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Review

Micronucleus test in tadpole erythrocytes: Trends in studies and new paths

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JOURNAL CLUB
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Giacomo Rossi

INTRODUCTION

It has been observed that greater than 70% of the world's amphibian species are in decline since the 1990s.

What could be the reason?

INTRODUCTION

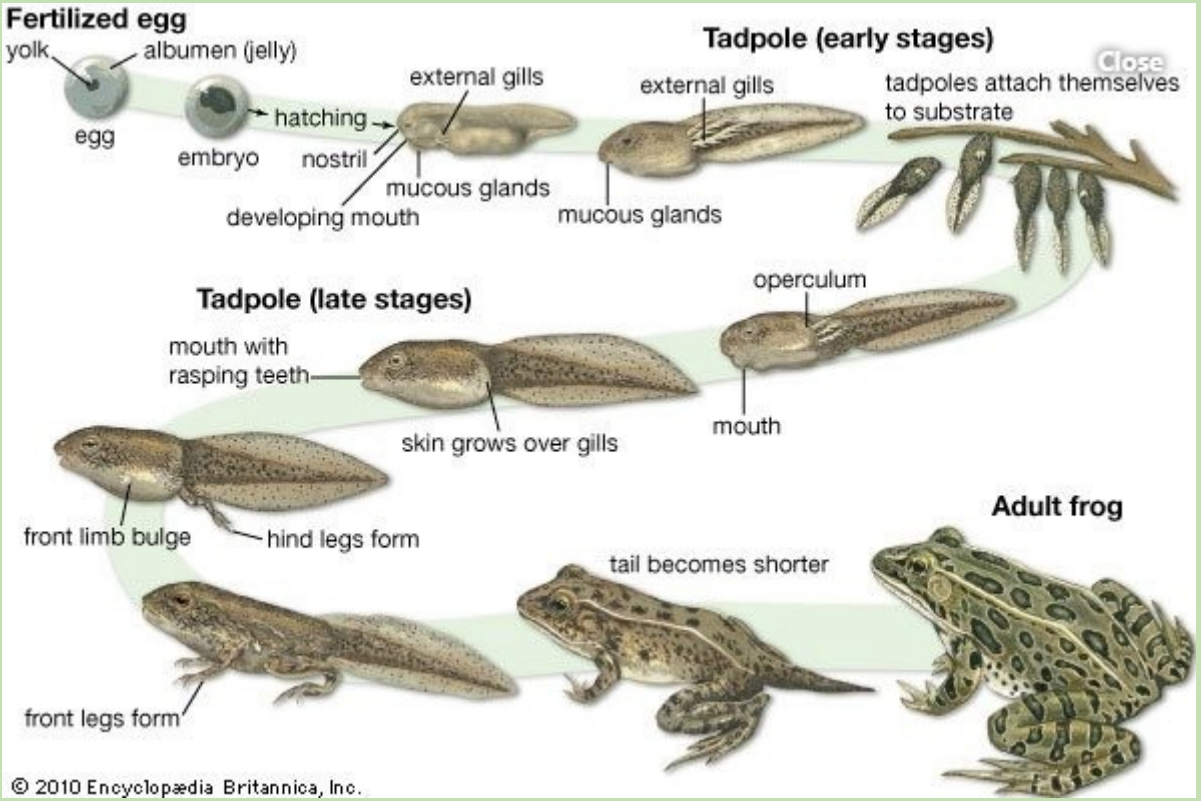
As amphibians are commonly found in agroecosystems, pollution by pesticides and herbicides is thought to be one of the primary determinants of this process.

Also industrial effluents containing heavy metals could been implicated, given their toxic effects on aquatic organisms, in particular amphibians.

INTRODUCTION

Amphibians are one of the most sensitive vertebrate groups to contamination by xenobiotic compounds, because of characteristics such as:

- biphasic life cycle
- limitations on their dispersal capacity
- semi-permeable skin
- adaptations for life in highly specific microhabitats



PURPOSE OF THE ARTICLE

Review the scientific literature up until the end of 2018 about the micronucleus test involving tadpoles.

The interest is to elucidate the number of published papers so far, evidencing the main genotoxic agents and the most common erythrocyte nuclear abnormalities (ENAs) in studies with tadpoles on a world scale.

WHAT IS THE MICRONUCLEUS TEST ?

The micronucleus (MN) test is based on the analysis of erythrocytes, according to specific criteria, such as the diameter of the micronucleus (which must be between 1/16 and 1/3 that of the principal nucleus), and its reflectivity and color, which must be the same as those of the principal nucleus.

The MN test is an important marker for environmental biomonitoring. It has been used to verify the possible existence of DNA damage resulting from chronic exposure to xenobiotic compounds in the target species, either in the laboratory or in the field. A significant increase in this damage, relative to a control sample, can provide a systematic measure of environmental alterations in a microhabitat.

It is simple, sensitive, reliable, and provides an immediate result.

ERYTHROCYTE NUCLEAR ABNORMALITIES

Although the micronucleus test has been applied to tadpoles for approximately three decades, it has been only a little over ten years since other ENAs are being scored, as complementary alternatives to MN analysis. Many ENAs have been scored, however, only eight have been frequently selected in the studies.

Some of these as binucleated cells, have their origin related to cell division, along with MN, while other abnormalities as the nuclear bud may be related to DNA amplification. However, there are still uncertainties abuot this.

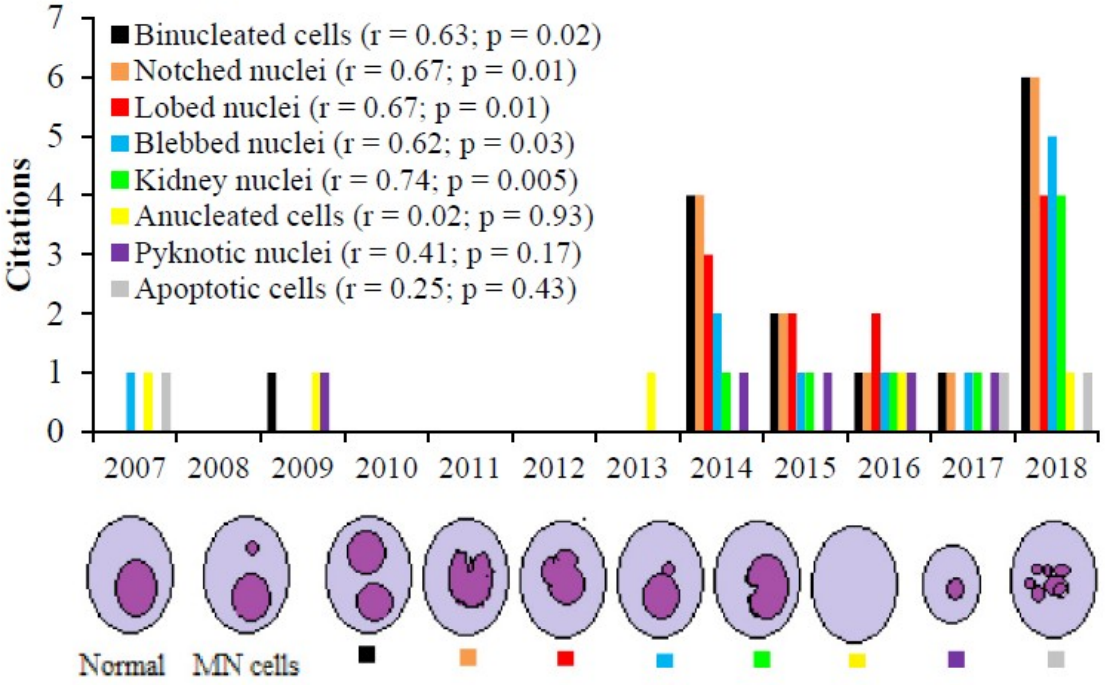


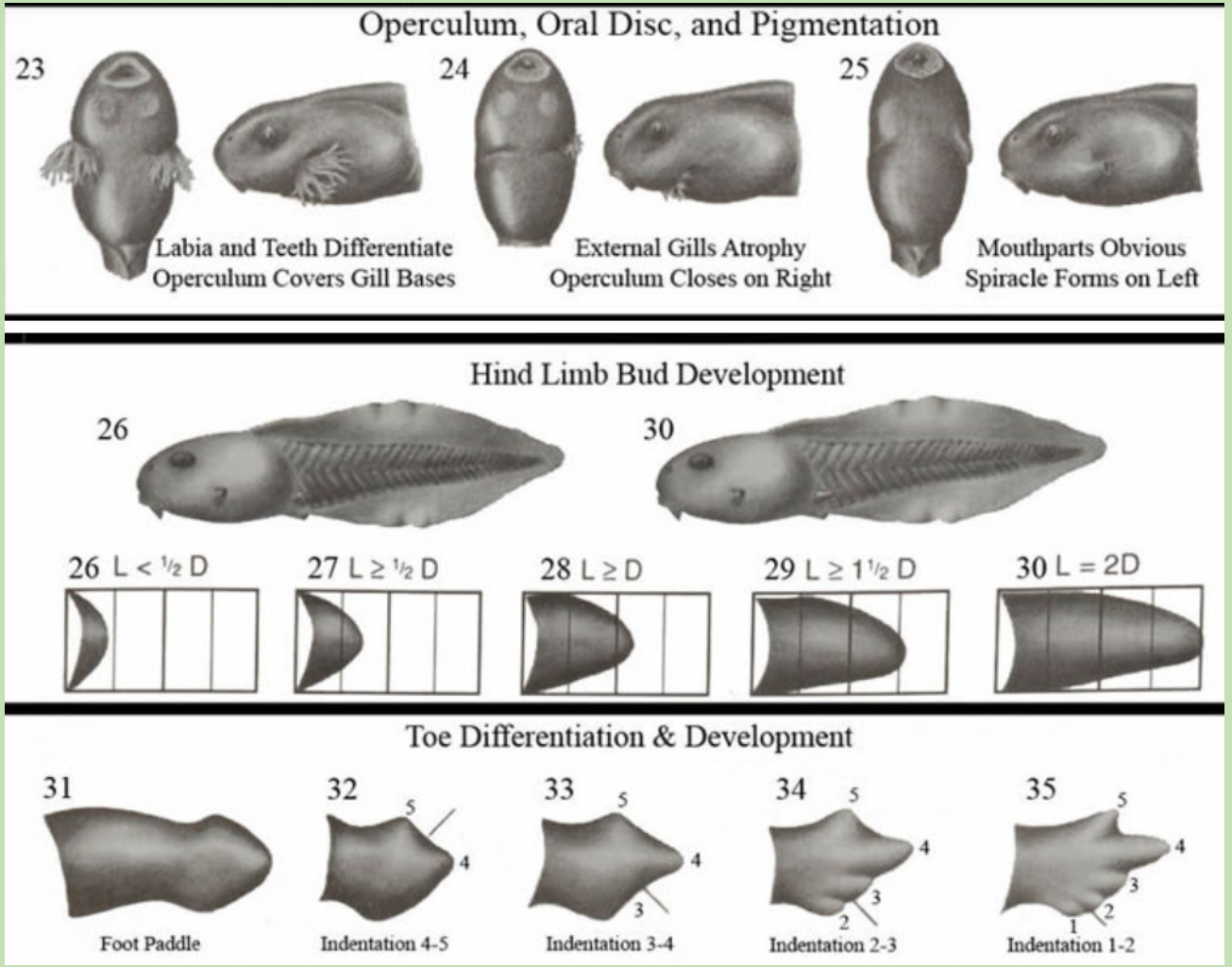
Fig. 3. The correlation of the main ENAs scored in the MN-ENAs test in tadpoles.

TADPOLES

Tadpoles are frequently used in toxicological research, and are excellent bioindicators, because they are more vulnerable than the adult phase, mainly due to the greater exposure to xenobiotics, which can easily penetrate the skin, mouth and gills in the aquatic environment, depending on the stages of larval development.

The embryonal and larval development of anurans can be classified into 46 stages («Gosner stages»), from fertilized embryo to the completion of metamorphosis.

Phases 24-33 are good choices for



NUMBER OF STUDIES

The micronucleus test as a biomarker of genotoxic damage has been carried out since 1987.

Although only 48 papers on this theme were selected (indexed in the Web of Science, Scopus and Scielo) there is a significant correlation for the growth of the studies over time.

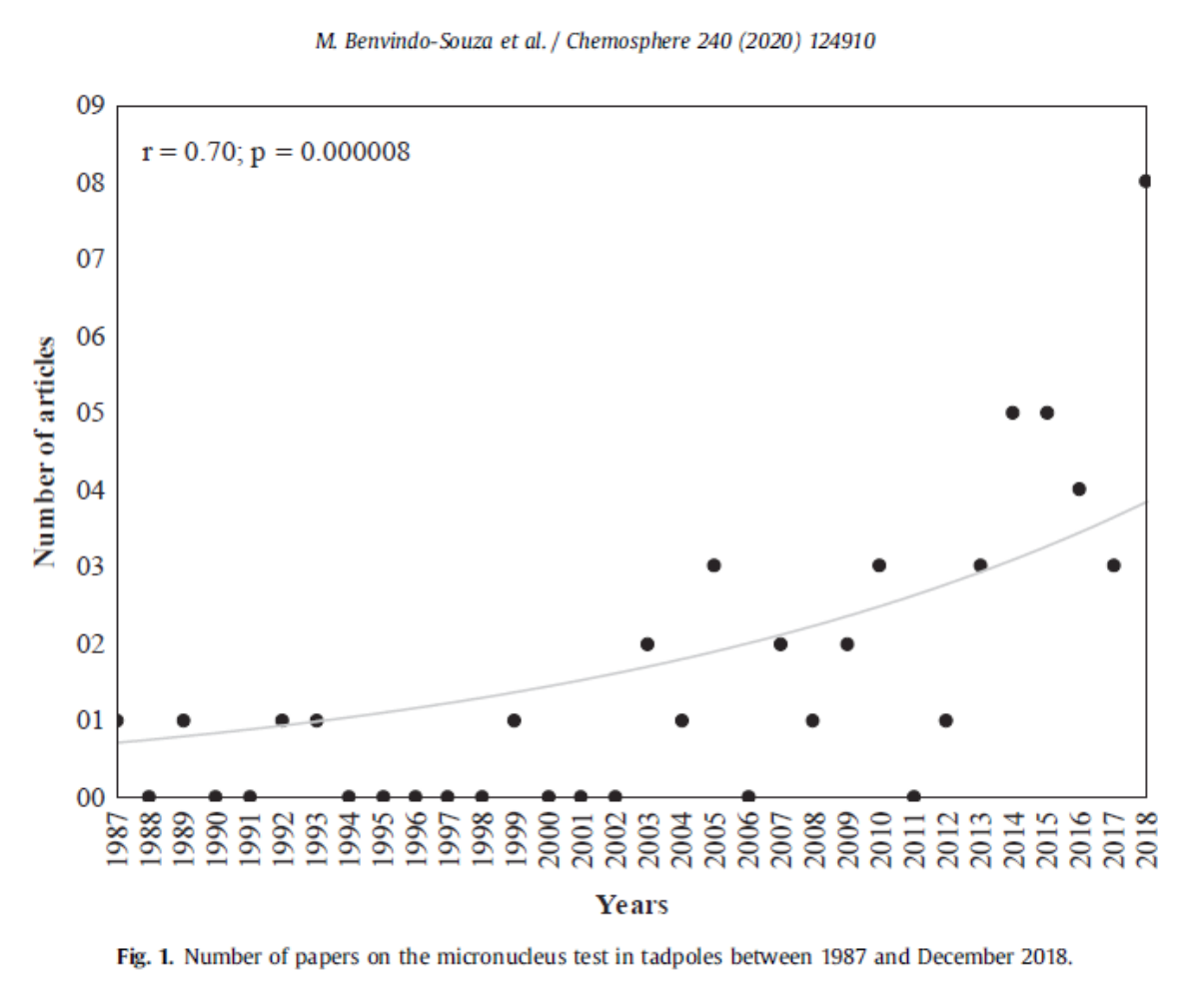


Fig. 1. Number of papers on the micronucleus test in tadpoles between 1987 and December 2018.

DISTRIBUTION OF STUDIES

The vast majority of the research was conducted at institutions in South America (65%) and Asia (19%), while a much smaller number of studies originated from Europe (12%) and North America 4%.

Argentina leads the research, followed by Brazil. In these countries, the expansion of agricultural land caused extensive losses and degradation in habitats; perhaps this is the great promoter of research with the micronucleus test there. Brazil also has the largest biodiversity of anurans in the world.

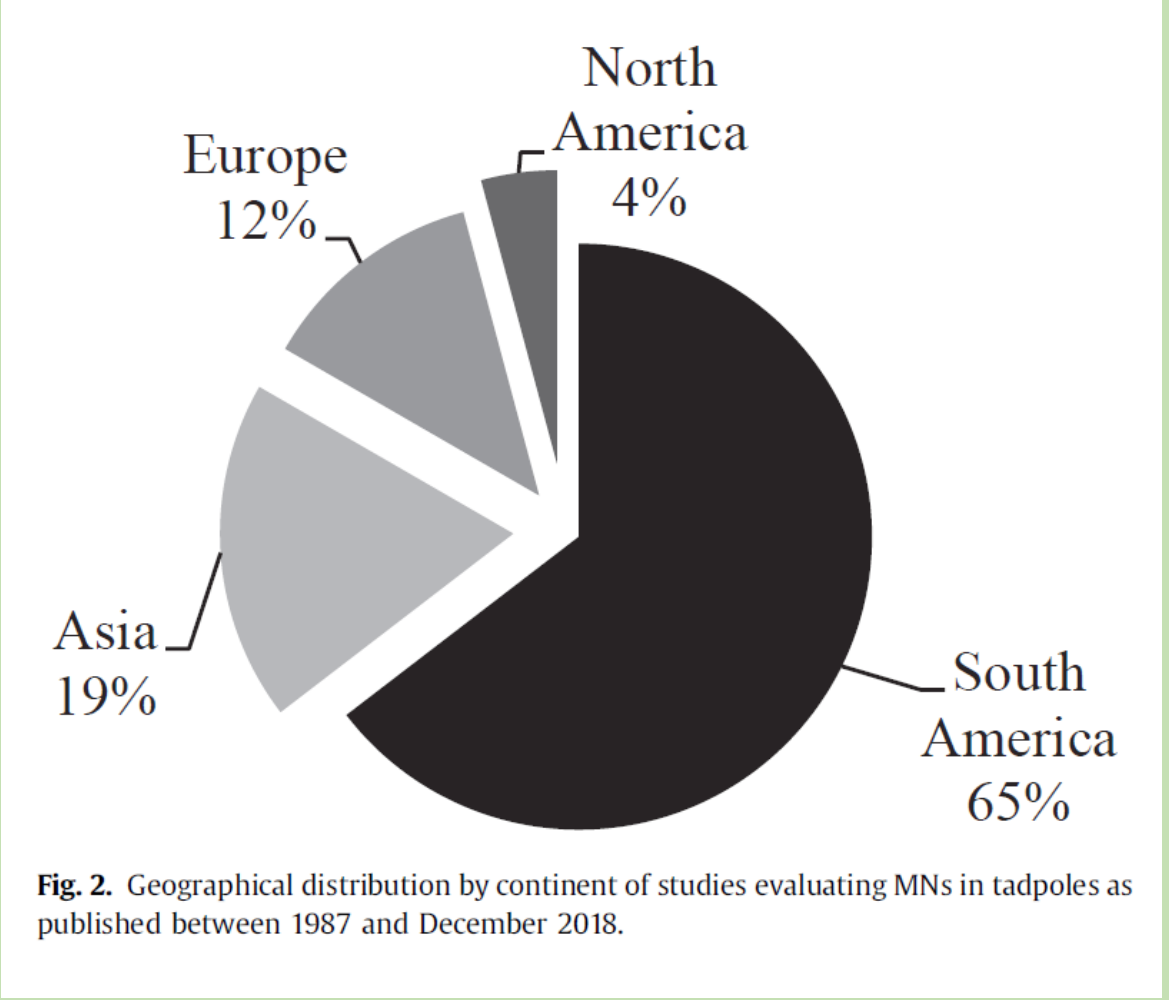


Fig. 2. Geographical distribution by continent of studies evaluating MNs in tadpoles as published between 1987 and December 2018.

TYPES OF XENOBIOTIC AGENTS

The studies investigated for:

- pesticides (56%)
- residues of mining (19%)
- metals and pesticides combined (8%)
- other genotoxic agents 17%

SOME PESTICIDES

Herbicides:

- **2,4-D herbicide** has a mutagenic effect on "*Rana catesbeiana*" tadpoles, even at low concentrations and for a short period of time;
- tadpoles of "*Dendropsophus minutus*", one of the most common species in South America, exposed to **atrazine** 18 mg/L showed higher frequencies of MNs regardless of the stage of development;
- low concentrations of **Roundup** (1, 2 and 3mg ae/L) induced the formation of MN in tadpoles erythrocytes at 24, 48, 72 and 96 h in a concentration-dependent manner; however there have not been found statistical significance for the frequency of MNs in erythrocytes of "*D. minutus*" after 96 h of exposure to **Roundup Original ® glyphosate** (0.28 mg ai/L, 1.0mg ai/L, 2.0 mg ai/L, 4.0 mg/L).

SOME PESTICIDES

Insecticide:

- **FASTAC 10 CE** induced mutagenicity with MN formation in “*Rana temporaria*” tadpoles, demonstrating that these larvae are more sensitive than “*Xenopus laevis*”;
- In the evaluation of the **endosulfan insecticide**, the MN frequency measured in erythrocytes of “*B. pulchella*” tadpoles showed no dose dependence at different concentrations (2.5, 5 and 10 mg/L);
- lower concentrations of **malathion** that extend into the sublethal range (0.5, 1.0 and 2.0 mg/L) induce MNs in erythrocytes of tadpoles at 24 h, 48 h, 72 h and 96 h in a manner concentration dependent;
- a concentration range of **pirimicarb** 0.005 - 0.39 mg/L. **Aficida** ® induced damage to DNA at the chromosome level by increasing the frequency of MNs and other ENAs, ie, lobed and carved nuclei and binucleate cells.

MINING ACTIVITY

Mines are source of physical, chemical, biological and landscape changes.

It’s difficult to establish a direct cause-and-effect relationship with a specific element because of environmental complexity and synergistic and/or antagonistic interactions between substances.

Examples:

- Fluorite mine → compounds or mixtures that are damaging the cells
- Iron and Manganese → potential to induce biological impacts
- Copper → only a modest effect on “*R. catesbeiana*” premetamorphic larvae
- Cadmium and Lead → damages also in adults of “*Amietophrynus regularis* ”

LACK OF KNOWLEDGE

16 species of anurans distributed in five families, 0.23% of the total number of described species.

Much of the research has been directed at "*R. catesbeiana*", a North American species resistant to xenobiotic agents, and few studies have been carried out on native species elsewhere.

Studies with more than one species are encouraged to confirm the response to genotoxic under experimental conditions and to generate strong conclusions.

Table 1
Species of anuran larvae studied for the micronucleus test.

Family/Species	N	Status of IUCN
Pipidae		
Xenopus laevis (Daudin, 1802)	4	LC
Ranidae		
Rana catesbeiana (Shaw, 1802)	15	LC
Rana temporaria Linnaeus, 1758	1	LC
Pelophylax nigromaculatus (Hallowell, 1861)	1	–
Pelophylax kl. esculentus (Linnaeus, 1758)	1	LC
Hylidae		
Boana pulchella (Duméril & Bibron, 1841)	7	LC
Boana cordobae (Barrio, 1965)	1	DD
Scinax nasicus (Cope, 1862)	1	LC
Dendropsophus minutus (Peters, 1872)	3	LC
Trachycephalus typhonius (Linnaeus, 1758)	1	LC
Bufonidae		
Rhinella arenarum (Hensel, 1867)	6	LC
Strauchbufo raddei (Strauch, 1876)	1	LC
Bufo gargarizans Cantor, 1842	1	LC
Dicroglossidae		
Quasipaa spinosa (David, 1875)	1	VU
Euphlyctis cyanophlyctis (Schneider, 1799)	2	LC
Fejervarya limnocharis (Gravenhorst, 1829)	2	LC

N = Citation number. LC = Least Concern, DD = Data Deficient and VU = Vulnerable.

CONCLUSIONS

- Application of the the micronucleus test has a significant increase over the years.
- Other erythrocyte nuclear abnormalities have been investigated, but there are still uncertainties about their classification and molecular cause.
- Pesticides, followed by heavy metals, are the genotoxic agents that drive the scientific interest, because of agriculture expansion and its strong use of chemicals.
- South America leads the publications in particular due to the contribution of Argentina and Brazil.
- Vulnerability of Anuras larval stages to chemical and physical agents is undeniable, but we need more studies to establish if there is a correlation with the global decline of this animals.