

Evidence from morphological and genetic data confirms that *Colossendeis tenera* Hilton, 1943 (Arthropoda: Pycnogonida), does not belong to the *Colossendeis megalonyx* Hoek, 1881 complex

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Abstract Within the Pycnogonida, genetic studies have revealed that *Colossendeis megalonyx* Hoek (Challenger Report, Zoology, 3(X), 1–167, 1881), consists of a complex of several cryptic or overlooked species. *Colossendeis megalonyx* is a typical Southern Hemisphere species complex distributed primarily on the continental shelves in the Antarctic and Subantarctic. However, a different *Colossendeis* species with a completely different geographic distribution range, *Colossendeis tenera* Hilton (Journal of Entomology and Zoology, Pomona College, Claremont, 35(1), 2–4, 1943), was considered a subspecies of *Colossendeis megalonyx* by Turpaeva

(Trudy Instituta Okeanology "P. P. Shirshova", Akademy Nauk SSSR, 103, 230–246, 1975). *Colossendeis tenera* occurs predominantly along the Pacific Coast of North America from the Bering Sea to central California. Prominent differences between these two currently distinct species are found in body proportions and other characters that were interpreted by Turpaeva as a possible case of pedomorphosis induced by deep-sea conditions. In this study, we tested the hypothesis that *Colossendeis tenera* belongs to the *Colossendeis megalonyx* complex by analyzing available and novel sequence data (CO1 and H3) of both *Colossendeis megalonyx* and *Colossendeis tenera* as well as a similar, apparently closely related species, *Colossendeis angusta* Sars (Archiv for Matematik og Naturvidenskab, 2, 237–271, 1877). We compared morphometric data and SEM of the ovigera of these species. Our results clearly indicate that *Colossendeis tenera* and *Colossendeis angusta* are not a part of the *Colossendeis megalonyx* complex. A sister-group relationship of *Colossendeis tenera* and *Colossendeis angusta* is strongly supported, but *Colossendeis tenera* is not clearly resolved as monophyletic with respect to *Colossendeis angusta*. This work highlights the need for further examination of the variation found in the *tenera-angusta* clade. It also gives a first hint of the phylogenetic affinities of species within *Colossendeis*.

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Introduction

Pycnogonids or sea spiders are a group of marine arthropods that occur from shallow to deep-sea habitats and from polar

to tropical regions. Despite considerable efforts to resolve the arthropod tree of life (Regier et al. 2010; Meusemann et al. 2010), it is not clearly resolved whether pycnogonids are the sister group to other Chelicerata or to all other arthropods. The taxonomy and systematics within the pycnogonids have also changed considerably over the last century (Hedgpeth 1947; Fry 1978; Munilla León 1999; Arango 2002; Arango and Wheeler 2007; Bamber 2007). Within the Pycnogonida, the Colossendeidae are one particularly species-rich (more than 100 species according to Bamber and El Nagar 2012) and broadly distributed clade. Their phylogenetic position within the group was controversial but recent data add support for their basal position within the Pycnogonida (Dietz et al. 2011). Most of the species in the family are classified in the genus *Colossendeis* Jarzynsky, 1870, which is not clearly monophyletic since the polymerous genera *Decolopoda* Eights, 1835, and *Dodecolopoda* Calman & Gordon, 1933, appear to group within *Colossendeis* (Krabbe et al. 2010).

Colossendeis tenera Hilton, 1943, was very briefly described from the Washington and Oregon coasts by Hilton, and a more detailed description was provided by Hedgpeth (1943). Subsequent descriptions confirmed its presence along the Pacific Coast of North America from the Bering Sea (Bowers Bank, Child 1995) to central California (Farallon Islands, Child 1994) (see Fig. 1). Other records of *C. tenera* are from Honshu (Japan) and the Kuriles (Turpaeva 1975) so *C. tenera* is best characterized as a circum-North Pacific species with only very limited occurrence in the Southern Hemisphere on the South American coast (one specimen reported off the Peruvian coast was

determined as *C. tenera* by Turpaeva 1975) (Fig. 1). *C. tenera* has been recorded in depths from 225 m (this publication) to 5,200 m (Turpaeva 1975) and can therefore be considered a eurybathic species.

While most authors regarded *C. tenera* as a distinct species, Turpaeva (1975) classified it as a subspecies of the otherwise Southern Hemisphere species *C. megalonyx* Hoek, 1881. This synonymization was based on the similar general appearance of the two species as noted by Hedgpeth (1943) and the lack of sufficient diagnostic characters in the denticulate spines of the ovigers according to Turpaeva's examinations (Turpaeva 1975).

Colossendeis angusta Sars, 1877, is a species of interest in the context of *C. tenera* affinities given the known general similarities between the species already reported in Hedgpeth (1943). Another species worth further examination and briefly discussed herein is *C. gracilis* Hoek, 1881, which is often synonymized with *C. angusta* (e.g., Fry and Hedgpeth 1969).

Colossendeis megalonyx is a Southern hemisphere circum-polar eurybathic species complex distributed in depths of 3–4,900 m on the continental shelves of Antarctica, the Subantarctic islands, southern South America, South Africa, and Madagascar (Munilla and Soler-Membrives 2009; Griffiths et al. 2011) (see Fig. 1). Recent molecular analyses (Krabbe et al. 2010) have shown that *C. megalonyx* is a complex of at least six lineages that can be clearly differentiated by mitochondrial sequences and probably represent overlooked species. Nevertheless, this *C. megalonyx* complex forms a clearly supported monophyletic group within the Colossendeidae.

Examples of species that spread from the Southern Ocean to other distant geographical regions are known in benthic

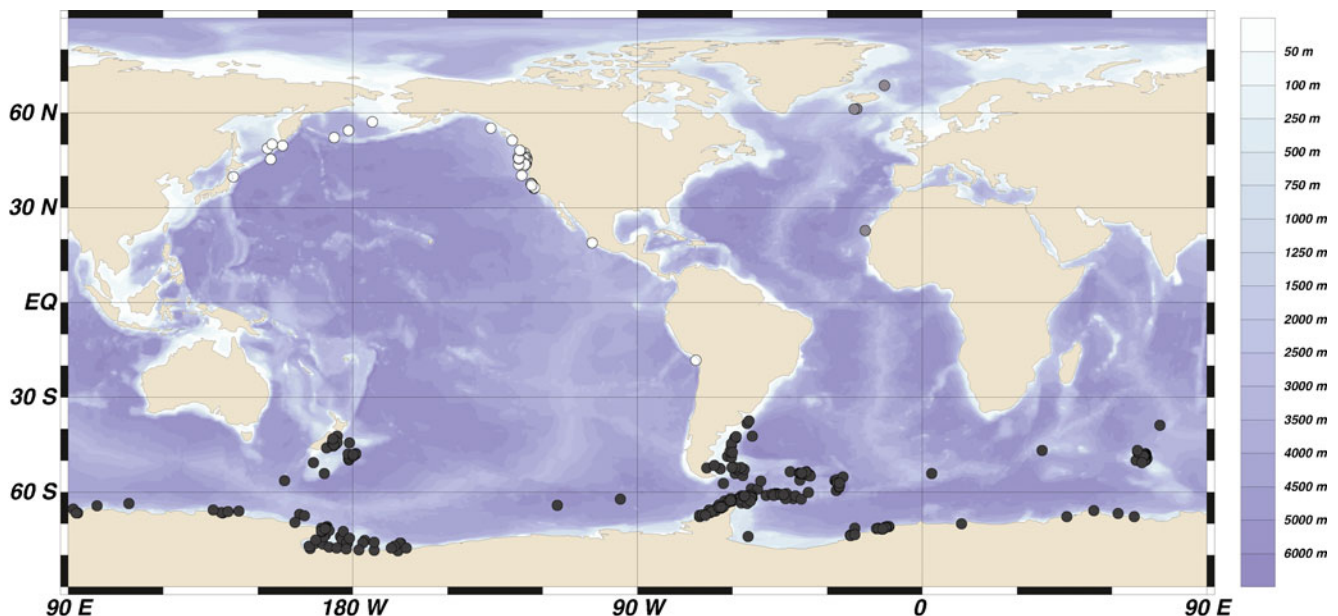


Fig. 1 Reported distribution ranges of *C. megalonyx* (black) from the ScarMARbin database (access data 25.4.2012), *C. tenera* (white), and the locations of the *C. angusta* specimens analyzed in this study (gray). Map: Ocean Data View version 4

invertebrates (Strugnell et al. 2008). If the inclusion of *C. tenera* within the *megalonyx* complex is confirmed, it would be another example of wide geographic distribution and extend distribution of the *megalonyx* complex to the Northern hemisphere.

The aim of this study was to investigate the relationship of *C. tenera* and the *megalonyx* complex while also examining affinities with *C. angusta*. We analyzed mitochondrial and nuclear sequence data to assess phylogenetic affinities of *C. tenera*, and we also examined morphological data including new SEM data on the morphology of the ovigera of all three species.

Material and methods

Morphology

Ethanol-preserved specimens were analyzed under a stereomicroscope (Olympus BX40) and a Zeiss DSM 950 scanning electron microscope (SEM). Since the ovigeral spine fields are of particular taxonomic importance (see, e.g., Fry and Hedgpeth 1969; Cano and López González 2007), we analyzed ovigeral spine fields in samples from five of the six *Colossendeis megalonyx* clades, in two *C. tenera* specimens, and one *C. angusta* specimen (Table 1). For the SEM pictures we first dried samples of the ovigera by adding hexamethyldisilazane (HMDS) in a rising concentration every 15 min. Second, the samples were air-dried underneath an outlet. Third, the samples were carbon-glued on cylindrical aluminum blocks and sputter-coated (180 s) with a 99-nm-thick palladium-gold layer and then analyzed under the SEM.

To study differences in other morphological traits, we measured 59 specimens of Antarctic and Subantarctic *C. megalonyx* (8 clade A, 15 B, 10 C, 14 D, 12 E, see [Online Resource 4](#)), 2 *C. tenera* specimens, 1 from British Columbia and 1 from the Pacific coast of Mexico, and 10 *C. angusta* specimens, 9 from the Northeast Atlantic and 1 from the West African coast. Specimens were determined using published keys and descriptions (Fry and Hedgpeth 1969 and Child 1995 for *C. megalonyx*, Hedgpeth 1943 for *C. tenera*, Sars 1891 for *C. angusta*). *C. tenera* and *C. angusta* were differentiated mostly by the presence of eyes and lack of anaxial insertion of the ninth palp article in the former species. Most of the *C. megalonyx* material was part of the molecular study by Krabbe et al. (2010) representing five of the six clades described based on mitochondrial sequence data (Table 1). We took body, proboscis, and leg measurements, which are widely used in the literature, i.e., length of the proboscis, trunk, abdomen, and the individual articles of the third leg. We compared our measurement data with those reported previously for *C. tenera*, *C. megalonyx*, and *C. angusta* (Hedgpeth 1943 for *C. tenera*, Calman 1915

and Cano and López González 2007 for *C. megalonyx*, Fage 1956 for *C. angusta* and Minnaard and Zamponi 1984 for *C. gracilis*).

To compare relative lengths, all values were also expressed as proportions of the trunk length. The relative length of the proboscis and the length of coxae 1-3, the femur, tibia 1, tibia 2, propodus, tarsus, and terminal claw were used as variables in a principal component analysis (PCA) with the STATISTICA version 10 software (StatSoft Inc.). The lengths of the three coxae were summed since measurements of the single segments were not available for comparison for all material. Further morphological traits were not consistently available for all specimens and therefore were not used.

Molecular phylogenetic analyses

We generated cytochrome oxidase 1 (CO1) sequences for the *C. tenera* specimen from Mexico and for the nine Northeast Atlantic *C. angusta* (GenBank accession numbers KC462557-KC462566). Other CO1 sequence data used in this article had been produced in a previous study from the Bochum's laboratory (Krabbe et al. 2010). Additionally, we retrieved relevant *Colossendeis* CO1 sequences from Genbank ([Online Resource 1](#)). Some sequences labeled as *Colossendeis* in Genbank were not included as we could not validate the species identification.

We tested the affinities of *C. tenera* within Colossendeidae analyzing a total of 44 CO1 sequences (545 bp) with Bayesian and maximum likelihood analyses. *Rhopalorhynchus* Wood-Mason, 1873, which is classified as a separate subfamily within Colossendeidae by Bamber and El Nagar (2012), was used as an outgroup. Multigene Bayesian and ML analyses were performed with a subset of ten taxa for which the nuclear gene histone 3 (H3) data were also available. We produced new H3 sequences from representatives of the six *C. megalonyx* clades (Krabbe et al. 2010). GenBank accession numbers: KC456423-KC456506. In these concatenated analyses, *Colossendeis macerrima*, which grouped in a basal position within *Colossendeis* in the CO1 tree, was used as an outgroup. An analysis using CO1 and H3 as well as the mitochondrial ribosomal genes 12S and 16S was also performed (see [Online Resource 4](#)).

For the Bayesian analyses, the program MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was used, and the appropriate model of evolution was determined with MrModeltest 2.3 (Nylander 2004) using the Akaike information criterion (AIC). For the multigene analyses, the model was partitioned by individual gene. The analysis was run for 10,000,000 generations with two independent runs with four chains each and a sample frequency of every 100th generation. Results were checked for convergence (splits frequencies of the likelihoods < 0.01) and the consensus tree calculated, discarding the first 25 % of the trees

Table 1 Ranges of morphological measurements of *C. tenera*, *C. angusta*, and *C. megalomyx* specimens, including both our own measurements and those from the literature (lengths ratio relative to trunk are shown if not listed otherwise). For a full list of the individual measurements, see [Online Resource 4](#)

Species	Description	Trunk (in mm)	Proboscis	Abdomen	1 st coxa	2 nd coxa	3 rd coxa	Sum of coxae	Femur	Tibia 1	Tibia 2	Tarsus	Propodus	Terminal claw
<i>C. tenera</i>	Male paratype	6.5	1.308	0.123	0.154	0.231	0.231	0.615	2.769	2.308	1.692	0.731	0.538	0.615
<i>C. tenera</i>	Female paratype	7	1.393	0.143	0.143	0.179	0.214	0.536	2.857	2.429	1.643	0.571	0.429	0.536
<i>C. tenera</i>	(Mexico)	6.8	1.544	0.147	0.206	0.294	0.294	0.794	2.324	2.132	1.618	0.721	0.691	0.765
<i>C. tenera</i>	(British Columbia)	10.366	0.964	0.115	0.130	0.153	0.135	0.418	1.954	1.742	0.728	0.284	0.260	0.291
<i>C. angusta</i>	(West Africa)		0.863	0.233	0.137	0.137	0.123	0.397	1.918	1.904	1.233	0.521	0.438	0.575
<i>C. angusta</i>	(Norwegian Sea)	7.91–9.04	0.906–0.988	0.177–0.217	0.128–0.15	0.113–0.133	0.102–0.122	0.356–0.403	2.053–2.321	1.861–2.15	1.284–1.507	0.468–0.575	0.411–0.417	0.508–0.565
<i>C. angusta</i>	(Iceland)	6.43–8.01	0.905–0.966	0.174–0.25	0.15–0.173	0.125–0.173	0.109–0.15	0.384–0.48	2.223–2.435	2.012–2.199	1.362–1.499	0.494–0.595	0.484–0.569	0.602–0.771
<i>C. megalomyx</i>	Holotype (Calman 1915)	11	1.818	0.255	–	–	–	0.636	2.068	1.909	1.614	0.932	0.727	0.636
<i>C. megalomyx</i>	Clade A	9.6–12.9	1.402–1.534	–	0.190–0.208	0.116–0.188	0.116–0.179	0.434–0.563	1.51–2.009	1.51–1.947	1.354–1.86	0.543–0.893	0.527–0.759	0.279–0.411
<i>C. megalomyx</i>	Clade B	11.14–13.83	1.439–1.725	–	0.179–0.219	0.11–0.172	0.11–0.16	0.416–0.542	2.052–2.61	1.835–2.314	1.594–1.994	0.86–1.114	0.638–0.804	0.46–0.638
<i>C. megalomyx</i>	Clade C	10.76–13.37	1.504–1.864	–	0.198–0.232	0.142–0.204	0.138–0.196	0.491–0.603	1.992–2.536	1.698–2.105	1.419–1.864	0.768–1.06	0.519–0.693	0.403–0.569
<i>C. megalomyx</i>	Clade D	11.4–13.65	1.53–1.878	–	0.184–0.236	0.16–0.195	0.147–0.193	0.509–0.607	2.105–2.77	1.851–2.42	1.64–2.187	0.93–1.307	0.724–1.602	0.44–0.579
<i>C. megalomyx</i>	Clade E	11.52–13.41	1.704–1.895	–	0.182–0.221	0.154–0.181	0.127–0.166	0.492–0.563	2.088–2.685	2.02–2.434	1.777–2.16	0.978–1.241	0.712–0.883	0.42–0.552

as burn-in. Posterior probabilities were calculated from the remaining trees. For ML analyses, the program RAxML version 7.0.3 (Stamatakis 2006) was used with the model GTR+G for finding the best tree, and bootstrap analyses were performed using 1,000 fast replicates with the GTR+CAT approximation. The multigene analysis was partitioned by gene.

Results

Morphometric comparison of *C. tenera* and *C. megalonyx*

The proportions of the proboscis, trunk, abdomen and leg articles in *C. tenera* analysed by us and from the literature differ considerably from those in *C. megalonyx*, both according to our own and to published data (Table 1, Online Resource 4).

According to the measurements of Hedgpeth (1943), *C. tenera* differs considerably from *C. megalonyx*. The absolute length of the trunk is much shorter in *C. tenera* than in *C. megalonyx*. The ratios of proboscis/trunk and abdomen/trunk are lower in *C. tenera* than in *C. megalonyx*. Coxa 1 is proportionally shorter, coxa 3, the femur and tibia 1 are longer, the tarsus and propodus are again shorter, and the claw is again longer. The sixth joint of the oviger is longer than in *C. megalonyx*.

The *C. tenera* specimen from British Columbia differs from all others in its large absolute size, relatively shorter proboscis, and short legs. The legs are relatively shorter than in any other specimen of *C. tenera*, *C. angusta/gracilis*, or *C. megalonyx* known to us. The short proboscis is comparable to the West African *C. angusta* specimens. In general shape, the proboscis agrees with the illustration of Hedgpeth (1943). Due to the short legs, the proportions of some leg articles are more similar to *C. megalonyx*, but the short coxa 1, tibia 2, tarsus, propodus, and claw fall outside its range of variation, as do the proboscis and abdomen.

The Mexican *C. tenera* specimen has proportions somewhat more similar to *C. megalonyx* than those described by Hedgpeth (1943). Only the short abdomen, the long coxae 2 and 3, the short tarsus and the long claw fall outside the range of variation for *C. megalonyx* (Table 1). The proportions therefore differ noticeably from those of the northern *C. tenera* specimens. The proboscis is longer and has a very different shape, being strongly inflated distally and more similar to species such as *C. scotti* Calman, 1915, than to other specimens of *C. tenera* or *C. megalonyx*.

When length of leg articles are expressed as percentage of the total leg length, coxa 1 is shorter in *C. tenera* than in *C. megalonyx*, coxa 3, the femur and tibia 1 are longer, and tibia 2, the tarsus, and propodus are again shorter. The claw/propodus ratio is higher in *C. tenera* than in *C. megalonyx*. In the British Columbia specimen, coxa 2 and 3, the femur,

and tibia 1 are longer than in *C. megalonyx*, and tibia 2, the tarsus, and propodus are shorter. It should be noted that in this specimen, both third legs were broken off, and the distal articles from tibia 2 on were measured on the fourth pair of legs, implying that the rather extreme values for this specimen may not necessarily be reliable because the fourth leg is often shorter than the third leg. In the Mexican specimen, coxae 2 and 3 are relatively longer than in *C. megalonyx*, tibia 2 and the tarsus are shorter, and the claw is longer. In all *C. tenera* and *C. angusta* specimens, the claw is longer than the propodus, while it is shorter in *C. megalonyx*.

The specimens we examined agree in most of the distinctive characters mentioned by Hedgpeth (1943) and Child (1994, 1995), such as a seventh palp article not longer than broad, claws as long or longer than the propodus, and a proportionally short abdomen. However, all of them have a rather low ocular tubercle that does not resemble the figure of Hedgpeth (1943). There is no indication that the tubercle is broken off, as claimed by Hedgpeth (1943) for the Washington specimen. Confirming previous reports, in all specimens of *C. tenera* we examined the eyes are unpigmented. The Washington and Oregon specimens agree with Hedgpeth's (1943) description in having front eyes somewhat larger than back ones, but in the specimen from British Columbia, the size difference is much more extreme.

Morphometric comparison of *C. tenera* and *C. angusta*

The West African specimen of *C. angusta* falls outside the range of variation of the specimens from the Northeast Atlantic. Relative to trunk length, the proboscis, femur and tibia 2, and the total leg length are shorter (Table 1, Online Resource 4). Other measurements fall into the range of variation for the North Atlantic specimens. Comparing leg articles relative to total leg length, the femur is slightly shorter and the claw is longer in the West African specimen.

All measured *C. tenera* specimens have a shorter abdomen than *C. angusta*, and all except the British Columbia specimen have a shorter proboscis. In the *C. tenera* specimens reported by Hedgpeth (1943), all leg articles except for coxa 1, the propodus, claw, and tarsus of the female specimen are longer than in *C. angusta* relative to trunk length. Relative to total leg length, only coxa 3 is consistently longer in both *C. tenera* specimens than in *C. angusta*. In the Mexico specimen, all three coxae, tibia 2, the tarsus, and propodus are longer than in *C. angusta* relative to trunk length. Relative to total leg length, all three coxae, the tarsus, and propodus are longer, and the femur and tibia 1 are shorter than in *C. angusta*. In the British Columbia specimen, all leg articles except for the three coxae are shorter than in *C. angusta* relative to trunk length. Relative to total leg length, all three coxae, the femur and tibia 1 are longer, and tibia 2, the tarsus, propodus, and claw are shorter.

In the *C. angusta* specimen recorded by Fage (1956), the proboscis, abdomen, femur, and tibia 1 and 2 are longer than in the specimens measured by us. Except for the abdomen, this shows greater agreement with the *C. tenera* paratypes, although tibia 1 and 2 are even longer. In the *C. gracilis* specimen described by Minnaard and Zamponi (1984), the proboscis is much longer than in *C. tenera* or our *C. angusta* specimens, but the short abdomen and long leg articles agree more with *C. tenera*, although coxa 1 is longer but femur and tibia 1 are somewhat shorter.

Principal component analyses (PCA)

In a PCA using the proportions of the leg segments relative to the trunk (Fig. 2), factor axis 1 represents 46.56 % of the total variation. It was primarily determined by the variables tibia 2 and tarsus and with almost equal proportions by the variables proboscis, propodus, and first coxa (see [Online Resource 3](#) for factor loadings and eigenvalues). The terminal claw had the smallest loading for factor 1. Factor axis 2, representing 21.14 % of the total variance, was primarily determined by the variables terminal claw, femur and tibia 1. Together, factors 1 and 2 explain 67.7 % of the total variance. Factor 3 adds an additional 15.35 % (based almost completely on coxae 2 and 3) but brings no further resolution to the data. The PCA based on factors 1 and 2 shows a clear distinction between the *C. megalonyx* specimens and all other specimens. The *C. tenera* specimen from Mexico and

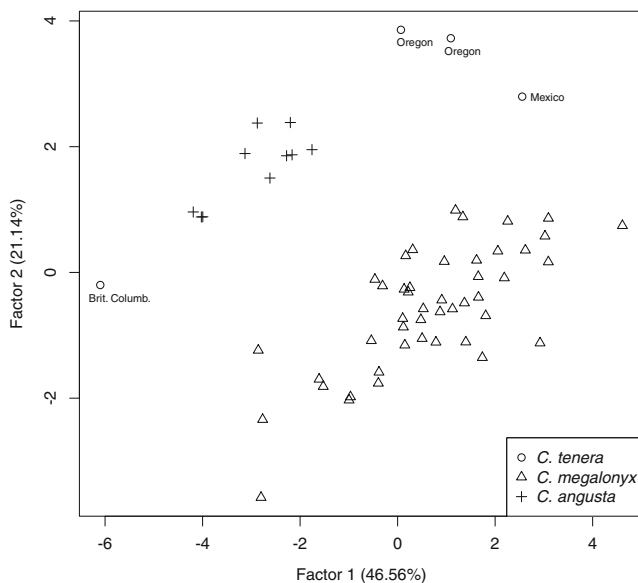


Fig. 2 Results of the principal component analyses for specimens of *Colossendeis tenera*, *C. angusta*, and *C. megalonyx*. PCA scores for the first two factors with the highest eigenvalues are plotted on both axes. Analysis was based on the different leg segments and the proboscis length relative to the length of the trunk. See [Online Resource 3](#) for further details

the paratypes cluster together in the upper right sector of the PCA, whereas the specimen from British Columbia falls within the opposite sector, with no similarities to the other specimens analyzed. It fits closer to the *C. angusta* specimens, however, which all form one dense group in the plot.

SEM analyses of ovigera *C. tenera* and *C. megalonyx*

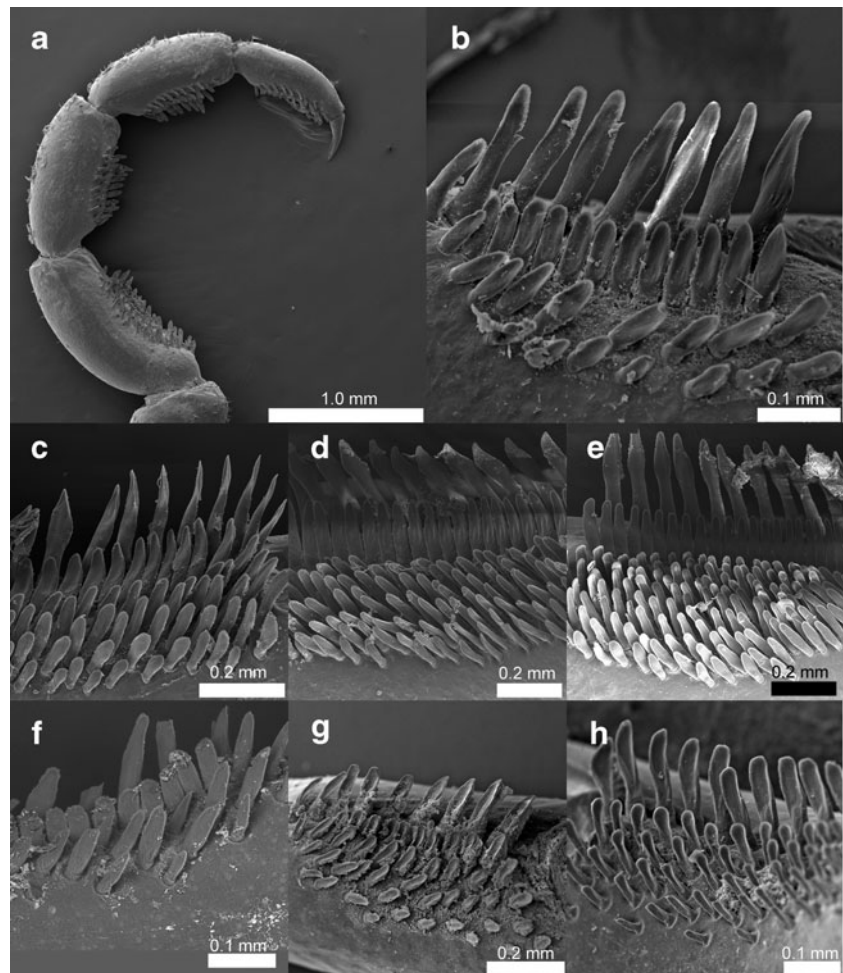
Analyses of the SEM pictures showed that the ovigeral spine configuration of *C. tenera* (Fig. 3a,b,f) is broadly similar to *C. megalonyx* (Fig. 3c-e) in having one row of long spines, one row of medium spines, and several rows of short spines, which are shaped rather similarly in both species. Our SEM data of *C. megalonyx* also agree well with those of Cano and López González (2007). However, even though ovigera from different *C. megalonyx* clades show consistent differences (Spaak et al. unpublished data), they are much more similar to each other than to those of *C. tenera*. In the examined specimens of *C. megalonyx*, the short spines of the strigilis are very densely placed in three to nine rows, which are not clearly segregated from each other, while those of *C. tenera* are much less numerous and are placed in only two clearly segregated rows in the Mexican specimen (Fig. 3a,b) or in about two irregular rows in the British Columbia specimen (Fig. 3f). As far as we could discern the morphology of the Washington and Oregon specimens from our light-microscopic observations, they are more similar to the British Columbia specimen. The original description of *C. megalonyx* by Hoek (1881) shows only two to three rows of short spines, but the spines are densely spaced, unlike in *C. tenera*.

SEM analyses of ovigera *C. tenera* and *C. angusta*

The ovigeral spine fields of North Atlantic *C. angusta* (Fig. 3g, h) specimens are rather different from those of both *C. tenera* (Fig. 3b,f) and *C. megalonyx* (Fig. 3c-e). In the Norwegian Sea specimens, the spines of the medium row are much less closely spaced than in the other species, and the short spines are also rather sparse and placed in about three to four irregular rows, with spines gradually getting smaller endally (Fig. 3g). In contrast to other specimens we examined, the spines are oriented orthogonally to the length of the oviger article. Medium and short spines are rather similar, but can still be clearly distinguished by their orientation and the arrangement of medium spines in one regular row. In the specimens from the Icelandic coast, there is no clear distinction between medium and short spines. The spines are rather sparse, become smaller endally, are oriented regularly, and placed into four to five irregular rows (Fig. 3h).

The ovigera of *C. gracilis* figured by Hoek (1881) agree much better with *C. tenera*, having a clearly distinguished

Fig. 3 Scanning electron microscopic pictures of ovigeral spine fields in *Colossendeis tenera* from Mexico (**a,b**), of different *Colossendeis megalonyx* specimens (**c–e**), of *Colossendeis tenera* from British Columbia (**f**), and of *Colossendeis angusta* from Iceland (**g,h**)



row of densely placed medium spines and one or two irregular rows of sparsely placed short spines.

Molecular phylogenetic analyses

For the CO1 data set, the model HKY+I+G was specified by MrModeltest, while GTR+I+G was specified for the multi-gene data set (Fig. 4).

The results of the both ML and Bayesian analyses clearly showed that *C. tenera* is not a member of the *C. megalonyx* complex, agreeing with the morphological data presented in this article. Monophyly of the *megalonyx* complex excluding *C. tenera* was highly supported in all analyses. A clade consisting of *C. tenera* and the morphologically similar *C. angusta* was strongly supported in the analyses of the CO1 data set (BS = 95, PP = 1). Remarkably, *C. tenera* itself was not resolved as monophyletic, and the sequence from British Columbia grouped closer to *C. angusta* than the one from Mexico but with poor support (BS = 75, PP = 0.69). While the position of the *C. tenera/angusta* clade was poorly resolved in all analyses, it did not group as

sister to the *C. megalonyx* complex in any analyses. CO1 and multi-gene analyses also generally agreed on the phylogeny of Colossendeidae, including the grouping of the ten-legged *Decolopoda* within the genus *Colossendeis*, as previously found by Krabbe et al. (2010). The H3 sequences of *C. megalonyx* generally confirmed the phylogeny resulting from CO1 sequences (Krabbe et al. 2010), although they did not distinguish the sister clades A/F, B/C, and D/E.

The genetic distances between the two *C. tenera* specimens whose CO1 sequences were included in this analysis were remarkably high (9 % uncorrected distance), possibly indicating that *C. tenera* is itself part of a species complex consisting of at least two species, which would also include *C. angusta* and possibly other species. We also found clear differentiation within *C. angusta*, with the specimens from near the Icelandic coast forming one closely related group with ca. 3 % genetic distance to another group consisting of the Norwegian Sea specimens and one of the two from Newfoundland. Both groups appeared to be genetically mostly homogenous, with individual sequences differing

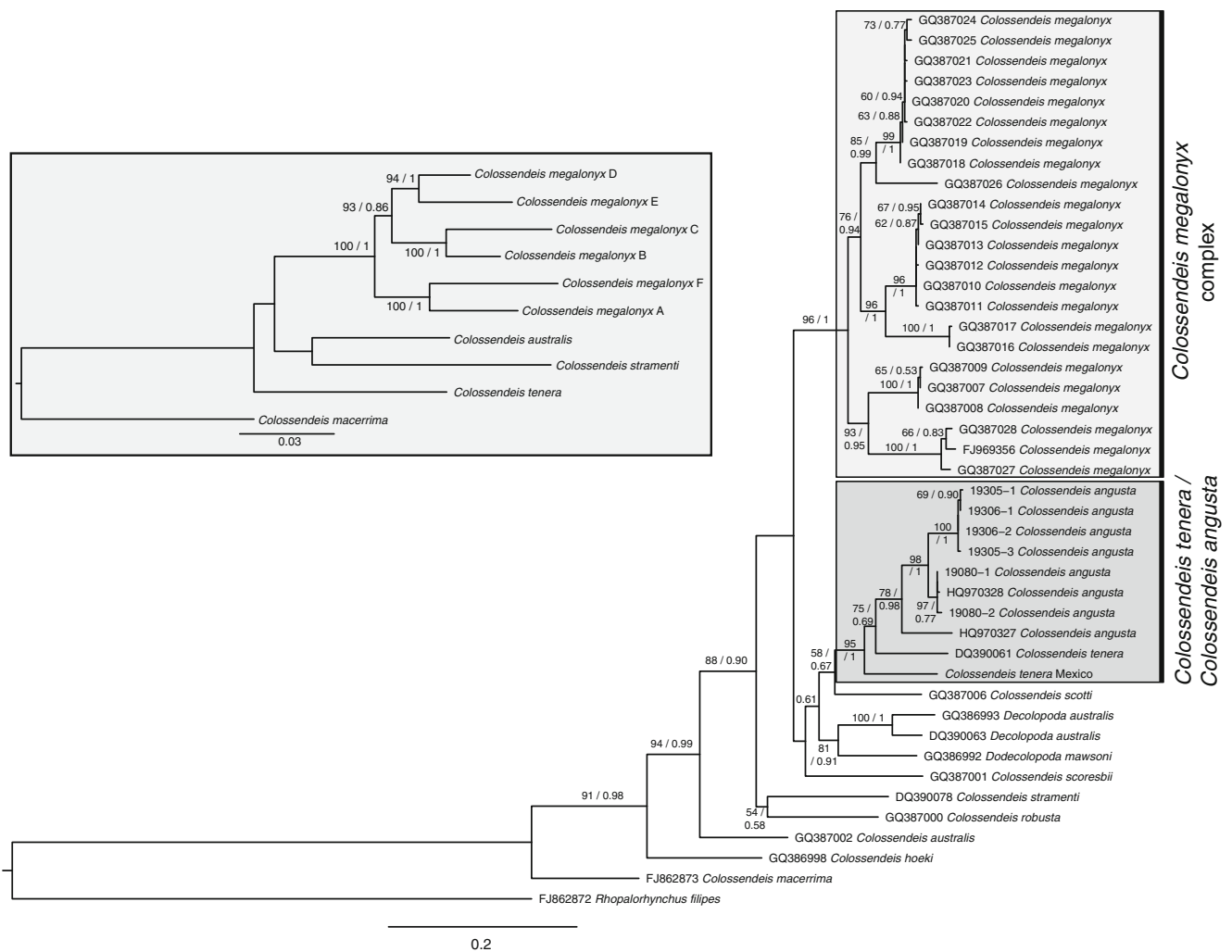


Fig. 4 Phylogenetic tree 1: Large tree: Phylogenetic tree based on maximum likelihood analysis of 545 bases of the CO1 gene of 44 specimens of Colossendeidae. ML bootstrap and Bayesian posterior probability support (if >50/0.5) are drawn on branches. Small tree:

Phylogenetic tree based on maximum likelihood analysis of 323 bases of H3 and 560 bases of CO1 of 10 representative specimens of Colossendeidae. ML bootstrap and Bayesian posterior probability support (if >50/0.5) are drawn on branches

only by single nucleotide substitutions. The other specimen from Newfoundland was placed basal to both groups, with about 5.7–7 % genetic distance to them.

Discussion

Morphology and DNA separate *C. tenera* from *C. megalonyx*

Data from both morphology and gene markers unequivocally support the view that *C. tenera* and *C. megalonyx* are distinct taxa and reject the hypothesis that *C. tenera* is a subspecies of *C. megalonyx* as suggested by Turpaeva (1975). They also provide further evidence for the distinction of the *C. megalonyx* clades identified by Krabbe et al. (2010), about which more detailed work is

in preparation. However, the analyzed data raise further interesting questions on the validity and distribution of *C. tenera*.

The records described by Turpaeva (1975) extending the distribution of *C. tenera* have not been considered by other authors who discussed the species (Child 1994, 1995). However, it is interesting to note that *C. tenera* is otherwise not known from Japanese waters (Hedgpeth 1949; Nakamura and Child 1983; Nakamura and Child 1991; Y. Takahashi, personal communication), suggesting that the single specimen reported by Turpaeva (1975), if correctly identified, does not represent a regular occurrence of the species.

Turpaeva (1996) mentions differences in the length ratio of the terminal claw to the propodus, with specimens from deeper waters having proportionally longer claws. Also, the abdomen

of deep-sea specimens was found to be longer than in shallow-water specimens. We cannot confirm this, as the specimens examined by us had long claws and short abdomina irrespective of the depth in which they were found. The Peruvian specimen had the eye tubercle situated further behind than the North Pacific specimens (Turpaeva 1975). The specimens we examined had a much lower ocular tubercle than described by Hedgpeth (1943), Turpaeva (1975), and Child (1994, 1995), but agreed with the description of Hilton (1943) that it is not pointed. The British Columbia specimen sequenced by Arango and Wheeler (2007) also had front eyes much larger than back ones and a relatively shorter proboscis, i.e., about the same length as the trunk (as described by Child 1994), as well as much shorter legs. These differing characters led to the separation of the British Columbia *C. tenera* from the others in the PCA. As our genetic study has revealed the presence of probable unrecognized lineages within *C. tenera*, these characters may distinguish several distinct geographically and/or bathymetrically segregated clades when more specimens are available. The claw and abdomen characters were interpreted by Turpaeva (1996) as a result of pedomorphosis, which was hypothesized to be part of a supposed general trend of deep-sea pycnogonid forms developing pedomorphic characters. The relatively longer legs and lower number of ovigeral spines of *C. tenera* compared to *C. megalonyx* were also attributed to pedomorphism. Our own results cannot confirm a correlation between a deep-sea habitat and decreasing number of ovigeral spine rows, as the *C. megalonyx* clades known from the deepest waters (C and E) do not have the lowest numbers of ovigeral spine rows (in fact, E has the highest number). Also, the total leg length of *C. tenera* as a proportion of the body length does not fall outside the range of variation for *C. megalonyx* (except in the British Columbia specimen, in which the legs are in fact shorter), but the proportions of the leg articles are different. Although we generally appreciate that pedomorphic traits can evolve under certain conditions (see Diz et al. 2012 for an example), we do not regard this as a reasonable explanation here.

C. tenera and *C. angusta*

Our molecular results indicate that *C. tenera* is closer to *C. angusta* than to *C. megalonyx*. The two species were already compared by Hedgpeth (1943), and *C. tenera* was claimed to differ from *C. angusta* by its longer proboscis, a higher ocular tubercle, and the presence of eyes. Our own measurements also showed that the proboscis is shorter in North Atlantic and West African *C. angusta* than in *C. tenera* (except for the British Columbia specimen), and this is also true for Hoek's (1881) original description of *C. gracilis* (relative length 1.08–1.11). However, the relative proboscis lengths of *C. tenera* and *C. angusta* seem to overlap considerably (see Results for *C. tenera*). Relative proboscis

lengths of 1.38 (Fage 1956) and even 1.72 (Minnaard and Zamponi 1984) have been recorded for *C. angusta/gracilis* specimens. The ocular tubercle is variable within *C. angusta* (Bamber and Thurston 1995), but the original description (Sars 1877, 1891) mentions a very high tubercle similar to Hedgpeth's (1943) illustration of *C. tenera*. However, we found that our *C. tenera* specimens had a much lower, rounded tubercle, apparently not broken off. Our *C. angusta* specimens from the Norwegian Sea show a high, pointed ocular tubercle, similar to the original description, while in those from near the Icelandic coast the tubercle is much lower and pointed, and in those from West Africa it is low and rounded. This difference within North Atlantic *C. angusta* is consistent with the genetic data showing differentiation between the Norwegian Sea and Iceland specimens. The extremely short eighth palp article and long claws, which are characteristic of *C. tenera* among the *Colossendeis* species from the North American Pacific coast (Child 1995), were present in all our specimens of both *C. tenera* and *C. angusta*. On the other hand, *C. angusta/gracilis* adults are known for their ninth palp articles inserted anaxially on the eighth ones (e.g., Hoek 1881; Sars 1891), which was also observed in the specimens we examined. This palp configuration is not present in *C. tenera*, and apparently not present in juveniles of *C. angusta* either (Meinert 1899).

Unlike other *Colossendeis* species from the same region, *C. tenera* is mostly found with its legs raised vertically above the trunk (Child 1994). All specimens of *C. angusta* examined by us also show this condition, which has not to our knowledge been described in the literature for that species. It is also present in other *Colossendeis* species such as *C. macerrima* Wilson, 1881, but only rarely in *C. megalonyx* (Dietz, personal observation). This is a typical position adopted by pycnogonids while swimming, which has been recorded in diverse taxonomic groups (e.g., Clark and Carpenter 1977), but it could also be a post-mortem artifact.

The presence of well-developed eyes is another difference between *C. angusta* and *C. tenera*, although Gordon (1944) mentions small eyes present in *C. gracilis*, a possible synonym of *C. angusta* (Fry and Hedgpeth 1969). Minnaard and Zamponi (1984) also reported the presence of eyes in *C. gracilis*. We examined *C. angusta* specimens from the North Atlantic and West Africa, and eyes appeared to be absent or at least vestigial in all of them. Another character that differs between *C. tenera* and most specimens of both *C. angusta/gracilis* and *C. megalonyx* examined and described in the literature is the very short abdomen in *C. tenera*, which however agrees with the measurements reported by Minnaard and Zamponi (1984) for *C. angusta/gracilis* from unspecified locations. Turpaeva (1996) reported that in both *C. angusta* and *C. tenera* the abdomen length is variable,

with specimens from deeper waters having longer abdomina. Additionally, *C. angusta/gracilis* specimens retaining fully developed chelifores have often been described (e.g., Hoek 1881; Meinert 1899; Fage 1956; Turpaeva 1996, one of the West African specimens examined by us), but this is not known for *C. tenera*.

The position of *C. tenera* relative to *C. angusta* is complicated by the status of the latter as a very widespread and variable deep-water species of the Northern Hemisphere and its possible synonymy with its Southern Hemisphere counterpart *C. gracilis*. According to Stock (1963), *C. gracilis* differs from *C. angusta* by the presence of a highly conical ocular tubercle (variable in North Atlantic *C. angusta* according to our own observations and Bamber and Thurston (1995), and actually present in the type material of *C. angusta* according to Sars (1891); also variable in specimens currently attributed to *C. tenera*), shortness of the three distal palp articles, small body size, and slender legs, characters that seem to fit *C. tenera*. In the *C. angusta* we examined, all characters mentioned by Stock (1963), except for the ocular tubercle of some specimens, agreed with *C. gracilis*. Also within *C. angusta* s. str., noticeable differences exist between different geographical regions (Appellöf 1912), as our own observations of the ocular tubercle also indicated. According to Bamber and Thurston (1995), the variability within specimens attributed to *C. angusta/gracilis* is so complicated that it is currently impossible to clearly distinguish multiple species. We consider it would be rather improbable that all records assigned to *C. angusta/gracilis* in the taxonomic literature can be classified as a single species, as this would mean that *C. angusta* has a worldwide distribution from the Arctic to the Southern Ocean, which is a very rare distribution pattern for marine benthic organisms (but see, e.g., Pawlowski et al. 2007).

The ovigeral spine configuration would seem to contradict the hypothesis that *C. tenera* (Fig. 3b, f) is closely related to *C. angusta* (Fig. 3g,h), as it shares more characteristics with *C. megalonyx* (Fig. 3c-e). While our *C. megalonyx* specimens showed a higher number of spine rows than *C. tenera*, the original descriptions of *C. megalonyx* (Hoek 1881), *C. rugosa* (Hodgson 1907), and *C. m. arundorostri* (Fry and Hedgpeth 1969), as well as the Scotia Sea specimens described by Turpaeva (1975), indicated that there are also members of the *C. megalonyx* complex with a lower number of ovigeral spine rows than those sampled either by us or by Cano and López González (2007). However, it must be noted that the number of spine rows increases with ontogeny (Fry and Hedgpeth 1969). The ovigeral spines of the Mexican specimen of *C. tenera* (Fig. 3a,b) differed from those of the *C. tenera* specimens from further north (Fig. 3f) and from both *C. angusta* and *C. megalonyx* in that the small spines are placed regularly in two distinct rows, not

irregularly in two to three rows. The specimen from Peru pictured by Turpaeva (1975) agrees with the northern specimens in this. Schimkewitsch (1893) mentioned that the specimens attributed by him to *C. gracilis* from the Central and South American Pacific coast had three irregular rows of small spines instead of two in Hoek's (1881) specimens, which agrees with *C. tenera*, but also with the *C. angusta* specimens examined by us. While the spine configuration of *C. angusta*, especially the Iceland specimens, seems strikingly different from that of *C. tenera* in the lack of a distinct row of closely spaced medium spines, such a row also occurs in a number of other *Colossendeis* species, such as *C. australis* or *C. scotti*. It is also clearly present in Hoek's (1881) illustration of *C. gracilis* from the Southern Indian Ocean. The differing morphology of North Atlantic *C. angusta* should therefore probably be considered autapomorphic and therefore not evidence for a closer relationship between *C. tenera* and *C. megalonyx*.

Schimkewitsch (1893) described a variety of *C. gracilis* from the Mexican Pacific coast that he named *C. gracilis* var. *pallida*. This was distinguished from the typical form of the species by its longer proboscis, a very high ocular tubercle with unpigmented eyes, a pale yellow color, more developed setae on the distal palp articles, and shorter ovigera. The first two of these characters are the same that Hedgpeth (1943) used to distinguish *C. tenera* from *C. angusta*. The color is the same as in the specimens from British Columbia, Oregon, and Mexico examined by us, but not the specimen from Washington, which is dark red. However, it also agrees with the North Atlantic *C. angusta* specimens. The West African *C. angusta* we examined show a much more intense yellow color. Sars (1891) and Möbius (1902) mention that living specimens of North Atlantic *C. angusta* have an intense brick red color, which however vanishes in ethanol. According to Wilson (1881) the color among US East Coast specimens varies "from straw yellow to nearly white." The setae on the palps are also mentioned and figured in Hedgpeth's (1943) description of *C. tenera*. This similarity indicates that *C. gracilis* var. *pallida* might be a senior synonym of *C. tenera*. The Mexican *C. tenera* specimen sequenced here falls within the distribution range of *C. gracilis* var. *pallida* as given by Schimkewitsch (1893). However, Schimkewitsch (1893) did not illustrate the specimens except for the distal palp articles or report any detailed measurements, and without examining the relevant specimens this suggestion cannot be tested.

The apparent non-monophyly of *C. tenera* with respect to *C. angusta* specimens from the North Atlantic, the strong genetic differentiation within *C. angusta*, and the morphological diversity observed within both species raises the question whether any distinction can be drawn between the two species, or whether they are both part of a species complex, which is in need of a profound and thorough

traditional taxonomic review. Examination of more material from different geographical areas with both morphological and molecular methods would be necessary to test this hypothesis and investigate the geographical and bathymetric distribution of possible undescribed species.

In conclusion, our results support the hypothesis that *C. tenera* and *C. megalonyx* are two morphologically and molecularly distinct species complexes. The two sequences of *C. tenera*, however, are very different from each other and form a monophyletic group together with *C. angusta*, which is also similar morphologically. Within both nominal species there appears to be a large amount of morphological variability. Hence, future studies with more material will need to analyze *C. tenera*, *C. angusta*, and related species in more detail.

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Ethical standards The experiments comply with the current laws of the country in which they were performed.

Conflict of interest The authors declare that they have no conflict of interest.

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