

# Morphological and genetic data clarify the taxonomic status of *Colossendeis robusta* and *C. glacialis* (Pycnogonida) and reveal overlooked diversity

LARS DIETZ, SVEN PIEPER, MEIKE A. SEEFELDT & FLORIAN LEESE \*

Ruhr-Universität Bochum, Lehrstuhl für Evolutionsökologie und Biodiversität der Tiere, Universitätsstraße 150, 44801 Bochum, Germany; Lars Dietz [lars.dietz@rub.de]; Sven Pieper [sven.pieper@rub.de]; Meike A. Seefeldt [meike.seefeldt@rub.de]; Florian Leese [florian.leese@rub.de] — \* Corresponding author

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## Abstract

*Colossendeis robusta* Hoek, 1881, originally described from the Kerguelen shelf, is considered as one of the most widespread Antarctic pycnogonids. However, the taxonomic status of this and similar species has long been unclear, as synonymy of *C. glacialis* Hodgson, 1907 and several other species with *C. robusta* has been proposed. Here we test the synonymy of *C. robusta* and *C. glacialis* with two independent molecular markers as well as comprehensive morphometric measurements and SEM data of the ovigeral spine configuration. We show that *C. robusta* and *C. glacialis* are clearly distinct species, and our results also indicate the existence of another previously unrecognized Antarctic species, *C. bouvetensis* sp.n., as well as an Antarctic lineage closely related to the endemic Kerguelen group *C. robusta* s.str. We find evidence for strong regional differentiation within each species. Our results suggest that diversity of Antarctic pycnogonids is still underestimated.

## Key words

*Colossendeis robusta*, *Colossendeis glacialis*, *Colossendeis bouvetensis*, Southern Ocean, Pycnogonida, phylogeography, integrative taxonomy.

## 1. Introduction

The Southern Ocean hosts a remarkably high diversity of benthic invertebrate species (CLARKE & JOHNSTON 2003; GUTT et al. 2004; GRIFFITHS 2010), of which a large proportion has radiated *in situ* after the thermal isolation by the Antarctic Circumpolar Current (ACC) (HELD 2000; CONVEY et al. 2009; LECOINTRE et al. 2013). Many of these show a remarkably high degree of endemism (GUTT et al. 2004; PEAT et al. 2006; BRANDT et al. 2007). A great number of species has only been recognized in the context of recent molecular studies (see JANOSIK & HALANYCH 2010; ALLCOCK & STRUGNELL 2012 for reviews).

One taxon that shows a uniquely high degree of endemism in the Southern Ocean are the sea spiders (Pyc-

nogonida or Pantopoda; MUNILLA & SOLER MEMBRIVES 2009; GRIFFITHS et al. 2011). Pycnogonids are exclusively marine arthropods, which occur in all oceans (ARNAUD & BAMBER 1987). The systematic position of the Pycnogonida in the arthropod tree of life is still unresolved (DUNLOP & ARANGO 2005; REGIER et al. 2010; GIRIBET & EDGE-COMBE 2012). However, their internal phylogeny has been studied, but not completely resolved, using genes and morphology (MUNILLA LEÓN 1999; ARANGO & WHEELER 2007; BAMBER 2007; NAKAMURA et al. 2007; ARABI et al. 2010; DIETZ et al. 2011). Within the Pycnogonida, there are over 1,300 species in 10 families (ARNAUD & BAMBER 1987; BAMBER & EL NAGAR 2014). Almost 20% of

these species (264 species) occur in the Southern Ocean (ARANGO & WHEELER 2007; MUNILLA & SOLER MEMBRIVES 2009), of which 50% are endemic to this region. The family Colossendeidae Hoek, 1881, in particular of the genus *Colossendeis* Jarzynsky, 1870, is especially abundant in the Southern Ocean. 36 of the known 75 species of *Colossendeis* occur in the Southern Ocean, 26 of which are endemic, rendering the region a hotspot of endemism (MUNILLA & SOLER MEMBRIVES 2009). For 55 species of Antarctic and Subantarctic pycnogonids a circumpolar distribution is currently assumed (MUNILLA LEÓN 2001; MUNILLA & SOLER MEMBRIVES 2009; GRIFFITHS et al. 2011).

However, in view of the occurrence of several cryptic or overlooked species reported for this group (MAHON et al. 2008; KRABBE et al. 2010; WEIS & MELZER 2012), consistent with reports from other groups (e.g. HELD & WÄGELE 2005; WILSON et al. 2007; LEESE & HELD 2008; THORNHILL et al. 2008; BRANDAO et al. 2010), both the actual number of species as well as their distribution ranges have to be regarded with caution. Reports of much smaller horizontal distribution ranges for some species (e.g. HELD & WÄGELE 2005; KRABBE et al. 2010) as well as much narrower vertical distribution ranges (SCHÜLLER 2011) challenge the traditional biogeographic concepts of circumpolarity and eurybathy (but see HEMERY et al. 2012). An important point when testing biodiversity estimates as well as biogeographic concepts is that species are correctly delimited. A combination of mitochondrial (DNA barcoding) and nuclear DNA markers coupled with morphological approaches has shown strong potential when studying pycnogonids (ARANGO & BRENNES 2013; DIETZ et al. 2013).

The morphology of *Colossendeis* species mostly resembles that of other Pycnogonida (KING 1973; ARNAUD & BAMBER 1987; CHILD 1995; ARANGO 2002; ARANGO & WHEELER 2007; CANO & LOPEZ-GONZALEZ 2007) except that adults lack chelifores. The trunk, comprising most of the body, consists of cephalic and leg-bearing segments. Four pairs of lateral processes carry the walking legs (each consisting of eight articles and a terminal claw), and at the posterior end of the trunk there is a short abdomen. The anterior part of the trunk, the cephalosoma, carries a pair of palpi, a pair of ovigera, the first pair of walking legs, the proboscis and the ocular tubercle carrying the eyes. In the genus *Colossendeis* there are both eye-bearing and eyeless species. In other pycnogonids, the ovigera are used by the male to carry the eggs, however, in *Colossendeis* no egg-carrying males have been found, and they probably serve as cleaning appendages (KING 1973; BAIN 2003). The last four ovigeral articles bear a complex arrangement of spines and together form the strigilis, the morphology of which can be diagnostic for particular species (CANO & LOPEZ-GONZALEZ 2007). The three post-cephalic leg-bearing segments are fused. The individuals of *Colossendeis* feed on bryozoans, cnidarians, sponges, small mollusks and small polychaetes (ARNAUD & BAMBER 1987; BRABY et al. 2009). Chelifores are completely absent in adult specimens of

*Colossendeis* but they are probably present in the larval development and sometimes still seen in subadults (e.g. HOEK 1881).

Within *Colossendeis* there are several taxonomic controversies resulting from different authors' views on the synonymy of certain species. One of these debates concerns *Colossendeis robusta* Hoek, 1881, and *Colossendeis glacialis* Hodgson, 1907, two morphologically similar species with reported circumpolar distribution. *C. robusta* was originally described from Kerguelen but later also reported to be geographically widespread in Antarctic waters (FRY & HEDGPETH 1969; MUNILLA & SOLER MEMBRIVES 2009), whereas *C. glacialis* was first documented in the Ross Sea but subsequently also in other regions of the Southern Ocean (e.g. PUSHKIN 1993). However, some authors regard *C. glacialis* as a junior synonym of *C. robusta* (HODGSON 1927; FRY & HEDGPETH 1969; CHILD 1995) and explain the observed morphological differences by intraspecific, mostly ontogenetic variation. The (mostly) larger species *C. robusta* is argued to be described based on more fully grown individuals. Other authors disagree with the synonymization of *C. glacialis* and *C. robusta* and continue to treat both species as being distinct (PUSHKIN 1988; STIBOY-RISCH 1993; CANO & LOPEZ-GONZALEZ 2007). Claimed differences between the two species are summarized in Table 1. In both species, according to the literature (HODGSON 1907a; CALMAN 1915; STIBOY-RISCH 1993; CANO & LOPEZ-GONZALEZ 2007), the lateral processes of the trunk are separated from each other by about half their diameter. The ocular tubercle is a broad and large cone with four eyes, one pair anterior and posterior eyes, which are placed very closely together. The palps consist of the usual 10 articles, of which the distalmost five are of similar length, but the seventh article is slightly longer than the more distal ones. The oviger is also divided into 10 articles, the four distal articles are approximately of the same size and combine to form the strigilis. The femur and the tibia 1 are approximately of the same length (HOEK 1881; HODGSON 1907a; CALMAN 1915; PUSHKIN 1993; STIBOY-RISCH 1993; CHILD 1995; CANO & LOPEZ-GONZALEZ 2007).

Although various differences between *C. glacialis* and *C. robusta* have been claimed (Table 1), no systematic measurements of numerous specimens testing for significance of the differences have yet been performed. Also, no molecular study has yet been performed that would enable to test the possible distinctiveness of the morphological characters with an independent source of data. Besides, it has never been tested whether the Antarctic specimens assigned to *C. robusta* really represent the same species as the holotype from Kerguelen. In a recent work, DIETZ et al. (2013) have shown the potential of combining molecular and morphological analyses to resolve controversial taxonomic and phylogeographic questions and identify unrecognized species inside the genus *Colossendeis*. In particular the mitochondrial gene cytochrome c oxidase subunit I (COI), which is often referred to as the "standard barcoding gene", evolves faster than most nuclear genes in many animal phyla and is of-

**Table 1.** Claimed differences in morphological characters between *C. robusta* and *C. glacialis*.

Body part	<i>Colossendeis robusta</i>	<i>Colossendeis glacialis</i>	Reference
Legs	Femur, tibia 1 < tibia 2	Femur, tibia 1 > tibia 2	CALMAN (1915), STIBOY-RISCH (1993)
Palps	Last 5 articles elongate	Last 5 articles rounded	HODGSON (1927)
Proboscis, palps, legs	Not spinous	Spinous	FRY & HEDGPETH (1969), STIBOY-RISCH (1993)
Palps	Article 6 longest of the last distal 5	Article 7 longest of the last distal 5	PUSHKIN (1988)
Legs	Claw > ½ propodus	Claw < ½ propodus	PUSHKIN (1993)
Body	Larger, robust	Smaller, gracile	STIBOY-RISCH (1993)
Proboscis	Shape D''':2 (FRY & HEDGPETH 1969)	Shape B''':1 (FRY & HEDGPETH 1969)	STIBOY-RISCH (1993)
Palps	Article 9 shorter than 8 and 10	Last 3 articles equally long	STIBOY-RISCH (1993)

ten easy to amplify, which makes it an ideal candidate for distinguishing species and disentangling their phylogeographic history (HEBERT et al. 2003, 2004; FRÉZAL & LEBLOIS 2008). However, as data based on a single gene may be misleading, independent data, i.e. nuclear genes, are required. One fragment of the nuclear genome widely used for phylogenetic analyses in animals is the Internal Transcribed Spacer (ITS) region (FRITZ et al. 1994; SCHLÖTTERER et al. 1994; ODORICO & MILLER 1997; CHEN et al. 2002; CHENG et al. 2006). This consists of the 5.8S rRNA and the non-coding regions ITS1 and ITS2, which separate the nuclear rRNA genes 18S, 5.8 S and 28S and are cut off during splicing. For pycnogonids there is only one recent study, which has clearly demonstrated the utility of this marker to address species-level questions (ARANGO & BRENNEIS 2013).

In this study, we analyzed sequence data from the COI and ITS genes as well as measurements to test the distinction between *C. glacialis*, *C. robusta*, and other possible species similar to them. The aims of the integrative taxonomic study were threefold: First, we wanted to test whether the two morphologically similar *C. robusta* and *C. glacialis* are conspecific (FRY & HEDGPETH 1969; CHILD 1995) or two distinct species (STIBOY-RISCH 1993; CANO & LOPEZ-GONZALEZ 2007). As a second aim, we tested for further evidence of unrecognized or cryptic species within *C. robusta* and *C. glacialis*. In particular we tested whether Antarctic specimens assigned to *C. robusta* belong to that species by studying specimens from the type locality around the Kerguelen Islands. Thirdly, we investigated whether regional intraspecific variation can be detected in our dataset.

## 2. Material and methods

### 2.1. Sampling

The material for the morphological research comes from the research expeditions ICEFISH 2004, Polarstern expedition ANT XIV/2, REVOLTA I and III, and POKER II “L’Austral”. Samples from the ICEFISH 2004 expedition

on the research vessel Nathaniel B. Palmer (<http://www.icefish.neu.edu>) come from South Georgia and the South Sandwich Islands (n = 12) and Bouvet Island (n = 14). Samples from the Antarctic Peninsula (n = 8) and the Eastern Weddell Sea (n = 1) are from RV Polarstern expedition ANT XIV/2 (KATTNER 1998). Samples from Terre Adélie (n = 3) are from the REVOLTA expeditions and those from Kerguelen (n = 13) are from the POKER II expedition. For the catch different bottom trawls (Blake, Otter and Agassiz trawl) were used. Samples were taken from depths between 100 and 648 meters. The captured animals were fixed directly in 96% ethanol. An overview of the sequenced specimens with their geographical origin is given in Table 2. Specimens from the POKER and REVOLTA expeditions are located in the Muséum National de l’Histoire Naturelle (MNHN), Paris, France, under the catalog numbers IU-2007-4795 to 5069 and IU-2013-15805 to 15812, respectively.

### 2.2. Species determination

Specimens were determined using a light microscope and according to the identification keys of CHILD (1995), PUSHKIN (1993), the original descriptions of the species, and the results of the study of STIBOY-RISCH (1993).

### 2.3. Molecular genetic analysis

DNA was isolated from muscle tissue (extracted from the tibia 1 with sterile tools), using the Qiagen QIAamp DNA mini Kit according to the manufacturer’s instructions. Part of the mitochondrial gene region cytochrome C oxidase subunit I (COI) and the nuclear ribosomal DNA region ITS were amplified. For this PCR was performed with a mix containing 2.5 µl of 10 × PCR buffer (HotMaster), 2.5 µl dNTP mix (2 mM), 0.125 µl each of the two primers (100 pmol/µl) per gene segment, 0.1 µl Taq polymerase (HotMaster; 5 U/µl) and 1–3 µl DNA. The difference to 25 µl was filled up with HPLC water. Primers used for the amplification of the gene segments are listed in Table 3. PCR conditions for the COI/ITS gene

**Table 2.** Overview of specimens, their sampling locations, COI clade assignment and COI haplotype number in this study. Printed in bold are specimens for which COI sequences for the analysis were obtained from GenBank. For these specimens, no morphological measurements could be obtained.

Specimen	Sampling location	Latitude	Longitude	Depth [m]	Species	Clade (COI)	Haplotype (COI)	ITS
300-1.4	Eastern Weddell Sea	70°50'28.79"S	10°35'16.80"W	268	<i>C. glacialis</i>	1	4	×
257-2.4	Eastern Antarctic Peninsula	64°54'45.00"S	60°39'0.61"W	158	<i>C. glacialis</i>	1	2	×
AGT42/164	South Shetlands	62°7'59.99"S	57°40'0.01"W	555	<i>C. glacialis</i>	1	3	
AGT42/175-7	South Shetlands	62°19'0.00"S	58°42'0.00"W	496	<i>C. glacialis</i>	1	27	×
<b>HM381691</b>	<b>South Shetlands</b>	<b>61°10'12.00"S</b>	<b>56°0'21.60"W</b>	<b>148</b>	<b><i>C. glacialis</i></b>	<b>1</b>	<b>2</b>	
<b>HM381692</b>	<b>South Shetlands</b>	<b>62°29'27.60"S</b>	<b>61°25'19.20"W</b>	<b>122</b>	<b><i>C. glacialis</i></b>	<b>1</b>	<b>34</b>	
<b>HM432370</b>	<b>Eastern Weddell Sea</b>	<b>71°7'8.40"S</b>	<b>11°26'13.20"W</b>	<b>228</b>	<b><i>C. glacialis</i></b>	<b>1</b>	<b>4</b>	
211-5.1	Shag Rocks	53°24'31.79"S	42°40'41.99"W	317	<i>C. glacialis</i>	2	9	×
211-6.3.2	Shag Rocks	53°24'48.60"S	42°40'1.81"W	315	<i>C. glacialis</i>	2	5	×
290T27-1	Shag Rocks	53°27'38.88"S	41°15'40.68"W	191	<i>C. glacialis</i>	2	6	
290T27-2	Shag Rocks	53°27'38.88"S	41°15'40.68"W	191	<i>C. glacialis</i>	2	7	
30BT14-2	Shag Rocks	53°20'3.48"S	41°28'48.00"W	146	<i>C. glacialis</i>	2	10	
45BT24	South Georgia	54°15'0.00"S	35°32'60.00"W	100	<i>C. glacialis</i>	2	11	
<b>HM426185</b>	<b>South Georgia</b>	<b>53°36'39.60"S</b>	<b>37°52'40.80"W</b>	<b>224</b>	<b><i>C. glacialis</i></b>	<b>2</b>	<b>12</b>	
PA_E006 (GQ386997)	South Sandwich Islands	56°8'60.00"S	27°20'24.00"W	336	<i>C. glacialis</i>	2	12	
PB_E005 (GQ386998)	South Georgia	54°15'0.00"S	35°32'60.00"W	100	<i>C. glacialis</i>	2	14	
PR_E006	Shag Rocks	53°27'40.32"S	41°15'38.16"W	193	<i>C. glacialis</i>	2	13	
PR_E010	Shag Rocks	53°27'55.44"S	41°13'27.12"W	195	<i>C. glacialis</i>	2	5	×
PS_E011	Shag Rocks	53°27'40.32"S	41°15'38.16"W	193	<i>C. glacialis</i>	2	8	
IU-2013-15805	Terre Adélie				<i>C. glacialis</i>	6	28	×
IU-2013-15808	Terre Adélie				<i>C. glacialis</i>	6	29	×
IU-2013-15812	Terre Adélie				<i>C. glacialis</i>	6	1	
<b>HM381674</b>	<b>Ross Sea</b>	<b>74°42'0.00"S</b>	<b>164°5'60.00"E</b>	<b>20</b>	<b><i>C. glacialis</i></b>	<b>6</b>	<b>1</b>	
286-1.1.2	Eastern Weddell Sea	70°51'18.00"S	10°35'21.01"W	224	<i>C. glacialis</i>	[only ITS]		×
226-7.3	Eastern Antarctic Peninsula	64°54'50.40"S	60°36'37.80"W	216	<i>C. robusta</i>	3	18	
257-2.1	Eastern Antarctic Peninsula	64°54'45.00"S	60°39'0.61"W	158	<i>C. robusta</i>	3	18	×
<b>Ch126.1</b>	<b>Western Antarctic Peninsula</b>	<b>67°43'36.42"S</b>	<b>69°18'6.18"W</b>		<b><i>C. robusta</i></b>	<b>3</b>	<b>32</b>	
<b>Ch231.1</b>	<b>Ross Sea</b>	<b>76°20'28.38"S</b>	<b>170°51'1.78"W</b>		<b><i>C. robusta</i></b>	<b>3</b>	<b>33</b>	
<b>HM381689</b>	<b>South Shetlands</b>	<b>61°0'39.60"S</b>	<b>55°46'30.00"W</b>	<b>162</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>18</b>	
<b>HM381690</b>	<b>South Shetlands</b>	<b>61°0'57.60"S</b>	<b>55°56'24.00"W</b>	<b>274</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>17</b>	
<b>HM426429</b>	<b>Ross Sea</b>	<b>74°35'25.80"S</b>	<b>170°16'33.60"E</b>	<b>283</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>16</b>	
<b>HM432386</b>	<b>Eastern Weddell Sea</b>	<b>71°6'18.00"S</b>	<b>11°32'2.40"W</b>	<b>175</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>18</b>	
<b>HM432414</b>	<b>South Orkneys</b>	<b>61°0'3.60"S</b>	<b>45°51'54.00"W</b>	<b>240</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>15</b>	
<b>HM432416</b>	<b>South Orkneys</b>	<b>61°0'3.60"S</b>	<b>45°51'54.00"W</b>	<b>240</b>	<b><i>C. robusta</i></b>	<b>3</b>	<b>18</b>	
IU-2007-4795	Kerguelen	48°24'S	70°36'E	119–124	<i>C. robusta</i>	10	30	
IU-2007-4797	Kerguelen	48°24'S	70°36'E	119–124	<i>C. robusta</i>	10	31	
IU-2007-4798	Kerguelen	48°24'S	70°36'E	119–124	<i>C. robusta</i>	10	30	
IU-2007-4800	Kerguelen	48°24'S	70°36'E	119–124	<i>C. robusta</i>	10	30	
IU-2007-4842	Kerguelen	47°34'S	70°19'E	161–162	<i>C. robusta</i>	10	30	
IU-2007-4870	Kerguelen	50°27'S	71°42'E	585–589	<i>C. robusta</i>	10	31	×
IU-2007-4902	Kerguelen	47°32'S	69°42'E	179–180	<i>C. robusta</i>	10	31	
IU-2007-5039	Kerguelen	48°40'S	70°23'E	117–118	<i>C. robusta</i>	10	30	×
IU-2007-5043	Kerguelen	48°48'S	70°09'E	103–104	<i>C. robusta</i>	10	30	
IU-2007-5044	Kerguelen	48°48'S	70°09'E	103–104	<i>C. robusta</i>	10	30	
IU-2007-5058	Kerguelen	48°11'S	70°09'E	133–139	<i>C. robusta</i>	10	30	
IU-2007-5063	Kerguelen	48°32'S	70°35'E	114–117	<i>C. robusta</i>	10	31	
IU-2007-5069	Kerguelen	48°57'S	69°59'E	100–103	<i>C. robusta</i>	10	31	
257-2.5	Eastern Antarctic Peninsula	64°54'45.00"S	60°39'0.61"W	158	<i>C. drakei</i>	4	14	
233-3.1.2	Eastern Antarctic Peninsula	65°33'27.61"S	61°37'17.40"W	324	<i>C. drakei</i>	4	12	
233-3.1.1	Eastern Antarctic Peninsula	65°33'27.61"S	61°37'17.40"W	324	<i>C. drakei</i>	4	13	
226-7.2	Eastern Antarctic Peninsula	64°54'50.40"S	60°36'37.80"W	216	<i>C. bouvetensis</i>	5	2	×
59BT40	Bouvet Island	54°12'36.00"S	3°6'48.96"E	465	<i>C. bouvetensis</i>	5	10	×
59OT45	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
66OT97	Bouvet Island	54°14'5.64"S	3°18'41.04"E	190	<i>C. bouvetensis</i>	5	11	
81OT58	Bouvet Island	54°17'39.12"S	3°8'34.80"E	169	<i>C. bouvetensis</i>	5	8	×
<b>HM426194</b>	<b>Eastern Weddell Sea</b>	<b>71°4'51.60"S</b>	<b>11°32'13.20"W</b>	<b>295</b>	<b><i>C. bouvetensis</i></b>	<b>5</b>	<b>2</b>	
<b>HM426263</b>	<b>South Sandwich Islands</b>	<b>57°40'15.60"S</b>	<b>26°27'57.60"W</b>	<b>630</b>	<b><i>C. bouvetensis</i></b>	<b>5</b>	<b>4</b>	
<b>HM426326</b>	<b>Eastern Weddell Sea</b>	<b>71°6'18.00"S</b>	<b>11°32'2.40"W</b>	<b>175</b>	<b><i>C. bouvetensis</i></b>	<b>5</b>	<b>3</b>	
<b>HM426374</b>	<b>Ross Sea</b>	<b>76°11'35.16"S</b>	<b>176°17'45.60"E</b>	<b>447</b>	<b><i>C. bouvetensis</i></b>	<b>5</b>	<b>5</b>	

Table 2 continued.

Specimen	Sampling location	Latitude	Longitude	Depth [m]	Species	Clade (COI)	Haplotype (COI)	ITS
HM426402	Ross Sea	72°20'22.20"S	175°31'55.20"E	950	<i>C. bouvetensis</i>	5	5	
HM426402	Ross Sea	72°20'22.20"S	175°31'55.20"E	950	<i>C. bouvetensis</i>	5	5	
HM426434	Ross Sea	74°6'40.32"S	170°47'45.60"E	632	<i>C. bouvetensis</i>	5	7	
HM432368	Eastern Weddell Sea	71°7'8.40"S	11°26'13.20"W	228	<i>C. bouvetensis</i>	5	3	
HM432391	South Sandwich Islands	57°40'37.20"S	26°25'26.40"W	301	<i>C. bouvetensis</i>	5	4	
PA_E003 (GQ386999)	South Sandwich Islands	56°8'60.00"S	27°20'24.00"W	336	<i>C. bouvetensis</i>	5	4	
PF_E008 (GQ387000)	Bouvet Island	54°20'25.08"S	3°13'13.08"E	648	<i>C. bouvetensis</i>	5	11	
PQ_E007	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
PQ_E008	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
PQ_E010	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
PQ_E011	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
PQ_E012	Bouvet Island	54°12'48.96"S	3°6'10.80"E	458	<i>C. bouvetensis</i>	5	11	×
PR_E003	Bouvet Island	54°12'48.96"S	3°6'8.64"E	458	<i>C. bouvetensis</i>	5	6	×
PR_E004	Bouvet Island	54°12'48.96"S	3°6'8.64"E	458	<i>C. bouvetensis</i>	5	11	×
PR_E005	Bouvet Island	54°12'48.96"S	3°6'8.64"E	458	<i>C. bouvetensis</i>	5	11	×
PS_E010	Bouvet Island	54°20'25.08"S	3°13'13.08"E	648	<i>C. bouvetensis</i>	5	9	×
HM426382	Ross Sea	72°35'25.08"S	175°20'31.20"E	475	<i>C. bouvetensis</i>	7	1	
HM426381	Ross Sea	72°35'25.08"S	175°20'31.20"E	475	<i>C. bouvetensis</i>	8	24	
HM426401	Ross Sea	73°14'53.52"S	178°43'26.40"E	753	<i>C. bouvetensis</i>	8	26	
HM426411	Ross Sea	75°37'27.12"S	167°19'15.60"E	474	<i>C. bouvetensis</i>	8	25	
HM426433	Ross Sea	72°1'24.60"S	173°10'48.00"E	814	<i>C. bouvetensis</i>	8	23	
HM426327	Eastern Weddell Sea	71°6'18.00"S	11°32'2.40"W	175	<i>C. bouvetensis</i>	9	21	
HM426375	Ross Sea	76°12'7.20"S	176°14'52.80"E	447	<i>C. bouvetensis</i>	9	19	
HM426383	Ross Sea	73°7'28.20"S	174°19'12.00"E	321	<i>C. bouvetensis</i>	9	19	
HM426389	Ross Sea	76°35'38.40"S	176°49'40.80"E	365	<i>C. bouvetensis</i>	9	20	
HM432388	Eastern Weddell Sea	71°6'18.00"S	11°32'2.40"W	175	<i>C. bouvetensis</i>	9	22	
HM432397	Eastern Weddell Sea	70°50'6.00"S	10°34'44.40"W	274	<i>C. bouvetensis</i>	9	22	

were: denaturation at 94°C for 120 s (both genes) followed by 38/37 cycles of 94°C denaturation for 20 s (both), annealing for 30 s at 46°C/55°C, extension at 65°C for 60 s/80 s followed by a final extension at 65°C for 5 min/10 min, respectively.

PCR products were purified enzymatically: For each 10 µl of the PCR product 0.75 µl ExoI (exonuclease I 20 U/µL) and 1.5 µl FastAP (Fast Alkaline Phosphatase 1 U/µL) were added. The reaction was carried out in a thermocycler at 37°C for 15 min and inactivated by incubation at 85°C for 15 min. Purified PCR products were sequenced by GATC (Cologne, Germany) bidirectionally by the Sanger method.

The resulting COI and ITS sequences were edited with Geneious version 5.6.6 (DRUMMOND et al. 2012). The amplified gene fragments were checked by a BLAST search against the NCBI GenBank to exclude contamination. For COI, sequences from all individuals listed in Table 2 (except for 286-1.1.2, for which amplification was unsuccessful) and 22 sequences from GenBank, representing other colossendeid species, were used. For ITS, sequences from all 25 individuals for which PCR amplification was successful were used (Table 2). Additionally, an ITS sequence for *C. megalonyx* clade E was generated from the 454 data published by LEESE et al. (2012). Contigs were assembled from the reads with Geneious, and the contig representing the ribosomal operon

was identified with BLAST search. The ITS region was identified by alignment with the *C. robusta* sequences.

Both COI and ITS sequences were aligned with the program MAFFT (KATO & STANDLEY 2013) as implemented in Geneious using algorithm autodetection with a gap opening penalty of 1.53 and offset value of 0.123. For the ITS alignment, poorly aligned regions were removed with the program Gblocks using less stringent parameters (TALAVERA & CASTRESANA 2007). In both cases, the program RAXML 7.0.4 (STAMATAKIS 2006) was used to calculate a maximum likelihood tree with the model GTR+G. Bootstrap support was calculated using 1000 replicates with the approximation GTR+CAT. MrBayes 3.2.1 (RONQUIST et al. 2012) was used to calculate a Bayesian tree with 5,000,000 MCMC generations, of which the first 25% were discarded as burn-in. Models of evolution for Bayesian analysis were chosen with jModeltest 2.1.2 (DARRIBA et al. 2012) with the Akaike Information Criterion (AIC). For COI the model GTR+I+G was chosen, and for ITS SYM+G was preferred. For the COI trees, the sequence from *Rhopalorhynchus filipes* Stock, 1991 was used as an outgroup taxon, and the ITS trees were rooted with sequences of *Nymphon australe* Hodgson, 1902 and *Pseudopallene constricta* Arango & Brenneis, 2013 published by ARANGO & BRENNIS (2013). With the TCS 1.21 program (CLEMENT et al. 2000), parsimony networks of the COI haplotypes were calculated

**Table 3.** Primers used for amplification of the mitochondrial (mtDNA) and the nuclear (nDNA) gene regions.

Gene region	Primer	Sequence	Source
COI (mtDNA)	LCO 1490	5' GGT CAA CAA ATC ATA AAG ATA TTG G 3'	FOLMER et al. (1994)
	HCO 2198	5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3'	FOLMER et al. (1994)
ITS (nDNA)	ITSRA2	5' GTC CCT GCC CTT TGT ACA CA 3'	WÖRHEIDE (1998)
	ITS2.2	5' CCT GGT TAG TTT CTT TTC CTC CG 3'	WÖRHEIDE (1998)

**Table 4.** Morphometric measurements taken for the examined specimens.

Body part		Measurement
Trunk	Length	From proximal edge of the trunk to distal edge of the 4 <sup>th</sup> lateral processes
Trunk	Height	Centrally between 2 <sup>nd</sup> and 3 <sup>rd</sup> lateral processes vertical
Trunk	Widest point	Width of the 2 <sup>nd</sup> lateral processes
Trunk	Width between 2 <sup>nd</sup> and 3 <sup>rd</sup> lateral processes	Centrally between 2 <sup>nd</sup> and 3 <sup>rd</sup> lateral processes parallel to the axial plane
Proboscis	Length	Proximal to distal edge
Proboscis	Diameter	Averaged (2 distances) widest point
Abdomen	Length	Proximal to distal edge
Abdomen	Width	Widest point at the proximal end
Ocular tubercle	Height	Base to tip of the ocular tubercle
Ocular tubercle	Width	Width at ocular tubercle base
Ocular tubercle	Front height	Top of eyes to tip of ocular tubercle; both anterior and posterior
Ocular tubercle	Eye size	Vertical longest distance of the eye
Palpi	Length	Proximal to distal edge; all 10 articles; unilaterally
Oviger	Length	Proximal to distal edge; all 10 articles; unilaterally
Oviger	Width	Widest point, all 10 articles; unilaterally
Basal element	Diameter	Diameter widest point, unilaterally
Lateral processes	Distance	Average distance between the neighboring lateral processes at the distal end
Lateral processes	Width	Average width of lateral processes 1–4 at the distal end
Legs	Length	Longest distance from proximal to distal edge; coxae I–III, femur, tibia I–II, tarsus, propodus, claw, Averaged length of left and right
Legs	Width	Widest point; coxa I–III, femur, tibia I–II, tarsus, propodus, claw; averaged length of left and right

with a connection limit of 95%. Genetic distances for the COI data were calculated with PAUP 4.0b10 (SWOFFORD 2002) using the Kimura 2-parameter (K2P) correction.

## 2.4. Morphological analyses

Body measurements were carried out using the digital caliper “IP54 Water-resistant Digital Caliper” model “Digimatic”, calibrated to one hundredth of a millimeter. All animals were used except for three of the Kerguelen specimens and one specimen from the Antarctic Peninsula (226-7.3) as, despite damage during storage, transport, trawling or the preceding genetic analysis all limbs could be at least partly measured. However, in some cases, distal leg articles, in particular tibia II, tarsus, propodus and claw were missing. Overall, if all limbs were present, up to 124 measurements per specimen were taken. Relative values expressed as proportions of the trunk length were used for analyses to avoid biases caused by different absolute sizes. All measurements are shown in Table 4.

Using the program Statistica (StatSoft), the morphological measurements were first tested for normal distribution using the Lilliefors test. As many measurements

were found to be not normally distributed, a non-parametric unifactorial Kruskal-Wallis ANOVA was used to test for significant differences between molecular clades. Significance was assessed using a comparison of the paired groups' mean ranks. A principal component analysis (PCA) with those values available in all individuals was performed to visualize the clustering of specimens based on morphological data.

For SEM analyses the specimens AGT 42/164 (*C. glacialis* clade 1), PA\_E006 and 29OT27-2 (*C. glacialis* clade 2), 233-3.1.1 (*C. drakei*), 257-2.1 (*C. sp. cf. robusta*), 226-7.2 and PF\_E008 (*C. bouvetensis* sp.n.) were used. Ovigeras of the specimens were dried by adding hexamethyldisilazane (HMDS) in a rising concentration every 15 min. For electrical conductivity the material was sputtered with gold for 180 s. SEM pictures were taken with the scanning electron microscope DSM 950 (ZEISS) and documented with the software program Digital Image Processing System (DIPS). Image quality was increased through the image editing program Adobe Photoshop CS3. The light microscopic pictures of museum material (MNHN IU-2013-15812 and MNHN IU-2007-5063) were taken under an Olympus SZX16 with the camera type Olympus SDF Plapo 1.6XPF using the software CELL<sup>D</sup>.

**Table 5.** Mean Kimura 2-parameter distances between COI sequences within clades (diagonal) and between different clades (below diagonal).

	<i>C. glacialis</i> 1	<i>C. glacialis</i> 2	<i>C. glacialis</i> 6	<i>C. robusta</i> 3	<i>C. robusta</i> 10	<i>C. stramenti</i>	<i>C. drakei</i>	<i>C. bouvetensis</i> 5	<i>C. bouvetensis</i> 7	<i>C. bouvetensis</i> 8	<i>C. bouvetensis</i> 9
<i>C. glacialis</i> 1	0.36										
<i>C. glacialis</i> 2	4.79	0.62									
<i>C. glacialis</i> 6	3.17	4.96	1.07								
<i>C. robusta</i> 3	16.44	14.85	17.07	1.22							
<i>C. robusta</i> 10	17.23	14.35	17.94	4.6	0.09						
<i>C. stramenti</i>	17.28	15.43	17.91	10.54	10.9	0					
<i>C. drakei</i>	16.83	16.17	17.14	9.17	10.87	9.68	0.25				
<i>C. bouvetensis</i> 5	18.26	16.23	17.29	10.25	11.53	12.23	11.27	0.9			
<i>C. bouvetensis</i> 7	17.19	15.82	16.67	10.09	11.91	11.16	10.42	5.15	0		
<i>C. bouvetensis</i> 8	16.73	15.17	15.29	10.53	11.24	10.99	10.56	4.01	5.28	1.05	
<i>C. bouvetensis</i> 9	17.06	16.53	15.83	10.05	12.2	11.87	9.3	5.76	4.87	5.23	0.53

### 3. Results

#### 3.1. Molecular analysis

##### 3.1.1. Phylogenetic results

In the phylogenetic trees based on the COI sequences, the examined sequences fall into ten clearly separated clades, seven of which (clades 1–6, 10; Fig. 1) are represented in our material, while the remaining three are known only from GenBank sequences. Kimura 2-parameter distances between the clades are given in Table 5. Clades 1, 2 and 6 are closely related to each other and consist of (and include all) specimens identified as *C. glacialis*, with interclade genetic distances ranging from 3.2 to 5%. Clade 1 (n = 7) consists of specimens from the Antarctic Peninsula and Eastern Weddell Sea, clade 2 (n = 12) of specimens from the Shag Rocks, South Georgia, and the South Sandwich Islands, and clade 6 (n = 4) is represented by specimens from the Ross Sea and Terre Adélie. While support for intraspecific relationships in the tree are generally poor, there is no support for a close relationship of *C. glacialis* with *C. robusta* s.str. The genetic distances between *C. glacialis* and *C. robusta* and related taxa range from 14.4 to 18.3%.

Specimens from clade 3 (n = 10) came from very different regions of the Antarctic, i.e. South Orkneys, Antarctic Peninsula, Eastern Weddell Sea, and Ross Sea, thus showing a wide distribution. Clade 3 forms a strongly supported sister-group relationship with clade 10 (n = 13), which includes the specimens from Kerguelen identified as *C. robusta* s.str. (4.6% interclade distance). Clade 4 (n = 3) includes specimens from the Antarctic Peninsula identified here as *C. drakei* Calman, 1915. There is one grouping with good statistical support (posterior probability 1, ML bootstrap 84) that includes clades 3, 10 and 4 as well as *C. stramenti* Fry & Hedgpeth, 1969 (DQ390078; sister to *C. drakei*). Specimens determined first as *C. robusta* (clade 5) clustered together with sequences of high similarity from GenBank (clades

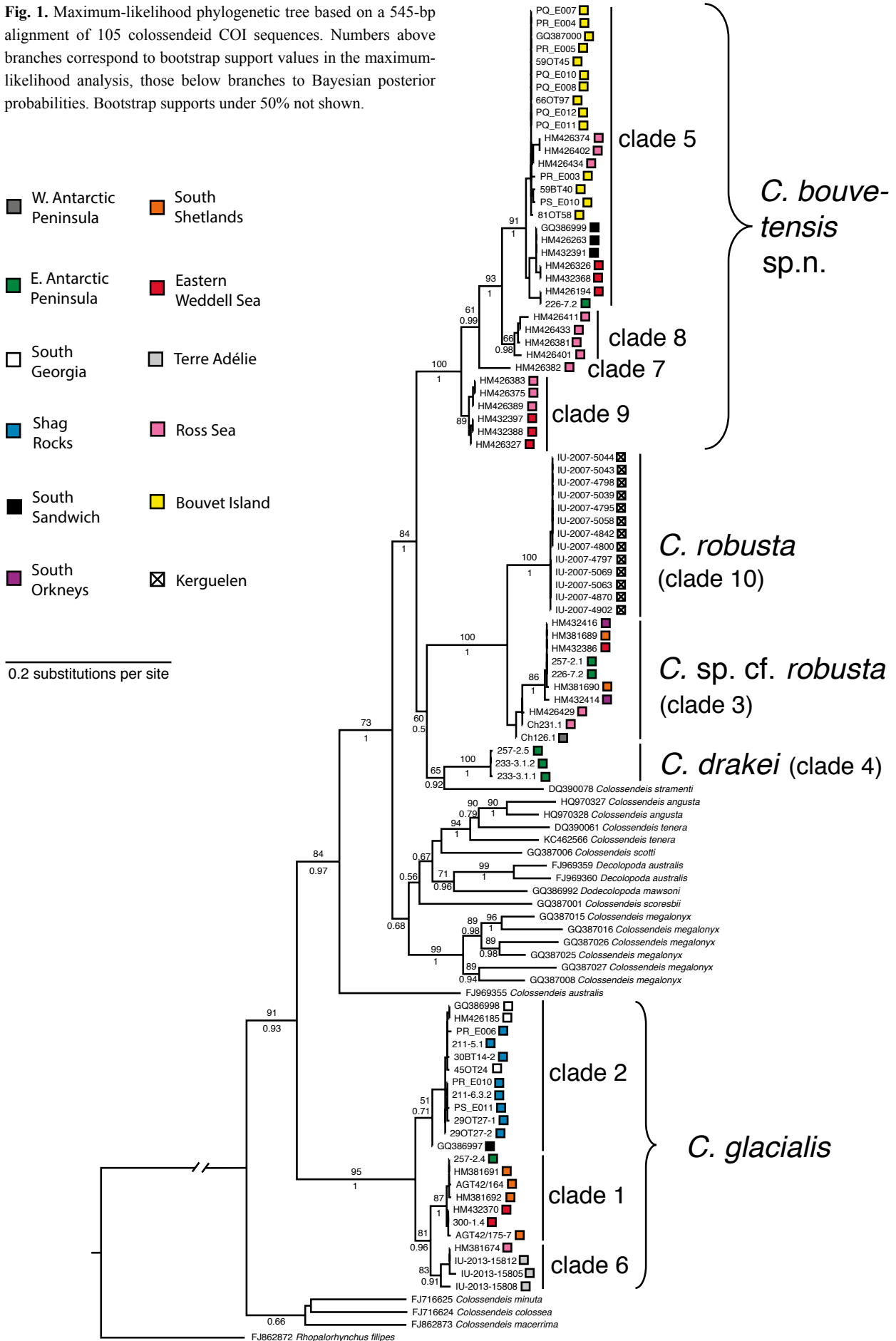
7, 8 and 9) and form one well-supported (posterior probability 1, ML bootstrap 100) and distinct group. Clade 5 (n = 23) is represented by specimens from Bouvet Island, the South Sandwich Islands, the Eastern Weddell Sea and the Ross Sea. Clades 7 (n = 1) and 8 (n = 4) are known only from the Ross Sea, and clade 9 (n = 6) is known from the Ross and Eastern Weddell Seas. Interclade distances between these four clades range from 4 to 5.8%, while the pairwise distances to *C. robusta* s.str. (clade 10) range from 11.2 to 12.2%. Members of clades 5, 7, 8 and 9 are assigned to *C. bouvetensis* sp.n. in this study.

The phylogenetic trees based on the ITS data (Fig. 2), although only present for a subset of specimens, show the same topology as the COI data (Fig. 1) in that *C. glacialis* (COI clades 1, 2 and 6) groups basally and is clearly separated from *C. bouvetensis* (COI clade 5) and from *C. robusta* and *C. sp. cf. robusta* (COI clades 3 and 10), which are closely related to each other. The COI clades 1, 2 and 6 are not recovered within *C. glacialis*. We did not succeed in obtaining ITS sequences from *C. drakei*.

##### 3.1.2. Phylogeographic results

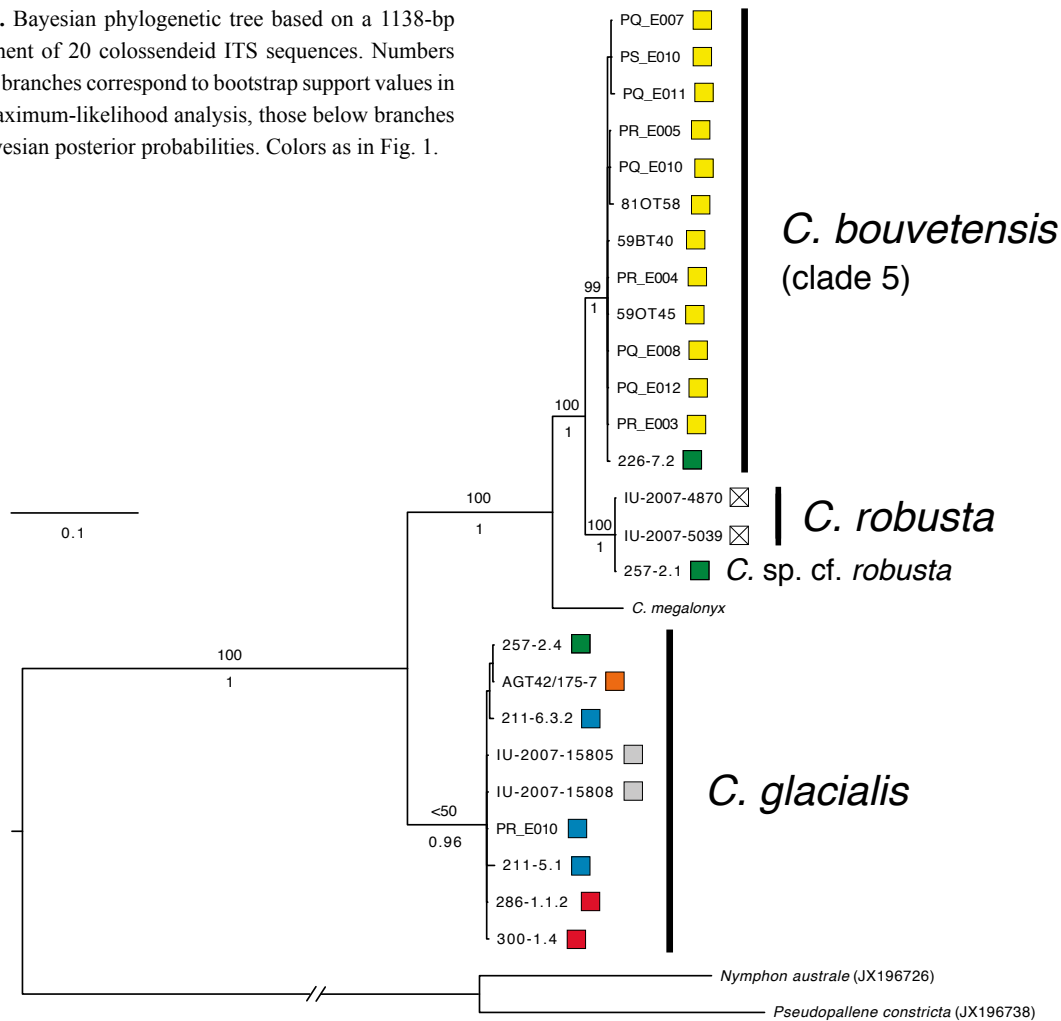
The TCS analysis result in separate networks for all ten clades at the 95% connection limit. Within each clade with specimens from different regions, geographical partitioning was obvious (Fig. 3). In clade 1, the Eastern Weddell Sea specimens group separated from those from the Antarctic Peninsula and South Shetlands. In clade 2, no shared haplotypes are found between Shag Rocks and South Georgia, although haplotypes from both regions are not separated in the network. The single specimen from South Sandwich is widely separated from the others. Clade 3 is divided into two groups, one including sequences from the Ross Sea and one sample from the Western Antarctic Peninsula, the other including sequences from the South Orkneys, South Shetlands, Eastern Antarctic Peninsula, and Eastern Weddell Sea. Clade 5 shows a separation into three groups, one of which is found in the Ross Sea and Bouvet Island, the second in the South Sandwich Islands and Eastern Weddell Sea, and

**Fig. 1.** Maximum-likelihood phylogenetic tree based on a 545-bp alignment of 105 colossendeid COI sequences. Numbers above branches correspond to bootstrap support values in the maximum-likelihood analysis, those below branches to Bayesian posterior probabilities. Bootstrap supports under 50% not shown.





**Fig. 2.** Bayesian phylogenetic tree based on a 1138-bp alignment of 20 colossendeid ITS sequences. Numbers above branches correspond to bootstrap support values in the maximum-likelihood analysis, those below branches to Bayesian posterior probabilities. Colors as in Fig. 1.



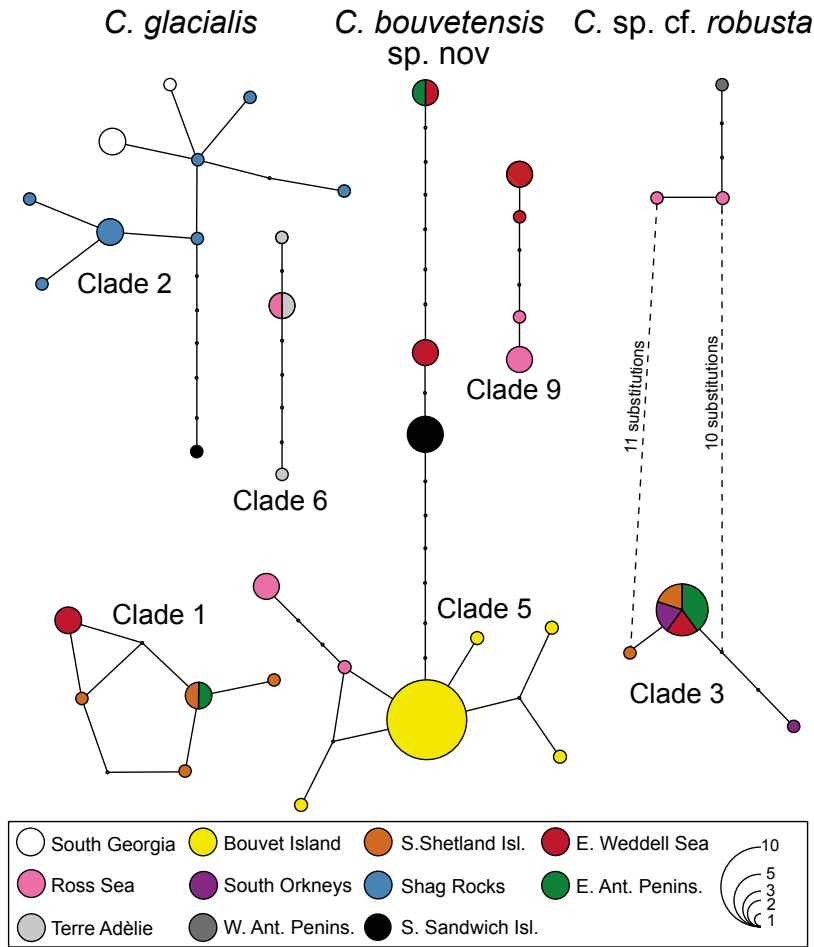
the third in the Antarctic Peninsula and Eastern Weddell Sea. Except for the latter group, no haplotypes are present in more than one geographical area. Clade 9 shows a division between the Ross and Weddell Sea specimens.

### 3.2. Morphological analyses

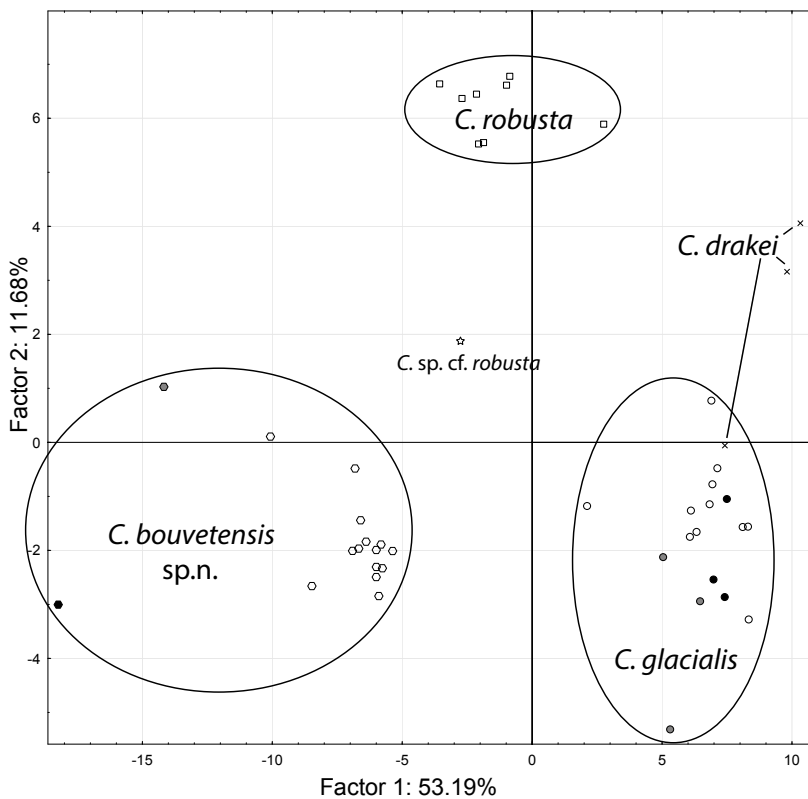
#### 3.2.1. Morphometric measurements

All measurements, as well as a list of significantly different measurements, are given in the supplementary material. The number of significantly different measurements between each pair of clades is given in Table 6. No significant differences were found between clades 1, 2 and 6, all of which were determined as *C. glacialis*, except for the larger anterior front height of the ocular tubercle in clade 6 compared to clade 2. Clade 1 has a somewhat shorter proboscis than clade 2, with clade 6 being intermediate. There are some other slight differences but none of them are significant at the  $p < 0.05$  level. Clade 10 (*C. robusta* from Kerguelen) differs from *C. glacialis* in many respects. Compared to *C. glacialis*, the

absolute length of the trunk is much greater, and the tibia 2 is longer. The ovigera and palps are in general longer and the ocular tubercle is lower. Compared to *C. bouvetensis*, the absolute trunk length is greater, the femur is longer and the distal palp articles are longer. In most of these characters, clade 3 (*C. sp. cf. robusta*), agrees with clade 10. However, it differs in the proportions of the palps and the ovigera, which are overall longer than in clade 10, in having a higher ocular tubercle with larger eyes, and in having somewhat longer distal leg articles. As clade 3 is represented by only one individual, the significance of its differences to the other clades could not be tested. It should be noted that the animal is a juvenile with chelifores and lacking genital pores. However, it shows some noticeable differences from other clades. Clade 5, here named *C. bouvetensis*, is the most divergent of the groups examined here and significantly differs from all other clades except for clade 3 in at least 25 characters. The animals are absolutely larger and all leg articles broader than *C. glacialis* and *C. drakei*. The proboscis is longer, the ocular tubercle broader, the 10<sup>th</sup> palp article shorter and all ovigeral articles longer and broader than in all others, except for article 6, which is shorter. Coxa 3 is longer, femur and tibia 1 shorter than in



**Fig. 3.** Statistical parsimony networks of COI haplotypes for *C. glacialis* clades 1 and 2, *C. sp. cf. robusta*, and *C. bouvetensis*. Diameters of circles represent number of specimens per haplotype, colors represent geographical origin of specimens. Unsampled (i.e. hypothetical) haplotypes are represented by dots.



**Fig. 4.** Results of principal component analysis of morphometric measurements. Squares: *C. robusta*. Star: *C. sp. cf. robusta*. Crosses: *C. drakei*. White circles: *C. glacialis* clade 2. Grey circles: *C. glacialis* clade 1. Black circles: *C. glacialis* clade 6. White hexagons: *C. bouvetensis*, Bouvet Island. Grey hexagons: *C. bouvetensis*, South Sandwich Islands. Black hexagons: *C. bouvetensis*, Antarctic Peninsula.

**Table 6.** Number of measurements that are significantly different between the COI clades.

	<i>C. glacialis</i> 1	<i>C. glacialis</i> 2	<i>C. glacialis</i> 6	<i>C. robusta</i> 3	<i>C. robusta</i> 10	<i>C. drakei</i>	<i>C. bouvetensis</i> 5
<i>C. glacialis</i> 1	—						
<i>C. glacialis</i> 2	0	—					
<i>C. glacialis</i> 6	0	1	—				
<i>C. robusta</i> 3	0	0	0	—			
<i>C. robusta</i> 10	13	28	10	0	—		
<i>C. drakei</i>	1	3	2	0	9	—	
<i>C. bouvetensis</i> 5	18	77	25	0	26	40	—

others. Propodus and claw are longer than in *C. glacialis*. Clade 4 (*C. drakei*), is most similar to *C. glacialis*, with only 1–3 significantly different measurements, the only consistent one being the longer fifth ovigeral article, but 40 are significantly different to clade 5. In general, all leg articles are more slender than in all other examined specimens, the trunk is shorter, the femur is longer, the 7<sup>th</sup> palp article is shorter, the 5<sup>th</sup> oviger article is longer than in *C. glacialis* and *C. sp.*, the 10<sup>th</sup> palp article is longer than in *C. glacialis* and *C. bouvetensis*, and the propodus and claw are longer than in *C. glacialis*.

In the principal component analysis (Fig. 4), *C. robusta*, *C. sp. cf. robusta* and *C. bouvetensis* clearly formed separate clusters. *C. glacialis* and *C. drakei* group apart from the other groups but the space occupied by them overlaps. The PCA did not clearly separate the northern, southern and East Antarctic groups of *C. glacialis* (clades 1, 2 and 6). Interestingly, the two measured *C. bouvetensis* specimens from the Antarctic Peninsula (226-7.2) and South Sandwich Islands (PA\_E003) grouped apart from a large cluster formed by all measured Bouvet Island specimens. Factor 1 of the PCA, explaining 53.2% of the variance, was correlated most strongly with measurements of the width of leg and oviger articles but also e.g. the length of the femur and tibia 1, and factor 2, explaining 11.7%, was correlated most strongly with the absolute length of the trunk and the height of the ocular tubercle.

### 3.2.2. SEM data

The examined specimens of *C. glacialis* show three clearly distinct types of ovigeral spines (Fig. 5D,K), including from ectal to endal one row of long spines, one row of medium spines, and irregularly placed short spines placed in two not clearly distinct rows, agreeing with the illustrations of CANO & LOPEZ-GONZALEZ (2007). This condition is similar to *C. tenera* and *C. megalonyx* (CANO & LOPEZ-GONZALEZ 2007; DIETZ et al. 2013). In the specimen 29OT27-2 (*C. glacialis* clade 2; Fig. 5A,D) the long spines show a constriction in the middle, which is not apparent in the specimen AGT42/164 (*C. glacialis* clade 1; Fig. 5H,K) and IU-2007-15812 (*C. glacialis* clade 6; Fig. 7N). A similar configuration is also shown in the *C. drakei* specimen 233-3.1.1 (Fig. 5G,J), which however differs by the orientation of the medium spines being perpendicular to the length of the oviger article. In con-

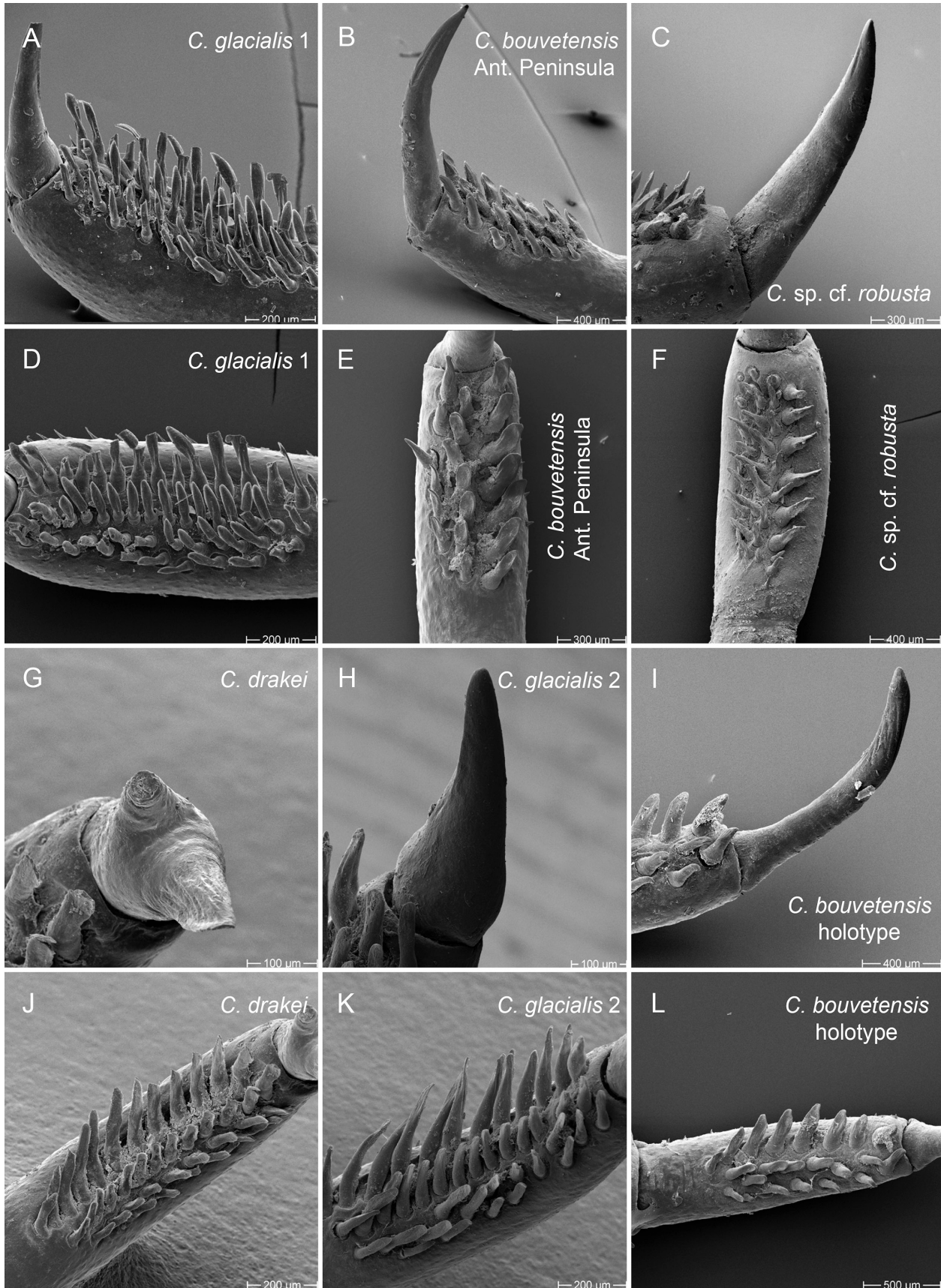
trast, the configuration in *C. bouvetensis* strongly differs (Fig. 5B,E,I,L). The long spines are much less closely spaced and less pointed than in *C. glacialis*, a distinct row of medium spines is absent, and there are about two rows of irregularly placed short spines which are somewhat sparser than in *C. glacialis*. In the specimen 257-2.1 (*C. sp. cf. robusta* Fig. 5C,F), all spines insert on a ridge running along the oviger article. Endally of the row of long spines, there are irregularly spaced spines of decreasing length, and the most endal ones point endally much more strongly than in other specimens. A similar condition appears to be present in *C. robusta* s.str. (Fig. 7O,P), where however the long spines are oriented parallel to the length of the article, not perpendicular as in 257-2.1. The ovigeral claw is rather short compared to the terminal article in *C. glacialis*, much longer and more slender in *C. bouvetensis*, and intermediate in *C. robusta* s.str. and 257-2.1. In *C. drakei* it appears to be bifurcated.

## 4. Taxonomy

*Colossendeis bouvetensis* sp.n.,  
Dietz & Leese, 2015

The new species is attributed to the family Colossendeidae because of its long proboscis, absence of chelifores (in adults), presence of 10-articled palps and ovigera, and absence of auxiliary claws. Within the Colossendeidae, it belongs to the genus *Colossendeis* because of its lack of visible segmentation, non-reduced abdomen, and presence of only four pairs of legs.

**Description.** Trunk length about 9–11 cm. Body and extremities slightly setose. Ocular tubercle slightly broader than high, conical with a rounded tip, with well developed and pigmented eyes. Lateral processes separated by about 2/3 their own diameter. Proboscis cylindrical with slight dilations in the middle and at the distal end, straight, directed slightly downwards (type B<sup>'''</sup>: 1 according to FRY & HEDGPETH 1969), about the same length as the trunk.



**Fig. 5.** Ovigeral claw (top) and spine configuration (bottom) of examined *Colossendeis* specimens. **A,D:** 29OT27-2 (*C. glacialis* clade 1). **B,E:** 226-7.2 (*C. bouvetensis*, probably juvenile, Antarctic Peninsula). **C,F:** 257-2.1 (*C. sp. cf. robusta*, probable juvenile, Antarctic Peninsula). **G,J:** 233-3.1.1 (*C. drakei*). **H,K:** AGT42/164 (*C. glacialis* clade 2). **I, L:** PF\_E008 (holotype of *C. bouvetensis*, Bouvet Island).

**Table 7.** Morphological differences between the species examined in this study. Characters unique for *Colossendeis bouvetensis* are marked in bold.

	<i>C. glacialis</i>	<i>C. drakei</i>	<i>C. robusta</i> s.str.	<i>C. sp. cf. robusta</i>	<i>C. bouvetensis</i>
Trunk length (mm)	8–10.5	6.5–9	14–20	>12	8–11
Ocular tubercle	Higher than broad	Higher than broad	Broader than high	Higher than broad	Broader than high
Proboscis : trunk length ratio	0.7–0.95	0.8–0.85	0.9–1.05	0.85	0.95–1.05
Proboscis shape	A':1 to B''':1	B''':1	B':2 (distally much more dilated than in other species)	B''':1	B''':1
Spinousness of body	Slightly to very spinous	Not spinous	Not spinous	Not spinous	Slightly spinous
Distance between lateral processes	~1.1 diameters	~0.7 diameters	~0.75 diameters	~0.7 diameters	~0.7 diameters
Ratio 3 <sup>rd</sup> : 5 <sup>th</sup> palp article	1.2–1.6	1.45–1.65	1.3–1.5	1.15	<b>1.7–2.2</b>
7 <sup>th</sup> palp article	Longest of distal 5	Not longest of distal 5	Longest of distal 5	Longest of distal 5	Longest of distal 5
Ratio 10 <sup>th</sup> : 9 <sup>th</sup> palp article	0.75–1.25	1–1.15	0.8–1	0.95	<b>0.3–0.7</b>
Ovigeral strigilis	3 types of spines, ~2 rows of short spines	3 types of spines, ~2 rows of short spines	2 types of spines, 3–4 rows of short spines	2 types of spines, ~3 rows of short spines	2 types of spines, ~2 rows of short spines
Ovigeral claw	Short, robust	Short, bifurcated?	intermediate	intermediate	<b>Long, thin</b>
Femur length : width ratio	8.5–14	13–17	~9	~9	<b>~7</b>
Tibia 1 : femur ratio	~1	~0.85	~0.93	~0.9	~1
Tibia 2 : femur ratio	~0.8	~0.75	~1	~1	<b>~1.2</b>
Propodus : tarsus ratio	0.6–0.8	0.8–0.95	~0.75	~0.8	~0.9
Claw : propodus ratio	0.3–0.6	0.7	0.65–0.85	0.75	0.7–0.85

No chelifores. Abdomen straight or bent slightly downwards, length about 25–30% of trunk.

Palps ten-articled. Third palp article longest, approximately twice as long as fifth one. Last five palp articles short and of approximately similar length, somewhat longer than broad, 7<sup>th</sup> the longest, 10<sup>th</sup> the shortest.

Ovigera ten-articled, fourth and sixth article longest. Ovigeral strigilis formed by last four articles, which bear one ectal row of longer spines and about two irregular endal rows of shorter spines. Ovigeral claw long and thin, curved, about the length of the last article.

Legs: All leg articles tubular without special thickened regions. Three coxae about equally long, together about 40% as long as femur. Tibia 1 about as long as femur, tibia 2 15–20% longer than either. Femur about 7 times as long as broad. Last three leg articles together as long as femur. Propodus about 90% as long as tarsus, claw about 75% as long as propodus, slightly curved.

*Measurements of holotype (in mm)*: length of trunk 9.86, proboscis 10.38, abdomen 2.82. Length of palp articles: 1: 0.46, 2: 0.47, 3: 5.81, 4: 0.75, 5: 2.83, 6: 1.19, 7: 1.49, 8: 1.09, 9: 1.32, 10: 0.82. Length of oviger articles: 1: 0.88, 2: 0.98, 3: 0.81, 4: 8.03, 5: 3.32, 6: 6.87, 7: 2.81, 8: 2.44, 9: 2.16, 10: 1.77. Length of articles of third leg: coxa 1: 1.45, coxa 2: 2.08, coxa 3: 1.99, femur: 13.98, tibia 1: 14.09, tibia 2: 16.56, tarsus: 5.32, propodus: 4.8, claw: 3.26.

**Differential diagnosis.** Compared to *C. robusta*, the animal is smaller and the body and extremities are more setose. The wide dilation of the distal part of the probos-

cis is absent. The ratio of 3<sup>rd</sup> to 5<sup>th</sup> palp article length is higher and the tenth palp article is shorter. The ovigeral strigilis has more rows of short spines and the ovigeral claw is more slender and somewhat longer. The leg and oviger articles are relatively broader. The tibia 2/femur and propodus/tarsus length ratios are higher.

Compared to *C. glacialis*, the proboscis is longer and the ocular tubercle is lower. The lateral processes are less widely separated. The ratio of third to fifth palp article length is higher and the tenth palp article is shorter. The ovigeral strigilis lacks a distinct row of medium spines and the ovigeral claw is much longer and more slender. The leg and oviger articles are relatively much broader. The tibia 2/femur and propodus/tarsus length ratios are higher, and the claw is proportionally longer.

Compared to the otherwise very similar species *C. lilliei* Calman, 1915, the lateral processes are more widely separated, the proboscis is relatively shorter, and the intersegmental suture lines are not well visible.

The differences between the species examined here are given in Table 7.

**Derivatio nominis.** After Bouvet Island, the location where the holotype and most other specimens we examined were found.

**Distribution.** So far known from the Eastern Antarctic Peninsula, South Sandwich Islands, and Bouvet Island. Specimens from the Ross and Eastern Weddell Seas not examined by us also seem to belong to *C. bouvetensis* based on COI sequence data.

**Material. Holotype:** specimen PF\_E008, female. Type locality: near Bouvet Island, 54°20'25.08"S, 3°13'13.08"E, 648 m depth. Label: '76 OT [Otter Trawl] 50 | 28.06.04 | Bouvet | PF\_E008'. This specimen has been deposited in the collection of the Zoologische Staatssammlung München (voucher ID ZSMA20159502). Illustrations: Fig. 5I: ovigeral claw, 5L: ovigeral spine configuration, 7D: proboscis and trunk, 7E: ocular tubercle, 7F: palps. – **Paratypes:** 226-7.2, 59BT40, 59OT45, 66OT97, 81OT58, PA\_E003, PQ\_E007, PQ\_E008, PQ\_E010, PQ\_E011, PQ\_E012, PR\_E003, PR\_E004, PR\_E005, PS\_E010. Localities where the specimens were found are listed in Table 2. Illustrations of specimen 226-7.2: Fig. 5B: ovigeral claw, 5E: ovigeral spine configuration, 6D: proboscis and trunk, 6E: ocular tubercle, 6F: palps.

## 5. Discussion

### 5.1. Molecular phylogenies

Our phylogenetic analysis of the COI gene supports the presence of several distinct species groups. One such monophyletic group includes *C. robusta*, *C. drakei*, *C. stramenti*, and several other species. Clearly, *C. glacialis* is not part of that group, but instead appears to be basal to all other “longitarsal” *Colossendeis* species examined here (Fig. 1). Hence, it is concluded that *C. robusta* and *C. glacialis* are not particularly closely related within the genus *Colossendeis*. The fact that ITS sequence data show the same species-level groupings as COI leads to the conclusion that there is no detectable hybridization between *C. glacialis* and *C. robusta* in our data, supporting the view that they are distinct species. However, the lack of resolution between the different *C. glacialis* clades that were well-resolved with COI suggests that ITS data have less resolution, and therefore ITS may not be as useful on the intraspecific level as COI.

We find that the clade 10 *C. robusta* specimens from the type locality (Kerguelen) are related to an Antarctic group (clade 3) with apparently circumpolar distribution. Both groups can be regarded as belonging to the species *C. robusta*. However, they are clearly distinct from another widespread species that has until now not been distinguished from *C. robusta*. This previously overlooked species is here described as a new species, *Colossendeis bouvetensis* sp.n.

### 5.2. Population structure

Similar to the Southern Ocean pycnogonids *C. megalonyx* (KRABBE et al. 2010) and *Nymphon australe* Hodgson, 1902 (MAHON et al. 2008; ARANGO et al. 2011), *C. robusta* and *C. glacialis* show a strongly geographically partitioned population structure. This is expected in benthic animals without a planktonic dispersal stage (THATJE 2012). *C. bouvetensis*, *C. robusta* and *C. glacialis* occur in most of the regions from which sequences were

available, although only *C. glacialis* seems to occur on Shag Rocks/South Georgia and only *C. bouvetensis* is known from Bouvet Island. Within *C. glacialis*, three different geographically separated clades are present, as clade 2 is present on Northern Scotia Arc islands, clade 1 on the Weddell Sea continental shelf, and clade 6 in East Antarctica. It can be assumed that these clades originated in different glacial refugia, the one of clade 2 located in the Scotia Arc, and the other two in different places on the Antarctic shelf. Hence, it appears that the Antarctic Peninsula shelf was probably not recolonized from the Subantarctic islands in this case, although we lack data from the more southern Scotia Arc islands (South Orkneys and South Shetlands). The strong differentiation of the one sequenced South Sandwich Islands individual also suggests that the South Sandwich Islands might have been a separate refugium from South Georgia and the Shag Rocks, while there appears to have been exchange between populations from the two latter locations based on the COI haplotype networks. In *C. bouvetensis*, the highest diversity was found in the Ross Sea, where individuals from four different clades (5, 7, 8, 9), possibly interpretable as separate species, were found, in one case even occurring sympatrically. Clade 9 is found only in the Ross and Eastern Weddell Sea, possibly supporting the hypothesis of an ice-free connection between these seas during previous interglacials (BARNES & HILLENBRAND 2010). Clade 5, the most widespread clade, shows a remarkable distribution. It can be separated into three subgroups, the first of which is found in Bouvet Island and the Ross Sea. This distribution is puzzling, as Bouvet is geographically very remote from the Ross Sea and the clade is not found at intermediate locations such as the Eastern Weddell Sea. However, we lack data from large parts of East Antarctica as well as some Subantarctic islands in the Indian and Pacific Ocean. The second group is found in the South Sandwich Islands and Eastern Weddell Sea, which can be explained by north-south dispersal of unknown direction. The third group is found on both sides of the Eastern Weddell Sea, similar to *C. glacialis* clade 1. Obviously, more specimens from more sampling points are needed to test explicitly for competing population genetic and phylogeographic hypotheses.

### 5.3. Morphological data

The specimens identified here as *C. bouvetensis* differ from *C. robusta* both according to our own measurements and published descriptions of the holotype and other specimens of *C. robusta*. The absolute size of the specimens is much larger in *C. robusta* (trunk length 15.57–19.99 mm in *C. robusta*, 8.22–11.14 mm in *C. bouvetensis*). The proboscis in *C. bouvetensis* is cylindrical with only slight dilations in the middle and the end (Fig. 6D, 7D), not bottle-shaped as in *C. robusta* (Fig. 7M). The femur/tibia 2 ratio is 0.97–1 in *C. robusta*,

while it is only 0.83–0.88 in *C. bouvetensis*. The relative width of the legs is greater in *C. bouvetensis* (length-width ratio of the 3<sup>rd</sup> femur 6.46–7.41 in *C. bouvetensis*, 8.63–9.46 in *C. robusta*). The ratio between the lengths of the 3<sup>rd</sup> and 5<sup>th</sup> palp articles is higher in *C. bouvetensis* (1.57–2.26) than in *C. robusta* (1.3–1.56). The 10<sup>th</sup> palp article is relatively shorter in *C. bouvetensis* than in *C. robusta*. These and other significant differences corroborate the molecular data and support the classification of *C. bouvetensis* as a separate species.

The specimens from Kerguelen agree well with published descriptions of *C. robusta* from that location, including the holotype (HOEK 1881; MÖBIUS 1902; CALMAN 1915; STIBOY-RISCH 1993). The proboscis has a similar shape, and both absolute measurements of body size and the relative measurements of the leg and palp articles agree more with *C. robusta* from Kerguelen than with any other species examined here. The leg is relatively shorter in our specimens (6.07–6.98 times trunk length, 7.52 in the holotype according to CALMAN 1915, 8 in the specimen reported by MÖBIUS 1902), but this may be explained if these authors measured trunk length from the insertion of the proboscis to that of the abdomen, instead of to the end of the 4<sup>th</sup> lateral processes as we did. According to STIBOY-RISCH (1993), the ratio is 6.62 in the holotype. However, according to the measurements of STIBOY-RISCH (1993), the palp in the holotype is much shorter (1.26 times trunk length excluding the basal article) than in our specimens (1.47–1.57). In all our specimens, as well as those reported by CALMAN (1915) and BOUVIER (1913) the propodus/claw ratio is 1.18–1.47, while in the holotype it is 2 (CALMAN 1915), more similar to *C. glacialis*. The drawing given by MÖBIUS (1902) agrees with our specimens in this respect. Palp article 9 is significantly shorter than articles 8 or 10 in the holotype, which is not the case in our specimens, in which article 10 is always slightly shorter than 8 and 9. Despite these differences, our specimens from Kerguelen are closer to the holotype of *C. robusta* than any other specimens we examined, and are here identified as *C. robusta*. CHILD (1995) noted that *C. robusta* specimens from Heard Island (part of the Kerguelen Plateau) are larger and have a distally more inflated proboscis than those from Antarctica, which is consistent with them belonging to *C. robusta* s.str. This appears to be an endemic species from the Kerguelen Plateau.

The two specimens 257-2.1 and 226-7.3 group in a separate clade (clade 3) together with other sequences from specimens not examined by us. Specimen 257-2.1 is unique in having chelifores, and the lack of genital pores indicates that it is an immature specimen despite its large size. Specimen 226-7.3, which could not be completely measured, is even larger, agreeing with the Antarctic *C. robusta* specimens reported by CALMAN (1915) and BOUVIER (1913). In some measurements, such as the femur/tibia 2 ratio, clade 3 resembles *C. robusta*, including the type, more than any other Antarctic specimens we measured. The legs are also relatively longer than in all specimens of *C. bouvetensis*, but fall into the range of variation of *C. robusta*. However, the proboscis is shorter

than in *C. robusta*, which agrees more closely with *C. bouvetensis* in this respect. The specimens also have a high and pointed ocular tubercle (Fig. 6H), while it is lower in *C. robusta* from Kerguelen (Fig. 7K, agreeing with STIBOY-RISCH 1993), similar to *C. bouvetensis*. Besides, the specimens are covered in fine spines, which also disagrees with *C. robusta*. In general, the morphological data agree with the molecular data that clade 3 is most closely related to, but distinct from, *C. robusta*. However, as we lack detailed measurements from adult specimens, we refrain from naming it as a new species, although we suggest that many previous records of *C. robusta* from Antarctica, such as those of CALMAN (1915) and BOUVIER (1913) as well as those specimens measured by FRY & HEDGPETH (1969) not referable to *C. glacialis*, belong to this taxon.

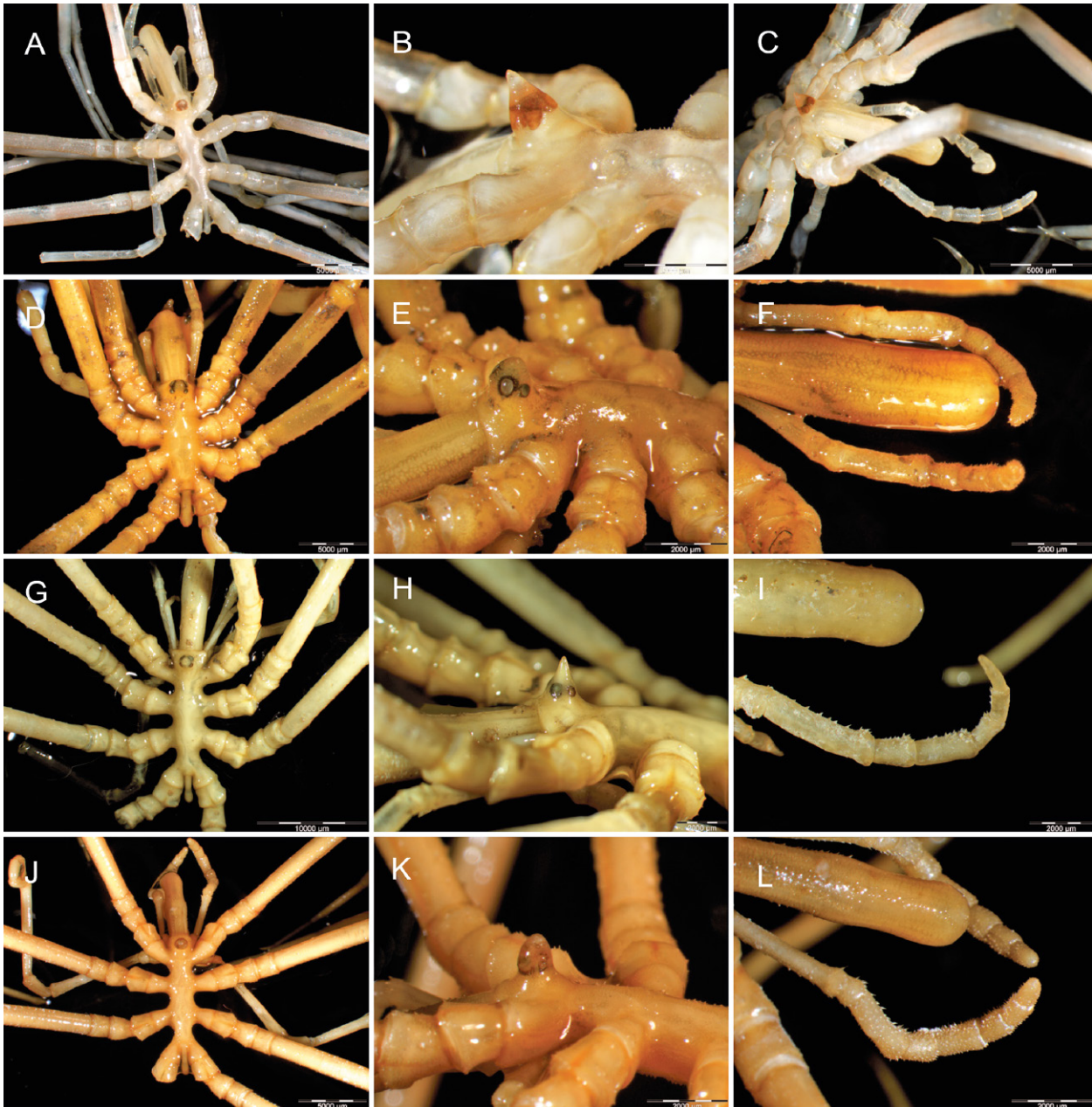
Our specimens of *C. glacialis* appear to be consistent with previous descriptions of that species. The differences found between clades 1 and 2 raise the question whether these clades can be recognized as separate species. For those differences that were also recorded by CALMAN (1915) and STIBOY-RISCH (1993) for the holotype and by CANO & LOPEZ-GONZALEZ (2007), whose specimen also came from the Ross Sea, their values agree with those of clade 2 and clade 6 specimens. As the holotype is from East Antarctica, where so far only clade 6 is known, the latter might retain the name *C. glacialis*. In this case, as discussed below, the name *C. gracilipes* Bouvier, 1913 would probably be available for clade 1, while a new species name would be required for clade 2. However, as the morphological and molecular differences may not be enough for recognizing a new species and can also be explained as intraspecific geographic variation, we do not erect a new species here.

The specimens here identified as *C. drakei* differ from the original description (CALMAN 1915) in their smaller relative lengths of proboscis, abdomen, and palps. However, this could be due to a different way of measuring the trunk, as already discussed for *C. robusta*. As in one of our specimens (257-2.5) the legs are relatively much shorter than in the others, the leg lengths of the types fall into the range of variation of our specimens. Identification of our specimens as *C. drakei* was based primarily on the proportions of the distal palp articles, which agree well with the original description but differ from all other similar Antarctic species in that article 7 is not the longest of the distal 5 articles.

#### 5.4. Comparison with previous work

According to FRY & HEDGPETH (1969) and STIBOY-RISCH (1993), *C. glacialis* shows much more spiny legs than *C. robusta*. We can confirm this, but the degree of spinousness appears to be variable within both *C. glacialis* and *C. bouvetensis* so that it cannot be used to distinguish between those species. The observation of STIBOY-RISCH (1993) that the tibia 2 is the longest leg article in *C. ro-*





**Fig. 6.** Proboscis and trunk (left column), ocular tubercle (middle column) and palps (right column) of examined *Colossendeis* specimens. A–C: 29OT27-2 (*C. glacialis* clade 1). D–F: 226-7.2 (*C. bouvetensis*, probable juvenile, Antarctic Peninsula). G–I: 257-2.1 (*C. sp. cf. robusta*, probable juvenile, Antarctic Peninsula). J–L: AGT42/164 (*C. glacialis* clade 2).

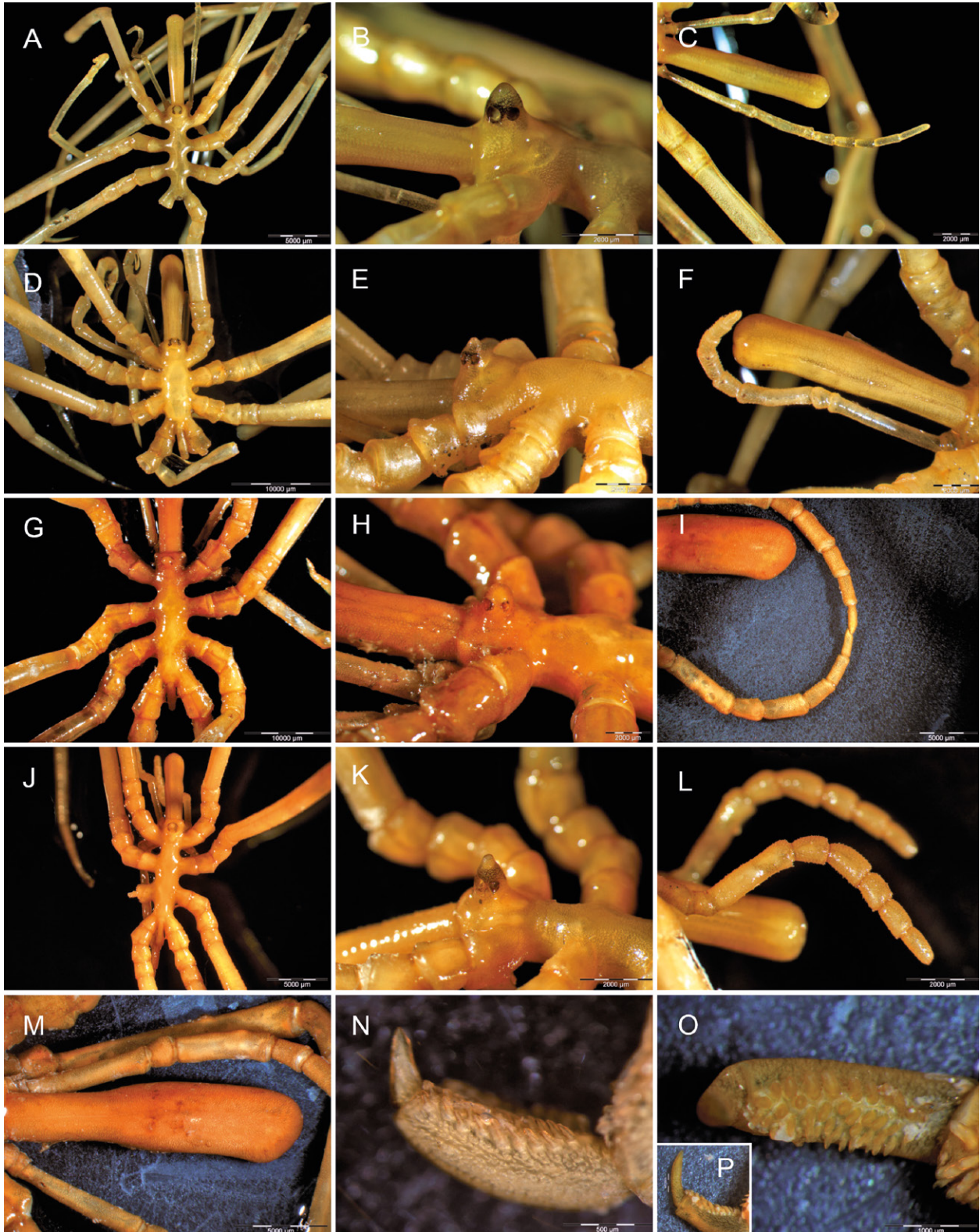
*busta* also agrees with our data, but the tibia 1 is not always the longest in our specimens of *C. glacialis*, as the femur is about equally long. Also agreeing with STIBOY-RISCH (1993), the three last palp articles are about equally long, although we cannot confirm that the second to last palp article is significantly shorter in *C. robusta*; instead, the last article is the shortest in our specimens. No specimens among our material could be assigned to the species *C. acuta* described by STIBOY-RISCH (1993).

According to the key of HODGSON (1927) the last three palp articles are elongated in *C. robusta* but rounded in *C. glacialis*. While they are indeed not longer than wide

in our *C. glacialis* clade 1 (Fig. 6C) and 6 (Fig. 7L) specimens, they are elongated in the clade 2 specimen (Fig. 6L).

CANO & LOPEZ-GONZALEZ (2007) described the ovigeral spine configuration of *C. glacialis*, which agrees well with our observations of that species. They also agree with the original description by HODGSON (1907a). The shape of the long spines in their Ross Sea specimen agrees more with our clade 1 than clade 2 specimen. The configuration with three distinct types of spines also occurs in *C. drakei* (this study), *C. megalonyx*, *C. tenera* (DIETZ et al. 2013), *C. australis* and *C. scotti* (CANO & LOPEZ-GONZALEZ 2007). In *C. bouvetensis* (this study),





**Fig. 7.** Proboscis and trunk (left column), ocular tubercle (middle column) and palps (right column) of examined *Colossendeis* specimens. **M:** Proboscis. **N,P:** Last oviger article and claw in side view. **O:** Last oviger article in endal view. **A–C:** 233-3.1.1 (*C. drakei*). **D–F:** PF\_E008 (holotype of *C. bouvetensis*, Bouvet Island). **G–I,M,O–P:** IU-2007-5063 (*C. robusta*, Kerguelen). **J–L,N:** IU-2103-15812 (*C. glacialis* clade 6).

*C. angusta* (DIETZ et al. 2013), *C. wilsoni* and *C. lilliei* (CANO & LOPEZ-GONZALEZ 2007) there is no distinction between medium and short spines. Although the spines in

the holotype of *C. robusta* as illustrated by HOEK (1881) are extremely worn, they show no evidence of a separate row of medium spines. This also agrees with the con-

dition in our clade 3 specimen, which is closely related to *C. robusta*. According to our phylogenetic analysis, a configuration similar to that in *C. glacialis* appears to be ancestral for this group of *Colossendeis* species and has been reduced multiple times. Interestingly, *C. wilsoni* and *C. lilliei*, both species rather similar in overall appearance to *C. bouvetensis*, show a similar spine configuration and shape of the ovigeral claw, suggesting that they are related more closely to it than *C. glacialis*.

In the key of PUSHKIN (1993) *C. glacialis* is differentiated from *C. robusta* and other species by the claw of the leg being less than half as long as the propodus. Although this ratio is indeed consistently lower in *C. glacialis* than in *C. robusta* and other species in our data, there are some specimens of *C. glacialis* in which it is slightly larger than 0.5. In the holotype of *C. robusta*, it is ca. 0.52 (STIBOY-RISCH 1993), lower than in any of our specimens of that species. Our results also disagree with the claim (PUSHKIN 1988) that the 6<sup>th</sup> palp article (5<sup>th</sup> in Pushkin's terminology) is the longest of the distal five articles in *C. robusta*. The 7<sup>th</sup> article is the longest in all of our specimens except for those assigned to *C. drakei*, and this is also the case in the holotype of *C. robusta* (STIBOY-RISCH 1993).

*C. robusta* and *C. glacialis* have been synonymized by FRY & HEDGPETH (1969), which has been accepted by CHILD (1995). The synonymization was based on the claim that the recorded differences show too much variability to be useful distinguishing characters and are partially explained by ontogeny. However, the data presented by FRY & HEDGPETH (1969) actually support the presence of two distinct species in their dataset. The femur/tibia 2 ratio shows a bimodal distribution consistent with our data, with a smaller ratio in *C. robusta*. Specimens assigned to *C. bouvetensis* have an even lower femur/tibia 2 ratio than any of those given by FRY & HEDGPETH (1969) and elsewhere in the literature (CALMAN 1915; CANO & LOPEZ-GONZALEZ 2007). Proboscis length compared to leg length is significantly larger in *C. robusta*. According to FRY & HEDGPETH (1969), the data is consistent with a single-species explanation if one assumes a sudden increase in proboscis length at a certain point during ontogeny, as specimens with a relatively shorter proboscis (here interpreted as *C. glacialis*) are typically smaller. However, their graph actually shows two larger specimens with a proboscis/leg ratio more typical of *C. glacialis*. Our data in general agree with those of FRY & HEDGPETH (1969) in finding that the ratio is typically larger for *C. robusta* as well as *C. bouvetensis*, and we also identify a smaller specimen (226-7.2) as *C. bouvetensis* showing that the ratio does not change significantly with increasing size. However, the range of variation for those characters is so great in *C. glacialis* that it would be difficult to differentiate those species based on this character alone. Notably, Fry and Hedgpeth's data include holo- and paratypes of both species, which agree in their measurements with the specimens examined by us. From their data, we conclude that most of the *C. robusta* specimens examined by FRY & HEDGPETH (1969) actually belong to *C. glacialis*,

and that *C. bouvetensis* was probably not represented in their samples.

## 5.5. Comparison with other similar species

BOUVIER (1913) described the species *Colossendeis gracilipes* based on two specimens from the South Shetlands and Antarctic Peninsula. The species was synonymized with *C. glacialis* by CALMAN (1915), as the only differences to that species are a narrowing of the proboscis at the base and a longer last palp article. The former character is not apparent as a consistent difference in our specimens, but in the latter *C. gracilipes* agrees well with clade 1 specimens, which is consistent with its geographical origin. The holotype of *C. glacialis* from the Ross Sea is more similar to clades 2 and 6 in that character. In the other measurements given by BOUVIER (1913), *C. gracilipes* also agrees with *C. glacialis* and the synonymization appears to be correct.

HODGSON (1907b) described the species *Colossendeis patagonica* from the Patagonian Atlantic coast based on an incomplete specimen. It is sometimes synonymized with *C. glacialis* (e.g. BAMBER & EL NAGAR 2014) which however is not otherwise known from Patagonia (PUSHKIN 1993; MUNILLA & SOLER MEMBRIVES 2009). The relative lengths of palp articles and the presence of only three rows of ovigeral spines differ from *C. glacialis*. As the specimen was incomplete and no illustrations were published, it is difficult to identify with another species. However, synonymy with *C. glacialis* appears to be unlikely.

CHILD (1995) synonymized *C. lilliei* Calman, 1915 with *C. robusta*. The species was originally distinguished from *C. robusta* by Calman by the lateral processes being placed much closer together, the presence of visible intersegmental suture lines, a proportionally longer proboscis, and a smaller femur/tibia 2 ratio. In the latter character, it agrees with *C. bouvetensis*, but it differs in the other mentioned characters. None of the specimens examined by us could be determined as *C. lilliei*. As the differences to *C. robusta* appear to be consistent, we regard the synonymy as unlikely. However, *C. lilliei* appears to be related to *C. bouvetensis* based on the ovigeral characters, which are virtually identical in the two species. Also, the proportions of the palp articles, especially the length of the 3<sup>rd</sup> relative to the 5<sup>th</sup> article and the shortness of the 10<sup>th</sup> article, are similar to *C. bouvetensis* but differ from the other examined species. The leg proportions are also most similar to *C. bouvetensis*. In conclusion, while *C. lilliei* appears to be distinct from *C. bouvetensis*, these species are probably closer to each other than to *C. robusta* or *C. glacialis*.

The Patagonian species *Colossendeis smirnovi* Pushkin, 1988 was synonymized with *C. drakei* by CHILD (1995), but the proportions of the palp articles do not agree with it and appear to be more similar to *C. gla-*

*cialis*. *C. smirnovi* was differentiated from *C. glacialis* by PUSHKIN (1988) by a lower width/length ratio of the femur, different relative lengths of the last five palp articles and the claw being longer than half of the propodus. However, his measurements of the femur for both species fall into the range of variation for our *C. glacialis* clade 2 specimens. The claw is longer than half of the propodus in some of our *C. glacialis* specimens as well as in one specimen described by CALMAN (1915) and the relative lengths of the palp articles are variable in *C. glacialis*. Despite the similarity, however, we regard synonymy of *C. smirnovi* with *C. glacialis* as doubtful as the latter species has not to our knowledge been recorded from Patagonia, although some specimens may have been misidentified as *C. drakei*.

Some other Southern Ocean *Colossendeis* species with proportions similar to *C. robusta* and *C. glacialis* have been described. *C. wilsoni* Calman, 1915 is similar especially to *C. lilliei*, but differs from all other *Colossendeis* species by having only 9 palp articles. *Notoendeis germanica* Hodgson, 1915 (later placed into the genus *Colossendeis* by HODGSON 1927) appears to be similar to *C. wilsoni* but was described as having a fully segmented body, which would be unique within the Colossendeinae. *C. stramenti* Fry & Hedgpeth, 1969 from the South American shelf differs from all other species in having a highly reduced ocular tubercle without eyes. *C. grassus* Pushkin, 1993 resembles *C. lilliei* in having the lateral processes closely placed together but differs in the form of the ocular tubercle. *C. avidus* Pushkin, 1970 has leg proportions similar to *C. glacialis* but a significantly longer and differently shaped proboscis. It appears to be very similar to *C. acuta*, and we regard these species as probably synonymous. *C. bruuni* Fage, 1956 from the Kermadec Trench and *C. curtirostris* Stock, 1963 from South Africa have a short proboscis similar to *C. glacialis*, but a claw longer than the propodus, which differs from all similar Antarctic species and especially from *C. glacialis*. As none of those species appears to be present in our material, we cannot assess their validity in the present study.

## 5.6. Conclusions

The results of this study strongly support the hypothesis that *C. robusta* and *C. glacialis* are separate species and not conspecific as suggested by several authors; they are not even closely related within *Colossendeis*. The integrative taxonomic approach identified a new species, *C. bouvetensis*, previously not distinguished from *C. robusta*, which has a broad geographic distribution. There might be further overlooked species in the *C. robusta* group, yet, more data are needed to show this. We also show that, while there is an endemic grouping in the Kerguelen region that appears to include the holotype of *C. robusta*, there is a related widespread grouping in the Antarctic that may be included in that species if a broad species concept is used. Within *C. glacialis*, *C. robusta*

and *C. bouvetensis*, we find regional intraspecific variation suggesting survival in multiple refugia during the Pleistocene glaciations.

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## Electronic Supplement Files

at <http://www.senckenberg.de/arthropod-systematics>  
("Contents")

**File 1:** dietz&al-colossendeis-asp2015-electronicsupplement-1.xls  
— **Table ES1.** Morphometric measurements of examined *Colossendeis* specimens in mm. P = Palp, O = Oviger, L1–4 = Walking leg 1–4, C1–3 = Coxae 1–3, F = Femur, T1–2 = Tibiae 1–2, TA = Tarsus, PR = Propodus, CL = Claw. L = length, H = height, W = width. Lateral 12 = distance between first and second lateral processes. a = anterior, p = posterior.

**File 2:** dietz&al-colossendeis-asp2015-electronicsupplement-2.xls  
— **Table ES2.** Morphometric measurements of examined *Colossendeis* specimens, expressed as proportion of trunk length. P = Palp, O = Oviger, L1–4 = Walking leg 1–4, C1–3 = Coxae 1–3, F = Femur, T1–2 = Tibiae 1–2, TA = Tarsus, PR = Propodus, CL = Claw. L = length, H = height, W = width. Lateral 12 = distance between first and second lateral processes. a = anterior, p = posterior.

**File 3:** dietz&al-colossendeis-asp2015-electronicsupplement-3.xls  
— **Table ES3.** Measurements that are significantly different ( $p < 0.05$ ) between different clades. P = Palp, O = Oviger, L1–4 = Walking leg 1–4, C1–3 = Coxae 1–3, F = Femur, T1–2 = Tibiae 1–2, TA = Tarsus, PR = Propodus, CL = Claw. L = length, H = height, W = width. Lateral 12 = distance between first and second lateral processes. a = anterior, p = posterior. < and > signs refer to clade listed second compared to first.