The host preferences of *Nuttalliella namaqua* (Ixodoidea: Nuttalliellidae): A generalist approach to surviving multiple host-switches

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Abstract *Nuttalliella namaqua* has been described as a "living fossil" and the closest extant species to the ancestral tick lineage. It was previously proposed that the *Nuttalliella* lineage parasitized reptile-like mammals in the Permian and had to switch hosts several times due to mass or host lineage extinctions. Extant hosts include girdled lizards and murid rodents. The current study extends knowledge on the extant host range of *N. namaqua* using gut-meal analysis of field collected specimens. Nymphs and females can parasitize a variety of reptiles that includes skinks, geckos and girdled lizards. Blood-meal from a hyrax was also detected in a specimen suggesting that *N. namaqua* could parasitize a broader range of mammals than the previously suggested murid rodents. Rather than being host specific, *N. namaqua* is proposed to be a generalist and the ability to switch and parasitize multiple hosts allowed it to survive multiple mass and host lineage extinctions.

Keywords *Ixodida*, *Nuttalliella namaqua*, ● host ● lizards ● mammals ● blood-meal ● generalist

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Introduction

Host specificity allows parasites to occupy unique niches and prevents competition for the same resources. The survival of the parasite is, however, intimately linked with that of the host and when a host becomes extinct, parasites that cannot adapt to new hosts will also succumb to a similar fate (Koh et al. 2004). A striking example of this are ticks, with at least sixty-three species being considered endangered (Mihalca et al. 2011). A generalist host strategy allows for parasites to switch hosts, parasitize different hosts in different geographic areas, extend geographic ranges independent of host restricted habitat and facilitate host finding in host deficient environments (Krasnov et al. 2008). In terms of ticks, host specificity has not been considered to be an important factor in the evolution of blood-feeding behaviour in ticks (Klompen et al. 1996).

The Ixodida (ticks) are composed of three families, Argasidae (soft ticks ~200 species), Ixodidae (hard ticks ~ 700 species) and the monotypic Nuttalliellidae (Barker and Murrell, 2004; Guglielmone et al. 2010). *Nuttalliella namaqua* has been described as a missing link between the families, since it possess features unique to either hard or soft ticks (Bedford, 1931). Adults and nymphs possess a pseudo-scutum reminiscent of the scutum of ixodids, but a leathery integument similar to argasids, and like the latter feeds fast (Bedford, 1931; Mans et al. 2011). Larvae possess a true scutum and resemble ixodids, and like the latter exhibit prolonged feeding with a slow and rapid engorgement phase (Latif et al. 2012; Mans et al. 2012). Males possess a scutum that covers most of the dorsal side, reminiscent of ixodid males (Latif et al. 2012). Several features are unique to *N. namaqua*, notably ball and socket-joints in adults and nymphs and secretion of excess blood-meal derived water via the malpighian system (Bedford, 1931; Mans et al. 2011). Systematic analysis suggested a basal relationship to the hard and soft tick families and it has been proposed that *N. namaqua* is a "living fossil" that dates from the Late Carboniferous-Early Permian (Mans et al. 2011; Mans et al. 2012). This would imply that this species underwent numerous host switches during its evolution. It is therefore not surprising that the host status of *N. namaqua* remains enigmatic and controversial.

The majority of adult and nymphal ticks have been collected off the host in a variety of natural habitats that include under a stone, from the ground, from the nest of the striped swallow (*Hirundo abyssinica unitatis*), from an abandoned eagle nest, from a rock crevice and from a rock face (Bedford, 1931; Keirans et al. 1976; El Shoura et al. 1984; Mans et al. 2011). Ten females were collected from slender-tailed meerkat (*Suricata suricatta hahni*) and one from Brants' karoo rat (*Parotomys brantsi*) (Keirans et al. 1976). Based on these collection sites, it was suggested that the preferential host could be rock hyraxes (*Procavia capensis*), swallows, rodents and meerkat (Keirans et al. 1976). The possibility that *Agama* or other lizards could be candidate hosts

was also considered (Hoogstraal, 1985). Efforts to feed females and nymphs on chickens, pigeons, rabbits, rats or mice were, however, not successful (Hoogstraal, 1985; El Shoura, 1990). Gut meal analysis from a field-collected *N. namaqua* female indicated the presence of nucleated red blood cells and DNA from girdled lizards (*Cordylus*), while nymphs and adult females were successfully fed on lizards (Mans et al. 2011). In contrast, numerous larval ticks were found on a variety of small murid rodents (*Micaelamys namaquensis*, *Aethomys chrysophilus* and *Acomys spinosissimus*), suggesting that these were the natural hosts of larval *N. namaqua* (Horak et al. 2012). Larvae could also be successfully fed on both mice and lizards and moulted to nymphs (Latif et al. 2012; Mans et al. 2012). The question was raised whether the female analysed in the previous study (Mans et al. 2011), could have incidentally fed on a lizard and that reptiles would not be natural hosts for female ticks, even if successful feeding could be completed in the laboratory. This was investigated by gut meal analysis of additional field collected nymphs and females and the results indicated that all specimens fed on different lizards, suggesting that *N. namaqua* is a generalist.

Materials and Methods

Tick collection, dissection, blood smear preparation and genomic DNA extraction

The *N. namaqua* specimens used in the current study were collected as previously described from the same localities (Mans et al. 2011). All necessary collection and transport permits were obtained from the Veterinary Authorities (Permit number: SP2011/02/02/01). In addition permission to collect ticks from Krymekaar and Voëlklip was granted by the owner, Mr. A. van Heerden.

Tick dissection and gut preparation

Tick guts were processed as previously described (Mans et al. 2011). Briefly, ticks were embedded in molten wax and their dorsal cuticle removed under 0.9% saline solution by dissection. Guts were removed in an intact form and ruptured on a microscope slide. Half were used to prepare a blood smear that was dried for Giemsa staining. The other half was used for DNA extraction using the Qiagen Blood kit according to the manufacturer's instructions.

Tick gut meal analysis for identification of host mitochondrial DNA

The 16S rRNA gene for lizards were amplified, cloned and sequenced as previously described (Mans et al. 2011). Briefly, primers used were for the 16S gene from reptiles and include the 16SF.1 and 16SR.0 primers (Whiting et al. 2003). For each tick, ten clones were sampled and consensus sequences derived that was used to search the non-redundant database using BLASTN analysis (Altschul et al. 1990). Mammalian DNA was amplified using the L14841 and H15149 primers for the cytochrome b gene (Kocher et al. 1989), and cloned, sequenced and analysed as for the 16S gene.

Results

Micro-habitats of *N. namaqua*

In the current study, adult females as well as nymphs were collected from a variety of micro-habitats that included the underside of a rock overhang exposed to the elements, a crevice in the ground packed with rocks and dirt that will not be accessible to large animals, on the wall of a hyrax den and under a flint with a clearing space of less than 1 cm (Table 1).

Gut meal analysis for hosts of *N. namaqua*

To extend the previous gut meal analysis performed on a single field collected tick specimen, eight additional field collected female ticks were analysed (Table 1). Blood smear analysis of the gut contents indicated that all ticks possessed nucleated red blood cells, which indicated that they recently fed on reptile or avian hosts. Amplification, cloning and sequencing of the 16S ribosomal RNA gene indicated that these ticks fed on a variety of different lizards that included girdled lizards (Cordylid family), skinks (*Mabuya*) and geckos (*Pachydactylus*). It should be noted that at least five of the lizard species did not have sequences in the database that would allow species identification, but could be assigned to lizard genera. Amplification of the mammalian cytochrome b gene was negative for most of the samples, suggesting that these specimens did not feed on any mammalian hosts. However, one specimen yielded sequences corresponding to that of a hyrax (*Procavia capensis*) as well as a gecko. Seven of the tick specimens only possessed one species of lizard DNA. In contrast, two specimens, including the previous described female, contained the blood-meal of three to four lizard species.

Discussion

Analysis of gut meal content to identify the hosts of blood-feeding arthropods has been used for a number of different ecto-parasites using RFLP markers (Oshaghi et al. 2006), reverse line blot (Scott et al. 2012), PCR (Ngo and Kramer, 2003, Kent and Norris, 2005), proteomics (Wickramasekara et al. 2008) and immunological methods (Clausen et al. 1998). For most of the above methods of host detection, host reference material is necessary (Laskay et al. 2012). Since the host range of *N. namaqua* is not known, amplification with universal primers for lizards and mammals, followed by cloning and sequencing were considered the most prudent approach as evident by the discovery of at least five unique lizard species that could not be identified.

In a number of tick species, multiple hosts including mammalian, could be detected (Scott et al. 2012). In the case of *N. namaqua*, the predominant hosts detected in females were single species of lizards. However, in a limited number of samples multiple species were detected, including the mitochondrial DNA of a hyrax. These ticks most probably had multiple feeding events and stored their blood-meals over prolonged periods as observed for argasids (Mans et al. 2011). Recently, it was shown that *N. namaqua* females could feed multiple times, that red blood cells can be stored in an intact form between molting events and that the blood-meal may be stored for more than six months without digestion (Mans et al. 2012). This supports the notion of storage and detection of blood-meal from multiple hosts.

N. namaqua has been found in Tanzania and a wide area of southern Africa that included the Karoo, Namaqualand, Kalahari and the Soutpansberg area of the Northern Limpopo province (Keirans et al. 1976; Mans et al. 2011; Horak et al. 2012). Lizard hosts identified thus far included the Karoo girdled lizard (*Karusasaurus polyzonus*), the western skink (*Mabuya sulcata*) and Bibron's gecko (*Pachydactylus bibroni*). In addition, a number of unidentified species closely related to Weber's gecko (*Pachydactylus weberii*), the Namaqua gecko (*Pachydactylus namaqua*) and members of the Cordylid family were detected. The girdled lizards and geckos have specific distributions in the arid North-Western region of southern Africa, while *Mabuya sulcata* is widely distributed across southern Africa (Broadley, 2000; Bauer and Lamb 2005; Stanley et al. 2011). It is therefore likely that *N. namaqua* parasitizes different reptile species in other geographic regions.

The habitats where most adult and nymphal *N. namaqua* were collected (under rocks, the underside of rock overhangs, under flint and on rocks within a ground crevice) are accessible to lizards and it would seem to be unlikely habitats frequented by rodents. If eggs were laid in these environments, larvae would probably parasitize lizards. Even so, reports of larvae feeding on mice were reported even though it is not clear what the

prevalence is on mice in general (Horak et al. 2012; Mans et al. 2012). It is furthermore intriguing that a number of females were found on burrowing mammals, such as meerkat and Brant's Karoo rat (Keirans et al. 1976), given that females feed fast (Mans et al. 2011). One possible explanation would be that these females were indeed feeding on these mammals when the animals were killed. These animals and ticks were collected on a trip to Namaqualand and South-West Africa conducted on behalf of the Transvaal Museum in 1937 by Austin Roberts and Vivian FitzsSimons (FitzsSimons, 1938). The trip extended from 6 March – 4 September 1937 and sites reported for *N. namaqua* collections (Keirans et al. 1976), correspond with the same dates visited during the trip, i.e. Kobos, Rehoboth (19-21 July 1937) and Port Nolloth (19-21 August 1937). From five *Suricata suricatta hahni* collected on the trip, two were infested with *N. namaqua*, while one out of three *Parotomys brantsi* collected were infested (Roberts, 1937). It would seem unlikely that these were incidental findings, especially since one meerkat was infected with nine female ticks. This extends the potential mammalian hosts for *N. namaqua* to murid rodents, meerkat, Brant's Karoo rat and hyraxes.

For nine specimens sampled, using gut blood meal analysis, nine different lizard genotypes were obtained, although the same genotype was found in different specimens. No lizard host of preference could, however, be assigned. Similarly, larvae were found on three different murid rodent species with similar infestation rates (Horak et al. 2012). The data for *N. namaqua* suggest a wide geographic distribution as well as host preference. Tentatively, it may be concluded that larvae may generally feed on rodents (no data exist for captured lizards), while nymphal and adult ticks prefer reptiles with no particular host preference. More likely, however, is the possibility that this tick is a generalist, given the fact that it parasitizes at least 14 different mammalian and reptile hosts (Krasnov et al. 2010), as well as birds (Keirans et al. 1976) (Table 2). As such, host preference might be determined by the specific habitat in which the tick finds itself at any given moment. It is therefore premature to conclude that the natural hosts of immatures or adults may be exclusively mammals or reptiles (Mans et al. 2011; Horak et al. 2012). In this regard, the generalist approach seems to hold for many argasid and ixodid tick species (Cumming, 2004; Klompen et al. 1996; Wells et al. 2012; Nava and Guglielmone, 2013).

As *N. namaqua* is monotypic and basal to the Ixodida, it has been suggested that this tick is a "living fossil" that dates from the time of the origin of the Ixodida and that some of its earliest hosts were reptile-like mammals (Mans et al. 2011). Given the molecular clock age estimations for the *Nuttalliella* genus (>280 MYA) it would be clear that this genus fed on reptiles long before the origin of mammals (Mans et al. 2011; Mans et al.

2012). It is therefore likely that the host preference of *N. namaqua* changed over temporal time so that extant mammals and lizards would be current preferred hosts. Similarly, ixodids and argasids changed hosts many times over their evolution, so that host specificity is likely to be temporal and determined by ecology as much as host availability (Klompen et al. 1996; Estrada-Pena et al. 2010). In this regard, those lineages unable to adapt to new hosts would have become extinct and a generalist approach to host specificity (as suggested for *N. namaqua*) would be the most optimal survival strategy. This correlates with considerations that host specificity was not a major driving force in tick evolution (Klompen et al. 1996).

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Table 1 Gut meal analysis of field collected *N. namaqua*. Indicated are the locality and the habitat from which ticks were collected, the presence of nucleated red blood cells in the gut, the best BLASTN hit and the number of clones sequenced in parenthesis, as well as the percentage identity to the best BLASTN hit. The Genbank accession numbers for the different clones are also indicated. Sequences from Nn1 were reported in a previous study (Mans et al. 2011). *These sequences considered to be closely related species to the best BLAST hit.

Sample	Locality/ Gender	Habitat	Nucleated RBC	Best BLASTN hit (number of clones)	Identity (%)	GI number
Nn1	Voëlklip / Female	In ground crevice	Yes	*Karusasaurus polyzonus* (12)	99	334562344
				Cordylus ukingensis (5)	88*	334562343
				Cordylus cordylus (6)	94*	308096005
				Ninurta coeruleopunctatus (3)	88*	334562345
Nn2	Krymekaar/ Female	Underside of rock overhang	Yes	*Pachydactylus weberi* (10)	87*	JQ739170
				Procavia capensis (2)	97	KC907408
Nn3	Krymekaar/ Female	Underside of rock overhang	Yes	*Pachydactylus weberi* (10)	87*	JQ739170
Nn4	Krymekaar/ Female	Hyrax den	Yes	*Pachydactylus bibronii* (10)	99	JQ739169
Nn5	Voëlklip/ Female	In ground crevice	Yes	*Pachydactylus bibroni* (10)	99	JQ739169
Nn6	Krymekaar/ Female	Under flint	Yes	*Mabuya sulcata* (10)	98	JQ739172
Nn7	Voëlklip/ Female	In ground crevice	Yes	*Pachydactylus bibronii* (4)	99	JQ739169
				Pachydactylus namaquensis (3)	95*	JQ739171
				Pachydactylus weberi (1)	87*	JQ739170
Nn8	Krymekaar/ Female	Under flint	Yes	*Pachydactylus bibronii* (10)	99	JQ739169
Nn9	Krymekaar/ Female	Underside of rock overhang	Yes	*Pachydactylus weberi* (10)	87*	JQ739170

Table 2 Potential hosts described for *N. namaqua*. *In the case of blood-meal analysis the minimum number of animals that were parasitized is indicated, assuming that each tick fed independently. In the case of the birds, ticks were found in two independent nests.

Animal	Number of ticks	Life stage	Number of animals	Collection method	Reference
Mammals					
Suricata suricatta hahni	10	Adult	2	On host	Roberts, 1937
Parotomys brantsi brantsi	1	Adult	1	On host	Roberts, 1937
Aethomys chrysophilus	58	Larvae	6	On host	Horak et al. 2012
Acomys spinosissimus	9	Larvae	3	On host	Horak et al. 2012
Micaelamys namaquensis	154	Larvae	10	On host	Horak et al. 2012
Procavia capensis	1	Adult	1*	Blood-meal	This study
Reptiles					
Karusasaurus polyzonus	1	Adult	1*	Blood-meal	Mans et al. 2011
Cordylus cf. ukingensis	1	Adult	1*	Blood-meal	Mans et al. 2011
Cordylus cf. cordylus	1	Adult	1*	Blood-meal	Mans et al. 2011
Ninurta cf. coeruleopunctatus	1	Adult	1*	Blood-meal	Mans et al. 2011
Pachydactylus cf. weberi	3	Adult	3*	Blood-meal	This study
Pachydactylus bibronii	4	Adult	4*	Blood-meal	This study
Pachydactylus cf. namaquensis	1	Adult	1*	Blood-meal	This study
Mabuya sulcata	1	Adult	1*	Blood-meal	This study
Birds					
Hirundo abyssinica unitatis	2	Adult	2*	Nest	Keirans et al. 1976