The Weevils *Argentinorhynchus breyeri, A. bruchi* and *A. squamosus* (Coleoptera: Curculionidae), Candidates for the Biological Control of Waterlettuce (*Pistia stratiotes*)

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Abstract

Eleven species of apparently monophagous weevils, genera *Neohydronomus*, *Pistiacola* and *Argentinorhynchus*, occur in South America plus 7 species of oligophagous insects of other groups. Biological control of waterlettuce was successful in Australia and South Africa using the weevil *Neohydronomus affinis* Hustache from South America. In the US, *N. affinis* was released in Florida during April 1987 but after a promising beginning, it failed to provide control. Other insects with potential for BC are the aquatic weevils *Argentinorhynchus* spp. with six species (presumed strictly monophagous) in the Neotropical Region from south-central Mexico to Argentina. Three species occur in Argentina, *A. bruchi* Hustache, *A. breyeri* Brethes and *A. squamosus* Hustache. All adults feed at night cutting round holes in the leaves. Larvae of the three species have different food niches: *A. breyeri* feed on leaf primordia of the center of *Pistia* rosettes, *A. bruchi* tunnels the upper part of the rhizome and *A. squamosus* feeds in the lower rhizome. Pupation is the most critical stage of the cycle and until now, it was a mystery for all species of *Argentinorhynchus*. The full grown larva abandons the plant and buries itself in the bottom of the water body. The further dessication of the pond seems important for the normal development of the pupa in the subterraneous cell. This implies an adaptation for sporadic population of waterlettuce. In the laboratory, *A. breyeri* showed potential to kill plants if enough larvae develop in one plant.

Keywords: waterlettuce, biological control, weevils *Argentinorhynchus*

Waterlettuce (*Pistia stratiotes* L.) is a troublesome floating weed that infests many waterways in southeastern U.S. and other tropical and subtropical areas of the world. Several attemps to control the plant biologically were succesfull in Australia, Papua New Guinea, South Africa, Zimbabwe and Botswana. In Florida, USA, it was estimated that the waterlettuce populations nearly quadrupled during 1982-1985 and about $650,000 are spent annually for waterlettuce control. Despite the release of the weevil *Neohydronomus affinis* and the moth *Spodoptera pectinicornis* (Hampson), control has not been achieved. Additional agents would be desirable. Because of cold weather and/or monsoon-type of climate with a defined dry season, vegetative populations of waterlettuce often do not persist during winter. On those shallow isolated water bodies that are regularly subjected to alternate wet and dry regimes that become infested with waterlettuce, presumably from seed germination, control is necessary to prevent further spread of the weed and to facilitate access to water. In these circumstances, populations of biocontrol agents may not be present early in the year. This may require annual re-introductions of control agents or better adapted agents for sporadic populations of waterlettuce.

A series of insects attack waterlettuce in South America and several appear to have it as their only host (Bennett 1975). Among these, the weevil *Neohydronomus affinis* has been proved to be highly specific for waterlettuce and is being utilized in many countries around the world. Other insects with potential and presumed narrow host range are *Argentinorhynchus* spp. and *Pistiacola* (=*Onychylis*) *cretatus* (Champion). Preliminary research conducted on these insects indicated that they are specific enough to warrant further studies (Cordo *et al.* 1978, Cordo *et al.* 1981). In the summer 1997-1998 we commenced the studies of the weevil *Argentinorhynchus* spp. We report here the result of the investigations.

Methods and Materials

All tests were conducted using adults (and their offsprings) collected in Chaco and Formosa Provinces, in an approximate quadrangular area formed by the cities of Resistencia, Saenz Peña, Ibarreta and Formosa in the Chaco phytogeographical region of northeastern Argentina. Tests were conducted in a walk-in-chamber maintained at constant 28° C and 14 hours photo phase from 3 fluorescent tubes of 40 Watts each, and in a glasshouse with fluctuating day-night temperatures of 20° to 38° C. Eggs and larvae for the tests were obtained by placing the adults on plants of *Pistia* floating in a nutrient solution made with one teaspoon of Iron Chelate and one teaspoon of 15N-15P-15K soluble fertilizer every 10 liters of water. Individual or group plants were confined in 500 ml clear plastic cups or in 1000 ml clear food containers with a white lid or in a 20-liter-bucket covered with plastic mosquito screening in the greenhouse. Development of the larval stadia was determined by periodically dissecting plants containing larvae of known age and measuring the head capsules. For pupation, full developed last instar larvae were collected from the bottom of rearing containers and placed on different moist, wet or soaked substrata as follows: regular sifted garden soil, fine construction sand, toilette paper and pasteurized sphagnum peat moss. Pasteurization was attained by pouring boiling tap water into a one-gallon plant pot containing the peat moss. Copper chloride 1% was used, 2-3 drops per larva, for protection against fungi that were common in the rearing.

Results and Discussions

Literature Review. *Origin of the Plant.* The center of origin of waterlettuce is not clear. It was described from specimens from Ceylon, but is was known for its medicinal properties in Egypt in 77 A.D. (Bennett 1975). Some authors as Stoddard (1989), suggested a North American origin because the plant was blocking waterways in Florida during the mid-1700s. European fossils record of *Pistia siberica* (probably same as *P. stratiotes*) seeds, 12-25 MYA, represented the most recent fossil record of waterlettuce thus far reported. However, the temperate climate of Europe during historic times has prevented the establishment of permanent waterlettuce populations there, despite recent introductions and periodic recurrences of introduced populations. Holm *et al.* (1977), suggested an African origin for waterlettuce because of the prevalence of sexual reproduction in African plants and absence of sexual reproduction elsewhere. However, now it is known that sexual reproduction is common through the range of distribution of the plant (Dray and Center 1989, Harley 1990). Bennett (1975) and Cordo *et al.* (1978), suggested that waterlettuce originated in South America because of the extensive plant-feeding fauna with many host specific insects associated with *Pistia* there. Although the determination of the geographical region in which it originated is controversial, there are no host specific insects in the North American fauna (except perhaps *Petrophila drumalis* (Dyar)) suggesting that Florida waterlettuce populations originated from transplanted African or South American stocks or both.

Surveys for Natural Enemies. Although extensive exploratory surveys have never been conducted on waterlettuce, collections in the Americas, Africa and Asia detected about 46 organisms associated with waterlettuce. Of these, 25 species were found in the Americas, 13 in Asia and 8 in Africa. These organisms are now enumerated, with an indication of their presumed host specificity. The letters (MO) following the organism name means monophagous, that is, the feeding preferences are restricted to single species or species in subgenus or genus; (OL) means oligophagous or restricted to several genera of the same family or related families; and (PO) means polyphagous or a wide preference for a variety of genera of unrelated families. In the Americas, Bennett 1975, Baranowski and Bennett 1979, Cordo and DeLoach 1982, Poi de Neiff 1983, Dray *et al.* 1988 O'Brien and Wibmer 1989a, b, and Wibmer and O'Brien 1989 listed the following insects: Col., Curculionidae, *Argentinorhynchus breyeri* Brethes (MO), *A. bruchi* (Hustache) (MO), *A. squamosus* (Hustache) (MO) , *A. bennetti* O'Brien and *Wibmer* (MO), *A. kuscheli* O'Brien and *Wibmer* (MO), *A. minimus* O'Brien and *Wibmer* (MO), *Neohydronomus pulchellus* Hustache (MO), *N. affinis* Hustache (MO), *N. elegans* O'Brien and *Wibmer* (MO) , *Pistiacola* (*=Onychylis*) cretatus (MO), *P. fasciatus* O'Brien and *Wibmer* (MO), *Ochetina bruchi* Hustache (OL), *Onychylis* sp. nr. nigrirostris (OL), *Tanysphyrus lemnae* Fabr. (OL); Lepidoptera, Pyralidae, *Samea multiplicalis* (Guenee)(PO), *Synclita obliteralis* (Walker) (PO), *Petrophila drumalis* (Dyar)(MO); Orthoptera, Acrididae, *Paulinia acuminata* De Geer (OL); Homoptera, Aphidae, *Rhopalosiphum nymphaea* (PO); Homoptera, Cicadellidae, *Draeculacephala inscripta* Van Duzee (PO); Homoptera, Pseudococcidae, *Pseudococcus* sp. (PO), *Planococcus citri* (Risso) (PO); Homoptera, Delphacidae, Genus?(OL); Heteroptera, Lygaidae, *Valtissius* sp. (OL), *Lipostemmata humeralis* Berg (OL). In India and Indonesia Zetter and Freeman 1972 , Sankaran 1974, Chaudhuri and Janaki Ran 1975, Mangoendihardjo and Soerjani 1978, and Joy 1978 mentioned the following: Lep., Pyralidae, *Nymphula responsalis* Walker (PO); Lep., Noctuidae, *Spodoptera* (*=Namangana*) *pectinicornis* (Hampson) (MO), *Erastroides curvifasciata* Hapmson (PO), *Proxenus hennis* Swinhoe (*=S. pectiniformis*) (OL), *Spodoptera litura* F.(PO), *S. mauritia* Boisd. (PO); Homopt., Aphididae, *Rhopalosiphum nymphaea* (PO); Homopt., Menoplidae, *Nisia atrovenosa* Lethierry (OL); Diseases, *Cercospora* sp. (OL), *Sclerotium rolfsii* (PO), *Phyllosticta stratiotes* (MO). In Thailand, Gonzalez 1978 mentioned: Acari, Homocaligidae, *Annerossella knorri* Gonzalez (PO); Acari, Oribatidae, Genus? (PO); Acari, Phytoselidae, Genus? (PO). For Nigeria, Obeng 1969, Pettet and Pettet 1970, and Scheibelreiter and Apaloo 1971-72, mentioned the following: Lep., Noctuidae, *Prodenia* sp. (PO), *Pseudocamalodus fletcheri* (PO), *Angionochus lividus* (PO), *Microvelia* sp. (PO); Homopt., Aphididae, *Rhopalosiphum nymphaea* (PO); Mollusca, *Bulinus* (*Physopsis*) *rohltsi* (PO), *B. torskalii* (PO), *Anisus coretus* (PO); Diseases, Virus. Most of the highly specialized organisms are in South America where 11 species of apparently strictly monophagous weevils occur, plus 6 species of oligophagous insects of other groups.

Control Agents. Biological control of waterlettuce was successful in Australia. They released *Neohydronomus affinis* near Brisbane in March 1982 and by 1984 dramatic reductions of waterlettuce coverage were observed here and in two other sites. They later documented similar reductions in other sites and successful biological control in Papua New Guinea. Biological control of waterlettuce was first used in 1985-86 with great success in Africa seasonal pans in the northern part of the Kruger National Park. Complete control occurred within 10 months. It was later introduced into Zimbabwe and Botswana apparently with similar results.

In the US *N. affinis* was released in Florida during April 1987 at Kraemer Island on the south end of Lake Okeechobee. By April 1989 waterlettuce coverage decreased to about 5%. At Torrey Island a similar reduction was noted in May 1990, three years after the release of *N. affinis* in July 1987. However, waterlettuce has since reinvaded both sites probably due to seed germination. Releases at a third site, a canal in St. Lucie county, failed to provide control and the insects disappeared. The moth *Spodoptera pectinicornis* (Lep.: Noctuidae), from Thailand, India, Indonesia, Bangladesh and Papua New Guinea, found to be monophagous in host range studies, was released in Florida in late 1990. By the summer of 1993 field colonies had not yet established despite the release of over 200,000 individuals (Center 1994).

The weevils Argentinorhynchus spp. The aquatic weevil *Argentinorhynchus* has six species in the Neotropical Region from south-central Mexico to Argentina. They are placed in two sister groups: *A. breyeri*, *A. minimus* and *A. kuscheli* in one, and *A. squamosus*, *A. bruchi* and *A. bennetti* in the second group. All species are associated with waterlettuce and although *A. kuscheli* was collected on *Nymphaea* during a flood, waterlettuce is the probable host plant. Some of them are sympatric and synchronic in several localities and it is not uncommon to find at least two species on the same plant together with *Ochetina bruchi* and *Neohydronomus* spp. The following have been collected together during the day and at night: *A. bennetti* and *A. squamosus* in Mexico; *A. bennetti*, *A. breyeri* and *A. minimus* in Venezuela and *A. breyeri*, *A. bruchi* and *A. squamosus* in Argentina (O´Brien and Wibmer 1980, 1989).

O´Brien and Wibmer (1980, 1986) give precise location for 521 adults of the six species of *Argentinorhynchus*. Although the geographical distribution ranges from Mexico to Argentina, the absence of species in Central America places a question mark on the natural occurrence of *A. squamosus* and *A. bennetti* in Mexico. Both species were collected in one site only, which makes the question stronger. *Pistia* occurs in Central America as many collections of *Neohydronomus* spp. clearly document. Thus, the absence of *Argentinorhynchus* spp. there, is not due to the absence of the plant but to the lack of suitable and unknown environmental factors. South America is the center of specialization of the genus. All six species of *Argentinorhynchus* occur there and except *A. bennetti*, all species occur in Bolivia. This country is also the only known place for *A. kuscheli*. With three species each, Argentina, Paraguay, Brazil and Venezuela follow. As for the number of sites where found, Argentina(48) is first, Bolivia (30) second, Venezuela (23) third, Paraguay (8) fourth, Brazil (6) fifth followed by other countries with 3 and less as Peru, Colombia, Uruguay, Mexico and Panama. Among the six *Argentinorhynchus* species, *A. breyeri* appears the most common, being present in 64 sites of 8 countries. Next are *A. squamosus*, *A. bruchi*, *A. minimus*, *A. bennetti* and *A. kuscheli* in that order. In 1972, Bennett found the larvae of a yellow-spotted weevil, *A. bruchi*, feeding in the crown of *Pistia* that weakened the plants so that they broke easily when disturbed. He collected it, together with *A. breyeri* and *A. squamosus* from *Pistia* in Asunción, Paraguay and Formosa Province in Argentina (Bennett 1975).

Cordo *et al.* (1978) studied in Argentina the biology and laboratory host range of *A. bruchi*. They concluded that the insect has the capacity to cause extremely heavy damage to *Pistia*. The females have very high fecundity and can produce large populations of larvae quickly. However, they were unable to produce pupae and adults in the laboratory because the full grown 4th. instar larvae drowned in the water of the rearing container. Adults fed and oviposited only on waterlettuce among 26 plant species tested. Because *A. bruchi* was very rare in the field and causes no important damage to waterlettuce, they proposed that high predation on the exposed eggs and very special requirements for pupation, were the main reasons for the insect paucity.

Research at SABCL. In preliminary laboratory studies, *Argentinorhynchus breyeri* had less mortality, produced many offsprings and showed potential to kill its host plant. Thus, it was selected as the most promising candidate among the three species of *Argentinorhychus* present in the country and studies were therefore focussed on this species.

Argentinorhynchus breyeri

Field occurrence. In December 1997, 30 adults were collected at 6 *Pistia* sites out of 10 sites examined. In January 1998, 59 adults were collected in 7 *Pistia* sites out of 15 sites examined. Thus, the presence of the weevil in both trips averaged 52% ,that is, half the sites visited had adults of *A. breyeri*. This abundance does not compare badly when confronted with presence of other common phytophagous of waterlettuce, *Neohydronomus affinis* (78%), *Pistiacola* (=*Onychylis*) cretatus (43%), and *Ochetina bruchi* (65%). However, capture efforts were biased in favor of *Argentinorhynchus*, so this data should be analyzed with caution. Considering both trips together, the total number of adults of *A. breyeri* was 89 collected on 2,750 plants examined which is equivalent to one adult every 31 plants. Curiously, no larva was found in any collecting site.

Unexpectedly, the relative abundance of *A. breyeri* (89 adults in 13 sites), was lower than *A. squamosus* (195 adults in 14 sites) although much larger than *A. bruchi* (16 adults in 3 sites). This abundance contradicts what was expected from the literature references. However, the current data is partial and could only reflect the particular climatic conditions of the season.

Adult. Adults of *A. breyeri* are smaller than the other 2 species, black with 6 whitish squared-spots on the elytra. These spots disappear with age so it is common to collect weevils without the spots. The rostrum is thickened distally and only slightly curved. They measured 4.14 mm long by 2.31 mm wide. No anatomical differences were found between the sexes. They are nocturnal, resting during the day usually on the underside of the older leaves of waterlettuce rosettes. At night they can be found feeding and mating on the upper side of medium-age leaves. They prefer to feed in the distal third of the leaves of medium age. In the laboratory, 78% of the feeding holes were in that part of the leaf. The feeding holes are circular, all the way through the leaf.

Egg. Females laid their eggs singly on the lower leaf surface (97%), externally, loose, in an upright position among the dense hairs of the inflated aerenchyma at the base of the leaf. Only 3,4% of the eggs were laid on the upper side of the leaf. The eggs are ovoid, 0.57± 0.03 SD mm long by 0.35 ± 0.02 mm wide (mean of 50) measured two days after being laid. They are yellow when laid and they turned orange-brown after a day or two. At a temperature of 28 ± 2.74° C, eggs hatched in a mean of 4.74± 0.56 days (range 4-6 days). *A. bruchi* also laid the eggs externally, singly and in the lower surface of the leaf but the eggs were glued to the leaf hairs. In contrast with the other two species, *A. squamous* laid the eggs inside holes in the aerenchyma of the upper leaf surface, covered with plant gums, 1-2 eggs per hole.

Larva. The larvae emerged from one end of the egg and entered the leaf immediately after hatching, the empty chorion remained attached to the leaf hairs. Three instars could be distinguished by the width of the head capsule. The 3rd. instar was spent partly in the plant (9.17 days) and partly buried in the substrate (11.50 days). The duration of the larval stage on the plant was 17.96 ± 2.14, range 15-20 days at 28 ± 2.74 °C. In all cases the fully developed larvae abandoned the plant and dropped to the bottom of the growing container. Fully-developed third instar larvae spent 11.5 ± 1.52 days (range 10-15 days) buried in the substrate before pupating. In the greenhouse similar results were obtained. Sixty-eight larvae were found in the bottom of growing containers 25 days after adults were placed on the plants at 25.12 ± 5.04° C, that is 2.3 days longer than egg and larval stages in the life cycle in the chamber (22.7 ± 2.7 days, range 19-26 days) at 28 ± °C. Another group of 74 larvae were collected from the bottom of the container 29 days after adults were placed on the plants. The temperature of this period was lower than the first batch, 24.81 ± 4.61°C.

The larval feeding behavior is interesting. After reaching the central part of the rosette, the first-instar larva remains in vertical position sticking its caudal end up. It keeps that position while feeding on the leaf primordia until development is completed and then abandon the plant. In one occasion twelve last instar larvae were found in the center of a 12 cm-diameter plant. They were packed together head down, caudal up and submerged because the central rosette was partially sunken due to the larval damage. The larval morphology appears to have adaptions for this type of feeding. The body is sub-cylindrical with pairs of dorsal spiracular spurs in abdominal segments 1 through 8. These spurs are present in *A. bruchi* and *A. squamosus* as well and have a respiratory function because they are connected to the tracheal system. In *A. breyeri*, the last pair of spurs are conspicuously more developed than the rest and this seems to correspond with the feeding behavior. The last pair of spurs would be better adapted to take air when the larva is in a upright position and the whole larval body is submerged. In *A. bruchi* and *A. squamosus* the last pair of spurs are of similar size as the anterior ones, which seems to follow the pattern described. The larvae of the two species develop in the rhizome, thus there in no advantage in having the last pair of spines more developed. Nevertheless, the caudal segments of *A. bruchi* and *A. squamosus* are clearly different although it is unknown if they respond to functional adaptations.

Pupa. The requirements for pupation were a mystery for *A. bruchi* whose last instar larvae drowned in the water of the container after dropping from the plants. Full-grown larvae of *A. breyeri*, collected from the bottom of the rearing container, buried themselves rapidly when placed over wet or soaked substrates. One hour and ½ after placing 35 bottom-dropped larvae on 7 different substrates, 68.6% of them buried themselves completely into the substrates. Three and ½ hours after the beginning of the test, all larvae were buried. Larvae placed on soaked construction sand were unable to dig in the substrate. Three out of five larvae placed on moist tissue paper remained uncovered 3 ½ hours after beginning of test. However, when more water was added, soaking the substrate, they disappeared into it in 2 hours. Overall, soaked substrates were better accepted than wet ones.

In the greenhouse, 139 bottom-dropped larvae were collected in February and June, 98 from plants exposed to 62 adults for periods of 7-10 days. These larvae, placed on tissue paper and peat moss with varied degrees of moisture, produced 48 (34.5%) pupae in 18.9 days and 15 (10.8%) adults in 17.6 days. However, most emerged adults appeared subnormal, some had a very short life and others took an unusually long time to achieve the black normal color. When the more practical substrates found were tested with different degrees of moisture, 7 (9.2%) adults emerged from the 76 larvae tested. The number of adults emerged from moist tissue paper (2), from soaked peat moss (2) and from moist peat moss (3), was similar although slightly higher from the moist peat moss. Larvae placed in tap water, as controls, were unable to pupate although they survived a maximum of 48 days in this condition. The 3 pupae and 2 adults obtained in the soaked peat moss showed the inherent capacity of some larvae to continue the development in anaerobic conditions.

The overall pupation and adult emergence rates of *A. breyeri* in the different conditions tested were low. Combining all tests, 265 bottom-dropped larvae produced 72 (27%) pupae and 24 (9%) adults which seems to have ample margins for improvements. One of the meliorations is reducing the frequency of examinations of the buried larvae and pupae. The constant disturbance, daily in cases, of the larval and pupal cells in the substrates, obviously had a deleterious effect on the immatures. Peat moss was an excellent substrate for methodological purposes. Its hydric and antiseptic properties make it very convenient although its acidity could be unsafe for the rearing. No evidence in this regard was found during the described studies. Nevertheless, other substrates need to be tested.

Pupation in *A. breyeri* and perhaps in the entire genus *Argentinorhynchus* is highly complex and a very ineresting process. After completing their development on the plant, the fully grown larvae abandon the plant and drop to the bottom of the water body or container. Then they buried themselves in the muddy bottom for pupation. The rest of the cycle is speculated, based on laboratory evidence. The optimal condition for the pupation at the bottom would be a slow desiccation of the water body that would induce the buried larva to pupate. The desiccation, total or partial, would permit all buried larvae or just the ones buried on the edges, to have more oxygen for development. Overwintering pupae or teneral adults would be stimulated to emerge from the subterraneous cells when the first rains of the season start filling the pans again. In rainy years, when the pans do not dry out, emergence of adults would be reduced but still some edge-buried larvae could produce adults. Besides, based on laboratory evidence, it is possible that totally submerged larvae could survive and produce adults. This hypothetical cycle shows an adaptation of the weevils *Argentinorhynchus* to sporadic populations of their host plant. In the Chaco region of Argentina, Paraguay and Bolivia, most rains fall during the summer months, November through March. The winter and spring are much drier and due to these conditions, temporary, shallow, water bodies are common. Waterlettuce and the three species of *Argentinorhynchus* of Argentina, are widespread in the Chaco more than in any other phytogeographical region of the country. Since *A. bruchi* and *A. squamosus* also have the same larval behavior of abandoning the plant for pupation, it is likely that all species in the genus share the same adaptation for sporadic waterlettuce populations. However, this evolutionary trend is for the moment theoretical and needs to be proved.

Damage to the Plant. Damage produced by adults in the field is easily spotted through the round, big feeding holes on the waterlettuce leaves. However, the damage of the three species of *Argentinorhynchus* is superficially very similar, so visual estimates of adult damage most likely will include damage of all species present. Number of feeding holes produced by all species ranged from 1-2 feeding holes per plant in low populations of weevils, to 1-2 feeding holes per leaf in places with noticeable damage, to 6 feeding holes per leaf in highly attacked plants.

In the laboratory, the potential of *A. breyeri* larval damage to kill plants of *Pistia* was observed in the greenhouse. Forty-three days after 15 adults were placed on 10 small plants (10 cm diameter, 7-8 leaves), 74 larvae were collected from the bottom of the container. These larvae fed in the leaf primordia in the central rosette, causing intense damage. Due to this damage, the plant had an empty center, usually covered by green algae and partially sunken. They were unable to recover from the damage and slowly all died in the next weeks.

The larval damage of *A. breyeri* is different to the larval damage of *A. bruchi* and *A. squamosus*. While the first feed on the meristematic tissues of the central part of the *Pistia* rosettes, the second feeds in the upper part of the rhizome and the third in the medium and lower part of the rhizome. Therefore, *A. breyeri* has the potential to kill plants if enough larvae develop in one plant. In one occasion, 12 full-grown larvae were observed feeding in one medium-sized plant. After that damage the plant died.

Host Specificity. No studies were conducted in the laboratory to find out the degree of specificity of *A. breyeri* to its host plant. However, preliminary testing of *A. bruchi* suggested that this species is highly specific to waterlettuce and there is no reason to suspect that *A. breyeri* will behave differently. All species of *Argentinorhynchus* of Argentina are only known to attack *Pistia*. They are sympatric and synchronic on waterlettuce in the Chaco region and their niches are clearly separated by where the eggs are laid and by the part of the plant where the larvae feed. Chances are overwhelmingly high that *A. breyeri* is a strictly monophagous species.

Argentinorhynchus bruchi and A. squamosus

These two species occur with *A. breyeri* in the Chaco region. Often, the three species occur synchronously on *Pistia* although the general abundance, according to literature records, decreases from *A. breyeri* to *A. bruchi*. However, the total number of adults collected during the summer in northern Argentina was higher for *A. squamosus* (195) than *A. breyeri* (89) and *A. bruchi* (16). Since the research focused on *A. breyeri*, for the reasons expressed, only limited studies were carried out on *A. bruchi* and *A. squamosus*.

A. bruchi. The biology of this weevil and preliminary host range was studied by Cordo *et al.* (1978). Therefore, only data to be used for comparison with the other two species, were taken. It appears as the least common *Argentinorhynchus* in Argentina and the only one that occurs at the southernmost range of *Pistia* in Argentina. It is the only species that occurs in Uruguay. The location of eggs and the larval feeding behavior clearly separates the three species of *Argentinorhynchus* of Argentina. *A. bruchi* lay the eggs on the underside of the leaves attached to the dense hair mat by a sticky substance and the larva feeds in the upper part of the rhizome.

A. squamosus. The adults of this species are the largest of the three *Argentinorhynchus* of Argentina. It was the most common of the season as well. They were easily collected at night in one locality of Chaco. Sixty-four adults were collected at night in an area of 15x4 m, one hour after sunset, 21:30 to 22:30. Most adults were feeding on the central, young leaves. Many were mating, 20 couples were collected.

The larva, conspicuously longer than the larvae of the other two species, was found for the very first time in the field. One last instar larva was found tunneling in the lower part of the rhizome and several of various ages were in the middle to low part of the rhizome. Adults of the three species were found at the spot but larvae of *A. bruchi* and *A. breyeri* were not found.

In the laboratory, the rearing of *A. squamosus* was impaired by the absence of rhizomes of the appropriate size on the *Pistia* plants in culture. Placing adults on small to medium sized *Pistia* and waiting for the full-grown larva to fall to the bottom of the containers was not as successful as with the other two species of *Argentinorhynchus*. This technique also failed when 69 adults were placed in a concrete pool in the back yard covered with medium size *Pistia* plants. Although the plants were well fed with nitrogen, phosphorus, potassium and iron during the summer, no larvae were observed at the bottom of the pool. Adults of different origins and kept in different containers, were decimated by a fungal disease superficially alike Beauveria. Although the fungus was also observed in *A. bruchi* and *A. breyeri*, is was much more destructive in *A. squamosus*.

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