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The Karyotype of Blainville's Beaked Whale, *Mesoplodon densirostris*

Ross Brookwell[a] Kimberly Finlayson[b] Jason P. van de Merwe[b]

[a]Cytogenetics Department, Sullivan Nicolaides Pathology, Bowen Hills, QLD, Australia; [b]Australian Rivers Institute, Griffith University Gold Coast, Southport, QLD, Australia

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Blainville · Beaked whale · Cetacea · Chromosome · G-band

Abstract
The karyotype of the Odontocete whale, *Mesoplodon densirostris*, has not been previously reported. The chromosome number is determined to be 2n = 42, and the karyotype is presented using G-, C-, and nucleolar organizer region (NOR) banding. The findings include NOR regions on 2 chromosomes, regions of heterochromatic variation, a large block of heterochromatin on the X chromosome, and a relatively large Y chromosome. The karyotype is compared to published karyograms of 2 other species of *Mesoplodon*.

Introduction

The infraorder Cetacea is divided into 2 subgroups, the Mysticeti, or baleen whales, and the Odontoceti, or toothed whales. Ziphiidae, or beaked whales, is a family within the Odontoceti group. There are currently 22 recognised species of beaked whales. Fifteen of these are contained within the genus *Mesoplodon*, and only 3 of these species have been karyotyped: *Mesoplodon europaeus* [Arnason et al., 1977], *M. carlhubbsi* [Arnason et al., 1977; Kurihara et al., 2017], and *M. stejnegeri* [Kurihara et al., 2017]. These 3 species have an X chromosome with a large distal heterochromatic block on the long arm and also have 42 chromosomes, whereas most cetaceans have 44 [Arnason et al., 1977]. The only other known cetacean karyotypes with 42 chromosomes, apart from the beaked whales, are the sperm whale, *Physeter macrocephalus*, the pygmy sperm whale, *Kogia breviceps* [Arnason and Benirschke, 1973], the North Atlantic right whale, *Eubalaena glacialis* [Pause et al., 2006], and the bowhead whale, *Balaena mysticetus* [Jarrell, 1979]. As the sperm and pygmy sperm whales are Odontocete whales, and the North Atlantic right whale and bowhead whale are Mysticete whales, the occurrence of only 2 chromosome numbers within the Cetacea suggests a relatively simple relationship between sub-groups. However, reference to the evolutionary tree, as it is currently understood [see figure 9 in Gatesy et al., 2013], indicates that the evolutionary pathway is more complex. Therefore, karyotyping of more cetacean species, in conjunction with comparative molecular and chromosome painting data, will assist our understanding of these relationships. We present the karyotype of *M. densirostris* from a single individual male, which also has 42 chromosomes, the large X chromosome, and a relatively large Y chromosome compared to that of those cetaceans studied so far.

Materials and Methods

Tissue Source and Cell Establishment

A kidney sample from a juvenile, male *M. densirostris* that stranded at Moonee Beach on February 3, 2017 was provided by Dolphin Marine Conservation Park, Coffs Harbor, NSW. There was no obvious cause of stranding, and the animal tested negative

karger@karger.com
www.karger.com/cgr

Ross Brookwell
Cytogenetics Department, Sullivan Nicolaides Pathology
24 Hurworth Street
Bowen Hills QLD 4006 (Australia)
Ross_Brookwell@snp.com.au

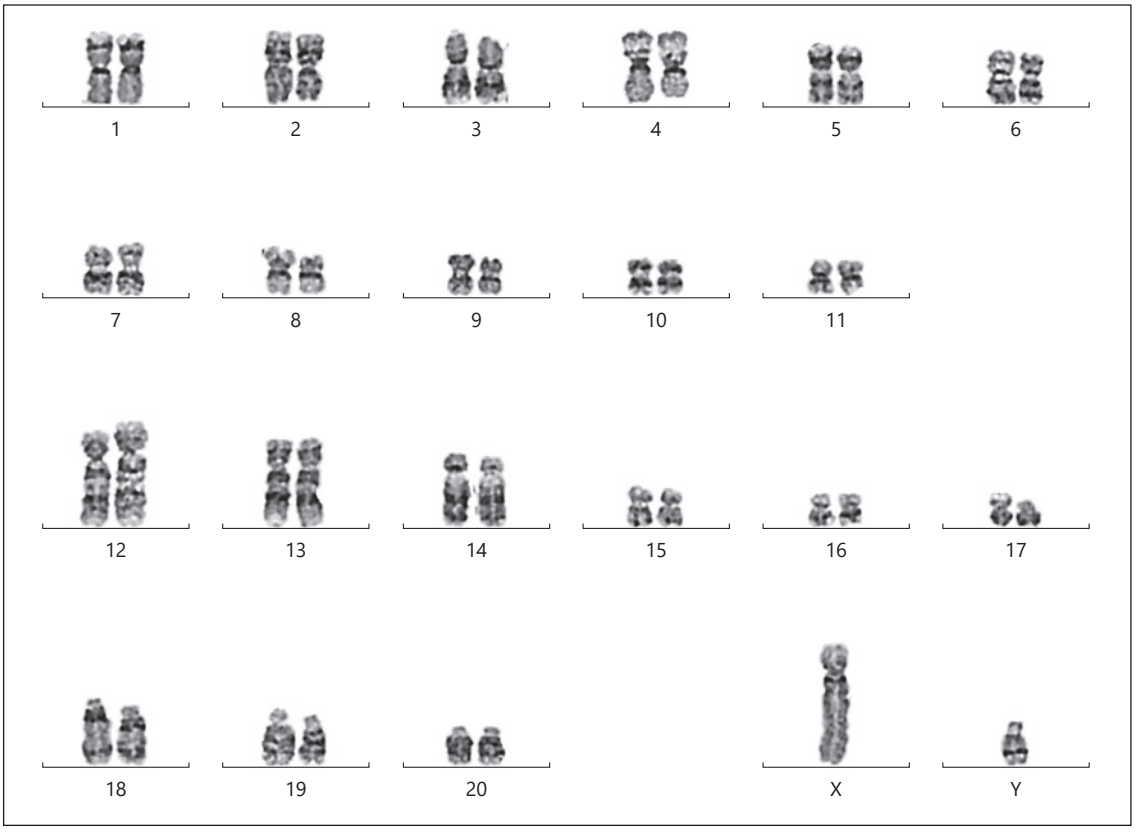

Fig. 1. G-banded karyotype. Note the heteromorphic regions in the long arm of pair 2, the long arm of pair 4, and the short arms of pairs 8, 14, and 17.

to morbillivirus, leptospirosis, and toxoplasmosis. The sample was kept at 4°C until establishment of the cell culture the following day. The kidney sample was washed several times in DMEM media with 10% fetal bovine serum, 1% penicillin/streptomycin (10,000 U/mL stock), and 1% amphotericin B (250 μg/mL stock). Tissue was cut into 1–3 mm pieces in fresh DMEM media, then transferred into a 25 cm^2 flask and arranged evenly on the bottom of the flask. The flask was inverted and incubated at 37°C, 5% CO_2 for 24 h. Following this, the flask was returned upright, 5 mL of fresh media was added, and the flask was returned to the incubator. Tissue pieces were detached and removed when cells reached about 70% confluency. Cells were cryopreserved at passage 2 at a concentration of 1×10^6 cells/mL in DMEM media described above supplemented with 10% dimethyl sulfoxide until ready to be used.

Species Identification

DNA was isolated from about 2×10^6 cells using the Qiagen DNeasy Blood and Tissue kit according to the manufacturer's protocol for cultured cells. DNA was sent to the Griffith University DNA Sequencing Facility for taxonomic validation. Briefly, approximately 660 bp of the mitochondrial *COI* gene was amplified by Platinum Taq DNA polymerase (Invitrogen) using the following primers: forward 5–3′ ATTCAACCAATCATAAAGATATTGG and reverse 5–3′ TAAACTTCTGGATGTCCAAAAAATCA [Hebert et al., 2004]. PCR amplicons were cleaned using ExoSap-IT (Applied Biosystems) and underwent bidirectional sequencing. The resulting sequences were classified using the Barcode of Life Database (v4, BOLD http://www.boldsystems.org/).

Karyotyping

A flask of cells at passage 4 was sent to the cytogenetics laboratory at Sullivan Nicolaides Pathology for karyotype analysis. The cells were subcultured and grown in Amniomax II medium (Gibco) in 25 cm^2 flasks. At around 80% confluence, cells were harvested by adding colchicine (100 μg/mL) for 2 h, removing the cells from the flask surface with trypsin (Trypsin/EDTA 1×, Sigma), and treating them with hypotonic solution (0.075 M potassium chloride) for 10 min at 37°C. A 3% acetic acid prefix solution was then added at a 1:9 ratio before standard 3:1 methanol:glacial acetic acid fixation. Slides were prepared by dropping the fixed suspension onto dry slides cleaned beforehand in ethanol. G-banding [Seabright, 1971] was performed after overnight incubation at 60°C. The trypsin solution used was 5 mL stock solution (2.5 g of 1:250 trypsin powder in 500 mL PBS without calcium and magnesium, dissolved on a magnetic stirrer) in 45 mL of PBS. Slides were then stained with Wright's/Giemsa stain (Kinetik).

The G-banded slides were processed by a Metafer slide scanner (Metasystems), and suitable cells were karyotyped using the Ikaros karyotyping system (Metasystems). Slides were processed for

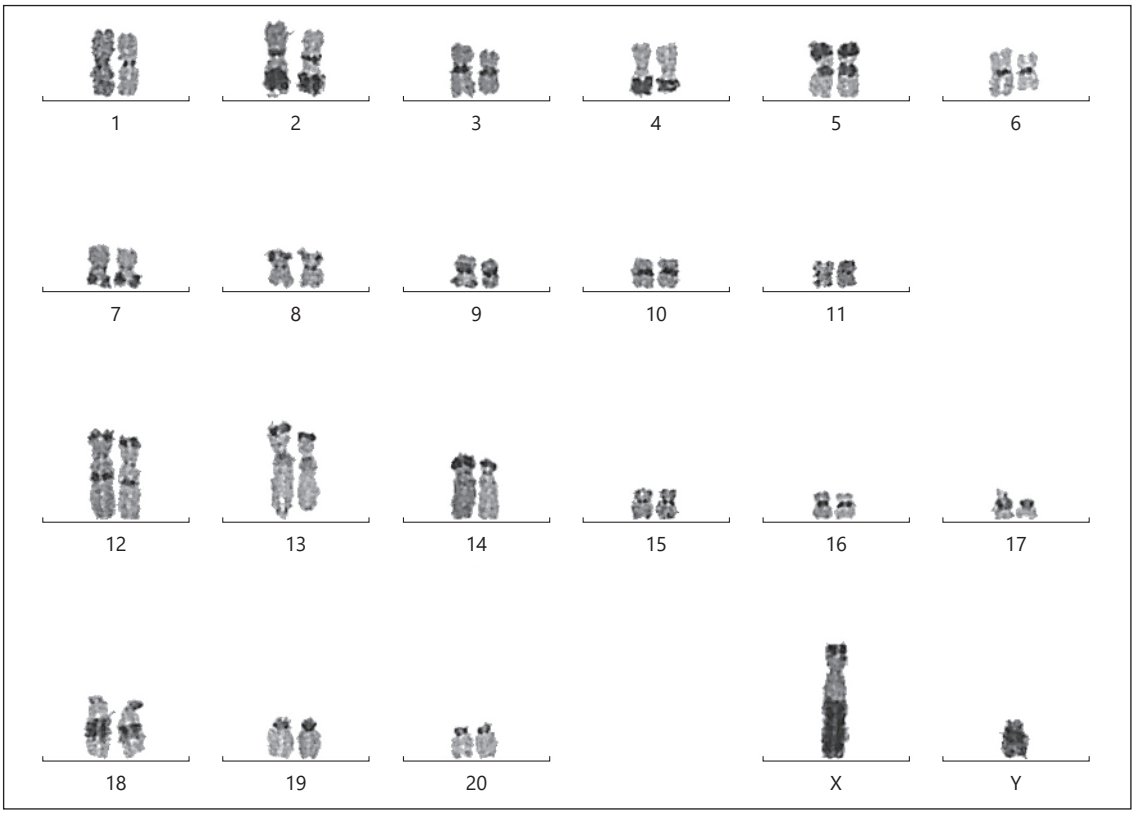

Fig. 2. C-banded karyotype. Assignment of chromosomes is based on a "best fit" with the G-banded cells, taking account of the likely concurrence of G-band heteromorphism and C-band positive heteromorphic regions.

CBG-banding using a barium hydroxide-based procedure [Sumner, 1972] and for NOR staining using silver nitrate [Howell and Black, 1980].

Results

Species Identification

Species identification was confirmed to be *M. densirostris* with a 99.41% match of the partial *COI* gene sequence.

Karyotype

The karyotypes are presented in a format to allow comparison to previous publications of cetacean species and show that pairs 1–11 are metacentric/near metacentric, pairs 12–17 submetacentric, and pairs 18–20 are subtelocentric/acrocentric. The X chromosome has an elongated long arm, and the Y chromosome is relatively large in comparison to previously published cetacean species.

G-banding shows the presence of several heteromorphic regions: on the long arm of the 2nd pair, the long arm of pair 4, and the short arms of pairs 8, 14, and 17 (Fig. 1) (online suppl. Fig. 1–5; for all online suppl. material, see www.karger.com/doi/10.1159/511730).

C-banding allows matching of the heteromorphic regions with C-band variation in pairs 2, 4, 8, and 14 (Fig. 2) and locates the heterochromatin on the Y. While the C-band positive region in the distal long arm of pair 2 does appear heteromorphic, G-banding indicates a variation in the proximal long arm in addition. This apparent polymorphism may be due to euchromatic variation or other unidentified factors.

NOR-banding (Fig. 3) shows the presence of NOR^{+ve} regions on the distal long arm of pair 13 and the short arm of pair 17; in the latter case a difference in size of NORs is noted (one is not visible in Figure 3, see online suppl. Fig. 6), explaining the size heteromorphism noted in G-banding.

The X chromosome has heterochromatic regions on the distal short arm and distal long arm, the latter being of significant size. The Y chromosome has a prominent dark band in the long arm by G-banding; in C-banding

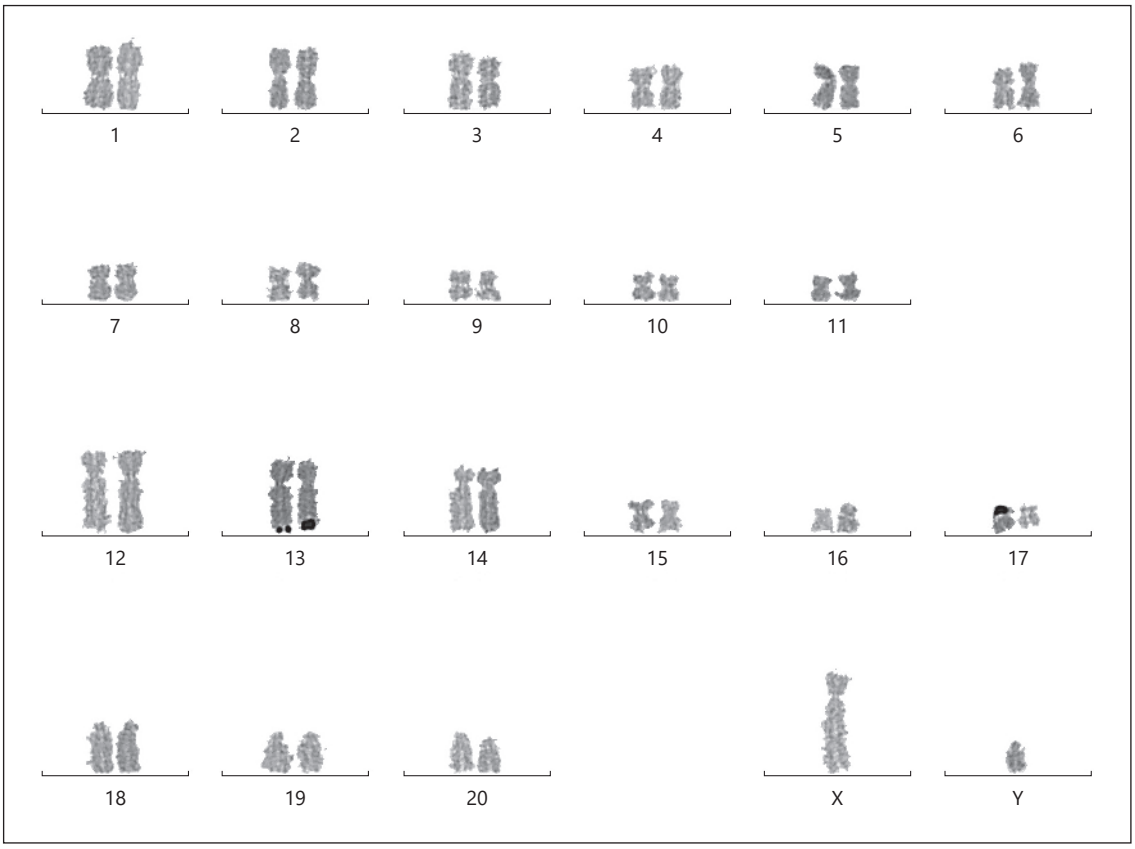

Fig. 3. NOR-banded karyotype. Positive regions are seen on 13q and 17p. One homologue of 17p shows no signal (absent in some cells, very small in others).

this region appears to be less dark than the rest of the long arm. Further examples of C-banded Y chromosomes are available in online supplementary Figure 7.

Discussion

While drawing conclusions concerning the species is limited by availability of material from just one individual, there are several points of interest raised. The karyotype has 42 chromosomes and an X chromosome with a large block of heterochromatin on the distal long arm, as seen in the other 3 species of *Mesoplodon* published so far. A similar X chromosome morphology has also been observed in the Mysticete fin whale, *Balaenoptera physalus* [Arnason, 1974], but not in the sei whale, *B. borealis* [Arnason, 1974], or the minke whale, *B. acutorostrata* [Arnason et al., 1977]. It is also present in the humpback whale, *Megaptera novaeangliae* [Burkard et al., 2015]. This X chromosome morphology is unusual, but not necessarily useful in establishing taxonomic relationships, as the heterochromatic blocks can vary considerably within individuals. For example, in the specimen of *B. physalus* in the paper by Arnason [1974], one X has lost much of the heterochromatic segment, and such variation would not be expected to have an effect on the phenotype. Heterochromatin can appear as dark, intermediate, or light areas by G-banding; in this individual the heterochromatic regions appear mainly as pale staining. The size variation of heterochromatin in cetaceans has been shown to be due to amplification of a small number of specific types of heterochromatin, as detailed by Arnason and Widegren [1984, 1989] and Kulemzina et al. [2016].

The location of NOR regions appears likely to be on the same 2 chromosomal regions demonstrated in *M. europaeus* in the study by Arnason [1981].

A composite of the karyotypes of 3 species of *Mesoplodon* [*M.densirostris*, *M.europaeus,* and *M.carlhubbsi*, the last 2 taken from figures in Arnason et al. [1977]] is pre-

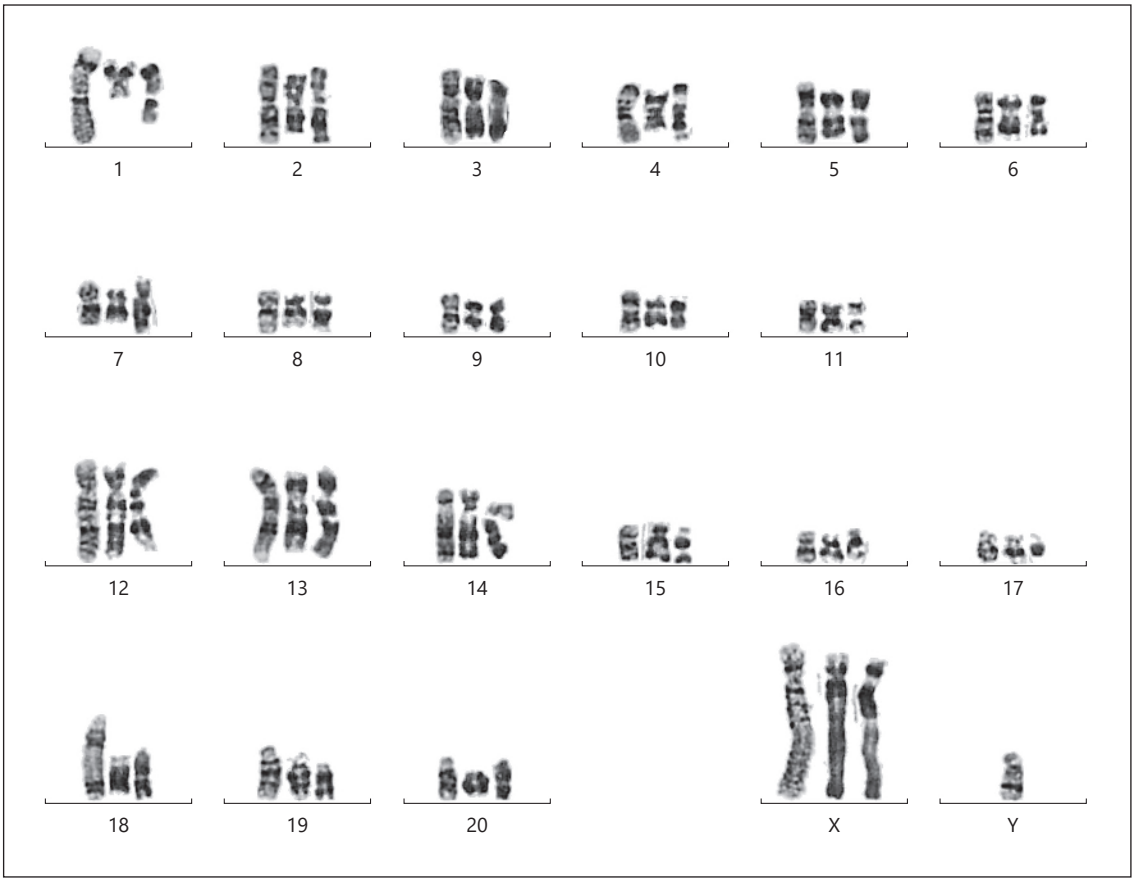

Fig. 4. Composite karyogram with 1 homologue of each pair from 3 species of *Mesoplodon* (left: *M.densirostris,* middle: *M.europaeus,* right: *M.carlhubbsi).* The latter 2 karyograms are from Arnason et al. [1977]. Note that this is necessarily a subjective assessment. While some chromosomes show recognisable features in common, in others placement is based on size or convenience alone.

sented in Figure 4. This shows the basic similarity between the chromosomes of these species. Of interest is that the X chromosome heterochromatin, despite heterochromatic blocks generally being regarded as a continuously variable feature, appears to be of very similar size. The different amounts of heterochromatin seen interstitially in the long arm of chromosome 18 of these individuals show how this can affect the appearance of the chromosome. While there is a great degree of similarity between these karyotypes, bearing in mind that G-band appearance is superficial and may not reflect underlying DNA homology, there are significant differences. Most noteworthy are chromosomes 1 and 4, where there are long stretches of G-band, and apparently C-band [Arnason, 1981] negative material in the current species that are not apparent in the other 2.

A further composite of the karyotypes of 4 species of cetaceans, *M. densirostis, E. glacialis* (2n = 42) [Pause et al., 2006], *Tursiops truncatus, and M. novaeangliae* (2n = 44) is presented in online supplementary Figure 8 and shows that certain chromosomes appear to be recognizable in all 4 species. This is in line with the high degree of karyotypic conservation postulated in marine mammals by Arnason [1972], based on chromosome number and arm ratio measurements. This comparison does point to a large number of chromosomal rearrangements distinguishing these species. However, chromosome painting studies show a high level of conservation of genetic segments in cetaceans [Bielec et al., 1998; Kulemzina et al., 2009] and in mammals more widely [Nie et al., 2012].

In other studies of cetaceans, the Y chromosome is variable, but generally small. The largest Y chromosome published to date is that of individuals of *B. acutorostrata* [Arnason, 1974; Arnason et al., 1977], where the Y is similar in size to the smallest autosome. With regards specifically to the genus *Mesoplodon*, the individuals of *M. europaeus* and

M. carlhubbsi in the study of Arnason et al. [1977] were female. In the paper by Kurihara et al. [2017], the Y chromosomes of *M. stejnegeri* and *M. carlhubbsi* appear to be small and metacentric. In the current individual of *M. densirostris*, the Y is significantly larger. It stains mainly positively with C-banding, there is a less intense region on the proximal short arm, and the prominent dark G-band on the long arm also coincides with a less intense C-band region, suggesting that this may contain some active genetic material. As the available karyotypes from *Mesoplodon* are from single individuals, and the Y chromosome, being mostly heterochromatic, can have considerable intraspecific morphological variability, not too many conclusions can be drawn; however, the Y structure does look unusual.

Conclusion

This study presents the karyotype of Blainville's beaked whale, *M. densirostris*. The results show a karyotype consistent with other published species in the genus and locate the heterochromatic regions and NORs of this species. Comparative analysis with other species of *Mesoplodon* confirmed a high degree of similarity within the genus.

Statement of Ethics

Ethical approval is not required for this type of research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

K.F. and J.P.v.d.M. performed the molecular work and wrote the tissue source and species identification sections, R.B. performed the cytogenetic work and wrote the introduction, karyotyping section and the discussion.

References

Arnason U. The role of chromosomal rearrangement in mammalian speciation with special reference to Cetacea and Pinnipedia. Hereditas. 1972;70(1):113–8.

Arnason U. Comparative chromosome studies in Cetacea. Hereditas. 1974;77(1):1–36.

Arnason U. Localization of NORs in cetacean karyotypes. Hereditas. 1981;95(2):269–75.

Arnason U, Benirschke K. Karyotypes and idiograms of sperm and pygmy sperm whales. Hereditas. 1973;75(1):67–74.

Arnason U, Widegren B. Different rates of divergence in highly repetitive DNA of cetaceans. Hereditas. 1984;101(2):171–7.

Arnason U, Widegren B. Composition and chromosomal localization of cetacean highly repetitive DNA with special reference to the blue whale, Balaenoptera musculus. Chromosoma. 1989;98(5):323–9.

Arnason U, Benirschke K, Mead JG, Nichols WW. Banded karyotypes of three whales: Mesoplodon europaeus, M. carlhubbsi and Balaenoptera acutorostrata. Hereditas. 1977;87(2):189–200.

Bielec PE, Gallagher DS, Womack JE, Busbee DL. Homologies between human and dolphin chromosomes detected by heterologous chromosome painting. Cytogenet Cell Genet. 1998;81(1):18–25.

Burkard M, Whitworth D, Schirmer K, Nash SB. Establishment of the first humpback whale fibroblast cell lines and their application in chemical risk assessment. Aquat Toxicol. 2015;167:240–7.

Gatesy J, Geisler JH, Chang J, Buell C, Berta A, Meredith RW, et al. A phylogenetic blueprint for a modern whale. Mol Phylogenet Evol. 2013;66(2):479–506.

Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proc Natl Acad Sci USA. 2004;101(41):14812–7.

Howell WM, Black DA. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method. Experientia. 1980;36(8):1014–1015.

Jarrell GH. Karyotype of the bowhead whale (Balaena mysticetus). J Mammal. 1979;60:607–10.

Kulemzina AI, Trifonov VA, Perelman PL, Rubtsova NV, Volobuev V, Ferguson-Smith MA, et al. Cross-species chromosome painting in Cetartiodactyla: reconstructing the karyotype evolution in key phylogenetic lineages. Chromosome Res. 2009;17(3):419–36.

Kulemzina AI, Proskuryakova AA, Beklemisheva VR, Lemskaya NA, Perelman PL, Graphodatsky AS. Comparative chromosome map and heterochromatin features of the gray whale karyotype (Cetacea). Cytogenet Genome Res. 2016;148(1):25–34.

Kurihara N, Tajima Y, Yamada TK, Matsuda A, Matsuishi T. Description of the karyotypes of Stejneger's beaked whale (Mesoplodon stejnegeri) and Hubbs' beaked whale (M. carlhubbsi). Genet Mol Biol. 2017;40:803–7.

Nie W, Wang J, Su W, Wang D, Tanomtong A, Perelman PL, et al. Chromosomal rearrangements and karyotype evolution in carnivores revealed by chromosome painting. Heredity (Edinb). 2012;108(1):17–27.

Pause KC, Bonde RK, McGuire PM, Zori RT, Gray BA. G-banded karyotype and ideogram for the North Atlantic right whale (Eubalaena glacialis). J Hered. 2006;97(3):303–6.

Seabright M. A rapid banding technique for human chromosomes. Lancet. 1971;2(7731):971–972.

Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 1972;75(1):304–306.