# Cytotaxonomy of the myobatrachid frogs of the genus *Limnodynastes*[1]

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*Summary.* 4 of the 5 species of *Limnodynastes* of the *peroni* group show 2n = 24. Only *salmini* has 2n = 22, thus resembling the species of *Platyplectron* (the *dorsalis* and *ornatus* complexes) which all show 22 chromosomes. This 2nd group also includes species *(ornatus)* with very low DNA amounts. From a karyological point of view, the species of the *Platyplectron* (plus eventually *salmini*) group appear more differentiated than the species of the *peroni* group, from which they have probably arisen.

The Australian frogs currently assigned to the genus *Limnodynastes* Fitzinger 1843 (family: Leptodactylidae, or more recently, Myobatrachidae) are readily separable into 2 groups of species on the basis of their general body form, and the shape of the inner metatarsal tubercle[2-5]. In the 1st, the so-called *peroni* group, frog-like anurans, without any special burrowing adaptation on their foot (species: *peroni, tasmaniensis, fletcheri, convexiusculus,* and *salmini*) are included. The 2nd group are more toad-like, burrowing anurans, showing in various degrees a shovelshaped metatarsal tubercle. It includes the *dorsalis* complex (species[6]: *dorsalis, dumerilii, terraereginae, interioris*) and the *ornatus* complex (species, or perhaps only subspecies[3], *ornatus* and *spenceri*). The recently described *Limnodynastes depressus*[7] is known from only 1 specimen and its generic placement is suspect. It is not considered here.

Using the principles of numerical and 'Hennigian' taxonomy, Liem[8], and Heyer and Liem[5] have recently re-considered the myobatrachid frogs, and have assigned these 2 groups of species to different, though related, genera. The *peroni* group constitutes the genus *Limnodynastes sensu stricto,* while the *dorsalis* and *ornatus* complexes are placed in the revived genus *Platyplectron.* The relative position of both genera within the myobatrachid radiation is presented by these authors[5] in the form of several alternatives. In the 'preferred' phyletic tree, *Limnodynastes* and *Platyplectron* are a sister-group of *Adelotus* near to the top of 1 of the 2 main branches of the Myobatrachidae.

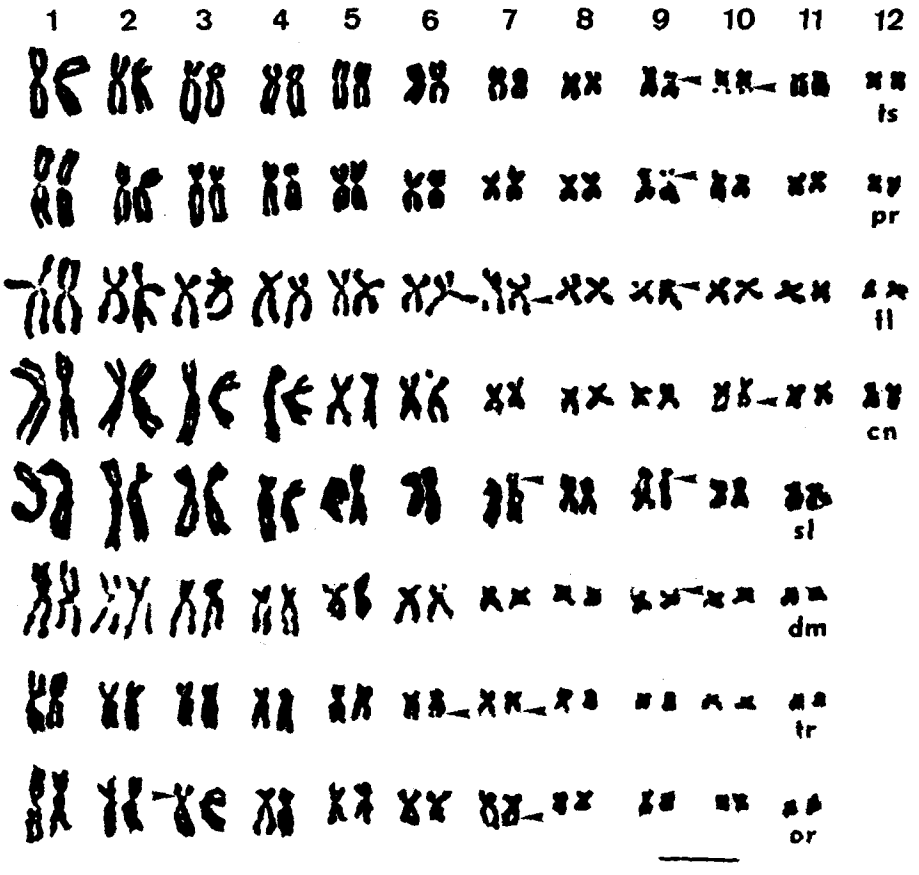
We have obtained karyological data from specimens from Queensland and New South Wales belonging to all the main groups of *Limnodynastes.* We present here our results and a short systematic comment on them. The species studied and the karyological results are listed in the table. The karyotype of each species is presented in the figure (for a comparison, we have added the karyotype of *tasmaniensis,* not studied by us, which is taken from a paper by Ullerich[9] with some minor changes in the linear arrangement of the chromosomes). The chromosomes have been prepared using the current squash techniques on the gonads and on some tissues rich in mitotic plates, from animals previously colchicinized. In collaboration with Dr E. Olmo, we have also obtained quantitative data on the nuclear DNA content (in pg) of some of the species. The DNA has been measured through histophotometrical methods[10] on erythrocytes from blood smears stained with Feulgen reagent. With respect to the previous literature on the subject, we are aware of only a few papers dealing with the chromosomes of species of *Limnodynastes*[9,11-13].

4 of the 8 species here examined show a diploid number of 24 chromosomes. They are the species currently assigned to the *peroni* group *(tasmaniensis, peroni, fletcheri, convexiusculus),* with the exception of *salmini* which shows only 22 chromosomes (figure). The 24-karyotypes, composed of bi-armed, meta-, submeta- or subtelo-centric chromosomes, are morphologically very similar in the 4 species. Some interspecific differences can be found in the absolute chromosome size (dependent on the amount of nuclear DNA, which is different between *fletcheri* on one side and *tasmaniensis* and *peroni* on the other) and especially in the localization of the heterochromatic areas (very probably the nucleolar organizers (NO). The NOs are on the shorter arms of the 9th pair of chromosomes in *tasmaniensis, peroni* and *fletcheri,* on the longer arms of the 10th pair in *tasmaniensis* and *convexiusculus,* and on the longer arms of the 7th pair in *fletcheri.* The distribution of the NOs is not especially diagnostic at the specific level in many Anurans[14]. However, in this group of species with 24 chromosomes, it shows some correlation to the morphological characters that Moore[3] has used to interpret the relationships within the *peroni* group. The species *salmini* (if it does belong to this group with its 22 chromosomes) maintains the interstitial NOs on the shorter arms of the 9th pair characterizing all the species of the group except *convexiusculus.* In the species of the *Platyplectron* group (2n = 22), only *dumerilii* shows NOs on the 9th pair, but they are distally localized on the shorter arms of the chromosomes, which are smaller than the corresponding chromosomes of the *peroni* group (figure). Relatively small NOs are frequently localized on the 6th and 7th pairs of *terraereginae* and on the 3rd and 7th pair of *ornatus.*

In the *dorsalis* complex, *dumerilii* (perhaps the most generalized species[6]) has larger chromosomes than *terraereginae.* The smallest chromosomes are those of *ornatus,* though this character is not clearly appreciable in the figure, provided that we have selected a well 'flattened' metaphase the better to illustrate the chromosome morphology. Karyological differences between the species of the *dorsalis* and the *ornatus* complexes can be found in the size of the 7th pair of chromosomes, which are larger in *ornatus.* From this point of view, *salmini,* if included in the *Platyplectron* group for its diploid number, would differ from the species of both *dorsalis* and *ornatus* complexes, especially in the morphology of the 7th pair, in that it is subtelocentric and with a short NO on the smaller arms.

The amount of nuclear DNA in the species of the *peroni* group is relatively high in *fletcheri* (7.6 pg), while it is lower and nearly equal in *tasmaniensis* (about 4.3 pg) and in specimens of *peroni* coming from New South Wales (4.5 pg). However, in specimens of the latter species coming from Queensland, we have found only 2.6 pg of DNA. It is

| Species of *Limnodynastes* | 2n | DNA (pg) |
|---|---|---|
| *Peroni* group | | |
| *tasmaniensis*[9] | 24 | 4.3 |
| *peroni* | 24 | 4.5 |
| *fletcheri* | 24 | 7.6 |
| *convexiusculus* | 24 | |
| *salmini* | 22 | |
| *Platyplectron* group | | |
| *dumerilii* | 22 | 6.5 |
| *terraereginae* | 22 | 3.9 |
| *ornatus* | 22 | 1.9 |




The karyotypes of *Limnodynastes tasmaniensis* (ts), *peroni* (pr), *fletcheri* (fl), *convexiusculus* (cn), *salmini* (sl), *dumerilii* (dm), *terraereginae* (tr), and *ornatus* (or). The bar is 10 µm long. The arrows point to the most frequently found heterochromatic areas (NOs).

thus possible that different populations (or subspecies?) of *peroni* may show different genome sizes. Apart from the last case, the DNA values shown by this group of species is not far from the mean values in the nuclear DNA of the Anurans, which is about 5 pg in the majority of the studied species[10]. We have not studied the DNA of *convexiusculus* and *salmini*. However, from their chromosome sizes, it can be inferred that these species have DNA values not far from the mean of the Anurans.

In the *Platyplectron* group, *dumerilii* (6.5 pg) has more DNA than *terraereginae* (3.9 pg). The species *ornatus* has only 1.9 pg of DNA, which is near to the lowest amounts found in the Anurans and typical of some species of the pelobatid *Scaphiopus* (about 1.6 pg). It seems probable that the genome size of the Amphibians is at least partially related to adaptive factors[14,15]. In the Anurans, the species showing the least DNA values, have quicker cleavage and developmental rates[16], thus being able rapidly to leave the temporary waters of arid climates. The karyological situation of *ornatus* supports this theory, since this species is characterized by a rapid egg development and may reproduce in relatively dry environmental conditions.

In conclusion, the karyological results do not fully coincide with the taxonomic subdivision currently accepted for the species of *Limnodynastes*. These data introduce the possibility that *salmini* belongs to the *Platyplectron* group as a 3rd complex with a 22-karyotype. A 2nd possibility is that *salmini* has independently gained its 22-karyotype in parallel to that of the species of *Platyplectron*, though retaining many anatomical features of the *peroni* group.

One of the most generalized tendences found in the karyotype evolution of the Anurans is towards a reduction in the diploid number, especially in the groups showing between 26 and 22 chromosomes[14]. The present results may thus suggest that the *Platyplectron* (plus eventually *salmini*) group is karyologically more advanced than the species of the *peroni* group. On phenetic bases, it seems highly probable that the 1st of the 2 groups has arisen from the 2nd, a hypothesis that finds support at a karyological level. However, from this viewpoint the picture is complicated by the fact that 24-chromosome karyotypes are relatively frequent within the myobatrachids. It also occurs in the genera *Notaden* and *Adelotus*[13], and the latter may be related to one or both groups of *Limnodynastes*[5].

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