KOREAN JOURNAL OF ICHTHYOLOGY, Vol. 21, No. 2, 87-92, June 2009
ISSN: 1225-8598

Received: May 28, 2009
Revised: June 19, 2009
Accepted: June 21, 2009

Seasonal Variation of Skin Structure in a Ricefield-dwelling Mud Loach *Misgurnus mizolepis* (Cobitidae) from Korea

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ABSTRACT **Histological investigation of seasonal changes of the mucus cells of ricefield-dwelling mud loach *Misgurnus mizolepis* was carried out on the skin in three regions of the fish: dorsal, lateral, and occipital. Although there were no significant differences in general morphology, the mucus cells of the epidermis showed a severe change in size and number by skin region. The density of the mucus cells was generally the highest in winter and the lowest in summer in all three skin regions. In particular, during two distinct periods, March to April when water is supplied to the ricefields, and August to September when the ricefields become dry after harvest, showed that the change in density and distribution of the mucus cells in both lateral and occipital regions is greater than during any other period. Moreover, the mucus cells for the dorsal and occipital regions temporally increased in size in June during the period of the highest water temperature of the year. Therefore, we may consider these seasonal changes as part of a protective adaptation to overcome extreme environmental conditions and to increase uptake of oxygen by cutaneous respiration.**

Key words : Mucus cell, ricefield, *Misgurnus mizolepis*, cutaneous respiration

INTRODUCTION

The mud loach *Misgurnus mizolepis* has a wide distribution from almost all regions of Korea to China mainland, and inhabits swamp-like regions such as a stagnant pond, irrigation canal, and ricefield (Kim *et al.*, 2005). In the ricefields, the water would traditionally be kept in the field through the four or five-month cropping period to prevent invasion by weeds and to enhance rice growth. After harvest, the water is drained out of the field to prepare for the following cultivation of rice, late autumn to the next early spring. Through a year, the mud loach inhabiting such a ricefield was subjected to a severe seasonal drought. At this time, they are frequently faced with a difficulty in conducting their respiration by the deficiency of water and dissolved oxygen.

Some fishes have developed additional respiratory apparatus to adapt an oxygen-poor environment as using the following organs: skin (Cobitidae, Gobiidae, Synbranchidae, Channidae), intestine (Cobitidae), branchial chamber (Channidae, Gobiidae), swim bladder (Dipnoi) and others (Jakubowski, 1958; Liem, 1967; Mittal and Munshi, 1971; Graham *et al.*, 1977; Graham, 1997; Park and Kim, 1999, 2001; Park *et al.*, 2000, 2001, 2003, 2005; Park, 2002; Sayer, 2005; Jang, 2007). In particular, the epidermal mucus cells were one of the evidences for supporting cutaneous respiration (Mittal and Munshi, 1971; Shephard, 1994; Park *et al.*, 2001). Secreting a great amount of mucus cell is also correlated with a burrowing and an amphibious habit (Liem, 1967), and has a possibility of physical protection (Fletcher and White, 1973; Shephard, 1994). Meanwhile, Oh and Park (2008) reported that the mucus cell of *M. anguillicaudatus*, the other species of genus *Misgurnus*, have greatly increased in winter and decreased significantly in summer. As the skin morphology could be changed under a dynamic environment (Sadovy *et al.*, 2005), it is necessary to examine the skin structure for an annual cycle. Therefore, the aim in present study is to investigate a seasonal variations on the skin structure of *M. mizolepis* in the ricefield, and to discuss an adaptation of mud loach to the

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extreme environment.

MATERIALS AND METHODS

Three specimens of *Misgurnus mizolepis* (total length: 96.5±9.2 mm, 81.3～111.0 mm) living in the ricefields were monthly collected using an inducing-net in farming season and picking up from the burrows in drought season from March 2008 to February 2009, Jeollabuk-do, Korea. The specimens were fixed in 10% formalin solution and skin tissues were taken from three regions of dorsal, lateral and occipital (Fig. 1). The skin samples were dehydrated by a standard ethanol series, cleared in xylene and embedded in paraffin. The 5 μm thick-sectioned preparations were deparaffinezed and subsequently stained with hematoxylin-eosin dye (Presnell and Schreibman, 1997). General morphology of the skin was analyzed using an Axio imager A1 microscope (Carl Zeiss, Germany) and the Axio Vision (Ver. 4.5, Germany). Analysis key points were as follows: the ratio of surface area of mucus cells layer and mucus cells in surface area of the epidermis layer per 1 mm length; the number of mucus cells in epidermis layer per 1 mm length. Analysis of variance (ANOVA) was statistically conducted on the seasonal change of mucus cell by Duncan's multiple range test

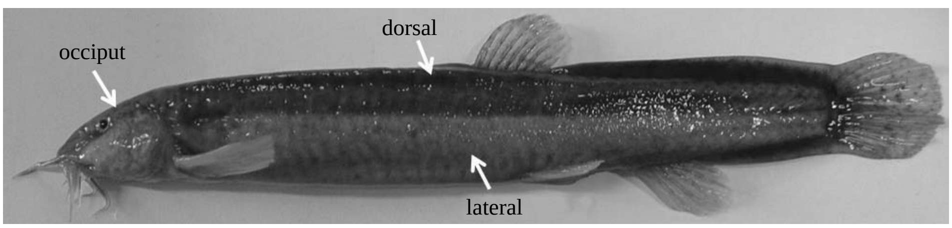

Fig. 1. Tissue sampling regions of *Misgurnus mizolepis*.

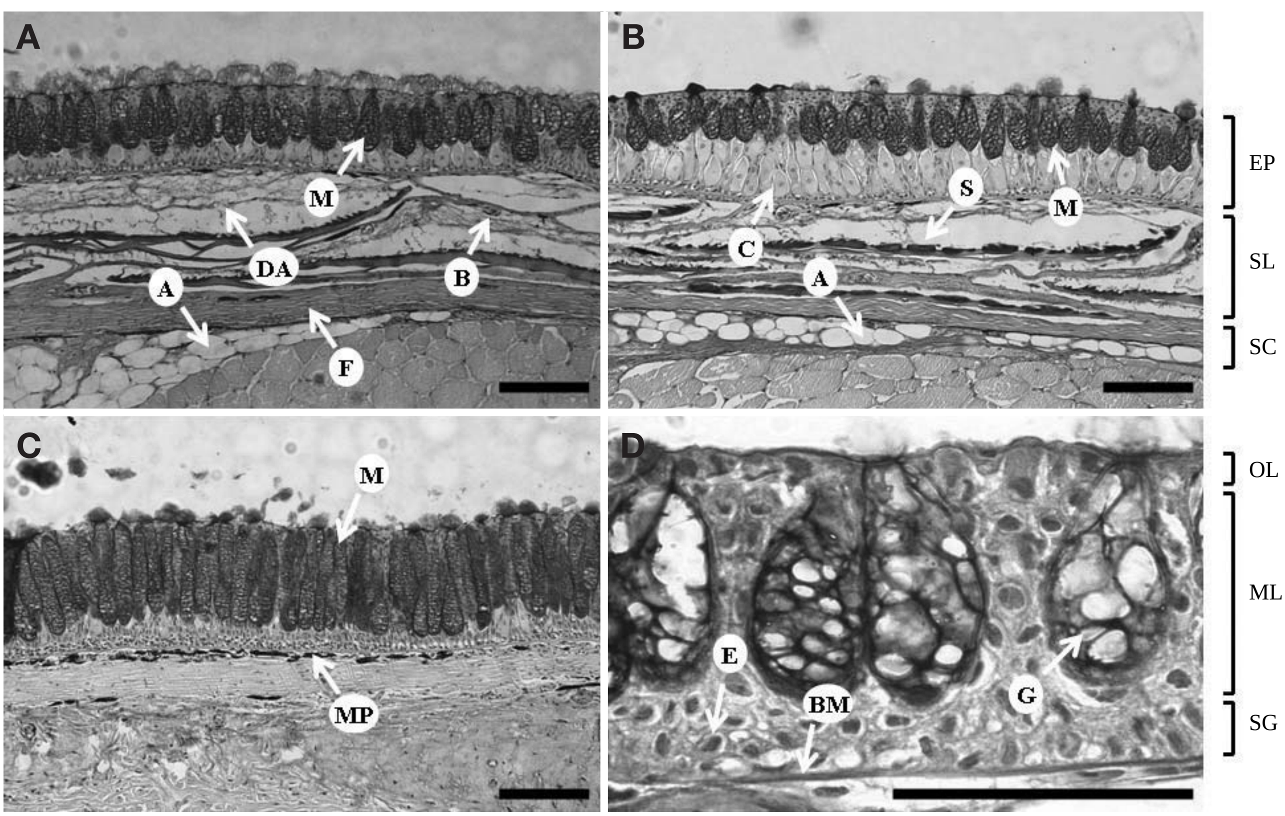

Fig. 2. General morphology of the skin of *M. mizolepis* in three regions of dorsal, lateral and occipital. H-E staining. (A) dorsal region, July 2008; (B) lateral region, September 2008; (C) occiput, September 2008; (D) enlargement of mucus cell from dorsal region, October 2008. A, adipose cell; B, blood capillary; BM, basement membrane; C, club cell; DA, definite area; E, epithelial cell; EP, epidermis; F, fibroblast cell; G, granule of mucus; M, mucus cell; ML, middle layer; MP, melanophore; OL, outermost layer; S, scale; SC, stratum compactum; SG, stratum germinativum; SL, stratum laxum. Bars indicate 100 μm, but (D) is 50 μm.

(DMRT) which the significance level was 95% confidence limit.

RESULTS

1. General morphology of skin

A skin of *Misgurnus mizolepis* is composed of epidermis and dermis in all three regions of dorsal, lateral and occipital (Fig. 2). The epidermis could be divided into three layers: outermost layer of superficial region occupied by flattened epithelial cells, the middle layer having mostly gland cells (mucus cell and club cell), and stratum germinativum of very thin layer of cuboidal cells lined with basement membrane (Fig. 2D). The dermis consisted mainly of connective tissue. In the dorsal and lateral regions, this layer is separated into two layer due to the inserted scales, so-called stratum laxum showing loose connective tissue and stratum compactum showing dense and regularly arranged connective tissue (Fig. 2B). There are a definite area between the basement membrane and the scales (Fig. 2A). The area was basophilic and presented in some free space in which scales are lodged. The scales consisted of two distinct layers, the upper bony layer and the inner fibrillary plate. The occiput has an only stratum compactum due to the lack of scales (Fig. 2C). Many blood capillaries and melanophores were to be seen in this layer.

2. Seasonal change of mucus cell

In the surface area of the epidermis layer per 1 mm length, the relative surface area of mucus cell layer and mucus cell, and the number of mucus cells generally increased in winter and decreased in summer (Fig. 3).

In the dorsal region, the surface area of mucus cell layer was highest in October (77.5±3.4 μm) and lowest in May (44.6±9.8 μm). The mean value somewhat increased in June and maintained to August, and finally showed distinctive increase between September and Octover ($P<0.05$). The surface area of mucus cell showed similar changing pattern to that of the surface area of mucus cell layer, but it did not increase around June (Fig. 3A).

In the lateral region, the surface area of mucus cell layer was greatest in January (68.9±1.0 μm) and lowest in June (30.6±5.6 μm). The surface area of mucus cell was maximum in December (49.3±7.9 μm) and minimum in July (7.7±1.3 μm). All the three mean values were significantly different ($P<0.05$) between August and September where the sudden increases occurred (Fig. 3B).

In contrast to the above two regions, the occipital region showed dynamic and fluctuant patterns in the surface area of mucus cell layer and mucus cell, which showed two distinct curves from early spring period (March to April) and early autumn period (August to September). The steep gradient meaned great change rates in the surface area of mucus cell layer, which it was 32.0% in the early spring period and 14.1% in the early autumn (Fig. 3C). In the surface area of mucus cell, it showed a abrupt change to 30.3% in the early spring and 30.2% in the early autumn. The number of the mucus cell was also in

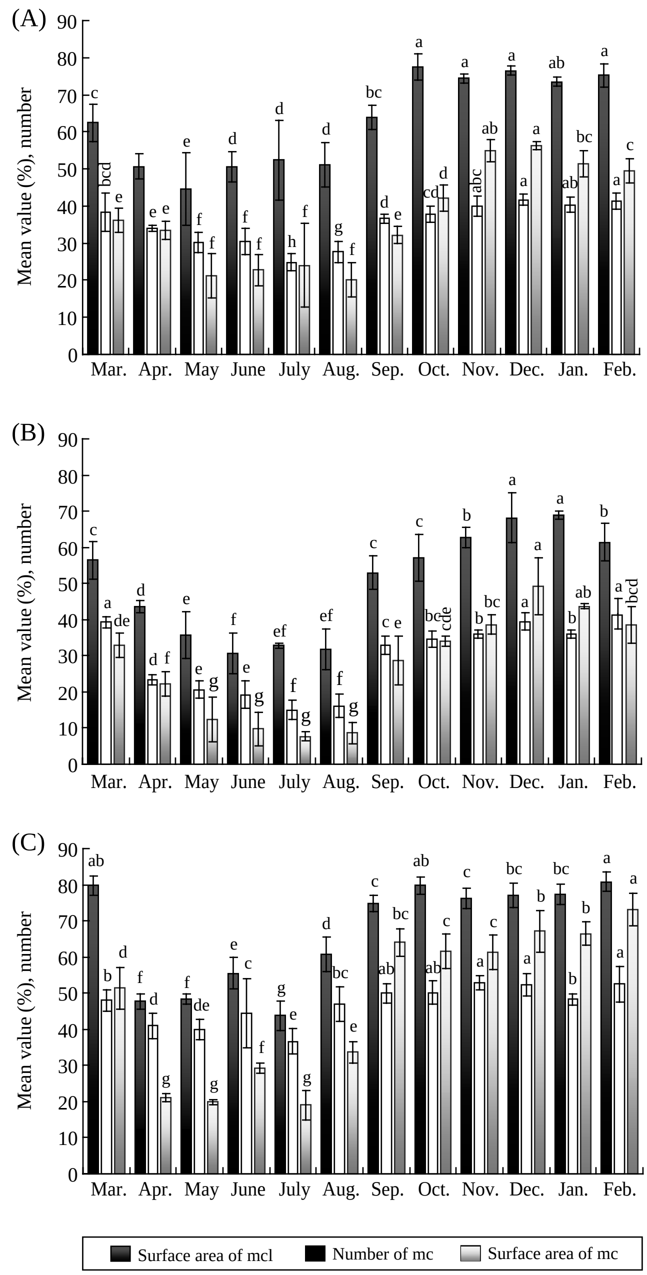

Fig. 3. Monthly change of relative surface area of the mucus cell layer and the mucus cell, and the number of mucus cells in the surface area of the epidermis layer per 1 mm length in *M. mizolepis* from March 2008 to February 2009. (A) dorsal region, (B) lateral region, (C) occiput region. Abbreviations: mc, mucus cell; mcl, mucus cell layer. Same alphabets above the bars mean not significantly differences ($P>0.05$).

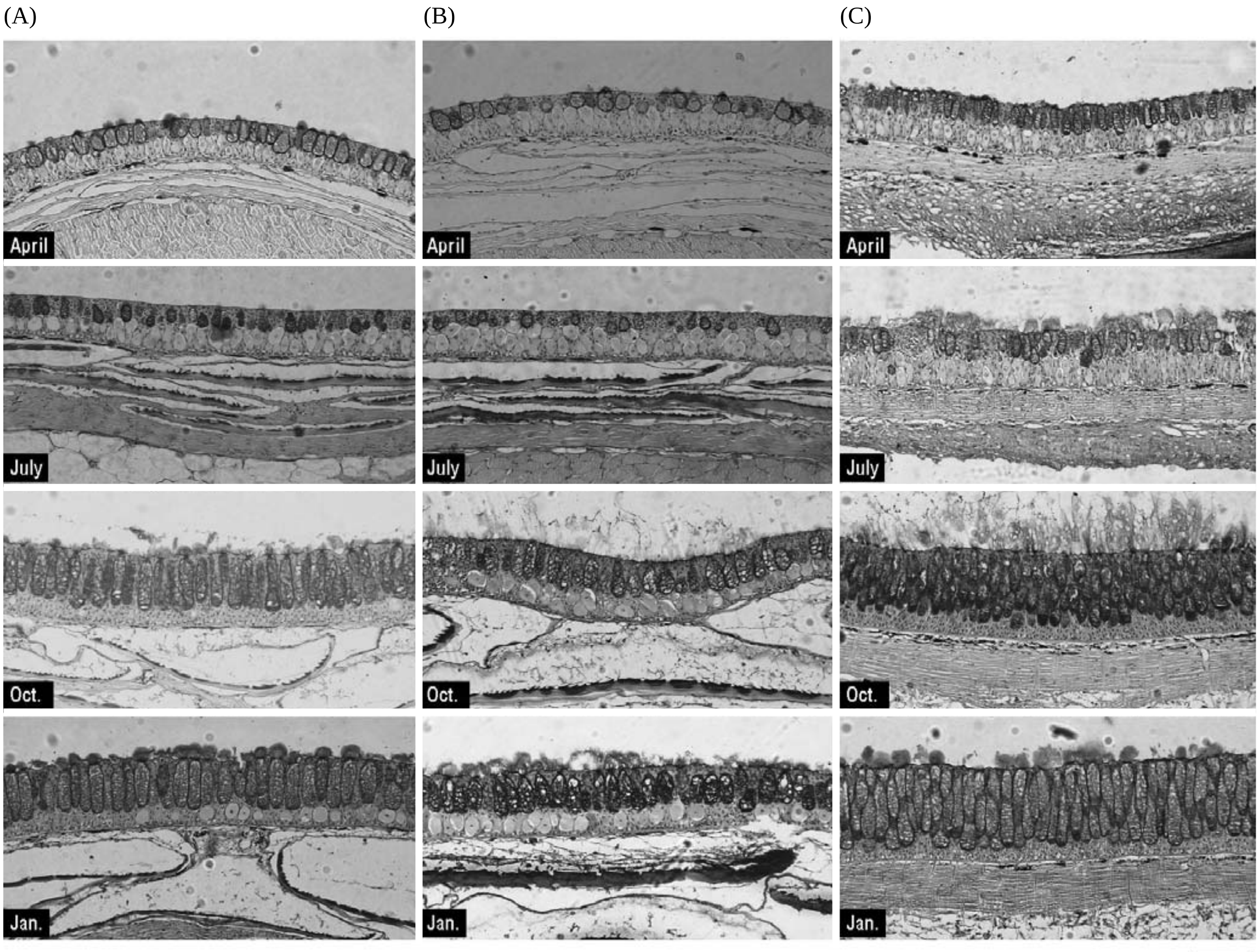

Fig. 4. Seasonal variations of the skin structure of *M. mizolepis* from March 2008 to February 2009. H-E staining. (A) dorsal region, (B) lateral region, (C) occiput region. Bar indicates 100 μm.

a similar tendency. There were sudden increases in all three mean values ($P < 0.05$) in June (Fig. 3C).

3. Change of mucus cells in shape

As shown in Fig. 3, the mucus cells dynamically changed in response to seasonal change. In summer, the mucus cells were mostly small-sized spherical shape where is located at the superficial layer of the epidermis. As it was getting colder, however, the mucus cells became large and elongated oval form occupying over an half area of the epidermis layer. Moreover, the dermis layer was filled with a hematoxylin-positive material in definite area, and then the area was enlarged as it is close to cold climate (Fig. 4).

DISCUSSION

The skin of air breathing fish has generally certain features such as a thicker epidermis due to a large glandular cell (*Misgurnus*, *Monopterus*), intraepithelial capillaries (*Periophthalmus*), and a well developed vascularization (*Heteropneustes*). These structures may be related to the amphibious habit of the fishes (Jakubowski, 1958; Liem, 1967; Johansen, 1970; Mittal and Munshi, 1971; Mittal and Banerjee 1974; Whitear, 1986; Park and Kim, 1999, 2001). Of them, the mucus has been well known as undergoing various functions such as respiratory gas exchange, osmoregulation, chemical and physical protection, disease resistance, and filter feeding (Liem, 1967; Shephard, 1994). The physical protection against friction during burrowing is achieved by lubricating their skin with mucus released from an enlarged mucus cell (Liem, 1967; Shephard, 1994; Fishelson, 1996).

The traditional rice farm has been periodically executing water supply for spring (March to April) and drainage for late autumn to the following early spring to the ricefield. In that case of drainage out of water, the field's environment becomes very unstable and hypoxia facing imperfect respiration for *M. mizolepis*. At the period for water supply, the density and size of the mucus cell decreased largely and sharply. Meanwhile at the drainage period extending late autumn to the follow-up early spring, they again increased dynamically and drastically. Such drastic changes for size of the mucus cell were not occurring in *M. anguillicaudatus* living in muddy bottom of the stream (Oh and Park, 2008). According to our study, the epidermal mucus cell for *M. mizolepis* was not so much during the period of having plenty of water. However during drainage of water, they frequently may face any difficulty of conducting their respiration by the deficiency of water. In such case, they dig to make the holes. In our field observation, when they are in the burrow, the water was already dried up and the skin of *M. mizolepis* is directly exposed to air. Finally, the gas exchange through the gill was unavailable any more. At this period, the density and size of the mucus cell on the skin became higher and greater than other period. In summer (especially in June), there were temporal increase in the size of mucus cell in the dorsal and occipital regions (Fig. 3A, 3C), unlike the result of Oh and Park (2008). In fact, the water temperature at the same time suddenly increased up to 28°C. So *et al.* (2008) reported that the respiration rate of fish increases in the high temperature and it may be possible that these sudden increase of mucus cell is for uptake more oxygen.

The dynamic increase in the size and the density of the mucus cell eventually let skin thicken. In particular, the size and abundance of mucus cells plays an important role in supporting and maintaining the normal relationship of the cutaneous respiration (Jakubowski, 1958; Liem, 1967; Johansen, 1970; Mittal and Munshi, 1971; Mittal and Banerjee 1974; Park and Kim, 1999, 2001) and Rogers (1961) reported that 1 g of mucopolysaccharides could bind or release 200 g or 500 g of water. Park *et al.* (2001) suggested that *M. mizolepis* could respire with skin due to its thick epidermis resulted from the enlarged glandular cells of mucus cell and club cell, a definite area in the dermis, and lack of scale. However, Park *et al.* (2001) did not give more information on mucus cell responding to environmental condition surrounding the habitat. An air breather, *Monopterus albus*, could respire with integument more efficiently in air than in water and the mechanism of cutaneous respiration is achieved by a diffusion of oxygen across the mucus coat of the epidermis (Liem, 1967). We found that the mucus cell during drought season (particularly at the holes) was being enlarged and the mucus might be excreted on the skin surface to make a mucus coat. Consequently, the mucus cell of *M. mizolepis* is considered as a cutaneous respiratory unit under a “stress” for oxygen demand, and it can be an adaptation for surviving in ricefield, an extreme environmental condition.

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논에 서식하는 미꾸라지 피부구조의 계절적 변화

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요 약 : 논에 서식하는 미꾸라지의 등, 체측, 후두부 피부조직에 대한 조직학적 연구를 통해 계절변화에 따른 점액세포의 변화여부를 조사하였다. 계절이 변화함에 따라 표피조직의 일반적인 형태에는 변화가 없었으나 상피조직에 존재하는 점액세포의 단위면적당 개수와 크기가 유동적으로 변화하였다. 비록 점액세포의 발달정도는 겨울철에 크게 증가하고 여름철에 감소하는 경향을 보였지만, 농지경작을 위해 관개를 실시한 봄(3~4월)과 추수를 위해 물을 배수시켜 미꾸라지의 피부가 공기에 노출되었던 가을(8~9월)에 점액세포의 월별 변화율이 가장 크게 조사되었다. 또한 농지 수온이 급격하게 높았던 6월에 점액세포가 일시적으로 발달하는 모습이 관찰되었다. 계절에 따른 점액세포의 변화는 유동적인 농지환경에 대한 능동적 행동이자 피부호흡을 하기 위함으로 여겨진다.

찾아보기 낱말 : 논, 미꾸라지, 점액세포, 피부호흡