Internal oral anatomy: a comparative analysis of the phyllomedusinae (Anura:Hylidae)

Erin B. Hines

Follow this and additional works at: https://scholarship.richmond.edu/honors-theses

Part of the Biology Commons

Recommended Citation
https://scholarship.richmond.edu/honors-theses/1038

This Thesis is brought to you for free and open access by the Student Research at UR Scholarship Repository. It has been accepted for inclusion in Honors Theses by an authorized administrator of UR Scholarship Repository. For more information, please contact scholarshiprepository@richmond.edu.
Internal Oral Anatomy: A Comparative Analysis of the Phyllomedusinae (Anura: Hylidae)

by

Erin B. Hines

Honors Thesis in Department of Biology University of Richmond Richmond, VA

April 30, 1997

Advisor: Dr. Rafael de Sá
INTERNAL ORAL ANATOMY: A COMPARATIVE ANALYSIS OF THE
PHYLLOMEDUSINAЕ
(ANURA: HYLIDAE)

Erin Hines and Rafael O. de Sá
Department of Biology, University of Richmond, Richmond, Va 23173

Abstract: The internal oral anatomy of phyllomedusines has only been described for Agalychnis callydrias using light microscopy. Herein, we compare the internal oral anatomy of the genera Phyllomedusa, Agalychnis, Pachymedusa, and Phasmahyla, using SEM methodology. Tadpoles of ten species were prepared using standard SEM techniques; these larvae ranged from Gosner stages 34-39. Phasmahyla has a unique prenarial arena with three well developed pre-narial arena papillae, whereas the other genera possess the prenarial arena papillae in a semicircular arrangement with the opening directed posteriorly. Pachymedusa differs from Phyllomedusa and Agalychnis by having a low, straight-edge, median ridge; also, its buccal floor arena is limited by two converging rows of narrow, attenuate papillae. Species of Phyllomedusa differ from Agalychnis, and from other genera, in having numerous and well-developed projections extending from the mid-dorsal velum. Minor species-specific differences were found within Phyllomedusa. These preliminary results suggest that characteristics of the internal oral anatomy may be useful in understanding the evolution of this group. Furthermore, they may provide additional support to Cruz's (1990) recent recognition of 6 genera within the Phyllomedusinae.

INTRODUCTION

Members of the subfamily Phyllomedusinae span in range from Mexico to Argentina, and consist of 6 genera: Agalychnis, Pachymedusa, Phyllomedusa, Hylomantis, Phasmahyla, and Phrynomedusa. The latter three genera have recently been resurrected from Phyllomedusa by Cruz (1990), and occupy habitats from Costa Rica to Uruguay. Pachymedusa dacnicolor inhabits the Pacific lowlands of Mexico, whereas Agalychnis is distributed throughout Southern and Central America, south to the Amazonian Ecuador.

The goal of this project is to identify, describe, and compare the internal oral anatomy of Phyllomedusinae tadpoles using scanning electron microscopy. Available for
analysis in this study were tissue samples from one species of *Pachymedusa (P. dacnicolor)*, one species of *Agalychnis (A. callydrias)*, two species of *Phasmahyla (P. guttata and P. cochranae)*, and six species of *Phyllomedusa (P. tomopterna, P. tetraploidea, P. tarsius, P. boliviana, P. hyperchondrialis, and P. vaillanti)* for a total of ten species representing four of the six genera. The only previous data available of this kind reported for this group was done by Wassersug (1980) on *Agalychnis callydrias* using light microscopy. Accumulation of this data may be used in conjunction with further descriptions of subsequent species to identify organisms based on the oral morphology of their larval stages. It is possible, as well, that the character identifications may be used in systematic analysis and classification of the Hylid family.

**MATERIALS AND METHODS**

Tadpoles were staged according to Gosner's (1960) table for staging anuran embryos and larvae and terminology used to describe features of the oral cavity follows that used by Wassersug (1976). Tadpoles ranging form stage 34-39 were dissected in order to separate dorsal and ventral surfaces of the oral cavity to facilitate SEM analysis. All specimens fixed in 10% formalin were prepared using standard SEM preparation techniques. Specimens were subjected to an ultrasonic wash for 15 minutes, fixed in a 3-4% glutaraldehyde solution for 3-4 hours at room temperature, put through three 15 minute washes in 0.1 M phosphate buffer, followed by secondary fixation for two hours in a 1% solution of osmium tetroxide, after which three additional 15 minute washes in 0.1 M phosphate buffer were performed. Next, all specimens were dehydrated through the use of 15 minute changes in a graded ethanol series as follows: 35%, 50%, 70%, 80%, 95%, and three changes of 100%. Specimens were critical point dried in CO₂, mounted on aluminum discs and sputter coated with gold/palladium, 30 nanometers thick, using a Hummer VII sputtering system. Features of dorsal and ventral internal oral anatomy
were examined using a Hitachi S-2300 scanning electron microscope at 15 kV and were photographed using Polaroid 55 positive/negative film. Additionally, a light microscope was used to check over any areas that may have been unclear in SEM photographs. Specimens were stored in a vacuum sealed container when not in use.

RESULTS

Agalychnis callydrias

Dorsal:

The prenarial arena is characterized by a semi-circular ridge with distinct marginal projections. The ridge is transected medially by a gap that gives way posteriorly to a medial projection containing 2-4 pustulations. The narens are about 0.65 mm in diameter. They have reduced narial valve projections on the medial corners of the posterior narial walls, but have numerous, tiny prenarial papillae running the length of the anterior walls. The postnarial arena is characterized by two distinct types of papillae that lie on a lateral ridge behind the narens. The postnarial papillae are the anterior-most of the two and originate behind the medial corner of the nares, projecting anteromedially. They are finger-like and dotted with smaller papillae at the tips. The posterior-most of the two are greatly reduced in size, being only slightly larger than pustulations, and extend medially. A pair of lateral ridge papillae, one on each side of the median ridge, extend medially. They are more angular and blunt in shape than the postnarial papillae and have uneven, pustulated margins. The postnarial arena also contains a cluster of 5-10 papillae in the region covered ventrally by the median ridge. Some of these pustulations are long and accunimate, while others were mere pustulations.

The rounded median ridge transects the buccal roof a little anterior to the midline and contains several secondary papillae on the anterior margins. The BRA is distinguished by what appears to be two rows of BRA papillae on each side. These papillae are elongate and extend medially. They lie in the posterior-most 1/3 of the BRA with the smaller papillae in the lateral row (2 to 4 each side) and the larger medial (2 to 4 each side). Within the boundaries of these papillae, and scattered randomly outside them, are numerous (~ 90-100) pustulations, the majority of which lie in the direct center of the arena. Clusters of 15-20 lateral roof lie on the lateral walls of the arena. The BRA terminates posteriorly with the glandular zone which is highly concentrated with
secretory pits. The pits are, for the most part, equal in size from anterior to posterior, maybe getting slightly smaller towards the dorsal velum. Where the dorsal velum converges medially are a few (4-6 each side) small, knobby projections randomly distributed.

**Ventral:**

The anterior-most region of the buccal floor is characterized by a series of infralabial papillae on the lateral walls, approximately 5-7 each side. The major papillae is large and cup-like (rounded ventrally and concave dorsally), and its is fringed with subpapillae. Surrounding this major, medial papilla, anteriorly and posteriorly, are clusters of pustulations. A pair of acuminate lingual papillae sit symmetrically about the midline of the buccal floor. In one specimen examined the tips were bifurcated, in another they were nipple-like.

The BFA is bound laterally by a single row of finger-like BFA papillae, about 5-6 each side. These begin approximately at the level of the buccal pocket and continue to about 2/3 the length of the floor, with the larger papillae appearing posteriorly. The smaller papillae are less uniform in alignment but similar in size and shape. They blend into a region of variably sized papillae and pustulations that seem to be homologous to prepocket papillae; but, due to the lack of a concrete separation, cannot be labeled as such. The BFA itself is scattered with approximately 40+ pustulations. The BFA gives way to the velum which shields the anterior portion of the 6 branchial baskets. The velar margin is characterized by 14 marginal projections, 6 on each side of the median notch. The medial-most four (8 total) are aggregated close together in front of the esophageal funnel and seem to share a common origin. The lateral-most three are associated with their respective filter cavities I, II and III.

**Pachymedusa dacnicolor**

**Dorsal:**

The prenarial arena contains the typical semi-circular arch of papillae transected medially by a gap that gives way posteriorly to a second, central ridge of papillae. Ten to twelve pustulations are present in the beak region directly anterior to this set of ridges.

The nares are characterized by fairly broad prenarial papillae on the anterior walls (concentrated medially) and flap-like prenarial papillae concentrated laterally. Narial valve projections are present on the medial-most corners of the posterior walls. The postnarial arena is characterized by a series of distinct papillae. The first of the series are the postnarial papillae which are, by far, the most distinct. They are finger-like with pustulated margins and extend medially and slightly anteriorly. Projecting from the
same ridge as the post-narial papillae are two smaller, but distinct, club-like sub-papillae. Behind and lateral to the ridge are the lateral ridge papillae that may, or may not, be bifurcated, but which are usually, to some degree, pustulated at the tips. In this species, the lateral ridge papillae are broad laterally with a sloping anterior margin, giving the effect of a right triangle. The postnarial arena that lies between and below the nares and stops at the median ridge contains 3-5 subpapillae.

The median ridge is a flat, horizontal flap with numerous spur-like secondary papillae running along its margin. The buccal roof arena (BRA) is bordered at the posterior lateral corners by 3-4 jagged, spur-like papillae, per side. The region delimited by these papillae houses 65-90 BRA pustulations. Clusters of elongate lateral roof papillae can be found on the lateral walls of the BRA, along with numerous smaller pustulations.

The BRA ends posteriorly with the glandular zone which is comprised of a carpet of secretory glands, leading into the dorsal velum. The margin of the dorsal velum is uneven, and as it gives way to the esophageal funnel medially, the lateral corners of the pressure cushions are characterized by 5 or 6 large, rounded projections.

**Ventral:**

The anterior-most region of the buccal floor houses a series of infralabial papillae. The most prominent papillae is the most medial and shaft-like in structure, sometimes bifurcating at the base. These extend anteromedially and are bordered on both sides by terminally pustulated subpapillae (approximately 3 per side). The lingual papillae are dorsoventrally flattened, fairly uniform in length from base to tip, and sit symmetrically about the midline.

Twenty to twenty-four (10-12 per side) finger-like BFA papillae make up the lateral boundary of the BFA. They are organized roughly as a straight line with the largest two lying centrally, sharing a common base. Anteriorly the row gives rise not only to smaller BFA papillae, but to a cluster of smaller subpapillae and pustulations that seem to be homologous to a region of pre-pocket papillae. The BFA that lies between the two lines of BFA papillae is concave medially and very sparsely dotted with pustulations. The bulk of the BFA pustulations are concentrated at the posterior-most region of the buccal roof, giving way to the velum. The velum is comprised of a series of marginal projections. The lateral-most two on each side are densely packed with secretory tissue and are associated with respective branchial baskets. Surrounding the medial notch is a cluster (one on each side) of bifurcated projections.
**P. boliviana**

**Dorsal:**

The prenarial arena in this species is highly distinct in that it is characterized by two semi-circular projections. The anterior-most ridge is not as distinct as the ridge behind it. It is m-shaped with the widest area being the medial juncture of the lateral wings with its with gradually decreasing posteriorly. Unlike this ridge, the posterior-most projection is highly papillated at its margins. The overall shapes are similar; however, the latter has a distinct medial gap that gives way to a central, anterior-posteriorly directed ridge containing 3 or 4 papillae.

The slit-like narens are slightly shorter than 1 mm in diameter. Their anterior walls have distinct spur-like prenarial papillae on the medial-most corners and flap-like prenarial papillae on the lateral. Distinct narial projections that are about half the width of the diameter are located on the medial-most corner of the posterior wall. The post-narial arena is characterized by a pair of postnarial papillae, one on each side, which are long and finger-like and project anteromedially. The surface of these papillae are smooth except for the tiny pustulations found at the tips. Behind and lateral to these papillae is a pair of lateral ridge papillae which, like the postnarial papillae are generally smooth until the tips. Six to seven post-narial pustulations are located in the region of the postnarial arena that is shaded by the median ridge.

The median ridge separates the post-narial arena from the BRA. Its overall shape is trapezoidal with a transecting medial gap. The marginal walls surrounding this gap are slightly fringed; however, the remainder of the edge and ridge surface is smooth. Posterior to the median ridge is the BRA, the anterior-most, lateral corners of which contain clusters of lateral roof papillae (approximately 5 of which are true papillae and 10-15 mere pustulations). The center of the buccal roof, the BRA, is bounded by a single row of conical BRA papillae, approximately three to four on each side, with the largest lying centrally and slightly laterally. Over 100 pustulations occur in and around the BRA, with the densest concentration centrally. These pustulations continue into the glandular zone posteromedially, which is comprised of densely packed secretory pits. Where the dorsal velum converges medially, leading into the esophagus, is a distinct region of about 20-25, elongate rounded projections.

**P. hyperchondrialis**

**Dorsal:**

The prenarial arena is distinguished by a pustulated semi-circular ridge that is transected medially by a gap. The gap gives way posteriorly to a projection that extends
anteriorly to posteriorly and whose anterior diameter is about twice that of the posterior tip. The narens are about 0.65 mm in diameter and have well defined narial valve projections on their posteriomedial walls which extend anteriorly to partially cover the narial openings. No prenarial papillae seem to be present on the anterior walls.

Distinct postnarial and lateral ridge papillae characterize the postnarial arena. The postnarial papillae extend anteromedially from an origin behind and to the center of the nares. They are finger-like with a rounded tip, and have a total length approximately equal to the nares. The lateral ridge papillae are flattened and fan-like with an uneven margin. They originate behind, and slightly lateral to, the prenarial papillae, but extend medially to only about one quarter their length. The postnarial arena also has about 3-5 random pustulations on the floor of the roof in the region between the nares and the median ridge.

Halfway back on the buccal roof is the anteriorly directed, semicircular median ridge which is slightly serrated on its anterior margin. The BRA consists of distinct BRA papillae (two in the specimen analyzed- one on each side) and are located in the lower 1/3 of the roof. They are more elongate than the pustulations that are numerous throughout the BRA, the most dense of which are posteriomedial as they gradually thin out laterally and anteriorly. Clusters of 10-15 lateral roof papillae are present on the lateral walls of the buccal roof.

The glandular zone is comprised of numerous secretory pits which are larger anteriorly and gradually get smaller posteriorly towards the dorsal velum. The dorsal velum in this species is highly papillated at the margins and lightly pustulated on the interior surface. Where the velar surfaces converge medially lies a distinct crescent-shaped protuberance that is concave posteriorly with an uneven margin.

Ventral:

A pair of cylindrical, terminally pustulated infralabial papillae characterize the beak cavity and are surrounded by two to four noteworthy pustulations. A pair of lingual papillae that sit on the anterior-most portion of the buccal floor and are symmetrical about the midline, and are uniform in diameter from base to rounded tip without subsequent papillations. The buccal floor arena (BFA) is characterized by two rows of BFA papillae per side that circumscribe the outer limits of the BFA. One or two larger, broader papillae comprise the lateral row, whereas smaller, narrower ones characterize the inner one. The interior surface of the BFA is scattered with numerous pustulations (75-80), the majority of which are concentrated posteriorly. As the pustulations thin out anteriorly, they seem to merge with a region of papillae comparable in location to the
pre-pocket papillae, but are not fully separated in this species. These papillae are knobby and variable in size, numbering between 10-15.

The BFA pustulations continue to some degree into the velum only stopping at the glandular zone at the very margin of the velum. The velum is further characterized by a medial notch that is bordered on both sides by a pair of knobby marginal projections that seem to share a common origin. Six other marginal projections make up the velar surface, three on each side of the notch. These projections hang above a respective branchial basket I, II, or III.

**P. tarsius**

**Ventral:**

Extralabial papillae, with three poorly differentiated projections, are found on either side of the lower beak. Four infralabial papillae (2 on each side) project into the prelingual space on each side. The anterior-most papillae is the most conspicuous with its highly pustulated anterior margin and broad, flattened surface. A second pair is considerably smaller and nipple-shaped and extends medially into the cavity. A pair of acuminate lingual papillae sit symmetrically about the midline of the anterior tip of the buccal floor. The anterior quarter of the buccal floor arena (BFA) is free of papillae, where the lateral posterior three quarters exhibit 7-8 BFA papillae that extend medially. The three posterior-most papillae are largest with papillae 1 and 2 of this trio sharing a common base. There is a gradual reduction in size anteriorly, where the anterior-most cluster of papillae may be homologous to pre-pocket papillae, but aren’t delimited as such.

**Dorsal:** The prenarial arena consists of a semi-circular ridge, concave posteriorly, that is transected medially by a gap followed by a medial extension of the ridge. The ridge contains numerous projections, ~ 4-5 per side and 1-2, medially. The nares are ~ 0.75 mm in diameter. A flap of prenarial papillae is present at the lateral corners of the anterior walls, and continue to fringe the edge to the medial corners. No distinct narial valve projections are present. The ridge directly behind the nares contains a pair of anteromedially directed postnarial papillae that are uniformly broad until an abrupt tapering at the tips. These tips may be further pustulated. Directly behind the postnarial papillae is a pair of reduced subpapillae. The postnarial ridge ends laterally with a pair of fan-like lateral ridge papillae that extend medially with an uneven margin. The median ridge lies central to these papillae and is pyramidal in shape with a slightly pustulated tip. The BRA is bounded laterally by 3-4 conical, medially projecting BRA papillae per side. The region enclosed by these papillae, the buccal roof arena, is characterized by a dense
mass of pustulations that may stray beyond the BRA papillae, laterally, but only a few in number. Due to marred specimens, the presence of lateral roof papillae cannot be determined, however the remaining tissue suggests their existence. The dorsal velum begins anteriorly with the glandular zone and gives way posteriorly, and medially, to a dense region projections. The larger, fleshier projections are found laterally, and gradually reduce medially into the esophageal funnel.

**P. vaillanti**

**Dorsal:** The prenarial arena is characterized by a semicircular ridge (concave posteriorly) that is transected medially by a gap. The top of the ridge contains small, irregularly-sized pustulations (3-5 per side of the gap). Medially, within the semicircle, are 1-2 more prenarial pustulations. The nares are approximately 3/4 of a mm in diameter. The anterior walls are rough with tiny pustulations, while the posterior walls have reduced narial valve projections. Between the nares and the median ridge is a ridge with two distinct papillae; the anterior-most, nearest to the nares, are the post-narial papillae which extend anteromedially. These papillae, one on each side, are club-shaped with a highly textured, folded surface. Directly behind them on the ridge is a pair of subpapillae, greatly reduced in size with pustulated tips. Slightly behind where the ridge meets the lateral walls is a pair of fan-shaped lateral ridge papillae, one on each side. These papillae extend anteromedially and have a fringed, uneven margin. On the floor between the two nares extending to the median ridge are numerous tiny postnarial arena papillae. The median ridge that separates the narial arenas from the buccal roof is rounded with an uneven margin.

The buccal roof arena is delimited laterally by 2-3 attenuate BRA papillae per side. Scattered in and amongst the region bordered by these papillae are numerous tiny pustulations (~ 60-75) that span the entire buccal roof, anteriorly from the median ridge, posteriorly to the dorsal velum, and laterally to just short of the lateral roof papillae. These latter papillae exist in clusters of about 6 larger, attenuate papillae and 7-10 smaller pustulations, per side. The crescent-shaped dorsal velum provides the posterior border of the roof and is characterized by a glandular zone densely packed with secretory pits of varying sizes. Unfortunately, in the two specimens analyzed of this species, the region of the roof below the pressure cushions was destroyed, and we were, therefore, unable to analyze this species for the special characteristics thought to be present in this region for this genera.
**Ventral:**

The infralabial papilla in this species is broadly flattened with a sloped, pustulate dorsal edge and a straight ventral edge that converge to a rounded, pustulate tip. The papillae are much larger in this species and appear folded to fit within the confines of the beak cavity. Directly behind these papillae on the ventral walls of the beak cavity are several more, reduced papillae and pustulations in random arrangement. The lingual papillae sit at the most anterior region of the tongue anlage and appear elongate and tapered at the tip. The buccal floor arena is bordered laterally by a series of BRA papillae. These papillae start as 2 rows, 2 papillae in each row, then converge at the level of the buccal pocket to form a single row of longer, slimmer papillae that extend medially towards the buccal roof arena. No distinct pre-pocket arena are present, however, scattered in and amongst the more anterior BRA papillae are several smaller, subpapillae that may be homologous to pre-pocket papillae. They cannot be defined as such due to lack of a clear differentiation. The internal area bordered by the BRA papillae is, for the most part, smooth with only a few (5-10) tiny pustulations scattered amongst it.

The velum is characterized by a median notch that is immediately surrounded on both sides by a fairly large, broad velar projection. Evenly spaced to the lateral sides of these projections are two more velar projections per side, each aligned with respective filer cavities I and II.

**P. tomopterna**

**Dorsal:** The prenarial arena is characterized by a semicircular ridge (concave posteriorly) that is transected medially by a gap. The top of the ridge contains small, irregularly-sized pustulations (3-5 per side of the gap), with the larger ones situated medially. Medially, within the semicircle, are 1-2 more prenarial pustulations located on a smaller ridge. The anterior walls of the nares have an uneven margin dotted with numerous tiny pustulations. Clear prenarial papillae (as described by Wassersug 1976) are present on the lateral anterior walls forming a posteriorly extended flap in the lateral corner. Distinct smooth, rounded narial valve projections are present. A conspicuous postnarial ridge is present directly behind the nares and gives way medially to well-developed post-narial papillae, one on each side, directed anteromedially. On the remainder of the ridge, between the postnarial papillae and the point where the ridge meets the lateral wall, is a smaller subpapillae. A fringed, slightly rounded median ridge separates the narial arenas from the BRA and is bordered laterally by a pair of triangular-
shaped lateral ridge papillae, that, like the median ridge, contain numerous secondary papillae on the anterior surface.

The buccal roof arena is delimited laterally by a single row of attenuate, tapered BRA papillae (~4 or 5 per side). Numerous tiny pustulations, approximately 45-60, are found scattered randomly within the region defined by the BRA papillae. Far lateral to the BRA, clusters of numerous lateral roof papillae are found. These papillae are of varying sizes with 5-10 being well defined, conical papillae and the remaining 10-15 as mere pustulations. The BRA is bordered posteriorly by a crescentric ring of secretory pits that form the glandular zone. The dorsal velum has a fringed anterior margin with a number of other various pustulations scattered amongst its folds.

**Ventral:** The most anterior projection of the floor are the infralabial papillae which, in this species, are multiple branching and highly pustulate at the tips. They extend posteromedially from the inner lateral walls of the lower beak. On the anterior-most region of the tongue anlage, is a pair of pointed lingual papillae that curve anteriorly and are symmetrical about the midline. Posterior to the anlage, the BFA is partially circumscribed by a single row of attenuate and bifurcated papillae. The circumscription ends approximately 3/4 of the way back on the buccal floor, approximately in line with filter cavity I. The internal region delimited by these papillae is scattered with numerous small pustulations, the majority of which are concentrated posteromedially.

Anterolaterally, the rows of BFA papillae merge with clusters of prepocket papillae which number around 30-40. In many cases it is difficult to determine where the prepocket papillae end and the BFA papillae begin, however, the pre-pocket papillae tend to be more square and stubby that the longer attenuate BFA papillae. The buccal floor is bordered posteriorly by the ventral velum which is transected medially by the median notch. Directly on either side of the notch is a broad pustulate velar projection. Moving laterally in evenly spaced intervals are 3 more velar projections per side, each associated with a respective filter cavity I to III.

**P. tetraploidea**

**Ventral:**

A pair of attenuate infralabial papillae is located within the buccal cavity. They are terminally pustulated curve slightly anteromedially. A pair of subpapillae sits behind them and is compressed and reduced in size with a blunt tip. Still further posterior is a cluster of pustulations and subpapillae. The anterior-most region of the buccal floor contains a pair of attenuate lingual papillae whose tips veer slightly laterally. The BFA is delimited by a single row of BFA papillae. The anterior-most of which is bifurcated and
appears as 2 papillae sharing a common base. There are 4 papillae total on each side, where the medial one (anterior to the bifurcated pair) is largest and most distinct with a base easily four times the tip. The remaining posterior papillae are reduced and conical in shape. Approximately 40 tiny pustulations populate the buccal floor, the majority of which are clustered anteromedially. They become more sparse anteriorly and are absent altogether around the level of the buccal pockets. Clusters of prepocket papillae are present but not easily differentiated from the BFA papillae.

Due to preparation error the ventral velum in the 2 species prepared is incomplete, but it appears that a pair of large nobular papillae flank the median notch and 3 velar projections are evenly spaced along the velar margin, each in association with a respective branchial basket I to III.

**Dorsal:** The prenarial arena is characterized by a semicircular ridge (concave posteriorly) that is transected medially by a gap. The top of the ridge is dotted randomly with small, irregularly shaped projections (~ 6-7 per side). Medially, within the semicircle, are 2 more projections. The internal nares have a fringed anterior margin, with a flap-like region of prenarial papillae at the lateral corner. Distinct smooth, rounded narial valve projections are present at the medial corners of the posterior narial wall. Between the nares and the median ridge on a diagonal ridge are 2 papillae; the anterior-most, the postnarial papillae, are long, attenuate and papillated at the margins. They extend anteromedially past the medial corners of the nares. Behind the postnarial papillae is a single, majorly reduced subpapillae. Posterior to this ridge are the medially extended lateral ridge papillae which are roughly triangular in shape with an uneven anterior margin and tip. The median ridge separating the narial arenas and the buccal roof is flattened laterally and develops into a generally smooth, rounded mound medially. A cluster of postnarial pustulations (~10) exist on the floor between the ridge and the nares.

The BRA is bounded laterally by a single row or conical BRA papillae (~2-5 per side). The majority of the roof is covered by a dense mass of tiny pustulations that extend as far anteriorly as the median ridge and posteriorly to about 0.5 mm from the glandular zone leading into the dorsal velum. The buccal roof is also characterized by lateral roof papillae, most of which are tiny pustulations with a few scattered pointed subpapillae among them. The dorsal velum is distinct in this species, like most of its genera, in that the mid-dorsal velum is characterized by several rows of numerous, well-developed projections extending posteriorly toward the glottis. The projections are variable in size and shape with the larger, broader ones lateral and gradually reducing medially.
**Phasmahyla guttata**

**Ventral:** Like *P. cochranae*, the distance between the lower beak and the tongue anlage is minimal. The infralabial papillae are level with the base of the lingual papillae, and, in many cases, the two are close enough to make contact. There are two pairs of infralabial papillae; the anterior-most project medially and then immediately curve posteriorly. The posterior-most pair are considerably reduced, yet still distinct, broad, rounded papillae. Rather than papillae, per se, a better description would be projections, as they appear as continuations or extensions of the lateral wall of the beak. The lingual papillae, at the anterior-most tip of the anlage, are conical and bulbous at the tips, occupying the majority of the beak cavity. The BFA is outlined laterally by a single row of tapering, finger-like BFA papillae that curve slightly medially at the tips. In general, the papillae tend to be largest medially, and number at about 5-6 per side. The smaller anterior ones fade into a region of subpapillae and pustulations that appears to be homologous to pre-pocket papillae; however, a clear boundary between the two areas is not evident. The region of the buccal floor demarcated by the BFA papillae is densely packed with pustulations starting at about the same level of the buccal pockets, anteriorly, and extending as far, posteriorly, as the two medial velar projections. Unfortunately, due to poor specimens, the next pair of velar projections moving laterally, and thought to be associated with filter cavity I, were not present, however, their existence is assumed due to genera comparison and tissue scarring. The velar projection associated with filter cavity II is present.

**Dorsal:** The prenarial arena in this genera is highly distinct. It contains three well-developed pre-narial arena papillae that are arranged in a manner whereby the two lateral papillae diverge from a medial point in the arena that gives way anteromedially to a third smooth, rounded projection. Little, or no, secondary papillation exists in this region. The nares are characterized by distinct prenarial papillae on the medial corners of the anterior walls. These smooth, rounded papillae extend anteromedially. There is also a papillated flap-like region at the lateral corners of the anterior wall that more closely resemble Wassersug’s description of “pre-narial papillae” (1976). No clear narial valve projections are present. Postnarial papillae are highly conspicuous; they are located on a ridge behind the nares and extend anteromedially. Unlike their tapered counterparts in other related genera, they are thick and finger-like with a rounded or lightly papillated tip. The median ridge is smooth and rounded and gives way laterally to projections of the lateral wall, or possibly of the buccal floor, that may be homologous to lateral ridge papillae. These projections may have an uneven anterior margin, and are directed anteriorly and slightly medially. The BRA is bound laterally on each side by a raised,
pustulated ridge that is parallel to the lateral walls of the roof. The portion of the buccal roof enclosed by these ridges is also randomly dotted with numerous pustulations (~25-30). Approximately 4-5 finger-like lateral roof papillae are present in clusters on the lateral walls of the BR, one on each side, and are surrounded by numerous smaller pustulations. The buccal roof folds posteriorly, immediately before giving way to the dorsal velum. The flap that results from this folding is highly pustulate. The pressure cushions lateral to the velum have an undulating anterior margin.

*Phasmahyla cochranae*

**Ventral:**

The boundary between the lower beak and the tongue anlage is minimal in comparison to other Phyllomedusines, to the point where the lingual papillae abut the interior walls of the beak in many cases. The most anterior projections of the buccal cavity are the infralabial papillae; the most anterior pair extend posteromedially and slightly dorsally in an elevated, skewed L formation. The second pair is considerably reduced, with the base twice the width of the tip, projecting medially. The lingual papillae are positioned slightly asymmetrical about the midline with one slightly behind the other. The tips may be bifurcate or bulbous. The BFA is bounded on both sides by a single line of finger-like papillae directed medially. The papillae have a slight bend at the tip giving them a hook-like appearance (~7 on each side). Within the BFA are numerous (70-80) pustulations, the majority of which lie in the lower half of the BFA, posteromedially, towards the velum. The only pustulations present in the anterior half outline the upper 4 or 5 BFA papillae. Scattered in and amongst the BFA pustulations are tiny dark specks of pigment. Again, these are absent, or few in number, where the pustulations are scarce. The velum is characterized by two marginal projections on each side of the medial notch that are associated with respective branchial baskets I and II. The broad projections that form the notch are approximately twice the diameter of the lateral marginal projections.

**Dorsal:**

The prenarial arena in this genera is highly distinct. It contains three well-developed pre-narial arena papillae that are arranged in a manner whereby the two lateral papillae diverge from a medial point in the arena that gives way anteromedially to a third smooth, rounded projection. The nares are approximately 0.75 mm in diameter and have distinct features as well; the anterior walls are characterized by a smooth, rounded projections on the medial corners, here called prenarial papillae. However, these are not homologous to the prenarial papillae described by Wassersug(1976). There is a
papillated flap-like region at the lateral corners of the anterior wall that more closely resemble Wassersug’s description of “pre-narial papillae”. No clear narial valve projections or post-narial arena papillae are present. Postnarial papillae are highly conspicuous; they are located on a ridge behind the nares and extend directly anteriorly. Unlike tapered counterparts in other related genera, they are thick and finger-like with a rounded tip.

The narial arenas are separated from the remainder of the buccal roof by a smooth, rounded median ridge, devoid of secondary fringe. Lateral ridge papillae are not present, per se, but outgrowths of the lateral walls opposite the median ridge may be homologous. The BRA is bound laterally on each side by a raised, pustulated ridge that is parallel to the lateral walls of the roof. The portion of the buccal roof enclosed by these ridges is also randomly dotted with numerous pustulations (~ 25-30). Approximately 4-5 finger-like lateral roof papillae are present in clusters on the lateral walls of the BR, one on each side, and are surrounded by numerous smaller pustulations.

The lip-like dorsal velum is highly pustulated with one longer, hook-like papillae present at the approximate level of the ridges on the buccal roof.

DISCUSSION

Prenarial arena: The prenarial arena in Agalychnis, Pachymedusa, and all Phyllomedusas studied was characterized by varying degrees of an M-shaped ridge that differed only in the number of papillae and small variations in length and shape. For example, P. tomopterna has a relatively short ridge with 3-4 papillae per side, whereas A. callydrias and P. boliviana are longer with 5-6 papillae per side. Specimens from Phasmahyla, on the other hand, have a unique prenarial arena, where the ridge is more V-shaped with a medial knob and no secondary pustulations, save for possible terminal ones at the tips of the “V”.

Nares: The overall shape of the nares is similar among all specimens, and size generally varied with stage. However, features of the nares, like the presence/absence of narial valve projections and prenarial papillae did vary significantly among the species.

• Prenarial papillae- Prenarial papillae as Wassersug describes them (1976) are present to some degree in all the specimens studied. In addition to this lateral papillated flap on the anterior wall, Phasmahylas have a conspicuous, enlarged medial projection on the anterior wall that resembles the narial valve projection, but that cannot be named as
such due to location. It is possible that this is a trait unique to the genera, and, therefore, useful in identification purposes.

- Narial valve projections- *Agalychnis*, *Pachymedusa*, and all *Phyllomedusa* specimens have them in some degree, though they are considerably reduced in *Pachymedusa dacnicolor*, *P. vaillanti*, and *P. tarsius*. They are completely absent in the *Phasmahylas*.

**Postnarial arena:** Postnarial papillae are present in all 4 of the genera, where size and shape is highly variable within and between species. The ridge between the postnarial papillae and the lateral ridge papillae contains one or two subpapillae in all but *Phasmahyla*, in which this ridge is drastically reduced, if not completely absent.

**Lateral ridge papillae:** These papillae are distinct in *Agalychnis*, *Pachymedusa* and all *Phyllomedusas*, and like the postnarial papillae, have a high degree of variability in size and shape, and may or may not be papillated at the margins. These papillae are absent in the *Phasmahylas* though remnants appear as lateral extensions of the median ridge, expanding the medial flap to the lateral wall.

**Median ridge:** It is hard to distinguish species by the median ridge due to great inter- and intraspecific variability in degree of secondary papillation, shape and length of extension, bifurcation, etc. However, unique differences can be discerned among genera. For example, the median ridge of *Phyllomedusas* and *Agalychnis* tend to be rounded to some degree, whereas *Pachymedusa* is flattened with extensive papillation of the anterior margin. *Phasmahyla*, as previously mentioned, is generally smooth and rounded with its flap extended to the lateral wall, meshing the ridge with the lateral ridge papillae.

**BRA papillae:** The BRA papillae among *Agalychnis*, *Pachymedusa*, and *Phyllomedusa* are similar overall with minor variations in size, shape and number. In *Phasmahyla*, on the other hand, there are no clear BRA papillae; rather, the BRA is delimited by a set of parallel ridges running in the anterior-to-posterior direction with numerous pustulations scattered on and between them.

**Dorsal Velum:** The region of the dorsal velum shows a lot of variation between the genera, and, may be a useful character in classification. For example, *Agalychnis* and *Pachymedusa* have continuous velums, where the region between the pressure cushions heading into the esophagus is relatively smooth with a few secondary papillae at the medial corners of the cushions. This same area in the *Phyllomedusa* species contains conspicuous projections and protuberances: *P. boliviana*, *P. tetraploidea*, and *P. tarsius* each have numerous rows of densely packed papillae. In *P. hyperchondrialis*, these rows of papillae converge to a crescent-shaped protuberance that opens posteriorly. This region was torn in *P. tomopterna* and *P. vaillanti* during processing, and, therefore, can’t
be described, though the remnants seem to suggest the presence of such projections. The dorsal velum of the *Phasmahylas* forms a lip at the base of the buccal roof and contains many secondary projections and pustulations.

**Infralabial papillae:** According to Wassersug (1980, 1988), two infralabial papillae are typical for Hylids, and I found this trend to be true in all the specimens analyzed. The only possible exception is *Pachymedusa dacnicolor* who appeared to have greater than two, however, these subsequent papillae may only represent subpapillae due to the reduction in size. Unfortunately, only one species *Pachymedusa* of was analyzed, so I am unable to make a comparison with other individuals in the genera. For all other specimens, size and shape varied greatly within and between species, therefore, these papillae are not ideal characters for identification purposes.

**Lingual papillae:** All genera studied have two. The differences arise in the relative positioning of the lingual papillae and the tongue anlage upon which they sit to the horny beak. In *Agalychnis*, *Pachymedusa*, and *Phyllomedusa* species, the tongue anlage, in general, sits between and below the beak, keeping a clear, marked separation between the two regions. The narrow, acuminate lingual papillae extend dorsally into the beak’s cavity. In *Phasmahyla*, the anlage is pushed anteriorly, and/or the beak posteriorly to the point where there is little physical space between the two regions. The lingual papillae are considerably wider and larger, filling up the anterior-most region of the cavity and causing contact between themselves and the infralabial papillae. The shape of the lingual papillae is also different; they are wide at the base and gradual taper until a secondary expansion dorsally to form a bulbous tip.

**BFA papillae:** Though, in general, all species were similar, a few marked differences can be noted among the genera. In *Pachymedusa* and *Phasmahyla* there are multiple rows (2-3) of long, finger-like BFA papillae (7-10 per side), whereas in *Agalychnis* and *Phyllomedusa*, there is generally only one row with 5-8 per side. The real difference from the former two is that the latter tend to be broader and flatter, and extend medially, creeping closer to the floor’s surface. In all the species, the larger papillae tend to be medial within the line-up and they may bifurcate at the base, giving the impression of two. Overall, the BFA papillae are not good determinations of a species due to high variability within overall similarity.

**Ventral velum:** There is little differentiation among species. In general, the velum is separated into right and left halves by a median notch around which two or more conspicuous posterior projections extend. In addition, there is usually one projection associated with each filter cavity.
CONCLUSION

Our analysis of 10 species from four of the six Phyllomedusinae genera has revealed a high degree of similarity between characters and minor and major variations among species and genera. Wassersug and Heyer (1988) found variations in the following characters: infralabial papillae, lingual papillae, BFA papillae and the median ridge, to be important in delimiting taxa. While these characters would aid in separating the Phyllomedusinae from other subfamilies, they are not sufficient in differentiating genera within this family, as the overall degree of similarity in these traits is too high. Through careful analysis and comparison, we suggest that the following characters may be more useful: post-narial and narial arena structures, the dorsal velum region, and positioning of the lingual and infralabial papillae to the horny beak. Analysis of these variables led us to support Wassersug’s 1980 suggestion that what was then Phyllomedusa cochranae, P. guttata, and P. jandaia, should be placed in a genus separate from Agalychnis callydrias and Phyllomedusa trinitatibus based on complex internal morphology. We, therefore, also endorse Cruz’s resurrection of the Phasmahyla genus in 1990, which includes P. guttata and P. cochranae.

We believe that the extensive analysis of the internal oral morphology of Phyllomedusinae tadpoles using SEM techniques has proven its great potential for identifying organisms at the genera level, though identification at the species level seems impractical. Of course, further research, using a greater number of species from a more complete taxonomic unit is necessary to get sound results. Our study was hindered greatly by the fact that only one species from Agalychnis and one from Pachymedusa was available, and that 2 of the 6 genera were not represented. With more complete studies, however, it is possible that the character identifications could be utilized in future systematic analyses and classifications of the Hylid family.
Agalychis callhydrias
(x30 15kV)

← Dorsal

Ventral ⇒
Pachymedusa dacnicolor
(x30 15kV)
Dorsal
Pachymedusa docmico/cor
(x 30 15 kV)
Ventral
Phasmatula guttata: Dorsal
(x 30 15 kV)
Phasmahyla guttata: Ventral
(x30 - 15kV)
Phasmatyra cochranae: Dorsal
(x 30, 15 kV)
Phasmahyla cochranae: Ventral
(X30 15kV)
Phyl lorem lorum boliviana (x30 15 KV)

⇒ Dorsal

Ventral ⇒
Phyllomedusa hyperchondrialis
(x30 15kV)
Dorsal
Phyllomedusa hyperchondrialis
(x30 15kV)
Ventral
Phyllomedusa tarsius
(x30 15 kV)
Dorsal
Phyllomedusa tarsius
(x30 15kV)
Ventral
Phyllomedusa tetraploidea
(x30 15kV)
Dorsal
Phyllomedusa tetraploidea
(x30 15KV)
Ventral
Phyllomedusa tomothoptera (X30 15 kV)

← Dorsal

Ventral
Phyllomedusa vaillantti
(x30 15kV)

← Dorsal

Ventral ➞
LITERATURE CITED


