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Overseas Biological Control Laboratories
U.S.D.A, Agricultural Research Service

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In This Issue

Introduction

OBCL is a group of overseas laboratories that support the domestic research carried out by USDA-ARS with the aim of "finding solutions to agricultural problems that affect Americans every day from field to table".

The **Australian Biological Control Laboratory (ABCL)** is based in Brisbane, Australia. The facility is run through a Specific Cooperative Agreement between USDA-ARS and Australia's Federal research body, CSIRO. This has been a long term relationship originating in 1985. Contact: Matthew Purcell, matthew.purcell@csiro.au

The **European Biological Control Laboratory (EBCL)** is based in Montpellier, France, and has a satellite laboratory in Thessaloniki, Greece. It has a permanent staff of 1 American and 7 foreign scientists, 9 technicians and 5 administration/ support. Contact: Lincoln Smith, Link.Smith@ars.usda.gov

The **Foundation for the Study of Invasive Species (FuEDEI)** is based in Hurlingham, Argentina and is operated as a nonprofit research organization. Contact: Guillermo Cabrera Walsh, gcabrera@fuedei.org

The **Sino-American Biocontrol Laboratory (SABL)** is based in Beijing, China. Contact: Liu Chenxi, liuchenxi@caas.cn

Australian Biological Control Laboratory - ABCL

Cold tolerant weevils to control Giant Salvinia

by Matt Purcell, Brad Brown and Ryan Zonneveld

Cyrtobagous salviniae is a weevil species discovered by CSIRO in South America that has successfully controlled giant salvinia (*Salvinia molesta*) where it has become invasive. ABCL staff collected a population of *C. salviniae* in southern Australia in 2012 where it has persisted as the most southern site in Australia since its introduction in the early 1980's. These weevils were used in cold tolerance studies at Texas A&M University. Results indicate that the population from Camden, near Sydney, in temperate NSW were more cold tolerant than the weevils in culture in Texas.

Louisiana State University (LSU) had problems releasing *C. salviniae* weevils in north Louisiana as they didn't survive their harsh winters. All weevils released in north Louisiana in the winter of 2010, and most weevils released in the winter of 2013, did not survive. The research team headed by Dr. Seth Johnson and Dr. Rodrigo Diaz have planned on conducting further cold tolerance studies using weevils from Camden including: super cooling point, lethal temperature determined in temperature controlled alcohol baths, and survival after exposure of 5h and 10h to maximum low temperatures in refrigerated incubators.

Initially, hundreds of weevils were collected from Camden by ABCL staff and shipped to quarantine facilities at LSU for cold tolerance studies. Unfortunately these insects were collected in late autumn, failed to oviposit in quarantine

tanks, and no substantial colony could be maintained. More recently the weevils virtually disappeared from the site, the likely culprit being the accidental introduction of insecticide into the aquatic system by local land owners upstream from the collection site. However in January 2017 more than 100 weevils were collected from uncontaminated sites upstream and these were successfully shipped to LSU. The weevils were young and, being mid-summer, likely to readily oviposit for colony establishment in quarantine. Cold tolerance studies should be underway in the near term.

Pond heavily infested by giant salvinia (*Salvinia molesta*) at Camden near Sydney in NSW, Australia.

Matthew Purcell collecting the weevil *Cyrtobagous salviniae* for shipment to colleagues at Louisiana State University (LSU).

Biological control of ear leaf Acacia

by Ryan Zonneveld, Jeff Makinson, Christine Goosem, Matt Purcell

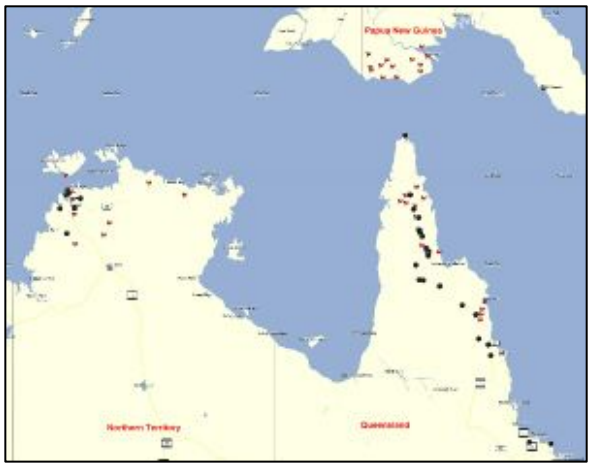
Ear leaf Acacia (*Acacia auriculiformis*) is native to northern Australia from northern Queensland through to northern regions of the Northern Territory. Outside of Australia it also occurs naturally in Papua New Guinea and Indonesia. It was introduced into Florida in 1932 and is now recorded in over 24 natural areas in Dade, Broward, Palm Beach, Martin, Collier and Lee Counties. It has the potential to become a serious invasive weed in the US. ABCL works collaboratively with Dr. Greg Wheeler at the USDA ARS Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, FL in a biological control program to control this invasive tree.

Ear leaf Acacia, *Acacia auriculiformis*.

Initially, a major objective was to locate *A. auriculiformis* sites in the native range to conduct surveys for herbivores. The taxonomy of this species and its relatives is complex and trees need to be seen in flower or with seed pods for identification as they are key characters. Areas surveyed thus far have been between Cape York and Townsville in Queensland with most sites being between Iron Range and Cairns. Sites around Darwin in the Northern Territory have also been surveyed. Many promising insects have been collected and efforts are currently focused on rearing and identification before a short list of candidates are prioritized for intensive study.

A second major objective was to collect specimens for DNA analysis so that Dr. Paul Madeira from IPRL can determine the origin of the

invasive *A. auriculiformis* in the USA. This could have implications for finding the most effective biological control agents. We contacted the Australian Tree Seed Centre (CSIRO) and seeds were obtained from 42 sites in the native range of Australia and Papua New Guinea. Plants have been geminated from these seeds and samples preserved on silica gel for genetic characterization by Dr. Madeira. These specimens have been supplemented with 38 dried samples taken from pressed herbarium specimens held at the Queensland Herbarium and the Australian National Herbarium and specimens collected directly from the field by ABCL staff. The combined coverage of specimens is shown on the map. Once the area of origin is determined, surveys will be refocused accordingly.



Collection sites of ear leaf Acacia to determine the area of origin of this invasive tree in the USA.

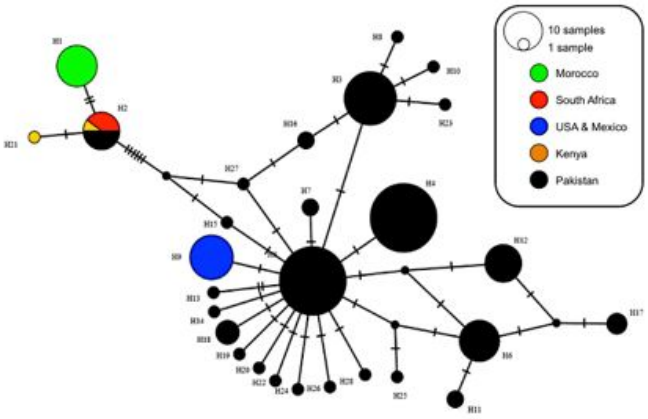
European Biological Control Laboratory - EBCL

Geographic Origin of Bragada Bug

by Lincoln Smith, Marie-Claude Bon, René Sforza

Bagrada bug (*Bagrada hilaris*) first appeared in California in 2008 and is spreading across the southern U.S. and Mexico. It attacks many crop species, but is especially damaging to cole crops (broccoli, cabbage, mustards, etc.). In order to develop an effective biological control program we need to determine the geographic origin of the insects so that we know where to go to find prospective biological control agents. Bagrada bug specimens were collected in the USA, Morocco, S. Africa, and Pakistan with the help of many colleagues: Darcy Reed (UC Riverside), Brian Hogg (USDA-ARS) and Charlie Pickett (CDFA) in California, Riaz Mahmood (CABI) in Pakistan, and by René Sforza with with Schalk Schoeman (ARC-ITSC) in South Africa and Ahmed Mazih (IAV) in Morocco.

Marie Claude Bon analyzed DNA and compared the COI barcode sequences with those obtained from public databases including the *BOLD* DNA Barcode Reference Library and the *GenBank* sequence database provided by the National Center for Biotechnology Information (NCBI), which included additional samples from Pakistan, Kenya and Mexico. All the Bagrada bug specimens from the USA share the same barcode sequence (haplotype), which suggests that the insects come from one origin. A similar pattern was observed with nine additional genes sequenced by Blake Bextine's group at University of Texas. The USA haplotype is most similar to one of the most prevalent haplotypes in Pakistan. Specimens from Kenya, South Africa and Morocco are more distantly related to the USA specimens. Thus, the USA infestation probably originated from, or near, Pakistan, thus this is a high priority region to explore for prospective biological control agents.



Evolutionary network showing genetic relatedness among *COI* barcode haplotypes. Each circle represents a different haplotype, the color corresponds to country, the diameter is proportional to abundance, and the cross hatches indicate the number of mutations (nucleotide changes) between haplotypes. The USA samples are very closely related to those from Pakistan.

EBCL obtained two species of parasitoids from Pakistan: *Trissolcus hyalinipennis* and *Gryon* sp. from CABI cooperators. Both species are now

being studied at EBCL and at ARS laboratories in California (Brian Hogg) and Mississippi (Walker Jones). We have analyzed the *COI* barcode sequences in these parasitoids to confirm their identity, and we are collaborating with a taxonomist, Elijah Talamas (Gainesville, FL), to determine the identity of *Gryon* sp.

Giant reed

by Gaylord Desurmont, Javid Kashefi and Lincoln Smith

Giant reed (*Arundo donax*) has invaded waterways and riparian habitats in many parts of the semiarid west, obstructing water flow, reducing biodiversity, and interfering with security along the Mexican border. It is also a problem in other countries, including South Africa. Dr. Angela Bownes (ARC, S. Africa) contacted EBCL and John Goolsby (ARS, Mission, TX) to obtain the scale insect, *Rizaspidiotus donacis*, for her to evaluate in S. Africa. EBCL scientists collected infested arundo roots and shipped them to S. Africa and met with Dr. Bownes to introduce her to colleagues at the University of Alicante in Spain, and showed her techniques for collecting the scale and other arundo insects.

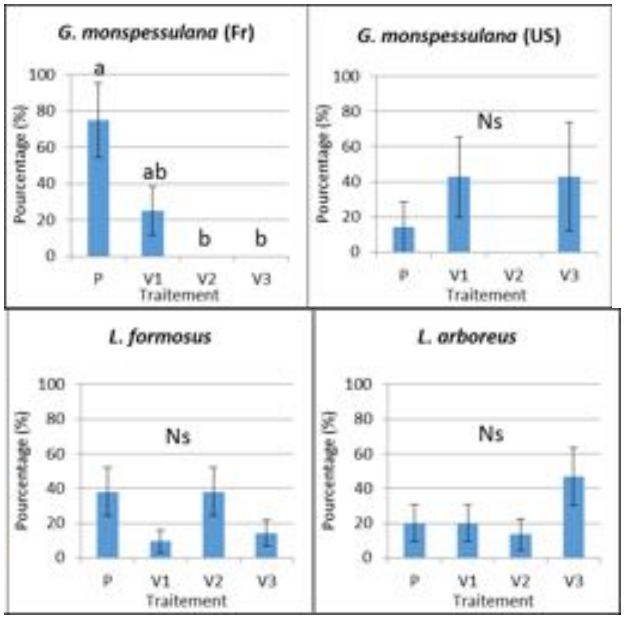
EBCL scientists and colleagues from BBCA, Italy continue making monthly collections of arundo shoots infested with the leaf-mining fly, *Cryptonevra* sp. for host specificity studies by John Goolsby (ARS) in Texas.

French broom psyllid

by Chloé Descombe (MS student, PURPAN Toulouse) and Gaylord Desurmont

French broom (*Genista monspessulana*) is an invasive shrub in the Pacific Western USA that is a target for biological control. The psyllid *Arytinnis hakani* is a successful biological control agent in Australia where it appears to cause heavy defoliation and mortality of its target weed. It has been considered for introduction in California, but some concerns exist regarding its host specificity, as *A. hakani* has been shown to complete at least partial development on several species of North American lupines (*Lupinus* spp., Fabaceae) in the laboratory. In order to determine if *A. hakani* is attracted to lupines we studied its olfactory preferences. In a 4-arm olfactometer, adult psyllids were given the choice between a plant and three empty arms. The plant was either the target weed, French broom, or a non-target lupine species, which was either undamaged or infested by psyllids for seven days. Results showed that psyllids responded poorly in the olfactometer setting (only 31% on the insects tested made a choice among the different treatments), and did not show significant preferences for plant odors. However, there was a distinct trend toward odors of a French population of French broom, whereas and American population and the two lupine species were unattractive.

French broom psyllid *Arytinnis hakani* nymphs feeding on French broom.



Olfactory preferences of the French broom psyllid in a 4-arm olfactometer when given the choice between a plant (P) and three empty arms (V1, V2, V3). The plant was either *Genista monspessulana* (French population), *G. monspessulana* (US population), *Lupinus formosus*, or *L. arboreus*. Means followed a different letter are statistically different. Ns = non-significant.

Chemical analyses of these plants showed that French broom plants emit very low amounts of volatiles when undamaged or after 7 days of infestation by *A. hakani*, which may explain the overall poor response of psyllids to plant odors.

The results do not support the idea that *A. hakani* would be innately attracted to odors of lupine plants in nature, but whether or not this psyllid uses plant volatiles to detect its host plants remains unclear. In 2017 similar experiments will be conducted with blooming *G. monspessulana* and *Lupinus* plants, which presumably emit considerably higher amounts of volatiles.

Biological Control of Cattle Fever Tick

by Javid Kashefi, John Goolsby and Lincoln Smith

Cattle fever ticks (*Rhipicephalus microplus* and *R. annulatus*) vector babesiosis and anaplasmosis to cattle in the southern USA. They have been eradicated to the Mexican border, but the ticks are developing resistance to acaricides and infested wildlife that are difficult to treat. Thus we need to find a more sustainable control method.
R. annulatus occurs in the Balkan region, but little is known about natural enemies that help to control its population. EBCL scientist Javid Kashefi has begun collecting ticks on cattle and sheep in Crete and the Balkan Region to initiate the search for parasites or predators of these ticks. Survey methods were developed in collaboration with John Goolsby (ARS, Edinburg, Texas).

Javid Kashefi collecting ticks from sheep in the Balkan region. John Goolsby developed survey techniques.

Integrated management of Mosquitoes

by Alexandra Chaskopoulou

Dr. Kenneth Linthicum (Director of USDA-ARS Center for Medical, Agricultural & Veterinary Entomology, Gainesville, FL) and Dr. Seth Britch (USDA-ARS Research Entomologist) visited Thessaloniki last May to conduct experiments on vector control in collaboration with EBCL scientists. The objective of this research is to evaluate and optimize residual and Ultra Low Volume (ULV) spray applications against mosquito and sand flies of medical importance. Treatments were applied to cloth barriers made of military material to simulate military construction. Similar treatments are being conducted simultaneously in other countries with different climatological profiles in order to determine the impact of weather on the efficacy of the spraying treatments. This project is partly funded by the US Department of Defense and results will be of high importance for the protection of US troops deployed oversees.

Testing effectiveness of residual formulations applied on simulated barriers used by Dept. of Defense to protect personnel from mosquitoes and sand flies in Greece.

Insect biodiversity in a mosquito-breeding rice field ecosystem

by Alexandra Chaskopoulou and Xavier C. Sierra

Rice fields have the potential to conserve biodiversity and may provide important alternative habitats for insect species originating from natural wetlands. However, little information is available on the impacts of vector control treatments on nontarget insects in rice paddies. To determine whether vector control affects insect diversity a survey for aquatic insects was initiated in the rice field region of North Greece, where no records of aquatic insects are available in the literature. A masters student, Xavier C. Sierra, from University of Lleida conducted this research under the supervision of EBCL scientists. More than 30 species of aquatic insects were detected in the region including five agricultural pests and

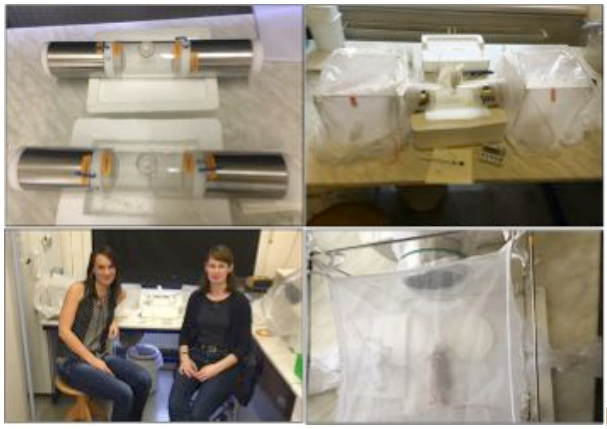
promising mosquito predators. The rice water weevil (*Lissorhoptrus oryzophilus*), an invasive alien rice pest, was collected in Greece for the first time (**Figure B**). We used DNA barcodes to identify species, some of which had never been characterized before.

Masters student Xavier Sierra surveying insect biodiversity in rice fields in northern Greece. The rice water weevil (*Lissorhoptrus oryzophilus*), an invasive alien pest, which was recorded for the first time in the Balkan region (lower right).

Evaluation of Sand Fly Control in the Czech Republic

by Alexandra Chaskopoulou

Sand flies are a major pest of people and animals; however, they are difficult to rear in the laboratory, and most pest species are not native to the USA. Spatial repellents such as transfluthrin have been widely used for the control of flying insects. However, no products have been tested against sand flies of the genus *Phlebotomus*. EBCL researchers teamed with Charles University Department of Biology to determine whether transfluthrin can provide effective spatial repellency against *Phlebotomus* sand flies. A modification of the WHO spatial repellency bioassay was used, and dual choice tubes with metallic chambers were constructed by EBCL researchers to perform dose response bioassays. The tubes were constructed in the EBCL Greece lab and were sent to Prague for testing against several sand fly species. Experiments are currently being conducted by postdoc researchers at Charles University under the supervision of Dr. Chaskopoulou (EBCL) and Dr. Volf (Charles University). Preliminary data show that transfluthrin has a significant spatial repellency effect on *Phlebotomus* sand flies.



Dual choice tubes constructed by EBCL scientists that were used to conduct WHO spatial repellency bioassays of transfluthrin on sand flies by collaborating scientists at Charles University, in the Czech Republic.

Visiting/temporary scientists at EBCL in early 2017 include:

Floriane Chardonnet (EBCL, France) - biology and rearing of *Psyttalia ponerophaga* for biological control of olive fly.

Francesca Marini (BBCA, Italy) - biology and rearing of *Psyttalia lounsburyi* for biological control of olive fly.

Guillaume Matel (EBCL, France), studying life history of *Gryon* sp.

Ioannis Giantsis (American Farm School, Greece) - molecular genetics of mosquitoes and sandflies.

Matthew Augé (BBCA, Italy) - life history and behavior of *Trissolcus hyalinipennis*.

Steve Novak (Professor, Boise State U.) - genetics of Ventenata, Medusahead and cheatgrass.

Foundation for the Study of Invasive Species - FuEDEI

Cactus moth

by Guillermo Logarzo and Laura Varone

The overall objective of the cactus moth project is to develop an integrated management program for *Cactoblastis cactorum* (Lepidoptera: Pyralidae) populations invasive in North America. Although this insect is a highly effective biological control agent of non-native cactus in Australia and South Africa, it is considered a serious pest of native *Opuntia* species in the southeastern USA, where it was accidentally introduced. This moth is spreading westward, threatening both wild and cultivated *Opuntia* in Mexico. Our research is being carried out in collaboration with Dr. Stephen Hight (USDA-ARS) at the Center for Medical, Agricultural and Veterinary Entomology, Tallahassee, FL. *Cactoblastis cactorum* sex pheromones combined with biological control is the current strategy being developed for controlling the insect. Pheromone baited traps were developed that are an effective monitoring tool for *C. cactorum* presence. Pheromones were also deployed in large scale field studies to evaluate the potential of using this synthetic compound for mating disruption. Trials conducted in multiple Argentine cactus plantations demonstrated that localized population reduction of moths and infestations could be obtained using this approach. We are also measuring damage levels to fruit and cladodes from *C. cactorum* attack in *Opuntia ficus-indica* plantations in the moths' Argentine native range. This study aims at measuring the economic damage level for the moth by determining the level of moth damage that causes economic income or yield loss equal to the cost of insect control.

Biological control studies are focused on the improvement of the rearing technique of the larval parasitoid *Apanteles opuntiarum* (Hymenoptera: Braconidae), the most promising classical biological control candidate for *C. cactorum*. Field exploration in Argentina for parasitoids of cactophagous Lepidoptera, indicated that the host range of this wasp is restricted to *C. cactorum* and *C. doddi*. Field collections of cactophagous larvae parasitized by the congeneric and sympatric parasitoid *A. alexanderi* included the host species *C. bucyrus, Sigelgaita nr. chilensis, Tucumania* sp., *Tucumania tapiacola* and *Salambona* sp. If a narrow host range for *A. opuntiarum* is confirmed in quarantine with North American cactus-feeding species, this parasitoid could be released as a biological control agent for *C. cactorum* with little or no risk to non-target species. Parasitoid female behavior is also being studied to determine the factors that affect search, encounter, and attack of larval *C. cactorum* hosts.

Cladodes and fruit of *Opuntia ficus-indica* (top left); *Cactoblastis cactorum* larva (top right); insect-damaged cladode (bottom right); female parasitoid *Apanteles opuntiarum* searching for hosts around holes where young larvae recently entered a cactus cladode.

Water primrose, *Ludwigia* spp.

by Cristina Hernández and Ana Faltlhauser

The South American water primroses, *Ludwigia grandiflora*, *L. grandiflora* subsp. *hexapetala*, *Ludwigia peploides*, and *L. p.* subsp. *montevidensis* (Onagraceae, Section Jussiaea, ex-Oligospermum), are invasive in many watersheds of the United States and Europe. They are particularly invasive in the West Coast watersheds, so the research is carried out in collaboration with Dr. Brenda Grewell at the USDA-ARS Exotic and Invasive Weeds Research Lab. in Albany, California. These plants become dominant species in shallow waters and marshes, leading to local loss of biodiversity by developing dense, monospecific stands. In their native range these species tend to grow in small patches of a few hundred square feet, and are usually mixed with other native plant species. Nevertheless, they are considered weedy in rice fields, where they grow stouter stands that foul machinery, possibly

because of fertilizer use and insecticides eliminating natural enemies.

Twenty specialized insect species were found breeding on *L. g*. subsp. *hexapetala* in surveys in central and northeastern Argentina. Among these, the thrips *Liothrips ludwigi* (Thysanoptera: Phlaeothripidae) was given high priority due to its damaging effects on the plant in the field and apparently narrow host range. Its biology, host specificity and potential impact were evaluated in the laboratory on 25 other plant species including five species of *Ludwigia* and other species in the Onagraceae family. The thrips affected the development of *L. g. hexapetala* under laboratory conditions and only bred on *L. grandiflora* and *L. peploides*. Other promising candidates are being evaluated.

Ludwigia grandiflora subsp. *hexapetala* growing in a pool at FuEDEI. In spite of its name, 5-petal forms are more usual in its South American range

Damage of *Liothrips ludwigi* on *Ludwigia peploides* subsp. *montevidensis* tips. The bright red nymphs are clearly visible, but the black adults are more difficult to spot.

Recent Publications by EBCL

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Desurmont, G.A., Zemanova, M.A., Turlings, T.C.J. 2016. The gastropod menace: slugs on brassica plants affect caterpillar survival through consumption and interference with parasitoid attraction. Journal of Chemical Ecology 42(3): 183-192.

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Hogg, Brian N., Patrick J. Moran and Lincoln Smith. 2016. Multi-generational impacts of the psyllid *Arytinnis hakani* (Hemiptera: Psyllidae) on growth and survival of the invasive weed *Genista monspessulana*. Biological Control 100: 87-93.

Hogg, B.N., Smith, L., Moran, P.J., Daane, K.M. 2016. Post-establishment assessment of host plant specificity of *Arytainilla spartiophila* (Hemiptera: Psyllidae), an adventive biological control agent of Scotch broom, *Cytisus scoparius*. Biocontrol Science and Technology 26(7): 995-1008.

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Kim, M-S., Lee, H-L., Ku, D-S., Hérard, F., Gould, J.R., Williams, D.W., Kim, I-K., Hong, K-J. 2016. Discovery of *Spathius ibarakius* Belokobylskij et Maeto (Hymenoptera: Braconidae) as a larval ectoparasitoid of citrus longhorned beetle in Korea. Korean J. Appl. Entomol. 55(3): 285-291.

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