Testing an alternative capture-analysis-release approach to document the reptile fauna of Hon Ba Nature Reserve, central Vietnam

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Abstract

Collecting or not collecting voucher specimens for identification and preservation is still a big controversy between experts around the world. In Vietnam, herpetological research currently involves the collecting of animals and voucher specimens. Three animals can be collected legally for each species. The importance of voucher specimens is universally accepted. However, some groups, especially turtles and snakes, are very rare in nature. Hence, alternative methods for reptile fauna need to be suggested and considered. Here, we test an exhausted "capture-analysis-release" approach that allows for successful fauna researches on reptiles without killing animals but original data for each taxon can be checked morphologically and genetically. The approach applied successfully to 28 of 30 recorded reptiles, including snakes, agamids, geckos, and skinks from Hon Ba NR, Vietnam. Based on this success of 93%, we suggest that this prior approach should be applied strictly to turtles, big, rare, well-known or well-identified species, and small and vulnerable populations in Vietnam. For other reptiles, except for tiny species which should not be applied the technique to, it is reasonable to reduce number of collected animals for each taxon in reptile fauna research from three to zero or one. This initial result can be scientific basis for policy managers in Vietnam in the future to reduce number of legal animal collected in faunal researches in protected areas.

Key words: CAR approach, herpetofauna, photography-based taxonomy.

INTRODUCTION

In recent years, collecting or not collecting voucher specimens for identification and preservation in museum is still a big controversy between experts around the world. Minteer *et al.* (2014) supposed that collecting specimen can be one of the reasons for extinction risk of rare species (occurred in great auk) and small, vulnerable and often isolated populations. Instead of killing animal for identification and preserved specimens, they suggested alternative methods, including high-resolution photography, audio recording, and nonlethal sampling. Immediately, Minteer and his colleagues were strongly opposed by hundreds of experts (Rocha *et al.* 2014; Krell & Wheeler 2014) who protect traditional collecting technique. Lately, Pape (2016), supported by 34 signatories, argued for a new technology that allows species can be name based on photos without preserved type specimen. Immediately, the opinion was strongly opposed by nearly 500 experts (Ceriaco *et al.* 2016; Jager 2016) who also support traditional collecting method. We herein keep out of the arguments. Instead of naming species, we mainly focus on identifying known species in the filed by testing a new technique for herpetofauna research in Vietnam. Our purposes are (1) to know if reptiles in Vietnam can be identified in the field without killing and (2) to develop new tools for confirming taxonomy and data obtained in the field.

Vietnam is a biodiversity hot-spot. Herpetological researches in Vietnam currently involve the collecting animals for identification in lab and the deposition of specimens in museums. Legally, three or more animals for each species are allowed to collect for research purpose (Official gazette no. 512+522, date 22 May 2014, by the Vietnam Government Office). The importance of voucher specimens is universally accepted. However, many independent research groups often collect specimens repeatedly at the same area. Most of small animals, such as insert, crustacean, small fish, mollusca, etc. are dense and therefore collecting three or more specimens may not affect seriously their population. However, some animal groups, especially snakes and turtles, are very rare in nature. For example, we conducted eight field trips within three years at a nature reserve in southern Vietnam and saw 20 of 22 snake species (91.9%) only one time. This implies that population sizes of these snakes are very small. Hence, an alternative approach for biodiversity researches would promote conservation. With regard to this, we test and suggest a new detailed approach that promotes herpetological research on reptiles without killing them or limiting killed animals.

Following the trend in conservation, in the future Vietnam may reduce or stop collecting known reptile specimens in protected areas. Hence, it is necessary to test this un-voucher approach making scientific basis to change the current law in future.

This work was conducted at Hon Ba Nature Reserve (~ 19,000 ha, highest peak 1578 m a.s.l.), Khanh Hoa Province, southern Vietnam with three field trips, about ten working days for each trip. The reptile fauna in Hon Ba Nature Reserve (NR) has been received less concern. In 2010, a new gecko (*Cyrtodactylus yangbayensis* Ngo & Onn 2010) was described from the area. Vassilieva (2015) recently recorded 24 reptile species in Hon Ba without morphological description. Hence, the reptile fauna there has been underestimated.

MATERIAL AND METHODS

"Capture-analysis-release" (CAR) approach was used and described as follows.

Capture. Field surveys were conducted essentially at night along streams and trails in forest using strong lights. Animals were collected by hand or using a stick for venomous snakes. At each sampling site, one animal was kept in one plastic bottle or box with small holes for air exchanging; the same codes for the site were marked in the bottle/box and in GPS. Co-ordinates, habitat, and other ecological data were recorded. The route was also tracked with GPS to use when releasing animals. All living samples were taken back to the camp and kept far from ants, rats, and other potential predators.

Analysis. This step was done at the camp and in the daytime by at least two people. Living animals were treated one by one.

- *Photographing*: This work is very important to get "voucher" photos. Technically, this practice requires a good camera with macro lens and flash and aperture is set to as high as possible value, perfectly higher than f/20, to get a deep clear field. The work includes the following steps:

(1) Lay a field or museum number beside the living animal and take a photo covering both the number and animal.
(2) Remove the tag and continue photographing the animal in different angles. Keep in mind that all photos following the photo with the number are of the same specimen. Basically, photos of dorsal, lateral, and ventral views of the animal must be taken. An artificial

photo “studio” with microhabitat should be prepared for each species to get natural photos and prevent animals from escaping.

(3) Photograph for scale counting characterizing: All required characters for scale, such as supralabial, infralabial, loreal, dorsal, subcaudal, etc. must be taken for scale counting and describing. Most of the characters are shown completely on one photo. However, some characters (for instance, ventral, subcaudal, mid-body scale rows, etc.) may not be performed on one photo. In the case, the area with interested character is split into parts using pen so that each part corresponds with one shot of photo (Figure 1). For example, a photo taking all ventrals or subcaudals of a snake may not clear for counting. Hence, the venter should be split into parts and taken photo the part in turn (Figures 2B–D). During the photographing, the aperture can be changed to obtain clear photos because the objects (e.g. the dorsum and venter of skink) may be different in light.

A suitable number of split points are given dependent on the analyzed animal. Figure 1 illustrates how to split and take photo of mid-body scale rows in typical snake and lizard in Vietnam. For snakes (Figure 1A), body scale rows exclude the wide ventral. Two sites (indicated by arrows) on dorsal-lateral sides of the body are marked and therefore three different photo angles need to be taken. For snakes with narrow ventrals, more split sites and photo shot angles are required. In the simple case with enlarged vertebral scale row, two photos on two lateral sides can be satisfiable. Similarly, figures 1B and 1C apply to typical skinks (genera *Lygosoma*, *Sphenomorphus*, *Scilcella*, *Eutropis*, etc.) and agamids (genera *Acanthosaura*, *Calotes*, *Pseudocalotes*, etc.) in Vietnam.

(4) Photograph for measurement: each measurement is taken photo showing both the character and the number obtained (Figure 3).

Camera systems used in this work were Canon 700D with 60 mm macro lens and Nikon D300 with 60 mm macro lens.

- *Morphological analysis*: Materials for identification must be ready in the field. Electric book or paper (portable document format - PDF) are more convenient than printed literature. Measurement and scale counting of morphological characters were done simultaneously with photographing. Because of no voucher specimens available for checking again in future, all measurement and scale counting are taken with photos in case reading errors occur. For each animal group, a suitable data sheet was prepared in advance to obtain all necessary characters of the specimens.

Measurements were taken with a digital caliper (nearest 0.1 or 0.01 mm) except for snout-vent length and tail length which were measured with a tape caliper (nearest 1 mm). Scale counting was done by eye (for big snakes) or using camera with macro lens and a stereo microscope (for gecko scale and tiny skinks or blind snakes). Because of having photo, all scale characters and measurements can be checked and counted repeatedly on computer.

- *Marking and taking tissue*: Marking animal by taking a piece of tissue for DNA analyses was done finally. Because the animal is released, the mark will eliminate the repeated sampling. For lizards, one finger or toe was cut and tip of tail was also taken for tissue because most of lizard tails can be regenerated. For snakes, a subcaudal scale for tissue and marking was removed. Scissors and clamp were cleaned by alcohol 70% and wound was treated by antiseptic (povidine 10%) to prevent infection. Tissues were stored in 95% alcohol.

Release. Animals were released at the site of its capture based on the codes in bottle, route, and GPS. Before releasing, all photos must be saved as two copies on different drives in case electronic error occurs.

RESULT

A total of 53 specimens of 30 reptile species (including 10 snakes and 20 lizards) were recorded from Hon Ba NR and used to test the "capture-analysis-release" approach. We successfully identified 28 of the 30 living recorded reptiles (93%) in the field without killing the animals. List of all recorded species and morphological data of each specimen obtained in the field were showed in Appendix.

Snakes

- *Scale counting and photographing*: Scalation of all 18 animals of 10 snake species (including three venomous snakes, see Appendix) were counted successfully in the field. Photos showing all these characters were also taken successfully and the scale counting as well as scale characters can be checked again on computer using these "voucher" photos.

Figure 2 shows a successful application of photography-based approach to scale counting on living venomous snake (*Trimeresurus vogeli* David, Vidal & Pauwels). Ventrals, subcaudals as well as body scale rows are hardly showed on one photo. However, we split the ventrals into three parts and took three corresponding photos to show all ventrals clearly. The ventrals therefore can be checked on computer without voucher specimen. Similarly, we split subcaudals and mid-body scale rows into two and three parts, respectively, to count the scales successfully.

- *Measurement*: In the field, we successfully measured all 18 captured animals (see Appendix) and showed all the measurements on photos (data not showed). Each measurement shows one value on the ruler and we simply took photo including both the character recorded and the value. An illustration for measurements of juvenile cat snake (*Boiga multomaculata* [Boie]) was showed in Figure 3.

- *Identification*: In the field, we identified successfully 9 of 10 snake species (90%) without killing the animals. We failed to identify one snake (*Trimeresurus vogeli*) because the requirement of hemipenes which hardly be seen completely in living specimen (see Discussion). One adult male of the snake therefore was collected and killed for identification.

Lizards

- *Scale counting*: Scalation for identification and description of all 35 animals of 20 lizard species were counted and taken photos successfully in the field and can be checked on computer. Figures 4, 5, and 6 show successful applications of photography-based approach to scale counting of mid-body scale row on living agamid (*Pseudocalotes microlepis* [Boulenger]), sublamellae and ventral scale rows on living gecko (*Cyrtodactylus bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler), and mid-body scale rows on living skink (*Sphenomorphus* cf. *buenloicus* Darevsky & Nguyen).

- *Measurement*: We also successfully measured all captured animals in the filed and showed all the measurements on photos. Each photo includes both the character recorded and the result value.

- *Identification*: In the field, we identified successfully 19 of 20 lizard species (95%) without killing the animals. The species that failed to be identified completely is *Sphenomorphus* cf. *buenloicus* (see Discussion). The skink therefore was collected for the identification.

DISCUSSION

Surely, a list of 30 recorded reptile species from Hon Ba NR (see Appendix) is not the completed reptile fauna of the area. The limitation of species account is because we conducted just three field trips and our main purpose is to test the CAR approach.

Collecting animal

Collecting animal in CAR approach is similar to that in traditional herpetological survey. Animals are collected by hand, stick, pit-fall trap, etc. in daytime and at night. Other related data following the collected animal (e.g. coordinate, elevation, habitat, temperature, etc.) are also recorded. However, in CAR approach, all animals must be kept alive and each of them is confined in a separated bottle or box with information about sampling site. In addition, all collected animals are released after analyses. Hence, the "capture" step is easy to do but requires much time and detail.

Identifying in the field

In some cases, new species or unknown form is discovered when checking specimens in the preserved collection but not enough samples to described or confirmed. Identifying in the field will priorly realized known or unknown species to decide if more animals are collected. This is an advantage of the CAR approach in discovering new taxon.

Colour and pattern of living animal are important in identification. Initial photos showing these characters were done simply in the field for all recorded reptiles.

- *Scale counting in the field*: Traditionally, herpetologists usually do scale counting in the lab with preserved specimens. However, as showed above, scale counting for snakes and lizards can be done successfully in the field without killing animal. Moreover, these counts can be also checked not only in lab but also everywhere using the detailed and corresponding photos. Experientially, another advantage of counting scale in the field is that scale and skin on living reptiles, especially on geckos and agamids, are regular and smooth and so easy to count or observe whereas those on preserved specimen are usually wrinkled (e.g. *Acanthosaura*, *Cyrtodactylus*, *Gekko*, *Pseudocalotes*, etc.).

Unfortunately, potential tiny reptiles, such as worm skink (*Dibamus*) and blind snake (*Indotyphlops*) were not recorded during our three field trips. The main key characters for identification of these animals are head and body scale arrangement (Honda *et al.* 2001; Niyomwan *et al.* 2001). However, these characters can be observed in the field under a stereo microscope and good camera with macro lens. We tested successfully these with adult *Dibamus kondaoensis* Honda, Ota, Hikida & Darevsky from southern Vietnam.

- *Scale and measurement photographing*: Because of the absence of voucher specimens, photos showing scale counting and morphological or key characters are very necessary to check repeatedly the counting and characters on computer. All recorded animals from Hon Ba NR were successfully photographed scale counting and characters. However, tiny reptiles are challenge to photograph their scalation. We tested the method using *Indotyphlops braminus* (Daudin) (not from Hon Ba NR) and failed to show the scalation on our cameras because the animal is too small and black. Hence, once the characters can not be seen clearly in photo for checking via

computer, one specimen should be collected for identification or using better photo equipments to obtain higher quality photos. Measurement is easy to show via photos because it requires only the character measured and the result value on ruler. It is noted that head scalation of snake genera *Ovophis* and *Psammodynats* is difficult to show on photo because of the irregular pattern and high contract on head. However, these can be solved by changing the light and photographing angle appropriately.

- *Identification*: In most cases, identification is finished in the field based on scale counting, scale character, measurement, and coloration/pattern. We successfully identified 28 of 30 living reptile species (93%) from Hon Ba NR in the field without killing. The two remained species (one snake and one lizard) needed more morphological characters. Hemipenis morphology in some snake and skink groups is a required character for identification (Pope 1935; Smith 1943; Darevsky & Nguyen 1983).

The Vogel's pitviper *Trimeresurus vogeli* has been recorded from Thailand, Laos, Cambodia, and central Vietnam. It characterizes by lacking of red tail and having short hemipenis with 10–12 stout spines on its proximal two-thirds and the tips calyculate (David *et al.* 2001; Malhotra *et al.* 2004). In Hon Ba NR, *T. vogeli* is common in high elevation. We analyzed six individuals of this species (see Table A6 in Appendix) for identification. The snakes appear to be *T. vogeli* because of its green tail. However, we had to kill one adult male for hemipenis analyzing. The result showed that the snakes belong to *T. vogeli* (Figure 7).

Buonluoi forest skink (*Sphenomorphus buenloicus*) was described from Gia Lai Province, central Vietnam with a hemipenis forked near the tip (Darevsky & Nguyen 1983). The similar skinks recorded from Hon Ba NR agree with *Sphenomorphus buenloicus* on scalation. They, however, have unsymmetrical and deeply forked hemipenes. Hence, the name of this taxon is conformed to *buenloicus*. We are working on DNA analysis of this skink to confirm its taxonomy.

Recent phylogenetic studies (Nguyen *et al.* 2013, 2014) showed that *Cyrtodactylus irregularis* species complex includes many cryptic species or undescribed taxa and that most of known species distribute in limited areas. *Cyrtodactylus bidoupimontis* has been recorded only from Bidoup – Nui Ba National Park (Lam Dong Province) where is about 50 km from Hon Ba NR. Populations that are out of the type locality may be different taxa or lineages. In Hon Ba NR, *C. bidoupimontis* was found frequently but only from around the top of Hon Ba Mountain. To confirm the taxonomy of this gecko, we sequenced the partial COI gene and saw that the gecko from Hon Ba is slightly different (0.5%) from *C. bidouimontis* collected from type locality. Hence, this record is a new lineage and new distribution of the *C. bidoupimontis* in Vietnam.

Unfortunately, we did not record any species of turtle during our three field trips in Hon Ba NR. However, turtle in Vietnam is a big, rare, valuable, and well-known group and therefore easy to photo and identify in the field. For tissue, a piece of toe web or blood can be removed. Hence, we strongly recommend that the CAR approach should be applied strictly to turtle.

"Voucher" tissue for DNA analyses

The main weakness of traditional photography-based taxonomy (Marshall & Evenhuis 2015) is that there is no voucher specimen to check when confirmation or more morphological character is required or updated for identification. Meanwhile, current herpetofauna researches in Vietnam usually kill animals for identifying and preserved specimen checking. However, the trend of herpetofaunal research must reduce collecting animal for identification and preservation. The CAR approach can solve the problem reasonably by applying two important keys, including

"voucher" photos and tissue for DNA analyses. First, the approach generates detailed and clear photos of all required morphological characters for identification, including colour, pattern, scale counting, scalation character, and measurement. Second, tissue obtained from the approach can be used as "voucher" data to confirm and describe the animal as molecular level. If a new similar taxon is described based on additional characters which are not obtained from the release animals, the CAR approach suggests another solution. Instead of trying to get more morphological character, researcher can use the "voucher" tissue to sequence and compare to DNA sequence of the new taxon.

Collecting tissue for DNA from tip of tail, toe, scale or blood of living specimen before antisepsis may not affected seriously on the animals. (We did not monitor the survival of marked animals in the field.) This technique was applied successfully to some amphibians (Mendoza *et al.* 2012; Prunier *et al.* 2012). Our suggestion of using tissue from released animal for DNA sequence is an alternative and convincing option because DNA sequencing is easy to do and not expensive and molecular data is much more informative than morphological one. However, this activity may not suitable for living tiny animals, such as worm skink (*Dibamus*) and blind snake (*Indotyphlops*) because taking tissue as this way can be harmful to the animals. Hence, we recommend that the CAR approach should not apply to such tiny reptiles unless a good tissue collecting tools can be applied to.

Disadvantage and limitation of the CAR approach

The most inconvenience of the exhausted CAR approach is that it requires much time in the field for photographing, identifying, and releasing. Moreover, the approach needs at least two experts in the field as well as necessary equipments and materials. When applying the CAR approach, experts face much risk because they must work with living venomous snakes. It should be also noted that juvenile animals can be died during the analysis. During the practice, we had two accident juveniles, including *Cyrtodactylus bidoupimontis* and *Sphenomorphus* cf. *buenloicus*.

The CAR approach cannot be applied to tiny animals and species that require more morphological character from hemipenis for identification. In the case, one adult male should be collected to fully understand the morphology of the taxon. Moreover, if the taxon is estimated as a new or potential new species after analyzing morphologically in the field, one specimen should be collected to describe fully morphological characters. In fact, new reptile species descriptions based on only one specimen with DNA (Nguyen *et al.* 2014) or without DNA (Neang *et al.* 2012; Vogel *et al.* 2012) are accepted recently.

CONCLUSION AND SUGGESTION

For the 30 recorded reptile species from Hon Ba NR, scale counting, measuring, and photographing were successfully done in the field; identifying was successful with a ratio of 93% (28 of 30 species) without killing. Tissues for DNA sequence and detailed photos should be used as key tools to confirm the identified taxa.

This prior CAR approach can be applied to reptile fauna research in Vietnam, except for tiny species, with the following notes. First, one adult male of each taxon (if any) should be collected except for well-known and well-identified taxa as well as small and vulnerable populations. Second, one specimen of new or potential new species needs to be collected.

Following the trend in conservation, this initial result should be scientific basis for policy managers in Vietnam in the future to reduce number of legal animal collected for each species from three (officially at present) to zero or one, and strictly stop killing for preserved specimen from well-known and well-identified taxa as well as small and vulnerable populations.

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Appendix: Morphological characters obtained in the field of released reptiles from Hon Ba Nature Reserve, central Vietnam

Abbreviation. Measurements were taken with digital calipers to the nearest 0.01 mm on living animals. The following morphological characters modified from Anita *et al*. (2004, *Herpetol. J.* 14: 65–77), David *et al*. (2008, *Zootaxa* 1965: 1–49), Nguyen *et al*. (2011, *J. Herpetol.*, 45: 145–154), Rösler *et al*. (2011, *Zootaxa* 2989: 1–50), Hartmann *et al*. (2013, *Zootaxa* 3599: 246–260), Nguyen *et al.* (2013, *Zootaxa* 3737: 399–414), Ziegler *et al*. (2016, *Zootaxa* 4136: 553–566), and Nguyen *et al.* (2016, *Zootaxa* 4139: 261–273) were used for species identification. *Morphometric characters.* **SVL**: snout-vent length, measured from the tip of the snout to the vent; **TaL**: tail length, distance from vent the tip of tail; **HL**: maximum head length (from the tip of rostral to the posterior end of the jaw); **HW**: maximun head width (at the widest point of temporal region); **HH**: maximun head height (at the deepest point of temporal region); **ED**: eye diameter, greatest diameter of eye; **EN**: Eye-nostril distance, distance between anteriormost point of eyes and nostrils; **EE**: Eye-ear distance, distance from anterior edge of ear opening to posterior corner of eyes; **ES**: Eye-snout distance, distance between anteriormost point of eyes and nostrils; **TymD**: maximum diameter of tympanum; **Trunk**: distance from posterior junction of forelimb and body wall to anterior junction of hind limb and body wall (with the limbs held at right angles to the body); **FA**: forearm length, distance between palm and elbow; **Hlimb**: the length of tibia. *Scalations*. **SL**: supralabials; **IL**: infralabials; **IN**: internasals (scales between nasorostral in contact with rostral); **Nos**: nostril direction; **Lor**: number of loreal scales; **SubOc**: number of subocular scales; **PreOc**: number of preocular scales; **PosOc**: number of postocular scales; **Temp**: number of temporal scales; **DSR**: number of scale rows around body (at one head length after the posterior end of the jaw - at the mid-body - at one head length before vent for snakes; and at the mid-body for lizards); **PaVS**: paravertebral scales, number of paravertebral scales counted from parietal to scale above vent; **KS**: number of keels on dorsal scales; **V**: number of longitudinal ventral scales; **SC**: subcaudals along underside of tail from cloacal slit to tail tip; **ANAL**: number scales (plates) above the vent; **Ven**: scales across the belly at mid-body; **DAS**: number scales across dorsum; **PP**: precloacal pores; **FP**: femoral pores; **LF4**: subdigital lamellae under the fourth finger; **LT4**: subdigital lamellae under the fourth toe; **BlaB**: number of black bands on body + on tail. Values of paired characters were given in state of left/right or only left.

Sexual chararacters abbreviation: **juv**: juvernile; **subm**: subadult male, **m**: adult male; **f**: female, **subf**: subadult female. Note: –, missing data.

Testing an alternative capture-analysis-release approach to document the reptile fauna of Hon Ba Nature Reserve, central Vietnam

Figures

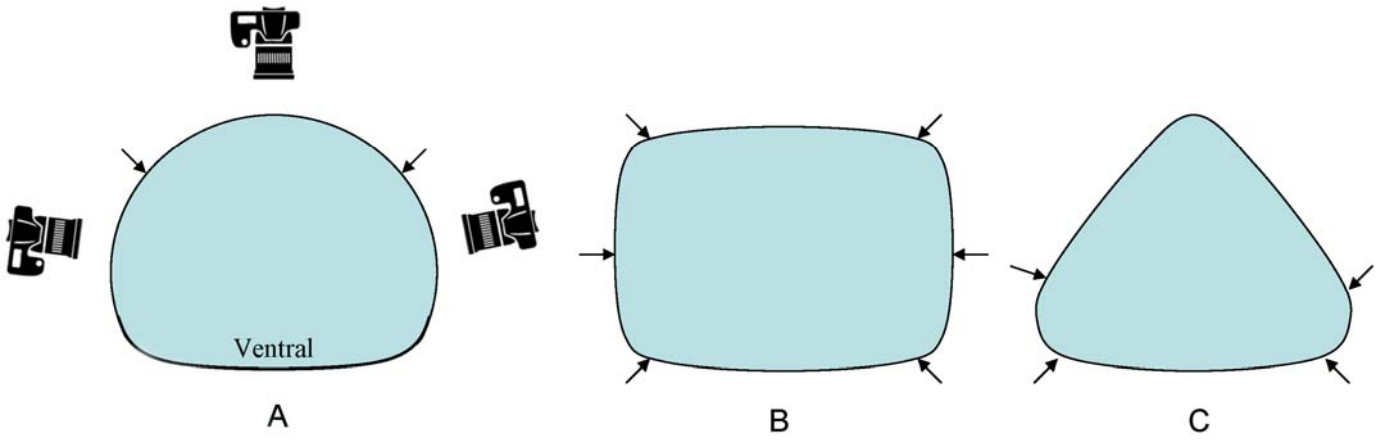

Figure 1. Illustration of splitting and photographing of body scale rows in snake and lizard at mid-body horizontal section. Split points are indicated by arrows and photo angles are represented by camera symbols. A, typical snake illustration: two marked sites and three photo angles are required. B, illustration of typical skink: six split points and six photo angles are required. C, illustration of typical agamid: four split sites are marked and a dorsal crest or vertebral scale row is usually present. Hence, five photo angles are required.

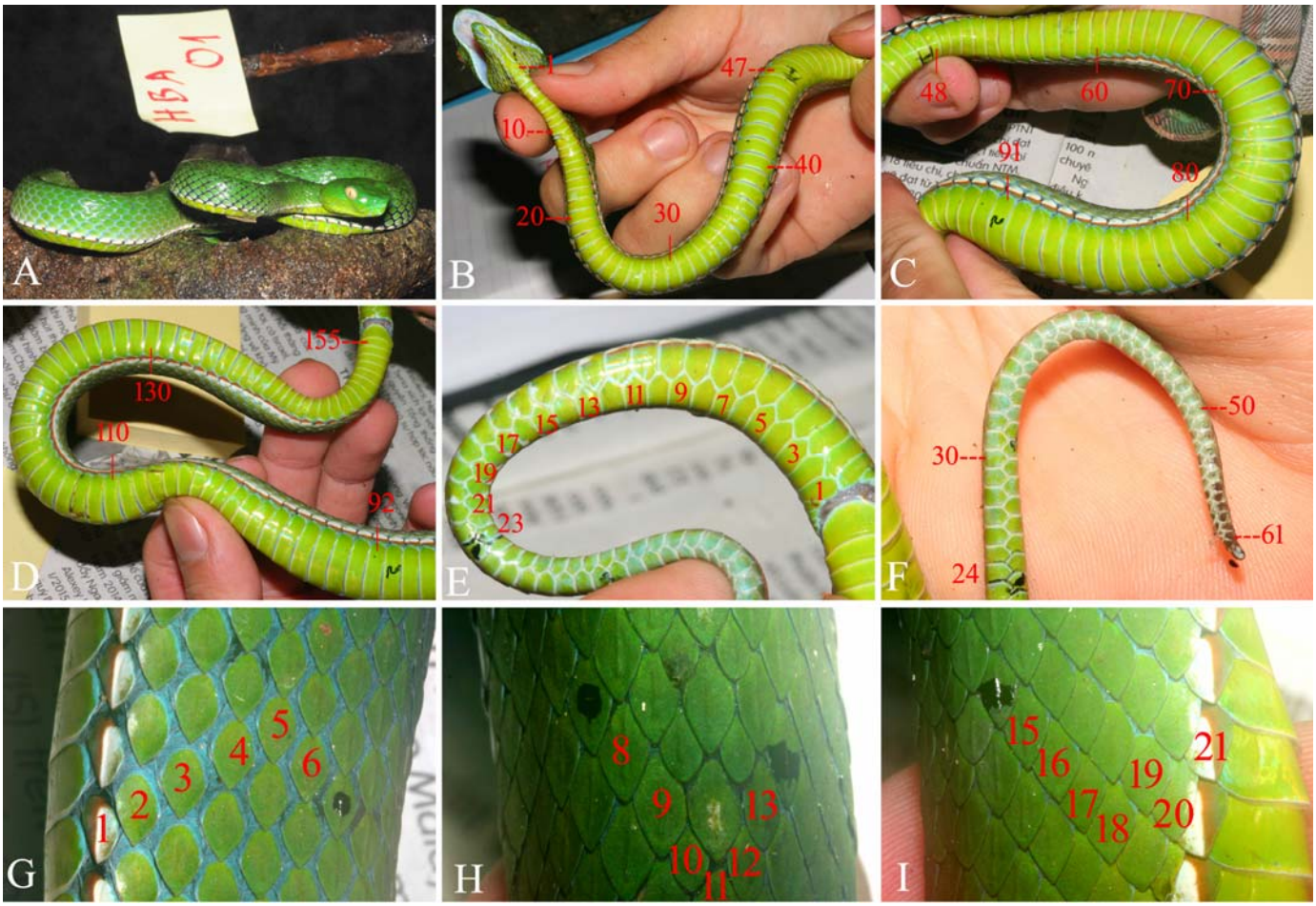

Figure 2. Scale counting based on photos applying to living venomous Vogel's pitviper (*Trimeresurus vogeli*), field no. HBA 01. A, living snake with field number. B–D, ventral scales were splitted into three parts by two black marks, 155 ventral scales in total. E&F, 61 subcaudals were splitted into two parts with one black mark. G–I, counting of 21 dorsal scale rows was done in three steps with two black marks. The snake was released after analyses.

Figure 3. Measurement of head of juvenile cat snake (*Boiga multomaculata*), field no. HBA 43. Characters measured are head length (13.8 mm), head width (8.0 mm), head hight (5.8 mm), eye diameter (3.0 mm), eye-nostril distance (2.2 mm), and snout length (4.5 mm).

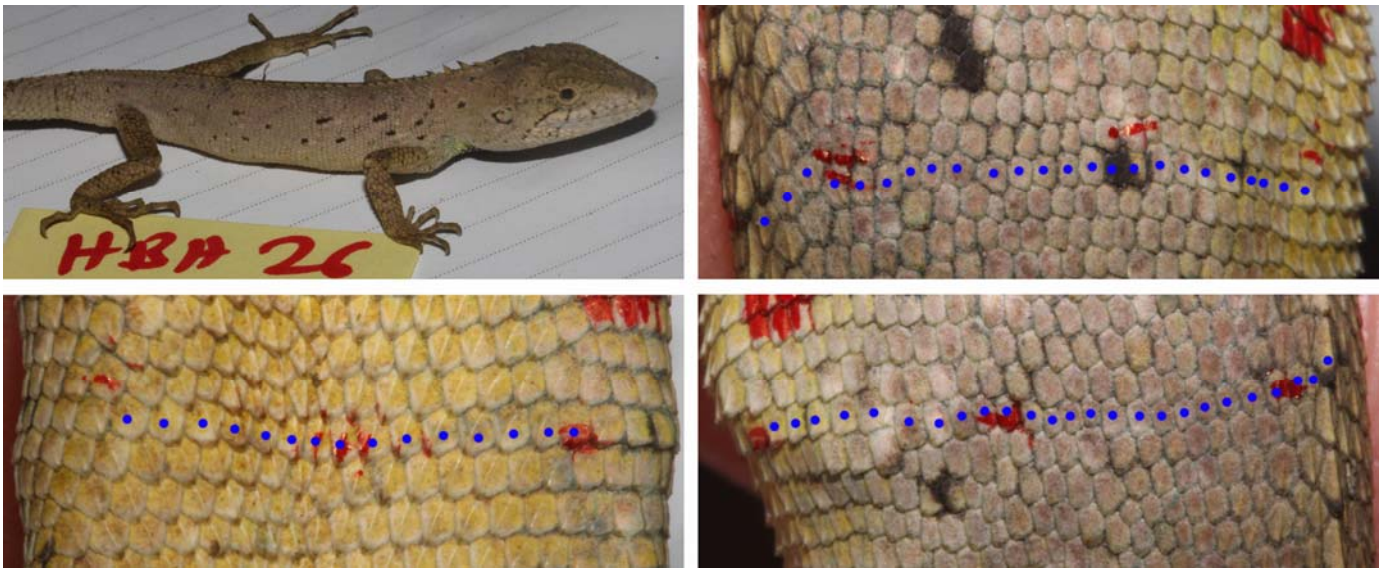

Figure 4. Scale counting based on photos applying to small-scaled forest agamid (*Pseudocalotes microlepis*), field no. HBA 26. The first split site is vertebral scale row, two others are marked by red inks at the end of blue dot rows. A total of 64 body scale rows as marked by blue dots are counted. This lizard was released after analyses.

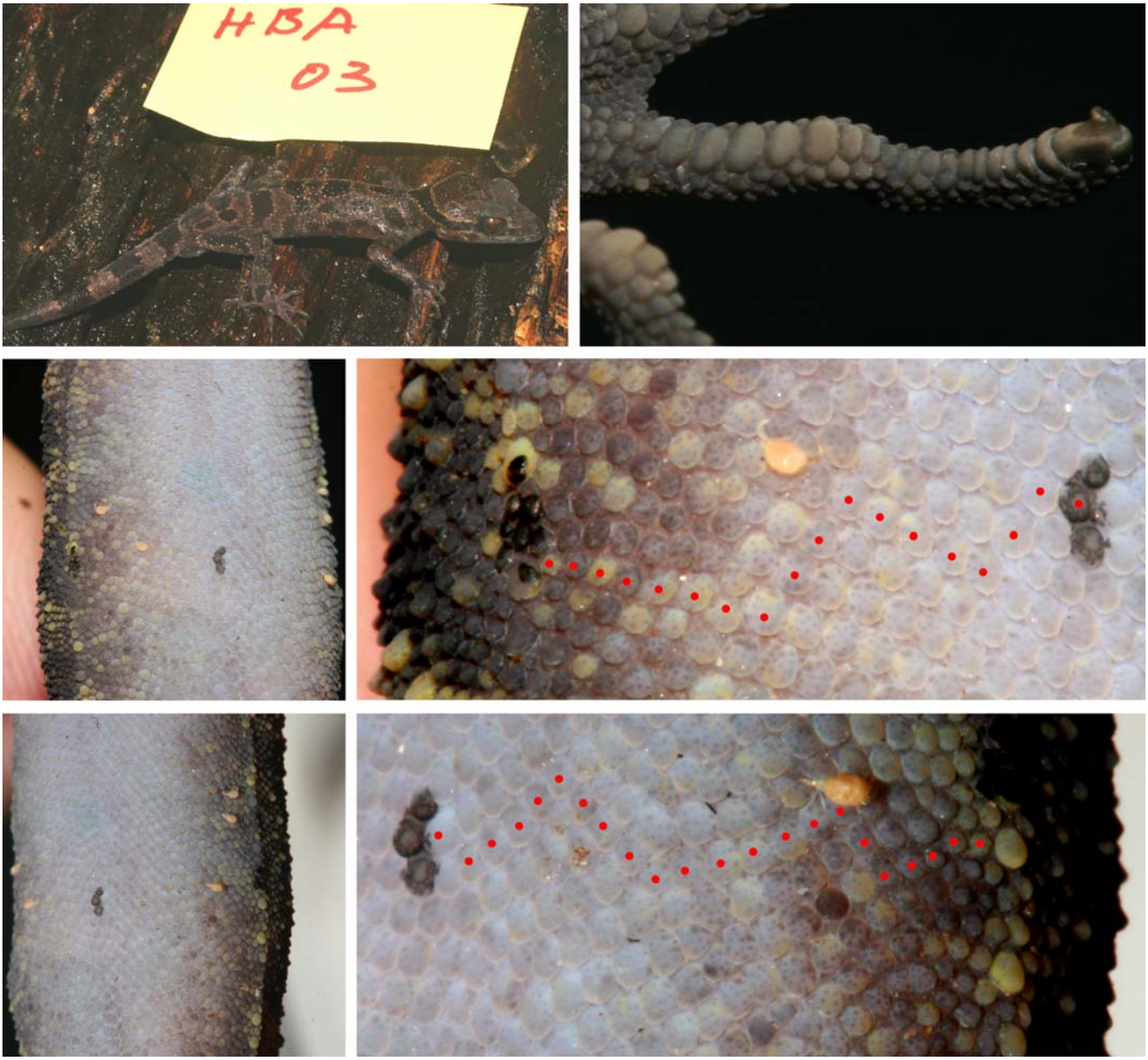

Figure 5. Scale counting based on photos applying to bent-toed gecko (*Cyrtodactylus bidoupimontis*), field no. HBA 03. Sublamellea under fourth toe and ventral scale rows are shown clearly on photos. Red dots are ventral counting between the two lateral-ventral folds, 40 ventral rows are counted. Black ink spots on venter are marked sites. The gecko was released after analyses.

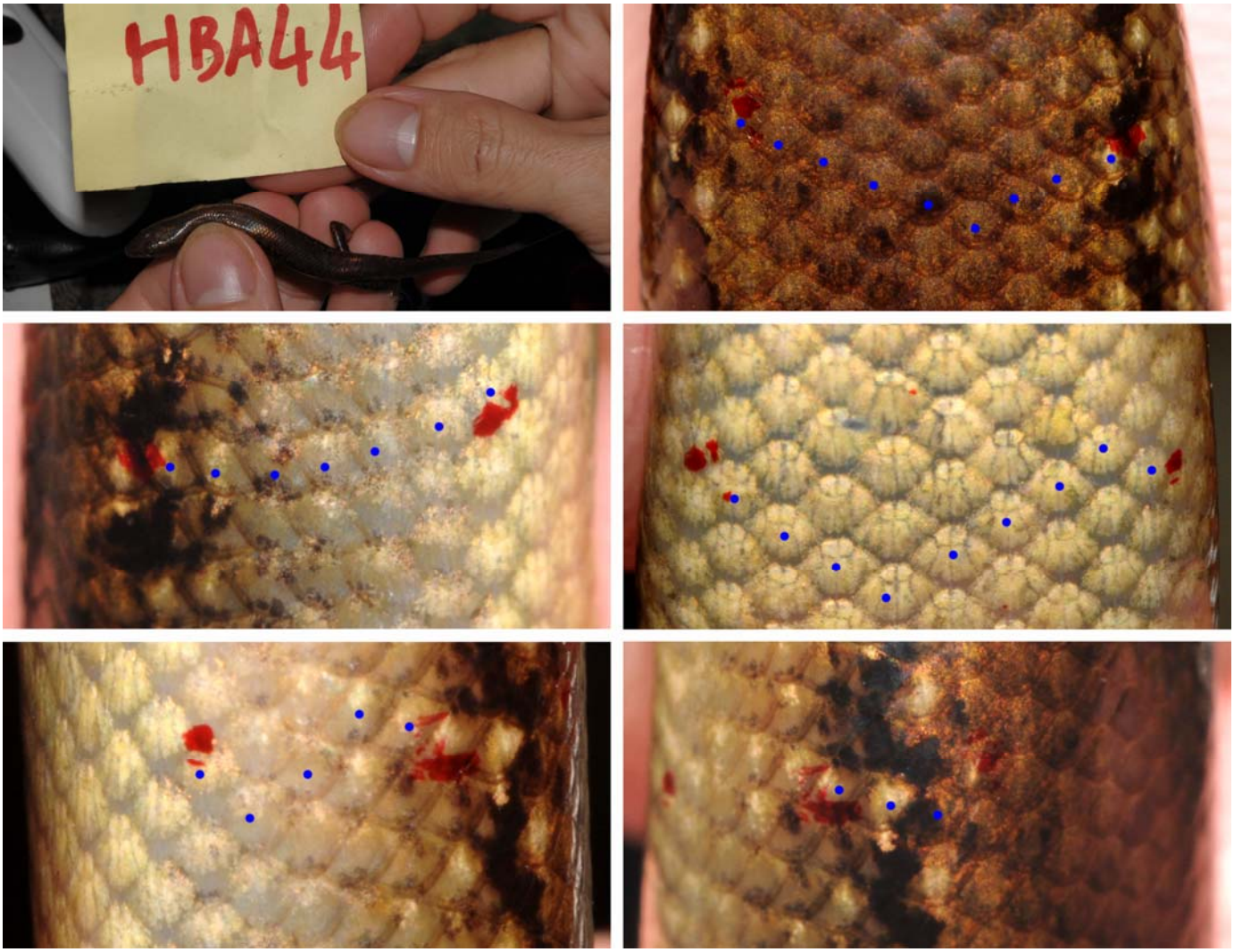

Figure 6. Scale counting based on photos applying to small skink (*Sphenomorphus* cf. *buenloicus*), field no. HBA 44. Red spots are split sites. Blue dots are body scale rows counting, 33 rows in total. The skink was released after analyses.

Figure 7. Identification of Vogel's pitviper (*Trimeresurus vogeli*) based on photos and preserved specimen. Green appearance, absence of red tail, first supralabial separated from nostril, and hemipenes short and spinose are key characters to distinguish the *T. vogeli* from other congeners. This fully everted hemipenis can not be seen in living specimen. The pitviper therefore was preserved for hemipenis morphological analysis.

Table A1. List of recorded reptiles in Hon Ba Nature Reserve (HBA = Hon Ba).

	Species name	**Field No.**	**No. of specimen**
	Agamidae		
1	*Acanthosaura capra* Günther	HBA 34	1
2	*Acanthosaura coronata* Günther	HBA 29, HBA 35,HBA 45	3
3	*Calotes mystaceus* Duméril & Bibron	HBA 41	1
4	*Calotes versicolor* (Daudin)	HBA 19	1
5	*Draco indochinensis* Smith	HBA 27, HBA 51, HBA 52	3
6	*Physignathus cocincinus* Cuvier	HBA 30, HBA 31, HBA 32	3
7	*Pseudocalotes microlepis* (Boulenger)	HBA 26, HBA 36	2
	Gekkonidae		
8	*Cyrtodactylus bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler	HBA 03, HBA 04, HBA 05, HBA 07, HBA 08, HBA 09	6
9	*Cyrtodactylus yangbayensis* Ngo & Onn	HBA 46	1
10	*Dixonius vietnamensis* Das	HBA 38	1
11	*Gehyra mutilata* (Weigmann)	HBA 49	1
12	*Gekko grossmanni* Günther	HBA 53	1
13	*Hemidactylus frenatus* Duméril & Bibron	HBA 48	1
14	*Hemidactylus platyurus* (Schneider)	HBA 50	1
	Lacertidae		
15	*Takydromus sexlineatus* Daudin	HBA 39, HBA 40	2
	Scincidae		
16	*Eutropis macularia* (Blyth)	HBA 37	1
17	*Eutropis multifasciata* (Kuhl)	HBA 21	1
18	*Lygosoma bowringii* (Günther)	HBA 47	1
19	*Sphenomorphus indicus* (Gray)	HBA 18, HBA 28	2
20	*Sphenomorphus* cf. *buenloicus* Darevsky & Nguyen	HBA 25, HBA 44	2

	Species name	Field No.	No. of specimen
	Colubidae		
21	*Amphiesma boulengeri* (Gressitt)	HBA 20, HBA 22	2
22	*Boiga multomaculata* (Boie)	HBA 43	1
23	*Calamaria pavimentata* Duméril, Bibron, & Duméril	HBA 13	1
24	*Enhydris plumbea* (Boie)	HBA 54	1
25	*Pareas hamptoni* (Boulenger)	HBA 11, HBA 14, HBA 15	3
26	*Psammodynastes pulverulentus* (Boie)	HBA 23	1
27	*Pseudoxenodon marcrops* (Blyth)	HBA 17	1
	Elapidae		
28	*Bungarus fasciatus* (Schneider)	HBA 42	1
	Viperidae		
29	*Trimeresurus vogeli* David, Vidal & Pauwels	HBA 01, HBA 02, HBA 06, HBA 10, HBA 16, HBA 24	6
30	*Ovophis monticola* (Günther)	HBA 33	1
	Number of specimens		**53**

Table A2. Morphological characters obtained in the field for agamids family Agamidae from Hon Ba Nature Reserve.

Filed No.	**Species name**	**Sex**	**SVL**	**TaL**	**HL**	**HW**	**HH**	**ED**	**TymD**	**EN**	**ES**	**Trunk**
HBA 34	*Acanthosaura capra*	subm	81	121	22.15	14.85	13.61	6.98	3.21	–	8.49	–
HBA 29	*A. coronata*	juv	63	88	18.23	13.53	11.03	3.67	3.71	–	–	32.57
HBA 35	*A. coronata*	m	72	11.5	21.17	14.68	11.64	3.75	3.28	4.03	7.54	35
HBA 45	*A. coronata*	m	77	116	22.2	16.1	13	4	3.3	6.4	9.9	–
HBA 41	*Calotes mystaceus*	subm	42	100	13.6	8.9	8.2	2.6	2	4.6	6.1	21
HBA 19	*C. versicolor*	subm	49.54	120.47	14.59	9.39	8.67	3.01	–	4.28	7.59	–
HBA 27	*Draco indochinensis*	subm	66	133	14.02	9.85	7.46	3.02	–	–	–	36.95
HBA 51	*D. indochinensis*	m	68	140	14.1	9.6	7.4	2.9	2.2	3.7	5.3	38
HBA 52	*D. indochinensis*	f	64	131	13.4	8.9	7.1	2.9	–	3.4	4.4	39
HBA 30	*Physignathus cocincinus*	f	110	329								
HBA 31	*P. cocincinus*	m	104	245								
HBA 32	*P. cocincinus*	f	138	375								
HBA 26	*Pseudocalotes microlepis*	subm	70	16.1	22.83	10.53	10.68	2	2.7	–	–	38.25
HBA 36	*P. microlepis*	m	81	183	24.2	10	10.1	4.3	2.8	6.5	10.4	43

Table 2. (continued)

Field No.	Species name	FA	HLimb	SL	IL	IN	Nos	DSR	V	ANAL	FP	LF4	LT4
HBA 34	*Acanthosaura capra*	17.46	23.07	12/12	14/13	6	side	92	56	16	0/0	16/–	–/25
HBA 29	*A. coronata*	13.94	17.79	12/12	12/10	8	side	103	–	–	0/0	16/–	24/–
HBA 35	*A. coronata*	14.47	19.01	11/–	13/–	7	side	108	55	–	0/0	16/–	23/–
HBA 45	*A. coronata*	–	22.3	10/11	10/11	6	side	–	56	16	0/0	15/–	–/21
HBA 41	*Calotes mystaceus*	8.3	11	10/11	9/9	6	side	52	76		0/0	18/18	28/30
HBA 19	*C. versicolor*	–	–	/12	/12	7	side	42	78		0/0	–/21	–/30
HBA 27	*Draco indochinensis*	12.34	13.02	12/10	9/9	8	up			24	0/0	26/–	–/29
HBA 51	*D. indochinensis*	11.6	12.5	10/10	11/10	7	up			20	0/0	28/–	32/–
HBA 52	*D. indochinensis*	11.1	11.7	9/8	11/9	6	up			25	0/0	–/29	–/34
HBA 30	*Physignathus cocincinus*	–	–	14/12	12/11	13	side			22	7/8	18/–	38/–
HBA 31	*P. cocincinus*	–	–	13/12	12/12	11	side			25	9/7	–/17	31/–
HBA 32	*P. cocincinus*	–	–	–/14	–/13	10	side			19	7/7	17/17	32/32
HBA 26	*Pseudocalotes microlepis*	9.98	12.89	8/7	9/9	10	side	64	–	15	0/0	19/18	26/25
HBA 36	*P. microlepis*	–	–	8/8	8/8	7	side	63	117	–	0/0	17/–	24/–

Table A3. Morphological characters obtained in the field for geckos family Gekkonidae from Hon Ba Nature Reserve.

Field No.	Species name	Sex	SVL	TaL	HL	HW	HH	ED	TymD	EN	ES	Trunk	FA	HLimb
HBA 03	*Cyrtodactylus bidoupimontis*	m	74	broken	23	15	–	–	–	–	–	34	11	13
HBA 04	*C. bidoupimontis*	juv	51	56	18	12	–	–	–	–	–	27	9	11
HBA 05	*C. bidoupimontis*	juv	60	regenerated	18	14	–	–	–	–	–	–	8	12
HBA 07	*C. bidoupimontis*	f	75	regenerated	22	16	–	–	–	–	–	–	11	14
HBA 08	*C. bidoupimontis*	m	72	regenerated	20	15	–	–	–	–	–	33	11	13
HBA 09	*C. bidoupimontis*	f	68	73	22	14	–	–	–	–	–	35	11	12
HBA 46	*C. yangbayensis*	f	84	regenerated	24.5	16.8	9.4	5.9	1.5	8.1	10.1	40	12.6	15.9
HBA 38	*Dixonius vietnamensis*	subm	38	52	11.5	7.3	4.3	2.4	1.1	3.4	4.8	22	5.6	6.5
HBA 49	*Gehyra mutilata*	f	55	56	14.9	11.2	5	3.4	1.2	4.6	6.2	30	7	7.8
HBA 53	*Gekko grossmanni*	m	88	103	25.1	16.9	8.8	5.3	3.9	7.8	9.8	45	12.4	15.9
HBA 48	*Hemidactylus frenatus*	f	48	regenerated	13.5	9.5	5.5	2.9	0.9	4.6	5.3	23	6.3	7.1
HBA 50	*H. platyurus*	m	53	regenerated	14.1	10.2	6	2.9	1	5	6.3	28	7.4	9.1

Table 3. (continued)

Field No.	Species name	SL	IL	IN	V	Ven	SC	ANAL	PP	FP	LF4	LT4
HBA 03	*Cyrtodactylus bidoupimontis*	11/13	10/10	3	–	40	granular	26	7	0/3	–/17	19/–
HBA 04	*C. bidoupimontis*	11/13	9/10	3	–	44	granular	28	6	0/0	17/–	19/–
HBA 05	*C. bidoupimontis*	12/11	9/9	4	–	45	granular	28	6	6/6	15/–	17/–
HBA 07	*C. bidoupimontis*	9/11	8/10	3	–	44	granular	25	2	3/0	14/–	17/–
HBA 08	*C. bidoupimontis*	13/12	10/11	3	–	44	granular	27	7	0/0	15/–	–/19
HBA 09	*C. bidoupimontis*	12/10	11/11	3	–	42	granular	27	0	9/4	14/–	–/16
HBA 46	*C. yangbayensis*	10/8	10/9	3	–	42	medial enlarged	28	0	7/7	15/–	20/–
HBA 38	*Dixonius vietnamensis*	8/8	6/6	3	88	20	59 (enlarged)	10	6	0/0	10/10	12/12
HBA 49	*Gehyra mutilata*	8/8	8/8	2	113	38	enlarged		0	0/0	–/6	–/8
HBA 53	*Gekko grossmanni*	16/15	12/12	2	143	30	99 (medial enlarged)	26	12	0/0	16/15	19/19
HBA 48	*Hemidactylus frenatus*	11/12	9/9	3	139	35	enlarged	granular	0	0/0	7/7	9/10
HBA 50	*H. platyurus*	11/11	8/8	3	115	48	enlarged	granular	10	14/12	7/7	8/8

Table A4. Morphological characters obtained in the field for lizards families Scincidae and Lacertidae from Hon Ba Nature Reserve.

Field No.	Species name	Sex	SVL	TaL	HL	HW	HH	ED	TymD	EN	EE	ES
	Lacertidae											
HBA 39	*Takydromus sexlineatus*	juv	49	241	13.2	5.5	4.6	2.4	1.7	3.8	3.3	5.5
HBA 40	*T. sexlineatus*	juv	32	regenerated	–	–	–	–	–	–	–	–
	Scincidae											
HBA 37	*Eutropis macularia*	m	50	broken	13.2	7.4	5.7	3.4	1	2.7	–	3.9
HBA 21	*E. multifasciata*	m	95.53	72	21.53	12.31	9.71	3.32	–	5.33	–	8.2
HBA 47	*Lygosoma bowringii*	f	43	regenerated	9.2	5	3.7	1.4	0.9	1.8	3.3	3.1
HBA 18	*Sphenomorphus indicus*	m	76	116	17.01	11.5	7.87	3.25	2.17	4.72	–	6.34
HBA 28	*S. indicus*	juv	54	113	–	–	–	–	–	–	–	–
HBA 25	*Sphenomorphus* cf. *buenloicus*	juv	28.9	54.4	–	–	–	–	–	–	–	–
HBA 44	*Sphenomorphus* cf. *buenloicus*	m	52	regenerated	12.3	7	4.8	2.6	1.9	2.7	4.2	3.8

Field No.	Species name	Trunk	FA	HLimb	SL	IL	IN	Lor	SubOr	DSR	PaVS
	Lacertidae										
HBA 39	*Takydromus sexlineatus*	26	6.3	7.4	6/6	6/5	2	–/2	3/3		–
HBA 40	*T. sexlineatus*	–	–	–	6/6	6/5	2	2/2	3/3		–
	Scincidae										
HBA 37	*Eutropis macularia*	27	6.4	5.7	8/–	9/–	1	2/–	4/4	32	41
HBA 21	*E. multifasciata*	–	–	–	7/7	7/7	3	–/2	4/4	32	42
HBA 47	*Lygosoma bowringii*	24	3.4	4.2	7/7	6/6	2	2/2	4/4	26	56
HBA 18	*Sphenomorphus indicus*	–	8.27	10.95	7/–	8/9	1	2/–	5/–	33	–
HBA 28	*S. indicus*	–	–	–	8/8	8/8	1	2/2	4/4	33	–
HBA 25	*Sphenomorphus* cf. *buenloicus*	–	–	–	–/8	–/6	1	–/2	–/4	–	–
HBA 44	*Sphenomorphus* cf. *buenloicus*	28	5.5	6.7	7/7	6/6	1	2/2	4/4	33	62

Table 4. (continued)

Field No.	Species name	KS	V	Ven	DAS	SC	ANAL	FP	LF4	LT4
	Lacertidae									
HBA 39	*Takydromus sexlineatus*		48	6	4	188	5	1/1	17/18	24/21
HBA 40	*T. sexlineatus*		46	6	4	regenerated	5	1/1	–/17	–/22
	Scincidae									
HBA 37	*Eutropis macularia*	5	43			broken	6	0	9/–	15/–
HBA 21	*E. multifasciata*	3	51			–	–	0	–	–
HBA 47	*Lygosoma bowringii*	0	53		8	regenerated	6	0	–/8	–/11
HBA 18	*Sphenomorphus indicus*	0	71			79 (medial englarge)	6	0	11/–	–/17
HBA 28	*S. indicus*	0	68			–	–	0	–/10	15/–
HBA 25	*Sphenomorphus* cf. *buenloicus*	0	–			–	–	0	10	17
HBA 44	*Sphenomorphus* cf. *buenloicus*	0	58			regenerated	4	0	–/10	18/18

Table A5. Morphological characters recorded in the field for snakes from Hon Ba Nature Reserve.

Field No.	Species name	Sex	SVL	TaL	HL	HW	HH	ED	EN	ES	SL	IL	IN	Nos	Lor	SubOr
	Colubridae															
HBA 20	*Amphiesma boulengeri*	f	430	18.2	15.8	6.82	5.74	2.56	2.53	5.27	9/9	10/11	2	side	1/1	1/1
HBA 22	*A. boulengeri*	juv	143	56	9.27	4.34	3.06	1.63	1.75	2.48	9/–	11/10	2	side	1/1	2/–
HBA 43	*Boiga multomaculata*	subm	390	91	13.8	8	5.8	3	2.2	4.5	8/8	11/11	2	side	1/1	1/1
HBA 13	*Calamaria pavimentata*	m	231	17	–	–	–	–	–	–	4/4	5/5	0	side	0/0	1/1
HBA 54	*Enhydris plumbea*	subm	232	35	12.4	8.7	5.7	1.8	–	–	8/8	10/10	0	up	1/1	1/1
HBA 11	*Pareas hamptoni*	m	366	120	15	10	7	3	2	5.5	7/7	8	2	side	1/1	1/1
HBA 14	*P. hamptoni*	juv	217	61	12	7	5	2.5	–	–	7/7	6/7	2	side	1/1	1/1
HBA 15	*P. hamptoni*	f	397	128	16.12	8.92	6.16	2.75	1.84	4.15	–	–	2	side	–	–
HBA 23	*Psammodynastes pulverulentus*	juv	–	–	–	–	–	–	–	–	9/10	8/8	2	side	1/1	1/1
HBA 17	*Pseudoxenodon marcrops*	juv	335	72	16.72	8	6.93	4.51	3.16	5.45	8/8	9/9	2	side	1/1	1/1
	Elapide															
HBA 42	*Bungarus fasciatus*	f	1080	95	31.1	21.2	13.2	4.6	4.6	8.7	7/7	7/7	2	side	0/0	1/1
	Viperidae															
HBA 01	*Trimeresurus vogeli*	m	300	–	–	–	–	–	–	–	9/10	13/13	4	side	2/2	1/1
HBA 02	*T. vogeli*	f	250	–	–	–	–	–	–	–	9/11	13/11	3	side	3/3	1/1
HBA 06	*T. vogeli*	f	630	100	–	–	–	–	–	–	11/10	11/13	4	side	1/1	1/1
HBA 10	*T. vogeli*	f	–	–	–	–	–	–	–	–	10/11	13/13	4	side	3/3	1/1
HBA 16	*T. vogeli*	f	557	102	31.16	22.03	12.54	4.17	7.05	9.07	10/10	12/11	4	side	2/2	1/1
HBA 24	*T. vogeli*	m	450	103	22.87	17.43	9.76	3.9	5.8	7.42	10/10	12/12	3	side	2/2	1/1
HBA 33	*Ovophis monticola*	juv	177	27	12.37	8.94	5.36	–	–	–	9/8	11/10	2	side	2/2	1/1

Table 5. (continued)

Field No.	Species name	PreOc	PosOc	Temp	DSR	DSRKee	V	SC	ANAL	BlaB
	Colubridae									
HBA 20	*Amphiesma boulengeri*[*]	1/2	3/3	1+1/1+1	19–19–17	except 1st	162	97 (paired)	2	0
HBA 22	*A. boulengeri*[*]	2/2	3/3	1+1/–	17–19–16	all keeled	162	98 (paired)	2	0
HBA 43	*Boiga multomaculata*	1/1	2/2	1+2/1+2	19–19–13	unkeeled	227	98 (paired)	1	0
HBA 13	*Calamaria pavimentata*	1/1	1/1	0/0	13–13–13	unkeeled	122	16 (paired)	1	0
HBA 54	*Enhydris plumbea*	1/1	2/2	1+2/–	19–19–15	unkeeled	125	33 (paired)	2	0
HBA 11	*Pareas hamptoni*	1/1	1/1	2+2/2+2	15–15–15	unkeeled	185	91 (paired)	1	0
HBA 14	*P. hamptoni*	1/1	1/1	2+2/2+3	15–15–15	unkeeled	190	81 (paired)	1	0
HBA 15	*P. hamptoni*	–	–	–	16–15	unkeeled	190	83 (paired)	1	0
HBA 23	*Psammodynastes pulverulentus*	1/2	2/3	2+2/2+2	19–17–15	unkeeled	163	51 (paired)	1	0
HBA 17	*Pseudoxenodon marcrops*	1/1	3/3	2+2/2+2	19–17–15	all keeled	155	59 (paired)	2	0
	Elapidae									
HBA 42	*Bungarus fasciatus*	1/1	2/2	1+2/1+2	15–15–15	unkeeled	220	34 (single)	1	20+4
	Viperidae									
HBA 01	*Trimeresurus vogeli*	3/3	2/3	irregular	21–19–13	all keeled	155	61 (paired)	1	0
HBA 02	*T. vogeli*	3/3	2/2	irregular	21–21–15	all keeled	160	56 (paired)	1	0
HBA 06	*T. vogeli*	3/3	3/2	irregular	23–21–15	all keeled	158	58 (paired)	1	0
HBA 10	*T. vogeli*	3/3	3/3	irregular	20–21–16	all keeled	159	61 (paired)	1	0
HBA 16	*T. vogeli*	3/3	2/2	irregular	24–21–15	all keeled	159	58 (paired)	1	0
HBA 24	*T. vogeli*	3/3	2/4	irregular	–21–15	all keeled	162	64 (paired)	1	0
HBA 33	*Ovophis monticola*	3/3	2/2	irregular	26–22–17	all keeled	148	36 (single)	1	0

([*]), HBA 20 and HBA 22 are slightly different from *Amphiesma boulengeri* by having more ventral scales (162 vs. 139–156) (David 2007, *Zootaxa* 1462: 41–60).