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Minirevision: Natural history of *Amynthas hawayanus* (Rosa, 1891)

Mini-revisão: História Natural de *Amynthas hawayanus* (Rosa, 1891)

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After using the earthworm, *Amynthas hawayanus* (Rosa,1891) as a research subject in neurophysiology for years, this author found three advantages in using it for this purpose over the use of the other subjects, such as human volunteers and patients, dogs, cats, rabbits, rats, frogs, fishes, Aplysia, silkworms and Ascaris The first advantage is its availability. It is the most widely distributed earthworm around the world perhaps only next to *Lumbricus terrestris*, L. (Stephenson, 1930, Gates,1937). It has no seasonal life cycle as most other invertebrates have. It can share the same ecological habitat with the research staff because it is a semi domesticated animal. One needs not to collect it in the ocean, mountain, forest or supply shop. No hospital staff or other persons to be convinced to obtain It. It takes no vacation. No school work hampers its presence in the laboratory and it does not excuse itself for absence due to business appointments. Its second advantage is low maintenance cost. A terrarium with correct eology and a monthly feeding is all it needs. No mail-order, no animal room, no employment of specialized personnel and no costly ration is needed (see detail in section 6). The third and the most important advantage is its originality in neurophyslology. We know next to nothing about its membrane exci-

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tability compared to what we know about that in the squid axon membrane. We know very little about its central and peripheral neuromuscular system organization compared to what we know about guinea-pig mesentery nervous system or Ascaris neuromuscular organization or frog somatic neuromuscular transmission. No spinal reflex arc was ever reported from his animal comparable to that of the cat. No neuronal circuit for its rhythmic locomotion, such as that of the leech, was ever studied. Only a few conditioning experiments were done in *Lumbricus terrestris* compared to the volume done on dog salivation, human motivation, bird-song learning and Aplysia gill-withdrawal modification. Equipped with knowledge from these above animals, each preliminary experiment on this animal yields unique mechanism comparing and enriching our contemporary knowledge accumud and formulated in neurophysiology from the other laboratory animals over the years. This advantage will be furtherly discussed in Section 4. There is a paradox in using this animal as neurophysiological subject, *i. e.,* its relatively unknown natural history in spite of its proximity to our daily life except from reports of some prominent authors (*e. g.*, STEPHENSON, 1930, GATES, 1937, ARAÚJO, 1962, RIGHI e KNEPPER, 1965). The purpose of this article is, therefore, to call attention on its natural history when this animal is needed as a neurophysiological subject and to compare a few results from experiments done in this animal and in some familiar species, such as *Lumbricus terrestris.*

2. TAXONOMY

Opisthopora is one of the orders in the class Oligochacta. Its two largest families are the Lumbricidae, to which *Lumbricus terrestris*, and Megascolecidae where the genus *Pheretima* is under present discussion. According to RIGHI e KNEPPER (1965), 17 synonyma were used for this species. It was originally called *Perichaeta hawayana* Rosa, 1891. But the name, *Pheretima hawayana* (Rosa, 1891), was best known from references in this century. Another synonym, *Amynthas hawayanus* (Rosa, 1891), was used in a taxonomical revision by SIMS and EASTON (1972) and this is the name used in this title of the article. A key to the

species genus *Pheretima* in North America was provided by GATES (1937) and was revised by RIGHI (1966) for the species most frequently found in Brazil Five species in this key, *Pheretima hawayana*, *P. californica*, *P. morisii*, *P. schmardae* and *P. indica*, were found around the City Curitiba where this author has his laboratory. The genus *Pheretima* was revised and divided into two genera, *Amynthas*, to which went *Amynthas hawayanus*, *A. morisii* and *A. indica*, and *Metaphire*, to which went *Metaphire californica* and *M. schmardae* (Sims and Easton, 1972). The only Lumbricidae species introduced into the local fauna during the recent years is *Eisenia foetida* (Savingy, 1826) of subfamily Helodrilus.

3. ANATOMY

Gross anatomy of *Amynthas* (or *Pheretima*) *hawayanus* was described in several review articles(ARAÚJO, 1962, RIGHI e KNEPPER, 1965, RIGHI, 1966). The most conspicuous external morphological character of the genus *Pheretima*, or genera *Amynthas* and *Metaphire*, is a ring of setae distributed in even distances around each segment, hence, its original name, *Perichaeta*, in contrast to the double setae distributed in a four-spoked wheel arrangement in Lumbricidae(Smith, 1917, Araújo, 1965). Its clitelium is in segments 14-15-16 instead of behind. the 2nd th, or 3rd, segment in Lumbicidae. Its internal anatomy useful for species identification includes several pairs of large spermathecae anterior to clitelium, in contrast to small spermathecae in Lumbricidae. The number and position of these spermathecae are listed in the species keys (GATES, 1937, RIGHI, 1966). The shape and position of its intestinal ceca are also used for identifying the species in the genus *Pheretima* (RIGHI, 1966) and are still used in the divided genera *Amynthas* and *Metaphire* (SIMS and EASTON, 1972). The longitudinal muscle cells are organized into Kästchenen (HESSE, 1894) in genera including *Pheretima* (HARMAN, 1960). It has an extensive peripheral nervous system. Intramural neurons are distinguishable from the surrounding muscle cells histologically (ZYENG, 1930). Its central nervous system consists of cephalic ganglia and a ventral nerve cord. The segmental ganglia in this ventral nerve cord are not as well marked externally as those of the leech. Its five giant axons,

equivalent to the S neuron and Rohde's fiber of the leech, are anastomose segments of neurites separated by septa. Neurons in both its central and peripheral nervous syatems are similar to those of the other animals histo1ogically (GÜNTHER, 1972) but were identified as bundles of thin processes with very small somata by intracellular Lucifer yellow-CH-filling (CHANG and ASSMÉ, 1989, CHANG, MARCHIORO and ASSMÉ 1991), similar to those of the other invertabrates, such as insects (TRUMAN and REISS, 1976). The muscle cells are spindie-shaped with oblique striations (Toida *et al.*, 1975) in histological preparations (HANSON, 1957, IKEMOTO, 1963, NISHIHARA, 1967) but appear also as bundles of thin processes when single muscle cell was identified in-vivo by intracellular Lucifer-Ye1low-CH-filling (CHANG and ASSMÉ, 1988), also similar to those of the other invertebrates (ALEVIZOS *et al*, 1989).

4. PHYSIOLOGY

Physlology of the earthworms, mostly *Lumbricus terrestris* and other Lumbricidae species, is descrlbed in several monographs (LAVERACK, 1963, MILL, 1978, PODESTA, 1982). A brief comparison of electroneurophysiology between *Amynthas hawayanus and Lumbricus terrestris* will be given in this section. The membrane excitability of longitudinal muscle cells is of the graded type. Their resting potentials are low and variable between 30 to 70 mV. They discharge spontaneously. Their evoked action potentials have graded amplitude according to stimulus intensity (HIDAKA *et al.*, 1969, CHANG, 1969, 1975) and conduct decrementally according to distance(ASSMÉ and CHANG, 1990). This graded excitability differs quantitatively, rather than qualitatively, among several species of *Pheretima* (CHANG and SCHULMAN, 1968, CHANG and CRUZ, 1968). Neuromuscular transmission of *Amynthas hawayanus* is cholinergic (HIDAKA *et al.*, 1969, CHANG and BRUNO, 1970, CHANG, 1975). Its nexal transmission between giant axon segments is electrical and rectifying but is non-rectifying to dye diffusion (Brink and Dewey, 1978). *Amynthas hawayanus* has a highly developed discriminability in its nervous system to peripheral mechanical and electrical stimuli. Some neurons in its nerve cord responded only to mechanical stimulus while others to electrical stimulus only (CHANG and

ASSMÉ, 1989, CHANG, MARCHIORO and ASSMÉ 1991) although it is still not clear whether this discriminability is a character of the peripheral receptor or of the neuron membrane. The peripheral receptors of *Amynthas hawayanus* are much less sensitive to serotonine (CHANG *et al*, 1996) than do receptors of the leech despite of the fact that *Lumbricus terrestris* has more serotonergic neurons (GRAS *et al.*, 1988) in its nerve cord than the leech has(Lent and Dickinson, 1988). No tail-flattening reflex of the Lumbricidae species(Gras, 1984) was observed in this species(CHANG, unpublished data). The Lumbricidae species can be conditioned associatively and non-associatively by olfactory, mechanical and optical stimuli (RATNER and MILLER, 1959, ABRAMSON and BUCKBEE, 1995, ABRAMSON *et al.*, 1996) but such conditioning is still under investigation in *Amynthas hawayanus* (CHANG *et al.*, 1997).

5. ZOOGEOGRAPHY AND ZOOHISTORY

Amynthas hawayanus (and many other *Pheretima* species), was originated from tropical and subtropical zones in Southeast Asia (STEPHENSON, 1930, GATES, 1937). Its original genus name, *Perichaeta*, was derived from its morphology (see section 3, Anatomy) and its species name was derived from a specimen collected from Hawaii in the Vienna museum identified by D. Rosa in 1891. It was introduced to South America from the Phillipines by early Spanish colonizers along with their crops. Due to its high adaptability, *Amynthas hawayanus* substituted the native earthworm species in South America and spread to the southern part of North America. This species was found as far north as Illinois state (STEPHENSON, 1930, RIGHI e KNEPPER, 1965) although HARMAN (1960) found only other Megascolecidae species there. Another highly adaptable species, *Metaphire* (or *Pheretima*) *schmardae*, was found as far north as Szechuan state in China (GATES, 1939). *Amynthas hawayanus*, instead of *Lumbricus terrestris*, became the easiest found species in California (CHANG, unpublished data, 1985). However, this human-aided dominance may be short-lifed. Another Lumbricicjae species, *Eisenia foetida* (SAVIGNY, 1826), was introduced to this country from Japan for its high humus productivity for agriculture in the late 20 th century after it was introduced to North

America by European colonists long time ago. This species is substituting *Amynthas hawayanus* in a much faster rate than the substitution of native species by *Amynthas hawayanus* earlier in this country. This author found only *Eisenia foetida* in Vale do Ribeiro in São Paulo state in 1992. *Eisenia foetida* has an unfortunate popular name locally, "minhoca da california" (Californian earthworm), which causes frequent confusion to *Metaphire* (or *Pheretima*) *californica*. No *Lumbricus terrestris* is known to have been introduced into Brazil despite of the recent commercial contact with North American countries.

6. Raising

Earthworms can be acquired from bait shops or suppliers., such as Carolina Biological supply Co. in the United States and it can be dug from gardens or farms in Brazil. But this author found it necessary to raise his own identified species with uniform nutritional and health care for laboratory controlled study in neurophysiology. For general knowledge of earthworm raising, a week-long course offered by he Brazilian Earthworm Raisers Association (Associação Brasileira de Minhocultura, Rua Floriano Peixoto 961, S/2, Itú, SP, Cep 13.300) is recommended. Brochures and catalogs of this course may be obtained c/o its superintendent, Dr. Victor C. del Mazo Suarez, in this address. The content of this course, however, emphasizes large-scaled raising of *Elsenia foetida* for the economical advantage of its humus to be exported to North America and Europe as fertilizer. Owing to the three advantages (Introduction) of using *Amynthas hawayanus* as neurophysiological subject, this author recommends small-scaled raising of this species self-sufficient for only one laboratory. An outdoor terrarium with a soil and decayed vegetation bedding of no more than 50 cm (one and half foot) depth is recommended. It needs indirect sunshine. Direct sunshine must be avoided by a partial roof or bush cover. Natural rain water is necessary but excessive flooding drowns the earthworm. It is not an aquatic animal. Indoor terrarium results often in fungus and parasites proliferation due to unnatural ventilation and humidity although protozoan and tapeworm parasites are relatively harmless. Instead of horse manure for feeding

Elsenia foetida, cow dung is prefered by *Amynthas hawayanus*. This cow dung is best collected dry from meadow instead of from cow-pen. Urine mixture causes unfavorable pH. This author found it also convenient to use laboratory animal (white rats, rabbits, etc.) excrements because they are always available. Residue of unconsumed rat ration is harmless but edible cabbage in rabbit ration is to be avoided because it frequently contains slug and caterpillar eggs. This animal does not coexist well with slugs and predator land leeches. Caterpillar causes irritational comments from laboratory colleagues. Carnivorous animal (dogs, cats) excrements are not recommended for their unpleasant smell. Although earthworms eat excrements of the other animals, they do not eat their own. A monthly renewal of this feeding is enough. The worm concentration raised in this terrarium is lower than that recommended by the course but it is enough for research experiments. This animal mass-reproduces in Spring and Autumn but also reproduces irregularly around the year. Therefore, one has a continuous supply of hatchling, immature and mature (clitellate) individuals for experiments.

RESUMO

A minhoca, *Amynthas hawayanus*, torna-se conveniente na pesquisa eletroneurofisiológica devido à sua ampla distribuição geográfica em várias partes do mundo. A despesa com a manutenção deste animal tem baixo custo. A vantagem mais importante na utilização deste animal consiste na originalidade dos resultados eletroneurofisiológicos quando comparados aos resultados obtidos com outros animais de laboratório. Este artigo tem como objetivo chamar a atenção de eletroneurofisiológistas que pretendem trabalhar com este animal. Esta espécie deve ser distinguida de outra espécie mencionada como *Lumbricus terrestris*, seguindo-se a cuidadosa identificação com a chave taxonômica especialmente, em países do Hemisfério Norte onde elas coexistem nas lojas de material biológico ou de iscas. O importante é criar a própria espécie identificada assegurando com isto o fornecimento continuo das amostras para os trabalhos de pesquisa bem como as condicões uniformes da sua ecologia. nutricão e saúde.

PALAVRAS CHAVE: *Amynthas hawayanus*, minhoca, história natural, taxonomia.

SUMMARY

The earthworm, *Amynthas hawayanus,* is a convenient subject for electroneurophysiological research because of its wide geographic distribution in several parts of the world. Its maintenance cost is low. The most important advantage in using this animal is the originality in its results obtained from electroneurophystological research compared to that from many of other laboratory animals. This article calls attention to several points for the electroneurophysiologists who would choose it as a research subject. It must be distinguished from another most often mentioned earthworm species, *Lumbricus terrestris,* by careful identification through taxonomical keys especially in the North Hemispheric countries where they often coexist in the same biological supply company and bait shop. It would also be important to raise one's own identified species to ensure a continuous supply of specimens in uniform ecological, nutritional and health conditions.

KEY WORDS: *Amynthas hawayanus*, earthworm, *natural history, taxonomy.*

RÉSUMÉ

Le ver de terre, *Amynthas hawavaynus,* est un modele convenient pour la recherche electroneurophisiologique car ils presentent une large distribution géographique, en effect, ils sont retrouvés en différentes part du Monde. En plus, le coût de manutention en laboratoire est tres bas. Le plus important avantage en utilizer ce modèle, chez ces animaux, l'originalité des résultats obtenus en recherche electroneurophisiologique comparée à d'autres animaux de laboratoire. Cet article a pour objective tirer l'attention de neurophisiologue on l'intention de travailler avec ces animaux. Cet espèce doit être distinguée de *Lumbricus terrestris* , et suivant attentivement des clés taxonomiques, especialement dans le Hemisphère Nord où les deux spèces peuvent co-exister dans un même niche ecologique ou encore dans des magazins de vente d'appât. Il est indispensable d'avoir une elevage au laboratoire pour avoir une foniture en continu et que cet espèce soi bien identifiée au départ. Il est également necessaire mantenir les conditions ecologiques, de nutrition et de sanité bien uniformisées.

MOTS CLÉS: *Amynthas hawayanus,* le ver de terre, l'histoire naturelle, taxonomie.

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