

# A novel trypanoplasm-like flagellate *Jarrellia atramenti* n. g., n. sp. (Kinetoplastida: Bodonidae) and ciliates from the blowhole of a stranded pygmy sperm whale *Kogia breviceps* (Physeteridae): morphology, life cycle and potential pathogenicity

S. L. Poynton<sup>1,\*</sup>, B. R. Whitaker<sup>2</sup>, A. B. Heinrich<sup>1,3</sup>

<sup>1</sup>Comparative Medicine, Johns Hopkins University School of Medicine, 459 Ross Building, 720 Rutland Avenue, Baltimore, Maryland 21205, USA

<sup>2</sup>Animal Health, National Aquarium in Baltimore, Pier 3, 501 East Pratt Street, Baltimore, Maryland 21202, USA

<sup>3</sup>Zoological Institute, Christian Albrechts University, Olshausen Strasse 40, 24098 Kiel, Germany

**ABSTRACT:** The successful 6 mo rehabilitation of a stranded juvenile pygmy sperm whale *Kogia breviceps* afforded the opportunity to study the poorly known protozoan fauna of the upper respiratory tract of cetaceans. Mucus samples were collected by holding either a petri dish or glass slides over the blowhole for 3 to 5 exhalations; preparations were examined as wet mounts, and then stained with Wrights-Giemsa or Gram stain. Blood smears were stained with Wrights-Giemsa. Unidentified spindle-shaped and unidentified broad ciliates, reported from the blowhole of the pygmy sperm whale for the first time, were seen only initially, while yeast-like organisms and bacteria were seen intermittently. Epithelial cells and white blood cells were often present in the blowhole mucus, but red blood cells were never seen. A novel trypanoplasm-like bodonid kinetoplastid biflagellate (Order Kinetoplastida) was commonly encountered in the blowhole mucus, but never in the blood. Both mature flagellates and those undergoing longitudinal binary fission were present. The elongate flagellate had a long whiplash anterior flagellum; the recurrent flagellum was attached along at least two-thirds of the body length, forming a prominent undulating membrane, and the trailing portion was short. The kinetoplast was irregularly fragmented. The flagellates were either free-swimming, or attached to host material via the free portion of the posterior flagellum. The prominent undulating membrane was characteristic of *Trypanoplasma*, while the fragmented kinetoplast was characteristic of some species of *Cryptobia*. For the novel bodonid kinetoplastid, with its unique combination of morphological features (prominent undulating membrane and fragmented kinetoplast), we propose the creation of a new genus *Jarrellia*. We believe this to be the first published description of a flagellate from a marine mammal, and among the first reports of a trypanoplasm-like flagellate from a warm-blooded host. We expect that a diversity of flagellates and ciliates are commonly present in the blowhole of cetaceans. Future studies on the identity of the protozoans and the health of their cetacean hosts, which are readily studied in captivity, are necessary to establish their status as commensals or parasites.

**KEY WORDS:** Bodonidae · Cetacean · Ciliate · *Cryptobia* · Protozoa · *Trypanoplasma*

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## INTRODUCTION

The protozoan fauna of cetaceans is little known (Dailey & Brownwell 1972, Dailey 1985, Dierauf 1991), due primarily to the challenges of sample collection.

Difficulties include wild hosts that are relatively inaccessible; many stranded animals are dead (thus delicate ciliates and flagellates may not survive, and there may be post-mortem contamination); and specialized procedures for detecting delicate protozoa have not

been routinely followed. Although recent handbooks and stranding response manuals (Dierauf 1991, Geraci & Lounsbury 1993, Kirk 1995) omit protocols for protozoan material while including protocols for collection and preservation of metazoans, recent texts on cetacean cytology do include protocols for protozoa (Campbell 1999, Sweeney et al. 1999). One would expect studies to show that marine mammals are suitable hosts for a diversity of protozoa, since other marine organisms, such as fish, are heavily infected (Lom & Dykova 1992, Woo 1995), and terrestrial mammals are also hosts for a broad range of protozoan species (Georgi & Georgi 1990).

Three groups of protozoans from cetaceans, namely ciliates, flagellates and apicomplexans, have been reported. The ciliates include the following: the prostomean *Haematophagus megapterae* from baleen plates (Woodcock & Lodge 1921, Evans et al. 1986); the phyllopharyngid *Kyaroikeus cetarius* from the blowhole (Sniezek et al. 1995), ciliates tentatively identified as *K. cetarius* from the blowhole, alveolar spaces and sub-bronchiolar mucosa (Woodard et al. 1969, Sniezek et al. 1995); and unidentified ciliates from the blowhole, skin lesions, lymph nodes and bronchioli (Howard et al. 1983, Dailey 1985, Schulman & Lipscomb 1997, 1999). Flagellates, though recently reported from the blowhole mucus of wild and captive cetaceans (Campbell 1999, Sweeney et al. 1999), including Atlantic bottlenose dolphins *Tursiops truncatus* and beluga whales *Delphinapterus leucas* (Arkush, Campbell, Dailey, Sweeney, Van Bonn pers. comm.), have never been studied in detail. Their identities remain unknown, with the exception of the unidentified kinetoplastid reported by Sweeney et al. (1999). Our knowledge of the apicomplexan fauna of cetaceans is more complete, with *Toxoplasma* and *Sarcocystis* reported from many internal organs (Cowan 1966, Owen & Kakulas 1968, Akao 1970, Dailey & Stroud 1978, Munday et al. 1978, Cruickshank et al. 1990, Inskeep et al. 1990, Migaki et al. 1990, Domingo et al. 1992, De Guise et al. 1993, Di Guardo et al. 1995). The relationships between the protozoa and their cetacean hosts are poorly understood. Although the ciliates of the baleen plates and blowhole are not reported to be pathogenic, ciliate infections of the skin, lungs and lymph have been associated with disease (Woodard et al. 1969, Schulman & Lipscomb 1999). Among the apicomplexa, *Sarcocystis* infections are of low pathogenicity (Cowan 1966, Owen & Kakulas 1968, De Guise et al. 1993), in contrast to *Toxoplasma* infections, which are frequently pathogenic and can be fatal (Cruickshank et al. 1990, Inskeep et al. 1990, Migaki et al. 1990, Domingo et al. 1992).

Additional work on the protozoans of cetaceans, particularly the ciliates and flagellates, is needed in order

to determine the range of organisms present and their role in disease (Dailey 1985, Campbell 1999). Captive cetaceans offer excellent opportunities to study their protozoan fauna, and we report now on one such study. The successful 6 mo rehabilitation of a stranded juvenile pygmy sperm whale *Kogia breviceps* (Physeteridae) at the National Aquarium in Baltimore, Maryland, USA, enabled us to monitor its health, including the fauna of the blowhole. This was an unusual opportunity to study a living member of this species. These uncommon whales live beyond the continental shelf in temperate and tropical oceans, feed in deep water, and are rarely seen at sea (Handley 1966, Odell et al. 1985, Caldwell & Caldwell 1989). Thus, strandings or fisheries by-catch are the only feasible means of obtaining individuals for study. Although pygmy sperm whales commonly strand along the east coast of the USA (Odell et al. 1985, Geraci & Lounsbury 1993), most of the animals are dead adults. The protozoan fauna of the pygmy sperm whale is largely unknown and appears restricted to a case of dermatitis with invasive ciliates (Schulman & Lipscomb 1999). In contrast, a diversity of metazoans has been reported including cestodes, nematodes, acanthocephalans, crustaceans, and a lamprey (Pendergraph 1971, Dailey & Brownell 1972, Caldwell & Caldwell 1989, McAlpine et al. 1997).

We now present the first published description of a flagellate from a cetacean, and describe the new bodonid kinetoplastid *Jarrellia atramenti* from the respiratory tract of the pygmy sperm whale. The observation of a flagellate with some morphological, life cycle, and habitat features characteristic of *Cryptobia* and some features characteristic of *Trypanoplasma* adds new information to the long-standing debate over the possible synonymy of the latter 2 genera. Although we believe this to be the first published description of a bodonid from a cetacean, other bodonids including the genera *Ichthyobodo*, *Cryptobia* and *Trypanoplasma* are well known as commensals and parasites of other aquatic animals including planarians, mollusks, leeches and fish.

## METHODS

**Maintenance of the whale.** On November 25, 1993, a stranded juvenile female pygmy sperm whale was rescued from an inlet near Brigantine, New Jersey, on the east coast of the USA, and brought to the National Aquarium in Baltimore. The Aquarium forms part of the Northeast Region Marine Mammal Stranding Network and is authorized by the National Marine Fisheries Service to rescue, rehabilitate and release stranded marine animals. On arrival, the whale was 1.8 m in length, weighed 94 kg, and was thought to be

no older than 9 to 18 mo. It was held in artificial seawater in a 100 000 gallon (454 545 l) pool. Although initially emaciated, disorientated, and unable to dive, the whale responded well to treatment, and on May 5, 1994, at 2.2 m in length and weighing 147 kg, it was transported to Marineland of Florida for final rehabilitation prior to release in the Gulf Stream, 49 km (30 miles) east of Cape Canaveral, Florida, on May 31, 1994.

**Examination of blowhole mucus.** Throughout rehabilitation and immediately prior to release, blowhole mucus samples were monitored for fungi, bacteria, protozoa, red and white blood cells, and epithelial cells. Samples were taken on 16 d: at 1 to 47 d intervals during rehabilitation in Baltimore (more frequently at the beginning of the rehabilitation period); on May 5, 1994, during transport to Marineland, Florida; and on May 29 and 30, 1994, prior to release off the coast of Florida (see Table 1).

Blowhole mucus was collected when the whale was restrained, either for a physical examination held on a thick foam pad on the poolside deck, or in a stretcher during transport. A sterile plastic petri dish (9 cm diameter) was held approximately 5 to 7 cm above the blowhole, to collect the mucus discharged during 3 to 5 exhalations. The mucus was then scraped from the dish with a cover slip and transferred to a glass slide. Some samples were collected directly onto glass slides. Preparations were kept moist by placing the slides in a covered petri dish containing a damp paper towel. The wet mounts were examined directly, and then smears were stained with Wrights-Giemsa for differentiation of protozoa and host cells (see below), or Gram-stained for detection of fungi and bacteria (see Table 1).

**Detailed examination of flagellates from the blowhole.** Morphology and motility of the flagellates were observed in wet mounts. To slow the movement of flagellates, a drop of viscous 'Protoslo' (Carolina Biological Supply Co., Burlington, NC, USA) was added to the preparation. Video-recordings were recorded at 5230× magnification. A Wrights-Giemsa stain was used to demonstrate morphometrics of the flagellates. The slides were air dried, fixed for 1 min in absolute methyl alcohol, air dried, placed in 10% neutral buffered formalin for 2 min, dried again and stained. Slides were flooded with Wrights-Giemsa for 2 min, followed by the addition of an equal volume of deionized water for 4 min, prior to rinsing, clearing and coverslipping. A grid was drawn on the underside of each slide to assist with location of individual flagellates. Measurements of the flagellates were made according to the recommendations of Lom & Dykova (1992), and based on 14 to 18 organisms. Morphometrics and paratype material were taken from the May 30 samples from Florida, since the flagellates were numerous, and morphologi-

cally indistinguishable from those seen earlier in Baltimore.

**Blood sampling.** Throughout rehabilitation and immediately prior to release, blood samples were taken for complete blood counts (CBC), as part of the health assessment of the whale. A total of 29 samples were taken, at 1 to 15 d intervals, with the most frequent sampling occurring at the beginning of the rehabilitation period.

Blood was drawn, using a 20-gauge needle, from the vessels in the fluke and placed in an EDTA vacutainer. Smears were stained with Wrights-Giemsa. After a portion of the slide was examined for CBC, the entire slide was examined for the presence of flagellates and ciliates.

## RESULTS

### Blowhole mucus

The most remarkable finding was the presence of the novel bodonid kinetoplastid flagellate *Jarrellia atramenti*, which was commonly encountered in the mucus from the blowhole. The flagellates were seen the second day after arrival in Baltimore and intermittently throughout the 6 mo of rehabilitation, and persisted until the whale's release in the waters off the coast of Florida (Table 1). Other organisms recovered from the blowhole were: (1) spindle-shaped ciliates and broad ciliates, seen only the second day after arrival in Baltimore; (2) yeast-like organisms, which were abundant in November and December, not detected in January and March, and rare in May; and (3) bacteria, which were seen intermittently throughout the rehabilitation period (Table 1). Small numbers of white blood cells were present intermittently in the blowhole samples in November, December, and May; higher numbers were present in January. Red blood cells were never seen in the blowhole samples. Epithelial cells, in contrast, were seen on most occasions (Table 1).

### Blood

No flagellates or ciliates were seen in any of the 29 blood smears examined during rehabilitation and immediately prior to release of the whale.

### Description of *Jarrellia atramenti* n. g., n. sp.

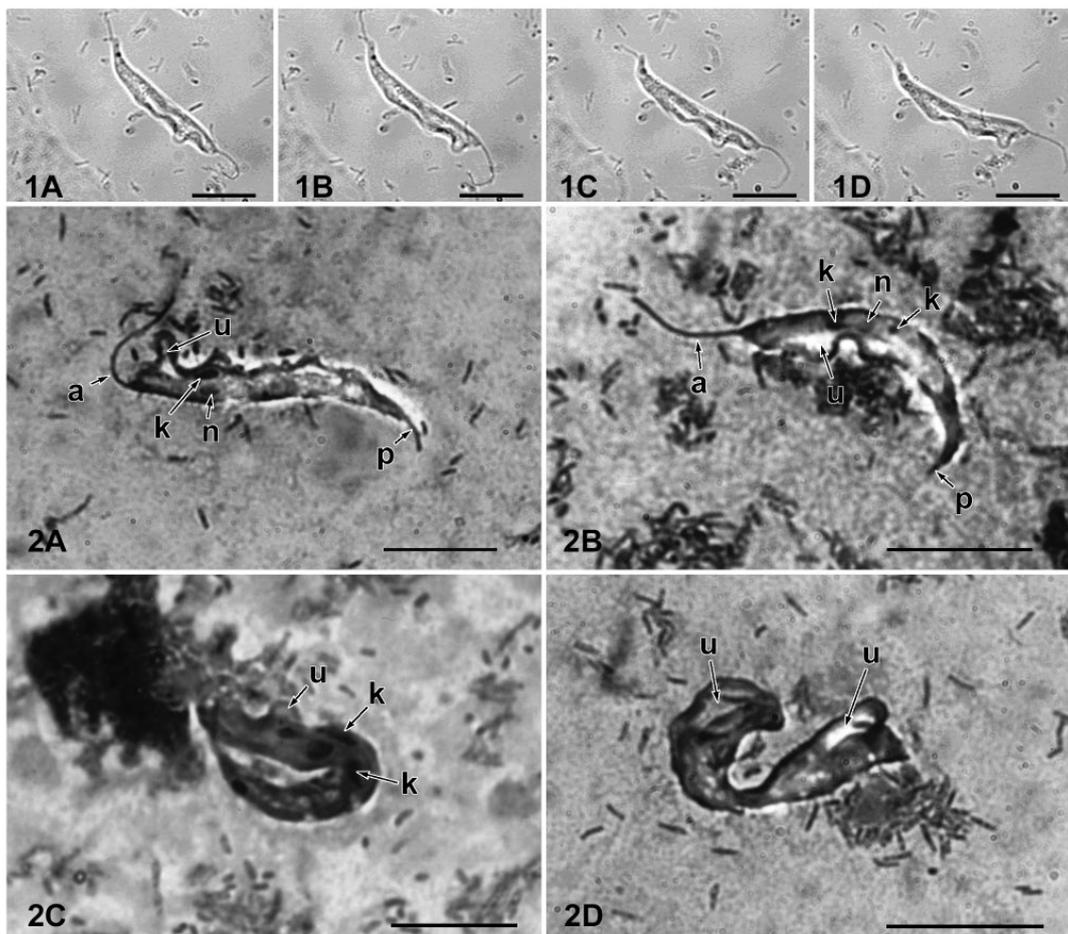
The flagellates swam with a gliding motility, using a vigorous whiplash action of the anterior flagellum and undulations passing posteriorly along the undulating membrane; the posterior flagellum trailed passively (Fig. 1A–D). Body shape was variable, ranging from

Table 1. Organisms and host cells in the mucus from the blowhole of a juvenile pygmy sperm whale undergoing rehabilitation. Data shown are presence (+) or absence (-). Wrights-Giemsa-stained preparations. Numerical superscripts next to dates show number of smears stained for study of morphometrics of the flagellates

	Nov 1993			Dec 1993						Jan 1994		Mar 1994 <sup>a</sup>		May 1994		
	27	29	30	3	6	9	12	16 <sup>3</sup>	30	18	27	14	2	5 <sup>4</sup>	29 <sup>2</sup>	30 <sup>2</sup>
<b>Organisms</b>																
Yeast-like organisms	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
Bacteria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flagellates	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	+
Ciliates	+ <sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Host cells</b>																
Red blood cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
White blood cells	-	-	+	+	-	-	-	-	-	+	+	-	-	+	+	+
Epithelial cells	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+	+

<sup>a</sup>Kinetoplastid flagellates also detected in wet mounts on March 24, 1994

<sup>b</sup>Two shapes of ciliate were observed, spindle-shaped and broad



Figs. 1 to 2. The bodonid kinetoplastid *Jarrellia atramenti* from the mucus of the blowhole of the pygmy sperm whale *Kogia breviceps*. Fig. 1 (A–D). Series of 4 images of an individual flagellate swimming in the mucus. Note the elongate body typical for the gliding motility phase, the whiplash action of the long anterior flagellum, the undulations passing posteriorly along the prominent undulating membrane, and the short free-trailing end of the posterior flagellum. Anterior end of flagellate lies to the right. Photographs taken from video images of wet mount. Scale bars = 10  $\mu$ m. Fig. 2 (A–C). Flagellates from a smear preparation stained with Wrights-Giemsa. Note the typical elongate body, with a rounded anterior end, pointed posterior end, long anterior flagellum (a), short trailing posterior flagellum (p), and prominent undulating membrane (u). The pale staining ovoid nucleus (n) is located in the mid-anterior region of the body, and the dark staining fragmented kinetoplast (k) is dispersed throughout the body in circular to oval masses. Scale bars = 10  $\mu$ m. Fig. 2 (D). Flagellate undergoing longitudinal binary fission. Note prominent undulating membrane (u). Smear preparation stained with Wrights-Giemsa. Scale bar = 10  $\mu$ m

elongate when swimming, to contracted and irregular when undergoing rapid wriggling movements in place. Flagellates were able to attach by their posterior flagellum to material from the respiratory tract of the host.

In Wrights-Giemsa stained smears, the flagellates were typically elongate, with a rounded anterior end and a pointed posterior end (Fig. 2A–C). The body was 18 to 30 ( $23.8 \pm 3.3$ )  $\mu\text{m}$  long and 2 to 5 ( $3.2 \pm 0.9$ )  $\mu\text{m}$  wide, and bore a prominent undulating membrane 1 to 3 ( $1.9 \pm 0.5$ )  $\mu\text{m}$  wide, which extended for most of the length of the body. The anterior flagellum was 5 to 10 ( $8.1 \pm 1.6$ )  $\mu\text{m}$  long, and the free end of the posterior flagellum was 2 to 4 ( $2.9 \pm 0.7$ )  $\mu\text{m}$  long. The nucleus, which stained pale pink, was approximately oval, 1 to 2 ( $1.6 \pm 0.5$ )  $\mu\text{m}$  long, and situated in the anterior one-third of the body; distance to the anterior end of the body was 5 to 11 ( $8.0 \pm 1.7$ )  $\mu\text{m}$ . The kinetoplast, which stained dark blue, was fragmented and variably dispersed throughout the body as a series of masses, typically 1 to 4 in number. These masses were of irregular shape and size (circular to oval and reaching approximately 1  $\mu\text{m}$  in diameter).

Flagellates undergoing division, via longitudinal binary fission, were present in the mucus (Fig. 2D). Bacteria were not recognized in the cytoplasm of the flagellates; the flagellate's feeding mechanisms are unknown.

### Taxonomic summary

*Jarrellia atramenti* n. g., n. sp.

Kinetoplastida, Bodonidae

*Diagnosis of genus.* Solitary bodonid kinetoplastid, with long whiplash anterior flagellum, and short trailing posterior flagellum; recurrent portion of posterior flagellum forms prominent undulating membrane extending at least two-thirds length of body. Body elongate during gliding swimming, contracted and irregular during rapid movements in place; attachment to host material by tip of posterior flagellum. Nucleus oval. Kinetoplast fragmented and variably dispersed throughout body, masses variable in number, irregular in shape (circular to oval) and irregular in size (Wright-Giemsa-stained preparations). Mature and longitudinally dividing individuals in mucus of blowhole of cetacean.

*Type species.* *Jarrellia atramenti* n. g., n. sp.

*Type host.* Pygmy sperm whale *Kogia breviceps* (Physeteridae).

*Type locality.* Marineland, Florida, USA.

*Other locality.* National Aquarium in Baltimore, Maryland, USA, subsequent to stranding near Brigantine, New Jersey, USA.

*Etymology.* The genus name *Jarrellia* is given in memory of Cheri Jarrell, a dedicated volunteer in the Marine Animal Rescue Program at the National Aquarium in Baltimore, whose collection and archival of samples allowed the novel flagellate to be described. The species name *atramenti* meaning 'of ink' or 'of Inky', is the genitive case taken from the Latin noun 'atramentum' meaning ink. The species name is given in recognition of the name 'Inky' that was given to the whale, acknowledging the unique ability of pygmy sperm whales to release clouds of dark reddish-brown 'ink' (liquid feces) when feeding and, or, threatened.

*Deposition of type material.* Paratype material, represented by a glass slide bearing a smear of Wright-Giemsa-stained organisms collected in Marineland, Florida, on May 30, 1994, immediately prior to the whale's release, has been deposited at the International Protozoan Type Slide Collection, at the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20506, USA. The accession number is USNM# 51555.

## DISCUSSION

### Identity of the flagellate

We believe this to be the first published description of a flagellate from a marine mammal. This also appears to be among the first reports of trypanoplasm-like kinetoplastids from warm-blooded hosts. Prior to the recent report of a kinetoplastid from cetaceans by Sweeney et al. (1999), bodonid trophozoites have been considered unable to survive the elevated body temperature of a mammal (Vickerman 1989); (species of *Bodo* reported from the feces or urine of man or other animals [Hassall 1859], have been either misidentified or were contaminants [Sinton 1912, Vickerman 1978]). The different morphologies of the single organism illustrated by Sweeney et al. (1999) and the organism we now describe, the body of the latter being at least twice as long as that of the former, suggest that multiple species of kinetoplastids inhabit cetaceans, and therefore cetaceans may be common hosts for some members of the suborder Bodonina. The bodonid kinetoplastid from the respiratory tract of the pygmy sperm whale possesses a unique combination of morphological features, namely a prominent undulating membrane and fragmented kinetoplast (Table 2), and thus could not be assigned to any genus described in the current key (Leedale & Vickerman in press). We therefore propose the creation of a new genus *Jarrellia* to receive this organism.

Although the motility and morphology, especially the prominent undulating membrane and elongate body, of

the new kinetoplastid most closely resembled that of *Trypanoplasma*, a genus reported from fishes and terrestrial salamanders, the kinetoplast structure, attachment to the host, life cycle and habitat were distinct. The new flagellate has a fragmented kinetoplast, whereas that of *Trypanoplasma* is rod-like. Furthermore, the new flagellate can attach to host material via a flagellum, which is not reported for *Trypanoplasma*. The new flagellate is believed to have a monogenetic (direct) life cycle in the mucus of the respiratory tract of the host, contrasting with the digenetic (indirect) life cycle of *Trypanoplasma*, which includes hematzoic stages. Although the kinetoplast structure, attachment to the host, life cycle, and habitat closely resembled that of *Cryptobia*, a genus known from planarians, mollusks, lizards and fish, the attachment of the recurrent flagellum was different. In the new flagellate, the recurrent flagellum is attached to the body forming a prominent undulating membrane, a feature not found in *Cryptobia*. We suggest that the prominent undulating membrane is a functional adaptation for propulsion in the viscous mucus of the blowhole.

The finding of a flagellate with some characteristics of both *Trypanoplasma* and *Cryptobia* is of particular interest in view of the debate over separation of the 2 established genera, or their possible synonymy. Continued separation is supported by distinct morphology (prominence of undulating membrane and shape of kinetoplast), life cycle and habitat (digenetic with transmission via a vector, and hematzoic; versus monogenetic and non-hematzoic) (Lom & Dykova 1992, Lukes et al. 1998, Leedale & Vickerman in press). Synonymy, initially advocated by Crawley in 1909, is supported by aspects of the life cycle, morphology and molecular phylogeny (Crawley 1909, Bower & Margolis 1983, Paterson & Woo 1983, Woo & Wehnert 1983, Woo 1994, 1995). In 2 of the 35 to 40 hematzoic species from fish (the pathogenic species *Trypanoplasma bullocki* and *T. salmositica*), there is an infective ectoparasitic phase on the body surface, which is morphologically similar to blood forms; and transmission can occur directly from fish to fish (Bower & Margolis 1983, Woo & Wehnert 1983). In addition, the blood forms of *T. salmositica* possess a functional contractile vacuole, an organelle initially believed to be confined to nonhematzoic pro-

Table 2. Comparison of morphological and biological features of the genera *Trypanoplasma*, *Cryptobia* and *Jarrellia*. Data for *Trypanoplasma* and *Cryptobia* taken from Leedale & Vickerman (in press), data for *Jarrellia* taken from present paper

	<i>Trypanoplasma</i>	<i>Cryptobia</i>	<i>Jarrellia</i>
<b>Morphological features</b>			
Undulating membrane	Prominent	Not prominent	Prominent
Kinetoplast <sup>a</sup>	Elongate	Single, compact, elongate or fragmented	Fragmented <sup>a</sup>
<b>Biological features</b>			
Attachment to host	Not known	Anterior or posterior flagellum	Posterior flagellum
Life cycle	Usually 2 hosts <sup>b</sup>	1 host	Probably 1 host
Habitat in vertebrate	Blood <sup>b</sup>	Gut, spleen, liver, gills	Mucus of respiratory tract
Hosts	Fish, terrestrial salamanders, leech vectors	Planarians, mollusks, leeches, lizards, fish	Cetacean

<sup>a</sup>The terms eukinetoplastic, pankinetoplastic and polykinetoplastic can be used to describe the distribution of the kinetoplast DNA in the mitochondrion (Vickerman 1977, 1989, Brugerolle & Mignot 1979, Leedale & Vickerman in press); however, some differences in definition and interpretation of these terms exist. Ultrastructural studies are needed to confirm which condition pertains in *J. atramenti*; light microscopy evidence suggests that the condition is pankinetoplastic (as understood and illustrated by Vickerman 1989)

<sup>b</sup>Direct transmission has been reported for 2 hematzoic species from fish, with infective ectoparasitic stages on the body surface of fish (Bower & Margolis 1983, Woo & Wehnert 1983)

tozoa (Paterson & Woo 1983). Recent comparisons of small ribosomal RNA gene sequences show that 4 hematzoic species of *Trypanoplasma* and *Cryptobia* from fish form a clade (Wright et al. 1999). In synonymizing the genera, *Cryptobia* is given priority since it was established in 1846 by Leidy, and *Trypanoplasma* was established in 1901 by Laveran and Mesnil.

Since Woo (1994) raised the possibility that some ectoparasitic kinetoplastid species might actually be the non-hematzoic phases of hematzoic species, we considered whether *Jarrellia atramenti* from the respiratory tract of the pygmy sperm whale was a phase of a flagellate also present in the blood. However, this appears unlikely because no flagellates were found in any of the 29 blood smears examined over a 5½ mo period. Since the dual life cycle of kinetoplastids (indirect and direct) appears rare (having been reported for only 2 species from fish), and our present evidence from the whale suggests only a direct life cycle, we favor erection of a new genus, rather than supporting the proposed synonymy of *Cryptobia* and *Trypanoplasma*. However, should subsequent research confirm the synonymy, and the subgeneric distinction proposed by Woo (1994) recognizing the basic biological differences between hematzoic and non-hematzoic species becomes widely accepted, the organism we

now describe would be known as *Cryptobia* (*C.*) *atramenti*.

Our assertion that the flagellate from the pygmy sperm whale is new is supported by the low probability that the whale was infected via other hosts or by free-living flagellates. The diet of the pygmy sperm whale is primarily composed of squid, and is supplemented with crabs and fish (Caldwell & Caldwell 1989, McAlpine et al. 1997). Of these potential hosts, kinetoplastids are not reported from squid or crabs (Hochberg 1990, Meyers 1990, Sindermann 1990, Leedale & Vickerman in press), but 3 genera, *Ichthyobodo*, *Cryptobia* and *Trypanoplasma*, are commonly reported from fish (Lom & Dykova 1992, Leedale & Vickerman in press). However, the morphology of *Jarrellia atramenti* is not consistent with that of any kinetoplastid previously described from fish. Of particular interest is the comparison with species of *Cryptobia*, since this was the genus to which the new flagellate was initially tentatively assigned. The prominent undulating membrane borne by this new flagellate distinguishes it from all 10 species of *Cryptobia* from fish recognized by Lom & Dykova (1992): these include 3 ectozoic species (*C. agitans*, *C. branchialis*, and *C. eilatica*) and 7 endozoic species (*C. congeri*, *C. coryphaenoideana*, *C. dahli*, *C. intestinalis*, *C. iubilans*, *C. stilbia*, and *C. trematomi*). It is unlikely that the organisms in the whale were opportunistic, normally free-living flagellates. Although 5 genera of free-living bodonid kinetoplastids are known, namely *Bodo*, *Dimastigella*, *Rhynchobodo*, *Rhynchomonas*, and *Procryptobia* (Leedale & Vickerman in press), the flagellate from the whale was readily distinguished from all of these, principally by its prominent undulating membrane and the absence of a distinct rostrum, snout, or proboscis.

During our consideration of *Cryptobia* spp. from fish, it was evident that many species are poorly described by light microscopy, particularly regarding the presence or extent of an undulating membrane. An undulating membrane is not present in any of the 5 species of *Cryptobia* from fish that have been studied by electron microscopy: these comprise *C. branchialis*, *C. dahli*, *C. eilatica*, *C. intestinalis* and *C. iubilans* (Brugerolle et al. 1979, Lom 1980, Nohynková 1984, Diamant 1990). For *Cryptobia* spp. described as having undulating membranes that appear indistinct by light microscopy, ultrastructural redescriptions are needed.

### Flagellate-host relationship

We believe that *Jarrellia atramenti* is a normal, benign component of the fauna of the respiratory tract of the pygmy sperm whale, consistent with the observations of unidentified flagellates from the blowhole

mucus of other cetaceans by Campbell (1999) and Sweeney et al. (1999). Evidence for this includes initial detection of the flagellates upon the whale's arrival, their persistence throughout the rehabilitation period, and their presence prior to release, when the animal was healthy and free of clinical signs of any respiratory ailments or other disease. Although the whale was initially in poor health, a condition that might have allowed an opportunistic pathogen to become problematic, this apparently did not occur, thus suggesting that the flagellates were commensals. Although occasional white blood cells and epithelial cells were present in the mucus of the blowhole, this is a normal cytological finding (Campbell 1999, Sweeney et al. 1999). Additional support for the proposed benign relationship comes from the similarity of *J. atramenti* to the bodonid kinetoplastid *Cryptobia* spp. affecting fish, in which all ectozoic species from the gills, and 6 of 7 endozoic species from the digestive tract, are commensal (Lom & Dykova 1992). In order to confirm that *J. atramenti* is a commensal, it is necessary to continue to demonstrate that it does not elicit a pathological reaction, and that it feeds upon detritus or bacteria, rather than depriving the host of sustenance. Although the feeding mechanisms of *J. atramenti* are not known, we did not recognize bacteria in the cytoplasm of the flagellate, suggesting that it obtains nutrients by pinocytosis, as do *Cryptobia* and *Trypanoplasma*; this is in contrast to the phagotrophic nutrition of most genera of free-living bodonids (Leedale & Vickerman in press).

We observed that *Jarrellia atramenti* could attach to host material via its posterior flagellum, a characteristic apparently unique to the flagellum of kinetoplastids (Vickerman 1989). Similar attachment mechanisms are known for *Cryptobia branchialis* and *C. eilatica*, which adhere to gill epithelial cells while feeding on bacteria and detritus in the surrounding water. In these flagellates, attachment is via a contact between a ridge on the flagellar membrane and the surface of the epithelial cell; there is no penetration of the epithelial cell or signs of damage to the host cell (Lom 1980, Diamant 1990). Such benign attachment mechanisms are in sharp contrast to the attachment of the pathogenic bodonid *Ichthyobodo*, in which the cytostome protrudes into the host cell and the integrity of epithelial cells is destroyed (Lom & Dykova 1992).

### Proposed life cycle of *Jarrellia atramenti*

We propose that *Jarrellia atramenti* has a direct life cycle. Flagellates were never found in the blood of the whale, despite frequent sampling throughout the rehabilitation period, which suggests that a hematophagous vector is not involved in the life cycle. Fur-

thermore, hematophagous ectoparasites are not known among the wide variety of organisms that attach to cetaceans (Dailey & Brownell 1972, Dailey 1985), although some ectoparasites, such as cyamid arthropods could damage the integrity of blood vessels.

We suggest that the principal mechanism of transmission is via an aerosol. The flagellates, expelled as an aerosol when the whale exhales, could be passed to another whale and then inhaled through the blowhole. A supplementary pathway is via ingestion with seawater and food, and subsequent migration of the flagellates from the oral cavity and esophagus, around the pillar-like epiglottis, and into the upper respiratory tract (Fig. 3). Regurgitation of food, a mechanism used by the pygmy sperm whale to remove indigestible squid pens from its stomach, may enhance this process. The longevity of the flagellates away from the host may be enhanced by their attachment to host material, such as mucus or epithelial cells from the respiratory tract, as has been documented for the freshwater ectozoic species *Cryptobia branchialis* (Bauer et al. 1973), and the marine endozoic species *C. dahli* (Khan 1991) and *Trypanoplasma salmositica* (Bower & Margolis 1983).

Since the host, *Kogia breviceps*, travels alone or in small groups (Geraci & Lounsbury 1993), the most significant transmission of flagellates probably occurs during mating and from mother to calf, rather than

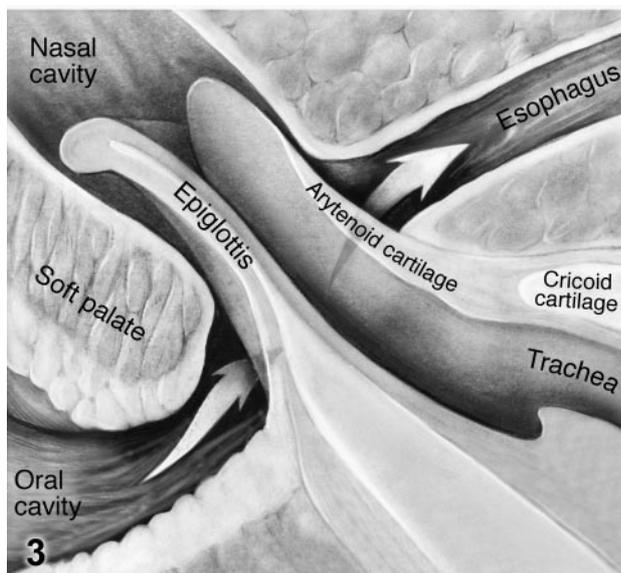


Fig. 3. *Kogia breviceps*. Anatomy of the epiglottis of the pygmy sperm whale. Ingested flagellates could pass from the oral cavity, around the pillar-like epiglottis, and up into the nasal cavity. Arrows show direction of movement of food, from the oral cavity, around the pillar-like epiglottis, to the esophagus. Illustration by Juan Garcia, Department of Art as Applied to Medicine, Johns Hopkins University School of Medicine

between schooling adults, as could happen in more social cetacean species. Although repeated infections of an individual cetacean are likely, an initial infection can probably be sustained without reinfection, since the flagellates can divide and multiply in the mucus of the blowhole (Fig. 2D). Retention of an initial infection throughout the life of the host has been reported for the bodonid kinetoplastid *Cryptobia dahli* infecting the stomach of lumpfish (Khan 1991).

### Ciliates

Ciliates are reported from the blowhole of a pygmy sperm whale for the first time. The 2 distinctive shapes of ciliates observed, namely spindle and broad, suggest a mixed species infection. The spindle-shaped ciliate may have been the phyllopharyngid *Kyaroikeus cetarius*, which has previously been reported from the blowhole of Atlantic bottlenose dolphins from the east coast of the USA, and from blowhole of killer whales *Orcinus orca*, false killer whales *Pseudorca crassidens*, and beluga whales housed at Sea World in Florida (Sniezek et al. 1995). The broad bodied ciliate may be similar to the unidentified ciliates reported from the blowhole of Atlantic bottlenose dolphins from Hawaii and California by Dailey (1985), or to the ciliate tentatively identified as a phyllopharyngid (chilodonellid) found in the blowhole of the same species in California (Arkush pers. comm.). The ciliates are expected to have direct life cycles.

The presence of ciliates in the blowhole mucus from the whale in this study raises the possibility that the respiratory tract may have been the source of ciliates that were associated with dermatitis in another pygmy sperm whale noted by Schulman & Lipscomb (1999). This possibility is consistent with the suggestion previously made by Dailey (1985) that the ciliates affecting the skin of bottlenose dolphins were probably opportunistic rather than active tissue invaders. It is unlikely that the ciliates associated with dermatitis were general surface commensals which opportunistically invaded the lesions, since ciliates have not been reported from undamaged skin of cetaceans.

### Recommendations for efficient sampling protocols for protozoa

The inconsistent presence of the protozoa in the blowhole mucus, with the flagellates being seen intermittently and the ciliates being seen only initially, prompted us to consider whether changes in our sampling protocols had affected their detection. We believe that the apparent absence of the flagellates in Decem-

ber (Table 1) was due to delays in preparing the samples, rather than to a period when the whale was uninfected. In December, samples were collected to monitor for the presence of yeast-like organisms in response to treatment, and thus emphasis was not placed on prompt fixation of flagellates. This delay was in contrast to prompt fixation in all other months. Our explanation is supported by examination of wet mounts, which showed that flagellates were more likely to be seen if the slides were examined within a few minutes of collection, and if there was a lot of mucus on the slides; this is consistent with recommendations for detection of ectoparasitic protozoans from fish (Lom & Dykova 1992). It is likely that during December, the flagellates, even if present, did not survive or remain intact during the extended time intervals between collection, examination and fixation. Thus, protozoan material for Wrights-Giemsa staining must be fixed promptly.

It was interesting to note that the ciliates were seen only in the initial sample, which was collected on the second day after the whale arrived in Baltimore. We believe that the lack of ciliates from subsequent samples was indicative of their absence from the whale, rather than their apparent absence due to delays in sample preparation. As discussed above, prompt sample preparation in the later months of rehabilitation allowed delicate flagellates to be seen; we also should have seen ciliates if they were present. We suggest that the ciliates were less tolerant than the flagellates of the changes in the microhabitat of the blowhole and in the air and water during stranding and rehabilitation. This finding highlights the importance of screening wild cetaceans for ciliate and flagellate infections in the mucus of the respiratory tract as soon as possible after rescue and beginning rehabilitation.

In order to further our understanding of the life cycle of kinetoplastids from cetaceans, subsequent studies should also include examination of the blood using the hematocrit centrifugation technique, in which the flagellates are concentrated in the buffy layer, thus allowing detection of lower density infections than is possible by examination of direct blood smears (Woo 1969). Morphological descriptions of the flagellates should be extended by the addition of scanning and transmission electron microscopy studies. Of particular interest at the ultrastructural level are the recurrent flagellum, undulating membrane and kinetoplast. In addition, molecular studies of nuclear and kinetoplast (mitochondrial) genes are strongly recommended, not only for their promise in distinguishing various kinetoplastids from one another (Figueroa et al. 1999), but also for the elucidation of phylogenetic relationships within the Bodonina (Lukes et al. 1998). Molecular studies will be enhanced by development of protocols for *in vitro* culture of kinetoplastids from cetaceans.

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**Note added in proof.** A further report of protozoans in cetaceans has recently come to our attention. An amoeba, resembling *Entamoeba*, and a flagellate similar to *Chilomastix* or *Hexamita*, were reported from the formalin-fixed colon contents of a blowhead whale, *Balaena mysticetus* (Balaenidae) by Heckmann et al. (1987).

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