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Permanent marking of a fossorial caecilian, *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae)

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Abstract

Despite the importance of permanent marking of animals for quantitative ecological studies, no such technique has been applied to any of the poorly-known caecilian amphibians. We evaluated four techniques (Panjet, freeze-branding, Elastomer Visible Implant tags and Soft Visible Implant Alphanumeric tags) of permanently marking a fossorial caecilian, *Gegeneophis ramaswamii* Taylor, in the southern Western Ghats, India. All the tested techniques are viable options for marking caecilians in the field but differ in their portability, ease and speed of application, and their suitability for batch and/or individual marking of animals. Panjet tattoos were deemed to be particularly effective and practical for batch marking, while Soft Visible Implant Alphanumeric tags offer good potential for individual marking.

Keywords: Ecology, Gymnophiona, *Gegeneophis ramaswamii*, permanent marking, soil

Introduction

Despite a widespread perception that caecilian amphibians (Gymnophiona) are rare (e.g. Gundappa et al., 1981), several studies have reported that at least some terrestrial caecilian species are locally abundant (Nussbaum and Pfrender, 1998; Oommen et al., 2000). However, there remains very little published information on the ecology of any caecilian species. We are largely ignorant of their ecological relations and their impact on the tropical soil communities they inhabit, and which in some places they appear to dominate (Oommen et al., 2000). As a precursor to meaningful quantitative ecological studies of terrestrial caecilians, it is necessary to identify those ecological techniques that can be readily applied to these animals. Marking of animals is an important ecological technique that, through mark-recapture methods, allows efficient estimation of population size and related parameters. In addition, individual marking enables the monitoring of individuals through time, providing information on, for example, growth, longevity and home range.

Murray and Fuller (2000) recently reviewed methods for marking vertebrates, and distinguished three categories: mutilation and scarrification, insertion or attachment of tags, and tagging using radiotransmitters. Marking techniques for amphibians have also been reviewed (Ferner, 1979; Donnelly et al., 1994; Nietfield et al., 1994). None of these reviews mentions the marking of caecilians and this is typical of ecological literature which pertains to 'amphibians' but which, because of the paucity of information on caecilians, deals only with frogs and salamanders. For studies of the latter two groups of amphibians, the removal of digits has been used to mark animals, although ethical concerns have led to this being discouraged by some workers. The lack of limbs and digits in caecilians precludes the use of toe-clipping for marking these animals. In the only report to date of individually identifying caecilians, Wright and Minott (1999) used natural variation in annulation patterns to distinguish between members of a small population of captive *Dermophis mexicanus*

(Duméril & Bibron) at Philidelphia Zoo. In this paper, we explore the applicability of several marking techniques to the fossorial Indian caecilian *Gegeneophis ramaswamii* Taylor which is abundant in a variety of cultivated areas in the southern Western Ghats, India (Oommen et al., 2000). These methods are intended to be permanent and could be used for either batch or individual marking.

Materials and methods

Gegeneophis ramaswamii specimens were dug from soil at Bonaccord Estate, Trivandrum District, Kerala, India (see Oommen et al., 2000 for further information on this locality) on 27 June 2000. Animals were transported to the Department of Zoology, University of Kerala and those that were uninjured and apparently healthy were housed in a soil-filled aquarium (600 × 300 × 300 mm) at ambient conditions. Handling was kept to a minimum to avoid stressing the animals. For some of the techniques, anaesthetic was used to prevent unnecessary pain and to subdue individuals during the marking procedure. Animals were placed into 750 ml of a 0.1% solution of tricane methane sulphonate (MS222, Sandoz) until they stopped swimming. As with most caecilians and terrestrial amphibians, *G. ramaswamii* are countershaded with a darker dorsal surface, and individuals were marked mid-ventrally so as to increase mark visibility. Marks were also positioned approximately halfway along the length of the body in order to avoid potential damage to the heart which is positioned further anterior. The skin in this region also lacks secondary annuli and scales, features that might be expected to complicate application and/or reading of marks. For each technique, five animals were selected from across the available spectrum of body size and maturity. After marking, animals were returned to the soil filled aquarium, maintained on a diet of worms and/or dead fish, and inspected for marks after 24 hours and subsequently on the 12 July, 29 August and 11 October, 2000. The specific marking techniques employed are given below.

Panjet (Wright Health Group Ltd., Dundee). Panjet marking uses a needleless tattoo gun to apply dye under pressure through a small aperture. This technique has successfully been used to mark anurans (see Wisniewski et al., 1979; Measey and Tinsley, 1998). A Panjet (fitted with the smallest supplied spring) was loaded with a 2% solution of Alcian Blue suspended in distilled water. Specimens were folded into paper towel to restrain them while exposing the area selected for marking. They were marked with the Panjet nozzle held 5 mm away from the skin, determined using the spacer provided. Potentially adverse effects to small individuals (< 100 mm) were avoided by increasing the distance between the nozzle and animal to approximately 20 mm. Anaesthesia was not induced because the technique is fast and the operator (GJM) was experienced.

Freeze branding. Marking by applying very cold metal brands to the skin has been used successfully on anurans (Daugherty, 1979; Measey, in press). Pieces of 1.3 mm thick copper wire were bent into Arabic numerals (5 mm high, 4 mm wide), leaving a free length of 50 mm for a handle. A brand was placed in liquid nitrogen until it stopped boiling. Specimens to be branded were gently dried with paper towel before brands were placed on the ventral surface for 1.5 seconds. Anaesthesia was not induced because the technique is fast and the operator (GJM) was experienced.

Elastomer Visible Implant (VIE) (Northwestern Technologies, Salisbury UK). VIE is a coloured flourescent elstomer that is injected as a liquid into or beneath translucent tissue, where it cures into a pliable solid tag. VIE was supplied in the form of an orange elastomer and a curing agent, which were mixed in the recommended 10:1 ratio. Once the two parts are mixed, tagging must be completed within 2 hours (at 20° C) or the mixture kept in a freezer to slow hardening. All VIE marked animals were first anaesthetised because the elastomer was administered using a hypodermic syringe (as supplied). Anaesthetised specimens were placed venter up on paper towel while approximately 0.05 ml of the prepared elastomer was injected sub-cutaneously.

Soft Visible Implant Alphanumeric (VIAlpha) Tags (Northwestern Technologies, Salisbury UK). Soft VIAlpha tags are coloured, flourescent, lettered and numbered, pliable tags that are injected into or beneath translucent tissue. The supplied VIAlpha tags were flourescent orange and measured approximately 2.8 mm by 1.2 mm and less than 0.1 mm thick. Each tag carried a unique combination of a letter and two-digit number marked in 1 mm tall black characters. The tags used in this study were supplied attached to a thin, water-soluble sheet of gelatin. One tag at a time was freed from the sheet and loaded into a supplied re-usable syringe and hypodermic needle with a flattened bevel tip. A sharpening stone (as supplied) was used to maintain the cutting edge of the needle tip after every two or so animals marked. Anaesthetised animals were held on paper towel, and the syringe inserted into the dermis at an annular groove and pushed anteriorly for a distance of approximately 5 mm. The syringe plunger was then

depressed to eject the tag and the needle withdrawn making sure that no part of the tag projected from the entry wound.

In addition, we considered using Passive Integrated Transponders (PIT tags) (AVID plc, Uckfield UK). The PIT tags investigated were cylindrical (approximately 12 mm long and 2.5 mm in diameter) and individually housed in a single-use syringe delivery system. In our judgement these PIT tags and their delivery syringes were unsuitable for marking *G. ramaswamii* and no further attempt was made to test them (see below).

Results

Four animals out of a total of 48 were injured during collection and were preserved and accessioned into the biodiversity collection of the Department of Zoology, University of Kerala.

Panjet. Panjet marking did not seem to cause undue disturbance to individuals. If marks hit an annular groove, there was a distinct swelling and discoloration of the area, but this subsided over the following 24 hours and no puncture mark was visible. Panjet marks were immediately visible and remained so throughout the study period (see Figure 1*a* and Table 1). It was necessary to check the quality of all marks immediately after marking because small animals were occasionally missed or imperfectly targeted.

Freeze branding. Animals that had been restrained with paper towel squirmed considerably more when a brand was placed on their skin, but this occurred whether the brand was at room temperature or had been cooled in liquid nitrogen. The prepared brands were too big for the slender bodies of some smaller animals, in which case scar patterns rather than recognisable numbers were produced. Brands were faintly visible immediately after marking but were clearly visible 15 minutes later. The skin blistered after 24 hours with no sign of skin punctures (see Figure 1*b* and Table 1). Freeze brands were clearly visible and distinct from other scars in all following assessments. Considerable difficulty was encountered in retaining liquid nitrogen because ambient laboratory temperatures were generally in excess of 30ºC.

VIE. Despite being anaesthetised, individuals reacted strongly to insertion of the hypodermic needle, although this effect lessened as the operator gained

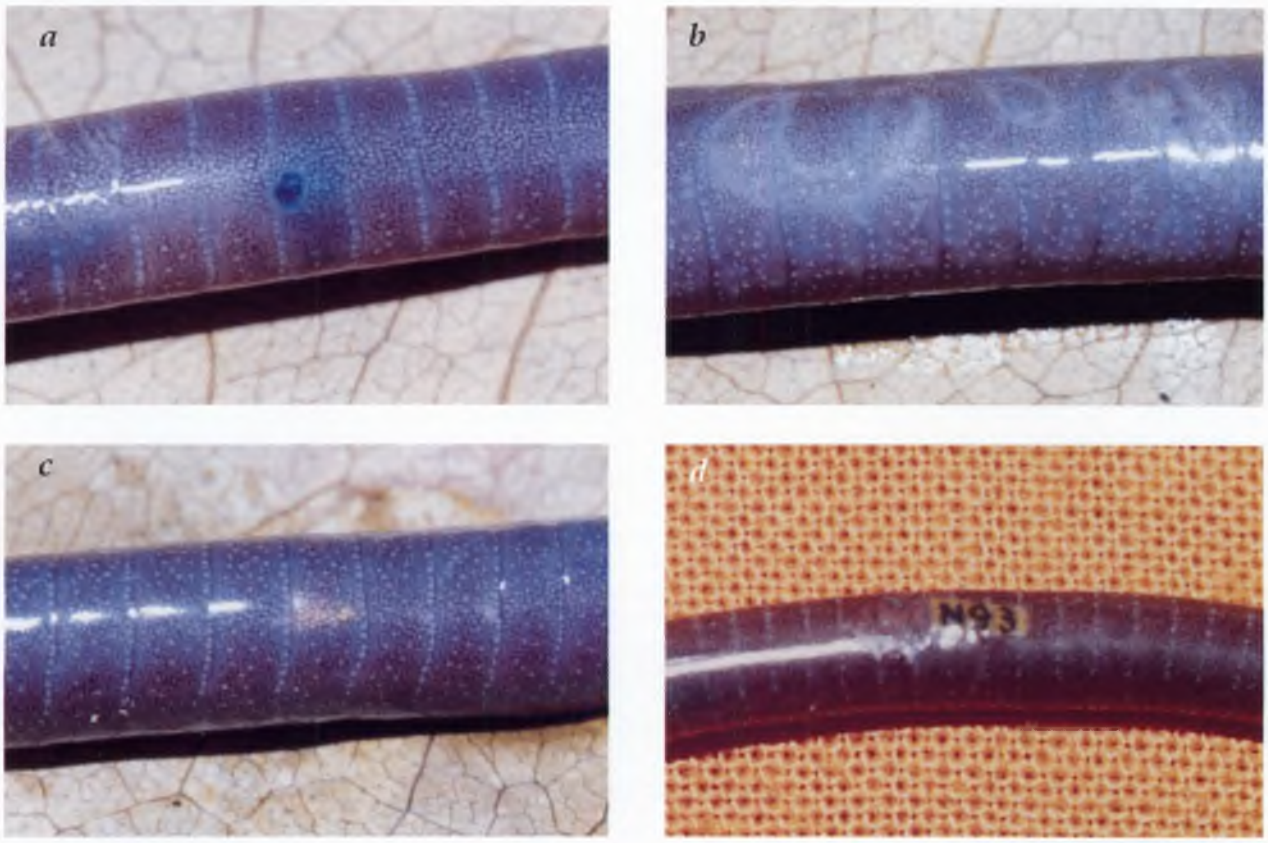

Figure 1. Marks applied to the ventral surface of the midbody region of *Gegeneophis ramaswamii*. In all cases the body diameter is between 6 and 10mm. ***a,*** Panjet tattoo; ***b,*** Freeze brand; ***c,*** Visible Elastomer Implant; ***d,*** Soft Visible Implant Alphanumeric tag.

Table 1 A summary of the different marking techniques tested on *Gegeneophis ramaswamii*.

	Panjet tattoo	Freeze brand	VIE	VIAlpha
Date of mark	26/06/00	28/06/00	28/06/00	18/08/00
Size range of animals marked (mm)	60 – 252	140 – 288	84 – 196	58 – 255
Anaesthetic	Optional	Optional	Required	Required
Average time taken to apply mark	5 seconds	10 seconds (per digit)	1 minute	Up to 5 mins
Portability	Good	Poor	Good	Good
Immediate effect on individual	Localised swelling where marked on annular groove	Animals disturbed by placement of brand	Animals disturbed by insertion of needle	Animals disturbed by insertion of needle
Immediate visibility of mark	Clear	Faint	Generally clear – better in sunlight	Generally clear – better in sunlight
Skin puncture	Yes	No	Yes	Yes
Visibility of mark on 12/7/00	Clear	Clear	Generally clear – better in sunlight	n/a
Visibility of mark on 29/8/00	Clear	Clear	Generally clear – better in sunlight	Generally clear – better in sunlight
Scars from marking seen on 29/8/00	No scar visible	Scar tissue formed in branded areas	No scar visible	Scar visible at needle insertion point
Visibility of mark on 11/10/00	Clear	Clear	Generally clear – better in sunlight	Generally clear – better in sunlight

experience. Marks were visible in strong daylight immediately after VIE was injected and on all subsequent inspections (see Figure 1*c* and Table 1). Unused elastomer was found to have set 24 hours after preparation at ambient temperature.

VIAlpha. After the initial needle puncture, tags were inserted into the dermis without adverse reaction from individuals. Tags were immediately visible. Puncture wounds remained visible after 24 hours but did not noticeably affect individual behaviour (see Figure 1*d* and Table 1) and had healed when next checked 11 days later. Tags were visible with the naked eye, but some required a hand-lens (× 10) to read or check the lettering and numbering. As the operator gained experience, tags were inserted closer to the epidermal-dermal border, and were consequently easier to read.

Discussion

Marking of animals is not necessary if individuals can otherwise be reliably distinguished. Amphibian workers have often relied on individuals having unique and easily recognisable body markings (see Donnelly et al., 1994) although, some such marks vary ontogenetically (e.g. Tilley, 1980). In Wright and Minott's (1999) study, 17 captive specimens of *Dermophis mexicanus* were distinguished on the basis of variation in individual annulation patterns. The ventral surface of each specimen was photocopied and identification consisted (p. 32) "of noting which annuli are incomplete, broken, or bent." Similar techniques have been successfully used for identifying laboratory maintained species of *Ichthyophis* (W. Himstedt pers. comm.). Wright and Minott (1999: 33) report that "similar annular disjunctions may not be present in all specimens of a given population of this species... or in other species, especially those that lack secondary or tertiary annuli (M. Wake pers. comm.)." In addition, ontogenetic variation in individual annulation patterns of caecilians has not been investigated and requires evaluation. Our taxonomic work suggests that while individual differences in annulation occur within many caecilian species, such differences may be very fine and easily missed during intensive field work involving many animals. We suspect that accurate recording of annulation patterns for later accurate identification (by sketching, photography or using handheld photocopiers) would

be prohibitively time-consuming under field conditions. Thus, although identification based on individual annulation patterns is adequate for a small number of individuals in laboratory conditions, it is likely to be impractical with larger populations of wild caecilians where previously unencountered individuals may be continually recruited or discovered. However, the use of such non-invasive techniques in field studies merits further investigation.

Where marking is needed, the preferred technique will depend on the objectives of the study, particularly whether individual marks, batch marks or a mixture are to be used (see Murray and Fuller, 2000). Practical considerations such as ease of use, and ethical issues relating to animal welfare and conservation (Cuthill, 1991) also need to be taken into account. In addition to being permanent and quick and easy to apply, Friend et al. (1994) advise that, ideally, marks should have a minimal effect on anatomy and physiology, must not influence behaviour, and must not make an animal more conspicuous. The results of this study are insufficient for a rigorous evaluation of the extent to which the different marking techniques satisfy these desiderata, particularly given the short time scale of the study. However, no serious damage or modification of behaviour was observed in any of the marked animals, and the wounds caused by injection of Panjet, VIE and VIAlpha marks showed no sign of infection and healed quite rapidly. *Gegeneophis ramaswamii* is seldom encountered above ground and thus the marking can be expected to have minimal effect on conspicuousness to predators.

For caecilians, our main ethical concern arises from the mortality inflicted during collection by digging. In this study, 8% of the *Gegeneophis ramaswamii* collected were injured during collection. This is unavoidable in the current absence of less destructive methods of sampling. Caecilians are rather poorly represented in museum collections and much remains to be learnt about their anatomy and systematics. Thus, any fatalities should be collected and deposited in a scientific repository where they can be used for diverse studies by present and future generations (Arnold, 1998). In our estimation, fatalities that occur during collection are unlikely to represent a serious threat to the survival of caecilian species that, like *G. ramaswamii*, are locally abundant. This is because the time and effort involved in digging means that only a small proportion of the available habitat can be investigated in any realistic study. Furthermore, we were studying animals from cultivated land, where digging and associated mortality are a 'natural' feature of the environment. In our view, the ethical concerns over fatalities of *G. ramaswamii* are offset at this time by the potential benefits that enhanced knowledge of the ecology of caecilians will have for their conservation.

Table 1 includes a summary of the properties of the four marking techniques with respect to a variety of practical considerations and the effect on individuals. It is useful for the operator if marks are immediately visible because this allows confirmation of successful tagging and may determine the positioning of subsequent marks. It should be noted that the speed of administering each mark greatly improved with increased operator experience, and that the two techniques requiring the greatest time to administer (VIE and VIAlpha) were being used for the first time by the operator.

The effectiveness of Panjet in permanently marking amphibians has been proved elsewhere (Wisniewski et al., 1979) and is believed to be the technique producing the longest-lasting recorded markings on wild amphibians (up to 14 years, Measey and Tinsley, 1998). There appear to be very few drawbacks with using Panjet in the batch marking of *Gegeneophis ramaswamii*, but creating unique marks for large numbers of individuals might be problematic. A large number of unique combinations could be devised using different numbers and positions of marks with respect to different annuli. However, the time required for marking and for reading would increase with complexity of these patterns, anaesthetic might be required, and errors in reading or applying marks might be expected to increase.

As in *Schistometopum thomense* (Barboza du Bocage) (Teodecki et al., 1998), *G. ramaswamii* have naturally occurring scars, at least some of which are probably bite marks from conspecifics. In this study, all freeze brands were readily distinguished from presumed bite marks. Freeze branding has long been used for marking amphibians (Daugherty, 1976) and has also been shown to be long lasting in field conditions (Measey, in press). The rate at which liquid nitrogen evaporates in tropical environments with high ambient temperatures is a potential serious practical limitation of the technique.

VIE and VIAlpha systems have thus far been extensively used only with fish, where their permanence is considered high (e.g. Haw et al., 1990; Niva, 1995). As far as we are aware, the only previously published applications of these methods to amphibians are in larval (Anholt et al., 1998) and adult (Nauwelaerts et al., 2000) anurans, and in plethodontid salamanders (Jung et al., 2000), although other flourescent dyes and pigments have been used successfully (e.g. Taylor and Deegan, 1982; Nishikawa and Service, 1988). The elastomers used in VIE and VIAlpha appeared to be biocompatable in our tests and the integument of *G. ramaswamii* showed

no undue reaction to them. Use of an ultra-violet (UV) light source is recommended by the manufacturers of both systems to increase the visibility of the fluorescent tags. Artificial UV illumination was not employed in this study, and although visibility was good under a range of light levels, it increased in bright sunlight. VIE tags have similar potential to, and suffer the same drawbacks as, Panjet for individual marking. Application of VIAlpha tags is relatively time consuming but single tags enable individual identification and of the methods used here we consider this to be the most promising for studies requiring individual rather than batch marking.

Large tags are expected to present problems for burrowing animals, such as caecilians, that use their skin in burrowing and in moving through burrows. Subcutaneous injection of large tags may therefore not be practical, hence our decision not to test the PIT tags. PIT tags have been used successfully to mark small frogs and salamanders through intraperitoneal injection (e.g. Fasola et al., 1993). However, this approach is probably not suitable for caecilians because it is likely to negatively affect their ability to maintain turgidity (and hence body shape, Gans, 1974) which may be essential for their locomotion. The much smaller VIAlpha tags we tested could be implanted subcutaneously and appeared to have no detrimental affects on the animals. The development of smaller PIT tags or radiotransmitters may enable these techniques to be applied to caecilians in the future.

In summary, Panjet, freeze branding, VIE and VIAlpha were all found to be successful techniques for the permanent marking of the caecilian amphibian *Gegeneophis ramaswamii*. Panjet was found to be the simplest and fastest method of administering marks. Both Panjet and VIE marking are suitable techniques for batch tagging, and both could probably be used to individually mark small numbers of animals. Freeze branding and VIAlpha are more suitable for individually marking larger numbers of animals, but the rapid rate of evaporation of liquid nitrogen and its relative lack of portability make freeze branding a less attractive method for marking in tropical field conditions. Donnelly et al. (1994: 83) recommended that "Marking methods should be tested in the laboratory and in the field prior to the initiation of any long term study." Our laboratory-based evaluations represent a first step in this procedure and allow some limited conclusions regarding the potential utility of the evaluated methods under field conditions. Field trials are now needed, and are currently underway for the two most promising techniques for batch and individual marking, namely panjet and VIAlpha.

A recent international summit of ecologists discussed the need to rectify "ecology's subterranean blind spot" (Copley, 2000) emphasising *both the* importance of the soil in terrestrial ecosystems and our relative ignorance of the organisms that inhabit soils and affect their physical and biological properties. This is particularly true for soil vertebrates, for which published ecological studies are largely restricted to mammals that inhabit permanent or semi-permanent burrow systems that can be readily identified and monitored (e.g. Bennett and Faulkes, 2000). Quantitative ecological studies of other soil vertebrates, such as caecilians and burrowing squamates, are expected to pose greater problems if only because of the difficulty associated with finding these animals. In the case of caecilians, the need to address the ecological blind spot assumes greater significance and urgency in the context of concerns over global amphibian declines which are as yet unrecorded for any caecilian (Houlahan et al., 2000). Permanent marking has the potential to allow quantitative monitoring of such poorly known aspects of caecilian biology as growth rates, longevity, and migration. Further work is needed to determine the applicability of marking techniques to other caecilian species, particularly those such as rhinatrematids and ichthyophiids that have more extensive squamation associated with their annuli. However, by demonstrating the applicability of marking techniques to *Gegeneophis ramaswamii* we hope to have brought meaningful quantitative studies of caecilian ecology and their role in tropical soil ecosystems a little closer.

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