Amphibia-Reptilia 11 (1990): 123-130, E. J. Brill, Leiden

# Comparative morphology of the adrenal gland in some species belonging to the family Lacertidae

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**Abstract.** The distribution and the ratios between noradrenaline (NA) and adrenaline (A) cells in eight species belonging to the family Lacertidae were studied. There are variations in the distribution of the two cell types within the gland and a wide range of variation in the NA/A cell ratio. Comparing their data with palaeontological, anatomical and biochemical evidence, the authors are inclined to consider *G. galloti* and *L. graeca* as the most ancient lizards among those studied and to ascribe *L. graeca* to the group *Lacerta* part II together with *L. dugesii*. *P. pityusensis* should also be ascribed to the same group on the basis of the results of this study. The other four species studied (*L. lepida, L. trilineata, L. viridis* and *L. schreiberi*) appear to be of fairly recent origin and should be ascribed to the group *Lacerta* part I, on the basis of the homogeneous distribution of the chromaffin cells inside the adrenal gland and their low NA/A cell ratios.

## Introduction

Early authors, who studied the morphology of the adrenal gland of Squamates, pointed out that steroidogenic tissue forms most of the gland, while chromaffin tissue constitutes a continuous dorsal ribbon that sends digitations into the steroidogenic parenchyma. Scattered between the steroidogenic cords there are small islets of chromaffin cells containing only adrenaline (A) cells. The chromaffin cells of the dorsal ribbon and those of its digitations, however, are only noradrenaline (NA) cells (Wright and Chester Jones, 1955, 1957; Chester Jones, 1957 a, b; Gabe and Martoja, 1961; Wassermann and Tramezzani, 1961, 1963; Gabe, 1970).

This description does not agree with that of Saint Girons (1976) for the adrenal gland of Anguimorpha, since this author notices the presence of A cells in the dorsal ribbon in these glands. Such an arrangement had already been observed in the adrenal gland of *Podarcis s. sicula* (formerly *Lacerta s. sicula*) (Varano et al., 1969) and confirmed in two subsequent papers (Varano and Laforgia, 1976; Laforgia and Varano, 1978).

Further studies on the morphology of the adrenal gland of Iguanidae, Gekkonidae and numerous other Squamates (Varano and Laforgia, 1982; Laforgia et al., 1982, 1983, 1985) and on the adrenal gland of some species of Cordylidae (Laforgia and

Varano, 1982) have shown that there is a great variability in the distribution of the two types of chromaffin cells and in the ratio between NA cells and A cells. The variability of the NA/A cells ratio has suggested a connection between the ancestry of the species and this ratio. Actually, older species have more NA cells in the adrenal while species of more recent origin have more A cells. Similar results were obtained by Ghosh (1977) in an extensive study on 28 avian species representing several orders and families: he found an extreme range of relative concentrations of adrenaline- and noradrenaline-storing cells, *Phalacrocorax* had 100% NA-containing cells, whereas many passerine birds had 95% A-containing cells. Ghosh (1977) found that the NA/A ratio related to avian phylogeny; thus birds of more primitive ancestry (cormorant, chicken, egret) had more NA cells, while recently evolved birds (passerine birds) had more A cells.

In this paper we have studied specimens of eight species belonging to the family Lacertidae, in order to evaluate whether within this family there also is a variation both in the morphology of the gland and in the NA/A cell ratio, which might be ascribed to their phylogenetic position.

## Material and methods

The adrenal glands of the following species were investigated: *Lacerta dugesii, Lacerta lepida, Lacerta viridis, Podarcis pityusensis, Gallotia galloti, Lacerta trilineata, Lacerta graeca, Lacerta schreiberi*. For each species, at least two specimens of each sex were examined. The animals were killed by decapitation. The adrenals were fixed in a mixture of potassium dichromate and sodium sulphate (buffered at pH 4.1 with acetate buffer, 5 M), to which 10% formaldehyde was added before use (Wood, 1963). The glands were embedded in paraffin, sectioned at 6-7 μ and stained with one of the following solutions which allow NA cells to be differentiated from A cells:

1) a mixture of eosine-aniline blue, buffered at pH 4 with acetate buffer, 5 M (Wood, 1963), staining NA cells gold and A cells orange-red
2) Giemsa solution modified according to Pearse (1960), staining NA cells dark green and A cells light green
3) Mallory trichromic stain. NA cells appear gold-yellow and A cells appear red.

The NA/A cell ratio was calculated from cell counts, using every tenth transverse section from the whole gland of each specimen.

The data reported here apply to both sexes since differences between sexes and between specimens were very slight and hence were not quantified.

## Results

The adrenal gland of all the species studied appears rather compact, surrounded by a thin capsule of connective tissue, with the exception of *Lacerta lepida* where the capsule is thicker. In all the species the two kinds of tissue that usually constitute the adrenal gland are easily distinguishable.

The steroidogenic tissue is formed by prismatic cells with a roundish generally basally displaced nucleus. The steroidogenic cells are arranged into sinuous anastomosing cords of two cell rows separated by small blood vessels (fig. 1). In *L. dugesii*, the steadily basal position of the nucleus clearly outlines the shape of the steroidogenic cords (fig. 2). The chromaffin tissue forms a compact ribbon along the dorsal margin of the gland and sends digitations between the steroidogenic cords in *L. lepida, L. viridis, L. trilineata, L. schreiberi* (figs 3, 4, 5), and *P. pityusensis*. In *L. dugesii* (fig. 6) the chromaffin cells of the dorsal ribbon show a tendency to concentrate at the two poles of the gland. Rather different, however, is the distribution of the chromaffin cells in *G. galloti* (fig. 7a) and *L. graeca* (fig. 7b). In these species the chromaffin cells constitute a single mass located at the cephalic pole of the gland.

Specific histochemical techniques for the differentiation of A and NA cells have allowed the identification of both cell types in all of the species. The A cells are the only constituents of the chromaffin islets in all the species. The islets are lacking in *L. graeca* (fig. 7b), are few and medium-sized in *L. viridis* (fig. 3), rather numerous and medium-sized in *L. schreiberi* (fig. 5), small and numerous in *L. trilineata* (fig. 4), and rare and small in all the other species.

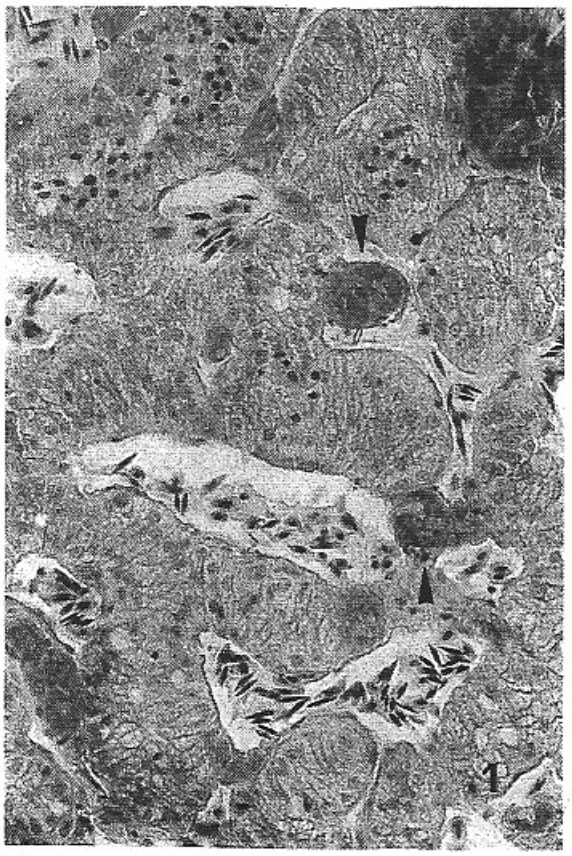
NA cells are the only constituents of the dorsal ribbon in *L. trilineata* and *P. pityusensis*; in the other species these cells are located in the outer rows of the ribbon and of the cephalic masses, while the A cells occupy the inner rows.

This variability in the distribution of the two kinds of chromaffin cells within the adrenal gland has an influence on the quantitative ratios between NA and A cells. The ratios observed in the species studied are the following:

| | |
|---|---|
| *Gallotia galloti* | 9/1 |
| *Lacerta graeca* | 8.6/1 |
| *Podarcis pityusensis* | 5/1 |
| *Lacerta dugesii* | 3/1 |
| *Lacerta lepida* | 2.5/1 |
| *Lacerta trilineata* | 2.4/1 |
| *Lacerta schreiberi* | 2.1/1 |
| *Lacerta viridis* | 2/1 |

## Discussion

The comparative study of the morphology of the adrenal gland in the Lacertidae examined has shown variation in the distribution of the two cell types within the gland and a wide range of variations in the NA/A cell ratio. In preceding studies carried out on species belonging to several families of Squamates (Laforgia and Varano, 1982; Varano and Laforgia, 1982; Laforgia et al., 1982, 1983, 1985) we have noticed that the species with a higher number of NA cells are those generally known as of more primitive ancestry, while in recently evolved species the number of NA cells shows a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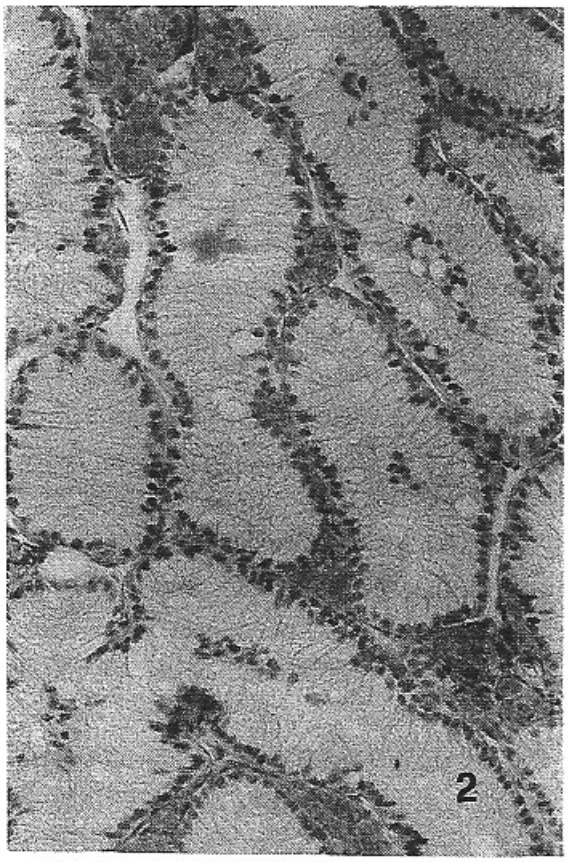
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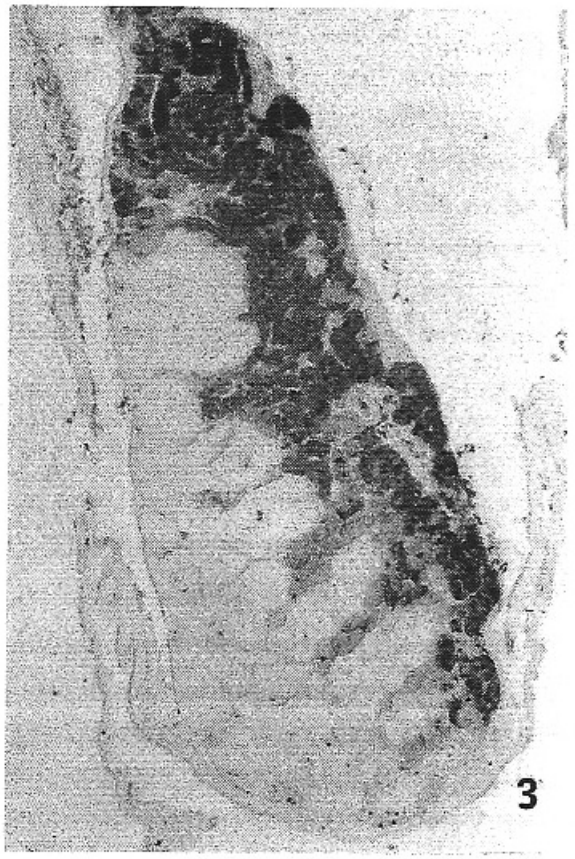
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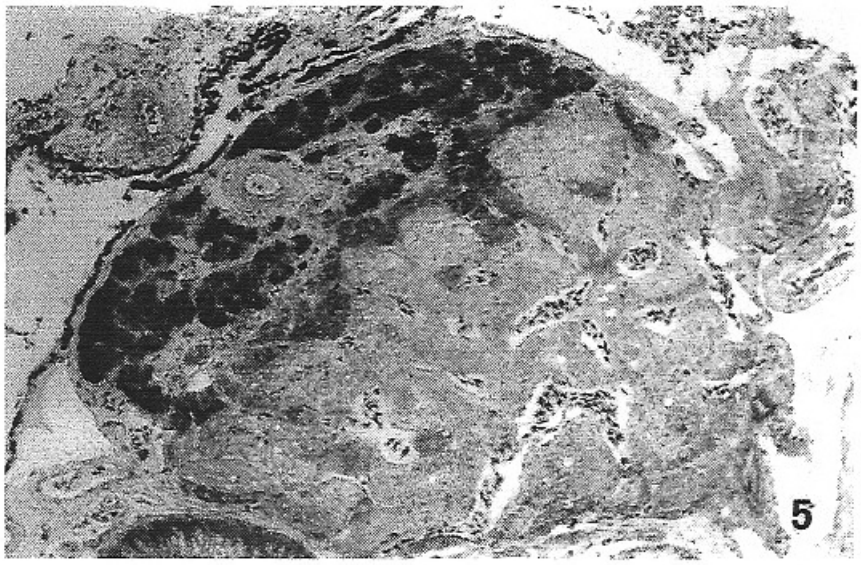


**Figure 5.** *Lacerta schreiberi*. Giemsa stain. Transverse section. × 80. Notice also in this species the dorsal ribbon of several cell strata with the NA cells (dark grey) in the outer layers and the A cells (light grey) in the inner layers and digitations.

tendency to decrease to a ratio 1/1, as observed in the genus *Podarcis* (Laforgia et al., 1985). According to this, among the lizards examined in this study, *Gallotia galloti* and *Lacerta graeca* should be the most ancient, while *Lacerta viridis* and *Lacerta schreiberi* should be the most recent.

Taxonomy and phylogenesis of Lacertidae and particularly of the genus *Lacerta* have been carefully studied by Böhme (1971) and later by Lanza et al. (1977). Lanza and Cei (1977), Mayer and Tiedemann (1982), Lutz and Mayer (1985), Busack and Maxson (1987) and Mayer and Lutz (1989). In 1973 Arnold suggested the division of the genus *Lacerta* into four groups: *Lacerta* part I, *Lacerta* part II, *Podarcis* and *Gallotia*. On the basis of this division, *G. galloti*, formerly *Lacerta galloti*, is ascribed to the genus *Gallotia*, which according to Lutz and Mayer (1985) is an older group separated from the others. Our observations on the morphology of the adrenal gland of the species and the NA/A cell ratio (9/1) lead us to consider *G. galloti* a very old species, very different from the others.

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**Figure 1.** *Lacerta schreiberi*. Mallory stain. Longitudinal section. × 185. Notice the steroidogenic cells arranged into anastomosing cords of two cell rows separated by small blood vessels. Notice also the small chromaffin islets (arrows).

**Figure 2.** *Lacerta dugesii*. Mallory stain. Longitudinal section. × 185. Notice the nuclei of the steroidogenic cells, steadily in basal position, which clearly outline the shapes of the cords.

**Figure 3.** *Lacerta viridis*. Giemsa stain. Longitudinal section. × 50. Notice the compact chromaffin ribbon sending digitations between the steroidogenic cords.

**Figure 4.** *Lacerta trilineata*. Mallory stain. Transverse section. × 80. Dorsal chromaffin ribbon has short digitations penetrating between the steroidogenic cords; the chromaffin islets (arrows) are numerous and small.

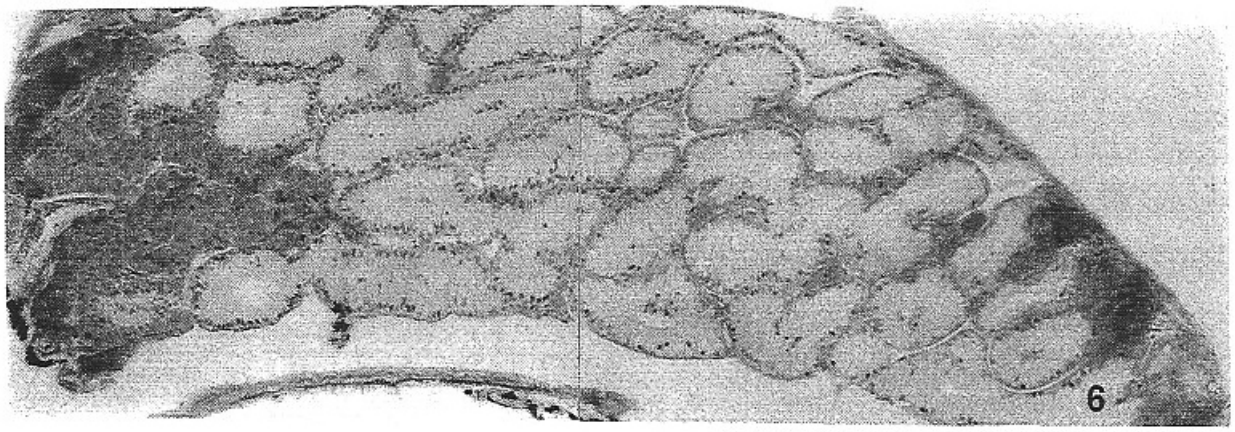


**Figure 6.** *Lacerta dugesii*. Mallory stain. Longitudinal section. × 80. In this species the chromaffin cells mostly concentrate at the two poles of the gland.

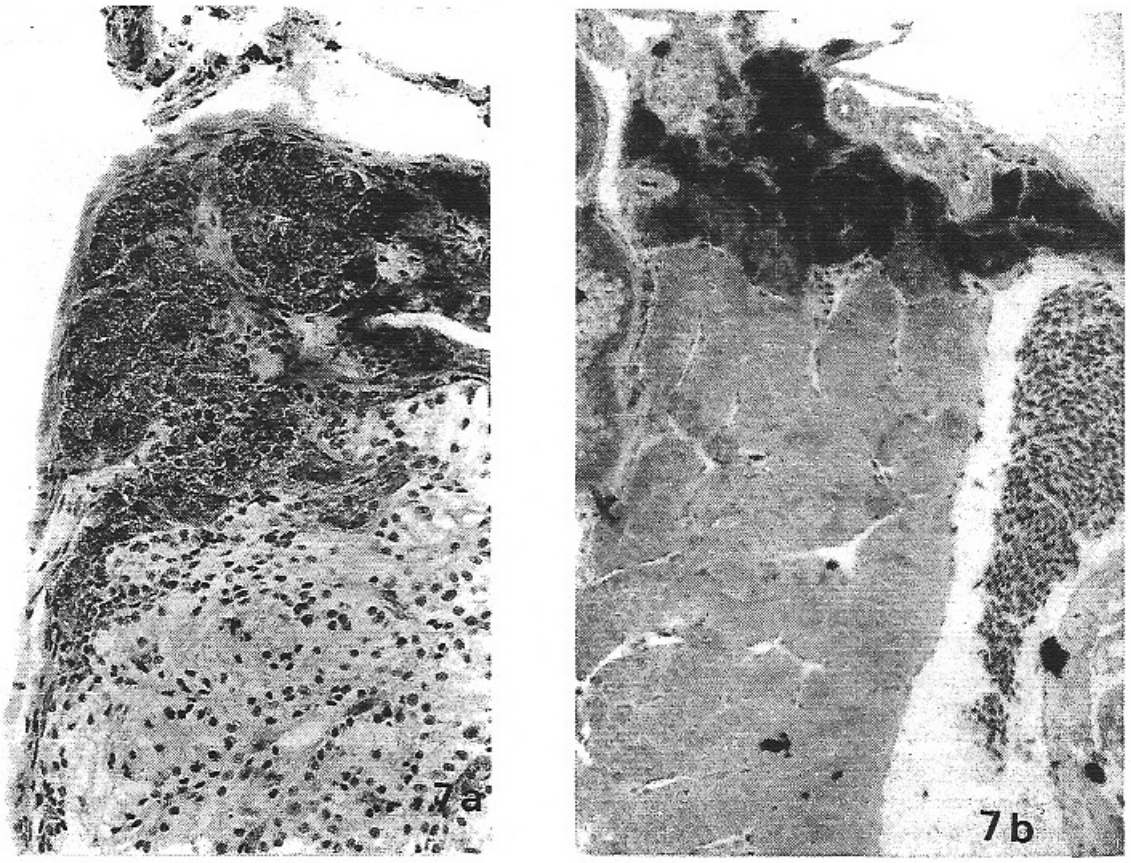


**Figure 7.** a) *Gallotia galloti*. Mallory stain. Longitudinal section. × 185. b) *Lacerta graeca*. Giemsa stain. Longitudinal section, × 125. In both species the chromaffin cells form a single mass located at the cephalic pole of the gland, completely separated from the interrenal cords.

There are different hypotheses on the position of *L. graeca*. Arnold (1973) ascribes this species to the group Lacerta part II, which according to this author is formed by more ancient lizards; in more recent papers *L. graeca* is successively considered: closely related to *L. oxycephala, L. horvathi* and *L. bedriagae* (Mayer and Tiedemann, 1982) in a very homogenous group; of unclear position but certainly on a lineage separate from *bedriagae* (Lutz and Mayer, 1985; Busack and Maxson, 1987); more closely related to the *Podarcis* group (Mayer and Lutz, 1989). Our studies show a very high NA/A cell ratio (8.6/1) in this species. This ratio, very similar to that of *G. galloti*, and the strong separation of the two tissues in the gland lead us to the conclusion that *L. graeca* is also an ancient species.

Until very recently, *L. dugesii*, as well as *P. pityusensis*, were ascribed to the genus *Podarcis* (Arnold, 1973); Arnold, instead, after the study of some bone structures and of the hemipenis of these species, is inclined to leave *P. pityusensis* in the genus *Podarcis* and to ascribe *L. dugesii* to the group "Lacerta part II". Mayer and Lutz (1989) in a study carried out with an immunological technique (micro-complement fixation) have found that *L. dugesii* has close relationship with *L. perspicillata*, but it seems to have diverged from the group *Podarcis* a fairly long time ago, longer than *L. graeca*. Our findings are in agreement with the findings of Arnold (1973) and of Mayer and Lutz (1989) for *L. dugesii*. *P. pityusensis* has a high NA/A cell ratio (5/1) and a distribution of the chromaffin cells typical of the genus *Lacerta* (with only NA cells in the dorsal ribbon); therefore, from this point of view, it seems more similar to this genus than to a species of the genus *Podarcis*, where the NA/A cell ratio is always very low (very close to 1/1). All the other species studied by us (*L. lepida, L. trilineata, L. schreiberi, L. viridis*) show a rather homogeneous distribution of the chromaffin cells inside the adrenal gland and also very similar NA/A cell ratios. The rather low value of the ratios indicates a fairly recent origin of all these species. These findings are supported by Arnold's conclusions. In fact, this author ascribes these species to the group "Lacerta part I" that he considers a recently evolved homogenous group.

## References

Arnold, E. N. (1973): Relationships of the palearctic lizards assigned to the genera *Lacerta, Algyroides* and *Psammodromus* (Reptilia: Lacertidae). Bull. Br. Mus. Nat. Hist. (Zool.). **25**: 291-366.

Böhme, W. (1971): Über das Stachelepithel lacertider Eidechsen und seine systematische Bedeutung. Z.f. Zool. Syst. Evolut. Forsch. **9**: 187-223.

Busack, L. D., Maxson, L. R. (1987): Molecular relationships among Iberian, Moroccan and South African lacertid lizards (Reptilia: Lacertidae). Amphibia-Reptilia **8**: 383-392.

Chester Jones, I. (1957a): Comparative aspects of adrenocortical-neurohypophyseal relationships. In: The Neurohypophysis. Heller, H., Ed., New York, Academic Press.

Chester Jones, I. (1957b): The adrenal cortex. Cambridge, Cambridge University Press.

Gabe, M., Martoja, M. (1961): Contribution à l'histologie de la glande surrénale des Squamata. Archs. Anat. Microsc. **50**: 1-34.

Gabe, M. (1970): The adrenal. In: Biology of Reptilia. Gans, C., Ed., New York, Academic Press.

Ghosh, A. (1977): Cytophysiology of the avian adrenal medulla. Int. Rev. Cytol. **49**: 253-284.

Laforgia, V., Varano, L. (1978): The influence of the interrenal steroidogenic tissue on the chromaffin cells

of the adrenal gland of *Lacerta s. sicula* Raf.: effects of ACTH administration during the winter. Cell and Mol. Biology **23**: 379-390.

Laforgia, V., Varano, L. (1982): Morphology and distribution of chromaffin cells in the adrenal gland of Cordylidae. A comparative study. J. Morph. **171**: 79-88.

Laforgia, V., Varano, L., Cavagnuolo, A., Putti, R. (1982): Ulteriori osservazioni sulla morfologia della ghiandola surrenale degli Squamati. I Geconidi e gli Iguanidi. Boll. Zool. **49** (suppl.): 101.

Laforgia, V., Varano, L., Cavagnuolo, A., Putti, R. (1983): La surrenale degli Agamidi: studio morfologico in alcune specie. Atti XXXIX Conv. Naz. Soc. It. Anatomia. 430-431.

Laforgia, V., Varano, L., Putti, R., Cavagnuolo, A., Capaldo, A. (1985): Morfologia della ghiandola surrenale in alcune specie della famiglia Lacertidae: uno studio comparativo. Atti XX Conv. Soc. It. Istoch., Milano.

Lanza, B., Cei, J. M. (1977): Immunological data on the taxonomy of some Italian lizards (Reptilia, Lacertidae). Mon. Zool. Ital. (N.S.) Firenze, **11**: 231-236.

Lanza, B., Cei, J. M., Crespo, E. G. (1977): Immunological investigations on the taxonomic status of some Mediterranean lizards (Reptilia, Lacertidae). Mon. Zool. Ital. (N.S.) Firenze. **11**: 211-221.

Lutz, D., Mayer, W. (1985): Albumin evolution and its phylogenetic and taxonomic implications in several Lacertid lizards. Amphibia-Reptilia **6**: 53-61.

Mayer, W., Lutz, D. (1989): Chemiosystematische Untersuchungen zur Phylogenese der Sammelgattung *Lacerta* (Reptilia: Lacertidae). Z. f. zool. Syst. Evolut. Forsch. (in press).

Mayer, W., Tiedemann, F. (1982): Chemotaxonomical investigations in the collective genus *Lacerta* (Lacertidae, Sauria) by means of protein electrophoresis. Amphibia-Reptilia **2**: 349-355.

Pearse, A. G. E. (1960): Histochemistry theoretical and applied. Boston, Little, Brown & Co.

Saint Girons, H. (1976): Comparative histology of the endocrine glands, nasal cavities and digestive tracts in anguinomorph lizards. In: Morphology and Biology of Reptiles, p. 205-216. A d'A. Bellairs and Cox, C. B., Ed., London, Academic Press.

Varano, L., Della Corte, F., Galgano, M. (1969): Brevi note sull 'ultrastruttura delle cellule a catecolamine di *Lacerta s.sicula* Raf. Atti Soc. Pel. sci. fis. mat. nat. **15**: 39-44.

Varano, L., Laforgia, V. (1976): Struttura e ultrastruttura dell'interrenale della lucertola (*Lacerta s. sicula* Raf.) in esemplari primaverili nomali e dopo somministrazione di ormoni estrogeni e di reserpina. Arch. Ital. Anat. Embriol. **81**: 245-262.

Varano, L., Laforgia, V. (1982): La morfologia della ghiandola surrenale degli Squamati: uno studio comparativo. Atti XXXVIII Conv. Soc. It. Anatomia. 407-408.

Wassermann, G. F., Tramezzani, J. H. (1961): Complete separation of adrenaline and noradrenaline secreting cells in a snake's adrenal gland. Acta Physiol. Latino-Am. **11**: 148-149.

Wassermann, G. F., Tramezzani, J. H. (1963): Separate distribution of adrenaline and noradrenaline secreting cells in the adrenal of snakes. Gen. Comp. Endocrinol. **3**: 480-489.

Wood, J. G. (1963): Identification of and observations on epinephrine and norepinephrine containing cells in the adrenal medulla. Am. J. Anat. **112**: 285-304.

Wright, A., Chester Jones, I. (1955): Chromaffin tissue in the lizard adrenal gland. Nature, Lond. **175**: 1001-1002.

Wright, A., Chester Jones, I. (1957): The adrenal gland in lizards and snakes. J. Endocr. **15**: 83-99.

*Received: May 19, 1989. Accepted: November 27, 1989*