1 We would like to thank the Referees for constructive review, that will help us to 2 improve the manuscript. Written below are our responses to the Referees' comments. The 3 comments were reproduced and are followed by our responses. Based on the comments, we 4 propose the changes of the manuscript. The revised version of the manuscript will be 5 prepared based on the decision of the Editor.

6

7 **Referee #1**

Referee's comment: The chronology of the core was based on 11 AMS-radiocarbon dated 8 9 mollusk shells. The data were shown in the previous publication (Pawłowska et al. 2014). The core depth vs. age relationship was completely chaotic, which is generally considered to 10 11 designate redeposition of the sediment. The authors had to discard 7 out of 11 dates to compile a sequence without obvious age inversions. Then the retained specimens were 12 13 assumed to be in situ, and the sediment sections containing the discarded specimens were interpreted to be redeposited. This is a weakly supported age model, but at least it was 14 15 published. In the new paper, the chronology is made even less convincing. The age model is implicitly replaced (p 3672 line 7 8). There is no explanation why the published age model is 16 discarded and which way the new model is more reliable. Moreover, the scatter of the 17 radiocarbon dates is disregarded, redeposition vanishes magically, and the authors do not 18 hesitate interpreting an uninterrupted sequence of climatic events. 19

20 *Response: As noted by the Referee, the previously published age model was weakly supported,*

however, it was sufficient for the study focusing on the direct comparison of microfossil and
molecular data. In the paleoceanographic reconstruction the dates should be as precise as
possible. Therefore, we decided to validate the age model with more sophisticated statistical
tools, instead of previously used linear interpolation. We agree with the Referees comments
regarding the age model, therefore, we propose to provide a more detailed description of the
age model (in the section Sediment dating):

"Four out of 11 samples were in chronological order and were used to establish an 27 28 approximated age model for the sediment core One sample contained post-bomb carbon, what indicate a post-1960 age. Six samples revealed age out of chronological order, 29 suggesting redeposition events. These samples occurred at sediment depths ~ 15-55 cm and ~ 30 80-115 cm and therefore, the data from these two intervals should be threatened with caution. 31 32 The age-depth model was made with the use of CLAM-R software (Blaauw, 2010). The age of the oldest layer was estimated to be ~ 965 AD. The sediment accumulation rate (SAR) in the 33 deepest part of the core (i.e., before 1800 AD; up to 120 cm) ranged from 0.1 to 0.125 cm yr 34

- ⁻¹. At ~ 1800 AD (120 cm), this rate increased to 1cm yr ⁻¹. In the upper layers (after ~ 1850
 AD; 70 cm), SAR decreased to 0.3cm yr ⁻¹."
- 3

Referee's comment: There are additional indications of sediment redeposition. Of the four retained dated specimens (Table 1), at least one is probably allochthonous. Hiatella arctica is shallow water species preferring active currents. This bivalve is unlikely to occur in muds at ca. 200mwd. The taxonomic composition of the dated bivalves is strange. I would expect the assemblage from fjord-basin muds to consist mainly of nuculanids and Thyasira.

9 Response: Hiatella arctica is widely distributed in a variety of Arctic settings. It is found primarily in shallow water, however, at e.g., Jan Mayen or East Greenland it has been also 10 11 found at the depths up to 270 m. In the North Atlantic H. arctica specimens have been found at depths down to 2380 m (Ockelmann, 1958; Meddelelser Om Grønland 122). The presence of 12 13 H. arctica in the study setting might be explained by active currents in the coring location. The study site was located close to the kind of sill (with a depth of approx. 135 m) between 14 15 Oceanografertangen and Hoferpynten, where, according to the mathematical model, the average near-bottom current speed is estimated to be 3.25 cm/s and maximal current speed is 16 17 up to 11.6 cm/s (Jakacki et al. 2015; Geophysical Research Abstracts Vol. 17, EGU2015-10520). 18

19

Referee's comment: The foraminiferal assemblage is strange too. If the bottom currents are sluggish and the sediments are muds, the assemblage contains way too high proportion of the sessile Cibicides lobatulus, and thus suggests redeposition. The extremely high number of foraminifera per gram in certain intervals (p.3674 line 5) may mean winnowing.

Response: As mentioned above, the bottom currents might be periodically active in the study 24 area, what might explain the presence of high number of C. lobatulus. However, C. lobatulus 25 might be associated with algae, hydroids or bryozoans (e.g. Dobson and Haynes, 1973; 26 Micropaleontol.). Ivanova et al. (2008; J. Foramin. Res.) suggested that C. lobatulus might 27 also survive inside the tubes of polychaetes. In our opinion, the variety of factors that might 28 affect the number of C. lobatulus in the study area precludes making any general conclusion. 29 30 We agree that winnowing might be one of the factors that affect foraminiferal abundance. However, our grain size data do not indicate sediment sorting. Therefore, 31

- 32 concluding about winnowing only based on the foraminiferal abundance seems speculative.
- 33

Referee's comment: Thus the radiocarbon dates and other evidence indicate that the core was retrieved from a redeposited package. Lobes of dislodged sediments are common under the flanks of the fjords of Svalbard. If the authors will insist their core is from a normal accumulation area, then instead of the single sentence "Four out of 11 samples were in chronological order and were used to establish anapproximated age model for the sediment core" (p. 3672) I recommend they provide more solid information on age control:

7 - Based on which data (bathymetry, seismics, else) the coring location was selected.

8 - What are the modern sediments at the location (based on box cores).

9 - On which basis the shells were selected for dating.

10 - Why some "shells identified to the highest possible taxonomic level" were identified to

11 "Bivalvia n.d." and "Gastropod n.d."? The mollusk fauna of Svalbard is comprehensively

12 studied (consult with Włodarska-Kowalczuk). What was wrong with these shells?

13 - Where the bivalve shells paired and did they have in-situ position?

14 Response: We agree with the Referee, that more detailed description of age model is

15 *necessary. As already mentioned, additional information will be added to the text.*

Indeed, fjords environment is dynamic and characterized by sediment reworking and redistribution by e.g. gravity flows (Elverhøi et al., 1983; Polar Res.) and bottom currents (Syvitski and MacDonald, 1982; Can. J. Earth Sci.). However, these processes influence mostly slopes and sills depostis. Moreover, in the periods of glacial advance/retreat the increased glacial meltwater discharge and suspension settling might result in creation of layer of unconsolidated sediment that could be easily resuspended and redeposited. In such case, signs of redeposition might be indicator of glacial-proximal environment.

The coring location was selected basing on the bathymetry and morphology of the sea 23 bottom. A flat seabed area has been chosen and checked with echo-sounder before coring. 24 The modern sediments are composed mainly of glaciomarine mud, with low sand content (less 25 than %; Pawłowska et al., in prep.). The information about the sediment type will be added to 26 the text. The core was dated based on all the shells found in the samples. Some shells were 27 28 fragmented, therefore the identification to species/generic level and the determination of shells position was not possible. The identification was performed by Maria Włodarska-29 Kowalczuk. 30

31

Referee's comment: I believe environmental DNA degrades rapidly with age. If the suggested
 age model is valid, then please demonstrate and discuss the deterioration of ancient DNA
 from the modern surface to the layers 1000 yr old at the bottom of the core.

Response: Indeed, some authors showed that DNA accumulates damage with time, thus, the
age of a sample might be a major factor that influences DNA preservation (Corinaldesi et al.,
2008; Mol. Ecol.). We would like to remind that in the palaeogenetic platform PALGENE (a
dedicated ancient DNA suite of laboratory), we could readily amplify c.a. 400 bp fragment
from our 1000 years old sample (see Pawlowska et al., 2014, Geobiol.). Although we did not
measure the degradation of DNA downcore, the fact that 400 bp fragments could be amplified
indicated that the DNA was preserved in relatively good conditions.

11 On the one hand, the Arrhenuis equation and the kinetics of well-known molecular mechanisms have been proposed to model the degradation of DNA molecules with time 12 13 (Willerslev et al. 2004; TREE). This model implies that a 100 bp molecule would easily survive a thousand years at the fjord temperatures of approx. 4 °C. On the other hand, other 14 15 authors indicate that there is no direct relationship between DNA preservation and time (e.g. Höss et al., 1996, Nucleic Acids Res.; Poinar et al., 1996, Science; Burger et al., 1999; 16 Electrophoresis). Several environmental conditions are key to preservation of DNA (Nielsen 17 et al. 2007; Environ. Biosafety Res.), which have not been extensively investigated in marine 18 sediment. Hence, enhanced DNA preservation is very likely in Arctic sediments because of 19 low temperatures and sediment mineralogical composition. Short DNA fragments can adsorb 20 to small sediment particles such as clay minerals, which are common in Hornsund. Adsorbed 21 22 DNA is more resistant to degradation by biotic and abiotic processes and remains detectable for extended periods of time (e.g., Franchi et al., 1999, Orig. Life Evol. Biosph.; Cai et al., 23 24 2006, Environ. Sci. .Technol.).

25

Referee's comment: There is 10-fold variation in the calculated sedimentation rate (Fig.3A).
Such large variation is not very plausible for the Late Holocene and is probably produced by
the imperfectness of the age model. In such a situation, derived variables, e.g. flux, calculated
via sedimentation rate become meaningless. Please replace the derived fluxes (IRD, foram
shells) with direct data (e.g. per g sediment).

Response: We do not agree with Referees' suggestion that the increase in sediment accumulation rate resulted from the imperfectness of age model. As discussed in the manuscript, the increase of the sediment accumulation occurred at the end of the LIA, when Svalbard glaciers reached their maximal Holocene extent. At that time, the tidewater glacier

fronts were probably located closer to the coring station than today, what caused the 1 2 increased sediment delivery and, in consequence, increase in sediment accumulation rate. Noticeably, the increase in the number of IRD per gram of sediment during the late LIA was 3 not followed by the increase in mean grain size, as it was observed in both precedent and 4 following periods. It is likely that the amount of fine-grained sediment delivered to the sea 5 bottom exceeded significantly the amount of coarse ice-rafted sediment (i.e., IRD) and 6 7 consequently, almost no change in mean grain size was observed. The adequate explanation 8 will be added to the text.

9 However, to provide more complete view of our data we decided to change the 10 figures' scale into sediment depth [cm] and to add the number of IRD grains per gram of 11 sediment to Figure 3. The information about number of foraminifera per gram of sediment is 12 already presented (Fig. 3G).

13

14 Ancient DNA

15 *Referee's comment:* Does this paper target the micropaleo community? I think it does. To be appreciated by the micropaleo auditorium, the paper, I believe, should have introduced a 16 concise overview that specifically answers the reader's most obvious question: Whether the 17 metagenomic technique provides a picture congruent to my fossil assemblages. To follow the 18 discussion the reader needs to feel how robust the metagenomic approach is, what the scale of 19 the mismatch between the fossil assemblage and aDNA in taxonomic and numerical 20 sensitivity is. The only relevant sentence in the Introduction provides insufficient information 21 "The study showed that aDNA record contained almost all of the species reported for 22 Hornsund from previous micropalaeontological investigations" (p.3668) and refers to the 23 previous paper (Pawłowska et al. 2014). Ok, I go to that paper. But I cannot find 24 comprehensive information. There is a rather confusing diagram; the description is too 25 general, non-specific, like the cited sentence above. And there is no control against the 26 fossilizable part of the assemblage that would show how accurate the technique is. 27

Response: Indeed, our research mainly targets the micropaleo community and the important aspects related to the ancient DNA data have been the focus of the previous paper (Pawłowska et al. 2014; Geobiol.). We agree with the Referee, that the match between micropaleontological and molecular data is one of the most important issues in paleogenetic studies. In the study of Pawłowska et al. (2014) we compared directly the results of micropaleontological and molecular analysis (for the comparison of frequencies of fossil specimens and aDNA sequences see Fig. 5 in the micropaleon paper) and we discussed the

possible reasons of the discrepancies between the records. It was not our intention to
replicate this discussion in the current paper. However, to provide a broader view of the
match between the fossil and molecular data we will add a more detailed description of
previous findings in the Introduction.

The paper of Lejzerowicz et al. (2013; Biol. Lett.) also demonstrated the poor match 5 6 between the microfossil and molecular views on the subsurface foraminiferal diversity. Such a 7 discrepancy is not surprising given the characteristics of these two approaches, which greatly differ in terms of studied material and analytical procedures. The accuracy of the molecular 8 9 methods is constantly improving with many respects and we developed an expertise for the 10 generation of foraminiferal high-throughput sequencing data (Pawlowski et al. 2014; Biol. 11 Bull.). The issue indeed relates to the match between the diversity obtained using DNA data and that obtained using morphological examination. The presence of monothalamous 12 13 foraminifera species that are not present in the microfossil record may affect the relative sequence abundances and the performance of the PCR method to enrich other species that 14 15 are expected from the microfossils examination. It has been recently discussed that species rarity and even species detection are affected by such skews when the diversity is high 16 (Youngblut et al. 2013, Appl. Environ. Microbiol.; Egge et al. 2015, Mol. Ecol.), especially 17 for the species that may exhibit sharply changing dominance patterns (Adams et al. 2013) 18 Microb. Ecol.). 19

20

Referee's comment: So I have to do this control myself, and I go to the data table (Supplement 2). The first surprise is that operational taxonomic units (OTUs) assigned to one species (e.g. Elphidium excavatum) are scattered through the list. This may indicate that nobody has really analyzed this table, because otherwise he would have certainly grouped OTUs of the same taxon together.

Response: According to the Referees comment, the Supplement 2 table was corrected and OTUs have been reordered.

28

Referee's comment: I choose the rotaliids, because they are least susceptible to postmortem decay, then lump all intervals, because the sediment package is dislodged, then select the most abundant fossil species in the census table (Supplement 1), and finally calculate their relative

32 frequencies. In order of abundance the principal rotaliids of the fossil assemblage are:

33 Elphidium excavatum 46 percent

- 1 Cassidulina reniforme 24
- 2 Nonion labradoricum 11
- 3 Cibicides lobatulus 9
- 4 Islandiella norcrossi/helenae 5
- 5 Buccella spp. 4
- 6 subtotal 100

7 The aDNA table shows numerous reads only for E.excavatum and C.lobatulus. Nonion 8 labradoricum is represented by a few reads, which is obviously an artifact. The other major 9 species are not detected. The control reveals that the aDNA technique fails to recognize 4 of 10 the 6 major species. Thus the technique fails to reveal the structure of the assemblage on the 11 species level. I suppose this conclusion applies equally to the monothalamids. I am not an expert and have no idea what is behind this poor performance: the incompleteness of the 12 13 modern foram DNA database; taxonomic or sequence mistakes in the modern database; the used SSU gene fragment is too long and degrades rapidly beyond recognition. Anyway, this is 14 15 an important result that should have been pronounced and discussed. The undetected rotaliid taxa are extremely numerous in the fjords. Their DNA is certainly out there, and it cannot just 16 17 disappear into thin air. A plausible assumption is their sequences are in the table but misidentified. I look into the massive reads of the exotic rotaliids. 18

19 - Globocassidulina biora is absent from the northern hemisphere. These numerous

- 20 reads may represent Islandiella norcrossi/helenae.
- 21 Pullenia carinata is absent or nearly so in the fjords. Its numerous reads most likely
- 22 are misidentified N.labradoricum.

23 - Cassidulina laevigata is nearly absent here. These numerous reads are probably misidentified

- 24 C. reniforme.
- 25 Cibicides wuellerstorfi does not dwell in the fjords. The numerous sequences are
- 26 probably misidentified, and then they may append to the C.lobatulus reads.
- 27 Epistominella exigua and E.vitrea occur in the fjords, but these numerous reads may
- be Buccella spp.
- 29 With these guesses the DNA frequencies of the principal rotaliids are:
- 30 Elphidium excavatum 10 percent
- 31 Cassidulina reniforme 35
- 32 Nonion labradoricum 11
- 33 Cibicides lobatulus 25
- 34 Islandiella norcrossi/helenae 3

- 1 Buccella spp. 15
- 2 subtotal 100

3 The correspondence to the fossil frequencies above is not perfect, but at least now it is

4 not a hopeless mismatch. The match perhaps could have been better if the sediments

5 were in situ.

6 Response: As above, we would like to refer to our previous paper (Pawłowska et al., 2014; 7 Geobiol.), where we show that it is possible to identify sequences of many rotaliids present in 8 the fossil record, but there was no match between the relative frequencies of sequences and 9 microfossils. In the article Pawłowska et al. (2014) we discussed the possible causes of 10 mismatch. The main of the presented paper adressed to the micropaleontological community 11 is to raise the attention to the importance of monothalamous foraminifera as 12 paleoenvironmental indicators.

We thank the Referee for the careful data re-analysis and for all the suggestions, but we do not agree on the relevance of replacing the attribution of the DNA sequences with that of the morphospecies on the basis of their ranks in terms of relative abundances. We are confident that our assignments are correct, given the available data in the reference sequence database:

- (i) the OTUs assigned to Cassidulina laevigata could correspond to C. reniforme, at
 least in the case of one of two types of sequences found in Faroe Islands. This
 would need to be verified by future SEM documenting of barcoded specimens.
- (ii) some OTU sequences are very closely related to Cibicides wuellerstorfi sequences
 but also to C.lobatulus. As it has been shown by Schweizer et al. (2008; Mar.
 Micropaleontol.) these two species are very closely related genetically. It is quite
 possible that all species identified as C. wuellerstorfii in our dataset belong to C.
 lobatulus or another closely related genotype.
- (iii) Globocassidulina biora certainly do not correspond to an Islandiella species. These
 reads might originate from a small cryptic species of Globocassidulina that has
 not been observed in larger fraction.
- (iv) the eDNA sequences of Pullenia carinata certainly do not correspond to Nonionella
 labradorica. Like above, this could be an indication of the presence of Arctic
 Pullenia closely related to the Antarctic species.
- 32 (v) the eDNA sequences of Epistominella exigua certainly do not correspond to
 33 a Bucella species, which phylogentically belong to a completely different clade.

- The obtained sequences are most probably of some small Epistominella species
 common in fjords.
- 3 *Referee's comment:* The aDNA shows that Stainforthia sp. is a major player in the assemblage
- 4 (Supplement 2). Its frequency in the fossil assemblage is severely underestimated probably
- 5 because of the small size (e.g. Stainforthia feylingi). A mesh size smaller than 100um
- 6 (which is commonly used in Svalbard) would have retained the important small taxa.

7 This may be a message that will reach the micropaleo community. Other comments habit to8 consider only those peaks that are supported by three or more data points.

- 9 Response: Indeed, the use of 100 µm mesh size might cause the underestimation of the
- 10 *abundance of smaller taxa, such as* Stainforthia *as well as* Epistominella *and probably some*
- 11 other small rotaliids. We already discussed this issue in the previous paper (Pawłowska et al.,
- 12 2014; Geobiol.). As highlighted above, our message is not to recover the exactly same
- 13 composition as fossil samples, but to show through aDNA that foraminiferal assemblage may
- 14 *comprise new paleo-indicators among soft-walled monothalamids.*
- 15

Referee's comment: The figures are of good quality. The figures will probably change after revision, so I will not speak now whether they all are necessary. The 'Years AD' scale is used in several figures. Its irregular increment is extremely confusing. I suggest the use of a core depth scale. The estimated ages can be shown on an additional age-model graph within each figure.

- *Response: We agree with the Referees suggestion. The figures scale will be changed from years AD to sediment depth.*
- 23
- 24 *Referee's comment:* The language is quite good but will need some amendment.

25 *Response: The manuscript will be corrected by a native speaker.*

26

27 *Referee's comments:* Minor matters

- The water depth at the coring location and its coordinates are never mentioned. There
- is a large distance discrepancy between the coring location shown in this paper (Fig.1)
- and in the previous one (Pawłowska et al. 2014). The M&M section reports that the
- 31 core was taken in the central part of the fjord (not clear whether it means along the axis
- 32 or between the flanks), in another place it is written that the core was taken under the
- 33 southern flank. Please, find out where the core was located.

- 1 Response: The Referee is right that the description of core location might be confusing. The
- 2 core was taken in the central part of the fjord, but not in the fjord axis. The adequate
- 3 explanation and the information about the coordinates and water depth is added to the text
- 4 *and to Figure 1.*
- 5 The Study Area section lacks information on the modern setting at the coring location.
- 6 Fig. 1: There must be at least two latitude marks.
- 7 Please provide captions for the supplements.
- 8 *Response: Supplements captions have already been provided with the manuscript.*
- 9 p.3666 line 10: "the distant position of the glaciers" is not very clear
- 10 p.3668 line 17: do not capitalize Eukaryotes.
- 11 line22: almost all species
- 12 lines 25-29: not specific, vague meaning
- 13 p.3669, line 10: a wide no-sill outlet
- 14 line 10: "facilitates its penetration by oceanic waters" is awkward. Rephraseline 11: awkward
- 15 "coastline is variable"
- 16 line 11: "basins, separated by sills" is geometrically unclear
- 17 p.3683 lines 3-9: not specific, vague meaning.
- 18 *Response: The text and figures will be corrected according to the Referees suggestions.*
- 19

20 **Referee #2**

- *Referee's comment:* Abstract Page 3666 line 12: The early LIA: : :.This is a strong claim since
 only one sample in this climate interval was analyzed for aDNA. Here, as well as throughout
 the manuscript there is a need to describe the growth or environmental requirements of
 described species in more detail.
- 25

Response: We agree with Referee that this is a strong claim, considering that it was based on only one sample. On the other hand, the sequences of Hippocrepinella hirudinea *and*Cedhagenia saltatus *made up approx.* 23% *of the monothalamid sequences in the above- mentioned sample, while in the other samples their abundance was rather minor. Therefore, the statement might be justified.*

31 Our knowledge about monothalamids ecology is limited, as they are usually not 32 included in the studies of modern foraminifera assemblages. Therefore, the ecological 33 interpretation of monothalamids data is often difficult or even not possible (see page 3680, lines 2-3; page 3682, lines 25-26). The need of increasing our knowledge about
 monothalamids ecology was one of the messages of the paper (page 3684, lines 6-7).

3

Referee's comment: Page 3666 line 17: Also here, only an expert would know what an increase in the relative abundance of these two species implies. In general: Are environmental sequences really that informative that you can say which exact species were present? I think that you can only describe environmental sequences at species-level if the corresponding microfossil is present. If not, it is a safer bet to stay at genus or family level.

9

10 Response: The taxonomic assignment of sequences strongly depends on the reference
11 database. When this database is rich, as in the case of Arctic foraminifera, the number of
12 assigned species might be high.

13 Indeed, to the paleontological purpose of the study, using species names is accessory because it cannot be ascertained whether a sequence assigned to a given species originated 14 15 from a microfossil assigned to this species, for which there might be no reference sequence available in the sequence database. We analyzed two different species entities: the 16 morphospecies based on microfossils and the OTU based on the SSU rDNA fragment. Both 17 species entities can be assigned to known species name and the several OTUs that could be 18 assigned to the same known species name may in fact represent different strains or genotypes 19 of this species name. Indeed, the 37f region does allow the assignment of environmental 20 sequences down to the species level, provided that the species is present in the reference 21 22 database. We chose to amplify and sequence a short fragment of the SSU rDNA corresponding to the hypervariable region 37f, which only exists among the foraminifera. 23 24 This region has been identified as an ideal barcode for species-level assignment of foraminiferal sequence (cf. Pawlowski and Lecrog, 2010; J. Eukaryot. Microbiol.). The 25 26 exactness of the sequence assignment also depends on the quality of the sequencing data and on the completeness of the reference database. We agree that to some extent, the variation in 27 28 the 37f region may be such that it may represent another species. This is why we accounted for the possibility of wrongly matching environmental sequences to distant species by taking 29 30 only the consensus of the taxonomies of all the reference sequences that match within a threshold of 5 differences, which complies with the Referees' thought. 31

32

Referee's comment: Introduction Page 3667 Line 13: However, to fully understand: : :.This
 paragraph seems to be out of place. Namely, this study does not result in the full

understanding of the consequences of climate changes in the Arctic. Please stick to claims and
aims that you have studied and discussed. The first few paragraphs should only discuss what
is known about past climate in the region. Then: what the big unknowns are, how forams can
help, limitations of the analysis of fossils, how aDNA can help, followed by what you did
here and a few lines about the major findings.

6

Response: The aim of the mentioned paragraph was to point at fjords' potential of providing high-resolution sedimentary record, which might provide valuable information about climate- driven environmental changes in the Arctic. We agree that the first sentence of the paragraph is irrelevant, therefore we propose to replace it with: "Fjords are unique form of coastline, that are affected from two directions: the glaciated land and the ocean, rendering the fjord system a sensitive indicator of climate change phenomena."

13

Referee's comment: Line 26: Therefore, it is crucial: : :This is a very big claim since a complete model of past environmental changes in the Arctic fjords is not provided with this study. Hence, the need for better structuring the introduction.

17

18 Response: The sentence emphasize the need of creation of a wide range of paleoceanographic 19 proxies sourcing from different research methods for better understanding past climate and 20 environmental changes. We have not claimed that we will provide a complete model of 21 paleoenvironmental changes in the Arctic. We propose to change the sentence to: "To study 22 accurately the climate-driven environmental variability in the past, it is crucial to create a 23 network of proxies carrying different but complementary information."

24

Referee's comment: Page 3668 Line 19: Metagenetics (the analysis of many genes) is a cool
but also vague term. Please be more specific about what "metagenomics" was performed (i.e.,
the identification of past foraminifera including non-fossilized taxa through PCR
amplification and sequencing analysis of preserved sedimentary taxonomic marker genes.

29 Response: Herein, the term metagenetics refers to genetic material obtained from
30 environmental samples. Therefore, we will replace the term with 'ancient environmental
31 DNA'.

Referee's comment: Page 3668 Line 25 and following: The ignorant reader might wonder why
 you can detect the DNA but not the microfossils. Please say a few words about why the DNA
 might still be present.

4

5 Response: We agree that there is a need to provide more information about the match
6 between the fossil and molecular data. Therefore, we propose to add a more detailed
7 description of previous findings in the Introduction:

8 "To include monothalamids into palaeoecological studies of the Arctic foraminifera 9 we analysed the ancient foraminiferal DNA record from the last millennium from Hornsund (Pawłowska et al., 2014). The study showed that aDNA record detected most of species 10 11 reported for Hornsund from previous micropaleontological investigations (e.g., Hald and Korsun, 1997; Pogodina, 2005).including species that dominate fossil assemblage (i.e., E. 12 13 excavatum, C. reniforme, C. lobatulus and N. labradorica; cf. However, the number of aDNA sequence reads and fossil specimens differed considerably. The richness of the foraminiferal 14 15 communities revealed by molecular analysis was much higher than in the fossil record, mainly due to the detection of high number of monothalamous species that were not preserved 16 during the fossilisation process as well as small-size species that are not retained on 17 micropaleontological sieves." 18

19

Referee's comment: Page 3669 Line 3: The Pawlowska et al., 2014 seems to be very important to cross read to fully explore this study. I was unable to get an electronic version despite being able to use the online libraries of two major universities. I strongly suggest to describe major findings and relevant methods from this paper in more detail also in this paper.

24

Response: We agree with the Referee that more detailed description of previous findings and
analytical methods will facilitate the understanding of the presented study. The adequate
information will be added to the Introduction and Material and methods. However, the .pdf
file of Pawłowska et al. (2014) paper can be obtained via Google Scholar.

29

32

Response: The Principal Component (PC) analysis showed the contribution of each
foraminiferal species in the assemblage, what enables us to identify the dominant species. The

Referee's comment: Page 3671 Line 14: Please describe in a bit more details what this
statistical approach exactly does.

taxa that favor similar environmental conditions may have high scores on one PC, indicating 1 2 their participation in the assemblage. The adequate explanation will be added to the Material and methods. 3

4

Referee's comment: Page 3671 Line 19: For reasons mentioned two comments ago: Please 5 provide a brief summary of these methods here. I don't think that the reader needs to be able 6 7 to cross read the 2014 paper to find out what methods have been used.

- 8
- 9

Response: As mentioned above, the additional information will be added to the text.

10

Referee's comment: Results Page 3673 Line 18: Spell out VPDB the first time. 11

12

13 Response: It has been corrected.

14

15 Referee's comment: Page 3674 Line 21 and following: It would have been nice to have seen a similar type of analysis to identify indicator taxa and their importance to explain 16 17 environmental stages for the molecular data. However, to do so you would need a much higher sampling resolution such as was the case for the microfossil work. I am not sure why 18 the sampling resolution for the aDNA data is not the same. Extracting DNA and subsequent 19 sequencing has become very cheap. It would have been a month or so extra work to get all the 20 DNA extracts, do the PCRs and to prepare the libraries for sequencing. I have more comments 21 22 about this later on.

23

Response: Statistical analysis (in our case PC analysis) is based on the quantitative data (i.e. 24 the absolute number of fossil specimens). The aDNA data is mainly qualitative, therefore, the 25 26 results of statistical analyses of molecular data will be strongly biased. The quantitative analysis of aDNA is still a challenging issue. The aDNA data should be interpreted carefully, 27 28 as it is not possible to establish the direct relationship between the number of specimens and the number of ribosomal sequences, due to the e.g., interspecific variability of number of 29 30 rDNA copies. In ancient DNA studies major difficulties arise also from DNA degradation and chemical modification. Therefore, the absolute number of sequences should be interpreted 31 32 with caution; however, it is possible to identify the dominant species based on the sequence proportion (Weber and Pawlowski, 2013; PLoS ONE). The aspects of qualitative and 33

quatitative molecular data analysis were discussed in the last paragraph of the discussion
 (see page 3682, line 29 and following).

We agree with the Referee, that higher sampling resolution and higher amount of data 3 will provide more complete view of changes in foraminiferal assemblages. However, the 4 presented as well as previous study (Pawłowska et al. 2014; Geobiol.) were the first attempts 5 to analyze the ancient foraminiferal DNA in the Arctic. We did not know if foraminiferal DNA 6 7 is preserved in Arctic marine sediments. Therefore, the chosen sampling resolution is not as 8 high as in case of micropaleontological analysis. As described in Material and methods, the 9 samples for molecular analyses were taken onboard, directly after taking the sediment core. For aDNA analysis, subsurface sediment samples were taken from the inner part of the core. 10 11 In order to prevent the disruption of the core structure and therefore cross-contaminations, the core tube was bored at each selected depth and ~ 5 grams of sediment was sub-sampled 12 13 using disposable spoons. Additional spoons were used to carefully remove the outer part of the core. This method does not allow to perform the subsampling with as high resolution as in 14 15 case of micropaleontological analysis. Later, the core was cut into 1-cm slices and the material was used for micropaleontological and sedimentological analyses (page 3670, lines 16 11-15). Therefore, it was not possible to increase the resolution of molecular sampling 17 thereafter. One of the possibilities to increase the amount of molecular data would be to 18 extract larger volume of sediments, what shall be done in the future studies. 19

20

Referee's comment: Page 3675 Line 10 and following: Please provide more detail in the
methods so that it becomes clear how the # of OTUs was determined. The reader should not
have to get a copy of the 2014 paper to understand this study.

24

25 *Response: As mentioned above, more detailed description will be added to the text.*

26

Referee's comment: Same page line 24: Are these the only possible most similar sequences
(i.e., top hit returns from BLAST)? Often several species or genera have the same sequence
similarity. Please make sure to be precise about the true taxonomic level that can be revealed
from the sequences. See also earlier comment about this.

31

32 *Response: We would like to remind the we did not use BLAST but global sequence alignments*

33 using the Needleman-Wunsch algorithm, based on which we calculated the distance by

34 counting each gap and substitution as a difference, allowing up to 5 differences to perform a

"species" level assignment (Pawlowski et al., 2014, Biol. Bull.; Esling et al. 2015, Nucleic 1 2 Acids Res.). This "species" level is accessory as explained above, and thus do not refer to any formal species nomenclature but rather serves to provide a gross taxonomic information since 3 (1) the reference sequence database is not complete for the Svalbard area, (2) species level 4 assignment for monothalamous foraminiferans are bound to revision and (3) it suffice to 5 6 reliably document diversity patterns observed based on the OTUs, that may entail a 7 taxonomic information more precise than that conveyed by the microfossils (cryptic species resolution). Within 5 differences, each of the eleven OTU sequences implicated in the 8 9 reviewer's question were matching one or several reference sequences, but when multiple 10 matches arose, then the taxonomies of all the matching reference sequences were congruent at the formal "species" level. In fact, only one of these 11 OTU sequences matched to more 11 than one reference sequence within 5 differences (one Micrometula reference sequence from 12 13 Scotland and another from Skagerrak).

14

Referee's comment: Page 3676 Line 8 and following: As mentioned earlier: This claim is
based on only one sample from that climate interval.

17

Response: As mentioned above – we agree that higher resolution of sampling will provide
more data and more complete view of the foraminiferal community. However, in the early LIA
(which encompasses two samples – at 150 m, dated to be ~ 1550 AD and at 125 m, dated to
be ~ 1800 AD), the percentage of sequences of Hippocrepinella hirudinea and Cedhagenia
saltatus was much higher than in the precedent and following periods. Therefore, the
statement might be justified.

24

Referee's comment: Discussion: Page 3677 Line 9: I think that the sampling resolution is too
low to make such claims. Please inform a bit more about what is known about the growth or
environmental requirements of Toxisarcon.

28

Response: As mentioned above (see the response to the first comment), monothalamous
foraminifera are important but largely ignored component of Arctic meiofauna. So far, three
species of Toxisarcon have been described: T. synsuicidica (Cedhagen and Pawlowski, 2002;
J. Foramin. Res.), T. alba (Wilding, 2002; J. Foramin. Res.) and T. taimyr (Voltski et al.,
2015; Mar. Biodiv.). However, only Wilding (2002) provided information about their possible

34 environmental preferences: the specimens were found buried or semi-buried in well

oxygenated sand. In Svalbard, Gooday (2005; Mar. Biol. Res.) found unidentified Toxisarcon
specimens in the inner parts of three fjords, dominated by glaciomarine mud. To conclude,
there is almost no data on Toxisarcon ecology and distribution patterns. Therefore, we were
able to suggest only the link between Toxisarcon occurrence and phytoplankton-originated
organic matter input (as presented in page 3677, line 15)

Referee's comment: Same page line 17: Is the d18O at 1600 AD really that different to link
this to an increase of melt water delivery etc?

9 Response: As written in the mentioned line, the peak was slight. Our interpretation was

10 supported by other proxies (IRD, foraminiferal fauna) as well as previous findings from

11 Hornsund fjord (Majewski et al., 2009).

12

Referee's comment: Page 3678 Line 8 and following: I don't see why this is obvious when looking at Fig. 3. When looking at the scale, Islandiella spp. seem to have never exceeded more than 3.5% of the total foram distribution.

16

17 *Response: As mentioned in the manuscript, the peaks were slight. However, the percentage of*

18 Islandiella spp (I. norcrossi and I. helenae) made up 11% of the total assemblage. However,

19 *the* Islandiella *spp. peak was not interpreted separately, but was supported by other proxies.*

20

Referee's comment: Page 3682 Line 25: This is true but with a substantially higher sampling resolution throughout the core, it would have been possible to perform an indicator species analysis to identify which taxa show a statistically significant response to the various environmental stages. This way even unnamed environmental sequences could potentially become proxies for certain conditions in comparable settings.

26

Response: Indeed, the statistically-supported analysis might allow to relate the presence of
environmental sequences to certain environmental variables. However, as explained above,
the proper statistical analysis of our aDNA data is not possible because of limited number of
sequences. The results of such analysis will be strongly biased and the interpretation will be
speculative. However, we hope, that further development of environmental aDNA research
will overcome these limitations ad will provide sufficient amount of data to perform statistical
analyses.

Referee's comment: Page 3682 final paragraph: This paragraph about the problems with 1 aDNA work is highly speculative. You don't actually have empirical proof that your DNA is 2 degraded and if this differs between intervals. The sediments analyzed here are relatively 3 young. A much higher sampling resolution (e.g. every other cm or so) combined with 4 statistical approaches will most likely reveal highly significant changes in the species 5 distribution as a result of major climate shifts. There will probably be less need to write a 6 7 negative and speculative paragraph about the things that can go wrong with the aDNA 8 approach. Right now this paragraph is totally out of place.

9

Response: We agree that higher sampling resolution and statistical analysis of data will 10 11 provide more detailed paleoenvironmental information. As discussed above, the statistical analysis of non-quantitative data will be strongly biased and interpretation will be 12 13 speculative. The final paragraph tackles the important issue of quantitation of aDNA data. In the modern environment, the establishment of the relationship between the number of 14 15 sequences and number of specimens is possible through the normalization of the results, according to, e.g., the interspecific variability of number of rDNA copies (Pawlowski et al., 16 2014; Biol. Bull.). In case of aDNA data additional difficulties arise from the degradation of 17 the material and the limited number of sequences, so the data quantitation is much more 18 complicated. The paragraph will be modified to emphasize the fact, that the presented aDNA 19 data can be only interpreted qualitatively and therefore, an ancient environmental DNA 20 approach should be used as a complementary source of information, supported by other 21 22 proxies.

23

Referee's comment: Table S2: Please make sure to identify the highest taxonomic level for
each OTU based on Blast results (e.g., if an OTU shows the same highest similarity with
multiple species use genus or even family level).

27

Response: Again, we would like to remind that we did not use BLAST because the short length of the environmental sequence reads makes it possible to employ global alignments. As explained in the article Pawłowska et al. (2014; Geobiol.) and several others (Pawlowski et al. 2014, Biol. Bull.; Pawlowski et al. 2014, Mol. Ecol. Resour.; Esling et al. 2015, Nucleic Acids Res.; Pochon et al., 2015; Marine Poll. Bull.), we did account for the cases when an OTU shows the same highest similarity with multiple species, by assigning the OTU to the taxon that makes consensus among all the taxonomies of multiple species. In fact, our

approach is even more conservative because we kept all the reference sequences that match within the threshold of 5 differences, and hence not only the reference sequences that would show the highest similarity. This means that if an environmental sequence is 100 % similar to a reference sequence belonging to genus A/species A but that there is a reference belonging to genus A/species B that is distant from A by less than 5 differences, then the environmental sequences would be assigned to genus A only, because of the conflict between species A and species B.

- Palaeoceanographic changes in Hornsund Fjord (Spitsbergen, Svalbard) over the last
 millennium: new insights from ancient DNA.
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1 Abstract

This paper presents a reconstruction of climate-driven environmental changes over the 2 millennium in Hornsund Fjord (Svalbard), based on sedimentological and 3 last micropalaeontological records. Our palaeo-investigation was supported by an analysis of 4 foraminiferal ancient DNA (aDNA), focusing on the non-fossilized monothalamous species. 5 The main climatic fluctuations during the last millennium were the Medieval Warm Period 6 (MWP, 1000 AD – 1600 AD), the Little Ice Age (LIA, 1600 AD – 1900 AD) and the Modern 7 Warming (MW, 1900 AD – present). Our study indicates that the environmental conditions in 8 Hornsund during the MWP and the early LIA (before ~ 1800 AD) were relatively stable. The 9 beginning of the LIA (~ 1600 AD) was poorly evidenced by the micropalaeontological record 10 but was well marked in the aDNA data by an increased proportion of monothalamous 11 foraminifera, especially Bathysiphon sp. The early LIA (~ 1600 AD - ~ 1800 AD) was 12 13 marked by an increase in the abundance of sequences of Hippocrepinella hirudinea and Cedhagenia saltatus. In the late LIA (after ~ 1800 AD), the conditions in the fjord became 14 15 glacier-proximal and were characterized by increased meltwater outflows, high sedimentation and a high calving rate. This coincided with an increase in the percentages of sequences of 16 Micrometula sp. and Vellaria pellucidus. During the MW, the major glacier fronts retreated 17 rapidly to the inner bays, which limited the iceberg discharge to the fjord's centre and caused 18 a shift in the foraminiferal community that was reflected in both the fossil and aDNA records. 19

The palaeoceanographic changes in the Hornsund fjord over the past millennium were driven mainly by the inflow of shelf-originated water masses and glacial activity. However, the environmental changes were poorly evidenced in the micropalaeontological record, but they were well documented in our aDNA data. We considerably increased the number of potential proxy species by including monothalamous foraminifera in the palaeoecological studies.

1 **1 Introduction**

The general outline of climate development over the last millennium is the Medieval 2 Warm Period (MWP), followed by cooling during the Little Ice Age (LIA) and warming in 3 the 20th and 21st centuries (Modern Warming; MW). In the European Arctic, the temperature 4 increase during the MWP and MW was correlated with the strong influence of the Atlantic 5 Water inflow and associated heat transport (Wanamaker et al., 2012). In contrast, the 6 weakening of the Atlantic Meridional Overturning Circulation (AMOC) and the lower heat 7 transport to the Arctic might have been responsible for the LIA cooling (Lund et al., 2006). 8 Changes in the Arctic Ocean heat budget were associated with significant changes in the 9 cryosphere, especially the gradual decreases in glacier mass balance and the extent of the sea-10 ice cover in the last century (e.g., D'Andrea et al., 2012; Jernas et al., 2013). 11

12 Fjords are a unique form of coastline that are under the influence of the glaciated land 13 and the ocean. Hence, fjord systems are sensitive indicators of climate change phenomena. 14 However, the greatest effort in studying the Holocene history of Svalbard has mainly focused 15 on the shelf area (e.g., Hald et al., 2007; Rasmussen et al., 2012; Łącka et al., 2015). There 16 have been only a few high-resolution studies of the sedimentary record of the Svalbard fjords 17 from the last millennium (e.g., Majewski and Zajączkowski, 2007; Majewski et al., 2009).

The environmental changes during the last millennium observed in the Svalbard shelf were correlated with the interplay of Atlantic and Arctic water masses (Kubischta et al., 2011; Jernas et al., 2013). The Hornsund fjord is strongly influenced by tidewater glaciers, and thus the sedimentary record in this fjord might indicate that enhanced melt-water delivery increased the sediment accumulation and restricted the sea productivity during the periods of glacial retreat. To accurately study climate-driven environmental variability in the past, it is crucial to create a network of proxies that carry different but complementary information.

Foraminifera are widely used as proxies of past and present environmental changes in 1 2 all types of marine environments. However, palaeoceanographic reconstructions have focused on multi-chambered hard-shelled taxa and have ignored soft-walled, monothalamous species, 3 which often dominate foraminifera assemblages in high latitude regions (Gooday, 2002). 4 Monothalamous foraminifera with organic or predominantly organic test walls are 5 traditionally defined as allogromiids (Gooday, 2002). However, morphological and molecular 6 evidence indicate that 'allogromiids' does not refer to a coherent taxonomic group but rather a 7 group what is scattered between several monothalamous clades (Pawlowski et al., 2002; 8 Lejzerowicz et al., 2013a). The group includes organic-walled ('naked') and agglutinated 9 forms of various shapes (Cedhagen et al., 2002). Monothalamous foraminifera with a test 10 build of agglutinated particles are referred to as 'saccamminids' or 'psammosphaerids.' The 11 term 'allogromiid' is sometimes applied to monothalamous taxa, irrespective of wall type. 12 13 Therefore, literature reports might include saccamminids and psammosphaerids in the allogromiids group (Gooday, 2002). 14

Previous studies have shown that it is possible to consider monothalamous and 15 polythalamous foraminifera (Lejzerowicz et al., 2013b) and other groups of non-fossilized 16 eukaryotes (e.g., Coolen et al., 2013, 2006; Boere et al., 2011) in palaeoecological surveys 17 using an ancient environmental DNA (aDNA) approach. To include monothalamids in 18 palaeoecological studies of Arctic foraminifera, we analysed the ancient foraminiferal DNA 19 record from the last millennium from Hornsund (Pawłowska et al., 2014). The study showed 20 that the aDNA record detected most of the species reported for Hornsund from previous 21 micropalaeontological investigations (e.g., Hald and Korsun, 1997; Pogodina, 2005), 22 including the species that dominate the fossil assemblage (i.e., E. excavatum, C. reniforme, C. 23 lobatulus and N. labradorica). However, the number of aDNA sequences read and fossil 24 specimens differed considerably. The richness of the foraminiferal communities revealed by 25

the molecular analysis was much higher than that in the fossil record, mainly due to the
 detection of a high number of monothalamous species that were not preserved during the
 fossilization process and small-size species that are not retained on micropalaeontological
 sieves.

The aim of this study was to reconstruct the climate-driven environmental changes over the last millennium in Hornsund, with decadal to multi-decadal resolution. The promising results of our previous study (Pawłowska et al., 2014) encouraged us to use our aDNA data to supplement the palaeoclimatic record based on traditional proxies. We evaluated the potential use of monothalamous foraminifera as palaeoceanographic proxies, showing that they might provide valuable environmental information that is complementary to the data obtained with traditional microfossil proxies.

12

13 2 Study area

Hornsund is the southernmost fjord of Spitsbergen. It is connected to the open sea by a wide no-sill outlet. The fjord's coastline encompasses several glacier - proximal basins that are separated by sills. In its central part, the water depth exceeds 200 m and varies from 55 m to 180 m in the glacier - proximal basins (Fig. 1).

The hydrology of the fjord is under the influence of two main exogenous water 18 masses: the Atlantic Water (AW) and the Arctic Water (ArW). The AW is warm and saline, 19 and its temperature and salinity are usually defined as \geq 3°C and \geq 34.9, respectively. The 20 ArW is colder and fresher, and its salinity varies along the Spitsbergen shelf due to the 21 freshwater outflows from fjords (Cottier et al., 2005). The AW and ArW mix over the 22 continental shelf to form the Shelf Transformed Water (STW), which has a temperature and 23 salinity of 1°C and 34.7, respectively. The STW mainly occupies the outer and central parts of 24 the fjord. The Local Water (LW) is formed directly in the fjord by convectional mixing during 25

cooling in the fall and winter or the interaction between the warmer fjord water and glacier
 fronts (Svendsen et al., 2002).

Seventy percent of the Hornsund catchment area is covered by glaciers (Hagen et al.,
1993). The melting of the eight major tidewater glaciers results in an important sediment
delivery to the fjord. The modern sediment accumulation rate varies from 0.5 to 0.7 cm yr⁻¹ in
the central and inner parts, respectively (Szczuciński et al., 2006).

During the last millennium, Hornsund was subjected to major environmental changes, including the MWP, cooling and glacial advances during the LIA, which culminated in the period from 1600 AD to 1900 AD, and warming and massive glacial retreats during the 20th and 21st centuries (MW; Ziaja, 2001; Pälli et al., 2003). These changes were correlated with the variability in the inflow of the cold ArW and warm AW and were recorded in foraminifera assemblages and the stable isotope compositions from foraminiferal tests (Majewski et al., 2009).

14

15 **3 Material and methods**

A 2 m long sediment core HF_2011 was taken with a gravity corer from the R/V 16 Oceania during a cruise in July 2011. The sampling station was located in the central basin of 17 the fjord, in a flat seabed area at a depth of 135 m (Fig. 1). The core was subsampled onboard 18 for aDNA analyses and frozen at -20°C until further analyses were conducted at the Institute 19 of Oceanology, Polish Academy of Sciences (Sopot, Poland), as described in Pawłowska et al. 20 (2014). After thawing at 4°C, the core was split into two parts longitudinally, and each half 21 22 was cut into 1 cm slices for micropalaeontological and sedimentological analyses. Carbonate shells were picked for accelerator mass spectrometry (AMS) ¹⁴C dating. 23

24

25 **3.1 Grain-size and stable isotope analyses**

The grain size analysis of the sediment slices was conducted using a Mastersizer 2000 1 laser analyser coupled with a HydroMU device (Malvern Instruments, Malvern, UK) and 2 supported by the wet sieving of fractions larger than 250 µm. The granulometric data were 3 analysed with the use of the GRADISTAT 8.0 software program (Blott and Pye, 2001). Dried 4 and weighted sediment fractions > 250 and 500 μ m were used for IRD analyses, and at least 5 500 mineral grains from each fraction were counted under a stereomicroscope. The IRD was 6 expressed as the number of grains per gram of sediment (grain g^{-1}) and number of grains per 7 square centimetre per year (grain $\text{cm}^{-2} \text{ y}^{-1}$). 8

Stable isotope analyses were performed on foraminiferal tests selected from 54
sediment layers. From each layer, 10 to 12 well-preserved specimens of *Cibicidoides lobatulus* were selected. The measurements were performed on a Finnigan-MAT 253 mass
spectrometer coupled to a Kiel IV carbonate preparation device (Thermo Fischer Scientific,
University of Florida). The resulting values were compared to isotopic standard NBS-19 and
expressed in standard δ notation relative to <u>Vienna Pee Dee Belemnite (VPDB)</u>.

15

16 **3.2 Foraminiferal counts and molecular analysis**

Prior to the analysis of the fossil foraminiferal assemblages, 74 selected sediment 17 samples were dried, weighed and wet-washed through sieves with 500 and 100 µm openings. 18 19 Each sample was divided using a dry microsplitter, and at least 300 specimens from each sample were counted. The foraminiferal counts were reported as percentages of the total 20 assemblage and as the number of individuals per square centimetre per year. The fossil 21 22 foraminifera assemblage was analysed with an orthogonally rotated (varimax) Q-mode Principal Component (PC) analysis, using commercially distributed software (SYSTAT 11). 23 24 Taxa with abundances > 2 % of the total assemblage in at least one sample were analysed. Each PC was defined by the dominant (and eventually accessory) species. The PCs were 25

referred to foraminiferal assemblages (FA) named after the dominant species. <u>The PC scores</u>
 <u>showed the contribution of the selected species to each PC. PC loadings higher than 0.4 were</u>
 <u>regarded as statistically significant (Malmgren and Haq, 1982).</u>

The analysis of molecular data from 12 selected layers was described in detail in 4 Pawłowska et al. (2014). Briefly, the total DNA of each of the 12 sediment samples was 5 6 extracted with a PowerSoil DNA kit (MoBio). A 3' SSU rDNA fragment including the 7 foraminifera-specific 37f hypervariable region (Pawlowski and Lecroq, 2010) was PCR amplified from environmental DNA. The SSU rDNA sequences were then obtained either 8 based on cloning and Sanger sequencing or after library-preparation and Illumina high-9 10 throughput sequencing. For the cloning-based Sanger sequencing, the environmental DNA was PCR amplified 11 with s14F3 forward primer combined with s17, s15.2 or s15ROTEX as reversed primers. The 12 13 sizes of the resulting fragments were of ca. 400 bp for s14F3/s17 and ca. 200 bp for both the s14F3/s15.2 and s14F3/s15ROTEX amplifications. Nested PCR was performed for samples 14 with s14F3/s17 with the use of a s14F1/s17 primer combination. Positive and controlled PCR 15 products of expected sizes were cloned and Sanger sequenced as in Pawlowski et al. (2011). 16 The resulting raw sequences were manually corrected and edited using Codon Code Aligner 17 and Seaview 4.0 (Gouy et al. 2010). 18 For high-throughput sequencing (HTS), s14F0 and s15 primers tagged with unique 19

20 sequences of 5 nucleotides were used. The size of the obtained fragment was ca. 100 bp. The

amplicons were quantified and pooled in equimolar quantities. A library preparation was
 performed using a TruSeq library-preparation kit (Illumina) and was loaded onto a HiSeq

23 instrument for a paired-end HTS run of 2*100 cycles at Fasteris SA (Plan-les-Ouates,

24 Switzerland). The processing of the HTS sequence data, including quality filtering, sample

25 demultiplexing, strict dereplication into unique sequences and operational taxonomic units

(OTUs) selection was realized according to Lejzerowicz et al. (2013a), except that unique
 sequences that were composed of up to 10 reads in a sample were removed. The results were
 presented as OTUs-to-samples tables and transformed in terms of the number of OTUs and

4 <u>the relative abundance (%) of sequences.</u>

5 **4 Sediment dating**

The age of the studied core was estimated based on high precision AMS ¹⁴C dating performed on bivalves shells, as presented in Pawłowska et al. (2014). Eleven shells identified to the highest possible taxonomic level were selected and processed on a 1.5 SDH-Pelletron Model 'Compact Carbon AMS' (Poznań Radiocarbon Laboratory, Poland). The dates were converted into calibrated ages using the CALIB Rev. 7.0.2 Beta calibration program (Stuiver and Reimer, 1993) and the Marine13 calibration dataset (Reimer et al., 2013). The difference ΔR in the reservoir age correction of 105 ± 24 was applied (Mangerud et al., 2006).

13 Four of the 11 samples were in chronological order and were used to establish an approximate age model for the sediment core. One sample contained post-bomb carbon, 14 which indicates a post-1960 age. Six samples revealed ages that were not in chronological 15 order, which suggests redeposition events (Table 1). These samples occurred at sediment 16 depths of ~ 15-55 cm and ~ 80-115 cm, and, therefore, the data from these two intervals 17 should be used with caution. The age-depth model was made with the use of the CLAM-R 18 software program (Blaauw, 2010; Fig. 2). The age of the oldest layer was estimated to be ~ 19 965 AD. The sediment accumulation rate (SAR) in the deepest part of the core (i.e., before 20 1800 AD; up to 120 cm) ranged from 0.1 to 0.125 cm yr $^{-1}$. At ~ 1800 AD (120 cm), the SAR 21 increased to 1 cm yr $^{-1}$. In the upper layers (after ~ 1850 AD; 70 cm), the SAR decreased to 22 0.3 cm yr^{-1} . 23

24

25 **5 Foraminifera as environmental indicators**

Due to the differences in the ecological tolerances of particular species, foraminifera are indicators of glaciomarine conditions, Atlantic and Arctic water masses and bottom currents. Herein, we followed the classification that Majewski et al. (2009) established based on ecological and palaeoenvironmental studies from Greenland, Svalbard, Novaya Zemlya and the Kara Sea region (see Majewski et al., 2009 and references therein).

The glaciomarine group comprised Cassidulina reniforme, Elphidium excavatum and 6 Quinqueloculina stalkeri. The characteristic species of the Atlantic water mass are 7 Nonionellina labradorica, Bolivina pseudopunctata, Buccella frigida, Adercotryma 8 glomerata, Ammotium cassis and Recurvoides turbinatus. We decided to exclude Reophax 9 10 fusiformis and Reophax pilulifer from this group because there were only 2 specimens of R. fusiformis in the HF_2011 core, and R. pilulifer was not reported. The Arctic water group was 11 composed of Islandiella norcrossi, Elphidium spp (excluding E.excavatum), Stainforthia 12 13 feylingi, Stainforthia loeblichii, Spiroplectammina biformis and Spiroplectammina earlandi. We decided to add Islandiella helenae to this group based on Kelly et al. (1999). The bottom 14 current indicator group consisted of Cibicidoides lobatulus and Astrononion gallowayi. 15

16

17 **6 Results**

18

19 **6.1 Sediment age and characteristics**

20 The sediment was composed mainly of glaciomarine mud, with low sand content. 21 Before 1600 AD (145 cm), the mean grain size fluctuated slightly, except for one peak at ~ 22 1450 AD (160 cm), which reached 4.5 φ . In ~ 1600 AD, the mean grain size increased 23 slightly to 6.2 φ . After 1800 AD (122 cm), it varied within a broader range of values and 24 presented three slight peaks between 1800 and 1850 AD (120 cm, 100 cm and 70 cm). A decrease in the mean grain size was observed from the mid to the end of the 20th century (25-0
cm; Fig. 3).

From 1000 AD to 1800 AD (200-122 cm), the IRD flux was relatively stable and did not exceed 2 grains cm⁻² y⁻¹. After that period, the IRD delivery increased considerably, reaching up to 28 grains cm⁻² yr⁻¹. From ~ 1900 to the end of the 20th century, the IRD flux varied from 0.24 to 10 grains cm⁻² yr⁻¹ (Fig. 3). There were three distinctive periods when the amount of IRD in the sediment considerably increased (Fig. 3): 1) at the transition from the MWP to the LIA (160-130 cm; up to 30 grains g⁻¹), 2) in the late LIA (115-80 cm; up to

9 <u>24 grains g^{-1}) and 3) in the early 20th century (60-20 cm; up to 24 grains g^{-1}).</u>

10 **6.2 Stable isotopes**

From 1000 to 1600 AD (200-145 cm), δ^{18} O showed relatively stable values varying 11 slightly from 2.63 ‰ vs. VPDB to 3.32 ‰ vs. VPDB. After 1600 AD, it fluctuated distinctly 12 from 2.23 to 3.50 ‰ vs. VPDB. Larger δ ¹⁸O values were observed before 1600 AD and in 13 the 20th century. The period from 1600 to 1900 AD (145-60 cm) was characterized by a 14 smaller δ^{18} O, with significant peaks at the beginning of the LIA (~ 1600 AD; 145 cm) and 15 during the late LIA (05 cm and 90 cm). The measured values of δ^{13} C varied from 0.54 ‰ vs. 16 VPDB to 1.59 ‰ vs. VPDB and fluctuated along the core. The most important fluctuations 17 occurred between ~ 1600 and 1900 AD (145-60 cm), with δ^{13} C values ranging from 0.54 to 18 1.48 ‰ vs. VPDB (Fig. 3). 19

20

21 6.3 Foraminiferal abundance and taxonomic composition revealed by the fossil record

The foraminiferal flux varied from 1 to 86 ind cm⁻² yr⁻¹. The most noticeable shift occurred at ~ 1800 AD (120 cm), when it increased from 2.8 to 81 ind cm⁻² yr⁻¹ (Fig. 3). The number of foraminifera per gram of sediment varied from 86 to 3838 ind g⁻¹. The highest values were observed before ~ 1850 AD (70 cm). After 1850 AD, the number of foraminifera
declined and did not exceed 1742 ind g⁻¹ (Fig. 3).

A total of 28,771 individuals were assigned to 72 species and 38 genera. Most of the 3 species belonged to Rotaliida (34), Textulariida (12) and Lagenida (12). The other species 4 were identified as Miliolida (10), Lituolida (2) and Globigerinida (Table S1 in the 5 Supplement). The most abundant species were *Elphidium excavatum*, *Cassidulina reniforme*, 6 7 Cibicidoides lobatulus and Nonionellina labradorica (Fig. 4). The fossil assemblage was strongly dominated by *E. excavatum* and *C. reniforme*, which together comprised up to 82 % 8 9 of the total abundance. The abundance of C. lobatulus and N. labradorica varied slightly 10 along the core, and no evident faunal changes were observed. The highest percentages of N. labradorica were noted after ~ 1800 AD and at the beginning of the 20th century (110 cm, 50 11 cm and 25 cm), when its relative abundance reached up to 25 %. The highest percentages of 12 C. lobatulus were noted before 1600 AD (145 cm), and a notable decrease in that species 13 occurred in the latter part of the 20th century (25-0 cm). The percentage of agglutinated taxa 14 did not exceed 25 % and reached its highest values between 1600 and 1800 AD (145-120 cm) 15 and after ~ 1930 AD (25 cm; Fig. 4). 16

The proposed 4-factor PC explained 98.5 % of the total variability of the tested 17 18 dataset. The most important PC analysis assemblages were (1) the E. excavatum FA, which explained 40.8 % of the total foraminiferal variance, (2) the C. reniforme FA, with E. 19 excavatum as an accessory species, which explained 34.8 % of the variance, (3) the N. 20 21 labradorica FA, with C. lobatulus as an accessory species, which explained 20.1 % of the variance, and (4) the C. lobatulus FA, which explained 2.8 % of the total variance (Table 2). 22 23 The HF_2011 core was dominated by the E. excavatum FA and the C. reniforme FA throughout. The E. excavatum FA showed the highest factor loadings during the LIA (i.e., 24 between 1600 and 1900 AD). In the uppermost part of the core, the E. excavatum factor 25

loadings decreased, and the role of the C. reniforme FA increased. The N. labradorica FA 1 was significant during the MWP and the early LIA (before ~ 1800 AD) and was not 2 significant during the late LIA (after ~ 1850 AD). The *N. labradorica* factor loadings started 3 to increase at the beginning of the 20th century. The *C. lobatulus* FA was significant only in 4 two layers dated to the MWP (Fig. 5). 5

6

7

6.4 Foraminifera in the ancient DNA record

8 The results of the aDNA analysis are described in detail in Pawłowska et al. (2014). 9 Herein, we summarize the results, focusing on monothalamous foraminifera.

We used Sanger and high-throughput sequencing (HTS) to obtain 717 and 8,700,815 10 sequences, respectively. A total of 394 operational taxonomic units (OTUs) were obtained 11 12 from the sequence clustering. The majority of the OTUs were assigned to Monothalamea (96 OTUs) and Rotaliida (93 OTUs). The remaining OTUs were assigned to Textulariida (33 13 OTUs), Miliolida (10 OTUs), Globothalamea (10 OTUs), Robertinida (1 OTU) and 14 15 Globigerinida (5 OTUs); 146 OTUs remained unassigned (Table S2). Although the sequences of the species that dominated the fossil record were present in most of the samples, their 16 abundances did not reflect the abundances in the fossil specimens (see Pawłowska et al., 17 2014). 18

The 96 OTUs assigned to monothalamids comprised 39.4 % of the sequences. The 19 20 percentage of monothalamous sequences varied along the core from 3.5 % to 65 %. (Fig. 5) There were 7 OTUs constituting more than 3 % of all the sequences in at least one sample. 21 They were referred to Bathysiphon sp. (clade BM), Micrometula sp. (clade BM, 2 OTUs), 22 23 Toxisarcon sp. (clade C) and monothalamous foraminifera of undetermined phylogenetic origin (3 OTUs; Table S2). 24

Monothalamid sequences were assigned to 14 clades, including 10 that were 1 represented by more than 5 % of the monothalamid sequences in at least one sample. The 2 assemblage of monothalamous foraminifera was strongly dominated by clade BM (genera 3 Micrometula and Bathysiphon), which together comprised up to 90 % of the sequences of 4 monothalamids (Fig. 5). *Bathysiphon* sp. was the most abundantly sequenced in the samples 5 spanning the MWP and the early LIA, whereas sequences of *Micrometula* sp. dominated in 6 the samples spanning the 20th century. The monothalamous assemblage during the MWP was 7 8 dominated by Toxisarcon sp. and environmental monothalamous sequences belonging to clade V. The early LIA (1600 - 1800 AD; 150-125 cm) was marked by an increased 9 proportion of sequences of Hipocrepinella hirudinea (clade D) and Cedhagenia saltatus 10 (clade O). The monothalamous assemblage during the beginning of the MW (~ 1900 AD; 50 11 cm) was strongly dominated by Micrometula sp. (which made up to 75 % of the 12 13 monothalamous sequences), together with Vellaria pellucidus. In the late MW, a high number of monothalamid sequences occurred that belonged to environmental clades or were of 14 15 undetermined phylogenetic origin (Fig. 6).

16

17 **7 Discussion**

18 Previous studies on the Svalbard Holocene history reported 'unstable environmental conditions' during the last thousand years (e.g., Berben et al., 2014; Groot et al., 2014), 19 reflecting the major climatic changes: the MWP (~ 900 - 1500 AD), the LIA (~ 1500 - 1900 20 AD) and the MW (~ 1900 - present) (Oerlemans, 2005). The Svalbard ice core records and 21 22 sediment records from the shelf adjacent to Hornsund suggested that prolonged cooling started ~ 1600 AD, and the most severe conditions occurred during the 19^{th} century (Isaksson 23 24 et al., 2003; Majewski et al., 2009). On the contrary, the reconstruction of the Earth's surface air temperature from Svalbard (Divine et al., 2011) constituted the cooling stage between 800 25

and 1800 AD, with no clear signs of the onset of the LIA. Our foraminiferal and
sedimentological records from Hornsund matched the trend described by Divine et al. (2011),
as it revealed a sharp change in environmental conditions at ~ 1800 AD.

4

5 7.1 The Medieval Warm Period and the early Little Ice Age (~ 1000 AD - ~ 1800 AD)

The period from ~ 1000 to ~ 1800 AD was characterized by low and stable fluxes of 6 IRD and foraminifera and slightly heavier δ^{18} O (Fig. 3). This might indicate the influence of 7 warmer and more saline waters, probably of Atlantic origin, and low glacial activity (Jernas et 8 al., 2013). The fossil foraminiferal assemblage was fairly stable during the MWP (i.e., before 9 1600 AD) and there was no clear evidence of faunal change. Conversely, the foraminiferal 10 aDNA record featured a high percentage of Toxisarcon sp. (Clade C) at ~ 1000 AD (Fig. 5). 11 As reported by Gooday et al. (2005), Toxisarcon sp. are commonly found in the Svalbard 12 13 fjords. In the case of our study, the increase in the Toxisarcon sp. percentage coincided with the peak of light δ^{18} O, followed by lighter δ^{13} C, which might suggest the presence of a highly 14 15 productive zone of frontal contact of the AW and ArW water masses. Voltski et al. (2014) 16 noted the presence of diatom frustules in the cytoplasm of Toxisarcon sp.. Therefore, we concluded that the occurrence of Toxisarcon sp. might be related to the phytoplankton-17 18 originated organic matter input.

Our data showed a slight peak of lighter δ^{18} O at 1600 AD (Fig. 3), which could indicate an increase in melt water delivery to Hornsund, but it was not followed by increases in sediment accumulation and IRD flux. Therefore, we concluded that lighter δ^{18} O at ~ 1600 AD showed the increased ArW inflow from the Barents Sea to the Svalbard shelf and Hornsund, which is in agreement with previous δ^{18} O and fossil foraminiferal records obtained from the outer fjord (Majewski at al., 2009). This event occurred within a period of significant climate changes connected to the transition from the MWP to the LIA. The Humlum et al. (2005) investigation of the frozen *in situ* vegetation below Longyearbyen
glacier (central Spitsbergen) indicated the advance of that glacier during the last ~1100 years.
Based on the terrestrial record from Hornsund, the WMP was interrupted 600 years ago by an
advance of glaciers (Marks and Pękala, 1986; Linder et al., 1990) that lasted until the
beginning of the 20th century.

6 The most pronounced changes that occurred at ~ 1600 AD in the HF_2011 fossil assemblage were slight peaks in the abundances of N. labradorica, I. norcrossi and I. helenae. 7 Moreover, an increase in the percentage of agglutinated taxa was noted between 1600 AD and 8 1800 AD (Fig. 4). Nonionellina labradorica is an AW indicator usually found in relatively 9 10 warm and saline waters (Lloyd, 2006; Majewski et al., 2009). However, the abundances of these three species seemed to be controlled more by the food supply than by water 11 temperatures (Hald and Korsun, 1997; Lloyd, 2006; Ivanova, 2008). The presence of 12 13 Islandiella spp might indicate a highly productive environment related to the Polar Front (Steinsund, 1994). This supports the evidence of the inflow of the colder and less saline ArW 14 15 at ~ 1600 AD, which changed the water mass balance and productivity in the fjord.

16 The foraminiferal flux before ~ 1800 AD was low (Fig. 3) and could be explained by the presence of species with low fossilization potential, e.g., agglutinated taxa (Wollenburg 17 18 and Kuhnt, 2000). The percentage of agglutinated taxa was relatively low during the MWP and increased significantly after 1600 AD (Fig. 4), which likely reflected the inflow of the 19 ArW and relatively low glacial activity (Hunt and Corliss, 1993; Hald and Korsun, 1997). Our 20 aDNA data suggests that the abundance of agglutinated foraminifera was higher than that 21 22 shown by the fossil record. Three agglutinated taxa were detected in both the fossil and aDNA record: C. crassimargo, Reophax spp and Spiroplectammina spp (Tables S1 and S2). 23 However, only C. crassimargo was detected by both approaches in the corresponding layers. 24 Reophax spp and Spiroplectammina spp sequences were recorded in all the examined 25

samples, but they were relatively rare or absent in the fossil material (Pawłowska et al., 2014), 1 probably due to the degradation of their tests. Korsun and Hald (2000) regarded S. biformis 2 and S. earlandi as typical for glaciomarine habitats. They noticed an increase in the 3 abundance of those agglutinated species off glacier. Korsun et al. (2005) and Hald and Korsun 4 (1997) reported *Reophax* spp and *Spiroplectammina* spp in the outer parts of the glacially fed 5 fjords of Svalbard and Novava Zemlya. Zajączkowski et al. (2010) noted a decrease in the 6 7 abundance of agglutinated foraminifera in Hornsund with increasing water turbidity. Thus, the presence of those species might indicate a glacier-distant environment. 8

Furthermore, the transition to the LIA between ~ 1600 and ~ 1800 AD was well 9 10 marked by the increase in the percentage of monothalamous foraminifera aDNA sequences (Fig. 6). Monothalamids are highly adaptable and occur in environments where conditions 11 may be extreme (Gooday, 2002; Sabbattini et al., 2010), which makes them effective 12 13 colonizers. It is likely that the change in the hydrology and productivity in Hornsund at \sim 1600 AD might have created a new ecological niche, which was effectively settled by 14 15 monothalamids. The monothalamous assemblage during the early LIA (from ~ 1600 to ~ 16 1800 AD) was dominated by taxa belonging to clade BM, mainly from genus Bathysiphon (Fig. 6). Gooday et al. (2005) reported Bathysiphon sp. in two glacial influenced fjords, Van 17 Mijenfjorden and Kongsfjorden, at glacier distant sites. Moreover, a sharp peak of heavier 18 19 δ^{13} C was noted in Hornsund at ~ 1600 AD, which might suggest the short-term suppression of primary productivity that resulted in the presence of degraded organic matter and 20 phytodetritus that seemed to be favourable for Bathysiphon sp. (Alve et al., 2010). The 21 presence of sequences of Bathysiphon sp. and agglutinated Spiroplectammina spp and 22 *Reophax* spp support our conclusion that at the onset of the LIA (~ 1600 - ~ 1800 AD), the 23 position of the glacier fronts was relatively distant to the fjord centre, which resulted in a low 24 SAR and a low IRD flux, whereas the fjords' water masses were influenced by the ArW. 25

The transition to the LIA (~ 1600 - ~ 1800 AD) was also marked by increased 1 percentages of sequences assigned to the monothalamid clade D (mainly Hippocrepinella 2 hirudinea) and to clade O (mainly Cedhagenia saltatus; Fig. 5). Cedhagenia saltatus is a 3 species recently found by Gooday et al. (2011) in the Black Sea. Little is known about the 4 environmental tolerance of C. saltatus. However, its presence in the area is strongly impacted 5 6 by human activity, which suggests that it is an opportunistic species that has a high tolerance 7 to environmental disturbance. *Hipocrepinella hirudinea* was noted in the fjords of Svalbard by Majewski et al. (2005) and Gooday et al. (2005). It appeared in the central and outer parts 8 of the studied fjords, mainly in the shallow water sites. Korsun et al. (2005) noted the 9 10 presence of the genus Hipocrepinella in Novaya Zemlya; however, it may not have referred to H. hirudinea. The scarce data on the ecological tolerances of H. hirudinea and C. saltatus 11 precluded making any general conclusions. 12

13

14 **7.2** The late Little Ice Age (~ 1800 AD - ~ 1900 AD)

15 The late LIA was characterized by an increased sediment accumulation rate and 16 strongly fluctuating IRD delivery (Fig. 3). These changes were linked to changes in the particulate matter flux, which in subpolar fjords was governed by glacial meltwater discharge 17 (Syvitski, 1989). Substantial amounts of suspended sediment and IRD might be released from 18 19 glaciers during rapid deglaciation and during glacial surges (Koppes and Hallet, 2002). Moreover, sediment might be stored in the proglacial zones of land-based glaciers, from 20 where could be eroded, particularly under conditions of increased glacial meltwater runoff 21 22 and increased precipitation (Szczuciński et al., 2009). Next, the sediment could be redeposited from the sublittoral zone by storm waves. During the LIA, glacial extent reached its Holocene 23 maximum (D'Andrea et al., 2012); thus, calving and melt water delivery could have occurred 24 close to the central part of the fjord. Noticeably, the increase in the IRD delivery during the 25

late LIA was not followed by an increase in the mean grain size, as was observed in both the
 precedent and following periods. It is likely that the amount of fine-grained sediment
 delivered to the sea bottom significantly exceeded the amount of coarse ice-rafted sediment
 (i.e., IRD) and, consequently, almost no change in the mean grain size was observed.

5 Our data showed a 20-fold increase in the foraminiferal flux at ~ 1800 AD (Fig. 3), 6 whereas the species diversity was relatively low due to the dominance of glaciomarine 7 species, especially *E. excavatum* and *C. reniforme* (Figs. 4 and 5). As a consequence of the 8 maximal range of the glaciers, conditions throughout the fjord became more glacier-proximal.

The aDNA revealed the dramatic increase in the percentage of sequences of 9 monothalamous foraminifera at ~ 1900 AD (Fig. 6). Previous studies revealed that the 10 distribution of monothalamids in Svalbard was closely related to the distance from the glacier 11 at the head of the fjord. The study conducted by Majewski et al. (2005) in Kongsfjorden and 12 13 Isfjorden showed a distinctive faunal gradient along the fjord axes, with three different monothalamous assemblages at subtidal, shallow-water and deep-water sites. Korsun et al. 14 15 (2005) and Korsun and Hald (1998, 2000) reported that allogromiids constituted up to 99 % 16 of living foraminifera in the stations close to the glacier termini in Novaya Zemlya and Spitsbergen. Sabbattini et al. (2007) attributed the occurrence of monothalamids in the 17 18 Svalbard region to inputs of fresh water and a high, changeable sedimentation rate. Gooday et 19 al. (2005) noted that the inner parts of Tempelfjorden and Kongsfjorden, fjords headed by tidewater glaciers, were dominated by organic-walled allogromiids and saccamminids. This 20 was reflected in our data as the percentage of allogromiids (mainly Micrometula sp.) 21 22 increased significantly from 40 % at ~ 1850 AD to 80 % at ~ 1900 AD (Fig. 5). Moreover, the late LIA and the early MW were marked by an increase of the percentage of sequences 23 assigned to clade E, mainly Vellaria pellucidis. Majewski et al. (2005) noted the presence of 24 Vellaria sp. in subtidal and shallow areas of the Spitsbergen fjords. The increase in the 25

percentage of *Micrometula* sp. and *V. pellucidis* in the period of the highest glacial activity
 suggests that those species were potential indicators of glacier-proximal settings.

3

4 7.3 The Modern Warming (~ 1900 AD – present)

5 The sedimentary record of the MW featured a decrease in the SAR and a lower but variable IRD flux (Fig. 3). Peaks in the IRD flux coincided with the increased mean grain size 6 (Fig. 3). This trend was opposite to that of the late LIA, where no clear correlation between 7 the IRD flux and mean grain size was observed. The post-LIA glacial retreat led to an 8 increased distance between the coring station and the main tidewater glacier front. In the 9 Spitsbergen fjords, fine-sized particles from glacial outflows are deposited close to the source 10 (Szczuciński et al., 2009); therefore, the HF 2011 station was impacted mainly by ice-rafted, 11 coarser particles. The IRD flux gradually diminished in the late 20th century, which was 12 13 probably a result of retreat of the tidewater glaciers' fronts to the inner bays, which limited iceberg drift to the fjord centre. The most noticeable changes in the fossil foraminifera 14 community occurred in the late 20th century, with the gradual increase of *B. frigida* and *I.* 15 16 norcrossi and the decrease of C. lobatulus (Fig. 4). Islandiella norcrossi and B. frigida typically occupied the distal sections of the glacial fjords of Svalbard and Novaya Zemlya 17 (Korsun et al., 2005; Hald and Korsun, 1997; Korsun and Hald, 2000; Pogodina, 2005). 18 19 Steinsund (1994) linked the presence of I. norcrossi and B. frigida with high productivity related to a polar front position and seasonal sea-ice cover. Thus, we concluded that since the 20 mid-20th century, Hornsund and the adjacent shelf remained under the influence of the AW, 21 which formed a frontal zone with local waters. This conclusion is supported by the PC 22 analysis, which showed that the significance of the N. labradorica FA was increasing during 23 the 20th century (Fig. 5). Cibicidoides lobatulus is a relatively shallow-water species and 24 takes advantage of vigorous bottom waters (Hald and Korsun, 1997; Lloyd, 2006); however, 25

it is an epiphytic species that needs a hard substrate to stay attached to the bottom surface. 1 Therefore, the decrease in the percentage of C. lobatulus could be connected to a decrease in 2 the near-bottom currents and a low IRD flux and, consequently, an increase in the fine 3 sediment fraction (Fig. 3). The increased abundance of species typical of glacier-distal faunas 4 was followed by a decrease in the abundance of species considered to be bottom current 5 6 indicators (Fig. 4). These results support our evidence for a rapid glacier retreat, coupled with 7 the decreasing influence of glaciomarine sedimentation and enhanced productivity. It was also reflected in the molecular record, where the number of OTUs and the percentage of 8 monothalamids decreased after \sim 1920 AD, reaching values similar to those during the MWP 9 10 (Fig. 6). The second half of the 20th century was marked by a significant increase in the 11 unassigned monothalamids sequences belonging to environmental clades. However, without an accurate identification of sequences, it is not possible to make any palaeoecological 12 interpretations. 13

The use of the approach based on aDNA allows the hidden diversity of benthic 14 foraminifera communities to be assessed and, therefore, the information based on traditional 15 palaeoceanographic proxies to be refined. However, current methodological biases associated 16 with the environmental DNA sequencing approach preclude comprehensive analyses of 17 18 sequence abundance data. The aDNA data should be interpreted carefully as it is not possible to establish the direct relationship between the number of 19 specimens and the number of ribosomal sequences. Some aspects of quantitative DNA 20 analyses were discussed in Weber and Pawlowski (2013). One of the conclusions was that 21 when the species is very abundant, its sequences are also numerous. Therefore, the genuinely 22 dominant species might be associated with high sequence occurrences in the sequencing data. 23 24

25 8 Conclusions and Perspectives

1 The main climatic fluctuations of the last millennium (the MWP, the LIA and the MW) were reflected in the fjord water mass balance and glacial activity. The marine 2 environmental conditions during the MWP and the early LIA were relatively stable, with a 3 low SAR and low IRD flux. The beginning of the LIA (~ 1600 AD) was poorly supported by 4 the fossil record, but it was well evidenced in the aDNA data. It was marked by the increased 5 percentage of sequences of monothalamous foraminifera, mainly Bathysiphon sp., which 6 supports our assumption that the terminal positions of the glaciers were relatively distant at 7 the onset of the LIA. The early LIA (~ 1600 - ~ 1800 AD) was also marked by high 8 9 percentages of H. hirudinea and C. saltatus. The late LIA (after ~ 1800 AD) was characterized by the increased proximity of tidewater glaciers' fronts, which increased 10 sedimentation from suspension and from the icebergs; thus conditions in the fjord centre 11 became glacier-proximal. The end of the LIA (~ 1900 AD) was marked by increased 12 percentage of Micrometula sp. and V. pellucidus. Those results revealed their potential as 13 indicators of glacier-proximal environments, which were characterized by melt water 14 outflows, a high sedimentation rate and increased calving. During the MW, the major 15 glaciers' fronts retreated rapidly to the inner bays, limiting the iceberg discharge to the fjord 16 centre and causing the shift in the foraminiferal community reflected in the fossil and aDNA 17 records. 18

The present study was the first attempt to implement an aDNA foraminiferal record for palaeoclimatic reconstruction. The data inferred from the molecular analyses correlated well with environmental changes. The aDNA record even revealed small environmental changes that were not clearly indicated by the fossil record. By including monothalamous foraminifera identified in the aDNA record, we considerably increased the number of potential proxy species. However, to fully benefit from this new source of information, it is essential to improve knowledge of the ecology of monothalamids. The positive results of the present study encourage further applications of ancient foraminiferal DNA sequences to reconstruct
 past environmental changes in polar regions.

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- 5

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1	Table 1. Raw AMS	¹⁴ C and calibrated	dates used for the	he age model (after Pawłowska et al.,

2 2014).

	Sediment depth [cm]	epth Material Raw AMS ¹⁴ C Calibrated years $BP \pm 2\sigma$		Years AD used in age model	
	8.5 33.5 48.5 56.5 70.5 89.5 106.5	Cilliatocardina cilliatea Bivalvia nd. Gastropod nd. Nuculana pernula Bathyarca glacialis Macoma calcarea Cilliatocardina cilliatea	105.58 (± 0.35) pMC 9990 (± 50) BP 610 (± 30) BP 880 (± 25) BP 580 (± 30) BP 765 (± 30) BP 760 (± 30) BP	-1035 10 605-11 040 40-240 315-485 1-150 230-420 230-420	1850
	109.5 122.5 166.5 173.5	Cilliatocardina cilliatea Gastropod nd. Hiatella arctica Macoma calcarea	735 (± 25) BP 615 (± 30) BP 1075 (± 30) BP 1145 (± 30) BP	180-380 40-250 500-630 540-670	1800 1450 1400
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- **Table 2.** PC scores and percent of total variance explained by four factor principal component
- 2 analysis. The contribution of each analysed species is shown, and species significant for
- 3 particular assemblages are marked in bold.

	PC 1	PC 2	PC 3	PC 4
Percent of total variance explained	40.8 %	34.8 %	20.1 %	2.8 %
Adercotryma glomerata	-0,1857	-0,13401	-0,75184	-0,06062
Buccella frigida	-0,40847	0,081688	-0,13456	-0,34934
Cassidulina reniforme	-1,44681	2,923605	0,894126	0,480619
Cibicidoides lobatulus	-0,22008	-1,29993	1,821983	2,252356
Cribrostomoides crassimargo	0,13799	0,418817	-0,63454	-1,15662
Elphidium bartletti	-0,06081	-0,41644	-0,64903	0,130446
Elphidium excavatum	3,21345	1,108558	0,281919	0,526077
Islandiella helenae	-0,39509	-0,39785	0,267723	0,366261
Islandiella norcrossi	-0,21596	-0,31512	-0,5112	0,091585
Nonionellina labradorica	0,124046	-0,71182	2,242218	-2,40609
Quinqueloculina stalkeri	-0,23152	-0,30084	-0,57036	0,05742
Recurvoides turbinatus	-0,15527	-0,33373	-0,6666	0,055335
Spiroplectammina biformis	-0,08015	-0,30205	-0,80459	0,020384
Spiroplectammina sp.	-0,07563	-0,32089	-0,78525	-0,00781

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Figure 1. Bathymetric map of Hornsund with sampling station HF 2011. The position of core
HR 3 studied by Majewski et al. (2009) is shown. Glaciers are shown in white. WSC – West
Spitsbergen Current, ESC – East Spitsbergen Current.



Figure 2. Age model of the studied core. The black line indicates the age-depth model
derived from a linear interpolation. The grey fields show the probability distributions of
calendar dates obtained by the calibration of individual ¹⁴C dates used for the age model (after
Pawłowska et al. 2014, modified).



Figure 3. Sediment accumulation rate (A), IRD delivery, expressed as IRD flux (B) and number of IRD grains per gram of sediment (C), mean grainsize (D), stable oxygen (E) and carbon (F) isotopes, flux of total fossil foraminifera (G) and number of foraminifera per gram of sediment (H). MWP: Medieval Warm Period, LIA: Little Ice Age, MW: Modern Warming. The time ranges of the MWP, LIA and MW are presented after Majewski et al. (2009).



Figure 4. The abundances of selected foraminifera species expressed as percentages (%) of the total assemblage. The foraminiferal taxa were grouped based on their ecological tolerances (see Sect. 5: Foraminifera as palaeoenvironmental indicators). MWP: Medieval Warm Period, LIA: Little Ice Age, MW: Modern Warming. The time ranges of MWP, LIA and MW are presented after Majewski et al. (2009).

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Figure 6. The relative abundance of the monothalamid sequences, expressed as the percentage of all foraminiferal sequences, and the composition of the monothalamid assemblage, expressed as percentages of sequences within clades. Clades that constitute more than 5 % of the monothalamid sequences in at least one sample are presented. 'Environmental clades' relate to foraminifera known only from environmental sequencing.

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9 Electronic supplementary material

Table S1. List of fossil foraminifera species and number of individuals in core HF_2011.

Table S2. OTU richness and number of foraminiferal sequences in core HF_2011.

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