BEAUFORTIA

SERIES OF MISCELLANEOUS PUBLICATIONS
INSTITUTE OF TAXONOMIC ZOOLOGY (ZOOLOGICAL MUSEUM)
UNIVERSITY OF AMSTERDAM

No. 280 Volume 21 December 28, 1973

A contribution to the intraspecific systematics of *Bufo bufo* (Linnaeus, 1758) (Amphibia)

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ABSTRACT

A biometrical analysis of preserved specimens of *Bufo bufo*, supplemented by a detailed examination of the shape of the skin warts, was performed in order to ascertain the taxonomic status of the form described as *B.b.spinosus*. Indications of a N.-S. clinal variation in the formation of wart thorns and in allometric relations of body elements were found. The validity of the form as a distinct subspecies seems accordingly very doubtful.

INTRODUCTION

Mertens & Wermuth (1960) mention as European subspecies of *Bufo bufo* (L.): *B.b. bufo* (L.), *B.b. spinosus* Daudin, *B.b. gredosicola* L. and *B.b. verrucosissimus* (Pallas). The latter two subspecies have a limited geographical range (Sierra de Gredos and Caucasus, respectively) and were not represented in the collections at hand, so that the investigation was limited to the two first-mentioned subspecies, the large geographical ranges of which are considered mutually exclusive: N. & Central Europe and the mediterranean Province, respectively. (However, Schinz, 1833, described *B. minutus* from Turin, which subspecies is considered by Mertens & Wermuth to be synonymous with *B.b. bufo*).

Originally several other forms were nomenclatorally distinguished. Daudin (1802a) discerned the "species" *B. vulgaris* and *B. cinereus*, from low-lying, moist environments and dry mountainous regions, respectively. Schinz (1833) described, apart from the above-mentioned *B. minutus*, the taxa *B. alpinus* and *B. palmarum*. These and other described and named forms — cf. also Bonaparte (1839) — are nowadays reduced to synonyms of the four above-mentioned subspecies, but point to the great variability within

Received: July 17, 1973

this species, especially as concerns the colour, the number and shape of warts, the form of the snout, etc. Koch (1872) discerned in the Unter-Main and Lahn districts four varieties : *typus, minor, medius* and *hybridus.* It is certainly justified to speak of a polymorphous species. (For comparison the great variability within the related species *B. fowleri* and *B. americanus* — cf. Blair, 1941 — may be mentioned). A critical appraisal of the many named "species" was already given by Bonaparte (1832—1841). He recognised "*B. palmarum*" from Sicily as a giant form of the same species as the one occurring in Rome, "*B. ferrugineus*" as a young, male specimen, etc. This author remarked that hundreds of figures would not be sufficient to show the many different forms and colours it (in casu *B. bufo* in Italy) adopts.

As to the currently still recognised subspecies *B.b. bufo* (L.) and *B.b. spinosus* Daudin the following remarks are relevant : these forms were initially not discerned by Daudin (1802a), but he inserted them in his second monograph published in the same year (Daudin, 1802b), from which I cite (:199—200): "Le crapaud épineux (*Bufo spinosus*) : Bosc vient de publier la description de cette espèce remarquable dans le Dictionaire d'histoire naturelle de Déterville : elle habite en France dans les pays montagneux, où il l'a observée plusieurs fois, et Latreille l'a aussi découverte aux environs de Brives et de Bordeaux. Il est vraisemblable que c'est à elle qu'il faut rapporter toutes les observations sur les crapauds monstrueux d'Europe, car elle a ordinairement quatre à cinq pouces de longueur, sur trois à quatre pouces de largeur. On ne la trouve jamais sur la terre, et les villageois croient qu'elle n'en sort jamais volontairement, et qu'on ne peut la découvrir q'en labourant la terre avec la bêche ou la charrue; c'est pourquoi je soupçonne qu'elle doit pondre ses œufs en terre dans des lieux humides auprès des sources souterraines. Ce crapaud est plus large et moins gros que le crapaud commun à pustules rousses, a le nez plus obtus, les pattes plus longues. Sa tête obtuse, aplatie, tuberculeuse et brune, a ses côtés plus pâles; le corps est brun en dessus, avec de grandes taches irrégulières plus pâles; le dessous est d'un gris blanc uniforme; il a ses pattes brunes en dessous, avec des taches plus pâles; les tubercules des côtés et du dessous antérieurs du corps, du dessus et du dessous des pattes, sont surmontés d'une épine obtuse, dure, de nature cornée, de couleur presque noire, et quelquefois divisée en deux ou en trois sur les côtés du cou".

In this description all properties are mentioned which have since been accepted as characteristic for the subspecies *B.b. spinosus.* But Sonnini & Latreille (1830) do not mention this taxon "*B. spinosus*". It is, moreover, noteworthy that the taxonomic features mentioned do not appear to be characteristic of the mediterranean province. Boulenger (1898) described specimens from China as follows : "warts sometimes with a horny point, that is often black, especially in old females". Mertens (1927: 47) remarked : "Obwohl die südwesteuropäischen Erdkröte sich nicht immer mit absoluter Sicherheit von der mitteleuropäischen unterscheiden läszt, erscheint es mir doch nicht unberechtigt sie als Lokalform von *Bufo bufo bufo* abzutrennen".

The cautious formulation of this remark may be stressed, as well as the neutral term "Lokalform" (cf. the term "form" in Mayr, 1947), although it is of course doubtful whether the author, in 1927, used the word in its present meaning. Leydig (1876: 22) stated: "Ich besitze ein etwa zweijähriges Exemplar, das bei Völs in Südtirol gefangen wurde und über und über rauch und stachelig im höchsten Grade ist. Solche Tiere gaben wohl Veranlassung zur Aufstellung des *Bufo spinosus* Daudin". The author continued with a description of the degree of development of the spinosity in 3 phases: 1) cuticular ridges, giving a "Zackenbildung" in the profile (cf. the types 2* and 4* of fig. 2), 2) larger, though still microscopic, spines and 3) big spines. The author continued (: 23): "Auch bei erwachsenen Tieren von *Bufo vulgaris* ist die Ausbildung der Hornhöcker eine individuell verschiedene". (He also stated, that the warts may suddenly appear on a completely smooth skin from one day to another). Schuster (1950: 25) stated: "Die Unterschiede zwischen den beiden Rassen der Erdkröte erscheinen bei der hohen Variabilität nicht gesichert". Hellmich (1956) found thorny warts in old female specimens of *spinosus* only. But Dürigen (1897: 469) described for Germany the frequent occurrence of spiny toads ("var. *spinosus*"), as well as specimens that are entirely without horny wart tips. Schreiber (1912: 212) gave the following description of the only subspecific form he discerned, viz. var. *maximus*, of which variety he considered *Bufo spinosus* Daudin and *B. palmarum* Cuvier (among others) as synonyms: "Verrucis praecipue temporum, gulae pedumque conice elevatis, mucronatis; tympano vix conspicuo; parotidibus turgidissimus". So this author omitted from the original description of Daudin the characters of the longer hind-legs and the blunter head, which features have proved to be the most problematic ones in the present investigation. The characters of the tympanum and the parotoid appear to be scarcely specific. The spinosity of the warts will be specially considered.

METHODS

A. Measurements: as the biometrical proportions in both sexes differ considerably, a reliable sex determination proved to be necessary. The sexing was based on the dimorphism mentioned below. (Kauri. 1954. pointed out the minute sexual dimorphism in *Rana esculenta* and disregarded it). In connection with an unequivocal determination of the sex, the supposedly strong allometric growth in juveniles and a change in proportions in specimens approaching maturity, only specimens with a body-length exceeding 40 mm were considered. (March, 1937, found in *Rana temporaria* a sudden change in the proportion of body-length : hind-leg in ♀♀ approaching maturity, with a body-length between 4.5 and 5 cm).

The error of 0.1 mm in the nonius measurements remained well within the tolerance put forward by Mayr, Linsley & Usinger (1953) of 1/20th of the range of variation.

The following measurements were taken, in accordance with Peters (1964):

1) body-length from snout-point to cloaca.

2) femur, measured from outside of knee to centre of cloaca, the femur being measured in a position perpendicular to the body-axis.

3) tibia, measured from knee to heel.

4) tarsus, measured outwardly from between the metatarsal tubercles up to and including the distal end of the tibia, in a position perpendicular to both the tibia and the foot.

5) foot, from the proximal end of the outward metatarsal tubercle to the end of the fourth toe.

For 2) up to and including 5) the right hind-leg was measured.

Following Schuster (1950) the forelegs, being too muscular with Salientia to be measured exactly, were disregarded.

6) length of head: not directly measurable from snoutpoint to occipital groove — cf. Kauri, 1959, re *Rana* —, as the latter is difficult to locate in *Bufo,* possibly because of the thicker skin. Koch (1872) measured the interorbital, the internostril and the eye-nostril distance in order to define the shape of the head. In preserved specimens the eyes are rather unsuitable for exact measurements, however. In the present paper the distance between the right corner of the mouth and the snoutpoint was measured, though any differences in this measure are relatively smaller than the actual differences in head length.

7) breadth of head: measured between the corners of the mouth, according to Goin & Netting, mentioned by Peters (1964). The index

$$\frac{\text{length of head}}{\text{breadth of head}} \times 100$$ defines the shape of the head.

Measurement of the diameter of the eyes and of the tympanum was relinquished because of their vague contours. Length and breadth of the parotoid were measured, however, and the form of the warts (according to fig. 2) was recorded. The colour and general aspect of the skin were also noted.

B. *Determination of sex:* visual secundary sex characteristics with a signal function appear to be indistinct. Mertens (1960) mentions the frequent "errors" that occur, males trying to copulate with another male. Differentiating characters useful in copulation are more or less conspicuous, however. Boulenger (1898) mentioned in this connection:

a) ♂♂ have longer hind-legs. This characteristic may be functional during copulation (fig. 1). The tarso-metatarsal joint reaches the tympanum or even the eye in ♂♂, but in ♀♀ the shoulder or the tympanum only. As the length of the hind-legs varies in different populations, only comparison within one population is possible. In ♀♀ from southern Europe the joint often reaches the eye. Stretching the leg of conserved specimens is often impossible, and the lateral bending of the spine or swollen flanks in museum specimens may cause additional difficulties in the determination of this characteristic. It is shown in the present paper, that the greater length of the hind-legs of ♂♂ is the result of the greater length of each of the 4 leg-parts, but that the difference is maximal in foot length + length of toe IV. When the parameter "length of the hind-leg" is defined as the reach of the heel-joint along the body, as is customary and applied in the present paper as well, this maximal differentiation escapes our notion!

b) In ♂♂ the webs comprise 2/3 of the length of the toes, in ♀♀ 1/2—2/3. This characteristic, which is also mentioned by Hellmich (1956), proved to be unreliable. Apart from the likelihood of seasonal fluctuation, no correlation between the relative dimensions of the webs in synchronically caught specimens and their sex could be established.

c) In ♀♀ the body is shorter. This is probably meant in relation to the breadth of the body. This measure was not ascertained, but in general the body of ♀♀ seems plumper, especially during egg-ripening, as was also stated by Dürigen (1897: 470).

d) In ♂♂ the skin is smoother, i.e. there are fewer warts and it seems, moreover, to be looser and flabbier. Within one population this often proves to be true, though the skin of ♂♂ is not thinner, if measured with a sliding-gauge.

e) The black to dark-brown horny crests on the upper and inner edge of the fingers of mature ♂♂ in spring (but visible until autumn) is of course the most reliable characteristic. These crests are functional during copulation (fig. 1). Remarkable is the fact, that ♀♀ have skin differentiations in corresponding places, viz., a smoother, yellowish, somewhat thickened zone, which may be compared with the finger-rims of ♀♀ of *Lechriodus melanopyga* and *Limnodynastes dorsalis* (compare the finger-rims in ♂♂ of *Crossodactylus gaudichaudii* and *Phyllobates nubicola*), and Hillenius (1962) suggested a function for these rims in ♀♀ of *Limnodynastes peronii*, namely, the beating of bubbles for the nest. Noble (1931) remarked in this connection (:121): "There are several possible ways by which a sex-linked or sex-limited character may lose this bondage to one sex and appear in both".

f) Greater body-length of ♀♀. This characteristic understandably has a comparative value only, unless one has an extremely large specimen in hand, which always proves to be female.

Koch (1872) mentioned the following additional characteristics:

g) In ♂♂ the inner metatarsal tubercle is often broader than the outward one. The former is better developed in the ♂♂. It was ascertained that it is pressed against the femur of the ♀ during copulation (fig. 1). This characteristic was found to hold true in 48 of the 67 ♂♂; and in 102 of the 161 ♀♀ the outward tubercle was broader than the inner one.

h) In ♂♂ the 3rd toe exceeds the length of the 1st toe by more than the length of the final phalanx (probably the 5th toe is meant). For practical reasons instead of this distance the total length of foot + 4th toe was measured. This measure proved to be significantly longer in ♂♂, and, as compared with the other parts of the leg, the sexual dimorphism was strongest in this part.

i) The red colour of the central parotoid-line and of the tops of the warts, occurring in juveniles, persists longer in ♀♀. On this characteristic (among

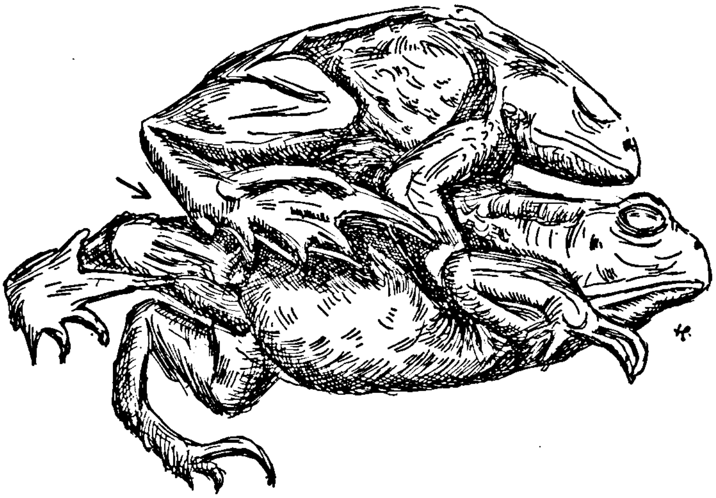

FIG. 1. Copulation of *Bufo bufo,* showing possible functional value of sexual dimorphism.

others) Koch (1872) based his variety "*minor*", which he described as a montane form. It was found that red wart-tops are most conspicuous in type 3 (fig. 2). This might be an indication of the occurrence of this type in drier environments, in agreement with the hypothesis worked out in the present paper, viz., that the development of the wart-types is connected with a stronger secretion of these skin-glands. It is remarkable, in this connection, that it is the most active part of the parotoid and of the warts which shows the red colour—turning yellow afterwards. Compare the microphotographs of ripe warts and parotoid-sections given by Funk Muhse (1909). Leydig (1876 : 58) remarked: "An jungen einjährigen Thieren von *Bufo vulgaris* ist die Spitze der Hautwarzen ebenfalls mit diesem roseroten Pigment geziert, selbst auf der Ohrdrüse sitzen Wärzchen von der gleichen Farbe". And furthermore: "Bei starker Einwirkung von Wärme und Sonnenschein werden die an sich schwach rötlichen Hauthöcker ganz lebhaft rot." This last remark concerned *Bufo viridis,* it is true, but it may also indicate a connection between colour and secretive action of the warts of *Bufo bufo.*

C. *Forms of warts discerned:* Fig. 2 shows the types discerned. The arrows suggest a supposed ontogenetic development. No such ontogenetic sequence could be ascertained in the present investigation and a climatic influence on the development is not apparent either, but an indication may be found in Funk Muhse (1909) who observed a continuous replacement of defunct glands in the warts by newly formed ones, showing all kinds of intermediate stages. The new gland has its own outlet on the same wart: "I have never seen small glands that were closely and definitely associated with the necks of large glands (ibid. : 354)". In this way the types 2a, 3a, 3b, 4a, 4b

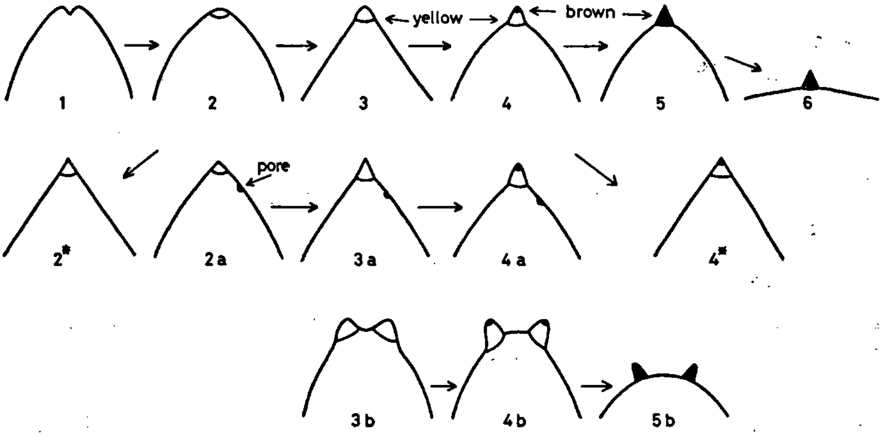

FIG. 2. Forms of warts discerned.

and 5b might be formed. The defunct gland collapses, and its outlet may become horny. This is sustained by the presence of new (?) outlets by the side of thorny wart-tops and by the occurrence of warts with more than one spine (the types 4a, 4b and 5b, respectively). The "most advanced" stages are generally found on the legs and the back. The predicate "spiny" was given to the types 4, 5 and 6.

The area of investigation was divided in 3 parts: I) The Netherlands north

TABLE 1. Distribution of spiny forms in the geographical areas.

Area	Sex	Total number of specimens	Number of spiny forms	do.in %	Number of specimens with type 5 or 6	do.in %
I)	♂	20	9	45	0	0
	♀	54	32	59	1	2
II)	♂	35	9	39	1	3
	♀	81	53	65	14	17
III)	♂	8	4	50	3	38
	♀	17	15	88	10	59
IV)	♂	1	0	0	0	0
	♀	5	2	40	0	0
V)	♂	3	3	100	1	33
		224				

of the river Waal, II) do. South of the river Waal + Belgium, northern France + Harz, III) Southern France + Spain + Italy, IV) Switzerland + southern Germany and V) the Balkan Peninsula. The distribution of spiny forms in these geographical areas is shown in table I. It is clear that the spinosity is increasing from N. to S. and that in all regions ♀ ♀ are more spiny than ♂ ♂. However, spinosity is by no means restricted to area III, where the subspecies "*spinosus*" is said to occur. The "75% rule" (Mayr, 1947: 107) is not fulfilled at all. This is in agreement with the above-mentioned data from different authors.

In order to ascertain whether the greater spinosity of the southern specimens was a result of their greater average bodylength, the wart-type per area was analysed per class of this parameter (table 2).

TABLE 2. Distribution of spiny forms per geographical area per class of body-length.

		bodylength in mm						
		40-50	51-60	61-70	71-80	81-90	91-100	101-110
area I								
spiny	♂	4	4	1	0	0		
	♀	9	7	8	6	2		
total number	♂	5	12	2	1	0		
	♀	16	12	16	7	3		
% spinosity	♂	80	33	50	0	0		
	♀	56	58	50	86	67		
	♂+♀	62	46	50	75	67		
area II								
spiny	♂	2	6	1	0	0	0	
	♀	5	17	15	14	1	1	
total number	♂	3	15	16	1	0	0	
	♀	11	20	21	26	1	2	
% spinosity	♂	67	40	6	0	0	0	
	♀	45	85	71	58	(100)	(50)	
	♂+♀	50	66	43	52	0	0	
area III								
spiny	♂	2	1	0	1	0	1	0
	♀	0	1	0	3	3	5	3
total number	♂	2	1	0	2	2	1	0
	♀	0	1	1	3	3	6	3
% spinosity	♂	(100)	(100)	0	50	0	100	0
	♀	0	(100)	0	100	100	83	100
	♂+♀	(100)	(100)	0	80	60	86	100

The greater spinosity is, accordingly, about uniformly divided among the classes of body-length, and may be looked upon as a habitat modification, possibly in such a way that a drier environment causes a quicker exhaustion of the glands, which then form thorns. The diversity in habitats within each district might explain the variability found. The alternative possibility of a genetic foundation is less probable in the light of the distribution pattern of the characteristic in question, but can only be ruled out by means of geographical translocation of specimens or by conditional breeding. The delimitation of a subspecies "*spinosus*" appears to be premature in any event, however. As to the tendency towards a higher percentage of spinosity towards the south the term "cline" might be appropriate (Huxley, 1939), which term is neutral in respect of the possible environmental or heriditary cause.

RESULTS

a) *Body-length*

The figures given in the preceding chapter show that

1) in all areas the ♀ ♀ are on the average longer, and their maximum length is also greater.

2) both ♂ ♂ and ♀ ♀ are on the average longer in more southwardly situated areas and the maximum length is also greater. This tendency is already quite manifest in district II, and not restricted to the supposed range of the subspecies „*spinosus*". It is possible that collectors, when travelling abroad, collect particularly the bigger specimens, or that in warmer, drier environments the smaller specimens are more inclined to take shelter owing to their relatively greater body surface area, but the greater maximum length cannot be explained in either way. If this difference in length would have a genetical basis, one might think of a loss of alleles from the centre of origin in the south, towards the more northern areas, colonised after the glacial ages (cf. Hillenius, 1959). Mertens (1953), however, assumed that some Amphibians survived the glacial ages in the north on nunataks, which populations, moreover, could have been enriched by several immigration waves from the south. This would result in a higher genetic variability. The alternative is a climatically induced modification. Kauri (1959) proved experimentally a growth-acceleration at higher temperature in *Rana esculenta*. Balcells (1955) observed copulations of *Bufo bufo* in Spain as early as January. The longer growing-season together with an accelerated growth might well explain the difference in body-length, which might, moreover, have an adaptive function in view of the smaller relative body surface area (Schuster, 1950).

b) *The relative length of leg-parts*

In all regions and for both sexes the relation between the length of each leg-part and the body-length can be expressed as a linear one with the functions shown in tabel 3, (in which y = leg-part, x = body-length). In addition the functional relations for all ♂♂ and all ♀♀ were computed (table 4). The results were tested for significance in a t-test (table 5), in which the values remaining below the 5% significance level are underlined.

TABLE 3. Relations between lengths of leg-parts and body-lengths per geographical area and per sex.

		function	standard deviation	
		y = ax+b	a	b
femur	♂ ar.I)	y = 0.44x − 2.53	0.07	3.61
"	♀ "	y = 0.36x + 0.44	0.02	1.08
"	♂ ar.II)	y = 0.55x − 7.81	0.03	2.06
"	♀ "	y = 0.41x − 1.85	0.01	8.69
"	♂ ar.III)	y = 0.43x − 0.71	0.03	2.10
"	♀ "	y = 0.41x − 2.11	0.05	4.02
tibia	♂ ar.I)	y = 0.34x + 1.73	0.04	2.44
"	♀ "	y = 0.33x + 0.06	0.01	6.84
"	♂ ar.II)	y = 0.47x − 5.81	0.04	2.53
"	♀ "	y = 0.35x − 0.97	0.01	9.13
"	♂ ar.III)	y = 0.42x − 1.74	0.02	1.27
"	♀ "	y = 0.38x − 1.22	0.03	2.60
tarsus	♂ ar.I)	y = 0.27x − 0.49	0.03	1.66
"	♀ "	y = 0.22x + 0.80	0.02	0.91
"	♂ ar.II)	y = 0.33x − 3.90	0.02	1.34
"	♀ "	y = 0.26x − 0.81	0.01	0.79
"	♂ ar.III)	y = 0.29x − 0.49	0.01	1.00
"	♀ "	y = 0.26x + 0.60	0.02	2.13
foot +	♂ ar.I)	y = 0.52x − 2.05	0.06	3.47
toe IV	♀ "	y = 0.41x + 0.19	0.02	0.99
"	♂ ar.II)	y = 0.67x −11.29	0.05	3.13
"	♀ "	y = 0.43x − 1.38	0.01	0.80
"	♂ ar.III)	y = 0.49x − 1.43	0.03	2.10
"	♀ "	y = 0.42x + 0.79	0.04	3.49

Conclusions

1) From the standard deviations in table 3 it follows that especially the coefficients *a* can be estimated with sufficient exactitude, which points to an isometric growth of each leg-part in respect of the body-length.

2) In each area and for each leg-part the estimated value of *a* is smaller in the case of ♀♀. This suggests that the shorter legs of the ♀♀ result from a relatively slower growth of each of the leg-parts separately.

3) The values of Δa in table 4 show a tendency towards a maximum sexual differentiation in the measurements of foot + toe IV.

TABLE 4. Overall relations between lengths of leg-parts and body-lengths per sex.

femur	♂♂ $y = 0.47x - 3.74$ ♀♀ $y = 0.41x - 2.03$	$\Delta a = 0.06$
tibia	♂♂ $y = 0.43x - 3.41$ ♀♀ $y = 0.38x - 2.55$	$\Delta a = 0.05$
tarsus	♂♂ $y = 0.32x - 2.98$ ♀♀ $y = 0.28x - 1.99$	$\Delta a = 0.04$
foot + toe IV	♂♂ $y = 0.50x - 1.48$ ♀♀ $y = 0.43x - 1.52$	$\Delta a = 0.07$

TABLE 5. Results of a significance test for the relations between lengths of leg-parts and body-lengths per sex.

area	tail probability in %			
	femur	tibia	tarsus	foot + toe IV
I (♂+♀)	18.2	84.2	31.2	3.6
II "	0.2	1.4	6.6	0.02
III "	82	33.4	34.8	21.8
all ♂♂ as compared to all ♀♀	0.8	1.4	3.6	0.2

4) From the differences in the coefficients *a* among the leg-parts of one sex in one area it is clear that the leg-parts show a mutual allometric growth-rate. In each area and in both sexes the growth-rate is greater for

foot + toe IV, followed by femur, tibia and tarsus, respectively. This means that during the ontogenetic development the most distal part exhibits the fastest growth, followed by the most proximal part, and the differential growth in ♂♂ is strongest in those leg-parts that exhibit a maximum growth-rate in both sexes. In other words, the importance of the leg-parts in sexual dimorphism shows the same sequence as their mutual growth-rate. This correspondence does not only prove to be a matter of sequence, but it also holds good quantitatively. By comparing the coefficients *a* (which can be considered as a measure for growth-rate) of the above mentioned functional relations one obtains:

for ♂♂: femur : tibia : tarsus : (foot + toe IV) = 47 : 43 : 32 : 50 and
for ♀♀: femur : tibia : tarsus : (foot + toe IV) = 41 : 38 : 28 : 43 ∽
47 : 43 : 32 : 49.

The resemblance in relative growth-rates in both sexes is striking and confirms the "law" of Huxley (1932), viz., that allometric growth in the case of genetic similarity proceeds according to a fixed relation, though the absolute rate of growth may differ on account of age, environment etc., or, as in this case, of sexual dimorphism.

A similar calculation for each district renders table 6.

TABLE 6. Relations between growth-rates of leg-parts per geographical area per sex.

area	sex	femur : tibia : tarsus : foot+toe IV
I	♂	0.44:0.34:0.27:0.52 ∽ 0.36:0.28:0.22:0.42
	♀	0.36:0.33:0.22:0.41
II	♂	0.55:0.47:0.33:0.67 ∽ 0.37:0.31:0.22:0.45
	♀	0.41:0.35:0.26:0.43 ∽ 0.35:0.30:0.22:0.36
III	♂	0.43:0.42:0.29:0.49 ∽ 0.33:0.32:0.22:0.37
	♀	0.41:0.38:0.26:0.42 ∽ 0.35:0.32:0.22:0.36

Apart from the femur-length of the ♂♂ in area III (which was the group with the smallest reliability, comprising only 8 specimens) these relations are strikingly similar, which can be considered an additional indication of genetical similarity throughout the considered area, including that of the alleged subspecies "spinosus".

The allometrically negative centre of growth in the hind-legs appears to be situated in the tarsus, with a progressive gradient in growth rate both in the distal and the proximal direction. It is to be expected (Huxley, 1932) that this gradient is also present within each leg-part.

5) The differences between the areas and between the sexes was sub-

TABLE 7. Results of a significance test for the relations between lengths of leg-parts and body-lengths per geographical area per sex.

Null-hypothesis	significance level	F (theoretic)	F calculated			
			femur	tibia	tarsus	foot + toe IV
a) no difference between a combined treatment of all ♂♂ in stead of a separation according to areas	5 % 2½% 1 % ½%	2.55 3.03 3.61 4.19	2.58	2.41	4.62	4.01
b) as a) for ♀♀	5 % 2½% 0.1%	2.44 2.88 4.91	2.83	8.42	9.25	2.64
c) as a) for ♂♂ + ♀♀	½% 0.1	3.84 4.86	4.40	9.09	13.1	0.944
d) no difference between a combined treatment of ♂♂ and ♀♀ of area I in stead of a separate one	0.1%	7.70	7.30	28.6	8.07	61.3 t
e) as d) for area II	½%	5.59	42.7 t	34.8 t	19.7	148 t
f) as d) for area III	10 % 5 % 1 % ½%	2.57 3.47 5.78 6.89	2.31	6.12	2.74	5.41
g) as d) for all areas together	½%	5.49	42.7 t	63.5 t	30.5 t	167 t

mitted to an F-test. The results are shown in table 7, in which the calculated values of F as well as the theoretically to be expected ones are shown for different significance levels. Cases of high significance are underlined. Those already proved to be significant in the t-test are marked "t".

It is clear that both the sexual dimorphism and the differences between the areas are significant at a verly low threshold. For foot + toe IV the value under c) seems to be contradictory to those under a) and b). The differences per area for ♂ ♂ and ♀ ♀ appear to compensate each other when the sexes are combined.

On the basis of the functional relations one can conclude accordingly, that ♂ ♂ show a maximum growth-rate in each legpart in area II (they exhibit higher values for *a*, see table 3). This is in accordance with Schuster (1950 :13): "Die einzelnen Beinteile sind sämtlich in wärmeren Gebieten relativ länger als in den nördlichen Arealen; nur die spanischen Tiere bilden eine Ausnahme. Die Unterschiede zwischen den mittleren Indices sind jedoch so gering, dasz sie hiernach nicht als belegt angesehen dürfen". In the present investigation the differences appeared to be significant, however. The change in this trend in the Spanish specimens mentioned by Schuster is analogous with the results concerning area III, where, with the exception of the tibia in

♀ ♀, the legparts appear to be shorter. Kauri (1959) found the same phenomenon in *Rana esculenta* (as far as identical with *Rana ridibunda*). It should be borne in mind that according to the original description of "*Bufo spinosus*" (Daudin, 1802b) the legs should be longer!

c) *The head-index*

The results of rank-correlation on tests for body-length versus head-index are given in table 8.

Table 8. Results of rank-correlation tests for body-length versus head-index.

	area I	area II	area III
Kendall	0.01	0.04	0.28
Spearman	0.02	0.05	0.11

Correlation between the 2 parameters seems evident, even in area III with 25 observations only. But the deviations of the coefficients are rather great (table 9); y = head-index, x = body-length).

Table 9. Relations between body-lengths and head-indices per geographical area.

area	sex	y = ax + b	standard deviation	
			a	b
I	♂	y = -0.12x+85.78	0.19	10.67
I	♀	y = -0.18x+87.93	0.04	2.37
II	♂	y = -0.06x+81.34	0.11	6.70
II	♀	y = -0.09x+80.75	0.03	2.00
III	♂	y = -0.16x+91.51	0.50	3.57
III	♀	y = -0.10x+85.39	0.09	8.01

The differences between the areas were again submitted to an F-test (table 10; the meaning of a-g as in table 7, significant cases underlined).

Summarising the results it can be seen that:

1) The head-index changes isometrically in relation to body-length in a negative way, i.e., the head grows blunter during development.

2) In each area the heads of ♀ ♀ are blunter. This can be considered an additional aid for sex-determination.

Table 10. Results of a significance test for the relations between body-lengths and head-indices per geographical area.

Null-hypothesis	significance level	F (theoretic)	F (calculated)
a	5 %	2.55	1.77
b	0.1%	4.91	6.76
c	0.1%	4.86	8.26
d	0.1%	7.70	0.515
e	0.5%	5.59	6.46
f	10 %	2.57	0.451
g	0.5%	5.49	5.93

3) From area I to II the head turns blunter, and from II to III becomes more pointed again, which reminds of the situation concerning the leg-parts and is again contradictory to Daudin (1802b).

d) *Parotoid-length and -breadth*

Both parameters show a linear relation with body-length, but neither between the sexes nor among the areas are the differences significant. This is in agreement with Blair (1941), who in 5000 American toads of 4 different species did not find significant differences for these characteristics and did not find a cline either. It also agrees with Funk Muhse (1909) who stated (:357): "Their (viz., of the parotoids) position and relative size are constant for the species. All the other warts are variable in size and location".

e) *Colour*

This variable characteristic did not show any regularity and was moreover changed and faded in preserved specimens. Contrasts between light and dark appear to be more pronounced in the southern regions, through the occurrence of conspicuous yellow pigments. (The parotoid often shows contrast colouring: light on the upper, dark on the lower half; possibly this has a warning function).

f) *The sexes*

The ♀ ♀ outnumber the ♂ ♂ in the collections (157 against 67). This is contrary to the results of Moore (1954), who counted a relation of 2 : 1 in favour of the ♂ ♂. However, of specimens run over on roads only 30—38% was ♂, which the author explained by their greater velocity. This might also be the explanation of the numbers of caught male and female specimens. A greater number of ♂ ♂ was also reported by Jungfer (1954), who mentioned a relation of 18.5 ♀ ♀ per 100 ♂ ♂.

SUMMARY AND CONCLUSIONS

1) Published records of *Bufo bufo spinosus* are vague or contradictory.

2) a reliable sex-determination based on secondary dimorphism is possible only by means of the horn-crests on the fingers, but there are a number of additional characteristics, which, especially in combination, may lead to a rather certain determination. The lower head-index of ♀♀ found in the present study is such an additional indication.

3) there are indications of an ontogenetic formation of wart-thorns from active warts, possibly in relation with climatic factors.

4) the percentage of thorny warts rises from north to south in a continuous way, but specimens with thorny warts occur in the most northernly area examined, and individuals with non-thorny warts occur in the south.

5) both the mean and the maximum body-length increase from north to south.

6) the leg-parts show an isometric growth in respect of the body-length.

7) the allometrically growing leg-parts show between them a fixed relation of growth-rates in both sexes and in each area studied. So the ontogenetic change in leg-form proves to be uniform, although the rate in which it is accomplished in relation to body growth differs.

8) the allometrical negative centre of growth (in relation to the other leg-parts) lies in the tarsus.

9) the gradient of growth-rate of the legs and of the head-index shows a bend in the mediterranean province in a negative and a positive sense, respectively.

10) the growth of the parotoid-length and -breadth is isometrical in respect of the body-length, but shows no differentiation according to sex or region.

Nos. 1), 3), 4), 5) and 7) do not plead in favour of a separate sub-species for the populations of the mediterranean province (viz. *Bufo bufo spinosus*). No. 9) may point to a genetic difference but is at variance with the original description of the form "*spinosus*".

ACKNOWLEDGEMENTS

The investigation was performed in the Herpetological Department of the Institute for Taxonomical Zoology of the University of Amsterdam, under supervision of Dr. D. Hillenius. Available was the material of *Bufo bufo* (L.) of this Institute as well as that of the "Rijksmuseum van Natuurlijke Historie" at Leyden, to which Institutes the author is greatly indebted.

For advise concerning the mathematical data processing he wishes to thank Dr. W. Molenaar, formerly of the "Stichting Mathematisch Centrum" at Amsterdam. Professor Dr. A. D. J. Meeuse corrected the English text.

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