

**NYCTERIDOPSYLLA QUADRISPINA LU AND WU, A JUNIOR SYNONYM
OF NYCTERIDOPSYLLA IAE BEAUCOURNU AND KOCK
(SIPHONAPTERA: ISCHNOPSYLLIDAE) AS DETERMINED BY
MORPHOLOGICAL AND DNA ANALYSES**

MICHAEL W. HASTRITER, SARAH E. BUSH, KATHARINA DITTMAR, S. S. HLA BU, AND
MICHAEL F. WHITING

(MWH) Monte L. Bean Life Science Museum, Brigham Young University, P.O. Box 20200, Provo, Utah 84602-0200, U.S.A. (e-mail: michaelhastriter@comcast.net); (SEB) Natural History Museum, The University of Kansas, Lawrence, Kansas 66045, U.S.A.; (KD) SUNY at Buffalo, Department of Biological Science, 109 Cooke Hall, Buffalo, New York 14260, U.S.A.; (SSH) Zoology Department, University of Mandalay, Myanmar; (MFW) Department of Biology, 693 Widsoe Building, Brigham Young University, Provo, Utah 84602, U.S.A.

Abstract.—*Nycteridopsylla iae* Beaucournu and Kock, 1992 and *Nycteridopsylla quadrispina* Lu and Wu, 2003 have heretofore been known only by the male and female sexes, respectively. The morphological differences in these two species are striking; however, a recent collection of male *N. iae* and female *N. quadrispina* from a single host individual of the Great Evening Bat (*I. io* Thomas), led us to suspect that *N. iae* and *N. quadrispina* represented the same species. Morphological similarities were noted and male and female exemplars were sequenced using five genes (18S, 28S, 16S, 12S, and COII). Across the five sequenced genes from both specimens (male and female), sequence identity was 100%. Considering morphological characteristics and DNA analysis, *N. quadrispina* is indisputably a junior synonym of *N. iae*. Myanmar is a new country record for *N. iae*, *Harpiocephalus harpia* (Temminck) is a new host record for *N. iae* and the preferred host is the Great Evening Bat, *I. io*.

Key Words: bat, flea, *Ia io* phylogeny

INTRODUCTION

The genus *Nycteridopsylla* is represented in China and Southeast Asia by seven species: [*N. dicondylata* Wang (China), *N. galba* Dampf (China, Japan), *N. iae* Beaucournu and Kock (China, Thailand), *N. liui* Wu, Chen and Liu (China), *N. nippono* Sakaguti and Jameson (Japan), *N. quadrispina* Lu and Wu (China), and *N. sakagutii* Jameson and

Suyemoto (Korea)]. The bat flea *N. iae* collected from the Great Evening Bat *Ia io* Thomas (Vespertilionidae) in Chiang Mai Province, Thailand was described from two male specimens in the absence of females. The female has never been described. Subsequently, Lu and Wu (2003) described *N. quadrispina* from a single female from the same bat host species from Hubei Province, China. Dr. Lu kindly provided two males of these specimens for DNA analysis as part of a

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larger endeavor to establish phylogenies in the order Siphonaptera based on molecular technology.

In 2007, Dr. Bush (SEB) collected four bat fleas from Shuipu Village, Libo County, Guizhou Province, China. These included two females and one male collected from one *I. io* (slide P-2163) and a single female collected from a second bat species *Harpiocephalus harpia* (Temminck) (slide P-1781). An additional female *N. quadrispina* was also collected in 2008 from *I. io* during Dr. Hla Bu's (SSHB) studies in Myanmar. The single male and four females were conspecific with the respective known sexes of *N. iae* and *N. quadrispina*. Since both a male and two females were collected from the same specimen and same host species, it was suspected that the males and females might belong to the same species. This provided an opportunity to compare these two species, each described from a single (and opposite) sex. A male from Guizhou Province and a female from Myanmar were submitted to Dr. Dittmar (KD) for DNA analysis and the male and other females were mounted on microscope slides. DNA and morphological comparisons of the bat fleas *N. iae* and *N. quadrispina* are the subject of this paper.

MATERIALS AND METHODS

Bats were collected in Guizhou Province, China by Dr. Bush (SEB) and from Northern Shan State, Myanmar by Dr. Hla Bu (SSHB). The method of capture, handling, and collection of ectoparasites from bats in China is described in Hastriter and Bush (2006). The single flea from Myanmar was removed from a hand collected male *I. io* in torpor condition during Dr. Hla Bu's studies of this bat species. To facilitate identification, fleas were mounted on glass slides (Hastriter and Whiting 2003). Gene sampling was done in accordance with Whiting et al. (2008), and primer se-

quences and protocols are given in Whiting (2002) and Dittmar et al. (2006). In addition to the genes (28S, 18S, and COII) sequenced by Whiting et al. (2008), two more genes—12S and 16S—were targeted. Two taxa were used as out-groups representing two families (Pulicidae: *Pulex irritans* Linnaeus and Leptopsyllidae: *Frontopsylla nakagawai borealosinica* Liu, Wu, and Chang). In-groups within the subfamily Ischnopsyllinae included: *Myodopsylla gentilis* Jordan and Rothschild, *Myodopsylla insignis* (Rothschild), *Myodopsylla palposa* (Rothschild), *N. iae*, *N. quadrispina* (herein theoretically probable female of *N. iae*) and *Sternopsylla distincta texana* (C. Fox) (Fig. 2).

RESULTS AND DISCUSSION

Nycteridopsylla iae Beaucournu and Kock, 1992
(Figs. 1–2)

Nycteridopsylla iae Beaucournu and Kock, 1992. Senckenbergiana Biology 72: 329.

Nycteridopsylla quadrispina Lu and Wu, 2003: 57. **New synonymy.**

Material Examined. China: Hubei Province, Badong, ex *I. io*, Lu Liang (1 ♂, DNA voucher, slide F-232); Guizhou Province, Libo County, Shuipu Village, (25°29.079'N 107°49.080'E), ex *I. io*, 15-IV-2007, S.E. Bush (1 ♂, 2 ♀, slide P-2163); same data except ex *H. harpia* (1 ♀, slide P-1781). Myanmar: Northern Shan State, Lagang Cave, Kui Kai, (23°32.576'N, 97°56.138'E), ex *I. io*, male, 1-I-2008, M. Roi Lum (1 ♀, DNA voucher, slide MH-859/F-293). DNA voucher specimens are deposited in the BYU flea collection. Other specimens remain in the senior author's collection.

Although there are differences in the shape of their antennae and the shape of their head capsules, sexual dimorphism

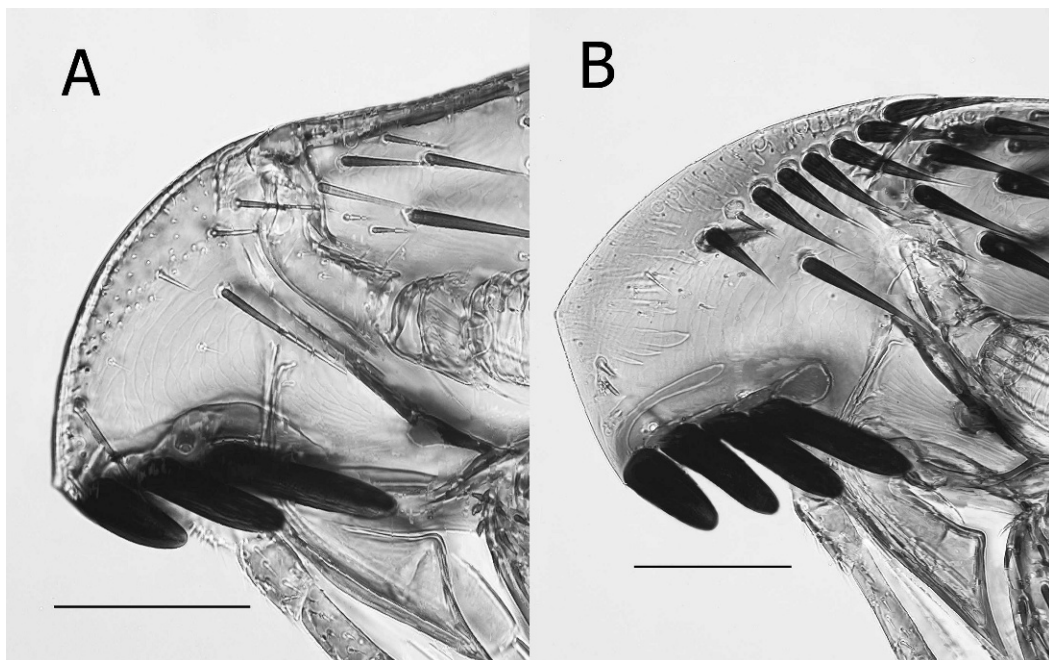


Fig. 1. A–B. *Nycteridopsylla iae*, anterior head and genal ctenidia. A. Male. B. Female (previously considered *Nycteridopsylla quadrispina*). Scale = 100 μ .

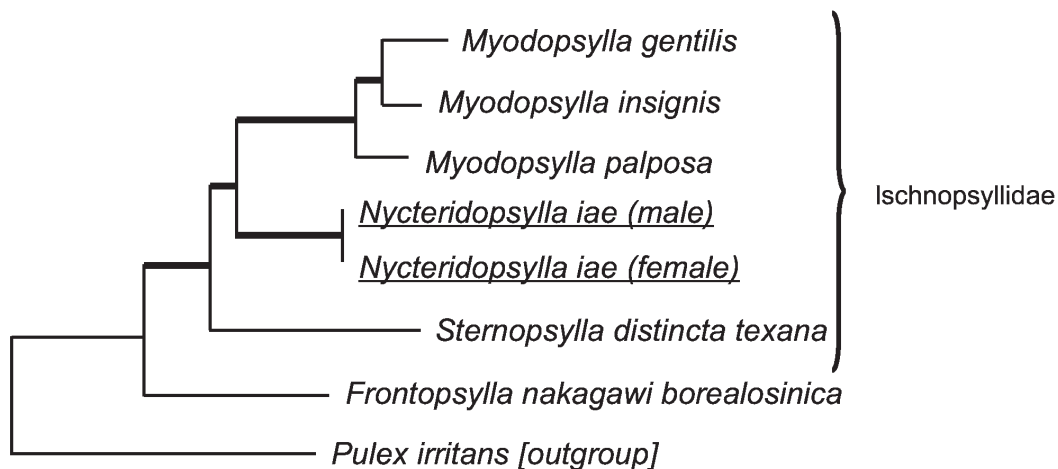


Fig. 2. Maximum Likelihood phylogram of taxa sequenced within the subfamily Ischnopsyllinae ($-\ln L = 23849.1$). The topology was derived from the concatenated dataset. **Pulex irritans** was identified as outgroup. Broad branches denote bootstrap supports $> 85\%$. GenBank accession numbers: Female *Nycteridopsylla iae*: COII - FJ422784, 28S - FJ422786, 12S - FJ422788, 16S - FJ422790, 18S - FJ422792. Male *Nycteridopsylla iae*: COII - FJ422785, 28S - FJ422787, 12S - FJ422789, 16S - FJ422791, 18S - FJ422793.

among bat fleas is most notably demonstrated in the genitalia and modified abdominal segments. The usual number of preoral genal spines within the subfamily (n = 119 species) Ischnopsyllinae Wahlgren is two. *Nycteridopsylla iae* and *N. quadrispina* are exceptional. *Nycteridopsylla iae* (known only by the male sex) possesses three preoral genal spines, while *N. quadrispina* (known only by the female sex) has four preoral genal spines (Fig. 1A–B). Setae adorning the head, thorax, and abdomen are considerably more spiniform in females of *N. quadrispina* than in males of *N. iae*. With these differences (somatic setae and preoral genal spines), one might conclude that *N. iae* and *N. quadrispina*, indeed represent different species. The male holotype of *N. iae* was not examined, but the setae of the head, thorax, and abdomen of males of *N. iae* from two areas in China (Hubei and Guizhou Provinces) are much stouter than those in the holotype illustrated by Beaucournu and Kock (1992) from Thailand. A comparison of the morphology of the head, preoral spines and nature of the setae of male and female sexes of *N. vancouverensis* Wagner with that of *N. iae* and *N. quadrispina* was conducted. The head, thorax, and abdominal setae of males and females of *N. vancouverensis* demonstrate a similar pattern in that although males have stout and somewhat spiniform setae (as does *N. iae*), the setae of females of *N. vancouverensis* and *N. quadrispina* are substantially stouter, darkly pigmented, and distinctly more spiniform than that of their male counterparts. Combined with the fact that the preoral genal spines (three in male, four in female) are unique in the subfamily Ischnopsyllinae and that the setae of both sexes are quite spiniform suggested to us that they might belong to the same taxon. It is noteworthy that Lu and Wu (2003) collected seven specimens of *Nycteridopsylla* Oudemans

(one female *N. quadrispina* and 6 males of *N. iae*) from only two *I. io* specimens. All seven specimens were removed from two bats placed in the same bag prior to examination for ectoparasites, thus it is impossible to determine if the female occurred on the same bat as any of the six male fleas (Lu Pers. Comm.). Thus, our record from Guizhou Province, China is the only record validating that accompanying males and females occur on the same host specimen.

Across the five sequenced genes (18S, 28S, 16S, 12S, and COII) from both specimens (male and female), sequence identity was 100%. Based on previous studies, this suggests that both specimens belong to the same species, despite their apparently different morphology. In order to assert their phylogenetic position, sequences were aligned with other known taxa representative of Ischnopsyllinae 18S sequences using the E-INS-i algorithm in MAFFT (Katoh et al. 2005). Maximum Parsimony analysis conducted with PAUP* clearly shows both *N. iae* sequences as nested within the subfamily Ischnopsyllinae (Fig. 2). Based on DNA analysis, *Nycteridopsylla quadrispina* is indisputably a junior synonym of *N. iae*. The GenBank accession numbers for the respective female and male sexes of *N. iae* for each sequenced gene are COII: FJ422784, FJ422785; 28S: FJ422786, FJ422787; 12S: FJ422788, FJ422789; 16S: FJ422790, FJ422791; and 18S: FJ422792, FJ422793.

Although *I. io* has been documented across a broad region of eastern Asia and Southeast Asia (northeastern India, Nepal, Myanmar, Laos People's Democratic Republic, southeastern China, Thailand and northern Vietnam), *N. iae* has only been reported in two Chinese Provinces, Myanmar, and Thailand. The occurrence of *N. iae* in Myanmar is a new country record. Its distribution likely follows what appears to be its primary host, *I. io*. *H. harpia* is a new

host record for *N. iae*. Future collecting efforts are needed to elucidate the importance of both *H. harpia* and *I. io* in the development and propagation of this unique bat flea and to elucidate the possible distribution in other regions where *I. io* occur.

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