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Diversity and distribution of North Atlantic Lepechinellidae (Amphipoda: Crustacea)

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North Atlantic lepechinellid amphipod species were investigated using morphological and molecular techniques based on material collected during two IceAGE expeditions in 2011 and 2013 (**Ice**landic marine **A**nimals: **G**enetics and **E**cology). The presence of eyes is reported for the first time for the family Lepechinellidae. Four lepechinellid species, *Lepechinella grimi*, *L. helgii*, *L. skarphedini* and *Lepechinelloides karii* were distinct across morphological, *COI* and 16S data. *Lepechinella arctica*, *L. norvegica* and *L. victoriae* were seen to be morphologically similar. No morphological or molecular separation was observed between *L. arctica* and *L. norvegica*, indicating that these taxa should not be considered separate species. Full illustrations of habitus and mouthparts are presented for *L. arctica* and a new synonymy is provided recognizing *L. norvegica* as a junior synonym of *L. arctica*. While *L. victoriae* shows little morphological variation from *L. arctica*, *COI* and 16S data show this taxon as genetically distinct. Furthermore, new geographic and depth ranges for North Atlantic and Arctic lephechinellids are provided.

ADDITIONAL KEYWORDS: Arctic – evolutionary systematics – *Lepechinella* – ontogeny – synonymy.

INTRODUCTION

The deep waters around Iceland include boreal, subarctic and arctic zones that hold discrete bodies of water. The North Atlantic Gateway (Jöst *et al*., 2019) refers to the region around Iceland, which is between the North Atlantic and the Nordic Seas (Arctic Ocean). This region is characterized by strong temperature and productivity gradients, an extensive geographic barrier and considerable depth and latitudinal ranges. The Greenland–Iceland–Faeroe (GIF) Ridge is a significant seafloor feature of the Icelandic region. It extends up to a saddle depth of 840 m (Hansen & Østerhus, 2000) and separates the deep North Atlantic from the deep Nordic Seas.

The first study of benthic organisms in the North Atlantic Gateway was from the Danish Ingolf expeditions (Wandel, 1899). As part of a broader programme, which also examined the Faeroe Islands and Greenland, the Ingolf expeditions explored waters around Iceland in 1895 and again in 1896 for a period of four months each year. The Ingolf expeditions were the first to use fine-mesh sampling gear to separate the smaller benthic invertebrates from the sediments, which led to the discovery of many small, unknown peracarid species (Wandel, 1899). The results of two recent IceAGE (Icelandic marine Animals: Genetics and Ecology) expeditions have similarly shown peracarids and, particularly, amphipods as an abundant group of small benthic invertebrates in the bathyal depth of the North Atlantic Gateway region of Iceland (Brix *et al*., 2018). Samples collected

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during the IceAGE expeditions were processed using modern standardized cold-chain protocols, providing collections of small benthic invertebrates, which are suitable for both morphological and molecular studies.

Prior to this study, most historic studies of Lepechinellidae had encountered specimens from only one or two trawl events per sea area (Barnard, 1973; Thurston, 1980; Sittrop & Serejo, 2009; Johansen & Vader, 2015). However, during two IceAGE expeditions, lepechinellids were collected in large numbers at ten stations, which constituted a quarter of all stations studied (Brix *et al*., 2018).

The family Lepechinellidae Schellenberg, 1926 is typified by elongate, slender pereopods and uropods with a body cuticle covered in long setae, and the placement of this taxa at family level was recently covered by Thurston & Horton (2019). There are currently five genera of Lepechinellidae (Horton *et al*., 2018), three of which (*Lepechinella* Stebbing, 1908; *Lepechinelloides* Thurston, 1980; *Lepesubchela* Johansen & Vader, 2015) have been reported in the North Atlantic (Thurston, 1980; Palerud & Vader, 1991; Johansen & Vader, 2015; Brix *et al*., 2018). The two remaining genera, *Lepechinellopsis* Ledoyer, 1982 and *Paralephechinella* Pirlot, 1933, are known from Madagascar and from the Equator to the Antarctic, respectively (Pirlot, 1934; Andres & Brandt, 2001). Of the 11 species of Lepechinellidae reported from the North Atlantic, the most species-rich genus in the area is *Lepechinella* with nine species, while the two remaining genera, *Lepechinelloides* and *Lepesubchela*, are represented each by single species there (Johansen & Vader, 2015).

Within the lepechinellids, morphological characters, including the epimeron shape, cuticular projections and setation, are known to vary with gender and growth stage (Barnard, 1973; Thurston, 1980). Furthermore, mature male lepechinellids develop aesthethascs along antenna 1 and 2. Assessment of lepechinellid species-level characters is hampered by the fragility of specimens, which present a number of challenges. The slender, elongate limbs are prone to breaking, the body cuticle is covered in dense, glass-like, slender setae making observation of somite margins difficult and the three-dimensional structure of the coxa and somites means observation and illustration are prone to parallax error. Based on these difficulties, species-level characters used by previous taxonomic works have been re-evaluated, with several now considered an interpretation error from illustrations.

In addition to the morphological studies, molecular methods are proving a powerful tool in recognizing hidden diversity for amphipods, which are morphologically conservative species (e.g. Fišer *et al*., 2015; Verheye *et al*., 2016; Delić *et al*., 2017; Grabowski *et al*., 2017). In other small marine crustacean studies, molecular work has provided great advances in joining males with females or larval stages with adult individuals for species where there is extreme morphological variability in gender and development (e.g. Bracken-Grissom *et al*., 2012; Grossmann *et al*., 2013; Błażewicz-Paszkowycz *et al*., 2014; Panasiuk *et al*., 2019). Considering the large variation in Lepechinellidae (Barnard, 1973; Thurston, 1980), molecular analysis provides another method to expand our knowledge.

Using an integrated taxonomic approach, this study examines the species collected during IceAGE expeditions and summarize the knowledge on the diversity and distribution of Lepechinellidae in the North Atlantic.

MATERIAL AND METHODS

The IceAGE1 expedition with RV *Meteor* took place in September 2011 and the IceAGE2 expedition with RV *Poseidon* in August 2013 (Brix *et al*., 2018). The material was sorted in the laboratories of the DZMB in Hamburg using a Leica 12.5 binocular microscope, following the protocols described by Riehl *et al.* (2014) with preservation ensuring an undisturbed cooling chain. For handling of the samples, each vial with amphipods was given a unique database number (DZMB-HH number), which was updated to a museum registration number for final long-term storage. All specimens selected as molecular vouchers are curated at the collection NTII of the Centrum für Naturkunde (CeNak), University of Hamburg, Germany, and given a ZMH-K number. The remaining lepechinellid material is registered in the crustacean collection of the Senckenberg Institute in Frankfurt am Main, Germany.

Material for comparison was borrowed from the Naturkunde Museum Berlin, Germany (ZMB numbers) and Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark (NHMD numbers).

Selected amphipod body lengths were measured from the tip of the rostrum to the end of the telson. To ensure accuracy, telsonic setal counts were made by mounting whole animals on slides and observing on a stereomicrosope before being returned to ethanol. Specimens were dissected in Euparal essence and 96% ethanol solution before being mounted as Euparal slide preparations. The pencil drawings were made using a LeicaM125 and an Olympus BX53. Illustrations were inked by hand using Rotring pens and converted to digital format using a HerbScan A3 scanner. Photos were taken with a Canon EOS 5 Mark III with a

Canon MP-E65 macro lens mounted for stacking and a Keyence 7000 digital microscope. Distribution maps were generated via the freeware QGIS. Illustrated plates, photographs and maps were assembled for publication using Photoshop CS6. Abbreviations on illustrations are: H, head; Md, mandible; LL, labium; Mx, maxilla; Mxp, maxilliped; P, pereopod; T, telson.

Twenty-two individuals from seven stations identified to five species and seven morphotypes (*Lepechinella arctica* Schellenberg, 1925, *L. grimi* Thurston, 1980, *L. skarphedini* Thurston, 1980, *L. victoriae* Johansen & Vader, 2015, *Lepechinelloides karii* Thurston, 1980) were used for genetic analysis (Tables 1, 2). Within *L. arctica*, two morphological forms (*L. arctica* '*sensu stricto*' and *L. arctica* 'form *norvegica*') were distinguished. Published cytochrome c oxidase subunit I gene (*COI*) sequences of 16 individuals (all five species listed above; Jażdżewska *et al.*, 2018) were supplemented by sequences obtained during the present study. Isolation of DNA from this additional material was performed using a Qiagen extraction kit (DNA-Minikit) according to the manufacturer's protocol. A fragment of the *COI* gene (*c*. 670 bp) was amplified using degenerated primers dgLCO-1490/dgHCO-2198 [Meyer *et al*. (2005), published sequences] and LCO1490-JJ/HCO2198-JJ [Astrin & Stüben (2008), newly obtained sequences; see Table 3]. The PCR conditions for the first primer pair followed Riehl *et al.* (2014), while for the second pair, Hou *et al.* (2007; see Table 4). Sequences were obtained using the BigDye sequencing protocol on an Applied Biosystems 3730xl sequencer by Macrogen Inc., Europe. Sequencing was conducted in both directions (three individuals were sequenced in forward direction only but the quality of the products was good enough to use them for further analysis). Sequences were edited using GENEIOUS 10.1.2 resulting in 20 sequences of length of 618–657 bp, excluding primers.

In order to provide additional molecular information for the species, 16S gene analysis was also performed. Amplification was done with the primer pair 16SFt_amp/16SRt_amp2 and used the cycling conditions of Lörz *et al.* (2018a, b; see Tables 3, 4). Sequencing was performed in both directions (in the Smithsonian and Macrogen Inc., Europe), but due to low quality of products, further study was based mainly on reverse strand. Sequence edition was done in GENEIOUS 10.1.2 and resulted in 16 sequences of length of 361–420 bp, excluding primers.

All molecular sequences recovered are deposited in GenBank (Table 1). Relevant voucher information, taxonomic classifications and sequences are accessible through the public dataset 'DS-NALEPE' (doi:10.5883/DS-NALEPE) in BOLD (www.boldsystems.org; Ratnasingham & Hebert, 2007).

The sequences were aligned with MAFFT v.7.308 algorithm with default settings (Katoh *et al.*, 2002; Katoh & Standley, 2013) in GENEIOUS 10.1.2. Uncorrected p-distance and the Kimura 2-parameter (K2P) model (Kimura, 1980) were used to determine sequence divergence in MEGA v.7.0.18 (Kumar *et al.*, 2016). For visual presentation of the results a Neighbour-Joining (NJ) tree was built based on p-distance using the default parameters (transition and transversion substitutions included and pairwise deletion). Node support was inferred with a bootstrap analysis (1000 replicates).

RESULTS

The study of IceAGE lepechinellids assessed 53 individuals from 11 stations. Significantly, of the 11 stations where lepechinellid species were present, seven of these stations had co-occurring Lepechinellid species (Figs 6–9). Eyes are reported for the first time in Lepechinellidae (Figs 1, 14). Pale yellow ommatidia were observed in specimens that had been preserved in ethanol for two years; after three years in preservative, the ommatidia in the same specimens could no longer be observed with light microscopy.

Species-level identification recognized seven morphotypes, six of which were attributed to the genus *Lepechinella* and one to *Lepechinelloides* (Table 1). Within *Lepechinella*, the morphotypes for *L. grimi*, *L. helgii* and *L. skarphedini* were distinct, while there was little variation in characters and significant overlap between material associated with the species attributable to either *L. arctica*, *L. norvegica* and *L. victoriae* (Figs 1–5, 14).

Prior to this study, *Lepechinella arctica* was only known from the holotype specimen, which is partially preserved as slide preparations with the head missing. There have been no full habitus, mouthpart or telson illustrations for this species. The limited original description of *L. arctica* has exacerbated problems in the definition and identification of this species. It is presented here as a whole animal (Fig. 1) and mouthpart illustrations are presented here for the first time based on the recently collected IceAge material (Fig. 2). A total of 14 lepechinellid specimens identified as *L. arctica* 'form *norvegica*' were present in stations 868, 879 and 979. All individuals between 2.4 and 3.2 mm have only a single carina on the pleonites and telson without lateral setae. A specimen measuring 3.8 mm has pleonites with double carina but without lateral setae on the telson. The larger specimens from 6.1 mm to 8 mm, have both the double carina on the pleonites and telson with lateral setae (Fig. 3). With multiple specimens in sample 879, it

Table 1. Summary of all North Atlantic Lepechinellidae with collection codes, accession numbers in BOLD, GenBank, Barcode Index Numbers (BIN, Ratnasingham & Hebert, 2013) and station identifiers. For station data seesee Table 2. GenBank accession numbers marked with asterisk (*) indicate sequences used in the paper by Jażdżewska *et al.* 2018.

Species	Process ID in BOLD	Collection code	Station	BIN	GenBank Accession numbers		No of ind.	Reference
					COI	16S		
Lepechinella arctica	AMPIV209-17	ZMH-K 56635	868	BOLD: ACV0699	MG264867*	MT081398	1	present study
Lepechinella arctica	AMPIV210-17	ZMH-K 56636	868	BOLD: ACV0699	MT048412	MT081389	1	present study
Lepechinella arctica	AMPIV219-17	ZMH-K 56637	868	BOLD: ACV0699	MG264803*	MT081393	1	present study
Lepechinella arctica	AMPIV218-17	ZMH-K 56638	868	BOLD: ACV0699	MG264830*	MT081396	1	present study
Lepechinella arctica	AMPIV217-17	ZMH-K 56634	869	BOLD: ACV0699	MG264759*	MT081388	1	present study
Lepechinella arctica		DZMB-HH 52294	879				1	present study
Lepechinella arctica		DZMB-HH 56230	879				1	present study
Lepechinella arctica		DZMB-HH 56231	879				1	present study
Lepechinella arctica		DZMB-HH 56232	879				1	present study
Lepechinella arctica	AMPIV067-17	ZMH-K 56622	879	BOLD: ACV0699	MG264747*	MT081386	1	present study
Lepechinella arctica	AMPIV068-17	ZMH-K 56623	879	NA	MG264876*		1	present study
Lepechinella arctica	AMPIV069-17	ZMH-K 56624	879	BOLD: ACV0699	MG264795*	MT081392	1	present study
Lepechinella arctica	AMPIV075-17	ZMH-K 56625	879	BOLD: ACV0699	MG264828*		1	present study
Lepechinella arctica	AMPIV076-17	ZMH-K 56626	879	BOLD: ACV0699	MG264753*	MT081387	1	present study
Lepechinella arctica	AMPIV079-17	ZMH-K 56627	879	BOLD: ACV0699	MG264812*	MT081395	1	present study
Lepechinella arctica		DZMB-HH 52669	1057				1	present study
Lepechinella arctica			St 41				1	Schellenberg 1925
Lepechinella arctica f. norvegica		DZMB-HH 56612	868				4	present study
Lepechinella arctica f. norvegica		DZMB-HH 56628	868				1	present study
Lepechinella arctica f. norvegica		DZMB-HH 52203	879				1	present study
Lepechinella arctica f. norvegica		DZMB-HH 56166	879				1	present study
Lepechinella arctica f. norvegica		DZMB-HH 56208	879				1	present study
Lepechinella arctica f. norvegica		DZMB-HH 56210	879				1	present study
Lepechinella arctica f. norvegica	AMPIV288-19	ZMH-K 56631	879	BOLD: ACV0699	MT048413	MT081397	1	present study
Lepechinella arctica f. norvegica	AMPIV289-19	ZMH-K 56632	879	BOLD: ACV0699	MT048415		1	present study
Lepechinella arctica f. norvegica	AMPIV212-17	ZMH-K 56633	879	BOLD: ACV0699	MG264817*		1	present study

Table 1. Continued

Species	Process ID in BOLD	Collection code	Station	BIN	GenBank Accession numbers		No of ind.	Reference
					COI	16S		
Lepechinella arctica f. norvegica		DZMB-HH 31185	979				1	present study
Lepechinella arctica f. norvegica		DZMB-HH 56195	979				1	present study
Lepechinella norvegica			R_578				3	Johansen and Vader, 2015
Lepechinella norvegica			R_11				1	Johansen and Vader, 2015
Lepechinella norvegica			1136_79				1	Johansen and Vader, 2015
Lepechinella chrysotheras			Gold seeker 1908				1	Stebbing 1908
Lepechinella chrysotheras			Thor st 99				3	Stephensen 1944
Lepechinella eupraxiella			Sadko				1	Gurjanova 1951
Lepechinella grimi		DZMB-HH 31341	963				1	present study
Lepechinella grimi	AMPIV204-17	ZMH-K 56628	967	BOLD: ADH4866	MG264805*	MT081394	1	present study
Lepechinella grimi	AMPIV205-17	ZMH-K 56640	967	NA	NA	MT081385	1	present study
Lepechinella grimi			7709_62				5	Thurston 1980
Lepechinella grimi			7709_66				1	Thurston 1980
Lepechinella grimi			7709_72				7	Thurston 1980
Lepechinella grimi			7709_73				4	Thurston 1980
Lepechinella grimi			7709_85				4	Thurston 1980
Lepechinella helgii		DZMB-HH 56117	983				1	present study
Lepechinella helgii			7709_62				4	Thurston 1980
Lepechinella helgii			7709_66				6	Thurston 1980
Lepechinella helgii			7709_72				69	Thurston 1980
Lepechinella helgii			7709_73				171	Thurston 1980
Lepechinella helgii			7709_85				2	Thurston 1980
Lepechinella schellenbergi			IngSt35				3	Stephensen 1944
Lepechinella schellenbergi			IngSt32				11	Stephensen 1944
Lepechinella skarphedini		DZMB-HH 52661	1054				1	present study
Lepechinella skarphedini	AMPIV202-17	ZMH-K 56639	1054	BOLD: ADH7185	MG264793*	MT081391	1	present study
Lepechinella skarphedini			7709_62				3	Thurston 1980

Table 1. Continued

Species	Process ID in BOLD	Collection code	Station	BIN	GenBank Accession numbers		No of ind.	Reference
					COI	16S		
Lepechinella skarphedini			7709_72				12	Thurston 1980
Lepechinella skarphedini			7709_73				49	Thurston 1980
Lepechinella skarphedini			7709_85				3	Thurston 1980
Lepechinella victoriae		DZMB-HH 52527	879				6	present study
Lepechinella victoriae	AMPIV214-17	ZMH-K 56620	963	BOLD: ADH4869	MG264781*	MT081390	1	present study
Lepechinella victoriae	AMPIV211-17	ZMH-K 56641	967	NA	NA	MT081400	1	present study
Lepechinella victoriae	AMPIV287-19	ZMH-K 56621	979	BOLD: ADH4869	MT048414	MT081399	1	present study
Lepechinella victoriae		DZMB-HH 52119	983				1	present study
Lepechinella victoriae		DZMB-HH 56149	983				1	present study
Lepechinella victoriae		DZMB-HH 56897	983				1	present study
Lepechinella victoriae		DZMB-HH 56898	983				1	present study
Lepechinella victoriae		DZMB-HH 56899	983				1	present study
Lepechinella victoriae		DZMB-HH 56695	1119				1	present study
Lepechinella victoriae			83.06.5.1				2	Johansen and Vader, 2015
Lepechinelloides karii	AMPIV208-17	ZMH-K 56629	967	BOLD: ADH6110	MG264778*		1	present study
Lepechinelloides karii	AMPIV203-17	ZMH-K 56630	979	BOLD: ADH6110	MG264794*		1	present study
Lepechinelloides karii			7709_62				8	Thurston 1980
Lepechinelloides karii			7709_66				4	Thurston 1980
Lepechinelloides karii			7709_72				34	Thurston 1980
Lepechinelloides karii			7709_73				41	Thurston 1980
Lepechinelloides karii			7709_85				6	Thurston 1980
Lepesubchela christinae			83.06.4.1				1	Johansen and Vader, 2015

Table 2. Station data for all records of Lepechinellidae in the North Atlantic

Station	Position start		Position end		Depth [m]	Collection Date	Reference
	latitude	longitude	latitude	longitude			
868	62° 09.140’ N	0° 15.510’ E	62° 10.300’ N	0° 15.860’ E	587.4	25-Jul-2013	present study
869	62° 16.200’ N	0° 01.210’ E	62° 16.450’ N	0° 01.810’ E	846.4	25-Jul-2013	present study
879	63° 06.100’ N	8° 34.320’ W	63° 05.620’ N	8° 36.220’ W	510.9	31-Jul-2013	present study
963	60° 02.730’ N	21° 28.060’ W	60° 02.730’ N	21° 29.880’ W	2749.4	28-Aug-2011	present study
967	60° 02.770’ N	21° 28.540’ W	60° 02.780’ N	21° 30.070’ W	2750.4	29-Aug-2011	present study
979	60° 21.480’ N	18° 08.240’ W	60° 20.720’ N	18° 08.600’ W	2567.6	30-Aug-2011	present study
983	60° 21.440’ N	18° 08.140’ W	60° 02.730’ N	18° 08.510’ W	2567.7	30-Aug-2011	present study
1054	61° 36.190’ N	31° 22.600’ W	61° 36.970’ N	31° 22.180’ W	2537.3	07-Sep-2011	present study
1057	61° 38.500’ N	31° 21.370’ W	61° 39.240’ N	31° 20.950’ W	2507.7	07-Sep-2011	present study
1119	67° 12.810’ N	26° 14.500’ W	67° 12.830’ N	26° 13.510’ W	696.9	14-Sep-2011	present study
Gold seeker 1908	59° 41’ N	03° 00’ W			850	NA	Stebbing, 1908
St 41	81° 20’ N	20° 30’ E			1000	NA	Schellenberg, 1925
Thor st 99	61° 15’ N	09° 35’ W			900	22-May-1901	Stephensen, 1944
IngSt35	65° 16’ N	55° 05’ W			682	18-Jul-1895	Stephensen, 1944
IngSt32	66° 35’ N	56° 38’ W			599	11-Jul-1895	Stephensen, 1944
Sadko	78° 02’ N	09° 12’ E			NA	1935	Gurjanova, 1951
7709_62	59° 58.8’ N	19° 58.2’ W	59° 58.8’ N	19° 58.8’ W	2714	01-May-1971	Thurston, 1980
7709_66	59° 59.4’ N	19° 53.5’ W	59° 58.3’ N	19° 53.5’ W	2712	03-May-1971	Thurston, 1980
7709_72	60° 05.7’ N	19° 43.3’ W	60° 06.8’ N	19° 42.5’ W	2663-2649	05-May-1971	Thurston, 1980
7709_73	60° 07.5’ N	19° 32.4’ W	60° 06.4’ N	19° 26.6’ W	2646-2636	05-May-1971	Thurston, 1980
7709_85	59° 58.6’ N	19° 53.4’ W	59° 58.1’ N	19° 54.3’ W	2708	07-May-1971	Thurston, 1980
R_578	70° 22.66’ N	17° 10.14’ E			706	02-Aug-2010	Johansen & Vader, 2015
R_11	71° 13.22’ N	21° 43.41’ E			301	01-Jun-2006	Johansen & Vader, 2015
1136_79	69° 27’ N	15° 20’ E			720-750	21-Aug_1979	Johansen & Vader, 2015
83.06.5.1	61° 38.2’ N	16° 27.7’ W			2355	5-Jun-1983	Johansen & Vader, 2015
83.06.4.1	59° 40.4’ N	09° 20.6’ W			1414	4-Jun-1983	Johansen & Vader, 2015

Table 3. Primers used in this study

Gene	Name	Sequence 5'-3'	Direction	Reference
COI	dgLCO1490	GGTCAACAAATCATAAAGAYATYGG	Forward	Mayer *et al*., 2005
	LCO1490-JJ	CHACWAAYCATAAAGATATYGG	Forward	Astrin & Stüben, 2008
	dgHCO2198	TAAACTTCAGGGTGACCAAARAAYCA	Reverse	Mayer *et al*., 2005
	HCO2198-JJ	AWACTTCVGGRTGVCCAAARAATCA	Reverse	Astrin & Stüben, 2008
16S	16SFt_amp	GCRGTATIYTRACYGTGCTAAGG	Forward	Lörz *et al*., 2018b
	16SRt_amp2	CTGGCTTAAACCGRTYTGAACTC	Reverse	Lörz *et al*., 2018b

appears that the small specimens have fewer carina and telsonic setae, and larger specimens develop more carina and setae, which was then followed up by molecular analysis.

Of the 22 individuals studied using molecular methods, 20 sequences of *COI* (from five species: *L. arctica*, *L. grimi*, *L. skarphedini*, *L. victoriae*, *Lepechinelloides karii*) and 16 sequences of 16S (from all species cited above, apart from *L. victoriae*) were obtained (Table 1). Although attempts were made to genetically test a number of smaller species, only one 'small' specimen (< 2.9 mm) of *L. arctica* 'form *norvegica*' was successfully sequenced. Across all species sequenced, the intraspecific diversity was low (ranging from 0.000 to 0.007, irrespective of the gene and distance measurement). At most, two haplotypes are distinguished (Table 5) in the majority of cases. The only exception is the 16S gene in *Lepechinella arctica*, where initially six haplotypes were recognized. However, they derived from ambiguous nucleotides in the sequences; when these sites are excluded from the alignment, only a single haplotype is recognized (Table 5). The distances between the lepechinellid species varies from 0.231 to 0.293 of p-distance and from 0.281 to 0.376 of K2P for the *COI* gene. In the case of 16S, they range from 0.161 to 0.265 and 0.181 to 0.327 for p-distance and K2P, respectively (Tables 6, 7). The highest interspecies distances were observed for *L. victoriae* and *Lepechinelloides karii* for *COI* and the pair *L. victoriae*/*L. skarphedini* for 16S. The lowest values were noted between *L. arctica* and *L. victoriae*, irrespective of the measure and the gene studied. The sequences of individuals originally identified as *L. arctica* 'form *norvegica*' share the same haplotypes with *L. arctica* '*s.s.*' (Fig. 10). The molecular grouping of this smaller specimen with the larger-body sized individuals for both *COI* and 16S support the morphological hypothesis that *L. arctica* becomes more carinate and setose with increasing growth stage (Fig. 3).

Based on morphological and molecular data from the currently study (Figs 1–3; Tables 5, 6), *L. arctica* is herein considered a senior synonym of *L. norvegica.* The following sections on the systematics of this group, taxonomically formalizes the findings from this integrative study.

DISCUSSION

Systematics

Family Lepechinellidae Schellenberg, 1926

Dorbanellidae Schellenberg, 1925: 205.
Lepechinellidae Schellenberg, 1926: 344; Barnard, 1932: 186; Dahl, 1959: 235; Andres & Brandt, 2001: 79; Sittrop & Serejo, 2009: 474; Johansen & Vader, 2015: 3.
Lepechinellinae Bousfield & Kendall, 1994: 31; Lowry & Myers, 2017: 57.

Type genus: *Lepechinella* Stebbing, 1908, by original designation.

Lepechinella arctica Schellenberg, 1926

(Figs 1–3, 13)

Dorbanella sp. Schellenberg, 1925: 206–207, fig. 6.
Lepechinella arctica Schellenberg, 1926: 394.
Lepechinella sp. Stephensen, 1938: 271–273.
Lepechinella arctica – Barnard, 1973: 10. — Barnard & Karaman, 1991: 269.
Lepechinella norvegica Johansen & Vader, 2015: 10–18, figs 5–9.
Non *Lepechinella arctica* – Gurjanova 1951: 674–677, fig. 465 (= *Lepechinella eupraxiella* Barnard, 1973: 17).

Type locality: Polar Basin north of Spitzbergen, 81°20'N, 20°30'E, 1000 m depth; station 41 of the Römer and Schaudinn expedition.

Material examined: Holotype, gender unknown, length unknown, ZMB 19848, three slides, pereon and pleon only (head and mouthparts missing), Polar Basin north of Spitzbergen, 81°20'N, 20°30'E, 1000 m depth; station 41 of the Römer and Schaudinn Expedition.

ZMH-K 56637, one specimen (Fig. 14); ZMH-K 56638, one specimen; ZMH-K 56636, one specimen; ZMH-K 56635, one specimen; DZMB-HH 56612, four specimens; DZMB-HH 56628, one specimen, IceAGE 2, POS 456, station 868, Norwegian Channel, North Atlantic (62°09.14'N, 000°15.51'E to 62°10.30'N, 000°15.86'E), 587.4 m, 25 July 2013.

Table 4. PCR conditions used for *COI* and 16S genes

Gene	Initial denaturation		Denaturation		Primers annealing		Elongation		No of cycles	Denaturation		Primers annealing		Elongation		No of cycles	Final elongation		Reference
	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)		Temp. (°C)	Time (s)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)		Temp. (°C)	Time (s)	
COI	94	600	96	60	45	45	72	60	5	93	60	50	45	72	60	35	72	300	Riehl *et al.* 2014
	94	60	94	30	45	90	72	60	5	94	30	51	60	72	60	35	72	300	Hou *et al.*, 2007
16S	95	120	95	30	50	30	72	45	35								72	300	Lörz *et al.*, 2018a, b

ZMH-K 56634, one specimen, IceAGE 2, POS 456, station 869, Norwegian Channel, North Atlantic (62°16.20'N, 000°01.21'E to 62°16.45'N, 000°01.81'E), 846.4 m, 25 July 2013.

DZMB-HH 56230, one male specimen, 8.3 mm, (Fig. 1C,); DZMB-HH 56231, one specimen; DZMB-HH 56232, one specimen; DZMB-HH 52294, one specimen; DZMB-HH 52203, one specimen; DZMB-HH 56166, one specimen; DZMB-HH, 56208, one specimen; DZMB-HH 56210, one specimen, ZMH-K 56622, one juvenile specimen, 6.1 mm (Fig. 1A); ZMH-K 56623, one specimen, 6.3 mm, (Fig. 1B); ZMH-K 56624, one specimen; ZMH-K 56625, one specimen; ZMH-K 56626, one specimen; ZMH-K 56627, one specimen; ZMH-K 56631, one specimen; ZMH-K 56632, one specimen; ZMH-K 56633, one specimen; IceAGE 2, POS 456, station 879, Faeroe Islands Ridge, North Atlantic (63°06.10'N, 008°34.32'W to 63°05.62'N, 008°36.22'W), 510.9 m, 31 July 2013.

DZMB-HH 31185, one specimen; DZMB-HH 56195, one specimen, IceAGE, ME 85-3, station 979, Iceland Basin, South Iceland, North Atlantic (60°21.48'N, 018°08.24'W to 60°20.72'N, 018°08.60'W), 2567.6 m, 30 August 2011.

DZMB-HH 52669, one specimen, IceAGE 1, ME 85-3, station 1057, South Iceland, Irminger Basin (61° 38.500'N, 31° 21.370'W to 61° 39.240'N, 31° 20.950'W), 2507.7 m, 7 September 2011.

Remarks: Well-developed eyes are clearly observable in two large male specimens at hand (Figs 1C, 14). This is the first report of eyes being present in *L. arctica*. Based on this broader range of material and re-assessment of the literature, no morphological difference can be seen between *L. arctica* originally described by Schellenberg (1925) and the more recently described *L. norvegica* Johansen & Vader, 2015. Based on morphological and molecular data from the currently study (Figs 1–3; Tables 5, 6), *L. arctica* is herein considered a senior synonym of *L. norvegica*. Although more than 13 characters were listed in Johansen & Vader, Table 1 (2015), these characters' differences contain frequent inconsistencies between the whole animal and parts illustrations, such as the coxa 1 and pereopod lengths. The illustrations do not match the measures given in the text and table, such as the telsonic cleft percentage. The variations in length or counts are often slight, as either 0.1 or 1 for mandible palp, pereopods 3 to 4, uropods 1 to 2, which we do not consider significant species-level characters.

Depth range: 301 to 2567 m.

Distribution: Iceland Basin, Faeroe Islands Ridge, Irminger Basin, Norwegian Channel (present study), Norwegian Sea (Johansen & Vader, 2015;

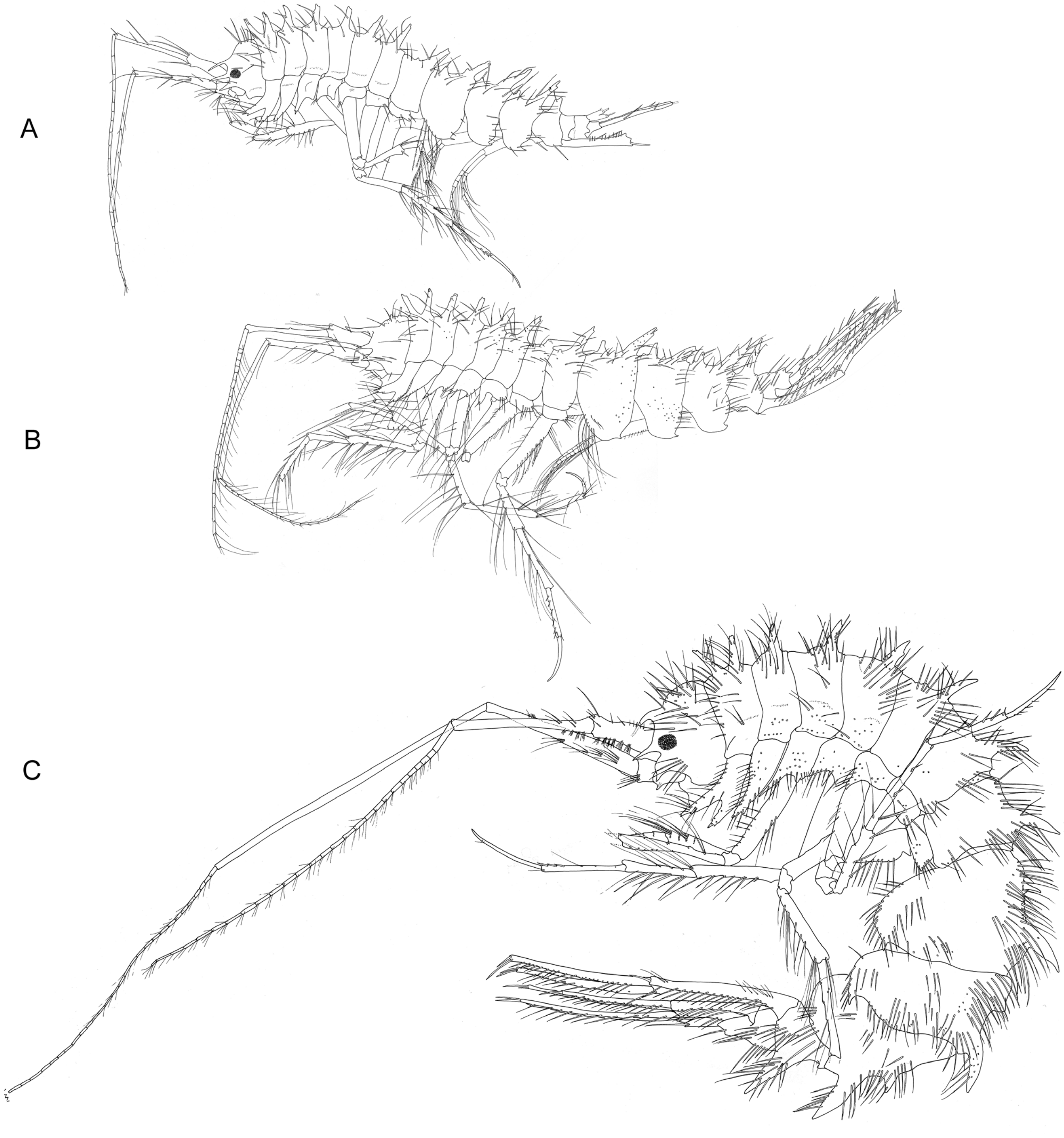

Figure 1. *Lepechinella arctica* (from top to bottom), 6.1 mm, ZMH-K 56622; 6.3 mm, ZMH-K 56623; terminal male with aesthetascs, 8.3 mm, DZMB-HH 56230, Station 879, Faeroe Island Ridge.

as *L. norvegica*), Polar Basin north of Spitzbergen (Schellenberg, 1925) (Fig. 7A; Tables 1, 2).

LEPECHINELLA CHRYSOTHERAS STEBBING, 1908

Lepechinella chrysotheras Stebbing, 1908: 191–193, pl. 27. — K.H. Barnard, 1925: 356–357. — Stephensen, 1944: 19. — J. L. Barnard, 1973: 14. — Barnard & Karaman, 1991: 269.

Type locality: North Atlantic, North Sea (between Orkney Islands and Shetland Islands), 59°041'N, 3°08'W, 850 m.

Figure 2. *Lepechinella arctica*, 8.3 mm, DZMB-HH 56230, Station 879, Faeroe Island Ridge, mandible, maxilla 1, labrum, maxilliped and telson. Scale bar 1 mm.

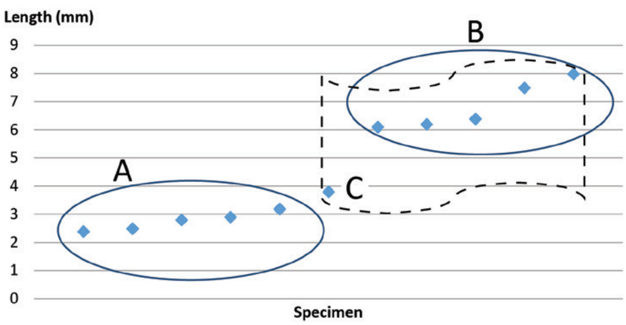

Figure 3. *Lepechinella arctica*, size versus morphotype for lepechinellids collected at IceAGE station 879 small specimens in group A, single carina, telson no lateral setae; B, specimens exceeding 6 mm body length: double carina, telson with lateral setae; C specimens having *COI* and 16S sequences confirming species affiliation.

Remarks: Lepechinella chrysotheras is not encountered in the samples from IceAGE expeditions. This species is only known from the holotype of Stebbing (1908) and three additional male specimens listed by Stephensen (1944). There are currently no reported female specimens *of L. chysotheras*. Stebbing (1908) does not list the institution where the type material is deposited and, unfortunately, no type material for *L. chrysotheras* was located at the NHM, London, which was the main repository of Stebbing's personal collection. Stebbing (1908) cited material as collected in the 'North Sea', by the RV *Goldseeker*, but this is a historic use of the name North Sea, as in more recent times, the term 'North Sea' is restricted to the region between the United Kingdom and the European mainland. Most importantly, the type locality listed in the formal taxonomic description of the species is given only as latitude and longitude coordinates. Following these coordinates, the collection locality is positioned between the Orkney Islands and the Outer Hebrides, hence outside the modern definition of the North Sea (a technical, but critical point in assessing regional listings and geographic species ranges).

Depth range: 850 to 900 m.

Distribution: North Atlantic, between Orkney Islands and Shetland Islands (Stebbing, 1908), South of the Faeroes (Stephensen, 1944) (Fig. 9B; Tables 1, 2).

Lepechinella eupraxiella Barnard, 1973

Lepechinella arctica Gurjanova, 1951: 674–678.
Lepechinella eupraxiella Barnard, 1973: 17.

Type locality: 78°02'N, 09°12'E (West of Svalbard), depth unknown; station sampled by the Icebreaker Sadko.

Table 5. Mean intraspecific distance (based on haplotypes) of *COI* and 16S sequences of studied species. The haplotypes recognized for 16S of *L. arctica* are associated with the presence of ambiguous nucleotides in the sequences. When these sites are removed, no variation within the 16S sequences is observed

	COI				16S			
	No of seq.	No of haplotypes	p-distance	K2P	No of seq.	No of haplotypes	p-distance	K2P
Lepechinella arctica	14	2	0.002	0.002	10	6 (1)	0.000	0.000
Lepechinella victoriae	2	2	0.006	0.007	3	2	0.003	0.003
Lepechinella grimi	1	1	NA	NA	2	2	0.006	0.006
Lepechinella skarphedini	1	1	NA	NA	1	1	NA	NA
Lepechinelloides karii	2	1	0.000	0.000	0	NA	NA	NA

Table 6. COI mean interspecies distances based on haplotypes between studied species (p-distance lower left, K2P upper right). The lowest values in bold, the highest values underlined.

K2P p-distance	*Lepechinella arctica*	*Lepechinella victoriae*	*Lepechinella grimi*	*Lepechinella skarphedini*	*Lepechinelloides karii*
Lepechinella arctica		**0.281**	0.282	0.290	0.366
Lepechinella victoriae	**0.231**		0.314	0.327	0.376
Lepechinella grimi	0.232	0.254		0.299	0.370
Lepechinella skarphedini	0.239	0.262	0.243		0.294
Lepechinelloides karii	0.287	0.293	0.291	0.243	

Table 7. Mean interspecies distances of 16S rRNA gene based on haplotypes between studied species (p-distance lower left, K2P upper right). The lowest values in bold, the highest values underlined.

K2P p-distance	*Lepechinella arctica*	*Lepechinella victoriae*	*Lepechinella grimi*	*Lepechinella skarphedini*
Lepechinella arctica		**0.181**	0.324	0.309
Lepechinella victoriae	**0.161**		0.303	0.327
Lepechinella grimi	0.263	0.249		0.304
Lepechinella skarphedini	0.253	0.265	0.250	

Remarks: *Lepechinella eupraxiella* Barnard, 1973 was not encountered in samples from the IceAGE expeditions.

It appears that the validation of *Lepechinella eupraxiella* is problematic. Known only from the original illustration by Gurjanova (1951) under the name *L. arctica*, Barnard's (1973) subsequently described the species based on literature assessment. Barnard nominated the specimen illustrated in Gurjanova's figure 465 as the type material, which provides only a partial illustration of the specimen. The species is known only from a single individual collected by Icebreaker Sadko in Polar Basin ('Полярный бассейн'). Given the statement of Gurjanova (1951, p. 675 and p. 678) that it fully agreed with the description and figure by Schellenberg (1925) of *L. arctica*, the sympatric distribution of both species as well as the erection of the species only on the basis of single figure one cannot exclude that *L. eupraxiella* is a junior synonym of *L. arctica*. The study of the holotype of *L. eupraxiella* may help solving this taxonomic problem.

Depth range: Unknown.

Distribution: West of Svalbard (Gurjanova, 1951; Barnard, 1973) (Fig. 7A; Tables 1, 2).

Lepechinella grimi Thurston, 1980

Lepechinella grimi Thurston, 1980: 73–78, figs 4–6. — Barnard & Karaman, 1991: 269.

Type locality: North Atlantic, Iceland Basin, 60°07.5'N, 19°26.6'W, 2646 m (Discovery station 7709#72).

Material examined: DZMB-HH 31341, one specimen, IceAGE, ME 85-3, station 963, Iceland Basin, South Iceland, North Atlantic (60°02.73'N, 021°28.06'W to 60°02.73'N, 021°29.88'W), 2749 m, 28 August 2011; ZMH-K 56640, one specimen; ZMH-K 56628, one specimen, IceAGE ME 85-3, station 967, Iceland Basin, South Iceland, North Atlantic (60°02.770'N, 21°28.540'W to 0°02.780'N, 21°30.070'W), 2750.4 m, 28 August 2011.

Remarks: The three individuals collected here increase the known specimens of *L. grimi* to 24 specimens, collected across seven stations.

Depth range: 2646 to 2750 m.

Distribution: Iceland Basin (Thurston, 1980; present study) (Fig. 8A, Tables 1, 2).

Lepechinella helgii Thurston, 1980

Lepechinella helgii Thurston, 1980: 70–73, figs 1–3. — Barnard & Karaman, 1991: 269.

Type locality: North Atlantic, Iceland Basin, 59°58.6'N, 19°58.2'W, 2714 m (Discovery station 7709#73).

Material examined: DZMB-HH 56117, one specimen, IceAGE, ME 85-3, station 983, Iceland Basin, South Iceland, North Atlantic (60°21.440'N, 18°08.140'W to 60°02.730'N, 18°08.510'W), 2567.7 m, 30 August 2011.

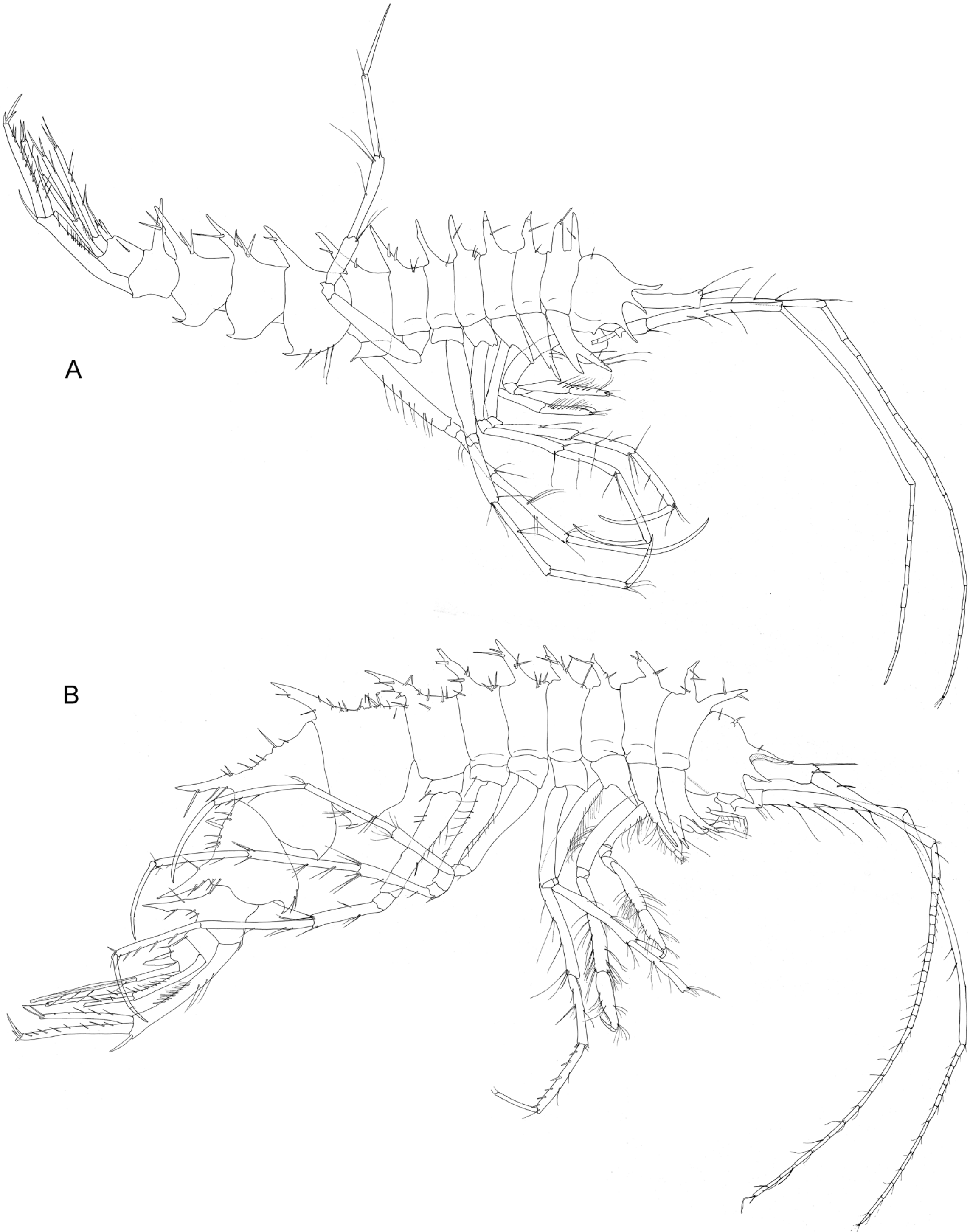

Figure 4. *Lepechinella victoriae*: A, juvenile, 3.7 mm, DZMB-HH 56149, station 983; B, female, 7.2 mm, ZMH-K 56620, Station 963, Iceland Basin.

Remarks: Thurston (1980) collected 252 specimens from five stations below 2600 m, the recent IceAGE collection added a single specimen from the Iceland Basin.

Depth range: 2568 to 2714 m.

Distribution: Iceland Basin (Thurston, 1980; present study) (Fig. 8B; Tables 1, 2).

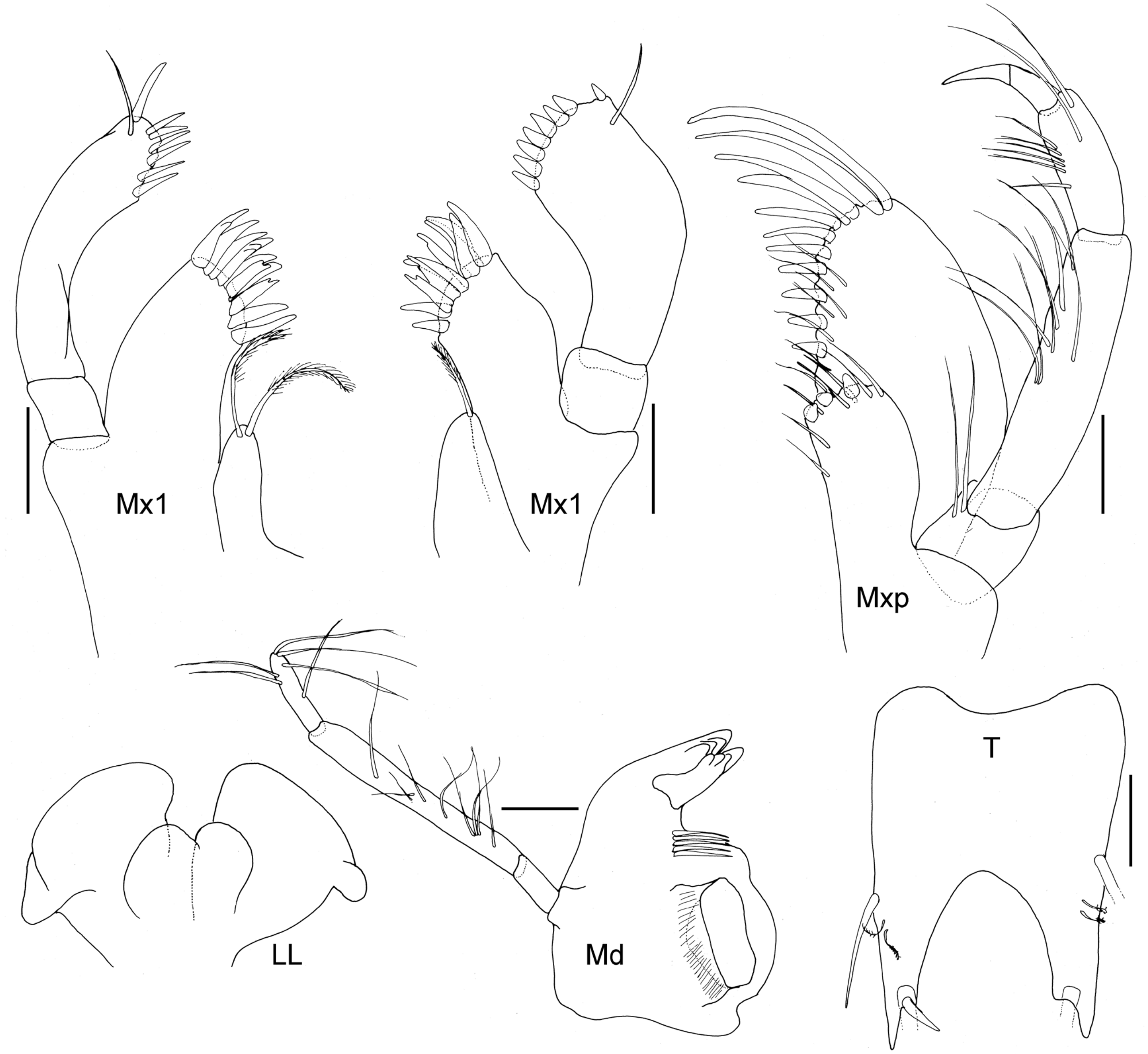

Figure 5. *Lepechinella victoriae*, female, 7.2 mm, ZMH-K 56620, Station 963, Iceland Basin, mouthparts and telson. Scale bar 1 mm.

LEPECHINELLA SCHELLENBERGI STEPHENSEN, 1944

Lepechinella schellenbergi Stephensen, 1944: 18–20, fig 11.
Lepechinella arctica—Barnard, 1973: 10. — Barnard & Karaman, 1991: 269.

Type locality: Arctic Ocean, western Greenland, 65°16.0'N, 55°4.60'W.

Material examined: NHMD-82314 (prev. ZMUC-CRU-4468): lectotype (five slides); NHMD-82315 (prev. ZMUC-CRU-4469): one male paralectotype (alcohol + two slides); NHMD-82316 (prev. ZMUC-CRU-4470): one female paralectotype (alcohol + two slides); NHMD-85863 (prev. ZMUC-CRU-8019): three paralectotypes (in alcohol); NHMD-85864 (prev. ZMUC-CRU-8020): six paralectotypes (in alcohol).

Remarks: Lepechinella schellenbergi was not present in IceAGE material, but for comparative purposes the type material was examined. The characters used to separate *L. schellenbergi* from *L. arctica* are not well observed, with the angle of the mandible susceptible to parallax error and molar surface subject to wear,

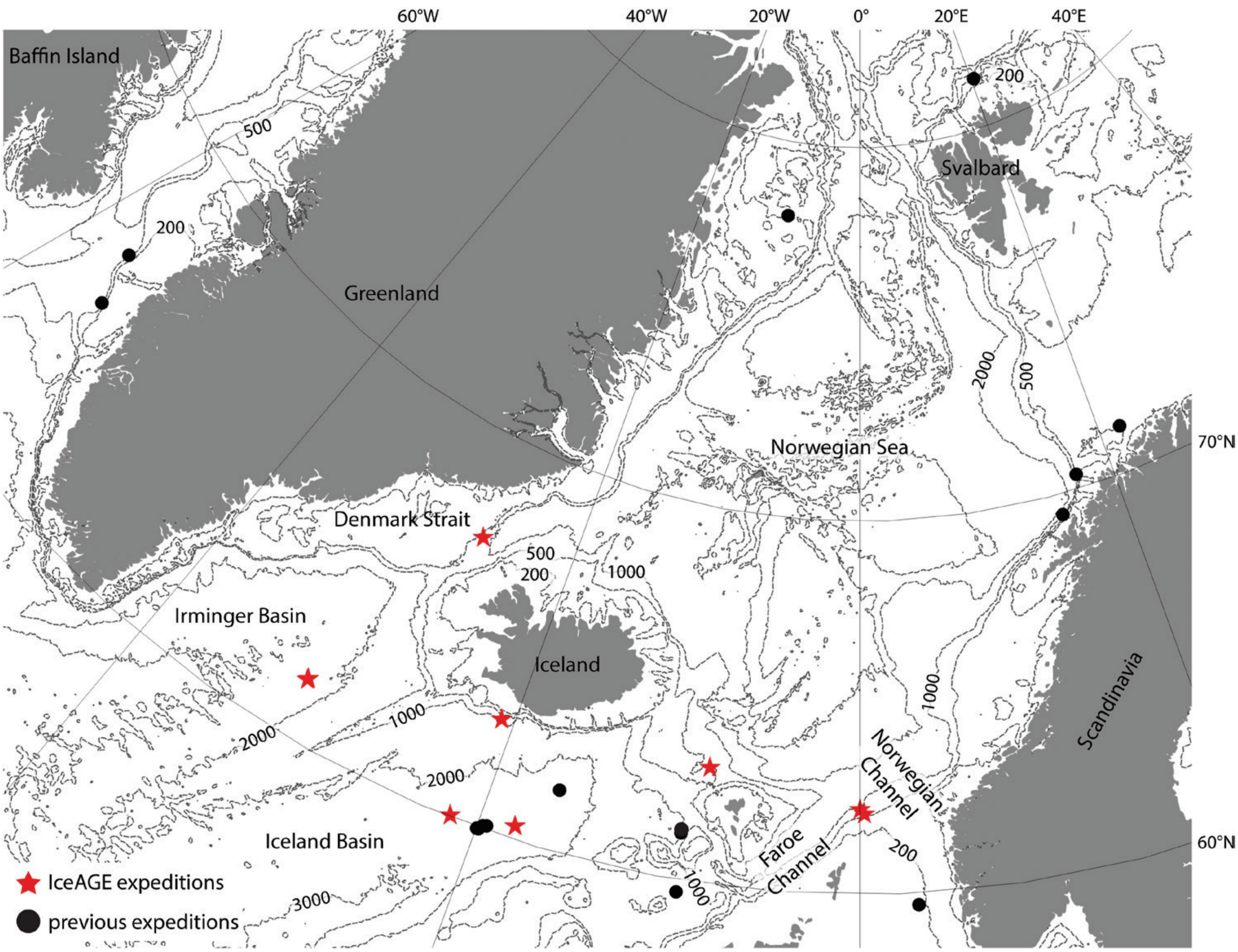

Figure 6. Distribution of Lepechinellidae from the North Atlantic sampling stations.

the variation of in rami length is only slight, listed as 0.7 vs. 0.8 by Johansen & Vader (2015) and too small to be reliable. We consider *L. schellenbergi* to be closely associated with *L. arctica*. *Lepechinella schellenbergi* is only known from the original collection of 14 specimens collected in western Greenland. The relationship of these two species would benefit from molecular study, but no suitable material for genetic analysis currently exists.

Depth range: 599 to 682 m.

Distribution: Western Greenland (Stephensen, 1944) (Fig. 7A; Tables 1, 2).

Lepechinella skarphedini Thurston, 1980

Lepechinella skarphedini Thurston, 1980: 78–81, figs 7–9. — Barnard & Karaman, 1991: 269.

Type locality: North Atlantic, Iceland Basin, 60°7.5'N, 19°32.4'W, 2646 m (Discovery station 7709#72).

Material examined: DZMB-HH 52661, one specimen; ZMH-K 56639, one specimen, IceAGE, ME 85-3, station 1054, Irminger Basin, south Iceland, North Atlantic (61°36.19'N, 031°22.60'W to 61°36.97'N, 031°22.18'W), 2537.3 m, 7 September 2011.

Remarks: The two specimens collected here provide additional records of *L. skarphedini*, extending the known distribution further north from the Iceland Basin to the Irminger Basin.

Depth range: 2450 to 2537 m.

Distribution: Irminger Basin (present study) and Iceland Basin (Thurston, 1980) (Fig. 9A; Tables 1, 2).

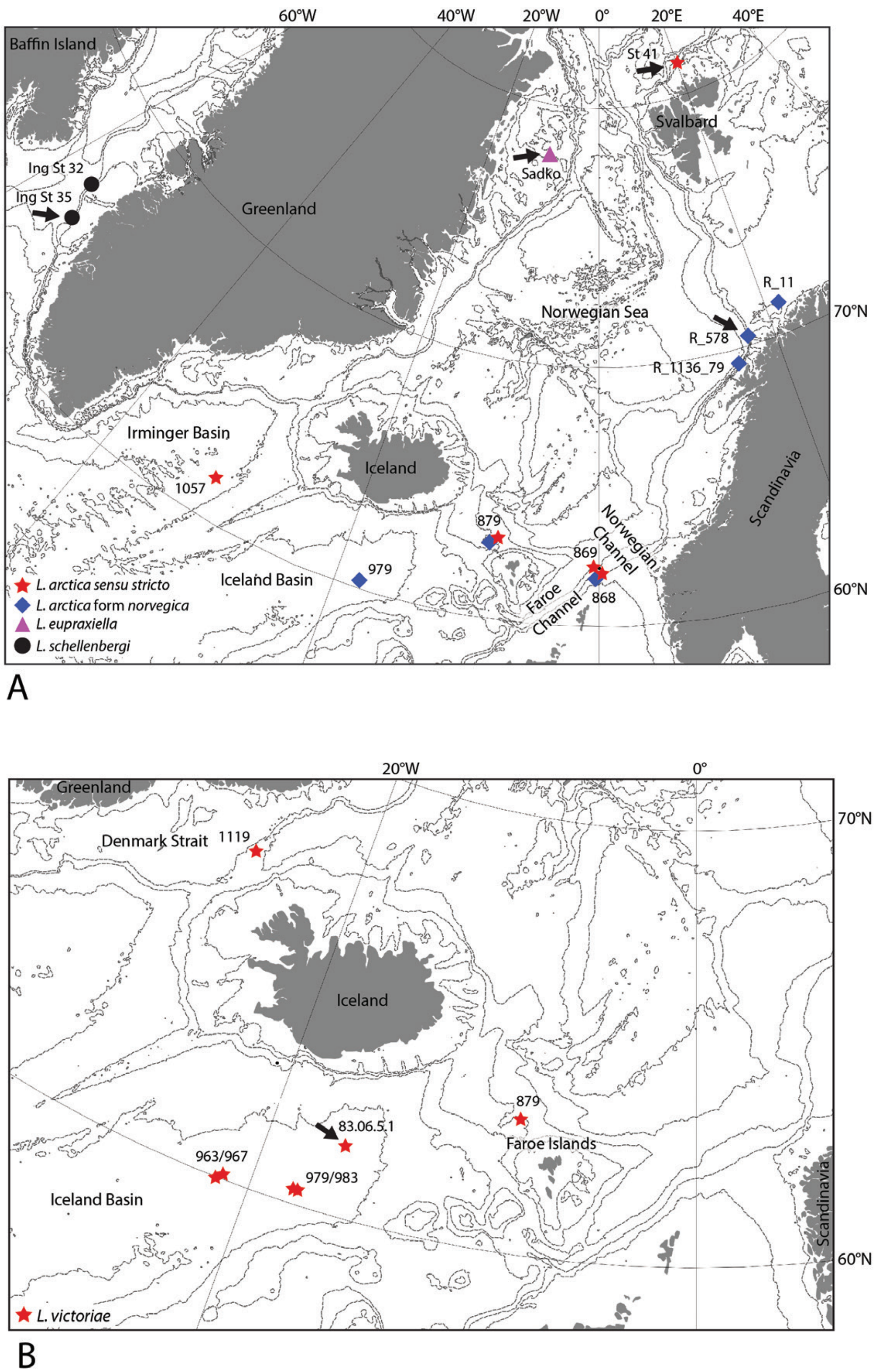

Figure 7. Distribution of: A, *Lepechinella arctica* ('sensu stricto', star; 'form *norvegica*', rhomb), *Lepechinella eupraxiella* (triangle) and *Lepechinella schellenbergi* (circle); B, *Lepechinella victoriae* (star). Numbers indicate stations codes (see Table 2 for station details), the arrows indicate the type localities.

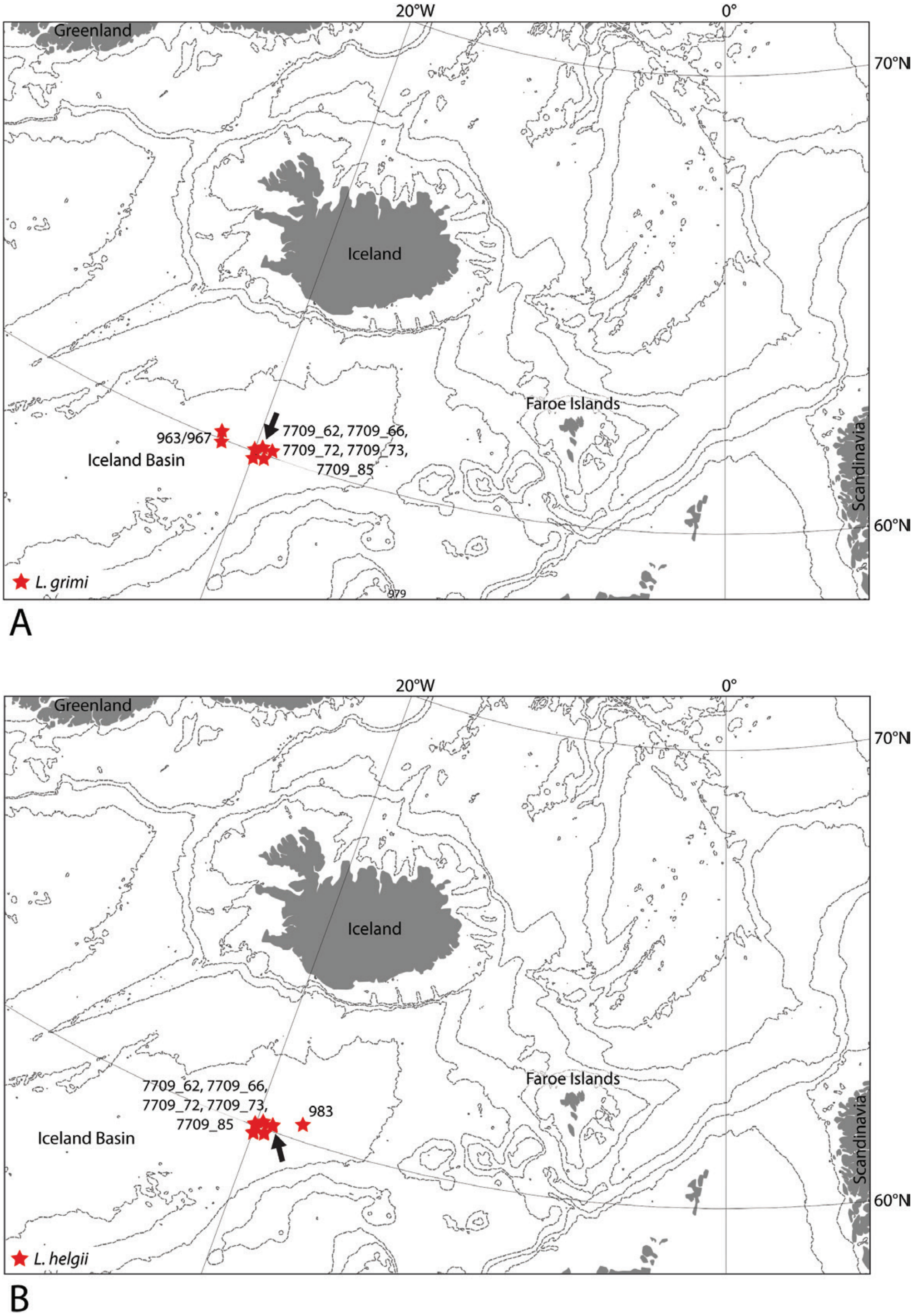

Figure 8. Distribution of: A, *Lepechinella grimi*; B, *Lepechinella helgii*. Numbers indicate stations codes (see Table 2 for station details), the arrows indicate the type localities.

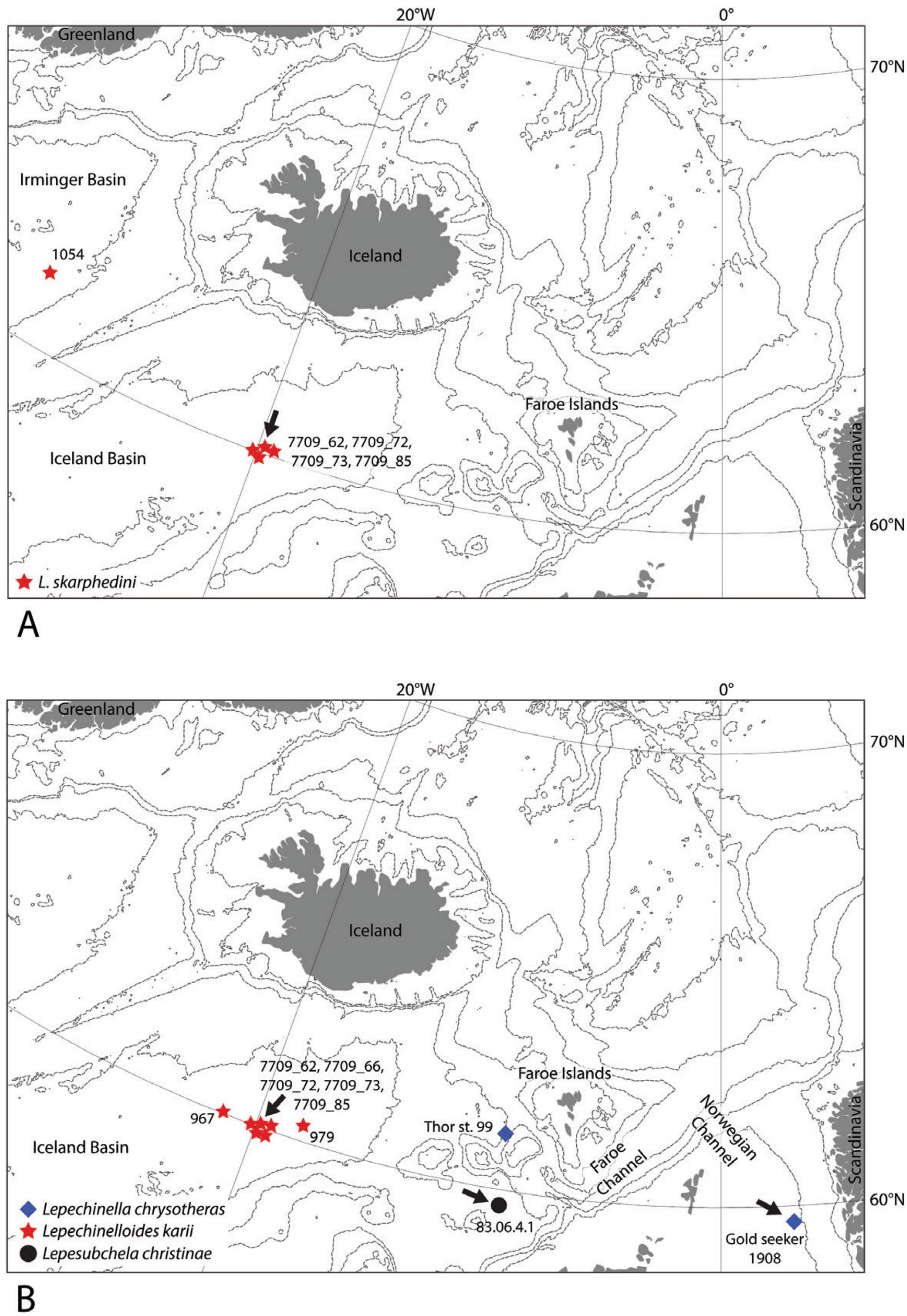

Figure 9. Distribution of: A, *Lepechinella skarphedini* (star); B, *Lepechinella chrysotheras* (rhomb), *Lepechinelloides karii* (star), *Lepesubchela christinae* (circle). Numbers indicate stations codes (see Table 1 for station details), the arrows indicate the type localities.

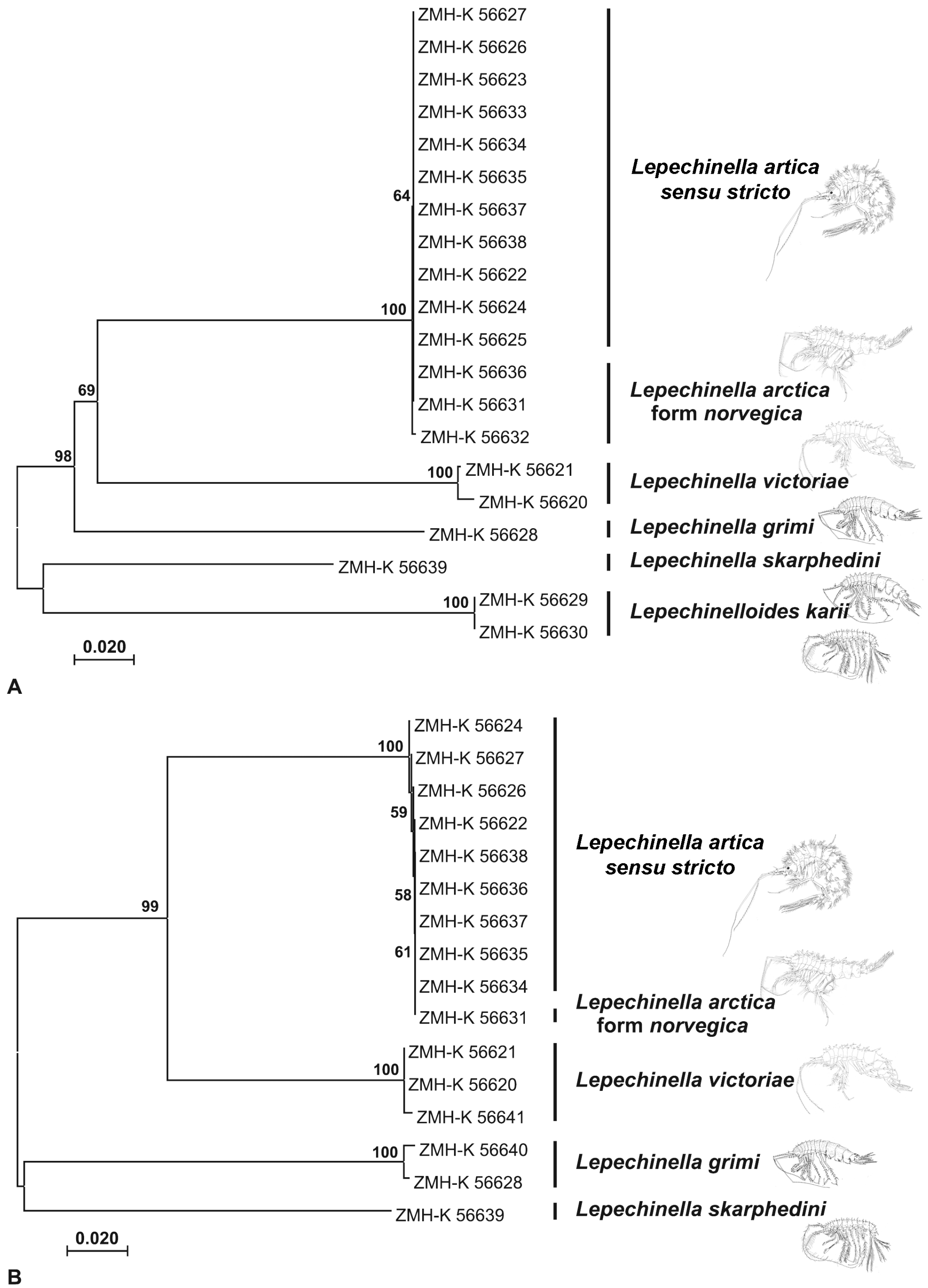

Figure 10. Neighbour-Joining tree of *COI* (A) and 16S (B) sequences based on p-distance. Bootstrap value – 1000 replicates.

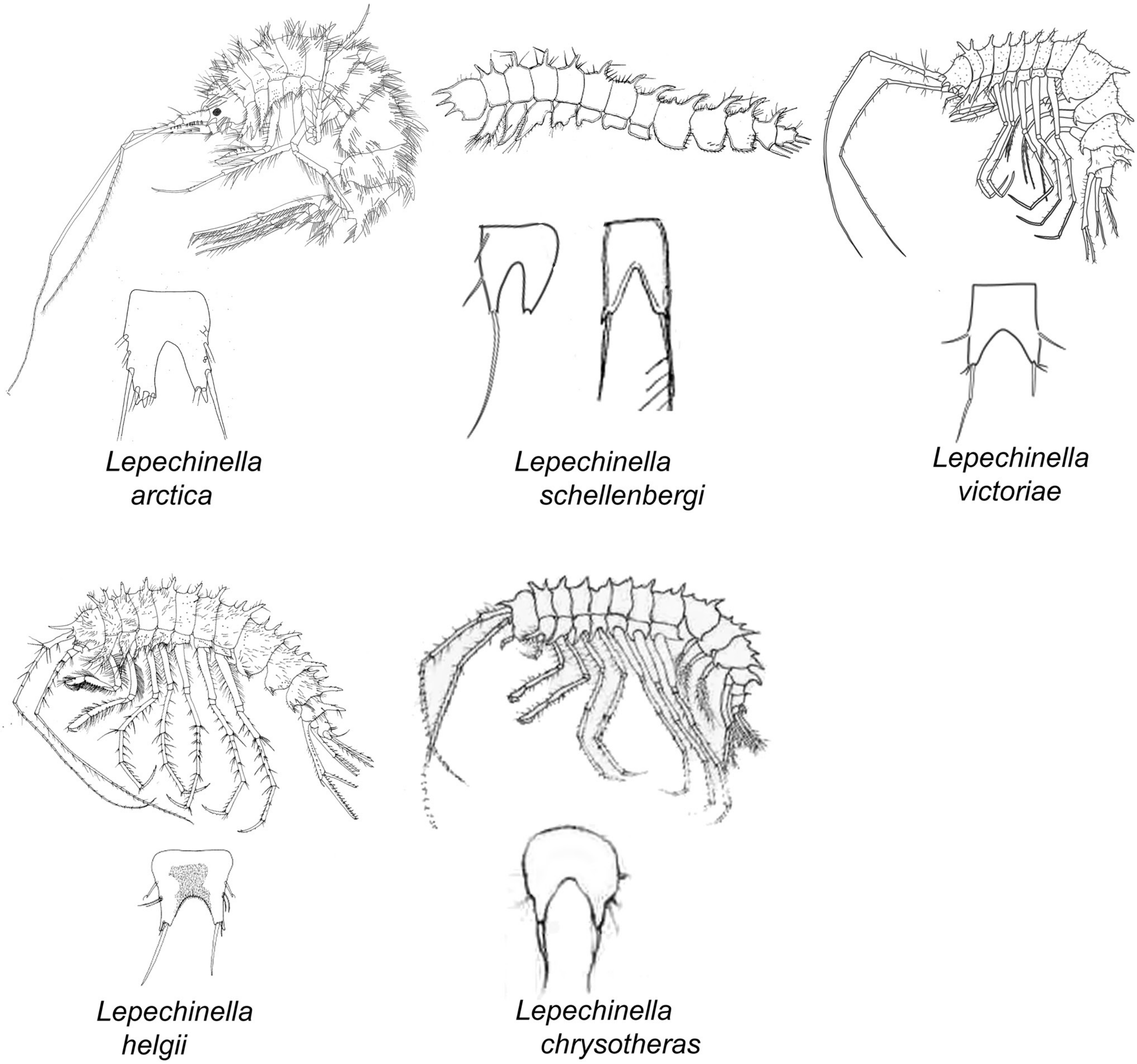

Figure 11. Field guide to North Atlantic lepechinellid species, part one of two.

Lepechinella victoriae Johansen & Vader, 2015

(Figs 4–5, 13)

Lepechinella victoriae Johansen & Vader, 2015: 23–28, figs 13–16.

Type locality: North Atlantic, south of Iceland, 61°38.2'N, 16°27.7'W, 2355 m.

Material examined: DZMB-HH 52527, six specimens, IceAGE 2, POS 456, station 879, Faeroe Islands Ridge, North Atlantic (63°06.10'N, 008°34.32'W to 63°05.62'N, 008°36.22'W), 510.9 m, 31 July 2013.

ZMH-K 56620, one specimen, 7.2 mm (Figs 4, 12), IceAGE, ME 85-3, station 963, Iceland Basin, south Iceland, North Atlantic (60°02.73'N, 021°28.06'W to 60°02.73'N, 021°29.88'W), 2749 m, 28 August 2011.

ZMH-K 56641, one specimen; IceAGE ME 85-3, station 967, Iceland Basin, south Iceland, North Atlantic (60°02.770'N, 21°28.540'W to 0°02.780'N, 21°30.070'W) 2750.4 m, 28 August 2011.

ZMH-K 56621, one specimen, IceAGE, ME 85-3, station 979, Iceland Basin, south Iceland, North Atlantic (60°21.48'N, 018°08.24'W to 60°20.72'N, 018°08.60'W), 2567.6 m, 30 August 2011.

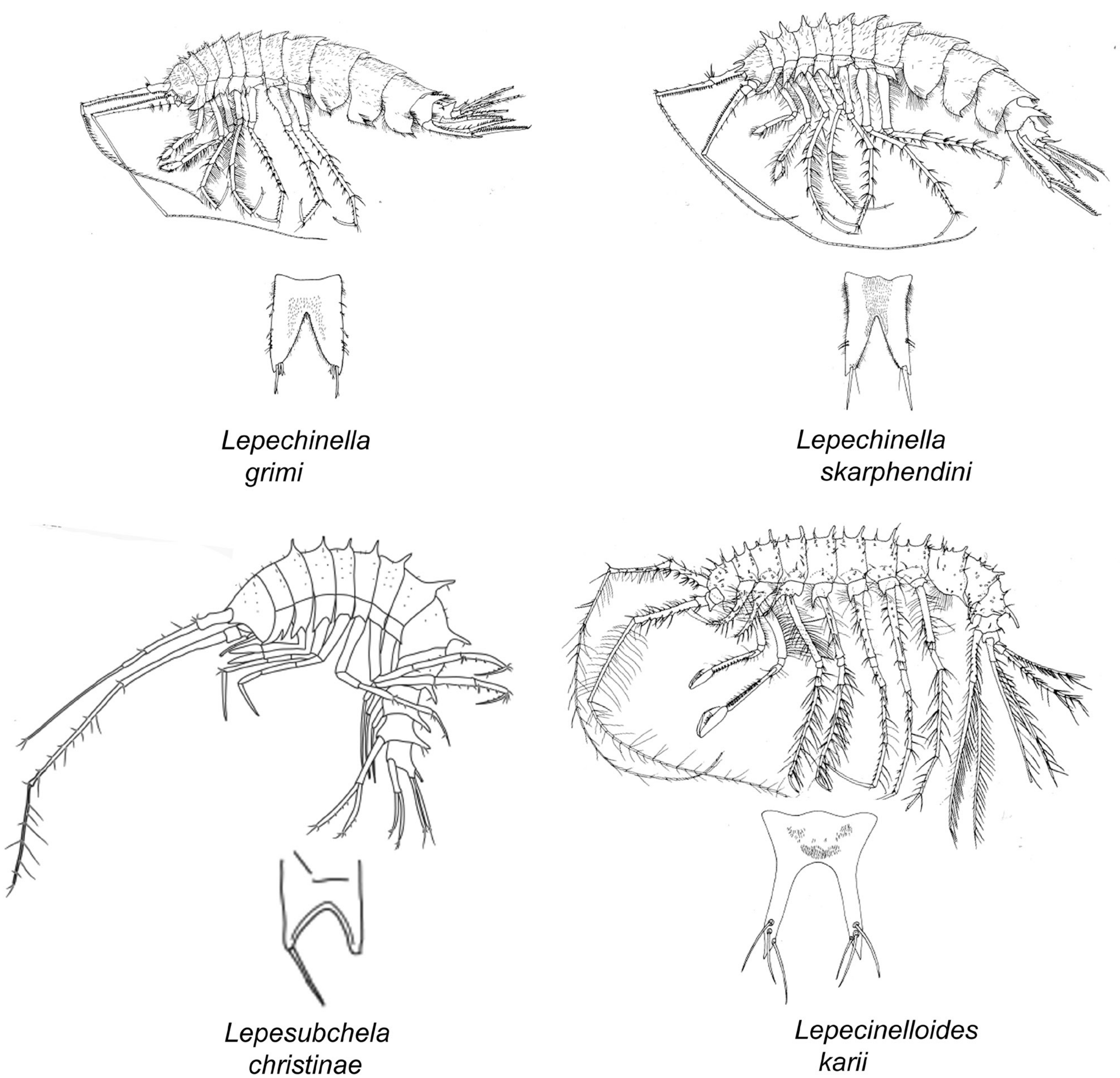

Figure 12. Field guide to North Atlantic lepechinellid species, part two of two.

DZMB-HH 56897, one specimen; DZMB-HH 56898, one specimen; DZMB-HH 56899, one specimen; DZMB-HH, 52119, one specimen; DZMB-HH 56149, one specimen (3.7 mm, Fig. 4), IceAGE, ME 85-3, station 983, Iceland Basin, south Iceland, North Atlantic (60°21.440'N, 18°08.140'W to 60°02.730'N, 18°08.510'W), 2567.7 m, 30 August 2011.

DZMB-HH 56695, one specimen, IceAGE, ME 85-3, station 1119, Denmark Strait, east Greenland, North Atlantic (67°12.81'N, 026°14.50'W to 67°12.83'N, 026°13.51'W), 696.9 m, 14 September 2011.

Remarks: Material reported here are larger specimens, 7.2 mm, (Figs 4, 5, 13), than the holotype ZMBN 99134, 5.5 mm. The IceAGE material includes additional juvenile and female specimens from sites close to the type locality 'south of Iceland' and at a similar 2000–m depth range. The rostrum shape, lateral cephalic lobe and head anteroventral lobe show some variation between juveniles, with more slender projections, and adults, which have broader and more sinusoidal projections (Fig. 4). The present study extends the known distribution of *L. victoriae*

Figure 13. *Lepechinella arctica*, 6.1 mm, ZMH-K 56624 and *Lepechinella victoriae*, 6.0 mm, ZMH-K 56620, photographed after preservation. Scale bar 1 mm.

north to the Denmark Strait and east to the Faeroe Islands Ridge. The collection data also broadens the bathymetric range from the middle bathyal (2355 m) to the shallower upper bathyal (at 679 m deep).

Depth range: 679 to 2750 m.

Distribution: Iceland Basin (Johansen & Vader, 2015; present study), Denmark Strait (present study), Faeroe Islands Ridge (present study) (Fig. 7B; Tables 1, 2).

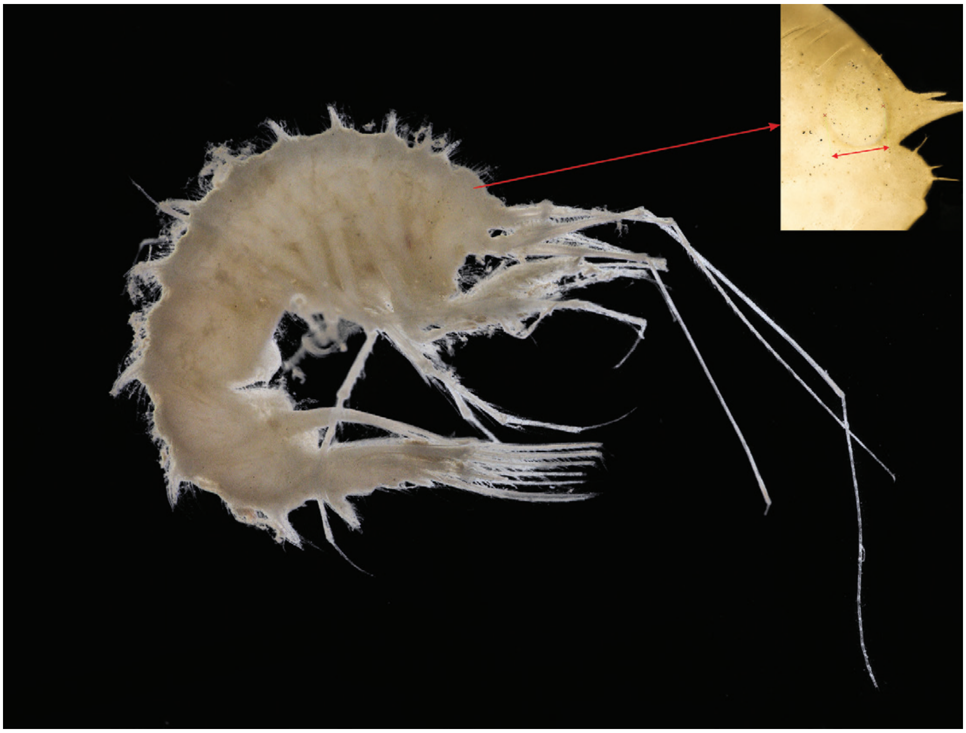

Figure 14. *Lepechinella arctica*, ZMK-H 56637, habitus lateral and eye including measurement of 179 µm. Scale bar 1 mm.

Genus *Lepechinelloides* Thurston, 1980

Lepechinelloides karii Thurston, 1980

Lepechinelloides karii Thurston, 1980: 83–86, figs 10–12. — Barnard & Karaman, 1991: 269.

Type locality: North Atlantic, Iceland Basin, 60°07.5'N, 19°26.6'W, 2646 m (Discovery station 7709#72).

Material examined: ZMH-K 56629, one specimen, IceAGE ME 85-3, station 967, Iceland Basin, south Iceland, North Atlantic (60°02.770'N, 21°28.540'W to 0°02.780'N, 21°30.070'W) 2750.4 m, 29 August 2011.

ZMH-K 56630 one specimen, IceAGE, ME 85-3, station 979, Iceland Basin, south Iceland, North Atlantic (60°21.48'N, 018°08.24'W to 60°20.72'N, 018°08.60'W), 2567.6 m, 30 August 2011.

Remarks: The two specimens recorded from the IceAGE expedition provide more recent collections of *Lepechinelloides karii* with a total of 95 specimens known from six stations in the Iceland Basin.

Depth range: 2150 to 2750 m.

Distribution: Iceland Basin (Thurston, 1980; present study) (Fig. 9B, Tables 1, 2).

Genus *Lepesubchela* Johansen & Vader, 2015

Lepesubchela christinae Johansen & Vader, 2015

Lepesubchela christinae Johansen & Vader, 2015: 28–32, figs 17–19.

Type locality: North Atlantic, south of Faeroe Islands, 59°40.4'N, 09°20.6'W, 1414 m.

Remarks: Lepesubchela christinae was not encountered in the IceAGE collections. This species is only known from the holotype specimen, a 4 mm individual that is here assumed to be a juvenile based on the comparatively small body size.

Depth range: 1414 m.

Distribution: south-west of the Faeroes (Johansen & Vader, 2015) (Fig. 9B; Tables 1, 2).

DISCUSSION

The IceAGE expedition sampled six out of 11 species of Lepechinellidae known from the North Atlantic. For five species, *Lepechinella grimi, L. helgii, L. skarphedini,* *L. victoriae* and *Lepechinelloides karii*, the new records are the first report of the species since their original description. Furthermore, our integrative taxonomic approach, using morphological characters and two mitochondrial genes, recognized limited variation between two species and has prompted *L. norvegica* to be synonymized with *L. arctica*.

How many lepechinellid species live in the North Atlantic?

Biodiversity has often been underestimated by overlooked, morphologically similar species (Vrijenhoek, 2009) (Figs 11, 12). Molecular analyses of the population structure and diversity of deep-sea benthic invertebrates have become more common within the last two decades and suggest that recently morphologically determined widespread species are likely to represent cryptic species or species complexes (e.g. Havermans, 2016). The present analyses demonstrate overestimated diversity for lepechinellid amphipods based on *COI* and 16S. The systematic findings from this work have provided a conservative approach and synonymized one species, *L. norvegica* with *L. arctica*, based on morphological and molecular analyses. Based on the results here, we postulate that other described species of lepechinellid may similarly show high morphological variation with growth stage and similar low genetic diversity across several molecular markers. Species, such as *Lepesubchela christinae* described from a single, small-body sized specimen, is likely a juvenile form of a described species (see Fig. 9B). Similarly, *Lepechinella chrysotheras* is only known from four specimens, and because the type material cannot be located, there is only the historic description to assess characters. Lastly, *Lepechinella schellenbergi* is highlighted as a potential morphological variation of *L. arctica* given the intraspecific variation and low genetic diversity observed in the IceAGE collections. Examination of the *L. schellenbergi* type material did not enable us to observe any morphologically distinguishing characters from *L. arctica*. Unfortunately, no DNA investigation of this taxon was possible.

In the North Atlantic, *Lepechinella arctica* (Fig. 7A) is reported from a broad depth range (from 510 to 2567 m) in various areas, including both sides of the Reykjanes Ridge, the Faeroe–Iceland Ridge, the Norwegian Channel and the north Norwegian coast to north of Spitzbergen. Ecological studies have reported *L. arctica* from the Arctic shelf (140–200 m) on silty substrates in the Kola section of the Barents Sea (Lyubina *et al.*, 2012; Zimina & Lyubina, 2016). It was also reported from a single individual collected in the lower bathyal (2770–2820 m) of the Kuril–Kamchatka Trench area (Kamenskaya, 1997). Future studies may reveal if *L. arctica* is truly a circumarctic species extending its distribution also to the Pacific.

Morphologically conservative but genetically distinct species

Lepechinella victoriae is morphologically similar to *L. arctica* (Figs 1, 2, 4, 5, 11–14). Prior to this study, the taxon *L. victoriae* was only known from two small specimens (holotype 5.5 mm, paratype 4.5 mm) from the type locality (see Fig. 7B). The additional IceAGE material provides the opportunity to investigate the ontogenic variation of the species and its molecular characterization. The molecular distances between the studied taxa for both mitochondrial genes greatly exceeds the values generally used for species delimitation in amphipods (Tables 6, 7) (e.g. Costa *et al.*, 2009; Lobo *et al.*, 2017; Tempestini *et al.*, 2018), supporting *L. victoriae* as a genetically distinct species. However, small specimens of *L. arctica* and *L. victoriae* cannot be distinguished morphologically, where individuals are under 4 mm (Fig. 3A) having a single carina and only apical setae on the telson. In adult *L. victoriae*, the single carina is maintained, whereas specimens larger than 6 mm of *L. arctica* (Fig. 3B) develop a double carinae and the telson is armed with additional lateral spines. A smaller specimen (under 4 mm) grouped with the larger body sized individuals for both *COI* and 16S indicate that the species becomes more carinate and setose with increasing growth stage (Fig. 3). This increase in carination in individuals with a larger body agrees with the findings of Barnard (1973) and Thurston (1980) for species of which high numbers of individuals are known, such as the Northern Atlantic species *L. grimi* and *L. helgii*.

Eyes fading with preservation

Well-developed eyes are clearly observable in two large male specimens of *Lepechinella arctica* (Fig. 1). The eyes are a tight, round cluster of pale yellow ommatidia. Close inspection of other adult and juvenile specimens show that ommatidia are fading from yellow to white in the ethanol preservative but are nonetheless observable. Both deep (2000 m+) and shallower (> 600 m) *Lepechinella* have eyes that are in contrast to the co-occurring deep-sea genus *Rhachotropis* S. I. Smith, 1883, which lacks eyes in deep-water species, while more shallow congeneric species are sighted. The family Lepechinellidae was thought to lack eyes prior to this study. This fading of pigment may explain why the presence of eyes has been overlooked in previous species reports.

Co-occurrence of species

Lepechinellid species from IceAGE material were seen to co-occur (Fig. 6), similar to the findings of Thurston, 1980, where four lepechinellid species were reported from the same station. Similar finding of congeneric species collected at the same station were reported also for other amphipod families in Icelandic waters (Weisshapel, 2000; 2001; Weisshappel & Svavarsson, 1998).

The distribution of lepechinellids lies across the gateway region of the North Atlantic, from the Arctic to the Greenland west coast and along the Greenland–Iceland–Faeroe Ridge towards the north Norwegian coast up to Svalbard. Most samples obtained for our study were from the deep Iceland Basin towards the Faeroe Islands and the Norwegian Channel (see also: Brix *et al*., 2018) and single stations in the Denmark Strait and the deep Irminger Basin (see Fig. 6). The depth variation between 400 and 800 m along the Norwegian coast channel is a recognized thermocline with a high species diversity seen in other amphipod families (Vader *et al*., 1997). The shelf-edge, especially in the Norwegian Channel, is particularly diverse in amphipod communities (Brix *et al*., 2018) and is thought to be influenced by the coincidence of a high fluctuation in temperature, with both, positive and negative values, indicating a varying depth for the thermocline.

CONCLUSION

Morphological observations of the lepechinellids in the IceAGE samples allow a detailed comparison of species-defining characters and reveal both convergent and divergent morphological and molecular characters. This outcome contrasts with previous molecular studies that largely show a trend of overlooked biodiversity with cryptic species. The IceAGE expedition samples reveal a previously overestimated biodiversity of North Atlantic lepechinellid amphipods.

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