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OF WILD FAUNA AND FLORA

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METHODOLOGIES FOR DIFFERENTIATING BETWEEN WILD AND CAPTIVE-BRED CITES-LISTED SNAKES

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Methodologies for differentiating between wild and captive-bred CITES-listed snakes

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Definitions

CITES source code “C” Captive-bred	Animals bred in captivity in accordance with Resolution Conf. 10.16 (Rev.), as well as parts and derivatives thereof, exported under the provisions of Article VII, paragraph 5.
CITES code “F” First generation captive-bred	Animals born in captivity (F1 or subsequent generations) that do not fulfill the definition of ‘bred in captivity’ in Resolution Conf. 10.16 (Rev.), as well as parts and derivatives thereof.
First-generation offspring (F1)	The progeny produced in a controlled environment from parents at least one of which was conceived in or taken from the wild [Definition adapted from Resolution Conf. 10.16 (Rev.)]
Second-generation offspring (F2) [or subsequent generation offspring F3, F4, etc.]	The progeny produced in a controlled environment from parents that were also produced in a controlled environment.
CITES code “W” Wild	Specimens taken from the wild.
Controlled environment	An environment that is manipulated for the purpose of producing animals of a particular species, that has boundaries designed to prevent animals, eggs or gametes of the species from entering or leaving it, and the general characteristics of which may include but are not limited to: artificial housing; waste removal; health care; protection from predators; and artificially supplied food. [Definition adapted from Resolution Conf. 10.16 (Rev.)]
Bred in captivity	Characteristic of animal specimens, as defined in Article I, paragraph (b), of the Convention, born or otherwise produced in a controlled environment, and applied only if: i) the parents mated or gametes were otherwise transferred in a controlled environment, if reproduction is sexual, or the parents were in a controlled environment when development of the offspring began, if reproduction is asexual; and ii) the breeding stock, to the satisfaction of the competent government authorities of the exporting country: A. was established in accordance with the provisions of CITES and relevant national laws and in a manner not detrimental to the survival of the species in the wild; B. is maintained without the introduction of specimens from the wild, except for the occasional addition of animals, eggs or gametes, in accordance with the provisions of CITES and relevant national laws and in a manner not detrimental to the survival of the species in the wild as advised by the Scientific Authority: 1. to prevent or alleviate deleterious inbreeding, with the magnitude of such addition determined by the need for new genetic material; 2. to dispose of confiscated animals in accordance with Resolution Conf. 10.7 (Rev. CoP15); or 3. exceptionally, for use as breeding stock; and C. 1. has produced offspring of second generation (F2) or subsequent generation (F3, F4, etc.) in a controlled environment; or 2. is managed in a manner that has been demonstrated to be capable of reliably producing second-generation offspring in a controlled environment. This definition applies to the specimens bred in captivity of species included in Appendix I, II or III, whether or not they were bred for commercial purposes. [Definition adapted from Resolution Conf. 10.16 (Rev.)

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Executive Summary

The international trade in snakes, including their parts and derivatives, involves many millions of individual animals of numerous species. Several of these snake species are listed in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates international trade in wildlife with the aim of preventing extinction. Snakes are traded for a variety of purposes, including for pets, medicines, leather goods and food. Many snake specimens entering trade are sourced from the wild, but an increasing number are being bred in captivity and many closed-cycle breeding facilities have been established for the purpose of supplying snakes and their parts and derivatives for trade. Under CITES, the source of snake specimens listed on an Appendix must be documented and communicated when specimens are exported and imported. Recently, however, reservations have been expressed about the potential misuse of CITES source codes, in particular the mis-declaration of wild snakes as captive-bred. Misuse of source codes is in contravention of CITES, may result in potentially detrimental trade if left unchecked, and can undermine mechanisms in place to ensure that trade is legal and sustainable.

Recognising the considerable difficulties in differentiating between wild and captive-bred snakes (and their parts and derivatives), in compliance with Decision 16.102 of the Sixteenth Meeting of the Conference of the Parties (Bangkok, 2013), the CITES Secretariat has commissioned a study on:

"Methodologies to differentiate between wild and captive-bred CITES-listed snakes in trade, including parts and derivatives, ensuring that the work is carried out in line with recommendations of the Standing Committee concerning source." http://www.cites.org/eng/cop/16/doc/E-CoP16-57.pdf

This report is the result of research aimed at addressing this Decision, and aims specifically to provide exporting and importing Parties with details of each method while examining their potential limitations, examples of their use and their applicability to snakes. The methods examined include:

- General health, appearance and behaviour of snakes
- Physical, chemical and thermal branding of snakes
- Passive Integrated Transponder (PIT) tags
- Use of snake eggshells
- Non-natural snake morphs
- Presence of gastrointestinal parasites
- DNA, genotyping and parentage assignment
- Stable isotopes

The report finds the most reliable and logistically feasible method (in terms of time and cost) for differentiating between wild and captive-bred snakes is the deliberate breeding of non-natural morphs that are not found in the wild. Facilities that breed non-naturally occurring morphs of snakes can be simply and cost-effectively monitored to unequivocally ensure captive origin. However, the method is not applicable for snake specimens that have already been processed into their parts and derivatives (e.g., gall, meat, fat). In these cases, the use of stable isotope methodologies provide promising forensic application for snakes, although it is noted that implementation time and costs may be prohibitive for some trade scenarios. After evaluating each of the methods, the major recommendations of the report are:

1) Methods are applicable on a case by case basis, which is strongly influenced by the type of trade taking place and the specific production scenario in the country of interest;

2) In some cases, Parties may choose to implement more than one method;

3) Although some methods are cost effective and easy to implement, reliability for the differentiation of source should be the primary concern when deciding on a suitable method;

4) The choice of method should be commensurate with the benefits of trade. Implementation of sophisticated methods may not be appropriate or economically viable for some corresponding trade situations and this should be reflected in the choice of method.

1.0 INTRODUCTION

1.1 Importance and CITES context

The international trade in snakes, including their parts and derivatives, is a multi million dollar industry. Snakes are often sourced from the wild, but are increasingly being sourced from captive breeding facilities that produce many thousands of individuals to meet demands for pets, medicines, leather goods and food. These trades are often critically important for the livelihoods of many people participating in them, and captive breeding has been promoted because it may reduce harvesting pressure on wild populations (Nogueira and Nogueira-Filho, 2011). However, there are also concerns that some captive breeding facilities are being used to launder wild-caught specimens and trade them as though they were captive-bred. When this occurs it can continue to place pressure on wild stocks through unregulated harvesting and can undermine mechanisms designed to ensure sustainable and legal trade.

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates trade in wild and captive-bred snakes listed in the CITES Appendices. Parties to CITES have acknowledged that illegal harvest and trade of wild snakes through captive breeding facilities undermines the rules of the Convention and may result in trade becoming detrimental to wild populations if left unchecked (Article IV). In compliance with Decision 16.102 of the Sixteenth Meeting of the Conference of the Parties (Bangkok, 2013), the CITES Secretariat has commissioned a study on:

"Methodologies to differentiate between wild and captive-bred CITES-listed snakes in trade, including parts and derivatives, ensuring that the work is carried out in line with recommendations of the Standing Committee concerning source." http://www.cites.org/eng/cop/16/doc/E-CoP16-57.pdf

This report aims to identify and describe these methods in detail. It also aims to acknowledge their potential limitations, examples of their use and score their applicability to snakes (and their parts and derivatives) in trade. Information on each method was gathered through consultation with relevant experts, scientists and CITES Management Authority staff. Parties are encouraged to also review other outputs that may provide useful guidance for differentiating wild from captive-bred snakes. E.g.,

http://www.cites.org/sites/default/files/eng/com/ac/27/E-AC27-Inf-17_0.pdf

1.2 Captive breeding scenarios for snakes

Captive breeding of CITES-listed snakes is becoming more common in many parts of the world, to supply both domestic and international markets. The situations in which captive breeding occurs are highly variable, and are influenced by the country of origin, the species of snake being bred, and the type of trade they are being bred for. For example, Indonesia has a small number of large and highly sophisticated commercial snake-breeding facilities producing snakes for the pet trade. By contrast, Viet Nam has hundreds of small household-sized satellite farms raising snakes for the skin and meat trades.

The methods used to differentiate between wild and captive-bred snakes will therefore be case specific. For example, some methods will be pertinent and reliable for application to snakes being bred in small numbers for pets, but become ineffective and costly when snakes are bred in the thousands for their meat or skins. Understanding the different scenarios in which snakes are being bred is important when deciding on the most appropriate method.

Fig. 1. Captive breeding scenarios for snakes ranging from intensive indoor facilities (top and right), small-scale households (bottom), and extensive outdoor farms (left).

1.3 What is an ideal method for differentiating between wild and captive-bred snakes?

When evaluating methodologies to differentiate between wild and captive-bred snakes, there are a number of important attributes that must be taken into consideration. Some of these relate to the types of trade and farming scenarios in the country of interest, and others relate to the logistical difficulty of implementation and reliability of the method. The most important attributes include:

Time to implement: An ideal method would be simple enough so it can be implemented almost immediately, without the need to establish a reference database and significantly alter existing infrastructure or system protocols.

Cost: The initial and ongoing cost of implementing the method should be as low as possible, both in terms of equipment expenditure and staff resources.

Suitability for live individuals, parts and derivatives: An ideal method should have applicability for snakes traded live (e.g., as pets) and for situations when their parts and derivatives are being traded (e.g., meat, skins, gall bladders, fat).

Suitability for large and small numbers: An ideal method should be equally applicable for use in situations where few farms breed small numbers of snakes (>100) and also where many farms breed large numbers of snakes (> 1000 snakes).

Labor intensity: An ideal method should not require many staff, large amounts of laboratory time or expertise to implement or apply.

Reliability: Most importantly, the method should be able to reliably differentiate between wild and captive-bred snakes.

1.4 What is the aim of this guide and how should it be used?

This guide aims to provide exporting and importing Parties with information on methods that can be used to differentiate between wild and captive-bred snakes and their parts and derivatives in trade. Some methods are very simple, cost effective and straightforward to implement, whereas others are more complex and require significant infrastructure, time and ongoing cost. Because of the range of captive breeding scenarios and trades for snakes (e.g., pets, skins, meat, etc.), some methods will be applicable for certain cases whereas others will not. Information is provided to guide Parties in selection of methods that are most appropriate for these needs. To do this, each methodology is broken up as follows:

How does it work – Provides background to the method and how it may be applied to differentiate between wild and captive-bred snakes.

What is the process – Describes a simplified step-by-step process for what is required to establish and implement the method.

Examples of use – Explains some of the ways in which the method has been used for similar purposes in the past, specifically for snakes and for other wildlife species.

Limitations – Describes situations where the method may be limited. Limitations are not restricted to the efficacy of the method for differentiating between wild and captive-bred snakes, but also for its suitability to different situations, its labor requirements and its costs.

Recommendations for snakes – Recommendations are provided on situations where the method would be most applicable.

Conclusion – A general conclusion is provided on how reliable the method is and its suitability for different types of trade (live, parts and derivatives). This conclusion is based on the scores given to a number of variables in the summary table at the end of each method. Scores have been assigned by the authors.

2.0 Available Methodologies to Differentiate Between Wild and Captive-bred Snakes

This section summarises available methodologies that can be used to differentiate between wild and captive-bred snakes. Some of the methodologies are more reliable than others, while some are only suitable for specific situations. It is the responsibility of each Party to evaluate and determine which methodology is most reliable, suitable and cost-effective for their trade situation.

2.1 General Health, Appearance and Behaviour of Individuals

How does it work?

Some species of snakes are better suited to captivity than others. In particular, wild snakes of many species are often poorly adapted to captive conditions and become easily stressed if proper captive care is not provided (McDiarmid et al. 2012). This can result in a number of symptoms, most notably:

- Failure to accept food for a prolonged period and/or regular regurgitation of food items;
- Continually attempting to escape and abrading (rubbing) the snout, even when the enclosure is covered;
- Unusual passivity or torpor;
- Skin folds and prominent vertebrae (suggesting emaciation due to food refusal);
- Lesions around the jaws, gums and lips; and/or
- Abnormal levels of aggression.

Wild snakes that have been brought into captivity also often exhibit scarring sustained from predators, combat bouts with rival males and/or when securing prey. In addition, lumps under the skin may be the result of skin worm parasites (most notably pentostomids), which cannot be acquired in captivity (Rataj et al. 2011). These and other ectoparasites (ticks, in particular) are most commonly found on wild snakes. Snakes that exhibit some or all of these physical and behavioral characteristics have a higher chance of being sourced from the wild than those that do not (**Fig. 2**).

Fig. 2. Green pythons (*Morelia viridis*) presenting with emaciation **(left)** and a scrub python (*Morelia amethistina*) with a heavy parasite load **(right)**, suggesting that they have been sourced from the wild.

What is the process?

- General examination of snake/s kept at captive-breeding facilities to determine if they are presenting one or more of these symptoms (for example, if a snake presented with several ticks then concerns would be raised), and

- General examination of snake/s upon arrival at airport (in exporting or importing country) to determine if they are presenting one or more of these symptoms.

Examples of use

This method is commonly used by customs officials to identify shipments of animals suspected to be of wild origin (e.g., see More et al. 2013). It is also used by CITES Management Authorities to identify facilities whose stock are suspected to have originated from the wild and may require additional review.

Limitations

- Snakes born in captivity can still often present with one or more symptoms mentioned above, especially if facility workers raising snakes lack knowledge about the husbandry and captive care requirements for the species and/or are mixing captive snakes with legally collected wild specimens;

- Behavioral symptoms can be difficult to observe – limited access to facilities, switching of captive with wild snakes and the need to examine closely may prevent correct identification of symptoms;

- The need to examine snakes closely may be difficult for staff lacking confidence with handling snakes;

- General examinations can be time consuming; therefore this method may not be applicable to facilities/shipments/cargo with many snakes;

- Can only be used for live snakes, most notably those traded as pets. Snakes bred in captivity for skins, meat and other derivatives can be examined when alive but tracing live snake to product is problematic; and

- Examining snakes may be stressful for the animals and discouraged by importers/exporters.

Recommendations for application to snakes

1) Although not an unequivocal means of differentiating between wild and captive-bred snakes, general behaviour and appearance can be used as an indicator for the identification of facilities that may be mis-declaring the source of snakes;

2) Examination of a sample of reportedly captive-bred snakes within a shipment may be enough to detect instances where mis-declaration of source is suspected; and

3) If mis-declaration of source is suspected then the more unequivocal means of determining source (detailed below) should be employed.

Conclusion

This method is not an unequivocal means of differentiating between wild and captive-bred snakes and does not have forensic application. For example, poor housing conditions (e.g., males kept with males in the same enclosure may fight during breeding season and therefore exhibit scaring), inadequate heating and cooling (e.g., snakes may become sick and exhibit respiratory disease), poor hygiene (e.g., snakes may spend many months in water and contract skin infections – scale rot, etc.), lack of appropriate food for the size of the snake or mixing with legally acquired wild specimens can result in each of the behavioral and health symptoms listed.

This method is only applicable for live snakes and may be difficult to implement for large shipments. It is thus most suitable for the trade in snakes for pets. It is not possible to use this method for snake parts and derivatives. The only cost associated with the method is the time required by staff from either the exporting or importing Party to inspect animals, and it would be a useful indicator for determining which facilities require additional monitoring to ensure their declared sources of snakes legitimate.

Table 1. Scores for attributes for the method observing general health, appearance and behaviour of individuals (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**2**	Can be implemented relatively quickly after training is provided to staff to adequately identify cases where a wild source may be suspected.
Cost	**3**	No cost is associated with this method, other than the resources and staff time required to physically inspect facilities and shipments.
Suitability for live snakes and parts	**0**	Only suitable for live snakes, thus of limited use once snakes are killed for parts and derivatives (e.g., skins, meat).
Suitability for large and small numbers of snakes	**1**	Can be time consuming to examine large numbers of snakes but may require examination of many snakes because some wild individuals will not always present symptoms.
Reliability	**0**	This method is subjective and should be used as an indicator only. It must be used in conjunction with additional methods.
Labor intensity	**1**	Labor intensity of this method is high but depends on the number of snakes in question. Relevant authorities would be required to handle snakes to perform a general examination.

2.2 PHYSICAL, THERMAL AND CHEMICAL BRANDING

How does it work?

Physical, thermal and chemical branding consists of physically marking the skin of a snake using a suitable tool or chemical substance. Hot branding may utilise heated metals, soldering irons, medical cauterizers, and lasers. Freeze branding utilises lead typewriter letters cooled in a mixture of acetone or ethanol with dry ice and tools using liquid nitrogen. Chemical branding can be achieved by "burning" solutions (e.g., silver nitrate and potassium permanganate) or by injection of pigment under the snake's skin (Brown and Parker 1976; Lewke and Stroud 1974; Winne et al. 2006).

The premise is that the brand heals to become a scar, which can be used to uniquely identify an individual snake (**Fig. 3**). By applying a brand to snakes born in captive breeding facilities and recording their identity in a database, authorities can trace an individual throughout its life to verify that it has indeed been bred and raised in captivity. In nearly all cases, the brand must be applied to the live individual. The resulting scar may be retained on the skin if the skin is removed, but will not be visible on parts and derivatives that are not marked directly (e.g., meat and gall bladders).

What is the process?

- The relevant authorities decide on a suitable method of branding (e.g., thermal or chemical);
- Establishment of a reference database for all the individual marks and/or codes;
- Live snakes in captive breeding facilities are restrained and uniquely branded;
- Facility owners report to relevant authorities when branded snakes or their parts and derivatives are sold; and
- During follow-up inspections, branded snakes are examined to verify their marks and/or codes.

Examples of use

Branding of domestic livestock has been used for thousands of years as a means of identifying their owner. A variety of branding methods are also used for ecological studies of snakes, but to the best of our knowledge are not used for assisting in the determination of source. A form of branding called scar buttoning is used to verify the source of *Caiman* species bred for the skin trade in Colombia. Caimans are marked on the tail skin, which slowly heals into a characteristic "scar button" over the course of the individual's life. In this way a wild specimen cannot be captured, marked, and then immediately passed off as a captive specimen (http://cites.org/sites/default/files/notif/E-Notif-2014-033.pdf). We are aware of no similar application in snakes.

Fig. 3. A scrub python (*Morelia amethistina*) with a specific ventral scale clipped and branded to form a scar.

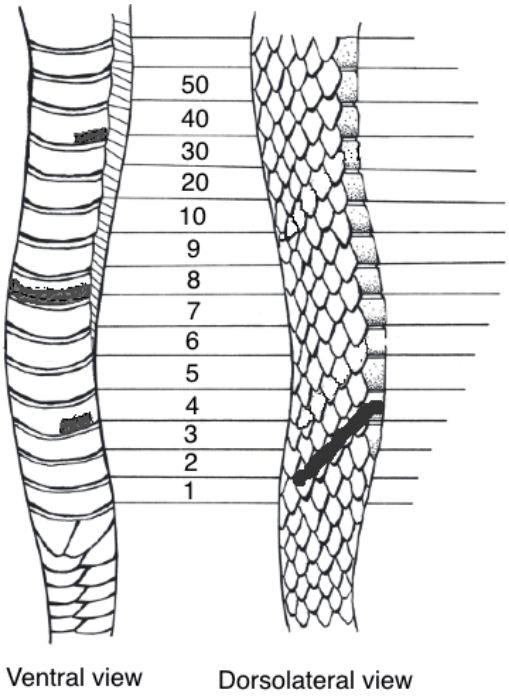

Fig. 4. A diagram for scale clipping and cauterizing snake scales for use in identification (adapted from Winne et al. 2006).

Limitations

- Facility owners can use the same methods to brand wild snakes and pass them off as captive bred provided sufficient time has passed for a scar to form;
- Not useful for snake parts and derivatives (such as gall, meat, fat);
- Unless relevant authorities are present when all snakes are processed then the method relies on honest reporting by the facility owner;
- A significant amount of manpower will be required to brand a large number of snakes, particularly in countries breeding thousands of snakes (and several species);
- Branding is not aesthetic for pets and skins and therefore may not be permitted by facility owners; and
- Some brands may be harmful to the health of the snake (particularly for small snakes)**.**

Recommendations for application to snakes

1) Marks and codes should only be administered by relevant authorities to minimise the risk of facilities branding wild snakes;
2) Relevant authorities should brand snakes soon after they are born to prevent facilities from branding larger wild individuals and to pass them off as captive-bred;
3) A database of brands for each species/facility combination should be kept by the relevant authorities to cross check against facility registers;
4) Snakes bred for skins may be branded on the head or tail, which are discarded from the exported skin and thus the brand does not reduce product value; and
5) Transportation records among facilities should include the unique mark and/or code of all snakes.

Conclusion

This method will be most suitable for snakes kept in small numbers within a few facilities, and is only suitable for exporting Parties because of the need to check facility stock against a database. Even with several fail-safes in place (e.g., branding snakes as soon as they are born at a facility), there is still scope for circumvention of this method (e.g., branding wild-caught snakes). Because of this, this method cannot be used to unequivocally differentiate between wild and captive-bred snakes. Logistic impediments also diminish the suitability of this method; costs will by high in terms of man-power, time and money needed to brand and record all snakes within a database, especially for Parties that have many facilities housing several different species in large numbers. Finally, this method may not be accepted by facility owners that require their snakes to be unmarked before export (unblemished skins or pets destined for high end markets).

Table 2. Scores for attributes for external markers (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**1**	Significant time will be required to brand all snakes at facilities.
Cost	**2**	The cost of tools is minimal, but considerable resources and staff time will be needed for ongoing branding, monitoring and database upkeep.
Suitability for live snakes and parts	**1**	Suitable for live snakes but only useful for their parts and derivatives if relevant authorities are present when branded snakes are killed.
Suitability for large and small numbers of snakes	**1**	Most suitable for small numbers of snakes.
Reliability	**1**	Only reliable for some scenarios and if considerable fail-safes are implemented to prevent circumvention of the method.
Labor intensity	**0**	Very labor intensive if large numbers of snakes in many different facilities are branded.

2.3 PASSIVE INTEGRATED TRANSPONDER (PIT) TAGS

How does it work?

A PIT tag is a small electronic microchip encased in biocompatible glass that varies in size (Gibbons and Andrews 2004). PIT tags are injected with a 12-gauge needle or inserted by surgical incision under a snake's skin or inside its body cavity (**Fig. 5**). They serve as a permanent coded marker that is as reliable as a fingerprint for identification of an individual snake. The implication of the term passive is that the tag is dormant until activated by a handheld reader with which the investigator scans a snake. If a PIT tag is present, the reader generates a close-range, electromagnetic field that immediately activates the tag, which transmits its number. This unique alphanumeric code permits a tagged individual to be distinguished from every other individual. The process is analogous to scanning bar codes in a grocery store (Gibbons and Andrews, 2004).

Using PIT tags, relevant authorities can uniquely mark and identify captive-bred snakes kept in breeding facilities. Snakes without PIT tags can be assumed to be of wild origin. The unique code allows authorities to determine which individual snakes have left the facility. Within CITES there is precedent for marking, with Res. Conf. 10.16 recommending that exports of captive-bred specimens listed in Appendix I be marked, with the mark recorded on the document authorizing the trade: http://www.cites.org/eng/res/all/10/E10-16R11C15.pdf.

What is the process?

- Establish a database of PIT tag codes;
- Visit facilities to tag and record all captive-bred snakes; and
- When snakes are traded, sold and/or killed, relevant authorities record the tag number of each snake to ensure their origin.

Examples of use

PIT tags have been used extensively for mark-recapture and population field studies involving wild snakes. They are commonly used for a variety of domestic pets as a means of registration and proof of ownership. For example, Broad headed snakes (*Hoplocephalus bungaroides*) are endangered in Australia due to collection for the pet trade. Small numbers are legally kept as pets in private collections and PIT tags have been used to uniquely identify individuals to prevent further collection and laundering of wild snakes.

Fig. 5. A Passive Integrated Transponder (PIT) tag inserted underneath the skin of a green python (*Morelia viridis*).

Limitations

- If injected under the skin, PIT tags are easily visible and can be removed captive-bred snakes and replaced under the skin of wild snakes;
- PIT tags can only be implanted within snakes above a certain size, meaning small wild snakes may be introduced into captivity and claimed to be captive-bred before tagging occurs;
- PIT tags can sometimes exit the body of the individual, or may migrate within the body;
- High purchase cost (USD $7-12 per tag) for each tag and tag readers;
- Low detection distance may require snakes to be removed from enclosures so the tag code can be recorded; and
- PIT tags have been known to result in the death of some individuals snakes.

Recommendations for application to snakes

1) PIT tags should only? be administered by relevant authorities to prevent misuse of tags;
2) This method should be used in cases where small numbers of snakes are housed in a few facilities; and
3) This method is most applicable for use on live snakes traded as pets.

Conclusion

PIT tags offer an improved method of marking compared to branding or scarring, and the use of alphanumeric codes means greater numbers of individuals can be identified. However, PIT tags are not a foolproof means of differentiating source and require intensive monitoring to be effective. Because they are injected underneath the skin, or within the body cavity, they are not suitable for determining the source of skins or other parts and derivatives (gall bladders, fat, meat). The overall cost and manpower needed to manage captive populations precludes the use of PIT tags in situations where farms are producing thousands of new snakes each year. By contrast, for species that are coveted as pets, and are kept in small numbers, application of PIT tags may be a simple and effective means of identifying specimens and monitoring captive breeding facilities, but can only be effectively applied within the exporting Party.

Table 3. Scores for attributes for internal markers (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**1**	Will take considerable time to establish a tag database and mark all snakes within facilities.
Cost	**0**	Very high ongoing costs, especially in situations requiring tagging of many snakes.
Suitability for live snakes and parts	**1**	Only suitable for live snakes (e.g., pets).
Suitability for large and small numbers of snakes	**1**	Only suitable for small numbers of snakes due to logistical and resource impediments associated with tagging and recording large numbers of snakes.
Reliability	**1**	Only reliable when accompanied by intensive monitoring.
Labor intensity	**0**	Very high ongoing labor cost due to the requirement to tag all new snakes produced at facilities.

2.4 EGGSHELLS

How does it work?

Many snake species are oviparous, meaning they lay a clutch of eggs. These eggs are leathery in texture and, after neonate snakes have hatched, keep their general shape and are consistent in dry mass with only minor compression and discolouring. Because a single egg corresponds to a single snake, the premise of this method is that if the animal has been bred in captivity there should be an associated eggshell to prove it. Eggshells of snake families vary in size and shape and consequently confusion between families is highly unlikely. For example, the eggs of pythons are roughly spherical or oval in shape and closely approximate a prolate spheroid. The eggshells of other snake families, such as Elapidae and Colubridae, are generally smaller and more elongate. Because of this, a species eggshell reference guide can be used to ensure that eggshells of one species are not substituted for another (e.g., Lyons and Natusch, 2011; TRAFFIC, 2013).

Instead of discarding the eggshells of captive-bred snakes, facilities could provide their respective CITES Management Authorities with an eggshell for each individual snake that is to be exported. These eggshells can be matched to the number of snakes to be exported to form a type of "quota", limiting the number of snakes to the number of eggshells and verifying provenance. These eggshells can be monitored by authorities within the exporting country or, additionally, exported together with the snakes. Logically, authorities would need only to examine a sample of the visually most "out of place" eggshells to ensure they originate from the species concerned.

What is the process?

- Gather information on known size and shape of eggshells for each oviparous snake species in question;
- Inspect facilities or shipments to confirm the number of eggs produced matches the number of snakes produced; and
- For each oviparous species of snake in question, accurately measure eggshell length, width or mass (using calipers and a weight scale) to ensure it matches eggs of the species being exported.

Examples of use

This method has been used to verify the origin of snakes in Asia. The Vietnamese CITES Management Authority has requested python farmers in Ho Chi Minh City to keep eggshells from captive-bred Burmese pythons (*Python molurus bivittatus*) to verify that they were indeed captive-bred and born at a facility. This method has also been successfully used by the CITES Management Authority of Thailand to verify records of Burmese pythons (*Python molurus bivittatus*) born at the country's only registered python facility. The CITES Management Authority conduct inspections during the breeding season and count the number of eggs produced by the facility, which are then used to calculate the facility's annual production figure.

Lyons and Natusch (2011) showed that this method could be successfully used to determine the origin of green pythons (*Morelia viridis*) in Indonesian breeding facilities. They measured 139 eggshells from five species of python bred at a facility in Indonesia and showed that each had a distinctive size and shape (**Fig. 6**). Simple measurement of eggshell length, width or mass is enough to differentiate between species of python.

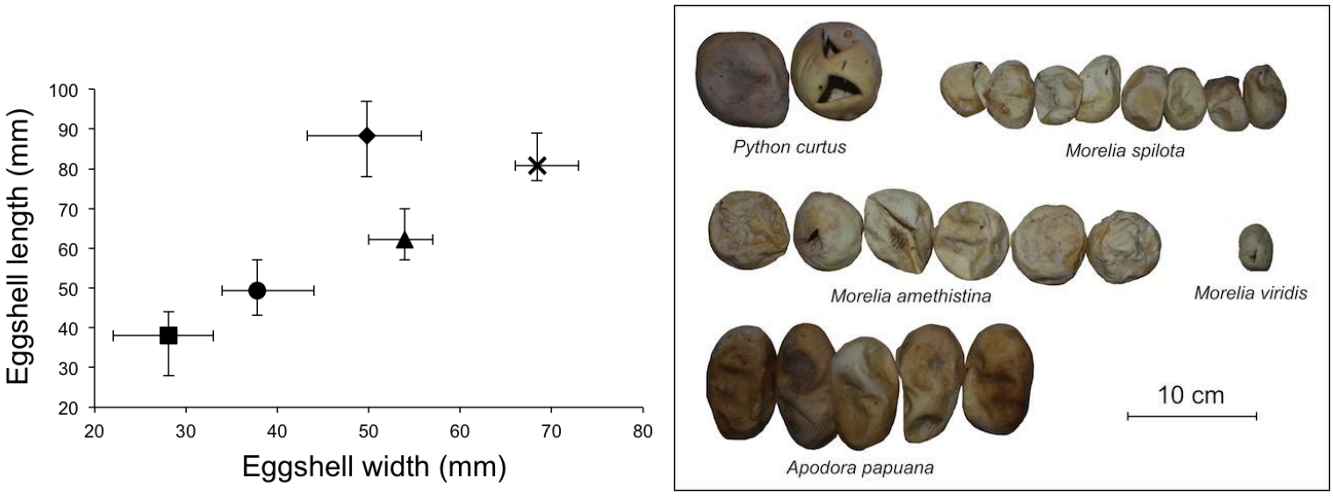

Fig. 6. (a) Length vs. width of pythonid eggshells, showing the distinctive shape of *Morelia viridis* (■), *Morelia spilota* (●), *Apodora papuana* (◆), *Morelia tracyae* (▲), and *Python curtus* (×). Error bars indicate the extremes of length and width for each species; **(b)** used Pythonid eggshells showing the distinctive size and shape of each.

Limitations

- Snakes that are bred may produce eggs but not a juvenile of export quality. This could potentially enable its eggshell to be attributed to what is in fact a wild snake;
- The eggshell method cannot be easily used for snake parts and derivatives (e.g., meat, fat) because products do not correspond to a single individual/egg;
- When exports of a single species comprise only few individuals (e.g., <100) eggshells from wild gravid snakes may be used to falsely prove captive origin; and
- If exported then CITES permits will be required for eggshells, which may require additional paperwork.

Recommendations for application to snakes

This method is most applicable for differentiating between captive-bred and wild snakes when:

1) The snakes being bred at the facility are oviparous;
2) “Whole” parts and derivatives (e.g., live snakes, whole skins, gall bladders) are traded, as each part or derivative corresponds to one snake;
3) Snakes are not bred in large numbers because it may be prohibitive to count thousands of eggshells and error margins increase.
4) Wild sourced snakes provide the entire supply to breeding facilities, as no eggshells would be available and therefore it would be straightforward to prove that snakes in the facility are wild rather than captive-bred.

Conclusion

This method has merit and could be used as an initial step in monitoring and enforcement to identify which facilities are providing fallacious breeding records. It would be most applicable to situations where facilities are mis-declaring the source of all snakes being exported, because simply the requirement to keep eggshells could not be satisfied. In addition, the method is cost effective (only requiring staff inspection time). Ultimately, the applicability of this method will depend greatly on the situation in the country in question, the species being monitored and the volume of trade. Because the applicability of the method across a broad range of scenarios is not comprehensive, we suggest it should be implemented in conjunction with another method in most situations.

Table 4. Scores for attributes of the eggshell method (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**2**	A reference guide for the size and shapes of eggshells must be established. Legislation would need to be modified to require facilities to keep eggshells to present upon application for export.
Cost	**3**	Relatively cost effective, as this method requires only an initial startup fee for an eggshell reference guide but does require staff time for ongoing monitoring.
Suitability for live snakes and parts	**2**	Best suited for live snakes or instances where snake parts and derivatives (e.g., skins, gallbladders) are traded whole. Suitability for snakes traded in parts (e.g., meat, fat) is minimal.
Suitability for large and small numbers of snakes	**1**	Use for small numbers of snakes may allow eggs from wild individuals to be used, while use for a large number may result in logistical impracticalities.
Reliability	**1**	Highly applicable for situations where facilities are exporting snakes but no breeding is occurring (wild snakes are being labeled as captive-bred). The reliability of this method depends on strict compliance and monitoring of facilities.
Labor intensity	**2**	Labor intensity is medium, but depends on the number of snakes exported. Relevant authorities would only be required to measure a sample of eggshells thus limiting handling time.

2.5 BREEDING NON-NATURAL MORPHS

How does it work?

All species of snakes exhibit a finite range of natural phenotypes. Because colour and pattern mutations in wild snake populations are extremely rare, the captive propagation of specimens exhibiting colours and patterns not displayed by wild conspecifics can therefore be used to distinguish between wild and captive-bred snakes. Replacement of all existing stock within captive breeding facilities with non-natural morphs would allow relevant authorities to inspect snakes to ensure that no natural morphs, that potentially represent wild-caught individuals, are present. Visually differentiating wild from captive animals using this method will be extremely rapid and straightforward given the obvious differences in pattern and coloration.

What is the process?

- Facilities can selectively breed or purchase snakes with non-natural colours and patterns; and
- These snakes become the breeding stock for a facility and can easily be checked by relevant authorities that are familiar with the appearance of natural morphs.

Examples of use

Although we are not aware of this method being directly used to differentiate between wild and captive-bred snakes, it is being used indirectly. Many facilities producing snakes for the pet trade focus on selectively breeding unique and non-natural morphs for sale. These can range from simple colour mutations to extreme hyper and hypo-melanism through to albinism. Because these morphs are extremely rare in the wild, authorities can be 100% certain that snakes exhibiting non-natural colours and patterns have originated from captive-bred stock.

This method can be used for snakes bred in captivity for their skins. Snake skins lose their natural coloration (and often the patterning) when the skin is tanned, thus reducing the relevance of the live specimen's skin color. For snake skin industries that covet the natural pattern of the snake (e.g., the Chinese market for traditional instruments), selective breeding can result in alteration of the snake's natural colour, but retention of its natural pattern. For example, the "Caramel" Burmese python morph exhibits natural patterning, but are instead caramel coloued rather than brown (**Fig. 7**).

Fig. 7. Naturally wild patterned Burmese (left) and reticulated (right) pythons (above) compared to selectively bred morphs of those same species (below) whose colours and patterns are non-natural. Note: photos taken at captive breeding facilities in range States.

Limitations

- Meat, gall bladders and other parts and derivatives cannot be identified as being wild or captive-bred; and

- Demand may be greatest for natural looking morphs (e.g., pets), resulting in some facility owners wanting to only keep and trade natural morphs.

Recommendations for application to snakes

1) Any non-natural morph chosen must be heritable (e.g., albinism, a recessive trait) to ensure the natural morph does not reappear in the captive population; and

2) Strict enforcement must be in place to ensure that captive non-natural morphs are not released into the wild population (although this should be already enforced for all captive-bred snakes due to possible introduction of exotic pathogens acquired in captivity, see Jacobson (1993)).

3) Training and guidelines on how to differentiate natural vs non-natural morphs may be needed for inspection authority staff.

Conclusion

Breeding and trading non-natural morphs of snakes that exhibit unusual coloration and pattern is a simple and cost-effective means of ensuring that those individuals were born in captivity. It is 100% reliable and the only cost is that of staff time to visually inspect facilities or shipments in either the exporting or importing Party, respectively. This method, however, does not have applicability for many parts and derivatives (e.g., meat, fat, gall bladders), unless the snake producing those parts has been visually sighted and verified to be captive-bred before it is killed. A disadvantage of this method is the time it will take captive breeding facilities to replace their existing captive stock with snakes exhibiting non-natural forms.

Table 5. Scores for the non-natural morph method (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**1**	May take considerable time before the method can be implemented because of the need for facilities to replace existing stock with non-natural morphs (particularly in countries with many thousands of snakes).
Cost	**3**	There is no cost associated with this method, other than the time required to visually inspect breeding facilities.
Suitability for live snakes and parts	**2**	Can easily be used for inspection of facilities producing snakes for any purpose. Can also be used for snakeskins, but not applicable for other parts and derivatives (e.g., meat, gall bladders).
Suitability for large and small numbers of snakes	**3**	Highly suitable for facilities housing only a few snakes to those with many thousands.
Reliability	**3**	Very high, but will not be applicable for parts and derivatives (e.g., meat, gall bladders).
Labor intensity	**3**	Minimal labour intensity involved, as it only requires brief visual inspection of snakes or their skins (in the facilities, or by customs in the exporting or importing Party).

2.6 PRESENCE OF GASTROINTESTINAL PARASITES

How does it work?

Many gastrointestinal parasites have complicated life cycles that require several hosts. Their indirect mode of reproduction means that certain life stages of parasites are unable to infect the host from which they came without transmission to one or several different hosts. However, there are also parasites that only require a single host to complete their life cycles.

The majority, if not all, of wild snakes are infected with parasite fauna that has been acquired throughout the course of their lives in the wild. That means the older a snake the higher the likelihood that it is infected. Gastrointestinal parasites can infect snakes through contaminated drinking water, from the soil, or through prey items that are also infected. By contrast, snakes bred in captivity usually do not have the opportunity to come into contact with parasites if they are given clean drinking water, live in relatively sterile enclosures and are fed captive sourced diets (e.g., laboratory rats).

We should expect, therefore, that the parasite loads of free ranging wild snakes would be much greater than captive-bred snakes. Of particular forensic application is the presence of parasites in snakes that require infection through an intermediate host (e.g., a snail or other invertebrate). It should not be possible for captive-bred snakes to acquire these parasites, indicating a wild origin for snakes presenting these infections.

What is the process?

- Establish an image database of known gastrointestinal parasites from wild snakes of the species of interest;
- Collect faecal samples from captive-bred snakes;
- Perform simple fresh smears and concentration procedures (flotation method) for diagnosis of eggs (helminthes and arthropods) and encysted protozoan stages;
- Examine samples under a microscope (calibrated to perform size measurements of particles) to check for the presence of parasites indicative of wild origin; and
- Compare with image database to confirm presence of parasites indicative of wild origin.

Examples of use

Several studies have shown that this method has the potential for differentiating between wild and captive-bred snakes, but at the time of writing this document it had not be applied or enforced in any snake breeding facility. Blood and faecal samples were taken from green pythons (*Morelia viridis*) exported from Indonesia to Germany and analysed for the presence of parasites with indirect lifecycles. Although the pythons were declared as captive-bred, they were infected with parasites requiring more than one intermediate host, suggesting that the snakes were in fact of wild origin (Ofner et al. 2012; More et al. 2013). To date, no studies have examined the efficacy of this method.

For this report, we examined and compared internal parasite faunas of wild reticulated pythons (*Python reticulatus*) to individuals that had been captive-bred and raised in captivity. This case study is presented in Box 1.

Box 1: Can internal parasites be used to differentiate between wild and captive-bred reticulated pythons (*Python reticulatus*)?

Research conducted by Sulaiman Ginting and Thomas Jäkel

Aim: To determine whether there are significant differences in the parasite faunas between wild and captive-bred reticulated pythons (*Python reticulatus*) from northern Sumatra, Indonesia.

Methodology: We randomly collected *P. reticulatus* scats from a facility in Medan, north Sumatra, Indonesia. All pythons were captive-bred and born at the facility and raised in large plastic containers or concrete cages, depending on their size. All enclosures are cleaned regularly to ensure the optimal health of the pythons. Pythons are fed on captive-bred laboratory rats (also bred at the facility) that are maintained on a standardised commercial laboratory diet. We also obtained scats from a random sample of wild *P. reticulatus* harvested in north Sumatra for the commercial skin trade.

We performed two types of scatological analyses on samples taken from the centre of python scats (to reduce contamination of samples, the outside of which may have been in contact with non-sterile surfaces). The first analysis involved directly examining wet smears on glass slides under a light microscope. The second analysis involved extracting parasites from fecal samples and concentrating them using a standard zinc flotation method. One gram of fecal sample was emulsified in a porcelain beaker in 16 ml of zinc sulphate solution and the suspension passed through brass wire into a 12.5 ml glass centrifuge tube. We placed glass coverslips over the centrifuge tubes and spun the samples in a centrifuge for two minutes. Coverslips were then removed together with their adherent concentrate and analysed under a light microscope.

Results: We compared internal parasite loads of 13 wild and 13 captive *P. reticulatus*. The results of our analyses are presented in Table 1, which clearly show a significant difference in the parasite faunas between the wild and captive-bred pythons examined.

Conclusion: The results of this case study reveal that the abundance and diversity of parasite fauna is much greater in wild than captive-bred pythons. In particular, the high prevalence of flagellates and ciliates in faecal samples from wild pythons may be a useful indicator for wild source. Of significant forensic interest is the Coccidian parasite *Sarcocystis singaporensis. S. singaporensis* was present in 100% of wild python samples, but none of the samples from captive-bred pythons. *Sarcocystis* sp. have indirect lifecycles requiring at least one intermediate host before finally infecting a definitive host. *S. singaporensis* is transmitted to pythons via infected wild rats and cannot be acquired in captivity. We conclude that examination of parasite faunas, and in particular the presence of specific parasites, can be a useful and definitive means of differentiate between wild and captive-bred snakes.

Table 1. Parasites of *Python reticulatus* detected in scats by combination of zinc sulphate flotation and wet smears. Sample sizes are presented in parenthesis.

	Captive-bred		**Wild-caught**	
Parasite taxa	Positive infections	%	Positive infections	%
Protozoa				
Flagellates	15 (27)	56	17 (17)	100
Ciliates	6 (27)	22	15 (17)	88
Amoebae	5 (12)	42	2 (13)	15
Entamoeba sp.	2 (12)	17	0 (13)	0
Endolimax	3 (12)	25	2 (13)	15
Coccidia	2 (12)	17	2 (13)	15
Caryospora sp.	1 (12)	8	0 (13)	0
Eimeria sp.	0 (12)	0	2 (13)	15
Cryptosporidium sp.	1 (12)	8	0 (13)	0
Sarcocystis singaporensis	0[#] (13)	7	13 (13)	100
Helminths				
Trematodes	0 (12)	0	1 (13)	8
Cestodes (*Bothridium* sp.)	1 (12)	8	4 (13)	31
Nematodes	3 (12)	25	11 (13)	85
Strongyloides sp.	3 (12)	25	0 (13)	0
Polydelphis & *Ophidascaris* sp.	0 (12)	0	4 (13)	31
Kalicephalus sp.	0 (12)	0	4 (13)	31
Capillarid eggs	0 (12)	0	2 (13)	15
Other	0 (12)	0	1 (13)	8
Arthropods (Pentastomes)				
Armillifer moniliformis	0 (12)	0	1 (13)	8

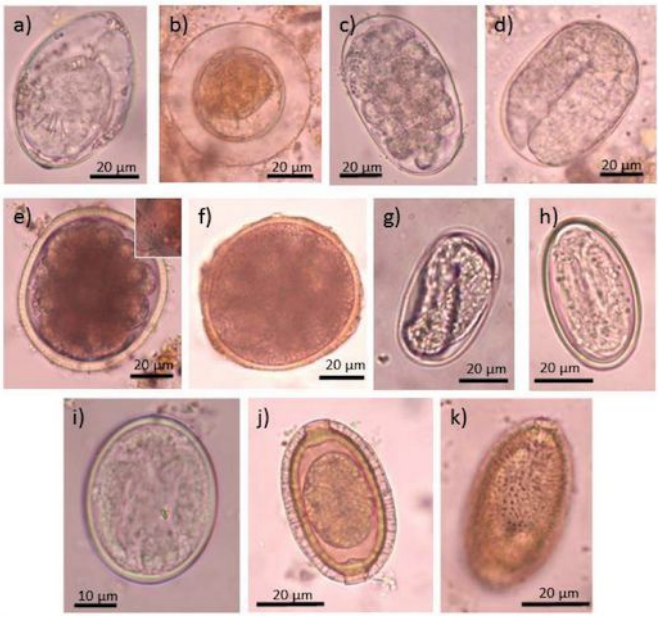

Fig. 8. An image database of parasites known to be indicative of wild origin is required, such as the one pictured.

Limitations

- Captive snakes that have been raised in natural environments or fed a diet comprising wild rats or other wild prey may likely become infected with the same parasites as wild snakes;
- Testing is useful only for live snakes and not parts and derivatives (e.g., skins, meat);
- This method is very labor intensive to test large numbers of snakes;
- Identification of parasites is complex and must be undertaken by a specialist; and
- Will not be applicable for juvenile snakes, which are usually free of parasites at birth. Wild hosts acquire parasites with increasing time of exposure.

Recommendations for application to snakes

This method is most applicable for differentiating between wild and captive-bred snakes when:

- Snakes are traded live for pets;
- Snakes are not bred in large numbers, as it may be prohibitive to take blood and faecal samples from thousands of snakes;
- Implementation of facility management protocols would ensure that captive-bred snakes are fed on prescribed diets that reduce opportunities for transmission and infection of certain parasites; and
- By sampling a small number of snakes in facilities, this method would provide an indication of which facilities are housing wild snakes and which are not.

Conclusion

This method has application for all CITES-listed snakes being traded internationally. Snake scat samples can be easily collected from facilities. Provided a sufficient image database of parasite faunas present in wild snakes is available, then samples can be compared to determine the likelihood of captive origin. Ideally, identification of a single, specific, parasite that is unequivocally associated with wild snakes will improve the efficacy of this method and significantly reduce the labor intensity and cost of ongoing sampling. It is most applicable to live snakes, which can be tested by either the exporting or importing Party, but less applicable for parts and derivatives (unless the live snake producing the parts is tested at the breeding facility in the exporting Party before it is killed). This method requires outsourcing to a laboratory (for microscopy) and cannot be conducted on site.

Table 6. Scores for attributes of presence of the gastrointestinal parasite method (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**1**	With experienced personnel this method can be implemented almost immediately, but relies heavily on a thorough knowledge of the types of parasites found in wild snakes.
Cost	**2**	The cost scat collection and employment of professional laboratory staff may be high if many samples are to be analysed. However, samples could be pooled to reduce analyses time.
Suitability for live snakes and parts	**1**	Only suitable for live snakes in captive breeding facilities.
Suitability for large and small numbers of snakes	**2**	Very suitable for small numbers of snakes, and may also have application for large numbers if specific indicator parasites can be quickly identified.
Reliability	**2**	Will be very reliable for situations where it is impossible for captive snakes to acquire certain parasites. It will not be reliable when captive snakes come in contact with modes of infection (e.g., captive snakes being fed wild rats).
Labor intensity	**1**	Reasonably labor intensive to collect scat samples from facilities and analyse in the laboratory.

2.7 DNA, GENOTYPING AND PARENTAGE ASSIGNMENT

How does it work?

All animals inherit their genetic makeup (DNA sequences of their genomes) from their parents. Some parts of the genome vary substantially between individuals and can be used to establish the genetic relationships among individuals within a single population, including determining an individual's parents. Offspring share a higher proportion of their genomes with their parents than they do with unrelated individuals in the same population. Thus, if a substantial part of the highly variable genome is examined we can establish if juvenile snakes are indeed the offspring of breeding stock in snake farms. Showing that a juvenile snake is not the offspring of the alleged breeding stock is straightforward and definitive with highly variable genetic markers, called microsatellites, or with more moderately variable markers, such as single nucleotide polymorphisms (SNPs). Genotyping, determining DNA sequences of a selected proportion of the genome, is readily done from any tissue that contains nucleated cells. For snakes, scales, shed skin, blood and/or internal tissue are suitable for genotyping. This also means that live snakes can be genotyped without detriment.

If the objective of the genotyping is to determine if snakes have been bred in captivity, then genotypes of the breeding stock are needed as an essential reference database. Genotyping only needs to be done once for each snake. Genotyping of offspring can be done independently of genotyping of breeding stock (i.e., at a later date or at a different facility). Suitable samples for genotyping can be taken from all offspring and stored indefinitely. To reduce costs, offspring genotyping could be done on a randomly selected set of individuals and if necessary for a particular case the remaining samples from that facility could then be genotyped as well. If the objective of the genotyping is to determine the source of snakes that have not been bred at a facility, then a comprehensive sampling of wild populations is required. This would be a substantially larger task, the size of which would be determined by what geographic scale of specificity is required.

What is the process?

- Collect and store samples for genotyping breeding stock;
- Develop and validate genetic markers and genotyping methods;
- Develop sample tracking, information handling and a data base system;
- Genotype breeding stock and store genotypes in database;
- Genotype offspring on demand and analyze with putative breeding stock; and
- Report parentage analysis.

Examples of use

Using microsatellites is widely employed in human forensics and parentage analysis and is thus conceptually, practically and statistically robust. It has been extended to forensics in a wide variety of domesticated and wildlife species, such as salmon, wolves, bears, dogs, cats, and elephants (Andreassen et al. 2012; Kraus et al. 2014; Martinsohn et al. 2009; Mucci et al. 2014; Schury et al. 2014; Wasser et al. 2008).

More recently, in DNA forensics for humans and wildlife species, genotyping of single nucleotide polymorphisms (SNPs) is being developed and applied (Glover et al. 2010; Moreno et al. 2014). Methods for genotyping SNPs vary as several technical approaches and instruments to implement them are available. SNPs can be genotyped with next generation DNA sequencing technology, which provides the ability to genotype large numbers of these markers in a less labor intensive manner (Nielsen et al. 2012;

Ogden et al. 2013), but this approach is yet to be developed widely for application in human and wildlife forensics.

Limitations

- Ongoing and relatively high cost of analysing samples;
- Ongoing monitoring, sampling and analysis of breeding stocks within facilities is required;
- Logistically difficulty to locate and test all breeding stock within many facilities;
- Establishing and maintaining a reliable sample and sample information tracking system from facility to laboratory; and
- Legal difficulties may occur when the putative parent or parents die and are not available to establish parentage.

Recommendations for application to snakes

1) Strategic consideration of the genotyping technology is required to deal with technological evolution. For example, microsatellite technology is readily implemented now but is not likely to be practical in several years as commercial facilities that do the final determination are likely to discontinue this service as next generation DNA sequencing technology replaces microsatellite genotyping.

2) Alternatives such as SNPs already exist but require initial development that the existing microsatellite approach has already achieved. However, the next generation DNA sequencing technology has many advantages over microsatellites for long-term projects such as monitoring parentage in facilities producing snakes. The advantages of next generation DNA sequencing data relate to its portability, straightforward interpretation and less labor-intensive technology.

3) Because of the cost of analysing samples, large volume imports should be randomly sampled to verify source.

Conclusion

This approach is most suitable for facilities producing small numbers of snakes. Countries where many farms are breeding and trading thousands of snakes will find the cost and logistical challenges too great to effectively implement this method. For example, in Indonesia there are facilities with breeding stock of five individual boelens python (*Morelia boeleni*) that produce approximately 50 juveniles each year; it would be feasible to genotype using next generation sequencing methods in this case.

However, there are other countries, such as Vietnam, that produce hundreds of thousands of snakes of a single species (e.g., Burmese python, *Python molurus bivittatus*) every year. These snakes are produced from a substantial number of breeders located in more than 400 facilities across the country. New breeding stock is introduced every year as older breeders become senescent. Given these sorts of numbers, the total cost of genotyping will be prohibitive. On the other hand processing very large numbers of snakes means that the cost will drop as parallel handling of large volumes of samples reduces individual costs and drives the introduction of mass processing approaches and robotic instrumentation. DNA technology could be useful for high value snake species exported in small numbers from a few farms (e.g., the previous example of *Morelia boeleni*), but it would be logistically difficult to use effectively when hundreds of thousands of snakes are involved for a range of other species.

Table 7. Scores for attributes of the DNA genotyping method (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**0**	The requirement to sample and analyse tissue from ALL parents in ALL farms will take a substantial amount of time.
Cost	**0**	High cost due to sampling many snakes and need to re-sample each generation.
Suitability for live snakes and parts	**3**	Suitable for live snakes or any part and derivative with viable DNA.
Suitability for large and small numbers of snakes	**1**	Very difficult for large numbers of snakes due to massive sampling efforts and costs, but offspring could be randomly sampled as a deterrent with follow up if illegal activity is detected in the initial sample. Useful for small numbers of captive snakes producing few offspring annually.
Reliability	**3**	If ALL snakes are sampled in ALL facilities then the method should be extremely reliable. The alternative is offspring are randomly sampled as a deterrent with follow up if illegal activity is detected in the initial sample
Labor intensity	**1**	Need to continually sample breeding stock, as breeding snakes are replaced, and establish database.

2.8 STABLE ISOTOPES

How does it work?

Isotopes are different forms of the same element with greater or fewer numbers of neutrons than their sister forms. For example, Carbon 12 (^{12}C), ^{13}C and ^{14}C are all isotopes of the element carbon. These distinctive isotopes occur at different ratios within all materials and this property can be utilised to differentiate between wild and captive-bred snakes. This succeeds because stable isotopes are propagated from one organism to another through food assimilation and growth (Rojas et al. 2007). For example, herbivores acquire an isotopic value from the plants they eat and this value is reflected up the food chain as predators consume herbivores (McCarthy and Waldren, 2000; Satterfield and Finney, 2002).

Diet is the primary determinant of animal isotopic compositions, and is perhaps the most obvious difference between wild and captive-bred specimens. For example, a wild snake may feed on a variety of prey such as lizards, birds and small mammals, while a captive snake is usually fed on a single food source (e.g., laboratory rats). Because these food items differ in their isotope ratios, measurement of these ratios can elucidate the source of the snake of interest. Thus stable isotopes offer a powerful tool for differentiating between wild and captive-bred snakes because isotopic compositions of tissues are a measurement of the assimilated (not merely ingested) diet, and reflect the short or long-term history of wild and captive snakes.

For this method to be implemented, tissue must be collected and analysed (usually with a mass spectrometer) from *known* wild and captive snakes. Once a database of wild and captive isotope ratios has been established, samples of unknown origin can be compared.

What is the process?

- Collection of tissue samples from *known* sources of wild and captive-bred snakes;
- Laboratory analysis of tissue samples using a mass spectrometer;
- Establishment of database with isotope ratios for wild and captive-bred snakes to allow comparison of tissue samples from an unknown source;
- Collection of tissue samples from snakes (and/or their parts and derivatives) of *unknown* source; and
- Laboratory analysis of tissue samples using a mass spectrometer and comparison to established database with isotope ratios for *known* wild and captive-bred snakes to verify the source of the tissue sample in question.

Examples of use

The most common use of stable isotopes in wildlife management has been to distinguish wild from farmed fish. A number of studies have done this successfully, most employing isotopes of Carbon and Nitrogen, for farmed and wild salmon (Dempson and Power, 2004), Australian Prawns (Carter et al. 2015) and Bream (Rojas et al. 2007). This method has also been used for other species such as free range vs. caged chicken eggs (Rogers 2009), wild vs. relocated cycads (Retief et al. in press) and farm bred vs. wild mink (Hammershoj et al. 2005). All studies utilising stable isotopes to distinguish wild from captive-bred specimens have relied on differences in the diets of individuals from different sources.

Limitations

- Depending on the species, it may be difficult and/or time consuming to collect tissue from known sources (e.g., uncommon cryptic snakes) when establishing a database of known isotope signatures;

- The cost of sampling may be prohibitive for some snake species (e.g., when the value of the snake is low); and

- May not be applicable where captive-bred snakes are fed wild prey items (e.g., wild rats in Viet Nam).

Recommendations for application to snakes

Stable isotopes analysis can be used for live snakes, skins, gall bladders, meat, bones, and fat.

1) It is essential to ensure that tissue samples collected to establish a database of *known* wild and captive-bred snakes are from 100% proven sources; and

2) Because of the cost of analysing samples, large volume imports should be randomly sampled to verify source.

Conclusion

This method has considerable potential for accurately determining the source of wild and captive-bred snakes. In the majority of cases, the reliability of this method is high and can be used to unequivocally detect instances of mis-declaration. For many snake species found in trade, stable isotopes analysis is straightforward to implement by exporting or importing Parties. However, the cost of sampling may be prohibitive. Analyses must be conducted by a specific isotopes laboratory and the cost of each sample can be high. Therefore, when large volumes of skins or meat are exported in a shipment it may be feasible only to analyse a sample of the skins or meat products. This method does not require pre-marking of wild snakes, and there is no possible means to remove or alter source by the collector; relevant authorities will be able to distinguish between wild and captive-bred sources for many years after collection from the wild.

Table 8. Scores for attributes for stable isotopes method (0 = poor, 3 = good).

Variable	Score	Explanation
Time to implement	**2**	A database of known isotope ratios with the tissue of interest from wild and captive-bred snakes needs to be established. Once established, tissues of unknown source can be compared to determine source.
Cost	**1**	Costs to determine isotope ratios from *known* wild and captive-bred snakes may be low to high. Ongoing costs for sampling the source of exported snakes will be high, with estimated prices starting from USD $35 per sample.
Suitability for live snakes and parts	**3**	Will not affect the survival of live snakes and can be used for skins, meat, fat and other snake parts and derivatives.
Suitability for large and small numbers of snakes	**2**	Highly suitable for small numbers of snakes and their parts and derivatives, but would require randomized sampling of large numbers due to the cost of analysis.
Reliability	**3**	Reliability is high, providing that a sufficient database is constructed to encompass possible variation among tissue samples.
Labor intensity	**2**	Depending on the species, collection of samples for database establishment can be simple or labour intensive. Once the database is established, collection of tissue samples and laboratory work are straightforward.

3.0 SUMMARY AND CONCLUSION

The suitability of all source differentiation methods is represented in Figure 9. The breeding of non-natural morphs appears to be the most reliable and cost and effort effective method for differentiating between both small and large numbers of snakes. However, this method is more limited in its applicability to parts and derivatives where examination of skins is not available (meat, gall bladders and fat). For forensic application, stable isotope methodologies offer a reliable means of differentiating between wild and captive-bred snakes in all forms (live animals and their parts and derivatives). However, isotope analyses can be costly for large numbers of snakes and require some time to implement.

A summary of where each method can be applied (in the exporting or importing Party) and if it can be conducted on site (e.g., at the facility or at customs) is provided in **Table 9**. Although some methods possess attributes such as minimal cost and low labor intensity, when choosing a suitable method Parties need to focus on those that are most reliable for their situations. For example, trade in parts and derivatives will often require sophisticated forensic methods (e.g., stable isotopes) to overcome the homogeneity of the product (e.g., meat). Other trades, such as the trade in small numbers of snakes for pets, may not require sophisticated methods to simply differentiate between small numbers of live snakes. In some cases it may be most beneficial to implement several methods in parallel.

Table 1. Summary of where each method can be implemented and if laboratory analysis is required.

Method	Exporting Party	Importing Party	Lab required
Health and appearance	Yes	Yes	No
External markers	Yes	No	No
PIT Tags	Yes	No	No
Eggshells	Yes	Maybe	No
Non-natural morphs	Yes	Yes	No
Parasites	Yes	Yes	Yes
DNA	Yes	No	Yes
Stable Isotopes	Yes	Yes	Yes

Implementation of methods should ideally be accompanied by a management plan, particularly with regard to dedicated funding. Although several techniques may be cost prohibitive for single facilities, establishment of nationally funded networks for inspection may allow more sophisticated techniques to be implemented. Additionally, small industry levies paid to regulatory bodies may allow spot-checks to be completed and may lead to establishment of more sophisticated certification systems for snake captive breeding facilities in general.

Although many of the methods presented herein will be useful for ensuring legal and sustainable trade in CITES-listed snakes, Parties should explore methods that are equivalent to (or lower than) the benefits of trade. Implementation of sophisticated methods may not be appropriate or economically viable for some corresponding trade situations and this should be reflected in the choice of method. Closely linking technical solutions to trade problems will help tease apart where and how these methods can be most effectively used. Thus, regulatory bodies wishing to implement one or more of these methodologies should undertake pilot studies to determine the efficacy of the method for their trade situation.

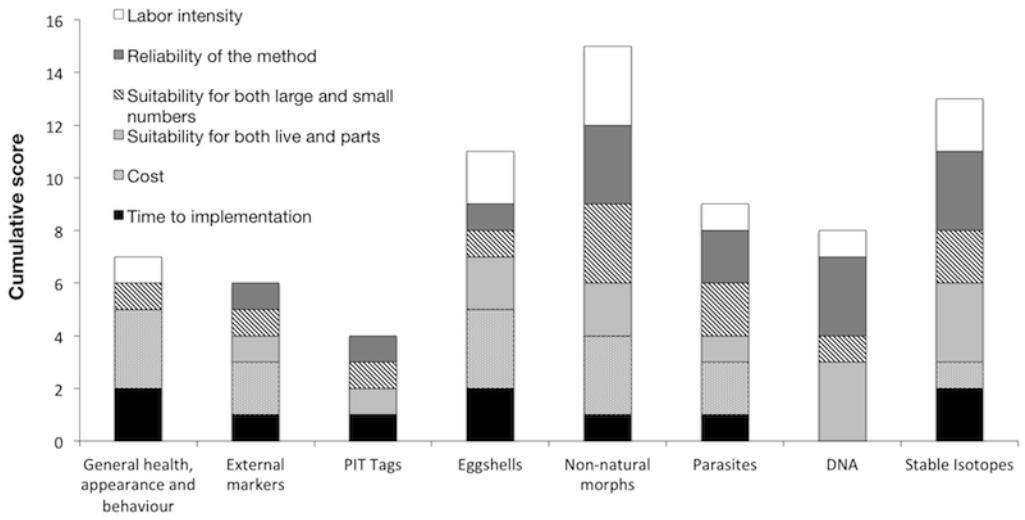

Fig. 1. A matrix of scores for attributes of each method used to differentiate between wild and captive-bred snakes. For each attribute, a score of 0 indicated that the method is poor, while a score of 3 indicates that it is very good (Scores assigned by the authors).

4.0 REFERENCES

Andreassen R, Schregel J, Kopatz A, Tobiassen C, Knappskog PM, Hagen SB, Kleven O, Schneider M, Kojola I, Aspi J, Rykov A, Tirronen KF, Danilov PI & Eiken HG 2012, 'A forensic DNA profiling system for Northern European brown bears (*Ursus arctos*)', *Forensic Science International: Genetics* vol. 6, pp. 798-809.

Brown WS & Parker WS 1976, 'A ventral scale clipping system for permanently marking snakes', *Journal of Herpetology* vol. 10, pp. 247-249.

Carter JF, Tinggi U, Yang X & Fry B 2015, 'Stable isotope and trace metal compositions of Australian prawns as a guide to authenticity and wholesomeness', *Food Chemistry* vol. 170, pp. 241-248.

Dempson JB & Power M 2004, 'Use of stable isotopes to distinguish farmed from wild Atlantic salmon, *Salmo salar'*, *Ecology of Freshwater Fish* vol. 13, pp. 176-184.

Freeland WJ & Fry K 1995, 'Suitability of Passive Integrated Transponder tags for marking live animals for trade', *Wildlife Research* vol. 22, pp. 767-773.

Gibbons WJ & Andrews KM 2004, 'PIT Tagging: simple technology at its best', *BioScience* vol. 54, no. 5, pp. 447-454.

Glover KA, Hansen MM, Lien S, Als TD, Høyheim B & Skaala Ø 2010, 'A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment', *BMC Genetics* vol. 11, p. 2.

Hammershøj M, Pertoldi C, Asferg T, Møller TB & Kristensen NB 2005, 'Danish free-ranging mink populations consist mainly of farm animals: evidence from microsatellite and stable isotope analyses', *Journal for Nature Conservation* vol. 13, pp. 267-274.

Hilderbrand GV, Jenkins SG, Schwatz CC, Hanley TA & Robbins CT 1999, 'Effect of seasonal differences in dietary meat intake on changes in body mass and condition in wild and captive brown bears', *Canadian Journal of Zoology* vol. 77, pp. 1623-1630.

Jacobson ER 1993, 'Implications of infectious diseases for captive propagation and introduction programs of threatened/endangered reptiles', *Journal of Zoo and Wildlife Medicine* vol. 24, no. 3, pp. 245-255.

Kraus R, vonHoldt B, Cocchiararo B, Harms V, Bayerl H, Kühn R, Förster DW, Fickel J, Roos C & Nowak C 2014, 'A single-nucleotide polymorphism-based approach for rapid and cost-effective genetic wolf monitoring in Europe based on noninvasively collected samples', *Molecular Ecology Resources* vol. 15, no, 2, pp. 295-305.

Lewke RR & Stroud RK 1974, 'Freeze branding as a method of marking snakes', *Copeia* vol. 1974, pp. 997-1000.

Lyons JA & Natusch DJD 2011, 'Wildlife laundering through breeding farms: illegal harvest, population declines and a means of regulating the trade of green pythons (*Morelia viridis*) from Indonesia', *Biological Conservation* vol. 144, pp. 3073-3081.

Martinsohn J & Ogden R 2009, 'FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing', *Forensic Science International: Genetics Supplement Series* vol. 2, no. 1, pp. 294-296.

McCarthy ID & Waldron S 2000, 'Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios', *Rapid Communications in Mass Spectrometry* vol. 14, pp. 1325-1331.

McDiarmid RW 2012, *Reptile biodiversity: standard methods for inventory and monitoring*, University of California Press, USA.

More G, Pantchev N, Herrmann DC, Vrhovec MG, Öfner S, Conraths FJ & Schares G 2014, 'Molecular identification of *Sarcocystis* spp. helped to define the origin of green pythons (*Morelia viridis*) confiscated in Germany', *Parasitology* vol. 141, no. 5, pp. 646-51.

Moreno F, Freire-Aradas A, Phillips C, Fondevila M, Carracedo Á & Lareu MV 2014, 'SNP variation with latitude: analysis of the SNPforID 52-plex markers in north, mid-region and south Chilean populations', *Forensic Science International: Genetics* vol. 10, pp. 12-16.

Mucci N, Mengoni C & Randi E 2014, 'Wildlife DNA forensics against crime: resolution of a case of tortoise theft', *Forensic Science International: Genetics* vol. 8, pp. 200-202.

Nielsen EE, *et. al.* 2012, 'Gene-associated markers provide tools for tackling illegal fishing and false eco-certification', *Nature Communication* vol. 3, p. 851.

Nogueira S., Nogueira-Filho, S. 2011, Wildlife farming: an alternative to unsustainable hunting and deforestation in Neotropical forests? *Biodiversity Conservation* vol. 20, pp. 1385–1397.

Öfner S 2013, 'The Reptile Rescue Center Munich, Germany: challenges and interesting cases', *20th Annual ARAV Conference*, 15-19 September, Indianapolis, Indiana, USA.

Öfner S., Baur, M.B., Blahak, S.C., Friz, T. Turbl, T. Pantchev, N & Hoffmann, RW 2012, 'Possibilities to differentiate wild born from captive-bred reptiles', *International Conference on Reptiles and Amphibian Medicine*, 13-14 May, Cremona, Italy.

Ogden R, Gharbi K, Mugue N, Martinsohn J, Senn H, Davey JW, Pourkazemi M, McEwing R, Eland C, Vidotto M, Sergeev A & Congiu L 2013, 'Sturgeon conservation genomics: SNP discovery and validation using RAD sequencing', *Molecular Ecology* vol. 22, pp. 3112-3123.

Rataj AV, Lindtner-Knific R, Vlahović K, Mavri U & Dovč A 2011, 'Parasites in pet reptiles', *Acta Veterinaria Scandinavica* vol. 53, p. 33.

Retief K, West AG & Pfab MF 2014, 'Can stable isotopes and radiocarbon dating provide a forensic solution for curbing illegal harvesting of threatened cycads?', *Journal of Forensic Sciences* vol. 59, no. 6, pp.1541-51

Rogers K 2009, 'Stable isotopes as a tool to differentiate eggs laid by caged, barn, free range, and organic hens', *Journal of Agricultural and Food Chemistry* vol. 27, pp. 4236-42.

Rojas JMM, Serra F, Giani I, Moretti VM, Reniero F & Guillou C 2007, 'The use of stable isotope ratio analyses to discriminate wild and farmed Gilthead Sea Bream (*Spares aurata*)', *Rapid Communications in Mass Spectrometry* vol. 21, pp. 207-211.

Satterfield SR & Finney BP 2002, 'Stable isotope analysis of pacific salmon: insight into trophic status and oceanographic conditions over the last 30 years', *Progress in Oceanography* vol. 53, pp. 231-246.

Schury N, Schleenbecker U & Hellmann AP 2014, 'Forensic animal DNA typing: allele nomenclature and standardization of 14 feline STR markers N', *Forensic Science International: Genetics* vol. 12, pp. 42-59.

TRAFFIC 2013. Inspection manual for use in commercial reptile breeding facilities in Southeast Asia. http://www.cites.org/sites/default/files/eng/com/ac/27/E-AC27-Inf-17_0.pdf

Wasser SK, Clark WJ, Drori O, Kisamo ES, Mailand C, Mutayoba B & Stephens M 2008, 'Combating the illegal trade in African elephant ivory with DNA forensics', *Conservation Biology* vol. 22, pp. 1065-1071.

Winne C, Willson J, Andrews K & Reed R 2006, 'Efficacy of marking snakes with disposable medical cautery units', *Herpetological Review* vol. 37, pp. 52-54.