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Pinniped Molecular Baraminology

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Abstract

Pinnipeds are a group of semi-aquatic animals which live on land, but hunt for food in the water. As such, they constitute an apobaramin, similar to bats, which are the only flying mammals. Differentiating between seals (Phocidae), sea lions, fur seals (Otariidae), and walruses (Odobenidae) is therefore an interesting task for baraminology. A morphology-based baraminology study showed discontinuity between phocids and all other pinnipeds. Hybridization results also show that many different phocid genera are capable of interbreeding.

Using a gamut of molecular baraminology tools, the mitochondrial DNA, whole genome sequences and proteomes of several dozen pinniped species were studied. The analysis of mtDNA sequence similarity shows that Phocidae, Otariidae, and Odobenidae form their own distinct groups The whole genome analysis shows discontinuity between Otariidae and Phocidae and also Odobenidae. However, discontinuity between Odobenidae and Phocidae is not so clear. Looking at differences in gene content shows discontinuity between Otariidae and the other two pinniped groups. Discontinuity also exists between Odobenidae and the majority of phocids, except for *Leptonychotes weddelli*. However, a closer examination of orthology groups unique to *L. weddelli*, *Odobenus rosmarus*, and the outlier species, *Mustela erminea*, show that *L. weddelli* shows continuity with phocids, whereas *O. rosmarus* shows discontinuity with this group.

Ultimately, the morphological evidence, hybridization data, and the results from the molecular baraminology analyses support three separate pinniped holobaramins at the level of the family. There also appear to be several phocid lineages in the subfamily Monachinae, based on mtDNA analysis, such as *Lobodon*, *Mirounga*, and *Monachus*.

Key Words: seals, sea lions, fur seals, walrus, hybridization, mitochondrial DNA, whole genome analysis, gene content method

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Introduction

Pinnipeds are interesting animals, living both on land and in the sea. They are carnivorous and usually live along the coastlines in the Northern and Southern Hemispheres. The name 'pinniped' is derived from the Latin 'pinna,' which means feather, and 'pedis,' which means foot. Thus, these animals are 'feather-footed.' Their bodies are covered in fur, and have thick layers of fat to protect themselves from the cold. Pinnipeds make up about 28% of the diversity of marine mammals, made up of 34–36 species in three families: Phocidae (seals), Otariidae (fur seals and sea lions) and Odobenidae (walruses). In Figure 1 we can see an example of a seal (A), a walrus (B), a fur seal (C), and a sea lion (D). Some scientists estimate that in the past, more species existed than we have today.

Figure 1. A. seal (Phocidae), B. walrus (Odobenidae) C. fur seal (Otariidae) D. sea lion (Otariidae)
Animal images: The web references for the images of the pinnipeds in Figure 1 are as follows: seal (A): publicdomainpictures.net/en/view-image.php?image=207005&picture=seal; walrus (B): commons.wikimedia.org/wiki/File:Close_up_of_head_of_young_bull_walrus_marine_mammal_in_water_odobenus_rosmarus.jpg; fur seal (C): openfotos.com/view/fur-seal-1360; sea lion (D): goodfreephotos.com/animals/mammals/california-sea-lion.jpg.php.

Walruses are characterized by their prominent, elongated upper-canine teeth, or tusks, and large size. Together with phocids, they do not have external ears, in contrast with otariids. Some argue for three species of extant walruses: *Odobenus rosmarus rosmarus* from the North Atlantic, *O. rosmarus divergens* from the North Pacific, and *O. rosmarus laptevi* from the Laptev sea. Two groups of walruses can be distinguished, namely the extinct Dusignathinae, and Odobeninae, which includes the living species and some more extinct species (Berta et al., 2015, pp. 27–50). A species of tuskless walrus, *Titanotaria orangensis*, has recently been discovered in the Capistrano Formation of Orange County, California (Magallanes et al., 2018).

Otariids and walruses have hind limbs that they can use to walk on land, whereas phocids do not. Both phocids and walruses use their hind limbs to propel themselves in the water, whereas otariids use their front legs. Phocids are characterized by thick mastoid bones, large endotympanic bones, an exerted pelvis, and large ankle bones. Within Otariidae, sea lions can be distinguished by their rounder snouts and shorter hair, as opposed to the more pointed snouts and thicker fur of fur seals.

Within Phocidae, there are two subfamilies, Phocinae, or the Northern seals, and Monachinae, or the Southern seals. Monachinae have 34 pairs of chromosomes, whereas Phocinae have either 32 or 34. The elephant seals, from the genus *Mirounga*, stand out among the other seals. There are two species, the Northern elephant seal (*Mirounga angustirostris*) and the Southern elephant seal (*Mirounga leonina*). They resemble walruses somewhat with their large, furless bodies, an elephant-like proboscis on the male, and the ability to pull themselves upright. They also have special whiskers, called vibrissae, to help them find food. Elephant seals differ from walruses based on their diet: they are deep-sea divers which feed on fish, cephalopods, sharks, and rays, whereas walruses prefer shallow water and eat shellfish, marine arthropods, some corals, and will also scavenge from other pinnipeds, birds, or even whales. Differences between walruses and elephant seals include facial features and flipper anatomy.

Based on these similarities and differences it is an interesting question as to whether elephant seals form a holobaramin with all other seals or with walruses. A ‘holobaramin’ denotes all species which constitute a single baramin, or created kind. Genetically, walruses have a diploid karyotype of 32 chromosomes, whereas Otariidae has 36. Within Phocidae, this number varies. In the genus *Phoca* the karyotype can be 32, whereas in *Erignathus* it can be 34. This suggests the presence of four possible pinniped holobaramins (Arnason, 1977). However, Árnason et al. propose that the 34 chromosome karyotype is ancestral to the 32 chromosome one, via chromosomal fusion (Arnason et al., 1977).

Pinnipeds also pose a very interesting question to the baraminologist. Bats are the only flying mammals, and thus display stark discontinuity with all other mammals. Similarly, since pinnipeds are semi-marine mammals, they also show discontinuity with all other mammals. Such a group is called an apobaramin, and may be composed on one or more holobaramins. Studying apobaramins is useful when determining the number of holobaramins within a set of species in a study. The big question here is, do pinnipeds form a single or multiple holobaramins?

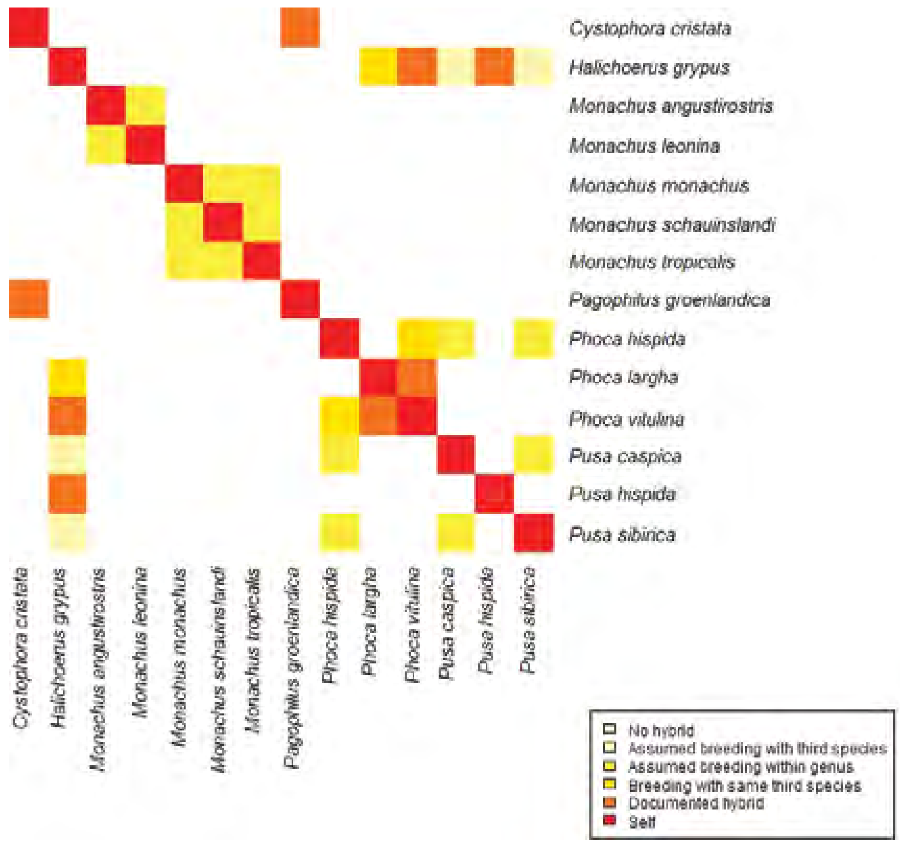

Figure 2. Hybridogram showing baraminic relationships between different species from the family Phocidae. All species can mate with themselves, as shown in red. Orange indicates documented hybridization between two given species. Dark yellow denotes two species both interbreeding with a third species. Yellow indicates breeding assumed within the same genus. Tan indicates that if a species can breed with another species, it is assumed that it can also breed with any other species from the genus that the second species belongs to. *Monachus tropicalis* is extinct.

Biblical Considerations

Pinnipeds are not specifically mentioned in the Bible, and it is also questionable as to which day they were created on, and whether they were on the Ark or not during the Flood in Genesis 6. Pinnipeds are adapted to moving around in the water, but mate and raise their young on land. Since they go into the water only temporarily to hunt for food, pinnipeds are most likely to have been created on Day 6 of Creation Week together with land animals. Thus, they could also have been taken on board the Ark during the Flood, especially since they breathe air through their lungs like other mammals. Due to their aquatic lifestyle, they could have possibly survived outside the Ark during the duration of the Deluge (Lightner et al., 2011). Whereas most seals live along ocean coastline, the existence of the Caspian seal (*Pusa caspica*) and the Baikal seal (*Phoca sibirica*) along the coasts of the inland Caspian Sea and Lake Baikal (McLaren, 1960) are testimonies to a global Flood that had once covered the Earth.

Previous Pinniped Baraminology and Phylogenetic Studies

Previous studies include analysis of a morphological data set including 196 characters for 21 pinniped species by Wood (2008, pp. 24–27). This study showed discontinuity between phocids and all other pinnipeds. While it also showed lack of continuity between the subfamilies Phocinae and Monachinae within Phocidae, it did not demonstrate any discontinuity between these two groups. This suggests that they are both monobaramins within the phocid holobaramin. A 'monobaramin' denotes either part of, or the entire holobaramin. This means it can also denote a specific lineage or subgroup within a holobaramin.

Similarly, Davis et al. (2004), who separate Odobenidae and Otariidae from Phocidae, show distinct differences between Monachinae and Phocinae. This was based on the analysis of the alignment and comparison of twelve protein-coding mitochondrial genes, including ATP synthase subunits 6 and 8, Cytochrome oxidase subunits I, II, and II, and NADH dehydrogenase subunits 1, 2, 3, 4, 4L, and 5.

Hybridization Data

Hybridization data is available for pinnipeds, but it is limited. Figure 2 shows the hybrid relationships between several species within Phocidae. There have been attempts to hybridize pinnipeds and non-pinnipeds, such as otters breeding with female seals (Heather et al., 2010), or male elephant seals attempting to breed with female fur seals (Best et al., 1981). However, in both cases, females usually died and no hybrids have been documented as yet. This is evidence that seals are discontinuous with otters (Mustelidae), and that probably otariids are discontinuous with phocids, suggesting that there are at least two holobaramins within pinnipeds.

Gray seals (*Halichoerus grypus*) hybridize with two other species: the harbor seal (*Phoca vitulina*) and the ringed seal (*Pusa hispida*) (Iverson et al., 1993; Savriama et al., 2018). Hybridization data also exists between the harp seal (*Pagophilus groenlandica*) and the hooded seal (*Cystophora cristatata*), producing live offspring (Moynagh, 2018). The harbor seal's breeding with the gray seal has been observed, but full-grown hybrids have not. However, there is no reason to doubt, based on Lightner's definition of hybridization success (i.e., several cell divisions in the zygote), that this is a successful hybridization (Lightner, 2007). Based on this, we can safely assume that the harbor seal and the ringed seal are related since they both breed with the gray seal. However, we can go a step further. Since breeding between species of different genera has been documented, we can extrapolate to state that all members of both genera are likely related. Thus, the gray seal is also related to the nerpa (*Pusa sibirica*) and the Caspian seal (*Pusa capsica*).

The genera *Pusa* and *Pagophilus* both used to be lumped into the genus *Phoca*. Therefore, it seems that several genera within Phocidae (*Phoca*, *Pagophilis*, *Pusa*, *Cystophora*, *Halichoerus*) are all related based on hybrid data, an indication of continuity within this family.

Principle of Analysis

The goal of this paper is to discern the number of baramins within the pinniped apobaramin. More specifically, do all pinnipeds make up a single holobaramin, or are phocids, otariids, and odobenids separate holobaramins within Pinnipedia? Do phocids belong to multiple holobaramins, as suggested by hybridization results? Are the subfamilies Monachinae and Phocinae separate baramins, or two separate lineages within Phocidae? As morphological comparisons might suggest, do walruses form a holobaramin with elephant seals?

Since morphological analyses have already been performed on pinnipeds, these studies were augmented with molecular studies to either challenge or confirm the previous results. The mitochondrial DNA (mtDNA) sequence is available for 27 pinniped species, and the whole genome sequence (WGS) for twelve. Therefore, these sequences could be compared with one another to elucidate molecular baraminic relationships between pinnipeds.

Materials and Methods

Sequences

MtDNA was downloaded for one species from the family Odobenidae, nine from Otariidae, and 17 from Phocidae from the National Center for Biotechnology Database (NCBI) Organelle Genome database at ncbi.nlm.nih.gov/genome/browse#!/organelles. The accession numbers for these sequences can be found in Supplementary data file 1, on the tab 'mtDNA.'

The proteomes for nine pinniped species and *M. erminea* were downloaded from the NCBI database. RefSeq proteins were selected for the comparison. The species' Latin name, family, number of proteins, mapped proteins, unique orthology groups, and taxid are available in the tab "Species, proteomes" in Supplementary File 3 online.

WGS for twelve pinniped species were also downloaded from the NCBI database at ncbi.nlm.nih.gov/genome. The accession number of these sequences are also listed in Supplementary data file 1, on the tab 'genomes.'

All supplemental data files and figures are available on github at github.com/csmatyi/pinnipeds.

Software

R version 4.0.3. was used to generate the heat maps in Figures 3, 4, and 5 using the heatmap command and the 'single' clustering method for mtDNA and gene content (GC) analysis, and the 'complete' method for the WGKS analysis. The fviz_nbclust function was used to create the elbow plots using the ‹wss› method. Significance levels were calculated in R using the ‹pnorm› function.

Protein sequences in the proteome data sets were mapped to Orthology Groups using the online Galaxy server

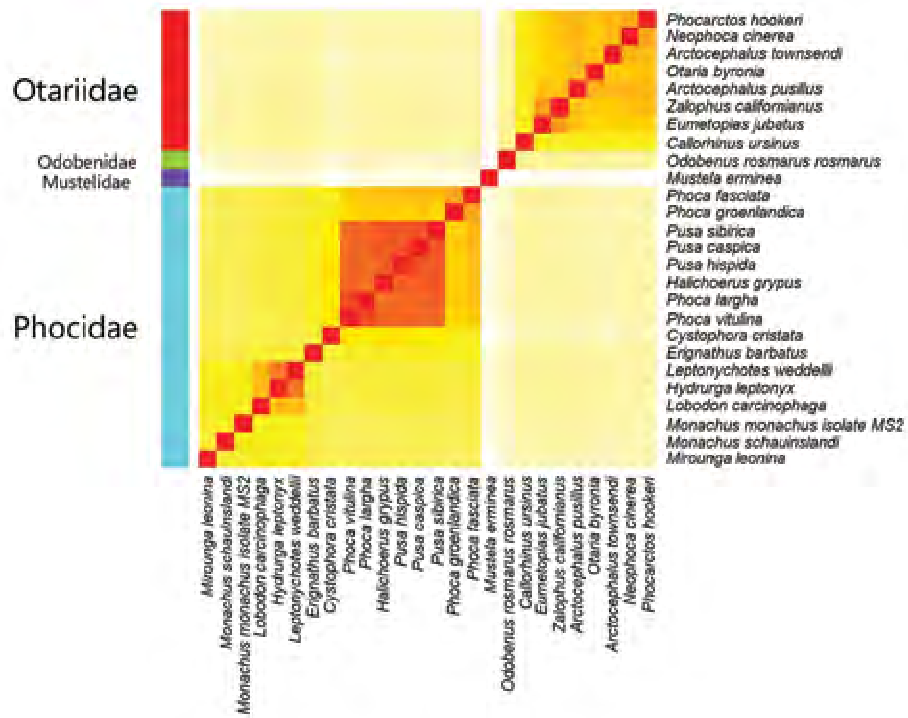

Figure 3 *(right)*. Heatmap showing baraminic relationships between Phocidae, Otariidae, Odobenidae, and *Mustela erminea*, the outlier species, based on mitochondrial DNA sequence similarity. Each colored pixel represents the global sequence similarity of a pair of species. Brighter, redder colors represent species which are more similar to one another, hence they are continuous with one another. Lighter, yellow colors represent species which are less similar to one another, and hence are discontinuous with one another.

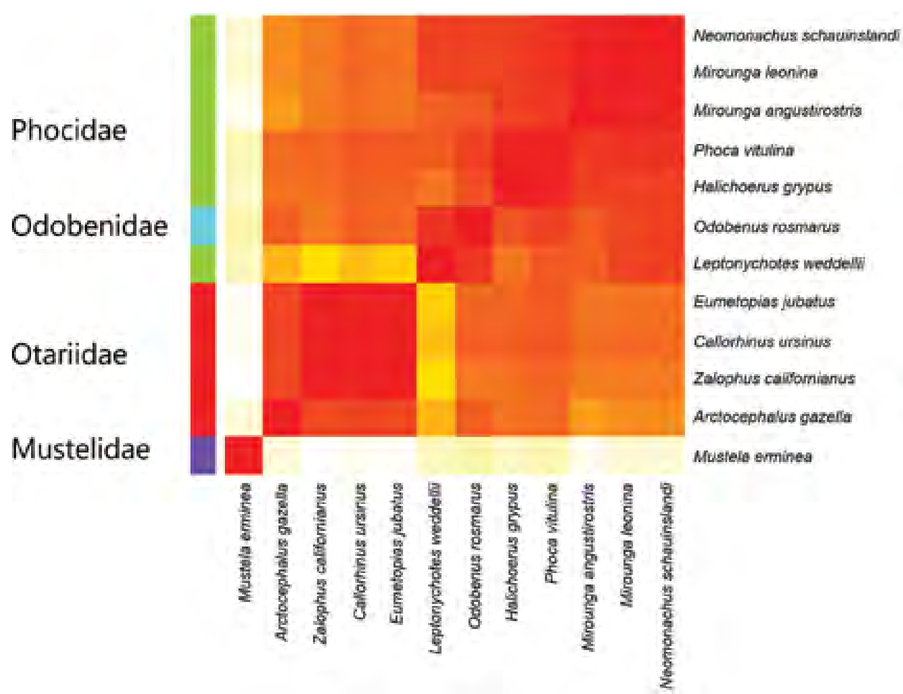

Figure 4 *(right)*. Heatmap showing baraminic relationships between Phocidae, Otariidae, Odobenidae, and *Mustela erminea*, based on the Whole Genome K-mer Signature analysis. Each colored pixel represents the Pearson Correlation Coefficient of a pair of species. Brighter, redder colors represent species which are more highly correlated with one another, hence they show continuity with one another. Lighter, yellow colors represent species which are less correlated with one another, and hence show discontinuity with one another.

tool at usegalaxy.org (Afgan et al., 2018). Comparisons between Orthology Group Identifiers (OGIs) between phocids and *L. weddelli*, *O. rosmarus*, and *M. erminea* were performed using the Venn diagram tool of the Vlaams Instituut voor Biotechnologie (VIB) at bioinformatics.psb.ugent.be/webtools/Venn.

Functional gene analysis

Functional analysis of the 119 proteins unique to *L. weddelli* was performed at the PANTHER database website at pantherdb.org. A tab-delimited PANTHER Generic Mapping file (NCBI accession plus PANTHER ID) was generated by performing an HMM search with them against all of the PANTHER HMM profiles, according to the protocol described in Mi et al. (2019). Version 3.3 (Nov 2019) of the HMM software was used (Eddy, 2011). The PANTHER HMM libraries were downloaded from ftp.pantherdb.org/panther_library/current_release (version 16.0), on March 30, 2021. Out of the 119 unique *L. weddelli* proteins, 112 were matched to 93 PANTHER functional IDs. These functional IDs are listed in Supplementary File 4 along with their functional classification. The Generic Mapping file was uploaded to the PANTHER database website for functional classification. Bar charts were produced showing the biological processes and protein classes that the 119 unique *L. weddelli* proteins mapped to.

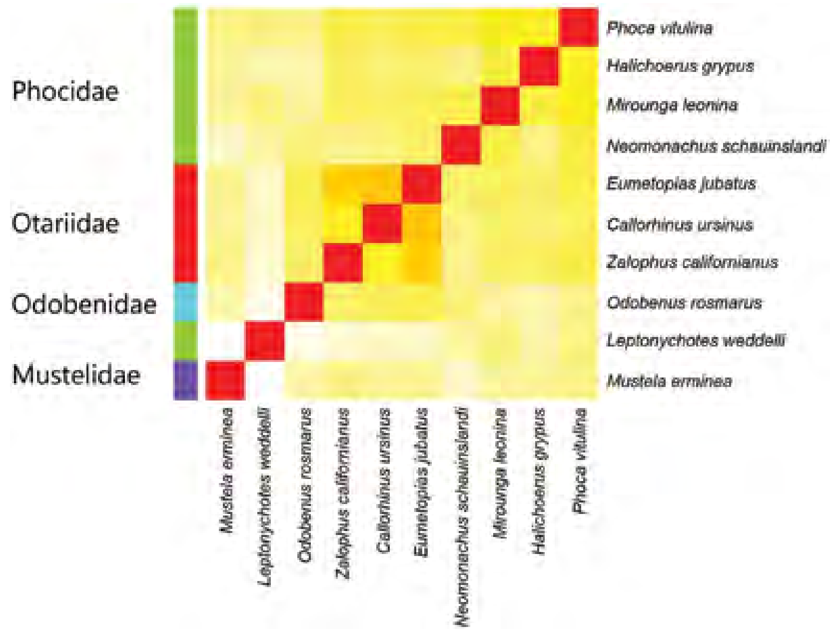

Figure 5. Heatmap showing baraminic relationships between Phocidae, Otariidae, Odobenidae, and *Mustela erminea*, based on the GC method analysis. Each colored pixel represents the Jaccard Coefficient Value of a pair of species. Brighter, redder colors represent species which are more similar to one another, hence they are continuous with one another. Lighter, yellow colors represent species which are less similar to one another, hence showing discontinuity with one another.

Results and Discussion

Mitochondrial analysis

A multiple alignment of 26 of the 27 pinniped mtDNA sequences was created and the sequence identity matrix was visualized in a heatmap in Figure 3. *Mustela erminea* (the short-tailed weasel) was used as an outlier. The identity matrix has a Hopkins clustering value of 0.784 which is good clustering quality.

The elbow plot in Supplementary Figure 1 shows a minimal total within sum of squares (TWSS) value at six clusters. However, the decrease from 0.279 at four clusters to 0.231 at five is an insignificant decrease of less than 5%. If we increase the number of predicted clusters to 5, then *O. rosmarus* clusters with *M. erminea*, and *Phoca groenlandica* and *Phoca fasciata*, are separated from the other *Phoca* species, clearly a bad clustering result. Thus, it appears that there are four baramins represented in the data set.

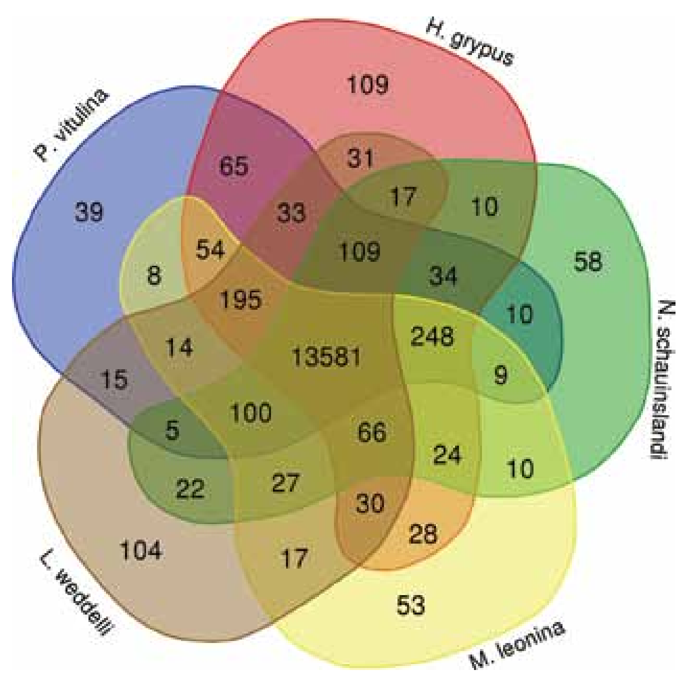

Figure 6. Venn diagram showing overlapping Orthology Group Identifiers between *P. vitulina*, *H. grypus*, *N. schauinslandi*, *M. leonina*, and *L. weddelli.*

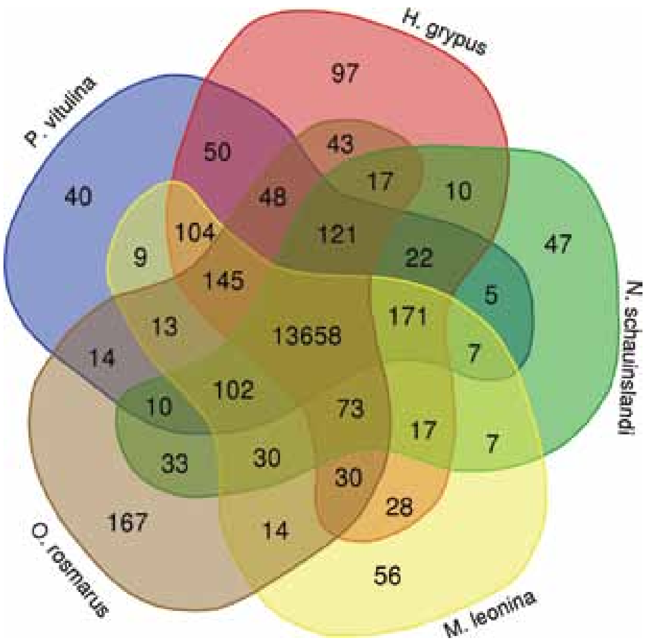

Figure 7. Venn diagram showing overlapping Orthology Group Identifiers between *P. vitulina*, *H. grypus*, *N. schauinslandi*, *M. leonina*, and *O. rosmarus.*

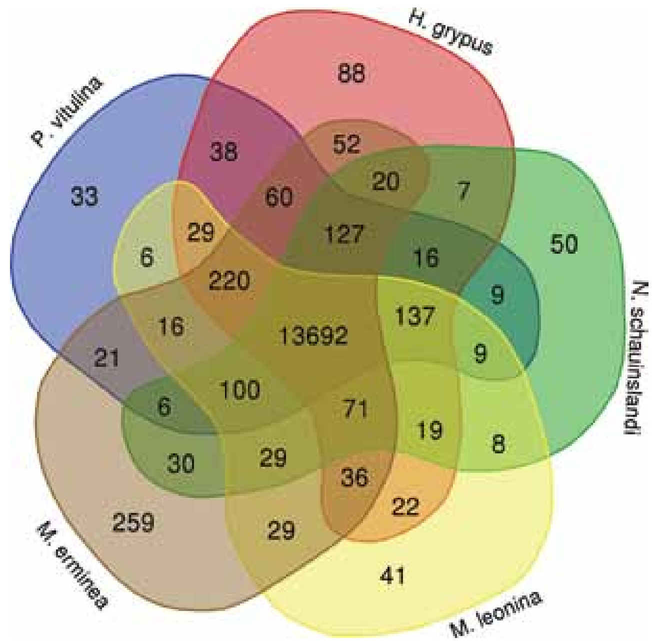

Figure 8. Venn diagram showing overlapping Orthology Group Identifiers between *P. vitulina*, *H. grypus*, *N. schauinslandi*, *M. leonina*, and *M. erminea.*

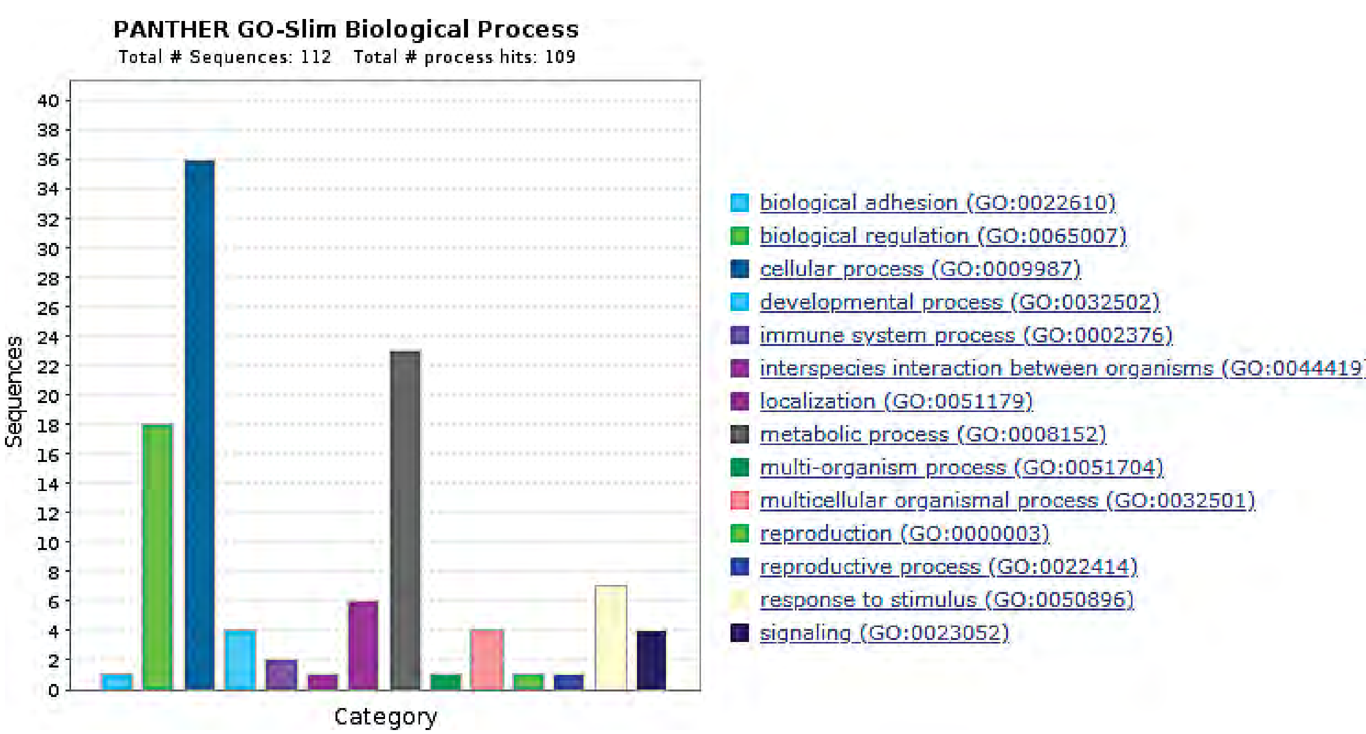

Figure 9. Different categories of PANTHER GO-Slim Biological Processes that the 119 unique *L. weddelli* proteins are enriched in.

The odobenid *O. rosmarus rosmarus* and the outlier, *M. erminea*, are the only singleton species within their own group.

The first larger group includes eight species of otarids: *Arctocephalus pusillus*, *Arctocephalus townsendi*, *Callorhinus ursinus*, *Eumetopias jubatus*, *Neophoca cinerea*, *Otaria byronia*, *Phocarctos hookeri*, and *Zalophus californianus*. This group is statistically significant at a p-value of 8.53E-26.

The second large cluster contains 16 phocids, including several species from ten genera in two subfamilies. From Phocinae: *Crystophora*, *Erignathus*, *Halicheorus*, *Phoca*, and *Pusa*. From Monachinae: *Hydrurga*, *Leptonychotes*, *Lobodon*, *Mirounga*, and *Monachus*. Apparently, Phocinae and Monachinae separate into two lineages based on the mtDNA results. Furthermore, three species of Lobodontine seals (the crabeater seal, *Lobodon carcinophaga*, the leopard seal, *Hydrurga leptonyx*, and the Weddell seal, *Leptonychotes weddelli*) also separate from the genera *Monachus* and *Mirounga*. Despite these different lineages, phocids apparently form a statistically significant holobaramin with a p-value of 4.83E-46.

Lastly, another mitogenomic study based on the alignments of the twelve heavy-chain mitochondrial genes (ATPase6, ATPase8, COI, COII, COIII, cytb, NADH1, NADH3, NADH4, NADH4L, NADH5, and NADH6) was performed by Arnason et al. (2002). 9,882 base pairs and 3,294 amino acids were aligned. These results showed significant discontinuity between pinnipeds and all other mammals, reinforcing the apobaraminic status of Pinnipedia. However, this study seemed to cluster Otariidae together with Odobenidae.

The sequence accession numbers and the results of the mtDNA analysis are available in Supplementary File 1.

Whole genome K-mer analysis

The WGS of six phocids (four otariids, *O. rosmarus*, and the outlier species, *M. erminea*) were downloaded from NCBI. A list of all species and the web address of their genome is listed in Supplementary File 2. The results of the WGKS analysis are also listed in this file: the Pearson Correlation Coefficient (PCC) matrix, the clusters, and the cluster statistics.

The PCC matrix has a Hopkins clustering value of 0.673, which denotes fair clustering. The PCC matrix was transformed into a heatmap to visualize baraminic relationships between the pinniped species and the outlier and can be seen in Figure 4. *M. erminea* clearly separates from all of the pinniped species. The four otariids also show statistically significant continuity within their own group and discontinuity with all other pinnipeds (p = 1.45E-4). The elbow plot in Supplementary Figure 2 shows a sharp drop between one to three

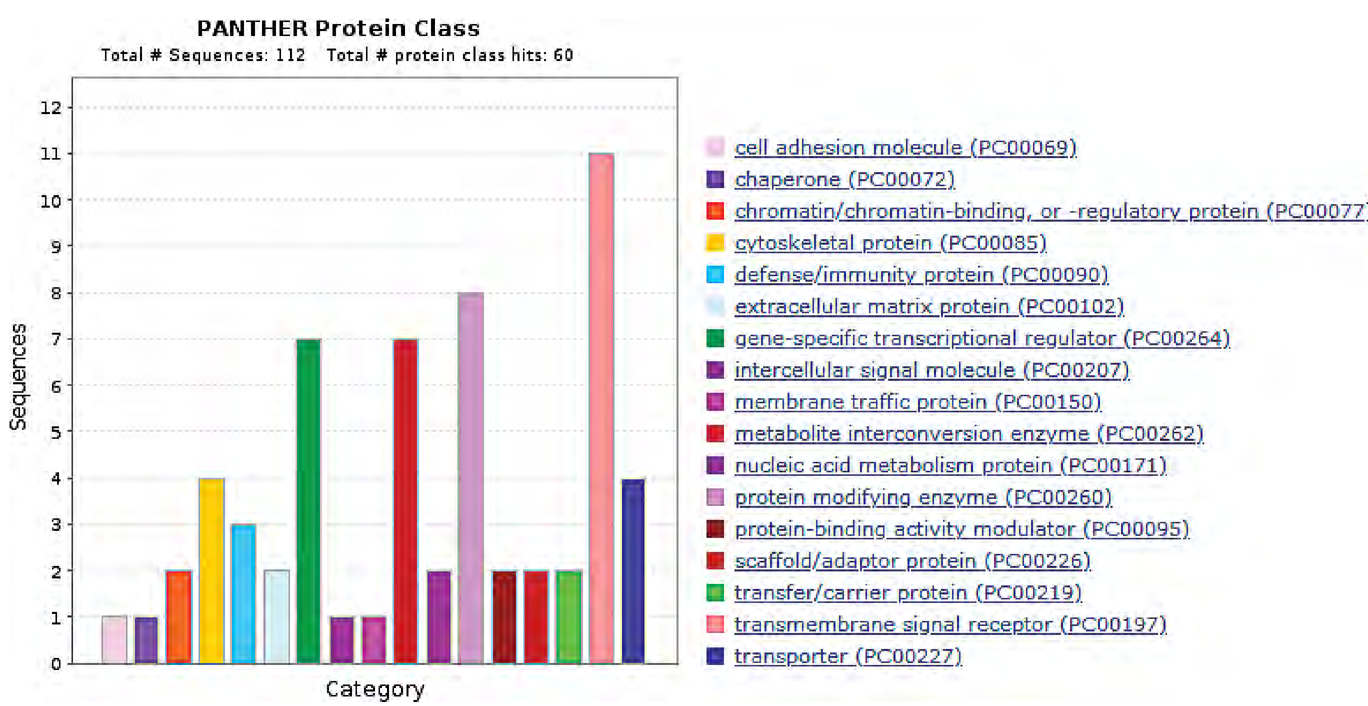

Figure 10. Different categories of PANTHER Protein Classes that the 119 unique *L. weddelli* proteins are enriched in.

clusters. However, the TWSS only decreases from 0.0015 to 0.0091 between three and four clusters. This difference (0.0076) is less than 5% of the TWSS value at one cluster (0.01835).

A more difficult question to answer is, do *O. rosmarus* and the phocids form a single holobaramin, or are they separate? The former result would contradict the mtDNA results where all three pinniped families were separate. To complicate things further, *O. rosmarus* is nested between *L. weddelli* and all other phocid species. Whereas *L. weddelli* has a mean PCC of 0.982±0.001 with the four otariids, it has a mean PCC value of 0.992±0.002 with the five other phocid species. However, it has a PCC value of 0.996 when paired with *O. rosmarus*. Supplementary Figure 2 seems to show an optimum of three clusters.

In order to clarify this discrepancy, further analysis is needed. Therefore, besides the WGKS algorithm, the Gene Content (GC) method was also used to analyze the pinniped species involved in this study.

Gene content analysis

The whole proteomes of five phocids (three otariids, *O. rosmarus*, and the outlier species, *M. erminea*) were downloaded from NCBI. The GC method was run according to the description in O›Micks (2017). The list of species, and the number of proteins, matched Orthology Groups (OG), Jaccard Coefficient Value (JCV) matrix, the putative clusters, and the cluster statistics are available in Supplementary File 3.

The Hopkins clustering statistic has a value of 0.529, which denotes acceptable, but not good clustering. The JCV matrix was visualized in a heatmap, seen in Figure 5. The elbow plot in Supplementary Figure 3 shows four optimal clusters. Statistics were calculated for five clusters. The three otariids (*C. ursinus*, *E. jubatus*, and *Z. californianus*) all form a statistically significant cluster, continuous among themselves and discontinuous with all other species. Four phocids (*H. grypus*, *M. leonina*, *N. schauinslandi*, and *P. vitulina*) group together, but are again conspicuously separated from *L. weddelli*, which was put into its own cluster, as was *O. rosmarus*.

Upon closer examination of the JCV matrix we can see that the mean JCV between *L. weddelli* and otariids is 0.937±0.001, whereas the mean JCV with phocids is 0.945±0.003. This would indicate that *L. weddelli* belongs to the phocid baramin. The JCV between *L. weddelli* and *O. rosmarus* is only 0.933. The mean JCV between *O. rosmarus* and all five phocids is 0.942±0.006. The mean JCV between *O. rosmarus* and the phocid species excluding *L. weddelli* is 0.945±0.003. This would seem to indicate that *O. rosmarus* forms a holobaramin with phocids. Just like with the WGKS results, the GC method also shows a discrepancy between *L. weddelli* and all other phocids.

The results of the GC analysis can be found in Supplementary File 3.

It is interesting to note that neither the mtDNA, nor the WGKS, nor the GC analysis showed that elephant seals are continuous with *O. rosmarus*. However, as we have seen here, the situation is not so clear with *L. weddelli*. Thus, closer attention was given to this species to see what genetic factors could be behind the special baraminic position of *L. weddelli*.

Examination of unique gene content of *L. weddelli*

In order to understand why *L. weddelli* is somewhat different from all other phocids genetically, those OGI were analyzed which belonged uniquely to *L. weddelli* compared to the phocids *H. grypus*, *M. leonina*, *N. schauinslandi*, and *P. vitulina*. *L. weddelli* had 104 such OGI›s mapped to 119 proteins, listed in Supplementary File 4.

Figure 6 shows the overlap of OGIs between *L. weddelli* and the other four phocids. As we can see, even another phocid species, *H. grypus* has more unique OGIs than does *L. weddelli* (109). When we compare the 104 unique OGIs of *L. weddelli* with the mean number of unique OGIs from the other phocids, we see that the z-score is only 1.28, which is statistically significant only at the 20.1% confidence level (see Table 1).

Figure 7 shows that *O. rosmarus* has 167 unique OGIs compared to the four

Table 1. Statistics showing differences in JCV values between four phocids and *Loptonychotes weddelli*, *Odobenus rosmarus*, and *Mustela erminea*.

Species	No. unique OGIs	Mean no. OGIs	St. dev.	Z-score	Conf. level
Leptonychotes weddelli	104	64.8	30.58	1.28	0.201
Odobenus rosmarus	167	60	25.52	4.19	2.79E-5 *
Mustela erminea	259	53	24.34	8.46	2.67E-16 *

*denotes statistically significant a difference between the given species and the four phocids

phocids. This corresponds to a z-score of 4.19, which is statistically significant at the 2.79E-3 % confidence level. This indicates that whereas *L. weddelli* does not contain too many discordant OGIs, *O. rosmarus* does. In comparison, as we can see in Figure 8, *M. erminea*, the outlier species, has 259 unique OGIs compared to the four phocids, corresponding to a z-score of 8.46 which is statistically significant at the 2.67E-14 % confidence level. Since *O. rosmarus* behaves just like the outlier *M. erminea*, this indicates that *L. weddelli* shows continuity with phocids, whereas *O. rosmarus* does not. This would mean that *L. weddelli* belongs to the phocid holobaramin, whereas *O. rosmarus* belongs to its own baramin.

The 119 unique *L. weddelli* proteins were analyzed at the PANTHER database according to the procedures described in the Materials and Methods section. Out of these 119 proteins, 112 mapped to 93 PANTHER functional categories. These were further analyzed for functional classification. Figures 9 and 10 show those biological classes and protein classes that the 119 unique genes were most enriched in. Figure 9 shows that many of the 119 unique genes mostly take part in biological regulation (GO:0065007), cellular processes (GO:0009987), and metabolic processes (GO:0008152). Figure 10 shows that these genes are most enriched in gene-specific transcriptional regulation (PC00264), metabolite interconversion (PC00262), protein modification (PC00260), and transmembrane signaling (PC00197).

Penso-Dolfin et al. (2020) analyzed 559 miRNA loci in *L. weddelli*, of which 146 (26.1%) were found to be unique to that species. These miRNA were expressed predominantly in the brain, the heart, muscle, and blood plasma. They were associated with physiological processes such as hypoxia, lipid metabolism, inflammatory signaling, Jak-stat signaling, and hypercortisolemia. The authors suggested that these physiological processes may aid in deep-sea diving. The Weddell seal is known to be able to dive down to 900 m, and stay submerged for at least 60 minutes, although another seal in this study, *M. leonina*, can dive up to 1000 m in depth. Figure 9 shows that both immune system (GO:0002376) and metabolic processes (GO:0008152) are associated with the 119 unique genes of *L. weddelli*. Figure 10 shows that several protein classes that these unique *L. weddelli* proteins belong to are also active in cell signaling: defense/immunity (PC00090), intercellular signaling (PC00207), membrane traffic (PC00150), metabolite interconversion (PC00262), and transmembrane signaling (PC00197).

The list of unique OGIs for *N. schauinslani*, *H. grypus*, *M. leonina*, *P. vitulina*, *L. weddelli*, *O. rosmarus*, and *M. erminea*, a list of the 119 unique *L. weddelli* proteins, and their PANTHER functional classification are available in Supplementary File 4.

Conclusion

Considering multiple lines of evidence, the three pinniped families each form a holobaramin. This is visible from the mtDNA results. The mtDNA analysis also shows that lobodontine and monachine seals, as well as seals from the genus *Mirounga* form a separate lineage from all other phocids. This is not so clear from the WGKS analysis, although these results show that otariids are fairly discontinuous with phocids and *O. rosmarus*. The GC method also shows that otariids separate from phocids and *O. rosmarus*, and that *O. rosmarus* also separates from phocids, except for *L. weddelli*. None of the studies show that *Mirounga* is in the same baramin as *O. rosmarus*. At best *O. rosmarus* shows genetic similarity with *L. weddelli*, although upon closer examination this does not seem to be the case, based on a statistically significant higher number of unique OGIs in the genome of *O. rosmarus* compared to *L. weddelli*.

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