Research Updates

Diamond mine or gold mine? Using eDNA to identify golden moles along the west coast of South Africa

Introduction

Golden moles (Chrysochloridae) represent one of the most threatened mammal groups in southern Africa (IUCN, 2021). The west coast of South Africa is an important but under-surveyed part of their range. Vast stretches of the coastal dune habitats have been severely impacted by alluvial diamond mining over the past century (Fig. 1), and rapidly developing residential areas and agriculture have continued to transform large sections of these habitats, particularly over the past two decades (Fig. 1a,b). This has created concern among conservationists for the conservation status and continued survival of golden mole species in these dune ecosystems. In spite of the prevailing diamond mining activities, we are interested in finding a shimmer of the "living gold" of the Chrysochloridae that potentially still exists here, and when we do, devising plans to protect it.

Figure 1. Mining and residential development on the west coast. Jacobsbay in 2003 **(a)** and 2021 **(b)** illustrating the increase in residential development (Source Google Earth). **c)** Approximate distribution of golden moles along the west coast. **d)** Habitat destruction resulting from diamond mining in the area north of Port Nolloth. Photos: Samantha Mynhardt.

In 2019 the Endangered Wildlife Trust's Drylands Conservation Programme (DCP) partnered with the University of Pretoria (UP) to investigate the presence and distribution of two of the most elusive golden moles, namely the Critically Endangered De Winton's golden mole (*Cryptochloris wintoni*) and the Endangered Van Zyl's golden mole (*Cryptochloris zyli*) (IUCN, 2021). Golden moles are considered highly elusive due to their behavioural ecology and the fact that they are exclusively subterranean. In the case of these coastal species, this is exacerbated by the fact that their foraging burrows, otherwise typically visible on the surface of the ground, do not remain visible in sand, especially in the windy coastal conditions characterising the west coast.

Detection and distribution mapping of elusive species is one of the major challenges of biodiversity surveys, which are crucial for the successful implementation of large-scale conservation efforts. For terrestrial mammals in particular, live trapping is still a common detection strategy, but may be invasive, labour-intensive and generally requires onerous permitting. In addition, the type of trap, bait and sampling design can strongly affect the detection probability of particular species (Bovendorp et al., 2017; Harkins et al., 2019). Camera trapping is increasingly used, but this approach is also labour-intensive and costly, and may require long-term coverage and demand substantial maintenance and post-processing (Burton et al., 2015). Camera trapping would also not be ideally suitable for detecting moles "swimming" sub-surface in dunes.

Terrestrial environmental DNA (eDNA) is poised as an effective alternative to existing monitoring approaches (Deiner et al., 2017; Leempoel et al., 2020). For animals, the premise of eDNA is that pieces of skin, hair, faeces or saliva are shed in the environment and that, by collecting environmental samples such as water or soil, we should be able to identify to which species the extracted DNA belongs (Leempoel et al., 2020). eDNA techniques have developed rapidly over the past decade, and particularly in the past three years (Rodríguez-Ezpeleta et al., 2021), and are now increasingly applied to aquatic samples (e.g. Dugal et al., 2021; Port et al., 2016; Ushio et al., 2017), and a handful of primarily proof-of-concept studies exist in which soil samples are used as a source of eDNA (Andersen et al., 2012; Leempoel et al., 2020; Taberlet et al., 2012). Since golden moles are morphologically cryptic, precluding the possibility of visual species identification, eDNA presents an opportunity for simultaneously detecting presence and identifying species.

We hope that by employing eDNA techniques to detect golden moles we could push the envelope on the use of terrestrial eDNA from soil (as opposed to aquatic eDNA) and that finding the golden moles, in particular De Winton's, would position these species as flagships for a renewed conservation focus on this coastline.

Our ongoing project

We have conducted two independent expeditions to the west coast to collect eDNA samples in the hope of locating and identifying golden moles from this region and learning more about the threats they face. The team consisted of Samantha Mynhardt (UP) and Cobus Theron (DCP), Jean-Pierre le Roux (DCP) and Esther Matthew (DCP), as well as Jessie, our trusted scent-detection dog (Fig. 2).

Our first trip was to Lambertsbay (Fig. 1), which represents the type locality for van Zyl's golden mole, and one of only two sites at which this species has been recorded (the other site being Groenriviermond, some 150 km further north along the coast; see Fig. 1)(Bronner & Asher, 2016b; Helgen & Wilson, 2001; Taylor et al., 2018). Lambertsbay also overlaps with the currently accepted distribution ranges of the Cape golden mole (*Chrysochloris asiatica*) and Grant's golden mole (*Eremitalpa granti*)(Taylor et al., 2018). The second trip was aimed at Port Nolloth and the adjacent stretches of coastline that have been impacted by diamond mining. Port Nolloth represents the type locality for De Winton's golden mole, known from only this locality, and which has not been recorded for more than 80 years (last recorded in the wild in 1937)(Bronner & Asher, 2016a). Although, categorized as Critically Endangered (IUCN, 2021), it is yet to be determined whether this assessment is due to extinction owing to threats posed by mining and habitat destruction, or simply due to the challenges associated with locating and identifying these animals.

Figure 2. The golden mole research team, left to right: Esther Matthew, Cobus Theron holding Jessie, our scent detection dog, Samantha Mynhardt and Jean-Pierre le Roux. The project is funded by Re:wild. Photo: Nicky Souness.

We found surprisingly high levels of golden mole activity in both regions of the west coast, although the effects of habitat transformation on the diamond mine sites were evident, and significantly less activity was found at these mining sites. We collected soil samples for eDNA from golden mole burrows wherever we found signs of recent activity, and also captured one specimen at Lambertsbay, through extensive trapping efforts, for the purpose of matching morphological characteristics to genetics (Fig. 3).

Figure 3. The golden mole specimen captured (and released) at Lambertsbay. Photo: Jean-Pierre le Roux.

Soil eDNA samples were collected by scraping soil from the inner walls of subsurface foraging burrows, wherever these were found to be intact, and from loose soil approximately 2cm below the surface (the depth at which moles typically burrow when foraging), wherever burrow traces were found in soft sand, which collapses as the moles move through it (Fig. 4). The soil samples are now in the laboratory at the University of Pretoria, where we are conducting eDNA extraction, following the methods of Taberlet *et al.* (2012) and Leempoel *et al.* (2020), and have already seen preliminary success in extracting, amplifying and sequencing mammalian eDNA.

Figure 4. Soil eDNA samples were collected by excavating subsurface foraging burrows by slicing neatly through them with a spade and scraping soil from the inner walls, wherever these were found to be intact. Photos: Endangered Wildlife Trust.

We aim to amplify two mitochondrial genes and one nuclear intron in each of the 100+ soil samples collected, using universal mammal primers that will facilitate amplification of all mammalian DNA in the samples, and thus enable identification of other mammalian species in addition to the golden moles. We will send these amplification products (amplicons) for next-generation amplicon sequencing at the Central Analytical Facility (CAF) at Stellenbosch University. This approach involves pooling multiple libraries (samples tagged with unique identifiers) onto a single sequencing chip. Since there is a cost involved in the tagging of libraries, the most cost-effective approach is to pool multiple samples from a single sampling site into a single library. Although this will not complicate species identification, since all unique sequences, and therefore all the mammalian species in the pooled library, will be individually detectable, the drawback to this approach is that we would not be able to pinpoint the exact GPS locations of samples that test positive for presence. However, the way that libraries have been structured will provide us with good insights into which regions require further investigation.

Figure 5. a) Moisture on the surface of the soil greatly enhances detectability of golden mole burrows, which leave a noticeable ridge on the surface. **b)** Surface ridges are less visible in soft dry sand, which collapses as moles move through it, although slightly deeper burrows, such as this one, may still remain intact. Photos: Samantha Mynhardt.

The time we have spent in these dune environments has really revealed to us the harshness of the habitat and the challenges that it holds for golden mole research and conservation. As a team, we found that moisture, either in the form of rain or early-morning dew, played a crucial role in our ability to detect golden mole activity. Moist sand results in higher visibility of subsurface foraging burrows, and also facilitates targeted eDNA collection, since burrows can be excavated and scraped rather than collecting soil from the approximate vicinity of the burrow (Fig.5).

The fact that very few pristine sites were encountered on either of the expeditions is disconcerting and points to an urgent need to firm up conservation planning and interventions for these species. Furthermore, we found that general public perceptions around golden moles are still highly prejudicial and persecutory; these perceptions often relate to the perceived damage that the sub-surface foraging burrows cause to lawns, particularly on golf courses, and the reaction is typically to seek out means to "eradicate" the animals. These attitudes need to be reversed in order to protect the species better.

Conservation impact

While we await sequencing results, it is clear that the eDNA technique is a potential game changer in the search for cryptic species. The fact that more than 100 good quality samples were collected in less than two weeks is a vast improvement over conventional approaches. The non-invasive nature of the approach is also preferable when working with critically endangered species.

Current land uses and ownership patterns are important considerations that will greatly impact the kind of conservation options that can be considered. We have surveyed across public lands, state land, private land and communal land. The variation in land use and ownership may mean that different models will need to be applied across the west coast.

Options for conservation of habitat would include: declaration of land as contract nature reserve under the provincial biodiversity stewardship process; Conservation Agreements; conservation servitudes; outright purchase or leasing of sites for conservation. In addition, there are also a number of actions we can promote amongst members of the public to ensure better protection of golden moles. These include: keeping domestic dogs leashed on beaches with high mole activity; highlighting the ecological importance of moles in gardens; discouraging the use of poisons in gardens; encouraging the establishment of indigenous gardens; providing more concise inputs in environmental processes concerning coastal developments and mining applications.

Conclusion

As a result of using eDNA, we have developed a highly efficient and effective way of detecting and identifying golden moles and mapping their distribution. This will facilitate planning of conservation action for the ecosystems in this region and for these species. The development of the approach and techniques have also pushed the boundaries of eDNA in applied conservation.

While there is a very good chance that follow-up work will be required for the eDNA study, the progress made in discovering the moles of the west coast is beyond our expectations. Some of our preliminary results already point towards a much more nuanced understanding of the species present on the coast.

References

Andersen, K., Bird, K. L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjær, K. H., . . . Willerslev, E. (2012). Metabarcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular Ecology, 21*(8), 1966-1979.

Bovendorp, R. S., MCCleery, R. A., & Galetti, M. (2017). Optimising sampling methods for small mammal communities in Neotropical rainforests. *Mammal Review, 47*(2), 148-158.

Bronner, G., & Asher, R. (2016a). A conservation assessment of Cryptochloris wintoni. In M. Child, L. Roxburgh, E. Do Linh San, D. Raimondo, & H. Davies-Mostert (Eds.), *The Red List of Mammals of South Africa, Swaziland and Lesotho*. South Africa: South African National Biodiversity Institute and Endangered Wildlife Trust.

Bronner, G., & Asher, R. (2016b). A conservation assessment of Cryptochloris zyli. In M. Child, L. Roxburgh, E. Do Linh San, D. Raimondo, & H. Davies-Mostert (Eds.), *The Red List of Mammals of South Africa, Swaziland and Lesotho*. South Africa.: South African National Biodiversity Institute and Endangered Wildlife Trust.

Burton, A. C., Neilson, E., Moreira, D., Ladle, A., Steenweg, R., Fisher, J. T., . . . Boutin, S. (2015). REVIEW: Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology, 52*, 675-685.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., . . . Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology, 26*(21), 5872-5895.

Dugal, L., Thomas, L., Reinholdt Jensen, M., Sigsgaard, E. E., Simpson, T., Jarman, S., . . . Meekan, M. (2021). Individual haplotyping of whale sharks from seawater environmental DNA. *Molecular Ecology Resources*. doi:10.1111/1755-0998.13451

Harkins, K. M., Keinath, D., & Ben-David, M. (2019). It's a trap: Optimizing detection of rare small mammals. *PLOS ONE, 14*(3), e0213201.

Helgen, K. M., & Wilson, D. E. (2001). Additional material of the enigmatic golden mole Cryptochloris zyli, with notes on the genus Cryptochloris (Mammalia: Chrysochloridae). *African Zoology, 36*(1), 110-112.

IUCN. (2021). The IUCN Red List of Threatened Species Version 2021-1. 1. Retrieved from http://www.iucnredlist.org

Leempoel, K., Hebert, T., & Hadly, E. A. (2020). A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. *Proceedings of the Royal Society B: Biological Sciences, 287*(1918), 20192353.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., . . . Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology, 25*(2), 527-541.

Rodríguez-Ezpeleta, N., Zinger, L., Kinziger, A., Bik, H. M., Bonin, A., Coissac, E., . . . Taberlet, P. (2021). Biodiversity monitoring using environmental DNA. *Molecular Ecology Resources,* 21(5), 1405-1409

Taberlet, P., Prud'homme, S. M., Campione, E., Roy, J., Miquel, C., Shehzad, W., . . . Coissac, E. (2012). Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology, 21*(8), 1816-1820.

Taylor, W. A., Mynhardt, S., & Maree, S. (2018). Family Chrysochloridae (Golden Moles). In R. A. Mittermeier & D. E. Wilson (Eds.), *Handbook of the Mammals of the World – Volume 8 Insectivores, Sloths and Colugos* (Vol. 8). Barcelona, Spain: Lynx Edicions

Ushio, M., Fukuda, H., Inoue, T., Makoto, K., Kishida, O., Sato, K., . . . Miya, M. (2017). Environmental DNA enables detection of terrestrial mammals from forest pond water. *Molecular Ecology Resources, 17*(6), e63-e75.

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