The origin of die skin colour of toothed whales (Odontoceti)

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Abstract

The origin of the colour of cetaceans (Odontoceti).
Originally the skin colour of mammals derives from melanocytes, which produce melanin-pigments of a variety of brownish colours, known from human beings. Whales possess such pigments too, but in addition the skin of cetaceans contains white, blue, red and yellow colours. Different forms of chromatophores were discovered in the skin of toothed whales, examined by light and scanning electro microscopy (SEM) and analysed by a microanalyzer. This is the first report of the existence of chromatophores in cetaceans.

Zusammenfassung

Die Herkunft der Hautfarbe der Zahnwale (Odontoceti).
Die Hautfarbe der Säuger entsteht durch Melanin, das in Melanozyten erzeugt wird. Durch die Einlagerung von Melaninpigmenten in der Haut entstehen dann nur bräunliche Farbtöne, so wie sie uns auch vom Menschen bekannt sind. Vergleichbare Melaninpigmente besitzen auch die Wale. Doch neben den genannten Farbtönen zeigt die Walhaut außerdem noch weiße, blaue, rote und gelbe Farbnuancen. In der Haut von Zahnwalen wurden verschiedene Formen von Chromatophoren entdeckt, mittels Licht- und Rasterelektronenmikroskop (REM) untersucht und analysiert. Dies ist der erste Nachweis von Chromatophoren in der Haut von Walen.

Introduction

Common names of some whales refer to their skin colour. Blue, Gray and White whales are widely known. “Brown fish” is the German name of the Harbour porpoise. Well known from whale watching are the yellow stripes of the Common dolphin.

The common skin colour of mammals originates from melanocytes (Fig. 1), cells in which the small dark granulous pigments named “melanin” are developed. Through small tubes the melanin-pigments leave their cells and are stored in the upper epidermal layers. This is

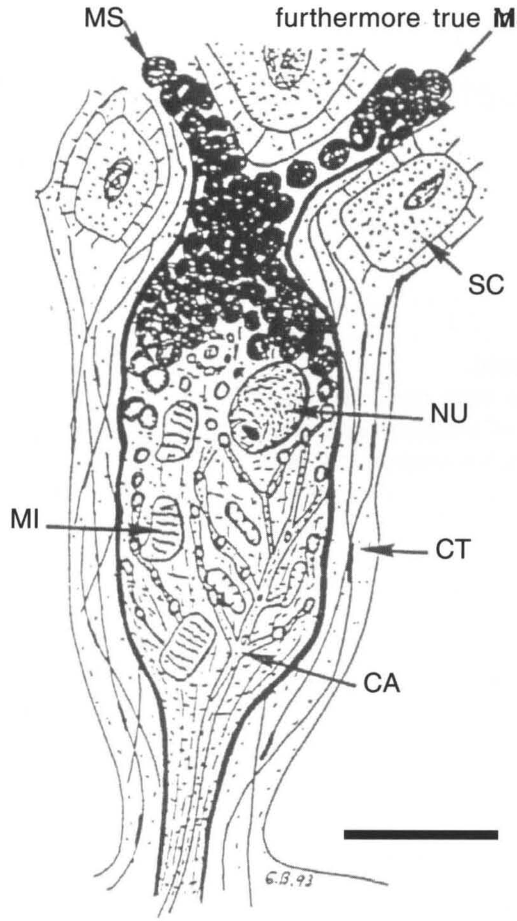


Fig. 1.
Schematic model of one melanocyte of a toothed whale,
bar 5 μm Capillar (CA), Connective tissue (CT), mitochondrion (MI), melanosome (MS), nucleus (NU), prickle cell (SC).

furthermore true whales special in back regions too, but the production of melanin-pigments is in the other regions of the skin not high enough to stain the skin, and furthermore, from the melanin only brownish colours arise, like in human beings. But the blue, red, yellow, and the bright white colours of whales cannot result from melanin.

The dark-brown colour of the back-side of whales mostly originates from melanin, while the white, red, brown, blue and yellow colours of the whale skin may be created by chromatophores. Their existence in different types will be described in this contribution. This paper is intended to prove the presence of such chromatophores in toothed whales in describing different types of such cells.

Material and methods

Skin pieces (5 to 5 centimetres) of beached whales, such as the Harbour porpoise (*Phocoena phocoena* L., 1758), Common dolphin *Delphinus delphis* L., 1758, Killer whale (*Orcinus orca* L., 1758), and Northern bottlenose whale *Hyperoodon ampullatus* (Forster, 1770) were cleaned after removing the blubber, in using a high pressure water beam. The skin was macerated in warm water of 40 degree Celsius. The epidermis disintegrated more quickly than the chromatophores, and they could be separated in distilled water by centrifugation.

The colour of pigments can only be examined in unfixed and unstained material, as most pigments are destroyed by the preparative solutions.

From the separated hard material coated by carbon (C), qualitative analyses of the elements were made by energy dispersal X-ray microanalysis (Edax) in a scanning electron microscope (SEM). Following elements were used for the analyses: natrium (Na), magnesium (Mg), silicium (Si), phosphorus (P), sulfur (S), chlorine (Cl), kalium (K), and calcium. Carbon (C) used for coating of the material is not considered here. The material used for SEM-photography was coated with gold.

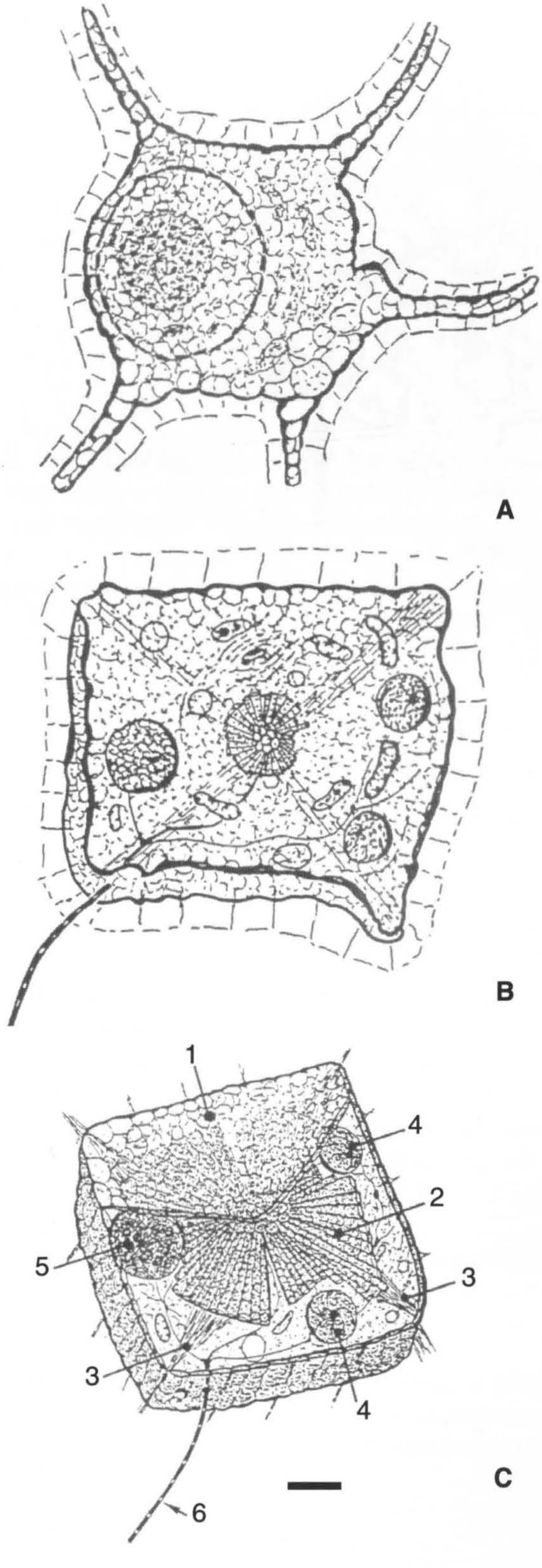
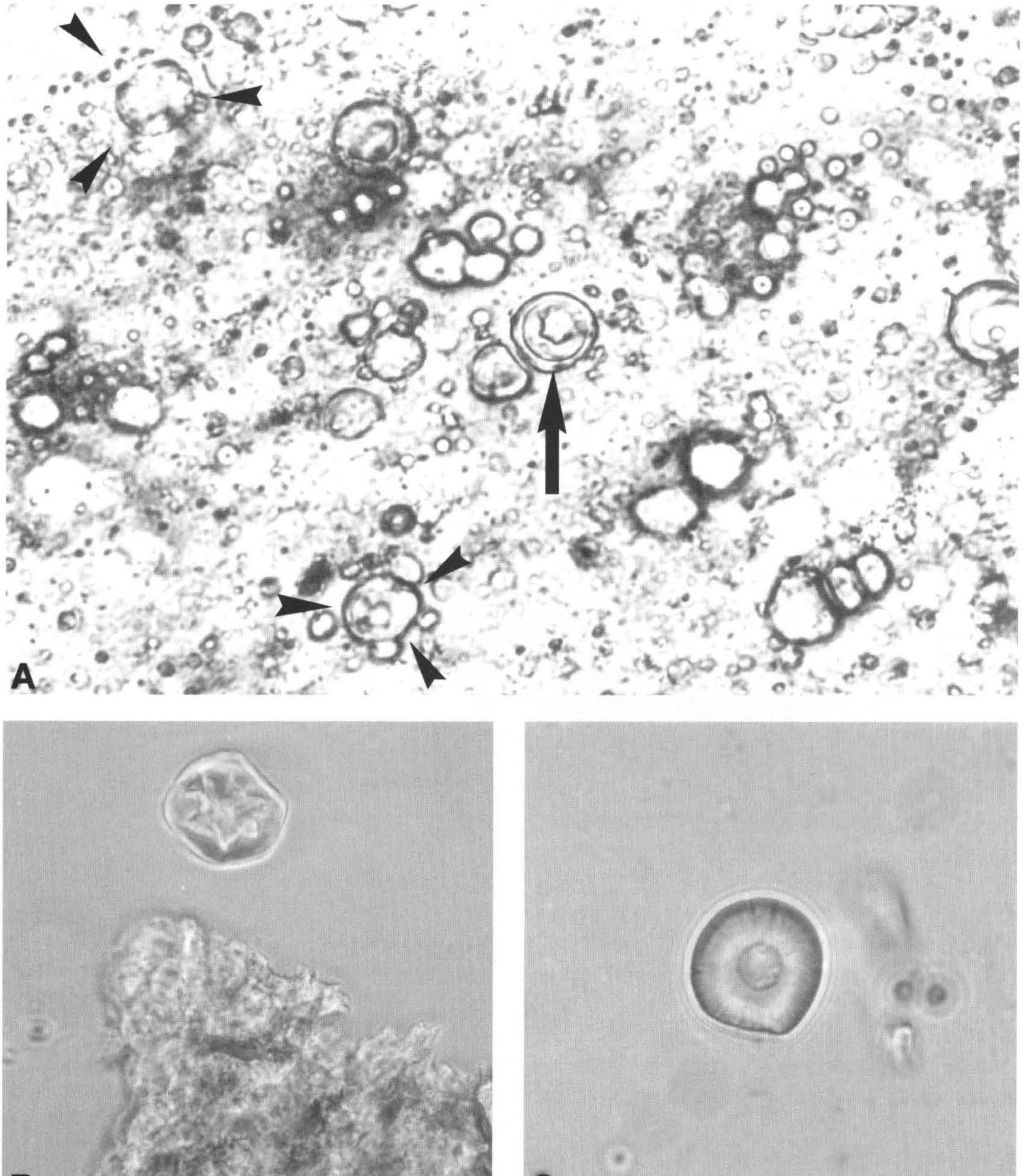


Fig. 2.
The development of a chromatocyte:
A: sclerocyte.
B: development of a star-like central plate.
C: schematic model of a star-like melanocyte, bar 10 μm.

1: membrane of the cell partially removed
2: star-like plate covered with pigments
3: tissue fibres keeping the plate in its position
4: nuclei
5: chromatoblast producing pigments
6: neurite


A
B
C

To distinguish lipid droplets from chromatophores, the fresh material was stained with 1 per cent osmium sulfate.

Using frozen unfixed microscopic sections, and fixed unstained and stained microscopic sections 3 to 5 μm thick and fixed in glutaraldehyd/formalin (2 : 2 per cent), the number and the development of the chromatophores were examined.

The drawings result from direct observations using SEM and light-microscopic photographs.

Results

In the dark back and the dark parts of the flukes and the flippers melanosomes dominate produced by melanocytes (Fig. 1). In contrast to the conditions in the skin of other mammals, where during the ontogenesis the melanocytes migrate into the upper epidermal layers (Spearman, 1973), the melanocytes of cetaceans remain on the epidermal basis (Stratum germanitivum), and only the melanin-pigments are stored in upper epidermal layers (Harrison, 1974).

Fig. 3., Page 134 Harbour porpoise: horizontal section through the epidermis, 10 em below the surface.

A: sclerocytes (◄), one chromatocyte with a star-like central plate (◄──), unstained, 400 x.

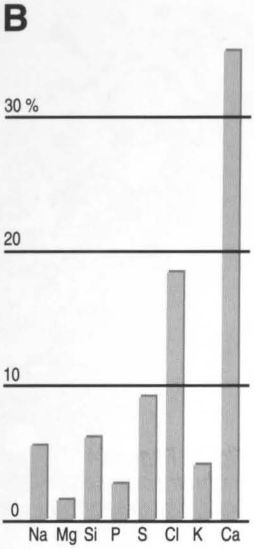
B: the separated chromatocyte with a star-like chromatophore, 1000 x.

C: Bottlenose dolphin, a separated chromatocyte with a roundish chromatophore, 1000 x.

Although melanocytes exist in all regions of the epidermis, they do not produce melanin in regions with white colour. In regions with other colours, the production of melanin is quite different, and has with its quantity of the melanin-pigments an influence of the colouring. Backs of Harbour porpoises with a little production of melanin have reddish colouring. White skin-regions with a little production of melanin have a greyish colouring. Sometimes the stripes of the Common dolphin have a bright yellow colouring, and sometimes their stripes are yellow-brownish. The reason of the colour-changing is still unknown.

Chromatocytes with a calcareous central corpuscle originate from skleroblasts, osteoblast-like cells (Fig. 2 A.), situated in the upper layers of the epidermis (Fig. 3). In the skleroblast, to identify by its bigger nucleus than in the other prickle cells, begins the calcification in the centre of the cell with small calcareous germs (Fig. 2 B.). Supplementing with small calcareous particles, the central plate or the roundish central corpuscle grows from the inside to the outside. Simultaneously a chromatoblast arises much bigger than the nucleus (Fig. 2 C: 4, 5), and is therefore clearly to differ.

The colour-pigments, produced by the chromatoblasts (Ballowitz, 1931), are installed on the calcareous central plate, or on the roundish central corpuscle, or between the spines of the spinous chromatophores.



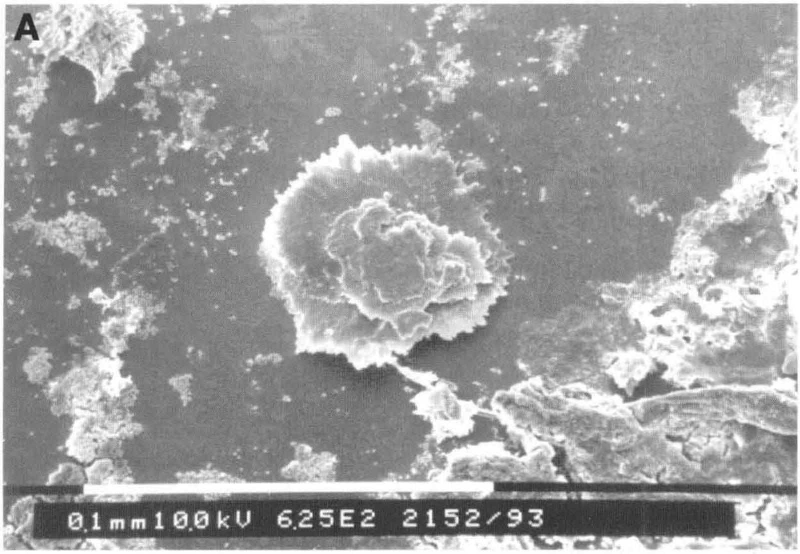


Fig. 4.
A: SEM photography of the star-like chromatophore.

B: the result of the electron microprobe analysis showing bone-like elements.

The roundish chromatophores (Fig. 3) have a diameter of up to 50 μm. The star-like chromatophores (Fig. 3 A, B) have a diameter of up to 80 μm and are held in their position by tissue fibres (Fig. 2 C: 3). The case-like chromatocyte is only little bigger than the plate and has a parakeratinous membrane, which is for a long time resistant against acid solutions of 10 per cent, and also against alkaline solution of 10 per cent.

With the outgrowings of the prickle cell layer, the chromatocytes come into the upper epidermal layer and the cells become thinner, and therefore the colour of the chromatophores becomes clearly visible on the outside.

Each type of chromatophore is determined by its distinct colour (Ballowitz, 1931): black = melanophore; red = erythrophore; yellow = xanthophore. The blue colour comes mostly from lipid droplets and therefore the cells are named lipidophores. Chromarophores with a white iridescent colour are named irridophores. Chromatophores can carry different pigments, and together with the reflected light in the membrane the whole spectrum of colours can appear.

Chromatocytes with blue colour (Fig. 7), mostly stored in the parakeratotic layer of the epidermis, are special lipid cells with a parakeratinous membrane. In the lipidophore the variation depends on the intensity of the colour and of the number of the pigment droplets. The lipidophores are clearly different from other lipid droplets which are also stored in the whole integument, by their bigger and darker chromatoblasts and their parakeratinous membranes. The difference of both, lipidophores and lipid droplets, becomes clearly visible in a solution of 1 per cent osmium-sulfate. The osmium solution

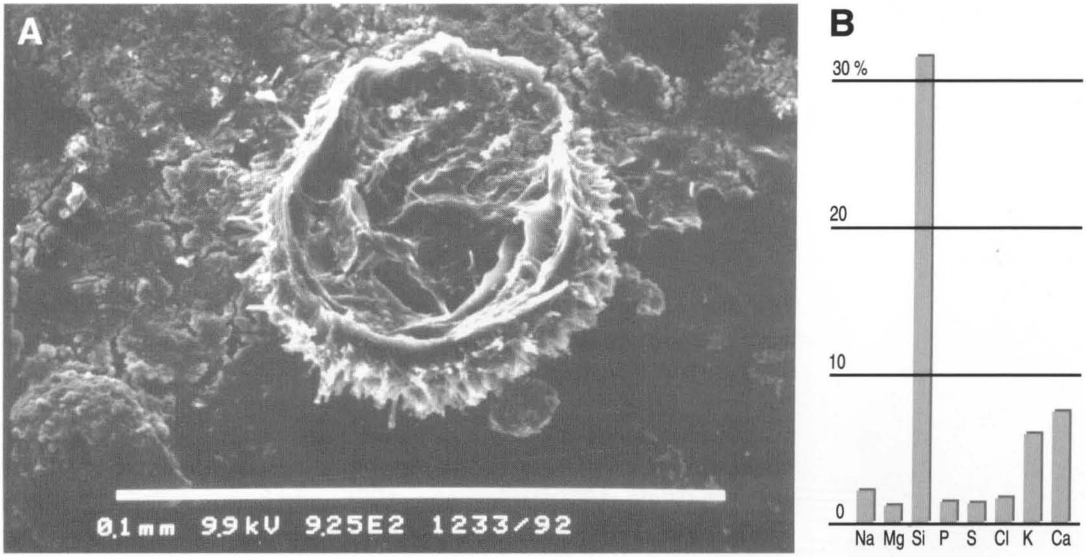


Fig. 5.
A: SEM photography of a partly destroyed spinous chromatophore

B: the result of the electron microprobe analysis, with the main element silicium, the spines have a keratineous character.

penetrates very quickly the cell membrane of the lipid droplets and the cell-plasma gets very quickly a brownish colour. The strong parakeratinous membrane of the chromarocytes prevents for long time the penetrating of the osmium-solution.

A third form of chromatocytes, situated in the light reflecting skin-regions of the Killer whale, are the irridophores, containing small cristals of the uric-acid. Bigger uric-cristals are stored between the spines of roundish central corpuscles (Fig. 7: 8) or on stamps of the lamp-like irridophores (Fig. 6). Such reflecting chromatocytes were also discovered in the skin of the upper and lower jaw tips of Harbour porpoises.

Conglomerations of spinous chromatophores, mainly combined with yellow pigments forming the yellow stripes of the common dolphin, have an extension of up to 1 mm.

A nervous supply was only proven in chromatocytes with a star-like chromatophore (Fig. 2) and in irridophores (Fig. 6). This indicates a control of the colouring by the central nervous system. A second allusion to a nervous influence may be the quick changing of the colour of the Amazonian river dolphins (Inia).

A changing of the colour can also be observed in fresh material by the melanophores (Fig. 7: 1), which change their colour through an extension of the branches.

The spinous chromatophores (Fig. 5), the star-like chromatophores and the roundish corpuscles (Fig. 4) were examined with microanalizer (Edax). Their main element is calcium and they have bone-like elements. But the spines of the spinous central corpuscle contain much silicium, and this is keratin-like.

In horizontal histological sections (Fig. 3 A) the total number of chromatophores can be up to 12 000 in one square centimetre.

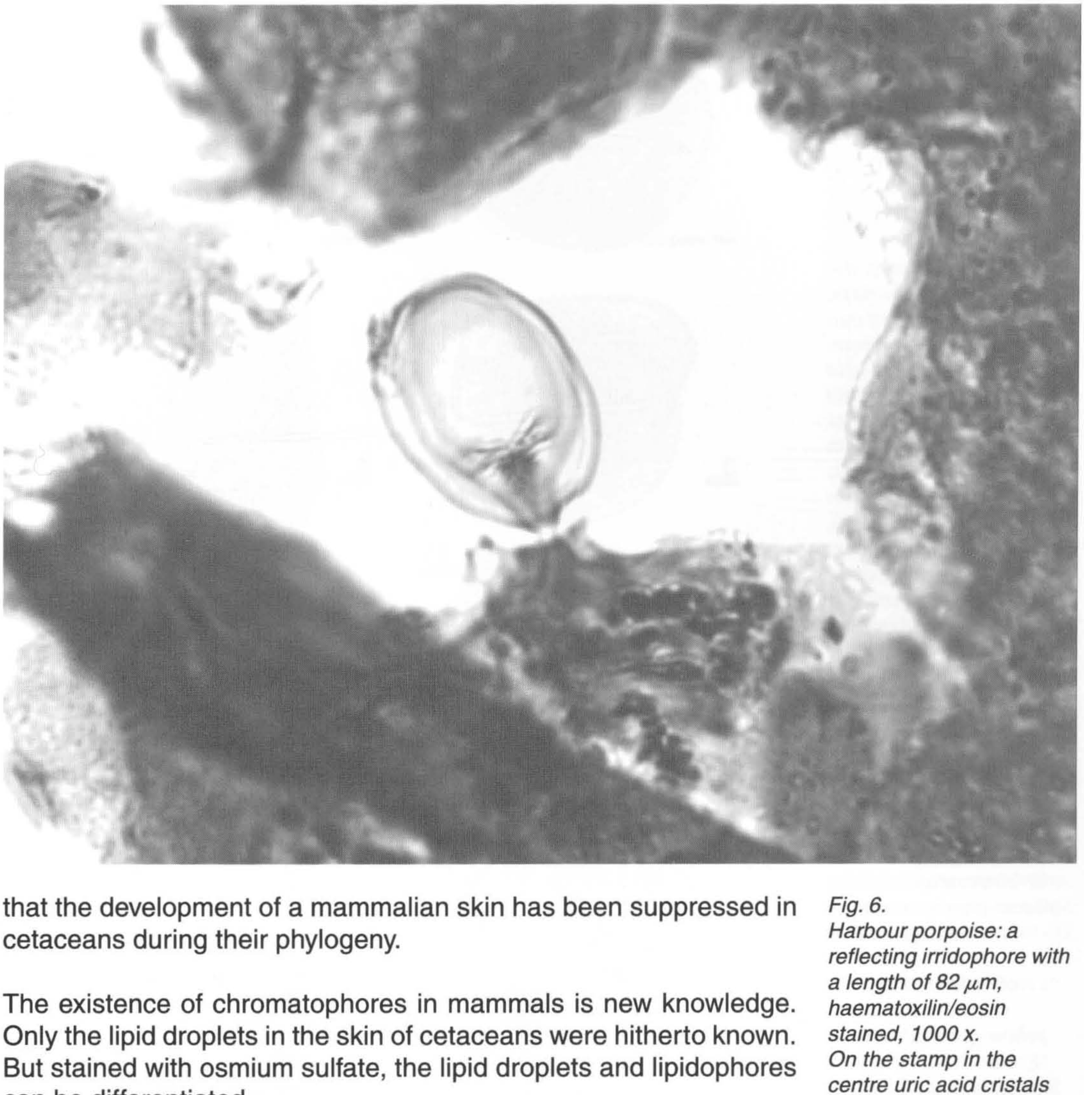
Discussion

The skin (integumentum commune) of cetaceans is quite different from the skin of lower vertebrates, and also not comparable to the mammalian skin (Spearman, 1973). The skin of cetaceans is also not fish-, amphibian-, or reptile-like: cetaceans possess their own sort of skin. Nevertheless it contains some characteristics of their ancestors, from fish up to early mammals. Beside calcareous concretions (Viale, 1979; Behrmann, 1996) and keratinous concretions (Behrmann, 1993) also the chromatophores of the cetacean ancestors seem to be preserved.

The colour of fishes, amphibians and reptiles originates from chromatophores (Romer, 1959). All mammals descend from warm-blooded reptiles *(Therapsida)*, and it is possible that anatomical remnants of reptiles or their phylogenetical ancestors are conserved in mammals. Particularly in cetaceans many active rudiments also named "constitutive marks" (Kull, 1980), are conserved.

The existence of chromatophores in cetaceans stands in contrariety to the textbooks. Up to day chromatophores have only been described in vertebrates lower than mammals (Ballowitz, 1931; Penzlin, 1980). Therefore all examinations and analyses have been repeated several times by the author, and all have ended with the same result. In cetaceans several marks of their ancestors have been preserved (Steinmann, 1909; Slijper, 1973), so that the preservation of chromatophores is not the only remnant on the cetacean ancestors. In the peripheral nerve system nervous end-corpuscles are situated, comparable to such of fishes, amphibians, reptiles and mammals (Behrmann, 1992). In its morphology the pineal organ *(Epiphysis cerebri)* is reptile-like (Behrmann,1990).

With an extension of up to one millimetre, clusters of chromatophores can be made visible by simple microscope, used by Kükenthal in the last century. Kükenthal (1893) assumed the existence of irridophores in the skin of toothed whales, but he was not able to prove it. Moreover Tomilin (1978) assumed a changing of skin-colour of toothed whales. One known characteristic of the mammalian skin is the existence of melanocytes, which migrate into upper epidermal layers. The remaining of the melanocytes on the basis of the epidermis indicates



that the development of a mammalian skin has been suppressed in cetaceans during their phylogeny.

Fig. 6.
Harbour porpoise: a reflecting irridophore with a length of 82 μm, haematoxilin/eosin stained, 1000 x.
On the stamp in the centre uric acid cristals are installed.

The existence of chromatophores in mammals is new knowledge. Only the lipid droplets in the skin of cetaceans were hitherto known. But stained with osmium sulfate, the lipid droplets and lipidophores can be differentiated.

If we assume that organs of cetaceans which were of no more use, have been regressed, we can also assume that all organs, of advantage for survival, were preserved or developed.

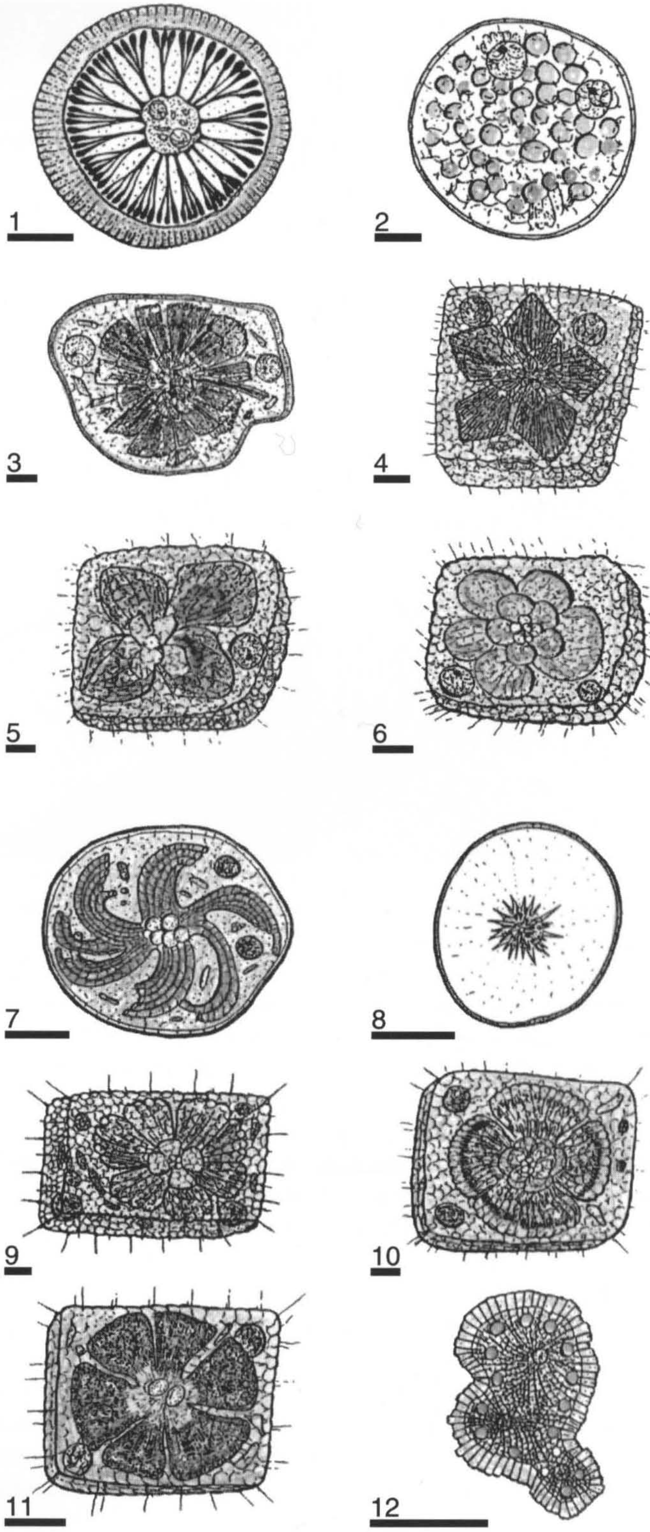
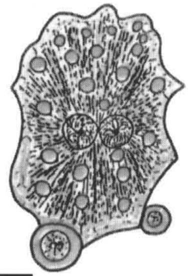


Fig. 7.
Chromatophores which were found in the skin of toothed whales, bar $10\ \mu m$.
Harbour porpoise: 1 - 5
Common dolphin: 6 - 12
Northern bottlenose whale: 13 - 22

1: melanophore, the branches carry black pigments and can change their form.
2: lipidophore, the lipid droplets vary from bright to dark blue.
3: chromatophore with blueish pigments.
4: chromatophore with grey-blueish pigments.
5: iridescent chromatophore with blue-green colour.
6: iridescent xanthophore with yellow and orange pigments.
7: erythrophore with red pigments.
8: irridophore with a white shining centre.
9: chromatophore with blueish pigments and a yellow centre.
10: chromatophore with blueish pigments and an orange centre.
11: iridescent chromatophore with brown-reddish and yellow-greenish colour.
12: chromatophore with blue-greyish and yellow-greenish colour Nothern bottlenose whale.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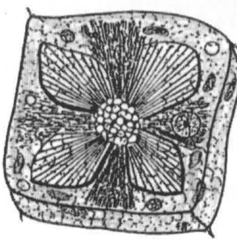
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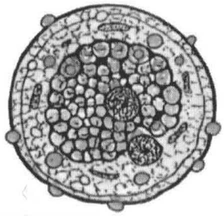
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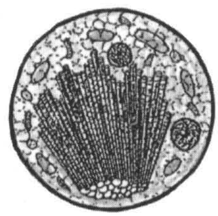
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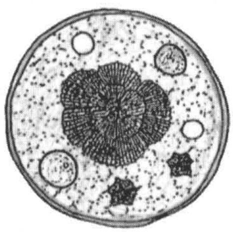
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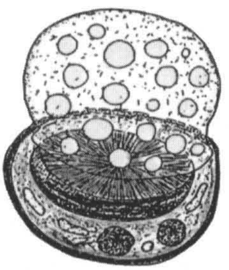
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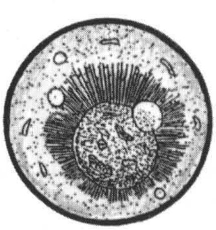
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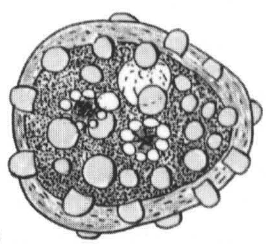
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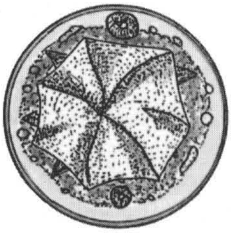
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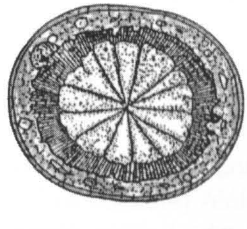
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13: blueish chromatophore with orange corpuscles on the surface of the membrane.
14: chromatophore with two forms of plates: the upper plate with black pigments, the lower plate with red-brownish pigments.
15: lipidophore with blue lipid droplets and orange grains outside the membrane.
16: melanophore with an excentric plate and blue lipid droplets.
17: melanophore with two chromatoplasts and two nuclei.
The orange-yellowish colour originates in the membrane.
18: chromatophore with blueish pigments covered by a gelatinous cupola with yellowish droplets.
19: xanthophore with a green centre and a big chromatoplast.
20: a blueish chromatophore with an orange-yellowish periphery and yellowish droplets on the surface of the membrane.
21: chromatophore with a blue colour and a star-like plate.
22: melanophore with a yellow-greenish periphery.

Acknowledgement

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