

# Species Diversity and Seasonal Distribution of *Culicoides* spp. (Diptera: Ceratopogonidae) in Jeju-do, Republic of Korea

Heung Chul Kim<sup>1</sup>, Glenn A. Bellis<sup>2</sup>, Myung-Soon Kim<sup>3</sup>, Terry A. Klein<sup>4</sup>, David Gopurenko<sup>5,6</sup>, Du-Cheng Cai<sup>7</sup>, Hyun-Ji Seo<sup>3</sup>, In-Soo Cho<sup>3</sup>, Jee-Yong Park<sup>3,\*</sup>

<sup>1</sup>5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247, USA; <sup>2</sup>Charles Darwin University, Darwin Northern Territory 0909, Australia; <sup>3</sup>Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Anyang 430-757, Korea; <sup>4</sup>Public Health Command Region-Pacific, Camp Zama, Japan, Address: 65th Medical Brigade, Unit 15281, APO AP 96205-5281, USA; <sup>5</sup>NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Private Mail Bag, Wagga Wagga, New South Wales, 2650, Australia; <sup>6</sup>Graham Centre for Agricultural Innovation, Locked Bag 588, Wagga Wagga, New South Wales, 2678, Australia; <sup>7</sup>College of Environment and Plant Protection, Hainan University, 58 Renmin Avenue, Haikou, Hainan, 570228, P. R. China

**Abstract:** Biting midges belonging to the genus *Culicoides* (Diptera: Ceratopogonidae) were collected by Mosquito Magnet<sup>®</sup> and black light traps at 5 sites on Jeju-do, Republic of Korea (Korea), from May–November 2013 to determine species diversity and seasonal distribution. A total of 4,267 specimens were collected, of which 99.9% were female. The most common species was *Culicoides tainanus* (91.8%), followed by *C. lungchiensis* (7.2%) and *C. punctatus* (0.6%), while the remaining 4 species accounted for <0.5% of all *Culicoides* spp. that were collected. High numbers of *C. tainanus* were collected in May, followed by decreasing numbers through August, and then increasing numbers through November when surveillance was terminated. Peak numbers of *C. lungchiensis* were collected during September, with low numbers collected from May–August and October–November. The presence of *C. lungchiensis* in Korea was confirmed by morphological and molecular analyses.

**Key words:** *Culicoides lungchiensis*, *Culicoides tainanus*, biting midge, Korea

Members of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae), or biting midges, are small (0.5–2.0 mm in length) blood feeding insects distributed worldwide, with many species impacting on human and veterinary health as nuisance biters and vectors of human and animal pathogens [1,2]. The first nationwide biting midge surveys in the Republic of Korea (Korea) were reported in 1974 [3,4], with seasonal abundance and host blood meal analysis of *Culicoides* spp. collected from cattle and poultry farms in Gyeonggi-do reported much later [5]. More recently, a brief history of the *Culicoides* fauna of Korea was reported by Kim et al. [6] along with new records of 3 *Culicoides* spp. (*C. nasuensis* Kitaoka, *C. pallidulus* Yu, and *C. jacobsoni* Macfie) [7], bringing the total number of confirmed re-

ords of *Culicoides* spp. to 31 in Korea.

Vector-borne disease surveillance was conducted by the Animal and Plant Quarantine Agency in collaboration with the Public Health Command Region-Pacific (PHCR-PAC) and the 65th Medical Brigade to determine the species composition and seasonal and geographical distributions for *Culicoides* spp. and associated pathogens using Mosquito Magnet<sup>®</sup> and black light traps at Jeju Island, a special self-governing Province in Korea, from May–November 2013. The surveillance was focused on international ports on Jeju Island to address the possible risk of exotic species of vector midges being newly introduced, as suggested by reports of biting midges being transported on boats [8] and on aircraft [9].

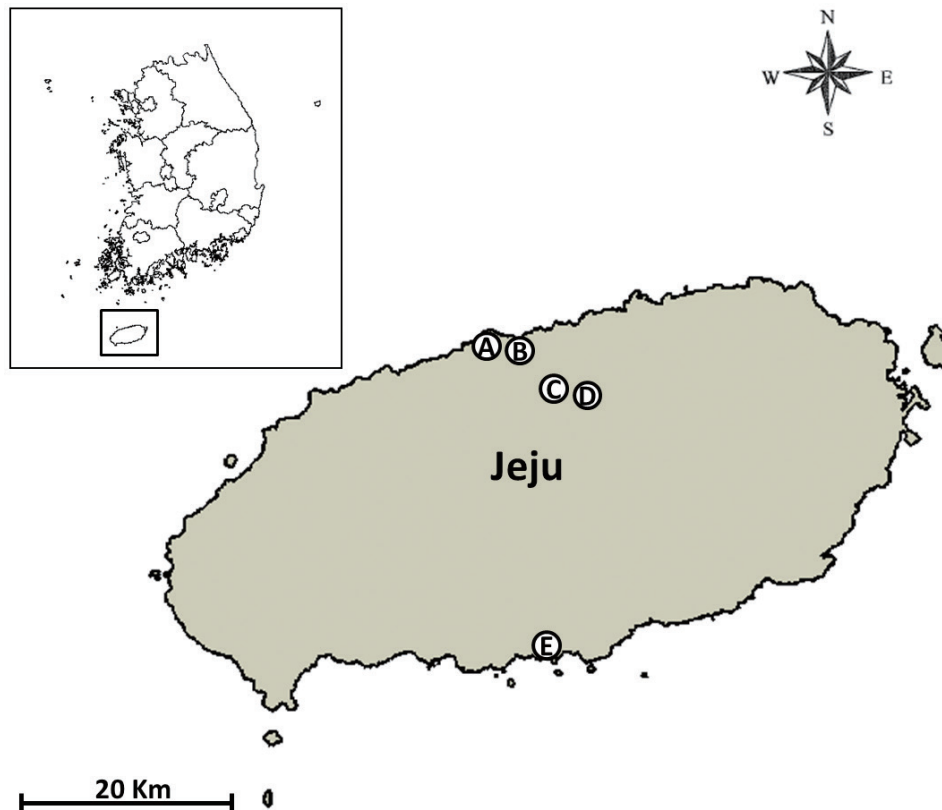
Three Mosquito Magnet<sup>®</sup> traps (Liberty Plus Model, Woodstream Corp., Lititz, Pennsylvania, USA) and 2 black light traps (Black Hole model, BioTrap, South Korea; <http://www.bio-trap.com>) were used for surveillance of mosquitoes, biting gnats (*Culicoides* spp.), and other biting flies at the Jeju airport cargo warehouse (126.498785 E, 33.506016 N), Jeju airport

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\*Corresponding author (parkjyunesy@korea.kr)

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**Fig. 1.** Survey sites for *Culicoides* spp. collected by Mosquito Magnet® and black-light traps at 5 sites from May-November 2013 in Jeju-do, a special self-governing Province, Republic of Korea (A: Airport cargo warehouse, B: Airport detection dog center, C: Cattle farm at Yonggang-dong, Jeju, D: Yonggang animal quarantine station, and E: Seogwipo port).

**Table 1.** Total number (%) of *Culicoides* spp., by species and collection site, collected by Mosquito Magnet® (MM) and black-light (BL) traps at 5 sites from May-November 2013 at Jeju-do, a special self-governing Province, Republic of Korea

Collection sites	Traps	<i>Culicoides arakawae</i>	<i>Culicoides circumscriptus</i>	<i>Culicoides kibunensis</i>	<i>Culicoides matsuzawai</i>	<i>Culicoides punctatus</i>	<i>Culicoides lungchiensis</i>	<i>Culicoides tainanus</i>	Total (%) <sup>a</sup>
Airport detection dog center	MM	0	1 (100)	0	0	0	0	0	1 (<0.1)
Airport cargo warehouse	BL	1 (100)	0	0	0	0	0	0	1 (<0.1)
Cattle farm at Yonggang-dong	BL	0	0	1 (0.02)	14 (0.3)	25 (0.6)	308 (7.3)	3,903 (91.8)	4,251 (99.6)
Yonggang animal quarantine station	MM	0	0	0	0	0	0	14 (100)	14 (0.3)
Seogwipo port	MM	0	0	0	0	0	0	0	0
Total (% of Total)		1 (0.02)	1 (0.02)	1 (0.02)	14 (0.3)	25 (0.6)	308 (7.2)	3,917 (91.8)	4,267

<sup>a</sup>Percent of the total number of *Culicoides* spp. for each collection site.

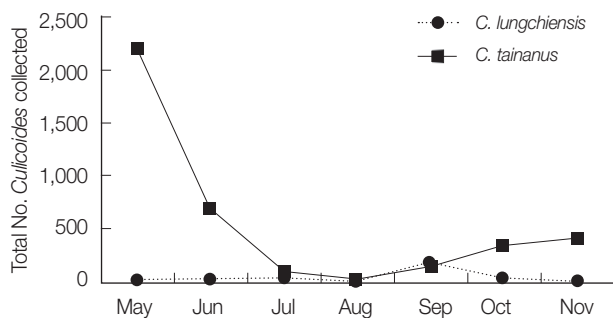
detection dog center (126.504718 E, 33.510294 N), a cattle farm at Yonggang-dong village, (126.586100 E, 33.455502 N), the Yonggang animal quarantine station (126.587995 E, 33.455616 N), and the Seogwipo seaport (126.564657 E, 33.455616 N) (Fig. 1). Traps were operated continuously, and specimens were collected twice per month from May-November 2013. Midges were processed as described previously [6]

and identified using the morphological keys and descriptions of Arnaud [10] and Wada [11] and the checklist for Korea [7].

A total of 4,262 female (99.9%) and 5 male (0.1%) *Culicoides* spp., belonging to 7 species, was collected. *Culicoides tainanus* Kieffer was the most commonly collected species (91.8%), followed by *C. lungchiensis* Chen & Tsai (7.2%), and *C. punctatus* (Meigen) (0.6%), while the remaining 4 species accounted

**Table 2.** Monthly prevalence of *Culicoides* spp. collected by Mosquito Magnet® and black-light traps at 5 sites from May–November 2013 in Jeju-do, a special self-governing Province, Republic of Korea

<i>Culicoides</i> species	MAY	JUN	JUL	AUG	SEP	OCT	NOV	Total (%)
<i>C. arakawae</i>	0	0	0	0	1 (100)	0	0	1 (0.02)
<i>C. circumscriptus</i>	0	0	1 (100)	0	0	0	0	1 (0.02)
<i>C. kibunensis</i>	1 (100)	0	0	0	0	0	0	1 (0.02)
<i>C. matsuzawai</i>	0	7 (50.0)	1 (7.1)	3 (21.4)	3 (21.4)	0	0	14 (0.33)
<i>C. punctatus</i>	8 (36.6)	5 (20.0)	10 (40.0)	1 (4.0)	1 (4.0)	0	0	25 (0.59)
<i>C. lungchiensis</i>	21 (6.8)	16 (5.2)	36 (11.7)	14 (4.6)	189 (61.4)	32 (10.4)	0	308 (7.2)
<i>C. tainanus</i>	2,197 (56.1)	702 (17.9)	81 (2.1)	13 (0.3)	168 (4.3)	350 (8.9)	406 (10.4)	3,917 (91.8)
Total (%)	2,227 (52.2)	730 (17.1)	129 (3.0)	31 (0.7)	362 (8.5)	382 (9.0)	406 (9.5)	4,267

**Fig. 2.** Seasonal distribution of 2 predominant species, *Culicoides tainanus* and *C. lungchiensis* collected from Mosquito Magnet® and black-light traps at 5 sites from May–November 2013 at Jeju-do, Korea.

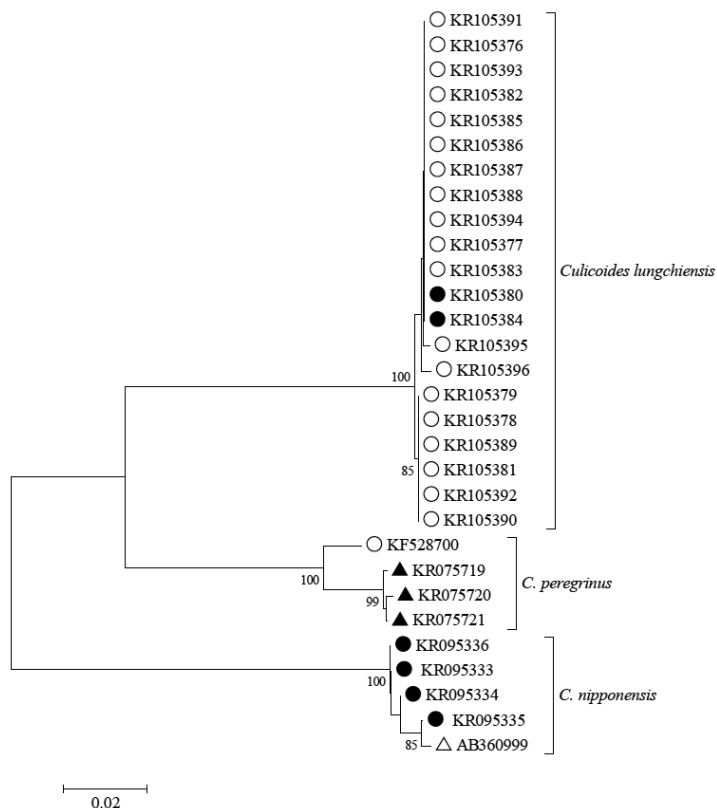
for 0.4% of all *Culicoides* spp. collected (Table 1). *C. tainanus* was collected only at 2 sites, a cattle farm at Yonggang-dong (3,903; 99.6%) and at the Yonggang animal quarantine station (14; 0.4%), while *C. lungchiensis* and *C. punctatus* were collected only at the cattle farm at Yonggang-dong (308 and 25, respectively). *C. tainanus* was most frequently collected during May, with decreasing numbers through August and then increasing numbers from August–November when collections were discontinued. These results coincided with that of Lee [2], where *C. tainanus* was the most common species collected at black light traps at 13 inland cowsheds (Taebaek, Yangyang in Gangwon-do, Incheon Metropolitan city, and Haenam in Jeollanam-do) in Korea. The dominance of *C. tainanus* at a cattle farm at Yonggang-dong, Jeju, agreed with the host preference of these species for cattle [2,12].

*C. lungchiensis* was collected in low numbers from May–August and November with high numbers collected during September (Table 2; Fig. 2). In contrast to the inland sites, only 2 specimens of any species were collected at the airport and none at the seaport suggesting that these sites are not conducive to the survival of *Culicoides*, which in turn reduces the risks

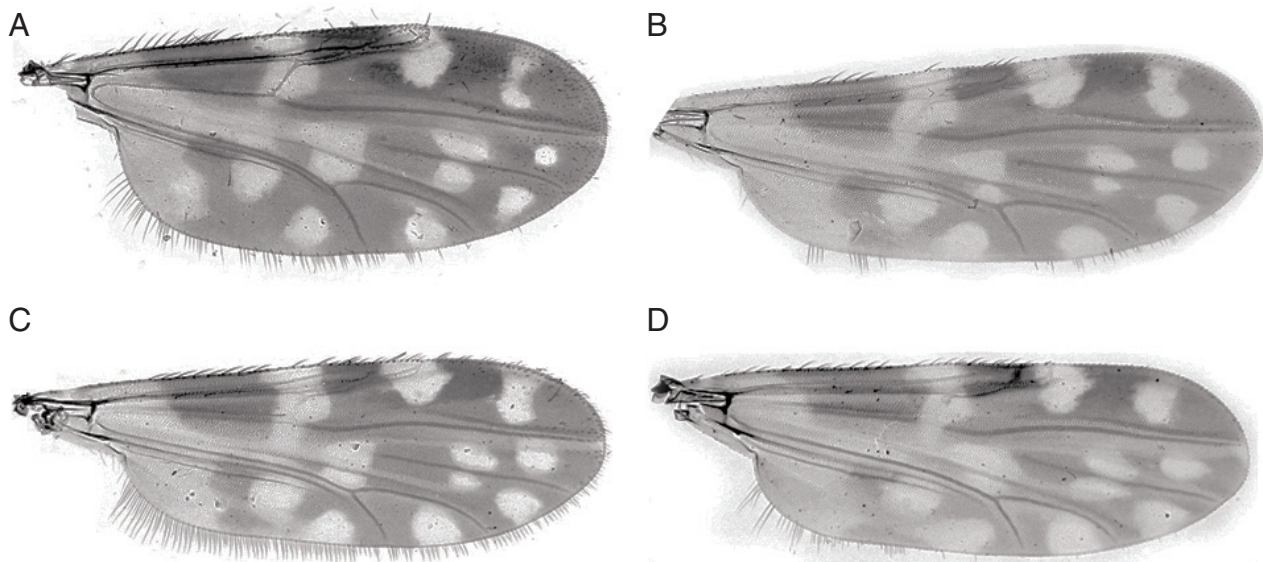
for the establishment of any new species arriving at these ports.

Seasonal studies of midge populations in Korea have only been investigated in months from April–November [2,5,6,12,13], and all showed that midge populations were already active at the start of sampling period and some additionally showed that some populations, like that observed here for *C. tainanus* on Jeju Island, were still active and even increasing at the end of the sampling period. Consequently, the onset and cessation of midge activity in Korea is not fully understood, and complete annual surveys of midge activity are needed to clarify peak periods of activity.

Novel DNA barcode data were obtained from a wide range of Asian and Australasian species including 2 female specimens of *C. lungchiensis* collected from Yonggang-dong on 15 May 2013 using methods reported in Bellis et al. [14]. Neighbor-joining analysis of DNA barcode sequences [646 bp portion of the mitochondrial cytochrome *c* oxidase I gene (COI)] recovered from the 2 Korean specimens demonstrated 99.7–100% similarity to *C. lungchiensis* (N=19) from Hainan and Zhejiang provinces in China (Fig. 3). No pre-existing *C. lungchiensis* COI sequence accessions were available for comparison at GenBank and queries of all *C. lungchiensis* sequences reported here returned a closest match of 86.7–87.6% sequence similarity to *C. peregrinus* from Australia and China (GenBank no. KF528700, KR075719–KR075721). *C. lungchiensis* is morphologically similar to *C. nipponensis* Tokunaga, and these 2 species were reciprocally monophyletic and differed by 18.8–19.8% at COI sequence comparisons (Fig. 3). *C. lungchiensis* and *C. nipponensis* sequences reported here were deposited in GenBank (accession no. KR105376–KR105396 and KR095333–KR095336, respectively), and associated specimen records are publically available as a dataset (<http://dx.doi.org/10.5883/DS-CLUNGCHI>) at the Barcode of Life Data Systems [15]. Vouchers of all of these novel specimens



**Fig. 3.** Neighbor-joining tree of COI DNA barcode sequences of taxonomically identified *Culicoides lungchiensis*, *C. peregrinus*, and *C. nipponensis*. Specimens from Korea and China indicated by closed and open circles, respectively; open and closed triangle indicate specimens from Japan and Australia, respectively. Scale bar equals 2% sequence difference (uncorrected). Bootstrap (10 K replicates) supports > 70% as indicated and estimated using MEGA version 6 [21]. Associated specimen records and sequence accessions available at <http://dx.doi.org/10.5883/DS-CLUNGCHI>.



**Fig. 4.** *Culicoides lungchiensis* Chen & Tsai. (A) Female wing. (B) Male wing. (C) Male wing variant. (D) *Culicoides nipponensis* Tokunaga, male wing.



are lodged in the Northern Territory Quarantine Insect Collection.

*C. lungchiensis* was first described as a subspecies of *C. peregrinus* Kieffer [16], but was elevated to a species status by Kitaoaka [17]. A range of morphological differences, including the absence of a sensory pit on the third palpal segment and the presence of only 1 pale spot in cell M4 (Fig. 4A, B) of *C. lungchiensis*, separates this species from *C. peregrinus*. The separation of these 2 species is here additionally supported by DNA barcode data (Fig. 3). Amongst the fauna of northeastern Asia, *C. lungchiensis* is most similar to *C. nipponensis*, which differs in the female mostly by the palpal ratio and the distribution of sensilla coeloconica (SCo) on the antennae and in the shape of the male aedeagus. Wada [11] also used the shape of the poststigmatic pale spot (distal margin angulate in *C. nipponensis*, rounded in *C. lungchiensis*) to separate females of these species. While this difference seems to be consistent in females, the wings of male *C. lungchiensis* from Lin An, Zhejiang province, China, vary in this character (Fig. 4B, C), and the distal margin of the poststigmatic pale spot of some specimens (e.g. Fig. 4C) is essentially indistinguishable from that of male *C. nipponensis* (Fig. 4D). Despite differences in the SCo distribution in females, males of both of these species have SCo distributed on flagellomeres 1, 11-13, and the shape of the aedeagus remains the most reliable means of morphologically distinguishing males of these 2 species.

The presence of *C. lungchiensis* on Jeju Island was confirmed by morphological and molecular analyses. This species was also reported from Japan (Honshu, Kyushu, Tsushima Is, Yakushima Is, Hateruma Is), Taiwan, and China (Liaoning, Fujian, Sichuan, Jiangxi and Yunnan, now including Zhejiang and Hainan) [11,18-20], so its presence in Korea is not surprising. Indeed, the number of specimens collected on Jeju Island is suggestive of a well established population, and it is likely that this species is also present on the Korean mainland as suggested by Wada [11]. The morphological similarity, particularly in male wing pattern, between this species and the widely reported *C. nipponensis*, may have resulted in specimens of *C. lungchiensis* being mistaken for *C. nipponensis* and overlooked in previous surveys. Retrospective morphological and DNA barcode analyses of mainland specimens earlier ascribed to *C. nipponensis* could be used to test this conjecture.

Evidence that *Culicoides* spp. were active when surveillance was initiated and terminated indicates the need for year-round surveillance that identifies levels of activity throughout the

year. In addition, further studies on the geographical and seasonal distributions, host attraction (e.g., placement of traps near human habitation and poultry, cattle, and swine farms), temporal biting activity, pathogen infection rates, and role as potential vectors of zoonotic pathogens that impact on human and animal health are warranted. Surveillance of both endemic and potentially imported exotic *Culicoides* spp. and their associated pathogens are an important part of the veterinary surveillance to identify potential vector populations and their distributions throughout Korea.

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## CONFLICT OF INTEREST

We have no conflict of interest related to this study.

## REFERENCES

1. Meiswinkel R, Venter GJ, Nevill EM. Vectors: *Culicoides* spp. Coetzer JAW, Tustin RC (eds.), Infectious Diseases of Livestock. Cape Town. Oxford University Press. 2004, pp. 93-136.
2. Oem JK, Chung JY, Kwon MS, Kim TK, Lee TU, Bae YC. Abundance of biting midges species (Diptera: Ceratopogonidae, *Culicoides* spp.) on cattle farms in Korea. J Vet Sci 2013; 14: 91-94.
3. Cho HC, Chong CS, Yu HS. Notes on biting midges of the *Culicoides* from South Korea. Rep. 5th Preventive Medicine Unit, 8th US Army, Yongsan, Korea. 1972, pp. 1-64.
4. Cho HC, Chong CS. Notes on biting midges of the *Culicoides* from South Korea, with special reference to unrecorded species and distribution. Korean J Parasitol 1974; 12: 45-75.
5. Kang CH, Yu HS. Seasonal abundance and host blood meal sources of the genus *Culicoides* (Diptera: Ceratopogonidae) from

- cattle and poultry farms in Gyeonggi Province, Korea. *Korean J Entomol* 1991; 21: 29-36.
6. Kim HC, Bellis GA, Kim MS, Chong ST, Lee DK, Park JY, Yeh JY, Klein TA. Seasonal abundance of biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae) collected at cowsheds in the southern part of the Republic of Korea. *Korean J Parasitol* 2012; 50: 127-131.
  7. Bellis GA, Kim HC, Kim MS, Klein TA, Lee DK, Gopurenko D. Three species of *Culicoides* Latreille (Diptera: Ceratopogonidae) newly recorded from the Republic of Korea. *Zootaxa* 2013; 3718: 171-182.
  8. Nie WZ, Li JC, Li DX, Grantz N. A preliminary report of introduced living biting midges (Diptera: Ceratopogonidae) found on entry ships arriving at Qinhuangdao Port, China. *Med Entomol Zool* 2005; 56: 359-361.
  9. Reye EJ. Report of a preliminary study of *Culicoides* infestation in French Polynesia. Report University of Queensland Department of Entomology. 1962, pp. 1-7.
  10. Arnaud P. The heleid genus *Culicoides* in Japan, Korea and Ryukyu Island (Insecta: Diptera). *Microentomol* 1956; 21: 84-207.
  11. Wada Y. *Culicoides* biting midges of Japan (Diptera: Ceratopogonidae). *Trans Nagasaki Biol Soc* 1999; 50: 45-70 (in Japanese).
  12. Lee YJ. Ecological studies in the genus *Culicoides* in Korea. M.S. Thesis. Incheon University. 1993, pp. 1-46 (in Korean).
  13. Kim HC, Bellis GA, Kim MS, Klein TA, Chong ST, Park JY. Seasonal abundance of *Culicoides* (Diptera: Ceratopogonidae) collected by Mosquito Magnet®, northern Gyeonggi Province, Republic of Korea. *Korean J Parasitol* 2014; 52: 57-62.
  14. Bellis GA, Dyce AL, Gopurenko D, Mitchell A. Revision of the Immaculatus Group of *Culicoides* Latreille (Diptera: Ceratopogonidae) from the Australasian biogeographic region with description of two new species. *Zootaxa* 2013; 3680: 15-37.
  15. Ratnasingham S, Hebert PDN. BOLD, the Barcode of Life Data System. *Mol Ecol Notes* 2007; 7: 355-364.
  16. Chen KC, Tsai LL. The bloodsucking midges (Ceratopogonidae) of Fukien. *Acta Entomol sinica* 1962; 11: 394-400 (in Chinese).
  17. Kitaoka S. Biting midges (Ceratopogonidae). In: Sasa M, et al. (eds), *Animals of Medical Importance in the Nansei Islands*. Shinjuku Shobo, Tokyo, Japan. 1977, pp. 187-200.
  18. Wada Y, Miyagi I, Takagi M, Tsuda Y. An annotated list of *Culicoides* biting midges in Yaeyama Islands in the southwestern part of Japan (Diptera: Ceratopogonidae). *Trop Med* 1996; 38: 61-68.
  19. Yu YX, Liu JH, Liu GP, Liu ZJ, Hao BS, Yan G, Zhao TS. Ceratopogonidae of China, Insecta, Diptera. Beijing, China. Military Medical Science Press. 2005 (1-2), pp. 1-1,699 (in Chinese).
  20. Liu YG, Chen HY, Liu XG, Xiong ZW, Guo XJ. Study on the species of midges of Meiling scenic area of Nanchang city. *Chin J Vector Biol Control* 2011; 22: 67-69 (in Chinese).
  21. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013; 30: 2725-2729.