PCR amplification and DNA sequence identification of an unusual morphological form of *Demodex cati* in a cat

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**Background** – Molecular characterization of *Demodex* mites is being used to identify mite species in dogs. This technique is now being applied to cat *Demodex* species, allowing for better characterization of the mites.

**Hypothesis/Objectives** – Molecular diagnostics will clarify the existence of diverse *Demodex* mites identified morphologically.

**Animals** – A cat with generalized demodicosis secondary to chronic steroid treatment for erythroid dysplasia.

**Methods** – Skin scrapings demonstrated large numbers of follicular mites consistent with *Demodex cati* as well as a morphologically different *Demodex* mite with a blunted abdomen. The 16S rRNA DNA was amplified by PCR, sequenced and compared with available *Demodex* sequences, including *Demodex cati*, *Demodex gatoi* and an unnamed *Demodex* sp.

**Results** – A single PCR product was obtained, the DNA sequence of which was an exact match with *D. cati*.

**Conclusions and clinical importance** – The shorter unnamed mite was not a different species in this case, but a different morphological form of *D. cati*. This report demonstrates the utility of molecular diagnostics to clarify the identity of mites that differ morphologically.

**Introduction**

Feline demodicosis is a parasitic condition affecting the skin of cats. Three mites, *Demodex cati*, *Demodex gatoi* and an unnamed mite, have been described morphologically and, in one case, characterized by molecular diagnostics.1–5 Recently, molecular studies of canine *Demodex* mites revealed *Demodex cornei*, a short-bodied mite morphologically distinct from *Demodex canis*, to be genetically indistinct from *D. canis*,6,7 emphasizing the importance of molecular characterization. The purpose of this case report is to describe molecular characterization of two morphologically distinct feline *Demodex* mites.

**Case report**

An 8-year-old male castrated domestic short hair cat was presented with the complaint of progressive alopecia of 3 months duration. The cat had a history 3 years prior of progressive anaemia and moderate pancytopenia that had responded poorly to prednisone and ciclosporin. PCR testing was negative for *Mycoplasma* spp.; tests for tick-borne diseases, feline leukaemia virus and feline immunodeficiency virus were negative. Bone marrow core biopsies from the ileum demonstrated erythroid hyperplasia, with dysplasia and incomplete maturation as well as myeloid and megakaryocytic hypoplasia. There was no evidence of lymphoid neoplasia. A diagnosis was made of erythroid dysplasia with myelodysplastic syndrome. The disease was treated with oral dexamethasone and epoetin alpha (Epogen®; Amgen, Thousand Oaks, CA, USA). At the time of presentation, the cat was receiving 0.25 mg (0.04 mg/kg) dexamethasone daily.

Dermatological examination revealed marked alopecia over the entire body, with easy epilation of hairs (Figure 1). The cat was not observed to be pruritic. A trichogram and skin scrapings both revealed large numbers of *Demodex* mites, and two morphological forms were noted. Long, slender mites consistent with

![Figure 1. Lateral trunk of an 8-year-old male castrated domestic short hair cat diagnosed with two morphological forms of feline demodicosis.](image-url)
D. cati were identified along with mites with blunted abdomens, longer than D. gatoi but shorter than D. cati (Figure 2). These mites were identified with the previously reported morphologically distinct Demodex. Scraped samples placed on slides in mineral oil contained both morphological forms. Material from scraped samples in mineral oil and scraped samples placed into a sterile tube underwent molecular analysis to determine whether the unidentified mite population represented a new unnamed species or a morphological variant of D. cati.

The cat was treated with weekly topical application of 80 mg moxidectin/imidacloprid (Advantage multi for cats; Bayer HealthCare LLC, Shawnee Mission, KS, USA) between the scapulae. Survey skin scrapings were performed monthly, and negative skin scrapings were achieved after 4 months. Dexamethasone oral therapy was decreased to every other day. Due to the ongoing steroid therapy, treatment for demodicosis was continued even after two negative consecutive monthly skin scrapings. PCR amplification parameters were 95 °C for 30 s and 68 °C for 5 min. The PCR product was visualized on an agarose gel and treated to remove primers. It was sequenced using the forward and reverse PCR primers at the Molecular Biology Resource Facility (University of Tennessee, Knoxville, TN, USA). The sequences were analysed using commercial software (Lasergene 10, SeqMan Pro; DNASTar Inc., Madison, WI, USA). BLAST was used to compare the sequence with all entries available in GenBank.

PCR amplification of DNA extracted from putative Demodex mites produced a single band of ~330 bp. A DNA sequence without any background was produced, suggesting the presence of a single amplified product. The product matched 100% with D. cati deposited in GenBank (JX193759) over the entire region that was compared. This corresponded to bases 1–329 relative to JX193759.

**Discussion**

Feline demodicosis is an uncommon dermatological disease of cats. Generalized demodicosis due to D. cati is usually associated with underlying immunosuppression, either endogenous or iatrogenic. In this case, the condition was presumed to be caused by chronic exogenous steroid administration.

To date, three distinct Demodex mites in cats have been described based on morphological criteria. Molecular techniques have been employed to identify canine Demodex mite species precisely. In two studies, morphologically distinct mites, the long-bodied mite (D. canis) and one with a short, blunted abdomen (D. cornei), were determined both to be D. canis based on molecular characterization. Recently, molecular techniques have been used for detection and differentiation of feline Demodex mites.

In the present case, the usefulness of molecular characterization of feline Demodex mites was demonstrated. Morphological evaluation suggested that there were two different species of mites, a long-bodied mite and one with a blunted abdomen that was intermediate in length between D. gatoi and D. cati. The authors suspected this to be the third unnamed species. It was not possible to separate the mites physically, so they were processed together, from both the dried scrapings of material and the mites in mineral oil. PCR yielded a single sequence of D. cati. It is possible that the primer set used in this study that amplified D. cati 16S rRNA DNA may fail to amplify DNA from other species of Demodex infecting the cat. However, the primers used are highly conserved among Demodex species, including D. gatoi, D. caprae, D. brevis, D. folliculorum, D. canis, D. injai and an unnamed species affecting cats. These include all Demodex species for which 16S rRNA gene sequences are currently available in GenBank. Thus, in this case, the authors showed that the mite with the blunted abdomen was likely not to be a distinct species but rather a morphologically different form of D. cati, similar to what has been described in the dog, with D. cornei shown to be a variant of D. canis rather than a distinct species. It is likely that as this technique is applied further, more mite species will be identified. This molecular characterization of the mites will aid in determining ancillary diagnostics and treatment approaches for each case, because underlying causes may differ.
References

Hypothese/Ziele – Die molekulare Diagnostik wird das Vorkommen verschiedener Demodex Milben, die morphologisch identifiziert werden können, verdeutlichen.

Tiere – Eine Katze mit generalisierter Demodikose, die sekundär nach chronischer Steroidbehandlung wegen erythroider Dysplasie auftrat.

Methoden – Hautgeschabsel zeigten eine große Anzahl von Follikelmilben, die wie Demodex cati aussahen, sowie eine morphologisch unterschiedliche Demodex Milbe mit einem stumpfen Bauch. Die 16S rRNA DNA wurde mittels PCR amplifiziert, sequenziert und mit vorhandenen Demodex Sequenzen, die Demodex cati, Demodex gatoi und eine unbenannte Demodex sp beinhalteten, verglichen.

Ergebnisse – Es wurde ein einziges PCR Produkt gewonnen, deren DNA Sequenz mit der von D. cati exakt übereinstimmte.

Schlussfolgerungen und klinische Bedeutung – In diesem Fall war die kürzere unbenannte Milbe nicht eine unterschiedliche Spezies, sondern eine unterschiedliche morphologische Form von D. cati. Dieser Bericht zeigt die Verwendung von Molekulardiagnostik, um die Identität von Milben abzuklären, die sich morphologisch unterscheiden.

要約
背景 - ニキビダニの分子生物学的特性は、ニキビダニの種を特定するために利用されている。この方法は、ニキビダニの種に対する検査を可能にし、これにより、ニキビダニの存在が明らかになる。

仮説/目的 - 分子学的な診断法により形態学的に識別された様々なニキビダニの存在が明らかになる。

供与動物 - 赤血球異形成のために長期的なステロイド治療で二次的な全身性毛包黴症を生じた1頭のネコ

方法 - 皮膚検査検査により Demodex catiと一致する多数の毛包内ダニ、形態学的に異なる長い腹部を持ったニキビダニが検出された。16S rRNA DNAをPCRで増幅し、配列を解析して、Demodex cati、Demodex gatoiならびに名前のつけられていな Demodex sp を含む利用可能な毛包黴の配列と比較を行った。

結果 - 1種類のPCR産物が得られ、そのDNA配列はD. catiと完全に一致していた。

結論および臨床的困難 - この症例において、名前のつけられていな短いダニは異なる種ではなかったが、D. catiと形態学的に異なっていた。この報告は形態学的に異なるダニの正体を明らかにするための分子生物学的診断法の有用性を示す了えた。