A sylvatic lifecycle of *Echinococcus equinus* in the Etosha National Park, Namibia

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**Abstract**

Various species of *Echinococcus* have been described in the past from wild mammals of sub-Saharan Africa. However, it is only recently that a few isolates have become available for molecular identification; therefore, the involvement of wildlife in the lifecycles of the various cryptic species within *Echinococcus granulosus* sensu lato is still only partially known. A preliminary survey was undertaken in Etosha National Park, Namibia, from August to October 2012. Faecal samples were obtained from 34 individual wild carnivores, and metacestodes were collected from carcasses of 18 culled herbivores. Single eggs and metacestode tissue were lyzed and identified from sequences of the mitochondrial *cox1* gene. In case of metacestodes, the *nad1* gene was additionally sequenced and haplotype networks were constructed.

*Echinococcus equinus* was found in lions (4 of 6), black-backed jackals (2 of 7) and Burchell’s zebras (11 of 12). The frequency of this parasite in the absence of domestic dogs, horses and donkeys strongly indicates its transmission in a wildlife cycle. Further, a variety of sequences were obtained from eggs and cysticerci from lions, cheetahs, caracals, spotted hyenas and oryx, which most closely clustered with species of *Taenia*. Only 3 of them, 2 of lion and 1 of hyena origin, could be allocated to *Echinococcus* sensu stricto. One was identified as *Echinococcus granulosus* (=*Taenia*) *regis*. Of the remaining two, one was confirmed in lions and the other in hyenas.

**1. Introduction**

Numerous records of *Echinococcus* adult and metacestode stages were reported during the second half of the 20th century from a wide range of wild African carnivore and herbivore species. In various surveys, mainly from eastern and southern Africa, two species of jackal, cape fox, African wild dog, spotted hyena, wild cat and lion were identified as definitive hosts, while cysts were found mainly in zebra, bush pig, warthog, hippopotamus, giraffe, buffalo and at least ten species of other boids (“antelopes”) (lit. in *Macpherson and Wachira*, 1997; *Hüttner and Romig*, 2009). High prevalence levels are known for buffalo (*Woodford and Sachs*, 1973), hippopotamus (*McCully et al.*, 1967), warthog (*Dinnik and Sachs*, 1969; *Woodford and Sachs*, 1973; *Eugster*, 1978), wildebeest (*Eugster*, 1978) and zebra (*Young*, 1975a), while sample sizes from carnivores were generally too small to determine reliable prevalences. Exceptions are golden and black-backed jackals (*Canis aureus, Canis mesomelas*) with prevalences of up to 30%, whose infections were assumed to have been obtained from scavenging livestock carcasses or discarded offal because they were obtained in areas where large wild herbivores are now rare or extinct (*Macpherson et al.*, 1986). Following the description of *Echinococcus felidis* Ortlepp, 1937 from South African lions, an original sylvatic lifecycle involving wild animals was proposed, involving lions (and possibly other wild carnivores) and large herbivore species. Even after being synonymized with *E. granulosus* (Nelson and Rausch, 1963), this parasite continued to be recognized as a subspecies, or the ‘lion strain’, mainly due to its presence in a member of the Felidae. Subsequently, most records from African wild mammals were tentatively associated with this strain (*Macpherson and Wachira*, 1997), although the diagnostic validity of adult worm morphology has been questioned, and identification of the cyst stage was not possible at that time. Only recently could *E. felidis* be reinstated as a distinct species, based on molecular characterization of deposited worm material from South Africa and recent parasite eggs of lion origin from Uganda (Hüttner et al., 2008). Currently, the presence of *E. felidis* is confirmed in lions (Panthera leo) and spotted hyenas (Crocuta crocuta) from various conservation areas of Uganda and Kenya (Hüttner et al., 2009; Kagendo et al., 2014), but to date only one metacestode, from an Ugandan warthog (*Phacochoerus africanus*), has been identified as *E. felidis*.
Therefore, the host range of this species is far from clear, particularly concerning intermediate hosts. In addition, lions and spotted hyenas, long thought to be exceptional hosts for Echinococcus spp., are now known to be frequently infected with E. granulosus sensu stricto as well (Kagendo et al., 2014). Apart from E. felidis and E. granulosus s.s., there are other candidates that may infect African wildlife. Echinococcus ortleppi had been identified in an unspecified species of zebra from Namibia (Obwaller et al., 2004), and E. equinus (the “horse strain”) was morphologically identified from dogs that had been fed with cysts from mountain zebra (Equus quagga burchellii) and 6 oryx (Oryx gazella) were culled and examined for larval stages of Echinococcus spp. and other metacestodes in lungs, liver, kidneys, spleen and skeletal musculature. Metacestode size (diameter) was measured and suspected Echinococcus cysts were examined microscopically for the presence of protoscolices. Subsequently, samples were stored in 70% ethanol at room temperature.

Three additional cyst samples of E. equinus from horses, one from Germany (Blutke et al., 2010) and two from Italy (kindly provided by Antonio Varcasia), were available for comparative analysis.

2. Materials and methods

2.1. Study area

The study was undertaken from August to October 2012 in Etosha National Park in northern Namibia. The area has been under protection as a game reserve since 1907, and was proclaimed as a national park in 1967. The park in its current form was established in 1966 and covers a total of approximately 22,000 km² and includes the Kruger National Park, South Africa. It is one of the most important reserves in Africa for the conservation of large carnivores. Elephant and black rhinoceros are present in the park, notable exceptions being African wild dogs (Lycaon pictus) and buffaloes (Syncerus caffer).

2.2. Collection of parasites

Parasite samples were obtained in the context of a relocation programme, where 6 lions (P. leo), 6 leopards (Panthera pardus), 4 cheetahs (Acinonyx jubatus), 6 spotted hyenas (Crocuta crocuta), 7 black-backed jackals (Canis mesomelas) and 5 caracals (Caracal caracal) were caught and kept temporarily in individual confinement for quarantine purposes. All handling of animals was performed by or under direct supervision of the wildlife veterinarian responsible for Etosha National Park, ensuring compliance with animal welfare regulations.

A faecal sample was obtained from each animal, prior to deworming. Faecal samples were frozen at −80 °C for one week for safety reasons and thereafter stored at −20 °C until used. During the same period 12 plains zebras (Equus quagga burchellii) and 6 oryx (Oryx gazella) were culled and examined for larval stages of Echinococcus spp. and other metacestodes in lungs, liver, kidneys, spleen and skeletal musculature. Metacestode size (diameter) was measured and suspected Echinococcus cysts were examined microscopically for the presence of protoscolices. Subsequently, samples were stored in 70% ethanol at room temperature.

2.3. DNA amplification and sequencing

Taeniid eggs were isolated from faeces via zinc chloride flotation (Mathis et al., 1996). Single eggs were separated with a pipette and transferred within 1 μl H2O into 10 μl of 0.02 M NaOH. The larval stage material was treated similarly, single protoscolices or approximately equal sized pieces of cyst material were transferred into 10 μl of 0.02 M NaOH. The taeniid eggs or larval material were lysed at 95 °C for 10 min and used directly in the PCR (Nakao et al., 2003a).

DNA amplifications were performed by nested PCR. The target of the PCR was a 1073–1078bp long fragment including the complete nad1 (NADH dehydrogenase subunit 1) gene. Used primers for the first amplification were forward 5′-TGG ACG TCA TGT GTA GCT TTA CTA-3′, reverse 5′-ATA TCA AAG TAA CCT GCT ATG CAG-3′ and for the second forward PCR 5′-TAT TAA AAA TAT TGA GTT TGC GTC-3′, reverse 5′-ATA TCA AAG TAA CCT GCT ATG CAG-3′. The second PCR reaction mixture was identical to the first, except for the primer.

The PCR reaction mixture contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 200 μM of each dNTP, 12.5 pmol of each external primer, 1.25 U Ampli-Taq Polymerase (Applied Biosystems) and 1 μl of the egg or larval lysate. Subsequent to the first PCR a nested PCR was performed using 1 μl of the first amplification product as template. The second PCR reaction mixture was identical to the first, except for the primers. All thermal reactions were performed for 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 30 s) and extension (72 °C for 60 s).

In addition to the nad1 gene, the cox1 (cytochrome c oxidase subunit 1) gene was amplified via nested PCR and sequenced from 19 cyst samples with zebra origin. The ingredients for the PCR reaction mixture were identical to the nad1 PCR except for the primer. The primers for the first PCR were forward 5′-GTG GAG TTA CGT CTA ATT TIG-3′ and reverse 5′-TAC CAC YTA CTU ATC AC-3′. The primer pair of the nested PCR was forward 5′-TCA AGT TTA CGT CTA ATT TIG TGT CAT-3′ and reverse 5′-GCA TGA TGC AAA AGGCAA ATA AAC-3′ as published previously (Hüttenrath et al., 2008). The condition during amplification was 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 30 s) and extension (72 °C for 90 s).

Amplification products were purified by using High Pure PCR Product Purification Kit (Roche) according to the manufacturer’s instructions. The purified PCR products were sequenced (GATC, Konstanz, Germany).

2.4. Data processing

Complete mitochondrial genomes, that are available for 11 taxa of Echinococcus spp. (Le et al., 2002; Nakao et al., 2002, 2007) and for Taenia solium (Nakao et al., 2003b), served as comparative sequences (accession nos. AB018440, AB086256, AB208063–4,
were identified in two and one lion, respectively. One hyena
2000 (of sufficient length to allow species identification. Eggs of
ualcarnivore, as well as the number of eggs, which yielded sequences
othertaeniidegg.
were found in faeces of four out of six lions and two out of seven
jackals. No other
taeniid eggs were isolated from any
other carnivores. In addition, faeces from all jackals contained eggs
of E. equinus spp. sequence was obtained from any
other taeniid egg. Taenia regis and Hydatigera (=Taenia) taeniaformis
were identified in two and one lion, respectively. One hyena
harboured a species whose sequence conformed to ‘lineage II’ of
an Ethiopian spotted hyena, which had tentatively been assigned to
Taenia crocutae (Terefe et al., 2014). Additional sequences from
taeniid eggs of lion, hyena, cheetah and caracal origin clustered with
taxa of Taenia, but were different from any other sequences depos-
ited in GenBank. In addition, faeces from all jackals contained eggs
of Trichuris and unidentified nematodes, and leopard faeces con-
tained eggs of Spirometra sp. and unidentified nematodes as well
as coccidia.

Due to the small amount of DNA in individual eggs, nad1 and
cox1 sequences were mostly incomplete, and the material was insufficient
to amplify cox1. Therefore the isolates from carnivores were not
included in the haplotype analysis.

3.2. Herbivores

All of the twelve zebras examined contained cysts morpholo-
gically suspected to be Echinococcus metacestodes; 34 cysts were
recovered for further examination. For eleven of the zebras (27
cysts), infection with E. equinus could be molecularly confirmed
(no amplification product could be obtained from two calcified cysts
of the remaining animal) (Table 2). Of the 27 molecularly charac-
terized cysts, 23 were located in the liver and four in the lungs.
All lung cysts were small (1–3 cm) and sterile, while liver cysts
ranged in diameter from 1 to 8 cm (mean 3.3 cm), eight of them
sterile (mean 3.0 cm), eleven fertile (mean 4.2 cm) and four calci-
fied. The size of calcified lesions ranged from 0.5 to 8 cm, but
most of these lesions were not collected or gave no amplification
product. In four of the twelve zebras, liver cysts were fertile. Severe
pathology was not apparent in any of the zebras, and even in case
of multiple infections cysts occupied only a small proportion of
the liver (Fig. 1). Spontaneous death of the cysts (with subsequent
calcification) seems to be common and occurs at a comparatively
small cyst size.

No Echinococcus metacestodes were found in six oryx exam-
ined. In contrast to the zebras, which harboured no other taeniid
metacestodes, cysticerci of three T. equina spp. were found in five
of the six animals. Two species could be identified as T. regis (in three
oryx) and T. crocutae (in one oryx), the third species did not
conform to any deposited sequence and to any of the sequences
obtained from carnivores.

| Host species | Number of isolated taeniid eggs | Number of successful amplified and sequenced eggs | Identified species (n eggs) |
|--------------|---------------------------------|--------------------------------------------------|---------------------------|
| Panthera leo 1 | 18                              | 11                                               | E. equinus (11)           |
| Panthera leo 2 | 1                               | 0                                                | –                         |
| Panthera leo 3 | 49                              | 10                                               | E. equinus (7), H. taeniaformis (1), T. regis (2) |
| Panthera leo 4 | 36                              | 7                                                | E. equinus (5), T. regis (2) |
| Panthera leo 5 | 5                               | 2                                                | E. equinus (2)            |
| Panthera leo 6 | 18                              | 2                                                | Taenia spp. (2)           |
| Canis mesomelas 1 | 1                      | 1                                                | E. equinus (1)            |
| Canis mesomelas 2 | 6                              | 2                                                | E. equinus (2)            |
| Canis mesomelas 3 | –                              | –                                                | –                         |
| Canis mesomelas 4 | –                              | –                                                | –                         |
| Canis mesomelas 5 | –                              | –                                                | –                         |
| Canis mesomelas 6 | –                              | –                                                | –                         |
| Canis mesomelas 7 | –                              | –                                                | –                         |
| Crocuta crocuta 1 | 26                        | 8                                                | Taenia spp. (8)           |
| Crocuta crocuta 2 | 10                        | 4                                                | Taenia spp. (4)           |
| Crocuta crocuta 3 | –                              | –                                                | –                         |
| Crocuta crocuta 4 | –                              | –                                                | –                         |
| Crocuta crocuta 5 | –                              | –                                                | –                         |
| Crocuta crocuta 6 | –                              | –                                                | –                         |
| Caracal caracal 1 | 18                        | 6                                                | Taenia sp. (6)            |
| Caracal caracal 2 | 24                        | 5                                                | Taenia sp. (5)            |
| Caracal caracal 3 | –                              | –                                                | –                         |
| Caracal caracal 4 | –                              | –                                                | –                         |
| Caracal caracal 5 | –                              | –                                                | –                         |
| Panthera pardus 1 | –                              | –                                                | –                         |
| Panthera pardus 2 | –                              | –                                                | –                         |
| Panthera pardus 3 | –                              | –                                                | –                         |
| Panthera pardus 4 | –                              | –                                                | –                         |
| Panthera pardus 5 | –                              | –                                                | –                         |
| Panthera pardus 6 | –                              | –                                                | –                         |
3.3. Phylogenetic and haplotype analyses of Echinococcus equinus

Comparative analysis of the E. equinus sequences (nad1 and cox1 genes) showed only a small number of polymorphic sites. Five haplotypes could be identified within the 894bp long sequence of the complete nad1 gene of the Namibian samples (submitted to GenBank under accession numbers KP161211–KP161216). A sixth haplotype was represented by the horse samples from Italy, whereas the German haplotype (and the sequence of the UK reference) was also present among our Namibian samples. The 1608bp long cox1 gene showed less variance with only 4 haplotypes (accession numbers KP161207–KP161210), including the Italian and German isolates (Tables 2 and 3, Fig. 2). Cox1 and nad1 haplotypes showed a high degree of correlation, so the combination of both did not result in a drastic increase of haplotype numbers (six variants from Namibia and one additional from each Italy and UK) (Table 2). The combined (concatenated) haplotype sequences were used for the phylogenetic ML analysis (Fig. 3).

4. Discussion

Cystic echinococcosis, a zoonosis of worldwide distribution, is typically associated with domesticated animals. Dogs are the principal definitive host, and a wide range of livestock species (cattle, yak, water buffalo, sheep, goats, pigs, camels, horses and donkeys) carry the cystic metacestode stage (Cardona and Carmena, 2013).

### Table 2
Location, size, fertility status and E. equinus haplotype variants of individual cysts.

| Sample | Organ, cyst diameter, fertility status | Haplotype | Combined |
|--------|--------------------------------------|-----------|----------|
|        |                                      | nad1     | cox1     |          |
| Zebra 1| Cyst 1 Liver, 2.5 cm, sterile         | I         | I        | A        |
|        | Cyst 2 Liver, 1.5 cm, sterile         | III       | II       | B        |
|        | Cyst 3 Liver, 1.3 cm, sterile         | I         | I        | A        |
| Zebra 2| Cyst 1 Liver, 6.0 cm, sterile         | II        | II       | C        |
|        | Cyst 2 Lung, 3.0 cm, sterile          | II        | –        | –        |
|        | Cyst 3 Lung, 11.0 cm, sterile         | II        | –        | –        |
| Zebra 3| Cyst 1 Lung, 8.0 cm, sterile          | III       | I        | B        |
|        | Cyst 2 Liver, 8.0 cm, fertile         | II        | II       | C        |
|        | Cyst 3 Liver, 8.0 cm, fertile         | II        | II       | C        |
| Zebra 4| Cyst 1 Liver, 2.5 cm, fertile         | IV        | III      | E        |
|        | Cyst 2 Liver, 2.0 cm, fertile         | II        | II       | C        |
|        | Cyst 3 Liver, 2.0 cm, fertile         | V         | IV       | G        |
| Zebra 5| Cyst 1 Liver, 1.5 cm, calcified       | –         | –        | –        |
|        | Cyst 2 Liver, 1.5 cm, calcified       | IV        | II       | D        |
|        | Cyst 3 Liver, 1.0 cm, sterile         | –         | –        | –        |
|        | Cyst 4 Liver, 1.0 cm, calcified       | –         | –        | –        |
|        | Cyst 5 Liver, 1.0 cm, sterile         | –         | –        | –        |
|        | Cyst 6 Liver, 1.5 cm, calcified       | IV        | II       | D        |
| Zebra 6| Cyst 1 Liver, 5.0 cm, calcified       | II        | –        | –        |
| Zebra 7| Cyst 1 Liver, 1.0 cm, calcified       | III       | –        | –        |
|        | Cyst 2 Liver, 1.0 cm, calcified       | –         | –        | –        |
| Zebra 8| Cyst 1 Liver, 8.0 cm, calcified       | –         | –        | –        |
|        | Cyst 2 Liver, 6.0 cm, calcified       | –         | –        | –        |
| Zebra 9| Cyst 1 Liver, 4.0 cm, fertile         | III       | I        | B        |
|        | Cyst 2 Liver, –, fertile              | IV        | III      | E        |
|        | Cyst 3 Liver, –, fertile              | III       | –        | –        |
| Zebra 10| Cyst 1 Liver, 2.0 cm, sterile         | III       | I        | B        |
| Zebra 11| Cyst 1 Liver, 4.0 cm, fertile         | II        | II       | C        |
|        | Cyst 2 Liver, –, fertile              | II        | II       | C        |
|        | Cyst 3 Liver, –, sterile              | –         | –        | –        |
| Zebra 12| Cyst 1 Liver, –, sterile              | III       | I        | B        |
| Germany| Cyst 1                              | III       | I        | B        |
| Italy | Cyst 1                              | VI        | III      | F        |
|        | Cyst 2                              | VI        | III      | F        |
| UK     | Ref. seq.                           | IV        | UK       | UK       |

### Table 3
Positions of nucleotide substitutions within the nad1 and cox1 gene of E. equinus.

| nad1 gene | cox1 gene |
|-----------|-----------|
| bp position | 321 | 381 | 818 | 695 | 888 | 393 | 1165 | 1455 | 1572 |
| NC_020374 | G   | T   | A   | T   | C   | G   | G   | T   | A   |
| Haplotype P | A   | T   | A   | T   | T   | Haplotype P | G   | A   | C   | G   |
| Haplotype NH | G   | T   | A   | C   | C   | Haplotype NH | G   | A   | C   | A   |
| Haplotype III | G   | T   | A   | C   | T   | Haplotype III | G   | A   | T   | A   |
| Haplotype IV | G   | T   | A   | T   | C   | Haplotype IV | T   | A   | C   | G   |
| Haplotype V | G   | C   | A   | T   | C   | Haplotype V | G   | T   | G   | T   |

Numbers indicate the position beginning from the start codon.
N = Namibia, G = Germany, I = Italy, UK = United Kingdom.
* Identical to reference sequence NC_020374.
In addition to this, a large number of wild mammal species are known to be suitable hosts, either being involved in primary or secondary “sylvatic” lifecycles, or as accidental hosts due to spill-over from domestic transmission (Carmena and Cardona, 2014). Occasional spill-over may occur wherever the parasite is present in dogs and livestock, and in some cases secondary cycles, involving wild mammals only, became established after anthropogenic introduction of the parasite, e.g. in Australia and possibly in eastern Africa (Jenkins and Morris, 2003; Kagendo et al., 2014). However, sylvatic lifecycles, which are assumed to be primary, i.e. having existed before the domestication of livestock and the subsequent dispersal of their parasites, appear to be restricted to two regions. One is the temperate to subarctic part of the northern hemisphere, with wolves and various cervids (particularly moose) as hosts (Rausch, 1995; Nakao et al., 2013b; Schurer et al., 2013), the other is sub-Saharan Africa, from where many species of large carnivores and ungulates have been recorded as hosts (Macpherson and Wachira, 1997; Hütten and Romig, 2009).

Here we report the presence of *E. equinus* in lions, black-backed jackals and plains zebras in northern Namibia. We think the transmission of the parasite in this region to be a genuine sylvatic lifecycle – without any involvement of domesticated animals – because domestic dogs, horses or donkeys are not present in the park or in the vicinity, and movement of animals in or out of the park is restricted by fencing. The small sample size of each animal species examined does not allow reliable prevalence estimates, but the high proportions of infection confirmed in lions (four of six) and zebras (eleven of twelve) strongly suggest a stable endemic presence of *E. equinus* in this ecosystem. Four of the twelve zebras harboured fertile cysts, but these cysts were small (mean diameter 4.2 cm). A large number of cysts were sterile (n = 12), and calcified cysts were also present, ranging in size from <1 cm to 8 cm. Thus, our data on cyst size and condition contrast with the description of *E. equinus* cysts from three Italian horses, all of which contained large, fertile cysts; small sterile or degenerated cysts were present in an additional three horses, but these cysts were identified as *E. granulosus* s.s. (Varvasia et al., 2008). Our cyst size and fertility data also contrast with infection data from Tunisian donkeys, whose cysts were invariably fertile whether belonging to *E. equinus* (n = 22) or *E. granulosus* s.s. (n = 13) (Boufana et al., 2014).

The parasite was completely absent in six oryx from the same area, a species, which is often seen grazing in mixed groups with zebras. A basic lifecycle between lions and zebras would therefore be highly plausible, with the jackals to become infected through scavenging on zebra carcasses killed by larger predators or by diseases (e.g. anthrax – Beyer et al., 2012). While a large number of other taeniid eggs were found in the carnivores examined, the absence of *Echinococcus* spp. was conspicuous. Unfortunately, our small sample does not allow definite conclusions on the absence of other *Echinococcus* taxa from Etosha National Park. However, even based on these limited data, the epidemiological situation appears fundamentally different from that in eastern Africa, where lions and spotted hyenas from Ugandan and Kenyan national parks and reserves were found to be frequently infected with *E. felidis* and *E. granulosus* s.s., but not with *E. equinus* (Hüttner et al., 2009; Kagendo et al., 2014). Previous records of echinococcosis in the Etosha National Park are restricted to one out of six examined giraffes (*Giraffa camelopardalis*) having cyst(s) of unreported fertility status and location (Krecek et al., 1990). The identity of this specimen cannot be established, but it raises the question of infectivity of *E. equinus* for giraffes. As the large mammal faunas of eastern and southern Africa are extremely similar, the differences of *Echinococcus* spp. lifecycles between Etosha National Park and East African conservation areas cannot be explained. Whether the situation in the Etosha ecosystem is representative for the region, or artificial due to a rather recent population bottleneck of host animals and their inhibited migratory movements (see below), is currently under investigation in similar studies in other parts of Namibia and southern Africa.

Black-backed jackals have not previously been reported before as host for *E. equinus*, but their host competence – as canids – is not surprising; numerous records of *Echinococcus* infections of this and
other species of jackals are reported from Africa (Macpherson and Wachira, 1997). Lions, however, have long been considered suitable hosts for *E. felidis* only, and the specific identity of *E. felidis* (as species, subspecies or strain) had for some time been justified by its adaptation to a felid as definitive host. Records of gravid infections with *E. granulosus* s.s. in Kenya have already shown that lions are competent host for a wider range of *Echinococcus* species, and our findings of *E. equinus* support this conclusion. Interestingly, leopards (*P. pardus*) and cheetahs (*A. jubatus*), whose prey range overlaps with that of lions, have neither in this nor in previous studies been positively identified as carriers of *Echinococcus* spp. (Macpherson and Wachira, 1997; Hüttner et al., 2009; Kagedondo et al., 2014). Therefore susceptibility to *Echinococcus* may be a specific feature of lions rather than other species of large cats.

*E. equinus* is considered to be globally distributed and specific for Equidae as intermediate hosts (although a captive primate has recently been found infected in the UK – Boufana et al., 2012). However, *Echinococcus* cysts in equids may also belong to other species, which makes molecular confirmation necessary (Varvasi et al., 2008; Boufana et al., 2014). So far, *E. equinus* has only been confirmed from domestic horses or donkeys in the United Kingdom (UK), Ireland, Germany, Italy, Spain, Tunisia and Egypt, and a dog in Kyrgyzstan (Bowles et al., 1992; Mwambete et al., 2004; Ziadepov et al., 2008; Blutke et al., 2010; Aboelhadid et al., 2013). With the exception of a captive zebra (*E. quagga*), born and raised in a UK zoo (Boufana et al., 2012), no molecular confirmation of *E. equinus* infection for any wildlife host has been previously reported. However, *E. equinus* was morphologically identified from worm material of dogs that had been fed cysts from mountain zebras (*E. zebra*) of unknown origin in Namibia (Kumaratilake et al., 1986). This latter record raises the question of the geographic extent of this sylvatic lifecycle in southern Africa. Etosha National Park has been entirely fenced for 40 years, so migration of larger mammals is restricted. The fence is not impenetrable, however, and a lion population to the west of Etosha, coexisting with mountain zebra, is believed to have originated from the park. Also, dispersal of the parasite could occur with smaller hosts (e.g. jackals). While the Etosha region is the southern and western limit of the plains zebra’s distribution, the northern subspecies of mountain zebras (*E. zebra hartmannae*) is much more widespread in Namibia, suggesting a wider distribution of *E. equinus*. In addition, 60% of plains zebras of the Kruger National Park in northern South Africa were found infected with *Echinococcus* cysts, which were successfully used in experimental infections of lions (Young, 1975a, 1975b). While this does not prove the existence of *E. equinus* in the Kruger National Park, it is certainly suggestive that this sylvatic lifecycle of *E. equinus* could be widespread at least in the northern parts of southern Africa.

There is no evidence to indicate this lifecycle is primary, i.e. having existed before the introduction of domestic animals into the region, or is the result of secondary establishment due to host switching from domestic dog–horse (or dog–donkey) transmission into wildlife. Horses, mules and donkeys, imported from Europe, were kept in large numbers in southern Africa until the early 20th century, and an occasional transmission from dogs to zebras or from horses to lions would not be improbable. Also, when considering the present distribution of the parasite, dynamic changes in the presence of wildlife over time has to be taken into account. Due to excessive hunting, the Etosha region was depleted of larger wild animals from the 1880s until after 1907, when the area became a game reserve and when animals (and their parasites) migrated back from elsewhere (Berry, 1997). Our panel of Namibian isolates showed some polymorphic sites on the mitochondrial cox1 and nad1 genes. Only a few samples from Italy (2), Germany (1) and UK (reference sequence) were available for comparison of the complete gene sequences used. The Italian cox1 haplotype, but not the nad1 haplotype, was represented in our Namibian panel. The reverse situation was found for the UK isolate, while the German haplotypes of both genes were present in the same combination in Namibia. The latter finding is intriguing (given the colonial history), but any conclusions on possible introduction routes cannot be drawn based on these few samples. In the only other study with a good sized panel of *E. equinus* samples (22 isolates of donkey origin from Tunisia) no polymorphism of partial cox1 and ef1a gene sequences was found (Boufana et al., 2014). The partial cox1 sequence of the Tunisian samples (acc. No. KF014645) is present in our haplotypes I, II and III. In summary, our samples do not provide evidence that *E. equinus* in Namibian wildlife shows unique haplotype patterns when compared to “domestic” isolates worldwide, or show differences in levels of diversity. To place the Etosha samples in a global context, a far larger number of isolates will have to be examined from other parts of southern Africa and of the world.

While this study focussed on *Echinococcus*, eggs or metacestodes of other taeniid species were found in lions, cheetahs, caracals, spotted hyenas and oryx. Of twelve taxa distinguished based on nad1 sequences, only *T. regis* (lions and oryx), *H. taeniaeformis* (lion) and *T. cf. crocutae* (“lineage II” of Terefe et al., 2014, in spotted hyena and oryx) could be identified to species level. The sequences of 9 other taxa clustered with *Taenia* spp., but did not give sufficient degrees of homology to allocate them to any molecularly characterized species. Only very few sequences are known from any sub-Saharan wildlife *Taenia*, and both molecular and morphological examinations indicate that the species diversity is far higher than previously assumed (Loos-Frank, 2000; Terefe et al., 2014). We have not attempted to discuss these preliminary *Taenia* data here, as a more comprehensive study on this genus in African wildlife is in progress.

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**Conflict of interest**

The authors declared that there is no conflict of interest.

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