

Mechanism of Lung Ventilation in the Caecilian *Dermophis mexicanus*

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ABSTRACT The pattern of lung ventilation in the terrestrial caecilian *Dermophis mexicanus* was investigated by recording pressure changes of buccal and pleuroperitoneal cavities and activity of the buccal musculature. This species uses a fairly typical sarcopterygian buccal pumping system to inflate its single lung. What distinguishes it from other amphibians is the large number of buccal pumping cycles that occur in each ventilatory cycle. Up to 29 buccal cycles were observed to occur in a single respiratory cycle, with a mean of 16.1 ± 3.0 buccal cycles. This long series of buccal cycles avoids the sarcopterygian pattern of rebreathing expired air because only the first buccal cycle pumps expired air back into the lung. The series of buccal cycles also generates pleuroperitoneal pressures that are three to ten times greater than those observed in other amphibians. We suggest that these high pleuroperitoneal pressures are necessary for the maintenance of body form and locomotor function in terrestrial caecilians. © 1995 Wiley-Liss, Inc.

Amphibians ventilate their lungs with a buccal pulse-pump that is derived from the aquatic buccal pump of their sarcopterygian ancestors (McMahon, '69). The pattern of ventilatory air flow has been described in anurans (de Jongh and Gans, '69; Gans et al., '69; West and Jones, '74; Macintyre and Toews, '76; Vitalis and Shelton, '90) and salamanders (Brainerd et al., '93). In both frogs and salamanders the ventilatory cycle begins with an expansion of the buccal cavity to draw fresh air in. Air from the lungs is then expired through the buccal cavity and out the nares (terrestrial species) or mouth (aquatic species). The nares or mouth are then closed, and the buccal muscles contract to pump a mixture of fresh and exhaled air into the lungs. This method of ventilation is characteristic of lungfishes, frogs, and salamanders and appears to represent the mechanism of the basal sarcopterygians (Brainerd et al., '93). We investigated the ventilation of the third group of amphibians, the caecilians, because we suspected they might breathe differently.

Caecilians are highly derived from the ancestral tetrapod configuration and are unusual in many respects. They are slender, limbless, girdleless, and largely tailless. Most caecilians are highly fossorial in their habits, and their anatomy reflects this lifestyle. In

addition to their long, limbless body, they have reduced eyes, a significantly reinforced and flattened cranial skeleton that is used as a ram during burrowing (Wake, '92), tentacles, and a unique tube-within-a-tube configuration of axial musculature that facilitates an axial telescoping or vermiform locomotion that is superficially similar to the movements of annelids (Gaymer, '71; Gans, '73).

The extent to which many of the specializations of caecilians appear to be adaptations to a fossorial lifestyle led us to question whether their mechanism of lung ventilation might also reflect a burrowing existence. Do caecilians breathe with the mixed-air buccal pump of the basal sarcopterygians, or have the constraints of a fossorial existence influenced the evolution of the caecilian ventilatory system? In this investigation we monitored activity of the buccal muscles and changes in buccal and pleuroperitoneal pressure to address the mechanism of lung ventilation in the caecilian *Dermophis mexicanus*.

MATERIALS AND METHODS

This investigation addressed the mechanism of ventilation in the caecilian *Dermo-*

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phis mexicanus. The species was chosen primarily for its large size, which we believed would facilitate measurement of muscle activity and ventilatory pressures. Animals were collected at Finca San Jeronimo, Chiapas, Mexico. Two preserved specimens were examined to clarify bone/muscle organization. The myology of this species is described by Bemis et al. ('83). Four live individuals were available for the physiological measurements. These subjects weighed 128, 158, 164, and 177 g. They were housed in the laboratory in individual plastic boxes in a mixture of moist soil and sand. Boxes were kept in a Biotronette environmental chamber (Labline Instruments, Melrose Park, Illinois) with temperature maintained at 27°C and a photoperiod of 14 h light: 10 h dark.

Pressure recordings

Ventilatory changes in buccal and pleuroperitoneal (i.e., body) cavity pressures were recorded in four individuals. Water-filled catheters were surgically implanted in the two cavities, and these were attached to either a Statham P23D (Schlumberger, Norcross, Georgia) or an Omega PX143 (Stamford, Connecticut) pressure transducer. Both transducers were sensitive to pressure changes of less than 0.10 cm of water and had a response time of 1 ms. Signals were amplified with bridge amplifiers, recorded on a Crown 700FM tape recorder (A.R. Vetter Co., Rebersburg, Pennsylvania), and printed on a Beckman R611 chart recorder (Fullerton, California). Catheters were made of polyethylene tubing 0.5 m in length with an inside diameter of 1.14 mm. The pressure transducers were calibrated through these recording catheters to avoid any damping of the signal. For implantation of catheters the animals were anesthetized by immersion in a 2% solution of ethyl p-amino benzoate. The buccal cavity catheter was implanted through the posterior, lateroventral floor of the buccal cavity so that only a few fibers of the interhyoideus muscle were disturbed. The pleuroperitoneal cavity catheter was inserted through the lateral body wall, one-third of the length of the body behind the head. Both catheters were secured in place with sutures to the skin.

We had hoped to measure pulmonary pressure, but this was not possible because of high pressures (see below). In this species, any puncture of the lung results in air leaking from the lung to the body cavity, expansion of the body cavity, and subsequent collapse of the lung. Due to the small size of the

trachea, we also found it impractical to monitor pulmonary pressure via the trachea.

Electromyography

In three of the subjects, electromyographic activity of the *M. interhyoideus*, *M. levator arcus branchialis*, *M. rectus cervicis*, and *M. geniohyoideus* was recorded simultaneously with changes in buccal and pleuroperitoneal pressure. The animals were anesthetized as described above, and patch electrodes were implanted on the surfaces of the muscles. Patch electrodes were used because they provide an electrically nonconductive barrier in one direction. If applied with care, this attribute of patch electrodes greatly reduces the risk of recording electrical signals from muscles adjacent to the intended muscle (Loeb and Gans, '86). This is particularly helpful in situations where the muscles of interest are small, thin, and packed closely together, as was the case here. Electrodes were constructed from Dow Corning 501-1 Silastic reinforced sheeting (Green Rubber Co., Cambridge, Massachusetts) and Teflon insulated 75 μ m diameter stainless-steel wire (Cooner Wire Co., Chatsworth, California) (Carrier, '89). The noninsulated portions of the two electrode wires were approximately 1 mm long and separated by roughly 1 mm. Electrodes were implanted through a single longitudinal incision made in the skin ventral to the retroarticular process of the lower jaw. Electrode wires were passed percutaneously to a single exit point along the midline of the back. Electrodes on the interhyoideus were placed on the medial surface of the muscle and faced laterally. Electrodes on the other three muscles were placed on the lateral surfaces of the muscles and faced medially. The electrical signals from the muscles were passed to Tektronix FM 122 preamplifiers (Pittsfield, Massachusetts), amplified 1,000 times, and filtered below 80 Hz and above 10 kHz. Signals were then amplified an additional ten times by a set of AC amplifiers and simultaneously stored on a Crown International 700 tape recorder and printed out on a Beckman 611 chart recorder.

RESULTS

Ventilatory changes in buccal and pleuroperitoneal pressure

In *Dermophis*, the ventilatory cycle begins with expiration. In our analysis, the first indication of expiration was a rapid increase in buccal pressure (Fig. 1), which was generally three to five times greater than the increase associated with routine olfactory

pumps. Then, within a tenth of a second the pressure of the pleuroperitoneal cavity dropped dramatically. On average, pleuroperitoneal pressure decreased for 1–2 seconds and dropped by 5.6 ± 1.2 cm of water. Throughout this period of declining pleuroperitoneal pressure, buccal pressure remained elevated above atmospheric, and the external diameter of the trunk decreased.

The expiratory phase was always followed by a series of pronounced buccal pumps (Fig. 1). As many as 29 and a mean of 16.1 ± 3.0 ($N = 40$, from four subjects) buccal pumps were observed to occur in rapid succession. Each of the pumps started as a rapid increase in buccal pressure. An initial peak was reached, and this was followed by a small but rapid drop in pressure. At this point, buccal pressure either leveled off or, more commonly, slowly rose to a second peak. The positive pressure phase lasted from 0.2–0.4 seconds and was followed by a rapid fall in pressure to subatmospheric levels. Buccal pressure remained subatmospheric for roughly 0.15 seconds and returned to atmospheric for a brief period, and then the buccal cycle repeated. A drop in buccal pressure to subatmospheric levels was not observed prior to the first pump of the ventilatory cycle but did occur before each subsequent pump. The peak positive pressure of the buccal pump

rose with each successive buccal cycle such that the last pump in the ventilatory cycle had the highest peak pressure. Associated with each buccal pump was a small rise in pleuroperitoneal pressure and a small but obvious increase in trunk diameter. Thus, the pleuroperitoneal pressure gradually rose in a stepwise fashion (Fig. 1). Generally, buccal pumping continued until the pleuroperitoneal pressure had risen to approximately the pressure recorded before the breath began.

When resting, individuals of this species maintained a fairly high pleuroperitoneal pressure (i.e., approximately 15 cm of water), and during locomotion recorded pressures were much higher. For example, during a 79 second period of slow, routine "exploratory" movements by one individual, pleuroperitoneal pressure varied dramatically, rising as high as 54 cm of water and dropping as low as 20 cm of water (mean = 33.8 ± 10.1 cm water; $N = 40$ samples). The highest pleuroperitoneal pressure that we recorded, 92 cm of water, occurred during an attempt by one subject to slowly climb the side of the glass cage in which it was enclosed. Although our recording system did not allow measurement of pleuroperitoneal pressures during rapid locomotor movements (the inertia of the water in the recording tubes produced a movement artifact during rapid locomotion that

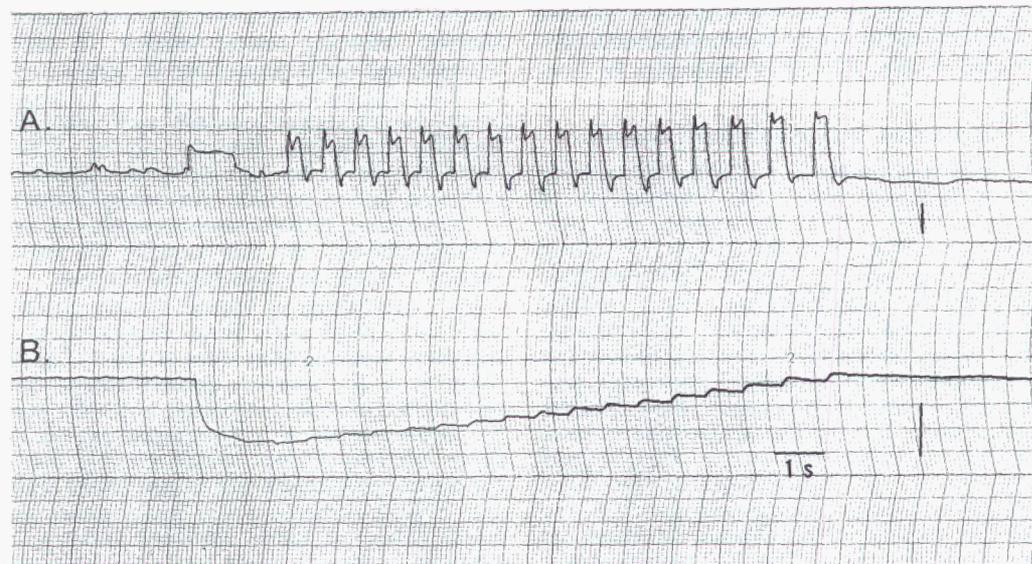


Fig. 1. Sample recording of changes in buccal (A) and pleuroperitoneal (B) pressure during a ventilatory cycle of *Dermophis mexicanus*. For each trace, the vertical bar represents a pressure of 5 cm of water. The baselines represent 0 cm of water (A) and approximately 12 cm of water (B).

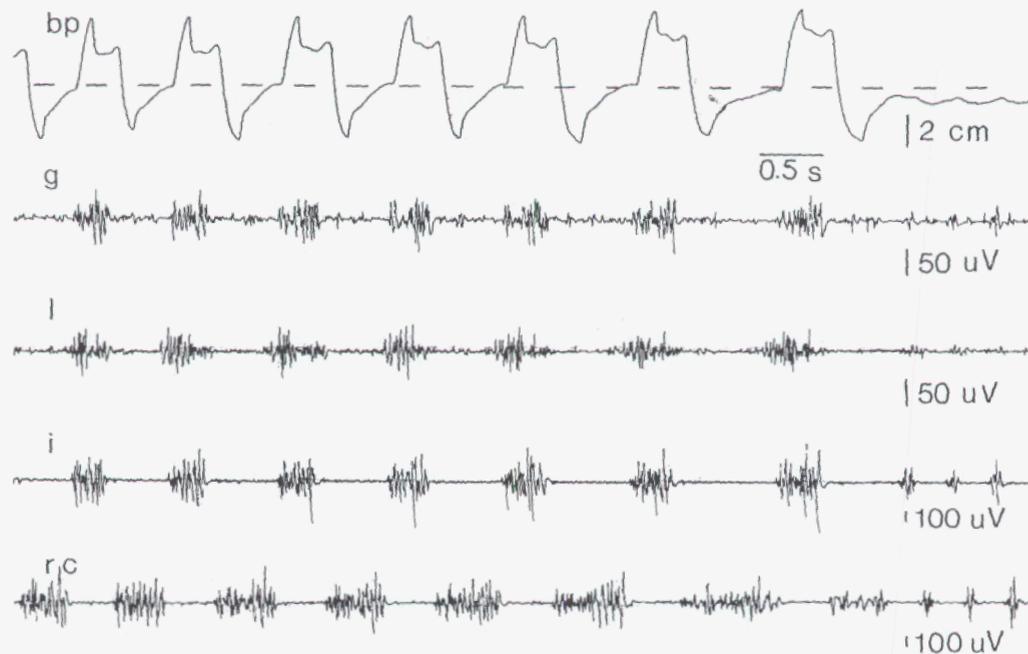


Fig. 2. Sample electromyographic recordings of the activity of four muscles associated with the inspiratory phase of ventilation in *Dermophis mexicanus*, plotted relative to changes in buccal pressure. bp, buccal pressure; g, M. geniohyoideus; i, M. interhyoideus; l, M. levator arcus branchialis; rc, M. rectus cervicis.

prohibited this), our impression from handling *Dermophis* is that the highest body turgor and presumably the highest pressures in the body cavity occur during digging behavior and escape efforts in response to handling.

Ventilatory activity of buccal muscles

The buccal muscles of *Dermophis* are described by Bemis et al. ('83). Examination of these muscles led us to suspect that five muscles participate in the inspiratory pump of the buccal cavity. The M. intermandibularis and M. interhyoideus form a ventral muscular sling around the floor of the cavity, which upon contraction raises the floor of the mouth and throat dorsally. This would, presumably, constrict the buccal cavity. The M. levator arcus branchialis is also able to raise the floor of the buccal cavity. This muscle originates in the dorsal fascia of the trunk behind the cranium and passes ventrally and medially to the interhyoideus, to insert on the hyoid apparatus. The M. geniohyoideus also reduces the volume of the buccal cavity but through a different action. The geniohyoideus runs from the symphysis of the lower

jaw to the ventral aspect of the hyoid arches. Activation of this muscle draws the hyoid arches rostrally, which swings them dorsally, thereby reducing buccal volume. In series with the geniohyoideus is the M. rectus cervicis. This muscle runs from the ventral aspect of the hyoid arches caudally and ventrally, thereby expanding buccal volume. Thus, there appear to be four muscles capable of reducing buccal volume (intermandibularis, interhyoideus, levator arcus branchialis, and geniohyoideus), and one muscle, the rectus cervicis, which increases buccal volume.

Our recordings of buccal muscle activity were consistent with these expectations in the three subjects that were studied (Fig. 2). Activity of the levator, geniohyoideus, and interhyoideus was associated with the positive pressure phase of the buccal pump. All three muscles became active shortly before the buccal pressure began to rise above ambient. Figure 3 displays the mean onset and offset times of these muscles relative to changes in buccal cavity pressure from one subject. Recordings from the other two subjects displayed the same pattern. Activity in the levator preceded that in the geniohyoi-

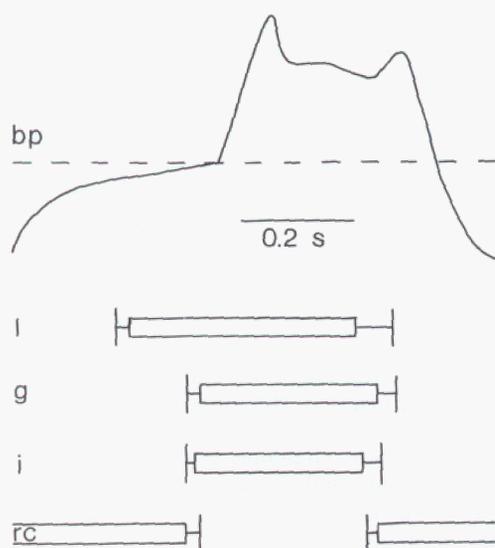


Fig. 3. Means and standard deviations of onset and offset times of ventilatory muscles calculated relative to the beginning of the positive pressure rise in buccal pressure during the inspiratory pulse-pump in *Dermophis mexicanus*. The buccal pressure trace (bp) is typical of those recorded, and the dashed line represents zero pressure. Abbreviations of muscles as in Fig. 2. For each muscle, the left edge of the box represents the mean time of the onset of muscle activity, and the right edge represents the mean offset time.

deus and interhyoideus by approximately 120 ms. The geniohyoideus and interhyoideus both became active about 30 ms before the initial rise in buccal pressure. All three of these muscles remained active throughout most of the positive phase but were silent by the time buccal pressure began to drop rapidly. Activity in the rectus cervicis was associated with the negative pressure phase of the buccal pump. It became active about 35 ms before the initial drop in pressure and stayed active throughout the negative pressure phase, ceasing activity some 50 ms before the rise in pressure.

DISCUSSION

In *Dermophis mexicanus*, the first indication that a ventilatory cycle is beginning is a rapid rise in buccal pressure, which is immediately followed by a drop in pleuroperitoneal pressure. As the pressure in the body cavity falls, the external diameter of the trunk is seen to decrease. We interpret these changes to represent the opening of the glottis and a passive flow of air out of the lungs, through the buccal cavity, and out the nares.

Until recently, animals utilizing a buccal pulse-pump for lung ventilation were believed to rely solely on the elasticity of the lungs to expel air during expiration. This has been shown to be the case in both lungfish (McMahon, '69) and anurans (de Jongh and Gans, '69). However, in the salamander *Necturus maculosus*, the transversus abdominis muscle is active during expiration to force air from the lungs (Brainerd et al., '93). Although we did not monitor the activity of the hypaxial muscles of *Dermophis*, all evidence suggests that trunk muscles are not involved in expiration. First, there does not appear to be any need for the hypaxial muscles to be involved in expiration. Pleuroperitoneal pressures are always well above atmospheric. Consequently, if both nares and glottis are open, air will flow from the lungs to the environment. Second, during expiration, pleuroperitoneal pressure is observed to decrease. If the hypaxial muscles were activated to force air from the lungs, pleuroperitoneal pressure would rise. Our recordings do not, however, exclude the possibility that pulmonary smooth muscle could contract or that supporting rings of cartilage in the lung could recoil to reduce pulmonary volume as Marcus ('23), Wake ('74), and Bennett and Wake ('74) have suggested. However, if elasticity of the lung did play a large role in expiration, we would expect to see subatmospheric pressures in the pleuroperitoneal cavity at the end of expiration, and this was not observed. Consequently, we suspect that air exits the lung solely as a result of the existing high pleuroperitoneal pressure.

If expiration is entirely passive in *Dermophis mexicanus*, then the inspiratory pump is ultimately responsible for the work of both inspiration and expiration. In general, the inspiratory pump of *Dermophis* is similar to what has been described for lungfish, anurans, and salamanders. As in other buccal pumpers, the rectus cervicis muscle shortens to expand the buccal cavity and fill it with fresh air, and the geniohyoideus, levator arcus branchialis, and interhyoideus muscles contract to reduce the buccal volume and thereby drive air into the lung. The pattern in *Dermophis* does differ from that of other amphibians in that the cycle does not begin with an active expansion of the buccal cavity to draw fresh air in. Instead, the buccal cavity expands passively in response to the positive pressure generated by expiratory flow of air from the lung. *Dermophis*, however, is

similar to lungfish and other amphibians in that there is not an expansion of the buccal cavity to draw in fresh air prior to the first contraction of the buccal cavity. The first buccal cycle pumps expired air back into the lung. Thus, *Dermophis* fits the sarcopterygian pattern of being a mixed-air buccal pumper (Brainerd et al., '93). However, in reality, very little expired air is returned to the lung because the subsequent 16 or so buccal cycles pump only fresh air.

Although amphibians and lungfishes generally inflate their lungs with a single buccal pump, anurans (de Jongh and Gans, '69; Vitalis and Shelton, '90), salamanders (Brainerd et al., '93), and lungfish (McMahon, '69) do occasionally use a short series of buccal pumps (e.g., 2–4) to inflate their lungs. Thus, caecilians are not unique in employing a series of inspiratory pulses of the buccal musculature. Furthermore, the higher number of inspiratory pumps used by *Dermophis* may, in part, be a consequence of the relatively small buccal cavity of caecilians as compared to that of most anurans and salamanders. However, *Dermophis* is distinguished from other buccal pumpers by the high pulmonary pressures they generate. Anurans generate pulmonary pressures of 2–5 cm H₂O (de Jongh and Gans, '69; West and Jones, '74; Macintyre and Toews, '76; Vitalis and Shelton, '90). Salamanders produce fairly high pleuroperitoneal pressures during expiration (i.e., 10–21 cm H₂O) but generally maintain pressures of only 2–5 cm of water (Toews and McRae, '74; Brainerd et al., '93). In contrast, *Dermophis* maintains a resting pleuroperitoneal pressure of 15 cm of H₂O and often generates pressures as high as 70–80 cm H₂O. These high pressures require a series of forceful pumps from the buccal musculature.

The large number of buccal cycles during each inspiration allows *Dermophis mexicanus* to achieve and maintain high pleuroperitoneal pressures. Our handling of other genera (e.g., *Gymnopsis*, *Oscacaecilia*) indicates that the high turgor pressure of *Dermophis* is typical of terrestrial caecilians. In addition to the long series of buccal pumps, there are two other characteristics of caecilians that facilitate high pleuroperitoneal pressures: the lung of caecilians is relatively large, filling a large proportion of the body cavity (Wake, unpublished observation), and the interhyoideus muscle is highly modified in caecilians. An anterior component originates on the hyoid

arch, as it does in fishes and other amphibians, and a posterior element originates on the large retroarticular process of the mandible. The muscle is also of greater thickness and extends more posteriorly than in other amphibians. Due to its position directly behind the retroarticular process, Nussbaum ('77, '83) suggested that the interhyoideus posterior adducts the lower jaw. Electromyographic observations confirm this (Bemis et al., '83). Although this is a unique function for the interhyoideus, it may not be the only action that is enhanced by the modifications. The retroarticular process provides a firmer anchor for origin of the muscle than does the hyoid. Furthermore, the posterior expansion of the muscle envelops the buccal cavity. Both of these modifications of the interhyoideus can be expected to enhance the power of the buccal pumping system.

Achieving and maintaining such high pressure must come at a relatively high energetic cost. Yet it is not immediately obvious how, and if, these pressures benefit the animals. High pulmonary pressure could simply be a means of achieving large tidal volumes. However, it is not clear what would be gained in terms of gas exchange, and large tidal volumes theoretically could be achieved without the presumably energetically costly high pressures.

The high pleuroperitoneal pressures do provide caecilians with a mechanism for maintaining general body form. Healthy caecilians have a circular cross-section and are very turgid when they are active. The circular cross-section and turgor disappear when they are anesthetized. This is not only a result of a loss of muscle tone but also a loss of pleuroperitoneal pressure. As animals come out of anesthesia, they gradually, over a period of several hours, pump themselves back up to their original pressure. Thus, Gans ('74) has suggested that a turgid body may be essential for locomotion in these animals. Caecilians progress by vermiform locomotion (Gaymer, '71; Gans, '73) and lateral undulation. Both forms of locomotion require the surface of the body to exert force against the substrate. As in other lissamphibians, the ribs of caecilians do not curve laterally. Consequently, there is nothing for the axial muscles to work against other than the internal pressure of the trunk when exerting force on the substrate. The observation that the highest pressures occur during locomotion is suggestive of the importance of coelomic pres-

sure to locomotion. Therefore, we suspect that the modifications of the caecilian ventilatory system (enlarged and modified interhyoid muscles, large lung, high number of buccal pumps for each inspiration, and high pulmonary pressure) do not necessarily improve gas exchange but that they enhance locomotor performance. The inspiratory phase of the ventilatory cycle of *Dermophis* can be likened to a hand pump inflating a bicycle tire. Each pump increases the volume of the tire, but a series of pumps is required to reach a pressure sufficient to support a rider.

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