Where Association Ends.

A Review of Associative Learning in Invertebrates, Plants and Protista, and a Reflection on its limits.

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Abstract

Since the beginning of the 21st century, the Minimal Cognition approach has emerged vigorously, focusing on the study of the adaptive behaviour of the simplest organisms, including bacteria, assuming that they are sentient and information-processing entities. Although Minimal Cognition has occasionally used Pavlovian methods to try to demonstrate Associative Learning, neither the Psychology of Learning nor the Comparative Psychology traditions are prominent in the movement. However, the Psychology of Learning approach, with its highly sophisticated experimental designs, has done a great deal of research on Associative Learning in animals and carried out several studies on plants and unicellular organisms. The present work offers a comprehensive review of these experimental results, along invertebrates, plants and unicellular (paramecia and the amoeba *Physarum policephalum*) showing that, while there are increasing instances of Associative Learning in many invertebrate *phyla* (and also many *phyla* with no data) there is no adequate evidence of it in unicellular protists (despite more than a century of experiments with paramecia and amoeba) or in plants (despite recent results that so claim). We then consider the alternative offered by Minimal Cognition and suggest some complementary ideas, from a Comparative Developmental Psychology approach, which we call "Minimal Development".

Keywords: Pavlovian Conditioning, Comparative Psychology, Minimal Cognition, Development, Invertebrate Learning, Plant learning, Unicellular Learning.

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Association between two events can be considered a basic way to acquire knowledge (or cognition). Consequently, studying association in invertebrates and other kingdoms (protists, plants) can provide useful information about the origin and evolution of cognition, allowing us know which organisms have shown evidence of learning by association. This review suggests that there is clear evidence of a wide array of associative phenomena in invertebrates, but this evidence is concentrated in a few species, since there is no conclusive evidence showing simple association in protists and plants. As a conclusion, it is argued that association seems to show some limitations as a basic form of describing any behavioural change due to experience (learning) in simple organisms and that psychology of learning can improve its scope by looking into the tradition of Comparative Psychology, which offers a framework based on phylogenetic and ontogenetic Developmental Psychology.

The aim of Classical Comparative Psychology was the study of intelligence in the animal kingdom in all the levels of complexity, that is to say, the natural foundation of knowledge, its evolution and its connection to human knowledge (see Figure 1). This project was made possible thanks to Darwin´s belief in the gradual evolution of mental faculties. Simple to say, hard to do.

Comparative Psychology faced several difficulties in this respect: it was initially based on very anecdotal psychology (Boakes, 1984), the variety of species employed as experimental subjects was very limited (Beach, 1950; Dewsbury, 2010; Shettleworth, 2009; Zucker, 2018), and, most importantly, the non-reductionist approach of Comparative Psychology to intelligence fitted neither Neo-Darwinism nor Behaviourism (Fernández and Sánchez, 1990; Fernández et al., 1994). Despite these shortcomings, Comparative

Psychology as a discipline did not disappear (Dewsbury, 1984; Burghardt, 2009; Gottlieb, 1998) and some central aspects of its contributions remain valid today, providing a valuable criticism to the mechanistic concept of instinct (Schneirla, 1957; Kuo, 1924; 1928; Lehrman, 1953). Also, the role of behaviour in evolution, a matter developed by Baldwin in his Organic Selection Theory (Baldwin, 1896; 1976) was never utterly discarded, in part due to its influence on Piaget´s theory of Phenocopy (Piaget, 1976) and also due to its widespread re-emergence in line with the crisis of Neo-Darwinism (Sánchez and Loredo, 2007).

The experimental research on learning or intelligence of the “lower” or “simple” organisms[1], apart from Jennings’ early work (Jennings, 1904; 1906), soon adopted Associative Learning methods, often Pavlovian Conditioning procedures. Although it was never a priority field of interest, the studies on Associative Learning and Pavlovian Conditioning in invertebrates and protists accumulated throughout the 20th century. Associative Learning became the main tool to study animal learning. It also became a complex domain comprising both the methods (a complex set of experimental procedures) and the theories developed in the last 50 years to explain the phenomenon itself (Rescorla and Wagner, 1972 and its ulterior developments).

Concerning the theoretical side, experimental research in learning has shown that nothing is simple in association (Dickinson, 2012; Heyes, 2012) because there are many complex cognitive processes involved in Pavlovian Conditioning. Some theories explain association as a result of differential stimulus processing and attentional processes (Rescorla

[1] Several names have been given to non-animal organisms (bacteria, protists, plants, and fungi). The term "inferior" was used by comparative psychologists of the late s. XIX and early s. XX (Jennings, 1906; Loeb, 1918) but is not accepted by the scientific community nowadays. The use of the term “previous” organisms has sometimes been suggested, but it is not correct, since it would only be valid for bacteria (3,500 Myr) and protists (2,000 Myr), given that plants (470 Myr) and fungi (450–500 Myr) appeared later than animals (750 Myr) according to Margulis and Chapman (2009). The terms "simple" or "lower" must be understood in relation to the complexity of animals and not in an absolute sense, and are sometimes used accordingly in this text, in the understanding that there is nothing simple in nature.

and Wagner, 1972; Mackintosh, 1975; Pearce and Hall; 1980), other theories have emphasised the role of memory in Associative Learning (Bouton and Moody, 2004), the processing of temporal information (Kirkpatrick, 2002; Gallistel and Gibbon, 2000; Balsam and Gallistel, 2009) or the comparison between learning and performance (Denniston et al., 2001). Finally, others have set out that association can be understood as decision-making processes (Schmajuk, 1987; Loy et al., 2009). These few examples illustrate the complexity of Associative Learning.

Concerning the methodological side, Pavlovian Conditioning comprises a set of very precise procedures, preparations, and practical prescriptions to study different learning phenomena (for instance, First-order conditioning, Second-order conditioning, Latent inhibition, Overshadowing, or Blocking) in many different species, mainly vertebrates, but also invertebrates and even plants or unicellular. The verification of some phenomena and not others in some organisms and not in others is also a very useful tool for the comparative study of the evolution of cognition.

Bearing all this in mind, and considering the central role that Associative Learning has recently been given to explain the origin of consciousness (Ginsburg and Jablonka, 2019), the central question is how far Associative Learning goes, not only in the various invertebrate *phyla* but also in other multicellular kingdoms (plants) and in Protist. In the following lines, we offer an overview of evidence for Associative Learning in invertebrates, and review the polemic evidence of Associative Learning in plants and unicellular (focusing on *Paramecium* and *Physarum polycephalum*). We conclude with some thoughts on the scope of Associative Learning and the convenience of completing this prolific tradition with the ideas from the Comparative Psychology and Developmental-Comparative traditions.

Associative Learning in Invertebrates

In recent years, the interest in learning processes in invertebrate species has dramatically increased (Figures 2 and 3), and some authors have claimed that invertebrate learning reveals some theoretical limitations in the current accounts of learning processes (Abramson and Wells, 2018). Even from a neural perspective of Associative Learning (Hawkins and Byrne, 2015), it can be assumed that learning in invertebrates is relevant. The research on *Aplysia californica* played an important role in clarifying the neural and biochemical bases of learning and memory (Abrams and Kandel, 1988). Most invertebrate taxa have a bilateral body plan and a bilateral central nervous system and most animals with confirmed Associative Learning are bilaterian and have brains (Ginsburg and Jablonka, 2019). Therefore, we would expect to find some form of learning throughout invertebrates. Nevertheless, the information available on the *phyla* in which Associative Learning (Classical Conditioning) has been demonstrated is scarce and dispersed (see Table 1).

According to Perry et al. (2013), habituation has been demonstrated in all *phyla*, and Associative Learning in all as well excepting chordates, maxillipods, myriapods and rotifers. However, this conclusion is not easy to interpret, given that categories used by Perry et al. (2013) belong to different taxonomic levels (including *subphyla* and not including some other *phyla*). If we compare them with the categories used by Ginsburg and Jablonka (2019, p. 333), in Perry et al.'s review there is a lack of 25 invertebrate *phyla*. Maxillipods and myriapods are *subphyla* of *Arthropoda,* a *phylum* where Associative Learning is widely demonstrated, especially in insects. Chordata includes, of course, all vertebrates. Where there is no evidence of Associative Learning is in the *subphyla* urocordata and cephalocordata. Ginsburg and Jablonka (2019) employed a classification of 33 *phyla* (including the vertebrate *phylum* which we will not be dealing with here). Both reviews agree on the *phyla* in which there is explicit evidence on Associative Learning (platyhelminthes, mollusc, annelida, nematoda, arthropoda and echinodermata). As regards tardigrades, it has been recently demonstrated (Zhou et al., 2019) that *Dactylobiotus dispar* showed curling behaviour of its bodies to blue light after pairing blue light with electrical shock, compared to numerous control conditions (unconditioned stimulus only, conditioned stimulus only, backward pairing, random pairing).

Considering the bilaterian invertebrate *phyla* (all of them except cnidaria, ctenophora, placozoa and porifera), the available evidence shows that Associative Learning has not been clearly demonstrated in slightly more than half of them (but we should not dismiss the important developments that are currently taking place in taxonomy regarding the number of *phyla*). In those in which Associative Learning has been demonstrated, the evidence is increasing (platyhelminthes –flatworms–, mollusc, annelida, arthropoda and nematoda). In non-bilaterian or basal species (Holland, 2011) the available evidence is scarce, not recent, and has never been replicated (cndiaria). It would be therefore useful to briefly review the literature to detect current advances, deadlocks and gaps.

The basal *phyla* include two groups without nervous systems: porifera (sponges) and placozoa, in which there is no evidence of learning at all. Two other basal *phyla* (ctenophora and cnidaria) have diffused nervous system networks called neural nets without a central brain. In ctenophora, no literature was found on learning (Cheng, 2021). Ginsburg and Jablonka, (2019, Table 7.1, p. 332) classified these three *phyla* (porifera, placozoa and ctenophora) as animals with "not known" nervous system and conclude that there is no feasible evidence of Associative Learning in them. In cnidaria the question is, however, open to debate (see Cheng, 2021, for a recent review). Haralson et al. (1975), using light as CS and shock as US, demonstrated conditioning -as distinguished from sensitization and pseudoconditioning- in anemones. Procedural controls included substitution of light alone, shock alone, and random light and shock in place of paired light-shock trials. Responses measured were electrical output and folding of the oral disc. The conditioned response was distinguished from the unconditioned response to light and the unconditioned response to shock in terms of response latency of both electrical and behavioural measures. Nevertheless, Haralson et al.'s experiment, in spite of clearly providing evidence of conditioning in these organisms, has never been replicated. Ginsburg and Jablonka (2019) assert that Torley (2007) conducted a literature search and interviews with specialists and that he was unable to find any other evidence of conditioning in cnidarians. However, a recent systematic review by Cheng (2021) retrieved two more studies providing evidence on conditioning: Ross (1965) and Hodgson (1981). Ross's experiments (1965) with sea anemones (*M. senile*) examined their reflex of mouth opening in response to a drop of squid extract place in its oral disk (US) presented after electrical stimulation to the base (CS). This treatment evoked mouth opening during presentations of CS alone, but the various controls required to establish Classical Conditioning as interpretation were not carried out. Similarly, Hodgson (1981) presented a wide variety of conditioning phenomena with both aversive shock and appetitive food in *C. gigantean*. According to Cheng (2021), these observations are far from being full demonstrations of conditioning due to the absence of a formal presentation of the data and relevant control conditions.

In bilaterian invertebrate *phyla* the evidence is uneven, with some *phyla* about which nothing has been published (see Table 1), others in which the evidence is inconclusive (rotifera, echinodermata) and others in which the amount, variety, complexity, and methodological requirements have been satisfied (platyhelminthes, mollusca, annelida, nematoda, arthropoda). In rotifers, habituation has been reported (Applewhite, 1968) but nothing more sophisticated has been shown in these animals (Ginsburg and Jablonka, 2019, p. 333). These authors, in their systematic review, show the phylogenetic relationships of major animal *phyla*, their braininess, and their ability to learn associatively (Ginsburg and Jablonka, 2019, Figure 7.13, p. 330). They notice that there are many *phyla* in which there is no evidence of Associative Learning, mainly because research about learning is extremely limited. They conclude that a brain seems to be a necessary although no sufficient condition for Associative Learning (Ginsburg and Jablonka, 2019).

Other types of learning, according to Perry et al. (2013), such as Reversal Learning, Latent Inhibition, Peak Shift, Operant Conditioning, Contextual Learning, or Concept Learning, are more limited to insects, crustaceans and molluscs.

In another review, Álvarez et al. (2017) employed the designs on cue interactions as a criterion to analyse the experimental evidence on invertebrate learning. According to these authors, there is abundant evidence that non-vertebrate animals learn associatively. For example, in insects (Giurfa, 2015; Hollis and Guillette, 2011) there are about 50 different species that have shown Associative Learning abilities. This indicates that procedures have been developed and adapted suitably for the study of Associative Learning in many different species, although a systematic analysis of Pavlovian Conditioning has not been carried out. Just in arthropods, molluscs and plathelminths, there is evidence of basic Pavlovian phenomena such as Conditioned Inhibition, Extinction, Latent Inhibition, Blocking or Overshadowing (for a review see Álvarez et al., 2017). Among these *phyla*, two species have monopolized most of the attention: the bee and the snail, in which nearly all associative processes have already been demonstrated (including contextual effects).

On the topic of context learning in insects, honeybees routinely learn to do different navigation or discrimination tasks in different contexts. They can learn to search in diametrically opposite locations to a landmark placed in two different contexts (Cheng, 2005), or make opposite choices for access at a feeding location and at the nest (Colborn et al. 1999). Collett et al. (2006) have pointed out that ants and bees have a rich store of navigational memories that are used in adequate mechanisms to be retrieved reliably. These insects seem to reduce possible retrieval errors by linking together the different parts of a memory and by associating a memory with its own spatial and temporal context. Cheng (2006) even called context-triggered servomechanisms the basis for arthropod navigation. This line of literature provides strong support for context learning in insects.

It is also important to note that there seems to be an extended bias against the possibility of learning abilities in invertebrates. For instance, Robert Lubow, an international authority in Latent Inhibition and an expert in attentional processes in Pavlovian Conditioning, discusses in a review about the phylogeny of this associative effect (Lubow, 2010) the possibility of that kind of learning in invertebrates. He refuses as evidence any result in which there is no record of CR during the pre-exposure phase. Based on this argument, he concludes that:

> The relative simplicity of the invertebrate nervous system makes that notion somewhat fanciful, perhaps residing more in the brains of the experimenters than in the relatively simple organisms that they study. The sensory world of the invertebrate may be so restricted that there is no meaningful distinction between stimulus and context. (Lubow, 2010, p. 217)

Many misconceptions converge here. The “nervous system of invertebrates” is a very broad category that includes many different types of nervous systems that can be very complex, showing, for instance, brain and behavioural lateralization (Frasnelli, 2013). The “sensorial world of invertebrates” (as a whole) is much more complex than the human sensorial world both in types of senses and in the stimulation ranges available. To give just one example, humans cannot see ultraviolet light, but many arthropods can. Marshall and Oberwinkler (1999; see also Marshall, et al. 2007, for a review) have found that some species of stomatopod crustaceans (mantis shrimp) have 12 different photoreceptor types, each sampling a narrow set of wavelengths ranging from deep ultraviolet to far red (300 to 720 nanometers) making the impressive colour-vision system in these stomatopod crustaceans unique.

Although it is true that contextual effects are less common in invertebrates, some evidence was already available in 2010, when Lubow published his review. In addition to the works already mentioned by Cheng and cols. (Cheng, 2005; 2006; Colborn et al., 1999; Collett et al., 2006), McComb et al. (2002), for instance, had shown contextual specificity of extinction (renewal) in *Lymnaea*. In recent years, some contextual effects in planarians and snails have been demonstrated. Prados et al. (2013) have shown cue competition effects in planarians, which involve similar demands than contextual effects. These authors showed that planarians were susceptible to basic conditioning in that they readily developed a conditioned response to a change in ambient luminance when it was consistently paired with an electric shock. With this procedure they showed that if the change in luminance was presented in a compound with a vibration stimulus during conditioning, subsequent tests revealed poor conditioning of the elements compared with control groups in which the animals were conditioned in the presence of the elements alone (overshadowing). Finally, and more relevant here, in Experiment 3, pre-training of one of the elements before compound conditioning resulted in blocking of learning about the other element. In snails, recent experiments in our laboratory indicate renewal using an appetitive Pavlovian Conditioning (Loy et al., 2020). In these learning experiments, snails eat a piece of carrot (US) while they are smelling a particular odour (CS). This experience produces an increase in the tentacle lowering response (CR), possibly an exploratory behaviour. Renewal is the recovery of an extinguished CR to the CS due to a change in context, similar to the recovery due to the passing of time after extinction (spontaneous recovery) or the re-experiencing of the US after extinction (reinstatement). These two extinction effects have already been demonstrated in preparations involving the lowering of tentacles in snails (Álvarez et al., 2014). In these experiments, an ABA paradigm was employed, so conditioning occurred in context A (light), but extinction took place in context B (darkness). When snails were placed back in context A and presented with the CS they showed recovery of the CR. These works indicate that invertebrates can effectively encode contextual stimuli.

Other misconceptions about leaning in invertebrates have been refuted by experimental results. For instance, it is usual to assert that the behaviour of ants is strongly controlled by pheromones and that they do not need to learn (Sasaki et al., 2014). However, there are many relevant behaviours directly determined by learning. For instance, foraging desert ants are known to learn a wide variety of cues to adequately navigate their environment (for a review see Freas et al., 2019). Ants display pre-foraging learning walks in which they learn the panorama around the nest, frequently looking back to it. These walks increase in duration and distance travelled with increasing experience. They also learn in their foraging trips, also looking back, different feeding-site panoramas. It is worth noting that ants do not only learn visual cues of their surroundings, but also magnetic, vibrational, olfactory and tactile cues. Furthermore, Nowbahari et al. (2009) reported experimental evidence that the ant *Cataglyphis cursor* displays rescue behaviour to free entrapped conspecifics. In the experimental preparation, conspecifics are ensnared with nylon thread and partially buried in sand. The ants are able to recognize the particular demands of this task, digging and transporting the sand to expose the nylon thread and then biting it. This biting behaviour, never reported before in the literature, shows that rescue behaviour is far more complex than other forms, as limb pulling and sand digging, which can be the result of a very simple mechanism, such as a chemical call for help. It is difficult to see how this mechanism could guide the behaviour of ants in such a precise way, as they go to a precise location of the nylon thread and bite just the thread.

Another widespread assumption is that the passive life of some species does not demand learning. Most insects chosen for Associative Learning studies have been those that move about their environment as they actively seek food, locate a host, evade a parasite or avoid some noxious stimulus (see Hollis and Guillette, 2015, for a review). Therefore, the extremely sedentary predatory behaviour of pit-digging antlions (Neuroptera: Myrmeleontidae) make them unlikely candidates for learning. These larvae dig pits and then sit at the bottom and wait, sometimes for months, for a prey to fall inside. Nevertheless, Hollis et al. (2011) experimentally paired a CS (some sand in the hole area) with the presence of a victim ant. Antlions that received this treatment used the preys more efficiently and pupated significantly sooner than the ones in the control group in which the CS and the presentation of a victim was not paired.

Learning plays an important role in a wide range of relevant biological functions in many species of invertebrates and even in situations in which we think that it would be unnecessary. For instance, the silkworm moth (*Bombyx mori*) is a monophagous insect, so we could assume that they do not need learning abilities to find food or choose the best site to oviposition. Nevertheless, Gámez and León (2018) have shown that moths that jointly experienced an odour (CS) and mulberry leaves (the preferred oviposition place for the moths) preferred to lay their eggs near the odour when it was present, whereas moths in which the odour and the mulberry leaves never appeared together showed no preference.

In conclusion, not only relatively simple associative abilities are present in invertebrate species. This is the reason why many authors have begun to use the term ‘cognition’ in order to describe the behavioural skills of some invertebrates such as insects (Giurfa, 2015) o cephalopods (Mather and Dickel, 2017). Phenomena like tool use (Loukola et al., 2017; Mhatre and Robert, 2018), face recognition (Chittka and Dyer, 2012; Avarguès-Weber et al., 2018), quantitative competence (Skorupski et al., 2017; Howard et al., 2019;

Giurfa, 2019), and learning by observation (Leadbeater and Dawson, 2017) are becoming common issues in psychology and neuroscience journals (Chittka et al., 2019). After the Darwinian revolution, concepts such as intelligence, culture, purposefulness, intentional behaviour, thinking, or language had to abandon the exclusive domain of the human being. Firstly, those concepts were assimilated in primatology (Beran et al., 2016); cetaceans (Chinea, 2017; Marino, 2004), dogs (Clark et al., 2019; Byosiere et al., 2018), and birds (Mettke-Hofmann, 2017; Güntürkün and Bugnyar, 2016; McMillan et al., 2015; Pepperberg et al., 2019). Currently, something similar is happening with cognition in invertebrate species and it has been shown that, for instance, insects display a variety of phenomena involving simple forms of tool use, attention, social learning of non-natural foraging routines, emotional states and metacognition, all phenomena that were once thought to be the exclusive domain of much larger-brained animals (Perry et al., 2017). The octopus is perhaps the most impressive case. In a recent review (Schnell and Clayton, 2019), examples are presented showing clear evidence of the outstanding cognitive skills of cephalopods. For instance, octopuses show a high behavioural flexibility to solve foraging problems (Mather and Dickel, 2017). They can suck, drill, rasp or even use water as a tool to extract their preferred preys from their protective armours.

In conclusion, Associative Learning, with all the psychological processes that it involves, is present among a remarkable number of current *phyla* of invertebrates. Tardigrades seems to be the latest addition (Zhou et al., 2019). However, there are still many *phyla* in which not a single species' learning capacities has been investigated.

The adaptive advantage that results from having learning skills has led Simona Ginsburg and Eva Jablonka to postulate it as an essential cue to understand the Cambrian explosion (Ginsburg and Jablonka, 2010), and as one of the main factors in the origin and evolution of consciousness (Ginsburg and Jablonka, 2019; Birch et al. 2020, Ginsburg and

Jablonka, 2021). For these authors, if the transition to life is marked by a system's capacity to manifest unlimited heritability (Maynard Smith and Szathmáry, 1995), the transition to consciousness is marked by five mayor learning capacities (corresponding to five mayor transitions) from learning in non-neural animals to human symbol-based cognition and cultural learning. Two of these transitions are focused on Associative Learning: the transition to animals showing elemental Associative Learning, and the transition to animals capable of Unlimited Associative Learning (UAL). UAL is an overt behavioural ability to attach motivational value to a compound natural (or ecological) stimulus and a new action pattern. This way of learning could have played a relevant role in the origin of consciousness (Ginsburg and Jablonka, 2021).

Comparing the possibilities of different *phyla* inside the vast amount of invertebrate species, from rotifers to octopuses, and taking into account the necessity of linking cognition abilities (included learning) to ecological demands (Loy et al., 2017), we have seen that learning abilities are very widely spread through the invertebrate *phyla* so far investigated, and that they are well tailored to particular goals. At the same time, the amount of available information on Associative Learning in invertebrate species is very limited, and much work remains to be done in the field of invertebrate Classical Conditioning.

Learning in Plants

Plants arise from eukaryotes cells (protists), and they represent the evolutionary line of one of the three ecological strategies in macroscopic organisms: production, along with absorption (fungi) and consumption (animals) (Margulis and Schwartz, 1982, p. 10).

Since 1960, the interest in studying learning in plants increased due to the possibility of learning in organisms without nervous system (for a review see Abramson and Chicas-Mosier, 2016; Adelman, 2018). Maher (2017) suggested that, although plants do not have representational minds, they could have minds in the more general sense of being alive and being autopoietic-and-adaptive, and several authors attribute cognition (understood as intelligence, mind, or in general, psychological processes) to plants based on their basic orientation trends (Calvo et al., 2020b; Gagliano et al., 2012; Mancuso and Viola, 2013) and communication abilities (Rhoades, 1983). The study of Dudley and File (2007) in the annual plant *Cakile edentula* showed that allocation of roots increased when groups of strangers shared a common pot, but not when groups of siblings shared a pot. Callaway and Mahall (2007) interpret these results as plants being able to discriminate and recognize their kin. However, this cannot be readily accepted as evidence of discrimination and recognition in plants, as the control conditions needed to rule out alternative explanations, such as changes in the soil due to the presence of stranger plants, are not included. As an example of communication in plants, the study of Rhoades (1983) in sitka willow (*Salix sitchensis*) showed that when plants were experimentally infected, they emitted signals that allowed their healthy neighbours to produce defences. However, although plants produce phytohormones as a response to an external threat and their neighbours detect them (Gális et al., 2009), that should not be straightforwardly accepted as evidence of intelligent or purposeful communication due to the absence of the typical controls employed by experimental learning psychology that aim to discard simpler explanations such a reflex reaction to the substance emitted by the other plant.

Abramson and Chicas-Mosier (2016) reviewed the work on habituation and conditioning in *Mimosa pudica* and concluded that there are few positive demonstrations with appropriate controls, and they have not been clearly replicated. Adelman (2018) reaches equivalent conclusions in a more recent review. In Table 2 the studies on learning in plants are shown.

One of the most important studies in habituation is Gagliano et al. (2014). Habituation was observed using a drag drop system to induce the defence response in *Mimosa pudica.* Plants were divided in two groups; in one of them plants received a high-light stimulation and in the other they received a lower-light stimulation. Firstly, during the habituation phase, plants received several presentations of drops. Next, a dishabituation test was carried out with a new stimulus that produced shakes. Finally, plants were again exposed to the drops, 6 days later. The results showed that leaves re-opened fast and stopped closing altogether. Furthermore, opening and closing of the leaves happened more rapidly in lower-light stimulation group. The same result was obtained in a replication when the test was conducted 28 days later. These results are interpreted as undoubted evidence of habituation in plants by Gagliano et al. (2018) and by Abramson and Chicas-Mosier (2016) but, according to Biegler (2018), Gagliano et al. (2014) carried out a specific stimulus test as a dishabituation test, in which shakes instead of drops were used to elicit the defensive response. Biegler argued that both stimuli should be counterbalanced to show that there are not differences between them in leaf-folding reflex. Therefore, this experiment did not include the correct dishabituation test and consequently sensory adaptation, or motor fatigue cannot be discarded. Thus, it is necessary to replicate the study.

Regarding Associative Learning, few studies show clear evidence of it in plants, and they all have several limitations (for a review see Adelman, 2018). For example, in Armus (1970), in which a Pavlovian Conditioning procedure was employed with *Mimosa pudica*, darkness (CS) was paired with a shake (US) and the results showed that darkness elicited the defensive response, which consisted of the leaflet folding and the stem drooping. However, darkness is not a neutral stimulus because it elicits the leaflet folding without being paired with the US. Furthermore, plants did not receive the treatment individually, so the results can reflect the interactions between the pairs of plants but not the behaviour for each one.

Gagliano et al. (2016) is the only study that shows how the neutral stimulus position predicts the location of a relevant stimulus (the light source) in pea seedlings *Pisum sativum cv* Massey gem. Experiment 1 consisted of a training session in which a group of plants was exposed to an airflow (CS) paired with light (US) in the same arm of the maze, while another group of plants was exposed to the airflow in the opposite arm to the light. Then, during the test, half of the subjects of each treatment were only exposed to the airflow and the other half were left intact as a control group. The results showed that plants which were exposed to the airflow paired with the light during training grew in the direction the airflow was presented during test while plants which were exposed to the airflow in the opposite arm to the light during training grew in the opposite direction to the airflow during the test session. Experiment 2, in which the influence of the time of the day in learning was tested, replicated the main results of Experiment 1. According to Adelman (2018), in Gagliano's study (2016) the CS could play the role as discriminative stimulus and the US could play the role as reinforcer, so the plant growth in one of the arms was reinforced by access to the light, showing Operant Conditioning rather than Classical Conditioning. However, in a general Operant Conditioning procedure, a subject must perform the response to receive the reinforcer and in this case the plants received the light regardless of their response (growth direction), so it cannot be considered strictly Operant Conditioning. Nevertheless, Markel's (2020) replication attempt failed to reproduce this result. Markel used a different type of pea (*Pisum sativum cv* Green Arrow) but incorporated a bigger sample size and a rigorously blind observation procedure (unlike Gagliano). Also, Markel points out that the criterion used to decide plant inclination was not fully operationalized in the study by Gagliano et al. (2016).

In sum, the limitations and other alternative explanations in the studies of orientation trends (Calvo et al., 2020b; Gagliano et al., 2012; Mancuso and Viola, 2013) and communication abilities (Rhoades, 1983) do not seem conclusive enough to claim that plants show Associative Learning, and can only contribute to polarize the debate to extreme positions, as those that extremely reject cognition in plants (Taiz et al., 2019). Furthermore, despite the positive evidence of Non-Associative Learning, many of the studies have not been replicated. Also, there is a lack of Pavlovian Conditioning evidence even with the few species of plants chosen, and this evidence usually shows non-replicated or opposite results as well as inappropriate experimental designs (see, for example, the comparison between the results in Gagliano et al. (2016) and Markel (2020)). Such a relevant result would modify our conception of an entire kingdom of the living beings, so it is necessary to replicate Gagliano's studies before the results in them are definitely accepted, considering the differences between plants and animals when proposing suitable experimental designs, as Adelman (2018) suggests.

Learning in simple (unicellular) organisms

Since its inception, Comparative Psychology has been interested in unicellular active life, such as various species of paramecia and amoebas (Jennings, 1906), now classified within the kingdom Protista.

Single-celled organisms are found spread over eukaryota and prokaryota forms of life. Apart from the aforementioned classic studies, the regulation of orientation in terms of chemotaxis has been considered such as coordinated behaviour in bacteria as *Escherichia coli* (Koshland, 1980). Defined as detection of variation in the concentrations of substances in the environment and response to them through movement, chemotaxis tells us about how prokaryota, as *E. coli*, are capable to orient themselves in order to move towards nutritious substances or move away if they result aversive (Sterling and Laughlin, 2015).

Due to its theoretical interest, the tradition has never ceased to look for evidence of unicellular learning, and this has been carried out by using experimental preparations of

Associative Learning, more or less simplified or adapted to the stimular "umwelt" of the organisms. Associative Learning methods allow rigorous analysis of the procedures, which has sometimes led to discovering methodological limitations that, once corrected, have promoted new research. Given the richness and continuity of this tradition, in which dozens of experiments have been carried out, mostly with paramecia, we will begin this section with a brief review of the most relevant results on Associative and Classical Conditioning. As we will see in the following lines, the efforts in the research on conditioning have been basically found in the eukaryotes *Paramecium* and *Physarum policephalum*.

Learning in Protista: *Paramecium*

This eukaryotic organism has been the most studied protist by means of Pavlovian Conditioning methods. Its natural environment is fresh water and it shows an approximately 0.05-millimetre size. 12 different species of paramecium (NCBI: txid5884) have been registered, and all of them present an oval shape and have a ciliary ensemble around its body.

From the early studies by Jennings (1904; 1906) to the most recent ones (Alipour et al., 2018), the same pattern can be detected about learning research in this eukaryote. The early works pointed out that learning in paramecia effectively occurred, but subsequent authors questioned the results by arguing the absence of control of several variables. For instance, the general theoretical position defended by Jennings (1906) or Binet (1889), which assumes very basic learning results through the successive trials and errors made by the paramecia, was contended by Loeb (1918), who defended that these behaviours were explainable only through mechanical tropisms. Nowadays, those rejections can be reconsidered in the light of recent developments (see Gershman et al., 2021, for a recent review).

Something similar can be found in the work of Gelber with *Paramecium aurelia*, (Gelber, 1952; 1958), who found a higher concentration of these organisms on the side of the preparation where they were fed with bacteria. Jensen (1957a; 1957b) tried to shed light into some experimental details and proposed that bacteria could cause chemical changes in the paramecium that modify the environment and could be attractive to others. This is an important point, which responds to the well-documented chemotaxis phenomenon in single-celled organisms (Koshland, 1980; Sterling and Laughlin, 2015). Two taxis mechanisms, depending on attractive or repellent chemical substances in the environment, have been studied in these organisms. Attractive concentrations cause changes in the polarization of the paramecium membrane, and a subsequent increase in swimming speed is usually observed (Van Houten, 1978). All in all, the main issue is whether chemotaxis is a mechanism of its own or if it reflects changes due to experience. This is essential to understand the debate between Jennings and Loeb, and to determine if Gelber's research demonstrates learning in *Paramecium* or not. It is clear that this controversy has not been solved yet, as proven by the current debates in the scientific community. Gershman et al. (2021), for example, conclude that Gelber's experiments convincingly demonstrate Pavlovian Conditioning in paramecia. However, Katz and Deterline (1958) failed to replicate Gelber's results. In a similar way, Lepley and Rice (1952) claimed to have found clear evidence of a tendency to turn to the opposite side of a T-maze when the paramecia had made an initial forced turn. These authors claimed that the results could be assumed as an example of Hull's reactive inhibition (Hull, 1943). However, neither Lachman and Havlena (1962) nor Harvey and Bovell (2006) replicated Lepley and Rice's experiment. Hanzel and Rucker (1972) and Huber et al. (1974) showed data about escape from tubes by paramecia as proof of the use of trial-and-error strategies. However, neither Applewhite and Gardner (1973) nor —in a more recent review—

Hinkle and Wood (1994) were able to accept such conclusions, according to their own results.

Applewhite (1979) reviewed and replicated some experiments about learning in protozoa from pattern learning to conditioning. He found no positive or reliable results in terms of conditioning in paramecia because they were obscured by the absence of proper control groups or inadequate control of physical changes in the preparations. An exception to this pattern of conflicting data is found in the most cited work on *Paramecium caudatum* learning by Hennessey et al (1979). These authors paired a vibration (CS) with a shock (US), a treatment that resulted in the CS producing a contraction motor conditioned response. Their experiments were appropriately designed and included replicas with different vibration intensities and adequate sensitization, pseudoconditioning and truly randomized control groups. From these results, if a reliable conditioning protocol is clearly confirmed and established, a whole biological field would be opened to work on protist behaviour. Surprisingly, despite being mentioned in many works as incontrovertible proof of conditioning in paramecia, the experiment by Hennessey et al. (1979) has never been replicated.

More recently, Armus et al. (2006a) trained a discrimination with cathodic stimulation, which is appetitive, paired with light or dark in a counterbalanced way. Three groups were used: an experimental group, which received cathodic stimulation (US) in a light or dark place (CS); a control group that never experienced the US, and a third group that received the same amount of cathodic shocks as the experimental groups but anywhere. The results revealed that the paramecia in the experimental group spent more time near the side that had been paired with the cathode, either illuminated or dark, than the other groups. Alipour et al. (2018) partially replicated the results of Armus et al. (2006a). They observed how paramecia linked high light intensities with cathodic shocks, but not dark stimulation.

This recent research reinterpreted Armus et al. (2006a) work as an unclear result, as they did not make a distinction between light and darkness as CS.

In essence, there are three studies presenting outstanding results on Associative Learning in paramecia (see Table 3 for a summary): Gelber, (1952; 1958), a polemic result which has not been successfully replicated, Hennessey et al. (1979), which was never replicated, and Armus et al. (2006a), which produced an artifactual result as the light-dark counterbalance was not properly analysed. However, Alipour et al. (2018) confirmed that there can be an association between intense light and cathodic stimulation in paramecia. Finally, it is important to highlight that there are no habituation works available on learning research in paramecia. This means that, although some classic learning paradigms have been observed in this protist, simpler phenomena have not been tested yet. Apart from chemotaxis, it is worth mentioning a study that showed flexible behaviour in paramecia benefiting from previous experience (Kunita et al., 2014). These authors have extensively studied a mathematical model to understand mobility and escape behaviour in paramecia. They placed paramecia in a capillary tube model that included an obstacle: an oil drop. Obstacles generate several avoidance back-swimming responses in paramecia (Short Backward Swimming or SBS), in conditions like narrow tubes that prevent them from turning. In Kunita el at. (2014), paramecia first showed SBS and failed attempts to avoid the drop. One minute after, SBS stopped in favour of increasing distance of back swimming (Long Backward Swimming or LBS). The authors consider this novel behaviour as evidence of intelligence. Also, Armus et al. (2006b) measured avoidance in paramecia that received an anode shock (aversive) paired with light or dark. During training, the paired group reduced progressively its time around the anode, while this measure was constant in the control group.

Learning in Protista: *Physarum polycephalum*

Physarum polycephalum is an eukaryota, traditionally included in the Protista kingdom and taxonomically classified in the *pyhlum* Amoebozoa for being able to move or feed through temporary projections of the cytoplasm called pseudopods in a vegetative phase of its life cycle. *Physarum* lives in a world of gradients, concentrations of attractants and repellents, and its behavioural responses are a direct consequence of their interaction with these gradients (Vallverdú et al., 2018).

Recently, *Physarum* has been claimed as the ideal organism to study Minimal Cognition. Some authors have pointed out that its ability to keep only the pseudopods that lead to a food source by the shortest path can be interpreted as evidence of cognitive processes such as problem solving, learning or memory (Smith-Ferguson and Beekman, 2019). The most renowned example could be maze-solving (Nakagaki et al., 2000), in which different pieces of the same plasmodium were distributed on a neutral agar surface with a labyrinth shape delimited by plastic walls. Four hours later, all the plasmodium pieces had collapsed into one that covered the entire surface of the maze. Then, two blocks of nutrient agar were placed in two points (entry-exit) of the maze. Four hours after the maximum elongation of the plasmodium, the way in which the pseudopods of the *Physarum* covered the labyrinth was observed. The shortest path was always preferred to the longest while there were no differences in choice between similar-length ones. Despite the undeniable utility of the results of this study for the field of engineering or mathematics, among others, this is not a genuine learning maze experiment, as the organism is allowed to maintain direct contact from the beginning with the reward (exit). The reconstructed plasmodium does not seek or decide within the labyrinth, but it simply expands and contracts over it depending on the absence or presence of externally provided food, fitting its surface to the minimum size necessary to transport these nutrients from one side to another (Ray et al., 2019).

If we analyse the literature on alleged Associative Learning in *Physarum* (see Table 4), we can conclude that, even today, it does not meet the minimum control requirements demanded in the Pavlovian Conditioning field. For example, Shirakawa et al. (2011) jointly presented an agar field at a temperature of 20°C (CS) with access to oat flakes (US) and a 25°C field without US, in an open field arrangement with free access to both sides. After training, a test choice was made in a limited field with one end at 20ºC and the other at 25ºC. Unfortunately, the control group was not described, it was only said “that was without the Associative Learning procedure” (p.102). We will assume that it was a naïve group. In any case, the pictures of the training phases and the bar graph of the distance travelled in the CS field by experimental and control *Physarums* are not enough to conclude the effectiveness of the conditioning treatment. A data analysis of occupied areas or distance travelled during each trial would have been a more accurate evidence of conditioning. Unfortunately, this information is not available. Similar objections, regarding analytical and procedural deficiencies, could be made to Saigusa et al. (2008) when they claimed that the *Physarum* is able to anticipate periodic environmental events marked by regular changes in temperature and humidity. In this study no data were provided about control groups kept at standard conditions along the trials, or maintained under the different conditions, or with changes from dry-colder to standard conditions.

The same methodological problems are found in other attempts to demonstrate Associative Learning on Amoebae *phyla*. An example is De la Fuente et al. (2019), who claimed to have found evidence of conditioned behaviour in *Amoeba proteus* and *Metamoeba leningradensis*. In their work, cells seem to associate an anode (negative galvanotaxis) with a peptide (positive chemotaxis). After training, the systemic movement of cells responded to the presence of an electric field by migrating towards the anode instead of migrating to the, supposedly unconditioned, cathode. However, as they conclude, their promising findings cannot be considered as Classical Pavlovian Conditioning since complete controls and parametric analyses for Classical Conditioning studies have not yet been performed.

Nevertheless, Associative Learning is not the only way of learning, and it cannot be ruled out that unicellular organisms can be benefit from their own experience. On the contrary, we can find a clear proof of habituation in these organisms in a study carried out by Boisseau et al. (2016). The authors landed the plasmodium in one side of a preparation with two chambers connected by a bridge. *Physarum* had to cross the bridge to reach food. When quinine, an aversive substance, was placed on the bridge, a reduction in the area of the bridge occupied by the plasmodium was observed as compared to other subjects without any substance. After five trials, the *Physarum* with quinine occupied the same amount of bridge area as the *Physarum* in the control group. On the sixth trial, the *Physarum* of the control group received quinine for the first time and, as a result, the area of the bridge occupied was drastically reduced if compared to the experimental group (see Boisseau et al., 2016, fig. 5). With this procedure and a suitable experimental design, they showed habituation and spontaneous recovery of the initial reaction after withdrawal of the habituated stimulus. They also showed specificity to stimulation (quinine-caffeine) of the habituation response, which excludes explanations based on sensory adaptation or motor fatigue. Boussard et al. (2019) have subsequently corroborated Boisseau et al.'s results, adding improvements to the procedure that offer a reliable and replicable experimental preparation to study habituation in slime mould.

In sum, only habituation has been clearly demonstrated in *Physarum polycephalum* (see Table 4 for a summary), although the only replica available has been made by the same research group. In spite of the effectiveness of the associative procedure for the study of learning in many animal species, the study of this topic on *Physarum* has not rendered any feasible results. There are some results that seem to show basic learning abilities, but it cannot be concluded that Associative Learning has been clearly demonstrated in this unicellular organism.

Associative Learning, Minimal Cognition and Minimal Development

More than a century of experimental work has not conclusively demonstrated association in single-celled organisms -as pointed out in Dussutour´s review of learning in single cell organisms (Dussutour, 2021)- while it does usually happen in animals, not only vertebrates, but also in a significant number of invertebrate *phyla*. Recent studies with plants, although promising, are not conclusive. Conditioning preparations show serious difficulties in building associations at unicellular level, given that the only work that can be considered as evidence of conditioning is the one by Armus et al. (2006a), as replicated by Alipour et al. (2018). Although more associative experiments can and should be done (it is evident that the interesting results by Gelber (1952; 1958) and Hennessey et al. (1979) should be replicated), this current study —though containing negative results— is already an important scientific achievement that should be taken into account by association psychologists. It is important to consider the possibility that the conditioning paradigm may reach its limits when it enters the realms of the unicellular. As there is no reason to suppose that association is the only possible operationalization of adaptive experiential processes or that it is the unique way to define learning or animal intelligence, it is worth considering other existing approaches. Minimal Cognition and the Developmental-Comparative tradition are the two to be briefly considered here, aiming to emphasize some aspects of both approaches that may be complementary to each other and to shed light on the aforementioned limitations of the associative paradigm.

In the 21st century the interest in the intelligence of the “lower” organisms, including protists and bacteria, has grown significantly, adopting the label “Minimal Cognition" (van Duijn et al., 2006; Lyon, 2015; Baluška and Levin, 2016; Vallverdú et al., 2018; Smith-

Ferguson and Beekman, 2019; Reber and Baluška, 2020). Some of the most prominent authors in this approach, as Lyon (2015) and van Duijn et al. (2006), are close to the Embodied Cognition thesis, rejecting the computational tradition and the concept of representation, and focusing on the complex mechanisms and sensory-motor adjustment processes found in that organisms, including bacteria. Other authors maintain a view of cognition that is closer to the information processing tradition, stressing that the basis of intelligence is signal processing, which is an intrinsic feature of life as “all living organisms require some form of information processing” (Smith-Fergusson and Beekman, 2019, p. 2), or “all interactions in a system are information” (Calvo et al., 2020a, p. 3). They also postulate that cognition would have arisen from the most basic trophic interactions of the organism with its environment, as appropriate food sources need to be detected, as well as internal states of need or satiation: “when a bacterium senses an attractive chemical in its environment, this signal is processed internally and induces a change in behaviour: the motor response” (Smith-Ferguson and Beekman, 2019, p. 2). Thus, for the latter authors, cognition, as information processing of signals, is considered coextensive with life.

Minimal Cognition often expresses the goal of building a sort of Cognitive Biology as an alternative of other traditions involved, such as Comparative Cognition or Comparative Psychology. Although it is not original in bringing intelligence to “lower” organisms (H. S. Jennings (1904) Lloyd Morgan (1896) and J. M. Baldwin (1976) did it with functionalist and Darwinian inspiration), it has collected and developed research on a vast amount of phenomena that normally are not associative but are adaptations related to experience and/or important physiological or biochemical features to understand experience in those simple organisms: complex sensory systems in bacteria or protists, rapid response processes in plants, signal transmission in non-nervous tissues, surprising adaptations in the *Physarum policephalum*, or evidence of memory in single cells. All these phenomena enrich and push the field beyond the traditional approach of Associative Learning. Nevertheless, Minimal Cognition does not offer a uniform and systematic approach to learning. Sometimes it uses procedures or (supposed positive) results coming from conditioning in plants, *Physarum* or paramecia (Smith-Fergusson and Beekman, 2019; Baluška and Levin, 2016), or habituation and sensitization in bone cells or plants (Baluška and Levin, 2016). It even claims that "acquisition of novel behaviors on unicellular species is associative in nature" (Reber and Baluška, 2020, p. 4). Sometimes Minimal Cognition tends to consider learning in a broad sense as "experience-modulated behavioral change" while conditioning is classified as a "narrowly human conception" (Lyon, 2015, p. 3). As an illustration of learning in the broad sense, Lyon collects experiments on non-associative learning in bacteria. In one of them, some cells were incubated in a medium with a lack of a fundamental nutrient. In a second phase of the experiment, these cells responded to new limiting conditions faster than control cells previously incubated in a medium with high concentration of the fundamental nutrient (Lyon, 2015; Hoffer et al., 2001).

The idea of "experience-modulated behavioural change", without the need for an association to take place, is important and it is not new either. The aforementioned Development-Comparative tradition -represented by the works of the zoologist and comparative psychologist H. S. Jennings (1904)- set things that way a long time ago, and is worth considering here, both to observe its overlap with Minimal Cognition, and also to emphasize its focus on a developmental approach that is not present in Minimal Cognition.

Jennings (1904) investigated the behaviour of unicellular cells in the late 19th and early 20th centuries and explained the phenomena by applying the notion of trial and error of the comparative zoologist and psychologist C. Lloyd Morgan (1896) and the evolutionary and developmental psychologist James Mark Baldwin (1976). For Jennings, the theoretical unit to understand unicellular behaviour is not a direct stimulus-response reaction ("forced by the stimulus", as Jacques Loeb's supporters said at the time), but a sequence of action through which the organism solves a basic adaptive problem, such as progressively approach a food source, progressively escape from a lighted area in *Stentor*, or completely adhere to a solid object while swimming in amoeba. The adaptive result comes from the repetition of the available and simple repertories of movement in a sequence continuously regulated by contextual experience (sensing food or light gradients, for example). So, it is not a single response but a temporal sequence where every step implies a relation to the previous one to secure the progress (Jennings, 1904).

A typical case is that of a paramecium that, by sensing an aversive chemical or light stimulus, performs the following sequence: first it stops or reverses slightly; then it tilts its front end and turns on its longitudinal axis, and then advances again, so that its direction has changed significantly in some degrees. The organism repeats the process until it detects a significant change in luminosity or chemical gradient and judges the current state as acceptable. Then, it stops making changes and stays in the acceptable zone (Jennings, 1904). By a similar method, the organism tries several angles until it detects the appetitive stimulus at a concentration sufficient to not change direction, move on, and eventually make contact with the appetitive stimulus (for example, food). In the case of an amoeba that swims and touches a solid object with one of its pseudopods, the rest of the pseudopods that fail to contact are contracted in the direction of the contact: the entire amoeba moves in that direction, inhibiting the emission of pseudopods, until it is fully attached (Jennings, 1904; Nakagaki et al., 2000). In all cases we find an organized sequence of trials whose consequences have to be felt or evaluated as partial successes or errors. The available repertoires of movement, typical of each species, are undoubtedly limited and stereotyped, but their modulation and sequencing are flexible and adjustable to context to some degree.

The behavioural process described by Jennings (1904) is the minimal expression of a developmental process, a “Minimal Development” as we could call it. Some improvements in the execution efficiency of the activity pattern can be identified, precisely due to its repeated exercise, regulated by experience. It is also a case of “experience-modulated behavioural change” in Lyon´s (2015) sense, and also a hierarchy of avoidance behaviour, as demonstrated in *Stentor roeseli* by Dexter et al. (2019). They can be improvements in the number of trials or in the time required to reach a valuable stimulus or to escape from a harmful one. It is the researcher who has to identify the parameters to be measured. These improvements are less lasting and more ephemeral (minutes, perhaps hours at most), if compared to long-term memory in animals (hours, days and even long-life periods), but they are improvements after all.

Jennings warned that the description of behavioural activities had to be made using meaningful terms, even if they were very basic, such as “attractive”, “aversive”, “detection”, or “error”. Those terms cannot be eliminated and replaced by the sole description of physiological or biochemical sequences because this would be, in Jennings’ words, “an endless circumlocution” (Jennings, 1904, p. 252). Accordingly, mechanical sequences (physiological or biochemical) can only make sense when understood in the framework of the global strategy of the organism to solve a general adaptive problem. He goes further and justifies his argument through the following analogy: if when dealing with human behaviour we assume that terms such as pleasure and pain are relevant (as correlated with physiological states that promote the escape of a large number of stimuli that have nothing physically in common) we cannot deny that they are also relevant when we deal with simpler behaviour. And if we deny it for the simplest organisms, we should be willing to deny it for the human case. In this way Jennings points to the idea that meaning belongs to the organic sphere: feeling or sensing are the ways in which the organism relates to its environment and the way in which we should approach the study of the behaviour of organisms, including humans. Modern theory of the Cellular Basis of Consciousness, agrees with Jennings in this regard and claims that "sentience was a property of the first form of life that emerged some 3.5 billion years ago" so "life and sentience are coterminous" (Reber and Baluška, 2020, p. 1). This is also the proposal of Ginsburg and Jablonka (2019), in their book on the origins and evolution of sentience or minimal animal consciousness.

What phenomena should we include in this category of Minimal Development? The trial-and-error orientation process described by Jennings probably constitutes one of the most important sets of behavioural adjustments in single cells, and it can lead to certain improvements, as he himself reported (Jennings, 1904). Recently, Dexter et al. (2019) have replicated these observations, demonstrating a hierarchy of avoidance behaviours in the ciliate *Stentor roeseli*. Sensitization and habituation, to the extent that they are found in unicellular organisms, would be clear examples of ephemeral improvements of execution efficiency of activity patterns. And so would be Lyon's examples of "experience-modulated behavioural change" already mentioned. But we also can find those improvements in phenomena that go unnoticed when only Associative Learning is considered. For example, let us consider the experiment by Armus et al. (2006a) described before. In this experiment, we can find a kind of escape facilitation due to repeated experience. A group of paramecia was repeatedly exposed to an aversive situation, from which they managed to escape. When exposed to the same situation after some time, that group escaped slightly faster than a control group of naïve paramecia (Armus et al., 2006a). This is an improvement in the execution of a basic behavioural pattern, achieved precisely by its repetition, which shows that this repertoire is not completely rigid but possesses some plasticity, some potential to take a developmental step. It is an adaptive achievement mediated by experience that may go unnoticed if we only pay attention to whether associative avoidance learning occurs. We could consider it, as we have said, as a case of Minimal Development, since it is produced through the active exercise of an available repertoire that adaptively transforms the repertoire itself in some parameter.

The term developmental (as used in Developmental Theory and Developmental Psychology) does not refer only to Piagetian child development or to the new contributions on life span, although human development has certainly received priority attention in the field. Developmental Psychology was born just to try to understand from a Darwinian point of view the phylogenetic origin and ontogenetic construction of higher faculties and abilities, such as abstract thinking, categorization, and voluntary action. For this reason, it was born as linked to Comparative Psychology, trying to offer some insight about the basic forms of learning in simple, even unicellular, organisms. *Mental development in the Child and the Race*, original published in 1894 (Baldwin, 1968) and *Development and Evolution*, originally published by Baldwin in 1902 (Baldwin, 1976), are the foundational works of this perspective, which provided part of the inspiration for Jennings's experimental work. In them Baldwin presents several learning processes that he calls Circular Reaction. The most elementary of them is the Organic Circular Reaction, which emphasizes, as we have been doing here, that it is by means of the contextual exercise of the organism's sensory-motor patterns that some adaptive novelty is achieved: a change, however minimal, in the pattern, even before we can speak of associations. These repertories depend entirely on its bodily organization, as Embodied Cognition stresses today, and are plastic enough to allow changes, including the learning of new habits (which imply new meanings in Jennings sense already mentioned) in more complex organisms.

A Minimal Development approach would be no more than the extension to unicellular organisms of that large existing Developmental-Comparative tradition that, for example, has also shown —against Lorenz's view— that instinct does not exist if by instinct we mean an innate motor pattern already adapted without any need for experience or development (Lehrman, 1953; Gottlieb, 1997; Moore, 2003). Also, this tradition has given rise to the Organic Selection Theory (Baldwin, 1896; 1976; Sánchez and Loredo, 2007), it has promoted theoretical approaches such as Development Systems Theory (Lickliter, 2008), and it has converged with epigenetics (Kuo, 1967; Moore, 2008) and with current evolutionary theories that give learning a critical role in evolution itself, like West-Eberhard's theory of evolution based on developmental plasticity (West-Eberhard, 2003).

The tradition of Associative Learning has developed the most accurate methods for the study of animal learning to date. Nonetheless, even considering all the research which is yet to be done in invertebrate, these methods seem to find their limits as they move away from the Animal Kingdom. Minimal Cognition meaningfully bursts into the scene but does not offer a uniform and systematic approach to learning, although some of its insights are clearly convergent with a Minimal Development approach such as Lyon's concept of learning in a broad sense or "experience-modulated behavioral change" (Lyon, 2015). The Developmental-Comparative tradition, however, substantially contributed to the study of the adaptive experience in unicellular and connected learning and evolution in a way that is now increasingly supported by current Evolutionary Developmental Biology and Epigenetics. The tentative idea of Minimal Development proposed here could help to focus future research on elementary behavioural change processes that associative methods do not seem to fully categorize and detect. Dialogue and contrast among the Associative Learning, the Minimal Cognition and the Developmental-Comparative approaches can provide mutual enrichment and perhaps some methodological and conceptual advancement in the complex field of the cognition and learning in "lower" organisms.

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Table 1

An overview of learning in invertebrates[2]

Invertebrate *phyla*	Ginsburg & Jablonka (2019) "Associative Learning"	Perry, et al. (2013) "Classical Conditioning"	"Learning" OR "Conditioning" AND *(phyla)* January, 2021
Gastrotricha			No
Platyhelminthes	*a*	Flatworm, 111	Jawad et al., 2018[1]
Ectoprocta/Bryozoa			No
Brachiopoda/Phoronida			No
Nemertea			No
Mollusc	*b*	Sea slug, 107,108 Pond snail,109 Land snail, 110 Cephalopods 100, 101	22/333
Annelida	*c*	Leech, 104,105 Earth worm, 106	5/41
Entoprocta			No
Cycliophora			No
Mesozoa-Orthonectida			No
Mesozoa-Dicyemida			No
Rotifera/Acanthocephala	*d* (habituation)		No
Gnathostomulida			No
Micrognathozoa			No
Chaetognatha			No
Kinorhyncha			No
Priapulida			No
Loricifera			No
Nematoda	*e*	*C. elegans*, 302,303 (26/867)	1/89
Nematomorpha			No
Tardigrada			Zhou et al. (2019)
Onychophora			No

[2] *Phyla* of invertebrates in which Classical Conditioning has been demonstrated according to data from the reviews of Perry et al. (2013), Ginsburg and Jablonka (2019), and ours, updated through a search in Scopus in January 2021. Ginsburg and Jablonka use the term "Associative Learning", Perry et al. "Classical Conditioning". Our search used the terms "conditioning" OR "learning" AND ("*phyla*"). We use the list of *phyla* by Ginsburg and Jablonka because it is more complete and updated than Perry's. The letters in the Ginsburg and Jablonka column reproduce the references that accompany the Table 7.1, on pp. 331-333, of their 2019 text. The numbers accompanying Perry's boxers are their references to those taxa. We have reviewed all the taxa and incorporated the newly found references (tardigrades, for example). In the taxa in which learning has already been clearly demonstrated, we have indicated the number of articles that contain the terms of our search and the number of those specifically found in the field of psychology (22 out of 333 in the case of the mollusks, for example, or 26 out of 867 in *C. elegans*, or 4/133 in arthropoda) as an indication of the relevance of learning psychology studies to these *phyla*. Some relevant points are commented through the notes.

Invertebrate *phyla*	Ginsburg & Jablonka (2019) "Associative Learning"	Perry, et al. (2013) "Classical Conditioning"	Learning" OR "Conditioning" AND *(phyla)* January, 2021
Arthropoda	*g* Perry et al. (2013)	Perry et al. 2013[2] Maxillipods Myriapods [3]	4/133
Urochordata			0/13
Cephalochordata			No
Hemichordata			No
Echinodermata	*l*	77 without control of sensitization [4]	0/14
Xenacoelomorpha			No
Cndiaria	*n* (habituation)	Sea anemona, 112[5]	1/27
Ctenophora			0/4
Placozoa			No
Porífera			0/16

Note. 1.- The work of Mohammed Jawad et al. (2018) shows very complex phenomena in planarians as a recent advance in the addiction model with contextual learning. 2.- Arthropoda is a *phylum*. In arthropoda many investigations of learning and conditioning with insects (ants, bees) and other crustaceans are grouped. Ginsburg and Jablonka cite Perry et al., 2013 as a reference to the review in arthropoda. Only maxillipods and myriapods show absence of conditioning tests but are *subphyla*. In any case, it remains so today: there is no demonstration of conditioning. 3.- There is one study (Schäfer, 1976) in *Lithobius forficatus l.* Although it appears in the search with the indicated terms (learning and myiriapods) it is in fact an experimental work on the maze behaviour of this centipede and the mouse. In the case of the centipede the author studied spontaneous orientation and bias but in the case of mice he studied spontaneous orientation and learning. It is definitely not a study on learning. 4.- The reference 77 in Perry et al. (2013) is conditioning in starfish (McClintock and Lawrence, 1982). It is quoted by 8 references, but no one is an experimental replication. 5.- The only reliable demonstration of conditioning in cnidiaria is Haralson et al. (1975) with sea anemones. This reference is quoted in 10 publications as an example of conditioning in cnidiaria. However, none of these publications is an experimental replication and only 4 belong to the category of the scientific field of psychology.

Table 2

Literature about Learning and Conditioning in Plants

Reference	Procedure	Psychological skill	Result	Replication
Pfeffer (1873)	Mechanical stimulation of leaflets in *Mimosa Pudica*	Habituation	Positive	Bose (1906) mechanical + electrical stimulation. The same results
Darwin (1880)	Mechanical stimulation of tendrils in *Passiflora gracilis*	Habituation	Positive	No
Pfeffer (1906)	The sundew tentacles stimulation in *Drosera* (sundew)	Habituation	Positive	No
Holmes & Gruenberg (1965)	Water drop and finger touch Tactile-shock *(Mimosa pudica)*	Habituation stimuli discrimination Conditioning	Positive Negative	No Holmes & Yost (1966). The same results
Haney (1969)	Tactile + Light-Dark or Dark-Light transition No Tactile + Light-Dark or Dark-Light transition	Conditioning	Light-Dark Less CR Dark-Light More CR Control Less CR	Levy et al. (1970) Tactile (Dark-Light)+ control. Opposite results
Armus (1970)	Light-Dark – "striking the main stem"	RT reduction to leaft-folding as a CR to darkness	Positive but problematic	No
Applewhite (1972)	Mechanical stimulation (dropping) and electrical stimulation in *Mimosa pudica*	Habituation	Positive	No

Reference	Procedure	Psychological skill	Result	Replication
Jaynes (1976)	Tactile + intense light in Mimosa (presumably *Mimosa pudica)*	Conditioning	Negative	No
Gagliano et al. (2014)	Defense response induced by drops in *Mimosa pudica*	Habituation	Positive	No
Gagliano et al. (2016)	Conditioning Fan-light (*P. sativum*)	Conditioned orientation	Positive	Markel (2020) *Pisum sativum* cv Green Arrow. Bigger sample size and rigorously blind observation. Negative results

Note. In this table studies on learning and conditioning in plants are shown. The first column shows the references followed by the experimental procedure used, the psychological skills involved, the main results and the replicas which support the data or not.

Table 3

Literature about Learning and Conditioning in Paramecium

Reference	Procedure	Psychological skill	Result	Replication
Jennings (1906)	Exposure to stimuli (light, heat, gravity, shocks)	Basic learning	Observation of trial-and-error behaviours	Negative by Loeb (1918)
Gelber (1952, 1958)	Conditioned place preference (bacteria as reinforcement)	Learning	Increased number of subjects in the conditioned place	Negative by Jensen (1957) and Kantz & Deterline (1958)
Lepley & Rice (1952)	Alternance behaviour T maze	Reactive Inhibition	Increased opposite side choose after forced turn	Negative by Lachman & Havlena (1962) and Harvey & Bovell (2006)
Hanzel & Rucker (1972) Huber et al. (1974)	Tube-escape	Escape	Enhanced escape behaviour	Negative by Applewhite & Gardner (1973) and Hinkle & Wood (1994)
Hennessey et al. (1979)	Conditioning (vibration-shock)	Anticipatory Avoidance Reaction (AAR)	Increased AAR	No replication
Armus (2006a)	Light- cathode Dark- cathode	Acquire placed preference	More time spent near cathode side	Partially positive in light- shock by Alipour et al. (2018)
Kunita et al. (2014)	Obstacle avoidance	Emergence of new behaviour in escape	Decreased distance with useless movement pattern, increasing the new	No replication

Note. In this table the studies on learning and conditioning in paramecium are shown. The first column shows the references followed by the experimental procedure used, the psychological skills involved, the main results and the replicas which support the data or not.

Table 4

Literature about Learning and Conditioning in Physarum polycephalum

Reference	Procedure	Psychological skill	Result	Replication
Nakagaki et al. (2000)	Maze-solving by an amoeba	Primitive intelligence	The largest path fades away	No replication
Saigusa et al. (2008)	Fixed periodic changes of temperature and humidity	The anticipation of impending environmental change	Periodic reduction of locomotive speed	No replication
Shirakawa et al. (2011)	Conditioning (paired low temperature and food)	Learning, memory, acquired place preference	Acquired reversed thermotactic property	No replication
Boisseau et al. (2016)	Re-exposure to quinine	Habituation	Change in pseudopod size	Boussard et al. (2019)

Note. In this table the studies on learning and conditioning in *Physarum polycephalum* are shown. The first column shows the references followed by the experimental procedure used, the psychological skills involved, the main results and the replicas which support the data or not.

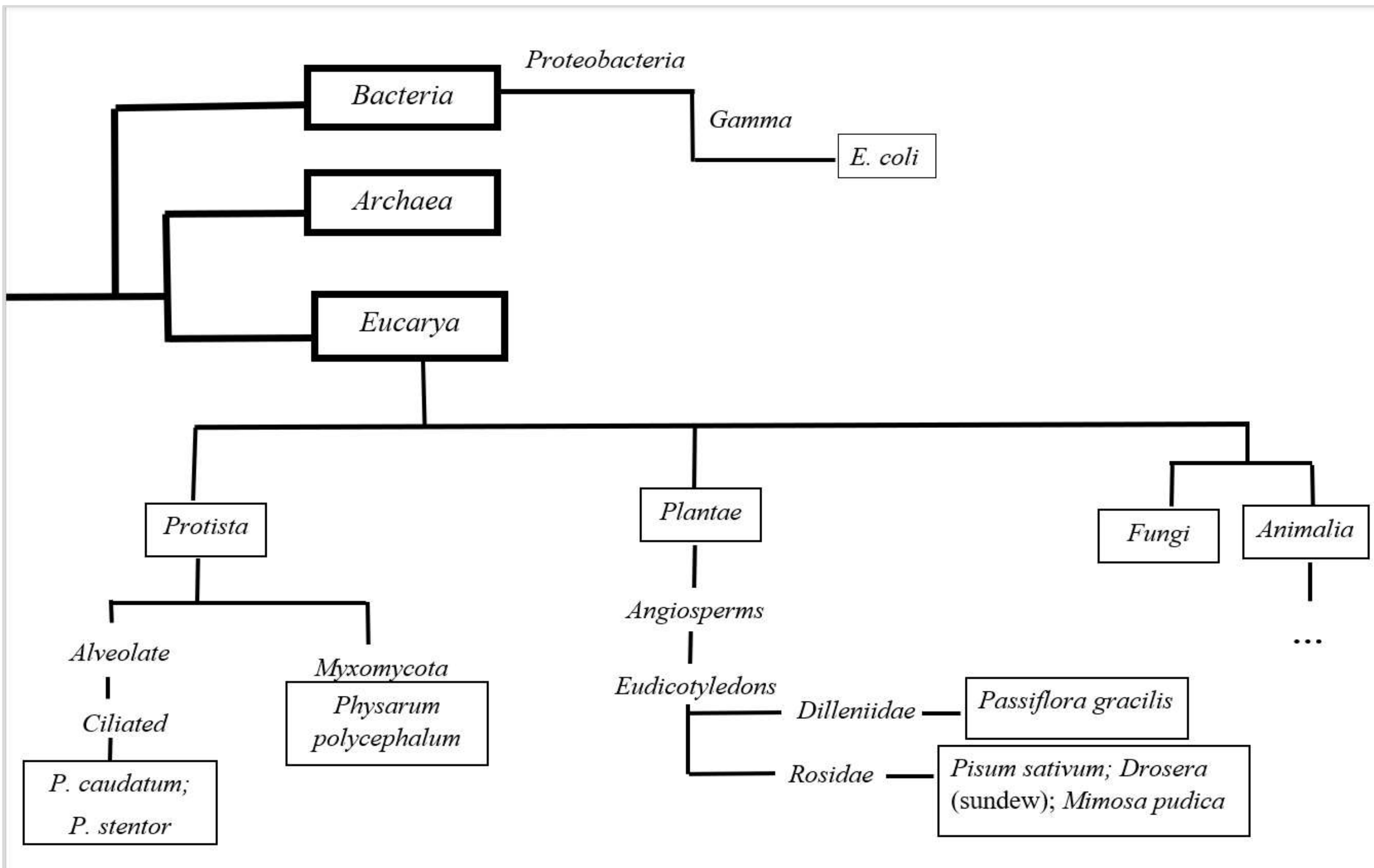

Figure 1. A phylogenetic map about the main organisms cited in the manuscript in which learning was studied, except for the *Animalia* kingdom which is represented in the following two figures. Based on Tudge (2000), Joseph et al. (2013) and Rungruangmaitree and Jiraungkoorskul (2017) to classify and compare the taxonomy of *Mimosa pudica* and *Pisum sativum* respectively.

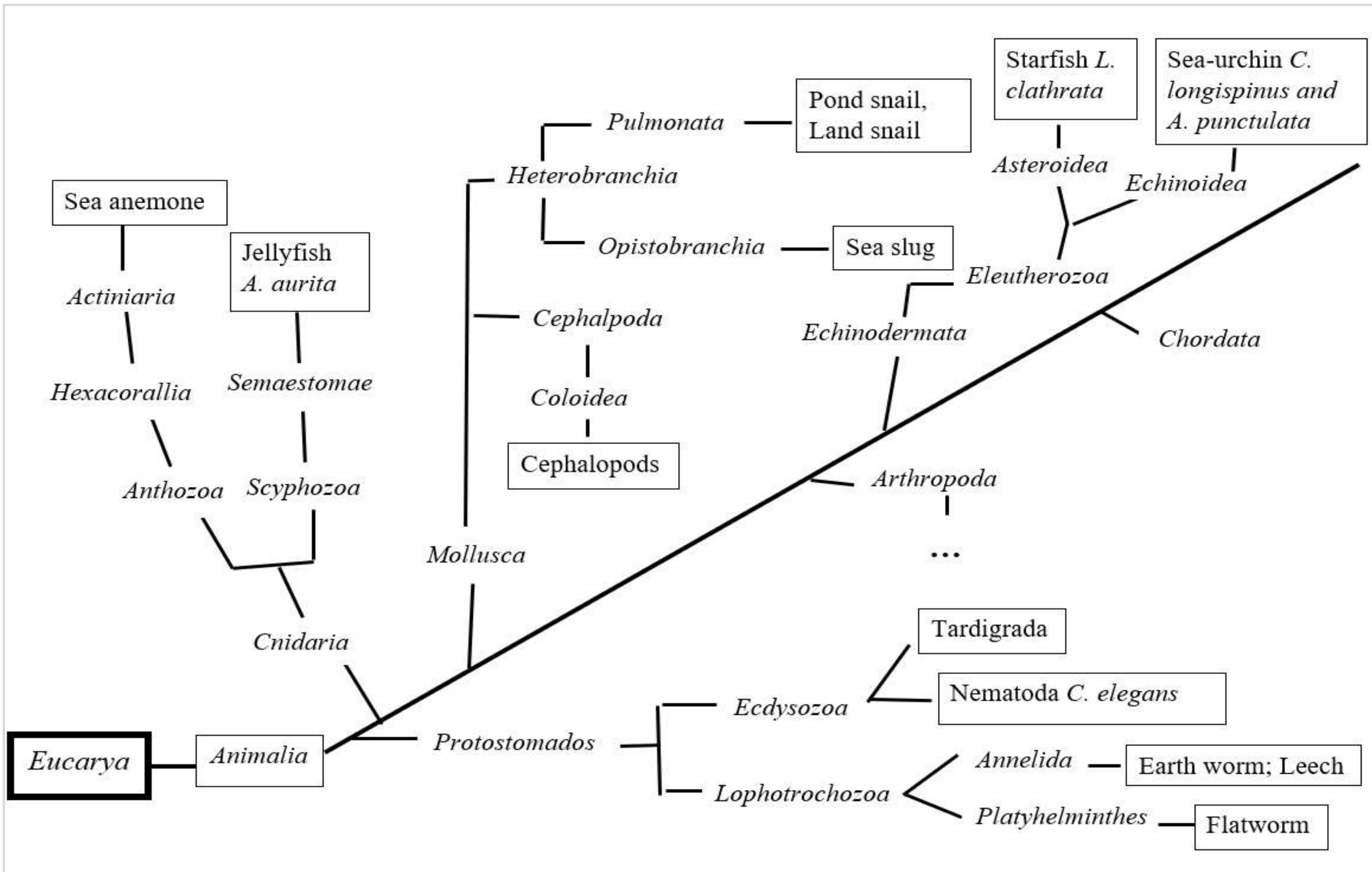

Figure 2. A phylogenetic map about the *Animalia* organisms cited the manuscript in which learning was studied. Based on Tudge (2000).

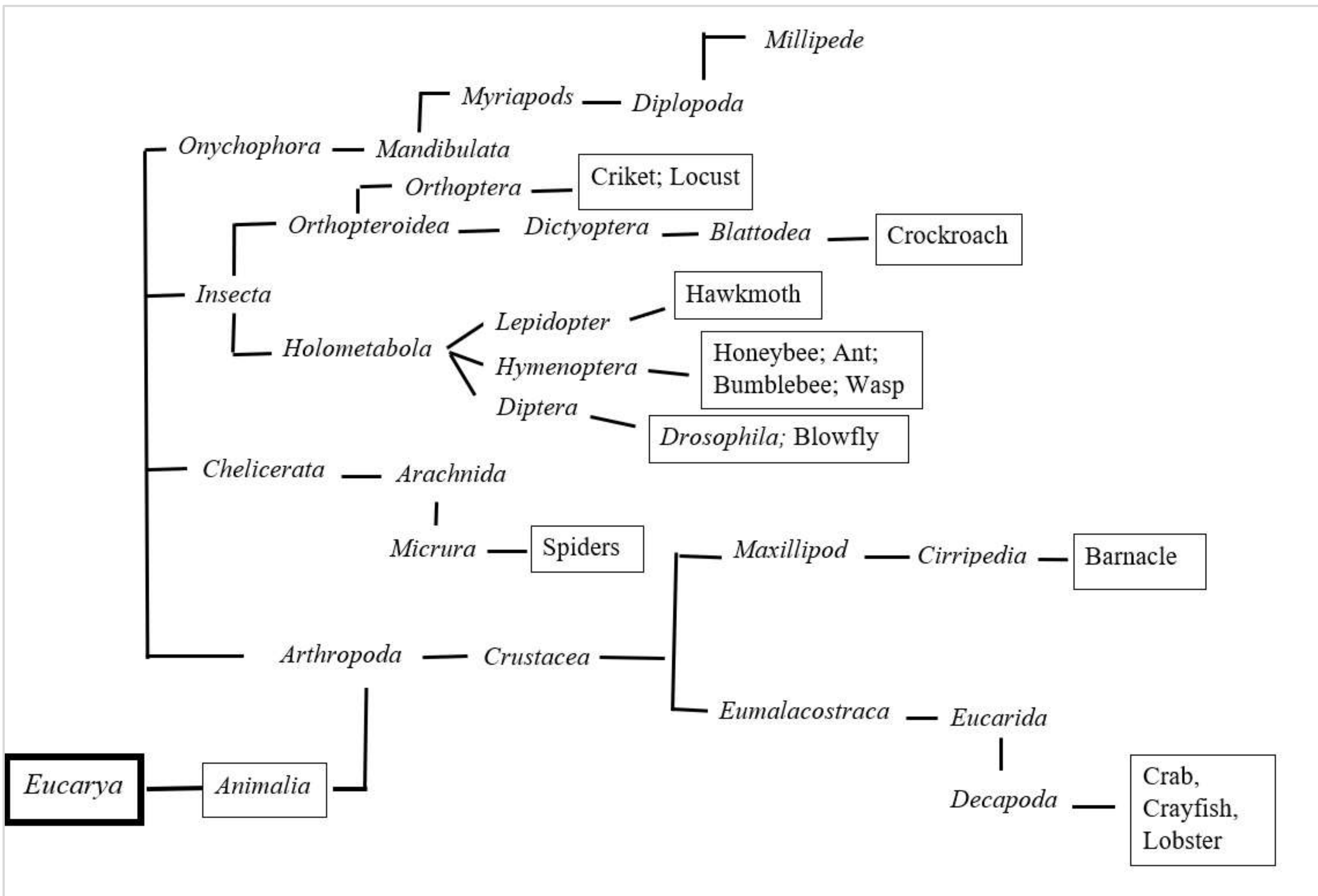

Figure 3. Continuation of the *Animalia* kingdom in which the representation of *Arthropoda phylum* is shown. Based on Tudge (2000).