



A second species in the epizoic diatom genus *Epipellis*:
E. heptunei sp. nov.

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Dedicated to Prof. Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday

Abstract

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Atlantic bottlenose dolphins housed in open ocean enclosures in San Diego Bay, California, developed non-pathogenic skin films consisting of a single diatom species belonging to the epizoic, monoraphid genus *Epipellis* R.W. Holmes. This diatom differs from the single known representative of this genus, *E. oiketis* R.W. Holmes, by its denser striae and marginal alveoli on both the raphe and araphid valves, a greater number of areolae rows on the marginal rim of the raphe valve, and the presence of an embossment of the external valve surface distal to the terminal raphe ends. Consequently, it is described here as a new species, *E. heptunei* Denys et Van Bonn. The new species occurs in more southern and warmer waters than *E. oiketis* and also appears to be the first endemic diatom reported from Atlantic bottlenose dolphins.

Introduction

Six diatom genera, all of which are raphid, are exclusively epizoic on the epidermis of marine cetaceans: *Epiphalaina* R.W. Holmes, S. Nagasawa et Takano, *Tursiocola* R.W. Holmes, S. Nagasawa et Takano, *Tripterion* R.W. Holmes, S. Nagasawa et Takano, *Plumosigma* Tak. Nemoto, *Bennettella* R.W. Holmes and *Epipellis* R.W. Holmes (see e.g. Holmes 1985, Holmes et al. 1993a, Denys 1997). In addition, a single species of the widespread, marine littoral araphid diatom genus *Licmophora*, *L. onassis* Hustedt, has only been reported from this habitat (Hustedt 1952). However, this diatom appears to be associated more with the hard substrate offered by barnacles growing on the host than with the skin itself. In addition, epidermal growths may also include numerous non-endemic diatom taxa (see e.g. Nemoto 1956).

Of the endemic genera only *Bennettella* and *Epipellis* are monoraphid, being morphologically similar to *Cocconeis* Ehrenberg, a common marine littoral and freshwater genus lacking any ceticolous representatives. Similar to *Cocconeis*, these

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diatoms live adnatly attached to the substrate by their raphe valve. Both genera were separated from *Cocconeis* by Holmes (1985) based on the following characteristics: (1) the structure of the alveolate araphid valve, which bears biseriate rows of perforations on the external surface and an unperforated internal surface with a single row of marginal alveolar openings, (2) the sigmoid raphe, and (3) the peculiar sternum, which is developed internally into a stauros-like, transverse structure with arms extending from opposite ends of the central nodule. *Epipellis* was distinguished from the closely related *Bennettella* by its lack of lateral ramifications of the internal raphe system and bifurcated, external, terminal raphe ends. Also, the cingulum appears to be more complex in *Bennettella*. So far, *Epipellis* includes only a single species, *Epipellis oiketis* R.W. Holmes (Holmes 1985), which was previously invalidly described as *Cocconeis orcii* Kliastorin (1962, fig. 4) from a killer whale (*Orcinus orca* L.) in the Bering Sea (Figs 1-3).

In January 1997 two Atlantic bottlenose dolphins, *Tursiops truncatus* (Montague), kept in an open-ocean enclosure complex in San Diego Bay, developed brownish skin films consisting of monospecific populations of a monoraphid diatom. Skin film development by the same taxon was subsequently observed also on other captive bottlenose dolphins housed in the bay. This taxon is described here as a second species belonging to *Epipellis*: *Epipellis heptunei* Denys et Van Bonn sp. nov.

Material and methods

Skin-film samples were taken (San Diego Bay, starting January 1997) with a bone curette and preserved with formalin. Aliquots were cleaned by boiling with 30 % H₂O₂, followed by distilled water rinses and settling. Permanent slides for light microscopy (LM) were prepared using Naphrax as the mounting medium. Observations were made with a Leitz Orthoplan microscope fitted with Nomarski interference optics. For scanning electron microscopy (SEM), cleaned material was filtered onto Nucleopore membrane filters, coated with gold, and observed with a Philips 550 SEM. For comparison, an isotype slide of *Epipellis oiketis* R.W. Holmes, collected from a Dall's porpoise, *Phocoenoides dalli* True, in the North Pacific (49°N, 173°E; BM 81045, Natural History Museum London), was examined. In spite of intensive enquiries (incl. National Marine Mammal Laboratory, Natural History Museum Los Angeles County, University of California Santa Barbara, California Academy of Sciences, Natural History Museum London, University of Bristol and persons involved with collection and investigation of the samples), no unmounted type material of this species could be retrieved for additional SEM study. Morphological terminology follows Holmes (1985) and Round et al. (1990).

Observations

Epipellis heptunei Denys et Van Bonn, sp. nov.

Figs 4-23

Diagnosis

Differt in LM a *Epipellis oiketis* R.W. Holmes in striis minus distinctis et magis densius positis (circiter 22 in 10 µm in valva araphide et valva raphofera) et in alveolis marginalibus magis densius positis (17-18 in 10 µm in valva raphofera, circiter 13 in 10 µm in valva araphide). In SEM criteria diagnostica additionalia sunt margo exterior

densius porosa valvae raphoferae cum ad maxime quattor seriebus irregularibus areolarum et inspissatio superficiei valvae externae apicalis ab terminationibus raphis polaribus.

Differs from *Epipellis oiketis* R.W. Holmes in LM by less distinct, denser striae (about 22 in 10 μm along the axial area of the araphid valve, not visible for most part of the raphe valve), as well as more closely spaced marginal alveoli (17–18 in 10 μm on raphe valve and about 13 in 10 μm on araphid valve). In the SEM, the densely poroid outer rim of the raphe valve, with up to four irregular rows of areolae, and the embossment of the external valve surface apically from the polar raphe endings are additional diagnostic criteria.

Holotype: CAS slide 219099 (California Academy of Sciences, San Francisco).

Isotypes: slides BM 100304 (Natural History Museum, London), BRM Zu5/29 (Alfred Wegener Institut, Bremerhaven).

Type locality: San Diego Bay, California, USA (32.7°N, 117.1°W); epidermis of the Atlantic bottlenose dolphin, *Tursiops truncatus*, 3 April 1997.

Non-mounted original material is deposited at California Academy of Sciences.

Etymology: the species is named after Heptuna, the dolphin on which it was observed for the first time.

Light Microscopy

The valves are broadly oval to almost circular in outline (Figs 4-9). Their length varies from 17.0 to 31.3 μm and their width from 15.0 to 28.6 μm (Table I). The sigmoid raphe sternum possesses linear lateral extensions which do not quite extend to the valve margin and are slightly offset from the valve centre. The marginal alveoli of the raphe valve (RV) are elongate (Figs 4-6). The striae are radiate, curved and somewhat wavy, and extend around the entire circumference of the valve. Their density is circa 22 in 10 μm , but they are only distinct along the marginal zone, where their punctuation can be resolved. Each stria terminates at a marginal alveolus, which are 17 to 18 in 10 μm . Small hyaline areas surround the terminal raphe endings, which are situated at some distance from the valve margin. The central nodule is well developed and varies in shape from more or less circular to almost rectangular in the largest valves. The raphe fissure is distinct, with large gradually expanded proximal endings and somewhat expanded apical terminations.

Table I. Comparison of selected morphometric characteristics of *Epipellis oiketis* and *E. heptunei*, based on Kliashtorin (1962), Holmes (1985) and own observations.

	<i>Epipellis oiketis</i>	<i>Epipellis heptunei</i>
length [μm]	17–47	17.0–31.3
width [μm]	16–42	15.0–28.6
marginal alveolar openings in 10 μm (RV)	10–14	17–18
marginal alveolar openings in 10 μm (AV)	9–10	circa 13
striae in 10 μm AV (along axial area)	12–16	circa 22

The araphid valve (AV) possesses a narrow, sigmoid axial area and a small more or less round central area (Figs 7-9). The striae of the AV are considerably more distinct than those of the RV, becoming more closely spaced towards the axial area and more strongly curved towards the poles. Their punctuation can only be resolved with some difficulty. As in the RV, a row of alveolar openings is present at the valve margin. The density of striae on the AV is similar to that on the RV (circa 22 in 10 μm along the axial area), but the number of alveoli is only circa 13 in 10 μm along the valve margin.

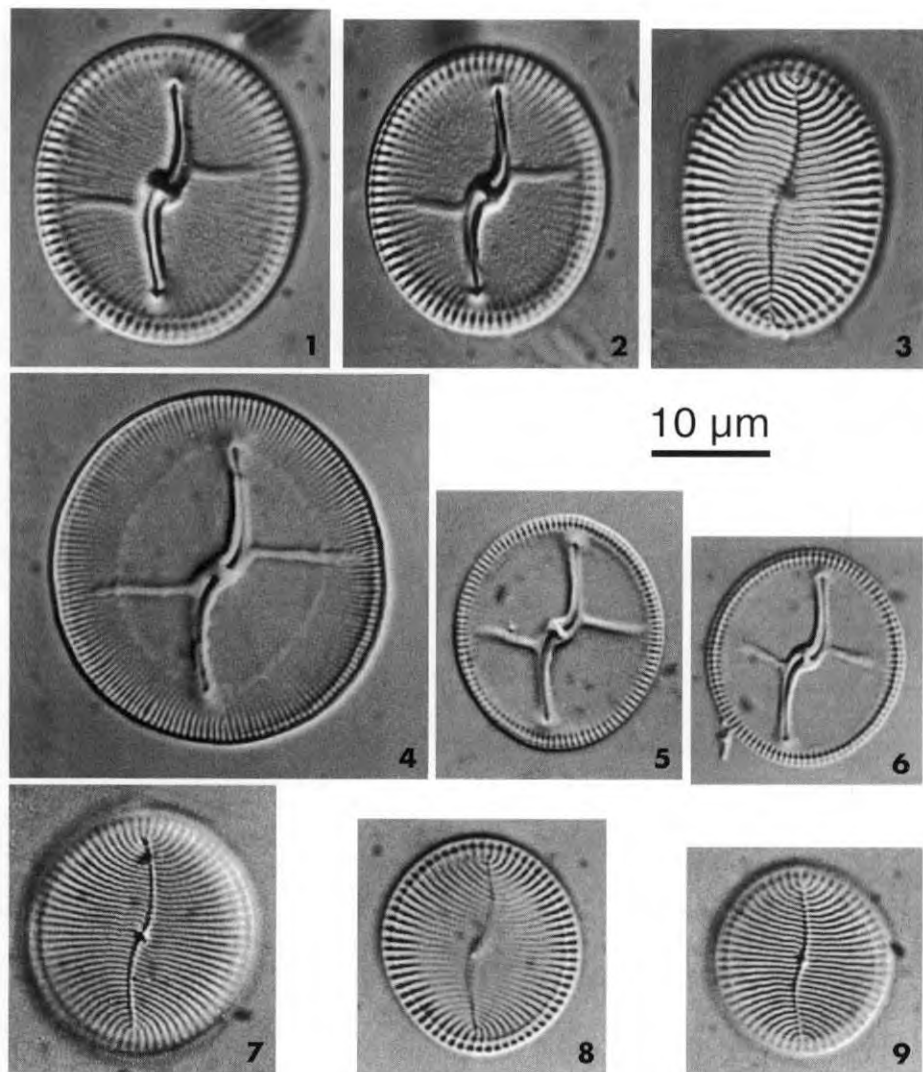
The cells possess a single plate-like chloroplast with entire margins.

Scanning Electron Microscopy

The structure of the RV is shown in Figs 10-13 and 16-21. The RV is concave externally (Figs 10-11). The marginal rim is more elevated than the valve surface and distinctly separated from it by a sharp angle (Figs 10-11, 16, 18). Its downward slope towards the narrow mantle is less steep. The shallow mantle is perpendicular to the valvar plane and sharply delimited (Figs 16, 18, 20). The valve face possesses biseriate rows of small, round areolae, which terminate shortly before reaching the marginal elevation and reappear on the inner slope of the marginal rim, leaving a narrow unperforated zone (Fig. 16). Areolae are also lacking along the sternum and its lateral extensions. The crest of the marginal rim is solid, but its downward outer slope bears approximately four rows of rather irregularly placed round areolae (Figs 16, 18). The mantle is furnished with a single row of narrow, slit-like openings, with a density of approximately 8 in 1 μm (Figs 13, 16, 18, 20). Externally, the raphe slit is narrow and filiform. The proximal ends are slightly dilated towards the exterior (Figs 10-11, 17). The distal raphe ends are simple, but an impression of slight bifurcation arises from the surrounding embossment of the valve surface, creating a short transverse funnel (Fig. 18).

The interior of the RV is concave as well, and shows a strongly developed sternum, rising gradually towards the center, where it develops into a thick central nodule (Figs 12-13). The central nodule expands roof-like over the valve surface at its margins. Near the poles, the raphe is situated in the middle of the sternum (Fig. 21), terminating simply and without helictoglossae. Towards the valve centre, the raphe fissures deflect to opposite sides of the central nodule and descend halfway down the sides of the sternum. The proximal raphe endings are thus markedly non-coaxial. Internally, the lateral extensions are distinctly elevated, unperforated siliceous thickenings of the valve surface which project from the sternum at the ends of the central nodule (Figs 12-13, 20). The interstriae rise slightly above the valve surface, separating biseriate rows of areolae (Figs 13, 19-21). The interstriae are often bifurcated towards the margin, and only single rows of pores occur within the bifurcations. The rows of areolae do not continue around the terminal raphe endings (Fig. 21). At the valve margin, the alveoli possess oblong openings towards the valve interior, separated by slightly vaulted struts (Figs 12-13, 19-21).

The AV is convex, with a fairly flat surface and gradually sloping margins (Figs 14-15). The exterior surface shows small round areolae in biseriate or – towards the poles – partly uniseriate rows (Figs 14, 22). Presumably, the areolae are closed by rotae, as in *Epipellis*



Figs 1-3. *Epipellis oiketis* R.W. Holmes; LM. Isotype slide BM 81045. Figs 1-2. Raphid valve. Fig. 3. Araphid valve.

Figs 4-9. *Epipellis heptunei* Denys et Van Bonn, sp. nov.; LM. Holotype slide CAS 219099. Figs 4-6. Raphid valve. Figs 7-9. Araphid valve.

oiketis, but no TEM observations were accessible. The axial area is demarcated only by an extremely narrow, unperforated zone. The valve margin is unperforated as well, except for a single row of pores that is just slightly set apart from the other aeolae. Areolae also overlie the transapical costae separating the elongate alveoli. These alveoli continue from the axial rib to the valve margin, where they open towards the interior through a single series of large, oblong openings (Figs 15, 23). Except for the marginal alveolar openings, the internal membrane covering the alveoli is unperforated.

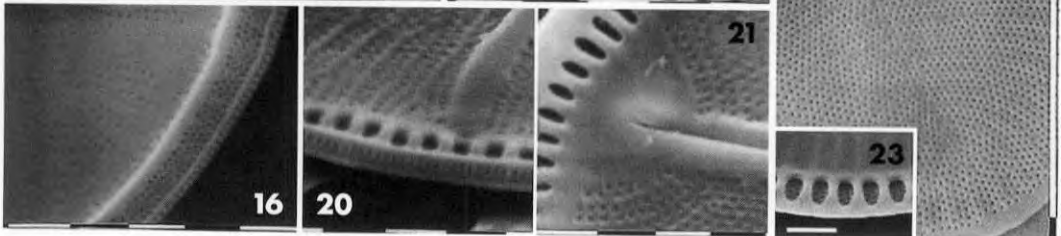
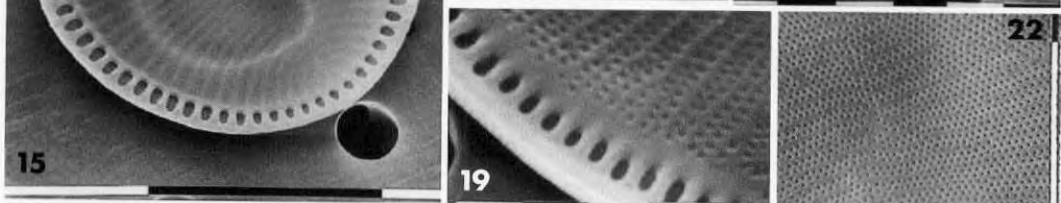
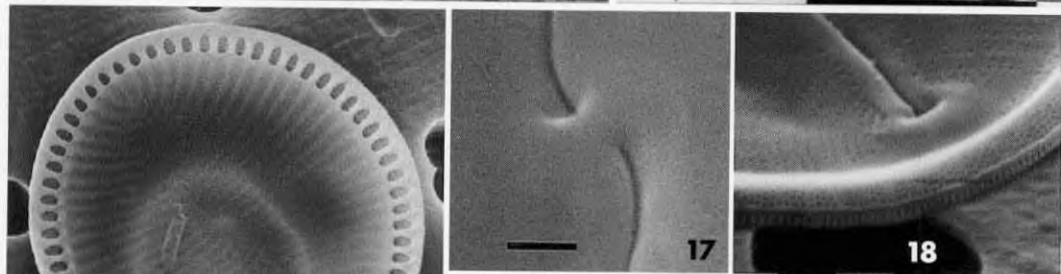
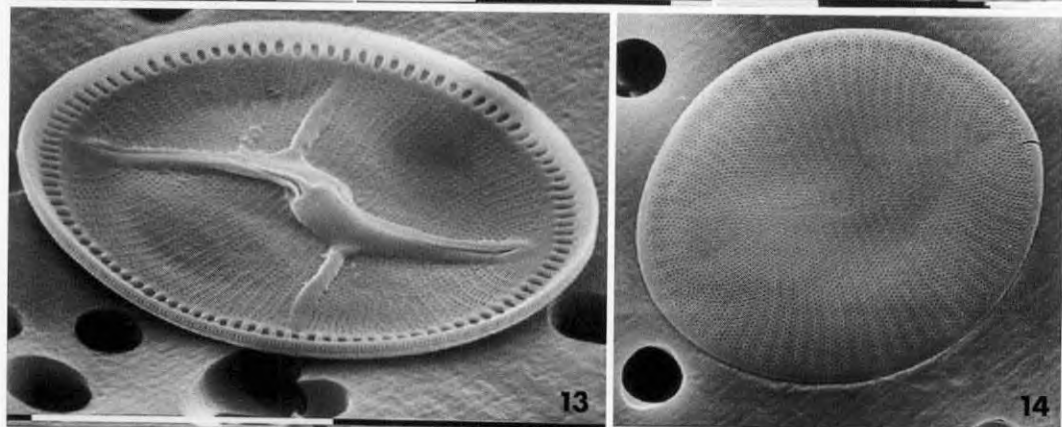
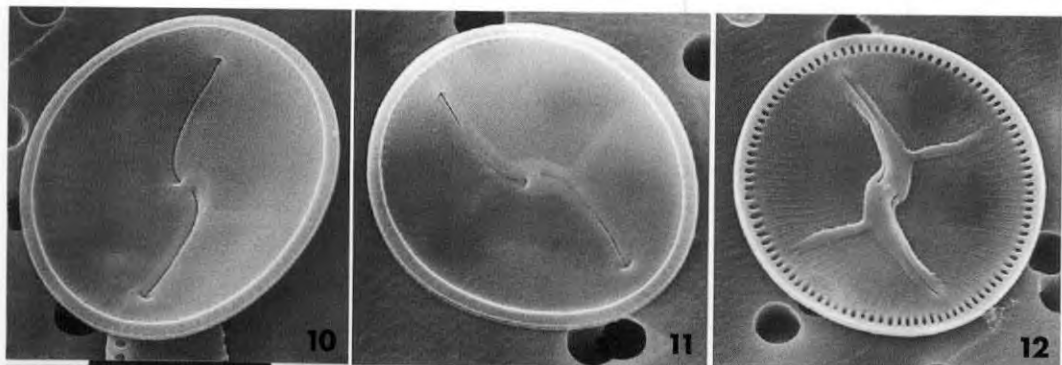
The cingulum rapidly dissociates from the valves during sample preparation. Only a single type of unperforated and open siliceous band circa 0.25 μm wide has been observed thus far.

Occurrence

Skin film development was first noted in January 1997 on two Atlantic bottlenose dolphins, each of which was over 30 years old. Since then, skin films of *Epipellis heptunei* were also observed on other Atlantic bottlenose dolphins housed in enclosures at the same pier, adults as well as juveniles. Some, but not all dolphins, were kept in contact with the older individuals. No films developed on dolphins kept at any of four other piers in the bay. Skin films occurred mainly at the dorsal and rostral trunk of the animals. Several animals had slight films around their blow holes and some *Epipellis* cells were also observed in blow hole swabs. Most of the diatoms appeared to be living at the time of collection. Apart from *Epipellis heptunei*, no other epizoic diatoms occurred, except for a single valve of *Fragilaria investiens* (W. Smith) Cleve-Euler observed on one slide. The presence of the latter, a common marine-littoral epiphyte, may be purely accidental. No clinical signs were associated with skin film development. Observations suggest that skin films are best developed in months when the water temperature is at its lowest (January-February). Minimum water temperatures at the location are usually slightly higher than 14 °C, whereas in summer temperatures rise to 20–22 °C. It is well known that diatom skin film development on other cetaceans is related to their stay in areas with cold water (Hart 1935, Nemoto et al. 1980). Salinity is quite stable throughout the year in the bay area, remaining at about 34.6 ‰. So far, *Epipellis heptunei* is known only from the type locality, which is approx. 32.7 °N. The taxon was not observed on migrating gray whales, *Eschrichtius robustus* (Lilljeborg), in San Diego Bay. *Epipellis oiketis*, which occurs in the Pacific as well, was observed only at latitudes above 36.5 °N on killer whales and Dall's porpoises (Holmes 1985, Holmes et al. 1989, Holmes et al. 1993a).

Figs 10-23. *Epipellis heptunei* Denys et Van Bonn, sp. nov.; SEM. Original material. (RV = raphid valve; AV = araphid valve).

Figs 10-11. External views of RV. Figs 12-13. Internal views RV. Fig. 14. External view of AV. Fig. 15. Internal view of AV. Fig. 16. Detail of margin and mantle of RV, external view. Note four rows of perforations on downslope of rim and single row on mantle. Fig. 17. Detail of external proximal raphe endings of RV. Fig. 18. Detail of external distal raphe end. Note the embossment of the valve surface distal to the raphe end. Fig. 19. Detail of valve margin of RV, internal view. Note bifurcated interstriae. Fig. 20. Detail of internal valve margin of RV. Note lateral extension of the sternum. Fig. 21. Detail of internal distal raphe end (RV). Fig. 22. Detail of AV areolae, external view. Fig. 23. Detail of internal valve margin of AV. Scale bars = 10 μm (Figs 10-15, 22), scale bars = 1 μm (Figs 16-21, 23).



Discussion

The diatom from Atlantic bottlenose dolphins in San Diego Bay clearly belongs to *Epipellis*, as it possesses all the features of this genus as described by Holmes (1985). It is very similar in morphology to *E. oiketis* and, obviously, closely related. Some differences in structure are nevertheless notable. In LM, a considerably coarser striation and alveolation pattern is obvious for *E. oiketis* (compare Figs 1-3 vs. 4-9). Table I lists quantitative valve features for both species and shows that no overlap exists with regard to these characteristics. Some additional differences are related to the valve ultrastructure. In *E. oiketis*, the slope of the marginal rim towards the mantle of the RV bears only two rows of perforations (illustrated by fig. 46 of Holmes 1985), whereas there are three to four rows of small poroids present in *E. heptunei*. Moreover, the external terminal raphe ends are set in a small depression in *E. oiketis*, but the valve surface remains flat in the immediate surrounding (figs 45, 46 of Holmes 1985). In contrast, the valve surface of *E. heptunei* possesses a lunate embossment distal to the raphe ends. While differences in the degree of valve silicification or the density of areolae and alveoli may be related to environmental conditions, this is less likely the case for the pattern of areolae on the marginal rim of the RV. Also considering practical advantages, a formal taxonomic separation of both morphs at the level of variety or species appears appropriate. As discussed by Cox (1997), the notion of variety has differed among diatomists. Together with the recognition that even minor phenotypic variation may accompany reproductive barriers, this has promoted the use of species rank as a 'less subjective' alternative. Cox (1997) suggests to use specific rank only where breeding barriers can be demonstrated and to designate morphologically and ecologically distinct populations where phenotypic variation is beyond environmental control as varieties. Although unequivocal within the classic biological species concept, it appears that a proliferation of varietal denominations would follow from such a stringent definition. For applied diatom studies (and paleontology) this concept appears less opportune. Even though dealing with allopatric populations, our inclination is to consider the *Epipellis* morphs as separate species at the moment in view of the congruity in morphological differences and the considerable gap between the ranges of all qualitative features listed in Table I, size excepted. Ultimately, culture and mating experiments will be required to corroborate this opinion, although these are likely to be problematic in the case of epizoic taxa.

Whereas other biota associated with cetaceans, such as Cyamidae (Amphipoda, 'whale lice') or barnacle species, demonstrate a high degree of host specificity, this does not appear to be the case for epizoic diatoms (Holmes et al. 1993b). However, this has not been demonstrated for all ceticolous taxa, as they have been recorded only rarely thus far. Further observations are necessary to establish whether taxa such as *Tripterion philoderma* R.W. Holmes, S. Nagasawa et Takano, observed on strap-toothed whales (*Mesoplodon layardii* Gray), *Epiphalaina radiata* R.W. Holmes, S. Nagasawa et Takano, on killer whales (Holmes et al. 1993b), *Epiphalaina aleutica* var. *lineata* Denys on Dall's porpoise (Denys 1997), and *Epipellis heptunei* may grow on other cetaceans as well.

Speciation in epizoid diatoms is likely to be influenced by the clonal nature of diatom reproduction and the high degree of isolation of their populations (Denys 1997). However, only a small number of taxa (only circa 15 at species or lower rank) appear to have adapted specifically to this lifestyle. Compared to other diatom genera, congeneric species usually demonstrate relatively little morphological differentiation, as exemplified also by *Epipellis oiketis* and *E. heptunei*. Even though it is quite likely that more extensive investigations and the application of narrower species concepts will yield additional taxa, the emerging picture is one of low taxonomic diversity. This seems to indicate that adaptations required for successful epizoid growth have emerged rarely and in a few different diatom lineages only. The relationship between epizoid diatoms and their hosts may not just be of a phoretic nature as culture experiments indicate their dependence on some organic substance present in the host epidermis, perhaps cysteine (Holmes et al. 1989). On the other hand, although inoculation of cetaceans by epizoid diatoms is considered to depend on bodily contact (Holmes et al. 1993a), gene flow between different populations may be more common than assumed, as incidental transfer could possibly occur through the water column as well. In organisms such as whale lice, the latter is less likely to occur, which may result in higher speciation rates. It appears unlikely that morphological investigations will suffice to address such questions. Possibly, genetic analysis of cryptic diversity in epizoid diatoms may yield further information on their origin and phylogeny, and perhaps their relationship to the evolutionary biology of their hosts.

The occurrence of *Epipellis heptunei* in San Diego Bay suggests that epizoid diatoms are not as limited in their distribution as supposed, but may be found on cetaceans in warmer seas as well. Here, their development into large populations forming macroscopic skin films may be limited by higher temperatures, however, and of less common occurrence. *E. heptunei* appears to be the first obligate epizoid diatom that is reported from Atlantic bottlenose dolphins. Further investigations of the endemic flora of this widespread cetacean are definitely required.

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References

- COX, E.J. (1997): Assessing and designating diatom taxa at or below the species level – a consideration of current status and some suggested guidelines for the future. – *Nova Hedwigia* **65**: 13-26.
- DENYS, L. (1997): Morphology and taxonomy of epizoid diatoms (*Epiphialina* and *Tursiocola*) on a sperm whale (*Physeter macrocephalus*) stranded on the coast of Belgium. – *Diatom Res.* **12**: 1-18.
- HART, T.J. (1935): On the diatoms of the skin film of whales and their possible bearing on problems of whale movements. – *Discovery Rep.* **10**: 249-282.

- HOLMES, R.W. (1985): The morphology of diatoms epizoic on cetaceans and their transfer from *Cocconeis* to two new genera, *Bennettella* and *Epipellicis*. – Brit. Phycol. J. **20**: 43-57.
- HOLMES, R.W., S. NAGASAWA & T. NEMOTO (1989): Epidermal diatoms on the Dall's porpoise landed at Otsuchi, Iwate, Japan. – Otsuchi Mar. Res. Centre Rep. **15**: 15-20.
- HOLMES R.W., S. NAGASAWA & H. TAKANO (1993a): The morphology and geographic distribution of epidermal diatoms of the Dall's Porpoise (*Phocoenoides dalli* True) in the Northern Pacific Ocean. – Bull. Natl. Sci. Mus. Tokyo, Ser. B **19**: 1-18.
- HOLMES R.W., S. NAGASAWA & H. TAKANO (1993b): A re-examination of diatom samples obtained from cetaceans collected off South Africa. – Bull. Natl. Sci. Mus. Tokyo, Ser. B **19**: 127-135.
- HUSTEDT, F. (1952): Diatomeen aus der Lebensgemeinschaft des Buckelwals (*Megaptera nodosa* Bonn.). – Arch. Hydrobiol. **46**: 286-298.
- KLIASHTORIN, L.B. (1962): The diatoms of the skin film of whales in the Far-Eastern Sea. – Akad. Nauk SSSR Trudy Inst. Okeanol. **58**: 314-321. (in Russian)
- NEMOTO, T. (1956): On the diatoms of the skin films of whales in the Northern Pacific. – Sci. Rep. Whales Res. Inst. **11**: 99-132.
- NEMOTO, T., P.B. BEST, K. ISHIMARU & H. TAKANO (1980): Diatom films on whales in South African waters. – Sci. Rep. Whales Res. Inst. **32**: 97-103.
- ROUND, F.E., R.M. CRAWFORD & D.G. MANN (1990): The diatoms. Biology and morphology of the genera. Cambridge University Press, Cambridge.

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