

## Features of the prenatal development of lungs in horseshoe bats (Rhinolophidae)

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**Abstract.** For the first time the prenatal development of lungs in horseshoe bats was investigated. The primary nature of dividing lungs into lobes at the early stages of development and the secondary lobeless structure of the lungs at later stages were described. The essential conclusion is that owing to the features of their morphological structure, horseshoe bats are especially vulnerable from the ecological point of view.

**Horseshoe bats, lungs, ontogenesis, ecology**

### Introduction

Hitherto published results of the definitive structure of horseshoe bats' lungs show that lungs are lobeless (Zhedenov 1957, Torubarova 1958, Kovalyova 1995). Nevertheless the division of right and left principal bronchi in the lungs of these animals is of the same type as in representatives of "plain-nosed" bats (Vespertilionidae) having lungs differentiated into lobes (Torubarova 1958).

According to the number of lobes in lungs as well as the degree of their separation, 15 types of the structure of lungs in mammals can be recognized (Zhedenov 1957). V. N. Zhedenov found in bats almost all kinds of transitions from typically lobed to lobeless lungs, and proposed a hypothesis that the presence of lobeless lungs in horseshoe bats is the secondary phenomenon resulting from merging of lobes. However the development of lobeless lungs has not yet been investigated.

Taking into account that there is no description of the embryology of bats' lungs in literature, the present paper is devoted to the research in the prenatal development of lungs in representatives of horseshoe bats. One of the main results of the work consists in the direct proof of the secondary nature of the lobeless lungs structure in connection with features of the rhinolophid ecology.

## Material and methods

Materials included serial microscopic sections of 10 mm in thickness representing the age series of 5 embryos which belonged to two species of horseshoe bats, *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* (Table 1).

According to the developmental stages defined in bats (Ledenev & Lihotop 1988), the material can be attributed to the 18th, 20th, 22nd, and 25th stage and to the stage of fetus, that corresponds to changing the crown-rump length (CRL) of embryos from 8.8 mm to 22.5 mm. The measuring method by Paetten (1959) was used.

To achieve an accurate orientation in naming the lobes in horseshoe bat embryonal lungs, adult specimens of vespertilionid bats were studied for comparison (in particular, *Nyctalus noctula*, Fig. 1). Names of the lung lobes were defined according to the nomenclature of the NAV (Paris, 1967).

Lungs of 10 adult individuals of horseshoe bats were prepared for morphometrical analysis.

The embryonic histological materials were stained with haematoxylin by the Mallori's method.

## Results

Lungs of adult individuals of horseshoe bats were symmetrical sacks, not divided into lobes (Figs 1, 2). The heart in the animals occupied a central position in the thoracic cavity between the right and left lung. The symmetry of definitive lungs in horseshoe bats was observed not only in external features, but also in the weight proportions. The left and right lung were of almost equal weight.

At the 18th stage of development, that corresponds to CRL 8.8 mm, *Rhinolophus hipposideros* embryos had lungs divided into lobes according to the division of the principal bronchi into the primary and secondary branch (Fig. 3). There were 4 and 3 lobes in the right and left lung, respectively.

The principal bronchus of the right lung was divided into 4 branches: the bronchus of an apex lobe (the cranial bronchus of the 1st order) was ramified at the base of the principal bronchus; somewhat more caudally both the bronchi of the heart lobe (the ventral lobe of the 1st order) and the bronchus of the additional lobe were separated at the same level from the principal bronchus; the remaining part of the principal bronchus entered the diaphragmal lobe (the caudal bronchus). The left principal bronchus was divided into two bronchi of the 1st order: the cranial and the

Table 1. Review of material examined

Stage	CRL [mm]	Weight [mg]	Plane	Species	No.
18	8.8	115	front.	<i>Rhinolophus hipposideros</i>	25
20	12.0	365	front.	<i>Rhinolophus hipposideros</i>	51
22	15.3	500	front.	<i>Rhinolophus hipposideros</i>	16
25	19.3	1760	sagit.	<i>Rhinolophus ferrumequinum</i>	12
fetus	22.5	2575	sagit.	<i>Rhinolophus ferrumequinum</i>	73

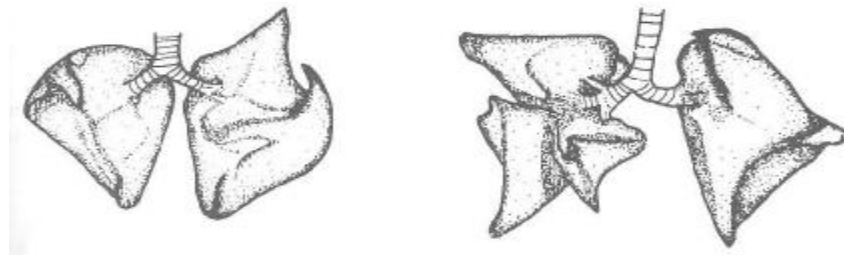


Fig. 1. Lungs of *Rhinolophus ferrumequinum* (left) and *Nyctalus noctula* (right).

caudal bronchus. The cranial bronchus of the 1st order was ramified into 2 bronchi of the 2nd order (the apex lobe bronchus was cranial, while the heart lobe bronchus was ventral). The caudal bronchus went into the diaphragmatic lobe. During this period of development the lung formed an aggregate of lobe bronchi growing into the mesenchymal primordium.

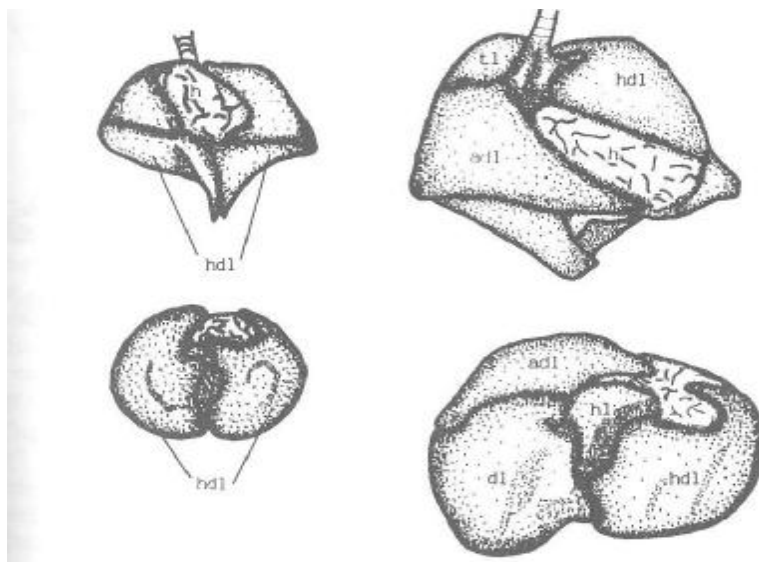


Fig. 2. Lungs of *Rhinolophus hipposideros* (left) and *Eptesicus serotinus* (right): costal (above) and diaphragmatic (down) surfaces. hdl – heart-diaphragmatic lobe; adi – additional lobe; dl – diaphragmatic lobe; hl – heart lobe; h – heart.

At the 20th stage of development of *Rhinolophus hipposideros* embryos, that corresponds CRL 12.0 mm, both the right and left lung displayed no division into lobes, and were compact (Fig. 4).

Lungs of embryos at the 22nd and 25th stages, that corresponds CRL 15.3 mm and 19.3 mm, respectively, represented compact formations, too. At these stages of development differentiation of wall cells in bronchi and alveoli was observed.

### Discussion

Representatives of horseshoe bats are notable for their stable dwelling sites: caves, grottos, lofts of the buildings with a high arch and a wide entrance, that enable to fly through easily and to hang up themselves by hind extremities. The bats practically do not use quadrupedal locomotion for moving on ground. Such a mode of life of horseshoe bats has been reflected in the structure of their locomoting organs, namely the thorax. Horseshoe bats have the thorax of a low mobility degree – “with constant toughness” in the Kovtun’s (1984) terminology – characteristic by extremely limited movement of ribs, reduced thoracic muscles, and prevailing respiration by the diaphragm (Kovalyova 1988).

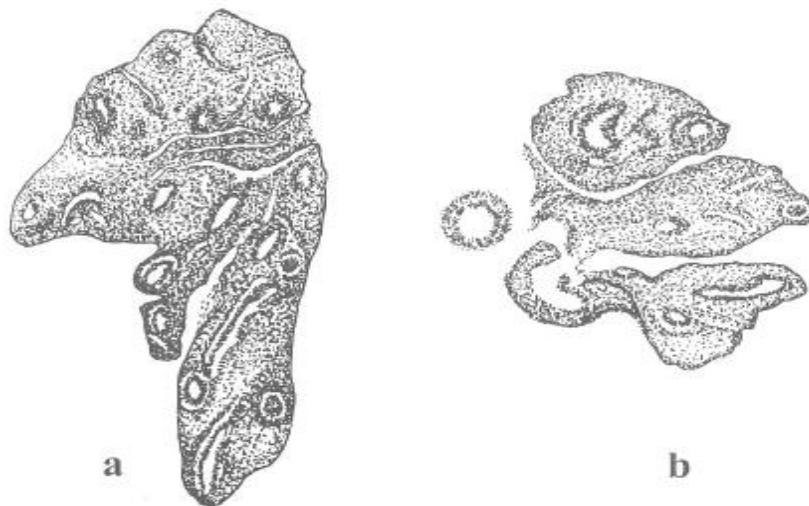


Fig. 3. Section through the right (a) and left (b) lungs of *Rhinolophus hipposideros* (CRL 8.8 mm).

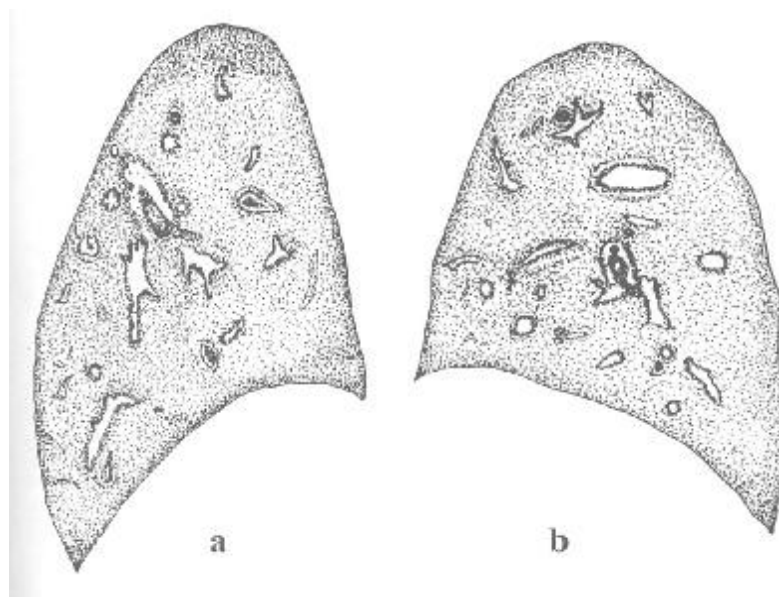


Fig. 4. Section through the right (a) and left (b) lungs of *Rhinolophus hipposideros* (CRL 15.3 mm).

The 18th stage of development of the bats is important for differentiation of the locomotory muscular system as well as for chondrogenesis of the axial skeleton, pectoral girdles and free extremities (Ledenev & Lihotop 1988, Štěrba 1990). At this stage of development of horseshoe bats, the lungs with the lobe structure were observed. Ledenev & Lihotop (1988) noted that the 21st stage is characterized, in particular, by forming the periosteal bone in the axial and girdle skeleton and in the free extremities. The lobeless structure of lungs was observed as early as at the 20th stage of development.

The comparison with published data shows that the so-called process of “merging” lobes in lungs starts prior to ossification of axial skeleton elements and is synchronous with chondrogenesis of respective structures, the thorax in particular. Therefore, forming of lobeless lungs is accompanied by development of a firm conjunction between some skeleton elements, resulting in the thorax with low mobility.

It should be noted that “merging lobes during the process of forming lobeless lungs”, was not properly interpreted in works of other morphologists. I have not

observed any trace of such merging lobes in lungs (Fig. 4). In my opinion, the process of forming lobeless lungs in horseshoe bats rather resembles blowing a rubber surgical glove to get a ball-shaped object.

So, the synchronized in prenatal development of the structures under consideration is not sure. Because the features of horseshoe bats' way of life are correlated with forming their thorax (Kovalyova 1988), one may conclude that the lobeless structure of lungs in the animals is connected with their ecology.

Thus, there are all grounds to consider horseshoe bats as ecologically very vulnerable animals, since their dependence on certain dwelling sites is related, in particular, to their morphology. Therefore the protection of horseshoe bats' dwelling sites is one of the most important measure of their conservation.

### Súhrn

**Charakteristiky prenatalného vývoja pľúc podkovárov (Rhinolophidae).** V práci sa po prvýkrát popisujú charakteristiky prenatalného vývoja pľúc podkovárov. Bolo zistené primárne rozdelenie pľúc týchto živočíchov do lalokov v skorých štádiách ontogenézy a sekundárne bezlaločná štruktúra pľúc v pozdejších štádiách. Na základe uvedeného sa v práci konštatuje, že v dôsledku charakteristík morfolologickej štruktúry patria z ekologického hľadiska podkováre k zraniteľným skupinám živočíchov.

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