Systematic applications for 21st century biosecurity solution - Research on the Brown Marmorated Stink Bug (BMSB) and the growing menace of other pest stink bugs.

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Introduction

This is the accessible text transcript of Australian Chief Plant Protection Officer webinar: **Systematic applications for 21st century biosecurity solution - Research on the Brown Marmorated Stink Bug (BMSB) and the growing menace of other pest stink bugs**.

Transcript

Webinar: **Systematic applications for 21st century biosecurity solution - Research on the Brown Marmorated Stink Bug (BMSB) and the growing menace of other pest stink bugs.**

Hosted by the Department of Agriculture, Fisheries and Forestry.

**Collins, Susie** 0:21

So before we get started, I would just like to acknowledge the traditional owners and custodians of country throughout Australia and acknowledge their continuing connection to land, waters and community. And I want to pay my respects to the people, cultures, elders, past and present, and I extend that recognition to traditional custodians of all the other lands that you are all joining us at this webinar today. So for those who don't know me, my name is Susie Collins. I'm the Assistant Secretary for Plant Health Policy in the Department of Agriculture, Fisheries and Forestry, and I'm just standing in today on behalf of Gabrielle Vivian Smith, who's otherwise occupied at other meetings.

So welcome, everyone, to today's ACPPO webinar.

Bit of housekeeping before we start. Please turn off your video and mute your microphone. That just helps with bandwidth, but if you are asking questions when we get to the Q&A part of the webinar we'd always love to see your face. So please turn your camera and microphone on. Then at that point the webinar itself is being recorded and will be available afterwards and if you want to access that recording, please let us know. We do have the chat function. So can you please use the chat function to ask your questions.

So the webinar, today's webinar reports on research on the Brown Marmorated Stink Bug and the growing menace of other stink bug pests. So this research has been conducted by an international team of specialists led by the University of NSW, and the outcomes will result in a phylogenomic backbone that covers the relationship and revised classifications of the genus, *Halyomorpha* and allied taxa. The project will also redescribe or provide a redescription of valid species and description of new species. And also investigate novel diagnostic keys to allow us to rapidly and reliably distinguish BMSB from other species that are related and other unrelated species.
Research outputs will meet the needs of our biosecurity system and our biosecurity practitioners and also the broader agricultural system.

We will be able to upskill our diagnosticians who are working on the front line and who are being exposed to these organisms and give us confidence that the taxonomic names and the species that we do intercept are all the most up to date and the most valid and the most accurate as they can be, and this all helps us make sure we’re managing biosecurity effectively and also making sure we can make decisions about our biosecurity risk management in a timely manner. So it's a really great piece of work that's really going to provide some strong foundations for our management of this particular pest.

So our speakers today:

Professor Gerry Cassis and Dr. Marcos Roca-Cusachs - I hope I've got that right, from the University of NSW, and Associate Professor Cristiano Schwertzner from the Federal University of Sao Paulo in Brazil. And apologies if I've mispronounced your names.
Professor Cassis is a systematic entomologist with 40 years of continuous post PhD research experience. He is an expert on the systematics of true bugs of the world and published over 202 hundred works on the Heteroptera species, he's a Eureka prize finalist and also a recipient of a Churchill Fellowship. So it's great to have Professor Cassis leading our webinar today.

Doctor Roca-Cusachs is a postdoctoral researcher currently working on the taxonomy and systematics of the Brown Marmorated Stink Bug with Gerry at the University of NSW. As an early career researcher, he's got an outstanding publication record over 70 plus research articles published and has also co-authored some books and book chapters. so again, great to have that experience and expertise on our webinar today.
And lastly, associate Professor Cristiano Schwertner is a systematic entomologist with more than 18 years of post-PhD research experience, and the main area of research is on the diversity and evolutionary history of true bugs and focusing particularly on stink bugs and relatives. So again, the international expertise that's involved as part of this project is really fantastic, so welcome Gerry, Marcos and Cristiano and I'll hand it over to you to start the presentation.

 **Gerry Cassis** 6:02

Thanks, Susie.

I'd like to first thank you for the kind introduction and it's a pleasure for us today to report to you on our work on invasive and pestiferous bugs in general, but also specifically about our current project on the Brown Marmorated Stink Bug and allied taxa, which are funded by DAFF. So the result of our research presented today are the product of our global stink bug team with colleagues from Brazil, the US, Czechia, India and Spain, which Marcos comes from, the University of Barcelona, and three of us will be presenting today.

I'll give a brief introduction to start with and Marcos will give the results. So far, the preliminary results on the Brown Marmorated Stink Bug and Cristian, who's an expert on Neotropical stink bugs, well, he's also an expert on stink bugs of the world, but he's speciality in what he's going to talk about a lot today is stink bugs that are pestiferous in soybeans in Brazil and more broadly in the Neotropical region. So I'm just moving along. So in terms of the introduction. Just to think about pentatomids in terms of the diagnostics, the shortfalls in terms of the classification, they’re hyper diverse lineage with 5000 plus species and these sort of taxa with many species in them often have historical issues with the classification. And it's no different with the Pentatomidae, which comprise taxa that are undescribed, and many species that are in the wrong genus. There are very few studies in this group that include molecular data and there's no phylogenomic studies at all. And so the specific classification is really mostly untested.

On the biological side, stink bugs are often prolific, they have high reproductive potential and the adults have diapause phase of their life cycle, and are very prone to being hitchhikers and often find both sexes of the same species captured together.

So recent studies indicate that the order Hemiptera is the most important invasive group, along with the Coleoptera, and the stink bugs are the most important family group in the Hemiptera suborder, Heteroptera, with 72 species of stink bugs that are now established outside their native range and 85% of stink bug introductions have occurred over the past sixty years.

So this is an important study that was produced by DAFF staff that was published in late 2023, which shows the incursions of Pentatomidae outnumbering many of the other groups. In fact, most of the other groups that are intercepted by DAFF on vessels that are bringing in cars and other machinery. So DAFF itself has about 5000 specimens in its collections, which we've borrowed, which have been identified to different levels in the taxonomy because the classification has got issues in terms of the availability of diagnostic keys, of the species that are identified in that lot of 199, there's 43 species that are considered pests in the literature.

So let's go to BMSB. BMSB belongs to the tribe Cappaeini, which was his first established by Atkinson in 1888 and it's had very little work done on it. There was a review of the of the higher classification of the Pentatomidae in the Seminal work in 2018 by David Rider and colleagues, including Cristiano Schwertner, that indicated there were doubts about the monophyly of the tribe and it remains that the Cappaeini have never been tested. Currently, it comprises 24 general and 151 species. So even though there's serious issues at the tribal level, there are also issues that are important at the genus group level and the species group level. This is a slide that has on your left a generic exemplars of the tribe Cappaeini, and on your right are exemplars of the genus *Halyomorpha*.

And you can see the more logical heterogeneity both at the genus and species level, but it suffice to say there are no generic keys to all the Cappaeini genera and there are no keys to all of the species of *Halyomorpha*. So not only is there no diagnostic protocol for BMSB, nor identification keys I would say, there is no international or national Australian diagnostic protocol for BMSB to separate males and females from other species in the genus *Halyomorpha*. And moreover, females are a major challenge to identify which is very common in stink bugs. So in this in effect what we have is a diagnosis diagnosticians are currently promised in identifying BMSB. So this goes back to the work that we've been doing with DAFF for six or seven years now, we run capacity building workshops. That includes keys from the subfamily, to the families within the superfamily Pentatomoidea, right down to genus level.

 In the first column you can see a picture of BMSB. The second column you can see an extract from our tribal key of the nominotypical subfamily Pentatominae and highlighted in yellow are the Cappaeini, appear in distinct parts of the key, which suggests the non-monophyly of the tribe. We could not produce keys to species in this manual, mainly because there's so many of them, but also because there are no keys that identify congeners, between each other, for many of the economic species, including BMSB. In the third column you can see what we call species pages that include: A habitus image, plus external genitalia of the male and female; now these are very useful for some diagnosticians, but what the problem is, is they don't offer a differential data for diagnosing between species in the same genus.

So DAFF was quite aware of the issues relating to these taxonomic impediments and came to us to see if we could revise *Halyomorpha* and the allied taxa that are in the Cappaeini. So in response to this, we have developed this research framework for what we call the 21st century solutions that basically; accumulates the data, does a review and accumulates specimens. We've done a forensic study of the type material, comparing it to the non-type material and we'd have two lines of evidence, the morphological and the molecular, which we're running in parallel and then we integrate into [this framework] to determine a robust classification, that has outputs that are on the information side, including monographs, differential diagnosis keys and manuals, and on the data size: vouchers, images, digital data, and sequences. So when we're using conventional methods to identify pests of importance and the characteristics that they possess, but we're also using new techniques, In particular, we're using Micro X-ray tomography for searching for new characters of the internal anatomy of the of these animals, plus the external characters, as well as looking at ultrafine details of the genitalia.

This is an image of the phallus of stink bug species, and we're able to now look at characters that we formally we couldn't readily homologize between species and in the third column here we're also using geometric morphometrics to distinguish closely related species. So in terms of the molecular side of our line of evidence, we've been evaluating DNA barcodes that are provided by public repositories (such as Bold and GenBank) and also sequences that were supplied by DAFF, but our main approach now is to use Ultra Conserved Elements [UCE’s] from phylogenomic scale of data using target enrichment, which we have at our disposal, Hemiptera bait kits that works really well and We've been using it for four or five years in my lab. This is used for testing monophyly of various taxonomic levels including the Cappaeini and the generic level for the *Halyomorpha*, as well as species within it. So it's a very useful tool for us to have robust results and match that with the morphological data sets. So that's the background in terms of where we stand in terms of BMSB and also the methods that we're using in our research framework. And now I'm going to pass on to Marcos to give us a report on the preliminary data associated with our research on the Brown Marmora Stink Bug.

**Marcos Roca-Cusachs** 16:47

Thank you very much, Gerry.

So as Gerry mentioned, I'm going to focus on the Part 2, which is the research on BMSB. The goal of this part of the presentation and also our part of the project is the production of a robust classification of the *Halyomorpha* complex in order to facilitate the rapid and accurate identification of all the species that are involved. My presentation on the project simultaneously are subdivided in five main names. I will not explain them here, but along the way, during the presentation.

 I'll be giving you insights to each one of these aims and some small results that have found simultaneously. So first of all the problem, the type species of *Halyomorpha* is a taxonomic mess. Not only has a confused taxonomic history due to inaccurate synonyms, misidentifications, no precise type locality, but additionally the species name was ignored for more than 100 years in the bibliography.

So despite this fact in the past 10 years, the number of publications regarding the species have exponentially grown, not only because the species is rapidly wide spreading along the globe — As you can see in in the map on the right —but also because it is a big economic pest. So in the face of its global biosecurity risk, the BMSB needs a robust classification and accurate diagnostic tools. So as part of the first aims, which would be the acquisition of research material and the study of the type specimens, we have been able to visit all the type depositories in Europe.

So these type specimens and also all the other known specimens, which would be around 6000 specimens, are currently housed at the National Museum in Prague, where one of our collaborators is, and also for those collections, mainly from outside Europe, that we have not been able to visit, we have acquired images of the type specimens. On the other hand, we have gathered here, at UNSW a dry and a wet collection both for morphological and molecular purposes. Additionally, as part of aim 1, we have gone to the field to acquire additional research material and this was thanks to Northern Australia Quarantine Strategy. We were able to go to some neighbouring countries from Australia which allowed us to get fresh material for molecular and morphological studies, additionally being able to verify the presence or absence of BMSB and any other cappaeines in the region.

As part of the preliminary results of these first aims, we can present that *Halyomorpha timorensis*, which is a species that was originally described from the island of Timor, only known from the type material, in this case one female, and even though this might sound redundant, the male is unknown, as Gerry has commented before in many pentatomids is the case, but in the case of *Halyomorpha* species, it is not possible to identify by means of the females, as they have no diagnostic characters that we know of yet, so it is currently a major concern for biosecurity in Australia.

However, after the examination of the types, examination of a lot of material, we are able to say now that the species may not occur in Timor and that the species was probably mislabelled at its origin. Additionally, as part of these preliminary results so far from parts one and two, we can recognize at least eight new species. In this set of images here, you can see two images of the related species from the Oriental region that are currently in *Halyomorpha*.

As for the aim three, which is the comparative morphological studies of target species, Gerry has mentioned before that we follow a classical line that would be by the use of SEM images, illustrations which would allow us to interpret all these structures and also optical microscopy, but on top of that, we have been acquiring additional information by doing microCT scans of either the whole body of some specimens or the genetic structures, allowing us to go in depth and not have to dissect and destroy part of the specimens. Here it's the phallus and of an *Halyomorpha halys* or BMSB.

As for the aim four In the phylogenomic studies as a preliminary part to of phylogenomics, we wanted to explore the availability of CO1 sequence data either in public databases or the ones that DAFF had produced and currently gave to us to be able to work with them. What we could recognize in some of these sequences that were deposited in public databases identified as BMSB that they did not cluster together with the big main cluster of *Halyomorpha* present, which could indicate that there is some issues in the identification of these sequences. So going on with that, we continued doing the phylogenomics of these *Halyomorpha* complex , the tribe Cappaeini and also all possible related taxa following the following protocol for creating all these phylogenomics based on UCE’s and ranking regions, on the right side you can see a very schematic tree of the data we have produced with these three areas marked, I will just focus in detail on these parts, which is the ones that we really want to know for our project, would be that these three parts tribe Cappaeini are not monophyletic and correspond to three independent lineages. The first group, the *Veterna* group with an African origin, related to some of the Neotropical carpocorines. The second group, which would be the *Cappaea* group or the true Cappaeini with East Palaearctic and Oriental distribution which hold the type of the tribe Cappaeini, and then, last but not least, the *Halyomorpha* group with Paleotropical distribution and then the focus of our study. I must say here, that all these results are preliminary and are still not published. So focusing on this *Halyomorpha halys* group, we can place it now here with all these other genera and species that belong to this Paleotropical group and then within this, let's say core group, we can find that *Halyomorpha* seems to be paraphyletic due to the fact that genus *Prytanicoris* clusters within the genus *Halyomorpha*.

However, *Halyomorpha* diagnostics at this point Is in review, getting sorted, *Halyomorpha* is recognized as a paleotropical genus and BMSB is closely related to the species that are present in this Australian and Oriental regions. So even though as part of these preliminary results that we have been obtaining from this 4 previous aims, we can now more or less define what the *Halyomorpha* core taxa would be, being able to recognize eight genera within. Despite the fact our for it comes for *Halyomorpha*, despite the fact that we have not been able to locate two of the types, we have for all the rest ,and therefore we can recognize 21 valid species in *Halyomorpha,* with eight new undescribed species, six new synonyms, and six species that have been transferred to other general. And all these leads us to the next aim which is still, in a very preliminary stage, and it's yet still work in progress, which would be the integrated taxonomic research. So now we are hypothesizing based not only on the molecular data, but also on all the evidence obtained from the morphological data, all this genus and species groups within *Halyomorpha*.

So this would be the end of my part and I would refer to Professor Cristiano's Schwertner that will continue with some other stink bugs.

**Cristiano** 26:35

Thank you, Marcos.

Well, for the third part of the presentation, I will add examples of stink bugs of economic importance and issues regarding classification, and identification and distribution of these species. The examples are based on information from studies we are developing. As mentioned before, stick bugs are one of the most important group of pest species, with almost 400 species reported in crops worldwide. At least 30 species were of major impact in the last 20 years. Top of them are also invasive as shown in the introduction.

Here I selected examples of important crops and the number of stink bugs associated to them, highlighting rice, soybean, wheat and corn, which are among the main produced food crops in the world. Those 30 plus species of major impact are included in four out of nine subfamilies and 9 of 45 Pentatominae tribes of the current classification. At least 70 crops in 33 plant families are attacked by stink bugs worldwide, and different levels of host association are known amongst stink bugs.

Some species are generalist and highly polyphagous like the global Brown Marmorated Stink Bug and the Green Vegetable Stink Bug [*Nezara viridula*], and the recent invasive Yellow Spotted Stink Bug [*Erthesina fullo*]. Nevertheless, some species are more selective in their diet, attacking hosts from one plant family or types of hosts like, fruit trees. In all these cases, damage can be severe and the managing and control of stink bugs are always a challenge. The correct identification of pests or invasive species is always key part for any program, and for groups including invasive species, diagnostic keys from non-pests, and native from non-native fauna is fundamental for stick bug identification, distribution records, the whole space associations are not always straightforward to reach and most instance diagnostic tools are not available to end users.

In the next slide, I will give 3 examples of major pests that have increasing importance in the last 30 years in the Western hemisphere, dynamic crop expansion scenario with continuing implications for pest dynamics. All examples are related to soybeans and important crop worldwide. Taxonomic research on these species were recently reviewed in a book chapters which includes information on the classification and diagnostics of these species.

Soybean major producer countries include Argentina, Brazil and US all in the Western Hemisphere or the Americas, a region with high biodiversity containing 1/3 of all stink bugs described in the world. In South America, soybean was introduced in the 30s with an explosive production growth in the last 50 years. Among the major soybean pests are the stink bugs, and together with the crop expansion, also the importance of these insects expanded.

For instance, we recently showed that five species represent 99% of the stink bugs in soybean fields in Brazil with the Neotropical Brown Stink Bug [*Euschistus heros*], the most abundant species, ranging from 53 to almost 90% abundance depending on the region we collect. The genus *Euschistus* is representative of economic Important stink bugs. Almost 70 species of *Euschistus* are known restricted to the Western Hemisphere. Some of the major pests of crops like corn, cotton, soybean and wheat. More than one species of *Euschistus* may occur in the same crop, and sometimes identifications are not easy to accomplish. It is important to notice that *Euschistus* species have been intercepted in cargo ships, including the least published by operational DAFF people recently, the Neotropical Brown Stink Burger *Euschistus* *heros* is widespread most regions of Brazil, also found in Argentina, Paraguay and Bolivia. Nonetheless, this species was not considered abundant in the 70s and the 80s, and currently large numbers are found in Central and Southeast of Brazil.

Recent studies have shown that two distinct populations can be recognized within n *Euschistus* *heros* a Northern and southern population with clear differences. Most important, this population's hybridized in central Brazil, a process that may be related to the soybean expansion, and with implication to the explosive nature of these species in the region. In Central Brazil *Euschistus* *heros* is also a pest of cotton and corn.

This result has implications for species identification as well, especially because we have recently found that at least three other species of the *Euschistus* that are associated with soybeans in Brazil in the northern region, where *Euschistus* *heros* is not common and indicating that the so-called Neotropical Brown Stink Bug may represent a complex of species. We also found evidence that at least one of these species produced fertile hybrids when crossed with *Euschistus* *heros*. We are currently reviewing the taxonomy and all information related to the species in *Euschistus* applying a similar framework that we are now developing for BMSB here.

My second example is the genus *Diceraeus*, a group related to the *Euschistus* and including two other main pest in South America*, Diceraeus furcatus* and *Dicereus melacanthu*s. Although occurring in partial sympatry on crops that you species do not overlap with *Diceraeus furcatus* found in South Brazil, Uruguay, Argentina and *Diceraeus melacanthu*s in central and southeastern regions. As the two species are very similar, we are current work on collecting, mapping and developing information to provide accurate identification of the species. During our studies, we noticed that *Diceraeus furcatus* ischanging its distribution, probably influenced by the expansion of agriculture scenario in Brazil. *Diceraeus furcatus* has now been commonly found in soybeans fields in central Brazil, and molecular evidence show that this expansion had occurred from South N region, with individuals collecting central Brazil, clearly derived from populations not commonly found on crop fields previously.

My last examples is on the genus *Piezodorus*, It's well known to some of you. One species *Piezodorus oceanicus* is endemic to the Australian region. It represents a monophyletic group of 11 species worldwide distribution. Some species are considered Pest of legumes and grass grains. Host plant records indicate high levels of polyphagy. Species group are recognized based on morphology, but species identification is still a challenge, especially in the [*Piezodorus hybneri* group. This group includes three cryptic species, including *Piezodorus oceanicus*, identifying mostly by the distribution range. Morphological identification is possible using internal genitalia, although morphological variation for *Piezodorus hybneri* and *Piezodorus guildini* were unknown. We have started to shed some light on this matter studying *Piezodorus guildini,* the only species recorded in the Western Hemisphere.

The species is considered originated in the Caribbean basin and currently invasive soybeans fields in North and South America. Indeed, our results show that all Brazilian populations of *Piezodorus guildini* were connected to one dispersal event from North to South, and there is also evidence that populations are still expanding. We also show that haplotypes from South Africa of available public databases clustered with *Piezodorus guildini* from Central America. Nonetheless, there is no official records for these species in the Afrotropical region. Currently we are evaluating the morphological variation we see in *Piezodorus guildini* using CO1 sequences and comparing them with the other two species of the *hybneri* group trying to clear the questions about the identity and the origin of *Piezodorus guildini*. These three samples here show the importance of a correct identification in stinkbug pests and how important gaps we still have in this matter.

**Gerry Cassis** 34:57

So thanks Cristiano for that excellent presentation, and Marcos as well.
So the conclusions are obvious in many ways, the sink bugs are growing by security risk worldwide, the data suggests that:

The biological attributes they have, including the polyphagy and high reproductive potential, makes them a recipe for incursions that could threaten agriculture in non-native areas.

The diagnostic knowledge of the Pentatomidae is in its infancy, no doubt, and this came up as a textbook case with BMSB and how these poor classifications hamper accurate and rapid identification.

The three points that we bolted here is that stink bugs such as BMSB have demonstrated how they provide diagnostic, or they don't provide, but they provide another way of putting is provide gaps that exist in the taxonomic literature, including the absence of diagnostic protocols and identification keys.

We have come to the conclusion that diagnostic gaps are best served by international research teams and coupled with capacity building. We could not have done this project if we didn't have an international team.

And lastly, a robust classification using an integrated systematic framework is the most effective way to produce fit for purpose diagnostic tools ,and what we've done here is we've attached our systematic framework to the questions that diagnostics, diagnosticians face, including the pathway to getting a rapid and accurate identification to a high level of confidence and we believe that the outputs that we're going to produce on the information and data side will do that.

Let's finish with a fun slide.

So there are over 5000 stink bugs, which we estimate about a third of them are brown in a variety of shades, and here are a few examples in this slide. This slide includes 3 BMSB images, including the type, other species of *Halyomorpha,* unrelated stink bugs, including Australian natives and stink bugs in other subfamilies. Our emphasis on developing tools at different taxonomic levels, we argue that this is the most authoritative way forward, and we have found it the best way to build capacity in frontline diagnosticians.

And we finish up with our acknowledgements to the Australian government, to DAFF, to the modern technologies and diagnostic tools program, Northern Australian Quarantine Service, Museum curators as well as Doctor Nik Tatanik, a collaborator from the Western Australian Museum.

So thank you very much and we're open to questions.

 **Collins, Susie** 37:51

Thanks, Gerry, Marcos and Cristiano.

That's very fascinating topic. I really like getting into the detail of taxonomy part because I don't understand a lot of it, but partly because it's such a fundamental capability that we need to support our biosecurity systems.

So what I liked about some of the things we were talking about was getting the foundations right, but making sure there's an operational tool at the end of the day so, I wondered if you before I open it up for questions from Alice, I want to if you wanted to comment on that because, you had some beautiful videos of the technology you were you were using on the internal anatomy of the bugs and the like. Do you think we will be able to translate those findings into something that that our operational field staff can actually use in, in more real time?

 **Gerry Cassis** 38:47

Well, the micro CTS are really just gathering new data and homologies for characters that we haven't seen before. Of course, those scans can be shared with other people, but it's the data that arises that from them that really counts, and it and it. It also demonstrates that we're trying to dig deep in, in solving some of these taxonomic problems which are which are really perplexing at time, and it is true for BMSB that it's one of the most poorly known groups in the Pentatomidae, in terms of its relationships with other taxa of the general, it belongs to and the tribe it belongs to. We believe that if we build this, this these results in a from a higher classification down to the species level, this will all be very useful and provide shortcuts for diagnosticians to make rapid identifications.

Cristiano, do you have any comments?

**Cristiano** 39:51

Well, regarding the CT scans, I believe that they can be useful for as a diagnostic tool as well. But of course we need to interpret and I think the outcome is the information that comes from all these tools that we are using.

 **Collins, Susie** 40:15

Thanks. So questions in the chat. I've got another one though.

Could you talk also about the importance of that international collaboration? And I was wondering how easy it was to find and then access all of those types specimens that I think was Marcos's presentation was showing.

 **Gerry Cassis** 40:36

So most of those types in *Halyomorpha* and in the Cappaeini are in Europe, there are a few types in the American Museum and the US National Museum in the US, and we've accessed them. Some of the material. We've actually borrowed it.
The type material was a fantastic piece of work by Petr Kment at the National Museum of Czechia. He's been going to many collections and multiple times to really important collections like the Natural History Museum, collections in in Paris, etc.
So we've done a pretty good job, I think to found all the type material aside from, is it Marcos it's just two species where the types have been lost?

 **Marcos Roca-Cusachs** 41:24

I guess as far as we know, there's only two species that we have not been able to locate the types.

 **Gerry Cassis** 41:29

So the work of Kment has been outstanding and we could not have done that without his expertise and his connections and his forensic approach to examining these type materials.
In the broader sense, in terms of the international group. I think we've amassed the best team we could possibly have amongst the stink bug systematists. We were thinking today how many stink bugs systematists there are in the world right now who are active, through professional entomologist, and it be around 30, but a lot of them are working at a very regional scale. So David Rider is the father, well the grandfather I’d say, of classification stink bugs alive today, he's done an enormous amount of work, and Kment works closely with Salini, and then Cristiano is working in the hotspot of stink bugs in the world.

There's 5000 species, about 1700 of them in Brazil, and you can see these.
Problems emerging in soybeans in South America are a real issue and it's an indicator of what pest could be coming next. I keep thinking about this genus *Euschistus* that Cristiano refers to and comprises 70 species including new species, with *Euschistus* *heros* intercepted by DAFF. We know that soybeans, for example, could be increased as a commodity crop in Australia, so it's a warning that there's another one coming down the pipe that could be of national importance.

 **Collins, Susie** 43:19

Thanks Gerry.

I was going to ask about the next one.

You already answered that.
So there's some comments in the chat about how our wonderful and amazing the slides were and maybe we should need to think about in terms of the future, how we best share that through infrastructure like peddle. But there is a question here about research on the odour variance of the different stink bugs and compared to Australian species, do you know of any research that's being done around that?

 **Gerry Cassis** 43:52

Cristiano.

Good.

 **Cristiano** 44:00

I was reading the chat. I was reading the chat and didn't pay attention closely to the question can you repeat please?

 **Collins, Susie** 44:09

It's about any research being done on the different odours and the variance in odours between different species.

 **Cristiano** 44:18

Different, sorry.

 **Gerry Cassis** 44:20

Stink gland.

Allomones.

 **Cristiano** 44:27

Yes. There's some research groups in Brazil that working on, for example *Euschistus heros*. So there's some research going on in in this matter. I think in in the US also there are at least two researchers that I know that work on, on chemicals, pheromones of stick bugs as well.
There's a reason with you on that topic as well.

 **Collins, Susie** 45:00

Excellent. Thank you.

Some other comments again about, it's great to see the integration between the traditional systematics and molecular work, and having that integrative approach that's that seems to have got lots of support as well.

And another question that’s been asked. Can we clarify so the *Halyomorpha* will have 29 recognized species in total and eight new species that are yet to be described. Is that correct?

 **Gerry Cassis** 45:30

Marcos, can you answer that question?

 **Marcos Roca-Cusachs** 45:41

I'm reading the comment just to get the numbers right.

 **Marcos Roca-Cusachs** 45:47

So what we can say now with the data we have is that we recognize 25 species of the ones that were already published, and there's 8 new ones. We still because we don't have, we are not have not been able to locate two of these types.
Those are bit not sure of them till we decide something about those two species.
But it would be around that, but also I do not discard the fact that the more sampling we do will bring up to like new species.

 **Collins, Susie** 46:29

Thank you.

So this question from Asrar. How many valid type species of Halyomorpha are present and do male specimens have a difference in their parameres?

 **Marcos Roca-Cusachs** 46:51

I don't understand the question.

How many type species of *Halyomorpha* there is?

Like there's one type species of *Halyomorpha* which is *Halyomorpha halys*, currently is a type species of the genus, and if that means how many valid species in general that would be responded to the with the question I said before.

As in for the male specimens, Yes - male specimens show some characters, which we are under investigation by integrating all these morphological information. both the parameters and the pygophore show some differences. However, the limits the variability within the species we are still under investigation for all of them to see the limits.

 **Collins, Susie** 47:44

Thanks. And Asrar, you got your hand up?

Did you want to clarify or ask a follow up?

 **Ahmad, Asrar** 47:48

Yeah, yeah.

Thanks Marcos.

I just like you know, you're talking about like the sourcing of those types of specimens. So how many species did you had? Is it only *Halymorpha halys*? Do you have other type specimens? and how many species do you think you know can be the which are valid and you're still working on that?

 **Marcos Roca-Cusachs** 48:13

I might not be completely understanding your question. So we checked all the type specimens of all the *Halyomorpha* species that were available to us.

 **Gerry Cassis** 48:25

Let's see.

 **Marcos Roca-Cusachs** 48:28

The amount of type specimens that we examined, I cannot tell you off by memory, it was as a lot, of some of this, I mean, many of the species are represented by just one main type, but in other cases the type series are very long and I do not remember this information of by hard.

 **Ahmad, Asrar** 48:51

Yeah, because I know I did some research there, like some other species of *Halyomorpha,* like *Halyomorpha* *picus* and *Halyomorpha* *halys*. So that's, you know, I just, like, want to clarify that *Halyomorpha* *picus*, is it a valid species? Like, is there any distinguishing character between two different species?

 **Gerry Cassis** 49:10

Yeah.

 **Marcos Roca-Cusachs** 49:10

Yes. And if you want to check actually one of the few species that has been recorded and where the diagnostic differences have been published with precisely *Halyomorpha halys.*

There is a paper by Petr Kment and collaborators from some years ago where they select the type or designate the type of *Halyomorpha* by selecting *Halyomorpha halys* and inside you'll find all that information.

It's probably the only two species that there is information regarding that sense.

 **Ahmad, Asrar** 49:55

Thanks for your help Marcos.

 **Collins, Susie** 49:59

Thanks. We've got time for another couple of questions. Boris, you've got your hand up. Go.

 **Lomov, Boris** 50:10

Sorry, I was just curious. What's the morphologically closest species to *Halyomorpha halys*? Is it another *Halyomorpha* species?

Because in the past we have intercepted something from Iran, and the only difference I could spot was just the gland, the, you know, the gland shape was different. So it was pretty close, but we couldn't identify it to species even after we did molecular sequencing. But so what is the closest kind of species, morphologically?

 **Marcos Roca-Cusachs** 50:51

I guess that's the question for me again.

With the information we have now, I cannot give you direct response right now, it's still like we are still finishing to produce this data. There is a handful of species that are closely related, though we are still resolving that part of the phylogeny. And additionally, it's information that it's not yet submitted or published and we cannot provide you with that information, but it's a complex of species very closely related that show a lot of morphological similarities, but then in also according in you said Iran, in like near to Africa countries there's several other very closely related general that are very similar to *Halyomorpha*.

 **Lomov, Boris** 51:56

Thank you.

 **Collins, Susie** 51:59

OK one last question, and apologies to those people whose questions we haven't got to, but it was around machine learning and AI have is are you implementing or any of that sort of technology or innovation into what you've been doing or do you see a use for more integration of machine learning and AI as part of the operationalization of what you're doing.

 **Gerry Cassis** 52:28

We actually had a discussion about that today. If the question would come up about AI and of course, we've done some of that in our work for other groups, we had a CRCP grant looking at use of AI as a diagnostic tool for separating insects of that are Important pests in terms of human health. And so we know how that works, but we also feel that he inclusion of AI right now is a little premature. Our experience has been that you have to accumulate a huge number of images to actually test the models, and sometimes you get sort of support values, confidence values around 95%, but the argument I would have is that. Is that sufficient for accurate and rapid identification?

The diagnosticians in Sydney have told me they're looking for a 99% level of confidence in terms of the use of these tools, the diagnostic tools and identifications that they're using.

So I think AI, we've thought about it. Maybe it can be used in terms of analysing the bioinformatics of the molecular data that might be of use, because that does take a lot of time, but right now it's not something we thought about integrating in the work that we're doing now. The basic work still really needs to be done.

 **Collins, Susie** 53:59

Excellent. Thanks.

 **Gerry Cassis** 53:59

You're not. We're not against it, but it's not part of our thinking right now.

 **Collins, Susie** 54:04

Not there yet. Great. Well, thank you very much everyone.

Please join me in thanking Gerry, Marcos and Cristiano for the fantastic presentation today. If you've got any questions or want to know more about this project, please reach out to us and there's the e-mail address is in the chat there. Now our next ACPO webinars in February, so look out for that. And between now and then, please have a good, safe summer break. Hopefully you'll get a chance to have a break. And we'll see you in February.

Thanks everyone.