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Comparative Immunohistochemical Study of the Distribution of CK 8/18 and CK-Cocktail in the Liver Epithelium of Three Vertebrate Species

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***Abstract*—**The purpose of this study, is to perform immunohistochemical comparison of the liver epithelium in three vertebrate species: *Hyla savignyi*, *Hemidactylus turcicus* and *Testudo graeca*, by using the antibodies CK8/18 and CK-cocktail. This assay determines the evolution of animal tissues by investigating the presence of cytokeratin and its density in these tissues. The tissues have shown different expression levels using CK-cocktail antibodies, where the bile ducts gave a strong signal at *Hyla savignyi*, and the cytoplasm of hepatocytes gave a medium signal. As for *Hemidactylus turcicus*, the bile ducts gave a medium signal and the cytoplasm of hepatocytes gave a slight signal. However, the bile ducts and the cytoplasm of hepatocytes signals were strong at *Testudo graeca*, which confirms the existence of cytokeratin in the liver epithelium of the studied species. On the other hand, the tissues have shown CK8/18 -negative signal in *Hyla savignyi* and *Hemidactylus turcicus*, in contrast to the tissues of *Testudo graeca*.

Keywords— Immunohistochemical study, liver, cytokeratin-cocktail, cytokeratin 8/18, Hyla savigni, Hemidactylus turcicus, Testudo graeca.

I. INTRODUCTION

Cytokeratin (CK) belongs to the family of intermediate filaments proteins within the cytoplasm of epithelial cells which is found in almost all epitheliums [1]. The diameters of the intermediate filaments range between 10-14 nm. Their diameters are in the middle between those two other major components of the cytoskeleton: fine actin filaments (7 nm) and thick myosin filaments (25 nm). Unlike actin and myosin filaments that present stability in chemical composition. The intermediate filaments present great variation in shape and composition, depending on species and living tissues [2].

Cytokeratin filaments extend within the epithelial cells from one side of the cell to the other side. The cytokeratin filaments existing in two adjacent cells are indirectly connected by intercellular communication devices called desmosome [3]. This distribution of filaments gives great resistance to tensile forces which epithelial tissues undergo. As a result, cytokeratin filaments are not directly involved in the cell movements as in myosin and actin filaments. In contrast, they play an essential structural role by providing mechanical strength to cells and tissues [4]. Cytokeratin consists of polypeptide chains, and has two types: the first is acidic with low molecular weight (LMW) (from CK 9 to CK 20), and the other is basic with high molecular weight (HMW) (from CK 1 to CK 8). The principle of numbering is based on the decrease in volume from high to low molecular weight. Cytokeratin is usually found in heterogeneous pairs of acidic and basic subunits with a similar volume. The mutations that weaken this structural framework increase the risk of cell rupture and cause a variety of disorders [5],[6],[7].

The distribution of cytokeratin is very limited, and it depends on location, type of the epithelium and extent of differentiation [8].

Cytokeratin filaments exist in most animal cells types [8], where the presence of cytokeratin has been confirmed in the central nervous system of several vertebrates groups [9],[10],[11], [12]. The second type (basic) of cytokeratin has appeared to be more common in nerve tissues compared with the first type (acidic) [13],[14]. The study of [15], has shown the development of the expression of the second type of cytokeratin in the spinal cord of different adult vertebrates by using immunohistochemical anti-CK. The second type of cytokeratin was stronger in lower vertebrates especially anura in comparison with higher vertebrates, and no expression of cytokeratin has been found in reptiles or birds, but it showed a weak expression in mammals. Also [16] tackled cytokeratin in vertebrate liver in a comparative study. The structure and composition of intermediate filaments isolated from the liver of different vertebrate classes have been studied using

electron microscopy and immunohistochemical methods. This study showed the presence of one protein only from type II (basic) of cytokeratin in all classes of vertebrates, whereas the lower vertebrates have two or even three types of proteins from type I (acidic) of cytokeratin which contribute to the composition of intermediate filaments of the liver.

This study is the first local and regional immunohistochemical comparison of the liver epithelium in some vertebrates species (amphibians and reptiles). We investigated the presence of cytokeratin and its density in the liver epithelium to determine the evolution of the animal tissues. For this purpose, we performed immunohistochemical assay using CK8/18 and CK-cocktail antibodies.

II. MATERIALS AND METHODS

1. Materials

Eight individuals of each species were collected from Latakia city in the period between (2017-2018):

- *Hyla savignyi* (Audouin,1829) from family: Hylidae.
- *Hemidactylus turcicus* (Linnaeus,1758) from family: Gekkonidae.
- *Testudo graeca* (Linnaeus,1758) from family: Testudinidae.

2. Methods

Samples were obtained alive, and then were transported to the laboratory of the faculty of sciences where they were anesthetized using chloroform. After dissecting these animals, liver was isolated and stored in a solution of Formalin 10% for at least 24 hours before the preparation of histological analysis. Tissue sections were prepared at the department of histopathology - Tishreen University Hospital. Dabbs method has been used to perform the immunological staining using CK-cocktail and CK8/18 antibodies [17]. CK-cocktail or AE1/AE3 is a combination of two different antibodies: AE1 detects cytokeratin (acidic) with a high molecular weight CK10,14,15,16 and low molecular weight CK19, whilst AE3 detects cytokeratin (basic) with a high molecular weight CK1,2,3,4,5,6 and low molecular weight CK7,8. CK8/18 or CAM 5.2 is a combination of antibodies that detect cytokeratin 8 and 18.

III. RESULTS

1. *Hyla savignyi*

1.1. Morphological characterization of the liver:

The liver of *Hyla savignyi* is located in the abdominal cavity near to the heart and lungs. It has a dark brown color and consists of two lobes: left and right. The left lobe is slightly larger than the right one and characterized by an incision in the lower limb. The gallbladder is large and located below the right lobe (Fig. 1).

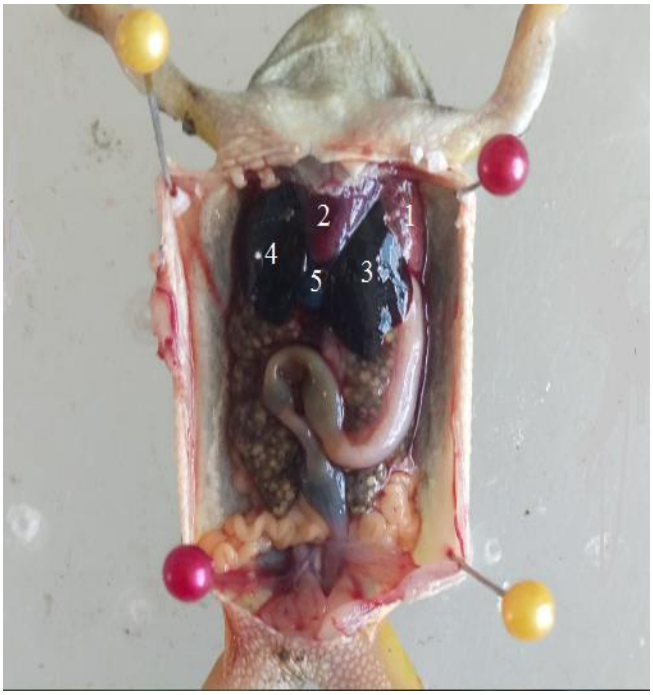

Fig 1. shows the viscera of *Hyla savignyi*: 1- Lung. 2- Heart. 3- Left lobe. 4- Right lob. 5- Gall bladder.

1.2. Immunohistochemical analysis of the liver:

The immunohisto analysis of liver of *Hyla savignyi* has shown strong signal (+3) (Tab.1) of the bile duct and medium signal (+2) (Tab.1) of cytoplasm of hepatocytes by using CK-cocktail antibodies (Figs. 2A,2B). The staining using CK 8/18, shows a negative signal in the liver parenchyma (Figs. 2C,2D).

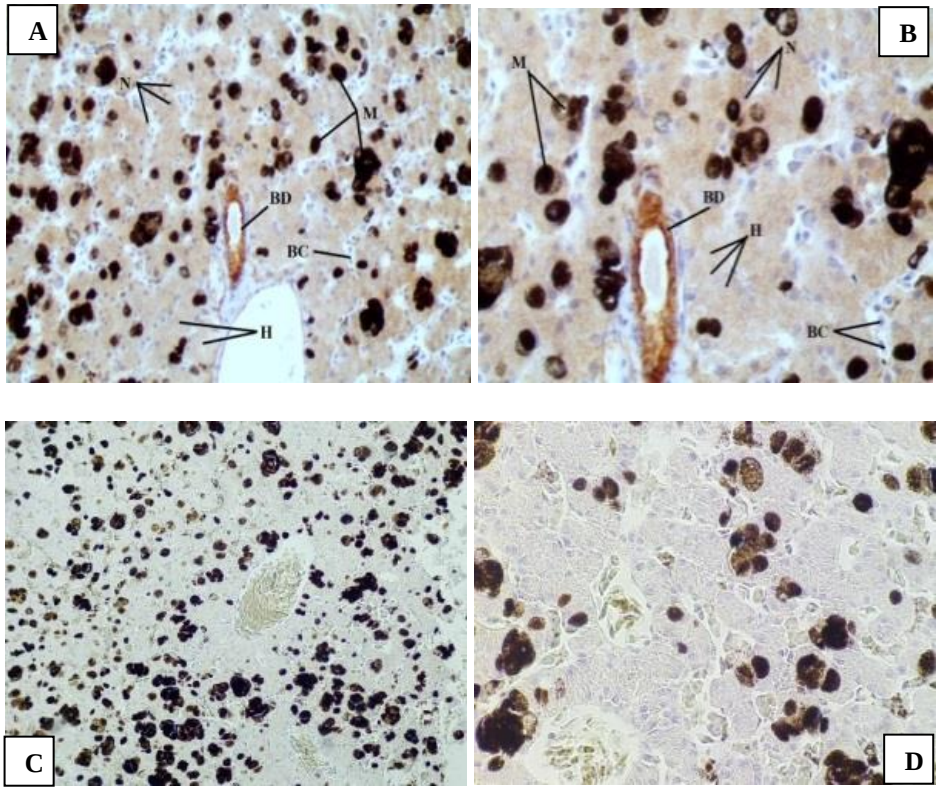

Fig 2: A cross section in the liver of *Hyla savignyi* : **A.** shows a signal of Hepatocytes (H) and Bile duct (BD) with CK-cocktail, Nuclei of hepatocytes (N), Blood cells (BC), Melanomacrophages (M). (CK-cocktail ×100). **B.** shows a signal of Hepatocytes (H) and Bile duct (BD) with CK-cocktail, Nuclei of hepatocytes (N), Blood cells (BC), Melanomacrophages (M). (CK-cocktail ×200). **C.** shows the non- signal of liver Parenchyma (CK8/18 ×100). **D.** shows the non- signal of liver Parenchyma (CK8/18 ×400).

2. *Hemidactylus turcicus*

2.1. Morphological characterization of the liver:

The liver of *Hemidactylus turcicus* is located in the abdominal cavity and covers the stomach completely. It consists of four lobes: right, left, middle and papillary, it has a protruding brown color with a large gallbladder attached to it (Figs 3,4).

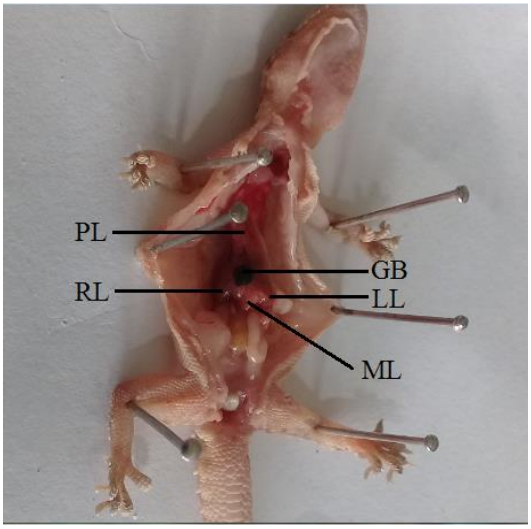

Fig 3: shows the viscera of *Hemidactylus turcicus*: Left lobe (LL). Right lobe (RL). Middle lobe (ML). Papillary lobe (PL). Gall bladder (GB).

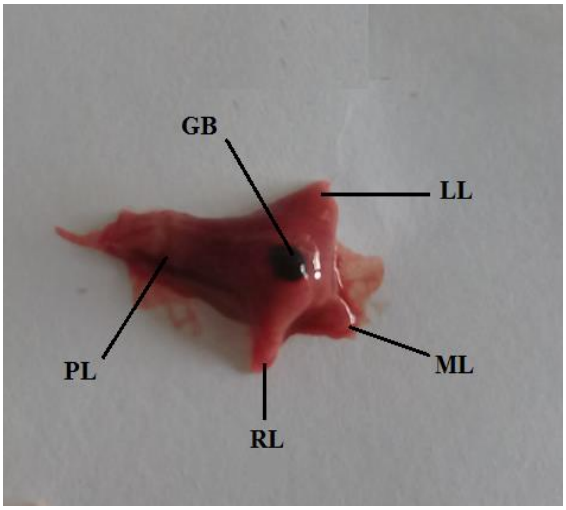

Fig 4: shows the liver of *Hemidactylus turcicus* : Left lobe (LL). Right lobe (RL). Middle lobe (ML). Papillary lobe (PL). Gall bladder (GB).

2.2. Immunohistochemical analysis of the liver:

The immunohisto examination of the liver of *Hemidactylus turcicus* by using CK-cocktail antibodies has shown a slight signal (+1) (Tab.1) of the cytoplasm of hepatocytes and medium signal (+2) (Tab.1) of the bile ducts, which are numerous and distributed within the hepatic tissue (Figs. 5E,5F). Staining by using CK 8/18 shows completely a negative signal in the liver parenchyma (Figs. 5G,5H).

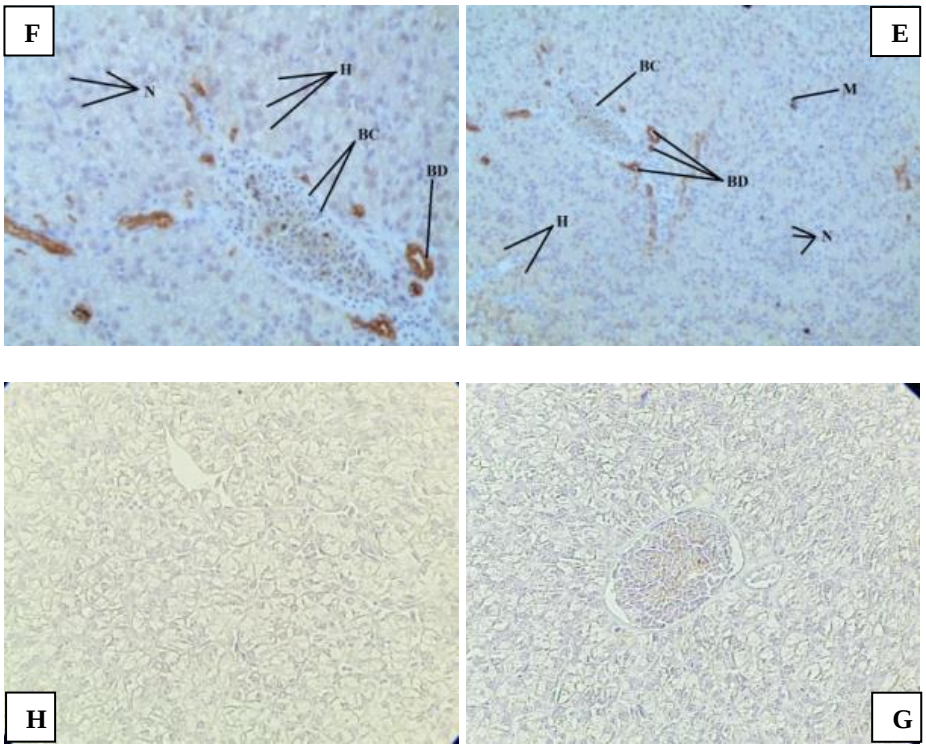

Fig 5: A cross section in the liver of *Hemidactylus turcicus* : **E.** shows both a slight signal to the cytoplasm of Hepatocytes (H) and a medium signal to the bile duct (BD) with CK-cocktail, Nuclei of hepatocytes (N), Blood cells (BC), Melanomacrophages (M). (CK-cocktail ×100). **F.** shows Hepatocytes (H), Bile duct (BD), Nuclei of hepatocytes (N), Blood cells (BC). (CK-cocktail ×200). **G.** shows a non-signal of liver Parenchyma (CK 8/18 ×100). **H.** shows a non-signal of liver Parenchyma (CK 8/18 ×400).

3. *Testudo graeca*

3.1. Morphological characterization of the liver:

The liver of *Testudo graeca* is situated in the abdominal cavity transverse to the long axis of the body. It has a dark brown color and consists of two lobes: right and left. The left lobe is slightly larger than the right one. The gallbladder is located below the right lobe (Figs 6,7).

Fig 6: shows the viscera of *Testudo graeca*: 1- Left lobe of liver. 2- Right lob of liver. 3- Heart. 4- Large intestine. 5- Small intestine.

3.2. Immunohistochemical analysis of the liver:

The immunohisto analysis of the liver of *Testudo graeca* by using CK-cocktail antibodies has shown extremely strong signal (+4) (Tab.1) of the bile ducts and strong signal (+3) (Tab.1) of the cytoplasm of hepatocyte. Moreover, the connective tissue and vascular epithelium have not been colored (Figs. 8I,8J). Staining by using CK 8/18 shows a strong positive signal for both the cytoplasm of hepatic cells (+3) (Tab.1) and bile ducts (+4) (Tab.1) (Figs. 8K,8L).

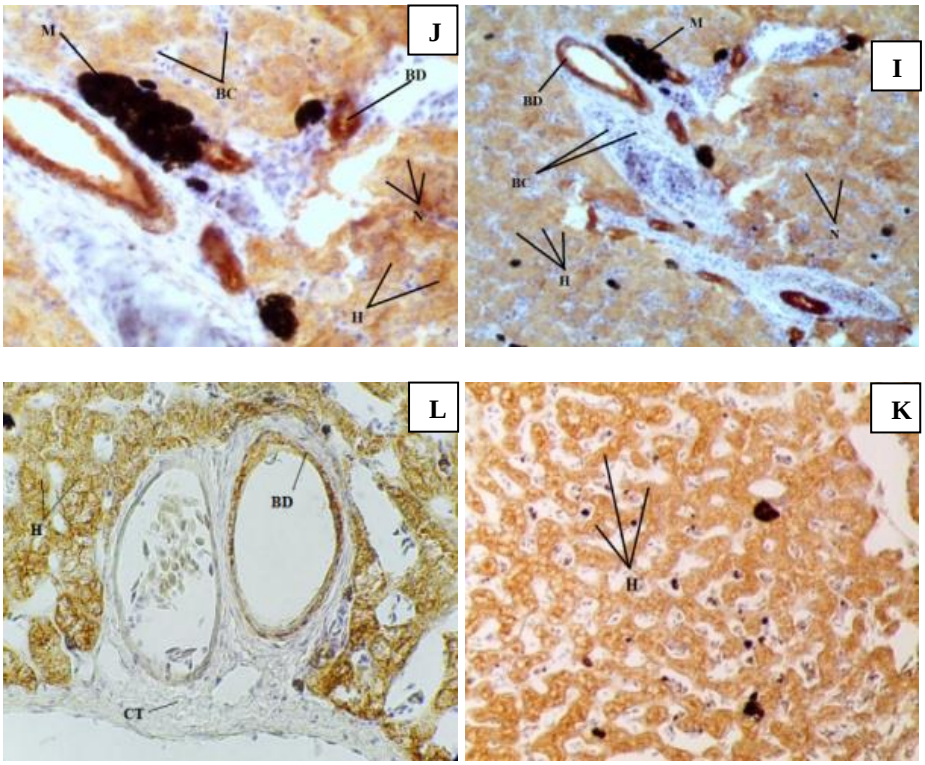

Fig 8: A cross section in the liver of *Testudo graeca* : **I.** shows a signal of Hepatocytes (H) and Bile duct (BD) with CK-cocktail, Nuclei of hepatocytes (N), Blood cells (BC), Melanomacrophages (M). (CK-cocktail ×100). **J.** shows Hepatocytes (H), Bile duct (BD), Nuclei of hepatocytes (N), Blood cells (BC). Melanomacrophages (M). (CK-cocktail ×200). **K.** shows a positive signal of Hepatocytes (H) (CK 8/18 ×100). **L.** shows a signal of Hepatocytes (H) and Bile duct (BD) with cytokeratin 8/18 , connective tissue (CT) (CK 8/18 ×400).

Tab.1. shows the distribution of CK-cocktail and CK8 / 18 in the cytoplasm of hepatocytes and bile ducts of the liver of the studied species.

Studied species	**CK8/18**		**CK-cocktail**	
	Bile duct	***cytoplasm of hepatocytes***	***Bile duct***	***cytoplasm of hepatocytes***
Hyla savigni	-	-	+++	++
Hemidactylus turcicus	-	-	++	+
Testudo graeca	++++	+++	++++	+++

* ++++ = strong signal, +++ = strong signal, ++ = Medium (moderate) signal, + = slight signal, - = negative signal.

IV. DISCUSSION

As mentioned above, this study is the first local and regional immunohistochemical study comparing CK-cocktail and CK8/18 in the liver of amphibians (*Hyla savigni*) and reptiles (*Hemidactylus turcicus* and *Testudo graeca*). Compared to the abundance of available data on intermediate filaments proteins in higher vertebrates there is little data about their variation and expression in amphibians and reptiles. Our results demonstrate that both cytoplasm of hepatocytes and bile ducts in the studied species showed different expression levels using CK-cocktail antibodies. Tissues treated with CK8/18 antibodies showed signal in *Testudo graeca*, unlike *Hyla savigni* and *Hemidactylus turcicus*. The results of our study add to the results of several previous studies. In [18] study, they performed histological comparison between the liver of two species of fish (Striped bass and Medaka) and the liver of four mammals species (Cat, Hedgehog, Groundhog, Sloth bear) using CK-cocktail antibodies. The cytoplasm of hepatocytes in Groundhog and Sloth bear showed positive signal using CK-cocktail, unlike Cat, Hedgehog, Striped bass and Medaka which showed negative signal. While bile ducts showed strong signal using CK-cocktail in Cat, Hedgehog, Groundhog, and Striped bass, the bile ducts in Sloth bear showed a medium signal and a slight signal in Medaka. In the study of [19] they conducted a comparative distribution study of different cytokeratin types (CK7, CK20, CK-cocktail) in the epithelial cells of the digestive system in three low vertebrate species (*Scyliorhinus canicula*, *Sparus aurata* and *Salamandra salamandra*). They confirmed the expression of

cytokeratin in the esophagus, stomach and intestines using CK-cocktail antibodies. The strongest signal appeared in *Sparus aurata* esophagus, whereas the weakest signal appeared in both *Salamandra salamandra* intestines and *Scyliorhinus canicula* stomach and intestines. [20] conducted an immunohistochemical study on the cytokeratin in both fish and amphibians using antibodies of cytokeratin. They confirmed that the staining using both cytokeratin acidic (AE1) and cytokeratin basic (AE3) in cartilaginous fish and bonefish showed a strong positive signal and the distribution pattern was uniform across all layers of the epidermis. In the anura (*Bufo viridis*, *Rana esculenta*), the AE1 is found mainly in the basal layers, while AE3 found in the skin. The study of [21] has explained the distribution of cytokeratin in the pads of the frog fingers *Philautus annanalii* using the antibodies of CK-cocktail. In this study they found that the strongest cytokeratin signal was in the middle rows to the basal cells of the skin, while the rest of the epidermis (i.e. The surface cell layer) showed a weak signal. In [22], they studied the organization and characterization of keratinocytes in the ovarian follicle of the lizard *podarcis sicula*. Comparative analysis was carried out by using immunoblotting analysis and immunological staining using the antibodies CK8, CK18. Both ovarian and intestinal follicles in this lizard showed that this creeping ovary follicle contains forms of cytokeratin like CK8 and CK18 in mammals and no CK19.

V. CONCLUSION

In our study as in the previous studies we confirm the presence of cytokeratin in amphibians and reptiles. Although it is difficult to compare the evolution of these vertebrates according to the distribution of CK-cocktail and CK8/18, yet we suggest that *Testudo graeca* is the highest developed species according to the distribution and abundance of CK-cocktail and CK8/18 in its liver epithelium. In *Hyla savigni* and *Hemidactylus turcicus* CK8/18 was completely negative, so depending on the distribution and abundance of CK-cocktail, *Hemidactylus turcicus* appeared to be the lowest developed species.

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