VERTEBRATE ZOOLOGY

68 (1): 21–26
19.4.2018

SENCKENBERG

Variability in pulmonary reduction and asymmetry in a serpentiform lizard: The sheltopusik, *Pseudopus apodus* (Pallas, 1775)

Markus Lambertz[1,2,*], Nils Arenz[1] & Kristina Grommes[1]

[1] Institut für Zoologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Poppelsdorfer Schloss, 53115 Bonn, Germany — [2] Sektion Herpetologie, Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany; lambertz@uni-bonn.de — * Corresponding author

Accepted 4.ix.2017.
Published online at www.senckenberg.de/vertebrate-zoology on 5.4.2018.

Editor in charge: Uwe Fritz

Abstract

Besides snakes, numerous lineages of squamates gave rise to limb-reduced and elongated (serpentiform) species, indicating the evolutionary success of this modification of the plesiomorphic lizard *Bauplan*. Concerted with a serpentiform habitus are several morphological adaptations, many of which also concern the structure and arrangement of the viscera, such as frequently a pronounced pulmonary asymmetry in which one lung is reduced or even absent. The European glass lizard or sheltopusik, *Pseudopus apodus*, is the largest species of the exclusively serpentiform Anguinae. Driven by pre-existing conflicting statements on pulmonary asymmetry, we examined the lungs of 14 sheltopusiks and compared the condition to 11 slow worms (*Anguis fragilis*). We consistently found the left lung pronouncedly shorter for the slow worm, but indeed a highly variable pulmonary asymmetry between left and right sides in the sheltopusik. This is the first verified case of such variability in pulmonary reduction for any serpentiform squamate and raises several questions about the underlying developmental program for this otherwise taxon-specifically conservative trait.

Kurzfassung

Neben den eigentlichen Schlangen haben zahlreiche Abstammungslinien der Squamaten Arten mit reduzierten Extremitäten und einem verlängerten (serpentiformen) Körperbau hervorgebracht, was den evolutionären Erfolg dieser Modifikation des plesiomorphen Echsen-Bauplans unterstreicht. Einhergehend mit einem solchen serpentiformen Habitus sind zahlreiche morphologische Anpassungen, von denen viele auch die Struktur und Anordnung der inneren Organe betreffen, wie beispielsweise häufig einer ausgeprägten Asymmetrie der Lungen, bei der eine rückgebildet oder sogar vollständig reduziert ist. Der Scheltopusik, *Pseudopus apodus*, ist die größte Art der ausschließlich serpentiformen Anguinae. Angetrieben von bestehenden aber widersprüchlichen Aussagen zur Asymmetrie der Lungen dieser Art haben wir die Lungen von 14 Scheltopusiks untersucht und die Befunde mit denen von 11 Blindschleichen (*Anguis fragilis*) verglichen. Bei der Blindschleiche haben wir konsistent eine verkürzte linke Lunge gefunden, während der Scheltopusik in der Tat ein hohes Maß an Variabilität der Lungenasymmetrie zwischen linker und rechter Seite aufweist. Dies ist der erste belegte Fall einer solchen Variabilität der Lungenreduktion eines serpentiformen Squamaten und wirft zahlreiche Fragen zum zu Grunde liegenden Entwicklungsprogramm dieses ansonsten taxonspezifisch konservativen Merkmalskomplexes auf.

Key words

Anguimorpha, Anguidae, Anguinae, legless lizard, lung anatomy, pulmonary morphology, respiratory biology, serpentiform habitus.

Introduction

Reduction or loss of the extremities is one of the most intriguing character traits that has evolved multiple times convergently among the Squamata, frequently even associated with the development of an elongated (serpentiform) habitus (e.g., Gans, 1975; Lande, 1978; Greer, 1991). Besides the true snakes (Serpentes), which con-

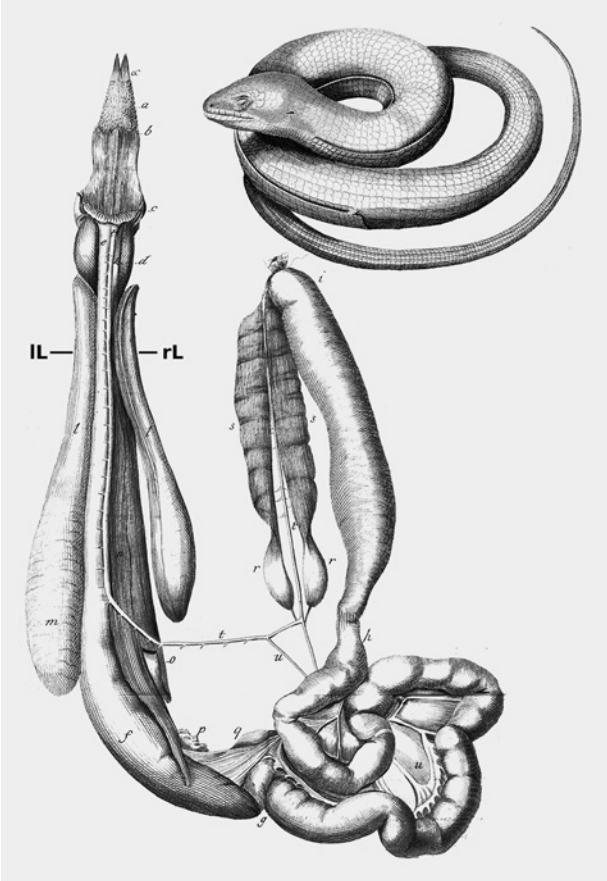

Fig. 1. Modified reproductions from the plates accompanying the original description of the sheltopusik by PALLAS (1775) showing the habitus of the species and a dorsal view of the viscera of an adult male specimen. If the dimensions of the engraving are correct, the right lung (rL) accounts for about 80% of the length of the left lung (lL). Original deposited at the *Universitäts- und Landesbibliothek Bonn*, Germany

stitute the most species-rich lineage of squamates, serpentiform species are found among almost all primary radiations of scaled reptiles and a number of clades, such as for instance the Amphisbaenia, even are exclusively comprised of such. Another one of such particularly serpentiform clades is the Anguinae (Anguimorpha: Anguidae), the slow worms and glass lizards (WIENS & SLINGLUFF, 2001). The currently recognized 20 species are grouped in five genera (*Anguis* LINNAEUS, 1758: 6 spp.; *Dopasia* GRAY, 1853: 7 spp.; *Hyalosaurus* GÜNTHER, 1873: 1 sp.; *Ophisaurus* DAUDIN, 1803: 5 spp.; *Pseudopus* MERREM, 1820: 1 sp.), and are distributed from North America over Europe, northern Africa and Central Asia up to Southeast Asia (UETZ *et al.*, 2017). The largest of all extant anguine species is the European glass lizard or sheltopusik, *P. apodus* (PALLAS, 1775), which inhabits a wide range from southeastern Europe, over northern Arabia up to Central Asia (OBST, 1978, 1981), and which is in the focus of the present paper.

The development and evolution of a serpentiform habitus is concerted with a number of morphological changes, including modifications concerning the general visceral structure and their topographical organization (GANS, 1975; LAMBERTZ, 2016). Many of the internal organs in such species are elongated as well, arranged sequentially, or even are reduced unilaterally if plesiomorphically paired. The reduction – up to the complete absence – of one lung thereby is a common trait found among many serpentiform species. Most snakes exhibit a significant reduction of their left lung and quite a few lack it entirely (BUTLER, 1895; WALLACH, 1998). Amphisbaenians, on the other hand, frequently exhibit a reduction of their right lung (BUTLER, 1895; PERRY, 1998; NAVEGA-GONÇALVES, 2009; NAVEGA-GONÇALVES & DA SILVA, 2013), which indicates that both alternatives (left vs. right reduction) are realized and apparently equally suitable adaptations to a serpentiform habitus. However, the individual pattern so far has to be considered strictly taxon-specifically conservative.

Already the first formal description of the sheltopusik by PALLAS (1775) provides information on pulmonary asymmetry also for this species, by indicating that here also the right lung is shorter and reduced if compared to the left one (fig. 1). CUVIER (1829), without providing details on left or right, confirmed this overall asymmetry of the lungs for this species. As did MILANI (1894), although asymmetry was noted to be less pronounced. However, his generally very accurate descriptions still seem to be in strong contrast to those of PALLAS (1775), since he consistently found the left lung to be shorter than the right one in the two specimens he examined. BUTLER (1895) agrees with MILANI (1894) in terms of the side of reduction, but described the left lung to account for only about 60% of the length of the right lung in the solitary specimen he examined. In his unpublished dissertation, BECKER (1993) also examined one sheltopusik and found the left lung to be pronouncedly shorter than the right one as well, which also agrees with the situation repeatedly documented for instance for the closely related slow worm *A. fragilis* LINNAEUS, 1758 (MECKEL, 1818; MILANI, 1894; MOSER, 1902; BECKER, 1993). The aim of the present study was to evaluate these conflicting statements on the asymmetry and reduction of the lungs in the sheltopusik.

Materials and Methods

We examined a total of 14 specimens of *P. apodus*. 11 deceased specimens obtained from herpetoculturists were dissected manually, and complemented by 2 preparations available in the teaching collection of the *Institut für Zoologie* (Bonn) and the data and specimen from BECKER (1993). Since the status of preservation of the uncatalogued specimens was not always ideal and several of the viscera were already partly decayed, we had to refrain from a quantitative approach to describe pulmonary asymmetry in some of them (see table 1) as the obtainable values appeared unreliable. We therefore restricted the assessment to a qualitative approach in such instances focusing only on which lung was longer

Table 1. Summary of specimens examined for the present study. SVL: snout-vent length; lL: length of the left lung; rL: length of the right lung; TC: uncatalogued teaching collection on display, *Institut für Zoologie*, Bonn; s: this lung shorter but no measurements available; n/a: not available.

Species	Specimen	Sex	**SVL** [mm]	**lL** [mm]	**rL** [mm]	Form of examination
Pseudopus apodus	uncatalogued	♀	248	s	n/a	dissection
	ZFMK 22316	♀	350	s	n/a	dissection: Becker (1993) [*ex errore* as ZFMK 27316]
	uncatalogued	♀	360	124	140	dissection
	uncatalogued	♀	373	142	139	dissection
	uncatalogued	♀	405	142	98	dissection
	uncatalogued	♀	450	183	218	dissection
	uncatalogued	♂	288	110	81	dissection
	uncatalogued	♂	344	103	104	dissection
	uncatalogued	♂	348	160	183	dissection
	uncatalogued	♂	349	113	172	dissection
	uncatalogued	♂	377	145	180	dissection
	uncatalogued	♂	401	s	n/a	dissection
	TC	♂	n/a	n/a	s	wet
	TC	n/a	n/a	n/a	s	dried
Anguis fragilis	uncatalogued	♀	120	18	38	dissection
	uncatalogued	♀	133	22	37	dissection
	uncatalogued	♀	167	27	42	dissection
	ZFMK 7325	♀	226	s	n/a	dissection: Becker (1993)
	uncatalogued	♂	130	34.5	64	dissection
	uncatalogued	♂	133	15	26.5	dissection
	uncatalogued	♂	141	21	40	dissection
	uncatalogued	♂	141	30	n/a	dissection
	uncatalogued	♂	183	35.5	60	dissection
	uncatalogued	♂	185	24	40.5	dissection
	uncatalogued	n/a	155	s	n/a	dissection: Lambertz *et al.* (2015)

in absolute terms. All measurements taken are based on uninflated and removed lungs in wet (alcohol preserved) condition. Several of these preserved lungs were dried after removal according to standard procedures (see e.g. Lambertz *et al.*, 2015), and photographed using a Canon EOS 5D Mark II digital camera equipped with a Canon EF 24-105 mm 1:4.0 IS USM lens. We furthermore examined 11 specimens of *A. fragilis* for comparison, 10 by dissection and again supplemented by the data from Becker (1993). Table 1 summarizes morphometric data on all individuals examined as well as information on their sex and the voucher numbers for the museum specimens, which are deposited in the herpetological collection of the *Zoologisches Forschungsmuseum Alexander Koenig*, Bonn, Germany (ZFMK).

Results and Discussion

The basic pulmonary morphology in the sheltopusik agrees with what has been described by the earlier authors for this species (Pallas, 1775; Cuvier, 1829; Milani, 1894; Butler, 1895; Becker, 1993): The sac-like lungs are paired and elongate and there is an asymmetry in terms of length when left and right sides are compared. Surprisingly, we found indeed specimens in which the right lung was shorter and reduced in comparison to the left one, but also several ones in which the situation occurred vice versa (fig. 2), whereas the left lung consistently was reduced in the slow worm. Further surprising is the fact that it appears that there is no clear pattern for this variability recognizable. Initially we expected a potentially sexually dimorphic situation, but we found both females in which either state was present, as well as males in which this was the case (table 1). Most of the sheltopusiks examined exhibited a smaller left lung, which is true both for the females and the males. Table 2 summarizes the ratios quantitatively. Albeit a slight pulmonary asymmetry was always present, some specimens exhibited only marginal differences in the lengths of their lungs. Regardless of the side, the average length of the shorter lung if compared to the longer one in the sheltopusik equaled about 1.5 times the ratio observed for the slow worm, in which there furthermore appeared to be a somewhat lower degree of variability for this ratio than in the former species (table 2). Comparing the shorter lung to snout-vent length (SVL) in the sheltopusik revealed a ratio that equals about 2 times the ratio observed for the slow worm (table 2), thereby indicating a more pronounced one-sided pulmonary reduction in the latter species. The sample size available to us does not qualify for a definitive and statistically sound statement on this, but it appears that in those sheltopusiks in which both lungs are almost of about equal length, the lungs seem to be

Fig. 2. Variable pulmonary reduction and asymmetry in the sheltopusik. Dried lungs in dorsal view. A slight asymmetry is always present, but there are specimens in which the left lung is reduced, ones in which the right one is reduced, and some in which the two lungs approximate the same length. Note that the imperfect preservation of the specimens available not always resulted in fully inflatable lungs, but that all measurements (see table 1) were taken in wet condition prior to drying and that the illustration in general reflects the obtained values. Scale bar equals 5 cm.

shorter (in relation to SVL) if compared to the longer one in those with a more pronounced asymmetry (table 1). The present report hence confirms that all previous authors that dealt with the subject were perfectly right in their conflicting descriptions of the lungs of *P. apodus* (PALLAS, 1775: right lung reduced; MILANI, 1894: left lung reduced but only slight asymmetry; BUTLER, 1895; BECKER, 1993: left lung reduced with pronounced asymmetry). The sheltopusik apparently exhibits an unexpected but highly variable asymmetry in the reduction of either its left or right lung.

The left lung of a large number of non-serpentiform lepidosaurs is smaller than the right one (COE & KUNKEL, 1906, CAMP, 1923, KLEIN *et al.*, 2005; LAMBERTZ *et al.*, 2015), which functionally might be correlated with the fact that the stomach occupies and dominates this portion of the coelomic cavity. A smaller left lung in turn might circumvent spatial constraints and ventilatory impediments after food uptake. If one of the lungs is reduced almost completely, such as in several snakes and amphisbaenians, it probably does not matter which one is maintained as the functional consequences seem virtually identical. A remarkable pathological specimen of the common snapping turtle, *Chelydra serpentina* (LINNAEUS, 1758), with unilateral pulmonary aplasia exhibited only a left but hypertrophied lung that furthermore showed indications for increased intrapulmonary branching (SCHACHNER *et al.*, 2017). We suggest that the suppressed formation of one lung in general might be genetically coupled with a pronounced development of the other lung. This hypothesis, aside from several other squamate examples and the suggestively shorter lungs in those sheltopusiks where the left and right side approximated the same length, would also be in agreement with the elongated singular lung of the Australian lungfish *Neoceratodus forsteri* (KREFFT, 1870) (SPENCER, 1898), and possibly

Table 2. Summary of quantitative parameters related to pulmonary asymmetry in the two serpentiform species studied. n: number of specimens available per analysis; SVL: snout-vent length; S.D.: standard deviation.

	Pseudopus apodus		***Anguis fragilis***	
	n	%	n	%
ratio of specimens with shorter left lung	14	64	11	100
ratio of females with shorter left lung	6	66.7	4	100
ratio of males with shorter left lung	7	71.4	6	100
ratio shorter lung / longer lung [mean ± S.D.]	9	82.9 ± 11.8	7	56.6 ± 5.2
ratio shorter lung / SVL [mean ± S.D.]	9	34.6 ± 6.8	8	17.1 ± 4.7

even the remarkable situation in the extant coelacanth *Latimeria chalumnae* Smith, 1939 (Lambertz, 2017). The sheltopusik undoubtedly would be an intriguing organism to further study this remarkable phenomenon of pulmonary asymmetry, both from the basic as well as applied point of view.

To the best of our knowledge, this is the first documented case of such variability in lung reduction and asymmetry for any species of serpentiform squamate in particular and even any reptile in general. The actual functional and developmental context of this variability in *P. apodus*, however, unfortunately remains obscure. Variation in visceral left-right asymmetry is also known as a pathological condition in humans, in which it usually is associated with primary cilia dyskinesia and high (> 50%) rates of male infertility, but at very low rates of prevalence (López-Gracia & Ros, 2007; Sutherland & Ware, 2009). The high degree and prevalence of pulmonary variability and left-right asymmetry observed even in our moderate sampling strongly argues against such a pathological condition in the sheltopusik and demands a different, but yet to be found explanation. As a first next step, we suggest that the phenomenon of variable pulmonary asymmetry in sheltopusiks should be examined in a biogeographical context in order to evaluate whether there are any patterns that correlate with the different subspecies/populations of this widely distributed anguine lizard (cf. Obst, 1978, 1981), which due to the uncertain origin of the specimens available to us could not be reliably addressed as of now.

Acknowledgements

We wish to thank Morris Flecks (ZFMK) for his help in the collections. We furthermore would like to thank Michael H. Hofmann (*Institut für Zoologie*, Bonn) for funding the research of ML and for providing the required laboratory infrastructure for the dissections.

References

Becker, H.O. (1993): Vergleichende Untersuchungen am respiratorischen Apparat anguimorpher Eidechsen: Eine stammesgeschichtliche Deutung. – Unpublished doctoral dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany: 212 pp.

Butler, G.W. (1895): On the complete or partial suppression of the right lung in the Amphisbaenidae and of the left lung in snakes and snake-like lizards and amphibians. – Proceedings of the Zoological Society of London **1895**: 691 – 712 + Pl. XL.

Camp, C.L. (1923): Classification of the lizards. – Bulletin of the American Museum of Natural History **48**: 289 – 481.

Coe, W.R. & Kunkel, B.W. (1906): Studies on the California limbless lizard, *Anniella*. – Transactions of the Connecticut Academy of Arts and Sciences **12**: 349 – 203 + Pls. XLI – XLVIII.

Cuvier, G. (1829): Le règne animal distribué d'après son organisation, pour servir de base a l'histoire naturelle des animaux et d'introduction a l'anatomie comparée. Nouvelle édition, revue et augmentée. Tome II. – Déterville, Paris: XV + 406 pp.

Daudin, F.M. (1803): Charactères des vingt-trois genres qui composent l'ordre des Ophidiens. – Magasin Encyclopèdique **5**: 433 – 438.

Gans, C. (1975): Tetrapod Limblessness: Evolution and Functional Corollaries. – American Zoologist **15**(2): 455 – 467.

Gray, J.E. (1853): Descriptions of some undescribed species of Reptiles collected by Dr. Joseph Hooker in the Khassia Mountains, East Bengal, and Sikkim Himalaya. – The Annals and Magazine of Natural History, including Zoology, Botany, and Geology, 2nd Series **12**: 386 – 392.

Greer, A.E. (1991): Limb Reduction in Squamates: Identification of the Lineages and Discussion of the Trends. – Journal of Herpetology **25**(2): 166 – 173.

Günther, A. (1873): Description of a new Saurian (*Hyalosaurus*) allied to *Pseudopus*. – The Annals and Magazine of Natural History, including Zoology, Botany, and Geology, 4th Series **11:** 351.

Klein, W., Reuter, C., Böhme, W. & Perry, S.F. (2005): Lungs and mesopneumonia of scincomorph lizards (Reptilia: Squamata). – Organisms Diversity & Evolution **5**: 47 – 57.

Krefft, G. (1870): To the Editor of the Herald. – The Sydney Morning Herald **9878**: 5.

Lambertz, M. (2016): Recent advances on the functional and evolutionary morphology of the amniote respiratory apparatus. – Annals of the New York Academy of Sciences **1365**: 100 – 113.

Lambertz, M. (2017): The vestigial lung of the coelacanth and its implications for understanding pulmonary diversity among vertebrates: new perspectives and open questions. – Royal Society Open Science **4**: 171518

Lambertz, M., Grommes, K., Kohlsdorf, T. & Perry, S.F. (2015): Lungs of the first amniotes: why simple if they can be complex? – Biology Letters **11**(1): 20140848.

Lande, R. (1978): Evolutionary Mechanisms of Limb Loss in Tetrapods. – Evolution **32**(1): 73 – 92.

LINNAEUS, C. (1758): Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, Cum Characteribus, Differentiis, Synonymis Locis. Tomus I. Editio decima. – Laurentii Salvii, Holmiæ [Stockholm]: [2] + 823 + [1] pp.

LÓPEZ-GRACIA, M.L. & ROS, M.A. (2007): Left-right asymmetry in vertebrate development. – Advances in Anatomy, Embryology and Cell Biology **188**: 1–121.

MECKEL, J.F. (1818): Ueber das Respirationssystem der Reptilien. – Deutsches Archiv für die Physiologie **4**: 60–89.

MERREM, B. (1820): Versuch eines Systems der Amphibien. – Christian Krieger, Marburg: XV + 191 pp. + 1 Pl.

MILANI, A. (1894): Beiträge zur Kentniss der Reptilienlunge. I. Lacertilia. – Zoologische Jahrbücher, Abtheilung für Anatomie und Ontogenie der Thiere **7**: 545–592 + Pls. 30–32.

MOSER, F. (1902): Beiträge zur vergleichenden Entwicklungsgeschichte der Wirbeltierlunge (Amphibien, Reptilien, Vögel, Säuger). – Archiv für Mikroskopische Anatomie und Entwicklungsgeschichte **60**: 587–668 + Pls. XXX–XXXIII.

NAVEGA-GONÇALVES, M.E.C. (2009): Anatomia visceral comparada de seis espécies de Amphisbaenidae (Squamata: Amphisbaenia). – Zoologia **26** (3): 511–526.

NAVEGA-GONÇALVES, M.E.C. & DA SILVA, J.R.M.C. (2013): Respiratory system of *Amphisbaena vermicularis* and *Amphisbaena microcephala* (Squamata, Amphisbaenia, Amphisbaenidae). – Iheringia, Série Zoologia **103**(1): 20–30.

OBST, F.J. (1978): Zur geographischen Variablität des Scheltopusiks, *Ophisaurus apodus* (Pallas). – Zoologische Abhandlungen, Museum für Tierkunde Dresden **35**(8): 129–140.

OBST, F.J. (1981): *Ophisaurus apodus* (Pallas 1775) – Scheltopusik, Panzerschleiche. – pp. 259–274, *in*: BÖHME, W., (ed.): Handbuch der Reptilien und Amphibien Europas, Band 1, Echsen (Sauria) I. Akademische Verlagsgesellschaft, Wiesbaden.

PALLAS, P.S. (1775): *Lacerta apoda* descripta. – Novi Commentarii Academiae Scientiarum Imperialis Petropolitanae **19**: 435–454 + Pls. IX–X.

PERRY, S.F. (1998): Lungs: Comparative Anatomy, Functional Morphology, and Evolution. – pp. 1–92, *in*: GANS, C. & GAUNT, A.S., (eds.): Biology of the Reptilia, Volume 19, Morphology G, Visceral Organs. Society for the Study of Amphibian and Reptiles, Ithaca, NY (Contributions to Herpetology **14**).

SCHACHNER, E.R., SEDLMAYR, J.C., SCHOTT, R., LYSON, T.R., SANDERS, R.K. & LAMBERTZ, M. (2017): Pulmonary anatomy and a case of unilateral aplasia in a common snapping turtle (*Chelydra serpentina*): developmental perspectives on cryptodiran lungs. – Journal of Anatomy **231**: 835–848

SMITH, J.L.B. (1939): A living fish of Mesozoic type. – Nature **143** (3620): 455–456.

SPENCER, B. (1898): Der Bau der Lungen von *Ceratodus* und *Protopterus*. – pp. 51–58 + Pl. IX–X, *in*: SEMON, R. (ed.): Zoologische Forschungsreisen in Australien und dem malayischen Archipel. Erster Band: *Ceratodus*. II. Lieferung. – Denkschriften der Medicinisch-Naturwissenschaftlichen Gesellschaft zu Jena **4**.

SUTHERLAND, M.J. & WARE, S.M. (2009): Disorders of left-right asymmetry: Heterotaxy and situs inversus. – American Journal of Medical Genetics **151C**(4): 307–317.

UETZ, P., FREED, P. & HOŠEK, J., Eds. (2017): The Reptile Database. – Available online at http://www.reptile-database.org, accessed on 13 May 2017.

WALLACH, V. (1998): The Lungs of Snakes. – pp. 93–295, *in*: GANS, C. & GAUNT, A.S., (eds.): Biology of the Reptilia, Volume 19, Morphology G, Visceral Organs. Society for the Study of Amphibian and Reptiles, Ithaca, NY (Contributions to Herpetology **14**).

WIENS, J.J. & SLINGLUFF, J.L. (2001): How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. – Evolution **55**(11): 2303–2318.