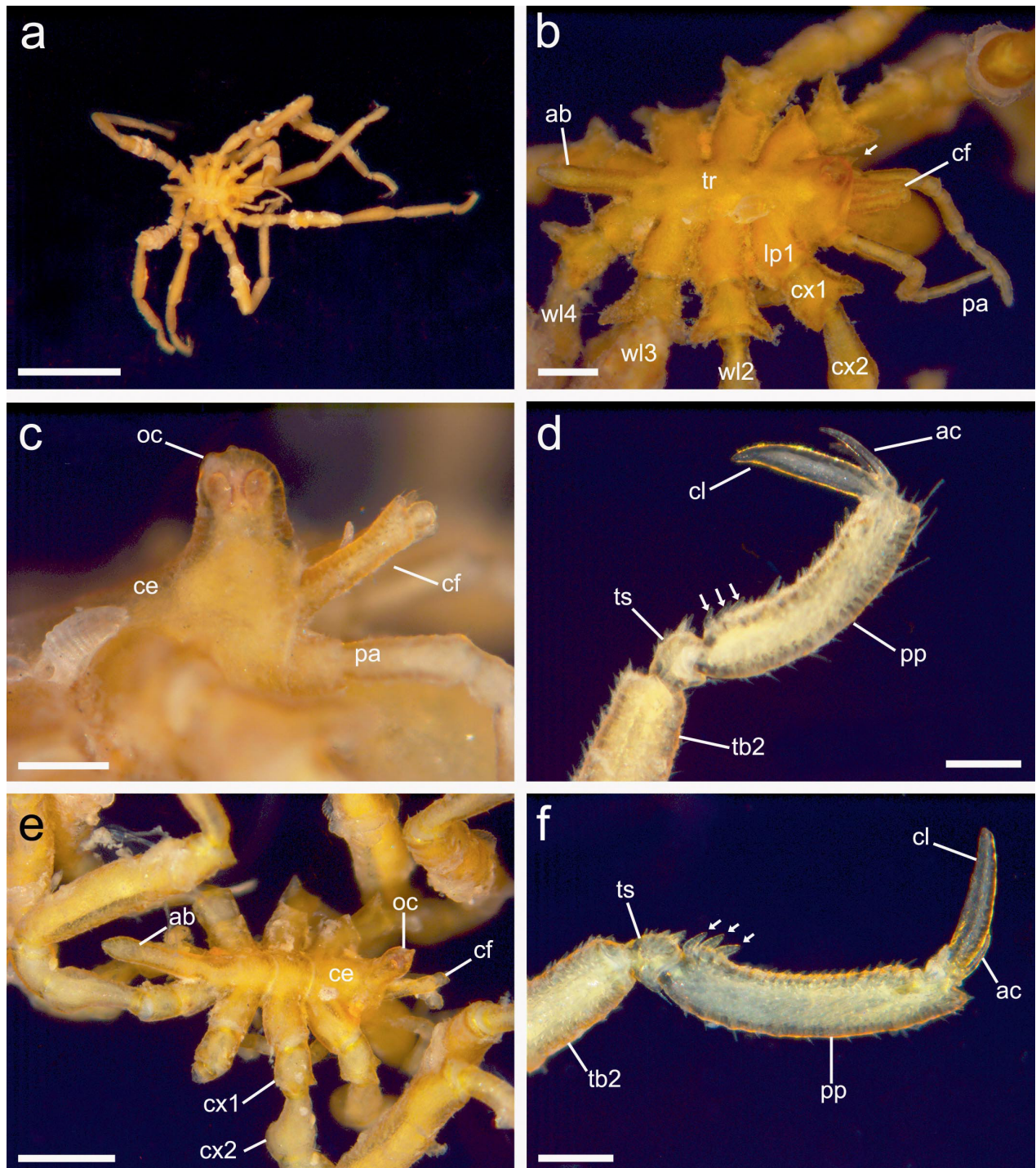
Synonym. Achelia intermedia Calman, 1915

Material examined. ZSMA20010087: 1 specimen; Antarctica, Weddell Sea; 11.04.2000; 08:10–08:19; FS Polarstern; Station 138-1; 71°8.90′ S, 013°12.80′ W; EBS; 765.0–840.0 m.

Remarks. Since *Achelia spicata* shows a very wide range of morphological variation, especially the more compact form of this species can easily be confused

Figure 2. a–d. *Achelia communis*; **a.** Dorsal view; scale = 3 mm; **b.** Dorsal view of trunk, note spine on cephalic segment (arrow); scale = 500 μm; **c.** Ocular tubercle; scale = 250 μm; **d.** Tarsus and propodus with claw and auxiliary claws of left 3rd walking leg, note sole spination (arrows); scale = 250 μm; **e–f.** *Achelia spicata*; **e.** Dorsal view of trunk; scale = 1 mm; **f.** Tarsus and propodus with claw and auxiliary claws of right 2nd walking leg, note sole spines (arrows); scale = 250 μm. **ab** – abdomen; **ac** – auxiliary claw; **ce** – cephalon; **cf** – chelifore; **cl** – claw; **cx** – coxa; **lp** – lateral process; **oc** – ocular tubercle; **pa** – palp; **pp** – propodus; **tb** – tibia; **tr** – trunk; **ts** – tarsus; **wl** – walking leg.

with *A. communis* on account of the closely spaced lateral processes, the shape of the proboscis with median swelling, and the tendency toward fusion of the third and fourth trunk somite (Fry & Hedgpeth 1969). However, besides the differences between the two species already mentioned by Child (1994), there are further discrepancies. Compared to *A. communis*, *A. spicata* lacks the two anterior cephalic spurs, the stout propodal spines are closely set and have a more thorn-like appearance (Fig. 2f), and in relation to the main claw also the auxiliary claws are conspicuously shorter (Fry & Hedgpeth 1969).

Ammothea Leach, 1814

Ammothea magniceps, Thompson, 1884

Figures 3a–c

Ammothea magniceps Thompson, 1884: 244, pl. XV, figs 1–5

Synonym. Achelia flynni Marcus, 1940

Material examined. ZSMA20100169: 1 specimen; Antarctica, Weddell Sea; 24.12.2003; 09:03–09:13; FS Polarstern; Cruise: PS 65; Station: 259-1; Exp.: ANT XXI/2; 70°56.57′ S, 010°31.98′ W – 70°57.00′ S, 010°33.02′ W; GSN; 300.0–333.0 m

Remarks. The examined specimen corresponds well with the description of Fry & Hedgpeth (1969). It can be distinguished from other species by several charac-
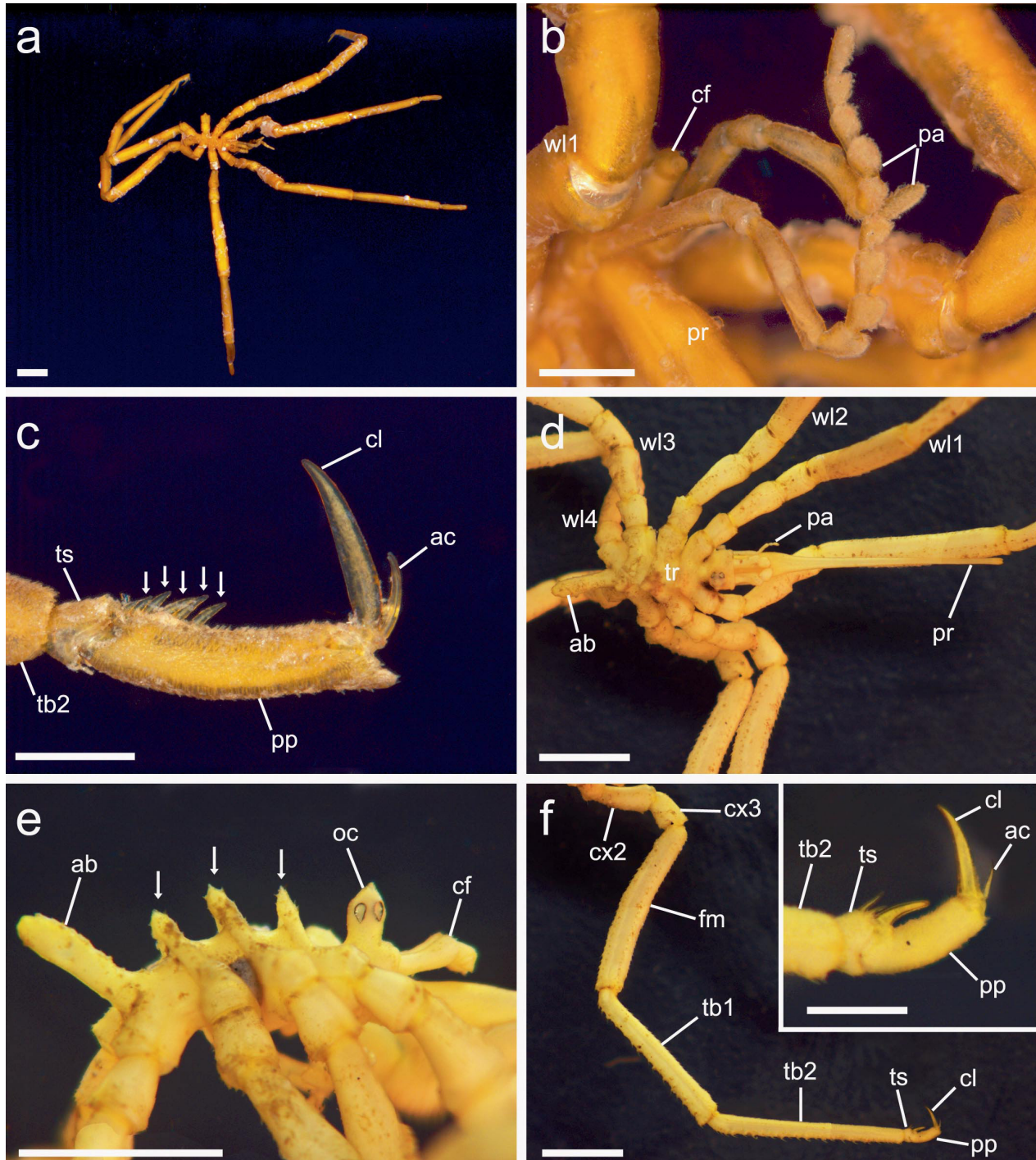
Figure 3. a–c. *Ammothea magniceps*; **a.** Dorsal view; scale = 3 mm; **b.** Detail view of palps; scale = 1 mm; **c.** Tarsus and propodus with claw and auxiliary claws of left 2nd walking leg, note sole spines (arrows); scale = 1 mm. **d–f.** *Ammothea longispina*. **d.** Dorsal view of trunk and proboscis; scale = 5 mm; **e.** Lateral view of trunk, note prominent body ridges (arrows); scale = 5 mm; **f.** Overview of right 3rd walking leg; scale = 5 mm. Insert: Detail of propodus with claw and auxiliary claws; scale = 1 mm. **ab** – abdomen; **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cx** – coxa; **fm** – femur; **oc** – ocular tubercle; **pa** – palp; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **tr** – trunk; **ts** – tarsus; **wl** – walking leg.

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ters: The palp is 9-segmented, segments 5–8 are asymmetrically conical (Fig. 3b), and the propodus bears 3–5 sole spines with the most proximal being smaller than the others (Fig. 3c).

To our knowledge this represents the first records of *Ammothea magniceps*, both from the Antarctic region and from depths of 300–333 m. This depth differs strongly from the previous records, all of which were from 0.5–24 m (Müller 1993).

Ammothea longispina Gordon, 1932
Figures 3d–f

Ammothea longispina Gordon, 1932: 101–103, figs 50–52

Material examined. ZSMA20080524: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m ZSMA20080578 – ZSMA20080579: each with 1 specimen (juvenile); Antarctica, Weddell Sea; 17.12.2007; 17:50–18:18; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/016-01; Exp.: ANDEEP-SYSTCO; 70°35.29′ S, 009°2.89′ W – 70°35.35′ S, 009°2.27′ W; Rauschert dredge; 486.3–488.4 m.

Remarks. The tapering proboscis in combination with the very short palps distinguish *Ammothea longispina* from all other congeneric species known from the Antarctic. The palps are also very short in *A. sextarticulata* Munilla, 1990 and *A. adunca* Child, 1994, but in these the proboscis is not styliform as in the species studied here (Fig. 3d). Compared to the total body size the legs appear rather short and stout, with the second tibia being the longest leg segment (Fig. 3f), which is in accordance with the descriptions of Fry & Hedgpeth (1969).

According to Gordon (1944), the anterior eyes are larger than the posterior ones in *A. longispina*. However, the individuals examined in this study all have eyes of about the same size (Fig. 3e). Furthermore, Child (1994) described the ocular tubercle as taller than the trunk cones. In our specimens, the trunk segmentation ridges show the same height as the ocular tubercle (Fig. 3e).

Cilunculus Loman, 1908

Cilunculus cactoides Fry & Hedgpeth, 1969
Figures 4a–c

Cilunculus cactoides Fry & Hedgpeth, 1969: 124–126, figs 205–206

Material examined. ZSMA20100165: 2 specimens; Antarctica; Antarctic Peninsula; 26.04.2000; 14:42–14:57; FS Polarstern; Cruise: PS 56; Station: 158-1; Exp.: EASIZ III; 63°4.70′ S, 057°31.60′ W – 63°4.50′ S, 057°32.00′ W; AGT; 94.0–95.0 m.

Remarks. The most obvious characteristic of this species is its very spinose appearance (Fig. 4b). This prominent feature differentiates it from all other *Cilunculus* species (Fry & Hedgpeth 1969; Pushkin 1993).

This is the first record from the Weddell Sea for *Cilunculus cactoides*. Previous sampling localities were the Scotia Sea, Antarctic Peninsula, East Antarctic

Zone (Munilla & Soler-Membrives 2008), and the Ross Sea (Fry & Hedgpeth 1969).

Colossendeidae
Colossendeis Jarzinsky, 1870

Colossendeis australis Hodgson, 1907
Figures 4d–e

Colossendeis australis Hodgson, 1907: 59, pl. IX, fig. 1, pl. X, figs 1–2
Material examined. ZSMA20100170: 1 specimen; Antarctica, Weddell Sea; 28.12.2003; 18:14–18:27; FS Polarstern; Cruise: PS 65; Station: 276-1; Exp.: ANT XXI/2; 71°6.44′ S, 011°27.76′ W – 71°6.64′ S, 011°27.28′ W; AGT; 268.0–277.0 m.

Remarks. The short and downcurved proboscis shows a conspicuous medial and distal inflation (Fig. 4e). This unique shape of the proboscis and the very short main claw serve to differentiate *C. australis* from other *Colossendeis* species (see Child 1995; Cano & López-González 2007), as does the robust body with the closely spaced lateral processes.

Colossendeis longirostris Gordon, 1938
Figure 4f

Colossendeis longirostris Gordon, 1938: 8 [key], 9–10, fig. 1
Material examined. ZSMA20060794: 1 specimen; Antarctica; W Antarctic Peninsula, Bellinghausen Sea; 30.03.2005; 16:14–16:49; FS Polarstern; Cruise: ANT XXII/2; Station: PS 67/154-7; Exp.: ANDEEP III; 62°31.81′ S, 064°38.31′ W – 62°31.33′ S, 064°40.52′ W; AGT; 3801.0–3813.0 m

Remarks. As the name implies, this species can be identified easily by its long proboscis (Fig. 4f). Besides that, the distal palp segment is inserted anaxially at a sharp angle (see Child 1995).

With a depth range of 3801–3813 m the present record is the deepest for *Colossendeis longirostris*. Previous documented depths did not exceed 3700 m (Munilla & Soler-Membrives 2008).

Colossendeis megalonyx Hoek, 1881
Figures 5a–d

Colossendeis megalonyx Hoek, 1881: 67–69, pl. IX, figs 1–3
Synonyms
Colossendeis arundorostris Fry & Hedgpeth, 1969
Colossendeis frigida Hodgson, 1907
Colossendeis orcadense Hodgson, 1909
Colossendeis rugosa Hodgson, 1907

Material examined. ZSMA20060788 – ZSMA20060790: each with 1 specimen; Antarctica; South Shetland Islands; 25.04.2002; FS Polarstern; Cruise: PS 61; Station: 252-1; Exp.: LAMPOS; 61°23.42′ S, 055°26.82′ W – 61°23.87′ S, 055°26.66′ W; AGT; 285.0–293.0 m. ZSMA20060791 – ZSMA20060792: each with 1 specimen; Antarctica; W Antarctic Peninsula, Bellinghausen Sea; 29.03.2005; 16:37–16:58; FS Polarstern: Cruise: ANT XXII/3; Station: PS 67/153-8; Exp.: ANDEEP III; 63°19.53′ S, 064°36.79′ W – 63°19.10′ S, 064°37.13′ W; AGT; 2069.0–2124.0 m. ZSMA20060793: 1 specimen; Antarctica; South Shetland Islands; 25.04.2002; FS Polarstern; Cruise: PS 61; Sta-
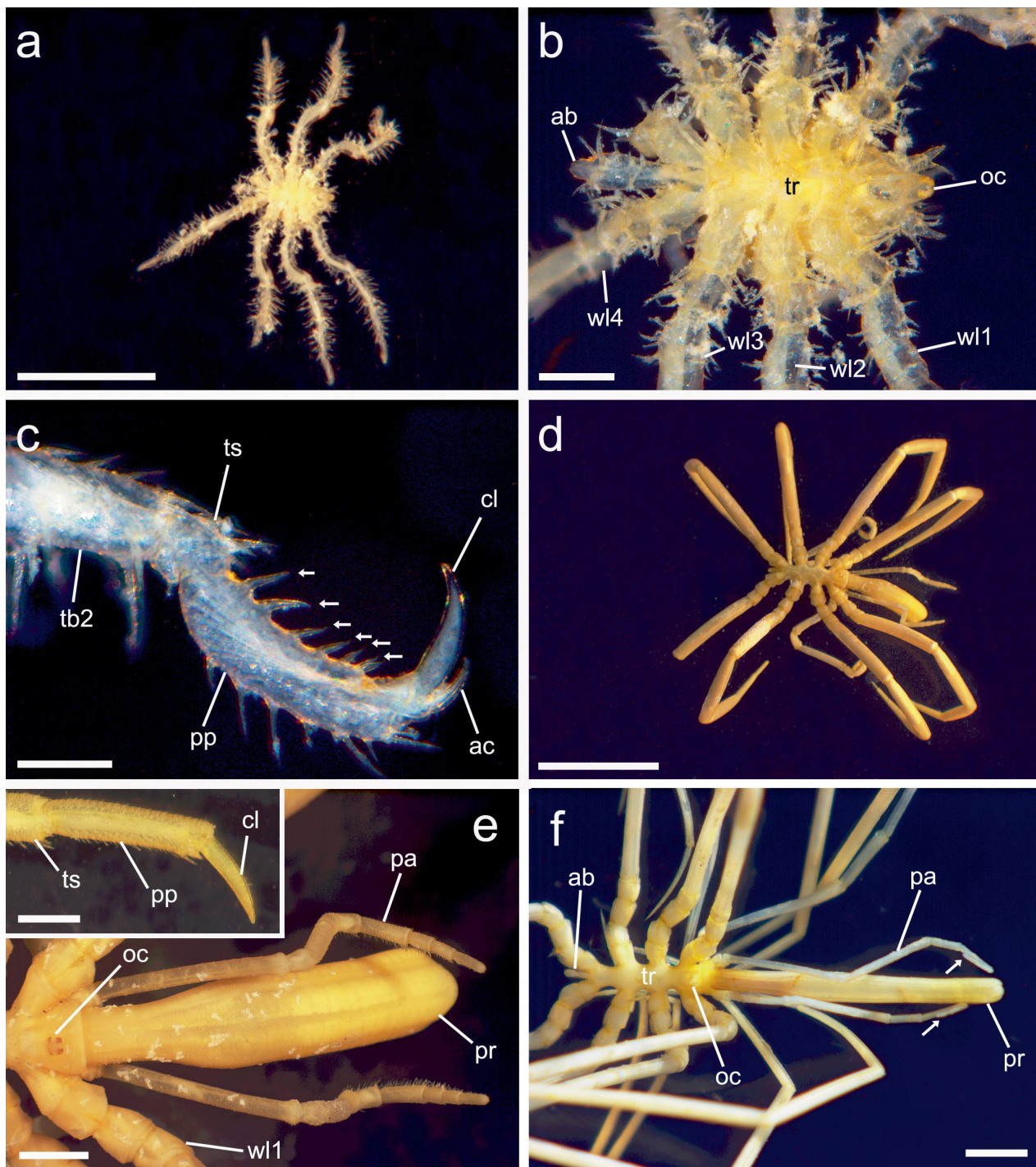
Figure 4. a–c. *Cilunculus cactoides*; **a.** Dorsal overview; scale = 3 mm; **b.** Dorsal view of trunk; scale = 500 μm; **c.** Tarsus and propodus with claw and auxiliary claws (right 2nd walking leg), note spines of inner margin of propodus (arrows); scale = 200 μm; **d–e.** *Colossendeis australis.* **d.** Dorsal overview; scale = 2 cm; **e.** Proboscis and palps; scale = 5 mm. Insert: Detail view of propodus and claw (right 4th walking leg); scale = 2 mm; **f.** *Colossendeis longirostris.* Dorsal view of trunk and proboscis, note recurved distal palp segments (arrows); scale = 3 mm. **ab** – abdomen; **ac** – auxiliary claw; **ce** – cephalon; **cl** – claw; **oc** – ocular tubercle; **pa** – palp; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **tr** – trunk; **ts** – tarsus; **wl** – walking leg.

tion: 252-1; Exp.: LAMPOS; 61°23.42′ S, 055°26.82′ W – 61°23.87′ S, 055°26.66′ W; AGT; 285.0–293.0 m. ZSMA20060824: 1 specimen; Antarctica; South Georgia, South Sandwich Islands; 09.04.2002; 16:25–16:45; FS Polarstern; Cruise: ANT XIX/5; Station: PS 61/164-1; Exp.: LAMPOS; 53°23.79′ S, 042°41.78′ W – 53°23.81′ W, 042°42.48′ W; AGT; 299.3–322.5 m. ZSMA20080516: 1 specimen; Antarctica, Weddell Sea; 22.12.2007; 10:10–11:22; FS Polarstern; Cruise: ANT XXIV/2; Station: PS 71/017-10; Exp.: ANDEEP-SYSTCO; 70°4.78′ S,

003°21.09′ W – 70°4.31′ S, 003°19.11′ W; AGT; 2084.7 – 2163.0 m. ZSMA20080517 – ZSMA20080518: each with 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–10:55; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.88′ S, 008°18.65′ W; AGT; 594.6–601.8 m. ZSMA20080521: 1 specimen; Antarctica, Weddell Sea; 04.01.2008; 01:45–01:56; FS Polarstern; Cruise: ANT XXIV/2; Exp.: ANDEEP-SYSTCO; 64°28.87′ S, 002°52.35′E – 64°28.79′ S, 002°52.74′E; AGT;

2150.4–2151.3 m. ZSMA20100172: 6 specimens; Antarctica; NW Antarctic Peninsula; 29.03.2005; 16:48–16:58; FS Polarstern; Cruise: PS 67; Station: 153-8; Exp.: ANT XXII/3; 63°19.21′ S, 064°37.07′ W − 63°19.10′ S, 064°37.13′ W; AGT; 2108.0–2124.0 m.

Remarks. According to the older literature, the palps are 9-jointed in this species (Loman 1923; Fry & Hedgpeth 1969), whereas in recent papers they are considered as "10-segmented" (Cano & López-González 2007). This is due to the small basal element, which is either identified as the first palp segment or not (Fig. 5b). Here, the palps are seen as 10-jointed in accordance with recent literature like (Fig. 5c) (e.g. Child 1995; Cano & Lopez-Gonzalez 2007).
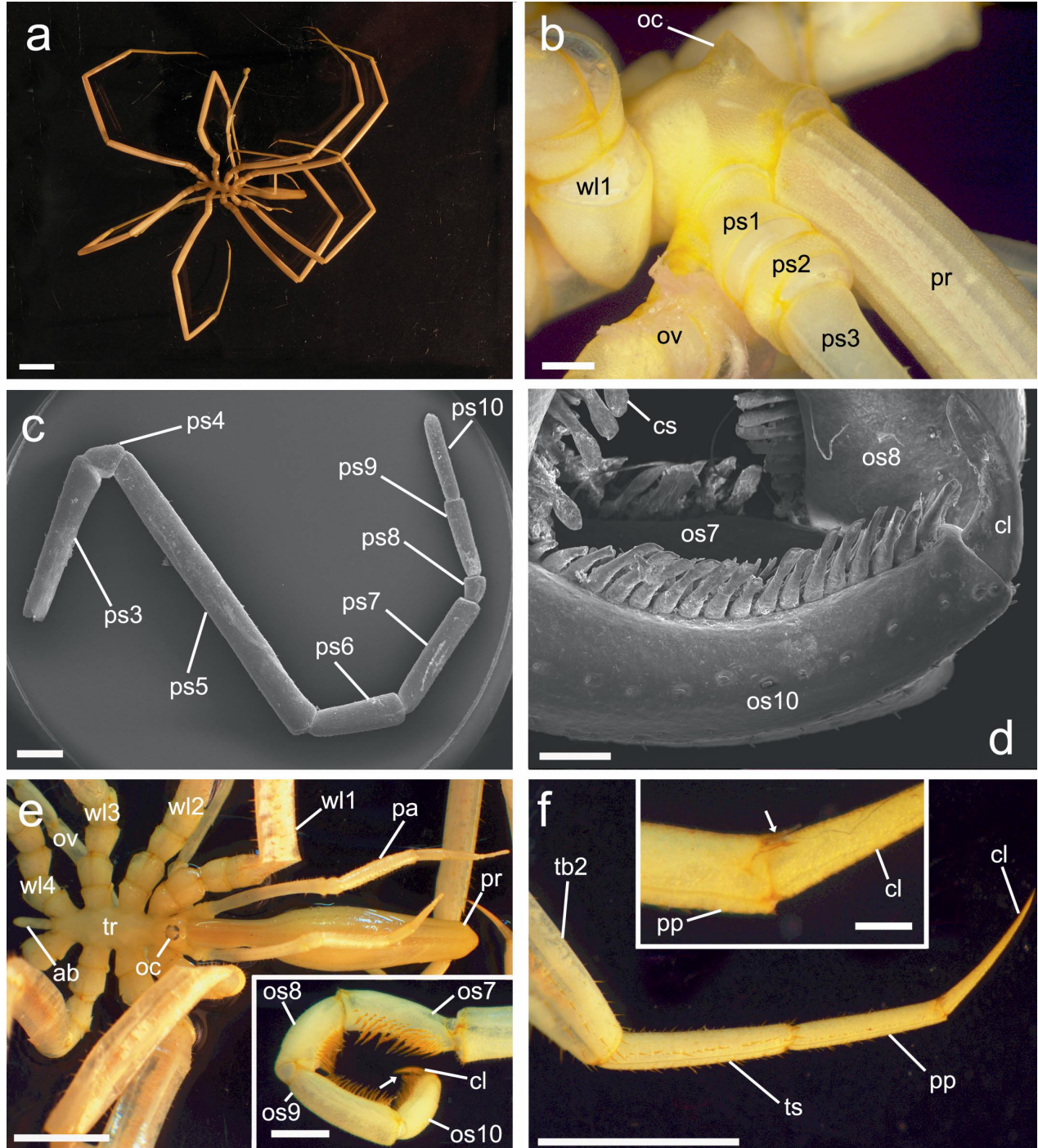
Figure 5. a−d. *Colossendeis megalonyx*; **a.** Dorsal overview; scale = 1 cm; **b.** Lateral view of ocular tubercle and first right palp segments; scale = 1 mm; **c.** Overview of right palp; scale = 1 mm; **d.** Distal oviger segment with terminal spatulate claw; scale = 200 μm; **e−f.** *Colossendeis tortipalpis*; **e.** Dorsal overview of trunk and proboscis; scale = 1 cm; Insert: Right distal oviger segments with compound spines, note subchelate claw (arrow); scale = 2 mm; **f.** Tarsus, propodus and claw of right 3rd walking leg; scale = 5 mm; Insert: Detail view of claw (right 3rd walking leg), note two spines on propodus (arrow); scale = 1 mm. **ab** − abdomen; **cl** − claw; **cs** − compound spine; **oc** − ocular tubercle; **os** − oviger segment; **ov** − oviger; **pa** − palp; **pp** − propodus; **pr** − proboscis; **ps** − palp segment; **tb** − tibia; **tr** − trunk; **ts** − tarsus; **wl** − walking leg.

In the examined individuals different developing stages of the ocular tubercle and eyes were noticed. According to Fry & Hedgpeth (1969), the eyes of *Colossendeis megalonyx* have variably developed ocular tubercles, but the shape of the ocular tubercle is always conical. In our material two individuals show a very low ocular tubercle with unpigmented eyes (Fig. 5b). In this context a detailed study on *C. megalonyx* by Krabbe et al. (2010) should be mentioned which deals with problematic taxonomic issues associated with this species name. This work presents five cryptic and one pseudocryptic mitochondrial lineages, suggesting that cryptic speciation occurred within the genus *Colossendeis*, but without 3 though without describing morphological correlates for the genetic lineages. Since the material of Krabbe et al. (2010) had been collected from Burdwood Bank, the South Sandwich Islands, Bouvet Island and the tip of the Antarctic Peninsula, the Weddell Sea specimens housed at ZSM represent a geographic complement. However, the specimens analysed here did not show any morphological discrepancies either. Slightly different ocular tubercle shapes do not seem to justify separation of our specimens from those described earlier.

Colossendeis tortipalpis Gordon, 1932
Figures 5e–f

Colossendeis tortipalpis Gordon, 1932: 12–15, figs. 2b–e, 3b, d, 4a

Material examined. ZSMA20080523: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–10:55; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.88′ S, 008°18.65′ W; AGT; 594.6–601.8 m

Remarks. This species can be distinguished from other representatives of its group by the last oviger segment being subchelate (Fig. 5e) (Child 1995). Another outstanding character is the anaxial articulation of the distal palp articles. There is only one other Antarctic species with tiny, recurved distal palp segments, namely *Colossendeis longirostris*. From this, *C. tortipalpis* can be distinguished by a larger, triangular eighth palp segment and a downcurved, medially more inflated proboscis (Child 1995). Additionally, according to Gordon (1932) the propodus measures about 1.4–1.7 times the length of the main claw. In our material, however, main claw and propodus are of almost equal size. Furthermore, Gordon described an occasional spine on tarsus and propodus, whereas the individual studied here shows two larger spines each on the distal ends of tarsus and propodus (Fig. 5f).

Nymphonidae
Nymphon Fabricius, 1794

Nymphon australe Hodgson, 1902
Figures 6a–d

Nymphon australe Hodgson, 1902: 257, pl. XL
Synonyms
Nymphon altioculatum Möbius, 1902

Chaetonymphon assimile Hodgson, 1908
Nymphon stylops Bouvier, 1911

Material examined. ZSMA20080556 – ZSMA20080570: each with 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m. ZSMA2010164: 1 specimen; Antarctica; Weddell Sea, between Vestkapp and Halley; 13.02.1996; 14:42–14:57; FS Polarstern; Cruise: Antarktis; Station: 39/11; Exp.: ANT XIII/3; 73°22.60′ S, 021°10.60′ W – 73°23.00′ S, 021°12.90′ W; GSN; 338 m

Remarks. Nymphon australe is the most frequently captured species in the Antarctic and exhibits a high level of morphological plasticity (Child 1995; Mahon et al. 2008). The *Nymphon australe* individuals examined here correspond well with the description by Gordon (1932) in the Discovery Reports. One of the most prominent characteristics is the much inflated 5th oviger segment in males (Fig. 6c), which is a typical feature in males of nymphonids (Arnaud & Bamber 1987). On the other hand, while Child's key (1995) gives trunk segments 3–4 as fused, all our examined individuals show distinct segment borders (Fig. 6b).

Nymphon biarticulatum (Hodgson, 1907)
Figures 6e–f

Nymphon biarticulatum Hodgson, 1907: 85, pl. IV, figs 2, 97, pl. X, fig. 12

Material examined. ZSMA2010088: 1 specimen; Antarctica, Weddell Sea; 31.03.2000; 19:09–19:39; FS Polarstern; Station: 65-1; 71°17.60′ S, 013°48.00′ W; GSN; 615.0–648.0 m

Remarks. This species is very closely related to *Nymphon brevicaudatum* Miers, 1875. Gordon (1932) even considered *N. biarticulatum* as "...only a more southern form of *Nymphon brevicaudatum*." However, the present species can be distinguished clearly by its much higher ocular tubercle and the 'club shaped' 5th oviger segment (Fig. 6f) (Child 1995).

Nymphon charcoti Bouvier, 1911
Figures 7a–c

Nymphon charcoti Bouvier, 1911: 1137

Material examined. ZSMA20042394: 1 specimen; Antarctica; Antarctic Peninsula, Bransfield Strait; 28.04.2000; 12:37–13:03; FS Polarstern; Cruise: ANT XVII/3; Station: 164-1; Exp.; EASIZ III; 63°4.90′ S, 059°32.90′ W – 63°4.70′ S, 059°32.70′ W; AGT; 858.0–859.0 m, ZSMA20080534 – ZSMA20080544: each with 1 specimen; Antarctica, Weddell Sea; 17.12.2007; 17:50–18:18; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/016-01; Exp.: ANDEEP-SYSTCO; 70°35.29′ S, 009°2.89′ W – 70°35.35′ S, 009°2.27′ W; Rauschert dredge; 486.3–488.4 m

Remarks. This species can be distinguished from other Antarctic *Nymphon* by the four terminal palp segments being subequal (Fig. 7b). Although *N. charcoti* can easily be confused with *N. unguiculatum* Hodgson, 1915, the latter species shows a more compact appearance with the lateral processes not as widely separated as in
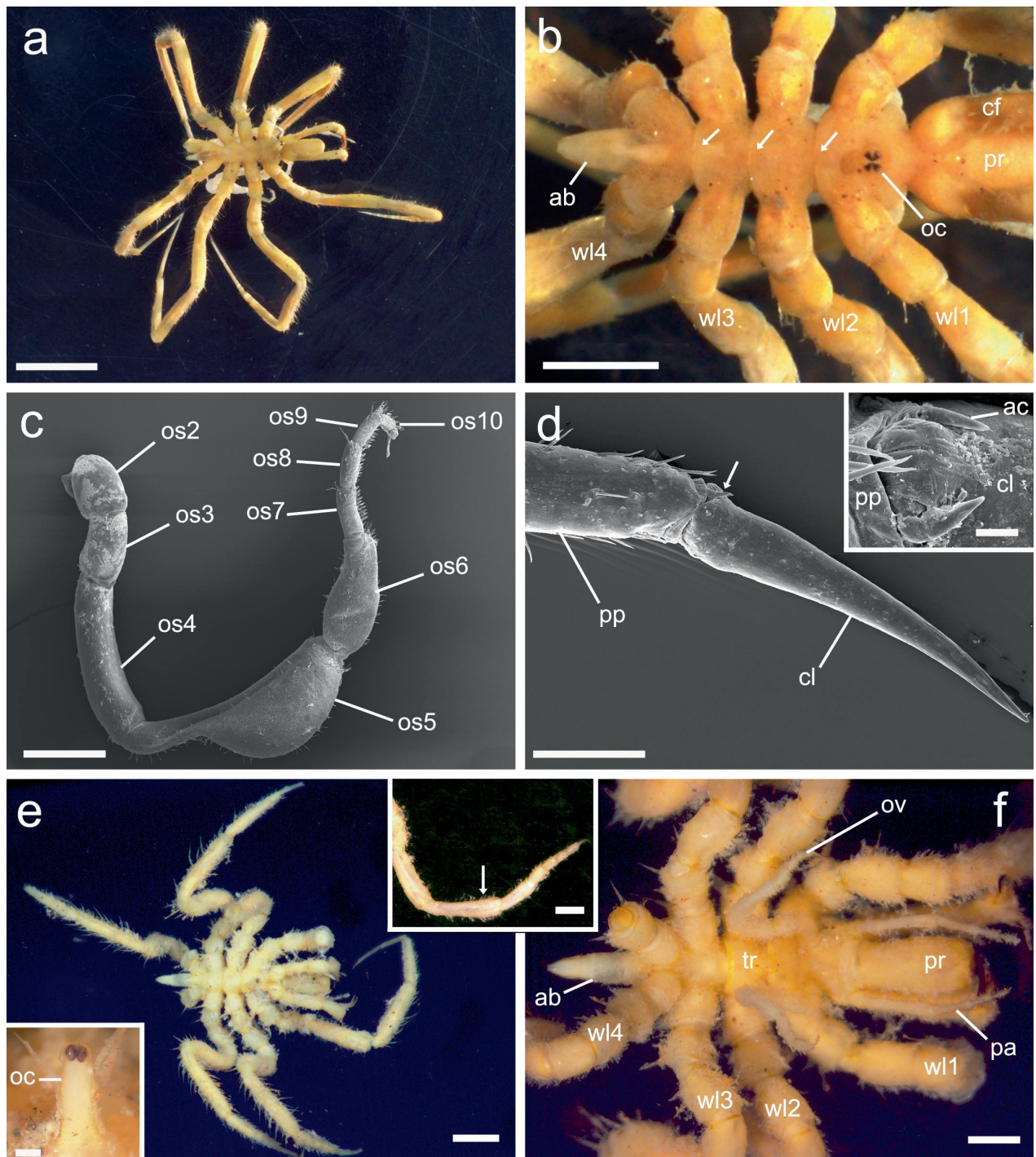
Figure 6. a–d *Nymphon australe*; **a.** Dorsal overview; scale = 5 mm; **b.** Dorsal view of trunk, note segment borders (arrows); scale = 200 μm; **c.** Male, overview of right oviger, note inflated oviger-segments 5 and 6; scale = 1 mm; **d.** Terminal claw of right 3rd walking leg with vestigial auxiliary claws (arrow); scale = 200 μm. Insert: Detail view of auxiliary claws; scale = 20 μm; **e–f.** *Nymphon biarticulatum*; **e.** Dorsal overview; scale = 2 mm. Insert lower left: Detail view of ocular tubercle; scale = 250 μm. Insert upper right: Right oviger with club shaped 5th oviger segment (arrow); scale = 500 μm. **f.** Ventral overview; scale = 1 mm. **ab** – abdomen; **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **oc** – ocular tubercle; **os** – oviger segment; **ov** – oviger; **pa** – palp; **pp** – propodus; **pr** – proboscis; **wl** – walking leg.

N. charcoti. This can be seen from figures published in PycnoBase (Bamber & Nagar 2011).

As mentioned in the literature, the lateral processes are separated by a considerable interval (Gordon 1932). According to Gordon, this interval is about 1.5 times the diameter of the lateral process, whereas some of the individuals studied here show a remarkable distance of up to 2.0 times the width of this process (Fig. 7a). Furthermore, the adult individual examined in this study shows conspicuous spines on the lateral processes. There are three such spines on lateral processes 1–2, and another two on lateral processes 3–4 (Fig. 7c). These spines have not been described in the literature.
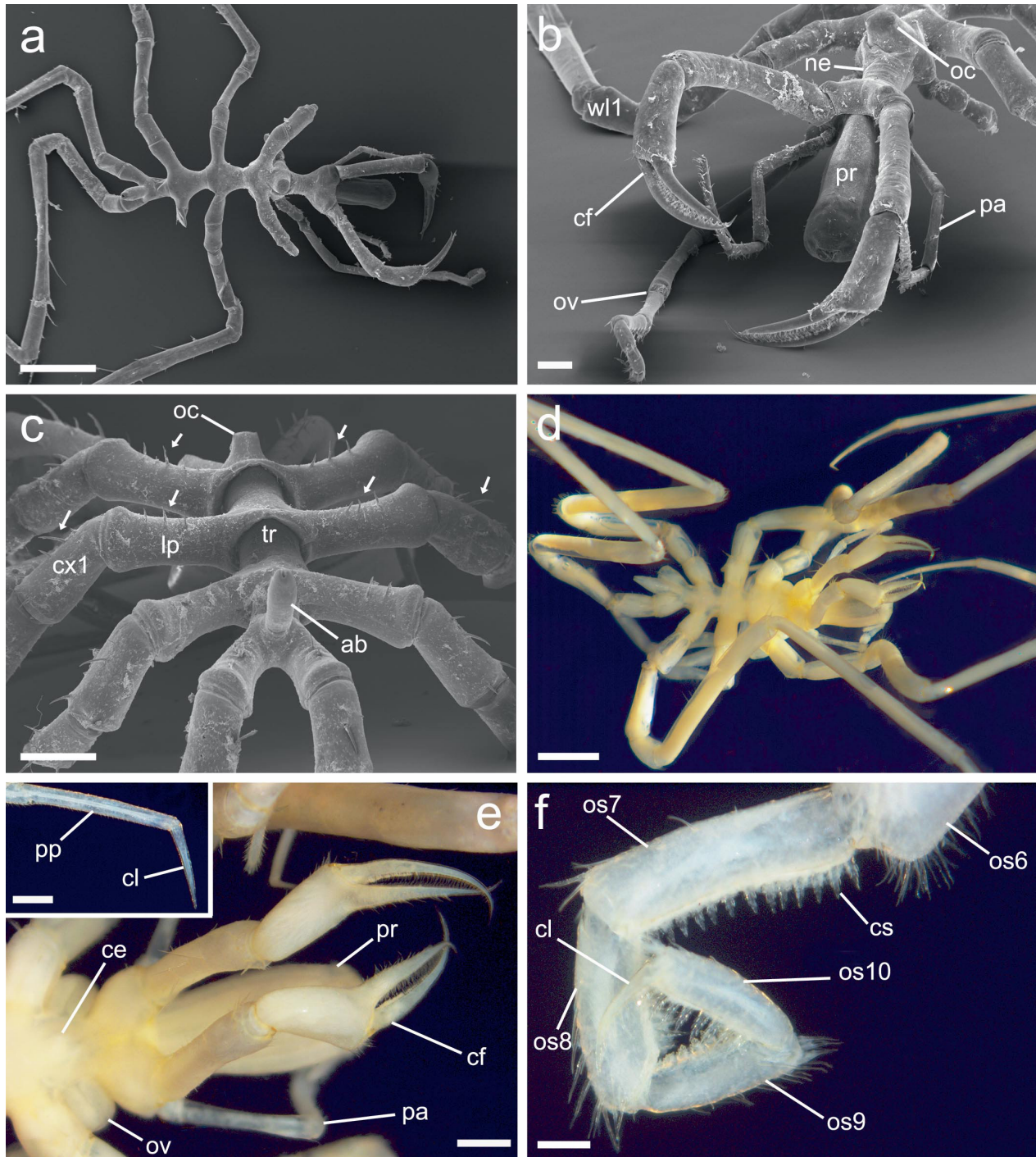
Nymphon compactum Hoek, 1881
Figures 7d–f

Nymphon compactum Hoek, 1881: 41–43, pl. II, figs. 6–8, pl. XV, fig. 10

Material examined. ZSMA20060797: 2 specimens; Antarctica; NW Weddell Sea, Powell Basin; 13.03.2005; 14:53–15:25; FS Polarstern;

Cruise: ANT XXII/3; Station: PS 67/121-7; Exp.: ANDEEP III; 63°35.66′ S, 050°42.86′ W – 63°34.65′ S, 050°41.68′ W; AGT; 2617.0–2618.0 m

Remarks. The short neck, absence of an ocular tubercle and the long main claw missing auxiliary claws serve to differentiate *N. compactum* from other *Nymphon* species (Fig. 7e). As mentioned in Child (1995), trunk and

Figure 7. a–c. *Nymphon charcoti*; **a.** Dorsal overview; scale = 1 mm; **b.** Frontal view; scale = 200 µm; **c.** Dorsal view of trunk, note spines on lateral processes and first coxae (arrows); scale = 1 mm; **d–f.** *Nymphon compactum*; **d.** Dorsal overview; scale = 3 mm; **e.** Dorsal view of cephalon and chelifores; scale = 1 mm; Insert: Detail view of propodus and claw (left 2nd walking leg); scale = 500 µm; **f.** Distal segments of left oviger; scale = 250 µm. **ab** – abdomen; **ce** – cephalon; **cf** – chelifore; **cl** – claw; **cs** – compound spine; **lp** – lateral process; **ne** – neck; **oc** – ocular tubercle; **os** – oviger segment; **ov** – oviger; **pa** – palp; **pr** – proboscis; **tr** – trunk; **wl** – walking leg.

lateral processes are glabrous and the chela bears 40–48 teeth on each finger.

Nymphon compactum was previously recorded from the Scotia Sea, South Africa and New Zealand (East of Auckland) (Müller 1993; Munilla & Soler-Membrives 2008). Here, we present the first record for the Weddell Sea.

Nymphon eltaninae Child, 1995
Figures 8a–b

Nymphon eltaninae Child, 1995: 14–16, fig. 2

Material examined. ZSMA20042385, ZSMA20042387 – ZSMA20042389, ZSMA20042391 – ZSMA20042393: each with 1 specimen; Antarctica; Antarctic Peninsula, Bransfield Strait; 28.04.2000; 12:37–13:03; FS Polarstern; Cruise: ANT XVII/3; Station: 164-1; Exp.; EASIZ III; 63°4.90′ S, 059°32.90′ W – 63°4.70′ S, 059°32.70′ W; AGT; 858.0–859.0 m. ZSMA20100173: 1 specimen; Antarctica; SW South Orkney Islands; 20.03.2005; 21:50–22:20; FS Polarstern; Cruise: PS 67; Station: 151-1; Exp.: ANT XXII/3; 61°45.51′ S, 047°7.49′ W – 61°45.31′ S, 047°7.84′ W; AGT; 1179.0–1187.0 m

Remarks. The original description of this species by Child (1995) is matched well by the examined specimens. The most outstanding characteristic are the lateral processes bearing many long, curved spines (Fig. 8a). On the other hand, there are some differences. According to Child (1995), the 3rd palp segment is longer than the 2nd and the oviger bears 33 denticulate spines. In our specimens, the 2nd palp segment is always longer than the 3rd (Fig. 8b) and the number of denticulate spines on the distal four oviger segments ranged between 23 and 30, but never reached 33. Also, our animals do not show a teardrop-shaped ocular tubercle as mentioned by Child (1995), because there is neither a basal constriction nor a pointed apex in lateral view.

Nymphon eltaninae was previously recorded from the Scotia Sea and the Ross Sea (Child 1995; Munilla & Soler-Membrives 2008). Here we present two further localities at which this species occurs, namely the Antarctic Peninsula and the South Orkney Islands.

Nymphon longicollum Hoek, 1881
Figures 8c–d

Nymphon longicollum Hoek, 1881; 40–41, pl. II, figs 1–5, pl. XV, figs 8–9

Material examined. ZSMA20060830: 1 specimen; Antarctica; NW-Weddell Sea, Powell Basin; 20.03.2005; 23:17–01:00; FS Polarstern; Cruise: ANT XXII/3; Station: PS 67/151-2; Exp.: ANDEEP III; 61°45.56′ S, 047°7.41′ W – 61°45.64′ S, 047°7.99′ W; AGT; 1179.0–1186.0 m

Remarks. The slight gap between the oviger and the first lateral process as well as the very broad-based ocular tubercle lacking eyes allow identification of this species as *Nymphon longicollum* (Figs 8c–d). While Child (1995) described the ocular tubercle in this species as tall, the ocular tubercle of the individual examined here appears as rather low.

Nymphon longicoxa Hoek, 1881
Figures 8e–f

Nymphon longicoxa Hoek, 1881: 38–39, pl. 2, figs 1–5, pl. XV, figs 8–9

Material examined. ZSMA20060796: 1 specimen; Antarctica; NW Weddell Sea, Powell Basin; 13.03.2005; 14:53–15:25; FS Polarstern; Cruise: ANT XXII/3; Station: PS 67/121-7; Exp.: ANDEEP III; 63°35.66′ S, 050°42.86′ W – 63°34.65′ S, 050°41.68′ W; AGT; 2617.0–2618.0 m. ZSMA20060826: 1 specimen; Antarctica; Weddell Sea, Kapp Norvegia; 20.02.2005; 17:32–17:50; FS Polarstern; Cruise: ANT XXII/3; Station: PS 67/074-7; Exp.: ANDEEP III; 71°18.60′ S, 013°59.11′ W – 71°18.40′ S, 013°58.14′ W; AGT; 1047.0–1066.0 m. ZSMA20060827: 1 specimen; Antarctica; E Weddell Sea; 20.02.2005; 15:50–16:08; FS Polarstern; Cruise: ANT XXII/3; Station: PS 67/074-6; Exp.: ANDEEP III; 71°18.42′ S, 013°58.22′ W – 71°18.28′ S, 013°57.31′ W; EBS; 1040.0–1048.0 m. ZSMA20100162: 1 specimen; Antarctica; N Carney Island; 28.02.1994; 21:06–22:30; FS Polarstern; Cruise: Antarktis; Station: 29/057; Exp.: ANT XI/3; 73°10.80′ S, 121°54.20′ W; AGT; 627.0 m

Remarks. This species has an extended, curled chela tip on the movable finger which is unlike any other species (Fig. 8f). Child (1982) compared its shape to that of a "pig's tail". Another important diagnostic character is the separation of the oviger implantation from the first lateral processes (Gordon 1932).

Nymphon longicoxa was previously recorded from the Scotia Sea, Bellingshausen Sea, Ross Sea, Rio de la Plata and from southeast of New Zealand (Müller 1993; Munilla & Soler-Membrives 2008). Here, we present the first record from the Weddell Sea.

Nymphon mendosum (Hodgson, 1907)
Figure 9a

Nymphon mendosum Hodgson, 1907: 30–32, 85, pl. IV, figs 3, 97, pl. X, fig. 13

Material examined. ZSMA20071581: 1 specimen; Antarctica; 03.04.2000; 15:37–15:56; FS Polarstern; Cruise: ANT XVII/3; Station: PS 56/102–1; Exp.: EASIZ III; 71°11.90′ S, 12°21.70′ W – 71°11.44′ S, 12°19.20′ W; GSN; 312–323 m ZSMA20100232: 1 specimen; Antarctica, Weddell Sea, N Kapp Norvegia; 02.02.1998; FS Polarstern; Cruise: ANT XV/3; Station: PS 48–77; Exp.: EASIZ II; 71°09.7′ S, 12°28.7′ W – 71°09.9′ S, 12°29.2′ W; AGT; 341.0–360.0 m

Remarks. The size of the laterodistal spines and their arrangement in *Nymphon mendosum* are unlike those in any other species (Child 1995). At first sight this species might be mistaken for *N. proximum*, but it differs in having the lateral processes much more separated, a longer abdomen and a glabrous trunk (Fig. 9a) (Child 1995).

Nymphon proceroides Bouvier, 1913
Figures 9b–c

Nymphon proceroides Bouvier, 1913: 90–94, figs 42–48

Material examined. ZSMA20010085: 1 specimen; Antarctica; Weddell Sea; 26.04.2000; 17:25–17:35; FS Polarstern; Station: 159–1; 62°55,00′ S, 57°39,50′ W; AGT; 214–218 m
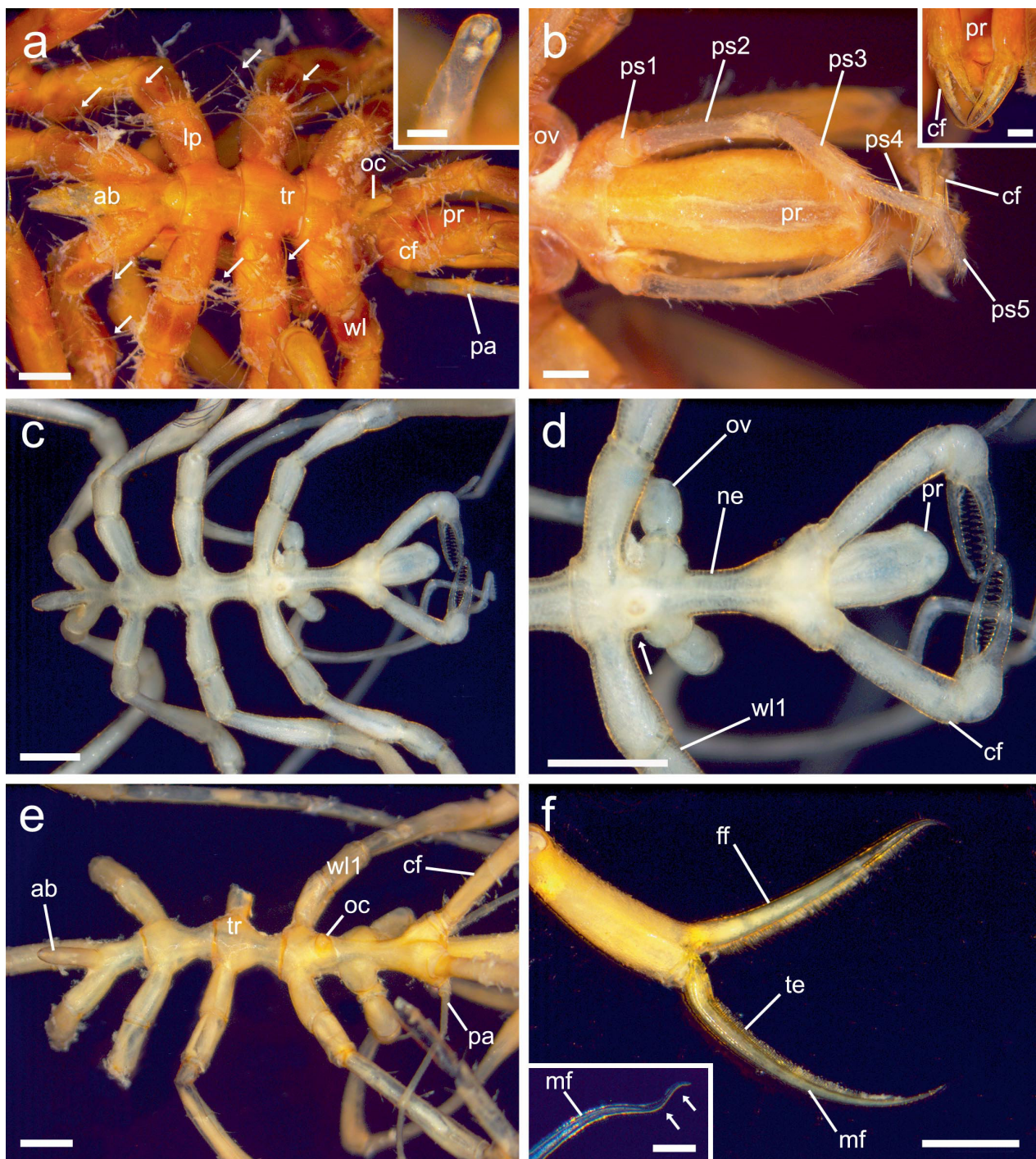
Figure 8. a–b. *Nymphon eltaninae*; **a.** Dorsal view of trunk, note long curved spines on lateral processes (arrows); scale = 1 mm. Insert: Detail view of ocular tubercle; scale = 200 μm; **b.** Ventral view of palps; scale = 500 μm. Insert: Detail view of chelifores; scale = 500 μm; **c–d.** *Nymphon longicollum*; **c.** Dorsal overview; scale = 1 mm; **d.** Dorsal view of cephalon, note gap between first pair of walking leg and oviger (arrow); scale = 1 mm; **e–f.** *Nymphon longicoxa*; **e.** Dorsal overview; scale = 1 mm; **f.** Left chelifore; scale = 1 mm. Insert: Detail view of tip of movable finger (right chelifore), note recurled chela tip (arrows); scale = 100 μm. **ab** – abdomen; **cf** – chelifore; **cx** – coxa; **ff** – fixed finger; **lp** – lateral process; **mf** – movable finger; **ne** – neck; **oc** – ocular tubercle; **ov** – oviger; **pa** – palp; **pr** – proboscis; **ps** – palp segment; **te** – teeth; **tr** – trunk; **wl** – walking leg.

Remarks. The most prominent characters which distinguish this species are the well separated lateral processes and the small number of teeth on the chela fingers (Figs 9b–c). Furthermore, the anterior body appendages appear very small in relation to the remainder of the species' habitus (see Child 1995).

While Child (1995) counted only 3–4 spinules on the oviger claw, the specimens examined here correspond better with the description of Gordon (1932), who mentioned 4–7 spinules on the terminal oviger claw.

This is the first record of *Nymphon proceroides* from the Weddell Sea. Previously mentioned local-
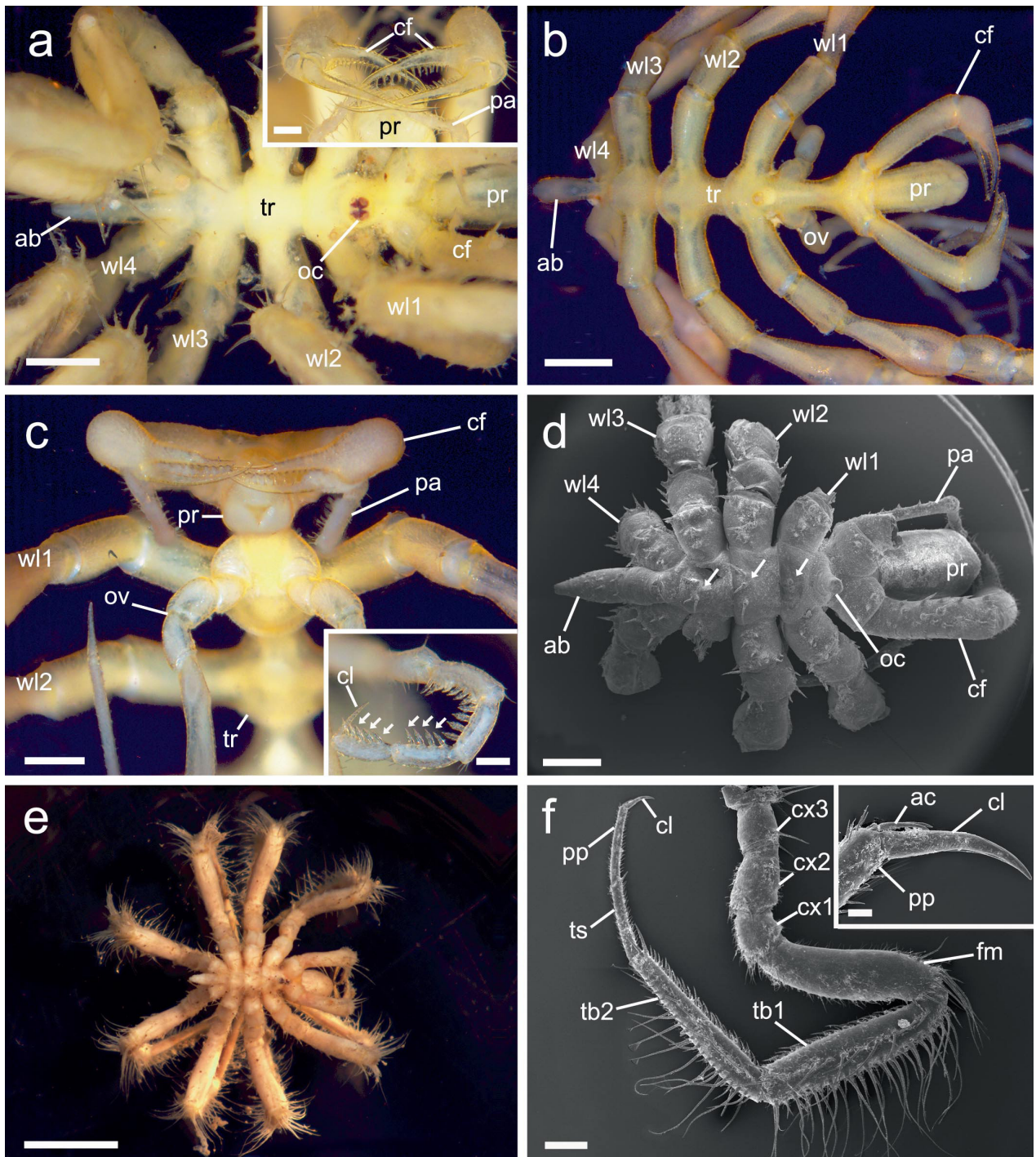
Figure 9. a. *Nymphon mendosum*; Dorsal view of trunk; scale = 1 mm. Insert: Detail view of chelifores; scale = 250 μm; **b**–**c.** *Nymphon proceroides*; **b.** Dorsal overview; scale = 1 mm; **c.** Frontal view; scale = 500 μm. Insert: Distal segments of right oviger, note compound spines (arrows); scale = 200 μm; **d.** *Nymphon proximum*; Dorsal overview, note spines on trunk (arrows); scale = 1 mm; **e**–**f.** *Nymphon villosum*; **e.** Dorsal overview; scale = 500 μm; **f.** Overview of right 3rd walking leg; scale = 1 mm. Insert: Detail view of claw and auxiliary claws; scale = 100 μm. **ab** – abdomen; **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cx** – coxa; **fm** – femur; **ne** – neck; **oc** – ocular tubercle; **ov** – oviger; **pa** – palp; **pp** – propodus; **pr** proboscis; **tb** – tibia; **tr** – trunk; **ts** – tarsus; **wl** – walking leg.

ities are the Scotia Sea, Antarctic Peninsula, east Antarctic zone, Palmer Archipelago and South Shetland Islands (Müller 1993; Munilla & Soler-Membrives 2008).

Nymphon proximum Calman, 1915

Figure 9d

Nymphon proximum Calman, 1915: 34–36, fig. 6

Material examined. ZSMA20091355, ZSMA20091356: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern;

Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYST-CO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597–601.8 m; ZSMA20091379, ZSMA20091380: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m; ZSMA20091381, ZSMA20091382: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m; ZSMA20080545–ZSMA20080550, ZSMA20080552, ZSMA20080553, ZSMA20080555: each with 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m

Remarks. In comparison to the closely related *Nymphon mendosum* (see above), the present species can be recognized by its closely set lateral processes, the moderately short abdomen and the conspicuous spines on the trunk (Fig. 9d) (Child 1995).

Nymphon villosum (Hodgson, 1907)
Figures 9e–f

Chaetonymphon villosum Hodgson, 1907: 26–28, 85, pl. IV, figs 1, 97, pl. X, fig. 11

Synonym. Chaetonymphon villosum Hodgson, 1907

Material examined. ZSMA20080525: 1 specimen; Antarctica, Weddell Sea; 17.12.2007; 17:50–18:18; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/016-01; Exp.: ANDEEP-SYSTCO; 70°35.29′ S, 009°2.89′ W – 70°35.35′ S, 009°2.27′ W; Rauschert dredge; 486.3–488.4 m; ZSMA20080527 – ZSMA20080530: each with 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m

Remarks. This species differs from most other nymphonids by its hairy appearance and its thickly set trunk. The tibiae are covered with extremely long and conspicuous setae with a well developed basal ring and a distal hair shaft (Figs 9e–f) (Gordon 1932).

Pentanymphon Hodgson, 1904

Pentanymphon antarcticum Hodgson, 1904
Figures 10a–b

Pentanymphon antarcticum Hodgson, 1904: 459, pl. XIV

Synonym. Pentanymphon minutum Gordon, 1944

Material examined. ZSMA20080531: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–10:55; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.88′ S, 008°18.65′ W; Rauschert dredge; 594.6–601.8 m; ZSMA20080532: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m; ZSMA20080533: 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–10:55; FS Polarstern; Cruise: ANT XXIV/2;

Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.88′ S, 008°18.65′ W; AGT; 594.6–601.8 m; ZSMA20100226: 1 specimen; Antarctica, Weddell Sea, N Kapp Norvegia; 02.02.1998; FS Polarstern; Cruise: ANT XV/3; Station: PS 48–77; Exp.: EASIZ II; 71°09.7′ S, 12°28.7′ W – 71°09.9′ S, 12°29.2′ W; AGT; 341.0–360.0 m

Remarks. The present material corresponds well with the descriptions in the literature (Gordon 1932, Child 1995). The species can be recognized by its five pairs of walking legs (Figs 10a–b).

Callipallenidae
Austropallene Hodgson, 1915

Austropallene cornigera (Möbius, 1902)
Figures 10c–d

Pseudopallene cornigera Möbius, 1902: 186, pl. XXVII, figs 14–20

Synonyms
Pseudopallene australis Hodgson, 1907
Pseudopallene cornigera Möbius, 1902
Cordylochele turqueti Bouvier, 1905

Material examined. ZSMA2010086: 1 specimen; Antarctica, Weddell Sea; 26.04.2000; 14:42–14:57; FS Polarstern; Station: 158-1; 63°4.70′ S, 057°31.60′ W; AGT; 94.0–95.0 m. ZSMA20071578: 1 specimen (juvenile); Antarctica; Antarctic Peninsula; 26.04.2000; 14:42–14:57; FS Polarstern; Cruise: ANT XVII/3; Station: PS 56/158-1; Exp.: EASIZ III; 63°4.70′ S, 057°31.60′ W – 63°4.50′ S, 057°32.00′ W; AGT; 94.0–95.0 m; ZSMA20080571: 1 specimen; Antarctica, Weddell Sea; 17.12.2007; 17:50–18:18; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/016-01; Exp.: ANDEEP-SYST-CO; 70°35.29′ S, 009°2.89′ W – 70°35.35′ S, 009°2.27′ W; Rauschert dredge; 486.3–488.4 m; ZSMA20080572 – ZSMA20080576: each with 1 specimen; Antarctica, Weddell Sea; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°18.68′ W; AGT; 597.0–601.8 m; ZSMA20100229: 1 specimen; Antarctica, N Antarctic Peninsula, Drake Passage, South Orkney Islands; 23.04.2002; FS Polarstern; Cruise: ANT XIX/5; Station: PS 61/238-1; Exp.: LAMPOS; 61°10.82′ S, 45°42.78′ W – 61°10.48′ S, 45°42.03′ W; 322.0–324.0 m.

Remarks. In this study it was possible to take SEM pictures of the tuft of hairs surrounding the mouth which had been mentioned in the literature as a "Borstenkranz" (Helfer & Schlottke 1935) (Fig. 10d) and seems to be unique in the genus *Austropallene*. Although the description of Gordon (1932) generally corresponds well with the SEM images, the absence of any reference to such a distinguishing mark as the hair tuft is quite notable.

Austropallene gracilipes Gordon, 1944
Figures 10e–f

Austropallene gracilipes Gordon, 1944: 39–41, figs 10a, 11a, 11c, 14c–d

Material examined. ZSMA20100231: 1 specimen; Antarctica, Weddell Sea, N Kapp Norvegia; 02.02.1998; FS Polarstern; Cruise: ANT XV/
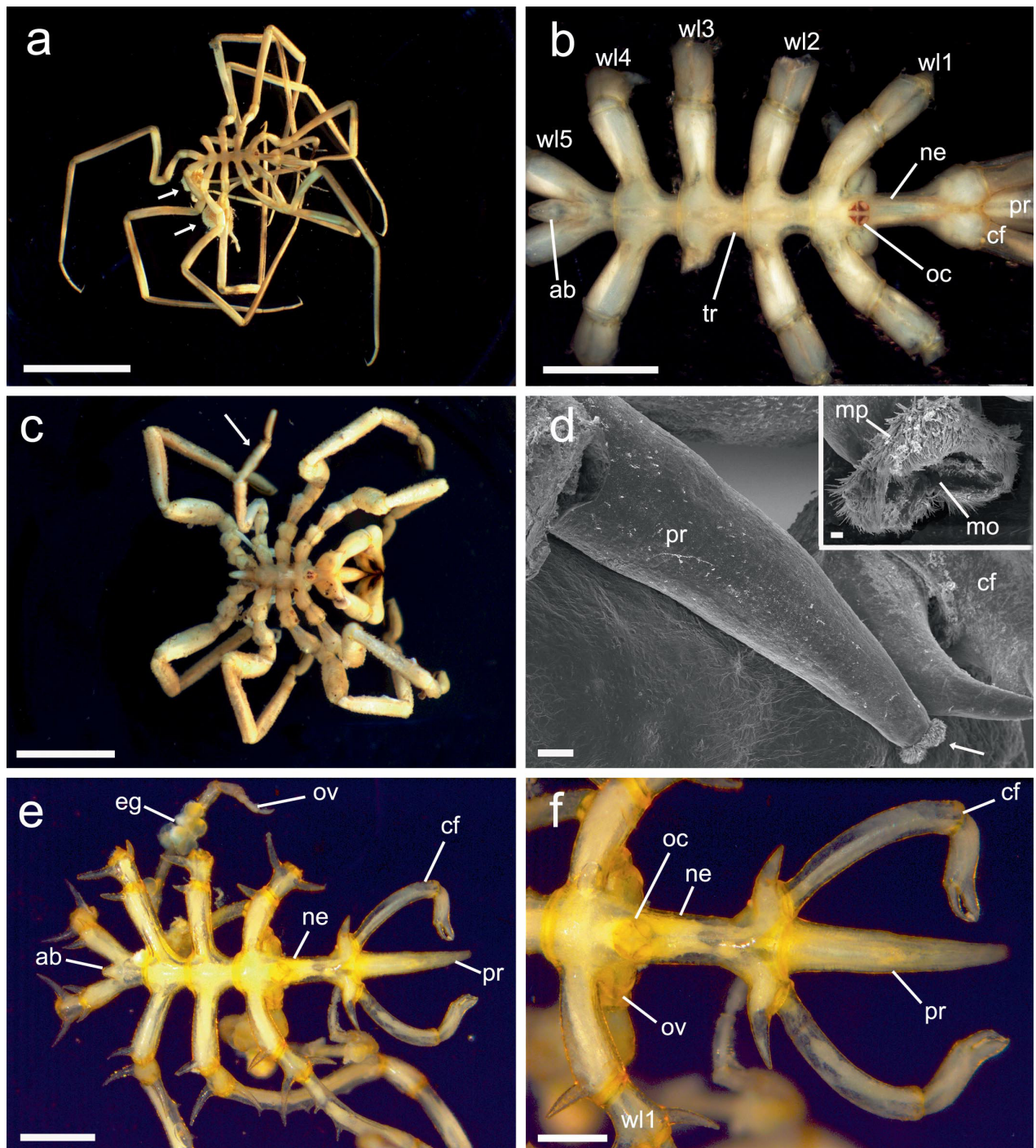
Figure 10. a–b. *Pentanymphon antarcticum*; **a.** Dorsal overview, note eggs (arrows); scale = 1 mm; **b.** Dorsal view of trunk, note five pairs of walking legs; scale = 1 cm; **c–d.** *Austropallene cornigera*; **c.** Dorsal overview, note smaller left 3rd walking leg (arrow); scale = 5 mm; **d.** Lateral view of proboscis, note prominent mouth protrusion; scale = 200 µm. Insert: Close up of mouth opening; scale = 20 µm; **e–f.** *Austropallene gracilipes*; **e.** Dorsal overview; scale = 1 mm; **f.** Detail view of cephalon; scale = 500 µm. **ab** – abdomen; **cf** – chelifore; **eg** – eggs; **mo** – mouth opening; **mp** – mouth protrusion; **ne** – neck; **oc** – ocular tubercle; **ov** – oviger; **pr** – proboscis; **tr** – trunk; **wl** – walking leg.

3; Station: PS 48–77; Exp.: EASIZ II; 71°09.7′ S, 12°28.7′ W – 71°09.9′ S, 12°29.2′ W; AGT; 341.0–360.0 m

Remarks. This species can be distinguished from other members of *Austropallene* by its slender build, the long neck and the presence of two spurs on each lateral process (Figs 10e–f). The unusually long and slender walking legs also are a characteristic feature (Gordon 1944).
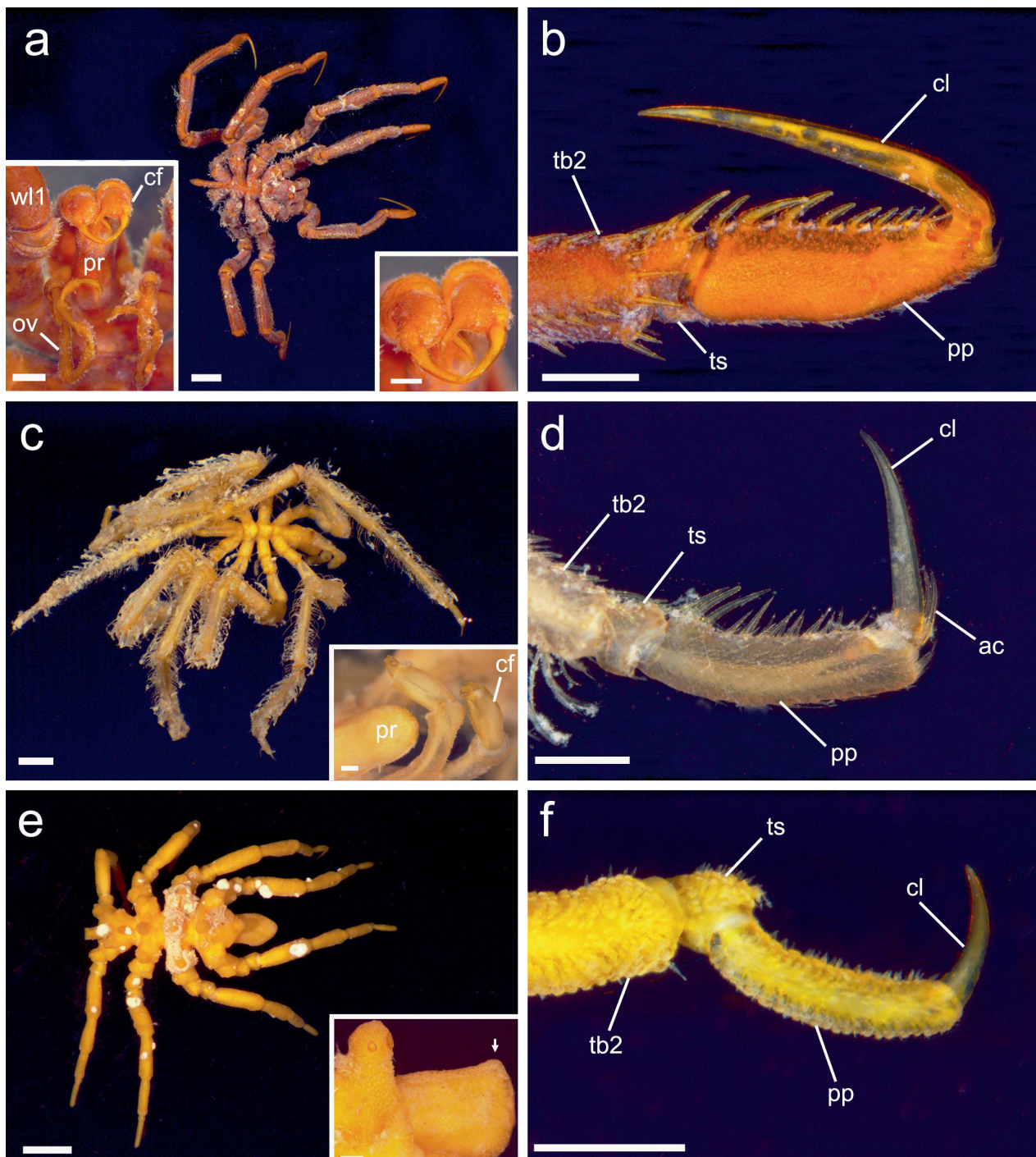
Pallenopsidae
Bathypallenopsis **Pushkin, 1993**

***Bathypallenopsis macronyx* (Bouvier, 1911)**
Figures 11a–b

Pallenopsis macronyx Bouvier, 1911: 1139
Synonym. Pallenopsis knipovichi Turpaeva, 1974

Figure 11. a–b. *Bathypallenopsis macronyx*; **a.** Dorsal overview; scale = 3 mm. Insert left: Detail view of ovigers and chelifores; scale = 1 mm. Insert right: Close up of chelifores; scale = 500 µm; **b.** Tarsus and propodus with claw (right 4th walking leg); scale = 1 mm; **c–d.** *Pallenopsis hodgsoni*; **c.** Dorsal overview; scale = 3 mm. Insert: Detail view of chelifores; scale = 500 µm. **d.** Tarsus and propodus with claw and auxiliary claws of right 2nd walking leg; scale = 1 mm; **e–f.** *Pycnogonum gaini*; **e.** Dorsal overview; scale = 3 mm. Insert: Lateral view of proboscis, note distal hump (arrow); scale = 500 µm; **f.** Tarsus and propodus with claw of right 3rd walking leg; scale = 1 mm. **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **ov** – oviger; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **ts** – tarsus; **wl** – walking leg.

Material examined. ZSMA20042386, ZSMA20042390: each with 1 specimen; Antarctica; Antarctic Peninsula, Bransfield Strait; 28.04.2000; 12:37–13:03; FS Polarstern; Cruise: ANT XVII/3; Station: 164-1; Exp.; EASIZ III; 63°4.90′ S, 059°32.90′ W – 63°4.70′ S, 059°32.70′ W; AGT; 858.0–859.0 m.

Remarks. The examined specimens differ from other representatives of the genus *Pallenopsis* by the short pro-

boscis and the lateral palp buds which appear as rather long and slender compared to other species (Child 1995). Furthermore, as described by Child (1995), the trunk, lateral processes and legs are covered with many plain and short setae (Fig. 11a).

Beyond that, two peculiar features must be pointed out that are of high taxonomic relevance. The first are

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the atypical chelae. Whereas in related species, e.g. in *Pallenopsis lateralia* Child, 1995 and *P. villosa* Hodgson, 1907, the fingers are placed laterally to the palm, in *P. macronyx* the fingers are carried as longitudinal extensions of the palm (Fig. 11a). The second peculiarity is the absence of auxiliary claws, as shown in Figure 11b.

Remarkably, these two features do not fit with those of representatives of the subgenus *Pallenopsis*, but instead correspond to what has been described as diagnostic for *Bathypallenopsis*. Originally introduced as a subgenus by Stock (1975), it was raised to genus rank by Pushkin (1993), who also transferred *Pallenopsis macronyx* to *Bathypallenopsis*. In spite of the latter, recent studies carried the species in the preceding way, as *Pallenopsis* (*P.*) *macronyx* (Child 1995; Munilla 2008; Bamber & Nagar 2011). However, the articulation of the chelae as well as the absence of auxiliary claws – which are conspicuous features of our specimens, too – have convinced us of following Pushkin's interpretation here.

Pallenopsis Wilson, 1881

Pallenopsis hodgsoni Gordon, 1938
Figures 11c–d

Pallenopsis hodgsoni Gordon, 1938: 16–17, figs 3a, 4d, 5d

Material examined. ZSMA20100163: 1 specimen; Antarctica; N Carney Island; 28.02.1994; 21:06–22:30; FS Polarstern; Cruise: Antarktis; Station: 29/057; Exp.: ANT XI/3; 73°10.80′ S, 121°54.20′ W; AGT; 627.0 m.

Remarks. This species corresponds well with the descriptions given by Gordon (1938) and Child (1995). Only the auxiliary claws show well visible differences. According to Child the auxiliaries are half the length of the main claw, but in the specimen examined here they are significantly shorter (Fig. 11d), more in agreement with the drawings by Munilla (1991).

Pycnogonidae
Pycnogonum Bruennich, 1764

Pycnogonum gaini Bouvier, 1910
Figures 11e–f

Pycnogonum gaini Bouvier, 1910: 30

Material examined. ZSMA20100171: 1 specimen; Antarctica; Weddell Sea; 05.01.2004; 15:21–15:31; FS Polarstern; Cruise: PS 65; Station; 336-1; Exp.: ANT XXI/2; 70°50.70′ S, 010°28.32′ W – 70°50.75′ S, 010°28.01′ W; AGT; 276.0–281.0 m.

Remarks. Following the key given in Child (1995), the defining difference between *Pycnogonum gaini* and the similar *P. diceros* is the presence of two dorsal tubercles on the proboscis in the latter species. The specimen examined here shows a single distal tubercle on the proboscis (Fig. 11e). *Pentapycnon charcoti* "lacks the more proximal of the two proboscis tubercles" (Child 1995)

but it differs from the examined specimen in the presence of five pairs of walking legs. Hence, our observation corresponds well with the description of Gordon (1944), that "the proboscis is enlarged distally and possesses a dorsal hump near the apex."

Rhynchothoracidae
Rhynchothorax Costa, 1861

Rhynchothorax australis Hodgson, 1907
Figures 12a–b

Rhynchothorax australis Hodgson, 1907: 57–58, 93, pl. VIII, fig. 3

Material examined. ZSMA20100167: 1 specimen; Antarctica; Antarctic Peninsula; 26.04.2000; 14:42–14:57; FS Polarstern; Cruise: PS 56; Station: 158-1; Exp.: EASIZ III; 63°4.70′ S, 057°31.60′ W – 63°4.50′ S, 057°32.00′ W; AGT; 94.0–95.0 m.

Remarks. This specimen corresponds well with the description given by Child (1995). It can be distinguished from other *Rhynchothorax* species by the arrangement of tubercles on the lateral processes and the first coxae. While *R. percivali* Clark, 1976 shows tubercles on both, the lateral processes and the first coxae, *R. australis* has tubercles on the first coxae, but none on the lateral processes (Figs 12a–b).
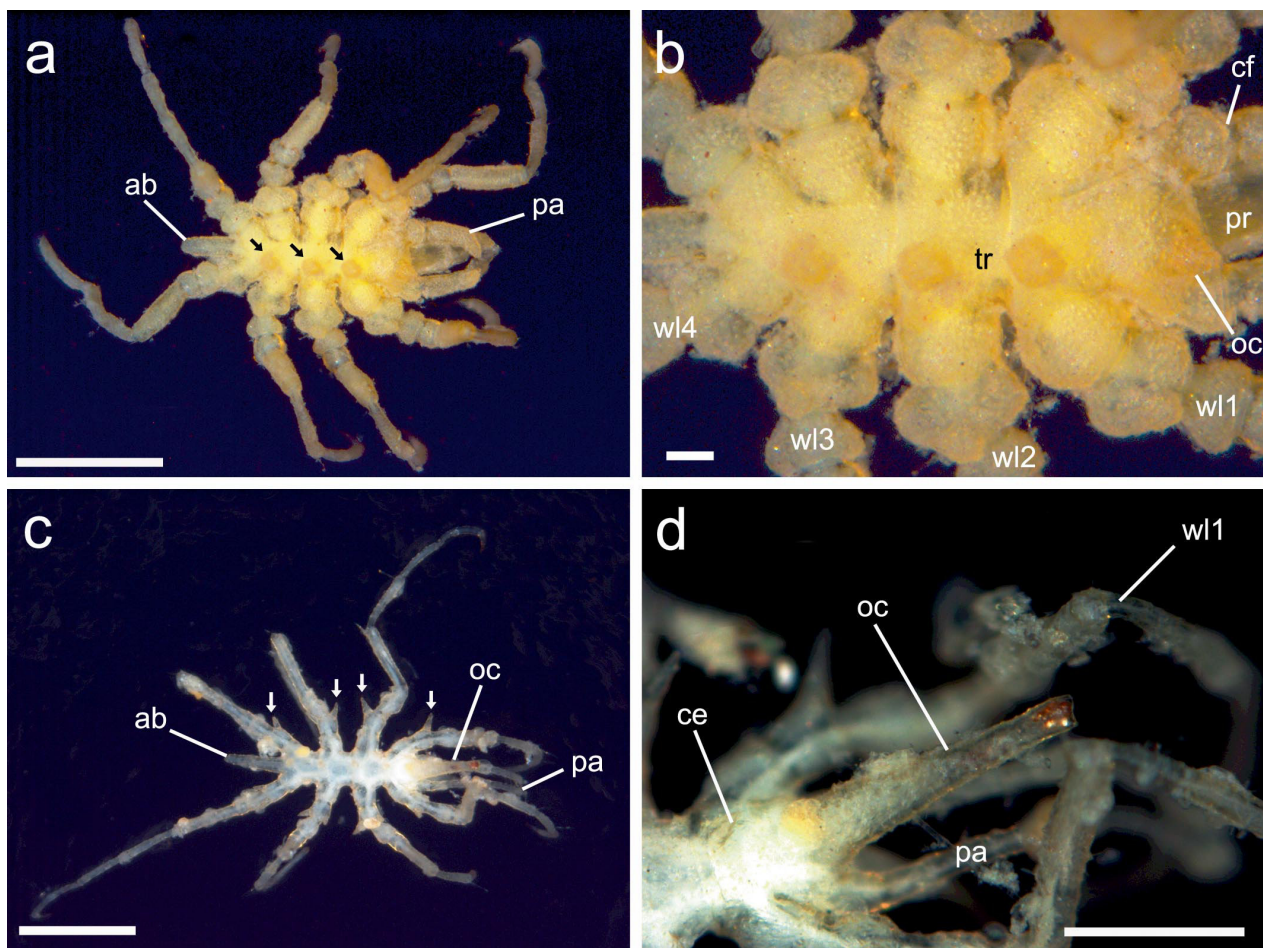
Austrodecidae
Austrodecus Hodgson, 1907

Austrodecus glaciale Hodgson, 1907
Figures 12c–d

Austrodecus glaciale Hodgson, 1907: 53, 93, pl. VIII, fig. 1

Material examined. ZSMA20080577: 1 specimen; Antarctica, Weddell Sea; 17.12.2007; 17:50–18:18; FS Polarstern; Cruise: ANT XXIV/2; Station: PS71/016-01; Exp.: ANDEEP-SYSTCO; 70°35.29′ S, 009°2.89′ W – 70°35.35′ S, 009°2.27′ W; Rauschert dredge; 486.3–488.4 m; ZSMA20100166: 1 specimen; Antarctica; Antarctic Peninsula; 26.04.2000; 14:42–14:57; FS Polarstern; Cruise: PS 56; Station: 158-1; Exp.: EASIZ III; 63°4.70′ S, 057°31.60′ W – 63°4.50′ S, 057°32.00′ W; AGT; 94.0–95.0 m; ZSMA20100192: 1 specimen; Antarctica, Weddell Sea, Atka Bay; 12.01.2008; 10:33–11:04; FS Polarstern; Cruise: ANTXXIV/2; Station: PS71/048-01; Exp.: ANDEEP-SYSTCO; 70°24.00′ S, 008°19.72′ W – 70°23.86′ S, 008°19.20′ W; AGT; 597.0–601.8 m; ZSMA20100228: 2 specimens; Antarctica, E Weddell Sea; 16.12.2003; FS Polarstern; Cruise: ANT XXI/2; Station: PS 65/173-1; Exp.: BENDEX; 70°56.82′ S, 10°31.76′ W – 70°56.77′ S, 10°31.17′ W; AGT; 279.0–296.0 m.

Remarks. The examined specimens correspond well with the descriptions of Stock (1957) and Child (1994). The long ocular tubercle (Fig. 12d) and the specific spine arrangement on the coxae (Fig. 12c), with one single tubercle on the coxae of the first pair of walking legs and two tubercles on the posterior three pairs of walking legs, separate this species from others in its genus.

Figure 12. a – b. *Rhynchothorax australis*; **a.** Dorsal overview, note trunk tubercles (arrows); scale = 1 mm; **b.** Detail view of trunk; scale = 100 µm; **c – d.** *Austrodecus glaciale*; **c.** Dorsal overview, note different spine arrangement on first coxae (arrows); scale = 1 mm; **d.** Ocular tubercle; scale = 500 µm. **ab** – abdomen; **ce** – cephalon; **oc** – ocular tubercle; **pa** – palp; **pr** – proboscis; **wl** – walking leg.

Note added in proof

After acceptance of the present paper, a new study by R. Bamber (2011) was published, giving new localities for 9 of the species studied here. Nevertheless the distribution range is not expanded remarkably by adding the King George Island as further location. However, the depth range could be expanded for Nymphon eltaninae. The shallowest depth ever measured for this species is now at 111 m.

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4. Paper II

Weis A & Melzer RR (2012a) Chilean and Subantarctic Pycnogonida collected by the 'Huinay Fjordos' Expeditions 2005-2011. *Zoosystematics and Evolution* 88 (2), 187-205.

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Chilean and Subantarctic Pycnogonida collected by the "Huinay Fjordos" Expeditions 2005–2011

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Abstract

The Chilean and Subantarctic pycnogonid material housed at the Bavarian State Collection of Zoology mainly collected by the "Huinay fjordos" expeditions between 2005 and 2011 is reviewed. It represents 12 species from 5 of the 11 pycnogonid families, namely: Ammotheidae, Callipallenidae, Colossendeidae, Pallenopsidae and Phoxichilidiidae. The animals were studied with light and scanning electron microscopy (SEM) in order to depict complete sets of the species' diagnostic features. Series of light microscopic pictures were used to generate extended depth of field pictures. The observed features are discussed and compared to results from previous literature and data on the species' geographic distribution are updated.

Introduction

The Pycnogonida or sea spiders are thought to represent a basally branching lineage within Chelicerata of primarily marine origin present in all oceans from the littoral zone to abyssal depths (Bamber 2007; Park et al. 2007). Currently these cryptic "nobodies" are containing more than 1300 species worldwide (Arango & Wheeler 2007; Munilla & Soler-Membrives 2008). Many species are almost perfectly hidden in the benthic organisms they feed on, moving so slowly that they are very hard to detect. At the moment many pycnogonid studies concentrate on the Antarctic area, since there they appear with remarkable species richness (Stiboy-Risch 1993). Beyond that also Hodgson (1927) considered this continent as the centre of speciation for pycnogonids ("Hauptquartier der Pantopoden"). Since the Southern Ocean has been described as a centre of pycnogonid geographic dispersal and evolutionary radiation (Hedgpeth 1947; Fry & Hedgpeth 1969; Munilla & Soler-Membrives 2008; Griffiths et al. 2011) we want to extend the spectrum to hitherto relatively unexplored Subantarctic regions. In this context, the 90.000 km long Southern Chilean coastline with its impressive fjord regions represents an interesting study area. The last extensive studies on Chilean fjord pycnogonids are those of Loman (1923a, b) on pycnogonids collected by

the Antarctic Swedish Expedition (1901–1903), and of Hedgpeth (1961) on the Lund University Chile Expedition which dates back to the 1950s. The Chilean fjord regions were completely covered by glaciers during the last ice age and were subsequently recolonized by benthic communities (Försterra 2009). Today this area is strongly influenced by precipitation, since numerous rivers and streams carry down fresh water from the mountains into the upper benthos zone. This leads to the formation of an uppermost low salinity layer attaining a thickness of up to 7 m creating specific living conditions. Below the transitional zone to the seawater, the halocline, various types of benthic communities are found including also pycnogonids (see Fig. 2) (Melzer et al. 2006).

The animals studied in this paper originate from a wide range of latitudes (30°–55° S) including several biogeographic regions: (1) The Peruvian or warm-temperate province of northern Chile ranging from Península Illescas (approx. 6° S) to Chiloé Island (42° S) (Häussermann & Försterra 2005), (2) the Magellanic or cold-temperate region ranging from Chiloé Island (42° S) to Tierra del Fuego (55° S) (Dall 1909; Carcelles & Williamson 1951; Stuardo 1964; Dell 1971; Brattström & Johanssen 1983), (3) the Subantarctic region generally considered as ranging from about 46° to 60° S, and (4) the Falkland Islands (52° S) that is either

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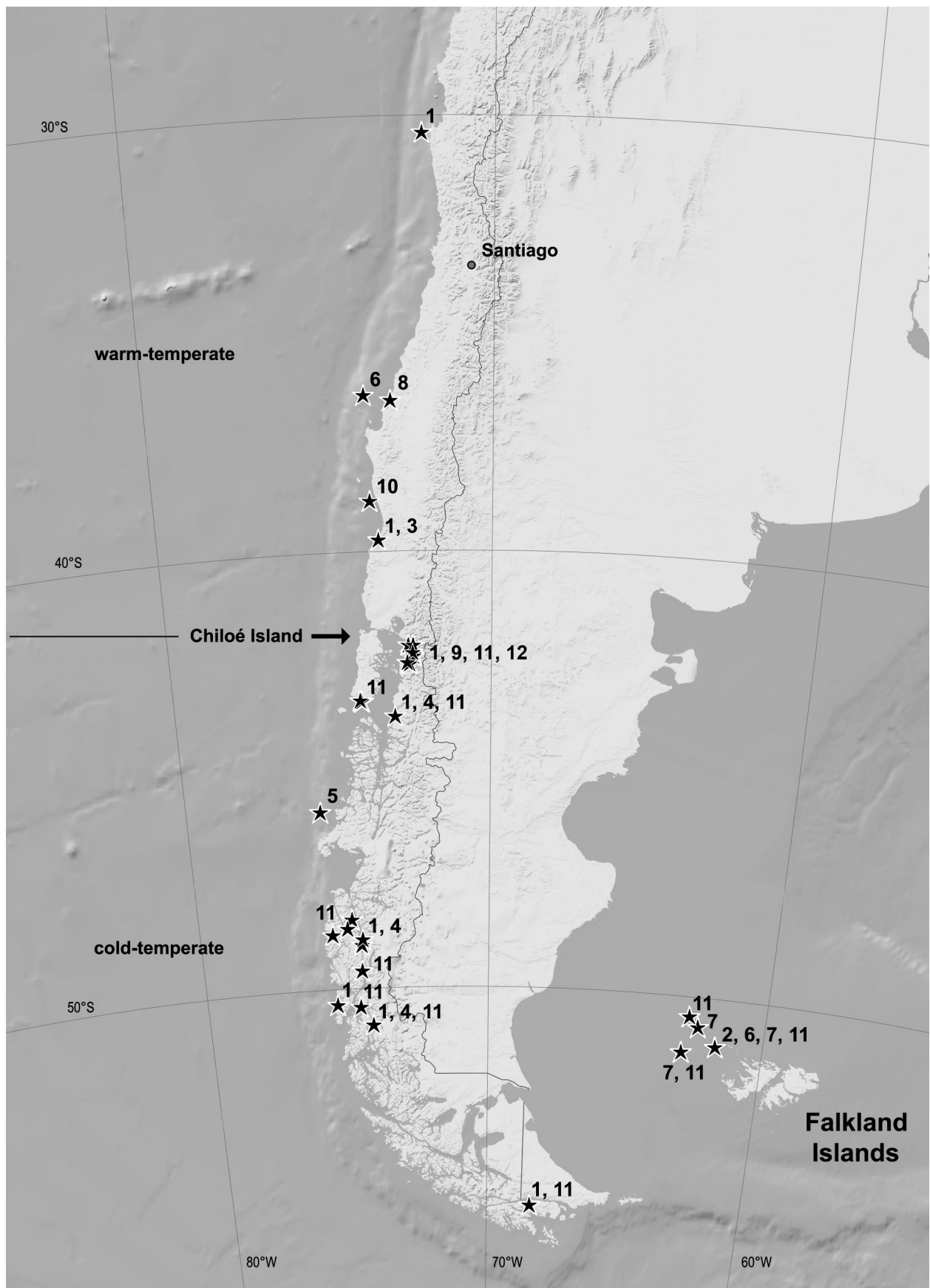
Figure 1. Overview of collecting sites of Chilean and Subantarctic pycnogonids deposited at the Bavarian State Collection of Zoology. Species are numbered as follows: **1.** *Achelia assimilis*, **2.** *Ammothea spinosa*, **3.** *Tanystylum cavidorsum*, **4.** *Tanystylum neorhetum*, **5.** *Colossendeis macerrima*, **6.** *Colossendeis megalonyx*, **7.** *Colossendeis scoresbii*, **8.** *Anoropallene palpida*, **9.** *Callipallene margarita*, **10.** *Pallenopsis notiosa*, **11.** *Pallenopsis patagonica*, **12.** *Anoplodactylus californicus*.
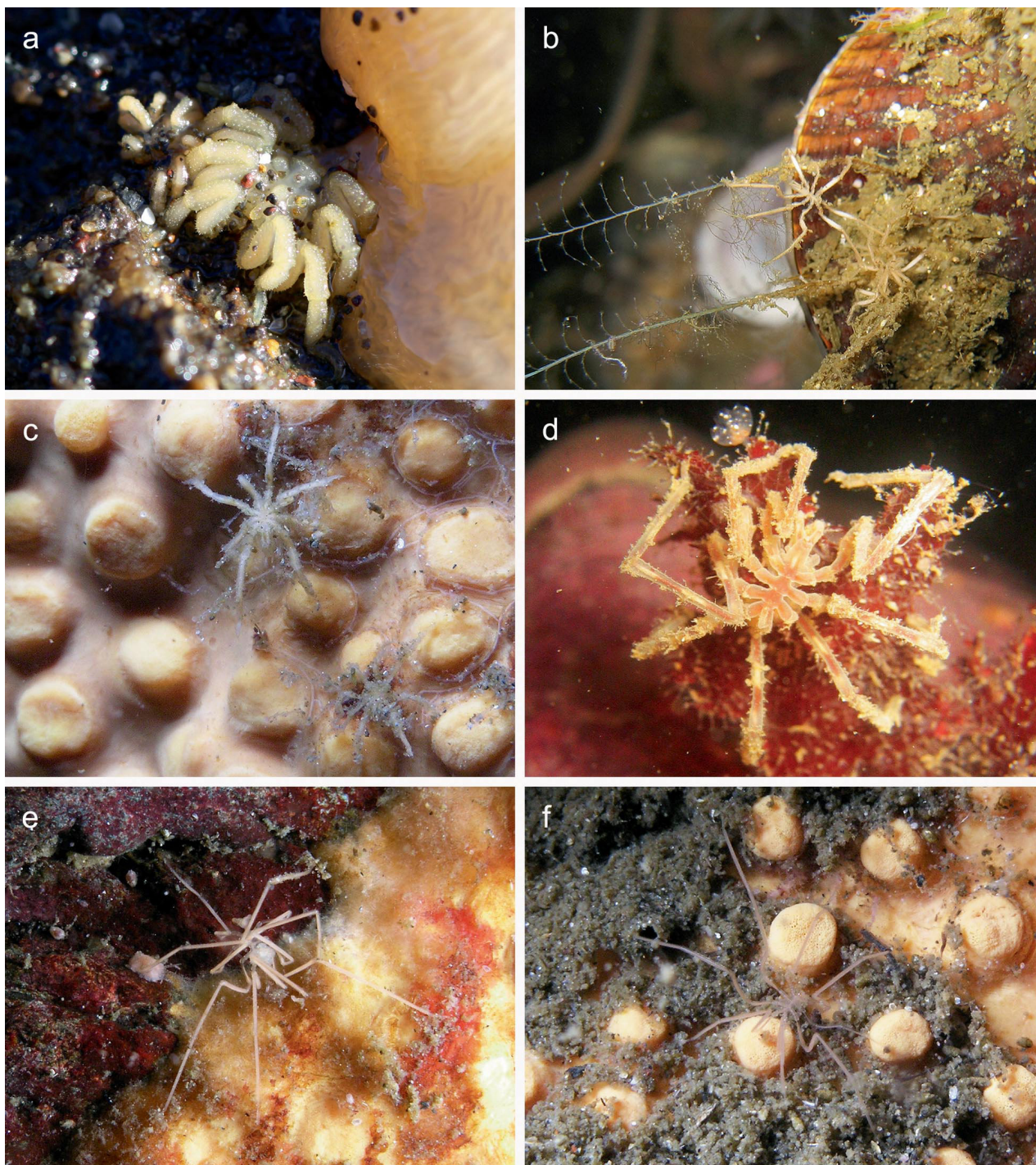
Figure 2. In situ pictures of Chilean fjord pycnogonids: **a.** *Tanystylum cavidorsum*; **b.** *Anoplodactylus californicus*; **c.** *Achelia assimilis*; **d.** *Pallenopsis patagonica*; **e, f.** *Callipallene margarita*; Underwater-photos a, c, e, f by Roland Meyer.

seen as a distinct region (Powell 1965) or as part of the Magellanic region.

This study displays samples predominantly collected from expeditions of the Huinay Scientific Field Station from 2005 to now. The few species found in the Chilean fjord regions so far are particularly interesting due to their extraordinary distribution patterns. Our new records expand the known geographic range for some of the species (see also Melzer et al. 2006; Melzer 2009).

This might correspond with the idea suggested in previous studies that wideranging Magellanic invertebrate taxa are extending their distribution range also far north due to the Humboldt Current (see Brattström & Johanssen 1983; Schrödl 2003).

Representatives of most of the Chilean fjord pycnogonids previously recorded are housed at the Zoologische Staatssammlung München (ZSM), distributed among 12 species. According to Sielfeld's (2003) spe-

cies list about 40 pycnogonid species have been found in the southeast Pacific Ocean including Chilean Patagonia so far, many of them being inhabitants of deep waters. The species figured in the present study were already mentioned in Sielfeld (2003) with the exception of *Colossendeis macerrima* Wilson, 1881, *C. megalonyx* Hoek, 1881 and *Anoropallene palpida* (Hilton, 1939). Here we document the morphology and sample locations of the different species housed at the ZSM contributing to a taxonomic and faunistic survey of Pycnogonida from the Chilean Fjords. Based on our catalogue of Antarctic Pycnogonida (Weis et al. 2011) we present the classification given in PycnoBase (Bamber & Nagar 2011) as a taxonomic backbone, and illustrate a general overview of the most prominent characteristics of the collected Chilean/Subantarctic pycnogonid species.

Material and methods

Sampling sites are mainly located in the Chilean fjord regions, but we also got some specimens from more northern areas (from Dr. Javier Sellanes López; Universidad Católica del Norte, Facultad De Ciencias Del Mar, Coquimbo, Chile) and the Falklands (from Dr. Vladimir Laptikhovsky; Falkland Islands Fisheries Department); details are given under the material examined section for each species. Figure 1 shows the location where the species were collected. Most of the material was collected by SCUBA diving during stays at the Huinay Scientific field station or "Huinay fjordos 3–10" expeditions between 2005 and 2011 (Försterra 2009) and fixed in either 75 % or 96 % ethanol.

Chilean/Subantarctic pycnogonids were determined using a variety of literature. Especially the works of Loman (1923a, b) and Hedgpeth (1961) must be pointed out since they are the only studies that concentrate on Chilean pycnogonids and provided many helpful drawings. Determinations were checked further using the more recent work of Sielfeld (2003) and Melzer (2009). Beyond that synonyms were looked up in PycnoBase (Bamber & Nagar 2011) and Müller's (1993) "World Catalogue and Bibliography of the recent Pycnogonida".

All specimens were documented using an Olympus SZX stereo microscope equipped with a Jenoptic Prog-Res C12 digital camera (2580 $\times$ 1944 px; 96 dpi; colour depth 24 bit) at different levels along the *z*-axis. In a following step these pictures were edited and combined to a single respective image with greater depth of field using the computer software CombineZ and/or Syncroscopy Auto Montage. Specimens used for SEM documentation (using a LEO 1430VP at 15 kV) were prepared according to methods described in Montoya Bravo et al. (2009).

Annotated catalogue

General remarks

A total of 12 Subantarctic/Chilean pycnogonid species could be identified. In all cases, the major morphological characteristics correspond well with the respective descriptions published earlier. The species most frequently recorded in our collection is *Achelia assimilis* (Haswell, 1884) with a total of 226 specimens.

Classification

Order Pantopoda Gerstäcker 1863
Suborder Eupantopodida Fry 1978
Superfamily Ascorhynchoidea Pocock 1904
Family Ammotheidae Dohrn, 1881
   Achelia assimilis (Haswell, 1884)
   Ammothea spinosa (Hodgson, 1907)
   Tanystylum cavidorsum Stock, 1957
   Tanystylum neorhetum Marcus, 1940
Superfamily Colossendoidea Hoek 1881
Family Colossendeidae Hoek, 1881
   Colossendeis macerrima Wilson, 1881
   Colossendeis megalonyx Hoek, 1881
   Colossendeis scoresbii Gordon, 1932
Superfamily Nymphonoidea Pocock 1904
Family Callipallenidae Hilton, 1942
   Anoropallene palpida (Hilton, 1939)
   Callipallene margarita (Gordon, 1932)
Family Pallenopsidae Fry, 1978
   Pallenopsis notiosa Child, 1992
   Pallenopsis patagonica (Hoek, 1881)
Superfamily Phoxichilidoidea Sars 1891
Family Phoxichilidiidae Sars, 1891
   Anoplodactylus californicus Hall, 1912

Ammotheidae

Achelia Hodge, 1864

Achelia assimilis (Haswell, 1884)
Figures 3a–f, 4a–d

*Ammothea assimilis* Haswell, 1884: 1026–1027, figs 5–9

Synonyms
Ammothea wilsoni Schimkewitsch, 1887
Achelia variabilis Stock, 1954
Achelia wilsoni Stock, 1957
Nymphopsis denticulate Gordon, 1932 (misidentification)

Material examined. Chilean fjord region, Comau fjord, Punta Huinay: 42°22′ S, 72°25′ W; 1 specimen; 04.05.2005; 18 m; ZSMA20051920; 1 ♀, 1 ♂; 04.03.2006; 15–25 m; ZSMA20111018, ZSMA20111019; 31 specimens; 05.03.2009; 20–30 m; ZSMA20100115, ZSMA20100116, ZSMA20111068; 1 ♂; 06.03.2009; 20–30 m; ZSMA20111069; 2 ♀♀, 2 ♂♂, 2 juv.; 12.03.2006; 5–10 m; ZSMA20111029, ZSMA20111033, ZSMA20111034, ZSMA20111037, ZSMA20111038, ZSMA20111040; 4 ♀♀, 7 ♂♂, 3 juv.; 14.03.2011; 25 m; ZSMA20111163–ZSMA20111171, ZSMA20111186, ZSMA20111187–ZSMA20111190; 6 ♀♀, 3 ♂, 5 juv.; 22.03.2011; 20–30 m; ZSMA20111311–ZSMA20111315; ZSMA20111539–ZSMA20111547. Chilean fjord region, Comau fjord, Huinay: 42°19.88′ S, 72°27.66′ W; 1 specimen; 09.03.2004; 10–25 m; ZSMA20051994. Chilean fjord region, Comau fjord, Huinay, Punta Gruesa: 42°24′ S, 72°25′ W; 5 specimens; 22.02.2005; 20–30 m; ZSMA20051921, ZSMA20051922, ZSMA20051931–ZSMA20051933; 3 ♀♀, 1 ♂, 24 juv.; 13.03.2011; 20 m; ZSMA20111132–ZSMA20111158, ZSMA20111162. Chilean fjord region, Comau fjord, Huinay, Swall: 42°19′ S, 72°27′ W; 2 ♂♂; 15.03.2011; 10–20 m; ZSMA20111193, ZSMA20111194. Chilean fjord region, Comau fjord, Huinay, Anti-Punta: 16 specimens; 21.02.2005; 5–36 m; ZSMA20051928–ZSMA20051930,
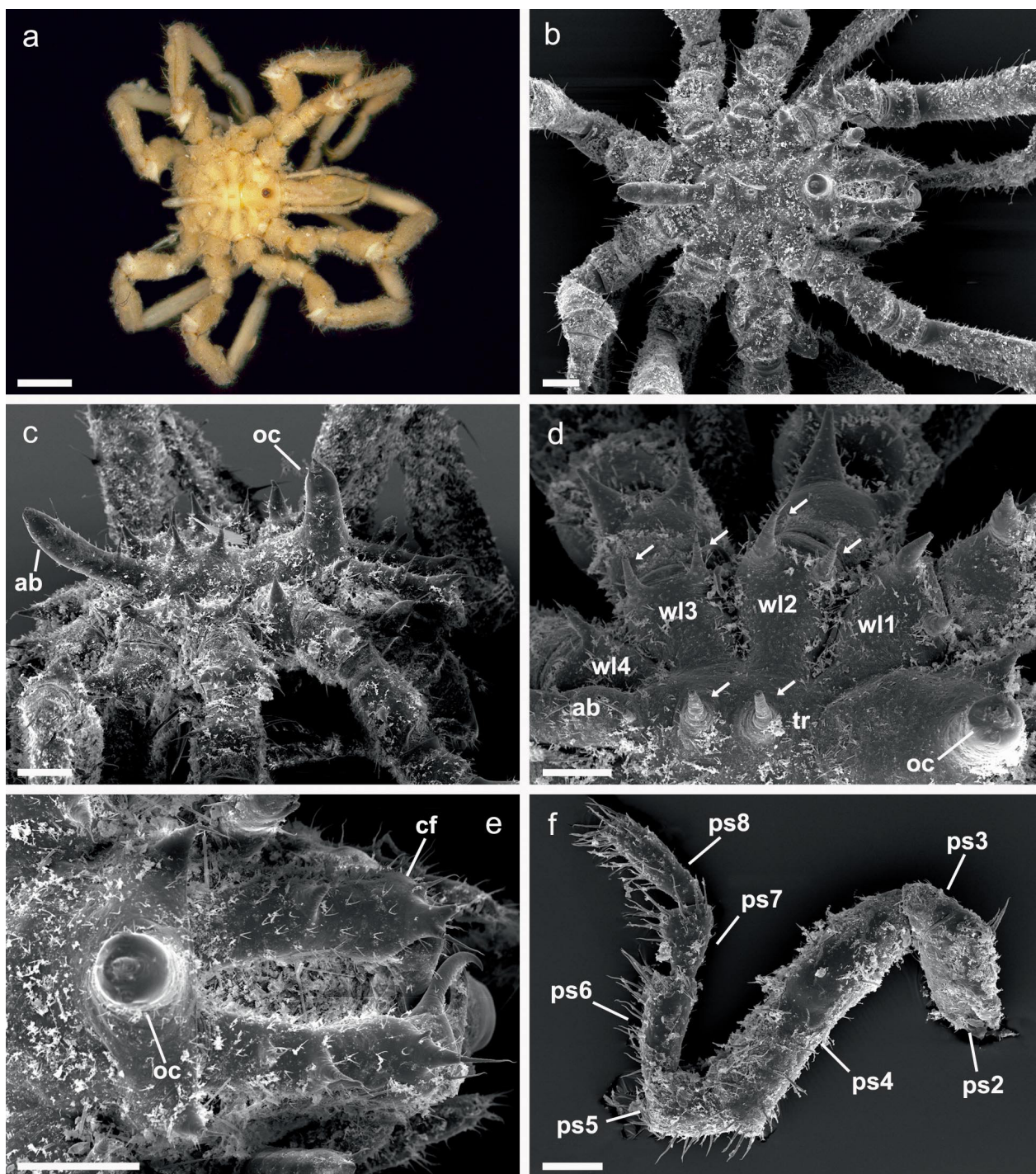
Figure 3. *Achelia assimilis*: **a.** Dorsal overview; scale = 1 mm; **b.** Dorsal view of trunk; scale = 200 μm; **c.** Lateral view of trunk; scale = 200 μm; **d.** Dorsal view of trunk, note spines on trunk, lateral processes and first coxae (arrows); scale = 200 μm; **e.** Dorsal view of cephalon; scale = 200 μm; **f.** Overview of left palp; scale = 100 μm. Abbreviations: **ab** – abdomen; **cf** – chelifore; **oc** – ocular tubercle; **ps** – palp segment; **tr** – trunk; **wl** – walking leg.

ZSMA20051946–ZSMA20051957, ZSMA20051993. Chilean fjord region, Comau fjord, Lilihuapi: 42°09′ S, 72°35′ W; 5 specimens; 24.02.2005; 5–36 m; ZSMA20051923–ZSMA20051927; 3 ♀♀, 1 ♂, 2 juv.; 26.03.2006; 17 m; ZSMA20111041–ZSMA20111046; 2 ♀♀, 1 ♂; 14.04.2006; 15–25 m; ZSMA20111047, ZSMA20111049, ZSMA20111050. Chilean fjord region, Comau fjord, Quintopeu: 42°09′ S, 72°26′ W; 15 specimens; 25.02.2005; 15–25 m; ZSMA20051934–ZSMA20051945, ZSMA20051978; 2 ♀♀, 2 ♂♂, 1 juv.; 27.03.2006; 17 m; ZSMA20111052, ZSMA20111054–

ZSMA20111057. Chilean fjord region, Fjord Renihué, Loberia: 42°34′50.0″ S, 72°33′14.6″ W; 13 ♀♀, 6 ♂♂, 1 juv.; 20.03.2011; 20 m; ZSMA20111256–ZSMA20111275. Chilean fjord region, Fjord Renihué, Cabudahue: 42°32′45.9″ S, 72°37′06.6″ W; 10 ♀♀, 10 ♂♂, 15 juv.; 20.03.2011; 20 m; ZSMA20111276–ZSMA20111310. Chilean fjord region, Fiordo corno: 1 ♀; 25.02.2005; 19–25 m; ZSMA20111021. Chilean fjord region, Madres Dios Archipelago, Canal Copihue: 50°20′23.1″ S, 75°22′39.2″ W; 1 ♀; 12.03.2006; 20 m; ZSMA20111001. Chilean fjord region, Hanover Area, Canal Pitt Chico: 50°50′ 07.1″ S,

74°08′20.9″ W; 1 ♂; 07.03.2006; 20 m; ZSMA20111010. Chilean fjord region, Raul Marin, Las Hermanas: 43°46.285′ S, 073°02.632′ W; 1 ♀; 11.03.2007; 16 m; ZSMA20111014. Chilean fjord region, Messier Channel and Fjords, Estero Denmann: 48°51′34.5″ S, 74°22′47.2″ W; 1 ♂; 11.03.2006; 5–15 m; ZSMA20111025. Chile, Tierra del Fuego, Fjord Ponsenby: 55°03′71.96″ S, 68°44′13.48″; 1 ♂, 1 juv.; 19.12.2010; 29 m; ZSMA20111341, ZSMA20111342. Chile, off Coquimbo: 30°22.893′ S, 71°57.759′ W; 1 ♂; 140 m; ZSMA20111076. Chile, Playa Chica: 39°43′10.3″ S, 73°24′11.8″ W; 3 ♀♀, 4 ♂♂; 07.03.2011; 0–1 m; ZSMA20111080–ZSMA20111082, ZSMA20111085–ZSMA20111087, ZSMA20111089; 1 juv.; 08.03.2011; 0–1 m; ZSMA20111094.

Remarks. With a total of 226 specimens *Achelia assimilis* is the most frequently found pycnogonid species in the Chilean fjords so far. As Hedgpeth (1961) already mentioned this species shows a variable appearance concerning the spination of the dorsal trunk and lateral processes. Our specimens show two (Fig. 3c) or three dorsal trunk spines (Fig. 3d) and further two spines on each of the lateral processes. Specimens with three dorsal trunk spines are well in accordance with the drawings provided by González and Edding (1990).

After Hedgpeth (1961) some spurs of the lateral processes are bifurcate. Here we could observe additional bifurcated spurs on the first coxa as well (see Fig. 3d). Beyond that there are also distinct spurs at the anterior margin of the cephalic segment (Fig. 3e). The remaining characteristic features like for example the ovigers (Fig. 4a–b) and walking legs (Fig. 4c–d) correspond well with the descriptions of the older literature.

Apart from being the most frequently collected species, *Achelia assimilis* shows also the largest distribution area of the pycnogonid species analysed in our study. *Achelia assimilis* is found in both of the above-mentioned climates, the warm-temperate and the cold-temperate zones of Chile (see Fig. 1). Müller (1993) summarized records of this species from tropical and temperate southwest Pacific and Indonesia, Malaysia, French Polynesia and Chile. Furthermore this species was reported for Australia (Child 1975; Arango 2003) and New Zealand (Munilla & Soler-Membrives 2008). Although the material from the Lund expedition contains specimens from about 53° S (see Hedgpeth 1961), with Tierra del Fuego being situated at 55° S, our collected specimens show the southernmost collecting site for *A. assimilis* (see Melzer 2009).

Ammothea Leach, 1814

Ammothea spinosa (Hodgson, 1907)
Figures 4e–f, 5a–c

Leionymphon spinosum Hodgson, 1907: 49–50, pl. VII, fig. 2

Synonyms
Ecleipsothremma spinosa Fry & Hedgpeth, 1969

Material examined. Subantarctic, Falkland Islands West: 51° 5′8.00″ S, 61°44′0.00″ W; 1 ♀; 06.02.2010; 174–176 m; ZSMA20111356.

Remarks. The present material corresponds well with the description of Fry and Hedgpeth (1969) and Child

(1994). At first sight this species might be mistaken for *Ammothea allopodes* Fry & Hedgpeth, 1969, but it differs in having prominent dorsal trunk ridges (Fig. 4e) and a tall ocular tubercle with a pointed cone (Fig. 5b). Furthermore the articulation of the scape with palm of *Ammothea spinosa* is synaxial and the first four palp segments are as long as the proboscis (Fig. 4f). Based on these characteristics, *A. spinosa* can be clearly distinguished from *A. allopodes*.

Ammothea spinosa has so far been collected from the Antarctic area, the Argentine Basin and the Magellanic region (Müller 1993; Child 1994). Thus the here examined individual from the Falkland Islands fits well in the previous described distribution pattern.

Tanystylum Miers, 1879

Tanystylum cavidorsum (Stock, 1957)
Figures 5d–f, 6a–d

Tanystylum neorhetum Stock, 1954: 149–151, figs 73–74

Synonyms
Tanystylum cavidorsum var. *steatopygidium* Hedgpeth, 1961

Material examined. Chile, Playa Chica: 39°43′10.3″ S, 73°24′ 11.8″ W; 1 ♂, 3 juv.; 07.03.2011; 0–1 m; ZSMA20111083, ZSMA20111084, ZSMA20111088, ZSMA20111090; 9 ♀♀, 10 ♂♂, 20 juv.; 08.03.2011; 0–1 m; ZSMA20111091–ZSMA20111093, ZSMA20111095–ZSMA20111130. Chile, Región de Magellanes y de la Antárctica Chilena, Puerto del Hambre: 53°36′ S, 70°55′ W; 1 specimen; 25.02.2002; ZSMA20111575.

Remarks. The most obvious characteristic of this species is the large rounded bulb-like base of the abdomen (Fig. 5e). This prominent feature differentiates it from all other *Tanystylum* species from this region. There is only one known congener from the study region with a similar bulb-shaped abdomen base, namely *Tanystylum oedinotum* Loman, 1923b. However, this species differs from *Tanystylum cavidorsum* in having a truncate conical proboscis (Child 1994), instead of a barrel-shaped one (Figs 5d, 6a).

In the literature *Tanystylum cavidorsum* is known from New Zealand, South Georgia, Possession Island, Crozet Island, South Sandwich Islands and Southern Chile with the northernmost discovery in Mehuin at 39° S (Clark 1977; Müller 1993; Child 1994). Our specimens (except ZSMA20111575) have been found in the warm-temperate region at Playa Chica, coinciding in terms of latitude (39° S) with the northernmost of the previously documented sample sites. Just as given in Hedgpeth (1961) we also found all 39 specimens in the tidal area between 0 and 1 meter water depth.

Tanystylum neorhetum (Marcus, 1940)
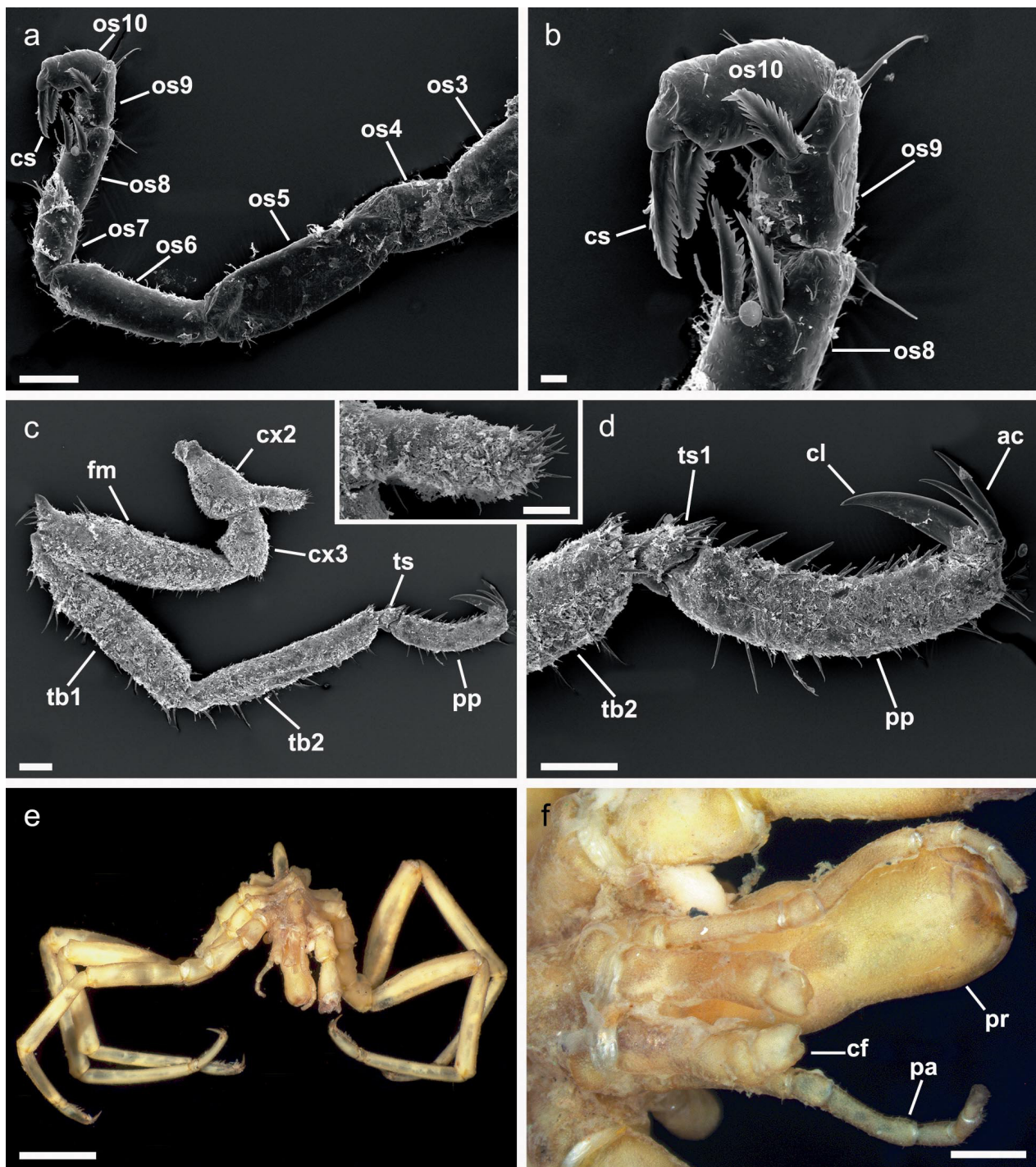Figures 6e–f, 7a–b

Clotenia dohrnii Pfeffer, 1889: 48

Synonyms
Tanystylum pfefferi Bouvier, 1913

Material examined. Chilean fjord region, Hanover Area, Canal Pitt Chico: 50°50′07.1″ S, 74°08′20.9″ W; 1 juv.; 07.03.2006; 20 m;

Figure 4. a–d. *Achelia assimilis*; **a.** Overview of right oviger; scale = 100 μm; **b.** Distal segments of right oviger; scale = 20 μm; **c.** Male, overview of left 4th walking leg; scale = 200 μm. Insert: Detail view of genital opening of second coxa; scale = 100 μm; **d.** Tarsus and propodus with claw and auxiliary claws of left 4th walking leg; scale = 200 μm; **e, f.** *Ammothea spinosa*; **e.** Frontal overview; scale = 5 mm; **f.** Dorsal view of palps; scale = 1 mm. Abbreviations: **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cs** – compound spine; **cx** – coxa; **fm** – femur; **os** – oviger segment; **pa** – palp; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **ts** – tarsus.

ZSMA20111011. Chilean fjord region, Raul Marin, Las Hermanas: 43°46.285′ S, 073°02.632′ W; 1 ♀, 1 ♂; 11.03.2007; 16 m; ZSMA20111013, ZSMA20111015. Chilean fjord region, Messier Channel and Fjords, Angostura Inglesa: 48°59′18″ S, 74°25′08″ W; 1 ♀; 11.03.2006; 15 m; ZSMA20111022.

Remarks. As Child (1994) already mentioned this is a very plain species. Compared to the species described

above it lacks a prominent bulb at the base of the abdomen and has a more cone-like proboscis with a rounded tip (Figs 6e, 7a). The only outstanding character of *Tanystylum neorhetum* might be the long and obliquely upwards pointing abdomen (Fig. 6f). While the ocular tubercle has been described as being not as tall as wide by Child (1994), the ocular tubercles of our specimens
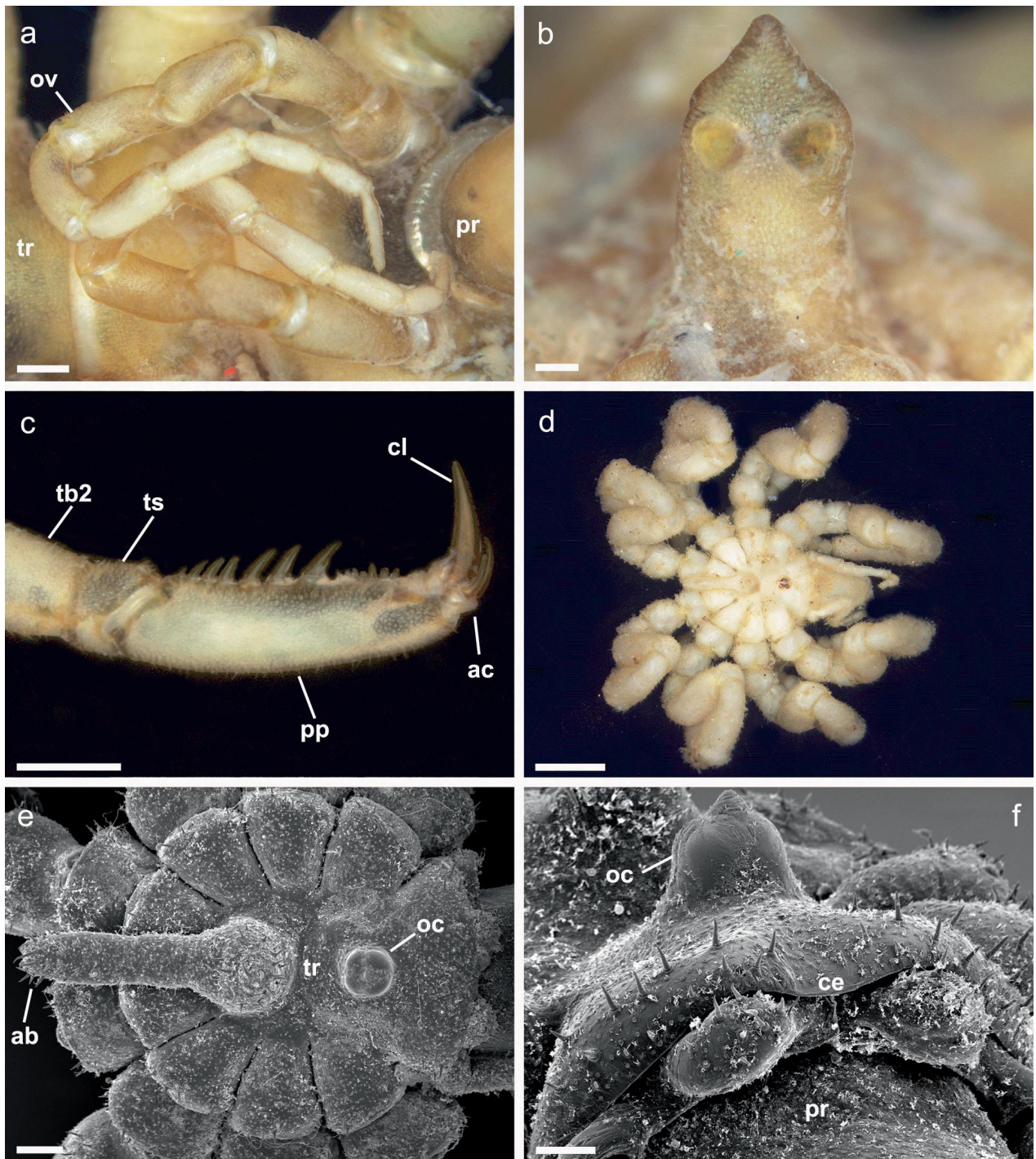
Figure 5. a−c. *Ammothea spinosa*; **a.** Detail view of ovigers; scale = 500 μm; **b.** Frontal view of ocular tubercle; scale = 250 μm; **c.** Tarsus and propodus with claw and auxiliary claws (left 1st walking leg); scale = 1 mm; **d−f.** *Tanystylum cavidorsum*; **d.** Dorsal overview; scale = 1 mm; **e.** Dorsal view of trunk; scale = 200 μm; **f.** Frontal view of cephalon; scale = 100 μm. Abbreviations: **ab** – abdomen; **ac** – auxiliary claw; **ce** – cephalon; **cl** – claw; **oc** – ocular tubercle; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **tr** – trunk; **ts** – tarsus.

have a much taller appearance (Fig. 6f). Since this species seems to be very variable in most respects (see Stock 1954; Hedgpeth 1961; Clark 1977), we anyhow determined these four specimens as *Tanystylum neorhetum* because the remaining characteristics are well in accordance with the literature.

Tanystylum neorhetum shows the widest known distribution pattern for any Subantarctic species of its genus (Child 1994). It has been collected from New Zealand, South Georgia, Kerguelen Islands, Macquarie Islands, Tristan da Cunha, Bouvet Islands, Southern Chile, Tierra del Fuego and the Falkland Islands (Müller 1993; Child 1994). Thus the collecting sites from the cold-temperate Chilean fjord regions from the specimens studied here are not surprising. After Child (1994) these diverse collecting localities suggest disper-
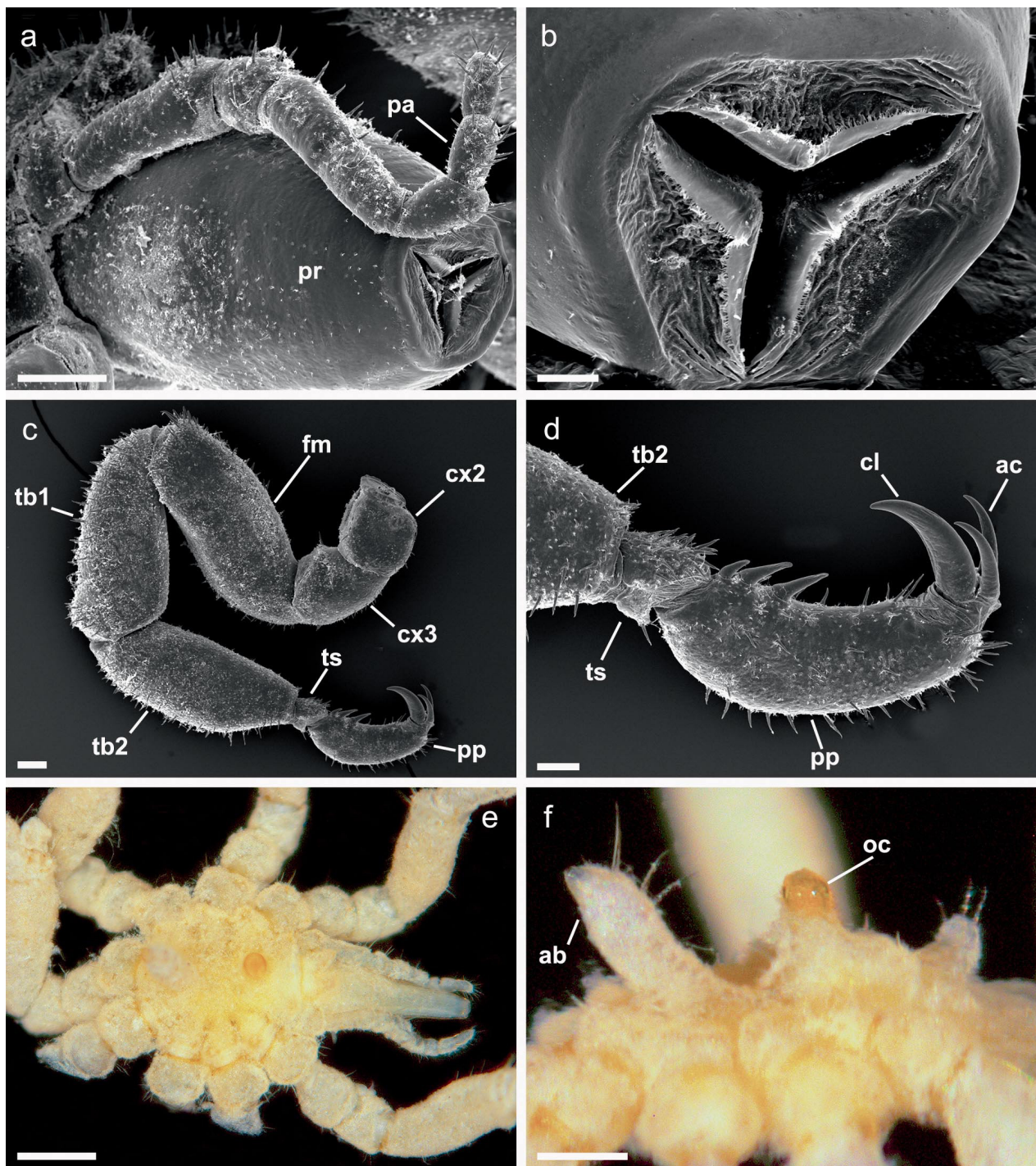
Figure 6. a−d. *Tanystylum cavidorsum*; **a.** Overview of right palp; scale = 200 µm; **b.** Close up of mouth opening; scale = 50 µm; **c.** Overview of right 4th walking leg; scale = 200 µm; **d.** Tarsus and propodus with claw and auxiliary claws of right 4th walking leg; scale = 100 µm; **e−f.** *Tanystylum neorhetum*; **e.** Dorsal view of trunk and proboscis; scale = 500 µm; **f.** Lateral view of trunk; scale = 250 µm. Abbreviations: **ab** – abdomen, **cl** – claw, **cx** – coxa; **fm** – femur; **oc** – ocular tubercle, **pa** – palp; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **ts** – tarsus.

sal dispensation of the species supported by the forces of the west wind drift around the southern hemisphere. The majority of the specimens are found in shallower depths between 0–115 m which is well in accordance with our findings.
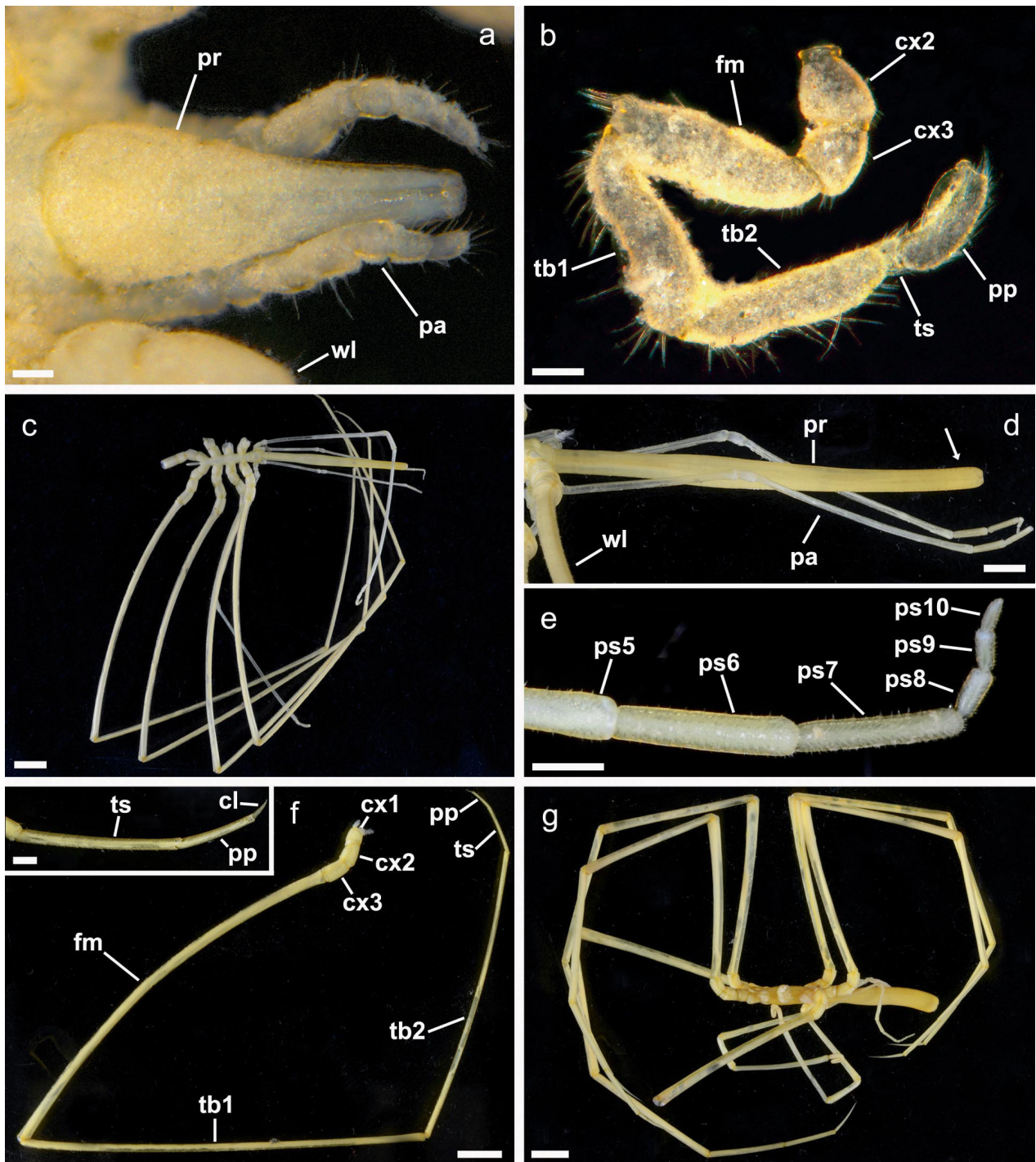
Colossendeidae

***Colossendeis* Jarzinsky, 1870**

***Colossendeis macerrima* Wilson, 1881**

Figures 7c−f

Colossendeis macerrima Wilson, 1881: 246–247, pl. 1, fig. 2, pl. 4, figs 9–12, pl. 5, fig. 32

Figure 7. a, b. *Tanystylum neorhetum*; **a.** Ventral view of proboscis; scale = 100 μm; **b.** Overview of left 2nd walking leg; scale = 250 μm; **c–f.** *Colossendeis macerrima*; **c.** Dorsal overview; scale = 6 mm; **d.** Lateral view of palps and proboscis, note upturned distal end of proboscis (arrow); scale = 3 mm; **e.** Distal segments of left palp; scale = 1 mm; **f.** Overview of left 2nd walking leg; scale = 6 mm. Insert: Detail view of tarsus and propodus; scale = 1 mm; **g.** *Colossendeis megalonyx*: Lateral overview; scale = 6 mm. Abbreviations: **cl** – claw; **cx** – coxa; **fm** – femur; **pa** – palp; **pp** – propodus; **pr** – proboscis; **ps** – palp segment; **tb** – tibia; **ts** – tarsus; **wl** – walking leg.

Synonyms
Colossendeis gigas-leptorhynchus Bouvier, 1937
Colossendeis japonica Hoek, 1898
Colossendeis leptorhynchus var. septentrionalis Caullery, 1896
Colossendeis spei Pushkin, 1970
Colossendeis villegentei Milne-Edwards, 1881

Material examined. Chile, off Peninsula de Taitao: 45°54.471′ S, 75°36.021′ W; 2 specimens; AGT; 510 m; ZSMA20111336, ZSMA20111337.

Remarks. The short propodus, tiny claw (Fig. 7f) and distally upturned long proboscis (Fig. 7d) serve to dif-

ferentiate *Colossendeis macerrima* from other Subantarctic *Colossendeis* species. As mentioned in Child (1995) the palps are longer than the proboscis (Figs 7c–d) with the 8th palp segment articulated synaxially (Fig. 7e) (see Fry & Hedgpeth 1969).

Colossendeis macerrima is a deep sea species with worldwide distribution and a recorded depth range of 121–4000 m. Thus the sample location of the two specimens studied here does not deviate from the literature.

Colossendeis megalonyx Hoek, 1881
Figures 7g, 8a

Colossendeis megalonyx Hoek, 1881: 67–69, pl. IX, figs 1–3

Synonyms
Colossendeis arundorostris Fry & Hedgpeth, 1969
Colossendeis frigida Hodgson, 1907
Colossendeis orcadense Hodgson, 1908
Colossendeis rugosa Hodgson, 1907

Material examined. Chile, Concepcion; 36°24.010′ S, 73°43.074′ W; 2 specimens; AGT; 769 m; ZSMA20111071, ZSMA20111338. Subantarctic, Falkland Islands West: 51°5′8.00″ S, 61°44′0.00″ W; 2 specimens; 06.02.2010; 174–176 m; ZSMA20111358, ZSMA20111364.

Remarks. The present material of four specimens is well in accordance with the descriptions in the literature (Fry & Hedgpeth 1969; Child 1995). *Colossendeis megalonyx* can be recognized by its down-curved proboscis, which is much longer than the trunk (Figs 7g, 8a), the 8th palp segment which is always shorter than the two more distal ones (Fig. 8a) and the claw being longer than half of the propodus length (Fig. 8a). As mentioned in our previous paper (Weis et al. 2011) different adult individuals show deviating shapes of the ocular tubercle and their eyes are found to show varying degrees of differentiation. In the specimens studied here the ocular tubercles are well developed presenting four dark pigmented eyes (Fig. 8a). In this context also the work of Krabbe et al. (2010) should be mentioned suggesting cryptic lineages exist within *C. megalonyx*.

Colossendeis megalonyx was originally described by Hoek (1881) from the South American shelf between Falkland Islands and Patagonia. Nevertheless this species is predominantly found around the Antarctica (see Munilla & Soler-Membrives 2008; Krabbe et al. 2010) and according to Child (1995) has one of the widest distributions known for a deep-water non-cosmopolitan species. Further sample localities have been the east coasts of South America and New Zealand and even as far north as South Africa (off southern Madagascar) (Child 1995). Two of our specimens were found in a more northern part of Chile near Concepcion, thus representing an interesting geographic complement.

Colossendeis scoresbii Gordon, 1932
Figures 8b–c

Colossendeis scoresbii Gordon 1932: 18–21, figs 5c, 6b, c, 7a, b

Material examined. Subantarctic, Falkland Islands West: 50°40′5.00″ S, 62°26′1.00″ W; 1 specimen; 09.02.2010; 160–165 m;

ZSMA20111347. Subantarctic, Falkland Islands West: 51°16′8.00″ S, 62°57′8.00″ W; 1 specimen; 05.02.2010; 171–174 m; ZSMA20111353. Subantarctic, Falkland Islands West: 51°5′8.00″ S, 61°44′0.00″ W; 1 specimen; 06.02.2010; 174–176 m; ZSMA20111362.

Remarks. In former times *Colossendeis scoresbii* was seen as a subspecies of *C. megalonyx* (Fry & Hedgpeth 1969). Currently *C. scoresbii* is regarded as a separate species (see Child 1995). This could be confirmed by recent studies done by Krabbe et al. (2010). Also morphologically there are some distinct differences to *C. megalonyx* like the shorter proboscis (Fig. 8b), the subequal tarsus and propodus as well as the extremely long propodal claw (Fig. 8c).

Beside the South Orkney Islands, Tierra del Fuego and the Ross Sea *Colossendeis scoresbii* is also found northwest of the Falkland Islands in 130–304 m (Child 1994). The specimens examined in this study were all collected west from the Falkland Islands between 160–176 m and agree therefore with the literature.

Callipallenidae

Anoropallene Stock, 1956

Anoropallene palpida (Hilton, 1939)
Figures 8d–e

Pallene palpida Hilton, 1939: 30

Synonyms
Anoropallene crenispina Stock, 1956
Anoropallene heterodenta Stock, 1956
Oropallene palpida Hilton, 1942
Oropallene heterodenta Hilton, 1942

Material examined. Chile, Bahia de Coliumo; 36°31′23.76″ S, 72°57′9.19″ W; 2 ♀, 1 ♂; May 1992; 3–5 m; ZSMA20010828.

Remarks. The most prominent characters which distinguish this species are the absence of auxiliary claws, palps which are only present in the male and the ventrally carried abdomen (Fig. 8d). Child (1979) mentions an abnormality in the chelifores in one of the females collected by B. W. Walker. In this specimen, the scapes show proximally distinct constrictions, which might point to a previous loss and regeneration of the chelifores (Child 1979). However the two females present in our material show the same "abnormality" regarding their chelifores (Fig. 8e), whereas the chelifores of the male appear without any such distinctive feature. Furthermore females of the species *Propallene stocki* Fage, 1956 show the constriction of the chelifores as well, which suggests, that this might be a characteristic feature of females in these two genera rather than an abnormality. To confirm this hypothesis, the chelifores of all other hitherto collected females of *Anoropallene palpida* should be analysed in detail as well. Unfortunately in the available literature there was no evident hint regarding detailed morphology of the females chelifores.
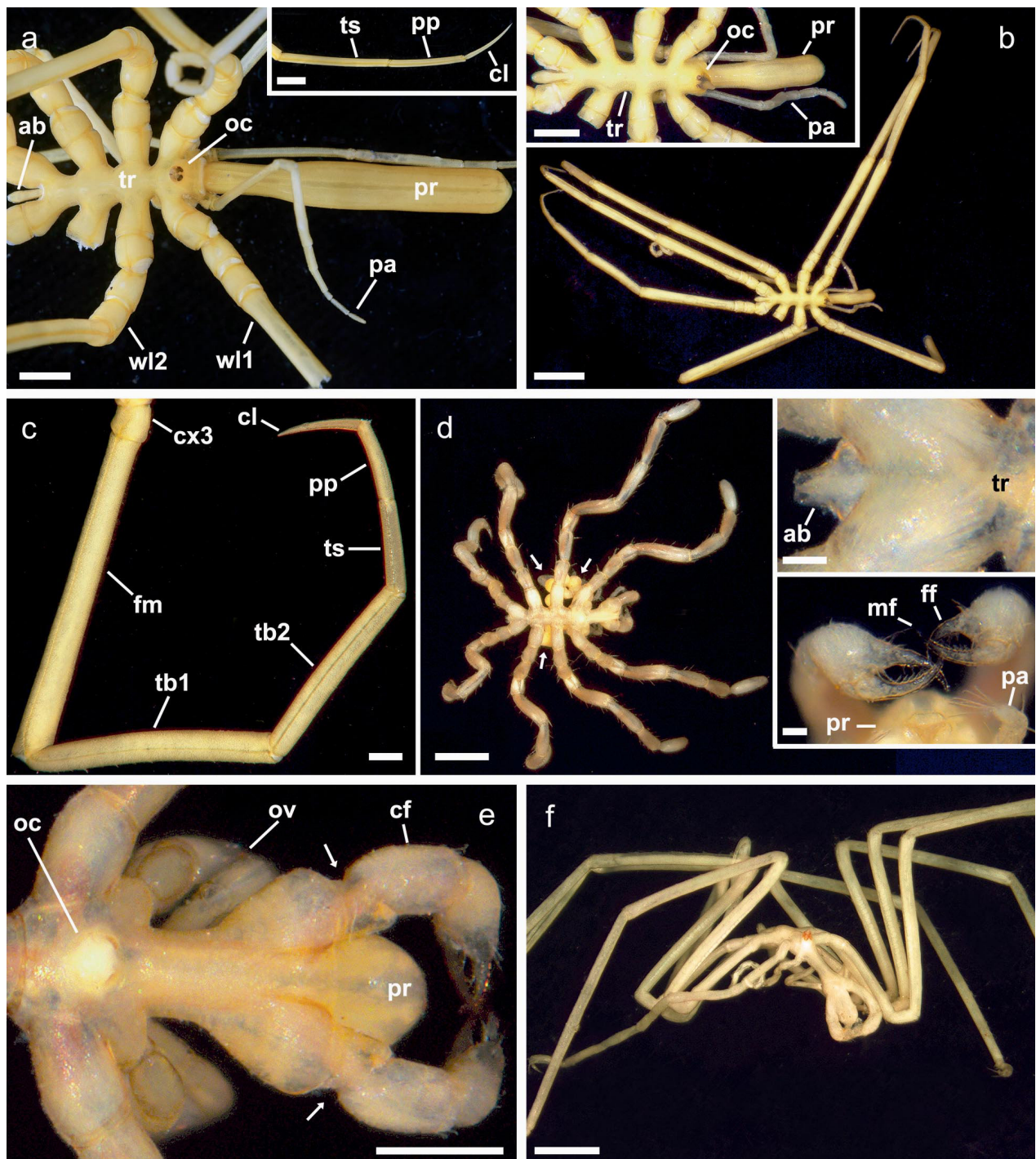
Figure 8. a. *Colossendeis megalonyx;* Dorsal view of trunk and proboscis; scale = 3 mm. Insert: Detail view of tarsus and propodus with claw of right 1st walking leg; scale = 3 mm; **b, c.** *Colossendeis scoresbii*; **b.** Dorsal overview; scale = 5mm. Insert: Dorsal view of trunk and proboscis; scale = 2 mm. **c.** Overview of right 2nd walking leg; scale = 1 mm; **d**–**e.** *Anoropallene palpida*; **d.** Dorsal overview, note eggs (arrows); scale = 1 mm. Insert upper right: Dorsal view of abdomen; scale = 100 μm. Insert middle right: Detail view of chelifores; scale = 100 μm; **e.** Detail view of cephalon, note constrictions of chelifores (arrows); scale = 500 μm; **f.** *Callipallene margarita*; Frontal overview; scale = 1 mm. Abbreviations: **ab** – abdomen; **cf** – chelifore; **cl** – claw; **cx** – coxa; **eg** – eggs; **fm** – femur; **ff** – fixed finger; **mf** – movable finger; **oc** – ocular tubercle; **ov** – oviger; **pa** – palp; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **tr** – trunk; **ts** – tarsus; **wl** – walking leg.

Pursuant to the literature *Anoropallene palpida* has been collected from southern California, the Panama Canal area, Mexico, Peru and Ecuador (Child 1992; Müller 1993). Even though the samples studied here were also collected in the warm-temperate region, with Bahia de Coliumo (Chile) our specimens extend the previously known distribution to the South. The southernmost sample location so far was South-East of Punta Lomas (Peru) (see Child 1992). The specimens examined here have been found about 2500 km more south.

Callipallene **Flynn, 1929**

Callipallene margarita **(Gordon, 1932)**

Figures 8f, 9a–c

Pallene margarita Gordon, 1932: 82–85, figs 40–41

Material examined. Chilean fjord region, Comau fjord, Punta Huinai: 42°22′ S, 72°25′ W; 1 ♂; 12.03.2006; 5–10 m; ZSMA20111039; 4 ♀♀,

1 ♂, 1 juv.; 04.05.2005; 18 m; ZSMA20051909, ZSMA20051914, ZSMA20111535–ZSMA20111538; 3 ♀♀, 6 ♂♂, 4 juv.; 14.03.2011; 25 m; ZSMA20111172–ZSMA20111175, ZSMA20111177, ZSMA20111178, ZSMA20111180–ZSMA20111185, ZSMA20111191; 4 ♀♀, 2 ♂♂; 22.03.2011; 20–30 m; ZSMA20111316–ZSMA20111318, ZSMA20111333–ZSMA20111335. Chilean fjord region, Comau fjord, Huinay, Punta Gruesa: 42°24′ S, 72°25′ W; 3 ♀♀, 1 ♂; 03.03.2009; 8–30 m; ZSMA20111058–ZSMA20111061; 4 ♀♀; 13.03.2011; 20 m;
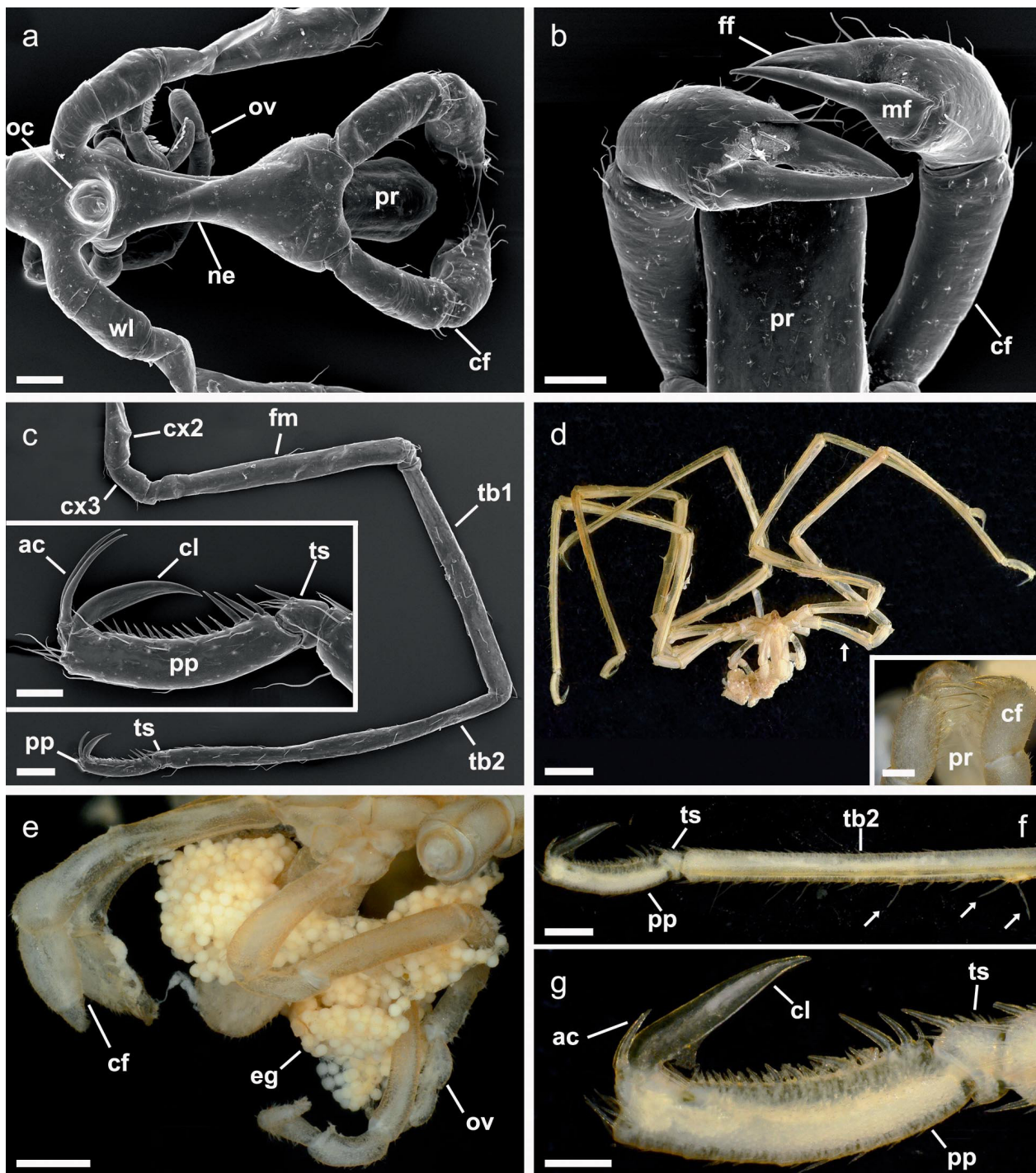
Figure 9. a–c. *Callipallene margarita*; **a.** Dorsal view of cephalon; scale = 100 µm; **b.** Ventral view of chelifores; scale = 100 µm; **c.** Overview of left 1st walking leg; scale = 200 µm. Insert: Detail view of propodus with claw and auxiliary claws (right 3rd walking leg); scale = 100 µm; **d–g.** *Pallenopsis notiosa*; **d.** Frontal overview, note long second coxa (arrow); scale = 3 mm. Insert: Dorsal view of chelifores; scale = 500 µm; **e.** Detail view of ovigers with eggs; scale = 1 mm; **f.** Right 2nd walking leg, note long setae (arrows); scale = 1 mm; **g.** Detail view of propodus with claw and auxiliary claws (right 2nd walking leg); scale = 500 µm. Abbreviations: **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cx** – coxa; **eg** – eggs; **fm** – femur; **ff** – fixed finger; **mf** – movable finger; **ne** – neck; **oc** – ocular tubercle; **ov** – oviger; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **ts** – tarsus; **wl** – walking leg.

ZSMA20111131, ZSMA20111159–ZSMA20111161. Chilean fjord region, Comau Fjord, Huinay, Swall: 42°19′ S, 72°27′ W; 2 ♀♀, 2 juv.; 04.03.2009; 20–30 m; ZSMA20111063–ZSMA20111066.

Remarks. The present material corresponds well with the descriptions in the literature (Gordon 1932; Hedgpeth 1961; Child 1994; Melzer 2009). The lack of both palps (Fig. 9a) and terminal oviger claw as well as the slender legs with long claws and auxiliary claws (Fig. 9c) allow identification of this species as *Callipallene margarita*.

As already mentioned in Melzer (2009) these specimens are the first described from SCUBA-accessible depths between 10–40 m. Also in accordance with our records *Callipallene margarita* is found in southern South America off the shores of both Chile and Argentina. Further sampling localities were Brazil, South Georgia and the Palmer Archipelago in high Antarctic waters.

Pallenopsidae

Pallenopsis Wilson, 1881

Pallenopsis notiosa Child, 1992
Figures 9d–g

Pallenopsis notiosa Child, 1992: 25–27, fig. 10

Material examined. Chile, Temuco: 38°50′ S, 73°38′ W; 3 ♂♂; 344 m; ZSMA20111077–ZSMA20111079.

Remarks. The original description of this species by Child (1992) is matched well by the examined specimens. *Pallenopsis notiosa* is characterized amongst others by its massive chelifores with anaxially articulated short fingers (Fig. 9d–e). Furthermore the immovable finger is slightly shorter than the movable one, which bears many short setae on its proximal third (Fig. 9d). Another important diagnostic character is the long second coxa measuring three times the length of the third coxa (Fig. 9d). Beyond that the cement gland tube is about half as long as the femoral diameter. According to Child (1992) the auxiliary claws are only about 0.4 times as long as the main claw. In our specimens they appear even somewhat shorter (Fig. 9g). The legs and particularly the propodi of the species studied here are very similar to those of *P. meinerti* Schimkewitsch, 1930 (see Stock 1975; Child 1992). But the slightly longer auxiliary claws and the pointed ocular tubercle in the latter species serve to separate it from *P. notiosa*. The ocular tubercle of *P. notiosa* possesses a rounded apex (see Fig. 9d).

To our knowledge, only two specimens of *P. notiosa* have been collected so far, off Bahia Las Canas (see Child 1992). The three males examined in our study have been collected in the warm-temperate region as well, nearly at the same area close-by Temuco, which is located only 3° more to the south. The depth of 344 m lies in the depth range given by Child (1992) which was between 290–450 m.

Pallenopsis patagonica (Hoek, 1881)
Figures 10a–e

Phoxichilidium patagonicum Hoek, 1881: 199, figs 6–9

Synonyms
*Pallenopsis glabra* Möbius, 1902
Pallenopsis hiemalis Hodgson, 1907
Pallenopsis meridionalis Hodgson, 1915
Pallenopsis moebiusi Pushkin, 1975

Material examined. Chilean fjord region, Western Katalalixar, Canal Adalberto: 48°36′28.7″ S, 74°53′55.7″ W; 1 ♀; 12.03.2006; 32 m; ZSMA20111016. Chilean fjord region, Western Katalalixar, Canal Castillo: 48°44′11.4″ S, 75°24′53.1″ W; 1 ♂; 12.03.2006; 15 m; ZSMA20111000; 1 juv.; 23 m; ZSMA20111005. Chilean fjord region, Messier Channel and Fjords, Paso del Abismo: 49°34′38.7″ S, 74°26′49.3″ W; 1 ♀, 1 juv.; 10.03.2006; 28 m; ZSMA20111023, ZSMA20111024. Chilean fjord region, Hanover Area, Canal Pitt Chico: 50°50′07.1″ S, 74°08′20.9″ W; 1 ♂; 07.03.2006; 25 m; ZSMA20111002. Chilean fjord region, Fjords of region X: 43°25′03.0″ S, 74°04′51.2″ W; 1 ♀, 1 ♂; 24.02.2008; 25 m; ZSMA20111003, ZSMA20111006. Chilean fjord region, Fjords of region X: 43°24′34.5″ S, 74°05′00.7″ W; 1 ♀; 24.02.2008; 9 m; ZSMA20111004. Chilean fjord region, Fjords of region X: 43°23′33.4″ S, 74°07′56.5″ W; 1 ♀; February 2008; 26 m; ZSMA20111009. Chilean fjord region, Raul Marin, Las Hermanas: 43°46.285′ S, 073°02.632′ W; 1 juv.; 11.03.2007; 22 m; ZSMA20111012; 1 ♀, 2 juv.; 12.03.2007; 25 m; ZSMA20111020, ZSMA20111026, ZSMA20111027. Chile, Anihue, Raul Marin, Balmaceda, Islas Tres Hermanas: 43°46′31.35″ S, 73°01′44.14″ W; 1 ♂; 17.01.2011; 19 m; ZSMA20111339. Chile, Tierra del Fuego, Canal Murray: 55°00.006′ S, 68°18.881′ W; 1 ♀; 22.12.2010; 28 m; ZSMA20111340. Subantarctic, Falkland Islands West: 50°26′4.00″ S, 62°46′5.00″ W; 1 specimen; 09.02.2010; 146–148 m; ZSMA20111348. Subantarctic, Falkland Islands West: 51°16′8.00″ S, 62°57′8.00″ W; 3 ♀♀, 1 juv.; 05.02.2010; 171–174 m; ZSMA20111349–ZSMA20111352. Subantarctic, Falkland Islands West: 51°5′8.00″ S, 61°44′0.00″ W; 4 ♀♀, 2 ♂♂; 06.02.2010; 174–176 m; ZSMA20111354, ZSMA20111355, ZSMA20111357, ZSMA20111359–ZSMA20111361.

Remarks. The almost glabrous appearance, well separated lateral processes and the long erect abdomen serve to differentiate *P. patagonica* from almost all other *Pallenopsis* species. The movable finger of the chelae bears a prominent proximal pad without conspicuous setae (Fig. 10c). In the male the cement gland tube is very short compared to other species of its group. Also the propodus with claw and auxiliary claws (Fig. 10e) is well in accordance with the description of the literature (Hedgpeth 1961; Child 1995; Gusso & Gravina 2001). Moreover some of the Chilean specimens show some kind of additional eyes on the ocular tubercle just below the usual two pairs (Fig. 10b), a characteristic that was already mentioned by Munilla and Stock (1984) for the species *Pallenopsis bulbifera* Munilla & Stock, 1984.

Another peculiarity we could observe among our material is the difference in size. The specimens from the Falkland Islands are conspicuously larger than those collected from the Chilean coast. While the Chilean specimens show a leg span of about 90 mm, the specimens from the Falklands reach a leg span of approximately 120 mm. But perhaps this could be ex-
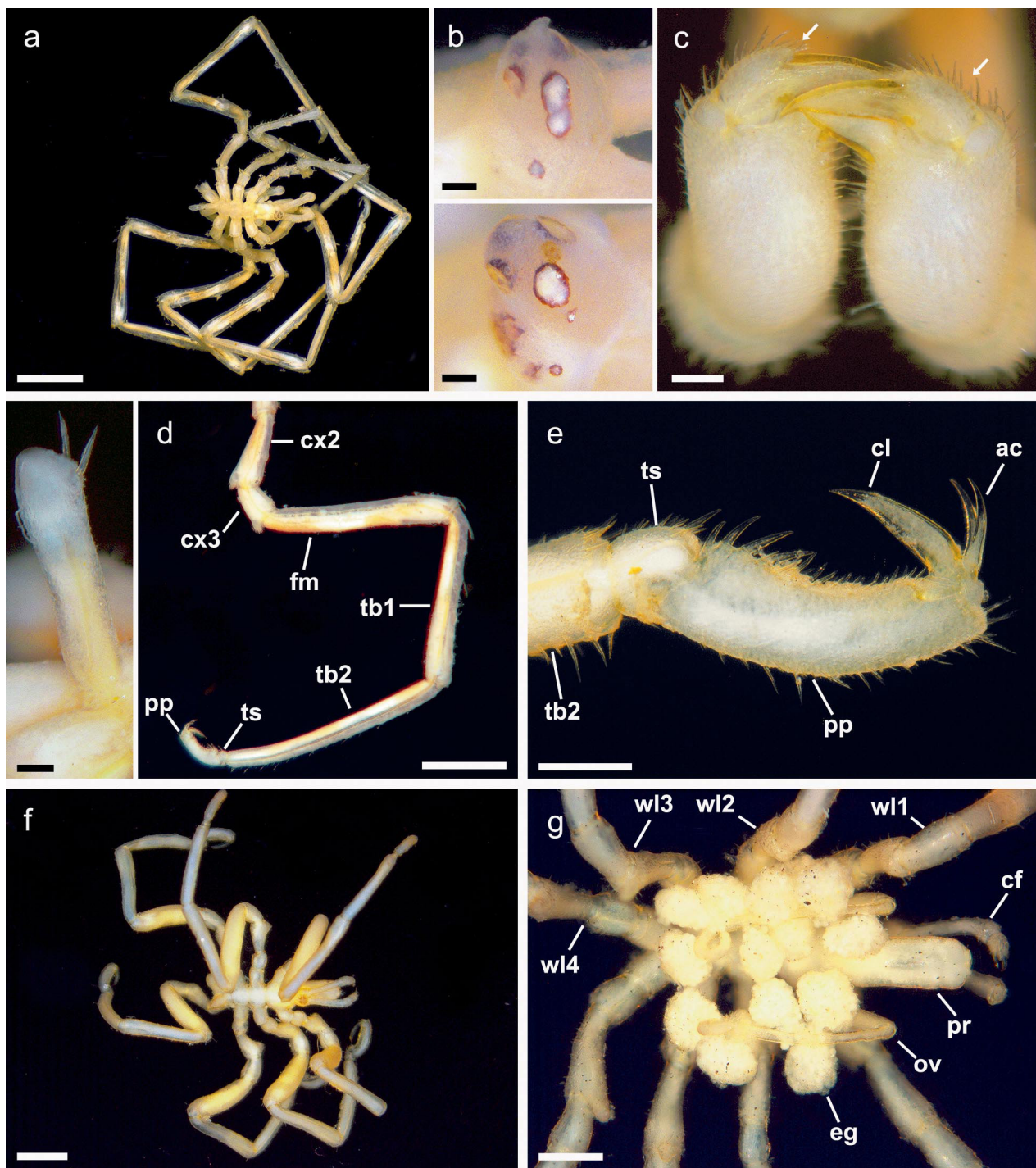
Figure 10. a–e. *Pallenopsis patagonica*; **a.** Dorsal overview; scale = 5 mm; **b.** Detail view of ocular tubercle from two specimens; scales = 250 μm each; **c.** Detail view of chelifores, note movable finger without conspicuous setae (arrows); scale = 500 μm; **d.** Left picture: Detail view of abdomen; scale = 250 μm. Right picture: Overview of right 4th walking leg; scale = 5 mm; **e.** Tarsus and propodus with claw and auxiliary claws of left 1st walking leg; scale = 500 μm; **f, g.** *Anoplodactylus californicus*; **f.** Dorsal Overview; scale = 1 mm; **g.** Male, ventral view of ovigers with eggs; scale = 500 μm. Abbreviations: **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cx** – coxa; **eg** – eggs; **fm** – femur; **ov** – oviger; **pp** – propodus; **pr** proboscis; **tb** – tibia; **ts** – tarsus; **wl** – walking leg.

plained by the deviating depth ranges. Whereas the Falkland specimens were captured between 146–176 m the Chilean individuals could only be collected during SCUBA dives at about 9–32 m. On the other hand the conspicuous size differences in adults could

also be an indication of possibly cryptic species. Previous collecting sites were the Magellanic region, Scotia Sea, Antarctic Peninsula, Ross Sea and several localities around the eastern sector of the Antarctic coast (Child 1994; Munilla & Soler-Membrives 2008).

Phoxichilidiidae

Anoplodactylus Wilson, 1878

Anoplodactylus californicus Hall, 1912

Figures 10f–g, 11a–g

Anoplodactylus californicus Hall, 1912: 91

Synonyms

Anoplodactylus californiensis Hedgpeth, 1941
Anoplodactylus carvalhoi Marcus, 1940
Anoplodactylus portus Calman, 1927
Anoplodactylus projectus Hilton, 1942
Anoplodactylus robustus Hilton, 1939 non Dohrn, 1881

Material examined. Chilean fjord region, Comau fjord, Punta Hui-nay: 42°22′ S, 72°25′ W; 3 ♀♀, 3 ♂♂; 12.03.2006; 5–10 m;
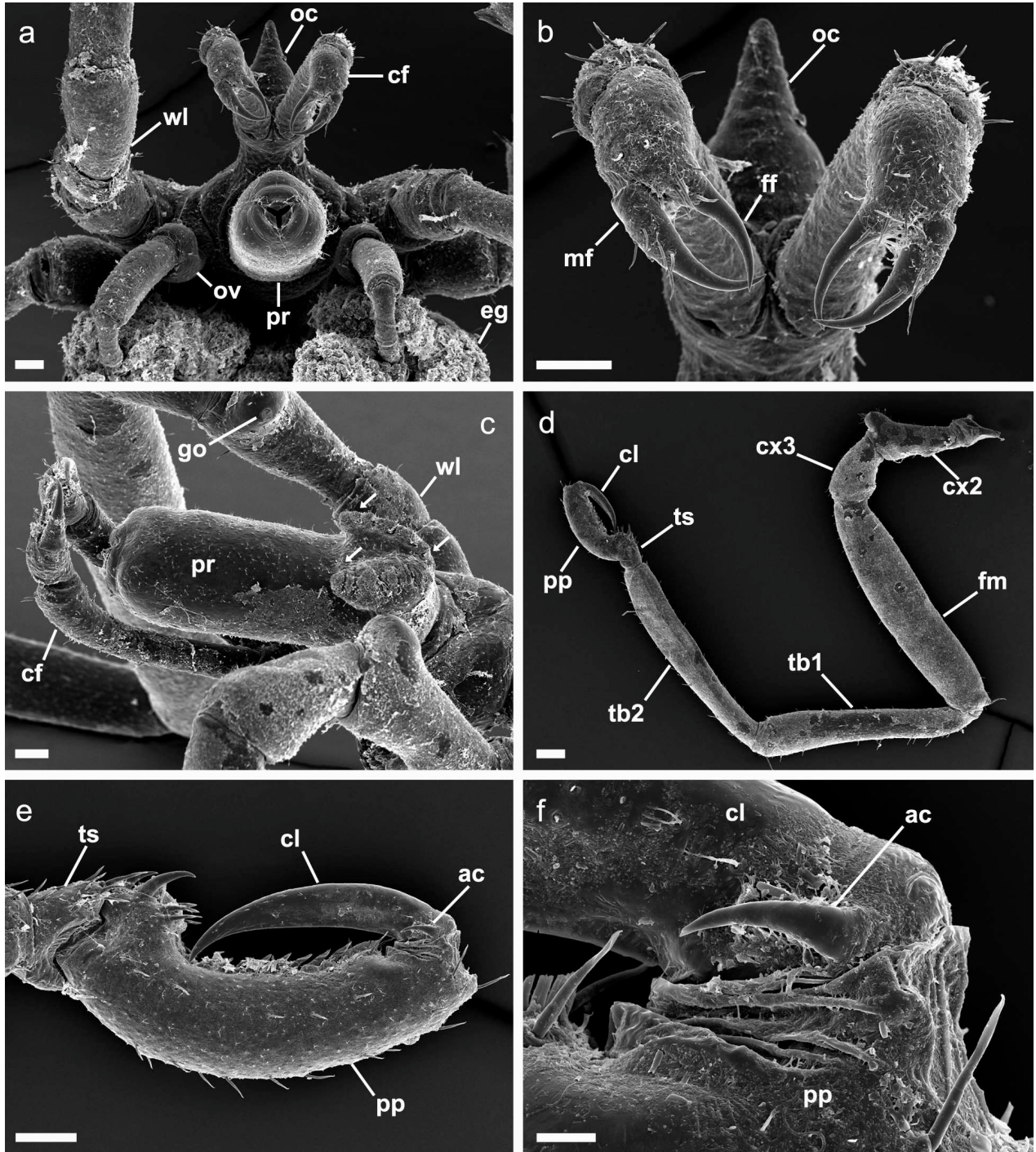
Figure 11. a–f. *Anoplodactylus californicus*; **a.** Male, frontal view; Scale = 100 μm; **b.** Detail view of chelifores; scale = 100 μm; **c.** Female, ventral view of proboscis, note alar process (arrows); scale = 100 μm; **d.** Overview of left 3rd walking leg; scale = 200 μm; **e.** Detail view of tarsus and propodus of left 4th walking leg; scale = 100 μm; **f.** Detail view of auxiliary claw of left 4th walking leg; scale = 20 μm. Abbreviations: **ac** – auxiliary claw; **cf** – chelifore; **cl** – claw; **cx** – coxa; **eg** – eggs; **fm** – femur; **ff** – fixed finger; **go** – genital opening; **mf** – movable finger; **oc** – ocular tubercle; **ov** – oviger; **pp** – propodus; **pr** – proboscis; **tb** – tibia; **ts** – tarsus; **wl** – walking leg.

ZSMA20111028, ZSMA20111030–ZSMA20111032, ZSMA20111035, ZSMA20111036; 1 ♂; 17.03.2006; 8 m; ZSMA20111051; 1 ♂; 14.03.2011; 25 m; ZSMA20111179; 2 ♀♀, 11 ♂♂, 1 juv.; 22.03.2011; 20–30 m; ZSMA20111319– ZSMA20111332; 4 ♂♂; 08.03.2004; 20–30 m; ZSMA20111343– ZSMA20111346. Chilean fjord region, Comau fjord, Huinay, Anti-Punta: 3 specimens; 21.02.2005; 7–30 m; ZSMA20051910, ZSMA20051911, ZSMA20051913. Chilean fjord region, Comau fjord, Playa Llonco; 42°20′ S, 72°27′ W; 1 specimen; 18.02.2005; 10–30 m; ZSMA20051912. Chilean fjord region, Comau fjord, Lilihuapi: 42°09′ S, 72°35′ W; 1 ♀; 14.04.2006; 15–25 m; ZSMA20111048; 1 ♀; 06.01.2005; 20 m; ZSMA20051967; 1 specimen; 07.03.2009; 10–20 m; ZSMA20111070. Chilean fjord region, Comau fjord, Quintopeu: 42°09′ S, 72°26′ W; 1 ♀, 4 ♂♂; 25.02.2005; 15–25 m; ZSMA20051915–ZSMA20051919; 1 ♂; 27.03.2006; 17 m; ZSMA20111053. Chilean fjord region, Comau fjord, Swall: 42°19′ S, 72°27′ W; 1 ♀; 04.03.2009; 20–30 m; ZSMA20111062; 26 ♀♀, 30 ♂♂, 1 juv.; 15.03.2011; 10–20 m; ZSMA20111192, ZSMA20111195–ZSMA20111250. Chilean fjord region, Comau fjord, X-Huinay: 42°19.894′ S, 72°27.661′ W; 1 ♀; 04.03.2009; 20–30 m; ZSMA20111067.

Remarks. The most obvious characteristic of this species is the female proboscis bearing a conspicuous alate process on the ventral side (Fig. 11c). Although a thorough description of this feature is discussed in Arango & Maxmen (2006) the wing-shaped form of this process is unique in the females of this species. The males of *C. margarita* can be distinguished from other species in this genus by their long sex pore tubercles on the second coxae, the tiny cement gland tube and the hairy oviger strigilis (Child 1992). All the specimens studied here match well with the descriptions of the literature (Hedgpeth 1961; Child 1992; Melzer 2009).

The specimens collected from our own sampling trips are some of the southernmost found *Anoplodactylus californicus,* tolerating even water temperatures below 10 °C (see Melzer 2009). According to Child (1994) this species has been collected from the Straits of Magellan south of Punta Arenas as well. Normally this species is found in the tropical and temperate North Atlantic and the Mediterranean Sea (Müller 1993).

Conclusions

Altogether 12 Subantarctic/Chilean pycnogonid species that are housed at the ZSM could be classified and documented regarding their species-specific morphological features, and also their biogeographical distribution. Since the Antarctic pycnogonid fauna is already well explored (see Arango & Wheeler 2007; Arango et al. 2011; Griffiths et al. 2011; Mahon et al. 2008; Munilla & Soler-Membrives 2008; Nielsen et al. 2009; Krabbe et al. 2010; Weis et al. 2011) our aim is to focus on hitherto relatively unexplored neighbouring regions. Out of these Chile is especially interesting because it can be divided in different zones: the warm-temperate (Peruvian), the cold-temperate (Magellanic) and Subantarctic regions. The latter is directly connected to the Antarctic, and it can be surmised that it

underwent postglacial recolonisation from various directions. Also recent molecular studies (Mahon et al. 2008; Krabbe et al. 2010) already show that cryptic speciation exists in Antarctic pycnogonids. Based on this, similar phenomena related to the examined material could also affect our Chilean fjord pycnogonids. To clarify the recolonisation issue, genetic analyses are needed in addition to the morphological examination.

As mentioned above the definition of the Subantarctic area and the Magellanic region differs greatly depending on the view/definitions of the different authors. In our point of view the Falkland Islands should be considered as being part of the Magellanic region, since they are greatly influenced by the Falkland current arriving from Tierra del Fuego and passing the Falklands on the West-side where our specimens were collected. Our material accrues from two different climates namely the temperate zone with Chiloé taken as the southern border (corresponding to the majority of the authors) and the cold-temperate zone enclosing the Magellanic region with Tierra del Fuego and the Falkland Islands.

In this connection it is important to note that our sample includes both species of a probably northern origin that extend far to the south and species with southern origin extending to the north. The first group is represented by *Anoplodactylus californicus* Hall, 1912 from the family Phoxichilidiidae, which is normally found in tropical and subtropical regions. This species was collected by the Lund expedition at about 41° S (Hedgpeth 1961), collected by SCUBA-dives in the Chilean fjord region at about 42° S (Melzer et al. 2006), and has been reported even for the Straits of Magellan at about 53° S by Child (1995). In addition, *Achelia assimilis* could be found at Tierra del Fuego (55° S). Most of the numerous records of this species are located in tropical, subtropical and temperate waters of the southwest Pacific as well as the coasts of Australia and New Zealand. However, along the Chilean coast this species seems to reach the southernmost limit of its distribution range. In the Antarctic region it has not been found to date (Munilla & Soler-Membrives 2008).

Furthermore to our knowledge, with Bahia de Coliumo (Chile) *Anoropallene palpida* (Hilton, 1939) shows also a more southern distribution pattern than previously recorded. So far this species has only been collected from southern California, the Panama Canal area, Mexico, Peru and Ecuador.

In the second group we find *C. megalonyx* with a more northern distribution pattern than previously recorded. This non-cosmopolitan species is predominantly found around the Antarctica and between the Falkland Islands and Patagonia, and our record at about 36° S (near Concepcion) is the northernmost collecting site for this species. This discovery from a warm-temperate zone could be explained by the Humboldt Current arising from the Antarctic and passing along the Chilean coast to the north probably supporting northward dispersal of Magellanic species as suggested by Brattström & Johanssen (1983).

Our sample size does not allow a detailed comparison of species composition/distribution between outer coast, channels, and inner fjords. However, in the inner fjords pycnogonids were not found above the halocline, i.e. in depth between 0 and 5–7 m. Conversely, at the outer coast where a low salinity layer is absent, pycnogonids were sampled already close to the water surface like for example *Achelia assimilis* and *Tanystylum cavidorsum* at Playa Chica. The fjord species thus seem to have a low tolerance for brackish water.

Beside the geographical aspects the species *A. palpida* displays also a morphologically interesting peculiarity. The abnormality regarding the female chelifores of *A. palpida* mentioned in the literature (Child 1979) could be observed in our specimens as well. This observation suggests the possibility that this might be rather a common characteristic of females of this species than an irregularity by chance.

Acknowledgements

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5. Paper III

Weis A & Melzer RR (2012b) How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity* 10(3), 361-374.

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Research Article

How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885)

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The present paper reports on a first attempt at resolving the taxonomy of Chilean Pycnogonida using a combination of DNA sequence and morphological data. In a subproject of the Marine Barcode of Life (MarBoL) campaign we analysed a fragment (about 657 base pairs) of the mitochondrial protein-coding gene COI (cytochrome c oxidase subunit 1) from 76 Chilean/Subantarctic pycnogonids. Since most molecular data on pycnogonids are from the Antarctic region, the new information constitutes a significant extension. The phylogenetic consensus tree displays 10 distinct, well-supported branches corresponding to the studied species, namely *Achelia assimilis* (Haswell, 1885), *Ammothea spinosa* (Hodgson, 1907), *Tanystylum cavidorsum* Stock, 1957, *T. neorhetum* Marcus, 1940, *Colossendeis macerrima* Wilson, 1881, *C. megalonyx* Hoek, 1881, *C. scoresbii* Gordon, 1932, *Callipallene margarita* (Gordon, 1932), *Pallenopsis patagonica* (Hoek, 1881), and *Anoplodactylus californicus* Hall, 1912. These represent four superfamilies, and five of the 11 existing pycnogonid families (Bamber & El Nagar, 2011): Ammotheidae Dohrn, 1881, Colossendeidae Hoek, 1881, Callipallenidae Hilton, 1942, Pallenopsidae Fry, 1978 and Phoxichilidiidae Sars, 1891. Within *Achelia assimilis*, four distinct subbranches correspond to the different geographic regions represented in our samples. While these include a total of 11 distinct haplotypes, the morphological differences among the corresponding specimens lie well within the variation described in the literature for this cosmopolitan species. Therefore, the four branches of *A. assimilis* might represent geographically limited subspecies rather than cryptic species. Repeated drastic glaciation of the fjord region during the Cenozoic resulting in alternating extinction and recolonization phases and the holobenthic lifecycle of sea spiders are discussed as the main factors resulting in the observed phylogeographic pattern. Standard barcoding sequences are confirmed as a suitable tool in addition to morphology for taxonomic analyses in Pycnogonida. The corresponding haplotype distribution patterns allow inferences on the biogeographical history of the relatively unexplored Chilean fjord region.

Key words: Ammotheidae, biogeography, COI, cryptic species, Pantopoda, Subantarctic

Introduction

In recent years, molecular approaches have increasingly been taken as an adjunct to classical taxonomy in marine benthic invertebrate groups. The results are used as the basis for taxonomic descriptions and revisions as well as for studies on speciation processes. Besides common groups such as echinoderms (Hunter & Halanych, 2008), molluscs (Wilson *et al.*, 2009; Joerger *et al.*, 2010) or crustaceans (Lefebure *et al.*, 2006; Raupach & Wägele, 2006; Oliveira-Biener *et al.*, 2010), pycnogonids are also gaining more research interest concerning molecular or phylogenetic studies (Mahon *et al.*, 2008; Nielsen *et al.*, 2009; Arabi *et al.*, 2010; Krabbe *et al.*, 2010; Masta *et al.*, 2010; Arango *et al.*,

2011; Dietz *et al.*, 2011). A particular study focus lies on the Southern Oceans, where pycnogonids appear with high species richness, endemism and wide geographic distribution (Munilla Leon, 2001; Mahon *et al.*, 2008; Munilla & Soler Membrives, 2008; Weis *et al.*, 2011). In adjacent areas, specifically in the South American Magellan region, Tierra del Fuego and the Chilean fjord region, pycnogonid research has included morphological analyses only (Loman, 1923*a*, 1923*b*; Hedgpeth, 1961; Sielfeld, 2003; Melzer *et al.*, 2006; Melzer, 2009; Weis & Melzer, 2012).

The 90 000 km long southern Chilean coastline was covered by glaciers during the last ice age 15 000 years ago, and was subsequently recolonized by benthic communities (Försterra, 2009). The fact that species could recolonize Chile by immigration either from the north or the south, combined with the lack of a planktonic stage in

pycnogonids (King, 1973; Arnaud & Bamber, 1987), makes them an interesting group to study in this region. Using integrative taxonomy (Dayrat, 2005; Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010), which combines morphological species determination by means of classical taxonomy with modern methodological developments like DNA barcoding, helps us to recheck species boundaries or search for cryptic species previously undetected by morphological analysis. According to Hebert *et al.* (2003*a*, 2003*b*) the mitochondrial gene cytochrome c oxidase I (COI) can serve as 'the core of a global bioidentification system for animals'. Advantages of choosing a mitochondrial gene over a nuclear gene are, for example, the absence of introns, the limited exposure to recombination, and the haploid mode of inheritance (Saccone *et al.*, 1999). Furthermore, the rapid evolution of the COI gene allows not only the discrimination of closely allied species, but also predictions concerning phylogeographic groups within a single species (Cox & Hebert, 2001; Wares & Cunningham, 2001).

The aim of the present study is to document for the first time COI sequences of pycnogonids from the Chilean fjords and surrounding areas. A special focus is directed at *A. assimilis*, which is one of the most abundant species in this region and shows a remarkable distribution pattern. We hypothesized that molecular data for this species from various sampling locations might give a first hint concerning speciation processes correlated with the last glaciation periods. A detailed description of the morphology of *A. assimilis* has been given in Weis & Melzer (2012).

Materials and methods

Specimens and vouchers

Specimens were collected by scuba-diving during several expeditions along the Chilean coastline organized by the Huinay Scientific Field Station between 2005 and 2011 (Huinay fjordos 3-10). In addition we received samples from more northern areas in Chile as well as from the Falkland Islands. The material was preserved in 96% ethanol to ensure high-quality DNA for genetic analysis. Prior to molecular analysis, species determination was made based on external morphology according to Loman (1923*a*, 1923*b*), Gordon (1932), Marcus (1940), Hedgpeth (1961) and Fry & Hedgpeth (1969), and rechecked with the more recent works of Pushkin (1993), Child (1992, 1994, 1995), Melzer (2009) and Weis *et al.* (2011).

All barcoded voucher specimens are kept at the Zoologische Staatssammlung München (ZSM) under specific museum voucher IDs (see also Table 1); their respective DNA extract aliquots are stored at the Canadian Center for DNA Barcoding (CCDB) and the ZSMs DNA bank facility. Tables 1 and 2 list collection data, BOLD and GenBank accession numbers of all 76 pycnogonid sequences produced in this study and of the chosen

outgroup taxa. Further specimen details can be accessed in Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007, http://www.boldsystems.org) under the project CFAP (Chilean Fjord Pycnogonids) as part of the 'Marine Life (MarBOL)' campaign. Morphological documentation for this paper was done using the following specimens: ZSMA20111055, ZSMA20111086 and ZSMA20111094 for light microscopy; ZSMA20111540, ZSMA20111545-ZSMA20111547 for SEM studies. The sequences for three additional haplotypes of *P. patagonica* and one of *A. assimilis* were accessed from GenBank (FJ969367-69, DQ390087) (see also Table 2).

DNA extraction and sequencing

Depending on the size of the individual, either whole legs or a piece of one leg was taken for DNA extraction. Sequencing was performed at the CCDB using the standard protocols of IBOL (http://dnabarcoding.ca/pa/ge/research/protocols). Specimen data, images and DNA sequences of the studied pycnogonids will be available from BOLD and GenBank.

Search for species boundaries

Intra- and interspecific distances were calculated (except for DQ390087, FJ969367-69, which were mined from GenBank) using the K2P distance model in BOLD. The search for barcoding gaps was performed with the freely available software ABGD (Automatic Barcode Gap Discovery) (Puillandre *et al.*, 2012).

Phylogenetic analysis

Altogether, 80 pycnogonid sequences and seven outgroup sequences were used for phylogenetic analysis. DNA sequences were aligned with Muscle using Geneious Pro version 5.5.4 (Drummond *et al.*, 2011). Aligned COI nucleotide sequences were translated into amino acids using the invertebrate mitochondrial genetic code to check for frameshift mutations or stop codons. Base pair frequencies were calculated with MEGA 5.05 (Tamura *et al.*, 2011). The alignment was tested statistically for substitutional saturation in DAMBE 5.2.69 (Xia *et al.*, 2003; Xia & Lemey, 2009).

Nucleotide composition, maximum parsimony (MP) and neighbour-joining (NJ) trees based on the Kimura 2-parameter (K2p) model (Kimura, 1980; Saitou & Nei, 1987) with bootstrap values (1000 replicates) were calculated using MEGA 5.05 software. Maximum likelihood (ML) was performed after 1000 replicates by ML bootstrap analysis under RaxML 7.0.4. Based on Modeltest by MEGA 5.0 the selected model was GTRCAT+I+G with proportion of invariable sites $= 0.389486$, and gamma shape parameter $= 0.99356$. The same model was chosen for

Table 1. Overview of collection data and registration of specimens included in this study.

Museum voucher ID	Species	Country/Region	Latitude	Longitude	Depth	BOLD ID
ZSMA20111001	Achelia assimilis	Chile; Region de Magallanes y de la Antarctica Chilena	50°20′23.1″S	75°22′39.2″W	20m	CFAP092-11
ZSMA20111085	Achelia assimilis	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP078-11
ZSMA20111086	Achelia assimilis	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP079-11
ZSMA20111087	Achelia assimilis	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP080-11
ZSMA20111089	Achelia assimilis	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP081-11
ZSMA20111162	Achelia assimilis	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP082-11
ZSMA20111163	Achelia assimilis	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP083-11
ZSMA20111164	Achelia assimilis	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP084-11
ZSMA20111166	Achelia assimilis	Chile; Region de los Lagos	42°22′S	72°25′W	25m	CFAP085-11
ZSMA20111256	Achelia assimilis	Chile; Region de los Lagos	42°34′50.0″S	72°33′14.6″W	20m	CFAP086-11
ZSMA20111257	Achelia assimilis	Chile; Region de los Lagos	42°34′50.0″S	72°33′14.6″W	20m	CFAP087-11
ZSMA20111258	Achelia assimilis	Chile; Region de los Lagos	42°34′50.0″S	72°33′14.6″W	20m	CFAP088-11
ZSMA20111259	Achelia assimilis	Chile; Region de los Lagos	42°34′50.0″S	72°33′14.6″W	20m	CFAP089-11
ZSMA20111341	Achelia assimilis	Chile; Region de Magallanes y de la Antarctica Chilena	55°03′71.96″S	68°44′13.48″W		CFAP090-11
ZSMA20111342	Achelia assimilis	Chile; Region de Magallanes y de la Antarctica Chilena	55°03′71.96″S	68°44′13.48″W		CFAP091-11
ZSMA20111356	Ammothea spinosa	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP048-11
ZSMA20111195	Anoplodactylus californicus	Chile; Region de los Lagos	42°19′S	72°27′W	10-20m	CFAP061-11
ZSMA20111196	Anoplodactylus californicus	Chile; Region de los Lagos	42°19′S	72°27′W	10-20m	CFAP062-11
ZSMA20111197	Anoplodactylus californicus	Chile; Region de los Lagos	42°19′S	72°27′W	10-20m	CFAP063-11
ZSMA20111198	Anoplodactylus californicus	Chile; Region de los Lagos	42°19′S	72°27′W	10-20m	CFAP064-11
ZSMA20111320	Anoplodactylus californicus	Chile; Region de los Lagos	42°22′S	72°25′W	20-30m	CFAP065-11
ZSMA20111321	Anoplodactylus californicus	Chile; Region de los Lagos	42°22′S	72°25′W	20-30m	CFAP066-11
ZSMA20111322	Anoplodactylus californicus	Chile; Region de los Lagos	42°22′S	72°25′W	20-30m	CFAP067-11
ZSMA20111323	Anoplodactylus californicus	Chile; Region de los Lagos	42°22′S	72°25′W	20-30m	CFAP068-11
ZSMA20111131	Callipallene margarita	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP069-11
ZSMA20111159	Callipallene margarita	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP070-11
ZSMA20111160	Callipallene margarita	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP071-11
ZSMA20111161	Callipallene margarita	Chile; Region de los Lagos	42°24′S	72°25′W	20m	CFAP072-11
ZSMA20111173	Callipallene margarita	Chile; Region de los Lagos	42°22′S	72°25′W	25m	CFAP073-11
ZSMA20111174	Callipallene margarita	Chile; Region de los Lagos	42°22′S	72°25′W	25m	CFAP074-11
ZSMA20111182	Callipallene margarita	Chile; Region de los Lagos	42°22′S	72°25′W	25m	CFAP076-11
ZSMA20111336	Colossendeis macerrima	Chile; Region de Asien del General Carlos Ibanez del Campo	45°54.471′S	75°36.021′W	510m	CFAP040-11
ZSMA20111337	Colossendeis macerrima	Chile; Region de Asien del General Carlos Ibanez del Campo	45°54.471′S	75°36.021′W	510m	CFAP041-11
ZSMA20111071	Colossendeis megalonyx	Chile; Region del Bio-Bio	36°24.010′S	73°43.074′W	769m	CFAP038-11
ZSMA20111358	Colossendeis megalonyx	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP044-11
ZSMA20111364	Colossendeis megalonyx	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP045-11
ZSMA20111347	Colossendeis scoresbii	Falkland Islands	50°40′5.00″S	62°26′1.00″W	160-165m	CFAP042-11
ZSMA20111353	Colossendeis scoresbii	Falkland Islands	51°16′8.00″S	62°57′8.00″W	171-174m	CFAP043-11
ZSMA20111362	Colossendeis scoresbii	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP046-11
ZSMA20111363	Colossendeis sp.	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP047-11
ZSMA20111000	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	48°44′11.4″S	75°24′53.1″W	15m	CFAP013-11
ZSMA20111002	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	50°50′07.1″S	74°08′20.9″W	25m	CFAP017-11

Table 1. (Continued)

Museum voucher ID	Species	Country/Region	Latitude	Longitude	Depth	BOLD ID
ZSMA20111003	Pallenopsis patagonica	Chile; Region de los Lagos	43°25′03.0″S	74°04′51.2″W	25m	CFAP006-11
ZSMA20111004	Pallenopsis patagonica	Chile; Region de los Lagos	43°24′34.5″S	74°05′00.7″W	9m	CFAP005-11
ZSMA20111005	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	48°44′11.4″S	75°24′53.1″W	23m	CFAP014-11
ZSMA20111006	Pallenopsis patagonica	Chile; Region de los Lagos	43°25′03.0″S	74°04′51.2″W	20m	CFAP007-11
ZSMA20111008	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	50°24′52″S	74°33′33″W	15-25m	CFAP026-11
ZSMA20111009	Pallenopsis patagonica	Chile; Region de los Lagos	43°23′33.4″S	74°07′56.5″W	26m	CFAP004-11
ZSMA20111012	Pallenopsis patagonica	Chile; Region de los Lagos	43°46′28.5″S	073°02′63.2″W	22m	CFAP008-11
ZSMA20111016	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	48°36′28.7″S	74°53′55.7″W	32m	CFAP012-11
ZSMA20111017	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	48°36′28.7″S	74°53′55.7″W	32m	CFAP025-11
ZSMA20111024	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	49°34′38.7″S	74°26′49.3″W	28m	CFAP016-11
ZSMA20111072	Pallenopsis patagonica	Chile; Region de Valparaiso	33°23′55″S	71°52′78.2″W	339m	CFAP023-11
ZSMA20111339	Pallenopsis patagonica	Chile; Anihue Raul Marin Balmaceda	43°46′31.35″S	73°01′44.14″W	19m	CFAP019-11
ZSMA20111340	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena	55°00′00.6″S	68°18′88.1″W	24m	CFAP018-11
ZSMA20111348	Pallenopsis patagonica	Falkland Islands	50°26′4.00″S	62°46′5.00″W	146-148m	CFAP027-11
ZSMA20111349	Pallenopsis patagonica	Falkland Islands	51°16′8.00″S	62°57′8.00″W	171-174m	CFAP034-11
ZSMA20111350	Pallenopsis patagonica	Falkland Islands	51°16′8.00″S	62°57′8.00″W	171-174m	CFAP035-11
ZSMA20111351	Pallenopsis patagonica	Falkland Islands	51°16′8.00″S	62°57′8.00″W	171-174m	CFAP036-11
ZSMA20111352	Pallenopsis patagonica	Falkland Islands	51°16′8.00″S	62°57′8.00″W	171-174m	CFAP037-11
ZSMA20111354	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP028-11
ZSMA20111355	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP029-11
ZSMA20111357	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP030-11
ZSMA20111359	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP031-11
ZSMA20111360	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP032-11
ZSMA20111361	Pallenopsis patagonica	Falkland Islands	51°05′8.00″S	61°44′0.00″W	174-176m	CFAP033-11
ZSMA20111090	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP053-11
ZSMA20111091	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP054-11
ZSMA20111102	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP057-11
ZSMA20111103	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP058-11
ZSMA20111104	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP059-11
ZSMA20111105	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP060-11
ZSMA20111110	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP055-11
ZSMA20111111	Tanystylum cavidorsum	Chile; Region de los Rios	39°43′10.3″S	73°24′11.8″W	0-1m	CFAP056-11
ZSMA20111011	Tanystylum neorhetum cf	Chile; Region de Magallanes y de la Antarctica Chilena	50°50′07.1″S	74°08′20.9″W	20m	CFAP049-11
ZSMA20111015	Tanystylum neorhetum cf	Chile; Region de los Lagos	43°46.285′S	073°02.632′W	16m	CFAP051-11

Table 2. Overview of pycnogonids and outgroup specimens mined from GenBank.

	GenBank ID	Species
Pycnogonids	DQ390087	Achelia assimilis
	FJ969367	Pallenopsis patagonica
	FJ969368	Pallenopsis patagonica
	FJ969369	Pallenopsis patagonica
Outgroups	AF216203	Limulus polyphemus
	AINV-019	Limulus polyphemus
	EU834780	Limulus polyphemus
	NC_003057	Limulus polyphemus
	AY731174	Mastigoproctus giganteus
	EU520643	Mastigoproctus giganteus
	NC_010430	Mastigoproctus giganteus

calculating Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Bayesian analysis was performed with 5.5 million Metropolis-coupled MCMC generations, with tree sampling every 200 generations and a burn-in of 6875. The figure of the recovered phylogenetic tree was edited with FigTree 1.3.1 and MEGA 5.0.

COI haplotype network

For the species *Achelia assimilis*, we constructed a statistical parsimony network with all 16 individual COI sequences, using TCS 1.21 (Clement *et al.*, 2000). To illustrate the number of mutation steps between the different

haplotypes in a single parsimony network, the maximum number of connection steps was raised to 69.

Morphological analysis

All studied pycnogonids were documented using a Wild M400 photomacroscope equipped with a digital camera (Nikon D700) by taking several shots focused at different levels along the z-axis. This series of pictures was then edited and combined to a single respective image with greater depth of field using the computer software CombineZ. Specimens used for scanning electron microscopy (SEM) documentation were transferred into 100% acetone for dehydration in three steps of about 20 minutes each. Subsequent critical-point drying was performed on a BAL-TEC CPD 030 at 40 °C and 80 bar. Afterwards specimens were sputtered with gold for three minutes using a Balzers Polaron E5100. Pictures (2048 × 1536 px; 72 dpi; colour depth 8 bit) were taken at 15 kV using a LEO 1430VP scanning electron microscope. Further editing and composition of both light microscopic and SEM pictures was done with Adobe Photoshop CS.

Results

Molecular and phylogenetic analysis

The 657-bp COI fragment alignment shows no gaps and includes 80 pycnogonid specimens (10 species), as well as three *Mastigoproctus giganteus* and four *Limulus polyphemus* sequences chosen as outgroups. A total of 10 Chilean/Subantarctic pycnogonid COI branches could be identified (Fig. 1). These correspond to the species *Achelia assimilis, Ammothea spinosa, Tanystylum cavidorsum, T. neorhetum, Colossendeis macerrima, C. megalonyx, C. scoresbii, Callipallene margarita, Pallenopsis patagonica* and *Anoplodactylus californicus*. The morphological determinations of the species are well in accordance with the molecular analysis, which showed 10 well-defined clusters within the studied pycnogonids. All branches received high bootstrap support, which indicates that the COI barcoding method is a suitable tool for resolving relationships of among these pycnogonids at species level.

Although the COI gene appears as inadequate for resolving relationships at taxonomic levels higher than species, some of the pycnogonid families studied here show distinct COI branches as well. For example, *Ammothea* Leach, 1814 and *Tanystylum* Miers, 1879, both genera belonging to the Ammotheidae, form a single COI branch. Similarly, all three examined *Colossendeis* species, viz. *C. macerrima, C. megalonyx* and *C. scoresbii*, are clustering together, representing the Colossendeidae with supported bootstrap values >95%. On the other hand, *Anoplodactylus californicus* and *Callipallene margarita* belong to different families (Phoxichilidiidae and Callipallenidae) and form distinct branches with high bootstrap values >99%.

Calculated mean base pair frequencies for A (29.7%), C (17.5%), G (15.0%) and T (37.8%) indicated a bias towards adenosine and thymine, which is characteristic for arthropods. The index of substitution saturation (Iss) was tested for the whole alignment as well as for the third codon position. Iss was always significantly lower than the critical Iss.c value, indicating only little substitution saturation. Our COI data showed no frameshift mutations or stop codons when sequences were translated using the invertebrate mitochondrial codon table.

Among our 76 pycnogonid sequences the mean interspecific distance was 18.83%. The lowest interspecific distance was found between *Colossendeis megalonyx* and *C. scoresbii* (13.36%), the maximum distance between *Callipallene margarita* and *Anoplodactylus californicus* (29.4%). Intraspecific distances ranged from 0.09% to 10.4% (mean 2.48%). *Achelia assimilis* and *Pallenopsis patagonica* showed the highest mean intraspecific values at 6.81% and 10.4%, respectively (see Fig. 2).

For the phylogenetic analysis we reconstructed three rooted phylograms (BI, ML and NJ) and one cladogram (MP). Since all trees were concordant in topology, Figure 1 shows the Bayesian inference tree only, but indicates the bootstrap values of the other trees as well. Since a Baysian tree is depicted, and the majority of the applied methods of analyses result in phylograms we name its components branches. The two outgroup species, *Mastigoproctus giganteus* and *Limulus polyphemus*, are clustering against all pycnogonid sequences with boostrap values >99%.

Although each studied species forms a single COI branch, *Pallenopsis patagonica* and *Achelia assimilis* both show four distinct and well-supported subbranches. The number of individuals representing the four different branches of *P. patagonica* varies between 1 and 15 specimens. Since *P. patagonica* seems to be a more complex case, a detailed analysis of this species, including more individuals from more geographic regions, will be the goal of a future publication.

The subbranches of *A. assimilis* are distinctly split by geographic origin. Four specimens from the Región de los Rios, eight from Región de los Lagos, and three specimens from a more southern Chilean area (>50 °S) cluster in a single branch each, with bootstrap values >93%. The sequence DQ390087 for *A. assimilis* from Australia contrasts to the three Chilean branches (bootstrap value >95%).

Given the high intraspecific divergence and high support for branches, we used ABGD to test whether these clusters comprise cryptic species. Based on the different calculated distance values (minimum 0.01, maximum 0.18) this analysis also shows four distinct branches, with three obvious barcode gaps in between (Fig. 3).

A parsimony network for the *A. assimilis* branch is shown in Figure 4. The COI data for the 16 specimens formed 11
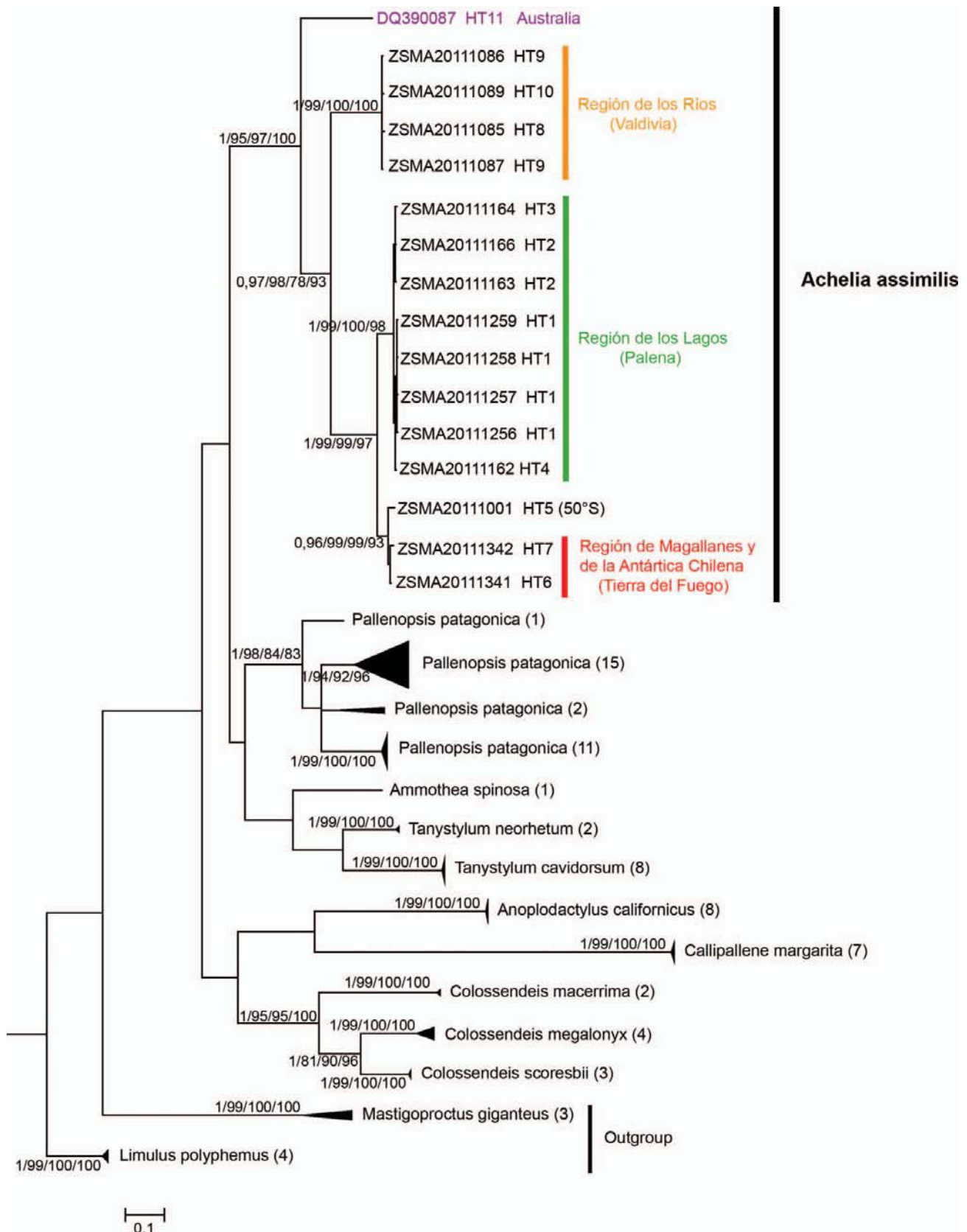
Fig. 1. Bayesian inference tree of cytochrome c oxydase I (COI) sequences, showing the placement of 80 pycnogonids, plus 7 outgroup specimens retrieved from GenBank. Numbers in brackets indicate the number of analysed individuals. Different haplotypes of *A. assimilis* are defined as HT1-HT11. Numbers above and below branches show posterior probability of BI and bootstrap values (>75%) of NJ, MP and ML analyses; branch length indicates substitutions per site.
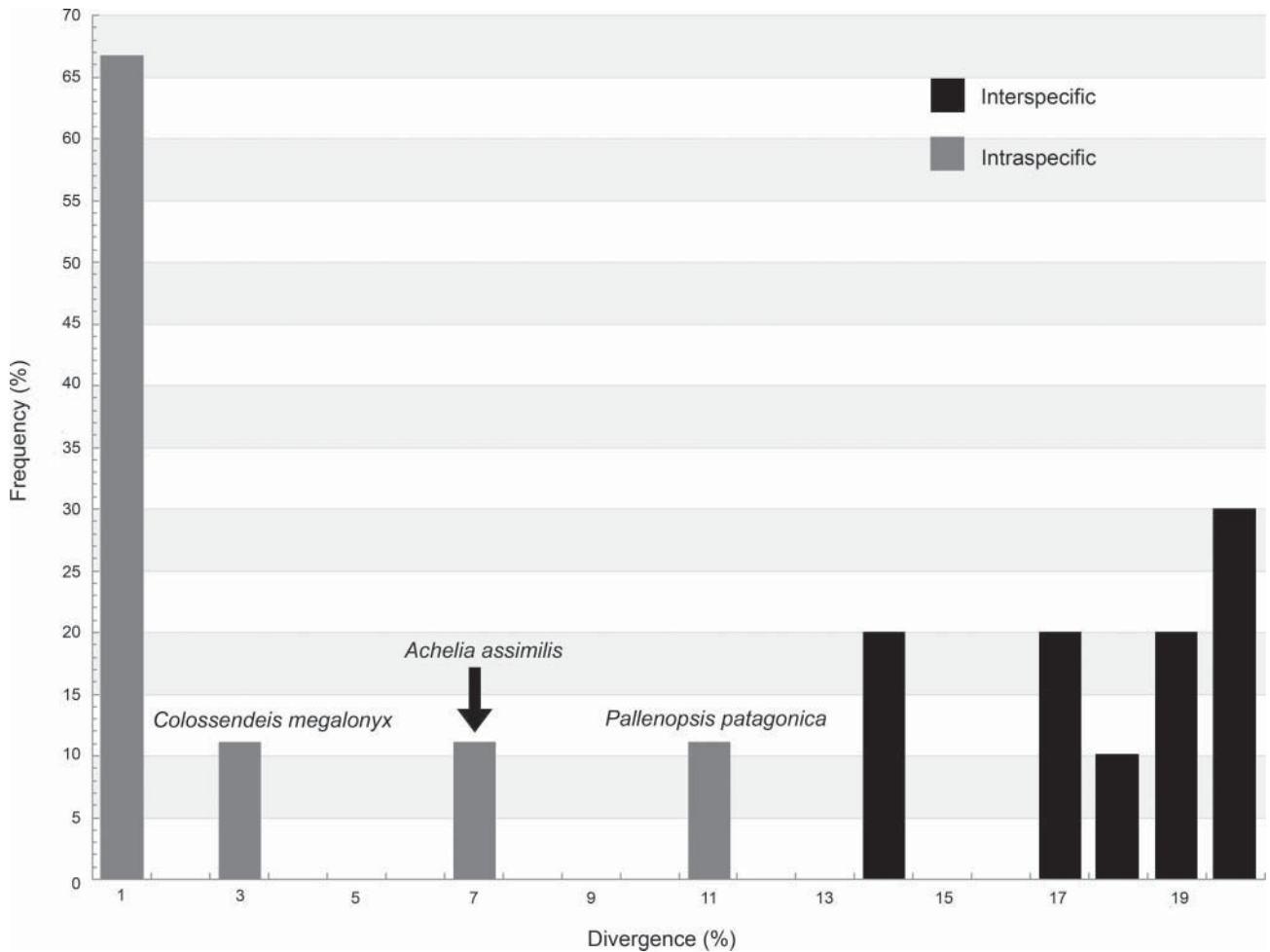
Fig. 2. Intraspecific and interspecific distance distribution among cytochrome c oxidase I sequences for 76 pycnogonids.

distinct haplotypes. Some haplotypes were represented by multiple individuals. Haplotype 1 comprised four individuals (ZSMA20111256-59), all collected from Reñihué fjord; haplotype 2 contained two individuals (ZSMA20111163,
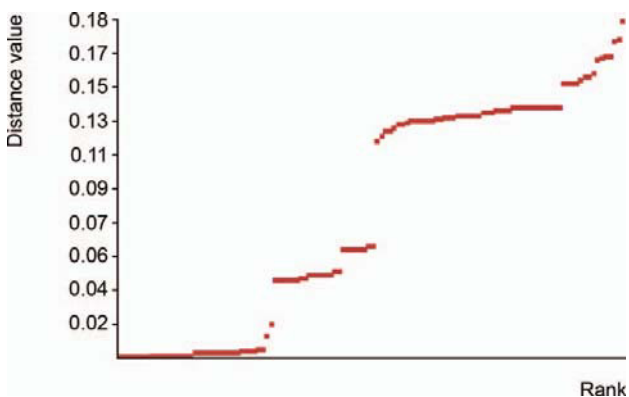

Fig. 3. Automatic Barcode Gap Discovery (ABGD) analysis for all 16 specimens of *Achelia assimilis* used in this study.

ZSMA20111166) from the Comau fjord; and sequences ZSMA20111086 and ZSMA20111087, both collected near Valdivia, showed the same haplotype (HT9). In the *A. assimilis* alignment we found a total of five amino acid replacement substitutions. Other illustrated replacement substitutions involved only amino acids with similar chemical characteristics. Specimens collected from Región de los Lagos showed four haplotypes (HT1–HT4); four *A. assimilis* from Región de los Rios represented three haplotypes (HT8–HT10) that differ by a maximum of two substitutions only, one of which involves a single amino acid substitution (HT2/HT3). Each of the three specimens from the Región de Magallanes y de la Antártica Chilena formed its own haplotype (HT5–HT7); the latter differed by either two amino acid substitutions (HT6/HT7) or a maximum of five substitutions (HT5/HT6). The Australian *A. assimilis* individual (HT11) differed by a minimum of 69 substitutions (HT11/HT5), including one amino acid replacement. Analysis of the 16 COI sequences of *A. assimilis* using TCS with 99% and 90% statistical parsimony connection limits,
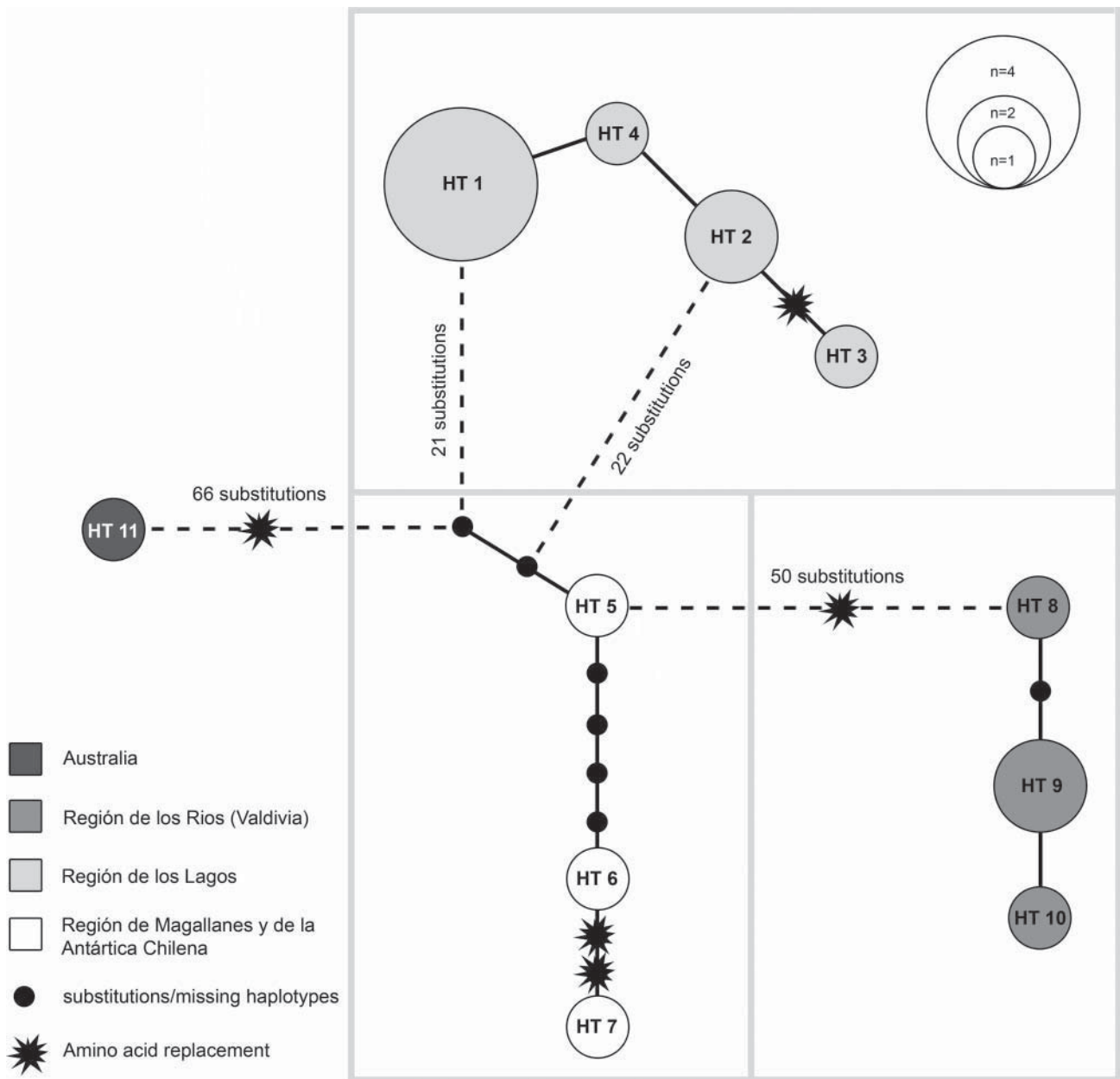
Fig. 4. Statistical parsimony network of 11 COI haplotypes (HT1-HT11) in *Achelia assimilis*. Sizes of the circles are proportional to the number of specimens. Lines represent the most-parsimonious relationships between haplotypes; black dots represent intermediate haplotypes missing in the sample set. Dashed lines show branches with a connection limit <90% (allowing a maximum number of 69 steps). Shades of grey indicate different geographic locations.

respectively, resulted in four separate networks representing the four subbranches already displayed in the phylogenetic tree (Fig. 1). Allowing a fixed connection limit at 69 steps we obtained a statistical parsimony network in which the four groups were connected by long branches illustrating high numbers of substitutions (including two amino acid substitutions between HT11/HT5 and HT5/HT8) between haplotypes from different geographical regions (Fig. 4).

Morphology

The members of *A. assimilis* show great morphological variation concerning the spination of the trunk, lateral processes and first coxae (Figs 5–8). Whereas the number of trunk spines is either two or three, the number of spines on the lateral processes and first coxae varies between two and five (Figs 6–8). However, based on the distinct characteristics of the species, for example the leg setae borne on
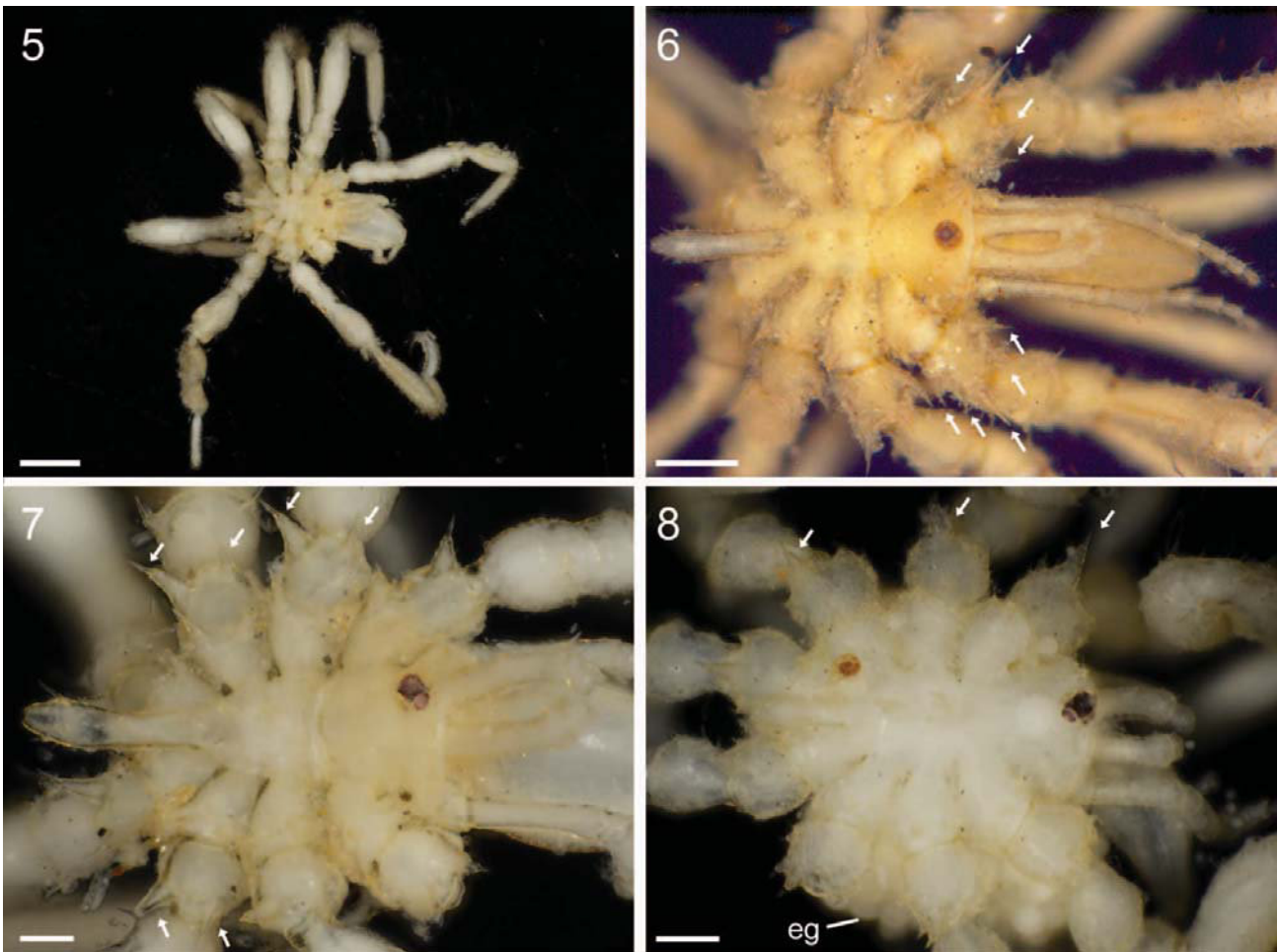
Figs 5–8. Light microscopy of *Achelia assimilis*. **5.** Dorsal overview; scale = 1 mm. **6–8.** Variation of spination of first coxae (arrows); scales = 500 μm, 250 μm and 250 μm, respectively.

rounded tubercles (Figs 12–13), the spination of the trunk (Figs 9–10), the auxiliary claws one-half to two-thirds as long as the terminal claw, and the long (see Weis & Melzer, 2012) and slender abdomen (Fig. 11), all studied specimens are clearly identified as *A. assimilis*. Furthermore, when we checked the leg length ratio of several individuals from different collection sites, the femur, tibia 1 and tibia 2 always showed proportions near 1:1:1. Detailed illustrations of legs and further *A. assimilis* characteristics can be found in Weis & Melzer (2012). Specimens from Playa Chica (Región de los Rios) are generally smaller but were sexually mature, as some of the males carry eggs (Fig. 8). The spines on the lateral processes and first coxae of these specimens are less prominent, but this too falls within the known variation in this species. Consequently, with all individuals showing the typical features of *A. assimilis* mentioned above, and without any morphological pattern that would correspond to the different molecular branches, we determined all corresponding specimens as *A. assimilis*.

Discussion

Our study demonstrates that DNA barcoding provides a reliable tool for identifying Chilean and Subantarctic pycnogonids to species level. Although it is generally assumed that the COI sequence is not adequate to resolve higher phylogenetic relationships, it is conspicuous that species from the same genus form clusters with high bootstrap support (>95%). *Tanystylum cavidorsum* and *T. neorhetum* form a distinct branch, as well as the three *Colossendeis* species (*C. macerrima*, *C. megalonyx*, *C. scoresbii*). This is not very surprising, as we studied a limited number of species from a relatively small geographic area, and pycnogonid species coverage by available DNA barcodes is still limited (158 species with barcodes in BOLD by June 2012 versus 1344 described pycnogonid species; Munilla & Soler Membrives, 2008). Thus, many more steps will have to follow to reveal DNA sequence divergences between closely related species and sister taxa in general worldwide. However, some of the taxa covered by this study deserve particular attention, since
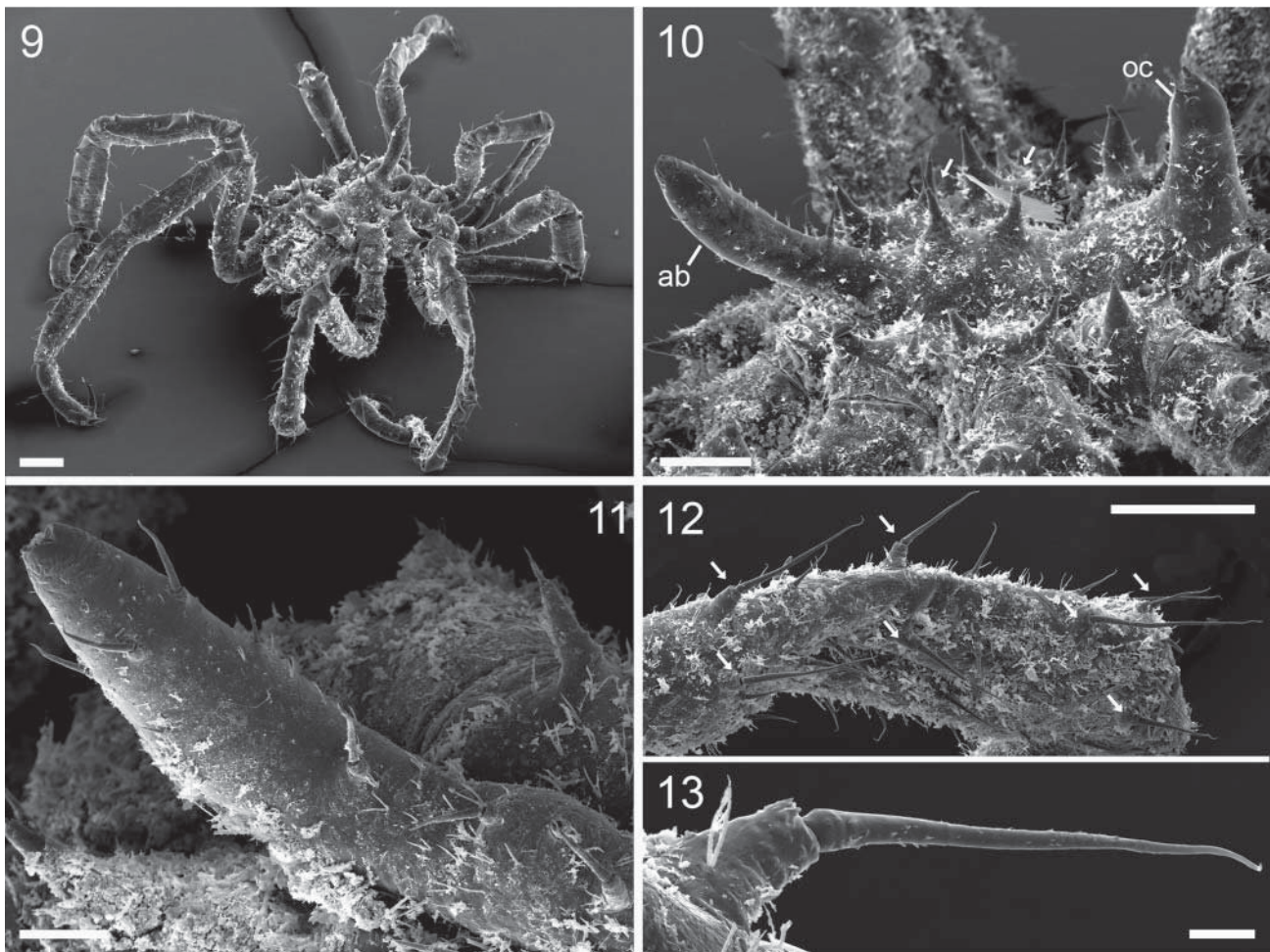
Figs 9–13. SEM of *Achelia assimilis*. **9.** Dorsal overview; scale = 200 μm. **10.** Lateral view of trunk, note prominent trunk spines (arrows); scale = 200 μm. **11.** Dorsal view of abdomen; scale = 100 μm. **12.** Overview of first tibia of right 2nd walking leg, note setae on rounded tubercles (arrows); scale = 200 μm. **13.** Detail view of setae situated on rounded tubercle; scale = 20 μm.

a body of comparable results has been published on them, and they exhibit high intraspecific divergence.

It is generally surmised that cases like ours of *Pallenopsis patagonica* (about 11% intraspecific divergence) and *Achelia assimilis* (about 7%) regularly reflect the presence of a species complex composed of previously undetected 'cryptic' species (Allcock *et al.*, 1997; Held, 2003; Raupach & Wägele, 2006; Leese & Held, 2008; Mahon *et al.*, 2008; Wilson *et al.*, 2009). *Colossendeis megalonyx* also shows relatively high intraspecific variation (about 3%), confirming the results of Krabbe *et al.* (2010).

While the four branches of *A. assimilis* receive high bootstrap support, we have not found any matching morphological differences. The only potentially significant exception might concern the specimens from Playa Chica (Región de los Rios), which are smaller and carry fewer and less prominent spines on the first coxae (Fig. 8). There are two descriptions of *A. assimilis* from Australia and the Tiahura barrier

reef lacking trunk spination (Müller, 1989; Arango, 2003). However, these and all other studied individuals show the characteristics typical for this very variable species (Hedgpeth, 1961; Fry & Hedgpeth, 1969), which has been discussed also under the synonyms *A. variabilis* Stock, 1954 and *A. wilsoni* Schimkewitsch, 1890. The only species bearing trunk spines like those in *A. assimilis* is *A. columnaris* Stock, 1992. The latter species, however, is found in Brazil, and can be distinguished from *A. assimilis* by the tall and pillar-shaped spurs on femur and tibiae (Stock, 1992).

On the other hand, our molecular analyses show four distinct COI branches representing four geographical regions, namely Región de los Rios, Región de los Lagos, Región de Magallanes y de la Antártica Chilena and Australia. We have constructed NJ, ML and BI trees (data not shown) including two other *Achelia* species, *A. bituberculata* Hedgpeth, 1949 (NC009724, AY457170) and *A. hoekii* (Pfeffer, 1889) (DQ390087), to test whether they fall within

or outside of our *A. assimilis* complex. As expected, *A. bituberculata* and *A. hoekii* each formed a branch of their own beside the studied *A. assimilis*, supporting the assignment of our specimens. Furthermore, a statistical parsimony analysis yielded four completely separated networks for the four *A. assimilis* branches, indicating definite geographical splitting.

In most DNA barcoding publications, the *A. assimilis* branches we found in the Chilean fjords would probably be referred to as cryptic species (see, e.g. Hebert *et al.*, 2004; Lefébure *et al.*, 2006; Krabbe *et al.*, 2010), due to the high intraspecific sequence divergence, tree statistics indices strongly supporting the branches, distinctly separated haplotype networks, and deep barcoding gaps between the branches. In recent publications intraspecific divergence greater than 3% has been interpreted either as suggesting the presence of cryptic species (Radulovici *et al.*, 2009) or as a threshold for species delineation (Hebert *et al.*, 2003*a*, 2003*b*). Our calculated intraspecific value of 6.81% for *A. assimilis* lies clearly above that proposed threshold. This corresponds well with Child's (1990) notion that 'there may possibly be more than one species hiding under the umbrella of this name', *A. assimilis*. Since there are already remarkable differences between the Chilean *Achelia assimilis* specimens, we suppose that if there are cryptic species, then the Australian specimen (DQ390087) would be the best candidate.

However, the geographically discrete distribution of our branches leads us to handle this point with care. According to classical biogeography-based taxonomy (e.g. Mayr, 1975) the branches showing distinct areas without overlap would be referred to as subspecies rather than as species. In addition, we have found no morphological evidence for differences at species level, only the already well-known variability of this species. Moreover, we cannot exclude with certainty any artefacts due to the small sample size.

What could be an evolutionary scenario for the origin of the branches, if we refer to them as phylogeographic units? The most probable explanation is that they are products of alternating extinction and colonization events from shelters and/or surrounding regions during the ice ages. For example, the last postglacial recolonization took place in the Chilean fjords after the end of the latest glaciation about 15 000 years ago. At that time the entire Chilean coastline (as far as about 30 °S), including the Chilean fjord regions and a large portion of the offshore shelf area, was covered by the Patagonian ice shield (Clapperton, 1993). Along the Chilean coast the Pacific Ocean has very steep slopes achieving depths of several kilometres. The absence of stepping stones in the Pacific Ocean, which would be essential for survival, explains why no shallow benthos communities were present; the same has been inferred for Antarctica. Thatje *et al.* (2005) hypothesized that during glacial periods, survival of benthic communities was possible only in the deep sea or in shelters on the continental shelf. Further-

more, they suggested that taxa with poor dispersal abilities might constitute cryptic species as a result of isolation in glacial shelters.

Although *A. assimilis* has also been recorded from a depth of 903 m off Peninsula Mitre (Argentina) (Child, 1994), it can be considered as a shelf species mostly occurring at shallow depths above 300 m (Hedgpeth, 1961; Müller, 1993; Arango, 2003). We therefore assume that postglacial recolonization of the Chilean fjords by *A. assimilis* occurred either from the deep sea (relatively unlikely) or from glacial refugia in the North and/or South.

Pycnogonids have holobenthic life cycles as they lack a pelagic larval stage, thus have relatively limited dispersal abilities (except for very rare drift events) compared with pelagobenthic animals such as decapod crustaceans. Therefore, both preconditions for cryptic species suggested by Thatje *et al*. (2005) apply to *A. assimilis* in the Chilean fjords. All this could explain the high intraspecific variation among our *A. assimilis* specimens and the patchy distribution of their branches. What might be the underlying mechanisms of these phenomena? In our view, the following two (or a combination of both) have to be considered: (1) As is indicated by high sequence divergence between the branches, pre-existing, 'old' lineages might have colonized the fjords after the last glaciation from different refugia, and (2) small colonist populations might have been highly susceptible to founder effects and/or genetic drift, which may also push divergence to higher and higher levels. Testing these hypotheses will require more *A. assimilis* specimens and sequence data from different regions along the Chilean coastline.

The advantages and disadvantages of DNA barcoding are still hotly debated and discussed (Hebert & Gregory, 2005; Will *et al.*, 2005; Birky, 2007; Taylor & Harris, 2012). Benefits of DNA barcoding are, for example, that it can be applied even to fragments of organisms, that it works for all life stages, and is much faster and cheaper than traditional systematics (Birky, 2007). However, molecular techniques are nothing more than another source of information; they cannot replace morphological analysis for assigning organisms to species (Birky, 2007). Therefore, different methods for species determination should be used in combined approaches such as 'integrative taxonomy' (Dayrat, 2005; Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010; see also Huxley, 1940). The present paper takes the first such step toward using both traditional (morphology) and modern (molecular) techniques for analysing pycnogonids from the Chilean coast and inner fjord regions.

Acknowledgements

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A. Weis and R. R. Melzer

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Associate Editor: Andrew Brower

6. Paper IV

Weis A, Meyer R, Dietz L, Dömel J, Leese F & Melzer RR (2013) *Pallenopsis patagonica* (Hoek, 1881) – a species complex revealed by morphology and DNA barcoding with description of a new species of *Pallenopsis* Wilson, 1881. *Zoological Journal of the Linnean Society*. Accepted.

1 *Pallenopsis patagonica* (Hoek, 1881) – a species complex revealed by
2 **morphology and DNA barcoding with description of a new species of**
3 *Pallenopsis* **Wilson, 1881**

4

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7

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14

15 Running headline: *Pallenopsis patagonica* – a species complex
16

17   **Abstract**

18

19   *Pallenopsis patagonica* (Hoek, 1881) is one of the most taxonomically problematic and

20   variable pycnogonid species and distributed around the southern South American coast, the

21   Subantarctic and Antarctic areas. We conducted a phylogenetic analysis of mitochondrial COI

22   sequences of 47 *Pallenopsis* specimens, including 39 morphologically identified as *P.*

23   *patagonica,* five *P. pilosa,* one *P. macneilli,* one *P. buphtalmus* and one *P. latefrontalis.*

24   Furthermore, we studied morphological differences between the different COI lineages using

25   light and scanning electron microscopy, including also material of Loman's and Hedgpeth's

26   classical collections as well as Hoek's type material of *P. patagonica* from 1881. The

27   molecular results unambiguously reveal that *P. patagonica* is a complex of several divergent

28   clades, which also includes *P. macneilli*, *P. buphtalmus* and *P. latefronalis.* Based on the

29   material available, two major clades could be identified, namely a "Falkland" clade, to which

30   we assign the nominal *P. patagonica*, and a "Chilean" clade, which is distinct from the

31   former. The latter we describe as new species, *P. yepayekae* Weis, 2013. All molecular results

32   are confirmed by specific morphological characteristics that are discussed in detail and

33   compared to *Pallenopsis* species closely related to the *P. patagonica* complex. Our results

34   reveal that *P. patagonica* is a species-rich complex that is in need for a thorough taxonomic

35   revision using both, morphological and genetic approaches.

36

37   Key words: biogeography – Chile – COI - cryptic species – Pallenopsidae – Pantopoda -

38   Subantarctic.

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Introduction

Pallenopsis patagonica (Hoek, 1881), from the material of the Challenger expedition, was, as the name implies, first sampled off southern South American coasts. It represents one of the most taxonomically problematic and variable pycnogonid species known to date. The complexity can already be recognized by the various synonyms that exist for this species, viz. *P. glabra* Möbius, 1902, *P. hiemalis* Hodgson, 1907, *P. meridionalis* Hodgson, 1915, *P. moebiusi* Pushkin, 1975 and *Bathypallenopsis meridionalis* (Hodgson, 1927) (Bamber & El Nagar, 2011). In addition, some valid species exist that are morphologically very similar to *P. patagonica*, e.g. *P. buphtalmus* Pushkin, 1993. *P. patagonica* is known from Antarctic and Subantarctic regions, mainly the Scotia Sea, Ross Sea, Antarctic Peninsula, South America including the Magellan Strait, but also from the Falkland Islands, South Georgia and the eastern sector of the Antarctic coast (Child, 1995; Gordon, 1932; Hedgpeth, 1961; Hodgson, 1907; Hoek, 1881; Loman, 1923a; Loman, 1923b; Marcus, 1940; Möbius, 1902; Müller, 1993; Munilla & Soler Membrives, 2008; Pushkin, 1975; Pushkin, 1993; Stock, 1975; Weis & Melzer, 2012b). Specimens can be found in depths ranging from 3 down to 4540 meters (Munilla & Soler Membrives, 2008).

To unscramble the complex taxonomy of *P. patagonica,* and to test whether all morphologically variable specimens available for our analysis represent a single species, we sequenced a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. This gene is variable and has been applied successfully for species-level taxonomy in pycnogonids (Krabbe *et al.*, 2010; Mahon, Arango & Halanych, 2008; Weis & Melzer, 2012a). Altogether 39 *P. patagonica* specimens were sampled from 33°-72° South and 11°-170° West (depth range between 3 and 466 m), with focus on the area around the southern tip of South America. Furthermore, morphology of all available specimens was studied in detail with light and scanning electron microscopy (SEM), demonstrating differences among samples from different localities. Morphological analyses include specimens from Loman's type material (SMNH Type 1293 and syntypes) of *P. tumidula,* one specimen of *P. patagonica* (SMNH-125527) from Hedgpeth's collections from the Swedish Museum of Natural History (Hedgpeth, 1961; Loman, 1923b), eight other specimens of *P. patagonica* (SMNH-125445, SMNH-125507, SMNH-125508, SMNH-125509, SMNH-125510), and one unidentified *Pallenopsis spec.* (SMNH-125514). In addition we also studied/consulted Hoek's type material of *P. patagonica* (BMNH 1881.38, three specimens) and *P. patagonica* var. *elegans* (BMNH 188.38, one specimen) which are kept in the Natural History Museum of London.

75   Furthermore, we analysed three *P. notiosa* Child, 1992 specimens*,* which are housed at the

76   Zoologische Staatssammlung München (ZSM) (Weis & Melzer, 2012b). Our morphological

77   data set includes a total of 61 specimens.

78   As mentioned in our previous study (Weis & Melzer, 2012a) the southern Chilean coastline

79   displays an interesting chance for studying speciation processes. Given that the last glaciation

80   ended only 15.000 years ago, and the low dispersal ability of pycnogonids, haplotypes of

81   cryptic species have already been linked with their geographical distribution, as was the case

82   for *Achelia assimilis* (Haswell, 1885) (Weis & Melzer, 2012a). Whether similar effects can be

83   found concerning the species *P. patagonica* is one aim of the present study.

84   Further molecular studies focusing on particular groups of pycnogonids have only explicitly

85   been done for the genus *Colossendeis* (Dietz *et al.*, 2011; Krabbe *et al.*, 2010) and *Nymphon*

86   (Mahon *et al.*, 2008; Arango, Soler-Membrives & Miller, 2011) so far. With *Pallenopsis* we

87   want to open the field for a further very complex, variously shaped group with focus on

88   southern South American coasts and surrounding areas.

89

90   **Material and Methods**

91

92   *Specimens and vouchers*

93

94   Specimens from the Chilean coast were collected by "SCUBA diving" during expeditions

95   organized by the Huinay Scientific Field Station between 2006 and 2011 (Försterra, 2009).

96   Additionally, we received material from the region of Valparaiso, a more northern area in

97   Chile, from the Falkland Islands, South Georgia and the Weddell Sea (see acknowledgments).

98   A detailed overview of the different sample locations of the studied individuals is given in

99   figure 1. Material was fixed in 96% ethanol to ensure high quality DNA for genetic analysis.

100   Pycnogonids were identified based on morphology using a variety of literature (Child, 1995;

101   Gordon, 1932; Gordon, 1944; Hodgson, 1907; Hoek, 1881; Möbius, 1902; Pushkin, 1975;

102   Pushkin, 1993; Stock, 1957; Stock, 1975; Weis & Melzer, 2012b). Furthermore, synonyms,

103   depth ranges and distribution patterns were taken from Müller's (1993) "World Catalogue and

104   Bibliography of the recent Pycnogonida"(Müller, 1993), Munilla and Soler-Membrives

105   (2008) and Pycnobase (Bamber & El Nagar, 2011). All barcoded voucher specimens are kept

106   at the Zoologische Staatssammlung München (ZSM) under specific Voucher ID's (see table

107   1) including also PpaE_001-008, PpaE_010, PpaA_001 and PxxE001-002; their respective

108   DNA extract aliquots are stored partially at the Canadian Center for DNA Barcoding

109   (CCDB), the ZSM's DNA bank facility and the Ruhr University Bochum. Collection data,

110   BOLD or GenBank accession numbers of all 39 pycnogonid sequences examined in this study

111   as well as chosen outgroup taxa are summarized and listed on table 1. Some of the specimen

112   details can further be accessed in Barcode of Life Data Systems (Ratnasingham & Hebert,

113   2007) under the project CFAP (Chilean Fjord Pycnogonids) as part of the "Marine Life

114   (MarBOL)" campaign. The sequences FJ969367-69 of *P. patagonica* from the Ross Sea were

115   accessed from GenBank (Nielsen, Lavery & Lorz, 2009). Furthermore, we used five Genbank

116   sequences of *P. pilosa* Hodgson, 1915 (PxxE001, PxxE002, KC 848052, KC848053,

117   KC848054,), one sequence of *P. buphtalmus* Pushkin, 1993 (HM426171), one *P. latefrontalis*

118   Pushkin, 1993 (HM426218), and *P. macneilli* Clark, 1963 (DQ390086) as outgroups. While

119   specimens PxxE001 and PxxE002 were checked for correct determination, we could not

120   access the outgroup specimens KC 848052, KC848053, KC848054 (deposited at the British

121   Antarctic Survey in Cambridge), HM426171, HM426218 and DQ390086.

122   For comparative morphological analyses in addition to our specimens used for dna

123   sequencing we investigated 18 specimens from historical collections housed at the Swedish

124   Museum of Natural History, and the British Museum of Natural History i.e. *P. tumidula*

125   (SMNH- Type 1293 and seven syntypes), *P. patagonica* (SMNH-125445, SMNH-125507,

126   SMNH-125508, SMNH-125509, SMNH-125510) and one unidentified *Pallenopsis* spec.

127   (SMNH-125514) from the Loman collection, as well as one *P. patagonica* (SMNH-125527)

128   sampled by the Lund University Chile expedition, determined by Hedgpeth (1949). Beyond

129   that we examined Hoek's type material from the Challenger expedition that include three

130   specimens of *P. patagonica* and one specimen designated as *P. patagonica* var. *elegans*

131   (BMNH 1881.38). Furthermore, we studied a related species *P. notiosa* (ZSMA20111077-

132   79), which is kept at the ZSM and discussed in a previous paper(Weis & Melzer, 2012b).

133   For morphological documentation we used the following specimens: ZSMA20111000,

134   ZSMA20111002, ZSMA20111004, ZSMA20111006, ZSMA20111009, ZSMA20111016,

135   ZSMA20111348, ZSMA20111350, ZSMA20111357, PpaE007 and PpaE010 for light

136   microscopy; ZSMA20111006, ZSMA20111009, ZSMA20111024, ZSMA20111349,

137   ZSMA20111359 and ZSMA20111360 for SEM studies.

138

139   *DNA extraction and sequencing*

140

141   Since all the studied individuals had a suitable size it was sufficient to take only a piece of leg

142   for DNA extraction. Here, muscle tissue from the tibia was extracted using the DNeasy Mini

143   Kit following the tissue protocol of the manufacturer. As a modification from the original

144   protocol, we used only 100 µl of AE buffer for elution. Amplification of a 657 bp fragment of

145   the Cytochrome c Oxidase subunit 1 gene (COI) was performed using standard Folmer

146   primers (Folmer *et al.*, 1994) in 25 ul reactions. Individual reactions consisted of 1x PCR

147   buffer (5Prime HotMaster), 0.2 mM dNTPs, 0.5 uM of each primer, 0.025 U/ul Taq (5Prime

148   Hotmaster), 1-3 ul extracted DNA (depending on yield), and was filled up to 25 ul with

149   molgrade H_2O. Cycle conditions were: initial denaturation at 94°C for 2 min followed by 36

150   cycles of 94°C for 20s, 48°C for 30 s, 65°C for 80 s. After a final extension at 65°C for 5 min

151   the reactions were stored at 4°C. Both, DNA extraction and PCR success were checked on a

152   1% TBE agarose gel. 10 µl PCR product were purified enzymatically with 0.5 µl Exonuclease

153   I (20 U/µl) and 1 µl FastAP (1 U/µl, Thermofisher), by incubating in a thermocycler at 37°C

154   for 15 min followed by 96 °C for 15 min prior to sequencing. Sequencing was conducted at

155   GATC (Konstanz, Germany) or performed partially at the CCDB using the standard protocols

156   of IBOL (http://dnabarcoding.ca/pa/ge/research/protocols).

157

158   *Phylogenetic analysis*

159

160   A total of 47 pycnogonid sequences were used for the phylogenetic analyses of the 657 bp

161   fragment of the cytochrome c oxidase I gene (COI). All 47 DNA sequences were aligned with

162   MUSCLE using GENEIOUS Pro version 5.5.4 (Drummond *et al.*, 2011). To check for

163   frameshift mutations or stop codons, the COI sequences were translated into amino acids

164   using the invertebrate mitochondrial genetic code (translation table 5). After the calculation of

165   "base pair" frequencies and uncorrected pairwise distances with MEGA 5.05 (Tamura *et al.*,

166   2011) we tested the alignment statistically for substitution saturation in DAMBE 5.2.69 (Xia

167   & Lemey, 2009; Xia *et al.*, 2003).

168   Using MEGA 5.05 software we calculated nucleotide composition, maximum parsimony

169   (MP), and since we were interested in shallow species level differences also Neighbor-Joining

170   (NJ) trees based on the Kimura 2 parameter (K2P) model (Kimura, 1980; Saitou & Nei, 1987)

171   with bootstrap values. For Maximum Likelihood (ML) and Bayesian inferences (BI) we first

172   identified the most appropriate substitution model using Modeltest2 and the Akaike /

173   Bayesian Information Criteria (Darriba *et al*. 2012). For ML we used the full set of 88

174   models, for MrBayes we used the reduced model search scheme (nst=1,2 and 6; +I, +G, +IG).

175   Just as for MP and NJ we used 1,000 replicates for the ML analysis under RAxML 7.0.4. The

176   1,000 rapid bootstraps were conducted by using the –x option (random seed number). Based

177   on jModeltest2 the best models according to both the AIC and the BIC was GTR+I+G and

178   used in RAxML and the Bayesian analyses with MrBayes 3.2 (Ronquist *et al.*, 2012).

179   Bayesian analysis was performed using four independent runs with 4 independent chains and

180   5 million Metropolis-coupled MCMC generations each. Every 500th tree was saved (10000 in

181   total). The four independent runs reached stationarity after $0.7 - 0.9$ million generations

182   (average standard deviation of split frequencies below 0.01) and thus the consensus tree was

183   calculated after discarding the first 25% of the trees as burn-in (1.25 million generations). The

184   figure of the recovered phylogenetic tree was made using FigTree 1.4.0.

185

186   *Search for species boundaries using DNA sequences*

187

188   To be independent from morphology, we decided to perform molecular analyses on the whole

189   dataset including also *P. macneilli, P. buphtalmus* and *P. latefrontalis.* To check for species

190   boundaries in our *P. patagonica* complex, we conducted a general mixed Yule-coalescent

191   (GMYC) analysis (Monaghan *et al.*, 2009; Pons *et al.*, 2006). As identical sequences cannot

192   be considered in GMYC analyses, we removed identical sequences, resulting in a dataset of

193   32 sequences. An ultrametric starting tree was obtained using BEAUTi and BEAST (both

194   version 1.6.1) (Drummond & Rambaut, 2007). The chain length for the Markov-Chain Monte

195   Carlo (MCMC) algorithm was set to 10 million generations, with sampling trees every 1,000

196   generations. Effective sampling sites and convergence of the parameter estimates was

197   inspected using Tracer (version 1.5). Using TreeAnnotator (version 1.6.1) a consensus tree

198   was obtained. The burn-in was set to 2500, rejecting the first 25% of the trees and the

199   posterior probability threshold was set to 0.5. The resulting ultrametric tree was subsequently

200   imported into the statistics software R 2.15.2 (available: http://www.R-project.org/ accessed

201   2012). GMYC analysis was conducted with the R package "SPLITS" (Species Limits by

202   Threshold Statistics, obtained from: http://r-forge.r-project.org/projects/splits). We used the

203   single and multiple threshold model for the inference of the number of entities with standard

204   parameters (interval = c(0,10)) and used a likelihood ratio test to select the appropriate model.

205   Furthermore, we used the freely available software ABGD (Automatic Barcode Gap

206   Discovery) (Puillandre *et al.*, 2012) for searching barcoding gaps between all 42 sequences

207   (sequences of *P. pilosa* were excluded) and for calculating their intraspecific

208   distance/variance.

209

210   *COI network*

211

212 Since networks are better suited to visualize the often reticulate relationships within as well as

213 among closely related species, we constructed a NeighborNet of all individual COI sequences,

214 using Splitstree version 4.12 (Huson & Bryant, 2006) and K2P-corrected distances.

215

Morphological analysis

217

218 Specimens were photographed using a Wild M400 photomacroscope equipped with a digital

219 camera (Nikon D700) by taking several shots focused at different levels along the z-axis. To

220 constitute a greater depth of field this series of pictures was then edited and combined to a

221 single   respective   image   using   the   computer   software   Helicon   Focus

222 (http://www.heliconsoft.com/). Specimens were prepared for SEM as described in Weis and

223 Melzer (2012a). The editing and composition of both light microscopic and SEM pictures was

224 performed with Adobe Photoshop CS.

225

Nomenclatural Acts

227 The electronic edition of this article conforms to the requirements of the amended

228 International Code of Zoological Nomenclature, and hence the new name contained herein is

229 available under that Code from the electronic edition of this article. This published work and

230 the nomenclatural acts it contains have been registered in ZooBank, the online registration

231 system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the

232 associated information viewed through any standard web browser by appending the LSID to

233 the   prefix   "http://zoobank.org/".   The   LSID   for   this   publication   is:

234 urn:lsid:zoobank.org:act:0E39E226-30C7-4853-A6A1-7DD2336F33FE.   The   electronic

235 edition of this work was published in a journal with an ISSN, and has been archived and is

236 available from the following digital repositories: PubMed Central and LOCKSS.

237

Results

239

Molecular and phylogenetic analysis

241

242 The 657-bp COI alignment of 47 pycnogonid specimens showed no gaps. Base pair

243 frequencies indicated an arthropod-typical bias towards adenosine and thymine: A 31.31 %, C

244 19.80 %, G 13.95 % and T 34.68 %. The value of substitution saturation (Iss), which was

245   calculated for the whole alignment as well as for the third codon position, was always

246   significantly lower than the critical Iss.c value. Iss being lower than Iss.c implies only little

247   substitution saturation for the analysed sequences. The 657 basepairs consisted of 410

248   conservative sites and 247 variable sites of which 202 were parsimony informative.

249   Translating the COI sequences into amino acid sequences using the invertebrate

250   mitochondrial codon table in Geneious showed neither frame shift mutations nor stop codons.

251   Phylogenetic trees constructed with the different approaches (BI, MP, NJ, ML) showed no

252   major differences, therefore we present the Bayesian tree (Fig. 2). Support values of the other

253   inferences are also shown on the branches. Minor differences are caused by ZSMA20111008

254   and ZSMA20111072, which are also showing bad bootstrap support. Both are slightly

255   changing their position within the tree but are never affecting any of the other "well

256   supported" clades.

257   Specimens of *P. patagonica* from Chile (ZSMA20111000, ZSMA20111002-06,

258   ZSMA20111009, ZSMA20111012, ZSMA20111016, ZSMA20111024, ZSMA20111339)

259   and the Falkland Islands (ZSMA20111348-51, ZSMA20111354-55, ZSMA20111357,

260   ZSMA20111359-61, PpaE004-008, PpaE010) cluster within two well-supported,

261   geographically distinct clades (Figs 2 and 3). Several specimens cluster outside these two

262   distinct groups, highlighting the complex nature of *P. patagonica*: ZSMA20111008,

263   ZSMA20111072 (both from 33°S) and [ZSMA20111017 (48°S) and ZSMA20111340

264   (Region de Magallanes)], [PpaE003 and ZSMA20111352 (Falklands)]. Specimens from the

265   Ross Sea (FJ969367-69) cluster together with one individual from the East Weddell Sea

266   (PpaA001), one from the Shag Rocks (PpaE001) and two Southern Ocean specimens assigned

267   to different species (*P. buphtalmus, P. latefrontalis*) forming an "almost Antarctic" clade. The

268   only specimen from South Georgia (PpaE002) clusters basally to the Falkland and "Antarctic"

269   clades. *P. macneilli* clusters with ZSMA20111008. The results reveal that specimens initially

270   identified as *P. patagonica* are genetically very heterogeneous and some show close affinities

271   to specimens identified as different species. Figure 3 shows the Neigbornet of all *Pallenopsis*

272   specimens including alternative connections.

273   The five specimens of *P. pilosa* selected as the outgroup cluster apart from all other 42

274   pycnogonids. The statistical support for the ingroup is good for the model-based inferences

275   (BI:1, ML: 88) but poor for the NJ and MP inferences (25 and 40, respectively). Interestingly,

276   the five *P. pilosa* specimens are genetically highly heterogeneous, hinting at further problems

277   with the taxonomy of other *Pallenopsis* specimens. In general, the mitochondrial COI

278 fragment is a suitable marker for uncovering lineages previously undetected by morphological

279 analyses (see also Weis & Melzer, 2012a).

280 To test whether these clusters comprise cryptic or overlooked species we calculated and

281 compared uncorrected pairwise distances between the different specimens/clades (see table

282 S1). Variation between clades or specimens are high with a maximum of 23.6 % uncorrected

283 genetic distance. Genetic distances between *P. patagonica* s. str. (Falkland Clade) and *P.*

284 *yepayekae* sp. nov. (Chile Clade) were high (4.5-5.3% and 14.9-19.1%), whereas the variation

285 within these clades was low (0-1.2 % and 0-3.5 %, respectively).

286 In addition, we analysed the distance data for distinct barcode gaps using ABGD. Including

287 all 42 studied pycnogonids (five specimens of *P. pilosa* excluded) no distinct barcode gap is

288 visible (Fig. 4). Although there is a large increase at the beginning of the slope, the two

289 horizontal lines are connected by several dots or small clusters of dots. However doing the

290 same analyses with the 11 specimens from the Chilean clade together with the 16 specimens

291 from the Falkland clade a barcoding gap becomes more obvious (Fig. 5). The two vertical

292 "clusters" are now clearly separated without any dots in between them. Using ABGD we

293 calculated the intraspecific distance of the same 42 specimens. Intraspecific distance varied

294 between 0 and 23 % (see Fig. 6).

295 For the tree-based assessment of hidden species, using the GMYC model with multiple

296 branching events (indicating the presence of several species) was preferred over the null

297 model (single coalescent branching model): Likelihood ratio test: $p<0.001$. This indicates the

298 presence of several species. We also compared the single-threshold model versus the

299 multiple-threshold model and found support for the single-threshold model $p=0.861$ (Chi

300 square 0.751 and 3 degrees of freedom). According to the single-threshold GMYC model, the

301 tree consists of 32 haplotypes split into three clusters (confidence interval: 3-5) and 15 distinct

302 GMYC species (ML entities; confidence interval: 11-16). The threshold between Yule

303 speciation and coalescence within populations is indicated by a vertical line in the lineage-

304 through-time plot (LTT) of the Bayesian tree in figure 7. According to this, all Falkland Clade

305 specimens and the Chile clade specimens represent GMYC species. Furthermore, [FJ969367

306 and FJ96968 (HT21)] and [ZSMA20111017 (HT11) and ZSMA20111340 (HT12)] and all

307 other 11 specimens represent distinct GMYC species.

308

309 **Morphology**

310

311 To check if the results of our sequence analyses i.e., that *P. patagonica* might be a complex of
312 several species, are paralleled by previously undetected morphological differences between
313 these clades we made a detailed analysis of all available specimens. Table S2 displays the
314 enormous morphological variance of the different clades/specimens with respect to their
315 general body size, length of the cement gland tube, leg setation and auxiliary claw length, but
316 they all fit in the traditional definition of *P. patagonica*. Since for most cases only one
317 specimen is available, and since these lack morphological differences that allow us to decide
318 whether they represent variations or putative species-specific features, their analysis will be
319 continued when more specimens are available. Thus we focused our analyses on the two
320 biggest clades, initially referred to as the Chile clade and the Falkland clade (including 11 and
321 16 specimens, respectively). Within each of the two clades we observed constant
322 morphological features, which is in accordance with the molecular results. Light microscopy
323 pictures of individuals from the Falkland Island and the Chilean clade are shown in figures 8
324 and 9. Furthermore, figure 10 displays detailed SEM studies of the cement gland ducts,
325 female ovigers and hairs of the second and third coxae from specimens of both clades.

326 Specimens from the Chilean coast seem to be smaller in their body size compared to the
327 specimens captured from the Falklands and South Georgia (except ZSMA20111352 and
328 PpaE003 from Burdwood Bank) (Figs 8A and 9 A). Whereas the shape of the proboscis is
329 cylindrical along its length for most specimens (Fig. 8B), individuals from the Chilean clade
330 show a distinct swelling at the middle of the proboscis (Fig. 9C). Also specimens
331 ZSMA20111008 (Region de Magallanes), ZSMA20111072 (Region de Valparaiso),
332 ZSMA20111352 (Falkland Islands) and PpaE003 (Burdwood Bank) show a light swelling at
333 about half the length of the proboscis. Almost all studied specimens bear an upwards erected
334 slender abdomen (except PpaE002 horizontal) with some short setae. The abdomen from
335 specimens from the Chilean clade is dorsodistally sloped. At the beginning of the slope a
336 rounded edge is found bearing two very prominent spines (Fig. 9D). In contrast specimens
337 from the Falklands and Antarctic area lack those spines, but show several randomly
338 distributed short setae on the abdomen (Fig. 8C). All examined individuals show a pointed or
339 slightly pointed ocular tubercle. Specimen ZSMA20111008 is the only one with a rounded
340 ocular tubercle.

341 Furthermore, whereas the length of the cement gland tubes in the Chilean pycnogonids is
342 about three times their diameter (Fig. 10B), specimens from the Falklands and Antarctic area
343 show a very short cement gland tube (Fig. 10A), which is sometimes only hardly visible.
344 Additionally females of the Chilean clade show a swollen 4th oviger segment which is not

345   noticeable in the females from the Falkland clade (Figs 8D, 9F, 10C-D). Furthermore, female

346   ovigers from the Chilean clade are eight- to nine-segmented (distal segments often fused)

347   compared to females of the Falkland clade, which exhibit a "ten-segmented" oviger (Figs

348   10C-D).

349   The proportion of the length of the different leg segments is similar throughout all studied

350   specimens, with tibia 2 being the longest. The number of heel spines on the propodus varies

351   between three and four (Fig. 8F). Concerning the leg setae, all individuals show setae being

352   not longer than the diameter of the segment on which they are situated (except

353   ZSMA20111017). The 11 specimens from the Chilean clade show numerous distinct small

354   and stout hairs on the distal ventral side of the second and third coxa (Fig. 9E). Though this

355   characteristic is weakly developed in juveniles, it is already discernable at that stage. This

356   characteristic is not visible or that prominent in any of the other studied specimens (Fig. 8E).

357   Furthermore, the setae themselves show remarkable differences. The setae on the second and

358   third coxae of the specimens from the Chilean clade bear several tiny hairs on their surface

359   (Fig. 10F), whereas the setae from the other specimens are "smooth" or rather normally

360   developed (Fig. 10E).

361   The length of the auxiliary claws varies between one third and one half the length of the main

362   claw without distinction between specimens from different areas. Only specimen

363   ZSMA20111008 from the Chilean fjord region at 50°S bears extremely short auxiliary claws

364   being one fourth the length of the main claw.

365   *P. patagonica* specimens from the Swedish Museum of Natural History determined by Loman

366   show similar morphological characteristics to those of our specimens from the Falkland clade.

367   Loman's specimens were collected by the Swedish South Polar Expedition (1901-1903) at the

368   Graham Region, South Georgia and the Falkland Islands. The undetermined *Pallenopsis*

369   (SMNH-125514) was collected at the Patagonia archipelago (Tierra del Fuego) 55°10'S,

370   66°15'W and is in good accordance in morphology with our Chilean clade. This specimen, an

371   ovigerous male, shows the characteristic hairs on the ventral side of the second and third

372   coxae, has a long cement gland tube (more than three times its width) and a proboscis with a

373   light swelling at half of its length.

374   The specimen of Hedgpeth (SMNH-125527) appears to be a female and was collected by the

375   Lund University Chile Expedition (1948-49) at Canal San Antonio 41°47'S, 73°15'W. This is

376   the exact region where samples from our Chilean clade are from. Also this specimen shows

377   the same morphological characteristics as our specimens from the Chilean fjords that are: a

378   nine-segmented oviger (with the 4th oviger segment swollen), a proboscis with a slight

379 swelling at the middle and prominent brush-like setae on the ventral side of the second and
380 third coxae.
381

382 **Reinvestigation of Hoek's type material**
383

384 Hoek's type material consists of three female specimens: one bigger specimen on which his
385 type determination is based and two smaller specimens which he designated as juveniles. The
386 three individuals were sampled from three different stations, namely station 304, 308 and 313
387 (located at 46°53'S, 75°11'W, 50°10'S, 74°42'W and 52°20'S, 68°0'W, respectively).
388 Unfortunately it is not known which specimen was captured from which sample site, since the
389 specimen labels don't contain this information. Whereas the bigger specimen and one of the
390 smaller ones are morphologically identical with the individuals of our "Falkland clade", the
391 other one resembles accurately the specimens from our "Chilean clade". It shows the distinct
392 prominent features which are (i) a proboscis slightly swollen at the middle, (ii) an "eight-" to
393 "nine–segmented" oviger with the fourth oviger segment thickened and (iii) several short
394 brush-like setae at the ventral side of the second and third coxae. Also the structure of these
395 hairs accords well with that described for the individuals of our "Chilean clade". The
396 abdomen shows the same shape bearing two spines on the rounded edge of the beginning of
397 the dorsodistal slope. One of the spines on the dorsal side is broken, the other one is not as
398 prominent as in most of the individuals from our "Chilean clade" but nevertheless clearly
399 visible.
400 Moreover Hoek's material also contains a specimen called *P. patagonica var. elegans* from
401 station 320 near the La Plata estuary in Argentine (37°17'S, 53°52'W). As Hoek already
402 mentions this individual resembles a variety of *P. patagonica*, i.e. our "Falkland clade", with
403 only a more slender appearance.
404

405 Results of our morphological analyses as well as our molecular data strongly indicate that the
406 "Chilean clade", i.e. the 11 specimens collected at the southern Chilean coast, represents a
407 new species that is described in the following.
408

409 *Pallenopsis yepayekae* **Weis spec. nov.** urn:lsid:zoobank.org:act:0E39E226-30C7-4853-
410 A6A1-7DD2336F33FE
411 Figs 9A-F, 10B, D, F, 11A-F
412

413 The new species can clearly be attributed to the genus *Pallenopsis* Wilson, 1881 by its slender

414 segmented body, cylindrical proboscis, rudimentary palps, "ten–segmented" ovigers in males

415 and slender legs with claws and auxiliary claws (Wilson, 1881).

416 Species description of *P. yepayekae* is based altogether on 14 specimens: 11 specimens

417 collected by the "Huinay Fjordos" expeditions 2006-2011, one specimen (SMNH-125514)

418 that was only determined to genus level by Loman (1902) and two further specimens that

419 were initially determined as *P. patagonica*, namely SMNH-125527 from Hedgpeth (1949)

420 and BMNH-1881.38 from Hoek (1881).

421

422 Types: Holotype: male (ZSMA20111002), Chile, Hanover area, Canal Pitt Chico,

423 50°50'07.1"S, 74°08'20.9"W, 25 m, 07.03.2006, leg. R. Melzer, M. Schrödl.

424 Paratypes:

425 4 males: ZSMA20111000, Chile, Western Katalalixar, Canal Castillo, 48°44'11.4"S,

426 75°24'53.1"W, 15m, 12.03.2006, leg. R. Melzer, M. Schrödl; ZSMA20111006, Chile, Fjords

427 of region x, Inio 4, 43°25'03.0"S, 74°04'51.2"W, 20m, 24.02.2008, leg. G. Försterra;

428 ZSMA20111339, Chile Anihue Raul Marin Balmaceda, Islas Tres Hermanas, 43°46'31.35" S,

429 73°01'44.14" W, 19m, 17.01.2011, leg. V. Häussermann; SMNH-125514, South Atlantic

430 Ocean, Argentina, Patagonia archipelago (Tierra del Fuego), 55°10'S, 66°15'W (St. no. 60 of

431 Swedish South Polar Expedition 1901-03), 100m, 15.09.1902, leg. J. C. C. Loman.

432 7 females: ZSMA20111003, Chile, Fjords of region x, Inio 4, 43°25'03.0"S, 74°04'51.2"W,

433 25m, 24.02.2008, leg. RF; ZSMA20111004, Chile, Fjords of region x, Inio 5, 43°24'34.5"S,

434 74°05'00.7"W, 9m, 24.02.2008, leg. NR; ZSMA20111009, Chile, Fjords of region x, Inio 3,

435 43°23'33.4"S, 74°07'56.5"W, 26m, 24.02.2008, leg. V. Häussermann; ZSMA20111016, Chile,

436 Western Katalalixar, Canal Adalberto, 48°36'28.7"S, 74°53'55.7"W, 32m, 12.03.2006, leg. R.

437 Melzer, M. Schrödl; ZSMA20111024, Chile, Messier Channel and Fjords, Paso del Abismo,

438 49°34'38.7"S, 74°26'49.3"W, 28m, 10.03.2006, leg. R. Melzer, M. Schrödl; SMNH-125527,

439 South Pacific Ocean, Chile, Canal Chacao, Canal San Antonio, 41°47'40"S, 73°15'40"W (St.

440 no. M109 of Lund University Chile Expedition 1948-49), 36m, 06.05.1949; BMNH-1881.38,

441 either from station 304, 308 or 313 of the H.M.S. Challenger expedition 1872-76 between

442 46°53'S, 75°11'W and 52°20'S, 68°0'W, between 82-320m, 31.12.1875-20.01.1876.

443 2 juveniles: ZSMA20111005, Chile, Western Katalalixar, Canal Castillo, 48°44'11.4"S,

444 75°24'53.1"W, 23m, 12.03.2006, leg. V. Häussermann; ZSMA20111012, Chile, Raul Marin,

445 Las Hermanas, 22m, 11.03.2007, leg. R. Meyer, K. Jörger.

446   Beside the specimens there are also DNA aliquots (including ten paratypes plus holotype)

447   stored under specific Voucher ID's at the Zoologische Staatssammlung München (see also

448   table 1) and at the Canadian Center for DNA Barcoding (CCDB).

449

450   Etymology:

451   In Kawésar language, yepayek is the name of the ciprés de las güaitecas (*Pilgerodendron*

452   *uviferum*). If one looks at the fine ramification of the branches of a cypress-like tree, the

453   similarity to the structure of the setae of the ventral side of the second and third coxae of the

454   new species described here becomes obvious. The name of the species also refers to the

455   Yepayek, ranger boat of the CONAF (Corporación Nacional Forestal) named after the tree,

456   which carried the scientists to the different places in the Chilean fjords sampled during

457   "Huinay fjordos" expedition # 3. It was the Yepayek and its always friendly and cooperative

458   crew to whom we owe the chance to collect this new species. Therefore, we decided to name

459   the species *Pallenopsis yepayekae* and also to keep in mind the adventurous trip through the

460   labyrinth of the Chilean fjords.

461

462   Diagnosis:

463   Compared to *P. patagonica* a rather small species of smooth habitus and in a few individuals

464   the legs show red stripes. Proboscis (Fig. 11B) with distinct swelling at the middle. Abdomen

465   (Fig. 11B) erect (about 45°) and dorsodistally sloped. The beginning of the slope shows a

466   rounded edge on which two very prominent spines are sited (Fig. 11B). Second and third

467   coxae with many conspicuous short brush-like setae on the ventral side (Fig. 11C). Oviger of

468   the females eight- to nine-segmented with the fourth oviger segment being swollen (Fig.

469   11E). Cement gland duct of males relatively long measuring about three times the length of its

470   diameter.

471

472   Description:

473

474   Male: Size moderate to small, leg span less than 60 mm. Trunk glabrous with distinct segment

475   borders, lateral processes separated by about 1/3 their diameter (Figs 11A and 11B). Ocular

476   tubercle at anterior portion of cephalic segment, slightly pointed (Fig. 11B). Eyes prominent,

477   pigmented with posterior ones smaller than anterior ones. Proboscis slightly directed

478   downwards, swollen at middle (Fig. 11B). Abdomen erect, somewhat extending beyond the

479　distal margins of the lateral processes, dorsodistally sloped, with two very prominent spines

480　on the dorsal side (Fig. 11B).

481　Chelifores with movable finger equipped with setose pad. Tips overlap when closed, inner

482　edges join when closed. Lateral palp buds have the form of short knobs (Fig. 11B).

483　Oviger "ten segmented", typical for genus (Fig. 11F). Distal segments more setose than

484　proximal segments, with setae pointing in various directions.

485　Legs (Fig. 11C) with several setae not longer than the diameter of the segment on which they

486　are situated. Coxae one and three subequal. Second coxa about twice length of third coxa.

487　Second and third coxae with many conspicuous short brush-like setae on the ventral side (Fig.

488　11C). Femur and tibia 1 of about equal size. Tibia 2 longest leg article. Tarsus short, armed

489　with one bigger spine on the ventral side. Propodus (Fig. 11D) slightly curved, with three to

490　four heel spines. Sole with many shorter spines. Claw robust, slightly curved, auxiliary claws

491　about 1/3 to ½ of main claw length.

492　Cement gland tube about three times as long as its diameter, medioventrally on femur on

493　slightly raised surface. Sexual pores on ventral side of second coxae of third and fourth pair of

494　legs.

495　Measurements (holotype, in mm): length of trunk (anterior margin of first trunk segment to

496　distal margin of 4th lateral processes), 4,82; trunk width (across first lateral processes), 2,94;

497　proboscis length, 2,29; abdomen length, 1,81; third leg, coxa1, 0,85; coxa 2, 2,58; coxa 3,

498　1,23; femur, 5,90; tibia 1, 5,49; tibia 2, 7,06; tarsus, 0,27; propodus, 1,44; claw, 0,76;

499　auxiliary claws, 0,50. Different leg segments were measured in natural posture.

500　Female: General habitus and size similar to male. Differences are only in the sexual

501　characters: oviger (Fig. 11E) eight-to nine-segmented with fourth oviger segment swollen;

502　distal oviger segments fused and less setose than in the male; all setae pointing distally.

503　Sexual pores on all second coxae on ventrodistal surface.

504

505　Distribution: Chilean fjord region 41°47'40"S - 55°10'S and 66°15' W - 75°24'53.1"W; depth

506　range 9-100 m.

507

508　Since Hoek's syntypes series of *P. patagonica* includes one specimen of *P. yepayekae* spec.

509　nov. a lectotype for *P. patagonica* has to be designated. Of the two specimens from the

510　BMNH-1881.38 material of the Challenger expedition, the larger specimen on which Hoek's

511　description is based shall be the lectotype, and the smaller specimen the paralectotype. The

512　lectotype of *P. patagonica* can clearly be distinguished from the new species *P. yepayekae*

spec. nov. by the following characteristics: abdomen without two prominent spines on the dorsal side, ten segmented oviger in females, second and third coxae without conspicuous short brush-like setae on the ventral side and a cylindrical proboscis without a swelling at the middle.

Discussion

The results of our study indicate great morphological as well as genetic variation in the examined individuals, indicating *P. patagonica –sensu lato* is a good example for studying species complexes.

To avoid circular reasoning by mixing morphology-based considerations and molecular results, all molecular analyses were done using the whole dataset, and checked against the morphological results later. Correspondingly morphology of the specimens was analysed without taking sequence-defined groupings into account. After the first morphological determinations all studied specimens could be assigned to *P. patagonica* according the hitherto existing definitions (Child, 1995; Gordon, 1932; Pushkin, 1975; Pushkin, 1993; Stock, 1957). We also decided to include available sequences of *P. macneilli, P. buphtalmus* and *P. latefrontalis* in our studies, owing to their close relationship to *P. patagonica.* Furthermore, since we did not have these three specimens at hand to check whether the determinations and the genetic data show their affinities to the *P. patagonica* complex, we treated them as neutrally as possible and considered them also as possible *P. patagonica* specimens.

Molecular analysis

Regarding the molecular results presented in this study, different clades are supported by high bootstrap or posterior probability values. Regarding all studied *Pallenopsis* specimens, two bigger clades can be clearly distinguised: on the one hand the Chilean clade with 11 specimens and on the other hand the Falkland clade comprising 16 individuals. This is not surprising, since already our morphological data put the Chilean and Falkland specimens in separated groups (see table S2).

Combining all evidence of our results, in particular the extremely high intraspecific distances of 23%, and also considering the high "intraspecific" variation of 10.4% for *P. patagonica*

546 reported in our previous study (Weis & Melzer, 2012a), we conclude that *P. patagonica*
547 might represent a large species complex, potentially hiding several undescribed new species.
548 In contrast to our previous study of *Achelia assimilis* (Haswell, 1885), where we assumed
549 subspecies due to their geographic pattern (possible allopatric speciation process), in *P.*
550 *patagonica* we find another case. As seen in the network and the phylogenetic tree, there is
551 geographic overlap between the single clades, i.e. haplotypes of different sub-networks are
552 present at the same location (see Fig. 3). The same pattern has been observed at several
553 locations for the giant sea spider *Colossendeis megalonyx* (Krabbe *et al.*, 2010). To confirm
554 this finding, more sequences from specimens from South Georgia, Antarctica and more
555 northern areas of the Chilean coast are required.
556 Again, like in other pycnogonids, in *P. patagonica* we observe very high interspecific
557 distances compared to other taxa (Hebert *et al.*, 2004; Lefebure *et al.*, 2006; Raupach *et al.*,
558 2010). Either the amount of undescribed species in Pycnogonida is higher than in other taxa,
559 or there is a peculiar "pycnogonid" phenomenon not understood at the moment.
560 Furthermore, the tree-based GMYC modelling analyses, a recently developed species
561 delimitation method (Monaghan *et al.*, 2009; Pons *et al.*, 2006), which has been used in
562 several groups of organisms (Barraclough *et al.*, 2009; Bode *et al.*, 2010; Esselstyn *et al.*,
563 2012; Williams *et al.*, 2012) reveal the presence of about 15 distinct GMYC species, of which
564 only two are represented by our two bigger clades (Falkland Island and Chile). This suggests
565 the presence of possibly unrecognized species. However, further sampling is needed to test
566 explicitly for this phenomenon.
567
568 **Morphological analysis**
569
570 Since for most of the clusters/clades only a few or even only one specimen is available at the
571 moment, more specimens from these scattered clades are needed to unravel this complex
572 phenomenon. However, there are enough specimens in the Falkland and the Chilean clade for
573 making conclusions regarding their species status. Since the original description of
574 *Pallenopsis patagonica* (Hoek, 1881) fits perfectly with the morphology of the 16 specimens
575 from the Falkland clade, they must be the *Pallenopsis patagonica sensu stricto.* Specimens
576 from the Chilean clade in contrast show several morphological and molecular differences,
577 which leads us to the description of a species new to science.
578 Specimens described by Hoek have a cylindrical proboscis without swelling at half of its
579 length and a "ten-segmented" oviger in females. The bigger female Hoek describes has a body

580 length of about 16 mm, which is similar to our specimens from the Falkland Islands, South
581 Georgia and Antarctica. Hoek mentions some small and stout hairs at the swollen extremity of
582 the second, third and fourth joint of the leg (meaning coxa 2, coxa 3 and femur, respectively).
583 Perhaps this could be the setae we describe in the specimens from the Falkland clade on the
584 ventral side of coxa two and three. However these hairs are not visible in his drawings (see
585 Hoek 1881, Plate XII, Figs 6-9), implying that they are not as prominent as for example in our
586 studied individuals from the Chilean coast. Hoek's specimens were captured by the H.M.S.
587 Challenger at station 304 (46°53'S, 75°11'W), station 308 (50°10'S, 74°42'W) and station
588 313 (52°20'S, 68°0'W). Fortunately two of our specimens, namely ZSMA20111008 and
589 ZSMA20111002, are from almost exactly the same location as Challenger station 308.
590 Regrettably Hoek did not mention which of the three specimens is from which sample
591 location. We assume that the only adult female, on which also his description and drawings
592 are based, has been captured east of Chile in the Atlantic at station 313, since this description
593 matches much better with our specimens from the Falkland Islands and surrounding area (see
594 above).

595 If one follows the first description given by Hoek (1881) under the synonym *Phoxichilidium*
596 *patagonicum*, the specimens from the Falkland Islands and Antarctica would match better
597 than those from the Chilean clade. Hoek focused his description only on the bigger individual
598 and denominated the smaller ones as juveniles, without giving them any more attention. In
599 our opinion these two specimens are adult females as well since both are already carrying
600 eggs inside the femur. After specific study one of the smaller females resembles exactly *P.*
601 *yepayekae* spec. nov. Furthermore, one of Hoek's sample location (station 308) falls exactly
602 in the area of the sample sites given for *P. yepayekae* spec. nov. Hence we assume, that this
603 individual of Hoek's material derives from station 308. Unfortunately we can not deduce
604 either from Hoek's descriptions nor from his material we have at hand which specimen was
605 captured at which station. The bigger specimen and the one that resembles *P. yepayekae* spec.
606 nov. are both kept in the same tube labelled with station 313 which is obviously wrong since
607 according to Hoek's original data these samples come from two different locations. Also the
608 sample site of the third specimen is not well documented.
609 Later on also Möbius (1902), Hodgson (1907), Hodgson (1915), Bouvier (1913), Calman
610 (1915), Loman (1923), Gordon (1932), Marcus (1940), Hedgpeth (1961), Pushkin (1975,
611 1993), Stock (1957) and Child (1994) described several further specimens and synonyms of
612 *P. patagonica*. The specimens were mainly captured from the Southern Ocean including
613 Bouvet and South Georgia, or from the Falkland Islands and the Atlantic coast of South

America. With every newly added description the species *P. patagonica* with its various existing synonyms became more and more diverse and variable. The morphological frame under which one could assign a pycnogonid to this species became broader and more ambiguous. Hence it is not surprising that in a broader sense, all our studied specimens match with the characterisation of *P. patagonica*.

To check if there are no other species hidden behind the 39 studied specimens, we choose *P. pilosa* as outgroup as well as *P. buphtalmus, P. latefrontalis* and *P. macneilli* and examined and compared the descriptions of other *Pallenopsis* species found in this area with our individuals. 1992 Child described two new *Pallenopsis* species from Chile, namely *P. notiosa* and *P. truncatula*. The latter one has very short auxiliary claws (about 0.15 the length of the main claw), well separated lateral processes, a glabrous abdomen, a very short cement gland tube in males and a ten segmented oviger in females. None of our individuals shows all of these features in combination. For example ZSMA20111008 is the only specimen bearing such short auxiliary claws, but in contrast to *P. truncatula* it has a rounded ocular tubercle, a setose abdomen and a femur being as long as tibia one (femur is shorter than tibia one in *P. truncatula*). Also *P. notiosa* can be excluded concerning our specimens, since it has a rounded ocular tubercle, well separated lateral processes and a very long second coxa (about three times coxa 3) (see Weis & Melzer, 2012b). Our specimens have a slightly conical or pointed ocular tubercle, only little separated lateral processes and a second coxa being about twice the length of the third coxa. ZSMA20111008 for example has a rounded ocular tubercle, but the other characteristics do not match. Furthermore, in neither of the two species Child mentions are there prominent hairs on the ventral side of the second and third coxae which occur in our Chilean specimens. *P. macneilli,* which is closest to ZSMA20111008 in the tree does not fit with our material due to its horizontal abdomen, relatively long auxiliary claws and also its distribution area which is located in Australia.

Two other interesting possible species could be *P. tumidula* Loman, 1923 and *P. candidoi* Mello-Leitao, 1923, since both seem to exhibit the short hairs on the ventral side of the second and third coxa. However the latter has an eight-segmented oviger in females and auxiliary claws clearly longer than half the length of the main claw, which differs from our specimens. Furthermore, *P. candidoi* is only sampled from South Georgia to South Brazil so far. *P. tumidula* is characterized and drawn by Stock (1957) with "Fiederdornen" on the ventral distal side of coxa two and three. He mentions that this feature makes *P. tumidula* clearly distinguishable from *P. patagonica*. Confusingly if one regards the original description of 1923, Loman neither mentions short hairs on the coxae nor shows them in his

648 drawings. Furthermore, the type material we had at hand from the Swedish Museum of

649 Natural History didn't show any prominent hairs on the coxae. Only our specimens from the

650 Chilean clade show this kind of "Fiederdornen" and in contrast to *P. tumidula* they have

651 eight- to nine-segmented ovigers in females, whereas Loman mentions a "ten-segmented"

652 oviger in his individuals. One drawing by Loman of a young female shows the last oviger

653 segments to be fused, which could be more consistent with our specimens. But this would

654 mean that all our specimens from the Chilean clade would be just juveniles, which can be

655 excluded for example by the visible eggs inside the femur in females, indicating an adult

656 state. Furthermore, Loman does not mention any setae on the abdomen. Besides several short

657 setae, our specimens show also two very prominent larger spines on the distal end of the

658 abdomen. Another fact that should be kept in mind is that *P. tumidula* has only been captured

659 from North Argentina so far. All this leads us to the decision that our specimens can not be *P.*

660 *tumidula*.

661 Concerning our specimens from the Falkland clade, on the first view one possible candidate

662 could be *P. kupei* Clark, 1971. However, the auxiliary claws, being more than half as long as

663 the main claw (Clark, 1971), and the Macquarie and New Zealand Plateau distribution of this

664 species (Child, 1995) separate it from *P. patagonica*.

665 Analysing Loman's *P. patagonica* collection and one *P. patagonica* specimen of Hedgpeth

666 from the Swedish Museum of Natural History furthermore confirms our considerations. Eight

667 specimens (SMNH-125445, SMNH-125507, SMNH-125508, SMNH-125509, SMNH-

668 125510) captured from the Graham region, South Georgia and Falkland Islands determined as

669 *P. patagonica* by Loman are perfectly in accordance with the morphology of our specimens

670 from the Falkland clade. In contrast the specimen SMNH-125527 determined as *P.*

671 *patagonica* by Hedgpeth, which was collected 41°47'S, 73°15'W, fits better with the

672 description of the specimens of our Chilean clade. This would mean this specimen is not a *P.*

673 *patagonica*, but a *P. yepayekae*. Furthermore, the only undetermined specimen by Loman

674 (SMNH-125514), which was collected at Tierra del Fuego (55°10'S, 66°15'W) shows the

675 same characteristics as *P. yepayekae,* here described as a new species. This also explains why

676 Loman determined this specimen only to genus level. He seemed to see the differences to *P.*

677 *patagonica*.

678 For *P. patagonica* however a broad variability concerning different characteristics is

679 discussed. Gordon (1932) notices that the gap between the lateral processes ranges from being

680 little separated to separated by about their own diameter. Furthermore, the spination of the

681 propodus varies greatly in numbers and length, bearing for example either two, three or four

682   spines (Gordon, 1944). Whereas Stock (1975) describes the propodus as more heavy and

683   robust, it is considered as long by Child (1995).

684   The length of the auxiliary claws is given as either one third the length of the main claw

685   (Stock, 1957), half the length of the main claw (Calman, 1915; Gordon, 1944; Hodgson,

686   1907; Möbius, 1902) or even longer (Pushkin, 1975; Pushkin, 1993). Except for one specimen

687   (ZSMA20111008) our studied specimens have auxiliary claws reaching one third to one half

688   the length of the main claw.

689   Whereas Stock (1957) remarks that *P. patagonica* lacks "Fiederdornen" (stellate setae) on the

690   second and third coxa of the legs, some kind of short hairs are mentioned in Pushkin (1975):

691   "…The few very small spines are located along the ventral surface of the 2nd and 3rd

692   segments. Similar spines surround the genital pore and form a small cluster on the ventral

693   dilatation of the distal part of the third segment." Here specimens from the Chilean clade are

694   distinguishable by their "Fiederdornen" from specimens from the Antarctic region or Falkland

695   Islands.

696   Another very variable characteristic affects the cement gland of the males. Whereas the

697   cement gland tube itself when present is always very short, the ventral pore can be on a flat

698   surface, on a broad raised surface or something in between (Child, 1995). Our specimens

699   show a mixture of everything: sometimes the cement gland tube is hardly visible (PpaE_001-

700   002, PpaA_001), short (specimens from the Falkland Islands) or three times its own width

701   (which is the case for the Chilean clade). Concerning the orientation of setae of the ovigers,

702   we could detect the same sexual dimorphism as mentioned in Bamber (2002). There are no

703   differences between *P. yepayekae* spec. nov. and *P. patagonica.*

704   Moreover the abdomen of *P. patagonica* can be long and erect or be shorter and horizontal

705   (Child, 1995). The only specimen with a straight horizontal abdomen is PpaE_001 from the

706   Shag rocks, near South Georgia. All other individuals have an upwards erected abdomen.

707   Since the morphological differences among the corresponding specimens lie well within the

708   broad variation described in the literature, we assigned all of our studied specimens (except

709   those assigned to *P. yepayekae*) tentatively to *P. patagonica*. However, this pronounced

710   morphological variability in many features indicates in parallel with our molecular results that

711   *P. patagonica* is a species complex.

712

713   **Conclusion**

714

715 To summarize our considerations, we could not assign our specimens (except *P. yepayekae*
716 described in the present paper) to any of the described/known *Pallenopsis* species other than
717 *P. patagonica* occurring near the studied area with sufficient certainty. It seems necessary to
718 attach, beyond the morphological description, also another level/source of information, i.e. a
719 dataset independent of morphology, as is done here. With our molecular data, this is the first
720 attempt/step to unravel the species complex of *P. patagonica* also with a wider set of
721 techniques. But also the molecular data confirm the variability of the species, resulting in
722 different clades supported by high bootstrap values.
723 As already discussed in our previous study (Weis & Melzer, 2012a), with focus on *Achelia*
724 *assimilis,* the distribution area of *P. yepayekae* corresponds well to the area covered by
725 glaciers during the last ice age. However, the *Pallenopsis* habitat extends to much deeper
726 waters (down to 3,500m) than for *Achelia* (about 900m) (Child, 1994). Therefore, the present-
727 day distribution was either achieved by recolonization from deeper waters or by leading-edge
728 recolonization from more northern, ice-free habitats. The diversity of different haplotypes
729 does not imply that there was a strong bottleneck, however, further specimens are needed to
730 verify this assumption. The extremely high genetic distances between the Falkland
731 "patagonica" clade and the Chilean "yepayekae" Clade indicates that these do not resemble
732 populations geographically isolated. Over a long geographic gradient, genetic distances within
733 *P. yepayekae* were low. Therefore, an allopatric speciation, possibly influenced by the
734 massive glaciations, may be a likely explanation for the speciation.
735 The morphological and molecular results strongly support the hypothesis that the specimens
736 from the Chilean clade represent a species new to science, described here as *Pallenopsis*
737 *yepayekae* spec. nov. The decision to errect *P. yepayekae* as a new species is also supported
738 by the number of eleven individuals, which do not differ strongly both, genetically and
739 morphologically. It is known from previous works (for example (Hebert *et al.*, 2004)) that in
740 less extensively studied invertebrate taxa (such as pycnogonids) hidden biological diversity,
741 in the form of cryptic or overlooked species, is often the rule rather than the exception. How
742 many further species may be hidden behind the *Pallenopsis* complex remains beyond the
743 scope of this paper. This will be an interesting question for further analyses with hopefully
744 more available specimens from the Southern Ocean.
745
746 **Acknowledgments**
747

765

766

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918   *to DNA and Protein Phylogeny.* : Cambridge University Press.

919 **Xia XH, Xie Z, Salemi M, Chen L, Wang Y. 2003.** An index of substitution saturation and
920   its application. *Molecular Phylogenetics and Evolution* **26:** 1-7.

921

922 **Figure 1. Map of sampling sites of Chilean, Antarctic and Subantarctic *Pallenopsis***
923 **specimens deposited at the Bavarian State Collection of Zoology. Sequences of**
924 **specimens from the Ross Sea were downloaded from GenBank.**

925

926 **Figure 2. Bayesian phylogenetic tree of COI sequences of 28 *P. patagonica* (Falkland**
927 **clade and others), 11 *P. yepayekae* sp. nov. (Chile clade), one *P. macneilli,* one *P.***
928 ***buphtalmus,* one *P. latefrontalis* and five *P. pilosa*, which serve as the outgroup.** Posterior
929 probabilities of the Bayesian inference and bootstrap values (>75%) of NJ, MP and ML
930 analyses are displayed above or below branches; different branch lengths indicate

931 substitutions per site. Different haplotypes of the studied specimens are defined as HT1-
932 HT29.

933

934 **Figure 3. NeighborNet of all individual COI sequences, using Splitstree and K2P-**
935 **correction method.**

936

937 **Figure 4. Automatic Barcode Gap Discovery (ABGD) analysis for 42** *Pallenopsis*
938 **specimens (*P. pilosa* excluded) used in the present study.**

939

940 **Figure 5. Automatic Barcode Gap Discovery (ABGD) analysis for 27** *Pallenopsis*
941 **specimens: 16 specimens from the Falkland clade vs. 11 specimens from the Chilean**
942 **clade.**

943

944 **Figure 6. Pairwise genetic distances (K2P) for COI sequences of** *Pallenopsis* **specimens**
945 **(*P. pilosa* excluded) used in the present study.**

946

947 **Figure 7. Lineage-through-time plot of the number of lineages (N) in the linearized**
948 **Bayesian haplotype tree (32 unique COI-barcode sequences).** Vertical line represents the
949 single threshold identified by the GMYC model between Yule speciation and coalescence
950 within populations. The number of GMYC species identified was 15.

951

952 **Figure 8. Light microscopy of** *Pallenopsis patagonica* **s. str. (Falkland clade).** A Dorsal
953 view; scale = 4 mm. B Ventral view of proboscis; scale = 2 mm. C Dorsal view of abdomen;
954 scale = 500 μm. D Right oviger (female); scale = 500 μm. E Detail view of second and third
955 coxa of left fourth walking leg; scale = 1 mm. F Tarsus and propodus with claw and auxiliary
956 claws of right third walking leg; scale = 500 μm.
957 A: PpaE007; B: PpaE010; C: ZSMA20111357; D-E: ZSMA20111350; F: ZSMA20111348.
958 **ac** - auxiliary claws; **cf** - chelifore; **cl** - claw; **cx** - coxa; **eg** - eggs; **fm** - femur; **os** - oviger
959 segment; **ov** - oviger; **pp** - propodus; **pr** - proboscis; **tb** - tibia.

960

961 **Figure 9. Light microscopy of** *Pallenopsis yepayekae* **spec. nov. (Chile clade).** A Dorsal
962 view; scale = 2 mm. B Lateral view of trunk; scale = 1 mm. C Ventral view of proboscis;
963 scale = 500 μm. D Detail view of abdomen, note two prominent spines (arrows); scale = 250

964 μm. E Detail view of second and third coxae of right walking legs, note several short and
965 prominent hairs (arrows); scale = 500 μm. F Left oviger (female); scale = 250 μm.
966 A: ZSMA20111009; B: ZSMA20111006; C: ZSMA20111000; D: ZSMA20111004; E:
967 ZSMA20111002; F: ZSMA20111016.
968 **ab** - abdomen; **cf** - chelifore; **cx** - coxa; **os** - oviger segment; **ov** - oviger; **pr** - proboscis; **tr** -
969 trunk; **wl** - walking leg.
970

971 **Figure 10. SEM of *Pallenopsis patagonica* (A, C, E) and *Pallenopsis yepayekae* spec. nov.**
972 **(B, D, F).** A. Detail view of cement gland tube of left first walking leg; scale = 200 μm. B
973 Detail view of cement gland tube of left second walking leg; scale = 100 μm. C Right oviger
974 (female); scale = 1 mm. D Right oviger (female); scale = 200 μm; insert: Detail view of distal
975 oviger segments (female); scale = 100 μm. E Detail view of hairs on third coxa of left fourth
976 walking leg; scale = 100 μm. F Detail view of hairs on second coxa of left second walking
977 leg; scale = 20 μm.
978 A: ZSMA20111360; B, F: ZSMA20111006; C: ZSMA20111349; D: ZSMA20111009, insert:
979 ZSMA20111024; E: ZSMA20111359.
980

981 **Figure 11. Drawings of *Pallenopsis yepayekae* spec. nov.** A Dorsal view. B Lateral view of
982 female and detailed view of abdomen. C Walking leg, with enlargement of setae of coxae two
983 and three. D Propodus with claw and auxiliary claws. E Female oviger. F Male oviger.
984

985 **Table 1. Summary of collection data and registration of specimens used in this study.**
986

987 **Supporting information**
988

989 **Table S1 Uncorrected pairwise distances between the different specimens/clades.** Chile
990 clade = ZSMA20111000, ZSMA20111002-006, ZSMA20111009, ZSMA20111012,
991 ZSMA20111016, ZSMA20111024 and ZSMA20111339. Falkland clade = PpaE004-008,
992 PpaE010, ZSMA20111348-51, ZSMA20111354-55, ZSMA20111357, ZSMA20111359-61.
993 (XLS)
994

995 **Table S2 Morphological characteristics of specimens that were available for**
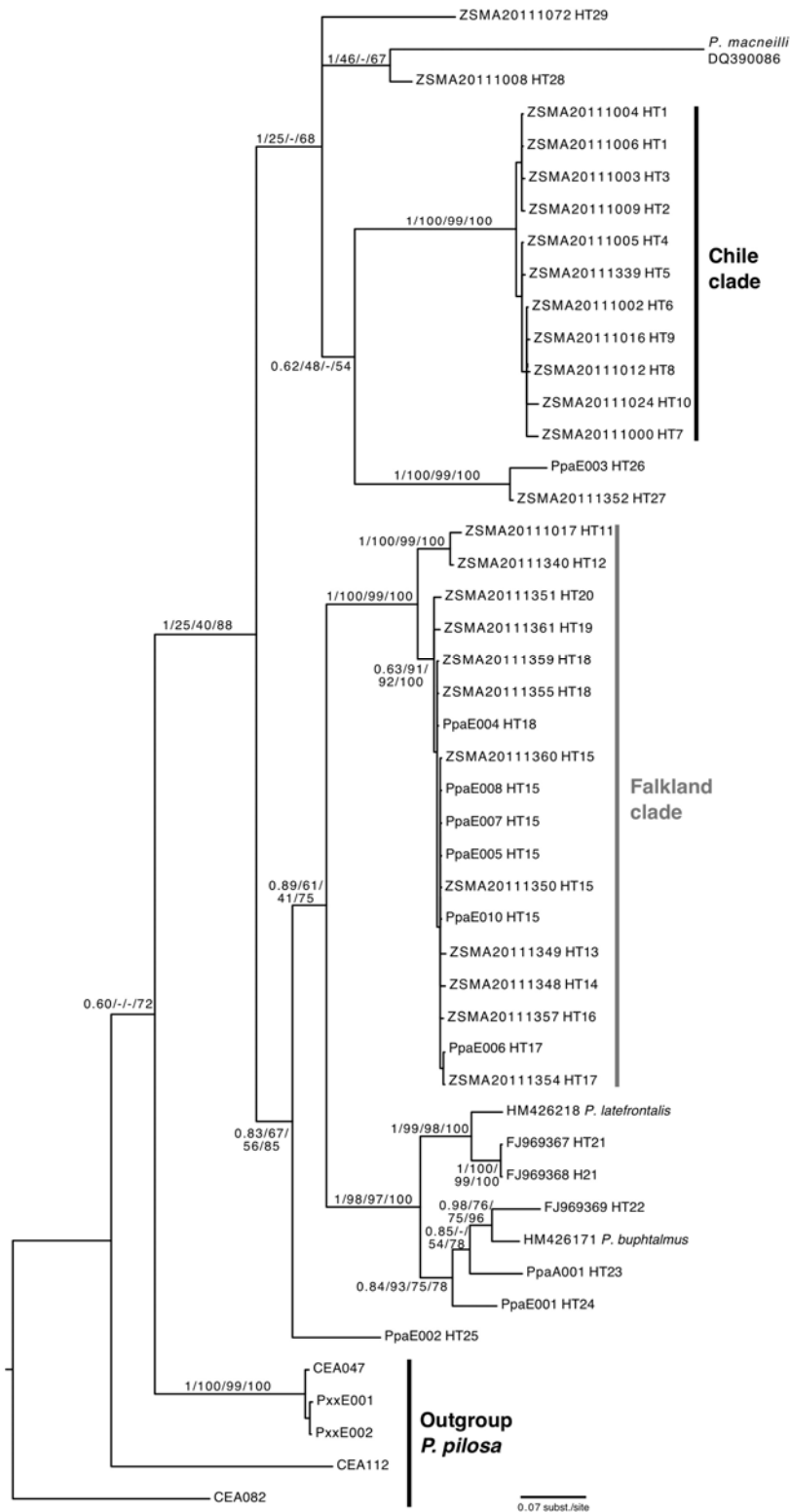996 **morphological studies.**

A: 0 = oval, 1 = slightly swollen at middle, 2 = swollen at middle; B: 0 = ≤ 7,5 mm, 1 = ≥ 8,5 mm; C: 0 = erected, 1 = horizontal; D: 0 = glabrous, 1 = two rows of lateral spines, two outermost spines not conspicuously larger, 2 = two outermost spines very prominent (about three times larger); E: 0 = ≤ 1/3 main claw lengths, 1 = 1/3-1/2 main claw lengths; F: 0 = rounded, 1 = slightly pointed, 2 = pointed; G: 0 = almost glabrous, 1 = few hairs, 2 = many prominent hairs; H: 0 = swollen, 1 = not swollen/straight; I: 0 = 10 oviger segments, 1 = < 10 oviger segments; J: 0 = hardly/not visible, 1 = ≤ 2 times its diameter, 2 = > 2 times its diameter; K: 0 = separated by less their diameter, 1 = separated ≥ their diameter. Chile clade = ZSMA20111000, ZSMA20111002-006, ZSMA20111009, ZSMA20111012, ZSMA20111016, ZSMA20111024 and ZSMA20111339. Falkland clade = PpaE004-008, PpaE010, ZSMA20111348-51, ZSMA20111354-55, ZSMA20111357, ZSMA20111359-61. (XLS)

Figure 1. Map of sampling sites of Chilean, Antarctic and Subantarctic *Pallenopsis* specimens deposited at the Bavarian State Collection of Zoology. Sequences of specimens from the Ross Sea were downloaded from GenBank.

Figure 2. Bayesian phylogenetic tree of COI sequences of 28 *P. patagonica* (Falkland clade and others), 11 *P. yepayekae* sp. nov. (Chile clade), one *P. macneilli*, one *P. buphtalmus*, one *P. latefrontalis* and five *P. pilosa*, which serve as the outgroup. Posterior probabilities of the Bayesian inference and bootstrap values (>75%) of NJ, MP and ML analyses are displayed above or below branches; different branch lengths indicate substitutions per site. Different haplotypes of the studied specimens are defined as HT1-HT29.

Figure 3. NeighborNet of all individual COI sequences, using Splitstree and K2P-correction method.
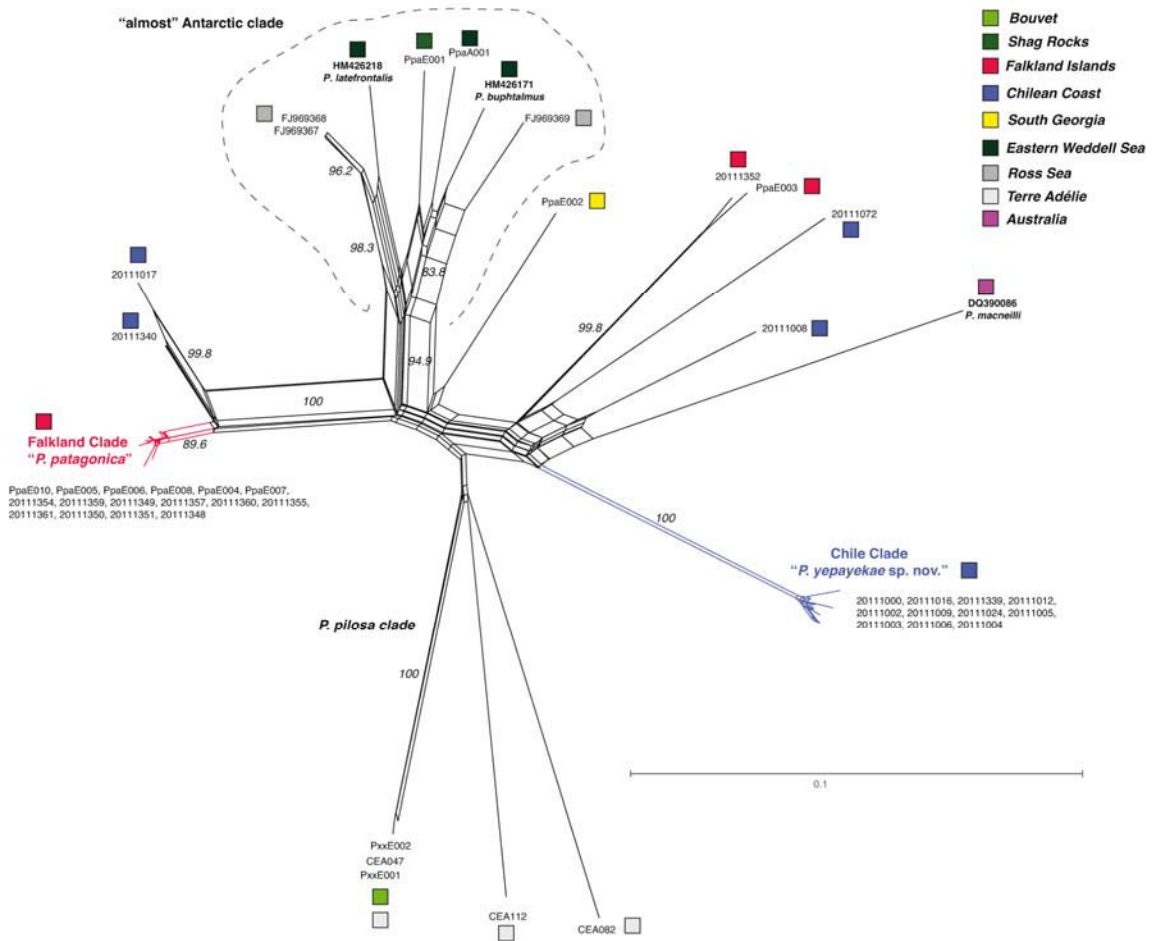
1031   **Figure 4. Automatic Barcode Gap Discovery (ABGD) analysis for 42 *Pallenopsis***
1032   **specimens (*P. pilosa* excluded) used in the present study.**
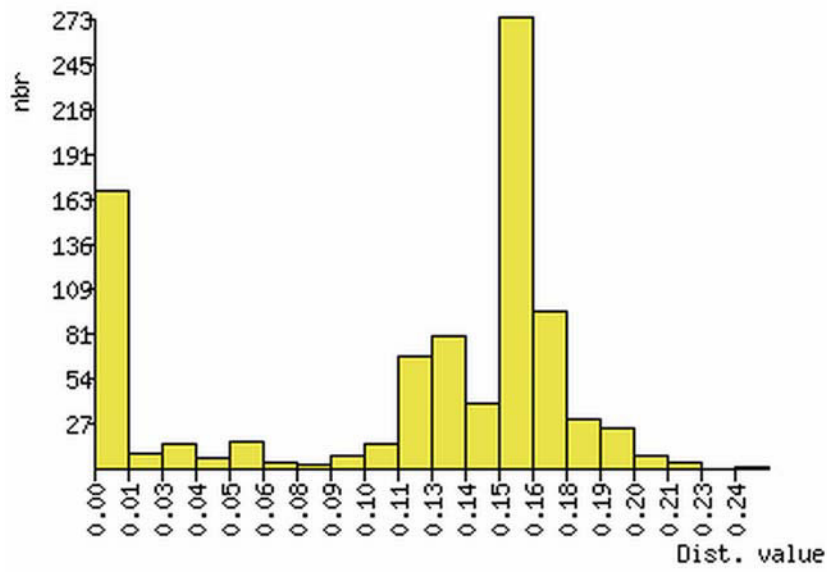1033
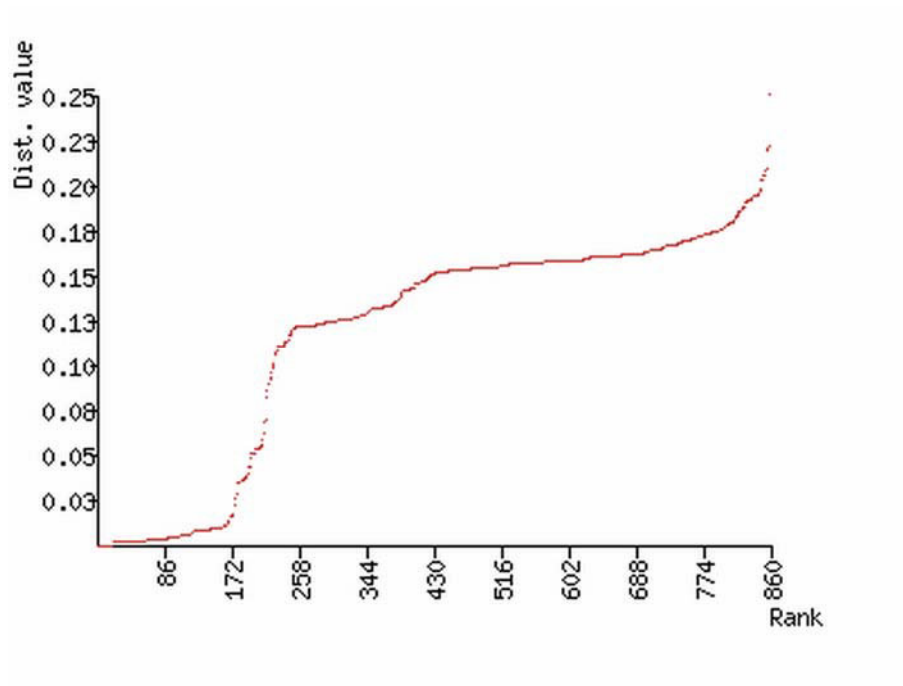
1034
1035

1036 **Figure 5. Automatic Barcode Gap Discovery (ABGD) analysis for 27** *Pallenopsis*
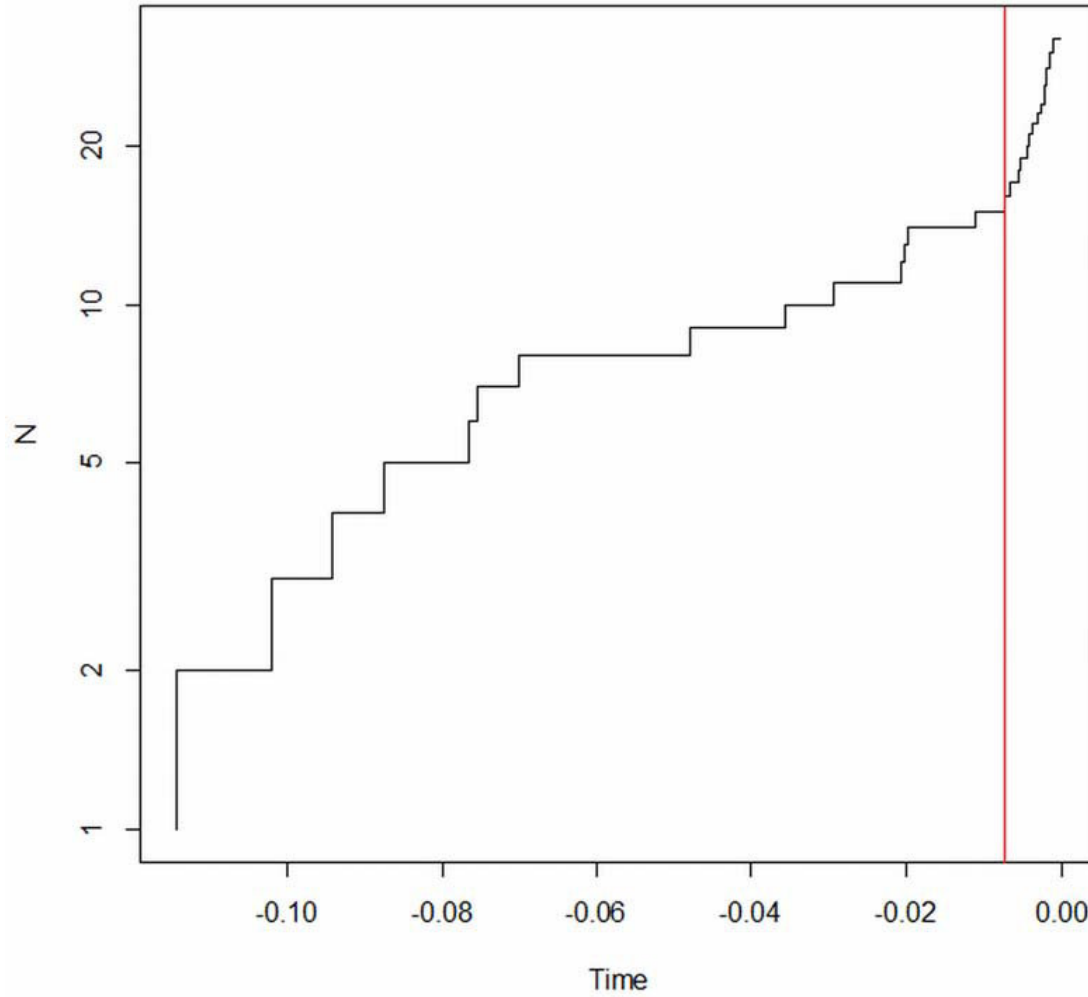1037 **specimens: 16 specimens from the Falkland clade vs. 11 specimens from the Chilean**
1038 **clade.**
1039
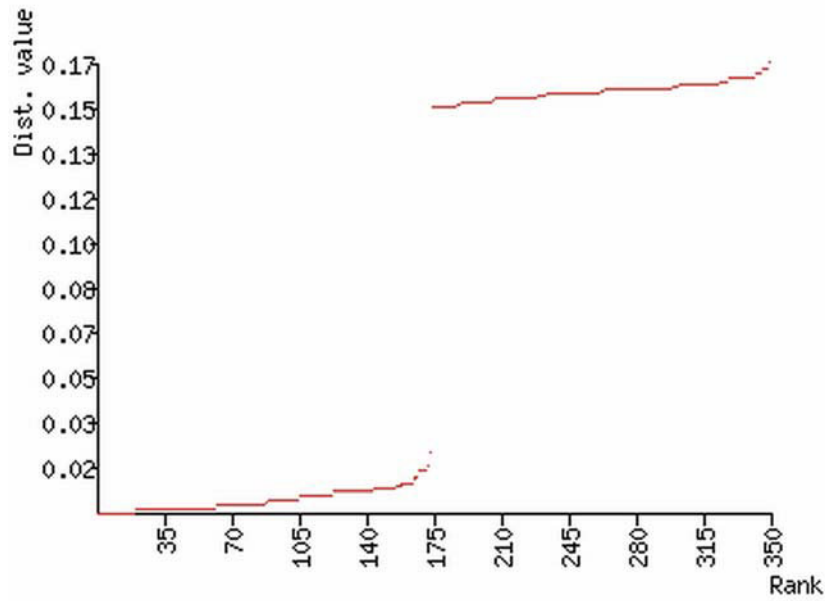
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1046 **Figure 7. Lineage-through-time plot of the number of lineages (N) in the linearized**
1047 **Bayesian haplotype tree (32 unique COI-barcode sequences).** Vertical line represents the
1048 single threshold identified by the GMYC model between Yule speciation and coalescence
1049 within populations. The number of GMYC species identified was 15.

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Figure 9. Light microscopy of *Pallenopsis yepayekae* spec. nov. (Chile clade). A Dorsal view; scale = 2 mm. B Lateral view of trunk; scale = 1 mm. C Ventral view of proboscis; scale = 500 µm. D Detail view of abdomen, note two prominent spines (arrows); scale = 250 µm. E Detail view of second and third coxae of right walking legs, note several short and prominent hairs (arrows); scale = 500 µm. F Left oviger (female); scale = 250 µm.
A: ZSMA20111009; B: ZSMA20111006; C: ZSMA20111000; D: ZSMA20111004; E: ZSMA20111002; F: ZSMA20111016.
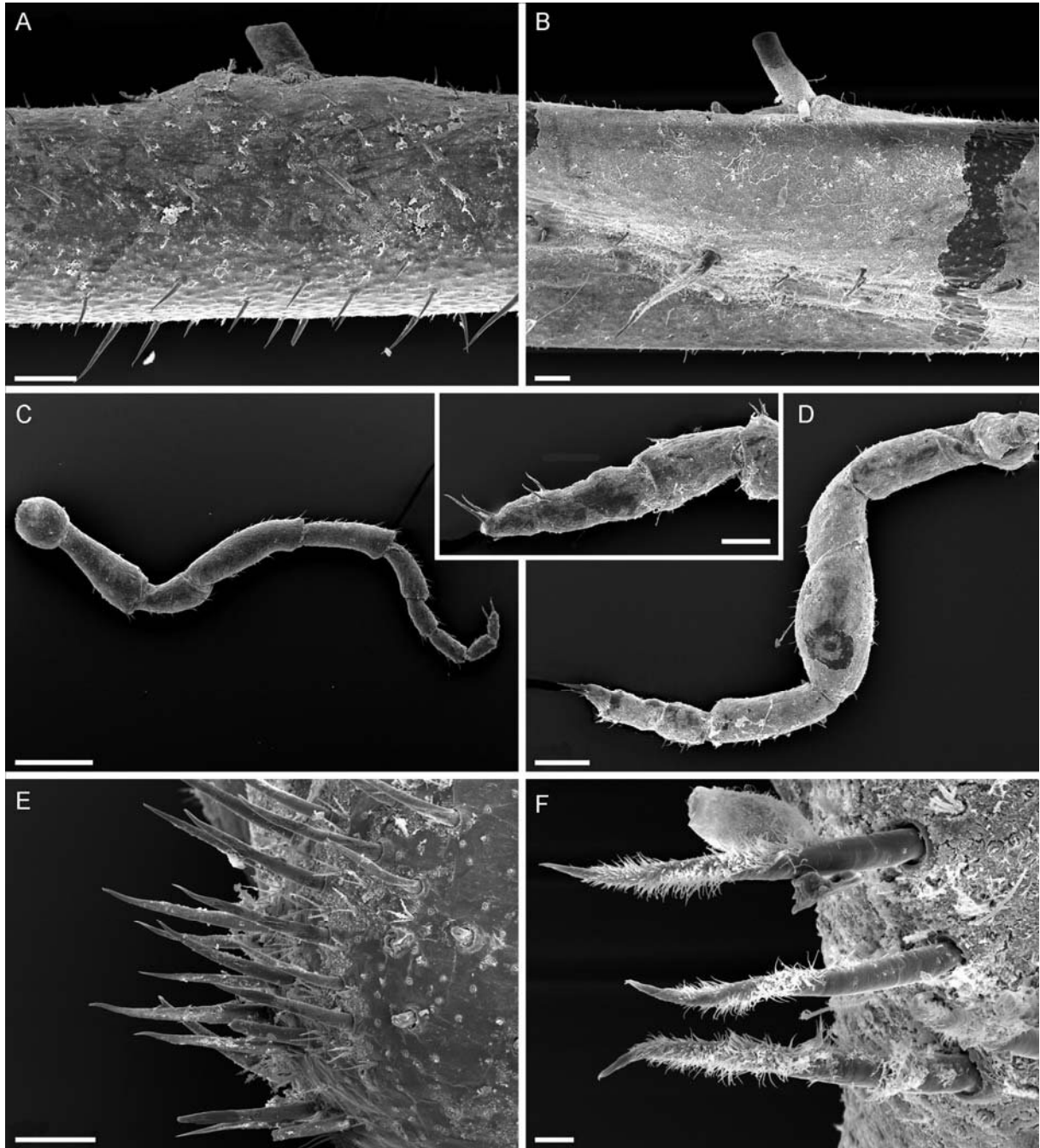**ab** - abdomen; **cf** - chelifore; **cx** - coxa; **os** - oviger segment; **ov** - oviger; **pr** - proboscis; **tr** - trunk; **wl** - walking leg.
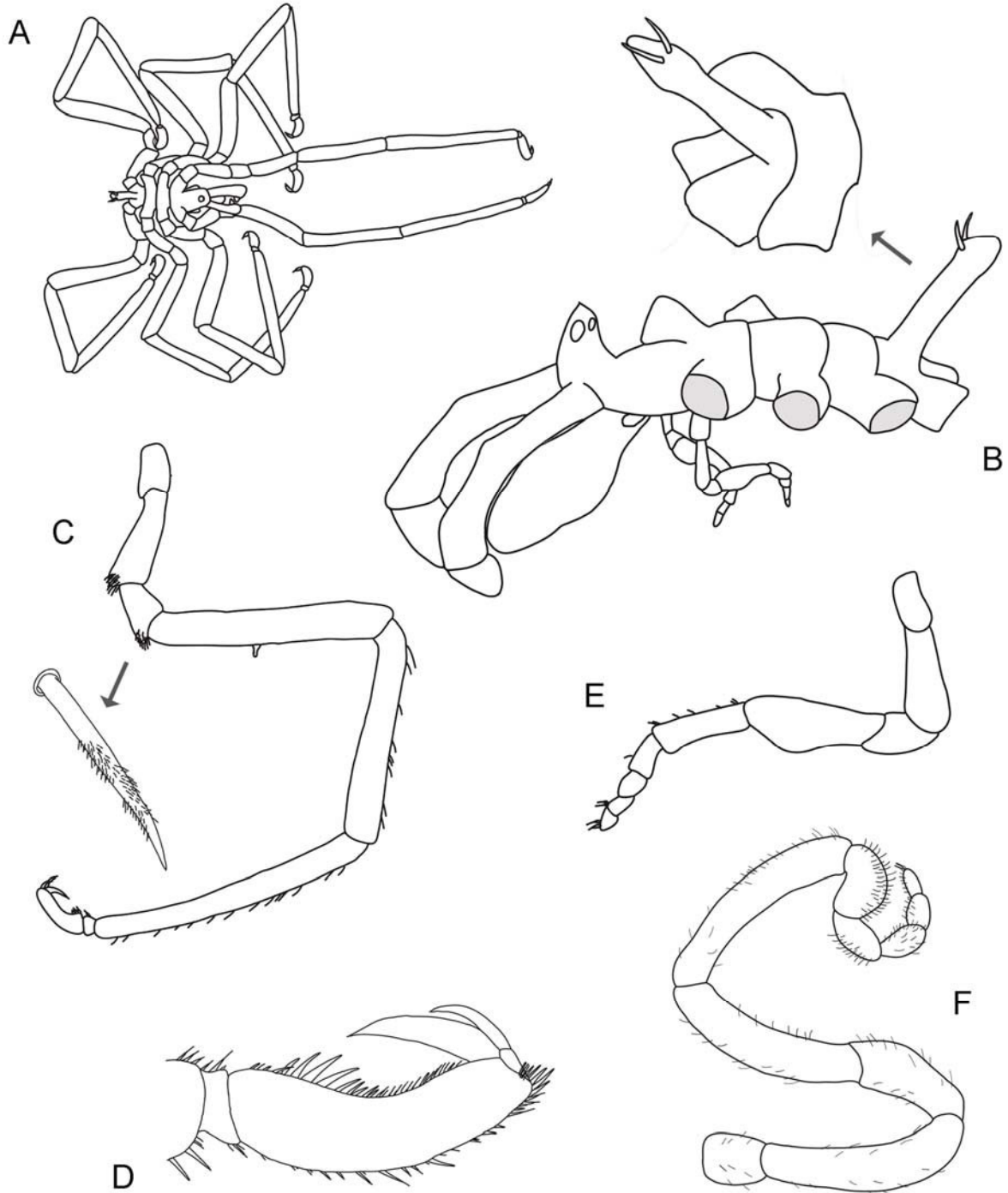
Figure 10. SEM of *Pallenopsis patagonica* **(A, C, E) and** *Pallenopsis yepayekae* **spec. nov.**
(B, D, F). A. Detail view of cement gland tube of left first walking leg; scale = 200 μm. B
Detail view of cement gland tube of left second walking leg; scale = 100 μm. C Right oviger
(female); scale = 1 mm. D Right oviger (female); scale = 200 μm; insert: Detail view of distal
oviger segments (female); scale = 100 μm. E Detail view of hairs on third coxa of left fourth
walking leg; scale = 100 μm. F Detail view of hairs on second coxa of left second walking
leg; scale = 20 μm.
A: ZSMA20111360; B, F: ZSMA20111006; C: ZSMA20111349; D: ZSMA20111009, insert:
ZSMA20111024; E: ZSMA20111359.

Figure 11. Drawings of *Pallenopsis yepayekae* spec. nov. A Dorsal view. B Lateral view of
female and detailed view of abdomen. C Walking leg, with enlargement of setae of coxae two
and three. D Propodus with claw and auxiliary claws. E Female oviger. F Male oviger.

Table 1. Summary of collection data and registration of specimens used in this study.

Voucher ID	Haplotype	Species	Country/Region
ZSMA20111000	HT 7	Pallenopsis yepayekae n. sp.	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111002	HT 6	Pallenopsis yepayekae n. sp.	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111003	HT 3	Pallenopsis yepayekae n. sp.	Chile; Region de los Lagos
ZSMA20111004	HT 1	Pallenopsis yepayekae n. sp.	Chile; Region de los Lagos
ZSMA20111005	HT 4	Pallenopsis yepayekae n. sp.	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111006	HT 1	Pallenopsis yepayekae n. sp.	Chile; Region de los Lagos
ZSMA20111008	HT 28	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111009	HT 2	Pallenopsis yepayekae n. sp.	Chile; Region de los Lagos
ZSMA20111012	HT 8	Pallenopsis yepayekae n. sp.	Chile; Region de los Lagos
ZSMA20111016	HT 9	Pallenopsis yepayekae n. sp.	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111017	HT 11	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111024	HT 10	Pallenopsis yepayekae n. sp.	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111072	HT 29	Pallenopsis patagonica	Chile; Region de Valparaiso
ZSMA20111339	HT 5	Pallenopsis yepayekae n. sp.	Chile; Anihue Raul Marin Balmaceda
ZSMA20111340	HT 12	Pallenopsis patagonica	Chile; Region de Magallanes y de la Antarctica Chilena
ZSMA20111348	HT 14	Pallenopsis patagonica	Falkland Islands
ZSMA20111349	HT 13	Pallenopsis patagonica	Falkland Islands
ZSMA20111350	HT 15	Pallenopsis patagonica	Falkland Islands
ZSMA20111351	HT 20	Pallenopsis patagonica	Falkland Islands
ZSMA20111352	HT 27	Pallenopsis patagonica	Falkland Islands
ZSMA20111354	HT 17	Pallenopsis patagonica	Falkland Islands
ZSMA20111355	HT 18	Pallenopsis patagonica	Falkland Islands
ZSMA20111357	HT 16	Pallenopsis patagonica	Falkland Islands
ZSMA20111359	HT 18	Pallenopsis patagonica	Falkland Islands
ZSMA20111360	HT 15	Pallenopsis patagonica	Falkland Islands
ZSMA20111361	HT 19	Pallenopsis patagonica	Falkland Islands
PpaE_004	HT 18	Pallenopsis patagonica	Falkland Islands
PpaE_005	HT 15	Pallenopsis patagonica	Falkland Islands
PpaE_006	HT 17	Pallenopsis patagonica	Falkland Islands
PpaE_007	HT 15	Pallenopsis patagonica	Falkland Islands
PpaE_008	HT 15	Pallenopsis patagonica	Falkland Islands
PpaE_010	HT 15	Pallenopsis patagonica	Falkland Islands
PpaE_001	HT 24	Pallenopsis patagonica	Subantarctic; West of South Georgia; Shag Rocks
PpaE_002	HT 25	Pallenopsis patagonica	Subantarctic; South Georgia
PpaE_003	HT 26	Pallenopsis patagonica	Subantarctic; Burdwood Bank
PpaA_001	HT 23	Pallenopsis patagonica	Antarctic; Eastern Weddell Sea
NIWA46256	HT 21	Pallenopsis patagonica	Antarctic; Ross Sea
NIWA46257	HT 21	Pallenopsis patagonica	Antarctic; Ross Sea
NIWA46258	HT 22	Pallenopsis patagonica	Antarctic; Ross Sea
HM426218		Pallenopsis latefrontalis	Antarctic; Eastern Weddell Sea
HM426171		Pallenopsis buphtalmus	Antarctic; Eastern Weddell Sea
DQ390086		Pallenopsis macneilli	Australia; Rocky Point, Torquay
PxxE001		Pallenopsis pilosa	Subantarctic; Bouvet Islands
PxxE002		Pallenopsis pilosa	Subantarctic; Bouvet Islands
CEA047		Pallenopsis pilosa	Antarctica
CEA112		Pallenopsis pilosa	Antarctica
CEA082		Pallenopsis pilosa	Antarctica

Latitude	Longitude	Depth	BOLD ID/GenBank ID
48°44'11.4"S	75°24'53.1"W	15m	CFAP013-11
50°50'07.1"S	74°08'20.9"W	25m	CFAP017-11
43°25'03.0"S	74°04'51.2"W	25m	CFAP006-11
43°24'34.5"S	74°05'00.7"W	9m	CFAP005-11
48°44'11.4"S	75°24'53.1"W	23m	CFAP014-11
43°25'03.0"S	74°04'51.2"W	20m	CFAP007-11
50°24'52"S	74°33'33"W	15-25m	CFAP026-11
43°23'33.4"S	74°07'56.5"W	26m	CFAP004-11
43°46'28.5"S	073°02'63.2"W	22m	CFAP008-11
48°36'28.7"S	74°53'55.7"W	32m	CFAP012-11
48°36'28.7"S	74°53'55.7"W	32m	CFAP025-11
49°34'38.7"S	74°26'49.3"W	28m	CFAP016-11
33°23'55"S	71°52'78.2"W	339m	CFAP023-11
43°46'31.35"S	73°01'44.14"W	19m	CFAP019-11
55°00'00.6"S	68°18'88.1"W	24m	CFAP018-11
50°26'4.00"S	62°46'5.00"W	146-148m	CFAP027-11
51°16'8.00"S	62°57'8.00"W	171-174m	CFAP034-11
51°16'8.00"S	62°57'8.00"W	171-174m	CFAP035-11
51°16'8.00"S	62°57'8.00"W	171-174m	CFAP036-11
51°16'8.00"S	62°57'8.00"W	171-174m	CFAP037-11
51°05'8.00"S	61°44'0.00" W	174-176m	CFAP028-11
51°05'8.00"S	61°44'0.00"W	174-176m	CFAP029-11
51°05'8.00"S	61°44'0.00"W	174-176m	CFAP030-11
51°05'8.00"S	61°44'0.00"W	174-176m	CFAP031-11
51°05'8.00"S	61°44'0.00"W	174-176m	CFAP032-11
51°05'8.00"S	61°44'0.00"W	174-176m	CFAP033-11
52°57'42"S	60°08'36"W	378m	KC794961
52°57'42"S	60°08'36"W	378m	KC794962
52°57'42"S	60°08'36"W	378m	KC794963
52°57'42"S	60°08'36"W	378m	KC794964
52°57'42"S	60°08'36"W	378m	KC794965
52°57'42"S	60°08'36"W	378m	KC794966
53°46'12"S	41°26'6"W	193m	KC794959
54°00'59"S	37°26'14"W	78m	KC794960
54°33'00"S	58°49'20"W	158m	KC794969
71°08'09"S	11°31'37"W	123m	KC794958
71°15'45"S	170°38'08"W	466m	FJ969367
72°00'81"S	170°46'47"W	235.5m	FJ969368
71°37'24"S	170°51'99"W	204.5m	FJ969369
71° 5' 31.23"S	11° 30' 28.8"W	302m	HM426218
71° 19' 1.2"S	13° 56' 31.2"W	848m	HM426171
38°20'38.07"S	144°19'12.77"E	0.5m	DQ390086
54°21'00"S	3°11'36"E	465m	KC794967
54°21'30"S	3°26'6"E	200m	KC794968
66° 23' S	140° 25' 43.87"E	743m	AAC7281
65° 52' 11.81"S	143° 0' 5.57"E	428m	AAC7183
65° 51' 9.32"S	144° 2' 23.15"E	1104m	AAC7182

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7. General Discussion and Results

The present thesis provides a detailed overview including plenty of explicit light- and scanning electron microscope pictures of Antarctic and Subantarctic pycnogonids. Beside a comprehensive discussion concerning their species' diagnostic features compared to previous literature, this work supplies beyond that an updated version of their geographic distributions. Both the Antarctic and the Subantarctic pycnogonid material are housed at the Bavarian State Collection of Zoology in Munich. This material also includes specimens from the Chilean coast and fjords that have been mainly collected during the "Huinay fjordos" expeditions between 2005 and 2011. Altogether 40 species from 9 of the 11 pycnogonid families are represented, namely:

Order **Pantopoda** Gerstäcker, 1863

Suborder **Eupantopodida** Fry, 1978

Superfamily **Ascorhynchoidea** Pocock, 1904

Family **Ammotheidae** Dohrn, 1881

  Achelia assimilis (Haswell, 1884)

  Achelia communis (Bouvier, 1906)

  Achelia spicata (Hodgson, 1915)

  Ammothea longispina Gordon, 1932

  Ammothea magniceps Thompson, 1884

  Ammothea spinosa (Hodgson, 1907)

  Cilunculus cactoides Fry & Hedgpeth, 1969

  Tanystylum cavidorsum Stock, 1957

  Tanystylum neorhetum Marcus, 1940

Superfamily **Colossendoidea** Hoek, 1881

Family **Colossendeidae** Hoek, 1881

  Colossendeis australis Hodgson, 1907

  Colossendeis longirostris Gordon, 1938

  Colossendeis macerrima Wilson, 1881

  Colossendeis megalonyx Hoek, 1881

  Colossendeis scoresbii Gordon, 1932

  Colossendeis tortipalpis Gordon, 1932

Superfamily **Nymphonoidea** Pocock, 1904

Family **Callipallenidae** Hilton, 1942

    Anoropallene palpida (Hilton, 1939)

    Austropallene cornigera Möbius, 1902

    Austropallene gracilipes Gordon, 1944

    Callipallene margarita (Gordon, 1932)

Family **Nymphonidae** Wilson, 1878

    Nymphon australe Hodgson, 1902

    Nymphon biarticulatum (Hodgson, 1907)

    Nymphon charcoti Bouvier, 1911

    Nymphon compactum Hoek, 1881

    Nymphon eltaninae Child, 1995

    Nymphon longicollum Hoek, 1881

    Nymphon longicoxa Hoek, 1881

    Nymphon mendosum Hodgson, 1907

    Nymphon proceroides Bouvier, 1913

    Nymphon proximum Calman, 1915

    Nymphon villosum Hodgson, 1907

    Pentanymphon antarcticum Hodgson, 1904

Family **Pallenopsidae** Fry, 1978

    Bathypallenopsis macronyx (Bouvier, 1911)

    Pallenopsis hodgsoni Gordon, 1938

    Pallenopsis notiosa Child, 1992

    Pallenopsis patagonica (Hoek, 1881)

    Pallenopsis yepayekae Weis nov. spec., in Weis et al. accepted

Superfamily **Phoxichilidoidea** Sars 1891

Family **Phoxichilidiidae** Sars, 1891

    Anoplodactylus californicus Hall, 1912

Superfamily **Pycnogonoidea** Pocock, 1904

Family **Pycnogonidae** Wilson, 1878

    Pycnogonum gaini Bouvier, 1910

Superfamily **Rhynchothoracoidea** Fry, 1978

Family **Rhynchothoracidae** Thompson, 1909

    Rhynchothorax australis Hodgson, 1907

Suborder **Stiripasterida** Fry, 1978

Family **Austrodecidae** Stock, 1954

     Austrodecus glaciale Hodgson, 1907

7.1. Morphological analyses and biogeographic remarks

7.1.1. Antarctic pycnogonid fauna

Concerning the species richness pycnogonids display an important component of the Antarctic and Subantarctic benthos (Chimenz Gusso & Gravina 2001). Up to now about 40.000 specimens have been found in the Antarctic and surrounding area (Munilla & Soler-Membrives 2008), an area which has previously been described as a centre of pycnogonid geographic dispersal and evolutionary radiation (Hedgpeth 1947, Fry & Hedgpeth 1969, Munilla & Soler-Membrives 2008, Griffiths et al. 2011). From the so far 264 recorded pycnogonid species recorded from Antarctic waters (representing 19.6% of the 1344 species described worldwide!) 108 are endemic (Munilla & Soler-Membrives 2008). According to the latter authors the main austral genera are *Nymphon* Fabricius, 1794 with 67 species of about 270 worldwide (Bamber & El Nagar 2013) (with *Nymphon australe* being the most frequently recorded species) and *Colossendeis* Jarzinsky, 1870 with 36 species of about 70 worldwide (Bamber & El Nagar 2013). This is in well accordance with the Antarctic collection (comprising 119 specimens) of the present study where *Nymphon australe* and *Colossendeis megalonyx* were the most abundant species (16 and 17 specimens respective). Furthermore this study presents to our knowledge the first record of *Ammothea magniceps, Cilunculus cactoides, N. compactum, N. eltaninae, N. longicoxa* and *N. proceroides* from the Weddell Sea. Beyond that *Ammothea magniceps* is recorded for the first time from the Antarctic and in depths between 300-333 m, much deeper than previously mentioned (see Müller 1993: 0.5-24 m). Also the depth ranges for some further species could be expanded like for example *C. longirostris* showing with 3800 m its deepest location ever measured. Although the major morphological characteristics correspond well with the respective descriptions published earlier, I could discover some minor discrepancies which could impede correct species determination. To avoid problems in future species determinations, I added "remark-sections" beneath each species description followed with high resolution pictures of the species' diagnostic features (see Paper I).

An example for these new findings is the most frequently found species *N. australe* showing distinct segment borders between segments 3-4 in all examined individuals. This is

contradictory to Child's key (1995) where the trunk segments 3-4 are described as fused. Furthermore for the species *N. charcoti* I could illustrate detailed SEM pictures of several spines on the lateral processes that have not been described in the literature so far. Beyond that for the genus *Austropallene* it was possible to take SEM pictures of the tuft of hairs surrounding the mouth which has previously been mentioned in the literature as a "Borstenkranz" (Helfer & Schlottke 1935).

7.1.2. Subantarctic and Chilean pycnogonid fauna

As already mentioned above many pycnogonid studies focus on the Antarctic area. Aim of my theses is to extend the spectrum to hitherto relatively unexplored Subantarctic regions. The 90.000 km long Southern Chilean coastline with its impressive fjord regions represents an interesting study area. However even in that, to some extent very isolated area pycnogonids have already been studied decades of years ago by Hoek (1881), Loman (1923a, b) and Hedgpeth (1961). Pycnogonid material was collected on the one hand during the H. M. S. Challenger Expedition (1872-1876), the Antarctic Swedish Expedition (1901-1903) and on the Lund University Chile Expedition which dates back to the 1950s. Now about 50 years later we resume the work adding plenty of new Chilean pycnogonid material and are able to study them with more modern techniques like SEM or molecular approaches (DNA barcoding).

A species list of pycnogonids that have been found in the southeast Pacific Ocean was published by Sielfeld (2003) including Chilean Patagonia and subtropical habitats. From these about 40 pycnogonid species many are inhabitants of deep waters (Melzer 2009). In contrast the pycnogonid material in the present study was mostly collected by SCUBA diving from shallower depths. Nine of the altogether 12 collected Subantarctic/Chilean species are already mentioned in Sielfeld (2003) (exceptions are *Colossendeis macerrima, C. megalonyx* and *Anoropallene palpida*). Although *C. megalonyx* was already described from the South American shelf by Hoek (1881), two of our specimens were found in a more northern part of Chile near Concepcion (36°S). Since this species is predominantly found in more southern region, especially the Antarctica (see Munilla & Soler-Membrives 2008) this locality represents the northernmost collecting site for this species. A northward dispersal of Magellanic species could be explained by the Humboldt Current arising from the Antarctic and passing along the Chilean coast (see also Brattström & Johanssen 1983).

On the other hand *Anoropallene palpida* has been collected about 2500 km more south (in Bahia de Coliumo) from its previous southernmost sample location South-East of Punta Lomas (Peru) (see Child 1992). Two further examples are *Anoplodactylus californicus* and *Achelia assimilis* which are both predominantly known from tropical and subtropical regions (Müller, 1993). The specimens collected from our own sampling trips are some of the southernmost found, with *Anoplodactylus californicus* from the Chilean fjord region at about 42°S and *Achelia assimilis* from Tierra del Fuego at about 55°S. The latter one resembles with a total of 226 specimens beyond that also the most frequently recorded species in the present study. Our samples include both species of a probably northern origin that extend far to the south and species with a southern origin extending to the north, confirming again the Chilean fjord region as a particular interesting study area for pycnogonids. A detailed overview of the most prominent characteristics and different distribution patterns is illustrated in Paper II.

7.2. DNA barcoding of Subantarctic/Chilean pycnogonida

Detailed morphological studies of specimens form the basis for further deeper analytical analyses using molecular techniques. Methodological developments like DNA barcoding has been introduced by Hebert et al. (2003) and may serve as "the core of a global bioidentification system for animals" and tie in where morphological analyses reach their limits. Especially where character combinations overlap or high variability within one species occurs another level of analyses is needed. Beyond that cryptic species can often be misidentified based on morphological traits alone (Baker & Gatesy 2002, Proudlove & Wood 2003). Therefore a more modern approach called integrative taxonomy (Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010) has reached public interest by combining morphological and molecular data to delineate and identify species. Like Grant and his colleagues (2011) stated: "Molecular taxonomy in combination with traditional taxonomic methods, is the way forward and offers the best chance of recording, and therefore protecting biodiversity."

The mitochondrial protein-coding gene COI from 76 Subantarctic/Chilean pycnogonids was analysed displaying 10 distinct, well-supported branches in the phylogenetic consensus tree, namely: *Achelia assimilis, Ammothe spinosa, Tanystylum cavidorsum, T. neorhetum, Colossendeis macerrima, C. megalonyx, C. scoresbii, Callipallene margarita, Pallenopsis patagonica* and *Anoplodactylus californicus*. The calculated trees received high bootstrap support and are in well accordance with the previous species determination based on

morphology, indicating that the COI barcoding method is a suitable tool for resolving relationships of pycnogonids at species level. Special focus however is directed on the most abundant species *Achelia assimilis* which is splitted up in four distinct lineages.

7.2.1. *Achelia assimilis*

As mentioned above *Achelia assimilis* belongs with a total of 226 specimens in our samples to one of the most abundant species in the Chilean fjord region. Interestingly the 16 barcoded specimens are divided into four subbranches corresponding to their different geographic distributions. Región de los Rios forms one branch with four specimens, Región de los Lagos one branch with eight specimens and three specimens from a more southern Chilean area (>50°S) cluster in a third branch (bootstrap values >93%). The single specimen of *A. assimilis* from Australia (DQ390087) contrasts to the three Chilean branches with a bootstrap value of >95%.

Whereas the morphological differences lie well within the variation described in the literature for this cosmopolitan species (Stock 1954, Hedgpeth 1961, Fry & Hedgpeth 1969), molecular analyses could distinguish 11 different haplotypes. Furthermore *A. assimilis* shows a high mean intraspecific value of 6.81% sequence divergence. Intraspecific divergences greater than 3% has been interpreted either to suspect the presence of cryptic species (Radulovici et al. 2009) or a threshold for species delineation (Hebert et al. 2003a, b). However it is hard to decide whether there are cryptic species already existent or there is ongoing allopatric speciation. Concerning the geographically discrete distribution of the four subbranches, according to Mayr (1975) they would be referred rather to as subspecies than species. Hence in this case it would be adventurous to speak of different species. A more probable explanation of the four phylogeographic units of *A. assimilis* might be given by the last ice age. About 15 000 years ago the entire Chilean coastline (as far as 30°S) was covered by the Patagonian ice shield (Clapperton 1993). During glacial periods survival of benthic communities was possibly only in the deep sea or in shelters on the continental shelf (Thatje et al. 2005). Beyond that Thatje and his colleagues suggested that taxa with poor dispersal abilities might constitute cryptic species as a result of isolation in glacial shelters. Limited dispersal abilities are also given for pycnogonids concerning their holobenthic lifecycle and lack of a pelagic larval stage. Furthermore the Pacific Ocean along the Chilean coast is known for its steep slopes achieving depths of kilometres and its absence of stepping stones, which would be essential for survival. Since *A. assimilis* can be considered as an almost exclusive

shelf species mostly occurring at shallow depths above 300 m (Hedgpeth 1961, Müller, 1993, Arango 2003) a postglacial recolonization of the Chilean fjords from the deep sea is relatively unlikely. Recolonization of *A. assimilis* must have been occurred from glacial refugia in the North and/or South. The high intraspecific variation among *A. assimilis* and the patchy distribution of their branches could be products of alternating extinction and colonization events from surrounding regions during the ice age. Furthermore founder effects and/or genetic drift are a common phenomenon concerning small colonist populations and can increase divergence among species/specimens.

7.2.2. Revision of the *Pallenopsis patagonica* complex

Pallenopsis patagonica represents one of the most taxonomically problematic and variable pycnogonid species and is known from the Antarctic, Subantarctic and South America including also the Falkland Islands. To unscramble this species complex the COI fragment of 39 *P. patagonica* specimens was sequenced, displaying two bigger clades which we named the "Falkland" and the "Chilean" clade. Supported also by thorough morphological analyses all 11 specimens from "Chilean" clade could be described as a species new to science: *Pallenopsis yepayekae* Weis nov. spec., in Weis et al. accepted, whereas the "Falkland" clade could be assigned to the "real" *P. patagonica* described by Hoek 1881. Beyond that one specimen of Hoek's type material which he originally described as a juvenile could be assigned to *P. yepayekae* Weis nov. spec., in Weis et al. accepted as well. Furthermore this specimen represents rather an adult female than a juvenile since there are already eggs visible inside the femur. In addition one specimen originally determined by Hedgpeth as *P. patagonica* (SMNH-125527) and one only to genus level determined specimen of Loman (SMNH-125514) could both be assigned to *P. yepayekae* Weis nov. spec., in Weis et al. accepted as well. Molecular and morphological data confirm the need for a taxonomic revision of *P. patagonica* as it is done in the present study. Even decades later the high variability of the species complex *P. patagonica* is evident and underpinned by DNA barcoding.

In contrast to *Achelia assimilis* the *Pallenopsis* habitat extends to much deeper waters (down to 3,500m). A recolonization of the Chilean fjords of *P. yepayekae* Weis nov. spec., in Weis et al. accepted after the last ice age from deeper waters cannot be excluded. Furthermore haplotypes of different *P. patagonica* sub-networks are present in the same location. This could suggest that *P. patagonica* was able to outlive glaciation by moving back to much

deeper refugia of/across the deep sea. This geographic overlap between the single clades could not be detected in the case of *A. assimilis*. A similar pattern as seen for *P. patagonica* has been observed at several locations around the Antarctica for the giant sea spider *Colossendeis megalonyx* (Krabbe et al. 2010). Perhaps pycnogonids that are able to live and survive also in greater depth are less susceptible to glaciation processes than smaller shallow depth species as *A. assimilis*. To our knowledge up to date no specimen of *A. assimilis* has been captured from the Antarctic area.

To test the different hypotheses concerning the recolonization after glaciation more specimens and sequence data from different regions along the Chilean coastline are needed. In *P. patagonica* we could observe very high interspecific distances compared to other taxa (Hebert et al. 2004, Lefebure et al. 2006, Raupach et al. 2010). If this phenomenon may be a peculiar "pycnogonid" phenomenon lies beyond the scope of this Thesis.

8. Conclusions and Outlook

There is a strong need to study biodiversity, because especially marine biodiversity is changing rapidly. The vast changing biodiversity has many factors some of them caused by nature itself (glaciations, climate change, invasive alien species, etc.) others by humans like pollution, overfishing or building of various salmon farms (aquaculturing) in areas where biodiversity has not yet been explored. In many cases we are going to destroy or extinct species, before they even have the possibility to be discovered or further to get to know their functional role in the complex ecosystem. We do not know to what extent such changes in biodiversity might lead to environmental or economic problems. As Boero (2010) already stated, the study of biodiversity in all its facets is needed, from phenotypes to genotypes, ecological niches, life cycles, populations and communities. Some work has already successfully been done, like for example the ten-year international research programme of the major marine biodiversity initiative the Census of Marine Life (CoML), which was completed in 2010. More than 2.700 scientists assembled more than 30 million species-level records, including 1.200 newly discovered species and established a baseline of the diversity, distribution and abundance of life in the world's oceans (see also www.coml.org). CoML estimates that there are 240.000 marine species known to science. In contrast recent estimates of the total number of living marine eukaryotic species range from 0.7 million (Appeltans et al. 2012) to 2.2 million (Mora et al. 2011). This indicates, that only a fraction of species is known, whereas the majority of marine eukaryotic species (at least 70%) are waiting to be

described. Moreover every second specimen collected from waters deeper than 3.000 m by the CoML belongs to a species new to science (Crist et al. 2009). The establishment of a better understanding/baseline knowledge of the marine living organisms and their role in ecosystem functioning provides the scientific basis for the protection and conservation of marine biodiversity.

Therefore main focus should lie on studying hitherto relatively unexplored regions which is the case for the Chilean fjord area. The present thesis takes the first such step by analysing and describing invertebrates from isolated and partly difficult to access areas, which might be neglected otherwise. For successful protection of the ecosystem and natural habitats within the fjords more knowledge about the marine biodiversity is needed and lies in focus for future explorations.

Pycnogonids with their remarkable holobenthic lifestyle offer an interesting and fascinating model group. In contrast to other invertebrates like for example decapod crustaceans, pycnogonids due to their low mobility in all stages of development are philopatric, i.e. constrained to a certain place (except for very rare drift events) and gene flow with individuals living in the neighbourhood is low. This fact constitutes an important precondition for speciation, already suggested by Thatje and his colleagues (2005). Accordingly COI-sequences of pycnogonids show very high intraspecific distances compared to other taxa (Hebert et al. 2004; Lefebure et al. 2006; Raupach, et al. 2010). More specimens will be necessary to clarify if this could be a specific "pycnogonid" phenomenon that is not understood at the moment.

Concerning the studied *Achelia assimilis* as well as the *Pallenopsis* complex cryptic species are no rarity in pycnogonids, making them to a suitable study taxon. Due to similar environmental conditions between the different fjords there might be no need for developing different morphological traits. Nonetheless there are enormous molecular differences resulting in several clades that could be explained by recolonization events from different glacial refugia. Small colonist populations could have been highly susceptible to founder effects which push further divergence.

Up to now only a small percentage of the over 1300 known pycnogonid species are found and described for the Chilean area. More specimens are needed to get a better overview of the pycnogonid diversity in that region and to detect more species potentially new to science. Due to molecular methods it is possible to detect considerably more (cryptic) species than by morphological approaches alone. Pycnogonids are philopatric due to their holobenthic lifecycle and low mobility, thus high amounts of endemism appears possible. This can be seen

by *Achelia assimilis*, which shows large genetic differences corresponding well to the different geographic regions (Paper III). All this shows the importance to explore and/or protect such relatively unexplored areas like the Chilean fjords, because the damage of only a small area could mean the death for several (pycnogonid) species. Although pycnogonids represent an interesting and suitable study object there are even more taxa waiting to be explored.

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