# FORAGING ECOLOGY OF SYMPATRIC HONEY BEES AND STINGLESS BEES: PALYNOLOGY AND HONEY METABOLITE APPROACH

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**STATEMENT LETTER**

I hereby declare that dissertation entitled "Foraging Ecology of Sympatric Honey Bees and Stingless Bees: Palynology and Honey Metabolite Approach" is original result of my own research supervised by supervisory committee and has never been submitted in any form at any institution before. All information from other authors cited here are mentioned in the text and listed in the reference at the end part of the dissertation.

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Bogor, August 2026

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# RINGKASAN

TIARA SAYUSTI. Ekologi *foraging* lebah madu dan lebah tanpa sengat simpatrik: pendekatan palinologi dan metabolit madu. Dibimbing oleh RIKA RAFFIUDIN, NINA RATNA DJUITA, R. R. DYAH PERWITASARI, dan IRMANIDA BATUBARA.

Lebah madu dan lebah tanpa sengat merupakan penyerbuk penting pada ekosistem tropis serta menjadi kelompok lebah utama yang dibudidayakan dalam sistem perlebahan di Indonesia. Meskipun hidup simpatrik pada lanskap *foraging* (pencarian makan) yang sama, lebah madu dan lebah tanpa sengat dapat menunjukkan pola aktivitas *foraging* harian, pemanfaatan sumber daya flora, dan karakteristik metabolit madu yang berbeda, yang berpotensi mengurangi kompetisi serta mendukung koeksistensi. Namun, studi mengenai ekologi *foraging* lebah simpatrik yang menghubungkan pola aktivitas temporal, pemanfaatan sumber daya flora, dan variasi karakteristik fisikokimia serta komposisi metabolit madu masih terbatas.

Oleh karena itu, penelitian ini bertujuan menganalisis ekologi *foraging* lebah madu dan lebah tanpa sengat yang hidup simpatrik melalui tiga pendekatan, yaitu: (1) membandingkan pola aktivitas *foraging* harian serta mengevaluasi pengaruh faktor lingkungan terhadap aktivitas *foraging*; (2) mengidentifikasi komposisi polen korbikula dan polen madu (*melissopalynology*) untuk mengevaluasi pemilihan sumber daya flora berdasarkan ketersediaan vegetasi di sekitarnya; dan (3) menganalisis karakteristik fisikokimia dan komposisi metabolit madu, serta mengevaluasi hubungan antara komposisi polen madu dengan karakteristik fisikokimia dan komposisi metabolit madu.

Penelitian ini dilakukan pada dua lokasi peternakan lebah di Indonesia yang melibatkan jenis lebah yang paling banyak dibudidayakan. Pengamatan aktivitas *foraging*, koleksi polen korbikula, madu, dan analisis vegetasi dilakukan di peternakan lebah Flora Nauli, Kota Pematang Siantar, menggunakan lebah madu *Apis cerana* dan dua spesies lebah tanpa sengat, yaitu *Heterotrigona itama* dan *Geniotrigona thoracica*. Analisis *melissopalynology*, sifat fisikokimia, dan komposisi metabolit madu dilakukan pada madu *A. cerana*, *Apis koschevnikovi*, dan *H. itama* asal peternakan lebah Nirun Mandiri, Kabupaten Belitung.

Aktivitas *foraging* diamati selama tiga hari berturut-turut dari pukul 07.00 hingga 16.00 WIB menggunakan dua koloni pada setiap spesies lebah. Hubungan antara aktivitas *foraging* dengan faktor lingkungan dianalisis menggunakan *Generalized Linear Models*. Sampel polen korbikula dan madu dikoleksi dan diproses menggunakan metode asetolisis. Polen korbikula dikoleksi setiap jam dari pukul 07.00 hingga 16.00 WIB sehingga diperoleh total 44 sampel polen korbikula dari tiga spesies lebah. Tipe polen diidentifikasi berdasarkan karakter morfologi dan dianalisis menggunakan kelimpahan relatif. Analisis vegetasi dilakukan untuk mengevaluasi ketersediaan sumber daya flora. Analisis *melissopalynology* pada sampel madu dilakukan dengan menghitung 300 butir polen per sampel untuk mengidentifikasi sumber flora. Parameter fisikokimia yang diukur meliputi kadar air, kadar gula, pH, *electrical conductivity* (EC), dan *total dissolved solids* (TDS), sedangkan profil metabolit madu dianalisis menggunakan LC–MS/MS.

Hasil penelitian menunjukkan adanya perbedaan pola aktivitas temporal yang signifikan antara lebah madu dan lebah tanpa sengat. *Apis cerana* menunjukkan puncak aktivitas lebih awal di pagi hari, sedangkan lebah tanpa sengat mencapai puncak aktivitas pada waktu yang lebih siang. Lebah tanpa sengat mempertahankan aktivitas pengumpulan polen yang lebih stabil dibandingkan *A. cerana*. Pola tersebut mengindikasikan adanya pembagian relung temporal yang berpotensi mengurangi kompetisi antarspesies dan mendukung koeksistensi. Tumpang tindih aktivitas ditemukan pada kedua spesies lebah tanpa sengat. Variasi pola aktivitas temporal berkaitan dengan respons spesifik spesies terhadap faktor lingkungan, yang dapat berkontribusi terhadap perbedaan pemanfaatan sumber daya flora.

Perbedaan pemanfaatan sumber daya flora terlihat pada komposisi polen korbikula, lebah tanpa sengat mengumpulkan polen yang lebih beragam dibandingkan *A. cerana*, dengan *H. itama* menunjukkan diversitas polen lebih tinggi dibandingkan *G. thoracica*. Madu lebah tanpa sengat juga mengandung tipe polen yang lebih beragam, sedangkan madu *A. cerana* didominasi oleh satu famili tumbuhan. Hasil ini menunjukkan bahwa lebah tanpa sengat cenderung memiliki pola *foraging* yang lebih generalis dibandingkan *A. cerana*. Perbandingan dengan vegetasi sekitar menunjukkan bahwa tipe polen dominan yang ditemukan tidak sesuai dengan tumbuhan dominan di lokasi penelitian. Hal ini menunjukkan bahwa pemanfaatan sumber daya flora oleh lebah tidak hanya ditentukan oleh kelimpahan vegetasi, tetapi kemungkinan juga dipengaruhi oleh faktor lain seperti fenologi pembungaan dan kesesuaian karakteristik bunga dengan morfologi lebah. Perbedaan pemanfaatan sumber daya antar lebah simpatrik dapat berkontribusi terhadap variasi karakteristik fisikokimia dan komposisi metabolit madu.

Konsisten dengan perbedaan pemanfaatan sumber daya flora antar spesies lebah di Peternakan Flora Nauli, variasi komposisi polen madu juga terlihat di Peternakan Nirun Mandiri, dengan *H. itama* menunjukkan jumlah tipe polen tertinggi. Sebaliknya, *A. cerana* dan *A. koschevnikovi* menunjukkan pola *foraging* yang lebih terbatas dan memanfaatkan *Acacia* sp. sebagai sumber flora dominan. Perbedaan tersebut juga terlihat pada sifat fisikokimia madu, *H. itama* menghasilkan madu dengan kadar air, EC, dan TDS tertinggi serta pH dan kadar gula terendah. Profil madu menunjukkan perbedaan kelimpahan relatif metabolit dan keberadaan sejumlah senyawa spesifik, termasuk berbagai senyawa yang belum teridentifikasi yang hanya ditemukan pada masing-masing spesies lebah.

Kebaruan penelitian ini terletak pada pendekatan integratif yang menghubungkan aktivitas *foraging*, pemanfaatan sumber daya flora berdasarkan analisis *melissopalynology*, serta karakteristik fisikokimia dan metabolit madu pada beberapa spesies lebah yang hidup simpatrik di Indonesia. Penelitian ini menunjukkan bahwa koeksistensi lebah madu dan lebah tanpa sengat pada lanskap yang sama didukung oleh perbedaan pola aktivitas temporal dan pemanfaatan sumber daya flora, yang selanjutnya berkontribusi terhadap variasi karakteristik fisikokimia dan komposisi metabolit madu. Hasil penelitian ini memberikan dasar ilmiah bagi pengelolaan sumber daya pakan lebah, pengembangan budidaya lebah secara berkelanjutan, konservasi penyerbuk dan keanekaragaman hayati, serta pengembangan fingerprint metabolit sebagai pendekatan autentikasi madu.

Kata kunci: *Apis koschevnikovi*, asam glukonat, *Heterotrigona itama*, pembagian sumber daya, polen predominan

## SUMMARY

TIARA SAYUSTI. Foraging ecology of sympatric honey bees and stingless bees: Palynology and honey metabolite approach. Supervised by RIKA RAFFIUDIN, NINA RATNA DJUITA, R. R. DYAH PERWITASARI, and IRMANIDA BATUBARA.

Honey bees and stingless bees are important pollinators in tropical ecosystems and represent the main groups of bees managed in beekeeping systems in Indonesia. Although they occur sympatrically within the same foraging landscape, honey bees and stingless bees may exhibit differences in daily foraging activity patterns, floral resource utilization, and honey metabolite characteristics, which may reduce competition and facilitate coexistence. However, studies on the foraging ecology of sympatric bees that link temporal activity patterns, floral resource utilization, and variation in physicochemical properties and metabolite composition of honey remain limited.

Therefore, this study aimed to analyze the foraging ecology of sympatric honey bees and stingless bees through three approaches: (1) comparing daily foraging activity patterns and evaluating the influence of environmental factors on foraging activity; (2) identifying corbicular pollen and honey pollen composition (melissopalynology) to assess floral resource utilization in relation to the availability of surrounding vegetation; and (3) analyzing the physicochemical properties and metabolite composition of honey, as well as evaluating the relationship between honey pollen composition and honey physicochemical and metabolite characteristics.

This study was conducted at two beekeeping sites in Indonesia involving the most commonly managed bee species. Observations of foraging activity, collection of corbicular pollen and honey samples, and vegetation analysis were carried out at the Flora Nauli beekeeping site in Pematang Siantar City, involving the honey bee *Apis cerana* and two stingless bee species, namely *Heterotrigona itama* and *Geniotrigona thoracica*. Melissopalynological analysis, physicochemical characterization, and metabolite profiling of honey were conducted using honey from *A. cerana*, *A. koschevnikovi*, and *H. itama* collected from the Nirun Mandiri beekeeping site in Belitung Regency.

Foraging activity was observed for three consecutive days from 07:00 to 16:00 h using two colonies per bee species. The relationship between foraging activity and environmental factors was analyzed using Generalized Linear Models. Corbicular pollen and honey samples were collected and processed using the acetolysis method. Corbicular pollen was collected hourly from 07:00 to 16:00 h, resulting in a total of 44 corbicular pollen samples from three bee species. Pollen types were identified based on morphological characteristics and analyzed using relative abundance. Vegetation analysis was conducted to evaluate the availability of floral resources. Melissopalynological analysis of honey samples was performed by counting 300 pollen grains per sample to identify floral sources. Physicochemical parameters measured included moisture content, sugar content, pH, electrical conductivity (EC), and total dissolved solids (TDS), while honey metabolite profiles were analyzed using LC–MS/MS.

The results showed significant differences in temporal activity patterns between honey bees and stingless bees. *Apis cerana* exhibited an earlier peak of activity in the morning, whereas stingless bees reached their peak activity at a later time of the day. Stingless bees maintained a more stable level of pollen foraging activity compared with *A. cerana*. This pattern indicates temporal niche partitioning, which may reduce interspecific competition and facilitate coexistence. Overlapping activity patterns were observed between the two stingless bee species. Variation in temporal activity patterns was associated with species-specific responses to environmental factors, which may contribute to differences in floral resource utilization.

Differences in floral resource utilization were reflected in corbicular pollen composition, with stingless bees collecting more diverse pollen types than *A. cerana*, and *H. itama* showing higher pollen diversity than *G. thoracica*. Honey from stingless bees also contained a wider diversity of pollen types, whereas *A. cerana* honey was dominated by a single plant family. These results indicate that stingless bees tend to exhibit a more generalist foraging pattern compared with *A. cerana*. Comparisons with surrounding vegetation revealed that dominant pollen types did not correspond to the dominant plant species in the study sites. This finding suggests that floral resource utilization by bees is not solely determined by vegetation abundance, but presumably influenced by other factors such as flowering phenology and the compatibility between floral traits and bee morphology. Differences in floral resource use among sympatric bees may contribute to variation in honey physicochemical properties and metabolite composition.

Variation in honey pollen composition was also observed at the Nirun Mandiri beekeeping site, with *H. itama* showing the highest number of pollen types. In contrast, *A. cerana* and *A. koschevnikovi* exhibited a more restricted foraging pattern and relied predominantly on *Acacia* spp. as the main floral resource. These differences were also reflected in honey physicochemical properties, with *H. itama* producing honey characterized by the highest moisture content, electrical conductivity (EC), and total dissolved solids (TDS), as well as the lowest pH and sugar content. The honey metabolite profiles differed in the relative abundance of metabolites and the presence of species-specific compounds, including several unidentified compounds detected exclusively in each bee species.

The novelty of this study lies in its integrative approach linking foraging activity, floral resource utilization based on melissopalynological analysis, and the physicochemical characteristics and metabolite profiles of honey from sympatric bee species in Indonesia. The findings demonstrate that the coexistence of honey bees and stingless bees within the same foraging landscape is supported by differences in temporal foraging patterns and floral resource utilization, which subsequently contribute to variations in the physicochemical characteristics and metabolite composition of honey. This study provides a scientific basis for the management of bee forage resources, the sustainable development of beekeeping, the conservation of pollinators and biodiversity, and the development of metabolite fingerprinting as an approach for honey authentication.

Keywords: *Apis koschevnikovi*, gluconic acid, *Heterotrigona itama*, predominant pollen, resource partitioning

# FORAGING ECOLOGY OF SYMPATRIC HONEY BEES AND STINGLESS BEES: PALYNOLOGY AND HONEY METABOLITE APPROACH

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in Animal Bioscience Study Program

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*Tiara Sayusti*

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