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.

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 CSIRO.
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The **European Biological Control Laboratory (EBCL)** is based in Montpellier, France, and has a satellite laboratory in Thessaloniki, Greece. Contact: Lincoln Smith, Link.Smith@ars.usda.gov / www.ars-ebcl.org

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 / www.fuedei.org

The **Sino-American Biocontrol Laboratory (SinoABL)** is based in Beijing, China.
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Previous newsletter editions are available at:
www.ars-ebcl.org

Overseas Labs moved to OIRP

Last fall the administration of the Overseas Biological Control Laboratories was moved from the Office of National Programs to the Office of International Research Programs (OIRP). Responsibility for the OBCLs shifted from Kim Hoelmer to Eileen Herrera, the Acting Director of OIRP, who then retired in December. Eileen briefly overlapped with the new Director of OIRP, Bryan Norrington, to help the transition. Bryan previously worked in the USDA Food Safety and Inspection Service as a Senior Food Defense Analyst, and later as the Acting Director of the Food Defense Assessment Staff. He also previously worked for the USDA Foreign Agricultural Service as an International Trade Specialist. Rosalind James continues to serve as the National Program Leader responsible for OBCL research programs

EBCL Sabbatic Program

This year EBCL is supporting two ARS scientists to conduct research in Europe. Dr. Bryan Rector (Reno, NV) will be in Europe for 6 months to conduct research on eriophyid mites associated with medusahead, cheat grass and red brome. Travel will include France, Spain, Portugal, Italy, Greece, Bulgaria, Serbia, Poland and Kazakhstan.

Dr. Lee Cohnstaedt (Manhattan, KS) will spend two months in Greece developing and testing light emitting diode (LED) traps that are attractive to olive fruit flies, mosquitoes and sandflies.

Australian Biological Control Laboratory - ABCL

Exploration for biological control agents of Round Leaved Tooth Cup

by Matt Purcell, Greg Wheeler, Bradley Brown and Phil Tipping

Round leaved tooth cup, *Rotala rotundifolia*, is becoming a serious weed in ponds and irrigation channels in south Florida and has the potential to be a more serious aquatic weed than hydrilla, *Hydrilla verticillata*. Like hydrilla, stem fragments can become new plants which has significant implications for disturbance through mechanical

control. Unlike hydrilla, the plants also form viable seeds.

Figure 1. A mating pair of *Altica* sp. adults on *Rotala rotundifolia* at Leping Village, Fujia Province, China.

ABCL has been conducting preliminary surveys in the native range of *R. rotundifolia* in mainland China and Hong Kong. In June 2017, a defoliating *Altica* sp. beetle (Fig. 1) was discovered at high altitude at Liping Village in Fujian Province *R. rotundifolia* located on a roadside seep. Heavy damage was observed caused by both adults and larvae (Fig. 2), which were collected for identification. What appeared to be the same beetle was also collected near Qinglongkou Lake in Guangxi Province in July in a roadside channel beside the road in flowing water. At both sites the adults and larvae defoliated the emergent portion of the plant including flowers (Fig. 3) and pupae were found in webbed cases on the leaves. The submerged part of the plant is not attacked.

Figure 2. *Altica* sp. larvae feeding on a flower spike of *R. rotundifolia* at Leping Village.

Figure 3. Defoliation of *R. rotundifolia* at Qinglongkou Lake in Guangxi Province.

Adults were sent to Dr. Chris Reid from the Australian Museum in Canberra, Australia and identified as *A. aenea*, the same species which attacks *Ludwigia peploides* in Australia. ABCL staff collected *A. aenea* from *L. peploides* near Brisbane and released them onto laboratory stocks of *R. rotundifolia* (a weed in Australia). After two generations, egg production and larval survival in the colony diminished dramatically suggesting *R. rotundifolia* may be an unsuitable host for Australian *A. aenea*. There is a possibility that the Chinese *Altica* species collected from *R. rotundifolia* could be a separate species. Mass field releases of *A. aenea* recruited from *L. peploides* in Australia are also planned at invasive *R. rotundifolia* sites near Brisbane to verify host acceptance.

Rotala rotundifolia was also observed in Longsheng County, Guizhou Province growing in a rice paddy (Fig. 4). No damage was observed at the field site but a weevil larva was collected from plant material processed in a berlese funnel.

Figure 4. *Rotala rotundifolia* growing in a rice paddy in Longsheng County, Guizhou Province.

Exploration for biological control agents of Chinese Tallow

by Matt Purcell, Greg Wheeler and Jialiang Zhang

Chinese tallow tree, *Triadica sebifera* is one of the worst invasive weeds of the southeastern USA. It impacts coastal wetlands, native forests, and natural areas turning them into closed canopy forests of tallow trees. Since 2006, ABCL has assisted the USDA ARS Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, Florida with foreign exploration for potential biological control agents of *T. sebifera* in the native range in mainland China. These surveys were performed with Dr. Greg Wheeler (IPRL) with the assistance of staff from the Chinese Academy of Sciences in Wuhan, Hubei Province (Fig.5).

Figure 5. Dr Greg Wheeler (USDA ARS IPRL) with Jialiang Zhang at a Chinese tallow site near Guilin in Guangxi, China.

Figure 6. A. *Schizomyia* species (Cecidomyiidae) gall on Chinese tallow, *Triadica sebifera*

One of the potential agents discovered is a midge, a *Schizomyia* species (Cecidomyiidae) which galls the stems and flowers. Galls (Fig. 6) are prevalent during the winter period at sites near Guilin in Guangxi Province and were collected and shipped to the IPRL quarantine over the last several years. However insufficient adults were reared from these galls to establish a quarantine culture for further testing and evaluation. In the winter of 2017, sites at Guangxi were revisited and surveys were expanded into Guizhou Province to boost gall collections and increase the chances of quarantine establishment of the midge. The surveys were very successful and hundreds of galls were hand carried to IPRL by Greg Wheeler. Colony establishment is pending and further surveys are planned for 2018.

Crested Floating Heart & Feathered Mosquitofern

by Matt Purcell

Crested floating heart, *Nymphoides cristata*, is a water lily spreading in Florida where it is now found in at least 7 counties and also occurs on Lake Okeechobee and in the Big Cypress Preserve (Fig. 7). This aquatic plant also occurs further north into South Carolina where it has significant coverage on Lake Marion. The weed is native to Asia and surveys for biocontrol agents are planned for 2018.

Figure 7. Crested floating heart, *Nymphoides cristata*

Feathered mosquitofern, *Azolla pinnata*, is problematic in northern Florida, particularly during the winter. This floating fern spreads rapidly over water surfaces through vegetative reproduction, forming dense mats which often turn a distinctive red color. Worldwide analysis of *A. pinnata* plants by Dr. Paul Madeira (USDA ARS IPRL, Fort Lauderdale) determined that the genetic match for the US genotype of *A. pinnata* in the native range was collected near Brisbane, Australia. A *Bagous* sp. weevil which severely damages this fern has been collected from this region and has been colonized for testing against native US *Azolla* species, including *A. filiculoides* and *A. caroliniana* (Fig. 8).

Figure 8. *Bagous* sp. weevil that damages feathered mosquitofern in Australia

Foundation for the Study of Invasive Species - FuEDEI

Prospects for classical biological control of *Tamarix* in Argentina

by Fernando Mc Kay, Guillermo Logarzo and Alejandro Sosa

The main mission of FuEDEI is to search for, evaluate, and export beneficial organisms for the biological control of weeds and insects to benefit agriculture and the environment in the USA and or other countries. Another important objective of FuEDEI is to cooperate on agricultural research and experimentation with researchers from USDA-ARS. The invasion of saltcedar (*Tamarix* spp.) in USA and Argentina, constitutes an opportunity for collaboration to work on shared invasive weed problems.

Biological control of *Tamarix* spp. has been rapid and highly successful in the USA after the introduction of four *Diorhabda* spp. leaf beetles. Piggybacking on the biocontrol program undertaken in the USA constitutes an opportunity for Argentina to reduce costs associated with the exploratory surveys, risk assessments and rearing procedures to implement biological control of *Tamarix* spp. in the country. Consequently, a minimal amount of additional host specificity testing would have to be conducted in Argentina to evaluate if *Diorhabda* beetles could be safely used as biological control agents against *Tamarix* spp. in Argentina.

Researchers from FuEDEI, the National University of Río Cuarto and the Exotic and Invasive Weeds Research Unit USDA-ARS, recently published a feasibility assessment study to implement a CBC program against *Tamarix* spp. in Argentina using *Diorhabda* species as biological control agents (Mc Kay et al. 2017). In Argentina there are four species of *Tamarix* distributed in arid, semi-arid and coastal areas of most provinces (Fig. 9, Fig 10). The taxonomic isolation of Tamarix spp. in Argentina, their widespread distribution, negative impact to natural areas and lack of impact from existing natural enemies all indicate that Tamarix is an ideal candidate for classical biological control (CBC) in Argentina.

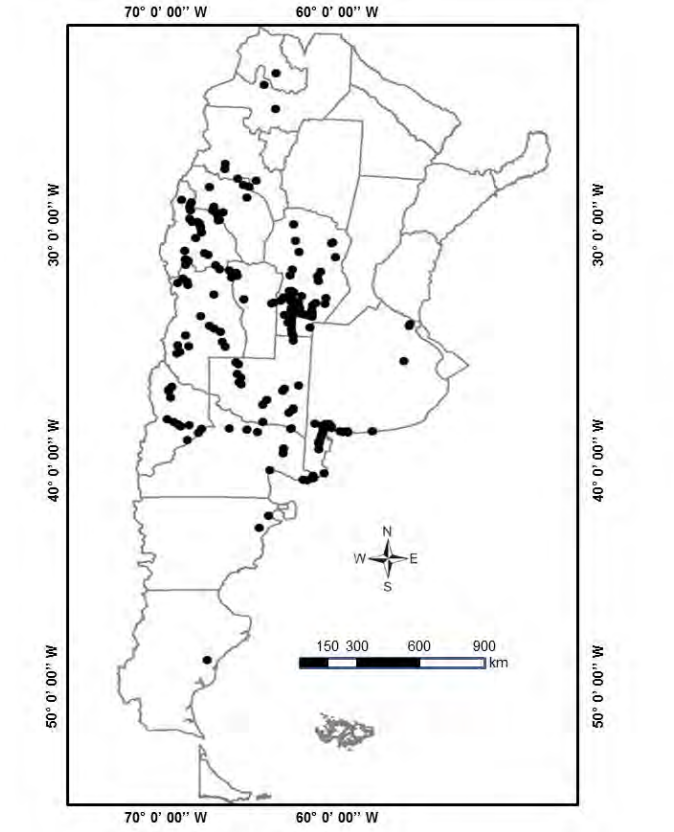

Figure 9. Distribution of *Tamarix* spp. in Argentina (from Natale et al. 2008).

Figure 10. Tamarix in Argentina. (Photo from: Ministry of environment and sustainable development).

To investigate Argentina's climate suitability for colonization by *Diorhabda* species, we modeled the potential distribution and resulting overlap of *D. carinulata*, *D. sublineata*, *D. elongata*, and *D. carinata* with their host *T. ramosissima*. MaxEnt modeling results for the exotic weed *T. ramosissima* and candidate biological control agents (*Diorhabda* spp.) indicated that *D. sublineata* may be the best candidate for Argentina (Fig. 11). This was concluded based on the fact that in the US it was found to be the species that was better adapted to the short critical day-lengths for diapause induction that occur in Argentina.

The implementation of a CBC program for Tamarix in Argentina is expected to contribute to the restoration of biodiversity and ecosystem services in protected areas of high conservation value and to the conservation of water resources in sensitive arid and semi-arid regions of Argentina.

Besides Tamarix, other target weeds, such as hawkweed (Hieracium pilosella), teasel (Dipsacus fullonum), knapweeds (Centaurea spp.), and privet (Ligustrum spp.), provide opportunities for future collaboration between the USA and Argentina for exchanging information on shared invasive species.

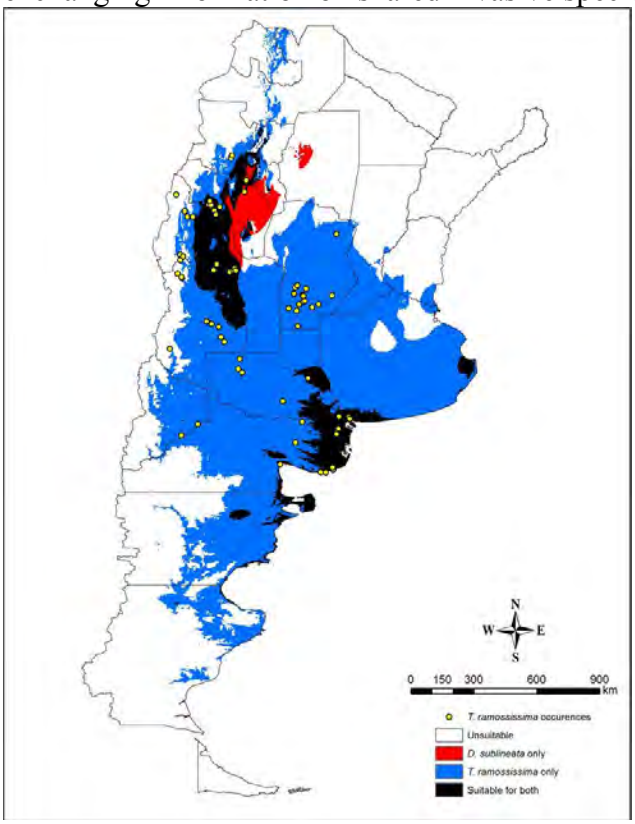

Figure 11. Habitat suitability predictions for *Tamarix ramosissima* and the specialized herbivore *Diorhabda sublineata* for Argentina based on maximum entropy modeling. Blue is *Tamarix*, red is *Diorhabda*, black is both.

References

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Natale E, Gaskin J, Zalba SM, Ceballos M, Reinoso H. 2008. Especies del género *Tamarix* (Tamarisco) invadiendo ambientes naturales y seminaturales en Argentina. Bol Soc Argent Bot 43: 137–145.

The invasive Tawny Crazy Ant in its native range

by María Belén Fernández and Luis Calcaterra

The invasive Tawny Crazy Ant, *Nylanderia fulva*, (TCA) is an ant native to South America that first appeared in Texas in 2002. Little is known about its biology or the geographic origin of the population invading the USA. The southernmost limits (temperate limits) of its distribution in Argentina are still undefined.

A project was recently initiated at FuEDEI in cooperation with local and foreign institutions using different approaches to better understand the ant's ecology and genetics. Objectives are to determine the distribution of TCA, the origin of its invasion in the US, and find natural enemies that could be used for its biocontrol. First surveys were conducted in April 2017. Nests of *Nylanderia* species were found across most northern Argentina and near areas of neighboring countries (Brazil, Paraguay and Uruguay), suggesting the genus is widely distributed in the region. However, it was most common in the Parana-Uruguay basin. The first genetic analyses (Fig. 12) revealed the presence of at least four distinct lineages that could correspond to four different species. The lineage of *N. fulva* introduced in the US only occurred in the lower subtropical latitudes. Thus, the source population of the invasions in the US might be located in this subtropical region, which includes several transatlantic ports from where TCA could have been introduced.

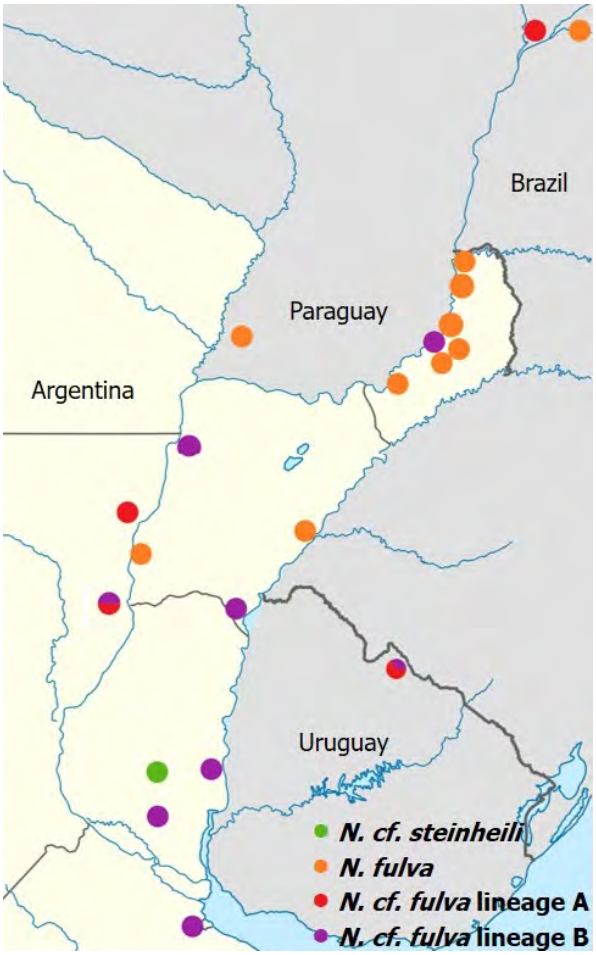

Figure 12. Distribution map of *Nylanderia* sp. samples from South America. Colors represent the four genetic lineages found in the Bayesian Analysis.

The three closest lineages (two of them more extended towards southern Argentina) do not show obvious morphological differences and could be cryptic or sibling species. The fourth lineage (less common) was distant from *N. fulva* and grouped with a population of *N. steinheili* present in the Caribbean, which confirms that *N. steinheili* could be present in Argentina; this species has been recently reported in Buenos Aires province (Fig. 13).

At present, *N. pubens* was not found during our surveys and could be restricted to the Caribbean region. Some colonies of the lineages *N. fulva* and its two sister lineages were up to 7 km wide (supercoloniality), had several queens per nest (polygyny), and/or reproduced by parthenogenesis (clonality), which are all features that are usually found in other highly invasive ant species originating from the same region, such as the little fire ant (*Wasmannia auropunctata*), the Argentine ant (*Linepithema humile*) and the fire ants (*Solenopsis invicta* and *S. richteri*). Finally, one microsporidian similar to *Myrmecomorba nylanderiae* (which was previously found in introduced populations of the TCA in the US) and phorid flies (putatively *Pseudacteon convexicauda*) were found during the surveys in northeastern Argentina. Further sampling will be conducted in native and anthropic areas in order to continue with this project.

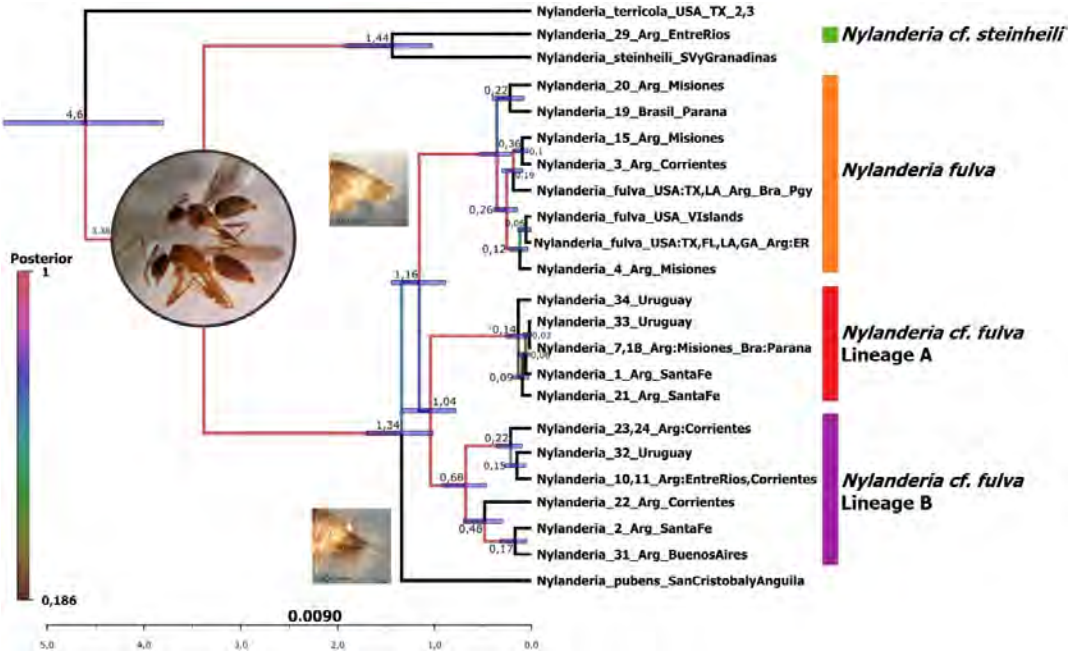

Figure 13. Bayesian analysis of COI partial sequence. Branch posterior probabilities are represented in colors (red = 1), values and error bars show coalescence time (million years), and insets show male morphology in different genetic lineages.

European Biological Control Laboratory - EBCL

DNA sequences of the nematode attacking the arundo leaf miner

by Marie-Claude Bon

The arundo leaf miner, *Lasioptera donacis*, has been approved for release in the U.S. and Mexico to control the invasive giant reed, *Arundo donax*, which is severely threatening riparian habitats throughout the southern half of the U.S. and Mexico. This agent has been imported from Mediterranean Europe to the USDA-ARS arthropod quarantine laboratory in Edinburg, Texas to rear flies to release. Pupae, which are the preferred stages for transatlantic shipment of this agent, were shown to be parasitized by a species of nematode, *Tripius gyraloura*, which was first described in 2014 by Dr. George Poinar from Oregon State University (Fig. 14).

Figure 14. Mature female of the nematode *Tripius gyraloura*.

However, the species description lacks molecular genetic data such as DNA sequence information, which could complement the morphological taxonomic description and contribute valuable diagnostic characters, especially for cryptic species. EBCL in collaboration with Dr. Goolsby and Anne Vacek, ARS USDA, Edimburg, TX, has extracted the DNA of this nematode and the 18S and 28S genes were sequenced (Fig. 15). These sequences, which have been deposited in the Genbank public database, can be used to identify the nematode, and could be used to develop a molecular method such as the Quantitative Polymerase chain reaction method (QPCR) to diagnosis presence of nematode infection in a more specific and straightforward manner than using microscopic observation.

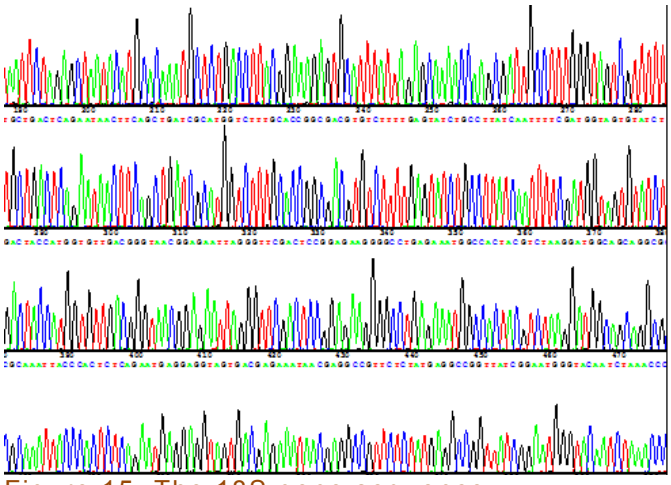

Figure 15. The 18S gene sequence chromatogram of the nematode attacking the arundo leaf miner.

The quest for oophagous mites against the viburnum leaf beetle

by Gaylord Desurmont and Elven Kerdellant

The viburnum leaf beetle (*Pyrrhalta viburni*) is a quickly spreading landscape and forest pest in North America. It was detected for the first time in the USA in Maine in 1994 and has since then spread steadily throughout the Northeast. It has also become established on the West Coast, particularly in the states of Washington and Oregon. The larvae defoliate native *Viburnum* shrubs and can kill full-grown plants after 2-4 years of infestation. The beetle has one generation per year and overwinters as eggs. Females lay egg masses (6-12 eggs) inside cavities that they create in young branches. Because eggs remain for 6 to 10 months before hatching, they are a particularly vulnerable stage for predators and parasitoids.

In the southern part of France, which is at the southern limit of the beetle's native range, we have observed high egg mortality and high densities of several types of mites inhabiting the egg masses. This prompted an observational study on the abundance and diversity of mites associated with viburnum leaf beetle egg masses on the native shrub *Viburnum tinus* at EBCL in 2016, in collaboration with mite taxonomists from the Centre de Biologie pour la Gestion des Populations (CBGP, Montpellier). Results showed a high diversity of mites inhabiting egg masses (>15

species, Fig. 16) belonging to different trophic levels, from generalist predators to mycophagous and detrivorous species. Egg masses seem to be a perfect habitat for these mites, which can find shelter and food within the cavities where the eggs are laid. Mite diversity and abundance were positively correlated with the number of intact egg masses present on a twig (Fig. 2). The link between egg mortality and these mites remains to be explored. In 2018, a follow-up study will investigate the effects of the most promising mite species on viburnum leaf beetle egg mortality in a series of observational and manipulative experiments.

Figure 16. Some types of mites discovered within viburnum leaf beetle egg masses in southern France.

Quadripartite meeting

by Lincoln Smith

Scientists from the European Biological Control Laboratory, CABI (Egham, England and Delémont, Switzerland), BBCA (Rome, Italy) and CSIRO (Montpellier, France) held a biannual "Quadripartite" meeting Feb. 8-9 at the Edmund Mach Foundation, in San Michele all'Adige, Italy. All four institutions conduct research on the discovery and evaluation of classical biological control agents of invasive arthropod pests and weeds. Each team presented updates on current projects and workshops were held on eriophyid mites, chemical ecology, annual grass targets, Access and Benefit Sharing (Nagoya Protocol), and new joint projects. The meeting helped to coordinate ongoing projects and to identify future targets.

Recent Publications by EBCL

Alexandridis, T.K., Tamouridou, A.A., Pantazi, X.E., Lagopodi, A.L., **Kashefi, J**., Ovakoglou, G., Polychronos, V. and Moshou, D., 2017. Novelty detection classifiers in weed mapping: *Silybum marianum* detection on UAV multispectral images. Sensors, 17(9): 2007. doi:10.3390/s17092007

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Kim, M.S., Kim, C.J., **Herard, F**., Williams, D.W., Kim, I.K., Hong, K.J., 2017. Discovery of *Leluthia honshuensis* Belokobylskij & Maeto (Hymenoptera: Braconidae) as a larval ectoparasitoid of the Asian longhorned beetle in South Korea. Journal of Asia-Pacific Biodiversity 11 (2018) 132e137.

Tamouridou, A.A., Alexandridis, T.K., Pantazi, X.E., Lagopodi, A.L., **Kashefi, J.,** Kasampalis, D., Kontouris, G. and Moshou, D., 2017. Application of multilayer perceptron with automatic relevance determination on weed mapping using UAV multispectral imagery. Sensors, 17(10): 2307. doi:10.3390/s17092007