

The use of visible implant elastomer to permanently identify caecilians (Amphibia: Gymnophiona)

BENJAMIN TAPLEY^{1*}, CHRISTOPHER J. MICHAELS¹, DAVID J. GOWER² & MARK WILKINSON²

¹Zoological Society of London, Regents Park, London NW1 4RY, UK

²The Natural History Museum, Cromwell Road, London, SW7 5BD, UK

*Corresponding author e-mail: ben.tapley@zsl.org

ABSTRACT - Identifying individual animals is important for studying populations and for the optimal management of individual animals in captivity. In the absence of natural markings that discriminate individuals, such identification may require animals to be marked by researchers. Amphibians are challenging subjects to mark due to their small size and sensitive, permeable and frequently shed skin. Visible Implant Elastomer (VIE) has been widely used to mark amphibians, but no long-term study has validated this technique in caecilian amphibians. We anaesthetised and attempted to VIE mark seven *Herpele squalostoma* and one *Microcaecilia unicolor* held at ZSL London Zoo. No specimens suffered ill effects of anaesthesia or VIE injection, but mean persistence of marks was 191 days in *H. squalostoma* suggesting that this marking technique is not suitable for identifying individuals of this species in the long-term. We were unable to inject VIE into the *M. unicolor* and/or the elastomer was not visible through the darkly pigmented skin. Further research is required to develop methods for long-term marking of a diversity of caecilians.

INTRODUCTION

Identifying individual animals facilitates the estimation of population size, and understanding population dynamics, home range, longevity and numerous other life history, ecological and biological characteristics (Donnelly et al., 1994). The identification of individuals is also important in the management of captive animals in laboratory, zoo and private settings. Furthermore, it may help understand and police the illegal wildlife trade (Buhlmann & Tuberville, 1998). Individuals of some amphibian species have distinctive naturally occurring markings that may aid in discriminating individuals and in their reidentification over time. Identifications based on photographic records of natural markings are minimally invasive and have been used to identify individual amphibians in all three extant amphibian orders (Hagström, 1973; Bailey, 2004; Kramer et al., 2001; Bradfield, 2004; Kenyon et al., 2009). However, many amphibians do not have distinctive or temporally stable individual markings (Wengert & Gabriel, 2006; Kraus & Allison, 2009), and in such cases more invasive marking techniques may need to be adopted (see review by Ferner, 2007). Amphibians are particularly challenging candidates to mark due to their relatively small size, the permeability and sensitivity of their frequently shed skin and their often complex life cycles (Heemeyer et al., 2007).

Caecilians (Gymnophiona) are elongate limbless amphibians (see Wilkinson, 2012 for an introduction). They can be difficult to study because most species burrow in soil (Gower & Wilkinson, 2005) and their limblessness and fossoriality means that they present fewer options for marking, and marks that have proven useful in other (anuran

and caudatan) amphibians may be shed or may impair marked animals. Some caecilian species have markings that have been suitable for the generation of individual photographic identifications (Kramer et al., 2001) and the natural variation in annulation patterns in *Dermophis mexicanus* (Duméril & Bibron, 1841) have been used to distinguish between members of a small population of captive animals (Wright & Minott, 1999).

Previous work has shown that some marking techniques may be appropriate for identifying individual caecilians. These include the use of Panjet (Wright Health Group Ltd., Dundee; Measey et al., 2001; Measey & Di Bernardo, 2003; Measey et al., 2003); freeze branding (Measey et al., 2001); soft visible implant alphanumeric tags (Measey et al., 2001; Measey et al., 2003; Gower et al., 2006) and visible implant elastomer (VIE; Measey et al., 2001). However, the long-term stability and visibility of any of these identifiers has not been determined for periods greater than 15 weeks (Measey et al., 2001; Measey & Di Bernardo, 2003; Measey et al., 2003) and the longer-term, viability of the markings is unknown.

Most field applications of individual identification require a longer persistence of marks and so further investigation to identify viable marking techniques for caecilians is required. Moreover, the morphology, ecology and life history of caecilians varies widely among species (e.g. Taylor, 1968; Wilkinson & Nussbaum, 2006; Gower & Wilkinson, 2005; San Mauro et al., 2014), and so an expansion of marking trials to more taxa is also necessary in order to better design appropriate marking techniques for Gymnophiona.

VIE is a liquid polymer that solidifies when mixed with a curing agent. The polymer is coloured and fluoresces under black light (UVA light) and can be injected superficially

into animals to create individual marks for identification. VIE is commonly used to mark amphibians and has gained popularity in recent years. Bailey (2004) reported a 100 % VIE mark retention rate in 36 marked salamanders (*Eurycea bislineata*) over 44 weeks. A 100 % VIE retention rate was also reported for laboratory housed *E. bislineata* over 15 weeks (Marold, 2001). Other studies have questioned the reliability of VIE for marking amphibians; Brannelly et al. (2013) reported that VIE tag movement occurred within one week in 50 % of the tags implanted into the toad *Nectophrynoides asperginis*, and VIE tag movement and loss was reported in a study by Brannelly et al. (2014) evaluating marking techniques for the tree frog *Litoria verreauxii alpina*.

Very few population parameter estimates for caecilians have been made and there have been limited attempts to test field methods representing barriers to further research and caecilian conservation (Gower & Wilkinson, 2005). Maintaining caecilians in captivity provides an opportunity to study caecilians and develop and validate methods that can be used to understand and conserve them (Wake, 1994; O'Reilly, 1996; Wilkinson et al., 2013; Maddock et al., 2014, Tapley et al., 2014, 2018; Rendle et al., 2014). To test the application of VIE as a method to permanently identify individual caecilians, we attempted to mark captive *Herpele squalostoma* (Stutchbury, 1836) and *Microcaecilia unicolor* (Duméril, 1863) with VIE and to determine how long marks remain visible.

MATERIALS AND METHODS

Ethics

This study was compliant with the BHS Ethics Policy (British Herpetological Society, 2017). Ethical approval to mark caecilians with VIE using the described methods was granted by the ZSL ethics committee (Project ZDZ17). The methods used to mark caecilians here, including anaesthesia and recovery did not, in this context, require a Home Office License as a) VIE is a routine marking technique for amphibians, and even though it has been little used in caecilians it is the most routine marking type for use in this group, and b) animals were marked opportunistically as part of a routine veterinary health examination for which they needed to be restrained and anaesthetised.

Study species

The Congo caecilian (*H. squalostoma*; Family Herpelidae) is a burrowing caecilian from lowland forests in south-eastern Nigeria, Cameroon, south-western Central African Republic, mainland Equatorial Guinea, Gabon, Congo, western Democratic Republic of Congo, and Bioko Island in Equatorial Guinea (IUCN SSC Amphibian Specialist Group, 2018). The species is oviparous (Kouete et al., 2013) and exhibits maternal dermatophagy; young receive extended parental care and have specialised deciduous teeth that they use to remove and eat the stratum corneum of maternal skin (Kouete et al., 2012). The black micro caecilian (*M. unicolor*; Family Siphonopidae) is a poorly known species (Bittencourt-Silva & Wilkinson, 2018) that is likely a dedicated burrower (Wilkinson et al., 2013, Bardua et al., 2019) and is known

with certainty only from French Guiana (Wilkinson & Kok, 2010). Reproductive mode is oviparity (San Mauro et al. 2014) and, based on other siphonopids, can be inferred to involve direct development (i.e. no larval stage) and maternal dermatophagy (Wilkinson et al., 2008, 2013).

Husbandry

In 2008, ZSL London Zoo acquired *H. squalostoma* via donation, the animals were long-term wild collected captives that had been imported directly to the UK by a licensed importer. A further four juveniles were loaned to ZSL London Zoo in October 2014. These individuals were legally collected as eggs by Marcel Koute from Nkong in the central region of Cameroon in June 2013 and hatchlings raised by MW. *Microcaecilia unicolor* specimens were legally collected from the Core Mountains at Camp Patawa between 2008 and 2010 by the authors (DG & MW) and transferred to ZSL London Zoo in 2013. Both *H. squalostoma* and *M. unicolor* were maintained at ZSL London Zoo as part of a collaborative project with the Natural History Museum's Herpetology Research Group aimed at refining methods for caecilian husbandry, developing and validating field methods, and discovering aspects of life history and behaviour.

Microcaecilia unicolor and *H. squalostoma* of unknown sex were housed in a dedicated, climate-controlled facility. Room temperature ranged from 24–27 °C. *Herpele squalostoma* were maintained in two groups in separate enclosures and *M. unicolor* were housed individually. All enclosures were glass and custom-made (56 x 56 x 35 cm) with slanted bottoms to create a humidity gradient. Part of the lid consisted of a fine mesh for ventilation. Specimens were provided with a 15 cm deep layer of Megazorb (Northern Crop Driers (UK) Ltd.) substrate (Tapley et al., 2014) a waste product from the paper making industry which contains unbleached, wood derived cellulosic fibre and inorganic pigment (Kaolin and calcium carbonate), which is sold for equine husbandry. Dry Megazorb was soaked in water for 24 hrs until saturated. Specimens were fed three times per week with live worms (*Lumbricus* sp. and *Eisenia* sp.). *Herpele squalostoma* were occasionally also offered freshly killed crickets (*Gryllus bimaculatus* and *G. assimilis*) left on the surface of the substrate.

Marking

To observe and monitor any potential detrimental effects of marking, *H. squalostoma* were marked in two batches, seven months apart. We also attempted to mark a single *M. unicolor*. Caecilians were anaesthetised by a ZSL veterinarian for a routine veterinary health examination. Caecilians were anaesthetised in either buffered tricaine methanesulfonate 1g/L (PHARMAQ Ltd., Hampshire, UK) or 4 % isoflurane (Zoetis Inc., New Jersey, USA) in oxygen in a plastic bag (details to presented elsewhere) in order to prevent injury during the marking process because unanaesthetised caecilians are extremely difficult to manually restrain. VIE elastomer and a curing agent (Northwest Marine Technology Inc., Shaw Island, Washington, USA), were prepared following the manufacturer's guidelines and mixed in a 10:1 ratio. Using an insulin syringe and needle (BD U-100 Insulin 0.3 mL /

cc), approximately 0.05 ml of the prepared elastomer was injected subcutaneously into the dorsal surface one third of the distance between head and terminus. Each caecilian was marked with a different coloured VIE. Elastomers were implanted between annular grooves. The needle was inserted perpendicular to the long axis of the body with the needle tip pointing towards the vertebrae, then rotated to a near-parallel orientation to the skin surface and advanced at a low angle of insertion underneath the skin for c. 8–10 mm. Even pressure was then applied to the plunger of the syringe to extrude VIE while the needle was slowly withdrawn, creating a linear mark. Pressure was removed from the plunger c. 2–3 mm from the injection site so that the trail of VIE stopped well before the injection aperture; failing to do so can result in the solidified mark being extruded through the aperture.

During the procedure, animals were laid out on an absorptive disposable bed pad soaked with amphibian Ringer's solution (Wright & Whitaker, 2001), and frequently rinsed with the same solution in order to avoid dehydration and damage to the skin. Animals recovered from anaesthesia in a container of shallow amphibian Ringer's solution and were not returned to their enclosures until they had a normal righting reflex and exhibited a normal response to aversive stimuli (a gentle pinch). Post-recovery, the marked caecilians were weighed (to the nearest 0.1 g), using Pesola spring scales before being released back into their original enclosures where they were housed in two groups. Animals were subsequently periodically checked for the presence of the VIE marks and were weighed each time they were checked. They were checked infrequently in order to minimise disturbance to the animals and to the substrate, including any possible burrow structures. The last date that a marking was recorded as being visible was used as the minimum estimate of mark retention.

RESULTS

All *H. squalostoma* were successfully marked (Figs. 1A & B), although the marking in one animal was not visible the day after marking. We were unable to mark the single *M. unicolor* because we were unable to get the needle to form a channel into which VIE could be freely injected. Attempts to mark further *M. unicolor* were not made as the method was deemed non-viable in this species based on the initial trial.

In *H. squalostoma*, VIE mark retention ranged from 0–422 days (Table 1). On average, marks remained visible for 191 days with a standard deviation of 169.9 days. Mean average

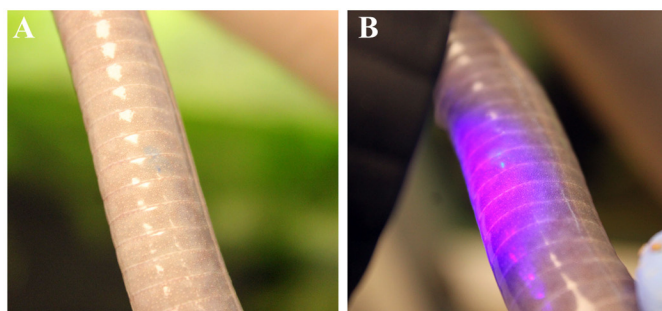


Figure 1. **A)** VIE marking in *H. squalostoma* in ambient light, **B)** VIE marking in *H. squalostoma* under black light

Table 1. VIE mark retention and weight change in *H. squalostoma*

Specimen ID	Colour of marking	Minimum duration mark visible (days)	Weight change over marking period (g)
ZRS16-08880	Orange	422	+35.9
ZRS16-08881	Pink	197	-5.1
ZRS16-08882	Green	42	+0.6
ZRS16-08883	Cherry red	422	+30.6
ZRS15-08651	Red	128	+26.6
ZRS15-08652	Green	0	-2.0
ZRS15-08653	Blue	128	+20.8

body weight change between being marked and the date that the mark was last observed was +15.3 g, and although both weight gains and losses were measured, losses were fewer and generally smaller. No animal showed any clinical signs associated with marking. The single *M. unicolor* also recovered well from the failed marking attempt (where the needle punctured the skin but no VIE was deposited) with no clinical signs of ill health observed.

DISCUSSION

Our data suggest that VIE tags might not be appropriate for marking *H. squalostoma* when individuals need to be identified in long-term studies. The colour of the mark could have been a factor but as only one animal was marked with each colour it is not possible to associate VIE colour and mark retention. In *H. squalostoma*, mark retention was highly variable between individuals. Furthermore, marks were not always easily visible, even when illuminated under a black light and many marks were found only after repeatedly inspecting the animal. This marking technique would be impractical for field use because anaesthesia is required for implantation, and ideally animals should be monitored for 24 hours after implantation to assess anaesthetic recovery status and mark retention (given that one mark was immediately lost in one of our marked individuals). Moreover, due to the difficulty in finding marks known to be present, differentiation between marked and unmarked animals may be problematic in the field even where marks persist. However, this could be ameliorated if all caecilians were marked in exactly the same location of the body.

The VIE marks in *H. squalostoma* did not migrate as reported in some marking trials of other amphibians (e.g. Brannelly et al., 2013, 2014). It is unclear how caecilians lost the marks in this study; no marks were found on a postmortem examination of an animal that died of natural causes over a year after the mark was last observed. This might indicate ejection, absorption, or micro-fragmentation of the VIE tag.

We were unsuccessful in our single attempt to mark a *M. unicolor*. Both *H. squalostoma* and *M. unicolor* have dermal scales in annular folds (Nieden, 1912; Taylor, 1968; Zylberberg & Wake, 1990), but squamation is much less extensive anteriorly in *H. squalostoma*, and this might be

causally related to the difficulty of inserting the elastomer in *M. unicolor*. Ichthyophiidae and Rhinatrematidae also exhibit extensive squamation (Colbert, 1955; Zylberberg et al., 1980), consequently VIE marking would likely be difficult in these taxa. The skin of *M. unicolor* is much more darkly pigmented than the skin of *H. squalostoma*, this could have obscured the visibility of any traces of VIE that might have been injected. We also found it difficult to track the applicator needle at a very shallow depth under the skin in this species.

While our sample size was small and limited to two species representing two of the ten caecilian families; this is the first attempt to validate long-term VIE marking in caecilian amphibians. This method is viable for shorter term studies of at least *H. squalostoma*, given that most animals retained marks and none showed any ill effects of having been anaesthetised and marked. However, its utility for the long-term study of any caecilian species is at best uncertain but warrants further research.

Dark skin pigmentation and possibly other morphological features, such as squamation, may preclude the efficient use of subcutaneous marking techniques in some caecilians. Thus alternative techniques should be developed to permanently identify individual *H. squalostoma* and *M. unicolor* for long-term studies. Natural variation in annulation patterns might be useful in this respect as they have in *D. mexicanus* (Wright & Minott, 1999).

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