



Issue 3 • September 2024

NO BEES
LIFE

EBA MAGAZINE

20 COUNTRIES

FROM WHICH EBA HAS MEMBERS
(27 beekeeping organizations)

In order of confirmation of the Statute of EBA

277.964 beekeepers



Serbia
Slovenia
North Macedonia
Bulgaria
Greece
Romania
Malta
Germany
Hungary
Ukraine
Montenegro
Lithuania
Bosnia and Hercegovina
Sweden
Croatia
Czech Republic
Poland
United Kingdom
Netherlands
Italy



GENERAL SPONSOR

OF THE
EUROPEAN BEEKEEPING ASSOCIATION



SAVA
INSURANCE
GROUP

AMONG GOOD PEOPLE



SAVA
ZAVAROVALNA
SKUPINA

V DRUŽBI DOBRIH LJUDI



SILVER SPONSOR

OF THE
EUROPEAN BEEKEEPING ASSOCIATION

VITA BEE HEALTH IS COMMITTED TO THE EUROPEAN HONEY BEE INDUSTRY

PROUD TO SUPPORT THE EUROPEAN BEEKEEPING ASSOCIATION

VITA
beehealth

www vitabeehealth.com
 @vitabeehealth
 vitabeehealth

SUPPORTING GLOBAL BEE HEALTH





BRONZE SPONSOR

OF THE EUROPEAN BEEKEEPING ASSOCIATION

DULCOFRUCT BEE NUTRITION SCIENCE: Specialized Producer Of Bee Feed And Supplements

DULCOFRUCT BEE NUTRITION SCIENCE is a company with a portfolio of over 17 bee nutrition products, that are the result of continuous development and diversification, combining innovation and perseverance. We study market needs and meet the demands of beekeepers with a diverse range of products that correspond to the highest standards of quality, safety and veterinary security.

We are dedicated to investing in **research and development** to bring ongoing innovation to the beekeeping industry and enhance the health and productivity of bee colonies.

Our **research and development** team conducts extensive studies continually to create products that respond to the latest scientific discoveries and the ever-evolving challenges of the beekeeping community.

Check out more on:

WEB: dulcofruct.com

Social media: Dulcofruct Bee Nutrition Science



A PLEA TO EUROPE'S POLITICIANS – STOP COUNTERFEIT FOOD!

Health is our greatest asset, and bee products have been proven to boost it. But BEWARE – only genuine natural bee products can positively impact your health, and counterfeit bee products can even be harmful!

Europe is awash with fake food and HONEY is one of the top three most com-

monly counterfeited foods. The European Beekeeping Association (EBA) is calling on all European politicians to work together to stop the flow of counterfeit food and protect the consumers. Bee products are a gift of nature, they represent the traditional pharmaceutical products used by our grandmothers. Unfortunately, almost a staggering half of the honey on the market is of counterfeit origin and this has left the beekeeping industry in an impossible situation.

Beekeeping is already being abandoned across Europe and soon there will be a shortage of bees, and the pollination service provided by bees will suffer as a result.

This will have a significant impact on agriculture, which will produce less food, and as a consequence we will see even more counterfeit food entering the European market.

**Are we really going to allow this?
I SINCERELY HOPE NOT!**

To this end, the EBA is preparing concrete proposals which will be sent in October to all agricultural ministries in Europe, to all MEPs and to the European Commission.



Their realisation will not require a huge amount of money, but it will require determination and a clear goal – allow Europeans to enjoy quality food from their local environment!

We will invite the whole agricultural sector to participate, as food has an undeniable impact on people's health, and it is proven that eating locally produced food is particularly important, as only natural food is "real" food. Only together can we stop the invasion of counterfeit and harmful food. That is why we are calling on all decision-makers in Europe: let us stand together and put aside political

and other divisions, because at the end of the day, we are all in this together and we all deserve to eat real food. We would therefore like to appeal to everyone to STOP fake food.

There is a lot of talk about security in Europe, but food security is also very important and can only be achieved by supporting European agriculture and beekeeping. Nevertheless, preserving European agriculture and beekeeping in the face of unfair competition from fake food is impossible!

We invite everyone who agrees with us to join us.

Please contact us at eba@ebaeurope.eu.

Boštjan Noč

President of the Slovenian Beekeeping Association and European Beekeeping Association





European
Beekeeping
Association

BEEKEEPERS' CALL TO CONSUMERS: LET'S PUT AN END TO FAKE HONEY TOGETHER!

Don't we Europeans really deserve to enjoy real food?

Can't we really come together, both food producers and consumers, and put an end to fake food on the market, which is indeed low-priced but at the same time often even harmful to health! Unfortunately for beekeepers and consumers, HONEY is one of the most commonly adulterated foods.

The European Beekeeping Association has almost 300,000 beekeepers from 19 European countries, and the main goal of this association is to protect the consumer from consuming fake bee products and, as a result, preserve bees and beekeeping!

Today, the bee is endangered in Europe, because unfortunately it cannot survive without the help of the beekeeper! Due to fake bee products on the market (according to the official data of the EU Commission, almost 50% of them and most of them are imported from outside the Europe) and thus unfair competition, the entire European beekeeping is at risk, the collapse of beekeepers means the collapse of bees and consequently the end of the pollination service of bees, which is a prerequisite for food production in Europe, as every third spoonful of food depends on bee pollination!

Food security in Europe can only be ensured by European farmers, honey (and mostly "honey", which is not real honey at all) can be imported, but not the bee pollination service!

The presence of bees and other pollinators is therefore necessary for the production of European food.

In order to preserve bees and beekeeping, we must immediately put an absolute STOP to all fake bee products and at the same time support beekeeping and agriculture by buying local bee products, which are proven to strengthen our health the best!

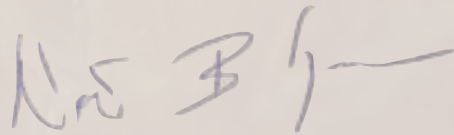
The new leadership of the European Union has been elected, the European Beekeeping Association publicly calls on all European Members of Parliament, European Commissioners and other decision-makers in the EU and in individual countries to protect the European beekeeper, to protect the European farmer, because without bees there will be no agriculture and to protect the European consumer against adulterated food.

The European Beekeeping Association will immediately contact the decision-makers in the European area, and we believe that together we will find a solution.

I am convinced that we all want and deserve to enjoy real food!

Boštjan Noč

President of the European Beekeeping Association
eba@ebaeurope.eu



GENERAL SPONSOR
OF THE EBA



SILVER SPONSOR
OF THE EBA



BRONZE SPONSOR
OF THE EBA

DR URŠKA RATAJČ APPOINTED TO THE COPA COGECOA'S HONEY WORKING PARTY

Dr Urška Ratajč, Head of the EBA's Scientific Committees, Appointed to the Copa Cogeca's Honey Working Party as Slovenian Beekeepers' Association Representative



In July, the Cooperative Union of Slovenia (Zadružna zveza Slovenije, ZZS) appointed Dr Urška Ratajč, a representative of the Slovenian Beekeepers' Association (Čebelarska zveza Slovenije, ČZS) and Head of the Scientific Committees of the EBA, to the Copa

Cogeca Honey Working Party. Copa Cogeca is an international organization that unites European farmers (Copa) and agricultural cooperatives (Cogeca) to advocate for the interests of the agricultural sector within the European Union. One of their specialized working groups focuses exclusively on honey. Slovenian Beekeepers' As-

sociation has previously collaborated successfully with Copa Cogeca through Cooperative Union of Slovenia particularly on key issues related to honey origin labeling.

We are confident that this fruitful cooperation will continue, and we look forward to working together to achieve significant advancements for beekeepers.

From before, the EBA already has a vice-president of the WP in its membership. This is the president of the DIB, Mr. Ellman Torsten from Germany.

We expect their performance in WP to be significant.





ANTI-DUMPING DUTY

A FEASIBLE SOLUTION TO LIMITING HONEY FRAUD THAT COLLIDES WITH INTERESTS

Summary

Dumping refers to the situation where a product is imported from third countries at unjustifiably low prices compared to domestic products, causing distortions in the domestic market. To address this issue, the European Union (EU) imposes additional duties to protect domestic products. These measures are provided for by EU legislation and are already applied to more than 50 different Chinese products imported into the EU. Imported honey arrives at prices significantly below the production cost of domestic honey, creating major market problems that have already been identified by the EU. According to these findings, the conditions for applying EU Regulations to protect domestic honey and ensure its true value are fully met. The solution of imposing anti-dumping duties on imported honey to limit fraud and its sale as domestic honey is feasible, is supported by existing EU legislation, and can provide an immediate and radical solution. However, it is not implemented because it conflicts with enormous interests that influence political will.

The problem

The coordinated action “From the Hives” of European Union (EU), emphatically revealed the large-scale fraud involving the importation and distribution of inferior honey in European countries, to the detriment of consumers and beekeeper producers.

Key points of the investigation include that most honey traded on the global market does not comply with the EU's legislated quality standards, that this honey is imported into European countries uncontrolled by EU borders and is traded without traceability. The coordinated action even spotted, that traders-distributors know that the honey they import is inferior, that there is collusion between accredited analysis laboratories and importing traders to upgrade the honey according to the EU's legislated control criteria, and that there is a need to develop methods for detecting modern fraud and harmonizing laboratory analysis methods.

In its report, the EU not only makes general observations but also provides specific findings for each country regarding the monitoring and

control system for the distribution of adulterated-inferior honey. The EU's observations mainly refer to the lack of controls at border stations, the fraud and uncontrolled distribution, the absence of honey balance, inadequate traceability, the lack of established official and validated methods for honey analysis, and the distribution of imported honey as domestic.

Alongside these feasible measures, the EU has legislation that gives it the ability to address the problem directly and effectively by imposing additional duties known as anti-dumping measures to protect the domestic product. This article discusses the possibility of imposing duties on cheap and inferior imported honey to protect European honey in accordance with existing legislation.

What are anti-dumping measures?

The word "dumping" is used as a term in international trade for cases where some countries dump products on the market at particularly low and unjustifiable prices. According to the definition, dumping occurs when a company exports a product at a price lower than its normal value in the domestic market or below the production cost in the importing country.

The European Union (EU) favors free trade, but when there is proven information about dumping to the extent that it disrupts the domestic market and creates unfair competition against businesses, the EU can resort to anti-dumping measures, which are mainly additional tariffs or requiring the exporter to sell their products at a minimum price.

This minimum price must be above the production cost of the specific product in the importing country. In countries that subsidize their exports, resulting in their product creating dumping conditions, corresponding countervailing duties are imposed.

These EU defense measures concern products entering from third countries. The EU Commission has mechanisms to check for possible circumvention of its trade defense measures. For example, it can check whether the honey imported from a member state is produced in that

country or if it comes from China or another third country.

Is this provided for in European legislation, and if so, is it applied to other products?

Regulation (EU) 2016/1036 on defense against dumped imports from third countries exists. This regulation has been amended by implementing regulations (EU) No. 2018/825 and 2020/1173. EU legislation follows the procedures provided by the World Trade Organization (WTO).

Since 2016, the EU has imposed anti-dumping duties on more than 50 different Chinese products, mainly aluminum, bicycles, cement, chemicals, ceramics, glass, paper, solar panels, and steel.



Are anti-dumping measures applied to honey in other countries?

The United States is the country that imports the largest quantity of honey in the world. As shown in Figure 1, honey is mainly imported from India, Vietnam, Argentina, Ukraine, Brazil, and Vietnam. China is completely absent as major honey-importing country, even though it is known to export the largest quantity of honey globally (Figure 1).

The absence of Chinese honey from the US market is due to the anti-dumping tariffs imposed

on this honey since 2000, tripling the price of Chinese honey and making it unprofitable for importing traders. In the past, the US imposed anti-dumping duties on honey from Argentina for a few years. These measures greatly help to adjust the price of honey to its true value, always according to the production cost in that country.

Are there conditions for imposing anti-dumping duties in Europe?

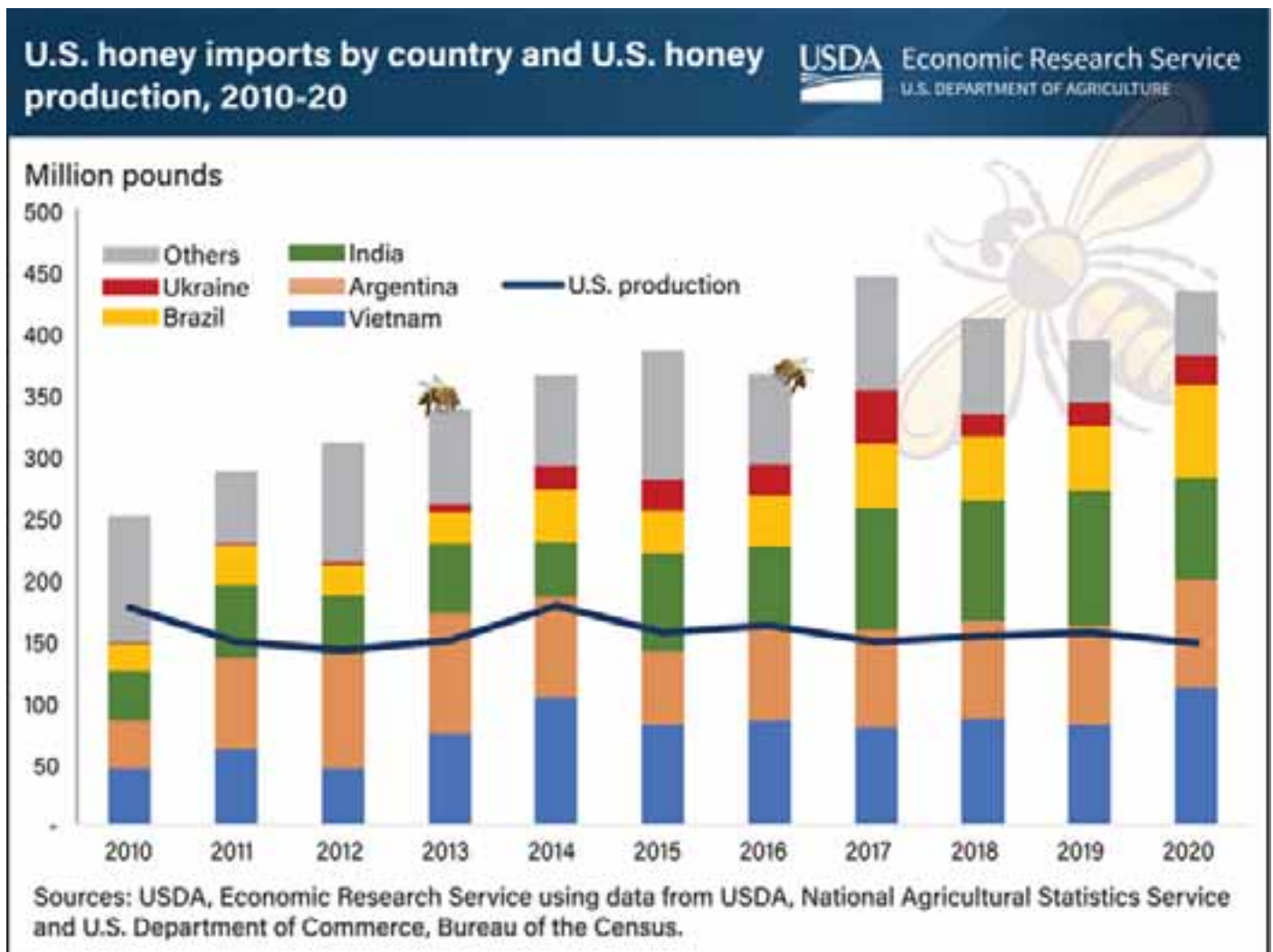
Dumping exists when a company exports a product at a price lower than its normal value in the domestic market or below the production cost.

The average cost of honey production, according to the annual beekeeping programs of the Union member states for the years 2017-18,

Figure 1. China is not among the countries that export large quantities of honey to U.S.A, although it is the world's largest exporting country, due to the anti-dumping measures that were implemented.

Chart is taken by

<https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=101457>



was 3.9 euros (Figure 2). Today, the cost of producing honey in European countries is likely to be much higher due to the higher price of oil needed for transporting hives, the sugar necessary for bee survival, and beekeeping supplies and tools in general. A possible increase in honey production costs could also be due to the observed lower honey yields caused by climate change (Figure 2).

However, even with the 2017-2018 data, the cost of honey production in European countries is much higher than the import prices of honey from third countries, which range from 0.80 to 2.54 euros, according to the EU's coordinated action 'From the hives, 2023' report."

As a result of cheap imported honey, there are distortions in domestic markets, unfair competition, profiteering at the expense of consumers, and the sale of imported honey with misleading labels regarding its geographic origin. This situation leads to disappointment and aban-

donment of the beekeeping profession, degradation of product quality, and more. This situation has been observed by the EU and has been publicly expressed in its reports.

According to the above, the conditions for the application of Regulations (EU) 2016/1036 and 2018/825 on anti-dumping measures for imported honey are fully met. In fact, the EU itself, through its reports and statements, fully justifies the need for protective measures for the domestic market through anti-dumping measures.

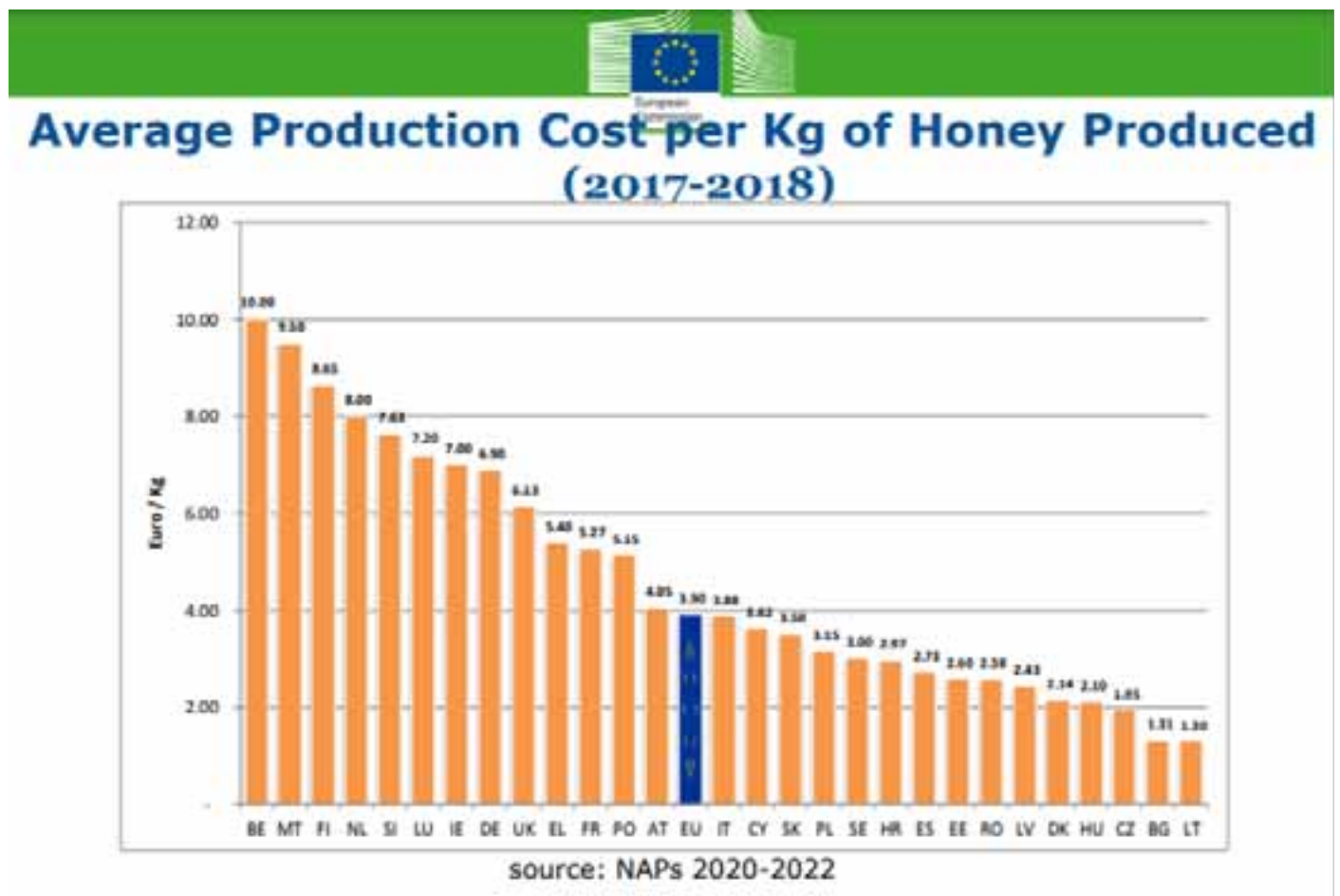
What obstructs the implementation of the feasible anti-dumping solution for honey?

The solution of imposing anti-dumping duties on imported honey to limit fraud is feasible, based

Figure 2. Figure 2. Honey production costs in European countries.

Chart is taken by

https://www.apiservices.biz/documents/articles-en/national_apiculture_programmes_eu.pdf





on existing legislation, and can provide an immediate and radical solution.

If a honey trader is forced to import honey at a price above the production cost of domestic honey, honey purchase prices will immediately rise.

An example: The Greek beekeepers, under the pressure of current needs, are forced to sell their honey to traders-processors at a price well below the production cost. Essentially, however, they are unknowingly participating in compulsory dumping, with the difference that their product is natural, pure, and worth much more. This real value will be reflected in the price of natural honey if anti-dumping duties are applied.

In my personal opinion, based on the actual events taking place today in the European Union, anti-dumping measures to protect domestic production are unlikely to be imposed. The reason is that these measures harm a) the enormous interests of the global industry producing adulterated and artificial honey, estimated at 2.5 billion

euros annually, b) the interests of traders importing cheap, inferior honey c) the collusion between exporter, importer, blender and their clients d) the EU-accredited laboratories that collaborate with traders of fake honey to adapt the imported honey to elude possible detection by official authorities e) the beekeepers or intermediaries that collaborate with the adulterating traders. They also harm the interests of many European countries that import cheap, inferior honeys and re-export it as upgraded European products.

Thus, this feasible solution, under the pressure of all the above interests, is removed from the agenda of proposals and discussions for potential solutions to the long-standing problem of distributing cheap, inferior honey from third countries.

Andreas Thrasyvoulou

Emeritus Professor

Aristotle University of Thessaloniki (AUTH)

Hellenic Republic (Greece)

JOIN THE EBA VIBER AND WHATSAPP GROUPS FOR FREE

Anyone interested can join the EBA Viber or WhatsApp groups free of charge to inform beekeepers about EBA activities, regardless of whether you are an EBA member or not, so that you can be timely and properly informed about

everything important in EBA and European beekeeping.

If you have the Viber or WhatsApp application installed, in order to join the EBA groups, you just need to click on the following link:

For Viber: <https://ebaeurope.eu/join-the-eba-viber-group-for-free>

For WhatsApp: <https://ebaeurope.eu/join-the-eba-whatsapp-group-for-free>



Investing in our region is investing in our future.

This is yours and our home. And that is why our dedication to Southeastern Europe goes beyond providing financial services. We are committed to fostering a thriving community, where every individual and business finds new opportunities.

NLB Group



Museum of Apiculture SLOVENIA

Celebrating the Rich and Exceptional
Heritage of Beekeeping



You're Welcome to Visit Us!

www.mro.si
Linhartov trg 1, 4240 Radovljica, Slovenia

IT IS POSSIBLE TO PROVE MISLABELING OF HONEY'S ORIGIN!

Slovenian experts distinguish between honey from Slovenia and honey from neighbouring countries, making it possible to prove mislabeling of honey's origin!

The Slovenian Beekeepers' Association has been working on the characterisation of bee products for years. We are the only country in Europe that places such an emphasis on this. So far, we have done an excellent job characterising honey, pollen, propolis and royal jelly, and the characterisation of bee venom is in its final stages.

The aim of characterisation is to determine the typical characteristics of Slovenian bee prod-

ucts, which helps us to distinguish bee products by their country of origin.

In Slovenia, we are already able to determine the region of production of many bee products, and in most cases, we can clearly identify whether the bee product is Slovenian or comes from neighbouring countries or beyond.

All of the above information is public, so it can also be used by the competent authorities for control. At the Slovenian Beekeepers' Associ-





ation, we are proud to use such characterisations, which are very important in detecting incorrectly labelled origins of bee products.

This capability allows us to better protect Slovenian consumers from being misled, as incorrect origin labelling is a form of consumer deception and, ultimately, fraud.

The Slovenian Beekeepers' Association is an initiator of accurate honey origin labelling throughout Europe, and we have called on the state of Slovenia to immediately implement the new EU Honey Directive and to tighten controls



**BEES
LIFE**



on the correctness of honey origin labelling. The characterisation of Slovenian bee products will enable the competent authorities in our country to be very successful in detecting mislabelling of the origin of bee products, especially in cases

where they are falsely labelled as being of Slovenian origin.

Boštjan Noč

President of Slovenian Beekeeping Association
President of European Beekeeping Association

BeeConn

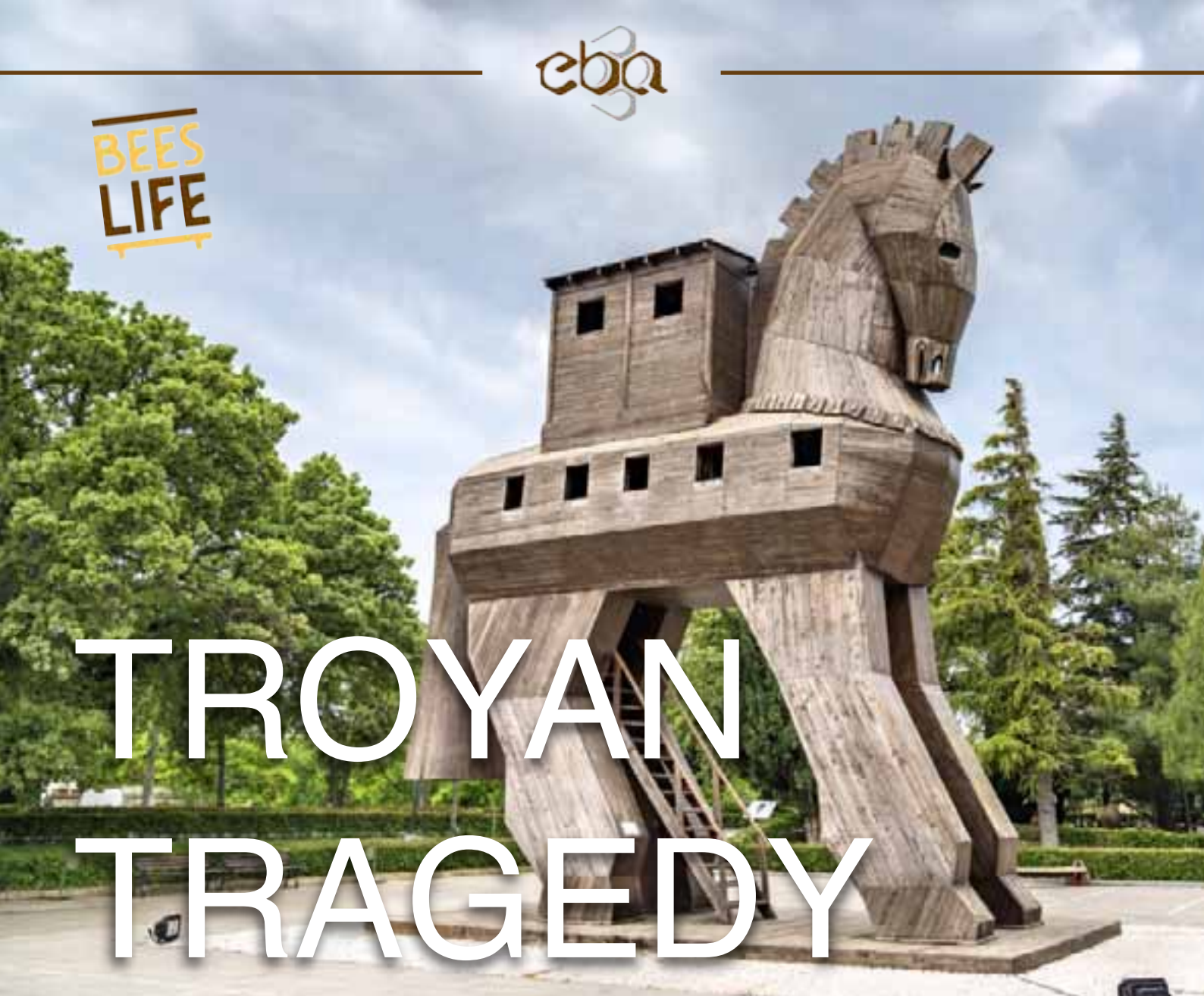
@beeconn.si
 beeconn.si

BeeConn

All you need for successful beekeeping!

A wireless monitoring device that enables beekeepers to regularly monitor and obtain current information about activities in the beehive.

Strip's d.o.o., Kandriše 7, 1252 Vače, Slovenia, +386 3 56 70 770 | info@beeconn.net | www.beeconn.net



TROYAN TRAGEDY OF BEE COLONY

The fall of Troy happened when Trojans brought a wooden horse into their fortress inside of which the enemy was skilfully hidden, after which Troy was destroyed.

The key role was played by the “gate”, spotted by the enemy as the weakness. The first thing they did after going out of the horse was to eliminate the gate keepers and open the gate. All this would not have happen if they had not taken the horse inside their fortress, i.e. the gate had not been open. Maybe the bee colony experiences the same destiny with Varroa. You wonder how?

Black bees

According to the findings of Taranov, considering foragers, the most important role in finding nectar is entrusted to the bees losing their tomentum and becoming darker in the first days of their forage.

He called them scouts and according to his findings, these bees are in charge of informing where and at what distance there is nectar in the nature. While waiting for the scout information, foragers are situated under or with the brood.



the honey and goes back to its colony mobilizing its foragers.

Carefully observing, we can spot black bees. They represent a type of invisible unmanned aerial vehicle informing the bombers about the weaknesses of the enemy, while the bombers (foragers) are strong and capable of carrying lots of load.

Earlier, the belief was that the black bees are in fact old bees and that they lose the tomentum due to aging, but later on, it was found that it is not correct (Taranov) and it can be easily determined by marking just emerged bees and observing after 19 days.

Taranov claims that the existence of scout bees is justified, because, if every bee would look for the source of nectar individually, they would unproductively spend food and energy i.e. this would cause an increased food consumption.

This means that when there is no nectar flow for a long time, and there is a subsequent increase in the number of scout bees, the increase in the food consumption is increased, as well. The assumption is that, due to the constant minus on the scale, a bigger mobilization of the "black scout bees" is performed because there is more need for those that search.

Before few years, many people noticed more "black bees" in the apiaries with bad nectar flow conditions.

The assumption is that colonies with bigger food supplies perform the mobilization of scout bees at much lesser intensity.

This warns us that without a scale there is no beekeeping. The important thing is that the beekeeper knows that in a very short period, this way, he can lose big quantities of food. In a natural habitat, when the scale is in plus all the time, for a short period, there is a big mobilization of bees receiving nectar.

Seeley claims that the number of bees receiving nectar, for one day only, increased from 550 to 2,000 after more than 15 waggle dances. This warns us that, when the flow goes weaker, the number of scout bees can also become increased in this short time.

It can happen that in only two to three days the scale goes rapidly into minus and there is the loss of significant food stores, even run out of bees in the hive, which naturally depends on the food stores and on the way our bees rob.

When a scout finds nectar and brings it in her honey sac, she goes straight in the centre of the foragers and among them, on the comb, performs signal movements, distributes a part of the brought nectar to them, then stops the signal movements and after the delivery of the load, flies out again.

Following her game, the foragers fly out toward the food source.

Taranov also states the behaviour of the scout bees when there is a lack of nectar in the nature.

At that time, they are attracted by the buzzing and the scent of nectar coming from the entrance of the neighbouring hives and they try to go in and reach it.

Nevertheless, they face the strong resistance of the guards, which, according to him, have the ability of telling a forager from a scout bee. If a scout bee comes into some other hive, it takes



The entrance is narrowed, but the fight of bees without killing and "silent" robbery still continues

It can happen that in a moment we run out of both food (in case the beekeeper does not come to the rescue) and bees.

Komarov and Avetisyan have determined that for one-hour flight a bee spends 100 mg of food or 1.66 mg per minute (determined by measuring with a special scale of high precision). When flying out of the nest at the distance of 4-4.2 km, each bee takes 6.8 -7 mg of food as a reserve and based on that they have concluded that if a bee flies 3 km away and does not fill the food reserve, she can not come back. How many times a scout bee needs to do the same if the quest attempt was a failure and if from day to day (in a period without a nectar flow) there are more and more of them? And it can also happen that she never comes back to the hive due to less and less food stores both in the colony and in the honey sac.

Last year, many people found empty hives during the summer, repeating the claims of experts that it is certainly Varroa and that the colonies left their habitats because of it, but here's one reason pointing that it does not necessarily mean that it is always it. And maybe it never has been, at least considering the summer period?

Castes

It has been noticed that during a strong flow bees visit weak honey plants, as well, and this raises the question (and maybe gives the answer) that among bees performing the foraging activity there are castes that even during a strong flow visit various plants, because, in case this would be only bees from one colony, it would have to be noticed that, in the peak of a strong forage, there is at least one colony with extremely poor performance, exactly as when the flow is poor.

Since this is not the case, we need to ask ourselves why during a strong flow all the colonies in an apiary collect nectar from poor nectar plants, as well.

During a strong nectar flow, scout bees in time accept the information from other scout bees that perform the navigation which is more dominant for a new, stronger flow. The strength of the nectar smell reduces the ability to recognize bees, that is why it is easiest to put the bees together (and reduce the fight to the minimum) during the bigger processing of nectar. Later on,



**BEES
LIFE**

when the nectar flow is ceasing and there is no bigger nectar processing, the smells are weaker and the mutual recognition of bees by all their scents and pheromones is much easier, therefore the putting together is made more difficult.

It can be dangerous if some scout castes are directed to the honey stores of some other colonies. They probably won't leave it in the beginning of a strong nectar flow, unless they accept the navigation of other scout bees. Furthermore, "silent" robbery is possible even when there is a strong flow, because "black scout bees" probably do not make any difference between the nectar in nature and the "comb nectar".

The existence of castes is spotted also when scout bees are looking for a space for the swarm, as well as in the moving of the swarm.

Thomas D. Seeley states that when a swarm is looking for a new habitat, the scout bees that choose the best place win, while the rest of the scout bees join the "dance" for the new "better" location. He also states that there is some kind of consensus, but the way he describes the behaviour during the moving of the swarm tells something different.

The moving of the swarm itself, which is never in one direction, shows that castes "go each their own way" and the question is whether information about the "better place" is adopted at all, because it is rather a web of circumstances. According to his findings, it is more likely that the first scout bee in quest comes, starts its dance attracting a bigger number of bees to accept her as the guide.

If no other scout bee comes during that time, the number of bees accepting the information becomes bigger and bigger. In case some other scout bee comes, it starts its own dance and also allures its "caste" and so on, every next scout bee in the same way. Lindauer (1955) states that the number of potential places for a swarm nesting can range from 13 to 34.

It can happen that there is a scout bee that has not gone to its scouting yet, thus she receives the same information and goes toward that place.

When she comes back, she repeats the same information, that way making a caste for the same place. When this swarm goes out, every caste has its own force (meaning the

physics term force in virtual sense – editor's note) and it goes its own way.

The resultant of these forces represents the direction of the swarm moving and it changes by each part of the second depending on the jerk, i.e. the change in direction and current strength of individual forces, sometimes disturbing the compactness of the swarm that way. That is why the swarm makes inarticulate movements when moving.

After all the bees from the swarm fill their honey sacs before going out of the swarm, this kind of moving can last only until the energy (honey) is spent. It can happen (according to Seeley) that the swarm temporary stops on a branch and waits for the final decision. But I would not say that it is because of some kind of consensus.

Maybe it rather depends on the queen because she does not have flying predispositions as a bee and cannot endure this kind of jerks and that kind of moving because she is heavy, only few days ago she laid eggs.

In case they do not come to the place chosen by the scout bees attracting the biggest number of bees, and the bees spend the majority of their energy, they won't have the strength to go on and the swarm stays where it is at the moment.

Sometimes the swarm divides in two. It is probably the case when two strong forces start to act in different directions.

If the Seeley's claim that the "better" place is chosen is true, and that there is some kind of consensus happening, we would not be the witnesses of some swarms inhabiting very bad places and with a little bit "better", more ideal space nearby, more appropriate for their habitat.

It happens that the swarm is situated on a branch near the apiary. The assumption is that it is the consequence of the queen's inability to fly, regardless the strong force directing to the destination.

Considering the foraging activity, there is also a valid justification that the nature itself is arranged in a way that in the majority of cases it has excluded the monoflora and that the diversity still prevails.

This kind of bee urge for diverse food is utterly natural, because it has been noticed that monotonous food can be harmful.

Silent robbery and horizontal transmission of Varroa

In a long nectarless period and with the increase in the number of scout bees, the “horizontal transmission of Varroa” (among various colonies) is accelerated, which becomes very dangerous for already exhausted colonies. The relation between the secreting of nectar and “horizontal transmission of Varroa” is spotted and has a logical explanation in the increase in the number of scout bees and can tell us that the least infection will happen when the flow conditions during the year are favourable, which tells us that the “horizontal” transmission has far more effects than the “vertical” one (transmission to the descendants).

Let’s look at the data from M. N. Kosarev. He observed the behaviour and Varroa infection in Burzan woods of Bashkiria and compared the infection in natural and tamed colonies in various periods of a year. He says: “In the beginning of the season, the level of infection in modern apiaries and in the woods with colonies in tree holes was similar (4-6%), in the beginning of June 1-3%, and in August increased to 8-20% averagely. Since the appliance of medication in colonies in natural habitats is hard to perform, these colonies are left without any treatment. In five years, from 1982 to 1986, only the samples of bees were taken so that the percentage of infection with Varroa could be determined”.

It is interesting that in June the level of infection decreases, which can tell us that the “vertical” transmission has not played a significant role.

On contrary, the level of infection is decreased, although there was a “horizontal” transmission at that time, as well, but much lesser.

Carefully observing, I noticed that, in a nectarless period, bees that participate in a silent robbery go freely in and out from one hive to another. A good deal of them certainly wears Varroa mites on themselves.

I have also noticed that the guards are hostile toward certain bees entering the hive, but still let them go in. It is inevitable that in bees, the same as in people, there is solidarity. Thus I have made



A black scout bee is waiting to come into the hive, while the guard is trying to send away a predecessor of hers



Without any fuss, the guard recognizes the scout bee and tries to send her away

the difference between the robbery and the silent robbery. Silent robbery can be present all the time. When foraging conditions are favourable, it is relative whether there is a silent robbery or not, but it is certain that the silent robbery is possible at that time, as well. I assume that the big number of scout castes, considering the foraging activity, lead toward more frequent horizontal transmission, especially based on the Lindauer’s data on the number of places scout bees find for the swarm settling.

It is realistically to say: when the foraging conditions become unstable, it is possible to have the horizontal transmission from about 30 places in the apiary and the surrounding. The number of

places, as well as the frequency itself, depends on the concentration of the colonies per area unit.

If the number of bees in a caste is small, than the occurrence is called “the silent robbery”, and in case a bigger number of bees respond to the “call” of scout bees, robbery, resistance and fight occur. It seems as if the nature says: “Moderateness – yes, voracity – no”.

Narrowed entrance and alighting-board

Many beekeepers keep the entrances narrowed during the year explaining that there is no robbery or the damage from it is lesser when they are not around. Is it necessary to have a large entrance and of which kind of shape it should be? Is there any damage when the entrance is lesser?

Has anyone ever dared make this kind of experiments, when any reasoning is excluded when you see the “struggle” and crowd of bees at the entrance? After all, it would be expensive to try something illogical with a bigger number of colonies.

Is there any harm from having a crowd at the entrance what so ever? Maybe it is vice versa, maybe there is some harm from the crowd, but generally speaking, it is probably symbolical! Maybe the crop will be somewhat lesser, but something else would not be there, either. Varroa mites!

When will bees, coming back from a forage, be more likely to recognize their colony? When there is a cloud of pheromone in front of the hive (due to the crowd) or when it is not there? Will they be more likely to get lost if they by mistake come across some other’s cloud of pheromone? Will they miss at all? Since the “robber bee” is accused of bringing Varroa mites in, does it mean that there will be lesser Varroa mites brought in, as well?

The same question stands for the drifting drones, although, according to the experiments of Livenec (1949) who observed the drone drifting, there are data that the drifting is very rare. True, the Carniolan race was the only one he did not include in the experiment, but the data are as follows: in Italian drones, drone drifting to other

colonies was 1.75%, with Bashkirian 1.47%, with Caucasian 0.85%. Analogue data have been provided also by Gubin and Halifman.

Many people believe that bees waste their time if their entrance is made more difficult. Nevertheless, maybe it is all relative? If a bee waits at the entrance for 5 seconds to go in, and there is a crowd, is this waste relative considering that the same 5 seconds will be wasted if there is less nectar in one flower than in another, therefore she wastes her efforts to use the flower, not knowing about its poor capacity. Since time is relative, this is relative, as well, but worth considering.

Let us ask the first question about the justification of alighting-board in hives. When the man made the alighting-board, did he think why bees needed the alighting-board in the first place? Do you think that a bee is not able to fly up from the spot? Do you think that she needs some kind of running start (like a plane) or her performances are more similar to a helicopter?

There is really no such a thing in nature. And it does not matter, and have you ever wondered do we really need to have the alighting-board? Do you think that, when a tired bee alights to the alighting-board, she will have a better rest in horizontal position? Or you think something else? That she has a lot of trouble with vertical position? Does she put “much more” efforts if she rests in vertical position (on a tree bark where there is not an alighting-board or on the external wall of the hive)?

When a “tired” bee comes back from a forage, she falls in front of the hive or on the alighting-board and rests for a while or immediately starts to crawl through the entrance, climbing up the internal wall of the hive, up to the top bar, and, not sooner than then, goes down to the frame. Those that do not stop and fall in front, directly fly in through the entrance. In this crawling bees do waste some time, but is this time longer than the time when after the rest they directly fly in through the circle entrance, and is it easier at all for them to take a short flight or to take a longer crawl? Who haven’t noticed this, please measure, but I assume that there won’t be any significant difference in time if there should be any?

But let’s ask ourselves whether sometimes this “waste of time” can be useful? Taranov says:

“When there is a lot of stored nectar in the nest, the receiving of nectar is slowing down, 10-12 receiver bees take it over from the foragers, spending a lot of time on taking the food over. At that time, there is a new type of signal movements of the scout bees on the comb and the informative signal to stop the foraging, although there is nectar in the flowers”. Meaning, the number of nectar receivers is increased first.

Does this increasing continue till the moment when all the bees take over the job of receivers, and there are no more of them available, and the scout bees keep looking for them? But, what if Taranov was wrong about this? What if he has drawn a wrong conclusion that bees by their dance request the stopping of the forage?

Maybe it was not the signal about stopping the collecting of nectar, but the signal that more receivers are needed. In one moment, there will be no more bees because they have all been engaged in receiving. But, the signal still continues, until, in one moment, the forage stops because receiving is very slow.

That way maybe the struggle and crowd at the entrance are not harmful due to the uniformity in the nectar delivery, because there is no use if the entrance is large – there will be no one to take over the brought nectar.

With a small entrance on a vertical plane (either circle or some other shape) the defence system is much better. There is a “bee web” and the access of intruders (scouts) is much more difficult there than at the lower entrance. When demonstrators are pushing the police cordon, they will push it away depending on the thickness, but also the width of the cordon. The same number of demonstrators will have much more difficulties to push away the same number of policemen if the street is narrower, because with narrowing of the street, the thickness of the cordon is becoming bigger and the resistance is becoming bigger, as well. We do not compare the man with the bee here, but we compare the resistances.

Findings of Ivan Brndušić

Ivan's several-years findings in nature and claims about the advantages of using the circle entrance are very interesting. Both Seeley and

Brndušić have noticed similar sizes of the openings that maybe currently exist the most in the nature. Seeley states that bees like the entrance placed on the southern side and opening that is smaller than 7.5 cm², which is about 3.08 cm in diameter, while in Brndušić it is 3.5 cm.



Brndušić states that bees propolize the circle opening reducing it themselves as needed, and we have already found enough reasons why they need to do that. The colonies he monitors in natural habitat resist Varroa. His hives have circle openings and his colonies have been surviving for many years without using any chemicals. It happens that in time some of them die, but he also says that when he once treats with chemicals, according to him, the colonies lose their resistance and die. The intensity of chemical effects on the nervous system of bees is very hard to determine. Do the preparations affect the ability of recognizing and for how long do these effects last? Taranov says: “Guard bees have the ability to discriminate foragers from scout bees”.

With people, various forms of chemical medicines have various effects on the nervous system. When a man takes heavy drugs such as heroin, he loses the ability of recognizing his surrounding and people around him.

We still do not know whether fluvalinat, kumafos, amitraz and other chemicals are heavy drugs for our bees, but there are some findings that queens suffer damages with bigger doses of chemicals.

In his monograph “Honey Bee Ecology”, Thomas D. Seeley states seven elements bees take into consideration when choosing a new habitat. First of all, it is the volume of the cavity, and secondly, the size of the opening.

What can also be favourable for Varroa mites in colonies in nature are the possibilities for finding appropriate habitat in nature, increasing the possibility of “horizontal” transmission if the choice is lesser, as well as the presence of weak colonies and concentration of colonies per area unit.

In his apiaries, Ivan Brndušić keeps the entrances open, applying the system of vertical defence and his colonies resist Varroa, but does it work for him if the nectar flow conditions become unstable and others surrounding him leave possibilities for more frequent horizontal transmission (common entrances), that his scout bees are going to use with his foragers without any sentimentality?

Is it sufficient that only one colony in his apiary has an inadequate opening (regardless that all the others have a narrowed one) to have all start going down headfirst, and favourable for Varroa? It’s because horizontal transmission is probably reciprocal, i.e. it is possible to bring Varroa mites in, but it is also possible to take them out.

Ivan Brndušić adds to all this: “If you chose circle entrances, you need to do this on all of the hives, because those colonies that remain with the entrance on the bottom board can be damaged and die from robbery.

In case the concentration of colonies per area unit is bigger, the frequency and speed of Varroa transmission becomes bigger and bigger, which gives an explanation why big apiaries can suddenly be destroyed. And another interesting thing for the end. The massive spreading of Var-

roa happened just at the moment when modern hives with their construction started to occupy apiaries worldwide. Has this speeded up the horizontal transmission, since beekeepers have been enabled to change the century-old system of defence, to control the narrowing and expanding of the entrance, interfering their own will into the job of bees? Is it possible that Varroa had existed before, as well, and no one had even cared because it was hardly noticeable in a small controlled number?

A big question remains, whether Varroa will go back to the controlled number if we would all have maximally narrowed entrances, if we apply “vertical” system of defence and “bee web”, just as it had been for centuries. True, for centuries, bee habitats had been hidden in the woods, and not on meadows exposed to the sunshine.

Lots of things point that silent robbery kills “silently”. With beekeeping conditions we have today, without the presence of big food supplies, with today’s system of defence, big concentration of colonies per area unit, conditions for this kind of occurrences are realistic. Judge for yourself, but before you judge, go into the nature, i.e. what is left of it. Observe natural habitats, entrances, systems of defence, and many other things. First of all, you will notice that the alighting-board and standard entrance has been invented by someone, because, in nature, you will not find any.



Milan Jovanović

37A/16 Radoja Krstića St, 37240 Trstenik

Republic of Serbia

+381 37 713 335, +381 629611513

3/114 May Street, Adelaide, South Australia

+61 451 549 929 (viber)

www.apiarium.rs

apiaryum@outlook.com



INFLUENCE OF PROBIOTIC FEED SUPPLEMENT ON THE GUT MICROBIOTA OF ADULT HONEYBEES (*APIS MELLIFERA* L.)

Abstract

Intestinal microbiota of honeybees can provide novel insights into the pathogenesis related factors involved in pathogen infection. Hence, we investigated the comparison of intestinal microbiota communities in control and *Nosema* spp. infected groups of honeybee colonies through high-throughput sequencing of the 16S rRNA. As a novel approach in therapy, we hypothesize that EM® probiotic for bees could potentially have an important therapeutic and immunomodulatory effect on honeybee colonies. The aim of our study was to evaluate its impact on gut microbiota composition of honeybees. The major genera were

detected, with *Lactobacillus* being the most abundant genus, followed by *Gilliamella*, *Snodgrassella* and *Bifidobacterium*. Inoculation with *Nosema* spp. spores made the relative proportions of *Bifidobacterium* lower, which was ameliorated by the EM® for bees application. In addition, EM® for bees applied treatments suppressed the increase in the number of *Nosema* spp. spores. This result indicates that continuous EM® for bees treatments shall clearly change bees' gut microbiome composition and mitigate the influence of *Nosema* spp. infection. *Snodgrassella alvi* was a major member of the honeybee gut microbiota and may be significantly increased by long-term EM® for bees treatment.

According to those re-sults, it would be possible with EM® for bees treatments protect honeybees from herbicide glyphosate negative effects in agricultural fields, by improving microbiome and immune func-tions.

Keywords: honeybee colony; *Apis mellifera* L.; bacteria; probiotics; EM® for bees, gut micro-biota, *Nosema* spp.; colony strength

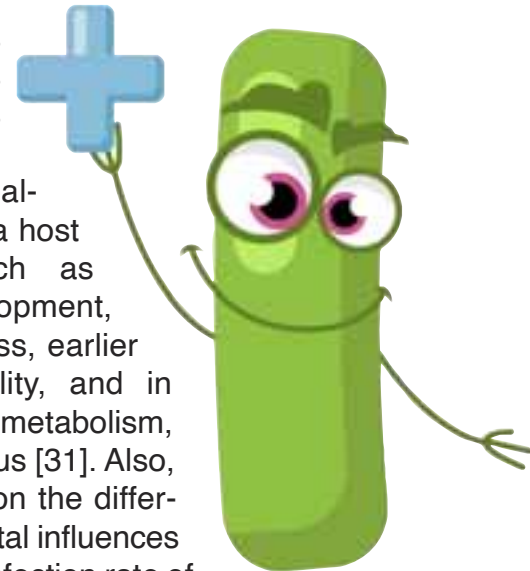
1. Introduction

Honeybee colonies (*Apis mellifera* L.) repre-sent the most important social insects. They have a crucial role in plant pollination [1] and are closely linked to global food production and natu-ral biodiversity balance maintaining. Therefore, their economic, ecological, and social values are enormous [2-3]. Due to their specific nutrition physiology and flight behavior patterns, they show complex interactions with environmental eco-systems and consequently with a diverse range of microorganisms [4]. In recent years, large-scale colony declines have been reported. These losses have been associated with unfavor-able pedo-climatic and forage frames, parasites and pathogens multiple infec-tions, pesticide in-toxications, and nutritional stress of different ori-gins [5-9]. All these factors are often combined by the beekeeper’s management practices [10-12]. Conse-quently, they appear in a meaningful and synergistic package approach, possibly causing different disturbances of adult honeybee gut microbiota composition.

The core gut microbiota of adult honeybees consists of complex bacteria communities with high genomic diversity, whose functionality is dic-tated by the host, environment, and social ex-changes, as well as microbial interactions among themselves [4, 13-14]. This microbiota is located in various parts of the gastrointestinal tract, in-cluding the crop (just a few microorganisms orig-inated from the environment, e.g. *Apilacobacillus kunkei*), midgut (mostly no stable microbiota), ileum (diverse microbial clusters; 108 bacterial cells, e.g. *Snodgrasella alvi*, *Gillimella apicola*, *Lactobacillus Firm-4* and *Firm-5*, *Bifidobacterium* spp., *Frischella perrara*, *Bartonella apis*, *Parasac-charibacter apium*, *Bombella apis*, *Apibacter ad-ventoris*, *Apibacter mensalis*) and rectum (109 bacterial cells, e.g. *Lactobacillus Firm-4*, *Lacto-*

bacillus Firm – 5, *Bifidobacterium* spp.) [14-18]. Bacterial symbionts or “good bacteria” in the honeybee gut have important functions for host nutrition, food digestion, metab-olism, devel-opment, weight gain, reproduction, behavioral physiology, and immunity through pathogen and insecticide resistance [19-25]. It is known that gut microorganisms significantly contribute to pollen digestion, which is the main source of proteins in honeybee nutrition. Therefore, microbiome com-position affects survival rates [26]. Also, gut microflora has a crucial role in vitamin, fatty acids, and amino acid synthesis [27], in the devel-opment and maturation or renewal of enterocytes [28], in the increasing anti-microbial peptide gene expression, pheromone production [29], and in forming of biofilm on gut epithelium as a mech-anical barrier against diseases causative agents [30].

Dysbiosis in honeybees is often defined as gut-intestinal microbial imbal-ance linked to a host deficiency such as deficient development, lower body mass, earlier workers mortality, and in general health, metabolism, and fitness status [31]. Also, in such situ-ation the differ-ent environmental influences (e.g., the high infection rate of *Nosema ceranae* spores or immune response suppression caused by oxidative stress) could change the composition of gut bacterial phylo-types and remaining microbiome components (e.g., fungi), leading to the appearance of visible clinical signs of opportunistic diseases and col-onies weakening [32-33]. Infections of honey-bees with the endoparasite *Nosema ceranae* and with simultaneous exposure to pesticides can sig-nificantly contribute to gut dysbiosis [34-35]. Also, previously published results showed that *N. ce-ranae* was included in the removal of *Serratia* spp. from bees’ gut, and a consequently signifi-cant increase in *Snodgrassella* spp. and *Barto-nella* spp. was determined [36]. Furthermore, glyphosate combined with *N. ceranae* spores’ in-



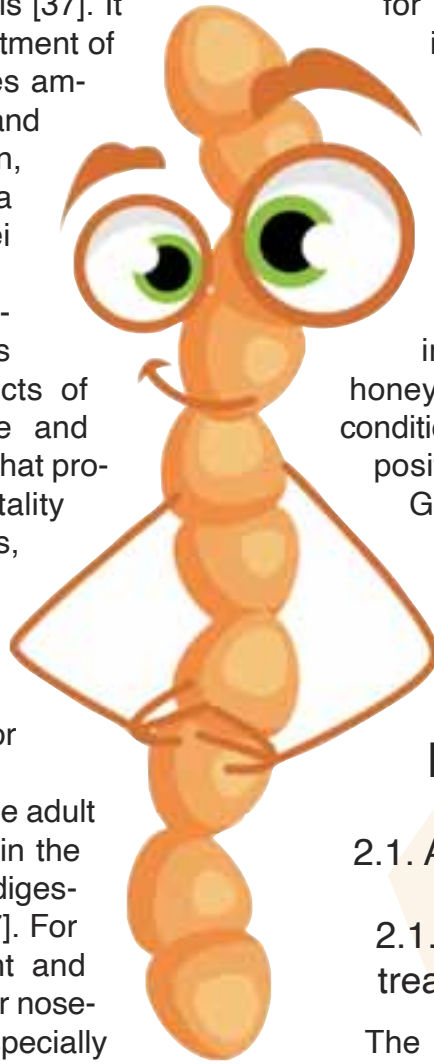
fection changes gut microbiome composition by de-creasing the average proportions of *Snodgrassella alvi* and *Lactobacillus apis* [37]. It is in-teresting that after in vivo treatment of colonies with oxalic acid, it reduces amplicon sequence variant richness, and alters gut microbiome composition, especially in the genus *Bombella* and bacteria *Lactobacillus kunkeei* [38].

Published studies on the impacts of used commercial probiotics and prebiotics on different aspects of honeybee health are incomplete and contradictory. Some studies show that probiotics increase adult bees' mortality and different pathogen loads, whereas others suggest that the application of probiotics has a positive effect on the protection against diseases, apian products gain, and the strength of colonies or wax glands development [39-46].

N. ceranae adversely affects the adult honeybee's health by parasitizing in the mid-gut epithelium and impairing digestion and absorption of nutrients [47]. For efficient beekeeping management and sustainable alternative strategies for nose-mosis type C, control is needed. Especially today because there is not a single registered authorized veterinary medicine product for nose-mosis control. To facilitate full therapeutic success there is a need for the appliance of bio-inspired honeybee colony protection products in form of feed supplements [48-53] and novel technology designs, based on natural in-gredients active against microsporidia pathogens. The other possibility is to trigger honeybees immune defense responses [54] and at the same time avoid the possibility of detection of hazard residual in honey and wax originating from treated hives.

One of the natural and bio-inspired ways to protect the honeybee's health and to produce safe apian products is the use of effective micro-organisms' culture EM® PROBIOTIC FOR BEES (hereinafter, EM® for bees). That commercially available product is a proprietary probiotic formulation owned and managed by the EM Research Organization in Okinawa, Japan. It contains

multiple species of lactic acid bacteria, yeast, and photosynthetic bacteria. After promising results for honeybee nose-mosis combating in the apiary and laboratory-con-trolled conditions [54], we hypothesized that EM® for bees could have an important effect on gut microbiome content. The aim of this study was to evaluate the therapeutic effects of EM® for bees on *Nosema* spp. infection levels and re-lation to honeybee colony strength, in apiary conditions. Also, the gut microbiota composition was obtained using Next Generation Sequencing (NGS) analyses, to check dif-ferences between experimental and control honeybee colony groups.



2. Materials and Methods

2.1. Apiary conditions

2.1.1. Field test design, feed treatments and bees sampling

The field experiment was conducted for 42 consecutive days (beginning on the 7th of July 2020) at the apiary situated in the continental part of Croatia (45°56'54.71" N, 16°37'46.06" E), after the main honey harvesting season. In order to perform the field test, approximately 12 homogeneous honeybee colonies (*A. mellifera carnica*, Pollmann, 1879) were accommodated in standard Langstroth Root (LR) hives acquired from the same apiary. Experimental groups were composed of colonies: TH1 - colonies were naturally infected with *Nosema* spp. spores; TH2 - colonies were treated with EM® for bees; TH3 - colonies were additionally infected with *Nosema* spp. spores before the start of treatment with EM® for bees; and TH4 – control, non-infected colonies without treatment.

Experimental honeybee colonies were additionally fed with a total of 300 mL sugar syrup (1:1 water-sugar; Virosecer, Croatia) supplemented with 5 % EM® for bees solution, consecutively for

10 days from initial sampling (start of experiment; sampling conducted prior to the first application session). Supplemented sugar syrup (TH2, TH3), as well as pure sugar syrup (TH1), were administered to honeybee colonies by spraying directly on frames (15 mL of diluted supplemented solution per frame) covered with adult honey-bees. The pertaining control honeybee colony (TH4) received only 300 mL of pure sugar syrup prepared and provided in the described way. The dose of EM® for bees was adapted according to the manufacturer's instructions. During the clinical inspection of honeybee colonies, approximately 60 forager adult bees per colony were collected from the hive entrance for microscope examination on the presence and quantification of *Nosema* spp. spores. Adult honeybee samples were collected into clean plastic receptacles by catching bees directly in front of hives entrances, using long tweezers. Each sample consisted of approximately 60 specimens (foragers) taken on the 7th of July, 17th of July, 27th of July and 20th of August 2020.

At the beginning of the study, none of the colonies showed clinical signs of brood diseases. Also, the last treatment against the mite *Varroa destructor* was carried out on 25th of June 2020 (Apitraz®, a.m. amitraz) to avoid the negative effects of mite parasitism on honeybee colony health. To the best of our knowledge, no insecticides were used in the surrounding area during the experiment.

2.1.2. Sampling of adult honeybee's guts

The full length of intestine of each individual adult honeybee ($n = 10$ specimens per pooled sample) was pulled out after a short exposure to a low temperature (10 min at 4 °C). For extraction purposes, a larger pair of forceps was used to hold the head and the thorax of each specimen and a smaller pair of forceps to hold the top of the last abdominal segment where the intestines were carefully pulled out. Immediately after, the esophagus and honey sac as well as midgut were removed by cutting them off. For examination of gut microbiome content, gut samples (ileum and rectum) were fixed in the Eppendorf tubes and cooled directly in a box full of ice during

transportation to the laboratory, where they were stored at -80 °C until further molecular analysis.

2.1.2. Clinical inspections of honeybee colonies and strength estimation

Clinical signs of diseases, the presence of the queen and the mortality of the honeybees were checked during each inspection of the honeybee colonies at the experimental apiary. The Liebefeld method for visual determination of the number of adult bees and brood amount was performed to estimate the strength of honeybee colonies [55]. The assessment of honeybee colony strength was done on the 1st (7th of July) and 42th (20th of August) day of the experiment. The estimation was done during the morning hours (around 10:00 a.m.), before the first massive forage flights of workers.

2.2. Laboratory examinations

2.2.1. Presence determination and quantification of *Nosema* spp. spore levels

Honeybees were counted in each sample. Their abdomens were separated, thoroughly crushed, and homogenized in a plastic container loaded with 1 ml of pure water per bee specimen. *Nosema* spp. spores were counted in each sample using a Malassez hemocytometer. The infection levels were calculated according to the World Organization for Animal Health (WOAH) guidelines, (former Office International des Epizooties, OIE) [56]. Each counting procedure was repeated three times. The counting equipment was carefully washed after each sample counting in order to avoid contamination with spores from the previous sample.

2.2.2. Gut microbiota processing and analysis

2.2.2.1. Extraction

All honeybee gut samples were processed by homogenization for 1 min, in sterile plastic tubes with the addition of 0.5 mL 1x TE (10 mM

Tris-HCl, 1 mM EDTA, pH 8). Total DNA was extracted using the DNeasy PowerSoil Kit (12888-50, Qiagen, Hilden, Germany) according to the manufacturer instructions. The DNA extractions were monitored with electrophoresis on a 1 % agarose gel to check purity, and then quantified using a NanoDrop 1000 spectrophotometer (ThermoScientific™, Waltham, MA, USA). The final concentration of the DNA sample was adjusted to 20 ng/μL.

2.2.2.2. Amplicon sequencing by NGS

The V3-V4 regions of Bacterial and Archaeal 16S rRNA were amplified using the Pro341F (CCTACGGGNBGCASCAG) / Pro805R (GACTACNVGGGTATCTAATCC) primers and dual-index method [57-58]. Barcoded amplicons were paired-end sequenced on 2x284-bp cycle using the Illumina MiSeq system with MiSeq Reagent Kit ver 3 (600 Cycle) chemistry. Paired-end sequencing reads were merged using a fastq-join program with default settings [59]. Only joined reads that had a quality value score of ≥ 20 for more than 99 % of the sequence were extracted using FASTX-Toolkit [60]. The chimeric sequences were deleted with usearch6.1 [61-63]. Nonchimeric reads were submitted for 16S rDNA-based taxonomic analysis using the Ribosomal Database Project (RDP) Classifier ver. 2.11 (attributing taxon – Phylum 0.8*1) and the TechnoSuruga Lab Microbial Identification database DB-BA ver 13.0 (TechnoSuruga Laboratory, Ltd., Japan) with homology for ≥ 97 % [64-65].

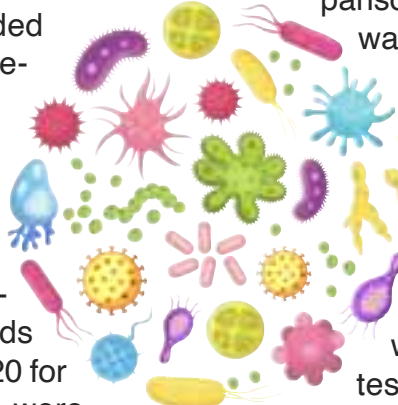
2.2.2.3. Sequence analyses

Secondary analyses were conducted based on the results of the core set of the previously mentioned database. For comparative analysis between samples, the software Megagenome@KIN (World Fusion, Japan) was used. With the purpose of analyzing sequence similarities among different Operational Taxonomic Units (OTUs), multiple sequence alignment was performed by using free available Metagenome Analysis Software (MEGAN) and R package

multi-comp software ver 4.0.5 environment for statistical computing and graphics [66]. For group comparison analyses the Linear discriminant analysis Effect Size (LEfSe) software with the pertaining website was used: <https://huttenhower.sph.harvard.edu/galaxy/>.

2.3. Statistical Analyses of colonies strength and *Nosema* spp. infection levels

Data was analyzed with Stata 13.1. computer program (Stata Corp, USA). The number of *Nosema* spp. spores detected on a certain date was compared between four groups using the Kruskal Wallis non-parametric test. Paired comparison between groups on the same date was done by Dunn test and the results are expressed as z and p values. The number of spores were log normalized and checked for normality of distribution using Shapiro Wilk test. Log transformed data were longitudinally compared between different time points of sampling within the same group using paired t-test. All results are presented as the main values and standard deviations. Statistical significance testing was conducted with a significance level of $\alpha = 0.05$ to define statistical differences (0.95 confidence interval).



3. Results

3.1. Apiary conditions

3.1.1. Estimated strength of honeybee colonies

Differences in the average number of honeybees per group during two estimation dates are shown in Figure 1. The statistically significant differences in honeybee colony strength between the control and experimental groups were determined on day 42 ($p < 0.001$; $F = 19,71$). A higher number of honeybees was estimated in groups treated with EM® for bees (TH2, TH3) compared to control groups (TH1, TH4). The colony strength of the TH1 and TH4 groups was similar.

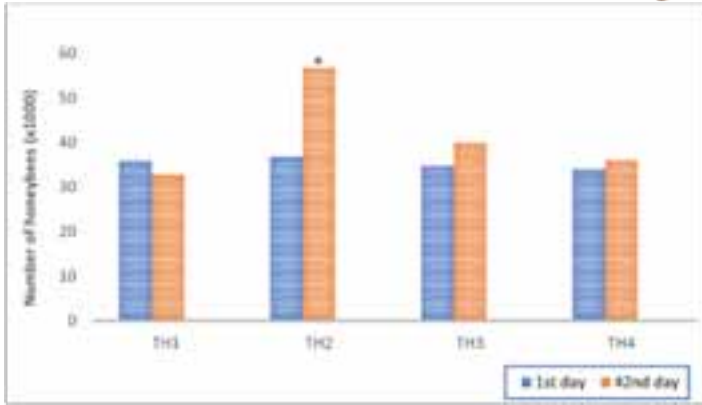


Figure 1. Honeybee colonies' strength variations between control (TH1, TH4) and experimental groups (TH2, TH3) by the estimation days (1st and 42nd day from the initial day of the experiment); the statistically significant difference for TH2 on the second day of estimation vs. TH1, TH3, TH4 second day of estimation; $p < 0.05$

3.2. Laboratory examinations

3.1.2. Determination of Nosema spp. infection levels in adult bee samples

Results of estimation of Nosema spp. infection levels are presented in Table 1. A decline in the number of Nosema spp. spores in adult bee samples collected in the colonies fed with EM® for bees (TH2; TH3) on the second ($p < 0.01$; $p < 0.001$) and third ($p < 0.01$; $p < 0.001$) sampling term through a statistically lower number of spores in comparison to initial sampling before experimental feeding was observed. Only after the last subsequent sampling day (day 44), the increase in the number of spores was estimated for both experimental groups of colonies. Opposite to the presented results, in control groups TH1 and TH4, the continuous increase in

Nosema spp. infection levels were confirmed for each subsequent sampling term, respectively.

Furthermore, in the experimental group TH2, the decreased number of spores compared to the initial spore count was 25.18 % on average on day 10; 96% on average on day 20; and 58.10 % on average on day 44. For group TH3 the reduction in spore counts in comparison with the initial sampling term at average, were as follows: 60.51 % on day 10; and 40.81% on day 20. Observed differences between groups (TH1, TH2, TH3, TH4) are statistically significant in every time point of control (Table 2 a., b., c., d.).

Table 1. The number of Nosema spp. spores per honeybee during supplemental feeding in apiary conditions; mean values \pm SD.

Group	Spores of Nosema spp. (x10 ⁹)			
	7 th July	17 th July	27 th July	20 th August
TH1 mean	2.77	4.26	8.57	15.08
(N=9) SD	1.23	2.67	6.19	3.65
TH2 mean	8.02	2.02	0.77	4.66
(N=9) SD	10.19	2.88	0.42	3.02
TH3 mean	14.31	8.66	5.84	22.91
(N=9) SD	8.71	5.68	4.71	11.88
TH4 mean	15.44	16.42	18.05	59.91
(N=9) SD	12.38	10.62	10.53	65.53
p (Kruskall Wallis)	0.001	0.0005	0.0001	0.0001

¹ sample from each colony was examined in triplicates

Table 2. Statistics of Nosema spp. spores burden differences between the same group for each sampling time point; 7th July (a), 17th July (b), 27th July (c), and 20th August (d)

TH	z	p	TH	z	p	TH	z	p	TH	z	p
1:2	-0.581781	0.2804	1:2	1.354283	0.0878	1:2	3.214710	0.0007	1:2	2.350559	0.0094
1:3	-3.020785	0.0013	1:3	-1.544554	0.0612	1:3	0.705668	0.2402	1:3	-1.387949	0.0826
1:4	-3.199795	0.0007	1:4	-2.585449	0.0049	1:4	-1.680162	0.0465	1:4	-2.350559	0.0094
2:3	-2.439005	0.0074	2:3	-2.898836	0.0019	2:3	-2.509042	0.0061	2:3	-3.738509	0.0001
2:4	-2.618014	0.0044	2:4	-3.939731	0.0000	2:4	-4.894872	0.0000	2:4	-4.701119	0.0000
3:4	-0.179010	0.4290	3:4	-1.040895	0.1490	3:4	-2.385830	0.0085	3:4	-0.962610	0.1679

(a)

(b)

(c)

(d)

3.1.2. The estimation of intestinal microbiota composition, richness and diversity

The number of OTUs and reads at the species level for each sampling date and treatment obtained from the amplicon sequence are shown in Figure 2. The number of OTUs and reads on 27th July increased in TH1, TH2, and TH3. However, the number of reads did not increase in TH4. The lifespan of adult summer honeybees is about 42 days, suggesting that most of the July 27th bees were old and most of the August 20th bees were bees of the next generation.

Figure 2. Comparison of the number of OTUs and reads on each treatment and sampling date. Each column in the graph is the average (n = 3) of the number of OTUs and reads on each treatment and sampling date. Values represent mean ± SEM.

The bacterial composition ratios (family, genus, and species) determined by samples of honeybee intestines for each examined sample

are shown in Figures 3.-5. In all groups of colonies and additional feeding regimes (TH1, TH2, TH3, TH4), the bacteria with the highest compositional ratios were the most common bacteria in the honeybee gut microbiome.

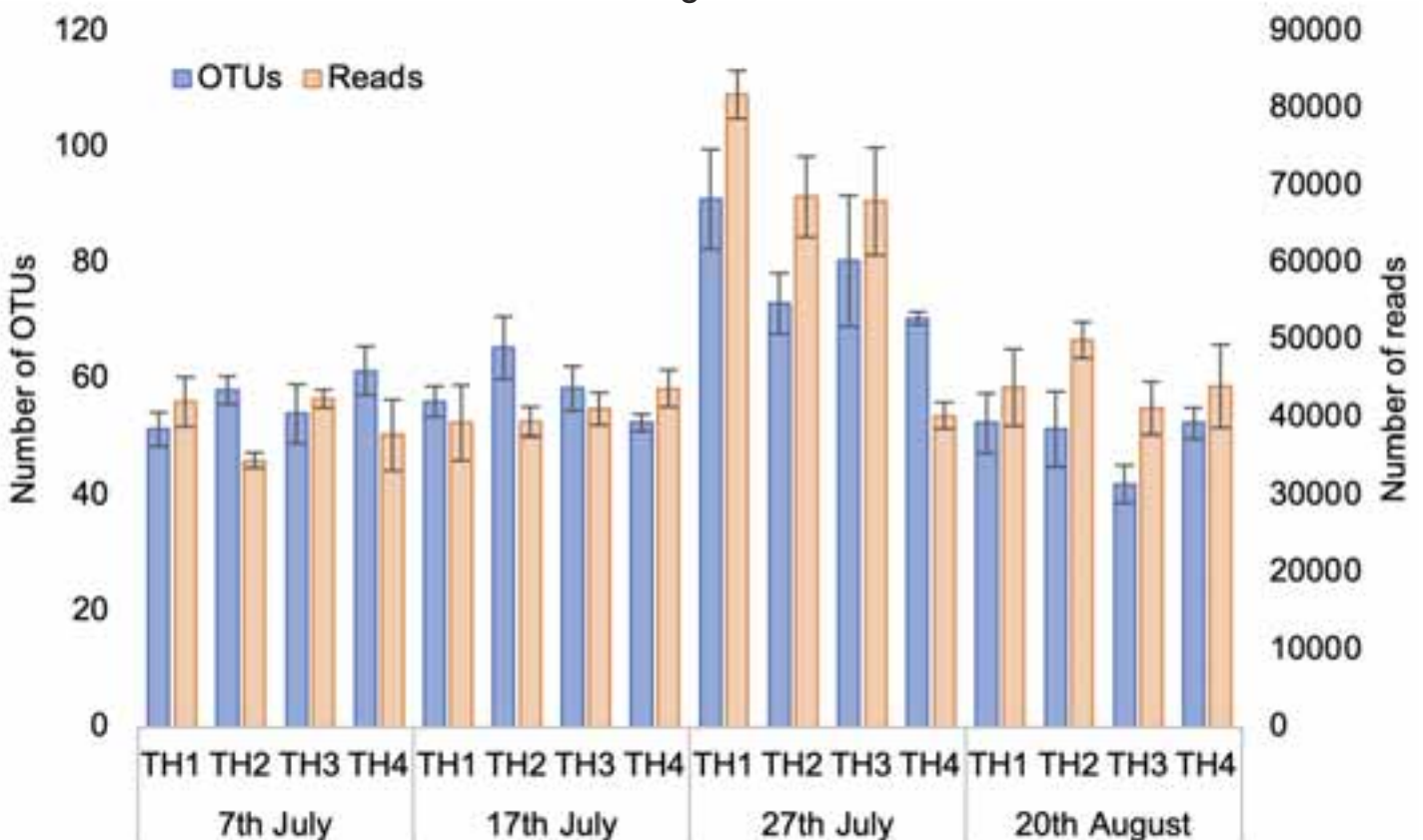
Figure 3. Relative abundance (family) of the gut microbiome in each treatment and on each sampling date.

Figure 4. Relative abundance (genus) of the gut microbiome in each treatment on each sampling date.

Figure 5. Relative abundance (species) of the gut microbiome in each treatment and on each sampling date.

The diversity index (Shanon index) using family-level data for all 48 samples is shown for each sampling date, which was significantly higher for TH3 compared to TH1 and TH4 for the 17th of July data, indicating that TH3 has a higher diversity of gut microbiota. No significant differences were found between treatments on the other sampling dates (Figure 6).

Figure 2



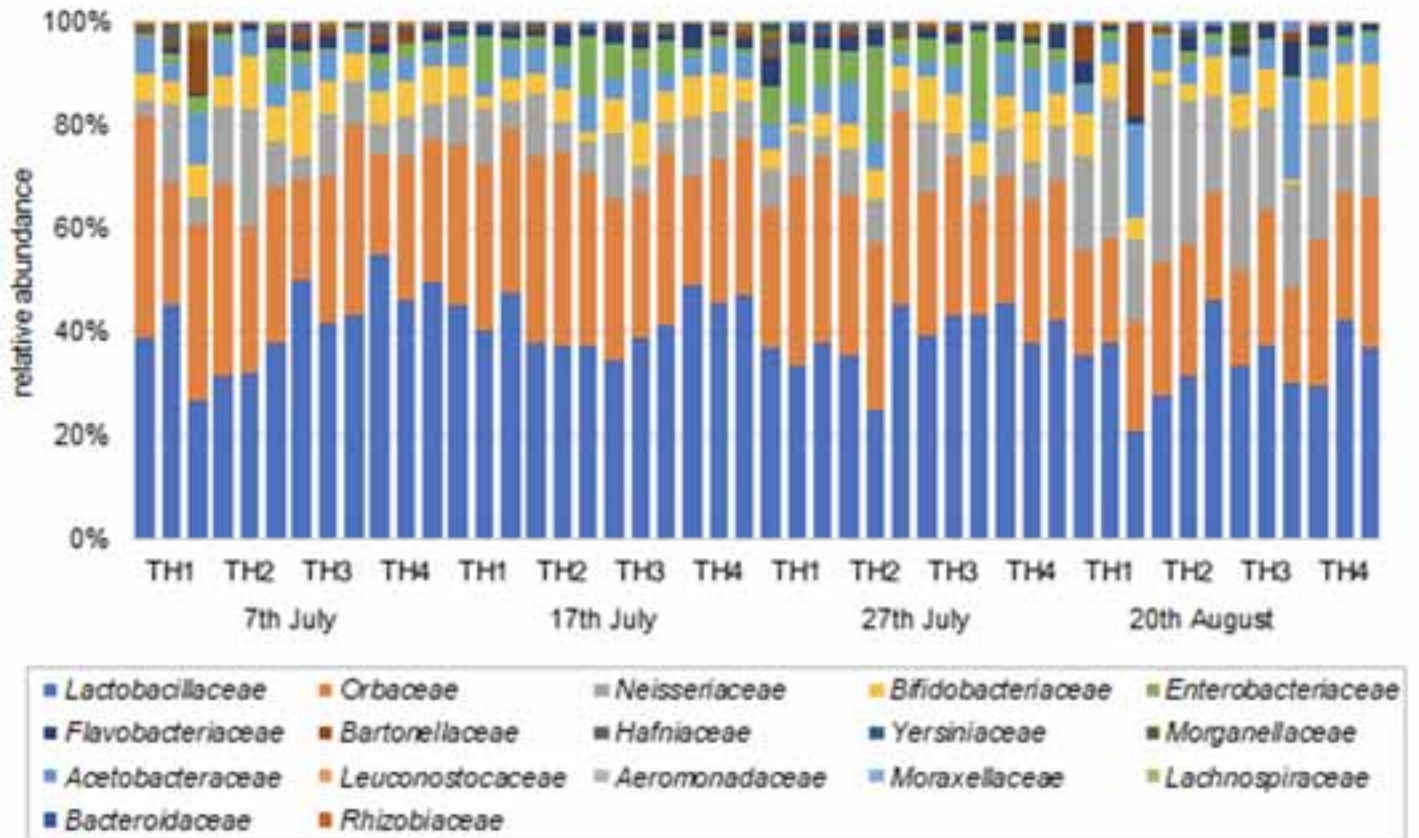


Figure 3

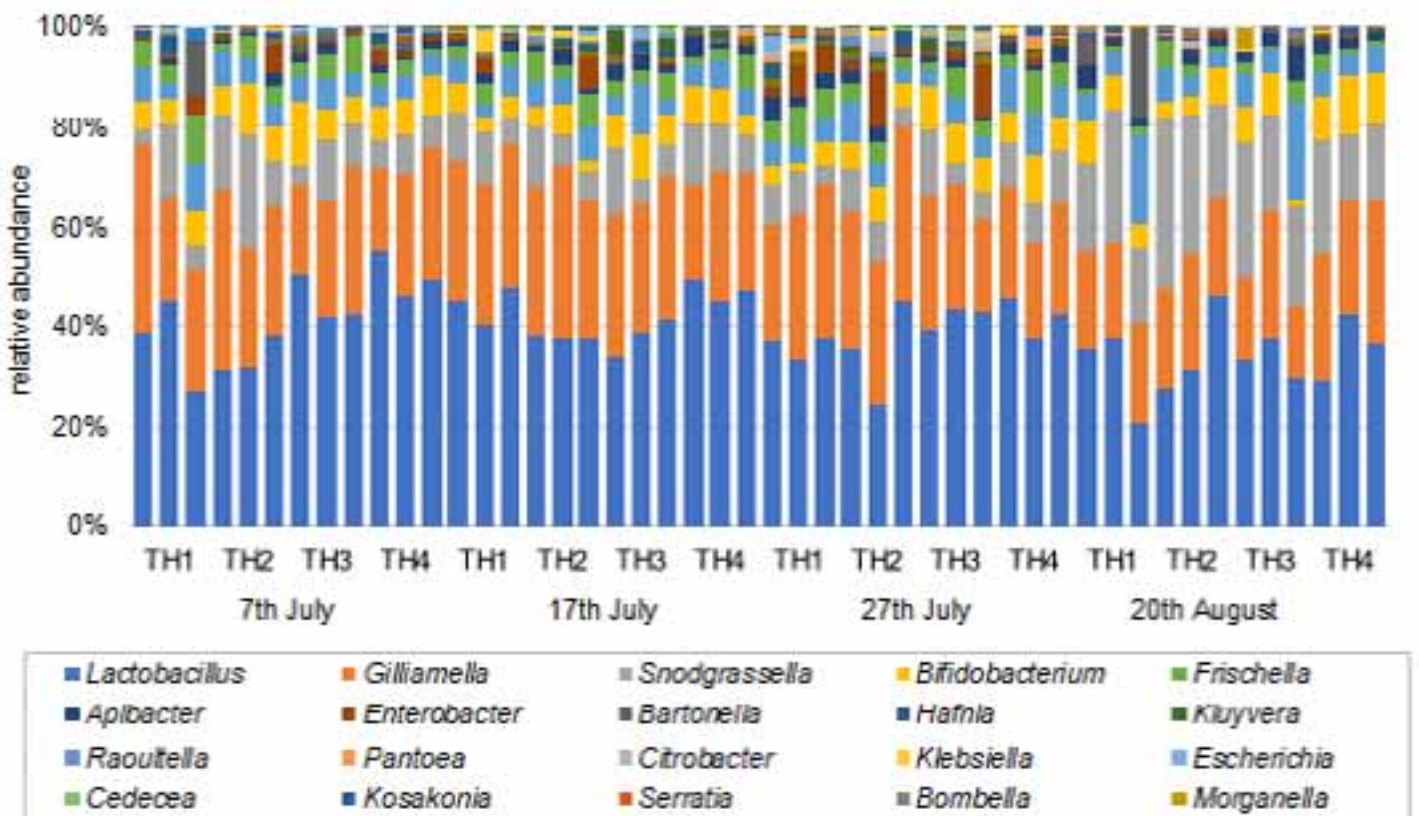


Figure 4

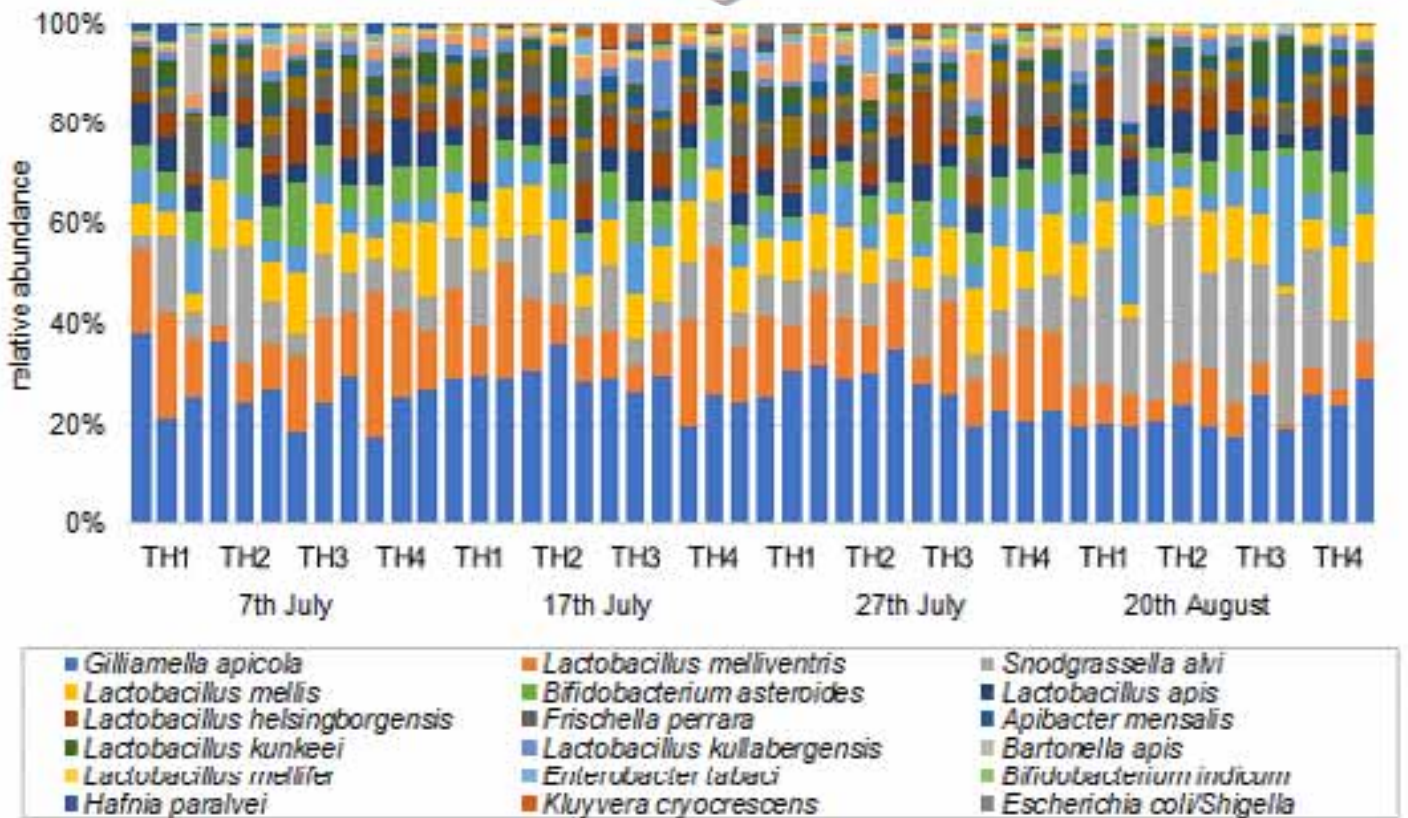


Figure 5

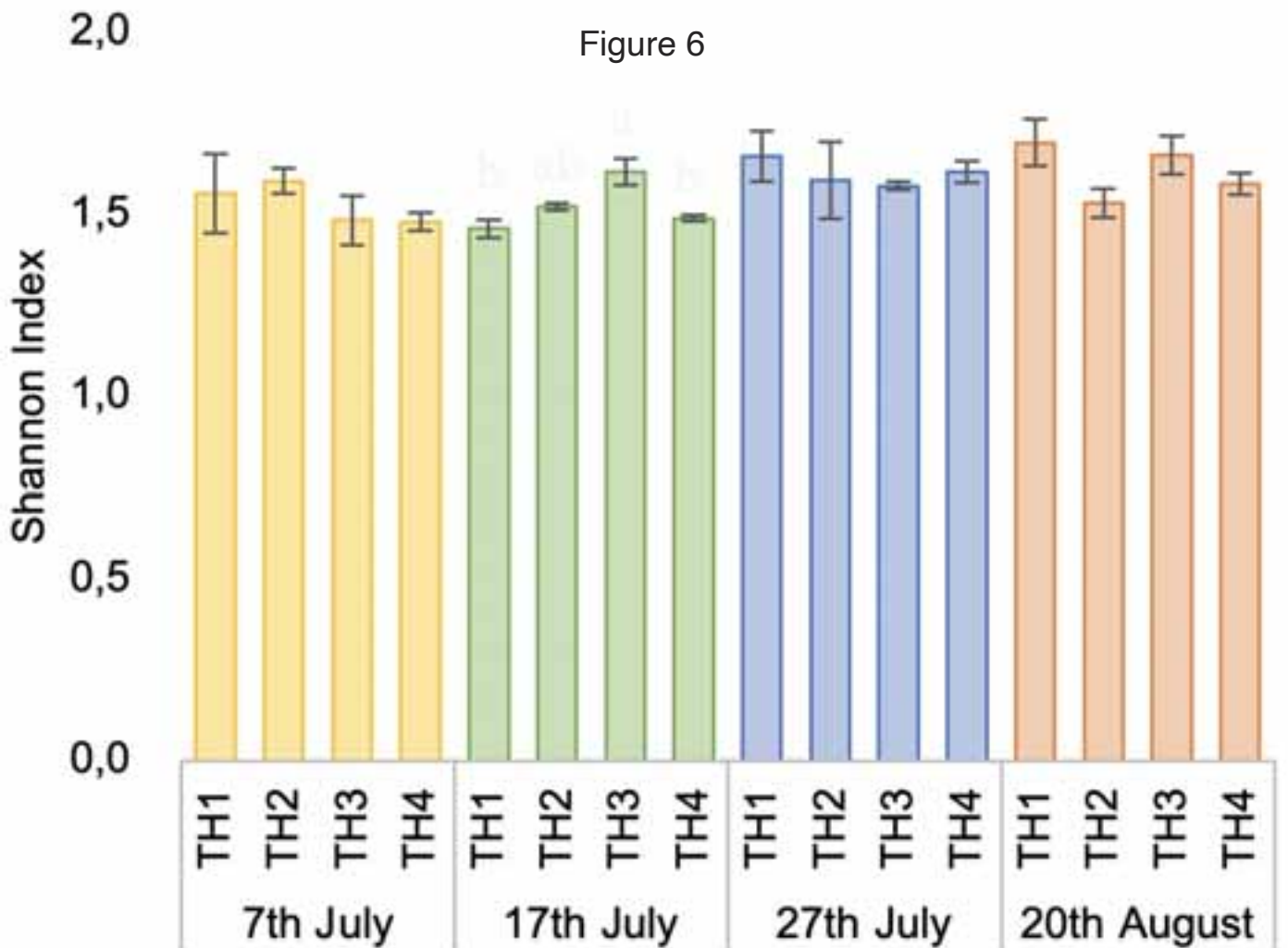


Figure 6

Figure 6. Diversity index (Shannon index) using family data: comparison of treatment intervals and collection dates. There is a significant difference between the days of collection. Values represent mean \pm SEM ($n = 3$). Letters above bars indicate treatments that differ significantly (Tukey HSD test, $\alpha = 0.05$).

The results of a Principal Coordinate Analysis (PCoA) visualizing the similarity of complex data (e.g., plotted figures) are shown in Figure 7.

The labels of each plot mean the number of *Nosema* spp. spores in the gut of bees.

PCoA analysis examined at the species level showed that the plots on the 20th of August samples were away from the other sampling date, further the 27th of July treatments except

for TH4 which was also plotted apart from the other plots.

The samples taken on 7th July and 17th July samples were plotted close together, while TH2 with EM was located slightly further away.

In the samples from 27th July, only TH4 plotted close to the aforementioned samples, while TH2 and TH3 (with EM® for bees treatment) plotted far from them.

This cluster is a relatively low spore count group for *Nosema* spp.

Figure 7. Principal Component Analysis (PCoA) using the species data comparison of treatment intervals and sample collection dates. Labels in each plot represent the number of *Nosema* spp. spores.

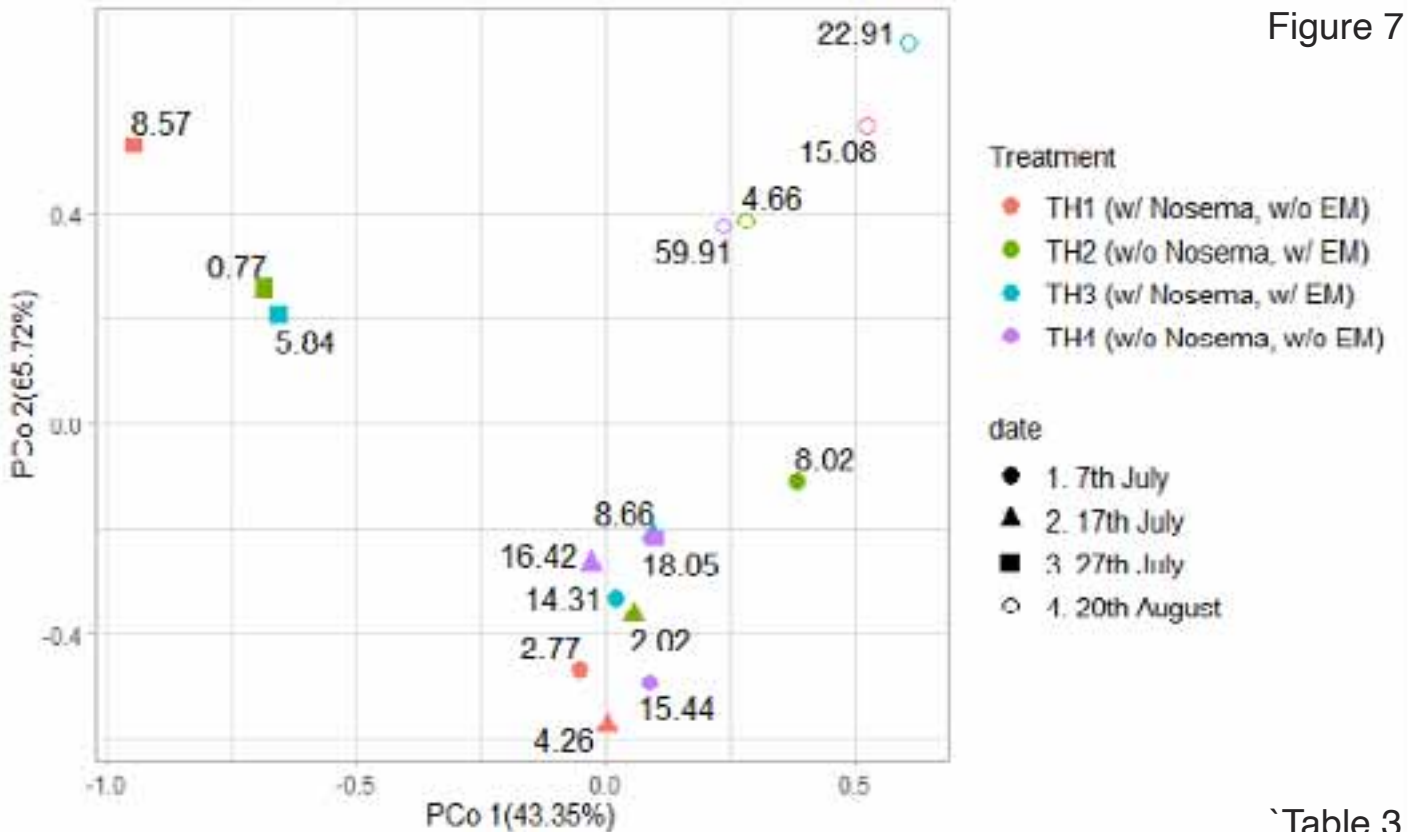


Figure 7

Table 3

GenBank identification	7th July				17th July				27th July				20th August			
	TH1	TH2	TH3	TH4	TH1	TH2	TH3	TH4	TH1	TH2	TH3	TH4	TH1	TH2	TH3	TH4
<i>Gilliamella apicola</i>	27.6	28.7	23.6	22.7	28.3	30.7	27.7	22.9	27.8	30.1	23.3	21.2	19.4	20.9	20.2	25.5
<i>Lactobacillus melliventris</i>	16.4	6.7	14.9	19.3	16.6	10.2	8.0	20.4	12.7	11.1	10.8	14.7	7.0	7.9	4.4	5.7
<i>Snodgrassella alvi</i>	7.5	15.6	8.2	6.8	8.5	7.9	7.9	9.4	6.8	7.0	7.7	9.2	20.1	27.0	23.9	17.1
<i>Lactobacillus mellis</i>	5.1	9.1	10.0	9.5	8.7	8.6	9.7	8.9	8.6	8.0	9.4	10.1	7.5	7.7	7.2	10.1
<i>Bifidobacterium asteroides</i>	5.1	7.0	7.3	6.6	3.7	3.5	6.3	5.7	2.4	4.6	6.8	6.3	6.0	4.3	5.2	9.8
<i>Lactobacillus apis</i>	6.7	5.3	5.5	7.4	4.1	4.4	5.9	4.3	4.0	4.4	5.5	4.2	5.7	7.7	4.0	7.2
<i>Lactobacillus helsingborgensis</i>	3.1	3.5	6.4	5.3	5.7	4.7	6.1	5.5	2.7	4.1	7.6	6.3	4.8	4.8	2.6	5.6
<i>Frischella perrara</i>	6.2	3.2	4.9	2.6	3.5	5.0	3.2	3.4	5.7	3.6	3.5	5.1	1.5	3.2	2.6	1.9
<i>Lactobacillus kimbladii</i>	1.4	3.8	3.3	2.5	3.1	2.4	1.7	2.4	3.4	1.9	3.5	2.7	1.7	2.4	1.9	1.9
<i>Apibacter mensalis</i>	0.5	1.2	1.5	1.3	1.7	1.8	2.5	2.7	3.3	2.0	1.4	2.3	2.4	1.6	4.6	2.4
<i>Lactobacillus kunkrei</i>	2.2	2.7	1.7	2.5	2.4	4.9	0.8	1.6	1.8	2.6	1.4	1.5	1.1	1.4	4.4	1.6
<i>Lactobacillus kullabergensis</i>	1.1	1.5	1.9	2.4	2.5	1.5	4.9	2.7	1.9	2.1	2.5	0.8	1.6	1.7	1.0	1.6

Table 3 shows the percentage of reads of major species to total reads for each treatment and day, with *Gilliamella apicola*, *Lactobacillus meliventris*, *Snodgrassella alvi*, *Lactobacillus melis*, and *Bifidobacterium asteroides*, accounting for a high percentage. The relative proportions of major species tend to vary between treatments and dates.

Table 3. Relative proportions (%) of the major species in the gut microbiome.

The effect of *Nosema* spp. spore inoculation and EM® for bees supplement application on the relative proportions of the major species is represented in Figure 8. *Gilliamella apicola*, which accounted for the highest proportion in all treatments, increased the proportion of the intestinal microbiome by inoculation with *Nosema* spores (TH1). On the other hand, of the treatments that were not inoculated with *Nosema* spores, TH4, where spores increased in the gut after infection with *Nosema*, had the lowest proportion of *G. apicola* until 27th July, while TH2 with EM application had the highest relative proportion of *G. apicola*, moreover kept the lowest number of *Nosema* spores in the gut (a). The relative proportion of *Bifidobacterium asteroides* in TH1 inoculated with *Nosema* spp. spores was lower than in TH4 without *Nosema* inoculation, and the proportion continued to decline until 27th July. Even with inoculation with *Nosema* spores, the relative proportion of *B. asteroides* was not low in TH3 with EM application and remained high until 27th July (b).

Figure 8. Effect of implemented treatments on the relative proportions of major species – (a) *Gilliamella apicola*, (b) *Bifidobacterium asteroides*. Values represent mean ± SEM (n = 3). Statistical analysis by Student’s t-test, * p < 0.05, ** p < 0.01, * p < 0.001**

Relative proportions of *Lactobacillus* species in the gut microbiome are shown in Figure 9. *L. melliventris* had a higher relative proportion in group TH4, which had the highest number of *Nosema* spp. spores in the gut, in comparison with the other treatments, and decreased after 17th July.

TH1 decreased similarly, but in group TH2 with only EM® for bees application tended to increase, albeit at a low proportion (a).

The relative proportion of *L. mellis* was lower in group TH1 with *Nosema* spp. spores inoculation than in group TH4, however significantly higher in TH3 with in-time EM® for bees application.

The proportion changes of *L. mellis* were low (b). The relative proportion of *L. heisingborgensis* was lower in group TH1 than in TH4 but tended to be higher in TH3 fed with EM® for bees.

The TH4 proportion increased until 27th July, and TH2 fed with EM® for bees showed the same trend, albeit at a lower proportion (c).

The relative proportion of *L. apis* was high on 7th July and then decreased (TH1, TH4).

In groups TH3 and TH2 treated with EM® for bees, the proportion changes were low until 27th July (d).

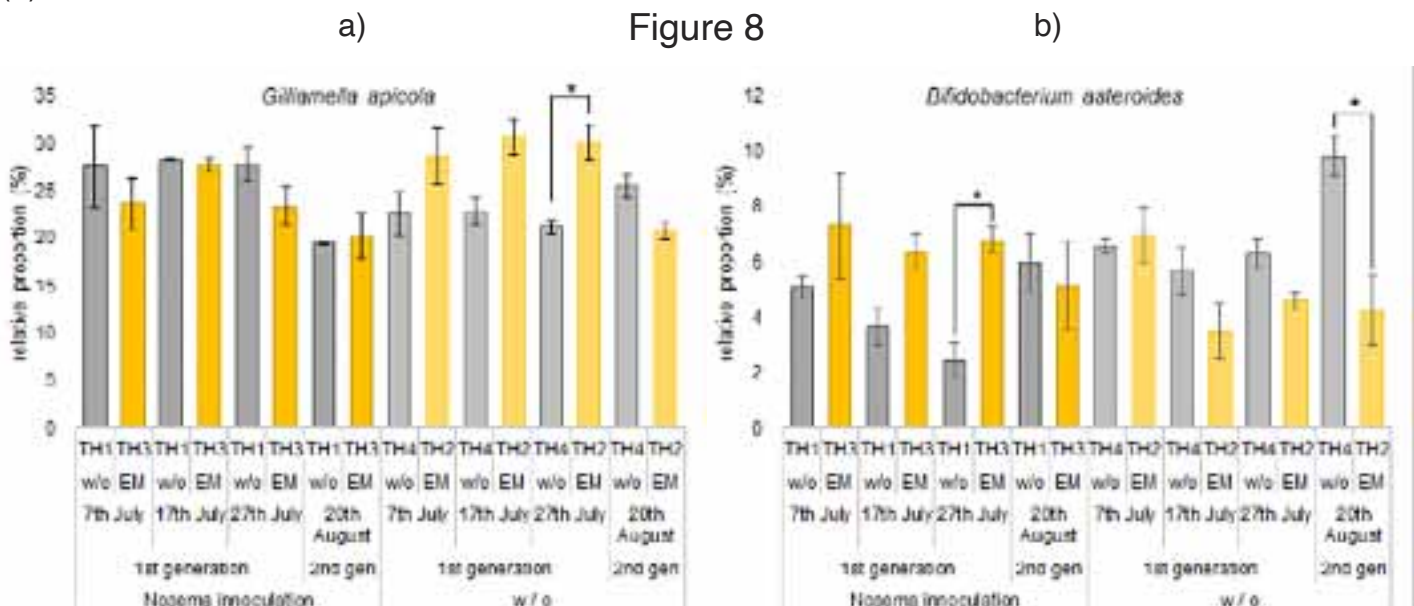


Figure 9. Effect of implemented treatments on the relative proportions of major species – (a) *Lactobacillus melliventris*, (b) *L. mellis*, (c) *L. heisingborgensis*, (d) *L. apis*. Values represent mean \pm SEM ($n = 3$). Statistical analysis by Student's *t*-test, * $p < 0.05$

lated with *Nosema* spp. spores, the proportion tended to be lower in TH3 with EM® for bees (a). *Apibacter mensalis* in the treatments with inoculation *Nosema* spores (TH1, TH3) increased in proportion until 27th July. In contrast, the treatments without inoculation (TH2, TH4) tend to be stable (b).

The relative proportion of *Frischella perrara* tended to be higher in TH1 inoculated with *Nosema* spp. spores than in TH4. The proportion of *F. perrara* increased until 27th July in treatments not inoculated with *Nosema* spores (TH4 and TH2). In the treatments (TH1, TH3) inocu-

Figure 10. The effect of implemented treatments on the relative proportions of major genus – (a) *Frischella perrara*, (b) *Apibacter mensalis*. Values represent mean \pm SEM ($n = 3$)

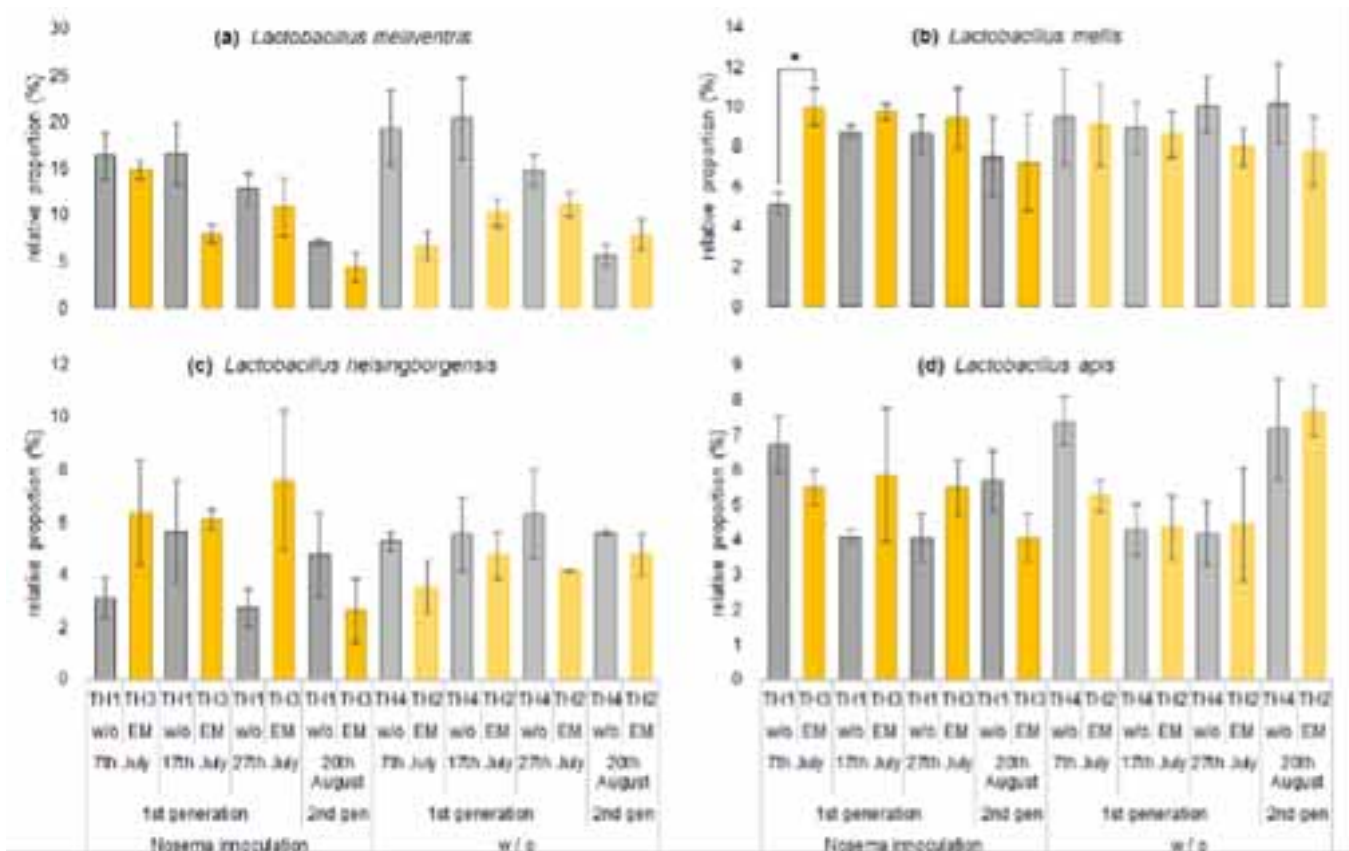


Figure 9

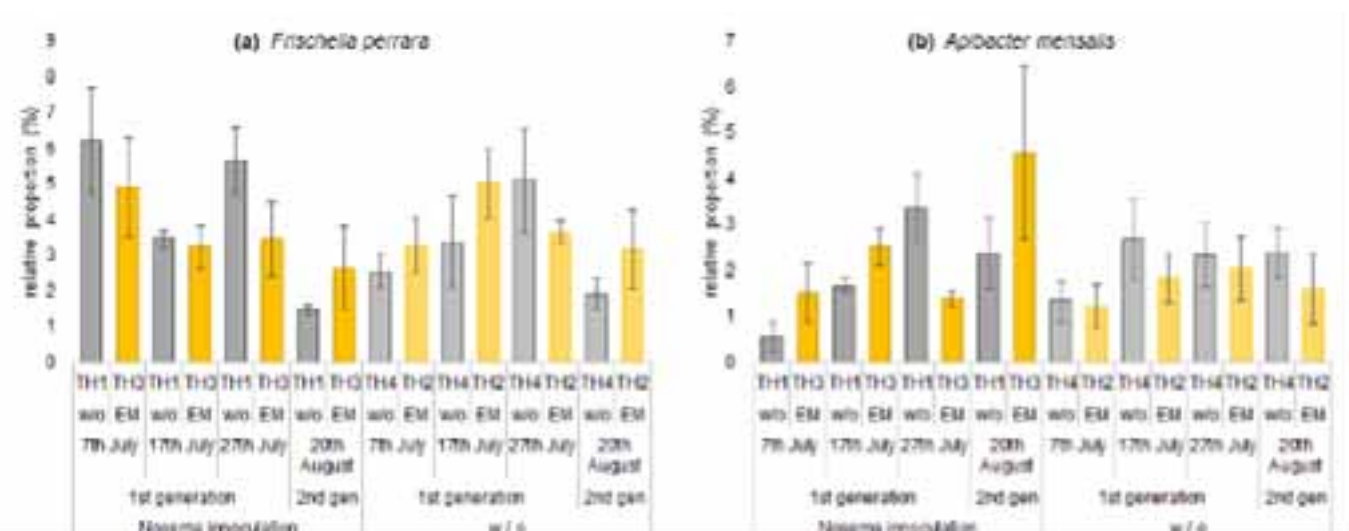
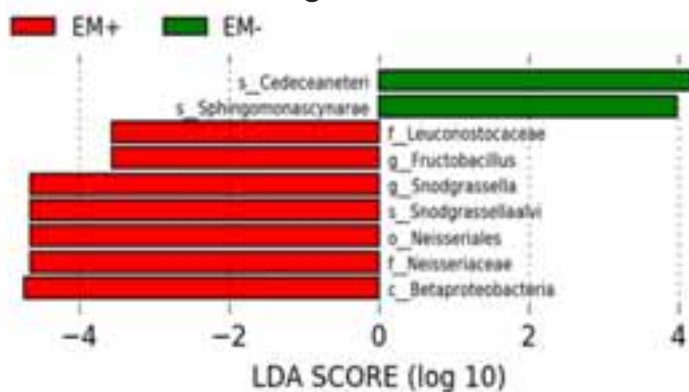


Figure 10

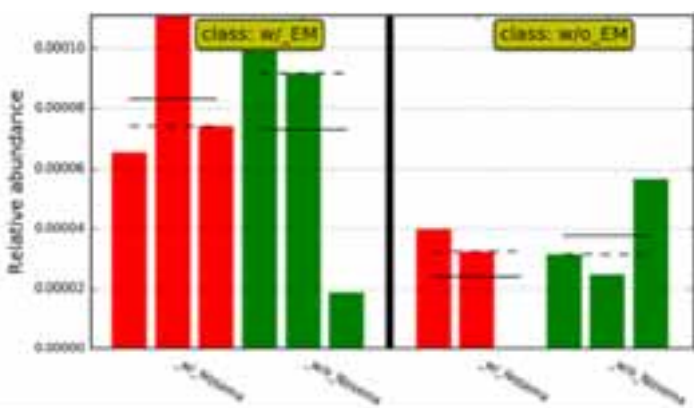
The results of LEfSe are shown in Figures 11 a and 11 b. LEfSe is an analytical method for extracting microbial groups correlated with differences between groups and for searching for microorganisms responsible for certain phenomena, but after analyzing all samples with and without EM® for bees treatment, it did not show any microorganisms characterizing the differences between the experimental and control groups. When LEfSe analysis was performed just on the samples collected on 20th of August, some microbial groups showed significant differences between the groups with and without EM® for bees treatment (TH2 and TH3, TH1 and TH4) (Figure 11).

The relative abundance of characteristic microorganisms that showed significant differences in the EM® for bees treatment are shown in Figure 11 b and c. The relative abundance of *Fructobacillus* and *Snodgrassella alvi* was significantly increased by supplemental treatment, regardless of the group with and without *Nosema* spp. infection.

Figure 11



(a)



(b)

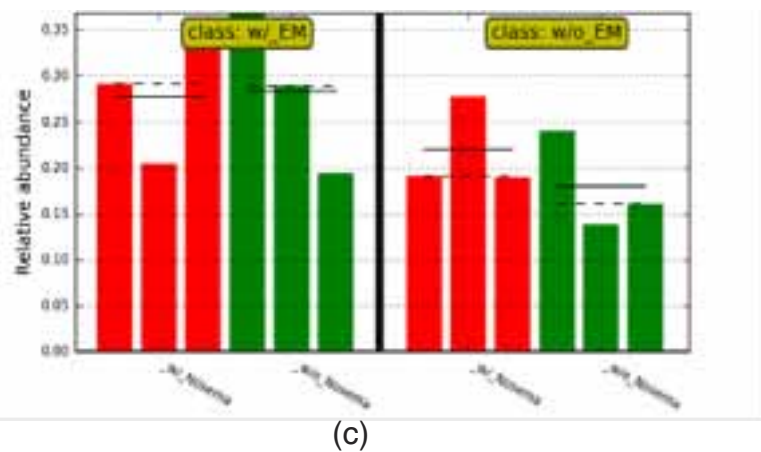


Figure 11. The results of LEfSe (LDA Effect Size) analysis for samples collected on 20th of August. The histogram of the LDA score showed the biomarkers with statistical differences between the groups (with or without EM® for bees treatment) (a). Histogram of the *Fructobacillus* (b) and *Snodgrassella alvi* (c) relative abundance in groups with and without EM® for bee treatments. Subclasses (with and without *Nosema* spp.) are differentially colored and the mean and median relative abundance of the biomarker are indicated with solid and dashed lines, respectively

4. Discussion

The application of EM® for bees has been confirmed by beekeepers as a good management practice in apiaries. Also, encouraged by the results of the previous study regarding EM® for bees impacts on nutritional adult honey bee physiology [54], we considered that is important to proceed with an investigation of the potential of this complex probiotic to change gut microbiota composition in adult bees originating from fed colonies.

The strength of colonies was expectedly different between experimental and control groups of honeybee colonies. In all observation terms, the EM® for bees-supplemented fed colonies were stronger, and the significant difference is most visible on the 42nd day of the experiment. These results were similar to previous scientific records [48-49, 67-70]. The field test was chosen since *Nosema* diseased adult bees fed with a natural protein food (pollen, especially beebread) show higher microbiota diversity and stability in

com-parison with those fed only carbohydrates [71]. In this study, it was again observed that EM® for bees applied as a supplement to sugar syrup decreased microsporidium *Nosema* spp. in the honeybee gut. In detail, in the first three sampling terms number of lower spore counts in fed colonies compared with controls was determined, which is in accordance with the results of Tlak Gajger et al. (2020) [54]. Only after the last sampling term on day 44, the increase in the number of spores was estimated for both experimental groups of colonies, probably due to an implemented novel feeding regime. In pertaining control groups (TH1 and TH4), the continuous increase in *Nosema* spp. infection levels were confirmed for each subsequent sampling term, respectively, which was within expected ranges under field, environmental, and study conditions.

According to previously published results, the *Lactobacillus* and *Bifidobacterium* microorganisms decrease invasion levels of nosemosis in *A. mellifera* [72], and in *A. cerana* [73].

The fed supplementation with *Parasaccharibacter apium* confirmed a good way to improve the resistance of adult bees to *Nosema* invasion [74]. Also, some other apiculture positive impacts like a decrease of Varroosis damage and successful honeybee queens production [75], maintaining of good colony vitality status [43,76] and increased honey production [75,77] were reported. In this study, *Nosema* spp. spore counts were higher in accidentally naturally infected groups of colonies TH4 than in colonies that were initially naturally infected with spores of *Nosema* spp. TH1 (Table 1), but the relative proportion of *Bifidobacteria* was lower in TH1, presumably due to longer *Nosema* spp. invasion.

It was inferred that when with *Nosema* spp. spores highly infected adult bees are young enough, the effect on the gut microbiota is greater and the relative proportion of *Bifidobacterium* is reduced. EM® for bees treatment was shown to have the potential to mitigate the effects of Nosemosis on the gut microbiota content.



BEES
LIFE

But, since high levels of *Nosema* spp. spores are detected also in group TH4, where the relative proportion of *Bifidobacterium* is kept relatively high, it is assumed that *Bifidobacterium* does not directly inhibit the formation of *Nosema* spp. spores.

When the samples were compared in time series, those taken on the 20th of August, the second generation of adult honeybees, showed a different microbiome content trend from the earlier taken samples (Figure 6). Also, when the data of each treatment area for the samples taken on the 20th of August were analyzed, there were significant differences in some microbial groups between the samples from the experimental and control groups (Figure 11). For example, *Snodgrassella alvi*, which has been shown to be a characteristic fungus in the honeybee colonies treated with EM® for bees (TH2 and TH3), was higher in the last sampling term (20th of August) in comparison with other treatments (Figure 11). *S. alvi*, which in this study showed significant differences in the experimental and control honeybee colony groups is a known major member of the honeybee gut microbiota. This bacterium is susceptible to the herbicide glyphosate, and research has shown that glyphosate consistently reduces this bacterium resulting in lower resistance to pathogens in honeybees [78], and causing metabolic level perturbation [79]. Also, the reduction of this bacterium may be a factor that decreases the immunity of honeybees [78].

The number of OTUs and reads obtained from amplicon sequencing increased on the 27th of July in groups TH1, TH2, and TH3. However, it did not increase in reads for group TH4 (Figure 2). Therefore, the number of intestinal *Nosema* spp. spores suppressed the increase in the number of gut bacteria more than the fresh natural infection with *Nosema* spp. spores. Then, in the group TH2 plot on the 7th of July was slightly off, suggesting that the adult honeybee intestinal microbiota was affected by EM® for bees application.

The plots of the sampled bee's gut microbiome in groups TH1, TH2, TH3, and TH4 on the 20th of August were away from the ones of the other sampling dates. Furthermore, the plots of experimental colonies from groups TH1 and TH3 that were invaded with *Nosema* spp. spores, and

the ones considered uninvaded (TH2 and TH4) were close together, respectively. Therefore, it is suggested that the gut microbiome of adult bees sampled on the 20th of August was affected by the invasion with *Nosema* spp. spores more than the level of invasion.

The number of intestinal *Nosema* spp. spores in group TH2 applied only EM® for bees remained lowest, and the relative proportions of *Gilliamella* were kept higher than in the other groups of colonies. On the other hand, the number of *Nosema* spp. spores remained highest in group TH4, and the relative proportions of *Gilliamella* were kept lower in the first three sampling terms (Figure 8 a). Therefore, EM® for bees application to honey bees increased the proportion of *Gilliamella* in the bee's gut microbiome, indicating that it may inhibit the development of *Nosema* spp. spores in the midgut.

In groups, TH1 and TH3 invaded with *Nosema* spp. spores, the level of invasion increased until the 27th of July in TH1 and decreased in TH3 (Table 1). *Bifidobacterium asteroides*, *Lactobacillus mellis*, *L. heisingborgensis* and *L. apis* were higher in group TH3 than in group TH1 until the 27th of July. We think that probiotics may have suppressed the increase of *Nosema* spp. spore levels in the intestine. Both of these proportions in group TH3 decreased on 20th August and *Nosema* spores increased, probably as a consequence of the long period past from initial feeding. On the other hand, group TH2 had the lowest number of *Nosema* spp. spores on all performed sampling terms after the 17th of July and the highest relative proportion of *Gilliamella apicola* until the 27th of July, and *Snodgrassella alvi* on the 20th of August.

The relative proportions of *Frischilla perrara* and *Apibacter mensalis* on the 27th of July were higher in group TH1 than in group TH3. Furthermore, the number of *Nosema* spp. spores were also higher. However, on the 20th of August, the proportions of these bacteria were higher in group TH3, and the number of spores was also higher in TH3. Thus, if invaded with *Nosema* spp. spores, these bacteria may be associated with the development of *Nosema* spp. spores in the intestine.

Fructobacillus fructosus, a member of the genus *Fructobacillus*, has been reported to ex-

hibit antagonistic activity against the pathogen bacterium *Paenibacillus larvae*, the causal agent of American foulbrood [80]. However, this species is not known from the results of LEfSe analysis in this study but represents a good topic for further research. It is known that hive structure, environment, and natural diet influence the assembly and maintenance of honey bee gut microbiota and facilitate future experimental designs [18]. According to our knowledge, this is the first research aimed to investigate the impacts of probiotic EM® for bees on adult bee's health and immunological condition by analyzing intestinal microbiome content.

5. Conclusions

There have been many reports from beekeepers that honeybee colonies become more resistant to diseases after using EM® for bees. According to the results of this study, it is also probable that the continued use of EM® for bees improves their gut microbiome and, consequently, immunity, by maintaining and increasing the level of *S. alvi*. If the higher level of *S. alvi* is supported by feed supplementation with EM® for bees, it may be possible for this beekeeping management practice to reduce the negative effect of glyphosate on honeybees.

Author Contributions: Conceptualization, I.T.G.; methodology, I.T.G., S.N. and T.N.; investigation, I.T.G., S.N., L.C. and T.N.; writing—original draft preparation, I.T.G.; writing—review and editing, I.T.G., S.N., L.C. and T.N.; visualization, I.T.G., S.N. and T.N.; funding acquisition, I.T.G.

All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.
Data Availability Statement: Data are presented in the manuscript.

Acknowledgments: The authors would like to thank B. Kefelja, I. Gajger, G. Husinec, N. Jo-vanović, S. Nagamoto and H. Tomii for their technical assistance.

Conflicts of Interest: The authors declare no conflict of interest. Although, the author T.N. is affiliated with the independent research laboratory of EMRO Japan. It should be stated that the re-searched product has been registered and is commercially available in the European market where the field experiment was done.

Ivana Tlak Gajger

Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Croatia
ivana.tlak@vef.unizg.hr

Srebrenka Nejedli

Department for Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Croatia
snejedli@vef.unizg.hr

Luka Cvetnić

Department for Bacteriology and Parasitology, Croatian Veterinary Institute, 10 000 Zagreb, Croatia
lcvetnic@veinst.hr

Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

References

1. Klein, A.M.; Vaissiere, B.E.; Cane, J.H.; Steffan-Dewenter, I.; Cunningham, S.A.; Kremen, C.; Tscharntke, T. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B Biol. Sci.* 2007, 274, 303–313.
2. Fontaine, C.; Dajoz, I.; Meriguet, J.; Loreau, M. Functional Diversity of Plant–Pollinator Interaction Webs Enhances the Persistence of Plant Communities. *PLoS Biol.* 2006, 4, e1.
3. Rollin, O.; Benelli, G.; Benvenuti, S.; Decourtye, A.; Wratten, S.D.; Canale, A.; Desneux, N. Weed-insect pollinator networks as bioindicators of ecological sustainability in agriculture. A review. *Agron. Sustain. Dev.* 2016, 36, 8.
4. Ellegaard, K.M.; Engel, P. Genomic diversity landscape of the honey bee gut microbiome. *Nat. Commun.* 2019, 10, 446.
5. Tlak Gajger, I.; Sakač, M.; Gregorc, A. Impact of Thiamethoxam on Honey Bee Queen (*Apis mellifera carnica*) Reproductive Morphology and Physiology. *Bull. Environ. Contam. Toxicol.* 2017, 99, 297–302.
6. Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E.L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 2015, 347, 6229.
7. Raymann, K.; Moran, N.A. The role of the gut microbiome in health and disease of adult honey bee workers. *Curr. Opin. Insect. Sci.* 2018, 26, 97–104.
8. Alaux, C.; Brunet, J.L.; Dussaubat, C.; Mondet, F.; Tchamitchan, S.; Cousin, M.; Brillard, J.; Baldy, A.; Belzunces, L.P.; Le Conte, Y. Interactions between

- Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* 2010, 12, 774–782.
9. Di Pasquale, G.; Salignon, M.; Le Conte, Y.; Belzunces, L.P.; Decourtye, A.; Kretzschmar, A.; Suchail, S.; Brunet, J.-L.; Alaux, C. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? *PLoS ONE* 2013, 8, e72016.
10. Gilioli, G.; Sperandio, G.; Hatjina, F.; Simonetto, A. Towards the development of an index for the holistic assessment of the health status of a honey bee colony. *Ecol. Indic.* 2019, 101, 341–347.
11. Sperandio, G.; Simonetto, A.; Carnesecchi, E.; Costa, C.; Hatjina, F.; Tosi, S.; Gilioli, G. Beekeeping and honey bee colony health: A review and conceptualization of beekeeping management practices implemented in Europe. *Sci. Total Environ.* 2019, 696, 133795.
12. Tlak Gajger, I.; Mañes, A.M.; Formato, G.; Mortarino, M.; Toporcak, J. Veterinarians and beekeeping: What roles, expectations and future perspectives? - a review paper. *Vet. Arhiv* 2021, 91, 4, 437-443. doi: 10.24099/vet.arhiv.1444
13. Zheng, H.; Steele, M.I.; Leonard, S.P.; Motta, E.V.S.; Moran, N.A. Honey bees as models for gut microbiome research. *Lab. Anim.* 2018, 47, 317-325.
14. Engel, P.; Martinson, V.G.; Moran, N.A. Functional diversity within the simple gut microbiome of the honey bee. *Proc. Natl. Acad. Sci.* 2012, 109, 11002–11007.
15. Vásquez, A.; Forsgren, E.; Fries, I.; Paxton, R.J.; Flaberg, E.; Szekely, L.; Olofsson, T.C. Symbionts as major modulators of insect health: Lactic acid bac-

teria and honeybees. *PLoS ONE* 2012, 7, e33188.

16. Nowak, A.; Szczuka, D.; Górczyńska, A.; Motyl, I.; Kręgiel, D. Characterization of *Apis mellifera* Gastrointestinal Microbiome and Lactic Acid Bacteria for Honeybee Protection—A Review. *Cells* 2021, 10, 701. doi.org/10.3390/cells10030701
17. Callegari, M.; Crotti, E.; Fusi, M.; Marasco, R.; Gonella, E.; Denoni, I.; Romano, D.; Borin, S.; Tsiamis, G.; Cherif, A.; Alma, A.; Daffonchio, D. Compartmentalization of bacterial and fungal microbiomes in the gut of adult honeybees. *npj Biofilms Microbiomes* 2021, 7, 42. doi.org/10.1038/s41522-021-00212-9
18. Anderson, K.E.; Ricigliano, V.A.; Copeland, D.C.; Mott, B.M.; Maes, P. Social Interaction is Unnecessary for Hindgut Microbiome Transmission in Honey Bees: The Effect of Diet and Social Exposure on Tissue Specific Microbiome Assembly. *Microbial Ecol* 2022. doi.org/10.1007/s00248-022-02025-5
19. Kešnerová, L.; Mars, R.A.T.; Ellegaard, K.M.; Troilo, M.; Sauer, U.; Engel, P. Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS Biol* 2017, 15, e2003467
20. Kwong, W. K.; Zheng, H.; Moran, N.A. Convergent evolution of a modified, acetate-driven TCA cycle in bacteria. *Nat. Microbiol.* 2017, 2, 17067.
21. Lee, F. J., Miller, K. I., McKinlay, J. B. & Newton, I. L. G. Differential carbohydrate utilization and organic acid production by honey bee symbionts. *FEMS Microbiol. Ecol.* 2018, 94, 1–10.
22. Kwong, W. K.; Moran, N.A. Gut microbial communities of social bees. *Nat. Rev. Microbiol.* 2016, 14, 374–384.
23. Wang, S.; Wang, L.; Fan, X.; Yu, C.; Feng, L.; Yi, L. An insight into diversity and functionalities of gut microbiome in insects. *Curr. Microbiol.* 2020, 77, 1976–1986.
24. Pernice, M.; Simpson, S.J.; Ponton, F. Towards an integrated understanding of gut microbiome using insects as model systems. *J. Insect Physiol.* 2014, 69, 12–18.
25. Jing, T.-Z.; Qi, F.-H.; Wang, Z.-Y. Most dominant roles of insect gut bacteria: Digestion, detoxification or essential nutrient provision? *Microbiome* 2020, 8.
26. Shi, W.; Syrenne, R.; Sun, J.-Z.; Yuan, J.S. Molecular approaches to study the insect gut symbiotic microbiome at the “omics” age. *Insect Sci.* 2010, 17, 199–219.
27. Lee, F.J.; Rusch, D.B.; Stewart, F.J.; Mattila, H.R.; Newton, I.L.G. Saccharide breakdown and fermentation by the honey bee gut microbiome. *Environ. Microbiol.* 2015, 17, 796–815.
28. Parker, A., Lawson, M.A.E., Vaux, L., Pin, C. Host-microbe interaction in the gastrointestinal tract. *Environ Microbiol.* 2018, 20, 2337-2353. doi.org/10.1111/1462-2920.13926
29. Dillon, R.J.; Vennard, C.T.; Charnley, A.K. A note: gut bacteria produce components of a locust cohesion pheromone. *J Appl Microbiol* 2002, 92, 759-763.
30. Vásquez, A.; Forsgren, E.; Fries, I.; Paxton, R.J.; Flaberg, E.; Szekely, L.; Olofsson, T.C. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One* 2012, 7, 3:e33188. doi: 10.1371/journal.pone.0033188
31. Maes, P.W.; Rodrigues, P.A.P.; Oliver, R.; Mott, B.M.; Anderson, K.E. Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honeybee (*Apis mellifera*). *Mol. Ecol.* 2016, 25, 5439–5450.
32. Anderson, K.E.; Ricigliano, V.A. Honey bee gut dysbiosis: A novel context of disease ecology. *Curr. Opin. Insect Sci.* 2017, 22, 125–132.
33. Morimoto, T.; Kojima, Y.; Toki, T.; Komeda, Y.; Yoshiyama, M.; Kimura, K.; Nirasawa, K.; Kadowaki, T. The habitat disruption induces immune-suppression and oxidative stress in honey bees. *Ecol. Evol.* 2011, 1, 201–217.
34. Paris, L.; Peghaire, E.; Moné, A.; Diogon, M.; Debroas, D.; Delbac, F.; El Alaoui, H. Honeybee gut microbiome dysbiosis in pesticide/parasite co-exposures is mainly induced by *Nosema ceranae*. *J Invertebr Pathol* 2020, 172, 107348. doi: 10.1016/j.jip.2020.107348
35. Rouzé, R.; Moné, A.; Delbac, F.; Belzunces, L.; Blot, N. The Honeybee Gut Microbiome Is Altered after Chronic Exposure to Different Families of Insecticides and Infection by *Nosema ceranae*. *Microbes Environ* 2019, 25, 34, 226-233. doi: 10.1264/jisme2.ME18169
36. Panjad, P.; Yongsawas, R.; Sinpoo, C.; Pakwan, C.; Subta, P.; Krongdang, S.; In-on, A.; Chomdej, S.; Chantawannakul, P.; Disayathanoowat, T. Impact of Nosema Disease and American Foulbrood on Gut Bacterial Communities of Honeybees *Apis mellifera*. *Insects* 2021, 12, 525. https://doi.org/10.3390/insects12060525
37. Castelli, L.; Balbuena, S.; Branchiccela, B.; Zunino, P.; Liberti, J.; Engel, P.; Antúnez, K. Impact of Chronic Exposure to Sublethal Doses of Glyphosate on Honey Bee Immunity, Gut Microbiome and Infection by Pathogens. *Microorganisms* 2021, 9, 845. https://doi.org/10.3390/microorganisms9040845
38. Cuesta-Mate, A.; Renelies-Hamilton, J.; Kryger, P.; Jansen, A.B.; Sinotte, V.M.; Poulsen, M. Resistance and vulnerability of honeybee (*Apis mellifera*) gut bacteria to commonly used pesticides. *Front. Microbiol.* 2021, 12, 717990. doi:10.3389/fmicb.2021.717990
39. Pătruică, S.; Mot, D. The effect of using prebiotic and probiotic products on intestinal micro-flora of the honeybee (*Apis mellifera carpatica*). *Bull. Entomol. Res.* 2012, 102, 619–623.
40. Pătruică, S.; Dumitrescu, G.; Stancu, A.; Bura, M.; Dunea, I.B. The Effect of Prebiotic and Probiotic Feed Supplementation on the Wax Glands of Worker Bees (*Apis Mellifera*). *Anim. Sci. Biotechnol.* 2012, 45, 268–271.
41. Pătruică, S.; Hutu, I. Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies. *Turk. J. Vet. Anim. Sci.* 2013, 37, 259–263.
42. Andrearczyk, S.; Kadhim, M.J.; Knaga, S. Influence of a probiotic on mortality, sugar syrup ingestion and infection of honeybees with *Nosema* spp. under laboratory assessment. *Med. Veter.* 2014, 70, 762–765.
43. Arredondo, D.; Castelli, L.; Porrini, M.P.; Garrido, P.M.; Eguaras, M.J.; Zunino, P.; Antúnez, K. *Lactobacillus kunkeei* strains decreased the infection by honey bee pathogens *Paenibacillus* larvae and *Nosema ceranae*. *Benef. Microbes* 2018, 9, 279–290.
44. Ptaszynska, A.A.; Borsuk, G.; Mułenko, W.; Wilk, J. Impact of vertebrate probiotics on honeybee yeast microbiome and on the course of nosemosis. *Med. Veter.* 2016, 72, 430–434.
45. Daisley, B.A.; Pitek, A.P.; Chmiel, J.A.; Al, K.F.; Chernyshova, A.M.; Faragalla, K.M.; Burton, J.P.; Thompson, G.J.; Reid, J. Novel probiotic approach to counter *Paenibacillus* larvae infection in honey bees. *ISME J.* 2020, 14, 476–491.
46. Stephan, J.G.; Lamei, S.; Pettis, J.S.; Riesbeck, K.; De Miranda, J.R.; Forsgren, E. Honeybee-specific lactic acid bacterium supplements have no effect on American Foulbrood-infected honeybee colonies. *Appl. Environ. Microbiol.* 2019, 85, e00606-19.
47. Dussaubat, C.; Brunet, J.-L.; Higes, M.; Colbourne, J.K.; López, J.; Choi, J.-H.; Martín-Hernández, R.; Botías, C.; Cousin, M.; McDonnell, C.; et al. Gut pathology and responses to the microsporidium *Nosema ceranae* in the honey bee *Apis mellifera*. *PLoS ONE* 2012, 7, e37017.
48. Tlak Gajger, I.; Vugrek, O.; Pinter, L.; Petrinc, Z. “Nozevit patties” treatment of honeybees (*Apis mellifera*) for the control of *Nosema ceranae* disease. *Am. Bee J.* 2009, 149, 1053–1056.
49. Tlak Gajger, I.; Kozaric, Z.; Berta, D.; Nejedli, S.; Petrinc, Z. Effect of the herbal preparation Nozevit on the mid-gut structure of honeybees (*Apis mellifera*) infected with *Nosema* spp. spores. *Vet. Med.* 2011, 56, 343–350.
50. Tlak Gajger, I. Nozevit aerosol application for *Nosema ceranae* disease treatment. *Am. Bee J.* 2011, 151, 1087–1090.
51. Tlak Gajger, I.; Smodiš Škerl, M.I.; Šoštarić, P.; Šuran, J.; Sikirić, P.; Vlanić, J. Physiological and Immunological Status of Adult Honeybees (*Apis mellifera*) Fed Sugar Syrup Supplemented with Pentadecapeptide BPC 157. *Biology* 2021, 10, 891. doi.org/10.3390/biology10090891
52. Tlak Gajger, I.; Ribarić, J.; Smodiš Škerl, I.M.; Vlanić, J.; Sikirić, P. Stable gastric pentadecapeptide BPC 157 in honeybee (*Apis mellifera*) therapy, to control *Nosema ceranae* invasions in apiary conditions. *J. Vet. Pharmacol. Therap.* 2018, 41, 614-621. doi.org/10.1111/jvp.12509
53. Tlak Gajger, I.; Ribarić, J.; Matak, M.; Svecnjak, L.; Kozaric, Z.; Nejedli, S.; Smodiš Škerl, I.M. Zeolite clinoptilolite as a dietary supplement and remedy for honeybee (*Apis mellifera* L.) colonies. *Veterinarni Medicina* 2015, 60, 696–705.
54. Tlak Gajger, I.; Vlanić, J.; Šoštarić, P.; Prešern, J.; Bubnić, J.; Smodiš Škerl, M.I. Effects on Some Therapeutical, Biochemical, and Immunological Parameters of Honey Bee (*Apis mellifera*) Exposed to Probiotic Treatments, in Field and Laboratory Conditions. *Insects* 2020, 11, 638. doi.org/10.3390/insects11090638
55. Delaplaine, K.S.; Van Der Steen, J.; Guzman-Novoa, E. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Res.* 2013, 52, 1–12.
56. OIE—Office International des Epizooties. Chapter 2.2.4., Nosemosis of Honey Bees. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*; OIE: Paris, France, 2013; Available online: <http://www.oie.int/international-standard-setting/terrestrial-manual/access-online> (accessed on 20 May 2022).
57. Takahashi, S.; Tomita, J.; Nishioka, K.; Hisada, T.; Nishijima, M. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. *PLoS ONE* 2014, 9: e105592. doi.org/10.1371/journal.pone.0105592
58. Nguyen, N.P.; Warnow, T., Pop, M., White, B. A perspective on 16S rRNA operational taxonomic unit clustering using sequence similarity. *npj Biofilms Microbiomes* 2016, 2, 16004. doi.org/10.1038/npjbiofilms.2016.4
59. Hisada, T.; Endoh, K.; Kuriki, K. Inter- and intra-individual variations in seasonal and daily stabilities of the human gut microbiome in Japanese. *Arch Microbiol* 2015, 197, 919–934.
60. Aronesty E. Comparison of sequencing utility programs. *Open Bioinforma J* 2013, 7, 1–8.
61. Gordon, A.; Hannon, G.J. FASTX-Toolkit FASTQ/A short-reads preprocessing tools [software]. Available from: http://hannonlab.cshl.edu/fastx_toolkit/index.html
62. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; Huttley, G.A.; Kelley, S.T.; Knights, D.; Koenig, J.E.; Ley, R.E.; Lozupone, C.A.; McDonald, D.; Muegge, B.D.; Pirrung, M.; Reeder, J.; Sevinsky, J.R.; Turnbaugh, P.J.; Walters, W.A.; Widmann, J.; Yatsunenko, T.; Zaneveld, J.; Knight, R. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010, 7, 335336.
63. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011, 27, 2194–2200.
64. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007, 73, 5261–5267.
65. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, et al. Comparison of the gut microbiome composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol* 2015, 15, 100.
66. Horthorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biometrical J.* 2008, 50, 346-363.
67. Audisio, M.C.; Benítez-Ahrendts, M.R. *Lactobacillus johnsonii* CRL1647, isolated from *Apis mellifera* L. bee-gut, exhibited a beneficial effect on honeybee colonies. *Benef. Microbes* 2011, 2, 29–34.
68. Sabate, D.C.; Cruz, M.S.; Benítez-Ahrendts, M.R.; Audisio, M.C. Beneficial effects of *Bacillus subtilis* subsp. *subtilis* Mori2, a honey-associated strain, on honeybee colony performance. *Probiotics Antimicrob. Assoc.* 2012, 4, 39–46.

69. Audisio, M.C.; Sabate, D.C.; Benitez-Ahrendts, M.R. Effect of *Lactobacillus johansonii* CRL1647 on different parameters of honeybee colonies and bacterial populations of the bee gut. *Benef. Microbes* 2015, 25, 1–10.

70. Alberoni, D.; Baffoni, L.; Gaggia, F.; Ryan, P.M.; Murphy, K.; Ross, P.R.; Stanton, C.; Di Gioia, D. Impact of beneficial bacteria supplementation on the gut microbiota, colony development and productivity of *Apis mellifera* L. *Benef. Microbes* 2018, 9269–9278.

71. Huang, S.K.; Ye, K.T.; Huang, W.F.; Ying, B.H.; Li, X.S.; Lin, H.; Li, J.H.; Chen, Y.P.; Li, J.L.; Bao, X.L.; Hu, J.Z. Influence of Feeding Type and *Nosema ceranae* Infection on the Gut Microbiota of *Apis cerana* Workers. *mSystems* 2018, 3, e00177-18.

72. Baffoni, L.; Gaggia, F.; Alberoni, D.; Cabbri, R.; Nanetti, A.; Biavati, B.; Di Gioia, D. Effect of dietary supplementation of *Bifidobacterium* and *Lactobacillus* strains in *Apis mellifera* L. against *Nosema ceranae*. *Benef. Microbes* 2016, 7, 45–51.

73. Wu, Y.; Zheng, Y.; Chen, Y.; Chen, G.; Zheng, H.; Hu, F. *Apis Cerana* Gut Microbiota Contribute to Host Health Through Stimulating Host Immune System and Strengthening Host Resistance to *Nosema ceranae*. *R. Soc. Open Sci.* 2020, 7, 192100.

74. Corby-Harris, V.; Snyder, L.; Meador, C.; Naldo, R.; Mott, B.; Andersom, K. *Parasaccharibacter apium*, gen. Nov., sp. Nov., improves honey bee (*Hymenop-*

tera: Apidae) resistance to *Nosema*. *J. Econ. Entomol.* 2016, 109, 537–543.

75. Audisio, M.C. Gram-positive bacteria with probiotic potential for the *Apis mellifera* L. honey bee: The experience in the northwest of Argentina. *Probiotics Antimicrob. Proteins*. 2017, 9, 22–31.

76. Yoshiyama, M.; Wu, M.; Sugimura, Y.; Takaya, N.; Kimoto-Nira, H.; Suzuki, C. Inhibition of *Paenibacillus* larvae by lactic acid bacteria isolated from fermented materials. *J. Invertebr. Pathol.* 2013, 112, 62–67.

77. Fanciotti, M.N.; Tejerina, M.; Benítez Ahrendts, M.; Audisio, M.C. Honey yield of different commercial apiaries treated with *Lactobacillus salivarius* A3iob, a new bee-probiotic strain. *Benef. Microbes* 2018, 9, 291–298.

78. Motta, E.V.S.; Raymann, K.; Moran, N.A. Glyphosate perturbs the gut microbiota of honey bees. *App Biol Sci* 2018, 115, 10305-10310. doi.org/10.1073/pnas.1803880115

79. Wang, B.; Habermehl, C.; Jiang, L. Metabolomic analysis of honey bee (*Apis mellifera* L.) response to glyphosate exposure. *Mol Omics*. 2022, 18, 635-642. doi: 10.1039/d2mo00046f

80. Zeid, A.A.A., Khattaby, A.M., El-Khair, I.A.A. et al. Detection Bioactive Metabolites of *Fructobacillus fructosus* Strain HI-1 Isolated from Honey Bee's Digestive Tract Against *Paenibacillus* larvae. *Probiotics Antimicrob.* 2022, 14, 476–485. doi.org/10.1007/s12602-021-09812-5

www.ander matt-biovet.de/de-de



Andermatt BioVet

MICRONUTRIENT DEFICIENCY AND THE ONSET OF CHALKBROOD DISEASE IN HONEY BEES

Abstract

Chalkbrood is a fungal disease caused by *Ascosphaera apis* that affects honey bee larvae, typically weakening the colony by reducing its population rather than causing colony death. Poor nutrition is known to contribute to the onset of honey bee diseases, but a direct link between dietary ingredients and chalkbrood symptoms has not been well established. Our study highlights significant differences in the elemental composition of larvae from hives with varying chalkbrood status. Chalkbrood mummies exhibited higher concentrations of macroelements such as sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K), and calcium (Ca), as well as some microelements like rubidium (Rb). However, they also had much

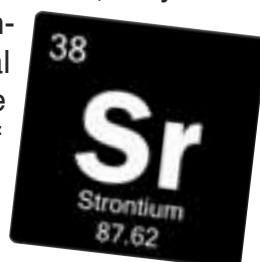
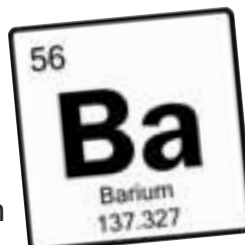
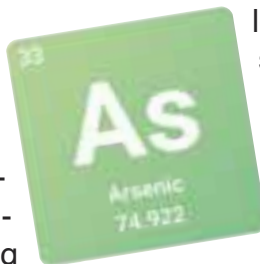
lower levels of essential microelements with known antifungal and antimicrobial properties, including boron (B), arsenic (As), strontium (Sr), silver (Ag), cadmium (Cd), antimony (Sb), barium (Ba), and lead (Pb). Infected larvae

showed reduced concentrations of these microelements compared to healthy larvae from disease-free hives.

This is the first study to reveal such differences, suggesting that the lack of certain elements may contribute to the onset of chalkbrood disease or that the infection itself alters larval nutrition.

Although the results are based on case studies rather than controlled experiments, they

emphasize the potential role of nutritional deficiencies in the development of chalkbrood and the need for further in-



investigation into the nutritional factors influencing honey bee health.

Introduction

Honey bees, crucial pollinators, are facing significant threats due to the reduction in the availability and diversity of pollen and nectar sources, largely driven by changes in land use. This decline in food resources can lead to malnutrition, which directly impairs bee health and indirectly affects their immune competence [1–3]. To meet their nutritional needs, honey bees rely on macro- and micro-nutrients obtained from nectar, pollen, and water [4]. A diverse, multifloral pollen diet is considered essential for maintaining honey bee health [5–8], though the specific role of micronutrients remains not fully understood [9]. Minerals are vital for the reproduction and development of both adult bees and larvae. Certain elements, such as sodium, potassium, calcium, magnesium, and phosphorus, are essential for honey bees, while others like aluminum, lead, cadmium, and barium can be toxic [10,11]. For instance, aluminum is not a proven nutrient and can replace magnesium in proteins, leading to conformational defects [12]. Lead affects enzyme and antioxidant activity, causes oxidative stress, and primarily impacts the nervous system [13]. Cadmium catalyzes the production of reactive oxygen species, thereby increasing oxidative stress and interfering with DNA repair, which can result in cell death [14]. Barium negatively influences potassium accumulation within cells, leading to hyperkalemia and membrane depolarization [15].

Supplemental feeding can provide colonies with additional essential minerals, though the exact requirements and optimal amounts for honey bees are still largely unexplored [4,16]. Honey bee larvae have some buffering capacity against changes in the colony's food supply [17], but the effects of larval nutrition on disease susceptibility in bees have not been extensively studied [18].

Chalkbrood is a disease of honey bee brood caused by the fungus *Ascosphaera apis* (Ascomycota: Eurotiomycetes: Ascospaerales). The disease is widespread globally, and evidence suggests that its incidence may be on the rise [19–22]. Typical symptoms include irregular wax capping over the brood and uncapped cells scattered across brood frames. Mummies are often visible in cells, at the hive entrance, or on the bottom board, having been removed by worker bees [23]. Initially, dead larvae are covered in a fluffy white mold and swollen to the hexagonal shape of the cell. Over time, they shrink into mummies, which may turn gray or black if spore cysts form [24]. Globally, chalkbrood reduces honey production by approximately 5-37%, due to decreased productivity in affected colonies [25]. This productivity loss is primarily due to a reduction in the workforce caused by the fungal infection [26]. Although chalkbrood is typically not lethal to the colony, it can hinder its development by reducing its population [27]. While adult bees are not susceptible to this pathogen, they can still transmit the disease within and between hives [20]. Genetic traits, such as hygienic behavior, play a role in preventing the onset of chalkbrood [28]. Environmental factors like temperature and humidity also contribute to the disease's development [29,30]. Chilling of brood cells 24 hours before or after sealing is a significant predisposing factor for chalkbrood [31]. The fungus is best understood as an opportunistic pathogen that is efficiently dispersed and widespread. Its presence in larvae does not necessarily lead to disease unless one or more predisposing conditions are present [24].

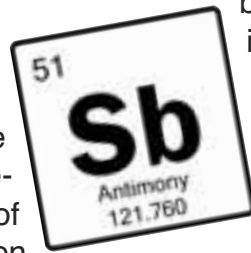
While adult bees are not susceptible to this pathogen, they can still transmit the disease within and between hives [20]. Genetic traits, such as hygienic behavior, play a role in preventing the onset of chalkbrood [28]. Environmental factors like temperature and humidity also contribute to the disease's development [29,30]. Chilling of brood cells 24 hours before or after sealing is a significant predisposing factor for chalkbrood [31]. The fungus is best understood as an opportunistic pathogen that is efficiently dispersed and widespread. Its presence in larvae does not necessarily lead to disease unless one or more predisposing conditions are present [24].

The development of healthy honey bee colonies relies on adequate nutrition [4], but no direct link between poor larval nutrition and chalkbrood has been established. Recent studies suggest that poor nutrition may exacerbate the negative effects of infectious viral and fungal diseases in honey bees [32]. Conversely, common bee pathogens and parasites can negatively affect the nutritional physiology of honey bees [33,34]. This dynamic holds the potential for

While adult bees are not susceptible to this pathogen, they can still transmit the disease within and between hives [20]. Genetic traits, such as hygienic behavior, play a role in preventing the onset of chalkbrood [28]. Environmental factors like temperature and humidity also contribute to the disease's development [29,30]. Chilling of brood cells 24 hours before or after sealing is a significant predisposing factor for chalkbrood [31]. The fungus is best understood as an opportunistic pathogen that is efficiently dispersed and widespread. Its presence in larvae does not necessarily lead to disease unless one or more predisposing conditions are present [24].

The development of healthy honey bee colonies relies on adequate nutrition [4], but no direct link between poor larval nutrition and chalkbrood has been established. Recent studies suggest that poor nutrition may exacerbate the negative effects of infectious viral and fungal diseases in honey bees [32]. Conversely, common bee pathogens and parasites can negatively affect the nutritional physiology of honey bees [33,34]. This dynamic holds the potential for

The development of healthy honey bee colonies relies on adequate nutrition [4], but no direct link between poor larval nutrition and chalkbrood has been established. Recent studies suggest that poor nutrition may exacerbate the negative effects of infectious viral and fungal diseases in honey bees [32]. Conversely, common bee pathogens and parasites can negatively affect the nutritional physiology of honey bees [33,34]. This dynamic holds the potential for



harmful feedback loops between poor nutrition and infectious disease, possibly contributing to a cycle of deteriorating bee health [32]. In this study, we investigated whether there are differences in the elemental composition of mummies and larvae from infected colonies compared to larvae from healthy colonies.

Materials and Methods

Honey bee larvae and chalkbrood mummies were collected from two apiaries in Vršac, Serbia. One apiary is located in an urban environment (45°06'35.3"N 21°18'27.0"E), while the other is situated in a rural area (45°08'14.9"N 21°20'04.2"E), approximately 3.7 km apart. The urban apiary hosts up to 15 hives and has been free of chalkbrood disease for the past ten years. In contrast, the rural apiary contains more than 60 hives, with an additional 100 hives nearby, and has occasionally experienced chalkbrood outbreaks.

Sampling took place on June 3, 2023. At the urban apiary, 25 larvae were collected from each of three hives. At the rural apiary, we sampled 25 mummies and 25 larvae from each of three hives exhibiting moderate clinical symptoms of chalkbrood, as well as 25 larvae from each of three hives that showed no symptoms of the disease. The larvae were approximately 5 days post-hatching, appeared healthy, and were collected from comb cells nearest to the capped brood, with the first signs of capping visible. Mummies were collected only from frames (not from the bottom board) and only from cells partially opened by bees, regardless of their color.

Sampling occurred during a nectar dearth, and infected hives had noticeably smaller honey and pollen reserves compared to healthy hives. The samples were pooled, resulting in four distinct pooled samples: (1) larvae from the urban disease-free apiary, (2) larvae from rural hives without chalkbrood symptoms, (3) larvae from rural hives with chalkbrood symptoms, and (4) mummies from rural hives with chalkbrood symptoms. Each pooled sample was analyzed in trip-

licate. Element concentrations were determined as described [35] using inductively coupled plasma mass spectrometry – ICPMS.

Results and Discussion

Chalkbrood mummies exhibited higher concentrations of macroelements like Na, Mg, P, S, K, Ca, and certain microelements such as Rb compared to other samples (Figure 1a, 1b, and 1d;). Conversely, the mummies had lower concentrations of B, As, Sr, Ag, Cd, Sb, Ba, and Pb (Figure 1d and 1e). Both mummies and larvae from infected hives contained significantly lower levels of Al, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, and Cs compared to larvae from uninfected hives within the same apiary and larvae from the disease-free urban apiary (Figure 1).

These findings suggest that larvae from infected hives may have consumed a more uniform diet rich in macroelements but deficient in microelements, compared to larvae from healthy hives, which likely had access to more diverse food sources. Previous research has highlighted the importance of food composition over quantity in honey bee health [36]. It is possible that bees from infected hives collected less or less diverse pollen, potentially due to

factors like genetic predispositions (e.g., pollen hoarding behavior) [28,37], or competition with bees from healthier, nearby hives [32,38]. Prior studies have also shown that bees from different hives in the same apiary can accumulate varying amounts of elements [35]. Since these elements are passed on through food to larvae, it is plausible that not all larvae receive the same elemental composition.

Even in well-functioning colonies, some larvae may be temporarily neglected by nurse bees, leading to a "hunger signal" that prompts feeding [42]. This uneven distribution of nutrients may mean that infected larvae attempt to compensate for deficiencies by increasing their "hunger signal," similar to how bees infected with *Nosema ceranae* increase their food intake to mitigate energetic stress [45,46].



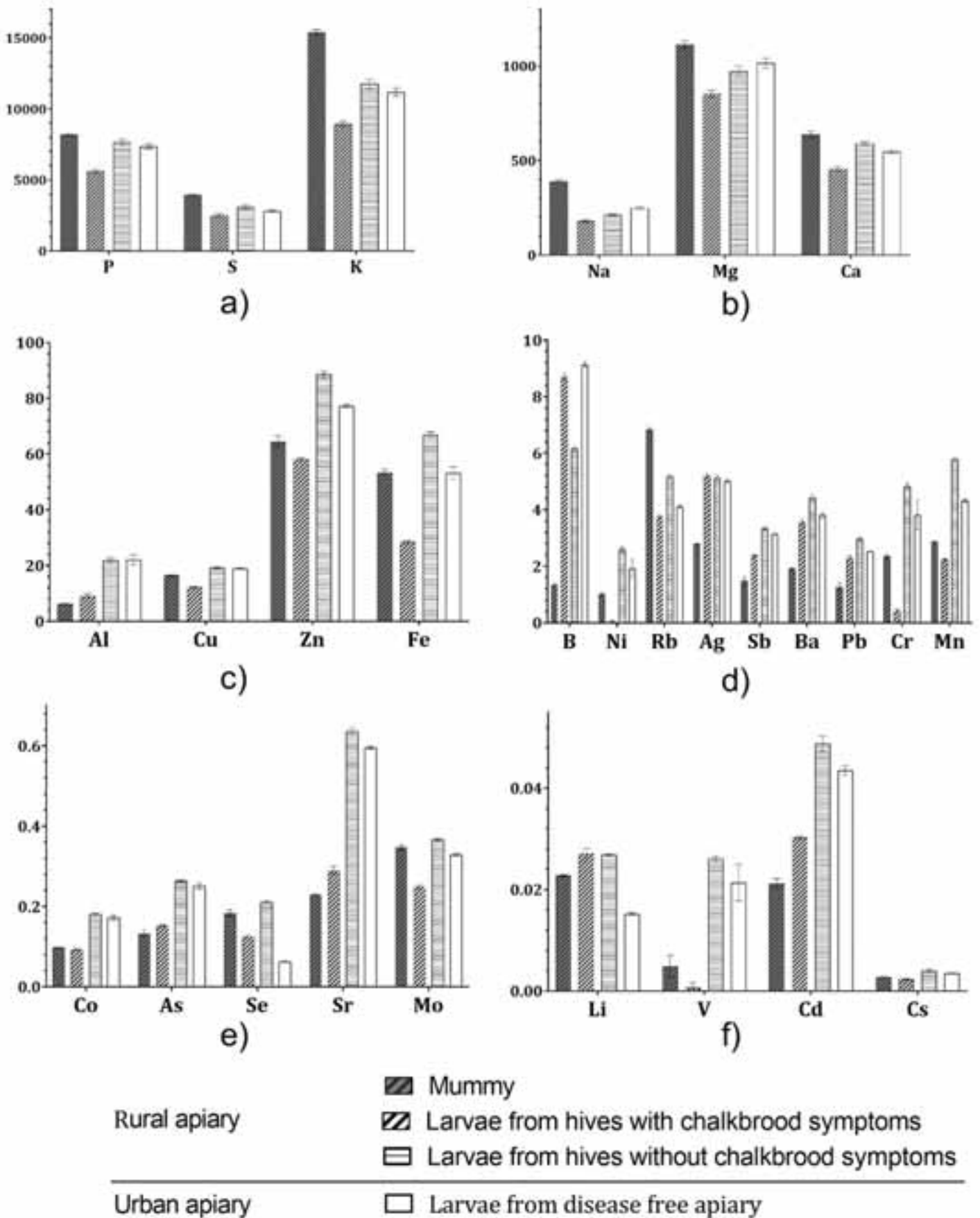


Figure 1. (a–f) Element concentrations (mg kg^{-1} dry weight) in mummies, larvae from hives showing chalkbrood symptoms and larvae from hives not showing chalkbrood symptoms from rural apiary and larvae from disease-free urban apiary

The data suggests that deficiencies in certain elements could either contribute to the onset of chalkbrood disease or result from the infection. Elements such as B, As, Sr, Ag, Cd, Sb, Ba, and Pb, which were found in lower concentrations in mummies than in larvae from the same colonies, are particularly noteworthy (Figure 1). It is important to note that not all larvae in an infected colony become mummified; only those with significantly lower levels of these elements did. Larvae from hives showing symptoms of chalkbrood also had lower concentrations of Al, Cu, Zn, Ni, Cr, Mn, Co, Mo, V, and Cs compared to larvae from healthy hives, reinforcing the hypothesis that chalkbrood is associated with poor nutrition.

The significantly lower boron concentration in mummies is notable because boron-containing compounds have shown strong antifungal activity against key fungal pathogens [47]. The low levels of boron and other elements like aluminum, zinc, and silver in mummies compared to healthy larvae suggest that nutrient deficiencies could impair the larvae's ability to resist *Ascosphaera apis*. Elements like zinc, which enhance antioxidant defenses in honey bees [51], and silver, known for its antimicrobial properties [54,55], were also found in much lower concentrations in mummies. This indicates that larvae receiving a diet deficient in these microelements may be more susceptible to mummification, as they lack the protective effects of these elements.

In conclusion, this study highlights a potential link between elemental deficiencies and the outbreak of chalkbrood. Future controlled studies are needed to further explore this connection and the role of specific elements in disease resistance.

Conclusions

Chalkbrood, caused by *Ascosphaera apis* spores, requires the presence of these spores in a colony before clinical symptoms appear. Within an infected colony, some larvae will develop into adult bees, while others will succumb and become mummies. Our findings reveal that the concentration of certain elements is higher in mummies compared to healthy larvae from the same colony, whereas the opposite is true for

other elements. This likely reflects differences in the nutritional quality of the food these larvae received, which is influenced by the composition of the pollen. Infected larvae destined to become mummies seem to be fed a diet richer in certain elements (P, S, K, Na, Mg, Ca, Cu, Zn, Rb, Cr, Mn, and Mo), while deficient in others (Al, B, Ag, Sb, Ba, Pb, As, Sr, Cd), some of which have antifungal properties. The absence of these antifungal elements in the diet could contribute to the onset of chalkbrood and the mummification of certain larvae within infected hives. However, more comprehensive studies involving different locations, timepoints, and larval ages are needed to draw more definitive conclusions.

For full text, refer to: Pavlović, R.; Brodschneider, R.; Goessler, W.; Stanisavljević, L.; Vujčić, Z.; Zarić, N.M. Micronutrient Deficiency May Be Associated with the Onset of Chalkbrood Disease in Honey Bees. *Insects* 2024, 15, 269. <https://doi.org/10.3390/insects15040269>

MSc. Ratko Pavlović, beekeeper
 Doctoral student in the field of bee nutrition at
 Faculty of Chemistry, University of Belgrade
 +381 63 879 0374, ratkopav@gmail.com



**Ratko Pavlović¹, Robert Brodschneider²,
 Walter Goessler³, Ljubiša Stanisavljević⁴,
 Zoran Vujčić¹, Nenad M. Zarić^{4,5}**

¹ University of Belgrade – Faculty of Chemistry, Department of Biochemistry, Belgrade, Serbia

² University of Graz, Institute of Biology, Graz, Austria;

³ University of Graz, Institute of Chemistry, Analytical Chemistry for Health and Environment, Graz, Austria

⁴ University of Belgrade - Faculty of Biology, Belgrade, Serbia;

⁵ Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology IFA-Tulln, University of Natural Resources and Life Sciences Vienna (BOKU), Tulln, Austria

References

1. Alaux, C.; Ducloz, F.; Crauser, D.; Le Conte, Y. Diet Effects on Honeybee Immunocompetence. *Biol Lett* 2010, 6, 562–565, doi:10.1098/rsbl.2009.0986.
2. Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E.L. Bee Declines Driven by Combined Stress from Parasites, Pesticides, and Lack of Flowers. *Science* (1979) 2015, 347, 1255957, doi:10.1126/science.1255957.
3. Danilíhlik, J.; Škrabišová, M.; Lenobel, R.; Šebela, M.; Omar, E.; Petřiválský, M.; Crailsheim, K.; Brodschneider, R. Does the Pollen Diet Influence the Production and Expression of Antimicrobial Peptides in Individual Honey Bees? *Insects* 2018, 9, doi:10.3390/insects9030079.
4. Brodschneider, R.; Crailsheim, K. Nutrition and Health in Honey Bees. *Apidologie* 2010, 41, 278–294, doi:10.1051/apido/2010012.
5. Di Pasquale, G.; Salignon, M.; Le Conte, Y.; Belzunces, L.P.; Decourtye, A.; Kretzschmar, A.; Suchail, S.; Brunet, J.-L.; Alaux, C. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? *PLoS One* 2013, 8, e72016.
6. Di Pasquale, G.; Alaux, C.; Le Conte, Y.; Odoux, J.-F.; Pioz, M.; Vaisière, B.E.; Belzunces, L.P.; Decourtye, A. Variations in the Availability of Pollen Resources Affect Honey Bee Health. *PLoS One* 2016, 11, e0162818.
7. Omar, E.; Abd-Ella, A.A.; Khodairy, M.M.; Moosbeckhofer, R.; Crailsheim, K.; Brodschneider, R. Influence of Different Pollen Diets on the Development of Hypopharyngeal Glands and Size of Acid Gland Sacs in Caged Honey Bees (*Apis Mellifera*). *Apidologie* 2017, 48, 425–436, doi:10.1007/s13592-016-0487-x.
8. Barroso-Arévalo, S.; Vicente-Rubiano, M.; Ruiz, J.A.; Bentabol, A.; Sánchez-Vizcaino, J.M. Does Pollen Diversity Influence Honey Bee Colony Health? *Spanish Journal of Agricultural Research* 2019, 17, doi:10.5424/sjar/2019173-13991.
9. Khan, K.A.; Ghramh, H.A.; Ahmad, Z.; El-Niweiri, M.A.A.; Mohammed, M.E.A. Honey Bee (*Apis Mellifera*) Preference towards Micronutrients and Their Impact on Bee Colonies. *Saudi J Biol Sci* 2021, 28, 3362–3366, doi:10.1016/j.sjbs.2021.02.084.
10. Ilijević, K.; Vujanović, D.; Orčić, S.; Purać, J.; Kojić, D.; Zarić, N.; Gržetić, I.; Blagojević, D.P.; Čelić, T. V Anthropogenic Influence on Seasonal and Spatial Variation in Bioelements and Non-Essential Elements in Honeybees and Their Hemolymph. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology* 2021, 239, doi:10.1016/j.cbpc.2020.108852.
11. Monchanin, C.; Blanc-Brude, A.; Drujont, E.; Negahi, M.M.; Pasquaretta, C.; Silvestre, J.; Baqué, D.; Elger, A.; Barron, A.B.; Devaut, J.-M.; et al. Chronic Exposure to Trace Lead Impairs Honey Bee Learning. *Ecolotoxicol Environ Saf* 2021, 212, 112008, doi:https://doi.org/10.1016/j.ecoenv.2021.112008.
12. Burge, P.S.; Scott, J.A.; McCoach, J. Occupational Asthma Caused by Aluminium. *Allergy* 2000, 55, 779–780, doi:https://doi.org/10.1034/j.1398-9995.2000.00641.x.
13. Al osman, M.; Yang, F.; Massey, I.Y. Exposure Routes and Health Effects of Heavy Metals on Children. *BioMetals* 2019, 32, 563–573, doi:10.1007/s10534-019-00193-5.
14. Rani, A.; Kumar, A.; Lal, A.; Pant, M. Cellular Mechanisms of Cadmium-Induced Toxicity: A Review. *Int J Environ Health Res* 2014, 24, 378–399, doi:10.1080/09603123.2013.835032.
15. Al osman, M.; Yang, F.; Massey, I.Y. Exposure Routes and Health Effects of Heavy Metals on Children. *BioMetals* 2019, 32, 563–573.
16. Bonoan, R.E.; TAI, T.M.; TAGLE RODRIGUEZ, M.; FELLER, L.; DAD-DARIO, S.R.; CZAJA, R.A.; O'CONNOR, L.D.; BURRUSS, G.; STARKS, P.T. Seasonality of Salt Foraging in Honey Bees (*Apis Mellifera*). *Ecol Entomol* 2017, 42, 195–201, doi:https://doi.org/10.1111/een.12375.
17. Schmickl, T.; Crailsheim, K. Inner Nest Homeostasis in a Changing environment with Special Emphasis on Honey Bee Brood nursing and Pollen Supply. *Apidologie* 2004, 35, 249–263.
18. Foley, K.; Fazio, G.; Jensen, A.B.; Hughes, W.O.H. Nutritional Limitation and Resistance to Opportunistic Aspergillus Parasites in Honey Bee Larvae. *J Invertebr Pathol* 2012, 111, 68–73, doi:https://doi.org/10.1016/j.jip.2012.06.006.
19. Gerdtz, J.R.; Roberts, J.M.K.; Simone-Finstrom, M.; Ogbourne, S.M.; Tucci, J. Genetic Variation of *Ascosphaera Apis* and Colony Attributes Do Not Explain Chalkbrood Disease Outbreaks in Australian Honey Bees. *J Invertebr Pathol* 2021, 180, 107540, doi:https://doi.org/10.1016/j.jip.2021.107540.
20. Aronstein, K.A.; Murray, K.D. Chalkbrood Disease in Honey Bees. *J Invertebr Pathol* 2010, 103, S20–S29, doi:https://doi.org/10.1016/j.jip.2009.06.018.
21. Rowland, B.W.; Rushton, S.P.; Shirley, M.D.F.; Brown, M.A.; Budge, G.E. Identifying the Climatic Drivers of Honey Bee Disease in England and Wales. *Sci Rep* 2021, 11, 21953, doi:10.1038/s41598-021-01495-w.
22. Sevim, A.; Akpinar, R.; Karaoğlu, Ş.A.; Bozdeveci, A.; Sevim, E. Prevalence and Phylogenetic Analysis of *Ascosphaera Apis* (Maassen Ex Clausen) LS Olive & Spiltoir (1955) Isolates from Honeybee Colonies in Turkey. *Biologia (Bratisl)* 2022, 77, 2689–2699, doi:10.1007/s11756-022-01114-7.
23. Jensen, A.B.; Aronstein, K.; Flores, J.M.; Vojvodic, S.; Palacio, M.A.; Spivak, M. Standard Methods for Fungal Brood Disease Research. *J Apic Res* 2013, 52.
24. Heath, L.A.F. Development of Chalk Brood in a Honeybee Colony: A Review. *Bee World* 1982, 63, 119–130, doi:10.1080/0005772x.1982.11097876.
25. Deneke, Y.A.; Dero, B.S.; Mekonnen, A.S. Review on Chalkbrood Disease of Honey Bee. *Vet Med Open J* 2023, 8, 47–55, doi:10.17140/VMOJ-8-176.
26. Evison, S.E.F. Chalkbrood: Epidemiological Perspectives from the Host-Parasite Relationship. *Curr Opin Insect Sci* 2015, 10, 65–70, doi:https://doi.org/10.1016/j.cois.2015.04.015.
27. Castagnino, G.L.B.; Mateos, A.; Meana, A.; Montejo, L.; Zamorano Iturralde, L.V.; Cutuli De Simón, M.T. Etiology, Symptoms and Prevention of Chalkbrood Disease: A Literature Review. *Revista Brasileira de Saude e Producao Animal* 2020, 21.
28. Gilliam, M.; Taber, S.; Lorenz, B.J.; Prest, D.B. Factors Affecting Development of Chalkbrood Disease in Colonies of Honey Bees, *Apis Mellifera*, Fed Pollen Contaminated with *Ascosphaera Apis*. *J Invertebr Pathol* 1988, 52, 314–325, doi:https://doi.org/10.1016/0022-2011(88)90141-3.
29. Flores, J.M.; Ruiz, J.A.; Ruz, J.M.; Puerta, F.; Bustos, M.; Padilla, F.; Campano, F. Effect of Temperature and Humidity of Sealed Brood on Chalkbrood Development under Controlled Conditions. *Apidologie* 1996, 27, 185–192, doi:10.1051/apido:19960401.
30. Yoder, J.A.; Nelson, B.W.; Main, L.R.; Lorenz, A.L.; Jajack, A.J.; Aronstein, K.A. Water Activity of the Bee Fungal Pathogen *Ascosphaera Apis* in Relation to Colony Conditions. *Apidologie* 2017, 48, 159–167, doi:10.1007/s13592-016-0461-7.
31. Puerta, F.; Flores, J.M.; Bustos, M.; Padilla, F.; Campano, F. Chalkbrood Development in Honeybee Brood under Controlled Conditions. *Apidologie* 1994, 25, 540–546, doi:10.1051/apido:19940604.
32. Dolezal, A.G.; Toth, A.L. Feedbacks between Nutrition and Disease in Honey Bee Health. *Curr Opin Insect Sci* 2018, 26, 114–119, doi:https://doi.org/10.1016/j.cois.2018.02.006.
33. DeGrandi-Hoffman, G.; Chen, Y.; Huang, E.; Huang, M.H. The Effect of Diet on Protein Concentration, Hypopharyngeal Gland Development and Virus Load in Worker Honey Bees (*Apis Mellifera* L.). *J Insect Physiol* 2010, 56, 1184–1191, doi:https://doi.org/10.1016/j.jinsphys.2010.03.017.
34. Goblirsch, M. Nosema Ceranae Disease of the Honey Bee (*Apis Mellifera*). *Apidologie* 2018, 49, 131–150, doi:10.1007/s13592-017-0535-1.
35. Zarić, N.M.; Brodschneider, R.; Goessler, W. Honey Bees as Biomonitors – Variability in the Elemental Composition of Individual Bees. *Environ Res* 2022, 204, doi:10.1016/j.envres.2021.112237.
36. Pavlović, R.; Dojnov, B.; Šokarda Slavić, M.; Pavlović, M.; Slomo, K.; Ristović, M.; Vujčić, Z. In Pursuit of the Ultimate Pollen Substitute (Insect Larvae) for Honey Bee (*Apis Mellifera*) Feed. *J Apic Res* 2022, 1–10, doi:10.1080/00218839.2022.2080950.
37. Hellmich II, R.L.; Kulinčević, J.M.; Rothenbuhler, W.C. Selection for High and Low Pollenhoarding Honey Bees. *Journal of Heredity* 1985, 76, 155–158, doi:10.1093/oxfordjournals.jhered.a110056.
38. Henry, M.; Rodet, G. Controlling the Impact of the Managed Honeybee on Wild Bees in Protected Areas. *Sci Rep* 2018, 8, 9308, doi:10.1038/s41598-018-27591-y.
39. Taylor, M.P.; Gillings, M.M.; Fry, K.L.; Barlow, C.F.; Gunkel-Grillion, P.; Gueyte, R.; Camoin, M. Tracing Nickel Smelter Emissions Using European Honey Bees. *Environmental Pollution* 2023, 335, doi:10.1016/j.envpol.2023.122257.
40. Gekière, A.; Vanderplanck, M.; Michez, D. Trace Metals with Heavy Consequences on Bees: A Comprehensive Review. *Science of the Total Environment* 2023, 895, 165084.
41. Zarić, N.M.; Brodschneider, R.; Goessler, W. Sex-Specific Element Accumulation in Honey Bees (*Apis Mellifera*). *Environmental Science and Pollution Research* 2024, doi:10.1007/s11356-024-32822-z.
42. Sagili, R.R.; Metz, B.N.; Lucas, H.M.; Chakrabarti, P.; Breece, C.R. Honey Bees Consider Larval Nutritional Status Rather than Genetic Relatedness When Selecting Larvae for Emergency Queen Rearing. *Sci Rep* 2018, 8, doi:10.1038/s41598-018-25976-7.
43. Huang, Z.Y.; Otis, G.W. Inspection and Feeding of Larvae by Worker Honey Bees (Hymenoptera: Apidae): Effect of Starvation and Food Quantity. *J Insect Behav* 1991, 4, 305–317, doi:10.1007/BF01048280.
44. Lindauer, M.; Watkin, B. Division of Labour in the Honeybee Colony. *Bee World* 1953, 34, 63–73, doi:10.1080/0005772x.1953.11094788.
45. Mayack, C.; Naug, D. Energetic Stress in the Honeybee *Apis Mellifera* from Nosema Ceranae Infection. *J Invertebr Pathol* 2009, 100, 185–188, doi:10.1016/j.jip.2008.12.001.
46. Sagili, R.R.; Metz, B.N.; Lucas, H.M.; Chakrabarti, P.; Breece, C.R. Honey Bees Consider Larval Nutritional Status Rather than Genetic Relatedness When Selecting Larvae for Emergency Queen Rearing. *Sci Rep* 2018, 8, doi:10.1038/s41598-018-25976-7.
47. Liu, C.; Steere, L.; McGregor, C.; Frederick, B.H.; Pastoor, T.; Zhou, Y.; Liu, C.T.; Cai, Y.; Zhou, H.; Xu, M.; et al. Exploring Boron Applications in Modern Agriculture: A Structure-Activity Relationship Study of a Novel Series of Multi-Substitution Benzoxaboroles for Identification of Potential Fungicides. *Bioorg Med Chem Lett* 2021, 43, 128089, doi:https://doi.org/10.1016/j.bmcl.2021.128089.
48. Avis, T.J.; Rioux, D.; Simard, M.; Michaud, M.; Tweddell, R.J. Ultrastructural Alterations in Fusarium Sambucinum and Heterobasidion Annosum Treated with Aluminum Chloride and Sodium Metabisulfite. *Phytopathology* 2009, 99, 167–175, doi:10.1094/PHYTO-99-2-0167.
49. Gauthier, M.; Aras, P.; Jumarie, C.; Boily, M. Low Dietary Levels of Al, Pb and Cd May Affect the Non-Enzymatic Antioxidant Capacity in Caged Honey Bees (*Apis Mellifera*). *Chemosphere* 2016, 144, 848–854, doi:https://doi.org/10.1016/j.chemosphere.2015.09.057.
50. Zarić, N.M.; Ilijević, K.; Stanislavljević, L.; Gržetić, I. Metal Concentrations around Thermal Power Plants, Rural and Urban Areas Using Honeybees (*Apis Mellifera* L.) as Bioindicators. *International Journal of Environmental Science and Technology* 2016, 13, 413–422, doi:10.1007/s13762-015-0895-x.
51. Zhang, G.; Zhang, W.; Cui, X.; Xu, B. Zinc Nutrition Increases the Antioxidant Defenses of Honey Bees. *Entomol Exp Appl* 2015, 156, 201–210, doi:https://doi.org/10.1111/eea.12342.
52. Hýbl, M.; Šipoš, J.; Krejčová, A.; Sodomová, K.; Polák, O.; Koláčková,

I.; Mráz, P. Preference of Pollinators over Various Forage Mixtures and Microelement Treatments. *Agronomy* 2022, 12, doi:10.3390/agronomy12020370.

53. Hussain, R.; Hasan, M.; Iqbal, K.J.; Zafar, A.; Tariq, T.; Saif, M.S.; Hassan, S.G.; Shu, X.; Caprioli, G.; Anjum, S.I. Nano-Managing Silver and Zinc as Bio-Conservational Approach against Pathogens of the Honey Bee. *J Biotechnol* 2023, 365, 1–10, doi:https://doi.org/10.1016/j.jbiotec.2023.01.009.

54. Pacheco, N.I.N.; Semerad, J.; Pivokonsky, M.; Cajthaml, T.; Filip, J.; Busquets-Fité, M.; Dvorak, J.; Rico, A.; Prochazkova, P. Effects of Silver Sulfide Nanoparticles on the Earthworm *Eisenia Andrei*. *Comparative Biochemistry and*

Physiology Part C: Toxicology & Pharmacology 2022, 257, 109355, doi:https://doi.org/10.1016/j.cbpc.2022.109355.

55. Sibiya, A.; Gopi, N.; Jeyavani, J.; Mahboob, S.; Al-Ghanim, K.A.; Sultana, S.; Mustafa, A.; Govindarajan, M.; Vaseeharan, B. Comparative Toxicity of Silver Nanoparticles and Silver Nitrate in Freshwater Fish *Oreochromis Mossambicus*: A Multi-Biomarker Approach. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 2022, 259, 109391, doi:https://doi.org/10.1016/j.cbpc.2022.109391.

BEES LIFE

Agricultural Institute of Slovenia

BEEKEEPING ACADEMY OF SLOVENIA

We provide non-formal education and training in the field of beekeeping.

Current educational programmes

- Apitourism
- Beekeeping with AŽ beehives
- Queen breeding and selection
- Guidelines for good hygiene habits in beekeeping
- Honeybee nutrition



Luxury anti-age cream with **BEE VENOM** and **COENZYME Q10**

REVOLUTIONARY SKINCARE

A rich blend of hyaluronic acid, natural antioxidants, vitamins and nourishing oils.

Contains the highest quality bee venom also called **natural botox**, as it is scientifically proven to reduce wrinkles.

With regular daily care, it deeply nourishes, intensely hydrates, reduces wrinkles and ensures a youthful, glowing skin.

REJUVENATED SKIN IN JUST 30 DAYS



More than just a cream, Elixir is a promise of eternal beauty, self-confidence and self-love.

LAMORIX®

Available at www.lamorix.com.

NEW SCALE

NEW DIMENSION OF BEEHIVE MONITORING

Stay connected with your beehive like never before and enjoy the peace of mind that comes with advanced monitoring and security!

We are excited to announce that our latest product is available - the BeeConn Lite GPS beekeeping scale for 374,99€! Here's what the BeeConn Lite GPS beekeeping scale offers:

Advanced monitoring with GPS precision

The BeeConn Lite GPS introduces a new level of beehive monitoring by integrating GPS functionality. This feature ensures that you can keep an accurate and real-time track of your hive's location. Enhanced security guaranteed!

Ideal for small-scale beekeeping

Perfectly suited for smaller beekeepers and beginners, the BeeConn Lite GPS is designed to

monitor a single hive. This makes it an accessible and manageable tool for those who are just starting out or maintaining a modest apiary.

Real-time data transmission

The scale sends data on the hive's weight, temperature, and humidity up to three times a day via SMS. This frequent and reliable data transmission allows you to stay informed about the condition of your hive, ensuring that you can respond promptly to any changes.

Security alerts and theft prevention

The built-in alerts for movements (for example animal attack) and potential thefts enable immediate responsiveness. If there's any unusual activity, such as movement or an attempted intrusion, you'll receive instant tracking data! If someone wants to break into the scale,



you will receive his location and telephone number.

This proactive features ensures that you can take action swiftly to protect your hive.

Solar-powered efficiency

The BeeConn Lite GPS is powered by an included solar panel - eco-friendly power source, ensuring sustainable and continuous operation. With no batteries required, it eliminates the need for battery replacements by relying on built-in capacitors.

Easy setup and communication

Setting up your BeeConn Lite GPS is straightforward, with simple SMS communication facilitating the process. This user-friendly approach ensures that even those with minimal technical experience can get their scale up and running quickly. Whether you're at home or away on vacation or



business trip, the BeeConn Lite GPS ensures that your beekeeping activities are never out of reach.

BeeConn - where technology meets tradition for the ultimate beekeeping experience!

Useful information:

Website:

www.beeconn.net

Instagram:

<https://www.instagram.com/beeconn.si/>

Facebook:

<https://www.facebook.com/beeconn.si/>

BeeConn LITE GPS

- 100% Wireless
- Compact aluminium casting
- High precision measurements
- Weight sensor
- For all types of hives
- SMS message 3x per day
- Precise location and scale movement tracking
- Autonomous system, no battery/electricity needed
- Real-time monitoring and data collection

ADVANCING BEE CONSERVATION: INNOVATIVE TECHNOLOGIES FOR PROTECTING HONEYBEE POPULATIONS WITH ILLUMINUM & BEESAGE

The protection of European honeybees is becoming increasingly critical as these vital pollinators face numerous threats from invasive species, diseases, and environmental changes. In response, a collaborative project involving Illuminum, BeeSage, and MG Sustainable Engineering is leveraging advanced technologies such as Geographic Information Systems (GIS), Internet of Things (IoT), and predictive analytics to develop innovative solutions for bee conservation.

The Challenge: Protecting European Honeybees

Honeybees play an essential role in pollinating crops and maintaining biodiversity, yet they are facing significant threats that jeopardize their survival. Among the most dangerous of these are invasive species like the Asian yellow-legged hornet (*Vespa velutina*), the Tropilaelaps mite,

Asian yellow-legged hornet

(*Vespa Velutina*)

Description of *Vespa velutina*
Vespa velutina, commonly known as the Asian hornet or yellow-legged hornet, is a species of hornet native to Southeast Asia. It is distinguished by its dark brown or black velvety body, with a distinctive orange-yellow band on the fourth abdominal segment. The legs are yellow-tipped, giving it the name "yellow-legged hornet." *Vespa velutina* is a predatory wasp that primarily feeds on other insects, particularly bees, posing a significant threat to beekeeping and local ecosystems in areas where it has been introduced.

Data Source Acknowledgment
 The data provided in this description was extracted from GAIA.ECO, a comprehensive environmental database offering detailed information on various species and their ecological impacts.



SIZE: 2 – 2,5 cm
REACH RADIUS: 5–20 km
LIFESPAN: 6–12 weeks






Filter the data

Visualize the spread

Find the nests

Picture 1

and the Small hive beetle. These pests are spreading rapidly across Europe, posing severe risks to bee colonies. Additionally, existing diseases and pests such as the Varroa mite, American foulbrood, and European foulbrood are further contributing to the decline of honeybee populations. The combination of these invasive species and ongoing threats has created a complex and urgent challenge for beekeepers and environmental scientists alike (picture 1).

While each European country has its own monitoring systems—typically basic GIS services focused on visualizing the spread of foulbrood—these systems mostly do not provide real-time data or fast enough warning systems to be truly effective. This limitation reduces their ability to manage and respond to the wide array of threats that bees are facing today in a timely manner.

The Solution: A Comprehensive Monitoring and Management Platform

Our project focuses on developing a sophisticated platform that integrates GIS and IoT technologies to monitor and manage bee health more effectively. Central to this platform will be a joint reporting system available through an app, po-

tentially iNaturalist, where users can report sightings of invasive species and diseases. The data collected through the app will be funneled into our platform, enabling real-time tracking, analysis, and the generation of warnings based on the test developments (picture 2).

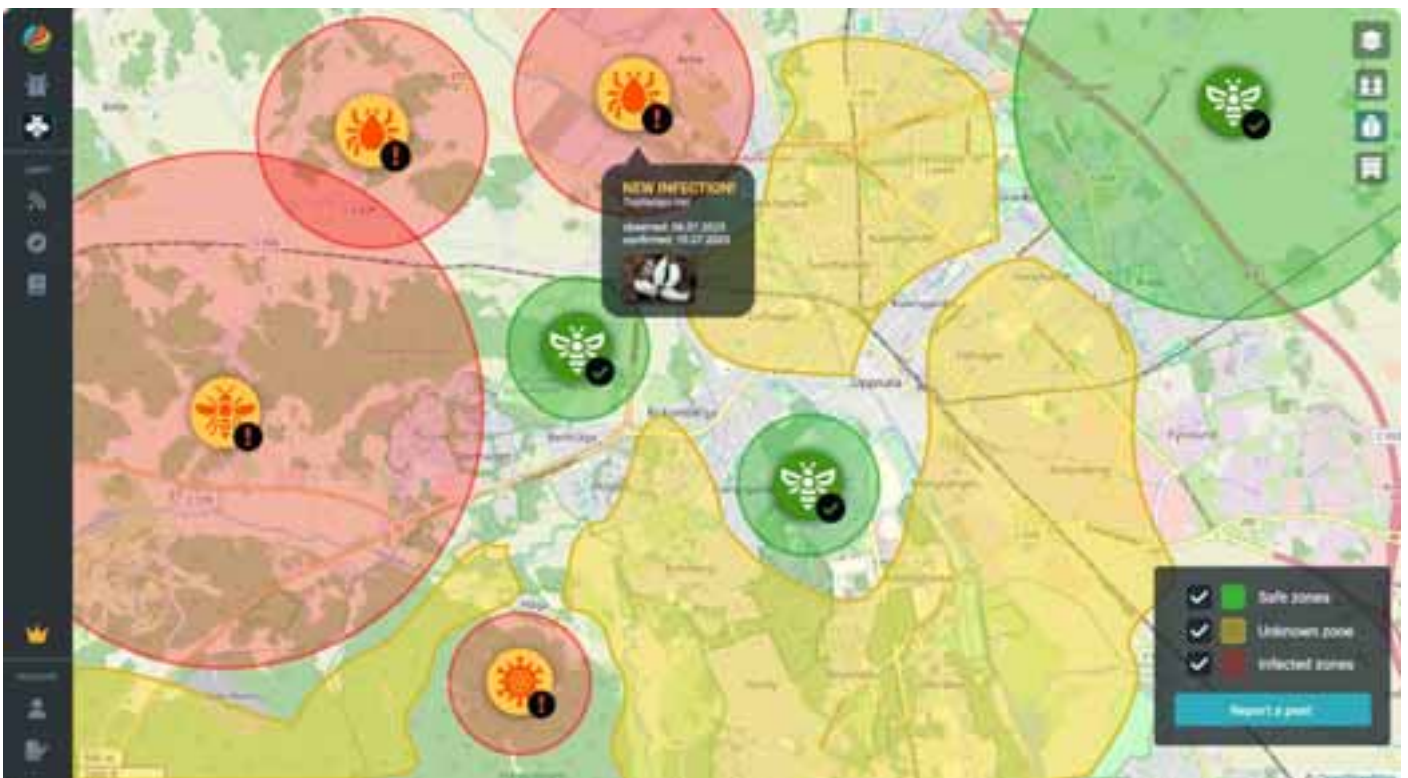
Because we understand that tracking is only effective when supported by comprehensive data, the reporting and warning components of this tracking system will be made freely available to everyone. This approach ensures that the system can gather the broadest possible dataset, facilitating more accurate analysis and enhancing the fight against the spread of invasive species and existing pests (picture 3).

The warning system and data analysis will be accessible to all users on the joint platform, connected to BeeSage's IoT network of hive users, with the aim of expanding to a Europe-wide user base.

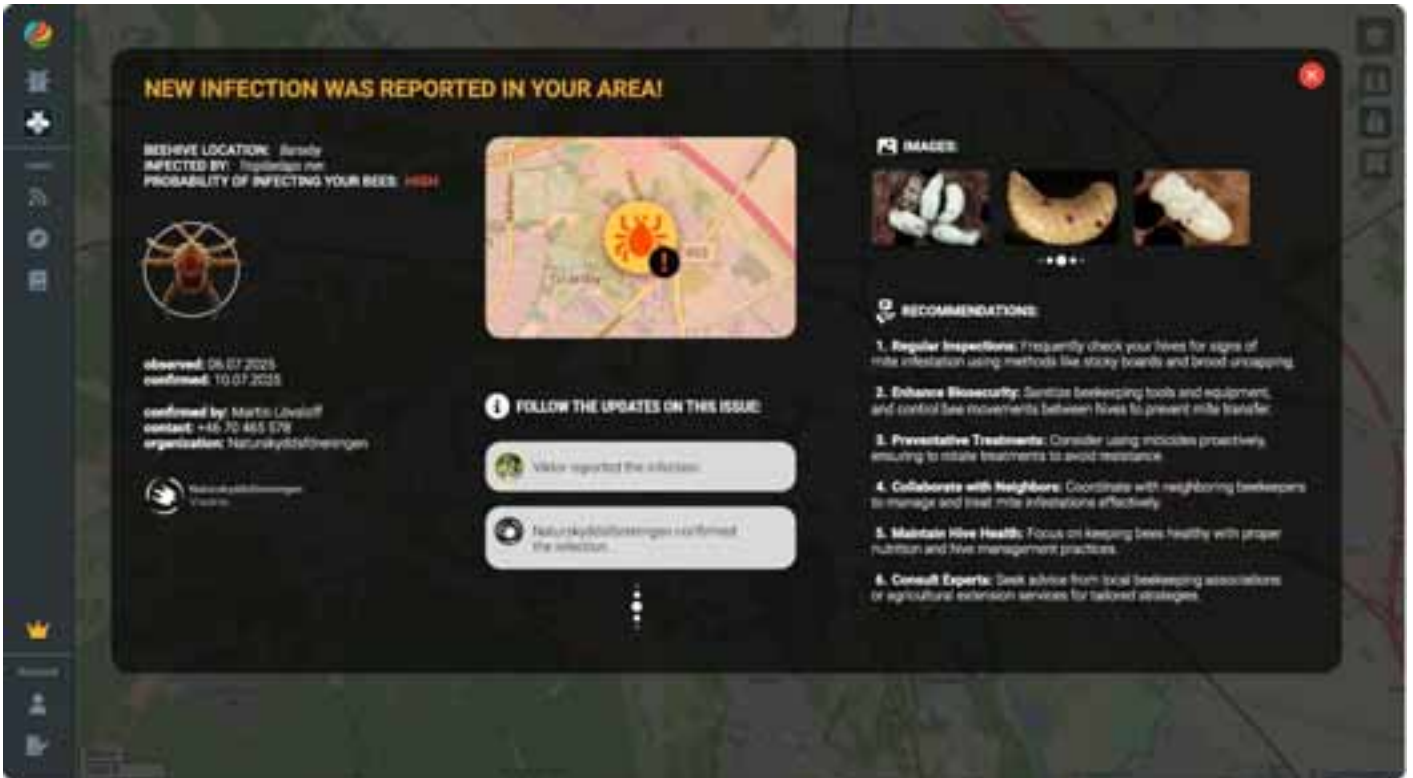
Key Features of the Platform

1. Real-Time Pest and Disease Tracking:

- Through the joint reporting app and integration with Gaia.eco, the platform will provide real-time tracking of invasive species and pests such as *Vespa velutina*, *Tropilaelaps*, the Small hive beetle, and others. The system will generate



Picture 2



Picture 3

timely warnings and offer comprehensive analysis to users across Europe, contributing to more effective management and response strategies.

3. Currently Testing/Building on BeeSage's Platform:

- Our project is being implemented on BeeSage's existing platform, which is known for its smart beehive scales and data-driven approach

to beekeeping. BeeSage offers tools that help beekeepers monitor hive weight, temperature, and other critical factors, ensuring productivity and sustainability. BeeSage is also developing a Smart Hive Node Sensor that will enable sound recognition and humidity monitoring, further enhancing the platform's ability to detect early warning signs of potential threats. The ultimate goal is



Picture 4

to expand this platform's reach to cover all of Europe, providing beekeepers across the continent with the resources they need to protect their hives effectively (picture 4).

Progress and Collaboration

Significant progress has been made in developing this platform, particularly during a recent event held in Uppsala in June 2024, where experts and participants collaborated to refine the system's features. This event also provided a valuable opportunity to gather feedback from researchers and industry professionals, further enhancing the platform's effectiveness.

Call to Action

As development continues, we invite all organizations, companies, beekeepers, and individuals interested in contributing to this project or utilizing our solutions to contact us. Your involve-

ment could play a crucial role in safeguarding honeybee populations and, by extension, our environment. For more information or to get involved, please reach out to us at info@illumium.se or ru@beesage.co.

Together, we can make a significant impact on bee conservation and ensure the sustainability of our agricultural ecosystems for future generations.

In addition to its benefits for beekeeping, the reporting system will also be invaluable in fruit cultivation and other agricultural fields where invasive pests can be tracked. By providing farmers with real-time data on the spread of these pests, the system will help them take proactive measures to protect their crops and improve overall agricultural productivity. This cross-industry application makes the platform a powerful tool not only for beekeepers but also for a wide range of stakeholders in the agricultural sector.

Žan Križnar

zan.kriznar@illumium.se



Medikoel

INHALATION FROM THE HIVE
With the inhalation sets API-AEROSOL I, API-AEROSOL II and API-AEROSOL Plus, you can perform aerosol apitherapy directly from the hive. You can choose between sets with natural draft and sets with forced draft.

HONEY PACKAGING IN SACHETS
We pack honey into convenient packaging, in 8-gram sachets. Let your honey in practical packaging be present everywhere, both in restaurants and in every hiker's backpack.

PROPO STEAM - PROPOLIS EVAPORATOR
The Propo Steam apitherapeutic device allows us to perform inhalation therapy by heating natural propolis. With the included accessories, the user can perform inhalations by themselves or with the help of an apitherapist, which are beneficial for respiratory system issues. Propo Steam is also suitable for cleaning airborne particles, microbes and organic solvents from the air. It pleasantly scents the space with the aroma of propolis.

Medikoel d.o.o., Jalnova cesta 2, 4240 Radovljica, Slovenia,
T: +386 4 537 85 10 • M: + 386 41 618 775 • info@medikoel.com • www.medikoel.com

INTERVIEW

CARLA MARINA MARCHESE

HONEY SOMMELIER



Introduction

Carla Marina Marchese, Writer, Honey Sensory Expert and Founder of Red Bee Honey.

She started beekeeping by accident, when being a female beekeeper was downright provocative (and unheard of), and now her company, Red Bee Honey, has grown – 24 years strong – into a sought-after national brand. A graduate of the School of Visual Arts – she had an international design career for 15 years– she became enthralled with bees and the terroir of honey after a visit to a neighbor’s apiary in 2000. She soon dove headfirst into beekeeping, taking courses, acquiring Italian honeybees and eventually making her own honey under her own brand. What sets her product apart -- aside from being the first Italian trained honey expert in the US - was her

design sensibilities and signature apothecary bottles, elevating honey to a high art.

After selling for 10 years at the New Canaan farmer's market as well as in other regional venues, her products are now sold nationally to celebrity chefs and the finest cheese and food stores.

On a visit to Montalcino, Italy, aka “The City of Honey,” she became passionate about the diverse flavor profiles of varietal honey then signed up for their honey courses. Red Bee flavors include bamboo, blueberry, clover and wildflower and are not to be poured in tea, per say -- Marchese describes her honeys like fine wines -- but are better suited for finishing and pairing, i.e. for drizzling on muffins, adding to salads or serving with cheeses.

Along with the local honey she produces on her Weston farm – Red Bee specializes in rare



hired to design giftware in China for a few years, it was that year I was invited to visit my neighbor's apiary. I did not know anything about bees yet after seeing how docile they were and tasting fresh honey from the hive I became intrigued. Soon after I got my own hives of honeybees and began a new unexpected journey.

Naturally, I began selling my own honey and Red Bee Honey was born.

Red Bee began as a luxury brand of honey with beautiful bottle and labels. I felt strongly that honey deserved to be regarded as a noble food and given the same respect as wine or olive oil. During this time, I was still employed as an artist and had begun illustrating my own collection of greeting cards featured a queen bee named Rosie cards under the Red Bee name, and have appeared in on the cover of American Bee Journal in 2003.

I never thought that my creativity would harmonize with honeybees but passions can take you on a journey that can change the course of your life. I combined my creative spirit with bees to make my passion into a business.

2. How is it to be a woman in a business where men are prevalent?

It can be a challenge for women to be accepted in the workplace, especially in businesses that have been traditionally dominated by men. Simultaneously, we are discussing honeybees



and unique honeys from around the country, all sourced ethically and with top-notch quality control.

In addition, Red Bee produces other honey-related products such as lip balms, soaps, body oils, beeswax candles and gifts.

Marchese, who's big on educational workshops, is the author of *Honeybee: Lessons from an Accidental Beekeeper* and the co-author with Kim Flottum, editor of *Bee Culture* magazine, *The Honey Connoisseur: Selecting, Tasting, and Pairing Honey*. She is a member of the Italian Register of Experts in the Sensory Analysis of Honey, founder of The American Honey Tasting Society and acts as a consultant with The National Honey Board, Slow Food USA and other commercial honey companies.

Carla Marina Marchese she accepted our invitation with pleasure and gave an interview, which we transcribe below.

1. Describe your evolution from a designer to a beekeeper?

After graduating from the School of Visual Arts, I began working as a freelance designer and illustrator for textiles, stationary and giftware clients. Although New York City is a very competitive city, I was determined to pursue a career in the arts. My passion for design, pattern and color led me to illustrate for children's books, educational materials and greeting cards. In 2000 I was



3. What is a „honey sommelier“?

I coined the term honey sommelier in my first book *HONEYBEE: Lessons from an Accidental Beekeeper*. It is a term I made up for the chapter about honey tasting to explain the work I am doing and my training in Italy. There is not a title for a honey expert so honey sommelier seemed to be the best representation of this work. My formal training in Italy and journey to becoming a honey sensory expert was the most challenging and rewarding opportunity in my life. Honey and wine have remarkable parallels that many people may not understand.

My personal definition of a honey sommelier is essentially someone who is formally trained and knowledgeable about all aspects about honeybees, and honey from the production, harvest and most importantly the sensory qualities of many different types of honey then is able to articulate its flavor profiles and how to pair them with food. I enjoy consulting with chefs, restaurants and food professionals by introducing them varietal and rare honeys, write tasting notes and pairing menus for each. I feel very blessed to be able to follow my passion and bring attention to the finer aspects of honey. Honey has not been 100% accepted by the culinary professionals but it is changing and I am working very hard so that honey can achieve the same respect rightfully as wine and olive oil.

4. Are the terms used in the description and analysis of honey taken from wine or are they authentic honey terms?

Many of these words used to describe honey can also be used to describe wine, cheese, olive oil, chocolate and even coffee. Humans can experience thousands of different aromas and flavors so it makes sense that some of the descriptors would overlap wine and other foods. The honey aroma and flavor wheel list only a few of the many words we use to describe honey, since each one of us have our own taste memories and food experiences, tasters are encouraged to be creative and find their own words. In some countries like Italy, the honeys produced there have been identified through chemical, pollen and sensory analyses and have specific

where the females perform most of the hive duties and is guided by the queen bee's pheromones. We find in ancient cultures like Egypt that women commonly held positions of great power so there are plenty of female role models celebrated for accomplishing important works in all aspects of life including beekeeping. With dedication and perseverance, anything is possible!

I have been honored to work closely alongside two very prominent men in beekeeping. My second book, *The Honey Connoisseur Selecting, Tasting, and Pairing Honey, With a Guide to More Than 30 Varietals* was co-authored with (Mr.) Kim Flottum, (editor of *Bee Culture* magazine). I was also invited to write the chapter on honey in the international best-selling book *Beekeeping for Dummies* for my mentor and friend Howland Blackiston. Mr. Blackiston is the neighbor who introduced me to honeybees in 2000.

Presently, my passion project is educating about honey through my organization, The American Honey Tasting Society, based upon my training in Italy as a honey tasting expert. My partners are Raffaele Dall'Olio and Gian Luigi Marcazzan both from Bologna, Italy who both come to the USA a few times a year to teach sensory training in honey.

My hope is that the next generation of young women will have more opportunities to follow their dreams and that my journey would open doors for women everywhere to show them that they can achieve anything in life that they want however they must be dedicated and work hard.

profiles in a database that was developed by their register of honey tasting experts.

5. Can the best tasters discover fake honey, as well as various additives in honey, such as flavor enhancers, artificial colors, extracts?

There are many ways that honey can be compromised or adulterated, the processes have become quite sophisticated that it has become much more difficult to identify honey fraud in the lab. Expert honey taster are trained to identify defects: fermentation, smoke, smoke, metallic or thymol that cannot be easily detected in the lab.

Those who are familiar with a specific varietal honey from specific floral source and region can make educated judgements and possibly be able to identify off flavors however, sensory analysis it is not about detecting adulteration as it is about identifying floral sources through sensory evaluation. Honey fraud is best confirmed by a qualified lab.

6. Can the consumers learn to analyze the taste of honey?

Yes, with formal training anyone can learn to the methods and techniques to be a honey sensory expert. However, like all sensory work, it requires practice, a firm commitment to expanding your tasting experiences and vocabulary, with a conscious dedication. Humans are not perfect or identical so each one of us has personal taste memories and experiences that will affect the evaluation process in different ways.

7. What are your experiences with many trips? Can you talk about the culture of consuming honey from certain nations in Europe, America, Asia?

I enjoy traveling to discover new cultures, art, food and especially the local honey.

Honey is a product of the local floral sources, climate, season and soil so you can learn much about a region from its honey but also the beekeepers and their traditions of how they produce, harvest and use honey into their food culture.

I find these stories fascinating and honey can reveal the true character and culture of a region. As I travel I learn the recipes of each culture, for example one of my favorite ways to eat honey is a *sedeas* which is a warm fried dough cake filled with cheese from Sardegna or Japanese *castella*, honey cake.

8. Have your trips in Italy affected your products and methods?

The inspiration for all of my Red Bee products is deeply influenced by my Italian roots. My love of high quality, design and craftsmanship are the essence of my culture and are woven into everything I do. Designers know that consumers buy with their eyes so products, including honey must be visually appealing but also delicious. Customers appreciate quality and good customer service and will return as loyal buyers.

9. Mixing honey with some food? What is the best way to do it?

I love the challenge of pairing a particular honey with interesting food and beverages by matching complimentary or contrasting flavors. I generally eat fresh foods and simple dishes that I prepare at home. I exclusively use honey because of its many flavors and health benefits. It is perfect with olive oil and vinegar over fresh





with all type of foods. There never is a wrong way to eat honey, if you like it then it is good!

... and what is your favorite combination?

Honeycomb is perfect for special occasions or celebrations I like it with triple crème cheeses like Robiola due Latti, crusty hand baked Italian peasant bread and a glass of sparkling Prosecco. Add some nuts, olives, fruits or vegetables... enjoy!

10. Please give our readers an exclusive recipe with honey?

Page 194 of The Honey Connoisseur Honey Struck Chocolate Truffles Recipe-see photo. My favorite healthy way to enjoy honey and chocolate.

From my kitchen to your readers.....Thank you!

Vlastimir Spasić

Editor in chief of "Serbian beekeeper"

+381 60 444 0110

srpskipcelar@gmail.com

Photos courtesy of Red Bee, LLC 2019

greens, a simple honey butter or mustard can be extremely versatile as well. I bake with very little sweetener then drizzle the honey on the warm cake or bread from the oven. I do prefer to use honey rather than sugar but when I find a delicious honey I like to enjoy it with cheeses on bread. Especially on warm toast with butter and nuts. Honey is very versatile and can be enjoyed

SIMPLE HONEY RECIPES

Honey Struck Chocolate Truffles

SERVES: 4 PREP TIME: 20 minutes

2 cups almonds, cashews, or walnuts
 8 cup cacao powder
 3 dates, dried and pitted
 ¼ cup buckwheat honey
 1 tablespoon coconut butter
 Pinch of sea salt
 Water, 2 tablespoons as needed
 Shredded unsweetened coconut

In a food processor, grind the nuts of your choice. Add the cacao powder, dates, honey, coconut butter, and sea salt and blend. Add enough water to make the mixture moist but not wet. Hand-roll the honey-chocolate mixture into small balls, the size of cherries. Roll the truffles in the shredded coconut and store in the fridge for 1 hour before serving.

BEES LIFE





A new book

Carla Marina Marchese

THE WORLD ATLAS OF HONEY

A beautifully illustrated global survey of the flavor of honey, *The World Atlas of Honey* includes profiles of more than eighty countries and the botanical sources of honey found in each.

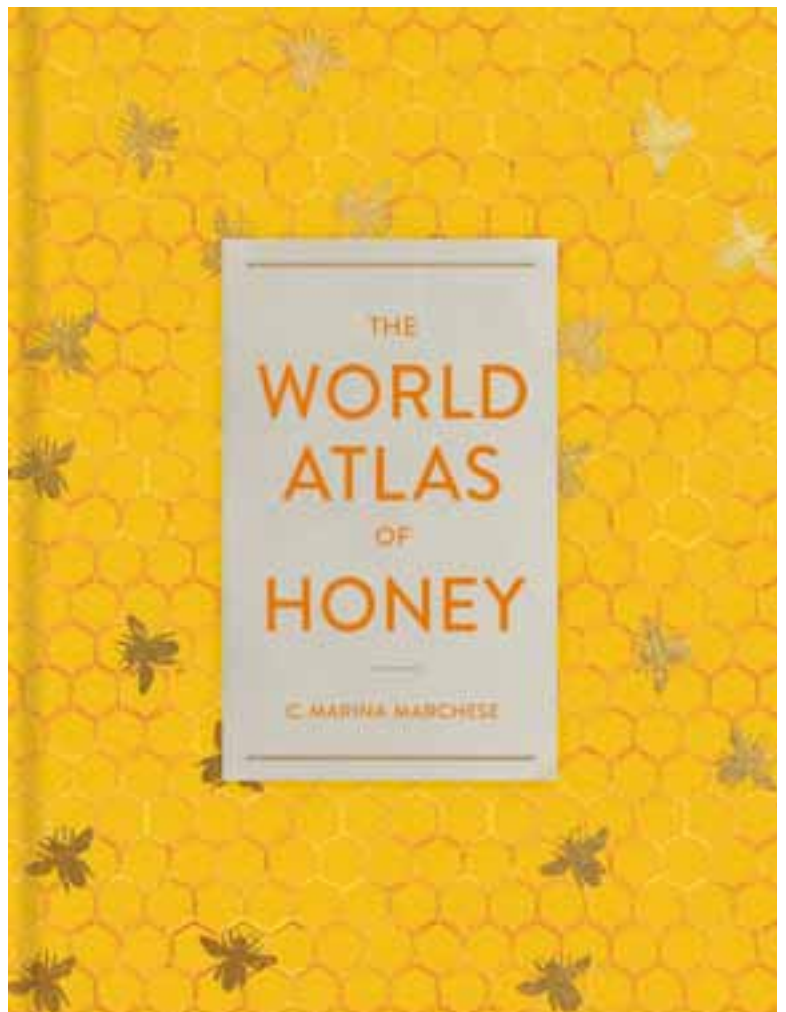
With text, illustrations, and photos, honey expert Carla Marina Marchese takes readers through the global history of honey production from the earliest beekeepers to today's harvests.

This colorful guide celebrates the exceptional range and diversity of honey, revealing how terroir – the environment in which a food is produced – influences honey's qualities just as it does for wine, olive oil, coffee, and chocolate.

The book also covers the methods used by honey sommeliers to taste and evaluate honey. Unique and authoritative, *The World Atlas of Honey* puts honey on the culinary map and elevates it to an epicurean treasure.

Link for more information and ordering
<https://www.ucpress.edu/book/9780520397705/the-world-atlas-of-honey>

Vlastimir Spasić





A new book

THE SECRETS OF MISS HONEYBEE

THE USE OF BEEHIVE PRODUCTS THROUGH THE STORY FROM EVERYDAY LIFE

Is the first intergenerational book written as a story and also a manual about apitherapy. It is appropriate for teachers of beekeeping, who are not apitherapists. It can be read and understood by the children who are old enough to read by themselves, it can be read by parents and grandparents, who will learn a lot new about the bees, how they and their products help us. This

is the first book on apitherapy for all the generations.

The book was very well accepted in all the target groups, especially by the teachers of beekeeping, because it helps them to explain what are all the bee products, and for what is the main point- how we can improve our health by using them in everyday life. It is the book where we introduce bee products to adults, so that they understand how those products can help the whole family to be more healthy on natural way – what apitherapy really is! This is the right way to introduce beehive products to everyone, so that the bees and their products will be respected and valued as they deserve. Remember – honey was medicine, before medicine was invented!

In the book we learn about the importance of the clean environment where we keep bees – only ecologically gathered bee products can be used in apitherapy.

We learn how important is that first we help bees by giving them food – we plant meliferous, honey plants and take care for fresh water.

We learn what is pollination and why (wild) pollinators are important insects in environment.

We learn about the Slovenian beekeeping which is peculiarity in the whole world (the bee-



house, the AZ hives, the beepanels as a cultural heritage and the way of life).

We learn about the biology of the bee family and beefamily through the year.

We learn what importance have bee products for the bees.

We learn how to behave near the beehouse / apiary and what to do when a bee sting us.

We learn what is apitherapy and who is apitherapist.

We learn how to gather the bee products correctly and how we store them (raw unheated honey, fresh pollen..).

We learn how we can mix them together so that they help us improve our health – better together.

We learn how to use bee products in everyday life for better immune system.

The book includes a special chapter at the end For Adults where apitherapeutical approach and bee products for health issues are explained even more detailed.

The book is originally written in Slovene, and translated into English and Czech language as it was recognized as very useful for young generations who get to know about the bees, but they don't know how to use their products in apitherapy and everyday life to improve the immune system.

Follow the link to the book:

<https://www.amazon.com/Secrets-Miss-Honeybee-Products-Everyday/dp/9610712770>



**BEES
LIFE**

**BEES
LIFE**



BEE TOURISM IN SLOVENIA AND AWARDING CERTIFICATION

Bee tourism in Slovenia is part of the apitourism programme, i.e. the type of tourism related to beekeeping and bee products.

Apitourism offers visitors the opportunity to learn about and experience the world of bees and beekeeping, and to delve into the beekeeping heritage of Slovenia and its natural beauty.

A timeline of the development of apitourism in Slovenia provides us with some interesting insights:

- For almost 20 years, the travel agency Ari-tours, in collaboration with the Slovenian Beekeepers' Association, has been developing the

apitourism partner product, which is promoted and marketed under the ApiRoutes brand. APIMONDIA, the International Federation of Beekeepers' Associations, appoints this brand as the coordinator of the APIMONDIA working group for apitourism. ApiRoutes assumes the leading role in promoting the development of apitourism around the world;

- The Slovenian Beekeepers' Association (hereinafter: the SBA) certifies apitourism service providers for the first time in 2013 and awards them certificates of excellence (rated from 1 to 3 bees);

- In 2016, the SBA establishes the Apitourism Providers Unit and brings together the recipients of certificates of excellence (the unit becomes the official point of contact for the state, the Slovenian Tourist Board and tourist agencies);

- In collaboration with the SBA, the Slovenian Professional Guides Association conducts the first training programme for beekeeping / tourist guides in the same year. The acquired knowledge leads to a specialisation of tourist guides, and a link between visitors and apitourism providers, which further improves quality and, consequently, leads to a more enjoyable experience for the visitor.

Apitourism, in the context of the rapid development of tourism, seeks to improve quality in order to respond to the needs of the modern traveller. Therefore, the quality of services offered by certified providers needs to be taken to the next level. At the beginning of 2024, the Anton Janša Beekeeping School conducted the first such training programmes, completion of which is one of the conditions for obtaining the "Top Bee" certificate of excellence. Taking into account the other conditions (uniqueness of the programme, accessibility for people with disabilities – Accessible Tourism, and knowledge of world languages), three apitourism providers earned an upgrade to their 1–3 bees rating categories in the spring.

The Anton Janša Beekeeping School also offers the "Top Bee" training programme abroad. The school provides examples of good practice to all those who want to improve the quality of their beekeeping, regardless of their geographical location outside the territory of Slovenia.

So why "Top Bee"? Because it represents the best of the best that apitourism providers can offer to their visitors. Quality in tourism is crucial for tourists' satisfaction and the success of a tourist





**BEE'S
LIFE**



**certifikat
odličnosti**

Certificate of Excellence

**Apiturizem
2024**

*čebelarstvo
Janez Kranjski*



ČEBELARSKA ŠOLA
ANTONA JANŠE



**certifikat
odličnosti**

Certificate of Excellence

**Apiturizem
2024**

*čebelarstvo
Janez Kranjski*



ČEBELARSKA ŠOLA
ANTONA JANŠE



Top Bee

destination or service. All of the above also apply to apitourism, which forms part of the larger tourism industry. It combines ecological, educational and cultural elements into an attractive, sustainable and high-quality tourist programme. Certified apitourism providers, especially those in the "Top Bee" category, represent the foundation of api-

tourism, an innovative Slovenian tourist product.

Dominika Koritnik Trepel

More about the Top Bee certificate category at the link: Slovenian Beekeepers' Association (czs.si)

LET'S CERTIFY



APITOURISM

ACROSS EUROPE!

CATEGORISATION OF APITOURISM – 1-3 BEES AND "TOP BEE"

Slovenia is the first and currently the only country to carry out certification of apitourism providers. Certificates of excellence ensure control over the range and quality of services and promote competitiveness. The first evaluation took place in 2011, with the first certificates awarded in 2013.

Certification is a very important element in the development of the beekeeping tourism offer, both in terms of quality and competitiveness. On one hand, apitourism is an opportunity to upgrade existing beekeeping farms, while it also provides young people and anyone who is fascinated by bees with the opportunity to start an in-

dependent journey or make some extra money.

The basis for the successful development of apitourism is the cooperation of beekeeping farms, providers of tourism products, tourist associations, local communities and the state, as well as their collaboration in planning, positioning and marketing their offer.

Apitourism in Slovenia is also supported by the Ministry of the Environment and Spatial Planning, Ministry of Economic Development and Technology, Directorate for Tourism and Internationalisation, WTO, Sector for Tourism and Ministry of Foreign Affairs.

1. SETTING THE CRITERIA – SCORING

Candidates vying to receive the 1-3 Bees or the Top Bee certificate are expected to show self-

initiative and innovation. The certification assesses the specialisation and focus of the activities of different providers, e.g. farms, museums, shops, and whether they meet the criteria to be certified as a good or excellent apitourism provider.

One, two or three bees indicate and rank the extent to which basic conditions were met, the orderliness of the provider's facilities and surroundings, organic or biodynamic beekeeping, the ability to present activities and products in an attractive fashion, innovative packaging, the ability to create an Api experience, elements of surprise, etc.

This also includes offerings of creative workshops: gingerbread baking, candle making, hive painting, culinary workshops, professional lectures and training, protective clothing for visitors, first aid, animation programme, music, multimedia, photo gallery, massage, chamber where you can smell the aroma of bee products, cosmetics, gift programme, museum, guided tours, honey plant and herb plantations, bee trail, product innovation and other offers.

The criteria for obtaining the Top Bee category represent an upgraded version of apitour-



ism. This category can be acquired exclusively after completing training to acquire additional knowledge, presenting a unique offering and its application in the actual offer and, consequently, obtaining additional points on the evaluation sheet of apitourism certification.

A candidate for Top Bee certification may proceed to the direct assessment for this category as soon as they have been verified and awarded 3 Bees by the committee and have completed the required training courses for the acquisition of additional skills (listed below), have satis-

**BEES
LIFE**



fied the requirements of points 3 and 4, and have practically equipped their apitourism facilities in accordance with the minimum requirements for the accommodation of guests with disabilities (knowledge acquired in the course of the training course, photographic evidence will suffice as proof of this).

2. TRAINING FOR THE ACQUISITION OF ADDITIONAL KNOWLEDGE

As mentioned above, additional knowledge is a prerequisite for obtaining the "Top Bee" certificate. Additional knowledge can be acquired during training, which includes several modules.

If the owners of an apitourism facility have not undergone additional training, they can obtain the 3 Bees certificate at most.

Of course, apitourism providers that have not applied for the "Top Bee" category can also participate in the training at their own request.

The training includes the following modules:

Module I: Beekeeper's presentation to an audience

To accurately portray the experience their apitourism facility provides, the beekeeper needs to have a good knowledge of the rules of behaviour and presentation.

The following content will help:

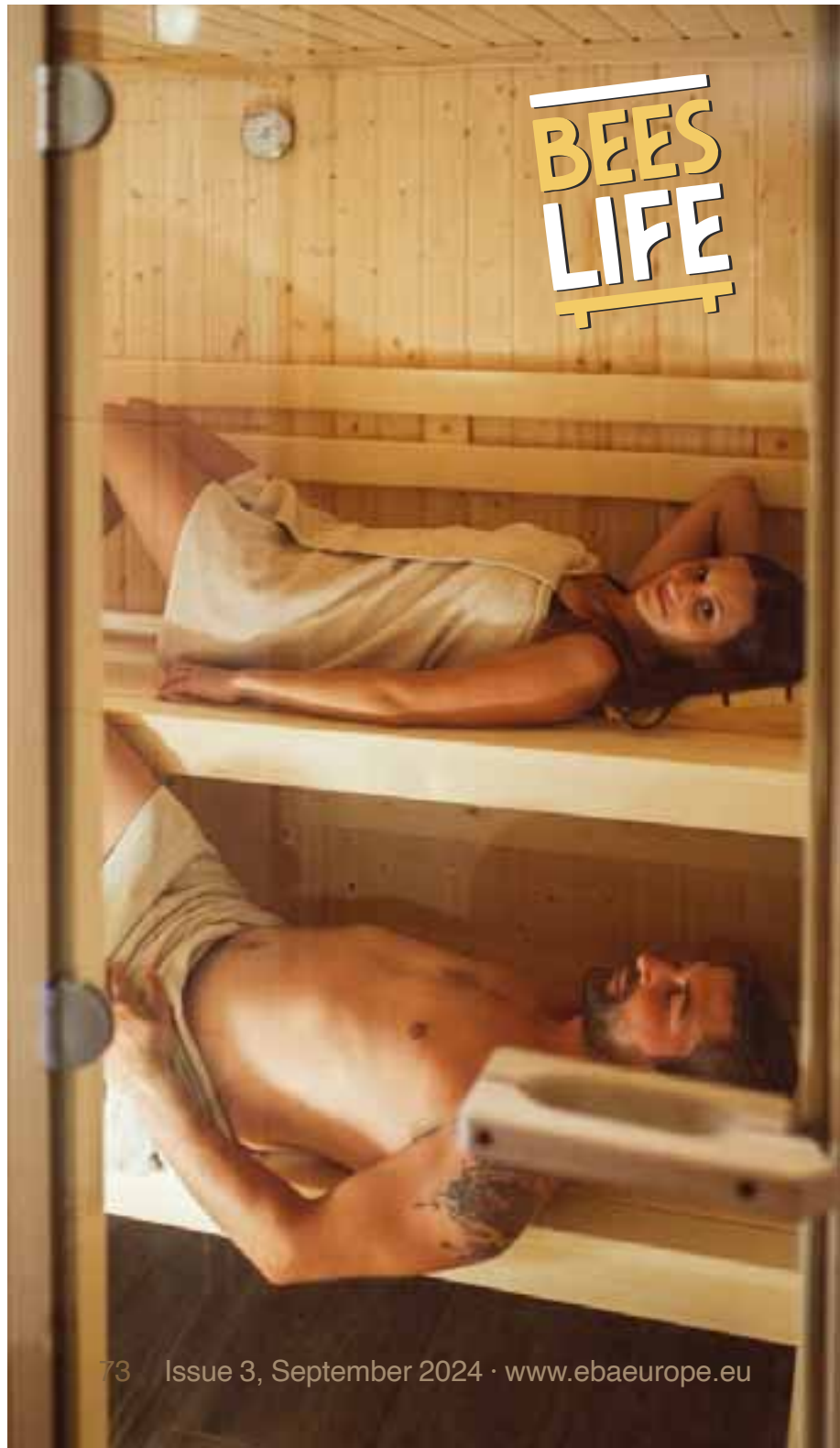
- Knowledge of performance and body language
- Appearance of the beekeeper – following "the suit maketh the man" principle
- Visitor involvement – group dynamics
- Knowledge of the local environment outside the area of their own apitourism (the beekeeper is also a promoter of the place where they live and work)

Module II: Accessible Apitourism

As equal stakeholders in tourism, apitourism facilities are also visited by people with disabilities (physical and sensory disabilities, people with mental health problems, people with intellectual disabilities, etc.).

Content that provides the beekeeper with enough information for a confident appearance in front of people with disabilities:

- Who are people with disabilities
- Skills of the correct approach and giving content to people with disabilities



- How to tailor your apitourism to the well-being of guests with disabilities

Module III: Honey garden

Apitourism must have a honey garden. Because we can always acquire new gardening skills and learn more about suitable plants, the following content is included:

- how to arrange a honey garden for the purpose of a perfect experience for visitors;
- important plants that are essential in the honey garden and their characteristics;
- urban honey garden

Module IV: Art and bees

Apitourism also includes certain forms of art. To upgrade your knowledge, the following content has been added:

- Bees in painting, sculpture, architecture
- Beehive panels as an element of cultural heritage
- Applied arts and apitourism

Module V.: Organic beekeeping and apitherapy

Apitherapy goes hand in hand with organic beekeeping. Bee products containing synthetic acaricides that have been used by a beekeeper to treat varroasis or other bee diseases are in no way suitable for treating humans.

Highlights:

- What are the basic conditions that a beekeeper must meet in order to be able to be an organic beekeeper
- How and which honey to use in wound treatment
- Dangers of improper honey massage
- Who can use bee venom in the treatment of rheumatoid diseases

- How to prevent pollen allergy if we want to help people with various health problems
 - Introduce the participants to the meaning of the famous quote by Hippocrates: "Let food be thy medicine and let medicine be thy food".

Note: The modules are adjusted according to the country or environment.

3. KNOWLEDGE OF WORLD LANGUAGES

To receive the "Top Bee" certificate, apitourism presenters must have a satisfactory knowledge of at least one world language (English, German, French, Italian, Spanish, Portuguese, etc.).

A secondary school or grammar school certificate showing that the presenter has completed four years of language education, or a certificate of completion of a language course at a certified language school is sufficient (showing that the presenter demonstrates language proficiency at the level of four years of secondary school education).

If the apitourism presenter does not show a satisfactory level of knowledge of the foreign language, the "Top Bee" category cannot be obtained and the presenter is placed in one of the 1-3 Bees categories.

Native speakers are exempt from this rule (they do not need proof of completed education at the level of a four-year secondary school, etc.).

The presence of a beekeeping guide to provide translation is not sufficient to achieve the "Top Bee" category.

Croatian, Macedonian, Serbian etc. are foreign and not world languages. Knowledge of these languages is not sufficient to qualify as a "Top Bee", but it is sufficient to qualify as a "1-3 Bee".

4. "TOP BEE" CATEGORY – OBLIGATION OF A UNIQUE OFFERING

To achieve the "Top Bee" category, the apitourism provider must present their own unique



(innovative) offering and regularly integrate it into their general offer.

This offer can include souvenir categories, accommodation, offers adapted for people with disabilities and for children, museum collections, bee trails, unique presentations of apitourism to guests, organisation of events, workshops, etc.

The apitourism provider is free to choose what they want to do. The only condition is that this additional product is unique enough.

The Slovenian Beekeepers' Association will start certifying beekeeping tourism globally in autumn 2024. The Slovenian Beekeepers' Association has already sent an initiative to Apimondia to authorise Slovenia to do the same.

For any questions regarding the costs of training and certification send an e-mail to:

barbara.dimc@czs.si
+386 17296102.

6. COMMITTEE DECIDING ON THE ACQUISITION OF A CATEGORY

The committee of 3-4 evaluators, which verifies the apitourism providers, consists of members from the Apitourism Section of the Slovenian Beekeepers' Association and other experts or external collaborators, as well as an active beekeeping tourist guide, a member of the KPTVS (they must have a broad knowledge of apitourism).



SLOVENIAN APIARY



BEES
LIFE

The apiary is architecture. As such, it embraces the bees, the hive, the apiary and the beekeeper. There are many bee species; apiaries rely especially on the honeybee.

The beehive is a functionally shaped unit that protects the bee family and may be set up individually, in rows or in stacks. A modern beehive allows assemblage and extension and makes beekeeping as easy as possible.

The apiary is architecture and, as such, primarily functions to house a stack of beehives. It is built to meet the requirements of its users, is solid and provides protection from the sun, cold, damp and intruders. It must be designed and set up to comply with the relevant rules and laws.

An apiary's construction is so simple that any tolerable carpenter can build it with readily available materials. In Slovenia, these are wood and stone, rarely brick. An economically constructed small-sized apiary is an assemblage of four vertical supports with an open front and a room for the beehives and beekeeper. As a rule, it has an

asymmetrical and pitched roof projecting in front with an arched panelled underside.

The apiary is located so as not to disturb or be disturbed, in a visible place easily accessible for the beekeeper. As a piece of functional architecture, its shape and material is in harmony with the architecture around it, so people accept it as part of nature itself.

The beekeeper is the man who keeps bees. With beehives, he protects them from the rain, sun and cold, throughout the year, and keeps all the implements and tools for work within reach. The apiary also gives the beekeeper a means of personal expression, as an artist. Elsewhere in the world, apiaries are more often sites than built structures, but the most important aspect of the Slovenian apiary is that it is a building that presents beehive fronts for viewing. The roof with its large projecting eaves provides ideal conditions for paintwork on the beehive front panels. There are two main types of Slovenian apiary: the central-Slovenian and the karstic-littoral – the first



wooden and the second predominantly stone or brick.

The potential for paintwork came with the introduction of angular, stacked hives with flat fronts. The paintwork mostly serves humans: they can distinguish the beehives by their paintwork rather than by number or position. The

painted beehives of the Slovenian apiary are a peculiarity in the beekeeping world, elevating its culture above other variants.

The apiary is the product of a simple unschooled builder, his skills passed on through heritage and experience; it knows no repetition and is original in its continuous reinvention.





A typical small-sized apiary has two vertical supports in cross section, an asymmetrical roof with large projecting frontal eaves and is elevated from the ground. The open beehive fronts allow every individual to fully express his inclinations and wishes and display all his skills through the visual image of the apiary.

The industriousness of bees has always been used as an attribute of people and associations; in Slovenia since 1671, even members of Dismus' Fraternity called themselves "apes", which is Latin for bees. Elements of beekeeping such as the bee, hive or apiary, are quite often found in mythology, literature and architecture.

The apiary itself often features in artistic depictions of folklore themes. The apiary as architecture thus embraces everything: industriousness, work, functionality, construction, beauty and, finally, art. With the apiary, we can proudly show both foreigners and our own posterity a high level of culture. As a part of Slovenian cultural heritage, however, we must first of all be familiar with it and respect and preserve it.

Slovenian Beekeepers' Association



THE CARNIOLAN BEE HOUSE

A most special house, called the Carniolan Bee House, has opened its doors in the medieval town centre of Višnja Gora in the municipality of Ivančna Gorica. With it, we want to revive the unique beekeeping story of one of Slovenia's most important beekeeping families, the Rothschutz, which is responsible for the present-day spread and visibility of the Carniolan bee.

Members of the Rothschutz family introduced the Carniolan bee to the world more than 150 years ago. Today, they tell the story of beekeeping, the interesting biology of the Carniolan bee, and, above all, they teach visitors from Slovenia

and abroad about the important impact bees have on the environment and on people.

The Carniolan Bee House is unique in the world as it is entirely dedicated to our native Slovenian bee – the Carniolan bee. Every single room is designed with it in mind.

The Carniolan Bee House is home to the Tourist Information Centre, where guests can learn all about what the house and the surrounding area have to offer. The café with a summer garden is a space with a selected range of honey products and products from local suppliers.

The ApiLab Centre of Innovative Technologies is dedicated to improving the competencies of small and medium-sized companies and provides various training courses.

The centrepiece of the Carniolan Bee House is the experiential exhibition that presents a holistic view of the Carniolan bee, including a timeline of its origins and an introduction to its habitat. You will learn about its excellent qualities that have made it so popular with beekeepers. The presentation of the Carniolan honey bee includes a demonstration of its external and behavioural characteristics and also brings the animal closer





BEES
LIFE



BEES
LIFE





BEES
LIFE



BEES LIFE

to you through unique microscopic images. The bee colony, or its members, which make up the superorganism, is also presented in detail. We can see them up close in special glass hives, which house seven bee colonies. Special visual images of the inside of the hive also provide an

unforgettable experience, bringing us closer to the workings of a bee colony. The beekeeping objects on display tell the story of the relationship between a beekeeper and a bee.

Bee products are also shown. At the final station, you will find thoughts and sayings about



bees from Slovenian folklore and literature, as well as an interactive quiz. You can test your knowledge through the quiz, which is designed to encourage interconnection and cooperation and to strengthen team spirit.

The presentation of the Carniolan honey bee is interwoven with the story of the Rothschütz beekeeping family, that lived in the nearby Podsmreka Castle near Višnja Gora in the second half of the 19th century.

Emil Rothschütz, its most important representative, sent over 100,000 hives of live bees to the world, within his global company »Carniolan Commercial Apiary«, thus helping to raise its profile and spread it.

He also contributed to its official recognition as a special subspecies of honey bee with the scientific name *Apis mellifera carnica*, Pollmann 1879, by providing dr. August Pollmann with the genetic material of the Carniolan bees.

The enormous contribution of Rothschütz family to the development of Slovenian beekeeping and to raising awareness of the importance of the Carniolan honey bee among the general and professional public is presented in the inter-

active exhibition through narratives of important members of this family as animated guides.

Another innovative feature is the overnight “Honeybee” accommodation in the form of honeycomb rooms.

This is a large wooden structure with a glass roof where people stay together but have their own privacy. The Carniolan Bee House brings together bees, people, and beekeepers under one roof. The Carniolan Bee House offers a wide range of lectures for the general public as well as professional training for beekeepers.

Everyone is welcome to try sleeping in a honeycomb or to experience the world of bees through various activities.

Petra Špehar & Maja Lampret

Photo author: Andrej Peunik

Carniolan Bee House

Mestni trg 2

1294 Višnja Gora

+386 1 44 40 410

info@hkc.si

www.hisakranjskecebele.si/en



**BEEES
LIFE**



THE MUSEUM OF APICULTURE SLOVENIA

A UNIQUE BEEKEEPING HERITAGE A SONG OF THE SOUL

FOR EVERY BEEKEEPER AROUND THE WORLD

The love for bees has a long-standing tradition in Slovenia. In fact, it is almost certain that few countries in the world possess such a rich natural and cultural heritage so deeply intertwined with beekeeping as ours.

The special care for bees in the past led to the creation of unique bee houses called

"apiaries," which can now be found in nearly every Slovenian village. These apiaries hold significant importance, serving as safe havens for a unique species of honeybee—the Carniolan bee (*Apis mellifera carnica*), the second most widespread honeybee in the world. This honeybee is renowned for its excellent traits, such as excep-





tional calmness, gentleness, adaptability, high honey yields, disease resistance, low winter food consumption, and rapid spring development. The Carniolan bee is legally protected, meaning that beekeeping in Slovenia is permitted exclusively with this species.

Since opening in 1959, the museum has specialized in preserving the heritage of Slovenian beekeeping by keeping records, collecting, storing, documenting, representing, and popular-

izing this tradition. The museum's exhibits cover several key topics defining Slovenian beekeeping: the indigenous subspecies bee, world-renowned beekeepers, painted beehive panels, bee hives, tools, crafts, and trade.

In the late 18th and early 19th centuries, due to the bee's remarkable qualities and high demand from foreign beekeepers, live Carniolan bees were exported worldwide at an accelerated pace. This period marked a boom in the trade of





Carniolan bees, which traveled to every corner of the globe, from Australia and Japan to South America and Canada, via mail, trains, ships, and even airplanes.

The original painted beehive panels, typically the front and often removable wooden part of the

hive, especially in "kranjič" hives, are unique to Slovenia. The Beekeeping Museum's collection of painted beehive panels, which includes 900 pieces, is the largest in Slovenia, both in terms of quantity and representation. The most famous is the panel depicting the Virgin Mary, dating back



KLANJIČ KLANJIČ

Kranjič je panj iz desk s nepremičnim sajem, ki ga je mogoče slagati v skladnici, pogosto je okrašen s poslikavo na sprednji deski.

Traditional kranjič hives are made of wooden planks with a fixed comb that can be stacked, often adorned with painted front panels.



keeping teacher, whose birthday, May 20, was declared World Bee Day by the United Nations in 2017. The museum dedicates a special corner to him, alongside many other significant beekeeping writers.

Among the distinctive hives, the "kranjič" and, since the early 20th century, the Alberti-Žnideršič hive, are characteristic of our beekeeping tradition. Beekeeping literature, exceptional beekeepers, unique beekeeping tools, and traditional crafts like candle making and honey-based treats are valuable aspects of our heritage, preserved since 1959 in the Beekeeping Museum—and proudly showcased in our new exhibition.

In 2021, we created a new permanent exhibition titled "Living Together: The Bee and Humankind." We collaborated with many partners, institutions, and individuals who recognize the importance of our beekeeping heritage and care about the Carniolan bee and its future. The new exhibition is designed holistically and interdisciplinarily, based on three pillars—heritage, experience, and education. It emphasizes the modern interpretation of museum artifacts, with more interactive content and the presentation of new aspects of beekeeping heritage. The author of the

to 1758, which is the oldest preserved painted beehive panel.

A special place in the history of Slovenian beekeeping belongs to Anton Janša, the first bee-



**BEES
LIFE**



In 2022, Slovenia received a UNESCO nomination for the inclusion of "Beekeeping in Slovenia, a Way of Life" into the Intangible Cultural Heritage list. We are also very proud that the Beekeepers' Association of Slovenia succeeded in establishing World Bee Day, celebrated on May 20th, the birthday of the Slovenian beekeeper Anton Janša, the first beekeeping teacher.

We pay special attention to the current challenges of beekeeping worldwide. To address this, we have developed the project "The World of Pollinators" (Ivan Esenko, Petra Bole, Andrej Šalehar), which highlights the contemporary issues faced by bees and beekeepers in the contemporary world. We have also published a book with the same title.

The Beekeeping Museum is one of the most visited museums in Slovenia. It is known across all continents of the world. Each year, we welcome visitors from more than 30 countries worldwide. We take great pride in our beekeeping heritage and warmly invite you to visit us in Radovljica. Welcome to visit us in Radovljica!

exhibition, Dr. Petra Bole, received the Valvasor Award for Outstanding Achievements in 2021 and the international GoingGreenGlobal Award.

The objective of the new exhibition project is to provide a broader perspective on beekeeping heritage in the context of contemporary global issues—particularly the bee as an endangered species, which could significantly impact the future of humanity. In defining the objectives for each theme, we used a three-level approach to break down the subject matter.

Dr. Petra Bole
 director
 +386(0)4 532 05 20
 +386(0)31 540 480



BEE PATH

IN LJUBLJANA



In 2016, Ljubljana, the capital of Slovenia, was a candidate for the prestigious European Green Capital of Europe title. The award is given every year to a city that achieves high environmental standards, sets high goals for further environmental improvement and sustainable development, and can serve as an example to other cities. At that time, Ljubljana won the competition of the following cities: Essen (Germany), Nijmegen (the Netherlands), Oslo (Norway) and Umeå (Sweden).

In preparation for this candidacy, the idea of the Bee Path in Ljubljana was born. Ljubljana has a rich and long tradition of beekeeping in the urban and suburban environment, where there are as many as 5 beekeeping societies with about 400 members. Modern urban beekeeping with hives on roofs and terraces began to develop only after 2011, when the beekeeper and head of maintenance at the Cankarjev dom congress and cultural centre, Franc Petrovčič, in agreement with the then director, placed some hives on the terrace. His model was mainly Paris, where beekeeping took place on the roof of the Paris Opera and some other buildings. The honey from Cankarjev dom was sent for analysis to a reference laboratory in Bremen and the analysis showed a complete absence of any pesticides or heavy metals. Interestingly, this document, along with all the others, was submitted to the European Green Capital Evaluation Committee. When, as an api-tourist or local tourist guide, I take a tour of the Bee Path in Ljubljana, I also mention this fact and add a little jokingly that it

was the "key document" that convinced the evaluation committee. Jokes aside: where there are bees, the environment is also healthy. And there are a lot of bees in Ljubljana: only within the motorway ring, on an area of 72 km², there are 125 apiaries or stands with almost 800 hives. In terms of the density of bees (see the article Urban Beekeeping Survey by William Blomstedt – American Bee Journal, June 2020), Ljubljana is certainly European, and perhaps even the world capital of bees.

On the one hand, the initiator of the Bee Path, Maruška Markovčič from the Municipality of Ljubljana, conceived the Bee Path as a movement of like-minded people who take care of the well-being of bees and other pollinators in the city through various activities. In addition to beekeepers and Ljubljana beekeepers' societies the movement also includes various institutions, schools, museums, companies and tourist providers. Today, it has over 40 members. Thanks to numerous activities within the Bee Path, Ljubljana has been declared the most bee-friendly municipality in Slovenia by the Beekeepers' Association of Slovenia three times (2017, 2019 and 2022). On the other hand, it is a journey through points related to bees and beekeeping.

The success of the Bee Path and its numerous activities has also been recognized internationally. In 2017, it received the European award – URBACT Good Practice Award. Ljubljana has thus been given the opportunity to apply for European funds to transfer its good



The new Bee Path map, edition 2024

practice to other European cities. In 2018-2019, five cities joined the Bee PathNet project led by Ljubljana: Hegyvidéki - 12th District of Budapest – Hungary, Amarante – Portugal, Bydgoszcz – Poland, Cesena – Italy and Nea Propontida – Greece. Later (2020-22), under the name Bee-PathNet Reloaded, four more cities joined: Bergamo – Italy, Osijek – Croatia, Sosnowiec – Poland and Bansko – Bulgaria.

In the almost ten years of its existence the Bee Path in Ljubljana – the grand opening and

the first guided hike took place in October 2015 – the path has also evolved and upgraded, especially with the addition of new apiaries and beehive stands. Today, it includes 14 points. We named the path: "The bees go buzzing one by one". On it – the total length is 7.7 km – hikers under the guidance of api-tourist guides can, among other attractions, see 7 apiaries or beehive stands (at the opening of the path there were only two). In this article, I will limit myself to a brief presentation of all apiaries and beehive stands

along this route, in the order in which they are also marked on the map above.

A guided tour of various locations in the city related to bees and beekeeping is definitely a slightly different experience of Ljubljana – its picturesque squares and streets, abundance of greenery, lively atmosphere on the one hand and pleasant tranquility on the other. So let's go from apiary to apiary on this path.

The Bee Path

1. Botanical Garden – Apiary "Step Closer" (AŽ hives) – since 2019 (No. 1 on the map)

The Botanical Garden in Ljubljana is the oldest cultural, scientific and educational institution in Slovenia with continuous operation since 1810. There are almost 6000 plant species in it. There was also the grand opening of the Bee Path at the beginning of October 2015 and the first hike along the Bee Path. The botanical garden did not yet have an apiary at that time. It was erected in 2019 on the basis of cooperation between the City of Ljubljana and the Faculty of Architecture, University of Ljubljana. It was designed by students at the seminar of Prof. Mihael Dešman. It is the winning project of setting up an urban apiary with AŽ hives as part of the "Urban Bee Homes" project. The pavilion consists of two parts, a room for observers and a room for bees and work with them. The units are separated by a glass barrier, which allows visitors a safe close-up view of the operation inside the apiary.



Apiary »Step Closer«

Photo: web, www.visitljubljana.si, 9.8.2024

2. Plečnik's House – Plečnik's apiary (AŽ hives) - since 1935 (No. 2 on the map)

Around 1935, the greatest Slovenian architect Jože Plečnik built this apiary in the garden behind his house for his nephew Karel Matkovič.

Matkovič worked there as a beekeeper until his death in 1971. It is a smaller, modest apiary with an original but simple design: he put five AŽ hives into a wooden box and lifted them off the ground by placing it on a cut concrete sewer pipe. He made a simple single-pitched roof on top, with a large eaves on all sides. The only decoration is ceramic panels with an eagle – also the work of J. Plečnik. The apiary was renovated in 2012, but it is not inhabited by bees.

The Plečnik House Museum is a member of the Bee Path – and itself worth a special visit.



Plečnik's apiary in Trnovo

Photo: web, www.odprtehiselovenije.org, 9.8.2024

3. Apiary of Beekeepers' Society Ljubljana (AŽ hives) – from 2023 (No. 3 on the map)

The apiary was set up by the oldest beekeepers' society in Ljubljana on the occasion of its 110th anniversary. In this case, too, the installation was the result of cooperation with the Faculty of Architecture, University of Ljubljana. The apiary project was prepared by students under the guidance of Prof. Mitja Zorc. The requirements of the society were that the apiary should be similar in shape to a typical Slovenian apiary, and that education and meetings of small groups should be carried out in a room that is separate from the space for working with bees. Outside observers who would accompany the beekeeper in his work with the bees should not have direct contact with the bees. Among the requirements was to ensure accessibility for people with disabilities – they should be provided with access to both the education space and the space for working with bees. The apiary is an important (ne-

west) addition to the Bee Path. It is located not far from Plečnik's house.



*The apiary of Ljubljana Beekeeping Society in Trnovo under Barjanska cesta
Photo: A.Süssinger*

4. Beehives on the terrace of the Jože Plečnik Gymnasium (LR hives) – from 2021 (No. 5 on the map)

The Jože Plečnik Gymnasium in the very centre of Ljubljana came up with the idea of set-



ting up beehives based on the story of greening two school terraces. A few years earlier, they decided to invite bees and other pollinators to the school, thus further contributing to the preservation of bees and increasing biodiversity in the city. Thus, first in 2015, the smaller lower terrace next to the classrooms was greened, and later an even larger, upper roof terrace. With the arrival of bees, the school also became part of the Bee Path. The beekeeping project is carried out in cooperation with the Barje Beekeepers' Society, which also conducts a beekeeping circle for students at the school. As a member of the Bee Path, the school offers announced visitors the opportunity to climb the terrace and see the hives accompanied by a guide. From there there is an exceptional view of the old town and the Ljubljana Castle. I can say: hives with a view.

5. Beehives on the terrace of Cankarjev dom (LR hives) – since 2011 (No. 6 on the map)

Cankarjev dom is the main Slovenian cultural institution. As it is written on their website, it nurtures a chosen ear not only for culture, all genres

Franc Petrovčič with an assistant on the terrace of Cankarjev dom

Photo web, www.bing.com, 9.8.2024



of art and the heritage of humanity in general, but also for nature. One of the roof terraces has been home to bees since 2011. As mentioned in the introduction, the beginner of modern urban bee-

Beehives on the terrace of the Jože Plečnik Gymnasium

Photo: Aleš Süssinger

keeping in Ljubljana, Franc Petrovčič, who takes care of the bees there. In 2014, he was also one of the initiators of the establishment of the youngest beekeepers' society in Ljubljana, the Urban Beekeeper. Cankarjev dom is also a member of the Bee Path, and tours of the hives are possible by prior arrangement.

The bees make their home on the terrace at the height of the third floor. Due to the perimeter walls, they are protected from the winds, which has a beneficial effect on wintering and the rapid spring development of families. The view for visitors is therefore a little less attractive compared to the terrace at the nearby J. Plečnik Gymnasium.

6. Tivoli Park – Beehives "Tivoli Bees" (LR hives) – since 2017 (No. 7 on the map)

Similar to the apiary in the Botanical Garden, this beehive stand was created as part of the Urban Bee Homes project. The conceptual design of the "Tivoli Bees" was the best solution for a stand with loading hives. The installation in Tivoli Park took place shortly after the completion of the project. The building elements of the stand consist of concrete pedestals and wooden beams. By combining these units, benches, stump bases and countertops can be created. Benches are kept at a safe distance from where it is possible to observe the bees flying out.



Beehives »Tivoli bees«

Photo web www.tivoliskimed.si, 9.8.2024

7. Castle Hill – replica of Plečnik's apiary from the Czech Republic (1927) – from 2021 (No. 14 on the map)

Slovenian architect Jože Plečnik also designed an apiary with 14 hives for the park at the presidential residence of Lány, the first Czech

president T.G. Masaryk. The plan for it dates back to 1925, and the installation was carried out two years later, in 1927. Plečnik highly appreciated this work, as he included a full-page photograph of the apiary in his monograph entitled *Architectura perennis* (Eternal Architecture), published in his honour in Ljubljana in 1941 - i.e. among his selected works.

The Barje Beekeepers' Society from Ljubljana is a member of the Bee Path and its president - the undersigned - when designing this trail, I proposed the installation of a replica in Ljubljana.

The road from idea to realization was quite long. It was necessary to find a suitable location, sponsors and donors of the installation and, of course, Plečnik's plan. The idea itself was met with overwhelming approval and willingness to cooperate.

The Barje Beekeepers' Society from Ljubljana is a member of the Bee Path and its president - the undersigned - when designing this path, I proposed the installation of a replica in Ljubljana. The road from idea to realization was quite long. It was necessary to find a suitable location, sponsors and donors of the installation and, of course, Plečnik's plan.

The idea itself was met with overwhelming approval and willingness to cooperate. The first to support the idea was the president of the Beekeepers' Association of Slovenia, Boštjan Noč, who provided the necessary start-up funds through the donor bank (SID Bank).

Art historian Dr. Peter Krečič, a great connoisseur of Plečnik's works, wrote an inaugural address for the opening ceremony at the beginning of July 2021, in which he stated, among other things:

»The initiators were driven by great enthusiasm to be able to experience this little masterpiece of art by Plečnik in Slovenia as well, but they approached it with a trembling hand, looking for the right sources, the right advisors and a skilled architect (Rok Žnidaršič, note A.S.), who knew how to transfer the nearly 100-year-old master's plans into a living artistic organism, to transfer the real Czech Plečnik among us.

The building itself is therefore a great achievement for Slovenians, it is quite sufficient for pure, unadulterated artistic pleasure, but at the

same time it will serve the same purpose as in the Czech Republic as an apiary.«

The apiary was given the status of an architectural monument and Prof. Janez Koželj, then Deputy Mayor and honorary patron of the opening ceremony, contributed the following record to the plaque at the apiary:

»In addition to the refurbishment of the Hradčany Castle and Gardens in Prague, Tomáš Garrigue Masaryk, the first president of the Czech Republic, also entrusted Jože Plečnik with furnishing the hunting mansion of the Fürstenberg family in Lány, which the state bought for the president's annual residence. As part of the arrangement of the castle park, the architect also set up an apiary in the economic part. He designed it in the form of a simple pavilion, which consists of prefabricated elements. The harmoniously proportioned composition of

the apiary, which is not inspired by either classical or folk architecture, works strictly and cleanly. In a similar way, but in a different form, Plečnik also designed his apiary in the garden of his home in Trnovo. On the initiative of the Beekeepers' Society Barje, Medprostor, an architectural studio, carried out and erected a replica of the apiary from Lány according to the architect's plans«.

The apiary stands on the southern slope of the Castle Hill, on a clearing under a mighty two-hundred-year-old plane tree. We named the clearing Balcony on the Marshes (Balkon na Barje) - for two reasons: in front of the apiary, a view of the Ljubljana Marshes (Barje) opens above the houses along Karlovška Street, and the apiary was set up and is managed by the



Apiary on the Castle Hill and the original in the Czech Republic (photo from 1941 – Architectura perennis)

Photo: A.Süssinger and web, 9.8.2024

Barje Beekeepers' Society. For the second year in a row, visitors can taste forest honey from this apiary. Honey from Plečnik's Apiary has a special label and can also be purchased as a souvenir in the Plečnik House Museum.

Architect Jože Plečnik has left an exceptional mark on Ljubljana with his works, and some of the works were included in the UNESCO World Heritage List in 2021. Hikers along the Bee Path can see many of these. They cross or see all three bridges that are included in this list: the Tri-

ple Bridge and the Cobblers' Bridge on the Ljubljana River and the Trnovo Bridge on the Gradaščica River. On Plečnik's so-called water axis along the Ljubljana River, the path also leads along Trnovo Embankment, i.e. the landscaped left bank past stone terraces and a row of willows. On the so-called land axis, they walk from Plečnik's house, cross the Trnovo Bridge, pass Križanke Complex and National and University Library, to Zvezda Park.

In 2022, Slovenian beekeeping was included in the UNESCO List of Intangible Cultural Heritage – as the only one so far – due to its tradition and all its specialties. Therefore, it can be said that Plečnik's architectural genius, Slovenian beekeeping and UNESCO symbolically intersect at the two Plečnik apiaries on the Bee Path.

You are invited to hike and visit the Bee Path in Ljubljana, where you will be able to see and

learn much more in the company of an api-tourist guide.



Aleš Süssinger

Vice-President of the Slovenian Beekeepers' Association

Responsible for beekeeping cultural heritage and api-tourism

President of Beekeepers' Society Barje
ales.sussinger@gmail.com

References:

- Brochure EU cities – good for BEES is good for PEOPLE, MOL, Ljubljana 2022
- www.urbact.eu/networks/beepathnet
- www.ljubljana.si/sl/moja-ljubljana/podezelje/cebela-v-ljubljani/cebela-pot/
- www.ljubljana.si/sl/moja-ljubljana/zeleno-prestolnica-evrope-2016/
- www.visitljubljana.com/en/visitors
- Brochure The Bee Path in Ljubljana, MOL 2024
- Brochure Urbana čebelja domovanja, MOL 2016
- Article An Urban Beekeeping Survey by William Blomstedt – American Bee Journal, June 2020
- www.ljubljana.si/sl/aktualno/urbani-cebelnjak-v-botanicnem-vrtu/
- <https://mgml.si/sl/plecnikova-hisa/o-plecnikovi-hisi/>
- zbornik 110 let Čebelarskega društva Ljubljana, ČD Ljubljana, 2023
- www.gjp.si/solski-vrt/
- www.cd-ljubljana.si/2023/11/03/drustveni-cebelnjak-cd-ljubljana/
- Article Plečnik's Contribution to the Development of the Slovenian Beehouse, Andej Hrausky, Magazine Piranesi, No. 46-47, 2022
- Brochure Plečnik's Ljubljana, Turizem Ljubljana, 2024
- www.whc.unesco.org/en/list/1643 - The works of Jože Plečnik in Ljubljana – Human Centred Urban Design
- www/ich.unesco.org/en/RL/beekeeping-in-slovenia-a-way-of-life-01857



strip's

Customised Innovations

APITOURISM INSPIRED BY SLOVENIA IS NOW IN TURKEY WITH “APITURROUTE” PROJECT

Beekeeping is an important sector for not only producing honey but also pollination and other bee products. The other bee products such as propolis, royal jelly, bee venom, bee bread are getting more popular for also apitherapy. The use of bee products in the fields of supportive medicine or cosmetics in human health attracts great attention in the treatment of many diseases, anti-aging and beauty, care and aesthetics fields. The increasing interest also brings to the agenda the issues of quality production of bee products and the beneficial use of their contents.

On the other hand, today, no matter what you produce, no matter how high quality and valuable it is, unless you promote it, it is not in demand or used incorrectly. For example, we, scientists, promote our scientific research by publishing articles in high-impact journals. Until now, it seemed that honey and other bee products produced in the beekeeping sector could also be promoted through marketing and advertisement. However, now consumers want to observe the production conditions of the food they will buy and experience it where it is produced. The processing of food alienates humans from nature. Experience

in the natural environment also corrects consumer misinformation.

In fact, the best method of promotion is to show the beekeeping profile of a country, the apiaries, the flower diversity in the country, the honey, propolis and plant resources produced in that region... "Apitourism" is the type of tourism, which was established years ago in Slovenia, the impact of apitourism, and whose impact factor is increasing, and which is the most effective method of existence of our age, aimed at the promotion of beekeeping and bee products. Apitourism is also the new entrepreneurial field of



Figure 1 a

beekeeping. The existence of Anton Jansa, who was the Europe's beekeeping leader, and Slovenia's unique traditional bee houses and production techniques have become interesting and instructive, causing the natural formation of apitourism. (Figure 1 a-b. Apitourism in Slovenia).



Figure 1 b

Apitourism in Turkey involves the presentation of traditional and modern techniques together. Turkey ranks 2nd in the world with 9 million hives and over 90,000 beekeepers and honey production. Turkey has 3 different phytogeographic regions. While there are 3000 endemic plant species in all of Europe, there are 3500 endemic species only in Turkey (Özkırım 2018, Beekeeping in Turkey). An average of 115,000 tons of honey is produced annually in Turkey. (TUIK, 2023). Honey production in Turkey is offered to consumers in the form of combed honey and filtered honey. Migratory beekeeping constitutes 75% of beekeeping activities in Turkey. (Özkırım, 2018).

The famous honeys in Turkey are Anzer honey, Castanea honey, Oak honey, Pine honey, Multifloral honey etc. There are several scientific studies which reveal that their benefits on human health. That's why apitherapy applications are being common in Turkey day by day.

Some governmental departments, municipalities, development agencies or EU have supported attractive projects to build up marketing places and apitherapy application centers in different areas. On the other hand, we think that apitherapy and apitourism are both interconnected and very different concepts.

The target participants of apitherapy includes patients and people who want to use bee prod-

ucts for therapeutic purposes. The target participants of Apitourism is completely healthy, aiming to travel and experience the nature, history and culture of different countries, as well as beekeeping in different geographies. For this reason, the only type of apitherapy that can be included in apitourism can be called as "Apicare"... This means that healthy people take care of themselves, mostly including topical applications, in order to rest and protect their health.

Apitourism in Turkey was created in 8 different routes in parallel with its seasonal diversity and beekeeping based on its historical background. In the apitourism activities developed as the ApiTURroute project, "api" represents the beekeeping area, "TUR" represents Turkey, and "route" represents the routes to be followed during the trips (Figure 2. The logo of ApiTURroute)



Figure 2

The ApiTURroute project was carried out in 3 stages:

1. Determination of routes and associating beekeeping activities with the history, nature and culture of the region

2. Certification of beekeeping units on the routes (beekeeping, museums, university laboratories, beekeeping associations, etc.) in accordance with certain criteria (Hacettepe University

Apitourism Certificate Program www.harum.hacettepe.edu.tr)

3. The certificated stations are recognized by tourism companies and tourism companies create the "apitourism" title in their website menus.

In determining the 8 routes in Turkey, the airports at the starting and ending points of the routes, different climatic features, different plants flora and different beekeeping structures were taken into consideration (Figure 3 a-f. Examples of different plants, beekeeping structures).



Figure 3 a-f

Famous honey produced in different geographical regions or interesting bee products and the colorful and different designs of the apiaries were primarily preferred. (Figure 4. The Routes of Apitourism in Turkey/ApiTURroute Project).

Within the scope of the ApiTURroute project, a visual archive of beekeeping in Turkey has been created, thanks to the promotional clips created with photographs and videos taken while determining the routes. In the creation of this



- 1 İstanbul-Şile-Kocaeli-Sakarya-Düzce
- 2 Rize-Ordu
- 3 Kars-Ardahan-Artvin
- 4 Bingöl-Erzincan-Tunceli
- 5 Afşin
- 6 Muğla
- 7 Balıkesir
- 8 Kırklareli

Figure 4.



**BEES
LIFE**

ApiTur - Kars Ardahan Artvin

ApiTURroute ile Apiturizm Kars Ardahan Artvin Rotası



Tur Programı	Tarihler ve Fiyatlar	Diğer Bilgiler	Bunlarda Önemli
---------------------	-----------------------------	-----------------------	------------------------

Figure 5 a

Figure 5 b

TAMZARA BAŞARDIK

Anasayfa > [İstanbul Apiturizm Buluşması](#)

Düzce ApiTurizm Buluşması

Apiturizm ve Apituroute Düzce buluşması

Rezervasyonlarınızı buradan yapabilirsiniz

Türkiye'de İLK DEFA Apiturizm bir Turizm firmasının resmi web sayfası menüsünde!

Tur Programı	Tarihler ve Fiyatlar	Diğer Bilgiler	Bunlarda Önemli
---------------------	-----------------------------	-----------------------	------------------------

API TURİZM ROTASI OLARAK DÜ

Apiturizmin tüm bileşenlerini bir araya getiren kural ve uygulama modüllerinin ortaya konulmasında ör

TAMZARATUR.COM

archive, the photo contest organized by ApiTURroute attracted the attention of professional photographers and tourism operators of the country and the world to apitourism.

Social media platforms created by ApiTURroute (you tube, instagram, gmail, apiTURroute web page etc.).(Figure 5 a-b. Instagram SS of apiTURroute account)

ApiTURroute ensures that its activities have a large number of followers at national/international level. The experiences in the photos and videos shared by the followers also lead to the formation of new followers (Figure 6 a-b. The photos shared by followers in their accounts). It is observed that when each participant on the ApiTURroute platform experiences a route, they are more willing to participate in other different routes. This situation also allows the formation of an apitourism tourist platform.



Figure 6 a

Apitourism was first implemented in Turkey this year performed on route no 1 Among the participants were medical personnel, lawyers, faculty members, art directors, photographers,



Figure 6 b

students and beekeepers (Figure 7 a-d The photos of the first trip of ApiTURroute in Düzce). The group of 12 people kept in touch with beekeepers. Apitourists wore their beekeeper suits for the first time. They saw the chestnut flower for the first time and observed the hives where chestnut honey was produced. However, they also visited Düzce University Beekeeping Application and Research Center (DAGEM) and received information about scientific studies on beekeeping. They visited historical places, museums, nature parks and waterfalls in Düzce province. Among the Apitourism passengers, there were participants who stayed in a tent for the first time in their lives and experienced the happiness of waking up in nature in the morning.

Other trips of ApiTURroute were organized to Kars-Ardahan-Artvin (route no.3) (Figure 8 a-c The photos of Kars-Ardahan-Artvin route) and Afşin (route no.5) (Figure 9 a-c The photos of



Afşin route). The near future goal of the trips, which are currently at a national level, is to host our guests from abroad in Turkey, which ranks 2nd in the world in the field of beekeeping.



Figure 8 a-c



Figure 7 a-d

Among our other goals is to create apitourism routes in cooperation with other hemisphere countries, since beekeeping is active in the same seasons in countries in the same climate zone. Thus, while it is winter in Turkey or Europe, joint apitourism tours will be organized in countries where the summer season is experienced.



Figure 3 g-h



Figure 7 e

Figure 9 a-c



Figure 3 i

We think that apitourism is a new field of initiative that will introduce beekeeping and directly affect all activities related to beekeeping (production, marketing, export, product development, etc.). Beekeeping sector that is always on display and in front of everyone's eyes will definitely develop and become more beautiful. Therefore, apitourism will be the most important field of activity contributing to this development in the near future.



Figure 10



Prof. Dr. Aslı Özkırım

MicroBeeotic Laboratory (MBL)
Department of Biology
Faculty of Science, Hacettepe University
Ankara-Turkiye

Director of
Bee and Bee Product Application and Research
Center, Hacettepe University, Ankara-Turkiye

Vice-President of
COLOSS Honey Bee Research Association

Editor-in-Chief
MELLIFERA Journal

ozkirim@hacettepe.edu.tr



Figure 3 j



Mustafa Ertekin

References

Özkırım, Aslı (2018). Beekeeping in Turkey: Bridging Asia and Europe. 10.1007/978-981-10-8222-1_2. 13)
<https://data.tuik.gov.tr/Bulten/Index?p=Hayvansal-%C3%9Cretim-%C4%B0statistikleri-2023-49681&dil=1>

DISCOVER

THE VARIETY OF FLAVOURS OF SPARKLING MEAD FROM SLOVENIAN BEEKEEPERS

Slovenia is located in the heart of Europe, at the crossroads of four major European geographical units: the Alps, the Pannonian Basin, the Dinaric Mountains and the Mediterranean.

This geographical and natural diversity allows a wide variety of flora to grow.

For this reason, Slovenia has excellent conditions for the development of beekeeping, which is valued both at home and abroad.

Slovenian beekeepers are proud of our high standards and good practices, which is also reflected in the production of honey drinks.

Beekeepers, with their passion and years of experience, produce honey that is also a culinary speciality - rich in aromas and full of flavours.

Slovenia produces a wide variety of honeys, such as acacia, flower, lime, chestnut, spruce, fir and forest, each with its own unique colour, flavour and aroma. It is this diversity in honey types that is the basis for creating magical honey drinks that delight the taste buds of every lover of quality honey drinks.

One of the oldest alcoholic beverages is mead, which is made by alcoholic fermentation



Variety of flavours in honey drinks

of a honey solution (water and honey). Its distinctive value and quality are built up with great patience and the beekeeper's excellent knowledge. Depending on the residual unfermented sugar, dry, semi-dry, semi-sweet and sweet meads are produced.

The real pride of Slovenian beekeepers is the sparkling mead, which was first introduced to the general public in 2005 in Maribor. Sparkling mead is made using the classic (Champagne) method from the highest quality honey. This noble drink combines sophistication and freshness, as the secondary alcoholic fermentation in the bottles enriches its taste and adds a sparkling effervescence.

Sparkling mead must contain between 9,5 and 15,0 vol. % alcohol and must have a minimum pressure of 3 bar. Depending on the residual of unfermented sugar, we produce dry, semi-dry, semi-sweet and sweet sparkling mead. Its effervescence and sparkle give it a special charm, while the harmony of flavours brings lightness, freshness, honeyed aroma, refreshing acidity, elegance and fullness. The process of making sparkling mead is long and demanding, but is rewarded by the unparalleled nobility of the finished product.

Sparkling mead is the queen of honey drinks, suitable for festive occasions and special moments that we want to mark with a special touch. In Slovenia, we offer a wide range of honey drinks, each made from the finest quality honey, which preserves and enriches its aroma through



Sparkling mead for special occasions

a carefully executed technological process. Slovenian beekeepers, with their knowledge, passion and dedication, create products from bee products that certainly deserve international recognition. With this, we spread the word about honey drinks from Slovenia, a country with a rich beekeeping heritage. Let our sparkling mead and other honey drinks become a symbol of Slovenian excellence and innovation, which inspires and connects people all over the world.

Tomaž Samec
Food safety adviser
for Slovenian Beekeepers' Association

**BEES
LIFE**

HONEY CONTESTS

AN OPPORTUNITY FOR BEEKEEPERS

If you want to find out whether your honey expresses the distinct sensory characteristics typical of its type and stands out from others, you might consider entering a honey contest. Every year, several Slovenian and international honey contests are held in Slovenia, where all Slovenian beekeepers can participate. Beekeepers from other countries can submit their samples in international and the European honey contest. These honey contests in Slovenia are carried out in accordance with the Rules of the Board of the Beekeepers' Association of Slovenia on sensory evaluation of honey.

The samples in a contest must meet high quality criteria, therefore analysis is performed. Water content and electrical conductivity are always tested, while certain samples are also tested for pesticide residue, authenticity and HMF

content. The types of honey evaluated at each contest depend on the submitted samples, with the most common varieties being multifloral, forest, acacia, linden, and chestnut honey. The honey judges are experts in the sensory properties of honey, having completed training at the Biotechnical Faculty, University of Ljubljana, and they renew their expertise every three years through a refresher course.

Each honey sample is assessed by an expert committee consisting of three to six members. Each judge independently evaluates the appearance, smell, taste, and aroma, assigning points in each category.

The final score for the sample is the average of the judges' scores, with a maximum of 30 points possible. Based on the points awarded, the honey sample can receive a gold, silver, or



bronze medal, or simply a certificate of participation.

If the number of samples for a specific type of honey is seven or more, the organizer may declare a champion for that type. This champion is the sample that scored the highest among all samples of the same type and therefore exhibits the most typical sensory properties for that variety of honey. Additionally, the sample with the highest overall score in the contest may be declared the overall champion by the competition organizer.

An award and title from a honey contest reflect the beekeeper's skill and dedication in producing top-quality honey and presenting it excellently. This recognition serves as a guarantee of quality for consumers and provides the beekeeper with a valuable marketing tool to command a higher price for the honey.

Different honey contests may be more or less known among consumers and the general public. This year, we aim to elevate the status of honey contests in Slovenia and highlight the significance of contest medals. To achieve this, we will host the first European Honey Contest in Koper,

where an internationally recognized expert committee will announce the top three honeys in each category. The winners will be revealed on December 7, 2024, during a professional and culinary event in Koper.

The best honeys will receive:

- The title of EUROPEAN CHAMPION 2024,
- unique plaques and awards,
- medal logos to mark the award-winning series of honey,
- special exposure in the European Parliament,
- analysis reports on high quality and safety,
- the right to use the flattering title for the next two years!

We invite all beekeepers and honey packers from European countries to participate!

More information at: <https://www.honey-contest.eu/>

Aljaž Debelak

Public advisory service in beekeeping
Slovenian Beekeeper's Association

aljaz.debelak@czs.si



The poster features a large hexagonal logo on the left containing a stylized bee. To its right, the text reads "European Honey Contest Koper - Slovenija". Below this, there are three smaller logos: the Slovenian Beekeeper's Association logo, the logo of the Municipality of Koper, and the logo of the Ministry of Agriculture, Forestry and Food. The central text in large, bold, orange letters says "Sample collection up to 16.9.2024". At the bottom, it says "More information on web site www.honey-contest.eu". The background is a light yellow with a faint, large illustration of a bee.



TRIBUTE TO BEES

Since childhood, I have been fascinated by bees...

I used to spend hours in the garden or in the park observing them.

Because I'm a musician, my emotions became music notes and I composed this string quartet in homage to bees.

It is called "Tribute to bees".

I built it in four parts:

The first part is about sadness, the second part about anger, the third part is a waltz of hope and the fourth part a love song for life.

Françoise Derissen and Bénédicte Chabot (violinists) and Fabienne Van Den Driessche (cellist) joined me to form the String quartet "Tribute to Bees".

We played our music in concert for the first time in spring 2024 in Brussel and in the garden of the Museum of fine Arts in Mons (Belgium).

Trough this music, we would like to raise to awareness the protection of bees, pollinators and biodiversity.

You can listen to us on You Tube: Tribute to bees – string quartet and on instagram: tribute_to_bees.

For "Tribute to bees"
Johanne Samek
composer and viola violin
player
in the string quartet
+32 499 16 99 99
johannesamek@yahoo.fr
Instagram: tribute_to_bees



**BEES
LIFE**

Ulster
Beekeepers
Association



ANNUAL CONFERENCE

Friday 14th to Saturday 15th February 2025

CAFRE, Greenmount Campus, 45 Tirgracy Road, Antrim BT41 4PS

READING OUR BEES THE NEXT CHAPTER

Two days of Science, Practical Beekeeping & Workshops covering:
Introductory Beekeeping, Microscopy, Working with Wax,
Encaustic Art, Skep Making, the ever popular
Mead Making, and more!

Featuring an Extensive Trade Exhibition

EXPERT SPEAKERS INCLUDE:

Prof Lars Chittka – Queen Mary University of London

Dan Basterfield – BBKA Master Beekeeper

Colm O'Neill – North Kildare Beekeepers Association

Ruth Wilson – AIPP Farmland Pollinator Officer

Dr Rowena Jenkins – Microbiologist, Swansea University

Ass. Prof Dalial Freitak – University of Graz, Austria

Richard Noel – Brittany Bee Farmer

Prof Robert Paxton – Martin Luther University, Germany

***Lock this important conference
into your diary now!***

**BOOKING OPENS ON 1st
OCTOBER 2024 ON-LINE AT:
<https://buytickets.at/ulsterbeekeepersassociation/1321240>**

For more information contact: Brian Grzymek – Conference Organiser

Email: bgrzymek@icloud.com or Visit our Website: www.ubka.org

XXVII

TRADITIONAL MANIFESTATION

DAYS OF NORTH MACEDONIAN BEEKEEPING, BITOLA 2024

FROM 10-12 OCTOBER AT SHIROK SOKAK IN BITOLA

PROGRAM:

10.10 Thursday

- 12.00 – Ceremonial opening of the event
- 12.30 – A culturally entertaining program
- 13.00 – Visit and introduction with the exhibitors at the event

11.10. Friday

- 09.00 – - Visit to the exhibition part of the event - Magnolia Square
- 12.00 – Announcement of the winners after the announced award-winning literary competition on the topic "Beekeeping" for participants from elementary schools
- 19.30 – Beekeeping evening – "Hotel Molika"

12.10. Saturday

- 09.00 – Visit to the exhibition part of the event - Magnolia Square
- 12.00 – Professional lecture
- 16.00 – Awarding of certificates of thanks to the participants of the Manifestation

TRAINING FOR PROFESSIONAL COOPERATION WITH KINDERGARTENS TO WORK ACCORDING TO APIPEDAGOGY

Date: Saturday 14 September 2024

Hours: 10:00 a.m. – 4:00 p.m

Location: Kočevje, Slovenia. The exact location will be provided to registrants 1 week before the event.

Who is it for? beekeepers and apitherapists

Apipedagogy is an innovative professional program formed by certain segments, including the apitherapy segment, environmental protection segment, API school for parents, Honey massage for children, etc.. It is supported by the Municipality of Ljubljana and the Ministry of Education.

In today's fast pace of life, it is sometimes a challenge for educators to create something fresh, to live in the bloom of power and at the same time to pay attention to contributing to their family and also to the parents of the children for whom they are responsible. In addition, everything that children experience in the first years of life shapes their entire life. That is why it is considered that the teaching profession is one of the professions with the greatest responsibility, as educators co-create the life path of all the children entrusted to us. Everyone who participates as external experts also enters this process with the same degree of responsibility.

API kindregarten is a pedagogical program that, through a healthy life in kindergarten with the help of the apitherapy segment, makes educators, children, and their parents aware of the quality effects of bee products on our health.

Kindergartens are encouraged to cooperate with external experts who are qualified to work

according to Apipedagogy. Reports from educators also testify that we are on the right path:

»Parents' satisfaction was high, as they noticed positive changes in their child's development, greater self-confidence and better social skills. Parents felt more involved in the life of the kindergarten and appreciated the approach of the API kindergarten program to the holistic development of children.«

»Joint efforts have created a cohesive work environment. The program has therefore brought positive changes for both children and employees, which motivates us for further research.«

»The children were extremely interested in participating in the project and were happy to participate. They also impressed their parents with certain activities and knowledge. They were most impressed by the wax massage, which they also performed at home on their parents and siblings.«

The training for professional cooperation with kindergartens to work according to Apipedagogy will contain a certificate of completion of the training, qualification for conducting API workshops, added values that educators need from beekeepers and apitherapists, and the secret of the apitherapeutic-pedagogical approach.



Training provider: Nina Ilič, Public Administration Graduate, Preschool Teacher, Food Technician, beekeeper, and Certified Apitherapist.

Welcome to the beautiful world of bees and children with us.

More information and application for education:

zavod.eneja@gmail.com.

Institute for the Development of Empathy and Creativity Eneja

Nina Ilič

Coordinator of the Network

of API Kindergartens

and Schools APIS RETIS

Professional manager of

apipedagogical programs,

Institute for the Development of

Empathy and Creativity Eneja

President of the Commission for

Junior Beekeepers of the

Beekeeping Association of Slovenia

Member of the board of the

Beekeeping Association of Slovenia

Member of the board

of the Bee Path Cities network



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 without 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:

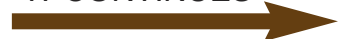
1. Fight against counterfeit honey that flooded the European market;
2. Introduction of incentives per beehive as agro-ecological programme;
3. Fight against the improper use of chemicals that are harmful to bees;

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

2	20 COUNTRIES FROM WHICH EBA HAS MEMBERS (27 BEEKEEPING ORGANIZATIONS)
6	A PLEA TO EUROPE'S POLITICIANS – STOP COUNTERFEIT FOOD!
8	BEEKEEPERS' CALL TO CONSUMERS: LET'S PUT AN END TO FAKE HONEY TOGETHER!
10	DR URŠKA RATAJC APPOINTED TO THE COPA COGECA'S HONEY WORKING PARTY
11	ANTI-DUMPING DUTY A FEASIBLE SOLUTION TO LIMITING HONEY FRAUD THAT COLLIDES WITH INTERESTS
15	JOIN THE EBA VIBER AND WHATSAPP GROUPS FOR FREE
17	IT IS POSSIBLE TO PROVE MISLABELING OF HONEY'S ORIGIN!
20	TROYAN TRAGEDY OF BEE COLONY
28	INFLUENCE OF PROBIOTIC FEED SUPPLEMENT ON THE GUT MICROBIOTA OF ADULT HONEYBEES (APIS MELLIFERA L.)
46	MICRONUTRIENT DEFICIENCY AND THE ONSET OF CHALKBROOD DISEASE IN HONEY BEES
53	NEW SCALE - NEW DIMENSION OF BEEHIVE MONITORING
55	ADVANCING BEE CONSERVATION: INNOVATIVE TECHNOLOGIES FOR PROTECTING HONEYBEE POPULATIONS WITH ILLUMINUM & BEESAGE
59	INTERVIEW: CARLA MARINA MARCHESE, HONEY SOMMELIER
64	A NEW BOOK: THE WORLD ATLAS OF HONEY
65	A NEW BOOK: THE SECRETS OF MISS HONEYBEE
67	BEE TOURIS IN SLOVENIA AND AWARDED CERTIFICATION
71	LET'S CERTIFY APITOURISM ACROSS EUROPE!
76	SLOVENIAN APIARY
79	THE CARNIOLAN BEE HOUSE
85	THE MUSEUM OF APICULTURE SLOVENIA A UNIQUE BEEKEEPING HERITAGE A SONG OF THE SOUL FOR EVERY BEEKEEPER AROUND THE WORLD
90	BEE PATH IN LJUBLJANA
97	APITOURISM INSPIRED BY SLOVENIA IS NOW IN TURKEY WITH "APITURROUTE" PROJECT
105	DISCOVER THE VARIETY OF FLAVOURS OF SPARKLING MEAD FROM SLOVENIAN BEEKEEPERS
107	HONEY CONTESTS AN OPPORTUNITY FOR BEEKEEPERS
109	TRIBUTE TO BEES
115	SPONSORSHIP REQUEST AND METHOD OF ADVERTISING IN THE MAGAZINE



EBA informative and professional monthly magazine “**NO BEES, NO LIFE**”

September 2024.

Issued since July 2024.

Publisher: **European Beekeeping Association (EBA)**

Head office: Brdo pri Lukovici 8, 1225 Lukovica, SLOVENIJA

eba@ebaeurope.eu

www.ebaeurope.eu

Downloading and printing texts from "NO BEES, NO LIFE" in other magazines and electronic media is allowed and free of charge, but it is mandatory to indicate the source of the text immediately below the title. Magazine sharing is preferred.

The contents of the texts and advertisements are the responsibility of the authors.

The responsibility for the correctness of the English language in the magazine lies with the authors of the texts.

The editor reserves the right to publish a larger advertisement than the size specified by the sponsorship package, if it improves the design of the magazine.

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.

The total number of pages in the magazine is not fixed.

There are no fees for published texts and photos.

Editor in chief of the electronic edition of the magazine:

MD Rodoljub Živadinović, Epidemiology Specialist, Apitherapist

apikult@gmail.com, +381 60 444 01 01 (Viber, WhatsApp, Signal, Telegram, WeChat)