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Cytochrome oxidase I sequences reveal possible cryptic diversity in the cosmopolitan symbiotic copepod *Nesippus orientalis* Heller, 1868 (Pandaridae: Siphonostomatoida) on elasmobranch hosts from the KwaZulu-Natal coast of South Africa

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ABSTRACT

Over the past decade, numerous molecular phylogenetic studies uncovered cryptic diversity within the Copepoda, yet very few investigations focused on symbiotic copepods. Here we report mitochondrial DNA *cytochrome oxidase* I diversity in the cosmopolitan elasmobranch symbiont *Nesippus orientalis* off the KwaZulu-Natal coast of South Africa. Analysis of partial *COI* sequences of copepods sampled from a diversity of shark hosts, revealed the presence of two divergent clades. Diversity within the clades does not appear to be structured based on host species, host individual, geographic locality or time of sampling. However, divergence between the two clades seems to be related to host species. Phylogenetic analyses of representatives from the two clades, along with *Nesippus* spp., *Caligus* spp. and *Lepeophtheirus* spp. outgroups, further supports the distinction between the two clades. Future molecular phylogenetic investigations of widespread copepod symbionts most likely will reveal far greater levels of biodiversity than currently recognized.

1. Introduction

The genus *Nesippus* Heller, 1868 includes five nominal species, i.e. *N. orientalis* Heller, 1868; *N. crypturus* Heller, 1868; *N. vespa* Kirtesinghe, 1964; *N. tigris* Cressey, 1967 and *N. nana* Cressey, 1970 (Boxshall and Halsey, 2004; Cressey, 1967, 1970). All species are cosmopolitan in their distribution and occur in the mouths, on the gill arches and in the nasal passages of a variety of elasmobranch hosts (Cressey, 1967, 1970; Dippenaar and Jordaan, 2006).

Nesippus orientalis can be easily distinguished from the other species by the presence of dorsal plates on the fourth thoracic segment (Cressey, 1967). They are commonly found on a number of shark species (Cressey, 1967, 1970; Dippenaar, 2004; Dippenaar and Jordaan, 2006), often in clusters on the gill arches and in the mouth (Dippenaar and Jordaan, 2006).

The life cycle of *N. orientalis* is unknown, however, Wilson (1907) already stated that the life cycles of representatives of Pandaridae are similar to those of the Caligidae and this suggestion is strengthened by the monophyletic grouping of Pandaridae and Caligidae (Huys et al., 2007; Dippenaar, 2009). The general life cycle of caligids consists of two free-living nauplius stages, followed by one infective copepodid stage and four parasitic chalimus stages, two parasitic preadult stages and the parasitic adults (Kabata, 1981). Even though small deviations from the general life cycle are found (see Lin and Ho, 1993), information about their life cycles (see Lin and Ho, 1993; Schram, 1993; Todd, 2007) can be used to deduce that the life cycle of *N. orientalis* individuals likely comprise a short free-living phase, while all other stages will be attached to a host. Information about the biology of members of the Caligidae (see Boxshall, 1974; Anstensrud, 1990; Todd, 2007) can be used to speculate that males of *N. orientalis* can probably

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copulate with several females, while the females may be polyandrous. Individuals of *N. orientalis* attach to the gill arches and in the mouth of their hosts and the females in particular do not seem to move vast distances due to their orderly way of attachment (Dippenaar and Jordaan, 2006). Based on these observations and the occurrence of young among adults (Dippenaar and Jordaan, 2006), it thus appears unlikely that they would move from one host to another, even when hosts come into physical contact with one another.

Since it is impractical to track the planktonic stages of marine invertebrate larvae in the water column (Todd, 1998), especially of those species that as adults live symbiotically on elasmobranch hosts, analyses of the degree and scale of genetic differentiation among populations offer an indirect method of accessing the scale of larval dispersal (Todd, 1998). Knowlton (1993, 2000) also recognized the potential of molecular genetics to identify sibling or cryptic species (i.e. species difficult to distinguish using traditional techniques), which often exist among members of the Crustacea and Copepoda (see e.g. Burton and Lee, 1994; Bucklin et al., 1999; Rocha-Olivares et al., 2001). In this respect, mitochondrial genomes contain markers suitable for studies of population genetics and evolution (Shao and Barker, 2007), with the cytochrome *c* oxidase subunit I (*CO*I) gene reported to be successfully employed for species recognition (Hebert et al., 2003; Waugh, 2007). Even though *CO*I markers get saturated quickly around 0.3 substitutions per site, determined with uncorrected pairwise distances, a fairly good approximation can still be made to delimit species using this method (Lefébure et al., 2006). Under the phylogenetic species definition, the *CO*I gene has a proposed species delimitation threshold of 0.16 substitutions per site in crustaceans (Lefébure et al., 2006), and has proven to be specifically efficient in copepods (Bucklin et al., 1999; Hill et al., 2001; Øines and Heuch, 2005).

Few studies have been conducted to assess the genetic variation within and between populations of symbiotic siphonostomatoids. A study on the genetic heterogeneity within populations of *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 parasitic on two host species, reported incipient sympatric speciation (De Meeüs et al., 1992). More recent studies on *Caligus elongatus* Nordmann, 1832 revealed the presence of two distinct genotypes possibly distinguishing two sibling species

Table 1

A list of the 43 *CO*I haplotypes (see Fig. 2), obtained from *Nesippus orientalis* specimens, collected from 19 different host individuals (host nr), host species, the localities (see Fig. 1) where the hosts were caught, the dates when they were caught and their GenBank accession Nos.

Haplotype	Host nr	Shark species	Locality	Coordinates	Date caught	GenBank Accession Nos.
Clade I						
GHDN01	1	*Sphyrna mokarran*	Durban	29.51S 31.00E	11/2000	GQ369463
GHDN02	1	*Sphyrna mokarran*	Durban	29.51S 31.00E	11/2000	GQ369464
GHDN03	1	*Sphyrna mokarran*	Durban	29.51S 31.00E	11/2000	GQ369465
BTRB01	2	*Carcharhinus limbatus*	Richards Bay	28.48S 32.06E	10/2002	GQ369466
BTRB02	2	*Carcharhinus limbatus*	Richards Bay	28.48S 32.06E	10/2002	GQ369467
BTRB03	2	*Carcharhinus limbatus*	Richards Bay	28.48S 32.06E	10/2002	GQ369468
ZMRB01	3	*Carcharhinus leucas*	Richards Bay	28.48S 32.06E	10/2002	GQ369469
ZMRB02	3	*Carcharhinus leucas*	Richards Bay	28.48S 32.06E	10/2002	GQ369470
Clade II						
GWRB01	4	*Carcharodon carcharias*	Richards Bay	28.48S 32.06E	10/2002	GQ369471
RTRB03	5	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369480
CHPE01	6	*Sphyrna lewini*	Port Edward	31.03S 30.14E	06/1998	GQ369502
CSMG02	7	*Carcharhinus brachyurus*	Margate	30.52S 30.21E	11/2004	GQ369505
GWLB01	8	*Carcharodon carcharias*	Leisure Bay	31.01S 30.14E	03/1999	GQ369474
RTRB06	10	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1997	GQ369483
RTRB02	5	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369479
RTRB04	5	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369481
RTSB03	11	*Carcharias taurus*	Scottburgh	30.17S 30.45E	11/2002	GQ369492
GWRB03	4	*Carcharodon carcharias*	Richards Bay	28.48S 32.06E	10/2002	GQ369473
GWLB03	8	*Carcharodon carcharias*	Leisure Bay	31.01S 30.14E	03/1999	GQ369476
RTRB09	12	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369486
RTRB01	5	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369478
RTZN01	13	*Carcharias taurus*	Zinkwazi	29.12S 31.27E	10/1998	GQ369493
SHDN01	14	*Sphyrna zygaena*	Durban	29.51S 31.00E	10/2002	GQ369500
GWLB02	8	*Carcharodon carcharias*	Leisure Bay	31.01S 30.14E	03/1999	GQ369475
SFSR02	9	*Isurus oxyrinchus*	Salt Rock	29.30S 31.15E	11/2002	GQ369495
RTSB02	11	*Carcharias taurus*	Scottburgh	30.17S 30.45E	11/2002	GQ369491
RTRB10	12	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369487
SHDN02	14	*Sphyrna zygaena*	Durban	29.51S 31.00E	10/2002	GQ369501
RTRB07	10	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1997	GQ369484
RTRB11	12	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369488
SHAB01	15	*Sphyrna zygaena*	Anstey's Beach	29.55S 31.01E	11/2002	GQ369497
SHMZ01	16	*Sphyrna zygaena*	Mzamba	31.05S 30.10E	08/1995	GQ369499
RTRB05	5	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369482
SHAB02	15	*Sphyrna zygaena*	Anstey's Beach	29.55S 31.01E	11/2002	GQ369498
CHAM01	17	*Sphyrna lewini*	Amanzimtoti	30.03S 30.53E	07/1998	GQ369503
RTSB01	11	*Carcharias taurus*	Scottburgh	30.17S 30.45E	11/2002	GQ369490
SFSR01	9	*Isurus oxyrinchus*	Salt Rock	29.30S 31.15E	11/2002	GQ369494
RTRB08	10	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1997	GQ369485
GWUM02	18	*Carcharodon carcharias*	Umhlanga Rocks	29.43S 31.05E	11/2000	GQ369477
CSMG01	7	*Carcharhinus brachyurus*	Margate	30.52S 30.21E	11/2004	GQ369504
SFSR03	9	*Isurus oxyrinchus*	Salt Rock	29.30S 31.15E	11/2002	GQ369496
RTRB12	19	*Carcharias taurus*	Richards Bay	28.48S 32.06E	11/1999	GQ369489
GWRB02	4	*Carcharodon carcharias*	Richards Bay	28.48S 32.06E	10/2002	GQ369472

Hosts: GH = Great Hammerhead, BT = Blacktip, ZM = Zambezi, GW = Great White, RT = Raggedtooth, CH = Scalloped Hammerhead, SH = Smooth Hammerhead, SF = Shorfin Mako, CS = Copper Shark; Localities: RB = Richards Bay, ZN = Zinkwazi, SR = Salt Rock, UM = Umhlanga Rocks, DN = Durban, AB = Anstey's Beach, AM = Amanzimtoti, SB = Scottburgh, MG = Margate, LB = Leisure Bay, PE = Port Edward, MZ = Mzamba.