twice by resuspension in PBS and centrifugation. Coupling to the iron-dextran was performed by suspending the cells in 1 ml of PBS and adding 1 ml of Imferon (Fisons Ltd, Loughborough, England) consisting of 50 mg iron as a complex of ferric hydroxide with dextrans of average mol. wt between 5000 and 7500. The mixture was held for 60 min at 22 °C. The reaction was stopped by dilution, and the mycoplasmas were centrifuged and washed 3 times with PBS. In control experiments, the binding sites of Con A were blocked by the specific inhibitor[8], α-methyl-D-glucopyranoside, final concentration 0.2 M. Thin sections of the cytochemically treated mycoplasmas were examined in the electron microscope[4] without conventional staining by uranyl acetate and lead citrate[6].

Results. After cytochemical treatment, the non-contrasted *Mycoplasma neurolyticum* (figure A) and *Mycoplasma gallisepticum* (figure B) cells showed a dense and homogeneous layer of discrete, electron-dense iron-dextran particles covering the entire membrane surface. Labelling of *M. gallisepticum* was obviously independent of the terminal bleb structure[2]. The cytochemical reaction was completely inhibited by the specific Con A-inhibitor, α-methyl-D-glucopyranoside.

Discussion. High electron-microscopic magnification is necessary for visualization of the electron-dense iron-dextran particles bound to the free valences of Con A which, in a first step, had been bound to mycoplasma surface carbohydrate structures. Due to the small size of the marker molecules, the iron-dextran technique presumably allows a more precise and perhaps stoichiometric demonstration of cellular carbohydrates than can be obtained by labelling with the large Con A-ferritin molecules[5]. On mycoplasma membranes, we regularly observed a lower density of marker molecules than could be detected on mammalian plasma membranes[6,7]. This result is fully consistent with the data of our agglutination experiments with lectins[3].

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Two new polyploid *Xenopus* species from western Uganda[1]

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Summary. 2 new species of the anuran genus *Xenopus* have been found in western Uganda: *X. ruwenzoriensis sp.n.* with the hexaploid chromosome number of 108 in the Semliki Valley, west of the Ruwenzori, and *X. species nova* with the tetraploid chromosome number of 72 in and near lake Bunyoni.

In May 1972 we had the occasion to collect frogs of the genus *Xenopus* (Pipidae, Anura) in the western part of Uganda. 2 of the 4 types encountered displayed the unusually high chromosome numbers of 2n = 75 and 2n = 108, which contrast with the 2n = 36 of most other *Xenopus* species[2]. Both types breed true, giving rise to fertile offspring of both sexes. Moreover, they are distinct on the basis of morphological and biochemical characteristics. Thus, these 2 forms must be considered as taxa in their own right.

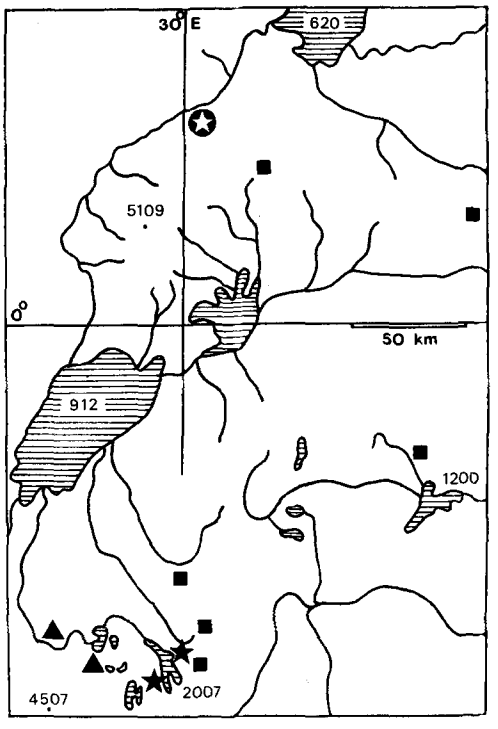
Xenopus ruwenzoriensis sp.n. (table, figures 1 and 2). From the Rain forest near Bundibugyo in the Semliki Valley at the foot of the Ruwenzori Mountain (1 °N, 30 °E; altitude 700 m), natives brought us 13 adult *Xenopus* (2♂ and 11♀) of a type similar to *X. fraseri*. 2 of the females differed from the others in their somewhat reddish dorsal colour and some minor morphological characteristics; they possessed 2n = 36 chromosomes and are most likely representatives of *X. fraseri* Boulanger. Attempts to cross these 2 females with males of *X. fraseri* and *X. ruwenzoriensis* were not successful.

The other specimens gave fertile offspring of normal sex ratio, which had, like their parents, a karyotype of 2n = 108 chromosomes. Correspondingly, the DNA content of erythrocytes is 2.6 times that of *X. laevis*[3]. During meiosis, chromosomes generally pair to form bivalents both in oocytes (Müller, unpublished results) and in spermatocytes[2]. These observations show that our small sample belongs to a true-breeding population of hexaploid *Xenopus*; we propose the name *Xenopus ruwenzoriensis sp.n.*.

X. ruwenzoriensis resembles strikingly *X. fraseri*. As in the latter, the prehallux is armed with a claw, tentacle length is at least half the diameter of the smallish eyes. The ventral body surface is whitish grey with few dark spots, while the ventral face of the thigh may be densely spotted on a yellowish background. The back is grey, often with a slightly darker pattern. A dark grey-brown transverse band between or behind the eyes can often be observed; it is most clearly visible in juvenile specimens. As compared to *X. fraseri*, *X. ruwenzoriensis* reaches greater maximal length in both sexes (table). The snout is relatively shorter and the mean number of lateral line organs around the eye is higher.

Xenopus sp. nova. 2 samples of clawed frogs were found around Lake Bunyoni: 3 ♀ and 4 ♂ were trapped by a native fisherman on the eastern shore of Lake Bunyoni near Kyabahinga (altidue 2007 m), the other sample of 28 ♀ and 45 ♂, all juvenile, were taken from an artificial fishpond (altitude 2130 m) near Kashasha (figure 1). The pond contained many hundreds of *Xenopus* tadpoles and newly metamorphosed froglets. Kashasha lies in a valley to the west of Lake Bunyoni, with which it is connected.

The adult *Xenopus sp.n.* (table) is a medium-sized frog without a claw on the prominent prehallux. The dorsal surface is immaculate, of uniformly olive to dull chocolate brown colour. The ventral surface of the belly and thigh is of a striking yellow ochre or dep orange colour, sometimes with small dark pigment spots. However, many specimens are also ventrally unspotted. The eyes are relatively small and far apart, the subocular tentacles are short and covered



with some melanophores. The large head and the heavily muscled hind legs give the impression of a stoutly built, short-legged *Xenopus*. During metamorphosis, the dorsal colour develops without any sign of distinct spots, yet a faint occipital transverse band may occur as in *X. fraseri*, *X. vestitus* and *X. ruwenzoriensis*.

On the ventral surface, the first dark spots may appear several weeks after metamorphosis. Some larval characteristics, i.e. the appearance of first melanophores on several body parts, and their pattern of distribution, are listed in the table (Vigny[4]). The chromosome number of 2n = 72, listed erroneously under *X. bunyoniensis* by Tymowska and Fischberg[2], and the DNA content of erythrocytes[3] is double that of *X. laevis*. Therefore, *Xenopus sp.n.* has to be considered as a tetraploid species, even though meiosis proceeds prevalently with bivalents as in the hexaploid *X. ruwenzoriensis*.

Xenopus sp.n. resembles no other *Xenopus* species and is very distinct from *X. laevis victorianus* Ahl and *X. laevis bunyoniensis* Loveridge, both of which occur in the same region. But its distinction from the newly described *X. vestitus*[5-7], which was found 10 km westward only to Lake

Fig. 1. Records of *Xenopus* species in western Uganda, spring 1972. ✪, *X. ruwenzoriensis*, ★, *X. species nova*, ■, *X. laevis victorianus*, ▲, *X. vestitus*[6]. Numbers indicate altitudes in meters above sea level.

Some descriptive and morphometric characters of *X. ruwenzoriensis* and *X. species nova*, compared with *X. fraseri* and *X. vestitus* respectively. Our sample of *X. fraseri* originated from the Cameroons

Character	Reference	Species *X. fraseri*	*X. ruwenzoriensis*	*X. vestitus*	*X. spec. n.*
Diploid chromosome number	2	36	108	72	72
DNA content in pg (erythrocytes)	3	6.4	16.3	12.8	12.6
Adult:					
Body length, maximal, in mm ♀	4	44 (9)*	57 (5)*	55 (83)*	55 (31)*
Body length, maximal, in mm ♂	4	35 (3)*	43 (2)*	48 (44)*	45 (49)*
Indices (for 5 ♀♀, in percent of body length):					
Eye diameter		4.6±0.4	4.9±0.1	3.8±0.3	3.7±0.3
Distance between the eye centers		17.2±1.4	17.7±1.3	14.5±1.2	17.3±1.3
Femur length		36.8±0.7	40.0±1.5	34.5±3.5	36.2±2.4
Tibia length		37.8±0.9	36.6±1.4	33.9±1.3	35.2±2.4
Foot incl. 5th toe, length		48.3±2.1	49.6±1.8	46.1±3.6	50.3±2.7
Lower fore limb incl. 1st finger		31.3±1.6	33.1±1.8	27.0±1.7	31.4±2.2
Number of lateral line plaques (same 5 ♀♀ as above)					
Around the eye $\frac{(L+H)}{2}$		8.2±1.0	11.0±1.2	10.6±1.6	11.1±1.7
On the lower jaw (L+R)		11.2±0.8	9.2±0.8	15.0 ±3.0	15.0±1.0
Dorsal between eye and cloaca $\frac{(L+R)}{2}$		19.0±0.3	19.0±1.2	23.0±2.5	22.0±1.9
Claw on prehallux		+	+	−	−
Egg diameter in mm	4	0.9−1.2	1.2−1.5	1.0−1.2	1.1−1.4
Larva: Stages[13] at which melanophores first appear on or over:					
Head, dorsally	4	35/37	34	35/36	34/35
Head, ventrally	4	47/48	46/47	46	54/55
Intestine	4	39	34	35/36	37
Proctodeum	4	45	45/46	45/46	48
Tail	4	39	37	38	38
Ventral fin	4	50	48	48	50
Melanophores distribution at stage 60					
Head ventral, uniform (u) or V-shaped (v)	4	u	u	u	v
Pigment-free zone distal of anus	4	−	−	−	+

*The number of individuals on which measurements are based is indicated in parentheses when applicable.

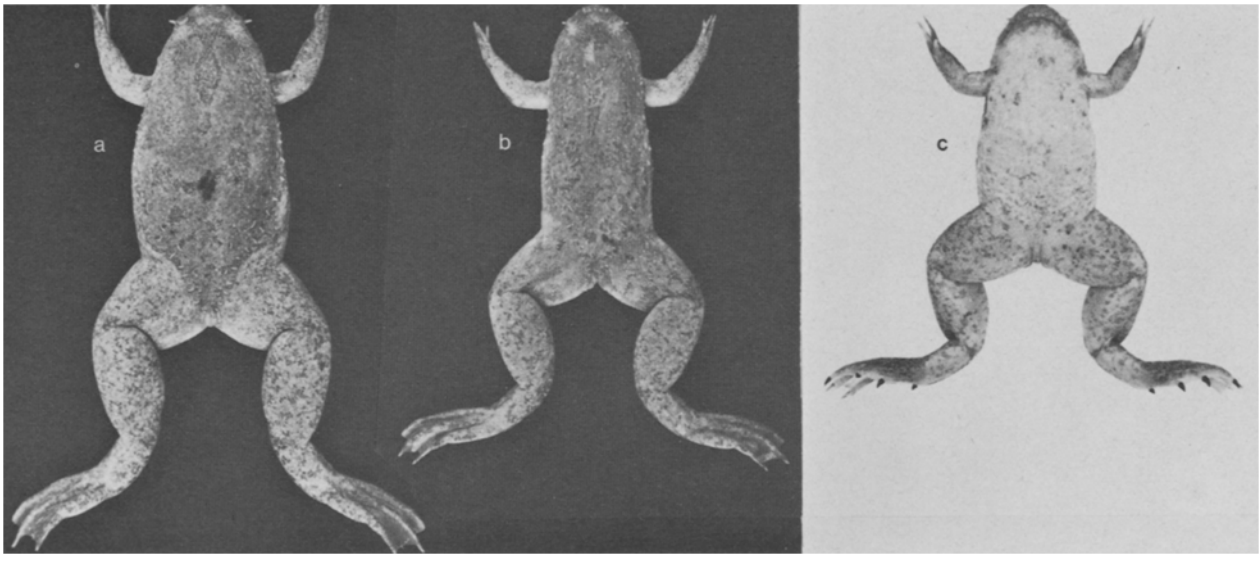


Fig. 2. a–c. *X.ruwenzoriensis. a* ♀ dorsal, *b* ♂ dorsal and *c* another ♀ ventral; ¾ natural size.

Bunyoni, is more crucial, especially as *X.vestitus* also turned out to be a tetraploid with a very similar karyotype[8]. Nevertheless, *X.vestitus* with its bronzy golden back, its light hood, and a dark transverse band across the neck, possesses a typical colour pattern not present in *Xenopus sp.n.*.

Furthermore, there are important differences between the 2 species with respect to electrophoretic mobility of hemoglobins (A.V. Muir and Z. Dobrowski, unpublished results) and lactate dehydrogenase iso-enzymes (by E. von Wyl and M.C. Chavane de Flacelière, unpublished results). Finally, hybrids between *X.sp.n.* and *X.vestitus,* produced in our laboratory, show a high number of univalents in oocytes[9], thus demonstrating a substantial degree of genetic divergence. All these observations indicate that a gene flow between these 2 species is unlikely or at least very limited. On the other hand, *Xenopus sp.n.* may be similar to specimens collected by de Witte[10] in the region of the Mokoto lakes in Zaïre and referred to by Tinsley[7]. A definitive name for the tetraploid *Xenopus sp.n.* cannot be proposed before a detailed comparison of these 2 collections has been worked out.

In a survey of *Xenopus* species of the region concerned, Tinsley[6] suggested that *X.vestitus* replaced *X.laevis bunyoniensis,* which was the only species recorded in Lakes Mutanda and Mulehe some 40 years ago. The sudden appearance of *X.sp.n.* at Lake Bunyoni, from which large samples, collected 40 years ago[10], contained *X.l.bunyoniensis* exclusively[7], suggests for this lake also a similar replacement of species.

So far, the known distribution of *X.sp.n.* is limited to the environs of Lake Bunyoni, de Witte's specimens mentioned above not considered. Lake Bunyoni is connected westwards with Lake Mutanda which today is occupied by *X.vestitus*[6]. To the east of Lake Bunyoni, only *X.laevis victorianus* is known to occur. Of this subspecies we found in fishponds near Kyanamira (altitude 1800 m, 10 km east of Lake Bunyoni) 4 ♀, 3 ♂, near Kitanga (altitude 1500 m) 46 ♀, 24 ♂ and near Kisiizi (altitude 1800 m) 13 ♀, 5 ♂.

The recent discovery of 3 new *Xenopus* species, *X.vestitus, X.ruwenzoriensis,* and *X.sp.n.,* all of which are polyploid and confined to a relatively restricted region of Central African highlands, may indicate a modern evolutionary trend in this genus. Compared with the number of diploid species, polyploid tetrapods are very rare indeed, and most of them show an aberrant mode of reproduction. Polyploidisation is therefore not considered to be a common mechanism of evolution in this class of animals. Nevertheless, the genus *Xenopus* with DNA contents ranging from 0.56 to 2.6 relative to *X.l.laevis*[3] can be taken as an example that polyploidisation may be of major importance in the evolution of some vertebrate groups. The fact that all of the polyploid *Xenopus* species discovered so far live in 1 limited area, a mountainous region on the border between forest and savanna, strongly suggests a close systematical relationship between the 3 types, although this is not evident from their actual phenotypes. On the other hand, the particular environment may have favoured a certain mechanism of speciation which repeatedly and independently could lead to the establishment of novel taxa. Hybridization seems to be the obvious mechanism, since it is known that hybrid *Xenopus* females often generate diploid gametes[11,12]. We suggest that different species with leaky reproductive isolation were brought together by drastic environmental changes. Such changes have indeed occurred several times during pleistocenic pluvials and interpluvials, and recently man's activity interfered with ecological conditions in this region.

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