POLISH POLAR RESEARCH
vol. 35, no. 2, pp. 225–242, 2014 doi: 10.2478/popore-2014-0015

# A multi-gene analysis reveals multiple highly divergent lineages of the isopod *Chelator insignis* (Hansen, 1916) south of Iceland

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**Abstract**: The eurybathic isopod species *Chelator insignis* shows a wide distribution south of Iceland. We analysed 51 specimens from shelf (213–305 m depth), slope (885–891 m and 1380–1390 m depth) and deep-sea habitats (2750 m) south of Iceland with different DNA markers. A fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) was studied for 47 specimens, 16S was studied for 36 specimens, and a fragment for the 18S rRNA gene could be amplified for 11 specimens. For the COI data, specimens clustered into five distinct lineages each separated by ≥20% uncorrected pairwise distances. Both the mitochondrial 16S and the nuclear 18S sequence data further support this deep divergence, suggesting the presence of overlooked species inside the nominal *C. insignis*. Populations on the shelf occurring east and west of the Reykjanes Ridge were genetically identical suggesting that this ridge is not a barrier to gene flow. However, populations from different depth ranges differed substantially. Our multi-gene analysis suggests that the newly found species likely have more narrow vertical distribution ranges and highlights a possible role of bathymetry in speciation processes.

Key words: Icelandic waters, Desmosomatidae, distribution, phylogeography, genetic diversity, DNA barcoding.

## Introduction

Iceland is located on top of the Greenland-Iceland-Scotland Ridge (GIS Ridge), which separates Nordic seas from the north Atlantic Ocean. The deepest passage is the Faroe Channel with a water depth of 840 m (Hansen and Østerhus

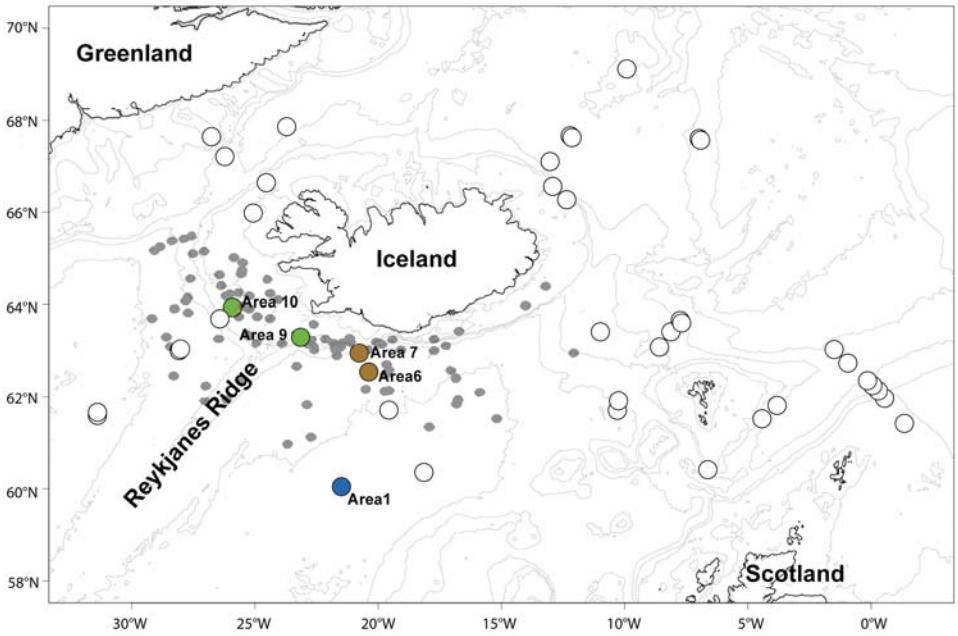


Fig. 1. Map of the sampling area showing the distribution of *Chelator insignis* south of Iceland according to Brix and Svavarsson (2010, grey dots) and at the EBS stations sampled during IceAGE1 and 2 (white dots) without any finding of *Chelator* in the subsamples and the EBS samples sorted so far. The labeling of areas (1, 6, 7, 9–10) indicates that *Chelator* specimens were available for molecular analyses (compare Table 1 for station and specimen information). The colours of the dots are according to the colour scheme of the clades in Figs 2–4: green, Reykjanes Ridge shelf clade; brown, slope clades; blue: deep sea.

2000). The GIS Ridge emerged 57–16 million years ago (Larsen 1983) and due to the lack of deep passages (except the Faroe Channel), it has been hypothesized to be an effective barrier to dispersal for the abyssal fauna and thus actively shaping the distribution of benthic species (compare Brix and Svavarsson 2010; Schnurr *et al.* 2014). Furthermore, the marine topography strongly influences the oceanographic conditions around Iceland (Stefánsson 1962; Hansen and Østerhus 2000; Malmberg and Valdimarsson 2003). While the Nordic seas are generally cold (bottom temperatures <0°C), the bottom waters to the south of the GIS Ridge are generally warmer (temperatures most often >2°C; Hansen and Østerhus 2000; see also Brix and Svavarsson 2010, fig. 2; Meißner *et al.* 2013, fig. 2; Ostmann *et al.* 2014). South of Iceland, the Reykjanes Ridge separates the eastern deep areas of the Iceland Basin and the western deep areas of the Irminger Basin (Fig. 1).

In abyssal habitats throughout the world's oceans, asellote isopods represent one of the most abundant crustacean taxa (Hessler and Sanders 1967; Hessler 1970; Brandt 1993; Wilson 2008). They are a particularly well-studied and important faunal element of the Nordic Seas, the Arctic Ocean and the North Atlantic Ocean (Sars 1897, 1899; Hansen 1916; Svavarsson 1982, 1984, 1988, 1997;

Svavarsson *et al.* 1990, 1993; Brix and Svavarsson 2010; Meißner *et al.* 2013; Schnurr *et al.* 2014). Approximately 70 described isopod species occur around Iceland (*e.g.*, Sars 1864, 1868, 1897, 1899; Stephensen 1915; Hansen 1916; Paul and George 1975; Siebenaller and Hessler 1977; Just 1980; Brandt 1993; Negoescu and Svavarsson 1997; Stransky and Svavarsson 2006; Svavarsson 1982, 1984, 1988; Brix and Svavarsson 2010, 1981; Schnurr *et al.* 2014). The most extensive information about isopods at the GIS Ridge was gathered during the Danish *Ingolf* Expedition in 1895 and 1896 (Hansen 1916).

Desmosomatidae Sars, 1897 (Isopoda, Asellota) are small (typically 2–5 mm) animals dwelling in the uppermost layer of the sediment (Hult 1941; Hessler and Strömberg 1989). Mature males, who swim better than females, may enter the water column in search for females. Desmosomatid shelf species are hypothesized to have evolved from deep-sea taxa (Brandt 1991). Shelf species of the Northern Seas, for example, are probably derived from deep-sea ancestors of the North Atlantic Ocean (Svavarsson *et al.* 1993). Around Iceland 20 desmosomatid species are known to occur with different distribution patterns (Brix and Svavarsson 2010).

South of Iceland *Chelator insignis* (Hansen, 1916) is the most common desmosomatid species, found at 94 stations studied during the BIOICE project (*Benthic Invertebrates of Icelandic Waters*) in 1991–2004 (43% of the stations analyzed; in all 6100 specimens; Brix and Svavarsson 2010; see Brix *et al.* 2014). This species has a wide geographical distribution, spanning at least over 4000 km in the North Atlantic Ocean. *Chelator insignis* has not been reported from Greenland, despite numerous studies (*e.g.*, Stransky and Svavarsson 2010). The type locality is in the Davis Strait (Hansen 1916) and Hessler (1970a) added findings from the Gay-Head Bermuda Transect. While redescribing *C. insignis*, Hessler (1970a) described two other deep-sea species of *Chelator*: *C. verecundus* Hessler, 1970 and *C. vulgaris* Hessler, 1970. Brix and Svavarsson (2010) recorded *Chelator insignis* south of Iceland restricted to three different water masses: Modified North Atlantic Water (MNAW); Labrador Sea Water (LSW); Iceland Sea Overflow Water (ISOW) (Brix and Svavarsson 2010: Fig. 2). The known depth range of this species is extensive, spanning from 136 to 2537 m (shelf to abyss) according to Brix and Svavarsson (2010). The deepest record in the present study is at 2750 m water depth.

*Chelator insignis* is frequently reported from shallower depths than the sill depth of the GIS Ridge in the BIOICE and IceAGE samples (about 45 localities at 620 m or less). Thus, the bathymetric range of the species would allow crossing the GIS Ridge. While *C. insignis* does not pass the channels of the GIS Ridge (Brix and Svavarsson 2010), it is distributed on both sides of the Reykjanes Ridge (Fig. 1), which separates the Iceland and the Irminger Basins. Brix and Svavarsson (2010) concluded that most desmosomatid and nannoniscid isopod species are restricted to water masses of a certain temperature and salinity, in the case of *C. insignis* three water masses (see above). Schnurr *et al.* (2014) observed no specific pattern in the distribution of munnopsid isopods with high swimming abilities. In

contrast to desmosomatids, munnopsids may be able to disperse actively as already suggested by Stransky and Svavarsson (2010).

The aims of this study were to use genetic information from both, nuclear and mitochondrial markers to test whether (1) there is evidence for unrecognized highly divergent lineages that may represent a cryptic species inside nominal *C. insignis*, (2) whether gene flow is restricted between populations to the east and west of the Reykjanes Ridge, and (3) whether there is a significant partitioning of genetic variation related to bathymetry.

## Material and methods

Samples were taken during IceAGE expeditions 1 and 2 (Fig. 1) with R/V *Meteor* (M85/3) and R/V *Poseidon* (POS456) in 2011 and 2013 using two types of epibenthic sleds (EBS, Rothlisberg and Pearcy 1977; Brenke 2005) and a large box corer. All samples were fixed in precooled (-20°C) 96% undenatured ethanol and treated as described in Riehl *et al.* (2014). The specimens from the EBS were sorted on board from subsamples. Additional specimens were sorted from subsamples of the upper one of the two nets (supra net) of the Brenke sled and from the box corer at the German Centre for Marine Biodiversity Research (DZMB) in Hamburg in 2013. The sorting process of the complete EBS samples from both expeditions is still in progress, but all subsamples taken during IceAGE1 are completely sorted and all *Chelator* specimens found have been determined to species level. Besides 51 *C. insignis* specimens, 4 specimens of *C. vulgaris* were collected. All specimens were individually separated into vials, given a voucher identification number (voucher ID) and a DZMB number according to the local Access 2010 database as collection reference. Specimens were subsequently stored at 4°C at the DZMB Hamburg and the DNA extract is stored at the Smithsonian Institution at -80°C.

As outlined in Brix *et al.* (2011), immediately after sample sorting on board and at the DZMB, up to three posterior legs were removed and placed in a separate vial for DNA extraction and amplification. Protocols for PCR are presented in Brix *et al.* (2014) and Riehl *et al.* (2014). PCR was performed using primers HCO/LCO and dgHCO/dgLCO for COI (Folmer *et al.* 1994; Meyer *et al.* 2005), Sar/Sbr for 16S (Palumbi *et al.* 1991; Tsang *et al.* 2009) and 18A1neu/1800neu for 18S (Raupach *et al.* 2004). Specimens were then determined to species level using a Leica MZ12.5 stereo microscope. Sequences (Table 1: 4 specimens of *C. vulgaris* and 51 specimens of *C. insignis*) were obtained according to the protocols described in Riehl *et al.* (2014) during two research visits at the Smithsonian Institution in October 2011 and November 2013. We sequenced the nuclear ribosomal small subunit (18S, complete sequence), the mitochondrial large ribosomal subunit (16S, fragment) and the mitochondrial cytochrome c subunit 1 gene (COI). Sequences were assembled with Geneious v. 6.1.2 (Drummond *et al.* 2011). Assemblies were manually inspected for

discrepancies. Consensus sequences of 18S, 16S and COI were aligned using MAFFT v. 7.017 (Katoh *et al.* 2002) with the E-ins-I option. Individual contigs were cropped to equal lengths within the alignment resulting in alignments of 1383 bp for 18S, 374 bp for the 16S and 504 bp for the COI gene. Maximum likelihood phylogenetic trees were calculated using RAxML v. 7.2.8 (Stamatakis 2008) for individual gene alignments as well as for the concatenated data set that included all 18S and the corresponding 16S/COI sequences. The GTRCAT model was used and branch support was calculated with 1000 fast bootstraps. For the 3-gene data set we partitioned the alignment into three sections for individual parameter optimization. Trees were visualised using FigTree v. 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

## Results

Sequencing success varied for the three genes (Table 1, Figs 2–4). For 18S we could obtain 13 sequences (11 for *C. insignis* and 2 for *C. vulgaris*), for 16S – 38 se-

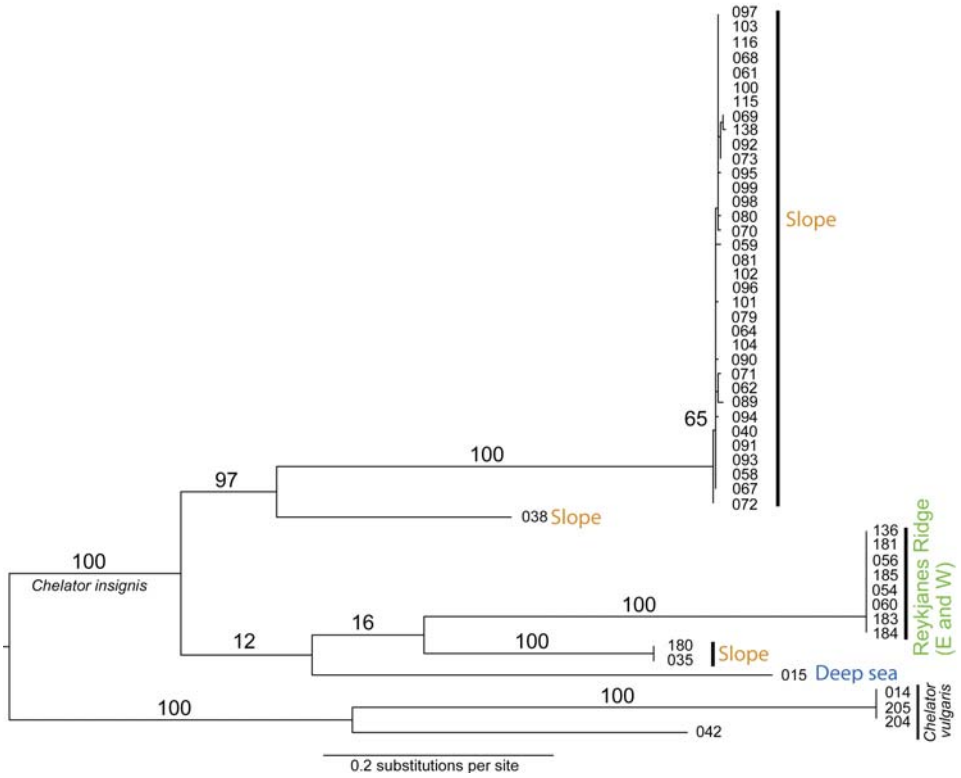


Fig. 2. Maximum likelihood phylogenetic tree for the 504 bp mitochondrial COI data set calculated with RAxML and the GTRCAT model of sequence evolution. Bootstrap support was calculated using 1000 fast bootstrap replicates. Labels indicate the vertical distribution ranges (shelf, slope, deep sea); numbers are related to the Voucher (IDesm).

Table 1

Specimens used for molecular analyses including information about working area, station, region, depth, gear, Voucher ID, GenBank accession number, DZMB number, species taxonomic information, latitude and longitude of the station where the specimens have been found.

| Area | Station | Region | Depth (m) | Gear | Voucher IDesm | DZMB HH no. | GenBank Accession No | Species | Latitude/ Longitude |
|---|---|---|---|---|---|---|---|---|---|
| 1 | #963 | Iceland Basin deep sea | 2749.4 | EBS | 204 | 34236 | COI:KJ937303 | *Chelator vulgaris* | 21°28.06'W/ 60°02.73'N |
| | | | | | 205 | 34237 | COI:KJ937305 | *Chelator vulgaris* | |
| | | | | | 042 | 19888 | COI:KJ710288 18S:KJ630819 | *Chelator vulgaris* | |
| 1 | #967 | Iceland Basin deep sea | 2750.4 | EBS | 014 | 19860 | COI:KJ710289 16S:KJ630813 18S:KJ630816 | *Chelator vulgaris* | 21°28.54'W/ 60°02.77'N |
| | | | | | 015 | 19861 | COI:KJ710302 16S:KJ937325 18S:KJ630817 | *Chelator insignis* | |
| 6 | #1003 | Iceland Basin slope | 1390 | GKG | 180 | 34212 | COI:KJ937306 18S:KJ630826 | *Chelator* cf. *insignis* | 20°21.18'W/ 62°33.50'N |
| 6 | #1006 | Iceland Basin slope | 1386.8 | EBS | 131 | 20174 | 16S:KJ937312 18S:KJ630824 | *Chelator insignis* | 23°23.33'W/ 62°33.05'N |
| | | | | | 039 | 19885 | 16S:KJ937311 | *Chelator insignis* | |
| 6 | #1010 | Iceland Basin slope | 1384.8 | EBS | 035 | 19881 | COI:KJ710278 16S:KJ630812 18S:KJ630818 | *Chelator* cf. *insignis* | 20°23.71'W/ 62°33.10'N |
| | | | | | 038 | 19884 | COI:KJ710294 16S:KJ630811 | *Chelator insignis* | |
| | | | | | 138 | 20181 | COI:KJ710292 18S:KJ630825 | *Chelator insignis* | |
| 7 | #1017 | Iceland Basin slope | 891.7 | EBS | 040 | 19886 | COI:KJ710280 16S:KJ937314 | *Chelator insignis* | 20°46.43'W/ 62°55.84'N |
| | | | | | 058 | 19904 | COI:KJ710306 16S:KJ630815 18S:KJ630820 | *Chelator insignis* | |
| | | | | | 059 | 19905 | COI:KJ578692/ KJ710310 16S:KJ937331 | *Chelator insignis* | |
| 7 | #1019 | Iceland Basin slope | 913.6 | EBS | 061 | 19907 | COI:KJ710309 | *Chelator insignis* | 20°44.61'W/ 62°56.32'N |
| | | | | | 062 | 19908 | COI:KJ710287 16S:KJ937320 | *Chelator insignis* | |
| | | | | | 064 | 19910 | COI:KJ710276 16S:KJ578669 18S:KJ630821 | *Chelator insignis* | |
| | | | | | 065 | 19911 | 16S:KJ937327 | *Chelator insignis* | |
| | | | | | 066 | 19912 | 16S:KJ937321 | *Chelator insignis* | |

Table 1 – *continued*.

| Area | Station | Region | Depth (m) | Gear | Voucher IDesm | DZMB HH no. | GenBank Accession No | Species | Latitude/ Longitude |
|---|---|---|---|---|---|---|---|---|---|
| 7 | #1019 | Iceland Basin slope | 913.6 | EBS | 067 | 19913 | COI:KJ710300 16S:KJ937324 | *Chelator insignis* | 020°44.61'W/ 62°56.32'N |
| | | | | | 068 | 19914 | COI:KJ710275 16S:KJ630814 | *Chelator insignis* | |
| | | | | | 069 | 19915 | COI:KJ710299 16S:KJ937323 | *Chelator insignis* | |
| | | | | | 070 | 19916 | COI:KJ710277 16S:KJ937310 | *Chelator insignis* | |
| | | | | | 071 | 19917 | COI:KJ578693/ KJ710296 16S: KJ578670 | *Chelator insignis* | |
| | | | | | 072 | 19918 | COI:KJ710286 | *Chelator insignis* | |
| | | | | | 073 | 19919 | COI:KJ710305 16S:KJ937328 | *Chelator insignis* | |
| | | | | | 074 | 19920 | 16S:KJ937319 | *Chelator insignis* | |
| | | | | | 079 | 19925 | COI:KJ710308 16S:KJ937330 | *Chelator insignis* | |
| | | | | | 080 | 19926 | COI:KJ710279 16S:KJ937313 | *Chelator insignis* | |
| | | | | | 081 | 19927 | COI:KJ710281 16S:KJ937315 | *Chelator insignis* | |
| | | | | | 089 | 20131 | COI:KJ710298 16S:KJ937322 | *Chelator insignis* | |
| | | | | | 090 | 20132 | COI:KJ710290 | *Chelator insignis* | |
| | | | | | 091 | 20133 | COI:KJ710293 | *Chelator insignis* | |
| | | | | | 092 | 20134 | COI:KJ710318 16S:KJ937336 | *Chelator insignis* | |
| | | | | | 093 | 20135 | COI:KJ710297 | *Chelator insignis* | |
| | | | | | 094 | 20136 | COI:KJ710315 | *Chelator insignis* | |
| | | | | | 095 | 20137 | COI:KJ710284 16S:KJ937317 18S:KJ630822 | *Chelator insignis* | |
| | | | | | 096 | 20138 | COI:KJ710313 | *Chelator insignis* | |
| | | | | | 097 | 20139 | COI:KJ710311 16S:KJ937332 | *Chelator insignis* | |
| | | | | | 098 | 20140 | COI:KJ710317 16S:KJ937335 | *Chelator insignis* | |
| | | | | | 099 | 20141 | COI:KJ710282 | *Chelator insignis* | |
| | | | | | 100 | 20142 | COI:KJ710285 16S:KJ937318 18S:KJ630823 | *Chelator insignis* | |

Table 1 – *continued.*

| Area | Station | Region | Depth (m) | Gear | Voucher IDesm | DZMB HH no. | GenBank Accession No | Species | Latitude/ Longitude |
|---|---|---|---|---|---|---|---|---|---|
| 7 | #1019 | Iceland Basin slope | 913.6 | EBS | 101 | 20143 | COI:KJ710301 | *Chelator insignis* | 020°44.61'W/ 62°56.32'N |
| | | | | | 102 | 20144 | COI:KJ710307 16S:KJ937329 | *Chelator insignis* | |
| | | | | | 103 | 20145 | COI:KJ710295 | *Chelator insignis* | |
| | | | | | 104 | 20146 | COI:KJ710303 16S:KJ937329 | *Chelator insignis* | |
| | | | | | 115 | 20157 | COI:KJ710312 16S:KJ937333 | *Chelator insignis* | |
| | | | | | 116 | 20158 | COI:KJ710314 16S:KJ937334 | *Chelator insignis* | |
| 9 | #1031 | Reykjanes Ridge west | 305.3 | GKG | 181 | 34213 | COI:KJ937309 18S:KJ630827 | *Chelator insignis* | 023°10.00'W/ 63°20.00'N |
| | | | | | 184 | 34216 | COI:KJ937304 | *Chelator insignis* | |
| | | | | | 185 | 34217 | COI:KJ937307 | *Chelator insignis* | |
| 9 | #1032 | Reykjanes Ridge west | 289.4 | EBS | 136 | 20179 | COI:KJ710283 16S:KJ937316 | *Chelator insignis* | 023°09.46'W/ 63°18.51'N |
| | | | | | 183 | 34215 | COI:KJ937308 18S:KJ630828 | *Chelator insignis* | |
| 9 | #1033 | Reykjanes Ridge east | 288.5 | EBS | 054 | 19900 | COI:KJ710304 16S:KJ630808 | *Chelator insignis* | 023°09.61'W/ 63°18.88'N |
| 10 | #1043 | Reykjanes Ridge east | 213.9 | EBS | 056 | 19902 | COI:KJ710291 16S:KJ630809 | *Chelator insignis* | 025°57.66'W/ 63°55.46'N |
| | | | | | 060 | 19906 | COI:KJ710316 16S:KJ630810 | *Chelator insignis* | |

quences (36 for *C. insignis* and 2 for *C. vulgaris*) and for COI – 51 sequences (47 for *C. insignis* and 4 for *C. vulgaris*). For several specimens PCR success was limited to one or two of the three markers, despite repeated trials and different primer combinations. We were only able to amplify and sequence all three markers for eight specimens. While three of four specimens assigned to *C. vulgaris* (IDesm204, IDesm 205, IDesm014) were genetically identical for the COI gene, one specimen of *C. vulgaris* (IDesm 042) was genetically very distinct from the other *C. vulgaris* specimens (24.8% uncorrected p-distance). However, the *C. vulgaris* sequences formed a well-supported clade (Bootstrap support of 100 for all three genes and in the concatenated data set, Figs 2–4) that was used as outgroup to root the trees.

Analyses of all three genes (Table 2, Figs 2–3) consistently supported the presence of multiple highly divergent lineages. Four distinct lineages were found for the 16S and the 18S data sets (Fig. 3). The COI data set also included a sequence from one specimen (IDesm038) from the slope that was highly divergent and made up a fifth lineage (Fig. 2). Divergences inside the different clades were low (0.0–0.8% for COI, 0.0–0.5% for 16S and 0.0–0.3% for 18S). Between the different *C. insignis*

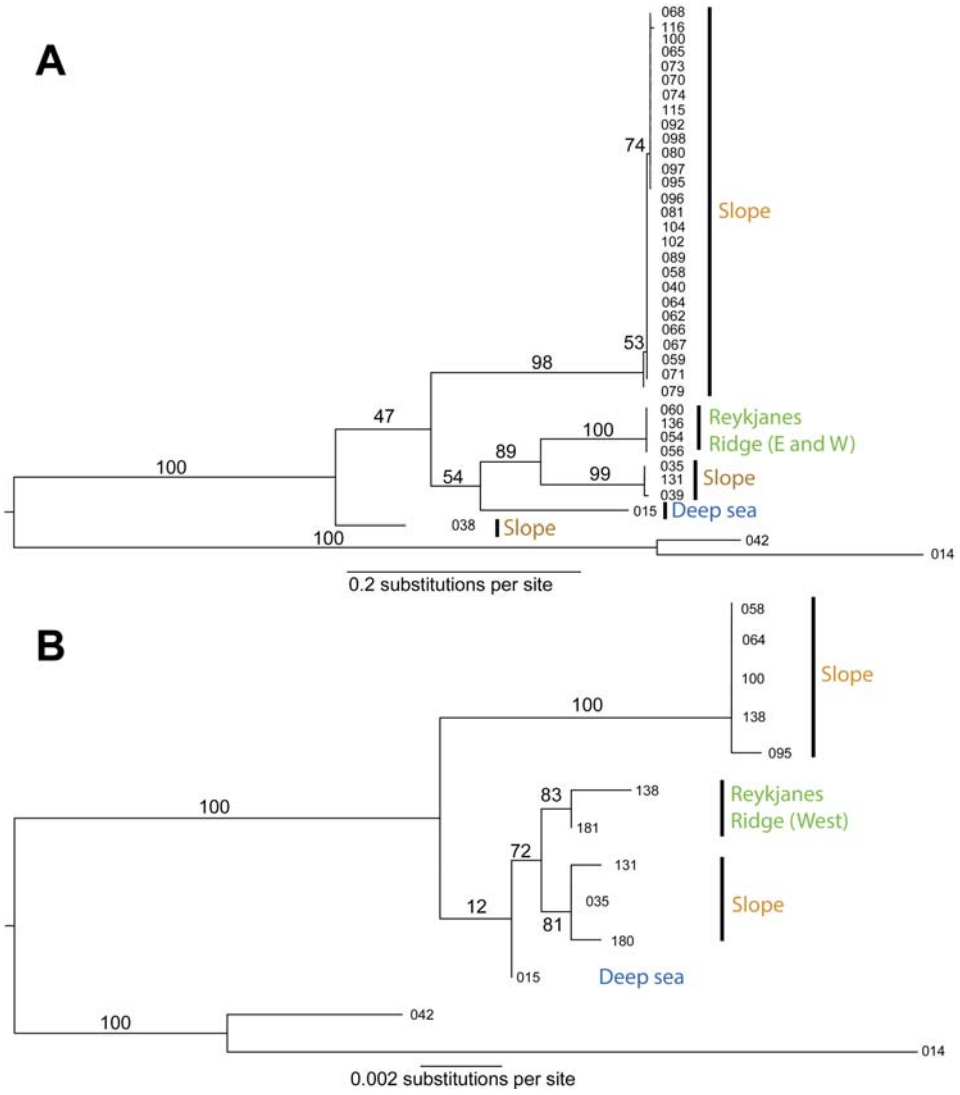


Fig. 3. **A**. Maximum likelihood tree for the 374 bp mitochondrial 16S. **B**. The 1383 bp nuclear 18S gene alignment. Bootstrap support was calculated with 1000 fast bootstrap with RAxML 7.2.8 and the GTRCAT model. Labels indicate the vertical distribution ranges (shelf, slope, deep sea); numbers are related to the Voucher (IDesm).

lineages, however, the divergences were high (≥20% for COI, >15% for 16S and 1–3% for 18S). Three genetically divergent clades were composed of at least two specimens while two lineages consisted of single specimens only (IDesm015 and IDesm038 for the two mitochondrial genes). Interestingly, IDesm015 is the only specimen of *C. insignis* found from deeper waters (2750 m). Bootstrap support for the divergent clades increased in the analysis of the concatenated data set (Fig. 4).

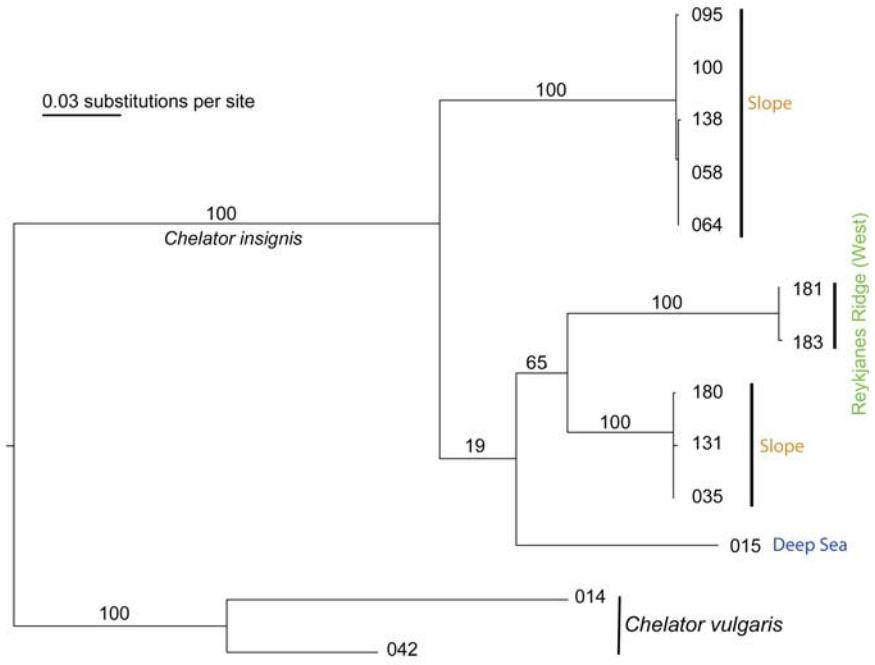


Fig. 4. Maximum likelihood tree for the concatenated gene alignment (2261 bp). Bootstrap support was calculated with 1000 fast bootstrap with RAxML 7.2.8 and the GTRCAT model. Independent partitions for the three genes were used for parameter inference. Labels indicate the vertical distribution ranges (shelf, slope, deep sea); numbers are related to the Voucher (IDesm).

The four highly divergent lineages found in all data sets are represented by different groups: (1) Shelf specimens from the Reykjanes Ridge from area 9 and 10, (2) slope specimens from area 6, (3) slope specimens from area 7, and (4) a single deep-sea specimen from area 1 (Table 2). In addition, for the 16S and the COI gene, the specimen of *C. insignis* (IDesm038) from the slope area 6 makes up an individual lineage (Figs 2–3).

The first slope clade consists of all specimens from area 7 and one specimen (IDesm138) from area 6 (Figs 2, 3). In COI, one specimen from slope area 6 (IDesm038) represents the sister group to the large slope clade (Fig. 2, bootstrap support of 97). This position, however, is not supported in the 16S data set (Fig. 3). All specimens from the shelf (area 10 west and area 9 east of the Reykjanes Ridge) form one well-supported group (Figs 2–4). No genetic variation was found in this clade for the 16S and COI gene and specimens IDesm181 and IDesm183 differed by only one substitution in the 18S gene (Fig. 3). Specimens IDesm180 (18S and COI), IDesm131 (only 16S and 18S), IDesm035 and IDesm039 (only 16S data) formed the last well-supported slope clade (bootstrap support of 100 for the concatenated data set and the COI gene, 99 for the 16S and 81 for the 18S gene, Figs 2–4). In this clade, specimens IDesm180 and IDesm035 could not be clearly assigned to *C.*

*insignis* based on morphology. Although the body shape (form of pereonite 5) hints to *C. insignis,* the setation and form of carpus and propodus of pereopod I resemble more *Chelator verecundus* Hessler, 1970. Therefore, IDesm180 and IDesm035 are indicated as "cf." (Table 1 and Figs 2–4). Table 2 summarizes the average uncorrected *p*-distances within and between the different stations.

Table 2

Average uncorrected pairwise genetic distances for the COI gene of *Chelator insignis* within and among stations. The depth and number of specimens per station (N) are indicated. Station numbers as in Table 1. Not for all stations with *C. insignis*, the COI gene could be amplified successfully. NA for the within station distance refers to values that are not available as only one specimen was found. Three boxes in the table indicate the deep-sea station, the four slope stations and the four shelf stations. All stations differed prominently from the deep-sea station. All shelf samples from both sides of the Reykjanes Ridge were genetically identical. Stations #1017 and #1019 from the slope were similar but different to the other two stations (#1010 and #1003). Colour code: white = average pairwise distances <1%; grey = >10%.

| Station | Depth [m] | N | # 967 | #1003 | #1010 | #1017 | #1019 | #1031 | #1032 | #1033 | #1043 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| # 967 | 2750 | 1 | NA | | | | | | | | |
| #1003 | 1390 | 1 | 22.4 | NA | | | | | | | |
| #1010 | 1385 | 3 | 23.3 | 14.9 | 22.0 | | | | | | |
| #1017 | 892 | 3 | 24.6 | 22.3 | 14.6 | 0.3 | | | | | |
| #1019 | 914 | 31 | 24.6 | 22.2 | 14.6 | 0.3 | 0.3 | | | | |
| #1031 | 305 | 3 | 22.2 | 20.0 | 22.6 | 23.9 | 24.1 | 0.0 | | | |
| #1032 | 289 | 2 | 22.2 | 20.0 | 22.6 | 23.9 | 24.1 | 0.0 | 0.0 | | |
| #1033 | 289 | 1 | 22.2 | 20.0 | 22.6 | 23.9 | 24.1 | 0.0 | 0.0 | NA | |
| #1043 | 214 | 2 | 22.2 | 20.0 | 22.6 | 23.9 | 24.1 | 0.0 | 0.0 | 0.0 | 0.0 |

## Discussion

Recent molecular studies on deep-water peracarids have shown that some of the widespread species with similar phenotype may consist of species flocks of morphologically similar but genetically distinct, *i.e.* cryptic species (*e.g.*, *Betamorpha fusiformis*, Raupach *et al.* 2007; *Eurythenes gryllus*, Havermans *et al.* 2013). Other species may show a uniform morphology and low intraspecific variation in genes despite a large bathymetrical and distributional range (*e.g.*, *Macrostylis roaldi*, Riehl and Kaiser 2012 and *Parvochelus russus,* Brix and Kihara 2014). Within *Chelator*, the specimens we examined exhibit a uniform morphology, but genetic divergence indicating the presence of overlooked or cryptic species (Brix *et al.* 2014: *C. aequabilis* Brix *et* Leese, 2014 and *C. rugosus* Brix *et* Riehl, 2014). Brix *et al.* (2014) found a *p*-distance of 10.4–15.1% in 16S and of 15.6–18.6% in COI between the *C. rugosus* and *C. aequabilis* haplotypes. They used *C. insignis* specimens of the present study from area 7 (all in the slope clade: IDesm059, IDesm064 and

IDesm071; see Figs 2–4), which showed uncorrected pairwise distances to *C. rugosus* of 26.8–27.5% for the COI gene and 22.9–25% for the 16S, and to *C. aequabilis* of 26.7–27.7% for COI and 22.9–25.6% for 16S (Brix *et al.* 2014).

DNA barcoding (Hebert *et al.* 2003) offers a promising approach for delimiting species. While Hebert *et al.* (2003) proposed a 3% threshold value it is obvious that delimitation is often difficult and different thresholds may exist. For example, Radulovici *et al.* (2009) found intraspecific divergences between 3.78–13.6%, but considered in particular the larger distances as evidence for cryptic species in amphipods. For asellote isopods only few studies are yet known that applied genetic distances for species delimitation. In case of the Haploniscidae, reported distances between morphospecies ranged from 9–20% sequence divergence (COI uncorrected *p*-distance, Brix *et al.* 2011) and from 25–28% between genera. The high between-group divergence was contrasted by intraspecific variability of below 1.8%. Comparable patterns were observed for Munnopsidae (Osborn 2009). In Macrostylidae, between-species distances of the 16S rRNA lay between 23–31% and were thus not smaller than inter-familiar distances while intraspecific diversity was close to zero (Riehl and Brandt, 2013).

For *Chelator insignis* (Figs 2–4), in-group variation within clades was low (0.0–0.8% for COI, 0.0–0.5% for 16S and 0.0–0.3% for 18S) and comparable to haploniscid species (Brix *et al.* 2011). Values between groups were high (≥20% for COI, >15% for 16S and 1–3% for 18S). The consistency of both, mitochondrial and the nuclear DNA markers, as well as the comparisons to known pairwise distances of other isopod species (Raupach *et al.* 2007, Osborn 2009, Brix *et al.* 2011) indicate that *C. insignis* consists of at least five highly divergent clades that we interpret as overlooked or cryptic species in the light of the published data. However, as long as these clades cannot be separated morphologically and are not described as separate species, species status remains provisional. A thorough morphological analysis, such as presented in Brix *et al.* (2014), has not been carried out for the *Chelator* specimens studied herein. It would be necessary to dissect and draw female and male representatives from all clades in detail as baseline for a morphological discussion. This is beyond the scope of the present study that focuses on the genetic polymorphisms.

In general, cryptic species are common in the deep sea and lead to the severe underestimation of true species diversity (Vrijenhoek 2009). While the detection of cryptic species is important for biodiversity studies, the processes that have lead to speciation are of primary interest to evolutionary biologists. Both, geographic distance and depth separating samples can influence genetic distances and thus population differentiation and speciation (Etter *et al.* 2005). The large genetic differences between specimens and populations from the shelf, the slope and the deeper waters in our study suggest that bathymetry and factors associated with depths may have had an important influence in speciation processes within this group. Similar findings have been made across different taxa (France and Kocher 1996; Rogers 2002;

Schüller 2011; Havermans *et al.* 2013). Explaining the distributional pattern of the ampeliscid amphipod fauna around Iceland, Dauvin *et al.* (2012) found bathymetry most important, but "the factor depth" encompasses other factors that change with depth, like total organic carbon within the sediment, availability of food (Altabet *et al.* 1991), hydrostatic pressure (Somero 1992) and dissolved oxygen concentration (Watling *et al.* 2013). Furthermore, for slope organisms, "the factor depth" parallels gradients of decreasing temperature and decreasing metabolic rates (Gage and Tyler 1991; Rogers 2002). Decreasing nutrient input might be the most important gradient as the rate of nutrient input from sinking phytodetritus decreases exponentially with depth (Etter *et al.* 2005). Those authors demonstrated also that bathyal species of gastropods showed more pronounced geographic variation than lower bathyal and abyssal species and stated that also genetic population structure in deep-sea gastropods appears to decrease with depth. For some peracarid crustaceans, like the amphipod *Eurythenes gryllus*, Havermans *et al.* (2013) showed that a clear distinction was observed between bathyal and abyssal samples with a genetic break occurring around 3000 m observing higher diversity in the abyss compared to the bathyal zone contrasting with the findings by Etter *et al.* (2005) for gastropods. Higher diversity in the abyss compared to the bathyal zone stands in contrast to the depth-differentiation hypothesis as reviewed in Rex and Etter (2010) as outlined in Havermans *et al.* (2013). Unfortunately, the data observed for *C. insignis* south of Iceland do not reach below 3000 m, and specimen numbers are too limited to explicitly test these hypotheses. However, in the case of *C. aequabilis* and *C. rugosus* (Brix *et al.* 2014) from the south Atlantic Ocean, high genetic distances in morphologically uniform species were observed at abyssal depths supporting the hypothesis of high diversity within peracarid crustaceans (amphipods and isopods) at abyssal depths. These two species even showed higher genetic distances in all three markers compared to the lineages found in *C. insignis* south of Iceland above 3000 m depth. Intraspecific as well as interspecific morphological variation seems to be generally low in *Chelator* (compare Brix *et al.* 2014) and the factors that distinguish species in this genus may be likely more related to differences in *e.g.* physiology of the species that are adapted to different depth ranges.

Unfortunately, we did not find specimens in areas 3 or 5 (Fig. 1). Sorted subsamples from these areas did not contain any *Chelator* specimen although the distribution of *C. insignis* (Brix and Svavarsson 2010, grey dots in Fig. 1) would hint at the presence of *C. insignis* in these areas. Possibly, further detailed sorting of available EBS material from these regions may uncover further yet overlooked material. Areas 3 and 7 cover the depth range between 2700 m and 1800 m that is not represented in our data at present. However, in the case of *C. insignis* it is obvious that there is no differentiation between populations on either side of the Reykjanes Ridge and apparently this ridge is not an efficient barrier for gene flow. This may partly be due to the topography of the ridge, as the Reykjanes Ridge is in many places more a seamount chain rather than a ridge. Between the seamounts,

frequently a soft bottom occurs, connecting the Labrador and the Iceland basins. As desmosomatid distribution is linked to the water masses around Iceland (Brix and Svavarsson 2010), we might hypothesize that the depth gradient in the stations of the present study (areas 1, 6, 7, 9 and 10) may correlate with different water masses. According to the CTD profiles from IceAGE1 (Ostmann *et al.* 2014; Sarah Schnurr, pers. comm.), all areas in the present study are in MNAW. Thus, the different clades cannot be explained by different water masses. The current system south of Iceland is, however, generally circling along the continental slope from East to West (see Hansen and Østerhus 2000) and MNAW is the most prominent water mass (see fig. 2 in Brix and Svavarsson 2010). Like the anthurid isopod *Astacilla boreaphilis* Stransky *et* Svavarsson, 2006, theoretically, *C. insignis* could pass the GIS Ridge and inhabit the north of Iceland, but like *A. boreaphilis* (Stransky and Svavarsson 2006), *C. insignis* is adapted to a temperature range fitting to the regime of North Atlantic water masses and seems to be not adapted to temperatures below 1°C. Even though our dataset contains over 50 specimens and is one of the largest datasets for deep-sea isopods, all genetic data sets of deep-sea isopods – including this one – have in common that they are still rather small and most likely do not cover the whole range of intraspecific variability. As a consequence, our interpretation of the patterns and processes need to be regarded with caution (Brix *et al.* 2014).

## Conclusion

Multilocus DNA marker analyses suggest the presence of overlooked or cryptic species inside the nominal *C. insignis*. This is indicated by high divergences (≥20% uncorrected *p*-distance for the COI gene) contrasted by low intraspecific variability (≤1%). There is no differentiation between specimens occurring to the east and west of the Reykjanes Ridge suggesting that this represents no barrier to gene flow. The different overlooked species seem to have rather limited vertical distribution ranges. Therefore our data suggest the existence of distinct species, identifying the investigated specimens as a complex comprising cryptic species with vertically restricted distribution. Given the limited sampling area and the number of specimens available, this study must be regarded as pioneer study aiming at describing the Icelandic marine biodiversity and understanding dispersal and colonisation processes of marine isopods. However, the results demonstrate clearly how little we know about the diversity, distribution and evolution of slope and deep-sea isopods.

**Acknowledgements**. — Captain Michael Schneider and the crew of R/V *Meteor* and Captain Klaus Ricke and the crew of R/V *Poseidon*, all scientists on board processing samples and standing up during the nights “to cuddle” samples are thanked for their valuable contribution. We also wish to thank all pickers and sorters on board providing fresh material and the ladies

from the Sorting Centre Sandgerði for their help in isopod determination, especially Sigrún Haraldsdóttir, and the technical assistance of the DZMB Hamburg for the sorting process at home. Karen Jeskulke was our right hand in producing the sequence data. Without her enthusiasm and all her efforts the quick production of sequences this study would not have been possible. Special thanks go out to Amy Driskell and Karen Osborn at the Smithsonian Institution making our research possible and supporting it in the framework of the *Barcoding deep-sea Isopoda* project. We also wish to thank the University of Iceland´s Research Centre in Suðurnes for kindly hosting people associated with this project during several workshops. Financial support for S.B. was provided by the German Science Foundation (DFG) under contracts BR 3843/2,3,4,5-1. F.L. was supported by DFG grant MA 3684/2-1.

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Received 20 February 2014
Accepted 2 June 2014