THE DISTRIBUTION AND AVERAGE SIZE OF GRANULAR GLAND IN POISONOUS ROCK FROG, *Odorrana hosii*

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ABSTRACT

Frog skin is reported to have potential in medical application especially the granular gland on the skin producing secretion containing peptide. The objective of this paper was to examine the distribution and average size of granular glands in *Odorrana hosii’s* skin. The skin histology was stained with Haematoxylin-Eosin to identify granular gland. Results revealed that the distributions of granular glands were statistically significantly different between six regions of the frog skin (F (5,234) =3.47, p=0.005) with dorsal central region of skin has the highest mean number of granular gland (2.22 ± 1.69). The average size of the granular glands was statistically significantly different between six skin regions (F (5,234)=4.04, p=0.012) with dorsal central region contained the biggest granular gland size (11.95 x 10^3 μm²). This study showed that the granular glands in *O. hosii* were significantly abundant in dorsal head region and the largest size was in the dorsal central region as compared to other regions of the skin. This implicates the dorsal head and central skin region can be used for peptides extraction due to the abundance and size of granular glands.

Key words: Skin histology, dorsal, ventral, granular gland, frog

INTRODUCTION

The skin is the primary defence mechanism of both animal and human. Some amphibians have the ability to secrete venom or toxin as one of the main defence mechanisms against predators (Toledo et al., 2011; Sharma et al., 2012). The amphibians pose a chemical defence mechanism mainly contributed by skin glands type, particularly granular gland, mostly producing chemical agent against microbial or parasite (Fox, 1994; Clarke, 1997; Rollins-Smith et al., 2005; Sharma et al., 2012). The granular glands of amphibian skin secrete a broad active biochemical such as alkaloids, peptides and steroids (Daly et al., 1987). This active biochemical advanced unconventionally in diverse anuran families throughout the world differing on its ecology and natural history (Toledo & Jared, 1995; Mallet & Joron, 1999; Sharma et al., 2012). Jared et al. (2009) reported that the parotid macro glands of the toad produced venom located over the dorsal head of the skin region and will be activated to expelled the venom after being bitten by predator. Whereas Lenzi-Mattos et al. (2005) reported another species of toad released the venom from inguinal macroglands located over the dorsal thigh region.

*Odorrana hosii* is commonly known as poisonous rock frog and can be found throughout Borneo, Peninsular Malaysia and Sumatra from sea level up to 1800 m and is categorised as least concern in the International Union for Conservation of Nature (Van-Dijk et al., 2004; Inger & Stuebing, 1997). The colour of dorsal side of skin is green, where the lateral sides are brown. The texture of the dorsal skin mainly fine pebbled with minimal fold on each side. The dorsal side of the lower limbs is light brown with dark crossbars and the belly is mainly silvery white as shown in Figure 1 (Inger &
This species is known to have poison secretion and it can produce a certain smell when stressed (Conlon et al., 2008). The secretion from the skin of O. hosii can harm or kill other frogs or other small animals (Inger & Stuebing, 1997).

One of the major interests in the application of frog skin-derived agents is the use of these agents for combating microbial infections. The previous finding shows the secretion from O. hosii contained eight peptides with differential antimicrobial activities. This antimicrobial property of the peptides extracted from this species caused further research hoping this discovery to supplement or replace antibiotics in future (Conlon et al., 2008).

Recent study has suggested that the poison from O. hosii is not harmful to the wounded rat but it helped the wound to heal faster compared with non treated wound (Sungif et al., 2015). This evidence warrants a deeper inquiry into the development of potent alternative antimicrobial agent from this frog skin. While there has been fairly good information on granular glands distribution of many frog species, however to the best of our knowledge there was no data published on O. hosii frog skin. In the current work, we present the distribution and average size of granular glands of the dorsal and ventral side of frog skin through microscopic analysis using Haematoxylin and Eosin Staining Protocol.

**MATERIALS AND METHODS**

Frogs were sampled from Matang Wildlife Centre and Batang Ai National Park, Sarawak, Malaysia under Park Permit No. 148/2014 given by Sarawak Forestry Department. The research was approved by the Sarawak Biodiversity Centre (SBC-RA-0094-AHR). The dorsal and ventral frog skins were peeled off and spread finely on filter paper followed by standard process, stain cut into section and stained with Haematoxylin-Eosin to identify granular gland as described by Brown (2002). The slides were examined under Olympus image capture microscope and measured using ImageJ software. The histology study was done to evaluate the number of granular glands, and their average gland size, at six different body regions (Figure 2).

We used skin fragments of 2.5 x 0.5 cm of six regions (Figure 2) of four individuals (two frogs from Batang Ai National Park and another two from Matang Wildlife Centre). For each individual, we sampled 10 skin sections in each of the six regions, and spaced apart from each other to avoid sampling in the same regions. We photographed each section under 40x magnification. The number of granular glands were counted and the size of the glands were measured in each section. The statistical analysis was done using the One-way ANOVA and followed by Tukey’s post-hoc test.
RESULTS AND DISCUSSION

Morphology of granular gland
Granular glands of *O. hosii* were found in the stratum spongiosum of the dermis, superficially with an enormous number of chromophores. Synctiums were observed in both Figure 3a and 3b. The granular glands are elliptical and densely filled with juxtaposed spherical granules (Figure 3a and 3b). The alveolus has a duct that connects to the skin external and bounded by a stratum of myoepithelial cells (Figure 3b).

Distribution of granular gland
*O. hosii* granular glands were distributed throughout the dorsal and ventral side of the skin (Figure 4 & Table 1). The dorsal central region of skin has the highest mean number of granular gland 

\[
\text{DH} = 2.22 \pm 1.69
\]

whereas ventral thigh region has the least mean of number of granular gland 

\[
\text{VT} = 1.02 \pm 1.23
\]

as shown in Table 1. There was a statistically significant difference of means number of granular gland between all skin regions tested as determined by one-way ANOVA 

\[
\text{F (5, 234)} = 4.043, P = 0.012
\]

as shown in Table 2. By using Post Hoc Tukey test, it shows four pairs of regions with significant difference; DH and VT with \(P = 0.021\), DH and DT with \(P = 0.001\), DH and VC with \(P = 0.002\) and DH and VT with \(P = 0.001\) while the rest were not significant.

Average size of granular gland
Table 3 shows the distribution of size of granular gland in six regions of the skin. The mean and standard deviation for the size of granular gland in the studied regions. DH shows the biggest granular gland size, 119.50 \(\times 10^2\) \(\mu m^2\) and VT shows the smallest gland size, 29.50 \(\times 10^2\) \(\mu m^2\). The average size of granular glands was statistically significantly different between the all skin regions as determined by one-way ANOVA 

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\text{F (5, 234)} = 4.043, P = 0.012
\]

as shown in Table 4. Using Post Hoc Tukey test, it shows four pairs of regions with significant difference; DH and VT with \(P = 0.021\), DH and DT with \(P = 0.001\), DH and VC with \(P = 0.002\) and DH and VT with \(P = 0.001\) while the rest were not significant.

The dorsum of head and trunk densely contained granular gland may be due to regions that normally exposed to the surrounding while the frogs are actively forage or resting, and might be an adaptation as defence strategy for the frogs. Thus more studies are needed to be done in terms of morphology and toxicology of frog skin, as well as behavioural observations on the field that might explain the findings. It will also contribute to further new knowledge on the biology of frog skin and their potentials in clinical application. We observed that the skin of *O. hosii* gives the morphological structure found in anurans as a whole with some similarities with human skin (Farquhar & Palade, 1965; Mills & Prum, 1984; Terreni et al., 2003).

Apart from the densely distribution of the granular gland over the dorsal head and central region of the skin, our finding also demonstrated that these regions had the biggest number of the granular gland compared to the other region, could be due to the size of the stratum spongiosum of the specific region which is bigger to accommodate the glands.
Fig. 4. Glands of *O. hosii*. a) Distribution of granular glands at dorsum. b) Distribution of granular glands at the ventral skin side. E, epidermis; GG, granular glands; MG, mucous glands; SG, seromucous gland; D, dermis. Bars: 200 μm.

Table 1. The statistic distribution of the number of granular glands in six regions of skin of *O. hosii*

<table>
<thead>
<tr>
<th>Region of skin</th>
<th>DH</th>
<th>DC</th>
<th>DT</th>
<th>VH</th>
<th>VC</th>
<th>VT</th>
<th>Pooled Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>240</td>
</tr>
<tr>
<td>MNGG</td>
<td>2.15</td>
<td>2.22</td>
<td>1.70</td>
<td>1.50</td>
<td>1.47</td>
<td>1.02</td>
<td>1.68</td>
</tr>
<tr>
<td>SS</td>
<td>260.00</td>
<td>309.00</td>
<td>202.00</td>
<td>182.00</td>
<td>201.00</td>
<td>101.00</td>
<td>1,255.00</td>
</tr>
<tr>
<td>SSD</td>
<td>1.39</td>
<td>1.69</td>
<td>1.49</td>
<td>1.54</td>
<td>1.71</td>
<td>1.23</td>
<td>1.56</td>
</tr>
<tr>
<td>SDM</td>
<td>0.22</td>
<td>0.27</td>
<td>0.24</td>
<td>0.24</td>
<td>0.27</td>
<td>0.19</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Note: DH: skin at the dorsal head; DC: middle of the back; DT: dorsal thigh; VH: ventral head; VC: the belly; VT: the ventral thigh; N: number of sample; MNGG: Mean of number of granular gland; SV: Sample variance; SSD: Sample std. dev.; SDM: std. dev. of mean.
Table 2. One-way ANOVA test of means number of granular glands between all skin region

<table>
<thead>
<tr>
<th>Region of skin</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>5</td>
<td>39.80</td>
<td>7.96</td>
<td>3.47</td>
<td>0.0048</td>
</tr>
<tr>
<td>Within</td>
<td>234</td>
<td>536.85</td>
<td>2.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>576.65</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: df: Degree of freedom. SS: Sums of square; MS: Mean square; F: F ratio.

Table 3. The statistic distribution of the size of granular gland in six region of the skin

<table>
<thead>
<tr>
<th>Region of skin</th>
<th>DT</th>
<th>DC</th>
<th>DH</th>
<th>VH</th>
<th>VC</th>
<th>VT</th>
<th>Pooled Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>33</td>
<td>33</td>
<td>30</td>
<td>21</td>
<td>22</td>
<td>164</td>
</tr>
<tr>
<td>MSGG (x10^2µm^2)</td>
<td>75.42</td>
<td>87.78</td>
<td>119.48</td>
<td>41.71</td>
<td>46.11</td>
<td>29.48</td>
<td>70.69</td>
</tr>
<tr>
<td>SV (x10^2µm^2)</td>
<td>509,864.87</td>
<td>681,285.05</td>
<td>1,035,943.70</td>
<td>48,514.99</td>
<td>28,142.78</td>
<td>31,658.05</td>
<td>528,949.79</td>
</tr>
<tr>
<td>SSD (x10^2µm^2)</td>
<td>71.40</td>
<td>82.54</td>
<td>101.78</td>
<td>22.03</td>
<td>16.78</td>
<td>17.79</td>
<td>72.73</td>
</tr>
<tr>
<td>SDM (x10^2µm^2)</td>
<td>14.28</td>
<td>14.36</td>
<td>17.72</td>
<td>4.02</td>
<td>3.66</td>
<td>3.79</td>
<td>5.68</td>
</tr>
</tbody>
</table>

Table 4. The one-way ANOVA test of the mean size of granular gland in six region of the skin

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>dF</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between the skin region</td>
<td>1,639,612,972.92</td>
<td>5</td>
<td>327,922,594.58</td>
<td>7.42</td>
<td>2.8125e-06</td>
</tr>
<tr>
<td>Within the skin region</td>
<td>6,982,268,696.48</td>
<td>158</td>
<td>44,191,574.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8,621,881,669.40</td>
<td>163</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: df: Degree of freedom. SS: Sums of square; MS: Mean square; F: F ratio.

Generally, anurans exhibit a homogenous distribution of skin glands alongside dorsal and ventral side (Toledo & Jared, 1995). Present work on O. hosii also reveals the the distribution of granular gland specifically abundant in dorsal head and trunk region which is similar to Rana pipiens (Bovberg, 1963). In term of the pattern of granular maturation, the comparable patterns had been reported for a variety of anurans and we observed the same pattern of the maturation in O. hosii (Delfino et al., 2002; Angel et al., 2003; Terreni et al., 2003).

CONCLUSION

Our research finding suggested that granular gland distribution in O. hosii is mostly abundant over the dorsal head and trunk region of the skin. Further study on the extraction of biochemical content in the granular gland must focus on the skin over the dorsal head and central region because that region contains the most number and the biggest size of granular gland compared to other regions.

ACKNOWLEDGEMENTS

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