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Climate of the Past Discussions

Interactive comment on "Palaeoceanographic changes in Hornsund Fjord (Spitsbergen, Svalbard) over the last millennium: new insights from ancient DNA" *by* J. Pawłowska et al.

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

Referee's comment: The chronology of the core was based on 11 AMS-radiocarbon dated 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 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 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 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 discarded and which way the new model is more reliable. Moreover, the scatter of the radiocarbon dates is disregarded, redeposition vanishes magically, and the authors do not hesitate interpreting an uninterrupted sequence of climatic events.

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 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, suggesting redeposition events. These samples occurred at sediment depths $\sim$ 15-55 cm and $\sim$ 80-115 cm and therefore, the data from these two intervals should be threatened with caution. 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 $\sim$ 965 AD. The sediment accumulation rate (SAR) in the deepest part of the core (i.e., before 1800 AD; up to 120 cm) ranged from 0.1 to 0.125 cm yr -1 . At $\sim$ 1800 AD (120 cm), this rate increased to 1cm yr -1 . In the upper layers (after $\sim$ 1850 AD; 70 cm), SAR decreased to 0.3cm yr -1."

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.

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 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 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 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 up to 11.6 cm/s (Jakacki et al. 2015; Geophysical Research Abstracts Vol. 17, EGU2015-10520).

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 area, what might explain the presence of high number of C. lobatulus. However, C. lobatulus might be associated with algae, hydroids or bryozoans (e.g. Dobson and Haynes, 1973; Micropaleontol.). Ivanova et al. (2008; J. Foramin. Res.) suggested that C. lobatulus might also survive inside the tubes of polychaetes. In our opinion, the variety of factors that might affect the number of C. lobatulus in the study area precludes making any general conclusion. 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, concluding about winnowing only based on the foraminiferal abundance seems speculative.

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: - Based on which data (bathymetry, seismics, else) the coring location was selected. - What are the modern sediments at the location (based on box cores). - On which basis the shells were selected for dating. - Why some "shells identified to the highest possible taxonomic level" were identified to "Bivalvia n.d." and "Gastropod n.d."? The mollusk fauna of Svalbard is comprehensively studied (consult with Włodarska-Kowalczuk). What was wrong with these shells? - Where the bivalve shells paired and did they have in-situ position?

Response: We agree with the Referee, that more detailed description of age model is 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 bottom. A flat seabed area has been chosen and checked with echo-sounder before coring. The modern sediments are composed mainly of

glaciomarine mud, with low sand content (less than %; Pawłowska et al., in prep.). The information about the sediment type will be added to the text. The core was dated based on all the shells found in the samples. Some shells were fragmented, therefore the identification to species/generic level and the determination of shells position was not possible. The identification was performed by Maria Włodarska-Kowalczuk.

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 Pawłowska 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. 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 (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 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; Electrophoresis). Several environmental conditions are key to preservation of DNA (Nielsen et al. 2007; Environ. Biosafety Res.), which have not been extensively investigated in marine sediment. Hence, enhanced DNA preservation is very likely in Arctic sediments because of low temperatures and sediment mineralogical composition. Short DNA fragments can adsorb to small sediment particles such as clay minerals, which are common in Hornsund. Adsorbed 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., 2006, Environ. Sci. .Technol.).

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 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 not followed by the increase in mean grain size, as it was observed in both precedent and following periods. It is likely that the amount of fine-grained sediment delivered to the sea bottom exceeded significantly the amount of coarse ice-rafted sediment (i.e., IRD) and consequently, almost no change in mean grain size was observed. The adequate explanation will be added to the text. However, to provide more complete view of our data we decided to change the figures' scale into sediment depth [cm] and to add the number of IRD grains per gram of sediment to Figure 3. The information about number of foraminifera per gram of sediment is already presented (Fig. 3G).

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 concise overview that specifically answers the reader's most obvious question: Whether the metagenomic technique provides a picture congruent to my fossil assem-

blages. To follow the discussion the reader needs to feel how robust the metagenomic approach is, what the scale of the mismatch between the fossil assemblage and aDNA in taxonomic and numerical sensitivity is. The only relevant sentence in the Introduction provides insufficient information “The study showed that aDNA record contained almost all of the species reported for Hornsund from previous micropalaeontological investigations” (p.3668) and refers to the previous paper (Pawłowska et al. 2014). Ok, I go to that paper. But I cannot find comprehensive information. There is a rather confusing diagram; the description is too general, non-specific, like the cited sentence above. And there is no control against the fossilizable part of the assemblage that would show how accurate the technique is.

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 mentioned 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 between the microfossil and molecular views on the subsurface foraminiferal diversity. Such a 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 methods is constantly improving with many respects and we developed an expertise for the generation of foraminiferal high-throughput sequencing data (Pawlowski et al. 2014; Biol. 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 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 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 (Youngblut et al. 2013, Appl. Environ. Microbiol.; Egge et al. 2015, Mol. Ecol.), especially for the species that may exhibit sharply changing dominance patterns (Adams et al. 2013 Microb. Ecol.).

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.

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 frequencies. In order of abundance the principal rotaliids of the fossil assemblage are: Elphidium excavatum 46 percent Cassidulina reniforme 24 Nonion labradoricum 11 Cibicides lobatulus 9 Islandiella norcrossi/helenae 5 Buccella spp. 4 subtotal 100 The aDNA table shows numerous reads only for E.excavatum and C.lobatulus. Nonion labradoricum is represented by a few reads, which is obviously an artifact. The other major species are not detected. The control reveals that the aDNA technique fails to recognize 4 of the 6 major species. Thus the technique fails to reveal the structure of the assemblage on the 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 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 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 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. - Globocassidulina biora is absent from the northern hemisphere. These numerous reads may represent Islandiella norcrossi/helenae. - Pullenia carinata is absent or nearly so in the fjords. Its numerous reads most likely are misidentified N.labradoricum. - Cassidulina laevigata is nearly absent here. These numerous reads are probably misidentified C. reniforme. - Cibicides wuellerstorfi does not dwell in the fjords. The numerous sequences are probably misidentified, and then they may append to the C.lobatulus reads. - Epistominella exigua and E.vitrea occur in the fjords, but these numerous reads may be Buccella spp. With these guesses the DNA frequencies of the principal rotaliids are: Elphidium excavatum 10 percent Cassidulina reniforme 35 Nonion labradoricum 11 Cibicides lobatulus 25 Islandiella norcrossi/helenae 3 Buccella spp. 15 subtotal 100 The correspondence to the fossil frequencies above is not perfect, but at least now it is not a hopeless mismatch. The match perhaps could have been better if the sediments were in situ.

Response: As above, we would like to refer to our previous paper (Pawłowska et al., 2014; Geobiol.), where we show that it is possible to identify sequences of many rotaliids present in the fossil record, but there was no match between the relative frequencies of sequences and microfossils. In the article Pawłowska et al. (2014) we discussed the possible causes of mismatch. The main of the presented paper adressed to the micropaleontological community is to raise the attention to the importance of monothalamous foraminifera as 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. (v) the eDNA sequences of Epistominella exigua certainly do not correspond to 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.

Referee's comment: The aDNA shows that Stainforthia sp. is a major player in the assemblage (Supplement 2). Its frequency in the fossil assemblage is severely underestimated probably because of the small size (e.g. Stainforthia feylingi). A mesh size smaller than 100um (which is commonly used in Svalbard) would have retained the important small taxa. This may be a message that will reach the micropaleo community. Other comments habit to consider only those peaks that are supported by three or more data points.

Response: Indeed, the use of 100 μm mesh size might cause the underestimation of the abundance of smaller taxa, such as Stainforthia as well as Epistominella and probably some other small rotaliids. We already discussed this issue in the previous paper (Pawłowska et al., 2014; Geobiol.). As highlighted above, our message is not to recover the exactly same composition as fossil samples, but to show through aDNA

that foraminiferal assemblage may comprise new paleo-indicators among soft-walled monothalamids.

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.

Referee's comment: The language is quite good but will need some amendment.

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

Referee's comments: - 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 core was taken in the central part of the fjord (not clear whether it means along the axis or between the flanks), in another place it is written that the core was taken under the southern flank. Please, find out where the core was located.

Response: The Referee is right that the description of core location might be confusing. The core was taken in the central part of the fjord, but not in the fjord axis. The adequate explanation and the information about the coordinates and water depth is added to the text and to Figure 1.

- The Study Area section lacks information on the modern setting at the coring location. - Fig. 1: There must be at least two latitude marks. - Please provide captions for the supplements. Response: Supplements captions have already been provided with the manuscript. p.3666 line 10: "the distant position of the glaciers" is not very clear p.3668 line 17: do not capitalize Eukaryotes. line22: almost all species lines 25-29:

not specific, vague meaning p.3669, line 10: a wide no-sill outlet line 10: "facilitates its penetration by oceanic waters" is awkward. Rephraseline 11: awkward "coastline is variable" line 11: "basins, separated by sills" is geometrically unclear p.3683 lines 3-9: not specific, vague meaning.

Response: The text and figures will be corrected according to the Referees suggestions.

Interactive comment on Clim. Past Discuss., 11, 3665, 2015.