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ECOLOGIA DO MOVIMENTO DE PERERECAS DA FAMILIA PHYLLOMEDUSIDAE NA RESERVA ECOLÓGICA DA MICHELIN

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Orientador: Dr. Mirco Solé

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“Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less”
Marie Curie

SUMÁRIO

A new spool-and-line attachment method to track short movements in phyllomedusid frogs (Anura: Phyllomedusidae)

INTRODUÇÃO

O movimento de um organismo é definido como uma mudança na localização espacial do indivíduo no tempo e é uma característica fundamental da vida, incentivada por diversos fatores que atuam em múltiplas escalas espaciais e temporais. Ele desempenha um papel importante na determinação do destino dos indivíduos; da estrutura e dinâmica das populações, comunidades e ecossistemas; e da evolução e diversidade da vida (Nathan et al., 2008). Os movimentos e comportamentos dos animais de pequeno porte são de grande interesse, pois eles podem fornecer informações importantes sobre sua ecologia. Por tanto, estudos sobre o comportamento e os padrões de movimento espacial dos indivíduos são necessários para uma compreensão completa da ecologia das espécies.

Diferentes métodos de rastreamento podem gerar grandes quantidades de dados ecológicos detalhados ao longo de vários dias (Heyer et al., 1994) e podem ser usados para examinar a área de residência, dispersão, padrões de atividade, preferências de habitat, e uso de micro habitats. Além disso, os avanços tecnológicos nos métodos de rastreamento melhoram nossa capacidade de abordar quatro questões fundamentais sobre o movimento dos organismos: (i) por que se movimentam? (ii) como se movimentam? (iii) quando e para onde se movimentam? e (iv) quais são as consequências ecológicas e evolutivas do movimento? (Nathan et al. 2008).

Os anfíbios são maioritariamente animais pequenos, noturnos, inconspícuos e que passam grande parte do dia inativos. O comportamento dos anfíbios fora dos tempos de atividade reprodutiva permanece pouco conhecido, exceto para algumas espécies. Para a maioria das espécies de anfíbios, os padrões de movimento e o uso do habitat não são muito bem conhecidos, especialmente os locais usados longe dos locais de reprodução (Rowley e Alford, 2007). O que eles fazem quando estão longe ou fora dos locais de reprodução, a que distância viajam, que comportamentos exibem, que habitats utilizam são aspectos que ainda não são bem estudados. Uma das técnicas mais valiosas para ganhar informação sobre o uso do micro-habitat, o alcance e o movimento de uma espécie é o rastreamento remoto de indivíduos no campo. Durante os últimos anos houve vários avanços nos dispositivos de monitoramento, entretanto, como observa

Ferner (2010), não existe um método geral ou ideal: os procedimentos que provavelmente funcionarão melhor variam com a espécie, o habitat e os objetivos do estudo.

O método mais frequentemente utilizado para estudar a ecologia e a preferência de habitat de anfíbios tem sido a coleta de dados descritivos de campo simples (Heyer et al. 1994; Duellman 2005; McDiarmid et al. 2012; Beirne et al. 2013). No entanto, este método fornece apenas conhecimentos ecológicos pontuais, que geralmente se limitam a fornecer pontos de dados únicos para indivíduos (Waddel et al, 2016). Pesquisas sobre o movimento de anfíbios começaram nos anos sessenta com métodos de rastreamento de rádio (Tester, 1963; Madison e Shoop, 1970) e se tornaram mais frequentes nos últimos anos devido à aplicação de diversos métodos de rastreamento de animais, tais como: transmissores de rádio de frequência muito alta (VHF) (Van Nuland e Claus, 1981; Hodgkison e Hero, 2001; Seebacher e Alford, 2002; Lemckert e Brassil, 2003; Muths, 2003; Blomquist e Hunter, 2007; Rowley e Alford, 2007; Indermaur et al, 2008; Schmidt et al., 2008; Madison et al., 2010; Gourevitch e Downie, 2018; Pašukonis et al., 2018, 2019), captura-recaptura (Sutherland et al, 2016), radar harmônico (HDF) (Rowley e Alford, 2007; Pašukonis et al., 2014; Beck et al., 2017), pós fluorescentes (Rittenhouse et al., 2006; Ramírez et al, 2017), e o método da linha "bobina de fio" (Dole, 1965; Duellman e Lizana, 1994; Lemckert e Brassil, 2000; Tozetti e Toledo, 2005; Gourevitch e Downie, 2018; Silvester et al., 2019).

Como foi mencionado, várias técnicas estão disponíveis para rastrear anfíbios; no entanto, seu uso é frequentemente limitado pelo tamanho da espécie focal e pelo método de fixação do dispositivo de rastreamento. A radio telemetria dos anfíbios e répteis tem sido historicamente limitada pelo tamanho e peso do transmissor. Seja implantado ou afixado na superfície do animal, a preocupação tem sido que a mobilidade do sujeito seria prejudicada pelo volume do transmissor (Fitch 1987; Richards et al. 1994). Mas os recentes avanços na tecnologia de radio telemetria, como a diminuição do tamanho do transmissor e o aumento da vida útil da bateria, facilitam o rastreamento de indivíduos menores (Berg et al., 2010). Quando se trata de herpetofauna, existem mais estudos de telemetria em répteis (Ujvari e Korsos, 2000; Fraga et al., 2013; Alexy et al., 2003; Pearson et al. 2003, Dodd e Barichivich 2007, Hoss et al. 2010, Jackson 2013, Bauder et al. 2016) do que em anfíbios, no entanto, diversos estudos de telemetria foram realizados em anuros, com transmissores externos (Rowley e Alford, 2007; Indermaur et al., 2008) ou implantados (Lamoureux et al., 2002; Johnson et al., 2007). Além disso, parece que o fato de um animal estar equipado com um transmissor não altera os padrões de movimento revelados pela localização de anfíbios no campo. As observações relativas ao comportamento de rãs monitoradas no campo suportam essa ideia. Em todas as espécies estudadas, os anfíbios com transmissores pareciam estar desinibidos no seu movimento, eram comumente observados em associação com indivíduos sem transmissores, e eram frequentemente observadas chamando e em amplexo (Rowley e Alford, 2007). Contudo, as espécies pequenas (<7 g; Rowley e Alford, 2007) e que usam buracos representam um desafio, pois as técnicas comumente usadas, como a radio telemetria, são muito pesadas, podem ferir o animal quando enterrado (Eggert, 2002; Graeter e Rothermel, 2007; Rowley e Alford, 2007), ou não são apropriadas para detectar o uso de habitat de resolução fina (Lövei et al. 1997; Birchfield e Deters 2005). Por esta razão, várias técnicas de monitoramento devem ser utilizadas e comparadas.

O método "spool-and-line" é considerado uma alternativa aos radio transmissores , uma vez que os custos são muito mais baixos e o tamanho da bobina pode ser adaptado ao peso do indivíduo que está sendo rastreado. Também permite mapear os movimentos dos anfíbios com mais detalhes (Vieira e Loretto, 2004). Esta metodologia já foi testada em anfíbios como *Lithobates pipiens* (Dole, 1965), *Mixophyes iteratus* (Lemckert e Brassil, 2000), *Leptodactylus labyrinthicus* (Tozetti e Toledo, 2005), *Leptodactylus rhodomystax*, *Oreobates quixensis* (Waddel et al. , 2016), *Rhinella marina* (Waddel et al, 2016; Silvester et al., 2019) e *Boana faber* (Oliveira et al., 2016). Recentemente também foi usada para rastrear *Phyllomedusa trinitatis* (Gourevitch e Downie, 2018).

Finalmente, outra alternativa para monitorar o movimento dos anfíbios é o uso de pós fluorescentes. Pós não tóxicos foram usados para rastrear pequenos animais, incluindo insetos (ex. Johansson 1959; Vardeman et al. 2007), mamíferos (ex. Lemen & Freeman 1985; Mullican 1988), e anfíbios (ex. Woolbright, 1985; Birchfield e Deters 2005; Ramirez et al. 2012). Este método de rastreamento provou ser inofensivo para os anfíbios (Rittenhouse et al. 2006; Orlofske et al. 2009) enquanto fornece dados detalhados sobre movimentos em pequena escala e uso de habitat (Eggert 2002; Graeter e Rothermel 2007).

OBJETIVOS

Objetivo geral

Estudar a área de vida de espécies de anfíbios da família Phyllomedusidae

Objetivos específicos

- Determinar um novo método de fixação de carretel para rastrear movimentos curtos em Phyllomedusidae.
- Testar a eficácia desta nova metodologia no campo em indivíduos de *Phyllomedusa burmeisteri*
- Medir a distância percorrida por indivíduos de *Phyllomedusa burmeisteri* usando esta metodologia

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Capítulo I

A new spool-and-line attachment method to track short movements in phyllomedusid frogs (Anura: Phyllomedusidae)

Aceito em Herpetology Notes

A new spool-and-line attachment method to track short movements in phyllomedusid frogs (Anura: Phyllomedusidae)

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Abstract

Monitoring the movements of amphibians has always been a challenge, especially as the effectiveness of the tracking mode depends not only on the environment and habit but also on the morphology of the individuals. These factors affect how tracking devices can be attached to individual amphibians. We developed and validated a minimally invasive attachment methodology for the spool-and-line method using *Phyllomedusa burmeisteri* as a model. We tested our methodology in the laboratory and in the field and did not observe any hinderance to movement over a four-day period. We propose the use of this technique to study movement ecology and habitat use for other anuran amphibians with similar morphology and movement behaviour.

Keywords. Amphibians, thread bobbin, movement ecology, tree frogs

Introduction

Studies on animal movement provide important information on migration, dispersal, homing activity, activity area, and site selection for reproduction (Eggert, 2002). Research focused on animal movement has a long history and is necessary to understand a species' ecology. For many years, the most common and affordable way to track wildlife was simply to follow and observe the movement and habits of an animal by collecting descriptive data (Altmann, 1974; Altmann and Muruthi, 1988; Heyer et al., 1994).

Research on amphibian movement started during the mid-sixties with radio tracking methods (Tester, 1963; Madison and Shoop, 1970), but it was only during the 80´s that van Nuland and Claus (1981) developed a tracking system specifically for anuran species. Since then, these and other methods have been commonly used: very high frequency (VHF) radio transmitters (Van Nuland and Claus, 1981; Hodgkison and Hero, 2001; Seebacher and Alford, 2002; Lemckert and Brassil, 2003; Muths, 2003; Blomquist and Hunter, 2007; Rowley and Alford, 2007; Indermaur et al., 2008; Schmidt et al., 2008; Madison et al., 2010; Gourevitch and Downie, 2018; Pašukonis et al., 2018, 2019), capture-recapture (Sutherland et al., 2016), harmonic direction finding (HDF) (Rowley and Alford, 2007; Pašukonis et al., 2014; Beck et al., 2017), fluorescent powders (Rittenhouse et al., 2006; Ramírez et al., 2017), and the line "thread bobbin" method (Dole, 1965; Duellman and Lizana, 1994; Lemckert and Brassil, 2000; Tozetti and Toledo, 2005; Gourevitch and Downie, 2018; Silvester et al., 2019).

Recently, radio telemetry studies have attracted enormous attention, because of advances in tracking technology and analytical methods (Kays et al., 2015). However, radio telemetry is not accessible to all researchers due to the high associated cost and because the weight of the transmitter should not exceed 10% of the tracked individual's weight (Richards et al., 1994; Pašukonis et al., 2019), and therefore this could be a limitation for its use in studying the movement ecology of small species.

The "spool-and-line" method could be considered a good alternative, since costs are much lower than transmitters, and the bobbin size can be adapted to the weight of the individual being tracked. It also permits to map the movements of amphibians in more detail (Vieira and Loretto, 2004).

The "spool-and-line" method has been tested in frogs such as *Lithobates pipiens* (Schreber, 1782) (Dole, 1965), *Mixophyes iteratus* Straughan, 1968 (Lemckert and Brassil, 2000), *Leptodactylus labyrinthicus* (Spix, 1824) (Tozetti and Toledo, 2005), *Leptodactylus rhodomystax* Boulenger, 1884, *Oreobates quixensis* Jiménez de la Espada, 1872 (Waddel et al., 2016), *Rhinella marina* (Linnaeus, 1758) (Waddel et al., 2016; Silvester et al., 2019) and *Boana faber* Wied-Neuwied, 1821 (de Oliveira et al., 2016). Recently it was also used to track *Phyllomedusa trinitatis* Mertens, 1926 (Gourevitch and Downie, 2018) but there were complications and the tree frogs became entangled in the line and this resulted in injuries. Our aim was to develop a new spool-and-line attachment technique and use it to track several individual treefrogs over a course of several days. One of the common problems with tracking amphibians is that the morphological aspects of the target species are often overlooked. When working with treefrogs, the challenges of their movement and their body morphology need to be considered.

We conceptualised a new harmless technique to attach thread bobbins to adult treefrogs using *Phyllomedusa burmeisteri* Boulenger, 1882 as a model.

Materials and Methods

The study was reviewed by the animal ethics committee (Comissão de Ética no uso dos Animais - CEUA) of the Universidade Estadual de Santa Cruz and the committee did not raise any concerns.

Study sites.—For logistics and proximity to our laboratory, fieldwork was first carried out in an agroforestry cocoa plantation located behind the campus of Universidade Estadual de Santa Cruz (UESC) in Ilhéus-Bahia, Brazil. Later, our experiments were taken into the field to a forest system formed by a mosaic of rubber tree plantations and primary and secondary forests at the Michelin Ecological Reserve (REM), municipality of Igrapiúna, northeastern Brazil. We selected both places since outside the rainy season *Phyllomedusa burmeisteri* is difficult to collect as they are dispersed throughout the habitat instead of conspicuously calling at water bodies. We collected frogs manually near streams and temporary ponds and in the case of the REM, where the species has been previously recorded (de Mira-Mendés et al., 2018).

Experimental phase.—We tested different prototypes in the form of harnessess and backpacks with various materials (elastic bands, linen, silicon, latex, nylon) in the laboratory. We started testing a harness-form prototype as this has been previously used with phyllomedusid frogs

(Gourevitch and Downie, 2018). However, individuals managed to remove the harness devices easily. Harnesses were most easily removed when the frogs were moving vertically; the harness type device together with the bobbin, created weight towards the back of the individuals when they were in an upright position and made the frog and the harness go backwards and the harness eventually fell off. Additionally, some materials that have been used previously on other species, injured the frogs, resulting in open wounds on the dorsal skin. When we found an adequate model, we tested it in the field. We considered it a suitable model when individuals failed to remove the device when frogs moved normally, jumped normally and if the material did not cause any visible injury soon after the device was attached.

Device construction.—After finding a suitable material, we created the tracking device with the microtube of a 23G Disposable Butterfly Needle (Tianjin Hanaco Xingda Medical Product CO LTD) by making two circles for the anterior legs of the individual (average diameter of 1.0 cm) and two other pieces for the dorsal and ventral part of the neck of the animal (Fig. 1A, B). One of the most important considerations was the need for the dorsal strut to be completely straight (Fig. 1D) so that no edge of the device directly touched the individual's skin. We bound the four pieces together with a medium cotton thread (Fig. 1E). The bobbins were nylon thread cocoon bobbins (HILTEX IND. E COM. DE FIOS LTDA) which were encapsulated in plastic wrapping (Fig. 1C) with a small hole at one end to allow the thread to unwind, and silver tape (Scotch 3M) to protect the bobbin from humidity and water. The wrapped bobbin was then attached to the device with silver tape (Fig. 1E). The whole device weighed less than 10% of the weight of each individual frog (Table 1). As the device was easily assembled, we had the pieces ready and constructed it in the field. Originally, all the bobbins were 5.0 g; we unwound them until we obtained smaller bobbins with the adequate weigh for each individual.

Field data collection.—We conducted our study in July, September and November 2019, and January and February 2020. Individuals were collected by active searches near temporary ponds and flooded areas during the night. Once an individual was located, coordinates were taken and we marked the exact place where we found the frog with marking tape. We then weighed the frog and measured the snout-vent length (SVL) and head width (HW). When we collected frogs in 2020, we also took a picture of the ventral pattern of each individual. We used a photo ID methodology (see Smith et al., 2019) and created a photo library to avoid recaptures. After the device was attached, we released the individual frog at night at its original capture site and observed its movements and behaviour for several minutes to ensure that it was able to move normally before walking away (Fig. 1F). As the thread was long enough for individuals to move and to be tracked daily (a 0.28 g bobbin holds 10 meters of thread), we monitored each individual once a day over a four-day period. We followed the trail of thread and whenever an individual was re-located, coordinates were noted, and the time of the observation recorded. As we were in an experimental phase, we collected the individual daily to check the device and to assess the frog for any injuries. We also observed the width of the bobbin and if we thought that the length of the remaining thread would not be sufficient to track the movement over the next 24-hr period, we changed the bobbin. We recorded information about the individual´s activity (if it was resting or moving), and any notes on behaviour observed during the encounter. The distance moved by the animals was determined by measuring the distance of the thread and the angle from the capture/recapture sites for every encounter. This information was not measured for all individuals since in the first instance, we were only testing the capacity and effectiveness of the animal to support the material and the device.

Results

We trialled our device on 15 individuals (Fig. 1) of *P. burmeisteri* over the entire sampling period (Table 1), and we were able to calculate the distance they moved for nine of them. After testing many prototypes, we developed a backpack prototype that was suitable for this species. However, the main challenge in finding an effective method was related to the angle of movement

of the shoulder and the absence of a body structure that 1 would help to hold the device (like a clearly defined neck, for example). We observed that the device did not affect the motion of the individuals when we released and monitored them in the laboratory and then in the field. We observed the individuals for some time after their release and they managed to move around on the ground as well as to climb trees and branches. The mean distance individuals moved was longer in the primary forest areas (n = 6, M = 12.24, SD = 8) than in the cacao plantation (n = 3, M = 5.17, SD = 4.25) (Table 1). The average number of days an individual could carry the device without having any injury was four days. After the fourth day, we started to observe slight skin colour changes, and in two cases, visible wounds. The wounds consisted of superficial cuts on the dorsal surface of the animal, specifically on the sides. Wounds were closely monitored after the devices were removed and resolved after two weeks with no issue. The photo ID technique was helpful in cases when two individuals shed their devices. We managed to re-capture both individuals the next day after the first release and continued with our study.

Discussion

This is the second study to use the thread bobbin methodology in phyllomedusid frogs and the first to propose a less invasive attachment methodology. One of the advantages of the device we have developed is that it allows to follow both vertical and horizontal movement of individuals, which is very important for this species, since they climb trees and are seldomly found on the ground. In the same way, even if the individual used the same route multiple times, we did not observe tangling of the thread or formation of knots, which allowed the frog to continue moving normally. This was one of the limitations found by Gourevitch and Downie (2018), where thread entanglement was thought to be caused by a particular individual using the same path over and over again; this restricted the frog's movements, sometimes bruising the waist of the frog, and this was fatal on one occasion. Fortunately, we did not lose any individuals during our study. However, two of our individuals developed minor injuries after the fourth day of monitoring. We resolved this issue and observed that it is important to create a "rectangle-shape" form of the device for the dorsal part. We also took care not to adjust the backpack too tightly so that individuals could move freely without the backpack applying undue pressure to the skin. Our device can be used only to monitor small movements over short periods of time, it is not suitable for long-term movement studies. We were unable to develop a device that was suitable for use over longer periods of time; even materials used in previous studies e.g. elastic bands (Waddel et al., 2016), thread (Tozetti and Toledo, 2005), tissue and latex; all resulted in injuries to the frogs within hours of attachment. Frogs attempted to remove the devices and the repeated movement of the device abraded the skin and open wounds developed.

One of the considerations when working with this species was that *P. burmeisteri* moves through aquatic habitats. Previous studies found the spool-and- line technique to be successful for medium to large terrestrial species that may occasionally use aquatic and semi-arboreal habitats (Waddel et al., 2016), and we did not find this to be an issue either. Aquatic or humid environments could be a limitation when using other techniques such as fluorescent powders, for example.

Previous studies have tested waist band attachment methodologies (Waye, 2001; Muths, 2003; Tozetti and Toledo, 2005; Gourevitch and Downie, 2018) and in most cases (Bufonids and Leptodactylids) they obtained positive results. However, it is important to create a specific backpack device for treefrogs to cater for their specific movement type and habitats. Attachment waist bands concentrated a lot of weight towards the waist of the frogs and this hinders movement. The waist of *P. burmeisteri* is much smaller than that of *Rhinella* or *Leptodactylus* (most species are considered large-bodied amphibians) in relative terms and as a result, individual frogs easily shed waist band devices when they started climbing.

One of the disadvantages of using the spool-and-line method to track phyllomedusid frogs is the weight of the thread bobbin. This is a limitation since the bobbin ends quickly and needs to be changed regularly (a 0.28 g bobbin holds 10 m of thread). This aspect has not been reported as a problem in other studies where they used quilting cocoons containing 300 m of cotton thread (Tozetti and Toledo, 2005).

Spool-and-line tracking has been incorrectly considered a disadvantageous alternative to radiotracking, but actually it provides a level of detail of the animal´s movements which is not possible with radiotracking. In fact, rainforest species use a complex three-dimensional structure (microhabitat use of burrows, logs, aquatic environments) and the thread bobbin method could yield information about the movements species make through all of these habitats in a three dimensional space (Waddel et al., 2016). Thus, both methods should be considered complementary. In many cases it is more useful to estimate the area of daily activity instead of the area of life in the traditional sense, which generally only estimates the area used during a single night of animal activity. Spool and line tracking allows the measurements of the movement pattern, its tortuosity, orientation, habitat selection and its intensity of use (Viera and Loretto, 2004). For all the variables mentioned above, spool-and-line can be considered a good alternative for rapid ecological surveys (days) and for animals with low to medium movement pattern (Waddel et al., 2016) or for long term but continuous (daily) movements since daily movements and microhabitat selection, as well as the search of retreat sites, can be obtained by using thread bobbins (de Oliveira et al., 2016) in a more precise way.

We have begun to test this device on other phyllomedusid frogs (Pithecopus sp. and Agalychnis sp.) and found that the device performs well. We encourage the use of this device in other medium to large arboreal frogs including Hylids (e.g. *Boana, Cruziohyla, Nyctimantis*) as well as frogs from other families (e.g. *Hemiphractidae, Pelodryadidae and Rhacophoridae*) with similar morphology to phyllomedusids (wider dorsum and a narrow waist), since these species are also known to have slow and precise movements, and they have a morphology that is more suited to our methodology than to waist band attachments.

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Table 1. Information on the individuals tracked (Weight of the individual, weight of the device (device and thread bobbin) and distance moved by the individuals during the study). Individuals collected at the agroforest are labelled with -UESC and the ones collected at the reserve with -MER. *Information not available since here we were testing the capacity and effectiveness of the animal to support the material and the device during the validation phase of our study.

Individual	Sex	Weight (g)	Weight of the device (g)	Distance (m)
P1-MER	Female	28.00	2.20	*
P2-MER	Male	23.00	1.90	*
P3-MER	Male	18.00	1.70	*
P4-MER	Female	27.00	2.50	*
P5-MER	Male	15.00	1.40	*
P6-MER	Male	17.00	1.60	16.71
P7-MER	Male	21.44	2.00	10.00
P8-MER	Male	18.58	1.70	3.70
P9-MER	Male	18.84	1.70	2.73
P10-MER	Female	22.41	2.10	18.32
P11-MER	Male	19.49	1.90	22.00
P12-UESC	Male	19.37	1.80	*

P13-UESC	Male	19.81	1.80	10.00
P14-UESC	Male	22.41	2.10	2.00
P15-UESC	Male	21.00	2.10	3.50

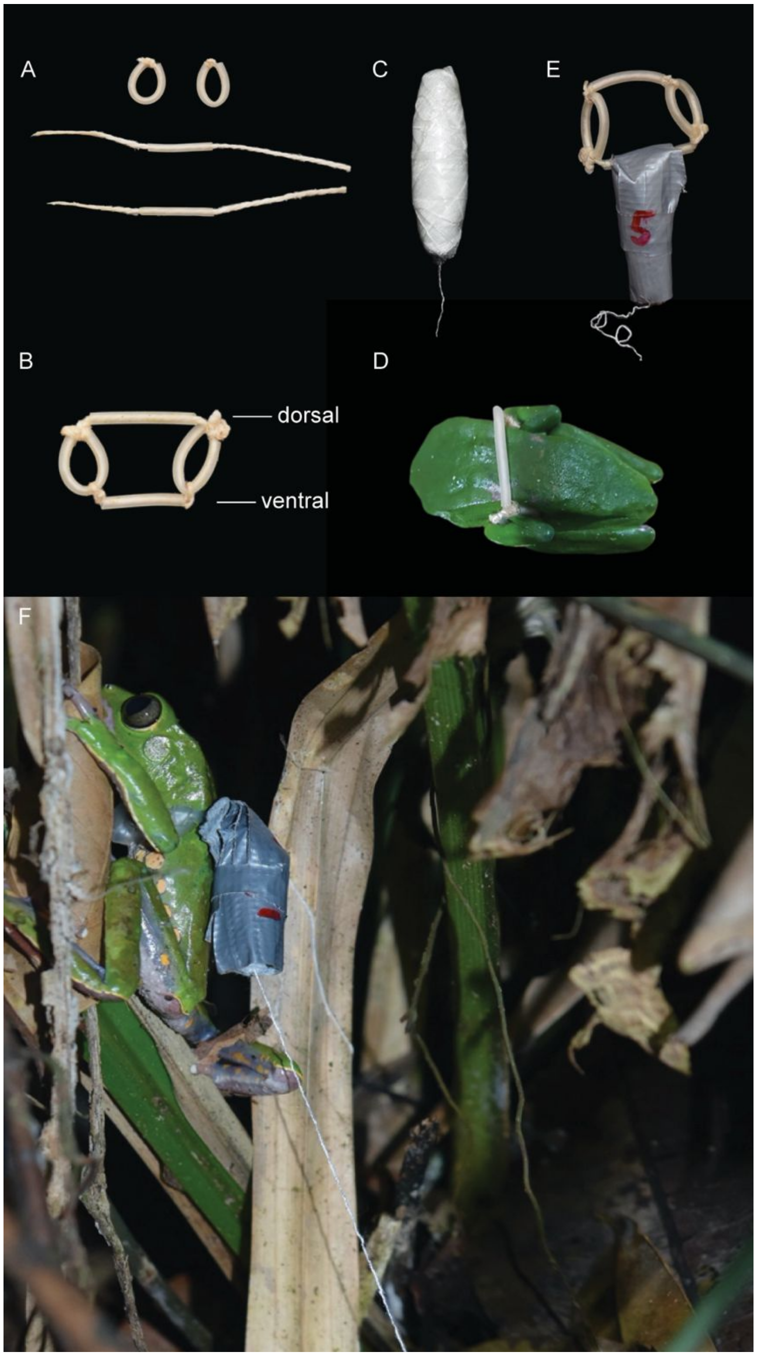

Figure 1. Device for bobbin attachment. (A) Parts of the device. (B) Armed device. (C) Thread-bobbin covered with plastic wrap. (D) Individual with device showing rectangle shape on the dorsum part. (E) Thread-bobbin attached to the device. (F) Individual released at its capture site after placement of the device on its back.