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NO BEES LIFE

EBA MAGAZINE

32 COUNTRIES

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(65 beekeeping organizations)

In order of confirmation of the Statute of EBA

430.584 beekeepers

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Romania
Malta
Germany
Hungary
Ukraine
Montenegro
Lithuania
Bosnia and Hercegovina
Sweden
Croatia
Czech Republic
Poland
United Kingdom
Netherlands
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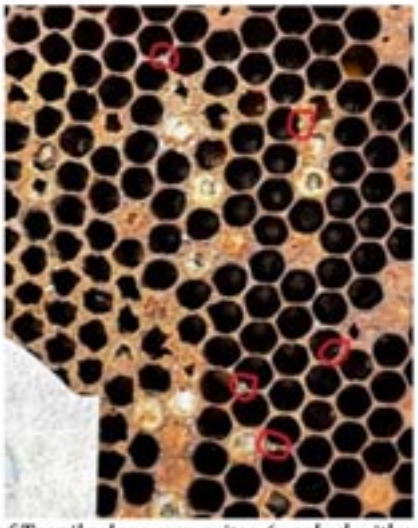
TROPILAELAPS, WHEN YOU KNOW WHAT TO LOOK FOR, IT IS EASIER TO PROTECT YOUR BEES

DON'T FEAR THE UNKNOWN. • KNOW THE MITE. • SPOT THE SIGNS. • ACT RESPONSIBLY.



Example of Tropilaelaps spp. mites (marked with red circles)

WHAT IS TROPILAELAPS spp.?

Tropilaelaps spp. is a parasitic mite of honey bees.

MAIN HABITAT: honey bee brood cells.

WHERE DOES IT FEED? On developing brood.

WHERE DOES IT REPRODUCE? In brood cells.

WHERE IS IT LESS COMMON? On adult bees, where it is mostly only temporary.

WHY IS IT IMPORTANT TO KNOW IT?

1 • HIDDEN
THE MITE LIVES IN BROOD
Because it spends much of its time in capped cells, it can easily be missed at low infestation levels.

2 • TRANSMISSION
MATERIALS ARE NOT RISK-FREE
Outside brood, the mite can also survive on materials associated with the bee colony.

3 • MOVEMENTS
MOVEMENT CAN MEAN SPREAD
Special attention should be paid to queens, colonies, brood, packages, pollen and used equipment.

HOW LONG CAN IT SURVIVE OUTSIDE BROOD?

UP TO 3-6 DAYS
DRY/ FRESH POLLEN
The source material reports survival for up to 3-6 days.

UP TO 6-9 DAYS
EMPTY COMBS/ADULT BEES
The source material reports survival for up to 6-9 days.

SEVERAL DAYS
OTHER MATERIALS
Other bee-related materials can also carry live mites.

IMPORTANT: THE ABSENCE OF BROOD DOES NOT MEAN THERE IS NO RISK. Source: Pettis, 2025. Presentation in China.

DO NOT CONFUSE IT WITH SOMETHING ELSE

A SMALL MITE IS NOT PROOF!
Various mites and other organisms may be found on the bottom board.
Finding a small mite alone does not confirm Tropilaelaps spp.

Tropi Look a-likes

Photo by AHPA Bee Unit York. UK Crown Copyright. OIE Terrestial Manual 2018.

(WOAH, 2018)

Comparison: Tropilaelaps mites with Varroa mites, the bee louse (Braula), and pollen mites.

LOOK WHERE IT LIVES. TROPILAELAPS spp. SHOULD BE LOOKED FOR PRIMARILY IN BROOD

Tropilaelaps spp. mite in the brood area.

WHY IS THERE A RISK OF MISSING IT?

- A sticky bottom board may miss the mite at low infestation levels.
- A sugar shake may miss low infestation levels.
- Examining or washing adult bees may miss the mite because brood is its main habitat.

Scan QR code to watch RBD method

RBD – RAPID BROOD DECAPPING

The Rapid Brood Decapping (RBD) method was developed specifically for rapid detection of *Tropilaelaps* spp. in capped brood. The procedure is simple but requires careful observation (Uzunov et al., 2024).

1	2	3	4	5
SELECT capped worker brood.	**ATTACH** a wax strip to a small area.	**PULL** the strip away quickly.	**RECORD** the entire procedure with your phone.	**SLOW DOWN** the video and review what happened.

RECORD. SLOW DOWN. WATCH AGAIN. Mites can leave opened cells very quickly.

HOW DO WE REDUCE THE RISK?

QUEENS
BUY RESPONSIBLY
Do not buy or use uncertified queens. For queens from potentially affected areas, follow official veterinary guidance.

POLLEN
KNOW THE SOURCE
Do not introduce potentially contaminated pollen directly into colonies. If the origin is uncertain, the source material recommends precautionary freezing for at least 10 days.

MOVEMENTS
CHECK BEFORE MOVING
Colonies, packages, brood, queens and used equipment should not be moved from potentially affected areas without checking current veterinary requirements.

COMB AND MEASURES
DO NOT IMPROVISE
Carefully inspect stored or new comb before introducing it. Use only officially approved products and methods and comply with legislation.

SUSPECT TROPILAELAPS MITES? DON'T WAIT – ACT.

STOP	DOCUMENT	DO NOT SPREAD	REPORT
Do not move the bee colony.	Take a photo or video.	Do not move colonies, brood, queens or equipment.	Contact the competent veterinary authority.

TOGETHER WE ARE STRONGER

Tropilaelaps spp. does not recognize national borders. Successful control therefore requires early detection, responsible movement, monitoring, scientific evidence and international cooperation.

KNOWLEDGE IS OUR FIRST DEFENCE AGAINST TROPILAELAPS spp.

Source: **Prof. Dr. Aslı Özkırım** — "People Fear What They Do Not Know – Tropilaelaps spp.: Know It, Detect It, Manage It."

August, 2026

HOW SHOULD EUROPE RESPOND TO A POSSIBLE INVASION OF THE TROPILAELAPS MITE?

The Tropilaelaps mite has not yet been detected in Europe, but the risk of its introduction is real because it is already present in southwestern Russia and Georgia, while suspected infestations have also been reported in Turkey.

The relatively recent invasion of honey bee colonies by the Small Hive Beetle (Aethina tumida) in Italy, followed by the destruction of dozens of apiaries by burning entire hives, remains a vivid and frightening memory for beekeepers. What will happen if the Tropilaelaps mite escapes containment and invades Cyprus, Greece, and other European countries? What do European regulations require for controlling its spread? What are the first measures that authorities and beekeepers should take? Will colonies be destroyed, as happened with Aethina tumida, or will different control measures be applied?

ESSENTIAL INFORMATION FOR BEEKEEPERS

Identification Inside the Hive

The Tropilaelaps mite is reddish-brown, with an elongated body, and moves extremely quickly across the comb. It has eight legs, although the two front legs are longer and constantly probe the surroundings, giving the impression of a six-legged insect rapidly moving its antennae. An experienced beekeeper can easily recognize the mite because of its characteristic speed of movement.

Biology of Tropilaelaps spp.

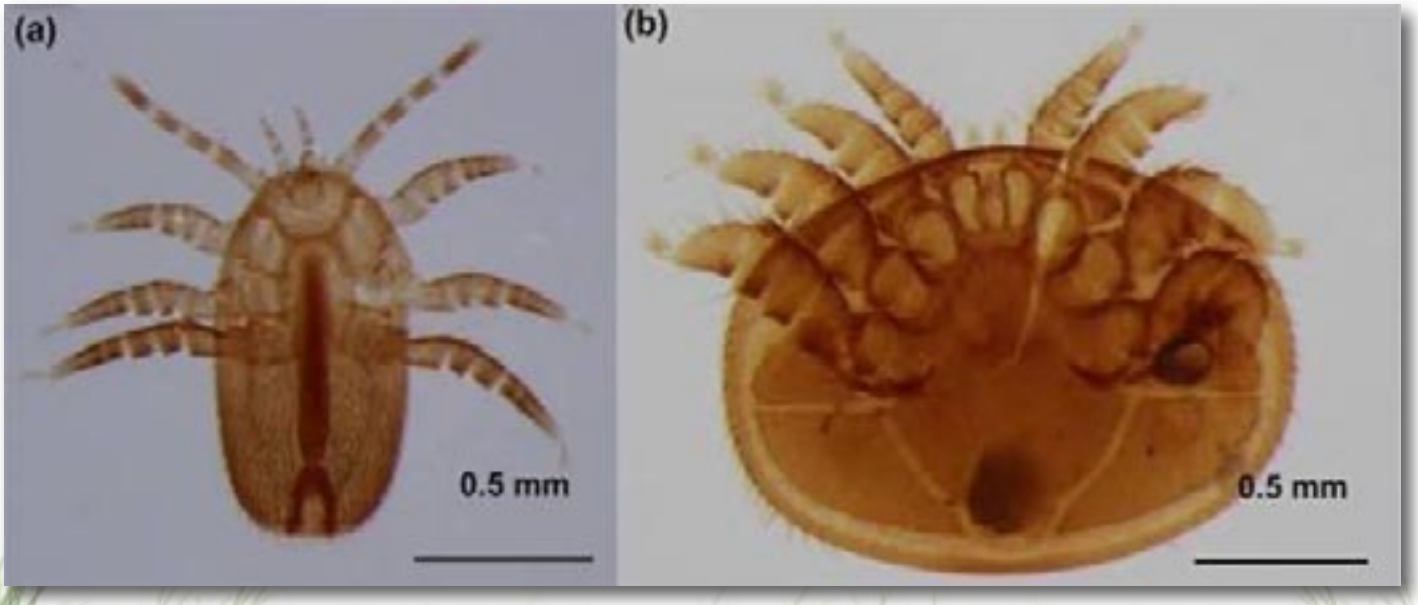
The female mite enters a brood cell 4–12 hours before capping. It moves over the larva and along the cell walls and begins laying eggs within 24 hours after the cell is capped. It gradually lays 4–5 eggs and is also capable of reproducing by parthenogenesis.

Its reproductive cycle lasts only 5–7 days, producing up to three female offspring per mite in worker brood.

Unlike Varroa destructor, the adult Tropilaelaps mite cannot parasitize adult bees because its mouthparts are unable to penetrate the hard exoskeleton of mature bees. It feeds exclusively on brood and survives for no more than three days on adult bees (Woyke 1987; Delfinado-Baker et al., 1992; Pettis et al. 2019).

Survival of Tropilaelaps spp. Outside Brood

Survival in empty combs. Tropilaelaps spp. cannot survive for long periods in empty combs. Compared with the hard-shelled Varroa mite, Tropilaelaps has a thinner and softer cuticle, making it more susceptible to water loss



and desiccation. At relative humidity (RH) levels below 70%, water loss increases rapidly, and mites may become severely dehydrated and die within approximately 12–48 hours (Khongphinitbunjong et al. 2019).

Under laboratory conditions, when empty combs are maintained at a constant high relative humidity (approximately 70% RH) and mild temperatures, moisture loss is significantly reduced. Under these favorable conditions, Tropilaelaps may survive for up to approximately 6 days. In contrast, in the drier conditions commonly found in comb storage areas, where relative humidity may fluctuate well below 60% RH, mites dehydrate much more rapidly and may survive for only 1–2 days.

Survival on Adult Bees. Tropilaelaps mites have limited survival on living adult bees. Most mites are expected to survive for only approximately 1–3 days on adult bees, largely because of grooming by the bees and the mites' susceptibility to desiccation (Rinderer et al. 1994; Pettis et al. 2019). In an active colony, particularly in broodless areas, Tropilaelaps spp. should not be considered capable of surviving longer than 3 days on adult bees.

Survival on Dead Host Material. Outside the brood, Tropilaelaps spp. may survive for approximately 7–8 days on dead host material, such as dead bees or dead brood (Gill et al. 2024). However, survival depends strongly on the condition and moisture content of the material. In the absence of suitable decomposing bee material, the mites lose water rapidly and die much sooner.

Survival in Pollen. Tropilaelaps spp. may survive for up to approximately 3 days in dry pollen under laboratory conditions of 25°C and 70% RH, with an average survival time of approximately 34 hours. The mites do not feed on pollen. Therefore, their survival in pollen is not due to pollen serving as a food source, but is instead determined primarily by starvation and the surrounding humidity conditions RH (Khongphinitbunjong et al. 2019).

Symptoms of Infestation

The symptoms closely resemble those caused by Varroa infestation:

- Increased brood mortality.
- Spotty brood pattern with sunken cappings and dead larvae or pupae.
- Adult bees with deformed wings and legs due to associated viral infections.
- Rapid decline of the colony population and, without treatment, colony collapse within a short period.
- Bees crawling in front of the hive entrance, unable to fly.

Why Is It So Dangerous?

The Tropilaelaps mite is particularly dangerous because of its extremely rapid reproductive cycle, its protection inside capped brood cells, and its ability to reproduce parthenogenetically.

Without immediate control measures, infestation can quickly lead to colony collapse.

Control Measures

A major biological weakness of Tropilaelaps is that it dies from starvation within 2–3 days when no capped brood is available. This characteristic can be exploited through brood interruption techniques, including:

- Artificial queen caging for 25 days.
- Creating nucleus colonies that remain broodless for at least 7 days.
- Splitting colonies into broodless packages for colony trading.
- Wintering colonies without brood.

During winter, the mite survives only in the small amount of brood maintained by some colonies. In spring, reinfestation of healthy colonies can occur rapidly. A proposed strategy is to move colonies during winter to elevations above 1,500 metres, where all colonies naturally become broodless, interrupting the mite's life cycle.

Chemical Control

Conventional hive acaricides are generally insufficient against Tropilaelaps. Among currently available treatments, formic acid is considered the only one capable of reaching mites

inside capped brood cells with meaningful effectiveness.

For this reason, an integrated pest management (IPM) approach is recommended, combining early detection, brood interruption, appropriate treatment, follow-up monitoring and prevention of reinfestation.

Education, early detection, and preventing the mite from spreading will be far more effective than relying on a “magic” chemical treatment.

Reinfestation

Even when colonies are successfully cleared of Tropilaelaps through brood interruption, they can quickly become reinfested by mites arriving from neighbouring colonies through robbing behaviour or drifting bees.

Reinfestation is therefore one of the greatest challenges in controlling the parasite. Treating a single colony without addressing neighbouring apiaries is not sufficient.

EUROPEAN LEGISLATION FOR THE PREVENTION AND CONTROL OF ANIMAL DISEASES

The European Animal Health Law — Regulation (EU) 2016/429 — is the primary legislation that applies directly across all EU Member States for the prevention and control of transmissible animal diseases.

Its complementary Delegated Regulation (EU) 2018/1882 classifies listed animal diseases into five categories.

Disease Categories Under EU Regulation 2018/1882

Category A – Diseases not normally present in the European Union and requiring immediate eradication measures as soon as they are detected.

Category B – Diseases that must be controlled in all Member States with the objective of eradicating them throughout the Union.

Category C – Diseases relevant to certain Member States, requiring measures to prevent their spread into regions officially free of the disease or operating eradication programmes.

Category D – Diseases requiring measures to prevent their spread through entry into the Union or through movements between Member States.

Category E – Diseases that require surveillance within the European Union.

Classification of Honey Bee Diseases

Under Regulation (EU) 2018/1882, major honey bee diseases are classified as follows:

Disease	EU Categories
Varroa destructor	C + D + E
American foulbrood	D + E
Aethina tumida	D + E
Tropilaelaps spp.	D + E

The legal provisions concerning the compulsory killing of animals (Articles 53–71 of Regulation 2016/429) apply only to Category A diseases. They do not apply to Tropilaelaps, since the mite is classified under Categories D and E.

Legislation Specifically Concerning Tropilaelaps

Delegated Regulation (EU) 2020/688, which supplements Regulation (EU) 2016/429, establishes health requirements for moving bees between Member States.

According to Article 48, colonies and hives intended for movement must show no signs of:

- American foulbrood,
- Aethina tumida,
- Tropilaelaps spp.

In addition, the regulation requires that:

- The apiary of origin must be located at the centre of a 100 km radius within which no Tropilaelaps infestation has been reported.
- No restrictions must be in force due to a suspected or confirmed Tropilaelaps outbreak within that area.

This means that the regulation imposes movement restrictions on bees between Member States based on the health status of the area of origin.

The 100 km radius is a surveillance and movement-control zone, not an automatic destruction or eradication zone.

Harmonising National Legislation with European Regulations

European Regulations, including Regulation (EU) 2016/429 and Delegated Regulation (EU) 2018/1882, apply directly and are legally binding in all EU Member States from the moment they enter into force.

Unlike EU Directives, they do not require transposition into national legislation through a separate law.

First Detection in an EU Member State

Authorities: The first confirmed occurrence of Tropilaelaps spp. in an EU Member State does not automatically entail, under Regulation (EU) 2016/429, the compulsory destruction of colonies in the affected apiary or in neighbouring apiaries, since the parasite is classified under Categories D + E.

For Category E diseases, Article 18(1)(b) of Regulation (EU) 2016/429 requires operators to inform the competent authority as soon as

possible whenever a Category D + E disease is suspected or detected.

Article 19 also provides for notification to the European Commission and the other Member States in cases requiring immediate notification, so that the necessary risk-management measures can be taken promptly.

The clear EU-level measures specifically applicable to Tropilaelaps are primarily **surveillance, notification and movement restrictions**, with the **100 km radius specified in Article 48 of Regulation (EU) 2020/688** being particularly important.

Beekeepers: Immediately stop any movement of colonies or distribution of beekeeping equipment to other beekeepers.

Do not attempt to conceal the finding, as the situation could otherwise become uncontrollable.

The colonies should not be destroyed; they should be saved.

Contact the Veterinary Services or specialist scientists at universities and research institutes immediately.

For laboratory confirmation of the species, samples of the mite (dead specimens), the beekeeper's details, the date, the location of the apiary, and information concerning any recent movements or purchases of bees or frames are required.

Does the Principle of Non-Destruction of Bees Also Apply to Aethina tumida?

Aethina tumida is classified under Categories D + E, which does not in itself imply the compulsory destruction of all colonies. Nevertheless, when the beetle was detected in Calabria, southern Italy, in 2014, a very large number of colonies were compulsorily destroyed by burning.

The decision to destroy the colonies in Italy was based on the national emergency framework and on European Union eradication rules, and did not violate EU law, for two principal reasons:

a) Implementing Regulation (EU) 2018/1882 and the Animal Health Law (Regulation (EU) 2016/429) did not yet exist when the beetle appeared in Italy in 2014.

b) When a disease or parasite appears for the first time in an area, Member States may, under the precautionary principle, implement an aggressive immediate-eradication strategy ("stamping out") before the parasite becomes permanently established. Therefore, even when a parasite belongs to Categories D + E,

as Aethina does, a Member State may invoke the precautionary principle and impose compulsory destruction of bees.

Can the Precautionary Principle Also Be Applied to Tropilaelaps?

Regulation (EU) 2016/429 is governed by the principle of proportionality (Article 5 TEU), according to which measures established or approved by the EU must be effective without causing excessive economic damage where less severe methods are available.

In the case of Tropilaelaps, interruption of egg-laying by restricting the queen, or removal/destruction of the brood alone, creates a brood break. When combined with approved acaricidal treatments, this is considered scientifically sufficient for the eradication of a local outbreak through targeted intervention.

Burning an entire apiary would therefore be a disproportionate measure against a parasite that dies within approximately three days—and at most seven days under particular conditions—in the absence of brood. The objective should be the preservation of the livestock.

Furthermore, Regulation (EU) 2018/1882 differentiates control measures according to the biological characteristics of each parasite.

There is a fundamental biological difference between Tropilaelaps mercedesae and Aethina tumida. The former depends entirely on the presence of capped brood for feeding and reproduction.

The latter can survive for months outside brood, fly considerable distances, feed on honey and pollen, and has larvae and pupae that develop in the soil outside the hive. A simple brood interruption therefore does not eradicate Aethina.

This is why, in Italy, burning of hives and disinfection of the soil were initially selected as control measures.

Consequently, although Tropilaelaps and Aethina belong to the same regulatory category under EU legislation, they should be managed in completely different ways when first detected in an EU Member State.

For Tropilaelaps, colonies are restricted and treated;

For Aethina, colonies may be destroyed.

Greece and the European Regulations: A Point of Divergence

Article 4(1) of Greek Joint Ministerial Decision (JMD) 44441/2026 (Government Gazette B′ 971/20.02.2026) provides for the compulsory destruction of colonies and hives in confirmed or suspected outbreaks involving the ectoparasites Tropilaelaps spp. and Aethina tumida.

According to the argument presented here, this provision of the JMD is in direct conflict with the European Regulations. As a matter of law, a national Joint Ministerial Decision cannot contradict or modify provisions of Regulation (EU) 2016/429 or Implementing Regulation (EU) 2018/1882.

The JMD concerns the framework for implementing national and EU legislation and regulates economic and administrative matters. EU legislation allows Member States to establish national rules concerning how and to what extent livestock owners are compensated. It does not, however, allow them to establish different animal-health requirements, different disease classifications—these are established by Regulation (EU) 2018/1882—or to set aside the surveillance and control obligations imposed by the Animal Health Law.

How Can the Beekeeping Sector Respond?

Because Article 4 of JMD 44441/2026 is presented as being in direct conflict with the European Regulations, beekeeping organisations may consider the following avenues:

- Administrative/Judicial appeal: The JMD may be challenged before the Council of State (StE) through an application for annulment on the grounds of violation of EU law.
- Complaint to the European Commission: Any citizen or organisation may submit a complaint to the European Commission concerning

incorrect application of EU law by an EU Member State.

• Direct application of EU law: Courts and administrative authorities are required to apply the European Regulation and to leave any conflicting provision of the JMD unapplied.

Under European Union law (Article 288 of the Treaty on the Functioning of the European Union — TFEU) and the case law of the Court of Justice of the European Union:

• European Regulations have direct and binding effect in all Member States from the date on which they become applicable.

• EU law takes precedence over any conflicting national provision, including laws, presidential decrees and ministerial decisions.

• Where a national act, such as a Joint Ministerial Decision, conflicts with an EU Regulation, the national provision is considered without legal effect and inapplicable to the extent that it conflicts with the Regulation.

CONCLUSIONS: PREPAREDNESS, NOT PANIC

The possible arrival of Tropilaelaps in Europe should be taken seriously, but it should not become a reason for panic among beekeepers. The experience with Aethina tumida has shown how frightening the first detection of a new pest can be, but Tropilaelaps has very different biological characteristics and therefore requires a different control strategy.

The most important advantage we have is knowledge. Tropilaelaps can be detected relatively easily by an experienced beekeeper because of its characteristic rapid movement, and its dependence on brood creates an important biological weakness that can be exploited through brood interruption and appropriate treatments.

If the mite is detected for the first time in an EU Member State, the priority should be rapid confirmation, notification, containment and coordinated action. Beekeepers should immediately stop movements of colonies and beekeeping material and inform the competent veterinary authorities or specialised scientific institutions. Concealing a finding would only allow the parasite to spread further and make control more difficult.

Most importantly, the first detection of Tropilaelaps should not automatically be equated with the destruction of entire apiaries. The regulatory classification of Tropilaelaps and its biological dependence on brood provide the basis for a targeted approach aimed at eliminating the parasite while preserving healthy colonies.

The greatest danger is not the first mite that is detected—it is the undetected mite that is allowed to spread.

For this reason, Europe needs an effective preparedness strategy based on surveillance, rapid diagnosis, beekeeper education, movement control, coordinated treatment and prevention of reinfestation. Beekeepers, veterinary authorities, researchers and beekeeping organisations must work together before the first confirmed European outbreak occurs.

We do not need to wait for Tropilaelaps to arrive before preparing for it. Early detection and informed action can make the difference between a manageable outbreak and an established European problem.

The message for beekeepers is therefore simple:

Be alert, report suspected findings, stop unnecessary movements, act quickly—and protect the colonies rather than destroying them unnecessarily.

Andreas Thrasyvoulou
Emeritus Professor
Aristotle University of Thessaloniki
GREECE

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THE "BUY LOCAL" CAMPAIGN IS EXPANDING TO ALL FOOD AND ACROSS EUROPE

At the August 25, I met with Elisabeth Werner, an Austrian economist and senior official of the European Commission, who has been Director-General of DG AGRI – the Directorate-General for Agriculture and Rural Development – since June 2025.

I presented her with the current challenges facing European beekeeping, particularly the insufficiently effective work of the Honey Platform. I am pleased that she showed understanding and promised to help address these issues.

Even more encouraging is the announcement that the European Union will launch a unified campaign promoting European food, with honey playing an important role.

We can be happy and proud that the idea behind our “Buy Local Honey” campaign is now expanding to European food as a whole – under the umbrella of the European Union. This is an important recognition of our work and proof that a good local idea can cross national borders and become a European story.

Boštjan Noč
President of the EBA

NEW HONEY LABELLING RULES – AND A CHANGE OF STRATEGY IN FRANCE

The first jars of honey displaying all countries of origin are now appearing on supermarket shelves. Following the amendment to the honey labelling rules under the so-called "Breakfast Directive", blended honeys made from honeys originating in different countries must now indicate all countries of origin, together with their respective percentage shares, on the label – and this information must appear "in the principal field of vision". In France, this new requirement appears to have prompted the country's leading honey packer to change its commercial strategy.

In Germany and other EU Member States, supermarket shelves already feature honey jars providing a transparent list of all countries of origin and their respective shares. Consumers can now see what was previously concealed behind indications such as "blend of honeys originating from EU and non-EU countries" – for example, 92% honey from Ukraine and 8% honey from Bulgaria.

Greater transparency for honey blends

The new provisions of the honey labelling rules have been applicable since 14 June. The Deutscher Imkerbund, Germany's leading beekeeping association with more than 134,000 members, together with other beekeeping organisations across Europe, had been calling for transparent origin labelling of honey for many years. On the labels we have seen so far, we have found up to four countries of origin.

A change of strategy by France's leading honey packer

France's leading honey packer, Famille Michaud Apiculteurs, appears to have changed its strategy. On new jars of honey now available in supermarkets, we found only a single country of origin indicated on its flagship "Lune de Miel" product. This development is quite remarkable, as it contradicts several previous statements made by representatives of the European honey-packing industry.

Previous industry arguments called into question

In a television report recently broadcast by a major mainstream channel, Managing Director Marie Michaud explained that the decision to stop blending honeys was a response to consumers' desire to find only honey from a single country of origin in the jars. Unless we are mistaken, consumers have never expressed such a demand (although some beekeepers certainly have!). What they have primarily called for is transparent origin labelling.

However, for honey originating from a single country, the indication of origin does not have to appear in the principal field of vision on the label and can therefore remain on the back of the jar. Thus, while French honey is clearly highlighted on the front of the jars with the French tricolour flag, Ukrainian or, for example, Bulgarian origin is only visible on the back.

This is, incidentally, also a strategy used by some German honey packers, who prominently display origins perceived by consumers as a sign of quality while other origins are effectively kept out of sight. Who has ever seen a jar of Chinese honey with the country of origin promi-

nently displayed on the front, complete with a flag?

Why does the approach taken by one of Europe' largest honey packers deserve closer attention?

1. For years, honey packers and their representatives have argued that the origin of honey was of no importance to consumers. It is therefore legitimate to ask why Famille Michaud Apiculteurs is now highlighting French origin on the front of its jars – both in France and abroad – while other origins have to be searched for on the label.

2. Honey packers have also argued that blending honeys from different origins was essential to compensate for market fluctuations and ensure a consistent taste. If one of Europe's largest honey packers is now abandoning such blends, this argument also loses credibility in practice.

Greater clarity for honey analysis?

The strategy adopted by Famille Michaud Apiculteurs raises questions for the industry that will presumably become increasingly difficult to answer.

At the same time, abandoning blends of honeys from several countries could significantly facilitate analyses aimed at detecting fraud. Several representatives of the beekeeping sector had in fact called outright for such blends to be banned, arguing that they complicated certain analytical procedures.

What happens next?

By changing its commercial strategy, Famille Michaud Apiculteurs has demonstrated to the whole of Europe that it is entirely possible to offer honeys with consistent characteristics without systematically relying on blends from multiple origins.

This approach shows that blending honey from five, six, even or more countries is not a structural necessity for meeting the supposed expectations of consumers. It opens the door to a broader discussion within the sector about the value of clearly identified origins, supply-chain transparency and the evolution of packing practices in ways that respect the integrity of the product and the work of the bees.

It is still too early to say whether honeys blended from five, six or seven different origins

will gradually disappear from supermarket shelves. What is clear, however, is that the period now beginning will be decisive in determining how – and whether – commercial flows will be reorganised.

Which strategies will European honey packers choose to pursue? This development could reshape part of the market by strengthening the value of clearly identified origins and putting business models based on international blends involving “suspicious” origins to the test.

In the meantime, a thought for all the beekeepers who are fighting to make their profession and their businesses sustainable.

Sebastian Spiewok
Deutscher Imkerbund e.V.

REQUEST TO COMMISIONER HANSEN

Subject: Request for clarification regarding subsidies per beehive

Dear Commissioner Hansen,

I am writing to you regarding recent information and media reports concerning support measures for the beekeeping sector.

We have recently noted reports regarding the non-approval by the European Commission of a Romanian national aid scheme aimed at providing €15 per bee colony.

To avoid any misunderstanding among beekeepers and ensure complete transparency, we would be grateful if you could kindly clarify the following points:

1. Is the recently rejected Romanian scheme separate from the per-hive/colony support measure mentioned in your official letter as taking effect from January 2026? (Ref. Ares (2026)2074780-24/02/2026)

2. Could you please clarify in detail how Member States can access and implement this support framework in practice, and what specific requirements must be met for approval?

We remain deeply committed to constructive dialogue and to ensuring that support measures effectively reach beekeepers across the European Union. Thank you very much in advance for your time, consideration, and guidance on this important matter.

Boštjan Noč
President of the European Beekeeping Association - EBA

Source:
https://agrointel.ro/356295/subventia-de-15-euro-familia-de-albine-blocata-la-bruxelles-aca-este-o-lovitura-foarte-mare-pentru-apicultura

European Beekeeping Association

Holy Mass *and* Meeting with all beekeepers *in a special audience* with the Pope

11 - 12 December 2026

Vatican

HOLY MASS FOR BEEKEEPERS

One year of hard work has paid off!

On 11-12 December, the Pope will receive beekeepers at a private audience. He will meet beekeepers from all over the world, at the initiative of the Slovenian Beekeepers' Association and the European Beekeeping Association. On this occasion, he will also receive a special candle made in Slovenia, in Polzela.

On Saturday, 12 December, a special Holy Mass for all beekeepers will be held at the Borgo Laudato Si'. The Mass will be held by Cardinal Baggio.

According to our information and the publicly available records of the Holy See, there has never before been an official private audience with a Pope dedicated exclusively to beekeepers. This makes the event particularly significant and truly historic.

We will present the importance of bees and bee products to the Pope and ask him to do his best to help us raise awareness in the world about the importance of bees and also about the importance of consuming real bee products. We also want to send a message to the world through him – let's buy local honey!

We encourage all beekeeping organizations around the world to organize visits to the Vatican and be part of a unique event…

We look forward to this very special event and to meeting you all in Rome!

Invitation & Logistics Guidance: Historic Vatican Events for Beekeepers (11 - 12 December 2026)

Dear Beekeeping Organisations,

Following our recent announcement regarding the historic audience with the Holy Father and the Holy Mass for Beekeepers, we are writing to provide preliminary operational guidance to assist you in planning participation for your members.

Please note that the date of the Holy Mass has changed from the date previously communicated through our social media channels. The confirmed date for the meeting with all the beekeepers in a special audience is Friday, 11 December 2026, in Vatican City and the Mass for the beekeepers will take place on Saturday, 12 December. We kindly ask you to take note of this change and update any information already shared with your members accordingly. For reference, the official communication from the Holy See regarding the date change can be found HERE.

Thank you for helping us spread the word!

KEY PROGRAM OVERVIEW

Friday, 11 December 2026 at 12:00 PM:
Official Meeting with all beekeepers in a special private audience with the Pope in the Vatican.

Saturday, 12 December 2026:
Special Holy Mass for beekeepers at Borgo Laudato Si' estate, celebrated by Cardinal Baggio.

DETAILED SCHEDULE & ASSEMBLY POINTS

Friday, 11 December 2026 – Meeting with all beekeepers in a special private audience with the Pope

Assembly time: 11 AM (at least 1 hour prior to the event)
Start time: 12:00 PM
Meeting location / Access point:: Vatican (the exact location will be communicated later)

Saturday, 12 December 2026 – Holly Mass at Borgo Laudato Si'

Assembly time: at least 1 hour prior to the event
Mass start time: Morning (the exact time will be communicated later)
Meeting location / Access point: Vatican (the exact location will be communicated later)

ORGANISATIONAL & TRAVEL LOGISTICS

To ensure smooth execution, each participating national/regional organisation is requested to independently organise travel and accommodation for its delegation.

As an example, the Slovenian Beekeepers' Association (ČZS) is organizing a 3-day trip to the Vatican via a selected travel agency for approximately 200 Slovenian beekeepers. We strongly recommend that other organisations partner with local travel agencies to arrange group transport, accommodation, and city transfers.

IMPORTANT GUIDELINES FOR ATTENDEES

Punctuality & Assembly:

All delegations must arrive at the designated meeting points at least 1 hour prior to the start of both events to allow time for security checks, registration, and group assembly.

Identification & Badges:

Further details regarding registration, entry passes, and dress code (or traditional beekeeping attire) will be communicated as soon as final protocols are confirmed by the Holy See.

We kindly ask you to share this preliminary information with your members so they can begin reserving travel arrangements.

We look forward to uniting beekeepers from around the world in Rome for this truly historic occasion!

Boštjan Noč

President of the European Beekeeping Association
and the Slovenian Beekeepers' Association

EMPOWERING EUROPEAN BEEKEEPING: INSIGHTS FROM B-THENET AND BEEXPERT MEETINGS IN LUKOVICA

From August 26 to 29, 2026, the headquarters of the Slovenian Beekeepers' Association and the European Beekeeping Association in Brdo pri Lukovica served as the center of European apiculture. The multi-day gathering brought together international researchers, advisors, policy leaders, and practical beekeepers to address pressing challenges in hive health, pest management, and sector policy.

BEEXPERT 1st Annual Meeting (August 26–27); Erasmus+ project

The event opened with the BEEXPERT 1st Annual Meeting, focusing on foundational bee biology, nutrition, and innovative management practices:

• Day 1 (August 26): Following morning internal meetings and welcome greetings from authorities, the afternoon centered on a major round table titled "Honey Bees, Nutrition and Beekeeping". Moderated by Prof. Dr. Asli Özdemir Özkırım (Hacettepe University), the panel featured contributions from Dr. Aygun Schiesser, Dr. Alessandra Giacomelli, Dr. Dan-

iele Alberoni, Prof. Dr. Ivana Tlak Gajger, Prof. Priyadarshini Chakrabarti Basu, Prof. Dr. Michel Bocquet, Prof. Robert Chlebo, Prof. Soraia I. Falcao, and Dr. Maja Smodis Skerl.

• Day 2 (August 27): Dr. Karen Power opened the morning with an overview of the BEEXPERT project, followed by Prof. Dr. Asli Özdemir Özkırım's presentation on ApiTUR-route, which connects apitourism, bee products, and the One Health framework. Prof. Janja Filipi delivered a technical talk on worker bee lifespans to bridge biological research with daily colony management, concluding with a

round table on innovating the beekeeping sector.

B-THENET European Beekeeping Event (August 27–28); Horizon project

The B-THENET European Beekeeping Event shifted the program toward active sanitary threats, field demonstrations, and European policy dialogue:

• Day 1 (August 27): The afternoon opened with a hybrid plenary lecture by Dr. Fani Hatjina on the emerging threat of the Tropilaelaps mite. This was followed by a four-hour practical workshop held at the Slovenian Beekeepers' Association apiary, where B-THENET advisors and BEEXPERT experts demonstrated best practices for Varroa monitoring to 50 registered in-person participants.

• Day 2 (August 28): The morning featured a hybrid plenary on invasive vespids and stressors led by Prof. Dirk de Graaf and Dr. Karen Power, alongside Apimondia's presentation and distribution of the Best Beekeeping Practices Manual: 101 Practices Adapted to 13 EU Beekeeping Contexts. The afternoon split into two parallel tracks: a policy dialogue hosted by BeeLife covering fake honey, generational renewal, gender balance, and invasive hornet roadmaps,

and an on-field excursion to Koper demonstrating management strategies for Vespa orientalis.

Saturday Excursion & Technical Tour (August 29)

The final day was dedicated to technical tours and field excursions across Slovenian apiaries, innovators, and cooperatives. Participants visited Strips (Beeconn) to learn about modern digital hive-monitoring technologies, followed by a visit to the Beekeeping Society Zagorje, where they explored their educational trail and a traditional Slovenian apiary. The journey continued with a cultural and culinary stop at the Zlati Grič wine cellar for a guided tour, before concluding with a showcase of local api-

tourism at Apitourism Žvikart. By integrating academic research, practical apiary demonstrations, and direct policy feedback, the combined meetings in Lukovica provided a clear roadmap for strengthening European apiculture.

EBA


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No bees,
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1ST AN
MEET
26–27
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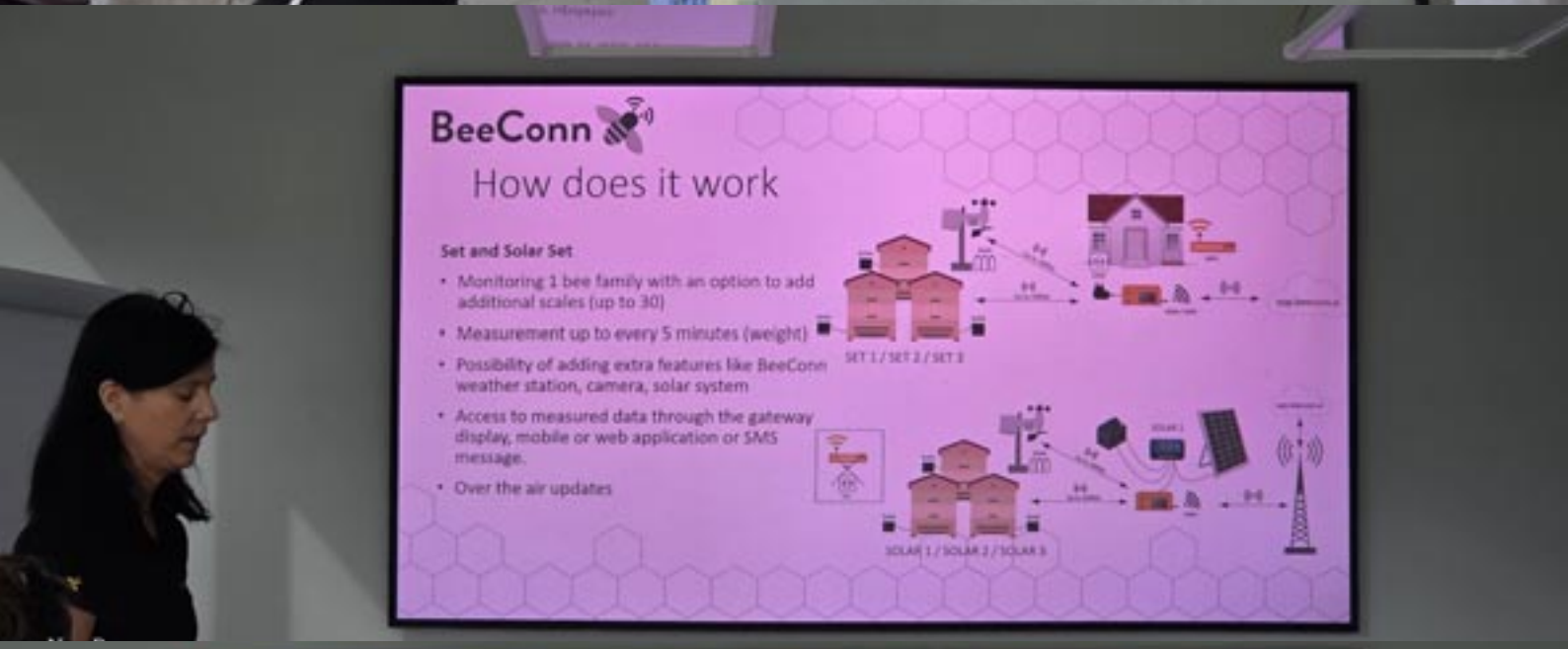
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Pesticide Residues in Honey in Kosovo* a Case Study

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University of Hohenhaim, Stuttgart, Germany, [2]

Higher Colleges of Technology, Sharjah, U.A.E.[3]

ABSTRACT

The objective of this study was to assess the the presence of some organochlorine and organophosphorus compounds pesticide residues (34) of locally produced honey collected from individual apiaries during August, 2017 from Peja region in Kosovo. In the present study, a total of 17 honey samples collected have been quantitatively screened by Gas Chromatograph-Electron Capture Detector (GC-ECD). The recovery rate ranged from 78.2 to 98.0%. The method provided limits of detection (LOD) in the range of 0.001-0.168 µg/kg. The limit of quantification (LOQ) was 0,003 µg/kg only for flumethrin 0,005 µg/kg). Residues of pesticides were found in a considerable number of honey samples 8 out of 17 or 47.1%. GC-ECD analysis demonstrated that the minimum and maximum level of coumaphos residue, as only present pesticide in samples was 3.4–39.1 µg/kg. Six samples (35.3%) exceeded the European Union maximum residue levels (EU MRLs). Positive samples with exceeded MRL Residue of Coumaphos 6/17 owing to its applications to control Varroa destructor, indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey. The obtained results of this study imply that more emphasis should be given to a continuous monitoring programme for pesticide residues in honey, accompanied by an education programme to beekeepers on proper hive management, at the national level to protect consumer health.

INTRODUCTION

Currently, Kosovo has 6,453 beekeepers with 70,664 bee-hives distributed in the territory of Kosovo with an average production for bee society 9.55 kg honey or 674 T / year whereas the import from 2014 is around 140 t / year. (MAFRD, 2015). Honey is a natural product of bees and is recognized as a food with nutritional properties and is known like a food with valuable therapeutic applications. However, bee products can also be a source of toxic substances, such as antibiotics, pesticides and heavy metals due to environmental pollution and misuse of beekeeping practices. Honey bees collect pollen and nectar from the surrounding flowers and then they may return to hives collecting significant amounts of toxic contaminants, in addition the honeybees are also exposed to pesticides and antibiotics administered by beekeepers as part of the hive to control some infestations such as Varroa destructor, Acarapis woodi and Paenibacillus larvae (Fell & Cobb, 2009; Genersch, Evans, & Fries, 2010); Residues of organochlorine insecticides in honey can possibly be dangerous for people, because of the insecticides mutagenic, teratogenic and embriotoxic activities. Coumaphos, is an organophosphate insecticide widely used to control varroosis in bee colonies and for the treatment of ectoparasitoses in livestock. Coumaphos targets cholinergic signaling through acetylcholine esterase inhibition and comprises the majority of excitatory neurotransmission in the Varroa's nervous system (Millar and Denholm 2007). In Kosovo honey has never been included in regular pesticide residues controls. The aim of our study was to estimate the amounts of residues from organochlorine insecticides in Kosovar honey.

Fig. 1 Interactions between pesticides, parasites and pathogen stressors in relation to honey bee colony collapses

MATERIALS AND METHODS

The objective of this study was to assess the the presence of some organochlorine and organophosphorus compounds pesticide residues (34) of locally produced honey collected from individual apiaries during August, 2017 from Peja region in Kosovo. In the present study, a total of 17 honey samples collected have been quantitatively screened by Gas Chromatograph-Electron Capture Detector (GC-ECD) at Apicultural State Institute, University of Hohenhaim, Germany. All honey samples were labeled either according to their botanical and geographical origin as suggested by the beekeepers. The samples were stored at room temperature in the dark until analysis. One honey sample was checked to be free of any of the targeted pesticides and it was used as blank honey for calibration curve.

RESULTS

Residues of pesticides especially coumaphos was found in a considerable number of honey samples 8 out of 17 or 47.1%. GC-ECD analysis demonstrated that the minimum and maximum level of coumaphos residue, as only present pesticide in samples was 3.4–39.1 µg/kg. Six samples or 35.3% of the detected pesticide exceeded the European Union maximum residue levels (EU MRLs). Positive samples with exceeded MRL Residue of Coumaphos 6/17 owing to its applications to control Varroa destructor, indicating that the chemicals used by apiculturists inside the hives in order to control disease are the main pollutants of the produced honey.

CONCLUSIONS

This research studied the presence of pesticides in honey for consumption in Kosovo. Overall, it was confirmed, the primary determined hypothesis, that there is presence of pesticides in honey. It is noteworthy that in these study the pesticide detected/quantified can present a risk to human health but also they pose a risk to bees as increased mortality rate in developing larvae, reduced longevity in adult bees which were exposed to coumaphos as immatures, and higher number of queen cells under construction in treated hives. Inevitably, the utilization of acaricides in apiculture has resulted in residues being frequently found in bee products. Finally in Kosovo there is no published study about the presence of pesticides in honey. The lack of information about pesticide residues in honey in Kosovo implies the necessity to determinate the pollution of those bees' products in the country. In that way, the aims of this study were to identify currently used pesticides and common pesticide management practices and to validate a multiresidue analysis methods. As this is the first ever study on possible presence of pesticides in honey in Kosovo, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of pesticide contamination in Kosovo especially in bee products.

Table 1. Honey samples analyzed to detect possible presence of pesticides in 2017

Honey	No. of samples	No. of positive samples	No. of negative samples	Positive samples Mean±SD
Local	17	8 (47.1%)	9 (52.9%)	11.81(±11.53)

Table 2. Coumaphos levels (µg/kg) in positive samples

Sample No.	Sample Code	Level (µg/kg)
1	518/17	6.8
2	520/17	9.9
3	522/17	7.4
4	523/17	39.1
5	524/17	12
6	527/17	3.4
7	528/17	3.6
8	530/17	12.3

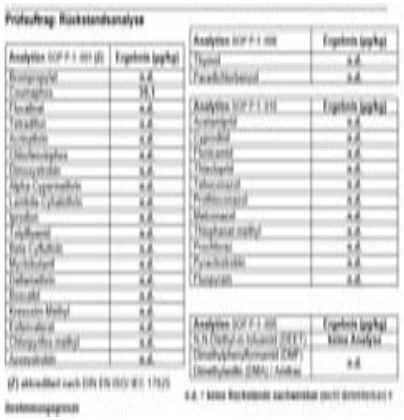

Table 3. The list of pesticides examined

Presented in DIFSC , October 29-31 2018, Dubai, UAE

*This designation is without prejudice to positions on status, and is in line with UNSC 1244 and the ICJ Opinion on the Kosovo declaration of independence

FIRST DETECTION OF LESS KNOWN HONEY BEE VIRUSES IN APIARIES OF REPUBLIC OF KOSOVO*

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Introduction

Apis mellifera plays an important role in pollination and food production and one of the major threat to its health is infection by a wide variety of pathogens, which are responsible for significant weakening and losses of bee colonies [1]. Among them, viruses often persist as unapparent simultaneous infections altering bee physiology, behaviour, immune response and lifespan. Of the numerous viral species identified to infect honey bees, seven of them (Acute Bee Paralysis Virus – ABPV; Chronic Bee Paralysis Virus – CBPV; Deformed Wing Virus – DWV; Black Queen Cell Virus – BQCV; Sacbrood Virus – SBV; Kashmir Bee Virus – KBV; Israeli Acute Paralysis Virus - IAPV) cause the most common diseases [2], while for other emerging viral pathogens, such as *Apis mellifera* filamentous virus (AmFV), Aphid Lethal Paralysis Virus (ALPV), Apis Rhabdovirus 1 and 2 (ARV-1, ARV-2), Bee Macula-like Virus (BMLV) and Lake Sinai Virus (LSV), recently discovered and signalled worldwide but poorly characterized, our knowledge is still limited [3]. However, in several countries, including the Republic of Kosovo, despite a consolidated beekeeping tradition, there is a lack of information regarding the health status of honey bees and the pathogens circulating in their colonies, especially viruses.

Aim of the study

Analyzing honey bee specimens collected during a passive surveillance program on weak honey bee colonies, and in collaboration with the Veterinary Laboratory of the Republic of Kosovo, we aimed to determine the occurrence and distribution of known and emerging honey bee viruses, updating the current epidemiological data about the spread of these viral species in some municipalities of the Republic of Kosovo.

Materials & Methods

Adult honey bees specimens were collected from 8 different apiaries of 8 municipalities of the Republic of Kosovo (Figure 1).

Figure 1. Map of the municipalities of the Republic of Kosovo. The municipalities from which the apiaries were sampled are highlighted in orange and are numbered as follows: Gjilan 1; Prizren 2; Suharekë 3; Junik 4; Kamenicë 5; Drenas 6; Lipjan 7; Skenderaj 8.

After pooling and homogenizing 5 bee specimens per apiary, total RNA and DNA extraction was performed using the NucleoSpin RNA kit (Macherey Nagel), and QIAamp® DNA Mini Kit (Qiagen), respectively. The presence of viral RNA and DNA was assessed using the methods, primers and protocols reported in Table 1.

Negative and positive controls were included in each PCR. Amplification products for IAPV, KBV and the emerging viruses (AmFV, ALPV, ARV-1, ARV-2, BMLV, and LSV) were first analyzed by capillary electrophoresis on LabChip GX Touch HT® (Perkin Elmer) and when the amplification products were present, they were sequenced using Sanger method. The obtained sequences were then aligned and compared with those available in the GenBank database, using BLAST software.

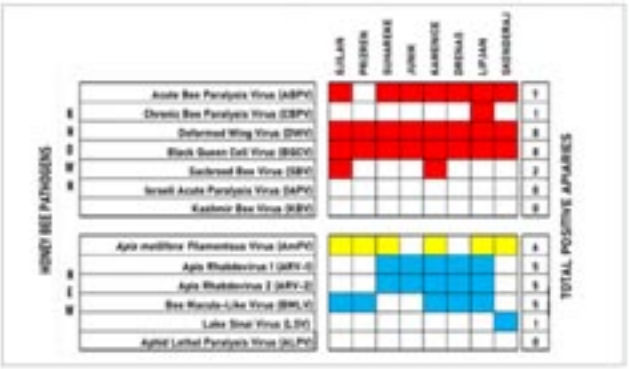
Table 1. Methods, primers and protocols' references for each virus investigated.

METHODS	VIRUS	PRIMER & PROTOCOL REFERENCE
Real time RT-PCR	ABPV CBPV DWV BQCV SBV	Bordin *et al.* [4]
One step RT-PCR	IAPV KBV	Bordin *et al.* [4]
PCR	AmFV	Hartmann *et al.* [7]
Two step RT-PCR	ALPV ARV-1 ARV-2 BMLV LSV	Wamonje *et al.* [8] Lavin *et al.* [9] Eliash *et al.* [10] de Miranda *et al.* [4] Hou *et al.* [11]

Results

The results obtained are summarized in Table 2. Among the known viruses, DWV and BQCV were the most prevalent, detected in all the apiaries analysed, followed by ABPV (7/8 apiaries), SBV (2/8 apiaries), and CBPV (1/8 apiaries). IAPV and KBV were not detected in any apiary. Of the less known viruses investigated, AmFV was found in 6 out of 8 apiaries, ARV-1 and ARV-2 (always in co-infection) and BMLV were detected in 5 out 8 apiaries, while LSV only in 1 apiary (Skenderaj municipality). No samples tested positive to ALPV. Several viral co-infections were found in all the apiaries, and the highest number occurred in the apiaries from Kamenicë e Lipjan municipalities (Eastern and Central Kosovo).

Table 2. Municipalities of the Republic of Kosovo tested positive for the known and emerging honey bee viruses investigated. Sample analysed by PCR are shown in yellow, by RT-PCR in blue and by real time RT-PCR in red.



For the emerging viruses, the comparison of the sequences, obtained after Sanger sequencing of the amplification products, with those available in the GenBank database highlighted the following percentages of identity: AmFV: 99,80%; ARV-1: 99.59%; ARV-2: 99,18%; BMLV: 98.83%; LSV: 88.31%.

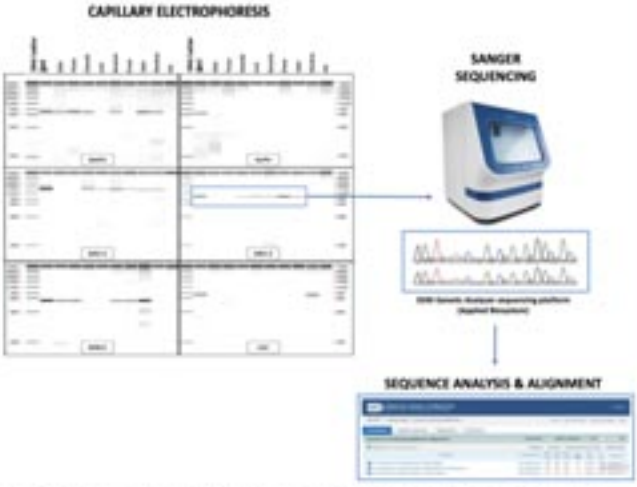


Figure 2. Capillary electrophoresis on LabChip GX Touch HT® of the PCR products for the six emerging honey bee viruses (AmFV, ALPV, ARV-1, ARV-2, BMLV and LSV). DNA ladder: from 100 to 7000 bp; PC: PCR positive control; NTC: PCR negative control. On the lower right, the results of alignment of ARV-2 sequences with those available in the GenBank database using BLAST.

Discussion & Conclusions

Although in recent years there have been clinical cases of suspected emergent honey bee diseases in some bee colonies of the Republic of Kosovo, they have never been investigated. This study revealed for the first time the presence of ten viral species (ABPV, CBPV, DWV, BQCV, SBV, AmFV, ARV-1, ARV-2, BMLV, LSV) in 8 apiaries from different municipalities. Most of the well known viruses detected were often present in co-infection also with other less known viruses. DNA virus AmFV occurred in several apiaries, in line with its endemic distribution in bee [7]. BMLV, recently reported in the USA and in some European countries, was highly detected [4]. Rhabdoviruses ARV-1 and ARV-2, which showed a near-global distribution [5,8] occurred simultaneously in our samples, suggesting either some sort of interdependence or a common transmission route [12]. Finally, LSV despite its global distribution [11], was detected only in one of the eight apiaries of the Republic of Kosovo.

In conclusion, this study proved for the first time the presence and distribution of several honey bee viruses, both known and less known, possibly responsible for honey bee diseases, in apiaries of the Republic of Kosovo. The results we obtained could be useful to update the epidemiological data on the health status of honey bee colonies in the Republic of Kosovo and to better design the future surveillance programs.

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insects

MDPI

Article

Megaselia scalaris and *Senotainia tricuspis* Infesting *Apis mellifera*: Detection by Quantitative PCR, Genotyping, and Involvement in the Transmission of Microbial Pathogens

Franca Rossi [1,*], Martina Iannitto [1], Beqe Hulaj [2], Paola Manocchio [1], Francesca Gentile [1], Ilaria Del Matto [1], Massimiliano Paoletti [1], Lucio Marino [1] and Luciano Ricchiuti [1]

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Simple Summary: Among the pests infesting the honeybee *Apis mellifera*, the parasitoid flies *Megaselia scalaris* and *Senotainia tricuspis* are not yet well characterized for the occurrence and harmfulness to bee colonies. These flies can attack the forager bee and lay eggs or larvae on its body, which will develop by feeding on the host tissues until it is killed. Their prevalence in different countries is still not well defined, so, in this study, rapid molecular methods were developed to facilitate their detection. The new methods were applied in a preliminary analysis of adult bees and hive debris from parts of Central Italy and the Republic of Kosovo*. Both flies were detected in the two countries and more frequently in Italy. The *M. scalaris* flies isolated from Italian apiaries were genotypically related to biotypes from distant countries. Single flies were shown to possibly transmit honeybee microbial pathogens, so their presence should be efficiently contrasted in apiaries.

check for updates

Citation: Rossi, F.; Iannitto, M.; Hulaj, B.; Manocchio, P.; Gentile, F.; Matto, I.D.; Paoletti, M.; Marino, L.; Ricchiuti, L. *Megaselia scalaris* and *Senotainia tricuspis* Infesting *Apis mellifera*: Detection by Quantitative PCR, Genotyping, and Involvement in the Transmission of Microbial Pathogens. *Insects* **2024**, *15*, 786. https://doi.org/10.3390/insects15100786

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Abstract: The *Megaselia scalaris* and *Senotainia tricuspis* parasitoid flies of the honeybee *Apis mellifera* were found to infest apiaries of different European and Mediterranean countries but their prevalence and impact on apiary health are little known. Therefore, in this study, quantitative PCR (qPCR)-based methods were developed for their rapid detection directly in hive matrices. The newly developed qPCR assays were targeted at the mitochondrial cytochrome oxidase subunit I (*COI*) gene for the *M. scalaris* and the cytochrome B (*cyt*B) gene for the *S. tricuspis*. The tests were preliminarily applied to 64 samples of adult honeybees and hive debris collected in the Abruzzo and Molise regions, Central Italy, and the Republic of Kosovo showing that both flies occur in the two countries and more frequently in Italy. The positive apiaries in Italy were re-sampled by capturing viable forager bees and isolating emerging flies to carry out the genotyping and analyses aimed at defining if these flies can transmit honeybee pathogens. Genotyping based on the *COI* and *cyt*B gene sequencing for *M. scalaris* and *S. tricuspis*, respectively, identified one *S. tricuspis* genotype and diverse genotypes of *M. scalaris* highly similar to those from distant countries. Some fly isolates harbored the DNA or RNA of honeybee microbial pathogens *Paenibacillus larvae*, deformed wing viruses A and B (DWVA and B), black queen cell virus (BQCV), chronic paralysis virus (CBPV), and *Nosema ceranae*. The results indicated that these parasites should be efficiently controlled in apiaries by using rapid detection methods to facilitate the large screening studies and early detection.

Keywords: parasitoid flies; *Apis mellifera*; *Megaselia scalaris*; *Senotainia tricuspis*; qPCR detection; occurrence in apiaries; genotyping; pathogen carriage

*This designation is without prejudice to positions on status, and is in line with UNSC 1244 and the ICJ Opinion on the Kosovo declaration of independence

1. Introduction

Parasitoid flies represent one of the threats to honeybee (*Apis mellifera*) pollinators worldwide. These flies are known to be present in many countries based on reports of sporadic detection, whereas their prevalence and extent to which they contribute to honeybee colony weakening, are still little known. Diptera able to attack honeybees are the scuttle flies, family Phoridae, *Megaselia scalaris* [1–6], and *Senotainia tricuspis*, a viviparous species of the flesh fly family Sarcophagidae, found in Europe, Mediterranean countries and the Middle East [7]. Other Phoridae able to parasitize honeybees were described in particular geographic regions, namely *Apocephalus borealis*, possibly involved in the colony collapse disorder (CDD) in North America [8], and *Melaloncha* spp. in Brazil [9]. Moreover, other *Megaselia* species such as *M. rupifes*, could exert parasitism towards honeybees [10–12].

M. scalaris is a generalist species able to cause myiases in a variety of living hosts, including humans [13], animals [14], and different arthropod species [15–18]. Indeed, it was proposed as a biocontrol agent against other pests [14,19]. It also infests inanimate organic matter and food commodities [20]. It was found to infest honeybees in the Abruzzo and Molise regions of Central Italy, with high infestation rates expressed as the ratio of fly larvae number to the number of infested bees [2]. The reports on honeybee infestation by this or other *Megaselia* species regard different European and extra-European countries [3–6,10,12]. The modeling of *M. scalaris* expansion favored by the ongoing climate changes indicated that this species currently finds optimal living conditions in regions of North and Sub-Saharan Africa, Southern Europe, and France with the potential to invade internal zones of the Balkans and Eastern Europe [21].

S. tricuspis was reported to cause heavy infestations with a high colony mortality rate in apiaries. This fly can lead to the collapse of a honeybee colony when it reaches an infestation rate above 70% [22]. *S. tricuspis* flies live in proximity of bee hives where they lie in wait for honeybees leaving for or returning from foraging. Then they attack the bees repeatedly according to a behavior that has been recently described in detail [22,23]. Since each female harbors several hundreds of larvae, it can infest a high number of bees [7]. In Tuscany, Central Italy, adults of *S. tricuspis* emerge during late spring and begin to infest honeybees at the end of May [22]. Larvae formed on bees finally leave the hosts and pupate in the soil. Adults can emerge from the soil either after overwintering, in early spring, or in June-July, since pupae formed in spring develop into adults within 15-20 days giving rise to a second generation in the same year [22].

The extent of the detrimental effects of the parasitoid flies on honeybee health is not yet fully known and requires further investigation. This can also depend on the laborious and time-consuming methods of detection currently used that are based on the observation of fly larvae development on the bodies of honeybees captured and kept alive in controlled conditions of temperature and humidity [2,7]. The time required to complete the analysis is of more than three weeks [2].

Therefore, this study was aimed at developing molecular detection methods based on a quantitative polymerase chain reaction (qPCR) to speed up the detection of parasitoid flies on apparently healthy honeybees. This could allow the detection of the infestation in an early phase and adopt countermeasures such as placing traps to reduce the fly infestation levels [22]. The detection methods developed in this study were characterized for specificity, inclusivity, and limit of detection (LOD) and were preliminarily applied to samples of both adult bees and hive debris collected in the Abruzzo and Molise regions of Central Italy and the Republic of Kosovo.

In addition, thirteen isolates of *M. scalaris* obtained from apiaries in Abruzzo and Molise were genotyped by sequencing a region of the mitochondrial cytochrome oxidase I gene *COI* and compared to biotypes from other world regions to obtain indications on genetic relatedness. This was not possible for *S. tricuspis* because of the unavailability of sequences for comparison.

Finally, fly isolates were analyzed for the presence of honeybee microbial pathogens to investigate their role in the transmission of infectious diseases.

2. Materials and Methods

2.1. Collection of Bees and Hive Debris

The samples of honeybees and hive debris were collected by beekeepers from 16 apiaries in the Abruzzo and Molise regions of Central Italy and 16 apiaries in the Republic of Kosovo during June–July 2021. The sample sites were uniformly distributed in the two geographical districts. Only one hive per apiary was sampled. At least 30 foraging bees to be examined by molecular tests were captured at the hive entrance and introduced in sterile 50 mL centrifuge tubes (Biosigma, Cona, VE, Italy). The same tubes were used to collect hive debris picked with sterile disposable spoons (Biosigma) from the hive bottom drawer. The samples were transported to the laboratory in refrigerated conditions within 48 h and stored at −80 °C before analysis. The samples collected in the Republic of Kosovo were soaked in RNAlater solution (Thermo Fisher Scientific, Rodano, Italy) before being transported by hand to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), branch of Campobasso, Italy, and analyzed.

Viable honeybees were collected in apiaries in Abruzzo and Molise in July 2022 (the sampling of viable bees could not be carried out in the year 2021 because of the COVID-19 pandemic activity limitations) and analyzed for the development of parasitoid flies, as described by Ricchiuti et al. [2].

2.2. Design of qPCR Tests Specific for M. scalaris and S. tricuspis

The experimental plan of the present study, as reported in Figure S1, included as the first phase the development of qPCR assays specific for *M. scalaris* and *S. tricuspis*. The PCR primers and probes used in this study were selected after a search of the nucleotide sequences available for *M. scalaris* and *S. tricuspis* in the public domain database accessed through the National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/, accessed on 30 August 2024). The representatives of the gene sequences available were aligned by Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome, accessed on 31 August 2024) to the nucleotide database limiting the search to *M. scalaris* or *S. tricuspis* taxa, respectively, so conserved regions could be identified for the species. Then all the oligonucleotides that could be designed in the conserved regions were aligned by Blastn to verify inclusivity for the highest number of biotypes and exclusivity towards other insect species. The Blastn alignments were carried out for up to 1,000 database entries. The oligonucleotides and probes were provided by Eurofins Genomics (Ebersberg, Germany). Those used in the test specific for *M. scalaris* were targeted on the mitochondrial *COI* gene and were MegaF2: 5′-ACTCTTTTATTAGCAAGAAGTA-3′, MegaR2: 5′-ATAGAAGAAATTCCGGCAA-3′, and MegaP2: 5′Cy5-TTCTAGAATTGCYCATAG-MBGEQ-3′, while those used in the test specific for *S. tricuspis* were targeted on the mitochondrial *cyt*B gene and were StrF1: 5′-GTTACTCCCGTTCACATT-3′, StrR: 5′-TATTGTTCAGAAATAGATTTTATTAATT-3′, and StrP3: 5′FAM-CTTCGTTCAATCCCCAAT-MBGEQ-3′. Other primers designed for sequencing purposes on the same genes were MegaF3: 5′-TAAGTATTATAATTCGAGCTGAA-3′ for *M. scalaris* and senseqF: 5′-AGATAATGCAACACTTACC-3′/senseqR: 5′-GTAAATTATCTCACCATTTAAC-3′ for *S. tricuspis*. For the amplification of the DNA extraction control the primers specific for *Tenebrio molitor* reported by Rossi et al. [24] were used.

2.3. DNA and RNA Extraction

The DNA extraction was carried out by two procedures differing in the amount of sample processed and, consequently, amounts of reagents and number of phases. In particular, two or twenty bees and 200 µL or 3 mL of hive debris were examined to ensure an acceptable sensitivity. The hive debris was measured approximately in volume because of the difficulty of manipulation for precise weighing and for the great variability in density among samples. The extraction procedure from two bees or 200 µL of hive debris was designated as "small-scale" and that from 20 bees or 3 mL of hive debris as "large-scale". In the small-scale procedure, the sample was transferred into a 2 mL Safe-Lock Eppendorf

Tube (Eppendorf, Milan, Italy) containing approximately 200 µL of unwashed glass beads of 200 µm diameter (Merck, Darmstadt, Germany) previously sterilized by autoclaving. The bee sample was crushed with a sterile pipette to facilitate the subsequent mechanical disruption by the bead beating. The sample of 200 µL of Macherey-Nagel lysis buffer T1 (Carlo Erba, Cornaredo, Italy) and 25 µg of the DNA extraction control constituted by homogenized *T. molitor* as described by Rossi et al. [24] were combined and the sample was disrupted in TissueLyser II (Qiagen, Milan, Italy) at 30 Hz for 3 min. The sample homogenate was centrifuged at 12,000× *g* for 10 min and the supernatant was transferred into a 2 mL Safe-Lock Eppendorf Tube containing 200 µL of Macherey-Nagel B3 buffer. After mixing, 210 µL of pure molecular biology grade ethanol was added and the DNA was allowed to precipitate for 10 min at room temperature. The whole sample suspension was transferred on a Macherey-Nagel NucleoSpin Tissue kit spin column (Carlo Erba) and DNA purification was completed according to the instructions. The DNA was eluted in two steps in a final volume of 60 µL by adding in each step 30 µL of elution buffer to the column.

In the large-scale procedure, the sample was transferred in a 5 mL screw-cap tube (Sarstedt Srl., Trezzano sul Naviglio, MI, Italy) containing approximately 1 mL of sterile unwashed glass beads of 200 µm diameter (Merck). To the 20 bees, 1 mL of Macherey-Nagel lysis buffer T1 (Carlo Erba) was added, and the bees were crushed with a sterile pipette. An additional 500 µL of lysis buffer T1 and 25 µg of the DNA extraction control were added and the sample was mechanically disrupted in the TissueLyser II instrument equipped with a 5 mL tube adapter. To the hive debris, 1.5 mL of T1 buffer and 25 µg of the DNA extraction control were added prior to disruption. The homogenate was then centrifuged at 12,000× *g* for 10 min and the supernatant was transferred into a 5 mL Eppendorf Tube containing 1.5 mL of B3 buffer. For the hive debris, the centrifugation was repeated by transferring the supernatant in a 5 mL Eppendorf Tube if the supernatant was not clear before mixing with B3 buffer. The sample suspension was mixed and 1.575 mL of pure ethanol was added. The DNA was allowed to precipitate for 10 min at room temperature and the sample was centrifuged at 14,000× *g* for 10 min. Most of the supernatant was discarded by gently aspiring with a micropipette and about 600 µL of it was left at the bottom of the tube. As for the small-scale extraction method, after the pellet resuspension, the sample was loaded on a spin column and the DNA purification was completed.

The small-scale extraction method was applied to six *M. scalaris* flies or one *S. tricuspis* fly to detect honeybee pathogens *Paenibacillus larvae*, *Melissococcus plutonius*, and *Nosema ceranae*.

The RNA was extracted from six *M. scalaris* flies or one *S. tricuspis* fly by the same procedure applied to the honeybees for virus detection by Rossi et al. [24].

2.4. Determination of the Limit of Detection (LOD)

The minimum number of target gene copies spiked in samples of bees and hive debris and detectable in 95% of the extraction replicates, i.e., the limit of detection (LOD) [25], was determined by using plasmid pUC57 containing synthetic DNA fragments (GenScript Biotech, Rijswijk, The Netherlands) with sequences identical to the gene regions between the primers. These fragments were quantified by the Qubit 3 Fluorometer (Thermo Fisher Scientific, Rodano, Italy) and the Qubit DNA HS Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions and were used to spike the honeybee or hive debris samples, which, in preliminary assays, tested negative in the qPCR assays to be evaluated in this study.

The LOD was determined also in terms of weight of fly per g of sample by spiking the honeybee and hive debris samples previously found to be negative for the fly-specific tests, with known amounts of *M. scalaris* or *S. tricuspis* homogenate obtained from the isolates made available in this study. The twenty replicates of spiked samples were used for DNA extraction with the two procedures described above.

2.5. qPCR Conditions

The quantitative PCR reactions were carried out in a QuantStudio 5 instrument (Thermo Fisher Scientific) with a PCR program comprising an initial denaturation at 95 °C for 5 min and 50 cycles of denaturation at 95 °C for 15 sec, and an annealing/elongation at 52 °C for 1 min. The qPCR reaction contained 1× Takara Premix Ex Taq Probe qPCR (Diatech LabLine, Jesi, AN, Italy), bovine serum albumin (Merck) 0.1 µg/µL, primers and probes for the target 0.2 µM, and primers and probes for the DNA extraction control 0.1 µM and 10 µL of DNA extract in a total reaction volume of 50 µL. The quantitative PCR tests were carried out to detect the microbial pathogens of honeybees in the fly isolates by previously reported tests. These comprised specific tests for *Paenibacillus larvae*, *Melissococcus plutonius*, *Nosema ceranae*, *N. apis*, acute paralysis bee virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wings virus A and B (DWVA and DWVB), and sacbrood virus (SBV) [24,26–28]. Synthetic DNA or RNA were used as positive controls in the PCR reactions and the negative amplification controls were represented by extracts obtained without adding a sample.

2.6. Sanger Sequencing

Sanger sequencing was carried out by Eurofins Genomics on the amplification products from the *M. scalaris COI* gene obtained with primers MegaF3: 5′-TAAGTATTATAATTC GAGCTGAA-3′ and MegaR2, and from the *cyt*B gene of *S. tricuspis* with the primer senseqF: 5′-AGATAATGCAACACTTACC-3′/senseqR: 5′-GTAAATTATCTCACCATTTAAC-3′. The same oligonucleotides were used as sequencing primers. The obtained sequences received the GenBank accession numbers PQ242635—PQ242647 for *M. scalaris* and PQ255542—PQ255544 for *S. tricuspis*.

2.7. Construction of a Phylogenetic Tree

For *M. scalaris* a phylogenetic tree was constructed including the sequences obtained in this study and one representative for each sequence variant retrieved by Blastn. The sequence variants to be included were selected by visually observing each pairwise matching in Blastn. For the tree construction the online tool "advanced phylogeny analysis", available at http://www.phylogeny.fr/simple_phylogeny.cgi, accessed on 31 August 2024, was used by choosing the functions "Phylogeny analysis" and "Advanced", which perform a multiple alignment using MUSCLE, an alignment curation using Gblocks, a construction of the phylogenetic tree by PhyML in default Approximate Likelihood-Ratio Test (aLRT), and a visualization of the phylogenetic tree by TreeDyn.

2.8. Statistical Analyses

The distribution of Ct values obtained at LOD for the samples artificially inoculated with *M. scalaris* and *S. tricuspis* was graphically represented by using PAST v4.03 statistical analysis software.

3. Results

3.1. Performance of the qPCR Methods in the Detection of M. scalaris and S. tricuspis

The GenBank database search for nucleotide sequences from the species *M. scalaris* and *S. tricuspis* retrieved 170 partial or complete sequences of the *COI* gene, one complete mitochondrion genome, and a whole genome in the stage of unassembled and not annotated scaffolds for *M. scalaris*. For *S. tricuspis* only four partial coding sequences for genes elongation factor 1-alpha (Ef1a) and mitochondrial genes *cyt*B, *COI*, and NADH dehydrogenase subunit 4, *ND4*, obtained in a phylogenetic study [29], were available. The Blastn analysis showed that the *M. scalaris* specific qPCR test designed by Furui et al. [20] is targeted at the mitochondrial *ATP6* gene for which only one representative is available in the GenBank database. Since no analysis of inclusivity was possible for this gene, it was decided to design a new test on the *COI* gene, for which many database entries are available.

For *M. scalaris* the first half of the *COI* gene was elected as the region for primer design since it exhibits higher variability among the species of Diptera as well as intraspecies. Moreover, the high number of sequences available for this gene could allow a reliable assessment of the inclusivity of the oligonucleotides. It was not possible to evaluate the inclusivity of the nucleotides designed for *S. tricuspis* because of the unavailability of sequences from different individuals.

None of the oligonucleotides evaluated for *M. scalaris* were specific only for this species, but the combination of MegaF2 with MegaR2 can ensure specificity since these primers differ in the fly species matched beyond *M. scalaris*. Primers targeted at *S. tricuspis* mitochondrial *cyt*B, StrF1 and StrR, appeared to be specific for *S. tricuspis* in in silico evaluation.

The sensitivity of the PCR tests was evaluated with the DNA extracts from the honeybee and hive debris samples contaminated with known copy numbers of plasmid pUC57 containing the synthetic DNA fragments identical to the target regions. In the small-scale extraction procedure, 100 copies of the target per g of sample were detected in all 20 replicates for both target organisms and both sample types. The lowest copy number detected in all replicates of the sample types with the large-scale extraction was 10 copies of the target sequence per g of sample. The cycle threshold (Ct) values at the LOD in the large-scale extraction, which showed higher sensitivity compared with the small-scale extraction method, varied between 30.26 and 32.16 for *M. scalaris* and between 28.36 and 30.02 for *S. tricuspis*.

3.2. *Analysis of Samples of Honeybees and Hive Debris for the Presence of M. scalaris and S. tricuspis*

The samples used for the detection of *M. scalaris* and *S. tricuspis* naturally present in the honeybees and hive debris were obtained from the sampling sites shown as black dots in Figure 1 in Abruzzo, Molise, and the Republic of Kosovo. The sites in which the target organisms were detected are circled.

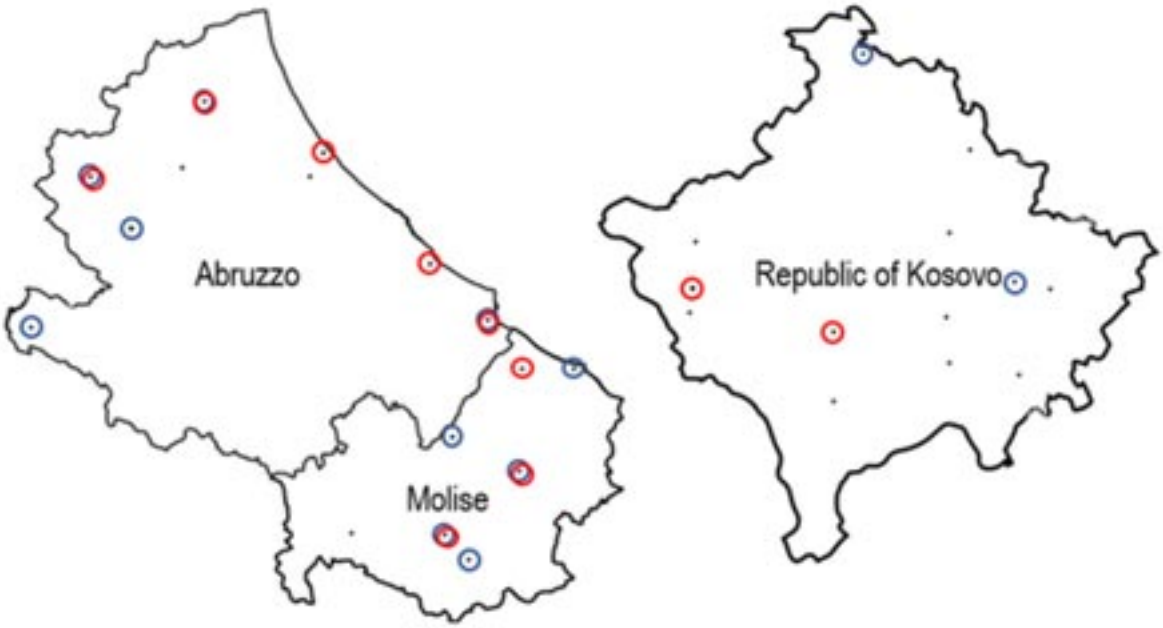

Figure 1. Sites of apiaries sampled in Abruzzo, Molise, and the Republic of Kosovo in June–July 2021. Black dots indicate the sites, blue circles indicate the sites positive for *M. scalaris*, and red circles those positive for *S. tricuspis*.

The samples were analyzed by using the large-scale extraction procedure and the Ct values were in most cases below the range of those observed at the LOD, indicating a low amount of fly material present on the apparently healthy honeybees and hive debris.

The Ct values obtained for samples are shown in Table 1, which also reports the towns of collection and sample type.

Table 1. Sites of sampling in order of collection date with the results of qPCR for parasitoid flies. Results are expressed as Ct because the assays give a presence/absence response.

Sampling Sites	Ct Values			
	Honeybee Samples		Hive Debris Samples	
	M. scalaris	*S. tricuspis*	*M. scalaris*	*S. tricuspis*
Italy				
Isola del Gran Sasso	-	-	-	-
L'Aquila	31.15	30.61	-	-
Termoli	37.18	-	-	-
Montereale	35.51	33.88	-	-
Oratino	35.62	37.12	38.29	
Mirabello Sannitico	34.12	-	36.32	-
Trivento	36.42	-	35.24	-
Morrone Del Sannio	34.37	-	-	28.09
Collecorvino	-	-	-	-
Fossacesia	-	23.15	-	31.14
Vasto	41.22	-	-	28.31
Sant'Agapito	-	-	-	-
Montenero Di Bisaccia	37.41	-	-	-
Torricella Sicura	-	34.37	33.25	-
Silvi	-	-	-	-
Pereto	41.26	-	37.06	-
The Republic of Kosovo				
Viti	-	-	-	-
Peje	-	-	-	-
Prishtine	-	-	-	-
Deqan	-	35.63	-	-
Junik	-	-	-	-
Mitrovice	35.15	-	-	-
Deqan	-	-	-	-
Peje	-	-	-	-
Novoberd	33.11	-	-	-
Prizren	-	-	-	-
Podujeve	-	-	-	-
Peje	-	-	-	-
Viti	-	-	-	-
Lipjan	-	-	-	-
Malisheve	-	38.92	-	-
Malisheve	-	-	-	-

It can be observed that both flies were more frequently detected in Abruzzo and Molise with 52.25% of the samples positive for *M. scalaris* and 31.25% for *S. tricuspis*. In Abruzzo and Molise the flies were also detected in hive debris in percentages of 37.5% for *M. scalaris* and 12.5% for *S. tricuspis*. In half of the sites positive for *M. scalaris*, the fly was detected both in bees and hive debris, while this was observed only for one among eight sites positive for *S. tricuspis*. For the samples from the Republic of Kosovo the flies were only detected in bees at percentages of 12.5% each. The results indicate that these organisms are more reliably detected using bees as sample type.

3.3. Isolation of Flies from Honeybees Captured in Positive Sampling Sites

Samples of viable bees were collected one year later from the molecular analyses in Abruzzo and Molise sites, in which *M. scalaris* and *S. tricuspis* were detected. Emerging fly larvae were observed for five of the samples, namely those taken in Oratino, Termoli, Montenero di Bisaccia, and Trivento for *M. scalaris*, and Vasto for *S. tricuspis*. For each sample four individual flies of those developed in adults were used for the DNA extraction, amplification and sequencing of a *COI* gene region and a *cyt*B region for *M. scalaris* and *S. tricuspis*, respectively. A total of thirteen sequences could be obtained for *M. scalaris* and three for *S. tricuspis*. The results show sequence variability for *M. scalaris* isolates from the same sample in two instances, though most of the isolates shared identical sequences.

The three sequences obtained for *S. tricuspis* were identical but divergent from the only database entry available.

Figure 2 shows a phylogenetic tree constructed with the *COI* gene regions of *M. scalaris*, in which the isolates from Abruzzo and Molise are compared with isolates from other countries.

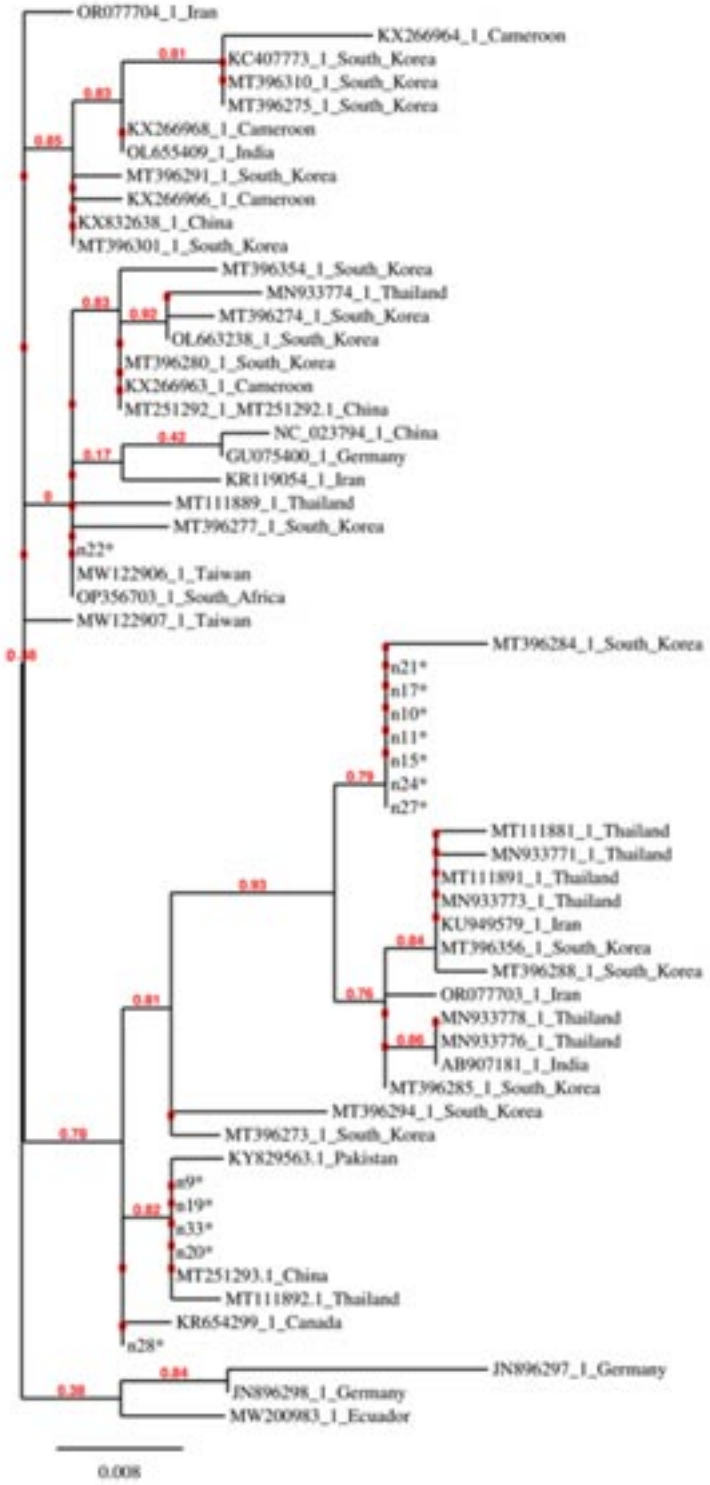

Figure 2. Phylogenetic tree constructed with partial *COI* sequences of *M. scalaris* isolates from this study (labelled with an asterisk) and one representative of each sequence variant retrieved by the GenBank database.

It can be observed that some isolates from this study, i.e., those that do not have an accession number in Figure 2, do not cluster closely and some of them are identical to biotypes from very distant geographical regions. Identity was also observed among other isolates from distant geographical regions, indicating the possibility that the *M. scalaris* flies might be transferred among countries through infested materials.

3.4. Re-Assessment of LOD in Terms of Weight of Fly Material per g of Sample

The availability of fly isolates allowed a better simulation of real sample analysis by using known weights of fly material to spike the bee and hive debris samples negative for the fly-specific qPCR tests. In this case, 10 µg of fly per g of bees or hive debris could be detected in 90% of replicates processed with the small-scale extraction method with Ct values between 30.56 and 33.24 for *M. scalaris* and 27.91 and 30.02 for *S. tricuspis*. With the large-scale extraction method 1 µg per g of bees or hive debris was detected in 95%/100% of the samples processed with Ct values between 31.37 and 34.01 for *M. scalaris* and 29.71 and 30.45 for *S. tricuspis*. Therefore, the large-scale extraction method proved again more sensitive and reliable than the small-scale extraction method for the detection of parasitoid flies in real samples of hive matrices, since in the preliminary analysis most field samples presented Ct values higher than the Ct values at the LOD of the large-scale method (Table 1).

Figure 3 shows the distribution of the Ct values at the LOD for 20 samples of honeybees and 20 of hive debris artificially inoculated with known amounts of *M. scalaris* and *S. tricuspis* fly isolates and submitted to DNA extraction with the small-scale and the large-scale extraction methods. The Ct values considered for the small-scale DNA extraction method regard the LOD for 90% positive samples (LOD_{90}).

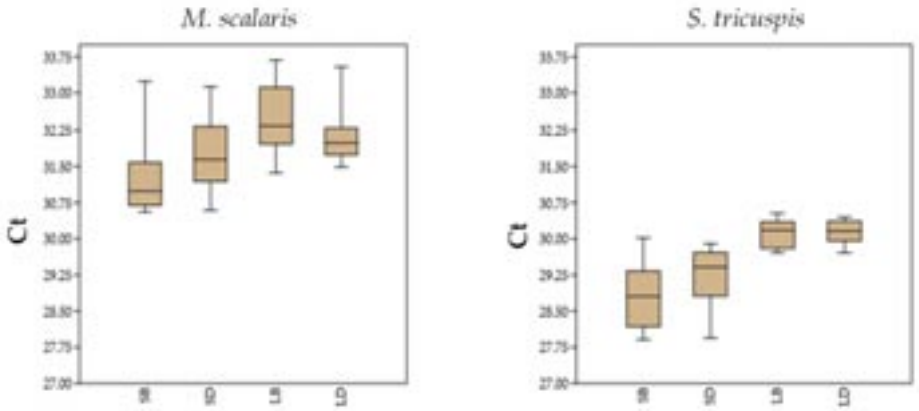

Figure 3. Distribution of the Ct values observed at LOD for *M. scalaris* and *S. tricuspis* isolate materials for the small-scale DNA extraction from honeybees (SB) and hive debris (SD) and the large-scale DNA extraction from honeybees (LB) and hive debris (LD).

3.5. Detection of Honeybee Microbial Pathogens on Parasitoid Flies

The DNA and RNA extracted with the small-scale extraction method from the pools of adult flies that emerged from the bees were used to analyze the presence of honeybee microbial pathogens in the parasites. *P. larvae* was detected in one *M. scalaris* sample, DWVA in four *M. scalaris* samples, BQCV and CBPV in two *M. scalaris* samples, and DWVB in one *M. scalaris* sample. *N. ceranae* was detected in two *S. tricuspis* samples. Viral pathogens and *P. larvae* were present at low levels, close to or below the respective LODs [24,26], and *N. ceranae* was detected with rather high Ct values, namely 34.78 and 35.02, thus at low levels, in the flies. Despite the levels of the detected pathogens being low, their presence indicated that the parasitoid flies could participate in their spread in apiaries.

4. Discussion

This study made available experimental procedures suitable to detect the parasitoid flies *M. scalaris* and *S. tricuspis* in hive matrices. For the *M. scalaris* a qPCR test targeted at

the mitochondrial *ATP6* gene was described before the one designed in this study [20], but the availability of just one sequence for this gene did not allow the analysis of inclusivity. Moreover, the previously available test was evaluated for specificity towards insects contaminating food commodities and not toward hive-associated insects [20]. For the new test targeted at the *COI* gene, the inclusivity and specificity could be evaluated in silico and appeared to be selective for the *M. scalaris* based on the database entries available at the time this study was carried out. No qPCR tests specific for the *S. tricuspis* were available before this study and, at this time, in silico evaluations excluded possible cross-reactions of the designed primer/probe system with other organisms.

The preliminary application of the developed methods to real samples indicated that the early detection of the infestation on apparently healthy honeybees is possible but the low levels of contamination observed, often below the LOD of 1 µg of fly material per g of sample, indicated that the large-scale DNA extraction method provides more reliable results by reducing the number of false negative results. The low levels of fly material detected on real samples are explainable by the fact that when the infestation is still not manifest only fly eggs or very small fly larvae are present on the bee's body.

Though the contamination levels were low, the developed methods showed some value in real sample analysis from distinct geographical contexts. A high percentage of sites infested by the flies was identified in the Central Italy regions, Abruzzo and Molise. The lower positive sample percentage identified in the Republic of Kosovo agrees with the distribution model of the infestation of apiaries by the *M. scalaris* proposed by Abou-Shaara and Darwish [21], which predicted a lower current prevalence of this pest in the internal Balkan countries compared to Italy. The presence of the *S. tricuspis* in that geographical region was also detected, so attempts to enlarge monitoring and isolate both flies in that region are worthwhile to understand if it is opportune to adopt measures to contrast their detrimental effects and further spread.

The methods suitable for targeted phylogenetic analyses of the *M. scalaris* and *S. tricuspis* that can be applied to a large number of isolates were devised in this study by designing end-point PCR primers specific for these flies. The tests can be used both for detection and to sequence the intraspecies discriminating regions of genetic markers. Indeed, in previous phylogenetic studies universal primers targeting *COI* or *cyt*B genes were used for the *M. scalaris* and *S. tricuspis* genotyping, respectively [4,17,18,29–37]. In particular, the primers specific for the *M. scalaris* reported here could be adopted for targeted studies on the occurrence and genotyping of this fly in fields other than apiculture.

The sampling of fly isolates by the method described by Ricchiuti et al. [2] showed the occurrence of the flies only in four out of the 16 samples sites that were positive in the qPCR-based tests, possibly because a new fly infestation was somehow hindered or because the latter has a higher sensitivity. Despite the low number of flies isolated, it was possible to obtain indications on their genotypic diversity. The possibility to exploit the sequenced gene regions in epidemiological studies was highlighted. Future investigations could be aimed at enlarging the number of fly isolates to reveal possible relationships between the genotype and the frequency and degree of apiary infestations.

The phylogenetic analysis based on the *COI* gene sequence of the *M. scalaris* showed that this generalist species in the Abruzzo and Molise regions is represented by different biotypes and that some of the biotypes are distributed worldwide. This indication could be exploited to investigate the reasons for the high adaptability of the most widespread biotypes by making available viable representatives and studying their behavior and distribution in the environment.

The detection of honeybee microbial pathogens in the parasitoid flies agrees with previous findings regarding the *A. borealis* and *M. scalaris* [3,8], thus confirming the implication of these pests in facilitating the spread of microbial bee diseases.

In conclusion, this study made available methods for the rapid detection of parasitoid flies in honeybees and hive debris that can be exploited to increase the knowledge of their prevalence and trends in geographic distribution. The application of these methods for early

detection at the apiary level will allow the adoption of measures such as the positioning of soil types that enhance fly larvae mortality rate around the apiary and chromotropic traps on the roof of the hives [22].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects15100786/s1, Figure S1: Phases of the experimental plan used in this study.

Author Contributions: Conceptualization, methodology, investigation, project administration, data curation, writing, review, and editing, F.R.; formal analysis, investigation, data curation, review, and editing, M.I., B.H., P.M., F.G., I.D.M., M.P., and L.M.; conceptualization, supervision, funding acquisition, review, and editing, L.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data obtained in the experiments carried out in this study are available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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An apiary is not merely a place where honey is produced. It holds another precious treasure: warm, aromatic air infused with the scents of honey, beeswax, propolis and other natural components of the beehive.

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A visit to an apitherapy chamber is more than an inhalation session. It is an opportunity to pause, listen to the bees and breathe in their rhythm. Visitors often associate the experience with greater respiratory comfort, deep relaxation and improved psychophysical well-being, although further scientific research in this field is still needed. For guided beehive air inhalation, the API AEROSOL II device can be used with portable hives placed inside an apitherapy chamber or connected directly to a beehive in the apiary.

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Matjaž Pogačnik

Member of the Apitherapy Commission of the Slovenian Beekeepers' Association

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Dear European partners of UNAF (French National Union of Beekeepers),

I am delighted to invite you to the European Beekeeping Congress Beecome 2026, which will take place in Agen from 1 to 4 October 2026. This event will bring together beekeepers, researchers and representatives of the French and European beekeeping sectors for conferences, round-table discussions and exchanges on the major challenges facing our sector — you will find the full programme of conferences attached.

This is a wonderful opportunity for your organization to be represented and to take part in the discussions that will shape the future of beekeeping.

The congress will open with an opening ceremony on Thursday 1 October at 11.00 am, in the Halle Marmande, Parc des Expositions Agora d'Agen, where I would be delighted to welcome you.

To enable your organization to participate fully, we are providing two 3-day passes to attend all the conferences, intended for representatives of your choice. To issue these passes in your representatives' names, please complete the form via the following link: https://forms.gle/HPmdXwGKe353RYcS6

Please do not hesitate to contact me if you have any questions, and I look forward to welcoming you to Agen.

Kind regards,

Christian PONS
President, UNAF


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1er au 4 octobre 2026
PROGRAMME DES CONFÉRENCES

JEUDI 1ER OCTOBRE 2026

APRÈS-MIDI

Agora · Amphithéâtre · Connexion

14H00 - 16H00	**Santé de l'Abeille : lutte contre Varroa, Tropilaelaps** *Maggie Gill, Entomologiste et apicultrice*	En partenariat avec LA FNOSAD
14H00 - 15H00	**Apicité et ASE : Valorisation avec les communes et partenaires** *Olivier Grima, Président de l'Agglomération d'Agen* *Gabriel Péna, Responsable label APIcité UNAF*	
14H00 - 15H00	**Les enjeux syndicaux - représentants de l'apiculture** *Christian Pons et Yves Delaunay (UNAF)* *Syndicats nationaux et européens*	
15H00 - 16H00	**Résultats Enquête Nationale Mortalité Hivernale (ENMHA) 2025-2026** *Carole Forfait et Laura Godard, Épidémiologistes plateforme ESA*	
15H00 - 16H00	**La marque GRF Gelée Royale Française** *Anais Rilcy, Animatrice communication et qualité au GPGR*	
16H15 - 17H15	**Économie de la ressource en eau chez l'abeille mellifère** *Pierre Le Bivic, Ingénieur d'études en Apidologie*	En partenariat avec LA FNOSAD
16H30 - 18H30	**Pesticides, abeilles, environnement** *Bernard Fau, Avocat à la Cour* *Jean-Marc Bonmatin, Chercheur chimiste et toxicologue au CNRS* *Philippe Grandcolas, Directeur scientifique adjoint du CNRS* *Noa Simon, Directeur scientifique et chef de projet chez BeeLife, Coordination européenne de l'apiculture* *Dr Michel Campano, Médecin généraliste et Co-président de l'AMLP (Alerte Médicale sur les Pesticides et les Perturbateurs Endocriniens)*	
16H45 - 17H45	**Séance Ciné-Club Apistoria : « La cité des abeilles »** *Michel Laporte, Trésorier Apistoria, bénévole Coopérative des Producteurs de Miel du Puy-de-Dôme ; référent Frelon GDSA63 ; apiculteur dans le Puy-de-Dôme.* *Christophe Perrier, Adhérent Apistoria, Médecin urgentiste*	
17H30 - 18H30	**S'adapter à l'impact climatique / Les microplastiques** *Etienne Bruneau, Vice-Président de Bee Life*	
18H00 - 18H45	**Cas cliniques de l'OMAA et santé des abeilles** *Karine Saget, Vétérinaire dans l'Aveyron et référente pour l'OMAA*	

VENDREDI 2 OCTOBRE 2026

MATIN

Agora · Amphithéâtre · Connexion

09H00 - 10H30	**Table ronde marché du miel français et mondial et lutte contre l'adultération** *Thomas Decombard, Président APIDIS* *Christian Pons, Président UNAF* *Norberto Luis Garcia, Universidad Nacional del Sur, Argentine* *Etienne Bruneau, ancien directeur du CARI*
09H00 - 10H15	**Biologie de l'abeille : Les abeilles ont-elles la bosse des maths ?** *Aurore Abagués-Weber, Chercheuse en neurosciences cognitives et éthologie*
09H00 - 09H45	**Des fleurs aux décisions : structurer l'action pour la biodiversité** *Fabien Kouachi, Directeur des partenariats Observatoire Français d'Apidologie*
10H00 - 10H45	**Les différents modèles de ruches utilisés en Afrique, ruches type Kenyane** *Alain Chevalier, Président d'Apiflordev* *Florent Huard, Apiculteur professionnel Bio en ruches kenyanes*
10H30 - 11H30	**La ruche basse consommation** *Damien Merit, Abeille Héraultaise, Apiculteur éleveur à Castres*
10H45 - 12H30	**Table ronde l'apiculture et la Communauté européenne : nouvelle PAC, mesures de soutien** *Noa Simon-Delso, Directrice scientifique et cheffe de projet chez Beelife* *Eric Sargiacomo, Député européen* *Stanislas Jas, Copa-Cogeca (AC)* *Andréa Rossi, Chargé de mission pollinisateurs Commission Européenne (AC)*
11H00 - 11H45	**La création de revenus apicoles pour les femmes libanaises** *Francine Vanlerberghe, Pilote du projet, Apicultrice* *Paul Bonnaffé, Apiculteur professionnel dans le Vaucluse*
11H45 - 12H45	**Maîtrise des mortalités hivernales : méthodes et résultats** *Gilles Grosmond, Docteur vétérinaire, auteur*
12H00 - 12H45	**Créer, valoriser et inspirer : une vie au service de la ruche** *Anne-Virginie Schmidt, Entrepreneure, autrice, cofondatrice des Miels d'Anicet*
12H45 - 13H30	**Traitement Varroa en Suisse** *Francis Saucy, Président Société Romande de l'Apiculture*
13H00 - 13H45	**Et si des bénévoles pouvaient bâtir un véritable centre dédié aux abeilles ?** *Loïc Leray, président du CETA*
13H00 - 13H45	**La résistance comportementale de Varroa destructor aux acaricides** *Théa Missud, Doctorante en éthologie, APINOV et CEBC-CNRS, La Rochelle Université*

VENDREDI 2 OCTOBRE 2026

APRÈS-MIDI

Agora · Amphithéâtre · Connexion

14H00 - 16H15 — **Sélection élevage en partenariat avec l'ANERCEA**

Julie Laur, Apicultrice-Eleveuse de reines Carnica en Ariège, GAEC de la Chouette
Ermanno De Chino, Apiculteur-éleveur en Sicile

14H00 - 15H00 — **Les innovations de Jacques Kemp**

Jacques Kemp, Membre d'honneur du Conseil d'Administration de l'UNAF, Apiculteur dans les Yvelines

14H00 - 14H45 — **L'utilisation des balances en apiculture**

Christian Lubat, Président et cofondateur BeeGuard
Paul Fert, Apiculteur professionnel dans le Béarn

15H00 - 15H45 — **L'apiculture solidaire et la protection forestière dans la région des plateaux au Togo**

Jean-Pierre Vincent, Vice-Président d'APIFLORDEV

15H15 - 16H15 — **Lutter contre le varroa : résultats des suivis d'efficacité des médicaments**

Florentine Giraud, Vétérinaire chargée de projet à la FNOSAD-LSA
Mickaël Throude, Chercheur en biologie moléculaire

En partenariat avec la FNOSAD

16H30 - 18H00 — **Apithérapie en partenariat avec l'AFA**

Marion Duris, Kinesitherapeute, Les massages au miel : du bien être à la thérapeutique
Philippe Garcia, vétérinaire, Intérêt de la propolis dans le traitement des MICI descarnivores domestiques
Pr Mesbah Lahouel, La propolis un enjeu pour demain
Florent Rouvier, Ingénieur recherche Observatoire Français d'Apidologie

16H30 - 17H30 — **La nutrition : premier rempart sanitaire de la colonie**

Abderrahim Hammaidi, Responsable technique Véto-pharma, Docteur Vétérinaire

16H00 - 16H45 — **Conférence e-facturing**

Jean-Marc Romilly, Expert-comptable et Commissaire aux comptes

16H45 - 17H30 — **Conférence « Quand les apiculteurs bâtissaient pour leurs abeilles »**

Bernadette Laporte, Présidente Apistoria
Nataly Perrier, Co-présidente Apistoria

17H45 - 18H30 — **Les abeilles sont-elles conscientes ? Attention et peur dans le cerveau d'un insecte**

Catherine Macri, Docteur en Neuroéthologie, IBPS-NeuroSU (Sorbonne)

17H45 - 18H30 — **Agroforesterie : une agriculture dans les règles de l'arbre, pour produire plus... Et polliniser les territoires**

Fabien Balaguer, Directeur exécutif Association Française d'Agroforesterie

SAMEDI 3 OCTOBRE 2026

APRÈS-MIDI

Agora · Amphithéâtre · Connexion

13H00 - 13H45	**L'Apiculture Douce : un modèle économique et éthique pour demain**	*Catherine Flurin, Apicultrice, Fondatrice de Ballot-Flurin*
14H00 - 15H45	**Tenter de s'adapter à l'impact climatique en apiculture**	*Fani Hatjina, Experte en santé de l'abeille mellifère – Dir. recherche à l'Institut des sciences animales d'ELGO « DIMITRA », Présidente Commission scientifique « Santé des abeilles » d'Apimondia et du conseil d'administration de l'International Bee Research Association (IBRA)* *Zahira Nedjraoui, Fondatrice et Présidente Beekeepers Foundation* *Anne Lavalette, Ingénieur chercheur en sciences du bois* *Emmanuel Ruffio, Ingénieur Chercheur en Thermique* *Anna Dupleix-Marchal, Ingénieure Chercheuse*
14H00 - 15H00	**L'apiculture en Outre-mer et au Sénégal**	*À la découverte des miels de La Réunion : origine, qualité et vertus naturelles* *Jimmy Chane-Ming, Chimiste analytique, Fondateur et le responsable du laboratoire Unité Analytique · GIP CYROI*
15H15 - 15H45	**Les miels en Afrique du Sud**	*Natasha Lyons, Apicultrice*
15H45 - 16H00	**Présentation du Congrès Apimondia de Dubaï et l'apiculture en zone aride**	*Zahira Nedjraoui, Fondatrice et Présidente Beekeepers Foundation*
16H00 - 16H45	**Sauvegarde des abeilles locales Sahariensis et Intermissa**	*Nabila Kabli, Chercheur en apidologie à l'INRA Algérie*
16H15 - 17H15	**Air des ruches**	*Patrice Percie du Sert, Chef d'entreprise, chercheur, apiculteur, enseignant* *Caroline Granziera, Administratrice UNAF Apicultrice, naturopathe*
17H00 - 17H45	**Rupture de couvain. Méthode, retours d'expérience, ateliers. En partenariat avec la FNOSAD**	*Louis Pister, apiculteur professionnel retraité et président de la FNOSAD-LSA*
17H30 - 18H30	**Apiculture Transhumante et apiculture sédentaire**	*Thierry Fedon, Apiculteur professionnel, co-gérant Maison Fedon* *François Peyrac, Apiculteur professionnel en Aveyron, vice-président des Compagnons du Miel*
18H00 - 18H45	**Protège ton abeille**	*Thérèse Moinnet, Présidente de l'Association Défi pour l'Environnement - Lions de France*

ΕΚΠΑΙΔΕΥΣΗ στην ΟΡΓΑΝΟΛΗΠΤΙΚΗ ΑΝΑΛΥΣΗ ΜΕΛΙΟΥ
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Level 1, 2026 in Athens

We are delighted to invite you to participate in the **2nd Honey Sensory Analysis Education Level 1**, which will take place in **Athens**.

This 2nd training event follows the highly successful 1st Honey Sensory Analysis Education - Level 1, which was held in **Thessaloniki** and brought together professionals from across Greece and abroad. The strong response and great interest from professionals encourage us to continue spreading specialized knowledge in honey sensory analysis, aiming to further support the development and promotion of Greek honey.

You can find **more information** about the 1st Education - Level 1 here:
https://chefstories.gr/1st-honey-sensory-analysis-education-in-greece/
Video: https://www.youtube.com/watch?v=uM0AcbNy7mM
Article by a graduate in the Gastronomos magazine: https://shorturl.at/ebL4n

The three-level educational scheme in honey sensory analysis has its roots in Bologna, Italy, where it was developed by **Bee Sources**.

Having trained hundreds of professionals internationally, Bee Sources collaborates with **Chef Stories**, and together they implement in Greece the first two levels of the Italian three-level educational program.

Location: Athens. The exact venue will be announced soon.

Dates & time: 18 - 22 November 2026, 09:00 - 17:00.

Description: Education – Level 1

This is an **intensive five-day training program** focused on developing skills in honey sensory analysis.

Participants will have the opportunity to:
- Train in honey sensory analysis techniques and in the recognition of aromas and flavors
- Become familiar with important varieties of Italian and Greek honey, their botanical origins, and their classifications
- Understand conditions such as crystallization, natural properties, and defects of honey
- Learn about European and Greek legislation regarding honey categories, quality, labeling, and marketing
- Learn how to describe and evaluate honey quality using methodological tools of sensory analysis
- Discover ways to use honey in cooking and pastry-making
- Explore current developments in research and laboratory methods for honey analysis

Note: The training activity will be conducted in English. If you require interpretation in Greek, please indicate this in your expression of interest.

ΕΚΠΑΙΔΕΥΣΗ στην ΟΡΓΑΝΟΛΗΠΤΙΚΗ ΑΝΑΛΥΣΗ ΜΕΛΙΟΥ

HONEY SENSORY ANALYSIS EDUCATION

Objective of the Honey Sensory Analysis Education

The objective of the Honey Sensory Analysis Education is to create a core group of trained sensory honey analysts who will be able to recognize, describe, evaluate, and communicate the origin, characteristics, and quality of honey in a unified and systematic way.

In the long term, the aim is for these trained honey sensory analysts, through their specialized knowledge, to contribute to the enhancement of the professional value chain and to the promotion of Greek honey as a high-quality product at both national and international levels.

The **2nd Honey Sensory Analysis Education Level 1** is addressed to:

- Beekeepers
- Honey producers and processors
- Honey trade networks and distributors
- Food & Beverage managers
- Nutritionists, dietitians, chefs, and pastry chefs
- Professionals from the agri-food and tourism sectors
- Retail store professionals
- Educators and researchers in relevant fields
- Journalists, food bloggers, and agri-food communication professionals

Next Education Levels: Level 2 & Level 3

Participants who successfully complete **Level 1** will have the opportunity to continue their training with **Level 2 Advanced**, which will take place in **Thessaloniki from 19–21 February 2027**, or in any other city where it may be organized by Bee Sources.

Subsequently, successful graduates will have the opportunity to take part in the **Level 3 Exams** in Bologna, Italy. Successful completion leads to inclusion in the **Italian National Register of Experts in the Sensory Analysis of Honey.**

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HONEY SENSORY ANALYSIS EDUCATION

Organization & Organizers

The Honey Sensory Analysis Education in Greece, Level 1 & Level 2, is organized by Chef Stories, a company providing consulting services as well as the design, organization, and implementation of specialized, high-quality gastronomy events.

Chef Stories is today a strategic partner of numerous businesses, organizations, and institutions in the sectors of food and beverages, retail, tourism, exhibitions, conferences, and development services, contributing its experience and expertise to the design and implementation of diverse projects that highlight and promote local gastronomy and Greek agri-food products.

Members of the Chef Stories team, Sylvia Koumedaki, Chef Stories Co-founder & Gastronomy Events Specialist, and Nana Zygoura, Business Development & Marketing Consultant and Trainer, have successfully completed the Honey Sensory Analysis Courses (Levels 1 & 2) in Italy Drawing from their personal experience, they have adapted the training content to the specific context and needs of the Greek honey market.

For this 2nd Honey Sensory Analysis Education Level 1, Chef Stories is collaborating with Bee Sources, an internationally recognized consulting and training company in the beekeeping sector Since 2005, Bee Sources has specialized in the development and dissemination of honey sensory analysis techniques.

Bee Sources systematically trains professionals who form part of the Italian National Register of Experts in the Sensory Analysis of Honey (https://www.albomiele.it/), a body of experienced analysts established in 1988 and officially recognized by the Italian Ministry of Agriculture since 1999.

Participation Fee - Level 1

The participation fee for the 2nd Honey Sensory Analysis Education – Level 1 is €900, payable in two installments.
Deposit: 500 €, until 25/08/2026
Final Payment: 400 €, until 20/09/2026.

Bank Account Details:
Piraeus Bank
IBAN: GR33 0171 5590 0065 5916 4159 697Account Number: 655916 4159 697
Account Holder: CHEF STORIES L.P.SWIFT/BIC: PIRBGRAA

The participation fee includes:
Training materials and notes, honey samples for tasting and analysis, a parallel activity, welcome lunch

Not included:
Transportation to and from **Athens** and accommodation.

ΕΚΠΑΙΔΕΥΣΗ στην
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ΑΝΑΛΥΣΗ ΜΕΛΙΟΥ
HONEY SENSORY ANALYSIS EDUCATION

Participation Process & Expression of Interest Form

In order to ensure the smooth conduct of the training program, the number of participants is **strictly limited**.

To secure your place:

1. Complete the expression of interest form at the following link: https://forms.gle/3cxicDHsn8H3cTYLA
2. After submitting the deposit, please send the payment receipt to the email: events@chefstories.gr
3. You will then receive the detailed program, additional instructions, and the payment receipt.

Participation registrations are managed by **Tania Georgiadou**
T. +30 2310471628
M. +30 6942980958

The Honey Sensory Analysis Education is an investment in personal and professional development and a step forward in promoting Greek honey as a product of high quality.

We look forward to welcoming you!

Kind Regards,

Sylvia – Ioanna Koumedaki & Nana Zygoura

ΕΚΠΑΙΔΕΥΣΗ στην ΟΡΓΑΝΟΛΗΠΤΙΚΗ ΑΝΑΛΥΣΗ ΜΕΛΙΟΥ

HONEY SENSORY ANALYSIS EDUCATION

INSTRUCTOR's CVs

Gian Luigi Marcazzan

Gian Luigi Marcazzan is a researcher and technical manager for honey quality control by chemical and sensory analysis at the Council for Agricultural Research and Economics (CREA) in Bologna, Italy for 26 years. He is the leader of the Honey Sensory group within the International Honey Commission, the leading organization to develop methods for honey quality evaluation. He is the President of the Italian Register of Experts in the Sensory Analysis of Honey with more than 25 years of experience as a teacher and professional honey taster From 2008, Gian Luigi works as a panel leader for the international honey competition BioMiel. Gian Luigi studies the composition of royal jelly and propolis to open up the knowledge on the composition in order to characterize and control the quality He is also a beekeeper and breeds bees for the production of swarms and honey.

Raffaele Dall'Olio

Raffaele Dall'Olio is a beekeeper and animal biologist with a master's degree in honeybee pathogens diagnostic, skilled in artificial insemination of honeybee queens. He has more than 10 years of experience in honeybee research and teaching focusing on genetic conservation of honeybee breeds, detection of pathogens and viruses, improving the quality of beekeeping products. He's a member of the international research networks COLOSS (on Colony Losses) and RNSBB (about Sustainable Bee Breeding). Raffaele has commercial experience with 150 hives in Tuscany, Italy and queen-rearing experience and manuka honey in New Zealand. As an internationally sought out speaker including Apimondia and the European Conference of Apidology, Raffaele has more than 10 years' experience as a teacher and professional honey taster and as a panel leader for the Italian National Register of Professional Honey Taster and member of official Panel Test at CRA-API lab since 2005 and in several Honey Contests. In 2015, he found "AsSenso", sensory analyses as an R&D tool for businesses. Raffaele also has written for national beekeeping magazines in Italy such as L'apicoltoreitaliano, Lapis and APOidea.

Mary Nikolaou

Mary Nikolaou was born and raised in Athens. After completing her studies as an Agronomist Technologist, she trained for a year at the Institute of Agricultural Sciences and Aristotle University of Thessaloniki in beekeeping and professional apiculture, establishing her first hives. Since 2012, she has been a beekeeper and honey producer, fully committed to quality and knowledge. Driven by her interest in understanding honey, she pursued further specialization in honey sensory analysis in Italy for two years. In 2023, she passed the exam at CREA, the Italian Ministry of Agriculture research center, earning the title of Expert in Honey Sensory Analysis, held by only 300 people worldwide. She is the first Greek member of the Italian National Register of Experts in Honey Sensory Analysis. Mary views honey as not merely food, but as a carrier of knowledge, experience, and culture. She is also a certified olive oil taster and collaborates with the Olive Oil Sensory Analysis Lab at the University of Peloponnese. In this first Greek training, she will teach Greek honey varieties alongside Italian experts, presenting the rich botanical and sensory profile of local honeys.

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HONEY SENSORY ANALYSIS EDUCATION

Nana Zygoura

Nana Zygoura is a Business Development and Marketing Consultant & Trainer. With studies in Marketing, Communication and Culture, she has had a long professional career as a director in Marketing and Communication, Client Service and New Business departments. Today she is an independent professional and supports companies, organizations and institutions in the design and implementation of development programs for agri-food, local, innovative and youth entrepreneurship. She also collaborates with Chef Stories company and together they design, organize and implement comprehensive programs and individual specialized events to highlight and promote Greek and local gastronomy. She is a certified adult trainer, a trained honey taste analyst in Italy, a graduate of the Butchers' School and has completed Level 3 of the Wine Studies of the international organization WSET. More about Nana Zygoura, on: www.zygoura.gr

chef stories
GASTRONOMY
STRATEGY & EVENT EXPERTS

BEESOURCES

crea
Consiglio per la ricerca in agricoltura

We are looking for new EUROPEAN CHAMPIONS in honey – a prestigious title for the next two years!

Sample submission

- **3 jars of honey (450 g each)**, properly labelled for sale.
- Register via the online form using the QR code.
- Attach the printed confirmation and proof of payment to the sample.
- Participation fee: **€70** per sample.
- Samples can be sent by post or delivered in person to:

Čebelarska zveza Slovenije, Brdo pri Lukovici 8, 1225 Lukovica, Slovenija.

Evaluation process

The honey will be evaluated by a panel of international honey experts.

- Evaluation is carried out in liquid form (crystallized honey will be properly liquefied beforehand).

A minimum of 7 samples per category is required

- If fewer samples are submitted, the commission will classify them based on electrical conductivity into: honeydew or multifloral.

All samples will be tested for **basic quality parameters** (≤ 18.6% moisture content, HMF ≤ 15 mg/kg). The top three honeys in each category will undergo additional analysis for **authenticity** and **safety**.

Online submission form with instructions for sample submission

Submit your samples by: 14.9.2026

AWARD CEREMONY IN KOPER, SLOVENIA 5 DECEMBER 2026

European Honey Contest
Koper - Slovenia

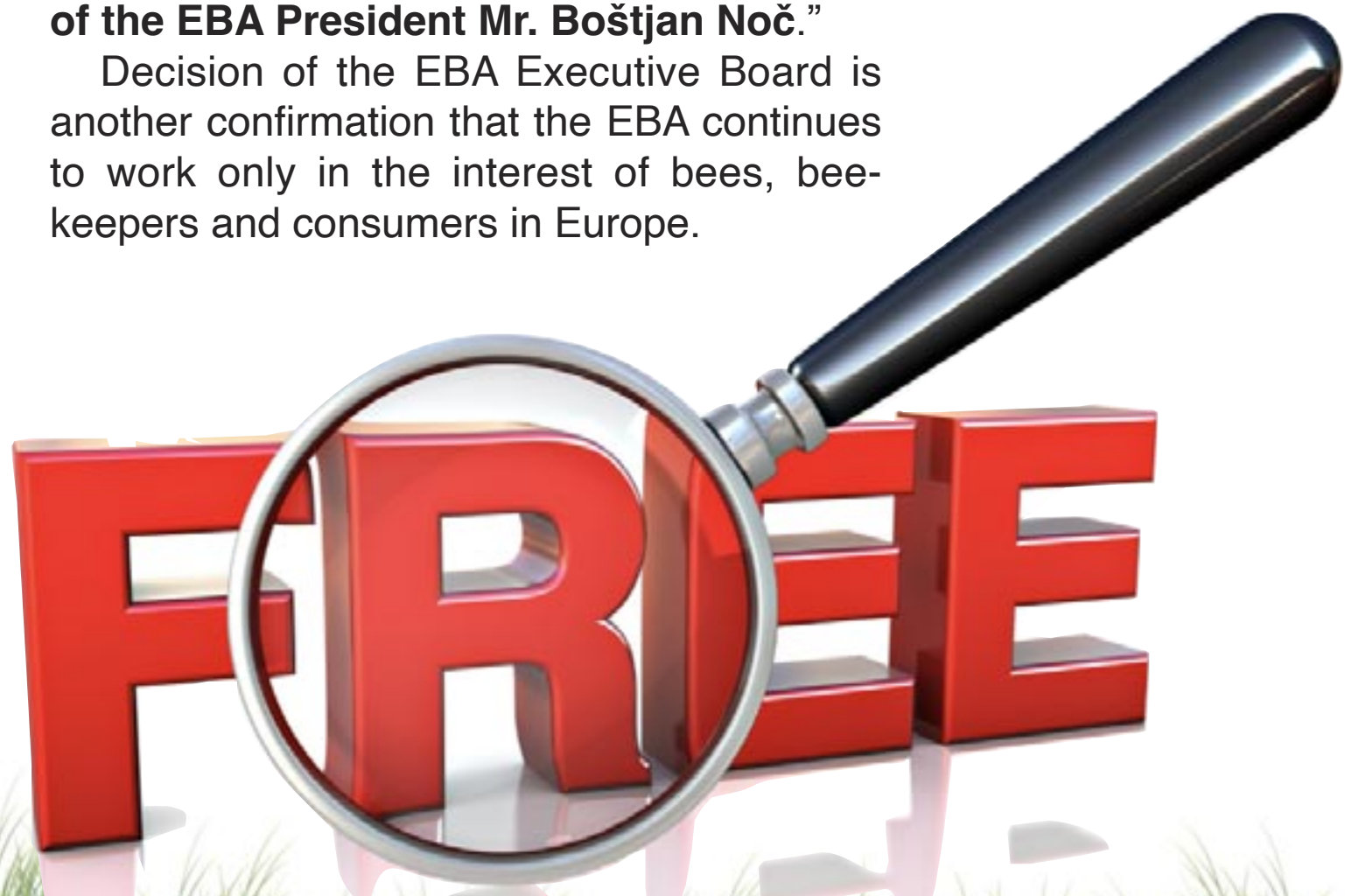
www.honey-contest.eu

Only authentic honeys with exceptional sensory characteristics typical of their variety will be awarded the top three distinctions and earn the right to promote this prestigious title.

TO THE EBA WITHOUT MEMBERSHIP FEE

At the meeting of the EBA Executive Board, on the proposal of the EBA President Mr. Boštjan Noč, an important decision was made regarding membership in the EBA in the upcoming period: "**Membership in the EBA is free for the duration of the mandate of the EBA President Mr. Boštjan Noč**."

Decision of the EBA Executive Board is another confirmation that the EBA continues to work only in the interest of bees, bee-keepers and consumers in Europe.



SPONSORSHIP REQUEST

AND METHOD OF ADVERTISING IN THE MAGAZINE

On behalf of the European Beekeeping Association (EBA),I am writing to seek your support in the form of sponsorship to help ensure the smooth and effective operation of our Association.

The EBA is dedicated to promoting and supporting beekeeping across Europe. The Association was founded out of necessity, as bees and beekeepers are essential for our ecosystem and society. Without beekeepers there are no bees, and whithout bees there is no pollination, leading to a lack of food on planet Earth.

EBA works for bees, beekeepers and consumers.

Our mission is to:

1. Fight against counterfeit honey that flooded the European market;
2. Introduction of incentives per beehive as agro-ecological programme;
3. Fight against the improper use of chemicals that are harmful to bees;

In return for your generous support, we offer various sponsorship benefits. We believe that this partnership would be mutually beneficial and would significantly contribute to the advancement of the european beekeeping sector.

ADVERTISING IN THE MAGAZINE:

1. Through sponsorship packages;
2. It is possible to pay for an ad only for 1/4 page (100 euros), for a larger area by agreement. The entire page cannot be obtained, it belongs only to the General Sponsor.

IT CONTINUES

EBA
sponsorship packages

GOLD sponsor - 5.000 euros:
Advertisement on the EBA website
Presentation at all EBA events, logo on all EBA correspondence
12 advertisements in the EBA monthly e-magazine in A4 page size

SILVER sponsor - 3.000 euros:
Advertisement on the EBA website
Presentation at all EBA events, logo on all EBA correspondence
12 advertisements in the EBA monthly e-magazine in half A4 page size

BRONZE sponsor - 2.000 euros:
Advertisement on the EBA website
12 advertisements in the EBA monthly e-magazine in the size of 1/4 A4 page

EBA SUPPORTER - 1.000 euros:
Advertisement on the EBA website
12 advertisements in the EBA monthly e-magazine in the size of 1/8 A4 page

These are basic packages, but we are open to different forms of cooperation, which we agree on individually. We would be delighted to discuss this opportunity further and explore how we can align our goals with your organization's values.

Thank you for considering our request. We look forward to the possibility of working together.

Yours sincerely,

Boštjan Noč
President of the European Beekeeping Association

NO BEES LIFE

EBA MAGAZINE

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