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Research history of *Nannophya* Rambur (Odonata: Libellulidae): A recently discovered species in addition to *Nannophya koreana* Bae in Korea

Dong Gun Kim*

Smith College of Liberal Arts, Sahmyook University, Seoul 01795, Republic of Korea

***Corresponding author**
Dong Gun Kim
Tel. 02-3399-1919
E-mail. ecology@syu.ac.kr

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Abstract: The *Nannophya* species in Korea was thought to consist of only *Nannophya pygmaea*. Previous studies on the species, including life history and development, conservation and restoration, habitat characteristics, genetic studies, distribution, behavior, and taxonomy have been conducted. However, a new *Nannophya* species, *Nannophya koreana*, was recently discovered in Korea. Moreover, this new species was found to inhabit both Korea and Japan. Thus, the previous studies should be re-evaluated in relation to the new species, *Nannophya koreana*, and the latter should be treated as an endangered species worldwide given the current population instability.

Keywords: *Nannophya*, *Nannophya pygmaea*, *Nannophya koreana*, new species, research history

INTRODUCTION

Nannophya is a genus belonging to the family Libellulidae, which includes the world's smallest dragonflies. They are commonly known as pygmy flies and are found in Asia and Australia. The genus of *Nannophya* includes the seven species *Nannophya australis*, *N. dalei*, *N. katrainensis*, *N. occidentalis*, *N. paulsoni*, *N. pygmaea*, and *N. koreana* (Corbet 1999; Bae *et al.* 2020). Of these, four species occur only in Australia, and one species, *N. katrainensis* is found in the Himalayas. However, there is limited information on *N. katrainensis*, and few records, so more research is required. *N. pygmaea* is found worldwide, and its distribution includes Australia, tropical and subtropical areas of Southeast Asia, China, Japan, India, and Korea, however, *N. pygmaea* in Korea and Japan recently have been discovered to be *N. koreana* (Kim *et al.* 2010; Bae *et al.* 2020).

N. koreana was first collected from Songnisan (MT.) in Korea by Professor Chang Whan Kim (Korea University, Korea) in 1957 (Kim *et al.* 2010; Bae *et al.* 2020). This species was designated as an endangered species, category level II by the Ministry of Environment of Korea in 1988 (Bae *et al.* 1999) and was listed in the Red Data Book of Korea (National Institute of Biological Resources 2013), but still known as the *N. pygmaea*. For the last 60 years, this species was known solely as *N. pygmaea*, and several intensive studies investigated its distribution and habitat, life history and development, and conservation and habitat restoration in Korea and Japan (Bae *et al.* 2020). The habitats of *N. koreana* (previously known as the *N. pygmaea*) are known from more than 20 localities throughout Korea, but now only a few sites are maintaining stable populations (Oh *et al.* 2017).

Recently, the *Nannophya* species group in Korea, Japan, and other countries was thoroughly investigated (Bae *et al.* 2020). Although the *Nannophya* species in Korea and

Japan have a mean genetic distance of 0.88%, this was regarded as an issue at the intraspecific level. As a result, the *Nannophya* species in Korea was hailed as a new species, *Nannophya koreana* (Bae *et al.* 2020).

Therefore, this study was conducted to evaluate all the previous studies, modify the species information, and utilize it as basic data for the preservation and protection of *Nannophya* species.

MATERIALS AND METHODS

For this study, text mining analysis was performed to identify research relevant to *Nannophya* species. For the data collection, we used several scientific web data library for broad research fields including taxonomy, ecology, genetic study and so on. The data analysis was carried out based on scientific articles and thesis that are searched for this study.

1. Data collection

In this study, we collected the Korean research related to *Nannophya* through the Research Information Sharing Services (RISS, http://www.riss.kr) and DBPIA (http://www.dbpia.co.kr/). In the case of foreign research, we collected the information through the Web of Science (http://www.webofknowledge.com/), Scopus (http://www.scopus.com/), and Wiley Online Library (http://www.onlinelibrary.wiley.com). The search keywords for the study were 'TITLE-ABS-KEY (*Nannophya*).'

2. Data selection

Of the many results, we selected only scientific journals and theses related to the *Nannophya* species. The tools used to extract keywords were R 3.6.2 ver, and the frequency was analyzed by extracting keywords from the title of each research paper. While analyzing frequency, stop word processing was performed for data purification, and keywords related to *Nannophya* were extracted.

RESULTS AND DISCUSSION

We extracted 16 studies from Web of Science, 15 studies from Scopus, 18 studies from Wiley online library, 18 studies from RISS, and 18 studies from DBPIA. For data analysis, we removed duplications and selected 31 studies, including four master dissertations and 27 scientific papers finally.

Twenty-one studies were conducted by Korean scientists and 10 by scientists from other countries. Among them, eight were Japanese, and the other two were Malaysian and Australian, respectively. The oldest study was conducted in 1978 and the most recent study in 2020. The largest number of studies were conducted in 2008 and 2010, with one or two studies being conducted each year on average (Fig. 1).

We extracted the 148 keywords through data purification processes such as stop word and misprint (Fig. 2). Species name showed a high frequency and locality, and other subject-related words showed a high frequency. Our results identified the study subject, area, and species. According to the research focus, the studies were divided into seven categories: distribution, behavior, life history and development, conservation and restoration, habitat characteristics, genetic studies, and taxonomy (Fig. 3, Table 1).

1. Distribution

This subject was covered by two studies, in Korea and Japan. Fujita *et al.* (1978) described the seasonal changes in population size and distribution of *N. pygmaea*. Bae *et al.* (1999) also added a new habitat from the southwestern part of Korea. Although Kim (1997) had reported a new habitat of *N. pygmaea* in Korea, we put this study in the conservation and restoration category because it focused on the protection and conservation of the population.

2. Behavior

In this subject, all studies were conducted by Japanese scientists Tsubaki and Ono (1985, 1986, 1987, 1995) and Tsubaki *et al.* (1994). They intensively studied the territorial site selection, mating, and guarding behavior after mating. All the studies were based on field observations in natural habitats of *N. pygmaea* in Japan.

3. Life History and Development

This subject included six studies, most of which, except a study by Siva-Jothy and Tsubaki 1994, were conducted from 2006 to 2010. Siva-Jothy and Tsubaki (1994) studied the sperm competition mechanism and the advantage of sperm re-positioning. Other studies focused on the effect of different temperature conditions on egg development

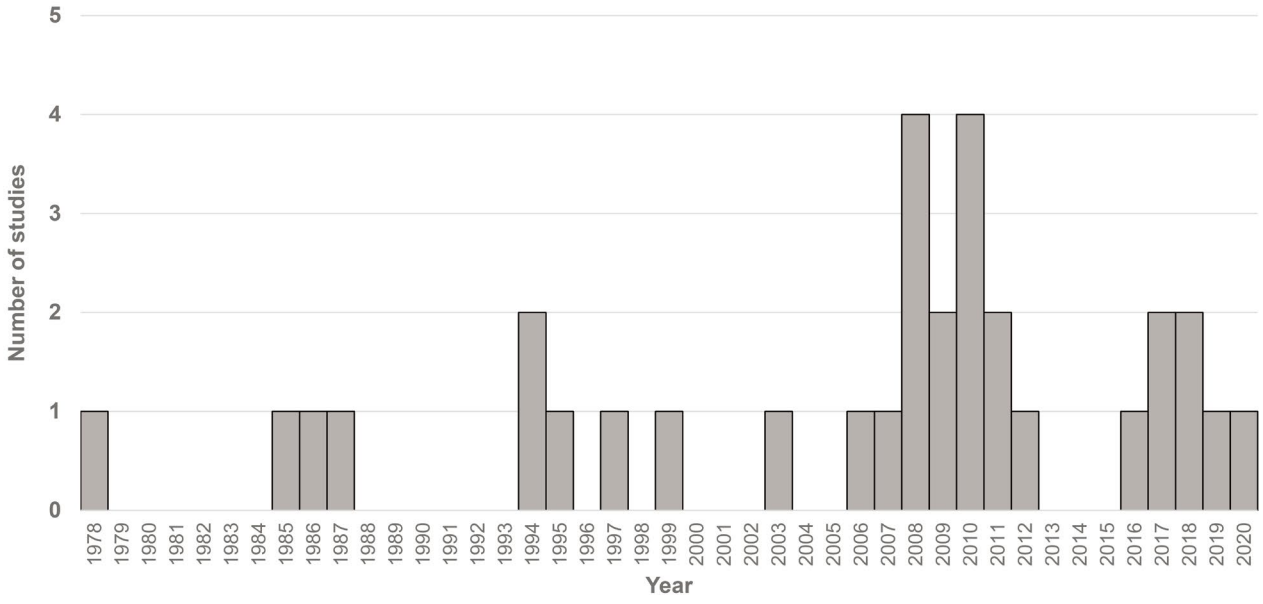

Fig. 1. The number of annual studies relevant to *Nannophya* species.

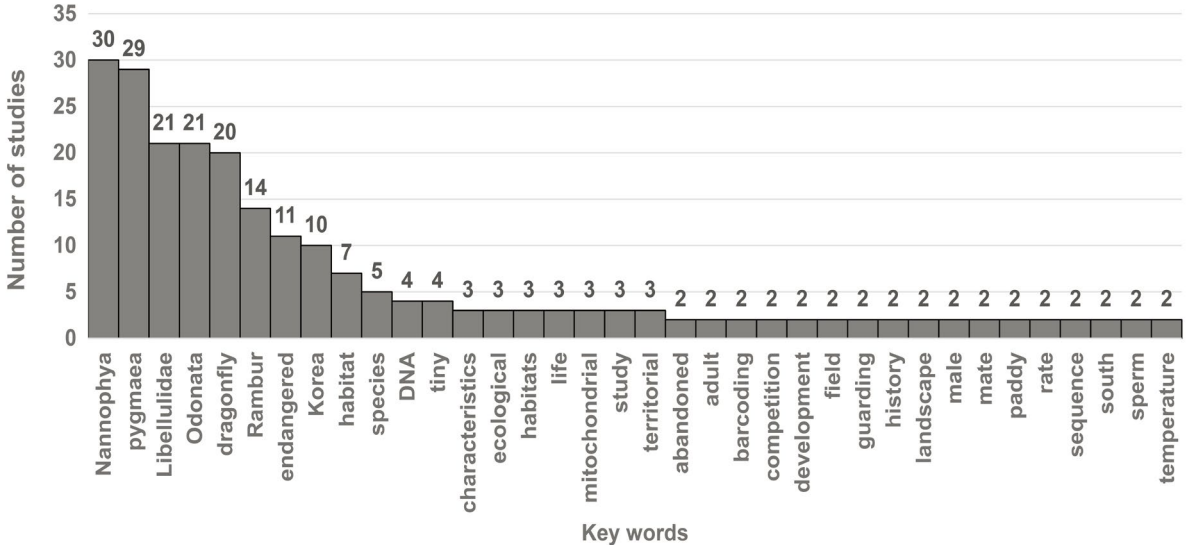

Fig. 2. The extracted keywords of the title and the cited number of studies.

and the growth rate of larvae (Kim *et al.* 2006; Kim 2008; Kim *et al.* 2009a, 2009b). Based on these studies, Kim *et al.* (2010a) investigated aspects of the life history of *N. pygmaea* in an abandoned paddy field in Korea. Through these studies, they discovered that the Korean population of *N. pygmaea* were univoltine species.

4. Conservation and Restoration

N. pygmaea was designated an endangered species by the Ministry of Environment of Korea. For these reasons, Korean scientists suggested protection and preservation of *N. pygmaea* and its natural habitat (Kim 1997; Oh *et al.* 2017). Furthermore, Park (2008) investigated the habitat

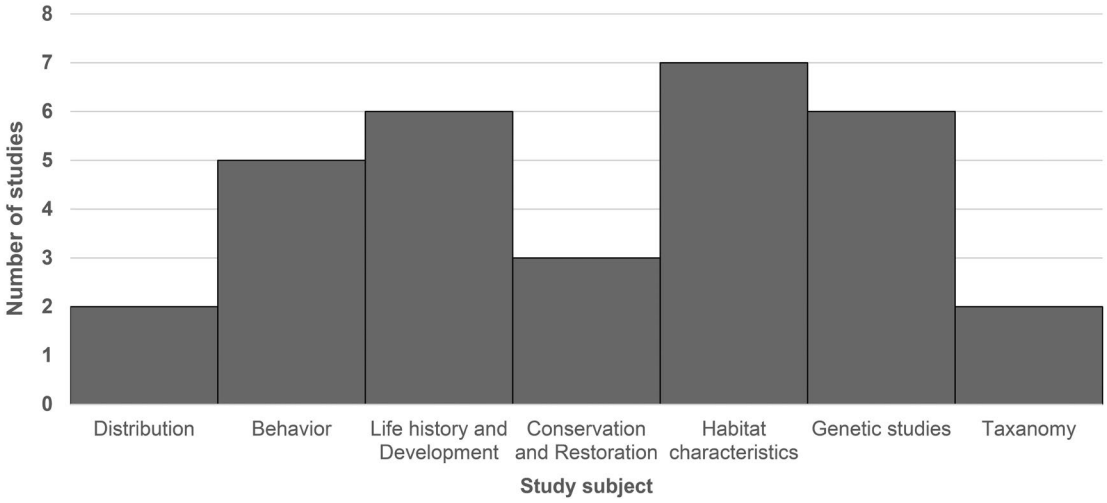

Fig. 3. The number of studies based on the research subject.

preferences of *N. pygmaea* and, based on the results, suggested the wetland construction model for restoration of *N. pygmaea* habitat.

5. Habitat characteristics

Habitat was the subject most intensively studied, and most research was carried out from 2008 to 2012. Most studies investigated the landscape properties, habitat size, vegetation types, water environments, water sources, and composition of the flora in the natural habitat of *N. pygmaea* (Lee *et al.* 2008; Yoon 2008; Kim *et al.* 2010b; Yoon *et al.* 2010, Kim 2011a; Kim 2011b; Cho *et al.* 2012).

6. Genetic study

Intensive studies on the genetics of *N. pygmaea* were recently conducted with the use of molecular techniques. Kim *et al.* (2007) firstly sequenced a portion of the mitochondrial COI gene, corresponding to the DNA barcode region (658 bp) using 40 individuals of *N. pygmaea* in Korea. Low *et al.* (2016) assessed the genetic diversity using the DNA barcode data of *N. pygmaea*. In addition, Wang *et al.* (2017) sequenced an additional mitochondrial gene (ND5) in the mitochondrial genome of *N. pygmaea*, and Okude *et al.* (2017) established an electroporation-mediated RNA interference (RNAi) procedure. Jeong *et al.* (2018) sequenced the complete 15,112-bp-long mitochondrial genome (mitogenome) of *N. pygmaea*, and Kim *et al.* (2018) developed 12 microsatellite markers using the NextSeq 500 platform. These studies have become important evidence for the discovery of a new Korean species of *Nannophya*.

7. Taxonomy

Before the study of Theischinger (2003), only five *Nannophya* species, including *N. australis*, *N. dalei*, *N. katrainensis*, *N. occidentalis*, and *N. pygmaea*, were known in the world. However, this study described a new *Nannophya* species from Australia and named it *N. paulsoni*.

Recently, Bae *et al.* (2020) described a new species from Korea based on morphology and mitochondrial cytochrome oxidase c subunit I (COI) gene sequences. This new species was named *N. koreana*.

According to Bae *et al.* (2020), the male and female adults of *N. koreana* can be morphologically distinguished from *N. pygmaea*, by characters such as the lateral synthorax stripe, anal appendage color, superior appendage teeth number, and basal wing pigmentation. Moreover, the body size of *N. koreana* is generally 1.2−1.4 times larger than that of *N. pygmaea*. Based on this study, *N. pygmaea* will have to be treated as a different species to *N. koreana* in Korea from now on.

Currently, *N. pygmaea* has stable populations worldwide, having the least concern designation in the IUCN (International Union for Conservation of Nature) red list (Karube 2009). However, the *Nannophya* species of Korea and Japan now include a new species, which is distinct from the existing *N. pygmaea*, and the conservation value of the Korean populations has consequently increased. In particular, the Korean population appears to be decreasing because

Table 1. Study subjects and summary of previous studies on *Nannophya* species

Study subjects	Study species	Summary	References
Distribution	*Nannophya pygmaea*	Seasonal changes in the population size and distribution in habitat	Fujita *et al.* 1978
	Nannophya pygmaea	Description of *Nannophya pygmaea* from the southwestern part of the Korean peninsula	Bae *et al.* 1999
Behavior	*Nannophya pygmaea*	Guarding behavior of male	Tsubaki and Ono 1985
	Nannophya pygmaea	Mating behavior such as competition for territorial sites and alternative mating tactics	Tsubaki and Ono 1986
	Nannophya pygmaea	Relationship between male size and mating success and effect of age on the male territorial system	Tsubaki and Ono 1987
	Nannophya pygmaea	Benefits of re-copulation and post-copulatory mate guarding behavior	Tsubaki *et al.* 1994
	Nannophya pygmaea	Territorial site selection of male	Tsubaki and Ono 1995
Life history and Development	*Nannophya pygmaea*	Sperm competition mechanism and advantage of sperm re-positioning	Siva-Jothy and Tsubaki 1994
	Nannophya pygmaea	Hatching rate of eggs under different temperature conditions	Kim *et al.* 2006
	Nannophya pygmaea	Master dissertation including study of Kim *et al.* 2006, 2009a, 2009b, 2010	Kim 2008
	Nannophya pygmaea	The nonlinear model of temperature in relation to egg development	Kim *et al.* 2009a
	Nannophya pygmaea	Larval development in an artificial wetland habitat	Kim *et al.* 2009b
	Nannophya pygmaea	Aspects of life history in an abandoned paddy field in Korea	Kim *et al.* 2010a
Conservation and Restoration	*Nannophya pygmaea*	Suggestions for protection and conservation of populations in Korea	Kim 1997
	Nannophya pygmaea	Master dissertation on wetland construction for habitat restoration	Park 2008
	Nannophya pygmaea	Suggestions for stronger protection in the preservation manual and in the species' native habitats in Korea	Oh *et al.* 2017
Habitat characteristics	*Nannophya pygmaea*	Investigation of landscape properties, habitat size, vegetation types, water environments and water sources in natural habitat.	Lee *et al.* 2008
	Nannophya pygmaea	Master dissertation to analyze the characteristics of the habitat and investigate the features of *Juncus effusus*, a plant species dominant in habitat	Yoon 2008
	Nannophya pygmaea	Environments and floral composition of the habitats in abandoned paddy fields	Kim *et al.* 2010b
	Nannophya pygmaea	Habitat characteristics of different successional stages	Yoon *et al.* 2010
	Nannophya pygmaea	Species relocation due to changes in habitat	Kim 2011a
	Nannophya pygmaea	Master dissertation on landscape and ecological characteristics of habitat in Korea	Kim 2011b
	Nannophya pygmaea	The ecological characteristics of the habitats in abandoned paddy fields	Cho *et al.* 2012
Genetic study	*Nannophya pygmaea*	Sequenced a portion of mitochondrial COI gene, corresponding the DNA barcode region (658bp)	Kim *et al.* 2007
	Nannophya pygmaea	The utility of DNA barcode data for assessing genetic diversity	Low *et al.* 2016
	Nannophya pygmaea	Sequenced an additional mitochondrial gene (ND5) in the mitochondrial genome	Wang *et al.* 2017
	Nannophya pygmaea	Established an electroporation-mediated RNA interference (RNAi) procedure	Okude *et al.* 2017
	Nannophya pygmaea	Sequenced the complete 15,112-bp-long mitochondrial genome (mitogenome)	Jeong *et al.* 2018
	Nannophya pygmaea	Developed 12 microsatellite markers using the NextSeq 500 platform	Kim *et al.* 2018
Taxonomy	*Nannophya paulsoi*	Described a new species of *Nannophya*	Theischinger 2003
	Nannophya koreana	Described a new species from Korea based on morphology and mitochondrial cytochrome oxidase c subunit I (COI) gene sequences	Bae *et al.* 2020

of increasing habitat loss caused by natural succession and climate changes, such as drought (Oh *et al.* 2017).

In conclusion, most studies on *Nannophya* species are limited to the *N. pygmaea* in Korea and Japan. Furthermore, the species was identified to be a new one named as the *N. koreana*. However, because the *Nannophya* species was designated as the endangered species in Korea and Japan, collecting its specimen and research is not sufficient. Thus, it would be reasonable to consider that *N. koreana* and *N. pygmaea* are existing in both countries although more studies are needed to clarify this ambiguity. Either way, we must strive to preserve and protect both *N. koreana* and *N. pygmaea*. In this regard, we suggest that the IUCN red list should be revised to include the species as Endangered (EN) or Critically Endangered (CR) in the threat level.

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